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Study on the Biological Characteristics and Scanning Electron Microscopy Features of Camellia guizhouensis

Ying Wang

wangyinghouzi@163.com

Guizhou Academy of Sciences

Shu Wang

Heilongjiang Polytechnic

Lin Xu

Guizhou Technology and Business Vocational University

Cheng Wan

Guizhou Academy of Sciences

Leijia Li

Guizhou Academy of Sciences

Jihong Wang

Guizhou Academy of Sciences

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Abstract

This study investigates the genetic relationship between *Camellia guizhouensis* S.Y.Liang et T.L.Ming (*C. guizhouensis*) and species of *Camellia* sect. *Chrysantha* (Theaceae), to provide taxonomic evidence for species identification based on the microscopic morphology of leaves and pollen, while supporting the sustainable and efficient utilization of *C. guizhouensis* as a characteristic tea resource. Comparative analyses were conducted on naturally occurring seedling populations in the native habitat of *C. guizhouensis* through regular fixed-point observations of new shoot emergence and the flowering and fruiting phenological stages. Scanning electron microscopy was used to examine the microscopic characteristics of mature leaves and pollen from both red-bud and green-bud phenotypes, including pollen germination apertures, exine surface ornamentation, and pollen grain size. The results showed that the leaf and pollen microstructures of both phenotypes shared the characteristic morphological features of species within *Camellia* sect. *Chrysantha*, indicating a close genetic affinity between *C. guizhouensis* and other members of this section. Furthermore, differences in pollen morphology confirmed that *C. guizhouensis* is taxonomically distinct from the morphologically similar *Camellia tunghinensis* (*C. tunghinensis*).

1. Introduction

Renowned as the "Oriental Magic Tea", the "Giant Panda of the Plant Kingdom", and the "Queen of the Tea Family", *Camellia chrysantha* (*C. chrysantha*) possesses extremely high ornamental, economic, scientific, and medicinal values. Plants in the *Camellia* sect. *Chrysantha* are rich in various antioxidant components (e.g., flavonoids, tea polysaccharides), and they offer lipid-lowering, antibacterial, antioxidant, anti-allergic, and anti-tumor efficacy, demonstrating great commercial potential [1–2]. *Camellia guizhouensis* (*C. guizhouensis*) is primarily distributed in the southern subtropical montane monsoon rainforests of Luodian County and Ceheng County in Guizhou Province [3]. To date, it is the only precious plant in the *Camellia* sect. *Chrysantha* is distributed north of the Tropic of Cancer at an altitude above 700 m, featuring excellent cold tolerance [4]. Since its aroma components are more intense than those of plants in the *Camellia* sect. *Chrysantha* from tropical areas such as Guangxi, *C. guizhouensis* has a dried flower product price of > 10,000 RMB/kg. Thus, less than 1,000 wild plants of *C. guizhouensis* exist due to excessive flower harvesting.

To date, few studies of *C. guizhouensis* have been reported. First, it tends to be located in remote mountainous areas. It was mistakenly identified as *Camellia indochiensis* Merr. in 1976 [4]. Indeed, its type specimens were successively collected by botanists from the Institute of Botany, Chinese Academy of Sciences, in Ceheng and Wangmo in 1958 and 1959. During the first National Herbarium Symposium held in Beijing in 1992, experts investigated the specimens mentioned above (*Theaceae* specimen No. 1260). They confirmed that it is a new species of the *Camellia* genus. *C. guizhouensis* is highly similar to *Camellia tunghinensis* (*C. tunghinensis*), while they differ in that *C. guizhouensis* has an early flowering period, pure yellow and solitary flowers at the branchlet tips, short pedicels (5–6 mm in length), and a

corolla diameter of 2.5-3 cm; the leaves are 5–9 cm long and 2-3.5 cm wide; the lateral veins are inconspicuous on the upper surface but highly detectable on the lower surface [4].

In this study, the similarities and differences between the red-bud and green-bud phenotypes of *C. guizhouensis* were examined at both the morphological and cytological levels to clarify the genetic relationship between *C. guizhouensis* and other species within *Camellia* sect. *Chrysantha*, and to determine whether the two phenotypes represent distinct species. The findings improve our understanding of the phenotypic variation and genetic diversity of *C. guizhouensis*, providing a valuable foundation for future research on its germplasm conservation, genetic resources, and breeding.

2. Materials and Methods

2.1 Plant Materials

C.guizhouensis(*Camellia guizhouensis* ShengYe.Liang et TianLu.Ming)were collected from Dating Village and Tianba Village in the Dating Community, Maojing Town, Luodian County, Guizhou Province (N25°16'01"–25°16'51", E106°57'55"–106°58'38"). Plant identification was performed by Wang Jihong, a senior taxonomist specializing in Theaceae at the Institute of Biology, Guizhou Academy of Sciences. The voucher specimen of this material has been deposited in Herbarium of Guangxi Institute of Botany（IBK）, the deposition number is IBK00118561. All samples were collected with prior informed consent from the land owner, and no endangered or protected species were sampled during this study.

All field collection activities strictly complied with the institutional, national and international guidelines and legislations for wild plant research, and fully followed the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Prior informed consent for sampling was obtained from the local village committee and landowners before field work. No protected or endangered species listed in the National Key Protected Wild Plants Catalogue of China, IUCN Red List or CITES Appendices were involved in this study.

2.2 Experimental methods

2.2.1 Investigation of biological characteristics

The biological characteristics of the specimens were investigated in Dating Village, Tianba Village, and Tingxi Village within the Dating Community of Maojing Town from July 2025 to May 2026. The study included flowering plants that were more than 10 years old. At each site, ten representative plants were selected for regular, fixed-point observations to monitor new shoot emergence and the phenological stages of flowering and fruiting.

2.2.2 SEM of leaves

For each bud phenotype, three plants were randomly selected, and 3–5 mature leaves were collected from each plant. The leaf surfaces were first cleaned with a soft brush, after which 2 mm × 2 mm

sections were excised from the same position adjacent to the midvein of each leaf. The samples were thoroughly rinsed with 95% ethanol, dehydrated in 100% ethanol, mounted on double-sided adhesive tape, vacuum-coated, and examined using a Hitachi S-800 scanning electron microscope.

2.2.3 SEM of pollen

For each bud phenotype, three plants were randomly selected, and 3–5 normally developed flowers at the pre-anthesis stage were collected from each plant. Fresh pollen was directly dispersed onto double-sided adhesive tape without previous treatment, vacuum-coated, and examined using a 6490LV scanning electron microscope to characterize pollen morphology, germination apertures, and exine surface ornamentation. The polar axis (P) and equatorial axis (E) lengths of the pollen grains were measured using the built-in measurement software of the scanning electron microscope. Pollen morphology was described following the terminology and classification standards presented in Introduction to Palynology by Wang Kaifa (1983) and Manual of Palynology by G. Erdtman.

3. Results

3.1 Biological characteristics

3.1.1 Biological characteristics

The specimens were identified as shrubs or small trees. The leaves were thinly coriaceous to nearly membranous, elliptic in shape, measuring 6–10 cm in length and 3.5–5.5 cm in width. The leaf apex was shortly acuminate with a blunt tip, whereas the base was broadly cuneate and occasionally nearly rounded. After drying, the adaxial surface remained green with a slight gloss, while the abaxial surface was glabrous and densely covered with fine black glandular dots. Five to six pairs of lateral veins were present, and together with the reticulate venation, they were visible on the adaxial surface and slightly raised on the abaxial surface. The leaf margins were bluntly serrate, and the petioles measured 7–12 mm in length.

The flowers were yellow, fragrant, and borne singly or in pairs at terminal or axillary positions, with diameters ranging from 2.5 to 4.5 cm. The glabrous pedicels were 6–7 mm long. Four to five ovate, glabrous bracts measuring 1–2 mm in length were present. The calyx consisted of five ovate, glabrous sepals, each 4–5 mm long with a rounded apex. The corolla comprised seven to eight oval, glabrous petals, each 1.0–1.5 cm long and 1.2–1.5 cm wide, with rounded apices and fused at the base for 3–4 mm. The stamens were arranged in multiple whorls and measured 1.2–1.5 cm in length, approximately equal to the petals. Their filaments were united at the base for approximately 6 mm to form a short tube, whereas the free portions of the filaments were glabrous. The ovary was also glabrous. Three glabrous styles, each approximately 1.3 cm long, arose from the ovary. The fruit was an oblate capsule, measuring 2–4 cm in height and 3–6.8 cm in width, with a thin pericarp. The seeds were nearly spherical and brown.

3.1.2 Phenological period

In its native habitat, *C. guizhouensis* produces two flushes of new shoots each year. Spring shoots begin to emerge from late January to early February, complete their growth between late February and late March, and develop into semi-lignified branches measuring 7.8–14.1 cm in length and more than 1.5 mm in thickness by late June. Autumn shoots emerge in early July, complete their growth from early August to early September, and become semi-lignified by late October, reaching lengths of 7.5–16.5 cm and thicknesses exceeding 1.5 mm. Compared with spring shoots, autumn shoots show a faster growth rate.

Flower bud differentiation begins in late November. Initial flowering occurs in early January of the following year, full bloom extends from mid- to late February, and the flowering period concludes in early March. Each plant produces, on average, more than 100 flowers, with an overall flowering duration of 40–50 days. Fruits mature between mid- and late October. However, the fruit set is relatively low, averaging approximately 20% of the total number of flowers, and the plants demonstrate a pronounced alternate-bearing habit.

3.1.3 Stress tolerance

C. guizhouensis is a shade-loving and highly shade-tolerant species with very limited tolerance to direct sunlight. Seedlings transplanted and cultivated around villagers' houses demonstrated poor growth under prolonged sun exposure, characterized by smaller, hardened, yellowish scorched leaves and slow development of new shoots. In particular, seedlings exposed to direct sunlight for only two days showed severe leaf scorching. After two weeks, the leaves had turned yellow, developed silvery scorched patches, and begun to abscise. Within three months, all affected seedlings had died from sun damage. Similarly, the upper leaves of mature plants exposed to direct sunlight showed scorching and became yellowish-green, accompanied by reduced retention of older leaves and overall poor plant vigor.

Although *C. guizhouensis* can adapt to a range of soil conditions, it thrives under high-humidity environments. During years of severe autumn drought, plants failed to produce autumn shoots and experienced substantial leaf abscission. Field surveys also revealed that some individuals were affected by red leaf blight, anthracnose, and southern blight. The principal insect pests were longhorn beetles and carpenter moths, whereas infestations by other insect species were infrequent.

3.2 SEM of leaves

The red-bud and green-bud phenotypes of *C. guizhouensis* showed highly similar microscopic leaf characteristics. In both phenotypes, stomata were confined to the abaxial (lower) leaf epidermis and were irregularly distributed. The stomatal apparatus was oval and consisted of two crescent-shaped guard cells with smooth outer stomatal ledges. Stomatal size was also highly consistent between the two phenotypes, measuring 32.86 × 32.32 μm² in the red-bud phenotype (Fig. 1) and 32.39 × 32.09 μm² in the green-bud phenotype (Fig. 2). These characteristics are consistent with the typical leaf epidermal microstructure reported for species within *Camellia* sect. *Chrysantha* (Theaceae).

The red-bud and green-bud phenotypes of *C. guizhouensis* share the diagnostic microscopic leaf features of *Camellia* sect. *Chrysantha*, with no significant differences observed in their leaf epidermal morphology. Together with the absence of appreciable differences in pollen morphology, these findings indicate that the red-bud and green-bud phenotypes belong to the same species.

Despite their overall similarity, the leaf micromorphology of the red-bud and green-bud phenotypes of C. guizhouensis differed in five respects. (1) The inner margin of the outer stomatal ledge was smooth in the red-bud phenotype but shallowly undulate in the green-bud phenotype. (2) Although both phenotypes demonstrated cuticles with coarse striations and ridges, their epicuticular wax ornamentation differed, consisting of fine granules in the red-bud phenotype and block-like granules in the green-bud phenotype. (3) Pronounced T-shaped thickening of the guard cells was evident in the green-bud phenotype but was absent or inconspicuous in the red-bud phenotype. (4) Epidermal hairs were thick and erect in the red-bud phenotype, whereas they were thin, short, and decumbent in the green-bud phenotype. (5) Although sparse glandular scales (heteromorphic stomata) were present on the abaxial epidermis of both phenotypes, they differed in abundance and morphology. The red-bud phenotype possessed fewer glandular scales, appearing as small nodular protuberances formed by clusters of several cells. The green-bud phenotype contained more numerous glandular scales that resembled individual, deeply sunken stomata.

3.3 SEM of pollen

The pollen morphology of the red-bud and green-bud phenotypes of *C. guizhouensis* was highly similar, supporting their classification as the same species. In both phenotypes, the pollen grains were prolate, with a narrowly elliptical outline in equatorial view and an obtusely triangular outline in polar view. The pollen possessed three (pseudo-)colporate germination apertures, with deeply sunken colpus margins extending longitudinally across the pollen grain. No ridges or protuberances were observed at the equatorial region. The exine demonstrated a smooth pseudo-reticulate ornamentation characterized by indistinct reticulate ridges and a few small, irregularly distributed perforations. The pollen grain size was (48.32–)46.53 × 25.62(–26.35) μm² in the red-bud phenotype (Fig. 3) and (49.47–)45.86 × 23.89(–25.76) μm² in the green-bud phenotype (Fig. 4).

Minor differences were observed in the germination apertures between the two phenotypes. In the green-bud phenotype, interrupted ridges were present at the equatorial region, with occasional inconspicuous protuberances, whereas these features were absent in the red-bud phenotype. Despite these subtle variations, the overall pollen morphology remained highly consistent, further indicating that the red-bud and green-bud phenotypes belong to the same species.

4. Discussion and conclusions

4.1 Biological characteristics

The shoot growth pattern of *C. guizhouensis* is characteristic of a shrub. The main stem branches at a relatively low position, typically producing three to four primary branches below 40 cm. In its native habitat, *C. guizhouensis* produces two flushes of new shoots annually. Spring shoots emerge from late January to early February and complete their growth between late February and late March, reaching lengths of 7.8–14.1 cm with stem diameters exceeding 1.5 mm. Autumn shoots begin to emerge in early July and complete their growth from early August to early September, attaining lengths of 7.5–16.5 cm and diameters greater than 1.5 mm. These observations differ from previous reports indicating that mature plants of *Camellia parvipetala* (*C. parvipetala*) [5] and *Camellia nitidissima* (*C. nitidissima*) introduced to Guilin produced only a single autumn flush [6–7]. However, Chen [8]similarly reported that *C. guizhouensis* introduced to Fujian demonstrated two annual shoot flushes. These findings suggest that *C. guizhouensis* possesses a distinctive growth pattern and considerable potential for cultivation and broader horticultural utilization.

In *C. guizhouensis*, flower bud differentiation begins in late November, followed by the onset of flowering in early January of the subsequent year. Peak flowering occurs from mid- to late February, and the flowering season concludes in early March. Each plant produces, on average, more than 100 flowers, with a flowering period lasting approximately 40–50 days. Preliminary analyses of the biochemical properties relevant to tea processing showed that the water extract contents of the flowers and leaves were 40.8% and 32.7%, respectively, accompanied by a relatively high phenol-to-ammonia ratio, indicating good suitability for tea fermentation. Market evaluations further suggest that flowers of *C. guizhouensis* possess a richer aromatic profile than those produced in lower-altitude, lower-latitude regions such as Guangxi. Therefore, a comprehensive characterization of its biochemical properties is needed to support the scientific processing, quality improvement, and efficient utilization of this valuable tea resource.

4.2 SEM features of leaves

Leaves represent one of the most important taxonomic characters in plant classification, and their microscopic morphological features have been widely used to distinguish taxonomically challenging groups in modern plant systematics [9–10]. Species within *Camellia* sect. *Chrysantha* share several characteristic leaf epidermal features. Stomatal apparatuses are restricted to the abaxial leaf surface and consist of two crescent-shaped guard cells with an elliptical or oval outline. The outer stomatal ledges are generally smooth, whereas the inner margins are nearly smooth, although shallowly undulate or dentate-shallowly undulate forms may also occur. The cuticle is predominantly striated, with some species additionally demonstrating granular or scale-like ornamentation. Variations in these leaf epidermal characteristics among species provide valuable taxonomic characters for distinguishing closely related taxa within the genus [11–13].

To our knowledge, this is the first comparative investigation of the leaf epidermal micromorphology of *C. guizhouensis* using scanning electron microscopy. The study was designed to clarify the genetic relationship between *C. guizhouensis* and species of *Camellia* sect. *Chrysantha*, determine whether *C.*

guizhouensis is conspecific with *C. tunghinensis*, and assess whether the red-bud and green-bud phenotypes of *C. guizhouensis* represent a single species.

In *C. tunghinensis*, the guard cells of the stomatal apparatus lack the characteristic T-shaped thickening at both poles, and neither glandular scales nor epidermal hairs are present. These distinct differences in leaf micromorphology support the conclusion that *C. guizhouensis* and *C. tunghinensis* are separate species. To date, the leaf epidermal micromorphology of more than 20 species within *Camellia* sect. *Chrysantha* has been described. Among these, lanceolate epidermal hairs have been reported only on the abaxial leaf surface of *Camellia pubipetala* (*C. pubipetala*). However, the outer stomatal ledges of *C. pubipetala* are rough, and diagnostic features observed in *C. guizhouensis*, such as glandular scales, are absent. These differences further distinguish *C. pubipetala* from *C. guizhouensis*.

Although *Camellia impressinervis* (*C. impressinervis*), *Camellia wumingensis* (*C. wumingensis*), and *C. parvipetala* share one characteristic with the green-bud phenotype of *C. guizhouensis*, namely, the presence of distinct T-shaped thickening at both poles of the guard cells, they differ in several other key leaf epidermal traits. These include the surface texture of the outer stomatal ledges, cuticular morphology, the shape of the inner margin of the outer stomatal ledges, and the size of the epicuticular wax granules [12–13]. These micromorphological differences indicate that none of these species is conspecific with the green-bud phenotype of *C. guizhouensis*. Although *Camillia chrysantha var. microcarpa* (*C. chrysantha var. macrocarpa*), *Camillia limonia form obovata* (*C. limonia form obovate*), *Camillia limonia* (*C. limonia*), *Camillia chrysanthoides* (*C. chrysanthoides*), *Camillia grandis* (*C. grandis)*, *Camillia longgangensis* (*C. longgangensis*), *Camillia pingguoensis* (*C. pingguoensis*), and *Camillia multipetala var. patens* (*C. multipetala var. patens*) share a common feature with the red bud phenotype of *C. guizhouensis* in that the guard cells of stomatal apparatus show no significant T-shaped thickening at poles [12–13], the stomatal apparatuses of *C. chrysantha var. macrocarpa*, *C. limonia form obovate*, *C. limonia*, *C. chrysanthoides*, *C. grandis* and *C. longgangensis* are elliptical in shape, and most of them have shallowly undulate or dentate-shallowly undulate outer stomatal ledges, blocky round or scales wax ornamentation granules, suggesting that they do not belong to the same species as the red bud phenotype of *C. guizhouensis*. Although *C. pingguoensis* and *C. multipetala* var. patens share an oval stomatal apparatus with the red-bud phenotype of *C. guizhouensis*, they differ significantly in several other leaf epidermal characteristics. *C. pingguoensis* possesses coarse outer stomatal ledges and a vermiform cuticle, whereas *C. multipetala* var. patens is characterized by coarse outer stomatal ledges, a shallowly undulate inner margin of the outer stomatal ledge, and block-like epicuticular wax granules. These distinct micromorphological differences indicate that neither *C. pingguoensis* nor *C. multipetala* var. patens is conspecific with the red-bud phenotype of *C. guizhouensis*.

4.3 SEM features of pollen

Pollen grains are the male reproductive structures that carry the genetic information of plants and generally exhibit greater morphological stability and stronger resistance to environmental influences than other plant tissues and organs [14]. Therefore, pollen morphology and exine architecture are highly

species-specific and have long been recognized as valuable taxonomic characters for the classification and identification of plant species and genera [15]. To date, the pollen micromorphology of more than 20 species within *Camellia* sect. *Chrysantha* has been described, and their overall characteristics are consistent with those of pollen grains found in the genus *Camellia* (Theaceae). In general, the pollen grains are medium-sized and range from spherical to oblate or prolate in shape. They are elliptical in equatorial view and trilobate-circular to obtusely triangular in polar view. The germination apertures are typically three (pseudo-)colporate, with a distinct colpus membrane showing an irregular outline and often protruding at the equatorial region. Exine ornamentation varies among species and may be verrucate (block-like), clavate-tuberculate (vermiform), or pseudo-reticulate [16–18].

In this study, SEM was employed to investigate the pollen morphology of the red-bud and green-bud phenotypes of *C. guizhouensis* collected from Dating Village, Maojing Town, Luodian County. The aim was to provide palynological evidence to support the taxonomic classification of *C. guizhouensis* and to clarify the genetic relationship between the two bud phenotypes. The results demonstrated that the pollen of both phenotypes exhibited the diagnostic morphological characteristics of species within *Camellia* sect. *Chrysantha*, indicating that *C. guizhouensis* shares a close genetic relationship with other members of this section.

Field investigations further showed that the red-bud phenotype is the dominant wild form of *C. guizhouensis*, whereas the green-bud phenotype accounts for less than 1% of the natural population. Compared with the red-bud phenotype, the green-bud phenotype is characterized by light green young shoots and tender buds, as well as pale yellow petals. Despite these phenotypic differences, the major taxonomic characters used in pollen classification. including pollen shape, size, colpus morphology, and exine ornamentation, provided no evidence that the two phenotypes represent distinct species. Only slight differences were detected in the pollen germination apertures: interrupted ridges at the equatorial region and occasional inconspicuous protuberances were observed in the green-bud phenotype but were absent in the red-bud phenotype. These minor morphological variations are more likely to reflect intraspecific variation than species-level divergence. However, further investigations are required to determine the extent and taxonomic significance of infraspecific differentiation within *C. guizhouensis*.

Pollen shape is a key taxonomic character for distinguishing closely related species within the same genus [15]. Based on published reports, the pollen of the 25 known species in *Camellia* sect. *Chrysantha* can be classified into three principal shape categories: spherical, oblate, and prolate. Xie et al. [16] and Wang et al. [17] reported that approximately 64.0% of these species possess spherical pollen. These include *C. impressinervis*, *C. chrysantha*, *Camellia nitidissima* form longistyla (*C. nitidissima* form longistyla), *C. chrysantha* var. macrocarpa, *Camellia micrantha* (*C. micrantha*), *Camellia multipetala* (*C. multipetala*), *C. grandis*, *C. longgangensis*, *C. pubipetala*, *Camellia euphlebia* (*C. euphlebia*), *C. tunghinensis*, *Camellia flavida* (*C. flavida*), *Camellia longzhouensis* (*C. longzhouensis*), *C. parvipetala*, *Camellia achrysantha* (*C. achrysantha*), and *C. limonia* form obovata. Liang [4] suggested, based on macromorphological characteristics, that *C. guizhouensis* and *C. tunghinensis* are closely related.

However, the present SEM observations revealed a clear difference in pollen shape: *C. guizhouensis* possesses prolate pollen, whereas *C. tunghinensis* has spherical pollen. This distinct palynological difference provides additional evidence that the two taxa should be regarded as separate species.

Pollen grain size is another important taxonomic character for distinguishing closely related species within the same genus [15]. Xie et al. [16] and Wang et al. [17] reported that *Camellia longruiensis* (*C. longruiensis*) has spherical to elliptical pollen measuring (33.90–)33.20 × 24.30(–24.60) μm; *Camellia ptilosperma* (*C. ptilosperma*) has oval pollen measuring (28.90–)28.80 × 23.50(–24.40) μm; *C. wumingensis* possesses nearly spherical to suboblate pollen with a size of (34.10–)30.90 × 26.60(–29.20) μm; and *Camellia fusuiensis* (*C. fusuiensis*) has oblate pollen measuring (26.1–30.6)27.9 × (28.7–33.1)31.6 μm. Although these species within Camellia sect. *Chrysantha* exhibit pollen shapes similar to those of *C. guizhouensis*, their pollen grains are substantially smaller than those of *C. guizhouensis*, which measure (48.32–)46.53 × 25.62(–26.35) μm in the red-bud phenotype and (49.47–)45.86 × 23.89(–25.76) μm in the green-bud phenotype. These differences in pollen size further support their recognition as distinct species.

Among the species of *Camellia* sect. *Chrysantha* with prolate pollen similar to that of *C. guizhouensis* are *Camellia xiashiensis* (*C. xiashiensis*) [(23.20–)21.40 × 21.1(–23.20) μm], *C. limonia* [(38.2–)35.5 × 34.4(–32.7) μm], *C. pingguoensis* [(28.30–)28.10 × 19.20(–19.70) μm], *C. chrysanthoides* [(34.1–)32.1 × 28.3(–30.9) μm], and *Camellia leptopetala* (*C. leptopetala*) [(42.3–)41.6 × 37.2(–39.9) μm]. In terms of pollen grain size, both the red-bud and green-bud phenotypes of *C. guizhouensis* possess significantly larger pollen grains than *C. xiashiensis* and *C. pingguoensis*. Although the pollen sizes of *C. limonia*, *C. chrysanthoides*, and *C. leptopetala* are more comparable, they remain distinguishable from *C. guizhouensis* when pollen size is considered together with other diagnostic palynological characteristics.

Pollen exine ornamentation is another important taxonomic character for distinguishing closely related species within the same genus [15]. Based on previous studies, the exine ornamentation of the 25 reported species in *Camellia* sect. *Chrysantha* can be classified into three principal types: verrucate (block-like), cerebriform (vermiform), and pseudo-reticulate [16–18]. Approximately 68.0% of these species exhibit verrucate or block-like ornamentation, including *C. impressinervis*, *C. chrysantha*, *C. chrysantha* var. macrocarpa, *C. longruiensis*, *C. micrantha*, *C. xiashiensis*, *C. flavida*, *C. wumingensis*, *C. grandis*, *C. longgangensis*, *C. limonia*, *C. euphlebia*, *C. chrysanthoides*, *C. fusuiensis*, *C. pingguoensis*, *C. tunghinensis*, and *C. ptilosperma*. Pseudo-reticulate ornamentation has been reported in *C. nitidissima* form longistyla, *C. multipetala*, *C. longzhouensis*, *C. leptopetala*, and *C. achrysantha*, whereas cerebriform (vermiform) ornamentation occurs in *C. pubipetala*, *C. limonia* form obovata, and *C. parvipetala*. Cerebriform ornamentation has also been observed on the pollen exines of *C. xiashiensis* and *C. limonia* [16–18].

Among the species with prolate pollen, *C. limonia*, *C. chrysanthoides*, and *C. leptopetala* possess pollen grains of sizes comparable to those of the red-bud and green-bud phenotypes of *C. guizhouensis*. However, *C. limonia* and *C. chrysanthoides* are readily distinguished by their verrucate (block-like) exine ornamentation, the presence of two half-colpi formed by equatorial protrusions, and a higher density of

uniformly distributed perforations [16–18]. These palynological features differ significantly from those of *C. guizhouensis*, indicating that these taxa are not conspecific with the red-bud or green-bud phenotypes of *C. guizhouensis*. *C. leptopetala* shows pseudo-reticulate exine ornamentation; however, its pollen morphology differs significantly from that of both the red-bud and green-bud phenotypes of *C. guizhouensis*. In *C. leptopetala*, protrusions at the equatorial region divide the colpi into two half-colpi, and the exine contains more numerous and larger perforations, together with abundant small cavities of varying depths between the perforations [17–18]. These features clearly distinguish it from *C. guizhouensis*. Among the 25 reported species of *Camellia* sect. *Chrysantha*, only *C. pingguoensis* shares the same pollen germination aperture pattern as the red-bud and green-bud phenotypes of *C. guizhouensis*, with three colpi extending across the entire pollen grain and deeply sunken colpus margins. However, its pollen grains differ significantly in size, indicating that C. pingguoensis is not conspecific with *C. guizhouensis*.

The pollen exine ornamentation of *C. tunghinensis* differs significantly from that of *C. guizhouensis*. In *C. tunghinensis*, the equatorial region bears distinct tuberculate protuberances, and a finely tuberculate ornamentation characterizes the exine. *C. guizhouensis* lacks equatorial protuberances and exhibits a pseudo-reticulate exine pattern. These pronounced differences in pollen micromorphology provide strong palynological evidence that *C. guizhouensis* and *C. tunghinensis* are taxonomically distinct species.

Declarations

Ethics approval and consent to participate This article does not contain any research involving people as objects of research.

Competing interests policy The authors have no competing interests , or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Author Contributions Statement W.Y. and W.J.H. designed the research. W.Y. and W.S. performed the research. L.L.J., W.C., and X.L. analyzed the data. W.Y. wrote the article. W.J.H. revised the article.

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Authors' information Ying Wang, E-mail wangyinghouzi@163.com.

Address correspondence to:

No.1 Longjiang St.

Huaxi Dist., Guiyang

Guizhou Prov., 550009

China

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Figures

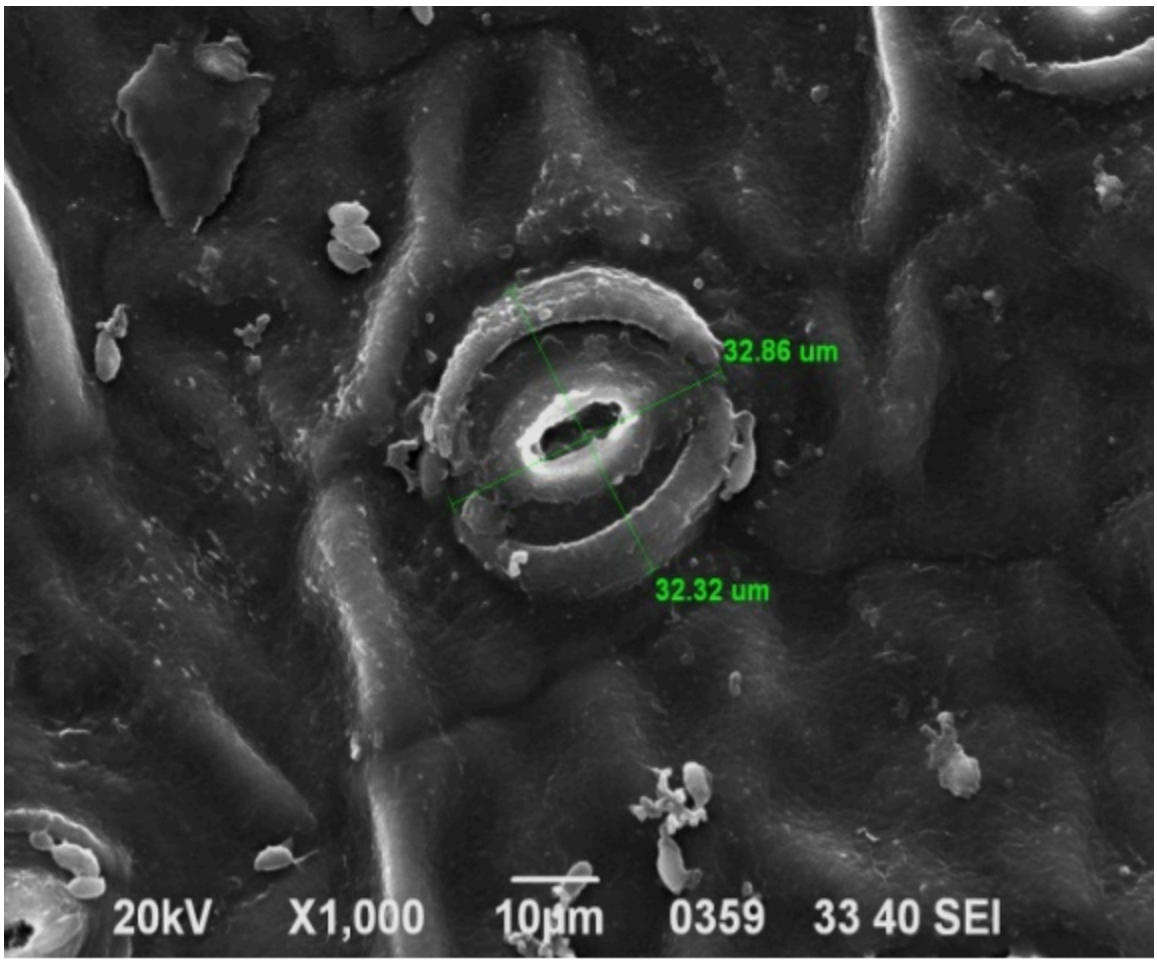


Figure 1

Scanning electron microscope image of red bud leaf

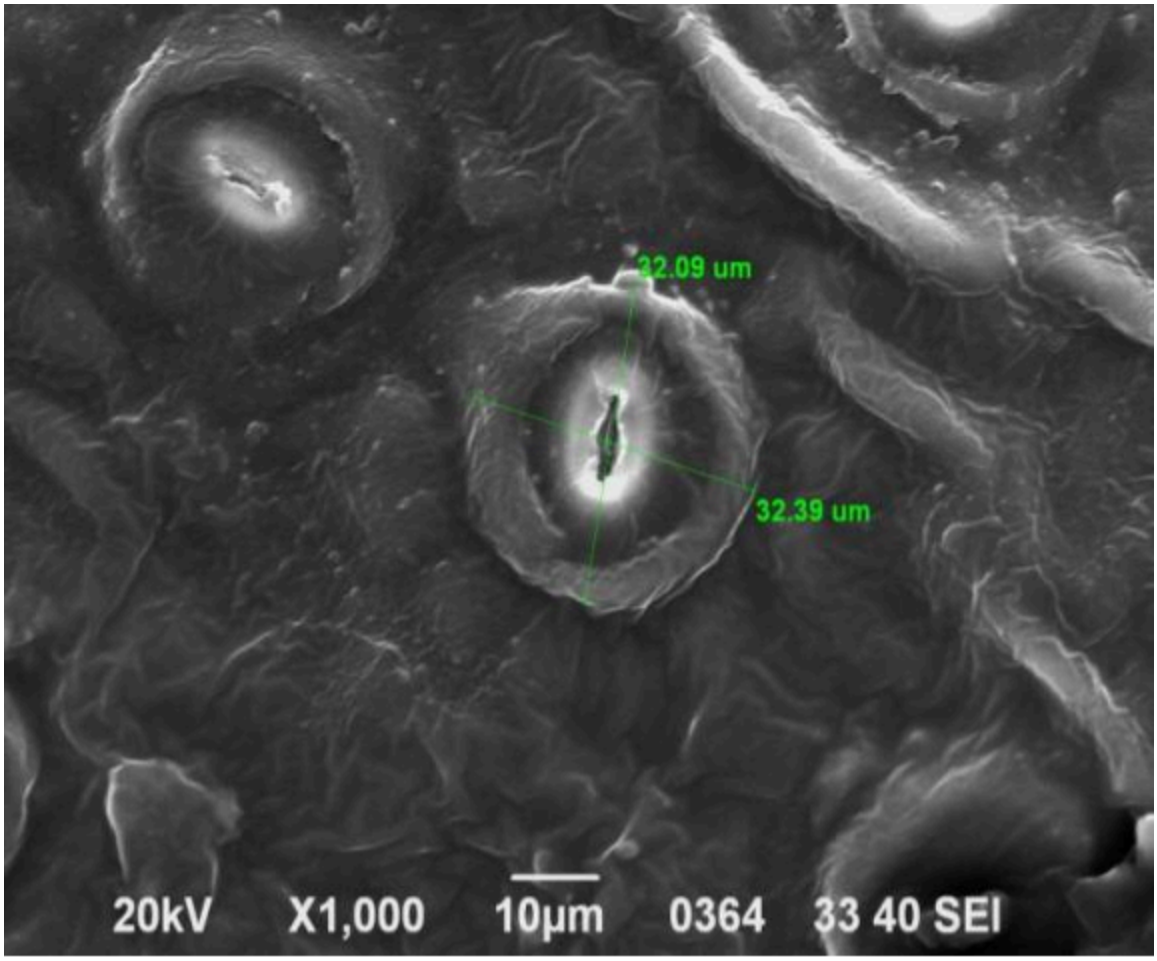


Figure 2

Scanning electron microscope image of green bud leaf

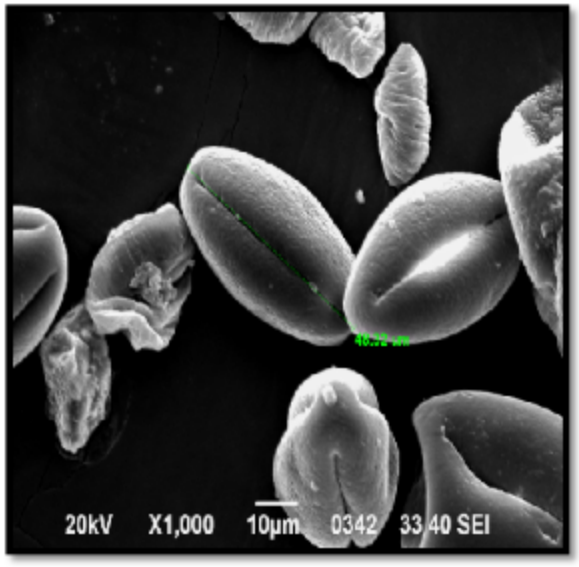


Figure 3

Scanning electron microscopy image of red bud pollen

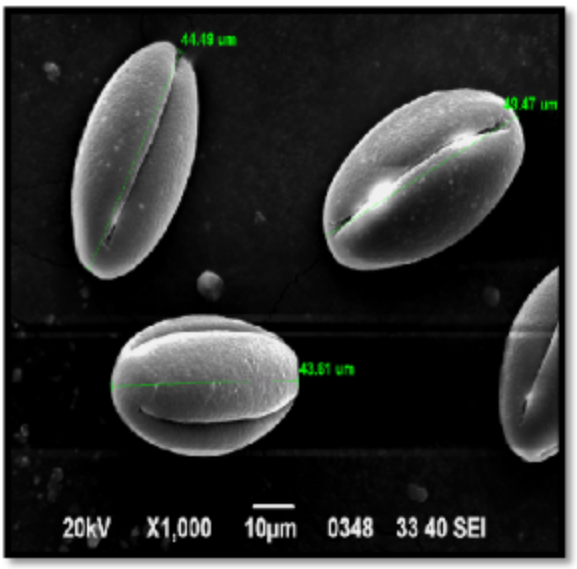


Figure 4

Scanning electron microscopy image of green bud pollen