



ARTICLE

Fractal dimension of the leaf vascular system of three *Relbunium* species (Rubiaceae)

Jorge Ernesto de Araujo Mariath^{1,3*}, Rinaldo Pires dos Santos¹ and Ricardo Pires dos Santos^{2,3}

Received: May 18 2009

Received after revision: December 29 2009

Accepted: January 15 2010

Available online at <http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/1247>

ABSTRACT: (Fractal dimension of the leaf vascular system of three *Relbunium* species (Rubiaceae)). Fractal analysis has been used as a powerful tool to characterize the complexity of plant structures. Tree ramification, root and venation systems are some examples of patterns studied using this geometry, and fractal dimension has quantified and graded the complexity of these structures. In this paper, the fractal dimension of the leaf vascular system was determined in three species of *Relbunium* (Endl.) Hook. f: *R. megapotamicum* (Spr.) Ehrendf., *R. hirtum* (Lam.) K. Schum. and *R. hypocarpium* (L.) Hemsl. The results showed significant differences in the fractal dimension of the three species (1.387 in *R. megapotamicum*, 1.561 in *R. hirtum* and 1.763 in *R. hypocarpium*), indicating that this type of measurement can be used as a taxonomic character to differentiate species and to quantify and grade the venation of leaves.

Key words: fractal dimension, leaf, *Relbunium*, venation.

RESUMO: (Dimensão fractal da vascularização foliar em três espécies de *Relbunium* (Rubiaceae)). Análise fractal tem sido usada como uma poderosa ferramenta para caracterizar a complexidade das estruturas vegetais. Ramificações de árvores, raízes e sistemas vasculares são alguns exemplos de padrões estudados usando essa geometria e a dimensão fractal tem quantificado e medido a complexidade dessas estruturas. Neste artigo, a dimensão fractal do sistema vascular foi determinada em três espécies do gênero *Relbunium* (Endl.) Hook. f: *R. megapotamicum* (Spr.) Ehrendf., *R. hirtum* (Lam.) K. Schum. e *R. hypocarpium* (L.) Hemsl. Os resultados mostram diferenças significativas na dimensão fractal das espécies (1,387 em *R. megapotamicum*; 1,561 em *R. hirtum*; 1,763 em *R. hypocarpium*), indicando que este tipo de medida pode ser usado como um caráter taxonômico para diferenciar espécies e quantificar o grau de venação foliar.

Palavras-chave: dimensão fractal, folha, *Relbunium*, venação.

INTRODUCTION

Leaf morphology has been studied since the origin of botany as a science, by using nomenclature to describe leaf shapes, such as needle-shaped, linear, lanceolate, obovate, ovate and oblong. These descriptive terms are still used for simple and compound leaves, as well as terms relating to the arrangement of the subunits (leaflets) of compound leaves (e.g., paripinnate, imparipinnate) and those that relate to the shape of the apex, base and margin of the leaf blade. Nevertheless, the diversity of shapes and the ambiguity of many terms make it difficult to clearly define and use these shapes as taxonomic characters and to impose a comparative analysis of non-homologous structures.

Traditionally, the vascular system of leaves has been described using two methods: descriptive terminology and biometry. In the first method, leaf shape, the arrangement of the first three orders of veins and the type of leaf margin were common systematic characters used to help define species of dicotyledons (Mouton 1970, Hickey & Wolfe 1975, Hickey 1970), and for the reason mentioned above this method has not been accurate. On the other hand, the analysis of many elements using biometrics produces a very accurate

idea of the shape of a leaf blade. This method allows for the construction of a representative figure of a leaf or leaflet analyzed, something that is impossible to make using descriptive terminology (Mouton 1976). Even in this last case, an analysis comparing a simple leaf to a leaflet (non-homologous structures) is possible. The most evident differences are the lack of the primary vein in the leaflet and the type of ramification. Biometrics could help highlight the differences among species or help distinguish taxonomic groups. Mouton (1976) concluded that using biometry to describe leaves would avoid the use of ambiguous terms to characterize them. As a consequence there would be a remarkable improvement in diagnosing species and the possibility of using this method in silviculture and paleobotany studies. In addition, the use of biometry in phytosociology would increase, adding data to the life forms of the Raunkiaer classification system, and would be very valuable for tropical floristic studies.

In biometric studies of plant morphology, fractal dimension (Df) has been used as an attribute to characterize the complexity of a structure by quantifying irregular and ramified processes. Compound forms by elements of topological dimension n , which define the

1. Plant Anatomy Laboratory, Department of Botany, Institute of Biosciences, Universidade Federal do Rio Grande do Sul (UFRGS). Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil.

2. Computer Engineering/Biotechnology Center of Sobral, Universidade Federal do Ceará (UFC). Campus Avançado de Sobral, Comandante Maurocílio Rocha Pontes 100, CEP 62042-280, Sobral, CE, Brazil.

3. CNPq Researcher.

* Author for correspondence. E-mail: jorge.mariath@ufrgs.br

regions of dimension $n + 1$ of the space, show $n < Df < n+1$, with $Df \in \mathbb{R}$ (set of real numbers), resulting in a fractional value for the fractal dimension. For example, when analyzing a ramified net made of lines ($n = 1$), which covers a flat surface ($n + 1 = 2$), there will be a fractional value for Df , between 1 and 2. Elements with a low level of complexity have fractal dimensions close to their topological dimension; however, elements with a high level of complexity tend to cover the spatial region that they define. This tendency, which is represented by the fractional values of the fractal dimension, is related to the complexity of the dynamic formation of the structure, and is an important topological attribute used for its characterization (Mandelbrot 1982, Chaves 1989, Weibel 1991).

There have been many studies about calculating fractal dimensions for plant structures. Morse *et al.* (1985) utilized fractal geometry in plant ecology by relating the Df of vegetation to arthropod distribution (structure of habitat scale). Tatsumi *et al.* (1989) and Fitter & Stickland (1992) calculated the Df of radicular systems. Berntson (1994) discussed how accurate the methods are for determining fractal dimensions. Turcotte *et al.* (1998) calculated the Df of the leaf vascular system of a leaf image of a *Sorbus* (Rosaceae) hybrid, a method reported by Merrill (1978) according to Horton's laws (Horton 1945). Vico *et al.* (1998) demonstrated through the study of the vascular net of chicken embryos that fractal geometry is the most appropriate method to characterize the evolution of this vascular system and suggested the possibility of using Df to characterize other vascular processes.

In this study we developed a method, using digital images of cleared leaves, to determine the fractal dimension of the leaf vascular system of three species of *Relbunium* (Endl.) Hook. f. (Rubiaceae): *R. megapotamicum* (Spreng.) Ehrend., *R. hirtum* (Lam.) K. Schum. and *R. hypocarpium* (L.) Hemsl. Our goal was to quantify the complexity of the leaf vascular system of these species, and to define the level of complexity so it could be used as a taxonomic character.

MATERIALS AND METHODS

The analyzed leaves of *Relbunium* (Fig. 1) were obtained by collecting 100 sample units from one population of each species (*R. megapotamicum*, *R. hirtum* and *R. hypocarpium*). The collections were made from cultivated plants in Porto Alegre, RS, Brazil. Adult leaves were collected indiscriminately at the apex, the middle and the base of each branch. The images of the vascular systems were made by clearing the leaves, drawing them, and digitizing the drawings for computer analysis.

To clear the leaves, fresh material was fixed in 96% ethanol and boiled in distilled water on a heating plate for 10 minutes. After this, each leaf was transferred and boiled for 10 minutes in a mix of 5% NaOH and 96% ethanol, 1:1 (v/v). Then, the material was washed

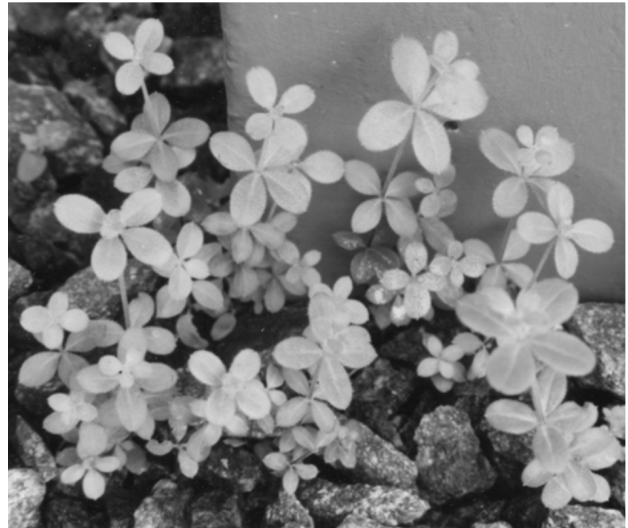


Figure 1. Image showing one of the analysed plants: *R. hypocarpium*.

in distilled water (5 times) and transferred to a sodium hypochlorite solution until it was transparent (1 hour). This was followed by a second wash in distilled water and then the material was put in 5% chloral hydrate for 10 min. The material was finally transferred into 70% ethanol (10 min), and then stained with 1% safranin in 80% ethanol (15 min). The stained material was then dehydrated in an ethanol series (96% and 100% for 10 min each) and transferred to xylene (5 min) and later mounted on histological slides with Canada balsam (Kraus & Arduin 1997, Strittmatter 1993). Figure 2 shows an image of the vascular system on an assembled slide.

The venation pattern (up to the fourth order) was obtained by drawings (0.1 mm pen) on white paper, using a Leitz Dialux 20EB bright field microscope, which was equipped with a drawing tube. The drawings were later digitized with a flatbed scanner, in black-and-white, and saved in BMP format (Bit Map Pixel).

Determination of the fractal dimension was made using a computer program developed in IDL (Interactive Data Language) by the authors of this paper. Initially the images were passed through an L-skeleton process, which was done using the same program, to reduce its structural shape to one pixel thick (Russ 1990). This step is necessary to properly analyze the fractals. The fractal dimension (Df) was obtained by the box-counting method (Feder 1988). This method consists of superimposing an image of the vascular system over tables divided into squares of l side (variable for each

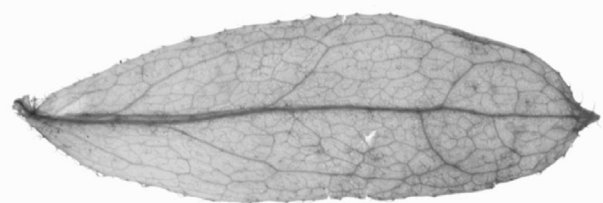


Figure 2. Image of the vascular system of a cleared leaf of *R. hypocarpium* mounted on a histological slide with Canada balsam.

table). Counting how many squares $N(l)$ that intercept at least one point (pixel) was made using the digital image. The minimum and maximum value for l as well as its variation range (Δl) were determined according to the methodology described by Vico (1998). The D_f value was estimated by the linear interpolation of the data $\log N(l)$ versus $\log(l)$, according to the following equation (where A is a constant):

$$\log N(l) = -D_f \times \log(l) + A$$

The statistical analysis used was based on descriptive statistics (central tendency and dispersion), sample sufficiency (for the determination of the correct sample size), a random iteration test (for the determination of random samples), Kolmogorov-Smirnov adhesion test (for the determination of the correct function to be used) and the meaning test (to verify the existence, or not, of significant differences between populations). All statistical analyses were made using an application created by the authors with IDL software.

RESULTS

The digital images of the cleared leaves showed the leaf venation patterns (Fig. 3) of the three species of *Relbunium* studied. Figure 4 illustrates the results of the $N(l)$ measurements using the graphic $\log N(l)$ vs. $\log(l)$ and the specific linear interpolation for the three species.

For D_f calculations (equal to the angular coefficient modulus of the linear interpolation), all regressions were $R^2 > 0.998$, showing that the images of the vascular system behaved as fractal objects for the range of resolution used.

The D_f results (Table 1) were the following: 1.387 (*R. megapotamicum*), 1.561 (*R. hirtum*) and 1.763 (*R. hypocarpium*). The statistical analysis pointed out a sufficient sample size, as well as the randomly selected samples that were collected. The Kolmogorov-Smirnov adhesion test indicated that the sample distribution

Table 1. Fractal dimension (D_f) for the three species of *Relbunium*, showing significant differences among the calculated values.

Species	D_f
<i>R. megapotamicum</i>	1.387±0.032
<i>R. hirtum</i>	1.561±0.031
<i>R. hypocarpium</i>	1.763±0.022

for each population adjusted conveniently to a normal distribution. As a result, the significance test used was a parametric test for two or more samples (populations); the significance range was 0.05. Significant differences among the populations (species) were found.

DISCUSSION

The leaf vascular system studied is basically a ramified three-dimensional structure made of xylem tracheary elements that are associated to the phloem in the mesophyll, between the two leaf surfaces. Based on the characteristics of a leaf (such as mesophyll thickness), the vascular system can “fold” itself into the three-dimensional space (3D) or it can follow rugosities and saliencies on the surface. The *Relbunium* leaves studied showed two important characteristics. First, the thickness and width of the vessels, in addition to variation in the different vascular orders, were considerably smaller than the linear dimensions of the leaves. Second, the leaf surfaces of the analyzed species did not show folds in 3D or any kind of measurable rugosities for the scale utilized. This allowed the tracheary elements to be treated like linear structures (topological dimension equal to 1) and the leaf surfaces to be treated like planes (topological dimension equal to 2) that are not curved and are easily transformed into digital images without significant loss of information. Consequently the fractal dimension of these structures should be between 1 and 2, as demonstrated.

The vascular pattern of each species showed qualitative differences in the level of venation complexity, which was lowest for *R. megapotamicum*, intermediate for *R. hirtum* and highest for *R. hypocarpium*. These

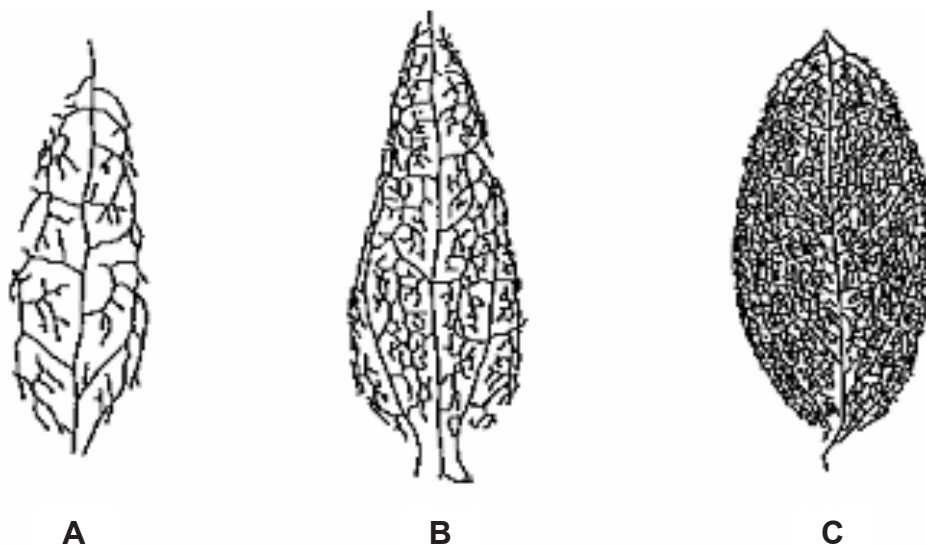


Figure 3. Digital images of cleared leaves of the three species of *Relbunium*. A. *R. megapotamicum*. B. *R. hirtum*. C. *R. hypocarpium*.

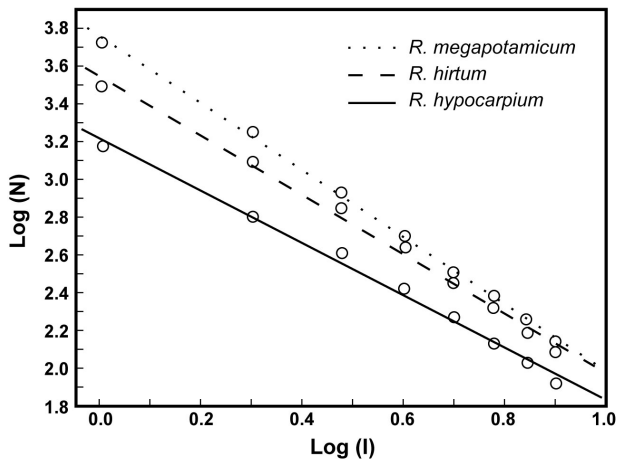


Figure 4. Graphic $\log N(l)$ vs. $\log (l)$ and the specific linear interpolation for the three species of *Relbunium*.

quantitative differences were represented by the Df values calculated. The low measures of dispersion (< 2%) showed how specific the fractal dimension was and the presence of subadjacent patterns in the vascular system for each species. According to these results we can define the leaf vascularization level as a complexity level of the vascular system using the fractal dimension.

It is important to point out that the application of the methodology used in this paper has well-defined limits. The vascular structures used to determine the fractal dimension have characteristics that allow them to be dimensionally reduced without creating major conflicts. The fractal analysis was made in two-dimensional space (2D). Nevertheless, if vascular structures that have relevant irregularities or uneven leaf surfaces and tracheary elements with dimensions that are not very reduced, this methodology should be modified so the fractal analysis is made in 3D.

CONCLUSION

Through fractal geometry we determined the fractal dimension of the leaf vascular system of three species of *Relbunium* (*R. megapotamicum*, *R. hirtum* and *R. hypocarpium*) and obtained the following values: 1.387 ± 0.032 , 1.561 ± 0.031 and 1.763 ± 0.022 , respectively. The fractal dimension proved to be a powerful taxonomic attribute that quantified the complexity of the leaf venation. This allowed the structure of the vascular system to be characterized and made it possible to differentiate the species using this character. A methodology to obtain images of the vascular system was developed based on leaf clearing, drawing of leaf veins and digitization of the drawings, which are specific for fractal analysis in 2D. This methodology proved to be efficient to analyze leaves because values such as thickness, width and curvature in 3D, of the tracheary elements, could be ignored without significant loss of information. The results obtained show that this methodology of using fractal geometry to quantify the vascular system of leaves, which results in a leaf morphology character,

increases the number of biometric variables that can be used in the characterization of plant groups.

ACKNOWLEDGMENTS

The authors would like to thank the teachers, the technicians and the students from the Plant Anatomy Laboratory (LAVeg, UFRGS) and from the Biotechnology Center of Sobral (NUBIS, UFC). All of these people assisted in infrastructure and technical support, and they gave important suggestions. We would also like to express our gratitude to Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) for financial support.

REFERENCES

- BERNTSON, G.M. 1994. Root Systems and Fractals: How Reliable are Calculations of Fractal Dimensions. *Annals of Botany*, 73: 281-284.
- CHAVES, M.G.F. 1989. Fenômenos de agregação. *Ciência Hoje*, 55: 27-32.
- FEDER, J. 1988. *Fractals*. New York: Plenum Press. 310 p.
- FITTER, A. H., STICKLAND T. S. 1992. Fractal characterization of root system architecture. *Functional Ecology*, 6: 632-635.
- HICKEY, L. J. & WOLFE, J. A. 1975. The bases of Angiosperm phylogeny: vegetative morphology. *Annals of the Missouri Botanical Garden*, 62: 538-590.
- HICKEY, L. J. 1979. A Revised Classification of the Architecture of Dicotyledonous Leaves. In: METCALFE, C. R. & CHALK, L. M. (eds). *Anatomy of the Dicotyledons. 1*: 25-39. Oxford: Clarendon Press.
- HORTON, R. E. 1945. Erosional development of streams and their drainage basins; hydrophysical approach to quantitative morphology. *Geol. Soc. Am. Bull.*, 56: 275-370
- KRAUS, J. E. & ARDUIN, M. 1997. *Manual Básico de Métodos em Morfologia Vegetal*. São Paulo: EDUR. 198 p.
- MANDELBROT, B. 1982. *The Fractal Geometry of Nature*. San Francisco: Freeman. 480 p.
- MORSE, D. R.; LAWTON, J. H.; DODSON, M. M.; WILLIAMSON, M. H. 1985. Fractal dimension of vegetation and distribution of arthropod body lengths. *Nature*, 314: 731-733.
- MOUTON, J. A. 1970. Architecture de la nervation foliaire. *Comptes Rendus du Quatre-Vingt-Douzième Congrès National des Sociétés Savantes*. Tome III.:165-176.
- MOUTON, J. A. 1976. La biometrie du limbe mise au point de nos connaissances. *Bulletin de la Société Botanique de France*, 123(3-4): 145-158.
- MURRILL, E. K. 1978. Comparison of nature leaf architecture of three types in Sorbus L. (Rosaceae). *Bot. Gas.*, 139: 477-453.
- RAUNKIAER, C. 1934. *Plant life forms*. London: Oxford University Press. 104 p.
- RUSS, J. C. 1990. *Computer-Assisted Microscopy-The Measurement and Analysis of Images*. New York: Plenum Press. 470 p.
- STRITTMATTER, C. G. D. 1973. Nueva tecnica de diafanización. *Soc. Arg. Bot. La Plata*, 15(1):126-129.
- TATSUMI, J.; YAMAUCHI, A.; KONO, Y. 1989. Fractal analysis of plant root systems. *Annals of Botany*, 64: 499-503.
- TURCOTTE, D. L.; PELLETIER, J. D.; NEWMAN, W. I. 1998. Networks with Side Branching in Biology. *J. Theor. Biol.*, 193: 577-592.
- VIGO, P. G.; KYRIACOS, S.; HEYMANS, O.; LOURYAN, S.; CARTILIER, L. 1998. Dynamic Study of the Extraembryonic Vascular Network of the Chick Embryo by Fractal Analysis. *J. Theor. Biol.*, 195: 525-532.
- WEIBEL, E. R. 1991. Fractal geometry: a design principle for living organisms. *Am. J. Physiol.*, 54: 250-258.