

# ***Streptoechites* gen. nov., a new genus of Asian Apocynaceae**

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## **ABSTRACT**

The new genus *Streptoechites* D.J.Middleton & Livsh., gen. nov. is described and the new combination *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh., comb. nov. is made. Analysis of chloroplast and nuclear loci leaves uncertain its phylogenetic position within tribe Apocyneae Rchb., but Templeton and winning sites tests reject with statistical significance its inclusion in *Sindechites* Oliv., *Epigynum* Wight or *Cleghornia* Wight, the three genera where it has been previously classified. In contrast, the monophyly of *Urceola* Roxb. and *Epigynum* is neither supported nor rejected, making any re-circumscription of these two genera premature. A close relationship between *Urceola* and the monotypic genus *Aganonerion* Pierre ex Spire, sampled for the first time in a molecular analysis, is supported.

## **RÉSUMÉ**

*Streptoechites* gen. nov., un nouveau genre d'Apocynaceae asiatiques. Le nouveau genre *Streptoechites* D.J.Middleton & Livsh., gen. nov. est décrit et la nouvelle combinaison *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh., comb. nov. est faite. L'analyse des loci nucléaires et chloroplastiques laisse incertaine la position phylogénétique de *Streptoechites* gen. nov. dans la tribu des Apocyneae Rchb., mais les tests de Templeton et des sites gagnants rejettent à un niveau statistique significatif la possibilité de l'inclure dans les genres *Sindechites* Oliv., *Epigynum* Wight ou *Cleghornia* Wight, où elle était classifiée autrefois. Par contre, la monophylie de *Urceola* Roxb. et de *Epigynum* n'est ni confirmée, ni rejetée, ce qui rend toute recombinaison de ces genres prématuée. Le genre monotypique *Aganonerion* Pierre ex Spire a été échantillonné pour la première fois dans une analyse moléculaire et se révèle très proche de *Urceola*.

## **KEY WORDS**

Apocynaceae,  
Apocyneae,  
*Aganonerion*,  
*Sindechites*,  
*Cleghornia*,  
*Urceola*,  
*Epigynum*,  
Templeton test,  
winning sites test,  
new genus,  
new combination.

## **MOTS CLÉS**

Apocynaceae,  
Apocyneae,  
*Aganonerion*,  
*Sindechites*,  
*Cleghornia*,  
*Urceola*,  
*Epigynum*,  
test de Templeton,  
test des sites gagnants,  
genre nouveau,  
combinaison nouvelle.

## INTRODUCTION

In the last twenty years there have been considerable changes in generic delimitation in Asian Apocynaceae Juss., especially in the paraphyletic subfamilies Rauvolfioideae and Apocynoideae Burnett (e.g., Leeuwenberg 1991; Middleton 1993a, b, 1994a, b, 1995, 1996a, b). More recent molecular systematic investigations (Simões *et al.* 2007, 2010; Livshultz *et al.* 2007; Livshultz 2010) have proved very useful in resolving questions around the status of *Micrechites* Miq. (Livshultz *et al.* 2007; Middleton 2006) and *Amphineurion* (A.DC.) Pichon (Livshultz *et al.* 2007; Middleton 2006), and in the generic limits of *Tabernaemontana* L. (Simões *et al.* 2010).

An unresolved issue is whether *Sindechites chinensis* (Merr.) Markgr. & Tsiang belongs in *Sindechites* Oliv., *Epigynum* Wight, *Cleghornia* Wight or perhaps to none of them. All genera have been fairly recently revised (*Sindechites* and *Cleghornia*: Xu [1988]; *Epigynum*: Middleton [2005]) but there is a contradiction among them in the treatment of *Sindechites chinensis*. Xu (1988) excluded it from *Sindechites* and placed it in *Epigynum*. Middleton (2005) excluded it from *Epigynum* and placed the species back in *Sindechites*, following the earlier, similar floristic treatments by Li *et al.* (1995) and Middleton (1999). This species was first published as *Epigynum chinense* Merr., but Merrill noted that it was rather different from the other *Epigynum* species, which at that time included only taxa that are all now treated as synonyms of the two rather similar species *E. griffithianum* Wight and *E. ridleyi* King & Gamble (see Middleton 2005). Tsiang & Markgraf in Tsiang (1936) made the combination in *Sindechites*. Although they gave no explanation as to why, Xu (1988), citing unpublished correspondence from Tsiang to Markgraf, noted that they did so due to the similarity in characters between *Epigynum chinense* and *Sindechites henryi* Oliv., specifically their pubescent stamens and the colleters inside the calyx. Xu (1988) suggested these characters were insufficient to delimit genera and noted they were also shared with *Cleghornia* and *Baissea* A.DC.

Li (1984), however, had used this very similarity to sink *Sindechites*, including *S. chinensis*, into *Cleghornia*. Xu (1988) suggested that *Sindechites chinense* did not fit well into either *Epigynum* or *Sindechites* but that until its position could be clarified it should be placed in *Epigynum*.

It would be fair to say, therefore, that delimitation of these genera has been rather volatile and that the generic placement of the species referred to in recent works as *Sindechites chinensis* is particularly open to debate.

*Sindechites henryi* Oliv. & Tsiang, the type species of the genus, and *S. chinensis* were both included in the phylogenetic analyses of Livshultz *et al.* (2007), incorporating chloroplast and morphological data, and Livshultz (2010), based on chloroplast sequences and the nuclear locus *phytochrome A*. Both species are part of a well-supported clade of predominantly Asian genera (tribe Apocyneae, the “Asian clade”), but the two species of *Sindechites* do not form a monophyletic group in any phylogenetic tree. *Sindechites henryi* is always placed as sister to *Amphineurion marginatum* (Roxb.) D.J. Middleton with strong bootstrap support in trees based on combined chloroplast and nuclear data (Livshultz 2010). *Sindechites chinensis* does not group with any of the other genera with which it has also been compared (Li 1984; Xu 1988): *Epigynum*, *Cleghornia* or *Baissea* (which is more closely related to subfamilies Asclepiadoideae and Secamonoideae than to any species of Apocyneae). Instead, *Sindechites chinensis* is placed in various positions in combined chloroplast and *phytochrome A* trees of Livshultz (2010): sister to a group of *Vallaris* Burm.f. and *Beaumontia* Wall. in the parsimony tree, sister to a clade of six genera including *Parameria* Benth. and *Epigynum* in the maximum likelihood tree. Neither position receives high bootstrap support, nor is either supported by any morphological characters.

The ambiguity of the phylogenetic position of *Sindechites chinensis* in analyses to date and the absence of morphological characters to support any one position over another allow alternative taxonomic judgements: leave the species in *Sindechites* (or in *Epigynum* or in *Cleghornia*) until

better support for its position is obtained or establish a new monotypic genus to accommodate it. This question is both theoretical and practical since the species is included in the soon-to-be published treatment of the family for the *Flore du Cambodge, du Laos et du Viêtnam*. To evaluate the strength of currently available evidence against inclusion of *S. chinensis* in *Sindechites*, *Epigynum*, and *Cleghornia*, we conducted Templeton (1983) and winning sites tests (Prager & Wilson 1988) to compare these three hypotheses to the alternative of segregating *S. chinensis* in a monotypic genus. We also compared the two alternative positions of *S. chinensis* obtained under different optimality criteria (Livshultz 2010) and evaluated the strength of evidence against monophyly of *Epigynum* and *Urceola* Roxb., two other genera of Apocyneae whose monophyly was also not supported in the analysis of Livshultz *et al.* (2007).

## MATERIAL AND METHODS

### TAXON SAMPLING (APPENDIX)

Thirty-seven species classified in tribe Apocyneae (representing 18 of 23 genera included by Endress *et al.* [2007]) were sampled. The monotypic genus *Aganonerion* Pierre ex Spire was sequenced for the first time.

**DNA EXTRACTION, AMPLIFICATION, SEQUENCING**  
DNA extraction procedures are described in Livshultz *et al.* (2007). The *trnL* intron and *trnL-trnF* spacer, and *rps16* intron were amplified from *Aganonerion polymorphum* Pierre ex Spire using primers listed in Livshultz *et al.* (2007). Part of the first exon of *phytochrome A* was amplified from *Epigynum ridleyi* and *Epigynum auritum* (C.K.Schneid.) Tsiang & P.T.Li using primers *PHYA* 2745F and 3560R (Livshultz 2010). PCR products were sequenced directly using Sanger sequencing as described in Livshultz (2010).

### SEQUENCE EDITING, ALIGNMENT, MATRIX ASSEMBLY

Sequencing traces were edited and contigs assembled with Sequencher v. 4.2 (Gene Codes Corpo-

ration, Ann Arbor, MI, U.S.A.). All sequences of Gentianales were downloaded from Genbank on June 9, 2011. All Apocynaceae sequences (new and previously published) for the chloroplast *trnL* intron and *trnL-trnF* spacer, *rpL16* intron, *rps16* intron, *matK* and 3' *trnK* intron, and the nuclear locus *phytochrome A* were assembled by eye into master alignments using Bioedit version 7.0.9.0 (Hall 1999). From these master alignments, alignments of Apocyneae sequences were extracted, further adjusted by eye, and reduced to one sequence per species. Where possible, a sequence published by Livshultz *et al.* (2007) or Livshultz (2010) was selected to represent the species. One region of the *rpL16* intron was considered unalignable (because of imperfect repeats) and excluded from analysis. Indels are treated as missing data. All loci were combined into a single data matrix (Study ID S12699 in Treebase). Genbank accession numbers for all included sequences and vouchers for newly generated sequences are listed in the Appendix.

### PARSIMONY ANALYSIS

Tree searches were conducted with NONA (Goloboff 1996) launched from Winclada (Nixon 2000) and consisted of 1000 random addition sequences with subtree pruning and re-grafting (SPR) followed by tree bisection-reconnection (TBR) swapping, holding a maximum of 10 equally parsimonious trees per search, and TBR swapping on all most parsimonious trees (MPTs) until the search swapped to completion. Bootstrap frequencies were calculated based on 1000 pseudo-replicate matrices, each analysed with a single random addition sequence and TBR swapping until a maximum of 20 trees were saved following recommendations of Freudenstein *et al.* (2004). Frequencies were calculated based on the strict consensus of all trees saved from each replicate, providing a conservative estimate of bootstrap support (Davis *et al.* 2004). Uninformative characters were deleted from the matrix prior to the bootstrap analysis. The root was placed between *Papuechites* Markgr. and *Anodendron* A.DC. and the rest of the Apocyneae, following the topologies obtained by Livshultz (2010).

TABLE 1. — Significance of support for most parsimonious topology (MPT; see Fig. 1) over alternative phylogenetic hypotheses based on winning sites tests. \*, indicates statistically significant difference.

MPT	Alternative hypothesis	Winning sites test			
		Characters with fewer steps on MPT	Characters with fewer steps on alternative tree	Sign test statistic	P (2-tailed exact binomial)
1 <i>Sindeclites chinensis</i> sister to <i>Vallaris</i> plus <i>Beaumontia</i>	<i>Sindeclites</i> monophyletic	15	3	0.83	P = 0.0075*
2 <i>Sindeclites chinensis</i> sister to <i>Vallaris</i> plus <i>Beaumontia</i>	<i>Sindeclites chinensis</i> plus <i>Cleghornia</i> monophyletic	35	7	0.83	P < 0.0001*
3 <i>Sindeclites chinensis</i> sister to <i>Vallaris</i> plus <i>Beaumontia</i>	<i>Sindeclites chinensis</i> plus <i>Epigynum</i> plus <i>Ichnocarpus</i> monophyletic	79	7	0.92	P < 0.0001*
4 <i>Sindeclites chinensis</i> sister to <i>Vallaris</i> plus <i>Beaumontia</i>	<i>Sindeclites chinensis</i> in clade with <i>Parameria</i> and <i>Epigynum</i>	12	7	0.63	P = 0.359
5 <i>Urceola rosea</i> sister to <i>Aganoneronion</i>	<i>Urceola</i> monophyletic	3	1	0.75	P = 0.625
6 <i>Ichnocarpus</i> nested in <i>Epigynum</i>	<i>Epigynum</i> monophyletic	3	1	0.75	P = 0.625

## SIGNIFICANCE TESTS

To evaluate the significance of support for groups in the most parsimonious trees over specified alternative topologies, Templeton (1983) and winning sites (Prager & Wilson 1988) tests were implemented in PAUP\*4.0b10 (Swofford 2003).

Most parsimonious constraint trees were constructed by adding group membership characters, weighted to 100, to the data matrix. These were then analysed with the same search strategy as the unconstrained matrix (see above).

For the Templeton and winning sites tests, one arbitrarily selected constraint MPT was compared to one arbitrarily selected unconstrained MPT. The null hypothesis of equal support for the two topologies was rejected at the 5% probability level.

The following alternative hypotheses were tested against the most parsimonious topology from the unconstrained analysis: 1) *Sindeclites* is monophyletic; 2) *Sindeclites chinensis* plus *Cleghornia* are monophyletic; 3) *Sindeclites chinensis* plus *Epigynum* and *Ichnocarpus* R.Br. are monophyletic; 4) *Sindeclites chinensis* is sister to a clade that includes *Parameria* and *Epigynum*; 5) *Urceola* is monophyletic; 6) *Epigynum* is monophyletic.

## RESULTS

The combined chloroplast and nuclear data matrix had 6727 aligned positions; 461 of these are parsimony informative. Parsimony analysis yielded six trees, length= 823 steps, CI = 65, RI = 79, strict consensus in Figure 1. *Sindeclites* is polyphyletic with *S. henryi* sister to *Amphineurion* (A.D.C.) Pichon (BS = 94%) and *S. chinensis* sister to *Beaumontia* plus *Vallaris* (BS = 72%). *Urceola* and *Epigynum* are also not monophyletic. *Urceola rosea* is sister to *Aganoneronion* (BS = 84%), and *Ichnocarpus frutescens* (L.) W.T.Aiton is sister to *Epigynum griffithianum* and *E. ridleyi* (BS = 63%).

Templeton and winning sites tests agreed with each other for all six hypotheses tested. Only the results of winning sites tests are shown (Table 1). The most parsimonious position of *Sindeclites chinensis* is significantly better supported than alternative topologies placing this species in a monophyletic *Sindeclites*, in *Cleghornia* or in *Epigynum* (Table 1; tests 1–3). The position of *S. chinensis* is not significantly supported over an alternative placement as sister to the large clade that includes *Parameria* and *Epigynum* (Table 1; test 4). Neither a monophyletic *Urceola* nor a monophyletic *Epigynum* can be rejected based on currently available data (Table 1; tests 5, 6).

TABLE 2. — Comparison of some morphological characters in *Streptoechites* gen. nov., *Cleghornia* Wight, *Epigynum* Wight and *Sindechites* Oliv.

	<b>Calyx colleters</b>	<b>Stamen insertion in corolla tube</b>	<b>Anthers with apical hairs</b>	<b>Disk shape</b>	<b>Ovary pubescence</b>	<b>Pedicel twisted in fruit</b>
<i>Streptoechites</i> gen. nov.	Marginal at base	Middle of tube	Yes	Bilobed	glabrous	Yes
<i>Cleghornia</i>	Marginal at base	Basal	No	Annular ring or somewhat 5-lobed	glabrous	No
<i>Epigynum</i>	Variable	Lower half	No	Annular ring or somewhat 5-lobed	Variable	No
<i>Sindechites</i>	Continuous at base	Upper half	Yes	Annular ring or somewhat 5-lobed	Pubescent	No

## DISCUSSION

Molecular phylogenetic studies have greatly advanced our understanding of Apocynaceae phylogeny, but relationships of many taxa still require further resolution (Livshultz *et al.* 2007; Simões *et al.* 2007; Livshultz 2010). Classifications should reflect our current understanding of relationships while respecting the need for taxonomic stability (Backlund & Bremer 1998). The affinities of *S. chinensis* have always been ambiguous, as demonstrated by its history of transfer among three genera. Its phylogenetic position remains obscure, with the most parsimonious position, sister to *Vallaris* plus *Beaumontia*, not better supported than a position sister to a large clade that includes *Epigynum* and *Parameria* as in the maximum likelihood topology of Livshultz (2010) (Table 1, test 4). However, these data do strongly reject inclusion of *S. chinensis* in *Sindechites*, *Cleghornia* or *Epigynum* (Table 1, tests 1–3). Segregation of *S. chinensis* into a monotypic genus best reflects both what we know and what we don't know about its phylogeny. In contrast, currently available data neither support nor reject the monophyly of *Urceola* and *Epigynum* with statistical significance (Fig. 1; Table 1, tests 5–6). Recircumscription of these genera based on these data would be premature. Templeton and winning sites tests proved much more useful for evaluating the strength of evidence for alternative circumscriptions than the bootstrap since all the clades tested have "moderate" bootstrap values in the range of 60–85% (Fig. 1).

The conclusion that must be reached is that a new genus, *Streptoechites* D.J.Middleton & Livsh.,

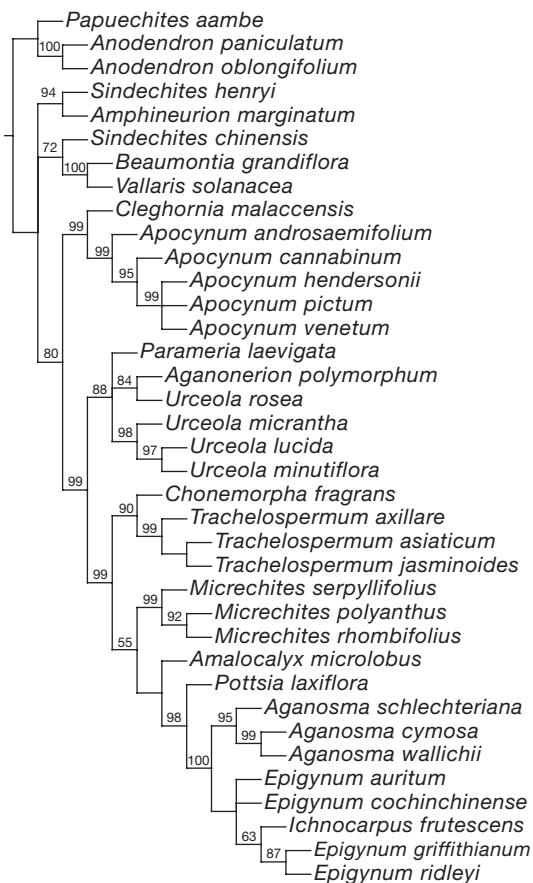


FIG. 1. — Phylogeny of Apocynaceae Juss. tribe Apocyneae Rchb. Strict consensus of six most parsimonious trees, length = 823 steps, CI = 65, RI = 79, based on combined chloroplast and PHYA datasets. Bootstrap support values > 50% shown above nodes.

gen. nov. is necessary to accommodate *Sindechites chinensis* which can be distinguished morphologically from the genera into which it has previously been placed using the characters in Table 2. It belongs to Apocynaceae, tribe Apocyneae.

## SYSTEMATICS

Family APOCYNACEAE Juss.

Genus *Streptoechites*  
D.J.Middleton & Livsh., gen. nov.

*A Sindechite calycum colleteribus in angulis calycis tantum praesentiis (in Sindechite in serie continua), staminibus circa medium corollae tubi insertis (haud in dimidio superiore ut in Sindechite), ovario glabro (in Sindechite pubescenti) et disco bilobo (haud quinquelobato vel annulo integro ut in Sindechite) differt. Ab Epigyno lobis corollae brevibus (in Epigyno multo longioribus), antheris cum pilorum caespitulō (in Epigyno caespitulō nullo), disco bilobo (in Epigyno quinquelobato vel annulo crenato) et pedicelilo fructifero torto (in Epigyno haud torto) differt.*

TYPUS. — *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh., comb. nov.

ETYMOLOGY. — Comes from the genus *Echites* P.Browne, from which many genera in subfamily Apocynoideae derive their name, and strepto-, meaning twisted and referring to the twisted pedicel in fruit.

### DESCRIPTION

Small to medium-sized woody climbers. Leaves opposite, petiolate. Inflorescence terminal or axillary, cymose, few- to many-flowered. Sepals 5, more or less free to base, with colleters at margins at base inside. Corolla 5-merous, lobes dextrorse in bud; mature corolla salverform; tube narrow, widening slightly around stamens; lobes short erect, slightly spreading. Stamens completely included; inserted in the middle of the corolla tube; anthers fertile in the upper half only, the lower half enlarged, sterile, laterally with lignified guide rails and sagittate appendages at the base, pubescent on the back and apex with a tuft of hairs; adnate to the style head. Disk irregularly 2-lobed, shorter to as high as the ovaries. Gynoecium 2-carpellate, apocarpous but apically united into a common style, glabrous;

style filiform, style head collared at base; ovules numerous. Fruit of paired slender follicles, glabrous, pedicels twisted when in fruit. Seeds linear; with an apical coma.

Only one species, *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh., comb. nov. in Hainan, Vietnam and Thailand.

*Streptoechites chinensis* (Merr.)  
D.J.Middleton & Livsh., comb. nov.  
(Fig. 2)

*Epigynum chinense* Merr., Philippine Journal of Science 23: 262 (1923); Xu, Wageningen Agricultural University Papers 88 (6): 25 (1988). — *Sindechites chinensis* (Merr.) Markgr. & Tsing, Sunyatsenia 3: 152 (1936); Kerr in Craib, Flora of Siamensis Numeratio 2: 471 (1939); Tsing & P.T. Li., Flora Republicae Popularis Sinicae 63: 170 (1977); P.T.Li et al., Flora of China 16: 188 (1995); Middleton, Flora of Thailand 7: 135 (1999). — *Cleghornia chinensis* (Merr.) P.T.Li, Guihaia 4: 192 (1984).

TYPUS. — China, Hainan, Yik Tsok Mau, McClure 9710 (lecto-, A, designated by Middleton (2005); isolecto-, A, BM, E, G, K, NY, P [P00495801], PNH, UC, US, Z; Figs 2; 3).

DISTRIBUTION. — Hainan, Vietnam and Thailand. Given its known distribution it is very likely also to be in Laos (Fig. 3).

### REDESCRIPTION

Branchlets glabrous or sparsely puberulent. Leaves: petiole 2-3.4 mm long, glabrous to puberulent; blade papery, ovate, 2.4-7.7 × 1.1-3.4 cm, 1.6-2.6 times as long as wide, apex acuminate, base rounded, few hairs on midrib beneath, 3-5 pairs of secondary veins strongly ascending, tertiary venation obscure. Inflorescence 2.5-5 cm long, glabrous or minutely puberulent; peduncle delicate, 0.8-2.6 cm long; pedicels 5-8 mm. Sepals ovate, 1.5-2 × 1-1.5 mm, apex acute; glabrous, ciliate; colleters present in axils. Corolla white; tube 9-15 mm long, 1.5 mm wide, outside puberulent at top of tube, pubescent around mouth and inside; lobes 2-3.5 mm long, pubescent outside. Stamens inserted at about half of corolla tube length from base; anthers 1.5-2.6 × 0.6-0.7 mm.

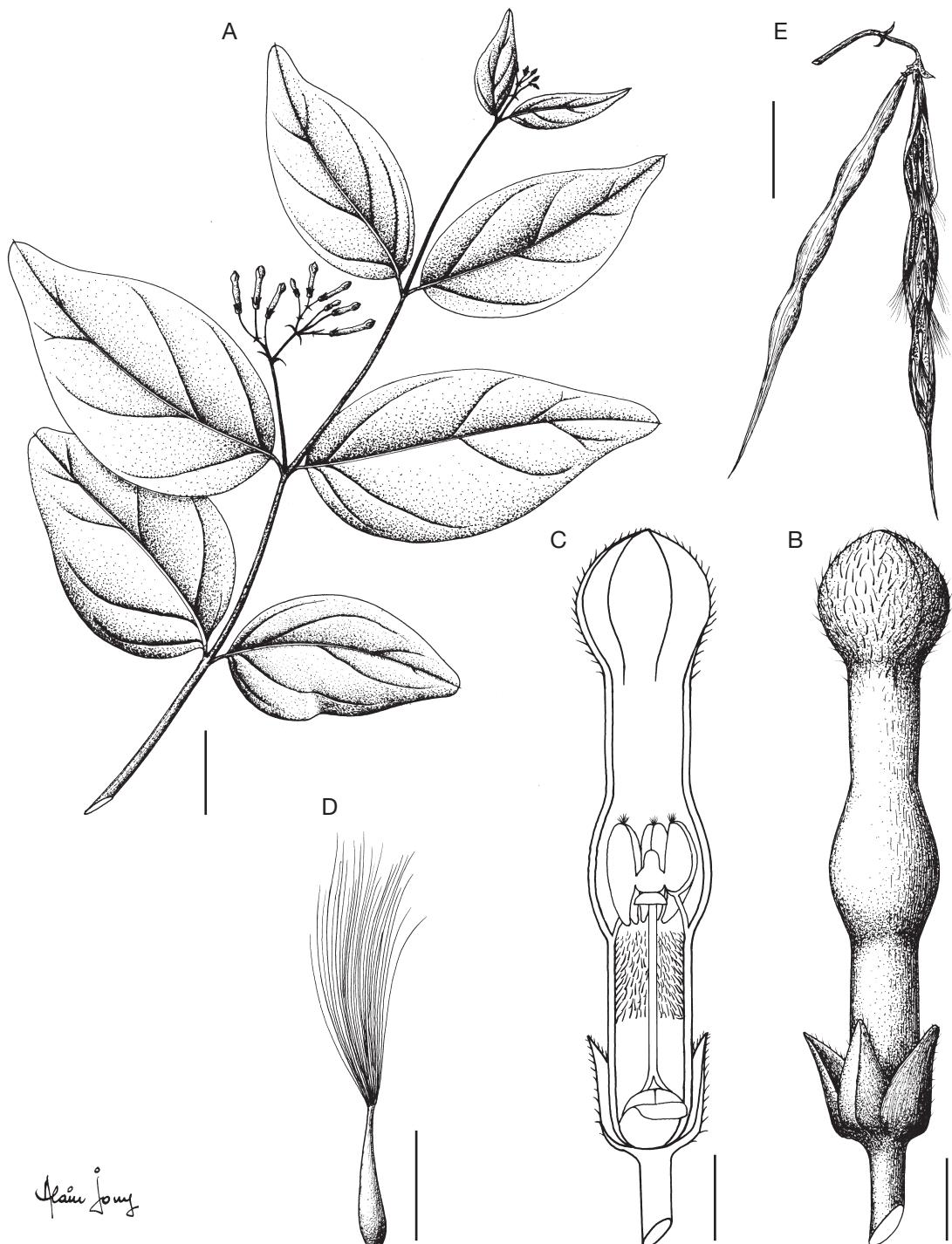


FIG. 2. — *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh., comb. nov.: A, habit; B, flower in bud; C, corolla dissection; D, fruit; E, seed. Drawn from Lau 1791 (P). Scale bars: A, 20 mm; B, C, 2 mm; D, 10 mm; E, 20 mm.

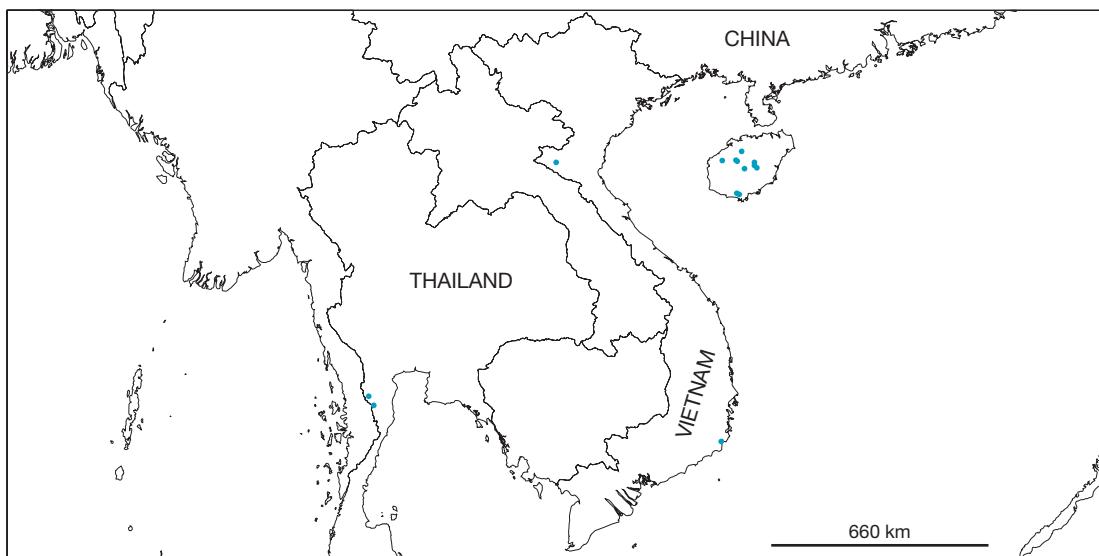


FIG. 3. — Distribution of *Streptoechites chinensis* (Merr.) D.J.Middleton & Livsh. comb. nov.

Disk 0.7–1 mm high, more deeply divided either side of ovaries so almost 2-lobed. Ovaries c. 1 mm long, glabrous; style and style head 4.8–6.8 mm long. Fruits weakly torulose, 8.9–17 cm long, 2.5–5 mm wide, glabrous, style twisted in fruit. Seeds narrowly elliptic, somewhat elongated at coma end, 13–18 × 1.4–1.7 mm; coma 2.4–3.4 cm long.

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## APPENDIX. — Continuation.

Species	<i>matK</i>	<i>rpL16</i> intron	<i>rpS16</i> intron	<i>trnL</i> intron and <i>trnL-</i> <i>trnF</i> spacer	phyto- chrome A	voucher for newly published sequences
<i>Trachelospermum jasminoides</i> Lem.	EF456294	EF456436	EF456578	EF456172	—	—
<i>Urceola lucida</i> Benth. & Hook.f.	EF456343	EF456512	EF456625	EF456226	GU901400	—
<i>Urceola micrantha</i> (Wall. ex G. Don) D. J. Middleton	EF456283	EF456510	EF456575	EF456157	—	—
<i>Urceola minutiflora</i> (Pierre) D. J. Middleton	EF456284	EF456385	EF456576	EF456159	—	—
<i>Urceola rosea</i> (Hook. & Arn.) D. J. Middleton	EF456285	EF456386	EF456603	EF456160	—	—
<i>Vallaris solanacea</i> (Roth) O. Kuntze	EF456286	EF456500	EF456559	EF456162	GU901401	—