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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: A gravid praying mantis just before she laid her ootheca—digital art on procreate. © Aakanksha Komanduri.



Elliptic Fourier analysis of leaf shape of *Callicarpa pedunculata* and *Callicarpa rubella* (Lamiaceae)

Jennifer S. Danila¹ & Grecebio Jonathan D. Alejandro²

¹The Graduate School, University of Santo Tomas, España Blvd., 1015 Manila, Philippines.

^{1,2}College of Science and Research Centre for the Natural and Applied Sciences, University of Santo Tomas, España Blvd., 1015 Manila, Philippines.

¹jennifer.danila.gs@ust.edu.ph (corresponding author), ²gdalejandro@ust.edu.ph

Abstract: Leaves play an important role in species discrimination. An elliptic Fourier analysis (EFA) based morphometric technique was used to assess divergence between the poorly differentiated species, *Callicarpa pedunculata* and *C. rubella*. Using leaf specimen images from herbarium collections, principal components (PCs) were extracted from the Fourier coefficients and used to describe leaf outline and leaf shape descriptors: circularity, aspect ratio, and solidity. The results indicate that symmetric (54%) and asymmetric (35%) components of the leaves of *C. pedunculata* and *C. rubella* are sources of shape variation, as shown in the width and leaf tips among the samples. MANOVA revealed significant interspecific differences ($P = 0.03$) between *C. pedunculata* and *C. rubella*. The jack-knife cross-validation showed 71% of correctly classified species both in *C. pedunculata* and *C. rubella*. Furthermore, the results of this study were able to reveal significant leaf shape descriptors like aspect ratio, circularity, and solidity as important diagnostic characters in discriminating *C. pedunculata* and *C. rubella*. Thus, in conclusion, leaf serrations, leaf size, and leaf lobes are important characteristics in discriminating between *C. pedunculata* and *C. rubella*.

Keywords: Aspect ratio, *Callicarpa*, circularity, correlation, evolution, geometric morphometrics, leaf, principal component analysis, solidity, symmetry.

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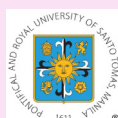
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Author details: JENNIFER S. DANILA is a graduate of Doctor of Philosophy major in Biology at the University of Santo Tomas. She has expertise in plant science including systematics, taxonomy, and biodiversity conservation. GRECEBIO JONATHAN D. ALEJANDRO is a professor at the College of Science and is currently the Director of the Office for Graduate Research, Graduate School, University of Santo Tomas (UST). He established the Thomasian Angiosperm Phylogeny and Barcoding Group (TAPBG) in the UST – Research Center for the Natural and Applied Sciences.

Author contributions: JSD—contributed to field collection, data analysis, discussion of results, and conclusion of the manuscript. GJDA—led the discussion, editing, and paper review for the manuscript.

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INTRODUCTION

Callicarpa is a genus of Lamiaceae characterized by branched hair; inflorescences axillary; flowers polysymmetric, 4(–5) merous; anthers porose; stigma peltate or capitate; and fruit a drupe (Linnaeus 1753; Munir 1982; Leeratiwong et al. 2009; Bramley 2013). Several species of *Callicarpa* have been classified and formally recognized from different parts of the world, including the Philippines and Borneo. *C. pedunculata* R. Br. and *C. rubella* Lindl. show extensive distribution in the southeastern Asian region, but both are geographically and taxonomically controversial. *C. pedunculata* is not found in Sumatra, Java and Borneo, while *C. rubella* is rather more extensive, occupying a wider range in the Asian continent. In contrast, *C. pedunculata* is widely distributed in the Philippines, while *C. rubella* is not present (BGCI 2024; Arvidsson 2020). Taxonomically, the relationship between the two taxa was not clear due to ambiguous morphological characters.

C. pedunculata and *C. rubella* were usually differentiated by their leaf size and presence of glandular hairs (Bramley 2013): *C. pedunculata* has wider leaves and lacks glandular hairs, while *C. rubella* has narrower leaves and hairs are present. Although its morphology has been previously described by Bramley (2013, 2019), *C. pedunculata* is easily confused with *C. rubella* due to misleading morphological characters. Likewise, several taxonomists have linked other species with *C. pedunculata* and *C. rubella*, e.g., the long-established *C. caudata* Maxim and doubtful *C. cuspidata* Roxb. were linked to *C. rubella* based on indumentum and leaf serrations (Roxburgh 1820; Lam & Bakhuizen 1921) and leaf bases (Bramley 2013), while *C. cuspidata* has been reported as a synonym of *C. pedunculata* (Munir 1982) which adds to the confusion between the two taxa. Likewise, no direct studies have identified the relationship between *C. pedunculata* and *C. rubella* to further separate or combine the two species. Thus, the taxonomic status of *C. pedunculata* and *C. rubella* was becoming uncertain due to the overlapping of morphological characters.

The taxonomic transcription among *C. pedunculata* and *C. rubella* and its closely related species were originally described by Roxburgh (1820) and revised by Munir (1982), but, according to Bramley (2013), they did not indicate any specimen or type to describe the species. Consequently, Bramley (2013), considered the description of Roxburgh (1820) and Munir (1982) unsuitable for correct identification due to lack of data and poor vouchering. In a previous study of *Callicarpa* in

Thailand and the Philippines (Leeratiwong et al. 2009; Bramley 2013), *C. rubella* was recognized as distinct from other *Callicarpa* species through its cordate or obliquely cordate leaf base, while *C. pedunculata* was defined by its attenuation to cuneate leaf bases. Currently, our knowledge of these two species is known only from collections made early in the twentieth century, and recent studies were mostly based on herbarium specimens. The lack of updated distribution listings and exhaustive data contributes to species taxonomic challenges. This also raises several questions on the current conservation status and taxonomic relationship of *C. pedunculata* and *C. rubella*. While *C. rubella* is thought to be absent in the Philippines, its current natural distribution is also difficult to determine with precision because of the potential impact of human use in different countries. In the southeastern Asian region, *C. pedunculata* and *C. rubella* were reported to have medicinal properties (Brown 1920; Tu et al. 2013) collected from twigs, roots, and leaves, while their fruits are used for human consumption. Thus, the natural distribution of most species may have been changed by its dispersal based on human actions affecting local or even global distributions (Di Marco & Santini 2015; Newbold et al. 2015). The change in the environment and distribution of species were highly influential in plant structures, especially on leaves which serve as indicators of environmental change (Gupta et al. 2019; Zhang & Li 2019).

In this paper, the authors discuss leaf morphometrics using a more comprehensive quantification of leaf shape, where measurements of individual parameters were obtained as a basis of species discrimination. This technique, elliptical Fourier descriptors (EFD) utilizes the sum of ellipses over contours to quantify outlines and silhouettes in an image (McLellan & Endler 1998; Hearn 2009; Godefroy et al. 2012), based on the instructions taken from Klein and Svoboda (2017) on geometric morphometric analysis. Aside from the typical leaf extraction, leaf shape descriptors: Circularity, measured as 4π (area/perimeter²) related to serrations and lobing; Aspect ratio (AR), the ratio of the major to the minor axis and influenced by length and width; and, Solidity, measured as area or convex hull and sensitive to leaf deep lobes (Cope et al. 2012) were incorporated into the downstream analysis. As leaf shapes vary among or within species, it is also important to quantify leaf shapes to understand broader aspects of plant adaptation to the environment (Chitwood, et al. 2014). Leaf morphological traits such as length, width, and veins are controlled by the environment, whether to

stabilize or to adjust to certain environmental conditions (Alonso-Forn et al. 2020). This study describes for the first-time accessions of *C. pedunculata* and *C. rubella* through leaf morphometrics, contributing to a better understanding of the species variation through leaf shapes. Furthermore, this study aimed to discriminate *C. pedunculata* and *C. rubella* leaf shape descriptors: circularity, aspect ratio (AR), and solidity between the two taxa, and predict the correlation among the three leaf descriptors.

MATERIALS AND METHODS

Study Site

A total of 46 individual herbarium samples of *C. pedunculata* and *C. rubella* were used in the study (Image 1). Twenty samples of *C. pedunculata* were collected in the secondary forests and forest edges of Palanan, Isabela in the Philippines while 26 samples of *C. rubella* were carried out from selected digital herbarium of AMD, FLMNH, K, MSU, NY, US, and USTH (Image 2) through online accessions in the Global Biodiversity Information Facility (GBIF) database via the web interface (Table 1). The online images and details were downloaded using the ‘Darwin Core Archive’ format which contains the URLs and information of the samples in GBIF (Table 9). On the other hand, samples



Image 1. The sample of *C. pedunculata* and *C. rubella* leaves used in the study from selected digital herbaria.



Image 2. Map of southeastern Asian region indicating the localities of selected *C. pedunculata* and *C. rubella* species used in the study: 1—China | 2—Indonesia | 3—Myanmar | 4—Taiwan | 5—Thailand | 6—Vietnam | 7—Philippines. (Map: www.scribblemaps.com).

from the fieldwork have undergone herbarium protocol from the securing of the permit for the collection of specimens, preparation of materials, pressing of the specimen, mounting in herbarium sheet, identification, and labeling to the deliberation of voucher specimen to the University of Santo Tomas Herbarium (USTH) in the Philippines.

Procedures

In this study, herbarium samples were the main source of datasets to build shape descriptors from the leaf outline. The collected digital images were subjected to leaf isolation using Adobe Photoshop version 22.0.0 (Adobe System San Jose, USA). After all leaves have been isolated from the scans, the software SHAPE (Iwata & Ukai 2002) which uses binary leaf outline image files in BMP format converts images to black and white. SHAPE converts the image outlines to chain code and then normalized EFDs. A maximum number of harmonics were set to 20 to recapitulate leaf shape and the normalization method was set to the longest radius for the initial orientation of the images. From the obtained

EFD coefficients, the analysis focused on coefficients *a* and *d*, as well as coefficients *b* and *c*. These correspond to the symmetric and asymmetric components of leaf shapes, respectively, following the approach outlined by Lexer et al. (2009). Subsequently, principal component analysis (PCA) was conducted on the EFD coefficients to identify variations in leaf shape across the entire set of leaf samples. Prinprint program was used to view the Eigen leaves or leaf contours of each principal component. Then an analysis of leaf shape descriptors was obtained using ImageJ version 1.52a, Java 1.8.0_112 (64-bit) (Ambramoff et al. 2004) software. After all images of *C. pedunculata* and *C. rubella* were measured based on AR, circularity, and solidity, the resulting data were imported to PAST version 4.06b software (Hammer et al. 2001) for further analysis.

Table 1. Populations and samples of *C. rubella* and *C. pedunculata* were used in this study.

Species	Localities	Accession number	Herbarium
<i>C. rubella</i> Lindl.	Myanmar	2648823	The New York Botanical Garden (NY)
	China	2787428	United States National Herbarium, Smithsonian Institution (US)
	China	FLAS 269814	Florida Museum of Natural History (FLMNH)
	China	FLAS 269815	Florida Museum of Natural History (FLMNH)
	Thailand	L 0534717	Naturalis Biodiversity Center (AMD)
	Thailand	L 0534080	Naturalis Biodiversity Center (AMD)
	Malaysia	L 2754590	Naturalis Biodiversity Center (AMD)
	Malaysia	L 2754591	Naturalis Biodiversity Center (AMD)
	China	L4212486	Naturalis Biodiversity Center (AMD)
	Malaysia	L0534846	Naturalis Biodiversity Center (AMD)
	Vietnam	P00991455	The New York Botanical Garden (NY)
	Taiwan	K000674727	Royal Botanic Gardens Kew (K)
	Indonesia	K000194757	Royal Botanic Gardens Kew (K)
	Indonesia	K000194756	Royal Botanic Gardens Kew (K)
	Vietnam	MW0756909	Moscow State University (MSU)
	Vietnam	MW075612	Moscow State University (MSU)
	China	103972	The New York Botanical Garden (NY)
	China	193971	The New York Botanical Garden (NY)
	China	103960	The New York Botanical Garden (NY)
	China	103959	The New York Botanical Garden (NY)
	China	103961	The New York Botanical Garden (NY)
	China	525329	The New York Botanical Garden (NY)
	Vietnam	2808318	The New York Botanical Garden (NY)
	Vietnam	2808046	The New York Botanical Garden (NY)
	Myanmar	3231815	The New York Botanical Garden (NY)
<i>C. pedunculata</i> R.Br.	Philippines	JDS001	University of Santo Tomas Herbarium (USTH)

Table 2. Eigenvalues and contribution of the first 10 principal components before data partitioning.

Component	Eigenvalue	Proportion (%)	Cumulative (%)
1	0.000682534	36.65	36.65*
2	0.000385342	20.69	57.34*
3	0.000166940	8.96	66.30*
4	0.000144030	7.73	74.04*
5	0.000106694	5.73	79.77
6	0.000085726	4.60	84.37
7	0.000063535	3.41	87.78
8	0.000048451	2.60	90.38
9	0.000029872	1.60	91.99
10	0.000025266	1.36	93.34

*Only the first four are significant based on the broken stick method.

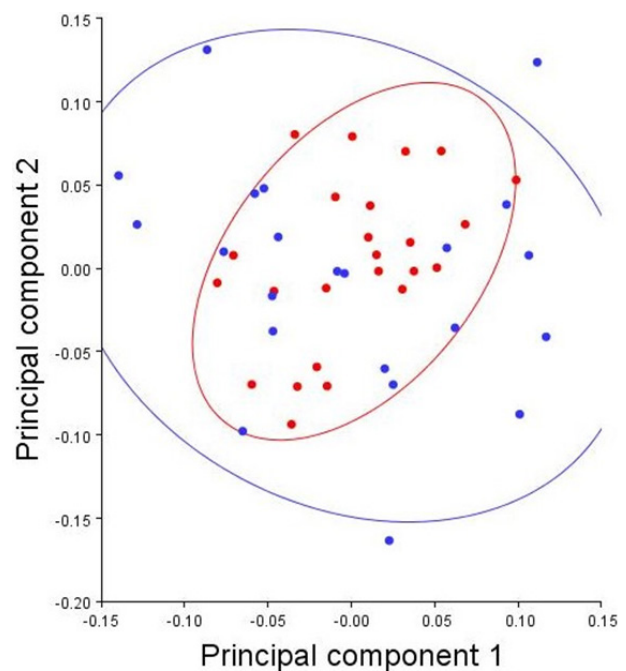


Figure 1. Principal component analysis based on variance-covariance matrix of the unpartitioned dataset from elliptic Fourier coefficients of *C. pedunculata* (red dots) and *C. rubella* (blue dots). The plot shows PC1 and PC2, which explained 39.26% and 22.17% of the total variation, respectively.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA)

Independent shape variables were identified by PCA of EFD. Table 2 shows the relative contributions of the first 10 PCs of the whole dataset are accounted

for 93% of the total variance while significant variations in the first four PCs (PC1, PC2, PC3, and PC4) equal to 74% cumulative variance based on broken stick method (MacArthur 1957). Most of the samples of *C. pedunculata* and *C. rubella* were densely overlapping than scattered in the scatter plot. (Figure 1). The

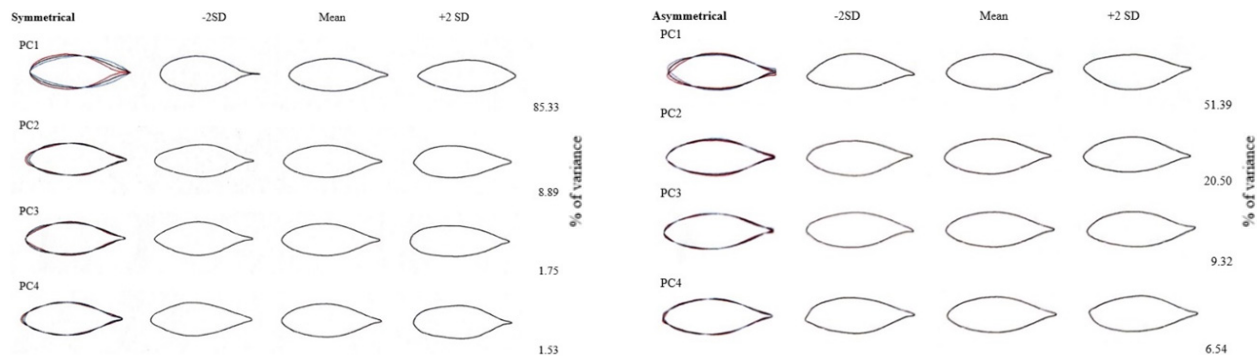


Figure 2. Leaf shape reconstructions using the elliptical Fourier descriptor (EFDs) along the first four PCs from the symmetric and asymmetric data. The first column shows the overlaid drawings of the next three columns along with each PC.

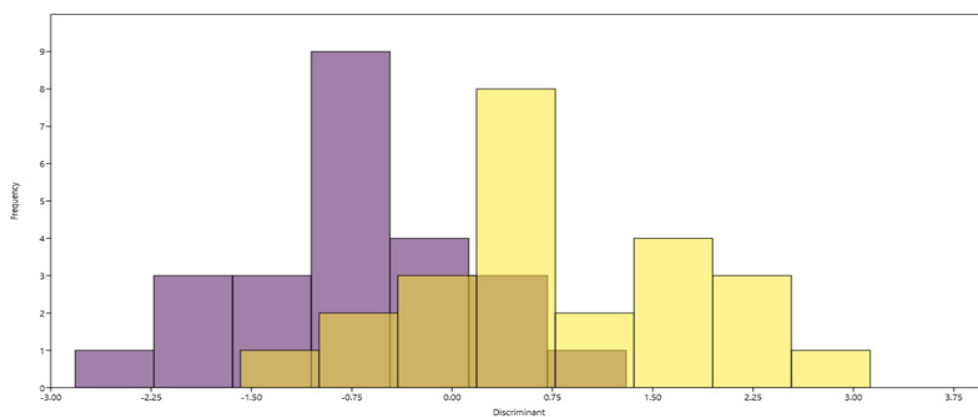


Figure 3. Discriminant analysis (DA) of the leaf shape of *C. pedunculata* and *C. rubella*. Blue bars *C. pedunculata*; Yellow bars *C. rubella*. Hotelling's $T^2 = 36.83$, $F = 2.2419$, $P = 0.08146$. Computed in PAST vers. 4.06b (Hammer et al. 2001).

Table 3. The relative contribution of symmetric and asymmetric components to leaf shape in two *Callicarpa* species.

Eigenvalues					
	PC1	PC2	PC3	PC4	Percentage contribution to overall shape *
Symmetric	6.76×10^{-4}	1.36×10^{-4}	1.13×10^{-4}	8.11×10^{-5}	54.01%
Asymmetric	3.80×10^{-4}	1.51×10^{-4}	6.88×10^{-5}	4.83×10^{-5}	34.80%

* Total percentage contribution from PC1 to PC4 only.

ordination plot of the two taxa in a two-dimensional space was highly defined by PC1 and PC2. It suggests that the plots of *C. pedunculata* and *C. rubella* are similar along PC1 and PC2, with positive values but few data points were positioned in the negative values in both PCs which results in overlap in the interspecific comparison suggesting similarities between the two taxa. Likewise, the similarity in leaf shape has been reflected in the discriminant analysis (DA), where there is no significant

difference between the means (Figure 3, Hotelling's $T^2 = 36.83$, $F = 2.2419$, $P = 0.08146$) of *C. pedunculata* and *C. rubella*. Additionally, the jack-knife cross-validation showed 71% of correctly classified species both in *C. pedunculata* and *C. rubella* (Table 4). Despite similarities in the ordination of plots between the two taxa, the comparisons showed relevant variations in their leaf mean shapes in multivariate analysis of variance (MANOVA) as the significant difference between the *C. pedunculata* and *C. rubella* exists based on leaf shapes (Wilk's $\lambda = 0.6196$, $F = 2.272$, d.f. = 10 and 37, $P = 0.03431$) (Figure 3).

The effects of shape variables in the Eigen leaves or leaf contours were determined based on the scores of the first four PCs to identify symmetric (54%) and asymmetric variations (35%). In Figure 4, symmetric variation highlights PC1 (85%) which explains leaf shape changes in width and leaf tips among samples of *C. pedunculata* and *C. rubella*. These variations were represented by discernible width expansion and transformation of leaf tips from acuminate to acute. Since PC1 accounts for the

Table 4. Cross-validation matrices from canonical variates analysis (CVA) of leaf shape in *C. pedunculata* and *C. rubella*.

	<i>C. rubella</i>	<i>C. pedunculata</i>	Total	% correct
A. confusion matrix without the jackknife				
<i>C. rubella</i>	20	4	24	83
<i>C. pedunculata</i>	5	19	24	79
Total	25	23	48	
B. confusion matrix with the jackknife				
<i>C. rubella</i>	17	7	24	71
<i>C. pedunculata</i>	7	17	24	71
Total	24	24	48	

Classification using PC scores computed from the original matrix. B. Jackknife classification.

Computed in PAST ver. 4.06b. (Hammer et al. 2001).

highest variations, it revealed that leaf tips and width expansion contribute to the overlapping of the two taxa. PC2 score (8.9%) describes cuneate, oblique to cordate leaf bases among samples, whereas PC3 (1.75%) and PC4 (1.53%) describe fine leaf changes along its margin that exhibit variations in the basal portion of the leaf. On the other hand, asymmetrical outline reconstruction shows basal and apical leaf variations on PC1 (51.4%) while remaining PCs (PC2 20.5%; PC3 9.32%; PC4 6.54%) revealed imperceptible variations across species. Thus, multivariate analyses were more restricted to the symmetric dataset due to the inadequate contribution of the asymmetric component.

In the recent study of two closely related genera,

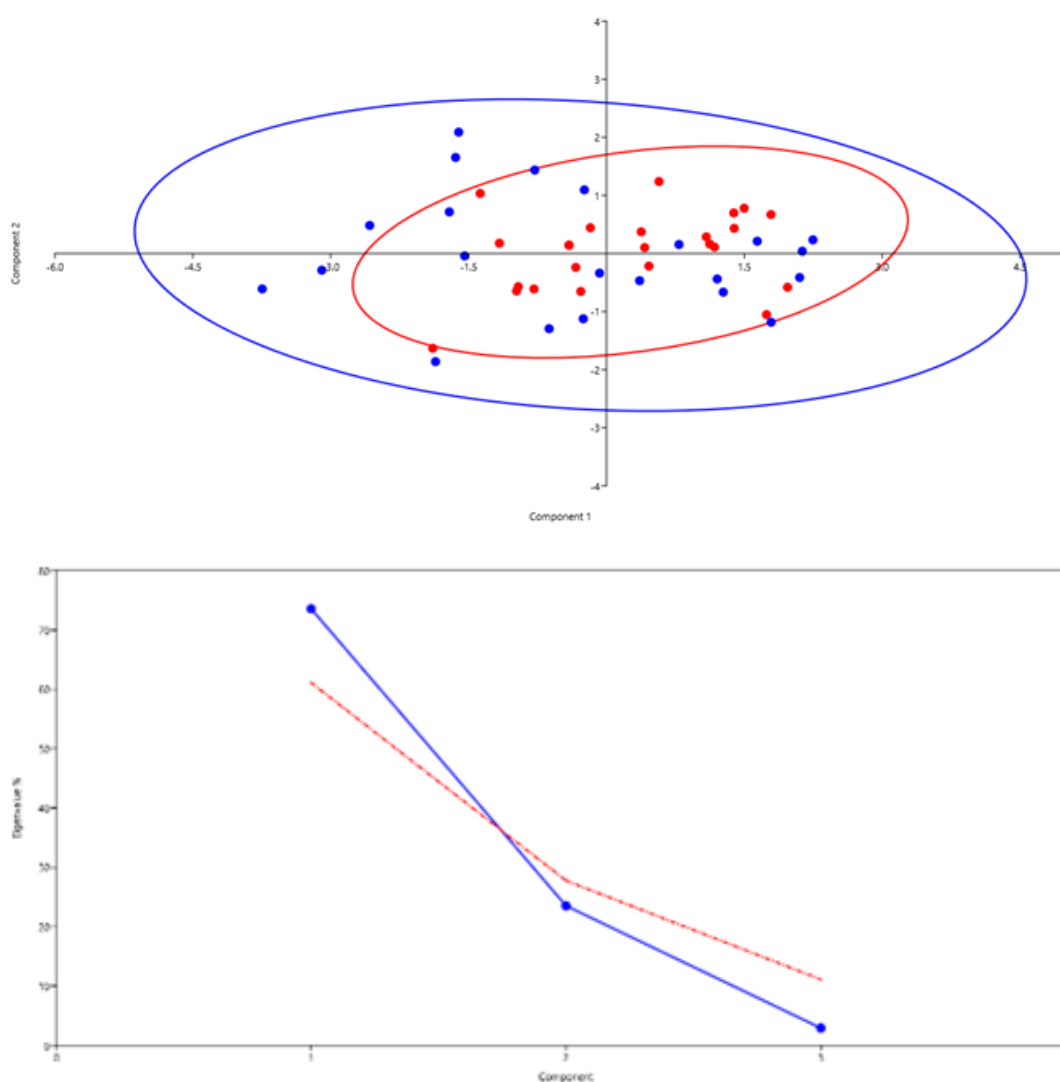


Figure 4. Principal component analysis (PCA) of the leaf of 46 individuals of *C. pedunculata* and *C. rubella*. (A) The plot of principal components 1 and 2 (PC1 and PC2) shows 95% confidence ellipses of the samples based on leaf shape descriptors. Red dots *C. pedunculata*, Blue dots *C. rubella*. (B) The broken stick method shows the retained number of principal components used in this study. (Red—Broken stick rule; Blue—Proportion of variance). Computed in PAST 4.06b software (Hammer et al., 2001).

Table 5. Leaf shape trait values across 46 selected species of (A) *C. pedunculata*, (B) *C. rubella*, and (C) overall accessions.

Trait	Range	Mean	SD	PCV (%)
Circularity				
(A)	0.36–0.57	0.47	0.06	13.51
(B)	0.29–0.57	0.44	0.09	19.68
(C)	0.29–0.57	0.45	0.08	16.6
Aspect ratio				
(A)	2.05–3.73	2.76	0.35	12.54
(B)	1.90–4.12	2.92	0.71	24.12
(C)	1.90–4.02	2.84	0.55	19.33
Solidity				
(A)	0.87–0.97	0.94	0.02	2.46
(B)	0.87–0.97	0.93	0.03	3.28
(C)	0.87–0.97	0.94	0.03	2.89

PCV, phenotypic coefficient of variation

Table 6. The eigenvalue of principal component analysis (PCA) of the leaf descriptors in 46 individuals of *C. pedunculata* and *C. rubella*.

PC	Eigenvalue	% variance	Eig 2.5%	Eig 97.5%
1	2.21911	73.970*	65.116	83.207
2	0.69498	23.166	14.095	32.331
3	0.0859082	2.8636	1.3203	4.5508

* Only the first PC is significant based on the broken stick method.

Table 7. Coefficients of correlation among PC1 to PC3 and the leaf shape descriptors

	PC 1	PC 2	PC 3
Circ	0.65380	-0.06060	0.75423
AR	-0.56176	0.62891	0.53749
Solidity	0.50692	0.77511	-0.37714

Table 8. Pearson correlation coefficients between three leaf-shape descriptors.

	Circularity	Aspect ratio	Solidity
Circularity		3.91×10^{-11}	6.64×10^{-7}
Aspect ratio	-0.8067		0.10703
Solidity	0.67839	-0.31056	

Callicarpa and *Geunsia*, the effect of environment and genetic factors were mentioned as the probable cause of the taxonomic overlap between the two taxa (Danila & Alejandro 2021). In geometric morphometrics, this overlap indicates morphological similarities among species and may occur due to the presence of hybrid

among samples (Adebowale et al. 2012). In recent years, there has been an increase in the number of hybrids in the genus *Callicarpa*, e.g., *C. japonica* Thunb. with *C. kochiana* Makino or *C. mollis* Siebold & Zucc., and *C. dichotoma* (Lour.) K.Koch with *C. kwangtungensis* Chun. (Yamanaka 1988, Tsukaya et al. 2003). The emergence of hybrids has brought several consequences in the population including introgression of plant traits or even the formation of new species which affect the interaction between plants and the environment (Orians 2000).

Despite the overlap, one clear finding in this study showed that symmetric variations on the leaf bases play a key role in determining leaf shape variations between *C. pedunculata* and *C. rubella*. In contrast to the symmetric variations, asymmetric PC1 also showed an interspecific variation focusing on the appearance of lobes in the basal portion of the leaf (Figure 2). In the leaf shape morphometric study conducted by Danila & Alejandro (2021) of the genus *Geunsia* and *Callicarpa*, the two taxa showed the possible occurrence of fluctuating asymmetry (FA). This results when the same species were unable to go through an identical development of the body organ on both sides resulting in uneven growth (van Valen 1962). Likewise, the occurrence of FA in leaves is a poor sign of environmental and genetic stress which happens when two closely related species mate and produce offspring (Sander & Matthies 2017). Hence, evidence of overlap in leaf shape variations and FA suggests that environmental and genetic factors affect variations in the leaf shape of *C. pedunculata* and *C. rubella*.

Analysis of Leaf Shape Descriptors

In this study, the first two principal components (PC1 and PC2) showed the most variation among the three leaf shape descriptors having 74% and 23%, respectively (Table 6). However, it shows that shape trends in most samples were mostly observed in PC1 (74%). The bar plot (Figure 5) and coefficient of correlation (Table 7) among PCs showed a significant relationship among the three leaf-shape descriptors. PC1 is more related to circularity (0.65380) and solidity (0.50692) but inversely related to AR (-0.56176) while PC2 is more associated with AR (0.62891) and solidity (0.77511) but inversely related to circularity (-0.06060). On the other hand, PC2 marked a high coefficient of correlation in solidity and AR, but the proportion of variability in PC2 is relatively low (23.17%). Therefore, the first principal component (PC1) was considered a statistically significant PC based on the broken stick method (MacArthur 1957) (Figure 4–6).

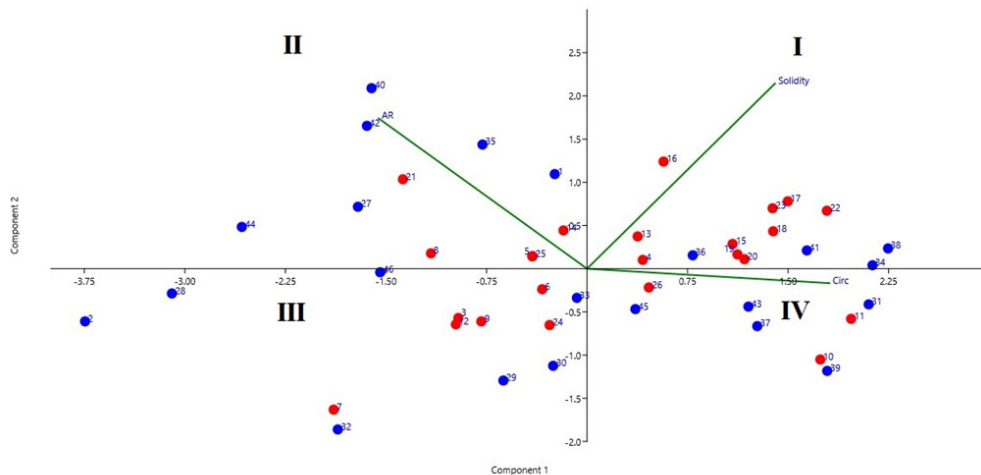


Figure 5. Principal component analysis (PCA) biplot showing the multivariate variation among the leaf descriptors of *C. pedunculata* (red dots) and *C. rubella* (blue dots) based on two principal components. Vectors (green line) indicate the direction and strength of each leaf descriptor to the overall distribution.

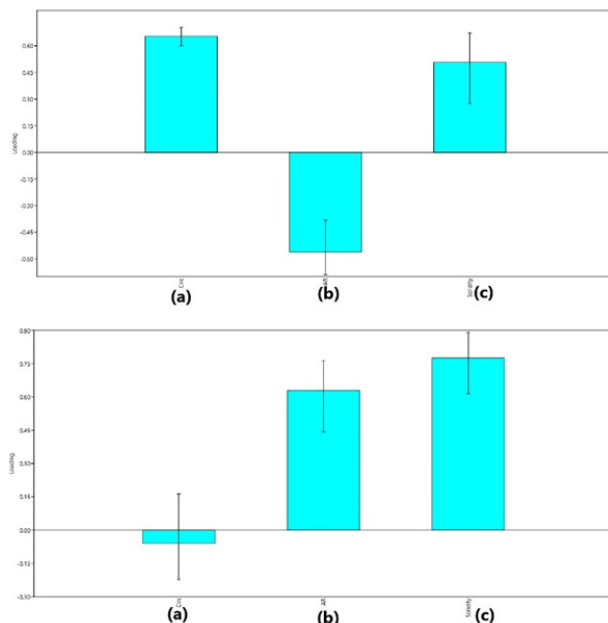


Figure 6. The barplot which represents factor loadings of the first two principal components shows the magnitude of each variable: (a) circularity, (b) aspect ratio, and (c) solidity.

The overall results showed that AR is the most variable leaf shape descriptor with a Phenotypic Coefficient of Variation (PCV; ((standard deviation/mean) $\times$ 100), estimates indicated the existence of a significant amount of variability among species, with 19.33% followed by circularity with 16.60% (Table 5). Additionally, both AR and circularity have a high distribution range of 1.90–4.02 and 0.29–0.57, respectively, meaning a high degree of variation was observed among samples. On

the other hand, solidity is the least variable with the narrowest distribution (0.87–0.97) and the lowest PCV of 2.89%. Almost all samples of *C. pedunculata* and *C. rubella* exhibited a high AR (>1.90) which manifested an increase in leaf width relative to the length, or vice versa. However, it shows that *C. rubella* has higher PCV values (24.12%) compared to *C. pedunculata* (12.54%) which indicates that the former has higher diversity in length-width ratio. While an increase in AR manifests an increase in the size of the leaf width relative to length, or vice-versa (Gupta et al. 2019). Some leaves of *C. rubella* were narrower but with high AR, that is, a larger major axis either on its length or width, affects the overall AR of the taxa. On the other hand, variations in circularity were observed in all accessions, where 30 samples indicated a low circularity (<0.50) while 16 samples had moderate circularity (0.50–0.57), meaning the lower the circularity values, the more prominent serrations are. Based on the observations, more specimens in *C. pedunculata* (45%) have more prominent serrations than in *C. rubella* (30%). Thus, these observations revealed that serrations and leaf size were useful in discriminating the two taxa. Moreover, the results showed a significant relationship between leaf serrations to leaf size, that is, as the leaf size increases, serrations decrease, or vice versa. Lastly, solidity showed a narrow distribution (0.87–0.97) and low PCV values (2.89%) indicating that most samples of *C. pedunculata* and *C. rubella* do not have lobed leaves. However, few accessions of *C. rubella* have been observed to show slightly rounded projections from the base of the leaf blade. Likewise, these samples of *C. rubella* were observed to have a lower solidity value representing cordate to oblique-cordate leaf bases. In

the study (Bramley 2013) of *Callicarpa* species in the Philippines, it has been noted that most *Callicarpa* species have either acute, acuminate, rounded, cuneate, oblique, or obtuse leaf bases which are all features of species with a high solidity (>0.87). Thus, we can conclude that solidity is also globally important as a diagnostic character to distinguish species between *C. pedunculata* and *C. rubella*.

Correlation among leaf shape descriptors

Figure 5 presents a biplot that simultaneously draws information from 46 individual samples of *Callicarpa* based on three leaf shape descriptors: AR, Circularity, and Solidity. The three leaf shape descriptors were positioned on the first, second, and fourth quadrants while data points of samples were distributed in all four quadrants based on their PCA scores. However, the distribution among individuals of *C. pedunculata* and *C. rubella* has found a minimal group differentiation due to a large degree of overlap. Although overlap has been observed among samples, the three leaf-shape descriptors produced a comparable level of relationship. In Table 8, the vectors of the variables circularity and solidity were closer to each other which suggests a positive correlation ($+0.6784$) between them. On the other hand, the greater distance close to 180 degrees found between circularity and AR suggests a negative correlation (-0.8067) while vectors of solidity and AR show almost an angle of 90 degrees which indicates that the variables were weakly correlated (-0.3106).

As mentioned above, AR and circularity were found to be the two most important variables in the discrimination of *C. pedunculata* and *C. rubella*. These leaf shape descriptors were highly influenced by length, width, and leaf margin. Since AR and circularity were found to be negatively correlated, variables like the length and width of the leaf were inversely proportional to the presence of serrations, that is, when the magnitude of the leaf decreases, the degree of serrations increases or vice versa. These observations exist among samples of *C. pedunculata* and *C. rubella*, where each taxon exhibits a corresponding trait relative to leaf serration and size. On the other hand, circularity and solidity indicate a moderate positive correlation that shows an impact of serrations in the projections of the leaf blade. Although a positive correlation was found between circularity and solidity the interval between the PCV values (circularity 16.6%; solidity 2.89%) is high, the two variables are related but exhibit different percentages in terms of their effects on the leaf shapes. This observation was evident among samples of *C. rubella* in the occurrence

of fine leaf lobes and discernible leaf serration. While the weak correlation was observed between solidity and AR where the former, unlike circularity, is little or not affected by serrations and leaf lobes (Figure 5).

Several studies (Thomas & Bazzaz 1996; Piazza 2005; Royer & Wilf 2006; Chitwood et al. 2013) have identified several factors in the evolution of leaf shapes and sizes, including the adaptation of plants to various types of environments. Likewise, different environmental factors showed a significant effect on morphological characters of closely related species (Jones 1995; Wolfe & Liston 1998; Royer et al. 2008). However, the adaptation mechanism in response to environmental variation in most species is still incomprehensible (Jump & Panuelas 2005). Since *C. pedunculata* and *C. rubella* have been identified to grow in a different environment, the two taxa showed distinct characteristics to discriminate the two species of *Callicarpa*. However, it also revealed that *C. pedunculata* and *C. rubella* showed similar leaf traits which can be considered as a plesiomorphic character of the two taxa. *C. pedunculata* has been described to show more serrations than *C. rubella*, while *C. rubella* exhibits a larger leaf size than *C. pedunculata* based on AR values. According to Peppe et al. (2011), leaf characters including sizes and shapes strongly correlate to environmental factors and prove that there is a biological basis for this relationship. The variations in serrations and leaf size between *C. pedunculata* and *C. rubella* are likely adaptations suited to specific environments. These distinctive features contribute to the species' ability to thrive in different ecological niches. To gain a more comprehensive understanding of the distribution and evolutionary relationships within the *Callicarpa* genus, it is strongly recommended to undertake a thorough phylogenetic study. This broader investigation will offer valuable insights into the geographic distribution of *Callicarpa* species and enhance our understanding of their adaptive evolution.

CONCLUSION

A statistically significant difference in leaf shape between *C. pedunculata* and *C. rubella* was observed, although there is considerable interspecific assessment, possibly due to environmental and genetic factors. Nevertheless, this study identifies aspect ratio and circularity as the two most informative variables in discrimination between the two species, emphasizing the importance of length, width, and leaf serrations as key diagnostic characteristics. The finding suggests

Table 9. Specimen examined. Authors and URLs of the digital images obtained from the online herbaria used in this study.

Author	HTTP url
Bijmoer, R., M. Scherrenberg & J. Creuwels (2021). Naturalis Biodiversity Center (NL) - Botany. Naturalis Biodiversity Center. Occurrence dataset https://doi.org/10.15468/ib5ypt accessed via GBIF.org on 2021-08-29.	https://www.gbif.org/occurrence/2516551448
	https://www.gbif.org/occurrence/2516532469
	https://www.gbif.org/occurrence/2516548469
	https://www.gbif.org/occurrence/2517253874
	https://www.gbif.org/occurrence/2516548469
MNHN & S. Chagnoux (2021). The vascular plants collection (P) at the Herbarium of the Muséum national d'Histoire Naturelle (MNHN - Paris). Version 69.223. MNHN - Museum national d'Histoire naturelle. Occurrence dataset https://doi.org/10.15468/nc6rxy accessed via GBIF.org on 2021-08-29	https://www.gbif.org/occurrence/2270292394
	https://www.gbif.org/occurrence/1852124824
	https://www.gbif.org/occurrence/2433456102
	https://www.gbif.org/occurrence/2433458107
	https://www.gbif.org/occurrence/1929638283
Ramirez, J., K. Watson, B. Thiers & L. McMillin (2021). The New York Botanical Garden Herbarium (NY). Version 1.38. The New York Botanical Garden. Occurrence dataset https://doi.org/10.15468/6e8nje accessed via GBIF.org on 2021-08-29.	https://www.gbif.org/occurrence/1930601756
	https://www.gbif.org/occurrence/1930296336
	https://www.gbif.org/occurrence/1930106241
	https://www.gbif.org/occurrence/1929663090
	https://www.gbif.org/occurrence/1929049006
	https://www.gbif.org/occurrence/1928131180
	https://www.gbif.org/occurrence/1929940867
	https://www.gbif.org/occurrence/1931232274
Royal Botanic Gardens, Kew (2021). Royal Botanic Gardens, Kew - Herbarium Specimens. Occurrence dataset https://doi.org/10.15468/ly60bx accessed via GBIF.org on 2021-08-29.	https://www.gbif.org/occurrence/912528324
	https://www.gbif.org/occurrence/912176780
	https://www.gbif.org/occurrence/912176785
Seregin, A. (2021). Moscow University Herbarium (MW). Version 1.195. Lomonosov Moscow State University. Occurrence dataset https://doi.org/10.15468/cpnhcc accessed via GBIF.org on 2021-08-29.	https://www.gbif.org/occurrence/3004116377
	https://www.gbif.org/occurrence/3004100339

leaf serrations and leaf size were important to *C. pedunculata* and *C. rubella*, respectively, and considered as an adaptive feature of the two taxa. Likewise, fine-scale variations in the basal region, e.g., presence of leaf lobes, also show significance in the discrimination of the two taxa. Thus, this research provides new experimental support for future taxonomic, genetics, or even ecological studies of *Callicarpa* species in the relevance of leaf size, leaf serrations, and leaf lobes.

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Mr. Jatishwor Singh Irungbam, Biology Centre CAS, Branišovská, Czech Republic.
Dr. Ian J. Kitching, Natural History Museum, Cromwell Road, UK
Dr. George Mathew, Kerala Forest Research Institute, Peechi, India
Dr. John Noyes, Natural History Museum, London, UK
Dr. Albert G. Orr, Griffith University, Nathan, Australia
Dr. Sameer Padhye, Katholieke Universiteit Leuven, Belgium
Dr. Nancy van der Poorten, Toronto, Canada
Dr. Kareen Schnabel, NIWA, Wellington, New Zealand
Dr. R.M. Sharma, (Retd.) Scientist, Zoological Survey of India, Pune, India
Dr. Manju Siliwal, WILD, Coimbatore, Tamil Nadu, India
Dr. G.P. Sinha, Botanical Survey of India, Allahabad, India
Dr. K.A. Subramanian, Zoological Survey of India, New Alipore, Kolkata, India
Dr. P.M. Sureshan, Zoological Survey of India, Kozhikode, Kerala, India
Dr. R. Varatharajan, Manipur University, Imphal, Manipur, India
Dr. Eduard Vives, Museu de Ciències Naturals de Barcelona, Terrassa, Spain
Dr. James Young, Hong Kong Lepidopterists' Society, Hong Kong
Dr. R. Sundararaj, Institute of Wood Science & Technology, Bengaluru, India
Dr. M. Nithyanandan, Environmental Department, La Ala Al Kuwait Real Estate. Co. K.S.C., Kuwait
Dr. Himender Bharti, Punjabi University, Punjab, India
Mr. Purnendu Roy, London, UK
Dr. Saito Motoki, The Butterfly Society of Japan, Tokyo, Japan
Dr. Sanjay Sondhi, TITLI TRUST, Kalpavriksh, Dehradun, India
Dr. Nguyen Thi Phuong Lien, Vietnam Academy of Science and Technology, Hanoi, Vietnam
Dr. Nitin Kulkarni, Tropical Research Institute, Jabalpur, India
Dr. Robin Wen Jiang Ngiam, National Parks Board, Singapore
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Dr. Spartaco Gippoliti, Socio Onorario Società Italiana per la Storia della Fauna "Giuseppe Altobello", Rome, Italy
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Dr. S.S. Talmale, Zoological Survey of India, Pune, Maharashtra, India
Prof. Karan Bahadur Shah, Budhanilakantha Municipality, Kathmandu, Nepal
Dr. Susan Cheyne, Borneo Nature Foundation International, Palangkaraja, Indonesia
Dr. Hemanta Kafley, Wildlife Sciences, Tarleton State University, Texas, USA

Other Disciplines

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Dr. Mandar S. Paingankar, University of Pune, Pune, Maharashtra, India (Molecular)
Dr. Jack Tordoff, Critical Ecosystem Partnership Fund, Arlington, USA (Communities)
Dr. Ulrike Streicher, University of Oregon, Eugene, USA (Veterinary)
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Dr. L.D. Singla, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India
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Dr. Bahar Baviskar, Wild-CER, Nagpur, Maharashtra 440013, India

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ravi@threatenedtaxa.org

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