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Amana kuocangshanica (Liliaceae), a new species from south-east China

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Amana kuocangshanica D. Y. Tan & D. Y. Hong is described as a new species from Mt. Kuocang, Zhejiang Province, south-east China. Morphological observations and a statistical analysis of the new species and its relatives show that it is most closely related to *A. anhuiensis* and *A. erythronioides*, but differs in having the lower leaf oblanceolate, the widest at two-thirds length from the base, tunics glabrous inside, and fruit beaks 5–7.5 mm long. The morphologies of the pollen, seeds, and epidermal cells of the leaves of this new species are described. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, **154**, 435–442.

ADDITIONAL KEYWORDS: morphology – scanning electron microscopy (SEM) – statistics – *Tulipa*.

INTRODUCTION

The genus *Amana*, with only four species in the Liliaceae (*s.s.*), is confined to East Asia. This genus has a number of morphological characters similar to those of the genus *Tulipa* L. and, indeed, many authors have placed it in *Tulipa* (Sealy, 1957; Mao, 1980; Ohwi & Kitagawa, 1992; Liang, 1995; Tamura, 1998; Shen, 2001). However, this group differs from the other members of *Tulipa* in having 2–3(–4) opposite or verticillate bracts in the upper part of the flowering stem and the style as long as the ovary proper. Therefore, the taxonomy of this taxon has long been controversial. Miquel (1867) described the first species in the group as *Orithyia edulis* Miq. because of its small size and the distinct slender style, often as long as the ovary itself. Baker (1874) included it in *Tulipa* L. subgenus *Orithyia* Baker, based on the style as long as the ovary and the woolly tunics. Honda (1935) segregated *T. edulis* (Miq.) Baker and *T. latifolia* (Makino) Makino from *Tulipa* to form a new genus, *Amana* Honda. Kitamura, Murata & Koyama (1980) and Satake *et al*. (1982) supported the separation of *T. edulis* and *T. latifolia* from the genus *Tulipa* on the basis of the small flowers, linear leaves, and bracts on the stem. Chen & Mordak (2000) placed it back in *Tulipa* L., but Wu *et al*. (2003) believed that this section would be better treated as a genus, *Amana,* on the basis of morphology and geographical distribution. Fay & Chase (2000) supported the separation of *Amana* from *Tulipa* on the basis of molecular and morphological data. Rønsted *et al*. (2005) placed *Amana* sister to *Erythronium*, with a bootstrap percentage of 70, based on *mat*K sequences. Tan *et al*. (2005) restored *Amana* Honda to generic rank on the basis of the results of a cladistic analysis of morphological characters of *Tulipa* and related taxa and molecular data (D.-Y. Tan, Z. Zhang, X.-Q. Wang & D.-Y. Hong, unpubl. data), which both showed that the *Amana* group and the other members of the genus *Tulipa* formed two independent clades.

In an expedition to Zhejiang Province, south-east China, to observe *Amana* in the field, an interesting population was found with a number of characters that did not fit any of the taxa described in the genus *Amana*. In addition, herbarium specimens from China and Japan [Jiangsu Institute of Botany (NAS), Hangzhou Botanical Garden (HHBG), Zhejiang University (HZU), and Institute of Botany, Chinese Academy of Sciences (PE)] were examined and analysed. As a

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result of this extensive comparison and analysis, the population is described here as a new species.

MATERIAL AND METHODS

Population sampling

The population was sampled twice at flowering time in 2002 and in fruit in 2003 on Mt. Kuocang, Zhejiang Province, China. More than ten individuals were collected randomly each time for morphological observations. In the same manner, six populations were sampled for *A. edulis* (Miq.) Honda, two for *A. erythronioides* (Baker) D. Y. Tan & D. Y. Hong, and one for *A. anhuiensis* (X. S. Shen) D. Y. Tan & D. Y. Hong from Zhejiang, Jiangsu, Shandong and Anhui Provinces. The vouchers are deposited in PE and XJA. In order to capture the complete range of variation of the taxa studied, extensive comparisons and analyses of herbarium specimens in NAS, HHBG, HZU, and PE and of living plants were made in 2003–04.

Scanning electron microscopy (SEM) observation

Pollen grains of the material under study were removed from fresh flowers and seeds from mature fruits in the field, acetolysed according to the procedure suggested by Erdtman (1966), washed first in 95% alcohol and then in absolute alcohol, sputtered with gold–palladium, and observed under a Hitachi S-800 scanning electron microscope. The terminology applied for the morphological descriptions of pollen and seeds generally follows Jessop (1975), Ness (1989), Punt *et al*. (1994) and Zarrei & Zarre (2005).

Morphological observations and statistical analysis

The leaves for observation were fixed in formalin–acetic acid–alcohol (FAA) in the field, and stained with safranin. Permanent preparations were sealed with Canada balsam, and then examined and photographed with an Olympus BH-2 microscope.

A statistical analysis was carried out to determine how the new taxon differs from its allies, and to evaluate the justification for describing it as a new species. Of the ten populations sampled, six were used in the analysis: two each for *A. edulis* and *A. erythronioides*, and one each for *A. anhuiensis* and the putative new species. The origin of the populations sampled and used in the statistical analysis is given in the Appendix. According to the method of Hong, Wang & Zhang (2004), 17 characters (Tables 1 and 2) were used for both cluster analysis (unweighted pair group method with arithmetic average, UPGMA) and principal coordinate analysis: four bistate (type of texture of the tunics, number of flowers, number of bracts, and stomata on the adaxial surface of the lower leaf); three ordinal (hairiness inside the tunic, shape of the lower leaf, widest position of the lower leaf); and ten continuous (length and width of the lower leaf, length of the bracts, length and width of the outer and inner tepals, length of the outer and inner stamens, length of the fruit beak). Statistical analysis was performed on ten living individuals of each population, with each individual treated as an operational taxonomic unit (OTU). Principal coordinate analysis was conducted using MVSP-Version 3.13b analysis software. All of the data were standardized to ensure that they more closely approximated a normal distribution.

RESULTS

The results of our observations on the morphology of the four species are shown in Table 2.

The morphological features of the leaf epidermis in the new species, *A. kuocangshanica*, are as follows: cells generally rectangular on both surfaces, anticlinal wall of epidermal cells undulate; on the adaxial surface, rectangular epidermal cells irregular with a density of *c.* 79 mm^{-2}; stomata absent (Fig. 1); on the abaxial surface, rectangular epidermal cells also

Table 1. Some morphological characters, states, and code numbers used in the statistical analysis

Character		Character states and their code numbers
Bistate	Texture of tunic type	Papery (0); thinly papery (1)
	Stomata of upper epidermis of leaves	Present (0); absent (1)
	Number of bracts	2 (0); 3 (1)
	Number of flowers	Solitary (0); >1 (1)
Ordinal multistate	Hairiness of outer bulb sheath	Dense (0); sparse (1); absent (1)
	Shape of lower leaf	Linear (0); lanceolate (1); oblanceolate (2)
	Widest position of lower leaf	At 1/2 length from the base (0); at 3/4 length from the base (1); at 2/3 length from the base (2)

Table 2. Comparison of diagnostic characters between ***Amana kuocangshanica***, *A. erythronioides*, *A. anhuiensis*, and *A. edulis*. Mean and standard deviation given for length/width ratio of lower leaves and length of fruit beak

Taxon	Population	Tunic texture	Hairiness of tunics inside	Branch of stem	Lower leaves: Shape	Lower leaves: Widest position	Lower leaves: Length/width ratio	No. bracts	No. flowers	Length of fruit beak (cm)
A. edulis	Zhe001	Papery	Dense	Present	Linear	1/2 length from base	20.17–49.38	2	≥ 1	0.45–0.90
	Zhe005	Papery	Dense	Present	Linear	1/2 length from base	22.38–57.14	2	≥ 1	0.45–0.80
A. erythronioides	Zhe002	Papery	Dense	Absent	Oblong	1/2 length from base	3.50–6.71	3	1	0.55–0.80
	Zhe006	Papery	Dense	Absent	Oblong	1/2 length from base	4.03–10.00	3	1	0.50–0.80
A. anhuiensis	Wan006	Thinly papery	Sparse	Absent	Oblanceolate	3/4 length from base	6.00–10.25	3	1	0.90–1.30
A. kuocangshanica	Zhe004	Thinly papery	Absent	Absent	Oblanceolate	2/3 length from base	7.12–14.25	3	1	0.50–0.75

irregular with a density of *c.* 72 mm^{-2}; stomata present, and guard cells *c.* 64 μm, with stomatal index *c.* 41.5% (Fig. 2).

The pollen grains of *A. kuocangshanica* are navicular, 52.2–56.7 × 26.1–28.3 μm, with P/E 0.50, the exine surface ornamentation reticulate, and the perforation dense and irregular (Figs 3, 4).

The seeds are triangular, 3.8 ± 0.5 × 1.8 ± 0.3 mm, with a length/width ratio of 2.19 ± 0.50. The surface cells are irregular and undulate (Figs 5, 6).

Figure 7 shows that all the OTUs form four clusters, corresponding to the four species, indicating that they are distinct. Figure 8 shows that all the OTUs are grouped into four distinct entities, which again correspond to the four species: *A. edulis, A. erythronioides, A. anhuiensis*, and *A. kuocangshanica*. *A. kuocangshanica* seems to form a natural group, distinct from all its allies, therefore justifying its description as a new species from morphological observations and statistics.

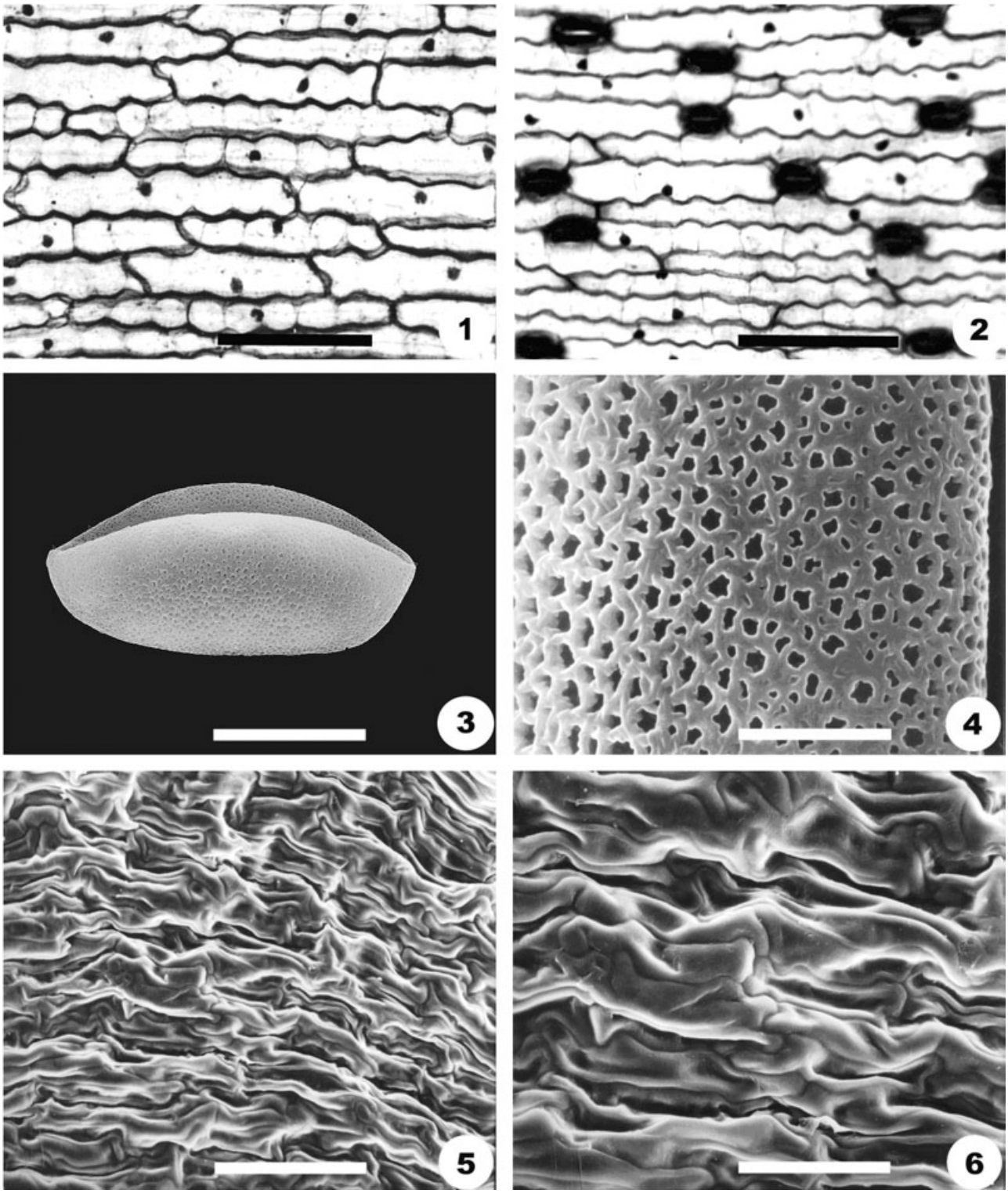
AMANA KUOCANGSHANICA

Amana kuocangshanica *D. Y. Tan & D. Y. Hong*, ***sp. nov.***

Type: China, Zhejiang Province, Mt. Kuocang, in bamboo forests or in bushes, 600–1100 m, 27.ii.2002, *D. Y. Tan & X. R. Li Zhe 004* (holotype, PE; isotype, XJA).

Diagnosis: Species nova *A. anhuiensi* (X. S. Shen) D. Y. Tan & D. Y. Hong & *A. erythronioidi* (Baker) D. Y. Tan & D. Y. Hong affinis, a quibus differt tunica intus glabra, foliis oblanceolatis, eo basali *c*. 2/3 a basi latissimo, fructus rostro 6.4 ± 0.8 mm longo (Fig. 9A–F).

Description: Perennial herbs; bulbs ovoid, 9–15(–19) mm in diameter; tunics yellow-brownish, thinly papery, glabrous inside. Stems (95–)118–200 mm tall, glabrous, simple. Leaves two, dark green or purplish-green, opposite, oblanceolate; the lower leaf 114–250 × 8–23 mm, with the widest part at two-thirds length from the base, the upper 104–245 × 4.5–11 mm. Bracts usually three, whorled, narrowly lanceolate, green or purple, 27–42 × 2.0–3.0 mm; pedicels 12–51 mm. Flowers solitary, funnel-shaped, tepals six, white, with a deep green or yellowish-green blotch at the very base inside and brownish-purple streaks on the back; outer tepals lanceolate, acute, 21–37 × 4–8 mm; inner tepals narrow elliptic, bluntly acute, 15–34 × 6–11 mm. Stamens six, the inner three slightly longer than the outer; filaments 4.0–6.5 × 1.5–2.0 mm, yellow, proximally dilated, gradually attenuate towards apex, glabrous; anthers 4.0–7.0(–10.0) × (1.0–)1.5–1.8 mm, purplish-brown with deep purple margins. Ovaries yellowish-green, constricted below



Figures 1–6. Photographs of micromorphological characters of ***Amana kuocangshanica***. Figs 1, 2. Light microscopy, leaf epidermis (scale bar, 200 μm). Fig. 1. Adaxial surface. Fig. 2. Abaxial surface. Figs 3, 4. Scanning electron microscopy, pollen morphology. Fig. 3. Pollen shape (scale bar, 20 μm). Fig. 4. Ornamentation of exine (scale bar, 3. 5 μm). Figs 5, 6. Scanning electron microscopy. Sculpture of the seed coat [scale bar, 300 μm (Fig. 5), 150 μm (Fig. 6)].

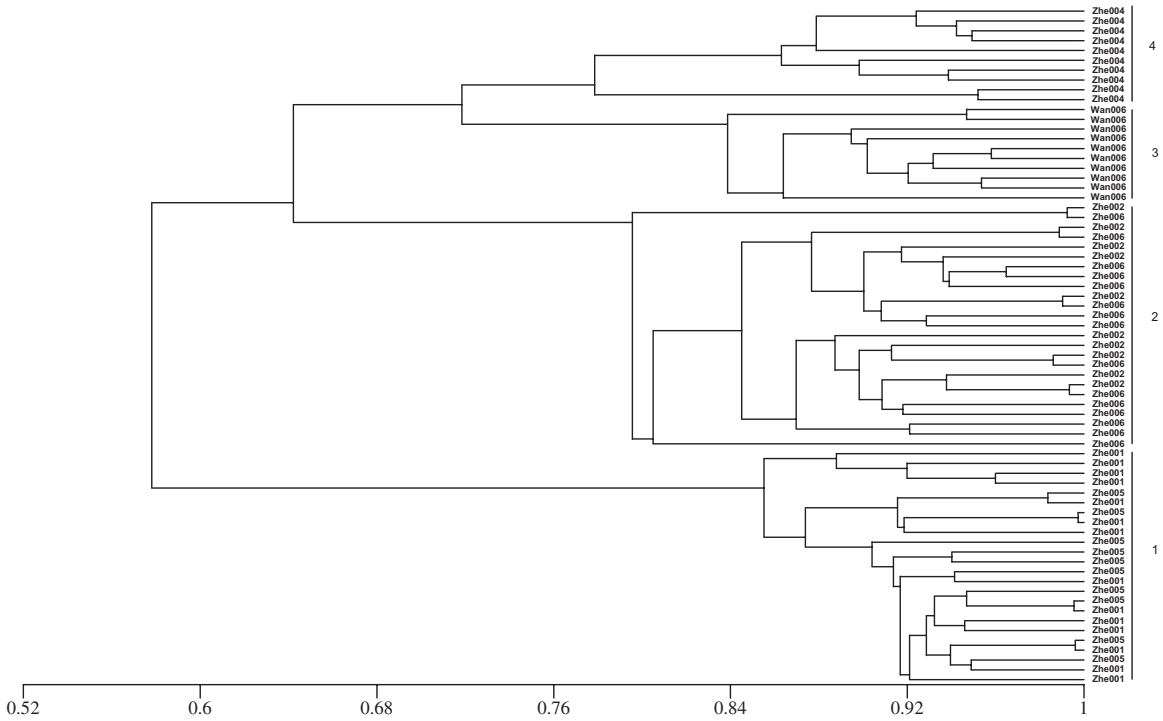


Figure 7. Cluster analysis (unweighted pair group method with arithmetic average, UPGMA). The operational taxonomic units (OTUs) and characters used are explained and described in the text. 1, *Amana edulis*; 2, *A. erythronioides*; 3, *A. anhuiensis*; 4, ***A. kuocangshanica***.

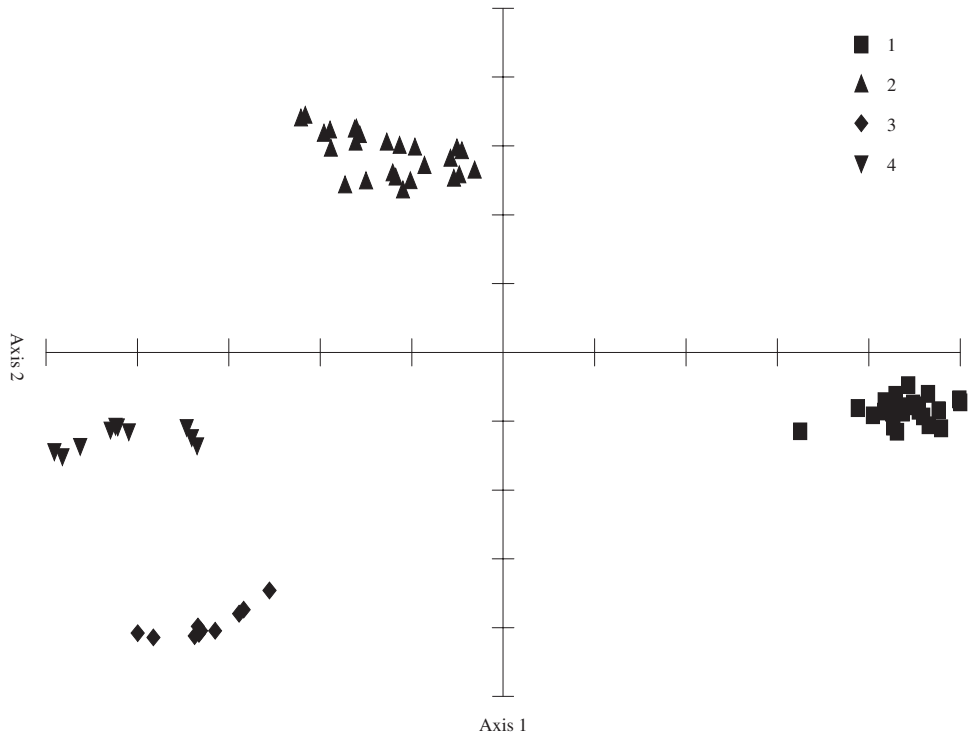


Figure 8. Principal coordinate analysis. The operational taxonomic units (OTUs) and characters used are the same as for Figure 7. Axis 1 represents 36.98% of the total variation, and axis 2 represents 18.69% of the variation. Numbers refer to taxa given in the legend of Figure 7.

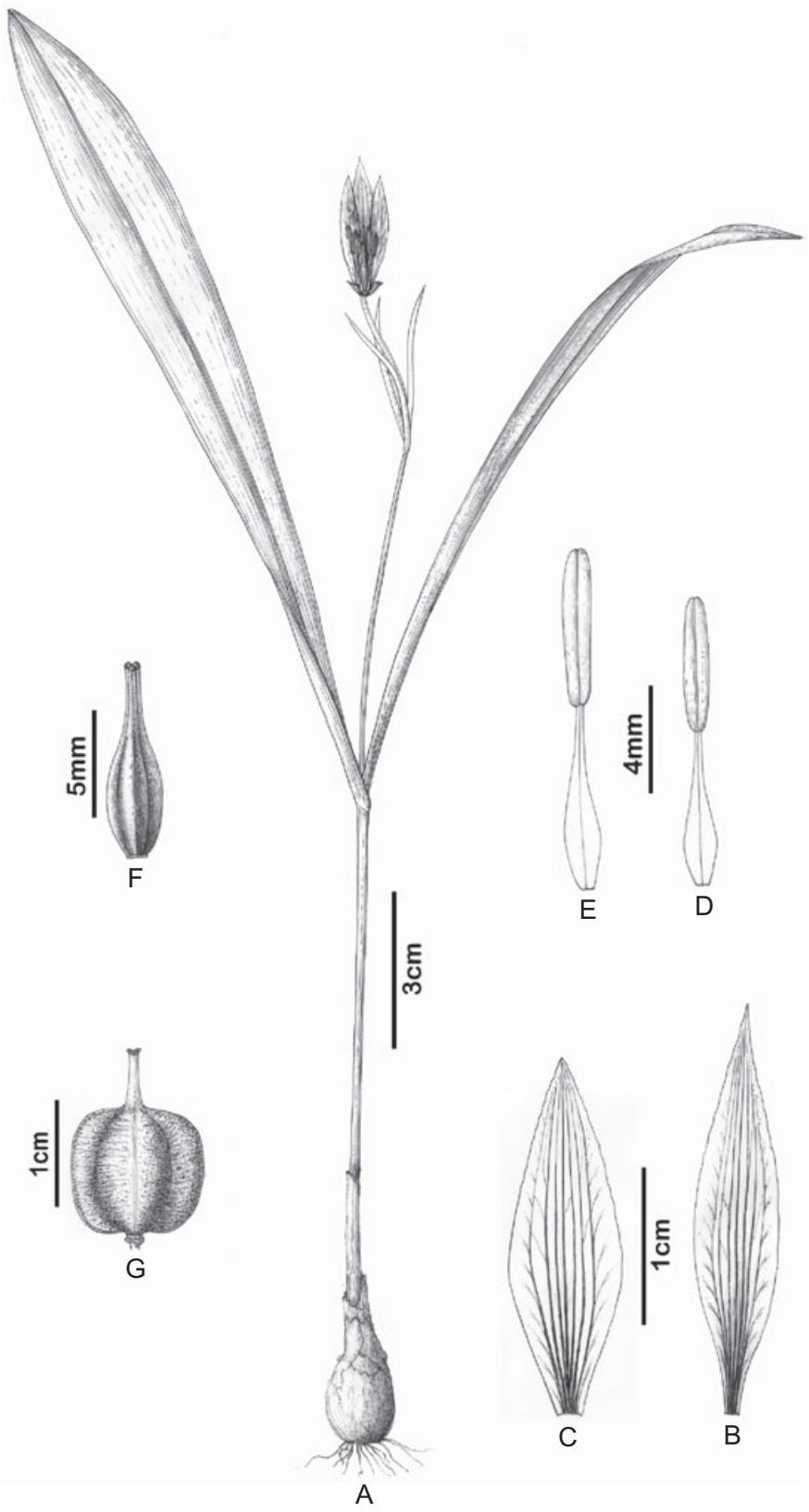


Figure 9. ***Amana kuocangshanica*** D. Y. Tan & D. Y. Hong: A, plant; B, one outer tepal; C, one inner tepal; D, one outer stamen; E, one inner stamen; F, gynoecium; G, fruit. Drawn by Mr Ying-Bao Sun and Miss Li-Xia Tan.

the style, 4.0–8.0 mm, styles 4.0–6.0 mm. Fruits triquetrous, 10–14 × 11–15 mm, the fruiting beak 5–7.5 mm. Flowers in February–March and fruits in April–May.

Distribution: Amana kuocangshanica is confined to Mt. Kuocang, Zhejiang Province, south-east China, where a large amount of individuals were seen in bamboo forests or in bushes at an altitude of 600–1100 m. It grows in moist places.

Notes: The leaf epidermis of *A. kuocangshanica* is similar to that of *A. erythronioides* in morphology. There are no stomata on the adaxial surface in the two species (Figs 3, 4). However, in *A. erythronioides*, the anticlinal walls of the epidermal cells on both the adaxial and abaxial surfaces are straight, the epidermal cells of the abaxial surface are rhombic, and their density is about 75 mm^{-2}. The length of the guard cells is about 58.5 μm and the stomatal index is about 38.7%.

The internal transcribed spacer (ITS), *trn*L-F and *mat*K sequences of the four species also reveal that this new species is closely related to *A. erythronioides* (D.-Y. Tan, Z. Zhang, X.-Q. Wang & D.-Y. Hong, unpubl. data).

The differences and relationships between the new species and the three other species are summarized in the following key.

1. Stems usually branched; leaves linear; flowers 1–5; bracts usually two, opposite, very rarely three or four, whorled. ..... *A. edulis* (Miq.) Honda
1. Stems simple; leaves oblong or lanceolate or oblanceolate; flowers solitary; bracts three or four, whorled.
   2. Tunics papery, densely villous inside; leaves oblong..... *A. erythronioides* (Baker) D. Y. Tan & D. Y. Hong
   2. Tunics thinly papery, villous or glabrous inside; leaves oblanceolate.
      3. Tunics glabrous inside; the widest position of lower leaf at two-thirds length from the base; fruit beak 5.0–7.5 mm long..... *A. kuocangshanica* D. Y. Tan & D. Y. Hong
      3. Tunics villous inside; the widest position of lower leaf at three-quarters length from the base; fruit beak 9.0–13 mm long..... *A. anhuiensis* (X. S. Shen) D. Y. Tan & D. Y. Hong

NEW COMBINATION

Amana erythronioides (Baker) D. Y. Tan & D. Y. Hong, comb. nov. – basonym. *Tulipa erythronioides* Baker in J. Bot., 13: 292. 1875.

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APPENDIX

Origin of sampled populations used in the statistical analysis

A. edulis

Zhe001. Hangzhou Botanical Garden, Zhejiang Province, China, forest, 10 m, 4.iii.2002, *D.Y. Tan* & *X. R. Li* (PE, XJA).

Zhe005. Mt. North Yandang, Leqing, Zhejiang Province, China, forest, 200 m, 28.ii.2002, *D.Y. Tan & X. R. Li* (PE, XJA).

A. erythronioides

Zhe002. Mt. Huading, Tiantai, Zhejiang Province, China, bamboo forest or bushes, 1080 m, 24.ii.2002, *D.Y. Tan* & *X. R. Li* (PE, XJA).

Zhe006. Mt. Siming, Yuyao, Zhejiang Province, China, bamboo forest or bushes, 700 m, 1.iii.2002, *D.Y. Tan & X. R. Li* (PE, XJA).

A. anhuiensis

Wan006. Mt. Tianzhu, Qianshan, Anhui Province, China, bamboo forest or bushes, 1200 m, 14.iii.2002, *D. Y. Tan & Z. Zhang* (PE, XJA).

A. kuocangshanica

Zhe004. Mt. Kuocang, Ninghai, Zhejiang Province, China, bamboo forest or bushes, 800 m, 27.ii.2002, *D.Y. Tan* & *X. R.. Li* (PE, XJA).