

Pollen morphology of the genus *Gossypium* and its systematic implications

Xiaoyan Cai (✉ cxycri@163.com)

Chinese Academy of Agricultural Sciences Cotton Research Institute

Yuqing Hou

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Heng Wang

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Yanchao Xu

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Jie Zheng

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Yuhong Wang

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Fang Liu

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Zhongli Zhou

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

Jinping Hua

China Agricultural University College of Agronomy and Biotechnology

Kunbo Wang

CAAS CRI: Chinese Academy of Agricultural Sciences Cotton Research Institute

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Abstract

Background

Plants develop a variety of pollen morphological features during long-term evolution, which are controlled by genes and are not easily affected by the external natural environment. Therefore, pollen morphology has great significance in plant taxonomy, evolution and identification. However, there is no detailed study on the pollen morphology of the *Gossypium* genus although some cotton species have been investigated in the scattered reports. In this study, the pollen morphology of 23 cotton species was comprehensively examined using scanning electron microscopy to evaluate the pollen diversity of the genus and its taxonomic significance.

Results

The common characteristics of *Gossypium* pollen are spherical, radially symmetrical, echinate, panporate and operculate. The pollen diameter ranges from 62.43 μm in *G. harknessii* to 103.41 μm in *G. barbadense*, revealing that there are great variations in pollen size among cotton species. Moreover, the exine sculpture is echinate. The exine echini shape is mostly conical or sharply conical and occasionally rodlike. Echini density is found maximum in *G. incanum* (173) and minimum in *G. gossypoides* (54), meanwhile, echini length varies from 3.53 μm in *G. herbaceum* to 9.47 μm in *G. barbadense*. In addition, all cotton species are divided into three clusters based on cluster analysis, which provides new understanding of the evolution and phylogeny of the *Gossypium* genus.

Conclusion

Although the pollen characteristics alone are insufficient to reconstruct taxonomic and systematic relationships within the genus *Gossypium*, our findings can enrich our knowledge of sporopollen morphology and fill the phenological gap of these taxa and will contribute to future systematic and phylogenetic studies of the *Gossypium* genus.

Introduction

The cotton genus (*Gossypium* L.), as the largest and most widely distributed genus in the *Gossypieae*, has more than 50 species (Fryxell, 1992). This genus contains 46 diploid species ($2n = 2x = 26$), five well-established tetraploid species and two new tetraploid species ($2n = 4x = 52$) (Grover et al., 2014; Wendel and Grover, 2015; Wang et al., 2018). All the diploid cotton species, which may have evolved from a common ancestor around 5–10 million years ago (MYA) that subsequently differentiated cytogenetically into eight genome groups (designated A up to G, and K) that differ in DNA content and chromosome size but not in chromosome number. These diploid species mainly distribute in the tropic and subtropic regions. The tetraploid cotton is formed around 1–2 MYA by hybridization between A-genome and D-genome ancestors, and subsequently underwent genome doubling. Subsequently, the AD-genome clade rapidly spread into the coastal tropical and subtropical regions and even distant islands (Wendel and Cronn, 2003). There are great variations in the morphology of *Gossypium* species, ranging from herbaceous perennials to subshrub, and

even to small trees. Moreover, the flowers of *Gossypium* are also different in size and color. The big size and milk white or bright yellow flowers are mainly observed in the AD genome group. The flower color of the G, C and K genome groups distributed in Australia is Rosy.

Cotton is not only an important economic crop in the world, but also a model plant for evolutionary studies (Wendel et al., 2012). Therefore, there are abundant of molecular phylogenetic work in *Gossypium* (Cronn et al., 2002; Wendel and Cronn, 2003; Grover et al., 2008; Xu et al., 2012; Wendel and Grover, 2015). Eleven single-copy nuclear loci, nuclear ribosomal DNA, and four chloroplast loci were used to evaluate the phylogenetic relationships of diploid cotton genome groups, revealing that the cotton genome groups radiated in rapid succession following the formation of the genus (Cronn et al., 2002). Targeted sequence capture of multiple loci in conjunction with both concatenated and Bayesian concordance analyses were used to reevaluate the phylogeny of allopolyploid cotton species, providing robust support for the *Gossypim* polyploidy clade (Grover et al., 2015). A comparative analysis of 19 *Gossypium* chloroplast genomes supports the resolution of the eight diploid genome groups into six clades and demonstrates contrasting evolutionary dynamics in different clades, with a parallel genome downsizing in two genome groups and a biased accumulation of insertion in the clade containing the cultivated cottons leading to large chloroplast genomes (Chen et al., 2016). Due to its rapid and global diversification, the cotton genus also has many difficult questions in their phylogenetic studies. In spite of these extensive efforts, several branch resolutions remained unclear.

Pollen morphological characteristics can provide a reliable basis for the origin, evolution, classification and identification of plants (Rosenfeldt and Galati, 2007; Erik, 2012; Baser et al., 2016; Mezzonato-Pires et al., 2018; Reunov et al., 2018; Grimsson et al., 2019; Zhang et al., 2021; Umber et al., 2022). *Sclerosperma mannii* and *S. walheri* pollen share the same distinct reticulate sculpture, suggesting that these two currently accepted *Sclerosperma* species are sister taxa of the same intrageneric lineage (Grimsson et al., 2019). Plant pollen individuals are small and large in number, forming unique morphological features during long-term evolution. These morphological features are controlled by genes and are not easily affected by the external natural environment. They have not only the common features of families and genera, but also species specificity. Therefore, the status of plants in the classification system can be determined by means of pollen morphology.

There is no detailed study on the pollen morphology of the *Gossypium* genus although some species *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum* were investigated in the previous studies (Jia et al., 1988; Liu et al., 1994; Lan and Xu, 1996). Jia et al (1988) studied the pollen grains of four cultivated cotton species by using the pollen wall stripping technique (PWST) and the scanning electron (SEM) and found the pollen grain size of *G. barbadense* is the biggest among the four cultivated cotton species. In this study, a comprehensive survey of the pollen morphology of 23 *Gossypium* species and 9 varieties was performed using the scanning electron microscopy (SEM) to explore the contribution and significance of pollen morphology in cotton taxonomy and phylogeny research.

Materials And Methods

Plant materials

A total of 33 germplasm accessions of *Gossypium* belonging to 23 *Gossypium* species were evaluated in the present study (Table I). All voucher specimens are deposited at the National Wild Cotton Germplasm Resources Nursery (Hainan, Sanya), Institute of Cotton Research, Chinese Academy of Agricultural Sciences. Pollen grains wrapped in anther were collected from the undissolved flowers that open on the day in the morning, and then fixed in 2.5% glutaraldehyde solution (in 0.2 mol/L phosphate buffer; PH 7.4) for 3 h and ashed in buffer at least three times, lastly soaked in the 2.5 glutaraldehyde solution and stored at 4 °C .

Scanning electron microscopy analysis of cotton pollen

Micro-morphological features of cotton pollens were examined using scanning electron microscopy (SEM) following to the protocol of Lan and Xu (Lan and Xu, 1996). Pollen grains were acetolysed and stepwise dehydrated in 50%, 70%, 90%, and 100% ethanol. Treated pollen grains then mounted onto the surface of polished aluminum stubs using double-sided adhesive tape. Each stub was sputter coated with a gold layer and taped to the object stage. Observation and micrographs acquisition were taken using a scanning electron microscope (Hitachi S-350) installed at the Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR, CAAS). Biometric measurements were made using Image-Pro plus 6.0 (Media Cybernetics, USA). For each sample, measurements were made on 20 mature pollen grains, which were correctly formed and chosen randomly.

Data exploration and statistical analysis

In this study, three quantitative pollen characters including pollen diameter, exine echini length, and exine echini density (echini number of a front-view of each pollen grain) were measured for at least 20 pollen grains of each specimen. For the qualitative characters, we paid special attention to the pollen shape, echini type, and the tuberculate bulge at the base of the exine echini. The tuberculate bulges at the base of exine echini are divided into three levels (little, medium, and obvious). All these pollen terminology follows Erdtman (Erdtman, 1987) and Punt et al (Punt et al., 2007). SPSS18.0 software was used for all statistical analyses. Relationships between the six traits assessed were examined using Pearson's correlation coefficient. After being standardized, they were used for cluster analysis based on the euclidean distance matrix using UPGMA (unweighted pair-group method with arithmetic mean) method by the NTSYS pc version 2.1 software.

Results

Pollen morphology

The results of SEM of the pollen morphology of 33 cotton specimens are shown in Fig. 1 and Table II. We found that the pollen of all test samples are spherical, radially symmetrical and panporate. Each pollen grain has more than eight apertures, which were almost operculate. However, the apertures number varies greatly among cotton species, and the most one existed in *G. gossypoides* (Fig. 1–25). In addition, the results showed that little variation has been observed in shape of pollen in all *Gossypium* species having spheroidal.

Pollen size is determined on the polar diameter (P) multiply by the equatorial diameter (E). The shape of all cotton species pollen grain are spheroidal, it is hard to distinct the polar and equatorial view of the cotton pollen grain. Therefore, we investigated the diameter of the spherical pollen to evaluate the pollen size.

Generally, pollen grains show great variations in size among cotton species. The highest mean value of pollen diameter is 103.41 µm in *G. barbadense*, and the smallest mean value of diameter is 62.43 µm in *G. harknessii* (Fig. 2A). Previous study has reported that the island cotton (*G. barbadense*) has the largest size of pollen grain followed by the upland cotton (*G. hirsutum*), *G. arboreum* and *G. herbaceum* (Jia et al., 1988). Our results also showed that the size of pollen grain for *G. barbadense* was obviously bigger than other cotton species. Moreover, we found that pollen grain size of allotetraploid (AD genome) cotton species with the mean value of P = 94.7 µm are prominently larger than the diploid cotton species (A-K genome) with the mean P value ranged from 69.66 µm (G genome) to 87.25 µm (K genome) (Fig. 2B).

Exine sculpture

Large variation in the shape, number and length of exine echini has been observed within the genus of *Gossypium*. Shape of echini is divided into sharply conical, conical and rod-like based on the sharpness of the echini top (Table II). The study shows that the shape of exine echini is sharply conical in 15 species, conical in 14 species, and rod-like in 3 species, which revealed that there are significant differences in exine echini shapes of studied species. The rod-like type of exine echini rarely existed in *G. harknessii*, *G. somalense* and *G. areysianum*, the latter two species belong to E genome group. In addition, the size of tuberculate form at the base of exine echini is various in *Gossypium* genus and can be classified into three categories, i.e. little obvious, medium obvious and very obvious (Table I).

Exine echini density varies from minimum in *G. gossypoides* (54), and maximum in *G. incanum* (173). There are great interspecific differences in echini density between cotton species, whereas little intraspecific differences are observed in echini density. In the *G. tomentosum* (AD₃), the numbers of exine echini of three varieties are 76, 64 and 57, respectively. Similarly, the echini numbers of two varieties from *G. mustelinum* (AD₄) are 89 and 87, respectively. Unexpectedly, the echini number of *G. incanum* (173) is much more than that of the other species from the E genome group included *G. stocksii* (118), *G. somalense* (100) and *G. areysianum* (106).

Length of echini reported in different species ranging from 3.53 µm in *G. herbaceum* to 9.47 µm in *G. barbadense*, which is consistent with the results of pollen size (Fig. 3A). Similarly, the exine echini length of the AD genome species is larger than all diploid species except for *G. rotundifolium* that belongs to K genome (Fig. 3B). In the allotetraploid, AD genome, the echini length of *G. hirsutum* (AD₁) is similar with the results of *G. tomentosum* (AD₃), meanwhile the echini length of *G. barbadense* (AD₂) is nearly the same to the echini length of *G. darwinii* (AD₄), which suggest that there are close relationships between *G. hirsutum* and *G. tomentosum*, *G. barbadense* (AD₂) and *G. darwinii* (AD₄), respectively. These results are completely consistent with the scientifically recognized phylogenetic classification. Based on the above results, we concluded that the morphological features of exine echini as well as pollen size have important phylogenetic and taxonomic implications.

Cluster analysis

Cluster analyses were conducted using the data that we have achieved as a result of morphometrical measurements taken from *Gossypium* species. Three clusters are distinguished in the cluster analysis of 33

samples belonging to 23 species (Fig. 4). Therefore, recognition of distinct groups is based on hierarchical phenogram, which is interpretable at genetic distance of 3.83. These levels are separated into three clusters which are assumed to represent distinct three morphotypes: Cluster I, a cluster consisting of five allotetraploid AD-genome species (*G. hirsutum*, *G. barbadense*, *G. tomentosum*, *G. mustelinum* and *G. darwinii*) and five diploid species (*G. klotzschianum*, *G. davidsonii*, *G. raimondii*, *G. schwendimanii* and *G. rotundifolium*). Cluster II, a cluster consisting of thirteen diploid species (*G. herbaceum*, *G. arboreum*, *G. anomalum*, *G. capitiviridis*, *G. thurberi*, *G. armourianum*, *G. harknessii*, *G. gossypoides*, *G. stocksii*, *G. somalense*, *G. areysianum*, *G. bickii*, *G. australe*). Cluster III, a cluster consisting of only one species (*G. incanum*).

The results of cluster analysis show that the genus *Gossypium* is divided into three pollen types (Fig. 4). The key to the pollen types and general pollen morphology of the genus *Gossypium* is listed (measurement is given as mean value in Table II). Type I: larger pollen size (almost diameters longer than 80 μm), longer exine echini length (almost longer than 6 μm), exine echini numbers ranging from 57 to 117, only exine echini shape is sharply conical type, the tuberculate bulge at the base of exine echini are little or medium. Type II: smaller pollen size (almost diameters shorter than 80 μm), shorter exine echini length (almost shorter than 6 μm), exine echini numbers ranging from 54 to 128, three types of exine echini shape were observed in this type, the tuberculate bulge at the base of exine echini are medium or obvious. Type III: smaller pollen size (diameter equal 72 μm), shorter exine echini length (4.21 μm), high exine echini density (173), and little tuberculate bulges at the base of exine echini.

Discussion

Despite a wide range of variation, the taxa of the *Gossypium* share a number of common features. The pollen grains of all species examined are monad, radially symmetrical, spherical, echinate, panporate and operculate (Saensouk and Saensouk, 2021). Pollen shape is determined based on the ratio of polar diameter (P) to equatorial diameter (E) and can be classed into five categories, i.e. oblate ($P/E < 0.50$), suboblate ($P/E = 0.5-0.88$), spheroidal ($P/E = 0.88-1.14$), subprolate ($P/E = 1.14-2.00$), and prolate ($P/E > 2.00$) according to Erdtman (1987). We find that pollen shape is spheroidal in all investigated species, which is congruent with the results of previous studies (Jia et al., 1988; Lan and Xu, 1996). Significant variation in size and sculpture of pollen shows great taxonomic potential in identification and delimitation of species (Ullah et al., 2018) In this study, there is significant interspecific differences in pollen size, but little intraspecific variation of pollen size was observed in most cotton species, indicating that pollen size can be useful index for taxonomy at (or above) the species level.

It is widely accepted that the pollen size usually increases with chromosome number. It has been reported that the pollen grains size of the tetraploid species in the genus *Skimmia* tend to be larger in comparison with other diploid species, though it is not always true (Fukuda et al., 2008). In this study, we also observe that the allotetraploids ($4n = 52$) with tetraploid had larger pollen size than the diploid cotton species ($2n = 26$) with diploid. The most widespread consequence of polyploidy in plants is the increase in cell size, caused by the larger number of gene copies. Consequently, polyploidy individuals may exhibit larger organs compared to their diploid counterparts, such as roots, leaves, tubercles, fruits, flowers and seeds (Sattler et al., 2016). Interestingly, it was also observed that the pollen size of diploid K genome species (*G. rotundifolium*) was larger than those of others diploid cotton species. That may be due to its larger genome sizes. It has been

reported that the genome size of K genome species (~ 2570 Mb) are even slightly larger than the AD genome Allotetraploids (~ 2400 Mb) (Wendel et al., 2012). Therefore, there may be a positive correlation between pollen size and chromosome dosage and genome size.

It has been pointed out that exine ornamentation of pollen grains plays a significant role within the tribe, family and between species in the same genus of *Brassicaceae* (Khalik et al., 2002; Erik, 2012). Baser et al. (2016) also stated that the ornamentation is useful to distinguish between closely related species in the same genus such as *Pelargonium endlicherianum* and *P. quercetorum*. From the investigated species in present study, we find that the pollen exine of all cotton species is densely covered with echini except for *G. armourianum*, whose pollen surface is covered with a thick layer of wax. According to the shape of exine echini, cotton pollen grains are separated in two main types with conical or rod-like exine echini, respectively. The most common shape of exine echini in *Gossypium* is conical (nineteen species) and rarely rod-like (three species). From the SEM photographs, it is common phenomenon that the base of the exine echini bulges into the tuberculate form, but the size of tuberculate form is varied among the investigated cotton species. Although *Gossypium* species has usually echinate exine ornamentation, most of them have different densities and lengths of exine echini. In addition, the *t* test results showed that cotton pollen size is highly correlated to the length of exine echini ($r^2 = 0.69$, $p < 0.001$) but low correlated to the number of exine echini ($r^2 = -0.04$, $p > 0.05$), which suggested that the cotton species with larger pollen size very possibly has longer exine echini. For example, in the diploid cotton species *G. schwendimanii* has larger pollen size, longer length but least number of exine echini. On the contrary, *G. incanum* has smaller pollen size, shorter length but most number of exine echini. Due to it is hard to remove the thick wax from the surface of pollen grain of *G. armourianum*, we can't observed any echini on the pollen grain exine though we have repeated the preliminary experiment for three times using the pollen grain collected from different years. Nevertheless, we speculated that it should have exine echini because having echinate sculpture is a conservative evolutionary feature for the family of Malvaceae and its sister species *G. harknessii* also has the normal exine echini. Thus, more materials are still needed for further study to observe the detailed pollen characteristics of *G. armourianum*.

Cluster analysis based on the six pollen traits show that all the allotetraploid species (AD genome group) are completely ranked into Cluster I, and the wild cotton *G. tomentosum* (AD₃) showed a closer relationship with the cultivated cotton *G. hirsutum* (AD₁) compared to the other allotetraploid cotton. However, there are also confused findings that the nine cotton species from D genome group are scattered into different cluster branches and *G. incanum* (E₄) is separated from the other E genome species (E₁, E₂, and E₃), which are out of our expectation. Although cluster analysis is not exactly coincident with the molecular phylogenetic tree proposed by Wendel and Cronn (2003), the results of cluster analysis would provide new information for the phylogenetic relationships of the *Gossypium* genus from the palynological perspective. Therefore, it is clear that pollen characteristic alone is insufficient to reconstruct taxonomic and systematic relationships within the genus *Gossypium*, but pollen variations are useful for further taxonomic revisions at the species level.

Conclusions

The present study is the first comprehensive investigation of the pollen morphology of *Gossypium* genus, and to date it has acquired the largest number of cotton species and SEM images. Pollen size, ornamentation and

echini feature resulted to be informative at a species level and contribute to a better understanding of their intergeneric relationships. The findings of this study enrich our knowledge of sporopollen morphology and shed new lights on the phylogeny and evolution of *Gossypium* species.

Declarations

Ethics approval and consent to participate

All the cotton germplasm resources used in this research were preserved in the National Wild Cotton Germplasm Resources Nursery (Sanya, China). Experimental research on plants in this study complied with institutional, national, or international guidelines and legislation. All the experiments were performed in accordance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Cai XY performed the experimental procedures and drafted the manuscript. Hou YQ and Wang H analyzed the data. Xu YC, Zheng J, and Wang YH carried out material management. Liu F, Zhou ZL, and Hua JP revised the manuscript. Wang KB designed the study. All authors read and approved the final manuscript.

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Authors' information

¹ State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang 450000, China

² Hainan Yazhou Bay Seed Laboratory, Sanya 572025, China / National Nanfan Research Institute of Chinese Academy of Agriculture Sciences, Sanya 572025, China

³ College of Agronomy and Biotechnology, China Agricultural University, No.2, Yuanmingyuan West Rd, Haidian District, Beijing 100193, China

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Tables

Table I The information of materials

S.No	Taxon	Genome group	Accession number	Geographical distribution/origin
1	<i>Gossypium hirsutum</i> Linnaeus	(AD) ₁	<i>G.hirsutum</i> (CRI12)	China
2	<i>Gossypium barbadense</i> Linnaeus	(AD) ₂	<i>G.barbadense</i> (CL)	Yunnan,China
3	<i>Gossypium barbadense</i> Linnaeus	(AD) ₂	<i>G.barbadense</i> (YM)	Yunnan,China
4	<i>Gossypium barbadense</i> Linnaeus	(AD) ₂	<i>G.barbadense</i> (XH7)	Xinjiang,China
5	<i>Gossypium tomentosum</i> Nuttall ex Seemann	(AD) ₃	<i>G.tomentosum</i> (LZ)	Hawaii Islands, USA
6	<i>Gossypium tomentosum</i> Nuttall ex Seemann	(AD) ₃	<i>G.tomentosum</i> (JFZ8)	Hawaii Islands, USA
7	<i>Gossypium tomentosum</i> Nuttall ex Seemann	(AD) ₃	<i>G.tomentosum</i> (01)	Hawaii Islands, USA
8	<i>Gossypium mustelinum</i> Miers ex Watt	(AD) ₄	<i>G.mustelinum</i> (LZ)	Brazil
9	<i>Gossypium mustelinum</i> Miers ex Watt	(AD) ₄	<i>G.mustelinum</i> (16)	Brazil
10	<i>Gossypium darwinii</i> Watt	(AD) ₅	<i>G.darwinii</i> (07)	Galapagos Islands
11	<i>Gossypium herbaceum</i> Linnaeus	A ₁	<i>G.herbaceum</i> (ZC1)	China
12	<i>Gossypium herbaceum</i> subs. <i>africanum</i> Hutchinson	A _{1-a}	<i>G.herbaceum</i> (africanum)	Africa
13	<i>Gossypium arboreum</i> Linnaeus	A ₂	<i>G.arboreum</i> (SXY)	Asia
14	<i>Gossypium arboreum</i> Linnaeus	A ₂	<i>G.arboreum</i> (Rozi)	Asia
15	<i>Gossypium anomalum</i> Wawra & Peyritsch	B ₁	<i>G.anomalum</i> (LZ)	Africa
16	<i>Gossypium capitis-viridis</i> Mauer	B ₃	<i>G.capitis-viridis</i> (LZ)	Cape Verde Islands
17	<i>Gossypium capitis-viridis</i> Mauer	B ₃	<i>G.capitis-viridis</i> (01)	Cape Verde Islands
18	<i>Gossypium thurberi</i> Todaro	D ₁	<i>G.thurberi</i> (LZ)	Arizona,USA
19	<i>Gossypium thurberi</i> Todaro	D ₁	<i>G.thurberi</i> (35)	Arizona,USA
20	<i>Gossypium armourianum</i> Kearney	D ₂₋₁	<i>G.armourianum</i> (LZ)	Mexica and California
21	<i>Gossypium harknessii</i> Brandegeee	D ₂₋₂	<i>G.harknessii</i> (LZ)	Mexica and California
22	<i>Gossypium davidsonii</i> Kellogg	D _{3-d}	<i>G.davidsonii</i> (LZ)	Mexica and

				California
23	<i>Gossypium klotzschianum</i> Andersson	D _{3-k}	<i>G.klotzschianum</i> (LZ)	Galapagos Islands
24	<i>Gossypium raimondii</i> Ulbrich	D ₅	<i>G.raimondii</i> (01)	Peru
25	<i>Gossypium gossypioides</i> (Ulbrich)Standley	D ₆	<i>G.gossypioides</i> (LZ)	Oaxaca, Mexica
26	<i>Gossypium schwendimanii</i> Fryxell & Koch	D ₁₁	<i>G.schwendimanii</i> (LZ)	Mexica
27	<i>Gossypium stocksii</i> Masters in Hooker	E ₁	<i>G.stocksii</i> (LZ)	Arabia
28	<i>Gossypium somalense</i> (Gurke) Hutchinson	E ₂	<i>G.somalense</i> (LZ)	North Africa
29	<i>Gossypium areysianum</i> Deflers	E ₃	<i>G.areysianum</i> (LZ)	South Yemen
30	<i>Gossypium incanum</i> (Schwartz) Hillcoat	E ₄	<i>G.incanum</i> (04)	South Africa
31	<i>Gossypium bickii</i> Prokhanov	G ₁	<i>G.bickii</i> (LZ)	Central Australia
32	<i>Gossypium australe</i> Mueller	G ₂	<i>G.australe</i> (LZ)	Australia
33	<i>Gossypium rotundifolium</i> Fryxell, Craven & Stewart	K ₂	<i>G.rotundifolium</i> (LZ)	Northwest Australia

Table II Pollen morphologies of *Gossypium* species

S.No	Accession number	Pollen shape	Pollen diameter (µm)	Echini length (µm)	Echini density	Echini shape	Tuberculate bulge
1	<i>G.hirsutum</i> (CRI12)	spheroidal	83.12 91.23 (87.07)	5.49 6.14 (5.78)	103 109 (105)	sharply conical	little
2	<i>G.barbadense</i> (CL)	spheroidal	98.02 108.05 (103.41)	7.88 8.64 (8.29)	81 95 (89)	sharply conical	little
3	<i>G.barbadense</i> (YM)	spheroidal	89.26 103.61 (96.09)	7.86 8.56 (8.20)	100 107 (104)	sharply conical	little
4	<i>G.barbadense</i> (XH7)	spheroidal	98.82 110.68 (103.41)	9.07 10.00 (9.47)	110 123 (117)	sharply conical	little
5	<i>G.tomentosum</i> (LZ)	spheroidal	94.86 104.29 (97.61)	5.53 5.96 (5.78)	74 80 (76)	sharply conical	little
6	<i>G.tomentosum</i> (JFZ8)	spheroidal	86.30 98.88 (91.12)	6.17 6.98 (6.54)	60 70 (64)	sharply conical	little
7	<i>G.tomentosum</i> (01)	spheroidal	87.67 100.66 (91.53)	5.51 5.88 (5.72)	52 62 (57)	sharply conical	medium
8	<i>G.mustelinum</i> (LZ)	spheroidal	86.39 97.55 (92.49)	8.35 9.32 (8.81)	81 96 (89)	sharply conical	little
9	<i>G.mustelinum</i> (16)	spheroidal	86.64 94.96 (91.08)	7.59 8.44 (8.01)	80 91 (87)	sharply conical	little
10	<i>G.darwinii</i> (07)	spheroidal	90.91 96.54 (93.28)	7.82 8.45 (8.12)	63 72 (67)	sharply conical	medium
11	<i>G.herbaceum</i> (ZC1)	spheroidal	65.93 73.47 (70.05)	3.30 3.91 (3.53)	102 114 (107)	conical	obvious
12	<i>G.herbaceum</i> (africanum)	spheroidal	67.05 84.43 (73.41)	6.25 7.25 (6.86)	121 139 (128)	conical	obvious
13	<i>G.arboreum</i> (SXY)	spheroidal	75.77 85.45 (80.36)	5.63 6.37 (5.97)	67 82 (74)	conical	little
14	<i>G.arboreum</i> (Rozi)	spheroidal	75.63 87.17 (81.16)	7.19 7.97 (7.69)	90 98 (94)	conical	little
15	<i>G.anomalum</i> (LZ)	spheroidal	69.63	4.76	73 81	conical	obvious

			76.22 (73.46)	5.67 (5.14)	(77)		
16	<i>G.capitis-viridis</i> (LZ)	spheroidal	66.94 70.47 (68.44)	4.55 4.88 (4.70)	78 90 (84)	conical	medium
17	<i>G.capitis-viridis</i> (01)	spheroidal	68.42 75.14 (71.69)	5.22 5.45 (5.36)	77 90 (83)	conical	medium
18	<i>G.thurberi</i> (LZ)	spheroidal	69.11 77.76 (73.93)	5.74 6.59 (6.19)	87 100 (93)	conical	little
19	<i>G.thurberi</i> (35)	spheroidal	71.55 75.65 (74.02)	5.90 6.67 (6.35)	89 100 (94)	conical	little
20	<i>G.armourianum</i> (LZ)	spheroidal	62.89 77.05 (69.73)	/	/	/	/
21	<i>G.harknessii</i> (LZ)	spheroidal	58.55 68.24 (62.43)	3.78 4.41 (4.07)	103 108 (106)	rodlike	little
22	<i>G.davidsonii</i> (LZ)	spheroidal	77.31 82.15 (79.50)	6.01 7.36 (6.78)	97 116 (103)	sharply conical	little
23	<i>G.klotzschianum</i> (LZ)	spheroidal	85.09 90.42 (87.22)	5.51 6.17 (5.90)	108 118 (113)	sharply conical	little
24	<i>G.raimondii</i> (01)	spheroidal	85.67 89.11 (86.71)	6.40 7.39 (6.83)	79 93 (86)	sharply conical	little
25	<i>G.gossypoides</i> (LZ)	spheroidal	75.65 81.40 (77.99)	5.35 5.96 (5.66)	50 60 (54)	conical	medium
26	<i>G.schwendimanii</i> (LZ)	spheroidal	82.03 86.17 (84.16)	7.85 8.25 (8.09)	62 72 (67)	sharply conical	medium
27	<i>G.stocksii</i> (LZ)	spheroidal	77.26 86.08 (81.98)	4.86 5.98 (5.34)	117 128 (121)	conical	obvious
28	<i>G.somalense</i> (LZ)	spheroidal	72.40 82.97 (79.14)	4.95 5.63 (5.36)	94 100 (96)	rodlike	obvious
29	<i>G.areysianum</i> (LZ)	spheroidal	66.26 74.39 (69.80)	5.23 6.09 (5.68)	106 119 (112)	rodlike	obvious
30	<i>G.incanum</i> (04)	spheroidal	67.28 77.92 (72.00)	3.70 4.68 (4.21)	165 178 (173)	conical	little

31	<i>G.bickii</i> (LZ)	spheroidal	63.08 76.30 (69.78)	4.91 5.87 (5.31)	75 87 (79)	conical	little
32	<i>G.australe</i> (LZ)	spheroidal	69.98 64.66 (64.66)	5.65 6.09 (5.88)	85 93 (90)	conical	little
33	<i>G.rotundifolium</i> (LZ)	spheroidal	83.29 92.25 (87.25)	7.05 7.93 (7.39)	69 78 (75)	sharply conical	little

Note: the datums in brackets represent the average values; /, the data is missing.

Figures

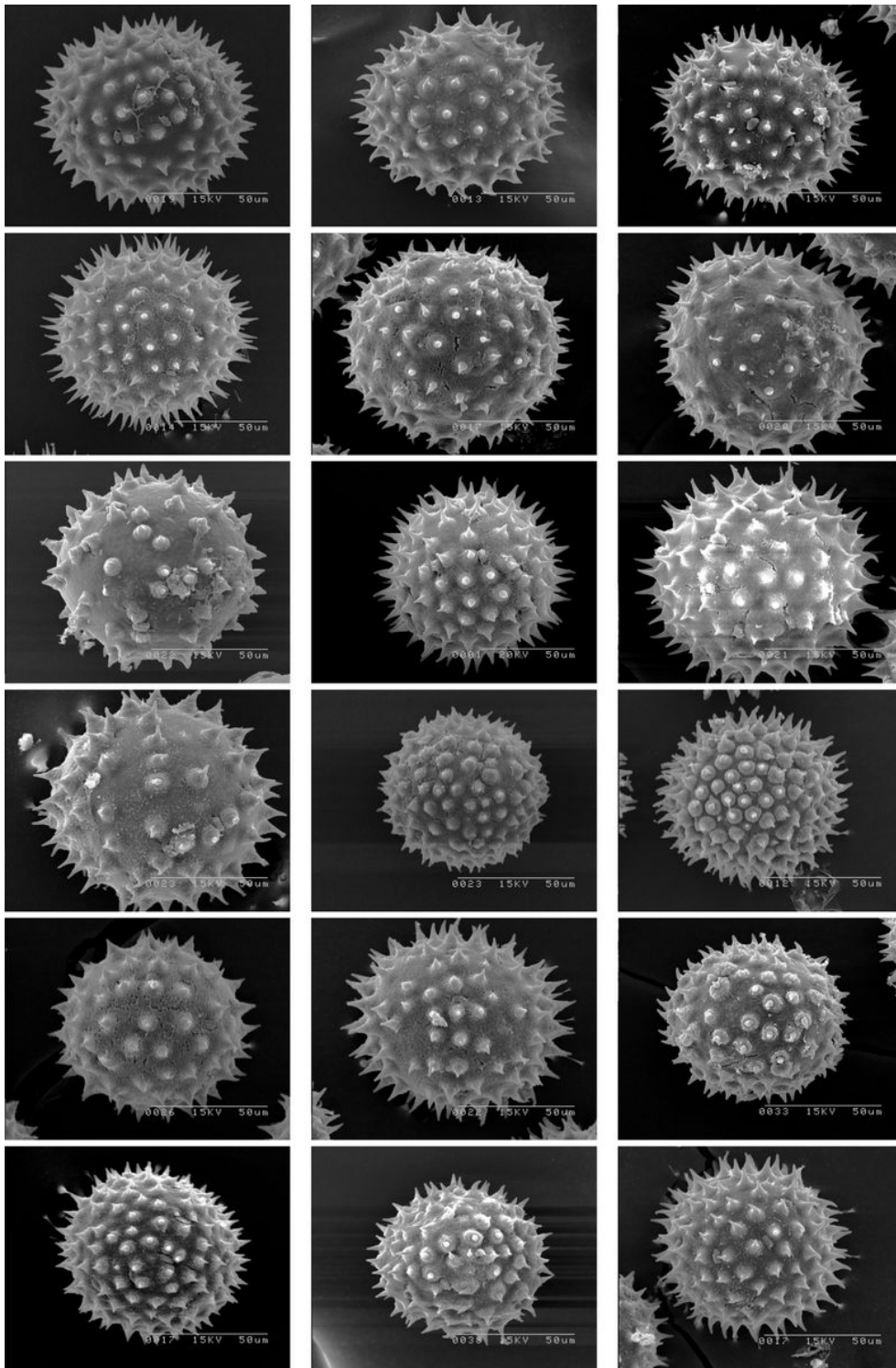


Figure 1

SEM micrographs of pollen grains in *Gossypium*.

1. *G.hirsutum* (CRI12), 2. *G.barbadense* (CL), 3. *G.barbadense* (YM), 4. *G.barbadense* (XH7), 5. *G.tomentosum* (LZ), 6. *G.tomentosum* (JFZ8), 7. *G.tomentosum* (01), 8. *G.mustelinum* (LZ), 9. *G.mustelinum* (16), 10. *G.darwinii* (07), 11. *G.herbaceum* (ZC1), 12. *G.herbaceum*(africanum), 13. *G.arboreum* (SXY), 14. *G.arboreum* (Rozi), 15. *G.anomalum* (LZ), 16. *G.capitis-viridis* (LZ), 17. *G.capitis-viridis* (01), 18. *G.thurberi* (LZ), 19. *G.thurberi* (35), 20. *G.armourianum* (LZ) 21. *G.harknessii* (LZ), 22. *G.davidsonii* (LZ), 23. *G.klotzschianum*

(LZ), 24. *G.raimondii* (01), 25. *G.gossypioide s*(LZ), 26. *G.schwendimani i*(LZ), 27. *G.stocksii* (LZ), 28. *G.somalense* (LZ), 29. *G.areysianum* (LZ), 30. *G.incanum* (04), 31. *G.bickii* (LZ), 32. *G.australe* (LZ), 33. *G.rotundifolium* (LZ).

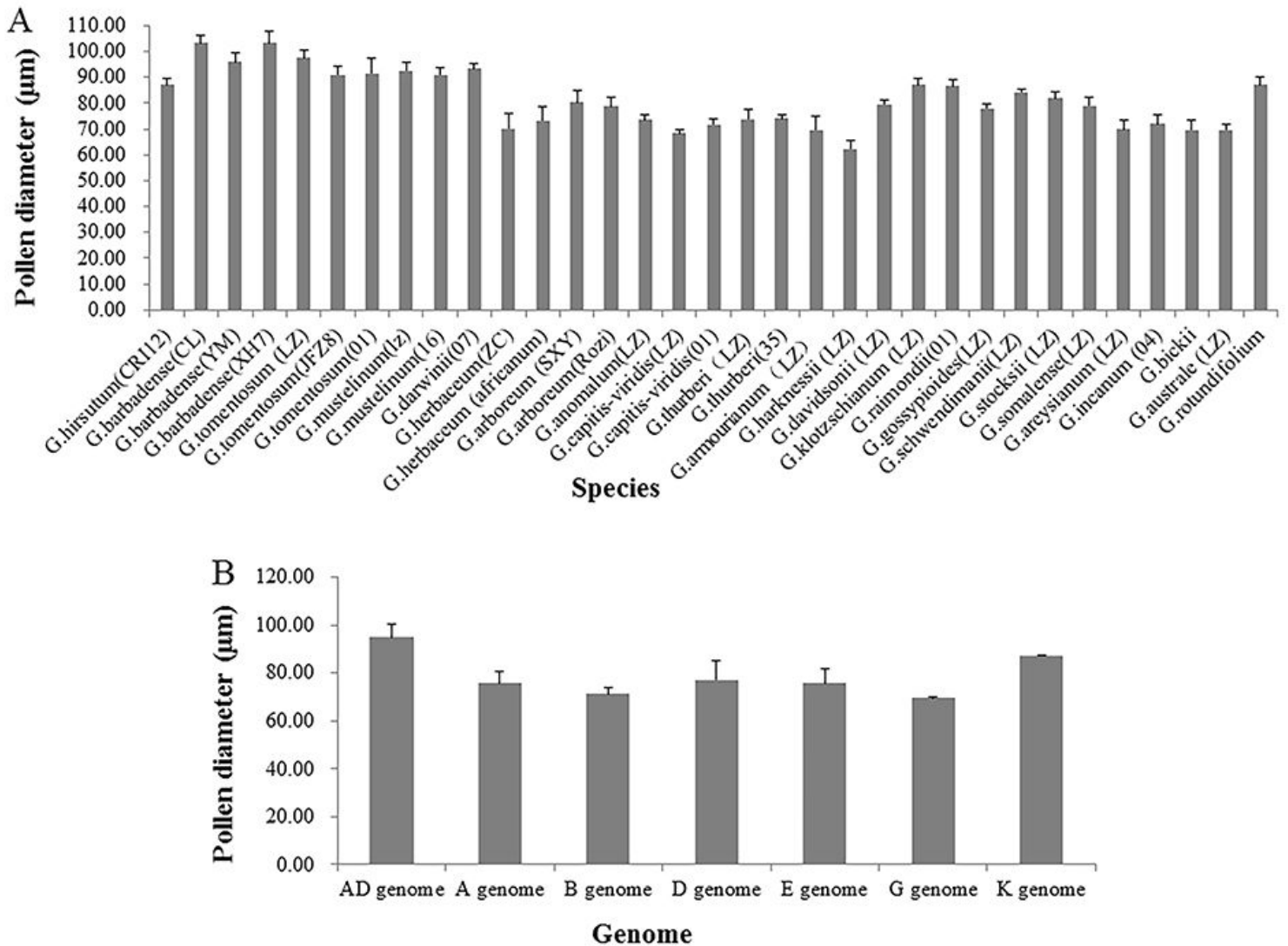


Figure 2

The diameter of pollen in the *Gossypium* species. A, Comparison of pollen diameter among cotton species. B, Comparison of pollen diameter among different genomes.

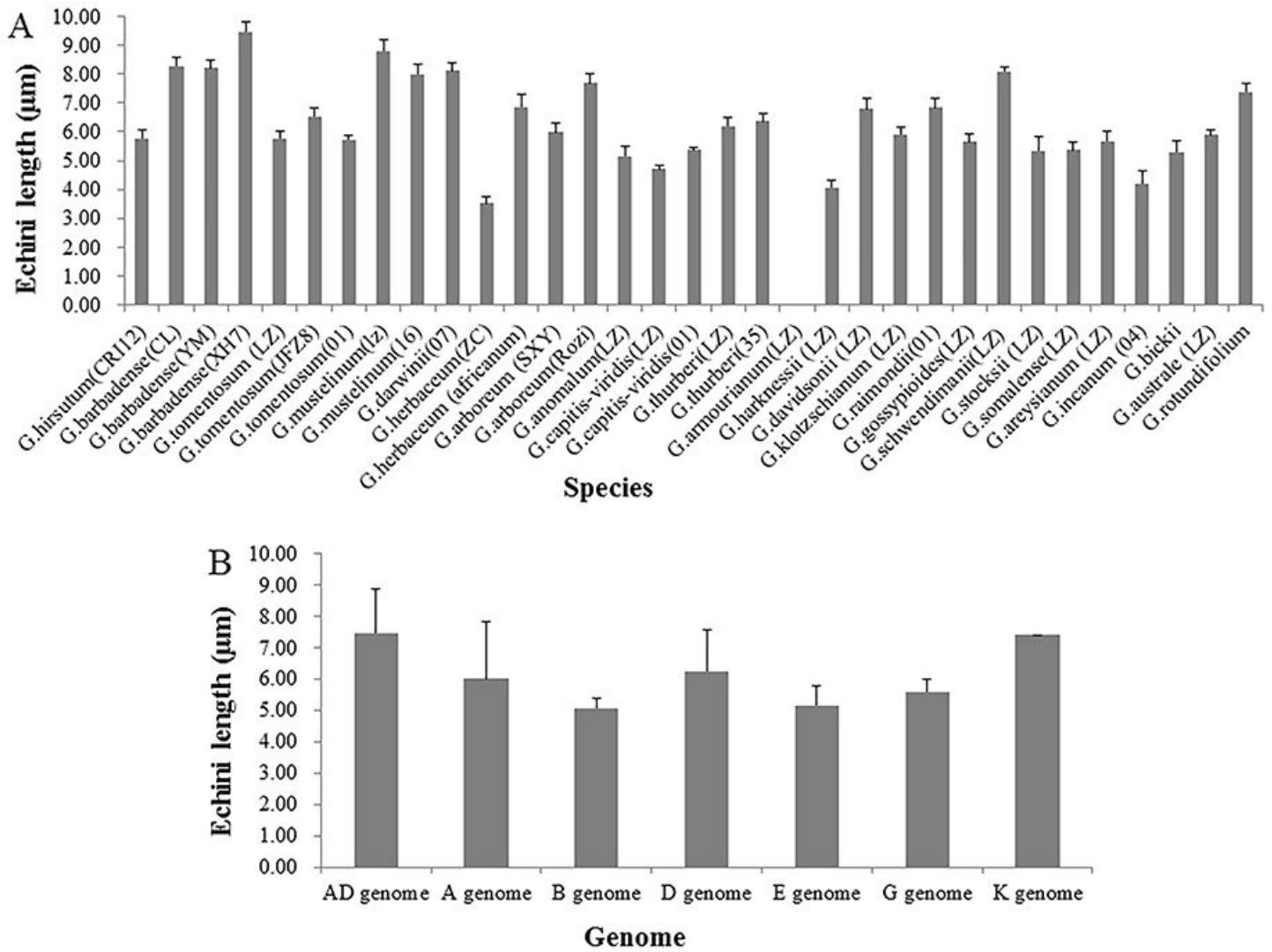


Figure 3

The length of exine echini in the *Gossypium* species. A, Comparison of exine echini length among cotton species. B, Comparison of exine echini length among different genomes.

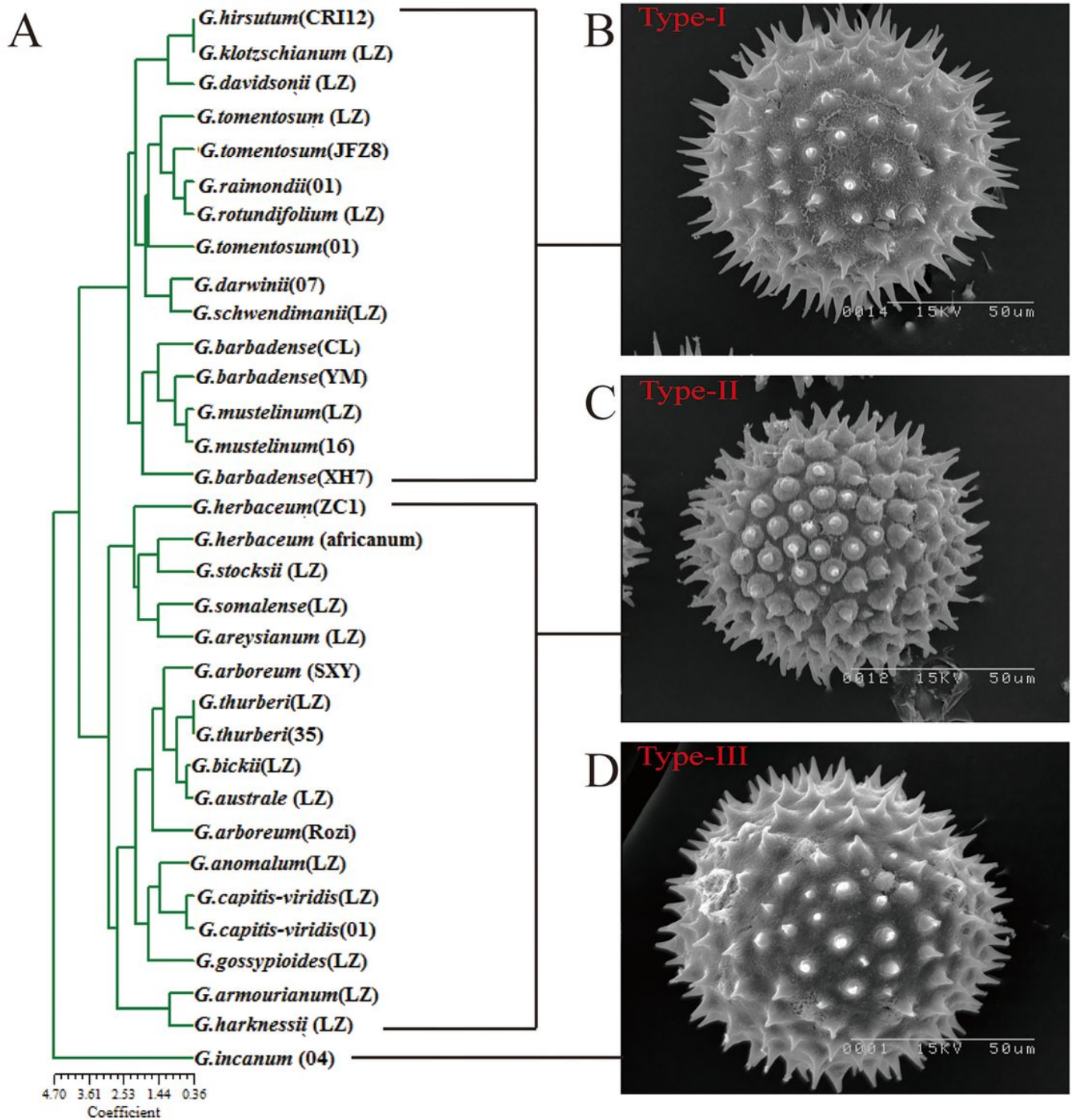


Figure 4

Dendrogram of *Gossypium* based on pollen characteristics (A). Three pollen types (right): Type I, big pollen size, long echini length, low echini density and little tuberculate bulges (B); Type II, small pollen size, short echini length, low echini density, and obvious tuberculate bulges (C); Type III, small pollen size, short echini length, high echini density, and little tuberculate bulges (D).