Berry and pip form covariation in wild and domesticated grapevines: eco-evo-devo implications and archaeobotanical perspectives

Vincent Bonhomme[1,&,@] , Sandrine Picq [1,2,&], Sarah Ivorra[1], Allowen Evin[1], Thierry Pastor[1], Roberto Bacilieri[3], Thierry Lacombe[3], Isabel Figueiral[1], Jean-Frédéric Terral[1] and Laurent Bouby[1]

[1] ISEM, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France. Équipe « Dynamique de la biodiversité, anthropo-écologie », Place Eugène Bataillon - CC065 34095 Montpellier Cedex 5, France.
[2] Laurentian Forestry Centre, Natural Resources Canada, 1055 Du P.E.P.S. Street, P.O. Box 10380, Québec, Quebec, G1V 4C7
[3] INRA, UMR 1334 AGAP, Equipe Diversité, Adaptation et Amélioration de la Vigne, F34060, Montpellier, France.
[&] these authors contributed equally to this work
[@] corresponding author: bonhomme.vincent@gmail.com +33 (0)4 67 14 41 60

Total word count:	6266
Abstract:	198
Introduction:	1070
Materials and Methods:	1586
Results:	1713
Discussion:	1897
Figure number:	10
Table number:	2
SI number:	2

Summary (198 words)

1. If grapevine phenotypic changes occurred during domestication and diversification processes are quite well known, particularly seed morphology used in archaeobiological studies, the functional causes and consequences behind these variations are still poorly understood. This study clarifies many aspects of size and shape (co)variation between pip and berry in both wild and domesticated *Vitis vinifera*.

2. The covariation of berry size, number of seeds per berry ('piposity'), pip size and pip shape were explored on 49 grapevine accessions sampled among Euro-Mediterranean traditional cultivars and wild grapevines. Consequences for archaeobotanical studies are discussed through the prediction of berry dimensions from archaeological pips from a Southern France Roman site.

3. For wild grapevine, the higher the piposity, the bigger the berry and the longer the pip. For wild and domesticated grapevine, the longer is the pip, the more it has a « domesticated » shape. Such covariations allow to infer the berry dimensions in archaeological context where only the pips are recovered.

4. This systematic exploration sheds light on new aspects of pip-berry covariation in both size and shape, draws the curtain on grapevine eco-evo-devo changes during domestication, and invites to explore the impact of cultivation practices on pip shape.

Keywords: archeobotany, berry and pip form covariation, cultivation practices, eco-evo-devo, plant domestication, traditional and modern morphometrics, *Vitis vinifera*, wine and table cépages.

Introduction

Grapevine (*Vitis vinifera* L.) is the most important fruit species in the modern world (FAO, 2017) and acquired a central economical and cultural role since Classical times, particularly in the Mediterranean area (Brun, 2003; McGovern, 2007). Grapevine has long been cultivated for its fruits, mostly used to be transformed into wine but also consumed fresh or dried. Its wild progenitor, *Vitis vinifera* subsp. *sylvestris*, has likely been first domesticated in the South Caucasian area (Imazio *et al.*, 2013), which provided the oldest wine making evidence (McGovern *et al.*, 2017) dated to early Neolithic period (~8000 BP). The existence of other domestication centres has also been argued (Grassi *et al.*, 2003; Arroyo-García *et al.*, 2006). Since the early times of domestication, grapevines varieties (or cépages) of *Vitis vinifera* subsp. *vinifera* have been selected, cultivated and propagated such as, today, several thousand varieties are recognized, and traditionally identified, by ampelography, the phenotypic description of grape varieties (Galet, 1988). Wild and domesticated grapes differ regarding their reproductive biology - wild plants being dioecious and cross-pollinated while most of the cultivars are hermaphrodite and self-pollinated, and an array of phenotypic changes with domesticated grape having bigger bunches and berries, higher diversity in their shape and skin colour, higher sugar content (Olmo, 1995; This *et al.*, 2006) .

Tracing back in time and space the origins and spread of these changes is a big challenge. Archaeobotanical material provides unique snapshots of ongoing domestication/diversification processes. Notwithstanding such material is often limited and frequently degraded, the quantitative and rigorous morphological description of preserved parts (mostly seeds), brought major insights into our understanding of the intertwined relationships between humans and domesticated plants in general (Zohary & Hopf, 1993; Jones *et al.*, 2000; Terral *et al.*, 2004, 2012; Willcox, 2004; Fuller, 2007; Ros *et al.*, 2014; Bonhomme *et al.*, 2015; Wallace *et al.*, 2018), including grapevine (Mangafa & Kotsakis, 1996; Terral *et al.*, 2010; Terral & Bouby, 2013; Orrù *et al.*, 2013; Bouby *et al.*, 2013; Pagnoux *et al.*, 2015; Ucchesu *et al.*, 2016; Karasik *et al.*, 2018). Molecular approaches on grapevine yielded limited information until now (Wales *et al.*, 2016; Bacilieri *et al.*, 2017), made difficult by the poor preservation of ancient DNA (aDNA), the major part of archaeobotanical grape seeds being indeed preserved by charring, especially in the prehistoric and early historic sites located in the South-Asian and Mediterranean areas.

Morphometrics on archaeological pips have first been criticized for the overlapping of seed shapes and the differential deformation of wild versus domesticated pips shape that could make the shape of domesticated pips more similar to wild pips (Smith & Jones, 1990). Experimental charring has allowed to validate resilience of identification even after distortions induced by charring (Ucchesu *et al.*, 2016; Bouby *et al.*, 2018). On the other hand, archaeological grapevine material is also found in waterlogged contexts and such conservation conditions possibly entail other, hardly testable, biases on shape.

It is well known that pips from wild and domesticate grapes differ in their form (that is size plus shape): wild grapes produce roundish pips with short stalks and cultivated varieties more elongated pips, with longer stalks (Levadoux, 1956). Such inter-(sub)specific, and also infra-specific, variations of form are now well known on archaeological grapevine pips (Terral *et al.*, 2010; Bouby *et al.*, 2013; Pagnoux *et al.*, 2015). However, the functional causes and consequences (if any) behind these variations in seed form are still poorly understood. If size, shape and colour of berries are phenotypic traits that have been selected by humans, seed shape was likely not a direct target of selective pressures. The form of pips may possibly be affected by: the berry size; the number of pips per berry, that implies sharing space and embryonic resources; the growing environment, including cultivation practices; the status of the plant (wild or domesticated); the grapevine variety for *Vitis vinifera* subsp. *vinifera*; and developmental stochasticity. Previous works suggested that pip size and the number of pips per berry are positively correlated to berry size (Negrul, 1960; Galet, 1988; Houel *et al.*, 2013). It was also evidenced that this correlation is stronger for wild grapevines than for domesticated varieties (Bouby *et al.*, 2013). Additionally, Houel and colleagues (2013) advocate that although seed number, seed weight and berry flesh weight are correlated at the intragenotypic level in domesticated grapevine, there is no global correlation at the inter-genotypic level.

A better understanding of the process of domestication relying on archaeological seeds goes hand in hand with a better understanding of the relationships between seed and berry forms. It is most likely that one of the first objective when cultivating grapevines was to obtain more numerous and bigger berries, as they are key yield factors for both wine and table production. It is therefore necessary to find out to what extent the shape changes observed in archaeological seeds imply changes in the size of berries. It is also important to know how this relation could be affected by simple cultivation of wild individuals and by domestication. Grape pips having a form typical of modern wild individuals have been abundantly and repeatedly found in ancient viticultural sites leaving open the question whether these

populations cultivated "true" wild grapevines or "weakly" domesticated forms (Bouby *et al.*, 2013; Pagnoux *et al.*, 2015).

This paper aims at exploring how the form of berries and the pips they contain covariate. A modern dataset composed of domesticated and wild grapevines allowed to compare patterns of covariation between these two *Vitis vinifera* compartments. To that respect, this paper considerably extents the previous work by Bouby and colleagues (2013) since it: i) enlarges the modern reference sampling, both in the cultivated and wild compartments; ii) tests the possible side effect of the number of pips per berry; iii) includes more berry measurements; iv) compares such length measurements with a geometric morphometric approach, namely outline analyses using elliptical Fourier transforms; v) tests the feasibility of archaeobotanical application.

This paper is divided into four parts: i) how does *size* (co)vary between pips and berries, and depending of the number of seeds?; ii) how does *shape* (co)vary between pips and berries and depending piposity?; iii) how much pip shape depends on berry size, number of seeds per berry, status, accession, and which practical consequences for archaeobotanical studies? iv) can we infer the dimensions of the (vanished) berry dimensions from the (recovered archaeological) pips?

Material and Methods

Nomenclature

Hereafter, *status* designs compartment (domesticated or wild); *accession* designs the variety or cultivar (or *cépage*) for cultivated grapevines and the individual for wild grapevines; synecdochically, a domesticated/wild pip/berry refer to the accession they were collected from; *usage* designs the typical destination of cultivated grapevine (wine or table); *cultivation* designs for wild grapevines whether they were cultivated in collection or collected *in natura*; *form* is used when *shape* and *size* are used in combination; "*piposity*" is short for "given a pip, the number of pips in the berry where it was sampled".

Modern and archaeological material

Modern reference material is composed of 49 accessions (30 domesticated and 19 wild) from Euro-Mediterranean traditional cultivars and wild grapevines (Table 1). Fourteen wild grapevines were collected at ripeness in their habitat and five were cultivated, along with

domesticated accessions, in the French National Grapevine Germplasm Collection (INRA *Domaine de Vassal*, Montpellier, France; https://www6.montpellier.inra.fr/vassal). Among domesticated accessions, 21 were wine varieties and 9 table varieties. For each accession, 30 normally developed berries have been haphazardly collected from a single, fully ripe bunch.

Archaeological material comes from wells of the Roman farm of Sauvian – La Lesse (US3022, US3063, US3171 and US3183) which has been extensively described elsewhere (Figueiral *et al.*, 2015). The waterlogged conditions of the two wells allowed very good preservation of numerous uncharred pips (N=205) while dating of the archaeological layers was provided by numerous chronologically characteristic pottery and coin remains (2025-1725 BP). This material provided an archaeobotanical application.

Traditional measurements

On modern material, the berry diameter ($berry_{Diameter}$), height ($berry_{Height}$) and mass ($berry_{Mass}$) were obtained before dissection (Table 1). Mass was not available for 9 accessions; these accessions were removed for further analyses when mass was involved. Then the number of pips (hereafter "piposity") was recorded and, if not unique, one pip was haphazardly chosen. In our dataset, a single berry from the variety "Kravi tzitzi" was found with 5 pips and the corresponding pip was discarded from further analyses. The dataset eventually consisted in 1469 pips (48×30 + 1×29) and was thus almost perfectly balanced between accessions.

Then, on each modern and archaeological pip, five length measurements were manually (Table 1, Figure 1) recorded using ImageJ by the same operator (L. Bouby): total length (pip_{Length}), length of stalk ($pip_{LengthStalk}$), position of the chalaza ($pip_{PositionChalaza}$), breadth ($pip_{Breadth}$) and thickness ($pip_{Thickness}$). All pips have been photographed in dorsal and lateral views by the same operator (T. Pastor) using an Olympus SZ-ET stereomicroscope and an Olympus DP 12 camera.

Statistical environment

All statistical analyses were performed using the R 3.5.1 environment (R Development Core Team, 2018) using the packages Momocs 1.3.0 for everything morphometrics (Bonhomme *et al.*, 2014; Bonhomme, 2018) and packages of tidyverse 1.2.1 for data manipulation and most graphics (Wickham & Grolemund, 2017). This stringent level was chosen to ensure marked differences for subsequent archaeobotanical application and ensure an overall alpha level below 0.05 when repeated tests were done (i.e., to apply a Bonferroni correction). All length

measurements were log-transformed to focus on relative changes and minimize size differences (Bouby *et al.*, 2013); the mass was cubic-root transformed for the same reason.

As preliminary analyses on modern material, differences between average piposity were tested using generalized linear model with Poisson error; differences in their distributions were tested using two-sided Fisher's exact tests on count data (Figure 2).

Testing the covariation between pip and berry size in relation to the number of pips

On modern material, three sets of differences in pips and berries measurements were tested within and between piposity levels using Wilcoxon rank tests (not affected by log). First, differences were tested between: (1) domesticated and wild accessions and reported on (Figure 3a); (2) then, the same differences were tested between wine and table varieties for domesticated accessions (Figure 3b); and finally (3) between cultivated wild individuals and those collected in their habitat (Figure 3c). Differences were also tested on the same three sets but without considering the number of pips (Figure A – SI).

Bivariate comparisons were then explored between the domesticated and wild accessions (piposity not considered), and tested using an analysis of covariance. When the "status" factor was found significant, separate regressions were then tested and, if significant, the adjusted r^2 was obtained and reported on Figure B (SI).

Testing the covariation between pip and berry shapes in relation to the number of pips

Outlines coordinates were obtained from photographs, centred, scaled, aligned and normalized for their first point before elliptical Fourier transforms (EFT). These preliminary steps removed positional, size, rotation and phasing differences between outlines and EFT could then be used without numerical normalization (Claude, 2008). EFT were performed on the two views separately, and the number of harmonics was chosen to gather 99% of the total harmonic power (8 for both views). Then 64 coefficients were obtained for each pip (2 views × 8 harmonics per view × 4 coefficients per harmonic).

To explore the overall variability of shape, a principal component analysis (PCA) was calculated on the full, raw matrix of Fourier coefficients (Figure 4). The first two PCs were used as synthetic shape variables (See Results). To test the effect of piposity and pip dimension on pip shape, the same approach than for length measurements was used with PC1 and PC2 as the response variables. To test the relation between shape and pip length (only pip_{Length} was used), analyses of covariance first tested if separate regressions were justified

(Figure 5a). Then Wilcoxon rank tests were used to test shape differences between and within piposity levels (Figure 5b).

To visualize shape differences between extreme piposity levels (1 and 4), and within subsets of interest, mean shapes for the dorsal and lateral views were calculated on the matrix of coefficients (Figure 6). These differences were quantified with the mean absolute difference (MD) between each sets of Fourier coefficients. To make these differences meaningful, they were divided by the mean difference of Fourier coefficients between cultivated and wild accessions with all piposity levels pooled. For each subset, MD was thus calculated as $(|\overline{\text{coefficients}_{\text{subset, 1-pip}}} - \overline{\text{coefficients}_{\text{subset, 4-pips}}}|)/(|\overline{\text{coefficients}_{\text{domesticated, all pips}}} - \overline{\text{coefficients}_{\text{wild, all pips}}}|)$. For example, a MD equals to 0 would indicate no difference between pips with a piposity of 1 and those with a piposity of 4; a MD greater than unit would indicate more differences relatively to differences that exist between cultivated and wild individuals (Figure 6).

Pip shape and size in relation to status, accession and piposity

To evaluate the respective contribution of berry dimensions, accession and piposity onto pip shape, a multivariate analysis of variance was used according to the following model: all Fourier coefficients ~ $\text{berry}_{\text{Height}}$ + accessions + piposity within accession. Since it was highly correlated to other berry measurements, only $\text{berry}_{\text{Height}}$ was used to describe berry dimensions. The contribution of each variable is the ratio of its sum of squares over the total sum of squares (thus including residuals). Again, this is tested on the different subsets of interest (Figure 7).

Linear discriminant analyses (LDA), implemented in the MASS package (Venables & Ripley, 2002) were used to evaluate whether piposity could preclude status and accession classification accuracy. Different combinations of predictors (sizes, shape and, sizes + shape) were also evaluated to compare their performance to classify the pips to their correct status and their original accession (Figure 8).

Given a combination of (status, accession) × (sizes+shape, sizes, shape), a leave-one-out cross-validation was used to assess status and accession classification accuracies. The principle of this method is to calculate a LDA on all-but-one pips of the sample and, then, to predict the classification of the omitted pip. This procedure is repeated for each pip of the sample. Classification accuracy was evaluated on all pips, to mirror the typical archaeobotanical context where the piposity cannot be known but also, on the four piposity levels (Figure 8).

To cope with unbalanced group structures, we also calculated a baseline for each subset that estimates the mean and maximum accuracy one can obtain by chance, using 10000 permutations (Evin *et al.*, 2013). If the accuracy observed is higher than the maximum value obtained using permutations, then the LDA can be considered to perform better than random, with an estimated alpha below 10^{-4}.

Predict the dimensions of the (vanished) berry dimensions from the (recovered archaeological) pips

To test whether *berry* dimensions can be inferred from the *pip* dimensions, separate multivariate regressions were calculated, on the reference material, for berry height and diameter (using the five length measurements on pips). For the sake of replicability, we decided to only use length measurements, that are straightforward to obtain from pips (whether modern or archaeological), which is not the case for Fourier coefficients. The difference between domesticated and wild grapevines regressions was first tested using an analysis of covariance: two regressions (one for cultivated, one for wild) were obtained for the berry height and two others for its diameter (Figure 9). These four regressions were fitted using stepwise regression with backward elimination based on the AIC, and started with full models ($berry_{Height}$/$berry_{Diameter}$ for wild/domesticated ~ pip_{Length} + $pip_{LengthStalk}$ + $pip_{PositionChalaza}$ + $pip_{Breadth}$ + $pip_{Thickness}$, all logged). Then, archaeological pips were classified into domesticated or wild using an LDA trained on the dimensions of modern pips. Pips with a posterior probability< 0.8 were filtered out. Finally, the berry height and diameter of this archaeological material were inferred using the corresponding models (Figure 10).

Results

Preliminary analyses on modern material

The average piposity, the number of pips per berry, is equivalent between domesticated and wild accessions (mean±sd: domesticated=2.01±0.891, wild=2.1±0.968; generalized linear model with Poisson error: df=1468, z=1.234, P=0.217 – Figure 2). Only the piposity distributions differed (Fisher's exact test: P=0.004), but this was likely due to a higher proportion of 4-pips berries in wild grapevines Fisher's exact test with 4-pips berries removed: P=0.6272). No difference were observed between cultivated (domesticated and wild) accessions and wild collected in their habitat (mean±sd: cultivated=2.06±0.931, in

natura=2.00±0.902; generalized linear model with Poisson error: df=1468, z=-0.676, P=0.499).

Covariation between pip and berry size in relation to the number of pips

The average piposity, the number of pips per berry, was slightly lower for cultivated grapevines yet not different (mean±sd: domesticated=2.01±0.891, wild=2.1±0.968; generalized linear model with Poisson error: df=1468, z=1.234, P=0.217 – Figure 2). Only the distributions differed (Fisher's exact test: P=0.004), but this was likely due to a higher proportion of 4-pips berries in wild grapevines Fisher's exact test with 4-pips berries removed: P=0.6272). No difference were observed between cultivated (domesticated and wild) accessions cultivated and (wild only) collected in their habitat (mean±sd: cultivated=2.06±0.931, in natura=2.00±0.902; generalized linear model with Poisson error: df=1468, z=-0.676, P=0.499).

Wild vs. domesticated. As expected, all berries and pips measurements were overall smaller for wild accessions compared to their domesticated counterparts (Wilcoxon one-tailed rank tests: all $P<10^{-8}$ – Figure A). Some pip dimensions were more contrasted between wild and cultivated grapevines than others ($pip_{LengthStalk} > pip_{PositionChalaza} > pip_{Length} > pip_{Thickness} > pip_{Breadth}$). Differences were even more marked on berries, domesticated grapevines having larger berries than their wild counterparts (Wilcoxon one-tailed rank tests: all $P \leq 10^{-16}$).

Whatever the piposity, the domesticated grapevines have the highest lengths (with four exceptions concerning the median, for 3-pips $pip_{Breadth}$ and for 4-pips pip_{Length}, $pip_{Breadth}$ and $pip_{Thickness}$ but, differences were non-significant – Figure 3a). $Pip_{LengthStalk}$ and $pip_{PositionChalaza}$ are found different for all piposity levels. For pip_{Length}, $pip_{Breadth}$ and $pip_{Thickness}$ are less contrasted between wild and domesticated grapevines, particularly when piposity is high. Overall, the highest the piposity, the lower the contrast between domesticated and wild. Indeed, for 1-pip berries, all lengths comparisons but $pip_{Thickness}$ were found different but, for 4-pips berries that is only true for $pip_{LengthStalk}$ and $pip_{PositionChalaza}$ (Figure 3a). Interestingly, the pip dimensions of wild grapevines increase faster with the piposity than their domesticated counterpart decrease. Indeed, for 1-pip berries, all lengths comparisons but $pip_{Thickness}$ were found different but, for 4-pips berries that is only true for $pip_{LengthStalk}$ and $pip_{PositionChalaza}$ (Figure 3a).

In wild grapevines, larger berries have more and larger pips (Figure 3a). Berries with 2 pips have higher berry diameter, height and mass than those with a single pip (no exception, all $P<10^{-9}$; similarly for 3 versus 2 (for $berry_{Mass}$, P was above the chosen alpha but with

P=0.0001), and less markedly for 4 versus 3 (all P<0.025). No differences in berry dimensions/mass along increasing piposity were found for the cultivated grapevines, excepted between 1-pip and 2-pip for $pip_{Thickness}$ (P=0.006).

Table vs. wine cultivars. Table grapes have globally significantly higher dimensions than wine varieties (Figure 3b). With increasing piposity, table varieties tend to have bigger berries which does not seem to be the case for wine varieties, but differences between low (1+2) and high (3+4) piposity levels were not significant. For pips, the only significant differences between low and high piposities were found for wine varieties and for $pip_{Breadth}$ and $pip_{Thickness}$ ($P<10^{-16}$).

Wild in cultivation vs. wild in natura. Similarly, wild grapevine pips and berries appear to be bigger when in cultivation than their counterparts growing *in natura* (again some P above the chosen alpha yet the highest P was for 4-pips with P=0.005 – Figure 3c). Besides these global differences, trends of all measured variables are similar along increasing piposity. The berry mass ratios, relatively to the berry mass of wild collected in their habitat, were on average, 6.4 for wine varieties, 15.6 for table ones and 1.8 for cultivated wild,.

Bivariate comparisons (Figure B, SI) indicate positive correlations between all pairwise dimensions (no exception among significant regressions). The total pip_{Length} appears to be the most consistent variable, between domesticated and wild grapevines: indeed, only the correlation with the $pip_{LengthStalk}$ show a significant interaction. Inversely, the correlations implying the length of stalk always show a significant interaction and thus the need for separate regressions. For pips dimensions, the best correlations were found between pip_{Length} and $pip_{PositionChalaza}$ (adj. r^2=0.787) among those with non-significant interactions and between $pip_{LengthStalk}$ and $pip_{PositionChalaza}$ (adj. r^2_{wild}=0.615, adj. $r^2_{domesticated}$=0.717) among those with significant interactions. Compared to pips dimensions, correlations between berry dimensions were much better (all significant and adj. r^2=0.864) and the three possible interactions were all significant.

Covariation between pip and berry shape in relation to the number of pips

The PCA obtained on the combined views shows that the first two PCs (Figure 5) gathered 69% of the total shape variation, and higher rank components clearly levelling off (PC1=43.0%; PC2=26.2%; PC3=6.7%; PC4=3.9%; etc.), only the first two PCs were thus

further used as two synthetic shape variables. Convex hulls illustrate that shape differences between wild and domesticated grapevines are mostly captured on PC1. Nevertheless, scores on both PC1 (Wilcoxon rank tests, $P<10^{-16}$) and PC2 ($P<10^{-16}$) were different. PC1 is a synthetic shape variable that summarizes how prominent is the stalk and how round is the pip. PC2 represents the circularity, a more global length/width ratio of pips, for the two views.

Regarding exploration of shape versus pip dimensions, pip length – correlated to all other measurements – is correlated itself with position on PC1. Two regressions were justified (Analysis of covariance: df=1, F=362.7, $P<10^{-16}$), their slope are identical (df=1, F=0.037, P=0.848) but, the intercept differed between wild and domesticated. These two regressions were significant yet r^2 were low (wild: $P<10^{-16}$, adj. r^2=0.195; domesticated: $P<10^{-16}$, adj. r^2=0.240 – Figure 5a). When PC1 and PC2 are considered jointly, two regressions were not justified (P=0.04) and the r^2 was even lower (P=0.04, adj. r^2=0.181 – Figure 5a). The longer the pip is, the more "domesticate" it looks like, particularly in terms of stalk prominence.

As concerns shape versus piposity, the latter is associated with shape changes on PC1 between wild and domesticated both overall (see above) and within levels (Wilcoxon rank tests, all $P<10^{-10}$ – Figure 5b). Within domesticated accessions, differences were never significant. Within wild accessions, differences were not found between pairs of successive piposity levels but those between 1-3, 2-3 and 2-4 (all with $P<10^{-8}$). For PC2, differences observed globally between domesticated and wild vanished for high piposities (1-pip: $P<10^{-12}$; 2-pips: $P<10^{-9}$; 3-pips: P=0.016; 4-pips: P=0.035 – Figure 5b). No differences within wild/domesticated and between successive piposities were found significant.

Average shapes reconstructed from the raw matrices of Fourier coefficients (Figure 6) illustrate these results. The mean absolute difference (MD) confirms that larger changes between extreme piposities are observed within wild grapevines (particularly for cultivated ones) and reveals that most of these changes affect the dorsal side of the pips.

Pip shape and size in relation to status, accession and piposity; consequences for archaeobotanical inference

The respective contributions of berry height, accession and piposity on the shape of pips (Figure 7) show that the accession is, overall and by far, the most correlated factor affecting pip shape. Among the different subsets, the accession factor has a higher impact on domesticated than on wild, and on cultivated wild accessions than on those collected in natura. By contrast, its contributions for wine and table domesticated varieties were similar.

Here again, piposity and berry height both affect the pip shape of wild accessions but have a limited (piposity) and very low (berry height) contribution for domesticated accessions.

Classification accuracies were compared using different training data and on different subsets (Figure 8). When different piposity levels were pooled, mirroring archaeobotanical admixtures, discrimination was very good at the status level (Figure 8a). As expected, size + shape performed better (95%), than shape (93.7%) and size (92.5%) alone. When these models were evaluated on piposity subsets, they all have an accuracy above 91%, except for 4-pips berries. In all cases, accuracies were much higher than what could be obtained by chance alone. Accuracies were lower at the accession level (Figure 8b) and when piposity levels were pooled size + shape (89.8%) outperformed shape alone (81.3%) and size alone (46.3%). The same model ranking was observed on piposity subset, except for 4-pips berries here again. Overall, accuracies were nevertheless much better than chance alone.

Application to the archaeological pips from the Roman site of Sauvian – La Lesse (Hérault, France): can we predict the dimensions of the (vanished) berry dimensions from the (recovered) pips?

On modern material, we used the size of pips to infer berry heights and diameters. Both regressions show a significant interaction of the status ($berry_{Diameter}$: df=1, F=8075, $P<10^{-16}$; $berry_{Diameter}$: df=1, F=7124, $P<10^{-16}$), and two regressions for berry diameter and two others for its height were obtained (Figure 9a, b). All were significant (all $P<10^{-16}$) yet the adjusted r^2 were quite low ($berry_{Diameter}$ adj. r^2_{wild}=0.583, adj. $r^2_{domesticated}$=0.468; $berry_{Height}$ adj. r^2_{wild}=0.609, $r^2_{domesticated}$=0.485). Final models all used pip_{Length} + $pip_{LengthStalk}$ + $pip_{Thickness}$ + $pip_{Thickness}$ predictors, except $berry_{Height}$ for wild that used $pip_{PositionChalaza}$ "instead" of $pip_{LengthStalk}$ (Table 2). On unlogged berry diameter and height, the relative deviations were obtained (Figure 9a, b). Mean relative deviation per accession for $berry_{Diamater}$ ranged from -13.8% to +10.5% for wild, and from -23.2% to +18.1% for domesticated; for $berry_{Height}$ they ranged from -13.3% to +13.7% for wild, and from -36.5% to +28.7% for domesticated. Then, these four models were applied on the archaeobotanical material after being classified at the wild/domesticated level using LDA. 46 pips (22%) were classified with a posterior probability < 0.8 and were filtered out. Among the remaining pips, 113 (71%) were classified as domesticated, 46 (29%) as wild. When compared to their modern analogues (Figure 10), the length of "domesticated pips" were closer to those of wine varieties than table varieties; the lengths of "wild pips" were intermediate between wild accessions collected in their habitat and those cultivated. For domesticated archaeological material, both inferred berry height and diameter were

intermediate between wine and table modern varieties yet closer to wine ones. Similarly, for wild archaeological material, inferred berry height and diameter were intermediate between wild collected in their habitat and those cultivated but closer to the former.

Discussion

405 This study contributes to understand Vitis vinifera phenotypic changes under domestication by clarifying many aspects of size and shape (co)variation between pip and berry in both wild and domesticated vines. It helps disentangle the interplay of the number of seeds per berry, berry dimensions, domestication, varietal diversity and cultivation practices onto the pip shape. We discuss implications for *Vitis vinifera* eco-evo-devo and perspectives for 410 archaeobotanical studies, where size and shape are largely used to discriminate seeds from wild and cultivated vines, and for which a possible application is proposed.

Patterns of covariation between the form of the pip, the form of the berry and the piposity

With four ovules per ovary, the theoretical maximum number of pips per berry is four, yet one (over almost 1500) was observed with five. Such abnormal piposities have already been 415 reported (T. Lacombe, *pers. comm.*). Most berries collected had two pips, and more than 70% of them had only one or two pips. This is in accordance with previous publication (Houel *et al.*, 2013). There were no differences either between domesticated or wild (Figure 2), or between cultivated wild individuals and those collected in their habitat. One could have expected, on average, higher piposity, for domesticated (since they have hermaphroditic 420 flowers), and more generally for cultivated accessions (since the pollen rain is expected to be lower, or even limiting, in the natural habitat).

Overall, and this is no surprise, wild pips and berries are smaller than their domesticated counterparts. Similarly pips and berries of wine varieties are smaller than those of table varieties, as pips and berries of wild vines collected in their habitat are smaller than those 425 from cultivated wild individuals. This study clarifies the possibly confounding effect of piposity reported by previous studies (Negrul, 1960; Olmo, 1995; Bouby *et al.*, 2013; Houel *et al.*, 2013). Among vertebrate dispersed plants, the reward (the fruit pulp mass) associate with a given seed mass is commensurate with work required to move it, and expected to scale relatively (Edwards, 2006). For wild grapevine, and vertebrate dispersed plant species in

general, fruit and pip dimensions/masses is furthermore constrained by their dispersers and by a general trade-off between seed size/number (Leishman, 2001).

On the piposity side, and again for all but wine varieties, the higher the piposity the longer the pip and the bigger the berry in which they develop (Figure 3). For these groups, it seems that more numerous pips are not limited by space or nutrients but rather contribute the development of bigger berries. The berry development is well known (Dokoozlian, 2000; Ristic & Iland, 2005) and can be divided into two phases of enlargement. The first, prior to anthesis, is a period of rapid berry growth mostly due to cell division. After anthesis, berry growth is largely due to cell enlargement. Coombe (1973) reported that 17 doublings of cell number occurred in the ovaries before anthesis, compared to only two after anthesis. Ojeda *et al.* (1999) suggested that seed growth may also increases cell mitosis in the developing berry. As suggested by Houel *et al.* (2013) auxins, cytokinins and gibberelins, upregulated shortly after fertilisation in grapevine ovaries, are likely to trigger fruit growth by cell expansion.

The absence of positive (or even negative) correlations between piposity, pip and berry dimensions for wine varieties remains unclear. Perhaps, the regulation, if any, is at the bunch or stock scale and, whether it has been selected (for example to concentrate sugars, aromas and flavours) or it is a by-product of another trait under selection for accessions destined to wine production. Since table varieties are bigger than wine varieties, the berry dimensions of the latter cannot be argued to have reached a developmental limit.

Finally, bivariate correlations concerning berry dimensions and mass are the best observed. This indicate robust allometries in berry sizes and mass, in other words that berry largely remain ellipsoid in shape, independently of their dimensions.

Morphometrics and domestication draw the curtain into grapevine eco-evo-devo

Domestication releases “natural” constraints by actively selecting for bigger seeds or fruits, for grapevine and for many cultivated plants in general (Fuller *et al.*, 2014; Bonhomme *et al.*, 2015), or by taking advantage of this trade-off with cultivation practices such as pruning. This may explain why cultivated wild accessions, have bigger berries for high piposities: the number of grapes is reduced, leading to bigger ones. Cultivation also releases most of the constraints of the wild, competition for water, taller plants competition for light, self-supporting and climbing costs, etc.

Such evidence of plastic and canalized phenotypic expression may be fuel for further eco-evo-devo studies. The latter brings a conceptual and experimental framework that relies on environmentally mediated regulatory systems to better understand ecological and

evolutionary changes (Sultan, 2007). Here, the norm of reaction of the pip size and shape, along increasing piposity and berry dimensions, is clearly different at the three investigated levels: between wild and domesticated, between wine and table, between cultivated wild and those collected in their habitat.

As seen from an eco-evo-devo perspective, domestication results in a change of phenotypic patterns that are selected or at least desirable. These phenotypic outputs arise from genetic background and signalling networks and that are rooted in phylogenetic history of the wild progenitor, constrained by physics, and modulated by environmental inputs. The aim of eco-evo-devo is to uncover the rules that underlie the interactions between an organism's environment, genes, and development and to incorporate these rules into evolutionary theory (Abouheif *et al.*, 2014).

Consequences for archaeological inference

Taken independently or in pairwise comparisons, some pip lengths differences between wild and domesticated appear more "robust" to increasing piposities than others, notably the length of stalk ($pip_{LengthStalk}$) and the position of the chalaza ($pip_{PositionChalaza}$ – Figure 3a, Figure A, SI). Their interest in discriminating domestication status has long been known and used, including when recovered archaeological material is charred (Smith & Jones, 1990; Mangafa & Kotsakis, 1996; Bouby *et al.*, 2013), and this has been experimentally tested (Bouby *et al.*, 2018).

Overall shape variability, as captured by the first two principal components, also tend to distinguish the wild and domesticated grapevines as already observed elsewhere (Terral *et al.*, 2010; Terral & Bouby, 2013). Yet the proper way to discriminate is using LDA and will be discussed below, the wild/domesticated differences are largely collinear with the largest shape variability components when an admixture of wild and domesticate pips is considered.

The piposity being positively correlated to pip length of wild accessions, the same pattern is observed: piposity is correlated with shape changes for wild accessions (Figure 5). Most of these changes affect the dorsal side of wild pips, that are much larger when piposity is high. This is illustrated and quantified in the Figure 6 where mean absolute difference indicate that there is ~70% as much difference, between 1- and 4-pips for wild accessions, than those observed between average wild and domesticated. Differences in extreme piposities are even larger than unit, that is larger than this "domestication gap", for the cultivated wild. This does not answer the question whether past vineyards cultivated "true"

wild grapevines or "weakly" domesticated forms (Bouby *et al.*, 2013; Pagnoux *et al.*, 2015) but it points out a possible mechanism underlying this confusion.

Pip shape being largely used in archaeobotany, it is crucial to point out which factors contribute to its variability, or at least covary with it. In this study, the main factor associated to pip differences was, by far, the accession and it was even more important for domesticated accessions than for wild ones (Figure 7). Relatively to accession, berry height and piposity poorly contributed to observed differences. This first confirms the usefulness of shape and its robustness to identify varieties (or morphotypes that are shape archetypes). It may also indicate that domestication, by creating (or revealing) agrobiodiversity, chiefly in traits of interest, may also favour pip shape diversification whether genes controlling it may be linked to those directly selected or the product of drift, that is non-adaptive.

Here we show the reliability of classification, independently of piposity. Indeed, classification accuracies at the status level were all good (Figure 8), even when the models trained on the pip admixture where evaluated on piposity subsets. Shape was nonetheless superior to size alone but, when considered jointly, classification were improved. Whenever possible, size should thus be included along morphometric coefficients and used jointly in classification models. Accuracies at the accession levels evidenced even more clearly the latter conclusions: the superiority of shape over dimensions, and the benefit of using them jointly when it comes to infra-specific classification.

Shape is overall more robust than sizes when models were evaluated on piposity subsets. The only exception, for both status and accession levels, were obtained on the 4-pips subset. This is likely due to the reported different piposity structure due to this 4-pips subset. This illustrates one limit of our experimental design, intended to reflect real-world admixtures rather than designing one where all levels of all studied factors would have been perfectly controlled and, primarily, balanced. That being said, such bias in the piposity structure is very unlikely to affect archaeobotanical identification when it includes shape, either at the (sub)specific level between wild and domesticated, or at the infraspecific level when one wants to identify accessions or morphotypes.

An application on archaeological material: inferring berry size from pip

Berry is home to the most selected traits, from the beginnings of domestication to varietal breeding and diversification times. Unfortunately, its dimensions cannot be quantified directly on archaeological material. The latter is indeed limited to pips, mostly charred or water-logged; fleshy parts are usually absent, or too degraded to allow any morphometric approach.

Thus, one falls back on actualistic inference trained on modern material, and this is what we tried for the first time here. Multiple regression on pip dimensions were obtained on our sample of modern domesticated and wild accessions. Berry diameter (Figure 9a) and height (Figure 9b) were far from perfectly predicted, particularly for the domesticated accessions; relative deviations from actual dimensions were nevertheless centered on zero and overall in the ±25% range.

Archaeological material from Sauvian – La Lesse presents an admixture of wild and domesticated type (Figueiral *et al.*, 2015). Berry dimensions inferred from pips of this site are intermediate between the wild growing in their habitat and those cultivated (Figure 10). This may suggest that past vineyards cultivated wild individuals. The berry dimensions inferred for domesticated varieties were closer to wine varieties than to table ones. This is congruent with the unequivocal wine production at this period and in this region (Figueiral *et al.*, 2010, 2015).

Conclusion

In our view, the main finding of this systematic exploration of berry and pip form covariation is that the more "pipose" is a wild grapevine, the bigger is the berry and the longer is the pip. For both wild and domesticated, the longer is the pip, the more its shape looks like "domesticated". Further studies will clarify the contribution of cultivation practices contribution on pip shape, largely used in archaeobotanical studies to better understand viticulture history. These findings on grapevine pave the way for dedicated studies to shed light on genetic, functional and evolutionary changes that occurred in *Vitis vinifera* between the seed, its reproductive unit, and the berry, its dispersal reward and main target of its domestication and amelioration. More largely, domesticated taxa appear as model of choice to better understand this interplay for other organisms.

Acknowledgements

This study is funded by ANR project "Viticulture: Vignes et vins en France du Néolithique au Moyen Âge. Approche intégrée en archéosciences" (PI: Laurent Bouby) and supported by the OSU-OREME (https://oreme.org/) that helped to the constitution of the wild grape pip collection. We are grateful to the grapevine germplasm collection of INRA, Domaine de Vassal (Marseillan-Plage, France) that provided all the pips from cultivated varieties and

cultivated wild grapes. We warmly acknowledge Michael Wallace for his help with English. This is ISEM contribution 2019-NNNN.

Author contribution

LB, SP, SI and JFT conceived and designed the experiments; SP, SI, TP and LB acquired data; VB, led the analyses with important contributions from SP, AE, JFT, LB and RB; VB led the manuscript writing with the help of all co-authors, and chiefly SP.

References

Abouheif E, Favé M-J, Ibarrarán-Viniegra AS, Lesoway MP, Rafiqi AM, Rajendhran R. **2014**. *Eco-Evo-Devo: The Time Has Come* (CR Landry et N Aubin-Horth, Éd.). Dordrecht: Springer Netherlands.

Arroyo-García R, Ruiz-García L, Bolling L, Ocete R, López MA, Arnold C, Ergul A, Söylemezoğlu G, Uzun HI, Cabello F, *et al.* 2006. Multiple origins of cultivated grapevine (Vitis vinifera L. ssp. sativa) based on chloroplast DNA polymorphisms. *Molecular Ecology* **15**: 3707-3714.

Bacilieri R, Bouby L, Figueiral I, Schaal C, Terral J-F, Breton C, Picq S, Weber A, Schlumbaum A. **2017**. Potential of combining morphometry and ancient DNA information to investigate grapevine domestication. *Vegetation History and Archaeobotany* **26**: 345-356.

Bonhomme V. **2018**. MomX repository.

Bonhomme V, Forster E, Wallace M, Stillman E, Charles M, Jones G. **2015**. The first shoots of a modern morphometrics approach to the origins of agriculture. *Web Ecology* **16**: 1-2.

Bonhomme V, Picq S, Gaucherel C, Claude J. **2014**. Momocs: Outline Analysis Using R. *Journal of Statistical Software* **56**.

Bouby L, Bonhomme V, Ivorra S, Pastor T, Rovira N, Tillier M, Pagnoux C, Terral J-F. **2018**. Back from burn out: are experimentally charred grapevine pips too distorted to be characterized using morphometrics? *Archaeological and Anthropological Sciences* **10**: 943-954.

Bouby L, Figueiral I, Bouchette A, Rovira N, Ivorra S, Lacombe T, Pastor T, Picq S, Marinval P, Terral J-F. **2013**. Bioarchaeological Insights into the Process of Domestication of Grapevine (Vitis vinifera L.) during Roman Times in Southern France (P Degryse, Éd.). *PLoS ONE* **8**: e63195.

Brun J-P. **2003**. *Le vin et l'huile dans la Méditerranée antique : Viticulture, oléiculture et procédés de fabrication*. Editions Errance.

Claude J. **2008**. *Morphometrics with R*. New York, NY: Springer New York.

Coombe BG. **1973**. The regulation of set and development of the grape berry. *Acta Horticulturae*: 261-274.

Dokoozlian N. **2000**. Grape berry growth and development. In: Raisin Production Manual. Oakland: University of California, Agricultural and Natural Resources, 30-37.

Edwards W. **2006**. Plants reward seed dispersers in proportion to their effort: The relationship between pulp mass and seed mass in vertebrate dispersed plants. *Evolutionary Ecology* **20**: 365-376.

Evin A, Cucchi T, Cardini A, Strand Vidarsdottir U, Larson G, Dobney K. **2013**. The long and winding road: identifying pig domestication through molar size and shape. *Journal of Archaeological Science* **40**: 735-743.

FAO. **2017**. *FAOSTAT*.

Figueiral I, Bouby L, Buffat L, Petitot H, Terral JF. **2010**. Archaeobotany, vine growing and wine producing in Roman Southern France: the site of Gasquinoy (Béziers, Hérault). *Journal of Archaeological Science* **37**: 139-149.

Figueiral I, Pomarèdes H, Court-Picon M, Bouby L, Tardy C, Terral J-F. **2015**. New insights into Mediterranean Gallo-Roman farming: a closer look at archaeological wells in Southern France. *Archaeological and Anthropological Sciences* **7**: 201-233.

Fuller DQ. **2007**. Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World. *Annals of Botany* **100**: 903-924.

Fuller DQ, Denham T, Arroyo-Kalin M, Lucas L, Stevens CJ, Qin L, Allaby RG, Purugganan MD. **2014**. Convergent evolution and parallelism in plant domestication revealed by an expanding archaeological record. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 6147-52.

Galet P. **1988**. *Cépages et vignobles de France* (Dehan, Éd.). Montpellier.

Grassi F, Labra M, Imazio S, Spada A, Sgorbati S, Scienza A, Sala F. **2003**. Evidence of a secondary grapevine domestication centre detected by SSR analysis. : 1315-1320.

Houel C, Martin-Magniette M-L, Nicolas SD, Lacombe T, Le Cunff L, Franck D, Torregrosa L, Conéjéro G, Lalet S, This P, *et al.* 2013. Genetic variability of berry size in the grapevine (Vitis vinifera L.). *Australian Journal of Grape and Wine Research* **19**: 208-220.

Imazio S, Maghradze D, De Lorenzis G, Bacilieri R, Laucou V, This P, Scienza A, Failla O. **2013**. From the cradle of grapevine domestication: molecular overview and

625 description of Georgian grapevine (Vitis vinifera L.) germplasm. *Tree Genetics & Genomes* **9**: 641-658.

Jones G, Valamoti S, Charles M. **2000**. Early crop diversity: A « new » glume wheat from northern Greece. *Vegetation History and Archaeobotany* **9**: 133-146.

Karasik A, Rahimi O, David M, Weiss E, Drori E. **2018**. Development of a 3D seed
630 morphological tool for grapevine variety identification, and its comparison with SSR analysis. *Scientific Reports* **8**: 6545.

Leishman MR. **2001**. Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. *Oikos* **93**: 294-302.

635 **Levadoux L**. **1956**. Les populations sauvages et cultivées des Vitis vinifera L. *Annales de l'amélioration des plantes* **6**: 59-118.

Mangafa M, Kotsakis K. **1996**. A New Method for the Identification of Wild and Cultivated Charred Grape Seeds. : 409-418.

McGovern PE. **2007**. *Ancient Wine The Search for the Origins of Viniculture*. Princeton
640 University Press.

McGovern P, Jalabadze M, Batiuk S, Callahan MP, Smith KE, Hall GR, Kvavadze E, Maghradze D, Rusishvili N, Bouby L, *et al.* 2017. Early Neolithic wine of Georgia in the South Caucasus. *Proceedings of the National Academy of Sciences* **114**: E10309-E10318.

Negrul A. **1960**. Evolucija razmera semjan i jagod u vinograda. *Izvaet TSHA*: 167–176.

645 **Ojeda H, Deloire A, Carbonneau A, Ageorges A, Romieu C**. **1999**. Berry development of grapevines: Relations between the growth of berries and their DNA content indicate cell multiplication and enlargement. *Vitis* **38**: 145-150.

Olmo H. **1995**. The Origins and Ancient History of Wine. In: McGovern P, éd. The Origins and Ancient History of Wine. Amsterdam: Gordon and Breach, 31-43.

650 **Orrù M, Grillo O, Lovicu G, Venora G, Bacchetta G**. **2013**. Morphological characterisation of *Vitis vinifera* L. seeds by image analysis and comparison with archaeological remains. *Vegetation History and Archaeobotany* **22**: 231-242.

Pagnoux C, Bouby L, Ivorra S, Petit C, Valamoti S-M, Pastor T, Picq S, Terral J-F. **2015**. Inferring the agrobiodiversity of Vitis vinifera L. (grapevine) in ancient Greece by
655 comparative shape analysis of archaeological and modern seeds. *Vegetation History and Archaeobotany* **24**: 75-84.

R Development Core Team. **2018**. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing, Vienna, Austria.*

Ristic R, Iland PG. **2005**. Relationships between seed and berry development of Vitis Vinifera L. cv Shiraz: Developmental changes in seed morphology and phenolic composition. *Australian Journal of Grape and Wine Research* **11**: 43-58.

Ros J, Evin A, Bouby L, Ruas M-P. **2014**. Geometric morphometric analysis of grain shape and the identification of two-rowed barley (*Hordeum vulgare* subsp. *distichum* L.) in southern France. *Journal of Archaeological Science* **41**: 568-575.

Smith H, Jones G. **1990**. Experiments on the effects of charring on cultivated grape seeds. *Journal of Archaeological Science* **17**: 317-327.

Sultan SE. **2007**. Development in context: the timely emergence of eco-devo. *Trends in Ecology & Evolution* **22**: 575-582.

Terral J-F, Alonso N, Chatti N, Capdevila RB i, Fabre L, Fiorentino G, Marinval P, Jordá GP, Pradat B, Rovira N, *et al.* 2004. Historical biogeography of olive domestication (Olea europaea L.) as revealed by geometrical morphometry applied to biological and archaeological material. *Journal of Biogeography* **31**: 63-77.

Terral J-F, Bouby L. **2013**. Domestication de la vigne (Vitis vinifera L.) et origines de cépages en France: apport de l'archéobiologie. *Food and History* **11**: 11-25.

Terral J-F, Newton C, Ivorra S, Gros-Balthazard M, de Morais CT, Picq S, Tengberg M, Pintaud J-C. **2012**. Insights into the historical biogeography of the date palm (*Phoenix dactylifera* L.) using geometric morphometry of modern and ancient seeds. *Journal of Biogeography* **39**: 929-941.

Terral J-F, Tabard E, Bouby L, Ivorra S, Pastor T, Figueiral I, Picq S, Chevance J-BB, Jung C, Fabre L, *et al.* 2010. Evolution and history of grapevine (Vitis vinifera) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Annals of Botany* **105**: 443-455.

This P, Lacombe T, Thomas MR. **2006**. Historical origins and genetic diversity of wine grapes. *Trends in Genetics* **22**: 511-519.

Ucchesu M, Orrù M, Grillo O, Venora G, Paglietti G, Ardu A, Bacchetta G. **2016**. Predictive method for correct identification of archaeological charred grape seeds: Support for advances in knowledge of grape domestication process. *PLoS ONE* **11**: 1-18.

Venables WN, Ripley BD. **2002**. *Modern Applied Statistics with S*. New York: Springer.

Wales N, Ramos Madrigal J, Cappellini E, Carmona Baez A, Samaniego Castruita JA, Romero-Navarro JA, Carøe C, Ávila-Arcos MC, Peñaloza F, Moreno-Mayar JV, *et al.* 2016. The limits and potential of paleogenomic techniques for reconstructing grapevine domestication. *Journal of Archaeological Science* **72**: 57-70.

Wallace M, Bonhomme V, Russell J, Stillman E, George TS, Ramsay L, Wishart J, Timpany S, Bull H, Booth A, *et al.* 2018. Searching for the Origins of Bere Barley: A

Geometric Morphometric Approach to Cereal Landrace Recognition in Archaeology. *Journal of Archaeological Method and Theory*.

Wickham H, Grolemund G. **2017**. *R for Data Science*. O'Reilly.

Willcox G. **2004**. Measuring grain size and identifying Near Eastern cereal domestication: evidence from the Euphrates valley. *Journal of Archaeological Science* **31**: 145-150.

Zohary D, Hopf M. 1993. *Domestication of plants in the world. The origin and spread of cultivated plants in west Asia, Europe and the Nile valley*. Clarendon Press, Oxford.

Tables and Figures legends

Table 1 Accessions used in this study. Dimensions are reported with mean±sd and given in mm, except for $berry_{mass}$ expressed in g.

Table 2 Estimates for pip lengths used to infer berry dimensions. Variables were all logged; so that $berry_{Diameter}$ (in mm and for wild) can be obtained with $\exp[0.65163 + 1.19914\times\log(pip_{Length}) + 0.10617\times\log(pipP_{ositionChalaza}) - 0.13263\times\log(pipBreadth) - 0.45449\times\log(pip_{Thickness})]$.

Figure 1 Dorsal and lateral views of a grapevine pip, here from the *Vitis vinifera* subsp. *sylvestris* wild" individual "Pic Saint-Loup 13", with indications of morphometric measurements: pip_{length} (total length), pip_{stalk} (length of the stalk), $pip_{chalaza}$ (position of the chalaza), pip_{width} (width) and $pip_{thickness}$ BL (thickness). Additionally, three measures were recorded on the berry: $berry_{Diameter}$ D (diameter), $berry_{Height}$ (height) and $berry_{Mass}$ (mass).

Figure 2 Distribution of the number of pips per berry for wild and domesticated grapevines.

Figure 3 Comparisons of (logged) lengths and (cubic-rooted) mass measurements. On rows are displayed different subsets: a) wild and domesticated grapevines, b) for domesticated accessions, table and wine varieties and, c) for wild accessions, those collected *in natura* and others cultivated as domesticated varieties. For each measurement, boxplots are displayed for each piposity level (number of pips per berry). Differences are tested using Wilcoxon rank tests and, if $P<10^{-5}$, displayed on top of brackets.

Figure A (SI) Comparisons of (logged) lengths and (cubic-rooted) mass measurements. On rows are displayed different subsets: a) wild and domesticated grapevines, b) for domesticated accessions, table and wine varieties and, c) for wild accessions, those collected *in natura* and others cultivated as domesticated varieties. Different piposity levels

are pooled (see Figure 3 for the detail). Differences are tested using Wilcoxon rank tests and all of them have a $P<10^{-5}$.

Figure B (SI) Bivariate pairwise plot between (logged) lengths and (cubic-rooted) mass measurements. For the sake of readability, only the wild versus domesticated status are displayed using different colours (green for wild; blue for domesticated). If two regressions are justified, then they are shown using the corresponding colours; otherwise a single regression line is showed in black. Then, for each regression, the correlations are tested and, if significant, the adjusted R^2 is displayed on the regression lines.

Figure 4 Principal component analysis on the joint matrices of Fourier coefficients obtained for the two views. The first two principal component gathered more than two third of the total variance. The component of shape variation they capture are illustrated with reconstructed shapes at each extreme of their range. Convex hulls of wild (green) and domesticated (blue) are shown.

Figure 5 a) Regressions PC1 and PC2 versus pip length for domesticated individuals, in shades of blue) and cultivation (for wild, in shades of green); b) Similarly, boxplots for each piposity levels.

Figure 6 Mean shapes calculated for all pips sampled from berry with 1 (blue) or 4 pips (red), for different subsets (in rows) and for the dorsal and lateral views (in columns). Between the two views, an index of shape differences between these two extreme piposity levels (0=identical shapes; unit=as much differences as between wild and domesticated average shapes).

Figure 7 Relative contribution of berry height, accession and number of pips per berry (coloured bars) onto the shape of pips for different subsets.

Figure 8 Classification accuracy (LDA leave-one-out) at the a) status and b) accessions levels. Models are trained (and evaluated) on the admixture of pips, then evaluated on piposity subsets. Different combinations of training data are used (Fourier coefficients of shape, lengths/mass measurements, both). Lines provide a random baseline and summarise 10000 permutations: solid line correspond to mean accuracy; dashed line to the maximal values obtained.

Figure 9 Regressions and predictions for berry dimensions from pip dimensions, obtained on modern material. The first row shows the predicted versus actual (logged) berry dimensions at the status level (all wild and domesticated accessions); the second row shows the relative deviation, at the accession level, and for real, unlogged, measurements. Columns are for berry diameter and height, respectively.

Figure 10 Distribution of pip lengths (observed for all), berry height and diameter inferred from pip dimensions for the archaeological material from Sauvian – La Lesse. Rows distinguish domesticated and wild grapes since separate regressions were required.

Table 1

			pip					berry		
accession	**status**	**dest./cult.**	**L**	**LS**	**PCH**	**BD**	**BL**	**bH**	**bD**	**bM**
Alvarinho	dom.	wine	5.18 ± 0.28	1.35 ± 0.19	2.66 ± 0.23	3.5 ± 0.21	2.5 ± 0.15	10.6 ± 0.81	10.93 ± 0.98	0.82 ± 0.21
Barbera	dom.	wine	6.73 ± 0.33	1.95 ± 0.14	3.58 ± 0.2	3.67 ± 0.23	2.7 ± 0.14	16.38 ± 1.07	15 ± 1.07	2.44 ± 0.49
Cabernet-Sauvignon	dom.	wine	5.93 ± 0.34	1.78 ± 0.16	3.09 ± 0.2	3.57 ± 0.22	2.65 ± 0.17	14.05 ± 0.89	13.47 ± 0.84	1.57 ± 0.27
Carignan	dom.	wine	6.85 ± 0.26	2.14 ± 0.21	3.85 ± 0.24	3.54 ± 0.22	2.73 ± 0.09	18.1 ± 0.83	16.85 ± 0.84	3.16 ± 0.41
Chardonnay	dom.	wine	5.28 ± 0.41	1.38 ± 0.2	3.06 ± 0.28	3.72 ± 0.28	2.78 ± 0.27	13.42 ± 1.24	12.9 ± 1.16	1.69 ± 0.41
Chevka	dom.	wine	5.97 ± 0.33	1.48 ± 0.13	3.05 ± 0.19	3.73 ± 0.19	2.53 ± 0.1	18.08 ± 1.17	16.51 ± 1.11	3.09 ± 0.58
Debina	dom.	wine	7.01 ± 0.48	1.73 ± 0.21	3.7 ± 0.3	4.21 ± 0.28	3.41 ± 0.19	19.61 ± 1.9	17.67 ± 1.58	3.89 ± 0.87
Feteasca alba	dom.	wine	5.19 ± 0.23	1.4 ± 0.13	2.65 ± 0.19	3.46 ± 0.19	2.5 ± 0.13	12.49 ± 0.79	12.99 ± 0.69	1.62 ± 0.26
Gaïdouria	dom.	wine	5.55 ± 0.37	1.61 ± 0.19	2.92 ± 0.23	3.8 ± 0.23	2.94 ± 0.14	16.51 ± 1.36	17.1 ± 1.3	3.46 ± 0.73
Grenache	dom.	wine	5.32 ± 0.29	1.46 ± 0.1	2.84 ± 0.17	3.38 ± 0.2	2.45 ± 0.14	15.91 ± 0.78	15.12 ± 0.66	2.3 ± 0.27
Kypreiko	dom.	wine	6.79 ± 0.39	2.05 ± 0.23	3.81 ± 0.26	3.99 ± 0.27	3.07 ± 0.12	21.38 ± 1.35	17.98 ± 1.16	4.26 ± 0.79
Mavrud	dom.	wine	6.9 ± 0.26	2.35 ± 0.18	3.93 ± 0.2	4.3 ± 0.19	3.19 ± 0.14	14.91 ± 0.9	14.53 ± 0.84	2.04 ± 0.31
Merlot	dom.	wine	6.02 ± 0.32	1.5 ± 0.15	3.01 ± 0.24	3.77 ± 0.24	2.75 ± 0.18	12.62 ± 1.04	12.83 ± 1.16	1.41 ± 0.31
Meslier Saint François	dom.	wine	5.51 ± 0.59	1.53 ± 0.2	3.05 ± 0.34	3.27 ± 0.31	2.61 ± 0.29	16.59 ± 1.64	15.54 ± 1.55	2.86 ± 0.77
Mourvèdre	dom.	wine	6.17 ± 0.41	1.51 ± 0.18	3.11 ± 0.27	3.73 ± 0.28	2.89 ± 0.2	15.71 ± 1.04	15.27 ± 1.16	2.28 ± 0.49
Muscat à petits grains roses	dom.	wine	5.23 ± 0.3	1.33 ± 0.19	2.95 ± 0.23	3.12 ± 0.18	2.39 ± 0.13	12.93 ± 1.16	13.82 ± 0.9	1.97 ± 0.28
Muscat noir à petits grains	dom.	wine	7.31 ± 0.29	2.27 ± 0.19	4.18 ± 0.26	4.2 ± 0.25	3.3 ± 0.16	20.07 ± 1.3	18.99 ± 1.39	
Pinot noir	dom.	wine	6.35 ± 0.31	1.77 ± 0.21	3.54 ± 0.2	3.93 ± 0.4	2.78 ± 0.19	13.87 ± 1.05	13.42 ± 1.19	1.83 ± 0.39
Roussanne	dom.	wine	6.57 ± 0.23	1.46 ± 0.15	3.39 ± 0.19	4.16 ± 0.23	3.09 ± 0.2	15.37 ± 0.77	14.91 ± 0.83	2.13 ± 0.32
Sauvignon	dom.	wine	6.53 ± 0.39	1.91 ± 0.19	3.49 ± 0.28	3.74 ± 0.18	2.68 ± 0.16	15.46 ± 1.07	13.45 ± 0.87	1.78 ± 0.3
Syrah	dom.	wine	5.39 ± 0.35	1.64 ± 0.18	3.14 ± 0.28	3.21 ± 0.17	2.54 ± 0.15	15.37 ± 0.91	13.51 ± 0.85	1.99 ± 0.29
Ain el Bouma	dom.	table	5.56 ± 0.41	1.9 ± 0.2	3.42 ± 0.23	3.53 ± 0.29	2.63 ± 0.14	21.11 ± 2.06	17.96 ± 1.8	4.51 ± 1.1
Chaouch blanc	dom.	table	6.95 ± 0.26	1.74 ± 0.16	3.86 ± 0.31	4.08 ± 0.25	3.44 ± 0.21	24.67 ± 1.85	22.55 ± 1.82	7.76 ± 1.59
Ferral tamara	dom.	table	6.79 ± 0.25	1.95 ± 0.18	3.52 ± 0.17	4.21 ± 0.18	3.29 ± 0.16	23.45 ± 1.22	19.73 ± 1.33	5.79 ± 0.83
Hadari	dom.	table	7.42 ± 0.38	1.89 ± 0.19	3.49 ± 0.28	4.2 ± 0.19	3.24 ± 0.15	21.54 ± 1.2	19.37 ± 1.03	4.73 ± 0.7
Hunisa	dom.	table	8.32 ± 0.39	2.27 ± 0.23	4.41 ± 0.27	4.94 ± 0.25	3.4 ± 0.2	30.28 ± 2.53	23.92 ± 1.68	9.87 ± 1.48
Kara papigi	dom.	table	6.58 ± 0.36	1.92 ± 0.2	3.21 ± 0.29	3.65 ± 0.22	2.8 ± 0.18	18.45 ± 1.67	16.29 ± 1.33	3 ± 0.73
Kravi tzitzi	dom.	table	7 ± 0.31	1.92 ± 0.21	3.51 ± 0.22	3.96 ± 0.17	3.09 ± 0.14	28.02 ± 2.11	19.37 ± 1.43	6.43 ± 1.19
Pembe Gemre	dom.	table	6.98 ± 0.33	2.11 ± 0.18	3.93 ± 0.26	3.84 ± 0.18	2.91 ± 0.09	16.46 ± 0.83	16.16 ± 1.02	3.07 ± 0.46
Sliva	dom.	table	6.98 ± 0.47	1.95 ± 0.29	3.67 ± 0.35	4.02 ± 0.2	3.23 ± 0.11	23.41 ± 1.65	19.8 ± 1.74	5.59 ± 1.28
Camp Saure	wild	in natura	5.23 ± 0.26	1.04 ± 0.15	2.46 ± 0.17	3.38 ± 0.17	2.5 ± 0.2	8.29 ± 0.83	8.15 ± 1.02	
Chalabre	wild	in natura	4.83 ± 0.28	0.92 ± 0.14	2.29 ± 0.17	3.59 ± 0.16	2.52 ± 0.25	7.68 ± 0.75	8.21 ± 0.92	
Col de la Babourade	wild	in natura	4.8 ± 0.56	0.91 ± 0.22	2.41 ± 0.31	3.24 ± 0.32	2.31 ± 0.27	8.26 ± 0.96	8.56 ± 0.93	
L'Escale (13)	wild	in natura	5.51 ± 0.38	1.07 ± 0.18	2.84 ± 0.22	3.58 ± 0.2	2.76 ± 0.2	9.44 ± 0.97	9.14 ± 1.06	
L'Escale (14)	wild	in natura	4.84 ± 0.32	0.82 ± 0.12	2.24 ± 0.18	3.32 ± 0.15	2.4 ± 0.17	7.72 ± 0.81	7.81 ± 0.95	0.32 ± 0.1
L'Escale (16)	wild	in natura	5.34 ± 0.28	1.18 ± 0.14	2.69 ± 0.17	3.6 ± 0.26	2.56 ± 0.23	7.74 ± 0.7	7.76 ± 0.92	0.35 ± 0.09
L'Escale (17)	wild	in natura	4.94 ± 0.27	1.02 ± 0.16	2.38 ± 0.16	3.61 ± 0.18	2.77 ± 0.19	7.37 ± 0.68	7.41 ± 0.85	0.33 ± 0.09
L'Escale (18)	wild	in natura	5.17 ± 0.46	0.72 ± 0.16	2.34 ± 0.24	3.95 ± 0.37	2.78 ± 0.33	7.45 ± 0.78	7.84 ± 0.8	
L'Escale (20)	wild	in natura	5.05 ± 0.24	0.89 ± 0.14	2.39 ± 0.17	3.48 ± 0.2	2.53 ± 0.21	8.05 ± 0.67	8.23 ± 0.87	0.37 ± 0.1
La Calmette (10)	wild	in natura	5.28 ± 0.31	0.89 ± 0.15	2.58 ± 0.23	3.7 ± 0.24	2.77 ± 0.27	7.98 ± 0.74	8.14 ± 0.82	
La Calmette (11)	wild	in natura	4.93 ± 0.42	1.15 ± 0.25	2.66 ± 0.31	3.33 ± 0.24	2.59 ± 0.24	7.66 ± 1.09	7.57 ± 1.14	
Pic Saint Loup (13)	wild	in natura	5.35 ± 0.29	0.98 ± 0.12	2.85 ± 0.21	3.75 ± 0.22	2.8 ± 0.3	8.75 ± 0.63	8.55 ± 0.73	0.42 ± 0.1
Pic Saint Loup (H)	wild	in natura	5.07 ± 0.44	0.75 ± 0.19	2.21 ± 0.27	3.78 ± 0.29	2.69 ± 0.26	7.67 ± 0.76	8.14 ± 0.88	
Rivel	wild	in natura	5.83 ± 0.55	0.97 ± 0.27	2.77 ± 0.28	4.08 ± 0.28	3.24 ± 0.26	7.8 ± 0.74	7.88 ± 0.95	0.38 ± 0.11
Ile de Ketsch (27)	wild	cultivated	5.68 ± 0.27	1.08 ± 0.15	2.63 ± 0.2	3.86 ± 0.27	2.84 ± 0.19	8.88 ± 0.72	8.75 ± 0.71	0.59 ± 0.11
cPalmA	wild	cultivated	5.78 ± 0.32	1.51 ± 0.19	3.06 ± 0.2	3.72 ± 0.14	2.66 ± 0.23	11.27 ± 1.3	10.78 ± 1.44	0.91 ± 0.33
Pic Saint Loup (13)	wild	cultivated	5.8 ± 0.34	0.91 ± 0.19	2.74 ± 0.25	4.12 ± 0.2	3.14 ± 0.25	8.79 ± 0.73	8.72 ± 0.96	0.52 ± 0.14
Pic Saint Loup (5)	wild	cultivated	6.05 ± 0.17	1.14 ± 0.09	2.82 ± 0.13	3.9 ± 0.18	2.79 ± 0.13	10.67 ± 0.57	10.9 ± 0.65	0.77 ± 0.12
Lambrusque noire	wild	cultivated	5.48 ± 0.3	1.06 ± 0.1	2.71 ± 0.2	3.85 ± 0.18	2.94 ± 0.25	9.12 ± 0.84	8.53 ± 0.96	0.54 ± 0.15

Table 2

log(Predicted)	subset	Intercept	Estimates of log(predictors)				
			pip_{Length}	$pip_{LengthStalk}$	$pip_{PositionChalaza}$	$pip_{Breadth}$	$pip_{Thickness}$
$berry_{Diameter}$	wild	0.65163	1.19914	–	0.10617	-0.13263	-0.45449
	domesticated	1.29219	0.49885	–	0.18310	-0.14986	0.51199
$berry_{Height}$	wild	0.67084	1.04167	–	0.27941	-0.16466	-0.33510
	domesticated	0.92824	0.81614	0.15772	–	-0.23220	0.61259

Figure 1

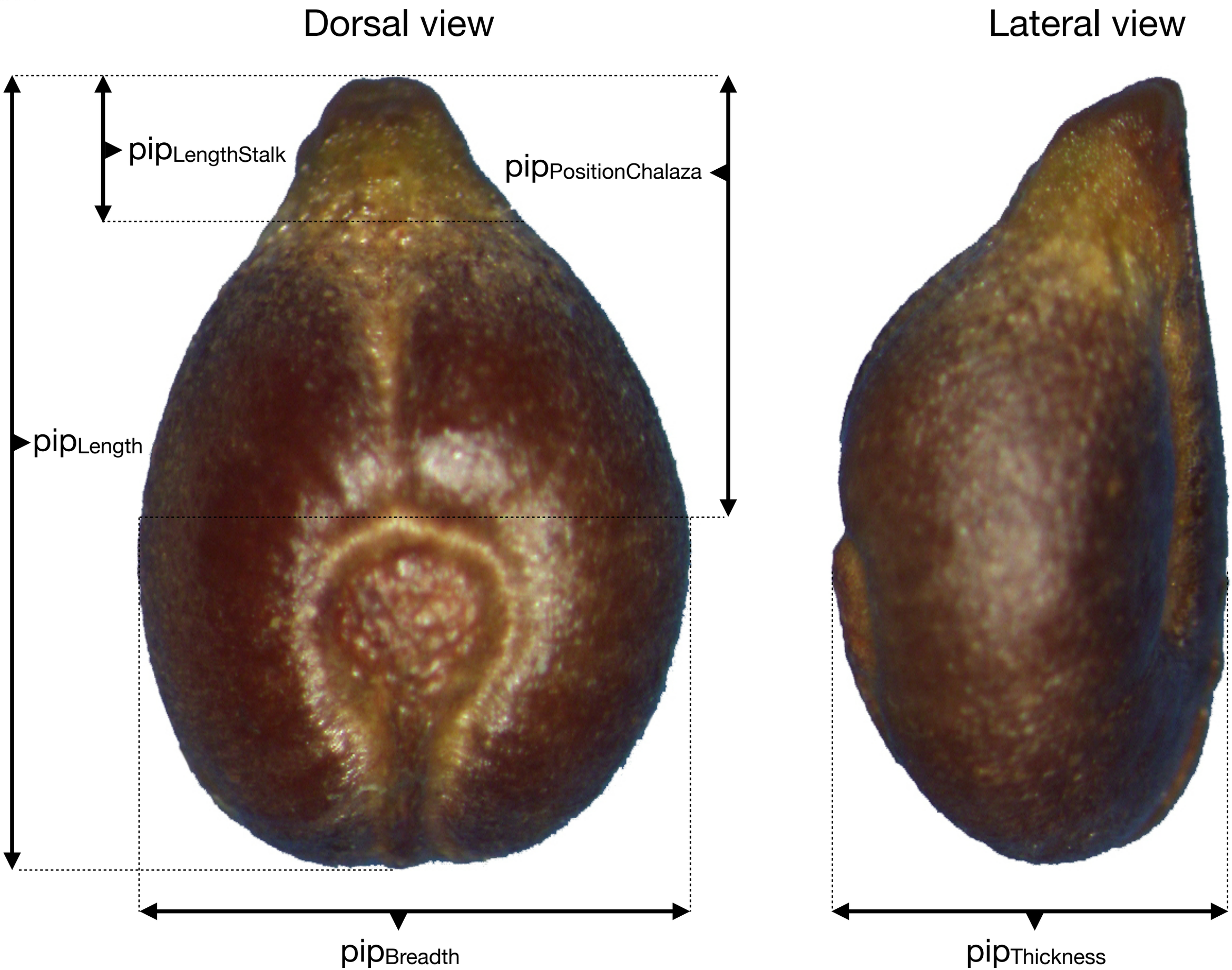

Dorsal view
Lateral view
pipLengthStalk
pipPositionChalaza
pipLength
pipBreadth
pipThickness

Figure 2

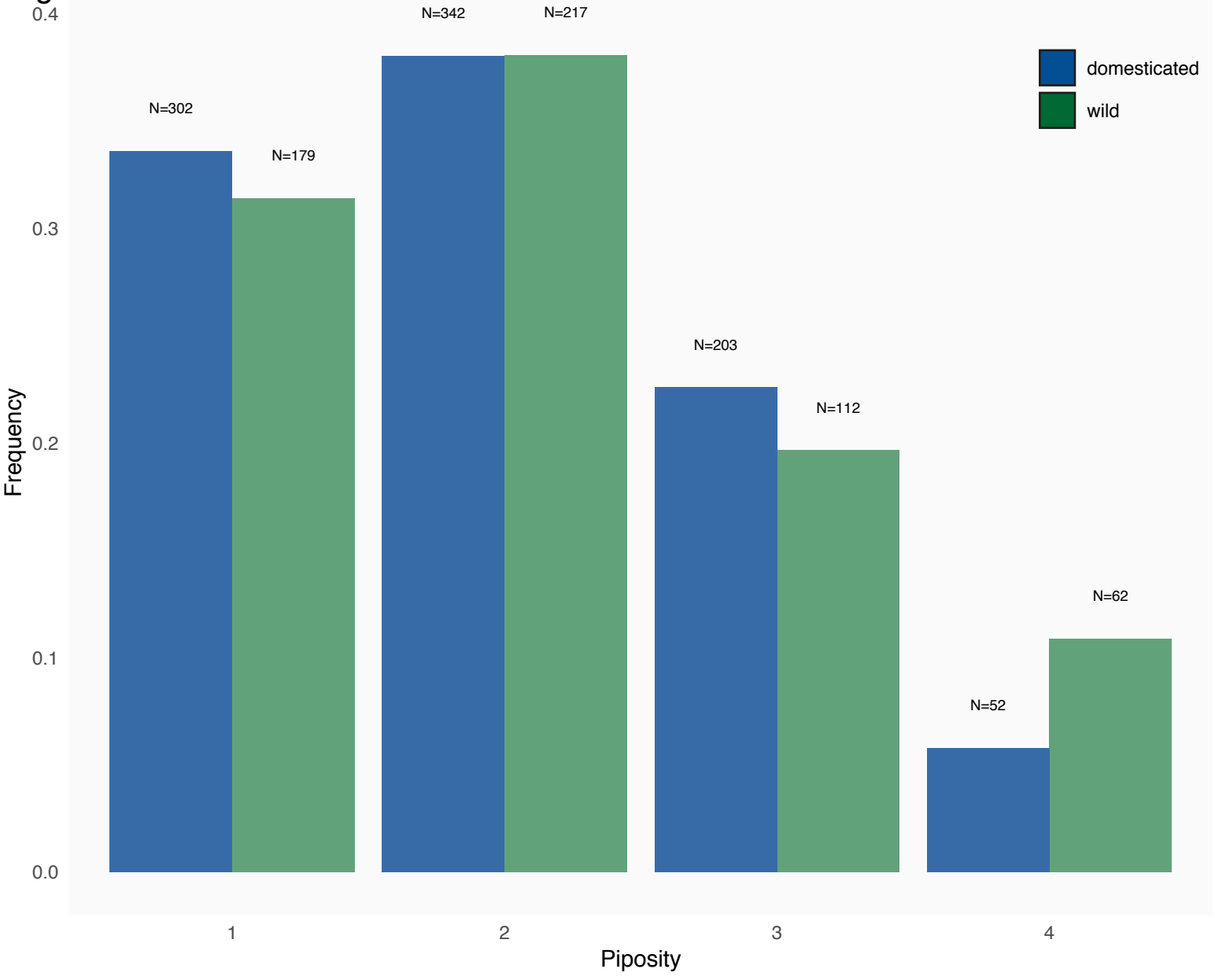

0.4
0.3
0.2
0.1
0.0
Frequency
N=302
N=179
N=342
N=217
N=203
N=112
N=52
N=62
domesticated
wild
1
2
3
4
Piposity

Figure 3

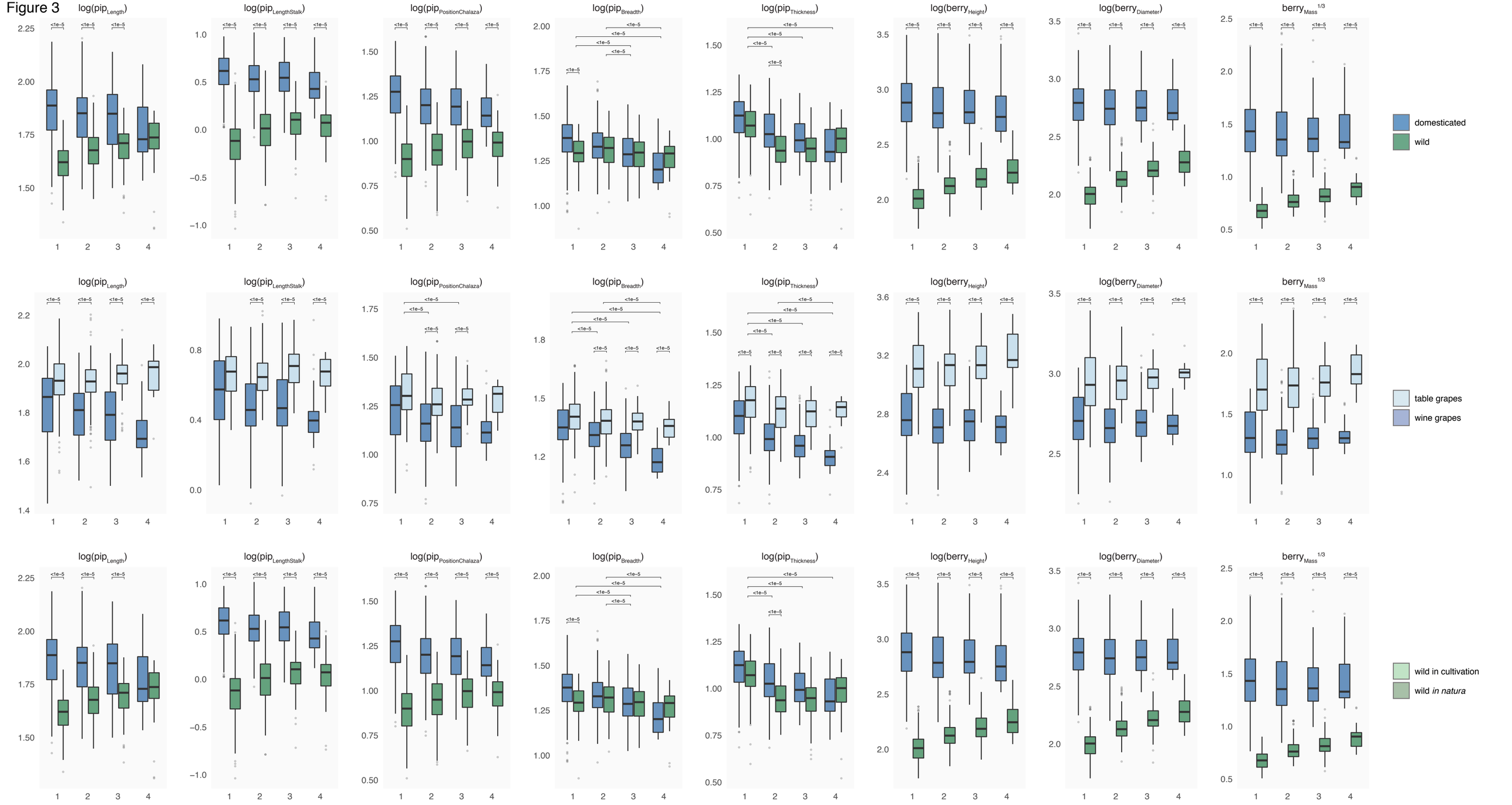

log(pip_Length)
log(pip_LengthStalk)
log(pip_PositionChalaza)
log(pip_Breadth)
log(pip_Thickness)
log(berry_Height)
log(berry_Diameter)
berry_Mass^1/3
domesticated
wild
table grapes
wild grapes
wild in cultivation
wild in natura

Figure A

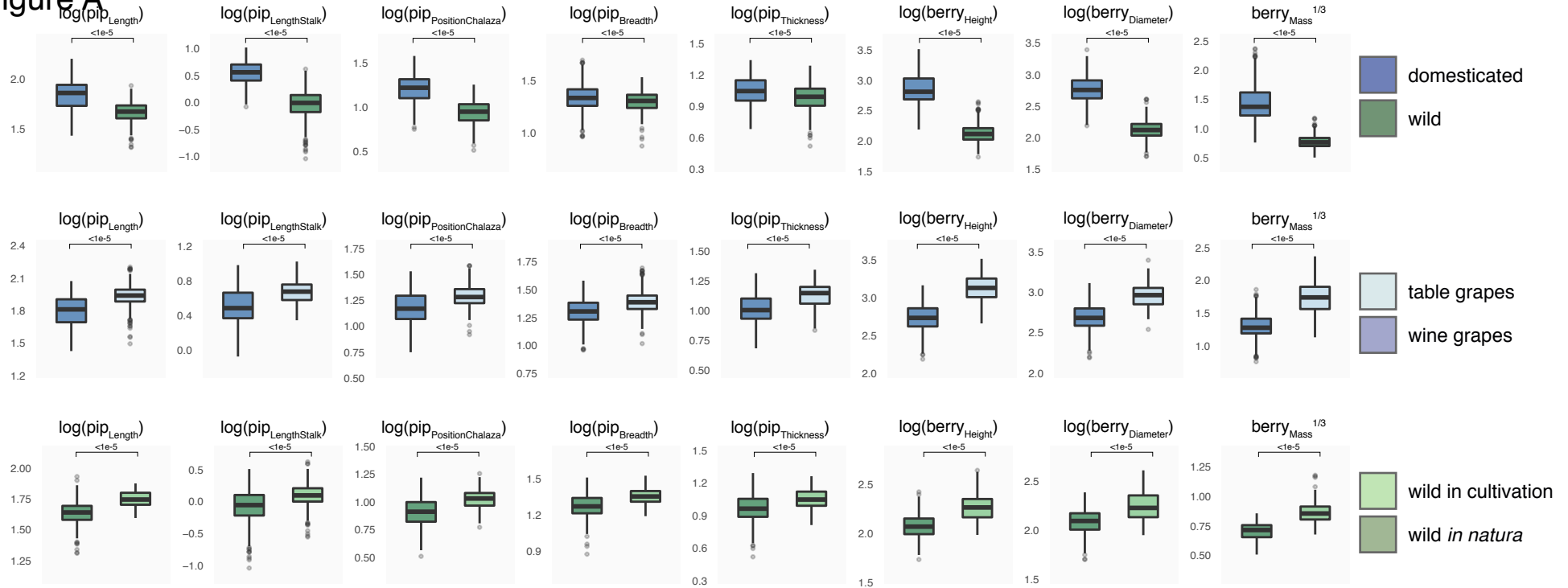
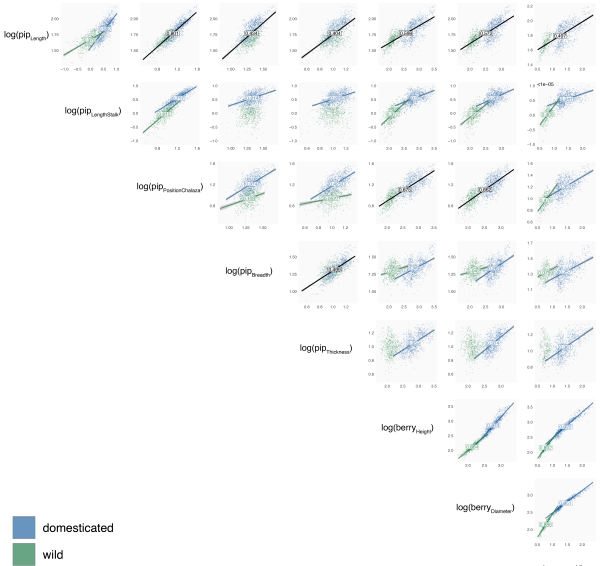

log(pip_Length)
log(pip_LengthStalk)
log(pip_PositionChalaza)
log(pip_Breadth)
log(pip_Thickness)
log(berry_Height)
log(berry_Diameter)
berry_Mass^1/3
<1e-5
domesticated
wild
table grapes
wine grapes
wild in cultivation
wild *in natura*

Figure B



log(pip_Length)
log(pip_LengthStalk)
log(pip_PositionChalaza)
log(pip_Breadth)
log(pip_Thickness)
log(berry_Height)
log(berry_Diameter)
berry_Mass^1/3
domesticated
wild

Figure 4

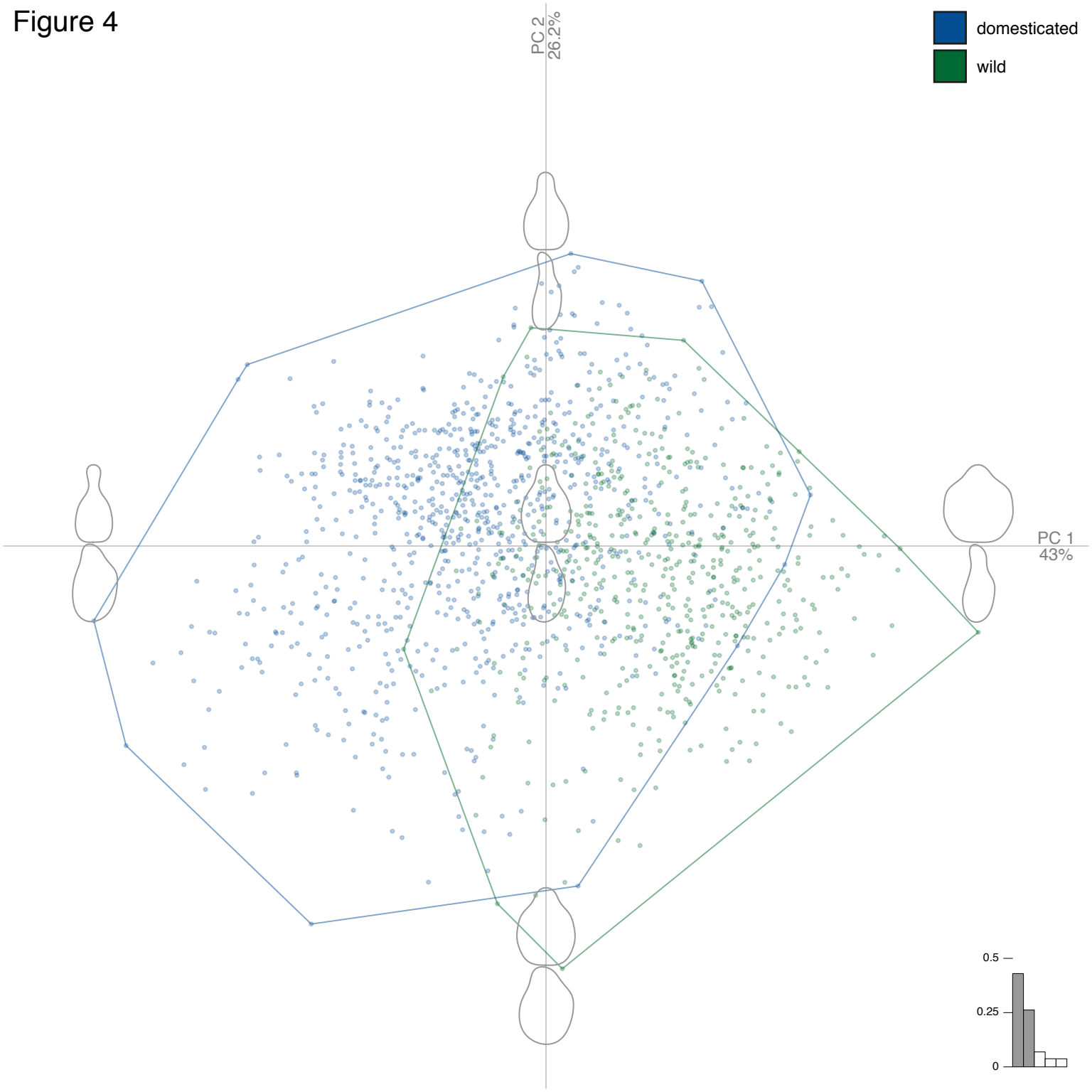


Figure 5

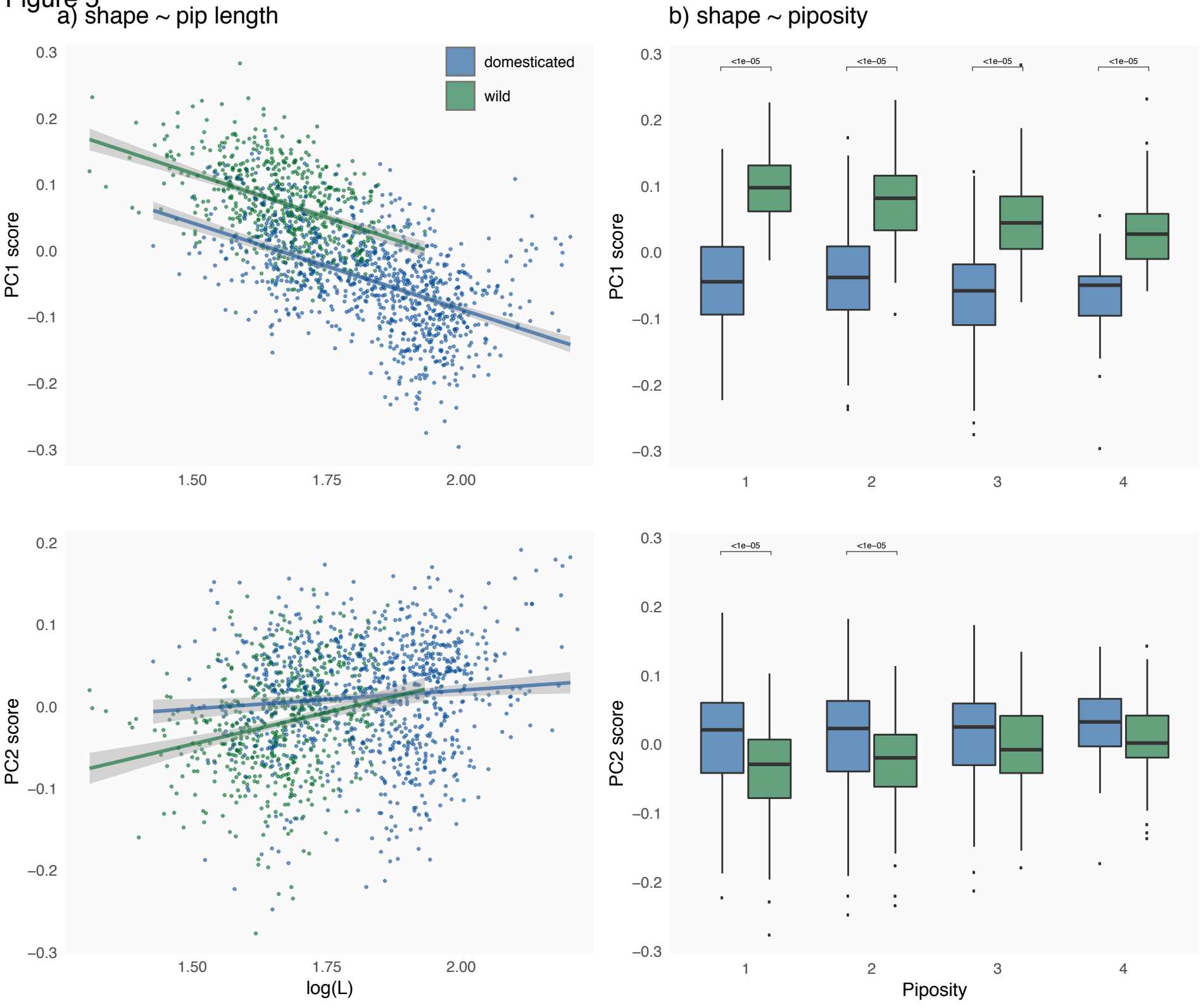

a) shape ~ pip length
b) shape ~ piposity
domesticated
wild
PC1 score
PC2 score
log(L)
Piposity
<1e−05
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
1.50
1.75
2.00
1
2
3
4

Figure 6

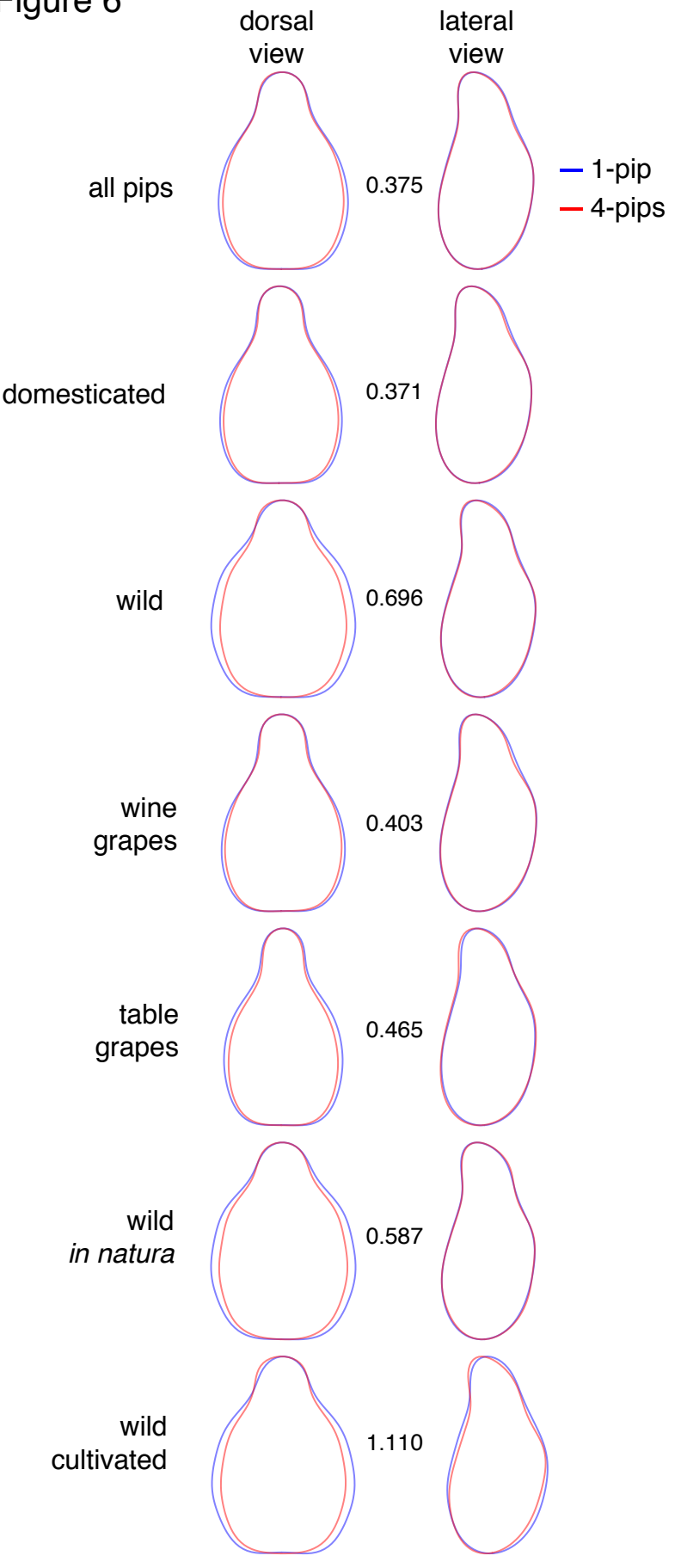

dorsal view
lateral view
1-pip
4-pips
all pips
0.375
domesticated
0.371
wild
0.696
wine grapes
0.403
table grapes
0.465
wild *in natura*
0.587
wild cultivated
1.110

Figure 7

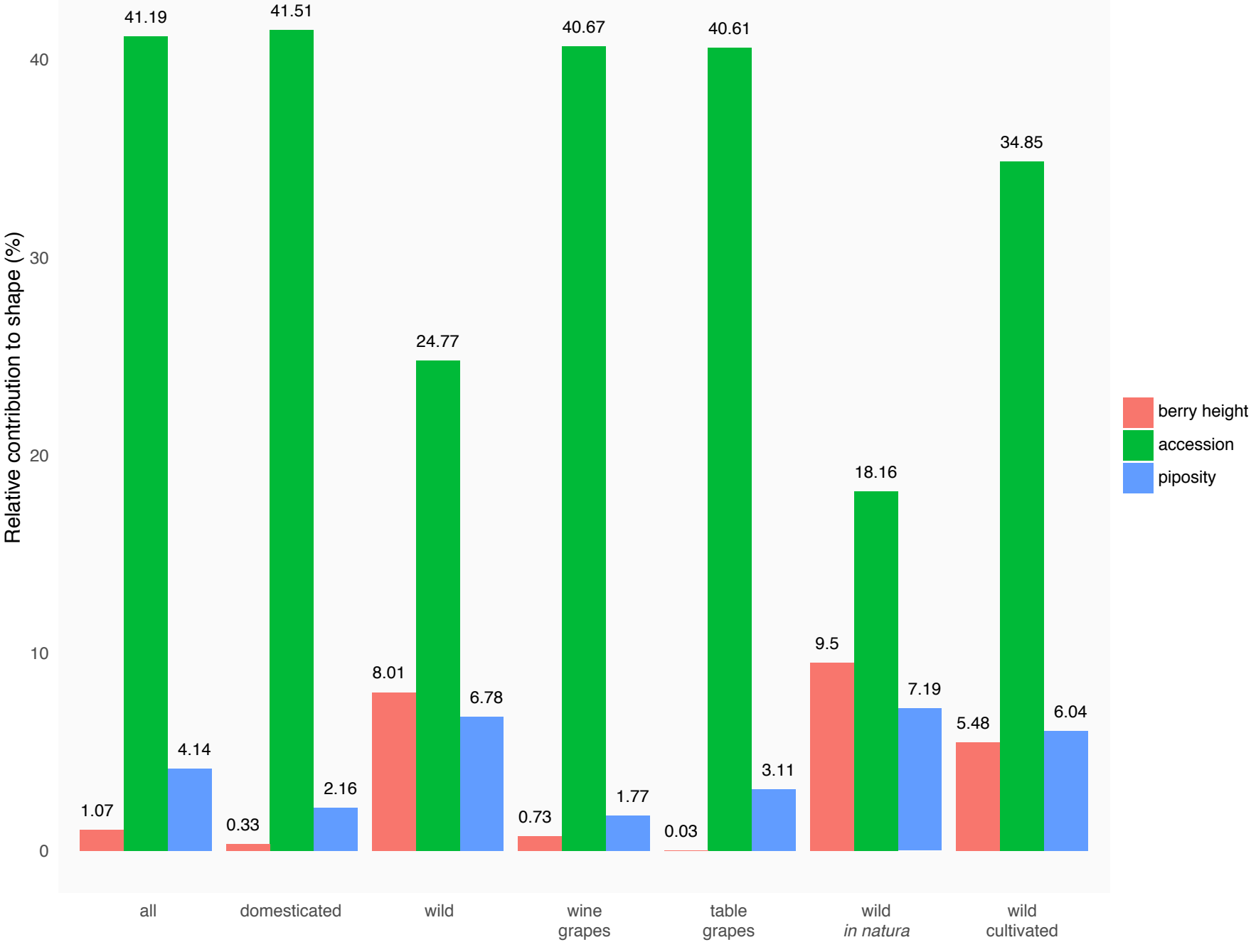

Relative contribution to shape (%)
0
10
20
30
40
41.19
41.51
40.67
40.61
34.85
24.77
18.16
9.5
8.01
7.19
6.78
6.04
5.48
4.14
3.11
2.16
1.77
1.07
0.73
0.33
0.03
all
domesticated
wild
wine grapes
table grapes
wild *in natura*
wild cultivated
berry height
accession
piposity

Figure 8

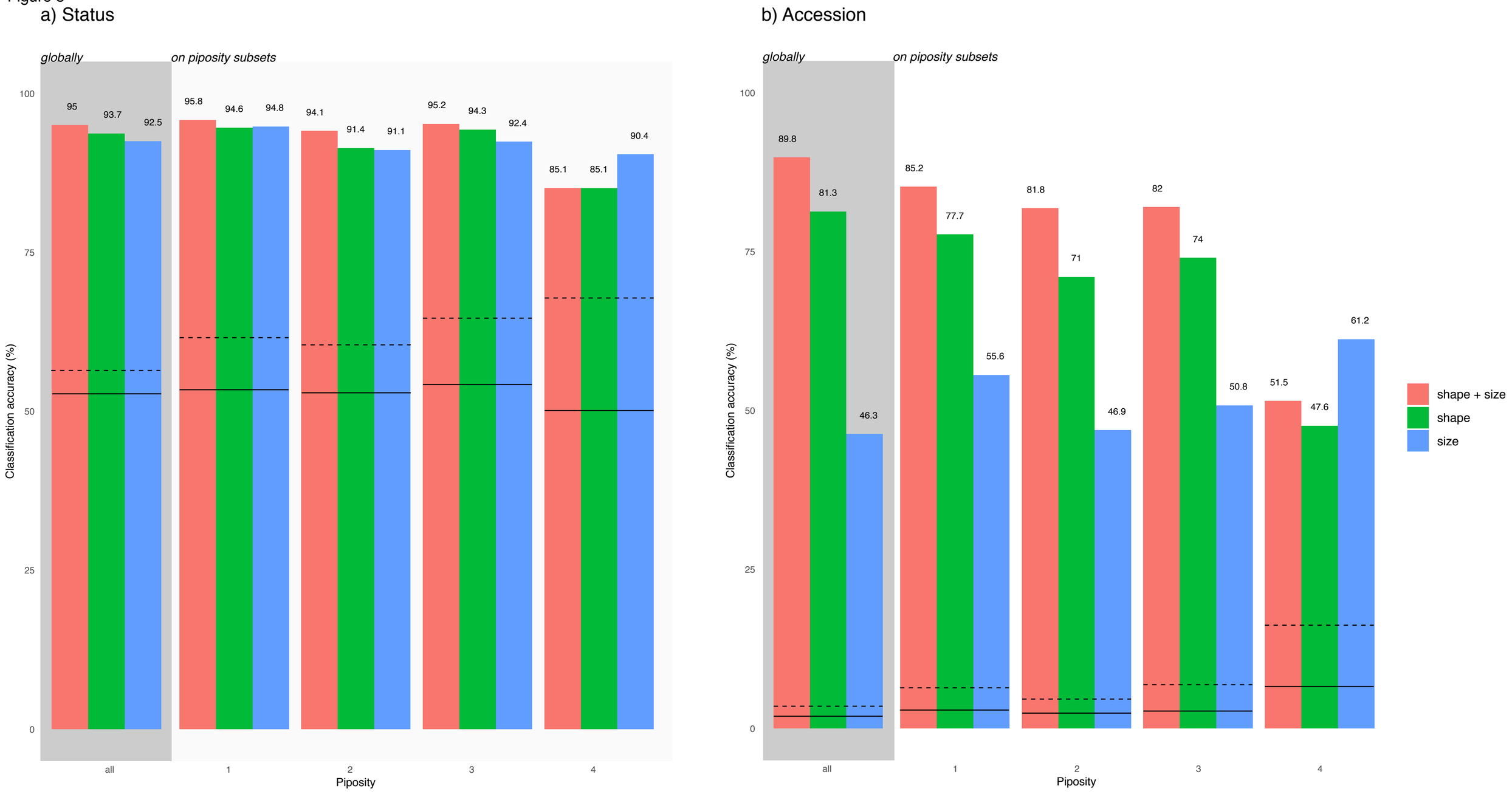

a) Status
globally
on piposity subsets
Classification accuracy (%)
100
75
50
25
0
95
93.7
92.5
95.8
94.6
94.8
94.1
91.4
91.1
95.2
94.3
92.4
85.1
85.1
90.4
all
1
2
3
4
Piposity
b) Accession
globally
on piposity subsets
Classification accuracy (%)
100
75
50
25
0
89.8
81.3
46.3
85.2
77.7
55.6
81.8
71
46.9
82
74
50.8
51.5
47.6
61.2
all
1
2
3
4
Piposity
shape + size
shape
size

Figure 9

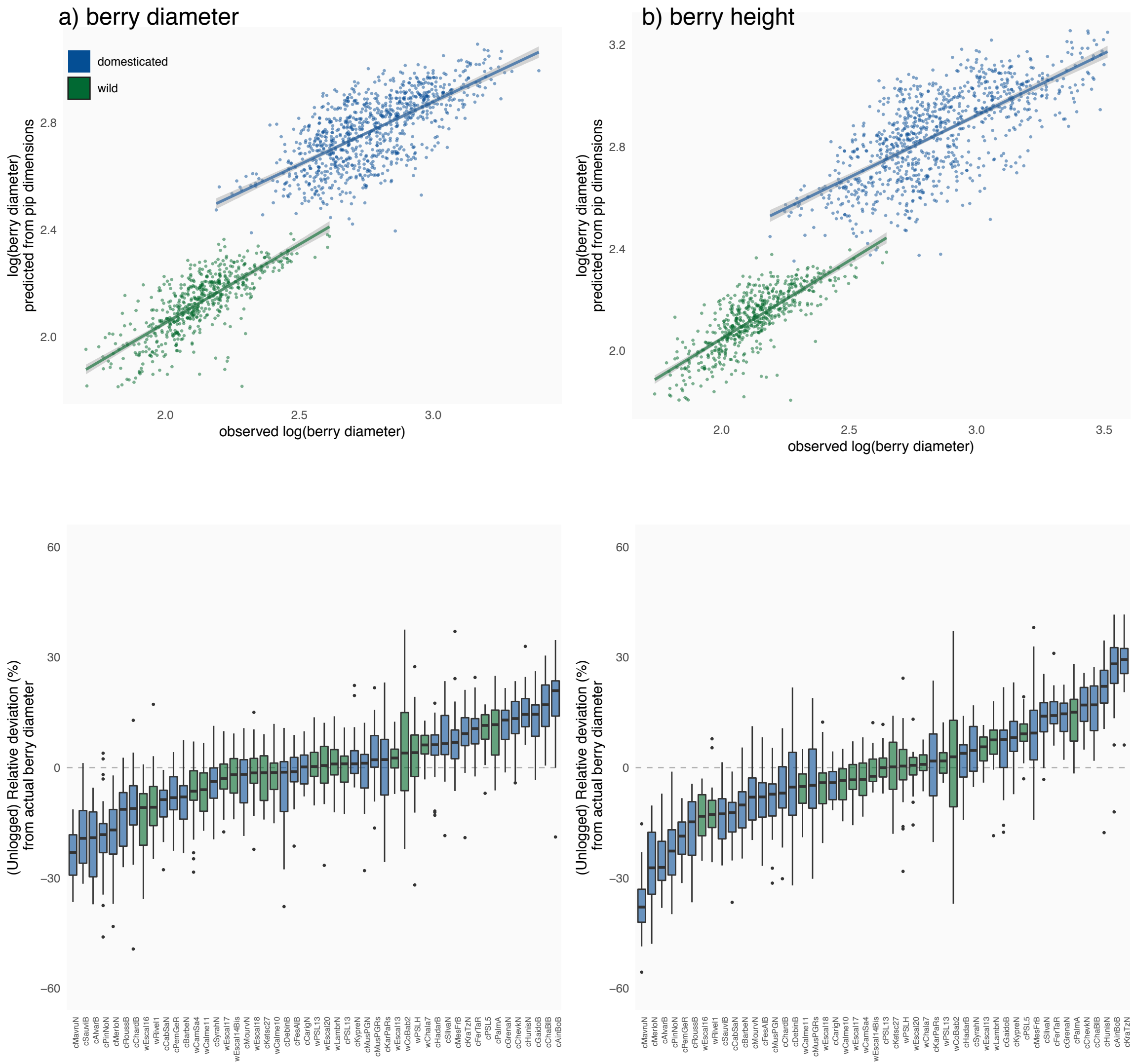
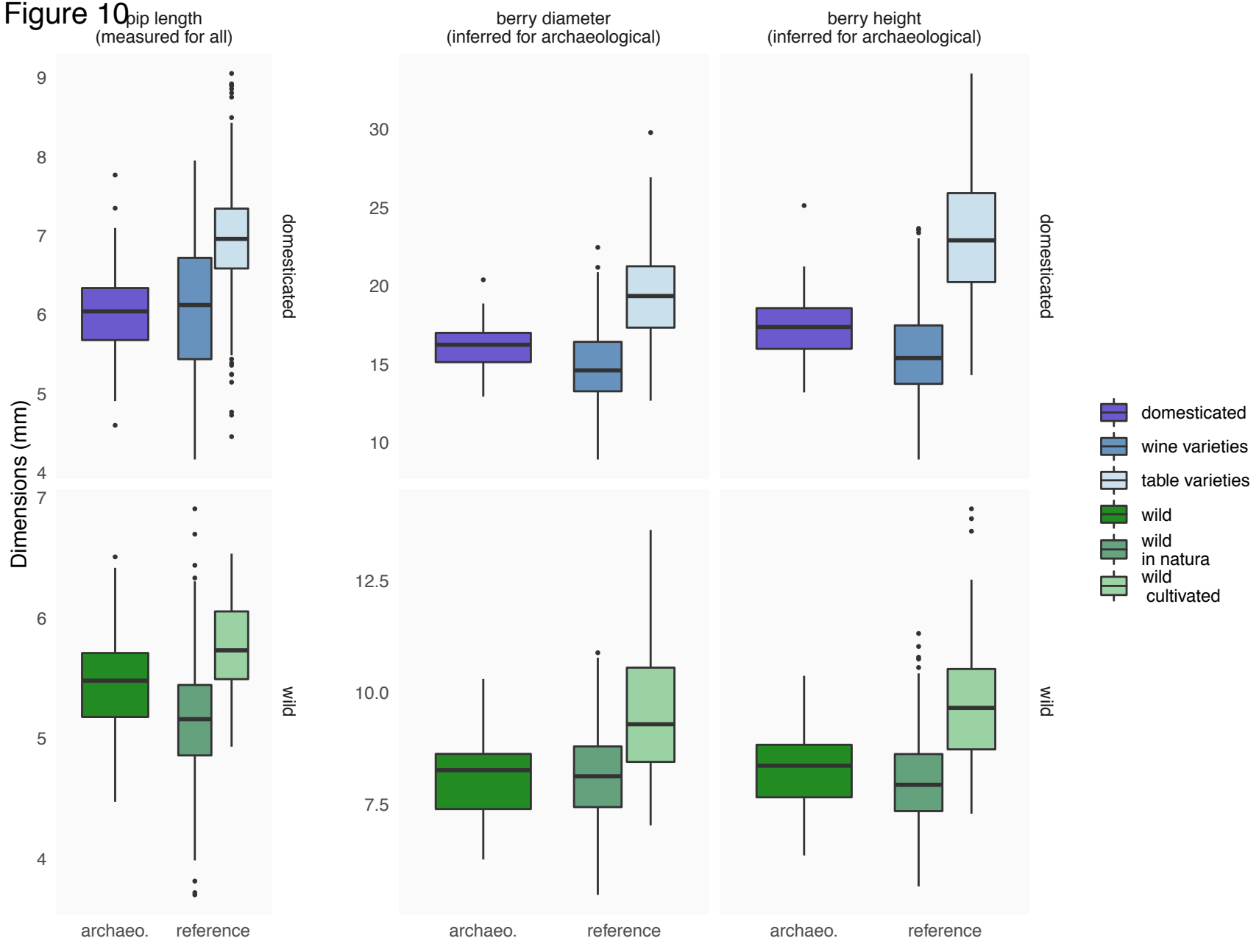

a) berry diameter
domesticated
wild
log(berry diameter) predicted from pip dimensions
observed log(berry diameter)
b) berry height
(Unlogged) Relative deviation (%) from actual berry diameter
cMavruN
cSauviB
cAlvarB
cPinNoN
cMerloN
cRoussB
cChardB
wEscal16
wRivel1
cCabSaN
cPemGeR
cBarbeN
wCamSa4
wCalme11
cSyrahN
wEscal17
wEscal14BIs
cMourvN
wEscal18
cKetsc27
wCalme10
cDebinB
cFesAlB
cCarigN
wPSL13
wEscal20
wLambrN
cPSL13
cKypreN
cMusPGN
cMusPGRs
cKarPaRs
wEscal13
wCoBab2
wPSLH
wChala7
cHadarB
cSlivaN
cMesFrB
cKraTzN
cFerTaR
cPSL5
cPalmA
cGrenaN
cChevkN
cHunisN
cGaidoB
cChaBlB
cAinBoB

Figure 10


pip length
(measured for all)
berry diameter
(inferred for archaeological)
berry height
(inferred for archaeological)
Dimensions (mm)
domesticated
wild
archaeo.
reference
domesticated
wine varieties
table varieties
wild
wild
in natura
wild
cultivated