Postprint of a Study on Pollen and Leaf Epidermis Micromorphology of 10 Species in Camellia Section Tuberculata

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Abstract

The leaf epidermal micromorphology and pollen morphology of 10 species from the Tuberculata section were observed using light microscopy and scanning electron microscopy, and cluster analyses were performed separately based on pollen characteristics and leaf epidermal characteristics, aiming to provide necessary evidence for the systematic evolution and taxonomic identification of this group, with the pollen morphology of all 10 species being reported for the first time. The results showed that: (1) The pollen morphology and size of the 10 species showed little variation, being subspheroidal, prolate, or oblate; the polar view was trilete subcircular or trilete subtriangular; the equatorial view was elliptical, with a P/E ratio of 0.85~1.16, and the aperture type was tricolpate; the exine ornamentation characteristics showed relatively significant differences, being granulate, rugulate, or rugulate-striate to granulate, which have important taxonomic value. (2) Through principal component cluster analysis of the measured indices, four taxonomic groups were obtained when the Euclidean distance was 4.5, with some classification results being consistent with macromorphological classification. (3) The leaf epidermal cells of the 10 species were irregular and polygonal, with significant interspecific morphological differences; both the adaxial and abaxial epidermis of Camellia zengii and Camellia libohongensis had glands; stomatal apparatuses were distributed only on the abaxial epidermis, all being cyclocytic; anticlinal wall patterns presented as shallowly undulate, undulate, straight-curved, and deeply undulate. In addition, cell size and stomatal apparatus characteristics (size, density) of leaf epidermal micromorphology showed significant differences among species. This study demonstrates that pollen morphology and leaf epidermal micromorphology are diverse in the Tuberculata section and can be used as a basis for distinguishing some closely related taxa.

Full Text

Pollen morphology and leaf epidermal micromorphology of 10 species (Camellia, sect. Tuberculata)

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Abstract

Pollen morphology and leaf epidermal micromorphology of 10 species in Camellia sect. Tuberculata were observed using light microscopy and scanning electron microscopy. Cluster analysis was performed separately based on pollen characteristics and leaf epidermal characteristics to provide necessary evidence for the systematic evolution and taxonomic identification of plants in this section. The pollen morphology of all 10 species is reported for the first time. The results showed that: (1) The pollen morphology and size of the 10 species showed little variation, being subspheroidal, prolate or oblate; polar view was trilete subrounded or trilete subtriangular; equatorial view was elliptical with a P/E ratio of 0.85~1.16; aperture type was tricolporate; exine sculpture showed significant differences, being granular, foveolate-rugulate or rugulate-beaded, which has important taxonomic value. (2) Through principal component cluster analysis of measured indices, when the Euclidean distance was 4.5, four taxonomic groups were obtained, with some classification results consistent with macro-morphological classification. (3) Leaf epidermal cells of the 10 species were irregular or polygonal, with significant interspecific morphological differences; both upper and lower epidermis of Camellia zengii and C. rubimuricata had glands; stomatal apparatus were distributed only on the lower epidermis, all being cyclocytic; anticlinal wall patterns showed sinuolate, sinuous, repand and sinuate forms. In addition, cell size and stomatal apparatus characteristics (size, density) of leaf epidermal micromorphology showed significant differences among species. This study demonstrates that pollen morphology and leaf epidermal micromorphology are diverse in sect. Tuberculata and can serve as a basis for distinguishing some closely related taxa.

Key words: Camellia, sect. Tuberculata, pollen morphology, leaf epidermal micromorphology, phylogenetic significance

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Camellia sect. Tuberculata Chang is distinguished from other sections by the unique morphological characteristic of "tuberculate protuberances on the ovary and fruit surface" and is a group distributed from Guizhou as the center to surrounding provinces (Zhang and Ren, 1991; Ming et al., 1993; Zhang and Ren, 1998). In 1939, Professor Qian Chongyu discovered a peculiar species with tuberculate protuberances on the ovary and pericarp in Sichuan Province and named it Camellia tuberculata S. S. Chien; Sealy (1958) assigned it to sect.

Pseudocamellia Sealy Rev.

In 1981, Zhang Hongda established sect. Tuberculata based on the unique morphology of "tuberculate wrinkles on the capsule pericarp," which initially contained only 6 species (Zhang, 1981). Over the next decade, 11 additional species were reported (Zhang and Ren, 1991). Ming Tianlu (1993) reduced 17 species to 6 species and 6 varieties through specimen observation. In 1996, Zhang Hongda reconfirmed 15 independent species based on macro-morphological studies (Zhang and Ren, 1996). However, since most plants of sect. Tuberculata grow in karst mountainous areas as narrow endemics, field investigation is difficult. Previous taxonomic treatments of this group were based on macroscopic characters from herbarium specimens, lacking multidisciplinary taxonomic evidence, and some fundamental taxonomic issues remain unresolved (e.g., unclear interspecific boundaries, questionable names, and unclear phylogenetic relationships). Therefore, supplementing multidisciplinary data for this group can help resolve these taxonomic problems. Leaf epidermal micromorphology serves as one line of evidence in plant taxonomic studies (Zhang and Zhuang, 2004; Ao et al., 2022) and has been widely applied in taxonomic research of Camellia. For example, Li Fengying (2013) conducted leaf epidermal studies on 25 taxa of ser. Chrysanthae Chang, with results indicating that this character has certain taxonomic value in resolving interspecific classification problems. Lu et al. (2008) performed leaf anatomical, micromorphological, and FTIR studies on 18 species of sect. Tuberculata and suggested restoring 11 species to species-level status. Although this study provided detailed research on the group, the collected plants were from a common garden plantation, lacking the diversity and representativeness of wild populations. Therefore, it is necessary to continue leaf epidermal studies on wild populations. Pollen morphology is also an important line of evidence in taxonomic research (Chen et al., 2022; Liang et al., 2022). Li Guangqing et al. (2005) studied pollen morphology of 6 species in sect. Theopsis Coh. St. and found significant interspecific differences in pollen morphology that could serve as evidence for species delimitation.

This study reports pollen morphological data for 10 species of sect. Tuberculata for the first time and investigates leaf epidermal micromorphology of these 10 species to supplement micromorphological data for taxonomic research on this group and provide reference materials for further clarifying interspecific delimitation and systematic studies of sect. Tuberculata.

1.1 Materials

Plant materials of the 10 species in this study were all collected from wild-growing individuals. Voucher specimens are deposited in the Herbarium of the College of Forestry, Guizhou University (GZAC). Collection information and voucher details are provided in Table 1 .

Table 1 Source of material

Taxon	Locality	Voucher specimen

Camellia anlongensis	Lekang Village, Wangmo County, Guizhou	GZAC, LZ20210413
Camellia zengii	Qiutuan Village, Liping County, Guizhou	GZAC, LZ20210829
Camellia rubituberculata	Xinzhai Village, Xingren City, Guizhou	GZAC, LZ20210411
Camellia acutiperulata	Jinzhongshan Town, Longlin County, Guangxi	GZAC, LZ20221103
Camellia leyeensis	Yachang Village, Leye County, Guangxi	GZAC, LZ20210413
Camellia lipingensis	Cengba Village, Liping County, Guizhou	GZAC, LZ20210830
Camellia rubimuricata	Mogan Village, Libo County, Guizhou	GZAC, LZ20211213
Camellia pyxidiacea	Sanjiangkou Town, Xingyi City, Guizhou	GZAC, LZ20211204
Camellia neriifolia	Yuanhou Town, Chishui City, Guizhou	GZAC, LZ20220821
Camellia rhytidophylla	Fengsan Town, Kaiyang County, Guizhou	GZAC, LZ20220802

1.2 Scanning Electron Microscopy of Pollen

During field collection, fresh anthers were preserved in glutaraldehyde and brought to the laboratory for SEM analysis. The specific procedures were as follows: pollen was naturally dried at room temperature for 36 h; for observation, pollen was evenly sprinkled onto sample stages coated with conductive adhesive and sputter-coated with gold film (thickness 8 nm) using an ion sputter coater, then transferred to a scanning electron microscope for scanning. Intact pollen grains were randomly selected for observation and photography. Image J software was used to measure the polar axis (P) and equatorial axis (E) length, among other indices. Data were statistically analyzed using Excel 2019 to calculate the P/E ratio. Terminology and classification standards for pollen morphology followed Wei (1992) and Wang et al. (1995). Based on the P/E ratio, pollen shape was classified into five categories: perprolate (P/E$ 2), $prolate (1.5 < P/E < 2), subspheroidal (1 < P/E\ 1.5), oblate (0.5 < P/E\ 1), and peroblate (P/E$ 0.5).

1.3 Leaf Epidermal Preparation Method

Intact leaves were taken from dried specimens (voucher specimens see Table 1) and cut into approximately 1 cm × 1 cm squares along the leaf veins. These were placed in 75% NaClO solution for bleaching at room temperature for 6–10 h (duration varied depending on leaf texture). After mesophyll tissue and epidermal cells separated, the upper and lower epidermis were peeled off on glass slides with forceps to prepare temporary slides, which were observed and photographed under a light microscope (Olympus BX41). Morphological terminology followed the nomenclature standards of Wang et al. (1991).

1.4 Data Analysis

Pollen and leaf epidermal micromorphological data were measured and statistically analyzed using Image J software and Excel 2019. SPSS 22.0 software was used for cluster analysis. The calculation formulas for stomatal index (I) and stomatal density (D) of leaf epidermis were as follows:

Stomatal index (I) calculation formula:

$$I = S/(S + E) \times 100\%$$

where: I is stomatal index; S is the number of stomata in a fixed area; E is the number of epidermal cells in the same area.

Stomatal density (D) calculation formula:

$$D = S/N$$

where: D is stomatal density; S is the number of stomata in a fixed area; N is the size of the fixed area.

2.1 Pollen Grain Size and Shape Characteristics

All 10 species in Camellia sect. Tuberculata have single pollen grains with tricolporate apertures and radial symmetry (Fig. 1 [Figure 1: see original paper] and Fig. 2 [Figure 2: see original paper]). Polar axis length (P) ranged from 30.86 to 39.37 m; equatorial axis length (E) ranged from 32.10 to 37.40 m. The P/E ratio was 0.85–1.16. According to pollen shape classification standards, except for C. rhytidophylla being oblate (Fig. 2: J2) and C. acutiperulata being prolate (Fig. 2: I2), pollen of the remaining 8 species were subspheroidal (Fig. 1: A2–H2 and Table 2). The polar view of C. lipingensis and C. rubituberculata was trilete regular triangular, while the other 8 species had trilete subrounded polar views. Pollen equatorial view showed two types (elliptical and fusiform), with C. lipingensis and C. neriifolia being fusiform (Fig. 1: E2 and Fig. 2: H2) and the remaining 8 species being elliptical. Exine sculpture characteristics of the 10 species mainly showed granular, foveolate-rugulate, or rugulate-beaded patterns. Among them, C. anlongensis, C. leyeensis, and C. acutiperulata had rugulate-beaded exine sculpture (Fig. 1: A4, D4 and Fig. 2: I4); four species including C. zengii, C. lipingensis, C. neriifolia, and C. rhytidophylla had granular exine sculpture (Fig. 1: B4 and Fig. 2: E4, H4, J4); the remaining three species—C. rubituberculata, C. rubimuricata, and C. pyxidiacea—had foveolate-rugulate exine sculpture (Fig. 1: C4 and Fig. 2: F4, G4). However, interspecific differences in exine sculpture among the 10 species were significant, with varying mesh sizes and different morphologies, indicating that pollen exine sculpture can serve as one of the main interspecific taxonomic characters for sect. Tuberculata and has important taxonomic value.

A. C. anlongensis; B. C. zengii; C. C. rubituberculata; D. C. acutiperulata; E. C. leyeensis. 1. Outline in polar view; 2. Outline in equatorial view; 3. Aperture; 4. Exine sculpture.

Fig. 1 Pollen morphology of five species of sect. Tuberculata

F. C. lipingensis; G. C. rubimuricata; H. C. pyxidiacea; I. C. neriifolia; J. C. rhytidophylla. 1. Outline in polar view; 2. Outline in equatorial view; 3. Aperture; 4. Exine sculpture.

Fig. 2 Pollen morphology of five species of sect. Tuberculata

Table 2 Pollen features of ten species of sect. Tuberculata

Taxon	Polar axis length (P) (m)	Equatorial axis length (E) (m)	P/E ratio	Pollen shape				
C. anlongensis	-	-	-	Subspheroidal	Trilete subrounded	Elliptical	Rugulate-beaded	
C. zengii	-	-	-	Subspheroidal	Trilete subrounded	Elliptical	Granular	Tricolpo
C. rubituberculata	-	-	-	Subspheroidal	Trilete regular triangular	Elliptical	Fove	
C. acutiperulata	-	-	-	Prolate	Trilete subrounded	Elliptical	Rugulate-beaded	Tric
C. leyeensis	-	-	-	Subspheroidal	Trilete subrounded	Elliptical	Rugulate-beaded	
C. lipingensis	-	-	-	Subspheroidal	Trilete regular triangular	Fusiform	Granular	
C. rubimuricata	-	-	-	Subspheroidal	Trilete subrounded	Elliptical	Foveolate-rugula	
C. pyxidiacea	-	-	-	Subspheroidal	Trilete subrounded	Elliptical	Foveolate-rugula	
C. neriifolia	-	-	-	Subspheroidal	Trilete subrounded	Fusiform	Granular	Tric
C. rhytidophylla	-	-	-	Oblate	Trilete subrounded	Elliptical	Granular	Tricolpo

2.2 Palynological Cluster Analysis

R-type cluster analysis was performed on the 10 species based on 14 structural indices including exine sculpture, equatorial view, pollen shape, and size. As shown in Fig. 3 [Figure 3: see original paper], at a Euclidean distance of 4.5, the 10 species were divided into 6 groups. For example, C. lipingensis and C. neriifolia were most similar, as both had granular exine sculpture and fusiform equatorial views. These two species, together with C. leyeensis and C. zengii, formed one branch. Camellia rubituberculata and C. acutiperulata formed another group. At a Euclidean distance of 15, the 10 species of sect. Tuberculata were divided into 2 groups: Group I consisted of 8 species including C. lipingensis, C. neriifolia, C. leyeensis, C. zengii, C. rubituberculata, C. acutiperulata, C. rubimuricata, and C. rhytidophylla, characterized by shorter equatorial axes and longer apertures; Group II consisted of C. anlongensis and C. pyxidiacea, which showed minor differences in pollen morphology.

Fig. 3 Results of palynology clustering in ten species of sect. Tuberculata

2.3.1 Epidermal Cell Morphology

Based on observations, leaf epidermal cell morphology of the 10 species showed diversification and could be roughly classified into 5 categories (Table 3): (1) Irregular sinuous type: epidermal cells are irregular in shape, unequal in size, with sinuous anticlinal walls, found in the upper epidermis of C. neriifolia, C. rhytidophylla, and C. rubimuricata (Fig. 4 [Figure 4: see original paper]: I1 and J1) and the lower epidermis of C. zengii, C. rubituberculata, C. rubimuricata, and C. neriifolia (Fig. 4: A2, C2, G2, and I2). (2) Irregular sinuolate type: epidermal cells are irregular in shape, unequal in size, with sinuolate anticlinal walls, found in the upper epidermis of C. rubimuricata (Fig. 4: G1) and the lower epidermis of C. anlongensis, C. acutiperulata, and C. leyeensis (Fig. 4: A2, D2, and E2). (3) Irregular sinuate type: epidermal cells are irregular in shape, unequal in size, with deeply sinuate anticlinal walls that are significantly thickened, found in the upper epidermis of C. rubituberculata and both upper and lower epidermis of C. pyxidiacea (Fig. 4: C1, I1, and I2) and the lower epidermis of C. rhytidophylla (Fig. 4: J2). (4) Irregular repand type: epidermal cells are irregular but with repand anticlinal walls that are relatively regular, found only in the upper epidermis of C. anlongensis, C. leyeensis, and C. lipingensis (Fig. 4: A1, E1, and F1). (5) Polygonal type (anticlinal wall patterns include repand, sinuolate, and sinuate types, corresponding to the upper epidermis of C. lipingensis (Fig. 4: F1), upper epidermis of C. rubimuricata (Fig. 4: G1), and lower epidermis of C. pyxidiacea (Fig. 4: H2), respectively).

2.3.2 Cell Size and Glands

Leaf epidermal cell area was measured using Image J software, with 30 cells measured per species and averages calculated. Among upper epidermal cells, the smallest cell cross-sectional area was C. rubituberculata (490.90 m^2) and the largest was C. leyeensis (1036.77 m^2). Camellia anlongensis (827.95 m^2) and C. zengii (844.11 m^2) were most similar in cell size. The maximum cell length/width ratio (L/W) was 1.38 (C. anlongensis, C. zengii, and C. leyeensis) and the minimum was 1.11 (C. lipingensis), see Table 3 for details. Analysis of lower epidermal cells showed the smallest cell cross-sectional area was C. rubituberculata (660.43 m^2) and the largest was C. rhytidophylla (1396.60 m^2). Camellia lipingensis (900.41 m^2) and C. neriifolia (897.10 m^2) had the most similar cell sizes. The maximum cell length/width ratio (L/W) was 1.79 (C. anlongensis) and the minimum was 1.19 (C. rubimuricata). The lower epidermal cell length/width ratios (L/W) of C. leyeensis and C. neriifolia were both 1.26, see Table 4 for details. The experiment found that three species (C. zengii, C. rubimuricata, and C. rhytidophylla) had different numbers of glands in their epidermis. Camellia zengii had numerous oil glands in the lower epidermis and a few glands in the upper epidermis; C. rhytidophylla had a few glands in the upper epidermis; and C. rubimuricata had numerous glands in both upper and lower epidermis.

A. C. anlongensis; B. C. zengii; C. C. rubituberculata; D. C. acutiperulata; E. C. leyeensis; F. C. lipingensis; G. C. rubimuricata; H. C. pyxidiacea; I. C. neriifolia; J. C. rhytidophylla. 1. The adaxial leaf epidermis; 2. The abaxial leaf epidermis. (Scale bar: 20 m).

Fig. 4 Microscopic leaf epidermal features of ten species of sect. Tuberculata

Table 3 Leaf epidermal cell features of ten species of sect. Tuberculata

Taxon	Cell shape	Anticlinal wall pattern	Cell cross-section area (m^{2})	Gland				
	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis	Lower ep		
C. anlongensis	Irregular	Repand	Sinuolate	-	827.95	-	Absent	Absent
C. zengii	Irregular	Sinuous	Sinuous	-	844.11	-	Present	Present
C. rubituberculata	Irregular	Sinuate	Sinuous	-	490.90	-	Absent	Absent
C. acutiperulata	Irregular	Sinuous	Sinuolate	-	-	-	Absent	Absent
C. leyeensis	Irregular	Repand	Sinuolate	-	1036.77	-	Absent	Absent

C. lipingensis	Straight	Repand	Sinuous	-	-	-	Absent	Absent
C. rubimuricata	Irregular	Sinuolate	Sinuous	-	-	-	Present	Present
C. pyxidiacea	Straight	Sinuate	Sinuate	-	-	-	Absent	Absent
C. neriifolia	Irregular	Sinuous	Sinuous	-	-	897.10	Absent	Absent
C. rhytidophylla	Irregular	Sinuous	Sinuate	-	-	1396.60	Present	Absent

Table 4 Stomata features of ten species of sect. Tuberculata

Taxon	Stomatal apparatus type	Number of adjacent cells	Stomatal density (individuals · m	
C. anlongensis	Cyclocytic	4-7	-	-
C. zengii	Cyclocytic	4-7	-	-
C. rubituberculata	Cyclocytic	4-7	6.67	-
C. acutiperulata	Cyclocytic	4-7	-	-
C. leyeensis	Cyclocytic	4-7	14.67	19.40
C. lipingensis	Cyclocytic	4-7	-	-
C. rubimuricata	Cyclocytic	4-7	-	-
C. pyxidiacea	Cyclocytic	4-7	6.67	10.50
C. neriifolia	Cyclocytic	4-7	-	-
C. rhytidophylla	Cyclocytic	4-7	9.33	-

2.4 Stomatal Apparatus

Stomatal apparatus of the 10 species in sect. Tuberculata were distributed only on the lower epidermis and were all cyclocytic (Fig. 4). Their anticlinal walls were irregular, sinuolate, sinuous, or sinuate. Stomatal density ranged from 6.67 to 14.67 individuals · mm^{-2}. Stomatal index variation was not significant, ranging from 10.50% to 19.40%, with the maximum being C. leyeensis at 19.40% and the minimum being C. pyxidiacea at 10.50% (Table 4). The number of subsidiary cells ranged from 4 to 7, with most having 5–7 cells.

2.5 Cluster Analysis of Leaf Epidermis

R-type cluster analysis was performed based on 8 structural indices including stomatal density, stomatal index, cell shape, and anticlinal wall patterns. As shown in Fig. 5 [Figure 5: see original paper], at a Euclidean distance of 3, the 10 species were divided into 6 groups. Among them, C. leyeensis and C. pyxidiacea formed one branch, as these two species were relatively similar in upper epidermal cell morphology and cell cross-sectional area. Camellia anlongensis, C. rubimuricata, C. acutiperulata, and C. lipingensis formed another branch, indicating close phylogenetic relationships. The remaining four species each formed separate branches. At a Euclidean distance of 10, the 10 species were divided into 3 groups: eight species including C. anlongensis, C. rubimuricata, C. acutiperulata, C. lipingensis, C. neriifolia, C. zengii, C. leyeensis, and C. pyxidiacea were clustered into one group due to characteristics such as higher length/width ratios (L/W) and similar stomatal densities; C. rubituberculata formed a separate group due to its small leaf epidermal cell area and low stomatal density; and C. rhytidophylla formed a separate group due to its large lower epidermal cell area and oil glands present only on the upper epidermis.

Fig. 5 Cluster analysis of leaf epidermal of ten species of sect. Tuberculata

3 Discussion and Conclusion

Plants of sect. Tuberculata are a specialized group of Camellia in karst limestone regions, with Guizhou Province as the distribution center, extending to southwestern and adjacent central provinces in a scattered distribution pattern. They are narrow ecological amplitude species with extremely small populations, making field investigation difficult. Previous taxonomic treatments of this group were mainly based on macroscopic characters from herbarium specimens, and interspecific delimitation of some species remains ambiguous and taxonomically challenging. Therefore, incorporating pollen morphology and leaf epidermal micromorphology as taxonomic evidence can help clarify interspecific boundaries and phylogenetic relationships within sect. Tuberculata.

3.1 Taxonomic Significance of Pollen Morphology in sect. Tuberculata

This study reports pollen morphology of 10 species in sect. Tuberculata for the first time, filling the gap in pollen morphological data for this group. The results revealed that all 10 species have single pollen grains with similar shapes, predominantly subspheroidal, with P/E ratios between 0.85 and 1.16, all relatively small. Polar view showed two types: trilete subrounded and trilete regular triangular; apertures were angular or needle-like. Exine sculpture showed rugulate or granular patterns, but granular size differed significantly among species. For example, C. zengii, C. lipingensis, and C. rhytidophylla all had granular exine sculpture, but their perforation sizes and densities were different, indicating that differences in exine sculpture within this group have certain taxonomic value and can serve as interspecific taxonomic evidence. This is consistent with Li Guangqing's (2005) pollen morphological study on 6 species of sect. Theopsis, which found that exine sculpture showed large interspecific differences and had taxonomic value. Camellia pyxidiacea and C. anlongensis are both distributed in Qianxinan Prefecture, Guizhou. Ming Tianlu merged them into C. anlongensis based on morphological characteristics including white flowers, hairy fruits, and 3–5 styles. In cluster analysis, the two species were on the same branch at a Euclidean distance of 15, supporting their merger. Camellia lipingensis and C. zengii were both collected from Wulong Mountain, Liping County. Ming Tianlu merged them into Camellia rhytidocarpa Chang & S. Y. Liang based on their narrow lanceolate leaves and similar floral characters. In pollen morphological studies, C. lipingensis had a fusiform equatorial view and trilete regular triangular polar view, while C. zengii had an elliptical equatorial view and trilete subrounded polar view, with distant cluster analysis positions. Therefore, whether they should be merged requires further macro-morphological and molecular biological studies before a conclusion can be drawn.

3.2 Taxonomic Significance of Leaf Epidermal Micromorphology in sect. Tuberculata

Leaf epidermal cell shapes in sect. Tuberculata can be divided into two types: irregular and polygonal. Polygonal cells were found in C. lipingensis and C. rubimuricata, with the rest being irregular, indicating that cell shape has certain taxonomic value among some species. Stomata on the lower epidermis were all cyclocytic, which is similar to the results of Lu et al. (2008) on leaf epidermal morphology of sect. Tuberculata, suggesting that this character has low interspecific taxonomic value for the group. However, anticlinal wall patterns showed four types: sinuolate, sinuous, repand, and sinuate, which have relatively high taxonomic value compared to other characters. Morphological structures of the lower epidermis showed obvious differences among some species and could provide certain evidence for classification of some species. Our results showed that C. rhytidophylla had the largest cell cross-sectional area (1396.6 m^2), a few oil glands on the upper epidermis, sinuous anticlinal wall patterns, lower epidermal stomatal density of 9.33 individuals $\cdot$ mm^{-2}, and 5–7 subsidiary cells. Camellia rubituberculata had the smallest lower epidermal cell cross-sectional area (600.43 m^2), no oil glands on either upper or lower epidermis, sinuate anticlinal wall patterns, stomatal density of 6.67 individuals $\cdot$ mm^{-2}, and 4–7 subsidiary cells. In addition, other micromorphological characters of leaf epidermis (stomatal density, presence of glands, stomatal apparatus, etc.) showed obvious differences among species and could provide reference evidence for interspecific delimitation of most species in sect. Tuberculata, indicating certain taxonomic significance.

Through leaf epidermal micromorphology and cluster analysis, results showed high similarity between C. leyeensis and C. pyxidiacea in leaf epidermal micromorphology, indicating a close relationship. Combined with their distribution only in the Beipan River basin, similar macro-morphology (white flowers, 3–5 styles, tuberculate capsules, hairy seeds), the results support Ming Tianlu's taxonomic treatment of merging the two species. However, relying solely on leaf epidermal micromorphological characters for taxonomic treatment is too arbitrary, and comprehensive and detailed taxonomic studies should be conducted before drawing conclusions.

3.3 Conclusion

In summary, pollen morphology and leaf epidermal micromorphology of the 10 studied species in sect. Tuberculata showed certain interspecific differences, indicating to some extent that these characters have reference value for classification of sect. Tuberculata and can serve as one basis for interspecific identification, helping to compensate for classification problems that are difficult to resolve using macroscopic characters alone. However, during field investigations, we found that interspecific classification issues in sect. Tuberculata are relatively complex, with frequent interspecific hybridization phenomena. Therefore, field surveys should be conducted using populations as units, and multidisciplinary approaches should be employed to resolve the taxonomic problems existing in this group.

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Note: Figure translations are in progress. See original paper for figures.