

# A taxonomic study on pollen morphology characteristics, chromosome karyotype characteristics, floral and fruit pigment compositions of 13 species and hybrids of *Lonicera* L

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## Research Article

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**A taxonomic study on pollen morphology characteristics, chromosome karyotype characteristics, floral and fruit pigment compositions of 13 species and hybrids of *Lonicera* L.**

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**Abstract:** In this paper, the pollen morphology characteristics, chromosome karyotype characteristics, floral and fruit pigment compositions of 13 species and hybrids of *Lonicera* L. were analyzed by means of scanning electron microscopy, root tip squash method, HPLC-MS and GC-MS, and then they were classified separately to discuss their genetic relationship and taxonomic relationship.

According to the morphological taxonomy, *Lonicera* L. distributed in northern are divided into two categories by whether the branchlets are hollow or not. In this experiment, *Lonicera chrysantha* Turcz. ex Ledeb., *Lonicera maackii* (Rupr.) Maxim., *Lonicera ruprechtiana* Regel., *Lonicera tatarica* Linn. and *Lonicera korolkowi* 'Zabelii'. belong to the category of hollow branchlets; and *Lonicera edulis* Turcz. and *Lonicera tatarinowii* Maxim. belongs to the category of branchlets with solid white pith. Through the analysis of pollen morphology characteristics, chromosome karyotype characteristics, floral and fruit pigment compositions, and using the above four indexes for clustering, *Lonicera ruprechtiana* Regel. and *Lonicera tatarica* Linn. can always be clustered together. Five hybrids such as *Lonicera korolkowi* 'Zabelii' × *Lonicera maackii* (Rupr.) Maxim., *Lonicera korolkowi* 'Zabelii' × *Lonicera tatarica* Linn. No. 8 et al. can basically clustered with their parents, which is conform to the morphological taxonomy. These results indicate that the analysis of pollen morphology characteristics, chromosome karyotype characteristics, floral and fruit pigment compositions, has certain value and significance for the study of system classification and genetic relationship of *Lonicera* L., and the conclusions of this study partially support morphological taxonomy. Among the four clusters, the clustering result of pollen morphology characteristics was in the highest agreement with morphological taxonomy.

**Key words:** 13 species and hybrids of *Lonicera* L., Pollen morphology characteristics, Chromosome karyotype characteristics, Floral pigment compositions, Fruit pigment compositions, Classification relations

## **1. Introduction**

*Lonicera* L. is rich in resources and widely distributed in north temperate zone. There are about 200 species of *Lonicera* L. in the world, 98 species in China and 11 species in Heilongjiang Province (Yancheng Tang and Liangqian Li 1994; Zhengyi Wu et al. 2003). *Lonicera* L. have ornamental values such as flowers, fruits, leaf colors and tree postures and have ecological adaptability such as cold tolerance, shade tolerance, humidity tolerance, drought tolerance and salt and alkali tolerance. Most of them also have medicinal and healthy care functions. Therefore, in recent years, the introduction, application and cross breeding of new varieties of *Lonicera* L. have been widely carried out, and a large number of new varieties superior to their parents have been cultivated. Beijing took the lead in introducing varieties such as "mint" *Lonicera*, "golden flame" jinghong *Lonicera*, "beauty" jinghong *Lonicera* and "scarlet" brown *Lonicera* from abroad, and carried out cross breeding with local *Lonicera* L. After extensive introduction and breeding, many new cultivars and varieties of *Lonicera* L. were produced, resulting in unclear genetic relationship of some varieties or uncertain origin of some varieties, So the genetic relationship and taxonomic relationship of these *Lonicera* L. varieties are confused, and the taxonomic relationship between these *Lonicera* L. varieties can not be clarified by traditional morphological taxonomy. At present, scientific methods are indeed needed to explore the classification of these new *Lonicera* L. varieties.

The research showed that pollen, chromosome, floral pigment and fruit pigment of a species are all genetic material of this species, which can be stably transmitted to offspring, reflecting the genetic

information of species and also are the basis for classification of species(Attar et al. 2018; Ullah et al. 2018; Xiong et al. 2019). Talebi, SM et al. analyzed the pollen morphology of 19 species of *Onobrychis* Miller(Fabaceae), and considered that their pollen morphology had taxonomic significance for the division of subfamilies, subgenera and species(Talebi et al. 2020).Xin Wang et al. analyzed the karyotypes of 7 species of *Rudbeckia*, and considered that their karyotypes were of great significance for identifying the genetic relationship and evolution degree of *Rudbeckia* (Xin Wang et al. 2021). Yizeng Lu analyzed the anthocyanin components of *Camellia*, and the domestic and foreign *Camellia* varieties were divided(Yizeng Lu et al. 2007). Shufang Yan et al. made a cluster analysis of the anthocyanins in the fruits of 18 species of *Malus hupehensis*, and providing a basis for the identification of *Malus hupehensis*(Shufang Yan et al. 2016).

Due to extensive introduction and breeding, the classification relationship of *Lonicera* L. varieties is unclear, and the classification of cultivars and varieties only by means of morphological and anatomical features has obvious limitations. Literature review shows that there is no report on the application of pollen characteristics, chromosome karyotype characteristics, floral and fruit pigments components clustering to identify and classify *Lonicera* L.in the meanwhile. This paper intends to analyze the genetic relationship of 13 species and hybrids of *Lonicera* L. in Heilongjiang Province by cluster analysis of pollen morphological characteristics, chromosome karyotype characteristics, floral and fruit pigments components. The phylogenetic relationship was determined and compared with morphological taxonomy in order to provide scientific methods for the classification of species and varieties of *Lonicera* L.

## 2. Materials and Methods

### 2.1. Experimental materials

13 species and hybrids of *Lonicera* L. (8 species, 5 varieties) were selected as experimental materials, which were from Heilongjiang Forest Botanical Garden and Northeast Agricultural University campus. Their growth environment was basically the same. They were 4-year-old, and these plants with good growth potential and pure flower color and fruit color (Table 1).

**Table 1.** Information of experimental materials

| NO | Latin name                                  | Typical morphological characteristics of flowers and fruits                  |
|----|---------------------------------------------|------------------------------------------------------------------------------|
| 1  | <i>Lonicera maackii</i> (Rupr.) Maxim.      | white corolla at first and then yellow<br>red berry                          |
| 2  | <i>Lonicera ferdinandi</i> Franch.          | white corolla at first and then yellow<br>red berry                          |
| 3  | <i>Lonicera korolkowi</i> ‘Zabelii’.        | purple-red corolla<br>red berry                                              |
| 4  | <i>Lonicera tatarica</i> Linn.              | white corolla at first and then pink<br>red berry                            |
| 5  | <i>Lonicera ruprechtiana</i> Regel.         | white corolla at first and then yellow<br>orange berry at first and then red |
| 6  | <i>Lonicera chrysantha</i> Turcz. ex Ledeb. | yellow-white corolla<br>red berry                                            |
| 7  | <i>Lonicera tatarinowii</i> Maxim.          | deep purple corolla<br>red berry                                             |
| 8  | <i>Lonicera edulis</i> Turcz.               | yellow-white corolla<br>deep blue berry covered with white powder            |

|    |                                        |                                        |
|----|----------------------------------------|----------------------------------------|
| 9  | <i>Lonicera korolkow</i> ‘Zabelii’.×   | white corolla at first and then yellow |
|    | <i>Lonicera maackii</i> (Rupr.) Maxim. | red berry                              |
| 10 | <i>Lonicera korolkow</i> ‘Zabelii’.×   | white corolla at first and then yellow |
|    | <i>Lonicera tatarica</i> Linn. No.1    | red berry                              |
| 11 | <i>Lonicera korolkow</i> ‘Zabelii’.×   | rose-red corolla                       |
|    | <i>Lonicera tatarica</i> Linn. No.2    | red berry                              |
| 12 | <i>Lonicera korolkow</i> ‘Zabelii’.×   | pink corolla                           |
|    | <i>Lonicera tatarica</i> Linn. No.4    | red berry                              |
| 13 | <i>Lonicera korolkow</i> ‘Zabelii’.×   | rose-red corolla, white in center      |
|    | <i>Lonicera tatarica</i> Linn. No.8    | red berry                              |

(Note: Due to the abortion of *Lonicera tatarinowii* Maxim. in the Heilongjiang Forest Botanical Garden, its fruit cannot be collected, and the fruit color composition and cluster analysis did not include this species.)

## 2.2. Experimental method

Morphological characteristics of *Lonicera* L. pollen: sampling and fixation, dehydration and replacement, drying and coating, measurement and photographing according to scanning electron microscope method (Al-Hakimi et al. 2018; Bosen Xu and Jing Yang 2007). In a sunny day morning from May 20-30 in 2020, 30 mature anthers (without crack) from the flowers about to open in *Lonicera* L. were collected. Before the sample being coated, the anthers were gently broken with a dissecting needle to pick out the pollen (Borsch et al. 2018). Thirteen pollen morphological indexes were selected, including 8 quantitative indexes (polar axis length, equatorial axis length, pollen size, polar axis length/equatorial axis length, germinating pore length, germinating pore width, average protrusion height, protrusion density) and 5 qualitative indexes (pollen shape, equatorial plane view, polar plane view, germinating pore shape, exine ornamentation). When the indexes are quantified, the quantitative indexes take their values, and the qualitative indexes are assigned value to 1, 2,...n, according to their different forms. In the calculation, the values of each index are directly input (Xiaoxiao Wu et al. 2018). Among them, ES-2030 (Hitachi) freeze-drying instrument was used for freeze-drying samples, E-1010 (Hitachi) ion sputtering coating instrument was used for plating metal film on the surface of samples, and S-3400n (Hitachi, Japan) scanning electron microscope was used for observing, measuring and photographing pollen morphological indexes.

Chromosome karyotype characteristics of *Lonicera* L.: Refer to the root tip squashing method for sampling, fixation, dissociation, staining, measuring and photographing (Parrish et al. 2021; Wu et al. 2021). When the root length of *Lonicera* L. seeds was up to 0.5-1.0 cm, 20 root tips were taken and pressed into slices; the numbers of clear and scattered chromosomes in the cells was counted when more than 85% of the cells have a constant and consistent chromosome numbers in 20 cells in each species *Lonicera* L., then the photos were taken under the 100 times oil lens (Ferreira et al. 2020; Ruiyang Chen 2003; Yonggang Qiao et al. 2020). The chromosome length and arm ratio were calculated and chromosome type and karyotype classification were judged according to the average chromosome of 10-20 cells selected; when the indexes are quantified, the quantitative indexes take their values, and the qualitative indexes are assigned value to 1, 2,...n, according to their different forms. In the calculation, the values of each indexes are directly input. When nikon-e200 biological microscope was used to examine the chromosomes, chromosome types, average relative length, average arm ratio and karyotype types were judged according to LiMaouxue, Levan and Stebbins respectively (Lee et al. 2020; Sinela et al. 2017; Wicker 1991).

Floral and fruit pigment compositions of *Lonicera* L.: According to HPLC-MS(Chai et al. 2021), 13 kinds of fresh flowers and mature fruits of *Lonicera* L. were collected in a sunny morning from April to September in 2020. 5g petal and 5g fruits were grounded into powder by liquid nitrogen in the mortars, then 0.1% acidic methanol solution (methanol: water = 80:20, v/v) was added. The filtrate was filtered by filter paper and 0.22µm microporous membrane and the components will be identified by machine after ultrasonic-assisted extraction at 40°C for 30 min and extraction at room temperature in the dark conditions for 24 h.

The model of chromatographic column is ACQUITY UPLC BEH C<sub>18</sub> whose size is 100mm × 2.1mm × 1.7µm. The detection was done for 25 minutes under column temperature 40°C, injection volume 5µL and flow rate 0.4 mL/min by mobile phase A (0.1% formic acid aqueous solution) and mobile phase B (pure acetonitrile); The elution procedure is as follows: 5%-15% concentration of B at 0-3 min; 15% concentration of B at 3-3.5 min; 15%-30% concentration of B at 3.5-6 min; 30% concentration of B at 6-6.5 min; 30%-70% concentration of B at 6.5-12 min; 70% concentration of B at 12-12.5 min; 70%-100% concentration of B at 12.5-18 min; 100% concentration of B at 18-25 min; 100%-5% concentration of B at 25-25.5min; 5% concentration of B at 25.5-29 min.

Mass spectrometry conditions of pigments: positive ion mode, scanning range of primary mass spectrometry and secondary mass spectrometry are 100-1200 M/Z and 50-1200 M/Z respectively, nitrogen is used in each gas path. The Scan Type of ESI-MS and ESI-MS<sup>2</sup> is TOF. The Ion Spray Voltage Floating (ISVF) of ESI-MS and ESI-MS<sup>2</sup> is 5500 V. The Source Gas 1 (GS1) of ESI-MS and ESI-MS<sup>2</sup> is 50 psi. The Source Gas 2 (GS2) of ESI-MS and ESI-MS<sup>2</sup> is 50 psi. The Curtain Gas (CUR) of ESI-MS and ESI-MS<sup>2</sup> is 35 psi. The Temperature (TEM) of ESI-MS and ESI-MS<sup>2</sup> is 550°C. The Declustering Potential (DP) of ESI-MS and ESI-MS<sup>2</sup> is 90 V. The Collision Energy (CE) of ESI-MS is 10V, and ESI-MS<sup>2</sup> is 35V. The Collision Energy Spread (CES) of ESI-MS<sup>2</sup> is 15V.

The results were analyzed by Masterview and Peakview software.

The assignment value method of non-quantitative character is adopted while quantifying pigment components, (0=Absent, 1= Present)

### 3. Results

#### 3.1. Taxonomic relationship of 13 species and hybrids of *Lonicera* L. based on pollen characteristics.

##### 3.1.1. Pollen morphological characteristics and value assignments of 13 species and hybrids of *Lonicera* L.

The qualitative and quantitative morphological indexes of *Lonicera* L. pollen are shown in Table 2,3.

**Table 2.** Pollen morphological characteristics and value assignments of 13 species and hybrids of *Lonicera* L.

| Latin name                             | Pollen shape        | Equator view           | Polar view       | Germinating pore shape   | Exine ornamentation                                                           |
|----------------------------------------|---------------------|------------------------|------------------|--------------------------|-------------------------------------------------------------------------------|
| <i>Lonicera maackii</i> (Rupr.) Maxim. | near spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong and<br>convexity1 | unsmooth pollen exine surface, with uneven-distributed spinous ornamentation1 |
| <i>Lonicera ferdinandi</i> Franch.     | near spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong and<br>convexity1 | unsmooth pollen exine surface, with uneven-distributed spinous ornamentation1 |

|                                                |                     |                        |                        |                  |                      |     |                                                                                   |
|------------------------------------------------|---------------------|------------------------|------------------------|------------------|----------------------|-----|-----------------------------------------------------------------------------------|
| <i>Lonicera</i><br>'Zabelii'.                  | <i>korolkowi</i>    | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera tatarica</i> Linn.                 |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br>Regel.                      | <i>ruprechtiana</i> | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera chrysantha</i> Turcz.<br>ex Ledeb. |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>tatarinowii</i> Maxim.   |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera edulis</i> Turcz.                  |                     | near<br>spherical<br>1 | triangular<br>circle 1 | ellipse2         | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>korolkow</i> 'Zabelii'.× |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera maackii</i> (Rupr.)<br>Maxim.      |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>korolkow</i> 'Zabelii'.× |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera tatarica</i> Linn.No.1             |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>korolkow</i> 'Zabelii'.× |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera tatarica</i> Linn.<br>No.2         |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>korolkow</i> 'Zabelii'.× |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera tatarica</i> Linn.<br>No.4         |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera</i><br><i>korolkow</i> 'Zabelii'.× |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |
| <i>Lonicera tatarica</i> Linn.<br>No.8         |                     | near<br>spherical<br>1 | triangular<br>circle 1 | near circle<br>1 | oblong<br>convexity1 | and | unsmooth pollen exine surface, with uneven-<br>distributed spinous ornamentation1 |

**Table 3.** Pollen morphological characteristics of 13 species and hybrids of *Lonicera* L. ( $\bar{X} \pm SD$ )1)

| Latin name | Polar       | equatorial | pollen | Polaraxis       | germinati | germinatin | average   | protrusion |
|------------|-------------|------------|--------|-----------------|-----------|------------|-----------|------------|
|            | axis length | axis       | sixze  | length/eq       | ng pore   | g pore     | protrusio | density    |
|            | P (μm)      | lengthE    | (P×E)  | uatoria         | length    | width      | n height  | (one/per   |
|            |             | (μm)       | (μm)   | axis            | (μm)      | (μm)       | (μm)      | area)      |
|            |             |            |        | length<br>(P/E) |           |            |           |            |

|                                             |            |           |         |          |           |           |          |           |
|---------------------------------------------|------------|-----------|---------|----------|-----------|-----------|----------|-----------|
|                                             | 46.80±1.42 | 42.50±2.3 | 46.80(  | 1.10±0.0 | 16.5±0.41 | 3.55±0.26 | 1.90±0.1 | 19.50±0.3 |
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 3          |           | ±1.42)  | 3        |           |           | 6        | 6         |
|                                             |            |           | ×42.50  |          |           |           |          |           |
|                                             |            |           | (±2.33) |          |           |           |          |           |
|                                             | 58.60±2.77 | 53.50±3.8 | 58.60(  | 1.10±0.0 | 18.8±0.37 | 5.16±0.16 | 1.50±0.2 | 25.46±0.3 |
| <i>Lonicera ferdinandi</i> Franch.          | 2          |           | ±2.77)  | 3        |           |           | 4        | 3         |
|                                             |            |           | ×53.50  |          |           |           |          |           |
|                                             |            |           | (±3.82) |          |           |           |          |           |
|                                             | 39.30±3.83 | 37.43±2.5 | 39.30(  | 1.05±0.0 | 12.3±0.25 | 2.23±0.10 | 2.00±0.2 | 28.92±0.3 |
| <i>Lonicera korolkowi</i> 'Zabelii'.        | 3          |           | ±3.83)  | 4        |           |           | 9        | 9         |
|                                             |            |           | ×37.43  |          |           |           |          |           |
|                                             |            |           | (2.53)  |          |           |           |          |           |
|                                             | 41.20±4.81 | 39.28±3.5 | 41.23(  | 1.05±0.0 | 12.8±0.30 | 2.57±0.20 | 2.20±0.2 | 29.00±0.2 |
| <i>Lonicera tatarica</i> Linn.              | 4          |           | ±4.81)  | 3        |           |           | 9        | 3         |
|                                             |            |           | ×39.28  |          |           |           |          |           |
|                                             |            |           | (±3.54) |          |           |           |          |           |
|                                             | 42.10±3.34 | 39.07±1.9 | 42.17(  | 1.08±0.0 | 17.9±0.31 | 1.95±0.07 | 2.30±0.2 | 24.56±0.1 |
| <i>Lonicera ruprechtiana</i> Regel.         | 9          |           | ±3.34)  | 7        |           |           | 2        | 5         |
|                                             |            |           | ×39.07  |          |           |           |          |           |
|                                             |            |           | (±1.99) |          |           |           |          |           |
|                                             | 40.30±5.53 | 38.50±2.0 | 40.30(  | 1.05±0.1 | 11.3±0.23 | 3.20±0.11 | 2.10±0.2 | 23.04±0.1 |
| <i>Lonicera chrysantha</i> Turcz. ex Ledeb. | 4          |           | ±5.53)  | 0        |           |           | 2        | 0         |
|                                             |            |           | ×38.50  |          |           |           |          |           |
|                                             |            |           | (±2.04) |          |           |           |          |           |
|                                             | 33.50±3.60 | 31.80±2.7 | 33.50(  | 1.05±0.0 | 0         | 0         | 1.10±0.2 | 35.28±0.1 |
| <i>Lonicera tatarinowii</i> Maxim.          | 8          |           | ±3.60)  | 2        |           |           | 2        | 9         |
|                                             |            |           | ×31.80  |          |           |           |          |           |
|                                             |            |           | (±2.78) |          |           |           |          |           |
|                                             | 40.90±4.79 | 32.40±2.9 | 40.90(  | 1.26±0.0 | 10.6±0.27 | 1.89±0.16 | 1.20±0.2 | 29.51±0.2 |
| <i>Lonicera edulis</i> Turcz.               | 6          |           | ±2.78)  | 6        |           |           | 2        | 6         |
|                                             |            |           | ×32.40  |          |           |           |          |           |
|                                             |            |           | (±2.96) |          |           |           |          |           |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 38.03±3.91 | 36.13±3.1 | 38.03(  | 1.05±0.0 | 13.6±0.20 | 2.22±0.04 | 2.60±0.0 | 21.60±1.9 |
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 7          |           | ±3.91)  | 2        |           |           | 5        | 3         |
|                                             |            |           | ×36.13  |          |           |           |          |           |
|                                             |            |           | (±3.17) |          |           |           |          |           |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 38.90±3.14 | 34.23±1.7 | 38.90(  | 1.14±0.0 | 11.8±0.41 | 2.21±0.13 | 1.90±0.1 | 31.00±0.1 |
| <i>Lonicera tatarica</i> Linn. No.1         | 6          |           | ±3.14)  | 4        |           |           | 6        | 8         |
|                                             |            |           | ×34.23  |          |           |           |          |           |
|                                             |            |           | (±1.76) |          |           |           |          |           |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 39.60±2.41 | 35.23±2.5 | 39.60(  | 1.12±0.0 | 14.3±0.48 | 2.22±0.11 | 2.50±0.2 | 37.50±0.2 |
| <i>Lonicera tatarica</i> Linn. No.2         | 6          |           | ±2.41)  | 2        |           |           | 2        | 6         |
|                                             |            |           | ×35.23  |          |           |           |          |           |
|                                             |            |           | (±2.56) |          |           |           |          |           |

|                                                |            |           |               |          |           |           |          |           |
|------------------------------------------------|------------|-----------|---------------|----------|-----------|-----------|----------|-----------|
| <i>Lonicera</i><br><i>korolkow</i> ‘Zabelii’.× | 36.43±2.98 | 34.13±2.4 | 36.43(±2.98)  | 1.07±0.0 | 13.5±0.30 | 2.06±0.06 | 2.20±0.1 | 40.52±0.2 |
| <i>Lonicera tatarica</i> Linn. No.4            |            | 4         | ×34.13(±2.44) | 1        |           |           | 4        | 3         |
| <i>Lonicera</i><br><i>korolkow</i> ‘Zabelii’.× | 38.43±4.05 | 38.03±1.9 | 38.43(±4.05)  | 1.01±0.0 | 13.4±0.34 | 2.47±0.10 | 2.20±0.2 | 37.91±0.1 |
| <i>Lonicera tatarica</i> Linn. No.8            |            | 8         | ×38.03(±1.98) | 6        |           |           | 4        | 1         |

The data in the table show that the pollens of 13 species(varieties) of *Lonicera* L. are near spherical, triangular circle in polar plane, and subround or ellipse in equatorial plane, with three-pored, furrow-shaped, ellipse and convex germination pore. Pollen exine surface is unsmooth, with uneven-distributed spinous ornamentation. The pollen is a medium type of pollen, with a size of 33.50×31.80 μm to 58.67×53.50 μm and P/E between 1.01 and 1.26. The length of polar axis is between 33.50 μm to 58.67 μm, the length of equatorial axis is between 31.80 μm to 53.50 μm, the size of germinating pore is 0~18.85 μm×0~5.16 μm. The pollen of *Lonicera* L. belongs to the more evolved type because its exine surface is not smooth, with uneven-distributed spinous ornamentation.

### 3.1.2. Cluster analysis of pollen morphological characteristics of 13 species and hybrids of *Lonicera* L.

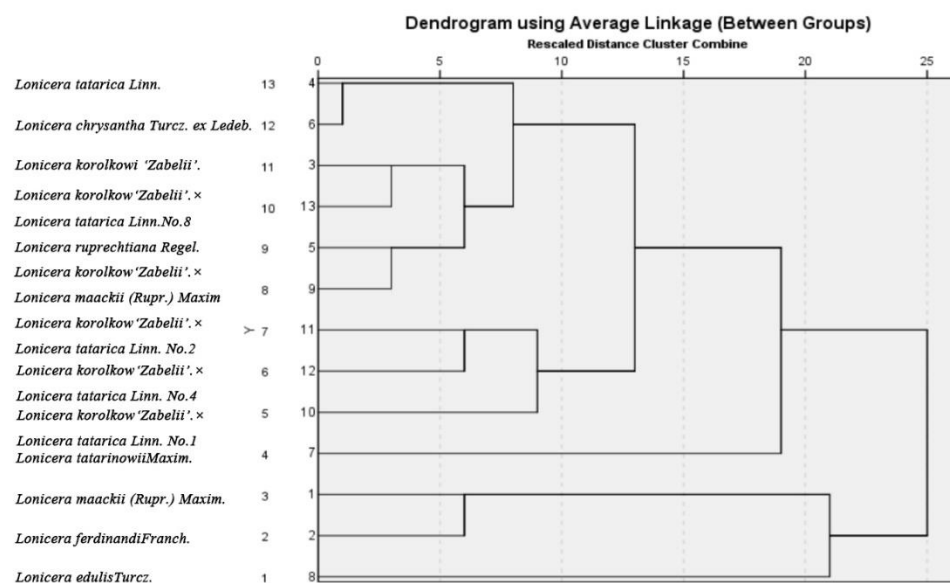
13 pollen indexes of 13 species and hybrids of *Lonicera* L. were selected and the results of principal component analysis were carried out by non-quantitative trait assignment method (Table 4).

**Table 4.** Five principal components and its accumulative contribution rates

| Principal components             | Y1     | Y2     | Y3     | Y4     | Y5     |
|----------------------------------|--------|--------|--------|--------|--------|
| Characteristic value             | 2.79   | 1.726  | 1.089  | 0.95   | 0.755  |
| Contribution rate (%)            | 34.872 | 21.581 | 13.607 | 11.871 | 9.436  |
| Cumulative contribution rate (%) | 34.872 | 56.453 | 70.061 | 81.932 | 91.368 |

As shown in Table 4, the cumulative contribution rate of principal components reached 91.37% through principal component analysis, which could represent most information of pollen traits of *Lonicera* L. and could be used as the inclusion items in cluster analysis.

Cluster analysis was conducted on the pollen morphological characteristics of 13 species and hybrids of *Lonicera* L. and taxonomic tree diagram was drawn (Fig 1).



X-axis indicate genetic distance, Y-axis indicate 13 species and hybrids of *Lonicera* L.

**Fig 1.** The clustering map of Pollen characters of 13 species and hybrids of *Lonicera* L.

Clustering results show that at the genetic distance of 25.0, the 13 species are divided into four groups: the first group consists of *Lonicera tatarica* Linn., *Lonicera chrysantha* Turcz. ex Ledeb., *Lonicera korolkowi* 'Zabelii'. , *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn.No.8, *Lonicera ruprechtiana* Regel. and *Lonicera korolkowi* 'Zabelii'. × *Lonicera maackii* (Rupr.) Maxim. The second group consists of *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn. No.2, *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn. No.4 and *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn. No.1. The third group only consists of *Lonicera tatarinowii* Maxim..The fourth group contains *Lonicera maackii* (Rupr.) Maxim., *Lonicera ferdinandi* Franch., and *Lonicera edulis* Turcz..

The pollen of *Lonicera* L. is similar in shape and exine ornamentation, so it can be distinguished according to the quantitative indexes of pollen. In the first group, the polar axis length of *Lonicera* L. pollen all ranged from 38.03  $\mu\text{m}$  to 42.17  $\mu\text{m}$ , and P/E ranged from 1.01  $\mu\text{m}$  to 1.08  $\mu\text{m}$ . The pollen sizes of the second group are similar, P/E ranged from 1.07  $\mu\text{m}$  to 1.14  $\mu\text{m}$ . The average size ( $P \times E$ ) of pollen in the third group is smallest. In the fourth group, the average protrusion height of the pollen exine ornamentation was similar, which was less than 2.00  $\mu\text{m}$ .

### 3.2. Taxonomic relationship of 13 species and hybrids of *Lonicera* L. based on chromosome karyotype characteristics.

#### 3.2.1. Chromosome karyotype characteristics and value assignments of 13 species and hybrids of *Lonicera* L.

The statistical results in Table 5. are as follows:

**Table 5.** The chromosome karyotype characteristics and value assignments of 13 species and hybrids of *Lonicera* L.

| Latin Name | Karyotype formula | Average relative length | Average arm ratio | Asymmetric al karyotype coefficient/ % | Arm ratio > 2 Lt/St ratio/ % | Centromeric index | Karyotype |
|------------|-------------------|-------------------------|-------------------|----------------------------------------|------------------------------|-------------------|-----------|
|------------|-------------------|-------------------------|-------------------|----------------------------------------|------------------------------|-------------------|-----------|

| /%                                                                           |          |           |      |       |       |      |       |      |  |
|------------------------------------------------------------------------------|----------|-----------|------|-------|-------|------|-------|------|--|
| <i>Lonicera maackii</i> (Rupr.) Maxim.                                       | 12m+6sm1 | 2.66-3.89 | 1.49 | 59.19 | 11.11 | 1.95 | 40.91 | 2A 1 |  |
| <i>Lonicera ferdinandi</i> Franch.                                           | 10m+8sm2 | 3.45-7.30 | 1.65 | 59.85 | 0.00  | 2.12 | 37.39 | 1B 2 |  |
| <i>Lonicera korolkowi</i> 'Zabelii'.                                         | 14m+4sm3 | 2.41-8.05 | 1.50 | 59.82 | 11.11 | 3.34 | 40.79 | 2B 3 |  |
| <i>Lonicera tatarica</i> Linn.                                               | 18m4     | 3.45-7.30 | 1.27 | 55.47 | 0.00  | 2.01 | 44.64 | 1B 2 |  |
| <i>Lonicera ruprechtiana</i> Regel.                                          | 16m+2sm5 | 2.78-5.76 | 1.48 | 56.43 | 0.00  | 2.07 | 40.58 | 1B 2 |  |
| <i>Lonicera chrysantha</i> Turcz. ex Ledeb.                                  | 8m+10sm6 | 2.14-4.15 | 1.72 | 63.03 | 11.11 | 1.94 | 37.09 | 2A 1 |  |
| <i>Lonicera tatarinowii</i> Maxim.                                           | 14m+4sm3 | 2.95-7.33 | 1.54 | 60.98 | 11.11 | 2.48 | 40.06 | 2B 3 |  |
| <i>Lonicera edulis</i> Turcz.                                                | 14m+4sm3 | 1.81-3.90 | 1.49 | 59.15 | 22.22 | 2.15 | 41.00 | 2B 3 |  |
| <i>Lonicera korolkow</i> 'Zabelii'. × <i>Lonicera maackii</i> (Rupr.) Maxim. | 14m+4sm3 | 2.46-4.93 | 1.47 | 58.61 | 11.11 | 2.00 | 41.24 | 2B 3 |  |
| <i>Lonicera korolkow</i> 'Zabelii'. × <i>Lonicera tatarica</i> Linn. No.1    | 14m+4sm3 | 2.52-5.31 | 1.46 | 59.68 | 11.11 | 2.68 | 41.39 | 2B 3 |  |
| <i>Lonicera korolkow</i> 'Zabelii'. × <i>Lonicera tatarica</i> Linn. No.2    | 16m+2sm5 | 3.45-7.30 | 1.32 | 56.54 | 0.00  | 2.57 | 43.36 | 1B 2 |  |
| <i>Lonicera korolkow</i> 'Zabelii'. × <i>Lonicera tatarica</i> Linn. No.4    | 14m+4sm3 | 2.72-5.87 | 1.39 | 58.20 | 11.11 | 2.16 | 42.73 | 2B 3 |  |
| <i>Lonicera korolkow</i> 'Zabelii'. × <i>Lonicera tatarica</i> Linn. No.8    | 14m+4sm3 | 2.23-4.48 | 1.43 | 58.94 | 0.00  | 2.01 | 41.64 | 1B 2 |  |

The chromosome number of 13 species and hybrids of *Lonicera* L. in the experiment is  $2n = 18$ , which is composed of middle centromeric chromosome (m) and near middle centromeric chromosome (sm).

Because of primitive 1B, 2B and 2A of karyotype types, indicating that 13 species and hybrids of *Lonicera* L. is not evolution. There are on no obvious evolutionary difference between 13 species.

### 3.2.2. Cluster analysis of chromosome karyotype characteristics of 13 species and hybrids of *Lonicera* L.

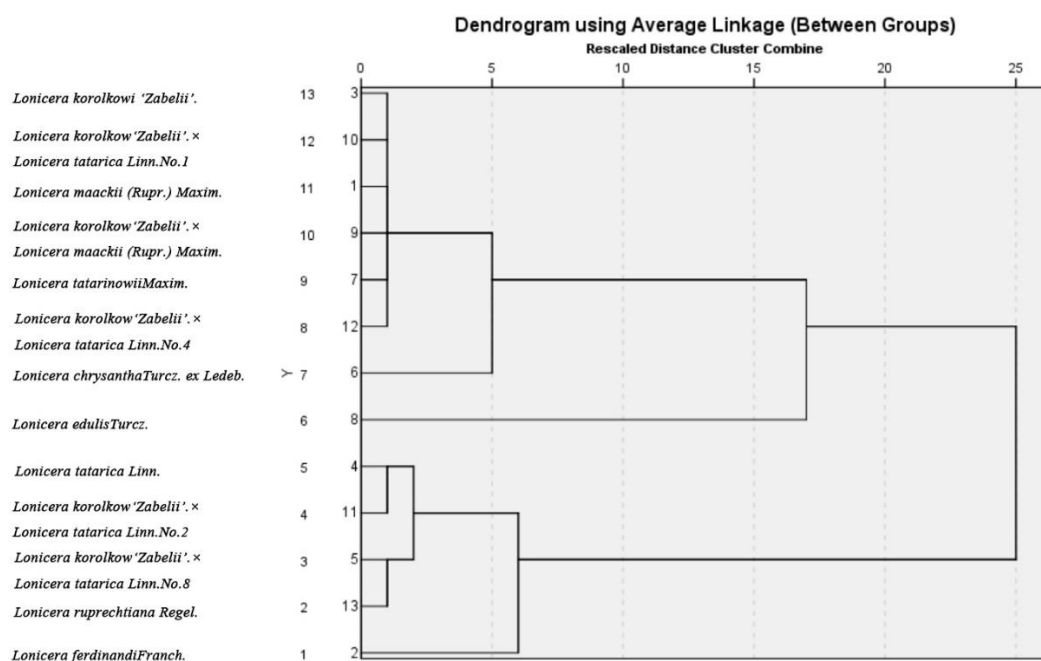
Three principal components were analysed according to the assignment value method of non-quantitative traits based on nine chromosomes indexes of 13 species and hybrids of *Lonicera* L. (Table 6).

**Table 6.** Three principal components and its accumulative contribution rates

| The principal component          | Y1     | Y2     | Y3     |
|----------------------------------|--------|--------|--------|
| Characteristic value             | 3.235  | 1.426  | 1.013  |
| Contribution rate (%)            | 53.916 | 23.76  | 16.882 |
| Cumulative contribution rate (%) | 53.916 | 77.678 | 94.561 |

As shown in Table 6., the contribution rate of principal components is 94.56%, indicating that the information contain in principal components could fully reflect most of the information of the karyotype parameters of *Lonicera* L., and could be used as the inclusion items in cluster analysis.

Cluster analysis was conducted on the chromosome karyotype characteristics of 13 species and hybrids of *Lonicera* L. and taxonomic tree diagram was drawn (Fig.2).



**Fig 2.** Cluster analysis of chromosome morphological characteristics of 13 species and hybrids of *Lonicera* L.

Clustering results show that at the genetic distance of 25.0, the 13 species are divided into three groups: the first group consists of *Lonicera korolkowi* 'Zabelii'. , *Lonicera korolkow*'Zabelii'. x *Lonicera tatarica* Linn.No.1, *Lonicera maackii* (Rupr.) Maxim., *Lonicera korolkow*'Zabelii'. x *Lonicera maackii* (Rupr.) Maxim., *Lonicera tatarinowii*Maxim., *Lonicera korolkow*'Zabelii'. x *Lonicera tatarica* Linn.No.4 and *Lonicera chrysantha*Turcz. ex Ledeb.. The second group only consists of *Lonicera edulis*Turcz.. The third group consists of *Lonicera tatarica* Linn., *Lonicera korolkow*'Zabelii'. x *Lonicera tatarica* Linn.No.2, *Lonicera korolkow*'Zabelii'. x *Lonicera tatarica* Linn.No.8, *Lonicera ruprechtiana* Regel. and

*Lonicera ferdinandi* Franch..

The average arm ratio of *Lonicera* L. in the first group is relatively large, and the ratio of arm ratio  $> 2$  is 11.11. The karyotype of *Lonicera* L. in the third group are 1B, and the ratio of arm ratio  $> 2$  is 0.

### **3.3. Taxonomic relationship of 13 species and hybrids of *Lonicera* L. based on floral pigment compositions.**

#### **3.3.1. Floral pigment compositions analysis and value assignments of 13 species and hybrids of *Lonicera* L.**

26 floral pigment compositions were identified from 13 species and hybrids of *Lonicera* L. and non-quantitative trait assignment method was adopted (including a certain composition being assigned as 1, excluding a certain composition being assigned as 0). The results are shown in Table 7.

**Table 7.** The value assignments of floral pigment compositions 13 species and hybrids of *Lonicera* L. (0=Absent, 1= Present)

| <i>Lonicera</i> L.<br>species               | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---------------------------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera ferdinandi</i> Franch.          | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1  | 1  | 1  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> ‘Zabelii’.        | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.              | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera ruprechtiana</i> Regel.         | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera chrysantha</i> Turcz. ex Ledeb. | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0  | 1  | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarinowii</i> Maxim.          | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 1  | 1  | 0  | 0  |
| <i>Lonicera edulis</i> Turcz.               | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 1  | 0  |
| <i>Lonicera korolkow</i> ‘Zabelii’.×        | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkow</i> ‘Zabelii’.×        | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.1          | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkow</i> ‘Zabelii’.×        | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.2          | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkow</i> ‘Zabelii’.×        | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1  | 1  | 1  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.4          | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1  | 1  | 1  | 0  | 0  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkow</i> ‘Zabelii’.×        | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1  |
| <i>Lonicera tatarica</i> Linn.No.8          | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 1  |

(Note: 1 quercetin-3-o-rutside 2 hexyl quercetin-3-o-glucoside 3 quercetin-3-o-glucoside 4 kaempferol-3-o-rutside 5 kaempferol-3-o-glucoside 6 geraniol-3-o-rutside 7 Geranium rutside 8 Geranium glucoside 9 paeoniflorin glucoside 10 vinblastin 11 isorhamnetin-7-o-rutside 12 delphinidin Sophorae 13 quercetin-3-o-sambuoside 14 quercetin-3-o-digluoside 15 3-hydroxyphlorin 16 Geranium -Malonyl glucoside 17 delphinidin - arabinose 18 cyanidin - Sophora 19 Petunia - Sophora 20 isorhamnetin-3-o-glucoside 21 isorhamnetin 22 kaempferol-3-o-sambuoside 23 ferulic acid glucoside 24 delphinidin - hexyl glucose 25 delphinidin - malonyl glucose 26 mallow glucoside)

According to the results analysis in Table 7., 26 pigment components were detected from 13 species and hybrids of *Lonicera* L., including 16 flavonoid pigments and 10 anthocyanin pigments. Quercetin-3-o-rutoside, Quercetin-3-o-glucoside, Kaempferol-3-o-rutoside, Kaempferol-3-o-glucoside, Geranium-rutoside, Paeoniflorin-glucoside are common components in most *Lonicera* L. Paeoniflorin-glucoside is the main anthocyanin component in *Lonicera* L., which accounts for the largest proportion of anthocyanin in *Lonicera* L.as well as there generally contains flavonoid pigment components in flowers in *Lonicera* L.

### 3.3.2. Cluster analysis of floral pigment components of 13 species and hybrids of *Lonicera* L.

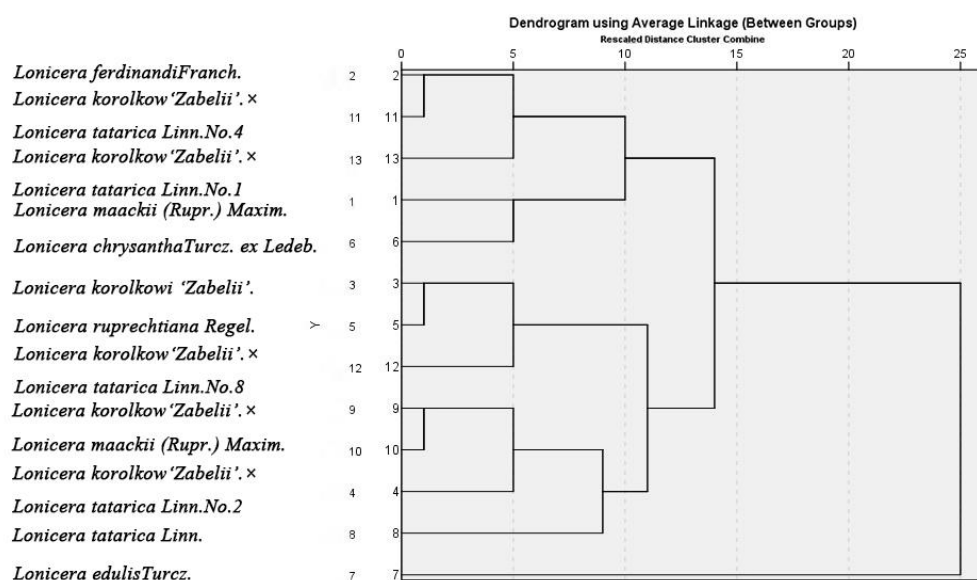
The floral pigments components of 13 species and hybrids of *Lonicera* L. were analyzed by principal component analysis (Table 8.).

**Table 8.** Eight principal components and its contribution rates

| The principal component          | Y1     | Y2     | Y3     | Y4     | Y5     | Y6     | Y7     | Y8     |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Characteristic value             | 6.676  | 4.669  | 3.229  | 2.349  | 1.665  | 1.591  | 1.25   | 0.627  |
| contribution rate (%)            |        |        |        |        |        |        |        |        |
| Contribution rate (%)            | 29.025 | 20.301 | 14.039 | 10.41  | 7.241  | 6.916  | 5.433  | 2.728  |
| Cumulative contribution rate (%) |        |        |        |        |        |        |        |        |
|                                  | 29.025 | 49.326 | 63.365 | 73.775 | 81.016 | 87.932 | 93.365 | 96.093 |

Table 8. shows that the contribution rate of principal components reaches 96.09%, which indicates that the information contained in principal components can fully reflect most of the information of floral pigment components of *Lonicera* L. and can be used as an entry option for cluster analysis.

Cluster analysis was conducted on the floral pigment components of 13 species and hybrids of *Lonicera* L. and taxonomic tree diagram was drawn (Fig 3.).



**Fig 3.** The clustering map of pigment components of 13 species and hybrids of *Lonicera* L.

Clustering results show that at the genetic distance of 10.0, the 13 species are divided into three groups: the first group consists of *Lonicera ferdinandi*Franch., *Lonicera korolkow*'Zabelii'.×Lonicera tatarica

Linn.No.4, *Lonicera korolkow*‘Zabelii’. $\times$ *Lonicera tatarica* Linn.No.1, *Lonicera maackii* (Rupr.) Maxim. and *Lonicera chrysantha* Turcz. ex Ledeb. The second group consists of *Lonicera korolkowi* ‘Zabelii’., *Lonicera ruprechtiana* Regel., *Lonicera korolkow*‘Zabelii’. $\times$ *Lonicera tatarica* Linn.No.8, *Lonicera korolkow*‘Zabelii’. $\times$ *Lonicera maackii* (Rupr.) Maxim., *Lonicera korolkow*‘Zabelii’. $\times$ *Lonicera tatarica* Linn.No.2, *Lonicera tatarica* Linn. and *Lonicera edulis* Turcz.. The third group only consists of *Lonicera tatarinowii* Maxim..

The first group contains quercetin-3-o-rutside, kaempferol-3-o-rutside, kaempferol-3-o-glucoside and geranium rutside. The flower color of the second group is reddish and contains paeoniflorin glucoside, vinblastin, isorhamnetin-7-o-rutside, geranium malonyl glucose and delphinidin arabinose, which contain more anthocyanin pigments than the other two groups. In the third group, *Lonicera tatarinowii* Maxim. has different flower color from other *Lonicera* L. there are four anthocyanins in this group: orhamnetin, kaempferol-3-o-sambuoside, ferulic acid glucoside and delphinidin hexyl glucose.

### **3.4. Taxonomic relationship of 12 species and hybrids of *Lonicera* L. based on fruit pigment components.**

#### **3.4.1. Fruit pigment components analysis and value assignments of 12 species and hybrids of *Lonicera* L.**

23 fruit pigment components were identified from 12 species and hybrids of *Lonicera* L. and non-quantitative trait assignment value method was adopted (including a certain composition being assigned as 1, excluding a certain composition being assigned as 0). The results are shown in Table 9.

**Table 9.** The value assignments of fruit pigment compositions 12 species and hybrids of *Lonicera* L. (0=Absent, 1= Present)

| <i>Lonicera</i> L.<br>species               | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---------------------------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera ferdinandi</i> Franch.          | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> 'Zabelii'.        | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.              | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0  | 1  | 1  | 0  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera ruprechtiana</i> Regel.         | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0  | 1  | 1  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera chrysantha</i> Turcz. ex Ledeb. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera edulis</i> Turcz.               | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera maackii</i> (Rupr.) Maxim.      | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 1  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0  | 1  | 1  | 0  | 0  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 1  | 1  |
| <i>Lonicera tatarica</i> Linn.No.1          | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1  | 1  | 1  | 0  | 0  | 1  | 1  | 0  | 1  | 1  | 1  | 1  | 1  | 1  |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.2          | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.4          | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera korolkowi</i> 'Zabelii'.×       | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0  | 1  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| <i>Lonicera tatarica</i> Linn.No.8          | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0  | 1  | 1  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

(Note: 1 vinblastin 2 quercetin-3-o-rutinoside 3 hexyl quercetin-3-o-glucoside) 4 quercetin-3-o-glucoside 5 kaempferol-3-o-rutinoside 6 kaempferol-3-o-glucoside 7 geranyl lignin-3-o-rutinoside 8 Geranium rutinoside 9 geranium malonyl glucoside 10 delphinidin arabinose 11 cyanidin Sophorae 12 paeoniflorin glucose 13 chlorogenic acid 14 isorhamnetin-

-7-o-rutside 15 quercetin-3-odiglucoside 16 isorhamnetin-3-o-glucoside 17 malvacin glucoside 18 geranium glucoside 19 trefoil glycoside 20 quercetin-3-o-mulberry glycoside 21 delphinidin sophoricose 22 delphinidin hexyl glucoside 23 cyanidin arabinoside)

According to the result analysis in Table 9., 23 pigment components were detected from 12 species and hybrids of *Lonicera* L., including 11 flavonoid pigments and 12 anthocyanin pigments. Vinblastin, quercetin-3-o-rutside, quercetin-3-o-glucoside, kaempferol-3-o-glucoside and paeoniflorin-glucoside are the common components in most *Lonicera* L. Paeoniflorin glucoside accounts for the largest proportion of anthocyanins in *Lonicera* L., which is the main component of fruit pigments in *Lonicera* L..

### 3.4.2. Cluster analysis of fruit pigment components of 12 species and hybrids of *Lonicera* L.

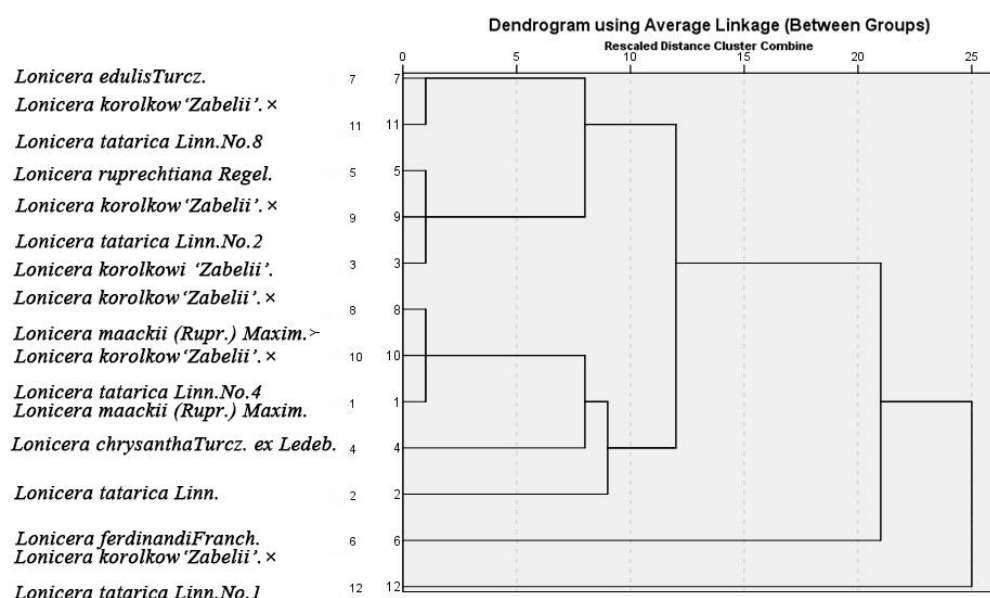
The 23 fruit pigments components of 12 species and hybrids of *Lonicera* L. were analyzed by principal component analysis (Table 10.).

**Table 10.** Seven principal components and its contribution rates

| Principal components             | Y1     | Y2     | Y3     | Y4     | Y5     | Y6     | Y7     |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Characteristic value             | 6.356  | 4.585  | 2.52   | 1.961  | 1.471  | 1.232  | 1.121  |
| Contribution rate (%)            | 30.265 | 21.831 | 11.999 | 9.338  | 7.006  | 5.865  | 5.338  |
| Cumulative contribution rate (%) | 30.265 | 52.096 | 64.095 | 73.433 | 80.439 | 86.304 | 91.642 |

Table 10. shows that the contribution rate of principal components reach 91.64%, indicating that the information contained in the principal components can fully reflect most of the information of the fruit components of *Lonicera* L. which can be used as the inclusion items in cluster analysis.

Cluster analysis was conducted on the fruit pigment components of 12 species and hybrids of *Lonicera* L. and taxonomic tree diagram was drawn (Fig4.).



**Fig 4.** The clustering map of pigment components of 12 species and hybrids of *Lonicera* L.

Clustering results show that at the genetic distance of 10.0, the 12 species are divided into three groups: the first group consists of *Lonicera edulis*Turcz., *Lonicera korolkow*'Zabelii'.×*Lonicera tatarica* Linn.No.8, *Lonicera ruprechtiana* Regel., *Lonicera korolkow*'Zabelii'.×*Lonicera tatarica* Linn.No.2, *Lonicera korolkowi* 'Zabelii'. , *Lonicera korolkow*'Zabelii'.×*Lonicera maackii* (Rupr.) Maxim., *Lonicera korolkow*'Zabelii'.×*Lonicera tatarica* Linn.No.4, *Lonicera maackii* (Rupr.) Maxim., *Lonicera tatarica*

Linn. and *Lonicera ferdinandi* Franch.. The second group only consists of *Lonicera chrysantha* Turcz. ex Ledeb.. The third group only consists of *Lonicera korolkowii* 'Zabelii' × *Lonicera tatarica* Linn. No. 1..

The first group contains vinblastin, quercetin-3-o-rutinoside and kaempferol-3-o-glucoside. The second group contains only four anthocyanins. In the third group, there are unique trilobatin, quercetin-3-o-sambuoside, delphinidin Sophora sugar, delphinidin hexoyl glucoside and cyanidin arabinoside in the *Lonicera korolkowii* 'Zabelii' × *Lonicera tatarica* Linn. No. 1, so it was grouped into one group separately. According to the clustering results of floral pigment components: in the first group, *Lonicera korolkowii* 'Zabelii'. and *Lonicera tatarica* Linn. are the parents of *Lonicera korolkowii* 'Zabelii' × *Lonicera tatarica* Linn. No. 2, *Lonicera korolkowii* 'Zabelii' × *Lonicera tatarica* Linn. No. 4 and *Lonicera korolkowii* 'Zabelii' × *Lonicera tatarica* Linn. No. 8.; *Lonicera korolkowii* 'Zabelii'. and *Lonicera maackii* (Rupr.) Maxim. are the parents of *Lonicera korolkowii* 'Zabelii' × *Lonicera maackii* (Rupr.) Maxim. Both parents and hybrids are clustered in one group. And *Lonicera korolkowii* 'Zabelii'. , *Lonicera ruprechtiana* Regel., *Lonicera tatarica* Linn. and *Lonicera maackii* (Rupr.) Maxim. are clustered in one group, which is consistent with the morphological taxonomy.

### **Discussion and conclusion**

#### **The taxonomic relationship of pollen morphology characteristics of 13 species and hybrids of *Lonicera* L.**

Jamzad Z and Hasani nejad M proved that surface ornamentation is an important basis for classification in this genus by studying the morphological characteristics of 29 kinds of pollen of two subgenera of *Scutellaria* and identifying the types of pollen surface ornamentation (Jamzad and Hasani-Nejad 2014). Li Huaicheng et al. divided 17 species of *Desmodium* Desv. into 4 groups according to the cluster analysis on the pollen (Huaicheng Li et al. 2018). The research results on the palynological characters of *Desmodium* have certain guiding significance for the classification of its subordinates.<sup>[18]</sup> In this paper typical characteristic indexes of 13 species and hybrids of *Lonicera* L. are selected for cluster analysis. The results that both parents and hybrids, *Lonicera korolkowii* 'Zabelii'. , *Lonicera ruprechtiana* Regel., *Lonicera tatarica* Linn. and *Lonicera chrysantha* Turcz. ex Ledeb as well as *Lonicera ferdinandi* Franch. and *Lonicera edulis* Turcz. are clustered in one group are consistent with morphological taxonomy, indicating that the results of pollen morphological classification are highly consistent with the morphological classification of *Lonicera* L. and providing a palynological basis for the classification of *Lonicera* L.

#### **The taxonomic relationship of Chromosome karyotype characteristics of 13 species and hybrids of *Lonicera* L.**

Chuanmei Xu et al. clarified the genetic relationship of 10 species of *Carya cathayensis* according to their karyotypes (Chuanmei Xu 2017) . In this paper, 8 typical characters of chromosome morphology of 13 species and hybrids *Lonicera* L. were selected for cluster analysis.

The results that both parents and hybrids, *Lonicera maackii* (Rupr.) Maxim. and *Lonicera tatarinowii* Maxim. as well as *Lonicera tatarica* Linn. and *Lonicera ruprechtiana* Regel. belonged to the category of hollow branchlets are clustered in one group are consistent with morphological taxonomy, showing that the chromosome morphological classification is partly consistent with the morphological classification of *Lonicera* L., which indicates that the results of this study are scientific.

#### **The taxonomic relationship of floral pigment components of 13 species and hybrids of *Lonicera* L.**

Masaji et al. divided the genetic relationships of 9 species of *Curcuma* into two categories according to 11 kinds of anthocyanins detected from them (Koshioka et al. 2015). In this paper, 13 species and hybrids *Lonicera* L. are divided into three groups according to 26 anthocyanins.

The results that both parents and hybrids as well as *Lonicera korolkowi* ‘ZabTelii’, *Lonicera ruprechtiana* Regel. and *Lonicera tatarica* Linn. are clustered in one group are partly consistent with the morphological taxonomy of *Lonicera* L., indicating that the floral pigment components has a certain scientific value for the classification of *Lonicera* L..

#### **The taxonomic relationship of fruit pigment components of 12 species and hybrids of *Lonicera* L.**

Wang Y et al. divided 44 firework trees into three types according to the anthocyanins which identified from the plants(Wang et al. 2018).In this paper, 21 anthocyanins identified from 12 species of *Lonicera* L. were clustered,12 species and hybrids of *Lonicera* L. are divided into three groups. Both parents and hybrids are clustered in one group. And *Lonicera korolkowi* ‘Zabelii’, *Lonicera ruprechtiana* Regel., *Lonicera tatarica*Linn. and *Lonicera maackii* (Rupr.) Maxim. are clustered in one group, which is consistent with the morphological taxonomy.

In morphological taxonomy, the berries of *Lonicera edulis*Turcz. are long ellipse in shape and deep blue with a coat of powder in color. The berries of the other 11 species (varieties) *Lonicera* L. are spherical in shape and red in color. Although there are great differences in morphology, *Lonicera edulis*Turcz. is divided into corresponding groups during clustering in this paper. Ying Zhu et al. believed that *Lonicera edulis*Turcz. belongs to the blue-fruited subgroup of *Lonicera* L., which is far away from other *Lonicera* L.. This view explains one phenomenon that *Lonicera edulis*Turcz. belongs to *Lonicera* L., but far away from the other *Lonicera* L. in the clustering results(Ying Zhu et al. 2009).

In this paper, 13 species and hybrids of *Lonicera* L. were clustered according to pollen morphology characteristics, chromosome karyotype characteristics, floral pigment and fruit pigment components. It is found that the cluster results that *Lonicera korolkowi* ‘Zabelii’, *Lonicera ruprechtiana* Regel., *Lonicera tatarica* Linn. and *Lonicera chrysantha*Turcz. ex Ledeb. as well as *Lonicera ferdinandi*Franch. and *Lonicera edulis*Turcz. are clustered in one group are most consistent with morphological taxonomy. Five hybrids such as *Lonicera korolkow*‘Zabelii’.×*Lonicera maackii* (Rupr.) Maxim. and *Lonicera korolkow*‘Zabelii’.×*Lonicera tatarica* Linn.No.8 all can basically be clustered with their parents. Among the four clusters, *Lonicera ruprechtiana* Regel and *Lonicera tatarica* Linn. are always clustered together, which is consistent with morphological taxonomy, indicating that pollen morphology characteristics, chromosome karyotype characteristics, floral pigments and fruit pigments components have certain scientific significance for the study of systematic classification and genetic relationship of *Lonicera* L. However, it is found that the classification results of *Lonicera* L. at these four levels are not completely consistent, there being some differences, indicating that the evolution of *Lonicera* L. at the levels of secondary metabolites, palynology and cell biology is not very consistent.

#### **Supplementary Information**

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##### **Author Contributions**

Da LongLi: Determination of flowers and fruits pigment components

Xiaoou Zhai: Observation and measurement of pollen morphological

Li Fang: Sampling and photographing of chromosome karyotype

Liwei Sun and Xing Ji: Data curation

Sijia Zhang: Image processing

Yujiao Zhang: Cluster analysis

Liu Huimin\*: Writing - original draft

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**Availability of data and materials**

The datasets generated during and/or analyzed during the current study are available in supplementary table fle.

**Declarations****Ethics approval and consent to participate**

Yes, we agreed and give consent for participation.

**Consent for publication**

Authors give consent for publication of this manuscript.

**Competing interests**

The authors declare no conflicts of interest that are relevant to the content of this article.

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## appendix

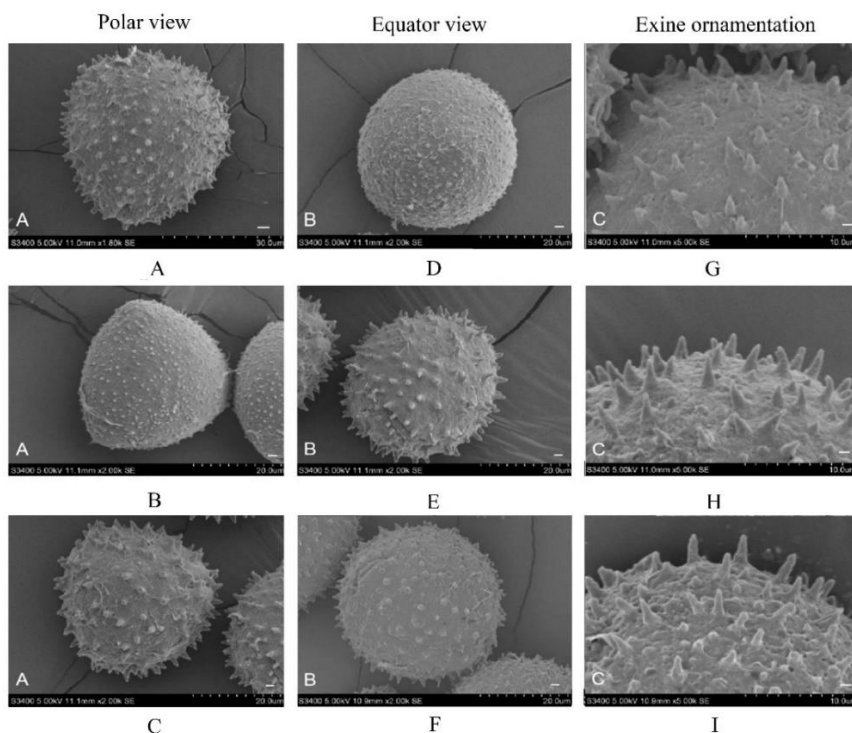


Plate. I | Pollen morphology and exine ornamentation characteristics of 9 species of *Lonicera* L.

(A: *Lonicera tatarica* Linn. B: *Lonicera tatarinowii* Maxim. C: *Lonicera korolkowi* 'Zabelii'. × *Lonicera maackii* (Rupr.) Maxim.; D: *Lonicera tatarinowii* Maxim.; E: *Lonicera korolkowi* 'Zabelii'. × *Lonicera maackii* (Rupr.) Maxim.; F: *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn.No.4; G: *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn.No.4; H: *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn.No.8; I: *Lonicera korolkowi* 'Zabelii'. × *Lonicera tatarica* Linn.No.2)

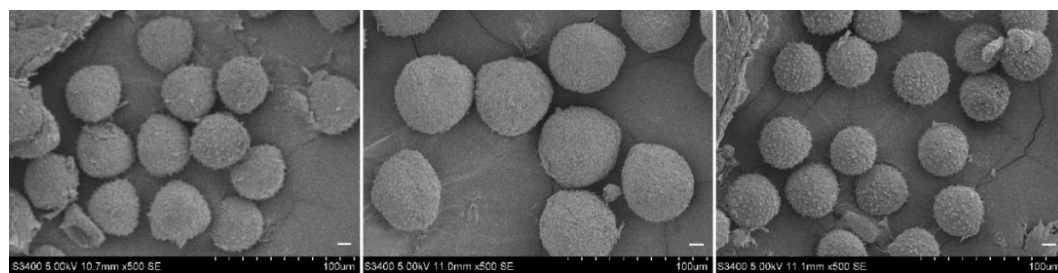


Plate. II | Pollen group view under SEM of three species of *Lonicera* L.

(A: *Lonicera maackii* (Rupr.) Maxim.; B: *Lonicera ferdinandi* Franch.; C: *Lonicera korolkowi* 'Zabelii'.)

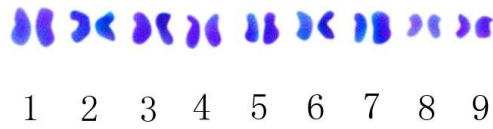
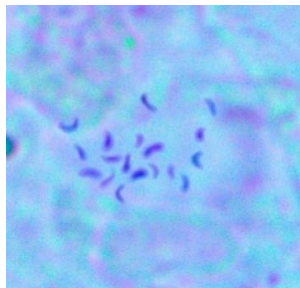
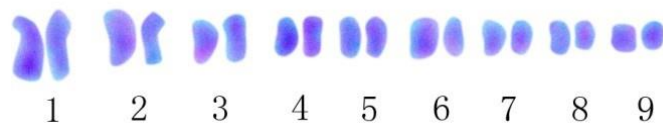
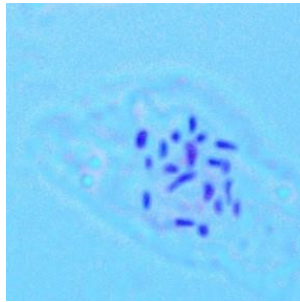
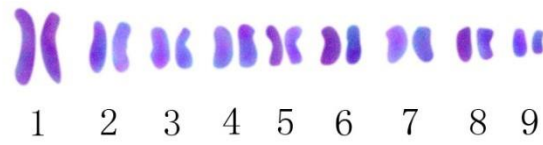
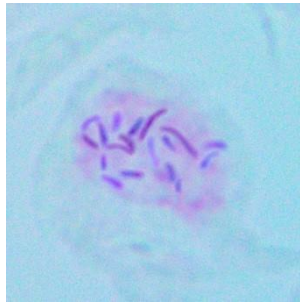


Plate. III Chromosome characteristics of three species of 3species *Lonicera* L.

(A: *Lonicera korolkowi* 'Zabelii'.; B: *Lonicera tatarinowii* Maxim.; C: *Lonicera korolkowi* 'Zabelii'.)