Leaf epidermal micromorphological characteristics of Chinese *Aucuba* and their taxonomic significance^{1}

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Abstract

To reveal the micromorphological characteristics of the leaf epidermis of Aucuba and explore their taxonomic significance, thereby providing relatively objective morphological evidence for species delimitation in the genus, this study investigated 10 species and 7 varieties of Aucuba in China. The micromorphological characteristics of the leaf epidermis were systematically observed using light microscopy and scanning electron microscopy. The results showed that: (1) The stomatal apparatuses of Aucuba plants are all distributed on the abaxial surface of the leaves, with shapes ranging from nearly circular to elliptical. The guard cells are reniform, overlying 5~7 unspecialized epidermal cells and surrounded by these epidermal cells, with no subsidiary cells. This reveals a new stomatal type: Platanus-Typestoma. (2) Cluster analysis based on leaf epidermal micromorphology showed that Aucuba in China can be divided into 2 groups: in Group I, the cuticular surface of the leaf epidermis is relatively smooth, papillae are present on the adaxial surface, the anticlinal walls of epidermal cells are mainly shallowly sinuate and sinuate, and the stomatal apparatuses are mainly nearly circular; in Group II, abundant wax is attached to the cuticular surface of the leaf epidermis, the anticlinal walls of epidermal cells are mainly sinuate and deeply sinuate, and the stomatal apparatuses are mainly subcircular and elliptical. This result supports the taxonomic treatment of the "green-flowered tree group" and the "red-flowered shrub group" based on integrated morphological characteristics. (3) This study clarified some interspecific relationships and taxonomic treatments, such as supporting the treatment of A.grandiflora and A.cavinervis as independent species, and not supporting the treatment of the narrow-leaved Aucuba as a variety of A.chinensis. In summary, leaf epidermal micromorphological characteristics have important taxonomic value at the group level for Aucuba plants in China and have certain reference value for the identification of some species within the genus.

Full Text

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Leaf epidermal micromorphological characteristics of Chinese *Aucuba* and their taxonomic significance[1]

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Abstract: To reveal the micromorphological characteristics of the leaf epidermis in the genus *Aucuba* and to explore their taxonomic significance, thereby providing relatively objective morphological evidence for species delimitation in this genus, this study examined 10 species and 7 varieties of Chinese *Aucuba*. Leaf epidermal micromorphological characters were systematically observed using light microscopy and scanning electron microscopy. The results showed that: (1) The stomatal apparatuses of *Aucuba* plants are all distributed on the abaxial leaf surface, and their shapes range from nearly circular to oval. The guard cells are kidney-shaped, overlie 5–7 undifferentiated epidermal cells, and are surrounded by these epidermal cells; no subsidiary cells are present. This reveals a new stomatal-apparatus type—the Platanus-type stoma. (2) Cluster analysis of leaf epidermal micromorphology showed that Chinese *Aucuba* can be divided into two groups: in Group I, the surface of the leaf epidermal cuticle is relatively smooth and has papilla-like protrusions near the axis; the anticlinal walls of epidermal cells are mainly shallowly undulate and undulate, and stomatal apparatuses are mainly nearly circular. In Group II, more wax is attached to the cuticular surface of the leaf epidermis; the anticlinal walls of epidermal cells are mainly undulate and deeply undulate, and stomatal apparatuses are mainly subcircular and oval. This result supports the taxonomic treatment, based on integrated morphological characters, of the "green-flowered shrub group" and the "red-flowered shrub group." (3) This study clarifies some interspecific relationships and taxonomic treatments, such as the tendency to treat *Aucuba grandiflora* and *A. cavinervis* as independent species, and it does not support treating narrow-leaved *Aucuba* as a variety of *Aucuba chinensis*. In summary, leaf epidermal micromorphological characters have important taxonomic value for Chinese *Aucuba* at the group level and have certain reference value for the identification of some species within the genus.

Keywords: *Aucuba*; species identification; leaf epidermal micromorphology; systematic taxonomy; stomata

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Leaf epidermal micro-morphology of genus *Aucuba*

(Garryaceae) in China and its taxonomic significance

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Abstract: To reveal the micro-morphological characteristics of leaf epidermis in the genus *Aucuba*, explore their taxonomic significance, and provide objective morphological evidence for species classification within this genus. This study examined 10 species and 7 varieties of Chinese *Aucuba* using light microscopy (LM) and scanning electron microscopy (SEM). The results are as follows: (1) Stomatal apparatuses in *Aucuba* occur exclusively on the abaxial leaf epidermis. They range from nearly round to oval in shape, lack subsidiary cells, and consist of kidney-shaped guard cells surrounded by 5-7 unspecialized epidermal cells. This represents a novel stomatal type

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termed the "*Platanus*-Type" . (2) Cluster analysis of micro-morphological traits revealed that Chinese *Aucuba* species can be divided into two groups: Group I exhibits a relatively smooth cuticular surface, papillae adaxial epidermis, sinoulate to sinuous anticlinal walls, and predominantly round stomatal apparatuses; Group II is characterized by cuticles with abundant wax, sinuous to sinuate anticlinal walls, and sub-round to oval stomatal apparatuses. These groupings align completely with the traditional morphological classification of "green-flowered tree group" and "red-flowered shrub group" . (3) Leaf epidermal features supported certain taxonomic revisions, such as recognizing *A. grandiflora* and *A. cavinervis* as distinct species, but do not support the recognition of *A. chinensis* var. *angusta*. In summary, the micro-morphological traits of leaf epidermis have significant taxonomic value at the group level in Chinese *Aucuba* and provide a useful reference for the identification of certain species within the genus.

Key word: *Aucuba*, species identification, leaf epidermal micro-morphological features, taxonomic significance, stoma

The genus *Aucuba* Thunb. belongs to Garryaceae (Angiosperm Phylogeny

Group et al., 2016). It comprises approximately 10 species and 8 varieties worldwide (POWO, 2025), is mainly distributed in East Asia, South Asia, and Southeast Asia, and is endemic to Asia. China is the modern center of distribution for the genus, with about 10 species and 7 varieties (Xiang & Boufford, 2005), distributed throughout all provinces and regions south of the Yellow River basin (Editorial Committee of *Flora Reipublicae Popularis Sinicae*, 1990). Plants of *Aucuba* are evergreen throughout the year and bear red fruits in winter, making them excellent ornamental foliage and fruiting plants for landscaping, as well as pollution-resistant tree species (Xiang & Boufford, 2005). Among them, variegated Japanese laurel (*A. japonica* Thunb. var. *variegata* Dombrain) has been widely cultivated ornamentally for centuries in temperate regions such as East Asia, Europe, and North America, while *Aucuba chinensis* Benth. is commonly used as an ornamental tree species in parks in southwestern China. At the same time, plants of this genus also have certain medicinal value: 6 species and 3 varieties of the genus can be used medicinally (Editorial Committee of *Chinese Materia Medica*, State Administration of Traditional Chinese Medicine, 1997; Sichuan Provincial Food and Drug Administration, 2011; Ai Tiemin, 2013).

Because of their distinctive morphological characteristics, plants of *Aucuba* are readily recognized among the eudicots. The principal diagnostic features of the genus are: branches entirely emerald green, with conspicuous annular bud-scale scars; leaves opposite, with toothed margins; plants dioecious, with paniculate cymes; flowers unisexual and 4-merous; staminate flowers with two-loculed anthers, pistillate flowers with an inferior ovary and a single anatropous ovule; and drupes red. However, species of the genus are highly similar in morphology and their leaf forms are variable, making morphology-based classification difficult and complicated (Xiang & Boufford, 2005), and hindering effective conservation and utilization of species and germplasm resources. At present, infrageneric classification of *Aucuba* relies on unstable vegetative characters. For example, *Flora Reipublicae Popularis Sinicae* (Editorial Committee of *Flora Reipublicae Popularis Sinicae*, 1990) and *Flora of China* (Xiang & Boufford, 2005) delimit species using characters that vary considerably, such as leaf margin, leaf shape, and the presence or absence of spots on the leaf surface, but they do not conduct a systematic and in-depth investigation of patterns of morphological trait variation among species. *Aucuba* is dioecious, and it is therefore difficult to observe all characters of a species on the same individual, especially complete reproductive traits. In addition, the genus is recently differentiated (Huang et al., 2022), and its evolutionary history may have involved extensive hybridization, incomplete lineage sorting, and polyploidization events. Therefore, relying solely on macromorphological characters makes it difficult to resolve its taxonomic problems.

Wilkinson proposed that leaf epidermal morphological characters are usually relatively stable among species (Metcalfe & Chalk, 1957). Although leaf epidermal characters are influenced to some extent by environment and climate (Geng Qianqian et al., 2019), they can still directly reflect morphological differences among different taxa and provide valuable taxonomic evidence. Therefore, leaf

epidermal characters have important taxonomic value for understanding relationships among genera and among species within a genus. At present, studies at home and abroad on the leaf epidermal micromorphology of *Aucuba* plants in taxonomy and systematics are uncommon; only *Aucuba chinensis* (*A. chinensis*) (Peng Chuangye et al., 2008) and Emei *Aucuba* [*A. chinensis* (*A. omeiensis* Fang)] (Song Liangke et al., 2007) have been subjected to simple pharmacognostic observations of leaf epidermal micromorphology. Therefore, comprehensive observations of leaf epidermal micromorphological characters across the genus are urgently needed in order to discover new taxonomic evidence for species delimitation in this genus.

In this study, mature leaves of Chinese *Aucuba* plants were used as the research material. Micro-morphological characters were observed using light microscopy (LM) and scanning electron microscopy (SEM), micro-morphological image data were obtained, and cluster analysis and principal component analysis were carried out by combining numerical taxonomic methods with micro-morphological information. This

This study further improves the data on leaf epidermal micromorphology of Chinese *Aucuba*, providing reference evidence for exploring the taxonomy and phylogenetic relationships of the genus. It will also address the following questions: (1) what characteristics the leaf epidermal micromorphology of Chinese *Aucuba* possesses; (2) whether *Aucuba* can be subdivided infragenerically based on leaf epidermal micromorphology; and (3) whether interspecific differences exist in the leaf epidermal micromorphology of *Aucuba*, and, on this basis, what possible taxonomic significance can be revealed.

1 Materials

The experimental materials were collected in the wild or introduced and cultivated in botanical gardens, covering all species of *Aucuba* in China, comprising 10 species and 7 varieties. Voucher specimens are deposited in the Herbarium of Guangzhou University of Chinese Medicine (GUCM); detailed source information is shown in Table 1. The classification follows *Flora of China* (Xiang & Boufford, 2005), and this study additionally collected materials of *Aucuba omeiensis*, *A. grandiflora* C. Y. Wu, and *A. cavinervis* C. Y. Wu ex T. P. Soong for analysis.

Table 1 Collection information for *Aucuba* species

Species name	Locality	Voucher
Chinese aucuba*Aucuba chinensis*	Dongguan City, Guangdong Province, China	Y. TongTY23102301
Emeishan aucuba*A. chinensis* (*A. omeiensis*)	Emeishan City, Sichuan Province, China	Z. Y. Lin, *et al.*LLFZ20240318002-3

Species name	Locality	Voucher
Dense-flowered aucuba*A. confertiflora*	Baise City, Guangxi Zhuang Autonomous Region, China	Y. TongTY20081412
Loquat-leaved aucuba*A. eriobotryfolia*	Mang City, Yunnan Province, China	Z. C. Fan, *et al.*LLF20240717001-1
Japanese aucuba*A. japonica*	Nanjing City, Jiangsu Province, China	Z. Y. Lin, *et al.*LLFZ20240322001-1
Variegated Japanese aucuba*A. japonica* var. *variegata*	Guilin City, Guangxi Zhuang Autonomous Region, China	Z. C. Fan, *et al.*LLF20240724001
White-spotted-leaved aucuba*A. albopunctifolia*	Nanchuan District, Chongqing City, China	Z. Y. Lin, *et al.*LLFZ20240322001-1
Narrow-spotted-leaved aucuba*A. albopunctifolia* var. *angustula*	Nanchuan District, Chongqing City, China	Z. Y. Lin, *et al.*LLFZ20240321002-4
Obcordate-leaved aucuba*A. obcordata*	Qingyuan City, Guangdong Province, China	X. E. YeQYLZ13
Few-flowered aucuba*A.filicauda* var. *pauciflora*	Fuzhou City, Jiangxi Province, China	Z. C. Fan & Y. Q. LanLF20241017005
Thick-stalked aucuba*A. robusta*	Laibin City, Guangxi Zhuang Autonomous Region, China	Y. TongTY20080713
Fine-toothed aucuba*A. chlorascens*	Lincang City, Yunnan Province, China	Z. C. Fan, *et al.*LLF20240714001-2
Himalayan aucuba*A. himalaica*	Baoshan City, Yunnan Province, China	Y. J. GuoGYJ202103025001
Densely hairy aucuba	Shizhu Tujia Autonomous County, Chongqing City, China	Z. Y. Lin & S. Y. Zhang
A. himalaica var. *pilossima*	Shizhu Tujia Autonomous County,Chongqing City, China	LZ20240322008
Oblanceolate-leaved Aucuba*A. himalaica* var. *oblanceolata*	Emeishan City, Sichuan Province, ChinaEmeishan City, Sichuan Province, China	Z. Y. Lin et al.LLFZ20240318001-9

Narrow-leaved Aucuba*A. chinensis* var. *angusta*	Emeishan City, Sichuan Province, ChinaEmeishan City, Sichuan Province, China	Z. Y. Lin et al.LLFZ20240318001-8
Long-leaved Aucuba*A. himalaica* var. *dolichophylla*	Beibei District, Chongqing City, ChinaBeibei District, Chongqing City, China	J. LiLJ23022201
Slender-tailed Aucuba*A. filicauda*	Guilin City, Guangxi Zhuang Autonomous Region, ChinaGuilin City, Guangxi Zhuang Autonomous Region, China	Z. C. Fan et al.LLF20240723001-7
Concave-veined Aucuba*A. filicauda* (*A. cavinervis*)	Wenshan City, Yunnan Province, ChinaWenshan City, Yunnan Province, China	Y. TongTY23022509
Large-flowered Aucuba*A. chinensis* (*A. grandiflora*)	Hanoi, VietnamHà Nội, Vietham	Y. TongTY20231228

2 Methods

2.1 Preparation and observation of leaf epidermal slides

Mature leaves were collected from the plants, and small pieces of approximately 1 cm × 1 cm were cut from the middle portion of each leaf. The pieces were placed in distilled water and heated in a 90 °C water bath for about 30 min, then soaked in cold water for 10 min. They were then transferred to a 5% NaClO solution and left in the dark in a fume hood for several hours, depending on leaf texture. After the material had decolorized, it was rinsed thoroughly with clean water. The upper and lower epidermis were separately peeled off and placed on slides, stained with 1% safranin for 15 min, rinsed, and mounted with drops of distilled water to prepare temporary water-mounted slides. The prepared slides were observed and photographed under an OLYMPUS CX23 light microscope, and measurements were made using kopa capture and Image J FIJI software. Results are expressed as "mean ± standard error."

2.2 Observation and photography with scanning electron microscopy

Leaf samples of approximately 1 cm × 0.5 cm were cut, and the leaf surface was gently brushed with a paintbrush dipped in 75% ethanol. After natural drying, the upper and lower epidermis were separately affixed to specimen stubs bearing double-sided conductive adhesive tape. After platinum coating of the

leaf samples under vacuum using a Mini Coater ion sputter coater, they were observed and photographed with a JSM-IT210 scanning electron microscope. The terminology used follows Dilcher (1974), Fahn (1982), and Barthlott (1998).

2.3 Data processing

IBM SPSS Statistics 27.0 was used to standardize quantitative character data, including epidermal cell length and width, stomatal length and width, stomatal length-to-width ratio, stomatal index, and stomatal density, together with assigned qualitative character data, including upper epidermal cuticular pattern, upper epidermal anticlinal wall pattern, lower epidermal anticlinal wall pattern, stomatal shape, relative position of the outer ledge and guard cells, inner margin of the outer ledge, distribution of non-glandular hairs, hair-cover density of lower epidermal cells, and hair-cover density along the leaf axis on the lower epidermis. The standardized data for the above 18 taxonomic characters were then imported into Origin 2024 for principal component analysis, and systematic cluster analysis was performed using the UPGMA method based on Euclidean distance.

3 Results and Analysis

3.1 Morphological characteristics of the leaf epidermis

The micromorphological characteristics of the leaf epidermis of 10 species and 7 varieties of Chinese plants of the genus *Aucuba*, as well as the corresponding data, are shown in Tables 2 and 3.

Table 2. Micro-morphological characteristics of leaf epidermis of genus *Aucuba* (qualitative traits)

Taxon	Adaxial epidermis—Cuticle	Adaxial epidermis—Anticlinal wall	Abaxial epidermis—Anticlinal wall	Abaxial epidermis—Non-glandular trichomes distribution	Abaxial epidermis—Vein trichomes density	Abaxial epidermis—Epidermis trichomes density	Abaxial epidermis—Stomata—Inner margin of outer rim	Abaxial epidermis—Stomata—Relative to the guard cell	Abaxial epidermis—Stomata—Shape
Peach-leaf aucuba	Papillae	Sinuous	Sinuolate	None	None	None	Nearly entire	Raised	Nearly round

Taxon	Adaxial epidermis—Cuticle	Adaxial epidermis—Anticlinal wall	Abaxial epidermis—Anticlinal wall	Abaxial epidermis—Non-glandular trichomes distribution	Abaxial epidermis—Vein trichomes density	Abaxial epidermis—Epidermis trichomes density	Abaxial epidermis—Stomata—Inner margin of outer rim	Abaxial epidermis—Stomata—Relative to the guard cell	Abaxial epidermis—Stomata—Shape
Aucuba chinensis	Papillae	Sinuous	Sinuolate	None	None	None	Straight	Raised	Nearly round
Emei peach-leaf aucuba	Papillae	Sinuous	Sinuolate	None	None	None	Nearly entire	Raised	Nearly round
A. chinensis (*A. omeiensis*)	Papillae	Sinuous	Sinuolate	None	None	None	Straight	Raised	Nearly round
Dense-flowered aucuba	Papillae	Sinuous	Sinuous	None	None	None	Nearly entire	Raised	Nearly round
A. confertiflora	Papillae	Sinuous	Sinuous	None	None	None	Straight	Raised	Nearly round
Loquat-leaf aucuba	Papillae	Sinuous	Sinuous	None	None	None	Nearly entire	Raised	Nearly round
A. eriobotryifolia	Papillae	Sinuous	Sinuous	None	None	None	Straight	Raised	Nearly round

Taxon	Adaxial epidermis—Cuticle	Adaxial epidermis—Anticlinal wall	Abaxial epidermis—Anticlinal wall	Abaxial epidermis—Non-glandular trichomes distribution	Abaxial epidermis—Vein trichomes density	Abaxial epidermis—Epidermis trichomes density	Abaxial epidermis—Stomata—Inner margin of outer rim	Abaxial epidermis—Stomata—Relative to the guard cell	Abaxial epidermis—Stomata—Shape
Japanese aucuba	Nearly smooth	Sinuate	Sinuous	None	None	None	Sinuous	Raised	Subround
A. japonica	Absent	Sinuate	Sinuous	None	None	None	Sinuous	Raised	Subround
Variegated Japanese aucuba	Nearly smooth	Sinuous	Sinuous	None	None	None	Sinuous	Raised	Subround
A. japonica var. *variegata*	Absent	Sinuous	Sinuous	None	None	None	Sinuous	Raised	Subround
Spotted-leaf aucuba	Nearly smooth	Sinuate	Sinuate	None	None	None	Nearly entire	Level	Subround
A. albopunctifolia	Absent	Sinuate	Sinuate	None	None	None	Straight	Level	Subround
Narrow-spotted-leaf aucuba	Nearly smooth	Sinuate	Sinuate	None	None	None	Nearly entire	Level	Subround

Taxon	Adaxial epidermis—Cuticle	Adaxial epidermis—Anticlinal wall	Abaxial epidermis—Anticlinal wall	Abaxial epidermis—Non-glandular trichomes distribution	Abaxial epidermis—Vein trichomes density	Abaxial epidermis—Epidermis trichomes density	Abaxial epidermis—Stomata—Inner margin of outer rim	Abaxial epidermis—Stomata—Relative to the guard cell	Abaxial epidermis—Stomata—Shape
A. albopunctifolia var. *angustula*	Absent	Sinuate	Sinuate	None	None	None	Straight	Level	Subround
Obcordate-leaf aucuba	Nearly smooth	Sinuous	Sinuous	None	None	None	Nearly entire	Level	Subround
A. obcordata	Absent	Sinuous	Sinuous	None	None	None	Straight	Level	Subround
Few-flowered peach-leaf aucuba	Nearly smooth	Sinuous	Sinuous	Veins	Present	None	Nearly entire	Level	Nearly round
A. filicauda var. *pauciflora*	Absent	Sinuous	Sinuous	Veins	Present	None	Straight	Level	Nearly round

Thick-stalked aucuba	nearly smooth	deeply sinuate	sinuous	leaf veins	present	none	sinuous	level	oval
A. robusta	Absent	Sinuate	Sinuous	Vein	Have	None	Sinuous	Level	Oval
Fine-toothed aucuba	nearly smooth	deeply sinuate	sinuous	leaf veins	present	none	nearly entire	level	sub-round
A. chlorascens	Absent	Sinuate	Sinuous	Vein	Have	None	Straight	Level	Sub-round
Himalayan aucuba	nearly smooth	deeply sinuate	deeply sinuate	veins and epidermis	dense	dense	sinuous	raised	sub-round
A. himalaica	Absent	Sinuate	Sinuate	Vein and epidermis	More	More	Sinuous	Rise	Sub-round
Densely hairy aucuba	nearly smooth	deeply sinuate	deeply sinuate	veins and epidermis	relatively many	dense	nearly entire	level	sub-round
A. himalaica var. *pilossima*	Absent	Sinuate	Sinuate	Vein and epidermis	Many	More	Straight	Level	Sub-round
Oblance-leaved aucuba	nearly smooth	sinuous	sinuous	veins and epidermis	dense	dense	sinuous	raised	sub-round
A. himalaica var. *oblanceolata*	Absent	Sinuous	Sinuous	Vein and epidermis	More	More	Sinuous	Rise	Sub-round

Narrow-leaved aucuba	nearly smooth	sinuous	deeply sinuate	veins and epidermis	dense	dense	sinuous	raised	sub-round
A. chinensis var. *angusta*	Absent	Sinuous	Sinuate	Vein and epidermis	More	More	Sinuous	Rise	Sub-round
Long-leaved aucuba	nearly smooth	deeply sinuate	deeply sinuate	none	none	none	nearly entire	raised	sub-round
A. himalaica var. *dolichophylla*	Absent	Sinuate	Sinuate	None	None	None	Straight	Rise	Sub-round
Thread-tailed aucuba	nearly smooth	deeply sinuate	deeply sinuate	veins and epidermis	dense	present	nearly entire	raised	sub-round
A. filicauda	Absent	Sinuate	Sinuate	Vein and epidermis	More	Have	Straight	Rise	Sub-round
Raised-vein aucuba	nearly smooth	deeply sinuate	sinuous	none	none	none	nearly entire	level	oval
A. filicauda (*A. cavinervis*)	Absent	Sinuate	Sinuous	None	None	None	Straight	Level	Oval
Large-flowered aucuba	nearly smooth	deeply sinuate	deeply sinuate	none	none	none	sinuous	level	sub-round

A. chinensis (*A. grandiflora*)	Absent	Sinuate	Sinuate	None	None	None	Sinuous	Level	Sub-round

Table 3. Micro-morphological characteristics of the leaf epidermis of genus *Aucuba* (quantitative traits)

Taxon	Adaxial epidermis — Cell length (μm)	Adaxial epidermis — Cell width (μm)	Abaxial epidermis — Cell length (μm)	Abaxial epidermis — Cell width (μm)	Abaxial epidermis — Stomata length (μm)	Abaxial epidermis — Stomata width (μm)	Abaxial epidermis — Stomatal index (%)	Abaxial epidermis — Stomatal density (ind. · mm^{-2})

Taxon	Adaxial epidermis — Cell length (μm)	Adaxial epidermis — Cell width (μm)	Abaxial epidermis — Cell length (μm)	Abaxial epidermis — Cell width (μm)	Abaxial epidermis — Stomata length (μm)	Abaxial epidermis — Stomata width (μm)	Abaxial epidermis — Stomatal index (%)	Abaxial epidermis — Stomatal density (ind. · mm^{-2})
Peach-leaved aucuba *Aucuba chinensis*	69.906$±5.787|47.729±6.215|61.509±6.910|41.078±6.762|35.605±1.710|32.830±1.722|13.208±2.380|137 *leavedaucuba* < *br* > ∗*Aucubachinensis*∗ (∗*A.omeiensis*∗)|73.846±9.709|49.847±8.457|59.469±6.396|38.187±5.133|39.989±2.608|36.944±2.728|1 *floweredaucuba* < *br* > ∗*A.confertiflora*∗ |64.543±7.638|41.299±4.952|57.120±7.331|34.244±4.520|34.450±1.848|31.739±1.179|13.391±1.312|163 *leavedaucuba* < *br* > ∗*A.eriobotryifolia*∗ |79.482±10.509|54.692±7.790|71.369±12.885|47.874±9.205|36.659±2.706|33.126±3.030|12.894±2.464|9 *br* > ∗*A.japonica*∗ |74.746±9.156|50.959±5.094|58.747±7.183|38.231±6.296|34.365±1.707|28.797±2.178|11.159±0.681|166 *br* > ∗*A.japonica*∗ *var.* ∗ *variegata*∗ |79.312±8.972|57.008±4.345|72.026±9.761|49.266±6.682|35.444±1.922|29.904±2.164|10.233±2.435|87.5 *leavedaucuba* < *br* > ∗*A.albopunctifolia*∗ |116.186±17.094|85.358±12.852|68.231±9.454|43.953±6.383|37.676±1.844|30.019±2.449|16.691±1.879| *leavedaucuba* < *br* > ∗*A.albopunctifolia*∗ *var.* ∗ *angustula*∗ |96.150±13.159|72.045±10.806|67.473±12.416|46.210±9.117|37.287±3.216|29.636±2.270|14.846±1.369| *leavedaucuba* < *br* > ∗*A.obcordata*∗ |76.769±5.979|56.712±7.914|52.802±7.344|36.006±6.783|32.146±2.277|26.673±1.650|14.208±1.913|196 *floweredpeach*— *leavedaucuba* <								

Thick-stalked au-cuba *A. robusta*	82.472$±9.670|53.792±5.578|67.422±7.459|46.427±8.736|34.537±1.841|26.568±1.901|12.236±0.758|166 *toothedaucuba* < *br* > ∗*A.chlorascens*∗ |71.618±9.728|47.677±5.490|50.941±9.280|31.812±4.414|31.520±1.948|24.944±2.199|15.209±1.337|231 *br* > ∗*A.himalaica*∗ |76.224±13.309|50.009±9.760|61.893±11.266|41.838±8.502|31.041±2.076|25.608±2.169|9.882±1.932|11 *br* > ∗*A.himalaica*∗ *var.* ∗ *pilossima*∗ |91.905±8.806|63.928±7.888|62.961±10.476|40.031±6.630|32.074±2.175|27.250±2.281|10.500±1.469|15 *leavedaucuba* < *br* > ∗*A.himalaica*∗ *var.* ∗ *oblanceolata*∗ |92.833±15.185|63.508±8.107|56.569±9.199|35.404±5.896|32.819±2.374|28.292±2.944|9.176±1.485|109 *leavedaucuba* < *br* > ∗*A.chinensis*∗ *var.* ∗ *angusta*∗ |87.015±10.149|66.915±6.646|53.788±6.532|33.994±4.903|35.428±1.529|29.495±2.796|9.251±1.668|135 *leavedaucuba* < *br* > ∗*A.himalaica*∗ *var.* ∗ *dolichophylla*∗ |101.509±16.767|72.588±13.777|74.022±12.924|50.745±11.722|38.820±3.239|32.359±3.703|12.994±2.49 *tailedaucuba* < *br* > ∗*A.filicauda*∗ |78.440±11.357|57.263±8.567|63.533±6.767|45.551±6.434|37.000±3.311|29.333±2.518|14.667±3.180|15 *veinedaucuba* < *br* > ∗*A.filicauda*∗ (∗*A.cavinervis*∗)|66.782±7.216|48.118±5.917|53.184±7.889|35.016±5.186|35.233±2.350|26.473±1.554| *floweredaucuba* < *br* > ∗*A.chinensis*∗ (∗*A.grandiflora*∗)|97.889±12.281|72.370±7.861|79.220±11.358|57.602±6.416|40.114±1.685|31.507±1.6

3.2 Characteristics of Leaf Epidermal Cells

3.2.1 Leaf epidermal morphology, size, cuticle type, and anticlinal-wall pattern

As shown in Fig. 1 and Fig. 2, the leaf epidermal cells of Chinese *Aucuba* species are all irregular in shape. The adaxial epidermal cells measure (64.543 ∼ 116.186) μm × (41.299 ∼ 85.358) μm; among them, those of variegated *Aucuba* are the largest, while those of fine-toothed *Aucuba* are the smallest. The abaxial epidermal cells measure (50.941 ∼ 79.220) μm × (31.812 ∼ 57.602) μm; among them, the abaxial cells of large-flowered *Aucuba* are the largest, and those of fine-toothed *Aucuba* are the smallest. Overall, in Chinese *Aucuba*, the adaxial epidermal cells are larger than the abaxial epidermal cells. According to the degree of undulation of the anticlinal walls, they can be roughly divided into shallowly sinuate, sinuate, and deeply sinuate types. The anticlinal walls are closely interlocking, with no intercellular spaces. On the adaxial surface, only in the central surface of the epidermal cells of *Aucuba japonica*, Emei *Aucuba*, densely flowered *Aucuba*, and loquat-leaved *Aucuba* are there spherical projections, with thick cell walls, i.e., papillate projections.

3.2.2 Wax ornamentation of the leaf epidermis

Barthlott et al. (1998) conducted scanning electron microscopy analyses of the leaves of at least 13,000 species of seed plants and proposed 23 wax types. This study found that the epidermal cells of most Chinese *Aucuba* species possess waxes (Fig. 3–Fig. 6). Seven types of wax ornamentation occur on the adaxial surface: nearly smooth layers, plates, platelets, threads, crusts, a syntopism of plates and threads, and a syntopism of plates and platelets. Four types of wax ornamentation occur on the abaxial surface: plates, threads, a syntopism of plates and threads, and a syntopism of platelets and threads. Overall, plate-like and thread-like waxes predominate.

A. *Aucuba chinensis*; B. *A. omeiensis*; C. *A. confertiflora*; D. *A. eriobotryifolia*; E. *A. japonica*; F. *A. japonica* var. *variegata*; G. *A. albopunctifolia*; H. *A. albopunctifolia* var. *angustula*; I. *A. obcordata*; J. *A. filicauda* var. *pauciflora*. 1. Adaxial surface; 2. Abaxial surface.

A. *A. chinensis*; B. *A. omeiensis*; C. *A. confertiflora*; D. *A. eriobotryifolia*; E. *A. japonica*; F. *A. japonica* var. *variegata*; G. *A. albopunctifolia*; H. *A. albopunctifolia* var. *angustula*; I. *A. obcordata*; J. *A. filicauda* var. *pauciflora*. 1. Adaxial surface; 2. Abaxial surface.

Fig. 1 Leaf epidermal characters of *Aucuba* under light microscopy (I)

K. Thick-stalked *Aucuba*; L. Fine-toothed *Aucuba*; M. Himalayan *Aucuba*; N. Densely hairy *Aucuba*; O. Oblanceolate-leaved *Aucuba*; P. Narrow-leaved *Aucuba*; Q. Long-leaved *Aucuba*; R. Slender-tailed *Aucuba*; S. Impressed-veined *Aucuba*; T. Large-flowered *Aucuba*. 1. Adaxial surface; 2. Abaxial surface.

K. *A. robusta*; L. *A. chlorascens*; M. *A. himalaica*; N. *A. himalaica* var. *pilossima*; O. *A. himalaica* var. *oblanceolata*; P. *A. chinensis* var. *angusta*; Q. *A. himalaica* var. *dolichophylla*; R. *A. filicauda*; S. *A. cavinervis*; T. *A. grandiflora*. 1. Adaxial surface; 2. Abaxial surface.

Fig. 2 Leaf epidermal characteristics of *Aucuba* under light microscopy (II)

A. *Aucuba chinensis*; **B.** *A. omeiensis*; **C.** *A. confertiflora*; **D.** *A. eriobotryifolia*; **E.** *A. japonica*. **1.** Adaxial surface; **2, 3.** Abaxial surface.

Fig. 3 Leaf epidermal characteristics of *Aucuba* under scanning electron microscopy (SEM) (I)

F. *A. japonica* var. *variegata*; G. *A. albopunctifolia*; H. *A. albopunctifolia* var. *angustula*; I. *A. obcordata*; J. *A. filicauda* var. *pauciflora*. 1. Adaxial surface; 2, 3. Abaxial surface.

Fig. 4. Leaf epidermal characteristics of *Aucuba* under SEM (II)

K. *A. robusta*; **L.** *A. chlorascens*; **M.** *A. himalaica*; **N.** *A. himalaica* var. *pilossima*; **O.** *A. himalaica* var. *oblanceolata*.
1. Adaxial surface; **2, 3.** Abaxial surface.

Fig. 5 Characteristics of the leaf epidermis in *Aucuba* under SEM (III)

P. *Aucuba chinensis* var. *angusta*; Q. *A. himalaica* var. *dolichophylla*; R. *A. filicauda*; S. *A. cavinervis*; T. *A. grandiflora*. 1. Adaxial surface; 2, 3. Abaxial surface.

P. *A. chinensis* var. *angusta*; Q. *A. himalaica* var. *dolichophylla*; R. *A. filicauda*; S. *A. cavinervis*; T. *A. grandiflora*. 1. Adaxial surface; 2, 3. Abaxial surface.

Fig. 6 Leaf epidermal characters of *Aucuba* under scanning electron microscopy (SEM) (IV)

3.3 Types and Characteristics of Stomatal Apparatus

The types of stomatal apparatus in Chinese *Aucuba* are consistent across the genus, and their characteristics are shown in Fig. 7. The stomata are all distributed on the abaxial surface of the leaf blade and are hypostomatic, with a random distribution. The guard cells are kidney-shaped; no "T-shaped" thickening was observed at the two poles; there are no subsidiary cells. Some epidermal cells adjacent to the guard cells are positioned beneath the guard cells and partially surround them. The cuticular layer on the surface of the guard cells is nearly smooth. Except for peach-leaved *Aucuba*, Emei peach-leaved *Aucuba*, dense-flowered peach-leaved *Aucuba*, loquat-leaved *Aucuba*, sunken-veined peach-leaved *Aucuba*, and oblanceolate-leaved *Aucuba*, all the others possess wax, and the wax ornamentation is mainly flake-like. The stomatal shape varies from nearly circular to elliptical. The inner margin of the outer stomatal ledge is nearly entire or undulate, and the outer stomatal ledge is raised

above, or level with, the guard cells. In Chinese *Aucuba*, stomatal size is (29.550–40.114) μm × (24.893–36.944) μm; among them, the stomata of narrow-spotted-leaved *Aucuba* are the largest, while those of fine-toothed peach-leaved *Aucuba* are the smallest. The stomatal index ranges from 9.176% to 16.691%, being highest in spotted-leaved *Aucuba* and lowest in oblanceolate-leaved *Aucuba*. Stomatal density ranges from 87.536–271.187 ind. · mm^{-2}, being highest in few-flowered peach-leaved *Aucuba* and lowest in variegated aoki.

A. Abaxial surface; B. Transverse section; S_1. Stoma sectioned perpendicular to the pore; S_2. Stoma sectioned parallel to the pore.

A. Abaxial surface; B. Transverse section; S_1. stoma sectioned perpendicular to the pore; S_2. Stoma sectioned parallel to the pore.

Fig. 7 Schematic diagram of leaf anatomical characteristics of *Aucuba*

Fig. 7 Schematic diagram of leaf anatomical characteristics of *Aucuba*

3.4 Trichomes

In Chinese *Aucuba*, trichomes on mature leaves occur only on the abaxial surface, and all are non-glandular trichomes, specifically unicellular trichomes; they are distributed both along the leaf veins and on the epidermal surface. On this basis, they can be divided into three categories: (1) species without trichomes on the abaxial surface, namely peach-leaved *Aucuba*, Emei peach-leaved *Aucuba*, dense-flowered peach-leaved *Aucuba*, loquat-leaved *Aucuba*, large-flowered peach-leaved *Aucuba*, aoki, variegated aoki, spotted-leaved *Aucuba*, narrow-spotted-leaved *Aucuba*, obcordate-leaved *Aucuba*, and long-leaved *Aucuba*; (2) species in which trichomes on the abaxial surface occur only along the veins, namely sunken-veined peach-leaved *Aucuba*, fine-toothed peach-leaved *Aucuba*, few-flowered peach-leaved *Aucuba*, and thick-peduncled peach-leaved *Aucuba*; and (3) species in which trichomes on the abaxial surface occur over the entire leaf surface, namely Himalayan *Aucuba*, oblanceolate-leaved *Aucuba*, narrow-leaved peach-leaved *Aucuba*, densely hairy peach-leaved *Aucuba*, and slender-tailed peach-leaved *Aucuba*.

3.5 Principal Component Analysis

Principal component analysis (Table 4) showed that there were four principal components with eigenvalues greater than 1, with contribution rates of 30.243%, 28.112%, 18.833%, and 8.128%, respectively. The cumulative contribution rate reached 85.316%, indicating that these components already represented most of the information in the original characters; therefore, the first four principal components were selected as the newly selected principal components. The first principal component contained information from eight characters: stomatal width, stomatal length-to-width ratio, stomatal shape, cuticular-layer pattern of the upper epidermis, anticlinal-wall pattern of the upper epidermis, anticlinal-wall pattern of the lower epidermis, distribution of non-glandular trichomes, and

trichome density along the veins of the lower epidermis. The second principal component contained information from five characters: upper epidermal cell length, upper epidermal cell width, lower epidermal cell length, lower epidermal cell width, and stomatal length. The third principal component contained information from four characters: stomatal index, stomatal density, relative position of the outer stomatal ledge and guard cells, and trichome density on lower epidermal cells. The fourth principal component contained information from one character: the inner margin of the outer stomatal ledge. From the factor loadings of the original 18 characters on the four newly selected principal components, it can be seen that all 18 micromorphological characters of the leaf epidermis have relatively high loadings on the first four principal components, indicating that the above characters have certain taxonomic significance for species delimitation in Chinese *Aucuba*.

From the loadings of the 18 leaf epidermal micromorphological traits (the index items in Table 4) on the principal components, it can be seen that all 18 traits have relatively high loadings on the first four principal components. Therefore, a two-dimensional scatter plot was constructed based on the first two principal components (Fig. 8). The plot shows that all individuals are distributed in an intermingled pattern. Although they cannot be effectively distinguished at the species level, the 10 species and 7 varieties of this genus can be clearly divided into two major groups, namely Group I and Group II.

Table 4. Loading matrix based on principal component analysis of 18 leaf epidermal micromorphological traits

Index	PC1	PC2	PC3	PC4
Adaxial epidermis cell length	0.442	0.716	0.124	0.419
Adaxial epidermis cell width	0.442	0.706	0.074	0.436
Abaxial epidermis cell length	0.059	0.908	0.202	-0.145
Abaxial epidermis cell width	0.118	0.882	0.144	-0.208
Stomatal length	-0.258	0.818	0.102	-0.017
Stomatal width	-0.706	0.535	0.342	0.093

Index	PC1	PC2	PC3	PC4
Stomatal length-width ratio	0.784	0.192	-0.451	-0.207
Stomatal shape	0.810	0.085	-0.214	-0.354
Stomatal index	-0.293	0.380	-0.701	0.347
Stomatal density	0.057	-0.568	-0.777	0.108
Cuticle patterns of adaxial epidermis	-0.905	-0.007	0.202	0.045
Anticlinal wall patterns of adaxial epidermis	0.646	0.409	-0.273	-0.296
Anticlinal wall patterns of abaxial epidermis	0.733	0.356	0.113	0.355
Inner margin of outer rim	0.431	0.019	0.523	-0.552
Relative position of outer rim and guard cells	-0.387	-0.038	0.772	-0.157
Non-glandular trichome distribu-tion	0.626	-0.548	0.372	0.209
Abaxial epidermis cell trichome density	0.592	-0.497	0.483	0.212

Index	PC1	PC2	PC3	PC4
Abaxial epidermal cell trichome density	0.510	-0.371	0.682	0.298

Abaxial epidermis leaf vein trichomes density				
Eigenvalue	5.444	5.060	3.390	1.463
Cumulative contribution (%)	30.243	58.355	77.188	85.316

Note: Underlined numbers indicate the characteristic component with a higher load in the principal component.

Note: Underlined numbers indicate the characteristic component with a higher load in the principal component.

Fig. 8 Scatter plot of principal component analysis of leaf epidermal micromorphological characteristics of *Aucuba*

Fig. 8 Scatter plot of principal component analysis on leaf epidermal micromorphological characteristics of *Aucuba*

3.6 Cluster analysis

Cluster analysis was conducted on 18 taxonomic characters, including the length and width of leaf epidermal cells and the length and width of stomata. The constructed clustering tree is shown in Fig. 9. At a Euclidean distance of 6.5, the plants of this genus formed two major branches, Group I and Group II. The main differences in leaf epidermal characteristics between the two major branches are as follows: Group I, consisting of peach-leaved *Aucuba*, Emei peach-leaved *Aucuba*, dense-flowered peach-leaved *Aucuba*, and loquat-leaved *Aucuba*, is characterized by papillate protrusions in the cuticle of the adaxial leaf epidermis, anticlinal wall patterns mostly shallowly undulate or undulate, and stomata nearly circular in shape; Group II, consisting of Japanese aucuba, variegated Japanese aucuba, large-flowered peach-leaved *Aucuba*, spotted-leaved *Aucuba*, narrow spotted-leaved *Aucuba*, Himalayan *Aucuba*, densely hairy peach-leaved *Aucuba*, oblanceolate-leaved *Aucuba*, filiform-tailed peach-leaved *Aucuba*, long-leaved *Aucuba*, narrow-leaved peach-leaved *Aucuba*, thick-stalked peach-leaved *Aucuba*, fine-toothed peach-leaved *Aucuba*, few-flowered peach-leaved *Aucuba*, obcordate-leaved *Aucuba*, and sunken-veined peach-leaved *Aucuba*, is characterized by relatively abundant waxy deposits on the cuticle of the adaxial leaf epidermis, anticlinal wall patterns mostly undulate or deeply undulate, and stomata mostly subcircular or elliptical in shape.

Visible terminal labels in Fig. 9 (top to bottom):

- Aucuba, *Aucuba chinensis*
- Emei aucuba, *A. chinensis* (*A. omeiensis*)
- Dense-flowered aucuba, *A. confertiflora*
- Loquat-leaved aucuba, *A. eriobotryifolia*
- Large-flowered aucuba, *A. chinensis* (*A. grandiflora*)
- Narrow spotted-leaved aucuba, *A. albopunctifolia* var. *angustula*
- Spotted-leaved aucuba, *A. albopunctifolia*
- Long-leaved aucuba, *A. himalaica* var. *dolichophylla*
- Japanese aucuba, *A. japonica*
- Variegated Japanese aucuba, *A. japonica* var. *variegata*
- Stout-stalked aucuba, *A. robusta*
- Densely hairy aucuba, *A. himalaica* var. *pilossima*
- Oblanceolate-leaved aucuba, *A. himalaica* var. *oblanceolata*
- Narrow-leaved aucuba, *A. chinensis* var. *angusta*
- Himalayan aucuba, *A. himalaica*
- Slender-tailed aucuba, *A. filicauda*
- Obcordate-leaved aucuba, *A. obcordata*
- Few-flowered aucuba, *A. filicauda* var. *pauciflora*
- Concave-veined aucuba, *A. filicauda* (*A. cavinervis*)
- Fine-toothed aucuba, *A. chlorascens*

Fig. 9 Dendrogram of *Aucuba* based on 18 leaf epidermis micro-morphological characteristics

4 Discussion and Conclusions

4.1 Leaf epidermal morphological characteristics of Chinese *Aucuba*

This study reports the leaf epidermal micro-morphological characteristics of 10 species and 7 varieties of *Aucuba* in China. The results show that, although plants of this genus differ in anticlinal wall pattern, cuticular pattern, wax ornamentation, stomatal-apparatus shape, and other aspects, they share the following characteristics: the epidermal cells are all irregular in shape; stomatal apparatuses are distributed only on the abaxial surface of the leaf blade and are randomly distributed; the guard cells are kidney-shaped; no "T-shaped" thickening is found at either pole, and subsidiary cells are absent. These features show a certain stability and support the view that Chinese *Aucuba* is a natural taxonomic group.

Wax is a layer of hydrophobic and lipophilic compounds on the plant surface. As an interface structure mediating interactions between plants and the external environment, it has basic functions such as reducing water loss and resisting external stress (Samuels et al., 2008), and it is ecologically important for interactions between plants and the environment (Barthlott et al., 1998). Frölich & Barthlott (1988) considered that wax ornamentation of epidermal cells could serve as a taxonomic character for certain plants, and found that different wax types could serve as taxonomic characters for some monocotyledons. For exam-

ple, Hennig et al. (1994) discussed the phylogenetic relationships among plants of Magnoliidae, Ranunculidae, and Hamamelididae on the basis of wax types. However, modern studies indicate that the chemical composition, structure, and coverage density of plant epidermal wax may change with alterations in the external environment or in intrinsic mechanisms (Baker, 1974; Ni Yu et al., 2014). Therefore, although the wax patterns of Chinese *Aucuba* are diverse, further observation and confirmation are still needed before leaf epidermal wax can be applied to distinguish species of *Aucuba*.

4.2 Stomatal-apparatus characteristics of Chinese *Aucuba*

As early as 1957, Metcalfe & Chalk (1957) had systematically applied stomatal-apparatus characters to plant classification. Stomatal apparatuses are genetically stable and are among the important characters in plant taxonomy. Metcalfe & Chalk (1965) were the first to report that the stomatal type of *Aucuba* is ranunculaceous, i.e., irregular; later scholars, such as Takhtajan (2009) and Xiang (Kubitzki, 2016), also supported the view that the stomatal-apparatus type of *Aucuba* plants is irregular. However, Peng Chuangye et al. (2008) and Song Liangke et al. (2007) reported that the stomatal-apparatus types of *Aucuba chinensis* and *Aucuba omeiensis* are cyclocytic, i.e., whorled. The results of the present study differ from those obtained by most scholars. On the one hand, this may be because previous research methods involved only transverse-section observations of the leaf epidermis, affecting the accurate judgment of the observations; on the other hand, differences among researchers in the criteria used to delimit stomatal types can also lead to inconsistent determinations. In this study, fresh leaves and specimens of all plants in the genus were examined by transverse and longitudinal observations of the leaf epidermis, and the Dilcher system (1974) was adopted. Stomatal types were delimited according to the number and position of subsidiary cells, among other criteria. It was found that the epidermal cells surrounding the guard cells of this stomatal apparatus are not in the same plane as the guard cells, but rather

located below the guard cells, encircling the guard cells. On this basis, it was inferred that this type of stoma may represent a new mode of stomatal development. Therefore, the stomatal apparatus type in *Aucuba* is considered to differ from previous understanding and to represent a new stomatal apparatus type.

In Chinese *Aucuba*, stomata occur only on the abaxial surface, with a mean stomatal index of 12.751% and a mean stomatal density of 147.136 ind. $\cdot$ mm^2. They are slightly raised and attached to 5–7 small epidermal cells, with no subsidiary cells. Some of the epidermal cells adjacent to the guard cells are located beneath the guard cells, and some surround the guard cells; the anticlinal walls of the epidermal cells beneath the guard cells are straight or shallowly undulate. This phenomenon can be distinguished only under the light microscope and cannot be observed under the scanning electron microscope. This special stomatal apparatus type was first discovered by Baas (1969) in a study of the leaf epidermis of the chestnut-leaved plane tree (*Platanus kerrii* Gagnep.), and it was

considered that this stomatal apparatus did not conform to any stomatal pattern proposed by Metcalfe & Chalk (1957). Baas (1969) speculated that, if this plant were subsequently fossilized, only typical irregular stomata would be observed, and that this might result from the absence or incomplete development of the cuticular flanges and cuticular furrows of the epidermal-cell anticlinal walls beneath the guard cells. By studying the individual development between guard cells and subsidiary cells, Fryns-Claessens & Cotthem (1973) proposed the Fryns-Claessens & Cotthem stomatal system, and considered that the positional relationships of the undifferentiated subsidiary cells around the stomata observed by Baas were complex: some were beneath the guard cells and some encircled the guard cells. This situation does not conform to the developmental characteristics of typical mesogenous, mesoperigenous, or perigenous stomata, making it difficult to determine their developmental type.

This stomatal pattern has 5–7 epidermal cells encircling the guard cells, and the cells near the guard cells are not differentiated compared with normal epidermal cells. Morphologically, this stomatal pattern is similar to the irregular type, but differs in that the epidermal cells around the guard cells are not in the same plane as the guard cells; rather, they are located below and encircle the guard cells. Therefore, the authors consider this to be a special irregular stomatal type. Because it was first reported from *Platanus*, we name it the Platanus-type stoma (*Platanus*-Type stomata). At present, there is no definitive conclusion regarding the developmental origin of this stomatal type. Meanwhile, whether Platanus-type stomata also occur in other angiosperms besides *Platanus* and *Aucuba*, how this type of stoma originates and develops, and whether it represents adaptive evolution to a particular ecological environment are all scientific questions worthy of further study.

4.3 Taxonomic significance of leaf epidermal micromorphology in Chinese *Aucuba*

4.3.1 Application of leaf epidermal micromorphological characters at the infrageneric grouping level Tetsuo Ohi (2018) used four chloroplast gene fragments (*rbcL*, *ndhF*, *psbA*, *matK*) and seven chloroplast intergenic spacers (*atpB-rbcL*, *trnH-psbA*, *trnT-trnL*, *trnL-trnF*, *accD-psaI*, *trnK* intron, *psbA-trnK*), combined with nuclear gene fragments such as ITS, as well as flow cytometry for ploidy determination in *Aucuba*. The study found that *Aucuba* was divided into two branches: branch 1 included the "green-flowered-tall tree" species, and branch 2 included the "red-flowered-low shrub" species. It further recommended treating the above two lineages as two subgenera of *Aucuba* —subgenus *Aucuba*, with *Aoki* as the type species, and a newly established subgenus with *Aucuba* as the type species. Li Juan et al. (2023) used chloroplast genome sequences to construct a phylogenetic tree for six species of *Aucuba*, and likewise divided *Aucuba* into two highly supported evolutionary lineages—Clade I, with green flowers and a tree habit, and Clade II, with red flowers and a shrub habit—overall similar to the results of Tetsuo Ohi. In the present

study, clustering results based on 18 leaf epidermal micromorphological characters are consistent with the molecular results, and Chinese *Aucuba* can be divided into two groups, corresponding to the green-flowered tree group and the red-flowered shrub group. The former includes peach-leaved aucuba, Emei aucuba, dense-flowered aucuba, and loquat-leaved aucuba; their shared micromorphological characters are the presence of papillate projections in the cuticle of the adaxial leaf epidermis, anticlinal wall patterns mostly shallowly undulate or undulate, and nearly circular stomata. The remaining species are assigned to the red-flowered shrub group; their shared micromorphological characters are the presence of relatively abundant wax attachments in the cuticle of the adaxial leaf epidermis, anticlinal wall patterns mostly undulate or deeply undulate, and stomata mostly subcircular or elliptic. This demonstrates that the use of leaf epidermal micromorphological characters for classification at the group level has relatively significant taxonomic significance.

4.3.2 Identification of leaf epidermal micromorphological characters at the species level *Flora of China* (Xiang & Boufford, 2005) merged large-flowered aucuba into *Aucuba*. In the present study, the adaxial cuticle of *Aucuba* has papillate projections, the anticlinal wall pattern is undulate or shallowly undulate, and the stomata are nearly circular with the outer stomatal ledges protruding.

arising from the guard cells; whereas in large-flowered *Aucuba*, the cuticle on the adaxial surface is flat, the anticlinal wall pattern is deeply sinuate, the stomata are suborbicular, and the outer stomatal ledges are level with the guard cells. Therefore, the authors tend to treat large-flowered *Aucuba* as an independent species. Wang Fazuan (1949) published a new variety, narrow-leaved *Aucuba*, and Wu Zhengyi, in *Flora Yunnanica* (Kunming Institute of Botany, Chinese Academy of Sciences, 1992), raised narrow-leaved *Aucuba*, *Aucuba angustifolia* (Rehd.) C. Y. Wu, to species rank. Judging from the present study, in narrow-leaved *Aucuba* the cuticle on the adaxial surface is flat; the anticlinal wall pattern is sinuate or deeply sinuate; the stomata are suborbicular; and trichomes are densely distributed over the entire leaf surface. Thus, the authors consider that narrow-leaved *Aucuba* should not be treated as a variety of *Aucuba chinensis*, although its systematic position still requires further investigation. At the same time, narrow-leaved *Aucuba* and oblanceolate-leaved *Aucuba* are similar in such characters as epidermal hairs, anticlinal wall pattern, and stomatal apparatus, indicating that the two are closely related; however, their relationship still needs to be determined by integrating other morphological evidence.

On the other hand, *Flora of China* (Xiang & Boufford, 2005) also merged concave-veined *Aucuba* into slender-tailed *Aucuba*. In slender-tailed *Aucuba*, the anticlinal wall pattern is deeply sinuate, the stomata are elliptic, the outer stomatal ledges protrude from the guard cells, and its trichomes are distributed over the entire leaf surface and are dense; whereas in concave-veined *Aucuba*, the anticlinal wall pattern is sinuate or deeply sinuate, the stomata are suborbic-

ular, the outer stomatal ledges are level with the guard cells, and the trichomes are distributed only along the leaf veins and are relatively sparse. The two are clearly different. Therefore, from the perspective of leaf epidermal morphology, concave-veined *Aucuba* is more appropriately treated as an independent species.

In *Flora Sichuanica* (Editorial Committee of *Flora Sichuanica*, 1981), oblanceolate-leaved *Aucuba* was treated as a variety of Himalayan *Aucuba*, whereas Qi Chengjing (2000) considered the characters of oblanceolate-leaved *Aucuba* to be stable and distinctive and therefore unsuitable for treatment as a variety of Himalayan *Aucuba*; consequently, he raised oblanceolate-leaved *Aucuba*, *A. oblanceolata* (Fang et Soong) C. J. Qi, to species rank. Judging from the results of the present study, oblanceolate-leaved *Aucuba* and Himalayan *Aucuba* are similar in leaf epidermal characters, differing only slightly in the anticlinal wall pattern of the upper epidermis. The existing observations of the leaf epidermis are insufficient to distinguish oblanceolate-leaved *Aucuba* from Himalayan *Aucuba*.

Flora Sichuanica (Editorial Committee of *Flora Sichuanica*, 1981) also treated Emei *Aucuba* [*A. chinensis* Benth. subsp. *omeiensis* (Fang) Fang et Soong] as a subspecies of *Aucuba chinensis*, and *Flora Reipublicae Popularis Sinicae* (Editorial Committee of *Flora Reipublicae Popularis Sinicae*, Chinese Academy of Sciences, 1990) continued this taxonomic treatment. *Flora Yunnanica* (Kunming Institute of Botany, Chinese Academy of Sciences, 1992) treated Emei *Aucuba* (*A. omeiensis* Fang) as an independent species, whereas *Flora of China* (Xiang & Boufford, 2005) merged Emei *Aucuba* into *Aucuba chinensis*. Li Juan et al. (2023), after constructing a phylogenetic tree, considered Emei *Aucuba* to be more closely related to dense-flowered *Aucuba*. In the micromorphological results of the leaf epidermis, *Aucuba chinensis*, Emei *Aucuba*, and dense-flowered *Aucuba* are all similar in cuticular pattern and stomatal-apparatus characters; however, dense-flowered *Aucuba* differs slightly from *Aucuba chinensis* and Emei *Aucuba* in its anticlinal wall pattern. Thus, on the clustering tree based on leaf epidermal micromorphology, *Aucuba chinensis* is more closely related to Emei *Aucuba*. These three taxa all belong to Group I and are extremely similar in leaf macromorphology and overall plant morphology; there are no obvious differences in leaf texture, leaf margin, leaf base, or leaf blade shape. These three species may represent the same taxon, but their final taxonomic treatment still awaits further study after obtaining evidence from chromosomes, palynology, genetics, and other lines of evidence.

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References:

AI TM, 2013. Medicinal Flora of China [M]. Beijing: Peking University Medical Press, 7: 279–287. [Ai Tiemin, 2013. Chinese Medicinal Flora [M]. Beijing: Peking University Medical Press, 7: 279–287.]

Angiosperm Phylogeny Group IV, 2016. An update of the Angiosperm Phy-

logeny Group classification for the orders and families of flowering plants: APG IV [J]. Botanical Journal of the Linnean Society, 181(1): 1–20.

BAAS P, 1969. Comparative anatomy of *Platanus kerrii* Gagnep. [J]. Botanical Journal of the Linnean Society, 62(4): 413–421.

BAKER E, 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera* [J]. New Phytologist, 73(5): 955–966.

BARTHLOTT W, NEINHUIS C, CUTLER D, et al., 1998. Classification and terminology of plant epicuticular waxes [J]. Botanical Journal of the Linnean Society, 126(1): 237–260.

DILCHER DL, 1974. Approaches to the identification of angiosperm leaf remains [J]. Botanical Review, 40(1): 1–157.

Editorial Board of Flora Sichuanica, 1981. Flora Sichuanica: Vol. 1 [M]. Chengdu: Sichuan People's Press: 474–475. [Editorial Board of Flora Sichuanica, 1981. Flora Sichuanica: Vol. 1 [M]. Chengdu: Sichuan People' s Press: 474–475.]

Editorial Committee of Chinese Materia Medica of the State Administration of Traditional Chinese Medicine, 1997. Chinese materia medica [M]. Shanghai: Science and Technology Press, 5: 734–737. [Editorial Committee of Chinese Materia Medica of the State Administration of Traditional Chinese Medicine, 1997. Chinese Materia Medica [M]. Shanghai: Science and Technology Press, 5: 734–737.]

FAHN A, 1982. Plant Anatomy [M]. New York: Pergamon Press: 144–164.

Editorial Committee of the Flora of China, Chinese Academy of Sciences, 1999. Flora Reipublicae Popularis Sinicae: Vol. 56 [M]. Beijing: Science Press: 37–51. [Editorial Committee of the Flora of China, Chinese Academy of Sciences, 1999. Flora Reipublicae Popularis Sinicae: Vol. 56 [M]. Beijing: Science Press: 37–51.

FRÖLICH D, BARTHLOTT W, 1988. The micromorphology of epicuticular waxes and the system of monocotyledons [J]. Tropische und subtropische Pflanzenwelt, 63: 1–135.

FRYNS-CLAESSENS E, VAN COTTHEM W, 1973. A new classification of the ontogenetic types of stomata [J]. Botanical Review, 39(1): 71–138.

GENG QQ, YANG JY, LI FS, et al., 2019. Adaptive characteristics of leaf epidermis micromorphology of *Caragana* sp. in different climates and environments in Inner Mongolia Plateau [J]. Chinese Journal of Applied and Environmental Biology, 25(2): 281–290. [Geng Qianqian, Yang Jiuyan, Li Fengshi, et al., 2019. Adaptive characteristics of leaf epidermal micromorphology of geographical replacement species of the genus *Caragana* on the Inner Mongolia Plateau to different climatic environments [J]. Chinese Journal of Applied and Environmental Biology, 25(2): 281–290.]

HENNIG S, BARTHLOTT W, MEUSEL I, et al., 1994. Micromorphology of epicuticular waxes and the systematics of Magnoliidae, Ranunculidae, and Hamamelididae [J]. Tropische und subtropische Pflanzenwelt, 90: 5–60.

HUANG Y, FAN L, HUANG J, et al., 2022. Plastome Phylogenomics of *Aucuba* (Garryaceae) [J]. Frontiers in Genetics, 13: 753719.

KUBITZKI K, 2016. The Families and Genera of Vascular Plants Volume XIV [M]. Berlin: Springer: 37.

Kunming Institute of Botany, Chinese Academy of Sciences, 1992. Flora Yunnanica: Vol. 5 [M]. Beijing: Science Press: 1–140. [Kunming Institute of Botany, Chinese Academy of Sciences, 1992. Flora Yunnanica: Vol. 5 [M]. Beijing: Science Press: 270–281.]

LI J, TONG JY, FAN ZC, et al., 2023. Chloroplast genome structure characteristics and phylogenic analysis of genus *Aucuba* [J]. Guihaia, 43(11): 2008–2023. [Li Juan, Tong Jiayun, Fan Zhichao, et al., 2023. Structural characteristics of chloroplast genomes and phylogenetic analysis of plants of the genus *Aucuba* [J]. Guihaia, 43(11): 2008–2023.]

METCALFE CR, CHALK L, 1957. Anatomy of the dicotyledons: Vol I [M]. Oxford: Clarendon Press: 97–165.

METCALFE CR, CHALK L, 1965. Anatomy of the dicotyledons: Vol II [M]. Oxford: Clarendon Press: 736.

NI Y, SONG C, WANG XQ, 2014. Investigation on Response Mechanism of Epidermal Wax on *Arabidopsis thaliana* under Cold Stress [J]. Scientia Agricultura Sinica, 47(2): 252–261. [Ni Yu, Song Chao, Wang Xiaoqing, 2014. Response mechanism of epidermal wax in *Arabidopsis thaliana* under low-temperature stress [J]. Scientia Agricultura Sinica, 47(2): 252–261.]

OHI T, 2018. Approach to adaptive and phylogenetic evolution of *Aucuba* from genome information [R/OL]. https://kaken.nii.ac.jp/ja/grant/KAKENHI-PROJECT-15K07181/.

PENG CY, LIN J, DONG K, 2008. Pharmaceutical research on *Aucuba chinensis* [J]. Journal of Li-shizhen Traditional Chinese Medicine, 158(10): 2374–2375. [Peng Chuangye, Lin Jing, Dong Ke, 2008. Pharmacognostic study of *Aucuba chinensis* [J]. Lishizhen Medicine and Materia Medica Research, 158(10): 2374–2375.]

POWO, 2025. Plants of the World Online [DB/OL]. Facilitated by the Royal Botanic Gardens Kew, https://powo.science.kew.org/.

QI CJ, LIN QZ, 2000. Woody Flora of Hunan [M]. Changsha: Hunan Science and Technology Press: 550. [Qi Chengjing, Lin Qinzhong, 2000. Woody Flora of Hunan [M]. Changsha: Hunan Science and Technology Press: 550.]

SAMUELS L, KUNST L, JETTER R, 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells [J]. Annual Review of Plant Biology, 59(1):

683.

Sichuan Food and Drug Administration, 2011. Standards for Traditional Chinese Medicine Materials in Sichuan Province (2010) [S]. Sichuan: Sichuan Science and Technology Press: 497–500. [Sichuan Provincial Food and Drug Administration, 2011. Standards for Chinese Medicinal Materials of Sichuan Province: 2010 edition [S]. Sichuan: Sichuan Science and Technology Press: 497–500.]

SONG LK, WEI Y, JIANG SY, et al., 2007. Research on *Aucuba omeiensis* Fang, a distinctive plant of Sichuan [J]. Special Wild Economic Animal and Plant Research, 117(3): 19–20. [Song Liangke, Wei Yi, Jiang Shuyang, et al., 2007. Study on *Aucuba omeiensis*, a distinctive medicinal material of Sichuan [J]. Special Wild Economic Animal and Plant Research, 117(3): 19–20.]

TAKHTAJAN A, 2009. Flowering Plants [M]. Berlin: Springer: 455–457.

WANG FT, 1949. Plantae novae generis *Aucuba* [J]. Contributions from the Institute of Botany, National Academy of Peiping, 6(4): 201–208.

XIANG QY, BOUFFORD DE, 2005. Flora of China [M]. Beijing: Science Press: 222–226.

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