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Sun and shade leaf variability in *Liquidambar chinensis* and *Liquidambar formosana* (Altingiaceae): implications for palaeobotany

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Many factors influence leaf anatomy and morphology in the crown of a tree, particularly those resulting from microclimatic differences between the periphery and the interior of the crown. These influences can be so strong that single species can produce different leaf forms in which shade and sun leaves exhibit consistently distinctive morphological and epidermal character sets. Here we show, using *Liquidambar* as a model system, that the principal morphological characters for distinguishing shade and sun leaves in two modern *Liquidambar* spp. with different lamina types (entire in *L. chinensis* and lobate in *L. formosana*) are the leaf lamina length to width ratio, the degree of development of venation networks, tooth size and tooth shape. The main epidermal characters are ordinary cell size and anticlinal wall outlines. Many fossils, however, are only preserved as impressions and morphological characters alone have been used to distinguish shade and sun leaf morphotypes. To evaluate the utility of our approach, populations of fossil *Liquidambar* leaves from the Eocene of southern China, preserved only as impressions, were categorized into sun and shade morphotypes. Recognition that sun and shade leaf morphological diversity exists in fossil populations will enable palaeobotanists to identify more reliably foliar polymorphisms that would otherwise be used to describe, incorrectly, different species.

ADDITIONAL KEYWORDS: epidermal characters – fossil leaves – leaf morphology – statistics.

INTRODUCTION

Numerous previous studies have revealed that variations in leaf characters arise from adaptations to different growth habitats (e.g. Larcher, 1976; Pandey & Nagar, 2002; Sack *et al.*, 2006). However, this variation is often overlooked when erecting new species of leaf fossils, leading to an inflation of palaeobiodiversity (e.g. Samsonov, 1964; Golovneva, 2004). Leaf morphology and anatomy can strongly be affected by position in the crown, in which crown shape and density determine the gradient of solar radiation flux from its periphery towards its centre and therefore the microclimates and small scale spatial and temporal variations that it hosts (e.g. Zalensky, 1904; Larcher, 1976). For instance, the humidity and wind speed experienced by a leaf also depend on its position in the crown and, with radiation balance, these factors affect leaf temperature, boundary layer thickness and evapotranspiration. Because these types of constraints on leaf performance have remained subject to time-stable laws of physics (radiation, diffusion gradients,

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hydraulics and mechanics) throughout evolution, and leaf form is highly adaptive reflecting functional optimization (e.g. Pigliucci, 2003), studies of within-crown morphological and epidermal character variation in modern plants have the potential to be applied to palaeobotanical material in order to resolve the characters that have genuine value for dispersed leaf fossil systematics and can be used in palaeoenvironmental reconstruction.

Traditionally, Altingiaceae comprised three genera: *Liquidambar* L. (with eight species), *Altingia* Noronha (with eight species) and *Semiliquidambar* H.-T.Chang (with three species), and differences in the structure of reproductive organs have been demonstrated for these genera (Pigg, Ickert-Bond & Wen, 2004; Ickert-Bond, Pigg & Wen, 2005, 2007). Leaves of *Liquidambar* were described as mostly three- to five-lobate and those of *Altingia* as unlobed, whereas leaves of *Semiliquidambar* include both unlobed and lobate morphotypes. Recently, Ickert-Bond & Wen (2013) combined the three genera in *Liquidambar*, the genus with nomenclatural priority, based on molecular data and analysis of morphological characters (fruit anatomy and pollen morphology). This idea had been discussed previously in light of cladistic morphological analysis and molecular data in a series of papers (Shi *et al.*, 2001; Ickert-Bond, Pigg & Wen, 2005, 2007; Ickert-Bond & Wen, 2006) and in a number of earlier works on morphology of *Altingia* and *Liquidambar* (Blume, 1828; Lindley, 1836; Oken, 1841; Bentham & Hooker, 1865). Ickert-Bond & Wen (2013) produced a synopsis of Altingiaceae with a sole genus *Liquidambar* and a key for the identification of the 15 *Liquidambar* spp.

Lobate leaves assigned to *Liquidambar* are widely represented in the Palaeogene and Neogene of North America and Asia (Knowlton, 1902; Endo & Morita, 1932; Brown, 1933; Hu & Chaney, 1940; MacGinitie, 1941; Chaney & Axelrod, 1959; Suzuki, 1961; Huzioka, 1972; Onoe, 1974; Huzioka & Uemura, 1979; Wolfe & Tanai, 1980; Uemura, 1983; Ozaki, 1991; Maslova, 1995; Zhang & Lu, 1995; Meyer & Manchester, 1997; Stults & Axsmith, 2011; Xiao *et al.*, 2011; Maslova *et al.*, 2015). These leaves can be recognized with confidence due to their semicraspedodromous to festooned semicraspedodromous secondary venation and concave/retroflexed teeth with more prominent basal sides. Where there is a representative selection, sun and shade leaf morphotypes can be easily distinguished (Maslova *et al.*, 2015), and existing data on the leaf epidermal characters facilitate this (e.g. Xiao *et al.*, 2011, 2013). However, epidermal characters are not always preserved in fossils and in such cases only meso-scale and larger morphological characters obtained from modern analogues can be used for recognizing sun and shade leaf morphotypes.

Previously we undertook a study of sun and shade leaves in modern *Platanus acerifolia* Willd. (Platanaceae) with the aim of revealing typical sun and shade morphological and epidermal characters that could be used in a practical and reliable way to distinguish sun and shade forms in fossil platanaceous leaves (Maslova, Gordenko & Volkova, 2008; Maslova, Volkova & Gordenko, 2008). Subsequently, these results were used to distinguish sun from shade leaf morphotypes in fossil platanaceous *Ettingshausenia sarbaensis* N.Maslova & Shilin (Maslova & Shilin, 2011).

Here, we report morphological and epidermal character variability in populations of unlobed leaves of *L. chinensis* and lobate leaves of *L. formosana*. We focus on those leaf characters that are often available for study in palaeobotanical material, such as shape of a lamina, length/width (L/W) ratio, venation type and thickness of veins of all orders, margin type of the leaf lamina, the shapes of ordinary epidermal cells, peculiarities of their anticlinal walls and stomata type. In general, leaves of *L. chinensis* are characterized by a rather stable morphology, and therefore it is especially important to recognize fine differences in the leaf morphology of sun and shade leaves at both macro and micro scales. Previously Xiao *et al.* (2011) examined epidermal characters of sun and shade leaf morphotypes of *L. formosana,* but data on the macromorphology of such leaves are absent. Here we fill this gap because clear differences in the morphology of typical sun and shade leaf morphotypes of *L. formosana* can be observed and used in palaeobotanical studies for both systematics and palaeoenvironmental reconstruction (Maslova *et al.*, 2015). Knowledge of variation in typical sun and shade leaves characters helps achieve a reliable systematic attribution of fossil leaves. The variability range of leaf morphological characters observed for modern angiosperms can also be used to determine past climates (e.g. Wolfe, 1993; Yang *et al.*, 2015).

MATERIAL AND METHODS

DATA COLLECTION

Leaves of the evergreen *L. chinensis* and deciduous *L. formosana* were chosen for their distinct unlobed and lobate shapes, respectively. Material was collected in the South China Botanical Garden and the South Campus of Sun Yat-sen University, Guangzhou, China, in September 2013. Specimens of each species were collected from one mature tree, situated in a more-or-less open space without crown overlap with adjacent trees. Shoots were cut from the inner (shade), middle (intermediate) and outer (sun) parts of the crown at *c.* 3 m above the ground.

Terminology and methods

For leaf descriptions we use terms from the *Manual of leaf architecture* (Leaf Architecture Working Group, 1999; Ellis *et al.*, 2009). The ratio of the lamina length to its width (L/W) was measured using maximum leaf lengths and widths. Epidermal characters were studied for four leaves collected in the inner part of the crown (shade leaves) and compared with those (four leaves) from the periphery of the crown (sun leaves), using central parts of the lamina. The cuticles from these leaves were prepared from leaf fragments by immersing them in nitric acid with added potassium chlorate for 24 hours, followed by treatment with potassium hydroxide for complete removal of the leaf mesophyll. Stomatal density (SD), defined as the number of stomata per leaf unit area (1 mm^2), and the density of epidermal cells including subsidiary cells of the stomata (ED), defined as the number of epidermal cells per leaf unit area (1 mm^2), were both measured. SD and ED values are reported as an arithmetical mean ± one standard deviation.

Epidermal characters analysed in this study are given in Appendix 1.

Statistical analysis

The analysis of morphological variability was carried out for 150 leaves of each species with 50 samples for each type of leaf (sun, shade and intermediate); enough to evaluate differences using basic statistical procedures (Greenwood & Sandomire, 1950; Cooke, 2005). The Shapiro–Wilk normality test of our data shows that they exhibit a non-normal distribution. We use interquartile range (IQR) as a robust measure for our non-parametric data. Box plots characterize samples using IQR represented by the size of the grey shaded box, the median (horizontal line in the box) and the mean (black dot in the box). Whiskers are conventionally extended to minimum and maximum of the data values (Spear style). Notches show the 95% confidence interval (CI) for the median, given by median ± 1.58 × IQR√n (Krzywinski & Altman, 2014). Quantitative analyses were performed and box plots generated using R software (version 3.1.2) with IDE Rstudio (R Core Team, 2014; RStudio Team, 2015). The values of the statistical parameters are provided in Appendix 2.

Palaeobotanical material

The plant fossils studied here were collected from the oil-shale-bearing Maoming Basin (Huangniuling Formation), located in southwest Guangdong Province, South China (more information is given in Maslova *et al.*, 2015). The leaves (92 specimens) are preserved as impressions. No cuticle could be obtained from the plant remains. Collections MMJ3 are housed at the Museum of Biology of Sun Yat-sen University, Guangzhou, China.

Equipment

Leaves were photographed using a digital camera Nikon Coolpix 8700. Photographs of fine venation details were made with a Leica M165 stereomicroscope equipped with a Leica DFC420 digital camera. The scanning electron microscope (SEM) study was carried out by means of a Tescan SEM.

RESULTS

Leaf morphology of *Liquidambar chinensis*

Leaves are simple, entire, with caducous stipules and a petiole up to 13 mm long (Fig. 1). The lamina ranges in shape from elliptical to obovate or oblong and varies from 62 to 135 mm in length and 30 to 65 mm in width, with a L/W ratio of 1.8–2.8. The apex angle is acute or obtuse, and the apex shape is acuminate. The base angle is acute, and overall the base shape is cuneate. The margins are revolute, serrate or crenate and serrate, but occasionally entire near the base. The teeth are mostly irregularly spaced, tiny and inconspicuous, with a simple apex. There are three to six teeth per 1 cm of a leaf margin. The tooth shape is concave/retroflexed, with a longer basal side. The primary venation is pinnate, with a straight midrib. The secondaries are semicraspedodromous to festooned semicraspedodromous and there are seven to 12 on each side. They are irregularly spaced with inconsistent vein angles. The tertiaries are opposite percurrent, sinuous or irregularly reticulate.

Epidermal characters of *Liquidambar chinensis* leaves

Ordinary epidermal cells of the upper surface of shade leaves are polygonal (Fig. 2A, B), measuring 26.7–40.0 × 20.1–32.0 µm, with sinuous anticlinal walls forming 'ameboid' outlines where the sinus depth reaches 10 µm (Fig. 2B). Anticlinal walls are thinner than those in sun leaves. Ordinary epidermal cells of the upper surface of sun leaves are polygonal, more rarely rectangular (Fig. 2C, D), 21.7–36.6 × 13.3–16.7 µm, with straight anticlinal walls. Ordinary epidermal cells of the lower surface of shade leaves are rectangular, more rarely polygonal (Fig. 3A, B), 28.8–59.6 × 14.4–27.9 µm, with greatly sinuous anticlinal walls forming ameboid outlines where the sinus depth reaches 10 µm (Fig. 3B, C). Ordinary epidermal cells

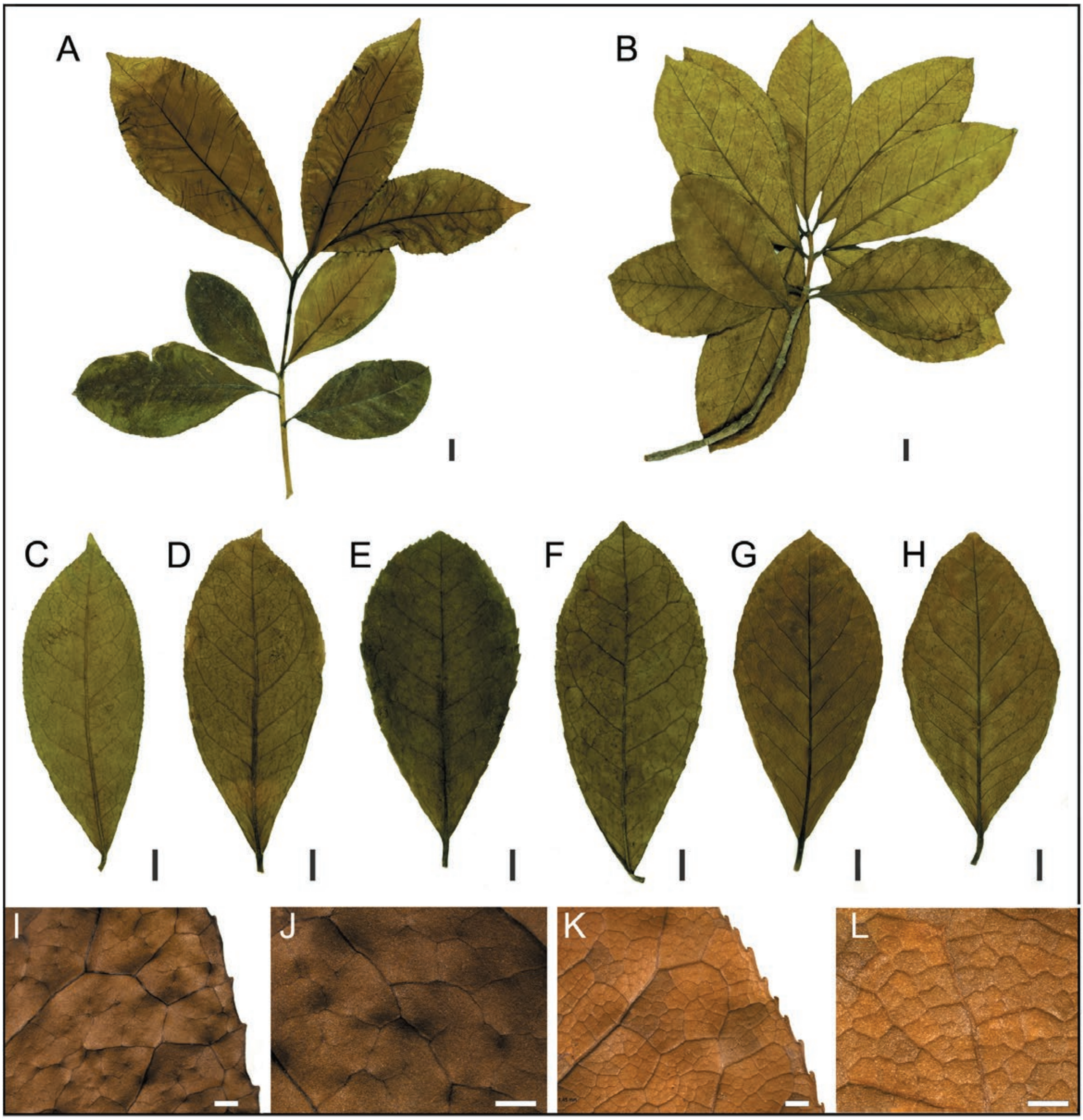

Figure 1. Shoots and leaves of *Liquidambar chinensis*. A, Shoot from inner crown (shade). B, Shoot from peripheral crown (sun). C–E, Shade leaves (C and E are cited from figs 47 and 44, respectively, in Maslova *et al.*, 2015). F–H, Sun leaves. I, Lamina margin of shade leaf with rather small, rare and irregular teeth (Maslova *et al.*, 2015, fig. 46). J, Detail of shade leaf lamina with inconspicuous tertiary veins. K, Detail of sun leaf margin showing more numerous and regularly distributed teeth (Maslova *et al.*, 2015, fig. 45). L, Detail of sun leaf lamina with prominent tertiary veins. Scale bar, 10 mm (A–H) and 2 mm (I–L).

of the lower surface of sun leaves are polygonal, more rarely tetragonal (Fig. 3D, E), 21.2–57.7 × 9.6–28.8 μm, mostly with straight or slightly curved anticlinal walls (Fig. 3E, F). The density of ordinary epidermal cells (ED) is 3000 ± 131 per 1 mm^2 for shade leaves and 3755 ± 99 per 1 mm^2 for sun leaves. Stomata are paracytic, widely oval or rounded, with stomatal density (SD) measured as 625 ± 48 per 1 mm^2 of the leaf surface in shade leaves and 802 ± 43 per 1 mm^2 of the leaf surface in sun leaves.

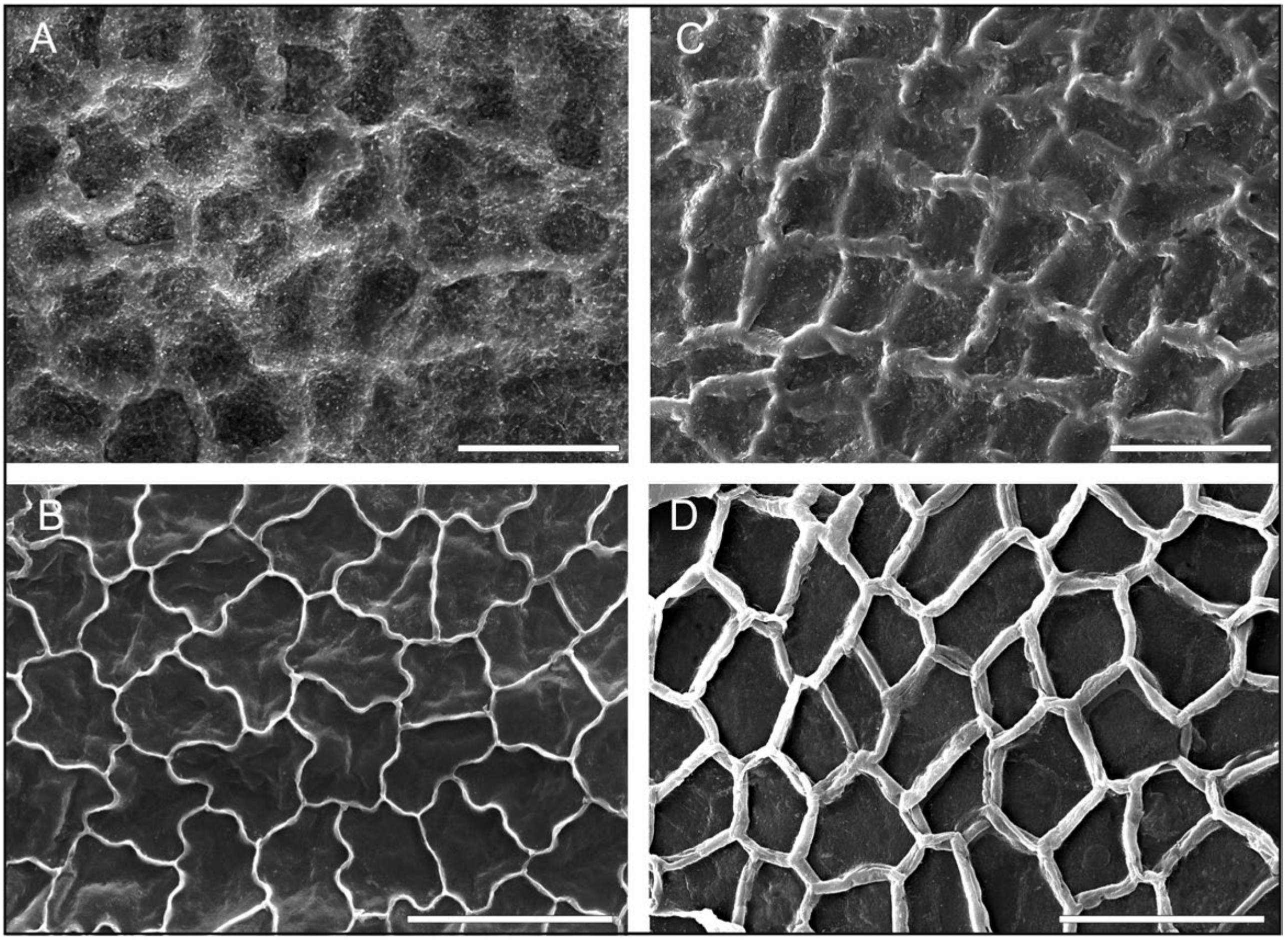

Figure 2. Epidermal characters of leaves of *Liquidambar chinensis*, SEM. A, B, Cuticle of upper surface of shade leaf, outer (A) and inner (B) views showing sinuous anticlinal walls of ordinary epidermal cells. C, D, Cuticle of upper surface of sun leaf, outer (C) and inner (D) views showing straight anticlinal walls of ordinary epidermal cells. Scale bar, 50 μm.

Leaf morphology of *Liquidambar formosana*

Leaves are simple, palmately trilobate (Figs 4A–F, 5A, B), with caducous stipules and a petiole up to 85 mm long. The lamina is ovate or elliptic in shape, varies from 158 to 174 mm in length and 47 to 51 in width, with a L/W ratio of 0.59–1.35. The shape of the central lobe is triangular or narrowly triangular. Apices of the lobes are acuminate in shape, often with a drip tip, and the apex angle is acute. The base angle is reflex or obtuse, the base shape is cordate, convex or rarely truncate. The margin is serrate from the base or slightly above. Teeth are mostly regularly spaced. They are small, with a simple apex and rounded sinuses, concave/retroflexed in shape, with a longer basal side, and there are four to six per 1 cm of the leaf margin. Venation is basally actinodromous, with three primaries. Lateral primary veins are equal to the midvein in thickness and are either mostly straight or occasionally slightly curved. Secondaries occur in five–eight pairs, are alternate to subopposite, often irregular relative to one another, and semicraspedodromous or festooned semicraspedodromous. Tertiaries are percurrent or reticulate (Fig. 5C–F). Fourth order veins are alternate percurrent. We did not study epidermal characters of sun and shade leaf morphotypes of *L. formosana*, because they were previously examined by Xiao *et al.* (2011).

Variability of morphological characters of sun and shade leaf morphotypes in *Liquidambar chinensis*

The variability of morphological characters of entire *Liquidambar* leaves is studied here for the first time. Morphological characters of the lamina of *L. chinensis* are rather stable and show only small variability. The study of a large selection of shade and sun leaves revealed the following pattern: the lamina shape of sun leaves is exclusively elliptic, whereas obovate or oblong laminae were also observed for shade leaves.

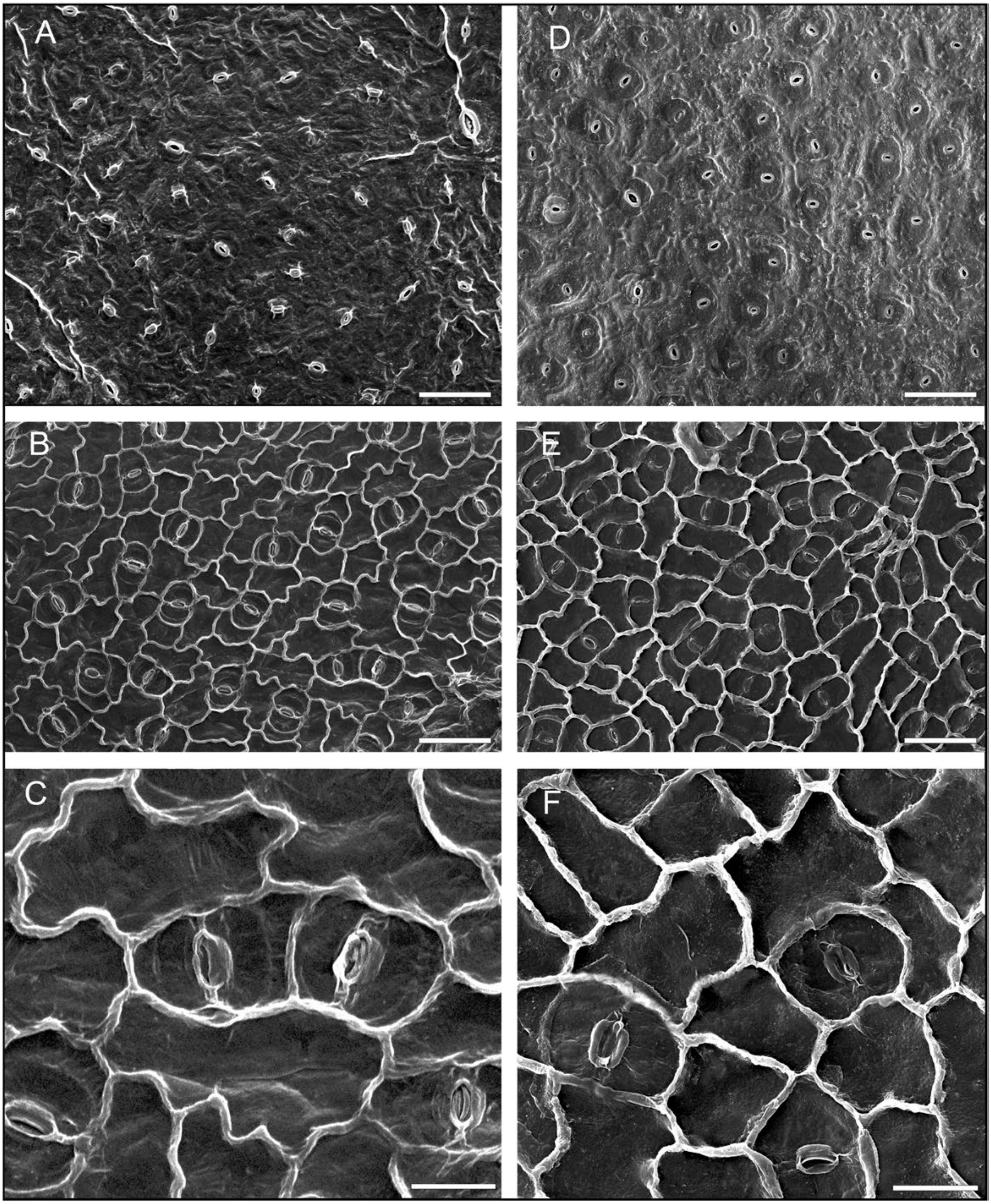

Figure 3. Epidermal characters of leaves of *Liquidambar chinensis*, SEM. A, B, C, Cuticle of lower surface of shade leaf. A, Outer view, thin folded cuticle, borders of ordinary epidermal cells are imperceptible. B, C, Inner view showing paracytic stomata and sinuous anticlinal walls of ordinary epidermal cells. D, E, F, Cuticle of lower surface of sun leaf. D, Outer view, cuticle is thick, borders of ordinary epidermal cells are distinct. E, F, Inner view, showing paracytic stomata and straight anticlinal walls of ordinary epidermal cells. Scale bar, 50 μm (A, B, D, E) and 20 μm (C, F).

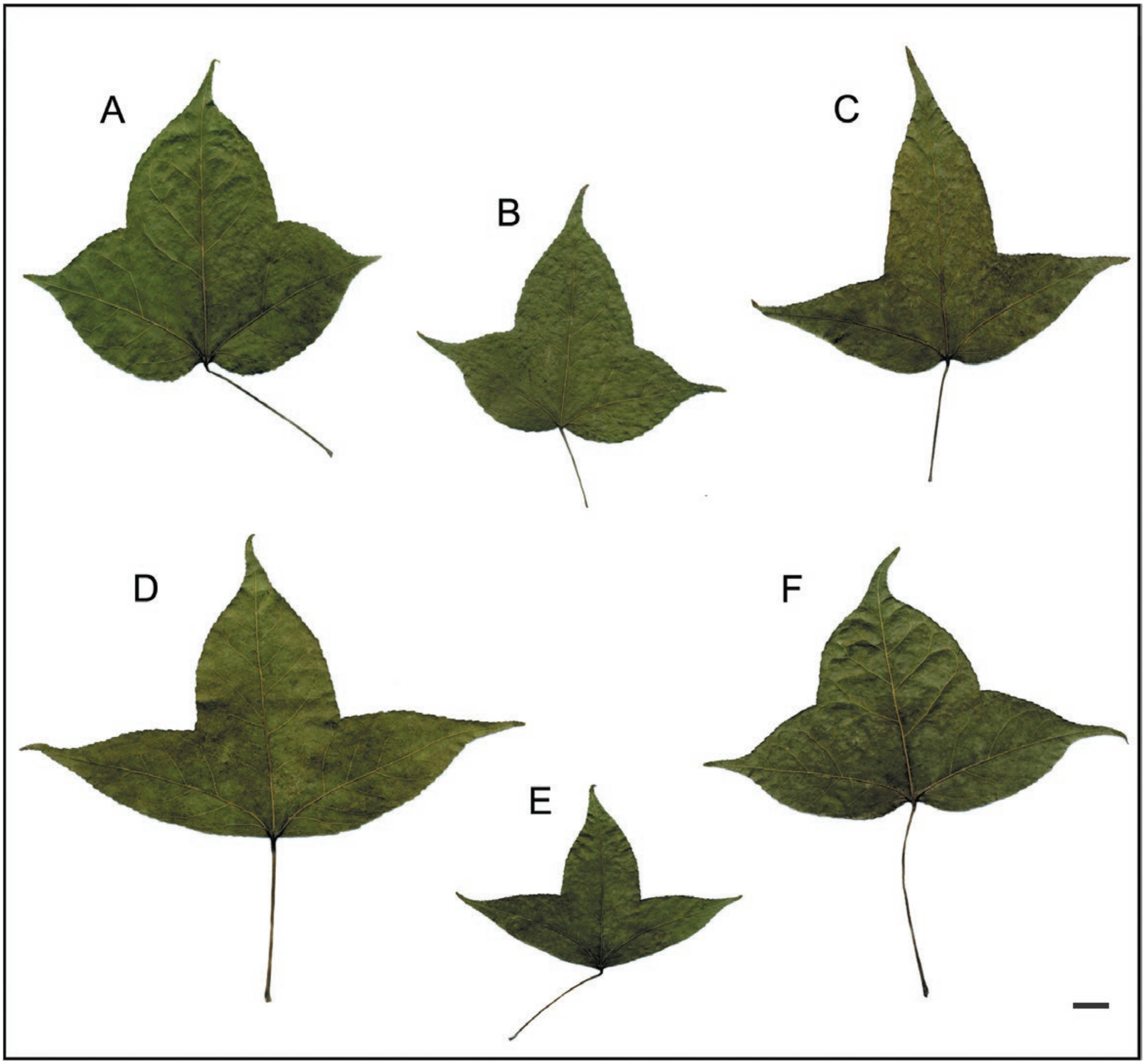

Figure 4. Leaves of *Liquidambar formosana*. A–C, Shade leaves with higher L/W ratio. D–F, Sun leaves with lower L/W ratio, lobe apexes are distinctly acuminate. Scale bar, 10 mm.

The mean value of the petiole length, *c*. 9 mm, is almost the same in shade and sun leaves; however, in shade leaves there is slightly greater variation. The petiole length varies from 5 to 13 mm [interquartile range (IQR) 3 mm] in shade leaves, and from 6 to 12 mm (IQR 1 mm) in sun leaves (Fig. 6).

The typical sizes of shade and sun leaves show some difference: on average the laminae of shade leaves are longer (106.5 ± 21.9 mm versus 102.1 ± 7.7 mm) and narrower (46.2 ± 8.2 mm versus 51.9 ± 4.5 mm) than those of sun leaves. The IQR of the lamina length and width is larger (21.0 and 6.8, respectively) in shade leaves than in sun leaves (4.0 and 3.0, respectively) (Fig. 7A, B). Thus, such characters as lamina length and width show more variation in shade leaves. The IQR of linear dimensions of shade leaves exceeds completely (lamina length) or significantly (lamina width) that of sun leaves (Fig. 7A, B). This implies that sun leaves have mostly (>50% of those sampled) similar values to shade leaves. Leaf sizes in the central part of the crown (transitional areas between shade and sun leaves) generally correspond to those of shade leaves, and their size variation is greater than that of sun leaves (Fig. 7A, B).

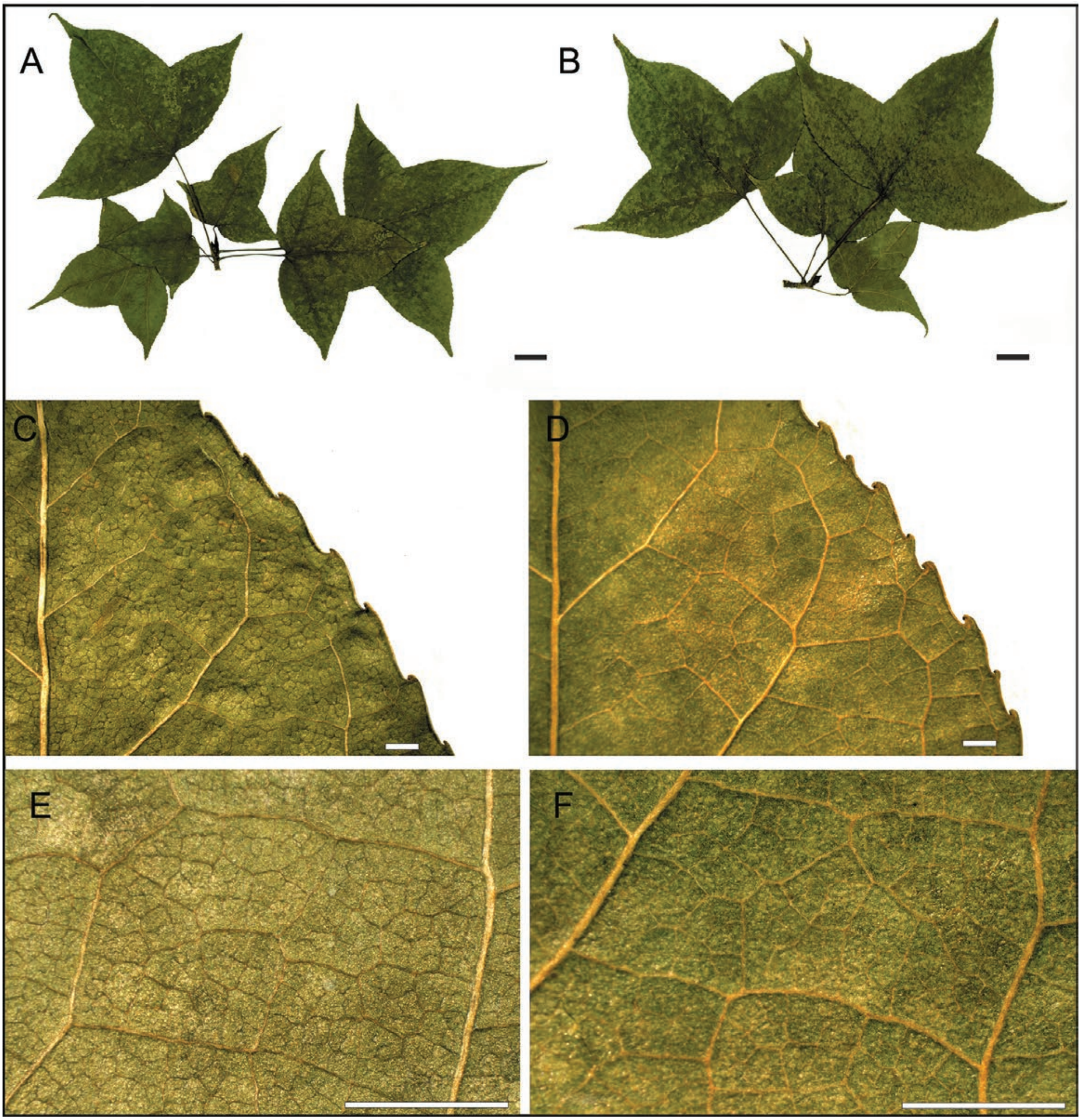

Figure 5. Shoots and leaf morphological features of *Liquidambar formosana*. A, Shoot from inner crown part (shade). B, Shoot from peripheral crown part (sun). C, Lamina margin of shade leaf with small teeth. D, Lamina margin of sun leaf with relatively large teeth. E, Detail of shade leaf lamina with inconspicuous tertiary veins. F, Detail of sun leaf lamina with prominent tertiary veins. Scale bar, 10 mm (A, B), 3 mm (E, F) and 1 mm (C, D).

The ratio of lamina length and width (L/W) emerged as a useful character for distinguishing sun and shade leaves. The variation of the L/W ratio is lower in sun leaves (IQR 0.06) and higher in shade leaves (IQR 0.20). The average value of the L/W ratio is higher in shade leaves (2.30) than in sun leaves (1.97), whereas for leaves from transitional areas this value is close to that of shade leaves (2.21) (Fig. 7C). L/W ratios close to 2 occur in both sun and shade leaves, but the percentage of such values in shade and transitional leaves is small (< 25%) (Fig. 7C).

On average, shade leaves of *L. chinensis* have three to five teeth and sun leaves have five to six teeth per 10 mm of the lamina margin indicating that shade teeth are larger than sun teeth. There are also differences in the tooth shape: in shade leaves they are concave/retroflexed with approximately equal flanks (Fig. 1I), whereas in sun leaves the teeth are concave/retroflexed with a much larger proximal flank.

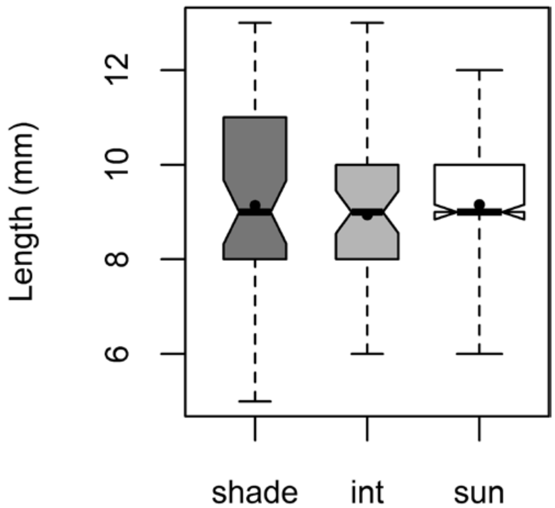

Figure 6. Variation in petiole length of shade (dark grey box plot), sun (white box plot) and intermediate (light grey box plot) leaves of *Liquidambar chinensis*, mm. A black point in the box indicates the arithmetic mean.

There is also a difference in venation between shade (Fig. 8A) and sun (Fig. 8B) leaves of *L. chinensis*. Secondary veins are prominent on the surface of sun leaves and less distinct in shade leaves. A significant difference was observed in the network of higher order veins. Fine veins are less distinct in shade than in sun leaves. However, the average number of secondary veins is almost equal in both leaf groups.

Variability of morphological characters of sun and shade leaf morphotypes in *Liquidambar formosana*

Leaves of *Liquidambar formosana* are exclusively trilobate. The average petiole length in *L. formosana* shade leaves is less (44.2 mm) than that in sun leaves (53.7 mm) (Fig. 9). However, as in *L. chinensis*, there is a greater variability of this character in shade leaves: the IQR is 23.0 mm for shade leaves and 8.8 mm for sun leaves.

Dimensional features of shade and sun leaves of *L. formosana* show significant differences. Typical shade leaves reveal a considerably greater range of variation in the lamina length (IQR 24.8 mm) in comparison to typical sun leaves (IQR 16.0 mm) (Fig. 10A, B), whereas the average value of this character is higher for sun leaves (99.0 and 82.7 for sun and shade leaves, respectively). The same trend was observed for the lamina width: this character shows higher variation in shade leaves (IQR 33.5 mm versus

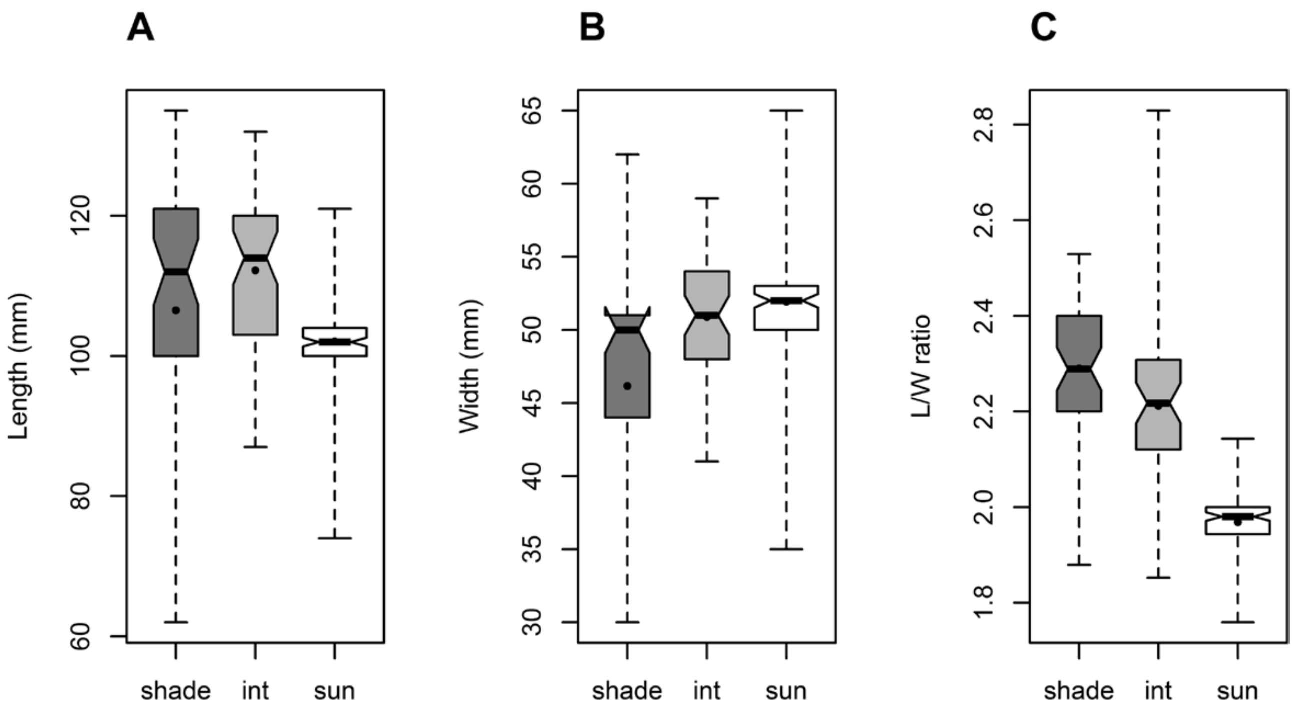

Figure 7. Size variation of shade (dark grey box plot), sun (white box plot) and intermediate (light gray box plot) leaves of *Liquidambar chinensis*. A, Length, mm. B, Width, mm. C, L/W ratio. A black point in the box indicates the arithmetic mean.

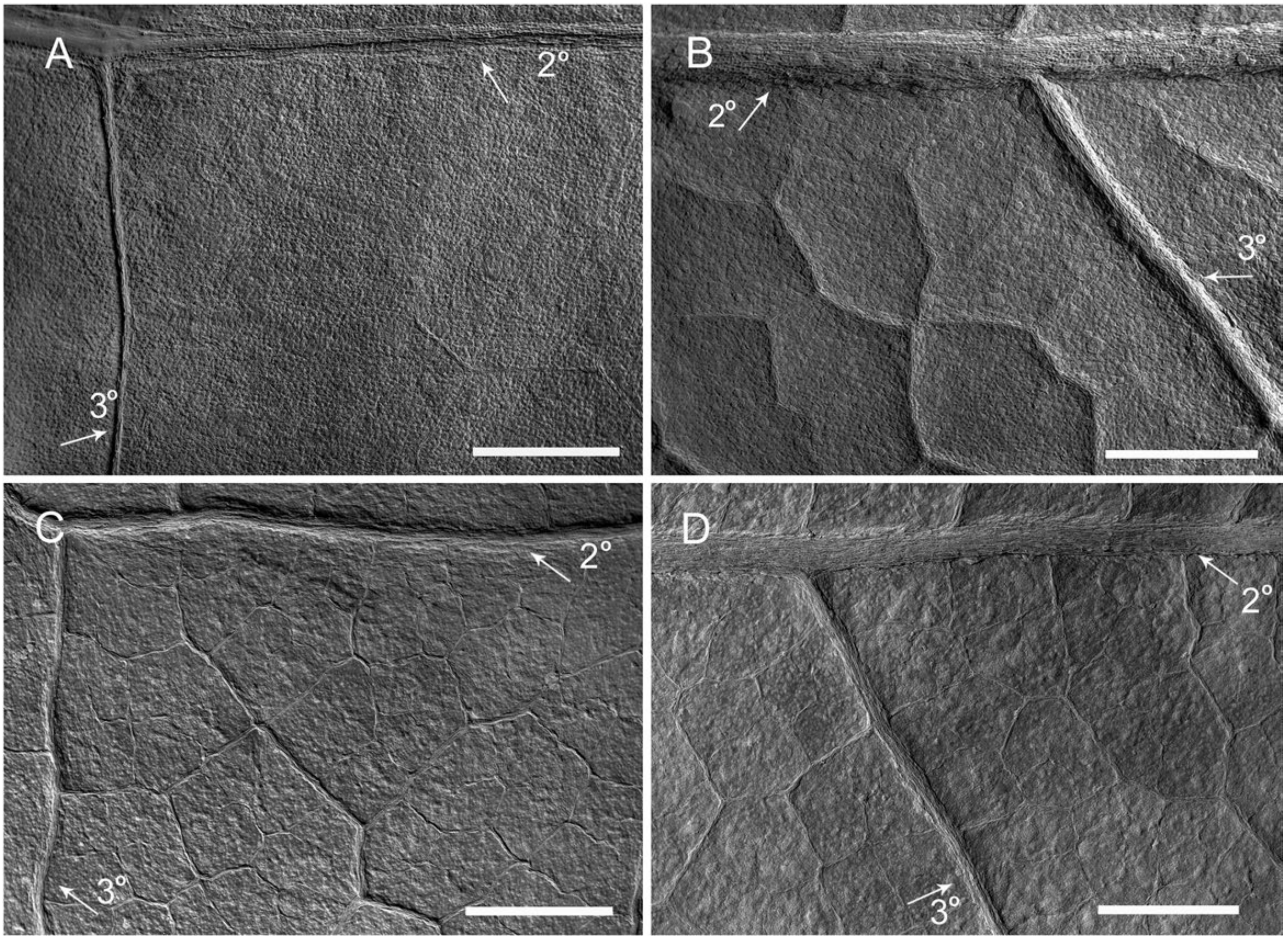

Figure 8. Details of leaf lamina venation of shade (A, C) and sun (B, D) leaves, SEM. A, B, *Liquidambar chinensis*. C, D, *Liquidambar formosana*. Scale bar, 1 mm. Arrows indicate the secondary (2°) and tertiary (3°) veins.

25.8 mm in sun leaves), whereas the average value is higher in sun leaves (140.2 mm versus 97.5 mm in shade leaves). Evidently in both species shade leaves show a larger variation in dimensional features. The IQR of the L/W ratio is higher in shade leaves (0.26 versus 0.04 in sun leaves) (Fig. 10C). The average value of the L/W ratio is higher in shade leaves (0.87 versus 0.71 in sun leaves), i.e. shade morphotypes tend to be more elongate. It should be noted that some leaves that developed in the middle part of the crown have the same L/W ratio (0.71) as most sun leaves (Fig. 10C). These leaves comprise an intermediate group.

Lobe sizes in shade and sun leaf morphotypes show less variation. The IQR of the central lobe length is 15.0 mm in shade leaves and 13.8 mm in sun leaves (Fig. 11A), whereas the IQR of the central lobe width is 12.3 mm in shade leaves and 8.0 mm in sun leaves (Fig. 11B). A clearly visible extension in the lobe tips was generally observed in the sun leaves.

In *L. formosana* there are again differences in tooth size with teeth on shade leaves being larger than those of sun leaves. Typically, there are four to five teeth per 10 mm of the lamina margin in shade leaves, and five to six teeth over the same length in sun leaves. As in the case of *L. chinensis*, there are also some differences in the tooth shape, which is concave/retroflexed with approximately equal length flanks in shade leaves (Fig. 5C), and concave/retroflexed teeth with longer proximal flanks than distal flanks in sun leaves (Fig. 5D). The differentiation of vein orders is less conspicuous in shade leaves (Figs 5F, 8C) in comparison to sun leaves (Figs 5E, 8D).

DISCUSSION

Factors determining differences between shade and sun leaves

Variability of leaf morphological characters in the crowns of large trees results from variations of a range of ecological and environmental factors (e.g. Zalensky, 1904; Givnish, 1988; Sun *et al.*, 2003;

Tsukaya, 2005; Rozendaal, Hurtado & Poorter, 2006; Sack *et al.*, 2006; Maslova, Volkova & Gordenko, 2008; Xu *et al.*, 2009; Rubio de Casas *et al.*, 2011; Witham, Marchiano & Reynolds, 2014). Leaves from the inner and peripheral parts of the crown experience different temperatures, light and exposure, whereas leaves from different crown heights experience different hydrostatic constraints because gravity limits the delivery of water to the upper levels. Thus, both horizontal and vertical environmental gradients give rise to a diversity of macromorphological and microstructural leaf characters in the same crown, and in general terms this is reflected by Zalensky's law (Zalensky, 1904). According to Zalensky, the leaves of woody plants developing in the upper region of a crown or near the shoot apex are more exposed to sunlight and experienced less favourable hydrological conditions, and consequently exhibit generally more xeromorphic features than leaves elsewhere on the plant. Solar radiation, particularly its intensity and qualitative composition, is an important factor that influences leaf morphology and anatomy, as shown by Rubio de Casas *et al.* (2011) for *Olea europaea* L., in which particular morphological features of sun leaves were correlated with the intensity of direct radiation, whereas those of shade leaves were mostly influenced by diffuse solar radiation. Both solar radiation deficit and excess result in leaf growth modifications. Leaves in the inner crown part (shade leaves) adapt to diffuse radiation and appear to reflect genetic differences in the population better than sun leaves. Meanwhile, sun leaves are subjected to allometric variation in a greater degree (Rubio de Casas *et al.*, 2011).

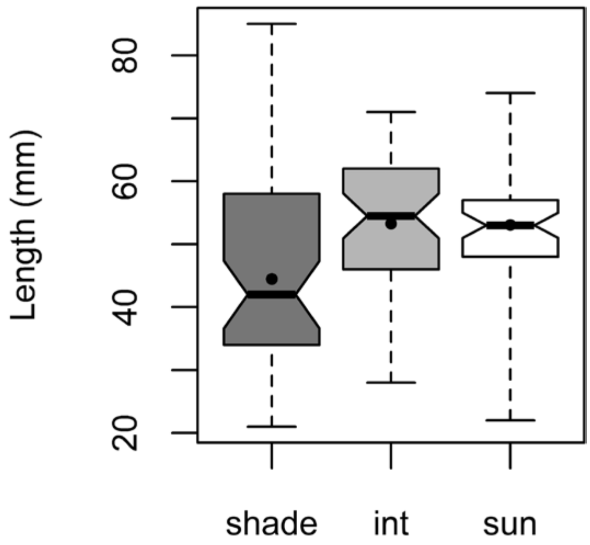

Figure 9. Variation in petiole length of shade (dark grey box plot), sun (white box plot) and intermediate (light grey box plot) leaves of *Liquidambar formosana*, mm. A black point in the box indicates the arithmetic mean.

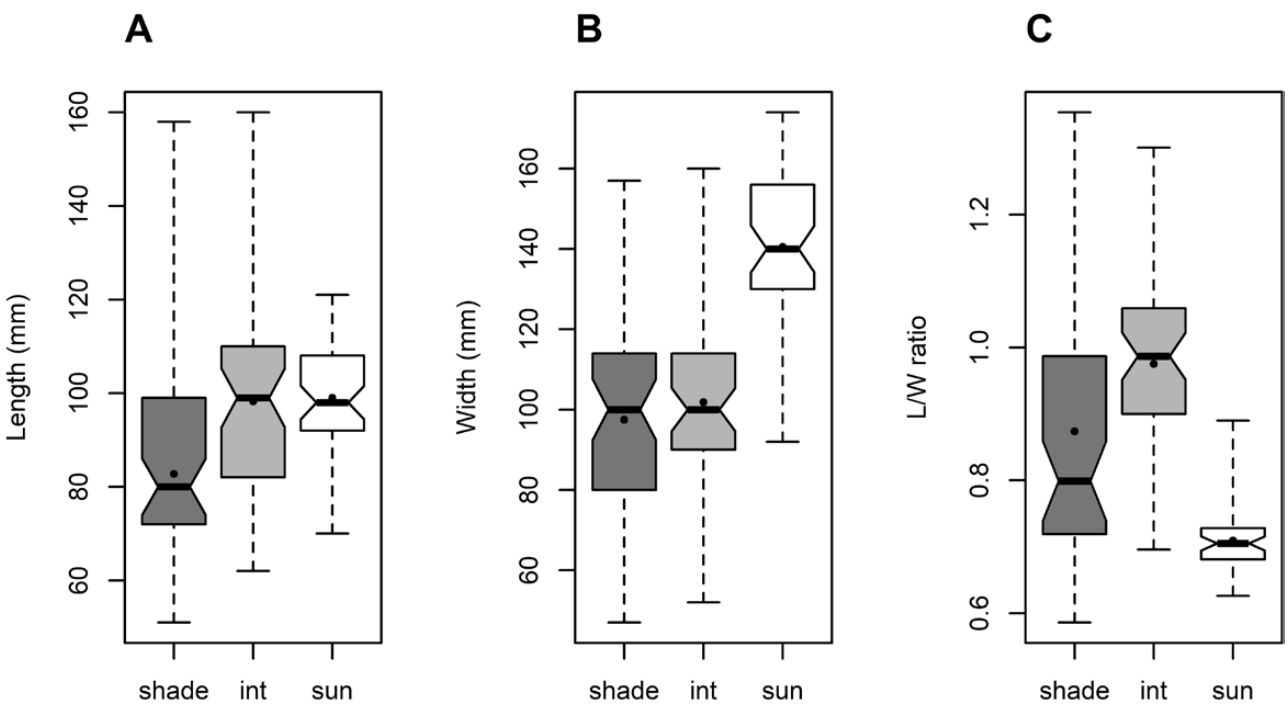

Figure 10. Size variation of shade (dark gray box plot), sun (white box plot) and intermediate (light grey box plot) leaves of *Liquidambar formosana*. A, Length, mm. B, Width, mm. C, L/W ratio. A black point in the box indicates the arithmetic mean.

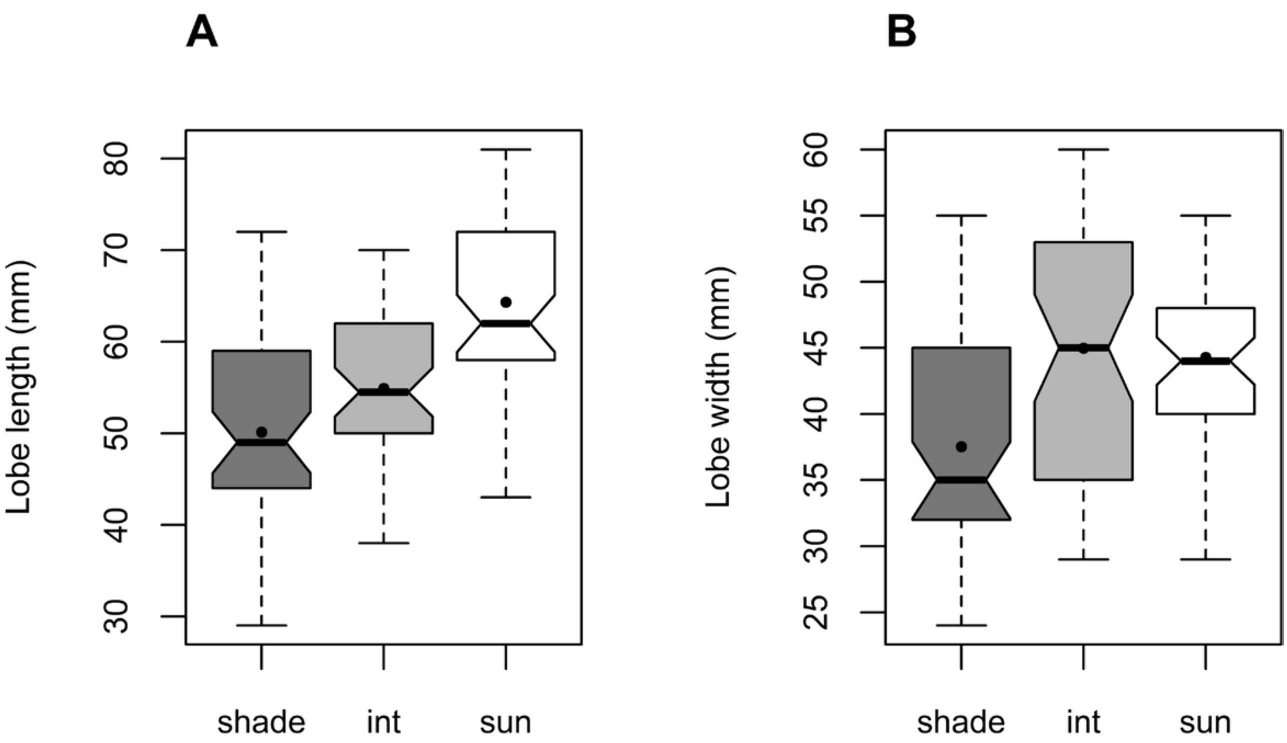

Figure 11. Lobe size variation of shade (dark grey box plot), sun (white box plot) and intermediate (light grey box plot) leaves of *Liquidambar formosana*. A, Lobe length, mm. B, Lobe width, mm. A black point in the box indicates the arithmetic mean.

Characteristic morphological features of shade and sun leaves of *Liquidambar chinensis* and *L. formosana*

Shade leaves of *Liquidambar chinensis* have laminae that are narrower, exhibit a variety of lamina shapes (elliptic, obovate, oblong), have less conspicuous venation networks and smaller teeth. Sun leaves of *L. chinensis* are exclusively elliptic, with a more conspicuous venation network and larger teeth.

Variability of lobing in *Liquidambar* was earlier studied for *L. styraciflua* L. (Smith, 1967), but then the emphasis was on leaf morphological variability along unevenly aged shoots at different ontogenetic stages. Here we studied morphological variability of *L. formosana* leaves experiencing different microclimatic conditions within the crown. Shade leaves of *L. formosana* are narrower than sun leaves, have less conspicuous venation networks and smaller teeth. Sun leaves of *L. formosana* have generally more extended lobe tips, a prominent venation network and larger teeth. Average values of lamina length and width of intermediate morphotypes in *L. formosana* correspond to those of typical shade leaves. In sun leaves these values are less variable.

The following trends in the development of morphological characters in groups of shade, sun, and intermediate leaves of *L. chinensis* and *L. formosana* are evident from statistical analyses: (1) leaf dimensions are more variable in shade leaves than in sun leaves; (2) laminae with a higher L/W ratio prevail in shady conditions; (3) leaves in the crown centre (intermediate between the shade and sun leaves) are more similar to shade leaves in their dimensional features; (4) sun leaves have somewhat longer lobes in comparison with those in shade and intermediate groups and (5) an average value of the petiole length is independent of the leaf position in the crown; however, this parameter shows greater variation in shade leaves.

Some of our data contradict conclusions presented by Rubio de Casas *et al.* (2011) for *Olea europaea*. In that species, the dimensions of sun leaves were reported to maximally reflect allometric variation, whereas shade leaves are more uniform. According to Rubio de Casas *et al.* (2011) low size variation in populations of shade leaves results from their development in more diffuse and uniform radiation, and the differences in dimensional features between shade and sun leaves are mostly defined by variations of sun leaf morphotypes. In contrast, our data for two *Liquidambar* spp. demonstrate more dimensional variation amongst inner crown shade leaves. This can be explained by the selective advantages conferred by occupying the optimal position in leaf arrangement (variation of the petiole length) or it can result from

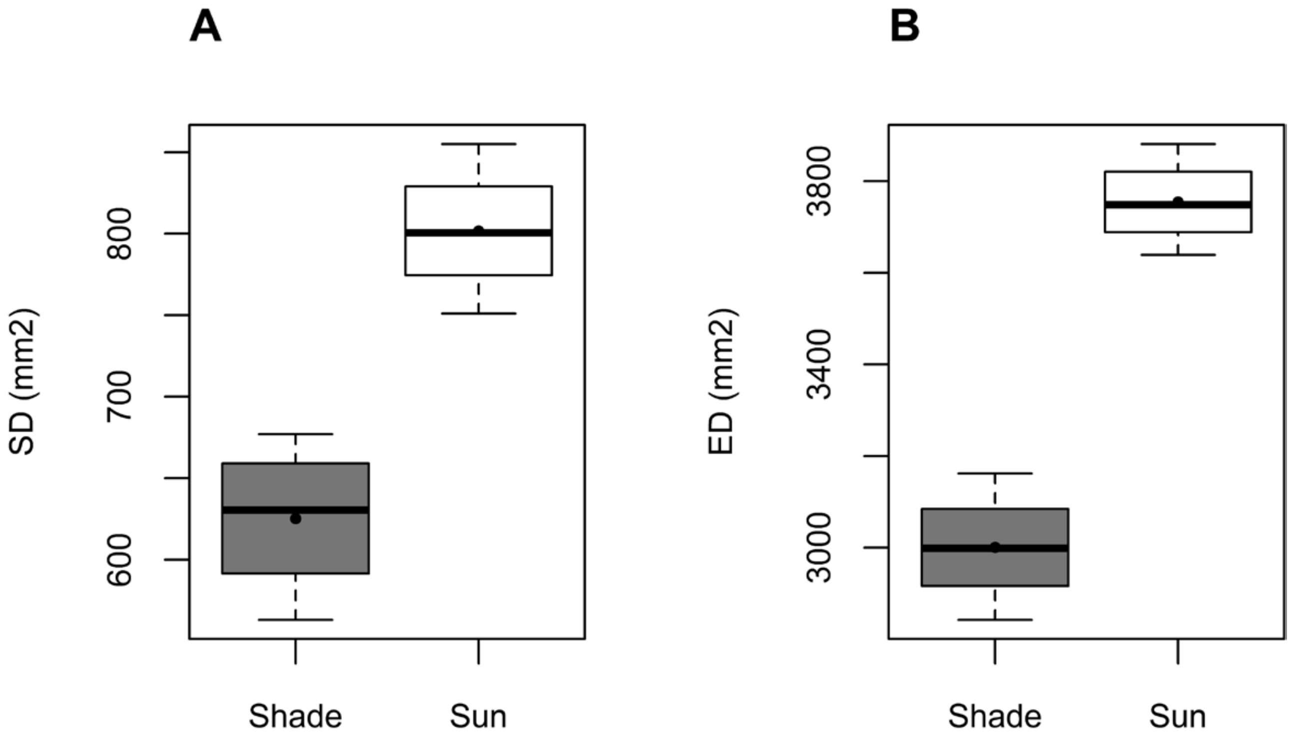

Figure 12. Variation of stomatal (SD) and epidermal cell (ED) density of shade (dark grey box plot) and sun (white box plot) leaves of *Liquidambar chinensis*. A, Stomatal density, per 1 mm^2. B, Epidermal cell density, per 1 mm^2. A black point in the box indicates the arithmetic mean.

relatively different conditions of sun exposure in the massive crown of a large tree. Rubio de Casas *et al.* (2011) showed that sun leaves of *O. europaea* differ in having a narrower lamina in comparison to shade leaves, and this was explained by an adaptation to provide the most effective light penetration to the inner crown part. Our data do not support this explanation, with the narrower forms (higher L/W ratio) observed in the inner part of the crown both for both entire leaves of *L. chinensis* and lobate leaves of *L. formosana*. The same trend was revealed for lobate leaves of *Platanus acerifolia* (Maslova, Volkova & Gordenko, 2008). These differences can be explained by the fact that *Liquidambar* trees have much denser crowns than olives. We interpret this as meaning that leaves in the inner part of the crown require greater morphological plasticity, allowing them to more effectively utilize the available sunlight.

Epidermal characters of shade and sun leaves of *Liquidambar chinensis* and *L. formosana*

Previous publications have documented epidermal characters of shade and sun leaves in several tree species, and these studies provide useful comparators for the work presented here (Zalensky, 1904; Balsamo *et al.*, 2003; Sun *et al.*, 2003; Herrick, Maherali & Thomas, 2004; Kouwenberg, Kürschner & McElwain, 2007; Wu *et al.*, 2009; Xiao *et al.*, 2011 etc.). Leaves of *L. chinensis* are dorsiventral, i.e. epidermal features of lower and upper surfaces of shade and sun leaves differ considerably. The cuticles of sun leaves are thicker than those of shade leaves, the anticlinal walls of ordinary epidermal cells have distinctive shapes (strongly sinuous in shade leaves versus straight or slightly curved anticlinal walls in sun leaves) and thicknesses (the anticlinal walls are thicker in sun leaves). The ED of shade leaves is lower than that of sun leaves (Fig. 12). As shown by previous studies (Kürschner, 1997; Sun *et al.*, 2003; Herrick, Maherali & Thomas, 2004; Xiao *et al.*, 2011 etc.), ED in sun leaves is often higher than in shade leaves due to the reduced cell size. The stomata are paracytic and the SD is higher in sun leaves than in shade leaves (Fig. 12).

Xiao *et al.* (2011) showed a similar pattern in the epidermal features of shade and sun leaves of *L. formosana*. The differences between shade and sun morphotypes are particularly marked in respect of the outline of anticlinal walls of ordinary epidermal cells and in the ED and SD values. In *L. formosana* the upper epidermis is a better differentiator between sun and shade leaves than the lower epidermis, particularly in respect of the degree of anticlinal wall undulation. Xiao *et al.* (2011) suggested that

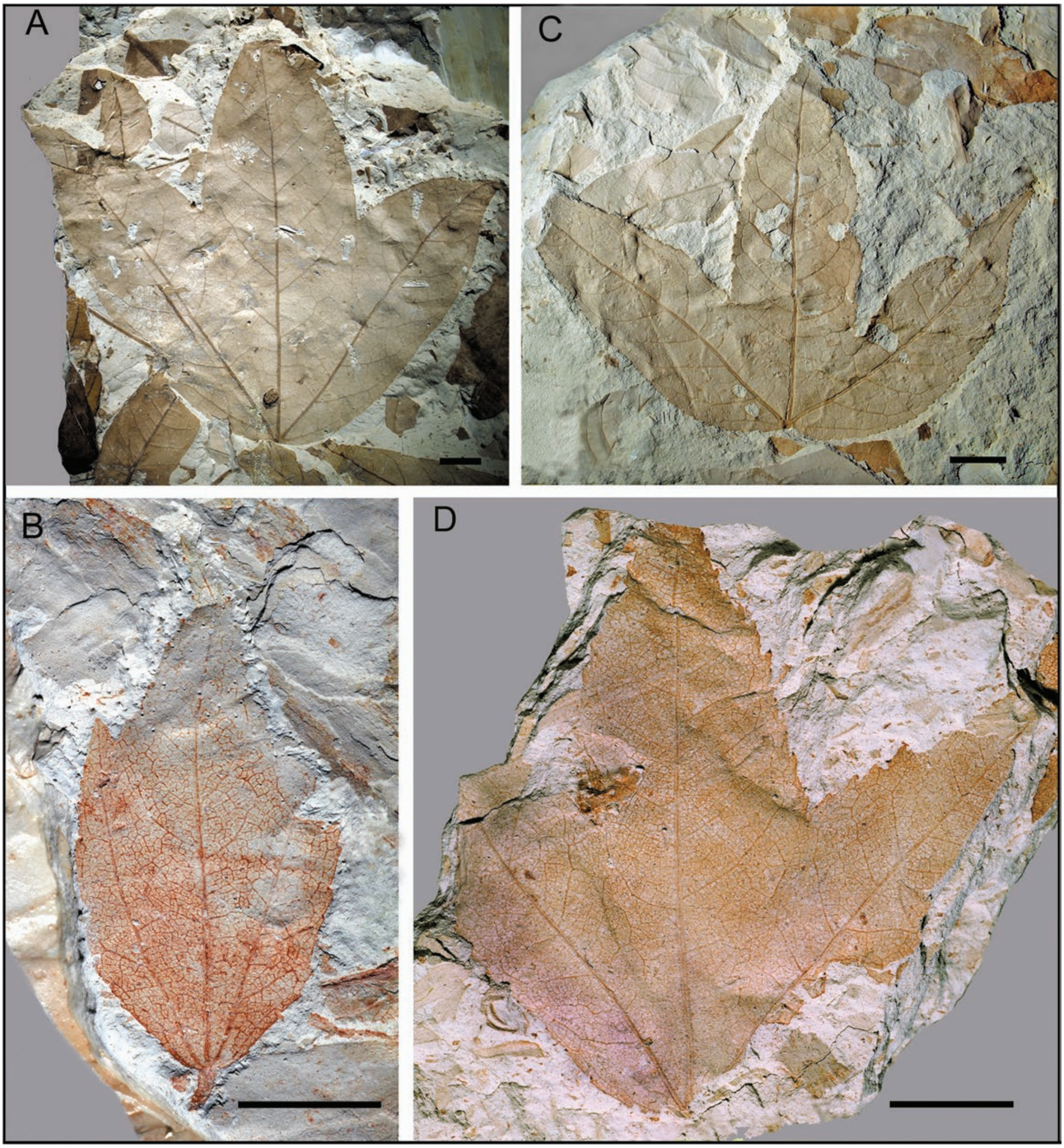

Figure 13. Fossil leaves of *Liquidambar*, Eocene, Maoming Basin, South China. A, Shade lobed leaf of *L. maomingensis*, MMJ3-151a-1 (Maslova *et al.*, 2015, fig. 29), L/W ratio is about 1, venation inconspicuous, teeth small. B, Shade lobed leaf of *Liquidambar* sp., MMJ2-2–279, L/W ratio is more than 1, venation inconspicuous, teeth small. C, Sun lobed leaf of *L. maomingensis*, MMJ3–129a-1 (Maslova *et al.*, 2015, fig. 10), L/W ratio is < 1, venation prominent, teeth relatively large. D, Sun lobed leaf of *Liquidambar* sp., MMJ2-2-114a, L/W ratio is *c*. 1, venation prominent, teeth relatively large. Scale bars, 10 mm.

this may represent a more sensitive response of the upper epidermis to environmental variations. Xiao *et al*. (2011) also concluded that anatomical features are more variable in sun leaves than in shade leaves, suggesting sun leaves may be more sensitive to environmental changes.

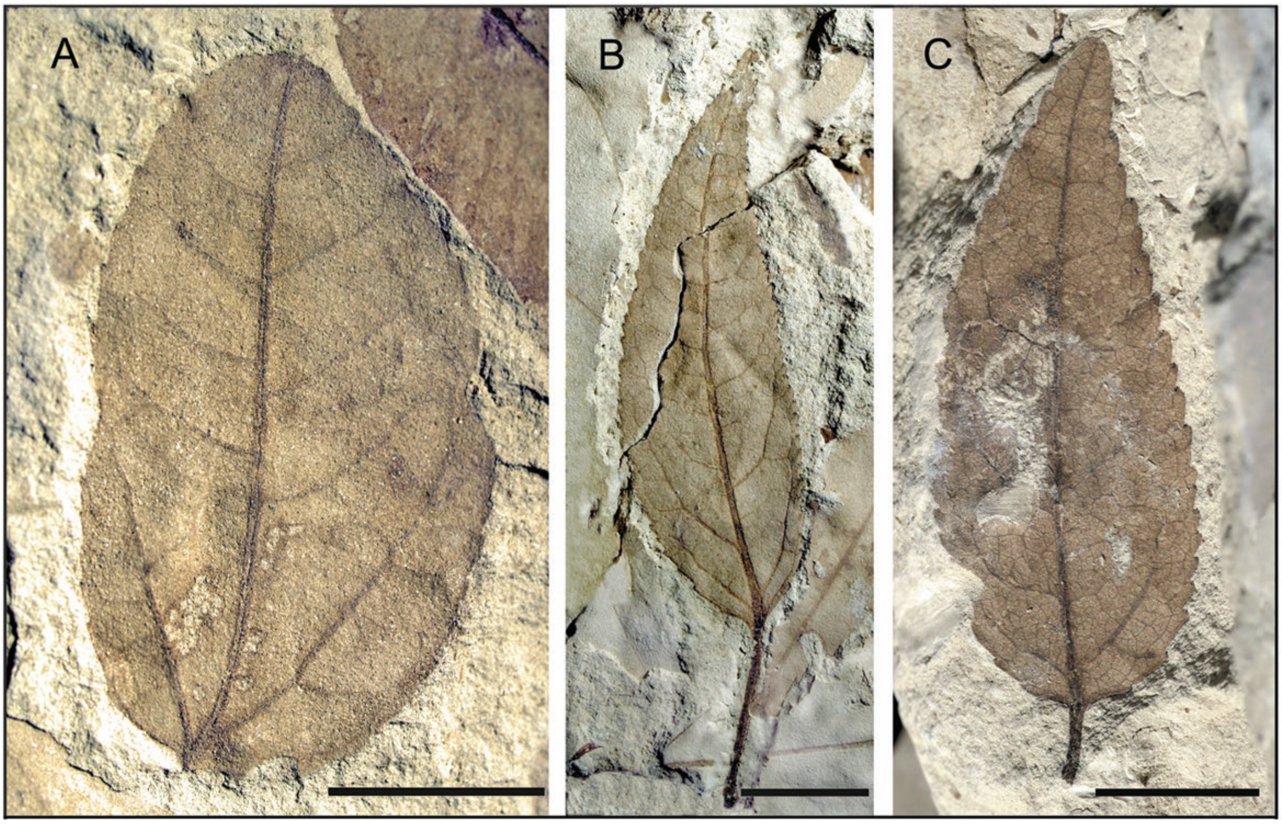

Figure 14. Fossil entire leaves of *Liquidambar maomingensis*, Eocene, Maoming Basin, South China. A, B, Shade leaves with small teeth and inconspicuous venation, MMJ3-159a-1 and MMJ3-162-1 (Maslova *et al.*, 2015, figs 37 and 35), respectively. C, Sun leaf with prominent venation and relatively large teeth, MMJ3-463-2. Scale bars, 10 mm.

Epidermal characters of *L. chinensis*, with the previously published data for *L. formosana* (Xiao *et al.*, 2011), reveal distinctive shade and sun variability. The observed trends are similar in both the modern species we studied. Shade leaves principally differ from sun leaves in possessing sinuous anticlinal walls of ordinary epidermal cells (especially on the upper lamina surface) and relatively larger ordinary cells on both lamina surfaces (Fig. 2C). There are also differences in the ED and SD values: both are lower in shade leaves. Lower values of ED and SD in shade leaves are also evident in *L. styraciflua* L. (Herrick, Maherali & Thomas, 2004).

Implications for palaeobotany

Taxonomic determination of fossil leaves is usually based on gross morphological (including venation) features and sometimes epidermal characters, which are more rarely preserved. The number of fossil leaf taxa in a given locality can be overstated when different morphotypes are considered to be separate taxa without regard to intra-species variability (e.g. Samsonov, 1964; Golovneva, 2004). A large selection of fossil leaves of the same taxon allows the range of variations to be properly evaluated and potentially reveals which specimens represent sun or shade forms (e.g. Barbacka *et al.*, 1998; Wu *et al.*, 2009; Maslova *et al.*, 2015). Often, poor preservation means that the study of epidermal characters is impossible, but it is important in clarifying systematic affinities of the leaves and understanding variation due to environmental factors (e.g. shade or sun leaves). In the absence of epidermal features, data on possible variability within morphological characters are critical for correct systematic assignment. Criteria used to define a fossil leaf environmental morphotype (shade/sun) can only be derived from large samples of modern analogues where the studied material is unlimited and the environmental factors can be determined.

Morphological and epidermal characters of shade and sun leaves of two modern *Liquidambar* spp. with different lamina forms (unlobed with pinnate venation in *L. chinensis* and palmately trilobate with basally actinodromous venation in *L. formosana*) illustrate different morphological responses to sun and shade environments. Note that transitional shade/sun leaf morphotypes tend to have strongly varying morphological characters, whereas shade/sun end members have more stable morphologies, facilitating discrimination between shade and sun forms.

The patterns of morphological responses to sun and shade seen in living trees enable us to distinguish shade and sun leaves in fossil *Liquidambar*. Using data obtained from modern species helps distinguish sun and shade leaves within populations of polymorphic leaves of *L. maomingensis* N.Maslova, Kodrul, Song & Jin from the upper part of the Huangniuling Formation, Eocene (locality MMJ3), Maoming Basin, South China (Maslova *et al.*, 2015). The variational range of leaves of *L. maomingensis* includes two main morphotypes: trilobate and entire and is a unique feature of this species. The specimens of *L. maomingensis* did not preserve epidermal features, and therefore only morphological characters were used to distinguish shade from sun leaves. In *L. maomingensis* the most variable characters are the lamina shape (lobed or entire), lamina size (length, width and L/W ratio), lobe shape and size, tooth shape and size and the degree of development of secondary and tertiary veins.

Based on modern *L. formosana* leaves we can distinguish shade (Fig. 13A, B) and sun (Fig. 13C, D) morphotypes among lobate leaves of *L. maomingensis* and *Liquidambar* sp. from the lower part of the Huangniuling Formation. In *L. maomingensis* we interpret shade leaves to exhibit the following characters: the L/W ratio is *c.* 1.22 (i.e. the lamina length exceeds its width), secondary and tertiary venation is inconspicuous, the laminae are only weakly dissected and the teeth are small. In the group leaves that we consider to have been sun leaves the L/W ratio is *c.* 0.6 (i.e. the lamina width greatly exceeds the length), venation is pronounced (secondary and tertiary veins are more prominent), the laminae are dissected and the teeth rather large.

Leaves of *Liquidambar* from the lower part of the Huangniuling Formation (locality MMJ2-2) corresponding to a new species (a study in progress) were preserved as impressions and no epidermal data could be obtained. However, many of these leaf impressions (48 specimens consisting of more-or-less complete impressions and numerous leaf fragments from thin fossil leaf litter layers with dense accumulations of *Liquidambar* leaves) allow us again to distinguish between shade (Fig. 13B) and sun (Fig. 13D) leaves. However, not all leaf specimens show clear sun and shade features, but exhibit intermediate morphologies with a mixture of features and may represent leaves with intermediate positions in the ancient tree crowns.

Shade and sun morphotypes were also recognized in the population of entire leaves of *L. maomingensis*. The shade morphotype includes leaves with a less pronounced venation network and small widely spaced teeth. The L/W ratio of shade leaves is higher than that of sun leaves (Fig. 14A, B versus Fig. 14C). The sun morphotype includes leaves with a conspicuous venation network and larger teeth.

Trees from different habitats such as lowland forests or highland areas also show similar differences in epidermal and some gross morphological characters (e.g. degree of development of the venation network etc.) (Zalensky, 1904). Variability of epidermal characters may also be used for distinguishing sun and shade morphotypes among fossil leaves (e.g. Poole *et al.*, 1996; Kürschner, 1997; Poole & Kürschner, 1999; Guignard, Bóka & Barbacka, 2001; Kouwenberg, Kürschner & McElwain, 2007; Maslova & Shilin, 2011; Xiao *et al.*, 2011). Such variability has also been used to distinguish groups of sun and shade trilobate leaves of *L. miosinica* Hu & Chaney from the Miocene, Zhejiang Province, eastern China (Xiao *et al.*, 2011). These authors demonstrated the same variability of epidermal characters for *L. miosinica* as for modern *L. formosana* and a higher variability of epidermal characters was observed for sun leaves both in modern and fossil *Liquidambar* spp.

Xiao *et al.* (2011) considered that sun leaves exhibit more morphological variation on the crown periphery and are more influenced by open atmosphere environmental conditions than shade leaves, which occupy the inner part of the crown with a buffered microclimate. We observed the opposite for morphological characters of two modern *Liquidambar* spp.: shade leaves are more variable than sun leaves. We think that the observed diversity in the quantitative characters (e.g. petiole length, length and width of the lamina) of shade leaves results from leaf optimization towards maximizing photosynthetic return where light levels are low but spatially variable, and structural and hydrological constraints that might otherwise limit morphological variety are less severe. Despite more marked changes in light intensity and other factors during the day, sun leaves are mostly not light limited and can afford to produce smaller, more robust forms and thus converge within a limited morphological range. As in case of lobate leaves of *L. formosana*, variations in the morphological and epidermal characters of entire sun and shade leaves of *L. chinensis* can be useful for future palaeobotanical studies.

CONCLUSIONS

By recognizing that shade and sun leaf morphotypes are distinct in modern *Liquidambar* spp., irrespective of overall leaf form and that similar morphotypes exist in populations of Eocene leaves of *L. maomingensis*, we demonstrate that it is possible to distinguish sun and shade leaves in the fossil record. Principal morphological characters are the L/W ratio, the degree of development of the venation network and tooth size and shape. The main epidermal characters are the size of ordinary epidermal cells and the outline of anticlinal cells. Identifying character variation typical of sun and shade morphotypes in modern taxa and recognizing similar character suites in fossil leaves will result in more accurate systematic determinations and help to avoid erroneous and inflated perceptions of diversity in ancient ecosystems.

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REFERENCES

Balsamo RA, Bauer AM, Davis SD, Rice BM. 2003. Leaf biomechanics, morphology, and anatomy of the deciduous mesophyte *Prunus serrulata* (Rosaceae) and the evergreen sclerophyllous shrub *Heteromeles arbutifolia* (Rosaceae). *American Journal of Botany* **90:** 72–77.

Barbacka M, van Konijnenburg-van Cittert JHA. 1998. Sun and shade leaves in two Jurassic species of Pteridosperms. *Review of Palaeobotany and Palynology* **103:** 209–221.

Bentham G, Hooker JD. 1865. *Genera plantarum*. London: Reeve and Williams & Norgate.

Blume CL. 1828. *Flora Javae nec non insularum adjacentium*. Brussels: J. Frank.

Brown RW. 1933. A Cretaceous sweet gum. *Botanical Gazette* **94:** 611–615.

Chaney RW, Axelrod DI. 1959. *Miocene floras of the Columbia Plateau*. Part II, systematic considerations. *Carnegie Institution of Washington Publication* **617:** 135–229.

Cooke R. 2005. *The history of mathematics: a brief course*. *2nd edn*. Hoboken: Wiley-Interscience.

Ellis B, Daly DC, Hickey LJ, Johnson KR, Mitchell JD, Wilf P, Wing SL. 2009. *Manual of leaf architecture*. Ithaca: Cornell University Press.

Endo S, Morita H. 1932. Notes on the genera *Comptoniphyllum* and *Liquidambar*. *Science reports of the Tohoku Imperial University. 2nd series, Geology* **15:** 41–53.

Givnish TJ. 1988. Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology* **15:** 63–92.

Golovneva LB. 2004. *The Late Cretaceous flora of Siberia*. Doctoral Dissertation in Biology, Komarov Botanical Institute.

Greenwood JA, Sandomire MM. 1950. Sample size required for estimating the standard deviation as a per cent of its true value. *Journal of the American Statistical Association* **45:** 257–260.

Guignard G, Bóka K, Barbacka M. 2001. Sun and shade leaves? Cuticle ultrastructure of Jurassic *Komlopteris nordenskioeldii* (Nathorst) Barbacka. *Review of Palaeobotany and Palynology* **114:** 191–208.

Herrick JD, Maherali H, Thomas RB. 2004. Reduced stomatal conductance in sweetgum (*Liquidambar styraciflua*) sustained over long-term CO_2 enrichment. *New Phytologist* **162:** 387–396.

Hu HH, Chaney RW. 1940. *A Miocene flora from Shantung province, China: Part I. Introduction and systematic considerations*. Washington: Carnegie Institution of Washington.

Huzioka K. 1972. The Tertiary floras of Korea. *Journal of the Mining College, Akita University. Ser. A, Mining Geology* **5:** 1–83.

Huzioka K, Uemura K. 1979. The *Comptonia-Liquidambar* forest during middle Miocene Daijima age in Japan. *Report of the Research Institute of Underground Resources, Mining College, Akita University* **45:** 37–50.

Ickert-Bond SM, Pigg KB, Wen J. 2005. Comparative infructescence morphology in *Liquidambar* (Altingiaceae) and its evolutionary significance. *American Journal of Botany* **92:** 1234–1255.

Ickert-Bond SM, Pigg KB, Wen J. 2007. Comparative infructescence morphology in *Altingia* (Altingiaceae) and discordance between morphological and molecular phylogenies. *American Journal of Botany* **94:** 1094–1115.

Ickert-Bond SM, Wen J. 2006. Phylogeny and biogeography of Altingiaceae: evidence from combined analysis of five non-coding chloroplast regions. *Molecular Phylogenetics and Evolution* **39:** 512–528.

Ickert-Bond S, Wen J. 2013. A taxonomic synopsis of Altingiaceae with nine new combinations. *PhytoKeys* **31:** 21–61.

Knowlton FH. 1902. *Fossil flora of the John Day basin, Oregon*. Washington: Government Printing Office.

Kouwenberg LLR, Kürschner WM, McElwain JC. 2007. Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy and Geochemistry* **66:** 215–241.

Krzywinski M, Altman N. 2014. Visualizing samples with box plots. *Nature Methods* **11:** 119–120.

Kürschner WM. 1997. The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/*Q. pseudocastanea* Goeppert) — implications for their use as biosensors of palaeoatmospheric CO_2 levels. *Review of Palaeobotany and Palynology* **96:** 1–30.

Larcher W. 1976. *Ökologie der Pflanzen*, *2nd edn*. Stuttgart: Ulmer.
Leaf Architecture Working Group. 1999. *Manual of leaf architecture: morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms*. Washington: Leaf Architecture Working Group.
Lindley J. 1836. *A natural system of botany*. London: Longman.
MacGinitie HD. 1941. *A Middle Eocene flora from the central Sierra Nevada*. Washington: Carnegie Institution.
Maslova NP. 1995. *Liquidambar* L. from the Cenozoic of eastern Asia. *Paleontological Journal* **29 (1A):** 145–158.
Maslova NP, Gordenko NV, Volkova LD. 2008. Structural epidermal features of leaves of *Platanus acerifolia* Willd. (Platanaceae) and significance of cuticular analysis for the determination of Cretaceous *Platanus*-like leaves. *Botanicheskii Zhurnal* **93:** 982–995 [In Russian].
Maslova NP, Kodrul TM, Song Y, Volkova LD, Jin J. 2015. *Liquidambar maomingensis* sp. nov. (Altingiaceae) from the late Eocene of South China. *American Journal of Botany* **102:** 1356–1370.
Maslova NP, Shilin PV. 2011. The new species *Ettingshausenia sarbaensis* (Angiospermae) from the Cenomanian-Turonian of western Kazakhstan in light of the problem of classification of dispersed cretaceous *Platanus*-like leaves. *Paleontological Journal* **45:** 459–473.
Maslova NP, Volkova LD, Gordenko NV. 2008. Morphological variability of leaves of *Platanus acerifolia* Willd. (Platanaceae) and approaches to the determination of dispersed Cretaceous *Platanus*-like leaves. *Botanicheskii Zhurnal* **93:** 825.
Meyer HW, Manchester SR. 1997. *The Oligocene Bridge Creek flora of the John Day Formation, Oregon*. Berkeley, London: University of California Press.
Oken L. 1841. *Allgemeine Naturgeschichte für alle Stände*. Stuttgart: Hoffmann'sche Verlags-Buchhandlung.
Onoe T. 1974. A Middle Miocene flora from Oguni-Machi, Yamagata prefecture, Japan. *Reports, Geological Survey of Japan* **253:** 1–64.
Ozaki K. 1991. *Late Miocene and Pliocene floras in central Honshu, Japan*. Yokohama: Kanagawa Prefectural Museum.
Pandey S, Nagar PK. 2002. Leaf surface wetness and morphological characteristics of *Valeriana jatamansi* grown under open and shade habitats. *Biologia Plantarum* **45:** 291–294.
Pigg KB, Ickert-Bond SM, Wen J. 2004. Anatomically preserved *Liquidambar* (Altingiaceae) from the middle Miocene of Yakima Canyon, Washington State, USA, and its biogeographic implications. *American Journal of Botany* **91:** 499–509.
Poole I, Kürschner WM. 1999. Stomatal density and index: the practice. In: Jones TP, Rowe NP, eds. *Fossil plants and spores: modern techniques*. London: Geological Society, 257–260.
Poole I, Weyers JDB, Lawson T, Raven JA. 1996. Variations in stomatal density and index: implications for palaeoclimatic reconstructions. *Plant, Cell and Environment* **19:** 705–712.
Pigliucci M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters* **6:** 265–272.
R Core Team. 2014. *R: a language and environment for statistical computing*. Available at: https://www.r-project.org.
Rozendaal DMA, Hurtado VH, Poorter L. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology* **20:** 207–216.
RStudio Team. 2015. *RStudio: integrated development for R*. Boston: RStudio, Inc. Available at: http://www.rstudio.com.
Rubio de Casas R, Vargas P, Pérez-Corona E, Manrique E, García-Verdugo C, Balaguer L. 2011. Sun and shade leaves of *Olea europaea* respond differently to plant size, light availability and genetic variation. *Functional Ecology* **25:** 802–812.
Sack L, Melcher PJ, Liu WH, Middleton E, Pardee T. 2006. How strong is intracanopy leaf plasticity in temperate deciduous trees? *American Journal of Botany* **93:** 829–839.
Samsonov SK. 1964. New data on Upper Cretaceous flora of Karatau. In: *Biostratigraphy and paleogeography of Meso-Cenozoic oil and gas-bearing regions of the south-east of the USSR*. Moscow: Nauka, 31–35. [In Russian].
Shi S, Huang Y, Zhong Y, Du Y, Zhang Q, Chang H, Boufford DE. 2001. Phylogeny of the Altingiaceae based on cpDNA *matK*, PY-IGS and nrDNA ITS sequences. *Plant Systematics and Evolution* **230:** 13–24.
Smith RF. 1967. The leaf dimorphism of *Liquidambar styraciflua* L. *The American Midland Naturalist* **77:** 42–50.
Stults DZ, Axsmith BJ. 2011. Filling the gaps in the Neogene plant fossil record of eastern North America: new data from the Pliocene of Alabama. *Review of Palaeobotany and Palynology* **167:** 1–9.
Sun B, Dilcher DL, Beerling DJ, Zhang C, Yan D, Kowalski E. 2003. Variation in *Ginkgo biloba* L. leaf characters across a climatic gradient in China. *Proceedings of the National Academy of Sciences of the United States of America* **100:** 7141–7146.
Suzuki K. 1961. *The important and characteristic Pliocene and Miocene species of plants from the southern part of the Tohoku District, Japan*. Fukushima University, Faculty of Art and Science.
Tsukaya H. 2005. Leaf shape: genetic controls and environmental factors. *The International Journal of Developmental Biology* **49:** 547–555.
Uemura K. 1983. Late Neogene *Liquidambar* (Hamamelidaceae) from the southern part of northeast Honshu, Japan. *Memoirs of the National Science Museum of Tokyo* **16:** 25–36.
Witham E, Marchiano M, Reynolds D. 2014. Relationship between canopy location and tensile strength of leave of *Heteromeles arbutifolia* in Malibu, CA. *All Undergraduate Student Research:* Paper 146. Available at: http://digitalcommons.pepperdine.edu/sturesearch/146.
Wolfe JA. 1993. A method of obtaining climatic parameters from leaf assemblages. *U. S. Geological Survey Bulletin* **2040:** 1–71.
Wolfe JA, Tanai T. 1980. *The Miocene Seldovia Point flora from the Kenai Group, Alaska*. Professional Paper 1105.
Wu J, Sun B, Liu Y-S, Xie S, Lin Z. 2009. A new species of *Exbucklandia* (Hamamelidaceae) from the Pliocene of China and its paleoclimatic significance. *Review of Palaeobotany and Palynology* **155:** 32–41.

Xiao L, Sun B, Li X, Ren W, Jia H. 2011. Anatomical variations of living and fossil *Liquidambar* leaves: a proxy for paleoenvironmental reconstruction. *Science China Earth Sciences* **54:** 493–508.

Xiao L, Yang H, Sun B, Li X, Guo J. 2013. Stable isotope compositions of recent and fossil sun/shade leaves and implications for palaeoenvironmental reconstruction. *Review of Palaeobotany and Palynology* **190:** 75–84.

Xu F, Guo W, Xu W, Wei Y, Wang R. 2009. Leaf morphology correlates with water and light availability: what consequences for simple and compound leaves? *Progress in Natural Science* **19:** 1789–1798.

Yang J, Spicer RA, Spicer TEV, Arens NC, Jacques FMB, Su T, Kennedy EM, Herman AB, Steart DC, Srivastava G, Mehrotra RC, Valdes PJ, Mehrotra NC, Zhou Z-K, Lai J-S. 2015. Leaf form–climate relationships on the global stage: an ensemble of characters. *Global Ecology and Biogeography* **24:** 1113–1125.

Zalensky VR. 1904. Materials to numerical anatomy of various leaves in the same plants. *Izvestiya Kievskogo Politekhnicheskogo Instituta* **4:** 1–209. [In Russian].

Zhang Z, Lu A. 1995. Hamamelidaceae: geographic distribution, fossil history and origin. *Chinese Science Abstracts Series B* **33:** 313–339.

Appendix 1 Anatomical features of sun and shade leaves of extant *Liquidambar chinensis*.

Characteristic		Sun	Shade
Lower epidermis	SD (n/mm²)	802 ± 43	625 ± 48
	ED (n/mm²)	3755 ± 99	3000 ± 131
	Ordinary epidermal cells: anticlinal wall	mostly straight or slightly curved	greatly sinuous, up to 10 µm
	shape	polygonal, more rarely tetragonal	rectangular, more rarely polygonal, up to ameboid
	Size	21.2–57.7 × 9.6–28.8 µm	28.8–59.6 × 14.4–27.9 µm
Upper epidermis	anticlinal wall	straight	sinuous, up to 10 µm
	Shape	polygonal, more rarely rectangular	polygonal, up to ameboid
	Size	21.7–36.6 × 13.3–16.7 µm	26.7–40.0 × 20.1–32.0 µm

SD = stomatal density; ED = epidermal cell density; Numerical values are expressed as mean value ± standard deviation.

Appendix 2 Values of statistical parameters in shade, intermediate and sun leaves of *Liquidambar chinensis* and *L. formosana*

Character	Parameter	*Liquidambar chinensis*			*Liquidambar formosana*		
		Shade leaves	Int. leaves	Sun leaves	Shade leaves	Int. leaves	Sun leaves
Length, mm	maximum	135.00	132.00	121.00	158.00	160.00	121.00
Length, mm	average	106.52	112.20	102.05	82.72	98.26	99.00
Length, mm	minimum	62.00	87.00	74.00	51.00	62.00	70.00
Length, mm	variation range	73.00	45.00	47.00	107.00	98.00	51.00
Length, mm	IQR	21.00	16.50	4.00	24.75	28.00	16.00
Length, mm	standard deviation	21.87	9.85	7.74	21.19	21.60	12.37
Width, mm	maximum	62.00	59.00	65.00	157.00	160.00	174.00
Width, mm	average	46.16	50.88	51.92	95.95	101.86	141.45
Width, mm	minimum	30.00	41.00	35.00	47.00	52.00	92.00
Width, mm	variation range	32.00	18.00	30.00	110.00	108.00	82.00
Width, mm	IQR	6.75	5.75	3.00	33.50	24.00	25.75
Width, mm	standard deviation	8.17	4.24	4.45	25.46	20.32	21.24
L/W ratio	maximum	2.53	2.83	2.14	1.35	1.30	0.89
L/W ratio	average	2.30	2.21	1.97	0.87	0.98	0.71
L/W ratio	minimum	1.88	1.85	1.76	0.59	0.70	0.63
L/W ratio	variation range	0.65	0.98	0.38	0.77	0.61	0.26
L/W ratio	IQR	0.20	0.19	0.06	0.26	0.15	0.04
L/W ratio	standard deviation	0.14	0.18	0.07	0.19	0.16	0.05

Appendix 2 Continued

Character	Parameter	*Liquidambar chinensis*			*Liquidambar formosana*		
		Shade leaves	Int. leaves	Sun leaves	Shade leaves	Int. leaves	Sun leaves
Petiole length, mm	maximum	13.00	13.00	12.00	85.00	71.00	74.00
Petiole length, mm	average	9.14	8.94	9.16	44.23	53.26	53.72
Petiole length, mm	minimum	5.00	6.00	6.00	21.00	28.00	22.00
Petiole length, mm	variation range	8.00	7.00	6.00	64.00	43.00	52.00
Petiole length, mm	IQR	3.00	2.00	1.00	23.00	15.50	8.75
Petiole length, mm	standard deviation	2.39	1.73	1.31	15.55	10.36	8.89
Lobe length, mm	maximum	-	-	-	72.00	70.00	81.00
Lobe length, mm	average	-	-	-	50.12	54.90	64.32
Lobe length, mm	minimum	-	-	-	29.00	38.00	43.00
Lobe length, mm	variation range	-	-	-	43.00	32.00	38.00
Lobe length, mm	IQR	-	-	-	15.00	11.50	13.75
Lobe length, mm	standard deviation	-	-	-	12.29	8.37	9.57
Lobe width, mm	maximum	-	-	-	55.00	60.00	55.00
Lobe width, mm	average	-	-	-	37.52	44.98	44.28
Lobe width, mm	minimum	-	-	-	24.00	29.00	29.00
Lobe width, mm	variation range	-	-	-	31.00	31.00	26.00
Lobe width, mm	IQR	-	-	-	12.25	17.25	8.00
Lobe width, mm	standard deviation	-	-	-	9.04	8.84	5.40

Abbreviations: int. – intermediate; IQR – interquartile range.