Nimis P. L., Vignes Lebbe R. (eds.)
Tools for Identifying Biodiversity: Progress and Problems – pp. 251-256.
ISBN 978-88-8303-295-0. EUT, 2010.

Geometric morphometrics as a tool to resolve taxonomic problems: the case of *Ophioglossum* species (ferns)

Sara Magrini, Anna Scoppola

Abstract — A modern method, geometric morphometrics, was used to clarify the taxonomic position of the European *Ophioglossum* species: *O. azoricum*, *O. lusitanicum*, and *O. vulgatum*. The identification of these *taxa* by traditional methods is rather difficult, due to different taxonomic interpretations. Sterile leaf shapes were investigated using a landmark-based method and the Fourier analysis of outlines. Both methods highlight the shape and the base of the leaf as an important diagnostic character.

Index Terms — geometric morphometrics, landmark, *Ophioglossum*, outline analysis.

1 Introduction

Geometric morphometrics [1], [2] is a technique for multivariate shape analysis that preserves the integrity of biological shape, avoiding its reduction to linear and angular measures that do not include information concerning the geometric relationships of the entire subject. allowing to identify the anatomical areas of morphological remodelling. Two different kinds of geometric morphometrics are most widely used: landmark-based methods such a Thin Plate Splines analysis [3], and Fourier analysis of outlines [4]. The theoretical formulation has been developed in recent decades by the synthesis of multivariate analysis methods of covariance matrices and methods for direct visualization of changes in the biological shape [1], [2], [5]. Multiple applications exist in the biomedical field, in paleontology, anthropology and zoology. In contrast, geometric morphometrics was almost absent from botanical research until a few years ago [3], with a few exceptions for *Dactylorhiza* [6], *Quercus* [7], and other groups.

S. Magrini is with the Tuscia Germplasm Bank, University of Tuscia, Viterbo, I 01100. E-mail: magrini@unitus.it.
A. Scoppola is with the Viterbo Botanical Gardens, University of Tuscia, Viterbo, I 01100. E-mail: scoppola@unitus.it.

Many groups of plants still require more intensive taxonomic studies. One of them is the genus *Ophioglossum* C. Presl, an ancient fern genus of about 25-30 species of *Ophioglossaceae*, with a cosmopolitan but primarily tropical and subtropical distribution. The simple morphology of the sporophyte - a single leaf or a few - has limited the number of characters available for building classifications on morphological ground and clarifying relationships, forcing investigators to rely on details of frond size or sporangia number as diagnostic markers. Problems associated with the evaluation of these often subtle differences gave rise to different taxonomic interpretations of the genus [8]. Furthermore, the number of taxonomic studies of *Ophioglossum* species is relatively low in Europe [9], [10], so that many questions concerning *Ophioglossum azoricum* C. Presl and its relationships with the other species (*O. vulgatum* L. and *O. lusitanicum* L.), remain unresolved. Our study aims at testing a modern method - geometric morphometrics - for resolving some of these problems and clarifying the taxonomic position of these *Ophioglossum* species.

2 Materials and Methods

2.1 Collecting materials

We have analysed three species: *O. azoricum*, *O. lusitanicum*, and *O. vulgatum*, as well as some critical populations from Tuscany (Moriglion di Penna and Monte Pianello, Lucca) and Latium (Selva del Lamone, Viterbo). We used 100 *exsiccata* from 9 European herbaria. We photographed with a digital camera all the sterile leaves in the specimens of Cagliari (CAG), Florence (FI), Genoa (GE), Pisa (PI), Siena (SIENA), Viterbo (UTV), and Valencia (VAL), and we collected images from the website of Paris (P), and Vienna (W). We obtained over 203 images, standardized with a resolution of 300 dpi and a size of 800x1000 pixels, prepared using *Adobe Photoshop 7.0.* As a first step, every foreign element was eliminated from the picture, thereby isolating the sterile leaf, and then the contrast with background was maximized.

2.2 Landmark-based method

The *TPS - Thin-Plate Splines* software package was used for landmark-based morphometrics analysis: *TpsDig2.12* to digitize landmarks [11] and *TpsRelw 1.42* for statistical analysis [12]. In our case, we could find only 3 homologous points (landmarks), due to the shape of the leaf, with an entire margin, and the lack of evident veining. A grid of 11 equally spaced horizontal lines was superimposed on all images, taking care to position it perpendicular to the longitudinal axis of the frond and to align the apex and the base with the top and the bottom line of the grid, respectively, to digitize other 18 semi-landmarks (points defined relatively to other landmarks). The next step was the development of matrix of landmark coordinates through *TpsRelw 1.42*. This program operates through an algorithm used to describe the mechanical deformation of thin metal foils (thin plate spline or TPS), building linear combinations of the landmark coordinates.

The new variables - called “shape variables” - allow the creation of deformation grids that visually illustrate the change in shape compared to the average shape of the sample (*consensus*). We have analysed the entire dataset and the partial datasets corresponding to each species and population to defy inter- and intraspecific variability, respectively, and we have performed a Relative Warps Analysis on the covariance matrix.

2.3 Outline Analysis

For Outline Analysis we used the software package *SHAPE 1.3* [13]. based on the methodology of Elliptic Fourier descriptors, which allows to describe - in terms of harmonics - each type of two-dimensional shape with a closed outline. All images were saved in .bps format (24bit) and were converted to binary with *Chain Coder* before tracing the outlines in *Chain-code*, a coding system that describes the geometrical information on the shapes. Then, the *Chain-code* file was transformed into a Normalized Elliptic Fourier file with *Chc2Nef*, using 20 harmonics on 77 components. The matrix of the harmonic coefficients underwent normalization based on the first harmonic, to transform the data into shape variables. Subsequently, a PCA was performed on the variance-covariance matrix of normalized coefficients (Elliptic Fourier Descriptors) using *PrinComp*, that gives a graphical output of the average shape ± the standard deviation.

3 Results

We report the results of the Relative Warps Analysis (RWA) on the entire dataset. The analysis of the first two RWs (accounting for 85.10% and 6.07% of the total variance, respectively), shows a clear separation between *O. vulgatum* and *O. lusitanicum* along RW1, while in the centre of the plot several samples of *O. azoricum* are very close to *O. lusitanicum*. The samples from Lamone (LAM) and M. Pianello (PIA) overlap with *O. azoricum*, while those from Moriglion di Penna (MOR) are distributed along RW1 in the areas of *O. vulgatum* and of *O. azoricum*. Moving along RW1 from negative values, corresponding to *O. lusitanicum*, to the positive values of *O. vulgatum*, the associated deformation grids show a vertical contraction and an expansion in width, particularly in the proximal part of the leaf (Fig. 1).

The results of the Outline Analysis of the entire dataset (Fig. 2a) are consistent with those from RWA: PC1 (accounting for 88.33%) shows the 2 extreme shapes diversified in the proximal part of the leaf, corresponding to *O. lusitanicum* for the negative end, to *O. vulgatum* for the positive end. The results for the single species show a lower variability in *exsiccata* of *O. azoricum “typus”* (PC1=56.15%) (Fig. 2b), and of *O. lusitanicum* (PC1=61.34%) (Fig. 2e), while the greater variability is in *O. vulgatum* (PC1=70.67%) (Fig. 2d), confirming the difficulty of a correct identification by traditional morphological methods due to the different taxonomic interpretations.

The results were used to identify the critical samples from LAM, PIA and MOR. The LAM outlines (Fig. 2f) have a low variability (PC1=64.15%), they are akin to *O. azoricum* outlines (Fig. 2b-c), as the PIA samples (Fig. 2g), which have a greater variability (PC1=80.67%). On the contrary, the MOR samples (Fig. 2h) are diversified (PC1=91.54%), showing 2 extreme shapes, corresponding to *O. vulgatum* (Fig. 2d) and *O. azoricum* outlines (Fig. 2 b-c).

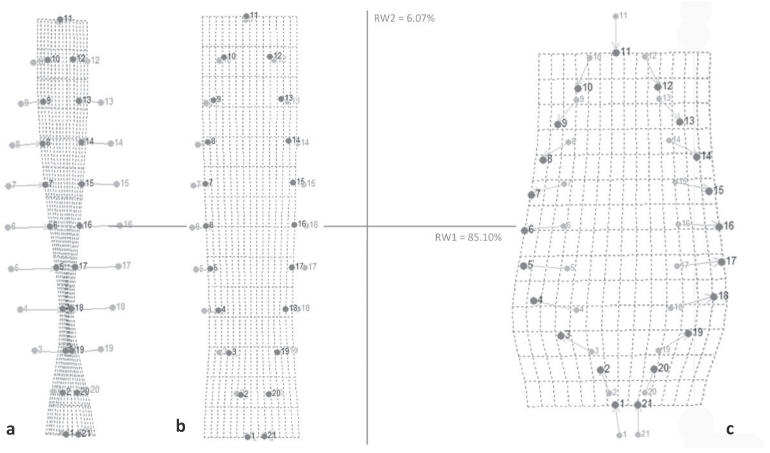

Fig. 1 – Deformation grids along the first Relative Warp with vectors of deformation relative to average shape of fronds, showing the anatomical areas of morphological remodelling.

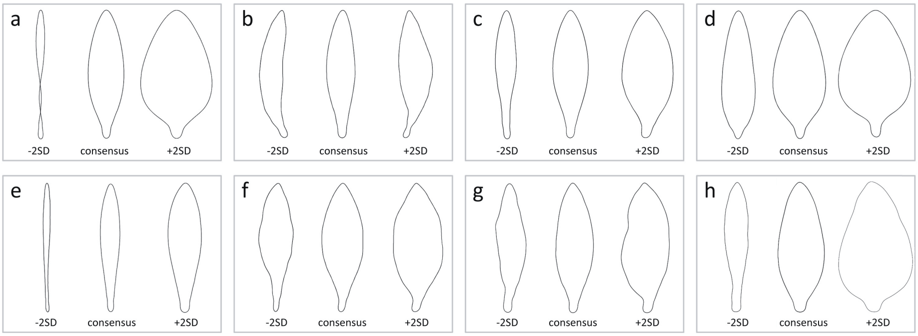

Fig. 2 – Results from Outline Analysis. First principal component of: (a) the entire dataset, PC1=88.33%; (b) *O. azoricum typus*, PC1=56.15%; (c) *O.azoricum*, PC1=64.98%; (d) *O. vulgatum*, PC1=70.67%; (e) *O. lusitanicum*, PC1=61.34%; and *Ophioglossum sp.* from: (f) LAM, PC1=64.15%; (g) PIA, PC1=80.67%; (h) MOR, PC1=91.54%.

4 Discussion

The geometric morphometrics analysis gave results that lead to a better characterization of the 3 *Ophioglossum* species.

The landmark-based analysis does not completely discriminate among the three species. It clearly separates *O. lusitanicum* from *O. vulgatum*, but *O. azoricum* and *O. lusitanicum* are widely overlapping.

More information derives from the deformation grids that highlight a diagnostic character like the relative width of the leaf, especially in its proximal part, as confirmed by the Outline Analysis. The graphical outputs of the PCA of outlines show the differences between the three species, confirming shape and base of the leaf as the main diagnostic characters. *O. vulgatum* is well distinct from the other two species by the shape of the lamina, which is from lanceolate to broadly ovate with a large round and attenuated base. *O. azoricum* and *O. lusitanicum* have a more or less cuneate base, but they are differentiated in the leaf shape: from lanceolate to narrow ovate in *O. azoricum*, lanceolate-linear in *O. lusitanicum*. Geometric morphometrics also allows to identify the critical samples: LAM and PIA samples can be referred to *O. azoricum*, as confirmed by both Relative Warp and Outline Analysis. Instead, the Outline Analysis of MOR samples shows a higher variance, although this could be attributed to the variability of *O. vulgatum;* probably in this site *O. azoricum* and *O. vulgatum* coexist.

5 Conclusion

The study led to a more accurate characterization of the three species, with the identification of valid diagnostic characters, and the presumable discovery of 2 new sites of *O. azoricum* in Italy, M. Pianello (Lucca) and Selva del Lamone (Viterbo), even if other analyses (e.g. molecular and caryological) are suggested. The research will continue with the revision of all samples that showed abnormal or out-of-range values, and by increasing the dataset with *exsiccata* from other herbaria. These results also will allow understanding and defining the actual distribution of *Ophioglossum* species in Italy and Europe.

Acknowledgement

The authors wish to thank the curators of the cited herbaria and dr. L. Peruzzi (University of Pisa) for providing images or exsiccata for this study.

References

[1] F. Bookstein, *Morphometric Tools for Landmark Data*. Cambridge Univ. Press, 1991.

[2] F. J. Rohlf and L. F. Marcus, "A Revolution in Morphometrics". *Trends Ecol. Evol.*, vol. 8, pp. 129-132, 1993.

[3] D. C. Adams, F. J. Rohlf and D. E. Slice, "Geometric Morphometrics: Ten Years of Progress Following the 'Revolution'". *Ital. J. Zool.*, vol. 71, pp. 5-16, 2004.

[4] R. J. Jensen, K. M. Ciofani and L. C. Miramontes, "Lines, Outlines and Landmarks: Morphometric Analyses of Leaves of *Acer rubrum*, *Acer saccharinum* (*Aceraceae*) and Their Hybrid". *Taxon*, vol. 51, pp. 475-492, 2002.

[5] L. R. Rabello, B. Bordin and S. Furtado, "Shape Distances, Shape Spaces and the Comparison of Morphometric Methods". *Trends Ecol. Evol.*, vol. 15, pp. 217-220, 2000.

[6] A. B. Shipunov and R. M. Bateman, "Geometric Morphometrics as a Tool for Understanding *Dactylorhiza* (Orchidaceae) Diversity in European Russia". *Biol. J. Linnean Soc.,* vol. 85, no. 1, pp. 1-12, 2005.

[7] V. Viscosi, O. Lepais, S. Gerber and P. Fortini, "Leaf Morphological Analyses in Four European Oak Species (*Quercus*) and Their Hybrids: a Comparison of Traditional and Geometric Morphometric Methods", *Plant Biosyst.*, vol. 143, no. 3, pp. 564-574, 2009.

[8] W. D. Hauk, C. R. Parks and M. W. Chase, "Phylogenetic Studies of Ophioglossaceae: Evidence from rbcL and trnL-F Plastid DNA Sequences and Morphology". *Mol. Phyl. Evol.*,

vol. 28, pp. 131-151, 2003.
[9] A. M. Paul, "The Status of *Ophioglossum azoricum* (Ophioglossaceae: Pteridophyta) in the British Isles". *Fern Gaz*., vol. 13, pp. 173-187, 1987.
[10] L. Peruzzi, G. Cesca and D. Puntillo, "*Isoëtes* (Isoetaceae), *Ophioglossum* and *Botrychium* (Ophioglossaceae) in Calabria (S Italy): More Karyological and Taxonomical Data". *Caryologia*, vol. 56, no. 3, pp. 355-359, 2003.
[11] F. J. Rohlf, *TpsDig2, Digitize Landmarks and Outlines, Version 2.12*. Dep. of Ecology and Evolution, State University of New York at Stony Brook, 2007.
[12] F. J. Rohlf, *TpsRelw, Version 1.42*. Dep. of Ecology and Evolution. State University of New York, Stony Brook, 2005.
[13] H. Iwata and Y. Ukai, "SHAPE: a Computer Program Package for Quantitative Evaluation of Biological Shapes Based on Elliptic Fourier Descriptors.", *J. Heredity*, vol. 93, pp. 384-385, 2002.