Epidermal structures and stomatal parameters of Chinese endemic *Glyptostrobus pensilis* (Taxodiaceae)

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Glyptostrobus pensilis K. Koch, the only living species, is endemic to southern China. Epidermal structures of *G. pensilis* have been studied on leaves collected from Guangzhou, southern China, the native locality of the species, and from Hangzhou, eastern China, the cultivated locality. Leaves are linear, linear-subulate and scale-like. Epidermal cells are rectangular and elongate parallel to the mid-vein on areas lacking stomata, and short, with rounded corners, on intrastomatal areas. Stomatal bands lie parallel to the mid-vein on both surfaces of leaves. Commonly the stomata have five or six subsidiary cells. Stomatal parameters (density and index) of the same surfaces of linear leaves from Guangzhou and Hangzhou show no statistically significant differences ($P > 0.05$). Considering the stomatal parameters of the same surfaces of linear-subulate leaves between the two localities, the stomatal index of the abaxial surfaces reveals no significant differences ($P > 0.05$), while the stomatal index of the adaxial surfaces and the stomatal density of both surfaces exhibit significant differences ($P < 0.05$). Intra-individual variation in stomatal index is smaller than that in stomatal density based on the coefficient of variability of stomatal parameters of the same areas of leaves. When studying the correlation between stomatal parameters of *G. pensilis* and atmospheric CO_2 concentrations, the stomatal parameters of linear leaves are mostly significant, and stomatal index is more useful than stomatal density. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, **146**, 153–162.

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INTRODUCTION

The conifer family Taxodiaceae traditionally includes nine genera. They are widely distributed in north temperate to subtropical regions of both the Old and New Worlds, with only one genus in the Southern Hemisphere (Page, 1990; Yu, 1994, 1995). *Taxodium* is distributed southwards from north-eastern USA to Florida, Mexico and Guatemala; *Cunninghamia* occupies southern China; *Taiwania* occurs in southern China, including Taiwan, and northern Myanmar; *Cryptomeria* occurs in China and Japan; *Sequoia* and *Sequoiadendron* occur in western North America; *Glyptostrobus* and *Metasequoia* grow in southern and central China; *Arthrotaxis* is endemic to Tasmania. All the genera in Taxodiaceae are either monotypic or consist of small and closely related species-groups (e.g. *Arthrotaxis*), and the geographical distribution of genera is one of considerable disjunction.

As an endemic Chinese genus, *Glyptostrobus* has a unique living species, *G. pensilis* K. Koch, which is naturally distributed within the Chinese provinces of Guangdong, Guangxi, Fujian and Yunnan (Fig. 1; Ying & Zhang, 1994). Trees of *G. pensilis* are also cultivated in the provinces of Jiangxi, Hunan, Sichuan, Jiangsu, Zhejiang, Anhui, Henan, Shandong, Shanghai, Hong Kong, Taiwan (Xu & Li, 1959; Jiangsu Institute of Botany, 1977; Zheng & Fu, 1978; Xu & Yu, 1980; Chen, 1992; Liu, Lu & Ou, 1994; Ying & Zhang, 1994; Huang, 1995; Yu, 1995; Chen, Yu & Miao, 1997; Han *et al.*, 1997; Ru *et al.*, 1999; Li & Zhang, 2001).

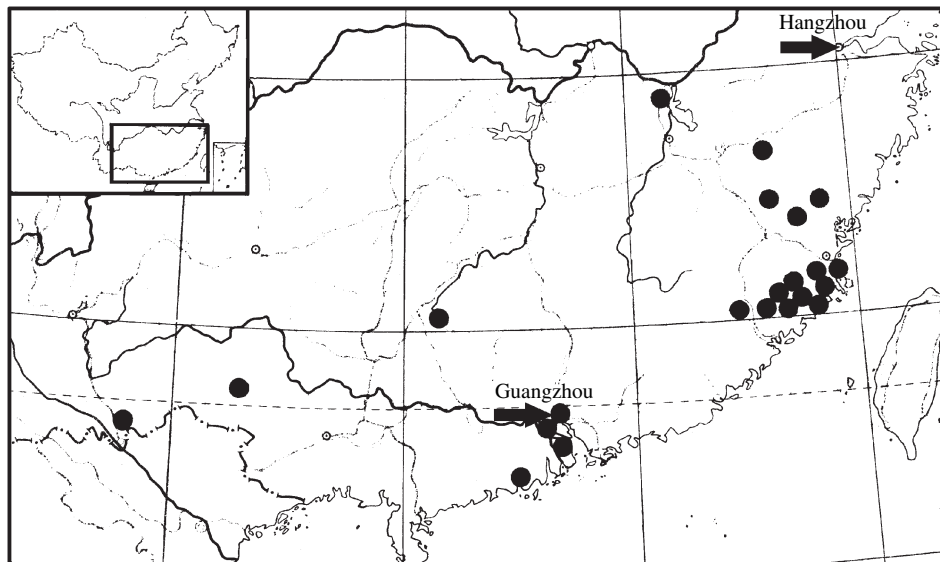*Corresponding author. E-mail: lics@ns.ibcas.ac.cn

Figure 1. The natural distributions of *Glyptostrobus pensilis* (based on map 6 in Ying & Zhang, 1994). The arrows show the localities from which the specimens were collected.

In the fossil record of *Glyptostrobus*, *G. europaeus* (Vickulin *et al*., 2003), *G. lineatus* (Alvin & Boulter, 1974), *G. nordenskioeldii* (Chandrasekharam, 1974; Christophel, 1976), *G. comoxensis* (Rouse, 1967), and *G. vachrameevii* (Sveshnikova, 1967) have been common in North America, continental Europe and eastern Asia since the Late Cretaceous period (Florin, 1963; Sveshnikova, 1963, 1967; Becker, 1969; Dorofeev, 1974; Christophel, 1976; Schneider, 1990; Mai, 1995; Yu, 1995). They are generally preserved as fragments of foliar shoots. In China, compression fossils of leaves and shoots, identified as *G. europaeus*, and the wood of *G. pensilis* were found from Upper Cretaceous to Upper Tertiary rocks in the provinces of Jilin, Xinjiang, Heilongjiang, Liaoning, Yunnan and Hubei (Editorial Group on Cenozoic Plants from China, 1978; Guo & Li, 1979; Shenyang Institute of Geology & Mineral Resources, 1980; Guo *et al*., 1984; Li & Yang, 1984; Tao & Xiong, 1986; Xiong, 1986; Guo & Chen, 1989; Yang *et al*., 1996; He & Tao, 1997; Guo, 2000).

The extent of intraspecific variation is relatively high in the macromorphology of leaf and branch structures in several genera of Taxodiaceae (Liu, Li & Wang, 1999; Ma & Gu, 2000; Ma *et al*., 2000a; Stockey, Rothwell & Falder, 2001; Ma & Li, 2002a, b). To a certain extent, leaf epidermal characters are reflected in the structural pattern of the cuticle (Alvin, 1970; Boulter, 1970, 1971; Alvin & Boulter, 1974; Cutler, Alvin & Price, 1982) and the cuticle is the standard source of valuable cellular information in leaf-compression fossils (Kerp & Krings, 1999). Thus, epidermal features might offer additional and decisive insights into taxonomic parameters.

A few papers have described simple epidermal structures of *G. pensilis* (Florin, 1922, 1931; Sveshnikova, 1963; Zheng & Fu, 1978; Yao & Hu, 1982; Vickulin *et al*., 2003). In this paper, the epidermal characters of three kinds of leaves of *G. pensilis* were observed under both scanning electron microscopy (SEM) and light microscopy (LM). Statistical analyses were performed to check the differences in stomatal parameters between the abaxial and adaxial surfaces of the same type of leaves from the same localities, and also to check the differences in stomatal parameters for the same kind of leaves in Guangzhou and Hangzhou. The coefficient of variabilities (CV) of stomatal density and index were compared and show that the intra-individual variation in the stomatal index is smaller than that in the stomatal density. The new data obtained from this study will provide reference material for reconstructing palaeoenvironments using the 'Nearest Living Relatives' approach (Woodward, 1987; McElwain & Chaloner, 1995; Sun, Chen & Li, 1999; Li, Wang & Sun, 2001).

MATERIAL AND METHODS

Some of the leaves investigated in this study were collected from *G. pensilis* specimens in the arboretum of Guangzhou (23°11′N, 113°22′E), Guangdong Province in January 2002. The remaining leaves were studied from a mature tree in the arboretum of Hangzhou (30°15′N, 120°06′E), Zhejiang Province in November 2000 and August 2002 (Fig. 1). All the leaves were macerated in >30% hydrogen peroxide (H_2O_2) and 99% glacial acetic acid (CH_3COOH) at a ratio of 1:1. Epi-

dermal photographs were taken under SEM (Hitachi S570) and LM (Olympus BX50).

The sizes of stomata were measured on both the abaxial and adaxial surfaces of linear, linear-subulate and scale-like leaves ($N = 50$ on each surface). The numbers of subsidiary cells of a stoma were determined for 200 stomatal apparati. Stomatal density is the number of stomata per unit area of the leaf surface. Stomatal index = $100S/(E + S)$, where S = number of stomata per unit area and E = number of epidermal cells of the same unit area. The areas used for calculating stomatal density and index were 0.5×0.3 mm^2 in linear leaves and 0.5×0.2 mm^2 in linear-subulate leaves. One hundred measurements of such areas were made on each of the abaxial and adaxial surfaces of linear and linear-subulate leaves. The values of stomatal size and parameters (mean ± SD) were obtained from the leaves collected from Guangzhou and Hangzhou, and also from these populations combined. Statistical analyses (independent-samples *t*-test) were performed using SPSS v.10.0 software. Statistically significant differences were assumed when a two-tailed *P*-value was <0.05. The values of CV (CV = SD/mean) of stomatal density and index were obtained in order to compare intra-individual variation between stomatal density and index.

LEAF DESCRIPTION AND COMPARISON

Trees of *G. pensilis* are semi-evergreen and monoecious. Their inflorescences develop in February to March, and seeds mature in October to November. Leaves are spirally arranged and comprise three morphotypes: linear, linear-subulate and scale-like. The linear leaves are flat, thin and two-ranked on annual branchlets of young trees and budding branchlets of mature trees (Fig. 2A). The linear-subulate leaves are quadrangular in cross-section and radially spreading on annual branchlets of mature trees (Fig. 2B). The branchlets with linear and linear-subulate leaves often fell off as a unit in winter. The scale-like leaves, relatively thick and persistent for 2–3 years, are distributed radially on main, perennial and fertile branches (Fig. 2C).
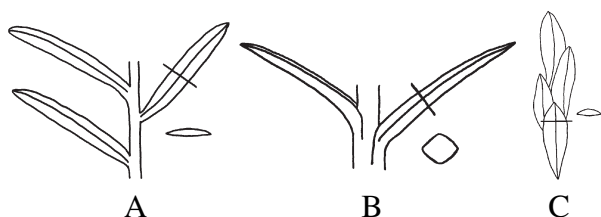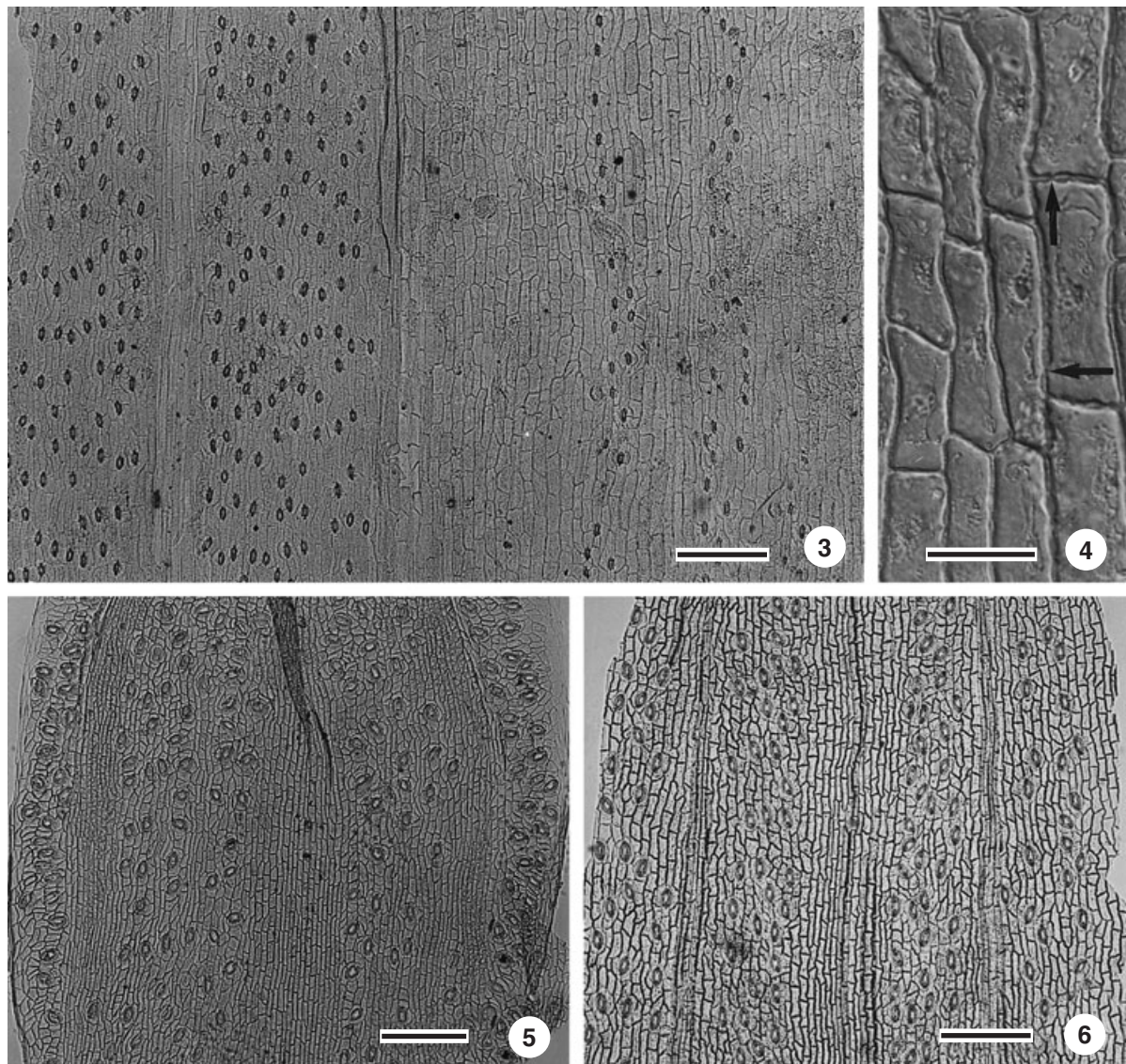

Figure 2. Leaves of *Glyptostrobus pensilis*. A, linear leaves; B, linear-subulate leaves; C, scale-like leaves.

The epidermal structures on both the abaxial and adaxial surfaces of the three morphotypes are very similar, which defines the leaves as amphistomatic. The epidermal cells are elongated within the non-stomatal areas, while they are rectangular with slightly rounded cell-corners on the intrastomatal zones (Figs 3, 5, 6). Pits can be seen clearly in the walls of epidermal cells both on non-stomatal areas (Fig. 4) and on intrastomatal zones (Figs 7, 8). In linear leaves, epidermal cell length in non-stomatal areas is 131.6 ± 31.1 μm (Guangzhou 129.3 ± 25.0 μm, Hangzhou 134.0 ± 36.4 μm) and width is 31.6 ± 5.4 μm (Guangzhou 31.1 ± 5.6 μm, Hangzhou 32.0 ± 5.1 μm), with the ratio of length to width (L:W) varying from 1.7:1 to 13.5:1 (on average 4.2:1). The statistical difference in epidermal cell size between Guangzhou and Hangzhou is not significant ($P > 0.05$). The shape and size of epidermal cells on the stomatal areas are irregular in comparison with those of non-stomatal areas (Figs 3, 5, 6). The shape and size of epidermal cells in an area of high stomatal density are more irregular than in an area of lower stomatal density (Figs 7, 8).

Stomata on both the abaxial and adaxial surfaces of the three morphotypes of leaves are very similar in structure, and are also similar on the inner surfaces of both the abaxial and adaxial epidermis. On linear leaves, stomata are 45.2 ± 3.5 μm long and 22.8 ± 2.6 μm wide on their abaxial surface, and 45.9 ± 3.3 μm long and 23.0 ± 2.1 μm wide on their adaxial surface. On linear-subulate leaves, stomata are 47.7 ± 4.0 μm long and 23.7 ± 1.9 μm wide on their abaxial surfaces, and 47.5 ± 3.9 μm long and 24.0 ± 2.1 μm wide on their adaxial surfaces. On scale leaves, stomata are 47.5 ± 3.4 μm long and 24.4 ± 3.2 μm wide on their abaxial surface, and 47.2 ± 3.2 μm long and 24.0 ± 3.4 μm wide on their adaxial surfaces (Table 1). Stomata pores are elliptical (Figs 7, 8, 11). The long axes of stomatal pores are mostly parallel to the mid-vein in linear and linear-subulate leaves (Figs 3, 6), but most stomata are oblique or perpendicular to the mid-vein in scale-like leaves (Fig. 5). Guard cells have thickened walls, especially on outer margins. The thickened outer margins of cell walls are banana shape (Figs 7, 8). The thickened walls of guard cells form polar lamellae that produce protruding and curved ends at the two poles of the stomata (Figs 7, 8, 13). Commonly the stomata have five (49.5%) or six (32%) subsidiary cells, sometimes four (15%) and occasionally seven (3.5%). For Guangzhou and Hangzhou, respectively, 51% and 48% of the stomata have five subsidiary cells, 33% and 31% have six, 14% and 16% have four, and 2% and 5% have seven (Figs 7, 8, 12).

Stomata are located on each side of the mid-vein and distributed on both the abaxial and adaxial surfaces of leaves (Figs 3, 5, 6). The numbers of stomata

Figures 3–6. Epidermis of leaves of *Glyptostrobus pensilis*. Fig. 3. Epidermis of linear leaf collected from Guangzhou: left, abaxial surface; right, adaxial surface. Scale bar = 10 μm. Fig. 4. Epidermal cells of linear-subulate leaf collected from Hangzhou. The arrows show the pits. Scale bar = 30 μm. Fig. 5. Epidermis of scale-like leaf collected from Guangzhou. The middle part of the figure is the abaxial surface. Scale bar = 10 μm. Fig. 6. Epidermis of linear-subulate leaf collected from Hangzhou. The middle part of the figure is the abaxial surface. Scale bar = 10 μm.

on the abaxial surface are greater than those on the adaxial surface in linear leaves, as the stomatal band, about 0.3 mm in width, on the abaxial surfaces comprises 7–10 lines of stomata on each side of the midvein (Fig. 3, left), while the band on the adaxial surface has only 1–3 lines of stomata (Fig. 3, right). On each surface of the quadrangular linear-subulate leaves, the numbers and distribution of stomata are almost the same. The stomatal band comprises 4–6 lines of stomata and occupies about 0.2 mm in width (Fig. 6). There are 2–5 lines of stomata in each sto-matal band on both the abaxial and adaxial surfaces of scale-like leaves (Fig. 5).

In the stomatal area, the stomatal density of linear leaves is 136 ± 24 mm^{-2} on the abaxial surface and 46 ± 14 mm^{-2} on the adaxial surface and that for linear-subulate leaves is 118 ± 26 mm^{-2} on the abaxial surfaces and 112 ± 24 mm^{-2} on the adaxial surfaces. The stomatal index of linear leaves is 13.88 ± 1.81 on the abaxial surface and 8.99 ± 1.81 on the adaxial surface, and for linear-subulate leaves 13.32 ± 2.32 on the abaxial surfaces and 13.06 ± 2.14 on the adaxial sur-
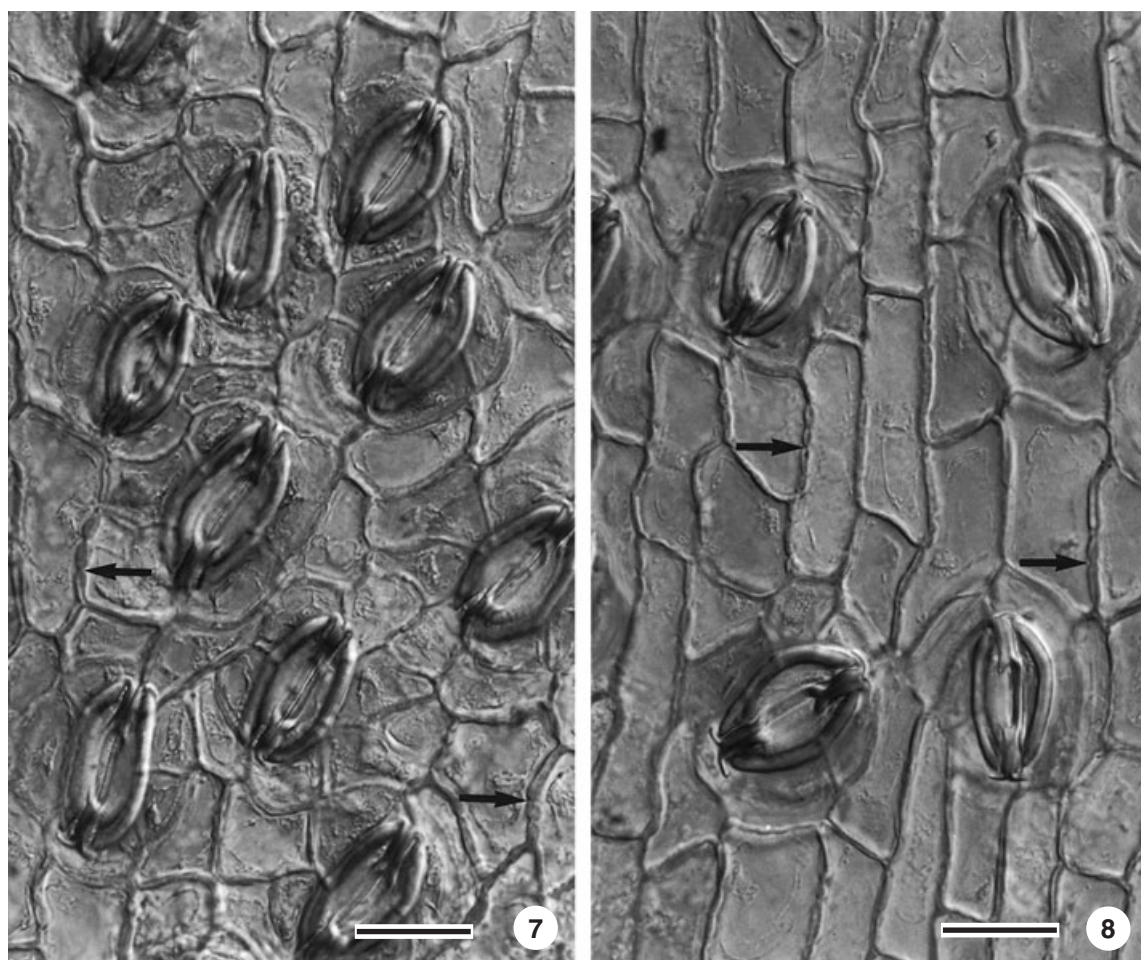
Figures 7–8. Stomata from linear-subulate leaves collected from Hangzhou. Arrows indicate pits. Scale bars = 30 μm.

Table 1. Stomatal size of *Glyptostrobus pensilis*

	Linear leaves		Linear-subulate leaves		Scale leaves	
	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface
Stomatal length (μm)	45.1 ± 3.2 (G)	45.5 ± 3.3 (G)	45.4 ± 3.7 (G)	46.1 ± 3.2 (G)	46.0 ± 2.9 (G)	46.2 ± 2.2 (G)
	45.3 ± 3.8 (H)	46.2 ± 3.3 (H)	49.9 ± 2.9 (H)	48.9 ± 4.1 (H)	49.1 ± 3.1 (H)	48.3 ± 3.6 (H)
	45.2 ± 3.5 (C)	45.9 ± 3.3 (C)	47.7 ± 4.0 (C)	47.5 ± 3.9 (C)	47.5 ± 3.4 (C)	47.2 ± 3.2 (C)
Stomatal width (μm)	22.9 ± 2.1 (G)	22.9 ± 1.7 (G)	23.1 ± 1.6 (G)	22.8 ± 1.5 (G)	21.9 ± 1.3 (G)	22.0 ± 1.4 (G)
	22.6 ± 3.1 (H)	23.2 ± 2.6 (H)	24.3 ± 2.0 (H)	25.1 ± 1.9 (H)	27.0 ± 2.3 (H)	26.0 ± 3.6 (H)
	22.8 ± 2.6 (C)	23.0 ± 2.1 (C)	23.7 ± 1.9 (C)	24.0 ± 2.1 (C)	24.4 ± 3.2 (C)	24.0 ± 3.4 (C)

G, Guangzhou, South China; H, Hangzhou, East China; values obtained from 50 leaf surface samples. C, combined Guangzhou and Hangzhou results obtained from 100 leaf surface samples.

faces (Table 2). The results of a *t*-test indicate that the statistical differences of stomatal parameters between the abaxial and adaxial surfaces of linear leaves from the same localities are significant ($P < 0.05$, Table 3). The statistical differences in stomatal density between the abaxial and adaxial surfaces of linear-subulate leaves from Guangzhou are significant ($P < 0.05$, Table 3), but those from Hangzhou are not significant ($P > 0.05$, Table 3). The statistical differences of stomatal index between the abaxial and adax-

Table 2. Stomatal density and index of *Glyptostrobus pensilis*

	Linear leaves		Linear-subulate leaves	
	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface
Stomatal density (mm^{-2})	138 ± 25 (G)	45 ± 15 (G)	112 ± 24 (G)	104 ± 19 (G)
	135 ± 23 (H)	47 ± 13 (H)	125 ± 27 (H)	119 ± 25 (H)
	136 ± 24 (C)	46 ± 14 (C)	118 ± 26 (C)	112 ± 24 (C)
Stomatal index	13.94 ± 2.07 (G)	9.12 ± 1.99 (G)	13.02 ± 2.20 (G)	12.52 ± 2.07 (G)
	13.82 ± 1.53 (H)	8.86 ± 1.61 (H)	13.62 ± 2.41 (H)	13.60 ± 2.09 (H)
	13.88 ± 1.81 (C)	8.99 ± 1.81 (C)	13.32 ± 2.32 (C)	13.06 ± 2.14 (C)

G, Guangzhou, South China; H, Hangzhou, East China; values obtained from 100 leaf surface samples. C, combined Guangzhou and Hangzhou results obtained from 200 leaf surface samples.

Table 3. Statistical analyses of differences in stomatal parameters between the abaxial and adaxial leaf surfaces of *Glyptostrobus pensilis* from Guangzhou and Hangzhou

	Guangzhou		Hangzhou	
Abaxial and adaxial surfaces of:	Stomatal density	Stomatal index	Stomatal density	Stomatal index
Linear leaves	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Linear-subulate leaves	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Table 4. Statistical analyses of differences in stomatal parameters of *Glyptostrobus pensilis* from Guangzhou and Hangzhou

Leaf type	Surface	Stomatal density	Stomatal index
Linear	Abaxial	$P > 0.05$	$P > 0.05$
	Adaxial	$P > 0.05$	$P > 0.05$
Linear-subulate	Abaxial	$P < 0.05$	$P > 0.05$
	Adaxial	$P < 0.05$	$P < 0.05$

Figure 9. Coefficients of variability (CV) for stomatal density (■) and stomatal index (□) of abaxial (ABL) and adaxial (ADL) surfaces of the linear leaves, and abaxial (ABS) and adaxial surfaces (ADS) of linear-subulate leaves collected from Guangzhou.

ial surfaces of linear-subulate leaves from the same localities are not significant ($P > 0.05$, Table 3). The differences in stomatal parameters of the same surfaces of linear leaves between Guangzhou and Hangzhou are not significant ($P > 0.05$; Table 4). The statistical differences of stomatal density of the same surfaces of linear-subulate leaves between Guangzhou and Hangzhou are significant ($P < 0.05$; Table 4). The statistical differences of stomatal index of the abaxial surfaces of linear-subulate leaves between Guangzhou and Hangzhou are not significant ($P > 0.05$, Table 4), but those of the adaxial surfaces of linear-subulate leaves are significant ($P < 0.05$; Table 4). The values of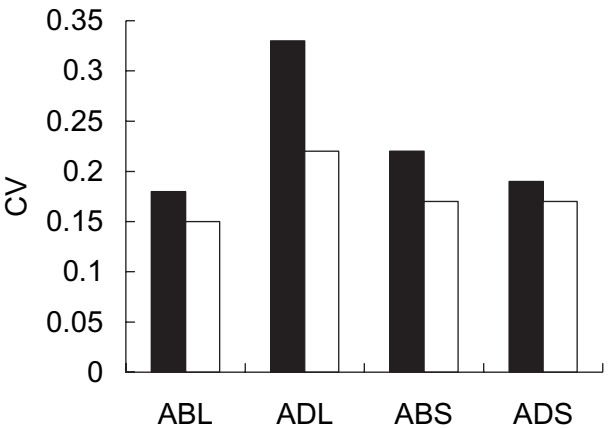 CV of stomatal index are lower than those of stomatal density from the same areas on each surface of the same type leaves from the same localities, which indicate that the stomatal index shows a lower intra-individual variation and hence is more useful for palaeoecological reconstruction than stomatal density (Figs 9, 10).

DISCUSSION

In genera of Taxodiaceae, some morphological features of branches, leaves and cones are similar (Stebbins, 1948; Chaney, 1951; Christophel, 1976; Ma & Gu, 2000). It is thus important to compare the epidermal structures of fossil species with their 'Nearest Living Relatives' (McElwain & Chaloner, 1995) in an attempt
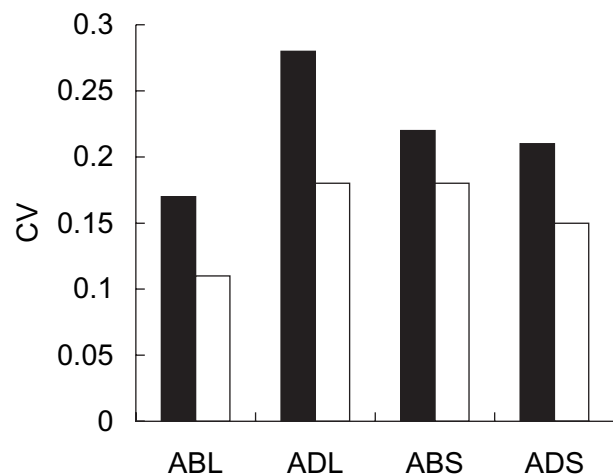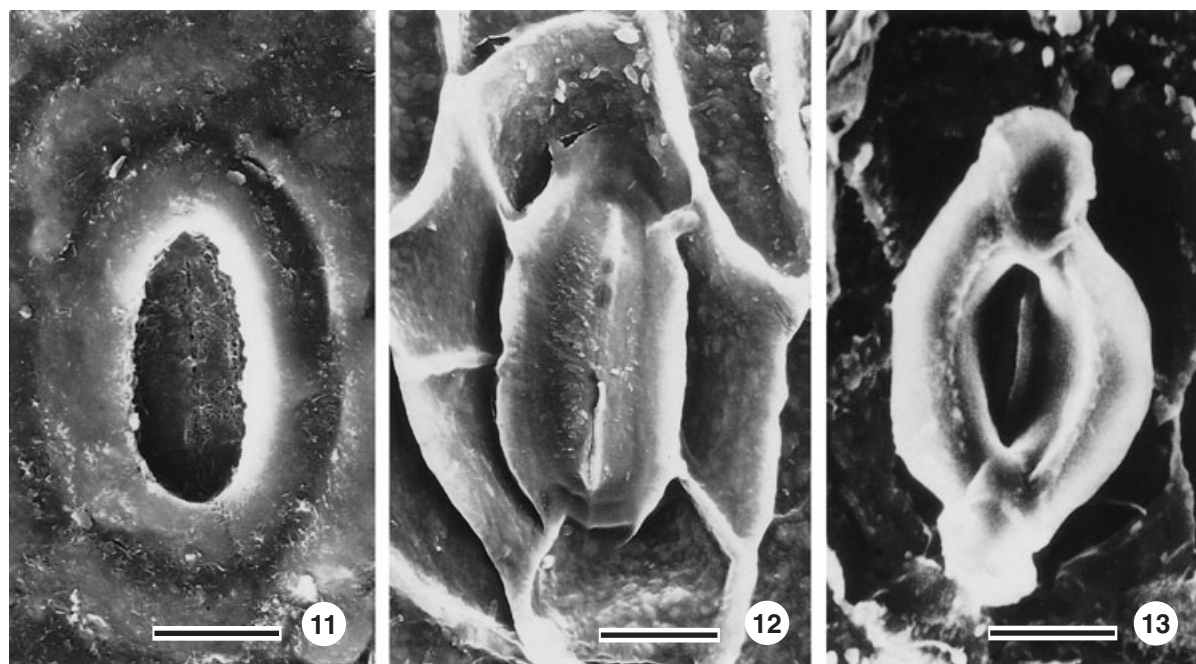
to identify fossil leaf-compressions accurately. In order to distinguish fossil specimens of these genera, there is a need to study characters of both epidermal cells and stomata of leaves in living Taxodiaceae.

Previous descriptions of stomata of *G. pensilis* were very brief and solely for the abaxial surfaces of linear leaves (Zheng & Fu, 1978). Stomata of *G. pensilis* were also shown on both abaxial and adaxial surfaces of leaves (amphistomatic) with 4–7 subsidiary cells (Yao & Hu, 1982). Sveshnikova (1963) reported that *G. pensilis* has two types of leaves, subulate (or needle-like) and scale-like, both amphistomatic and with 4–6 (7) subsidiary cells. Florin (1922, 1931) recognized that the stomata have 4–6 subsidiary cells. In a recent comparative study on fossil *Glyptostrobus*, cuticular micropapillae were also found on cuticle of *G. pensilis*. These details of cuticle micromorphology are considerably better expressed in a fossil species in comparison with the living species *G. pensilis* (Vickulin *et al.*, 2003). According to the present work on *G. pensilis*, stomata are located on each side of the mid-vein and distributed on both the abaxial and adaxial surfaces of the three types of leaves. The number of stomata on the adaxial surface are fewer than those on the abaxial surface in linear leaves, but they are approximately the same on the different surfaces of the other two kinds of leaves. Most commonly the stomata have five or six subsidiary cells, and sometimes also four or seven.

Stomatal guard cells in *G. pensilis* have thickened walls. The lignified thickenings of guard cells were not

Figure 10. Coefficients of variability (CV) for stomatal density (■) and stomatal index (□) of abaxial (ABL) and adaxial (ADL) surfaces of the linear leaves, and abaxial (ABS) and adaxial surfaces (ADS) of linear-subulate leaves collected from Hangzhou.

Figures 11–13. SEM images of stomata. Scale bars = 50 μm. Fig. 11. Stomata of outer surface of a linear leaf collected from Hangzhou. Figs 12, 13. Stomata of inner surface of linear leaves collected from Hangzhou.

described in detail by previous researchers, exceptions being Boulter (1970, 1971) and Ma & Li (2002a, b), presumably because the thickenings were removed to some extant by the different maceration procedures (Isherwood, 1965; Alvin & Boulter, 1974; Kerp, 1990; Vickulin, 1999; Ma *et al*., 2000b; Ma & Li, 2002a, b). Thickened walls of guard cells were also observed in *Sequoia* (Chaturvedi, 1993; Ma & Li, 2002a), *Metasequoia* (Liu *et al*., 1999; Leng *et al*., 2001; Ma & Zhang, 2003), *Taiwania* (Chaturvedi, 1993), *Taxodium* (Chaturvedi, 1993) and *Cryptomeria*, *Sequoiadendron*, *Arthrotaxis* (Boulter, 1970).

Recently, the authors (Vickulin *et al*., 2003) have studied *G. europaeus* leafy shoots collected from the early Miocene Kaydagul Formation, central Kazakhstan. The shoots have two kinds of leaves, needle-like ones similar to the leaves of *Cryptomeria* and squamate ones similar to cupressoid forms. The former leaves are equivalent to linear-subulate leaves while the latter ones are described as scale-like leaves in this paper. No linear leaves were found in *G. europaeus*. The two kinds of leaves in the fossil are amphistomatic, with similarly organized epidermal cells and stomata on both the abaxial and adaxial surfaces of leaves. The epidermal cells are quadrangular, with the ratio L:W being 1–3 in *G. europaeus*. The ratio L:W of epidermal cells on non-stomatal areas of linear leaves of *G. pensilis* is on average 4.2. The stomatal structures of *G. europaeus* are the same as those of the living species of *G. pensilis*. Stomata usually have five subsidiary cells and very rarely four or six in *G. europaeus*, but very often five or six in *G. pensilis*. Stomatal pores are randomly orientated coaxial or at a slight angle towards the leaf axis, but very rare orthogonal orientation in *G. europaeus*, while the long axes of stomatal pores are mostly parallel to the mid-vein in linear and linear-subulate leaves, and oblique or perpendicular to the mid-vein in the scale-like leaves of *G. pensilis*. Similar stomatal patterns were also found in Eocene specimens of *G. europaeus* from Fushun, Liaoning Province, China (Florin, 1922; Editorial Group on Cenozoic Plants from China, 1978). There are no further details of leaf epidermis in fossil *Glyptostrobus* for comparison with those of living *G. pensilis* (Sveshnikova, 1963, 1967; Rouse, 1967; Alvin & Boulter, 1974; Chandrasekharam, 1974; Zhilin, 1974, 1989; Christophel, 1976; Stuchlik *et al*., 1990; Worobiec, 1995; Vikulin & Zhilin, 1998).

Considering stomatal parameters and atmospheric CO_2 concentrations, a rise in CO_2 concentrations is correlated with a decline of stomatal parameters; thus, stomatal parameters can be used as an indication of ambient CO_2 concentrations (Woodward, 1987; Sun *et al*., 1999). Stomatal density and index are equally likely to be inversely related to CO_2 concentration, but the latter is not the only factor that influences the stomatal density and index. Stomatal density varies with changes in solar radiation, temperature and water status (Beerling, 1999). Stomatal index, in contrast, is sensitive only to factors affecting cell initiation, of which CO_2 appears to be one. Thus, even if stomatal density and stomatal index show similar responses for a given species, stomatal index should yield more accurate estimates of CO_2 levels (Royer, 2001).

The variation of stomatal parameters of linear leaves is smaller than that of linear-subulate leaves of *G. pensilis,* according to *t*-test results of stomatal parameters between Guangzhou and Hangzhou (Table 4). The variation of stomatal index of the abaxial surfaces is smaller than that of the index of the adaxial surface, and also is smaller than that of the density of the abaxial and adaxial surfaces in linear-subulate leaves according to *t*-test results (Table 4). Thus, in considering a correlation between stomatal parameters of *G. pensilis* and atmospheric CO_2 concentrations, the stomatal parameters of linear leaves should be selected.

Intra-leaf variations of stomatal density and index are present in many species (Royer, 2001). As we obtained the values of stomatal density and index of *G. pensilis* from the same areas of leaves in the present paper, the intra-individual variation of stomatal index is smaller than that of the density by comparing the CV of stomatal density with the CV of index (Figs 9, 10).

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