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Article

Morphological and Molecular Evidence for a New Species Within *Styrax* (Styracaceae) from Karst Area in Southwest Guangxi, China

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Abstract: *Styrax chongzuoensis*, a novel endemic species with a narrow distribution in limestone regions of Chongzuo, Guangxi, China, is described herein. Careful comparison of this species with all other *Styrax* species in China, as well as those in adjacent Vietnam, shown that it is essentially different, and is somewhat similar to *S. argentifolius* in leaf morphology, but significant differences exist due to its habit (deciduous shrubs vs. evergreen trees), thickly chartaceous (vs. coriaceous) leaf blade, inflorescence shape, and flowers number. To further ascertain the taxonomic position of *S. chongzuoensis*, we sequenced and assembled its plastomes. Notably, phylogenetic analyses based on the complete chloroplast genome clarify the taxonomic position of *S. chongzuoensis* in *Styrax*. Overall, the integration of morphological and phylogenetic evidence indicates that *S. chongzuoensis* actually represents a new species. Colour plates of *S. chongzuoensis* was illustrated, a distribution map and conservation assessment of this species were also provided.

Keywords: China; Guangxi; plastome; phylogenetic analysis; Styracaceae

1. Introduction

Styrax Linnaeus (1753: 444) comprises ca. 140 species of trees and shrubs, mainly distributed across tropical and subtropical region in eastern and southeastern Asia, the New World and the Mediterranean region [1–3]. In China, initial records in Flora of China recorded about 30 species, 7 varieties [4,5], which were subsequently revised to 30 species and 3 varieties in recent studies [6–9].

Styrax is an economically important genus with multiple utilization values. Most *Styrax* species produce white, fragrant flowers that are highly valued for ornamental purposes in garden landscapes [10,11]. Additionally, resin extracts from certain species have been widely utilized in the production of spices and medicinal products, further enhancing their commercial significance.

As part of a comprehensive monograph study on Chinese *Styrax* species, we conducted extensive field investigations, particularly in the limestone areas of Guangxi and Yunnan provinces revealed a previously undescribed species, *S. hwangiae* [3]. Subsequent fieldwork in October 2022, two shrubby *Styrax* individuals with fruiting stage were discovered on limestone outcrops in Chongzuo, Guangxi. These plants exhibited distinctive traits, including thickly chartaceous lanceolate leaves often with asymmetrical base and infructescence bearing few fruits, which differs from all other Chinese *Styrax* species. Subsequent field observations during flowering season were conducted to collect materials for further research. Combined morphological and molecular analyses revealed that it represents an undescribed species, which formally propose here as *S. chongzuoensis*.

2. Methods and Material

2.1. General Morphology

Living specimens of the putative new species were observed in their natural habitat within a karst mountain population in Chongzuo, Guangxi, China. The overall morphology including the plant habit, stems, leaves, flowers, and fruits were photographed by a digital camera, and then observed and measured with a ruler. Comparisons of the new species with other *Styrax* in China and adjacent Vietnam were conducted using specimens examination and literature study.

2.2. Species Sampling, DNA Extraction and Data Collection

We selected 22 species of *Styrax* including our putative new species, of which 18 species from series *Cyrta*, 3 species from ser. *Benzoin*, and one from ser. *Valvate*. Among all the *Styrax* species, 21 of them are distributed in China and Vietnam [2,3,7,8,12]. Two *Symplocos* species were designated as outgroup. Whole-plastome sequencing was performed for *Styrax chongzuoensis*, while sequences for the other species were extracted from GenBank. The voucher information and GenBank numbers of the materials used in this study are shown in Table A1.

The total genomic DNA of the new species *Styrax chongzuoensis* was extracted from silica-dried leaves using the modified CTAB method. DNA integrity was assessed via 1% (w/v) agarose gel electrophoresis, and DNA quality was evaluated using a NanoDrop spectrophotometer 2000 (Thermo Scientific, Waltham, MA, USA). The DNA samples were subsequently sent to Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) for library construction. Paired-end (2×150 bp) libraries were constructed using Nova-PE150 strategy, generating over 2 Gb of genome skimming data for each samples.

The raw reads were processed using Trimmomatic v. 0.39. to remove unpaired and low-depth reads, thereby enhancing the accuracy and quality of the assembly. The filtered data was assembled into the complete chloroplast (cp) genomes and nuclear ribosomal DNA (nrDNA) sequences with GetOrganelle v. 1.7.7. The assembly results were visualized and manually corrected using Bandage v.5.6.0. The complete cp genomes were annotated by CPGAVAS2.

2.3. Phylogenetic Analysis

Sequence alignment was conducted with MAFFT 7.450 [13]. Maximum likelihood (ML) analysis was implemented in IQ-TREE v2.1.3 [14], and bootstrap analysis was conducted with 20 000 ultrafast bootstraps. The best-fit BIC model was determined by ModelFinder [15]. Bayesian inference (BI) was constructed by MrBayes 3.2.6 [16,17] with 3,000,000 generations, sampling every 1,000 generations, which ultimately is needed to ensure the assessment of convergence within the valid range (mean standard deviation of split frequency < 0.01 and effective sample size > 200), and that the discarded. The sampled data are "burn-in" the first 25% of the final processing and are used to estimate the posterior probabilities (PP). Finally, strong bootstrap support (MLBS) was percentile (MLBS) values ≥70 [18] and PP values ≥0.95 [19] were considered strong support. Finally, the phylogenetic trees were visualized by Figtree1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

3. Results

3.1. General Morphology

Styrax chongzuoensis bears unique morphological traits within *Styrax*. It somewhat resembles *S. argentifolius* in leaf shape. However, this species shows clear differences in growth patterns and physical features. Unlike the evergreen and tree-like *S. argentifolius*, *S. chongzuoensis* is deciduous and shrubs. Its leaves thickly chartaceous with dense yellow-brown stellate tomentum on the underside, contrasting with the thicker leathery leaves of *S. argentifolius* that bears silver-gray scale-like coverings abaxially. Additionally, the flowering and fruiting structures show further distinctions. *Styrax chongzuoensis* often with single flower, sometimes 2 or 3 flowers cluster at branchlet tips (vs. 3–9 flowered cluster into axillary or sternal racemes), and develops elongated pointed fruits compared to the nearly round, short-tipped fruits of *S. argentifolius*.

3.2. Phylogenetics

Our maximum likelihood (ML) and Bayesian inference (BI) approaches yielded highly similar tree structures that align closely with prior research [9]. Both analyses strongly supported the placement of *Styrax chongzuoensis* within the *Styrax* sect. *Cyrta* lineage, with maximum statistical confidence (MLBS = 100%, PP = 1.00). Genetic data from chloroplast coding DNA sequences revealed this species forms a distinct evolutionary branch that clusters closest to ser. *Benzoin* group members. These DNA findings strongly support observed physical differences in plant characteristics, establishing *S. chongzuoensis* as a unique evolutionary entity (Figure 6).

4. Taxonomic Treatment

Styrax chongzuoensis M. Tang, R. Zhang & W.B. Xu, sp. nov

Figures 1–4

Chinese name: “chóng zuò ān xī xiāng”(崇左安息香)

Type: Tiandeng Mountain, Pairu village, Zuozhou county, Chongzuo city, Guangxi Zhuang Autonomous Region, China, in sparse forests on limestone karst hilltops, alt. 270 m, 27 May 2014, WB Xu & YS Huang CZ0921 (holotype, IBK!; isotypes, IBK, JXAU!). Figure 2.

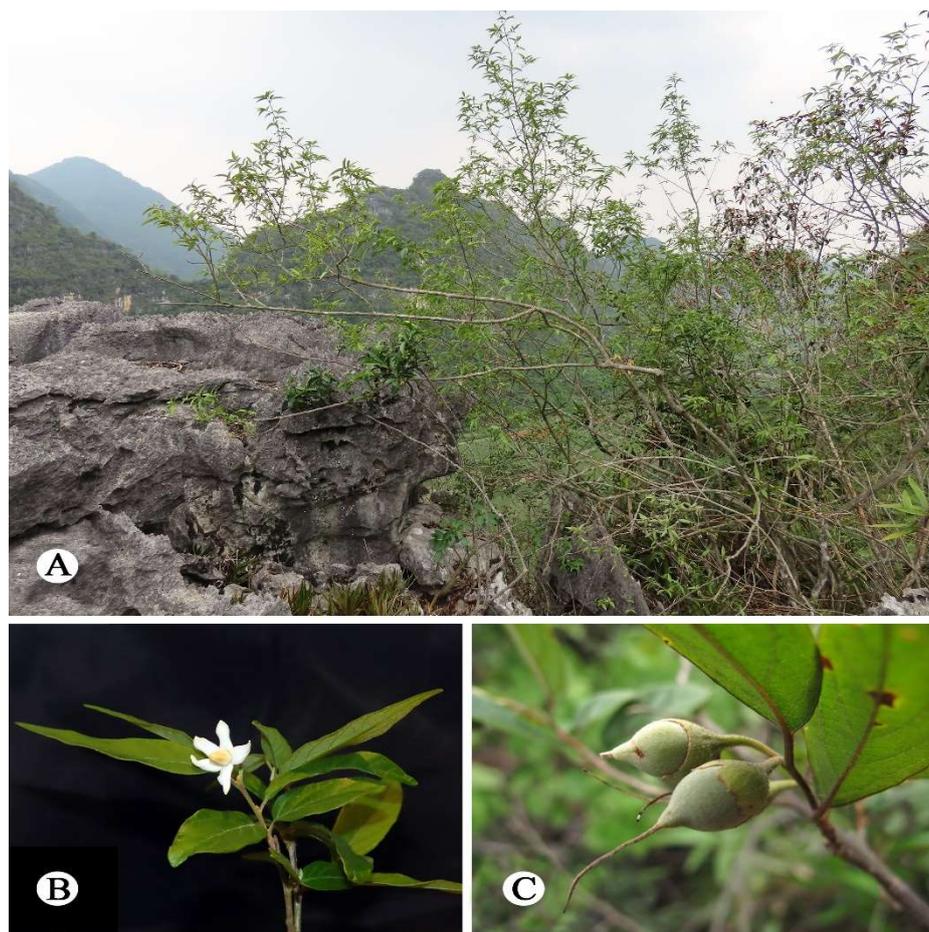


Figure 1. *Styrax chongzuensis* in the wild. (Jiangzhou district, Chongzuo city, Guangxi Zhuang Autonomous Region, China): (A) Habitat and habit; (B) Inflorescence; (C) infructescence. Photographed by Weibin Xu.



Figure 2. Holotype (A) and paratype (B) sheets of *Styrax chongzuensis*. Tiandeng Mountain, Pairu village, Zuozhou county, Chongzuo city, Guangxi Zhuang Autonomous Region, China, WB Xu & YS Huang CZ0921 (holotype, IBK!), CZ0928 (paratype, IBK!).

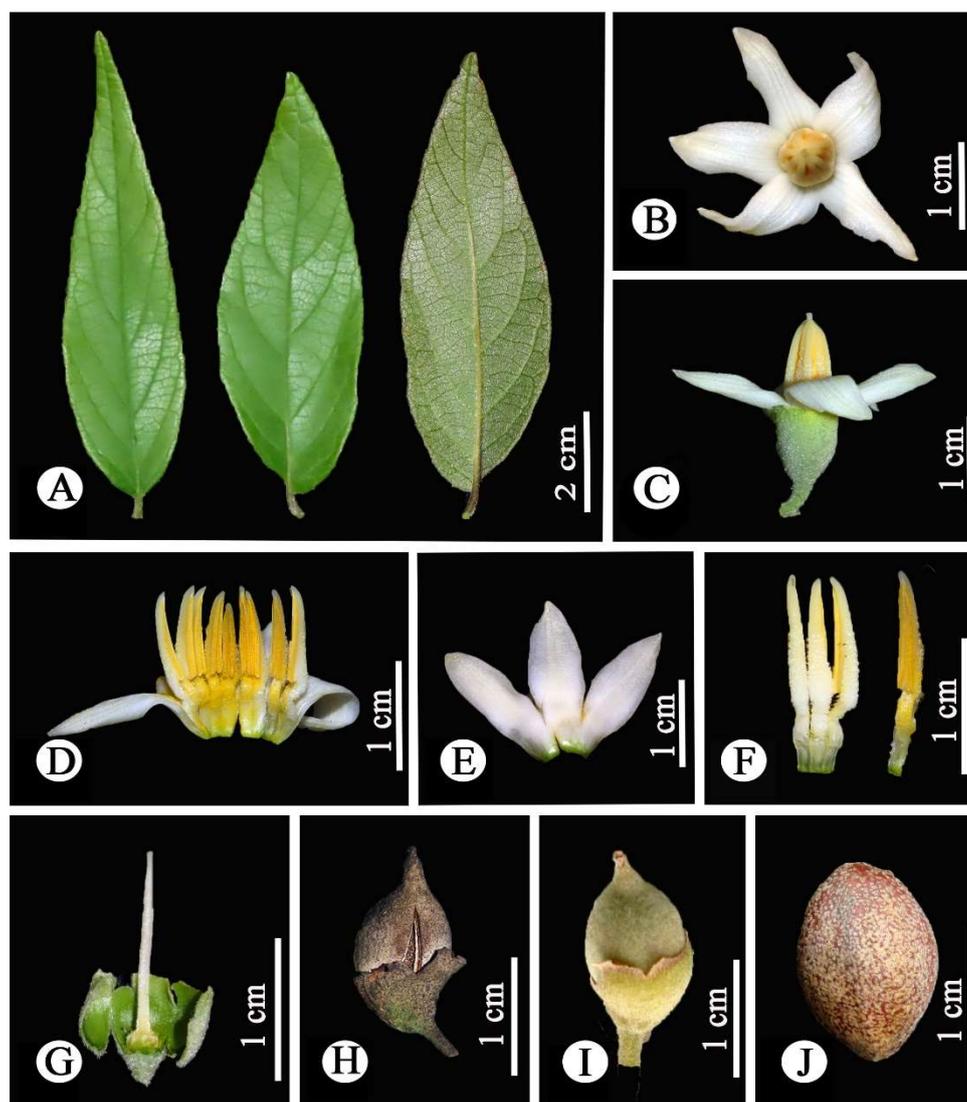


Figure 3. *Styrax chongzuoensis*. (A) Leaf blade; (B) Flower (apical view); (C) Flower (lateral view); (D) Opened corolla plus androecium, ventral view; (E) Opened corolla (abaxial view); (F) Stamens; (G) pistil; (H) Fruit (ripeness); (I) Fruit (immature); (J) Seeds. Photographed by Weibin Xu.

Description: Deciduous shrub or small tree, 2–5 m tall; bark gray or dark gray. Leaves thickly chartaceous, narrowly long ovate or elliptic-lanceolate, 5–15 cm long, 2.5–6.5 cm wide, entire, apically acuminate, base broadly cuneate, often asymmetrical, adaxially glabrous, abaxially densely covered with yellowish-brown stellate or scalelike tomentum, lateral veins 5–8 per side; petiole 5–10 mm long, densely covered with yellowish-brown hairs. Inflorescences terminal or axillary, often single flower, or racemes, 2 or 3 clustered at branchlet tips; peduncle, pedicel, bracteoles and calyx densely covered with yellowish-brown hairs; flowers white, bracteoles subulate or nearly subulate, ca. 3.0 mm long; calyx cup-shaped, corolla lobes 4–5, elliptic obovate or narrowly oblong obovate, 10–15 mm, 3–3.5 mm wide, valvate in bud; stamens 9–11, slightly shorter than corolla, filaments curved at middle, style 10–13 mm long; fruit ovate or slightly broadly ovate, apiculate, cusp longer and slightly curved; seeds ovate, brown, covered with rusty-brown stellate trichomes.

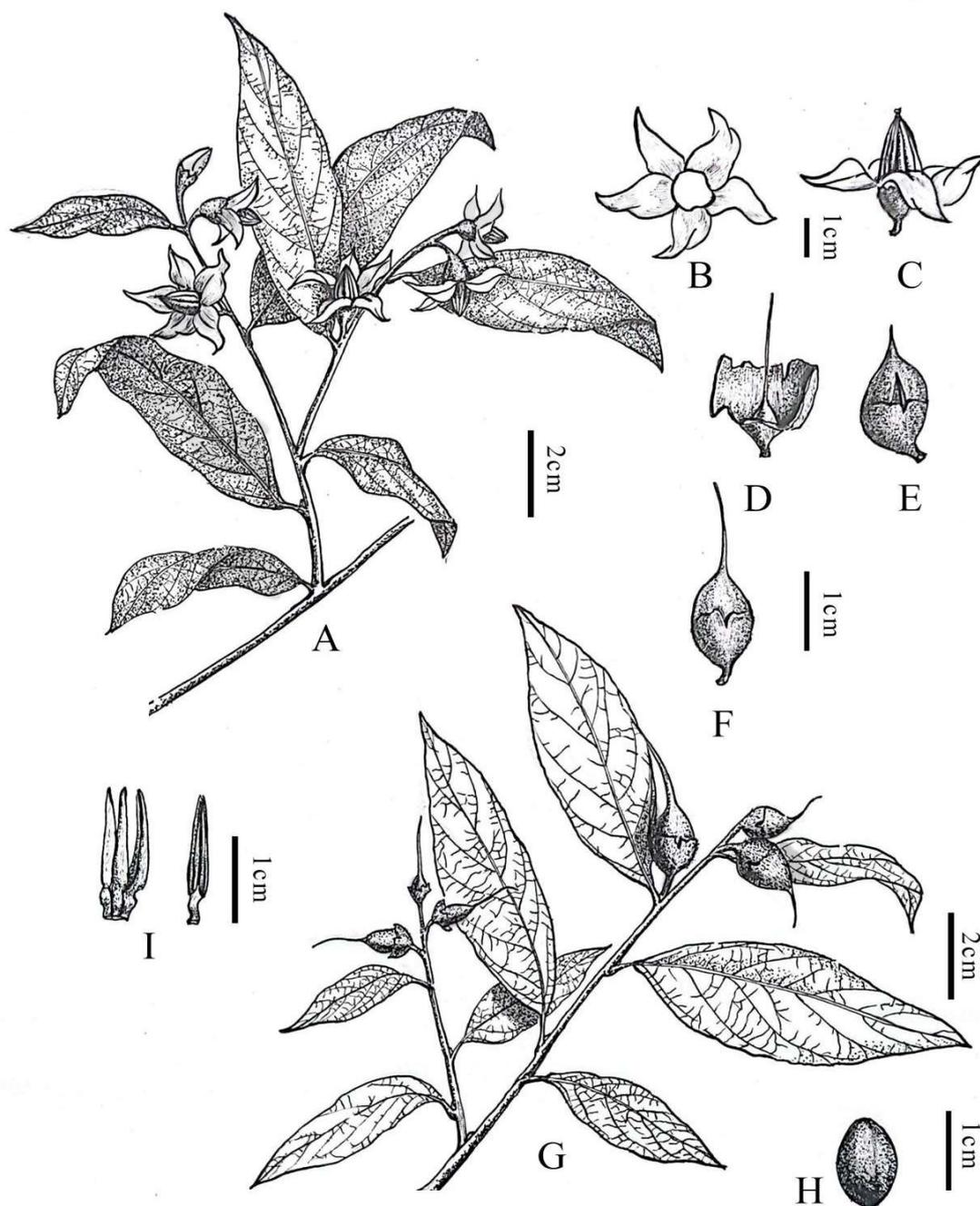


Figure 4. Line drawing of *Styrax chongzuoensis*. (A) Flowering branch; (B) Corolla; (C) Flower (lateral view); (D) Sepals and stigma; (E) Mature fruit; (F) Fruit; (G) Fruiting branches; (H) Seed. Illustration by Guoxing Peng based on living field-collected material.

Phenology: Flowering from April to June; fruiting from August to October.

Etymology: This species is named after its type locality, Chongzuo city, Guangxi Zhuang Autonomous Region, China.

Distribution and habitat: *Styrax chongzuoensis* is endemic to Chongzuo city, Guangxi Zhuang Autonomous Region, China (Figure 5). It grows in sparse forests of limestone in karst mountains tops at altitudes of 270 meters.



Figure 5. Distribution map of *Styrax chongzuensis* (★).

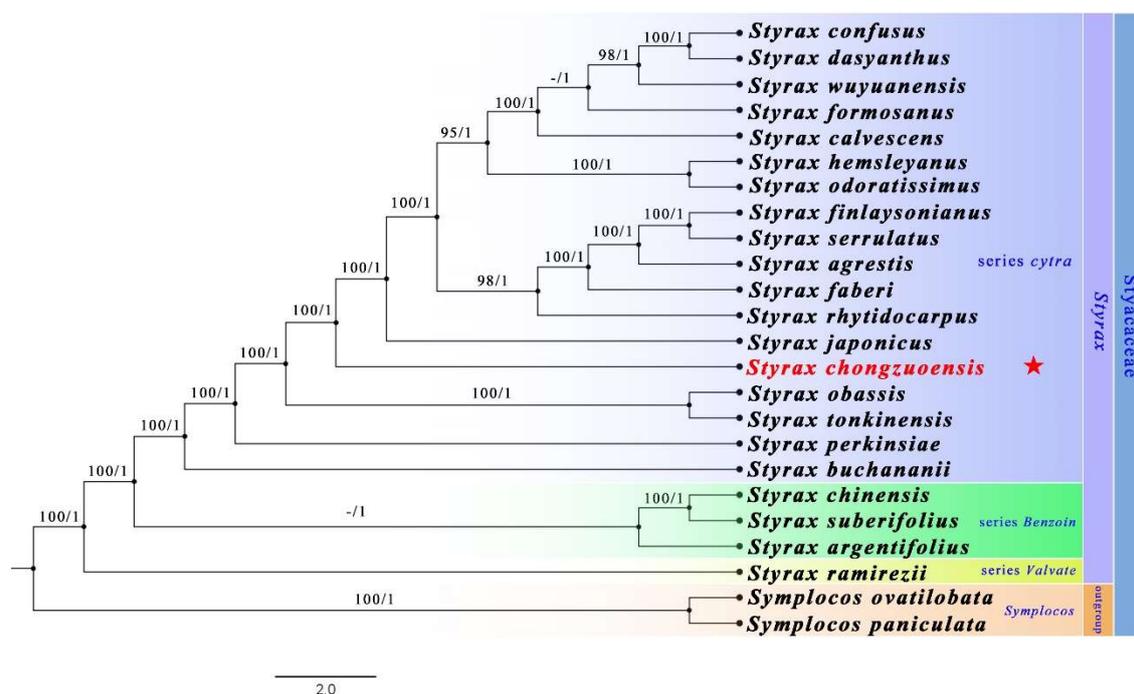


Figure 6. Maximum likelihood tree for the Styracaceae based on the CDS dataset, with *Styrax chongzuensis* highlighted in red color (★). Bootstrap values (MLBS \geq 70) and posterior probabilities (PP \geq 0.95) are labeled above the branches.

Diagnosis: *S. chongzuensis* is similar to *S. argentifolius* in leaf shape, but differs in the fact that *S. chongzuensis* is: 1) deciduous (vs. evergreen), 2) leaf blades thickly chartaceous (vs. coriaceous), 3) leaf abaxially densely covered with yellowish-brown stellate tomentum (vs. silvery-gray scaly tomentum), 4) inflorescences often single flower, or 2–3 clustered at branchlet tips (vs. axillary or sterminal racemes, 3–9 flowered), and 5) fruits with elongated tip (vs. apex shortly rostrate). (Table 1 and Figure 7)

Table 1. Comparison between *Styrax chongzuoensis* and *Styrax argentifolius*.

Character	<i>Styrax argentifolius</i>	<i>Styrax chongzuoensis</i>
Growth form	Evergreen tree, 5–15 m	Deciduous shrub or small tree, 2–5 m
Leaf texture	Leathery	Thickly chartaceous
Leaf blade	Elliptic, ovate-lanceolate or elliptic-lanceolate, base symmetrical	Narrowly long ovate or elliptic-lanceolate, base often asymmetrical
Leaf indumentum	Adaxial: glabrous, shiny; Abaxial: silvery-gray/light brown scalelike hairs	Adaxial: glabrous; Abaxial: yellowish-brown stellate/scalelike tomentum
Petiole (length)	7–12 mm, brown scalelike hairs	5–10 mm, silvery-gray scaly hairs
Inflorescence	Terminal/axillary racemes with 3–9 flowers	Axillary single flowers, sometimes 2 or 3 clustered at branchlet tips
Floral indumentum	Early stage: silvery white-gray scalelike hairs; Later: yellow-brown scalelike hairs	Dense brown scalelike hairs (peduncle, pedicel, bracteoles, calyx)
Fruit	Subglobose, ca. 2.6 cm diam., gray scalelike hairs, wrinkled when dry	Ovate/broadly ovate with long curved tip, apiculate
Seed	Subglobose, light brown, glabrous	Ovate, brown, covered with rust-brown stellate hairs

Conservation status: The Banli Area, Jiangzhou District, Chongzuo, Guangxi *White-headed Langur* Nation Reserve, is the first site in China where *Styrax chongzuoensis* was recorded. It is understood that the Guangxi Chongzuo *White-headed Langur* Nation Reserve is a wildlife-type nature reserve, and the peaked rocky mountains of the reserve are mainly dominated by shrubs, small trees and thorny vine species, with a fragmented community structure of vegetation and a very fragile ecology. At present, the population of *S. chongzuoensis* was found within a radius of 1 kilometer and does not exceed 10 plants, which can only be found in the extremely narrow distribution area of the karst mountain tops in the region. However, due to the surrounding adjoining farmland and villages, the low elevation of the mountain, the very sparse vegetation, the fragile ecological environment, and the existence of a certain degree of anthropogenic interference, it may face deforestation or other unforeseen habitat disturbance and destruction, and the species is at a high risk of extinction. However, as it is unclear whether there are new populations distributed in similar habitats in the region, according to the IUCN Criteria [20], it is considered Data Deficient (DD) until more information becomes available.

Additional specimens examined: Pairu Village, Zuozhou county, Chongzuo city, Guangxi Zhuang Autonomous Region, China. alt. 240 m, 13 Apr. 2021, *WB Xu et al. 14236* (paratypes, IBK!, JXAU!); same locality. alt. 270 m, 31 Oct. 2021, *WB Xu & YS Huang CZ0921* (paratypes, IBK!, JXAU!).

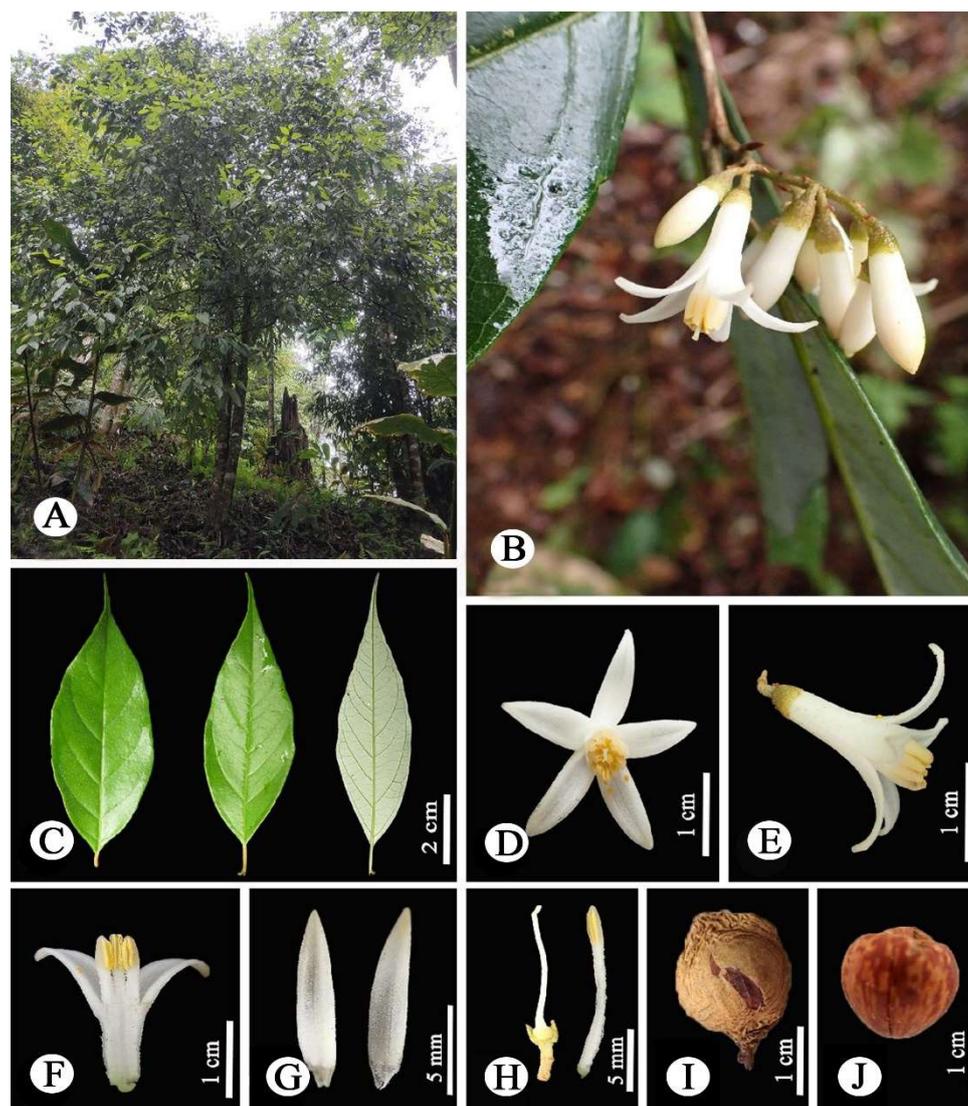


Figure 7. *Styrax argentifolius*. (A) Habitat and habit; (B) Inflorescence; (C) Leaf blades; (D) Flower (apical view); (E) Flower (lateral view); (F) Opened corolla plus androecium, ventral view; (G) Corolla lobes (left: front view; right: back view); (H) Stamens (right) and pistil (left); (I) Fruit; (J) Seeds. Photographed by Rui Zhang.

5. Notes

The genus *Styrax* likely contains undescribed or cryptic species in understudied regions, particularly karst areas requiring prioritized investigation. These limestone landscapes exhibit exceptional edaphic and topographic heterogeneity [21], creating specialized microhabitats that foster plant diversification and speciation. This is evidenced by recent discoveries of endemic taxa in Orchidaceae, *Begonia*, *Conandron* and *Primulina* [22–25].

Recent botanical surveys in the karst regions of Guangxi and Yunnan have revealed three novel species and one new regional record within the genus *Styrax* [3]. Notably, specimens of the newly described *Styrax hwangiae* M. Tang & W. B. Xu were previously misclassified as *S. chinensis* Hu & S. Y. Liang—a species widely distributed across southern China and northern Southeast Asia. However, *S. hwangiae* exhibits distinct morphological characteristics including smaller leaves, calyces with branched stellate hairs, and beaked fruit apices [3]. The Southeast Asian karst zone, particularly the areas bordering southern/western Yunnan and southwestern Guangxi, contains extensive limestone formations covering approximately 10% of the region's landmass. This geologically unique area represents one of the world's most significant biodiversity hotspots, harboring exceptional concentrations of endemic flora and fauna. Its distinctive ecosystem makes it particularly valuable

for studying the evolutionary patterns and biogeographical distribution of *Styrax* species. Despite its ecological significance, current field research efforts in these karst habitats remain insufficient [26,27]. Our findings suggest that additional undocumented *Styrax* taxa may exist in these understudied regions, highlighting the urgent need for intensified botanical surveys focused on limestone-specific vegetation communities.

Author Contributions: G.P.: software, formal analysis, writing—original draft, writing—review, editing; T.L.: formal analysis, visualization; Y.W.: formal analysis, visualization; L.Z.: software, formal analysis, writing—review, editing; R.Z.: investigation, visualization; Y.H.: investigation, funding acquisition; L.Z.: writing—review, editing. W.X.: investigation, funding acquisition; M.T.: conceptualization, supervision, funding acquisition, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding authors.

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Conflict of Interest: The authors have declared that no competing interests exist.

Ethical Statement: No ethical statement was reported.

Appendix A

Table A1. Voucher information and GenBank accession of species used in our study.

Accession no.	Taxon	Voucher information	Herbarium
BL14236	<i>Styrax chongzuoensis</i>	Xu WB et al, BL14236	JXAU
MT644192	<i>Styrax agrestis</i>	Poilane E., #18626	P
PQ276582	<i>Styrax argentifolius</i>	W. T. Tsang, #30238	A
PQ276583	<i>Styrax buchananii</i>	Buchanan, E.M., #51	E
MN560141	<i>Styrax calvescens</i>	A. Henry, #721	A
MT648752	<i>Styrax chinensis</i>	-	-
MT700478	<i>Styrax confusus</i>	A. Henry, #3450	A
MT700479	<i>Styrax dasyanthus</i>	C. Silvestri, #1770	A
MN335255	<i>Styrax faberi</i>	H. F. Hance, #13738	GH
PQ276585	<i>Styrax finlaysonianus</i>	-	-
MZ285742	<i>Styrax formosanus</i>	-	-
MN733525	<i>Styrax hemsleyanus</i>	Wilson, E.H., #2574a	HBG
PP853567	<i>Styrax japonicus</i>	Nebel, O., #Herb. J. Goltz s.n. (1900-05-25)	JE
MN560143	<i>Styrax obassia</i>	-	-
MN368610	<i>Styrax odoratissimus</i>	Champion, J.G., #138	K
MZ285734	<i>Styrax perkinsiae</i>	E. H. Wilson, #2576	A
NC041138	<i>Styrax ramirezii</i>	Pringle, C.G., #6848	G/K
PQ066665	<i>Styrax rhytidocarpus</i>	-	-
MZ152917	<i>Styrax serrulatus</i>	s.coll., #s.n.	K
MZ285747	<i>Styrax suberifolius</i>	P. G. Farges, #1487	A
MZ285744	<i>Styrax tonkinensis</i>	Augustine Henry, #12006	MO
OP581012	<i>Styrax wuyuanensis</i>	-	-

MF770705	<i>Symplocos ovatilobata</i>	F. C. How, #71750	A
MG719832	<i>Symplocos paniculata</i>	Siebold, PF von, #s.n.	L

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