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1 **Comparison of image acquisition techniques** 2 **and morphometric methods to discriminate** 3 **between *Vitis vinifera* subspecies and** 4 **cultivars**

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11

12 **Abstract** For decades, it has been known that the morphology of wild *Vitis vinifera*
13 (grape) pips differ from domesticated ones. Based on these differences, computerised
14 image analysis and morphometrics allow us to obtain excellent results to identify the
15 domestication status of archaeological grape pips. However, various image analysis
16 methodologies and morphometric methods have been applied in previous studies for this
17 purpose. Our study has examined, for the first time, the different techniques of digital
18 image acquisition (digital camera vs scanner) of grape pips, with the aim of testing the
19 identification performance using both traditional and geometric morphometric features to
20 distinguish wild from domestic grape pips. The performance of different morphometric
21 methods established that all image acquisition techniques and morphometric methods
22 correctly distinguished between wild and domestic grapes. The use of the combined
23 geometric morphometric features of both dorsal and lateral views of the pips provided the
24 best accuracy in distinguishing individual cultivars.

25 **Keywords** *Geometric morphometrics · Seed image analyses · Grape pip*
26 *morphology · Traditional morphometric features*

27 **Introduction**

28 *Vitis vinifera* ssp. *sativa* (DC) Hegi (grapevine) is currently one of the most
29 cultivated perennial fruit plants in the world, with eight million ha grown, mostly
30 for wine production (Myles et al. 2011; Wen et al. 2018). The currently cultivated
31 grape varieties are the result of long-term selection that began with the
32 domestication process of their wild ancestor, *Vitis vinifera* ssp. *sylvestris* (C.C.

33 Gmel.) Hegi. One of the most distinctive differences between the two subspecies
34 is hermaphroditism, which allows the domestic grape to self-pollinate and be
35 more productive (This et al. 2006). Genetic studies on modern cultivated and wild
36 grapes support the hypotheses of a single, or two main domestication events
37 which occurred in western Asia (Arroyo-García et al. 2006; Myles et al. 2011;
38 Dong et al. 2023).

39 Archaeobotanical studies indicate that the first domestication probably took place
40 in southwest Asia in the period between the 6th and 3rd millennia BCE. Thanks to
41 the widespread presence of wild grapes there, some researchers have indicated
42 that the southern Caucasus area is the most probable place where this event could
43 have occurred (Zohary et al. 1995, 2012; De Lorenzis et al. 2015).

44 The morphological characters of ancient grape pips found in archaeological
45 contexts can provide valuable information about their status, wild or
46 domesticated, to explain the beginning of grape domestication. The process of
47 domestication caused phenotypic changes in their wild relatives such as the shape
48 of the pips (seeds), so that domesticated grapes generally have elongated pips with
49 a long beak, while wild grapes have smaller, roundish pips with a short beak.

50 Several studies have focused on the development of methods to discriminate
51 between them through the measurement of morphometric parameters. The simple
52 breadth/length ratio proposed long ago by Stummer (1911) proved to be quite
53 inefficient in correctly distinguishing wild from domestic grape pips, particularly
54 with charred specimens (Logothetis 1970, 1974; Smith and Jones 1990;
55 Bonhomme et al. 2022). Subsequently, Mangafa and Kotsakis (1996) did several
56 carbonisation experiments on modern wild and domestic grape pips with the aim
57 of distinguishing them. Based on various distance measurements computed using
58 discriminant analysis, their work led to the proposal of four mathematical
59 formulae regarded as the most efficient for distinguishing domestic from wild
60 *Vitis*. Unfortunately, when this method was applied to French domestic grape
61 varieties, it proved not entirely accurate, as the proposed formulae needed to be
62 recalibrated for the larger number of grape varieties in France (Bouby and
63 Marival 2001).

64 In recent years, further studies have used analysis of the outlines of the pips to
65 distinguish wild from domestic grapes (Terral et al. 2010). This method uses
66 elliptic Fourier transforms (EFT), which converts the outline of an object into

67 shape descriptors (Fourier coefficients = EFDs) (Kuhl and Giardina 1982).
68 Subsequently, EFT results have been used as geometric morphometric features in
69 multivariate analyses to distinguish the different morphological types (Terral et al.
70 2010). This approach has been highly effective in distinguishing wild grape pips
71 from domestic ones (Pagnoux et al. 2015, 2021; Bouby et al. 2021). A recent
72 study compared different methodologies for this separation, EFT, Stummer
73 indices and length variables derived from the formulae proposed by Mangafa and
74 Kotsakis (Bonhomme et al. 2022). The authors concluded that, except for
75 Stummer's indices, length variables and EFT were able to correctly identify wild
76 from domestic grape pips and the best performance was obtained from EFT.
77 At the same time, other studies have used traditional morphometric features
78 (TMf), such as area, perimeter, width and height, and shape parameters, such as
79 roundness, circularity and convexity, to separate wild grapes from domestic ones
80 in both modern and archaeological material (Orrù et al. 2013; Ucchesu et al. 2015,
81 2016; Sarigu et al. 2022; Coradeschi et al. 2023).

82 Some of the methods mentioned above have also been used to go beyond species
83 identification, attempting to recognise groups of domestic grape varieties or
84 particular wild grape populations (Terral et al. 2010; Orrù et al. 2012, 2013;
85 Pagnoux et al. 2015, 2021; Ucchesu et al. 2015, 2016; Bouby et al. 2021).

86 Morphometric approaches usually rely on image analysis. In some of the cited
87 works, images were obtained with a stereomicroscope fitted with a digital camera
88 (Terral et al. 2010; Bouby et al. 2013, 2021; Pagnoux et al. 2015, 2021;
89 Bonhomme et al. 2022), while in other works, a flatbed scanner was used (Orrù et
90 al. 2013; Ucchesu et al. 2015, 2016). In both cases, the original images were
91 digitally processed to separate the objects (pips) from the background of the
92 image, so as to isolate the objects of interest from everything else.

93 The final stage of the process is the creation of binary images of the objects that
94 are ready to be measured so that grey scale or colour images are converted into
95 binary line images with only two pixel values, either black, usually the
96 background, or white. This phase of the digital image processing may require
97 different amounts of time, depending on the acquisition method and the
98 experience of the operator. It is not known how the method of obtaining images
99 may influence the results of morphometric analyses for accuracy or
100 reproducibility and the identification of the samples. Furthermore, the different

101 methods of image acquisition in combination with a traditional morphometric
102 approach and outline analysis have not yet been compared to discover which of
103 the two methodologies has the best performance in characterising grape pip
104 morphology.
105 Therefore, in this work, our objective was twofold. First, we compared the
106 reproducibility and performance of both image acquisition techniques using the
107 same samples of modern grape pips, both wild and domestic, and the same
108 morphometric methods (EFT and TMf). Second, we tested the performances of
109 both morphometric methods to discriminate between *Vitis vinifera* subspecies and
110 cultivars using the same grape pip samples and images from them.

111 **Material and methods**

112 ***Vitis* accessions**

113 Assessment and comparison of the errors of image acquisition techniques were
114 made using a set of three grape cultivars (Girò scuro, Monica and Trebbiano
115 Romagnolo) and one wild accession (Sos Forrighesos) with five pips for each of
116 the four accessions (Evin et al. 2020).
117 Further comparisons of morphometric methods and image acquisition techniques
118 were done using modern grape pips from 15 cultivars of *V. vinifera* ssp. *sativa* and
119 11 individuals of *V. vinifera* ssp. *sylvestris* (ESM Table S1). Domestic grape
120 samples were collected from the Institut national de la recherche agronomique
121 (INRAE), Vassal-Montpellier Grapevine Biological Resource Centre,
122 Ampelographic collection of Vassal-Montpellier (Marseillan-Plage, France) and
123 from the Agenzia per la Ricerca in Agricoltura della Regione Sardegna (AGRIS)
124 germplasm collection in Ussana (Sardinia, Italy).
125 Wild material came from the collection of the Institut des Sciences de l'Évolution
126 de Montpellier (ISEM-CNRS), University of Montpellier (ESM Table S1).
127 Following previous work (Terral et al. 2010), 30 pips were randomly selected
128 from each domestic and wild accession, in total, 780.

129 **Image acquisition techniques**

130 All pips were photographed in dorsal and lateral views using a stereomicroscope
131 (Olympus SZ-ET) with a digital camera (Olympus DP21).

132 In parallel, the images of the same grape pips were also acquired following the
133 method described in Orrù et al. (2012), using a flatbed scanner (Epson Perfection
134 V550), with a digital resolution of 600 dpi for a scanning area not exceeding
135 5,100×7,019 pixels. Due to the impossibility of placing the pips on the scanner
136 glass on their lateral side, only an images of the dorsal side were scanned.
137 To minimise errors during the acquisition of the grape pip images, only two
138 trained people did this work, one using the digital camera and the other used the
139 scanner.
140 The digital processing, which included creation of binary images, both from the
141 digital camera photos and the scans, was done by a single person.
142 Moreover, the time taken for the image acquisition and processing of 30 pips was
143 also measured.

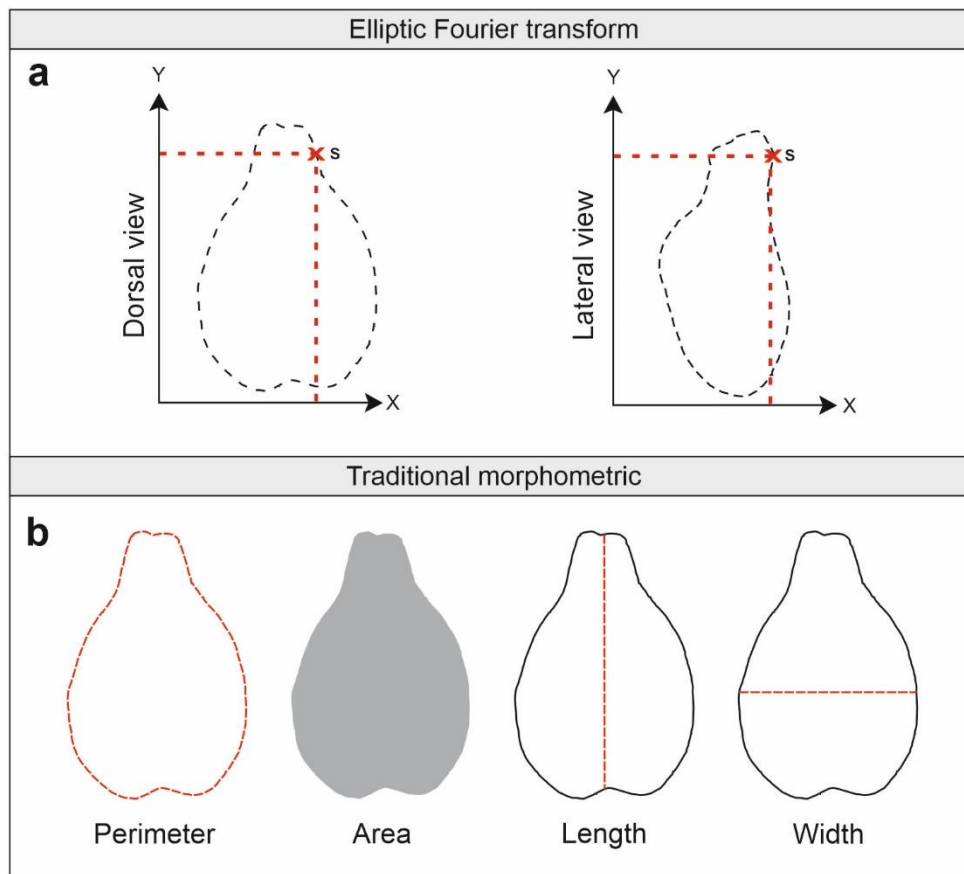
144 **Morphometric methods**

145 ***Elliptic Fourier transforms***

146 All grape pip images were converted to black silhouettes, the binary images. Pip
147 outline analyses were done of these images using the elliptical Fourier transforms
148 (EFT) method (Terral et al. 2010; Bonhomme et al. 2014, 2021). This method
149 converts the contour geometry into Fourier coefficients, for which outline
150 coordinates (x, y) of 160 equidistant points were sampled on the outline of each
151 grape pip. The outlines were normalised before calculating the EFTs using their
152 centroid size and considering the position of the first point above the centroid
153 (Fig. 1a).

154 Based on previous work (Terral et al. 2010; Bonhomme et al. 2021), we used only
155 the coefficients from the six first harmonics to describe each view (24
156 coefficients). Six harmonics allowed a good compromise between shape
157 description accuracy (more than 95% of the total harmonic power) and
158 measurement errors, which increased with harmonic rank. The flatbed scanner
159 could only obtain dorsal views of the pips. The microscope and digital camera
160 also photographed the lateral (side) view of the pips. In this case, each one was
161 then described by 24 coefficients for the dorsal view and another 24 for the lateral
162 view. All EFT analyses used Momocs v. 1.4.0 (Bonhomme et al. 2014).

163



164
 165 **Fig. 1 a**, diagram of elliptic Fourier transform (EFT) measurement of 160 reference points; **b**,
 166 principal traditional morphometric features (TMf) measurement of length, width, perimeter and
 167 area

168 ***Traditional morphometrics***

169 The same images used for EFT were measured for 20 morphometric parameters
 170 (nine of size and 11 of shape) using Image J 1.53k image processing and analysis
 171 software (Abràmoff et al. 2004). Of the 20 morphometric parameters, only those
 172 that were least correlated with each other ($R < 0.85$) were selected for a total of
 173 nine parameters (Fig. 1b; ESM Table S2).

174 **Repeatability tests**

175 Image acquisition was repeated three times for each pip, on different days with the
 176 flatbed scanner and digital camera. The variability between repeated image
 177 acquisitions was studied using principal component analysis (PCA). The
 178 percentage of measurement errors was assessed using an analysis of variance
 179 (ANOVA) (Claude 2008 Evin et al. 2020). Mean squares were used to estimate
 180 the proportion of variance associated with the replication measurements. The error

181 percentage was calculated as the ratio of the variance between replicates (within
182 variance) and the total sum of the variance between replicates and specimens (sum
183 of within and among variance).

184 **Exploring morphological variation**

185 To explore the overall morphological variations, we used principle components
186 analysis (PCA) for the traditional morphometric parameters, as well as for the
187 Fourier coefficients. To evaluate the respective performances of morphometric
188 methods and image acquisition techniques in identifying subspecies and cultivars,
189 we performed linear discriminant analysis (LDA) mainly on PCA scores, which
190 are not correlated. In parallel, we also performed LDA directly on the raw
191 traditional morphometric parameters and Fourier coefficients.

192 We used a balanced reference material for the LDA in which an identical number
193 (N=2430) of pips of cultivated and wild grapes were randomly selected.

194 Statistical analyses were done using the MASS package (Venables and Ripley
195 2002) in the R environment (v. 4.0.0) (R Development Core Team 2020).

196 **Results**

197 **Working times**

198 In the examination of working times for obtaining images of 30 pips, the scanner
199 required 5 min to acquire only the dorsal view and the digital camera took a total
200 of 30 min for both dorsal and lateral views. Subsequently, the total image
201 processing time was 1 min for 30 scanned images and 20 min for 60 photographed
202 images (Table 1).

203

Method	Time image acquisition (min)	Time image segmentation (min)	Total time (min)
Scanner (dorsal view)	5	1	6
Digital camera (dorsal view)	15	10	25
Digital camera (lateral view)	15	10	25

204

205 **Table 1** Time taken for processing 30 pips

206 ***Repeatability tests***

207 The first biplot of the PCA (81.44% of the total variance) on traditional
208 morphometric features (TMf) scanned in dorsal views of four cultivated and one
209 wild grape pips showed that the differences between the accessions and pips were
210 more marked than between the three repeated image acquisitions of single pips
211 (ESM Fig. S1).

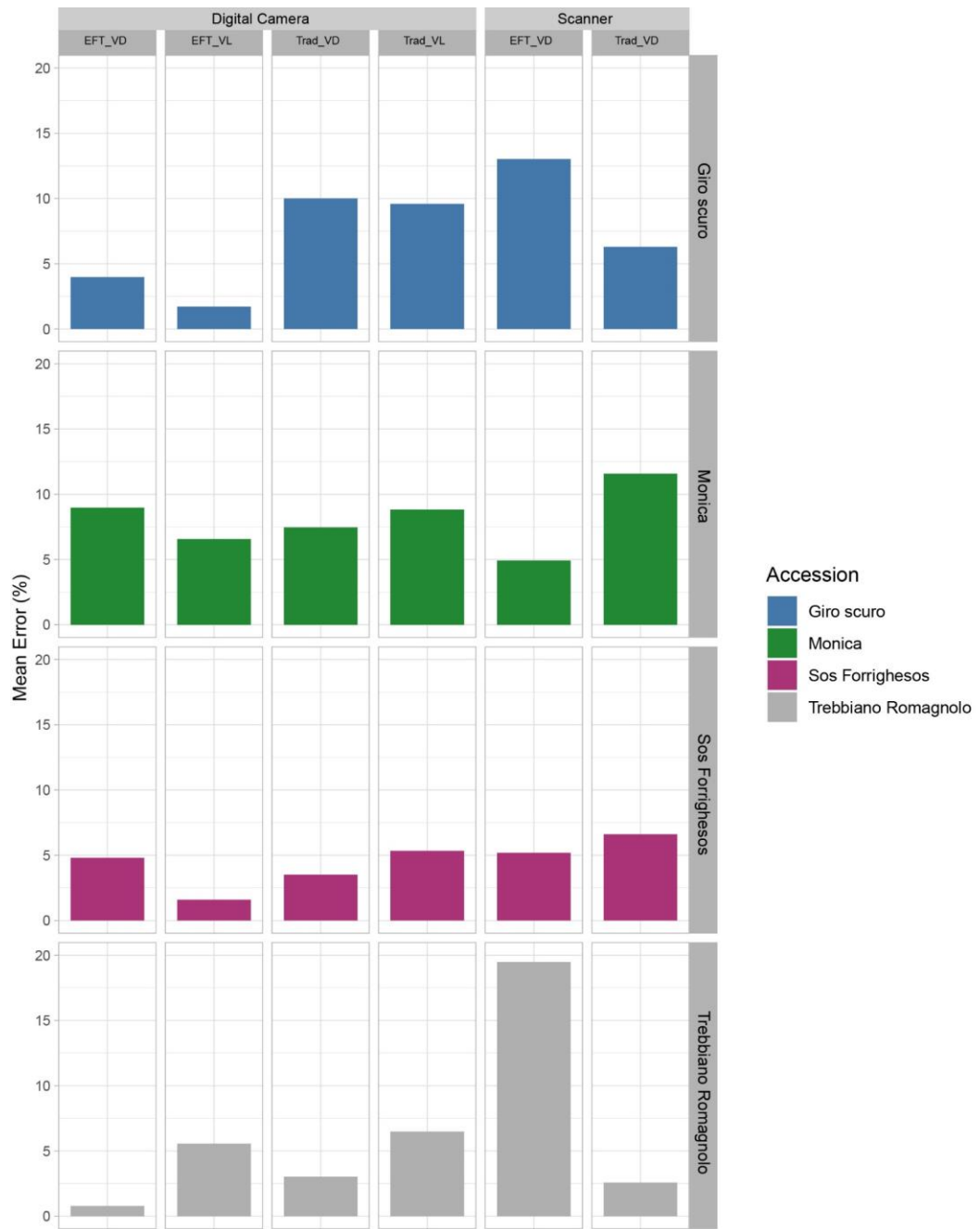
212 Similarly, the PCAs of the TMf measured on the dorsal and lateral views of the
213 pips when photographed showed larger differences between accessions and
214 between pips within accessions than between the three repetitions of
215 photographing single pips (ESM Figs. S2a and b).

216 The same approach was repeated using the Elliptic Fourier Transform (EFT)
217 parameters on images from both the scanner and digital camera. As with the TMf
218 results, the PCAs on shape descriptors of scanned dorsal views (ESM Fig. S3) and
219 when photographed (ESM Fig. S4) showed that the differences between
220 accessions and pips were generally greater than the three repeated image
221 acquisitions from both scanning and photography. The discrimination between
222 pips was, in some cases, less marked from the scanned images.

223 Using Elliptic Fourier Transform (EFT) parameters measured in lateral view with
224 a digital camera, PCA again showed that the differences between the various
225 accessions and pips were more marked than between the three repeated image
226 acquisitions (ESM Fig. S5).

227 In addition, we calculated the percentage of measurement error according to the
228 six combinations of acquisition techniques and morphometric methods for the
229 four grape accessions. The error associated with each image acquisition technique
230 and morphometric method varied noticeably, according to the particular grape
231 accessions (Fig. 2). However, it was usually less than 10% (ESM Table S3). The
232 measurement error was slightly lower for photos than with scans. For the photos,
233 the measurement error was slightly lower for the lateral than for the dorsal view.
234 However, the observed differences between morphological techniques and
235 methods were small and varied from one cultivar to another (ESM Table S3).

236



237

238 **Fig 2** Comparison of measurement error for the six combinations of acquisition techniques and
 239 morphometric methods with the four grape accessions, names on right. D, dorsal view; L, lateral
 240 view

241 **Comparison of morphometric methods (TMf vs EFT) using**
 242 **a scanner**

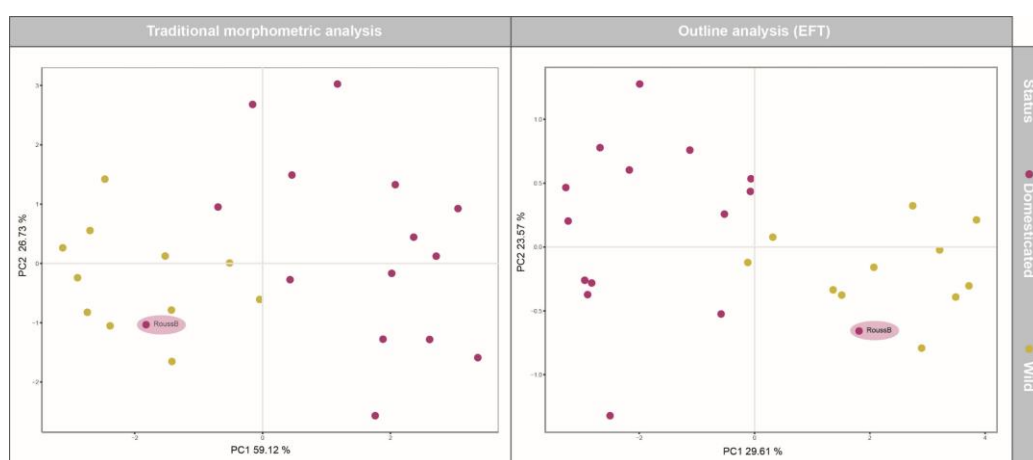
243 We then carried out principle components analyses (PCA) on the measurements
 244 obtained from both methods, traditional morphometric features (TMf and elliptic
 245 Fourier transform (EFT), of the 15 cultivars and 11 wild varieties in dorsal view.

246 Both PCAs showed a clear distinction between domestic and wild accessions,
247 except for the cultivar Roussanne (Fig. 3a, b).

248 **Comparison of morphometric methods (TMf vs EFT) using** 249 **the digital camera**

250 Further PCAs were done on the measurements with TMf and EFT in dorsal,
251 lateral and both views, obtained with a digital camera (Fig. 4a–f). All analyses
252 showed a clear differentiation between domestic and wild accessions, with the
253 exception of the cultivar Roussanne (RoussB, Fig. 4).

254



255

256 **Fig. 3** Principal component analysis of morphological variation between grape pip accessions
257 using a scan of the dorsal view, and using **a**, TMf; **b**, EFT. RoussB, cultivar Roussanne; purple
258 dots, cultivated grape; yellow dots, wild

259

260 **LDA analyses of traditional morphometric parameters and** 261 **elliptic Fourier coefficients using a scanner and digital** 262 **camera**

263 Finally, linear discriminant analyses (LDAs) were used to compare the
264 performance of image acquisition techniques (scans vs photos) using TMf and
265 EFT for status (domesticated/wild) and cultivar identification.

266 Figure 5 shows that classification according to domesticated or wild status using
267 both TMf and EFT from both acquisition techniques is at least 83% correct.

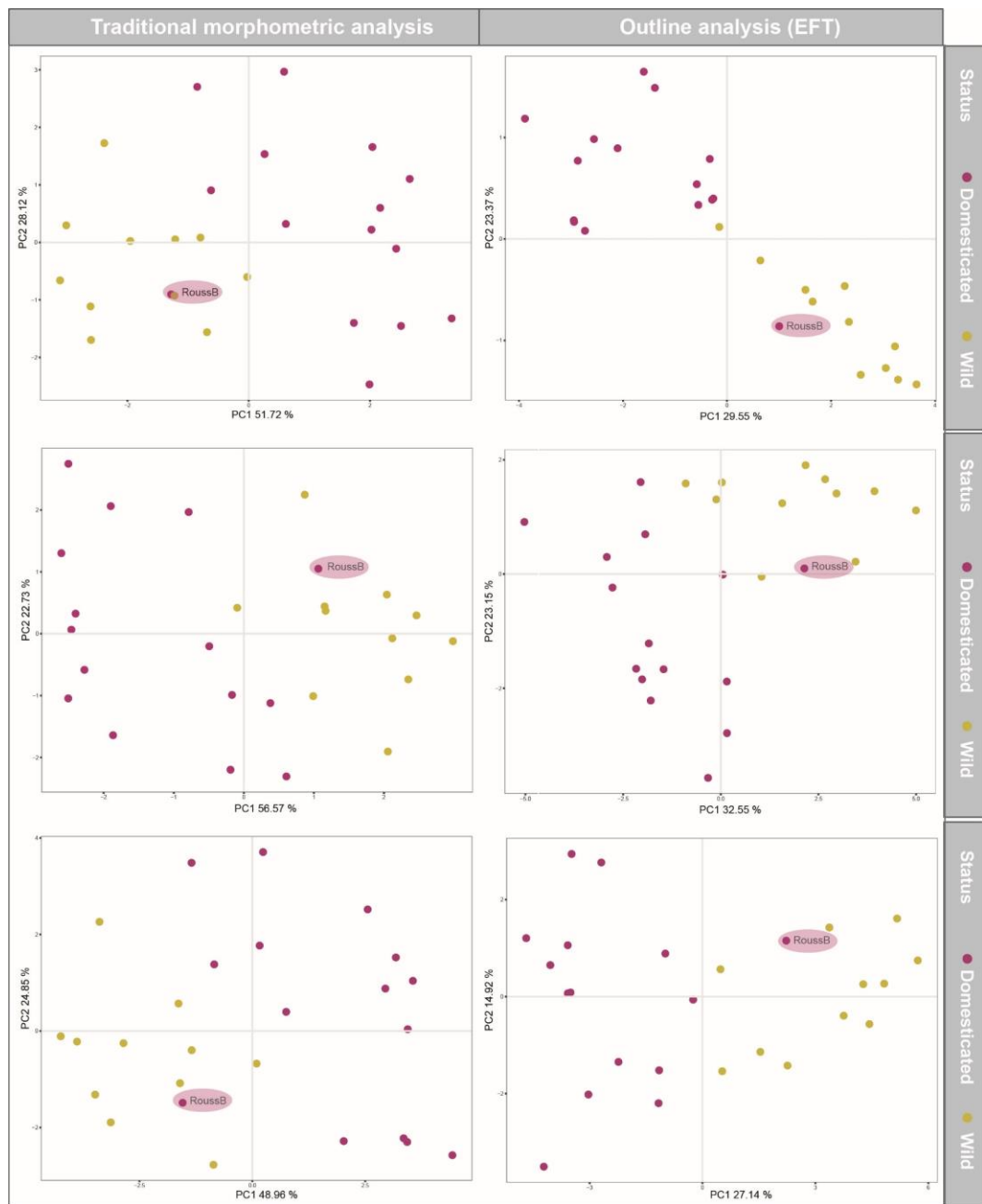
268 Overall, the percentages of correct identification are similar for both technical
269 acquisition and morphometric methods (Fig. 5). The photos provided

270 measurements in both lateral and dorsal views, which increased the accuracy of
271 identification of wild and domestic grape pips by a few percentage points when
272 using traditional measurements and EFT coefficients (Fig. 5a–d). LDAs of the
273 raw shape descriptors (Fig. 5b, d, f, h) generally gave slightly higher percentages
274 of identification than LDAs based on principal components.
275 All image acquisition techniques and morphometric methods also yielded good
276 results for identification of the cultivars (Fig. 5e–h). However, in this case, there
277 were greater differences between the different combinations; higher correct
278 identifications were obtained using EFT coefficients from photos than from the
279 scans. Moreover, the highest identification percentages were achieved when both
280 dorsal and lateral views of the pips were combined, especially when the LDA was
281 done on the raw EFT coefficients (Fig. 5h).
282 Overall, the accuracy of cultivar identification was clearly increased when using
283 EFT and the combination of dorsal and lateral views or lateral views rather than
284 when using traditional morphometrics on dorsal views only.

285 **Discussion**

286 The use of different morphometric methods and image analysis techniques on
287 both modern and archaeological samples raises the question of which is the best
288 option for an accurate description of pip morphology and, more especially, to
289 accurately distinguish wild from domestic grape pips. Bonhomme et al. (2022)
290 showed how the choice of morphometric methods could influence the results of
291 distinguishing between wild and domestic accessions, and so could provide
292 different identifications of archaeological pips.

293 Our study examined, for the first time, the different techniques of digital image
294 acquisition (digital camera vs scanner) in combination with a traditional
295 morphometric approach (TMf) and outline analysis (EFT), with the aim of testing
296 their performances in distinguishing wild from domestic grape pips and to make
297 further progress in the identification of grape varieties. We measured the errors
298 from the repeated image acquisitions of the same samples both with photos and
299 scans. The results for both image acquisition techniques and morphometric
300 methods showed that the differences between the various accessions and pips
301 were more marked than the errors in the repeated image acquisitions. It was also
302 found that the measurement error was slightly lower for photos than with scans.



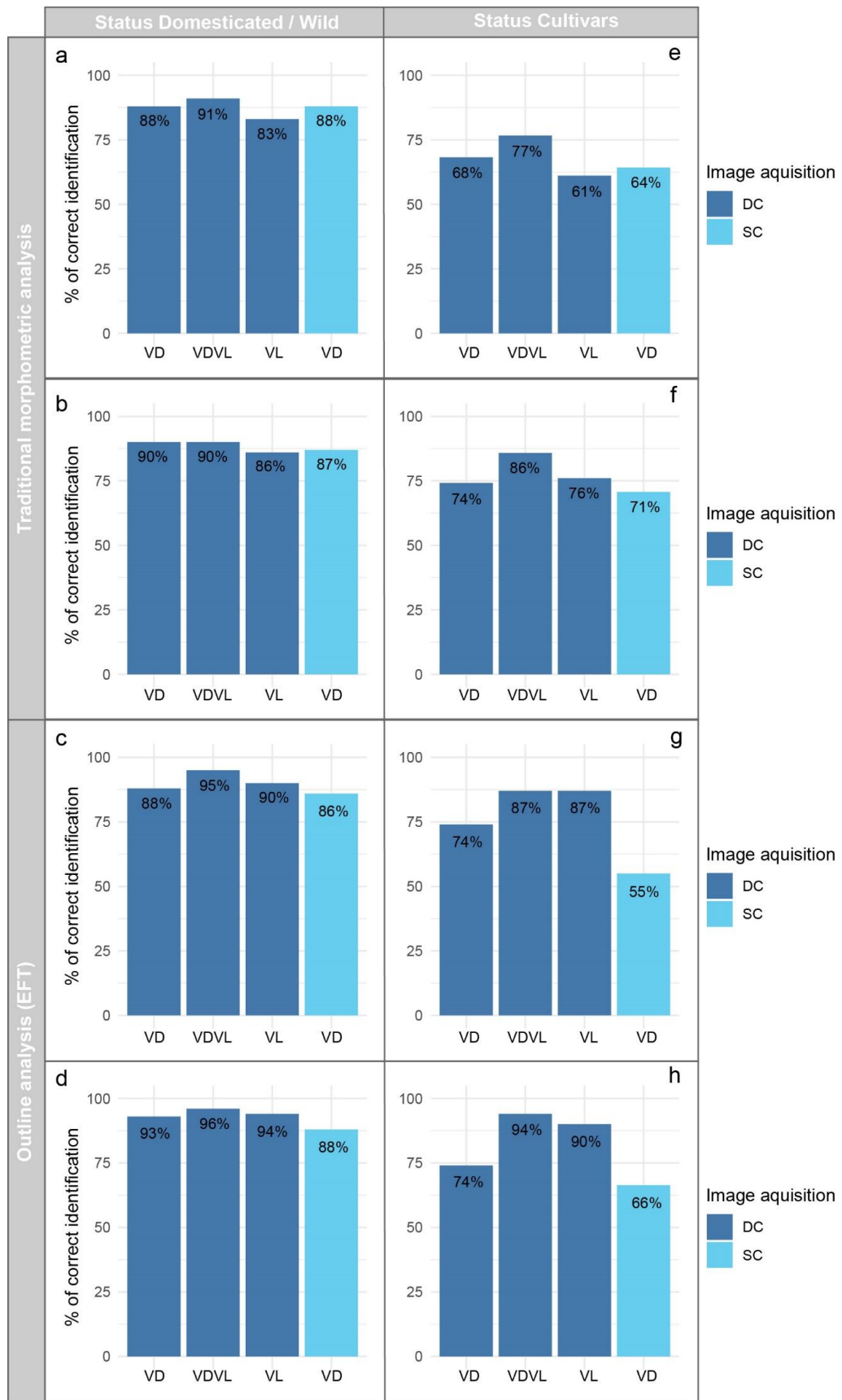
304

305 **Fig. 4** Principal component analyses of morphological variation between grape pip accessions
 306 using a digital camera and TMf and EFT of dorsal (D, **a, d**), lateral (L, **b, e**) and both views (D+L,
 307 **c, d**). RoussB, cultivar Roussanne

308

309 This slight difference could be due to the difficulty of arranging irregularly shaped
 310 pips in exactly the same position on the rigid surface of the scanner glass. In
 311 contrast, when using a digital camera, each pip was placed on a soft surface,
 312 which allowed the operator to adjust it to the best position. However, the error rate
 313 was always less than 10% and did not prevent the use of any method.

314



315
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318
319

Fig. 5 Bar plot of linear discriminant analysis (LDA) results comparing the performance of image acquisition techniques (SC, scanner vs DC, digital camera) using either Elliptic Fourier transform outline analysis or traditional morphometric features for identification; **a-d**, domesticatd; **e-h**, cultivars

320

321 The performance of the identifications with LDA allowed us to establish that both
322 image acquisition techniques and morphometric methods were able to separate
323 wild from domestic grape pips with similar accuracies, always close to 90%.

324 This is similar to the results of previous works where both traditional
325 morphometric parameters and outline analysis correctly distinguished wild from
326 domestic pips (Bouby et al. 2013; Bonhomme et al. 2022). In the future, we
327 should seek to further improve the statistical procedure of identification by
328 repeating the LDA thousands of times on a random sample of balanced reference
329 datasets, in order to assess the variability and accuracy of the result.

330 The digital camera provided a somewhat higher accuracy due to the possibility of
331 using pip measurements in both dorsal and lateral views.

332 It can be concluded that the use of a scans to obtain only dorsal view of the pips is
333 enough to be able to distinguish between wild and domestic pips, especially when
334 processing many archaeological samples.

335 However, to further investigate the morphological variation among domestic
336 grape varieties, our results showed that it is preferable to use photos and the EFT
337 method.

338 The use of TMf and EFT measurements of photographic images proved more
339 accurate in identifying individual cultivars than with scans.

340 More significantly, the combination of dorsal and lateral views of the pips was
341 shown to give better identifications, especially when using EFT parameters rather
342 than traditional measurements, and it gave the best performance in distinguishing
343 cultivars. This shows that the lateral view adds important morphological
344 characteristics that help to distinguish between each cultivar. Using EFT
345 parameters on data from both dorsal and lateral views is therefore the most
346 suitable approach when aiming to explore morphological variability of domestic
347 shapes from archaeological remains.

348 The analysis of times taken for capturing images of 30 pips using either a scanner
349 or a digital camera has provided additional insights into the efficiency of these
350 two methods, showing some significant differences for both image acquisition
351 methods and subsequent processing times.

352 Obtaining dorsal views of the pips is much faster when using a scanner, requiring
353 only 5 min per 30 pips. However, the digital camera, which provided a more
354 comprehensive dataset from both dorsal and lateral views, and more detailed and

355 repeatable images, required a substantially longer time of 30 min. This is mostly
356 due to the time taken to position each individual pip under the microscope.
357 Similarly, the processing phase, involving the formation of outline images of the
358 pips, took differing amounts of time. The scanner images were quickly processed,
359 taking only 1 min per pip. In contrast, the photos took longer to process, 20 min.
360 This extra time is due to the handling of 30 images individually, corresponding to
361 the number of pips to be analysed.
362 These results suggest that the scanner is especially well suited for quickly
363 including many pips in a single image to discriminate between wild and
364 domesticated morphotypes. The significant increase in acquisition time with a
365 digital camera and its ability to record both dorsal and lateral views of the pips is
366 especially worthwhile if the aim is for a more precise description of seed shape,
367 which is needed to analyse diversity within species or between cultivated
368 varieties.
369 Consideration should be given to the fact that these results obtained for grape pips
370 may be different when dealing with other species and different seed shapes.
371 Consequently, similar tests should be done for each newly investigated taxon.

372 **Conclusions**

373 The correct identification of archaeological grape pips allows us to investigate the
374 long history of grapevine domestication. It is known that the morphology of wild
375 grape pips differs from those of cultivated grapes due to the domestication
376 process. Based on these differences, computerised image analysis has obtained
377 excellent results for identifying archaeological grape pips. This study compares
378 different image analysis methodologies, starting with imaging techniques and
379 morphological methods used for the separation of wild from domestic pips and for
380 the identification of individual cultivars. The results have shown that the
381 acquisition method by scan or photo did not affect the good separation of wild
382 from domestic pips. The morphometric data, both traditional and geometric,
383 measured on images obtained from both photos and scans, were able to correctly
384 distinguish between the wild and domestic grapes, achieving very similar overall
385 percentages of correct identification. However, the photos also provided the
386 lateral view of the pips, which in combination with the dorsal view, gave the best

387 performance for exploring the morphological variability of cultivated grape
388 varieties.

389 **Supplementary Information** The online version contains supplementary
390 material.

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398 study.

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486 **Figure and Table legends**

487 **Fig. 1 a**, diagram of elliptic Fourier transform (EFT), measurement of 160 reference points, (s)
488 starting point of the outline; **b**, principal traditional morphometric features (TMf), measurement of
489 length, width, perimeter and area

490

491 **Fig. 2** Comparison of measurement error for the six combinations of acquisition techniques and
492 morphometric methods with the four grape accessions, names on right. D, dorsal view; L, lateral
493 view

494

495 **Fig. 3** Principal component analysis of morphological variation between grape pip accessions
496 using a scan of the dorsal view, and using **a**, TMf; **b**, EFT. RoussB, cultivar Roussanne; purple
497 dots, cultivated grape; yellow dots, wild

498

499 **Fig. 4** Principal component analyses of morphological variation between grape pip accessions
500 using a digital camera and TMf and EFT of dorsal (D, **a**, **d**), lateral (L, **b**, **e**) and both views (D+L,
501 **c**, **d**). RoussB, cultivar Roussanne

502

503 **Fig. 5** Bar plot of linear discriminant analysis (LDA) results comparing the performance of image
504 acquisition techniques (SC, scanner vs DC, digital camera) using either Elliptic Fourier transform
505 outline analysis or traditional morphometric features for identification; **a-d**, domesticatd; **e-h**,
506 cultivars

507

508 **Table 1** Time taken for processing 30 pips

509

510

511 **Conflicts of interest**

512 The authors have no competing interests to declare that are relevant to the content of this article.

513