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Studies of the pollen characteristics and the taxonomic significance of *Impatiens* from the Yunnan–Guizhou Plateau

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ABSTRACT

Impatiens is rich in germplasm resources, with more than 260 species in China. A study on the pollen micromorphology of *Impatiens* by scanning electron microscopy (SEM) showed that the pollen characteristics were richly diverse, and there was some correlation among the characteristics. In addition, the micromorphological index can be used for an effective cluster analysis of *Impatiens*. However, there was high interspecific similarity in some *Impatiens*, and the classification of *Impatiens* cannot be accurate to the species using pollen characteristics. Based on the classification conditions of *Impatiens*, the characteristics of the pollen structure were found to be useful to classify *Impatiens* into subgenera or smaller groups. The macroscopic characteristics and the number of sepals (NS) were used as references, which enabled the conclusion that there were 11 indices in the pollen micromorphological index, and the taxonomic effect was greater than the NS. In summary, the pollen micromorphology of *Impatiens* plays an important role in the classification of *Impatiens*. The purpose of this study was to explore this characteristic of *Impatiens*, which has some reference significance to supplement the pollen characteristics and palynological classification of *Impatiens*. The goal for this research was to aid in the interspecific identification and genetic breeding of *Impatiens*.

Keywords: *Impatiens*, micromorphology, palynology, pollen, SEM

Abbreviations: CGP, characteristics of germination pores; E1, equatorial major axis length; E2, equatorial short axis length; EO, epidermis ornamentation; EV, equatorial view; GPL, germination pore length; GPW, germination pore width; L/W, germination pore length/germination pore width; MD, mesh density; MF, mesh feature; NS, number of sepals; P, polar axis length; PV, polar view; RF, ridge feature; RW, ridge width; SEM, scanning electron microscopy.

INTRODUCTION

There are two genera of the Balsaminaceae, and one is *Impatiens*. This genus is rich in germplasm resources, with more than 1 000 species in the world and more than 260 species in China (Luo et al., 2022). However, the classification of *Impatiens* is very challenging because it is difficult to make and preserve specimens (Yu et al., 2016). The primary classification methods of *Impatiens* are traditional taxonomy and molecular biology or a combination of both. In molecular biological classification, ITS sequences (Eddie et

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al., 2003; Yuan et al., 2004) and the chloroplast genomes (Luo et al., 2022) are commonly used for classification. Compared with molecular biology, traditional taxonomy primarily classifies plants based on morphology and palynology. The classification of plants based on pollen characteristics is considered palynology. Plants of the same genus or different genera are crossed by self-pollination or through the actions of insects (Attique et al., 2022), wind and other factors (cross-pollination) (Zavada and Hackley, 2022). This carries the genetic material of the male parent, which is a necessary condition for plants to continue their offspring. Thus, during the process of plant evolution, the pollen structure is complex to protect the genetic material (Zavialova and Nosova, 2019). To adapt to the environment, plants have formed a special pollen structure, which helps preserve the pollen. There are some differences in pollen characteristics among different plants, which is the basis of pollen classification.

Optical microscopy and scanning electron microscopy (SEM) are the primary tools to observe pollen morphology, which greatly aid the development of palynology. Researchers promoted the development of plant taxonomy by extracting pollen characteristics from families such as Campanulaceae (Khansari et al., 2012), Combretaceae (El Ghazali, 2022) and Paropsieae (Mezzonato-Pires et al., 2022). The study of plant pollen helps determine that pollen characteristics can be used as a basis for plant classification (Tuler et al., 2017; Umber et al., 2022). The importance of plant classification and pollen characteristics at the intergeneric level has been emphasised, and it has been shown that pollen data can verify the information of pollen evolution on the true stamen pedigree. In addition, it provides some explanations for the direction of plant evolution.

Among *Impatiens* plants, Yu et al. (2016) made an accurate analysis by creating two subgenera of *Impatiens* and dividing them into seven groups. Pollen micromorphology was an important index for this classification of *Impatiens*. Studies on the pollen morphological characteristics of *Impatiens* by SEM helps identify *Impatiens*, provide basic pollen information for breeding and improve the rate of success of breeding.

MATERIALS AND METHODS

Information collection and preservation methods of plant materials

The plant materials were primarily derived from field studies and sampling, and the collected information was recorded (Annex 1). The change in height of flower colour among the species of *Impatiens* increases the difficulty of classifying them (Chen, 1978). In this study, we used 1, 2, 3 and higher numbers when necessary to mark *Impatiens* of the same species but different colours. In the same type of *Impatiens*, more than two types of mature pollens were collected and stored in a test tube or collection tube filled with FAA fixatives.

Scanning electron microscope

The pollen of 35 species of *Impatiens* was observed by SEM. The pollen samples were prepared by first soaking the material and then gently removing the anthers with tweezers and dissection needles. The anthers were placed in a 2 mL collection tube and dehydrated with an alcohol gradient of 30%, 45%, 60%, 75%, 90% and 95%. The solution was replaced with anhydrous alcohol twice for 1 h at a time. The anthers were then removed with tweezers and dissected using a needle, so that the pollen was evenly distributed on the sample table with conductive glue. Under the vacuum conditions of an ion sputtering instrument (Cressington Scientific Instruments, Watford, UK), the gold spray was coated for 2–3 min. It was observed by a Zeiss scanning electron microscope (Zeiss, Jena, Germany).

Image analysis

The pictures were analysed using Image J (NIH, Bethesda, MD, USA), and the morphological characteristics were named based on the pollen research literature (Yu et al., 2016; Mazari et al., 2017; Hu et al., 2020; Raza et al., 2020). There were slight modifications such as pillow shape, which was named so because its shape is similar to that of ancient Chinese porcelain. When the mesh density (MD) was 12 × 1 000-fold magnification, the number of mesh was regarded as the MD.

Data statistics and analysis

The statistical data of Microsoft Excel YEAR (Redmond, WA, USA) were used, and SPSS 26.0 (IBM, Inc., Armonk, NY, USA) was used for correlation, principal component and system clustering analyses (Pérez-Gutiérrez et al., 2015; Zafar et al., 2022). The number of sepals (NS), which is one of the important bases for the classification of *Impatiens*, was added as a reference index to compare the importance of pollen characteristics during the analysis of indices.

RESULTS

Statistics of pollen characteristics

Based on the study and analysis of pollen micromorphology of 35 species of *Impatiens*, the data of 19 characteristics indexes were obtained, including pollen morphology (Table 1), pollen germination pore characteristics, pollen size and mesh characteristics (the data sheet is provided in Annex 2). The pollen characteristics were primarily divided into two parts, qualitative and quantitative traits.

There were six indices of qualitative characteristics (Figure 1), which included epidermis ornamentation (EO), polar view (PV), equatorial view (EV), characteristics of germination pores (CGP), mesh feature (MF) and ridge feature (RF). The characteristics under each index were different, and the proportion was also different, indicating that the characteristics

Table 1. Statistics of pollen micromorphological characteristics of *Impatiens* in the Yunnan–Guizhou Plateau.

N	Species	Epidermal ornamentation	Shape	
			PV	EV
1	*I. siculifer*	Reticulate	Rectangular circle	Oval
2	*I. pinetorum*	Reticulate	Pillow	Oval
3	*I. rectangula*	Reticulate	Rectangular circle	Oval
4	*I. ruiliensis*	Reticulate	Rectangular circle	Oval
5	*I. holocentra*	Reticulate	Rectangular circle	Oval
6	*I. siculifer var.*	Reticulate	Rectangular circle	Oval
7	*I. racemosa*	Reticulate	Rectangular circle	Oval
8	*I. uliginosa1*	Reticulate	Rectangular circle	Oval
9	*I. uliginosa2*	Reticulate	Rectangular circle	Oval
10	*I. uliginosa3*	Reticulate	Rectangular circle	Oval
11	*I. uliginosa4*	Reticulate	Rectangular circle	Oval
12	*I. cyathiflora*	Reticulate	Rectangular circle	Oval
13	*I. dicentra*	Reticulate	Rectangular circle	Oval
14	*I. dicentra var.*	Reticulate	Oval	Oval
15	*I. noli-tangere*	Reticulate	Rectangular circle	Oval
16	*I. corchorifolia*	Reticulate	Rectangular circle	Oval
17	*I. delavayi*	Reticulate	Rectangular circle	Oval
18	*I. guizhouensis*	Reticulate	Triangular circle	Triangular circle
19	*I. auriculata*	Corrugated reticulation	Triangular circle	Triangular circle
20	*I. monticola var.*	Reticulate	Rectangular circle	Oval
21	*I. chlorosepala*	Reticulate	Oval	Oval
22	*I. xanthina*	Reticulate	Rectangular circle	Near circle
23	*I. monticola*	Reticulate	Rectangular circle	Oval
24	*I. yui*	Reticulate spinous granule	Rectangular circle	Oval
25	*I. rubrostriata*	Reticulate	Rectangular circle	Oval
26	*I. loulanensis*	Reticulate	Oval	Oval
27	*I. arguta1*	Reticulate	Rectangular circle	Oval
28	*I. arguta2*	Reticulate	Rectangular circle	Oval
29	*I. arguta3*	Reticulate	Rectangular circle	Oval
30	*I. fanjinica*	Reticulate	Pillow	Oval
31	*I. fanjinica var.*	Reticulate	Pillow	Oval
32	*I. reptans*	Reticulate	Pillow	Oval
33	*I. reptans var.*	Reticulate	Pillow	Oval
34	*I. pianmaensis*	Reticulate	Rectangular circle	Oval
35	*I. pianmaensis var.*	Reticulate	Rectangular circle	Oval

EV, equatorial view.

of pollen qualitative indices were rich, which aided the interspecific classification of *Impatiens* plants (Figure 2). There were 14 quantitative characteristic indices. The NS was the reference group, and the others included the number of germination pores (NGP), germination pore length (GPL), germination pore width (GPW), germination pore length/germination pore width (L/W), MD, ridge width (RW), polar axis length (P), equatorial major axis length (E1), equatorial short axis length (E2), pollen volume (V) and P/E1, P/E2 and E1/E2. There were differences among the species, and there was an obvious difference between the maximum and minimum values (Table 2), such as volume. The maximum value was more than 14 000 μm^3, the minimum value was approximately 4 000 μm^3 and the average value was approximately 8 000 μm^3. Based on the aforementioned analysis described, the pollen characteristics of *Impatiens* were rich in diversity, which can provide positive conditions to classify the members of this genus.

Correlation analysis of pollen characteristics

There was some correlation among the pollen characteristics (Figure 3). Among the 19 traits of pollen, there were significant correlations among some indices, such as the SN and polar morphology, equatorial morphology, number of germination holes, MD, RW, polar axis length, equatorial minor axis length, P/E1, P/E2 and E1/E2. With reference to the NS, there was some correlation between pollen characteristics, and there was also some correlation with the NS. *I. guizhouensis* and *I. auriculata* were used as examples to show that the correlation was significant for plant classification. In the classification index, there was some relationship

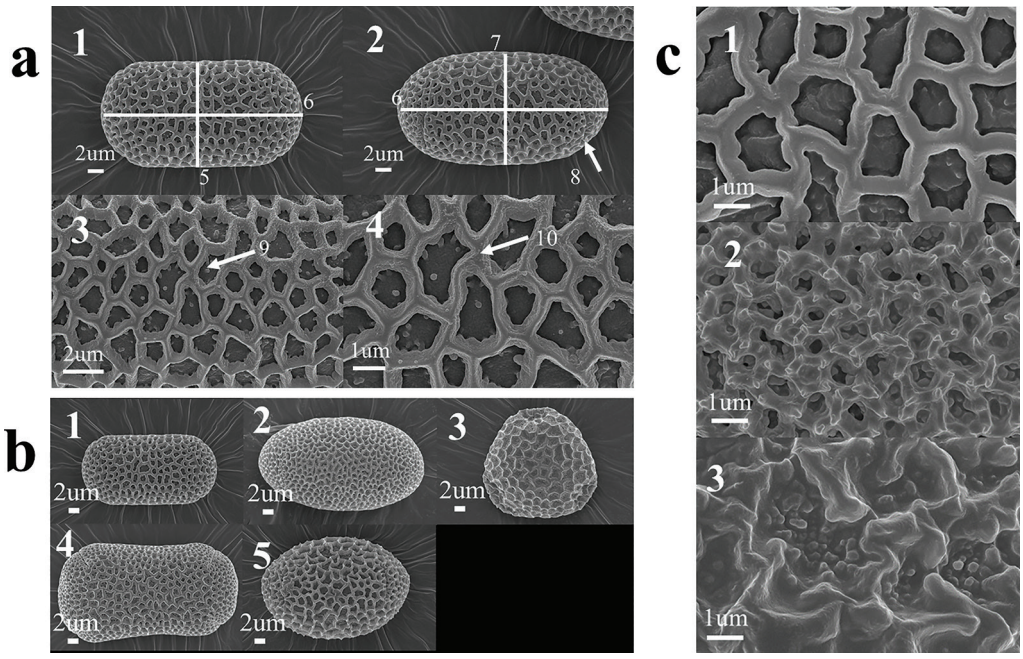

Figure 1. SEM image of *Impatiens* pollen. (A) Observation method and measurement index marking of *Impatiens* pollen: 1 PV; 2 EV; 5 polar axis; 6 equatorial major axis; 7 equatorial minor axis; 8 sprouting hole; 9 mesh; 10 mesh; 1 and 2 mirror multiple, 3 000, scale bar, 1 μm; 3 mirror multiple is 7 000, scale bar, 1 μm; 4 mirror multiple is 3 000, scale bar, 2 μm. (B) Pollen morphology of *Impatiens*: 1 rectangular circle, 2 oval, 3 triangular circle, 4 pillow shape, 5 nearly circular. (C) *Impatiens* pollen ornamentation: 1 reticulate pattern, 2 wavy reticulate pattern, 3 reticulate spinous granule. EV, equatorial view; SEM, scanning electron microscopy.

between the index and the index. The correlation showed that the pollen characteristics of *Impatiens* plants may be accompanied, and as the basis of plant identification, pollen characteristics can promote the acquisition of species information.

Principal component analysis and systematic cluster analysis

Principal component analysis

According to the principal component analysis of 20 characteristic indexes (Table 3), there were 5 main components, and the contribution rate of each component was more than 10%. The cumulative contribution rate of 5 components is 80.45% > 60%, and all characteristic indexes were included. It showed that the selected current index was of significance to the clustering results among the cluster members. The results showed that the selected characteristic indexes were of significance to the classification of *Impatiens*. The next step of systematic cluster analysis can be carried out. The composition matrix showed that the effect of each index on the clustering results of *Impatiens* was P, P/E1, E2, equatorial morphology, E1/E2, the number of germination holes and so on. The mesh condition and the width of germination holes had the least effect on the classification of *Impatiens*. Among the 19 traits of pollen, 11 had more taxonomic effect than the NS. Sepal was an important classification index of *Impatiens*, so these 11 pollen characteristics played an important role in the classification of *Impatiens*.

Systematic cluster analysis

In the clustering results of the system (Figure 4), with the red dotted line as the reference line, the system clustering had three branches, and the yellow reference line analysis had six branches. *I. guizhouensis* and *I. auriculata* were the first branch, and both types of pollens were the three-groove type. These pollens were shaped as triangular circles. They were all typical plants of the subgenus *Impatiens* and were reasonably classified together. However, the yellow reference line analysis indicated that they should clearly be separated because of their significant differences in the quantitative data of pollen EO, L/W and pollen size. *I. yui* remained a separate branch in which no reference line was used. Its EO and ridge characteristics were unique, but its quantitative index also differed significantly from that of other species.

The other species were uniformly clustered into one branch (third branch) under the red reference line, and the clustering distance was short, indicating that there was only a small difference among the species. With reference to the yellow dotted line, the third branch under the red reference line was further divided into three more careful branches, namely the fourth, fifth and sixth branches that contained 5, 4 and 23 species, respectively, and the varieties or same species of *Impatiens* were all divided together. On closer inspection, the *Racemosae* group was in the same branch (a small branch of the sixth branch), while the *Fasciculatae* group and the undetermined group of *Impatiens* gathered in another

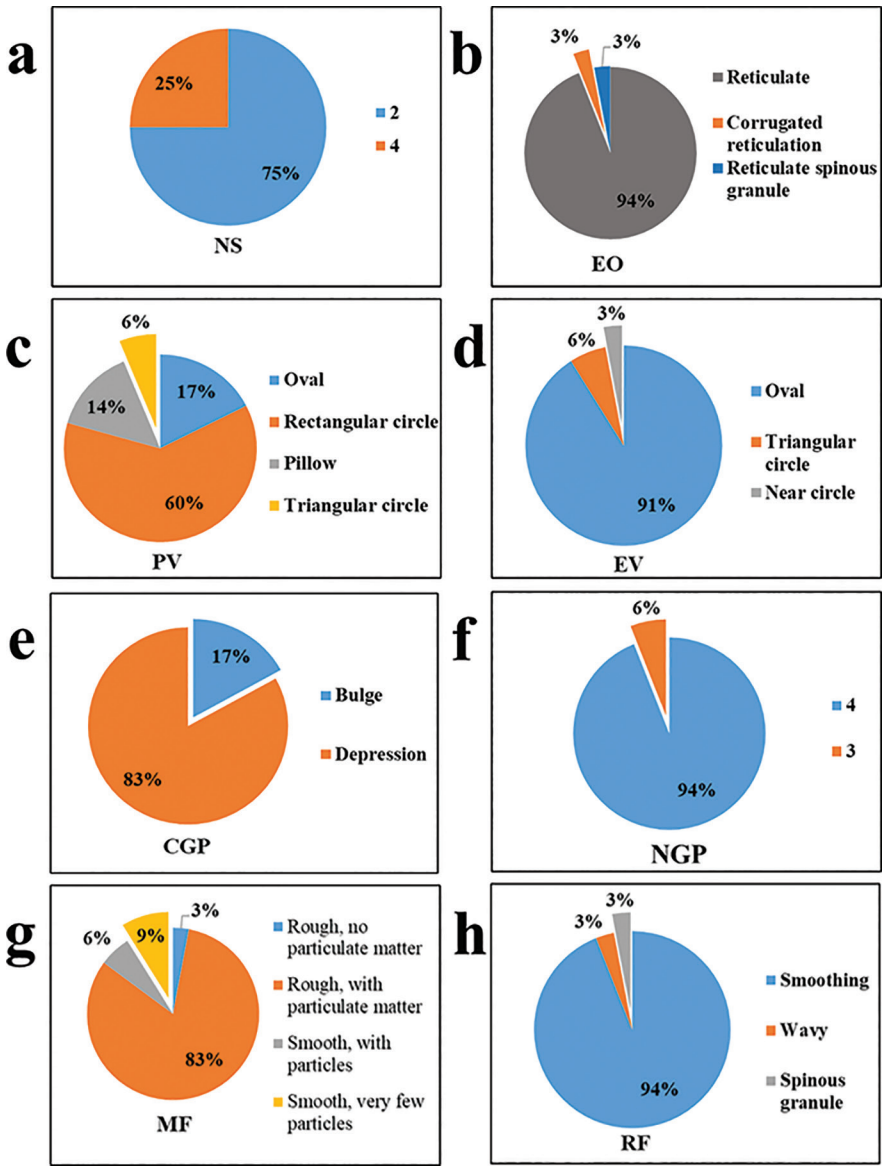

Figure 2. Proportion analysis of pollen characteristics of *Impatiens*. (A) Proportion analysis of SN of *Impatiens*. (B) Analysis on the proportion of EO of *Impatiens.* (C) Percentage analysis of PV morphological characteristics of *Impatiens*. (D) Percentage analysis of EV morphological characteristics of *Impatiens.* (E) CGP proportion analysis of pollen of *Impatiens*. (F) Analysis on the proportion of NGP of *Impatiens*. (G) Analysis on the proportion of MF of pollen. (H) Proportion analysis of reticulate RF of pollen. CGP, characteristics of germination pores; EO, epidermis ornamentation; EV, equatorial view; MF, mesh feature; NGP, number of germination pores; RF, ridge feature; SN, sepal number.

small branch. The clustering results of qualitative and quantitative traits were highly similar to those of the full characteristics (Figure 3). In summary, 35 species of *Impatiens* were systematically clustered with 19 pollen characteristics, and the results were satisfactory.

Impatiens grouping analysis

Based on the quantitative data analysis between groups of *Impatiens* (Figure 5; the data sheet is shown in Annex 3), no significant differences in the quantitative index of germination pore between groups were identified, which further verified that the quantitative index of the germination hole in the composition matrix played a low role in the classification of *Impatiens*. There were some differences in other quantitative indices among the groups, but there was no difference in the quantitative characteristics of pollen in some *Impatiens* groups, such as the *Impatiens* and *Racemosae*. The combination of Figure 4 showed that the pollen characteristics of *Impatiens* had some significance in the grouping of *Impatiens*.

The situation of the *Impatiens*, *Uniflorae* and *Fasciculatae* groups was relatively clear, but there was a relatively large difference between the two types of pollen in the *Scorpioidae* group owing to the unique pollen characteristics of *I. yui*. The similarity of the

Table 2. Analysis on the characteristics of pollen quantitative index of *Impatiens.*

Index	Average value	Maximum value	Minimum value
Polar axis length (P) (μm)	16.55	24.48	13.29
Equatorial major axis length (E1) (μm)	29.49	36.65	23.63
Equatorial short axis length (E2) (μm)	16.39	24.42	12.61
Pollen volume (V) (μm^3)	8277.23	14760.12	3958.54
P/E1	0.57	0.96	0.46
P/E2	1.01	1.23	0.93
E1/E2	1.83	2.22	1.06
GPL (μm)	7.96	11.64	5.37
GPW (μm)	0.38	1.58	0.09
L/W	27.35	105.78	5.45
MD	27.77	64.67	5.33
RW (μm)	0.54	0.94	0.3

GPL, germination pore length; GPW, germination pore width; L/W, germination pore length/germination pore width; MD, mesh density; RW, ridge width.

	NS	EO	PV	EV	NGH	CGP	GPL	GPW	L/W	MF	MD	RF	RW	P	E1	E2	V	P/E1	P/E2	E1/E2
NS		0.33	0.04	0.00	0.00	0.09	0.17	0.14	0.06	0.35	0.00	0.06	0.02	0.01	0.25	0.04	0.06	0.00	0.04	0.01
EO	0.33		0.16	0.06	0.05	0.04	0.28	0.07	0.00	0.34	0.17	0.00	0.12	0.01	0.41	0.00	0.01	0.02	0.18	0.01
PV	0.04	0.16		0.00	0.00	0.09	0.01	0.08	0.15	0.32	0.27	0.01	0.01	0.00	0.41	0.00	0.00	0.00	0.05	0.01
EV	0.00	0.06	0.00		0.00	0.23	0.17	0.45	0.23	0.41	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.04	0.00
NGH	0.00	0.05	0.00	0.00		0.26	0.12	0.46	0.19	0.33	0.01	0.00	0.01	0.00	0.05	0.00	0.00	0.00	0.02	0.00
CGP	0.09	0.04	0.09	0.23	0.26		0.47	0.28	0.04	0.29	0.39	0.28	0.43	0.04	0.08	0.01	0.01	0.30	0.06	0.04
GPL	0.17	0.28	0.01	0.17	0.12	0.47		0.05	0.27	0.15	0.09	0.46	0.44	0.00	0.01	0.01	0.00	0.10	0.13	0.16
GPW	0.14	0.07	0.08	0.45	0.46	0.28	0.05		0.00	0.23	0.36	0.08	0.29	0.23	0.23	0.12	0.12	0.43	0.18	0.28
L/W	0.06	0.00	0.15	0.23	0.19	0.04	0.27	0.00		0.48	0.14	0.00	0.20	0.01	0.13	0.00	0.00	0.10	0.17	0.03
MF	0.35	0.34	0.32	0.41	0.33	0.29	0.15	0.23	0.48		0.04	0.34	0.01	0.42	0.47	0.23	0.35	0.43	0.09	0.16
MD	0.00	0.17	0.27	0.01	0.01	0.39	0.09	0.36	0.14	0.04		0.31	0.00	0.07	0.34	0.09	0.16	0.04	0.38	0.07
RF	0.06	0.00	0.01	0.00	0.00	0.28	0.46	0.08	0.00	0.34	0.31		0.01	0.00	0.27	0.00	0.00	0.00	0.14	0.00
RW	0.02	0.12	0.01	0.00	0.01	0.43	0.44	0.29	0.20	0.01	0.00	0.01		0.00	0.32	0.00	0.01	0.01	0.48	0.02
P	0.01	0.01	0.00	0.00	0.00	0.04	0.00	0.23	0.01	0.42	0.07	0.00	0.00		0.04	0.00	0.00	0.00	0.25	0.00
E1	0.25	0.41	0.41	0.04	0.05	0.08	0.01	0.23	0.13	0.47	0.34	0.27	0.32	0.04		0.01	0.00	0.02	0.02	0.07
E2	0.04	0.00	0.00	0.00	0.00	0.01	0.01	0.12	0.00	0.23	0.09	0.00	0.00	0.00	0.01		0.00	0.00	0.06	0.00
V	0.06	0.01	0.00	0.00	0.00	0.01	0.00	0.12	0.00	0.35	0.16	0.00	0.01	0.00	0.00	0.00		0.00	0.14	0.00
P/E1	0.00	0.02	0.00	0.00	0.00	0.30	0.10	0.43	0.10	0.43	0.04	0.00	0.01	0.00	0.02	0.00	0.00		0.02	0.00
P/E2	0.04	0.18	0.05	0.04	0.02	0.06	0.13	0.18	0.17	0.09	0.38	0.14	0.48	0.25	0.02	0.06	0.14	0.02		0.40
E1/E2	0.01	0.01	0.01	0.00	0.00	0.04	0.16	0.28	0.03	0.16	0.07	0.00	0.02	0.00	0.07	0.00	0.00	0.00	0.40	

Figure 3. Correlation analysis of pollen characteristics. More intense colour indicates a higher correlation. CGP, characteristics of germination pores; E1, equatorial major axis length; E2, equatorial short axis length; EO, epidermis ornamentation; EV, equatorial view; GPL, germination pore length; GPW, germination pore width; L/W, germination pore length/germination pore width; MD, mesh density; MF, mesh feature; NS, number of sepals; P, polar axis length; PV, polar view; RF, ridge feature; RW, ridge width.

Clavicarpa was low. Interestingly, there was also a high degree of similarity among the six species of *Impatiens* that were not grouped. The grouping analysis of the similarity between *Impatiens* further determined the classification between them, verified the results of systematic clustering and supported the superiority of *Impatiens* grouping.

DISCUSSION

Impatiens germplasm resources

Impatiens is rich in germplasm resources, and there are approximately 120 species in the Yunnan–Guizhou Plateau (Luo et al., 2022). In this study of some areas of Yunnan and Guizhou, 48 species of *Impatiens* were collected, which is, of course, only a fraction of the local species. During the process of collection, the preliminary investigation of *Impatiens* shows that the there was a high interspecific coefficient of variation of *Impatiens*, particularly in the variation of flower colour. Three species of Lycos's with different flower colours, and five species of Lycos's with different flower colours were found. Their names were marked with 1, 2, 3 and 4 to distinguish them. The variation in height of *Impatiens* has been confirmed for a long time (Chen, 1978).

Pollen characteristics of Impatiens

We observed the pollen of *Impatiens* (Figure 1 and Figure 2). The results showed that the pollen of *Impatiens*

Table 3. Principal component analysis table of pollen micromorphological characteristics of *Impatiens*.

Bartlett sphericity test		$F = 0.000$				
Composition		1	2	3	4	5
Contribution rate (%)	Contribution rate	29.53	15.53	13.90	10.97	10.53
	Cumulative contribution rate	29.53	45.06	58.96	69.93	80.45
Characteristic index	P	0.92	0.13	0.29	−0.10	−0.05
	P/E1	0.87	−0.37	−0.04	0.12	−0.23
	E2	0.87	0.38	0.15	−0.21	−0.10
	EV	0.85	−0.45	−0.03	0.04	−0.09
	E1/E2	−0.85	0.07	0.16	0.00	0.37
	NGP	0.84	−0.45	0.01	0.12	−0.05
	V	0.79	0.45	0.32	−0.21	0.02
	RF	0.74	0.16	−0.39	0.30	−0.02
	PV	0.68	−0.21	0.28	0.14	0.34
	RW	0.56	−0.09	−0.21	−0.51	0.20
	EO	0.54	0.47	−0.33	0.45	−0.06
	NS	0.53	−0.33	−0.06	−0.09	0.43
	E1	0.05	0.70	0.51	−0.33	0.29
	P/E2	0.09	−0.65	0.28	0.30	0.15
	CGP	0.18	0.58	−0.07	−0.13	−0.52
	L/W	0.48	0.57	−0.31	0.38	0.28
	GPL	0.33	0.12	0.81	0.15	0.02
	MD	−0.33	0.39	0.21	0.67	−0.08
	MF	0.08	0.05	−0.35	−0.63	−0.01
	GPW	−0.22	−0.35	0.42	−0.10	−0.58

CGP, characteristics of germination pores; EO, epidermis ornamentation; EV, equatorial view; GPL, germination pore length; GPW, germination pore width; L/W, germination pore length/germination pore width; MD, mesh density; MF, mesh feature; NS, number of sepals; PV, polar view; RF, ridge feature; RW, ridge width.

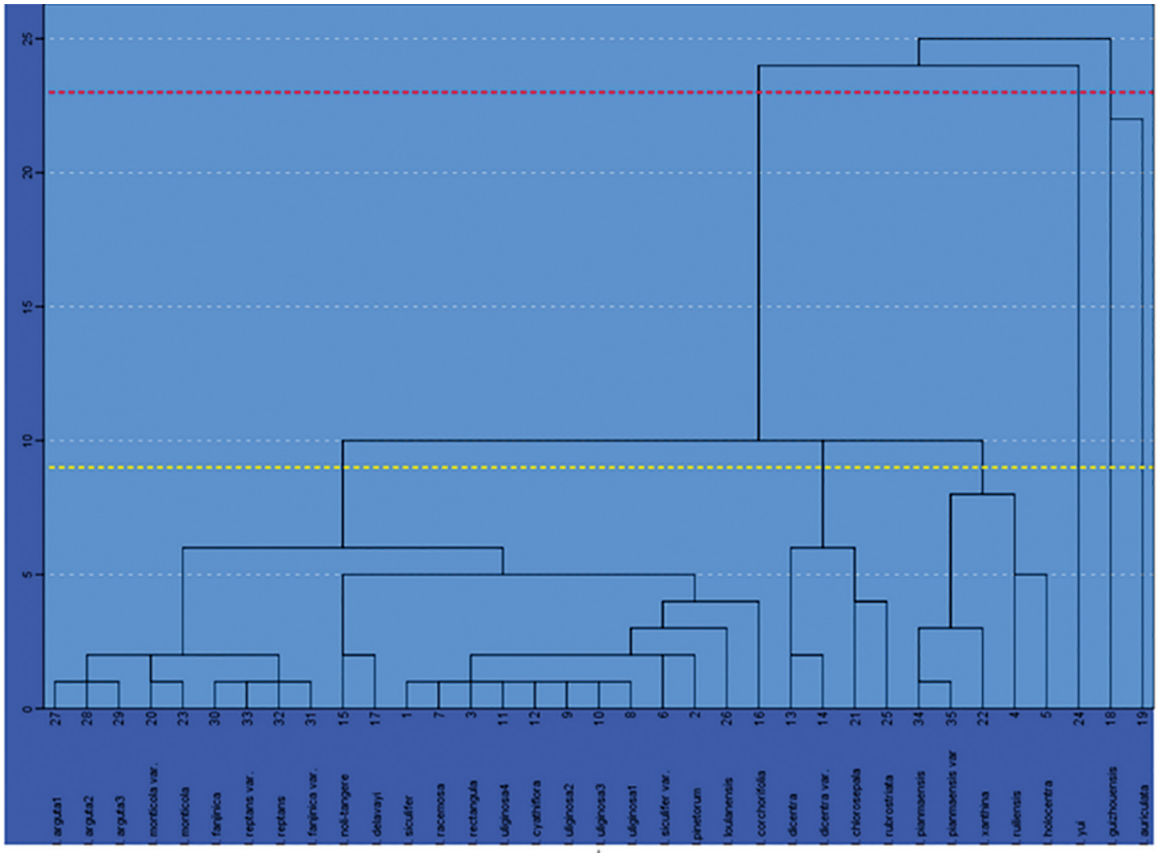

Figure 4. Phylogenetic tree of pollen micromorphology of *Impatiens* based on systematic cluster analysis. The red dotted line is 23, and the yellow dashed line is 9. The serial number values are consistent with those in Table 1.

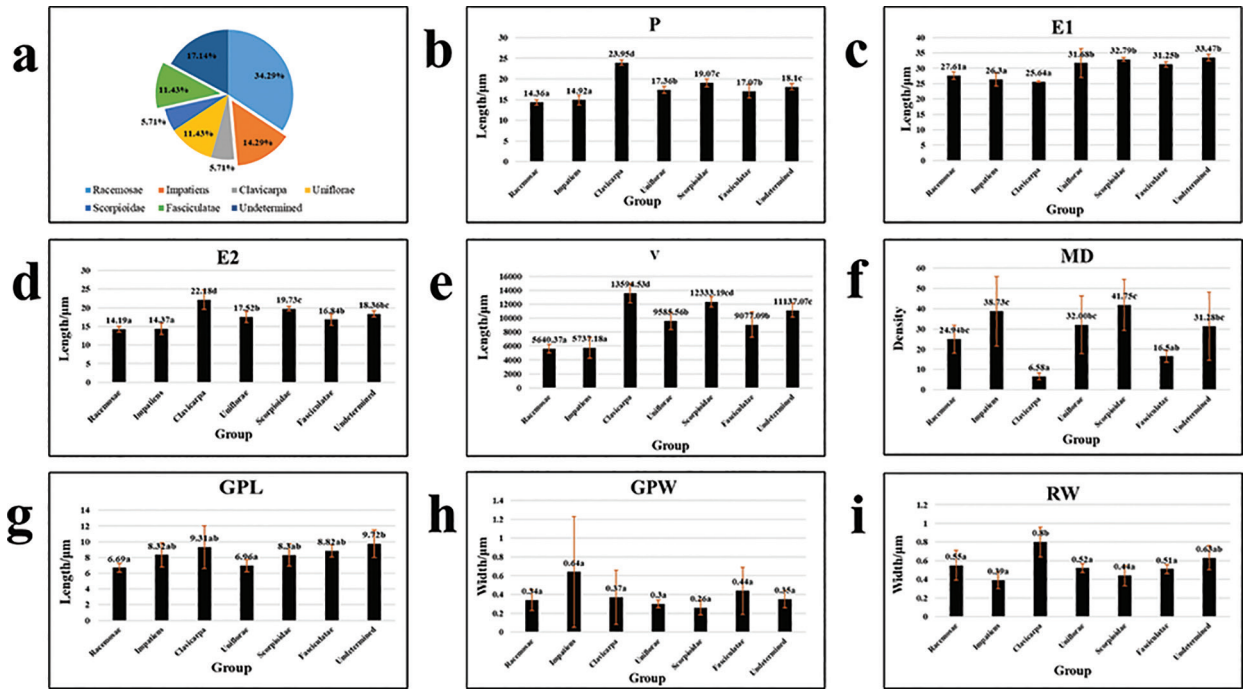

Figure 5. Based on grouping analysis of Impatiens. (a) Proportion of 35 species of Impatiens. (b) Analysis of inter-group differences based on polar axis length (P). (c) Analysis of the difference between groups based on the equatorial major axis length (E1). (d) Analysis of differences between groups based on the length of equatorial minor axis length (E2). (e) Analysis of differences between groups based on pollen volume (V). (f) Analysis of differences between groups based on MD. (g-h) Analysis of the difference between groups based on the length and width of Germinating pore (GPL/GPW). (i) Analysis of difference between groups based on RW. All abbreviations used in the table must be explained, also letters (a–c). MD, mesh density; RW, ridge width.

was primarily divided into three-groove and four-groove types, and there was a significant correlation between the number of pollen germination pores and its morphology (Figure 4). The pollen characteristics included four pollen germination pores of rectangular and oval shapes and three of triangular circles. Interestingly, *Impatiens* with three-groove pollen had four sepals, and *Impatiens* with four-groove pollen had two or four sepals. This phenomenon was used as the difference between primitive and more evolved species of *Impatiens* plants. As shown, the pollen of the original species of *Impatiens* was the three-groove type with four sepals; the over-species of *Impatiens* pollen was the four-groove type with four sepals, and the evolutionary species of *Impatiens* pollen included the four-groove types with two sepals (Yu, 2012; Yu et al., 2016). The EO of pollen was the reticulate type (Janssens et al., 2012), and the MD of evolutionary species was higher than that of the original species. The addition of reticulation on the pollen surface could contribute to plant pollination and fertilisation and protect the genetic material in plant pollen. The reticulate pattern increases the structural strength and surface friction coefficient of pollen, which helps them interact with insects and increases the ability of insects to carry the pollen (Attique et al., 2022). In our classification based on pollen quantitative characteristics, we found that polar axis length, equatorial axis length and pollen size played an important role in the classification of *Impatiens*, which was similar to the results of most plant pollen studies (Zafar et al., 2022).

The effect of pollen characteristics on the classification of Impatiens

The PCA showed that 11 of the 19 pollen characteristics were more effective than the NS in the classification of *Impatiens* (Figure 2 and Table 2), indicating that the pollen characteristics of *Impatiens* play an important role in the classification of *Impatiens*. Pollen characteristics played an important role in plant classification during the research and development of palynology and have been applied to the classification of many plants (Khansari et al., 2012; Pérez-Gutiérrez et al., 2015; Ullah et al., 2022). During the process of interspecific clustering (Figure 4), the results of pollen classification of *Impatiens* were satisfactory, but the difference between *Impatiens* that was highly similar was too low to subdivide some *Impatiens* that only differed slightly. During the study of pollen morphology, it was also explained that the characteristics of pollen structure can support the classification between genera and higher levels of taxonomy (Umber et al., 2022). We used pollen characteristics as the basis of interspecific classification, and the results showed that, to some extent, pollen

characteristics supported the interspecific classification of *Impatiens*. In summary, the pollen of *Impatiens* had some positive effect on the classification of *Impatiens*, whether qualitative or quantitative. However, the identification of specific species of *Impatiens* also required the support of other characteristics (Lu, 1991). This also indirectly showed that there was a small difference among some species of *Impatiens*, and the coefficient of variation of *Impatiens* pollen was not as high as those of other morphological indicators (Lens et al., 2005). This has important reference value for the study of ancient plants and plant evolution (Lens et al., 2012).

Analysis of the effect of Impatiens pollen on grouping

Based on the *Impatiens* grouping system proposed by Yu et al. (2016), the interspecific similarity (Figure 6) and grouping of *Impatiens* were analysed using pollen morphological characteristics and quantitative data (Figure 7). In this study, the materials that were collected involved two subgenera. There were 33 species of the *Impatiens* subgenus, which can be divided into six groups. In addition to these groups, six species of *Impatiens* did not belong to any group. They were designated undetermined groups. In the grouping, the *Racemosae* group had the most materials with 12 species. According to the interspecific similarity and the grouping of *Impatiens*, all the plants of the subgenus *Clavicarpa* were found to be the three-furrow type with a triangular circle. The pollens of *Impatiens*, *Racemosae* and *Fasciculatae groups* were all rectangular or oval. In the *Scorpioidae* group, there was low similarity between the two species of *Impatiens*, and they also differed greatly in terms of their shape and size. Among the 35 species of *Impatiens*, *I. yui* was the only one with a pollen epidermis. Interestingly, we had not determined that the basic situation of the pollen of the six species of *Impatiens* was highly consistent. The pollen morphology was pillow-shaped, and it was possible that there was some evolutionary relationship between them. Compared with the other groups, the pollen P/E1 of *Impatiens* in the *Uniflorae* group was smaller, and the pollen looked slender. According to the analysis of the grouping form of *Impatiens*, pollen has been demonstrated to play some role in the grouping system proposed by Yu et al. (2016), which supported the classification of the two subgenera. However, there were some differences in pollen morphology among the seven groups of *Impatiens*, and the classification system of *Impatiens* may require further refinement. This is particularly true for the *Scorpioidae* group.

Analysis of the classification results of Impatiens by pollen

The clustering results of 35 species of *Impatiens* showed that compared with the results of molecular markers, the clustering results of the original species were partially consistent with those of molecular markers, and the clustering distance of the same class of *Impatiens* was very short, or directly clustered together. This shows that the pollen morphology is similar to that of molecular markers or other morphological markers in interspecific classification (Yu et al., 2016). With

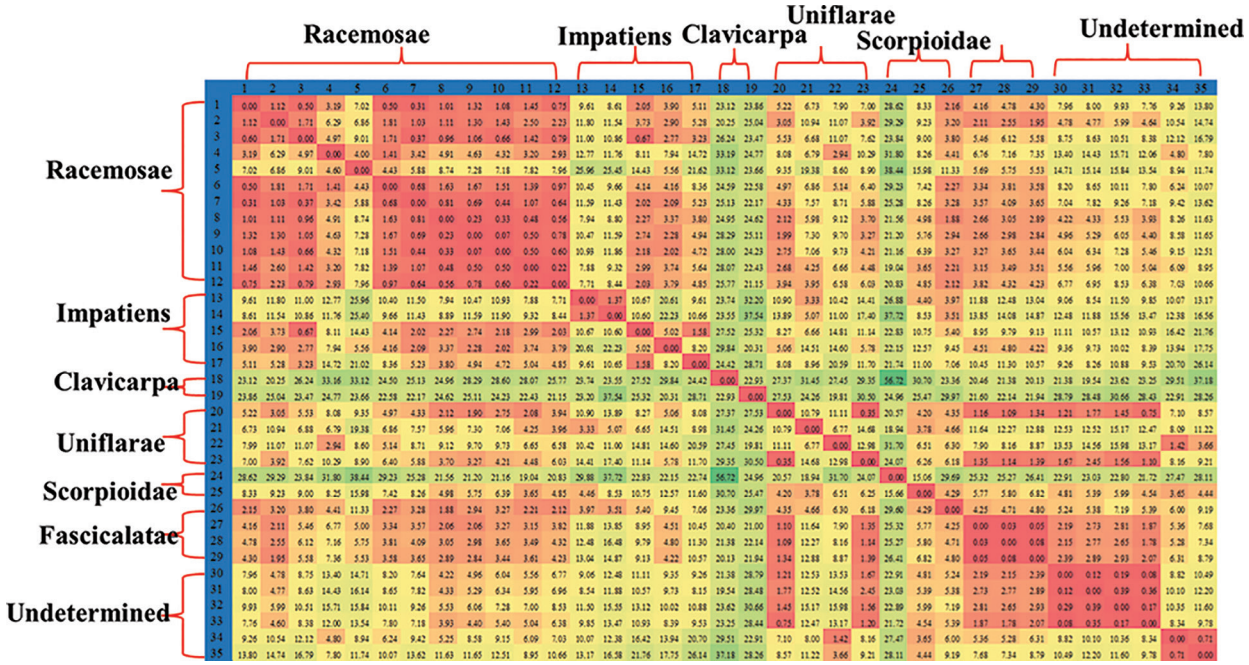

Figure 6. Heat map of interspecific similarity of *Impatiens*. The number in the blue area is the same as the species serial number in Table 1. More intense colour indicates higher similarity.

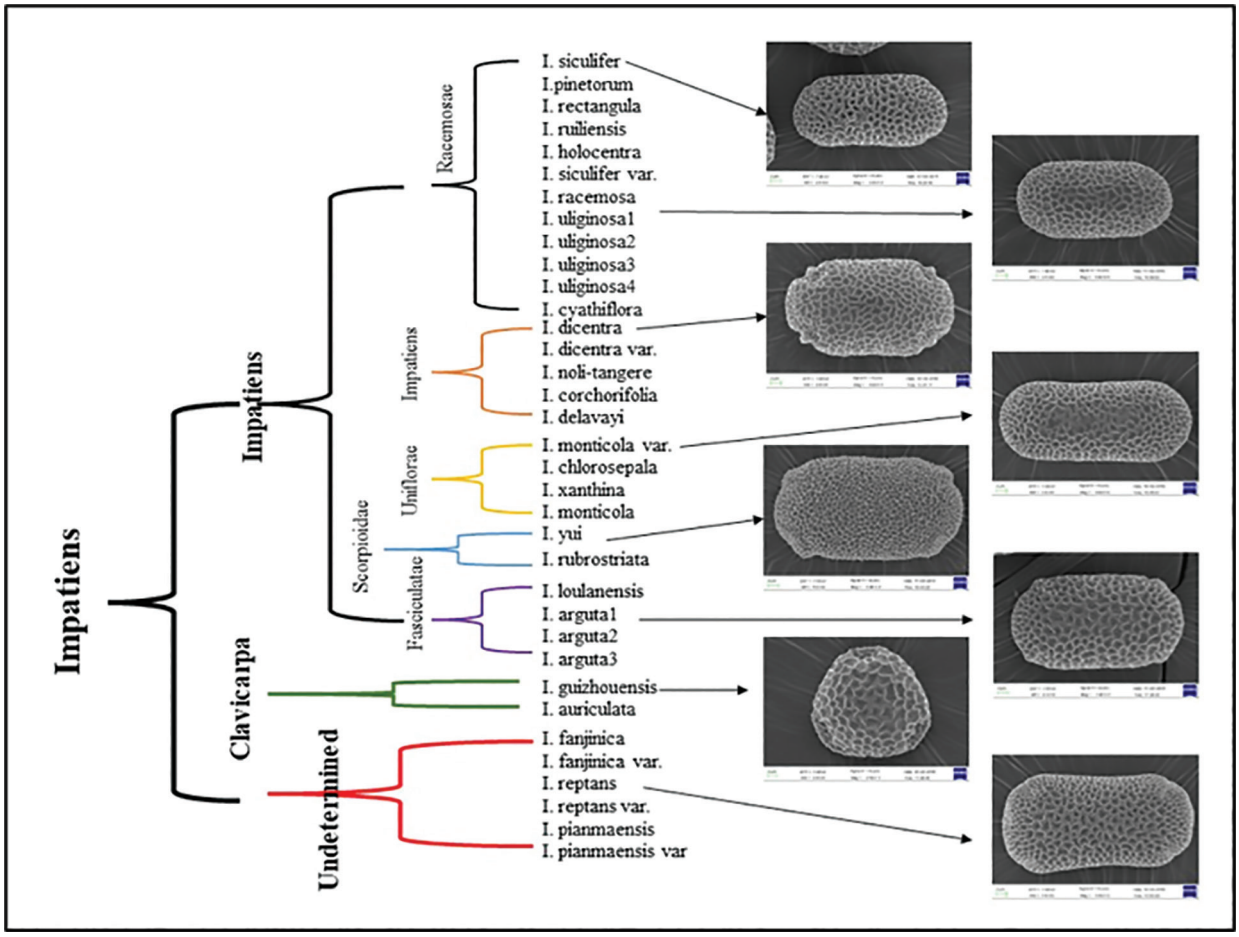

Figure 7. Pollen characteristics of *Impatiens* based on grouping.

the continuous improvement in the molecular marker technology, increasing numbers of researchers utilised the chloroplast genome (Janssens et al., 2006; Luo et al., 2022) or a gene fragment (Eddie et al., 2003; Swenson et al., 2007; Wang et al., 2014) to study the classification of *Impatiens*. The species of *Impatiens* is rich, and it was difficult to make and preserve specimens. The preservation of specimens greatly improved the classification of *Impatiens*. However, there was only a continuous breakthrough when traditional taxonomy was used, and the classification index was continuously refined. The combination of these taxonomic studies with those that utilised molecular markers and other technology overcame the classification problem of *Impatiens* and built a more detailed classification system of balsam.

The traditional taxonomy of *Impatiens* not only includes the observation of pollen by SEM but also involved the classification and analysis of *Impatiens* using micromorphological features, such as the leaf epidermis (Cai, 2007) and seeds (Martínez-Ortega and Rico, 2001; Janssens et al., 2009; Dadandi and Yildiz, 2015). This was a good way to combine macro- and micromorphologies (Yu et al., 2016) to find a more detailed classification system of *Impatiens*, but the refinement and integration of classification indicators should be more flawless.

CONCLUSIONS

The pollen of 35 species of *Impatiens* was studied. The analysis of pollen characteristics indicated that there was some correlation among the traits. A total of 19 pollen characteristics, except for sepals, were counted, and the qualitative characteristics showed that the pollen of *Impatiens* appeared rich. The comparison of quantitative characteristics showed differences among the *Impatiens* species. A PCA with sepals as the reference showed that the characteristic indices of *Impatiens* were highly important for taxonomy. In contrast, the order of taxonomic effect on *Impatiens* was P, P/E1, E2, EV, E1/E2, number of germinating pores, volume, reticulate ridge characteristics, PV, RW and epidermis decoration. The results of the classification of *Impatiens* based on pollen characteristics were reliable, and they showed that the pollen characteristics of *Impatiens* species were significant to the interspecific classification of *Impatiens*. However, the analysis of interspecific similarity showed that relying on the characteristics of pollen was not enough to support the clear classification of *Impatiens*.

Under the condition of *Impatiens* grouping, it was concluded that the new *Impatiens* classification system was reliable, but there were some shortcomings. Thus, it was necessary to further improve the classification system. In summary, pollen micromorphology played a positive role in the classification of *Impatiens*, but it also had some deficiencies.

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AUTHOR CONTRIBUTIONS

H.H. – data analysis and article writing. H.H. and H.M. – article instructor. Other authors participate in the collection and processing of samples.

CONFLICT OF INTEREST

The authors declare no conflict of interest about the publication of this research work.

REFERENCES

Attique, R., Zafar, M., Ahmad, M., Zafar, S., Ghufran, M., Mustafa, M. R., Yaseen, G., Ahmad, L., Sultana, S., Nabila, Zafar, A., and Majeed, S. (2022). Pollen morphology of selected melliferous plants and its taxonomic implications using microscopy. *Microscopy Research and Technique*, *85*, 2361–2380.

Cai, X. (2007). A study on micromorphological characters of leaf epidermis of six species in *Impatiens* L. *Journal of Natural Science of Hunan Normal University*, *30*, 68–71.

Chen, Y. L. (1978). Notulae de genere *Impatiens* L. florae Sinicae. *Journal of Systematics and Evolution*, *16*(2), 36.

Dadandi, M. Y., and Yildiz, K. (2015). Seed morphology of some *Silene* L. (Caryophyllaceae) species collected from Turkey. *Turkish Journal of Botany, 39*, 280–297.

Eddie, W. M., Shulkina, T., Gaskin, J. F., Haberle, R. C., and Jansen, R. K. (2003). Phylogeny of campanulaceae S. Str. Inferred from its sequences of nuclear ribosomal DNA. *Annals of the Missouri Botanical Garden*, *90*, 554–575.

El Ghazali, G. E. (2022). The genus *Buchenavia* Eichl. and its taxonomic affinity to the genus *Terminalia* L. (Combretaceae): Insight from pollen morphology – A review. *Review of Palaeobotany and Palynology*, *301*, 104644, doi: 10.1016/j.revpalbo.2022.104644.

Hu, Z., Zhao, Y., Zhao, C., and Liu, J. (2020). Taxonomic importance of pollen morphology in *Veratrum* L. (Melanthiaceae) using microscopic techniques. *Microscopy Research and Technique*, *83*, 865–876.

Janssens, S. B., Knox, E. B., Huysmans, S., Smets, E. F., and Merckx, V. S. (2009). Rapid radiation of *Impatiens* (Balsaminaceae) during pliocene and pleistocene: Result of a global climate change. *Molecular Phylogenetics and Evolution*, *52*(3), 806–824.

Janssens, S. B., Wilson, Y. S., Yuan, Y. M., Nagels, A., Smets, E. F., and Huysmans, S. (2012). A total evidence approach using palynological characters to infer the complex evolutionary history of the Asian *Impatiens* (Balsaminaceae). *Taxon*, *61*(2), 355–367.

Janssens, S., Geuten, K., Yuan, Y. M., Song, Y., Küpfer, P., and Smets, E. (2006). Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. *Systematic Botany*, *31*(1), 171–180.

Khansari, E., Zarre, S., Alizadeh, K., Attar, F., Aghabeigi, F., and Salmaki, Y. (2012). Pollen morphology of *Campanula* (Campanulaceae) and allied genera in Iran with special focus on its systematic implication. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *207*(3), 203–211.

Lens, F., Dressler, S., Vinckier, S., Janssens, S., Dessein, S., Van Evelghem, L., and Smets, E. (2005). Palynological variation in balsaminoid Ericales. I. Marcgraviaceae. *Annals of Botany*, *96*(6), 1047–1060.

Lens, F., Eeckhout, S., Zwartjes, R., Smets, E., and Janssens, S. B. (2012). The multiple fuzzy origins of woodiness within Balsaminaceae using an integrated approach. Where do we draw the line? *Annals of Botany*, *109*(4), 783–799.

Lu, Y. Q. (1991). Pollen morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic implications. *Journal of Systematics and Evolution*, *29*(4), 352.

Luo, C., Huang, W., Yer, H., Kamuda, T., Li, X., Li, Y., and Huang, H. (2022). Complete chloroplast genomes and comparative analyses of three ornamental *Impatiens* species. *Frontiers in Genetics*, *13*, 816123, doi: 10.3389/fgene.2022.816123.

Martínez-Ortega, M. M., and Rico, E. (2001). Seed morphology and its systematic significance in some

Veronica species (Scrophulariaceae) mainly from the Western Mediterranean. *Plant Systematics and Evolution*, *228*, 15–32.

Mazari, P., Liu, Q., Khan, M. A., Sadia, S., and Ahmad, L. (2017). Pollen morphology and pollen fertility estimation of three medicinal plant species of *Hypericum* L. from Kaghan Valley, Northern Pakistan. *American Journal of Plant Sciences*, *8*(12), 3073.

Mezzonato-Pires, A. C., De Souza Freitas, G. H. G., Mendonça, C. B. F., and Gonçalves-Esteves, V. (2022). The systematic value of pollen morphology in the tribe Paropsieae (Passifloraceae sensu stricto). *Review of Palaeobotany and Palynology*, *299*, 104606, doi: 10.1016/j.revpalbo.2022.104606.

Pérez-Gutiérrez, M. A., Suárez-Santiago, V. N., Fernández, M. C., Bonillo, M. J. S., and Romero-Garcia, A. T. (2015). Pollen morphology and post-tetrad wall development in the subfamily Fumarioideae (Papaveraceae). *Review of Palaeobotany and Palynology*, *222*, 33–47.

Raza, J., Ahmad, M., Zafar, M., Athar, M., Sultana, S., Majeed, S., and Hussain, A. (2020). Comparative foliar anatomical and pollen morphological studies of Acanthaceae using light microscope and scanning electron microscope for effective microteaching in community. *Microscopy Research and Technique*, *83*(9), 1103–1117.

Swenson, U., Bartish, I. V., and Munzinger, J. (2007). Phylogeny, diagnostic characters and generic limitation of Australasian Chrysophylloideae (Sapotaceae, Ericales): Evidence from ITS sequence data and morphology. *Cladistics*, *23*(3), 201–228.

Tuler, A. C., Da Silva, T., Carrijo, T. T., Garbin, M. L., Mendonça, C. B., Peixoto, A. L., and Gonçalves-Esteves, V. (2017). Taxonomic significance of pollen morphology for species delimitation in Psidium (Myrtaceae). *Plant Systematics and Evolution*, *303*, 317–327.

Ullah, F., Ahmad, M., Zafar, M., Parveen, B., Ashfaq, S., Bahadur, S., and Luqman, M. (2022). Pollen morphology and its taxonomic potential in some selected taxa of Caesalpiniaceae observed under light microscopy and scanning electron microscopy. *Microscopy Research and Technique*, *85*(4), 1410–1420.

Umber, F., Zafar, M., Ullah, R., Bari, A., Khan, M. Y., Ahmad, M., and Sultana, S. (2022). Implication of light and scanning electron microscopy for pollen morphology of selected taxa of family Asteraceae and Brassicaceae. *Microscopy Research and Technique*, *85*(1), 373–384.

Wang, W., Li, H., and Chen, Z. (2014). Analysis of plastid and nuclear DNA data in plant phylogenetics-evaluation and improvement. *Science China Life Sciences*, *57*, 280–286.

Yu, S. X. (2012). *Balsaminaceae of China*. Beijing: Peking University Press.

Yu, S. X., Janssens, S. B., Zhu, X. Y., Lidén, M., Gao, T. G., and Wang, W. (2016). Phylogeny of *Impatiens* (Balsaminaceae): Integrating molecular and morphological evidence into a new classification. *Cladistics*, *32*(2), 179–197.

Yuan, Y. M., Song, Y. I., Geuten, K., Rahelivololona, E., Wohlhauser, S., Fischer, E., and Küpfer, P. (2004). Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon*, *53*(2), 391–404.

Zafar, A., Zafar, M., Ahmad, M., Khan, A. M., Mahmood, T., Kilic, O., Fatima A., Habib D., Sultana S., Majeed S., Attique R., and Nabila. (2022). Microscopic (LM and SEM) visualization of pollen ultrastructure among honeybee flora from lower M argalla H ills and allied areas. *Microscopy Research and Technique*, *85*(10), 3325–3338.

Zavada, M. S., and Hackley, P. C. (2022). The effect of diagenesis and acetolysis on the preservation of morphology and ultrastructural features of pollen. *Review of Palaeobotany and Palynology*, *302*, 104679, doi: 10.1016/j.revpalbo.2022.104679.

Zavialova, N., and Nosova, N. (2019). The morphology and ultrastructure of Jurassic in situ ginkgoalean pollen. *Geobios*, *53*, 77–85.

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ANNEX 1

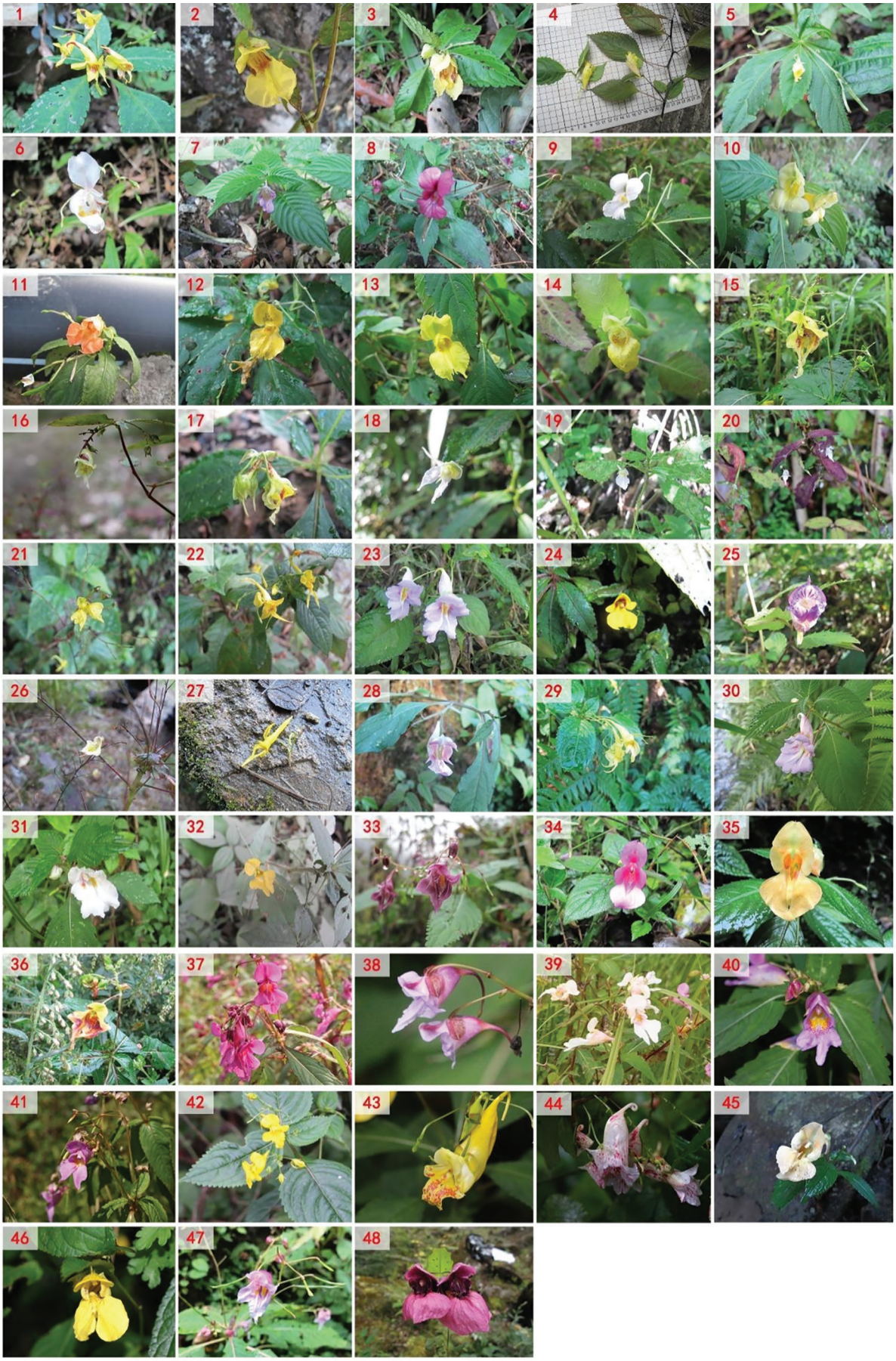
Supplementary Table 1. Information of 48 species of *Impatiens* in the Yunnan–Guizhou area.

N	Species	T	Area	Latitude and longitude	Height (cm)	Altitude (m)	Habitat characteristics
1	*Impatiens.siculifer*	2017.10	Fanjing Mountain, Guizhou	E108.700569, N27.919654	40–50	530	Ditch edge
2	*Impatiens.dicentra*	2017.10	Fanjing Mountain, Guizhou	E108.742382, N27.868716	40–120	530	Ditch edge
3	*Impatiens.dicentra var1.*	2017.10	Fanjing Mountain, Guizhou	E108.721257, N27.892699	30–40	530	Ditch edge
5	*Impatiens lasiophyton*	2017.10	Fanjing Mountain, Guizhou	E108.768975, N27.809401	30–50	870	Roadside
4	*Impatiens.dicentra var2.*	2017.10	Fanjing Mountain, Guizhou	E108.792546, N27.802755	30–50	530	Ditch edge
6	*Impatiens guizhouensis*	2017.10	Fanjing Mountain, Guizhou	E108.825316, N27.856424	20–30	870	Damp place
7	*Impatiens stenosepala*	2017.10	Fanjing Mountain, Guizhou	E108.819567, N27.79662	25–35	730	Roadside
8	*Impatiens fanjinica*	2017.10	Fanjing Mountain, Guizhou	E108.809219, N27.856935	30–100	540	Damp place
9	*Impatiens fanjinicavar.*	2017.10	Fanjing Mountain, Guizhou	E108.791396, N27.81707	80–100	610	Damp place
10	*Impatiens monticola*	2017.10	Suiyang, Guizhou	E107.206313, N28.246177	32–74	817	Ditch edge
11	*Impatiens chlorosepala*	2017.10	Suiyang, Guizhou	E107.206323, N28.246167	28–65	817	Roadside
12	*Impatiens reptans*	2017.10	Suiyang, Guizhou	E107.206313, N28.246177	40–70	1560	Roadside
13	*Impatiens reptans var.*	2017.10	Suiyang, Guizhou	E107.206303, N28.246168	30–80	1560	Roadside
14	*Impatiens noli-tangere*	2017.10	Suiyang, Guizhou	E107.206313, N28.246177	20–30	1260	Roadside
15	*Impatiens loulanensis*	2017.10	Liupanshui, Guizhou	E104.768578, N25.980954	80–120	1741	Ditch edge
16	*Unconfirmed species 1*	2017.10	Gaoligong Mountain, Yunnan	E98.718522, N25.964222	60–130	2230	Ditch edge
17	*Impatiens pinetorum*	2017.10	Gaoligong Mountain, Yunnan	E98.721274, N25.963222	60–80	2350	Damp place
18	*Impatiens pianmaensis*	2017.10	Gaoligong Mountain, Yunnan	E98.718522, N25.964222	40–80	2240	Damp place
19	*Impatiens pianmaensis var.*	2017.10	Gaoligong Mountain, Yunnan	E98.719801, N25.959622	40–80	2240	Damp place
20	*Unconfirmed species 2*	2017.10	Gaoligong Mountain, Yunnan	E98.689201, N25.975916	42–75	3001	Damp place
21	*Impatiens rectangula*	2017.10	Gaoligong Mountain, Yunnan	E98.684315, N25.965522	40–80	2177	Roadside
22	*Impatiens ruiliensis*	2017.10	Gaoligong Mountain, Yunnan	E98.872203, N26.925063	40–80	1228	Damp place
23	*Impatiens arguta*	2017.10	Gaoligong Mountain, Yunnan	E98.848631, N26.899806	30–50	1246	Roadside
24	*Impatiens xanthina*	2017.10	Gaoligong Mountain, Yunnan	E98.871053, N26.899291	20–30	1621	Damp place
25	*Impatiens gongshanensis*	2017.10	Gaoligong Mountain, Yunnan	E98.362313, N27.902298	40–60	1929	Damp place

(*Continued*)

Supplementary Table 1. Continued.

N	Species	T	Area	Latitude and longitude	Height (cm)	Altitude (m)	Habitat characteristics
26	*Unconfirmed species 3*	2017.10	Gaoligong Mountain, Yunnan	E98.365702, N27.880526	30–60	2826	Damp place
27	*Impatiens holocentra*	2017.10	Gaoligong Mountain, Yunnan	E98.345292, N27.881675	30–40	2293	Damp place
28	*Impatiens yui*	2017.10	Gaoligong Mountain, Yunnan	E98.345436, N27.877844	20–40	2297	Damp place
29	*Impatiens siculifervar.*	2017.10	Gaoligong Mountain, Yunnan	E98.342849, N27.877077	50–90	1409	Roadside
30	*Impatiens arguta*	2017.10	Gaoligong Mountain, Yunnan	E98.353844, N27.873246	30–60	1321	Ditch edge
31	*Impatiens arguta*	2017.10	Gaoligong Mountain, Yunnan	E98.357869, N27.86507	30–60	1321	Ditch edge
32	*Impatiens racemosa*	2017.10	Gaoligong Mountain, Yunnan	E98.332572, N27.88142	20–60	1522	Roadside
33	*Impatiens cyanantha*	2017.10	Anlong, Yunnan	E105.351878, N25.109422	80–100	1777	Roadside
34	*Impatiens napoensis*	2017.10	Anlong, Yunnan	E105.346129, N25.091099	40–50	1347	Roadside
35	*Impatiens chlorosepala var.*	2017.10	Wangmo, Yunnan	E106.049528, N25.215218	30–50	821	Damp place
36	*Impatiens loulanensisvar.*	2017.10	Anlong, Yunnan	E105.35389, N25.106543	80–120	1741	Roadside
37	*Impatiens uliginosa*	2017.10	Kunming, Yunnan	E102.776044, N25.090989	60–80	2151	Ditch edge
38	*Impatiens uliginosa*	2017.10	Kunming, Yunnan	E102.777769, N25.087062	60–80	2250	Ditch edge
39	*Impatiens uliginosa*	2017.10	Kunming, Yunnan	E102.775397, N25.088502	60–80	2430	Ditch edge
40	*Impatiens uliginosa*	2017.10	Kunming, Yunnan	E102.784224, N25.342856	20–35	2151	Roadside
41	*Impatiens uliginosa*	2017.10	Kunming, Yunnan	E103.197322, N24.648467	40–80	2560	Ditch edge
42	*Impatiens cyathiflora*	2017.10	Kunming, Yunnan	E102.62844, N24.983997	30–50	2500	Damp place
43	*Impatiens auriculata*	2017.10	Kunming, Yunnan	E102.630165, N24.982949	40–80	2460	Damp place
44	*Impatiens rubrostriata*	2017.10	Wenshan, Yunnan	E104.76302, N23.131053	34–45	2433	Damp place
45	*Impatiens monticola var.*	2017.10	Wenshan, Yunnan	E104.750947, N23.127863	46–85	2628	Roadside
46	*Impatiens corchorifolia*	2017.10	Wenshan, Yunnan	E104.774518, N23.13318	55–68	2227	Roadside
47	*Unconfirmed species 4*	2017.10	Wenshan, Yunnan	E104.622741, N23.131053	30–54	2475	Damp place
48	*Impatiens delavayi*	2017.10	Wenshan, Yunnan	E104.610093, N23.108186	54–63	2765	Damp place



Supplementary Figure 1. Flower morphology of 48 species, including varieties, of *Impatiens* from Yunnan and Guizhou. The serial number is the same as that of attached Supplementary Table 1.

ANNEX 2

Supplementary Table 2. Pollen size characteristics of *Impatiens* in the Yunnan–Guizhou Plateau.

N	Size						
	P (μm)	E1 (μm)	E2 (μm)	V (μm^3)	P/E1	P/E2	E1/E2
1	13.62 ± 0.04	26.35 ± 0.05	13.25 ± 0.02	4754.72 ± 27.43	0.52 ± 0.02	1.03 ± 0	1.99 ± 0.03
2	15.21 ± 0.60	28.92 ± 0.02	13.64 ± 0.03	5997.89 ± 239.4	0.53 ± 0.02	1.11 ± 0.05	2.12 ± 0.01
3	13.65 ± 0.02	25.35 ± 0.04	13.02 ± 0.03	4504.63 ± 7.64	0.54 ± 0.01	1.05 ± 0	1.94 ± 0.01
4	13.52 ± 0.02	26.48 ± 0.04	14.22 ± 0.03	5093.98 ± 11.04	0.51 ± 0.00	0.95 ± 0	1.86 ± 0.00
5	14.92 ± 0.04	27.64 ± 0.02	14.27 ± 0.03	5886.88 ± 21.83	0.54 ± 0.02	1.05 ± 0.01	1.94 ± 0.01
6	14.28 ± 0.02	26.98 ± 0.03	13.95 ± 0.02	5377.12 ± 16.82	0.53 ± 0.01	1.02 ± 0	1.93 ± 0.01
7	15.13 ± 0.03	27.65 ± 0.02	13.97 ± 0.02	5843.45 ± 4.87	0.55 ± 0.01	1.08 ± 0.01	1.98 ± 0.00
8	14.82 ± 0.03	29.12 ± 0.04	14.73 ± 0.04	6354.67 ± 15.26	0.51 ± 0.01	1.01 ± 0.01	1.98 ± 0.01
9	13.92 ± 0.03	29.12 ± 0.02	14.15 ± 0.03	5735.06 ± 20.11	0.48 ± 0.00	0.98 ± 0.01	2.06 ± 0.01
10	13.84 ± 0.04	28.35 ± 0.03	14.05 ± 0.04	5514.04 ± 20.92	0.49 ± 0.00	0.98 ± 0.01	2.02 ± 0.01
11	14.64 ± 0.03	28.54 ± 0.01	15.74 ± 0.02	6578.75 ± 19.17	0.51 ± 0.01	0.93 ± 0	1.81 ± 0.00
12	14.75 ± 0.01	26.84 ± 0.03	15.26 ± 0.03	6043.21 ± 4.99	0.55 ± 0.00	0.97 ± 0.01	1.76 ± 0.01
13	16.6 ± 0.03	28.24 ± 0.02	16.59 ± 0.02	7776.2 ± 14.94	0.59 ± 0.01	1 ± 0	1.7 ± 0.01
14	15.15 ± 0.01	28.18 ± 0.04	14.97 ± 0.02	6391.76 ± 7.03	0.54 ± 0.00	1.01 ± 0	1.88 ± 0.01
15	13.29 ± 0.02	23.63 ± 0.01	12.61 ± 0.03	3958.54 ± 16.44	0.56 ± 0.01	1.05 ± 0.01	1.87 ± 0.01
16	14.96 ± 0.02	27.09 ± 0.05	14.42 ± 0.04	5841.87 ± 21.14	0.55 ± 0.01	1.04 ± 0.01	1.88 ± 0.01
17	14.61 ± 0.04	24.37 ± 0.04	13.25 ± 0.03	4717.5 ± 19.7	0.6 ± 0.00	1.1 ± 0.01	1.84 ± 0.01
18	24.48 ± 0.01	25.5 ± 0.04	19.93 ± 0.03	12440.06 ± 25.84	0.96 ± 0.01	1.23 ± 0.00	1.28 ± 0.00
19	23.44 ± 0.03	25.79 ± 0.02	24.42 ± 0.03	14760.11 ± 30.71	0.91 ± 0.00	0.96 ± 0.00	1.06 ± 0.00
20	16.96 ± 0.03	34.8 ± 0.02	16.83 ± 0.04	9930.21 ± 5.15	0.49 ± 0.00	1.01 ± 0.01	2.07 ± 0.01
21	16.98 ± 0.05	26.97 ± 0.02	16.92 ± 0.01	7749.09 ± 20.81	0.63 ± 0.00	1 ± 0.01	1.59 ± 0.01
22	18.54 ± 0.04	28.3 ± 0.03	19.8 ± 0.03	10386.99 ± 44.18	0.66 ± 0.01	0.94 ± 0.00	1.43 ± 0.00
23	16.96 ± 0.04	36.65 ± 0.55	16.53 ± 0.03	10275.97 ± 166.77	0.46 ± 0.01	1.02 ± 0.01	2.22 ± 0.03
24	19.91 ± 0.05	32.22 ± 0.02	20.24 ± 0.02	12985.32 ± 18.53	0.62 ± 0.00	0.98 ± 0.01	1.59 ± 0.00
25	18.22 ± 0.03	33.34 ± 0.04	19.22 ± 0.03	11674.4 ± 11.48	0.55 ± 0.01	0.95 ± 0.00	1.74 ± 0.01
26	14.62 ± 0.05	30.07 ± 0.02	14.51 ± 0.02	6381.1 ± 22.92	0.49 ± 0.01	1.01 ± 0.01	2.07 ± 0.01
27	17.88 ± 0.02	31.49 ± 0.01	17.66 ± 0.03	9943.29 ± 24.86	0.57 ± 0.00	1.01 ± 0.00	1.78 ± 0.01
28	17.84 ± 0.04	31.97 ± 0.02	17.9 ± 0.02	10206.21 ± 29.62	0.56 ± 0.01	1 ± 0.01	1.79 ± 0.01
29	17.95 ± 0.03	31.49 ± 0.02	17.3 ± 0.08	9777.76 ± 57.43	0.57 ± 0.01	1.04 ± 0.01	1.82 ± 0.01
30	17.96 ± 0.03	33.64 ± 0.03	17.95 ± 0.04	10844.94 ± 34.53	0.53 ± 0.00	1 ± 0.00	1.88 ± 0.01
31	18.09 ± 0.06	32.47 ± 0.03	17.75 ± 0.02	10427.14 ± 37.16	0.56 ± 0.00	1.02 ± 0.01	1.83 ± 0.00
32	17.87 ± 0.03	34.44 ± 0.03	17.97 ± 0.02	11056.38 ± 14.29	0.52 ± 0.01	0.99 ± 0.01	1.92 ± 0.00
33	17.04 ± 0.04	33.25 ± 0.03	17.79 ± 0.03	10078.45 ± 30.12	0.51 ± 0.01	0.96 ± 0.00	1.87 ± 0.01
34	19.13 ± 0.03	32.05 ± 0.02	18.77 ± 0.03	11511.42 ± 33.33	0.6 ± 0.00	1.02 ± 0.00	1.71 ± 0.00
35	18.53 ± 0.03	34.95 ± 0.01	19.92 ± 0.03	12904.06 ± 33.01	0.53 ± 0.01	0.93 ± 0.00	1.75 ± 0.01

The serial number is the same as that of Supplementary Table 1.

Supplementary Table 3. Characteristics of pollen germination pores of *Impatiens* in the Yunnan–Guizhou Plateau.

N	Germinating pore				
	Number	Characteristics	L (μm)	W (μm)	L/W
1	4	Depression	6.16 ± 0.03	0.54 ± 0.02	11.42 ± 0.37
2	4	Depression	6.59 ± 0.04	0.48 ± 0.02	13.84 ± 0.38
3	4	Depression	6.59 ± 0.58	0.29 ± 0.02	22.78 ± 2.62
4	4	Depression	6.2 ± 0.02	0.44 ± 0.03	14.12 ± 0.83
5	4	Depression	7.45 ± 0.02	0.21 ± 0.01	35.51 ± 1.60
6	4	Depression	6.53 ± 0.03	0.45 ± 0.03	14.54 ± 0.82
7	4	Depression	5.37 ± 0.02	0.25 ± 0.02	21.55 ± 1.68
8	4	Depression	7.3 ± 0.02	0.35 ± 0.01	20.68 ± 0.63
9	4	Depression	7.39 ± 0.03	0.28 ± 0.02	26.76 ± 1.4
10	4	Depression	6.87 ± 0.06	0.25 ± 0.02	27.9 ± 1.66
11	4	Depression	6.91 ± 0.03	0.28 ± 0.02	24.78 ± 1.84
12	4	Depression	6.87 ± 0.02	0.29 ± 0.02	23.78 ± 1.71
13	4	Bulge	10.34 ± 0.05	0.84 ± 0.05	12.39 ± 0.61
14	4	Depression	8.59 ± 0.05	1.58 ± 0.04	5.45 ± 0.09
15	4	Depression	6.64 ± 0.03	0.31 ± 0.02	21.21 ± 0.95
16	4	Depression	6.94 ± 0.06	0.11 ± 0.02	61.95 ± 7.62
17	4	Depression	9.1 ± 0.05	0.36 ± 0.01	25.53 ± 0.69
18	3	Depression	11.64 ± 0.03	0.61 ± 0.01	18.98 ± 0.19
19	3	Depression	6.97 ± 0.03	0.12 ± 0.02	57.1 ± 7.09
20	4	Depression	7.54 ± 0.03	0.28 ± 0.03	27.07 ± 2.37
21	4	Bulge	6.04 ± 0.04	0.3 ± 0.03	20.00 ± 1.51
22	4	Depression	6.57 ± 0.02	0.35 ± 0.02	19.00 ± 1.06
23	4	Depression	7.69 ± 0.01	0.26 ± 0.01	29.99 ± 1.30
24	4	Bulge	9.52 ± 0.05	0.09 ± 0.00	105.78 ± 0.51
25	4	Bulge	7.08 ± 0.03	0.23 ± 0.02	30.42 ± 1.83
26	4	Depression	8.72 ± 0.03	0.81 ± 0.02	10.82 ± 0.26
27	4	Depression	8.81 ± 0.03	0.32 ± 0.02	27.28 ± 1.18
28	4	Depression	8.93 ± 0.02	0.31 ± 0.01	28.51 ± 0.52
29	4	Depression	8.83 ± 0.03	0.31 ± 0.01	28.2 ± 0.56
30	4	Depression	10.23 ± 0.03	0.44 ± 0.04	23.18 ± 1.75
31	4	Depression	11.09 ± 0.03	0.44 ± 0.02	25.24 ± 1.07
32	4	Depression	11.1 ± 0.02	0.37 ± 0.02	29.76 ± 1.2
33	4	Depression	10.89 ± 0.04	0.37 ± 0.02	29.22 ± 1.57
34	4	Bulge	7.52 ± 0.03	0.25 ± 0.02	30.58 ± 2.02
35	4	Bulge	7.49 ± 0.01	0.24 ± 0.03	31.98 ± 3.97

The serial number is the same as that of Supplementary Table 1.

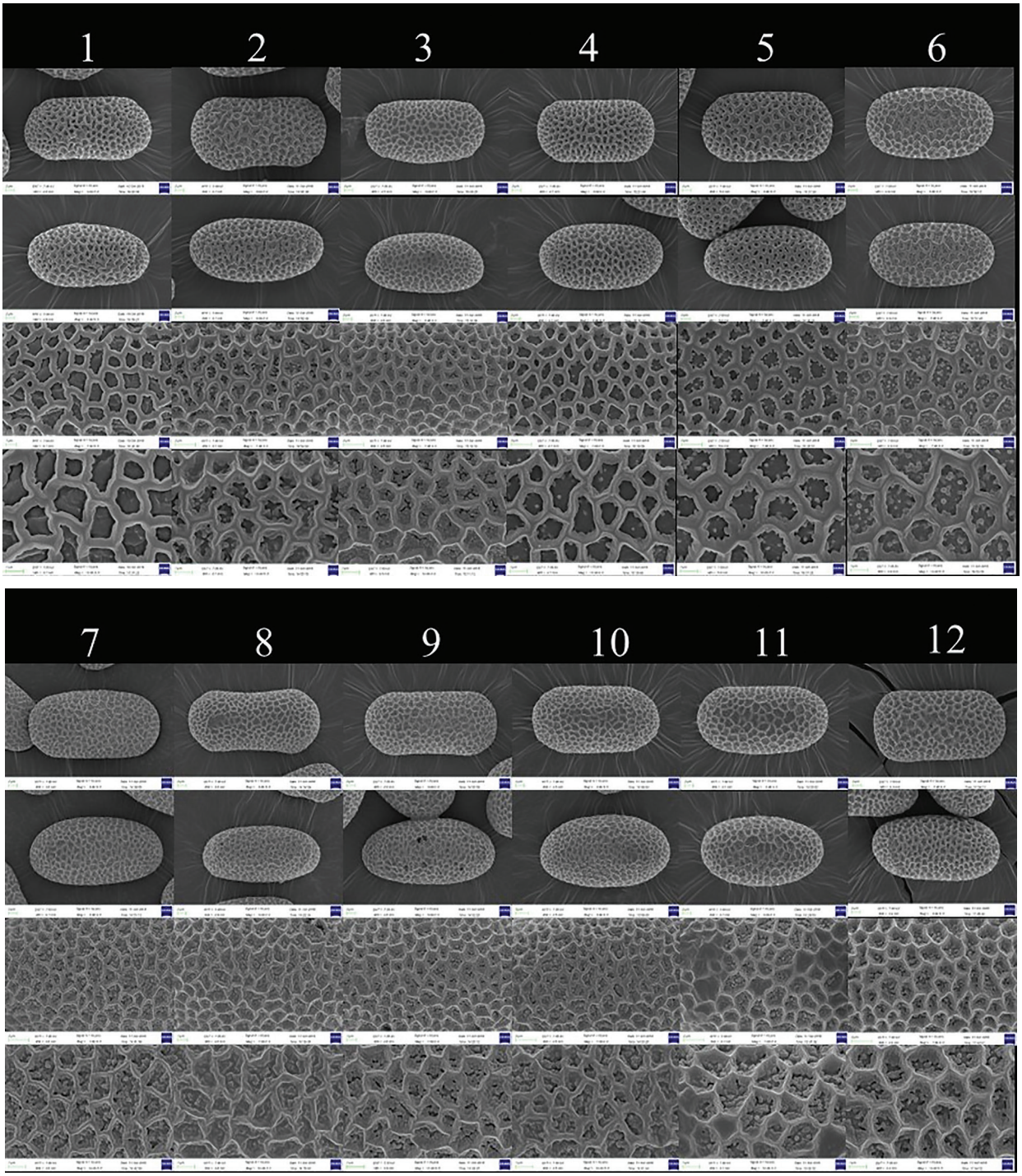
L/W, germination pore length/germination pore width.

Supplementary Table 4. Pollen mesh characteristics of *Impatiens* in the Yunnan–Guizhou Plateau.

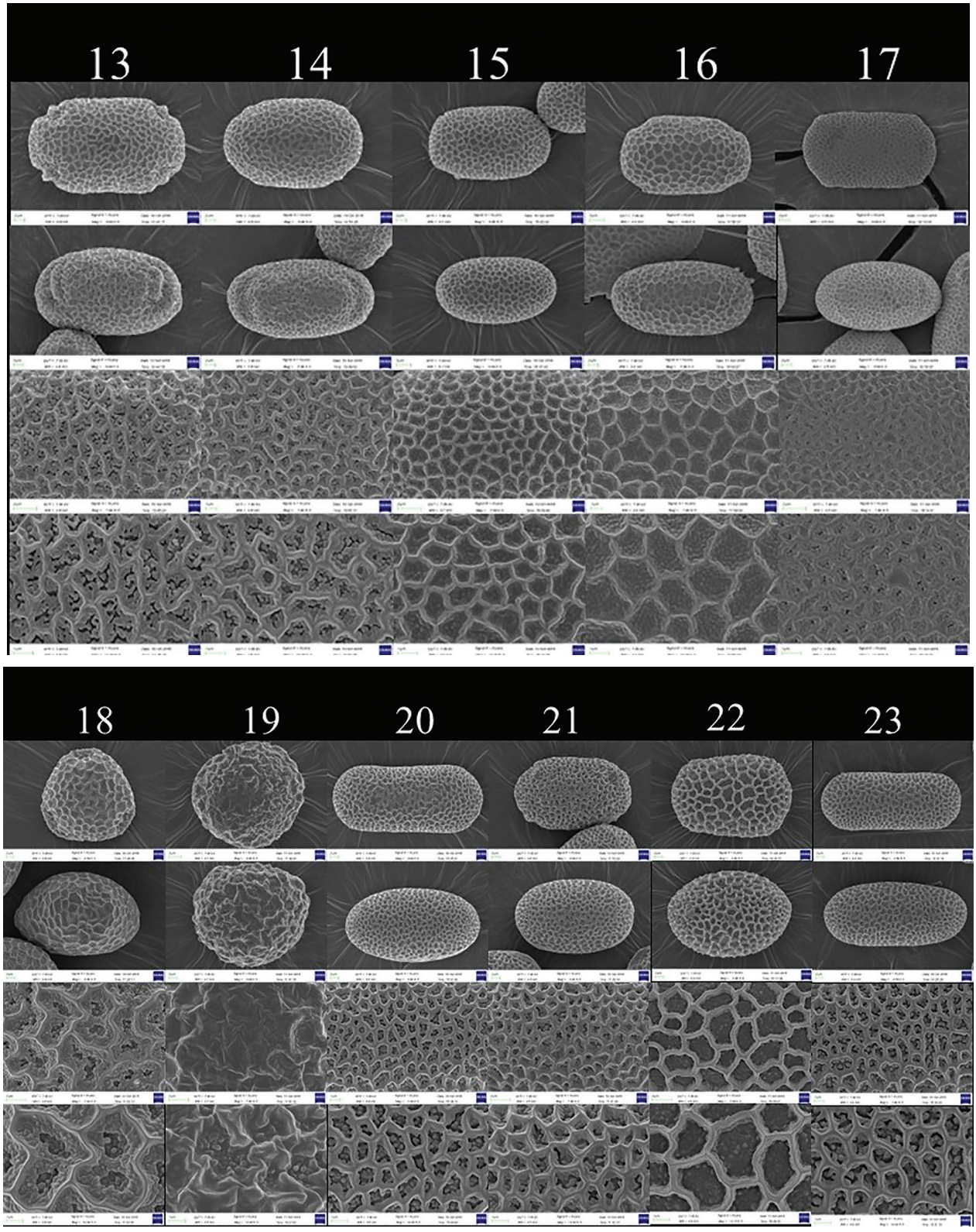
N	MF	MD	RW (μm)	RF
1	Rough, no particulate matter	16.33 ± 0.58	0.72 ± 0.02	Smoothing
2	Rough, with particulate matter	24.33 ± 1.53	0.57 ± 0.02	Smoothing
3	Rough, with particulate matter	33.33 ± 1.53	0.46 ± 0.01	Smoothing
4	Smooth, very few particles	23.33 ± 0.58	0.59 ± 0.01	Smoothing
5	Smooth, very few particles	15.67 ± 0.58	0.88 ± 0.02	Smoothing
6	Rough, with particulate matter	15.00 ± 1.00	0.73 ± 0.02	Smoothing
7	Rough, with particulate matter	20.00 ± 1.00	0.45 ± 0.03	Smoothing
8	Rough, with particulate matter	31.67 ± 0.58	0.39 ± 0.02	Smoothing
9	Rough, with particulate matter	31.00 ± 1.00	0.44 ± 0.02	Smoothing
10	Rough, with particulate matter	30.00 ± 1.00	0.42 ± 0.01	Smoothing
11	Rough, with particulate matter	32.00 ± 1.00	0.44 ± 0.02	Smoothing
12	Rough, with particulate matter	26.67 ± 0.58	0.44 ± 0.01	Smoothing
13	Rough, with particulate matter	28.33 ± 1.53	0.36 ± 0.02	Smoothing
14	Rough, with particulate matter	30.33 ± 1.53	0.53 ± 0.02	Smoothing
15	Rough, with particulate matter	47.33 ± 1.15	0.34 ± 0.01	Smoothing
16	Rough, with particulate matter	23.00 ± 1.00	0.42 ± 0.02	Smoothing
17	Rough, with particulate matter	64.67 ± 2.08	0.3 ± 0.02	Smoothing
18	Rough, with particulate matter	5.33 ± 0.58	0.67 ± 0.02	Smoothing
19	Rough, with particulate matter	8.00 ± 1.00	0.94 ± 0.02	Wavy
20	Rough, with particulate matter	41.67 ± 1.53	0.53 ± 0.01	Smoothing
21	Rough, with particulate matter	43.67 ± 2.08	0.46 ± 0.03	Smoothing
22	Smooth, very few particles	12.67 ± 0.58	0.59 ± 0.02	Smoothing
23	Rough, with particulate matter	30.00 ± 1.00	0.51 ± 0.02	Smoothing
24	Rough, with particulate matter	52.33 ± 3.06	0.54 ± 0.02	Spinous granule
25	Rough, with particulate matter	31.67 ± 2.08	0.35 ± 0.02	Smoothing
26	Rough, with particulate matter	12.00 ± 1.00	0.44 ± 0.02	Smoothing
27	Rough, with particulate matter	17.67 ± 0.58	0.52 ± 0.01	Smoothing
28	Rough, with particulate matter	18.33 ± 0.58	0.54 ± 0.01	Smoothing
29	Rough, with particulate matter	18.00 ± 1.00	0.56 ± 0.03	Smoothing
30	Rough, with particulate matter	42.67 ± 0.58	0.55 ± 0.02	Smoothing
31	Rough, with particulate matter	43.00 ± 1.00	0.54 ± 0.03	Smoothing
32	Rough, with particulate matter	44.33 ± 2.08	0.57 ± 0.02	Smoothing
33	Rough, with particulate matter	38.00 ± 1.00	0.54 ± 0.02	Smoothing
34	Smooth, with particles	10.00 ± 1.00	0.84 ± 0.01	Smoothing
35	Smooth, with particles	9.67 ± 1.53	0.76 ± 0.02	Smoothing

The serial number is the same as that of Supplementary Table 1.

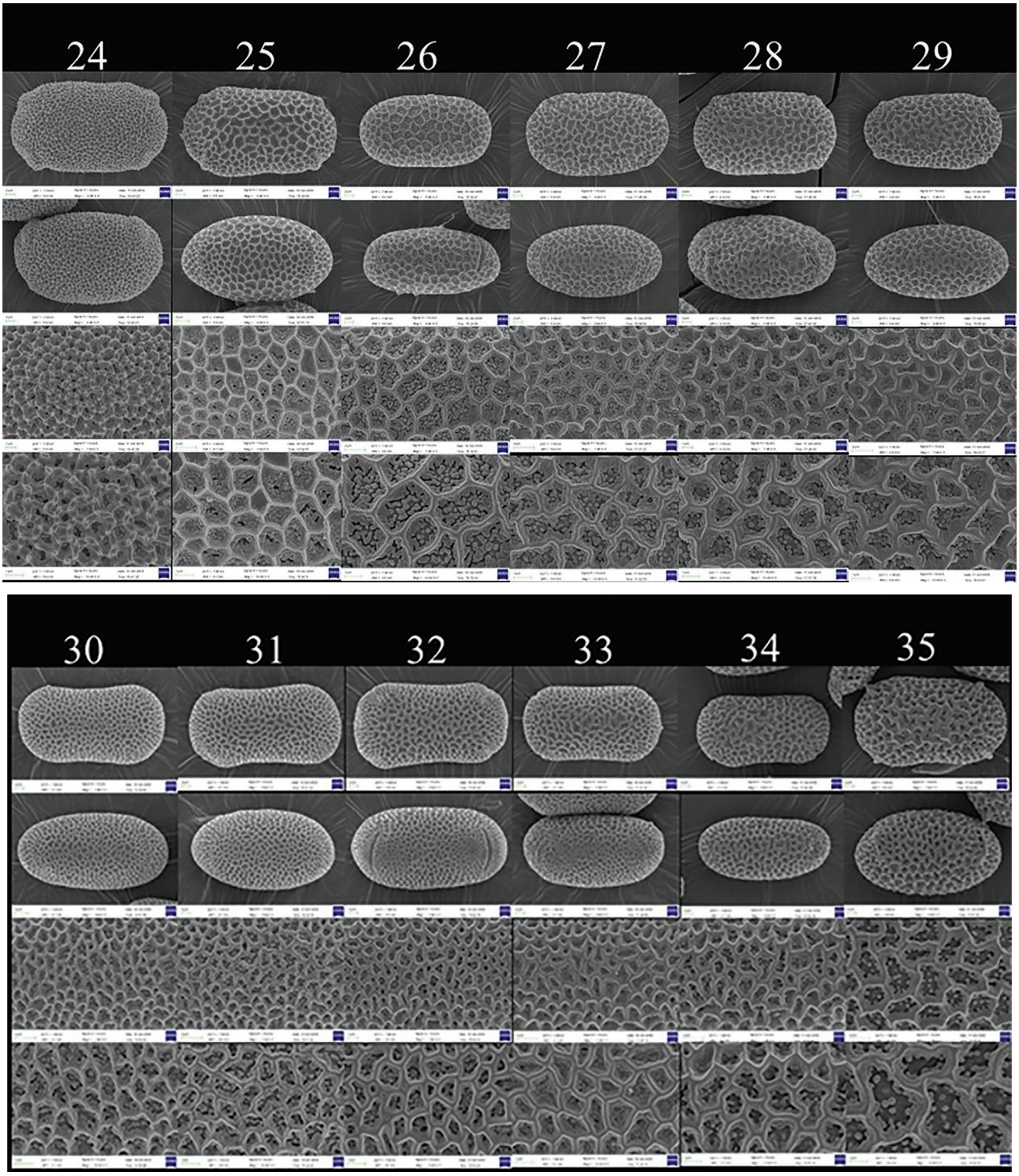
MD, mesh density; MF, mesh feature; RF, ridge feature; RW, ridge width.



Supplementary Figure 2. Continued.



Supplementary Figure 2. Continued.



Supplementary Figure 2. Pollen micromorphological map. From top to bottom, PV (3 000-fold, ruler 2 μm); EV (3 000-fold, ruler 2 μm); epidermis (7 000-fold, ruler 2 μm); epidermis (12 000-fold, ruler 1 μm). PV, polar view.

ANNEX 3

Supplementary Table 5. Based on the grouping category of *Impatiens*: analysis of the difference of quantitative characters of pollen among groups.

Group	Size							MD	RW	Germinating pore		
	P	E1	E2	V	P/E1	P/E2	E1/E2			L	W	L/W
Racemosae	14.36 ± 0.62 a	27.61 ± 1.23 a	14.19 ± 0.77 a	5640.37 ± 621.58 a	0.52 ± 0.02 a	1.01 ± 0.05 ab	1.95 ± 0.1 b	24.94 ± 6.86 bc	0.55 ± 0.16 a	6.69 ± 0.59 a	0.34 ± 0.11 a	21.47 ± 7.05 a
Impatiens	14.92 ± 1.19 a	26.3 ± 2.17 a	14.37 ± 1.55 a	5737.18 ± 1483.27 a	0.57 ± 0.03 a	1.04 ± 0.04 ab	1.84 ± 0.08 b	38.73 ± 17.12 c	0.39 ± 0.09 a	8.32 ± 1.54 ab	0.64 ± 0.59 a	25.31 ± 21.91 a
Clavicarpa	23.95 ± 0.61 d	25.64 ± 0.18 a	22.18 ± 2.61 d	13594.53 ± 1350.08 d	0.94 ± 0.03 b	1.1 ± 0.16 b	1.17 ± 0.13 a	6.58 ± 1.64 a	0.8 ± 0.16 b	9.31 ± 2.7 ab	0.37 ± 0.29 a	38.23 ± 22.35 a
Uniflorae4	17.36 ± 0.79 b	31.68 ± 4.76 b	17.52 ± 1.53 b	9585.56 ± 1239.67 b	0.56 ± 0.1 a	0.99 ± 0.04 ab	1.83 ± 0.37 b	32 ± 14.23 bc	0.52 ± 0.05 a	6.96 ± 0.79 a	0.3 ± 0.04 a	24.02 ± 5.36 a
Scorpioidae	19.07 ± 0.98 c	32.79 ± 0.64 b	19.73 ± 0.58 c	12333.19 ± 753.2 cd	0.58 ± 0.04 a	0.97 ± 0.02 a	1.66 ± 0.08 b	41.75 ± 12.63 c	0.44 ± 0.11 a	8.3 ± 1.41 ab	0.16 ± 0.08 a	68.16 ± 43.37 a
Fasciculatae	17.07 ± 1.63 b	31.25 ± 0.82 b	16.84 ± 1.57 b	9077.09 ± 1805.97 b	0.55 ± 0.04 a	1.01 ± 0.02 ab	1.87 ± 0.14 b	16.5 ± 3.01 ab	0.51 ± 0.05 a	8.82 ± 0.08 ab	0.44 ± 0.25 a	23.7 ± 8.61 a
Undetermined	18.1 ± 0.7 bc	33.47 ± 1.11 b	18.36 ± 0.85 bc	11137.07 ± 997.89 c	0.54 ± 0.03 a	0.99 ± 0.04 ab	1.83 ± 0.08 b	31.28 ± 16.75 bc	0.63 ± 0.13 ab	9.72 ± 1.74 b	0.35 ± 0.09 a	28.33 ± 3.39 a