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Intraspecific Pollen Morphology Variation and Its Responses to Environmental Factors of Wild *Cathaya argyrophylla* Chun Et Kuang Endemic to China

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Abstract: Studying the pollen morphology of this remnant and endemic wild species of *Cathaya argyrophylla* can be of use for paleobiologists. During this study, 23 genotypes sampled from four natural populations in two regions of Hunan Province, China. A total of 460 pollen grains were analyzed for seven quantitative and seven qualitative traits (including five new traits). Three quantitative traits (B, P, and A) (Width of the saccus (B); Length of the polar axis (P); Length of the saccus (A)) and four qualitative traits (O-CO, B-SD, O-CSR, and B-SU) (Pollen corpus outlined in the polar distal view (O-CO); Whether the outline of two sacci was distinct or not in the polar proximal view (B-SD); Roughness degree between corpus from the polar proximal view and the sacci from the polar distal view (O-CSR); Whether the overall size of two sacci was uniform or not (B-SU)) were the diagnostic pollen features that could possible to differentiate one population and classified 23 samples into two, three, or four clusters. Furthermore, 24 environmental factors were evaluated and precipitation factors effected more on pollen morphology than geographic and temperature factors, which including annual precipitation (bio12), precipitation of wettest month and driest month (bio13, bio14), precipitation seasonality (bio15) and monthly averaged precipitation in May (05-precip). The main precipitation and temperature factors exhibited positive and negative correlation with pollen size (B and E (Equatorial diameter (E))), respectively. This article provides deeper insight into intraspecific variability of pollen grains of *C. argyrophylla*, which have been investigated for the first time. In addition, the insights gained from this study could assist with the seed breeding and population reproduction of the endangered *C. argyrophylla* tree.

Keywords: pollen morphology; intraspecific variability; palynology; cluster analysis; environmental factors; *Cathaya argyrophylla*

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1. Introduction

Cathaya argyrophylla Chun et Kuang., the only species in the *Cathaya* genus, was once widely distributed in Eurasia during the Tertiary period, and now is a "living fossil" tree with a fragmented and isolated population distribution in south-central China due to climate change. *C. argyrophylla* with straight trunk and silver needles have a high ornamental value, but it is in danger of extinction as their low setting percentage and hard natural reproduction. Moreover, it is designated as a vulnerable species (VU) and totals 3018 individuals in four mountain regions [1–3]. Thus, it's really essentially for us to pay attention to protect and reproduce this endangered species of *C. argyrophylla*.

Evidence suggests that pollen morphology is controlled by genes with stable heritability, the production and pollen morphology are key features of pollination biology. Pollen

production may be strongly constrained by environmental factors and this might be one of the major forces limiting the fitness of plants [4–6], yet the relationship of intraspecific variability in the pollen morphology and environmental factors have rarely been conducted in tree *C. argyrophylla*. Pollen size may be considered as a superior character because plants with large pollen sired more seeds with higher viability than those from smaller pollen [7], and larger pollen grains provide a stronger germination ability [8]. Pollinators can preferentially collect pollen from those species with relatively smaller-sized pollen grains of bee-pollinated plants [9]. However, for those species with bisaccate pollen of wind-pollinated plants, the sacci size perhaps have effects on pollination.

Furthermore, pollen is diverse in shape and exine sculpturing that is useful for plant classification studies. Quantitative traits were used frequently to describe pollen features, P (The length of the polar axis), P/E (Pollen shape) and A/B (Saccus shape) were the most important quantitative pollen features of *Abies alba* [10]. P, E (Equatorial diameter), Exp (Thickness of the exine along the polar axis and equatorial diameter), Le (Length of the ectoaperture), and d (Distance between the apices of two ectocolpi), as well as P/E, Exp/P, Le/P, and d/E ratios, all have the significant relationship between each other and provide important evidence for interspecific classification [11]. We cannot distinguish pollen at the individual level but its geographical sources or plant populations on the basis of its features. Given that most qualitative traits could not be quantified, they are useless in conventional classification schemes, precluding their ability to serve as criteria. Yet combining and encoding quantitative and qualitative pollen traits to applied to classification has not been analyzed in this context.

The pollen related reports in tree *C. argyrophylla* were mostly conducted on fossil pollens but without detailed values [12,13]. A description of fresh pollen grains of *C. argyrophylla* was founded in Zhang's 1989 report: Zhang measured four pollen quantitative traits (P, E, A (saccus length), and B (saccus width)) and distinguished *Cathaya* from *Pinus* by the presence of spines on the pollen surface, but *Cathaya* is more similar to *Cedrus* and *Picea* spp. [14]. Another report of morpho-anatomical investigation was conducted on cultivated *C. argyrophylla*, which was a 2.5 m high and 14-years-old individual that potted and overwintered in a temperature house in Germany [15]. Research on fresh pollen grains of wild *C. argyrophylla* remains incomplete for lacking of high quality images. Pollen morphological variation within populations is one way of conveying intraspecific variation, yet no research has yet investigated intraspecific variability of wild *C. argyrophylla*'s pollen.

This study aims to fill a knowledge gap concerning pollen morphology and its responses to environmental factors in *C. argyrophylla* which from four populations of two regions in Hunan Province, China. In this work, qualitative and quantitative pollen traits are combined used to describe and classify 23 tree-level pollen samples into several clusters, with the objective being to demonstrate the utility of novel qualitative traits. 24 environmental factors were used to evaluate the effects on quantitative pollen traits. Therefore, this study makes a major contribution, in being the first to clarify intraspecific variability of pollen and the association between pollen morphology and environmental factors of *C. argyrophylla*. The reported findings can provide more scientific evidence for how to reproduce and manage with *C. argyrophylla*.

2. Materials and Methods

2.1. Plant Materials

We collected pollen from the 23 selected wild *C. argyrophylla* trees from four natural populations (Mapigu, SJD-M; Luanyangang, SJD-L; Simaoping, BMS-S; Jiaopenliao, BMS-J) in two regions (Shajiaodong Natural Reserve and Bamianshan Natural Reserve) of Hunan Province. Three male cones of each genotype were collected randomly from different inflorescences and mixed them together as a single sample representing the genotype (Table S1). From each population, two to nine individuals were selected. Pollen grains were dried in room conditions for 1 day and collected in sealing bag without moisture and stored in a 4 °C refrigerator before the SEM observations. Morphological observations were

carried out with a scanning electron microscope (Helios Nanolab G3 UC) after spraying with metal (SCD 500). Each individual (sample) was composed of 20 randomly selected, mature, and correctly formed pollen grains produced from the same tree. In total, 460 pollen grains were studied.

2.2. Coding the Qualitative Traits

Clear trait differences that could reflect characteristics of species were selected for quantification and encoded using the scalar quantity method [16]. Overall, 14 traits were encoded to describe pollen morphology (Table 1), consisting of seven numerical characters ("N") not coded and directly calculated from the raw data. Three binary characters ("B") were encoded with "0" and "1": the code "1" was used for a positive state and "0" for a negative state. Four ordered multistate characters ("O") were encoded with the consecutive positive integers 1, 2, 3 . . . . We employed B-GU, B-SU, B-SD, O-CO, and O-CSR (Table 1) as five new qualitative traits for the first time. The description terminology used in this paper follows Wang et al. [17], Wrońska-Pilarek et al. [10], Punt et al. [18], and Halbritter et al. [19].

Table 1. Characters and codes of *Cathaya argyrophylla*.

No.	Characters	Code Type	Code Details
1	Equatorial diameter(E)	N	/
2	The length of the polar axis (P)	N	/
3	Pollen shape classes (P/E ratio)	N	/
4	The length of the saccus (A)	N	/
5	The width of the saccus (B)	N	/
6	A/B ratio	N	/
7	The length of the germinal anacolpus (G)	N	/
8	Whether the width of the germinal anacolpus was uniformed or not in polar distal view (B-GU)	B	Yes, 1; No, 0
9	Whether the overall size of two sacci was uniformed or not (B-SU)	B	Yes, 1; No, 0
10	Whether the outline of two sacci was distinct or not in polar proximal view (B-SD)	B	Yes, 1 (Diploxylon-type); No, 0 (Haploxylon-type)
11	The pollen corpus outlined in the polar distal view (O-CO)	O	Suborbicular, 1; Ellipse, 2; Subcordate, 3
12	Overall roughness of pollen (O-OR)	O	Not rough, 1; Rough, 2; Very rough, 3
13	Ornament of exine surface (O-EX)	O	Granulate, 1; Micro-striate, 2; Mixture of granulate and micro-striate,3
14	The roughness degree between corpus from the polar proximal view and the sacci from the polar distal view (O-CSR)	O	All not rough, 1; All rough,2; Much rougher in pollen corpus, 3

Note: Diploxylon-type means describing the bisaccate pollen grains in which the outline of the sacci in the polar view is discontinuous with that of the corpus, so grains appear to consist of three distinct, more or less, oval parts. Haploxylon-type means the Bisaccate pollen in which the outline of the sacci in the polar view is more or less continuous with that of the corpus, so grains look more or less smooth and ellipsoidal in form [18].

2.3. Environmental Datas

In this study, environmental factors included three geographical variables (altitude, slope, and aspect) and 21 climatic variables (Table 2). The 21 factors were downloaded from WorldClim version 2 (www.worldclim.org (accessed on 29 July 2021)) with a 2.5 min spatial resolution (bio1-bio19) and ERA5-Land monthly averaged data (05-precip and 05-temp) (https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-land-monthly-means?tab=form (accessed on 14 April 2021)). We used ArcGIS v.10.8 to extract climatic variables of each sample.

Table 2. Climatic variables.

Variables	Description	Units
bio1	Annual mean temperature	°C*10
bio2	Mean diurnal range (Mean of monthly (max temp—min temp))	°C*10
bio3	Isothermality (bio2/bio7) × (100)	/
bio4	Temperature seasonality (standard deviation ×100)	°C*10
bio5	Max temperature of warmest month	°C*10
bio6	Min temperature of coldest month	°C*10
bio7	Temperature annual range (bio5—bio6)	°C*10
bio8	Mean temperature of wettest quarter	°C*10
bio9	Mean temperature of driest quarter	°C*10
bio10	Mean temperature of warmest quarter	°C*10
bio11	Mean temperature of coldest quarter	°C*10
bio12	Annual precipitation	mm
bio13	Precipitation of wettest month	mm
bio14	Precipitation of driest month	mm
bio15	Precipitation seasonality (coefficient of variation)	/
bio16	Precipitation of wettest quarter	mm
bio17	Precipitation of driest quarter	mm
bio18	Precipitation of warmest quarter	mm
bio19	Precipitation of coldest quarter	mm
05-precip	Monthly averaged total precipitation in May (×1000)	mm
05-temp	Monthly averaged 2 m temperature in May (−273.15)	°C

2.4. Statistical Analysis

The Shapiro-Wilk's normality test was used to test the normality of distribution for each of the pollen traits [20]. A multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) was carried out to compare the effects of the four wild populations upon variation in the observed quantitative and qualitative traits (quantified and encoded). Then, the minimal and maximal, the mean values, and the coefficients of variation of quantitative traits were calculated for each pollen-source tree individual. Pollen shape class (P/E ratio) was assigned according to the scheme proposed by Erdtman [21]: peroblate (less than 0.50), oblate (0.51–0.75), suboblate (0.76–0.88), oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), and subprolate (1.15–1.33). Bivariate relationships were assessed with quantitative traits and qualitative traits, respectively, based on Pearson's r correlation coefficient, using the means per pollen sample, and summarized in a heatmap.

The raw data were standardized (STD) for eliminating different dimensions. Applying a principal component analysis (PCA) within the dataset of 14 pollen traits [22,23], then R-type and Q-type cluster analyses were carried out [24]. These results were used to reveal whether the five new qualitative pollen traits contributed to distinguishing tree individuals (genotypes) or source locations. All these analyses were conducted in SPSS Statistics 19.0.

Based on Pearson correlation coefficient, SPSS Statistics 19.0 was used to evaluate correlation of 24 environmental factors and seven quantitative traits. Pollen morphology matrix and environmental matrix on the basis of Manhattan distance are conducted Mantel test in R Studio using "vegan" package [25].

3. Results

3.1. General Morphological Description of Pollen

A description of pollen grain morphology of the 23 studied samples of *C. argyrophylla* is given below and illustrated in the SEM photographs (Figure 1). In general, the pollen grains of *C. argyrophylla* were isodiametric, heteropolar monads. They have two sacci, with less distinct crista and a thick sexine. Lines of pollen corpus and sacci were unclear and no concave angle formed between the pollen corpus and sacci in the equatorial view.

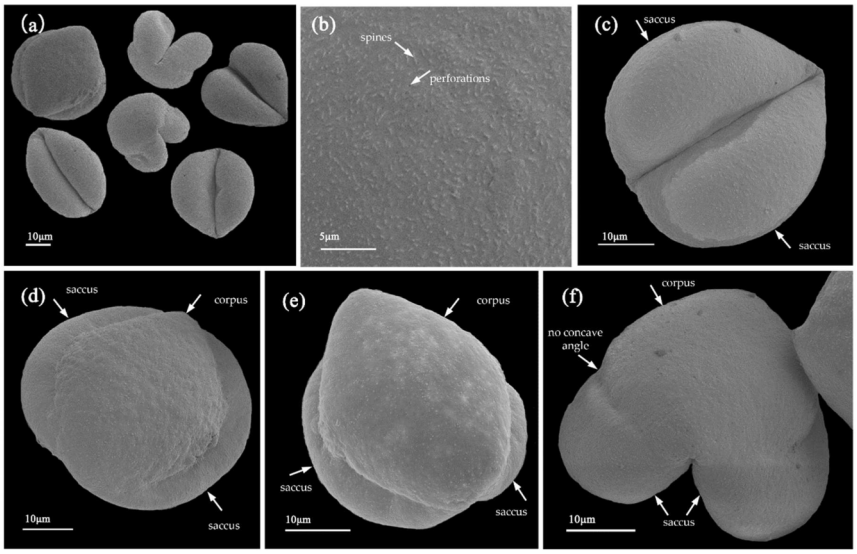


Figure 1. *C. argyrophylla* pollen grains. (**a**) Pollen grains of *C. argyrophylla* in the polar and equatorial view. (**b**) Mixture of granulate and striate ornamentation and perforation on the pollen exine surface. (**c**) Two sacci in the polar distal view. (**d**,**e**) Polar proximal view. (**f**) Two sacci in the equatorial view, no concave angle.

3.1.1. Description on Quantitative Traits of Pollen

Morphological observations for quantitative features are summarized in Table A1. According to Erdtman's pollen size classification scheme, in 460 measured pollens, the polar axis (P) of pollen corpus spanned from 28.32 to 51.68 μm (Figure 2a). In 23 samples, the average length of P was 40.16 μm (36.47~43.95 μm). The length of P in the BMS-J population was the shortest overall, ranging from 36.37 to 40.34 μm, whereas that of the BMS-S population was largest overall, ranging from 38.76 to 44.71 μm (Table A1).

In 460 measured pollen grains, the equatorial diameter (E) of pollen corpus varied between 23.58 and 49.78 μm (Figure 2b). The great majority of pollen grains were medium-sized (99.57%). In 23 samples, the average length of E at the sample level was 35.07 μm (29.83~43.49 μm). The length of E in the BMS-J population was the largest overall, it ranging from 36.48 to 40.57 μm. The E value ranged most (29.87 to 38.11 μm) in the BMS-S population and least (31.56 to 35.24 μm) in the SJD-L population, while the SJD-M ranged from 31.41 to 37.98 μm (Table A1).

The P/E ratio varied between 0.69 and 1.67 in 460 measured pollens (Figure 2c). For all 23 samples of *C. argyrophylla*, the P/E ratio was 1.17, on average, and ranged from 0.91 in sample 21 of the BMS-J population to 1.36 in sample 9 of the SJD-L population. The pollen shape was frequently subprolate (52.17%) and prolate-spheroidal (21.74%), and rarely oblate-spheroidal (13.04%) or prolate (13.04%). Among the populations, BMS-J was distinct in terms of its P/E ratio (0.94~1.07), while BMS-S harbored the widest range for the ratio, with values between 1.08 and 1.50; those of SJD-M and SJD-L were, respectively, 1.10~1.29 and 1.17~1.30 (Table A1).

In 460 measured pollens, the length of the base of the saccus (A) was ranged from 26.93 to 48.52 μm (Figure 2e), the width of the base of the saccus (B) was ranged from 8.67 to 24.62 μm (Figure 2f), the A/B ratio was ranged from 1.47 to 4.40 (Figure 3d). At the individual sample level, mean length of A was 37.39 μm, while mean width of B was 16.24 μm, the mean A/B ratio was 2.32. The BMS-J population was distinct from the others in having the narrowest range for the A/B ratio (1.95~2.25), being widest (2.07~3.12) in the BMS-S population; for SJD-M it was 2.13~2.88 and for SJD-L it was 2.19~ 2.61 (Table A1).

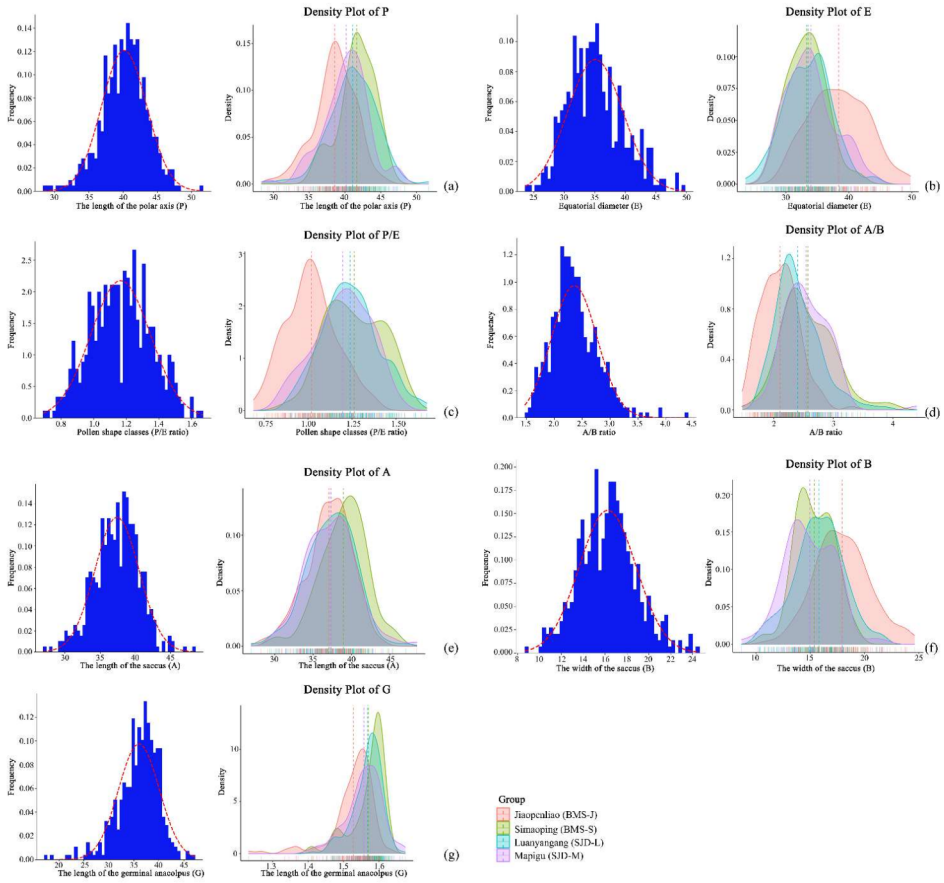


Figure 2. The frequency and density plots of seven pollen traits of *C. argyrophylla* based on 460 measured pollen grains. Shown is the range value of P, E, P/E, A, B, A/B, and G for each individual and population and the most frequent value of them, with populations ranked as follows: (**a**) BMS-S (35.76~46.11 μm) > SJD-L (28.32~51.68 μm) > SJD-M (29.41~47.74 μm) > BMS-J (29.17~45.70 μm). (**b**) BMS-J (29.20~49.78μm) > SJD-M (26.80~44.11μm) > SJD-L (23.58~44.26μm) > BMS-S (27.67~41.46μm). (**c**) BMS-S (0.91~1.55) > SJD-L (0.90~1.67) > SJD-M (0.86~1.52) > BMS-J (0.69~1.45). (**d**) BMS-S (1.73~3.90) > SJD-M (1.53~4.40) > SJD-L (1.78~3.89) > BMS-J (1.47~3.31). (**e**) BMS-S (30.27~45.37μm) > SJD-M (29.88~48.52μm)/SJD-L (26.93~47.11μm) > BMS-J (28.21~43.87μm). (**f**) BMS-J (11.04~24.62μm) > SJD-L (10.31~21.60μm) > BMS-S (10.81~18.99μm) > SJD-M (8.67~21.67μm). (**g**) BMS-S (25.73~42.46μm) > SJD-L (25.93~46.38μm) > SJD-M (25.12~47.26μm) > BMS-J (17.05~43.20μm).

We examined the length of germinal anacolpus (G) in the polar distal view of pollen. In 460 measured pollens, G was ranged from 17.05 to 47.26 μm (Figure 2g). The mean of G at the sample level was 36.01 μm. The width of G was nonuniform and this frequently observed in the BMS-J population, whereas the uniform state was more common in the SJD-M and SJD-L populations.

3.1.2. Description on Qualitative Traits of Pollen

We employed five new qualitative traits to describe pollen morphology of *Cathaya* according to observation (Figure 3, Table 1).

Adopting a proximal view of the pollen grains, the outlines of their sacci were more or less distinct (B-SD). Two sacci in the polar distal view presented the same or different size (B-SU) and the germinal anacolpus in the polar distal view was found with either a uniform or nonuniform width (B-GU), The width in the middle of germinal anacolpus ranged from 1.48 to 3.20 μm. The pollen corpus outlined in the polar distal view was mostly elliptic in SJD-M and SJD-L populations, but predominantly circular or subcordate in the BMS-S

and BMS-J populations (O-CO). By contrast, in the proximal view, the outline of the pollen corpus was mostly elliptic and rarely circular.

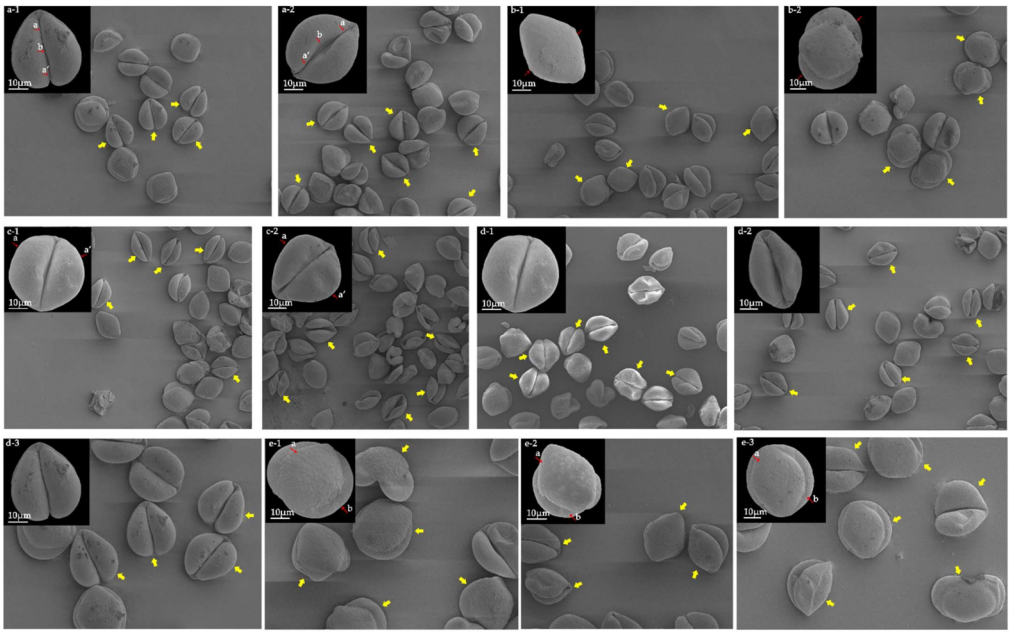


Figure 3. Description for five new qualitative traits of pollen, the yellow arrow indicate typical pollen grains. Anacolpus was uniform or not in the polar distal view (B-GU): (**a-1**) Anacolpus was uniform in the polar distal view given the subequal values among a, b, and a′ (Yes, 1). (**a-2**) Anacolpus was nonuniform in the polar distal view as the b was wider than a or a′ (No, 0). Whether the outline of two sacci was distinct or not in the polar proximal view (B-SD): (**b-1**) The outline of two sacci was not distinct in the polar proximal view (Haploxylon-type) (No, 0). (**b-2**) The outline of two sacci was distinct in the polar proximal view (Diploxylon-type) (Yes, 1). Whether the overall size of two sacci was uniform or not (B-SU): (**c-1**) The overall size of two sacci (a and a′) was uniform (Yes, 1). (**c-2**) The overall size of two sacci was nonuniform because the saccus was larger for a′ than a (No, 0). The pollen corpus outlined in the polar distal view (O-CO): (**d-1**) Suborbicular (suborbicular, 1). (**d-2**) Ellipse (ellipse, 2). (**d-3**) Subcordate (subcordate, 3). Roughness degree between the corpus from the polar proximal view and the sacci from the polar distal view (O-CSR): (**e-1**) The corpus is rougher than sacci in the polar distal view (much rougher in pollen corpus, 3). (**e-2**) The corpus and sacci are not rough (all not rough, 1). (**e-3**) The corpus and sacci are all rough as evinced by the many verrucae on the surface (all rough, 2).

The outline of pollen exine in the polar distal view was uneven; the exine surface was psilate or rough, with perforations, being granulate or striate, or a mixture of granulate and striate. We noticed that samples 4, 7, and 16 had less perforation on the exine surface, while the BMS-J samples featured more perforations. The saccus presented as reticulate or granulate on the surface. The exine surface of pollen having a mixture of granulate and striate was rarely observed in samples 10, 16, and 23, but samples 9 and 12 were striate, with all others being granulate. Then, we used two qualitative traits to, respectively, describe the overall roughness degree of pollen (we used O-OR as a qualitative trait) and the roughness degree between the corpus (from the polar proximal view) and saccus (from the polar distal view), given the differences we noticed between the latter two (O-CSR). We found that pollen in the BMS-J population was rougher in its corpus than saccus; although not a trait able to classify populations of *C. argyrophylla*, few samples could be distinguished nonetheless.

3.2. Intraspecific Pollen Variability of the Studied Samples

The MANOVA results uncovered significant differences among the 23 *C. argyrophylla* samples for seven quantitative traits (Wilk's $\lambda = 0.088$; $F = 2.395$; $p < 0.01$) and seven qualitative traits (Wilk's $\lambda = 0.0540$, $F = 3.170$, $p < 0.01$). The univariate ANOVAs for the five quantitative traits—E ($F = 5.704$), P/E ($F = 7.672$), B ($F = 7.745$), A/B ($F = 7.004$) and G ($F = 6.972$)—confirmed the variability of the studied samples at a high level of statistical significance ($p < 0.01$), with one trait (P; $F = 4.326$) significant at $p < 0.05$, leaving just one trait (A; $F = 1.930$) non-significant ($p > 0.05$). The mean, range, and coefficients of variation (CV) for the observed traits indicated high variability among the tested samples, for which significant differences were found in terms of six quantitative traits (P, E, P/E, B, A/B, and G) (Table A1). The ANOVAs for two new qualitative traits (O-CO: $F = 15.653$; B-SD: $F = 23.872$) confirmed the variability of the studied populations was extremely significant ($p < 0.001$), alongside one new trait (O-CSR: $F = 3.752$) that was significant at $p < 0.05$, whereas four traits—B-SU ($F = 1.411$), B-GU ($F = 2.626$), O-OR ($F = 1.917$), O-EX ($F = 1.028$)—were all statistically non-significant ($p > 0.05$). According to the pairwise multiple comparisons between populations based on three significant new traits, the new trait B-SD can significantly distinguish the BMS-J population from the other three *C. argyrophylla* populations ($p < 0.01$). Similarly, the new trait O-CSR was able to distinguish BMS-J from the SJD-L and BMS-S populations, and the new trait O-CO distinguished BMS-J from both SJD-M and SJD-S populations, and likewise BMS-S from either SJD-M or SJD-S.

For seven quantitative traits (Figure 4a), the correlation analysis revealed only two correlation coefficients that were not statistically significant, namely for E vs. A and A vs. B. Trait E was positively correlated with B (0.85) yet negatively correlated with P (−0.54), P/E (−0.94), G (−0.68), and A/B (−0.79). Trait P was negatively correlated with B (−0.49) and positively correlated with P/E (0.79), A (0.75), G (0.88), and A/B (0.62). Trait P/E was negatively correlated with B (−0.80) and positively correlated with A (0.51), G (0.84), and A/B (0.83). Trait A was negatively correlated with B (−0.28) and positively correlated with both G (0.63) and A/B (0.54). Trait B was negatively correlated with G (−0.67) and A/B (−0.96). Trait G was positively correlated with A/B (0.76). For seven qualitative traits (Figure 4b), the correlation analysis revealed that trait O-CSR was positive correlated with O-CR (0.84) and negative correlated with B-SD (−0.64), B-GU (−0.47), and O-EX (−0.42). Trait O-CR was negative correlated with B-SD. Trait B-GU was negative and positive correlated with O-CO and B-SD, respectively. Trait B-SD was negative correlated with O-CO.

3.3. Clustering Analysis of 23 Pollen Samples

The PCA of 14 pollen traits (seven quantitative and seven qualitative traits) yielded four principal components (PCs), which altogether explained 84.69% of the total variation in the data. The PC1 explained 53.51%, for which the most important variables were O-CO, B-SD, and B. The PC2 explained 12.57% and the important variables for it were P and A. The PC3 explained 11.29%, whose important variables were O-OR and O-CSR. The PC4 explained 7.32% and the sole important variable was B-SU.

The results of R-type clustering analysis showed that pairings of traits P and M, P/E and B-SD, E and B, O-OR and O-CSR clustered together with strong correlations (Figure 5a). We retained P, P/E, B-SD, E, and O-CSR in addition to the other remaining six variables, which were then treated in the regression sense of independency (we retained P/E and B-SD because both were important traits to distinguish pollen samples). Thus, 11 pollen traits were conducted for the follow-up Q-type clustering analysis. Its results were as follows (Figure 5b):

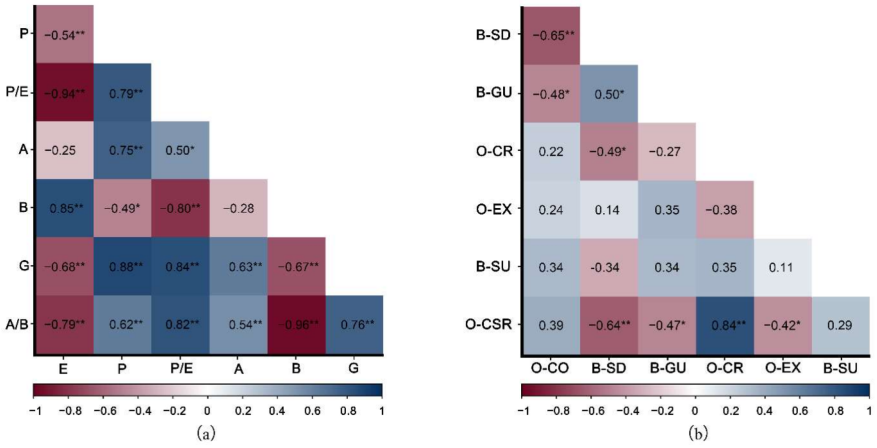


Figure 4. Heatmaps for Pearson's linear correlation coefficients between observed quantitative and qualitative traits (Table 1) of *C. argyrophylla*. The heatmap shows the magnitude of a phenomenon as color in two dimensions: a positive or negative number, respectively, denotes a positive or negative correlation between row and column traits (P, length of polar axis; E, equatorial diameter; A, length of saccus; B, width of saccus; G, length of germinal anacolpus; and the A/B and P/E ratios; B-SD, whether the outline of two sacci was distinct or not in polar proximal view; B-GU, whether the width of the germinal anacolpus was uniformed or not in polar distal view; B-SU, whether the overall size of two sacci was uniformed or not; O-OR, overall roughness of pollen; O-CSR, the roughness degree between corpus from the polar proximal view and the sacci from the polar distal view; O-CO, the pollen corpus outlined in the polar distal view; O-EX, ornament of exine surface). * $p < 0.05$, ** $p < 0.01$.

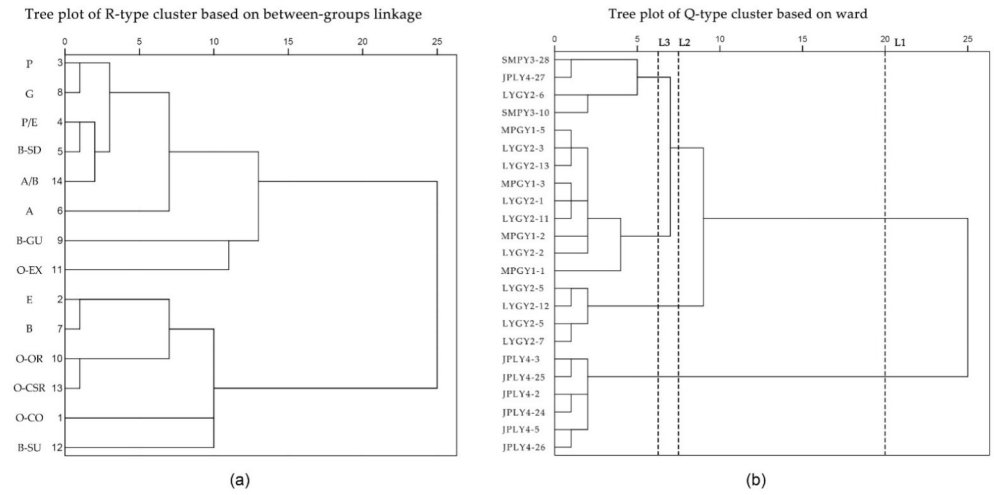


Figure 5. Tree plot of R-type cluster and Q-type cluster of *C. argyrophylla*. (**a**) R-type conducted on 14 pollen traits based on between-groups linkage method (P, length of polar axis; E, equatorial diameter; A, length of saccus; B, width of saccus; G, length of germinal anacolpus; and the A/B and P/E ratios; B-SD, whether the outline of two sacci was distinct or not in polar proximal view; B-GU, whether the width of the germinal anacolpus was uniformed or not in polar distal view; B-SU, whether the overall size of two sacci was uniformed or not; O-OR, overall roughness of pollen; O-CSR, the roughness degree between corpus from the polar proximal view and the sacci from the polar distal view; O-CO, the pollen corpus outlined in the polar distal view; O-EX, ornament of exine surface). (**b**) Q-type conducted on 23 genotypes based on ward method (Table S1).

(1) The 23 pollen samples could be classified into two clusters based on the grade bond line L1 (D = 20.0), for which the main distinguishing traits were O-CO, B-SD, B, P/E, and A/B. The first cluster contained six samples (18, 21, 17, 20, 19, and 22) collected

from the same population (BMS-J). The second cluster was composed of the remaining 17 samples.

(2) The 23 pollen samples could be classified into three clusters based on the grade bond line L2 (D = 7.5). The first cluster still harbored the six samples from the BMS-J population. The remaining 17 samples were classified into two clusters based on three main distinguishing traits: P/E, B-GU, and B-SU. The second cluster comprised four samples (sample 9, 13, 5, and 11), The third cluster consisted of the remaining 13 samples.

(3) The 23 pollen samples could be classified further into four clusters, based on the grade bond line L3 (D = 6.25). The first cluster was unchanged (samples 18, 21, 17, 20, 19, and 22). The second cluster had the same four samples as before (samples 9, 13, 5, and 11), with the remaining 13 samples collected from Shajiaodong Natural Reserve now classified into two clusters based on the main classifying traits of P, A, G, and O-CSR. The third cluster had four samples (samples 16, 23, 10, and 15).

Via five new qualitative traits combined with the results of the R-type and Q-type clustering analyses, we can confirm that three new pollen traits (O-CO, B-SD and O-CSR) could serve as key classifiers for populations of *C. argyrophylla*. Admittedly, however, the new traits B-GU and B-SU did not contribute to distinguishing among populations but they did help to distinguishing a portion of individual trees. We also found that the new qualitative trait B-SD was equivalent to the quantitative trait P/E as well as trait O-CSR and O-ORF, but clearly O-CSR outperformed O-OR.

3.4. Effects of Environmental Factors on Pollen Morphology of C. argyrophylla

Based on Pearson correlation analysis, all quantitative traits except trait A exhibited a significant correlation with a few environmental variables (Table 3). Precipitation factors were higher response to pollen size compared to temperature (bio1–bio10) and geographical factors (altitude and aspect), but slope and bio11 have no effect on any quantitative traits. E, P/E, G, B, and A/B were responded to all precipitation factors, particularly on B ($p < 0.01$, $|r|$: 0.573–0.674), then on E ($p < 0.05/0.01$, $|r|$: 0.487–0.643). The main precipitation factors with larger r were bio12, bio13, bio14, and bio15, they were exhibited negative correlation with *p*, P/E, G, and A/B. Except for bio17 and 05-precip, the rest of the precipitation factors all exhibited positive correlation with B and E. The main temperature factors including bio1, bio4, bio5, and bio6 were exhibited negative correlation with B and E. Trait B and E were affected by 21 and 15 environmental factors, respectively. Mantel test showed significant correlation between pollen morphological distance and environmental distance (r = 0.3093, $p < 0.01$).

Table 3. Correlation coefficient (r) values between pollen quantitative traits and environmental factors.

Quantitative Traits	Environmental Variables		
	Geog	**Temp**	**Precip**
E	altitude (0.487 *)	bio1 (−0.481 *); bio4 (−0.480 *); bio5 (−0.542 **); bio6 (−0.483 *); 05-temp (0.547 **)	bio12 (0.562 *); bio13 (0.573 **); bio14 (0.583 **); bio15 (0.643 **); bio16 (0.528 **); bio17 (−0.487 *); bio18 (0.516 *); bio19 (0.543 **); 05-precip (−0.547 **)
P	aspect (−0.501 *)	/	bio12 (−0.417 *); bio13 (−0.421 *); bio14 (−0.452 *); bio15 (−0.522 *)
P/E	altitude (−0.467 *); aspect (−0.454 *)	bio1 (0.456 *); bio4 (0.476 *); bio5 (0.528 **) bio6 (0.460 *); 05-temp (−0.550 **)	bio12 (−0.562 **); bio13 (−0.571 **); bio14 (−0.592 **); bio15 (−0.665 **); bio16 (−0.528 **); bio17 (0.462 *); bio18 (−0.513 *); bio19 (−0.547 **); 05-precip (0.550 **)

Table 3. *Cont.*

Quantitative Traits	Environmental Variables		
	Geog	**Temp**	**Precip**
B	altitude (0.644 **)	bio1 (−0.661**); bio2 (−0.427 *); bio3 (0.427 *); bio4 (−0.576 **); bio5 (−0.665 **); bio6 (−0.668 **); bio7 (−0.548 **); bio8 (−0.531 **); bio9 (−0.452 **); bio10 (−0.536 **); 05-temp (0.577 **)	bio12 (0.609 **); bio13 (0.640 **); bio14 (0.614 **); bio15 (0.674 **); bio16 (0.590 **); bio17 (−0.611 **); bio18 (0.593 **); bio19 (0.573 **); 05-precip (−0.577 **)
A/B	altitude (−0.592 **)	bio1 (0.609 **); bio4 (0.493 *); bio5 (0.587 **); bio6 (0.628 **); bio7 (0.472 *); bio8 (0.477 *); bio9 (0.414 *); bio10 (0.473 *); 05-temp (−0.478 *)	bio12 (−0.514 *); bio13 (0.551 **); bio14 (−0.519 *); bio15 (−0.602 **); bio16 (−0.499 *); bio17 (0.522 *); bio18 (−0.505 *); bio19 (−0.475 *); 05-precip (0.478 *)
G	altitude (−0.440 *); aspect (−0.541 **)	bio1 (0.431 *); bio4 (0.461 *); bio5 (0.502 *); bio6 (0.438 *); 05-temp (−0.530 **)	bio12 (−0.542 **); bio13 (−0.549 **); bio14 (−0.587 **); bio15 (−0.643 **); bio16 (−0.510 *); bio17 (0.433 *); bio18 (−0. 495 *); bio19 (−0.529 **); 05-precip (0.530 **)

Note: Pearson's linear correlation coefficients between observed quantitative traits (P, length of polar axis; E, equatorial diameter; A, length of saccus; B, width of saccus; G, length of germinal anacolpus; and the A/B and P/E ratios) of *C. argyrophylla* and environmental factors (Geog, geographic factors; Temp, temperature factors; Precip, precipitation factors). Details in Tables 1 and 2. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

4.1. Pollen Morphology and Diagnostic Features

C. argyrophylla with its distinct sacci were thus grouped into *Pinus*-type according to Erdtman [26]. Based on the classification in the book of Flora of China (7th edition) [17], yet Zhang [14] thought that a controversial classification if pollen morphology was considered, because *Tsuga* without distinct sacci but frill is designated a member of Abietoideae together with tree genera that have distinct sacci. We found *Cathaya* with bisaccate pollen and agree with Zhang's suggestion that *Tsuga* and *Larix* should form separate subfamilies and that other genere of Pinaceae with distinct sacci should be merged together into one subfamily based on pollen morphology.

In our study, we discovered a few useful diagnostic features. Although most of these features are mentioned by other palynologists, five (B-GU, B-SU, B-SD, O-CO and O-CSR) we describe here for the first time. The diagnostic features were O-CO, B-SD, B, P, A, O-OR, O-CSR, and B-SU, of which O-CO, B-SD and O-CSR were the three novel traits that can distinguish some of the groups. One of the best diagnostic features for fossil *Cathaya* pollen grains is the connection between the saccus and the corpus [13]. However, we found that the outline of sacci were either distinct or not in the polar proximal view (B-SD). Furthermore, this new trait contributed immensely to a robust classification of the studied samples; hence, the description of Diploxylon-type and Haploxylon-type can further an understanding of pollen morphology. Research on fossil pollen of *C. argyrophylla* noted that its sacci are seen to originate at the margin of the corpus regardless of the relative size of sacci in the polar view [13].

Our results corroborate Zhang's research reported mean values of P (45 µm), E 38 µm), A (42 µm) and B (25 µm) albeit with slightly lower values in our research [14]. On average, most of the pollen grains were medium-sized in this study and consistent with the conclusions of cultivated *Cathaya* [15]. In this study, a lower coefficient variation was found in *C. argyrophylla* compared to other species of Pinaceae, such as *Abies alba* (50.00~128.00 µm), *A. concolor* (51.00~100.00 µm), *A. nordmanniana* (51.00~100.00 µm) and species in *Cedrus* and *Picea* [10,27–30]. The pollen shape class (P/E ratio) was frequently subprolate and prolate-spheroidal in our research. Pollen grain shape of *C. argyrophylla* has not been the object of detailed investigations yet. In other studies, we may find more results presenting

P/E ratio without concluding the exact shape [31,32], but we can also find some work on *A. alba*, *Ginkgo biloba*, and *Sambucus nigra* where methods according to Erdtman are used to describe the exact shape [10,33,34]. The sacci shape (A/B) was first used as a new trait on study of *Abies alba* [10] and we applied it here to describe the sacci shape of *C. argyrophylla* for the first time. Among our studied samples, they mostly produced an elongated and flattened saccus (1.8—2.7 times longer than wider). It should be clarified that we followed the description in Zhang [14] and defined the longer side as the length of saccus, denoted "A", whereas in the above *A. alba* study longer side was defined as the width of saccus and that instead denoted "A".

4.2. New Qualitative Pollen Traits and Clustering

In using five new qualitative traits, we obtained interesting results. Firstly, in the majority of studies on pollen morphology, the description relied less on qualitative traits except pollen exine. Qualitative traits can also make a significant contribution, we once used some new qualitative traits to describe the petals morphology on classification research of Hibiscus syriacus cultivars, and we acquire effective results (The new ordered multistate character of red center line to red center relationship was one of the main criteria and basis for the classification of *Hibiscus syriacus* cultivars) [35]. Qualitative traits are used more often in pollen morphology classification of horticulture cultivars [36,37]. Thus, in our study, B-SD, B-SU, B-GU, O-CO, and O-CSR were derived and three of these new traits were shown helpful for distinguishing the pollen samples collected from the BMS-J population. We also confirmed that the traits B-SD, P/E, and A/B clustered together, based on a R-type clustering analysis, and that evidently pollen of *C. argyrophylla* displays a more distinct outline of two sacci (diploxylon-type), often accompanied by smaller values of P/E and A/B. Comparing the four *C. argyrophylla* populations, BMS-J presented a high degree of intraspecific differences, with a smaller P, A/B, and G, and higher E and B than the three other populations, along with a P/E ratio of almost 1. Further, BMS-J pollen grains are frequently subcordate for trait O-CO, have a distinct outline of sacci in trait B-SD, have much rougher sacci for trait O-CSR, an uneven germinal anacolpus for trait B-GU, and uniform size of two sacci for trait B-SU. These new qualitative traits enable a more intuitive classification and could prove useful for pollen classification in other Pinaceae genera, such as *Abies*, *Picea*, and *Cedrus*, whose pollen grains are similar in shape to *Cathaya*'s.

As demonstrated by the Q-type clustering analysis, the results confirmed that those 11 traits could serve as robust classification criteria of the 23 pollen samples of *C. argyrophylla* we studied. Evidently, the advantages of employing qualitative traits are pronounced, as they facilitated the samples' classification on the basis of Q-type clustering. Given that most qualitative traits could not be quantified, they are useless in conventional classification schemes, precluding their ability to serve as criteria. In the Q-type cluster (L2, D = 7.5), the new qualitative traits B-GU and B-SU helped to classify four samples (9, 13, 5, and 11) and another 13 samples (16, 23, 10, 15, 4, 8, 14, 3, 6, 12, 2, 7, and 1) into two independent clusters when they are could not be carried out with quantitative trait P/E alone. In the same way, the novel qualitative trait O-CSR helped to classify the remaining 13 samples into another two independent clusters—samples 16, 23, 10, and 15 with samples 4, 8, 14, 3, 6, 12, 2, 7, and 1—despite being not so easily distinguished by three quantitative traits (P, A, and G). Many palynologists used the Q-type cluster approach to identify classification criteria for tree species, especially in research on cultivars' classification [38–40]. O-OR and O-CSR were both descriptors of pollen roughness, but trait O-CSR could also convey the roughness degree of corpus and sacci in tandem, as we observed for sample 6; it was rough as gauged by trait O-OR but much rougher in the corpus according to trait O-CSR.

4.3. Implications for Siring Success Association between Pollen Size and Environmental Factors in Tree C. argyrophylla

Pollen sacci can help to fly during pollination that especially for wind-pollinated plants such as *C. argyrophylla*. Pollen size often depends on the value of equatorial diameter

(E). From our study, the width of the saccus (B) was correlated with E positively ($p < 0.01$, r = 0.85). Larger pollen is often accompanied by B and E's higher values, such as pollen grains in BMS-J. However, pollen size varies greatly among flowering plant species. It has been shown to influence the delivery of sperm cells to eggs, which imples pollen size is an excellent male "quality trait." [7,41,42]. From this point of view, we thought it would have the same effects on *C. argyrophylla*. Then, we have been observing the seed setting rates per tree of four populations in two regions since 2019. The highest value of seed setting rates and good quality seeds occurred in the region of Bamianshan Natural Reserve, especially in the BMS-J population, which has a larger pollen size. Pollen size may correlate with its germination ability, viability, and pollination patterns, and these need further studies on *C. argyrophylla* to figure out the influencing mechanism.

Nevertheless, populations with larger pollen sizes in Bamianshan Natural Reserve are always living in the habitat with higher precipitation (bio15, 52.33 mm) and smaller temperature (bio5, 27.73 °C) values than the Shajiaodong Natural Reserve (bio15, 48.00 mm; bio5, 29.05 °C). So it proved that precipitation and temperature factors mainly exhibited a significant positive and negative correlation with pollen size. Moreover, the pollination time of *Cathaya* is from May 10 to 30. Pollen viability was stable in dry and low-temperature conditions [43], so the high precipitation in May perhaps be insufficient for pollination. As we can see from the data of 05-precip (negative correlation) and 05-temp (positive correlation), Bamianshan Natural Reserve region often with smaller 05-precip and larger 05-temp which helped to fruit. Then, we also approve Wang's opinion that there was a low variation of pollen viability among individuals within populations but significant differentiation between populations within regions and among regions [43]. The distribution areas of wild *Cathaya* are usually rich in precipitation. However, we have not yet considered pollen size, particularly when sampling within the population for *ex-situ* conservation of *C. argyrophylla*. Seed sources perhaps could be sampled from individuals of the BMS-J population. In conclusion, the genetic resources with larger pollen size and planting regions with higher precipitation, lower temperature, and 05-precip all played important roles and helped sire success.

5. Conclusions

1. The most important and main pollen features of the studied *C. argyrophylla* samples are traits B, P, A, O-OR and the new traits, O-CO, B-SD, O-CSR, and B-SU. Accordingly, the 23 tree-level samples could be divided into two, three, or four clusters. By contrast, at the population level, the pollen of population BMS-J is very different from the other three populations.
2. Precipitation factors were higher response to pollen morphology compared to temperature and geographic factors, the main precipitation (bio12, bio13, bio14, bio15 and 05-precip) and temperature factors (bio1, bio4, bio5, and bio6) were exhibited positive and negative correlation with pollen size (E and B), respectively.
3. This is the first study on the intraspecific pollen morphology and variability of wild *C. argyrophylla* and its correlation with environmental factors. and these results can help for seed breeding and reproduction in endangered tree *C. argyrophylla*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13050651/s1, Table S1: Geographical location of studied *Cathaya argyrophylla* genotypes.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Minimal, maximal, and mean value as well as coefficient of variation (cv, in %) for P, E, P/E, A/B, A, B and G.

Samples	P			E			P/E			A/B		
	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv
1	38.62	34.22–43.38	6.42	38.35	29.68–44.11	9.31	1.02	0.84–1.46	13.59	2.63	1.53–4.40	20.40
2	39.63	29.41–46.87	10.41	33.06	26.80–39.90	10.45	1.21	0.87–1.52	12.17	2.38	1.74–3.08	16.17
3	41.21	34.79–47.74	7.74	34.46	29.32–43.51	8.58	1.20	0.93–1.42	10.90	2.43	1.83–3.22	12.40
4	41.07	37.31–46.59	5.75	32.36	28.80–36.69	6.78	1.27	1.12–1.42	6.94	2.42	2.04–3.08	12.28
5	40.23	33.84–42.96	5.94	31.53	28.23–37.59	8.14	1.28	1.06–1.50	8.61	2.64	2.21–3.04	8.24
6	40.65	33.48–45.25	7.39	33.56	28.30–39.64	9.45	1.22	0.97–1.47	12.41	2.42	1.80–3.25	15.88
7	39.67	33.39–44.31	6.68	36.14	30.99–43.76	8.69	1.10	0.90–1.27	8.50	2.10	1.78–2.48	9.11
8	39.37	28.32–45.60	8.85	33.03	26.58–39.06	9.44	1.20	0.94–1.53	11.11	2.33	1.80–3.05	12.35
9	43.95	37.87–48.31	5.61	32.77	25.41–40.01	11.06	1.36	1.06–1.67	11.99	2.55	2.00–3.33	14.65
10	43.75	39.90–51.68	6.06	35.37	28.84–44.26	8.40	1.24	0.90–1.47	8.97	2.36	1.89–3.14	11.91
11	40.08	30.71–47.05	10.04	29.83	23.58–35.08	9.96	1.35	1.05–1.64	10.57	2.55	2.07–3.89	18.75
12	41.02	35.92–45.78	5.12	33.84	27.13–43.82	11.31	1.23	0.92–1.48	10.43	2.28	1.84–2.76	11.99
13	42.28	36.44–46.26	5.14	33.08	26.47–39.87	12.61	1.30	0.94–1.61	13.93	2.53	1.94–3.53	15.32
14	38.90	35.26–42.86	5.43	34.05	28.76–41.23	8.48	1.15	0.91–1.36	9.94	2.19	1.73–2.93	11.50
15	42.92	38.90–46.11	3.68	31.90	27.67–37.12	8.79	1.36	1.06–1.55	9.70	2.74	1.99–3.90	14.60
16	40.34	35.76–45.39	6.35	34.70	31.31–41.46	7.13	1.17	0.90–1.45	10.57	2.34	1.71–3.17	14.22
17	36.47	29.17–41.68	7.52	39.24	35.01–43.55	5.79	0.93	0.69–1.13	10.67	1.91	1.47–2.38	11.33
18	37.72	30.43–43.14	9.13	41.08	32.25–49.78	11.01	0.93	0.71–1.11	12.25	1.94	1.67–2.43	10.28
19	39.57	32.11–45.70	8.31	37.90	31.50–42.56	9.29	1.05	0.77–1.26	11.79	2.16	1.63–2.72	12.78
20	38.13	35.21–41.92	4.92	38.01	32.89–46.42	8.57	1.01	0.85–1.18	8.88	2.06	1.69–2.49	10.42
21	39.28	32.89–43.21	6.96	43.49	33.75–48.45	7.50	0.91	0.77–1.26	12.62	1.83	1.56–2.38	12.67
22	39.17	33.58–43.87	6.00	34.53	29.20–39.80	9.24	1.14	0.95–1.40	11.10	2.26	1.85–2.86	11.65
23	39.55	36.15–44.74	5.52	34.41	30.70–40.25	8.40	1.16	1.00–1.34	8.16	2.41	2.04–3.31	11.33

Samples	A			B			G		
	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv
1	37.23	29.88–48.52	12.11	14.15	8.67–21.67	19.68	35.95	25.21–47.26	14.88
2	37.10	30.37–40.88	7.44	15.57	11.54–18.66	15.10	36.15	27.70–45.71	13.42
3	38.09	33.39–45.28	8.28	15.69	11.45–20.54	12.22	36.63	29.68–45.49	10.63
4	37.03	30.92–42.88	7.66	15.32	11.62–18.08	11.91	36.10	30.24–42.06	8.57
5	37.21	31.37–41.41	6.87	14.08	10.31–17.03	12.02	36.85	30.61–41.15	7.73
6	36.60	30.10–41.32	9.91	15.13	10.43–19.94	14.77	36.36	28.50–41.26	10.79
7	35.78	30.51–40.50	6.38	17.07	13.86–21.57	10.16	36.03	29.48–41.00	7.15
8	36.41	26.93–44.09	8.90	15.66	11.71–18.95	12.51	35.64	25.93–41.53	9.23
9	38.65	33.13–42.71	7.70	15.15	10.81–21.37	14.28	38.19	29.92–42.29	7.38
10	38.84	33.84–47.11	6.96	16.46	10.77–21.60	13.14	39.40	36.47–46.38	5.48
11	37.50	29.37–44.84	11.01	14.70	10.31–17.03	16.50	37.15	28.32–43.75	11.06
12	37.05	31.47–40.37	6.82	16.24	11.39–20.13	14.26	37.80	30.38–42.97	7.78
13	38.46	33.77–42.40	5.50	15.22	10.39–18.98	14.93	38.41	30.92–41.96	6.57
14	36.59	32.79–41.23	6.56	16.69	12.05–21.16	12.07	34.95	29.60–39.29	7.41
15	40.41	37.03–45.37	4.37	14.77	10.81–18.57	11.77	39.74	36.75–42.46	3.28
16	37.50	30.27–44.67	8.45	16.02	13.43–18.99	8.98	35.14	25.73–41.05	11.81
17	35.54	28.21–39.91	8.89	18.58	16.49–21.09	6.96	32.77	21.57–37.59	12.45
18	37.19	30.18–42.19	8.66	19.12	14.01–24.62	13.86	31.41	17.05–39.93	19.74
19	38.10	29.09–43.87	9.36	17.67	13.04–21.40	11.61	34.23	18.53–43.20	15.41

Table A1. *Cont.*

Samples	A			B			G		
	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv
20	36.56	33.16–41.12	6.04	17.76	15.37–23.76	11.74	34.83	30.35–39.10	6.83
21	37.54	30.86–42.10	7.86	20.56	16.47–23.91	9.74	34.11	27.60–39.92	8.96
22	37.29	33.35–40.88	5.83	16.49	13.04–20.37	11.28	35.23	25.64–38.60	9.09
23	37.24	31.70–41.37	6.60	15.44	11.04–18.91	11.49	35.24	31.74–38.16	5.50

References

1. Romero, M.; Rangelyagui, C.; Cassinello, J.; Cuzin, F.; Jedeidi, T.; Masseti, M.; Nader, I.W.; Clerq, K.D. *IUCN Red List of Threatened Species*; Version 2013.2; International Union for Conservation of Nature and Natural Resources: Grand, Switzerland, 2013.
2. Pan, Z. Conservation and Utilization of *Cathaya argyrophylla* germplasm resources. *Mod. Agric. Sci. Technol.* **2009**, *11*, 13–15.
3. Zongqiang, X.; Qingmei, L. Seed Characteristics of Endangered Plant *Cathaya argyrophylla*. *Acta Phytoecol. Sin.* **2000**, *24*, 82–86.
4. Ejsmond, M.J.; Wrońska-Pilarek, D.; Ejsmond, A.; Dragosz-Kluska, D.; Kozowski, J. Does climate affect pollen morphology? Optimal size and shape of pollen grains under various desiccation intensity. *Ecosphere* **2011**, *2*, 117. [CrossRef]
5. Srivastava, J.; Manjunatha, B.R.; Balakrishna, K.; Prajith, A.; Manjunatha, H.; Jose, J.; Kumar, N. Quantitative pollen-based reconstruction of the vegetation diversity in response to the late-Holocene climate change near Karwar, south-west coast of India. *Quat. Int.* **2021**, *599–600*, 95–106. [CrossRef]
6. Caliskan, O.; Bayazit, S.; Kilic, D.; Ilgin, M.; Karatas, N. Pollen morphology and variability of caprifig (*Ficus carica* var. *caprificus*) genetic resources in Turkey using multivariate analysis. *Sci. Hortic.* **2021**, *287*, 110283. [CrossRef]
7. Mccallum, B.; Chang, S.M. Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. *Am. J. Bot.* **2016**, *103*, 466–468. [CrossRef]
8. Chang, S.; Li, C.; Jiang, Y.; Long, Y.; Yin, J. Characteristics of the pollen morphology and viability of Bougainvillea (Nyctaginaceae). *Sci. Hortic.* **2021**, *277*, 109732. [CrossRef]
9. Hao, K.; Tian, Z.-X.; Wang, Z.-C.; Huang, S.-Q. Pollen grain size associated with pollinator feeding strategy. *Proc. R. Soc. B* **2020**, *287*, 20201191. [CrossRef]
10. Wrońska-Pilarek, D.; Dering, M.; Bocianowski, J.; Lechowicz, K.; Hauke-Kowalska, M. Pollen Morphology and Variability of *Abies alba* Mill. Genotypes from South-Western Poland. *Forests* **2020**, *11*, 1125. [CrossRef]
11. Wrońska-Pilarek, D.; Wiatrowska, B.; Bocianowski, J. Pollen morphology and variability of invasive *Spiraea tomentosa* L. (Rosaceae) from populations in Poland. *PLoS ONE* **2019**, *14*, e0218276. [CrossRef]
12. Campo, M.V.; Sivak, J. Structure alveolaire de l'ectexine des pollens à ballonets des Abietacées. *Pollen Spores* **1972**, *14*, 115–141.
13. Liu, Y.S.; Basinger, J.F. Fossil *Cathaya* (Pinaceae) pollen from the Canadian high arctic. *Int. J. Plant Sci.* **2000**, *161*, 829–847. [CrossRef]
14. Jintan, Z. Studies on Pollen Morphology of Pinacea in China. *Bull. Bot. Res.* **1989**, *9*, 12.
15. Nimsch, H.; Dorken, V.M. Morpho-anatomical investigations of cones and pollen in *Cathaya argyrophylla* Chung & Kuang (Pinaceae, Coniferales) under systematical and evolutional aspects. *Feddes Repert.* **2014**, *125*, 25–38.
16. Xu, K.X. *Numerical Taxonomy*; Science Publishing: Beijing, China, 1994.
17. Wang, F.X.; Qian, N.F.; Zhang, Y.L.; Yang, H.Q. *Pollen Flora of China*, 2nd ed.; Science Publishing: Beijing, China, 1995; pp. 5–13.
18. Punt, W.; Hoen, P.P.; Blackmore, S.; Nilsson, S.; Le Thomas, A. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.* **2007**, *143*, 1–81. [CrossRef]
19. Halbritter, H.; Silvia, U.; Grímsson, F.; Weber, M.; Frosch-Radivo, A. *Illustrated Pollen Terminology*, 2nd ed.; Springer: Vienna, Austria, 2018.
20. Shapiro, S.S.; Wilk, M.B. An Analysis of Variance Test for Normality. *Biometrika* **1965**, *52*, 591–611. [CrossRef]
21. Erdtman, G. The Acetolysis Method. A Revised Description. *Sven. Bot. Tidskr.* **1960**, *54*, 561–564.
22. Abdi, H.; Williams, L.J. Principal Component Analysis. *WIREs Comput. Stat.* **2010**, *2*, 433–459. [CrossRef]
23. Martinović, S.; Vlahović, M.; Gajić-Kvaščev, M.; Vuksanović, M.; Glišić, D.; Volkov-Husović, T. Principal component analysis of morphological descriptors for monitoring surface defects induced by thermal shock. *J. Eur. Ceram. Soc.* **2021**, *41*, 426–429. [CrossRef]
24. Parks, J.M. Part 2: Applications of Multivariate Statistics in Geology||Cluster Analysis Applied to Multivariate Geologic Problems. *J. Geol.* **1966**, *74*, 703–715. [CrossRef]
25. Ting, W.; Zhen, W.; Shufeng, L.; Zhanming, Y.; Xiaoxian, R.; Yingjuan, S. Association between spatial genetic variation and potential distribution in tree fern *Alsophila gigantea* (Cyatheaceae) in Hainan Island, China. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2021**, *49*, 12407.
26. Erdtman, G. Palynology. (Book Reviews: Pollen and Spore Morphology. Plant Taxonomy-Gymnospermae, Bryophyta, vol. 3). *Science* **1966**, *151*, 979–980. [CrossRef]
27. Halbritter, H.; Heigl, H.; Auer, W. PalDat—A Palynological Database. Available online: https://www.paldat.org/pub/Picea_abies/306140 (accessed on 9 December 2021).

28. Halbritter, H. PalDat—A Palynological Database. Available online: https://www.paldat.org/pub/Cedrus_atlantica/300586 (accessed on 9 December 2021).
29. Halbritter, H. PalDat—A Palynological Database. Available online: https://www.paldat.org/pub/Abies_nordmanniana/300648 (accessed on 15 May 2020).
30. Halbritter, H. PalDat—A Palynological Database. Available online: https://www.paldat.org/pub/Abies_concolor/300647 (accessed on 15 May 2020).
31. Gudeski, A. Morphological characteristics of the pollen grains of Abies alba from populations in Macedonia and of *A. cephalonica* from Parnis in Greece. *God. Zb. Na Zemjod.-Smarskiot* **1974**, *26*, 133–147.
32. Bagnell, C.R., Jr. Species distinction among pollen grains of Abies, Picea, and Pinus in the rocky mountain area (A scanning electron microscope study). *Rev. Palaeobot. Palynol.* **1975**, *19*, 203–220. [CrossRef]
33. Sedlackova, V.H.; Grygorieva, O.; Brindza, J. Study of Morphological Characteristics of Pollen Grains of *Sambucus nigra* L. *Agrobiodiversity Improv. Nutr. Health Life Qual.* **2018**, *2585–8246*, 277–284. [CrossRef]
34. Korszun, S.; Klimko, M. Microsporangia and pollen morphology of Ginkgo biloba cultivars. *Dendrobiology* **2014**, *71*, 83–92. [CrossRef]
35. Xiao, F.; Wang, X.H.; Wang, Y.Q.; Liu, C.L.; Xie, L.S.; Ren, X.Y. Numerical Classification and Principle Component Analysis of 27 Hibiscus Cultivars. *J. Cent. South Univ. For. Technol.* **2019**, *39*, 59–64.
36. Ma, S.L.; Lu, Y.M. Classification and phylogenetic analysis of Chinese hawthorn assessed by plant and pollen morphology. *Genet. Mol. Res. Gmr* **2016**, *15*, gmr8739. [CrossRef]
37. Ma, Z.H.; Glc, B.; Zhang, D.X. Pollen morphology of *Callicarpa* L. (Lamiaceae) from China and its systematic implications. *Plant Syst. Evol.* **2016**, *302*, 67–88. [CrossRef]
38. Zhang, L.; Tang, H.; Liu, W.L.; Zhu, X.X.; Li, Y. Studies on numerical taxonomy of the traditional Paeonia Rockii tree cultivars in Northwest regions. *J. Cent. South Univ. For. Technol.* **2011**, *31*, 7.
39. Han, X.Y.; Zhang, Y.L.; Niu, L.X.; Aamp, N.; University, F. Morphological traits based taxonomy of 39 tree peony cultivars. *J. Northwest A F Univ. (Nat. Sci. Ed.)* **2014**, *42*, 128–136.
40. Zhang, J.; Huang, D.; Zhao, X.; Hou, X.; Di, D.; Wang, S.; Qian, J.; Sun, P. Pollen morphology of different species of Iris barbata and its systematic significance with scanning electron microscopy methods. *Microsc. Res. Tech.* **2021**, *84*, 1721–1739. [CrossRef] [PubMed]
41. Mendoza, J.E.; Balanza, V.; Cifuentes, D.; Bielza, P. Selection for larger body size in *Orius laevigatus*: Intraspecific variability and effects on reproductive parameters. *Biol. Control* **2020**, *148*, 104310. [CrossRef]
42. Xia, J.; Lu, J.; Wang, Z.; Hao, B.; Wang, H.; Liu, G. Pollen limitation and Allee effect related to population size and sex ratio in the endangered *Ottelia acuminata* (Hydrocharitaceae): Implications for conservation and reintroduction. *Plant Biol.* **2013**, *15*, 376–383. [CrossRef] [PubMed]
43. Wang, H.W.; Deng, H.S.; Tan, H.M.; Qing, K.; Zhang, D.H.; Ge, S. Pollen viability and variation in *Cathaya argyrophylla*. *J. Plant Ecol.* **2007**, *31*, 1199–1204.