

This assignment is my own work, presented in my own words, ALL sources of information have been cited and any direct quotations are contained within quotation marks.

Critique of Klingenberg et al.'s (2012) study on *Potentilla* leaf plasticity and evolution using geometric morphometrics

Introduction

The diversity of leaf shapes and sizes, has been extensively studied in order to understand plant evolution (Brown et al., 1991; Ackerly and Donoghue, 1998), the form and function of plant ecotypes (Viscosi et al., 2009; Silva et al., 2012), plant development (Burns and Dawson, 2006), hybridization (Jørgensen and Mauricio, 2005), gene regulations (Uchida et al., 2010), and phenotypic plasticity (Smith, 1978; Ackerly et al., 2002). Geometric morphometrics, referred to as GM, (Rohlf and Archie, 1984, Zelditch et al., 2004; Klingenberg, 2013) has revolutionised morphological data collection using landmarks on digital images (Adams et al., 2004). Shape can be defined as all the geometric features of an object, except for its size, position and orientation (Bookstein, 1996). This shape data can be extracted using GM.

Klingenberg et al.'s (2012) study gives an insight to the patterns of cinquefoil (*Potentilla*, Rosaceae) leaf morphological integration, role of allometry and fluctuation asymmetry. They investigated the different shapes of compound leaflets, whole leaves and the variation within taxa and among taxa with the use of GM.

I chose this paper because I attended the Introduction to Geometric Morphometrics course led by Chris Klingenberg where this data set was part of one of the practical sessions. Therefore, I was able to re-analyse and re-evaluate this study using their original data. I also have experience using image analysis and using MorphoJ (Klingenberg, 2011) and I will be following Mensch and Kording's (2017) writing guide to critique this paper.



Figure 1. The leaves of *Potentilla* species greatly vary and consist of both entire leaves and pinnate leaflets. All of these species were used in the chosen article. Pictures from missouriplants.com.

Title of the paper and authors

The title 'Developmental plasticity, morphological variation and evolvability: a multilevel analysis of morphometric integration in the shape of compound leaves' is 132 characters long and does not mention the use of geometric morphometrics or the study organism. It has been shown that short titles, consisting of less than 94.5 characters are cited more often (Paiva et al., 2012) so a title such as 'A multilevel analysis of *Potentilla* compound leaf shapes using geometric morphometrics and phylogenetics' would be 92 characters and would include the most relevant information related to the article.

The authors of the chosen article are Chris Klingenberg (Klingenberg, 2016, 2014 and 2010), an expert in geometric morphometrics and Sascha Duttke (Hetzl et al, 2016; Duttke, 2015) specialising in gene regulation and reprogramming. Simon Whelan working in phylogenetic studies (Bogusz and Whelan, 2017; Whelan, 2008), and Minsung Kim researches genes involved in leaf development (Spencer and Kim, 2017; Kim et al, 2008).

Abstract critique

The abstract begins with describing the context of the study, their focus and approach (Figure 2). It gives an overall view of their methods, study organism, main findings and their implications. It ends with concisely reporting what their data suggests. It is an indicative abstract (Orasan, 2001), does not describe their study and methods step by step but follows the 'concise and precise' maxim described by Mack (2012). It is easy to read, consistent with the body of the paper and contains the main keywords. However, their phylogenetic bootstrap test did not reveal any patterns which they describe with circumlocution (Figure 2).

The structure of compound leaves provides flexibility for morphological change by variation in the shapes, sizes and arrangement of leaflets. Here, **we conduct** a multilevel analysis of shape variation in compound leaves to explore the developmental plasticity and evolutionary potential that are the basis of diversification in leaf shape. **We use the methods** of geometric morphometrics to study the shapes of individual leaflets and whole leaves in **20 taxa of *Potentilla*** (sensu lato). **A newly developed test** based on the bootstrap approach suggests that uncertainty in the molecular phylogeny precludes firm conclusions whether there is a phylogenetic signal in the data on leaf shape. For variation among taxa, variation within taxa, as well as fluctuating asymmetry, **there is evidence** of strong morphological integration. The patterns of variation are similar across all three levels, **suggesting** that integration within taxa may act as a constraint on evolutionary change

Figure 2. Abstract of Klingenberg et al's (2012) paper follows the general outline of context (green), their approach (blue), describing their methods (yellow), main findings (purple) and conclusion (dark blue). The abstract is clear and concise but they describe their non-significant results with circumlocution (underlined).

Introduction critique

This section begins with a broad overview of the topic (e.g. diversity of leaves), citing seven papers about leaf variation on a single plant and the role of mutations. Then the writers focus on compound leaves and cite five papers about the regulation and causation of their forms. The gap in scientific knowledge is identified about the evolution of compound leaves.

A brief overview introduces the range of morphometric methods used for leaf shape studies mentioning 11 references about using landmarks for outline analysis and geometric morphometrics (Jensen et al., 2012; Jones, 1992; Benschmihen et al., 2008), leaf radian measurements (Meade and Parnell, 2003), 3D allometric space (Langlade et al., 2005), and LeafAnalyser software (Weight et al., 2008). This overview is not critical about the cited methods. A gap is identified in compound leaf morphometric studies compared to the number of single leaf studies. The approach to compound leaf analysis and potential characters are described which again justifies their choice of subject. They describe the causation and importance of fluctuating asymmetry in plant leaf development using four references, two of which is by Klingenberg (Klingenberg, 2003; Klingenberg and Gidaszewski, 2010).

In the last sections they introduce studies done on animals with hypotheses and predications (Figure 3) and point out that these questions have not been studied for plants before. Hence, this is novelty of their research. A detailed description of their approach is given, describing the levels in their multi-level approach, what is their study organism, what are their questions and their topic for discussion (Figure 2). There is no outline of their major findings at the end of the introduction. The total of 36 references were cited, out of which 14 is by Klingenberg or Kim. This suggests that they have extensive experience in this subject.

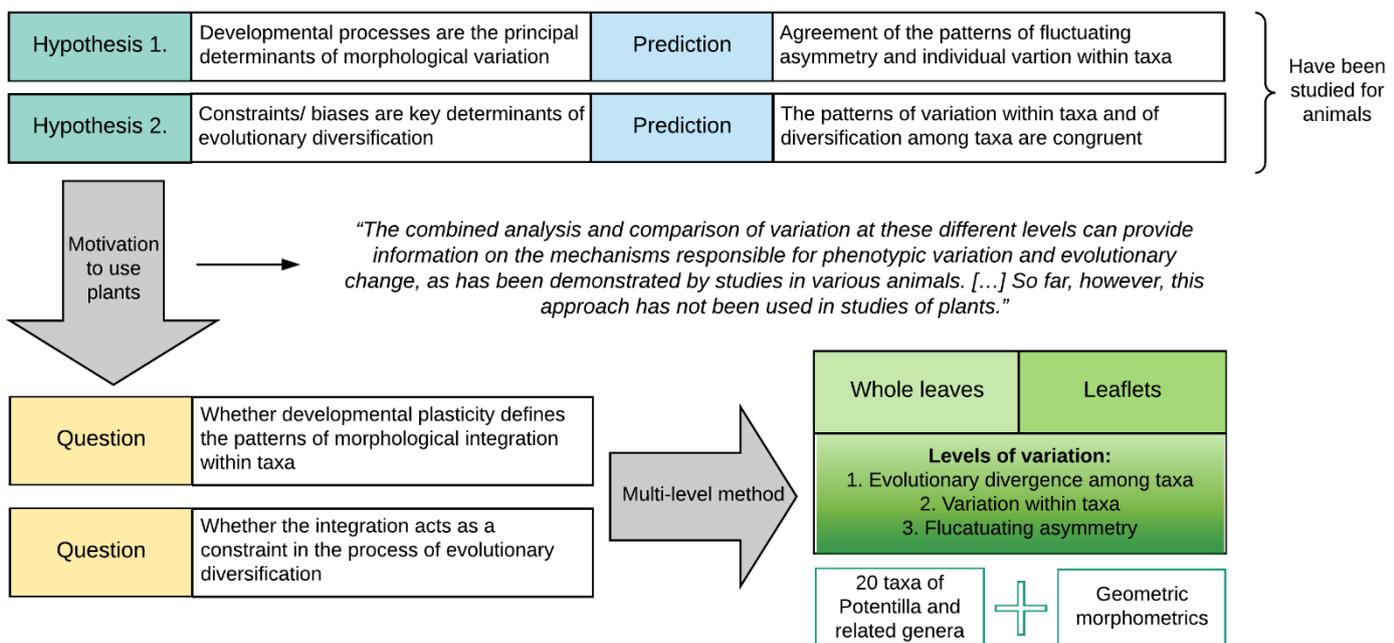


Figure 3. The introduction section describes their hypotheses and predictions based on previous studies done on animals, they clearly state their questions and outline their methods and approach clearly.

Materials and Methods critique

This sections begins with describing the data collection in the three botanic gardens. Selection of samples (what plants, which leaves) is precisely described which is crucial for justifying uniform sampling. They report finding two cultivars and a synonym taxa within their samples. Then landmark positions are clearly described both in text and on illustration which is required to be reported for landmark-based studies (Viscosi and Cardini, 2011). However, they fail to mention the software itself they used for the landmark digitization (tpsUtil and tpsDig). This would not adhere the repeatability of the study as the positions are well defined but the chosen reference object and positioning the camera and lights would enhance the repeatability of the study. The number of landmarks, 25 and 15, are appropriate for analysing 200 images considering four degrees of freedom are lost during the GPA (Viscosi and Cardini, 2011; Goodall, 2001). The 20 taxa is detailed in the Supporting Information (SI) file – which shape data was obtained from which taxa. However, SI also reveals that 14 taxa came from Edinburgh, three from Kew and three Freiburg botanical gardens raises the question if there was enough randomization to choose these plants as morphological representatives of each taxa. If each taxa would have been collected at separate locations, the mean shape would have been more representative and not location specific. Also, a control group (unrelated taxa) is missing from the data set which would have been useful to visualise on the graph outputs.

In a separate subsection, obtaining the phylogenetic data is described. The nature of *Potentilla* genetics is discussed briefly. This description could have been included in the introduction to give background information to the study organism. The methods of obtaining the genetic information is highly redundant in this section. There is no mention of their sample preparation, DNA extraction method, PCR and sequencing or any references to justify not mentioning these. Within the text a five

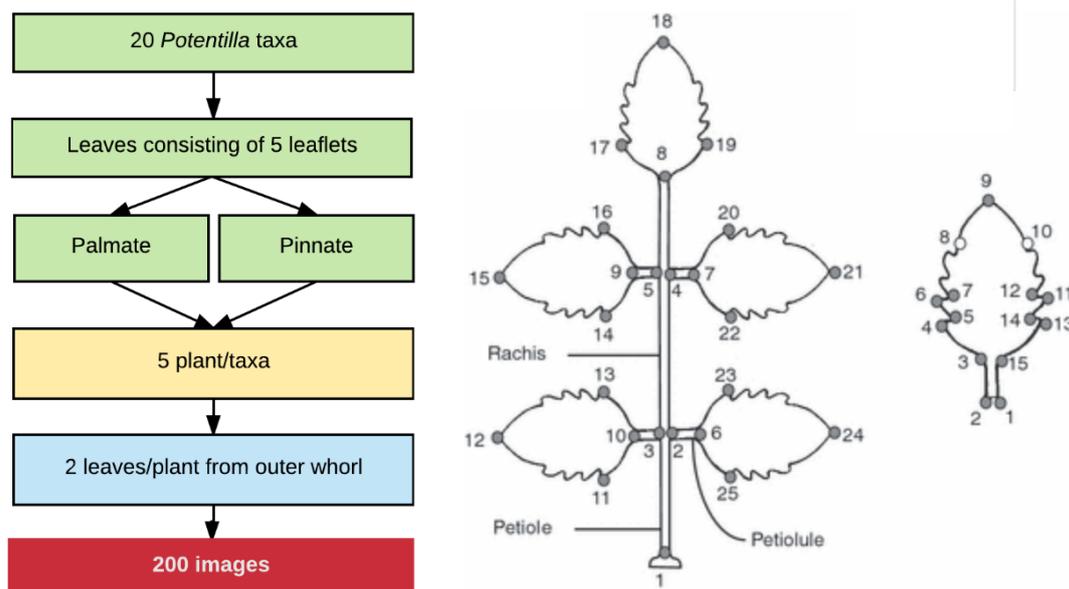


Figure 4. The experimental design, samples (right) and landmarks (left) are clearly defined in the Methods and Materials section.

PCAs on different levels is clearly written and in a logical way. By following these methods the analysis can be easily repeated in MorphoJ. The method is justified to have appropriate statistical power. Fourteen out of the total of 42 citations were from Klingenberg in this section implies that they in terms of the methods, the authors used more information from other studies than in the introduction.

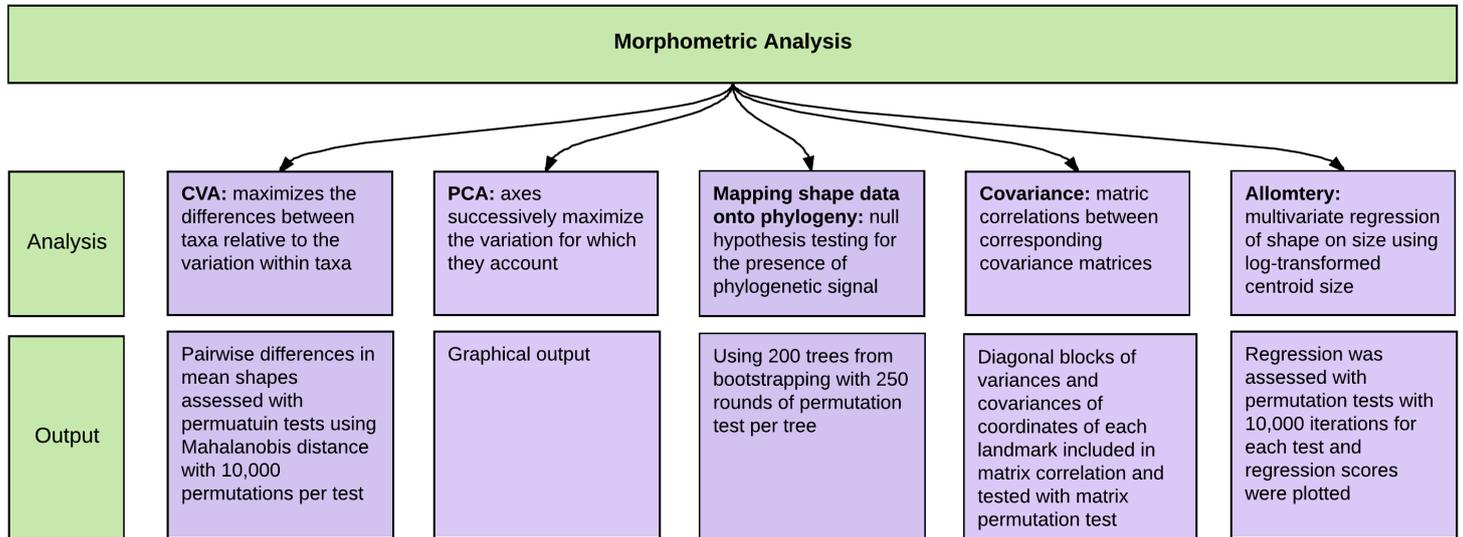


Figure 6. The methods used for morphometric analysis and their output. Text is quoted directly from the text on the graph. This flowchart would have helped the reader to see a concise overview of all the analyses used in this paper.

Results critique

The morphometric analysis of whole leaves is described precisely, describing the Mahalanobis distance ranges which is part of descriptive statistics, reporting P-values obtained from the analyses described in the Methods section. However, the total number of samples is not reported even though it varied among different analyses. The results of the CVA is described in terms of what morphological characters can be found on the axes, which can be visualised really effectively in MorphoJ, however the authors neglect to include the explained variance percentages of total variation that CV axes represent. The output of the phylogenetic analysis is ambiguously reported. There is no description which taxa's branch is weak and questionable exactly (Figure 7). Mapping the phylogeny into the morphospace produced by the morphometric analysis of the whole leaves did not reveal any predicted patterns. The P-value of this analysis is 0.059 which is clearly not statistically significant but the authors aim to justify it. Also, using their original data, following their methods of exporting and performing the phylogeny mapping, the output is not identical to the ones used in the paper (Appendix 1).

In terms of reporting PCA results, they are mentioning the explained variance percentages in an ambiguous way such as '*PC1, which account for under half the total variance*', and not reporting eigenvalue distribution.

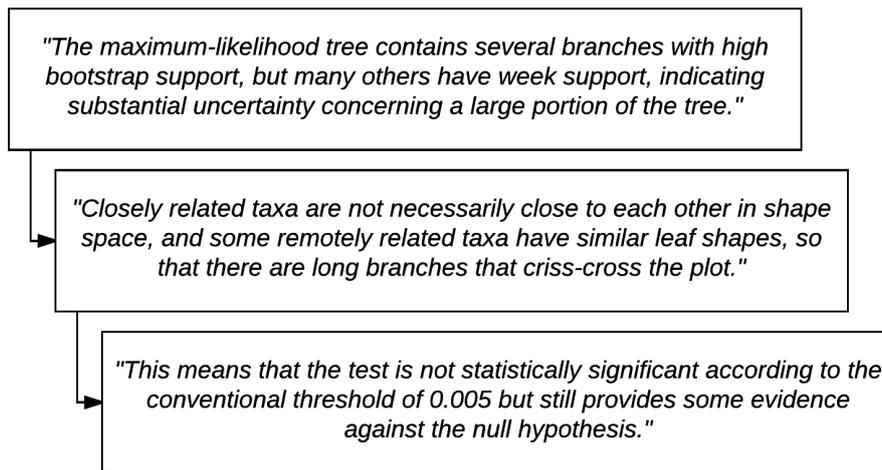


Figure 7. The phylogenetic signal is ambiguously reported. The non-significant results instead of being clearly reported, are attempted to be explain with circumlocution.

Discussion critique

The discussion begins a summary of the results and contrasting the study's findings to other published research (Figure 8). The most words in this section is used to reflect on their non-significant results for finding a phylogenetic signal. The statistical power is justified but the *'results of the permutation test for the ML tree for the original sequence data should therefore be interpreted with caution'*. Each subsection of the results is interpreted. After describing the patterns of fluctuating asymmetry, the discussion considers the implications of their finding in biological terms and compare their findings to previous studies. In the last paragraph, developmental genetic studies and the mechanism of leaf morphology regulatory systems are examined finishing with acknowledging a limitations as *"it is premature [...] to invoke specific changes in leaf shape within or among species"* and suggestion for future studies, *"studies that combine specific genetic changes with the quantitative analysis of shape are a step in the direction"*. The discussion is missing one of Vintzileos and Ananth's (2010) points that are crucial to be included in the discussion which is a *'clear and concise conclusion of the meaning of the study as it relates to practice or future research'*.

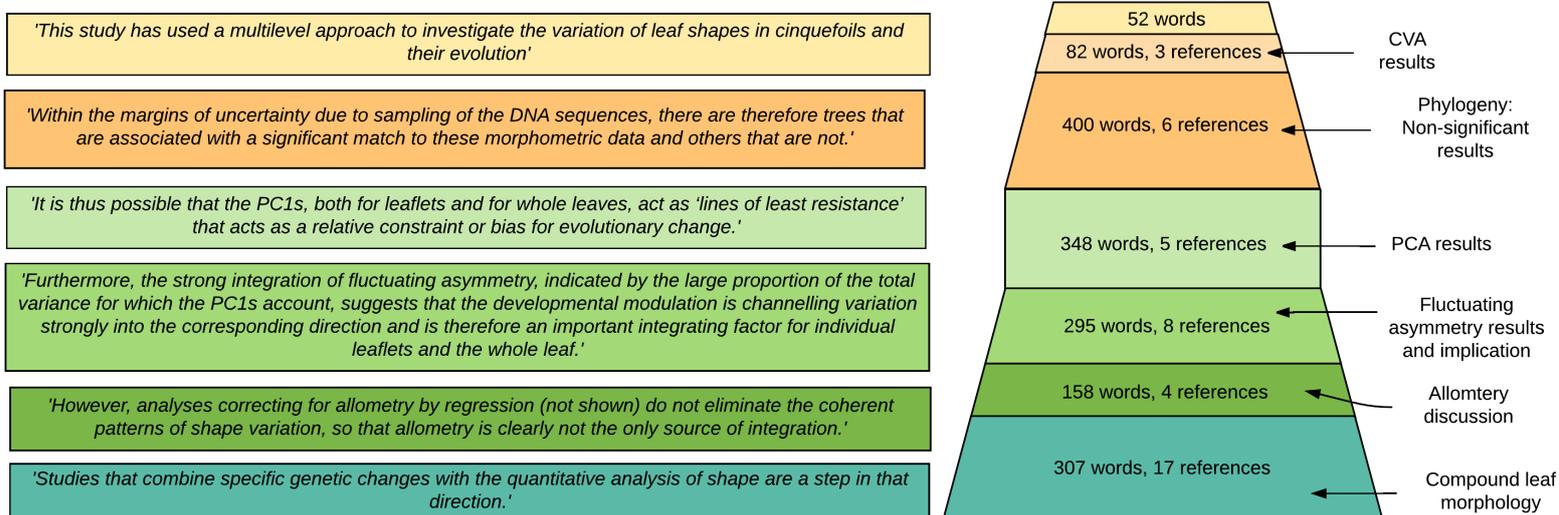


Figure 8. Structure of the discussion showing the amount of words and citations used for different paragraphs. The width of the graph on the right represents how broadly discussed the sections are and the quotations on the left are examples from the text.

Illustrations critique

There are a total of 21 graphs and one illustration in this paper which makes the article easy to follow and understand. The number of graphs can also emphasize the advantage of using geometric morphometrics – all shape variation can be calculated and visualised. The captions explain the graphs precisely and the article's findings can be understood by only looking at graphs. The ambiguous way of describing PCAs within the text can be excused, as the percentages of the total variance explained by the axes is illustrated on the graphs along with this the leaf shape changes. Graphs produced by mapping the morphospace onto the phylogeny are also clearly labelled, the taxa names are not over-stacked.

Conclusion

The chosen article by Klingenberg et al. (2012) follows the general outline of a scientific article in a logical order but describes the morphometric analysis in more detail than the phylogenetic analysis. Methods used for obtaining the five loci information is redundant and although they state their assumptions, they do not discuss the importance of conducting a study of a more genetically stable plant group.

I recommend a longer, more critical literature review for this paper as this factor was shown to be crucial for high impact articles (Sun and Linton, 2014; Figure 9). They only deter from the 10 paper structuring rules (Mensch and Kording, 2017) by not reporting their results at the end of the introduction and in the discussion they do not follow the 'Our strength', 'What it is useful' and 'The difference made' ending but disperse these points among different paragraphs.

Overall, this paper gives an insight what tests can be used in MorphoJ software rather than presenting significant results in terms of evolution of the compound leaf shape. For example, Viscosi et al. (2012) and Openshaw and Keogh (2014) follow the same methods in MorphoJ for plant ecotype and lizard head shape evolution studies.

One of highlighted novelty in Klingenberg et al.'s (2012) study is to use compound leaves. However, to test if entire leaves morphology has a phylogenetic signal in MorphoJ, *Passiflora* genus could be an appropriate choice. The genus consists of morphologically different leaves and both landmark and EFA data is available from Chitwood and Otoni (2017) and the clades' phylogeny is consistent with their flower's size (Muschner et al., 2003; Yockteng and Nadot, 2004), which could potentially lead to significant results using the same methods as Klingenberg et al. (2012).

Another impactful study by Chitwood et al. (2012) was similar about leaf shape development and evolution, with a multi-level Elliptic Fourier Analysis (EFA) for three *Solanum* wild tomato leaflets from 726 plants. They showed that proximal, distal and terminal leaves respond in a different way in terms of their outline to environmental variables and there are heteroblastic differences as well. This research gives more insight to developmental plasticity and the underlying mechanisms but lack the phylogenetic aspects.

In order to study overall morphological integration, single leaf, compound leaf and flower morphology should be collected for a genetically stable group where gene regulation is well researched. The use of a combination of different software and data, not only using MorphoJ software and landmark-based data, expands the possibilities of analyses and this paper is a stepping stone and motivation for future studies.

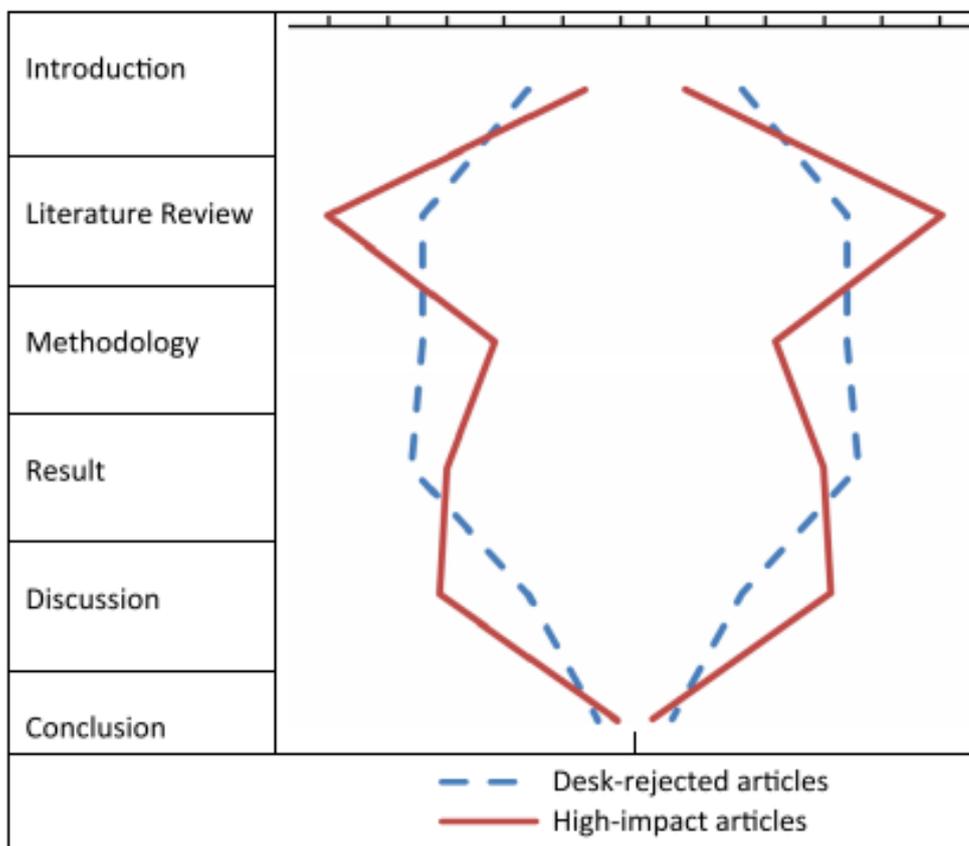
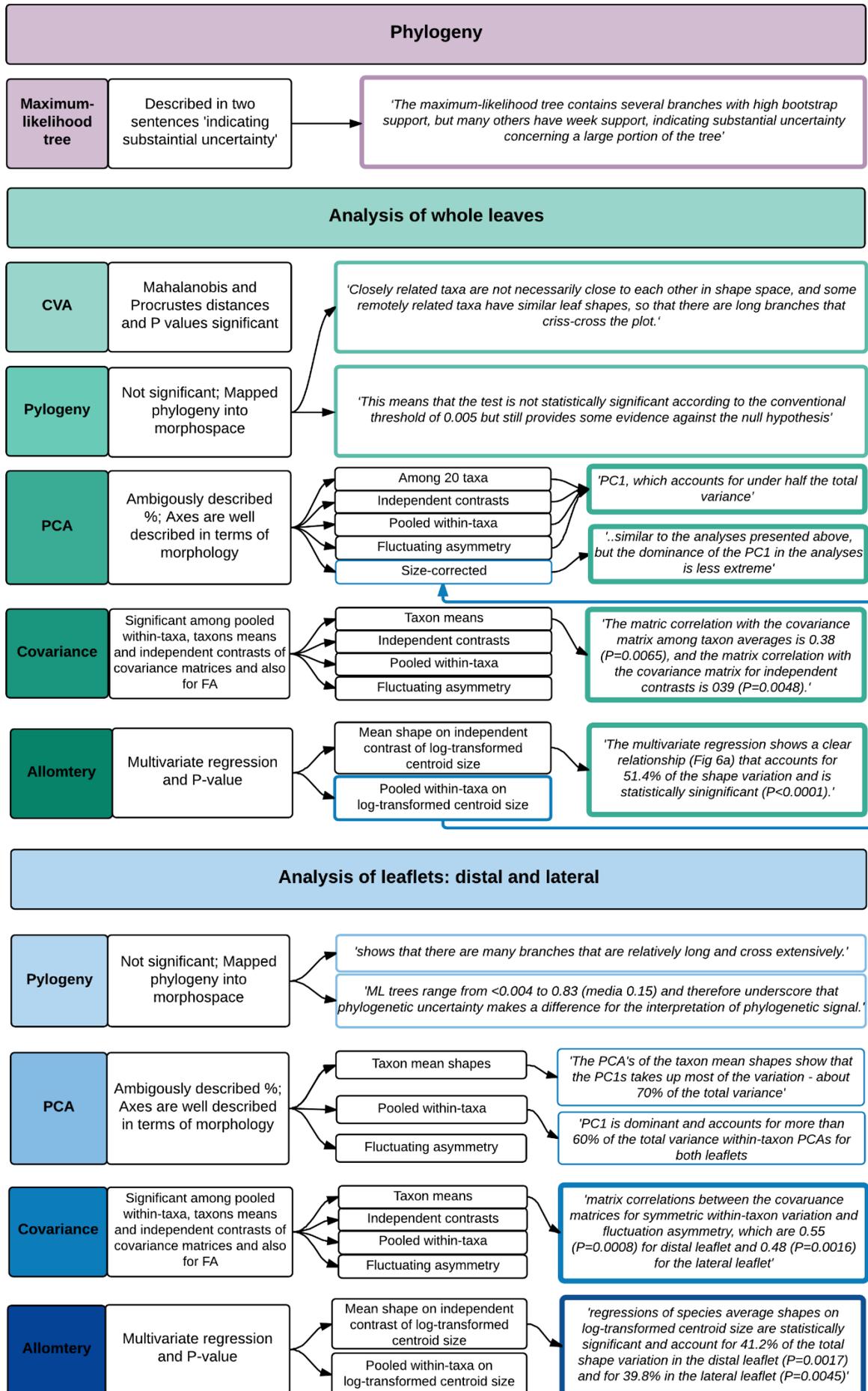


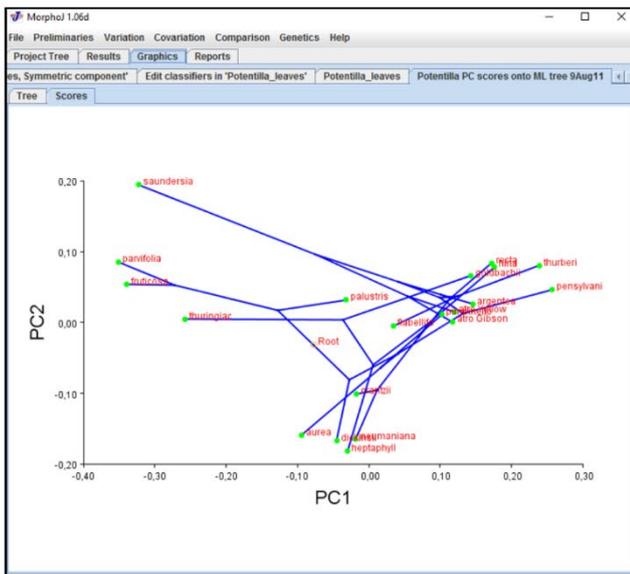
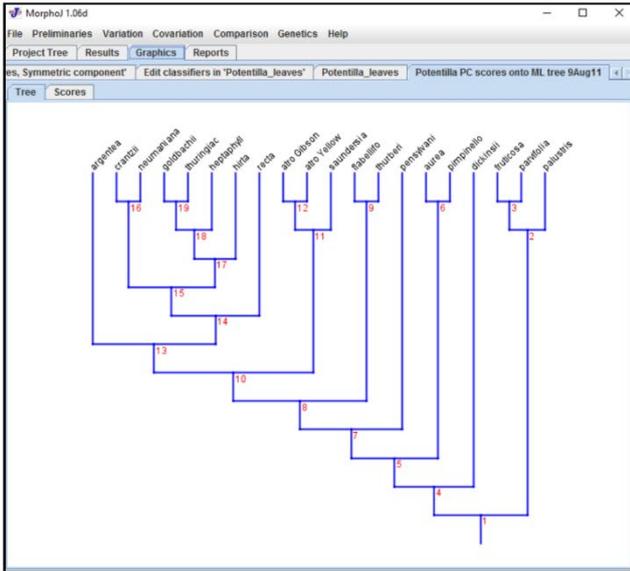
Figure 9. Different average structures compared of desk-rejected papers (blue) versus high-impact papers (red) by Sun and Linton (2014). The horizontal axis represents percentages of relative number of words for each section.

Appendix

Appendix 1. Complete overview of levels of analyses, different data sets and the results reported in the text.



Appendix 2. Re-running the shape mapping onto the phylogeny in MorphoJ using the original data set produces different graph output than reported by Klingenberg et al. (2012).



A screenshot of the 'Regression analysis' dialog box in MorphoJ 1.06d. The 'Name for the analysis:' field contains 'Regression: Potentilla ICs of shape onto ICs of logCS'. The 'Dependent variable(s):' field is empty. The 'Independent variable(s):' field contains 'IndContrasts: Potentilla_Leaves'. The 'Datasets:' section lists 'IndContrasts: Potentilla_Leaves' and 'IndContrasts: Potentilla_Leaves'. The 'Data matrices:' section lists 'Centroid size', 'Symmetric component', and 'Asymmetry component'. The 'Variables:' section lists 'Symmetric component', 'Centroid Size', and 'Log Centroid Size'. The 'Perform permutation test' checkbox is checked, and the 'Number of rounds:' is set to 10000. The 'Execute' button is highlighted.

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