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Research Article

Intrafamilial Pinna Shape Variations of Fern Species Under Family Thelypteridaceae and Nephrolepidaceae Using Elliptic Fourier Analysis

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Abstract. Fern species under family Thelypteridaceae and Nephrolepidaceae tend to have similarities when it comes to leaf morphology. Due to their cohesive morphological appearance, species under these two families are hard to distinguish from the other. While qualitative descriptions in leaf shape could aid in understanding the identification, evolution and development of ferns, it is argued that the quantitative description of the shape of the pinna is a good measure in the classification of ferns by delineating species. This study was therefore conducted to quantitatively describe the pinna shape of five species of ferns under family Thelypteridaceae and four species under family Nephrolepidaeceae by Elliptic Fourier Analysis (EFA). Significant shape variations were observed within and between species specifically in width, apex and base of the pinna. Species found on shaded habitat were observed to have broader pinna lamina compared to elongated and narrow pinna of fern species found on open areas. Light availability is argued to possibly influenced the pinna shapes of the ferns. Results of this study have shown the importance of EFA in the quantitative description of biological shape that aid in understanding the relationships of the fern species.

Keywords. Classification; Leaf apex; Leaf base; Habitat; Morphology

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1. Introduction

Ferns are pteridophytes that thrive in a variety of environment ranging from moist shaded parts of the forest, grasslands and roadsides. Many fern species thrive cohesively in their natural environment sharing similar frond morphology resulting to misidentification. Insights on their classification, evolution, phylogeny and morphology [1–3] still are erratic due to their morphological similarities with many genera [4]. Examples of these ferns are Nephrolepidaceae and Thelypteridaceae [5]. Nephrolepidaceae comprise only of the genus *Nephrolepis* which is hard to categorize because it is formerly associated with other fern families. Thelypteridaceae on the other hand, is a large group with 20 genera and about 1000 species [6]. The differences between species are minor and unclear because they are only described qualitatively. They are therefore good subjects to be quantitatively described to determine the extent of their morphological differentiation. Since many species within these two families prefer distinctly differing habitats and may have affected the general morphology of the fronds [7], their leaflets called pinna were chosen to be quantitatively described based on their shapes. The pinna is one of the morphological characters that aids in the classification of ferns as well as in detecting variation between species [7, 8] thus was examined in this study. Outline-based geometric morphometric (GM) tool of Elliptic Fourier analysis (EFA) was used in the description of the shape of the pinna since it uses outlines to analyze and visualize the shape and their variations in the different species of ferns.

2. Materials and Methods

Mature fern leaves or fronds were selected at random from five fern species belonging to Thelypteridaceae family and four fern species under family Nephrolepidaceae (Figure 1). Species of fern collected were identified using taxonomic and pictorial keys.= The ferns' frond leafy segment of the blade called pinna which bear the sori" was detached and theimages were directly taken using a Brother DCP-J100 scanner. The images were grouped according to species and were converted to Bitmap image to obtain its outline. Shape variations were evaluated using the elliptic Fourier descriptors that are generated through the use of the SHAPE software which consist of four programs; ChainCoder, Chc2Nef, PrinComp and PrinPrint [9]. The ChainCoder process the converted Bitmap images to obtain chain codes. The chain codes generated were used for the normalization and calculation of Elliptic Fourier descriptors in Chc2Nef [10]. Princomp software was employed for the principal component analysis which subsequently display the principal component analysis results, principal component contours and the calculated the principal component scores as the output. To visualize variations among the ferns pinna, PrinPrint program was applied [11]. This program reconstructs the contours using the elliptic Fourier coefficients and visualizes the pinna shape for each of the principal components. Scatter plot, box plots and cluster analysis were done to have a graphical presentation of the results which were generated using the Paleontological Statistics Software Package (PAST). Multiple

Analysis of Variance and Canonical Variate Analysis (MANOVA/CVA) using the principal components scores were also performed to determine variations between the shapes of the pinna of the different fern species.

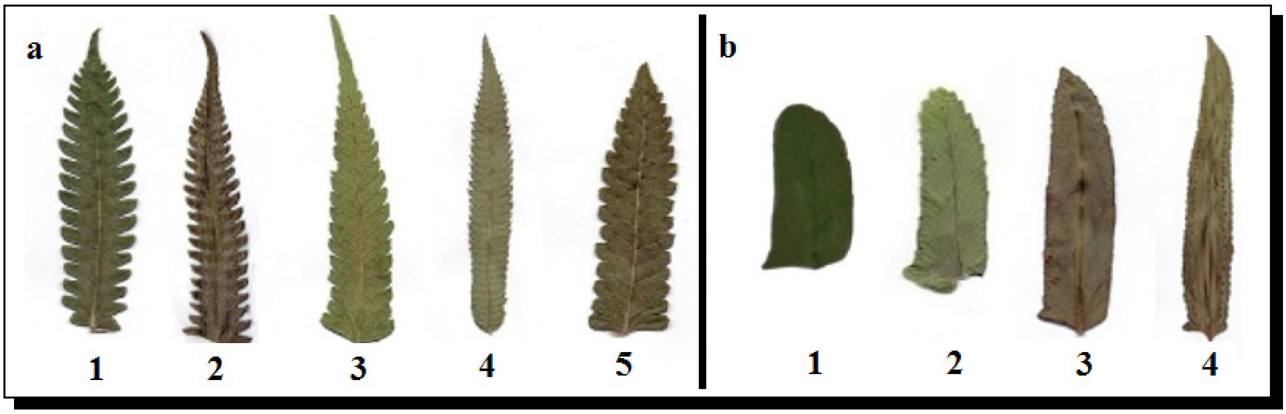

Figure 1. Sample images of (a) Thelypteridaceae species (1) *Christellaparasitica* (2) *Christellaarida* (3) *Pneumatopteris sp.* (4) *Sphaerostephanosunitus* (5) *Sphaerostephanos* sp.; (b) Nephrolepidaceae species (1) *Nephrolepissp. 1* (2) *Nephrolepiscordifolia* (3) *Nephrolepissp. 2* (4) *Nephrolepishirsutula*

3. Results and Discussion

Results of EFA of the pinna shapes from five species of Theleypteridaceae and four species of Nephrolepidaceae showintrafamily variations. Of the 4 significant principal components, 81.99% account for the total variance for Thelypteridaceae and only 1 significant component with 87.95% variance for Nephrolepidaceae (Table 1). These principal components from the Fourier coefficients were used to describe leaf outline among the fern species.

Table 1. The Eigenvalues and percentage variance explain by each significant principal component for the variation in pinna shape

Family Thelypteridaceae				Family Nephrolepidaceae			
Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)	Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)
PC1	5.11E-03	50.35	50.35	PC1	1.71E-02	87.95	87.95
PC2	1.80E-03	17.77	68.14				
PC3	7.99E-04	7.88	76.01				
PC4	6.07E-04	5.99	81.99				

Figure 2 shows the reconstructed contours of pinna shapes. Variations between the populations are summarized in Table 2. The pinna shape varies between the species within the 2 families. Most of the observed variations are on the pinna width, apex and base based on the reconstructed contour for each of the significant principal components. For Thelypteridaceae, the pinna shape ranges from subulate with tapering apex to oblong with acute apex. Slender, elongated and broad pinna was also observed in the population of the Thelypteridaceae species. For the family Nephrolepidaceae, pinna shape varies from subulate with acuminate apex to oblong with rounded apex and auriculate base.

Table 2. Description of leaf shapes of the five Thelypteridaceae and four Nephrolepidaceae species under based on the significant Principal Components

Principal components	**Description**
	Thelypteridaceae
PC1 (50.35%)	Observed variations are based on the width and shape of lamina as well as the shape of the leaf apex. Populations of *C. arida*, *S. unitus* and *Pneumatopteris sp*. tend to (-) deviation; the individuals approaching negative deviation have slender assymetricalsubulate lamina with long tapering apex *C. parasitica* and *Sphaerostephanos sp.* differ from the rest of the population having broader and oblong shape lamina that is symmetrical with acute serrated apex.
PC2 (17.77%)	Variations are based on apical and basal shape. Individuals under *C. arida* and *S. unitus* approaches (-) deviations having asymmetrical acuminate leaf apex and rounded base; Populations under *C. parasitica* and *Sphaerostephanos sp.* approaches the mean shape with rounded base shape and acuminate serrated apex; *Pneumatopteris sp*. tend to (+) deviation having an asymmetrical tapering tip and asymmetrical base
PC3 (7.88%)	Populations under *S. unitus* approaches the mean lanceolate shape in contrast to the populations under *Christella* which approaches (+) deviation having linear pinna shape with rounded base and serrated acuminate apex; The rest of the population approaches (-) deviation with acuminate apex tapering gradually and asymmetrical base marked by different shape on either side of the midline
PC4 (5.99%)	All the populations of the five species tend to (-) deviation having acuminate apex and truncate base
	Nephrolepidaceae
PC1 (87.95%)	Major variations on the pinna width, shape, apex and base. *N. cordifolia*and *Nephrolepis sp.1* tend to the (+) deviation having oblong shape and broader pinna lamina with rounded apex and auriculate one one side of the base; *N. hirsutula* approaches (-) deviation with subulate and slender lamina with acuminate and convex base *Nephrolepis sp.2* deviate from other species which tend to the mean shape which is elliptical with obtuse apex and asymmetrical *base*.

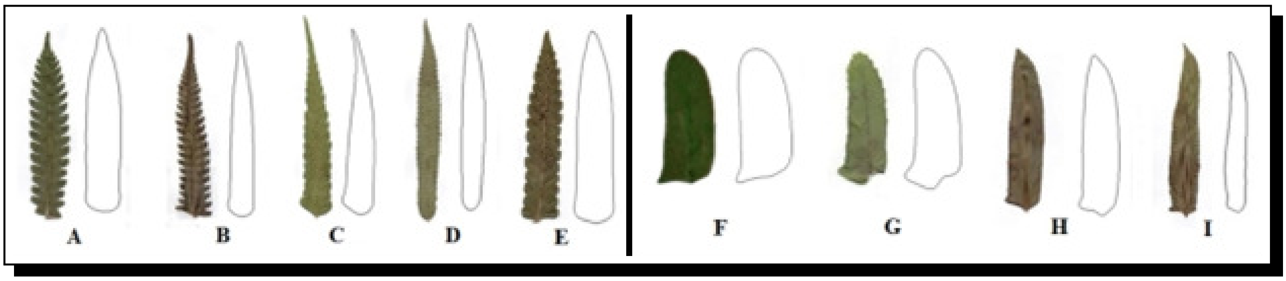

Figure 2. Mean Shape of fern pinnae [A-E] Thelypteridaceae (A) *C. parasitica* (B) *C. arida* (C) *Pneumatopteris sp*. (D) *S. unitus* (E) *Sphaerostephanos sp.* [F-I] Nephrolepidaceae (F) *Nephrolepis sp. 1* (G) *N. cordifolia* (H) *Nephrolepis sp.2* (I) *N. hirsutula*

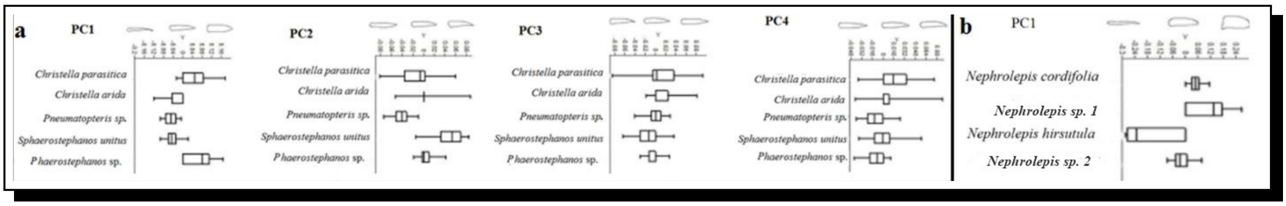

Figure 3. Box Plots demonstrating the difference in pinna shapes among the fern species under family Thelypteridaceae (a) and Nephrolepidaceae (b)

Multivariate analysis of variance (MANOVA) and cluster analysis of the principal component scores showed significant variation between species in each of the family. Canonical Variance of analysis (CVA) scatter plot (Figure 4a,b) and cluster analysis (Figure 4c,d) illustrate the significant interspecies variations of the pinna shape. Fern species that are found on shaded areas have broader pinna lamina while ferns that are found in the open field were observed to have slender pinna.

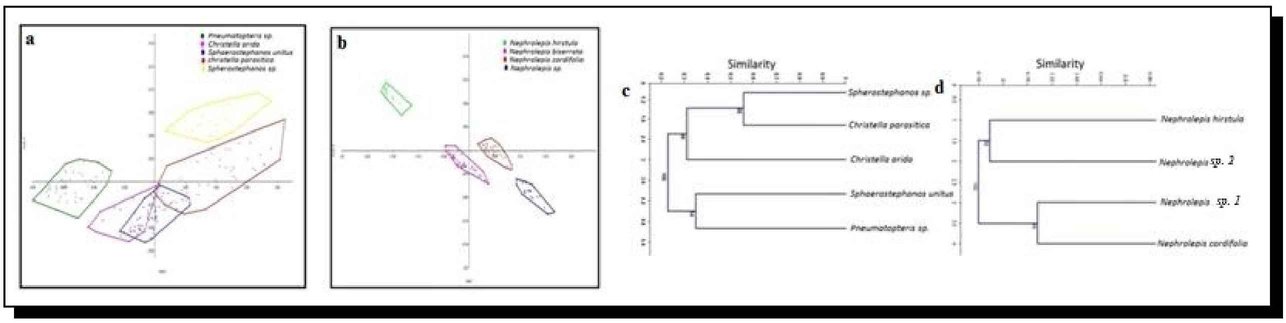

Figure 4. Scatterplots and cluster analysis of principal component scores demonstrating the significant difference among the pinna shape of the fern species under (a,c) Thelypteridaceae (b,d) Nephrolepidaceae

The results of this study which showed differences in the shape of the pinna within and between species of the two families can be argued to be not only genetically determined but can also be attributed to which habitat they were surviving [12, 13]. Leaves that are highly sensitive to environmental changes such as sunlight may acquire phenotypic plasticity [7]. Light is one of the environmental factors that contribute to morphological adaptation in leaves. Shade-avoidance syndrome is a paradigm for leaf plasticity which allows them to absorb adequate light to carry on photosynthesis [8]. The plasticity is observed on leaf dimension which increases in low light conditions such as those collected fern species growing on forest floors. Leaf shape tends to be narrower in sun exposed areas and broader in the shade. These explain why fern species found in shaded areas tend to have broader pinna lamina compared to other species that were found on partially shaded to exposed areas. The variations in the pinna shape of fern species inhabiting different microhabitats could provide insights on the growth and development in ferns.

4. Conclusion

Elliptic Fourier analysis of the pinna of the different species of ferns clearly visualized and facilitated the quantitative descriptions of the variations in the shape of the pinna. Likewise, understanding the possible relation of the habitat to the shape variation of the pinna between the species revealed the patterns of variation and covariation can be linked to differences in the light condition of the different habitats.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All the authors contributed significantly in writing this article. The authors read and approved the final manuscript.

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