

# *The Cinderella discipline: morphometrics and their use in botanical classification*

Article

Accepted Version

Christodoulou, M. D. ORCID: <https://orcid.org/0000-0003-0968-2311>, Clark, J. Y. and Culham, A. ORCID: <https://orcid.org/0000-0002-7440-0133> (2020) The Cinderella discipline: morphometrics and their use in botanical classification. *Botanical Journal of the Linnean Society*, 194 (4). pp. 385-396. ISSN 0024-4074 doi: 10.1093/botlinnean/boaa055 Available at <https://centaur.reading.ac.uk/91282/>

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To link to this article DOI: <http://dx.doi.org/10.1093/botlinnean/boaa055>

Publisher: Oxford University Press

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1 **REVIEW**

2 TITLE: The Cinderella Discipline: Morphometrics and their use in botanical classification

3

4 SHORT TITLE: Morphometrics and their use

5

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17

18 **ABSTRACT**

19

20 Between the 1960s and the present day, the use of morphology in plant taxonomy  
21 suffered a major decline, in part driven by the apparent superiority of DNA-based  
22 approaches to data generation. However, in recent years computer image  
23 recognition has re-kindled the interest in morphological techniques. Linear or geometric  
24 morphometric approaches have been employed to distinguish and classify a wide  
25 variety of organisms; each has strengths and weaknesses. Here we review these  
26 approaches with a focus on plant classification and present a case for the  
27 combination of morphometrics with statistical/machine learning. There is a large  
28 collection of classification techniques available for biological analysis and selecting the  
29 most appropriate one is not trivial. Performance should be evaluated using  
30 standardised metrics such as accuracy, sensitivity, and specificity. The gathering and  
31 storage of high-resolution images, combined with the processing power of desktop  
32 computers, makes morphometric approaches practical as a time- and cost-efficient  
33 way of non-destructive identification of plant samples.

34

35 Keywords: Plant taxonomy, geometric morphometrics, linear morphometrics, statistical  
36 learning, machine learning, identification, classification, neural networks.

37

38 In his keynote address during the 50<sup>th</sup> anniversary of botany MSc training at the  
39 University of Reading, Prof Vernon Heywood described a steady decline in the state of  
40 botany teaching in the UK with a resulting loss of skills in the next generation of scientists.  
41 With few institutions in the country offering training for young botanists, more and more  
42 researchers enter plant taxonomy through the field of molecular systematics, never  
43 learning the classic skills of a traditional botanist. Although great progress has been  
44 made in the development of molecular tools, increasing the insight gained from  
45 laboratory methods, what used to be the beating heart of botany - morphology - has  
46 lost some of its appeal. In our view this is because morphological data coding cannot  
47 readily be made into a clear data generation pipeline in the same way as much  
48 molecular data can. We believe this to be because morphology requires more in-  
49 depth knowledge and understanding of the organism prior to data collection than is  
50 required for DNA sequencing and that morphological variation is open-ended rather  
51 than with a fixed range of states as in DNA data. Whilst morphological data have lost  
52 favour in the construction of plant classification systems they have gained popularity in  
53 the study of evolution from variation in gross morphology of the centropogonid clade  
54 (Lobelioideae: Campanulaceae) (Lagomarsino *et al.*, 2017), speciation despite  
55 consistent floral morphology in *Myrcia* DC. (Vasconcelos *et al.*, 2019) though to  
56 detailed morphometric analysis of traits related to environment in *Vriesea* Lindl.  
57 bromeliads (Neves *et al.*, 2020).

58

59 The power of some of the more modern developments in morphometrics and statistical  
60 learning however can provide botanists with an extra toolbox to help them describe  
61 and quantify the variation that surrounds them. In this review we aim to make a case  
62 for the value of morphometrics, especially in combination with more sophisticated  
63 statistical methods, in a botanist's analytical toolbox - not to replace molecular  
64 techniques but to add to them. Morphology is often one of the most directly accessible  
65 and intuitive data sources for taxonomic research. In botanical taxonomy,

66 morphological characterization is the foundation of taxon description and  
67 identification, albeit often found in the formal and stylised format present in Floras and  
68 monographs. There is an opportunity for modern botanical taxonomy to explore the  
69 rapidly advancing field of morphometrics which already has some notable examples  
70 ranging from automatic leaf outline identification of *Passiflora* L. species (De Oliveira  
71 Plotze & Martinez Bruno, 2009), the tooth margin algorithm for *Tilia* L. leaf identification  
72 (Corney *et al.*, 2012), and the use of leaf venation architecture for major angiosperm  
73 clade recognition (Wilf *et al.*, 2016). Some computerised systems, starting with an  
74 existing classification of taxa, can use machine learning to handle the routine  
75 identification work, and then refer intransigent problems to a human expert (Clark,  
76 Corney, & Wilkin, 2017).

77

78 One of the principal arguments presented against morphological data is the potential  
79 for high levels of ambiguity. This ambiguity can be caused by a variety of factors such  
80 as inaccurate character definition (Assis, 2009) and difficulty in establishing homology  
81 (Schneider, Smith, & Pryer, 2009). Morphological data collection can be further  
82 complicated by plasticity of features (Perkins, Martinsen, & Falk, 2011), homoplasy  
83 (Schneider *et al.*, 2009), low numbers of characters (Giribet, 2010), and missing  
84 character states (Jenner, 2004). In some organisms such as parasites, reduced body  
85 plans can make characterisation of features even more difficult and lead to a very  
86 limited dataset (Perkins *et al.*, 2011). These concerns are neither exaggerated nor trivial  
87 and many are thoroughly discussed in the morphological literature. They do not,  
88 however, necessarily imply a lower quality of data produced by morphological work in  
89 comparison with other data sources (Jenner, 2004).

90

91 As most botanical researchers are question-driven rather than method-driven, we have  
92 structured our recommendations using general outlines on what kind of questions each  
93 combination of morphological and statistical tools can answer, with the aim of

94 promoting more thorough morphological investigation in botanical research. We have  
95 split this into two sections - Developmental hypotheses and Classification hypotheses.  
96 Under Developmental hypotheses we include all studies that may require the  
97 description of shape or size of a plant either to compare between treatments or to  
98 study how characters change along a particular gradient. For these we give an outline  
99 of morphometric tools available. Under Classification hypotheses we include all studies  
100 where the researcher is asking questions of taxon membership (e.g. are these two  
101 groups in the same taxon?) or questions of identification (e.g. what is the minimum set  
102 of diagnostics to accurately identify a sample?). These also require morphometric tools,  
103 such as those described under the developmental hypotheses, but can be taken  
104 further by combining them with machine learning techniques. There is a difference in  
105 terminology between the use of the word classification in biology and in computer  
106 science. Although the term is clearly defined in a taxonomic setting as the formal  
107 structure in which taxa are placed, in machine learning it means something much  
108 more general: it is the attribution of objects to a particular group. This is why  
109 identification in the machine learning context falls under classification, and therefore is  
110 included here under Classification hypotheses.

## 111 **DEVELOPMENTAL HYPOTHESES**

112 Plant growth and development studies already rely heavily on morphological  
113 measurements - size for example is often included as a proxy to an organism's  
114 developmental stage. These studies often focus on examining how the organism  
115 changes as it progresses through the various life stages. These could range from  
116 progression from seed to flower for an annual, or even development of fruit on a tree  
117 during the growing season.

118

119 Even though it is very commonly used, size itself is a complex and often unappreciated  
120 concept. Often researchers fail to explore the separation between shape and size,  
121 confounding the two and losing some of the clarity that can be obtained through their  
122 investigation. For Developmental hypotheses, we argue that the crucial point for insight  
123 is not the separation of size and shape just for the sake of it - it is for the researcher to  
124 either knowingly combine them or distinguish between the two based on the  
125 hypothesis in question. We believe that by cautiously selecting measurements that do  
126 not distinguish shape from size, a researcher can gain insight on changes in either size  
127 or shape during a developmental process based on how they use them. For example,  
128 the length of apple fruit through the growing season, plotted against time from anthesis  
129 can give insight on how size develops as time progresses (Atay, Pirlak, & Atay, 2010).  
130 The ratio between length and width for the same fruit provides an indication of the  
131 development of shape (Bollard, 1970). Both length and width independently are size  
132 metrics, but in combination they describe shape.

133

134 In the context of describing morphology, there are two mainstream methods: linear  
135 and geometric morphometrics. An essential distinction between them is that linear  
136 morphometrics do not actively separate size from shape, whereas geometric  
137 morphometrics do. We have structured the remainder of this section to describe these  
138 two techniques and have illustrated them using biological examples.

139

140 The traditional approach to morphometrics involves the measurement of distances  
141 between points deemed to be characteristic of shape and form. Measurements such  
142 as height, length, width, and diameter all fall under the general categorisation of **linear**  
143 **morphometrics**. These measurements are intuitive, easy to understand and to interpret,  
144 and have been in the biological toolbox for as long as the toolbox itself has existed.  
145 Linear morphometrics are quick to collect, low cost, easy to interpret, and often  
146 sufficient for biological description. Sanchez et al. (2011) compared the growth  
147 development of baobab seedlings of different origins using a variety of morphometric  
148 measurements ,such as length and diameter of roots, to establish that plants originating  
149 from drier environments grew to a smaller size even under optimal greenhouse  
150 conditions. Richardson et al. (2011) the studied fruit development patterns of kiwi from  
151 anthesis to ripening using amongst other character a collection of linear  
152 morphometrics, such as pericarp diameter. Zhang et al. (2015) performed a  
153 comparative study of the developmental patterns of Sweet cherry floral parts, using  
154 linear measurements such as pedicel length, establishing a correlation between floral  
155 morphology and environmental conditions during growth such as temperature.

156

157 For morphometric studies that require the description of very subtle shape characters,  
158 linear morphometrics may not be the most appropriate tool. The reason for this is  
159 because distance measurements, although excellent for summarising shape and size  
160 descriptions, often lack context. To correct for this, more linear measurements can be  
161 collected, creating a more complete dataset for each object. When the shape of  
162 interest is of biological form, it becomes crucial to be able to establish and quantify  
163 even the subtlest of differences. To be able to achieve this through linear  
164 morphometrics would involve an extensive collection of measurements and a  
165 generous amount of luck, as one may simply fail to measure the precise point where  
166 differences between taxa occur. Furthermore, within an evolutionary framework it is

167 more appropriate to view form as a whole since organisms evolve as a whole. To  
168 counter these concerns, morphometric theory progressed to what is often described as  
169 modern morphometrics, more accurately known as geometric morphometrics.

170

171 **Geometric morphometrics** allow the study of the shape of an organism as a whole,  
172 rather than as a collection of separate components. In contrast to linear  
173 morphometrics, by studying all the selected landmarks of a sample together, even  
174 subtle changes in geometry can be quantified and analysed using geometric  
175 morphometrics. Kendall's shape definition forms the basis of geometric morphometrics  
176 (Zelditch *et al.*, 2004). This clear separation of shape from position, orientation and size  
177 corresponds to an intuitive concept of shape. In practical terms, to achieve this  
178 separation there is a strong analytical reliance on multivariate techniques (Klingenberg  
179 & Monteiro, 2005). The way this is performed in geometric morphometrics is through the  
180 use of landmark coordinates (Van Bocxlaer & Schultheiß, 2010). A landmark is a  
181 recognizable point on the organism that, together with other landmarks, can be used  
182 to summarise the form of the organism (Zelditch *et al.*, 2004). As opposed to focusing  
183 on distance measurements, as is done in linear morphometrics, shape is summarised  
184 through the Cartesian coordinates of selected landmarks (Walker, 2000). By always  
185 analysing these coordinates together in a multidimensional space, shapes can be  
186 scaled, moved and rotated without losing any information (Goodall, 1991). Although  
187 the selection of appropriate landmarks can be difficult, this multivariate approach  
188 provides great flexibility for manipulation and statistical analysis.

189

190 After landmark selection, the recording of coordinates for all samples (a process  
191 referred to as "sample digitisation") creates the initial dataset to be used for analysis.  
192 The samples in this dataset are not, however, comparable if their coordinates have not  
193 been standardised. This is because regardless of how carefully and methodically  
194 digitisation occurred the samples are bound to not be fully aligned. Furthermore,

195 differences in sizes between samples will affect the position of the landmarks on the  
196 Cartesian axes, confounding shape comparison. To correct for this, the samples can be  
197 standardised using a Procrustes superimposition (Rohlf & Slice, 1990). Named after the  
198 mythical ancient Greek bandit who trimmed or stretched his victims to fit an iron bed,  
199 the process superimposes the samples using the landmarks to correct for orientation  
200 and alignment (Stegmann & Gomez, 2002). It then proceeds to stretch or shrink some  
201 samples aiming for all samples to be perfectly superimposed (Zelditch *et al.*, 2004). We  
202 have illustrated the steps of this process in Figure 1.

203

204 Selecting appropriate landmarks to summarise a shape is perhaps the most crucial  
205 aspect of geometric morphometrics. The reason for this is that if the choice of  
206 landmarks is poor, then any subsequent analysis will reflect that. Through the process of  
207 landmark selection, the overall shape of the organism in question is summarised using a  
208 small number of representative landmarks. Selecting representative landmarks is a  
209 subjective exercise that relies on in-depth knowledge and understanding of anatomy  
210 and biology of the organism in question. This is because not all landmarks are created  
211 equal. A wisely chosen landmark can summarise shape appropriately and provide  
212 adequate information for biological inference. A poorly selected landmark will at best  
213 add high levels of noise to the dataset or, at worst, result in misleading patterns.

214

215 Ideally, landmark selection requires four criteria that ensure quality: **repeatability**,  
216 **consistency of position, adequacy** and **homology** (Zelditch *et al.*, 2004). **Repeatability**  
217 refers to the potential of locating the selected landmark accurately on a specimen  
218 multiple times (Zelditch *et al.*, 2004). If a landmark is difficult to locate or its position is  
219 relatively vague, then samples that have no significant biological differences may be  
220 found to be different as an artefact of poor landmark choice. **Consistency of position**  
221 refers to the relative positions between landmarks (Zelditch *et al.*, 2004). If two  
222 landmarks switch relative positions between different specimens then their comparison

223 can lead to statistical outliers that may affect the findings and analyses. **Adequacy**  
224 refers to the number and position of landmarks used to summarise a form (Zelditch *et*  
225 *al.*, 2004). Although more is not always better in terms of landmark selection, including  
226 too few landmarks will not lead to a representative dataset. Even though repeatability  
227 can be quantified, and consistency of position detected, adequacy is a harder  
228 criterion to evaluate. This is because adequate coverage can be highly subjective. The  
229 concept relies on finding the golden mean between oversampling the specimen  
230 (where too many landmarks can lead to higher noise levels in the dataset) and  
231 undersampling (losing possible detectable variation between specimens).

232

233 **Homology** in landmark selection has both geometric and biological aspects. Two  
234 landmarks are considered homologous in two specimens if there is a degree of  
235 correspondence between them. This correspondence can be purely a geometric  
236 attribute (e.g. the tips of the Giza pyramids are geometrically homologous) or a  
237 biological attribute (e.g. the forelimbs of bats and primates). Although all four criteria  
238 are important for landmark quality, establishing homology is crucial. It is only through  
239 the use of homologous landmarks that the shapes studied are truly comparable. If the  
240 landmarks used are not homologous between the organisms in the study then there is  
241 no logical support for their comparison and the results can be highly misleading  
242 (Klingenberg, 2008). Although homology is considered one of the most crucial aspects  
243 in landmark selection, exactly how it can affect a given study depends on the nature  
244 and scope of the study itself. In general, the ability to identify homology can severely  
245 limit the quantity of potential landmark candidates.

246

247 These constraints imposed by homology increase the popularity of outline methods of  
248 analysis (Macleod, 1999). By replacing homologous landmarks with regularly spaced  
249 points along a curve, outline analysis sidesteps the issue of homology and can be used  
250 in cases where landmarks are sparse or hard to define (Macleod, 1999). Outline data

251 can then be analysed using Fourier harmonics (or possible variations such as Elliptical  
252 Fourier) or Eigenshape analysis (Macleod, 1999; Bonhomme & Claude, 2014). Although  
253 outline analysis is a popular and successful alternative to landmark analysis, the  
254 assumption that it bypasses homology issues may be misplaced. The reason for this is  
255 that outline methods are not completely independent of landmark correspondence  
256 assumptions (Klingenberg, 2008). That is because as with landmark methods, outline  
257 coordinates require a superimposition technique, such as Procrustes superimposition,  
258 prior to analysis (Bonhomme & Claude, 2014). This means that the outline points that  
259 are recorded are treated as actual homologous landmarks. This may appear minor,  
260 but as the superimposition process assumes a certain correspondence between points  
261 on the outline, it can result in increased levels of noise in the dataset. Furthermore,  
262 analytical approaches such as Elliptic Fourier Analysis also assume a certain degree of  
263 homology between outline points. It can therefore be argued that the principal  
264 difference between the two approaches is that in landmark analysis the homology  
265 criterion is explicit whereas in outline analysis it is implied and often ignored.

266

267 The choice between linear and geometric morphometrics for an analysis is not trivial as  
268 one technique is not necessarily superior to the other. Linear morphometrics are quick,  
269 intuitive and cost effective and often robust enough to not introduce noise in the  
270 analysis. They fail when separation of shape and size becomes important and when  
271 subtle changes in morphology are crucial - this is where geometric morphometrics  
272 excel. Selecting the appropriate method for the question in hand is always a  
273 challenging aspect of scientific discovery, although familiarity with both methods,  
274 combined with understanding of the studied organism helps when deciding which  
275 technique may provide more insightful findings. As a final point, it is not always  
276 necessary to choose one over the other, for example, Christodoulou et al. (2018)  
277 combined linear and geometric morphometrics to describe shape differences  
278 between apple cultivars with greater accuracy.

## 279 **CLASSIFICATION HYPOTHESES:**

280 Although classification in biology has a different meaning than in machine learning (a  
281 subset of statistical learning), this collection of hypotheses relies on grouping objects  
282 based on similarities between measured characters. These can include studies of  
283 morphological similarities between geographically distinct populations, segregation  
284 between species and hybrids, or revision of taxonomic limits.

285

286 Both linear and geometric morphometrics have been used for such studies. Compton  
287 and Hedderson (1997), in their taxonomic revision of the limits of *Cimicifuga foetida* L.  
288 s.l. (now *Actaea cimicifuga* L.), included 17 length variables, resulting in the detection  
289 of four geographically distinct species. Blanco-Dios (2007) used multivariate analysis of  
290 17 linear morphometric characters to contrast the morphology of hybrid populations  
291 between *Armeria beirana* Franco and *A. pubigera* (Desf.) Boiss. with that of their  
292 progenitors, detecting clear differences between the groupings. Da Costa et al. (2009)  
293 used distance measurements for both vegetative and reproductive parts to study the  
294 variation within the *Vriesea paraibica* Wawra complex. After statistical analysis, they  
295 proceeded to recognise four species within the complex (*V. paraibica*,  
296 *V. interrogatoria* L.B.Smith, *V. eltoniana* E.Pereira & Ivo, and *V. flava* A.F. Costa, H.  
297 Luther & M.G.L. Wanderley), for which they provided a taxonomic treatment. Returning  
298 to the genus *Actaea* L., Gardner et al. (2012) used linear morphometrics to quantify the  
299 variation within *Actaea racemosa* L., establishing that between-population variation  
300 was similar to within-population variation. In a study of the *Andropogon lateralis* Nees  
301 complex, Nagahama et al. (2014) used 19 linear morphometric measurements to  
302 successfully distinguish both species and hybrids within the complex. Shipunov and  
303 Bateman (2005) used geometric morphometrics to explore the diversity of lip shapes of  
304 *Dactylorhiza* Neck. ex Nevski orchids, studying both hybridization patterns and  
305 taxonomy in Russian populations. Volkova and Shipunov (2007) used similar tools to  
306 investigate the variation between three *Nymphaea* L. species in Russia and Siberia,

307 finding the species delimitation to be robust. Viscosi et al. (2009) successfully used  
308 geometric morphometrics on oak leaves to distinguish between four species. Savriama  
309 et al. (2012) presented a new methodology quantifying symmetry and asymmetry of  
310 corolla shape in *Erysimum mediohispanicum* Polatschek (now *Erysimum grandiflorum*  
311 subsp. *mediohispanicum* (Polatschek) Romo), establishing symmetry to be a  
312 fundamental character for floral variation within the taxon. Finally, Fernández-  
313 Mazuecos et al. (2013) used geometric morphometrics to study the role of flower  
314 specialisation for speciation in *Linaria* Mill. subsect. *Versicolores* (Benth.) Wetst. finding  
315 corolla tube differences to correlate with divergent pollination strategies. In a  
316 comparison of leaf shape of *Anacardium microcarpum* Ducke with *A. occidentale* L.  
317 using geometric morphometric descriptors, Vieira et al. (2014) established that  
318 although the leaves do present statistically significant differences, overlap between  
319 taxa and populations prevent them from being used as unique identifiers.

320

321 Analytically, methods from statistical/machine learning can offer great insight for this  
322 type of hypothesis. There are two broad sections in statistical/machine learning:  
323 supervised learning and unsupervised learning. We are excluding deep learning  
324 methods here, as the topic is too large for an adequate description within this review  
325 and the approaches are rather different. The review on the topic by Angermueller et  
326 al. (2016) offers a good overview of the major issues. Furthermore, deep learning is  
327 primarily aimed at processing huge amounts of multivariate data (so called 'big data'),  
328 and here we are more concerned with the utilisation of relatively small datasets, often  
329 with only a few data records per taxon, which is more realistic for consideration by  
330 practising botanists.

331

332 **Supervised learning** focuses on using combinations of characters to circumscribe  
333 known groups (classes) and then applying this knowledge to predict the class  
334 membership of an unknown sample (Tarca et al., 2007). This is essentially 'identification'

335 in the biological sense, if the classes represent named taxa. The classic example of  
336 supervised learning is Anderson's *Iris* dataset analysed by Fisher using Linear  
337 Discriminant Analysis (LDA) (Fisher, 1936). The original dataset contained measurements  
338 from 150 flowers belonging to three *Iris* species (50 flowers each of *I. setosa* Pall. ex Link,  
339 *I. versicolor* Thunb. and *I. virginica* L.), For each flower, length and width measurements  
340 of two tepals (one inner, and one outer tepal), as well as species, were recorded.  
341 When this dataset was analysed using LDA, discriminant functions were established for  
342 each species based on the lengths and widths of the tepals. These could then be used  
343 to establish the species of an unknown *Iris* sample using only length and width tepal  
344 measurements (provided it belonged to one of the three species). The factor that  
345 makes this example part of supervised learning is the prior knowledge of class  
346 membership, in this case *Iris* species, used for the design of the discriminant functions  
347 (Fogel, 2008).

348

349 **Unsupervised learning**, by contrast, has no prior knowledge of class membership, and  
350 the analysis aims to explore patterns in the data and create natural groupings (Fogel,  
351 2008). Such groupings can then be used as justification for delimitation of traditional  
352 ranked taxa such as species. This is essentially 'classification' in the biological sense.  
353 Cluster analysis (clustering), for example, is a case of unsupervised learning. Table 1  
354 summarises a selection of both supervised and unsupervised techniques, more  
355 extensive descriptions of which can be found in Appendix A.

356

357 Table 1 showcases botanical applications of machine learning. The combination of  
358 machine learning and morphometrics for classification has much more prominent  
359 examples outside of botany. We aim for this review to increase the uptake of these  
360 techniques in botany. In the meantime, we present some non-botanical examples here  
361 for illustration purposes. Santana et al. (2014) studied bee classification using the  
362 forewings of male members of five *Euglossa* species. This was performed by using 18

363 landmarks on the wing venation together with colour change variables, followed by  
364 comparisons between classification techniques including linear discriminant analysis  
365 and a modified neural network. The neural network outperformed the other classifiers,  
366 with an accuracy of 87.6%. da Silva et al. (2015) used more classes than Santana et al.  
367 (2014), studying 26 subspecies of *Apis mellifera* while still using the same 18 landmarks  
368 on wing venation. Their focus was on the performance of feature selection and their  
369 conclusion was that a Naïve Bayes classifier outperforms other classification techniques,  
370 with 65% mean accuracy on cross-validation (da Silva et al., 2015).

371

372 Van Bocxlaer and Schultheiß's (2010) gastropod study was one of the first in zoology to  
373 combine machine learning with morphometrics, their focus was primarily on comparing  
374 landmark analysis with outline analysis. For their gastropod dataset they found that  
375 outline analysis outperformed landmark analysis by 3%, reaching 78% accuracy when  
376 using a Support Vector Machine (SVM) classification (Van Bocxlaer & Schultheiß, 2010).  
377 The high success rate of the outline analysis is likely due to the presence of three-  
378 dimensional ornamentation on the shell surface. Also, the theory of outline methods for  
379 biological shape analysis is not as robust as landmark analysis, as discussed briefly in  
380 earlier sections.

381

382 Guisande et al. (2010) describe new software designed to identify fish species, using  
383 Classification and Regression Trees (CARTs) and linear morphometrics. The structure of  
384 the software is such that the user is required to make linear measurements on their  
385 sample, following a certain protocol, and the measurements are then used to classify  
386 the sample. This makes it similar to a multi-access key rather than a tool for automatic  
387 identification. For multi-access keys, success rates can be established by testing the key  
388 on the target audience and recording how successful was their navigation of the key.  
389 Guisande et al. (2010) did not perform this test and only tested accuracy using samples  
390 they had measured themselves.

391

392 In the field of anthropology, Velemínská et al. (2013) used semi-landmarks to study the  
393 greater sciatic notch (which is part of the pelvis bones) aiming to correctly classify the  
394 sex of the individual. Their best performing classifier was a Support Vector Machine that  
395 achieved a 92% accuracy. Instead of using a completely independent test set, the  
396 accuracy was quantified using a leave-one-out cross-validation approach on the  
397 learning set. The absence of a separate test set can lead to overestimating the  
398 accuracy of the classification as briefly discussed earlier.

399

400 The orthodontics paper by Yu et al. (2014) is based on the unusual premise of  
401 predicting attractiveness on malocclusion patients (patients with misaligned teeth). By  
402 using 101 landmarks on patient images combined with a Support Vector Machine, they  
403 achieved an accuracy of attractiveness prediction of 72%. This work is interesting  
404 because it is the only example in the literature where geometric morphometrics have  
405 been combined with the regression approaches of statistical learning, rather than the  
406 classification ones. This is because the attractiveness measure used was based on a  
407 (subjective) score from 69 orthodontics experts, therefore the prediction was a  
408 continuous measurement rather than a class.

409

#### 410 **Model evaluation**

411 There is a large collection of classification techniques available for biological analysis  
412 and selecting the most appropriate technique is not trivial. The reason for this is that  
413 there is no single classification technique that consistently outperforms all others  
414 regardless of the dataset studied. In machine learning this concept is referred to as the  
415 "No free lunch" Theorem. Stated formally by Wolpert and Macready (1997), the  
416 theorem suggests that the performance of all classifiers is equal when the totality of  
417 possible problems is considered. This means that for every classifier available there exists  
418 a possible problem where that classifier outperforms every other classifier. In practical

419 terms, this makes selecting a classifier for a study harder as the only way to establish the  
420 appropriateness of the technique is after the training of the classifier. Due to this, the  
421 most common approach to classification problems is to train a variety of different  
422 classifiers and then select the one that performs best (Fogel, 2008). This strategy makes  
423 performance evaluation the focus of the classification analysis. To this extent a series of  
424 metrics have been proposed in the literature, summarised in Table 2.

425

426 All the metrics presented in Table 2 rely on describing classification success through the  
427 use of a set of samples, however selecting the set that is used is not straightforward. In  
428 most biological situations there is a limited amount of data available for study, making  
429 each individual sample valuable to the study. With a limited dataset, therefore, the  
430 decision on the appropriate "spending" of the data is not an easy one to make. This  
431 makes pilot studies that can inform power analyses (to estimate appropriate sample  
432 sizes) a crucial aspect of experimental design (McDonald, 2014).

433

434 There are three stages in machine learning that require data: training, validating and  
435 testing (Olden, Lawler, & Poff, 2008). During the first stage the classifier is primarily  
436 trained to the problem in question. If the whole dataset is used at this stage then it will  
437 have to be re-used for both validating and testing, leading to potential overfitting and  
438 unrealistically high performance metrics (Olden *et al.*, 2008). This is because the  
439 classifier would have knowledge of the full dataset at the training stage, therefore  
440 when validating occurs (which is the process that verifies that appropriate tuning  
441 parameters have been selected during training), overfitting is more likely as none of the  
442 validating samples will be new. When the classifier is then tested using known samples,  
443 the performance will appear improved due to this overfitting effect. The peril from this is  
444 that when the classifier is applied to truly unknown samples, the confidence in the  
445 resulting class could be misplaced. To avoid this, common practice involves partitioning  
446 the initial dataset to a training set (including a validation set) and a testing set. In this

447 case the testing set is used solely for establishing the final, unbiased, performance of  
448 the classifier (Olden *et al.*, 2008). As this partition reduces the data available for training  
449 and validating, partitioning the training dataset further may not be realistic as an  
450 inappropriately small training set will create an inappropriate and untrustworthy  
451 classifier.

452

453 In order to reduce overfitting during the validating process, cross-validation (CV) can  
454 be used instead. In cross-validation the training dataset is partitioned, creating a  
455 training set (in the strict sense) and a validation set (Olden *et al.*, 2008). Training  
456 commences and is terminated when the performance with respect to the validation  
457 set begins to reduce. The validation set is thus used as a dummy 'test' set. After the  
458 classifier is trained and validated the two datasets are re-combined and re-partitioned  
459 creating a new training and validation dataset. The learning process is repeated again  
460 from the start until either a predefined number of data partitions, or all possible data  
461 partitions, have been used for training. In biological applications of machine learning,  
462 multifold (K-fold) cross-validation is commonly used to help avoid overfitting (Olden *et*  
463 *al.*, 2008). During that process the training dataset is partitioned into K equal sets, with K-  
464 1 of these recombined to create the training set and the last one used to validate. This  
465 process is repeated K times for all possible (or sensible) combinations of training and  
466 validation sets. More recently this technique has been slightly modified to include  
467 further repetitions; for example, in M repetitions of K-fold cross-validation the process of  
468 K-fold cross-validation already described is repeated M times. An example using two  
469 repetitions of 5-fold cross-validation is illustrated in Figure 2.

470

471 Throughout this paper, we have explained and illustrated the many strengths of  
472 morphometric study including the ability to train and evaluate a system, to conduct  
473 power analysis on trial data sets to help decide on appropriate sample sizes and the  
474 crucial element of reproducible measurement. Morphometric approaches can offer to

475 build strong and reproducible systems of classification and these can be combined  
476 with DNA derived data to give a holistic synthesis that might improve the stability and  
477 decrease the subjectivity of plant classification, especially at the species level. In short,  
478 when botanists and horticulturalists catch up with other disciplines we expect to see  
479 use of morphological data in the construction of more robust botanical classification  
480 systems.

481

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