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A View from a Different Angle: Investigating the Significance of Rattan Spines from a Small Mammals' Visual Point of View Using ImageJ

(Pandangan dari Sudut Lain: Menyelidik Signifikan Duri Rotan dari Sudut Pandangan Visual Mamalia Kecil Menggunakan ImageJ)

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ABSTRACT

*Rattans are one of the most unique and economically important plants for most tropical countries. There is however, a lack of interest in the specific study of the rattan spines. In this paper, we tested a new hypothesis concerning the functional role of rattan spines. We proposed that rattan spines also serve as a visual deterrent against herbivores or seed predators. In our proposed method we used an Imaging software, ImageJ, to measure the spine area of four species of rattan (*Calamus insignis, Myrialepis schortechinii, Plectocomiopsis geminiflorus *and* Calamus caesius*) from two different orientations (root to shoot and vice versa). Our results showed that rattan spines were very heterogeneous and highly variable between different species. One common trait that the rattan spines share is that spine area measurements of shoot to root (ShR) are larger than root to shoot (RH) orientation. We propose that the downwards spine angle might be specifically designed to discourage climbing leaf and seed predators.*

Keywords: Anti-herbivory; ImageJ; rattan; seed predators; small mammals; spines

ABSTRAK

*Rotan adalah tumbuhan yang mempunyai keunikan dan kepentingan daripada segi ekonomi bagi kebanyakan negara tropika. Namun begitu, kurang perhatian diberikan pada kajian duri rotan. Dalam kertas ini, kami menguji hipotesis baru mengenai fungsi peranan duri rotan. Kami mencadangkan bahawa duri rotan berfungsi sebagai penghalang visual untuk anti-herbivor atau haiwan perosak biji benih. Kami menggunakan applikasi pengimejan, ImageJ untuk mengukur luas permukaan duri bagi empat spesies rotan (*Calamus insignis, Myrialepis schortechinii, Plectocomiopsis geminiflorus *dan* Calamus caesius*) daripada dua sudut pemerhatian (dari arah akar ke pucuk dan sebaliknya). Hasil keputusan menunjukkan duri rotan adalah amat bervariasi dan berbeza bagi setiap spesies. Satu persamaan yang didapati ialah luas permukaan duri dari arah pucuk ke akar (ShR) adalah lebih luas berbanding dari akar ke pucuk (RH). Kami mencadangkan bahawa ini disebabkan oleh sudut duri rotan lebih condong ke arah bawah, kemungkinan berfungsi untuk menghalang pergerakan memanjat oleh haiwan perosak biji benih.*

Kata kunci: Anti-herbivor; duri; haiwan perosak biji benih; ImageJ; mamalia kecil; rotan

INTRODUCTION

Rattan is one of the most economically and ecologically important plants in many Asian and African tropical countries. This palm genus has around 600 species, the majority of them climbers and many of them are spiny (Dransfield 1979). To date, there were numerous publications concerning the unique morphology and characteristics of rattans (Hamid & Suratman 2010). However, a distinctive feature of the leaf sheath that is most important taxonomically has not been given attention concerning its function. The spines of the leaf sheaths have regular shapes and therefore are reliable for species identification. The number and arrangement of spines varies from one species to another and from genus to genus and even between individual stems of the same species. The size varies from minute spicules scarcely one mm long to huge papery spines 30 cm or more in length (Dransfield 1979). The spines are sometimes singly, or in various groupings from a few to whorled. *Calamus polystachys* and several species of *Daemonorops* have extremely fine black spines in pairs, one pointing upwards and the other downwards. The empty spaces between the spines are utilised by ants as galleries. Spine attachments are sometimes simple and slender or swollen, almost bulbous or with margins. The texture of the spines also varies from soft and papery to woody, tough or very brittle (Dransfield 1979; Hourt 2008). The basic shape also ranges from crescent, conical or needle-like. Most of the spines are light green to yellow in colour but also varies in color from red, black, grey to a straw colour. Even though the spines are heterogeneous, most taxonomic studies only provide descriptive comparisons without an in-depth methodological explanation.

In this short communication, we present a new method to investigate the morphology of rattan spines from the perspective of climbing mammalian herbivores.

METHODS

To the best of our knowledge, there are no specific methods that focus directly on the morphology of the rattan spines. As for a demonstration for our proposed method, we tested our methods on four different rattan species: *Calamus insignis, Myrialepis schortechinii, Plectocomiopsis geminiflorus* and *Calamus caesius*. All samples were gathered from Pulau Bendong, Temenggor (5°31′28.66″N, 100°20′50.39″E), Malaysia.

We conducted our study to test the hypothesis that a small mammal would actually perceive the thorns with the perspective of its direction of climbing the rattan along the stems from below rather at a 90^0 angle. Normally, a researcher would typically examine the thorn configuration from a straight lateral view depending on the direction of rattan growth. We hypothesize that small climbing mammals, for example *Tupaia glis*, would most likely view the rattan spines from a upward-climbing point of view (POV). Figure 1 illustrates our hypothesis. We observed that different viewing angles could produce different area distribution and colour composition of the rattan spines. Viewing the spines from a different POV does not change any physical aspect of the spine itself, but rather creates a different visual perspective that may serve as a deterrent to small mammals. We based our hypothesis on the varying design and arrangement of the spines among different rattan genera and species. Some rattans have tough and strong needle-like spines, whilst certain genera have soft papery spines. It is possible that the soft, brittle and papery spines might not provide much mechanical defence, but if viewed from a different angle, the amount, arrangement and surface area of the spines might present a repelling view to small climbing mammals that have already experienced better defended rattans.

(a)

(b)

FIGURE 1. (a) lateral side view of *Calamus caesius* and (b) upward climbing POV of small mammals

We took samples of the rattan stems; five individuals for each species. Each stem sample was about 20 cm long. Extra care was given not to damage the arrangement and shape of the spines. The samples were taken at 1.5 m distance from the ground. We then marked the orientation of growth of the rattan on each sample, from root to shoot (RS) and from the shoot to the root (ShR). By using a special custom made clamp, we placed the stem vertically on a white plastic background. A scale bar was placed alongside the sample. We took digital pictures from the top angle using an Olympus E-5 digital camera fitted with a 35 mm macro lens at aperture priority = F 7.1, ISO 200. The samples were rotated to arrive at the orientations resulting in two sets of images per sample (RS and ShR). Initially, we had problems focusing on the rattan spines image sharpness, but this was resolved using the image processing software, ImageJ, a freeware developed by the National Institute of Health USA (NIH; http://rsbweb.nih.gov/ij/).

First, we converted the high resolution images to 8 bit images and manipulated the colour threshold setting producing a black and white image of the original picture. We then drew a straight selection line across the 1 cm scale bar and readjusted the appropriate scale in pixel (using set scale menu command). Using the circle selection command, we carefully selected the circumference area of the rattan stem area ignoring the spines. After the selection was made we then measured the analyze particles command to measure the area. The advantage of using ImageJ is that we can process the image even with varying clarity. The problem of image sharpness was resolved by manipulating the image threshold settings. Even though the spines at the bottom end were out of focus, we were able to capture spine arrangement for the whole length of the sample. Using the analyze particle command; we measured the circle area of the rattan stem (designated as circle area). We then used the freehand selection command and carefully selected the outline of the whole rattan by tracking the outlines of the spines. Using the same analyze particle command, we measured the whole area of the rattan image (designated as whole area). By subtracting the circle area from the whole area, we acquired the area measurement of the rattan spines. The whole process was repeated twice for each picture to assess the area measurement for RS and ShR angle. The results were then analysed with two-way ANOVA, with view angle (RS and

ShR) as first variable and species as the second variable. Data was analyzed for normality and data transformation (log) was performed when needed. All calculations were conducted using SPSS 19.

RESULTS

There is a significant difference in the average spine area of all species between different angles (Table 1). ShR view has a larger area size, 6.89±4.26 cm^2 compared to RS angle, 4.58±2.36 cm^2 (ANOVA, F=128.96, df=1, p<0.05). There is also a significant difference for the spine area measurement among different species of rattans (ANOVA, F=386.12, df=3, p<0.05). The different angle of view also significantly affected the spine area measurements between each species (ANOVA, F=6.87, df=3, p=0.001). Figure 2 shows an example of the outline images generated by the ImageJ software for both viewing angles.

TABLE 1. Mean value for the spine area measurements (in cm^2) for four different species of rattan

	Calamus insignis	*Myrialepsis schortechinii*	*Plectocomiopsis geminiflorus*	*Calamus caesius*
RS	5.67 ± 0.51^{a}	7.68 ± 0.50^{b}	3.18 ± 0.50^{c}	1.79 ± 0.30^{d}
ShR	$12.03 \pm 0.65^{\alpha}$	$19.12 \pm 0.45^{\beta}$	$12.24 \pm 0.24^{\gamma}$	$2.65 \pm 0.13^{\delta}$

Letters in superscript denotes significant differences between each species for each viewing angle

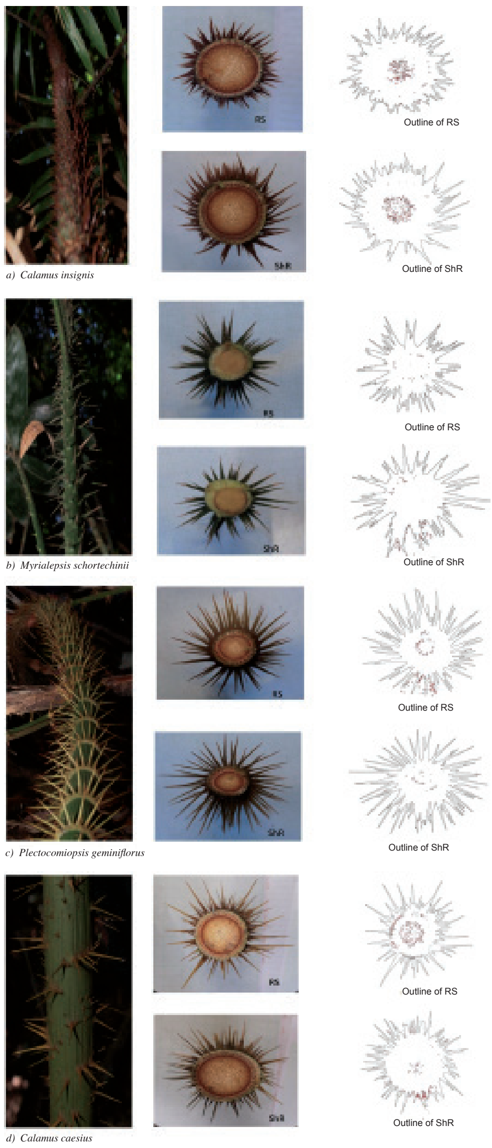

FIGURE 2. Rattan spine pattern viewed from multiple angles and also outline drawings produced from ImageJ. The small red dots within the ImageJ drawings are 'artefacts'- small inconsistence features. These artefacts however, do not affect the overall calculations

Discussion

As shown in our results, rattan spines are very heterogeneous and highly variable between different species. One common trait that the rattan spines share is that spine area measurements of ShR are larger than RS view. This can be attributed to the spine's angle on the rattan stem. Most spines are pointed downward towards the root or the ground. This creates an interesting visual illusion; accordingly the spine area seems larger when viewed from top (shoot) to bottom (root) or vice-versa. We initially suspected that the RS view would give a higher spine area measurement value, but the downwards spine angle might be specifically to discourage climbing leaf and seed predators. The arrangement of these armaments could also be linked to the specific seed-dispersal agent, i.e., primates are better seed dispersers than rodents, since rodents tend to consume seeds although their seed hoarding habit may enhance seed-dispersal distance from mother plants (Hamid & Suratman 2010; Pimentel & Tabarelli 2004; Silva & Tabarelli 2001).

To the best of our knowledge, this paper is the first to suggest the spine visual and angle pattern analysis. The methods are very simple and the software used is free. ImageJ had been previously used in other visual comparisons and heteroblastic comparison of leaves (Fadzly & Burns 2010; Fadzly et al. 2009). We hope to encourage further research in spine/thorns analysis (of other species of plants) in the future. There are many other variables and patterns that could be tested. For instance, we suggest manipulating the length of the stem which in turn could reveal different thorns' pattern. Based on our results, different points of views change the rattan spines appearance. Another component that also changes (albeit through different lighting conditions) from different POV is the colour of the rattan spines itself. From a lateral POV, the spines of *Myrialepis schortechnii* are dull and almost imperceptible dull red. Viewing the same spine arrangement from an upward climbing small mammal POV, the spines colours turn almost menacing red. This phenomenon could be studied using colour spectrometric analysis using portable handheld spectrometers. Spines and thorns in palms (Tomlinson 1990) and in other taxa are generally described as physical defences for plants against herbivory (Grubb 1992; Halpern et al. 2007; Ronel & Lev-Yadun 2012). Recently, spines and thorns were also linked to aposematic warning colours (Lev-Yadun 2009, 2001).

Here we studied only a small number of specimens since it was sufficient to demonstrate the technical ability and advantage of the method we used. Further analyses and more samples have to be collected and analyzed in order to come out with broader taxonomic and ecological/evolutionary conclusions.

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