Morpho-anatomical characteristics and ultrastructure of pistils and stamens in 10 wild species of *Musa*

YANG Tianyan, TIAN Qin, ZHAO Ziai, MENG Zhencheng, ZHANG Jianchun, ZHI Jiazeng

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Abstract

Musa L. is the type genus of Musaceae, with abundant germplasm resources, and serves as an important gene pool for banana genetic breeding. However, interspecific delimitation within this genus is unclear, its classification system remains controversial, and studies on the micromorphology of pistils and stamens are scarce. To clarify the micromorphological characteristics of pistils and stamens in wild Musa species and evaluate their taxonomic value, this study used 10 wild species belonging to sect. Musa (n=x=11) and sect. Callimusa (n=x=10/9/7) as materials, and systematically observed the macromorphology, anatomical structure, and ultrastructure of their pistils and stamens using stereomicroscopy, paraffin sectioning, and scanning electron microscopy. The results showed that: (1) Stamen characteristics: in species of sect. Musa, anther length was greater than or nearly equal to filament length, whereas in M. splendida of sect. Callimusa, anther length was much shorter than filament length. Pollen grains were all subspheroidal, and the exine ornamentation could be divided into two main types, "cerebriform-tuberculate" and "fine-granular/frosted," with significant differences between sections. The largest pollen diameter was found in M. splendida of sect. Callimusa [(133.13 ± 8.05) μm], and the smallest in M. velutina of sect. Musa [(73.04 ± 6.19) μm]. (2) Pistil characteristics: stigmas of sect. Musa species were mostly spoon-shaped, while that of sect. Callimusa (M. splendida) was capitate. The stigmas of all species were wet stigmas, and styles were mostly solid, with only M. balbisiana of sect. Musa possessing an open stylar canal. The morphology and arrangement of stigmatic papilla cells, as well as the state of secretions, could stably distinguish species and different developmental stages (receptive stage, elongation stage, and senescence stage). This study concludes that the micromorphological characteristics of pistils and stamens in Musa (anther/filament length ratio, pollen exine ornamentation, stylar canal type, stigmatic papilla cell morphology, etc.) are highly genetically conserved and stable, and can serve as important morphological evidence for infrageneric sectional classification (sect. Musa and sect. Callimusa) and interspecific identification. This study provides key fundamental data for the evaluation of Musa germplasm resources, the further improvement of systematics and taxonomy, and parent selection in banana genetic breeding.

Full Text

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Abstract: *Musa* L. is the type genus of Musaceae, with abundant germplasm resources, and is an important gene bank for banana genetic breeding. However, species delimitation within this genus is ambiguous, its classification system remains controversial, and micromorphological studies of pistils and stamens are scarce. To clarify the micromorphological characteristics of pistils and stamens in wild *Musa* species and evaluate their taxonomic value, this study used ten wild species from sect. *Musa* (n=x=11) and sect. *Callimusa* (n=x=10/9/7) as materials. Stereomicroscopy, paraffin sectioning, and scanning electron microscopy were used to systematically observe the macromorphology, anatomical structure, and ultrastructure of their pistils and stamens. The results showed that: (1) stamen characteristics: in species of sect. *Musa*, anther length was greater than or nearly equal to filament length, whereas in *M. splendida* of sect. *Callimusa*, anther length was much shorter than the filament. Pollen grains were subspheroidal in all species. Exine ornamentation could be divided into two main types: "cerebroid-tuberculate" and "fine-grained/frosted," with significant differences between sections. The largest pollen diameter occurred in *M. splendida* of sect. *Callimusa* [(133.13 ± 8.05) μm], and the smallest in *M. velutina* of sect. *Musa* [(73.04 ± 6.19) μm]. (2) Pistil characteristics: most species in sect. *Musa* had spatulate stigmas, whereas *M. splendida* of sect. *Callimusa* had a capitate stigma. All species had wet stigmas; the styles were mostly solid, and only *M. balbisiana* of sect. *Musa* had an open stylar canal. The morphology, arrangement, and secretion state of stigmatic papilla cells could stably distinguish species and different developmental stages (receptive stage, elongation stage, and senescence stage). This study suggests that the micromorphological characteristics of pistils and stamens in *Musa*—including the anther/filament length ratio, pollen exine ornamentation, stylar canal type, and stigmatic papilla-cell morphology—are strongly conserved genetically and stable in character, and can serve as important morphological evidence for distinguishing infrageneric sections (sect. *Musa* and sect. *Callimusa*) and species. This study provides key basic data for the evaluation of *Musa* germplasm resources, further improvement of systematic taxonomy, and selection of parents in banana genetic breeding.

Keywords: *Musa*; pistil; stamen; micromorphology; taxonomy; morphological anatomy

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Morpho-anatomy and ultrastructure of pistil and stamen in ten wild species of *Musa* (Musaceae)

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Abstract: The genus *Musa* L., the type genus of Musaceae, possesses abundant wild germplasm resources and serves as a crucial gene bank for banana genetic breeding. However, species delimitation within this genus remains ambiguous, leading to taxonomic controversies, and micromorphological studies on its stamens and pistils are scarce. This study aims to clarify the micromorphological characteristics of stamens and pistils in wild *Musa* species and evaluate their taxonomic significance. Using ten wild species representing sect. *Musa* (n=x=11) and sect. *Callimusa* (n=x=10/9/7), we systematically observed the phenotypic, cellular, and ultrastructural features of stamens and pistils using stereomicroscopy, paraffin sectioning, and scanning electron microscopy (SEM). The results were as follows: (1) Stamen characteristics: In sect. *Musa*, anther length was greater than or nearly equal to filament length, whereas in *M. splendida* (sect. *Callimusa*), anther length was much shorter than the filament. Pollen grains were subspheroidal in all species. The exine ornamentation was classified into two main types: "cerebroid-tuberculate"and "fine-grained/frosted" , with significant differences between the two sections. The largest pollen diameter was observed in *M. splendida* [(133.13 $\pm$ 8.05) μm], and the smallest in *M. velutina* [(73.04 $\pm$ 6.19) μm]. (2) Pistil characteristics: Most species in sect. *Musa* possessed a spatulate stigma, while *M. splendida* had a capitate stigma. All stigmas were

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of the "wet" type. The styles were predominantly solid, except for *M. balbisiana* (sect. *Musa*), which had an open stylar canal. The morphology, arrangement, and secretory state of stigma papillary cells could reliably distinguish species and different developmental stages (receptive, elongation, senescence). The study concludes that the micromorphological characteristics of stamens and pistils in *Musa* (anther/filament length ratio, pollen exine ornamentation, stylar canal type, stigma papillae morphology) are highly conservative and stable. These traits serve as valuable morphological evidence for infrageneric classification (sect. *Musa* vs. sect. *Callimusa*) and species identification. This research provides fundamental data for germplasm evaluation, taxonomic refinement, and parental selection in banana genetic breeding programs.

Keywords: *Musa*, pistil, stamen, micromorphology, taxonomy, morpho-anatomy

The genus *Musa* L. belongs to Musaceae of the order Zingiberales and is the core group in Musaceae with the largest number of species and the greatest diversity (Chen Wenna, 2014). Worldwide, about 70–80 wild species and variant groups have been recorded; their natural distribution is centered in tropical Asia, extending westward to the eastern Himalayas, eastward through Southeast Asia and the Malay Archipelago, and southward to northern Australia (Ning Fu et al., 2022; Rajeesh et al., 2024). China lies at the marginal region of the natural distribution of *Musa* plants; there are currently about 29 wild and domesticated species, with resources mainly distributed in tropical and subtropical regions of South and Southwest China, such as Yunnan, Guangxi, Guangdong, and Hainan (Ma Jinshuang, 2020). *Musa* plants have extremely high economic value. The cultivated varieties of banana (or plantain), which are staple foods for more than 400 million people worldwide, mostly originated through intra- or interspecific hybrid evolution from the wild ancestral small-fruited banana (*M. acuminate*) and wild banana (*M. balbisiana*) of the genus *Musa*, forming the abundant varieties or types in the banana industry (Kitavi et al., 2020). At the same time, after long-term natural selection, many wild *Musa* species possess resistance genes and are important original materials for genetic breeding and superior gene pools for genetic improvement (Li Weiming et al., 2018; Editorial Committee of *Flora of China*, Chinese Academy of Sciences, 1981). In addition, many wild species have high ornamental value because of their beautiful bracts.

Since the establishment of the genus *Musa* in 1753, the infrageneric classification system has long been controversial and has undergone repeated revisions. Early classifications were mainly based on macromorphology; Sagot (1887), Baker (1893), and others proposed preliminary frameworks. In the mid-twentieth century, Cheesman (1947) and Simmonds (1960, 1962), combining morphology and cytology, proposed a five-section classification system based on chromosome numbers, forming a clear overall framework. Until 2005, the framework of the five sections of *Musa* was widely accepted and has been used to the present, namely sect. *Musa* (Sect. *Musa*, 2n=22), sect. *Rhodochlamys* (Sect. *Rhodochlamys*, 2n=22), sect. *Australimusa* (Sect. *Australimusa*, 2n=20), sect. *Callimusa* (Sect. *Callimusa*, 2n=20), and sect. *Ingentimusa* (Sect. *Ingentimusa*, 2n=14; 2n=18). However, molecular systematic studies in recent years have shown that these five sections do not form monophyletic groups. Molecular phylogenetic studies based on nuclear and chloroplast genes have proposed a new infrageneric dichotomous classification system, merging the original five sections into two major evolutionary branches: sect. *Musa* with chromosome base number x=11 and sect. *Callimusa* with chromosome base numbers x=7, 9, and 10.

This system has also been supported by studies of male-flower morphology and has become the current mainstream classification framework in the academic community (Häkkinen, 2013; Chen Wenna, 2014; Inta et al., 2023).

In addition, the germplasm resources of the genus *Musa* are abundant; interspecific hybridization is frequent, polyploidy is common, and infraspecific variation is rich, leading to frequent disputes over species identification and classification, and the taxonomic status of many groups has long been ambiguous. For example, tree banana (*M. wilsonii*) was a new species published by Tutcher (1902) based on a plant of the genus *Musa* introduced from Yunnan and cultivated in the Hong Kong Botanical Garden. When Cheesman (1947) established the genus *Ensete*, he treated this species as *Ensete wilsonii*. Simmonds (1960) considered *E. wilsonii* to be a synonym of *Ensete glaucum* (*E. glaucum*). Liu Aizhong (2001) and others treated *E. wilsonii* as a synonym of *M. itinerans*. Häkkinen (2013) supported treating *E. wilsonii* as a synonym of *M. itinerans*, and further subdivided *M. itinerans*, treating it as a synonym of *M. itinerans* var. *xishuangbannaensis*, while also treating *E. wilsonii* as a synonym of *E. glaucum* and reducing it to the variety *E. glaucum* var. *wilsonii*.

At present, taxonomic and systematic studies of the genus *Musa* integrate methods such as morpho-anatomy, cytology, palynology, cladistics, and molecular biology. In morpho-anatomy, most analyses focus on phenotypic characters of structures such as flowers, seeds, and leaves (Feng Huimin et al., 2011; Zhang Qian, 2020; Inta et al., 2023), while reports on their micromorphological structures are limited to seeds (Jiang Yan, 2005) and leaves (Liu Aizhong, 2001). Floral organ traits are highly genetically conserved and have stable phylogenetic signals; they are core traits for the delimitation of closely related groups, infrageneric groupings, and precise species identification, and have been widely applied in taxonomic revision studies of multiple plant groups. Fan Xiaojing et al. (2021) used micromorphological characteristics such as epidermal ornamentation, stomata, and trichome ornamentation of floral structures including petals, column heads, and filaments as a basis for identifying different tea cultivars. Li Wenjuan (2021) used scanning electron microscopy, light microscopy, and transmission electron microscopy to compare and analyze differences among five species of *Lysimachia* in morphological characters of stamens, individual development, structures of anthers and pollen, epidermal micromorphology, and filament glandular structure, providing evidence for further exploration of the divergence patterns of stamens in Myrsinaceae and their possible new functions. Zhu Lin et al. (2023), through observation and measurement of pollen morphological characters, found that the ornamentation of the outer pollen wall of the yellow-flowered form of *Dolichos lablab* was similar to that of *Dolichos lablab*, while it differed markedly from that of *Macrotyloma uniflorum*, providing evidence for the study of the wild yellow-flowered type.

the problem of classification attribution of *Musa*, providing a basis for species identification and attribution in the genus. However, systematic micromorphological classification studies on the macromorphology, micromorphology, and ultrastructure of pistils and stamens in *Musa* are still lacking, making it impossible to provide morphological support for the latest two-section classification system and difficult to resolve the long-standing classification controversies and species-identification problems in this genus.

On this basis, this study used 10 representative wild species and germplasm resources from the two major evolutionary groups of *Musa* as research materials, covering nine species of sect. *Musa*—[*Musa itinerans*, *Musa velutina*, *M. ruiliensis*, *M. itinerans* var. *formosana*, *M. tonkinensis*, *M. acuminata*, *M. balbisiana*, *M. yunnanensis*, and *M. ornata*]—and one species of sect. *Callimusa* (*Musa splendida*). Using stereomicroscopy, paraffin sections, and scanning electron microscopy, we systematically observed the macromorphological types and micromorphological and ultrastructural characteristics of their pistils and stamens. The aim was to screen for traits of wild species in sect. *Musa* and sect. *Callimusa* that have stable taxonomic value in the macromorphological types, cellular structures, and ultrastructures of pistils and stamens; to clarify the morphological identification basis for the two major sections; to elucidate morphological differentiation differences among species and among infraspecific groups; to fill the gap in studies of floral organ micromorphology in *Musa*; and to provide reliable morphological theoretical support for the improvement of the infrageneric classification system, precise identification of wild germplasm, and genetic-breeding research in *Musa*.

1 Materials and Methods

1.1 Materials

Ten wild species of *Musa* were selected (Table 1). Among them, *Musa yunnanensis* was collected from Fugong County, Nujiang Lisu Autonomous Prefecture, Yunnan Province, and the other nine species were collected from the provincial characteristic tropical fruit tree germplasm resource nursery (banana nursery) of the Honghe Institute of Tropical Agricultural Sciences, Yunnan Province. Voucher specimens were identified morphologically and then preserved in the herbarium of the Honghe Institute of Tropical Agricultural Sciences, Yunnan Province. For each species, 3–5 mature plants with normal growth and no pests or diseases were selected. During full bloom, five female flowers and five male flowers that opened on the same day were collected from the upper part of each inflorescence, mixed, and used for subsequent experiments to reduce individual variation.

Table 1 Information of research materials

Section	Species	Locality	Voucher No.	Collection date
Sect. *Musa*	*Musa itinerans*	Hekou, Yunnan	HHSBJ-Mi-01	2025-05-10

Section	Species	Locality	Voucher No.	Collection date
Sect. *Musa*	*Musa velutina*	Hekou, Yunnan	HHSBJ-Mv-03	2025-06-12
Sect. *Musa*	*Musa ruiliensis*	Hekou, Yunnan	HHSBJ-Mr-02	2025-04-18
Sect. *Musa*	*Musa itinerans* var. *formosana*	Hekou, Yunnan	HHSBJ-Mif-01	2025-05-20
Sect. *Musa*	*Musa tonkinensis*	Hekou, Yunnan	HHSBJ-Mt-04	2025-07-05
Sect. *Musa*	*Musa acuminata*	Hekou, Yunnan	HHSBJ-Ma-02	2025-06-25
Sect. *Musa*	*Musa balbisiana*	Hekou, Yunnan	HHSBJ-Mb-03	2025-08-01
Sect. *Musa*	*Musa yunnanensis*	Fugong, Yunnan	HHSBJ-My-01	2025-09-15
Sect. *Musa*	*Musa ornata*	Hekou, Yunnan	HHSBJ-Mo-02	2025-07-18
Sect. *Callimusa*	*Musa splendida*	Hekou, Yunnan	HHSBJ-Ms-01	2025-05-28

1.2 Methods

1.2.1 Phenotypic observation and morphological measurement

Fresh female and male flowers were collected, placed under an OLYMPUS SZX16 stereomicroscope for observation and photography. ImageJ software was used to measure the anther length and filament length of the stamens, as well as the morphological characteristics of the pistil stigma. Fifteen samples were measured for each species ($n = 15$).

1.2.2 Paraffin sections

Fresh pistil stigmas, the middle part of the style, and the middle parts of stamen anthers and filaments were cut into small pieces of 0.5 cm × 0.5 cm, immediately placed in FAA fixative (50% ethanol:formalin:glacial acetic acid = 90:5:5), and fixed for 48 h. After dehydration through an ethanol gradient (70%, 85%, 95%, 100%), clearing with xylene, and paraffin embedding, continuous sections were cut with a Leica RM2235 microtome at a thickness of 8–10 μm. The sections were stained with safranin–fast green (H.E) or safranin–solid green and mounted in neutral resin. They were observed and photographed under a Leica DM2500 optical microscope. No fewer than 10 observations were made for each sample.

sections.

1.2.3 Scanning electron microscope observation

Pollen: Mature anthers were fixed in 2.5% glutaraldehyde, dehydrated through a graded ethanol series, critical-point dried, and ion-sputter coated with gold; pollen morphology and exine ornamentation were then observed under a ZEISS EVO LS10 scanning electron microscope. For each species, 10 pollen grains were randomly selected to measure the lengths of the polar axis and equatorial axis (n=10).

Stigma: Fresh pistil stigmas were fixed, dehydrated, dried, and gold-coated using the above method, and the overall morphology of the stigma and papilla-cell characteristics were observed under the scanning electron microscope.

1.3 Data analysis

Excel 2016 was used for data collation, and the mean and standard deviation ($\bar{x} \pm s$) of pollen diameter were calculated.

2 Results and analysis

2.1 Phenotypic characteristics of pistils and stamens

As shown in Fig. 1, there were obvious differences in the phenotypes of pistils and stamens among the 10 wild species. In the *Musa itinerans* group, the stigmas of the pistils in 9 species were mostly spatulate, among which the stigma of *M. ornata* was extremely small and rod-shaped. In the *Musa acuminata* group, the pistil stigma of *M. splendida* was capitate. With respect to stamens, the anthers of all species in the *Musa itinerans* group were oblong, and their length was greater than or approximately equal to the filament length. In the *Musa acuminata* group, the anther length of *M. splendida* was significantly shorter than the filament length (Fig. 1: C; Table 2).

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itinerans* var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I. *M. yunnanensis*; J. *M. ornata*; Top. Pistil; Bottom. Stamen.

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itinerans* var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I. *M. yunnanensis*; J. *M. ornata*; **Top.** Pistil; **Bottom.** Stamen.

Fig. 1 Phenotypic structure of stamens and pistils in ten *Musa* species

Table 2 Comparison of anther and filament lengths of stamens in ten *Musa* species

Section	Species	Anther length (mm)	Filament length (mm)	Ratio (A/F)
Sect. *Musa*	*M. itinerans*	12.5 ± 0.6	10.1 ± 0.5	1.24
Sect. *Musa*	*M. velutina*	8.9 ± 0.4	8.5 ± 0.4	1.05
Sect. *Musa*	*M. ruiliensis*	11.2 ± 0.5	11.8 ± 0.6	0.95
Sect. *Musa*	*M. itinerans* var. *formosana*	11.3 ± 0.6	12.1 ± 0.6	0.93
Sect. *Musa*	*M. tonkinensis*	14.1 ± 0.7	12.3 ± 0.6	1.15
Sect. *Musa*	*M. acuminata*	10.9 ± 0.5	10.5 ± 0.5	1.04
Sect. *Musa*	*M. balbisiana*	13.8 ± 0.6	11.4 ± 0.6	1.21
Sect. *Musa*	*M. yunnanensis*	12.7 ± 0.6	9.8 ± 0.5	1.29
Sect. *Musa*	*M. ornata*	9.6 ± 0.3	10.8 ± 0.3	0.88
Sect. *Callimusa*	*M. splendida*	6.5 ± 0.3	13.2 ± 0.7	0.49

Note: Ratio >1 indicates anther longer than filament, 1 indicates nearly equal, <1 indicates anther shorter than filament.

2.2 Microscopic and ultrastructural structure of stamens

2.2.1 Cellular structure of stamen anthers and filaments

Paraffin sections showed that, except for *M. ornata* (Fig. 2: J1), which was at the microspore mother-cell stage, the other nine species were all at the mature pollen-grain stage or anther dehiscence stage (Fig. 2: A1–I1). The transverse section of the anther was a typical four-locule structure, and the anther chambers were filled with round or nearly round mature pollen grains. Pollen-grain diameter differed significantly among species (Table 3), with *M. splendida* (Sect. *Callimusa*, Fig. 2: C1) being the largest [(133.13 ± 8.05) μm] and *M. velutina* (Sect. *Musa*, Fig. 2: B1) the smallest [(73.04 ± 6.19) μm]. The transverse-sectional structure of the filament varied among species; the transverse-sectional shape ranged from nearly circular, elliptical, and square to irregular. The epidermis was mostly 1–2 layers of cells and thick-walled; beneath it were thin-walled cells and vascular bundles (Fig. 2: A2–J2).

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itinerans* var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I.

M. yunnanensis; J. *M. ornata*; 1. Anther; 2. Filament.

Fig. 2 Cellular structure of anthers and filaments in ten *Musa* species

Table 3 Comparison of pollen morphology and size in ten *Musa* species

Section	Species	Pollen shape	Pollen diameter (μm)	Exine ornamentation type	Exine ornamentation characteristics
Sect. *Musa*	*M. itinerans*	Nearly spherical	79.29 ± 6.23	Cerebroid-tuberculate	Ridge-like protrusions connected into cerebroid form; surface extremely rough

Taxon	Shape	Size	Surface type	Surface ornamentation
				The ridges form a brain-like pattern, and the surface is extremely rough.
M. velutina	Nearly spherical	73.04 ± 6.19	Fine-grained	Layers of sheet-like structures are stacked to create a fine, undulating, matte texture.

Taxon	Shape	Size	Surface type	Surface ornamentation
M. ruiliensis	Nearly spherical	78.68 ± 12.17	Fine-grained	Laminate/ridge-like protrusions are connected, with clear and uniform grooves.
M. itinerans var. *formosana*	Near-spherical/spherical	88.57 ± 7.80	Fine-grained	Clump-like/lump-like protrusions intertwine with flocculent/filamentous structures, forming a non-uniform, multiscale composite texture.
M. tonkinensis	Nearly spherical	82.10 ± 6.98	Cerebroid-tuberculate	Lumps are piled up, with deep gullies and wide crevices; the surface is extremely rough.
M. acuminata	Nearly spherical	86.86 ± 5.09	Fine-grained	Fine folds and fluffy structures intertwine, creating gentle undulations.

Taxon	Shape	Size	Surface type	Surface ornamentation
M. balbisiana	Nearly spherical	81.41 ± 5.01	Compound	Smooth patches alternate with a dense network of grooves, creating a striking contrast.
M. yunnanensis	Nearly spherical	101.71 ± 8.04	Fine-grained	Strip-like/ridge-like protrusions interweave into a network, with gentle undulations.

	Purple-bracted banana *M. ornata*	–	–	–	Strip-like/ridge-like protrusions intertwine to form a mesh-like pattern, with gentle undulations. During the microsporangium stage, there is no mature pollen.
Sect. *Callimusa*	*M. splendida*	Nearly spherical	133.13 ± 8.05	Fine-grained	Net-like/grooved texture

2.2.2 Pollen ultrastructure

The scanning electron microscopy observations showed that the mature pollen grains of the 9 taxa studied were all nearly spherical and had no obvious germination apertures; their pollen exine ornamentation could be divided into three types: cerebriform, fine-grained/sand-like, and compound (Fig. 3, Table 3). Among them, the cerebriform type was represented by Akuan banana and Xuanfu banana (Fig. 3: A, F); the exine consisted of irregular ridge-like and block-like protrusions connected and stacked with one another, forming cerebrum-like coarse ornamentation resembling the cerebral cortex. The fine-grained/sand-like type was found in most species of sect. *Musa*—such as *M. coccinea* and small-fruited wild banana—as well as in *M. splendida* of sect. *Callimusa*; the exine was composed of fine, compact granules and short wrinkles, giving an overall sand-like texture, with the pollen surface of *M. splendida* being especially flat and uniform. The compound type was found only in wild banana (Fig. 3: H); its exine showed a two-dimensional structure in which smooth patches and dense grooved reticulate ornamentation were distributed alternately, and the ornamentation features were highly recognizable.

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itinerans* var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I. *M. yunnanensis*;
1. Pollen shape; 2. Pollen exine.

Fig. 3 Scanning electron microscope images of pollen and anther walls from nine *Musa* species

2.3 Micromorphology and ultrastructure of the stamen

2.3.1 Structure of the anther and filament cells

Paraffin sections showed that the anthers of all species were tetrasporangiate (Fig. 4: A1–J1; Table 4), with the surface covered by papillate cells. Most filaments were solid, with differing transverse-sectional shapes; the center consisted of thin-walled cells and vascular bundles, without a central canal (Fig. 4: A2–G2, I2, J2; Table 4). The size of the thin-walled cells and the arrangement of the vascular bundles also differed among species (Fig. 4: A2–J2). However, the filament of *M. balbisiana* had a conspicuous open filament canal in the center, formed by canal cells (Fig. 4: H2), which is a distinctive feature separating it from the other species.

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itinerans* var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I. *M. yunnanensis*; J. *M. ornata*; 1. stigma; 2. style.

Fig. 4 Cellular structure of pistil stigmas and styles in ten *Musa* species

Table 4 Main characteristics of pistil stigma and style in ten *Musa*

species

Section	Species	Stigma shape	Stylar canal type	Papilla cell morphology (receptive stage)
Sect. *Musa*	*M. itinerans*	Spatulate	Solid	Digitate/papillary, densely arranged

Section	Taxon	Stigma shape	Ovary type	Micromorphological characteristics of papilla cells
	Velvet banana *M. velutina*	Spatulate	Solid	Cell shrinkage during the aging process
	M. ruiliensis M. ruiliensis	Spatulate	Solid	Short, finger-like/papillary, arranged in a radial pattern
	Taiwan wild banana *M. itinerans* var. *formosana*	Spatulate	Solid	Highly elongated rod-like/filament-like
	M. tonkinensis M. tonkinensis	Spatulate	Solid	Long cylindrical/rod-shaped, arranged in a vertical mosaic pattern
	Dwarf wild banana *M. acuminata*	Spatulate	Solid	Short, round/hemispherical, arranged in a flat, inlaid pattern

Section	Taxon	Stigma shape	Ovary type	Micromorphological characteristics of papilla cells
	Wild banana *M. balbisiana*	Spatulate	Open	Irregular rod-shaped/finger-like (twisted and lodged after the elongation stage)
	Yunnan banana *M. yunnanensis*	Spatulate	Solid	Data missing
	Ornate banana *M. ornata*	Clavate	Solid	Short, round, or hemispherical, with a surface covered in secretions
Sect. *Callimusa*	Splendid banana *M. splendida*	Capitate	Solid	Extremely elongated, finger-like/thread-like

2.3.2 Ultrastructure of the stigma

Scanning electron microscopy observations further clarified the microscopic characteristics of the stigma and papilla cells. The stigma morphology was consistent with the results observed under a stereomicroscope (Fig. 5: A1–I1; Table 4). The morphology of the papilla cells and the characteristics of surface secretions showed marked differences among developmental stages and can be divided into three periods: during the receptive stage, represented by *M. itinerans* var. *formosana* (Fig. 5: A2) and *M. acuminata* (Fig. 5: G2), the papilla cells were densely packed, and a small amount of filamentous and granular secretions was distributed on the surface; during the elongation stage / post-pollination response stage, as seen in *M. splendida* (Fig. 5: C2) and Taiwan wild banana (Fig. 5: E2), the papilla cells were markedly elongated, finger-like or filament-like, and their surfaces were covered with abundant filamentous and reticulate secretions; during the senescence stage, represented by velvet banana (Fig. 5: B2) and pollinated wild banana (Fig. 5: H2), the papilla cells exhibited obvious wrinkling, collapse, lodging, and desiccation.

A. *M. itinerans*; B. *M. velutina*; C. *M. splendida*; D. *M. ruiliensis*; E. *M. itin-*

erans var. *formosana*; F. *M. tonkinensis*; G. *M. acuminata*; H. *M. balbisiana*; I. *M. ornata*; 1. Stigma; 2. Papilla cells.

Fig. 5 Scanning electron micrographs of the stigmas and papillae of nine *Musa* species

3 Discussion

3.1 The value of micromorphological characteristics of female and male 蕊 in the taxonomy of *Musa*

Morphological classification of *Musa* is mainly based on traits such as the state of the inflorescence, whether the male bud is imbricate, bract color, and the length of the compound tepal and free tepal (Li Xiwen, 1978). Inta (2023), by combining male-flower morphological characters with molecular phylogenetic research, strongly supported the classification system of Musaceae and *Musa*, and helped to screen and establish key characters for identifying *Musa*. As new species and new varieties of *Musa* have gradually been discovered and identified, compared with the bract morphology and plant habit on which traditional classification relies—characters that are easily disturbed by the environment—the above morphological traits alone can no longer accurately identify

determined. In contrast, pistil micromorphology is dominated by genetic material and exhibits stronger trait conservatism, giving it higher applied value in the infrageneric delimitation and species identification of *Musa*. In this study, 10 wild species of *Musa* belonging to two major groups were selected, and the micromorphological characteristics of their pistils were systematically observed and compared. The results showed that, in the Eumusa group, the stamen length of most species was greater than or close to the filament length, and the stigma was mainly hoof-shaped or rod-shaped; whereas the representative species of the Rhodochlamys group, *Musa ornata*, had stamens significantly shorter than the filaments and a capitate stigma. This result is consistent with Inta' s (2023) description that pistil stigmas are divided into hoof-shaped, rod-shaped, and capitate types, and that stamen and filament lengths are structural characteristics.

The results of this study showed that pollen diameter differed significantly among the nine *Musa* species, with the largest in *Musa ornata* (Rhodochlamys group) [(133.13 ± 8.05) μm] and the smallest in *Musa itinerans* (Eumusa group) [(73.04 ± 6.19) μm]. Pollen size was found to be related to genome type and ploidy: the pollen of genome group B (e.g., wild banana, mean 75.54 μm) was significantly larger than that of genome group A (e.g., *Musa acuminata*, mean 58.83 μm) (Sukkaewmanee, 2019). Notably, in this study the pollen diameters of *Musa acuminata* [(86.86 ± 5.09) μm] and wild banana [(81.41 ± 5.01) μm] differed only slightly, possibly because abnormalities occurred during meiosis (such as monads, dyads, or triads) or because external environmental factors affected pollen development; further study is still needed. Meanwhile, ornamentation of the pollen exine is also an important palynological basis for distinguishing the two groups. This study found that the pollen exine ornamentation of the Eumusa group was coarse, with developed verrucate protrusions, whereas the exine ornamentation of the Rhodochlamys group tended overall to be smooth. Although *Musa argentii* and *Musa laterita*, both belonging to the Eumusa group, had cerebriform verrucate pollen exines, they also differed from each other. The latter had coarser and deeper verrucate protrusions and grooves; whereas the unique "compound" pollen exine ornamentation and its characteristic open colpal channels in wild banana made it extremely easy to recognize within this group. Bai Tingyu et al. (2023), through scanning electron microscopic observations of pollen morphology in domestic *Musa* plants, found that there was an evident evolutionary gradient in pollen exine ornamentation in *Musa*, consistent with the results of the present study. From the perspective of broader plant classification studies, although pollen exine ornamentation types have limited significance for intrageneric grouping, they still have reference value for the identification of some taxa, and their value in distinguishing closely related species has been repeatedly confirmed (Liu Quanru et al., 1994).

3.2 Association of pistil micromorphological characteristics with reproductive biology and developmental stage

Temporal changes in stigma morphology and function are the result of long-term adaptation of plant pollination systems. This study found that, in *Musa*, the morphology of stigmatic papilla cells and the state of surface secretions are highly associated with floral organ developmental stage; this developmental pattern has also been reported in other angiosperm groups (Fan Xiaojing et al., 2021). In stigmas at the receptive stage, the papilla cells are plump, and the surface contains relatively little secretion; this structure facilitates pollen-grain adhesion. After pollination is completed, the stigmatic papilla cells elongate rapidly and simultaneously secrete large amounts of mucilage, providing a suitable environment for pollen water uptake, germination, and pollen-tube growth. After entering senescence, the papilla cells gradually shrink and degrade, and the stigma completely loses receptivity. Heslop-Harrison and Shivanna (1977) systematically summarized the regulatory mechanisms of stigma receptivity in angiosperms and pointed out that morphological changes in stigmatic papilla cells and the release of secretions are key indicators of receptivity acquisition. Edlund et al. (2004) further emphasized that stigma receptivity is a strictly regulated, stage-specific developmental process, and that its morphological and physiological states are closely related to the acquisition and loss of receptivity. Ren Zongxin and Wang Hong (2007), in their study of reproductive structures in Musaceae, pointed out that temporal changes in floral micromorphology are a reproductive strategy formed by plants through long-term adaptation to the pollination process. The dynamic changes in stigma identified in this study further enrich the basic research content of pollination biology in *Musa* and also provide morphological support for elucidating the reproductive characteristics of this genus.

Within *Musa*, Ssebuliba et al. (2006), through a systematic study, divided stigma receptivity in East African highland bananas into four stages and found that seed yield was highest at stage III (the stigma was pale brown, the papillae were plump, and secretions were abundant), whereas stages I and II had not yet reached the optimal receptive state and stage IV had completely lost receptivity, indicating that stage-specific changes in stigma micromorphology are closely related to breeding efficiency. In addition, Fortescue and Turner (2004) found that the pollen viability of wild banana and *Musa velutina* was significantly higher than that of *Musa acuminata*, suggesting that genetic differences may exist among different species in the developmental coordination between stamens and pistils; this also partly explains why mature pollen could not be obtained from some species in the present study.

3.3 Limitations and prospects of this study

This study is the first systematic investigation of the micromorphological characteristics of pistils and stamens in 10 wild *Musa* species, clarifying the micromorphological differentiation of pistils and stamens in this genus and providing micro-morphological data support for taxonomic revision and comprehensive evaluation of germplasm resources in *Musa*. Although certain research results were obtained, several limitations remain. First, the species coverage was relatively limited: the Rhodochlamys group included only one species, and representative materials of the Callimusa group, such as *M. textilis*, can be added in subsequent studies to further verify the within-group stability and specificity of pistil and stamen micromorphological characteristics in that group. Second, for some experimental materials (pollen of *Musa velutina* and stigmas of *Musa yunnanensis*), the sampling time did not match the organ developmental stage, resulting in the absence of relevant micromorphological data. Future studies may combine long-term phenological dynamic monitoring and standardize sampling criteria to ensure the validity and completeness of experimental samples. Third, this study conducted only qualitative morphological descriptions and did not perform quantitative analysis. In the future, continuous traits such as stamen length, filament length, pollen diameter, and the amount of pollen exine ornamentation may be used, together with discrete trait coding such as stigma type and colpal-channel type, to conduct principal component analysis and thereby test the robustness of the taxonomic conclusions.

4 Conclusion

In this study, systematic observations were conducted on the female and male floral phenotypes, micromorphology, and ultrastructure of 10 wild plant species of the genus *Musa*, clarifying the taxonomic value of micromorphological characteristics of female and male flowers in infrageneric grouping, species identification, and reproductive-development studies within *Musa*. The main conclusions are as follows:

(1) Multiple micromorphological traits of female and male flowers in *Musa*

plants show significant differentiation among groups. Among them, characters such as the flower anther/filament length ratio, ornamentation type of the outer pollen wall, and overall stigma morphology have high stability within the true banana group (sect. *Musa*) and the callimusa group (sect. *Callimusa*) and clear differences between groups. They can serve as core morphological evidence for rank delimitation below the genus level in *Musa*, providing microscopic structural evidence to validate and support the two-section classification system of *Musa*.

(2) Most fine micromorphological traits possess good potential for species identification. Traits such as the subtypes of ornamentation on the outer pollen wall (degree of roughness of cerebriform ornamentation, compound special ornamentation, etc.), the structural type of the stylar canal (solid and open types), and the morphology and arrangement patterns of stigma papilla cells show relatively high discriminatory power among species. They can effectively make up for the deficiencies of traditional macromorphological identification and can serve as key indicators for resolving difficulties in species identification within *Musa*.

(3) The morphological structure of stigma papilla cells shows evident spatiotemporal specificity during development. The external morphology of papilla cells and the distribution of secretions undergo regular dynamic changes along with the process of pistil development (receptive stage, elongation stage, senescence stage), and the two are highly coupled. This result indicates that unified sampling of developmental stages is an important prerequisite for obtaining reliable experimental data in taxonomic and reproductive-biological studies of *Musa*.

In summary, the micromorphological traits related to female and male flowers are highly stable and highly differentiated, and have very high taxonomic application value. Such traits can be effectively used for precise identification of wild germplasm resources of *Musa* and for revision of infrageneric classification systems, and can also provide important micromorphological reference evidence for parental screening in banana hybrid breeding.

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