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Amana hejiaqingii (Liliaceae), a New Species from the Dabie Mountains, China

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Abstract: In this paper, a new species from Central China, *Amana hejiaqingii* (Liliaceae), is described and illustrated. It resembles *A. anhuiensis* and *A. tianmuensis* but differs from *A. anhuiensis* as it has one white vein on its lower leaf and yellow anthers. It also differs from *A. tianmuensis* by possessing solitary pink flowers with longer and wider tepals. The principal coordinates analysis separated the three species based on morphological data. Cytological observation showed that *A. hejiaqingii* is diploid ($2n = 2x = 24$). Molecular phylogenetic analyses further supported its species delimitation.

Keywords: *Amana anhuiensis*; *Amana tianmuensis*; phylogeny; taxonomy

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1. Introduction

The genus *Amana* Honda (1935: 20; Liliaceae) is characterized by 2−3(–4) opposite or verticillate bracts at the upper part of flowering stem and a distinct beak of the fruit, which separates it from *Tulipa* L. (Tan et al., 2005 [1]). *Amana* consists of nine perennial herbaceous species and is endemic to East Asia (Ohwi and Kitagawa 1992 [2]; Chen and Mordak 2000 [3]; Shen 2001 [4]; Tan et al., 2007 [5]; Tan et al., 2008 [6]; Han et al., 2014 [7]; Li et al., 2017 [8]; Wang et al., 2019 [9]; Wang et al., 2022 [10]). *Amana* belongs to spring ephemerals, blooming in early spring. The above-ground tissues die after bearing fruit and become dormant underground (Struik, 1965 [11]). This genus mainly occurs in temperate deciduous or subtropical evergreen broadleaved/mixed forests. *Amana* is diploid, and its basic chromosome number is $x = 12$, except for two ploidy levels in *A. edulis*: diploid in northern populations and tetraploid in southern populations (Wang et al., 2022 [10]; Wu et al., 2022 [12]). This genus is confirmed to be in a monophyletic group and sister clade with *Erythronium* (Li et al., 2017 [8]). A suspected new species was found during fieldwork from 2016 to 2022 in the Dabie Mountains, China. Our morphological examination and phylogenetic analyses further verified that it is a new species. Therefore, we describe and illustrate it here.

2. Materials and Methods

2.1. Sampling, Sequencing and Assembly

The new species was investigated in eight localities in the Dabie Mountains bordering the Henan and Hubei provinces from 2016 to 2022 (Figure 1). Genomic DNA was extracted from silica-gel-dried leaves with modified CTAB reagent (Plant DNAzol, Shanghai, China), according to the manufacturer's protocol. Libraries were pooled and sequenced with 150 bp paired-end reads using the Illumina HiSeq X10 platform at China National

GeneBank (CNGB, Shenzhen, China). Raw reads were assembled into plastome sequences by GetOrganelle (Jin et al., 2020 [13]) with default settings. The newly generated sequences can be accessed on GenBank (Table 1). Voucher specimens were deposited at the Herbarium of Zhejiang University (HZU, acronyms according to Thiers et al., 2016 [14]).

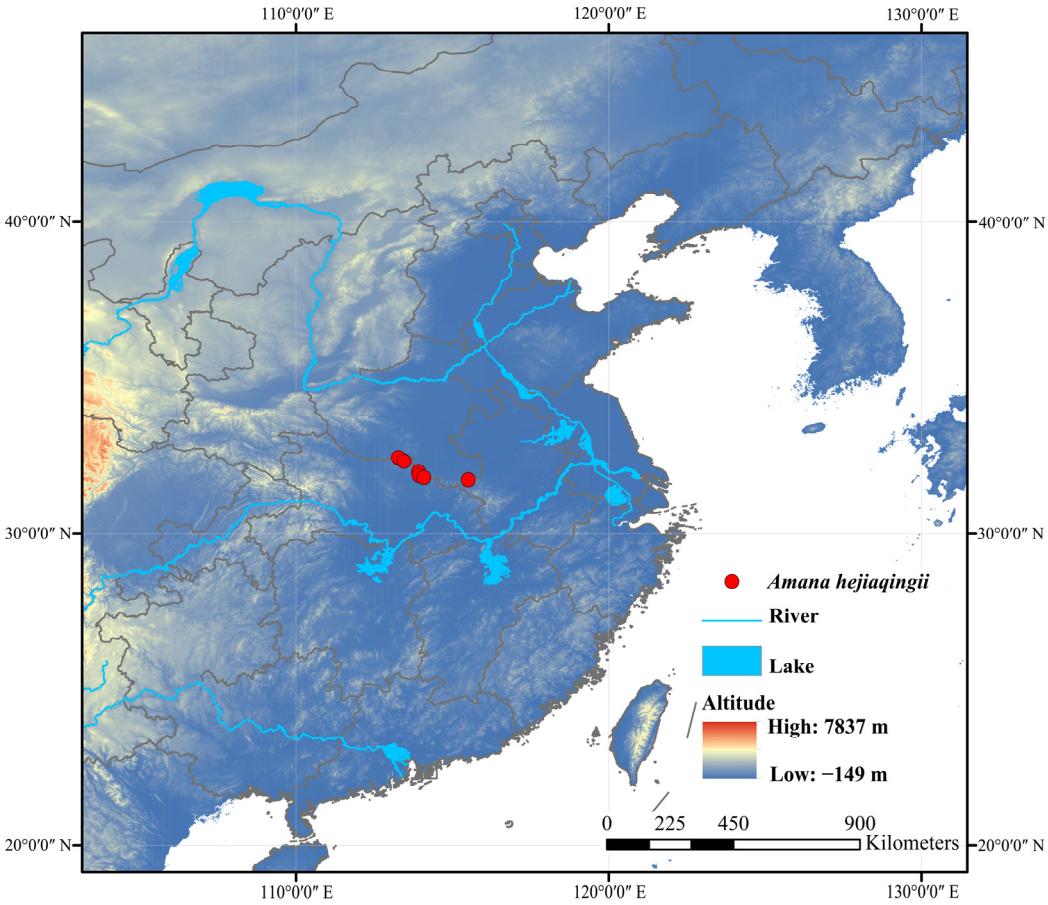

Figure 1. Distribution of *Amana hejiaqingii*. The red dots represent the known populations of *Amana hejiaqingii*.

2.2. Morphological Observation and Principal Coordinates Analysis

Morphological observations served as the basis of species description. Morphological traits of bulbs, bracts, leaves, flowers and fruits were measured and recorded. Herbarium specimens from CDBI, CSH, HHBG, HIB, HZU, IBSC, KUN, LBG, NAS, PE, WUK (acronyms according to Thiers et al., 2016 [14]) were examined for comparison. Additionally, a morphological comparison was made among *Amana anhuiensis* (X.S. Shen) D.Y. Tan & D.Y. Hong, *Amana tianmuensis* P. Li & M.Z. Wang and *Amana hejiaqingii*. Eleven traits were applied for principal coordinates analysis with R package *vegan* v.2.6.2 and *ggplot2* v.3.3.6 (Wickham 2016 [15]), including the position of the widest part of lower leaf (top: 0; upper: 1), number of white veins on lower leaf, bracts length, bracts width, shape of bracts (linear: 0; lanceolate: 1), color of flowers (white: 0; pink: 1), outer tepal length, outer tepal width, inner tepal length, inner tepal width and color of anthers (yellow: 0, light-purple: 1). A total of 52 individuals (7 of *A. anhuiensis* from three populations, 23 of *A. hejiaqingii* from six populations and 22 of *A. tianmuensis* from eight populations) were measured for analysis (Table S1).

2.3. Cytological Analysis

Actively growing droppers were collected in the field for chromosome counting. The dropper tip materials were pretreated in 0.1% colchicine for 4.5 h and then fixed in Carnoy's

Fluid (3 absolute alcohol: 1 glacial acetic acid, v/v) for 12–24 h. Afterwards, they were converted into anhydrous ethanol and stored at −20 °C in the refrigerator for further treatment. The fixed dropper tips were bathed in 37 °C enzyme solution (a mixture of 2% cellulase and pectinase (2:1)) for 1 h. After hydrolysis, the materials were rinsed with distilled water several times. Finally, stained tips with Carbol fuchsin and chromosome compression tablets were made for observation. Photos were captured by a SOPTOP DMCX40 microscope (SOPTOP, Ningbo, China).

Table 1. The plastome sequences of *Amana* accessions used in this research.

Accession Name	Accession Number	Location
Amana anhuiensis_CMQ2015075-7	KY401423	Tianzhushan, Qianshan County, Anhui Province, China
Amana anhuiensis_LJK62-1	MZ561649	Tianzhushan, Qianshan County, Anhui Province, China
Amana baohuaensis_ LP150012	MW929176	Maoshan, Jurong City, Jiangsu Province, China
Amana baohuaensis_LJK31-1	MZ561647	Maoshan, Jurong City, Jiangsu Province, China
Amana hejiaqingii_WMZ1483	ON764433	Liluocheng, Shangcheng County, Xinyang City, Henan Province, China
Amana hejiaqingii_WMZ1495	ON764434	Feiyundong, Xisaishan District, Huangshi City, Hubei Province, China
Amana edulis_CMQ16213	OL351567	Xilin Temple, Jiujiang City, Jiangxi Province, China
Amana edulis_LJK54-1	MW929177	Yuxiang Village, Huangshan City, Anhui Province, China
Amana edulis_LP161115-1	AB024388	Hangzhou Botanical Garden, Hangzhou City, Zhejiang Province, China
Amana edulis_LP161235-1	MW938051	Daheishan, Dalian City, Liaoning Province, China
Amana edulis_LP173029	OL351568	Cheyu Valley, Zhouzhi County, Shaanxi Province, China
Amana edulis_LP173055	OL351569	Dushan, Nanyang City, Henan Province, China
Amana erythronioides_LP150068-4	KY401421	Simingshan, Yuyao City, Zhejiang Province, China
Amana erythronioides_LJK26-1	MZ561646	Simingshan, Yuyao City, Zhejiang Province, China
Amana kuocangshanica_PNLI20141039-1	KY401426	Kuocangshan, Linhai City, Zhejiang Province, China
Amana kuocangshanica_LJK22-1	MZ561645	Kuocangshan, Linhai City, Zhejiang Province, China
Amana latifolia_LJK70-1	MZ561650	Koshikawa Botanical Garden, Tokyo, Japan
Amana latifolia_LP161225-2	KY401424	Koishikawa Botanical Garden, Tokyo, Japan
Amana nanyueensis_LP173011-2	MW876380	Hengshan, Hengyang City, Huan Province, China
Amana nanyueensis_LP196219-1	MW845753	Hengshan, Hengyang City, Huan Province, China
Amana tianmuensis_LP161203-2	MW876379	Jinhuashan, Jinhua City, Zhejiang Province, China
Amana tianmuensis_LJK42-1	MW876378	Tianmushan, Lin'an City, Zhejiang Province, China
Amana tianmuensis_CMQ16198-1	MW876377	Tianmushan, Lin'an City, Zhejiang Province, China
Amana wanzhensis_LJK44-1	MZ561648	Xianxia Town, Ningguo City, Anhui Province, China
Amana wanzhensis_LP150072-11	KY401422	Xianxia Town, Ningguo City, Anhui Province, China
Erythronium sibiricum	NC_035681	Xinjiang Province, China
Lloydia tibetica	MK673748	Taibaishan, Mei County, Shaanxi Province, China
Tulipa altaica	NC_044780	Tacheng, Urumqi, Xinjiang Province, China

Karyotype formula was based on the measurement of mitotic metaphase chromosomes photos. The degree of karyotype asymmetry (As.K%, Arano 1963), karyotypic symmetry division category (KA Type, Stebbins 1971 [16]), mean centromeric asymmetry index (MCA), coefficient of variation of chromosome length (CVCL), coefficient of variation (CVCI), and total haploid karyotype length (THL) were evaluated (Paszko 2006, [17] Peruzzi et al., 2009 [18], Peruzzi and Eroğlu 2013 [19], Peruzzi and Altınordu 2014 [20]). KaryoType software was used to measure the indices of karyotype asymmetry (Altınordu et al., 2016 [21]).

2.4. Phylogenetic Analyses

A total of 28 individuals representing 13 species were included. *Erythronium sibiricum* (Fisch. & C.A. Mey.) Krylov, *Tulipa altaica* Pall. ex Spreng. and *Lloydia tibetica* Baker ex Oliv. were used as outgroups based on their close phylogenetic relationships with *Amana* (Lu et al., 2021 [22]). The 76 common plastid CDS genes were extracted and concatenated for phylogenetic analyses in Geneious v.4.8.5 (Kearse et al., 2012 [23]). Phylogenetic reconstructions were conducted using IQ-TREE 2 (Minh et al., 2020 [24]) for maximum likelihood (ML) analyses with 1000 bootstrap replicates and Modelfinder (Kalyaanamoorthy et al., 2017 [25]) for gene partitions. Finally, 16 kinds of best partition

schemes were found. Bayesian inference (BI) was implemented on MrBayes v.3.2.7a (Ronquist et al., 2012 [26]) with GTR + I + G substitution model. The Markov chain Monte Carlo (MCMC) algorithm was run with two independent chains and default priors for 10,000,000 generations. Trees were sampled every 1000 generations.

3. Results

3.1. Morphological Comparisons

Amana hejiaqingii (Figures 2 and 3) is similar to *A. anhuiensis* (Figure 4) and *A. tianmuensis* (Wang et al., 2022 [10]) in bulbs, leaves and flowers. However, it differs from *A. anhuiensis* as it has lower leaves with a grayish-white midvein (vs. with more than one white vein), and yellow anthers (vs. light-purple) at maturity. Additionally, the widest part of the lower leaf of *A. hejiaqingii* is usually in the 2/8–3/8 position (upper part) of the leaf, while *A. anhuiensis* is usually at 1/8 position (top part) of the leaf. Meanwhile, it differs from *A. tianmuensis* as it has a solitary pink flower (vs. mostly solitary, sometimes two, white). Moreover, the outer and inner tepals of *A. hejiaqingii* were mostly longer and wider than *A. tianmuensis* (Table 2).

Table 2. Comparison of characteristics between *Amana anhuiensis*, *Amana hejiaqingii* and *Amana tianmuensis* (values in parentheses are averages).

Characters \ Species		*Amana anhuiensis*	*Amana hejiaqingii*	*Amana tianmuensis*
Bulbs	Diameter	1.13–1.72 cm	0.93–2.3 cm	0.7–1.8 cm
	Tunics	yellowish-brown, thinly papery	yellowish-brown, thinly papery	yellowish-brown, thinly papery
	Inside	sparely villous	glabrous, sometimes sparely villous	glabrous, sometimes sparely villous
Leaves	Color	green, with white veins	green, midvein grayish white	green
	Shape	oblanceolate	oblanceolate	oblanceolate
	Lower-leaf length (LL)	12.2–22.5 (17.05) cm	8.0–33.9 (19.91) cm	11.2–22.8 (16.5) cm
	Lower-leaf width (LW)	1.7–2.6 (2.17) cm	1.0–2.8 (1.71) cm	0.9–2.5 (1.7) cm
	LL/LW	5.5 < X < 10	6 < X < 20	5 < X < 14.5
	Upper-leaf length (LL)	11.0–21.4 (16.05) cm	7.7–34 (19.47) cm	9.5–23.4 (16.1) cm
	Upper-leaf width (LW)	0.7–1.85 (1.16) cm	0.5–1.4 (0.97) cm	0.6–1.9 (1.2) cm
	LL/LW	10 < X < 20	9 < X < 29.5	6.5 < X < 21
Bracts		3, verticillate, narrowly lanceolate	3, verticillate, linear	3, verticillate, linear
Bracts length		2.8–4.7 (3.5) cm	1.2–4.2 (2.6) cm	1.8–3.5 (2.6) cm
Pedicels		2.2–5.1 cm	0.5–4.1 cm	1.1–5.6 cm
Flowers		solitary; white or pink	solitary; pink	mostly solitary, sometimes two; white
Tepals	Outer length	2.9–4.3 (3.4) cm	2.4–4.4 (3.4) cm	1.8–3.4 (2.6) cm
	Outer width	0.4–0.6 (0.5) cm	0.6–1.1 (0.8) cm	0.4–0.9 (0.5) cm
	Inner length	2.7–4.3 (3.3) cm	2.1–3.9 (3.2) cm	1.8–3.2 (2.4) cm
	Inner width	0.7–1.2 (0.9) cm	0.6–1.0 (0.8) cm	0.4–1.1 (0.6) cm
Anthers		light-purple	yellow	yellow
Styles		0.5–0.7 cm long	0.4–0.6 cm long	0.5–0.6 cm long
Length of fruit beak		0.5–1.0 cm long	0.5–1.2 cm long	0.45–1.1 cm long

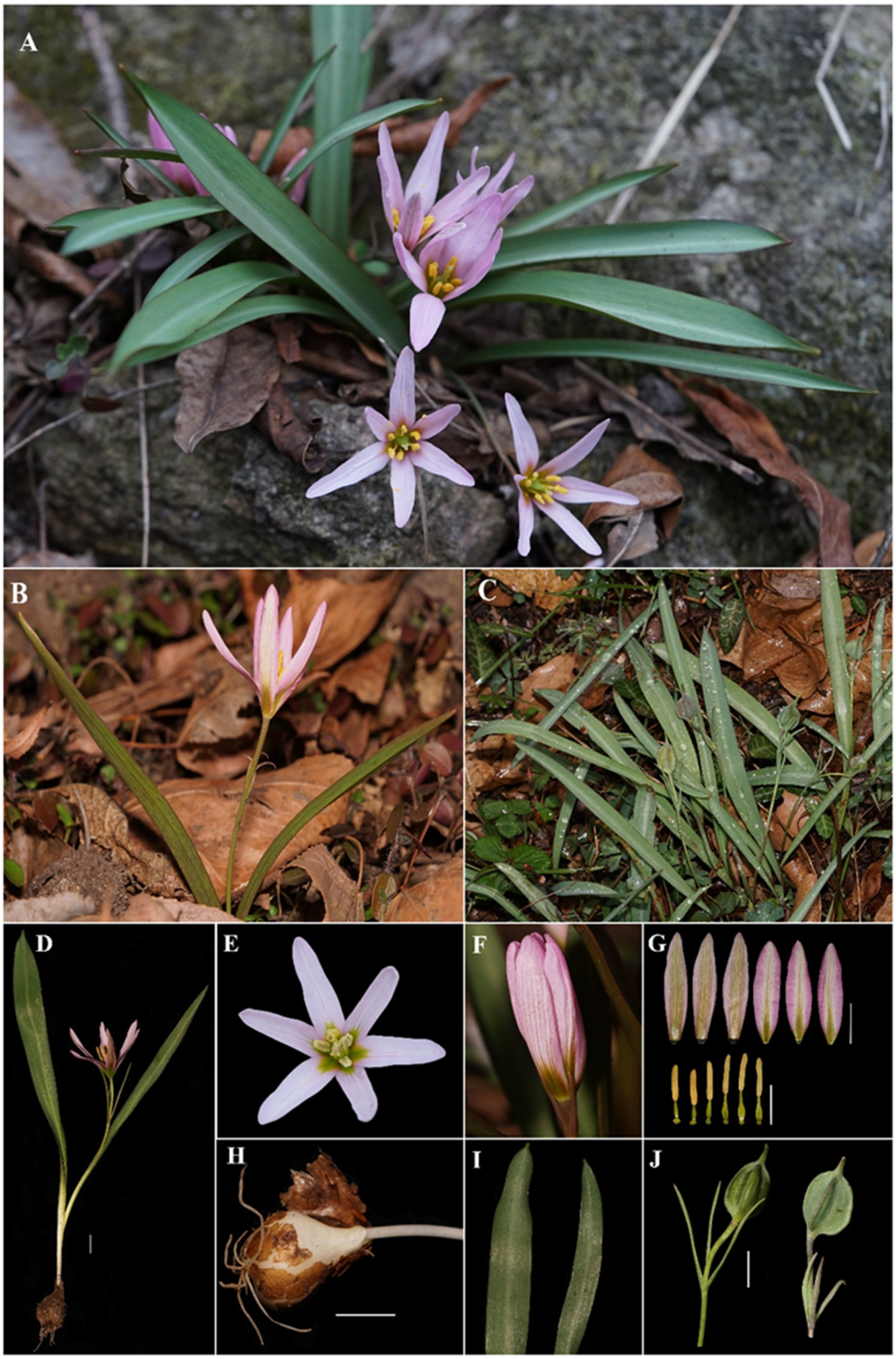

Figure 2. (**A**–**J**) *Amana hejiaqingii*: (**A**) Habitat, (**B**) Individual, (**C**) Population with fruits, (**D**) Whole plant, (**E**) The front view of the flower, (**F**) The side view of the flower, (**G**) Anatomy of flower, (**H**) Bulb, (**I**) Leaves, (**J**) Fruits. The white line segment represents a length of 1 cm.

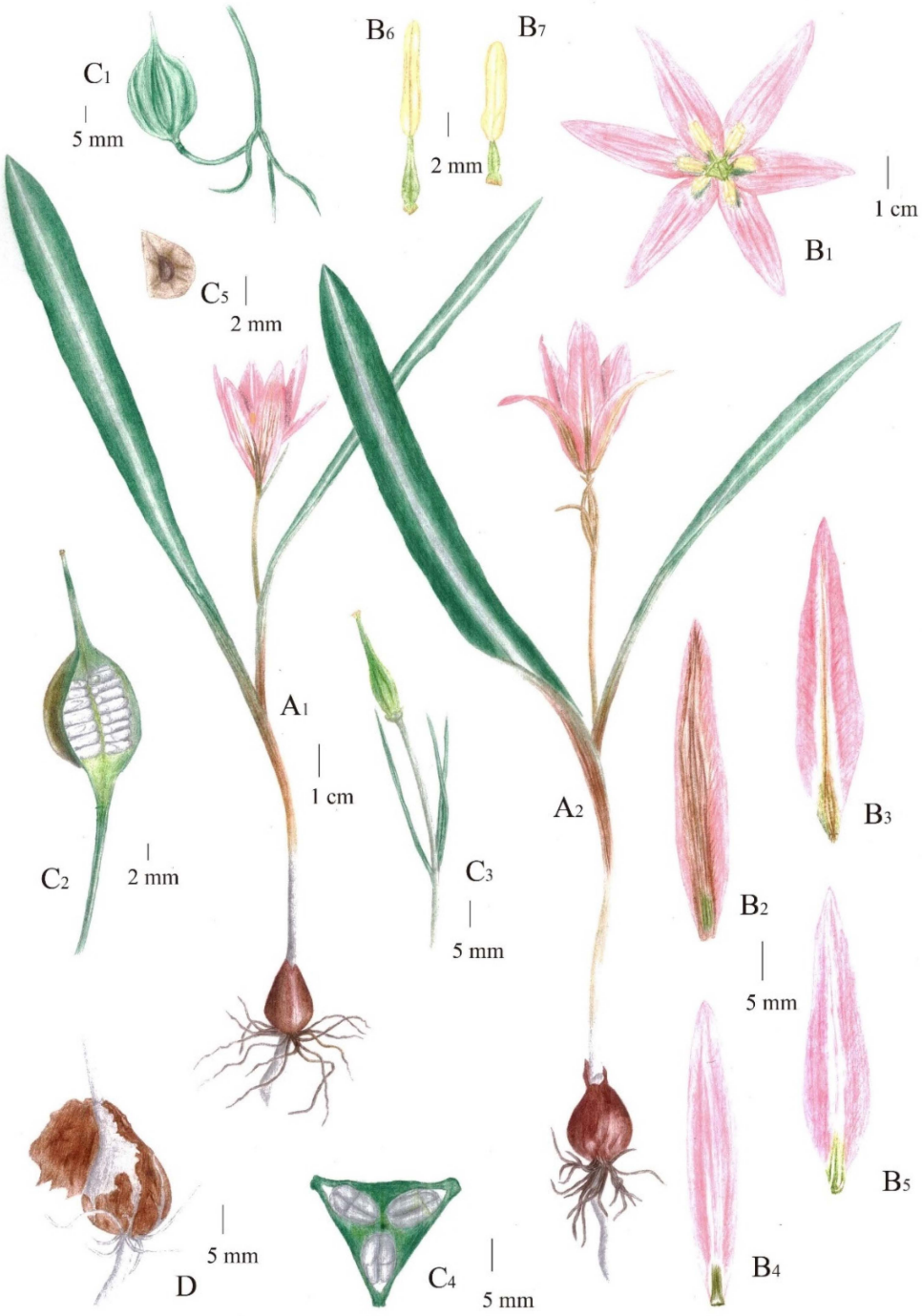

Figure 3. Illustration of *Amana hejiaqingii* (**A1**,**A2**) Flowering plants, (**B1**) Flower, (**B2**) Abaxial surface of outer tepal, (**B3**) Abaxial surface of inner tepal, (**B4**) Adaxial surface of outer tepal, (**B5**) Adaxial surface of inner tepal, (**B6**) Outer stamen, (**B7**) Inner stamen, (**C1**) Fruit, (**C2**) Longitudinal section of ovary, (**C3**) Immature capsule and bracts, (**C4**) Transverse section of ovary, (**C5**) Seed, (**D**) Bulb. Drawn by Xin-Jie Jin.

Figure 4. (**A**–**J**) *Amana anhuiensis*: (**A**) Habitat, (**B**) Population with flowers, (**C**) Population with fruits, (**D**) Whole plant, (**E**) The front view of the flower, (**F**) The side view of the flower, (**G**) Anatomy of flower, (**H**) Bulb, (**I**) Leaves, (**J**) Fruits. The white line segment represents a length of 1 cm.

3.2. Principal Coordinates Analysis

A principal coordinates analysis showed three separated clusters (Figure 5). The first principal coordinates (PCoA1) accounted for 31.77%, and the second principal coordinates (PCoA2) accounted for 13.34% of the total variance.

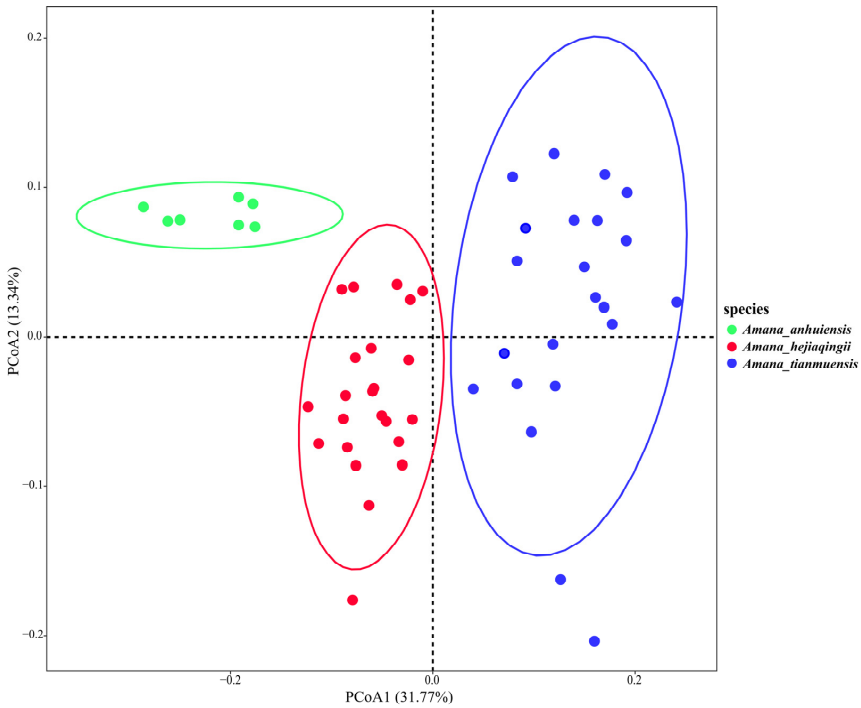

Figure 5. Principal components analysis plot of *Amana anhuiensis*, *Amana hejiaqingii* and *Amana tianmuensis*. The ellipse represents the 95% confidence interval.

3.3. Cytology Observation

A total of six populations were observed, and the numbers of metaphase chromosomes were $2n = 2x = 24 = 3$ m + 11 sm + 10 st. The length of chromosome varied from 5.75 to 10.05 µm. The ratio of the longest to shortest chromosome was 2.13. The total haploid length of the chromosome set (THL) was 99.10. The coefficient of variation of centromeric index (CVCI) was 18.83. The coefficient of variation of chromosome length (CVCL) was 11.60. The mean centromeric asymmetry (MCA) was 50.42. As.K% was 75.50. KA belonged to Stebbins's—3B (Figure 6).

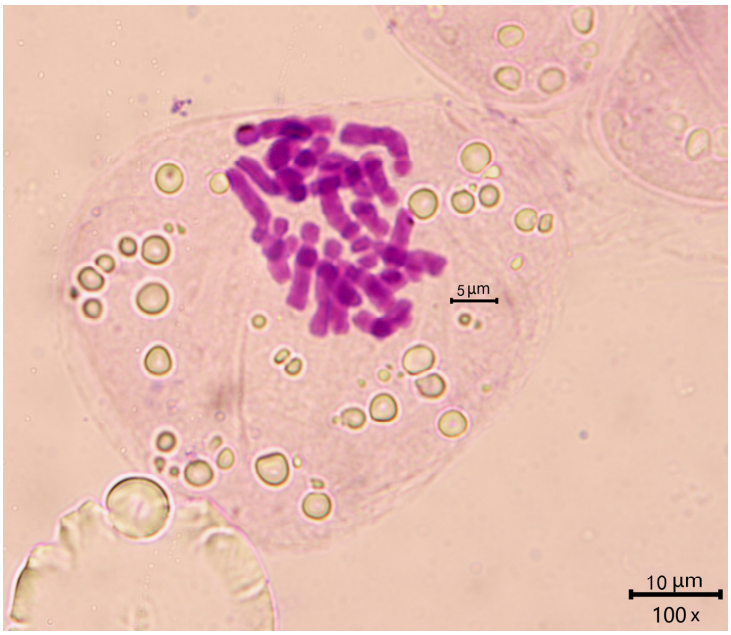

Figure 6. Chromosome number of *Amana hejiaqingii* ($2n = 2x = 24$).

3.4. Molecular Phylogeny

The complete plastome sequence length of *Amana hejiaqingii* was 151,513–151,516 bp (large single copy (LSC) 81,862–81,864 bp, small single copy (SSC) 17,107–17,112 bp and inverted repeat (IR) 26,271 bp with 36.7%, 34.6%, 30.1% and 42% GC content; Figure S1). The total alignment was 73,033 bp with 3065 variable sites. The phylogenetic tree based on plastid genes (Figure 7) revealed that *A. hejiaqingii* was distantly related to *A. tianmuensis* with robust supports (BIPP = 1.0, MLBS = 100). This finding is congruent with our ongoing phylotranscriptomic study on the genus *Amana*.

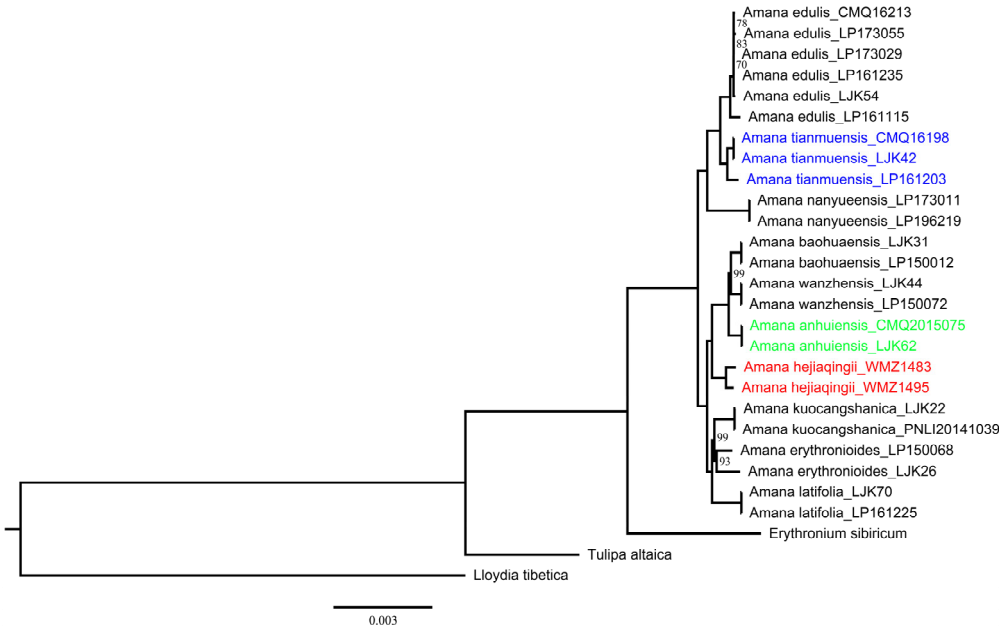

Figure 7. Phylogenetic tree of *Amana* based on plastid CDS sequences. All branches have Bayesian inference posterior probability (BIPP) =1.0. The numbers on the tree represent maximum likelihood bootstrap (MLBS) values. The unmarked branches all have BIPP = 1.0, MLBS = 100%.

4. Taxonomy

Amana hejiaqingii M.Z. Wang & P. Li, sp. nov. (大别老鸦瓣 is the Chinese name of Amana hejiaqingii. Figures 2 and 3)

Type: CHINA. Henan Province: Xinyang City, Shangcheng County, Fushan Town, Liluocheng (里罗城), in deciduous broadleaf forest, 31°43′14.4″ N, 115°30′12.5″ E, 487 m, 15 February 2022, *Pan Li* et al. *WMZ1722* (holotype HZU, isotype HZU, PE, KUN, CSH, HIB, WH, ZM).

Diagnosis: This new species resembles *Amana tianmuensis* P. Li & M.Z. Wang in possessing yellowish-brown, thinly papery bulb tunics, oblanceolate leaves and three verticillate linear bracts, but differs from it as it has solitary pink flower (vs. solitary, sometimes two, white) with longer wider tepals.

Description: Perennial herbs. Bulb ovoid, 0.9–2.3 cm in diameter, tunics yellowish-brown, thinly papery, glabrous, sometimes sparely villous inside. Stem 1.1–12.3 cm tall, glabrous, simple. Leaves usually two, opposite, green, adaxially middle grayish white, oblanceolate, glabrous; the lower leaf 8–26.6 × 1.0–2.8 cm, the upper 7.7–26 × 0.5–1.4 cm, and leaves can reach 34 cm long at fruiting stage. Bracts usually three, verticillate, linear, green, 1.2–4.2 × 0.1–0.3 cm, and bracts can be flat and slightly curved at fruiting stage. Scape 5.1–14.2 cm tall, glabrous, simple. Pedicel 0.5–4.1 cm. Flower solitary, funnel-shaped; tepals six, pink, with a yellowish-green blotch at the very base inside and green-yellowish or purple-red streaks on the back; outer tepals lanceolate, acute, 2.4–4.4 × 0.6–1.1 cm, inner tepals narrow elliptic, acute, 2.1–3.9 × 0.6–1.0 cm. Stamens six, two-wheeled, the inner three slightly longer than the outer; filaments yellowish-green, 3–13 mm long, proximally dilated,

gradually attenuate towards apex, glabrous. Anthers yellow, 5–15 mm long. Ovaries oval, yellowish-green, constricted below the style, 0.3–0.7 cm long. Style 0.4–0.6 cm long. Fruit capsule subglobose, triquetrous, 0.7–1.4 cm in diameter, apex long beaked, 0.5–1.2 cm long. Fl. January–March, Fr. March–May.

Distribution and habitat: Up to now, *Amana hejiaqingii* is found in eight localities across 233.7 km in the Dabie Mountains bordering Henan and Hubei provinces. It grows in moist deciduous broad-leaf forests on mountain slopes at elevations of 70–530 m. In future investigations, we think that more populations will be found in the area in and around these localities.

Etymology: The specific epithet is named in memory of Professor Jia-qing He, a Chinese botanist who was dedicated to plant investigation in the Dabie Mountains. He walked about 12,684 km and collected nearly 10,000 specimens during a 255-day fieldtrip in the Dabie Mountains, becoming the first person ever to make a comprehensive wild plant investigation of the Dabie Mountains.

Conservation Status: *Amana hejiaqingii* is distributed in several places in Henan and Hubei provinces, with thousands of individuals at each site, thus we suspect that *A. hejiaqingii* could be categorized as Least Concern (LC) according to IUCN criteria (IUCN Standards and Petitions Committee, 2022).

Additional specimens examined (paratypes): CHINA. **Henan** Province: Tongbai County, Huaiyuan Town, Huaiyuan Scenic Area (淮源风景区), 249 m, 7 March 2016, Fr., *Fuhe Chen LP161136* (HZU); Tongbai County, Chengjiao Town (城郊乡), 196 m, 24 February 2020, Fl., *Fuhe Chen LP207883* (HZU); Tongbai County, Tayuan Temple (塔院寺), 248 m, 8 March 2021, Fr., *Pan Li* et al. *WMZ1489* (HZU); *ibidem*, 139 m, 14 February 2022, Fl., *Pan Li* et al. *WMZ1711* (HZU); Tongbai County, Yuehe Town, Mt. Bijia (笔架山), 157 m, 8 March 2021, Fr., *Pan Li* et al. *WMZ1492* (HZU); *ibidem*, 105 m, 14 February 2022, Fl., *Pan Li* et al. *WMZ1712* (HZU); Xinyang City, Shihe District, Heilongtan Waterfall (黑龙潭瀑布), 185 m, 14 February 2022, Fl., *Pan Li* et al. *WMZ1714* (HZU); Shangcheng County, Liluocheng (里罗城), 416 m, 9 April 2017, Fr., *Xinglv Xie LP173093* (HZU); *ibidem*, 7 March 2021, Fr., *Pan Li* et al. *WMZ1483* (HZU). **Hubei** Province: Guangshui City, Wushengguan Town, Heilongtan Scenic Area (黑龙潭风景区), 529 m, 8 March 2021, Fr., *Pan Li* et al. *WMZ1487* (HZU); ibidem, 371 m, 14 February 2022, Fl., *Pan Li* et al. *WMZ1718* (HZU); Guangshui City, Santan Scenic Area (三潭风景区), 199 m, 14 February 2022, Fl., *Pan Li* et al. *WMZ1716* (HZU).

Key to the *Amana*:

1. Bracts usually two, opposite.	2
1. Bracts usually three, verticillate, or not verticillate (in this case degraded).	3
2. Lower leaves linear; flowers 1–5; anthers yellow at maturity (before releasing pollens).	*Amana edulis*
2. Lower leaves oblanceolate; flower mostly solitary, sometimes two; anthers purple at maturity (before releasing pollens).	*Amana nanyueensis*
3. Bracts usually not verticillate, degraded, 1–5 mm long.	*Amana wanzhensis*
3. Bracts verticillate, not degraded, longer than 1.2 cm.	4
4. Lower leaves linear, or linear to oblanceolate in the same population, always with a broad white band along the midvein above.	5
4. Lower leaves oblong or oblanceolate, sometimes with white veins or midvein, but not a broad white band.	6
5. Leaves green, linear, 0.4–1 cm wide; purple-red on the back of outer tepals.	*Amana baohuaensis*
5. Leaves green or brownish-green to purple, linear to oblanceolate, 1–2 cm wide; purple-red streaks on the back of outer tepals.	*Amana latifolia*
6. Anthers purple at maturity (before releasing pollens).	7
6. Anthers yellow at maturity (before releasing pollens).	8
7. Leaves green, with several white veins; bulb tunic sparsely villous in-side.	*Amana anhuiensis*
7. Leaves green, dark-green or purplish-green, without white veins; bulb tunic glabrous inside.	*Amana kuocangshanica*
8. Leaves oblong; bulb tunic densely villous-woolly inside.	*Amana erythronioides*
8. Leaves oblanceolate; bulb tunic glabrous or sparely villous inside.	9
9. Flowers pink, solitary.	*Amana hejiaqingii*
9. Flowers white, mostly solitary, sometimes two.	*Amana tianmuensis*

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/taxonomy2030022/s1, Figure S1: The complete plastome circle diagram of *Amana hejiaqingii*; Table S1: Morphological data of *Amana anhuiensis*, *A. hejiaqingii* and *A. tianmuensis*.

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