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Pollen morphology of Chinese *Begonia* (Begoniaceae) and its taxonomical significance

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Abstract: *Begonia* L. is the sixth biggest genus of flowering plants in the world and a major genus in the family Begoniaceae. It is complicated in current section delimitation and more morphological characters are needed for critical reevaluation within the genus. In present study, we selected 21 species from sections *Coelocentrum*, *Begonia*, *Platycentrum* and *Reichenheimia* of *Begonia* mainly distributed in China. Using the method of scanning electron microscopy (SEM), the pollen morphology was carefully examined and its systematic significance was discussed. The results showed that the common palynological characters of studies taxa were exhibited as monads, radially symmetrical, isopolar, 3-zono-colporate and perprolate to prolate. For non-metric multidimensional scaling (NMDS) analysis, nine stable pollen characters were selected and coded as unordered and unweighted. The results supported that these sections were not monophyletic groups and the useful palynological features for this study were the pollen sides, outline of the poles, colpus, exine ornamentation, and margo. According to the ornamentation of margo, the studied taxa can be divided into two groups: (A) without margo and psilate margo, (B) regulate, exquisitely regulate and coarsely regulate margo. In relation to Group B, the taxa with regulate and exquisitely regulate grains were gathered while the taxa with coarsely regulate margo were located between Group A and B. It indicated that the feature of margo was exhibited as a transition, from without margo and psilate margo, along coarsely regulate margo to exquisitely regulate. Therefore, it is worthy to pay more attention on the feature of margo and increase more taxa of the genus in further studies.

Keywords: SEM, characters coded, NMDS, margo, non-monophyletic groups

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国产秋海棠属(秋海棠科)花粉形态及其分类学意义

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摘要: 秋海棠属是世界有花植物第六大属, 是被子植物分类困难的类群之一, 亟需增加形态性状的比较研究, 以便于今后对该属开展分类学修订。该研究选取国产秋海棠属中较为常见的侧膜组、秋海棠组、单座组和二室组共 21 种, 应用扫描电镜观察花粉微形态, 探讨花粉形态对秋海棠属植物的分类学意义。

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结果表明：秋海棠属植物的花粉多为单粒花粉，辐射对称，等极，三孔沟，超长球形到长球形。选取 9 个稳定的花粉特征进行无序和不加权的性状编码，应用非线性多维标度分析对花粉特征矩阵进行聚类分析，结果支持这些组都不是单系类群需要重新修订，其中花粉边缘形状、极面观轮廓、萌发沟和花粉的外壁纹饰具有一定分类学意义，特别是塞缘特征具有重要的分类意义。根据塞缘特征可以将研究类群区分为 2 个类群：（A）无塞缘或塞缘光滑；（B）塞缘颗粒状。类群 B 中具规则颗粒状和精细颗粒状塞缘的种类聚在一起，而具粗糙颗粒状塞缘的种类位于类群 A 和 B 的中间，很可能是 2 个类群的过渡性状，这需要增加取样做进一步的研究。

关键词：扫描电镜，性状编码，非线性多维标度分析，塞缘，非单系类群

Begonia L. is estimated to have more than 1900 named species (Twyford et al, 2015), comprising nearly all the species of Begoniaceae (Forrest & Hollingsworth, 2003). The genus widely distributed in tropical and subtropical areas excluding Australia, within which most species occur as localized endemics in moist, shaded, forest or limestone habitats. This genus can be easily distinguished by their monoecious perennials, mostly fleshy stems, usually asymmetric leaf shape, numerous centripetal stamens, twisted stigma, numerous seeds and possess collar cells below an operculum of the seed.

Begonia has a complicated taxonomic history, especially in the delimitation of sections (Shui et al, 2002). Traditionally, the floral and fruit characteristics are used for sectional distinctions, such as perianth number, style number and morphology, locule number, placentae number, and mode of fruit dehiscence (Doorenbos et al, 1998; Shui et al, 2002; Gu et al, 2009). In the light of recently molecular phylogeny (Forrest et al, 2005), the current infrageneric classification has a certain diagnostic, but only poor predictive value within *Begonia* (Thomas et al, 2011). It is necessary to careful study of more qualitative characters for critical reevaluation within the genus.

Pollen grains are often of valuable assistance in delimiting taxonomic relationships imply useful characters for delimiting sections and species in *Begonia*. Van den Berg (1984) collected nearly all known African species of *Begonia* and found that pollen size, outline of the poles, and margo can provide significant information for the classification. Rajbhandary et al (2012) studied 28 species from Nepal and suggested that margo could be delimited section *Platycentrum* from all other sections. However, palynological studies are deficient in this massive genus, especially in China. Hence, we carefully describe pollen

morphology of main sections of *Begonia* in China and discuss their taxonomical significance.

1 Materials and Methods

The species of *Begonia* has strikingly morphological variation and exceedingly endemism in China. Shui et al (2002) recognized 150 species and divided into nine sections. Gu et al (2009) divided 173 species into seven sections. With relation to these sections, the species-rich sections are commonly adopted, e.g. *Coelocentrum*, *Begonia*, *Platycentrum* and *Reichenheimia*. We selected 21 species from these sections based on Gu's (2009) classification (Table 1). The herbarium specimens collected in China and deposited in KUN, IBK, and IBSC.

Mature male flowers were selected from herbarium collections. Pollen grains were mounted on the stubs, coated with gold in a sputter coater, and observed with a Hitachi S-4800 scanning electron microscope at 10 kV. The pollen size was measured on photo of SEM by the software Image J and the mean was calculated using SPSS 22. Terminology for pollen morphology is taken from Van den Berg (1984) and Punt et al (2007).

The stable characters were selected and coded for NMDS (Non-metric multidimensional scaling) analysis. Some characters were excluded due to the directed correlation between each other. For example, the pollen shape was exhibited as pointed and rounded. Casually, when the colpi were elongated and closely approached each other in the poles, the pollen was pointed. The colpi were normal, meanwhile the pollen was rounded. In this case, we selected one of these characters for NMDS analysis. Totally, nine pollen characters were selected and coded as unordered and unweighted (Table 3, 4).

Table 1 Materials examined among Chinese begonias species

| Taxa | World distribution | China distribution | Classification | | Voucher |
|---|--------------------|---------------------------|----------------------|----------------------|--|
| | | | Shui et al, 2002 | Gu et al, 2009 | |
| <i>Begonia. alveolata</i> T. T. Yu | China, Vietnam | Yunnan | <i>Diploclinium</i> | <i>Begonia</i> | Y. M. Shui, B91-527 (KUN) |
| <i>B. baviensis</i> Gagnepain | China, Vietnam | Guangxi, Yunnan | <i>Platycentrum</i> | <i>Platycentrum</i> | S. Q. Chen, 4980 (IBSC) |
| <i>B. edulis</i> H. Léveillé | China, Vietnam | Guangdong, Guangxi | <i>Platycentrum</i> | <i>Platycentrum</i> | Z. T. Li, 601930 (IBK) |
| <i>B. fangii</i> Y. M. Shui & C.I. Peng | China | Guangxi | <i>Coelocentrum</i> | <i>Coelocentrum</i> | Y. K. Li, 00095 (IBK) |
| <i>B. fimbristipula</i> Hance | China | S China | <i>Diploclinium</i> | <i>Begonia</i> | Z. Huang, 31018 (IBK) |
| <i>B. grandis</i> Dryander | China | Widely | <i>Diploclinium</i> | <i>Begonia</i> | J. X. Zhong, 83487 (IBK) |
| <i>B. hemsleyana</i> J. D. Hooker | China, Vietnam | Guangxi, Yunnan | <i>Platycentrum</i> | <i>Platycentrum</i> | Z. T. Li, 602381 (IBK) |
| <i>B. henryi</i> Hemsley | China | S China | <i>Reichenheimia</i> | <i>Reichenheimia</i> | S. W. Deng, 90840 (IBK) |
| <i>B. labordei</i> H. Léveillé | China | Guizhou, Sichuan, Yunnan | <i>Diploclinium</i> | <i>Begonia</i> | s. n., 50763 (IBK) |
| <i>B. lanternaria</i> Irmscher | China, Vietnam | Guangxi | <i>Coelocentrum</i> | <i>Coelocentrum</i> | Nonggang Expedition, 10694 (IBK) |
| <i>B. leprosa</i> Hance | China | Guangdong, Guangxi | <i>Leptosae</i> | <i>Begonia</i> | Z. X. Zhang & S. Z. Wang, 4103 (IBK) |
| <i>B. lipingensis</i> Irmscher | China | Guangxi, Guangdong, Hunan | <i>Platycentrum</i> | <i>Platycentrum</i> | J. F. Qin & Z. T. Li, 70904 (IBK) |
| <i>B. luzhaiensis</i> T. C. Ku | China | Guangxi | <i>Coelocentrum</i> | <i>Coelocentrum</i> | Z. Z. Chen, 53128 (IBK) |
| <i>B. mengtzeana</i> Irmscher | China | Yunnan | <i>Platycentrum</i> | <i>Platycentrum</i> | X. W. Li, 338 (KUN) |
| <i>B. ornithophylla</i> Irmscher | China | Guangxi | <i>Coelocentrum</i> | <i>Coelocentrum</i> | Nonggang Expedition, 12062 (IBK) |
| <i>B. parvula</i> H. Léveillé & Vaniot | China | Guizhou, Yunnan | <i>Reichenheimia</i> | <i>Reichenheimia</i> | M. K. Li, 00011 (IBSC) |
| <i>B. pedatifida</i> H. Léveillé | China | S China | <i>Platycentrum</i> | <i>Platycentrum</i> | Z.Z. Chen, 51847 (IBK) |
| <i>B. polytricha</i> C. Y. Wu | China | Yunnan | <i>Platycentrum</i> | <i>Platycentrum</i> | S. K. Wu, 50736 (KUN) |
| <i>B. setifolia</i> Irmscher | China | Yunnan | <i>Diploclinium</i> | <i>Begonia</i> | X. R. Liang, 68135 (IBK) |
| <i>B. taliensis</i> Gagnepain | China | Yunnan | <i>Diploclinium</i> | <i>Begonia</i> | R. C. Qin, 24680 (KUN) |
| <i>B. wilsonii</i> Gagnepain | China | Chongqing, Sichuan | <i>Reichenheimia</i> | <i>Reichenheimia</i> | X. L. Jiang & X. B. Zhang, 31964 (IBK) |

The NMDS plot was constructed with the PAST 1.81 software package (Hammer et al, 2007). The NMDS plot is accurately reflect the actual distances among the studied samples when stress index is between 0.05 and 0.2 (Clarke & Warwick, 1994; Lu et al, 2010). Correlation similarity index was a lower stress (0.1954) index than any other index available in the program, suggesting that the NMDS plot is reliable and can be used for this analysis.

2 Results and Analysis

2.1 General description of pollen morphology

Pollen grains of all studied taxa are monads, radially symmetrical, isopolar, and 3-zono-colporate. The main variation exhibits in size, shape, colpus, and exine ornamentation (Table 2, Plates 1-3). The further variable characters are the occurrence of margo along the colpus.

2.1.1 Pollen size and shape The mean size shows a

variance of $\pm 1-4 \mu\text{m}$ in polar axis. The biggest variance is found in *B. ornithophylla* with measurement ranged from $18.2 \mu\text{m} \times 8.4 \mu\text{m}$ to $24.5 \mu\text{m} \times 10.9 \mu\text{m}$. Excluding *B. ornithophylla*, all examined pollen rang from $16.4-23.8 \mu\text{m}$ in polar axis and $7.6-10.7 \mu\text{m}$ in equatorial axis. *B. edulis* has the smallest pollen, $16.4 \mu\text{m} \times 8.6 \mu\text{m}$. For NMDS analysis, we employ polar axis to represent pollen size and divided into two categories, as mean values in μm : (1) small ($\leq 20 \mu\text{m}$) and (2) medium ($> 20 \mu\text{m}$).

Pollen shape are mainly exhibited as perprolate (P/E > 2.00) in studied taxa. The prolate grains (P/E: 1.33-2.00) are discovered in *B. edulis*, *B. fangii* and *B. lanternaria*. These grains are nearly perprolate by having the P/E ratio around 1.9-2.0. The outline of the grains being always more or less elliptical, the sides are usually straight in most taxa. Some taxa are characterized by concave sides in sect. *Begonia* [*B. alveolata* (Plate 2:1), *B. fimbristipula* (Plate 3:4), *B. labordei* (Plate 2:3), *B. leprosa* (Plate 3:13),

Table 2 Characters of the pollen morphology of the examined *Begonia*

| Taxa | Polar axis (μm) | Equatorial axis (μm) | P/E ratio | Shape | Side | Outline of poles | Colpus | | Exine ornamentation | | |
|--------------------------|-----------------|----------------------|-----------|------------|----------|------------------|--------------------|--------------------|---------------------|-------------------------------|---------------------------------|
| | | | | | | | Equatorial view | Polar view | Equatorial view | Polar view | Margo |
| <i>Begonia alveolata</i> | 18.7±1.2 | 8.5±0.7 | 2.2 | Perprolate | Concave | Rounded | Linear, sunken | Approached | Coarsely striate | Striato-reticulate, perforate | Absent |
| <i>B. baviensis</i> | 17.8±1.3 | 8.3±0.2 | 2.1 | Perprolate | Straight | Rounded | Boatshaped, retuse | Approached | Finely striate | Striate | Absent |
| <i>B. edulis</i> | 16.4±0.9 | 8.6±0.3 | 1.9 | Prolate | Convex | Rounded | Boatshaped, retuse | Approached | Coarsely striate | Striato-reticulate, perforate | Thicken and psilate |
| <i>B. fangii</i> | 18.9±0.1 | 9.8±0.0 | 1.9 | Prolate | Convex | Pointed | Boatshaped, retuse | Closely approached | Coarsely striate | Striato-reticulate, perforate | Coarsely regulate, perforate |
| <i>B. fimbristipula</i> | 20.8±0.3 | 8.1±0.1 | 2.6 | Perprolate | Concave | Pointed | Boatshaped, sunken | Closely approached | Coarsely striate | Striato-reticulate, perforate | Exquisitely regulate, perforate |
| <i>B. grandis</i> | 23.4±1.7 | 10.1±0.6 | 2.3 | Perprolate | Straight | Rounded | Boatshaped, retuse | Approached | Finely striate | Striato-reticulate, perforate | Exquisitely regulate, perforate |
| <i>B. hemsleyana</i> | 18.6±0.8 | 8.2±0.4 | 2.3 | Perprolate | Straight | Rounded | Boatshaped, sunken | Approached | Coarsely striate | Striate, perforate | Absent |
| <i>B. henryi</i> | 25.1±0.1 | 9.9±0.3 | 2.5 | Perprolate | Straight | Rounded | Linear, sunken | Closely approached | Finely striate | Striato-reticulate, perforate | Exquisitely regulate, perforate |
| <i>B. labordei</i> | 22.2±0.8 | 8.8±0.1 | 2.5 | Perprolate | Concave | Rounded | Linear, sunken | Approached | Coarsely striate | Striato-reticulate, perforate | Regulate, perforate |
| <i>B. lanternaria</i> | 21.2±2.6 | 10.7±0.4 | 2.0 | Prolate | Concave | Pointed | Boatshaped, retuse | Closely approached | Finely striate | Striato-reticulate, perforate | Thicken and psilate, perforate |
| <i>B. leprosa</i> | 23.7±0.6 | 10.3±0.4 | 2.3 | Perprolate | Concave | Rounded | Boatshaped, retuse | Approached | Coarsely striate | Striato-reticulate, perforate | Psilate, perforate |
| <i>B. lipingensis</i> | 18.3±1.8 | 7.6±0.2 | 2.4 | Perprolate | Straight | Rounded | Boatshaped, retuse | Approached | Finely striate | Striate, perforate | Thicken and psilate |
| <i>B. luzhaiensis</i> | 22.0±0.1 | 9.4±0.0 | 2.3 | Perprolate | Concave | Rounded | Linear, sunken | Approached | Coarsely striate | Striato-reticulate, perforate | Regulate, perforate |
| <i>B. mengtzeana</i> | 21.6±0.6 | 9.3±0.6 | 2.3 | Perprolate | Straight | Rounded | Linear, sunken | Approached | Coarsely striate | Striate, perforate | Regulate, perforate |
| <i>B. ornithophylla</i> | 21.4±4.4 | 9.7±1.8 | 2.2 | Perprolate | Straight | Pointed | Boatshaped, sunken | Closely approached | Coarsely striate | Striato-reticulate, perforate | Coarsely regulate, perforate |
| <i>B. parvula</i> | 23.8±0.4 | 9.0±0.4 | 2.6 | Perprolate | Straight | Pointed | Linear, sunken | Closely approached | Coarsely striate | Striato-reticulate, perforate | Exquisitely regulate, perforate |
| <i>B. pedatifida</i> | 21.1±0.3 | 8.3±0.1 | 2.5 | Perprolate | Straight | Pointed | Boatshaped, retuse | Closely approached | Finely striate | Striate, perforate | Thicken and psilate |
| <i>B. polytricha</i> | 18.8±0.5 | 8.8±0.3 | 2.1 | Perprolate | Convex | Rounded | Boatshaped, retuse | Approached | Coarsely striate | Striato-reticulate, perforate | Regulate, perforate |
| <i>B. setifolia</i> | 22.4±0.0 | 8.5±0.3 | 2.6 | Perprolate | Concave | Rounded | Boatshaped, sunken | Approached | Finely striate | Striato-reticulate, perforate | Exquisitely regulate, perforate |
| <i>B. taliensis</i> | 19.9±2.4 | 9.0±0.3 | 2.2 | Perprolate | Concave | Pointed | Boatshaped, retuse | Closely approached | Finely striate | Striate, perforate | Regulate, perforate |
| <i>B. wilsonii</i> | 23.5±1.2 | 9.6±0.2 | 2.4 | Perprolate | Straight | Rounded | Boatshaped, retuse | Approach | Coarsely striate | Striato-reticulate, perforate | Coarsely regulate |

Table 3 Pollen characters and character states of *Begonia*

| No. | Character | Character states |
|-----|--|--|
| 1 | Size | 0: Small ($\leq 20 \mu\text{m}$); 1: Medium ($> 20 \mu\text{m}$) |
| 2 | Side | 0: Concave; 1: Straight; 2: Convex |
| 3 | Outline of poles | 0: Rounded; 1: Pointed |
| 4 | Shape of colpus | 0: Linear; 1: Boatshaped |
| 5 | Colpus membrane | 0: Sunken; 1: Retuse |
| 6 | Equatorial view of exine ornamentation | 0: Coarsely striate; 1: Finely striate |
| 7 | Polar view of exine ornamentation | 0: Striato-reticulate; 1: Striate |
| 8 | Margo | 0: Absent; 1: Present |
| 9 | Shape of margo | 0: Psilate; 1: Coarsely regulate; 2: Regulate; 3: Exquisitely regulate |

Table 4 Matrix of coded pollen character states for studied taxa

| No. | Taxa | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-----|--------------------------|---|---|---|---|---|---|---|---|---|
| 1 | <i>Begonia alveolata</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| 2 | <i>B. baviensis</i> | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | ? |
| 3 | <i>B. edulis</i> | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 4 | <i>B. fangii</i> | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| 5 | <i>B. fimbristipula</i> | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| 6 | <i>B. grandis</i> | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 |
| 7 | <i>B. hemsleyana</i> | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | ? |
| 8 | <i>B. henryi</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 3 |
| 9 | <i>B. labordei</i> | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| 10 | <i>B. lanternaria</i> | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| 11 | <i>B. leprosa</i> | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 12 | <i>B. lipingensis</i> | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| 13 | <i>B. luzhaiensis</i> | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| 14 | <i>B. mengtzeana</i> | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| 15 | <i>B. ornithophylla</i> | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| 16 | <i>B. parvula</i> | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 3 |
| 17 | <i>B. pedatifida</i> | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 18 | <i>B. polytricha</i> | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 1 | 2 |
| 19 | <i>B. setifolia</i> | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 3 |
| 20 | <i>B. taliensis</i> | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| 21 | <i>B. wilsonii</i> | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |

Note: Deleted character is coded as ?.

B. setifolia (Plate 1:13), and *B. taliensis* (Plate 1:16)] and in sect. *Coelocentrum* [*B. lanternaria* (Plate 1:2) and *B. luzhaiensis* (Plate 3:14)], in which the pollen has obvious

constriction around the ectoaperture. Some pollen is protruded around the ectoaperture and formed the convex side, e.g. sect. *Platycentrum* [*B. edulis* (Plate 3:2) and *B. polytricha* (Plate 3:16)] and *Coelocentrum* [*B. fangii* (Plate 3:3)].

2.1.2 Apertures The apertures are 3-zono-colporate formed by ectoaperture and endoaperture in studied taxa. The ectoaperture has three colpi and the endoaperture is situated at the equator in the colpus. The colpus is documented by nanograndulate, especially around the endoaperture. In most taxa, the colpus is broader at the equator than the polar, named boatshaped. The colpus is inflexed and formed narrow linear in *B. alveolata* (Plate 2:1, 5, 9), *B. henryi*, *B. luzhaiensis* (Plate 3: 14, 18, 22), and *B. mengtzeana* (Plate 2: 4, 8, 12). Sometimes, the margin of colpus is deeply inflexed lead to the membrane sunken. In studied taxa, the colpi are approached each other at the polar, while in some taxa the colpi are extremely elongated and approached each other closely at the poles, e.g. *B. fangii* (Plate 3:7), *B. fimbristipula* (Plate 3:8), *B. henryi*, *B. lanternaria* (Plate 1:6), *B. ornithophylla*, *B. parvula* (Plate 3:19), *B. pedatifida* (Plate 1:8), and *B. taliensis* (Plate 1:17).

2.1.3 Exine ornamentation The exine is shown a regular pattern of approximately parallel muri, defined as striate. Depending on the width of lirae and striae, the striate pattern can be designated as finely or coarsely striate. The finely striate owned more than ten straight and long lirae on each mesocolpia (Plate 1). The exine ornamentation is defined as coarsely striate by having short and less than ten lirae on each mesocolpia (Plates 2, 3).

The striate pattern is sometimes replaced by irregular ornamentation, especially towards the poles. Often perforate are intensively emerged on the poles, resulting in a fuzzy striato-reticulate appearance in most taxa, while the striate is exhibited in *B. baviensis* (Plate 1:5), *B. hemsleyana* (Plate 2:6), *B. lipingensis* (Plate 1:7), *B. mengtzeana* (Plate 2:8), *B. pedatifida* (Plate 1:8), and *B. taliensis* (Plate 1:17).

A striking feature is the presence of a margo: a zone along the ectocolpus showing a deviating

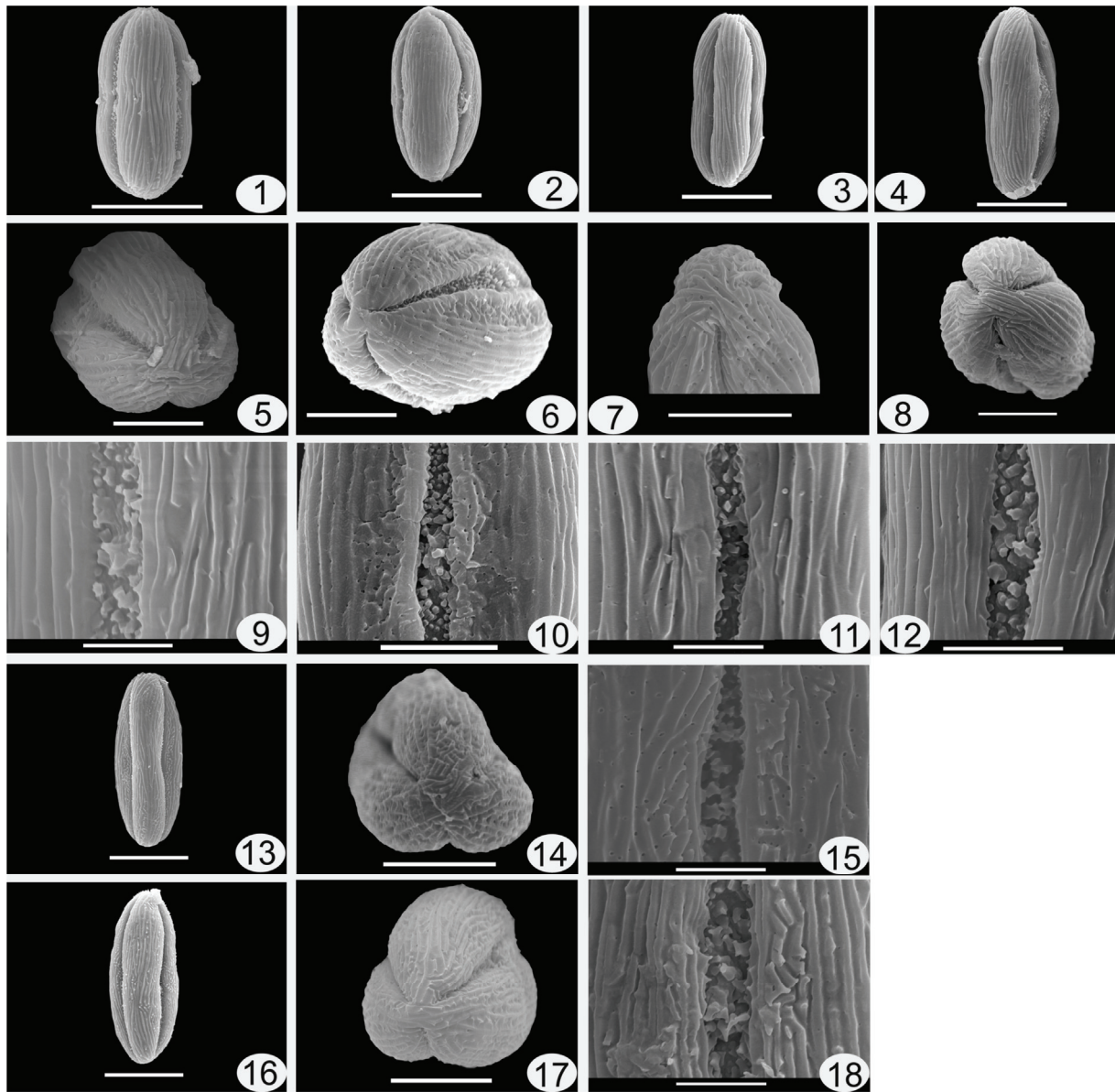


Plate 1 Pollen with finely striate on the surface 1, 5, 9. *B. baviensis*; 2, 6, 10. *B. lanternaria*; 3, 7, 11. *B. lipingensis*; 4, 8, 12. *B. pedatifida*; 13-15. *B. setifolia*; 16-18. *B. taliensis*. Scale bars: 10 μm in equatorial view and polar view, 2 μm in higher magnification of margo. The same below.

ornamentation from the remainder of the mesocolpium. The margo can be divided into four different types based on different ornamentation. (i) The margo is psilate and found in *B. edulis* (Plate 3:10), *B. lanternaria* (Plate 1:10), *B. leprosa* (Plate 3:21), *B. lipingensis* (Plate 1:11), and *B. pedatifida* (Plate 1:12). (ii) The margo is coarsely regulate and discovered in *B. fangii* (Plate 3:11), *B. ornithophylla*, and *B. wilsonii* (Plate 3:9). (iii) The margo is regulate, viz. *B. labordei* (Plate 2:11), *B. luzhaiensis* (Plate 3:22), *B. mengtzeana* (Plate 2:12), *B. polytricha* (Plate

3:24), and *B. taliensis* (Plate 1:18). (iv) The margo is exquisitely regulate, e.g. *B. fimbriatipula* (Plate 3:12), *B. grandis*, *B. henryi*, and *B. parvula* (Plate 3:23).

2.2 NMDS analysis

The studied species formed a domain near x axis in quadrant I and IV (Plate 4). In the domain, all studied sections are included; three species from section *Begonia* (*B. fimbriatipula*, *B. labordei*, and *B. setifolia*), one species from section *Platycentrum* (*B. mengtzeana*), one species from section *Coelocentrum* (*B. luzhaiensis*), and two

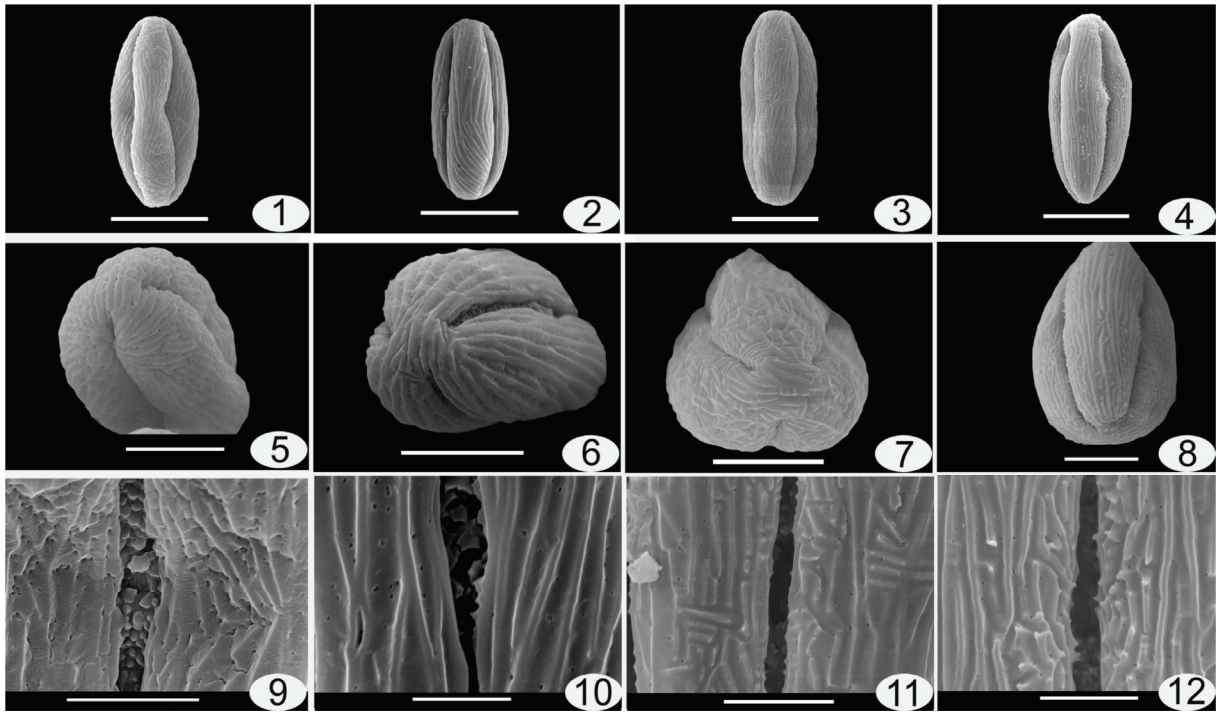


Plate 2 Pollen with coarsely striate on the surface 1, 5, 9. *B. alveolata*; 2, 6, 10. *B. hemsleyana*;
3, 7, 11. *B. labordei*; 4, 8, 12. *B. mengtzeana*.

species from section *Reichenheimia* (*B. henryi* and *B. parvula*). The remainder species are scattered in all quadrants. In section *Begonia*, *B. alveolata* and *B. leprosa* are scattered in quadrant III, while *B. taliensis* is located in quadrant I. The rest species of section *Platycentrum* are mainly located in quadrant II except for *B. pedatifida* in quadrant III and *B. polytricha* in quadrant I. Within studied species of section *Coelocentrum*, three species are dispersed out of the domain and located in quadrant II (*B. fangii*), III (*B. lanternaria*) and IV (*B. ornithophylla*). *B. wilsonii* is separated from the domain of section *Reichenheimia* and located near y axis in quadrant III.

All studied taxa are divided into two groups according to the character of margo. Group A is included the taxa with psilate margo [*B. edulis* (Plate 3:10), *B. lanternaria* (Plate 1:10), *B. leprosa* (Plate 3:21), *B. lipingensis* (Plate 1:11), and *B. pedatifida* (Plate 1:12)] or without margo (*B. alveolata* (Plate 2:9), *B. baviensis* (Plate 1:9), and *B. hemsleyana* (Plate 2:10)]. Group B is exhibited with regulate margo. Within these taxa, regulate and exquisitely regulate margo are gathered together in the domain. *B. polytricha* (Plate 3:24) and *B. taliensis* (Plate 1:18) is

located around the domain by having small pollen and regulate margo. While, the coarsely regulate margo is scattered between Group A and the domain in Group B, viz. *B. fangii* (Plate 3:11), *B. ornithophylla*, and *B. wilsonii* (Plate 3:9).

3 Discussion

Morphological and anatomical fruit and ovary characters have traditionally played an essential role as diagnostic characters and for infrageneric delimitation in *Begonia* (Irmischer, 1925; Warburg, 1894; Doorenbos et al, 1998). However, these characters were identified as highly homoplasious and had been overestimated in the past (Matolweni et al, 2000; Forrest et al, 2003; Forrest et al, 2005; Neale et al, 2006; Thomas et al, 2011). Pollen morphology should be to unearth the taxonomy of *Begonia* (Van den Berg, 1984).

The palynological characters of 21 studied taxa have common synapomorphy in *Begonia*, such as monads, radially symmetrical, isopolar, and 3-zono-colporate (Van den Berg, 1984; Rajbhandary et al, 2012). The variability

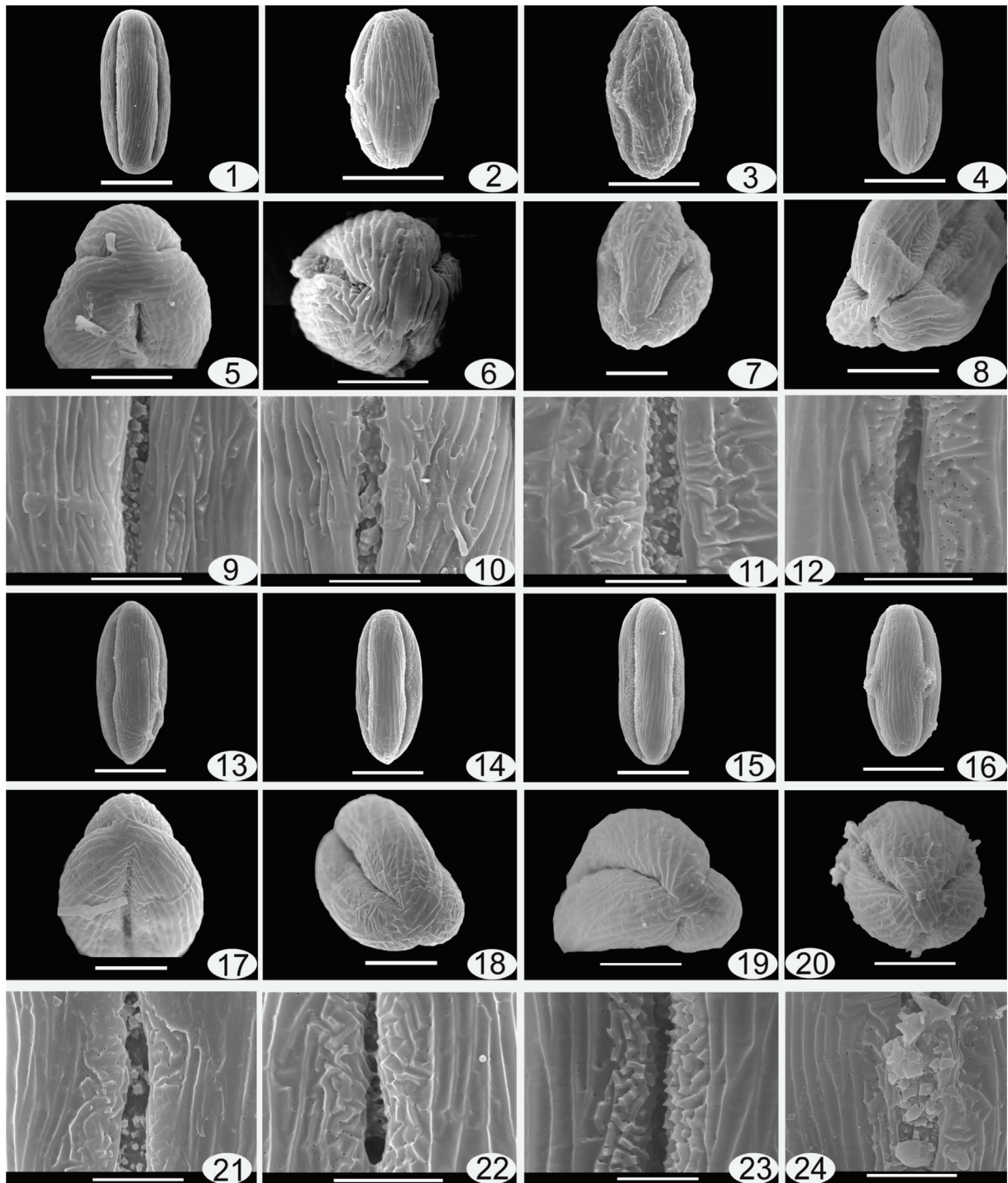


Plate 3 Pollen with coarsely striate on the surface 1, 5, 9. *B. Wilsonii*; 2, 6, 10. *B. edulis*; 3, 7, 11. *B. fangii*; 4, 8, 12: *B. fimbristipula*; 13, 17, 21. *B. leprosa*; 14, 18, 22. *B. luzhaiensis*; 15, 19, 23. *B. parvula*; 16, 20, 24. *B. polytricha*.

characters are displayed on pollen size, apertures, and exine ornamentation, especially the appearance of margo along colpus. The pollen data lend some support to the idea that the studied sections are not monophyletic and need revised (Thomas et al, 2011; Chung et al, 2014). Here, we have

further discussed the taxonomical significance of pollen characters within the studied taxa of genus *Begonia*.

Within studied species, the pollen varied from 16.4–23.8 μm with a variance of $\pm 1-4 \mu\text{m}$ in polar axis. For easy to demarcation, the pollen is divided into two

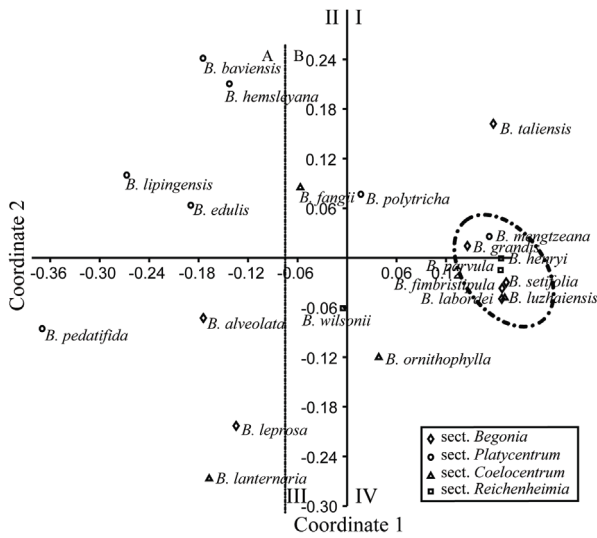


Plate 4 Non-metric multidimensional scaling (NMDS) plot of nine pollen characters sampled for 21 species of *Begonia* in China

types based on the mean of polar axis: $\leq 20 \mu\text{m}$ and $> 20 \mu\text{m}$. In fact, the measurements span the range in some species, e.g. *B. lanternaria*, *B. ornithophylla*, and *B. taliensis*. In relation to the different treatment (Reitsma, 1969) and aberrantly 2n pollen (Dewitte et al, 2009), it should be very careful to take pollen size as recognized characters in the genus.

The shape is highly similar to the studied taxa, while the sides are variable at species level. Van den Berg (1984) found that convex and straight sides imply rounded poles, while concave grains tend to possess or less pointed poles.

The different situations were observed in this study. The convex and straight grains exhibited pointed poles in *B. fangii* (Plate 3:3, 7), *B. ornithophylla*, *B. parvula* (Plate 3:15, 19) and *B. pedatifida* (Plate 1:4, 8). The concave grains exhibited rounded poles in *B. alveolata* (Plate 2:1, 5), *B. labordei* (Plate 2:3, 7), *B. leprosa* (Plate 3:13, 17) and *B. setifolia* (Plate 1:13-14). More species are needed to clarify the correlation between the sides and the outline of the poles.

The feature of aperture is stable as 3-zono-colporate in *Begonia*. The main variation is the shape and length of colpus. In most studied taxa, the colpi are elongated and approached to each other closely at the poles, especially within the boatshaped colpus. In relatively rare, the colpi anastomose at the poles as syncolpate grain (Van den Berg,

1984). In this study, the syncolpate grains is not discovered but the colpi are elongated and closely approach each other at the poles and the apocolpium resemble as a point in some taxa, e.g. *B. lipingensis* (Plate 1:7), *B. taliensis* (Plate 1:17), *B. fimbriatipula* (Plate 3:8). The feature of apocolpium can be used as discrimination at the species level combined with other characters and has limited classification significance at the section level.

The striate pollen is the common sculpture on the surface of the grains within *Begonia*. Two main types can be identified as finely and coarsely striate based on the width of the lirae and striae (Van den Berg, 1984; Rajbhandary et al, 2012). Perforate is minute holes situated in the tectum and common at the polar and in the margo. Especially, in coarsely striate grains, the sculpture is ornamented as striato-reticulate due to the sink around single hole at the poles. Most finely striate pollens are kept as striate at the poles in the presence of perforate, e.g. *B. baviensis* (Plate 1:5), *B. lipingensis* (Plate 1:7), *B. pedatifida* (Plate 1:8), and *B. taliensis* (Plate 1:17). The sculpture is very useful to identify different species, but it need further discussion about taxonomical significance at the section level under the context of recently phylogeny of the genus.

It is worthy to notice that margo, a zone along ectocolpus showing differentiated from the remainder of the sexine, the extra characters on the sculpture. Margo was discovered in *Begonia* from Africa and Nepal (Van den Berg, 1984, Rajbhandary et al, 2012). Rajbhandary et al (2012) stressed that margo could be used as a distinguished character for the separates of section *Platycentrum* from all other sections of Nepalese *Begonia*. In relation to China, the margo is found in all sections of *Begonia*. Furthermore, the feature of margo exhibited a clear transition, from without margo and psilate margo, along coarsely regulate margo to exquisitely regulate, based on NDMS analysis within this study. It is important to pay attention on the feature of margo within *Begonia* in the future studies.

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