Study on Phenotypic Variation and Diversity of Natural Polyspora Populations at Different Environmental Gradient, China

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Abstract

Polyspora pertains to Theaceae. It is a perennially green tree or shrub, flowering in winter. It served as an exceptional species of garden, mountain afforestation, and timber tree. It is primarily distributed in the tropical regions of Southeast Asian Islands and Indo-China Peninsula; eight varieties are spread across China, which is the northern periphery of the genus's distribution area, chiefly dispersed in subtropical evergreen broad-leaved forests. The phenotypic variability of *Polyspora* in China was relatively extensive, with leaf traits of *Polyspora longicarpa* exhibiting the most substantial variation, with an average coefficient of variation of 28.46%, flower characteristics of *Polyspora chrysandra* demonstrating a higher variation (16.26%), and capsule and seed traits of *Polyspora* displaying the most significant variation (20.15%); the phenotypic differentiation primarily originated from intergroups, and that of *Polyspora chrysandra* was slightly lower than that of intragroups, and that of *Polyspora speciosa* was not significantly distinguished between and within groups. The phenotypic differentiation between and within groups was not evident, and the phenotypic differentiation of *Polyspora longicarpa* and *Polyspora hainanensis* primarily originated from within groups; leaf length, leaf perimeter, leaf area, capsule length, and seed quality were the pivotal indicators of the phenotypic disparities of the species; leaf phenotypic variation of *Polyspora* was influenced by its own genetic factors and geographic environment, and the two roles were comparable; among the environmental factors, the bioclimatic factors and UV radiation had a more profound impact on the leaf phenotypes.

1. Introduction

Plant functional traits are comprehensive responses and adaptations of plants to habitat conditions and results of interactions between plants and environment. Coordinated expression of different plant functional traits has adaptive value. In limited resource competition, functional traits are how to realize coexistence, which is the core of our understanding of biodiversity control[1]. In the process of interacting with the environment, most plants adapt to the changes in the ecological environment by adjusting the morphological structure and internal physiological characteristics of plant organs. Plant leaf functional traits, for example, leaf area (LA), specific leaf area(SLA), leaf thickness(LT), leaf nitrogen content(LNC), and leaf phosphorus content (LPC) change as plant morphological, physiological and other characteristics adapt to the external living environment[2], that is, the result of a trade-off between different functional traits[3], and also the trade-off strategy adopted by plants to solve environmental heterogeneity adaptation by stable coexistence mechanism dependent on niche[4], which is manifested as plant phenotypic plasticity[5]. A morphological-physiological-phenotypic trait that indirectly affects individual fitness by affecting individual growth, reproduction, and survival[6]. Phenotypic traits are combinations of plant morphological characteristics, which are controlled by genetic and environmental conditions. After long-term natural selection and environmental stress, phenotypic variation usually occurs, which is also a survival strategy for plants to adapt to different habitats[7]. Phenotypic diversity focuses on the phenotypic variation of plant populations in different habitats. By studying plant phenotypic diversity, we can understand the genetic variation rules of plant populations and their response and adaptation mechanisms to the environment. This is also the initial way to select excellent plant varieties, which is of great significance for the protection and utilization of plants with potential value. Phenotype is the relatively fixed

morphological and structural characteristics of any organism, and the formation of phenotypic diversity is the comprehensive result of gene expression and long-term adaptation of plants to specific natural environments[8–10]. Phenotypic traits, as the expression form of plant genetic diversity, its geographical variation reflects the evolution mode of morphological adaptability along environmental gradient[11], which is of great significance for studying species variation and evolution mechanism. Grasping the inherent law mechanism of phenotypic variation of species and its response to environmental factors can be used as an important reference basis for exploring plant classification and evolution mechanisms and as a basis for introduction, domestication, and breeding improvement[12–13].

For a long time, the taxonomic community has paid little attention to *Polyspora*. A large number of misidentified specimens exist in the specimens, and many species within this genus are identified as the type species of *Polyspora axillaris*, or other species are identified incorrectly, resulting in inaccurate descriptions of the distribution of species in the genus in the existing flora and dendrology. So far, no investigation on germplasm resources of the genus *Polyspora* has been carried out in China, and the status quo of germplasm resources and the natural distribution of species of the genus are still unclear. After long-term natural selection and adaptive evolution, the morphological characteristics of interspecies and intraspecies of the genus *Polyspora* will show different degrees of differentiation. Due to the wide distribution in China, there may be some phenotypic variation under the synergistic effect of different environmental factors.

The genus *Polyspora* is the second most abundant taxon in Theaceae, mainly in South and Southeast Asia, and is a typical representative species of the Indo-Malay rainforest-Chinese subtropical broad-leaved evergreen forest. China is the northern distribution area of it, mainly distributed in Yunnan, Sichuan, Chongqing, Guangxi, Guangdong, and Fujian [14–17]. According to the taxonomic proposal of Yang[14], there are eight species in China, namely *Polyspora axillaris*, *Polyspora chrysandra*, *Polyspora speciosa*, *Polyspora kwangsiensis*, *Polyspora longicarpa*, *Polyspora tiantangensis*, *Polyspora hainanensis*, *Polyspora tonkinensis*. In the list of "Red List of China's Biodiversity(Higher Plants Volume, 2020)" [18] and "The Red List of Theaceae" [19], six species of *Polyspora* (*P. axillaris*, *P. chrysandra*, *P. speciosa*, *P. longicarpa*, *P.tiantangensis* and *P.hainanensis*) are listed on the list, which are in different degrees of endangered status. In recent years, domestic and foreign scholars have focused on classification and distribution[14–15], genetic differentiation and genome structure[20–21], chemical composition[22–23], community structure[24], and geographic distribution[25]. Studies on its phenotypic traits have rarely been reported.

In this study, we investigated the phenotypic traits of 32 wild populations of eight species on the basis of a resource survey of the genus *Polyspora* within China. The main objective of our study was to address three questions: (1) To clarify the diversity characteristics of phenotypic traits of each species through quantitative study of phenotypic traits of the genus *Polyspora*; (2) To analyze the phenotypic variation characteristics and the degree of population differentiation of the genus *Polyspora*, and to explore the environmental adaptability of leaf phenotypic traits, and the correlation between phenotypic and geographical environment factors; and (3) To explore morphological characters of *Polyspora* with phylogenetic signatures. The above problems were studied with a view to providing a reference for adaptive

evolutionary studies, germplasm conservation, species classification, and sustainable exploitation of *Polyspora*.

2. Materials and Methods

2.1.Study area and plant collection

According to the classification of Chinese *Polyspora*[14] and its distribution in China, based on an extensive review of literature and specimen records, consultation with relevant experts and scholars, and local guides, we chose the provinces of Yunnan, Guizhou, Sichuan, Chongqing, Guangxi, Guangdong, and Hainan, where the species and number of *Polyspora* species are concentrated, and determined the scope of the survey and the location in terms of counties to carry out the survey of wild germplasm resources. The survey was carried out in the provinces of Yunnan, Guizhou, Sichuan, Chongqing, Guangxi, Guangdong and Hainan. The sampling range covers all the concentrated distribution areas of the genus in China, covering different climatic zones and vegetation types. For key taxa, sampling was conducted at the model origin. For species with large variability, more materials of different variability types in different distribution areas were collected. According to the natural distribution status of *Polyspora* in the aforementioned provinces, and accordance with different elevations, habitats, community structures and other factors, 1 ~ 2 representative groups are delineated in the specific investigation area of each county and city, and sample lines are set to investigate the germplasm resources of *Polyspora* along the way, record the information of group size, habitat type, geographic coordinates, elevation, accompanying species, etc., and learn about vegetation types, soil types, and hydrothermal conditions of the investigation sites. type, hydrothermal condition. When there is no obvious geographic isolation such as mountains, rivers, valleys, and ravines, the minimum distance between groups of the same species is limited to 50 km or more; when there is obvious geographic isolation, the distance between groups is not limited. Within each group, more than 20 plants of the genus *Polyspora* were selected, and the distance between individuals was not less than 50 m. Individuals with less than 20 plants in the group or with obvious phenotypic differences within the group were collected regardless of the group size and sampling interval. Individuals with obvious phenotypic differences and stable traits were selected from each population, and some morphological data of leaves and flowers were measured in the field; capsules were collected and brought back to the laboratory to measure their morphological data, and after the capsules were dried and cracked, the morphological data of seeds were measured. Three to five fresh, well-grown annual leaves were collected from each plant, dried on silica gel and, stored in an ultra-low temperature refrigerator for subsequent studies.

According to the above sampling strategy, a total of 753 individuals were collected from 32 groups of *Polyspora* in China (Table S1), with an average group spacing of 744 km, more than 100 specimens, and a small amount of pollen and capsules, including 8 groups of *Polyspora axillaris*, 9 groups of *Polyspora chrysandra*, 4 groups of *Polyspora speciosa*, 5 groups of *Polyspora kwangsiensis*, 1 group of *Polyspora tonkinensis*, 2 groups of *Polyspora longicarpa*, 1 group of *Polyspora tiantangensis* and 2 groups of *Polyspora hainanensis*. According to the vegetation division of China, 10 populations were located in the Southern subtropical monsoon evergreen broadleaf forests(□Bii, □Aiii), 7 populations were located in the Northern subregion of evergreen broad-leaved forest in the central subtropical zone(□Aiia), 6 populations

were located in the Central subtropical evergreen broad-leaved forest(Bi), 2 populations were located in the Northern tropical monsoon forest(Bi), 4 populations were located in the Northern tropical semi-evergreen monsoon rainforests(Ai), and 3 populations were located in the Southern tropical humid rainforest monsoon forest(Aii).

- **Ethics statement**: The plant materials used in this study were obtained from wild plants and did not involve any endangered or protected species. The plant materials were identified by Chang-Le Ma and Zhi-Feng Fan. The authors declare no competing interests.
- **Plant guideline statement**: Experimental research and field studies on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

2.2 Plant Morphological Characteristics

According to Ye[26], Flora of China[27] and Bartholomew&Min[28], phenotypic characters related to leaf, flower and capsule morphology, capsule quality, seed quantity and seed quality were observed and coded. 10 plants (individual spacing no less than 50 m) of samples with good growth, no diseases and insect pests, obvious phenotypic differences and stable characters were selected from each population for phenotypic character determination. A total of 31 indicators were used for phenotypic analysis, of which 25 were measured directly and the remaining 6 were calculated (Table S2).

Five leaves in good condition were selected at each of the four orientations of the sample plants, 20 leaves were selected from each plant, and leaf length(PL), leaf width(LL) and petiole length(PL) were measured; 5 completely opened flowers were randomly selected from each plant, and flower character indexes such as flower diameter(FD), petal length(PEL) and petal width(PEW) were measured; 20 healthy and mature capsules were randomly collected from each plant, and seed data such as capsule length (CL), capsule stem length(CSL) and capsule diameter(CD) were taken back to the laboratory to measure capsule weight(CW) and seed weight(SW). In the above determination, the length, width and diameter are all measured by electronic vernier calipers (measurement accuracy: 0.01mm); mass is measured by electronic balance(measurement accuracy: 0.01g). After scanning leaves, leaf area(LA), leaf perimeter(LP), leaf base angle(LBA) and leaf tip angle(LTA) were measured with Digimizer software(version 6.4.0, MedCalc Software Ltd. Measurement accuracy: 0.01cm^2 or 0.01°).

Due to the influence of the climatic period, flowering and capsuleing pattern, and time of field survey, the number of flowers, capsules, and seeds in some groups was too small or absent to achieve the desired amount of data, for the sake of relative uniformity, the phenotypic data of floral organs were chosen from 16 groups (covering 5 species), the data of capsules and seeds were chosen from 23 groups (covering all the species), and the leaf morphology data were chosen from 32 groups (which had been collected according to the predetermined plan). All 32 populations were selected for the leaf morphology data (which were collected according to a predetermined schedule). The number of observations for the morphological data was 6,400 leaves, 800 flowers, 4,600 capsules and 4,600 seeds.

Table 1
Geographical environment factors

Serial number	Category	Geo-graphical environment factor
1	Bioclimatic factors	Annual Mean Temperature(bio01), Mean Diurnal Range (Mean of monthly) (bio02), Isothermality (bio02)/(bio07×100)(bio03), Temperature Seasonality (standard deviation ×100)(bio04), Max Temperature of Warmest Month(bio05), Min Temperature of Coldest Month(bio06), Temperature Annual Range (bio05-bio06)(bio07), Mean Temperature of Wettest Quarter(bio08), Mean Temperature of Driest Quarter(bio09), Mean Temperature of Warmest Quarter(bio10), Mean Temperature of Coldest Quarter(bio11), Annual Precipitation (bio12), Precipitation of Wettest Month(bio13), Precipitation of Driest Month(bio14), Precipitation Seasonality (Coefficient of Variation) (bio15), Precipitation of Wettest Quarter(bio16), Precipitation of Driest Quarter(bio17), Precipitation of Warmest Quarter(bio18), Precipitation of Coldest Quarter(bio19)
2	Terrain factors	Elevation(Elev), Aspect
3	Soil factors	Soil nutrient availability(sq1),Soil nutrient retention capacity(sq2)
4	UV-B radiation factors	Annual average ultraviolet intensity(uvb1), Seasonal variation of ultraviolet radiation(uvb2),Maximum monthly average ultraviolet(uvb3),Minimum monthly average UV(uvb4),sum of maximum monthly UV(uvb5),sum of minimum monthly UV(uvb6)
5	Aridity factors	Aridity index(AI), AI = MAP/(MAT + 33)[32], Lower AI means more drought
6	Vegetation factors	Vegetation types(Veg)
7	Geographical factors	Longitude(long.), Latitude(lat.)

2.3 Geographical environment factor

In order to reveal the correlation between phenotypic traits and habitats, redundancy analysis (RDA) and correlation analysis (CA) were performed using 33 geographical environment factors. Due to the limited number of flowers and capsule investigated, this study only conducted redundancy analysis and correlation analysis between leaf phenotypic traits and geographical environment factors. Geographical environment factors include 19 biological climate factors, 2 topographic factors, 2 soil complex factors, 6 ultraviolet radiation factors, 1 aridity index, 1 vegetation factor and 2 latitude and longitude factors (Table 1). 19 bioclimatic factors from Global climate and weather data (Worldclim, https://www.worldclim.org)[29], two soil composite factors were downloaded from the Harmonized World Soil Database V1.2 (HWSD, https://www.fao.org/) [30], six UV radiation factors from the Global UV-B Radiation Database (gIUV, https://www.ufz.de/gluv/)[31]and extract environmental variable values for each population using ArcGIS spatial interpolation. Latitude, longitude and elevation factors are based on GPS records during field survey, and aspect is based on ArcGIS 10.4 Spatial analysis functions are extracted based on altitude. Vegetation data were downloaded from Resource and Environmental Science

and Data Center, Chinese Academy of Sciences (RESDC, http://www.resdc.cn).

2.4 Morphological phylogenetic traits

- Bsae on phenotypic diversity analysis, phylogenetic analysis was carried out by combining their qualitative traits such as branchlets, terminal buds, petioles, presence or absence of hairs on leaves, leaf texture, leaf margins, and number of locules in the ovary. Referring to morphological study on the phylogenetic relationships. Phylogenetic studies showed that the consistency of characters within the genus was relatively high[33–34], and there were some distinct morphological shapes with evolutionary significance, which can be taken as a main reference for the discussion of the relationships between species of the genus *Polyspora*. It can be used as the main reference basis for exploring the interspecific relationships of this genus. In addition, leaf shape, leaf apex shape, sepal shape, and other traits are also different between species, but there is no obvious evidence to show which species is more primitive, or that they evolved in parallel. Combining qualitative morphological characteristics, 15 morphological traits were selected for phylogenetic analysis in our study, including 7 trophic traits and 8 reproductive traits, in which traits were coded as "0" for primitive, "1+" for evolved, and a few disordered traits were treated as equal in distance (Table S3).

The phylogenetic signal is an index that measures the degree of similarity of morphological traits of different species on the evolutionary tree[35]. The Blomgerg statistical value K represents the size of the pedigree signal. When it is close to 1, it means that the observed value of the trait is close to the predicted value of the Brownian motion model, and the trait has a certain degree of conservatism; when K is close to 0, it means that the trait evolution tends to be random; when the K value When it is significantly greater than 1, it indicates a strong phylogenetic signal[36]. We also compared other indices to determine morphological traits with phylogenetic signals.

2.5 Statistical Analysis

All phenotypic data were routinely data entered and statistically analyzed using Excel 2021. Characteristics and patterns of variation of each phenotypic trait among and within populations were calculated by nested ANOVA. Multiple comparisons were made using Duncan's new complex polar method. Based on ANOVA, the coefficient of variation of each phenotypic trait was calculated, and the larger the coefficient of variation, the greater the degree of trait dispersion and the higher the phenotypic diversity of the group. Principal component analysis was used to screen the main traits for phenotypic variation.

The linear model for nested ANOVA was: $Y_{ijk} = L + S_i + T_{(i)j} + E_{(ij)k}$, where Y_{ijk} is the k observation of the j individual plant of the i population, L is the total mean, S_i is the population effect (fixed), $T_{(i)j}$ is the effect of the individual plant within the population (random), and $E_{(ij)k}$ is the experimental error[37]. Phenotypic coefficient of variation: $CV(\%) = \sigma/\mu \times 100$, where σ is the standard deviation and μ is the mean. Phenotypic differentiation coefficient: $V_{ST} = (\delta^2_{t/s})/(\delta^2_{t/s} + \delta^2_{s})$, where $\delta^2_{t/s}$ is the between-population variance component and $\delta^2_{t/s}$ is the within-population variance component[38].

Because bioclimatic and UV factors may have strong autocorrelation within the study scale, 33 geographic environment factors were first tested for autocorrelation, and for pairs of environmental factors with

correlation coefficients |r|≥0.8, only those with greater biological significance were retained to form a more concise matrix of geographic environment factors (explanatory variable matrix) for each group. Due to the small number of leaf phenotypic traits, we did not down-weight them and included all 10 leaf phenotypic factors in the analysis as the response variable matrix. Then, the relationships between leaf phenotypic traits and geographic environment factors were analyzed using redundancy and correlation analyses, respectively, and the results of the two methods were summarized to explore the response relationships between phenotypic traits and environmental factors. Regression analysis was performed on paired variables with correlation coefficients |r|≥0.6 between *Polyspora* leaf phenotypic traits and geographic environment factors to explain the relationship between them.

Based on the results of the phenotypic survey, morphological trait characteristics were coded according to Table S2, a matrix of interspecific branching data within the genus was established, and a phylogenetic BI tree (Bayesian Inference) based on the morphological data was built using MrBayes, with the parameter set to build four Markov chains, and a randomized tree was used as the starting tree, running for a total of 2,000,000 generations, with every 100 generations were sampled once, 25% of the samples were discarded, and a consensus tree was constructed based on the remaining samples. Round-seeded lotus *Apterosperma oblata* was used as the outgroup.

Statistical analysis and visualization were performed using R (version 4.3.3). ANOVA and plotting were mainly performed using R packages “modelsummary”, “simplevis” and “ggplot2”. Correlation analysis and plotting were implemented using R packages “GGally”, “psych” and “corrplot”. Principal component analysis and plotting were implemented with the R package “factoextra”. Redundancy analysis was implemented with the R package “vegan”. Normality testing was performed with the R package “ggpubr”. Regression analysis was performed using the R package “ggpmisc”. Morphological phylogenetic signals were detected using the R package “phylosignal”, and *K*-value calculations and plotting were accomplished with the assistance of R packages “adephylo”, “ape” and “phylobase” .

3. Results

3.1.Leaf, flower, capsule and seed morphological diversity

The analysis of leaf phenotypic variability in the *Polyspora* showed that among the domestic *P. axillaris* species, the largest leaf blade was *P. speciosa*, with a mean leaf length of up to 227 mm, a leaf width of 64 mm, a leaf circumference of 562 mm, and a leaf area of 8,718 mm^2; the smallest leaf blade was *P. chrysandra*, with a leaf length of 123 mm, a leaf width of 42 mm, a leaf circumference of 299 mm, and a leaf area of 3,504 mm^2; and the leaves with greater roundness were *P. longicarpa*, and *P. chrysandra*. A greater roundness of leaves was found for *P. longicarpa* and *P. chrysandra*. The average coefficient of variation of leaf traits of each species ranged from 19.56–28.46%, with the largest degree of variation occurring in *P. longicarpa* and the smallest occurring in *P. tonkinensis*; the highest degree of variation was found in the leaf area of *P. axillaris* (52.46%), and the smallest degree of variation was found in the roundness of leaves of *P. tiantangensis*(9.3%). *P. hainanensis*, *P. kwangsiensis*, *P. speciosa*, *P. tiantangensis*, and *P. tonkinensis* all had

greater degrees of variation in leaf tips; *P. axillaris*, *P. chrysandra*, and *P. longicarpa* had the greatest variation in leaf area and the smallest variation in leaf roundness ((Table S4; Table 2).

Table 2
Coefficient of variation in the leaf phenotype of *Polyspora* (%)

Species	PL	LL	LW	LSI	PI	LTA	LBA	LP	LA	LR
P. axillaris	32.31	23.81	28.56	16.93	25.00	28.67	34.79	23.78	52.46	13.95
P. chrysandra	32.15	20.45	21.80	19.93	28.57	25.63	21.37	19.61	38.74	12.50
P. hainanensis	30.88	21.05	20.78	13.87	28.57	46.48	35.50	21.14	38.84	17.65
P. kwangsiensis	21.51	17.01	15.38	15.88	22.22	37.87	29.26	15.68	28.61	15.38
P. longicarpa	41.49	23.63	26.05	21.64	25.00	40.66	24.34	23.99	42.57	15.22
P. speciosa	22.27	17.63	19.22	12.22	20.00	36.00	22.41	17.24	31.89	11.76
P. tiantangensis	31.42	11.21	19.17	16.30	28.57	37.84	22.17	12.69	25.96	9.30
P. kwangsiensis	19.29	11.98	15.28	13.19	25.00	32.88	26.34	12.12	26.23	13.33

Table 3
Flower phenotypic characterization of *Polyspora*

Traits	*P. chrysandra*		*P. hainanensis*		*P. longicarpa*		*P. speciosa*	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
FD	68.80 ± 9.71 c	14.11	41.32 ± 2.90 d	7.02	130.75 ± 12.34 a	9.44	91.63 ± 6.64 b	7.25
PEL	35.42 ± 8.70 c	24.56	27.43 ± 2.65 d	9.66	69.72 ± 8.41 a	12.06	48.11 ± 3.29 b	6.84
PEW	28.91 ± 3.37 c	11.66	24.92 ± 2.48 d	9.95	60.43 ± 7.91 a	13.09	38.85 ± 3.97 b	10.22
PEN	6.10 ± 0.45 a	7.38	5.90 ± 0.31 b	5.25	6.00 ± 0.00 ab	0.00	6.00 ± 0.00 ab	0.00
SL	8.22 ± 1.94 b	23.60	5.68 ± 0.45 c	7.92	15.89 ± 2.45 a	15.42	9.71 ± 1.69 b	17.40

Note: The same lowercase letter following the value of a morphological trait for each species means that the trait does not differ significantly between species. Same as below.

Table 4
Capsule and seed phenotypic characterization of *Polyspora*

Traits	*P. axillaris*		*P.chrysandra*		*P.hainanensis*		*P.longicarpa*		*P.speciosa*	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
CL	23.94 ± 3.27 d	13.66	30.47 ± 4.07 b	13.36	22.85 ± 2.36 d	10.33	39.75 ± 4.51 a	11.35	26.95 ± 4.06 c	15.06
CSL	8.16 ± 1.38 d	16.91	8.96 ± 2.62 c	29.24	9.11 ± 1.02 c	11.20	14.71 ± 2.14 a	14.55	10.21 ± 1.95 b	19.10
CSTI	0.34 ± 0.05 c	14.71	0.29 ± 0.08 d	27.59	0.40 ± 0.06 a	15.00	0.37 ± 0.07 b	18.92	0.39 ± 0.08 ab	20.51
SW	0.11 ± 0.04 d	36.36	0.23 ± 0.05 b	21.74	0.12 ± 0.02 d	16.67	0.42 ± 0.03 a	7.14	0.17 ± 0.03 c	17.65
ST	1.52 ± 0.28 c	18.42	1.61 ± 0.28 bc	17.39	1.63 ± 0.31 b	19.02	1.75 ± 0.23 a	13.14	1.74 ± 0.29 a	16.67
SWL	12.85 ± 2.47 d	19.22	19.25 ± 2.47 b	12.83	16.33 ± 2.50 c	15.31	21.67 ± 3.56 a	16.43	17.24 ± 3.55 c	20.59
WL	8.55 ± 2.00 d	23.39	12.73 ± 2.44 b	19.17	11.17 ± 2.02 c	18.08	14.92 ± 2.91 a	19.50	12.10 ± 3.04 b	25.12
WD	4.39 ± 0.98 c	22.32	5.40 ± 0.72 b	13.33	4.69 ± 0.45 c	9.59	7.03 ± 0.79 a	11.24	5.68 ± 0.98 b	17.25
WSI	1.96 ± 0.32 c	16.33	2.38 ± 0.45 a	18.91	2.37 ± 0.29 a	12.24	2.14 ± 0.44 b	20.56	2.14 ± 0.43 b	20.09

Note: The same lowercase letter following the value of a morphological trait for each species means that the trait does not differ significantly between species. Same as below.

Due to the insufficient number of field observations and samples, the flower phenotypic differentiation only included 16 groups of four species, namely *P. chrysandra, P. hainanensis, P. speciosa* and, *P. longicarpa*, and the rest of the species did not obtain statistically significant phenotypic trait data owing to the small number of observations. Phenotypic characterization of the flowers revealed that, among the domestically produced species, *P. longicarpa* had the largest flowers, with a mean flower diameter of 130.75 mm, a petal length of 69.72 mm, a petal width of 60.43 mm, and a sepal length of 15.89 mm. The smallest flowers were found on *P. hainanensis*, with a mean flower diameter of 41.32 mm, a petal length of 27.43 mm, a petal width of 24.92 mm, and a sepal length of 5.68 mm, the vast majority of the flowers were the same size as those on *P. hainanensis*. Most of the flowers were 6-merous, with an 8-merous variant found in *P. chrysandra* and a 5-merous variant found in *P. hainanensis*. The flowering phenotype of *P. chrysandra* plants

was more variable, with an average coefficient of variation of 16.26%, and the most variable trait was petal length (24.56%). Except for the number of petals, the petal width of *P. speciosa* plants was the most stable, with a coefficient of variation of 6.84% (Table 3).

For the analyses of capsule and seed phenotypic traits, only 23 groups of 5 species were included, again due to insufficient sample data. The largest capsule and heaviest seed among the domestic species of *Polyspora* was *P. longicarpa*, with a mean capsule length of 39.75mm, a thousand-seed weight of 42g, and a wing length of 14.92mm; the smaller capsule were *P. hainanensis* and *P. axillaris*, with capsule lengths of 22.85 mm and 23.94mm, respectively, and thousand-seed weights of 12g and 11g, respectively, and the shortest seed wings were those of *P. axillaris*, with a mean value of 8.55 mm. The coefficient of variation for the seed phenotype was the highest for *P. axillaris* (20.15%), and the smallest was for *P. hainanensis* (14.16%). Among the traits, wing length had the highest variation, with an average coefficient of variation of 21.05%, and capsule length was the most stable (12.75%). The seed quality of *P. axillaris* had the largest variation, the coefficient of variation was 36.36%. The wing width of *P. hainanensis* had the smallest variation, with a coefficient of variation of 9.59% (Table 4).

3.2 .Population differentiation of phenotypic traits in the genus

To explore the ratio of variance components to variation for each trait, we calculated the nested variance component. The results showed that the phenotypic differentiation coefficients of each trait exhibited a wide range of variation, from 1.49–97.14%, among the domestically produced species of *Polyspora*, with 0% or 100% for individual traits due to an insufficient amount of data (Tables S5). The average phenotypic differentiation coefficient was 66.87%, for *P. axillaris*, 40.77%, for *P. chrysandra*, 40.31%, for *P. hainanensis*, 48.18%, for *P. speciosa*, was 35.11% for *P. kwangsiensis*, and 5.02% for *P. longicarpa*. The traits that were not listed for each species were missing data; only one population was investigated for *P. tonkinensis* and *P. tiantangensis*, and no population differentiation study was carried out. The phenotypic differentiation between populations was greater than that within populations for *P. axillaris*, the phenotypic differentiation between populations was slightly smaller than that within populations for *P. chrysandra*, the phenotypic differentiation between populations and within populations was not obvious for *P. speciosa*, and the main variations in *P. hainanensis*, *P. kwangsiensis*, and *P. longicarpa* came from within populations. Among the traits, the phenotypic differentiation coefficients for leaf length were generally small, and those for wing length, wing width, and seed weight were large.

3.3. Principal component analysis of phenotypic characters of *Polyspora*

To identify the key trait indicators of phenotypic differences among species and to assess the role of various phenotypic datasets in species delineation, principal component analysis (PCA) was performed on 32 groups of leaf phenotypic traits of 8 species, 23 groups of leaf and seed capsule traits of 5 species, and 16 groups of leaf, flower, and capsule phenotypic traits of 4 species. The PCA analysis of the 10 leaf traits showed that the cumulative contribution of the first three principal components reached 88.097%, with the first principal component accounting for 47.115% of the total variance, mainly including leaf length, leaf

perimeter, leaf area, petiole length, and leaf width; the second principal component accounted for 26.760% of the total variance, mainly including leaf basal angle, and leaf roundness; and the third principal component contributed 14.222% of the variance, mainly including the petiole index (Table 5). The top 5 leaf trait indexes in terms of contribution were: leaf length, leaf width, leaf circumference, leaf area and leaf roundness. Taking the top 2 principal components(PC1 and PC2) as coordinates, a two-dimensional scatter plot was obtained (Fig. 1). PC1 and PC2 could separate *P. chrysandra* and *P. hainanensis* completely, indicating that there were large differences in leaf shape between the two species; *P. tiantangensis* was completely contained within *P. longicarpa*, indicating that there was very little difference in leaf shape between the two species; and the information provided by the leaf traits was limited.

Table 5
Principal component analysis of leaf traits in *Polyspora*

Traits	Principal component		
	PC1	PC2	PC3
LL	**0.9734**	0.0693	-0.1165
LP	**0.9699**	0.1532	-0.1229
LA	**0.8692**	**0.4270**	-0.1611
PL	**0.7981**	0.1026	**0.5700**
LW	**0.7138**	**0.6543**	-0.1307
LTA	-0.6381	**0.3716**	0.2660
LSI	**0.3438**	-0.8573	-0.0286
LBA	-0.2698	**0.7716**	-0.0352
LR	-0.5500	**0.7423**	-0.0749
PI	0.1438	0.0844	**0.9733**
Eigenvalue	4.7115	2.6760	1.4222
Contribution rate(%)	47.1149	26.7599	14.2223
Cumulative contribution rate(%)	47.1149	73.8748	88.0971
Note: Numbers in bold represent the highest loadings along each axis.			

PCA analysis of 19 leaf and seed traits of five species showed that the first three principal components contributed 65. 37% cumulatively and the first six principal components contributed 84.47% cumulatively (Table S6). PC1 accounted for 30.94% of the total variance, mainly including leaf area, leaf circumference, leaf length, leaf width, seed wing length, capsuleing peduncle length, petiole length, and seed winged length; PC2 accounted for 22.34% of the total variance, mainly including capsule length, leaf roundness, seed mass, seed wing length, seed wing width, and leaf apical angle; and PC3 had a contribution of 12.08% to the variance, mainly including leaf basal angle. The top five contributing traits were leaf length, leaf

circumference, leaf area, seed wing length, and seed with wing length. Three two-dimensional scatter plots (Fig. 2, Fig. 3) were obtained with the coordinates of the top three principal components (PC1, PC2 and PC3) of the contribution value. PC1 and PC2 could basically separate *P. axillaris*, *P. chrysandra*, *P. hainanensis*, and PC3 could basically separate *P. longicarpa* from the other species; of the top three principal components, *P. speciosa* overlapped with the other species, indicating that leaf, capsule and seed traits could not be separated even if they were combined.

The results of the principal component analysis of the combined leaf, flower and capsule phenotypic traits of the four species showed that the first three principal components contributed 69.7% cumulatively, and the top five contributing traits were leaf length, leaf perimeter, leaf area, capsule length and seed mass. PC1 and PC2 separate the four species (Fig. 4).

3.4. Correlations between leaf phenotypes and geoenvironmental factors

Spearman's correlation analysis of 10 leaf quantitative traits and 18 geographic environment factors showed that most of the bioclimatic factors were weakly correlated with leaf phenotypic traits (Fig. 6). Isothermality (bio3) was significantly positively correlated with leaf basal angle, leaf apical angle and leaf roundness, and significantly negatively correlated with other leaf traits except leaf width; mean temperature of the coldest month (bio6) was significantly negatively correlated with leaf width, leaf area and leaf basal angle; annual temperature difference (bio7) was significantly positively correlated with leaf width, leaf area, leaf perimeter and petiole length; longitude was significantly negatively correlated with leaf basal angle, leaf roundness, and weakly correlated with the rest of leaf phenotypic traits; and the correlations with leaf phenotypic traits were weak. The correlation between latitude and leaf width, leaf area and other leaf traits was positive, indicating that the higher the latitude of the distribution of *Polyspora* in China, the larger the leaves were; altitude was positively correlated with leaf base angle and leaf roundness, and negatively correlated with the leaf shape index; the correlation between the soil factor and the leaf traits was weak; among all the geographical environment factors, the correlation between the ultraviolet radiation factor and the leaf phenotypic traits was the strongest, with the lowest monthly average ultraviolet radiation (uvb4) and the lowest monthly average ultraviolet radiation (uvb4) as the most significant. Among all the geographical environment factors, UV radiation had the strongest correlation with leaf phenotypic traits, with the lowest monthly average UV (uvb4) and the sum of the highest seasonal monthly average UV (uvb5) having a significant negative correlation with most of the leaf traits, and seasonal changes in UV (uvb2) having a positive correlation with most of the leaf traits, which indicated that UV radiation had a greater impact on the phenotypic traits of leaves of the genus Tabanica, with the stronger the radiation, the smaller the leaves, and the more stable the changes in UV, the more stable the leaf traits were. Besides, the results of CA analysis and RDA analysis on the relationship between leaf area and six geographic indicators were consistent.

Linear regression analyses of leaf phenotypic traits with correlation coefficients $|r| \geq 0.6$ with geographic environment factors were performed, and the results are shown in Fig. 7. Leaf petiole length showed strong negative correlations with isothermality, the sum of the lowest monthly mean UV and the highest seasonal monthly mean UV, with fits ranging from 0.37 to 0.71, and the strongest negative correlation was with the

lowest monthly mean UV, with a fit of 0.71. Leaf basal angle showed a highly significant negative correlation with longitude, and a significant positive correlation with isothermality, with fits of 0.31 and 0.18, respectively. Leaf roundness was weakly positively correlated with isothermality, with a goodness of fit of 0.14. These regression relationships between leaf traits and geographical environment factors indicated the geographically different distribution patterns of *Polyspora* in China.

3.5 Morphology-based phylogenetic analyses of *Polyspora*

The Bayesian phylogenetic tree based on 15 morphological traits showed that two nodes were strongly supported, with the basal taxon being *P. hainanensis*, the tropical component of the *Polyspora* in China, *P. speciosa*, *P. hainanensis*, and *P. kwangsiensis*, with the three species forming a weak sister (Clade 1); *P. tiantangensis* and *P. longicarpa*, diverged later and formed a sister relationship between the two (Clade 2); and all the other nodes of the morphological phylogenetic tree had a posteriori visual rate less than 0.5 (Figure. 8).

Figure 7 Linear regression relationship between leaf traits and geographical environmental factors of *Polyspora*

The K values of phylogenetic signal strengths for 15 morphological traits of 8 domestically produced species of the *Polyspora* were calculated by the Blomberg method, and the results are shown in Table S8. Significant phylogenetic signals were detected for style length, sepal length, capsule length, and wing length ($K > > 1$, $p < 0.05$); flower diameter, leaf length, and bract number also had some spectral signals.

4. Discussion

4.1 Characteristics of phenotypic variation

Phenotypic traits serve as crucial identifiers for germplasm characterization, preservation of species biodiversity, and selection of novel cultivars [39], featuring both resilience and dynamism [40], and are coerced by the distinct genetic materials and ecological milieu of a species [41]. Conventionally, a coefficient of variation exceeding 10% is deemed an appreciable disparity in traits [42, 43]. The outcomes of multivariate statistical scrutiny of 31 traits for leaves, flowers, capsules, and seeds of eight species of *Polyspora* indicated that the species of *Polyspora* exhibited significant divergence both inter-and intra-species, and were abundant in phenotypic diversity. The mean coefficient of variation for leaf traits was 24%, that for floral morphologies was 11.98%, and that for capsule and seed traits was 17.49%, all surpassing 10%. Among the leaf traits, the coefficient of variation for leaf apical angle was the highest, with a mean value of 35.76% across species. The leaf shapes of *Polyspora* range from obovate, lanceolate, oblong, narrow-oblong, etc. The leaf apex is considerably influenced by the leaf shape, primarily accountable for the substantial variation in the leaf tip angle, and manifests in the form of acuminate, acute, acute, slightly concave, convex, and rounded in various species and among diverse groups. Typically, phenotypic variation in nutritional traits is more susceptible to environmental influence than reproductive traits, which are relatively consistent. Comparison of phenotypic variation of leaves and flowers and capsules reveals that the findings of the current study also corroborate this pattern. The capsule is

cylindrical and dehiscent at maturity, and the seed possesses a terminal wing that exceeds the length of the seed itself, which are the most salient features that distinguish the genus from other plants in Theaceae.

The findings of the study on phenotypic diversification demonstrated that the manifestation of most *Polyspora* species predominantly emanates from within the group. The predilection of the plants in this genus for insect-borne distribution, coupled with a diminished pollen dispersal distance compared to wind-borne flowers, and the impediment of intergroup gene interaction likely contributes to the confinement of intergroup genetic divergence, resulting in the prevalence of intragroup phenotypic diversification. Furthermore, the seeds of this genus are flattened and aerially propelled in the superior part, and the seeds are lighter, which are conducive to wind-borne propagation, and to a certain extent, it can also stimulate the inter-group gene interchange, thereby leading to a heightened degree of inter-group divergence in some species. The phenotypic diversification of *Polyspora* primarily originates from the intergroup, and the marginal difference in phenotypic diversification between and within *P. speciosa* groups is a compelling evidence of this attribute.

Given the constraints of the survey data, only the leaf traits encompassed all species and all groups. The principal component analysis based on leaf traits revealed that leaf length, leaf width, leaf circumference, leaf area, and leaf roundness could encapsulate the primary characteristics of leaf traits in *Polyspora*. The initial two principal components can essentially distinguish *P. chrysandra* and *P. hainanensis*, as the leaf shape disparity between these two species is the most pronounced, *P. chrysandra* possesses an obovate leaf, *P. hainanensis* leaf shape is narrow and elongated, and *P. axillaris* falls under the transitional form of these two leaf shapes, and the three species are congregated together on the principal component analysis plot, the plant size of the three species is relatively diminutive, and the life type mirrors the transitional form of shrub to tree. The two-dimensional scatter plots of PC1 and PC2 illustrated that *P. tiantangensis* was entirely encompassed in *P. longicarpa*, implying that *P. tiantangensis* might be an intraspecific variant of *P. longicarpa* in terms of leaf morphology; the fact that *P. speciosa*, *P. kwangsiensis*, and *P. tonkinensis* were intertwined with each other signified that the disparities in leaf phenotypes between the three species were relatively minor, with the primary differences being *P. speciosa* had more deeply serrated margins of the leaves, and *P. tonkinensis* had more deeply serrated margins of the leaves. The primary distinction was that the leaf margin of *P. speciosa* was more deeply serrated, that of *P. tonkinensis* was more shallowly serrated, and that of *P. kwangsiensis* was sparsely serrated, occasionally nearly entire. Based on leaf traits, an increasing number of species could be delineated with the incorporation of capsule and flower phenotypic data; when utilizing the combined leaf, flower and capsule phenotypic data for principal component analysis, the top five contributing traits were, in order, leaf length, leaf perimeter, leaf area, capsule length, and seed mass, and the initial two principal components of the combined dataset could separate the four species more effectively, indicating that the more comprehensive the phenotypic traits, the more discernible the species boundaries.

4.2 Environmental adaptations of leaf phenotypic traits

Plant traits are the consequence of interactions between gene expression and environmental variables over prolonged evolutionary epochs[44]. Phenotype constitutes a fusion of diverse morphological traits and a direct manifestation of biological genetic variance[45, 46]. Alterations in growth space and milieu can

stimulate phenotypic adaptive responses in species[47, 48]. When phenotypes are correlated with environmental gradients, these responses are typically instigated by local adaptation, phenotypic plasticity, etc., and can influence gene flow and distribution patterns[49]. Nutritional traits tend to be under higher environmental selection pressure than reproductive traits. Leaves are the primary organs of plants for photosynthesis and material production, with the greatest contact area with the external environment and the most susceptible to environmental modifications[50]. Leaf traits are the products of long-term adaptive evolution of plants in specific habitats, which directly impact the fundamental functions of plants and directly reflect the survival strategies of plants. They are frequently utilized to monitor environmental alterations,such as pH and soil nutrient availability[51].

Plant leaf shape and size may exhibit distinct patterns of variation across taxa, with shape being generally conservative and variation usually induced by its own genetic material, whereas size is deemed to be highly variable and variation is primarily influenced by the environment. Plants in arid, high latitude, and high altitude regions possess small leaves, whereas high humidity, hot, and sunny conditions engender large leaf sizes [52]. Among the 10 leaf morphology indexes employed in this study, there were five each reflecting leaf shape and leaf size; leaf shape index, petiole index, leaf base angle, leaf tip angle, and leaf roundness represented leaf shape, and the remaining five indexes reflected leaf size. The average coefficient of variation of leaf shape was 23.61%, and the average coefficient of variation of leaf size was 24.40%, indicating that the leaf phenotypic variation of each species was influenced by its own genetic factors and geographic environment.

In adverse conditions, plants will mildly alter leaf morphogenesis to augment their capacity to acquire resources [53]. Among the geographical environmental factors, bio3, uvb4, and uvb5 are strikingly negatively correlated with most leaf phenotypic parameters of *Polyspora*. These parameters signify the resistance response and environmental adaptions of the leaves of *Polyspora*, which are petite in regions with elevated UV radiation, relative aridity, and cold. Latitude (Lat.), uvb2 and bio7 were significantly and positively correlated with most of the leaf phenotypic indices, primarily due to the influence of the four populations surrounding Chongqing of *P. speciosa*, which had the highest distribution latitude and the greatest variation in temperature and UV radiation, and which possessed the largest leaves within the domestic *Polyspora*, resulting in this unusual pattern, which should be determined by the species' inherent genetic material. Temperature factors such as bio3, bio6 and bio7 were profoundly correlated with leaf traits, while rainfall-related factors were not markedly correlated with leaf shape. Compared with rainfall, temperature was the pivotal factor influencing the variation of leaf traits in the genus *Polyspora*, which is consistent with the findings of the widely distributed timber tree species *Cunninghamia lanceolata* [50] in southern China, and *Litsea coreana* var. *sinensis* [54], which is also a dominant component of broadleaf evergreen forests. Overall, most of the bioclimatic factors were not markedly correlated with leaf phenotypes of *Polyspora*, and the variation was the result of the combined influence of its own genetic material and geographic environment factors, with the two types of factors playing comparable roles. To better understand and predict *Polyspora*'s response to global change and to enhance the understanding of the large-scale drivers of phenotypic plasticity, future in-depth studies at broader spatial scales and larger taxonomic scales are needed to better reveal key patterns and to uncover universal patterns.

Plants frequently encounter incident photons of ultraviolet radiation (UV-B, wavelength range 280–315 nm) during the process of locating light for photosynthesis[55]. Although these photons make up a minuscule percentage of solar radiation reaching the biosphere, ultraviolet radiation has a substantial influence on plants, leading to morphological alterations in plants. Moderate doses of UV-B radiation act as environmental signals. The UVR8 protein in plants perceives and initiates UV-B-induced plant photomorphogenesis, subsequently regulating plant development, such as suppressing hypocotyl elongation, stimulating cotyledon expansion, promoting flavonoid and anthocyanin accumulation, etc. Intense UV-B can damage DNA, instigate the accumulation of reactive oxygen species, impair photosynthesis and inflict harm on plants[56]. UV radiation can swiftly induce gene regulation, resulting in cumulative modifications in plant physiology and morphology, yet the resulting morphological modifications are gradual, which suggests that the transgenerational effects of solar UV-B radiation foster plant adaptability via morphological traits[57]. Leaves are deemed to be the most crucial target organ of ultraviolet radiation in plants. Plants shield sensitive tissues from UV-B radiation by thickening cuticle, waxy layer and trichomes and altering cell optical properties to reflect ultraviolet photons from leaf surfaces [58]. In this study, the correlation degree between uv and leaf phenotype was second only to bioclimatic factors. Uvb4 and uvb5 were significantly negatively correlated with most leaf morphological characters, indicating that the leaves of genus lapis were susceptible to uv radiation. Most of the species in the intense UV-B radiation zone exhibited thick leathery leaves, suggesting that *Polyspora* adapted to UV radiation stress primarily by modifying leaf epidermis structure and safeguarding mesophyll tissue. Both RDA and CA analyses revealed a robust negative correlation between leaf area and two UV factors (uvb4 and uvb5), i.e., leaf area diminishes with escalating UV radiation. This phenomenon was corroborated by physiological experiments of six woody plants in Mediterranean hard-leaf evergreen broad-leaved forest, which demonstrated that UV radiation induced increase of leaf palisade tissue thickness, reduction of leaf area and augmentation of leaf weight per unit area, thereby protecting photosynthetic structure and nucleic acid from damage [59].

Moreover, we determined that *P. axillaris* can cultivate into trees reaching up to 10 meters in height within tropical rainforests and monsoon evergreen broad-leaved forests, while on the leeward slope of Dangan Island in Zhuhai, primarily influenced by the typhoon, its mode of existence manifested as a low shrub, providing a more credible illustration of phenotypic plasticity. This suggests that aside from the leaf phenotype, the overall body size of plants in the genus *Polyspora* also possesses a superior capability to accommodate to the environment. Owing to constraints imposed by the volume of materials and data, this study solely examined the correlation between leaf morphological traits and geographical environmental factors, and initially unveiled the geographic variation pattern of leaf phenotypes in *Polyspora*, along with the primary environmental factors influencing leaf phenotypic variation. Nevertheless, the adaptive evolution of leaves is predominantly achieved through leaf function. Future studies on physiological traits (e.g. photosynthetic capacity, dark respiratory capacity), structural traits (e.g. fenestrated tissues, specific leaf weight), and chemical traits (e.g. N and P contents) of leaves in *Polyspora* should be conducted to associate leaf morphological traits with functional traits, scrutinize the leaf economic spectrum of *Polyspora*, and investigate the relationship between leaf functional traits and the environment, thereby elucidating the evolution of leaf adaptation and its mechanism of interaction with the environment.

5. Conclusion

In this research, predicated on an exhaustive survey of wild plant resources of *Polyspora* within China, we utilized ANOVA, principal component analysis, redundancy analysis, correlation analysis and cluster analysis to unveil the attributes of phenotypic variations and patterns of variation among the species of *P. longicarpa*, and probed into the predominant environmental factors influencing the comutation of leaf phenotypes. The outcomes indicated that the phenotypic variability of *P. longicarpa* was substantial, with *P. speciosa* exhibiting the greatest abundance in leaves, and *P. longicarpa* manifesting the most profusion in flowers, capsules and seeds. *P. longicarpa* was the most abundant in leaves, *P. chrysandra* was the most abundant in flowers, and *P. axillaris* was the most abundant in capsules and seeds. The degree of phenotypic differentiation varied among species, with the phenotypic differentiation in *P. axillaris* emanating primarily from intergroups, that in *P. chrysandra* being marginally smaller than within groups, that in *P. speciosa* being less pronounced between and within groups, and that in the remainder of the species emerging primarily from within groups. Leaf length, leaf perimeter, leaf area, capsule length and seed quality were the pivotal indicators of phenotypic disparities among species, and a more comprehensive dataset of leaves, flowers and capsules could more effectively delineate the species. Leaf phenotypic differences in *Polyspora* were influenced by a fusion of its inherent genetic factors and geographic environment, with both playing a comparable role; among the environmental factors, bioclimatic factors and ultraviolet radiation had a more profound influence on leaf phenotypes.

Declarations

Author Contributions: Conceptualization, C.M., and L.D.; Methodology, C.M., and J.Y.; Software, J.Y. and Z.F.; Investigation, C.M., Z.F., L.W. and J.Y.; Data curation, Z.F.; Writing-original draft preparation, Z.F., M.G. and J.Y.; Writing-reviewand editing, M.G., J.Y. and Z.F.; Visualization, Z.F.; Project administration, C.M. All authors have read andagreed to the published version of the manuscript.

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

Ethics statement: The plant materials used in this study were obtained from wild plants and did not involve any endangered or protected species. The plant materials were identified by Chang-Le Ma and Zhi-Feng Fan. The authors declare no competing interests.

Plant guideline statement: Experimental research and field studies on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

Data availability: The datasets analyzed during the current study are available from the manuscripts and supplementary materials.

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Figures

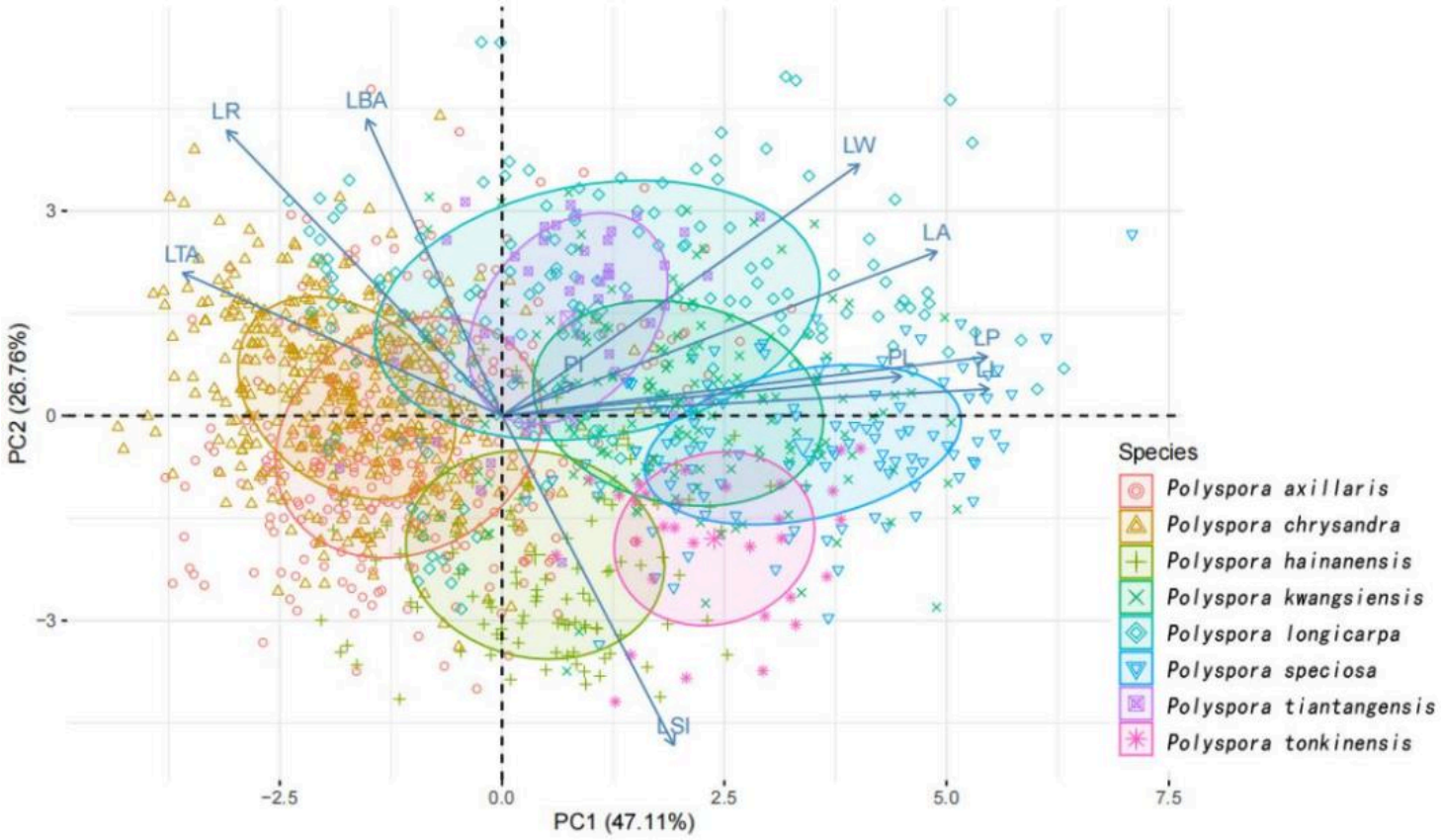

Figure 1

Principal component analysis of leaf traits in *Polyspora*

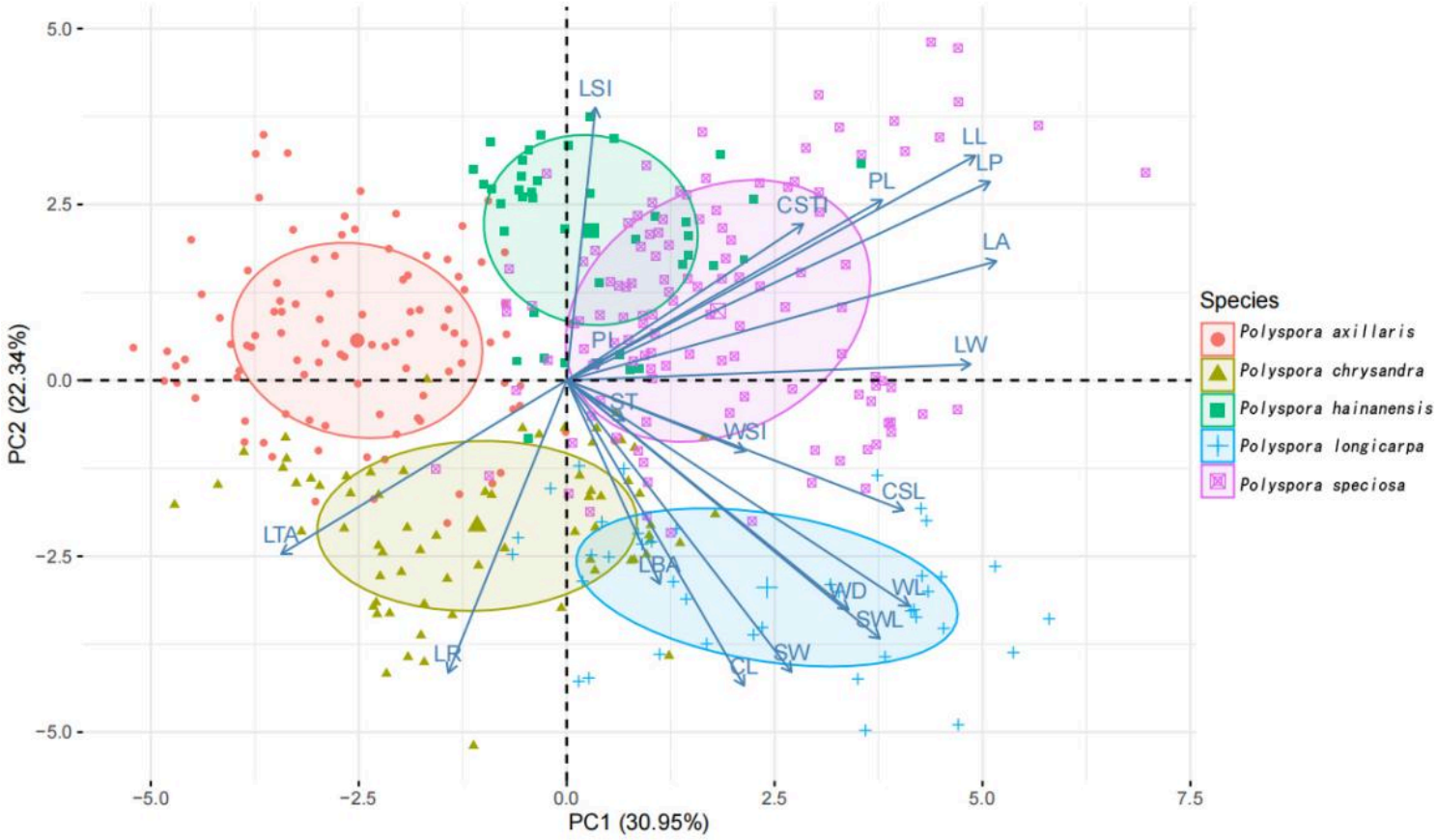

Figure 2

Principal component analysis of leaf, capsule, and seed traits in *Polyspora*(PC1-PC2)

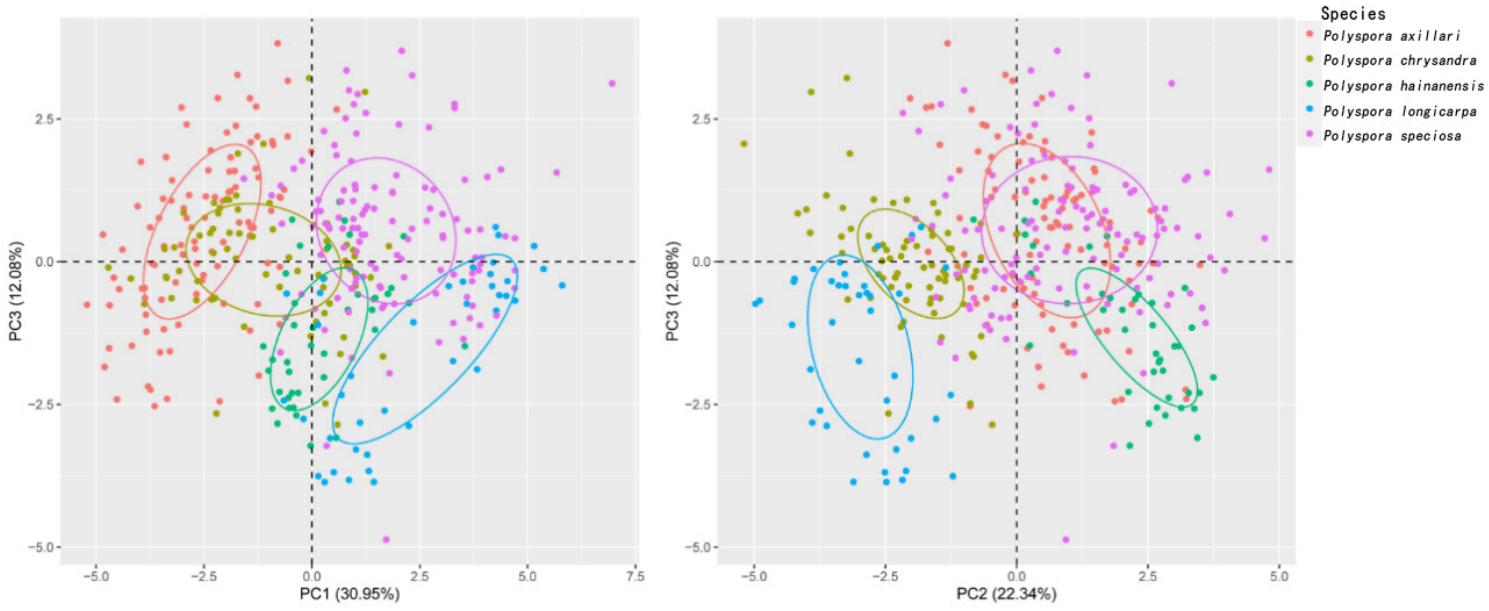

Figure 3

Principal component analysis of leaf, capsule, and seed traits in *Polyspora* (PC1-PC3; PC2-PC3)

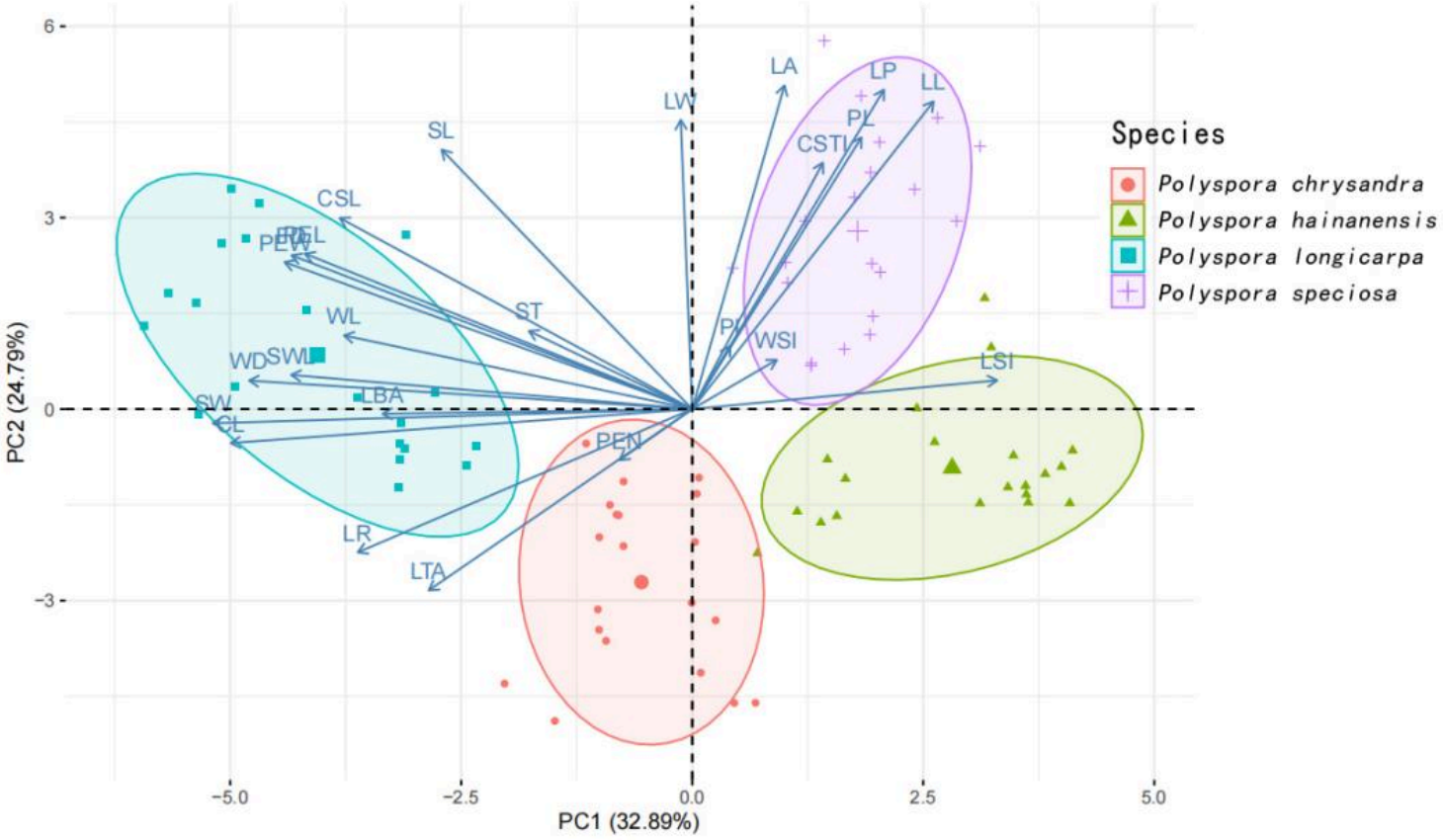

Figure 4

Principal component analysis of leaf, flower, capsule, and seed traits in *Polyspora*

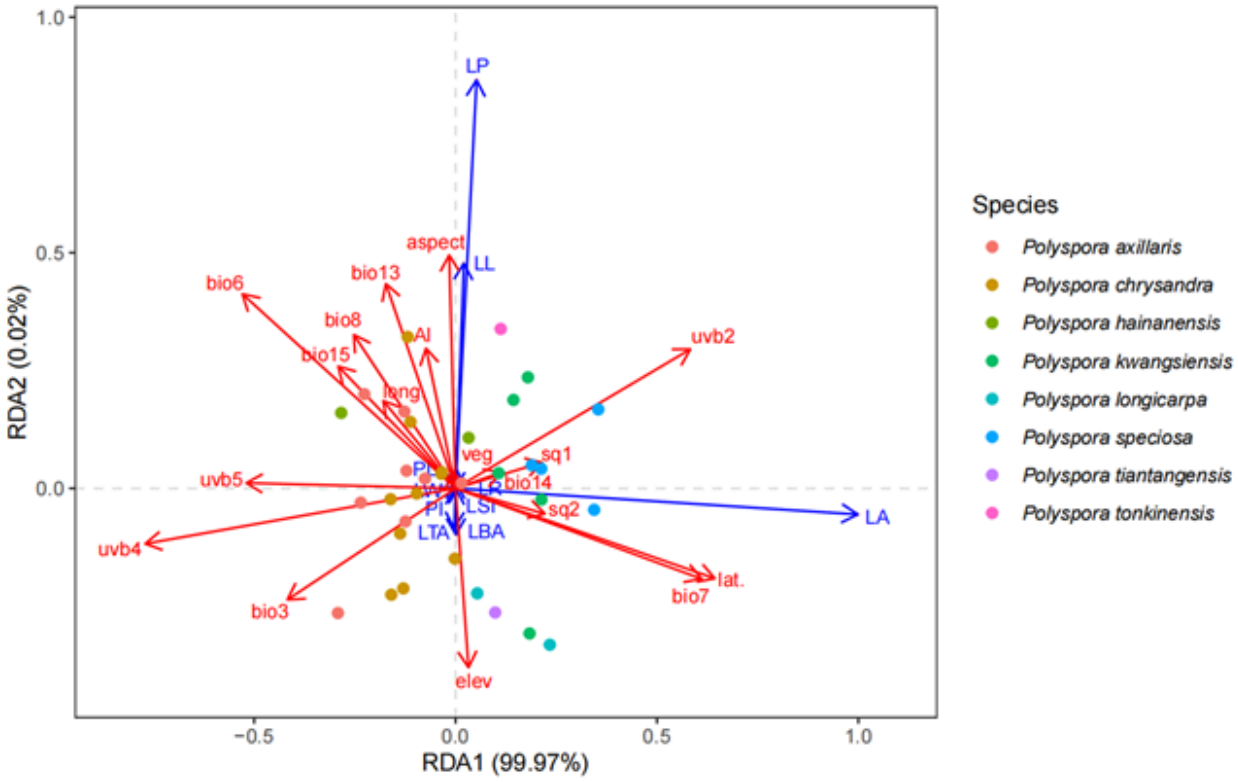

Figure 5

RDA of leaf phenotypic traits and geographical environment factors in *Polyspora*

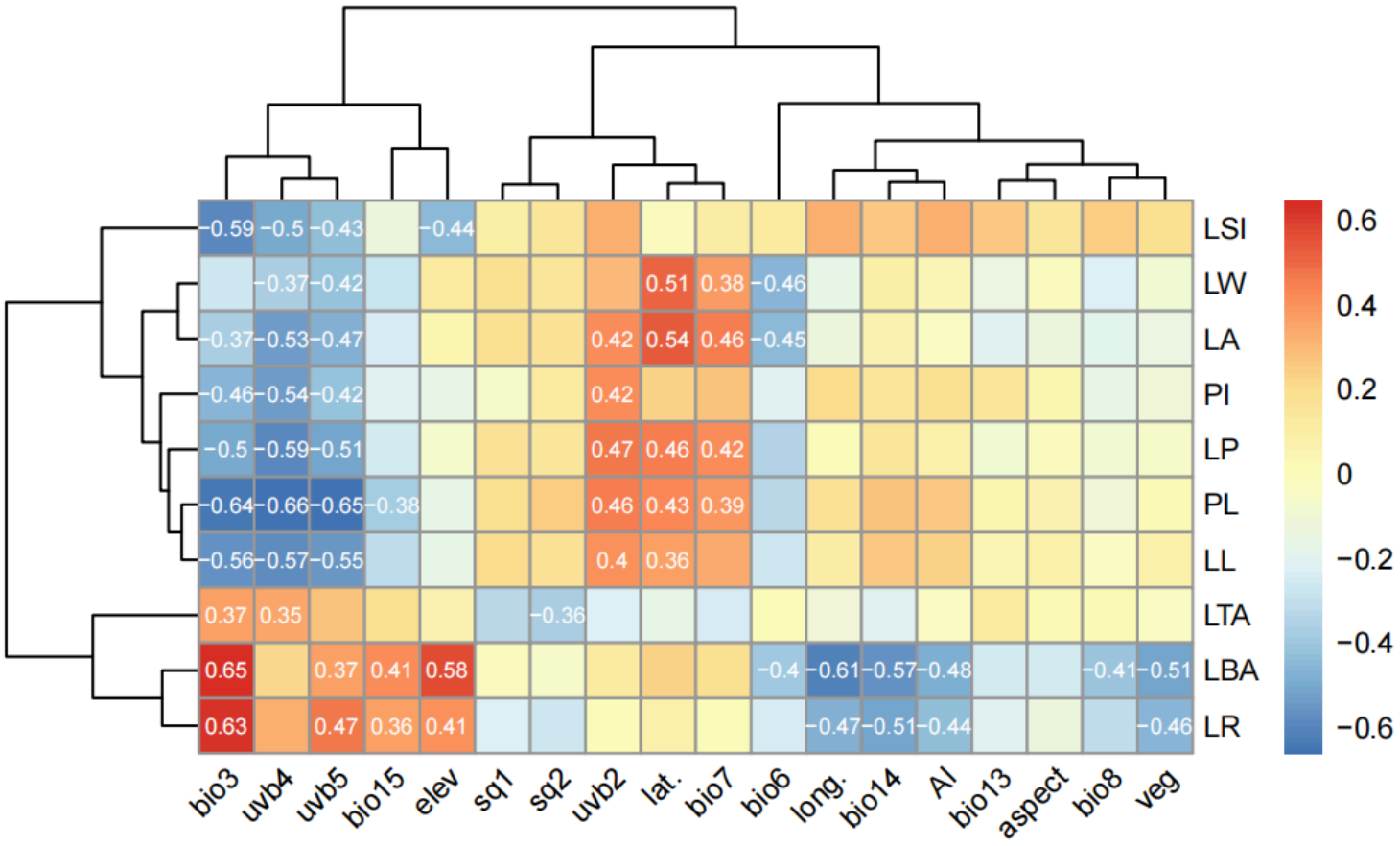

Figure 6

Correlation analysis between leaf phenotypic characters and Geo-graphical environment factors of *Polyspora*, the values in the squares show correlation coefficients with significance $p < 0.05$

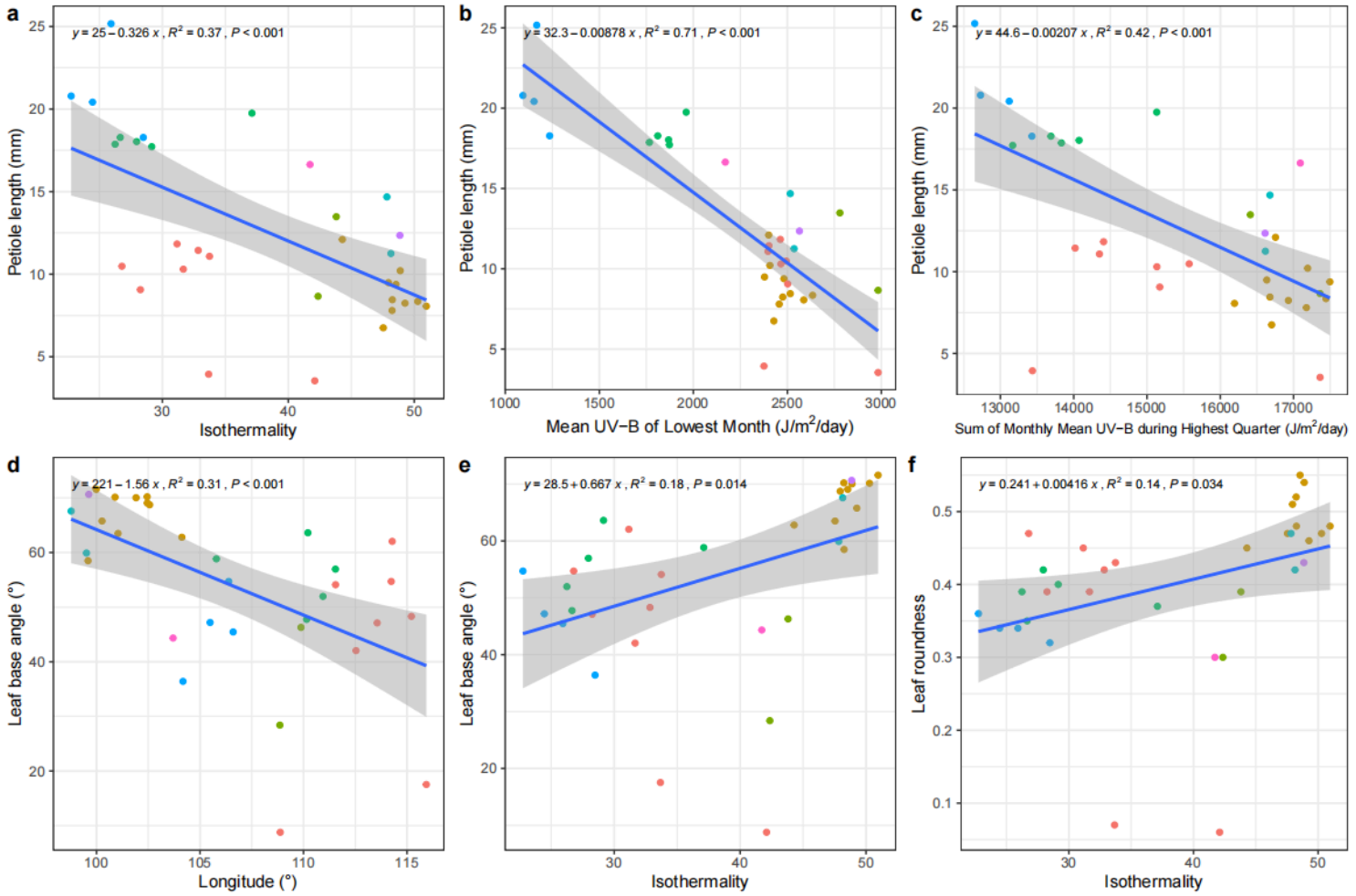

Figure 7

Linear regression relationship between leaf traits and geographical environmental factors of *Polyspora*

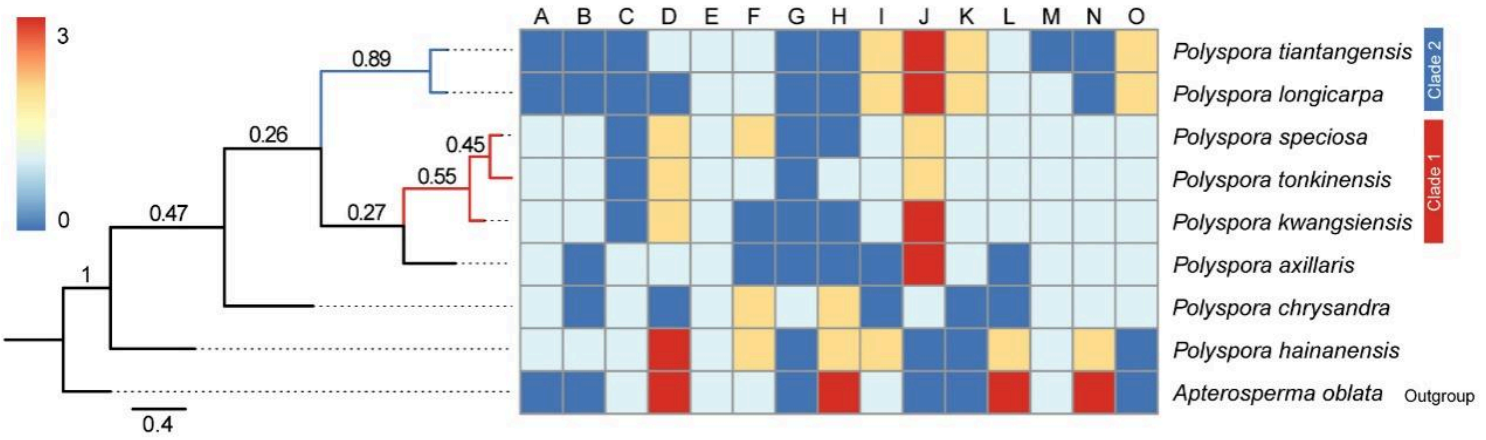

Figure 8

Phylogeny of *Polyspora* based on morphology. A-O are the codes for each morphological trait

Supplementary Files

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- Appendix.xlsx