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Superellipse and stomatal geometries of four Magnoliaceae species

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ABSTRACT

Stomata are essential for the exchange of gases and water between plants and the environment. The stomatal geometries of many plants appear to be elliptical; however, prior studies have not tested whether the geometries of stomata follow the ellipse equation. Many natural shapes are superellipses rather than ellipses, and the superellipse has an additional parameter n that can generate other shapes such as diamond, and rectangle. We randomly selected four Magnoliaceae species with 240 stomata for each species to test whether the stomatal geometries are superellipses or not. We find that the stomatal geometries were described well by the superellipse equation. The estimated values of parameter n in the superellipse equation for most stomata (943/960) were greater than two, indicating that the studied stomata are not true ellipses, but superellipses. Thus, the traditional calculation of stomatal area using the product of stomatal length and width multiplied by $\pi/4$ (i.e., the hypothesis of elliptical stomata) may underestimate its real area. This finding has important implications for accurately measuring stomatal area in these plant species, which in turn is crucial for understanding and quantifying stomatal shape and function.

KEYWORDS: curve fitting; Magnoliaceae; stomatal geometry; stomatal area; superellipse

Introduction

A study key point of biological geometries is the ability to model the shapes. With different environmental constraints, the biological geometries can exhibit significant diversity and complexity. Given the diverse and complex characteristics of biological geometries and their shapes, researchers have sought to develop a universal geometric formula that can be applied to a wide range of biological shapes (Preston, 1953; Dornbusch et al. 2011; Biggins et al., 2018; Buschmann and Borchers 2020; Narushin et al. 2021). Many natural geometries appear to follow classical geometry, such as diamonds, rectangles, circles, and ellipses. Lamé (1818) proposed a general equation referred to as the superellipse equation while studying crystal shapes that can contain the aforementioned classical geometries by introducing a parameter in the ellipse equation. The superellipse equation is actually a generalization of the ellipse equation, and its mathematical expression is:

$$\left|\frac{x}{a}\right|^n + \left|\frac{y}{b}\right|^n = 1 \quad (1)$$

where x and y represent the Cartesian horizontal and vertical coordinates, respectively; a and b are major semi-axis radius and minor semi-axis radius, respectively; n is a positive real number (Gielis 2003). Based on the superellipse equation, Gielis (2003) created a more generalized version (referred to as the Gielis equation for convenience hereinafter) that can be used to describe a wide range of natural shapes, such as flowers, tree rings, starfish shells, and cobwebs (Gielis 2017).

Prior studies have examined the validity of the superellipse equation in describing actual shapes of various plant organs. Tian et al. (2018) and Li et al. (2022) successfully applied the superellipse equation to fit the seed projected shape of two *Ginkgo biloba* cultivars and the leaf shapes of two *Michelia* species. Other studies have focused on fitting the cross-sections of plant stems. For instance, Shi et al. (2015) utilized the superellipse equation to fit the actual boundary data of tree-ring cross-sections from three species of conifers, and found that it outperformed the traditional circle equation in estimating the basal area increment. Huang et al. (2020) introduced a deformation parameter to the superellipse equation for describing the cross-sections of a square bamboo and identified two different types of cross-section shapes based on the value of n , which ranged from 1.5 to 3.0. These studies highlight the versatility and potential of the superellipse equation as a popular tool in biological shape analysis.

Stomata are specialized breathing pores that cover the surface of leaves and are formed by two guard cells, typically taking on a dumbbell or kidney shape (Nunes et al. 2019). They regulate gas exchange between the atmosphere and the intercellular spaces of leaves, and their behavior is of great importance for plant performance, agricultural production, and global carbon and water cycles (Hetherington and Woodward 2003; Bonan 2008). Stomatal traits, including stomatal size, density, and spacing, have been widely suggested to determine stomatal conductance and photosynthesis (Franks and Beerling 2009). Generally, there is a significant negative correlation between stomatal size and density across or within species, such that species with small stomata tend to have greater stomatal density (Franks and Beerling 2009; Xiong and Flexas 2020). A

higher density of smaller stomata can contribute to a faster stomatal response and allow for closer tracking of environmental variations (Xiong et al. 2022). For instance, in species typical of arid environments, stomata have smaller sizes and higher densities, allowing plants to avoid water loss through mechanisms such as stomatal closure (Toscano et al. 2018). A better understanding of stomatal geometry and its functional implications could provide valuable insights into plant adaptation and response to changing environmental conditions.

Some studies have assumed that stomatal shape conforms to the ellipse equation, and suggest calculating stomatal area using the product of stomatal length and width multiplied by $\pi/4$, which is derived from the ellipse's area formula (Ouyang et al. 2017; Xiong and Flexas 2020). However, prior studies seldom validate whether these geometric shapes truly conform to ellipses. In the present study, we aimed to test the stomatal geometries follow the superellipse equation or the ellipse equation using four Magnoliaceae species, and those geometries appear to have elliptical geometries. It is crucial to examine whether stomatal shapes conform to the superellipse equation, as this could provide valuable insights into stomatal structure and function.

Materials and methods

Leaf sampling

Healthy leaves from four Magnoliaceae species (*Magnolia denudate* Desr., *Magnolia stellata* (Siebold & Zucc.) Maxim., *Michelia chapensis* Dandy., and *Michelia martini* (H. Lév.) Finet & Gagnep. ex H. Lév) were collected between July and August 2022 when the leaves had reached at the maturation status. 40 mature and completely expanded leaves were selected for each species. *Magnolia denudata*, *M. chapensis*, and

M. martini were collected from the Nanjing Forestry University campus in Nanjing, and *M. stellata* was collected from Nanjing Botanical Garden Mem. For convenience, we will refer to these species as Md, Mc, Mm, and Ms hereinafter.

Lamina section sampling and data acquisition

For each leaf, a small lamina section of approximately 0.5 cm × 0.5 cm was selected along the widest part of the leaf, near the midrib and near the leaf margin, respectively.

Stomatal images (with a view field area 662 μm × 444 μm from each small lamina section) were acquired via utilizing the segregation method (Jiao et al. 2021). A Leica DM 2500 optical microscope (Leica Microsystems Shanghai, Shanghai) and LAS X Life Science Microscope software (version 3.4.2.18368, Leica Microsystems CMS GmBH, Wetzlar, Germany) installed on a desktop computer (Dell OptiPlex 3020, Xiamen, China) were utilized to observe and capture images on a temporary mount.

Take care to avoid leaf veins during photographing, and the exported images were saved as .TIF files.

For each of the four Magnoliaceae species, 240 stomata were randomly selected from the view field pool, resulting in a total of 960 stomata. The boundary of each stomatal profile were manually sketched using Procreate (version 5.2.9; Savage Interactive Pty Ltd, Hobart, TAS, Australia) on an iPad air3 (version 14.7.1; Adobe Systems Incorporated, Cupertino, CA, USA), and the output files were saved in .PSD format. The stomatal images were then cropped and converted to black and white .BMP images using Adobe Photoshop 2021 (version 22.4.2; Adobe Systems Incorporated, San Jose, CA, USA). Finally, the planar coordinates of the stomatal profile (i.e., the

projection of the stoma) in the Cartesian coordinate system were extracted using a MATLAB program proposed by Shi et al. (2018) and Su et al. (2019). The number of data points on each stomatal boundary is 500.

Models

In the polar coordinate system, the re-parameterized version of the superellipse equation can be rewritten as (Shi et al. 2015; Huang et al. 2020; Tian et al. 2020):

$$r = a \left(|\cos \varphi|^n + |\sin \varphi / k|^n \right)^{-1/n} \quad (2)$$

where r and φ are the polar radius and polar angle, respectively. $x = r \cos \varphi$, $y = r \sin \varphi$, and $k = b / a$, where a and b are the major and minor semi-axes, respectively. When the value of n in the superellipse equation is 2, it reduces to a typical ellipse equation. Apart from the three shape parameters (i.e., a , k , and n), there are additionally three location parameters, which include the x - and y -coordinates of the polar point, as well as the counterclockwise rotation angle from the x -axis. For more information on these parameters can be found in Shi et al. (2015).

Model fitting and data analysis

To evaluate the accuracy of the nonlinear fitting, the adjusted root-mean-square error (RMSE_{adj}) was used as a measure of the goodness of fit (Huang et al. 2020; Shi et al. 2020; Li et al. 2021).

$$\text{RMSE}_{\text{adj}} = \frac{\sqrt{\text{RSS} / N}}{\sqrt{S / \pi}} \quad (3)$$

where N and S represent the sample size (the number of data points on a stomatal boundary) and the area of a stoma, respectively.

To compare the values of the a , k , n , and $RMSE_{adj}$ among the four plant species, Tukey's honestly significant difference (HSD) test was employed (Hsu 1996). All statistical analyses were performed using the statistical software R (version 4.2.0; R Core Team, 2022).

Results

The stomatal shapes of the four Magnoliaceae species investigated exhibited a similar oval shape, as shown in Figure 1. Figure 2 depicts a comparison between the observed boundary data and predicted stomatal profile for each species. The superellipse equation was found to be a reliable predictor of stomatal shapes in all species, as evidenced by the close agreement between the predicted shape and the observations of boundary data from the experiment.

Figure 3a shows significant interspecific variability in the numerical value of \hat{a} among the four species. The mean of \hat{a} in Md was the largest among the four species, while the mean in Ms was the smallest ($p < 0.05$). The ratios of minor to major semi-axis (values for \hat{k}) ranged from 0.5 to 0.97 (Figure 3b). As the numerical value of \hat{k} approaches 1, the difference in length between the minor and major axes becomes smaller, indicating that the stomatal shape becomes more circular. Conversely, when the numerical value of \hat{k} approaches 0.5, the difference in length between the minor and major axes becomes larger, indicating that the elliptical shape is flatter, and thus the

stomatal shape becomes more elliptical. The numerical values of \hat{k} in Mc and Mm did not differ significantly from each other, but were higher than those in Md and Ms ($p < 0.05$). There was a significant difference in the value of \hat{k} between Md and Ms ($p < 0.05$). Additionally, the value of \hat{n} in Md and Ms was not significantly different from each other, but was higher than those in Mc and Mm ($p < 0.05$; Figure 3c). There was a significant difference in the value of \hat{n} between Mc and Mm ($p < 0.05$). The mean of \hat{n} of four species were all greater than 2, confirming that the stomatal geometries were hyperellipses rather than standard ellipses. All the adjusted RMSE values for the four species were smaller than 0.03, indicating that the mean absolute deviation between the actual and the predicted radii did not exceed 3% of the radius of the hypothesized circle whose area was equal to the stomatal area (Figure 3d).

Discussion

Stomatal function as gatekeepers, managing the flow of carbon dioxide and water vapour between the plant and the atmosphere while regulating the rate of water loss, ultimately impacting the plants transpiration, respiration, and photosynthesis (Giuliani et al. 2013; Henry et al. 2019; Gong et al. 2021). The stomatal geometries can vary largely across different plant species and groups, with differences in size, shape, and spatial distribution on lamina (Haworth et al. 2023; Xu et al. 2019; Shi et al. 2021; Jia et al. 2022). These variations in stomatal morphology can have significant implications for plant function. Across the diversity of plant species, leaves with a greater area of open stomatal pores tend to result in higher stomatal conductance, leading to greater rates of photosynthetic carbon dioxide assimilation and of transpiratory water loss (Henry et al.

2019; Gong et al. 2021). However, when the environment turns unfavourable, such as droughts, leaves tend to have smaller and denser stomata (Toscano et al. 2018; Henry et al. 2019). This adaptation mechanism allows plants to rapidly respond to decreasing leaf water potential, helping them maintain water balance and preventing damage to their tissues.

The hypothesis that stomatal shape conforms to an elliptical form has been widely accepted in previous studies. This approach has been used in developing models that predict the rate of gas exchange between plants and the atmosphere, as well as in estimating the maximum of stomatal conductance, a crucial parameter for determining gas exchange rates (Ouyang et al. 2017; Zhang et al. 2021). Furthermore, this hypothesis has been applied to quantifying the impact of environmental factors, such as light intensity, temperature, and humidity on stomatal conductance (Tian et al. 2016; Li et al. 2021; Xiong et al. 2022).

The present study found that leaf stomata shape can be accurately described by the superellipse equation. To determine whether the shape of a stoma follows a superellipse equation rather than a classical ellipse equation, the powers (represented by the values of n) were compared among four different species. If stomata followed the classical ellipse equation, the power would be equal to 2. However, the data showed that for the majority of stomata (943/960) the numerical values of n were greater than 2, suggesting that the stomatal geometries of these four species are specifically hypoellipses (Huang et al. 2020).

As we have discovered through this study, the assumption of elliptical stomata did not hold true for the four Magnoliaceae species. It is worth noting that a commonly used method for calculating stomatal area (S) is based on the assumption that stomata are elliptical in shape, with their major axis equal to the length of the guard cells (L) and their minor axis equal to the width of the guard cells (W ; Zhang et al. 2021; Xiong and Flexas 2020):

$$S = \frac{\pi}{4} LW \quad (4)$$

If a stomatal geometry is described by the superellipse equation, S is expected to be proportional to LW (Huang et al. 2020; Li et al. 2022). This relationship can be expressed using a proportional function that relates S to the product of L and W formed by two guard cells, where L and W approximately $2a$ and $2b$, respectively:

$$S = \frac{4^{-1/n} \sqrt{\pi} \Gamma(1+1/n)}{\Gamma(0.5+1/n)} LW \quad (5)$$

where Γ is the gamma function. It is apparent that if n is a constant or varies in a very small range, S can be approximated as proportional to LW , with a proportionality coefficient $\frac{4^{-1/n} \sqrt{\pi} \Gamma(1+1/n)}{\Gamma(0.5+1/n)}$. In this study, we used the arithmetic mean value of n to approximately calculate the proportionality coefficients for the four Magnoliaceae species. The proportionality constants for Md, Ms, Mc, and Mm species were found to be 0.8290, 0.8302, 0.8156, and 0.8209, respectively. Notably, all of these constants were found to be greater than the constant $\pi/4$ that was used in prior studies that hypothesize that stomata are classical ellipses for calculating stomatal area. This

suggests that using the assumption of elliptical stomata may underestimate stomatal area in the four studied species. In other words, the actual stomatal area in these species are larger than the values predicted by $\pi/4 \times LW$, which has important implications for our understanding of their gas exchange and water regulation capabilities. It is important to note that this result should not be extended to all "elliptical" leaf stomata. Further research on a species-by-species basis is necessary to fully understand the morphometry of stomata and determine the appropriate methods for estimating stomatal area in each species.

Conclusions

The purpose of the present study was to determine whether the stomatal geometries of four Magnoliaceae species could be regarded as ellipses or superellipses. The results show that the stomatal geometries in these species were adequately described by the re-parameterized superellipse equation. The adjusted root-mean-square errors for the 960 stomata are smaller than 0.03, indicating that the mean absolute deviation is less than 3% of the radius of a circle whose area is hypothesized to be equal to stomatal area. Furthermore, the mean of n , which determines the shape of a superellipse, for each of the four species was greater than two, signifying that the stomatal geometries of these species were hyperellipses. This finding has important implications for the accurate measurement of stomatal area in these plant species or closely related species, as the assumption of elliptical shapes may underestimate stomatal area.

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Author contributions

Qiyang Li: Writing – original draft (equal); Formal analysis (equal), Investigation (equal). Karl J. Nilas: Formal analysis; Supervision (equal); Writing – review & editing (lead). Ülo Niinemets: Formal analysis; Supervision (equal); Writing – review & editing (lead). Liuyue Zhang: Investigation (equal). Kexin Yu: Investigation (equal). Johan Gielis: Writing – review & editing (equal). Jie Gao: Writing – review & editing (equal). Peijian Shi: original draft (equal); Formal analysis; Supervision (equal).

Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure Legends

Figure 1. Representative stomata of four Magnoliaceae species. (a) *Magnolia denudate*. (b) *Magnolia stellata*. (c) *Michelia chapensis*. (d) *Michelia martini*. The stomata pointed by the arrows will be used to show the validity of the superellipse equation in Figure 2.

Figure 2. The observed shapes (gray lines) and the fitted shapes (red curves) of the stomata of the four Magnoliaceae species using the superellipse equation. $RMSE_{adj}$ is the adjusted root-mean-square error, which denotes the proportion of the mean deviation to the radius of a circle whose area is hypothesized to be equal to stomatal area

Figure 3. Comparisons of the estimates of the parameters \hat{a} (a), \hat{k} (b), \hat{n} (c), and $RMSE_{adj}$ (d) among the four Magnoliaceae species. The bold segments in the boxes represent the medians; the red snowflakes represent the means. The letters were used to indicate a significant difference of species means using Tukey's HSD at the 0.05 significance level. The numbers represent the coefficients of variation (in %).

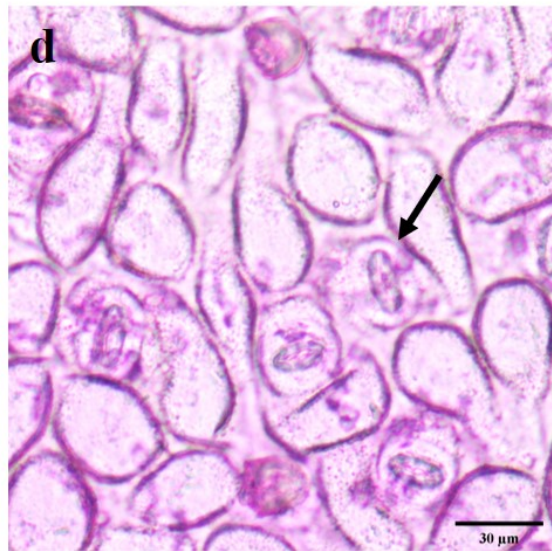
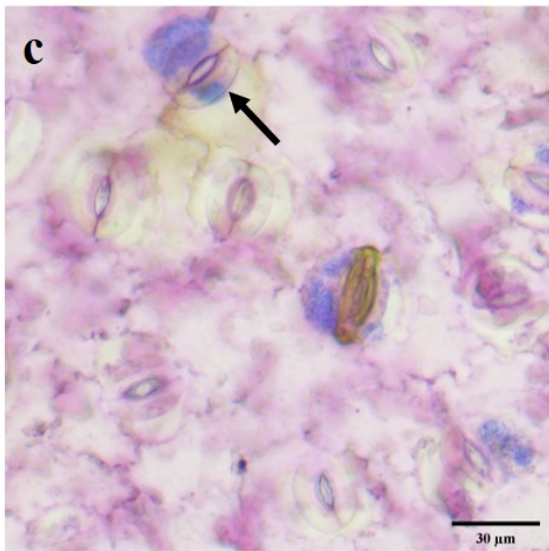
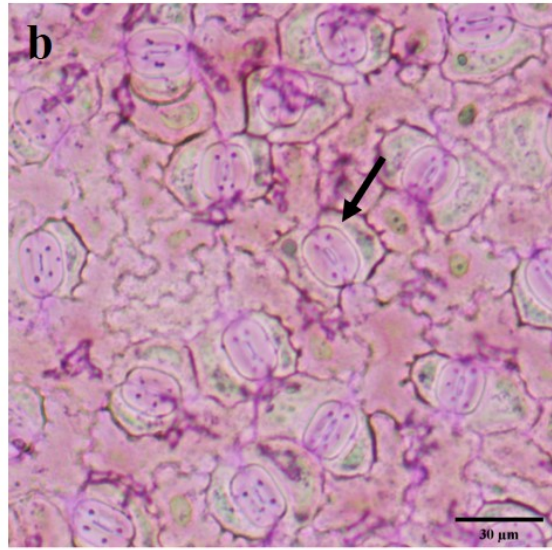
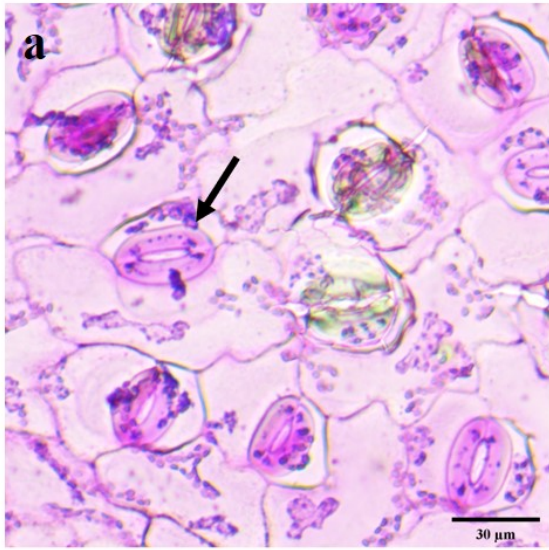


Figure 1

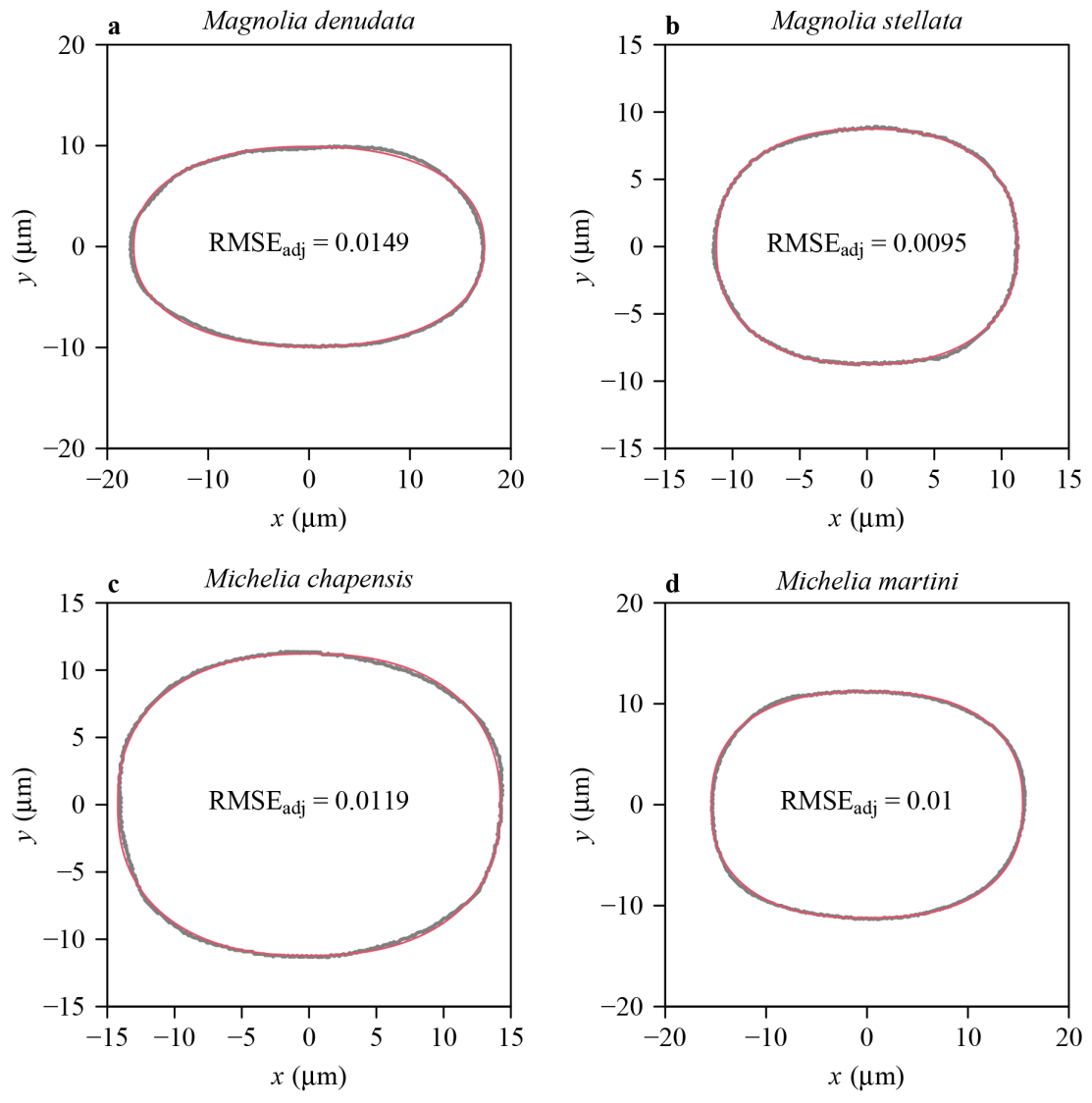


Figure 2

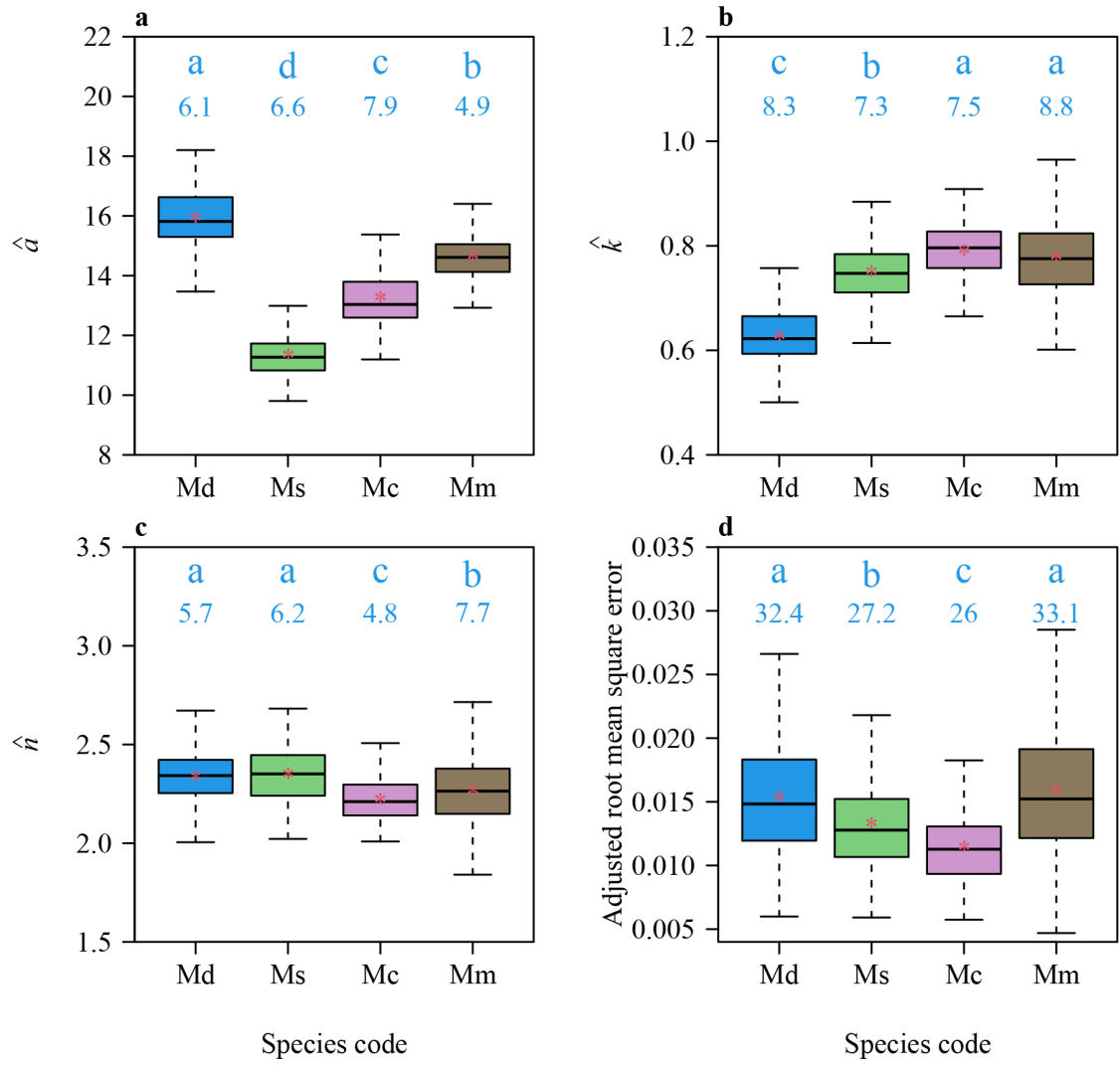


Figure 3