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Amana wanzhensis (Liliaceae), a new species from Anhui, China

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Abstract

Amana wanzhensis, a new species from Ningguo County, Eastern China, is described and illustrated. *A. wanzhensis* is similar to *A. erythronioides* in sharing villous tunics and oblanceolate leaves, but differs from it by having shorter bracts (0.1–0.5 cm long), yellow anthers, and deciduous tepals.

Keywords: ITS, Lilioideae, taxonomy, *trn*L intron, Tulipeae

Introduction

The genus *Amana* Honda (1935: 20) includes 4–5 species, endemic to Eastern Asia (Tan *et al.* 2007). This genus is overlapping with *Tulipa* Linnaeus (1753: 305) in many morphological character-states, and generic delimitation is confused. Many taxonomists considered *Amana* and *Tulipa* as synonyms (Sealy 1957, Mao 1980, Ohwi 1992, Tamura 1998, Shen 2001); however, other authors treated them as two distinct genera based on morphological characters and biogeography (Wu 2003), morphological (Tan *et al.* 2005) and molecular phylogenies (Peruzzi *et al.* 2009 and literature cited therein). *Amana* is characterized by 2–3(–4) opposite or verticillate bracts at the upper part of flowering stem, and is endemic to eastern Asia. *Tulipa* has no bracts on scape and distributed from Middle Asia to West Europe (Christenhusz *et al.* 2013).

There are four species of *Amana* in China (Wu 2003). Recently, one new species, *Amana kuocangshanica* D.Y.Tan & D.Y.Hong in Tan *et al.* (2007: 443) has been discovered.

During our fieldwork in Ningguo County, Ahui Province, China, in 2012, a unknown species with a lot of populations was discovered. Our further examination and analysis indicated that it was a new species by having a unique combination of character-states in *Amana*.

Materials and methods

Population sampling:—A total of 15 individuals of 4 *Amana* species were sampled, and at least two individuals were collected for each species (Table 1). All sequences from this study and two ITS region sequences (EU912095 and HE656028) from *A. erythronioides* (Baker) D.Y.Tan & D.Y.Hong in Tan *et al.* (2007: 441) were used as ingroups, while two sequences (JQ776498 and JQ280387) from *Tulipa saxatilis* Baker (1883: 168) was used to be combined one sequence as outgroup for the phylogenetic analysis. New sequences for *Amana* were produced in the present study, while all other sequences were retrieved from GenBank. The specimens and GenBank accession number in this study are listed in Table 1.

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TABLE 1. Specimens and GenBank accession number in this study.

Taxon and samples	Collection site	Voucher number	Haplotype number	GenBank accession number (Reference)	
				ITS	*trn*L intron
A. wanzhensis	Ningguo, Anhui, China	Han B.X. N2	Hap1	KJ402416 (In the present study)	KJ402424 (In the present study)
A. wanzhensis	Ningguo, Anhui, China	Han B.X. N3	Hap2	KJ402417 (In the present study)	KJ402425 (In the present study)
A. wanzhensis	Ningguo, Anhui, China	Han B.X. N4	Hap2	KJ402418 (In the present study)	KJ402426 (In the present study)
A. wanzhensis	Ningguo, Anhui, China	Han B.X. N5	Hap2	KJ402419 (In the present study)	KJ402427 (In the present study)
A. edulis	Chuzhou, Anhui, China	Han B.X. C1	Hap3	KJ402420 (In the present study)	KJ402428 (In the present study)
A. edulis	Chuzhou, Anhui, China	Han B.X. C2	Hap4	KJ402421 (In the present study)	KJ402429 (In the present study)
A. edulis	Chuzhou, Anhui, China	Han B.X. C3	Hap5	KJ402422 (In the present study)	KJ402430 (In the present study)
A. edulis	Chuzhou, Anhui, China	Han B.X. C5	Hap6	KJ402423 (In the present study)	KJ402431 (In the present study)
A. erythronioides	Huangshan, Anhui, China	Han B.X. F1	Hap7	EU912095 (From GenBank)	KJ402432 (In the present study)
A. erythronioides	Huangshan, Anhui, China	Han B.X. F2	Hap8	HE656028 (From GenBank)	KJ402433 (In the present study)
A. anhuiensis	Qianshan, Anhui, China	Han B.X. T1	Hap9	-	KJ402434 (In the present study)
A. anhuiensis	Qianshan, Anhui, China	Han B.X. T2	Hap9	-	KJ402435 (In the present study)
A. anhuiensis	Qianshan, Anhui, China	Han B.X. T3	Hap9	-	KJ402436 (In the present study)
A. anhuiensis	Qianshan, Anhui, China	Han B.X. T4	Hap9	-	KJ402437 (In the present study)
A. anhuiensis	Qianshan, Anhui, China	Han B.X. T5	Hap9	-	KJ402438 (In the present study)
Outgroup: *Tulipa saxatilis*	-	-	Hap10	JQ776498 (From GenBank)	JQ280387 (From GenBank)

DNA extraction, amplification, and sequencing:—Whole genomic DNA was extracted using the CTAB protocol from Rogers (1988). A partial nrDNA fragment of ITS region was amplified with the primers 17SE (5'-ACGAATTCATGGTCCGGTGAAGTGTTC G-3') and 26SE (5'-TAGAATTCCCCGGTTCGCTCGCCGTTAC-3') (Sun *et al.* 1994; Clennett *et al.* 2012), while a partial cpDNA fragment of *trn*L intron was amplified with the primers c (5'-CGAAATCGGTAGACGCTACG-3') and d (5'-GGGGATAGAGGGACTTGAAC-3') (Taberlet *et al.* 1991). These DNA fragments were amplified using a standard polymerase chain raction (PCR), and then the purified amplified products were sequenced using both forward and reverse primers on an ABI-PRISMTM 310 Genetic Analyzer (Applied Biosystems Information, USA). Sequences were edited and aligned manually using BIOEDIT version 7.0.9.0 (Hall 1999).

Phylogenetic analysis:—Test for homogeneity of the nrDNA ITS region and cpDNA *trn*L intron data was performed using HomPart command in PAUP* version 4.0 beta 10 (Swofford 2002), and this test was described as the incongruence-length difference test (Farris *et al.* 1995). The homogeneity of nucleotide base frequencies across taxa was checked using the chi-square test implemented in PAUP* version 4.0 beta 10 (Swofford 2002). For phylogenetic analyses, Bayesian inference (BI) and maximum parsimony (MP) were performed in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) and PAUP* version 4.0 beta 10 (Swofford 2002), respectively. For the Bayesian analysis, four partitions (i.e. partial ITS1, 5.8S, ITS2, and *trn*L intron sequences) were applied to the data, and models of molecular evolution were assessed for each partition using MrModeltest version 2.3 (Nylander 2004). The best-fit model (GTR) for partial ITS1, (K80) for 5.8S, (HKY) for ITS2, and (HKY) for *trn*L intron were selected by the Akaike Information Criterion (AIC) in MrModeltest version 2.3 (Nylander 2004). Four Markov Chains Monte Carlo (MCMC) samples were run for 5 × 106 generations. Two independent runs were performed to allow additional confirmation of the convergence of MCMC runs. Trees were sampled every 100 generations, providing 105 samples from the two runs. Analysis of the standard deviation of split frequencies between the two runs was used to determine that stationarity had been reached after 5 ×104 generations, which were typically discarded as burn-in, leaving 9.9 × 104 samples to estimate the consensus tree and the Bayesian posterior probabilities. For the MP analysis, bootstrap analyses (Felsenstein 1988) were performed with 1000 replicates. Gaps were treated as missing data, and all characters had equal weight.

Description of the new species

Amana wanzhensis L.Q.Huang, B.X.Han & K.Zhang, *sp. nov.* (Fig. 1)

Haec species nova ad A. erythronioides affinis, sed bracteis minoribus (0.1–0.5 cm longis), antherae luteae, tepalis deciduis differt.

Type:—China. Anhui Province: Ningguo City, Xianxia Town, 30°34' 79''N, 119°22'97"E, alt. 735 m, 18 March 2013, *B.X. Han & X.W. Song 2012125* (holotype, ACM!, isotype, PE!).

Perennial herbs; bulbs ovoid, 1.5–2.5 cm in diameter, tunics brown, papery, pilose inside. Stems 15–30 cm tall, glabrous, simple. Leaves 2, opposite, lanceolate, green, 15–30 cm long, 1–3 cm wide, entire, obviously vein. Bracts usually 3 in number, not whorled, ribbon, 0.1–0.5 cm long, floral deciduous. Flowers solitary, funnel-shaped; tepals 6, white, with a green blotch at the base and brown strips on the back; Stamens 6, two-wheeled, anthers 0.4–0.6 cm long, yellow, filaments 0.5–0.7 cm long, white. Oval ovaries, yellowish-green, 0.6 cm long, styles 1 cm long. Fruits triquetrous, 1–2 cm long, 0.5–1 cm wide. Flowers in February or March and fruits in March or April. The new species is closely related to *A. erythronioides*, but readily distinguished from it by having shorter bracts (0.1–0.5 cm long), yellow anthers, deciduous tepals.

Distribution and habitat:—*A. wanzhensis* is endemic to Xianxia Town, Ningguo City, Anhui Province, where it is widespread. It mostly grows in moist bamboo forests or meadow with elevation ranging from 600 to 800 m.

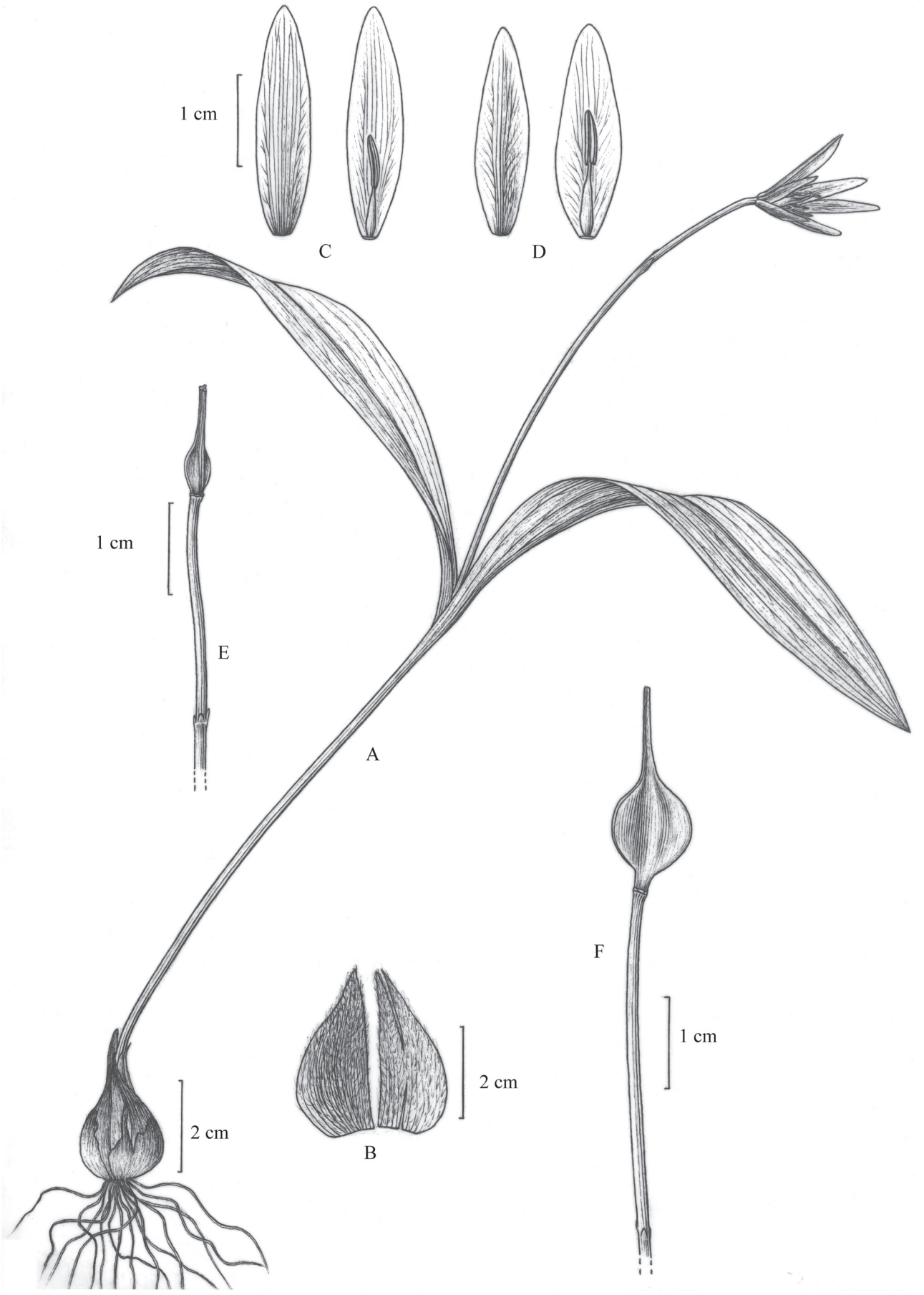

FIGURE 1. *A. wanzhensis* (A) plant, (B) tunics, (C) outer tepal, (D) inner tepal, (E) pedicel and ovary , (F) fruit. From holotype, drawn by Yun-Xi Zhu.

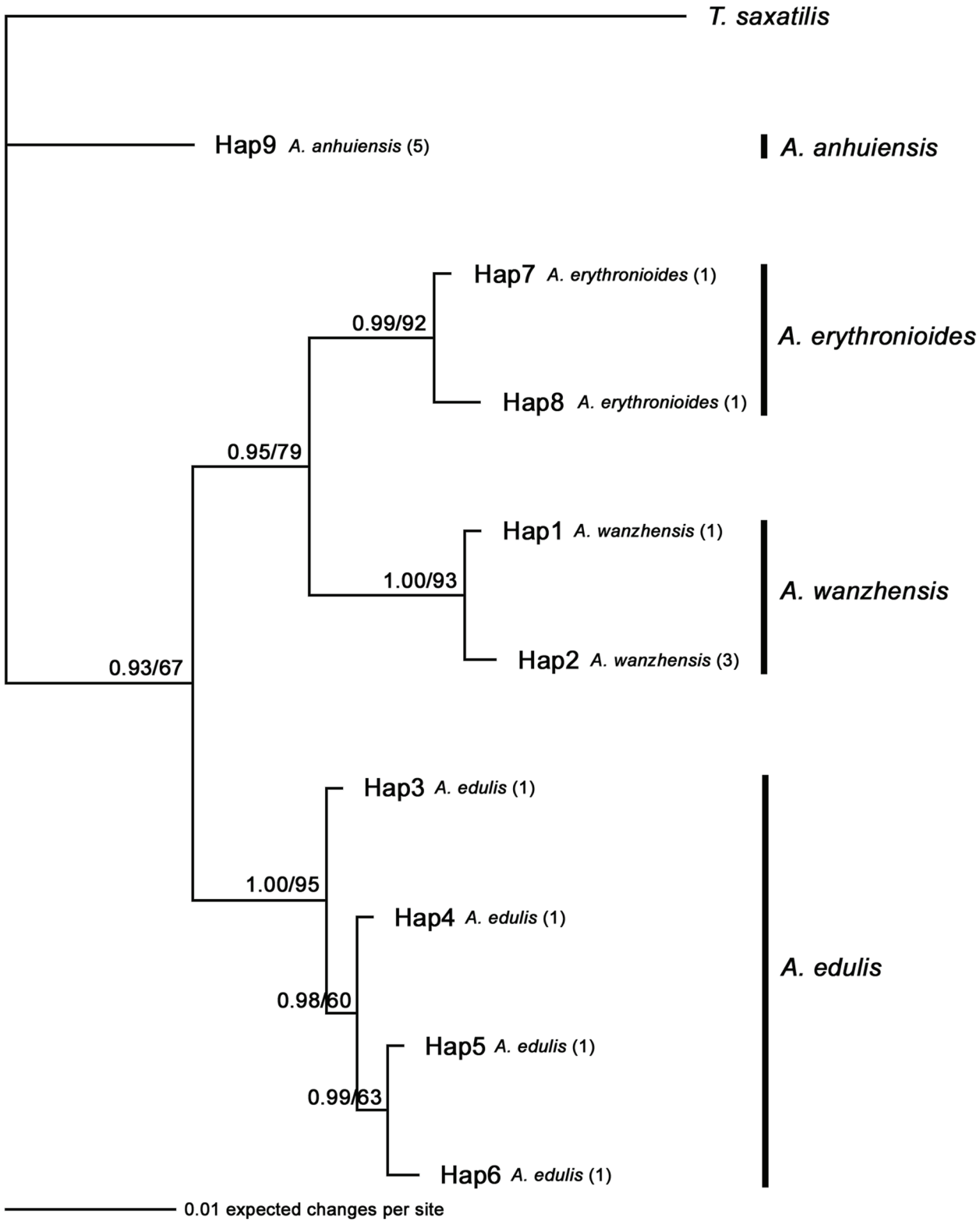

FIGURE 2. The partitioned Bayesian phylogenetic tree based on the combination of partial ITS regions and *trn*L intron. Numbers above the branches represent Bayesian posterior probabilities (PP) and MP bootstrap (BS) values. Taxa are haplotypes; all haplotype designations are listed in Table 1, followed by the species and numbers of individuals from each species having that haplotype [e.g., *A. anhuiensis* (5)].

Molecular phylogeny

Sequences, homogeneity between data partitions, and homogeneity of nucleotide base frequencies across taxa:—Twenty-three new sequences were obtained. Sequence lengths of partial ITS region and *trn*L intron were 617 bp and 497 bp, respectively. The combined alignment of partial ITS region and *trn*L intron was 1114 bp. Because the partition homogeneity test for the nrDNA ITS region and cpDNA *trn*L intron data showed character congruence (P = 1.00), we combined partial ITS region and *trn*L intron to obtain 16 combined sequences (including outgroup taxa) revealing 10 haplotypes (Table 1) for the phylogenetic analyses. A chi-square test indicates that there was no significant compositional heterogeneity of bases among these haplotypes (*chi-square* = 46.81, *df* = 27, $P > 0.01$), and thus the biasing effects on the phylogenetic analyses could be eliminated (Jermiin *et al.* 2004).

Phylogenetic analyses:—The Bayesian and MP analyses based on the combination of partial ITS regions and *trn*L intron resulted in almost identical tree topologies, and the posterior probability (PP) values from the Bayesian analysis were all higher than the bootstrap (BS) values from the MP analysis. Figure 2 showed the partitioned Bayesian tree along with the PP and BS values obtained by MP methods. All haplotypes from *A. erythronioides*, *A. wanzhensis*, and *A. edulis* formed a single moderately supported clade (PP = 0.93, BS = 67), while the haplotype corresponding to *A. anhuiensis* (Hap9) was found to be outgroup of this clade. All haplotypes from *A. erythronioides* formed a monophyletic group (PP = 0.99, BS = 92), while all haplotypes from *A. wanzhensis* formed another monophyletic group (PP = 1.00, BS = 93). *A. erythronioides* and *A. wanzhensis* were reciprocally monophyletic, and these two species formed a clade (PP = 0.95, BS = 79) that was sister to *A. edulis* from which all haplotypes formed a monophyletic group (PP = 1.00, BS = 95). Phylogenetic relationships estimated using BI and MP methods were almost identical, and both methods suggested that *A. wanzhensis* is an independent lineage, distinct from *A. wanzhensis*.

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