Annals of Botany 1–13, 2007
doi:10.1093/aob/mcm260, available online at www.aob.oxfordjournals.org

Stony Endocarp Dimension and Shape Variation in *Prunus* Section *Prunus*

LEANDER DEPYPERE[1,*], PETER CHAERLE[1], KRISTINE VANDER MIJNSBRUGGE[2] and PAUL GOETGHEBEUR[1]

[1]*Ghent University, Department of Biology, Research Group Spermatophytes, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium and* [2]*Research Institute for Nature and Forest, Scientific Institute of the Flemish Government, Gaverstraat 4, B-9500 Geraardsbergen, Belgium*

Received: 28 June 2007 Returned for revision: 1 August 2007 Accepted: 3 September 2007

• *Background and Aims* Identification of *Prunus* groups at subspecies or variety level is complicated by the wide range of variation and morphological transitional states. Knowledge of the degree of variability within and between species is a *sine qua non* for taxonomists. Here, a detailed study of endocarp dimension and shape variation for taxa of *Prunus* section *Prunus* is presented.
• *Methods* The sample size necessary to obtain an estimation of the population mean with a precision of 5 % was determined by iteration. Two cases were considered: (1) the population represents an individual; and (2) the population represents a species. The intra-individual and intraspecific variation of *Prunus* endocarps was studied by analysing the coefficients of variance for dimension and shape parameters. Morphological variation among taxa was assessed using univariate statistics. The influence of the time of sampling and the level of hydration on endocarp dimensions and shape was examined by means of pairwise *t*-tests. In total, 14 endocarp characters were examined for five Eurasian plum taxa.
• *Key Results* All linear measurements and index values showed a low or normal variability on the individual and species level. In contrast, the parameter 'Vertical Asymmetry' had high coefficients of variance for one or more of the taxa studied. Of all dimension and shape parameters studied, only 'Triangle' differed significantly between mature endocarps of *P. insititia* sampled with a time difference of 1 month. The level of hydration affected endocarp dimensions and shape significantly.
• *Conclusions* Index values and the parameters 'Perimeter', 'Area', 'Triangle', 'Ellipse', 'Circular' and 'Rectangular', based on sample sizes and coefficients of variance, were found to be most appropriate for further taxonomic analysis. However, use of one, single endocarp parameter is not satisfactory for discrimination between Eurasian plum taxa, mainly because of overlapping ranges. Before analysing dried endocarps, full hydration is recommended, as this restores the original dimensions and shape.

Key words: *Prunus* section *Prunus*, Eurasian plums, stony endocarps, dimension and shape variation, index values, mathematical descriptors, morphometrics, archaeobotany.

INTRODUCTION

With over 200 species, *Prunus* is the largest genus in the tribe Amygdaleae of the subfamily Spiraeoideae (Potter *et al.*, 2007). It contains many trees and shrubs and is an important component of Northern Hemisphere forest and desert communities (Mason, 1913; Wight, 1915; Fedorov *et al.*, 1971; Yü *et al.*, 1986; Browicz and Zohary, 1996). As well as the members that occur in the Northern Hemisphere, a significant number of species is found on tropical mountains worldwide (Kalkman, 1965; Brako and Zarucchi, 1993).

Prunus is economically very important and many species are cultivated worldwide for their fruits, such as sweet and sour cherries (*Prunus cerasus* and *Prunus avium*), apricot (*Prunus armeniaca*), almond (*Prunus dulcis*), peach (*Prunus persica*) and plums (several species of subgenus *Prunus*; Rehder, 1940; Moore and Ballington, 1990). *Prunus serotina* is valued for its timber (Elias, 1980) and several *Prunus* species are ornamentals, such as flowering cherries of subgenus *Cerasus* (Ingram, 1948; Krüssmann, 1986; Kuitert, 1999).

Traditionally, four genera (*Maddenia*, *Oemleria*, *Prinsepia* and *Prunus*) were included in the subfamily Amygdaloideae within the family Rosaceae (Rehder, 1940), but in the treatment of the Rosaceae by Takhtajan (1997), Amygdaloideae includes, next to *Amygdalus*, *Armeniaca*, *Cerasus*, *Laurocerasus*, *Padus* and *Prunus*, also the genera *Exochorda*, *Maddenia*, *Oemleria*, *Prinsepia* and *Pygeum*. However, with a few exceptions, during the last 30 years, the concept of a single genus *Prunus* has gained more favour (Bortiri *et al.*, 2001). Recently, on the basis of nucleotide sequence data from six nuclear and four chloroplast regions, Potter *et al.* (2007) have suggested a new phylogenetically based infrafamilial classification of the Rosaceae in which *Prunus* (including *Amygdalus*, *Armeniaca*, *Cerasus*, *Laurocerasus*, *Maddenia*, *Padus* and *Pygeum*) is included in the tribe Amygdaleae of the subfamily Spiraeoideae. According to Bortiri *et al.* (2006), the most widely accepted classification of *Prunus* is that from Rehder (1940) who distinguished five subgenera: *Amygdalus* (L.) Focke, *Cerasus* Pers., *Laurocerasus* Koehne, *Padus* (Moench) Koehne and *Prunus* L. However, subgeneric classification and relationships among groups are still far from being well understood (Bortiri *et al.*, 2001; Lee and Wen, 2001).

* For correspondence. E-mail Leander.Depypere@UGent.be

The taxonomic complexity of the subgenus *Prunus* has been stated by Hanelt (1997) and Nielsen and Olrik (2001). Recently, in their morphological analysis, Bortiri *et al.* (2006) demonstrated that the subgenus *Prunus* consists of sections *Prunus* (including *Prunus cerasifera*), *Prunocerasus*, *Armeniaca*, *Penarmeniaca*, *Piloprunus* and *Microcerasus*. According to Woldring (2000), Cherry plum (*Prunus cerasifera* Ehrh.), Damson (*Prunus insititia* L.), domestic plums (*Prunus domestica* L.), and Sloe (*Prunus spinosa* L.) are very closely related taxa. Following Bortiri *et al.* (2006), all these above-mentioned species belong to the Eurasian plums. These close relationships within the Eurasian plums have also been demonstrated by several other authors based on morphology (Hanelt, 1997; Kühn, 1999; Nielsen and Olrik, 2001) and have been confirmed by a number of studies based on molecular data (by, amongst others, Bortiri *et al.*, 2001; Aradhya *et al.*, 2004; Shaw and Small, 2004; Katayama and Uematsu, 2005).

Beside the unclear phylogenetic relationships between taxa of *Prunus* section *Prunus*, the morphological discrimination of these Eurasian plum taxa is also problematic. According to Woldring (2000), the identification of *Prunus* groups at subspecies or variety level is complicated by the very wide range of variation and transitional states between and within the different taxa. Woldring exemplified this by noting that *P. insititia* and *P. domestica* include such a wide range of forms with so many overlapping features that it is hardly possible to point out diagnostic features that clearly distinguish the two groups. This phenomenon can also be observed for individuals that are morphologically intermediate between *P. insititia* and *P. spinosa* (Woldring, 2000). Furthermore, Woldring argued that hybridization and subsequent back-crossing, and possibly also segregating F_2 progeny, leads to establishment of a variable aggregate of intermediates including types approaching the original parent species. As a result, the taxonomic status of these intermediates is unclear (see Körber-Grohne, 1996; Woldring, 2000; Hübner and Wissemann, 2004). Experimental proof that supports the assumptions about hybridization is still rather scarce. Christensen (1992) and Arnold (1997) argue that overlapping morphological characteristics increase the taxonomic complexity, which results in conflicting classifications.

Of all the characters used for identification, the features of the stones of *Prunus* taxa are the most stable ones (Woldring, 2000). The value of fruit stones for identification purposes of species and even varieties has been stated by various authors (see Van Zeist and Woldring, 2000; Nielsen and Olrik, 2001). According to Behre (1978), pit dimensions are very useful for the identification of *P. domestica*, *P. insititia* and *P. spinosa*. Röder (1940) demonstrated that the shape of the stones of modern plum cultivars, which finds expression in the index values, is characteristic of a particular variety. Behre (1978) and Van Zeist and Woldring (2000) use the following index values to define plums: 100B:L (defined here as 100SW/SL = 100 × stone width/stone length), 100T:L (100ST/SL = 100 × stone thickness/stone length) and 100T:B (100ST/SW = 100 × stone thickness/ stone width). Later, Schmidt-Tauscher *et al.* (1996, cited in Pollmann *et al.*, 2005) introduced a fourth index value $L^2/(T \times B)$ [here defined as $SL^2/(ST \times SW)$ = stone length^2/(stone thickness × stone width)] and Pollmann *et al.* (2005) demonstrated its usefulness in differentiating fruit stones of modern cultivars. However, in Taylor (1949) and Hedrick (1911), a series of varieties with morphologically identical endocarps are described and depicted.

Except by means of index values (see Van Zeist and Woldring, 2000; Pollmann *et al.*, 2005), stone shape has mainly been evaluated qualitatively and the resulting classifications are often based on rough estimates by visual judgment. This qualitative measure is inadequate for the evaluation of continuous shape variation, as it cannot eliminate the subjectivity of visual judgments, which result in unacceptable errors (Yoshioka *et al.*, 2004).

Brewer *et al.* (2006) developed an accurate and objective method for conducting phenotypic analyses combined with a concise and detailed set of descriptors and terms for fruit shape attributes. Because of their accuracy and objectivity, a selection of Brewer's descriptors is tested here for their effectiveness in expressing the dimensions and shape of *Prunus* endocarps.

In earlier *Prunus* studies, little attention was paid to sample size and variability. Körber-Grohne (1996) studied a variable number ($n = 11-40$) of individuals and fruits for each population. Hübner and Wissemann (2004) investigated five endocarps for each individual plant and ten individual plants per *Prunus spinosa* population. However, neither of these authors presented arguments for supporting these particular numbers of samples.

Intraspecific variability has been studied for several Eurasian plum species (for an overview see Kühn, 1999). Hübner and Wissemann (2004) studied the variability of qualitative and quantitative characters for *P. spinosa*, both at individual and population level by means of coefficients of variance.

Knowledge of the degree of variability within and between species is a *sine qua non* for taxonomic research. However, except for *P. spinosa* in Hübner and Wissemann (2004), no detailed study of intra-individual, intraspecific and interspecific variation of Eurasian plum taxa has been performed.

The aims of this study were threefold: (1) to identify the number of endocarps that has to be analysed to obtain a representative view of a Eurasian plum individual and species; (2) to describe and compare the intra-individual, intraspecific and interspecific variation of dimension and shape parameters for these taxa; and (3) to verify whether endocarp dimensions and shape of these taxa change with endocarp maturity and with changing levels of hydration.

MATERIAL AND METHODS

Sampling of Prunus *endocarps*

Endocarps of *Prunus cerasifera* and *P. spinosa* were supplied by several botanical gardens (Belgium, Czech Republic, Estonia, France, Mallorca, Romania, Russia and Slovakia). Most endocarps of *P. domestica* were collected by the National Orchard Foundation (NBS, Vliermaal, Belgium) and some were collected by P. Goetghebeur and P. Van der Veken (UGent, Ghent, Belgium). The majority of *P. insititia* endocarps originate from the private stock collection of Henk Woldring (RUG, Groningen, The Netherlands). Finally, all samples of the assumed hybrid taxon *P.* ×*fruticans* and some of *P. spinosa* and *P. insititia* were collected from the wild in Flanders by K. Vander Mijnsbrugge (INBO, Geraardsbergen, Belgium). Table 1 gives an overview of the endocarp samples for each *Prunus* taxon studied.

Morphometric analysis

Stone length (SL), width (SW), and thickness (ST) were measured with digital sliding calipers (Absolute Coolant Proof Digimatic sliding calipers IP-66, accuracy 0·01 mm; Mitutoyo, Andover, UK). The position of the measurements for SL, SW and ST follows Van Zeist and Woldring (2000) and is illustrated in Fig. 1. Index values were calculated according to Van Zeist and Woldring (2000): 100SW/SL (100 × width/length), 100ST/SL (100 × thickness/length) and 100ST/SW (100 × thickness/width). The 100ST/SL index value is a measure of the relative slenderness: the more slender the stone, the lower the index value. Roundness finds expression in the 100ST/SW value: stones with strongly domed sides show a low 100ST/SW value, while in rather flat stones this value is high (Van Zeist and Woldring, 2000). In addition, the index value $SL^2/(ST \times SW)$ ($length^2/thickness \times width$), given in Pollmann *et al.* (2005) was also calculated. In addition to these linear measurements and index values (illustrated in Fig. 2), a selection of Brewer's descriptors (Brewer *et al.*, 2006) were analysed. For this, the lateral side of *Prunus* endocarps were digitized with a scanner (Hewlett Packard Scanjet Scanner 4070), with a resolution of 200 dpi, and saved as a bitmap image in Adobe Photoshop. All images were inverted and saved as JPEG images, as required for use in Tomato Analyzer (Brewer *et al.*, 2006). 'Perimeter' and 'Area' are size parameters: endocarps with high values for Perimeter and Area are larger-sized than endocarps that have lower values for these parameters (Fig. 3). 'Triangle' is defined as the ratio of the proximal end width (w_1) to the distal end width (w_2). In this study, these widths were measured at the user-defined distances of, respectively, 10% and 90% from the proximal end (Fig. 3). A Triangle value greater than 1 indicates that the proximal end of the endocarp is wider than the distal end of the endocarp, while a value less than 1 indicates that the distal end is wider than the proximal end. 'Ellipse', 'Circular' and 'Rectangular' are functions that are related to homogeneity and uniformity, i.e. similarity of the object to the common shapes ellipse, circle and rectangle (Fig. 3). The closer the value to 1, the more similar the endocarp is to an ellipse, circle or rectangle (Brewer *et al.*, 2006). Rectangular is calculated as the ratio of the maximum area of the inscribing rectangle (S_{in}) to the minimum area of an enclosing rectangle (S_{out}; Fig. 3). 'Vasym' describes how asymmetric an endocarp is when divided along a vertical axis. The position of the vertical axis (m) is determined by detecting the left-most and right-most points of the endocarp and dividing it in half to find the centre. To compute Vasym, each row of pixels (L_i) is determined, and the midpoint of the row (m_i) is found. Next, the difference between m and m_i is calculated and recorded. Once every row is examined, the sum of the differences is determined and divided by the number of rows. Thus, the formula for Vasym is $(\Sigma|m - m_i|)$/number of rows (Fig. 3). Vertical asymmetry values of 0 signify a perfectly symmetric shape along the vertical axis (Brewer *et al.*, 2006). Table 2 presents an overview of all the characters that were studied, together with the references in which the parameters are used and/or described.

Statistical analysis

First, an explorative analysis of the dataset was performed by means of dot and box plots in order to eliminate outliers and to obtain a robust dataset. Normality was tested using the D'Agostino–Pearson K^2 test.

Estimation of sample size. The sample size (n) necessary to achieve a desired precision in estimating a population mean (μ), from a normally distributed population, is given by

$$n = [(st_{\alpha(2),(n-1)})/d^2] \quad (1)$$

where s is the sample standard deviation, $t_{\alpha(2),(n-1)}$ the critical value of t for $\alpha(2),(n - 1)$ and, d the half-width of the confidence interval (Zar, 1996). For the estimation of the sample size that is necessary to have a desired precision in estimating a population mean, two cases are considered: (1) the population represents an individual, and (2) the population represents a species. In this study, the desired precision was, for both cases, established as 5 % of the population mean ($d = 0{\cdot}05 \times$ mean). The value of n was achieved by iteration (Zar, 1996).

Intra-individual, intraspecific and interspecific variation. Variation at different taxonomic levels was studied by analysing the coefficients of variance (CV), which were interpreted following Rasch (1988, cited in Hübner and Wissemann, 2004), i.e. $CV < 10$ %, small variability; 10 % $< CV <$ 20 %, normal variability; $CV > 25$ %, high variability of the character studied. In order to investigate whether the number of endocarps (i.e. sample size) influences the variability, a distinction was made between five endocarps measured per individual and 30 endocarps measured per individual. Morphological variation among taxa was assessed using univariate statistics for each taxon and for each parameter studied.

Endocarp maturity. In order to study whether the endocarp parameters change with maturity, a between-group comparison (t-test) was performed for each character. The first group (group I) consisted of 30 mature-looking endocarps of an individual of *P. insititia* sampled in August 2006 and the second group (group II) consisted of 30 mature-looking endocarps of the same individual sampled one month later.

TABLE 1. Prunus *accessions used in this study. Voucher specimens are deposited in the Herbarium of the Ghent University (GENT), unless otherwise indicated. Numbers between brackets are the numbers of specimens studied for each taxon. Taxon names in the column 'source' are those given by the original collector*

Taxon, common name	Source	Collection no./voucher	Number of samples
P. insititia (82)	INS-DAL-92–2 (Weinkrieche, Werneck) / Woldring, The Netherlands	n/a*	10
	KRO-EEN-89 / Woldring, The Netherlands	n/a	10
	INS-ANAPHI-2001 'Gr':4 (oogst c. 1990) / Woldring, The Netherlands	n/a	10
	INS-Rue PERRIN-95-1 Gr:5 / Woldring, The Netherlands	n/a	10
	INS-DAL-92-6 / Woldring, The Netherlands	n/a	10
	BLA-NPV-90-6 Smal Bl. / Woldring, The Netherlands	n/a	10
	INS-GRON-03-4 [GRO-I] / Woldring, The Netherlands	n/a	10
	INS-AGELOO-03-1 / Woldring, The Netherlands	n/a	10
	Voeren – Remersdaal / K. Vander Mijnsbrugge 28/08/04, Belgium	n/a	2
P. ×*fruticans* (18)	Merelbeke / K. Vander Mijnsbrugge 16/10/01, Belgium	n/a	9
	Oosterzele / K. Vander Mijnsbrugge 15/10/01, Belgium	n/a	9
P. spinosa (35)	Heuvelland / K. Vander Mijnsbrugge 10/10/02, Belgium	n/a	10
	433 *P. spinosa* 'maxima' 20050626 Meshcherskoje, Russia	HBUG20050626	5
	432 *P. spinosa* 20050625 Meshcherskoje, Russia	HBUG20050625	5
	P. spinosa 20060006 Hort. Bot. Craiova, România	HBUG20060006	5
	P. spinosa 20051110 Hortus plantarum medicarum Bratislava, Slovakia	HBUG20051110	5
	2644 *P. spinosa* ssp. *fruticans* 20051109 Jardí Botànic de Sóller, Mallorca	HBUG20051109	5
P. cerasifera (26)	1213 *P. cerasifera* var. *cerasifera* 20060005 Hort. Bot. Craiova România	HBUG20060005	10
	118 *P. cerasifera* subsp. *divaricata* G 20050945 (S) Arboretum Novy Dvur Opava, Czech Republic	HBUG20050945	5
	757 *P. cerasifera* ssp. *divaricata* 20050566 Hort. Bot. Tallinn, Estonia	HBUG20050566	3
	758 *P. cerasifera* ssp. *divaricata* 20050570 Hort. Bot. Tallinn, Estonia	HBUG20050570	3
	454 *P. divaricata* 20050563 Jardin Botanique de l'Université Lous Pasteur de Strasbourg, France	HBUG20050563	5
P. domestica (202)	*Prunus* sp. 'Prune de Liège' 2811, Belgium	2811 – P. Goetghebeur	4
	P. domestica 'Susine Black Amber' 8526, Belgium	8526 – P. Goetghebeur	4
	P. domestica 'Susine Black Diamond' 8527, Belgium	8527 – P. Goetghebeur	4
	P. insititia var. *italica* (Reine Claude), Belgium	? – P. Van der Veken	4
	P. domestica 'Belle de Louvain' 10592, Belgium	10592 – P. Goetghebeur	10
	P. domestica 'Golden Drop' 10589, Belgium	10589 – P. Goetghebeur	11
	P. domestica 'Engelse Reine-Claude' NBS 2006-0050, Belgium	20060050 – L. Depypere	4
	P. domestica 'Reine-Claude Diaphane' NBS 2006-0049, Belgium	20060049 – L. Depypere	5
	P. domestica 'Reine-Claude de Bavay' NBS 2006-0047, Belgium	20060047 – L. Depypere	5
	P. domestica 'Reine-Claude Souffriau' NBS 2006-0048, Belgium	20060048 – L. Depypere	5
	P. domestica 'Reine-Claude Crottée' NBS 2006-0046, Belgium	20060046 – L. Depypere	6
	P. domestica 'Reine-Claude d'Althan' NBS 2006-0045, Belgium	20060045 – L. Depypere	5
	P. domestica 'Reine-Claude Conducta' 'NBS 2006-0043, Belgium	20060043 – L. Depypere	10
	P. domestica 'Mirabelle de Nancy' NBS 2006-0040, Belgium	20060040 – L. Depypere	5
	P. domestica 'Mirabelle de Nancy' NBS 2006-0039, Belgium	20060039 – L. Depypere	5
	P. domestica 'Mirabelle Herrenhaüser' NBS 2006-0041, Belgium	20060041 – L. Depypere	7
	P. domestica 'Mirabelle Herrenhaüser' NBS 2006-0042, Belgium	20060042 – L. Depypere	3
	P. domestica 'Enkele Bakpruim' NBS 2006-0033, Belgium	20060033 – L. Depypere	5
	P. domestica 'Belle de Louvain' NBS 2006-0005, Belgium	20060005 – L. Depypere	5
	P. domestica 'Belle de Louvain' NBS 2006-0019, Belgium	20060019 – L. Depypere	5
	P. domestica 'Anna Späth' NBS 2006-0086, Belgium	20060086 – L. Depypere	5
	P. domestica 'Prune des Princes' NBS 2006-0084, Belgium	20060084 – L. Depypere	5
	P. domestica 'Dubbele Bakpruim' NBS 2006-0101, Belgium	20060101 – L. Depypere	5
	P. domestica 'Queen Victoria' NBS 2006-0104, Belgium	20060104 – L. Depypere	5
	P. domestica 'Czar' NBS 2006-0109, Belgium	20060109 – L. Depypere	5
	P. domestica 'Kirke's Plum' NBS 2006-0067, Belgium	20060067 – L. Depypere	5
	P. domestica 'Betuwse Kwets' NBS 2006-0073, Belgium	20060073 – L. Depypere	5
	P. domestica 'Washington' NBS 2006-0065, Belgium	20060065 – L. Depypere	5
	P. domestica 'Wangenheims Frühzwetsche' NBS 2006-0066, Belgium	20060066 – L. Depypere	5
	P. domestica 'Pond's Seedling' NBS 2006-0085, Belgium	20060085 – L. Depypere	5
	P. domestica 'Utility' NBS 2006-0052, Belgium	20060052 – L. Depypere	5
	P. domestica 'Palokes' NBS 2006-0059, Belgium	20060059 – L. Depypere	5
	P. domestica 'Warwickshire Drooper' NBS 2006-0062, Belgium	20060062 – L. Depypere	5
	P. domestica 'Drongse Pruim' NBS 2006-0063, Belgium	20060063 – L. Depypere	5
	P. domestica 'Stanley' NBS 2006-0057, Belgium	20060057 – L. Depypere	5
	P. domestica 'Keizelpruim' NBS 2006-0055, Belgium	20060055 – L. Depypere	5
	P. domestica 'Sultan' NBS 2006-0054, Belgium	20060054 – L. Depypere	5
	P. domestica 'Scrazinsky' NBS 2006-0113, Belgium	20060113 – L. Depypere	5

* Not applicable.

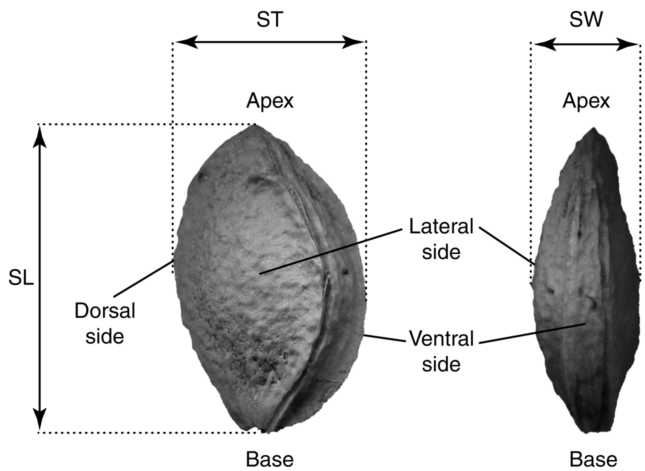


FIG. 1. Overview of the basic linear endocarp measurements. Left: lateral view; right: ventral view.

Endocarp hydration. The influence of the level of hydration on endocarp dimensions and shape was tested on ten endocarps of three different individuals belonging to three different species (*P. domestica*, *P. cerasifera*, *P. spinosa*). After measuring, the fresh endocarps were stored in plastic bags for a period of 5 months. After those 5 months, the endocarps were measured again and subsequently stored in an oven at 40 °C (day 1). Measurements were performed on days 2, 7, 8, 9 and 10. After measurements on day 14, the temperature of the oven was increased to 100 °C. The endocarps were measured again on days 15 and 17, and then they were immersed in water. After more than a week of immersion, the endocarps were removed from the water and measured (day 28) to check the influence of rehydration on the endocarp dimensions and shape. For each parameter, pairwise *t*-tests were performed between fresh endocarps and endocarps stored at 100 °C (measured at day 17) and between endocarps stored at 100 °C (measured at day 17) and fully rehydrated endocarps (measured at day 28).

RESULTS

Estimation of sample size

In order to estimate the number of endocarps that have to be measured to obtain a representative view of a particular population (henceforth referred to as the 'required sample size'), sample sizes were calculated for all parameters using the formula presented in eqn (1). In Table 3 the required sample sizes are summarized for five individuals belonging to five different Eurasian plum taxa (*P. domestica*, *P. insititia*, *P.* ×*fruticans*, *P. spinosa*, *P. cerasifera*).

The parameters SL, SW, ST, index values [except $SL^2/(ST \times SW)$], Perimeter, Ellipse, Circular and Rectangular had low required sample sizes (i.e. less than 10) for all individuals. The parameters Area and Triangle had required sample sizes varying from eight to 34 (Area, from eight for *P. spinosa* up to 19 for *P. cerasifera*; Triangle, from 13 for *P. spinosa* up to 34 for *P. cerasifera*). In contrast, required sample sizes of Vasym exhibited a high variability between taxa ($24 \leq n \leq 216$; see Table 3).

The sample sizes necessary to achieve a desired precision (i.e. mean value ±5%) in estimating a particular species mean are generally higher compared with those for estimating an individual mean [up to 22-fold for $SL^2/(ST \times SW)$ of *P. domestica*; compare Tables 3 and 4]. Parameters

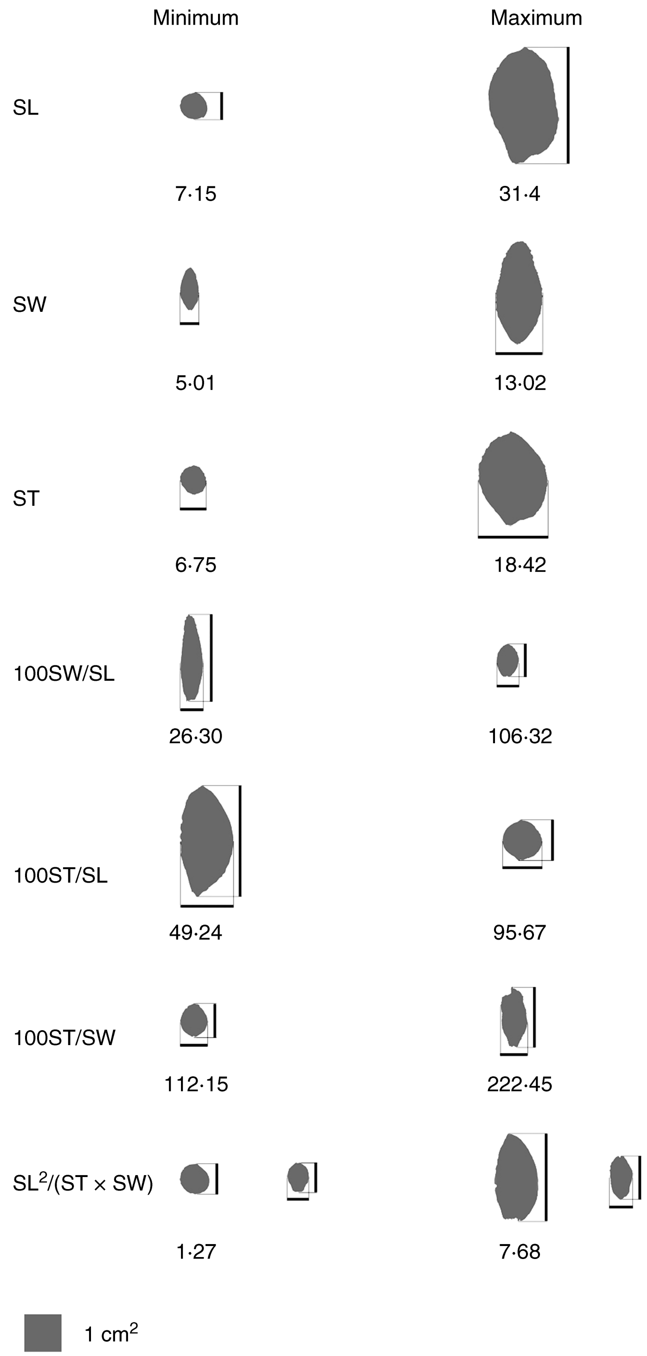


FIG. 2. Illustration of the linear measurements and index values, by means of minimum and maximum values of the reference samples. SL, ST, 100ST/SL: endocarps in lateral view; SW, 100SW/SL: endocarps in ventral view; 100ST/SW: endocarps in apical view; $SL^2/(ST \times SW)$: endocarps in lateral view (left) and in apical view (right). Values for SL, SW, ST are expressed in mm. Abbreviations used are explained in Table 2.

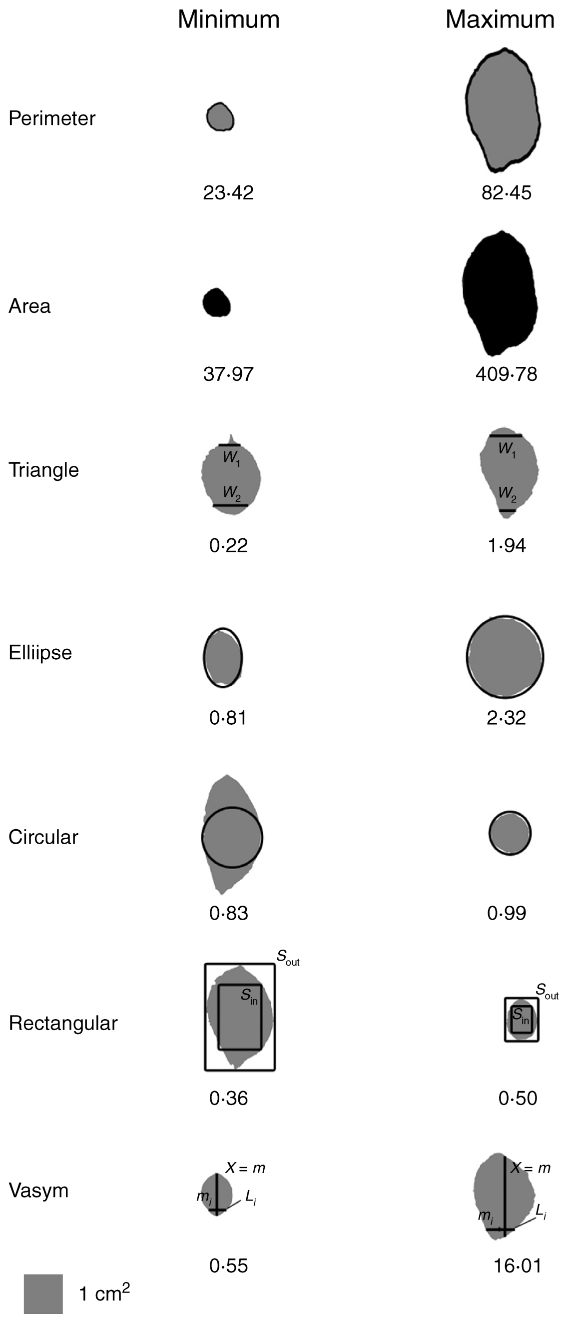


FIG. 3. Illustration of Brewer's parameters used in this study, by means of minimum and maximum values of the reference samples. For all endocarps the lateral view is displayed. Triangle = the ratio of the proximal end width to the distal end width, w_1/w_2; Ellipse and Circular: fitting precision R^2; Rectangular = the ratio of the maximum area of the inscribing rectangle to the minimum area of an enclosing rectangle, S_{in}/S_{out}; Vasym = $(\Sigma|m - m_i|)$/number of rows, where m is the position of the vertical axis (determined by detecting the leftmost and rightmost points of the endocarp and dividing it in half to find the centre), m_i is the midpoint of the row L_i. Values for Perimeter and Area are expressed in mm. Abbreviations used are explained in Table 2.

100ST/SW, Ellipse, Circular and Rectangular had the lowest required sample sizes ($n \leq 21$; Table 4) for all taxa studied. In contrast, for all taxa Vasym had very high required sample sizes ($n \leq 224$). For *P. domestica*, required sample sizes for 11 of the 14 parameters (Table 4) were higher than for the other species studied. In contrast, the assumed hybrid *P.* ×*fruticans* had very low required sample sizes except for Vasym.

TABLE 2. *Summary of characters used in this study, with the references in which the parameters are used and/or described*

Parameter	Abbreviation/ Formula	Reference
Endocarp Length	SL	Van Zeist and Woldring (2000)
Endocarp Width	SW	“
Endocarp Thickness	ST	“
100 × (Endocarp Width/ Endocarp Length)	100SW/SL	“
100 × (Endocarp Thickness/ Endocarp Length)	100ST/SL	“
100 × (Endocarp Thickness/ Endocarp Width)	100ST/SW	“
(Endocarp Length)^2/ (Endocarp Thickness × Endocarp Width)	SL^2/(ST × SW)	Pollmann *et al.* (2005)
Perimeter	Perimeter	Brewer *et al.* (2006)
Area	Area	“
Fruit Shape Triangle	Triangle	“
Fruit Shape Ellipsoid	Ellipse	“
Fruit Shape Circular	Circular	“
Fruit Shape Rectangular	Rectangular	“
Vertical Asymmetry	Vasym	“

Intra-individual variability

As indicated above, except for Vasym, the endocarp parameters that were studied exhibit a required sample size of at least approx. 5 and at most approx. 30 (Table 3). In Table 5 the intra-individual variability is expressed by means of coefficients of variance for all the parameters studied. SL, SW, ST, index values [except SL^2/(ST × SW) of *P.* × *fruticans*] and Ellipse were found to have a low coefficient of variance, both for the case of five and 30 endocarps measured. Perimeter, Area and Triangle were low-to-normally variable in both cases. Only when 30 endocarps per individual were measured did the parameters Circular and Rectangular have a low variability for all individuals studied (Table 5). The coefficients of variance of Vasym were neither consistent between taxa nor between cases (5 and 30 endocarps measured).

Intraspecific variability

In comparison with the variability on the individual level shown in Table 5b, coefficients of variance were higher at the species level (Table 6). Only the index value 100ST/SW, Circular and Rectangular had low coefficients of variance for all taxa studied. Parameters SW, ST, 100SW/SL, 100ST/SL, Perimeter, Ellipse and, except for *P. domestica*, also SL, Area and Triangle, had a low-to-normal variability. The index value SL^2/(ST × SW) was highly variable for *P. domestica* and *P. insititia*, and had a moderate variability for *P.* ×*fruticans*,

TABLE 3. *Sample sizes necessary to achieve a desired precision (±5 %) in estimating an individual mean of a normally distributed individual for five different individuals belonging to five different taxa of* Prunus. *Abbreviations used are explained in Table 2*

Parameter	*P. domestica*	*P. insititia*	*P. ×fruticans*	*P. spinosa*	*P. cerasifera*
SL	6	7	6	6	9
SW	5	6	7	6	7
ST	8	6	6	5	7
100SW/SL	6	7	7	6	5
100ST/SL	5	7	7	5	4
100ST/SW	7	4	4	4	4
$SL^2/(ST \times SW)$	8	16	20	12	10
Perimeter	6	5	6	4	8
Area	17	12	13	8	19
Triangle	33	17	23	13	34
Ellipse	5	3	4	3	3
Circular	3	3	3	2	3
Rectangular	6	5	6	6	4
Vasym	143	24	216	111	92

P. spinosa and *P. cerasifera*. The parameter Vasym was highly variable for all taxa studied.

Interspecific variability

Prunus domestica had the highest mean value of all the taxa studied for the parameters SL, SW, ST, 100ST/SW, $SL^2/(ST \times SW)$, Perimeter, Area and Vasym; however, this species also had the widest range of values for all of these parameters (Fig. 4). This resulted in an extensive overlap with the ranges of the other taxa.

Prunus spinosa had the lowest mean values for SL, SW, ST, $SL^2/(ST \times SW)$, Perimeter, Area and Triangle. Conversely, this taxon had a remarkable high mean value for 100SW/SL (91·2) in comparison with the other taxa studied (*P. domestica*, 42·1; *P. insititia*, 54·6; *P. ×fruticans*, 57·1; *P. cerasifera*, 50·1). Moreover, the range for 100SW/SL of *P. spinosa* (80·1–106·3) did not overlap at all with the ranges of the other taxa studied (26·3 for *P. domestica* to 70·4 for *P. insititia*).

Prunus insititia and *P. cerasifera* had very similar mean values for the parameters SL, SW, ST, 100SW/SL, 100ST/SW, Perimeter and Area; however, the mean values of 100ST/SL, Triangle, Ellipse and Vasym of these taxa were clearly distinct.

Focusing on the Sloe–Damson complex (*P. insititia*, *P. spinosa*, and *P. ×fruticans*), the mean values of nine of the 14 parameters studied for *P. ×fruticans* were intermediate between those of its putative parents (*P. spinosa* and *P. insititia*; Fig. 4). However, the mean values of the parameters 100SW/SL, $SL^2/(ST \times SW)$, Ellipse and Circular of *P. × fruticans* were very similar to those of *P. insititia*, while ST and Triangle resembled those of *P. spinosa* (Fig. 4).

Endocarp maturity

Of all parameters studied, only the mean value of Triangle showed a significant difference between the two defined maturity groups ($P \leq 0{\cdot}05$; Table 7). This parameter had a lower mean value for mature-looking endocarps of *P. insititia* sampled in September (group II) compared with mature-looking endocarps of the same individual sampled in August (group I). The mean endocarp length (SL) did not alter between the two maturity groups studied ($P = 0{\cdot}98$).

TABLE 4. *Sample sizes necessary to achieve a desired precision (±5%) in estimating a species mean of a normally distributed species for the five taxa studied. Abbreviations used are explained in Table 2*

Parameter	*P. domestica*	*P. insititia*	*P. ×fruticans*	*P. spinosa*	*P. cerasifera*
SL	83	29	5	19	13
SW	47	22	7	14	9
ST	43	16	5	13	11
100SW/SL	59	33	8	8	14
100ST/SL	32	20	6	9	13
100ST/SW	17	17	4	7	10
$SL^2/(ST \times SW)$	172	90	19	23	38
Perimeter	62	20	5	13	10
Area	179	62	9	43	27
Triangle	79	43	18	36	30
Ellipse	21	3	3	5	5
Circular	6	4	3	3	4
Rectangular	8	6	10	6	8
Vasym	224	251	273	237	268

TABLE 5. *Intra-individual variability for five individuals belonging to five different taxa of* Prunus *expressed by means of the coefficients of variability (CV) for each character used in this study; (a) for five endocarps and (b) for 30 endocarps per specimen*

Parameter	*P. domestica*	*P. insititia*	*P. ×fruticans*	*P. spinosa*	*P. cerasifera*
(a) Five endocarps/individual					
SL	5·87	2·65	4·54	3·99	2·11
SW	4·75	2·67	5·01	2·53	1·39
ST	6·09	4·00	2·57	2·95	3·48
100SW/SL	4·20	4·45	8·15	2·28	1·82
100ST/SL	2·23	5·29	5·58	1·93	3·10
100ST/SW	6·00	1·55	2·83	1·44	3·34
$SL^2/(ST \times SW)$	3·08	9·20	**13·59**	3·89	3·72
Perimeter	**17·14**	**11·30**	8·18	7·86	8·21
Area	2·49	0·88	2·15	1·40	1·10
Triangle	0·32	0·82	1·70	0·45	0·93
Ellipse	5·65	2·94	4·73	4·29	2·85
Circular	*37·75*	8·55	*23·81*	*23·88*	*21·28*
Rectangular	6·11	5·55	4·56	*53·58*	0·53
Vasym	3·27	2·63	3·09	*47·91*	0·56
(b) 30 endocarps/individual					
SL	4·66	5·06	4·56	4·30	6·17
SW	4·05	4·63	5·19	4·23	5·15
ST	6·08	4·44	4·90	3·40	5·33
100SW/SL	4·28	5·40	5·39	4·58	4·19
100ST/SL	3·66	5·08	5·38	3·51	3·23
100ST/SW	5·00	3·25	2·90	2·92	3·02
$SL^2/(ST \times SW)$	5·99	9·46	**10·66**	7·62	7·08
Perimeter	4·72	3·98	4·24	3·21	5·67
Area	9·55	7·64	8·11	5·97	**10·45**
Triangle	**14·07**	9·68	**11·54**	8·40	**14·22**
Ellipse	3·71	1·49	2·78	1·39	1·72
Circular	0·95	0·89	1·38	0·45	0·76
Rectangular	4·56	3·47	4·41	4·29	3·01
Vasym	*30·08*	**11·81**	*36·98*	*26·52*	*24·14*

Values in *italics* indicate a high variability (CV > 25 %); values in **bold** indicate a normal variability (10 % < CV < 20 %); CV < 10 % indicates a small variability (following Rasch, 1988; cited in Hübner and Wissemann, 2004). Abbreviations used are explained in Table 2.

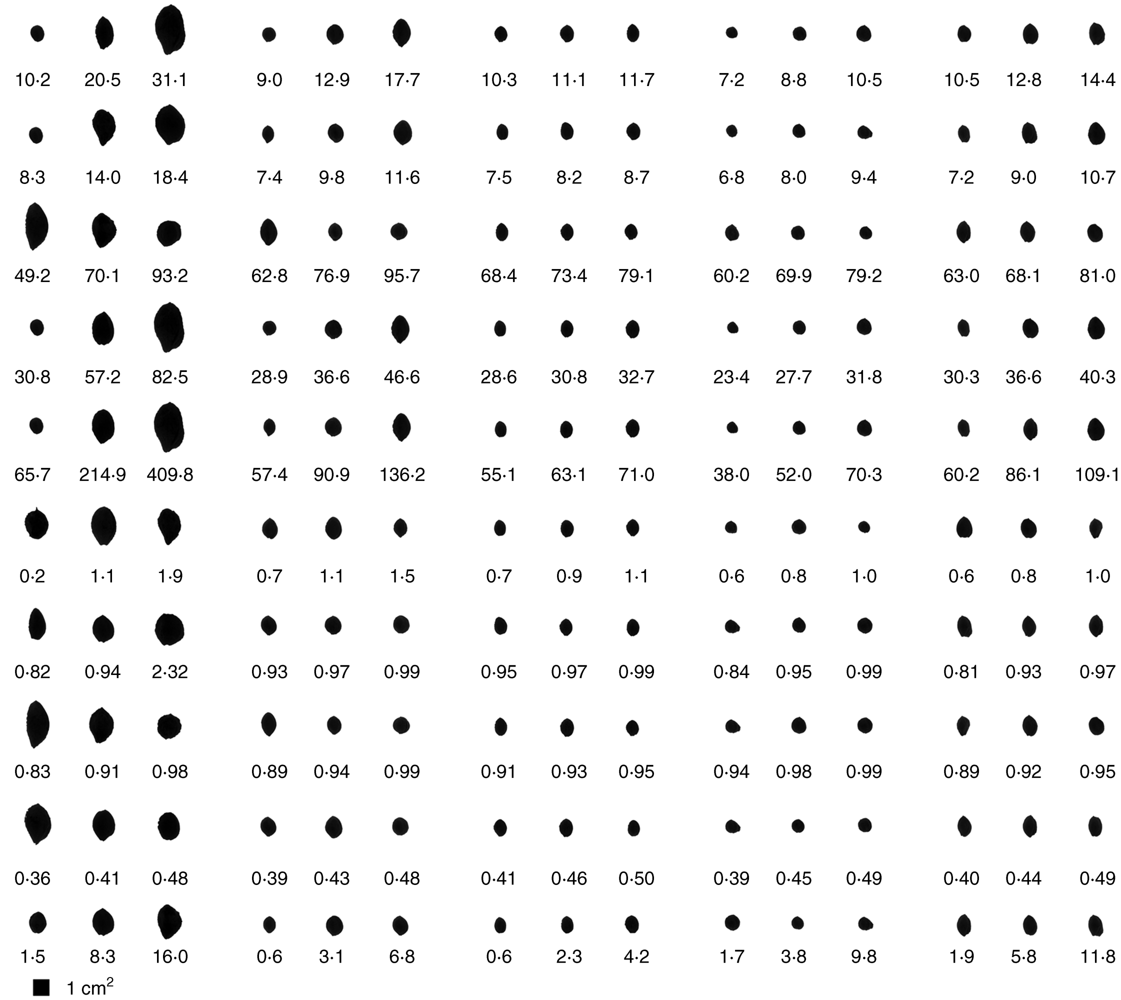
Endocarp hydration

Mean values of Perimeter and Area for the species *P. domestica* and *P. spinosa* showed a significant difference ($P \leq 0{\cdot}05$) between freshly sampled and dried (13 d at 40 °C plus 3 d at 100 °C) endocarps and between dried and fully rehydrated (11 d submerged in water) endocarps (Table 8). Figure 5 indicates that, after drying and subsequent rehydration, the mean Perimeter values returned almost completely the same values as the freshly sampled endocarps.

TABLE 6. *Intraspecific variability for five Eurasian plum taxa expressed by means of the coefficients of variability (CV) for each character used in this study*

Parameter	*P. domestica*	*P. insititia*	*P. × fruticans*	*P. spinosa*	*P. cerasifera*
SL	*22·82*	**13·16**	3·89	**10·23**	8·10
SW	**17·05**	**11·30**	5·27	8·75	6·64
ST	**16·24**	9·31	3·87	8·08	7·53
100SW/SL	**19·26**	**14·14**	6·07	5·99	8·66
100ST/SL	**13·90**	**10·76**	4·28	6·56	8·31
100ST/SW	9·58	9·72	3·09	5·47	6·92
$SL^2/(ST \times SW)$	*32·96*	*23·83*	**10·26**	**11·54**	**15·21**
Perimeter	**19·68**	**10·57**	3·45	8·36	6·74
Area	*33·70*	**19·73**	6·66	**16·25**	**12·61**
Triangle	*22·34*	**16·16**	9·89	**14·69**	**13·44**
Ellipse	**10·90**	1·18	1·12	3·41	3·74
Circular	4·31	3·10	1·40	1·15	2·34
Rectangular	5·84	4·70	6·76	4·86	5·93
Vasym	*37·67*	*39·86*	*41·66*	*38·78*	*41·21*

Values in *italics* indicate a high variability (CV > 25 %); values in **bold** indicate a normal variability (10 % < CV < 20 %); CV < 10 % indicates a small variability (following Rasch, 1988; cited in Hübner and Wissemann, 2004). Abbreviations used are explained in Table 2.



	P. domestica			*P. insititia*			*P.× fruticans*			*P. spinosa*			*P. cerasifera*		
	min	mean	max	min	mean	max	min	mean	max	min	mean	max	min	mean	max
SL	10·2	20·5	31·1	9·0	12·9	17·7	10·3	11·1	11·7	7·2	8·8	10·5	10·5	12·8	14·4
ST	8·3	14·0	18·4	7·4	9·8	11·6	7·5	8·2	8·7	6·8	8·0	9·4	7·2	9·0	10·7
100ST/SL	49·2	70·1	93·2	62·8	76·9	95·7	68·4	73·4	79·1	60·2	69·9	79·2	63·0	68·1	81·0
Perimeter	30·8	57·2	82·5	28·9	36·6	46·6	28·6	30·8	32·7	23·4	27·7	31·8	30·3	36·6	40·3
Area	65·7	214·9	409·8	57·4	90·9	136·2	55·1	63·1	71·0	38·0	52·0	70·3	60·2	86·1	109·1
Triangle	0·2	1·1	1·9	0·7	1·1	1·5	0·7	0·9	1·1	0·6	0·8	1·0	0·6	0·8	1·0
Ellipse	0·82	0·94	2·32	0·93	0·97	0·99	0·95	0·97	0·99	0·84	0·95	0·99	0·81	0·93	0·97
Circular	0·83	0·91	0·98	0·89	0·94	0·99	0·91	0·93	0·95	0·94	0·98	0·99	0·89	0·92	0·95
Rectangular	0·36	0·41	0·48	0·39	0·43	0·48	0·41	0·46	0·50	0·39	0·45	0·49	0·40	0·44	0·49
Vasym	1·5	8·3	16·0	0·6	3·1	6·8	0·6	2·3	4·2	1·7	3·8	9·8	1·9	5·8	11·8
SW	5·0	8·4	13·0	5·2	6·9	8·7	5·8	6·3	6·9	5·1	6·1	7·5	5·7	6·4	7·3
100SW/SL	26·3	42·1	64·7	40·5	54·6	70·4	49·6	57·1	63·1	80·1	91·2	106·3	42·5	50·1	58·4
100ST/SW	130·1	168·6	222·5	112·2	142·3	172·9	123·2	128·6	140·9	116·5	132·1	154·4	124·0	141·1	159·1
$SL^2/(ST \times SW)$	1·7	3·7	7·7	1·5	2·5	3·8	2·0	2·4	2·9	1·3	1·6	2·1	2·1	2·9	3·6

■ 1 cm^2

FIG. 4. Overview of the minimum, mean and maximum values with the corresponding endocarp images (lateral view) for each taxon and for all parameters used in this study. For SW and derived parameters, the minimum, mean and maximum values are shown without the corresponding endocarp images. Values for SL, SW, ST, Perimeter and Area are expressed in mm. Abbreviations used are explained in Table 2.

Remarkably, for the parameters Ellipse, Rectangular and Vasym, mean values for dried endocarps of *P. cerasifera* differed significantly from freshly sampled endocarps ($P \leq 0{\cdot}05$) but they did not change when dried endocarps were compared with fully rehydrated endocarps. For the three taxa studied, the mean values of 100ST/SW, Triangle and Circular did not change significantly with altering hydration conditions ($P > 0{\cdot}05$; Table 8).

DISCUSSION

In this study, in addition to linear measurements and index values that have been applied by various authors in order to discriminate between endocarps of several *Prunus* taxa (e.g. Behre, 1978; Körber-Grohne, 1996; Van Zeist and Woldring, 2000; Woldring, 2000; Nielsen and Olrik, 2001; Pollmann *et al.*, 2005), we also investigated the usefulness and effectiveness of recently described parameters (see Brewer *et al.*, 2006) to express the dimensions and shape of *Prunus* endocarps. Furthermore, in several taxonomic studies (e.g. Körber-Grohne, 1996; Woldring, 2000; Nielsen and Olrik, 2001) morphometric analyses have been carried out on an arbitrary number of endocarps, without any investigation as to whether the number selected was sufficient to obtain a representative view of the taxonomic unit studied. The sample size that is required to

TABLE 7. *Mean values and standard deviations of each character for group I and group II. Group I represents endocarps from an individual of* P. insititia *sampled in August 2006, while group II represents endocarps of the same individual, but sampled one month later*

Parameter	Group I (August)		Group II (September)			
	Mean	s.d.	Mean	s.d.	*t*-value	*P*
SL	12·0430	0·6099	12·0397	0·5718	0·0218	0·9827
SW	6·9653	0·3224	6·8957	0·3035	0·8618	0·3923
ST	9·8690	0·4379	9·6770	0·3637	1·8473	0·0698
100SW/SL	57·9293	3·1304	57·3471	2·7815	0·7614	0·4495
100ST/SL	82·0734	4·1719	80·4974	3·8800	1·5151	0·1352
100ST/SW	141·7743	4·6108	140·4334	4·2422	1·1722	0·2459
SL/(ST × SW)	2·1184	0·2004	2·1797	0·1940	−1·2040	0·2336
Perimeter	35·1546	1·3978	35·2823	1·3670	−0·3580	0·7218
Area	83·9513	6·4107	83·2574	6·0092	0·4326	0·6669
Triangle	1·0276	0·0994	0·9554	0·0761	3·1597	0·0025*
Ellipse	0·9123	0·0136	0·9055	0·0241	1·3557	0·1804
Circular	0·9528	0·0085	0·9499	0·0096	1·2475	0·2172
Rectangular	0·3749	0·0130	0·3737	0·0161	0·3149	0·7540
Vasym	7·9425	0·9381	8·1989	1·4947	−0·7960	0·4295

* Significant difference between group means. Abbreviations used are explained in Table 2.

achieve a desired precision in estimating a population is indispensable information and should be determined before starting an extensive sampling. That sample size is generally low when estimating for a population that represents an individual. It was found that at most eight endocarps had to be measured in order to obtain a desired precision (i.e. mean value ± 5 %) in estimating an individual mean for all linear measurements and all index values [except for $SL^2/(ST \times SW)$], and also for the parameters Perimeter, Ellipse, Circular and Rectangular. This means that, for example, in a study such as that of Hübner and Wissemann (2004), who considered only five endocarps per individual, it is possible that a representative view of an individual was not obtained, which could result in inaccurate conclusions. When focusing on the species level, the required sample sizes are in general higher in comparison with the individual level. When the population size or taxonomic level of biological entities increases, a bigger portion of the (naturally) occurring variation is included. As a consequence, the number of samples that has to be considered for a certain parameter in order to obtain a representative view of a taxonomic unit also increases. From our study, we can conclude that all linear measurements, index values and the parameters Perimeter, Area, Triangle, Ellipse, Circular and Rectangular have low required sample sizes, both on the individual and the species level, and they are thus potentially useful for further taxonomic analysis. However, of the index values studied, 100ST/SW should be preferred because of its low sample size ($n < 20$) while, for example, $SL^2/(ST \times SW)$ is less adequate due to its variable and/or high required sample size ($8 \leq n \leq 172$). The required sample sizes for the parameter Vasym were found to be highly variable and/or too high for further taxonomic use.

Knowledge of the degree of variability within and between species is a *sine qua non* for taxonomic research. However, except for *P. spinosa* in Hübner and Wissemann (2004), no meaningful study of intra-individual and intraspecific variation of Eurasian plum taxa has been performed. When all taxa studied are considered, our research

TABLE 8. P-*values of pairwise* t-*tests for a comparison between fresh endocarps and endocarps stored at 100 °C (measured at day 17) and between endocarps stored at 100 °C (measured at day 17) and fully rehydrated endocarps (measured at day 28) for three different individuals belonging to three different taxa*

Parameter	*P. domestica*		*P. spinosa*		*P. cerasifera*	
	Fresh vs. 100 °C	100 °C vs. rehydr.	Fresh vs. 100 °C	100 °C vs. rehydr.	Fresh vs. 100 °C	100 °C vs. rehydr.
100ST/SW	0·3346	0·4750	0·4442	0·7826	0·0774	0·0514
Perimeter	0·0223*	0·0212*	0·0089*	0·0037*	0·0604	0·0577
Area	0·0500*	0·0299*	0·0117*	0·0035*	0·0684	0·0549
Triangle	0·5106	0·9035	0·5495	0·4666	0·1818	0·9138
Ellipse	0·4049	0·7865	0·6273	0·9835	0·0006*	0·9240
Circular	0·5464	0·3725	0·9572	0·5581	0·8264	0·7862
Rectangular	0·0797	0·9632	0·2689	0·5098	0·0010*	0·8000
Vasym	0·3310	0·5740	0·6137	0·9179	0·0018*	0·5975

* Significant difference between groups. Abbreviations used are explained in Table 2.

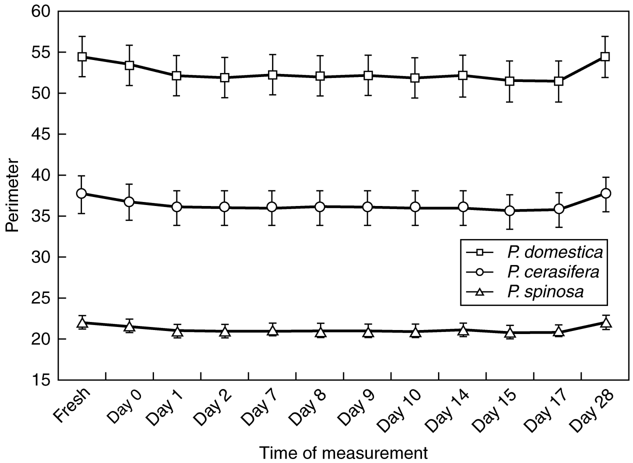


FIG. 5. Mean values of Perimeter for endocarps of three different individuals belonging to three different taxa (*P. domestica*, *P. cerasifera*, *P. spinosa*) exposed to different hydration conditions. For full explanation of treatments, see Materials and Methods.

demonstrates that, because of their low or normal variability both on the intra-individual and intraspecific level, the index value 100ST/SW and the parameters Perimeter, Area, Triangle, Ellipse, Circular and Rectangular are most useful for further analysis. However, if only one particular species is assessed, more parameters may show a low-to-normal variability both on the intra-individual and intraspecific level. The findings of Hübner and Wissemann (2004), who established that for *P. spinosa* the linear measurements and index values had a low-to-normal variability for populations and for individuals, have been confirmed in our study. The index value $SL^2/(ST \times SW)$ and the parameter Vasym were too variable on one or more taxonomic levels and therefore they should be omitted from further taxonomic analysis. The study of required samples sizes and of intra-individual and intraspecific variability both revealed that 100ST/SW, Perimeter, Area, Triangle, Ellipse, Circular and Rectangular are most appropriate for further taxonomic use.

When only five endocarps are measured, the coefficients of variance may be more biased than when 30 endocarps are measured per individual. This may result in different values for the variability. The high variability for a particular parameter may also be caused by the difficulties involved in measuring that parameter. For example, *P. spinosa* has small, rounded endocarps that are difficult to orientate uniformly during digitizing; even when being very careful, it is difficult to prevent minor deviations in the orientation of all the endocarps that are measured. Similar problems were experienced by Körber-Grohne (1996), who emphasized that the statistical usefulness of measurements of the small endocarps of *P. spinosa* had limitations. On the species level, the highest variability was observed for endocarps of *P. domestica*. This may be caused by the fact that, firstly, *P. domestica* includes a lot of intraspecific taxa (see, for example, Clapham *et al.*, 1962; Behre, 1978; Krüssmann, 1978; Scholz and Scholz, 1995; Lambinon *et al.*, 1998; Kühn, 1999) and, secondly, that in order to achieve a representative set of all these morphologically different types, this species had the highest sample size in the current study.

When considering only the mean values, some parameters seem to be obviously different between the taxa studied. However, they should not be used as diagnostic features because of their high required sample size and/or their high variability; when morphological variation for potential diagnostic characters is assessed among taxa using univariate statistics, the ranges show a large overlap between the different taxa studied. Therefore, instead of considering one single diagnostic parameter, multivariate morphometric analysis should be performed in order to select a combination of characters that enables separation of the taxa of interest.

Another aspect that could be important in the study of endocarp variability is the time of sampling and, inextricably bound up with that, the maturity of the endocarps. Our analysis revealed that only Triangle differed significantly ($P \leq 0{\cdot}05$) between endocarps of *P. insititia* sampled in August and those of the same individual sampled in September. Barabé *et al.* (1995) stated that in their study of *Prunus serotina* and *P. virginiana* fruits, endocarp growth occured in a single period without interruption and without there being a second growth phase towards the end of maturation. This may indicate that the studied endocarps were still differentiating during the sampling period. However, in their morphometric study of recently sampled and archaeological olive (*Olea europaea*) endocarps, Terral *et al.* (2004), demonstrated that no significant shape differences were observed between mature and immature stones. However, these studies did not include taxa of *Prunus* section *Prunus* and hence it would be very interesting to test the influence of maturation on endocarps of Eurasian plum taxa over a longer time period.

In archaeological studies (e.g. Behre, 1978; Van Zeist *et al.*, 1994; Van Zeist and Woldring, 2000; Pollmann *et al.*, 2005), contexts have been examined that contain endocarps of *Prunus*. In some cases, an attempt has been made to identify these endocarps based on identification keys designed for recent plant material. However, when endocarps are conserved for centuries in dry and/or humid conditions at different or changing temperatures, they may undergo severe transformation. However, Terral *et al.* (2004) demonstrated that the effect of carbonization (400 °C under an anaerobic atmosphere) on olive-stone shape was not significant and, consequently, that ancient specimens can be analysed together with wild, modern specimens and cultivars. In contrast, our study showed that both drying of freshly sampled *Prunus* endocarps and subsequent rehydration of dried endocarps could (depending on the species studied) influence particular endocarp parameters in a significant ($P \leq 0{\cdot}05$) way. Fully rehydrated endocarps had almost exactly the same dimensions and shape as freshly sampled endocarps. Therefore, for identification purposes it is necessary to fully rehydrate archaeological samples in order to be able to compare them with recent material.

In conclusion, before searching for diagnostic features between species, the required sample sizes should be determined. Next, intra-individual and intraspecific variability should be analysed and compared with the interspecific variability. In our study, some parameters were found to be very useful for further taxonomic research, based on their low sample size and low variability. In contrast, other parameters exhibited a high sample size requirement and/or high variability and we suggest omitting their use for taxonomic purposes. Because of their overlapping ranges, a single or a couple of parameters are not sufficient to discriminate between taxa of the section *Prunus*. Therefore, multivariate statistics would have to be performed in order to find a combination of parameters that allows the separation of the different Eurasian plum taxa being studied. In addition, some other factors may have an influence on endocarp dimensions and/or shape. The difference between the dimensions and shape of mature endocarps sampled with a time difference of 1 month was negligible. In contrast, the level of hydration had an influence on mature endocarp dimensions and shape. Therefore, before analysing, full rehydration of the endocarps is recommended, as this seems to restore the original dimensions and shape of freshly sampled endocarps.

ACKNOWLEDGEMENTS

Research was funded by a PhD grant of the Institute for the Promotion of Innovation through science and Technology in Flanders (IWT-Vlaanderen). We are grateful to H. Woldring for placing the endocarps of *P. insititia* at our disposal and for giving advice on the introduction; the different European Botanic Gardens for providing endocarp samples of *P. spinosa*, *P. cerasifera* and *P. domestica*; and the National Orchard Foundation (NBS, Nationale Boomgaarden Stichting, Vliermaal, Belgium) for providing endocarp samples of several domestic plum varieties.

LITERATURE CITED

Aradhya MK, Weeks C, Simon CJ. 2004. Molecular characterization of variability and relationships among seven cultivated and selected wild species of *Prunus* L. using amplified fragment length polymorphism. *Scientia Horticulturae* **103**: 131–144.

Arnold ML. 1997. *Natural hybridisation and evolution.* Oxford: Oxford University Press.

Barabé D, Daigle S, Labrecque M, St-Arnaud M. 1995. Allometry in the histogenesis of *Prunus* fruits (Rosaceae). *Canadian Journal of Botany* **73**: 299–306.

Behre KE. 1978. Formenkreise von *Prunus domestica* L. von der Wikingerzeit bis in die frühe Neuzeit nach Fruchtsteinen aus Haithabu und Alt-Schleswig. *Berichte der Deutschen Botanischen Gesellschaft* **91**: 161–179.

Bortiri E, Oh S-H, Jiang J, Baggett S, Granger A, Weeks C, *et al* 2001. Phylogeny and systematics of *Prunus* (Rosaceae) as determined by sequence analysis of ITS and the chloroplast trnL–trnF spacer DNA. *Systematic Botany* **26**: 797–807.

Bortiri E, Vanden Heuvel B, Potter D. 2006. Phylogenetic analysis of morphology in *Prunus* reveals extensive homoplasy. *Plant Systematics and Evolution* **259**: 53–71.

Brako L, Zarucchi JL. 1993. Catalogue of the flowering plants and gymnosperms of Peru. *Monographs in systematic botany from the Missouri Botanical Garden* **45**: 1–1286.

Brewer MT, Lang L, Fujimura K, Dujmovic N, Gray S, van der Knaap E. 2006. Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. *Plant Physiology* **141**: 15–25.

Browicz K, Zohary D. 1996. The genus *Amygdalus* L. (Rosaceae): species relationships, distribution, and evolution under domestication. *Genetic Resources and Crop Evolution* **43**: 229–247.

Christensen KI. 1992. Revision of *Crataegus* sect. *Crataegus* and nothosec. Crataeguineae (Rosaceae–Maloideae) in the Old World. *Systematic Botany Monographs* **35**: 1–199.

Clapham AR, Tutin TG, Warburg EF. (eds) 1962. *Prunus*. In: *Flora of the British Isles*, 2nd ed. Cambridge: University Press, 413–419.

Elias TS. 1980. The complete trees of North America. New York: Van Nostrand Reinhold Company, 1–948.

Fedorov AA, Komarov VL, Kostina KF, Kovalev NV, Krishtofovich AN, Linchevskii IA, Poyarkova AI. 1971. Rosaceae–Rosoideae–Prunoideae. In: Komarov VL. ed. *Flora of the USSR*, vol. 10. Moscow: Botanical Institute of the Academy of Sciences of the USSR, 380–448.

Hanelt P. 1997. European wild relatives of *Prunus* fruit crops. *Bocconea* **7**: 401–408.

Hedrick UP. 1911. *The plums of New York.* New York: Lyon Company.

Hübner S, Wissemann V. 2004. Morphometrische Analysen zur Variabilität von *Prunus spinosa* L.–Populationen (Prunoideae, Rosaceae) im Mittleren Saaletal, Thüringen. *Forum geobotanicum* **1**: 19–51.

Ingram C. 1948. *Ornamental cherries.* London: Country Life Limited.

Kalkman C. 1965. The Old World species of *Prunus* subg. *Laurocerasus* including those formerly referred to *Pygeum*. *Blumea* **8**: 1–174.

Katayama H, Uematsu C. 2005. Structural analysis of chloroplast DNA in *Prunus* (Rosaceae): evolution, genetic diversity and unequal mutations. *Theoretical and Applied Genetics* **111**: 1430–1439.

Körber-Grohne U. 1996. *Pflaumen, Kirschpflaumen, Schlehen–Heutige Pflanzen und ihre Geschichte seit der Frühzeit*. Stuttgart: Theiss.

Krüssmann G. 1978. *Handbuch der Laubgehölze*, Band III. Berlin and Hamburg: Verlag Paul Parey.

Krüssmann G. 1986. *Manual of cultivated broad-leaved trees and shrubs*, vol. 3. Portland: Timber Press.

Kühn F. 1999. Alte Pflaumen, lebende Zeugen mittelalterlichen Obstbaus. *Hamburger Werkstattreihe zur Archäologie* **4**: 70–77.

Kuitert W. 1999. *Japanese flowering cherries.* Portland: Timber Press.

Lambinon J, De Langhe J-E, Delvosalle L, Duvigneaud J. 1998. *Prunus*. In: *Flora van België, het Groothertogdom Luxemburg, Noord-Frankrijk en de aangrenzende gebieden (Pteridofyten en Spermatofyten)*, 3rd edn. Bouchout (Meise): Nationale Plantentuin van België, 337–342.

Lee S, Wen J. 2001. A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. *American Journal of Botany* **88**: 150–160.

Mason SC. 1913. The pubescent-fruited species of *Prunus* of the Southwestern States. *Journal of Agricultural Research* **1**: 147–179.

Moore JN, Ballington JR Jr. 1990. *Genetic resources of temperate fruit and nut crops*. Wageningen: International Society for Horticultural Science.

Nielsen J, Olrik DC. 2001. A morphometric analysis of *Prunus spinosa*, *P. domestica* ssp. *insititia*, and their putative hybrids in Denmark. *Nordic Journal of Botany* **21**: 349–363.

Pollmann B, Jacomet S, Schlumbaum A. 2005. Morphological and genetic studies of waterlogged *Prunus* species from the Roman *vicus Tasgetium* (Eschenz, Switzerland). *Journal of Archaeological Science* **32**: 1471–1480.

Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA, Campbell CS. 2007. Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution* **266**: 5–43.

Rehder A. 1940. *Manual of cultivated trees and shrubs hardy in North America*, 2nd edn. New York: The Macmillan Company.

Röder K. 1940. Sortenkundliche Untersuchungen an *Prunus domestica*. *Kühn-Archiv* **54**: 1–132.

Scholz H, Scholz I. 1995. Prunoideae. In: Conert HJ, Jäger EJ, Kadereit JW, Schultze-Motel W, Wagenitz G, Weber HE eds. *Gustav Hegi's Illustrierte Flora von Mitteleuropa*, Band IV Teil 2B. Berlin: Blackwell Wissenschafts-Verlag, 446–510.

Shaw J, Small R. 2004. Addressing the 'hardest puzzle in American pomology': phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. *American Journal of Botany* **91**: 985–996.

Takhtajan A. 1997. *Diversity and classification of flowering plants*. New York: Columbia University Press.

Taylor HV. 1949. *The plums of England*. London: Crosby Lockwood & Son.

Terral J-F, Alonso N, Capdevila RBI, Chatti N, Fabre L, Fiorentino G, *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.

Van Zeist W, Woldring H. 2000. Plum (*Prunus domestica* L.) varieties in late- and post-medieval Groningen: the archaeobotanical evidence. *Palaeohistoria* **39/40**: 563–576.

Van Zeist W, Woldring H, Neef R. 1994. Plant husbandry and vegetation of early medieval Douai, northern France. *Vegetation History and Archaeobotany* **3**: 191–218.

Wight WF. 1915. Native American species of *Prunus. Bulletin of the US Department of Agriculture* **179**: 1–75.

Woldring H. 2000. On the origin of plums: a study of sloe, damson, cherry plum, domestic plums and their intermediates. *Palaeohistoria* **39/40**: 535–562.

Yoshioka Y, Iwata H, Ohsawa R, Ninomiya S. 2004. Analysis of petal shape variation *Primula sieboldii* by elliptic Fourier descriptors and principal component analysis. *Annals of Botany* **94**: 657–664.

Yü TT, Lu LT, Ku TC, Li CL, Chen SX. 1986. Amygdaloideae. In: Yü T-t ed. Flora Reipublicae Popularis Sinicae, Vol. 38. Beijing: Science Press.

Zar JH. 1996. *Biostatistical analysis*, 3rd edn. New Jersey: Prentice Hall.