

# *Rubovietnamia nonggangensis* (Rubiaceae), a new species from China

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(Received December 8, 2008; Accepted June 18, 2009)

**ABSTRACT.** A new Rubiaceae species, *Rubovietnamia nonggangensis* F. J. Mou & D. X. Zhang, is described and illustrated from Guangxi, China. The somatic chromosome number ( $2n = 22$ ) and pollen morphology of the species are reported. The new species is characterized prominently by having dense hairs on many organs, such as young branches, leaves, and inflorescences, enlarged and foliaceous calyx lobes caducous after anthesis, and indehiscent and globose fruits with persistent yellowish annular floral disks on the apex. All morphological data support that it belongs to the genus *Rubovietnamia*, a genus distributed in Vietnam and southern China, currently classified in the tribe Gardenieae of the subfamily Ixoroideae.

**Keywords:** Chromosome number; Gardenieae; Molecular phylogeny; New species; Pollen morphology; Rubiaceae; *Rubovietnamia*; *Rubovietnamia nonggangensis*.

## INTRODUCTION

The genus *Rubovietnamia* Tirveng. was first described from northern Vietnam based on a single species *Rubovietnamia aristata* Tirveng. (Tirvengadam, 1998). Recently, it has been recorded from Guangxi and Yunnan in China (Zhang et al., 2007). *Rubovietnamia* is closely related to *Vidalasia* Tirveng. in the tribe Gardenieae (Rubiaceae), and is characterized by the indehiscent, few- to many-seeded and relatively large fruits, containing a juicy pulp formed from the septum and/or the placenta (Robbrecht and Puff, 1986). Tirvengadam (1998) described the sole species of the genus as follows: a shrub with reddish brown bark, its leaf apex often markedly aristate due to prolongation and the main nerve projected beyond the acumen on the undersurface of the limb; the deltoid stipules connate at base, caudate-acuminate at apex, subcoriaceous, provided with hairs and few small colleters mostly towards the base inside; the inflorescence a 2-4-flowered cyme or with a solitary flower; bracts and bracteoles minute and stipule-like; calyx foliaceous with a large base and aristate at apex; corolla tube much longer than the lobes, cylindrical with a ring of short hairs towards the base inside; anthers attached to the middle of the corolla tube and half-exserted; style slender and covered with short hairs with bifid and grooved stigma; ovary 1-celled; fruit globose and smooth; seeds ovoid to subglobose.

During a field trip in 2008 in the Nonggang Nature Reserve, Longzhou County, Guangxi Province in China,

a species characterized with creamy and fragrant corollas was found. Based on the leaf-like calyx lobes and indumentum, the specimens were putatively referred to as a new species of the genus *Rubovietnamia*.

## MATERIALS AND METHODS

### Morphology

The morphological characters of the new species were studied based on living specimens in their natural habitats and on plants growing in SCBG (South China Botanical Garden, the Chinese Academy of Sciences) and on specimens from herbaria in China (IBK [Guangxi Institute of Botany] and IBSC [the South China Botanical Garden, CAS]). The relevant literature concerning the genus was also reviewed. The field studies were carried out three times from May to July 2008 in Guangxi Province, China.

### Palynology

Pollen samples collected from dried specimen (*F. J. Mou 209-1*, IBSC) were prepared by acetolysis 10 min at 95-100°C (Erdtman, 1960). The unacetolyzed and acetolyzed pollens were rinsed twice respectively with ultrasound in 70% ethanol and stored in 70% ethanol. After drying in air, pollen grains were sputter-coated with gold-palladium and examined using a JSE-6360LV SEM at a voltage of 10 KV. More than twenty pollen grains were measured for acetolyzed pollen of the species. The terminology follows mainly Erdtman (1952) and Faegri and Iversen (1989).

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## Cytology

Mitotic chromosomes were investigated by using root tips from plants cultivated in the greenhouse of SCBG. Actively growing root tips were pre-treated with 0.002 mol/L 8-hydroxyquinoline solution while shielded from light for about 1.5 h at 4°C, washed with distilled water, fixed in fresh cold Carnoy's Fluid (ethanol : acetic acid solution = 3:1) in a mixture of water and ice for 2-3 h at 4°C, thrice washed with distilled water, and later hydrolyzed in 1 mol/L hydrochloric acid at 60°C for 7-8 min. After three more rinses in distilled water, root tips were stained in Carbol Fuchsin solution for more than 15 min and then squashed on the slide for light microscopy.

## Molecular phylogeny

Total DNA was isolated from fresh leaves of *Rubovietnamia nonggangensis* collected from plants growing in the greenhouse in SCBG (*F. J. Mou 230*, IBSC) using the modified 2 × CTAB method after leaf tissue was ground in liquid nitrogen (Doyle and Doyle, 1987). This study used two sequences of cpDNA, the *trnL-F* region (Taberlet et al., 1991) and the *rps16* gene intron (Oxelman et al., 1997). The *trnL-F* region was amplified with primers c (5'-CGAAATCGGTAGACGCTACG-3') and f (5'-ATTTGAACTGGTGACACGAG-3') (Taberlet et al., 1991). The *rps16* intron was amplified as described by Oxelman et al. (1997) with primers rpsF (5'-GTGGTAGAAAGCAACGTGCGACTT-3') and rpsR2 (5'-TCGGGATCGAATCAATTGCAAC-3'). The PCR amplification used a 5 min denaturing step at 94°C followed by 35 cycles of denaturing for 1 min at 94°C, primer annealing for 1.5 min at 52°C, and elongation for 1.5 min at 72°C, with a final 10 min elongation step at 72°C. The PCR products were sequenced using the same primer combination as for PCR amplifications. Sequences published in a previous study of Rubiaceae (Andersson and Rova, 1999; Persson, 2000; Rova et al., 2002; Bremer and Eriksson, 2009) were downloaded from GenBank and used in the present study (Table 1).

In consideration of the phylogeny of Gardenieae based on chloroplast DNA sequences from the *rps16* intron and *trnL* (U.AA)-F(GAA) intergenic spacer sequence (Andersson and Rova, 1999; Persson, 2000; Rova, et al., 2002; Bremer and Eriksson, 2009), thirty taxa (including the new species) included in "the Core Gardenieae clade" were selected as ingroups and three taxa (*Polysphaeria* sp., *Psydrax odorata* and *Ixora killipii*) from the tribes Octotropideae, Vanguerieae, and Ixoreae, respectively, were chosen as outgroups. A parsimony analysis was performed using a heuristic search with 100 random sequence addition replicates, and trees were limited to 10,000 during each replicate. Gaps were treated as missing data. Bootstrap values (BP) of the internal nodes were obtained with 500 replicates. In each replicate, we performed 10 random sequence addition replicates followed by a tree bisection-reconnection (TBR) swapping algorithm and keeping no more than 1000 trees per replicate.

**Table 1.** Species and GeneBank accession numbers (including previously published sequences).

Taxa	<i>trnL-F</i>	<i>rps16</i>
<i>Aidia micrantha</i> (K. Schum.) F. White	AF200974 <sup>1</sup>	AF201028 <sup>1</sup>
<i>Aoranthe castaneofulva</i> (S. Moore) Somers	AF200977 <sup>1</sup>	
<i>Atractocarpus stipularis</i> (F. Muell.) Puttock	AF200979 <sup>1</sup>	AF201031 <sup>1</sup>
<i>Benkara malabarica</i> (Lam.) Tirveng.	AF200981 <sup>1</sup>	AF201033 <sup>1</sup>
<i>Calochone redingii</i> (De Wild.) Keay	AF200986 <sup>1</sup>	AF201036 <sup>1</sup>
<i>Casasia calophylla</i> A. Rich.	AF200987 <sup>1</sup>	AF201037 <sup>1</sup>
<i>Catunaregam spinosa</i> (Thunb.) Tirveng.	AF200988 <sup>1</sup>	AF201038 <sup>1</sup>
<i>Ceriscoides sessiliflora</i> (Wall. ex Kurz) Tirveng.	AF200989 <sup>1</sup>	AF201039 <sup>1</sup>
<i>Deccania pubescens</i> (Roth) Tirveng.	AF200991 <sup>1</sup>	AF201041 <sup>1</sup>
<i>Duperrea pavettifolia</i> (Kurz) Pitard	AF200993 <sup>1</sup>	AF201042 <sup>1</sup>
<i>Euclinia longiflora</i> Salis.	AF200995 <sup>1</sup>	AF201043 <sup>1</sup>
<i>Gardenia volkensii</i> subsp. <i>spathulifolia</i> Stapf. & Hutch.	AF200996 <sup>1</sup>	AF201044 <sup>1</sup>
<i>Genipa americana</i> L.	AF200997 <sup>1</sup>	AF201045 <sup>1</sup>
<i>Kailarsenia ochreatea</i> (F. Muell.) Puttock	AF201002 <sup>1</sup>	AF201049 <sup>1</sup>
<i>Macrosphyra longistyla</i> (DC.) Hook. f. ex Hiern	AF201004 <sup>1</sup>	AF201051 <sup>1</sup>
<i>Massularia acuminata</i> (G. Don) Bullock ex Hoyle	AF201005 <sup>1</sup>	AF201052 <sup>1</sup>
<i>Morelia senegalensis</i> A. Rich. ex DC.	AF201007 <sup>1</sup>	AF201055 <sup>1</sup>
<i>Oligocodon cunliffeae</i> (Wernham) Keay	AF201008 <sup>1</sup>	AF201056 <sup>1</sup>
<i>Oxyceros longiflorus</i> (Lam.) Yamazaki	AF201010 <sup>1</sup>	AF201058 <sup>1</sup>
<i>Porterandia crosbyi</i> (Burkill) A.C. Sm. & S. Darwin	AF201012 <sup>1</sup>	AF201059 <sup>1</sup>
<i>Preussiodora sulphurea</i> (K. Schum.) Keay	AF201013 <sup>1</sup>	AF201060 <sup>1</sup>
<i>Rosenbergiodendron densiflorum</i> (K. Schum.) Fagerl.	AF201014 <sup>1</sup>	AF201061 <sup>1</sup>
<i>Rothmannia capensis</i> Thunb.	AM117340 <sup>2</sup>	AM117384 <sup>2</sup>
<i>Rubovietnamia nonggangensis</i> F. J. Mou & D. X. Zhang	GQ251031	GQ249894
<i>Sherbournia</i> sp.	AF201017 <sup>1</sup>	AF201064 <sup>1</sup>
<i>Sphinctanthus microphyllus</i> K. Schum.	AF201020 <sup>1</sup>	AF201066 <sup>1</sup>
<i>Sukumia longipes</i> A. C. Sm.	AF201022 <sup>1</sup>	AF201068 <sup>1</sup>
<i>Tamilnadia uliginosa</i> (Retz.) Tirveng.	AF201023 <sup>1</sup>	AF201069 <sup>1</sup>
<i>Tarennoidea wallichii</i> (Hook. f) Tirveng. & Sastre	AF201025 <sup>1</sup>	AF201070 <sup>1</sup>
<i>Trukia fitzalanii</i> (F. Muell.) Fosberg	AF201027 <sup>1</sup>	AF201072 <sup>1</sup>
<i>Ixora killipii</i> Standl.	AF201001 <sup>1</sup>	AF152659 <sup>3</sup>
<i>Polysphaeria</i> sp.	AF201011 <sup>1</sup>	AF152655 <sup>3</sup>
<i>Psydrax odorata</i> (Forst. f) A.C. Smith & S. P. Darwin	AF004031 <sup>4</sup>	AF152656 <sup>3</sup>

Note: <sup>1</sup> Persson (2000); <sup>2</sup> Bremer and Eriksson (2009); <sup>3</sup> Rova et al. (2002); <sup>4</sup> Andersson and Rova (1999).

## RESULTS AND DISCUSSION

### Morphology

The collections from Guangxi Province bears a superficial resemblance to *Rubovietnamia aristata* Tirveng. in having cymose inflorescences, a well-developed corolla tube and lobes and globose fruits (Figure 1A-G; Table 2). However, they can be distinguished easily from *R. aristata* in having much broader foliaceous calyx lobes, and the leaves, young branches and flowers (including pedicles, ovary, styles and calyx) densely covered with hairs. These specimens are thus proposed as a new species here.

### Palynology

The pollen grains of the new species are monads, oblate,  $23.72 \pm 0.82$  (22.1-25.3)  $\times$   $24.09 \pm 1.93$  (21.3-26.0)  $\mu\text{m}$  in size, 3-colporate and the perforate or foveolate sexine ornamentation with perforations of variable size. Apertures are c.  $5.7 \times 4.6 \mu\text{m}$  in size, with prominent pro-

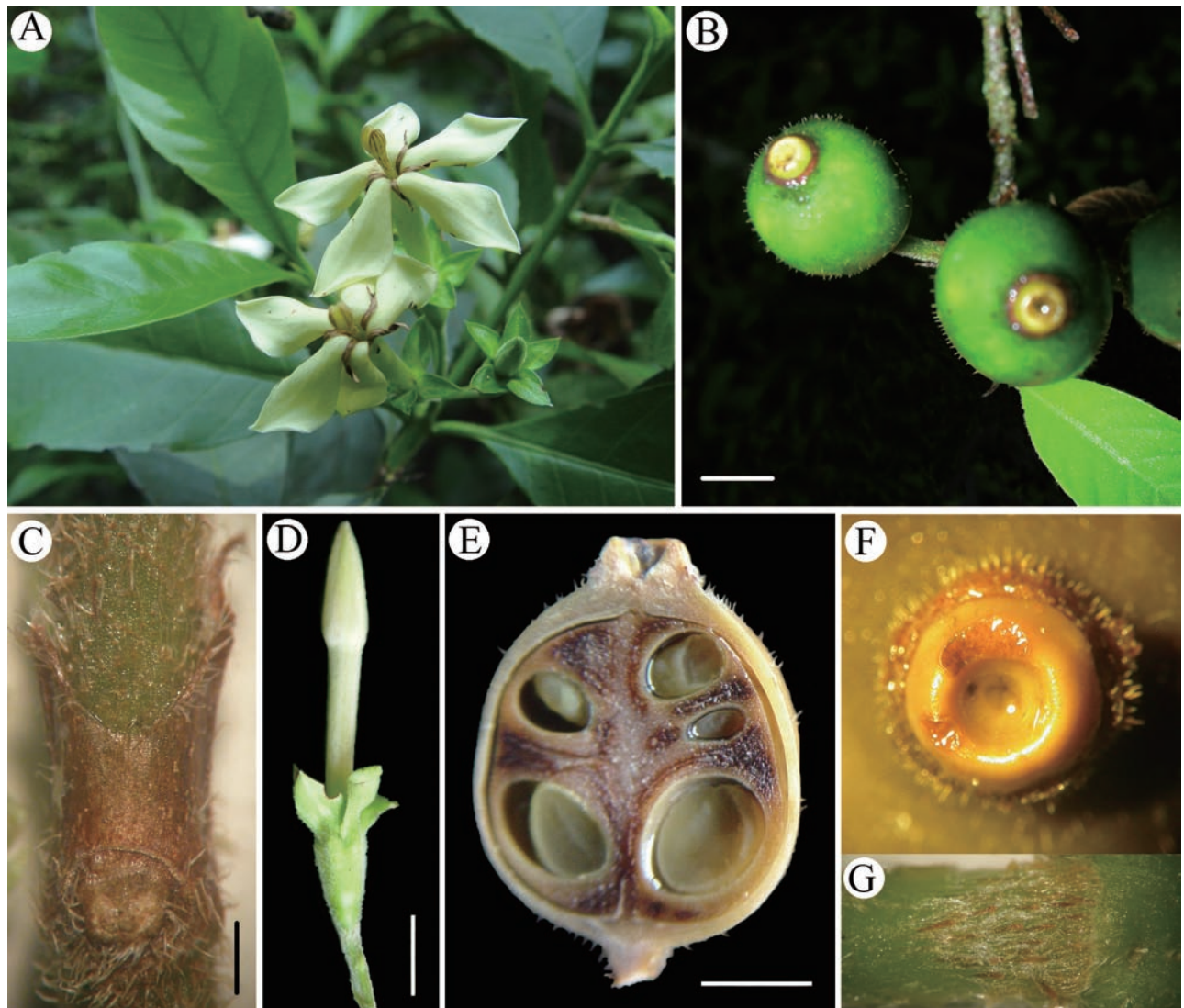
trudings (Figure 2A-E). The pollen grains of the new species are thus distinguished from *Rubovietnamia aristata*, which has heterogeneously reticulate sexine ornamentation (Tirvengadam, 1998). The exine ornamentation of pollen is usually reticulate, but also foveolate, rugulate, perforate and psilate in the subtribe Gardeniinae (Persson, 1993).

### Cytology

The mitotic chromosome number of the new species is  $2n = 22$  (Figure 3F), which is congruent with that of most taxa reported in the tribe Gardenieae, including the genera *Catunaregam*, *Massularia*, *Rosenbergiodendron*, *Rothmannia* (Gadella, 1982; Kiehn, 1985; Kiehn and Lorence, 1996), *Sukunia* (Kiehn, 1996.) and *Gardenia* (Bhattacharyya, 1958), while  $2n = 20$  or  $22$  have been reported in *Genipa* (Guerra, 1993; Pierozzi and Mendaçolli, 1997).

### Molecular phylogeny

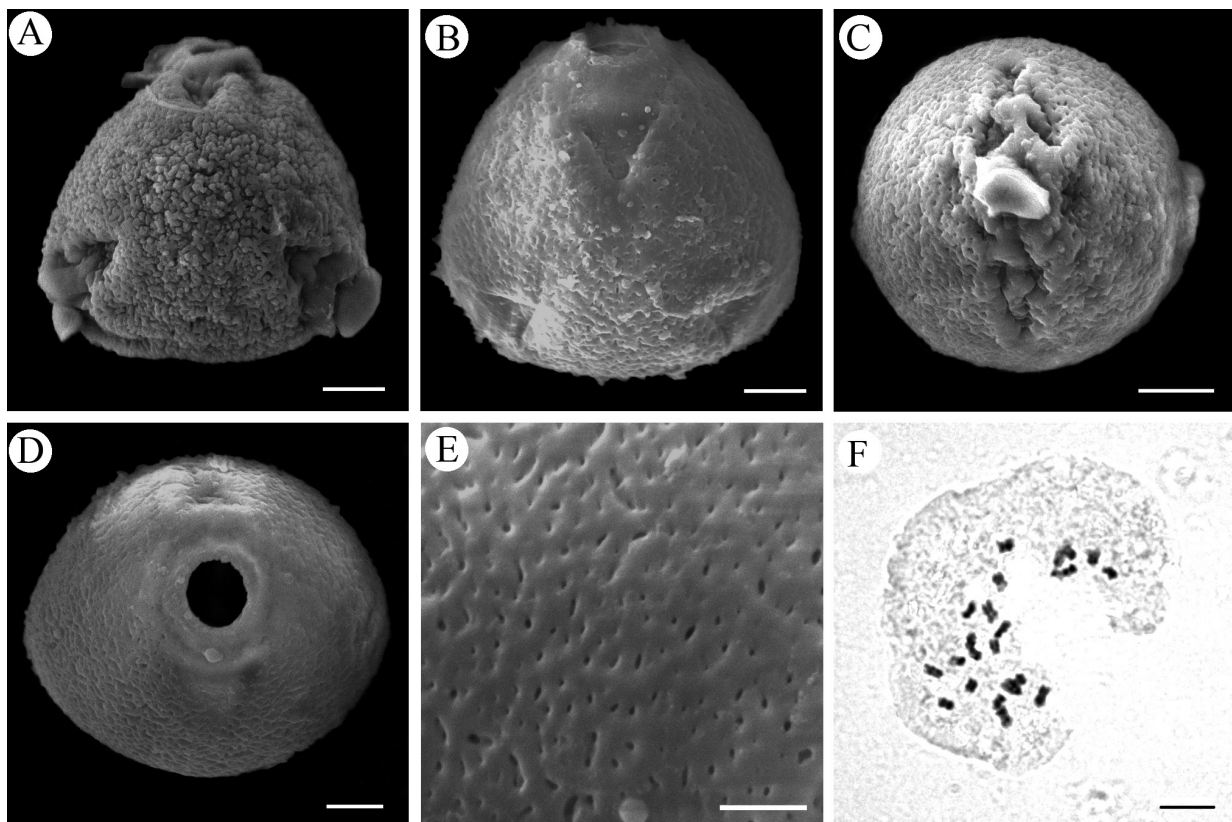
Each of the sequence addition replicates of the heuristic

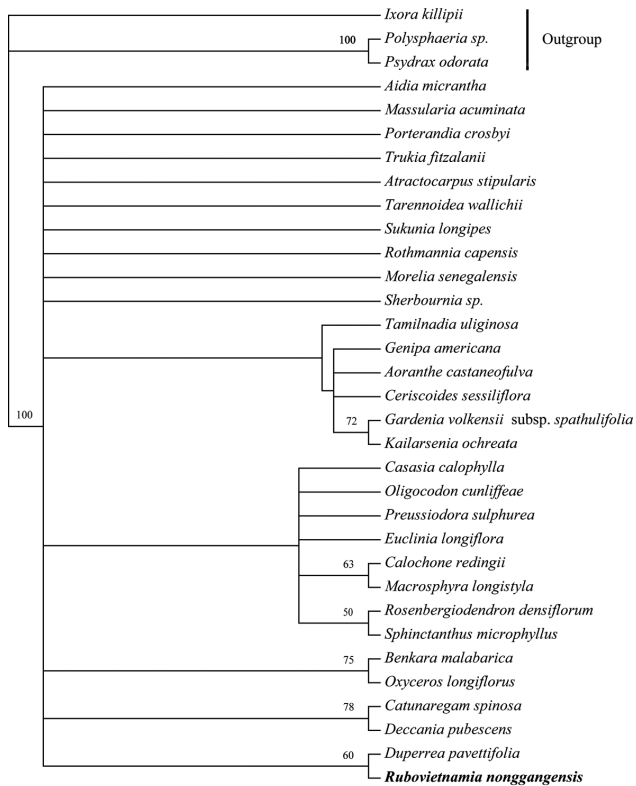


**Figure 1.** *Rubovietnamia nonggangensis*. A, Flowers; B, E, Fruits; C, Stipule; D, Bud; E, longitudinal section of a fruit; F, Floral disk on the fruit apex; G, Colleter on stem.

**Table 2.** A comparison of *Rubovietnamia nonggangensis* and *Rubovietnamia aristata*.

Taxa	<i>Rubovietnamia nonggangensis</i> F. J. Mou & D.X. Zhang	<i>Rubovietnamia aristata</i> Tirveng.
Young branch	Pubescent.	Glabrous.
Leaf	Densely pubescent on both sides, obovate, 8-18 cm × 3-5 cm, base cuneate, apex acuminate; petiole 5-10 mm long.	Elliptic or slightly oblanceolate, with unequal penultimate pairs, 4-12 cm × 1.5-4 cm; petiole 6-10 mm long.
Inflorescence	2-8 flowered cymes; pedicels hairy, up to 2.5 cm long.	1-4 flowered cymes; pedicel hairy, 1.7 cm long.
Calyx	Calyx tube campanulate, up to 8 mm long, hispid, widening at apex with enlarged lobes; calyx lobes 5, lobes elliptic-lanceolate, imbricate, both sides covered with dense hairs, apiculate, prominently nerved, up to 10 × 5 mm, deciduous.	Calyx tube campanulate, ca. 10 mm long; lobes broadly oval-oblong, valvate, ca. 7 mm, foliaceous with a large base, and aristate at apex, lob margin ciliate.
Stipule	Ca. 5 mm long, outside generally coated with thick hairs, terminating with two minute interpetiolar limbs. densely hirsute under stipule; late deciduous.	Ca. 8 mm long; connate near base, caudate acuminate at tip, inside covered with short hairs and small. colleters; early deciduous.
Corolla	Tube dilating gradually to the throat, up to 3.5 cm long, outside hairy; lobes 5, obovate to lanceolate, to 18 × 5.5 mm wide, pubescent outsides, glabrous inside for the most part.	Tube cylindrical, 2 cm; hairy outside and inside near base; lobes elliptic to slightly obovate; tip acute, 12×5-6 mm; glabrous adaxially, hairy abaxially; nerved.
Stamen	5, inserted at the corolla throat and between corolla lobes, sessile, exerted after anthesis; anther up to 5 mm long, linear.	5, inserted near throat, half exerted; anther 1.2 mm, oblanceolate.
Ovary	Inferior, 1-celled, with two parietal placentas; ovules numerous.	1-celled, ovules ca. 4.
Style	Cylindrical; 3.5-4 cm long, with glabrous basal part and pubescent in the mid.	Subcylindrical; slightly and shortly hairy, 2.5 cm long.
Stigma	Club-like, 4.5 mm long, 3 mm wide, with 2 lobes initially cohered, fluted, wholly exerted at anthesis.	Stigma bifid, grooved, ca. 5 mm long.
Fruit	Berry-like, globose, up to 2 × 1.5 cm, tomentose, crowned by the remnants of the calyx and large yellow disk, young with hairs and deciduous when maturity; seeds ca. 6.	Globose, up to 1.7 cm in diam, glabrous, topped by remnant of the calyx; seeds 2-4.

**Figure 2.** *Rubovietnamia nonggangensis*. A, Unacetolyzed pollen in polar view; B, Acetolyzed pollen in polar view; C, Unacetolyzed pollen in equatorial view; D, Acetolyzed pollen in equatorial view, showing aperture; E, Sexine ornamentation; F, Mitotic chromosome ( $2n=22$ ).



**Figure 3.** Strict consensus tree of the most parsimonious trees of Gardenieae based on combined sequences of two chloroplast markers.

search reached the maximum limit of the trees. The strict consensus tree had a step length of 268, with the consistency index (CI) = 0.8657 and the retention index (RI) = 0.7677. The topology of the strict consensus tree resembled that of Persson's (2000). Our results also suggested a sister relationship between *Rubovietnamia* and *Duperrea* (Figure 3). Gardenieae in a modern sense was first circumscribed by Robbrecht and Puff (1986) based on morphological data. However, several molecular studies have indicated that Gardenieae is not monophyletic (Andreasen and Bremer, 2000; Persson 2000; Bremer, 2009).

## DESCRIPTION OF NEW SPECIES

***Rubovietnamia nonggangensis*** F. J. Mou & D. X. Zhang, sp. nov.—TYPE: CHINA. Guangxi, Longzhou, Nonggang Nature Reserve, Longzhu, ca. 200 m a.s.l., in moist soil on slope of limestone hill, 1 June 2008, *F. J. Mou 221*, (Holotype IBSC; Isotype IBSC). 弄崗南茜

(Figures 1, 4)

*Species haec affinis R. aristatae, sed calycis lobis foliaceis elliptico-lanceolatis imbricatis differt.*

Shrub or tree without spine, up to c. 3 m high. Young stems covered with hairs and densely hirsute under stipules. Leaves obovate, 8-18 cm long, 3-5 cm wide, base cuneate, apex acuminate, thick chartaceous, densely

pubescent on both sides; main and lateral nerves visible above, very prominent beneath., secondary veins 7-14 pairs; petiole 5-10 mm long. Stipules 0.5 cm long, chartaceous, outside coated with thick hairs, terminating with two minute interpetiolar limbs. Inflorescences 2-8-flowered cymes, supra-axillary on lateral branches, with both bracts and bracteoles triangular, ca. 3-4 mm long; flowers 5-merous, very fragrant; pedicels up to 2.5 cm long in open flowers. Calyx tube campanulate, up to 8 mm long, hispid, widening at apex with enlarged (well-developed) lobes; calyx lobes 5, imbricate, elliptic-lanceolate, apiculate, prominently nerved and coated with dense hairs, up to 10 × 5 mm, deciduous. Corolla tubular, glossy, fleshy, waxy, cream white at anthesis, turning yellow with age and without spots in the tube; corolla tube dilating gradually to the throat, up to 3.5 cm long, very narrowly tubiform in the lower part and convex in the mid part, 5 mm wide at the mid-portion, outside hairy, inside largely glabrous except for a zone 1-1.5 cm above the base covered with hairs; lobes 5, obovate to lanceolate, to 18 mm long, 5.5 mm wide, contorted to the left in the bud stage, pubescent outsides, glabrous inside for the most part, reflexed at anthesis. Stamens 5, inserted just at the corolla throat and between corolla lobes, sessile; anthers linear, adnate, dorsifixed, up to 5 mm long, half of their length exerted more or less medifixed, exerted after anthesis; filaments very short to inconspicuous. Ovary inferior, unilocular, with two parietal placentas, numerous ovules each in 2 rows on an oblong-elliptic placenta fusing to form a compact mass when mature; style 3.5-4 cm long, with glabrous columnar basal part and pubescent at 2 cm above the base; stigma club-like, 4.5 mm long, 3 mm wide, with 2 lobes initially cohered together, fluted, wholly exerted. Floral disc annular. Fruits berry-like, globose, up to 2 × 1.5 cm, tomentose, crowned by the remnants of the calyx and a large yellow disk, lacking ribs; petiole up to 2.5 cm. Seeds up to 6.

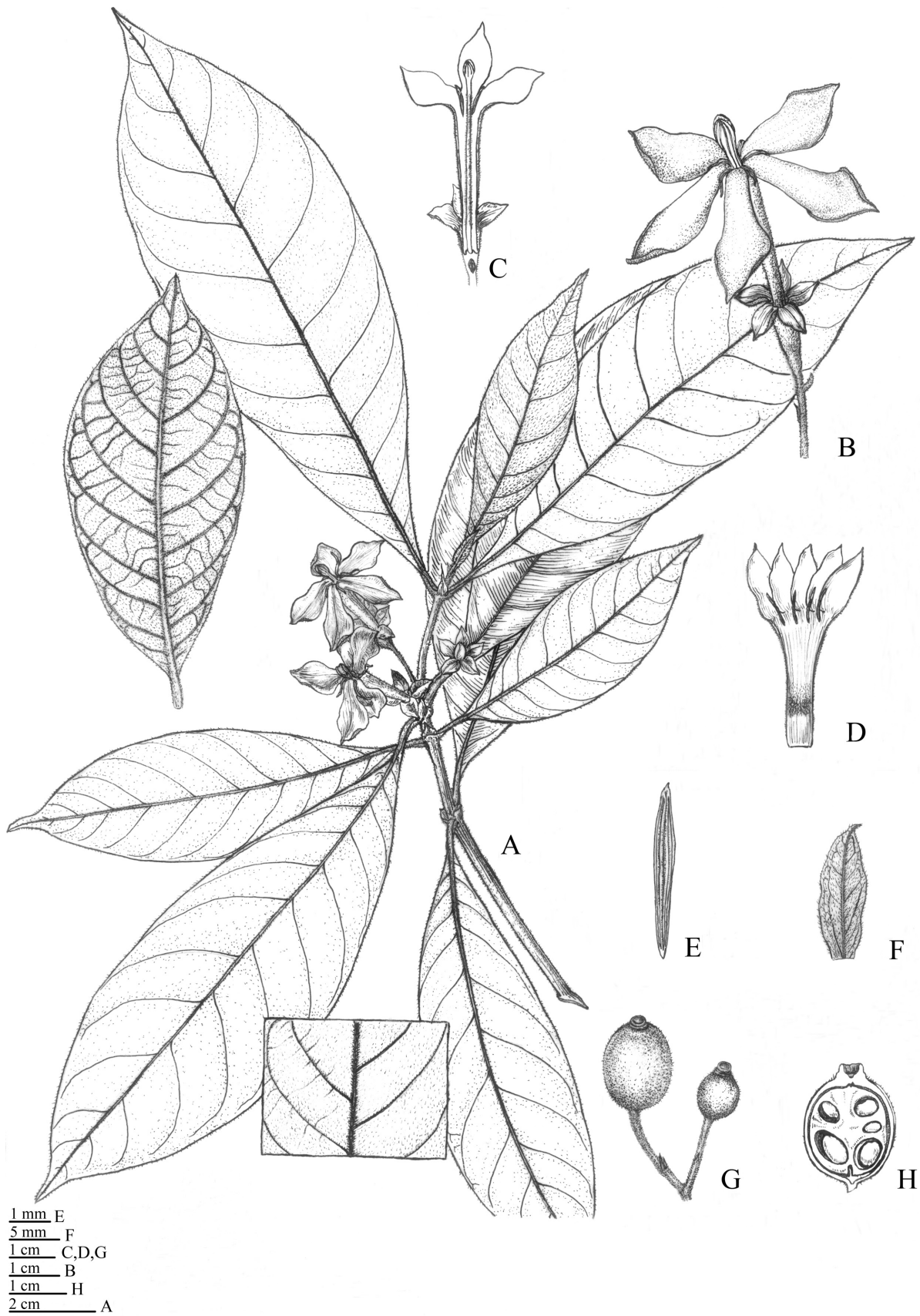
*Additional specimens examined.* CHINA. Guangxi, Nonggang Nature Reserve, Ningming, Longrui, *Nonggang Exp. 11897* (IBK); Guangxi, Nonggang Nature Reserve, Longzhou, Longhu, *F. J. Mou 209-1* (IBSC); Guangxi, Longzhou, Jinlong, China-Vietnam border, *F. J. Mou 230* (IBSC).

*Phenology and habitat.* Flowering from May to June; fruiting from July to October. It grows among the rocky crevices in the moist forests of limestone areas at an altitude of 200-400 m.

*Distribution.* The species is known from southern Guangxi in areas bordering Vietnam.

*Etymology.* The specific epithet derived from the name of its type locality.

**Acknowledgement.** The authors are indebted to Ms. Yun-Xiao Liu for the illustrations, Ms. Xiao-Ying Hu for technical assistance with SEM observation, and Dr. Wei Gong, Miss Yan Liu, and Miss Pei-Wu Xie for field assistance. This project was supported by the National Natural Sci-



**Figure 4.** *Rubovietnamia nonggangensis* F.J. Mou & D.X. Zhang. A, Branch in flower; B, C, Flower; D, Corolla; E, Stamen; F, Calyx lobe; G, H, Fruit & longitudinal section. (All materials from the holotype, *Feng-Juan Mou 221*).

ence Foundation of China (30970182, 30870367), and the Knowledge Innovation Program of the Chinese Academy of Sciences, Grant No. KSCX2-YW-Z-027.

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## 中國廣西茜草科越南茜屬一新種：弄崗南茜

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本文報導產自中國廣西壯族自治區南部石灰岩地區茜草科越南茜屬一新種：弄崗南茜 (*Rubovietnamia nonggangensis* F.J. Mou & D.X. Zhang)。本新種以幼莖，托葉，葉片，花序梗、花萼和花冠外側被毛以及葉狀花萼而與同屬其他種類明顯有別。其體細胞染色體數目為  $2n=22$ ，花粉為三孔溝，表面具穿孔紋飾。本文提供了彩色圖版、線繪圖和花粉圖片以資辨識。並將與其相近種之形態差異予以列表比較和區別，並確定其在梔子族中的系統發育關係。

**關鍵詞：** 染色體數；梔子族；分子系統發育；新種；花粉形態；茜草科；越南茜屬；弄崗南茜。