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REGULAR PAPER

Anna rubidiflora (Gesneriaceae), a new species from Guizhou, the southern part of China

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Background – *Anna* is a small genus of Gesneriaceae, occurring in China and Vietnam. During fieldwork in Guizhou, China, a representative of this genus was discovered which did not match the description of any of the three known species.
Methods – Herbarium taxonomical and morphological studies, SEM of pollen and seeds and molecular phylogenetic analyses were performed.
Key results – A new species, *Anna rubidiflora* S.Z.He, F.Wen & Y.G.Wei, a stenochoric species from the southern part of China (Kaiyang county of Guizhou province), is described and illustrated. It differs from the morphologically similar species *A. ophiorrhizoides* (Hemsl.) Burtt & Davidson by being a larger plant with undivided stem, its entire leaf blade margins, its abaxial greenish-white leaf blades with slightly purple nerves, its obovate-lanceolate calyx lobes with truncate and nearly rounded apex without nerves, and its reddish-purple corolla. The newly described species is illustrated both by a line drawing and photographs. A locality and distribution map is also presented. A phylogenetic analysis confirmed that *A. rubidiflora* is a member of *Anna* and a close relative of *A. ophiorrhizoides*.

Key words – *Anna*, endemic, Gesneriaceae, Guizhou province, new species, phylogenetic placement, taxonomy.

INTRODUCTION

Anna Pellegr. (Pellegrin 1930) was once regarded as an advanced genus in family Gesneriaceae, and was at the same time considered a primitive genus in tribe Trichosporeae, subfamily Cyrtandroideae (Wang & Pan 1990, Wang et al. 1998, Li & Wang 2004, Wei et al. 2010). In *Anna* species, the two somewhat elongated apices of the seed appear superficially similar to those in other genera and species of the Trichosporeae and *Anna* was placed in this tribe (Wang & Pan 1990, Wang et al. 1998). However, the seed apices in *Anna* are not homologous to the distinctly elongated appendages of other genera, such as *Aeschynanthus* Jack (Jack 1823) and *Lysionotus* D.Don (Don 1822), based on evidence from molecular phylogenetic work by Möller et al. (2009, 2011). Consequently, the tribe Trichosporeae was abandoned and the genus placed in the Advanced Asiatic and Malesian group of didymocarpoid Gesneriaceae genera (Weber 2004).

Anna was recognized by Pellegrin in 1930 based on *A. submontana* Pellegr. (Pellegrin 1930). The origin of the generic name is Hebrew, meaning graceful and charming, and was the name of the mother of Virgin Mary. It is a common German female first name (Weber & Skog 2007; see: The Genera of Gesneriaceae, http://www.genera-gesneriaceae.at/genera/anna.htm). As of 2010, this genus comprised three species, *A. submontana*, *A. mollifolia* (W.T.Wang) W.T.Wang & K.Y.Pan (Wang & Pan 1990) and *A. ophiorrhizoides* (Hemsl.) Burtt & Davidson (Burtt & Davidson 1955; see also Wang & Pan 1990, Li & Wang 2004, Wei et al. 2010), with a distribution in S and SW China. *Anna submontana* also occurs in N Vietnam (Li & Wang 2004, Wei et al. 2010). The two other species are endemic to China.

In October 2009, during fieldwork by some of the authors in the center of Guizhou province, S China, an unknown species of the genus *Anna* (Gesneriaceae) was found growing on a shaded and damp limestone cliff in a gorge. All mature plants were flowering at that time. The flowers resembled those of *A. ophiorrhizoides*, except that the colour of flowers was purplish-pink, the corolla tube larger and longer, and the number of flowers per cyme much lower than in *A. ophiorrhizoides*. Furthermore, the shape and characteristics of the leaf blade of these plants differed from *A. mollifolia* and *A. submontana*. The collected specimens were sent to Wang Wen-Tsai at the Institute of Botany, the Chinese Academy

of Science (CAS, PE) and one of the authors (Y.-G. Wei). After careful examination, they both ascertained that the plants represent a new species which has been provisionally described and illustrated as *A. rudibusflora* (Wei et al. 2010).

MATERIAL AND METHODS

Morphometric analysis

Observations were undertaken on fresh plants and dried specimens from the herbaria HGCM and IBK (abbreviations according to Fu 1993). Morphological data used for the description and studies on their variation were also obtained from *in-situ* examination of all living mature plants from the population in Kaiyang county, in the center of Guizhou province, S China. For light microscope studies (LM), fresh samples (flowers) from ten mature individuals were fixed in absolute alcohol-acetic acid (3:1 v/v), and herbarium materials were re-hydrated in distilled water with a drop of soap, for 30 min. All observations of specimens and fixed flowers were carried out using an Olympus BH2-RFCA microscope (Tokyo, Japan).

Pollen grains were acetolyzed according to Erdtman (1966) and critical point dried using a HCP-2 critical point dryer (Hitachi Limited,Tokyo, Japan). Mature seeds were extracted from capsules from plants growing at the type locality. For SEM, pollen grains and seeds were sputter-coated with gold using an IB-5, Ion Sputter Coater (Eiko Engineering Co. Ltd., Tokyo, Japan), and examined and photographed with a Quanta200 (FEI, Eindhoven, Netherlands) scanning electron microscope at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. The pollen terminology followed Erdtman (1966) and Punt et al. (2007).

Taxon sampling for molecular analysis

Sequences of ITS and *trn*L-F for three *Anna* and seventeen closely related species, according to a previous study (Möller et al. 2011), were downloaded from GenBank (see table 1), and used as outgroups to investigate the phylogenetic placement of *A. rubidiflora*. *Tetraphyllum roseum* was chosen to root the trees according to Möller et al. (2011).

Molecular analysis

Genomic DNA from *Anna rubidiflora* was extracted and purified from leaves desiccated in silica gel following the protocol described by Doyle & Doyle (1990) with some modifications to improve the DNA quality. PCR amplification of the *trn*L-F intron-spacer was performed using the primers *trn*L-C (CGAAATCGGTAGACGCTACG) and *trn*F-F (ATTTGAACTGGTGACACGAG) (Taberlet et al. 1991). PCR amplification of the ITS region was performed using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). The PCR amplification was carried out in a total reaction volume of 50 μL containing 1× reaction buffer, 2.5 μL $MgCl_2$ (50mM), 1.5 μL of each primer (10μM), 5 μL dNTPs

Table 1 – Species names and accession numbers of ITS and *trn*L-F sequences used for phylogenetic analysis. * indicates the new species.

Species	Voucher number	*trn*L-F	ITS
Anna mollifolia (W.T.Wang) W.T.Wang & K.Y.Pan		FJ501543	AF055050/ AF055051
Anna ophiorrhizoides (Hemsl.) B.L.Burtt & R.Davidson		HQ632937	HQ633034
Anna rudibiflora S.Z.He, F.Wen & Y.G.Wei*	*Shun-Zhi He* 090818	JN644338	JN644336
Anna submontana Pellegr.		FJ501542	FJ501362
Pseudochirita guangxiensis (S.Z.Huang) W.T.Wang		HQ632908	HQ633003
Pseudochirita guangxiensis (S.Z.Huang) W.T.Wang var. *glauca* Y.G.Wei & Yan Liu		HQ632909	HQ633004
Loxostigma glabrifolium D.Fang & K.Y.Pan		HQ632910	HQ633006
Loxostigma griffithii (Wight) C.B.Clarke		FJ501508	FJ501338
Petrocosmea kerrii Craib		FJ501502	FJ501334
Petrocosmea nervosa Craib		AJ492299	FJ501335
Briggsia longipes (Hemsl. ex Oliv.) Craib		FJ501545	GU350653
Briggsia mihieri (Franch.) Craib		FJ501544	FJ501363
Hemiboea fangii Chun ex Z.Yu Li		HQ632882	HQ632979
Hemiboea ovalifolia W.T.Wang		HQ632883	HQ632980
Hemiboea purpureotincta W.T.Wang		HQ632884	HQ632981
Hemiboea subcapitata C.B.Clarke		FJ501535	FJ501357
Lysionotus chingii Chun ex W.T.Wang		FJ501498	FJ501332
Lysionotus pauciflorus Maxim.		FJ501497	FJ501331
Lysionotus petelotii Pellegr.		FJ501496	HQ632974
Raphiocarpus sinicus Chun		HQ632877	HQ632973
Tetraphyllum roseum Stapf		FJ501434	HQ632950

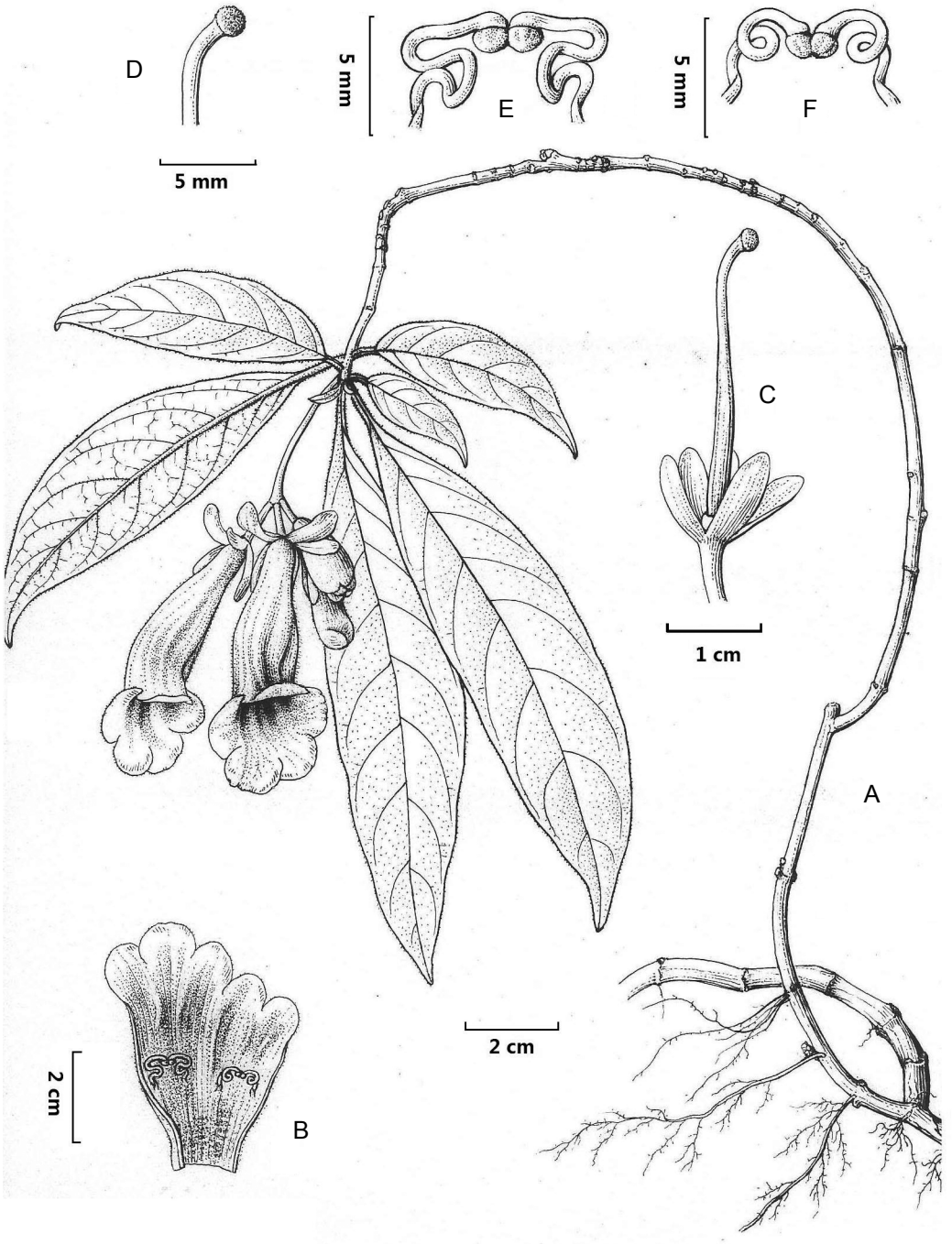

Figure 1 – *Anna rubidiflora* S.Z.He, Fang Wen & Y.G.Wei: A, habit; B, corolla opened to show stamens and staminodes; C, calyx and pistil; D, stigma and part of style; E, stamens; F, staminodes. Drawn by S.Q.He. Scale bars: A & B = 2 cm; C = 1 cm; D–F = 5 mm**.**

(2mM), 20–30 ng template DNA, 2 units of *Taq* Polymerase (TaKaRa Inc., Shiga, Japan) and distilled water up to the final volume. The thermocycling profile consisted of an initial denaturation step at 94°C, for 4 min, followed by 30 cycles of 45 s at 94°C, 45 s at 56–58°C (depending on primer combination), 1 min at 72°C, and final extension step of 10 min at 72°C. The amplification products were analyzed on 1% agarose gels, excised and purified using a DNA gel extraction kit (V-gene Biotechnology Co., Hangzhou, China). They were then directly sequenced in both forward and reverse directions using the PCR primers at the Sangon Biological Engineering Technology and Service (Shanghai, China). The Genbank accession numbers of ITS and *trn*L-F for *A. rubidiflora* are JN644336 and JN644338 respectively. Sequence alignment was carried out using the software CLUSTAL W ver. 1.83 (Thompson et al. 1994) and adjusted manually in BioEdit 5.0.9.1 (Hall 1999).

Figure 2 – Photographs of *Anna rubidiflora* S.Z.He, Fang Wen & Y.G.Wei at the type locality: A, habitat; B, stem with leaves and flowers; C, back of leaves; D, leaves; E, bracts; F, front view of flower; G, longitudinally opened flower showing stamens, staminodes and pistil.

To test the phylogenetic congruence between the ITS and *trn*L-F data, the incongruence length difference (ILD) test (Farris et al. 1994) was conducted in PAUP* ver. 4.0b10 (Swofford 2002), with 100 replicates, each with ten random additions with TBR branching swapping. The ILD test indicated no incongruence between the two data sets ($p = 0.190$).

A maximum parsimony analysis of the combined data set was performed with PAUP* ver. 4.0b10 using the branch-and-bound search algorithm with the MulTrees option on. To examine the clade robustness, a bootstrap analysis was run with 10000 replicates of bootstrapping using heuristic searches and TBR branch swapping. Other statistics, including tree length, consistency index (CI), retention index (RI), and homoplasy index (HI) were also calculated in PAUP*.

RESULTS AND DISCUSSION

Taxonomic treatment

Anna rubidiflora S.Z.He, F.Wen & Y.G.Wei, **sp. nov.**

Anna rudibusflora S.Z.He, Fang Wen & Y.G.Wei, nomen nudum (Wei et al. 2010: 674–675, figs 1–3).

Diagnosis – Differs from *Anna ophiorrhizoides* in its larger habit with unbranched stem, entire margins of the leaf blade, obovate-lanceolate calyx lobes with truncate and nearly rounded apex without nerves, reddish-purple corolla, glabrous filaments, single staminode c. 0.5 mm long, and capitate stigma, longer and thinner seeds (0.9–0.95 mm long,

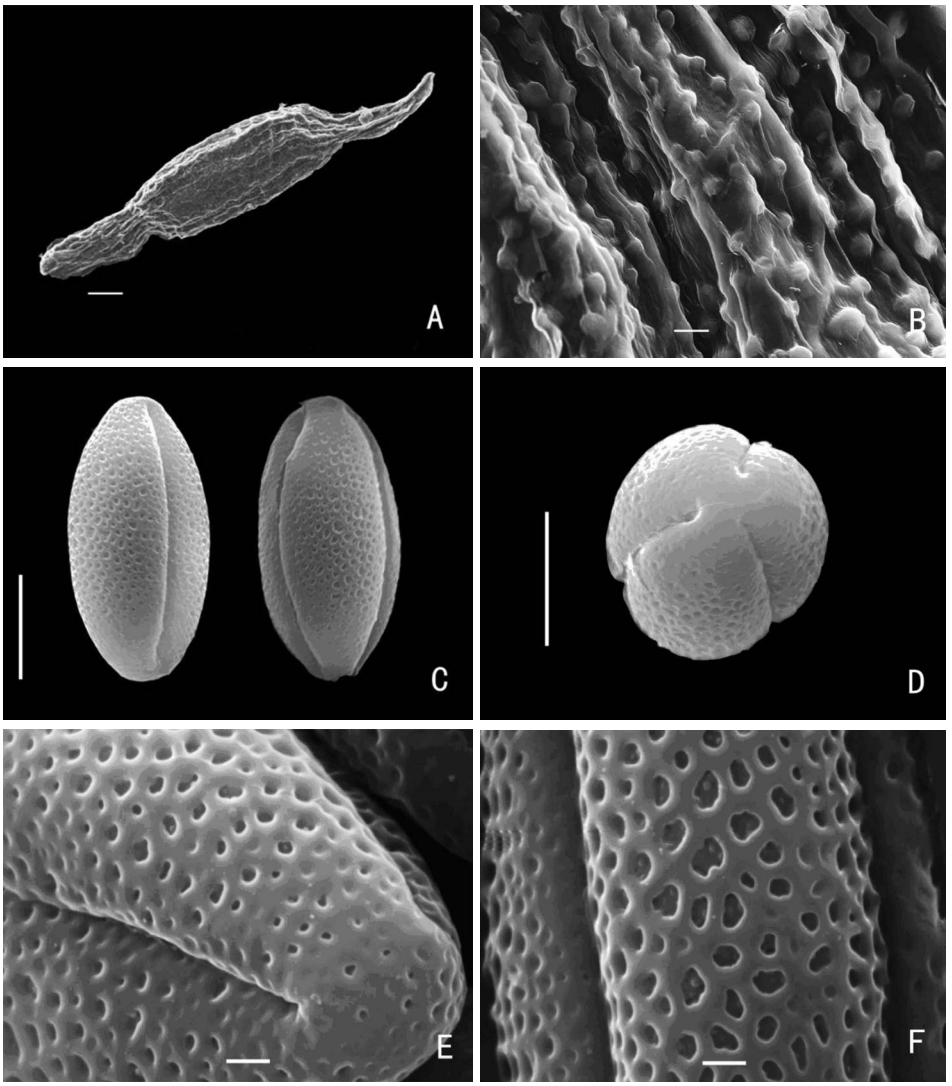

Figure 3 – Seed and pollen morphology of *Anna rubidiflora* S.Z.He, Fang Wen & Y.G.Wei (observations under SEM): A, seed; B, detail of ornamentation on the seed surface; C, two equatorial views of pollen grain; D, polar view of 3-colporate pollen grain; E, apocolpium; F, mesocolpium. Scale bars: A = 100 μm; B–D = 10 μm; E & F = 1 μm.

0.14–0.16 mm in diam.). – Type: China, center of Guizhou province, Zijiang gorge, Kaiyang county, grows on cliffs under forests along the road, alt. 1000–1020 m, 18 Aug. 2009, *Shun-Zhi He* 090818 (holo-: HGCM; iso-: IBK; image: electronic appendix).

Perennial subshrub. Rhizomes 6–10 cm long, 1–1.5 cm in diam. Stems 60–80 cm long, simple or rarely branched, inconspicuously angulate, glabrous. Leaves opposite; blades chartaceous, entire, slightly oblique, lanceolate or narrowly lanceolate, 5–12.5 × 1.5–2.8 cm, apex caudate acuminate or acuminate, base oblique cuneate, margins entire, adaxially appressed pubescent, abaxially pale green, appressed puberulent along veins, lateral veins 5–7 on each side, white, abaxially nerves gibbous; petioles short, 4–9 mm long, purplish red, pubescent. Cymes axillary, close to top, 2–3-flowered; peduncles 3–6 cm long, glabrous. Involucrum pale green, obovate, upper part clearly rotund, bracts close to deltoid rotund, white slightly purplish red tinged, c. 1 cm in diam., commonly deciduous before flowers open; pedicels c. 6 mm long, glabrous. Calyx lobes 5, deeply divided to base, segments obovate-oblong, apex rotund, margin entire, both sides glabrous, without nerve or extremely unapparent 5-nerved, c. 7 mm long, 3–4 mm in diam. Corolla reddish-purple, shortly glandular, 4.3–4.7 cm long; tube gibbose, 3.3–3.7 cm long, c. 0.8 cm in diam. at orifice; limb distinctly 2-lipped, adaxial lip 2-lobed, shorter than abaxial lip, lobes suborbicular, c. 7 mm long, c. 8 mm wide; abaxial lip 3-lobed, suborbicular or oblong, central lobe c. 8 mm long, c. 11 mm wide, lateral lobes c. 6 mm long, 9–10 mm wide. Stamens 4, the upper pair strongly 'Z'-shaped, c. 8 mm long, adnate to 2 cm above corolla base; the lower pair contorted from the center, c. 10 mm long, adnate to 1.8 cm above corolla base; four filaments glabrous and yellowish brown; anthers reniform, c. 1.2 mm long, chambers 2, fused on the top; 1 staminode, c. 0.5 mm long, adnate to 1.4 cm above corolla base. Disc c. 1.5 mm high, entire. Pistil linear, c. 2.8 cm long, c. 1.6 mm in diam., glabrous; ovary linear, c. 1.9 cm long; style short, c. 7 mm

long, stigma 1, capitate. Capsule linear, arc-shaped, dehiscing loculicidally. Figs 1 & 2.

Seed morphology – Seed long and thin, 0.9–0.95 mm long, 0.14–0.16 mm in diam.; appendage at both ends 0.18–0.21 mm long. Seed testa ornamentation reticulate, cells straight, lumina nearly rectangular, longitudinal length coinciding with the longitudinal axis of seed, with granular protuberances in the lumina and muri; protuberances 4.1–7.6 μm in diam. Fig. 3A & B.

Pollen morphology – Grains prolate-spheroidal, long or oblate, 3-colporoidate. Outline in polar view ± circular. Ectocolpi 14.64–15.05 × 7.42–7.53 μm; endoapertures laterally fused to form an endocingulum. Exine reticulate, muri smooth; width of muri variable, shape of perforations irregular, size variable 0.33–0.86 μm × 0.21–0.52 μm. Fig. 3C–F.

Etymology – The epithet *rubidiflora* refers to the reddish colour of the flowers by which the species can be clearly distinguished from other species in this genus that possess white or slightly yellow flowers.

Phenology – Flowering August to October, fruiting November to December.

Ecology – Until recently, only one locality with one population was known. Here, the plants grow in the undergrowth of a subtropical evergreen broad-leaf forest (but see Proposed Conservation Status). The plants occur at the edge of the forest where they receive brighter scattered light but not direct sunlight. The climate of the region, Kaiyang county, usually is moist in autumn and winter, but sporadically dry in spring and summer.

Biogeography of the genus

The genus consists now of four species, the new one included. All can be found in China, with three species being endemic to China, the center of diversity of *Anna* (fig. 4). The species are geographically very restricted, except for *Anna ophiorrhizoides* that can be found on the Yunnan-Guizhou Plateau, W Sichuan (Mt. Emei and Ebian county) and S & N Guizhou (Luodian county and Xishui county). *Anna mollifolia* is endemic to SE Yunnan and SW Guangxi, and *A. submontana* restricted to adjoining districts between S China and Vietnam (SE Yunnan, SW Guangxi of China and N Vietnam) (Wang & Pan 1990, 1998, Wei et al. 2010). The new species occurs in one county only, in Guizhou.

Notes

Anna rubidiflora is allied to *A. ophiorrhizoides*, but distinctly differs from the latter by the main characters given in table 2.

Phylogenetic position of the new species within the genus

The aligned combined ITS and *trn*L-F matrix had 1611 characters. Of these, 73 characters were excluded from analysis due to alignment ambiguities. Of the remaining 1538 characters 171 (11.12%) were variable but uninformative and 208 (13.52%) parsimony-informative, the rest invariable (1159 characters). The parsimony analysis yielded one most parsimonious tree of 674 steps length, with a CI of 0.7077 and RI of 0.6684 (fig. 5). The phylogenetic tree revealed several genus-specific clades supported with high bootstrap values. All

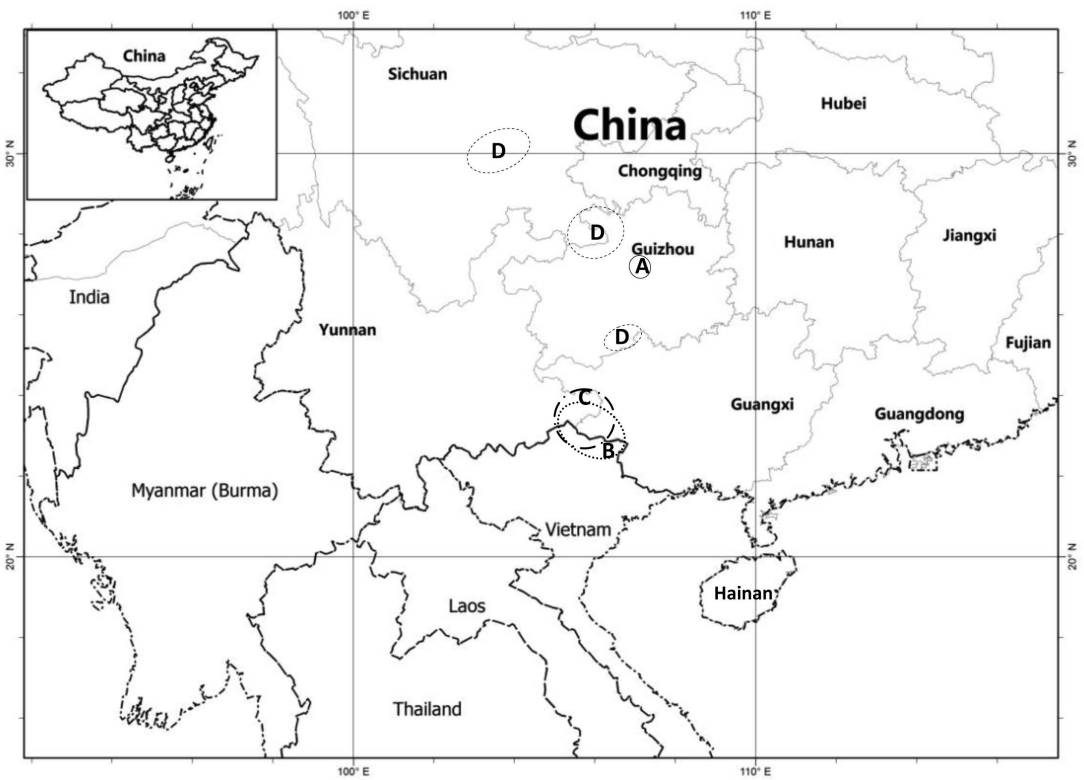

Figure 4 – Geographical distribution of: A, *Anna rubidiflora* S.Z.He, Fang Wen & Y.G.Wei; B, *A. submontana* Pellegr.; C, *A. mollifolia* (W.T.Wang) W.T.Wang & K.Y.Pan; D, *A. ophiorrhizoides* (Hemsl.) Burtt & Davidson.

Table 2 – Comparison of the diagnostic characters of *Anna rubidiflora* S.Z.He, F.Wen & Y.G.Wei and *A. ophiorrhizoides* (Hemsl.) Burtt & Davidson.

Characters	*A. rubidiflora*	*A. ophiorrhizoides*
Stem	60–80 cm long or longer; simple or rare branched; glabrous	30–60 cm long; with numerous branches; angulate, sparsely pubescent,
leaf blade	lanceolate, margin entire, lateral veins 5–6 on each side;	usually falcate, infrequently lanceolate, margin indistinctly denticulate, lateral veins 6–8 on each side;
Cyme	Terminal; 2–3 flowered	axillary, close to top; 3–4 flowered or more,
Peduncle	longer, 3–6 cm long or longer	shorter, 1.5–4 cm long
Bracts	cadusus, obovate-cordate, pale green, chartaceous, c. 1.2 × 1 cm	deciduous, sphaeroidus, pink, membranaceus, c. 1.5 × 1.3 cm
Calyx segments	segments membranaceus, obovate oblong, apex rotund, margin entire, both sides glabrous, without nerve or extremely unapparent 5-nerves, c. 7 mm long, 3–4 mm in diam.	obovate to oblong, 0.9–1.2 cm × 3–6 mm, outside glandular pubescent to glabrous, apex rounded, rarely emarginate
Bracts	4, pairwise opposite, larger pairs oblong-lanceolate, 1.2–1.4 × 0.42–0.45 cm, smaller pairs linear or linear-lanceolate, 9–10 × 1.2–2 mm	2, free, lanceolate to ovate, 4–10 × 1.6–5 mm
Indumentum of bracts	outside densely appressed pubescent but marge pubescent or ciliate	outside puberulent to glabrous
Corolla colour	fuchsia or purple, every lobe with 3 dark purple stripes	white or pale pink, lobes colour same as corolla
abaxial corolla lobes length	5–7 mm long	c. 9 mm
Flowering time	August–October	July–October

four species of *Anna* including *A. rubidiflora* formed a well-supported clade (BS = 98%), confirming the monophyly of the genus. It was sister to a clade including *Petrocosmea* (BS = 100%) and *Raphiocarpus sinicus*, though the latter with no branch support.

The new species *A. rubidiflora* and *A. ophiorrhizoides* shared a sister relationship (BS = 99%), as sister clade to *A. mollifolia* and *A. submontana* (BS = 89%). *A. rubidiflora* differed from *A. ophiorrhizoides* in nine substitutions in ITS and three in *trn*L-F, and three indels in each, ITS and *trn*L-F. This result indicates that *A. rubidiflora* and *A. ophiorrhizoides* differ substantially genetically from each other, and their closest relatives (fig. 5), supporting the independence of the new taxon as a new species.

Phytosociology

Anna rubidiflora grows, as presently known, only on moist crevices and rock face in one limestone gorge in Kaiyang County, the central of Guizhou province, China, at c. 1160 m altitude. The substrate of the type locality has a high content in calcium. The terrain of this limestone gorge is complex and the geological structure in this area unstable. The efflorescent rocks of cliffs in this gorge often collapse in summer and winter. The local microclimatic has recently changed dramatically on the background of global warming. Kaiyang County and Guizhou province have suffered severe droughts many times in recent years. This may indicate that this new species is facing serious survival stresses.

Phytosociologically, *Anna rubidiflora* grows in calciphilous plant communities with pantropical or tropical Asian distributions (Li 1996, Tu 1995, Wu 1979, 1991). The main accompanying plant species belong, among others, to *Ficus* Linn., *Buddleja* L., *Celtis* L., *Millettia* Wight & Arn., *Zanthoxylum* L., *Smilax* L., *Indigofera* L., *Bauhinia* L., *Sterculia* L., *Callicarpa* L., *Sapium* P.Browne, *Caesalpinia* L., *Rosa* L., *Viburnum* L., *Clematis* L., *Rubus* L., *Duchesnea* Sm., *Viola* L., *Pteris* L., *Adiantum* L., *Pilea* Lindl., *Elatostema* J.R.Forst. & G.Forst., *Aspidistra* Ker-Gawl., *Epimedium* L., *Arisaema* Mart.

Reproductive features

The population of *Anna rubidiflora* is being kept under continuous observation since 2009. One of our research team visited the locality for observations every month since March 2009, and this still continues for the time being. Seeds that were collected from capsules in the field for checking their morphology indicated that most (86%) were abnormally formed, so that it is not surprising that we hardly found any seedling near mature individuals.

Main reproductive method

According to our field observations, only eleven young plants, including six individuals from vegetative propagation through rhizomes were seen. We believe that vegetative and sexual propagations is significant for the survival of this species.

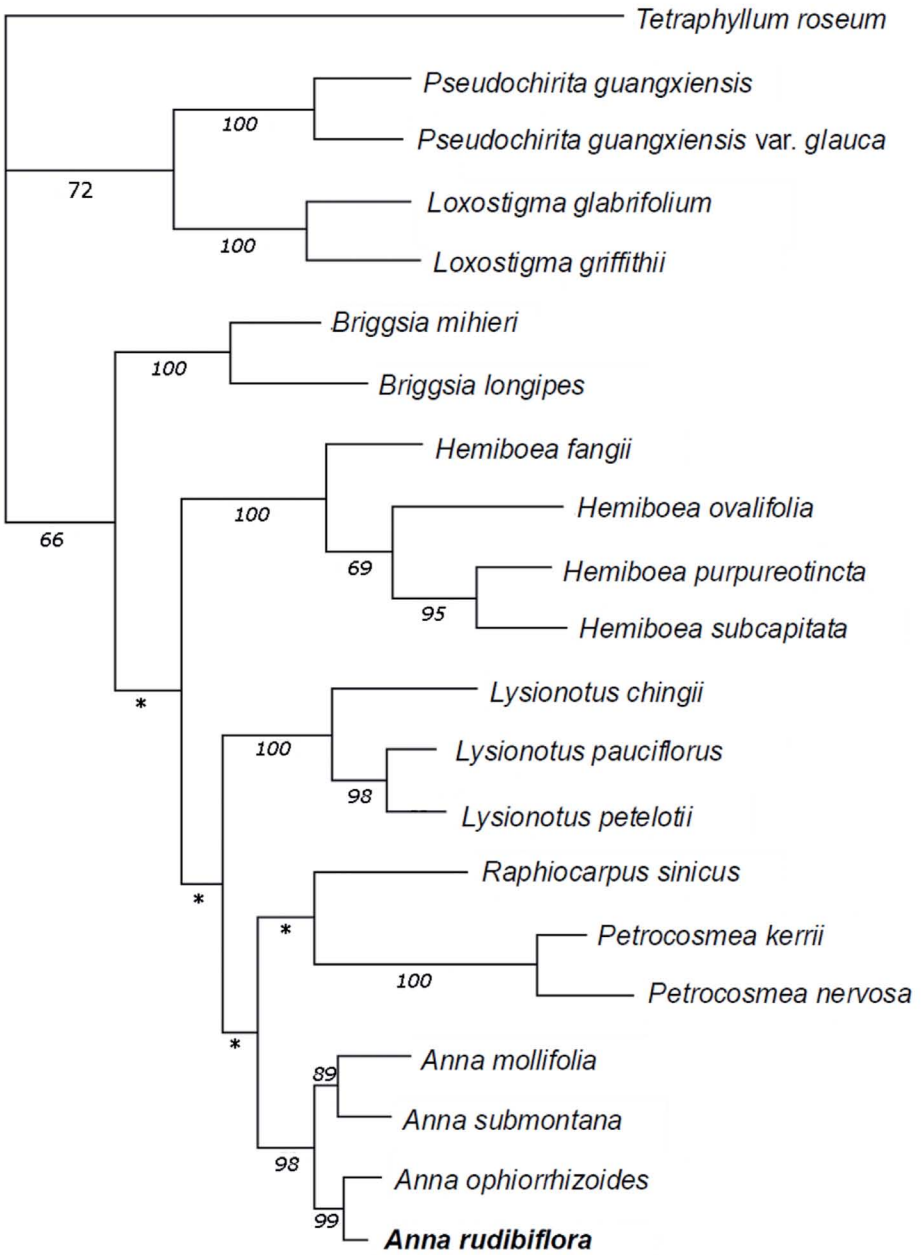

Figure 5 – Phylogram of the single most parsimonious tree resulting from a maximum parsimony (MP) analysis of combined ITS and *trn*L-F data. Numbers above and below branches indicate branch lengths in steps and bootstrap values > 50% respectively. * indicates branches with bootstrap values < 50%.

Proposed Conservation Status

Anna rubidiflora was only known from the type locality until very recently. It covers only an area of about 12 × 15 m^2 and includes twenty individuals. In July 2011, a second population was found in the same gorge but about two kilometers away from the first one. The habitat of the second population is similar to the first one but altitude is lower, c. 1140 m. The second one includes fewer plants, only ten individuals. According to the IUCN red list categories and criteria, version 3.1 categorization (IUCN 2001), the species should be categorized as Critically Endangered, CR A1ac, B1ab(i, ii, iv, v).

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of: (1) aligned combined ITS and *trn*L-F matrix used for phylogenetic analysis (FAS format); (2) sequence of ITS (Genbank accession number: JN644336) and *trn*L-F (Genbank accession number: JN644338) for *Anna rubidiflora* (text format); (3) image of the holotype specimen of *A. rudibiflora* (pdf).

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