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Pseudarthria panii (Fabaceae: Desmodieae), a new species from Asia, 120 years after its first collection

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Abstract

A new trifoliolate shrub, *Pseudarthria panii* (Fabaceae: Desmodieae), is described and illustrated. It resembles *Pseudarthria viscida*, but differs by its taller height, upright habit, late flowering, leaflets with an acuminate apex, longer light purple flowers, longer glabrous fruits, and more seeds. It occurs in the montane forest of China (Yunnan), Laos, Myanmar, Thailand, and Vietnam. Molecular evidence from the concatenated chloroplast fragments of *rbcL* and *matK* confirms its placement in *Pseudarthria*, which is a newly recorded genus from China, Laos, Thailand and Vietnam. The first collection of the new species can be dated back to ca. 120 years ago, i.e. Augustine Henry's collection in Yunnan during 1896–1899. A key to *Pseudarthria* is also presented.

Keywords: China, history of collection, legume, Leguminosae, taxonomy

Introduction

Pseudarthria Wight & Arnott (1834: 209) is a small genus of 4–6 species distributed in the Old World (Verdcourt 2000). Most of these species occur in seasonally dry tropical areas of Africa (Verdcourt 1971; Lock 1989), while only one species, *P. viscida* (L.) Wight & Arnott (1834: 209), is found in South and Southeast Asia (Baker 1879; Baker *et al*. 1963), where it is used in the treatment of fever, bronchial asthma, hemorrhoids, edema, diabetes mellitus, diarrhea and tuberculosis (Deepa *et al*. 2004). Traditionally, the genus was placed in the tribe Pseudarthrieae (Hutchinson 1964), subtribe Pseudarthriinae (Pramanik & Thothathri 1989) or subtribe Desmodiinae (Ohashi *et al*. 1981) of the tribe Desmodieae (Hutchinson 1964). However, molecular evidence revealed that it should be ascribed to the *Desmodium* group, one of three groups in the tribe Desmodieae (Nemoto & Ohashi 2003, Ohashi 2005, Jabbour *et al*. 2018).

The species of *Pseudarthria* are all pinnately 3-foliolate herbs, shrubs or subshrubs and closely resemble *Desmodium* Desvaux (1813: 122), but differ in their unsegmented fruits (Baker 1871). During a field survey in Yunnan Province, China, we found a distinct '*Desmodium*-like' species that morphologically did not match any of the known species from China, due to its non-articulated fruits with nearly straight sutures. Further morphological observations revealed that it represents an undescribed *Pseudarthria* species as described and illustrated below. Molecular phylogenetic analysis of the concatenated chloroplast fragments of *rbcL* and *matK* confirmed its position within *Pseudarthria*.

Materials and methods

Morphological studies

All morphological characters were examined in detail and compared with possible relatives based on the specimens from the Global Plants of JSTOR (http://plants.jstor.org/), HITBC, KUN, A, US and Kew herbaria.

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Molecular methods and phylogenetic analyses

For the phylogenetic analyses we used the two concatenated chloroplast fragments of *rbcL* and *matK* from 40 species from 17 genera of the tribe Desmodieae. All sequences except that of the new species were acquired from GenBank (Table S1 in Appendix). The voucher specimens (PZ02) of the new species were deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Total genomic DNA was extracted from 100 mg of silica gel-dried leaflets with a modified CTAB protocol (Doyle 1987) and sent to Novogene, Nanjing, China, for library construction and next generation sequencing. A DNA 250 bp insert size library was constructed. More than 3 Gb paired-end reads of 2×150 bp for this species were generated on the Illumina HiSeq XTen platform. The reads were assembled using Linux-OS SPAdes v3.10.1 (Bankevich *et al*. 2012) with k-mer = 127 into contigs. Contigs were filtered by python script slim_fastg_by_blast.py (Jin *et al.* 2018) and later connected into plastome using Bandage Ubuntu dynamic v.8.0 (Wick *et al*. 2015). The connected plastome sequence and reference *Lotus japonicus* downloaded from GenBank (NC002694) were aligned with MAFFT (Katoh *et al.* 2013) using default parameters to copy annotations, coupled with manual adjustment in Geneious v.9.1.4 (Kearse *et al.* 2012). We then extracted *rbcL* and *matK* sequences from the assembled plastome for phylogenetic analyses. Sequences of *rbcL* and *matK* were deposited in GenBank under the accessions MG674205 and MG674206.

Phylogenetic analyses

Sequences were aligned with MAFFT (Katoh *et al.* 2013) using default parameters (L-INS-I algorithm) in Geneious v.9.1.4. Alignments were subsequently analyzed visually and adjusted manually. Phylogenetic analyses were performed with standard Bayesian inference (BI) and maximum-likelihood (ML) methods. The best-fit model was determined by MODELTEST v.3.7 (Posada & Crandall 1998) with the Akaike Information Criterion (AIC) (Posada & Buckley 2004). The BI analysis was performed with MrBayes v.3.2 (Ronquist *et al*. 2012) with four Markov Chain Monte Carlo (MCMC) runs using a random starting tree, an invgamma rate model with six discrete categories and 10 million generations, with a sampling frequency of one every 1000 generations and 25% of the trees were discarded as burn-in. Stationarity was considered to be reached when the average standard deviation of split frequencies was below 0.01. The ML analysis was performed with command line RAxML v.7.2.8 (Stamatakis 2006) in Linux OS, including tree robustness assessment using 1000 replicates of rapid bootstrap (the "-f a" option) with the GTRGAMMA substitution model to assess branch support (Stamatakis 2006).

Results

Phylogenetic analyses

The ML and Bayesian analyses of the combined data set of two chloroplast fragments of *matK* and *rbcL* generated congruent topologies. The ML tree with ML bootstrap (BS) and Bayesian posterior probability (PP) values is shown in Fig. 1. The new species *Pseudarthria panii* and *P. hookeri* Wight & Arnott (1834: 209) formed a clade strongly supported in the ML analysis (BS = 98%, PP = 0.75; Fig.1). The phylogeny showed that *Pseudarthria* was retrieved as sister to a clade containing *Alysicarpus*, *Christia*, *Codoriocalyx*, *Desmodium*, *Hegnera*, *Mecopus*, *Melliniella* and *Uraria*, and that *Desmodium* was not monophyletic.

Taxonomy

Pseudarthria panii Rong Zhang, Ting-Shuang Yi & Bo Pan (潘勃), ***sp. nov.*** 百年假节蚂蝗 (Fig. 2–3)

Type:—CHINA. Yunnan: Xishuangbanna, Menghai County, Xiding, 21°55′36″N, 100°7′39″E, 1800 m, 13 December 2014, *Bo Pan XD001* (holotype: Barcode 1262264, KUN! isotype: HITBC!)

Diagnosis:—This species is similar to *Pseudarthria viscida* in the rhomboid-ovate leaflets and unjointed pods, but differs by its taller and upright growth form (vs. 0.6–1.2 m in height, diffuse or prostrate), late flowering in September (vs. May), leaflets with an acuminate (vs. acute) apex, light purple (vs. pink) and longer (14 vs. 5 mm) flowers, longer (30–60 vs. 9–20 mm) and glabrous (vs. sticky pubescent) pods, and more (5–12 vs. 2–6) seeds. This new species also resembles *Desmodium megaphylla*, a species widely distributed in Asia including China, especially under the flowering condition, but differs from it by the linear and unsegmented fruits.

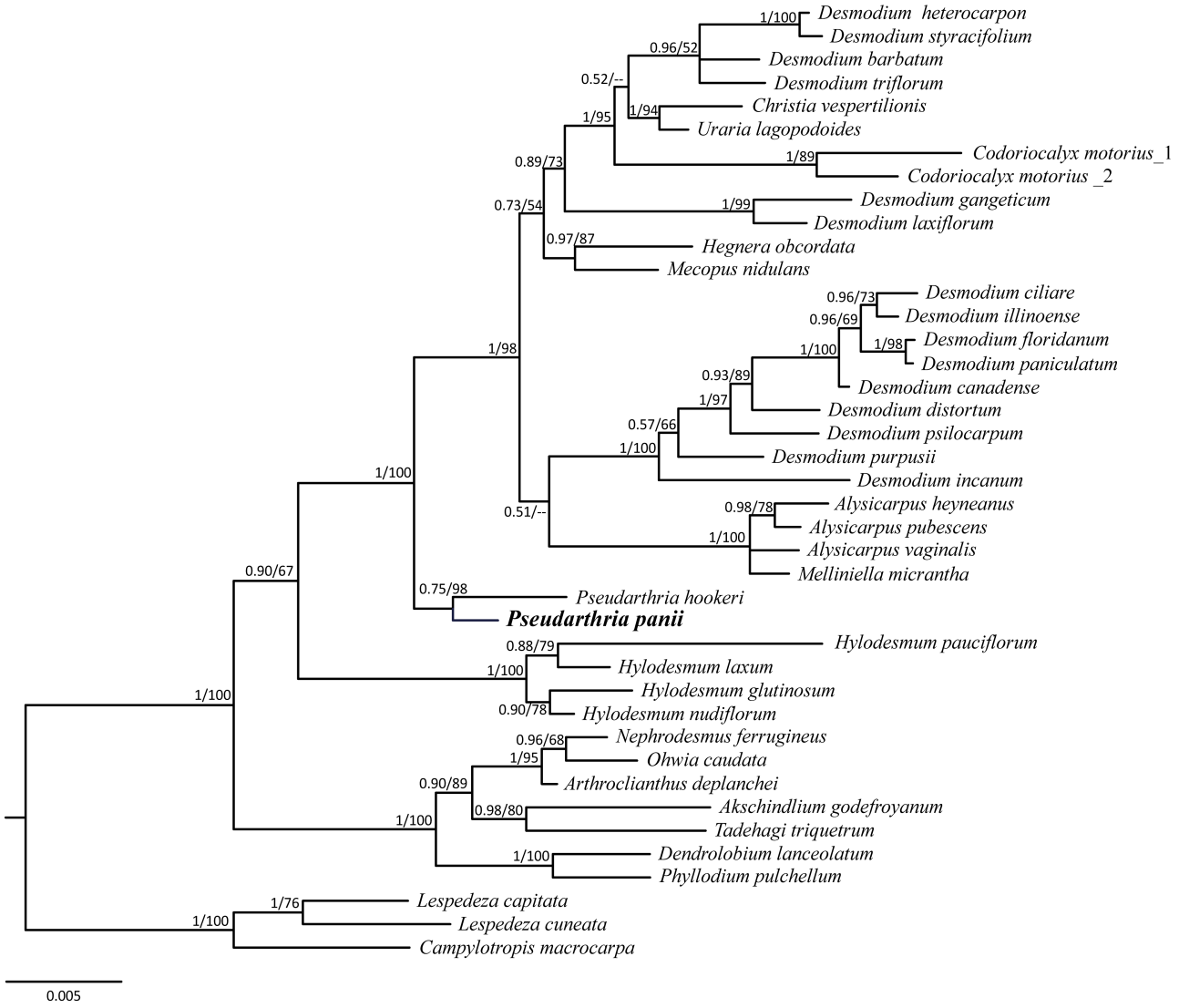

FIGURE 1. Phylogenetic relationships among 40 species from 17 genera of Desmodieae based on the combined data set of chloroplast *matK* and *rbcL*. The tree was constructed using the Maximum likelihood method. Numbers near the nodes are posterior probabilities and bootstrap percentages (PP, BP) from Bayesian analysis and Maximum likelihood, respectively. A dash (--) indicates the bootstrap <50% for ML tree. The new species in this study is shown in bold type.

Description:—Shrub with several clustered stems rising from one rootstock, 1.5–3.0 m tall, slightly zigzagged, and few branched. Old stem erect, terete, and glabrescent, while the young branchlets bending, more or less angled, and finely pubescent with small hooked hairs. Leaves two ranked, obviously getting smaller along the branch. Stipules triangulate, ca. 7 × 2 mm, caduceus late. Petioles 20–60 mm, covered with very short hooked hairs. Stipels linear, 2–4 mm. Petiolules shorter than the stipels, ca. 3 mm, pubescent with both long hairs and mixed with short hooked hairs. Leaflets 3, ovate, 30–120 × 15–60 mm, acuminate at apex, round at base, adaxial surface thin hairy with hooked hairs or glabrescent, but densely short pubescent abaxially; margins entire, revolved when dry. Terminal leaflets slightly bigger than or four times as big as the lateral ones. Secondary veins 4–6 pairs. Veins all sunken on the adaxial side. Main veins reach the margin. Inflorescences 50–140 mm long, much longer than the subtending leaf, 1–3 pseudoracemes clustered at the axil, ascending or pendulous. Each branchlet ends with a terminal inflorescence. Rachis of the inflorescence slender and short, 10–30 mm, pubescent with small hooked hairs. Bracts caducous. 2–3 flowers clustered at each node. Pedicels long and gracile, 10–15 mm, hooked hairy and soon glabrous. Bracteoles 2 below the calyx, minute. Calyx campanulate, 4 lobed, 4–5 mm, upper two teeth almost connate, lower lobe the longest. Corolla papilionaceous, light or pale purple. Standard orbicular to obovate, ca. 10 mm, with no auricles; wings lanceolate and incurved, ca. 14 mm, much larger than the keel; keel oblanceolate, ca. 10 mm; both wings and keel auriculate and clawed. Stamens 10, ca. 12 mm, vexillary stamen connate with the other 9 at base. Pistil linear, ca. 10 mm, with a dilated up-curved part and a

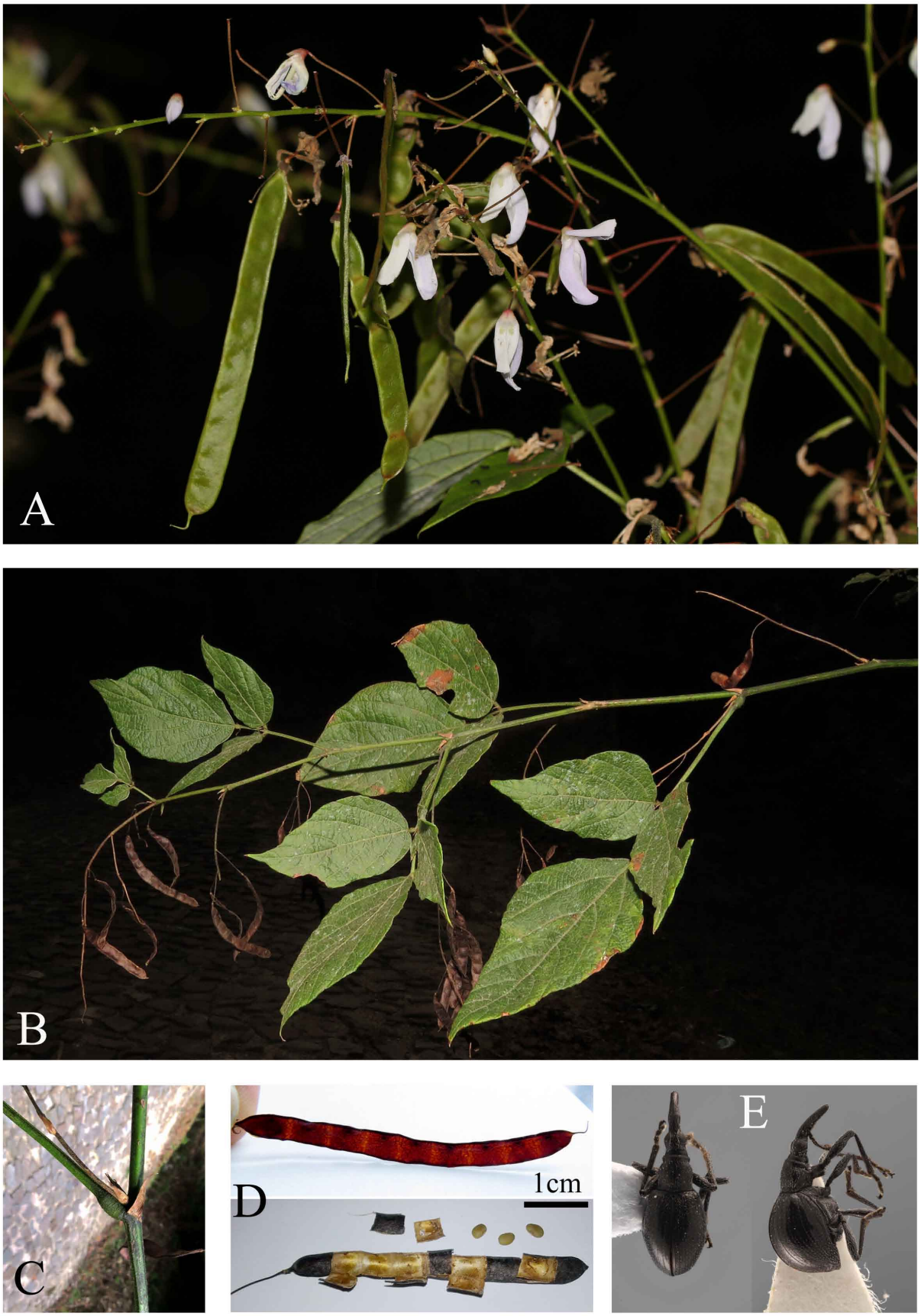

FIGURE 2. *Pseudarthria panii*. A. Flowering branch. B. Fruiting branch. C. Stipule on angulate stem. D. Fruits. E. *Conapium schenklingi*, weevil from the pod. A–D. Photos by Bo Pan. E. Courtesy of Dr. Zhi-Liang Wang.

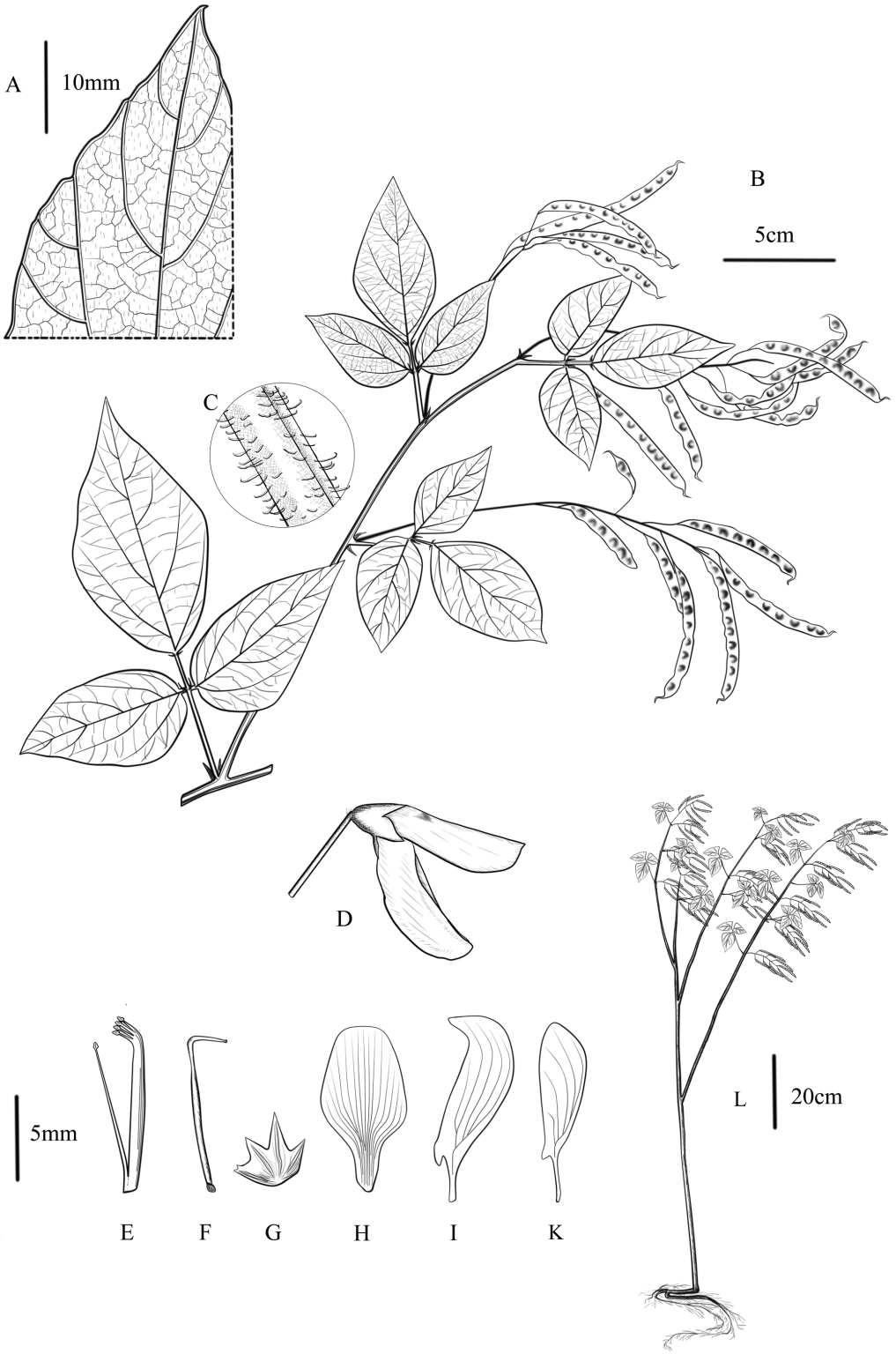

FIGURE 3. *Pseudarthria panii*. A. abaxial side of leaflet (revolved margin), B. fruiting branch, C. petiole clothed with small hooked hairs, D. flower, E. stamens, F. pistil, G. calyx, H. standard, I. wing, K. keel, L. habit. Illustrations by Bo Pan, from the holotype—CHINA. Yunnan: Xishuangbanna, Menghai County, Xiding, 13 December 2014, Barcode 1262264, *Bo Pan XD001* (KUN).

nectary disc at base; stigma capitate; pods linear and glabrous, 30–60 × 3–4 mm, with 5–12 seeds; both sutures almost straight, valves with conspicuous reticulate veins when young; indehiscent when ripe, persistent on the branches for a long time. Valves break into squares when torn but are still connected along the sutures. Seeds elliptic and compressed, ca. 2 × 3 mm.

Phenology and distribution:—The flowering period is very short, from late September to early October and fruiting occurs in October to December, with the fruits persistent on the branches until the following May. This species is distributed in the broad-leaved montane forests in China (Yunnan Province), Laos, Myanmar, Thailand and Vietnam (Fig. 4), at an elevation of 900–2000 m. Though we found a very limited number of specimens, we presume that it has more populations and a wider distribution in the wild because there have been few field surveys in this region and a very low rate of identification. *Pseudarthria panii* is highly scattered, and each known location has only 3–5 individuals. Its young leaves and fruits are always infested by pests, e.g. the pods of the Menghai population are seriously damaged by a weevil *Conapium schenklingi* Wagner (1914) (Fig. 3E), identified by Professor Marek Wanat (University of Wroclaw, Poland).

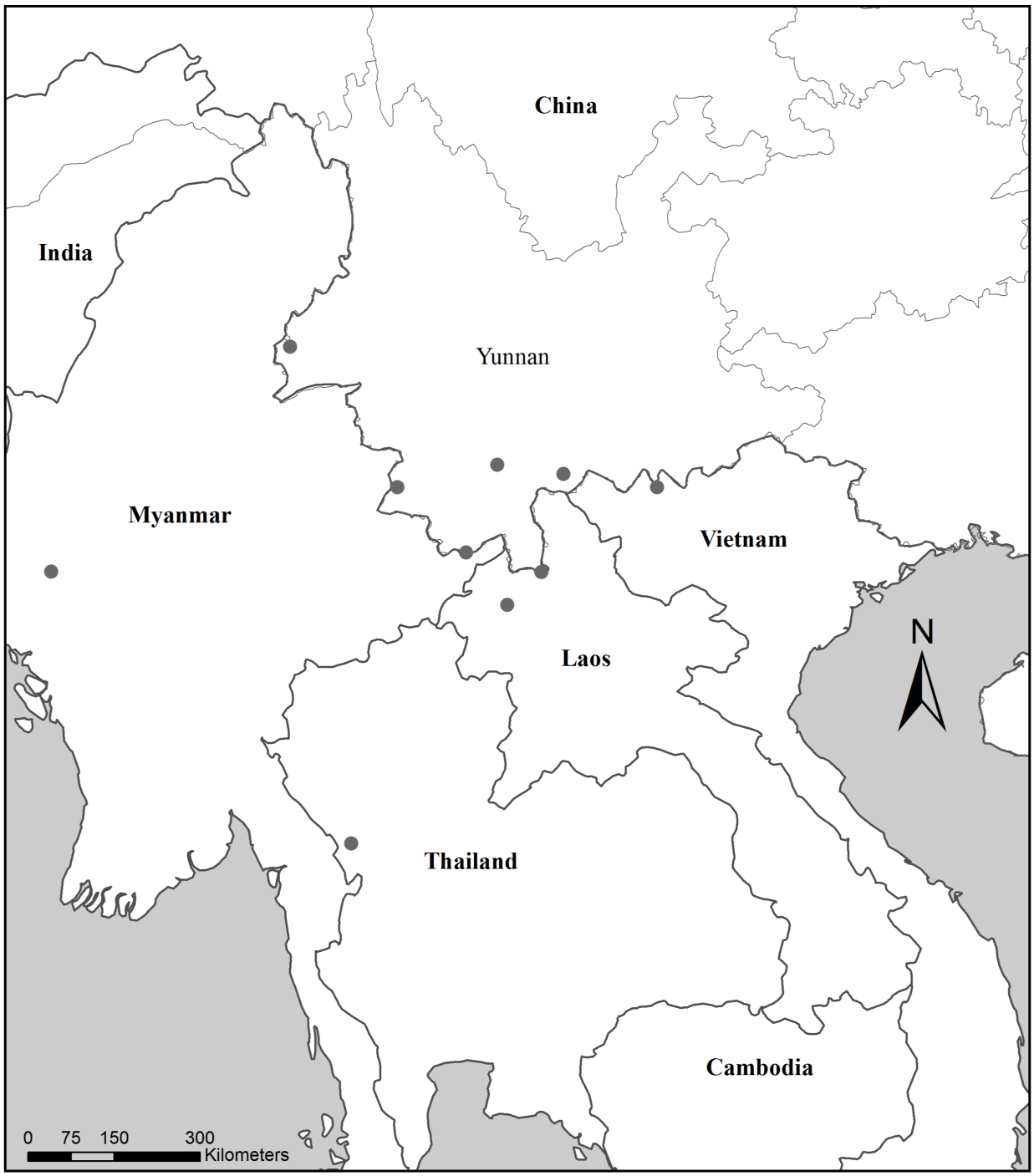

FIGURE 4. Geographical distribution of *Pseudarthria panii*. Prepared by Dr. Rui-Wu Zhou.

Etymology:—the epithet '*panii*' honours Lao-Cang Pan (1894–1962), who was the grandfather of the corresponding author Bo Pan. He was a carriage driver and horse veterinarian and has a large family and nurtured the love of nature among his children. His year of birth was during the period of Augustine Henry, an Irish Botanist, collecting in China. Henry collected plants in Taiwan in 1893–1894, and then Yunnan in 1896–1899 (Nelson 1983). Henry collected this *Pseudarthria* species (*No. 12483B*) in Simao, Yunnan Province, during 1898–1899, around 120 years ago, and considered it as '*Pueraria peduncularis*'. Here we propose a Chinese name '百年假节蚂蝗' for it; '百年' which means 'one hundred years', referring to the fact that it took more than 100 years to get a proper name. '假节蚂蝗' (http://duocet.ibiodiversity.net/index.php?title=豆科) derives from the Latin generic name, '*Pseud*—false' and '*arthria*— article', which means 'pods without articles'.

Additional specimens examined:—CHINA. Yunnan: Damenglong, Mengsheng, under thick woods, 1850 m, September 1936, *C.W. Wang 78285* (PE). Lüchun, Huanglianshan, Qimaba, 900–1200 m, 1 November 1995, *Su-Gong Wu et al. 1068* (KUN). No precise location record, hilly slopes, 1670 m, 21 November 1986, *86 Team* (KUN). Simao, 5000 ft, *A. Henry 12483B* (A). Ximeng, broad-leaved forest, 2000 m, 22 October 1989, *Guo-Da Tao* & *Xi-Wen Li 39759* (HITBC). Xishuangbanna, Mengla, Shangyong, 900 m, 22 October 1974, *Zeng-Hong Yang 12055* (HITBC). Yingjiang County, Xima, Dazhai, 1700 m, 2 November 1974, *Guo-Da Tao 13076, 12786* (HITBC). **LAOS.** 20 km south to the China-Laos border, 1700 m, 3 May 1993, *Guo-Da Tao 930222* (HITBC). **MYANMAR. Chin State:** Par Kung Village, Kanpetlet. 4000 ft, 18 December 2011, *Ling Shein Man 88022* (QBG). **THAILAND.** Tak, Tha Song Yang, Near Mon Kieo Lom, Mae Moei National Park, 17°31′25″N, 98°04′06″E, 820 m, edge of montane forest, 6 November 2010, *Pooma P. et al. 7590* (BKF). **VIETNAM.** Annam, 1500 m, 13 September 1940, *M. Poilane 30391* (P).

Key to *Pseudarthria* species:

1. Stems angulate; terminal leaflet ovate or rhomboid-ovate; plant from Asia 2
1. Stems sulcate; terminal leaflet oblong, oblong-lanceolate or obovate; plant from Africa 3
2. Shrub 1.5–3.0 m, upright; leaflet acuminate at apex; flowering in September to October, flower light purple and 14 mm long; pod glabrous and 30-60 mm long, seeds 5–12 ..... *P. panii*
2. Shrub 0.6–1.2 m, diffuse to prostrate; leaflet acute at apex; flowering in May, flower pink and 5 mm long; pod sticky pubescent and 9–20 mm long, seeds 2–6 ..... *P. viscida*
3. Lateral and terminal leaflets oblong-lanceolate ..... *P. crenata*
3. Terminal leaflet obovate, lateral leaflets ovate to elliptic 4
4. Racemes lax; pod 1–3-seeded ..... *P. fagifolia*
4. Racemes dense; pod many-seeded 5
5. Leaves glabrescent beneath; flowers in compact racemes ..... *P. confertiflora*
5. Leaves densely persistently pubescent beneath; flowers dense but not in compact racemes 5
6. Pod 4–5 mm wide, 5–6-seeded ..... *P. macrophylla*
6. Pod 3 mm wide, 6–10-seeded ..... *P. hookeri*

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TABLE S1. Accession number of *matK* and *rbcL* sequences of sampled species obtained from GenBank.

species name	*matK*	*rbcL*
Akschindlium godefroyanum (Kuntze) H. Ohashi	KX713079	KY702639
Alysicarpus heyneanus Wight & Arn.	KT222213	KT222229
Alysicarpus pubescens J.S.Law	KT222218	KT222236
Alysicarpus vaginalis (L.) DC.	JQ587509	KJ773259
Arthroclianthus deplanchei Hochr.	KX213147	KY702603
Campylotropis macrocarpa (Bunge) Rehder	AY386870	EU717277
Christia vespertilionis (L.f.) Bakh.f.	KF621098	KF621119
Codoriocalyx motorius (Houtt.) H. Ohashi	KF621099	KX527051
Codoriocalyx motorius (Houtt.) H. Ohashi	HM049526	KX344706
Dendrolobium lanceolatum (Dunn) Schindl.	AB924975	KY702645
Desmodium barbatum (L.) Benth.	EU717420	JQ591689
Desmodium canadense (L.) DC.	HQ593266	HQ590061
Desmodium ciliare (Willd.) DC.	KJ772716	KJ773442
Desmodium distortum (Aubl.) J.F.Macbr.	JQ587590	JQ591694
Desmodium floridanum Chapman	EF549994	KJ773443
Desmodium gangeticum (L.) DC.	KP093702	KX344694
Desmodium heterocarpon (L.) DC.	AB925045	KY702613
Desmodium illinoense A.Gray	KT456906	KX385961
Desmodium incanum DC.	JF501110	KJ773444
Desmodium laxiflorum DC.	KP094091	KP095045
Desmodium paniculatum (L.) DC.	KJ772717	KX385962
Desmodium psilocarpum A.Gray	AY386896	LT576814
Desmodium purpusii Brandegee	JQ587603	JQ591711
Desmodium styracifolium (Osbeck) Merr.	JN407126	JN407286
Desmodium triflorum (L.) DC.	KJ772719	KJ773446
Hylodesmum pauciflorum (Nutt.) H. Ohashi & R.R. Mill	EU717421	EU717280
Hegnera obcordata (Miq.) Schindl.	AB924977	KY702617
Hylodesmum glutinosum (Muhl. ex Willd.) H. Ohashi & R.R. Mill	KJ592990	KJ593475
Hylodesmum laxum (DC.) H.Ohashi & R.R.Mill	KF621102	KF621124
Hylodesmum nudiflorum (L.) H. Ohashi & R.R. Mill	KP642947	KP402657
Lespedeza capitata Michx.	KT456912	KJ773621
Lespedeza cuneate (Dum.Cours.) G.Don	EU717416	U74215
Mecopus nidulans Benn.	KX713102	KY702622
Melliniella micrantha Harms	KX713103	KY702623
Nephrodesmus ferrugineus Däniker	KX213176	KY702651
Ohwia caudata (Thunb.) H.Ohashi	KX713109	KX527365
Phyllodium pulchellum (L.) Desv.	HM049524	KY702658
Pseudarthria hookeri Wight & Arn.	JF270902	JF265559
***Pseudarthria panii* Rong Zhang, Ting-Shuang Yi & Bo Pan**	**MG674205**	**MG674206**
Tadehagi triquetrum (L.) H.Ohashi	JN407127	KX527094
Uraria lagopodoides Roxb.	KF621107	KF621128

Note: Accession numbers HM049526 and KX344706 were recorded as *Desmodium gyrans* (L. f.) DC. in GenBank, we use the accepted name *Codoriocalyx motorius* (Thunb.) H. Ohashi in this study (http://www.tropicos.org/Name/100356174); similarly, EU717421 and EU717280 as *Desmodium pauciflorum* (Nutt.) DC. in Genbank, we use the accepted name *Hedysarum pauciflorum* (Nutt.) H. Ohashi & R.R. Mill (http://www.tropicos.org/Name/50327477).