

# Taxonomic treatments of *Camellia* (Theaceae) species with secretory structures based on integrated leaf characters

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Received: 1 July 2009 / Accepted: 13 August 2010 / Published online: 15 September 2010  
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**Abstract** Multivariate analysis of leaf shape, anatomy, and Fourier-transform infrared (FTIR) data of 27 *Camellia* species with secretory structures (sects. *Archecamellia*, *Stereocarpus*, *Furfuracea*, *Chrysantha*), together with three species from related genera, *Gordonia* and *Tutcheria* (Theaceae), was conducted to clarify some taxonomic problems. Our results show that crystals occurring in adaxial epidermal cells are firstly observed in *Chrysantha* species, and the secretory structures described are in fact cork warts. Furthermore, we introduce a form coefficient ( $F_c$ ) to assess the shape of epidermal cells, since they are usually irregular and difficult to describe. Pearson correlation analysis indicates that  $F_c$  is useful to assess epidermal

cell shape. Principal component analysis (PCA) of leaf shape indicates that two species from section *Archecamellia* and two species from section *Stereocarpus* are significantly different from those in section *Furfuracea*. Cluster analysis of FTIR data visualizes the degree of affinity among the 30 species examined here, which is consistent with the cluster analysis (CA) of anatomical data, as illustrated in the dendrogram. Therefore, our study indicates that integrated leaf characters based on leaf shape, anatomy, and FTIR data are useful in the taxonomic treatment of *Camellia* species with secretory structures. Taxonomic controversies among the *Camellia* species with secretory structures could thus be successfully addressed using only a few intact or small portions of leaves. Moreover, our results tend to support that *Chrysantha* species should not be merged into section *Archecamellia*, and that section *Heterogenea* should not be recognized in taxonomic treatments of *Camellia* species with secretory structures.

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**Keywords** *Camellia* species · Secretory structures ·  
Integrated leaf characters · Multivariate analysis ·  
Taxonomic treatments

## Introduction

This is one of a series of studies on the genus *Camellia*, which hopes to improve our understanding of the taxonomic treatments of this genus (Lu et al. 2008a, 2008b; Lin et al. 2008). Secretory structures are common in the genus *Camellia* and are present in all species of sections *Archecamellia* Sealy, *Stereocarpus* (Pierre) Sealy, *Furfuracea* H.T. Chang, and *Chrysantha* H.T. Chang (Chang 1981, 1998). Notably, the above four sections represent groups of significant taxonomic controversy and uncertain affinities

**Table 1** Different taxonomic treatments of the genus *Camellia* (Sealy 1958; Chang 1981, 1998; Ming 1999, 2000)

Sealy (1958)	Chang (1981, 1998)	Ming (1999, 2000)
	Subgenus <i>Protocamellia</i> Chang	Subgenus <i>Thea</i> (L.) Chang
Sect. <i>Archeamellia</i> Sealy (7)	Sect. <i>Archeamellia</i> Sealy (3)	Sect. <i>Archeamellia</i> Sealy (18)
Sect. <i>Stereocarpus</i> (Pierre) Sealy (1)	Sect. <i>Stereocarpus</i> (Pierre) Sealy (5)	Syn. sect. <i>Chrysantha</i> Chang
Sect. <i>Heterogenea</i> Sealy (10)	Sect. <i>Protocamellia</i> Chang (5)	Sect. <i>Thea</i> (L.) Dyer (12)
Sect. <i>Piquetia</i> (Pierre) Sealy (1)	Sect. <i>Pleurocarpus</i> Chang (2)	Sect. <i>Cylindrica</i> Ming (1)
Sect. <i>Paracamellia</i> Sealy (6)	Sect. <i>Piquetia</i> (Pierre) Sealy (1)	Sect. <i>Piquetia</i> (Pierre) Sealy (1)
Sect. <i>Pseudocamellia</i> Sealy (2)	Subgenus <i>Camellia</i> Chang	Sect. <i>Corallina</i> Sealy (9)
Sect. <i>Camellia</i> (L.) Dyer (8)	Sect. <i>Oleifera</i> Chang (5)	Sect. <i>Theopsis</i> Coh. St. (19)
Sect. <i>Corallina</i> Sealy (5)	Sect. <i>Furfuracea</i> Chang (14)	Sect. <i>Eriandriia</i> Coh. St. (9)
Sect. <i>Calpandria</i> (Bl.) Coh. St. (2)	Sect. <i>Paracamellia</i> Sealy (16)	Sect. <i>Longipedicellata</i> Chang (5)
Sect. <i>Thea</i> (L.) Dyer (5)	Sect. <i>Pseudocamellia</i> Sealy (5)	Subgenus <i>Camellia</i> Chang
Sect. <i>Theopsis</i> Coh. St. (29)	Sect. <i>Tuberculata</i> Chang (15)	Sect. <i>Stereocarpus</i> (Pierre) Sealy (3)
Sect. <i>Camelliopsis</i> (Pierre) Sealy (6)	Sect. <i>Luteoflora</i> Chang (1)	Sect. <i>Heterogenea</i> Sealy (15)
Doubtful species (24)	Sect. <i>Camellia</i> (L.) Dyer (about 60)	Syn. sect. <i>Archeamellia</i> Sealy (sensu H.T. Chang 1981,1998)
	Subgenus <i>Thea</i> (L.) Chang	Syn. sect. <i>Stereocarpus</i> (Pierre) Sealy (sensu H.T. Chang 1981,1998)
	Sect. <i>Corallina</i> Sealy (12)	Syn. sect. <i>Furfuracea</i> Chang
	Sect. <i>Brachyandra</i> Chang (11)	Sect. <i>Paracamellia</i> Sealy (7)
	Sect. <i>Longipedicellata</i> Chang (2)	Sect. <i>Tuberculata</i> Chang (6)
	Sect. <i>Chrysantha</i> Chang (24)	Sect. <i>Camellia</i> (L.) Dyer (12)
	Sect. <i>Calpandria</i> (Bl.) Coh. St. (2)	Sect. <i>Calpandria</i> (Bl.) Pierre (2)
	Sect. <i>Thea</i> (L.) Dyer (32)	
	Sect. <i>Longissima</i> Chang (3)	
	Sect. <i>Glaberrima</i> Chang (3)	
	Subgenus <i>Metacamellia</i> Chang	
	Sect. <i>Theopsis</i> Coh. St. (49)	
	Sect. <i>Eriandriia</i> Coh. St. (14)	

Figures in parentheses are the numbers of species in the section

(Table 1). Based on morphological characters, Sealy (1958) first proposed the sections *Archeamellia* and *Stereocarpus*. He recognized seven species in section *Archeamellia* and one species in section *Stereocarpus*. H.T. Chang (1981) retained Sealy's (1958) sections *Archeamellia* and *Stereocarpus* in his taxonomic treatments. However, he transferred four species from section *Archeamellia* into a new section *Chrysantha*. Furthermore, he set up another new section *Furfuracea* characterized by a large leaf, rough capsule, and solitary, large flower. In 1996, sect. *Archeamellia* and sect. *Stereocarpus* were reviewed by Chang, in which the monotypic sect. *Stereocarpus* was maintained. However, in 1998, the taxonomic treatments of these two sections were returned to those of 1981. Ming (1999), based on Sealy's (1958) and Chang's (1981, 1998) work, produced a new taxonomic treatment. In his classification, Ming (1999) resurrected section *Heterogenea* from Sealy's classification, comprising section *Furfuracea* and several *Archeamellia* and *Stereocarpus* species. He also disagreed with section *Chrysantha* established in Chang's classification, and section *Chrysantha*

was merged with section *Pleurocarpus* into section *Archeamellia*. Moreover, section *Stereocarpus* was significantly revised, in that *C. dormoyana* was retained therein, and section *Luteoflora* and *C. pubipetala* (from section *Chrysantha*) were added. These taxonomic controversies of *Camellia* species with secretory structures indicate that only traditional morphological characters are insufficient for species classification in *Camellia* (Sealy 1958; Chang 1981, 1998; Ming 1999, 2000). Therefore, it is necessary to seek other evidence for reexamination of the classification of *Camellia* species with secretory structures.

The leaf is a vegetative organ whose morphology, anatomy, and biochemistry have proven to be of great use for taxonomic treatments in higher plants. Although leaf morphology, especially leaf macromorphology, had been regarded as a common diagnostic character in *Camellia* taxonomy, the difficulty is still apparent in current *Camellia* classifications, because of changing leaf morphology of plants and the difficulty and inconsistency of their character description (Sealy 1958; Chang 1981, 1998; Ming 1999, 2000). Thus, it is necessary to introduce a quantitative

method to describe leaf morphological characters to reduce the taxonomic confusion due to subjective descriptions (Meade and Parnell 2003). Multivariate analysis of leaf shape may have great potential to identify and classify *Camellia* species with secretory structures.

Additionally, leaves provide many anatomical characters which can be regarded as a classical source of data used in plant taxonomy (Stace 1966; Baranova 1972, 1987; Lubke and Phipps 1973; Upchurch 1984; Kong 2001; Yang and Lin 2005; Lu et al. 2008a, 2008b). Based on leaf anatomic characters, we successfully revised sections *Oleifera*, *Tuberculata*, and *Thea* of genus *Camellia* (Lin et al. 2008; Lu et al. 2008a, 2008b). However, little contribution has been made to the description of leaf anatomic characters in *Camellia* species with secretory structures (Ao et al. 2007).

Fourier-transform infrared spectroscopy (FTIR) is a rapid, simple, high-resolution analytical method which can provide biochemical profiles containing overlapping signals from a majority of the compounds that are present when whole cells are analyzed (Naumann et al. 1991; Mouille et al. 2003). Since plant leaves contain a wide range of different molecular components, FTIR data obtained from these structures may reveal species-specific biochemical profiles reflecting species relationships (Kim et al. 2004; Gorgulu et al. 2007). Recently, we have used this method to classify *Camellia* section *Tuberculata* (Lu et al. 2008a), section *Thea* (Lu et al. 2008b), and sections *Oleifera*, *Paracamellia*, *Camellia*, and *Thea* (Shen et al. 2008). Therefore, FTIR can be used to discriminate disputed *Camellia* species with secretory structures.

The goal of the present work is to conduct comparative leaf analysis of *Camellia* species with secretory structures to: (1) present integrated leaf characters including leaf shape, leaf anatomy, and leaf biochemistry, (2) assess these characters and the taxonomic significance of secretory structures, and (3) revise *Camellia* sections with secretory structures based on integrated leaf characters.

## Materials and methods

### Plant materials

Adult leaves fully exposed to sunlight of *Camellia* plants with secretory structures including 2 species from section *Arhecamellia* Sealy, 2 species from section *Stereocarpus* (Pierre) Sealy, 11 species from section *Furfuracea* H.T. Chang, and 12 species from section *Chrysantha* H.T. Chang were collected from the International *Camellia* Species Garden of Jinhua City (ICSG, 29°7'N, 119°35'S, altitude 40 m) for this study. Additionally, samples of two species of genus *Gordonia* Ellis and one species of genus

*Tutcheria* Dunn were included as outgroups. The examined samples had similar habitats to reduce any environmental and geographical effects on leaf anatomy and chemical composition. Vouchers were deposited at the Chemistry and Life Science College of Zhejiang Normal University (ZJNU) (Appendix 1). Leaves from three different individual plants of each species were analyzed.

### Principal component analysis of leaf shape

Thirty leaf samples from three different individual plants of each species were scanned using the WinFOLIA system (Regent Instruments Inc., Canada). Average measurements of area, perimeter, width, length, aspect ratio (width/length), and leaf form coefficient were obtained. For PCA, the average values were adjusted according to the formula:

$$D_j = \frac{nx_j}{\sum_{i=1}^n x_i}, \quad (1)$$

where  $D_j$  is the transformed value,  $n$  is the number of species examined, and  $x_j$  is the average value of the  $j$ th species ( $1 \leq j \leq n$ ).  $D_j$  is similar to Pearson's coefficient of variation (Briggs and Walters 1984), which allows comparison of data acquired in different units. Scatterplots were produced using the STATICA statistical package (STATICA for Windows, StatSoft) using PCA coordinates based on the transformed values ( $D_j$ ). We chose the "correlation (normalized var-covar)" option because the variables were measured in different units, which normalized all variables by division by their standard deviation.

### Cluster analysis of leaf anatomic characters

Leaf epidermal scrapings were macerated in 40% NaClO for 10 min at 35°C. After carefully removal of mesophyll tissues, pieces of epidermis were obtained and then dehydrated in a graded alcohol series and stained with safranin and fastgreen. Finally, they were mounted in neutral balsam and examined under an Olympus BX50 light microscope (Olympus Co., Tokyo, Japan). To check the constancy of the epidermal structure, at least five slides were made from five leaves for each species, respectively. Thirty abaxial and 30 adaxial epidermal cells from five slides were randomly selected to estimate their area and perimeter by using Dn-3 Micro-image analysis software (Ningbo Yongxin Optics Co., China). The formula used for estimating the form coefficient ( $F_c$ ) of epidermal cells was

$$F_c = \frac{16S}{P^2}, \quad (2)$$

where  $F_c$  is the form coefficient,  $S$  is the area, and  $P$  is the perimeter. Pearson correlation analysis (SAS 9.0 package) was conducted to test the relationships between  $F_c$  and

shape (irregular shape = 1, polygonal shape = 2) as well as the pattern [sinuous = 1, repand = 2, arched to straight (arc-str) = 3] of the anticlinal walls of the epidermal cells. It should be noted that the data of *C. granthamiana* and *C. albogigas* were not used in the Pearson correlation test, because both of them had various epidermal cells.

The samples for transection examination were cut into pieces and fixed in formalin:acetic acid:ethanol (FAA, 5 ml:5 ml:90 ml) solution. The samples were dehydrated in a graded ethanol series, embedded in paraffin, sectioned, mounted on glass slides, and stained with safranin and fastgreen. The sections were examined and photographed under an Olympus BX50 light microscope, while the thickness of palisade tissues and spongy tissues was estimated using Dn-3 Micro-image analysis software.

For the cluster analysis, 13 characters including various epidermal cells, the pattern of the anticlinal walls of adaxial and abaxial epidermal cells, form coefficient of adaxial epidermal cells, crystals, stomatal clusters, thickness of adaxial and abaxial epidermis, thickness of palisade tissues, thickness of sponge tissues, hypodermic crystals, hypodermis, and stone cells were selected (Tables 3, 4). The values representing character states were as follows: absent = 1, present = 2; sinuous = 1, repand = 2, sinuous & repand = 3, and arched to straight = 4. Additionally, for ready comparison of measurements with different units, the measurements of thickness of adaxial epidermis, abaxial epidermis, palisade tissues, and spongy tissues for cluster analysis were adjusted according to formula 1. Unweighted pair-group average (UPGMA) clustering was performed using the STATICA statistical package (STATICA for Windows, StatSoft) based on Euclidean distance using the transformed values.

#### Observation of secretory structures

Leaves of six species (two from section *Archechamellia* Sealy, two from section *Furfuracea* H.T. Chang, and two species from section *Chrysantha* H.T. Chang) were selected to study the secretory structures in the genus *Camellia* using semisections. Shortly after excision from the plants, samples were prefixed in a solution of 2.5% glutaraldehyde at 4°C, adjusted to pH 6.8 using 0.1 M phosphate buffer. Then, they were washed with the same buffer and postfixed in 1% osmium tetroxide. After dehydration through a gradual alcohol series from 30% to 100%, the samples were embedded in Epon812 (hard grade), and desiccated for 3 days in an oven at 60°C. Then, 1- $\mu$ m semisections were obtained using a Reichert-Jung ultramicrotome (Leica, Bensheim, Germany) and stained with methylene blue-azure. Five sections for each species were observed and photographed under both an Olympus BX50 light microscope and a Leica DM LB2 fluorescent microscope

(Hi-Tech Instruments Sdn. Bhd., Malaysia) with ultraviolet (UV)-light excitation.

#### Cluster analysis based on leaf FTIR data

The FTIR analysis followed a modified method described by Gorgulu et al. (2007) and Lu et al. (2008a, 2008b). Mature leaves were collected, and dried immediately in an oven at 70°C. Then, these samples were pulverized by light grinding in an agate mortar, and stored at 20°C until further use. FTIR analysis of the samples was carried out using a Nicolet FTIR spectrophotometer (Nicolet Nexus 870, American) with Omni-sampler attenuated total reflectance (ATR) accessory. The spectra, which were collected over wavenumbers ranging from 4,000 to 675  $\text{cm}^{-1}$ , were displayed in terms of absorbance as calculated from the reflectance-absorbance spectra using the Omnic (Thermo Nicolet Corp.) program (Nicolet version 7.0 package). The spectral resolution was 16  $\text{cm}^{-1}$ , and 256 scans of each sample were summed and averaged to improve the signal-to-noise ratio. The collection time for the spectrum for each sample was 60 s. Samples were run in triplicate, all of which were undertaken within a day period. The FTIR raw data were handled with five-point smoothing and 0–1 normalization using the Unscrambler procedure (9.6 package), and then subjected to multivariate analysis. Cluster analysis was performed on the dissimilarity matrix of Euclidean distances with UPGMA as the clustering algorithm using the STATICA statistical package (STATICA for Windows, StatSoft).

## Results

#### PCA based on leaf shape data

Leaf shape data of the *Camellia* species studied are listed in Table 2. The results of PCA showed that the two most important components, PC1 (eigenvalue 3.72) and PC2 (eigenvalue 1.16) accounted for 72.15% and 22.59% of the total variance, respectively. Other components, whose eigenvalues were much lower than the Jolliffe cutoff value (0.60), were considered insignificant in the light of Jolliffe's criterion (Jolliffe 1986). We therefore chose the first two components, PC1 and PC2, to display the data in the scatterplot (Fig. 1). Plants of the outgroup genera *Gordonia* and *Tutcheria*, usually possessing long-elliptic leaves, were well separated from the *Camellia* samples, bearing elliptic, oval, obovate leaves. It was further shown that leaf morphology was generally distinct between sections of the genus *Camellia*, though there was one species in section *Chrysantha* that clustered with the samples of section *Furfuracea*. It was interesting that the samples of section

**Table 2** Mean values of leaf area (cm<sup>2</sup>), perimeter (cm), vertical length (cm), horizontal width (cm), aspect ratio, and form coefficient

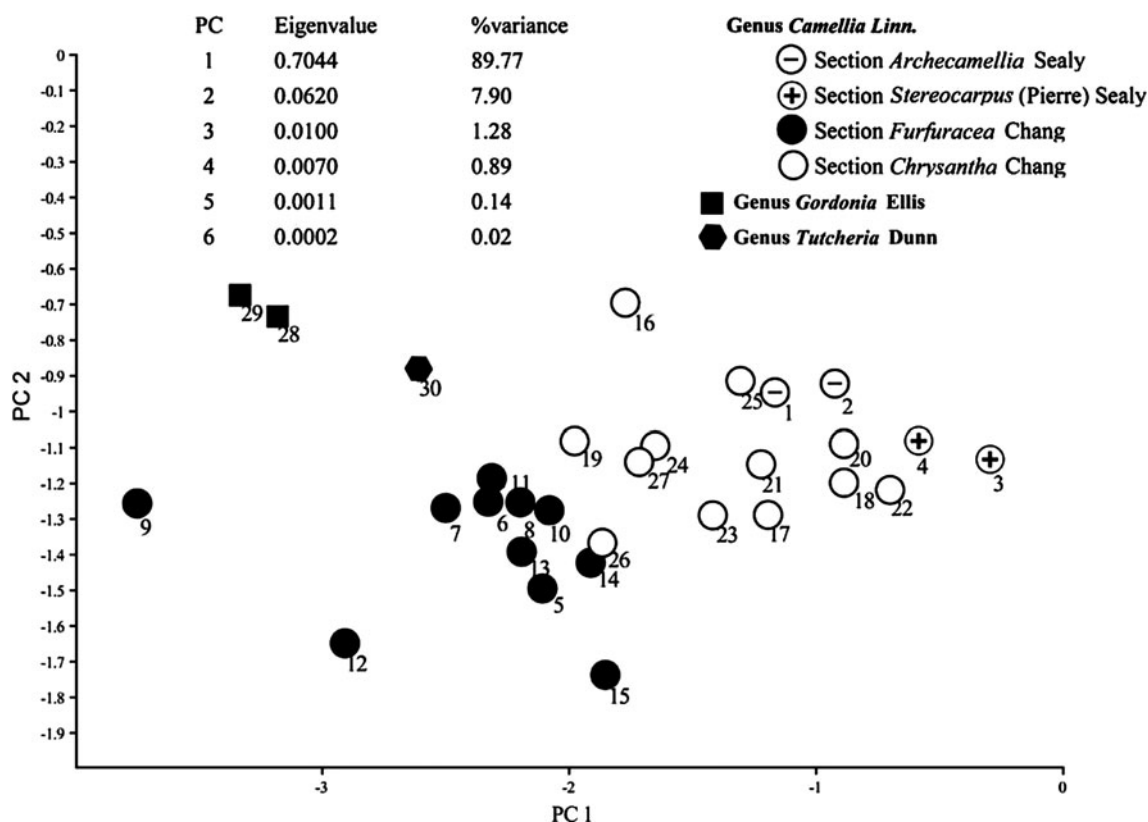
No.	Taxa	Area	Perimeter	Vertical length	Horizontal width	Aspect ratio	Form coefficient
Genus <i>Camellia</i>							
Section <i>Archechamellia</i>							
1	<i>C. granthamiana</i>	18.17 ± 2.78	20.79 ± 1.73	8.03 ± 0.79	3.43 ± 0.23	0.43 ± 0.02	0.53 ± 0.03
2	<i>C. albogigas</i>	11.98 ± 0.81	17.19 ± 0.60	6.30 ± 0.27	2.75 ± 0.06	0.44 ± 0.02	0.51 ± 0.02
Section <i>Stereocarpus</i>							
3	<i>C. liberistyla</i> Chang	2.60 ± 0.25	7.56 ± 0.35	2.68 ± 0.13	1.48 ± 0.08	0.56 ± 0.03	0.57 ± 0.03
4	<i>C. yunnanensis</i>	6.84 ± 0.84	12.79 ± 1.00	4.36 ± 0.27	2.42 ± 0.16	0.56 ± 0.04	0.53 ± 0.09
Section <i>Furfuracea</i>							
5	<i>C. pubifururacea</i>	43.87 ± 6.76	26.39 ± 3.33	11.30 ± 0.65	5.44 ± 0.47	0.48 ± 0.03	0.80 ± 0.08
6	<i>C. latipetiolata</i>	47.83 ± 2.10	32.05 ± 1.07	11.65 ± 0.33	5.87 ± 0.27	0.50 ± 0.03	0.59 ± 0.03
7	<i>C. crapnelliana</i>	53.87 ± 4.82	32.63 ± 2.11	13.06 ± 0.97	6.05 ± 0.16	0.46 ± 0.03	0.64 ± 0.03
8	<i>C. multibracteata</i>	44.71 ± 6.54	28.88 ± 4.87	12.70 ± 1.44	5.14 ± 0.26	0.41 ± 0.03	0.70 ± 0.20
9	<i>C. furfuracea</i>	92.04 ± 11.39	44.38 ± 1.79	17.68 ± 0.74	7.45 ± 0.65	0.42 ± 0.02	0.59 ± 0.03
10	<i>C. oblata</i> Chang	42.92 ± 1.22	29.28 ± 0.63	10.91 ± 0.19	5.53 ± 0.07	0.51 ± 0.01	0.63 ± 0.02
11	<i>C. gaudichaudii</i>	47.13 ± 2.38	30.65 ± 0.69	12.58 ± 0.58	5.40 ± 0.38	0.43 ± 0.05	0.63 ± 0.05
12	<i>C. gigantocarpa</i>	68.08 ± 6.54	32.86 ± 5.44	14.66 ± 1.40	6.87 ± 0.64	0.47 ± 0.08	0.84 ± 0.30
13	<i>C. octopetala</i>	45.98 ± 6.01	28.26 ± 1.15	11.60 ± 0.76	5.56 ± 0.36	0.48 ± 0.01	0.72 ± 0.08
14	<i>C. parafururacea</i>	37.93 ± 2.79	25.18 ± 3.54	10.69 ± 0.47	5.08 ± 0.17	0.48 ± 0.02	0.78 ± 0.18
15	<i>C. connatistyla</i>	38.98 ± 4.40	24.86 ± 4.51	9.05 ± 0.53	5.84 ± 0.28	0.65 ± 0.03	0.83 ± 0.24
Section <i>Chrysanthae</i>							
16	<i>C. euplesia</i>	29.44 ± 3.38	29.67 ± 4.45	11.26 ± 0.38	3.88 ± 0.20	0.34 ± 0.01	0.43 ± 0.10
17	<i>C. limonia</i>	19.73 ± 1.00	19.33 ± 0.10	7.39 ± 0.13	3.97 ± 0.20	0.54 ± 0.02	0.66 ± 0.04
18	<i>C. achrysantha</i>	11.73 ± 0.56	15.29 ± 0.90	5.96 ± 0.61	3.05 ± 0.17	0.52 ± 0.08	0.63 ± 0.07
19	<i>C. impressinervis</i>	37.60 ± 11.85	29.72 ± 4.29	10.74 ± 1.92	5.15 ± 0.67	0.48 ± 0.02	0.53 ± 0.02
20	<i>C. grandis</i>	11.94 ± 1.37	15.60 ± 1.37	6.30 ± 0.45	2.76 ± 0.12	0.44 ± 0.02	0.62 ± 0.04
21	<i>C. parvipetala</i>	19.51 ± 3.41	20.62 ± 2.51	7.55 ± 0.97	3.93 ± 0.23	0.52 ± 0.04	0.58 ± 0.04
22	<i>C. pinggaoensis</i>	9.07 ± 0.71	13.43 ± 0.64	5.02 ± 0.09	2.70 ± 0.21	0.54 ± 0.05	0.63 ± 0.01
23	<i>C. lunzhouensis</i>	24.77 ± 2.67	21.12 ± 1.92	8.82 ± 0.44	4.16 ± 0.29	0.47 ± 0.02	0.71 ± 0.12
24	<i>C. huana</i>	29.48 ± 4.80	26.22 ± 1.66	9.45 ± 0.78	4.77 ± 0.44	0.50 ± 0.04	0.54 ± 0.02
25	<i>C. liberofilamenta</i>	19.71 ± 3.08	22.48 ± 0.82	8.49 ± 0.44	3.67 ± 0.43	0.43 ± 0.04	0.49 ± 0.07
26	<i>C. fusuiensis</i>	36.49 ± 3.69	25.63 ± 4.47	10.60 ± 0.33	5.12 ± 0.39	0.48 ± 0.04	0.73 ± 0.22
27	<i>C. multipetala</i>	30.72 ± 1.25	26.04 ± 1.63	9.54 ± 1.08	4.81 ± 1.27	0.50 ± 0.08	0.56 ± 0.16
Genus <i>Gordonia</i>							
28	<i>G. acuminata</i>	66.89 ± 9.81	44.55 ± 4.72	18.03 ± 0.33	5.99 ± 0.92	0.33 ± 0.05	0.42 ± 0.03
29	<i>G. longicarpa</i>	70.31 ± 1.69	46.56 ± 0.75	19.11 ± 0.02	6.03 ± 0.31	0.32 ± 0.02	0.41 ± 0.00
Genus <i>Tutcheria</i>							
30	<i>T. hexalocularia</i>	52.73 ± 10.80	36.77 ± 3.16	15.03 ± 1.46	5.60 ± 0.56	0.37 ± 0.00	0.49 ± 0.02

*Archechamellia* were well separated from those of section *Chrysantha*, though the leaf morphology of section *Archechamellia* was similar to that of section *Chrysantha* and thus were difficult to distinguish from each other by naked eye.

#### Characters of the leaf epidermis

There were two types of epidermal cells in the adaxial epidermis of section *Archechamellia* (Fig. 2a; Table 3). One was relatively large with a repand anticlinal wall, the other small with a sinuous anticlinal wall. We defined this

character as various epidermal cell (VEC). VECs only occurred in section *Archechamellia* (Fig. 2a; Table 3), which made it distinguishable from the other sections. In section *Stereocarpus*, the anticlinal walls of the adaxial epidermal cells appeared sinuous (Fig. 2b; Table 3), while in section *Furfuracea*, all had arched to straight ones (Fig. 2c; Table 3). In section *Chrysantha*, four species shared sinuous anticlinal walls in the adaxial epidermal cells (Fig. 2d; Table 3), and another nine species bore repand ones (Fig. 2e; Table 3). Furthermore, the species examined in the genus *Gordonia* and *Tutcheria*, all had



**Fig. 1** PCA scatterplot based on the leaf shape data. Axes represent principle components 1 (PC1) and 2 (PC2). Each number code (see Table 2) represents one species, and related section or genus is indicated by each different symbol

arched to straight anticlinal walls in the adaxial epidermal cells (Fig. 2f, g). It was noticeable that all species examined in section *Chrysantha* had crystals in the adaxial epidermal cells (Fig. 2d, e), which had rarely been reported in the genus *Camellia*. In contrast, other sections lacked crystals in the adaxial epidermal cells.

The abaxial epidermal cells were similar among the sections in genus *Camellia*, which all had repand anticlinal walls and irregular shapes (Fig. 3a–d; Table 3). However, those of the species examined in genera *Gordonia* and *Tutcheria* had arched to straight anticlinal walls and polygonal shape (Fig. 3e, f; Table 3). Furthermore, hairs distributed on the leaf abaxial surface were only seen in *C. pubifurfuracea* (Fig. 3c), which made it distinct from the other species within section *Furfuracea*.

All species studied here were hypostomatic, and the stomata were all anisocytic, the guard cells surrounded by three subsidiary cells (Figs. 3a–f, 4a–x, 5a–x, 6a–f). Stomatal clusters, with more than one stoma adjacently arranged with common subsidiary cells, commonly occurred in all sections of genus *Camellia* (Figs. 3d, 4a–x, 5a–x, 6a–f), while they were rarely seen in *Gordonia* (Fig. 3e) and *Tutcheria* (Fig. 3f).

The secretory structures (Fig. 3b) in genus *Camellia* shared some identical features: (1) on fresh leaves in

surface view, the secretory structures appeared as brown spots; and (2) examined under LM, in surface view, they appeared as black holes, surrounded by two or three layers of small cells, which were more frequently stained red and thus easily differentiated from other epidermal cells.

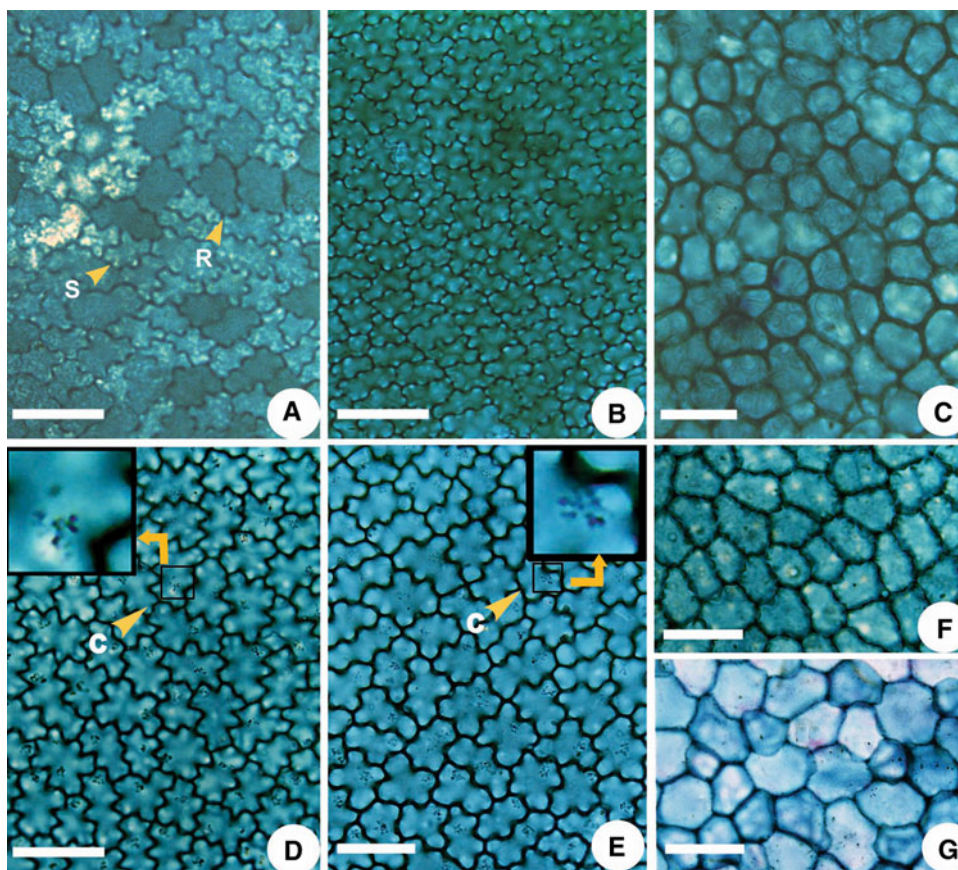
#### Leaf characters in transverse section

Discontinuous hypodermis was present in sections *Archecamellia* and *Furfuracea* (Fig. 7a, d; Table 4) of genus *Camellia*, but absent in sections *Stereocarpus* (Fig. 7b) and *Chrysantha* (Fig. 7e, f) and in genera *Gordonia* and *Tutcheria* (Fig. 7g, h). Hairs were only seen in *C. pubifurfuracea* (Fig. 7c).

All species examined had bifacial leaves. The number of layers of palisade cells varied from species to species (Table 4), and, more frequently, it was not easy to distinguish between palisade tissue and spongy tissue because of transition layers.

Stone cells were commonly present in all examined species of genus *Camellia* (Figs. 7b, 8a–aa), but few were seen in genera *Gordonia* and *Tutcheria*. Stone cells frequently occurred in palisade tissue, and some of them penetrated into spongy tissue.

All species examined had crystals in the mesophyll tissues, and only plants of section *Chrysantha* had crystals



**Fig. 2** The characters of adaxial epidermis in examined species. **a** *C. granthamiana* illustrating the general characters of section *Archechamellia*: having irregular shape and two distinct types of anticlinal walls, sinuous (arrowhead S) and repand (arrowhead R). **b** *C. liberistyla* illustrating the general characters of section *Stereocarpus*: irregular shape and sinuous anticlinal walls. **c** *C. pubifurcata* illustrating the general characters of section *Furfuracea*: polygonal shape and arc-str anticlinal walls. **d** *C. achrysantha*

illustrating the general characters of section *Chrysantha*: irregular shape and sinuous anticlinal walls. Arrowheads: C, crystals. **e** *C. parvipetala* illustrating the general characters of section *Chrysantha*: irregular shape and repand anticlinal walls. Arrowheads: C, crystals. **f** *G. acuminata* illustrating the general characters of genus *Gordonia*: polygonal shape and arc-str anticlinal walls. **g** *T. hexalocularia*: polygonal shape and arc-str anticlinal walls. Scale bar 50  $\mu$ m

in the adaxial epidermal cells (Fig. 7f). Some relatively large crystals were close under the adaxial epidermal cells, which differed from those embedded in the mesophyll tissues and were thus named hypodermic crystals (HC); they were also present only in section *Chrysantha* (Fig. 7e). These hypodermic crystals (HC) were confirmed in our semisections (Fig. 9e).

In paraffin sections, few diagnostic characters in secretory structures could be identified among the species. All secretory structures usually consisted of several layers of small, compressed cells (Figs. 7a, b, d, e, 8a–aa).

#### Semisection examinations and fluorescent investigations of secretory structures

The semisections, which had been stained by methylene blue-azure, illustrated that there were few variances in the secretory structures among species. All the secretory

structures consisted of loose, suberized tissues, surrounded by several layers of small cells. They were ellipsoidal, somewhat compressed in shape in the species of sections *Archechamellia* and *Furfuracea* (Figs. 9a, c), whereas spherical in shape in section *Chrysantha* (Fig. 9e). When examined under UV light, all emitted blue autofluorescence from the cell walls of the surrounded cells. This autofluorescence was very similar to that from stones cells (Figs. 9b, d, f).

#### Cluster analysis using data from epidermis and transverse sections

The dendrogram showed five homogeneous groups, conforming to sections *Archechamellia*, *Stereocarpus*, *Furfuracea*, and *Chrysanthae* and the outgroup genera sampled (Fig. 10). Additionally, the circumscriptions were indicated not only among the sections but also the genera,

**Table 3** Leaf epidermal characters

No.	Taxa	Abaxial epidermis					Adaxial epidermis			
		VEC	PAWEC	SEC	FCEC	Crystals	PAWEC	Hairs	Secretory	SC
Genus <i>Camellia</i>										
Section <i>Archecamellia</i>										
1	<i>C. granthamiana</i>	Present	Repand, sinuous	Irregular	1.80 ± 0.47	Absent	Repand	Absent	Present	Present
2	<i>C. albogigas</i>	Present	Repand, sinuous	Irregular	1.85 ± 0.40	Absent	Repand	Absent	Present	Present
Section <i>stereocarpus</i>										
3	<i>C. liberistyla</i>	Absent	Sinuous	Irregular	1.56 ± 0.23	Absent	Repand	Absent	Present	Present
4	<i>C. yunnanensis</i>	Absent	Sinuous	Irregular	1.62 ± 0.15	Absent	Repand	Absent	Present	Present
Section <i>Furfuracea</i>										
5	<i>C. pubifurfuracea</i>	Absent	str-arc	Polygonal	2.73 ± 0.13	Absent	Repand	Present	Present	Present
6	<i>C. latipetiolata</i>	Absent	str-arc	Polygonal	2.73 ± 0.12	Absent	Repand	Absent	Present	Present
7	<i>C. crapnelliana</i>	Absent	str-arc	Polygonal	2.43 ± 0.76	Absent	Repand	Absent	Present	Present
8	<i>C. multibracteata</i>	Absent	str-arc	Polygonal	2.82 ± 0.15	Absent	Repand	Absent	Present	Present
9	<i>C. furfuracea</i>	Absent	str-arc	Polygonal	2.86 ± 0.18	Absent	Repand	Absent	Present	Present
10	<i>C. oblata</i>	Absent	str-arc	Polygonal	2.76 ± 0.12	Absent	Repand	Absent	Present	Present
11	<i>C. gaudichaudii</i>	Absent	str-arc	Polygonal	1.80 ± 0.31	Absent	Repand	Absent	Present	Present
12	<i>C. gigantocarpa</i>	Absent	str-arc	Polygonal	2.11 ± 0.22	Absent	Repand	Absent	Present	Present
13	<i>C. octopetala</i>	Absent	str-arc	Polygonal	2.98 ± 0.57	Absent	Repand	Absent	Present	Present
14	<i>C. parafurfuracea</i>	Absent	str-arc	Polygonal	2.77 ± 0.14	Absent	Repand	Absent	Present	Present
15	<i>C. connatistyla</i>	Absent	str-arc	Polygonal	2.67 ± 0.41	Absent	Repand	Absent	Present	Present
Section <i>Chrysanthae</i>										
16	<i>C. euphlebia</i>	Absent	Sinuous	Irregular	1.92 ± 0.22	Present	Repand	Absent	Present	Present
17	<i>C. limonia</i>	Absent	Repand	Irregular	2.19 ± 0.22	Present	Repand	Absent	Present	Present
18	<i>C. achrysantha</i>	Absent	Sinuous	Irregular	1.94 ± 0.29	Present	Repand	Absent	Present	Present
19	<i>C. impressinervis</i>	Absent	Sinuous	Irregular	2.02 ± 0.33	Present	Repand	Absent	Present	Present
20	<i>C. grandis</i>	Absent	Repand	Irregular	2.71 ± 0.28	Present	Repand	Absent	Present	Present
21	<i>C. parvipetala</i>	Absent	Sinuous	Irregular	2.09 ± 0.38	Present	Repand	Absent	Present	Present
22	<i>C. pingguoensis</i>	Absent	Repand	Irregular	2.89 ± 0.24	Present	Repand	Absent	Present	Present
23	<i>C. lunzhouensis</i>	Absent	Repand	Irregular	2.39 ± 0.23	Present	Repand	Absent	Present	Present
24	<i>C. huana</i>	Absent	Repand	Irregular	2.53 ± 0.25	Present	Repand	Absent	Present	Present
25	<i>C. liberofilamenta</i>	Absent	Repand	Irregular	2.63 ± 0.27	Present	Repand	Absent	Present	Present
26	<i>C. fusuiensis</i>	Absent	Repand	Irregular	2.53 ± 0.34	Present	Repand	Absent	Present	Present
27	<i>C. multipetala</i>	Absent	Repand	Irregular	2.03 ± 0.21	Present	Repand	Absent	Present	Present
Genus <i>Gordonia</i>										
28	<i>G. acuminata</i>	Absent	str-arc	Polygonal	2.93 ± 0.27	Absent	str-arc	Absent	Absent	Absent
29	<i>G. longicarpa</i>	Absent	str-arc	Polygonal	3.02 ± 0.14	Absent	str-arc	Absent	Absent	Absent
Genus <i>Tutcheria</i>										
30	<i>T. hexalocularia</i>	Absent	str-arc	Polygonal	3.07 ± 0.19	Absent	str-arc	Absent	Absent	Absent

VEC various epidermal cells, PAWEC pattern of anticlinal walls of epidermal cells, FCEC form coefficient of epidermal cells, SC stomatal clusters, str-arc straight to arched, SEC shape of epidermal cells)

which was consistent with results of PCA based on leaf morphological data (Fig. 1).

Subtraction and cluster analysis based on leaf FTIR data

Comparisons of leaf FTIR spectra indicated that most differences were specific to the carbohydrate fingerprinting

region (wavenumbers 1,800–700 cm<sup>-1</sup>) (Kim et al. 2004). Figure 11a, b illustrates the variances of chemical compositions of the examined species in the four disputed sections. Most of the spectra had some characteristic absorbances that were constant within the same section or genus, but distinct among sections or genera (Fig. 11c). There were significant biomarkers in the spectra at



**Fig. 3** The characters of abaxial epidermis in examined species. **a–d** the abaxial epidermal characters of the *Camellia* species with secretory structures: repand anticlinal walls and irregular shape of epidermal cells.

**a** *C. granthamiana*.

**b** *C. liberistyla* showing secretory structures (SS).

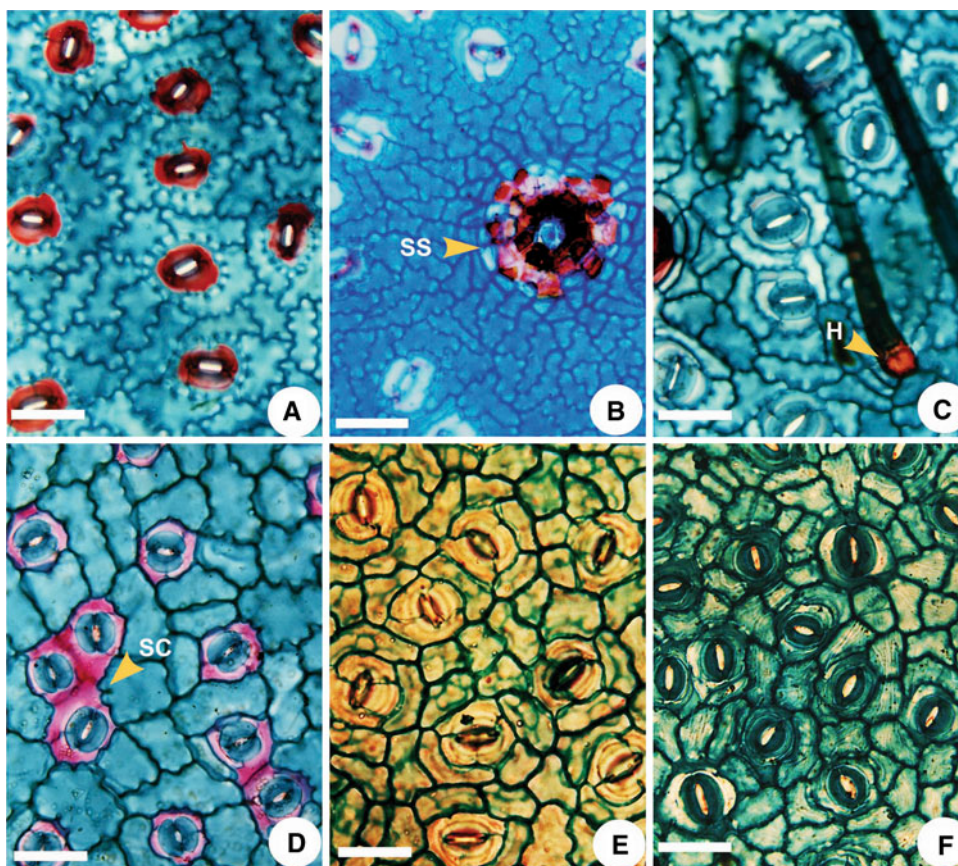
**c** *C. pubifurfuracea* showing hairs (H).

**d** *C. parvipetala* showing stomatal cluster (SC).

**e, f** *G. acuminata* and

*T. hexalocularia*, respectively:

polygonal shape and arc-str anticlinal walls. Scale bar 50  $\mu\text{m}$



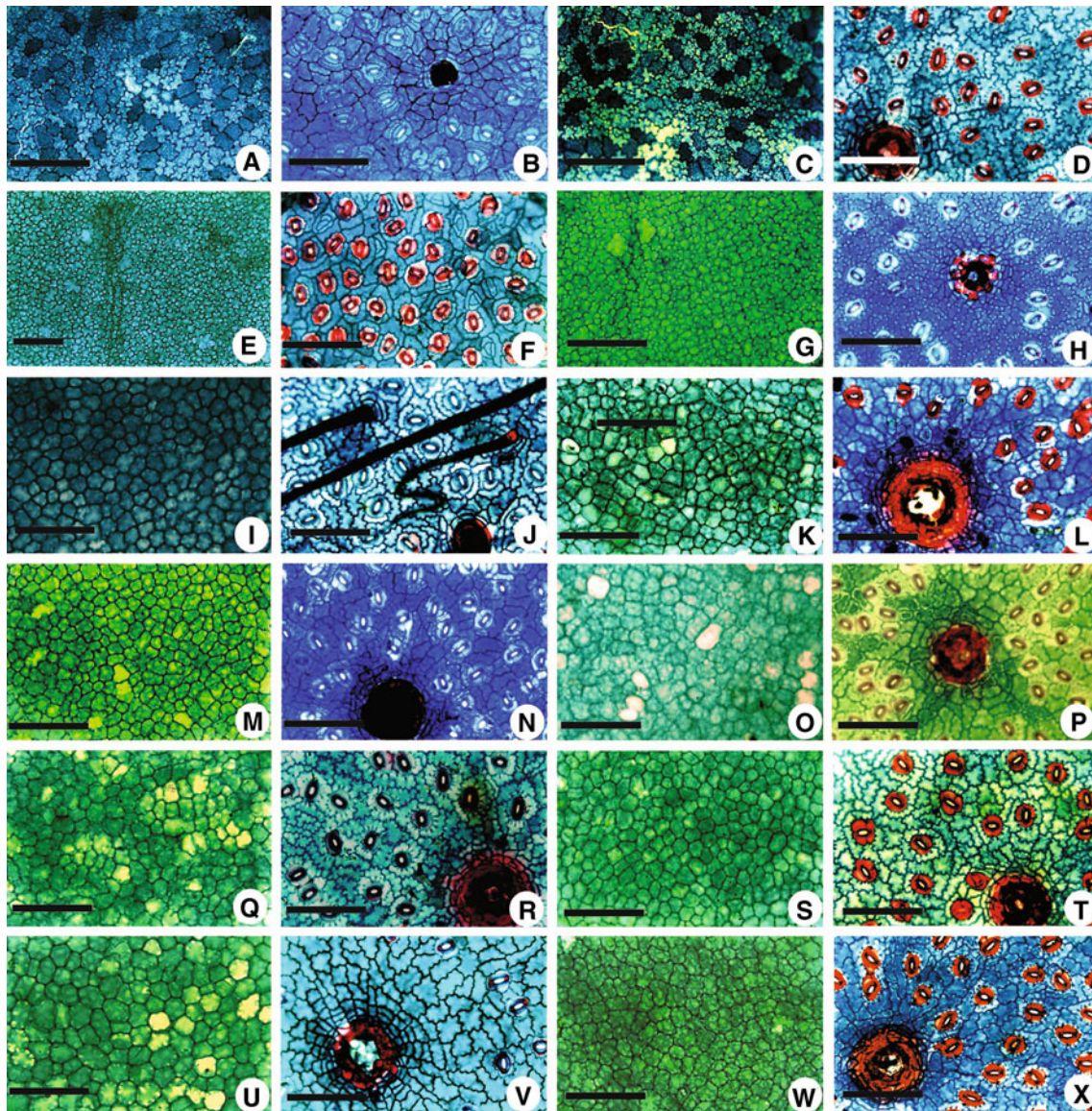
wavenumbers of 1,733, 1,651, 1,601, 1,555, 1,446, 1,415, 1,320, 1,271, 1,235, 1,145, 1,046, and 895  $\text{cm}^{-1}$  (Fig. 11c). The different spectra generated by digital subtraction of the spectra of the disputed sections showed that section C1 (*Archecamellia*), C2 (*Stereocarpus*), C3 (*Furfuracea*), and C4 (*Chrysanthae*) had marked differences (Fig. 11d). Differences at wavenumbers of 1,733, 1,651, 1,555, 1,446, 1,320, 1,235, 1,046, and 895  $\text{cm}^{-1}$  were found in the digital subtraction of the spectra of C1 (*Archecamellia*) from the spectra of C4 (*Chrysanthae*). Accordingly, digital subtractions of the spectra of C3 from C1, C3 from C2, and C2 from C1 also showed differences at wavenumbers of 1,733, 1,651, 1,601, 1,446, 1,320, 1,235, and 1,046  $\text{cm}^{-1}$ , of 1,733, 1,651, 1,601, 1,446, 1,415, 1,320, 1,271, 1,235, 1,145, 1,046, and 895  $\text{cm}^{-1}$ , and of 1,733, 1,651, 1,446, 1,320, 1,271, 1,046, and 895  $\text{cm}^{-1}$ , respectively (Fig. 11d). Furthermore, the clustering dendrogram (Fig. 12), resulting from the FTIR data by 0–1 normalization and five-point smoothing, illustrated the detailed relationships of the taxa examined. As shown in Fig. 12, generally, both genera and sections which were closely related grouped together, except for *C. parvipetala*, which clustered with section *Furfuracea*.

## Discussion

Taxonomic significance of integrated leaf characters in *Camellia* species with secretory structures

*Taxonomic significance of the secretory structures in Camellia plants*

Secretory structures have frequently been reported in species of *Camellia*, but the descriptions of these secretory structures varied and were even conflicting because of few anatomical studies (Keng 1962; Parks and Griffiths 1963; Chang 1996, 1998; Ye 1993; Ming 2000). Originally, Keng (1962) and Parks and Griffiths (1963) identified them as cork warts. Later, Chang (1996, 1998) described them as secretory structures, while Ye (1993) considered them as black glands, whereas Ming (2000) termed them brown glands. Furthermore, although the presence of secretory structures in *Camellia* species could serve as a diagnostic characteristic, it also brought controversy in the taxonomy of the genus *Camellia*. Chang (1981) merged *C. liberistyla* H.T. Chang and *C. yunnanensis* H.T. Chang into section *Stereocarpus* because of the presence of secretory structures in both species, while Ming (2000) transferred them



**Fig. 4** Characteristics of epidermal cells: adaxial epidermis on the left, abaxial epidermis on the right. **a, b** *C. granthamiana*. **c, d** *C. albogigas*. **e, f** *C. liberistyla*. **g, h** *C. yunnanensis*. **i, j** *C. pubifurfuracea*.

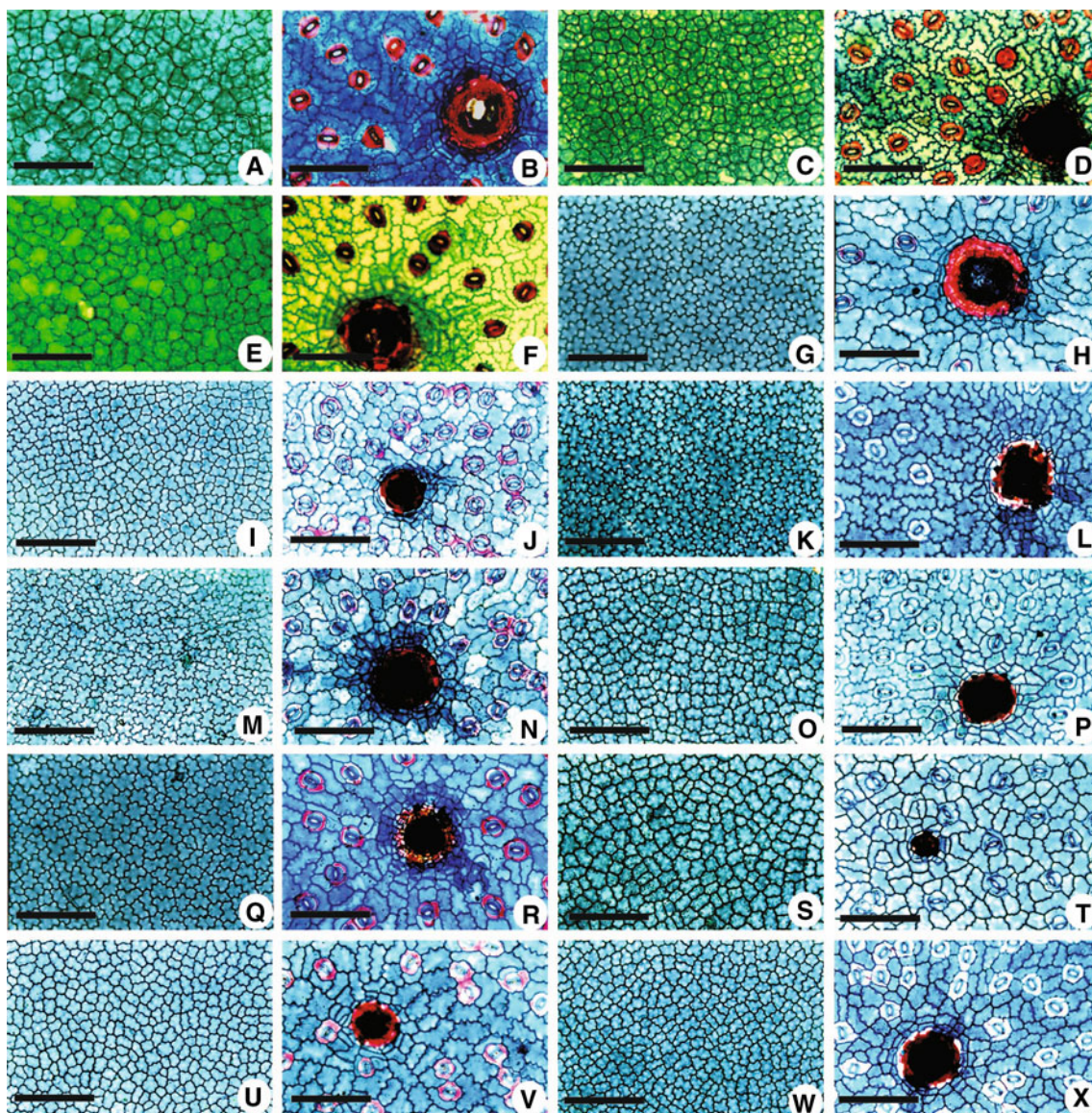
**k, l** *C. latipetiolata*. **m, n** *C. crapnelliana*. **o, p** *C. multibracteata*. **q, r** *C. furfuracea*. **s, t** *C. oblate*. **u, v** *C. gaudichaudii*. **w, x** *C. gigantocarpa*. Scale bar 100  $\mu\text{m}$

from section *Stereocarpus* into section *Heterogenea* because the secretory structures were overlooked when observed by naked eye. Ming (2000) also merged section *Chrysantha* H.T. Chang (1981, 1998) into section *Arche-camellia* Sealy based on comprehensive similarity in morphology, including presence of brown glands, in these two sections. Since it was very difficult to differentiate between the similar colors of the structures by naked eye (for example, dark, black, and brown), a comprehensive anatomical study of the secretory structures was conducted in the present study. Leaf anatomic characters showed that these structures were surrounded by several layers of relatively smaller cells, with ellipsoidal, and somewhat compressed shape in sections *Arche-camellia* and

*Furfuracea* (Fig. 9a, c), but spherical shape in section *Chrysantha* (Fig. 9e). Furthermore, when cross-sections of secretory structures were examined under fluorescent light, the cell walls had the same autofluorescence as stone cells (Fig. 9). These results indicated that the surrounding cells were different in shape among sections, but all may have thickened lignified walls, which supports well their identification as cork warts by Keng (1962) and Parks and Griffiths (1963).

#### Leaf shape analysis for classification

Analysis of leaf shape data using multivariate techniques such as PCA (Sneath and Sokal 1973) has been



**Fig. 5** Characteristics of epidermal cells: adaxial epidermis on the left, abaxial epidermis on the right. **a, b** *C. octopetala*. **c, d** *C. parafurfuracea*. **e, f** *C. connatistyla*. **g, h** *C. euphlebica*. **i, j** *C. limonia*.

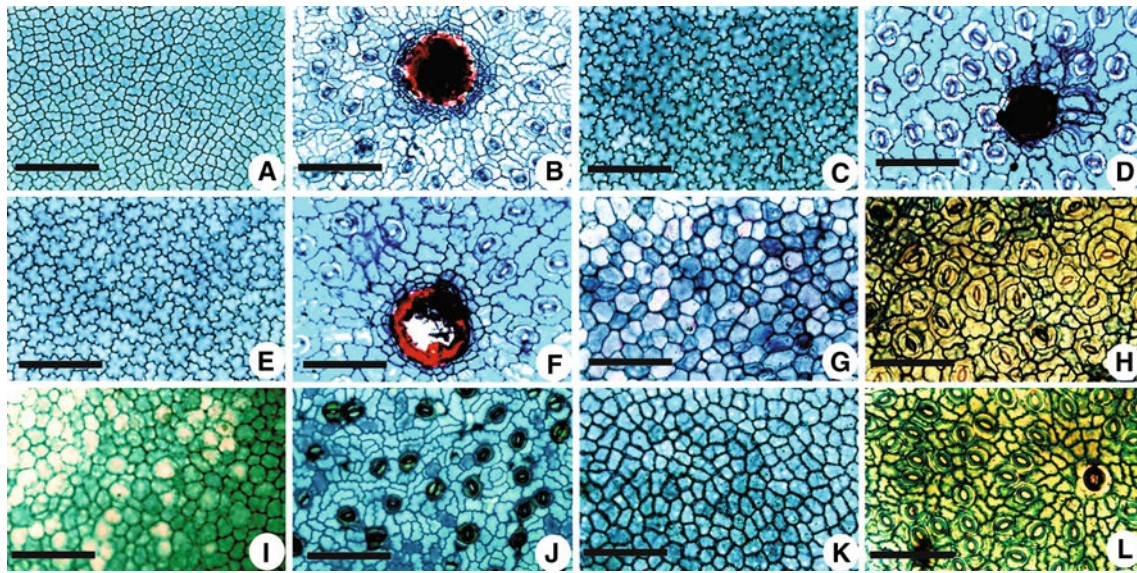
**k, l** *C. achrysantha*. **m, n** *C. impressinervis*. **o, p** *C. grandis*. **q, r** *C. parvipetala*. **s, t** *C. pingguoensis*. **u, v** *C. lunzhouensis*. **w, x** *C. huana*. Scale bar 100  $\mu$ m

successfully employed for investigation of taxonomic relationships in the past (Premoli 1996; Hodálová and Marhold 1998; Parnell and Needham 1998; Meade and Parnell 2003; Plotze et al. 2005). In the present work, quantitative leaf characters were analyzed by PCA. Our results showed that leaf  $F_c$  and other quantitative characters could well separate the disputed sections in *Camellia* with secretory structures. Figure 1 shows that *G. acuminata*, *G. longicarpa*, and *T. hexalocularia* were significantly separated from other species, indicating the difference between the outgroups (genus *Gordonia*, genus *Tutcheria*) and the *Camellia* species with secretory structures. Furthermore, most *Chrysantha* and *Furfuracea* species were

also separated from one another. Two species from section *Archechamellia* and two species from section *Stereocarpus* also formed a cluster in the PCA, respectively. These statistical analyses of leaf shape data thus revealed their potential as powerful tools for taxonomic differentiation in *Camellia* species with secretory structures.

#### Leaf anatomic characters for classification

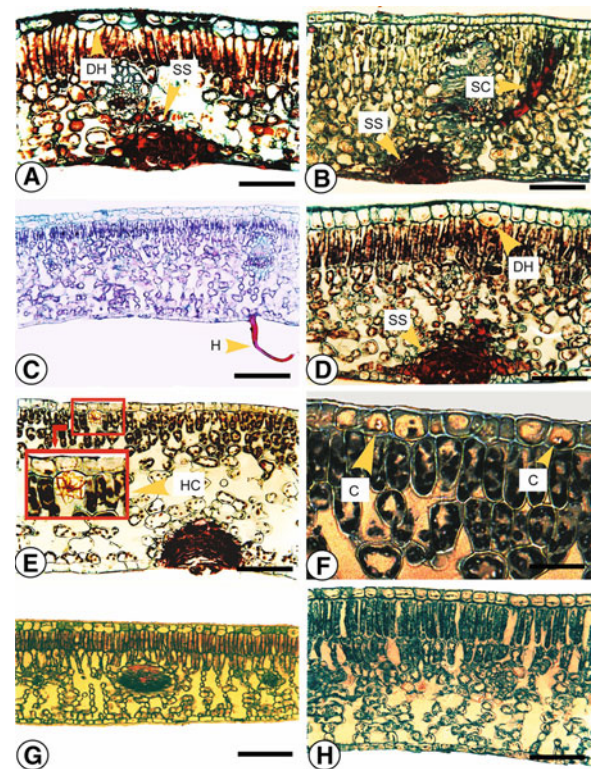
The study reported on herein was carried out to evaluate whether leaf anatomy could be used to provide additional data of taxonomic significance similar to our studies in other *Camellia* species (Lin et al. 2008; Lu et al. 2008a,



**Fig. 6** Characteristics of epidermal cells: adaxial epidermis on the left, abaxial epidermis on the right. **a, b** *C. liberofilamentata*. **c, d** *C. fusuiensis*. **e, f** *C. multipetala*. **g, h** *G. acuminata*. **i, j** *G. longicarpa*. **k, l** *T. hexalocularia*. Scale bar 100  $\mu\text{m}$

2008b). Leaf epidermal characters have proven to be of great use in identifying species, e.g., in Magnoliaceae (Baranova 1972), Sterculiaceae (Sterculiaceae) (Hussin and Sani 1998), Schisandraceae (Schisandraceae) (Yang and Lin 2005), and in *Camellia* sections *Oleifera* (Lin et al. 2008), *Tuberculata* (Lu et al. 2008a), and *Thea* (Lu et al. 2008b). Various epidermal cells and discontinuous hypodermis in the lamina were present in section *Archecamellia* and absent in section *Chrysantha*. Hypodermic crystals were only present in section *Chrysantha*. These characters thus partly support the treatments of *Camellia* species with secretory structures by Chang (1981, 1998) that section *Chrysantha* should not be merged into section *Archecamellia*.

Sinuuous anticlinal walls were found in adaxial epidermal cells in section *Stereocarpus* and arched to straight ones in *Furfuracea* species, which separated section *Stereocarpus* from section *Furfuracea*. This disagrees with Ming's merging of two *Stereocarpus* species with *Furfuracea* species as section *Heterogenea*. Although qualitative anatomic characters selected in the present work, which successfully classified the disputed sections in *Camellia* species with secretory structures, could not identify all species, multivariate analysis based on all quantitative and qualitative leaf characters (Tables 3, 4) clearly indicated that the species were different (Fig. 10). Figure 10 shows that four sections of *Camellia* species with secretory structures were significantly distant from the two out-groups, and these four sections also formed homogeneous groups, indicating their high homogeneity. These results support Chang's classification (1981, 1996) in which he recognized four sections.



**Fig. 7** Characters in transverse view. SS secretory structures (arrowheads). DH discontinuous hypodermis (arrowheads). **a** *C. granthamiana* illustrating the general characters of section *Archecamellia*. Scale bar 76  $\mu\text{m}$ . **b** *C. liberistyla* illustrating the general characters of section *Stereocarpus*. SC, stone cells (arrowhead). Scale bar 76  $\mu\text{m}$ . **c** *C. pubifurfuracea*. **h** hairs (arrowhead). Scale bar 152  $\mu\text{m}$ . **d** *C. oblate* illustrating the general characters of section *Furfuracea*. Scale bar 76  $\mu\text{m}$ . **e, f** *C. liberofilamentata* in section *Chrysantha*. **c**, crystals. **e** Scale bar 76  $\mu\text{m}$ . **f** Scale bar 19  $\mu\text{m}$ . **g** *G. acuminata*. Scale bar 152  $\mu\text{m}$ . **h** *T. hexalocularia*. Scale bar 76  $\mu\text{m}$

**Table 4** Leaf characters in transverse section

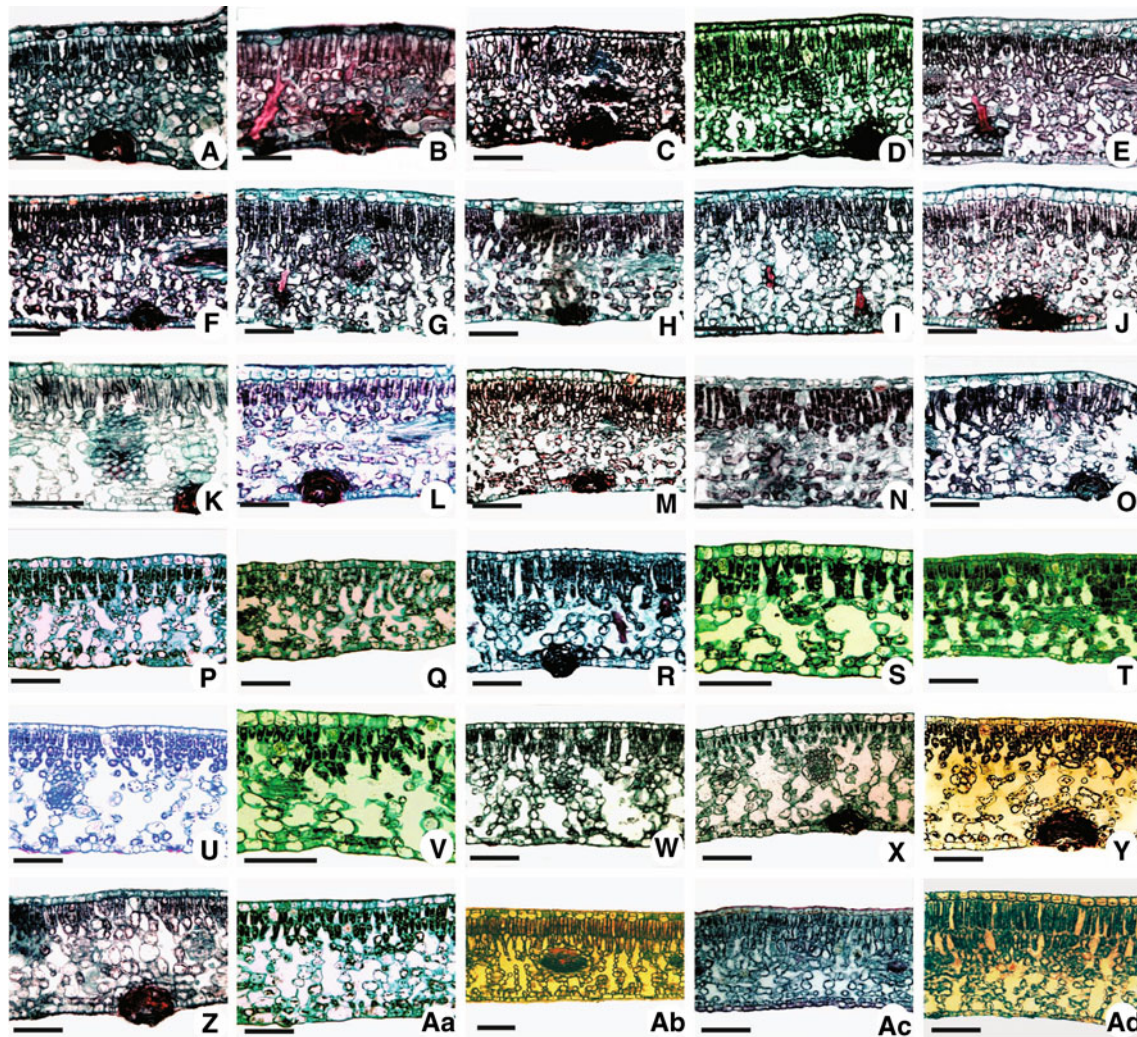
No.	Taxa	Adaxial epidermis		Abaxial epidermis Thickness (cm)	Mesophyll					
		Thickness (cm)	Hypodermis		Palisade tissue			Spongy tissue		
					HC	Thickness (cm)	Layers	Thickness (cm)	Stone cells	
Genus <i>Camellia</i>										
Section <i>Archecamellia</i>										
1	<i>C. granthamiana</i>	27.84 ± 2.35	Present	15.58 ± 3.25	Absent	74.3 ± 8.62	1–2	138.20 ± 18.25	Present	
2	<i>C. albogigas</i>	39.52 ± 6.18	Present	15.91 ± 1.63	Absent	67.29 ± 5.74	1	159.09 ± 11.84	Present	
Section <i>Stereocarpus</i>										
3	<i>C. liberistyla</i>	14.53 ± 2.86	Absent	11.66 ± 2.80	Absent	91.99 ± 14.41	2–3	140.26 ± 29.63	Present	
4	<i>C. yunnanensis</i>	15.23 ± 4.32	Absent	11.45 ± 2.44	Absent	93.22 ± 8.32	2–3	138.34 ± 16.88	Present	
Section <i>Furfuracea</i>										
5	<i>C. pubifurfuracea</i>	41.01 ± 3.14	Present	17.16 ± 2.40	Absent	60.06 ± 15.24	1–2	284.41 ± 33.89	Present	
6	<i>C. latipetiolata</i>	31.08 ± 3.28	Present	15.76 ± 1.12	Absent	92.56 ± 10.29	2–3	239.35 ± 17.69	Present	
7	<i>C. crapnelliana</i>	31.48 ± 5.42	Present	19.15 ± 2.87	Absent	90.50 ± 9.86	1–2	246.94 ± 23.42	Present	
8	<i>C. multibracteata</i>	33.21 ± 2.24	Present	17.29 ± 2.13	Absent	79.54 ± 11.28	1–2	200.04 ± 28.56	Present	
9	<i>C. furfuracea</i>	31.08 ± 4.51	Present	20.39 ± 2.18	Absent	92.04 ± 9.66	2–3	251.60 ± 15.91	Present	
10	<i>C. oblata</i>	37.00 ± 2.43	Present	19.51 ± 4.32	Absent	71.02 ± 5.94	1–2	173.82 ± 10.12	Present	
11	<i>C. gaudichaudii</i>	37.00 ± 1.17	Present	19.94 ± 2.47	Absent	79.66 ± 8.47	1	217.25 ± 34.69	Present	
12	<i>C. gigantocarpa</i>	34.96 ± 2.87	Present	18.23 ± 2.76	Absent	49.74 ± 6.44	1–2	183.65 ± 25.19	Present	
13	<i>C. octopetala</i>	36.30 ± 5.65	Present	19.87 ± 3.20	Absent	85.67 ± 12.52	1–2	221.63 ± 19.28	Present	
14	<i>C. parafurfuracea</i>	26.64 ± 1.95	Present	14.24 ± 1.16	Absent	77.69 ± 9.27	1–2	174.59 ± 14.56	Present	
15	<i>C. connatistyla</i>	34.38 ± 2.15	Present	16.94 ± 2.88	Absent	82.91 ± 6.85	1–2	223.85 ± 21.98	Present	
Section <i>Chrysanthae</i>										
16	<i>C. euphlebica</i>	26.10 ± 2.92	Absent	30.63 ± 5.69	Present	71.56 ± 10.75	1–2	155.14 ± 15.95	Present	
17	<i>C. limonia</i>	18.38 ± 0.77	Absent	21.97 ± 3.76	Present	62.57 ± 7.87	1–2	119.43 ± 9.87	Present	
18	<i>C. achrysantha</i>	21.39 ± 2.01	Absent	25.93 ± 5.33	Present	81.33 ± 24.47	1–2	126.67 ± 38.82	Present	
19	<i>C. impressinervis</i>	26.14 ± 1.12	Absent	14.44 ± 1.64	Present	94.21 ± 13.54	2–3	105.86 ± 11.93	Present	
20	<i>C. grandis</i>	19.08 ± 0.85	Absent	14.77 ± 1.20	Present	44.13 ± 6.56	1–2	111.29 ± 14.02	Present	
21	<i>C. parvipetala</i>	16.58 ± 0.95	Absent	27.90 ± 3.63	Present	110.07 ± 21.51	2–3	177.25 ± 24.86	Present	
22	<i>C. pingguoensis</i>	23.46 ± 1.97	Absent	18.29 ± 1.54	Present	61.99 ± 4.44	1–2	122.22 ± 21.26	Present	
23	<i>C. lunzhouensis</i>	23.91 ± 1.50	Absent	22.25 ± 2.53	Present	96.88 ± 8.82	2–3	155.75 ± 9.64	Present	
24	<i>C. huana</i>	21.03 ± 2.06	Absent	17.42 ± 1.77	Present	70.05 ± 6.18	1–2	133.84 ± 7.28	Present	
25	<i>C. liberofilamenta</i>	17.13 ± 1.50	Absent	19.69 ± 1.90	Present	79.48 ± 3.52	1–2	158.80 ± 7.20	Present	
26	<i>C. fusuensis</i>	30.72 ± 1.36	Absent	24.37 ± 5.62	Present	84.45 ± 7.50	2–3	206.40 ± 8.10	Present	
27	<i>C. multipetala</i>	20.56 ± 1.50	Absent	23.57 ± 2.22	Present	79.88 ± 13.35	2–3	137.81 ± 9.66	Present	
Genus <i>Gordonia</i>										
28	<i>G. acuminata</i>	34.84 ± 4.51	Absent	17.69 ± 1.41	Absent	125.94 ± 9.23	2	155.06 ± 11.78	Absent	
29	<i>G. longicarpa</i>	36.12 ± 3.67	Absent	16.24 ± 2.34	Absent	126.37 ± 4.75	2–3	156.51 ± 13.83	Absent	
Genus <i>Tutcheria</i>										
30	<i>T. hexalocularia</i>	21.83 ± 1.95	Absent	15.28 ± 2.63	Absent	75.27 ± 7.24	1–2	192.53 ± 15.35	Absent	

HC hypodermic crystals

Form coefficient of leaf epidermal cells and its taxonomic value

The shapes of leaf epidermal cells are usually constant within the same species, genus, or higher taxa, and thus are frequently used in taxonomy and classification (Kong 2001; Yang and Lin 2005). Various shapes of epidermal

cells can be distinguished in different taxa, for instance, elongated ones for many monocotyledons. However, use of this character in studying relationships between species or genera is still limited, because the shapes of epidermal cells are extremely diverse and thus very difficult to describe. Additionally, much confusion occurs because different taxonomists more often use different terminologies to



**Fig. 8** Leaf transverse sections of the examined species. **a** *C. granthamiana*. **b** *C. albogigas*. **c** *C. liberistyla*. **d** *C. yunnanensis*. **e** *C. pubifurfuracea*. **f** *C. latipetiolata*. **g** *C. crapnelliana*. **h** *C. multibracteata*. **i** *C. furfuracea*. **j** *C. obovate*. **k** *C. gaudichaudii*. **l** *C. gigantocarpa*. **m** *C. octopetala*. **n** *C. parafurfuracea*. **o** *C. connatistyla*.

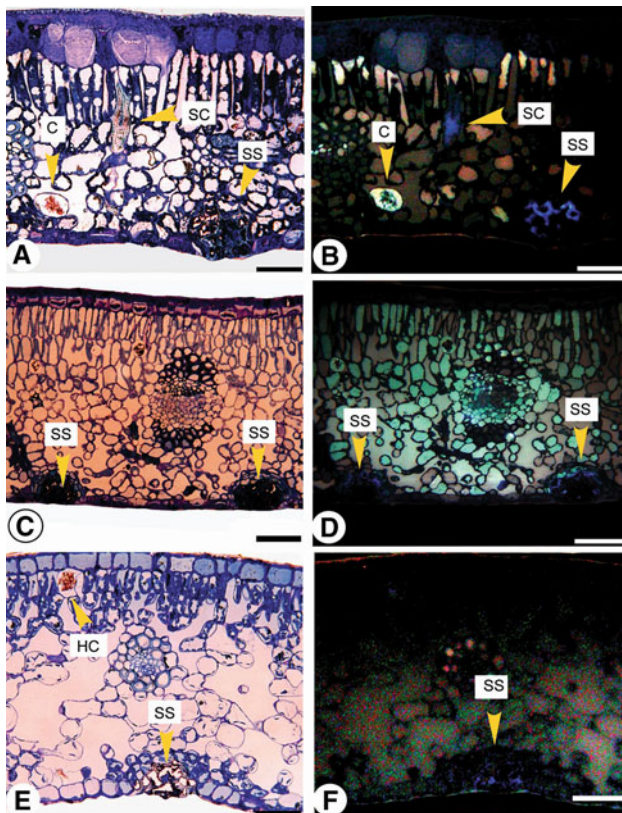
**p** *C. euphlebia*. **q** *C. limonia*. **r** *C. achrysantha*. **s** *C. impressinervis*. **t** *C. grandis*. **u** *C. parvipetala*. **v** *C. pingguoensis*. **w** *C. lunzhouensis*. **x** *C. huana*. **y** *C. liberofilamenta*. **z** *C. fusuiensis*. **aa** *C. multipetala*. **ab** *G. acuminata*. **ac** *G. longicarpa*. **ad** *T. hexalocularia*. Scale bar 50  $\mu$ m

describe even the same type (Kong 2001). Thus, it is desirable to develop a mathematical method to estimate the various shapes of epidermal cells reliably. In the present study, we propose a new nomenclature, i.e., the form coefficient ( $F_c$ ), to assess the shapes of epidermal cells. Usually, the  $F_c$  of polygonal cells is larger than that of irregular ones (Table 3). More often, the form coefficient is closely related to the pattern of anticlinal walls: cells with arched to straight walls > cells with repand walls > cells with sinuous walls (Table 3). It is possible to produce some interesting arguments because, to some extent, this value is also related to the ratio of width to length. We overlooked this parameter, because most epidermal cell shapes are irregular and thus an accurate ratio of width to length is very difficult to obtain. Despite this limitation, the form

coefficient is still useful to estimate the shapes of epidermal cells, because the Pearson correlation test indicates that there is a significant correlation between  $F_c$  and epidermal cell shape ( $r = 0.6517$ ,  $p < 0.001$ ) and an excellent correlation between  $F_c$  and the pattern of anticlinal walls of epidermal cells ( $r = 0.8203$ ,  $p < 0.001$ ).

#### Leaf FTIR analysis for classification

Kim et al. (2004) reported that FTIR was an excellent method for determining phylogenetic relationships between flowering plants based on its ease of use and quick results. Gorgulu et al. (2007) used FTIR to characterize and differentiate species in the genera *Acantholimon*, *Ranunculus*, and *Astragalus* and suggested that FTIR



**Fig. 9** Comparative semisection characters under light microscope (*left*) and fluorescent microscope (*right*) with UV-light excitation, showing that all secretory structures have similar autofluorescence to stone cells. SS, secretory structures (*arrowheads*). **a, b** *C. albogigas* illustrating the general characters of section *Archecamellia*. C, crystals (*arrowhead*). SC, stone cells (*arrowhead*). **c, d** *C. latipetiolata* illustrating the general characters of section *Furfuracea*. **e, f** *C. huana* illustrating the general characters of section *Chrysantha*. HC, hypodermic crystals. Scale bar 25  $\mu\text{m}$

could be introduced as a novel method for discrimination of plants based on their chemical structural differences at the level of genus, and even at the level of species living in different environmental conditions. Furthermore, FTIR had been successfully applied to solve the controversy regarding sections *Tuberculata* (Lu et al. 2008a), *Thea* (Lu et al. 2008b), *Oleifera*, *Paracamellia*, *Camellia*, and *Thea* (Shen et al. 2008) in genus *Camellia*. It was suggested that FTIR analysis combined with comparative leaf anatomical studies could be regarded as a reliable method for taxonomic treatments. In the present study, subtraction of the spectra among the disputed *Camellia* sections qualitatively illustrated their marked differences (Fig. 11c, d). Cluster analysis of leaf FTIR data quantitatively visualized the degree of affinity among the examined species (Fig. 12). These results were consistent with the leaf shape analysis and cluster analysis of leaf anatomic characters, in support of Chang's (1981, 1998) classification of *Camellia* species with secretory structures. This also indicated the

usefulness of FTIR for the taxonomic treatment of flowering plants.

#### Evidence from leaves for the classification of *Camellia* species with secretory structures

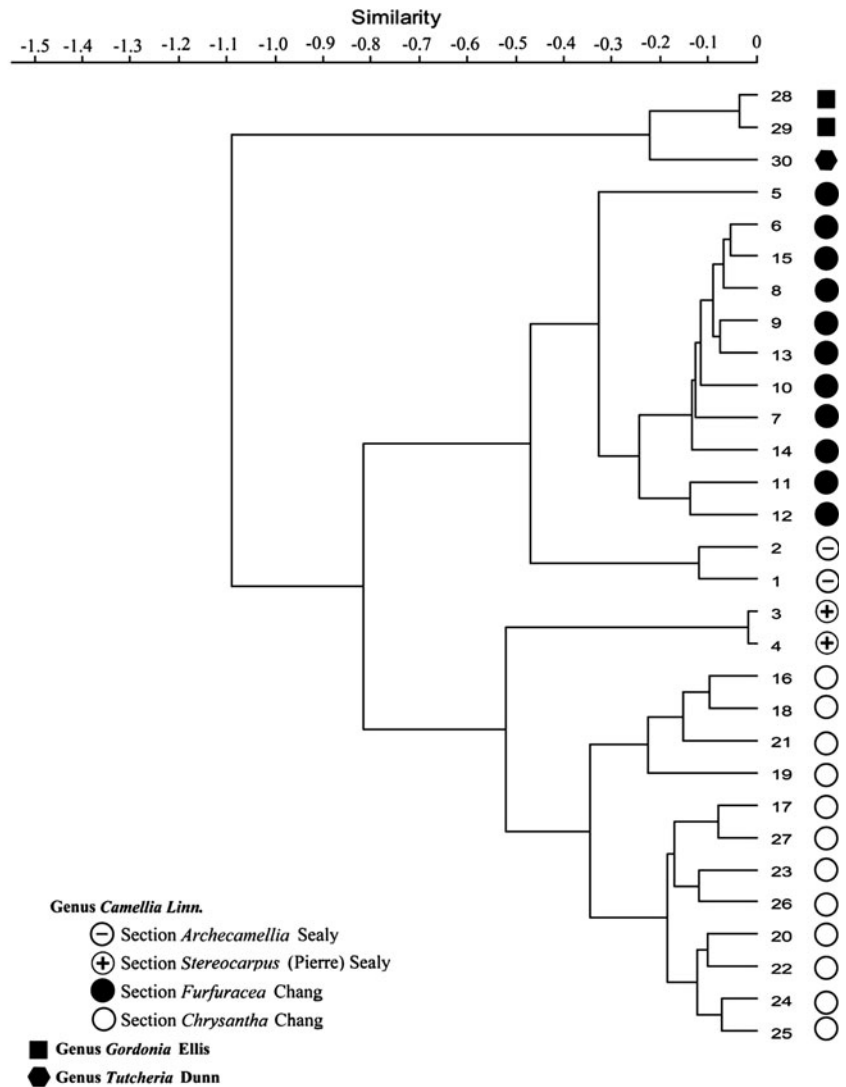
Leaf morphology has always played an important role in traditional plant taxonomy, particularly for identifying taxa in which variation in floral structures is uninformative or in which flowering specimens are infrequent owing to, for example, a limited flowering season (Stace 1989) or dioecy (Möller et al. 2007). However, it is not always possible to characterize plants by leaf morphology, because of various leaf morphologies under different environmental conditions and/or the disputed description of the same character. Therefore, recently, increasing attention has been paid to how to use other leaf characters to discriminate disputed species (Jensen et al. 2002; Meade and Parnell 2003; Neto et al. 2006; Gorgulu et al. 2007). In the present work, integrated leaf characters including leaf shape analyses, leaf anatomical investigations, and cluster analysis of leaf FTIR data succeeded in differentiating disputed species. Therefore, we propose a potential treatment in which integrated leaf characters could be applied in the future as a sensitive and reliable method to distinguish different taxa where only leaves or a small portion of leaves are available rather than the whole plant.

#### Taxonomic treatment of *Camellia* species with secretory structures

##### *Taxonomic position of the section Chrysantha* H.T. Chang

After section *Chrysantha* H.T. Chang was published by Chang in 1979, its taxonomic status remained controversial for many years (Ming and Zhang 1993, 1996; Ye 1993; Chang 1981, 1996, 1998; Chang and Bartholomew 1984). According to Chang (1998), most plants of this section are only distributed in a limited area of Guangxi Province in China, and just four of them occur in the north of Vietnam, adjacent to Guangxi Province. The species of this section are characterized by axillary, yellow flowers, distinct, persistent bracts and sepals, and 3–5 cleft styles. Yellow flowers are regarded as a key character of this section, distinguishing it from other *Camellia* sections (Ye 1993; Chang 1981, 1998). Ming and Zhang (1993, 1996) and Ming (2000) argued that, with the exception of yellow flowers, most features are similar to plants of section *Archecamellia* Sealy. Thus, he disagreed with Chang on the setup of section *Chrysantha* and transferred most species of section *Chrysantha* into section *Archecamellia*. However, notably, molecular evidence also did not support the merging of section *Chrysantha* into Sealy's section

**Fig. 10** Phenogram obtained from UPGMA cluster analysis using the data of leaf anatomy. Each number code (see Table 2) represents a species, and related section or genus is indicated by each different symbol



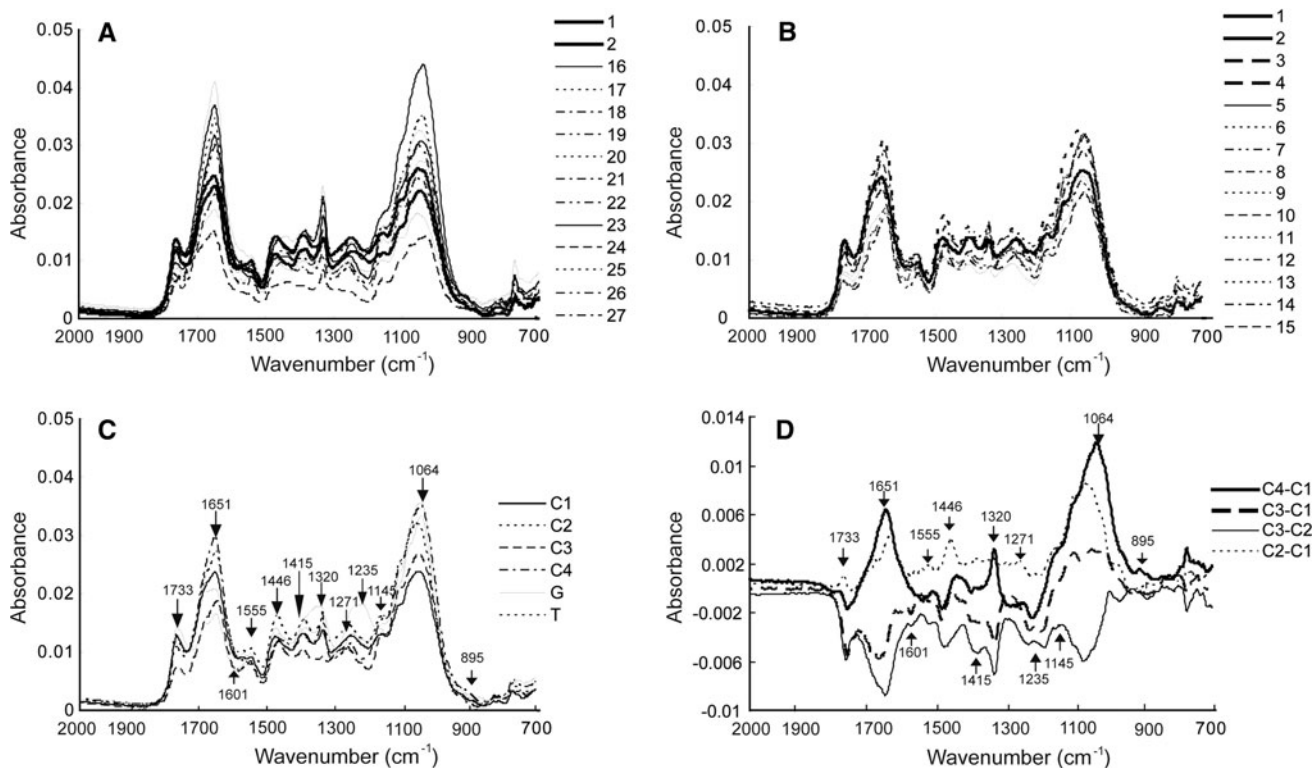
*Archeamellia* (Tang et al. 2001; Tang et al. 2006). In the present study, our investigations showed that many leaf morphological and anatomical features of section *Chrysantha* differ from those of section *Archeamellia* Sealy, such as: (1) various epidermal cells and discontinuous hypodermis were present in section *Archeamellia* Sealy but absent in section *Chrysantha*; (2) crystals only occurred in adaxial epidermal cells in section *Chrysantha* but were absent in section *Archeamellia*; (3) hypodermic crystals were usually seen in the former but not seen in the latter section; and (4) the shapes of the surrounding cells of the secretory structures were ellipsoidal, somewhat compressed in section *Archeamellia*, while they were spherical in section *Chrysantha*. Particularly, to date, few publications have described crystals occurring in adaxial epidermal cells in flowering plants, except in *Camellia* section *Chrysantha* in the present work (Figs. 2d, e, 7f). Moreover, our multivariate analysis using anatomic and FTIR data also successfully separated section *Chrysantha*

from section *Archeamellia* (Figs. 10, 12). Therefore, we suggest that section *Chrysantha* should not be merged into section *Archeamellia*.

#### *Delimitation of sections Archeamellia Sealy, Stereocarpus (Pierre) Sealy, and Furfuracea H.T. Chang*

Although sections *Archeamellia* and *Stereocarpus* were retained in Sealy's (1958), Chang's (1998), and Ming's (2000) classifications, conflicts still persisted. The number of species recognized in these two sections varied significantly, with an estimated 7 in *Archeamellia* and 1 in *Stereocarpus* by Sealy (1958), 3 in *Archeamellia* and 5 in *Stereocarpus* by Chang (1998), and 18 in *Archeamellia* and 3 in *Stereocarpus* by Ming (1999, 2000), indicating significant problems with species affinities. More and more new species were recognized after Sealy's work was published, thus the taxonomic treatments of Chang (1981, 1998) and Ming (1999, 2000) of the genus *Camellia* were





**Fig. 11** Leaf comparative FTIR spectra in the fingerprinting region ( $1,800\text{--}700\text{ cm}^{-1}$ ). Each number code (see Table 2) represents a species. **a** Comparative FTIR spectra of sections *Archecamellia* and *Chrysanthia*. **b** Comparative FTIR spectra of sections *Archecamellia*, *Stereocarpus*, and *Furfuracea*. **c** Average FTIR spectra of section *Archecamellia* (C1), section *Stereocarpus* (C2), section *Furfuracea*

(C3), section *Chrysanthia* (C4), genus *Gordonia* (G), and genus *Tucheria* (T). Arrows show different biomarkers in four sections and two outgroups. **d** Digital subtraction of the spectra of four disputed sections. Arrows indicate different biomarkers in four sections: *Archecamellia* (C1), *Stereocarpus* (C2), *Furfuracea* (C3), and *Chrysanthia* (C4)

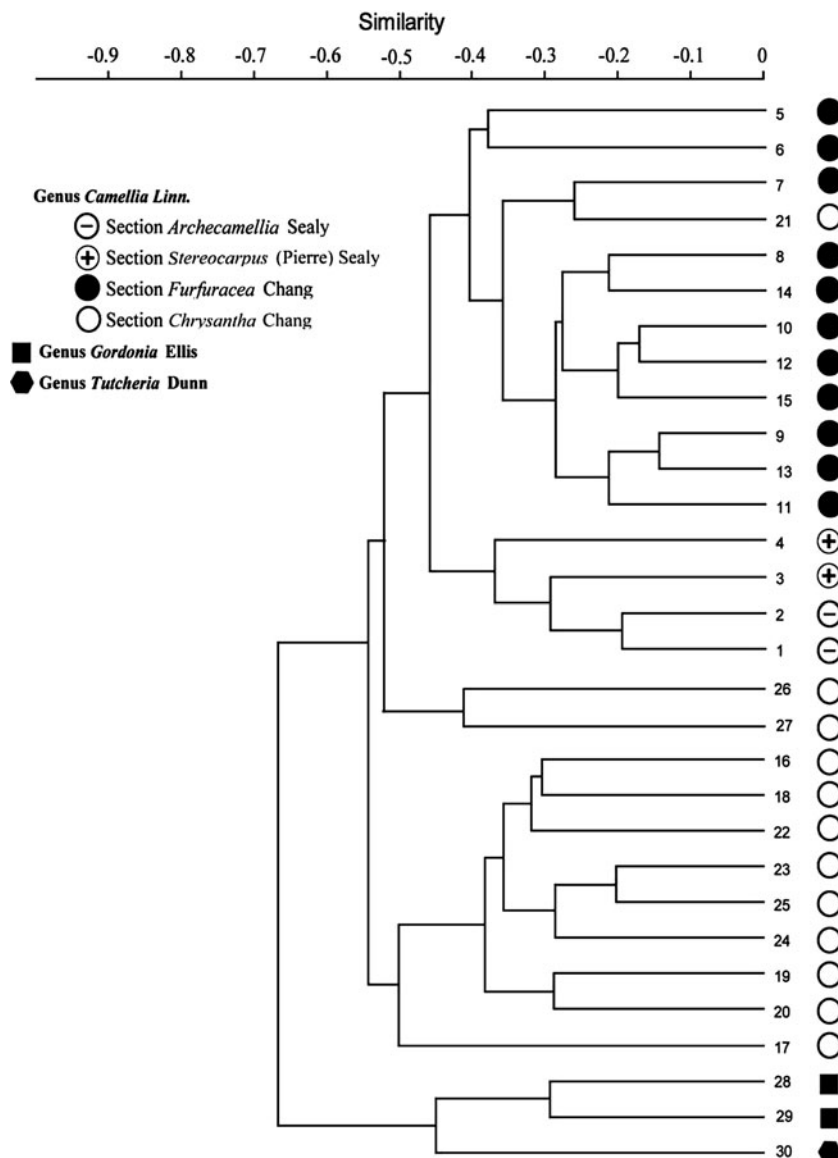
more frequently referred to in the current *Camellia* literature.

Chang (1981) revised section *Archecamellia* Sealy. He transferred two species from section *Archecamellia* Sealy into the new section, *Chrysanthia* H.T. Chang, treating *C. nitidissima* Chi as its type species, and described this section as characterized by axillary yellow flowers, distinct, persistent bracts and sepals, and 3–5 cleft styles. Three species were recognized in Chang's *Archecamellia*, which retained only one species, *C. peteloi*, from Sealy's *Archecamellia*, while another two species, *C. granthamiana* and *C. albogigas*, were newly added (Chang 1998). However, Ming disagreed with Chang's taxonomic treatments of the section. Ming (1999, 2000), highly influenced by Sealy's (1958) work, reinstated the merger of section *Chrysanthia* H.T. Chang into section *Archecamellia*. Moreover, Ming (1999, 2000) transferred *C. granthamiana* and *C. albogigas* from section *Archecamellia* in Chang's classification into section *Heterogenea* Sealy, which was absent from Chang's classification (1981, 1998) and comprised section *Furfuracea* plus several species from subgenus *Protocamellia* from Chang's classification (1981,

1998). In our study, *C. granthamiana* and *C. albogigas* were assessed based on leaf shape, anatomy, and biochemistry. Although *C. peteloi* was not examined in the present study due to its absence from China, results showed that these two species were significantly different from other considered *Heterogenea* species, such as *C. liberistyla* H.T. Chang, *C. yunnanensis* H.T. Chang, and *Furfuracea* species (Figs. 1, 10, 12). Thus, we did not approve of Ming's transfer of *C. granthamiana* and *C. albogigas* from section *Archecamellia* to section *Heterogenea*.

The diagnostic characters of section *Stereocarpus* described by Chang (1998) and Ming (1999, 2000) are terminal solitary flowers, extremely short pedicels, five locules in the ovary, and glands (secretory structures) in the leaf. However, *C. liberistyla* H.T. Chang and *C. yunnanensis* H.T. Chang were transferred from section *Stereocarpus* by Chang (1981, 1998) into section *Heterogenea* by Ming (1999, 2000), because of their white flowers and absence of brown glands (secretory structure) in the leaves. We examined leaf anatomical characters of the two species by light microscope and found that the two species also had secretory structures (Figs. 7b, 8c, d; Table 4). Furthermore,

**Fig. 12** Phenogram obtained from UPGMA cluster analysis using the data of leaf FTIR. Each number code (see Table 2) represents a species, and related section or genus is indicated by each different symbol



our multivariate analysis of leaf morphology, anatomy, and FTIR data also supported the separation between the two species and other *Heterogenea* species (Figs. 1, 10, 12).

Chang (1981) proposed section *Furfuracea* based on the characters of large leaf, rough capsule, and solitary, large white flower, but Ming (2000) considered that rough capsules also occurred in other sections, and he therefore denied this section and transferred most of the species into section *Heterogenea*, characterized by flowers without pedicels, calyx persistent until young fruit develop, five to three locules in the ovary, and glands present or absent. In Ming's classification (1999, 2000), section *Heterogenea* was recognized with 15 species, including section *Furfuracea*, 2 species from section *Archeamellia* Sealy (*C. granthamiana* and *C. albogigas*), 2 species from section *Stereocarpus* (*C. liberistyla* H.T. Chang and *C. yunnanensis* H.T. Chang),

section *Pseudocamellia*, and section *Protocamellia*. In the present study, we employed multivariate analysis of integrated leaf characters data to assess affinities of species and section in the disputed sections. The results illustrated that section *Furfuracea*, section *Archeamellia* Sealy, and section *Stereocarpus*, respectively, formed homogeneous groups, indicating their high homogeneity. Therefore, we agree with Chang (1981, 1998) in considering three groups as separate sections rather than merging them into a single section *Heterogenea* as done by Ming (1999, 2000).

**Acknowledgments** We thank the Institute of Physical Chemistry of Zhejiang Normal University for use of the Nicolet FTIR spectrometer. We are grateful to Prof. Cun-Gui Cheng and Ms. Dan-Ting Li for help with FTIR spectrometer operation and FTIR data analysis. This study was supported by grants from the Natural Science Foundation of Jiangsu Province, China (No. BK2006045) and Open the Foundation

of the Botany Key Discipline of Changshu Institute of Technology (No. 2008003).

## Appendix 1

Collection localities and vouchers of studied specimens

### Genus *Camellia* Linn. Section *Archecamellia* Sealy.

*C. granthamiana* Sealy, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 200610190201,02,03 (ZJNU); *C. albogigas* Hu, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 200610190101,02,03 (ZJNU); Section *stereocarpus* (Pierre) Sealy. *C. liberistyla* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 200612100301,02,03 (ZJNU); *C. yunnanensis* (Pitard) Coh. St., Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 200612100401,02,03 (ZJNU); **Section *Furfuracea* Chang.** *C. pubifurfuracea* Zhong, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610170901,02,03 (ZJNU); *C. latipetiolata* Chi, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171001,02,03 (ZJNU); *C. crapnelliana* Tutch., Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171101,02,03 (ZJNU); *C. multibracteata* H.T. Chang et Mo *ex* Mo, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171201,02,03 (ZJNU); *C. furfuracea* (Merr.) Coh. St., Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171301,02,03 (ZJNU); *C. oblata* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 200610221401,02,03 (ZJNU); *C. gaudichaudii* (Gagn.) Sealy, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171501,02,03 (ZJNU); *C. gigantocarpa* Sealy, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 200610221601,02,03 (ZJNU); *C. octopetala* Hu, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 200610221701,02,03 (ZJNU); *C. parafurfuracea* S. Y. Liang *ex* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171801,02,03 (ZJNU); *C. conmatistyla* Mo et Zhong, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.E. Tian* 200610171901,02,03 (ZJNU); **Section *Chrysantha* H.T. Chang.** *C. euphlebia* Merr. *ex* Sealy, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 2006031212301,02,03 (ZJNU); *C. limonia* C. F. Liang et

Mo, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 2006031219401,02,03 (ZJNU); *C. achrysantha* H.T. Chang et S.Y. Liang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *X.F. Wu* 2006031612401,02,03 (ZJNU); *C. impressinervis* H.T. Chang et S.Y. Liang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 2006031212501,02,03 (ZJNU); *C. grandis* (Liang et Mo) H.T. Chang et S.Y. Liang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *X.F. Wu* 2006031612601,02,03 (ZJNU); *C. parvipetala* J.Y. Liang et Su, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 2006031212801,02,03 (ZJNU); *C. pingguoensis* Fang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *H.B. Dai* 2006031219301,02,03 (ZJNU); *C. lunzhouensis* Luo, Jinhua International Camellia Species Garden, Zhejiang Province, China, *X.F. Wu* 2006031612901,02,03 (ZJNU); *C. huana* Ming et W.J. Zhang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *X.F. Wu* 2006031613001,02,03 (ZJNU); *C. liberofilamenta* H.T. Chang et C.H. Yang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *X.F. Wu* 2006031613101,02,03 (ZJNU); *C. fusuiensis* S.Y. Liang et X.J. Dong, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 2006011413601,02,03 (ZJNU); *C. multipetala* S.Y. Liang et C.Z. Deng, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 2006011413701,02,03 (ZJNU); **Genus *Gordonia* Ellis.** *G. acuminata* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 2006111219701,02,03 (ZJNU); *G. longicarpa* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 2006111219801,02,03 (ZJNU); **Genus *Tutcheria* Dunn.** *T. hexalocularia* Hu et Liang *ex* H.T. Chang, Jinhua International Camellia Species Garden, Zhejiang Province, China, *Q.F. Peng* 2006111220101,02,03 (ZJNU).

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