

Molecular phylogenetic and morphological analyses support recognition of a new species of Vincetoxicum (Apocynaceae, Asclepiadoideae) from eastern Thailand

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Research Article

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Abstract

An unknown Vincetoxicum species has recently been discovered in northeastern Thailand. It is a twiner that resembles in its morphology and ecology *V. flexuosum* var. *flexuosum*, a variable species widely occurring from tropical Asia to Australia. Comprehensive morphological examination showed that despite similarities in growth habit, leaf shape, branching of the inflorescence and flower colour, these two taxa exhibit substantial differences in both vegetative and reproductive traits that clearly distinguish one from the other, particularly shape of the flower bud and indumentum on the corolla lobes. In addition, we also evaluated phylogenetic relationships based on DNA sequence data for ITS, trnT-L, trnL, and trnL-F markers of this new Vincetoxicum sp. with congeners (including, inter alia, new sequences of V. flexuosum var. flexuosum and also those of V. flexuosum var. tenuis, the other variety occurring in Thailand). The analyses demonstrated that the new *Vincetoxicum* sp. is not closely related to the taxa recognized as V. flexuosum. The new species was instead retrieved as sister to a clade containing the African taxa, V. caffrum, V. lycioides and V. fleckii. Therefore, integrated analyses of morphology and molecular phylogeny revealed Vincetoxicum sp. to be a well-defined species clearly distinct from V. flexuosum, as well as from all other known congeners. The morphological similarity between the new Vincetoxicum sp. and V. flexuosum var. flexuosum likely resulted from convergence, leading to various taxonomic complications. We here describe it as a new species, V. sangyojarniae, sp. nov., and provide a detailed description, illustration, photographs, and comparison to the morphologically similar *V. flexuosum* var. *flexuosum*. A preliminary taxonomic reconsideration of the infraspecific taxa under V. flexuosum is also suggested.

Introduction

The genus *Vincetoxicum* Wolf has a complex taxonomic history. It has been wrongly placed in *Cynanchum* L. of the subtribe Cynanchinae K. Schum. (Hooker 1883; Forster 1991; Yamazaki 1993; Gilbert et al. 1995; Li et al. 1995) owing to some similarities in morphology (Hooker 1883; Forster 1991; Yamazaki 1993; Gilbert et al. 1995; Li et al. 1995). After several taxonomic revisions and molecular phylognenetic studies over the course of twenty years (Liede 1996; Liede 2001; Liede-Schumann et al. 2012; Liede-Schumann et al. 2016; Liede-Schumann and Meve 2018; Endress et al. 2018; Güven et al. 2019), the generic delimitation of *Vincetoxicum* has been clarified. All former genera of the subtribe Tylophorinae (Liede 2001), including *Tylophora* and the other smaller genera, except for *Pentatropis* R. Br. ex Wight & Arn., have been transferred to *Vincetoxicum* (Liede-Schumann and Meve 2018).

Vincetoxicum sensu lato, under its broadened circumscription, is largely characterized by clear latex (rarely yellowish or whitish in some species of the former *Tylophora*), twining or erect stems, small flowers with a gynostegial corona formed by five mostly separate staminal lobes, and small round pollinia (Forster 1991; Liede-Schumann et al. 2012, 2016). There are more than 200 species naturally distributed in Europe, Africa, Australia, and Asia. Some invasive species also occur in North America (Yamashiro et al. 2004; Tseng and Chao 2011; Liede-Schumann et al. 2016; Jiang et al. 2018; Liede-Schumann et al. 2018; Shah et al. 2018). In Thailand, the 14 known species are found from sea level up to an elevation of ca. 1,600 m a. s. l. (Thaithong et al. 2018; Kidyoo 2016, 2020).

In 2021, an unknown climbing *Vincetoxicum* was found in the protected area within Rajamangala University of Technology Isan Surin Campus, eastern Thailand. At first glance, it looks similar to *V. flexuosum* (R. Br.) Kuntze (Brown, 1810; Kuntze, 1891), a variable species widespread from tropical and subtropical Asia to northern Australia (Liede-Schumann et al. 2012). Owing to its considerable morphological variation, *V. flexuosum* has been recognized as a species complex (Schneidt 1999; Liede et al. 2002; Liede-Schumann et al. 2012). Recently, three

varieties have been assigned to this taxon, *V. flexuosum* var. *flexuosum*, *V. flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede and *V. flexuosum* var. *perrottetianum* (Decne.) Schneidt, Meve & Liede. The latter two varieties were formerly recognized as two separate species. In Thailand, *V. flexuosum* var. *flexuosum* and *V. flexuosum* var. *tenuis* are found in different regions (Thaithong et al. 2018). Our newly discovered *Vincetoxicum* species, although having several morphological traits that overlap with those of *V. flexuosum* var. *flexuosum*, there are consistent characters that can be used to distinguish one from the other. Detailed comparisons of the similarities and differences in morphology and ecology between these two taxa are provided. Moreover, to corroborate the separation of these two entities, their systematic positions based on ITS and *trn*T-L, *trn*L, and *trn*L-F DNA sequence data were investigated.

Materials and methods

Molecular phylogenetic analysis

To evaluate the taxonomic status of the unknown *Vincetoxicum* species and its phylogenetic relationships with congeners, we included new DNA sequences of one accession of this plant, eight of *V. flexuosum* var. *flexuosum*, and four of *V. flexuosum* var. *tenuis* (Blume) Schneidt, Meve & Liede, all newly collected from different locations throughout Thailand (see Table 1 for voucher numbers and sampling locations), in a phylogenetic analysis of the genus based on combined ITS (nuclear marker) and cpDNA datasets (Fig. 1). All sequences have been deposited in GenBank (Table 1). DNA extraction and sequencing protocols were performed as described in Kidyoo et al. (2021). Three accessions of *Pentatropis*, the remaining genus of the subtribe Tylophorinae, were downloaded from GenBank (Liede-Schumann et al. 2016) and used as outgroup taxa. In addition, the homologous sequences of 29 other *Vincetoxicum* spp. were downloaded from GenBank (Liede et al. 2002; Liede-Schumann et al. 2012, 2016; Xiong et al. 2020; see Table S1) for comparative analysis.

Phylogenetic analyses: MUSCLE sequence alignments were made using Mesquite v.3.51 (Maddison and Maddison 2019). PartitionFinder v.2.0.0 (Lanfear et al. 2016) was used to select a partition scheme and the nucleotide substitution models, based on the Akaike information criterion. Prior data blocks were defined by marker and codon position. The best-fit models for the combined ITS and cpDNA markers was GTR+I+G. Phylogenetic reconstructions based on the concatenated ITS and cpDNA datasets were performed using Bayesian inference with MrBayes v. 3 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Two analyses of four chains were run for 1,000,000 generations (after which the split deviation frequency value was lower than 0.01), sampling trees every 500 generations with 25 % burn-in samples discarded for each run. The remaining trees were used to build a majority-rule consensus tree and to calculate Bayesian posterior probabilities (PP). The consensus trees were visualized using Mesquite v.3.61 (Maddison and Maddison 2019).

Taxonomic study

Samples of the unknown *Vincetoxicum* species were collected from the plant's natural habitats in Surin Province during 2021 and 2022. Voucher specimens were prepared and deposited at BKF and BCU. Vegetative and reproductive structures of the newly collected specimens and of specimens preserved in herbaria (BK, BKF, QBG, BCU, K, L, BM, and P) were examined and compared. Furthermore, the similar *V. flexuosum* var. *flexuosum* was also included for comparison. Morphological characteristics were mainly observed under light microscopy (LM).

Results

Molecular phylogenetic analysis

GenBank accession numbers for the new sequences generated for this study are OP921231 to OP921246 for ITS and OP923868 to OP923893 for cpDNA (Table 1). Bayesian analyses of combined ITS and cpDNA sequences show that the specimens of *V. flexuosum* var. *flexuosum*, *V. flexuosum* var. *tenuis* and the new *Vincetoxicum* sp. sequenced from Thailand do not form a monophyletic clade (Fig. 2). With the exception of the new *Vincetoxicum* sp., all the other Thai accessions are grouped together with high support (PP = 1) in a sister-group position to the New Caledonian species, *V. biglandulosum* (PP = 0.57). This clade consists of two well-supported subclades, one of which comprises all the eight samples of *V. flexuosum* var. *flexuosum* from Thailand (PP = 1). In the second subclade, all accessions (four from Thailand and one from Borneo) of *V. flexuosum* var. *tenuis* are placed together with high support (PP = 1). The accessions of *V. flexuosum* var. *flexuosum* and *V.*

flexuosum var. perrottetianum from the Philippines and Malaysia are retrieved together in another well-supported clade (PP = 0.99). As for the new *Vincetoxicum* sp., it is retrieved as sister to the group comprising the African taxa *V. caffrum, V. lycioides* and *V. fleckii*, with moderate support (PP = 0.85). This group of four species is in turn sister, with moderate support (PP = 0.79), to the subclade constituted of *V. philippicum, V. cissoides* and *V. hainanense* from Philippines, Papua New Guinea and China, respectively. Overall, this phylogenetic analysis shows that the new *Vincetoxicum* sp. is not closely related to *V. flexuosum* var. *flexuosum* or any of its conspecific taxa studied here, both from Thailand and from other areas.

Table 1 Vouchers and accession numbers of DNA sequences of *Vincetoxicum* sp., *V. flexuosum* var. *flexuosum* var. *flexuosum* var. *tenuis* newly obtained from different regions throughout Thailand, and included in molecular phylogenetic analysis.

No.	me	Location	Habitat	Voucher	ITS	<i>Tm</i> T-L	<i>Tm</i> L <i>Tm</i> L-F
1	Vincetoxicum sp.	Surin province, 150 m a. s. l.	mixed deciduous disturbed forest	A. Kidyoo 80, BCU	OP921237	OP923887	OP923874
2	V. flexuosum var. flexuosum	Surin province, 150 m a. s. l.	mixed deciduous disturbed forest	A. Kidyoo 83, BCU	OP921232	OP923882	OP923869
3	V. flexuosum var. flexuosum	Sakon Nakhon province	dry dipterocarp forest	M. Kidyoo 1785, BCU	OP921239	OP923889	OP923876
		230 m a. s. l.					
4	V. flexuosum var. flexuosum	Loei province, 800 m a. s. l.	edge of evergreen forest	<i>A.</i> <i>Kidyoo</i> 60, BCU	OP921240	OP923890	OP923877
5	V. flexuosum var. flexuosum	Tak province, 800 m a. s. l.	edge of evergreen forest	M. Kidyoo 1697, BCU	OP921242	OP923892	OP923879
6	V. flexuosum var. flexuosum	Tak province, 625 m a. s. l.	limestone forest	M. Kidyoo 1696, BCU	OP921243	OP923893	OP923880
7	V. flexuosum var. flexuosum	Tak province, 850 m a. s. l.	limestone forest	A. Kidyoo 95, BCU	OP921236	OP923886	OP923873
8	V. flexuosum var. flexuosum	Kanchanaburi province, 890 m a. s. l	edge of evergreen forest	A. Kidyoo 88, BCU	OP921235	OP923885	OP923872
9	V. flexuosum var. tenuis	Rayong province, 0 m a. s. l.	mangrove forest	A. Kidyoo 85, BCU	OP921231	OP923881	OP923868
10	V. flexuosum var. flexuosum	Prachuap Khiri Khan province,	edge of evergreen forest	A. Kidyoo 97, BCU	OP921238	OP923888	OP923875
		15 m a. s. l.					
11	V. flexuosum var. tenuis	Phang Nga province,	mangrove forest	<i>A.</i> <i>Kidyoo</i> 68, BCU	OP921241	OP923891	OP923878
		10 m a. s. l.					
12	<i>V. flexuosum</i> var. <i>tenuis</i>	Trang province,	beach forest	<i>A.</i> <i>Kidyoo</i> 89, BCU	OP921233	OP923883	OP923870
		10 m a. s. l.					
13	<i>V. flexuosum</i> var.	Satul	mangrove	А.	OP921234	OP923884	OP923871

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tenuis	province,	forest	<i>Kidyoo</i> 90, BCU
	10 m a. s. l.		70, 200

Taxonomic study

The new *Vincetoxicum* sp. is similar to *V. flexuosum* var. *flexuosum* in growth habit, leaf shape, branching of the inflorescence and flower colour. However, thorough comparisons revealed substantial differences between these two entities in both vegetative and reproductive traits (Table 2). Further comparison with other known *Vincetoxicum* species led to the conclusion that this plant represents a distinct undescribed species. It is thus described here as a new species, named as *V. sangyojarniae* (Figs. 3, 4). Detailed morphological description, illustrations and photographs are provided in the section 'Taxonomic treatment'.

Table 2 Morphological and ecological comparisons of *Vincetoxicum sangyojarniae* sp. nov. and the similar *V. flexuosum* var. *flexuosum*.

	V. sangyojamiae	V. flexuosum var. flexuosum
Characters		
Habitat	mixed deciduous forest at ca. 100 m elevation	mixed deciduous to evergreen forest at 50–800 m elevation
Stem	glabrous or with trichomes arranged in one row near the nodes	glabrous or with trichomes arranged in 1–2 rows near the nodes
Leaf		
blade	ovate, ovate-lanceolate; apex acute to acuminate, base rounded to subcordate	ovate, ovate-lanceolate or elliptic; apex acute to acuminate, base acute, obtuse, rounded to subcordate
adaxial surface	glabrous	glabrous
abaxial surface	glabrous	glabrous
veins	obscure, lateral veins 7–9 pairs	obscure, lateral veins 4–6 pairs
petiole	sparsely pubescent along the upper side	sparsely pubescent along the upper side
Inflorescence	branched cyme, 2–4 zigzag rachises; 8–10 flowers at each node	branched cyme, 1–4 zigzag rachises; 4–8 flowers at each node
Peduncle	slender, 1.2–3 cm long, glabrous	slender, 2–4 cm long, glabrous
Flower bud	broadly pyramidal with obtuse apex	oblate with rounded apex
Flower	rotate, 5–6 mm in diameter	rotate, 5–6 mm in diameter
Pedicel	glabrous, 6–8 mm long	glabrous, 5–10 mm long
Corolla lobes	pinkish to reddish brown; ovate-triangular with acute apex; the distal half of adaxial surface densely covered with long white trichomes, abaxial surface glabrous	pinkish to purplish brown; ovate- triangular with acute to obtuse apex; glabrous on both surfaces
Gynostegium with staminal corona	broadly conical	broadly conical

Discussion

Molecular phylogeny and morphology support recognition of a new species

Considering ecological traits, both *Vincetoxicum sangyojarniae* and *V. flexuosum* var. *flexuosum* grow in lowland. The former is found at 150 m. The latter is more widespread, occurring from near sea level up to 800 m. In one location (Surin province), it has been found growing near *V. sangyojarniae*. The evidence from molecular phylogenetic analysis and comprehensive morphological comparisons clearly showed that *V. sangyojarniae* is a

well-defined entity and clearly distinct from *V. flexuosum* var. *flexuosum* and all the other varieties of this species. Morphologically, these two taxa are similar in several aspects. They possess a slender twining stem and branches, and ovate to lanceolate leaves that are glabrous on both surfaces. They produce branched cymes with zigzag rachises, and small red or reddish-brown flowers (5–6 mm in diameter) with a broadly conical gynostegium. The new species, however, can be clearly distinguished from *V. flexuosum* var. *flexuosum* by several consistent characters. It has broadly pyramidal flower buds with an obtuse apex, and corolla lobes that are pubescent on the adaxial surface, the distal half of which is covered with dense long white trichomes. On the contrary, *V. flexuosum* var. *flexuosum* has oblate flower buds with a rounded apex. Its corolla lobes are always completely glabrous throughout on both surfaces. These characteristics were found to be consistent in all samples from the eight populations studied. Detailed comparisons of the two species for each character are provided in Table 2.

Molecular phylogenetic relationships revealed that *Vincetoxicum sangyojarniae* is evolutionarily more closely related to several African species, i.e., the climber V. lycioides, and the non-twiners V. caffrum and V. fleckii, than to all varieties of *V. flexuosum*. Therefore, the similarity in morphology between *V. sangyojarniae* and *V. flexuosum* var. flexuosum observed in Thailand is likely the result of convergence. Overall, V. flexuosum var. flexuosum and the other varieties of this species included in this phylogenetic study were placed with high support in three distinct clades: (1) V. flexuosum var. flexuosum from different locations in Thailand, (2) V. flexuosum var. tenuis from Thailand and Borneo, and (3) V. flexuosum var. flexuosum from Malaysia and the Philippines and V. flexuosum var. perrottetianum from the Philippines. This result suggests that the taxonomic status as an infraspecific rank of V. flexuosum var. tenuis (Schneidt 1999; Liede et al. 2002; Liede-Schumann et al. 2012) might need to be reconsidered. Morphological examination of samples of this taxon obtained throughout its distribution range in Thailand revealed its substantial differences from *V. flexuosum* var. *flexuosum* both in ecological and floral traits (Fig. 5). While V. flexuosum var. flexuosum usually occurs in mixed deciduous to evergreen forests, V. flexuosum var. tenuis is rheophytic and restricted to wetlands. The former has oblate flower bud with a rounded apex (Fig. 5d). Its flowers are consistently characterized by the distally thickened inner corolla tube. This thickening forms a continuous ring at the corolla throat around the gynostegial corona (Fig. 5c). The latter, on the other hand, has flower buds that are oval in shape and obtuse at the apex (Fig. 5e). Its flowers lack the thickened ring on the inner surface around the corolla tube (Fig. 5f). For each of the two taxa, these qualitative traits are constant in all populations studied in Thailand. However, additional sampling in the other countries within the areas of distribution of these taxa is required to confirm whether the species rank of *V. flexuosum* var. *tenuis* should be reinstated.

Taxonomic treatment

Vincetoxicum sangyojamiae A. Kidyoo, **sp. nov.**:—**TYPE**: Thailand, Surin province, Muang district, Rajamangala University of Technology Isan Surin Campus, 150 m a. s. l., 31 Oct 2021, *A. Kidyoo 80* (holotype: BKF!; isotype: BCU!) (Figs. 3 and 4)

Diagnosis: Vincetoxicum sangyojarniae can be clearly distinguished from the similar *V. flexuosum* var. *flexuosum* by its broadly pyramidal flower bud with an obtuse apex and corolla lobes that are adaxially densely pubescent.

Etymology: The specific epithet 'sangyojarniae' is given in honour of Asst. Prof. Pacharaporn Sangyojarn, the senior botanist of the Faculty of Agriculture and Technology, Rajamangala University of Technology Isan, who first discovered and drew our attention to the plant.

Description: Slender climber, 1-5 m long with clear exudate in all vegetative parts. Stems and branches twining, terete; internodes 5–20 cm long, 1–2 mm in diameter, green to greyish green, with trichomes arranged in one row. Leaves opposite; petiole terete, slightly canaliculate and sparsely pubescent along the upper side, 8-16 mm long, 1.3-1.7 mm in diameter; blade membranous, ovate, ovate-oblong to oblong-lanceolate, $3.1-7.5 \times 0.9-3.9$ cm; leaf margins entire to ciliate; leaf base obtuse, rounded to subcordate, bearing 1-2 ovate-lanceolate glands on the adaxial surface, leaf apex acute to acuminate; adaxial surface green to dark green, glabrous except for scattered trichomes along the midrib, abaxial surface paler, glabrous; midrib prominent on the abaxial surface, lateral veins slightly visible on both surfaces, 7-9 on each side, at acute (45-60°) angles to the midrib. Inflorescence branched with 1-4 zigzag rachises, extra-axillary, 20-many-flowered; peduncle tiny, slender, 1.2-3 cm long, 0.7-0.9 mm in diameter, green, greenish brown to reddish brown, glabrous; pedicels green, greenish brown to reddish brown, 6-8 mm long, 0.4-0.5 mm in diameter, glabrous; bracts minute, attached at the base of the pedicel, triangularlanceolate with acute apex, green to greenish brown. Flower bud broadly pyramidal with obtuse apex. Calyx lobes ovate-triangular with acute to acuminate apex, $0.7-0.9 \times 0.6-0.7$ mm, greenish brown, glabrous, bearing an ovateoblong gland inside each lobe sinus. Corolla rotate, 6-6.5 mm in diameter, pinkish brown to reddish brown; tube 0.6-0.8 mm long, glabrous on both surfaces; lobes ovate-triangular, 2.2-2.5 × 1.8-1.9 mm, apex acute and slightly twisted; the distal half of adaxial surface densely covered with long white trichomes, abaxial surface glabrous; margins recurved. Gynostegium conical, 1.5–1.6 mm in diameter, 0.7–0.9 mm in length, sessile. Staminal corona lobes 5, inserted at the base of the stamens, fleshy, broadly ovate with rounded apex, half as long as the anther, $0.5-0.6 \times 0.5-0.6$ mm, reddish brown. Anthers obovate, yellow, tinted reddish brown at the center, with white membranous appendages, attached around the style-head. Pollinaria 5, pendulous, almost horizontally disposed with respect to the filament; pollinia ovoid, yellow, 0.16-0.19 × 0.12-0.13 mm; translator arms hyaline, 0.04-0.05 mm long; corpusculum ovate, reddish brown, 0.08-0.09 × 0.04-0.05 mm. Carpels ovoid, glabrous, 0.8-0.9 mm in length, ca. 0.3 mm in diameter; style-head truncate, pentagonal. Follicles cylindric-fusiform, green when young and turning brown with age, $7-8 \times 0.7-0.9$ cm. Seeds brown, ovate-oblong, $4-5 \times 2.5-3$ mm; coma white, 2-2.5 cm.

Phenology: Flowering from September to January.

Distribution and habitat. This new species is known only from the type locality. It grows in loam soil along roads in shady areas of disturbed mixed deciduous forest (Fig. 3a).

Additional specimens examined: Vincetoxicum sangyojarniae A. Kidyoo: THAILAND, Surin province: Muang district, Rajamangala University of Technology Isan Surin Campus, 150 m elevation, 31 October 2021, A. Kidyoo 81 (paratype: BCU!).

Conservation status: Vincetoxicum sangyojarniae is apparently rare, known from a single locality in Thailand. About 20 to 30 mature individuals were found growing in an area of 1500 m² that is frequently disturbed by human activities. Following IUCN (2019), a provisional conservation assessment of Endangered: B1ab(iii) is assigned owing to its extent of occurrence much smaller than 5,000 km², its restricted geographic distribution, and the small number of locations, which are susceptible to threats in the near future.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All DNA sequences that support the findings of this study are publicly available in GenBank at http://www.ncbi.nlm.nih.gov/genbank/.

Code availability

Not applicable.

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Figures

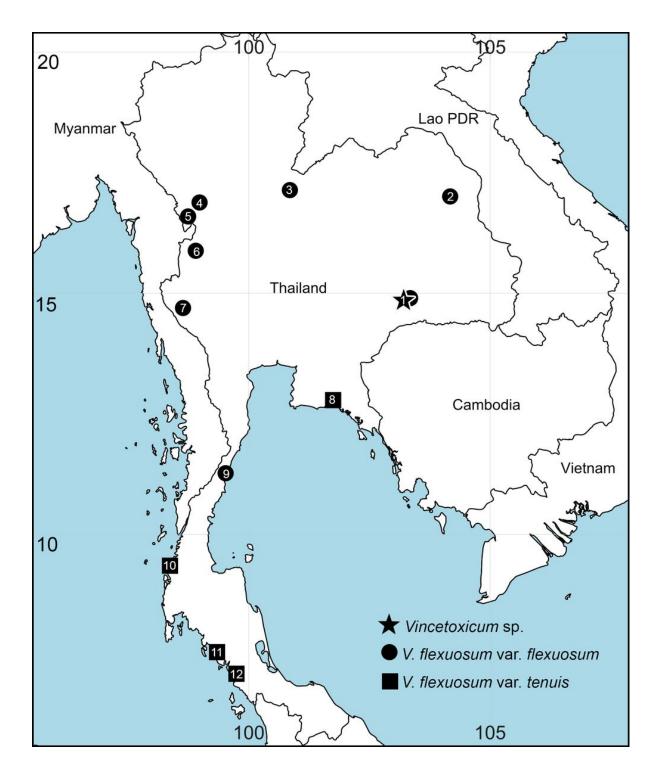


Figure 1

Map showing the sampling sites of *Vincetoxicum* sp. (star), *V. flexuosum* var. *flexuosum* (circles) and *V. flexuosum* var. *tenuis* (squares). The map was created using SimpleMappr (www.simplemappr.net)

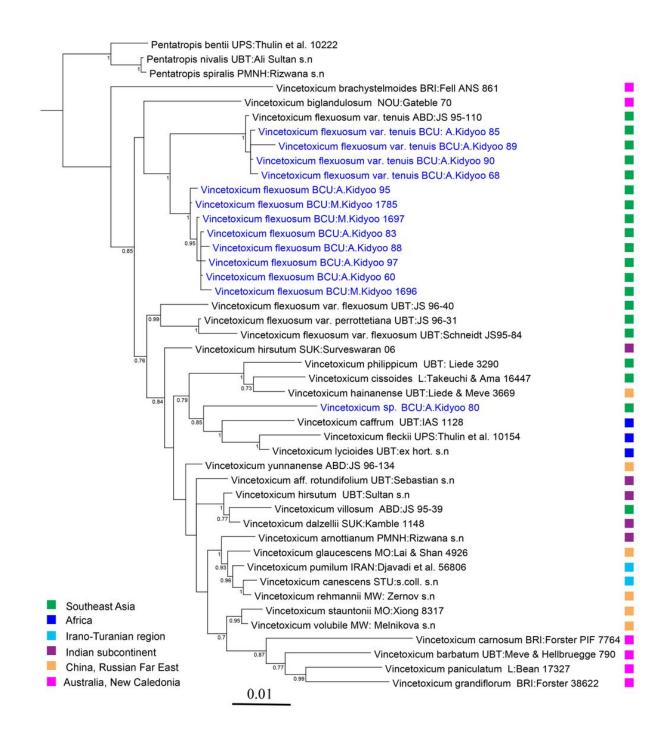


Figure 2

Bayesian phylogeny based on combined ITS and cpDNA data. Numbers next to the nodes are posterior probabilities (3 0.70, below branches). The thirteen accessions from Thailand are presented in blue. The accessions of the former *Tylophora*taxa were named following the new combinations and new names made by Liede-Schumann & Meve (2018).

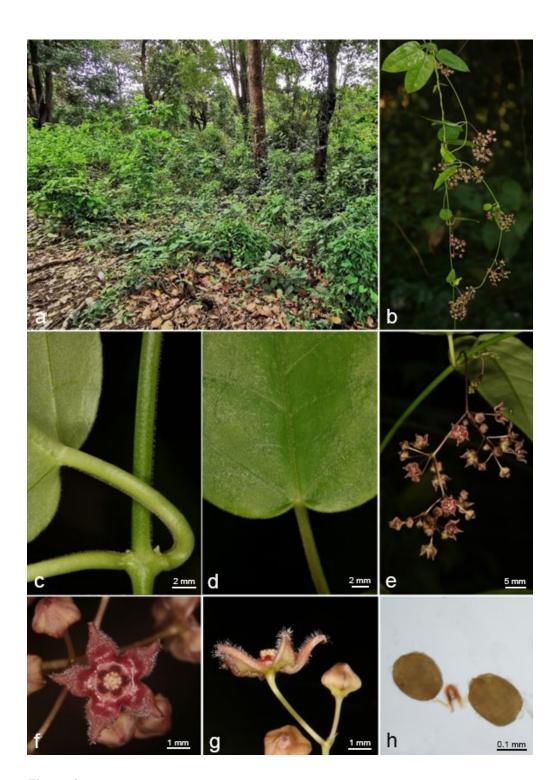


Figure 3

Photographs of *Vincetoxicum sangyojarniae* sp. nov.: **a** habitat; **b**flowering branch; **c** petiole; **d** leaf base with two glands on the adaxial surface; **e** Inflorescence; **f** flower in top view; **g**side view of flower bud and flower; **h** pollinarium. Photographs taken by A. Kidyoo.

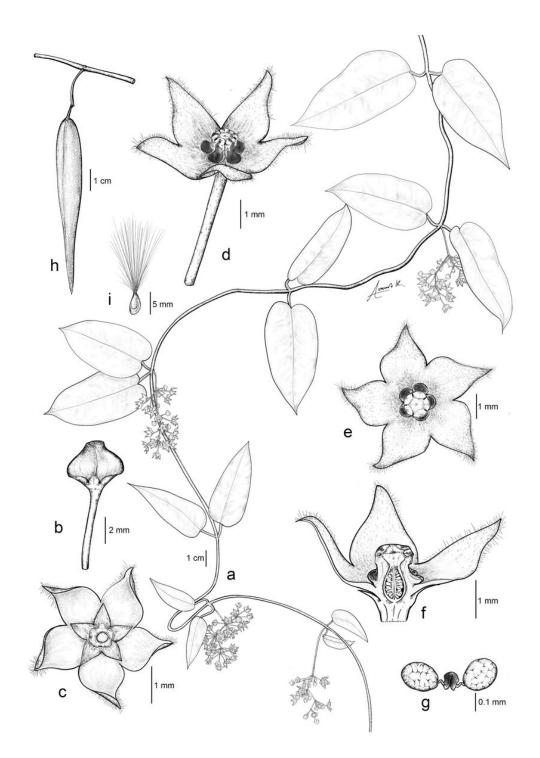


Figure 4

Illustration of *Vincetoxicum sangyojarniae* sp. nov.: **a** flowering branch; **b**flower bud; **c** bottom view of flower; **d** flower; **e** top view of flower; **f** longitudinal section of flower showing corona and gynostegium; **d** pollinarium; **h** follicle; **i** seed; a-g from *A. Kidyoo 80*, h-l from *A. Kidyoo 81*. Drawn by A. Kidyoo.



Figure 5

Photographs of *Vincetoxicum sangyojarniae* sp. nov. (a-b), *V. flexuosum* var. *flexuosum* (c-d) and *V. flexuosum* var. *tenuis* (e-f): **a, c, e** flowers in top view; **b, d, f** flower buds in side view. Photographs taken by A. Kidyoo.

Supplementary Files

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• TableS1.docx