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PRELIMINARY DATA ON STOMATAL DENSITY DISTRIBUTION IN LEAVES OF *GINKGO BILOBA* L.

Elizaveta M. GUNKO[1]***, Eugeny V. KARASEV***[2]***, Maja V. NILOVA***[1]

[1] Lomonosov Moscow State University, Moscow, Russia

[2] Kazan Federal University, Kazan, Russia

lizaveta_gunko@mail.ru

The relationship of atmospheric CO_2 concentration with leaf stomatal density and stomatal index is repeatedly revealed on experiments with recent plants and analysis of fossil data (Beerling et al., 1998; Chen et al., 2001; Royer, 2001, 2003; Sun et al., 2018). However, fossil samples are often fragmentary. In this regard, it is very important to understand the possible limits of variation of stomatal density and stomatal index throughout the leaf surface. *Ginkgo biloba* is a model object for identifying these patterns in modern and fossil material (Retallack, 2001; Quan et al., 2009; Barclay, Wing, 2016). The aim of this investigation is to quantify changes in stomatal density (SD) on the surface of the *G. biloba* leaf.

The leaves of *G. biloba* were collected in the greenhouse of the Tsitsin Main Botanical Garden RAS and herbarized. The epidermis was sampled from the entire abaxial surface of the lamina (Fig. 1a), as *G. biloba* leaf is hypostomatous. The leaf margin was cut off on the width of 0.2 mm for easier separation of epidermis after maceration. Leaf was macerated in the 5% alkali solution at 70–80 °C for 7–10 minutes, thoroughly purified from alkali to separate the entire abaxial epidermis thereafter. The epidermal plates were stained with 2% aqueous Safranin and embedded in glycerin-gelatin. A transparent film with a printed coordinate grid was used instead of a cover glass to obtain the coordinates of the field of observation. Stomata were counted under Nikon Eclipse Ci microscope and photographed with Nikon DS-Vi1 camera. The coordinate grid used to measure was created in the program Inkscape (https://inkscape.org). Our method of measuring stomatal density over the entire leaf area using a transparent grid can be used for any leaves with a flat leaf blade. We made a stomata distribution map and calculated the main statistics for different parts of the lamina. Such stomatal density maps have been constructed for a number of angiosperms (Poole et al., 1996, 2000; Weyers, Lawson, 1997; Lawson et al., 2002). All data analysis and plotting were performed with RStudio data analysis software (RStudio Team, 2015).

The area of the studied leaf was about 2100 mm^2. The measurements were taken from 247 fields of observation. The area of one field of observation for the calculation of stomata was 1 mm^2.

The stomatal density (SD) over the entire surface of the leaf varies from 26 to 55 per 1 mm^2, the average is 41.2 ± 5.7 per 1 mm^2. These values of SD are less than SD of the leaves obtained from other regions. For example, SD of the *Ginkgo biloba* leaves from China varies between 75–95 per 1 mm^2 (Sun et al., 2003). We believe that the lower SD values are related to the shade conditions or other growing conditions.

The coefficient of SD variation over the entire surface of the leaf is 13.8%. We calculated the area of the same SD values using the constructed map of stomata distribution. As a result, SD from 35 to 40 per 1 mm^2 occupies almost 80% of the lamina area, SD about 45 per 1 mm^2 takes about 10% of the area and SD less than 30 per 1 mm^2 and more than 50 per 1 mm^2 takes remaining 10% of the lamina area.

We distinguished the basal (lower), middle and upper parts of the lamina (fig. 1A). The SD average values are 41.4 ± 4.7, 40.3 ± 8.6, 40.3 ± 4.8 in the lower, middle and upper parts, respectively. The SD variation coefficient is 10–11% in the upper and middle parts and 20% in the lower part. The greater SD variability in the lower part is probably related to the greater thickness of the veins at the base of the leaf. On the contrary, the stomatal density is shown to increase generally from the leaf base to its tip in angiosperms (Salisbury, 1928; Zacchini et al., 1997; Royer, 2001).

The results of SD measurements in different parts of the leaf show that there are no statistically significant differences in SD values between different parts of the leaf (fig. 1B).

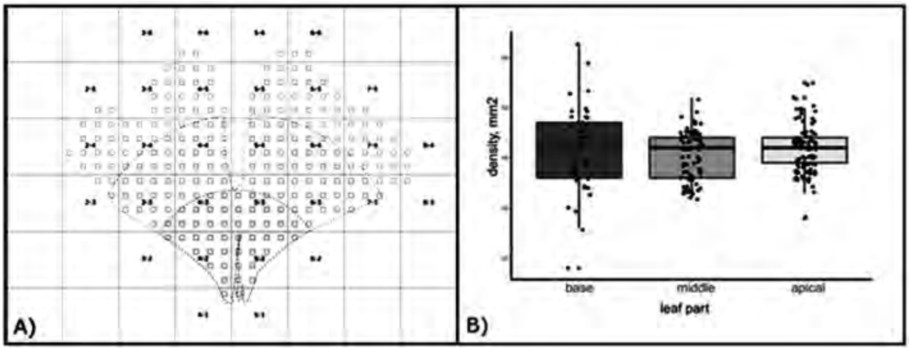

Figure. Stomatal density in different parts of the lamina.

The study was supported by the scientific program No. AAAA-A16-116021660045-2 of the Department of Higher Plants, Lomonosov Moscow State University.

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MUMMIFIED FOSSIL OF *KETELEERIOXYLON* FROM THE LATE PLIOCENE OF MAOMING BASIN, SOUTH CHINA, AND ITS PHYTOGEOGRAPHICAL AND PALEOECOLOGICAL IMPLICATIONS

Lu-Liang HUANG[1,2]***, Jian-Hua JIN***[1]***, Alexei A. OSKOLSKI***[2,3,4]

[1] Sun Yat-sen University, Guangzhou, China
[2] Nanjing Institute of Geology and Palaeontology CAS, Nanjing, China
[3] University of Johannesburg, Johannesburg, South Africa
[4] Komarov Botanical Institute RAS, Saint Petersburg, Russia

lssjjh@mail.sysu.edu.cn

In this paper, a new species *Keteleerioxylon maomingense* is described on the basis of mummified fossil wood from the late Pliocene Huangniuling Formation of the Maoming Basin, South China. Detailed anatomical study of well-preserved fossil wood confirms its close affinity to the extant conifer genus *Keteleeria* comprising three species distributed in central, southern, and southeastern China, northern Laos and in Vietnam. *Keteleerioxylon maomingense* is the most ancient fossil evidence of the occurrence of a taxa closely related to *Keteleeria* within the modern distribution area of this genus. This finding strongly suggests that the ancestors of extant *Keteleeria* ranged much further south in the late Pliocene than has been indicated by previous fossil records. Unlike more ancient fossil woods known from the Youganwo Formation of Maoming Basin (*Chadronoxylon maomingensis* and *Myrtineoxylon maomingensis*), *K. maomingense* has distinct growth rings confirming a progressive increase in rainfall seasonality in southern China from the middle to late Pliocene. The analysis of growth-rings in the fossil wood in comparison with those of modern *Keteleeria davidiana* suggests that in the late Pliocene of Maoming Basin there was humid subtropical monsoon climate with less pronounced rainfall seasonality than that seen in modern northeastern Vietnam.

This work was supported by the National Natural Science Foundation of China (Grant Nos. 41572011, 41210001, 41661134049); the grant of Natural Environment Research Council (No. NE/P013805/1); the joint Project of the National Natural