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Man, plant remains, diet: spread and ecology of *Prunus* L. in Sardinia

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Summary

Introduction

Plant-based diet is linked to man by a long history of agricultural traditions and food habits that, for many species, started during the Prehistory, when the first human groups began to produce the food as needed (Barnard 2004).

During the millennia that have preceded the development of agricultural techniques, the economy of human populations was mainly based on hunting and harvesting; adaptations to the dietary habits depended essentially upon the availability of plants and animals in the surrounding territory. Only later, with the perfection of techniques and tools, and with the selection of suitable preferred species, agriculture became the main form of economy: these alimentary choices greatly influenced the nature of agricultural systems (Peña-Chocarro et al. 1999; Mercuri 2008; van der Veen 2011).

The aims of the archaeobotanical research are to explore the way in which plant resources were used and managed by human groups (Peña-Chocarro et al. 2000). This discipline has an enormous potential to investigate and to elucidate the origin of plant domestication from a social, economic, technological, anthropological and historical perspective (Peña-Chocarro 1999; Peña-Chocarro and Zapata 2003; Peña-Chocarro 2007; Ibañez et al. 2008).

During the recent past, archaeobotanical studies have reached a significant position in archaeological knowledge. Recently Mercuri et al. (2014) summarized the state of art of archaeobotany research in Italy, pointing out that the multidisciplinary archaeobotanical approach centred on archaeological sites is one of the most specific and developed across Europe. In fact, through the recovery and analysis of macro-plant remains during recent archaeological research it is possible to outline a more realistic picture of the agrarian context in distant times (Agabbio et al. 2015). Sardinia for a long time was a gap on the map of European archaeobotanical research, indeed records are rather scarce and are mostly relates to casual findings (Michels and Webster 1987; Castaldi 1987; Sadori et al. 1989; Trump 1990; Costantini and Stancanelli 1997; Celant 1998, 2000, 2010; Costantini 2002).

Bakels (2002) began the first exhaustive work on archaeobotany in Sardinia, mainly focalized on Bronze Age period, which built up the bases for last newest researches about this period (Ucchesu 2014, Ucchesu et al. 2014a, 2014b, Sabato et al. 2015). Some further researches on macro-remains have been carried out on samples of Phoenician-Punic period (Montanari 2003; van Dommelen et al. 2008; van Dommelen and Finocchi 2008; Miola et al. 2009; Pérez Jordà et al. 2010). Further works have been carried out also for the Medieval Age (Becca et al. 2013; Bosi and Bandini Mazzanti 2013).

Ancient Fruits and Crop Wild relatives (CWR)

The impoverishment of the genetic heritage also affects tree and shrub species (80% of varieties of fruit species of Italian origin are considered to be at risk of extinction) mostly due to the progressive reduction of biodiversity (Bevilacqua 1996).

Furthermore, those cultivars that, in the last decades, have experienced a slow and progressive abandonment, much to the advantage of industrial fruit farming, are to be considered ancient fruits. Currently, the world of research is giving increasing attention to the ancient varieties, in order to reuse them in sustainable agriculture and biotechnology.

Italy and Sardinia hold a certainly rich heritage of cultivars because of their geological and agro-environmental heterogeneity (Agabbio 1994; Agabbio et al. 2015).

Despite the fact, the National-Regional Conference, 2008 has approved the "National Plan on Biodiversity of Agricultural Interest". In consideration of the extinction and progressive reduction of animal, plant and microbial genetic resources, this plan aims to provide guidelines for the preservation and enhancement of genetic resources in agriculture, in accordance with the existing legislation and with the principles of national and international policy documents.

The abandonment of traditional agriculture has caused negative environmental and social consequences, such as geological hazards, fragmentation of habitats, marginalization and

abandonment of historical cultivations, degradation of the landscape, extension of forests on previously cultivated territories, problems of depletion and pollution of soils, food-related risks and a general loss of landscape biodiversity (Biondi 2003). Ancient fruits must be considered as cultural heritage, because of the cultural identity they bear. With regard to the alimentary biodiversity, the entity of the loss of plant biodiversity is well documented for cereals, while little is known for arboriculture, in fact, many species of so-called minor fruits have almost disappeared (Bevilacqua 1996).

These changes mainly affected species with a short cycle, such as cherry, peach and plum, and had minor effects on species that survive longer such as olive tree. It may be of importance, for ancient fruits, to perform *ex situ* conservation of the germplasm, as these species may play a decisive role in the reinstatement of a sustainable agriculture, of an agriculture of typicality that opposes to the globalizing trends. The retrieval of marginal lands along with the issue of trademarks "Denominazione di Origine Protetta" (DOP) and "Indicazione Geografica Protetta" (IGP) might represent valid strategies to pursue sustainable quality and typicality, and contemporarily counteract the negative environmental impact (ISPRA 2010). Ancient fruits are just a topic of a wider subject in which historical, anthropological and archaeobotanical research should find their rightful space. At the regional level, several catalogue fields of fruit trees have been established. In Sardinia, for example, the "Agenzia per la ricerca in Agricoltura in Sardegna" (AGRIS) and the "Istituto di Scienze delle Produzioni Alimentari" (ISPA- CNR) hold the biodiversity of various fruit plant cultivars.

In order to preserve and enhance ancient fruits and new cultivars is important to consider the wild plant species that are close relations of domesticated plants, the Crop Wild Relatives (CWR), (Harlan and de Wet 1971; Maxted et al. 2006). In the context of CWR, Europe is an important centre of diversity of many crops and their wild relatives they are potential genetic resources for crop improvement and food security. Kell et al. (2005) report that there are between 50,000-60,000 crop wild relatives in the wild. About 10,739 of these are important Plant Genetic Resources For Food and Agriculture (PGRFA) and 700 of these, representing less than 0.26% of the world flora, are the most important in terms of global food security and the ones requiring urgent conservation measures. Climate change itself poses a major threat to CWR. The predicted rise in global temperatures over the next 50 years and the consequent changes in regional and seasonal rainfall patterns will have a significant impact on the survival of CWR, accelerating the reduction of suitable habitats and increasing the rate of habitat fragmentation with many predicted to be extinct by 2050 (Negri et al. 2007, Kell 2015)

Recent advances about CWR diversity in the European region, as well as in planning for its complementary conservation (both *in situ* and *ex situ*), provides a solid foundation for the development of a strategic approach to their conservation in Europe based on a range of commonly agreed and widely tested scientific concepts and techniques. To achieve sustainable conservation of CWR and maximize their sustainable exploitation in Europe, there is an imperative to develop an EU-led policy to harmonize their conservation, characterization and evaluation with existing biodiversity conservation and agricultural initiatives, and to develop new initiatives where necessary (Maxted et al. 2003).

Italy holds a great variety of wild progenitors of cultivated plants (Negri et al. 2007; Panella et al. 2012). The CWR checklist of Italy contains 7,128 species and the Italian priority list includes 797 species of which 123 are top priority, because they are related to food crops (FAOSTAT 2013). The Sicilian and Sardinian priority lists include 74 and 43 species, respectively, deserving the highest attention in planning a Plant Genetic Resources (PGR).

The International Union for Conservation of Nature (IUCN) has recently recognized the importance of wild progenitors and the lack of protection, thus establishing the Crop Wild Relative Specialist Group (CWRSG), in the context of the Species Survival Commission (SSC). On a similar basis, the European Cooperative Program on Genetic Resources

(ECP/GR), designed by Biodiversity International, has formed a working group to promote actions for the protection of wild progenitors (Kell et al. 2005, Hawkins et al. 2008).

The *Prunus* L. genus

The genus *Prunus* belongs to of the Rosaceae family and is a wide-ranging genus comprising about 400 species. Its 5 well-marked subgenera include plums and apricots, almonds and peaches, umbellate cherries, deciduous racemes cherries, and the evergreen racemes or laurel cherries (Thorne 1992).

Prunus is economically very important and many species are cultivated worldwide for their fruits, such as sweet and sour cherries (*Prunus cerasus* L. and *Prunus avium* (L.) L.), apricot (*Prunus armeniaca* L.), almond (*Prunus dulcis* (Mill.) D. A. Webb), peach (*Prunus persica* (L.) Batsch) and plums (*Prunus cerasifera* Ehrh., *Prunus salicina* Lindl., *P. domestica* L.). Several species of subgenus *Prunus* like *Prunus serotina* Ehrh. is valued for its timber (Elias 1980) and several *Prunus* species are ornamentals, such as flowering cherries of subgenus *Cerasus* L. (Ingram 1948; Krüssmann 1986; Kuitert 1999).

Prunus has many trees and shrubs and is an important component of Northern Hemisphere forest and desert communities (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 widely distributed in both the eastern and the western hemispheres north of the Equator.

The centre of origin is extended from Asia, Europe to America for different biotypes/landraces. Centres of origin for major plum species can be broadly designated as China for Japanese plum (*P. salicina*), southern Europe or western Asia for European plums (*P. domestica*), North America for American plum (*P. americana* Marsh.), western Asia or Europe for Damson plum [*P. domestica* subsp. *insititia* (L.) Bonnier & Layens], western and central Asia as well as Europe for cherry plum (*P. cerasifera*), (Crane and Lawrence 1956; Watkins 1995). Native places of other species have been identified as Italy, Greece and Yugoslavia for Italian plum (*Prunus cocomilia* Ten.), Europe and Asia for sloe (*P. spinosa* L.).

Linnaeus used four genera to include the species of modern species: *Prunus* L., *Amygdalus* L. *Cerasus* L. and *Padus* L. (simplified into *Amygdalus L.* and *Prunus* in 1758) (Linnaeus 1830). Since then, the various genera of Linnaeus and others have become subgenera and sections, as it is clearer that all the species are more closely related.

A recent DNA study of 48 species concluded that *Prunus* is monophyletic and is descended from some Eurasian ancestor. Historical treatments break the genus into several different genera, but this segregation is not currently widely recognised other than at the subgeneric rank (Bortiri et al. 2006). The taxonomic complexity of the subgenus *Prunus* has been stated (Nielsen and Olrik, 2001; Hanelt 1997). Recently, in their morphological analysis, Bortiri and co-workers demonstrated that the subgenus *Prunus* consists of sections *Prunus* (including *P. cerasifera*), *Prunocerasus* Koehne., *Armeniaca* L., *Penarmeniaca* Mason., *Piloprunus* Mason. and *Microcerasus* Webb. (Bortiri et al. 2006). According to Woldring (2000) *P. cerasifera*, *P. domestica* subsp. *insititia, P. domestica* and *P. spinosa* are very closely related taxa. These close relationships within the Eurasian plums have also been demonstrated by several other authors based on morphology (Hanelt 1997; Kuhn 1999; Nielsen and Olrik 2001) and have been confirmed by a number of studies according to molecular data (Aradhya et al. 2004; Shaw and Small 2004; Katayama and Uematsu 2005; Bortiri et al. 2006). 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 for the very wide range of variation and transitional states between and within the different taxa. Woldring exemplified this noting that *P. domestica* subsp. *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. domestica* subsp. *insititia* and *P. spinosa* (Fig. 2). Furthermore, Woldring (2000), argued that hybridization and subsequent backcrossing, and possibly segregating F2 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 (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.

The value endocarps for identification purposes of species and even varieties has been stated by various authors (Van Zeist and Woldring 2000; Nielsen and Olrik 2001). Commonly, species identification is done following official descriptors, based on morphological and physiological characters of the plant. According to Behre (1978), pit dimensions are very useful for the identification of *P. domestica*, *P. domestica* subsp. *insititia* and *P. spinosa*. Whatever of all the characters used for identification, the features of the endocarps of *Prunus* taxa are the most stable ones (Woldring 2000).

The plums are divided into European (*P. domestica, P. cerasifera*), Japanese (*P. salicina, P. imonii, P. ussuriensis*), and the North American species (*P. americana, P. nigra*). By far, the most important species of plum is *P. domestica* L. (Crane and Lawrence 1956). The species is likely derived from one or a combination of several Eurasian progenitors: *P. cerasifera*, *P. spinosa*; and *P. domestica* subsp. *insititia* (Crane and Lawrence 1956; Eryomine1990; Zohary and Hopf 2000). Other species known as plum include *P. americana* L., and *P. salicina* as well as various hybrids and several other wild species. Afterwards Eryomine (1990) proposed *P. spinosa* resulted from *P. cerasifera* x *Prunus microcarpa* C. A. Mey, while *P. domestica* is *P. spinosa* x *P. cerasifera.* Reynders-Aloisi and Grellet (1994) suggested *P. spinosa* itself carries the genome from *P. cerasifera* plus a second one from an unknown ancestor that was not *P. microcarpa* C. A. Mey.

However, cytogenetics and comparative morphology do not confirm this hypothesis (Zohary and Hopf 2000). Therefore, plum may result from polyploidy forms arising from cherry plums, forming a "*P. cerasifera* - *P. domestica* polyploidy crop complex". However, the possibility of secondary hybridisation with other species, including sloe, cannot be excluded (Zohary and Hopf 2000).

Botanical identification of *Prunus* species at taxonomic level is not always possible. Some years ago, the dimensional measurements of endocarps were done manually, generally by calipers, based on fixed categories officially recognized according on different methods of some authors (Woldring 2000; Pollmann et al. 2005; Depypere et al. 2007).

Currently, thanks to the new technologies applied to plant biology, computer vision techniques a more accurate, reliable and repeatable methods to distinguish wild species from cultivated ones applicable in many areas, including the agronomical field (Kilic et al. 2007; Rovner and Gyulai 2007; Venora et al. 2007, 2009a, 2009b; Mattana et al. 2008; Appelhans et al. 2011; Fawzi 2011; Grillo et al. 2011, 2012, 2013; Herridge et al. 2011; Smykalova et al. 2011, 2013; Orrù et al. 2012; Pinna et al. 2014; Santo et al. 2015) and the archaeobotanical one (Terral et al. 2010; Bouby et al. 2013; Orrù et al. 2013; Ucchesu et al. 2014; Pagnoux et al. 2015, Sabato et al. 2015b).

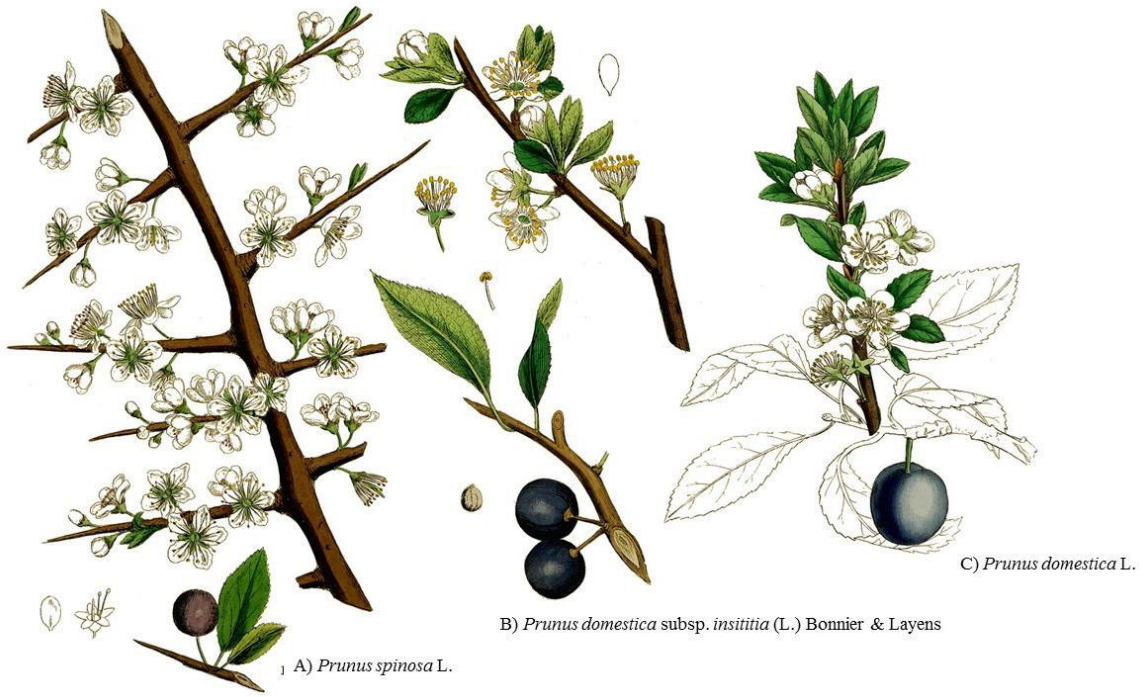

Fig. 1 Paint representation of the three species studied. (http://delta-intkey.com/angio/www/rosaceae.htm).

Prunus domestica L.

P. domestica is ranked as the second most important fruit tree crop in the temperate climate after apple. Historically is an ancient domesticated species cultivated now in temperate areas worldwide for its fruit. The species, originated in Asia Minor, was first recorded in cultivation by the Romans, and spread to Western Europe during the Crusades. *Prunus domestica* species comprises the following accepted subspecies *P. domestica* subsp. *insititia* and *P. domestica* subsp. *italica* (Borkh.) Gams. *P. domestica* trees grow 9 to 15 m tall, and has reddish-brown twigs with few or no spines; young twigs are often pubescent (covered with short, downy hair).

The petiole of 1-2 cm is densely pubescent. The leaves are oval to oblong, up to 10 cm long, somewhat serrated or with wavy margins. The flowers are white and 5-petalled and they occur singly or in clusters of 2 or 3. The fruit, up to 8 cm long, is called drupe and is composed of three distinct layers: an outer skin of exocarp, a fleshy mesocarp or middle layer, and a hard, woody layer (endocarp, usually called stone) surrounding a single large seed, which is discarded when eating.

The endocarp, in the different cultivars, can be smooth or wrinkled, round, globular or elliptical. The tips can be sharp or smooth with slightly conical base. The dorsal and lateral surfaces of the endocarp you have smooth, rough or crests (Agabbio 1994). Fruit colors vary considerably across varieties, ranging from green to yellow to red to purple to black, often with a glaucous (white waxy) bloom on the surface.

P. domestica is adapted to a wide range of climatic conditions. Regarding the soil, deep and well-drained soils with pH 5.5-6.5 give the best results. However, the plums are more tolerant of all stone fruits compared with the heavy soils and waterlogging. *P. domestica* cultivars are grafted on generative and vegetetative rootstocks. eside vigorous rootstocks, such as widely used Myrobalans (*P. cerasifera*) due to their good compatibility with most cultivars (Okie 1987), some interspecies hybrids are used as rootstocks due to their tolerance to heavy soils, nematodes and dwarfing effect. Among them, the most significant are Mariana 2624 (*P. cerasifera* x *P. munsoniana*), Citation (*P. salicina* x *P. persica*), and Jaspi (*P. salicina* x *P. spinosa*). Other rotstocks include St. Julien (*P. domestica* subsp. *insititia*), Wavit (*P. domestica*) and Brompton (*P. domestica*).

A variety of indices is used for plum maturity, depending on use, cultivar and location. European plums for fresh market are harvested based on skin color and firmness, although sugar content and sugar to acid ratio has been used. Flesh color, firmness, and sugar content are the most reliable indicators for plums (Milala et al. 2013).
FAO, in 2013, estimates that the total commercial harvest of plums and sloes was 12 million metric tons, harvested from 2.5 million hectares. China is the leading producer, responsible the largest share of the global harvest, followed by the U.S., Serbia, Romania, and Chile. Within the U.S., California produces more than 90% of the commercial harvest, with additional production in Idaho, Michigan, Oregon and Washington, for a total market value of over $80 million (FAOSTAT 2013).
In Sardinia there are about 23 traditional varieties of plum that are recognized in the field catalog of CNR (Nuraxinieddu–Oristano). It was created in the 80's, picking traditional plums from different localities of Sardinia for their characterization and conservation (Agabbio et al. 2015), (Fig. 2).
P. domestica is most important in Eastern Europe. In fact, it can be considered as indigenous species in the Balkans, thousands of local biotypes were grown here for centuries and being a part of the local culture (Botu et al. 2012). This is documented, for example, by the use of traditional local varieties in postal stamps.
Thanks to their proprieties, European plums have a much wider variety of uses. Plums are high in potassium and vitamins C and K, and are a good source of dietary fibre. They are eaten fresh, dried, or prepared into preserves and they are used in baked goods and puddings, or as a condiment alongside meat dishes. They are used in various alcoholic beverages, especially in Central and Eastern Europe (Serbia and Romania) where 80% of the plums go into the production of “slivovitz” or “tuica”.
In California, almost all European plums are dried for prunes. Plums are used for jelly/jam/preserves, plum brandy, pies, cakes, tarts, and in confectionery. A recent study revealed that the consumption of dried plums and fresh is effective in preventing and reversing bone loss (Hooshmand et al. 2011).

Fig. 2. The field catalog of CNR-ISPA (Nuraxinieddu-Oristano). A particular of *P. domestica* collection with some traditional varieties.

Prunus domestica subsp. *insititia* (L.) Bonnier & Layens

P. domestica subsp. *insititia*, damson or damson plum (Porcher 2012), also archaically called the "damascene" is an edible drupaceous subspecies of *P. domestica.* The name damson derives from the earlier term "damascene" and ultimately from the Latin *prunum damascenum*, "plum of Damascus". One commonly stated theory is that damsons were first cultivated in antiquity in the area around the ancient city of Damascus, capital of modern-day Syria, and were introduced into Europe by the Romans. The historical link between the Roman-era damascenum and the north and west European damson is rather tenuous despite the adoption of the older name, particularly as the damascenum described by the Roman authors has more of the character of a sweet dessert plum Nevertheless, remnants of damsons are sometimes found during archaeological digs of ancient Roman camps across England, and they have clearly been cultivated, and consumed, for centuries (Dalby 2003).

The exact origin of *P. domestica* subsp. *insititia* is still extremely debatable: it is thought to have originated in wild crosses, possibly in Asia Minor, between *P. spinosa* and *P. cerasifera* (Ayto 1990).

Despite this, tests on cherry plums and damsons have indicated that it is possible that the damson developed directly from of sloe (Ayto 1990). Damson has a shrub with thorny branches in the wildest or naturalized plants. Generally, it has a deeply furrowed stone, unlike bullaces, and unlike prunes cannot be successfully dried.

Most damson varieties can be identified by examining the endocarp, which varies in shape, size and texture. The flowers are hermaphrodite. The small and white anthesis takes place in early April in the northern hemisphere and are pollinated by bees. The fruits are harvested from late August to September-October, depending on the cultivar.

The fruit of damson can also be identified by its shape, which is usually oval and slightly pointed or pear-shaped; Damson color which goes from dark blue to indigo to almost black depends on the variety (Dalby 2003).

The main characteristic of the damson is its distinctive rich flavour: unlike other plums, it is both high in sugars and highly astringent. The skin of the damson can have a very tart flavour; particularly when unripe (the term "damson" is often used to describe red wines with rich yet acidic plummy flavours). The fruit is therefore most often used for cooking, and is commercially grown for preparation in jam and other fruit preserves (Greenoak 1983). Damson gin is made in a similar manner to sloe gin, although less sugar is necessary, as the damsons are sweeter than sloes. Some damson varieties are used to make slivovitz, a distilled plum spirit made in Slavic countries.

Prunus spinosa L.

P. spinosa is a wild species belonging to the *Prunus* genus. The specific epithet *"spinosa"* refers to the sharp spines or thorns that are characteristic of this plant.

An important plant for wildlife, its early spring flowers provide nectar for early emerging insects, and its branches create a spiny thicket, providing secure nesting sites for birds.

It is a deciduous thorny shrub native to Europe, western Asia and North West Africa. It is also locally naturalised in New Zealand and Eastern North America (Woldring 2000; Marakoglu et al. 2005). This small tree is commonly frequently found at the margin of deciduous forests. It often grows in hedgerows or thickets, where it can form dense stands. *P. spinosa* is insect pollinated and propagates vegetatively through root suckers. The anthesis takes place from February to April.

The fruits are spherical blue or purple - blue drupes between 10 and 15 mm of diameter, pruinose at maturity (Depypere et al. 2007). Seeds, enclosed in a woody endocarp, are dispersed by mammals and birds (Hübner and Wisseman 2004). The leaves appear after the flowers and are alternate, lanceolate and shortly petiolate; the upper surface is dull, glabrous

and dark green while the bottom are clear and pubescent and the margin is crenate or often dentate.

P. spinosa has an essential role in the taxonomy of the genus *Prunus.* Recent studies claim that that *P. spinosa,* is a CWR with secondary gene pool that have contributed to generate the domesticated form of plum although the two species are morphologically distinct (Crane and Lawrence 1956; Eryomine1990; Zohary and Hopf 2000; Zohary et al. 2012). Other genetic studies have shown that *P. spinosa* and *P. domestica* subsp. *insititia* have close relationships with the current European domestic plums (Nassi et al. 2003; Pollmann et al. 2005; Horvath et al. 2011; Athanasiadis et al. 2013). According to the criteria of the IUCN Red List sloe is not considered threatened because it is quite common in nature.

The use and the properties of sloe are varied and have been known from ancient times. Ethnobotany literature indicates their use principally for food (Parada et al. 2009; Łuczaj 2012; Pardo-de-Santayana et al. 2013; Pieroni and Quave 2014). Dioscoride in the first century. A. D. advised the leaves cooked in wine, in lotions and gargles to treat sore throat, gingivitis and tonsillitis. He considered the resin that oozes from the stem, drunk with wine, suitable to facilitate the expulsion of bladder stones. The drupes are constipated taste, astringent, sour; and more than a little sugar to contain tannin and different acids. Moreover, has been documented the decoction of the drupes used as a medicine for the treatment of many diseases, such as: biliary dyskinesia, gut, convulsive cough, urinary and cardiovascular disorders (Tiţă et al. 2009). In Sardinia, the consumption of the drupes as food, as medicine through decoction of flowers or drupe for the treatment of cough, as well as traditional use for wool dyeing, is well documented (Atzei 2003; Campanini 2009). The decoction of the leaves and flowers were used in swills and gargle to fight tooth (Vacca-Concas 1916).

Fig. 3 *Prunus spinosa L.* loc. Illorai (Sardinia)

PhD structure

The main goal of this PhD project is to improve the knowledge about the biodiversity of the ancient fruit species in Sardinia through morphometric characterization by image analysis techniques. In particular, we will seek to increase knowledge about the origin and use of wild and cultivated *Prunus* fruits in the diet of human communities of the past, and to seek relationships with traditional varieties of Sardinia.

The project will pursue the following specific aims described in four chapter listed below:

Chapter 1: is about the identification of plum varieties. In this chapter, image analysis techniques were applied to study endocarps variability of 22 *Prunus domestica* L. varieties from Sardinia. Digital images were acquired and analysed using a macro specifically developed to measure morpho-colorimetric endocarps features. The data were later statistically processed by Linear Discriminant Analysis (LDA).

The specific aim of this chapter is to measure endocarp descriptive features such as shape, size, surface color and texture by computer image analysis in order to:

- ✓ assess the existence of a relationship between the endocarp biometric features and the drupe color;
- ✓ implement a statistical classifier able to identify and classify each variety of *P. domestica*;
- ✓ identify plausible synonymy groups within the studied varieties.

Chapter 2: is about the identification by image analysis of *Prunus* L. endocarps from the Phoenician-Punic context of Santa Giusta (Oristano, Sardinia), dated between the 5th and the 2nd century BC).

The main objectives of this chapter are:

- ✓ investigate the domestication level of the waterlogged remains discovered in the amphorae of the archaeological site of Santa Giusta through image analysis system and the Linear Discriminant Analysis (LDA) method;
- ✓ exploring the possible relationships among archaeological remains, traditional varieties of *P. domestica* and *P. spinosa* populations present in Sardinia.

Chapter 3: describes the image analysis application on the waterlogged archaeological *Prunus* L. remains from the medieval context of Sassari (Via Satta) in Sardinia dated 1330-1360 AD.

The main goals of chapter 3 are:

- ✓ identify and characterize *Prunus* remains from Medieval Period by computer image analysis;
- ✓ compare the archaeological endocarps with the modern one.
- ✓ applied Linear Discriminant Analysis (LDA) to investigate the *status* of domestication of *Prunus* in the Medieval Period in Sardinia.

Chapter 4: describes the morphometric analysis on *Prunus spinosa* L remains from three Sardinian archaeological contexts. The sites under analysis are: Sa Osa (dated to the 12th-10th BC), Santa Giusta (Oristano, Sardinia), dated between the 5th and the 2nd century BC, and Via Satta (Sassari) dated back to the Middle Ages. The main goals of chapter 4 are:

- ✓ define the state of the art of *P. spinosa* remains in Sardinia;
- ✓ analyse the waterlogged endocarps from the archaeological sites of Sa Osa, Santa Giusta and Via Satta (SS) through the measurement of biometric features;
- ✓ explore the possible relationships among archaeological remains and the modern *P. spinosa* populations present in Sardinia.

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Chapter 1: Phenotypic identification of plum varieties (*Prunus domestica* L.) by endocarps morpho-colorimetric and textural descriptors.

Introduction

Plum, together with apple and pear, is one of the most important fruit in temperate regions of the world (Zohary et al. 2012). Plums are grouped into three main categories, *Prunus salicina* Lindl., *Prunus domestica* L., and [*Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens], cultivated before to the introduction of *P. domestica* (Crane and Lawrence 1956). Many authors are agree on the hypothesis that *P. domestica* derived from one or a combination of several Eurasian progenitors as *P. domestica* subsp. *insititia*, *P. cerasifera* Ehrh. and *P. spinosa* L. (Okie and Weinberger 1996; Faust and Surányi 1999; Zohary and Hopf 2012; Zohary et al. 2012). In addition, genetic studies have shown that *P. spinosa* and *P. domestica* subsp. *insititia* have close relationships with the current European domestic plums (Nassi et al. 2003; Pollmann et al. 2005; Horvath et al. 2011; Athanasiadis et al. 2013). *P. domestica* seems to have originated in Southern Europe or Western Asia between the Caucasus Mountains and the Caspian Sea overlapping with the centre of origin of *P. cerasifera*, and from there moved into Western Europe. The earliest archaeological remains of *P. domestica* in Europe is attributable to the Roman Period (Körber-Grohne 1996; Faust and Surányi 1999; Feemster and Meyer 2002; Zohary et al. 2012). During the Roman period, the domestic plum seems to appear and spread in western Europe (Janick 2005): in fact, plum seem to appear mostly in the Roman waterlogged archaeological context.

P. domestica fruits exhibit a great diversity in size, shape, color and taste. The endocarp can be smooth or wrinkled, round, globular or elliptical and it can be sharp or smooth with slightly conical base. The dorsal and lateral surfaces of the endocarp can also present crests (Agabbio 1994). There are significant differences in color among plum fruits. Usenik et al. (2009) demonstrated that there is a wide range of variability in the anthocyanins and chromatic parameters during fruit ripening of *P. domestica* varieties.

Commonly, variety identification is done following official descriptors, based on morphological and physiological characters of the plant. Until some years ago, the dimensional measurements of endocarps were done manually, generally by calipers, based on fixed categories officially recognized (Horvath et al. 2011). For this reason, in literature there are only studies on *P. domestica* endocarps based on classical morphologic and morphometric techniques (Körber-Grohne 1996; Van Zeist and Woldring 2000; Woldring 2000; Nielsen and Olrik 2001; Hübner and Wissemann 2004; Pollmann et al. 2005; Depypere et al. 2007). In the last two decades, a significant increase in image analysis applications has been highlighted in the plant biology research field and automatized system have the potential to replace human visual assessments.

Many recent papers testify the importance of the biometric features, measured by computer vision techniques, in taxonomic studies, to characterize and identify wild plant species (Rovner and Gyulai 2007; Kilic et al. 2007: Venora et al. 2007; Mattana et al. 2008; Appelhans et al. 2011; Fawzi 2011; Herridge et al. 2011; Grillo et al. 2012; Lo Bianco et al. 2015a; Pinna et al. 2014; Santo et al. 2015). This has stimulated research in many areas, including the agronomical field (Venora et al. 2009a, 2009b; Grillo et al. 2011; Smykalova et al. 2011, 2013; Lo Bianco et al. 2015b; Orrù et al. 2015; Sabato et al. 2015).

In this view, the aim of this study is to measure endocarp descriptive features such as shape, size, surface color and texture by computer image analysis in order to:

- ✓ assess the existence of a relationship between the endocarp biometric features and the drupe color;
- ✓ implement a statistical classifier able to identify and classify each variety of *P. domestica*;
- ✓ identify plausible synonymy groups within the studied varieties.

Material and Methods

Plant material

Samples of *P. domestica* endocarps referred to 22 varieties were collected from the field catalog of CNR-ISPA (Nuraxinieddu, OR-Sardinia). It was created in the 80's, picking traditional plums from different localities of Sardinia for their conservation and cultivation (Agabbio et al. 2015).

One to five trees of each variety were sampled, randomly collecting mature fruits at the time of the maximum concentration of sugar in the pulp. In order to reduce the environmental effects, the fruit sampling was conducted for three years (2012-2014). In additions, two commercial varieties, Mirabolano Giallo (MIB) and Mirabolano Rosso (MIR), were included in this study as outgroup, because phenotypically similar and the most representative at national level. Table 1 gives an overview of the endocarp samples used for this work.

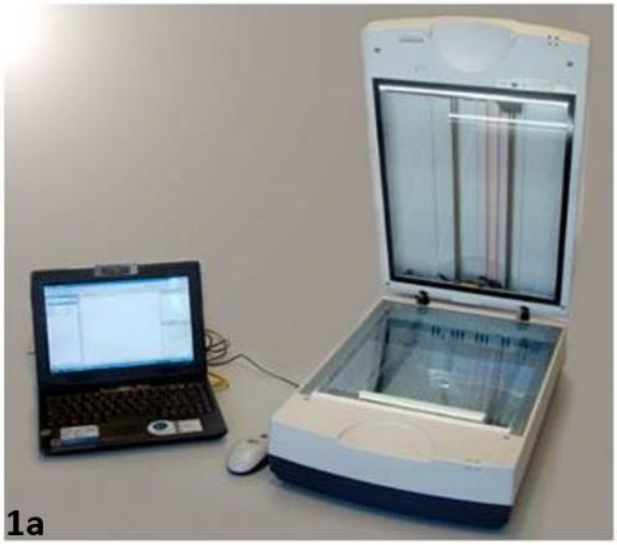
Code	Variety name	Locality	Endocarp amount	Drupe color
BOS	Bosana	Bosa	72	Y
CAD	Cariadoggia	Alghero	80	R
CAR	Cariasina	Medio Campidano	39	V
COR	Coru	Laconi	85	Y
COC	Coru ‘e Columbu	Laconi	80	Y
CRO	Croccorighedda	Laconi	85	Y
DOA	Dore A	Alghero	30	R
FAR	Fara	Bonarcado	100	O
GIB	Gialla di Bosa	Bosa	60	Y
GRO	Groga	Laconi	30	Y
LA1	Laconi A	Laconi	90	G
LA2	Laconi B	Laconi	90	Y
LA3	Laconi D	Laconi	85	R
LA4	Laconi E	Laconi	30	O
LA5	Laconi F	Laconi	70	V
MEL	Melone	Gonnosfanadiga	90	Y
LIM	Limuninca	Sassari	60	O
NES	Nero Sardo	Bosa	100	V
SAG	San Giovanni	Oristano	57	Y
SBO	Sanguigna di Bosa	Bosa	150	R/V
SAE	Sant'Elia	Nuoro	90	Y
SIG	Sighera	Gonnosfanadiga	90	R
MG1	Mirabolano Giallo	Commercial	90	Y
MG2	Mirabolano Rosso	Commercial	90	R

Tab. 1 Code, variety name, locality, amount and drupe color of studied endocarps of *P. domestica* varieties. Drupe color (G) green; (O) orange; (R) red; (Y) yellow and (V) violet.

Image analysys

Digital images of endocarps were acquired using a flatbed scanner (Epson Perfection V550 Photo), with a digital resolution of 400 dpi (Fig. 1). The images were processed and analysed using the software package KS-400 V. 3.0. (Carl Zeiss, Vision, Oberkochen, Germany). According to Shahin and Symons (2003), before the acquisition of the sample images, the scanner was calibrated for color matching, using a Q60 Kodak Color Input Target chart.

A macro, called *Prunus*.mcr specifically developed for the characterization of wild seeds (Bacchetta et al. 2008) and later modified to measure a further 20 morpho-colorimetric seed features (Mattana et al. 2008), was adapted to automatically perform the whole analysis procedure, reducing the execution time and contextual mistakes in the analysis process (Grillo et al. 2010). This macro was further enhanced adding algorithms able to compute the Elliptic Fourier Descriptors (EFDs) for each analysed endocarp, increasing the number of discriminant parameters (Tab. 2, Fig. 2).



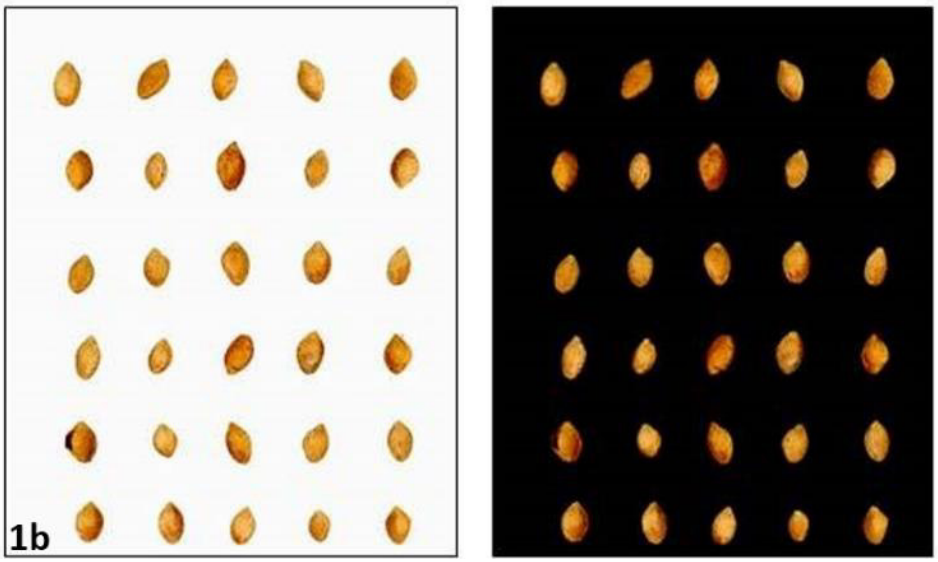


Fig. 1 a) Digital image acquisition system b) Endocarps images analysis with black and white background of the variety COR.

	Feature	Description
A	Area	Endocarp area (mm^2)
P	Perimeter	Endocarp perimeter (mm)
P_{conv}	Convex Perimeter	Convex perimeter of the endocarp (mm)
P_{Crof}	Crofton Perimeter	Crofton perimeter of the endocarp (mm)
P_{conv} /P_{Crof}	Perimeter ratio	Ratio between convex and Crofton's perimeters
D_{max}	Max diameter	Maximum diameter of the endocarp (mm)
D_{min}	Min diameter	Minimum diameter of the endocarp (mm)
D_{min} /D_{max}	Feret ratio	Ratio between minimum and maximum diameters
Sf	Shape Factor	Endocarp shape descriptor = (4 x π x area)/perimeter^2 (normalized value)
Rf	Roundness Factor	Endocarp roundness descriptor = (4 x area)/(π x max diameter^2) (normalized value)
Ecd	Eq. circular diameter	Diameter of a circle with equivalent area (mm)
F	Fiberlength	Endocarp length along the fiber axis
C	Curl degree	Ratio between D_{max} and F
$Conv$	Convessity degree	Ratio between P_{Crof} and P
Sol	Solidity degree	Ratio between A and convex area
Com	Compactness degree	Endocarp compactness descriptor = $[\sqrt{(4/\pi)}\ A\ ]/\ D_{max}$
EA_{max}	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
EA_{min}	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)
R_{mean}	Mean red channel	Red channel mean value of endocarp pixels (grey levels)
R_{sd}	Red std. deviation	Red channel standard deviation of endocarp pixels
G_{mean}	Mean green channel	Green channel mean value of endocarp pixels (grey levels)
G_{sd}	Green std. deviation	Green channel standard deviation of endocarp pixels
B_{mean}	Mean blue channel	Blue channel mean value of endocarp pixels (grey levels)
B_{sd}	Blue std. deviation	Blue channel standard deviation of endocarp pixels
H_{mean}	Mean hue channel	Hue channel mean value of endocarp pixels (grey levels)
H_{sd}	Hue std. deviation	Hue channel standard deviation of endocarp pixels
L_{mean}	Mean lightness ch.	Lightness channel mean value of endocarp pixels (grey levels)
L_{sd}	Lightness std. dev.	Lightness channel standard deviation of endocarp pixels
S_{mean}	Mean saturation ch.	Saturation channel mean value of endocarp pixels (grey levels)
S_{sd}	Saturation std. dev.	Saturation channel standard deviation of endocarp pixels
D_{mean}	Mean density	Density channel mean value of endocarp pixels (grey levels)
D_{sd}	Density std. deviation	Density channel standard deviation of endocarp pixels
S	Skewness	Asymmetry degree of intensity values distribution (grey levels)
K	Kurtosis	Peakness degree of intensity values distribution (densit. units)
H	Energy	Measure of the increasing intensity power (densitometric units)
E	entropy	dispersion power (bit)
D_{sum}	Sum of Density	Sum of Density values of the endocarp pixels (grey levels)
SqD_{sum}	Sum of the Squares of density	Sum of the Squares of density values (grey levels)

Tab. 2 List of morphocolorimetric features measured on endocarps, calculated according to Hâruta (2011), (excluding the Elliptic Fourier Descriptors - EFDs).

As described by Orrù et al. (2013), this method allows describing the boundary of the seed projection, as an array of complex numbers, which correspond to the pixels position of the seed boundary. About the use of number of harmonics for an optimal description of outlines, in order to minimize the measurement errors and optimize the efficiency of shape reconstruction, 20 harmonics were used to define the endocarp boundaries, obtaining further 78 parameters useful to discriminate among the studied varieties of *Prunus*.
Finally, the macro was further improved adding algorithms able to compute 11 Haralick's descriptors and the relative standard deviation values for each analysed endocarp (Tab. 3). These parameters are generally used to accurately describe the surface texture of an object based on grey tonal features (Haralick et al. 1973; Haralick and Shapiro 1991). A total of 135 morpho-colorimetric and textural features were measured.

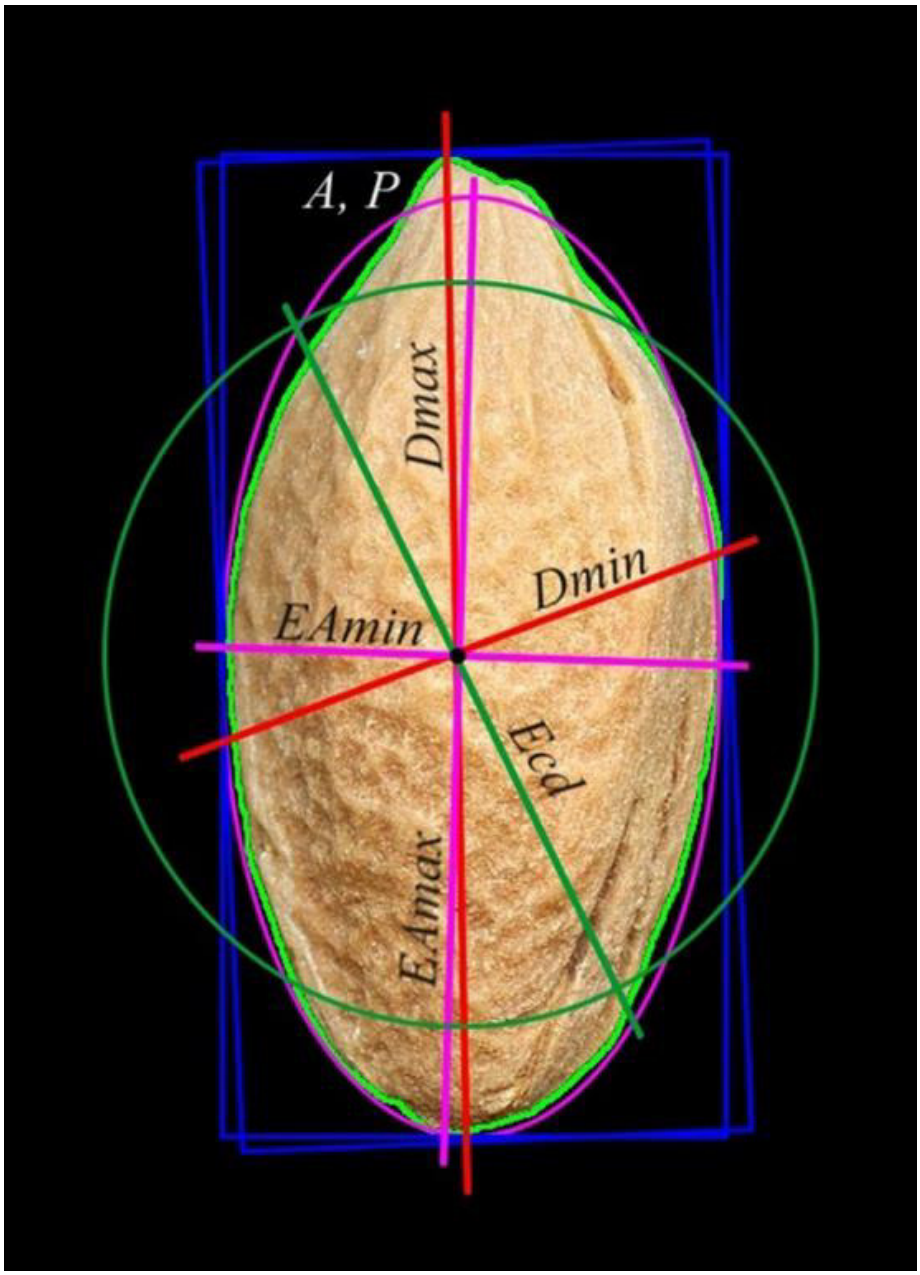


Fig. 2 Schematization of some morphometric parameters measured.

	Feature	Equation
Har 1	Angular second moment	$\sum_i \sum_j p(i,j)^2$
Har 2	Contrast	$\sum_{n=0}^{N_g-1} n^2 \left\{ \sum_{i=1}^{N_g} \sum_{j=1}^{N_g} p(i,j) \right\}, \lvert i,j \rvert = n$
Har 3	Correlation	$\frac{\sum_i \sum_j (ij) p(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y}$ where μ_x, μ_y, σ_x and σ_y are the means and the standard deviations of p_x and p_y.
Har 4	Sum of square: variance	$\sum_i \sum_j (i-\mu)^2 p(i,j)$
Har 5	Inverse difference moment	$\sum_i \sum_j \frac{1}{1+(i-j)^2} p(i,j)$
Har 6	Sum average	$\sum_{n=2}^{2N_g} i p_{x+y}(i)$ where x and y are the coordinates (row and column) of an entry in the co-occurrence matrix, and $p_{x+y}(i)$ is the probability of co-occurrence matrix coordinates summing to $x+y$.
Har 7	Sum variance	$\sum_{i=2}^{2N_g} (i-f_8)^2 p_{x+y}(i)$
Har 8	Sum entropy	$-\sum_{i=2}^{2N_g} p_{x+y}(i)\ log\{p_{x+y}(i)\} = f_8$
Har 9	Entropy	$-\sum_i \sum_j p(i,j)\ log[p(i,j)]$
Har 10	Difference variance	$\sum_{n=0}^{N_{g-1}} i^2 p_{x-y}(i)$
Har 11	Difference entropy	$-\sum_{n=0}^{N_{g-1}} p_{x-y}(i)\ log\{p_{x-y}(i)\}$

The basis for these features is the gray-level co-occurrence matrix (G in equation 1). This matrix is square with dimension Ng, where Ng is the number of gray levels in the image. Element $[i, j]$ of the matrix is generated by counting the number of times a pixel (p) with value i is adjacent to a pixel with value j and then dividing the entire matrix by the total number of such comparisons made. Each entry is therefore considered to be the probability that a pixel with value i will be found adjacent to a pixel of value j.

$$G = \begin{bmatrix} p(1,1) & p(1,2) & \cdots & p(1,N_g) \\ p(2,1) & p(2,2) & \cdots & p(2,N_g) \\ \vdots & \vdots & \ddots & \vdots \\ p(N_g,1) & p(N_g,2) & \cdots & p(N_g,N_g) \end{bmatrix} \quad (1)$$

Tab. 3 Haralick's descriptors measured as reported in Haralick et al. (1973).

Data analysys

The achieved results were used to build a database of morpho-colorimetric and texture features. Statistical elaborations were executed using IBM SPSS (Statistical Package for Social Science) software package release 16.0 (SPSS Inc. for Windows, Chicago, Illinois, USA), and the stepwise Linear Discriminant Analysis method (LDA) was applied to compare the *P. domestica* endocarps.

LDA method is commonly used to classify or identify unknown groups characterized by quantitative and qualitative variables (Fisher 1936, 1940; Sugiyama 2007), finding the combination of predictor variables with the aim of minimizing the within-class distance and maximizing the between-class distance simultaneously, thus achieving maximum class discrimination (Hastie et al. 2001; Holden et al. 2011; Alvin et al. 2012; Kuhn and Johnson 2013). The stepwise method identifies and selects the most statistically significant features among the 98 measured on each endocarp, using three statistical variables: Tolerance, *F*-to-enter and *F*-to-remove. The Tolerance value indicates the proportion of a variable variance not accounted for by other independent variables in the equation. *F*-to-enter and *F*-to-remove values define the power of each variable in the model and are useful to describe what happens if a variable is inserted and removed, respectively, from the current model. This method starts with a model that does not include any of the variables. At each step, the variable with the largest *F*-to-enter value that exceeds the entry criterion chosen ($F \geq 3.84$) is added to the model. The variables left out of the analysis at the last step have *F*-to-enter values smaller than 3.84, and therefore no more are added. The process is automatically stopped when no remaining variables are able to increase the discrimination ability (Venora et al. 2009b). Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them based on all others (SPSS 2006).

Results and Discussion

In order to assess the chance to discriminate among endocarps from drupe characterized by different gradient of color, a first statistical comparison was conducted comparing the five color categories reported in table 1. According to this analysis, an overall percentage of correct identification of 77.1% was reached, with individual performance ranged between 70.6% (violet) and 85.0% (green) (Tab. 4). This preliminary result proves the existence of a relationship between the color of the drupe and the endocarp biometrics. For this reason, the morpho-colorimetric variables selected by the LDA to discriminate among the drupe color, were investigated.

	V	**R**	**O**	**Y**	**G**	**Total**
V	**70.6 (254)**	2.3 (8)	15.7 (56)	10.7 (39)	0.7 (2)	100.0 (359)
R	14.5 (53)	**79.1 (283)**	5.8 (22)	0.6 (3)	-	100.0 (357)
O	7.9 (15)	1.1 (2)	**82.1 (156)**	7.9 (15)	1.1 (2)	100.0 (190)
Y	10.4 (69)	1.2 (8)	6.5 (44)	**75.4 (503)**	6.4 (43)	100.0 (667)
G	1.1 (1)	-	-	13.3 (14)	**85.0 (93)**	100.0 (90)
Overall						**77.1 (1,663)**

Tab. 4 Correct classification percentage among varieties grouped by drupe color. The percentages of correct identification are given in bold; the number of endocarps are given in parentheses. Drupe skin color (G) green; (O) orange; (R) red; (Y) yellow and (V) violet.

In table 5 the most discriminant five variables, over the 25 selected and used by the stepwise LDA, are reported. The first four variables are densitometric features, descriptive of the endocarp color, while the fifth is a shape descriptive variable. As a whole, 17 of the 25 chosen variables are color descriptive (data not shown). This achievement explains that the drupe color is related to the endocarp color.

	Tolerance	***F-to-remove***	***Wilks' lambda***
SqD_{sum}	0.002	126.714	0.096
D_{sum}	0.002	101.204	0.091
S_{mean}	0.017	55.403	0.083
E	0.119	55.290	0.083
Com	0.053	52.477	0.082

Tab. 5 Ranking of the best five discriminant morpho-colorimetric variables selected and used by the LDA.

In a recent paper Ucchesu et al. (2014), discussing that the archaeological seeds of *Vitis* from Sardinia were more similar to the white grape varieties rather than the black ones, proved that, a relation between the berry color and the seed morphology, exists. Although they do not evaluate the color of the seeds, the results of the present work confirm that the endocarp, or more extensively the seed, retain some characters, even if different from a species to another, also related to the drupe color.

Considering the achieved results, in order to evaluate possible similarities and differences, and contextually identify possible synonymy groups, the studied varieties of *P. domestica* were compared, distinguishing for the drupe colors (Tab. 6a; b; c). This analysis highlighted the discrimination power of the measured morpho-colorimetric features. Each of the five varietal color groups, given overall percentages of correct identification included from 94.3% (yellow, Tab. 6c) and 100% (orange and green, data not shown). In particular, the four violet varieties, showed performances ranged between 95% Cariasina (CAR) and 100% Sanguigna Bosa (SBO), registering an overall correct identification percentage of 97.3% (Tab. 6a). Very similar results were obtained for the five red varieties, with correct classification percentages included between 93% Bosana (BOS) and 100% Cariadoggia (CAD) and Laconi D (LA3), and an overall cross-validated performance of 97% (Tab. 6b). The three orange and the three green varieties were all perfectly identified, while the ten yellow drupe colored varieties showed correct identification percentages included between 81% Coru 'e Columbu (COC) and 100% Giallo di Bosa (GIB) and MIG. The high mutual misattribution percentages between the varieties Coru (COR) and COC (9.4% and 17.5%, respectively), suggest that these two nominal varieties could be synonyms of the same variety (Tab. 6c). Analysing the most discriminant variables used by the stepwise LDA, one more time it is possible to highlight the relevance of the colorimetric and densitometric features of the endocarp surface in the identification process (Tab. 7)

The predominance of parameters descriptive of color and densitometry is remarkable, especially for red drupes. Nevertheless, a few of dimensional parameters resulted discriminant for all the other colored groups, such as Feret ratio (D_{min} /D_{max}) and Compactness degree (*Com*) for Violet color drupes; Shape Factor (S_f), Perimeter ratio (P_{conf} /P_{croft}) and Curl degree (*C*) for orange drupes; Shape Factor (S_f) and Area (*A*) for yellow and green drupes. This highlights and confirms the existence of a direct relationship between the drupes color and the endocarps color (Tab. 7).

Finally, in order to confirm the plausible synonymy condition hypothesized for the varieties COR and COC, a further comparative analysis was conducted considering all the varieties together, without distinguishing for drupe color (Tab. 8). The 26 varieties were distinguished

with an overall percentage of correct identification of 86.1%, ranging between 60% COC and 100% Bosana (BOS) and LA3 proving to be easily detectable. Although the effect of all the varieties considered together, caused a significant reduction of the identification performance, the amplified mutual misidentification between the varieties COR and COC, confirms the theory that these two varieties could form a synonymy group. In addition, considering the percentages of correct identification of the varieties SB1 and SB2 in the global comparison, it is possible to assume that they have a parental line in common, although some differences exist, not only from the phenotypic point of view, such as flowering time, leaves, bearing shaft, disease resistance, differences in the chemical composition of anthocyanins and differences in ripening. Finally, the two commercial varieties, MIB and MIR were distinguished from other varieties with high values of classification respectively 94.4% for MIB and 85.6% for MIR.

	CAD	DOA	LA3	BOS	SIG	Total
CAD	**100.0 (80)**	-	-	-	-	100.0 (80)
DOA	-	**96.7 (29)**	3.3 (1)	-	-	100.0 (30)
LA3	-	-	**100.0 (85)**	-	-	100.0 (85)
BOS	-	1.7 (1)	-	**93.3 (68)**	3.3 (3)	100.0 (72)
SIG	-	-	1.1 (1)	2.2 (2)	**96.7 (87)**	100.0 (90)
Overall						**97.0 (357)**

a) Violet drupe color.

	CAR	LA5	NES	SBO	Total
CAR	**94.9 (37)**	-	2.6 (1)	2.6 (1)	100.0 (39)
LA5	1.4 (1)	**95.7 (67)**	2.9 (2)	-	100.0 (70)
NES	1.0 (1)	1.0 (1)	**97.0 (97)**	1.0 (1)	100.0 (100)
SBO	-	-	-	**100.0 (150)**	100.0 (150)
Overall					**97.3 (359)**

b) Red drupe color.

	COR	COC	CRO	GIB	GRO	LA2	MEL	SAG5	SAE	Total
COR	**89.4 (76)**	9.4 (8)	1.2 (1)	-	-	-	-	-	-	100.0 (85)
COC	17.5 (14)	**81.3 (65)**	1.3 (1)	-	-	-	-	-	-	100.0 (80)
CRO	1.7 (1)	-	**98.3 (84)**	-	-	-	-	-	-	100.0 (85)
GIB	-	-	-	**100.0 (60)**	-	-	-	-	-	100.0 (60)
GRO	-	-	-	-	**96.7 (29)**	-	-	3.3 (1)	-	100.0 (30)
LA2	-	-	-	2.2 (2)	-	**97.8 (88)**	-	-	-	100.0 (90)
MEL	-	1.1 (1)	-	-	-	1.1 (1)	**96.7 (87)**	1.1 (1)	-	100.0 (90)
SAG	-	-	-	-	5.3 (3)	-	3.5 (2)	**86.0 (49)**	5.3 (3)	100.0 (57)
SAE	-	-	-	-	-	-	-	3.3 (3)	**96.7 (87)**	100.0 (90)
Overall										**94.3 (667)**

c). Yellow drupe color.

Tab. 6 Percentages of correct identification of the three varietal color groups. The percentages of correct identification are given in bold; the number of endocarps are given in parentheses.

	V	R	O	Y	G
Number of cultivars	4	5	3	9	3
Number of steps	25	40	20	49	21
1st discriminant parameter	D_{min}/D_{max} (0.57; 94.104; 0.014)	Har5 (0.004; 36.669; 1,18E-06)	*Sf* (0.485; 94.811; 0.002)	*Sf* (0.052; 23.266; 1,51E-06)	*Bsd* (0.129; 54.446; 0.0014)
2nd discriminant parameter	Harsd7 (0.120; 35.831; 0.010)	Har11 (0.005; 188.985; 8,47E-08)	P_{conv}/P_{crof} (0.470; 68.462; 0.002)	G_{mean} (0.017; 23.243; 1,51E-06)	G_{mean} (0.018; 52.017; 0.0014)
3rd discriminant parameter	Har11 (0.098; 34.938; 0.010)	Har10 (0.004; 146.802; 7,14E-08)	*C* (0.693; 43.653; 0.001)	*Ssd* (0.175; 22.971; 1,50E-06)	L_{mean} (0.039; 39.716; 0.0012)
4th discriminant parameter	*Bsd* (0.144; 25.366; 0.009)	Har2 (0.004; 75.888; 4,90E-08)	Har1 (0.634; 28.725; 0.001)	*Bsd* (0.013; 22.796; 1,50E-06)	*A* (0.001; 36.118; 0.0012)
5th discriminant parameter	*Com* (0.176; 21.708; 0.009)	Har9 (0.021; 59.452; 4,38E-08)	Harsd8 (0.509; 23.341; 0.001)	*Rsd* (0.007; 21.093; 1,47E-06)	H_{mean} (0.061; 32.619; 0.0011)
Reached performance	97.3 %	97.0 %	100.0 %	94.3 %	100.0 %

Tab. 7 Ranking of the best five discriminant morpho-colorimetric variables selected and used by the LDA. The number of compared varieties, discriminant steps and performance of identification, are reported. For each parameter, the tolerance, *F*-to-remove and Wilks' lambda values are reported in parentheses. Drupe color (V) violet; (R) red; (O) orange; (Y) yellow and (G) green.

	BOS	CAD	CAR	COR	CCO	CRO	DOA	FAR	GIB	GRO	LA1	LA2	LA3	LA4	LA5	MEL	MIG	MIR	LIM	NES	PAR	SAG	SB1	SB2	SAE	SIG	Total
BOS	**100.0 (72)**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	100.0 (72)
CAD	–	**97.5 (78)**	–	–	–	–	–	–	–	–	–	–	2.5 (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	100.0 (80)
CAR	–	–	**82.1 (32)**	–	–	–	–	–	5.1 (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	5.1 (2)	5.1 (2)	–	2.6 (1)	100.0 (39)
COR	–	–	–	**78.8 (67)**	14.1 (12)	–	–	–	–	–	–	–	–	–	3.5 (3)	–	–	–	–	1.2 (1)	–	–	–	–	–	2.4 (2)	100.0 (85)
COC	–	–	–	30.0 (21)	**60.0 (55)**	–	–	–	–	–	–	–	–	–	–	2.5 (2)	–	–	2.5 (2)	–	–	–	–	–	–	–	100.0 (80)
CRO	–	–	–	–	–	**90.0 (55)**	1.7 (1)	–	–	–	–	–	–	–	5.0 (5)	–	–	–	3.3 (2)	–	–	–	–	–	–	–	100.0 (60)
DOA	–	–	–	–	–	–	**96.7 (29)**	–	–	–	–	–	–	–	–	–	–	3.3 (1)	–	–	–	–	–	–	–	–	100.0 (30)
FAR	–	–		–	–	–	–	**89.0 (89)**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10.0 (10)	100.0 (100)
GIB	–	–	–	–	–	–	–	–	**85.0 (51)**	1.7 (1)	–	–	–	–	–	–	–	1.7 (1)	–	–	–	–	5.0 (3)	5.0 (3)	–	1.7 (1)	100.0 (60)
GRO	–	–	–	–	–	3.3 (1)	–	–	–	**90.0 (27)**	–	–	–	–	–	–	–	–	–	–	–	–	3.3 (1)	–	–	3.3 (1)	100.0 (30)
LA1	–	–	–	–	5.6 (6)	–	–	–	–	–	**92.2 (83)**	1.1 (1)	–	–	1.1 (1)	–	–	–	–	–	–	–	–	–	–	–	100.0 (90)
LA2	–	–	–	–	–	–	–	3.3 (3)	–	–	–	**88.9 (80)**	–	–	1.1 (1)	–	–	–	–	–	–	–	–	4.4 (4)	–	1.1 (1)	100.0 (90)
LA3	–	–	–	–	–	–	–	–	–	–	–	–	**100.0 (85)**	–	–	–	–	–	–	–	–	–	–	–	–	–	100.0 (85)
LA4	–	–	–	–	–	–	–	–	–	3.3 (1)	–	–	–	**96.7 (29)**	–	–	–	–	–	–	–	–	–	–	–	–	100.0 (30)
LA5	–	–	–	–	–	5.7 (4)	1.4 (1)	1.4 (1)	1.4 (1)	–	–	–	–	1.4 (1)	**72.9 (51)**	–	–	–	–	1.4 (1)	–	5.7 (4)	2.9 (2)	1.4 (1)	4.3 (3)	–	100.0 (70)
MEL	–	–	–	–	–	1.7 (1)	–	–	–	–	–	–	–	–	–	**94.4 (85)**	–	–	–	–	2.2 (2)	2.2 (2)	–	–	–	–	100.0 (90)
MIG	–	–	1.1 (1)	–	–	–	3.3 (3)	–	–	–	–	–	–	–	–	–	**94.4 (85)**	1.1 (1)	–	–	–	–	–	–	–	–	100.0 (90)
MIR	–	–	4.4 (3)	–	–	–	2.2 (2)	–	1.1 (1)	–	–	–	–	–	–	–	–	**85.6 (77)**	–	–	–	–	3.3	1.1 (1)	–	2.2 (2)	100.0 (90)
LIM	–	–	–	–	–	1.7 (1)	–	–	–	–	3.3 (2)	–	–	–	–	–	–	–	**90.0 (54)**	5.0 (3)	–	–	–	–	–	–	100.0 (60)
NES	–	–	–	–	–	–	–	1.0 (1)	1.0 (1)	–	–	–	–	–	3.0 (3)	–	–	–	–	**88.0 (88)**	–	4.0 (4)	–	–	2.0 (2)	–	100.0 (100)
PAR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**94.4 (17)**	55.6 (1)	–	–	–	–	100.0 (18)
SAG	–	–	–	–	–	–	–	–	–	3.5 (2)	–	–	–	1.8 (1)	–	1.8	–	–	1.8 (1)	8.8 (5)	–	**68.4 (39)**	–	–	14.0 (8)	–	100.0 (57)
SB1	1.1 (1)	–	5.6 (3)	–	–	–	3.3 (2)	–	5.6 (6)	–	–	–	–	–	3.3 (3)	–	–	1.1 (1)	–	1.1 (1)	–	–	**67.8 (61)**	7.8 (7)	–	3.3 (3)	100.0 (90)
SB2	–	–	8.3 (5)	–	–	–	1.7 (1)	1.7 (1)	6.7 (4)	–	–	–	–	–	–	–	–	1.7 (1)	–	–	–	–	8.3 (5)	**68.3 (41)**	–	3.3 (2)	100.0 (60)
SAE	1.1 (1)	–	–	–	–	–	–	–	1.1 (1)	–	–	–	–	–	7.8 (7)	–	–	–	–	1.1 (1)	–	2.2 (2)	1.1 (1)	–	**84.4 (76)**	1.1 (1)	100.0 (90)
SIG	–	–	1.1 (1)	1.1 (1)	–	–	1.1 (1)	2.2 (2)	–	–	–	–	–	–	1.1 (1)	–	–	–	–	–	–	–	–	2.2 (2)	–	**91.1 (82)**	100.0 (90)
Overall																											**86.1 % (1836)**

Tab. 8 Comparisons among the analysed *P. domestica* varieties. The number of endocarps are given in parentheses.

Conclusions

A huge number of varieties characterizes Sardinian plums but due to the great anthropological and historical-cultural heterogeneity of the island, many varieties are the product of linguistic distortion creating a wide assortment of plum names (Agabbio 1994; Chessa and Nieddu 2003, 2005; Agabbio et al. 2015). Nevertheless, it was possible to hypothesize the existence of synonymy groups, the achievements discussed in this chapter confirm the extreme phenotypical, and more extensively biological diversity of *P. domestica*. Thanks to this study, for the first time, it was possible to investigate about the morphology and morphometry of plum endocarps of traditional varieties from Sardinia. The 135 morpho-colorimetric and texture features measured on the germplasm resulted a valid tool to achieve a clear discrimination among different varieties, also allowing the identification of possible synonymy groups. The obtained results prove, once again, that image analysis techniques can be considered as a useful tool in taxonomic investigations, also at varietal level. In this study according to Lo Bianco et al. (2015a), the Haralick's parameters resulted to be among the most discriminant, although different species can show different discriminant characters.

As for many other wild and cultivated species studied in the recent past (Kilic et al. 2007: Mattana et al. 2008; Venora et al. 2009a; Appelhans et al. 2011; Grillo et al. 2011, 2012; Smykalova et al. 2011; Orrù et al. 2012; Pinna et al. 2014; Lo Bianco et al. 2015a, 2015b), this method contributes to the cataloguing, conservation and improvement of the *Prunus* genus too. For those varieties of plums that have a particular economic value, this detective procedure could be used to define objective parameters useful in the attribution of European trademarks such as "Denominazione di Origine Protetta" (DOP) and "Indicazione Geografica Protetta (IGP). It could be helpful in germplasm banks, nurseries or in those institutions that deal with *ex situ* conservation of plant biodiversity. The currently proposed system results a good method for the quick and cheap identification of traditional plums for consumer satisfaction.

In the future, it would be interesting to assess whether these results would be confirmed by molecular analysis on the same traditional varieties.

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Chapter 2: Identification of *Prunus* L. endocarps from a Phoenician-Punic context (5th - 2nd century BC) by image analysis.

Introduction

In the Rosaceae family, the large genus *Prunus* includes about 400 species classified into five subgenera such as *Prunus*, *Amygdalus*, *Cerasus*, *Padus* and *Laurocerasus*, mainly distributed in temperate regions of the boreal hemisphere (Krussman 1986; Maynard et al. 1991; Aradhya et al. 2004; Yılmaz et al. 2009).

Cultivated *Prunus* includes: peach [*Prunus persica* (L.) Batsch], European plum (*Prunus domestica* L.), Japanese plum (*Prunus salicina* Lindl.), apricot (*Prunus armeniaca* L.), sweet cherry [*Prunus avium* (L.) L.], sour cherry (*Prunus cerasus* L.) and almond [*Prunus dulcis* (Mill.) D.A. Webb]. Except almonds, where the edible part is the seed, the others are consumed for their fleshy fruits (Janick 2005).

P. domestica (plum) is one of the most economic important fruits in temperate regions and represent the major crop in Europe and South-West Asia (Ramming et al. 1991; Watkins 1995; Körber-Grohne 1996; Zohary et al. 2012). The primary centre of *P. domestica* domestication has been identified in central Asia with others secondary centres in Eastern Asia, Europe and North America (Watkins 1995). In 2013, FAOSTAT estimated that the total commercial harvest of plums was 12.0 million tons cultivated from 2.5 million hectares. Today, China is the leading producer, responsible the largest share of the global harvest, followed by the U.S.A, Serbia, Romania and Chile (FAOSTAT, 2013).

Place of origin and domestication of plum is still under investigated. Crane and Lawrence (1952) and Watkins (1995) suggested that plum may be a polyploid derivative of a cross between diploid *P. cerasifera* and tetraploid *P. spinosa* L., that is a shrub with a distribution range, which extends from the west and middle Europe to Asia Minor; also, it is present in the Caucasus region and North Africa (Hegi 1995). However, as suggested by Zohary et al. (2012) the wild relative of *P. domestica* is an autopolyploid derived from *P. cerasifera* Ehrh. in which probably also partially contributed two others wild species such as *P. cocomilia* Ten. and *P. brigantino* Vill. Therefore, the possibility that *P. spinosa* have genetically contributed to generate the domesticated form of plum is unreliable because the two species are morphologically distinct. Nevertheless, it is not excluding that it might have contributed to domestic gene pool only through the secondary hybridization and later for introgression (Zohary et al. 2012).

Moreover, *P. domestica* subsp. *insititia* (L.) Bonnier & Layens (damson) is considered to be the ancestor of the modern plums (Woldring 2000; Zohary et al. 2012). In fact, recently genetic studies have shown that sloe, damson and plum have close relationships (Aradhya et al. 2004; Pollmann et al. 2005; Depypere et al. 2009; Horvath et al. 2011; Xuan and Spann 2011; Milošević and Milošević 2012; Athanasiadis et al. 2013). In addition, different authors investigated genetic relatedness from modern *Prunus* species concluding that the phylogenetic reconstruction is due to the several processes of speciation derived from hybridization occurred among them (Katayama and Uematsu 2005; Bouhadida et al. 2004, 2007; Yılmaz et al. 2009; Wünsch 2009; Horvath et al. 2011).

First archaeological evidence of *P. spinosa* endocarps has been documented in many archaeological sites, in western Mediterranean Basin, dating between Neolithic and the Bronze Age (Woldring 2000; Zohary et al. 2012). However, in archaeological sites dating between the Bronze Age and the Early Iron Age a large number of intermediate forms due to interspecific hybridization between sloe, damson and plum, were also documented (Pollmann et al. 2005). During the Roman period, the domestic plum seems to appear and spread in western Europe (Janick 2005), (for references see Tab. 1). Most of these remains consist of endocarps, whose identification to species level with the traditional

archaeobotanical methods is difficult due to morphological range variation within the different *taxa* (Woldring 2000; Pollmann et al. 2005; Depypere et al. 2007).
According to Horvath et al. (2011), the taxonomic classification of plum varieties is generally performed on the phenotypic characteristics of their flowers and fruits and it should be better to associate both morphological characteristics and molecular markers, as the phenotypic characteristics are not always reliable due to variations can occur in environmental conditions. As argued by Depypere et al. (2007) and Woldring (2000) and morphometric characteristics of the endocarps of *Prunus* would be the most stable of the all characters used for their identification. For this reason, in archaeobotanical studies, the characteristics of *Prunus* endocarps were successfully used for their classification (Pollmann et al. 2005; Zheng et al. 2014).
The oldest evidence of plum cultivation in western Mediterranean Basin was found in the cesspit under the temple of Fortuna in Pompeii in which an endocarp of plum, dated to 150 BC, was found (Zech-Matterne et al. 2015). Also from Pompeii in the House of the Orchard some paint representations of cultivated plums with yellow, blue and purple skin drupes dated back to 79 BCE were found (Jashemski and Meyer 2002).
Written sources report some descriptions of cultivated plum, for example Theophrast (κοκκυμηλέα) mentioned the name 'Prumnon' and Pliny (*Natural History*, 15.41-3) who describes several varieties of plums with fruits in yellow, red, violet, black, white or bright colors (Jashemski and Meyer 2002).
The recent discovery of several intact waterlogged endocarps in the Phoenician-Punic settlement of Santa Giusta (Oristano, Sardinia), dated in a range between the 5th and the 2nd century BC, brings into question about the spread of domesticated plums in the western Mediterranean Basin.
The main objective of this study is to investigate the domestication level of waterlogged remains, through the measurement of morphometric characters by the Linear Discriminant Analysis method (LDA) and exploring the possible relationships among archaeological remains, local cultivated plums and sloe populations present in Sardinia.

Archaeological context

The Phoenician-Punic settlement of Santa Giusta is located in the north-central part of the Gulf of Oristano (39° 51'57" N 8° 35'21" E). It has an almost circular shape with a maximum area of 900 hectares and a depth ranging between 40 to 150 cm (Fig. 1). The site is a waterlogged context. It is subjected to excavation since 2006, under the supervision of the Soprintendenza per i Beni Archeologici per le province di Cagliari e Oristano and the University of Cagliari. The underwater excavation allowed to recover several amphorae dating back to Phoenician-Punic period (5th - 2nd century BC), (Del Vais and Sanna 2009). Inside several amphorae and sediment, different materials were found, including animal bones and macro plant remains who were preserved in excellent condition thanks to the anaerobic conditions (Del Vais and Sanna 2009). The radiocarbon dating of these samples are still in progress.

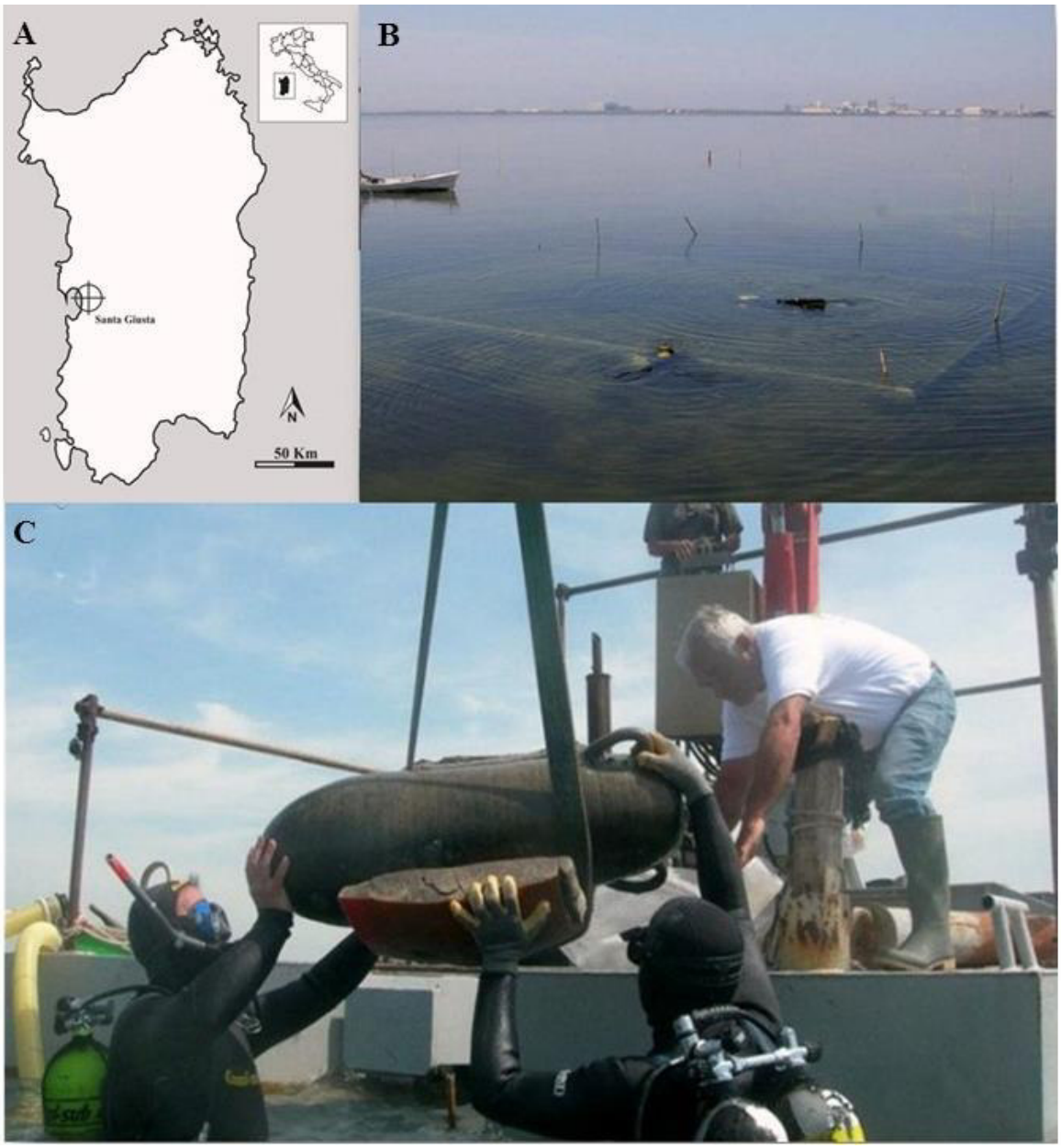

Fig. 1 A) Location of Santa Giusta lagoon B) the archaeological excavation area C) recovery of one of the amphorae containing *Prunus* endocarps under study.

Age	Taxon	Country	Site	Reference
5879-5074 cal BC	Damson endocarp	ITA	La Marmotta	Rottoli 1993
5633-4372 cal BC	Sloe endocarp	ITA	Sammardenchia	Rottoli 1999, 2005
5872-4547 cal BC	Sloe endocarp	ITA	Piancada	Rottoli 2005
5210-4355 cal BC	Sloe endocarp	ITA	Pavia di Udine	Pessina et al. 2004
5206-5050 cal BC	Sloe endocarp	ITA	Lugo di Romagna	Rottoli and Castiglioni 2009
4500-3500 cal BC	Sloe endocarp	ITA	Casalnoceto	Motella De Carlo and Venturino Gambari 2004
3800-3700 cal BC	Sloe endocarp	ITA	Lagozza di Besnate	Helbæk 1955; Castelletti 1976
4500-3500 cal BC	Sloe endocarp	ITA	Monte Covolo	Pals and Voorrips 1979
3500-2100 cal BC	Sloe endocarp	ITA	Monte Covolo	Castiglioni et al. 2008
2500-2250 cal BC	Sloe endocarp	ITA	Meduno	Castiglioni et al. 2003
1952-1778 cal BC	Sloe endocarp	ITA	Nola	Costantini et al. 2007
2111-1835 cal BC	Sloe endocarp	ITA	Riparo del Lauro	Bellini et al. 2008
1616-1464 cal BC	Sloe endocarp	ITA	San Lorenzo a Greve	Bellini et al. 2008
1500-1310 cal BC	Sloe charcoal	ITA	Terramara	Mercuri et al. 2006
1626-1434 cal BC	Sloe endocarp	ITA	Solarolo	Carra 2009
1270-1190 cal BC	Sloe endocarp	ITA	Duos Nuraghes	Bakels 2002
1286-1115 cal BC	Sloe endocarp	ITA	Sa Osa	Sabato et al. 2015
1443-1116 cal BC	Sloe endocarp	ITA	Scarceta di Manciano	Bellini et al. 2008
1091-1031 cal BC	Sloe endocarp	ITA	Stagno	Giachi et al. 2010
1091-1031 cal BC	Damson endocarp	ITA	Stagno	Giachi et al. 2010
800-700 BC	Sloe endocarp	ITA	Monte Trabocchetto	Arobba et al. 2003
700-600 BC	Sloe endocarp	ITA	Monte Polizzo	Stika et al. 2008
700-500 BC	Sloe endocarp	ITA	Mokarta	Stika et al. 2008
600/300 BC	**Sloe and plum endocarp**	**ITA**	**Santa Giusta**	**Present work**
150 BC	Plum endocarp	ITA	Pompei	Zech-Matterne et al. 2015
10 BC	Sloe endocarp	CHE	Vindonissa	Jacomet at al. 2002
10-15 AD	Damson and plum endocarp	CHE	Vindonissa	Jacomet at al. 2003
79 AD	Plum paint	ITA	Pompei	Jashemski and Meyer 2002
100 AD	Plum endocarp	DEU	Neuss	Knörzer 1970
100 AD	Plum endocarp	DEU	Aachen	Knörzer 1967
100-200 AD	Plum endocarp	ITA	Nuovastazione AV	Marchesini and Marvelli 2007
100-200 AD	Plum endocarp	ITA	Casalecchio di Reno	Marchesini and Marvelli 2007
100-200 AD	Damson endocarp Sloe endocarp	FRA	Gasquinoy	Figueiral et al. 2010
100-200 AD	Plum endocarp	ESP	Gabia	Rodriguez-Ariza and Moya 2010
200 AD	Plum endocarp	HUN	Tac-Gorsium	Hartyány et al. 1968
200-300 AD	Plum endocarp	FRA	Faulquemont	Preiss et al. 2005
200-300 AD	Damson and plum endocarp	FRA	Marseille	Bouby et al. 2011
300 AD	Damson, plum and sloe endocarp	CHE	Eschenz	Pollmann et al. 2005
300-400 AD	Damson and plum endocarp	BEL	Tienen	Cooremans 2008
400 AD	Plum endocarp	HUN	Balatonberény	Sági and Füzes 1967
600 AD	Plum endocarp	TUN	Carthage	Van Zeist et al. 2001

Tab. 1 The major records of *P. spinosa* (sloe), *P. domestics* subsp. *insititia* (damson) and *P. domestica* (plum) remains documented in the archaeological contexts in Western Europe. In chronological order from the earliest identifications until the 6th century AD, apart from the dating ranging from 800 BC to 600 AD the others chronology was calibrated with OxCal v4.2.3 (Bronk Ramsey and Lee 2013), r5, and the IntCal13 atmospheric curve (Reimer et al. 2013).

Materials and Methods

Archaeological samples

A total of 64 waterlogged endocarps were analysed in this study. The samples come from amphorae dated in a range between the 5th and the 2nd century BC. The endocarps were recovered through the wash-over technique using a fine mesh (0,25 mm), (Kenward et al. 1980). Subsequently, the samples were kept in distilled water and stored at +5 ° C.

Modern samples

Modern samples of *P. spinosa* were collected from 11 different localities in Sardinia (Tab. 2b Fig. 2) and the modern endocarps of *P. domestica* were collected from 22 traditional Sardinia varieties from the field catalog of CNR-ISPA (Nuraxinieddu, OR-Sardinia), (Tab. 2a, Fig. 2). One to five trees of each variety were sampled, randomly collecting mature fruits. Some of these were collected and selected from areas closest to the archaeological site, to evaluate the potential relationship between the varieties and archaeological remains. In order to reduce the environmental effects, the fruit sampling was conducted for three consecutive years from 2012 to 2014. In addition, two accessions of damson preserved in the Sardinian Germplasm Bank (BG-SAR) respectively (AN1) and (AN2) were added to the study.
All the material has been studied at the Biodiversity Conservation Centre (CCB) of Cagliari University (Atzeri et al. 2012).

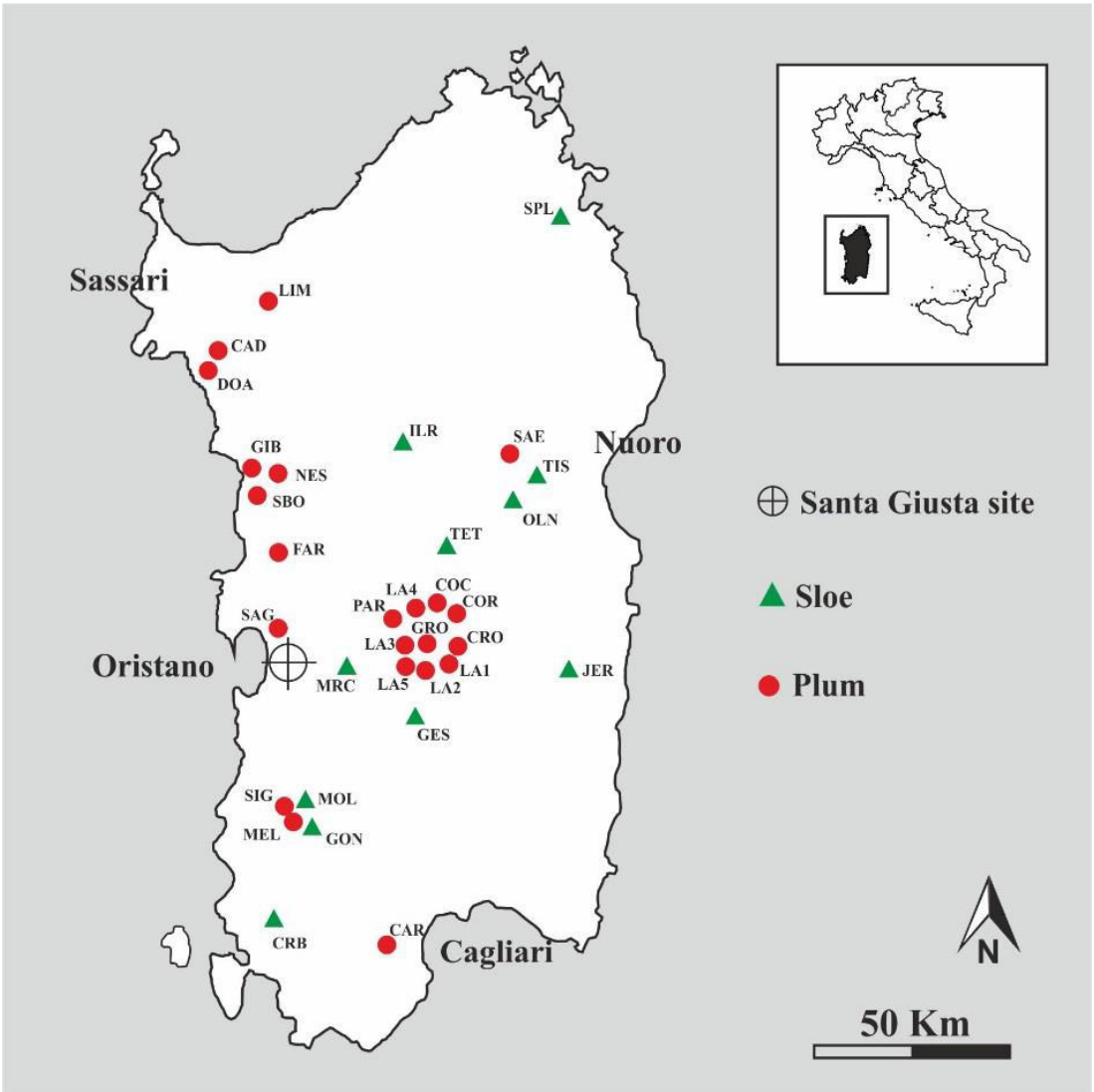

Fig. 2 Distribution of modern *P. spinosa* (sloe) and *P. domestica* (plum) samples selected for this study.

Image Analysis and Statistical analysis

Digital images of the modern and archaeological samples were acquired using a flatbed scanner (Epson Perfection V550), with a digital resolution of 400 dpi for a scanning area not exceeding 1024×1024 pixels. Image acquisition of modern endocarps was performed after the cleaning of the pulp. For minimizing shape variations, according to Depypere et al. (2007) image acquisition of the archaeological endocarps was performed on hydrated samples.

The images were processed and analysed using the software package KS-400 V. 3.0. (Carl Zeiss, Vision, Oberkochen, Germany). The morphometric parameters were obtained by the macro *Prunus*.mcr, specifically developed for the characterization of wild seeds (Bacchetta et al. 2008) and later modified to measure a further 20 morpho-colorimetric seed features (Mattana et al., 2008). This macro was adapted to perform automatically the whole analysis procedure, reducing the execution time and contextual mistakes in the analysis process (Grillo et al. 2010). Considering that endocarp color is altered in the archaeological samples, color and texture have been not considered in this research, but in order to increase the number of discriminant parameters and to accurately describe the shape of the analysed endocarps, the Elliptic Fourier Descriptors (EFDs) as described by Orrù et al. (2013) have been calculated.

Statistical elaborations were executed using IBM SPSS (Statistical Package for Social Science) release 16.0 (SPSS 2006), and the stepwise Linear Discriminant Analysis method (LDA). This method is commonly used to classify or identify unknown groups characterized by quantitative and qualitative variables (Fisher 1936, 1940; Sugiyama 2007). It allows to find the combination of predictor variables with the aim of minimizing the within-class distance and maximizing the between-class distance simultaneously, thus achieving maximum class discrimination (Hastie et al. 2001; Holden et al. 2011; Alvin et al. 2012; Kuhn and Johnson 2013). An overall of 98 morphometric features shown in table 3 were measured on 2,845 endocarps.

Code	Variety name	Locality	Endocarp amount	Drupe color
BOS	Bosana	Bosa	72	R
CAD	Cariadoggia	Alghero	80	R
CAR	Cariasina	Medio Campidano	39	V
COR	Coru	Laconi	85	Y
COC	Coru ‘e Columbu	Laconi	80	Y
CRO	Croccorighedda	Laconi	85	Y
DOA	Dore A	Alghero	30	R
FAR	Fara	Bonarcado	100	O
GIB	Gialla di Bosa	Bosa	60	Y
GRO	Groga	Laconi	30	Y
LA1	Laconi A	Laconi	90	G
LA2	Laconi B	Laconi	90	Y
LA3	Laconi D	Laconi	85	R
LA4	Laconi E	Laconi	30	O
LA5	Laconi F	Laconi	70	V
MEL	Melone	Gonnosfanadiga	90	Y
LIM	Limuninca	Sassari	60	O
NES	Nero Sardo	Bosa	100	V
SAG	San Giovanni	Oristano	57	Y
SBO	Sanguigna di Bosa	Bosa	150	R/V
SAE	Sant'Elia	Nuoro	90	Y
SIG	Sighera	Gonnosfanadiga	90	R

Tab. 2a General information on *P. domestica*, *P. spinosa* and *P. domestica* subsp. *insititia* samples utilised for the morphological comparison of archaeological endocarps from Santa Giusta. Drupe color (G) green; (O) orange; (R) red; (Y) yellow and (V) violet.

Code	Locality	Endocarp amount
CRB	Carbonia	55
GON	Gonnosfanadiga	61
MOL	Monte Linas	100
MRC	Monte Arci	200
TIS	Tiscali	70
ILR	Illorai	100
TET	Teti	46
GES	Gesturi	100
JER	Jerzu	100
SPL	San Pantaleo	52
OLN	Oliena	100

Tab. 2b General information on *P. spinosa* populations collected in Sardinia used for the comparison with the archaeological endocarps of Santa Giusta site.

	Feature	Description
A	Area	Endocarp area (mm^2)
P	Perimeter	Endocarp perimeter (mm)
P_{conv}	Convex Perimeter	Convex perimeter of the endocarp (mm)
P_{Crof}	Crofton Perimeter	Crofton perimeter of the endocarp (mm)
P_{conv}/P_{Crof}	Perimeter ratio	Ratio between P_{conv} and P_{Crof}
D_{max}	Max diameter	Maximum diameter of the endocarp (mm)
D_{min}	Min diameter	Minimum diameter of the endocarp (mm)
D_{min}/D_{max}	Feret ratio	Ratio between D_{min} and D_{max}
EA_{max}	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
EA_{min}	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)
Sf	Shape Factor	Endocarp shape descriptor = $(4\pi A)/P^2$ (normalized value)
Rf	Roundness Factor	Endocarp roundness descriptor = $(4A)/(\pi D_{max}^2)$ (normalized value)
Ecd	Eq. circular diameter	Diameter of a circle with equivalent area (mm)
F	Fiberlength	Endocarp length along the fiber axis
C	Curl degree	Ratio between D_{max} and F
$Conv$	Convessity degree	Ratio between P_{Crof} and P
Sol	Solidity degree	Ratio between A and convex area
Com	Compactness degree	Endocarp compactness descriptor = $[\sqrt{(4/\pi)A}]/D_{max}$

Tab. 3 List of 18 morphometric features measured on the endocarps, excluding the 80 Elliptic Fourier Descriptors (EFDs) calculated according to Hâruta (2011).

Results

A preliminary morphometric comparison among the 64 waterlogged archaeological endocarps of Santa Giusta was done. An overall correct identification percentage of 100.0 % were reached and the samples of Santa Giusta (*Prunus* SG) group were correctly identified in 100,0 % of the cases respectively (data not shown).

Starting from this preliminary result, using the data of the 98 morphometric variables measured by LDA, the archaeological remains were compared with the modern samples of *P. spinosa*, *P. domestica* and *P. domestica* subsp. *insititia*. From this comparison, an overall correct identification percentage of 100% were reached. This comparative analysis allowed to identify 53 endocarps as *P. spinosa* and 11 endocarps as *P. domestica* in the 100,0 % of the cases (Tab. 4).

Considering these achievements, the 11 archaeological endocarps identified as of *P. domestica* were one more time considered as unknown specimens and compared with the modern varieties of plum. In this case, the archaeological samples from Santa Giusta showed main similarities with the variety Sanguigna di Bosa (SBO) (Figs. 3, 4) in the 81.8% of the cases and with the variety Fara (FAR) in the 9.1 % of the cases. Only one endocarp was identified as *P. domestica* subsp. *insititia* (AN2), (Tab. 5).

Likewise, the 53 archaeological endocarps from Santa Giusta, identified as *P. spinosa*, were considered as unknown and compared with the modern populations of *P. spinosa* from Sardinia. These archaeological endocarps are very similar with those collected at Monte Arci (MRC) in the 90.6% of the cases (Tab. 6).

	P. domestica	***P. spinosa***	***P. domestica* subsp. *insititia***	Total
P. domestica	99.9 (1,661)	0.1 (2)	-	100.0 (1,663)
P. spinosa	-	**100.0 (984)**	-	100.0 (984)
P. domestica* subsp. *insititia	**12.0 (24)**	-	**82.0 (110)**	100.0 (134)
***Prunus* SG**	**17.0 (11)**	**83.0 (53)**	-	100.0 (64)
Overall				**100.0 % (2,845)**

Tab. 4 Identification percentage among the archaeological endocarps of *Prunus* from Santa Giusta site considered as unknown specimens. The numbers of endocarps analysed are in brackets.

Code	High value of variety classification	*P. domestica* SG	Total
BOS	60.0 (43)	-	100.0 (72)
CAD	72.5 (58)	-	100.0 (80)
CAR	69.2 (27)	-	100.0 (39)
COR	60.0 (51)	-	100.0 (85)
COC	40.0 (32)	-	100.0 (80)
CRO	64.7 (55)	-	100.0 (85)
DOA	83.3 (25)	-	100.0 (30)
FAR	64.0 (64)	**9.1 (1)**	100.0 (100)
GIB	50.0 (30)	-	100.0 (60)
GRO	53.3 (16)	-	100.0 (30)
LA1	44.4 (40)	-	100.0 (90)
LA2	77.8 (70)	-	100.0 (90)
LA3	83.5 (71)	-	100.0 (85)
LA4	73.3 (22)	-	100.0 (30)
LA5	50.0 (35)	-	100.0 (70)
MEL	83.3 (75)	-	100.0 (90)
LIM	66.7 (40)	-	100.0 (60)
NES	51.0 (51)	-	100.0 (100)
PAR	77.8 (14)	-	100.0 (18)
SAG	38.6 (22)	-	100.0 (57)
SBO	70.0 (105)	**81.8 (9)**	100.0 (150)
SAE	52.2 (47)	-	100.0 (90)
SIG	84.4 (76)	-	100.0 (90)
AN1	97.7 (86)	-	100.0 (88)
AN2	87.0 (40)	**9.1 (1)**	100.0 (46)
Overall			**70.1 % (1,815)**

Tab. 5 Correct classification percentages between modern endocarps of *P. domestica* varieties and archaeological one from Santa Giusta. The numbers of samples analysed are in brackets.

	CRB	GON	MOL	MRC	TIS	ILR	TET	GES	JER	SPL	OLN	Total
CRB	50.9 (28)	-	1.8 (1)	30.9 (17)	1.8 (1)	10.9 (6)	-	3.6 (2)	-	-	-	100.0 (55)
GON	-	-	3.3 (2)	29.5 (18)	1.6 (1)	-	-	26.2 (16)	21.3 (13)	3.3 (2)	14.8 (9)	100.0 (61)
MOL	5.0 (5)	-	44.0 (44)	13.0 (13)	4.0 (4)	25.0 (25)	1.0 (1)	1.0 (1)	5.0 (5)	1.0 (1)	1.0 (1)	100.0 (100)
MRC	7.0 (14)	1.0 (1)	1.5 (3)	66.0 (133)	1.5 (3)	2.0 (4)	-	4.0 (8)	12.0 (24)	-	5.0 (10)	100.0 (200)
TIS	4.3 (3)	-	7.1 (5)	42.9 (30	2.9 (2)	-	-	12.9 (9)	25.7 (18)	1.4 (1)	2.9 (2)	100.0 (70)
ILR	6.0 (6)	-	36.0 (36)	17.0 (17)	1.0 (1)	30.0 (30)	1.0 (1)	1.0 (1)	7.0 (7)	1.0 (1)	-	100.0 (100)
TET	-	-	17.4 (8)	28.3 (13)	2.2 (1)	2.2 (1)	4.3 (2)	6.5 (3)	32.6 (15)	-	6.5 (3)	100.0 (46)
GES	2.0 (2)	-	1.0 (1)	17.0 (17)	3.0 (3)	1.0 (1)	1.0 (1)	24.0 (24)	21.0 (21)	14.0 (14)	16.0 (16)	100.0 (100)
JER	1.0 (1)	-	6.0 (6)	39.0 (39)	-	3.0 (3)	2.0 (2)	14.0 (14)	30.0 (30)	-	5.0 (5)	100.0 (100)
SPL	-	1.9 (1)	-	5.8 (3)	-	-	1.9 (1)	26.9 (14)	5.8 (3)	42.3 (22)	15.4 (8)	100.0 (52)
OLN	1.0 (1)	-	3.0 (3)	32.0 (32)	1.0 (1)	1.0 (1)	-	14.0 (14)	12.0 (12)	11.0 (11)	25.0 (25)	100.0 (100)
***P. spinosa* SG**	-	-	-	**90.6 (48)**	3.8 (2)	-	-	-	3.8 (2)	1.9 (1)	-	100.0 (53)
Overall												**38.1 % (1,037)**

Tab. 6 Correct classification percentages between modern populations and archaeological samples of *P. spinosa*.

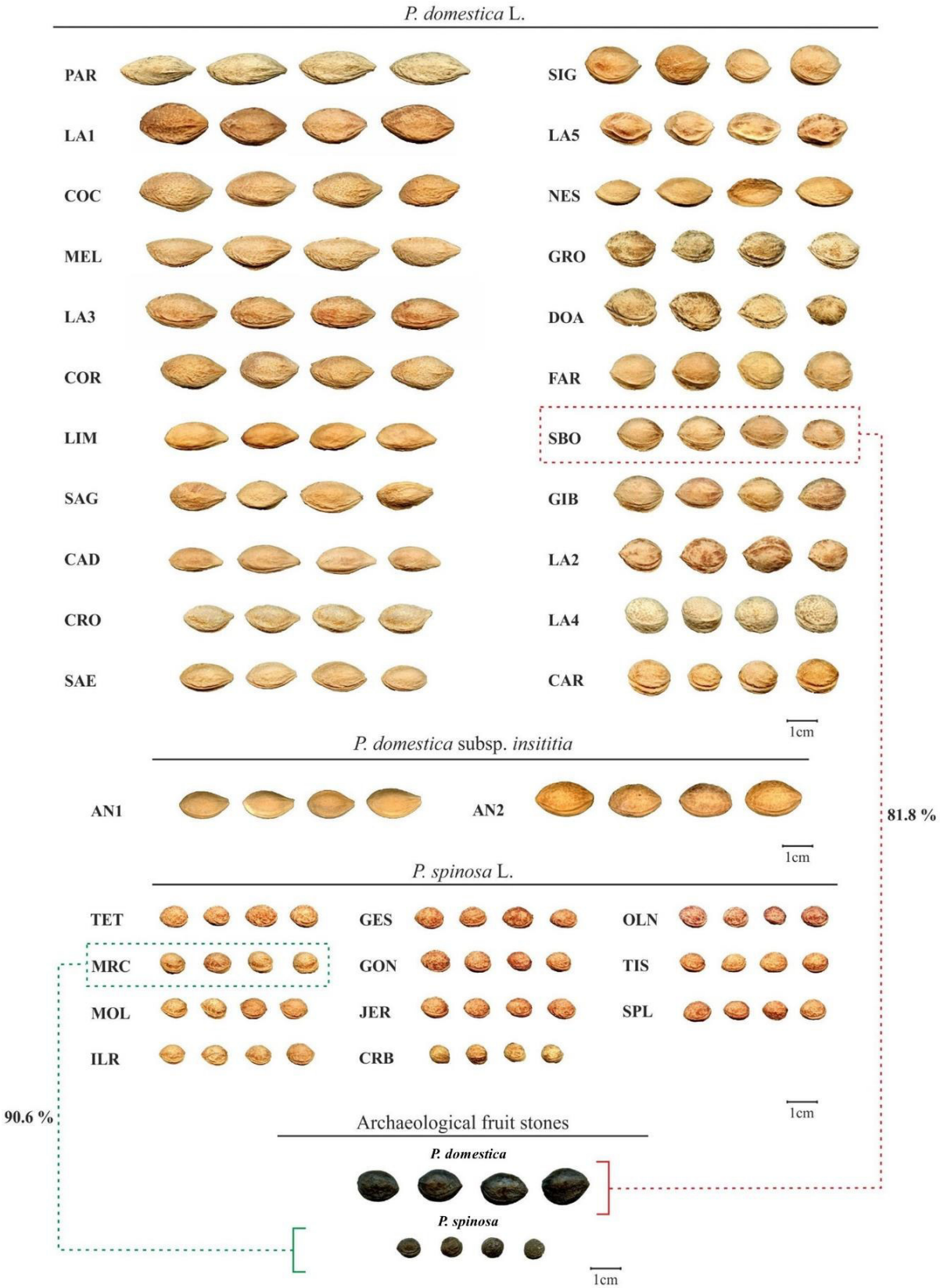

Fig. 3 Representation of the samples analysed. Below the types of endocarps identified at the Santa Giusta site.

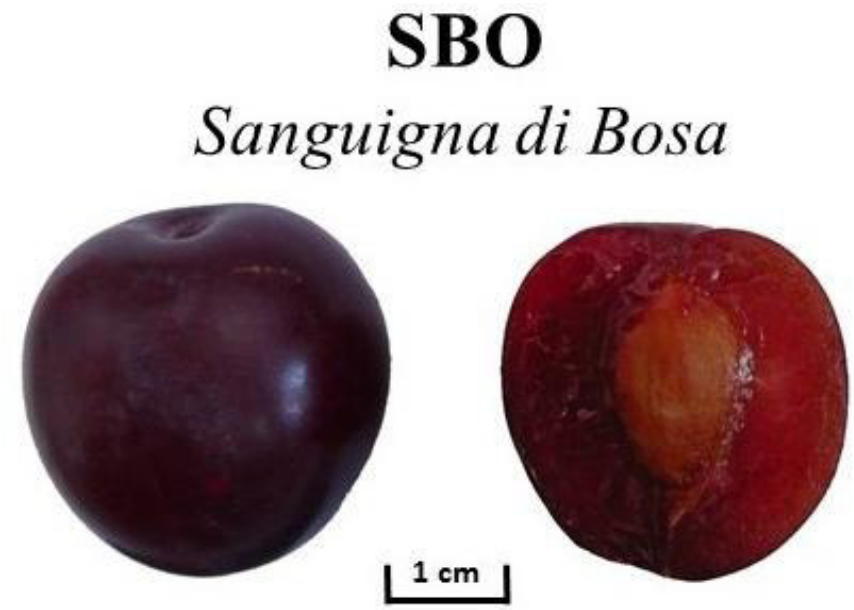

Fig. 4 The variety Sanguigna di Bosa (SBO) identified as the closest to the archaeological remains P. domestica from Santa Giusta.

Discussion

Introduction of fruit trees in the western Mediterranean Basin remains unclear, perhaps because fruits domestication has received much less attention than annual crop plants (Goldschmidt 2013). The identification of the place of origin of cultivated *Prunus* species is difficult due to their long history of cultivation to which the dispersion in different places by human kind is added (Pollmann et al. 2005). Therefore, *Prunus* species may have naturalized creating difficulties for the distinction between ancestrally wild population species to escape from cultivation (Kole and Abbott 2012). As suggested by Pollman et al. (2005), the attribution of the *Prunus* remains to a specific species is limited due to the imprecise classification of these groups.

From the results obtained through image analysis, it was possible identify correctly the *Prunus* remains of Santa Giusta site as cultivated and wild species.

From the 64 archaeological remains, 53 of these were classified as wild forms (*P. spinosa*), while the other 11 were classified in cultivated forms *(P. domestica)*: in particular, none of these endocarps was attributed to the wild forms and to the semi-cultivated form (*P. domestica* subsp. *insititia*).

During the Roman times, an increase of domesticated endocarps of plum in waterlogged contexts was observed (Zohary et al. 2012). This, suggest that the Roman people contributed to spread several varieties of plums in Western Europe (Pollman et al. 2005).

Despite this, according to these achievements, it could be possible suppose that first evidence of plum cultivation may be present in Sardinia at least from the 5th century BC, in the Phoenician Period.

The territories in the central-west coast of Sardinia has a key role in archeology. Likewise, from the same context of Santa Giusta, evidence of possible cultivated fruits from the such as almonds, olives and grapes were documented (Sabato 2015). Viticulture was already started between the 6th and the 3rd century BC, as shown by the discovery of stone structures for grape pressing in Terralba (Oristano), located a few kilometres from the Santa Giusta site (Pérez Jordà et al. 2010). As suggested by Stika and Heiss (2013), the increase in archaeobotanical records of different species of domesticated fruits is documented since the Bronze Age. Remains such as olive, grape, almond, fig, cherry, plum, suggests that the fruit trees domestication may have been started in the western Mediterranean Basin, in this period.

In fact, the recent discovery of cultivated grape and melon in the Bronze Age context in Sardinia, testified the first evidence of cultivated fruits in the western Mediterranean Basin in this period (Sabato el al. 2015; Ucchesu et al. 2014).
Probably, Phoenician people introduced agricultural knowledge of fruit trees in Sardinia around the 6th century BC. However, the place of origin of these cultivated fruits trees is still unknown.
A further result of this study is that the archaeological endocarps from Santa Giusta, identified as *P. domestica*, are similar to a traditional variety, actually cultivated in the territory of Bosa (northwest Sardinia).
Currently, this variety of plum with red and violet drupe color, is an important variety of Sardinia and is cultivated for its nutraceutical proprieties (Agabbio et al. 1994).
The close relationship highlighted by the comparative analysis between the archaeological and the modern samples of *P. spinosa*, allowed to hypothesize that the fruits found in Santa Giusta jars might have been gathered in the slopes of Monte Arci (location filled with obsidian), located at just 10 Km from the studied archaeological site.
The overall result allows assuming that *Prunus* remains contained inside the amphorae were collected nearby of the storage site and they were probably destined for other colonies.
The use of the fruits of sloe are varied. Ethnobotany literature indicates their use principally for food (Parada et al. 2009; Łuczaj 2012; Pardo-de-Santayana et al. 2013; Pieroni and Quave 2014). Moreover, has been documented the decoction of the drupes used as a medicine for the treatment of many diseases, such as: biliary dyskinesia, gut, convulsive cough, urinary and cardiovascular disorders (Tiţă et al. 2009). In Sardinia, the consumption of the drupes as food, as medicine through decoction of flowers or drupe for the treatment of cough, as well as traditional use for wool dyeing, is well documented (Atzei 2003; Campanini 2009).
Today, in Spain, sloe are used for the production of an alcoholic drink called *Patxaran* (Tardío and Pardo-de-Santayana 2014).
Other uses may be related to religious rituals. In some Punic tombs, charcoal remains of sloe, maybe used as fuel for the human body cremation or ritual offerings (Gómez Bellard et al. 1990). Also in the Roman cemeteries, the use of fresh fruit of sloe, damson and plum as ritual offerings has been well documented (Preiss et al. 2005; Cooremans 2008; Bouby et al. 2011; Rottoli and Castiglioni 2011).
Another interesting possible use of sloe could be connected to the yogurt starter. As reported by Girginoff (1959) and Michaylova et al. (2007), the bacteria of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* can easily grow on sloe, and according to Kültür (2008), the young shoots of sloe have traditionally used to produce yoghurt in Turkey.

Conclusions

In recent years many researchers have demonstrated the validity of seeds phenotypic characterization by LDA method in the archaeobotanical field for the discrimination of seeds of wild and cultivated species (Terral et al. 2010; Bouby et al. 2013; Orrù et al. 2013; Ucchesu et al. 2014; Pagnoux et al. 2015; Sabato et al. 2015).
This study demonstrates the validity of this approach also for the endocarps of the genus *Prunus*.
The discovery of well-preserved waterlogged cultivated endocarps of *Prunus* from the Phoenician-Punic settlement of Santa Giusta could be evidence that the introduction of primitive cultivated forms of plums in Sardinia have been started by the Phoenicians people. Moreover, these endocarps represent the oldest findings in Sardinia and they are the oldest evidence of cultivated plums in the western Mediterranean Basin. Some archaeological endocarps shown a greater affinity to traditional varieties of Sardinia. Moreover, the analysis demonstrated that during the 5th - 2nd century BC were present cultivated plums in Sardinia.

In addition, the phenotypic features of endocarps and the following applied LDA showed that the wild archaeological remains had close affinities to wild populations grown in Sardinia. We hope for the future new investigations to understand the history of beginning of fruit tree cultivation in western Mediterranean Basin and confirm these results with genetic analysis.

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Chapter 3: Image Analysis application on waterlogged archaeological *Prunus* L. remains from a Medieval Context.

Introduction

The genus *Prunus* L., genus of the Rosaceae family, includes plums and apricots, almonds and peaches, umbellate and deciduous racemes cherries and the evergreen laurel cherries mainly distributed in temperate regions of the boreal hemisphere (Krussman 1986; Maynard et al. 1991; Aradhya et al. 2004; Yılmaz et al. 2009).

Among these, *Prunus domestica* L. is one of the most economic important fruit in temperate regions of the world and represent actually the major crop in Europe and South-West Asia (Ramming and Cociu 1991; Watkins 1995; Körber-Grohne 1996; Zohary et al. 2012).

Understanding how wild species have contributed to the beginning of cultivated forms is difficult. As argued in [chapter 1] and [chapter 2], in recent years, genetic studies have shown that *Prunus spinosa* and *Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens have close relationships with the European domestic plums, considering damson as the ancestor of the modern domestic plum (Nassi et al. 2003; Pollmann et al. 2005; Horvath et al. 2011; Athanasiadis et al. 2013). However, due to the many processes of speciation the phylogenetic reconstruction, the origin and spread are still under study (Katayama and Uematsu 2005; Bouhadida et al. 2004, 2007; Yılmaz et al. 2009; Wünsch 2009; Horvath et al. 2011).

Archaeological evidence of *Prunus* remains are detected in many archaeological sites in Europe since the Neolithic/Bronze Age. The exact origin of the first domesticated forms of plum is extremely debatable (Pollmann et al. 2005). A common theory is that damson, *P. domestica* subsp. *insititia* and after plum, *P. domestica* species were antiquely cultivated in the area around the ancient city of Damascus (Syria), and only subsequently Romans (Dalby 2003) introduced them in Europe.

As suggested by Janick (2005), the domestic plum seem to appear and spread in Western Europe during the Roman Period by at least 100 BCE. The first endocarp currently recorded was dated back to 150 BC and was found in the cesspit under the temple of Fortuna in Pompeii (Zech-Matterne et al. 2015). *P. domestica* and *P. domestica* subsp. *insititia* remains seem to appear mostly in Iron Age-Roman Age waterlogged context, especially in Germany (Kreuz 2003) and probably in France (Wiethold 1994).

Nevertheless, the recent discovery of several intact waterlogged endocarps of *Prunus*, as discussed in [chapter 2] most probably related to *P. domestica,* in the Phoenician-Punic settlement of Santa Giusta (Oristano, Sardinia), dated between the 5th and the 2nd century BC, brings into question about the spread of domesticated plums in the western Mediterranean Basin.

Botanical identification of *Prunus* at taxonomic level is possible due to some traditional biometric classical analysis. The "pioneer" who studied the endocarps morphology and the morphometry of the genus *Prunus* was Behre in 1978 and until some years ago, the dimensional measurements of endocarps were done manually, generally by calipers, based on fixed categories officially recognized according on different methods of some authors (Woldring 2000; Pollmann et al. 2005; Depypere et al. 2007).

Currently, thanks to new computer vision technologies by image analysis applied to plant biology, it was possible to distinguish, in an accurate, reproducible and repeatable way, wild species from cultivated ones in the agronomical field (Kilic et al. 2007; Rovner and Gyulai 2007; Venora et al. 2007, 2009a, 2009b; Mattana et al. 2008; Appelhans et al. 2011; Fawzi 2011; Grillo et al. 2011, 2012; Herridge et al. 2011; Smykalova et al. 2011, 2013; Pinna et al. 2014; Santo et al. 2015) and in the archaeobotanical one (Terral et al. 2010; Bouby et al. 2013; Orrù et al. 2013; Sabato et al. 2014; Ucchesu et al. 2014; Pagnoux et al. 2015).

The main goals of this work are to:

- ✓ identify and characterize *Prunus* remains at specific level from Medieval Period by computer image analysis;
- ✓ compare the archaeological remains with the modern ones.
- ✓ applied Linear Discriminant Analysis (LDA) to investigate the *status* of *Prunus* domestication in the Medieval Period in Sardinia.

Archaeological context

In 2007, during the renovation of via Satta, in the core of the city-centre of Sassari, a Medieval well was discovered (Fig. 1). It was originally part of an open area or domestic courtyard, which has been dated between 1330-1360 AD according to the typology of majolica fragments from Pisa, Savona and Valencia widely diffused in this period (Biccone 2013). The sediment appeared very rich in waterlogged plant remains. Wood remains were studied at the University of Sassari (Becca et al. 2013), while macro plant remains were analysed by the Laboratorio di Palinologia e Paleobotanica of the University of Modena and Reggio Emilia. A total of 117 *taxa* have been identified (Bertacci 2012; Bosi and Bandini Mazzanti 2013).

A significant number of *Prunus* endocarps were recovered and thanks to their excellent state of preservation, it was possible to conduct specific morphometrical analyses for the characterization at the specific level.

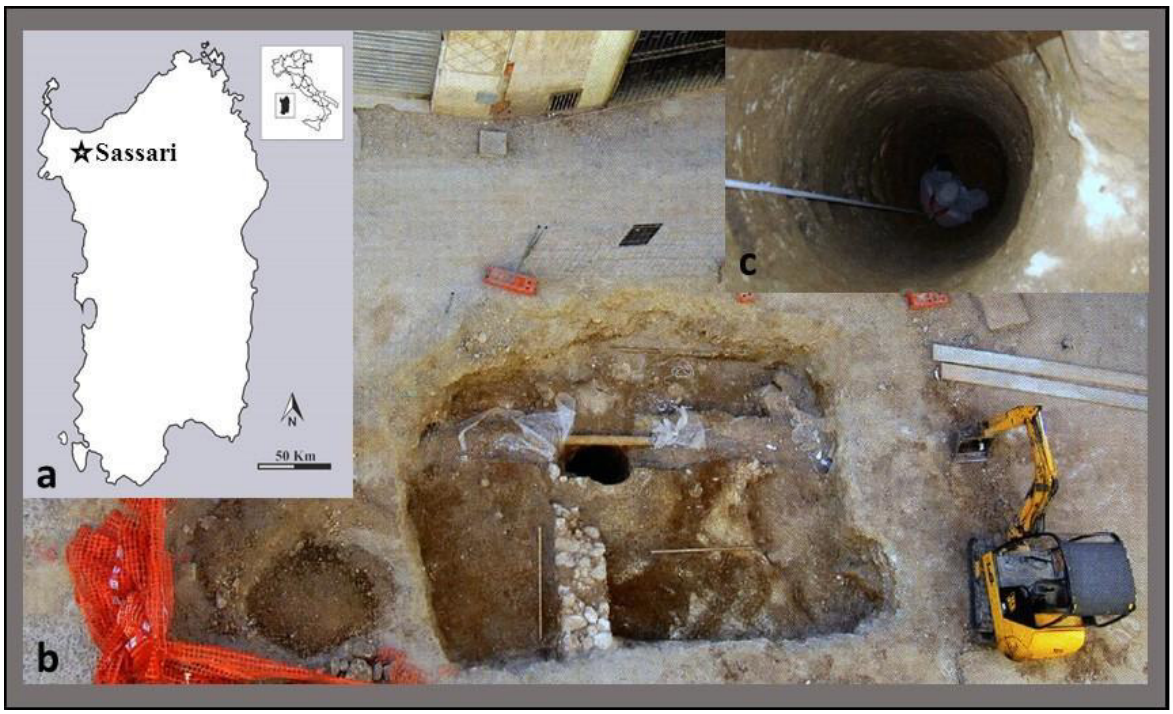

Fig. 1 a) Location of Sassari city in Sardinia; **b)** The archaeological excavation area of Via Satta; **c)** The well under study.

Materials and Methods

Plant material

A total of 341 full preserved archaeological *Prunus* endocarps have been selected for morphometric analysis. Broken or hard-distorted samples were not considered. In addition, 11 remains of *P. domestica* from the archaeological site of Santa Giusta (Oristano, Sardinia), dated back to the 5th- th2nd century BC, described in [chapter 2] were also used for the comparative analyses.

The samples of *Prunus* were analyzed according to the subdivision into four groups described in the work of Bertacci (2012), (Tab. 1). In order to be included into the classifier and then analysed, the archaeological remains were considered as unknown.

Likewise, modern endocarps of *P. spinosa* (984 samples) were collected from 11 populations of Sardinia (Italy) (Tab. 2a) and others *P. spinosa* samples (1146 endocarps) were collected by several European seed banks (Bulgaria, France, Germany, Italy, Portugal, Romania, Spain and Switzerland), (data not shown).

Modern samples of *P. domestica,* consists of 1663 endocarps from 22 traditional varieties of Sardinia, were collected from the field catalogue of CNR-ISPA (Nuraxinieddu, OR, Sardinia), (Tab. 2b). The varieties have been selected from several areas of Sardinia as shown in table 2. In order to evaluate the potential relationship between the cultivars and archaeological remains some varieties selected from areas closest to the city of Sassari. One to five trees, for each cultivar, were sampled for three consecutive years (2012-2014).

In addition, two varieties of damson referring to 134 endocarps and preserved in the Sardinian Germplasm Bank (BG-SAR), were added to the study.

The analyses were performed in the Biodiversity Conservation Centre (CCB) of the University of Cagliari (Atzeri et al. 2012).

Code	Endocarp amount	
SS_G1	17	
SS_G2	118	
SS_G3	70	
SS_G4	136	

Tab. 1 Amount of *Prunus* endocarps from Via Satta road (SS) used in the study. [(Division into groups according Bertacci (2012)].

Code	Locality	Endocarp amount
CRB	Carbonia	55
GON	Gonnosfanadiga	61
MOL	Monte Linas	100
MRC	Monte Arci	200
TIS	Tiscali	70
ILR	Illorai	100
TET	Teti	46
GES	Gesturi	100
JER	Jerzu	100
SPL	San Pantaleo	52
OLN	Oliena	100

Tab. 2a General information on *P. spinosa* populations collected in Sardinia for to the morphological comparison with the archaeological endocarps from Via Satta (SS).

Code	Variety name	Locality	Endocarp amount	Fruit skin color
BOS	Bosana	Bosa	72	R
CAD	Cariadoggia	Alghero	80	R
CAR	Cariasina	Medio Campidano	39	V
COR	Coru	Laconi	85	Y
COC	Coru ‘e Columbu	Laconi	80	Y
CRO	Croccorighedda	Laconi	85	Y
DOA	Dore A	Alghero	30	R
FAR	Fara	Bonarcado	100	O
GIB	Gialla di Bosa	Bosa	60	Y
GRO	Groga	Laconi	30	Y
LA1	Laconi A	Laconi	90	G
LA2	Laconi B	Laconi	90	Y
LA3	Laconi D	Laconi	85	R
LA4	Laconi E	Laconi	30	O
LA5	Laconi F	Laconi	70	V
MEL	Melone	Gonnosfanadiga	90	Y
LIM	Limuninca	Sassari	60	O
NES	Nero Sardo	Bosa	100	V
SAG	San Giovanni	Oristano	57	Y
SBO	Sanguigna di Bosa	Bosa	150	R/V
SAE	Sant'Elia	Nuoro	90	Y
SIG	Sighera	Gonnosfanadiga	90	R

Tab. 2b General information on cultivated plum collected in Sardinia for to the morphometric comparison with the archaeological endocarps under study.

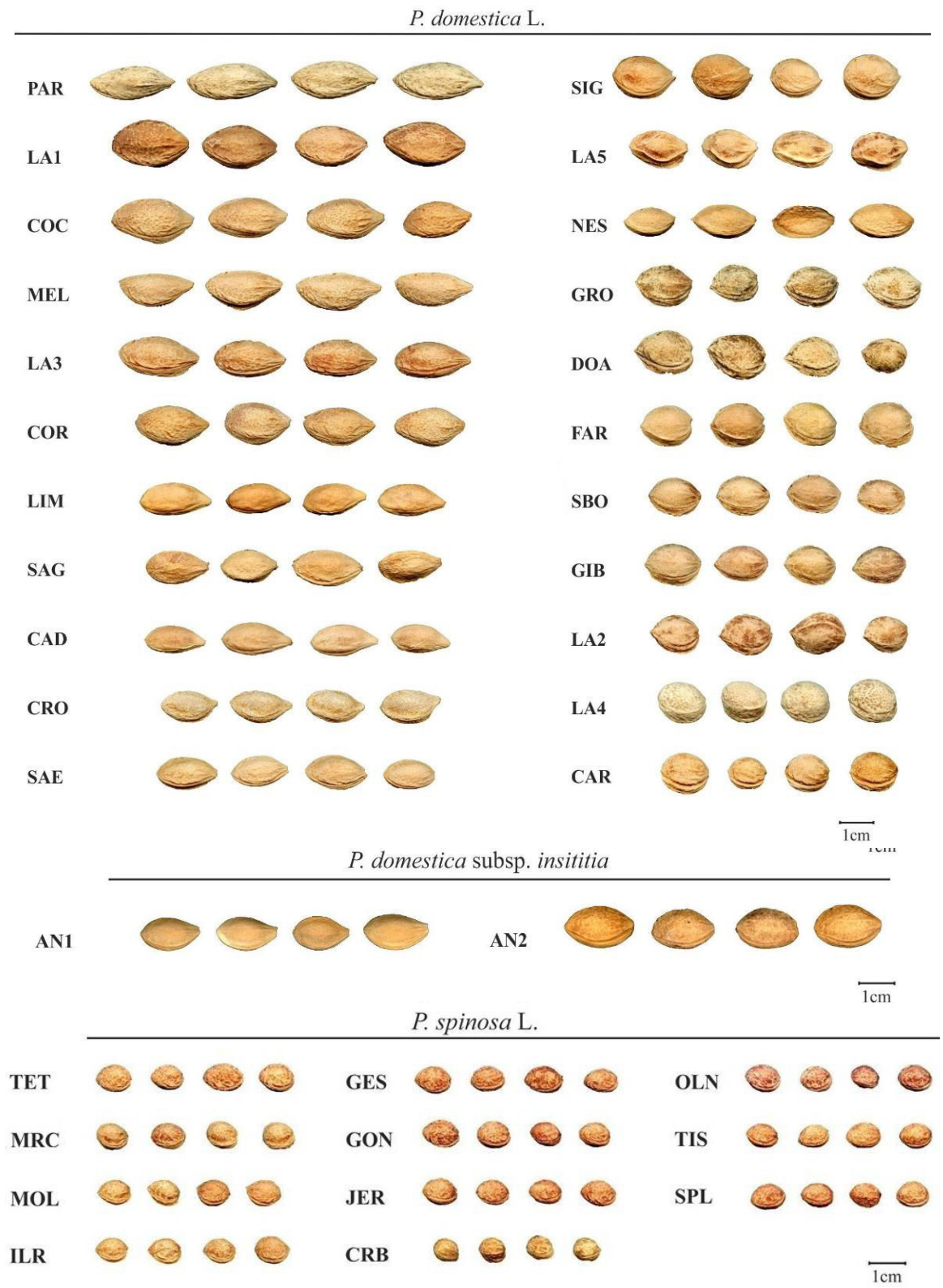

Fig. 3 Representation of cultivated *P. domestica*, *P. domestica* subsp. *insititia* and *P. spinosa* samples from Sardinia analysed in this study.

Morphological and statistical analysis

Digital images of the archaeological and modern samples were acquired using a flatbed scanner (Epson Perfection V550 photo), with a digital resolution of 400 dpi for a scanning area not exceeding 1024×1024 pixels. Image acquisition of modern endocarps was performed after the cleaning of the pulp. For minimizing shape variations, according to Depypere et al. (2007) image acquisition of the archaeological endocarps was performed on hydrated samples.

For each sample of endocarps, two images were acquired, with black and white background, and were analysed using the software package KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). The accuracy and speed of measurements was maximized by running an automated macro called *Prunus*.mcr, specifically developed for the characterization of this type of endocarps. Considering that endocarp color is altered in the archaeological samples, color and texture have been not considered in this research, but in order to increase the number of discriminant parameters and to accurately describe the shape of the analysed endocarps, the Elliptic Fourier Descriptors (EFDs) were also computed, as described by Orrù et al. (2013). The achieved results were used to build a database of morphometric features. A total of 98 parameters, describing size and shape, were computed (Tab. 4).

Statistical elaborations were executed using IBM SPSS (Statistical Package for Social Science) release 16.0 (SPSS 2006), and the stepwise Linear Discriminant Analysis method (LDA) was applied to compare the *Prunus* endocarps.

	Feature	**Description**
A	Area	Endocarp area (mm^2)
P	Perimeter	Endocarp perimeter (mm)
P_{conv}	Convex Perimeter	Convex perimeter of the endocarp (mm)
P_{Crof}	Crofton Perimeter	Crofton perimeter of the endocarp (mm)
P_{conv}/P_{Crof}	Perimeter ratio	Ratio between P_{conv} and P_{Crof}
D_{max}	Max diameter	Maximum diameter of the endocarp (mm)
D_{min}	Min diameter	Minimum diameter of the endocarp (mm)
D_{min}/D_{max}	Feret ratio	Ratio between D_{min} and D_{max}
EA_{max}	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
EA_{min}	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)
Sf	Shape Factor	Endocarp shape descriptor = $(4\pi A)/P^2$ (normalized value)
Rf	Roundness Factor	Endocarp roundness descriptor = $(4A)/(\pi D_{max}^2)$ (normalized value)
Ecd	Eq. circular diameter	Diameter of a circle with equivalent area (mm)
F	Fiberlength	Endocarp length along the fiber axis
C	Curl degree	Ratio between D_{max} and F
$Conv$	Convessity degree	Ratio between P_{Crof} and P
Sol	Solidity degree	Ratio between A and convex area
Com	Compactness degree	Endocarp compactness descriptor = $[\sqrt{(4/\pi)A}]/D_{max}$

Tab. 3 List of 18 morphometric features measured on the endocarps, excluding the 80 Elliptic Fourier Descriptors (EFDs) calculated according to Hâruta (2011).

Results

A first comparison was conducted among the archaeological endocarps samples. Using the 98 morphometric variables measured by image analysis techniques, the archaeological endocarps were classified reaching an overall correct identification percentage of 98.4%. In particular, the samples of SS_ G1 group were correctly identified in 94.1 % of the cases, SS_G2 group achieved the 98.2% of correct identification and the endocarps of the SS_G4 group were perfectly classified with a percentage of 100,0 %.
SS_G3 group was identified similar to SS_SG4 in the 86.2% of the cases and similar to SS_G2 in the 9.6% of the cases (Tab. 4).

	SS_G1	**SS_G4**	**SS_G2**	**SS_G3**	Total
SS_G1	**94.1 (16)**	-	5.9 (1)	-	100.0 (17)
SS_G2	0.9 (1)	0.9 (1)	**98.2 (109)**	-	100.0 (111)
SS_G4	-	**100.0 (136)**	-	-	100.0 (136)
SS_G3	-	**86.2 (60)**	9.6 (7)	4.2(3)	100.0 (70)
Overall					**98.4% (334)**

Tab. 4 LDA analysis results comparing the archaeological endocarps of *Prunus*.

In order to investigate the taxonomic level of the archaeological samples, a further statistical comparison was implemented among endocarps of the modern *taxa* and the archaeological sample groups, singularly considered as unknown (Tab. 5). For this comparative analysis, an overall correct identification percentage of 83.6% was reached. The 100.0% of SS_G4 archaeological endocarps samples were identified as *P. spinosa* and none of these were misattributed to cultivated forms. The 17 unknown archaeological endocarps of SS_G1 group were classified as *P. domestica* in the 64.7% of the cases and as *P. domestica* subsp. *insititia* in the remaining 35.3% of the cases. One more time, no misattributions resulted whit the wild species.
Finally, the unknown archaeological endocarps samples of SS_G2 group were mainly attributed to *P. domestica* and *P. domestica* subsp. *insititia* in the 46.8% and 51.4% of the cases, respectively. Only two over the 111 endocarps of SS_G2 were identified as *P. spinosa* (Tab.5).
Otherwise samples of SS_G3 and SS_G4 groups were attributed di *P. spinosa* with percentages higher than the 94.0 %.

	P. domestica	***P. spinosa***	***P. domestica* subsp. *insititia***	**Total**
P. domestica	**65.5 (1089)**	2.5 (42)	32.1 (532)	100.0 (1663)
P. spinosa	0.04 (1)	**99.4 (2117)**	0.6 (12)	100.0 (2130)
P. domestica* subsp. *insititia	13.4 (18)	0.7 (1)	**85.8 (115)**	100.0 (134)
SS_G1 as unknown	**64.7 (11)**	-	35.3 (6)	100.0 (17)
SS_G2 as unknown	46.8 (52)	1.8 (2)	**51.4 (57)**	100.0 (111)
SS_G3 as unknown	-	**94.2 (65)**	5.8 (4)	100.0 (70)
SS_G4 as unknown	-	**100.0 (130)**	**-**	100.0 (136)
Overall				**83.6 % (4261)**

Tab. 5 Identification percentage among the wild archaeological endocarps (SS_SPI) and the modern ones. The number of endocarps are given in parentheses.

The identified endocarps of *P. domestica* were one more time considered as unknown specimens and compared with the modern varieties of plum collected in Sardinia to investigate their connection state.
From this discrimination, a rating of 58.4% was achieved. The archaeologic endocarps of *P. domestica* were attributed to six modern cultivars with yellow-red drupe color coded as COR, CRO, LIM, SAG, SAE, SIG (Fig. 4, Tab. 2).
A further analysis has been performed comparing archaeological endocarps, identified as *P. spinosa,* with modern populations from different localities. The system was able to correctly discriminate the samples morphologically similar to the Sardinia specimens than the other European populations examined in this study (data not shown).
From the comparison with modern samples of Sardinia, the image system was able to classify 130 archaeological endocarps of *P. spinosa* similar to Monte Arci (MCR) for 70.4% and similar to Teti (TET) for the 25.1%. (Tab. 7).
A further comparison was conducted between the archaeological samples from Sassari identified by image analysis as *P. domestica* and the endocarps of the same species coming from the archaeological site of Santa Giusta in Oristano, described previously in [chapter 2]. The two archaeological groups were distinguished with percentages of correct classification of the 100.0 %. (Tab. 6)
Finally, archaeological *P. domestica* subsp. *insititia* samples from Via Satta were compared with the sample of *P. domestica* from the Phoenician-Punic Period of Santa Giusta: these results confirm that they are two different species (data not shown).

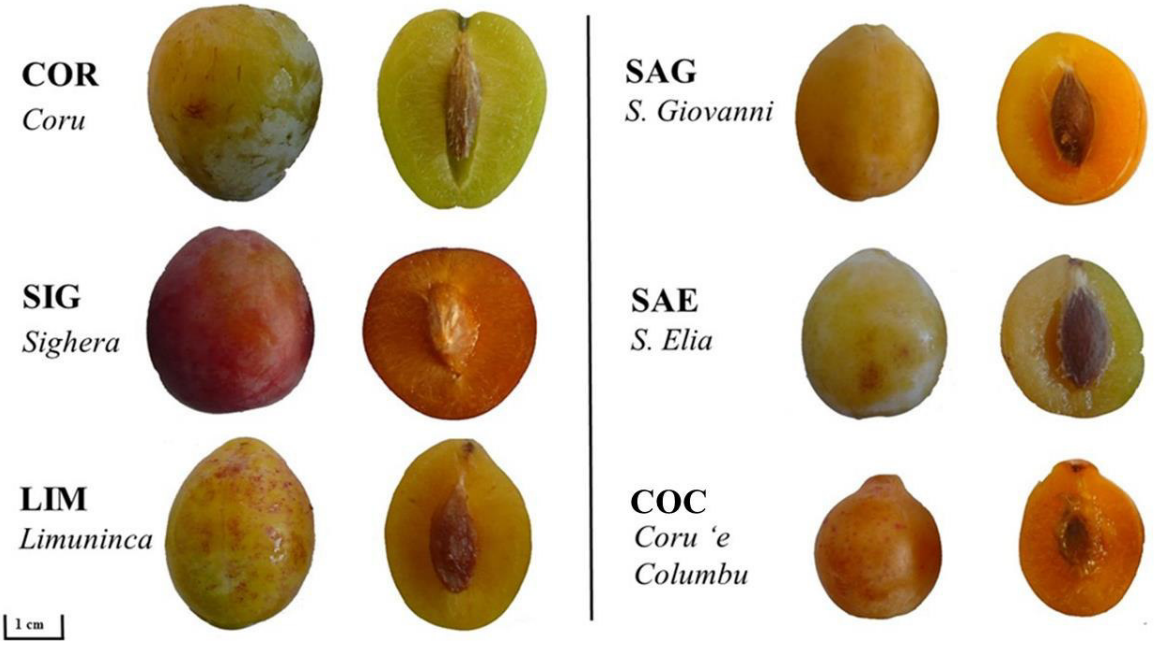

Fig. 4 Representation of probably modern varieties of *P. domestica* from the Sardinia field catalog more similar to the archaeological one investigated in this work.

	***P. domestica* SG**	***P. domestica* SS**	**Total**
***P. domestica* SG**	**100.0 (11)**	**-**	100.0 (11)
***P. domestica* SS**	-	**100.0 (17)**	100.0 (17)
Overall		-	**100.0 (28)**

Tab. 6 Comparison between *P. domestica* samples from SG (Phoenician-Punic Period) and Via Satta (Medieval Period).

	CRB	GON	MOL	MRC	TIS	ILR	TET	GES	JER	SPL	OLN	Total
CRB	50.9 (28)	-	1.8 (1)	30.9 (17)	1.8 (1)	10.9 (6)	-	3.6 (2)	-	-	-	100.0 (55)
GON	-	-	3.3 (2)	29.5 (18)	1.6 (1)	-	-	26.2 (16)	21.3 (13)	3.3 (2)	14.8 (9)	100.0 (61)
MOL	5.0 (5)	-	44.0 (44)	13.0 (13)	4.0 (4)	25.0 (25)	1.0 (1)	1.0 (1)	5.0 (5)	1.0 (1)	1.0 (1)	100.0 (100)
MRC	7.0 (14)	1.0 (1)	1.5 (3)	66.0 (133)	1.5 (3)	2.0 (4)	-	4.0 (8)	12.0 (24)	-	5.0 (10)	100.0 (200)
TIS	4.3 (3)	-	7.1 (5)	42.9 (30	2.9 (2)	-	-	12.9 (9)	25.7 (18)	1.4 (1)	2.9 (2)	100.0 (70)
ILR	6.0 (6)	-	36.0 (36)	17.0 (17)	1.0 (1)	30.0 (30)	1.0 (1)	1.0 (1)	7.0 (7)	1.0 (1)	-	100.0 (100)
TET	-	-	17.4 (8)	28.3 (13)	2.2 (1)	2.2 (1)	4.3 (2)	6.5 (3)	32.6 (15)	-	6.5 (3)	100.0 (46)
GES	2.0 (2)	-	1.0 (1)	17.0 (17)	3.0 (3)	1.0 (1)	1.0 (1)	24.0 (24)	21.0 (21)	14.0 (14)	16.0 (16)	100.0 (100)
JER	1.0 (1)	-	6.0 (6)	39.0 (39)	-	3.0 (3)	2.0 (2)	14.0 (14)	30.0 (30)	-	5.0 (5)	100.0 (100)
SPL	-	1.9 (1)	-	5.8 (3)	-	-	1.9 (1)	26.9 (14)	5.8 (3)	42.3 (22)	15.4 (8)	100.0 (52)
OLN	1.0 (1)	-	3.0 (3)	32.0 (32)	1.0 (1)	1.0 (1)	-	14.0 (14)	12.0 (12)	11.0 (11)	25.0 (25)	100.0 (100)
SS_G3/SS_G4				**70.4 (91)**	1.5 (2)		**25.1 (33)**		1.5 (2)	1.5 (2)		100.0 (130)
Overall												**38.1 % (1,052)**

Tab. 7 Correct classification percentages among modern populations of *P. spinosa* and archaeological endocarps of SS_G3 and SS_G4 from Via Satta SS. The numbers of endocarps analysed are in brackets.

Discussion

Thanks to the exceptional state of preservation of the archaeological remains of Sassari, it was possible to investigate and determine *Prunus* taxa present in the Medieval Period in Sardinia.

Based on results obtained the image analysis system was able to correctly classify, with high percentages, three species of *Prunus: P. domestica*, *P. spinosa* and *P. domestica* subsp. *insititia.*

Due to the insufficient number of archaeobotanical intact remains from archaeological sites, the reconstruction of the history of plum domestication in Europe, especially in Sardinia, results very difficult. Historical and archaeological information about the spread and the cultivation of fruits, in particular plum tree in of the area near Sassari, also like in the whole of Sardinia, are very poor if not entirely absent.

It is believed it was known in Roman times, while you are having reliable sources on its cultivation from the eighteenth century onwards, the period in which they were already present varieties still in culture and being part of the local varieties or old introduction. As documented by archaeological evidence and written sources in Roman times in Europe, an increase of domesticated fruits of plum in waterlogged contexts was observed (Pollmann et al. 2005; Zohary et al. 2012).

Based on the results obtained by image analysys, probably, the Phoenicians have introduced primitive cultivated forms of plums in the western Mediterranean Basin [chapter 2]. It seems that in the Phoenician-Punic context of Santa Giusta (Sardinia) could be evidence that the Phoenicians have introduced agricultural knowledge of fruit trees of plum in Sardinia around the 5th- 6th century BC.

The LDA analysis showed that the medieval forms of plum here described are perfectly distinguishable from the Phoenician-Punic remains of Santa Giusta, suggesting that *P. domestica* cultivars are phenotypically changed through time. This probably is due to the large chronological period that separates the two sites.

From the comparison between the archaeological endocarps with the modern ones, it was not possible to ascribe the 17 archaeological identified to a specific modern cultivar because few endocarps were available for the analysis, but some modern varieties of *P. domestica*, with yellow/red skin color, showed morphological affinity with the investigated archaeological remains. It means that some Medieval Sardinia plum fruits are very similar to some varieties currently cultivated and it could be assumed that the plums of Via Satta site probably maintained and preserved over time phenotypic characteristic still present today in the autochthonous varieties of Sardinia.

The large presence of *P. spinosa* and *P. domestica* subsp. *insititia* in the site of Via Satta shows that the use and the consumption of wild fruits and primitive forms of plum was widespread in the Middle Ages Sardinia although the spread and the utilisation is documented since the Neolithic/Bronze Age in many archaeological contexts in Middle Europe (Woldring 2000; Zohary et al. 2012).

Unfortunately, there is no information on the actual use of *P. spinosa* in the Medieval Period in Sardinia and probably its function has changed over time. Sardinia ethnobotany literature report numerous uses *P. spinosa* drupe and flowers as a medicine for the treatment of many diseases as antidiarrheal (the decoction of flowers), biliary dyskinesia, gut, convulsive cough, urinary and cardiovascular disorders, toothache, and oro-pharyngeal inflammation for the treatment of dysentery and cough (Atzei 2003; Campanini 2009).

Conclusions

The morphometric study presented in this work constitutes an innovative contribution to characterize past *Prunus* agrobiodiversity in methodological, taxonomical, bioarchaeological and historical perspectives in Europe, especially in the Mediterranean Basin.

The analyses indicated that the cultivation and use of *Prunus* was well established in Sardinia during the Medieval Period. In particular, different modern Sardinian autochthonous varieties of plum showed morphological and morphometrical affinity with the archaeological material. The combination of classic morphometric techniques associated with computer vision has allowed us to define with more precision the archaeological remains. In future, more archaeobotanical data may be helpful to provide important results. Finally, this study opens new and interesting perspectives on the assessment of plum agrobiodiversity at different taxonomic levels (species, subspecies and variety) and on the understanding of its cultivation and consumption history in the Mediterranean Basin.

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Chapter 4: Morphometric analysis on *Prunus spinosa* L. from archaeological Sardinian contexts.

Introduction

Prunus spinosa L., commonly known as sloe or blackthorn, is a deciduous thorny shrub native to Europe, western Asia and North West Africa. It is also locally naturalised in New Zealand and Eastern North America (Woldring 2000; Marakoglu et al. 2005). This thorny shrub or small tree is commonly frequently found at the margin of deciduous forests and often grows in hedgerows or thickets, where it can form dense stands. *P. spinosa* is insect pollinated and propagates vegetatively through root suckers. The fruits are spherical blue or purple-blue drupes between 10 and 15 mm of diameter, pruinose at maturity (Depypere et al. 2007). Seeds, enclosed in a woody endocarp, are dispersed by mammals and birds (Hübner and Wisseman 2004).

P. spinosa has an essential role in the taxonomy of the genus *Prunus.* Recent studies claim that *P. spinosa* have contributed to generate the domesticated form of plum although the two species are morphologically distinct and for this reason is considered a Crop Wild Relative (CWR), (Zohary et al. 2012). This is supported also by many authors who believe that *P. spinosa* together with *P. domestica* subsp. *insititia* and *P. cerasifera* Ehrh. contributed on the origin of *P. domestica* (Crane and Lawrence 1956; Eryomine 1991; Zohary and Hopf 2000). Other genetic studies have shown that *P. spinosa* and *P. domestica* subsp. *insititia* have close relationships with the current European domestic plums (Nassi et al. 2003; Pollmann et al. 2005; Horvath et al. 2011; Athanasiadis et al. 2013). However, according to Zohary et al. (2012) is not excluded the possibility that it might have contributed to domestic gene pool only through the secondary hybridization and later for introgression.

Archaeological evidence of *P. spinosa* remains have detected since the Prehistoric Period in many archaeological contexts in Middle Europe but the spread is documented until findings of the Middle Ages (Woldring 2000; Zohary et al. 2012). Table 1 shows an overview of the main discovery in Italy until the Phoenician-Punic Period.

Archaeobotanical data in Sardinia about *Prunus* remains are still scarce. The main reason is related to the condition of the archaeobotanical remains often found fragmented or charred and due to the scarcity of archaeological sites investigated. In fact, as suggested by Pollmann et al. (2005) waterlogged conditions is one of the rarest conservative methods of seeds in our latitudes but is that in which *Prunus* endocarps better are preserved.

The identification of *Prunus* species from archaeological contexts is not always easy. Through time, several authors have used different approaches to the study of archaeological endocarps of *Prunus* and until some years ago, the identification of the remains was made generally by calipers, based on fixed categories officially recognized (Behre in 1978; Woldring 2000; Pollmann et al. 2005; Depypere et al. 2007).

An evolution of these systems is represented by image analysis techniques that in recent years is being applied to archaeobotany (Terral et al. 2010; Orrù et al. 2012; Bouby et al. 2011, 2013; Sabato et al. 2014; Ucchesu et al. 2014; Pagnoux et al. 2015).

The main objective of this study is to:

- ✓ define the state of the art of *P. spinosa* remains in Sardinia;
- ✓ analyse the waterlogged endocarps from the archaeological sites of Sa Osa, Santa Giusta and Via Satta (SS) through the measurement of morphometric features;
- ✓ explore the possible relationships among archaeological remains and the modern *P. spinosa* populations present in Sardinia.

In this chapter are present new results and some comparisons based on the results described in the previous chapters.

Age	Type of remain	Country	Site	Reference
5633-4372 cal BC	endocarp	ITA	Sammardenchia	Rottoli 1999, 2005
5872-4547 cal BC	endocarp	ITA	Piancada	Rottoli 2005
5210-4355 cal BC	endocarp	ITA	Pavia di Udine	Pessina et al. 2004
5206-5050 cal BC	endocarp	ITA	Lugo di Romagna	Rottoli and Castiglioni 2009
4500-3500 cal BC	endocarp	ITA	Casalnoceto	Motella De Carlo and Venturino Gambari 2004
3800-3700 cal BC	endocarp	ITA	Lagozza di Besnate	Castelletti 1976; Helbæk 1955
4500-3500 cal BC	endocarp	ITA	Monte Covolo	Pals and Voorrips 1979
3500-2100 cal BC	endocarp	ITA	Monte Covolo	Castiglioni et al. 2008
2500-2250 cal BC	endocarp	ITA	Meduno	Castiglioni et al. 2003
1952-1778 cal BC	endocarp	ITA	Nola	Costantini et al. 2007
2111-1835 cal BC	endocarp	ITA	Riparo del Lauro	Bellini et al. 2008
1616-1464 cal BC	endocarp	ITA	San Lorenzo a Greve	Bellini et al. 2008
1500-1310 cal BC	charcoal	ITA	Terramara	Mercuri et al. 2006
1626-1434 cal BC	endocarp	ITA	Solarolo	Carra 2009
1270-1190 cal BC	endocarp	ITA	Duos Nuraghes	Bakels 2002
1286-1115 cal BC	endocarp	ITA	Sa Osa	Sabato et al. 2014
1443-1116 cal BC	endocarp	ITA	Scarceta di Manciano	Bellini et al. 2008
1091-1031 cal BC	endocarp	ITA	Stagno	Giachi et al. 2010
800-700 BC	charcoal	ITA	Monte Trabocchetto	Arobba et al. 2003
700-600 BC	endocarp	ITA	Monte Polizzo	Stika et al. 2008
600-300 BC	endocarp	ITA	Santa Giusta	Chapter 2

Tab. 1 The major records of *P. spinosa* (sloe) remains documented in the archaeological contexts in Italy. In chronological order from the earliest identifications until the 6th century AD Apart from the dating ranging from 800 BC to 600 AD the others chronology was calibrated with OxCal v4.2.3 (Bronk Ramsey and Lee 2013), r5, and the IntCal13 atmospheric curve (Reimer et al. 2013).

The archaeological sites studied

P. spinosa remains in Sardinia have been found actually only in four archaeological sites. One of these, the site of Duos Nuraghes have only charred endocarps (Bakels 2002; Ucchesu et al. 2014b), while the other three have waterlogged endocarps (Sa Osa, Santa Giusta and Via Satta, SS) presented in this work (Fig.1A).

The Duos Nuraghes site is located to the NE of Borore in an area of about 4600 sqm. The archaeological complex is composed of two "Nuraghi a Tholos" with a stratigraphy documenting occupation phases from the Early Bronze Age to the Iron Age. Plant remains were studied by Bakels (2002).

The archaeological site of Sa Osa (39°54′51″N 8°32′32″E, 6 m a.s.l.) (Fig. 1B) has been object of an intense excavation activity following the construction of a new road. It is located in the West-central area of Sardinia in the Gulf of Oristano, 2 km from the current coastline. The excavation seasons conducted between 2008 and 2009 by the Soprintendenza Archeologica per le province di Cagliari e Oristano and Università of Sassari confirmed the presence of several deep wells dug into the underlying sandstone with a large quantity of plant remains dated back to the Chalcolithic and Bronze Age (Usai et al. 2012). The most interesting of them is the well N (Fig. 1B). The structure emerged 2 m above sea level and

sandy brownish sediments characterized the first meter while from 1.40 m downwards the sediment is darker. It was excavated down to 4 m and yielded a huge amount of animal bones and plant macro remains (Usai 2011). The sediments come from the stratigraphic unit (US) 171 and US 172. These cavities probably had different functions (e.g. dwelling, quarry and water supply) and, at some point, were used either as refuse pits or for food storage (Usai 2011; Sabato et al. 2015). In fact, more than 50% of the sediment volume was made up of waterlogged seeds of fruits (Ucchesu et al. 2014a, b). The wide range of wild plants remains retrieved demonstrates the richness of the ancient local environment. The ^{14}C data on endocarp and seeds dates well N to the Late Bronze Age (1286-1115 2σ cal. B.C.; 1276-1088 2σ cal. B.C.).
As regards the site of Santa Giusta (Phoenican-Punic Period) and the site of Via Satta Sassari (Medieval Period), see [Chapter 2] and [Chapter 3] respectively.

Materials and Methods

Archaeological remains
Selected and waterlogged *Prunus* endocarps from the archaeological sites before described (except Duos Nuraghes) were studied (Tab. 2). The archaeobotanical remains of Sa Osa and Santa Giusta were extracted from the sediment with the wash-over technique using a fine mesh (0.25mm) to collect them (Kenward et al. 1980). Subsequently, the samples were kept in distilled water and stored at +5 ° C in the Germplasm Bank of Sardinia (BG-SAR) of the service centre and research Hortus Botanicus Kalaritanus (HBK) at the University of Cagliari (Atzeri et al. 2012). While, medieval *Prunus* remains of Via Satta (Sassari) site were selected and preliminarily analysed by the Laboratorio di Palinologia e Paleobotanica of the University of Modena and Reggio Emilia (Bertacci 2012; Bosi and Bandini Mazzanti 2013). Via Satta is the site that has provided the largest number of samples to analyzed.
The archaeological samples Sa Osa were preliminarily identified as belonging to the genus *Prunus* with uncertain or unknown Species. In this study these remains will be identified and classified at specific taxonomic level by image analysis system.
Table 2 shows the samples examined for this study. The total of the archaeological samples analysed were 198.

Modern samples
Modern wild plant material (2130 endocarps) were collected in the summer and autumn (2012-2014) from 11 populations of Sardinia (Italy) (Fig. 1, Tab. 3) and by several European seed banks (Bulgaria, France, Germany, Italy, Portugal, Romania, Spain and Switzerland) by *Index seminum* and exchanges (data not shown).
In order to compare the archaeological remains, modern samples of plum from the most representative autochthonous varieties of Sardinia from the field catalogue of CNR-ISPA (Nuraxinieddu, OR, Sardinia), were collected. In addition, 134 samples of damson collected in BG-SAR were added to the study.

Code	Taxon	Archaeological site	Age	Endocarp amount
SO	unknown	Sa Osa	Late Bronze Age	15
SG	*P. spinosa* [chapter 2]	Santa Giusta	Phoenican-Punic Period	53
SS	*P. spinosa* [chapter 3]	Via Satta (SS)	Medieval Period	130

Tab. 2 Amount of archaeological endocarps used in the study.

Fig. 1 A) Medieval site of Via Satta (SS); **B)** Sa Osa context; **C)** Santa Giusta site.

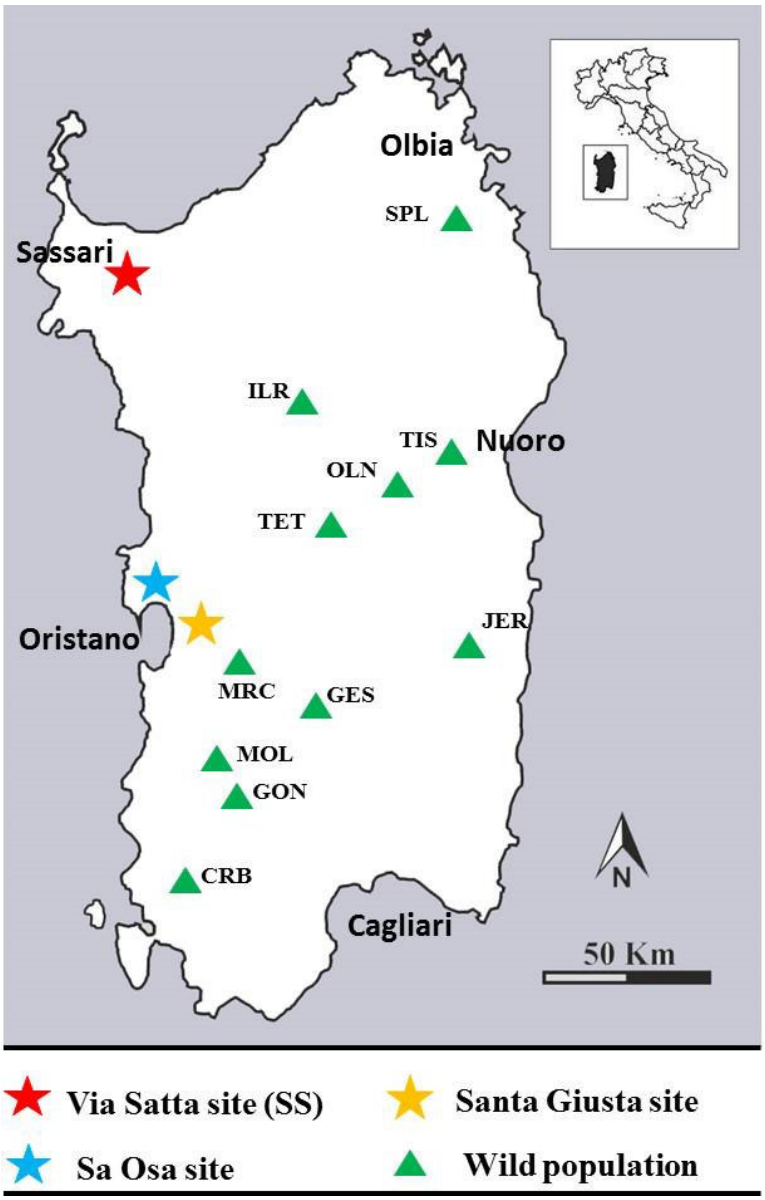

Fig. 2 Location of the archaeological sites investigated and distributions of *P. spinosa* samples selected for this study.

Code	Locality	Endocarp amount
CRB	Carbonia	55
GON	Gonnosfanadiga	61
MOL	Monte Linas	100
MRC	Monte Arci	200
TIS	Tiscali	70
ILR	Illorai	100
TET	Teti	46
GES	Gesturi	100
JER	Jerzu	100
SPL	San Pantaleo	52
OLN	Oliena	100

Tab. 3 General information on modern *P. spinosa* population collected in Sardinia utilised for the morphological comparison of archaeological endocarps.

Morphological and statistical analysis

The process Images were acquired using a flatbed scanner, with a resolution of 400 dpi, 24 bit and a scanning area not exceeding 1024 x 1024 pixel. Digital images were acquired for each sample, with black and white background and analysed using the software package KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). The accuracy and speed of measurements was maximized by running an automated macro, specifically developed for the characterization of *Prunus* endocarps developed from Bacchetta et al. (2008) and Mattana et al. (2008). Considering that seed and endocarp color is altered in the archaeological remains, color and texture have been not considered in this research, but in order to increase the number of discriminant parameters, the Elliptic Fourier Descriptors (EFDs) were also computed, as described by Orrù et al. (2013a), to accurately describe the shape of the analysed endocarps. A total of 98 parameters, describing seed size and shape, were computed (Tab. 1). Data were statistically elaborated applying the stepwise LDA (Linear Discriminant Analysis), following the same protocol described in [Chapter 2] and [Chapter 3].

	Feature	Description
A	Area	Endocarp area (mm^2)
P	Perimeter	Endocarp perimeter (mm)
P_{conv}	Convex Perimeter	Convex perimeter of the endocarp (mm)
P_{Crof}	Crofton Perimeter	Crofton perimeter of the endocarp (mm)
P_{conv}/P_{Crof}	Perimeter ratio	Ratio between P_{conv} and P_{Crof}
D_{max}	Max diameter	Maximum diameter of the endocarp (mm)
D_{min}	Min diameter	Minimum diameter of the endocarp (mm)
D_{min}/D_{max}	Feret ratio	Ratio between D_{min} and D_{max}
EA_{max}	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
EA_{min}	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)
Sf	Shape Factor	Endocarp shape descriptor = $(4\pi A)/P^2$ (normalized value)
Rf	Roundness Factor	Endocarp roundness descriptor = $(4A)/(\pi D_{max}^2)$ (normalized value)
Ecd	Eq. circular diameter	Diameter of a circle with equivalent area (mm)
F	Fiberlength	Endocarp length along the fiber axis
C	Curl degree	Ratio between D_{max} and F
$Conv$	Convessity degree	Ratio between P_{Crof} and P
Sol	Solidity degree	Ratio between A and convex area
Com	Compactness degree	Endocarp compactness descriptor = $[\sqrt{(4/\pi)A}]/D_{max}$

Tab. 4 List of 18 morphometric features measured on the endocarps, excluding the 80 Elliptic Fourier Descriptors (EFDs) calculated according to Hâruta (2011).

Results and Discussions

A total of 98 biometrics variables describing endocarp size and shape were measured and then analysed by stepwise LDA, to implement statistical classifiers able to distinguish the studied cases.

From the comparison between the archaeological endocarps from Well N of Sa Osa (SO) which were considered individually and added to the classifier as unknown group and the modern accessions of *P. spinosa, P. domestica* and *P. domestica* subsp. *insititia* samples, an overall percentage of 98.5 % were reached. These samples were identified correctly as *P. spinosa* in 100.0 % of the cases (Tab. 5).

As seen in previous chapters, regarding the endocarps of Santa Giusta (SG), these were identified correctly as *P. spinosa* in 100.0 % of the cases [chapter 2]. *P. spinosa* from the site of Via Satta in Sassari (SS) likewise were identified correctly as *P. spinosa* in 100.0 % of the cases [chapter 3], None of these groups has been identified as *P. domestica* or *P. domestica* subsp. *insititia* (Tab. 5).

	P. domestica	***P. spinosa***	***P. domestica* subsp. *insititia***	**Total**
P. domestica	75.4 (,1268)	2.5 (42)	22.1 (372)	100.0 (1,681)
P. spinosa	0.04 (1)	99.4 (2,117)	0.6 (12)	100.0 (2,130)
P. domestica* subsp. *insititia	13.4 (18)	0.8 (1)	85.8 (115)	100.0 (134)
SO as unknown	-	**100.0 (15)**	-	100.0 (15)
SG as unknown	-	**100.0 (53)**	-	100.0 (53)
SS as unknown	-	**100.0 (130)**	-	100.0 (130)
Overall				**98.5 %(4,143)**

Tab. 5 Identification percentage among the archaeological endocarps of *P. spinosa* from the three archaeological sites and the modern one of *P. spinosa* and *P. domestica* collected in Sardinia.

Based on the results obtained a comparison among the remains from the three archaeological sites examined was made in detail. A good percentage of classification of 74.3% was reached. In particular, the samples of SO group were correctly identified in the 80.0 % of the cases and endocarp of SG group in 100.0 % of the cases. Forty-two endocarps of SS were misattributed with SO with percentages of 32.3 % (Tab. 6).

	SO	**SG**	**SS**	**Total**
SO	**80.0 (12)**	-	20.0 (3)	100.0 (15)
SG	-	**100.0 (53)**	-	100.0 (53)
SS	32.3 (42)	-	**67.7 (88)**	100.0 (130)
Overall				**74,3 % (198)**

Tab. 6 Correct classification percentages *P. spinosa* remains from Sa Osa, Santa Giusta and Via Satta.

Likewise, to verify similarity with the modern sample collected in Sardinia, the three group, SO, SG and SS, were considered as unknown and compared with the modern populations of *P. spinosa* from Sardinia.

The archaeological endocarps of Santa Giusta (SG) are very similar with those collected at Monte Arci (MRC) in the 90.6% of the cases (Tab. 7). Sa Osa remains (SO) are similar with MRC in the 49.0 % and 32.8 % with CRB. Finally, samples of Via Satta (SS) are similar to MRC in the 70.4 % and to TET in the 25.1 % of the cases respectively.

	CRB	GON	MOL	MRC	TIS	ILR	TET	GES	JER	SPL	OLN	Total
CRB	50.9 (28)	-	1.8 (1)	30.9 (17)	1.8 (1)	10.9 (6)	-	3.6 (2)	-	-	-	100.0 (55)
GON	-	-	3.3 (2)	29.5 (18)	1.6 (1)	-	-	26.2 (16)	21.3 (13)	3.3 (2)	14.8 (9)	100.0 (61)
MOL	5.0 (5)	-	44.0 (44)	13.0 (13)	4.0 (4)	25.0 (25)	1.0 (1)	1.0 (1)	5.0 (5)	1.0 (1)	1.0 (1)	100.0 (100)
MRC	7.0 (14)	1.0 (1)	1.5 (3)	66.0 (133)	1.5 (3)	2.0 (4)	-	4.0 (8)	12.0 (24)	-	5.0 (10)	100.0 (200)
TIS	4.3 (3)	-	7.1 (5)	42.9 (30	2.9 (2)	-	-	12.9 (9)	25.7 (18)	1.4 (1)	2.9 (2)	100.0 (70)
ILR	6.0 (6)	-	36.0 (36)	17.0 (17)	1.0 (1)	30.0 (30)	1.0 (1)	1.0 (1)	7.0 (7)	1.0 (1)	-	100.0 (100)
TET	-	-	17.4 (8)	28.3 (13)	2.2 (1)	2.2 (1)	4.3 (2)	6.5 (3)	32.6 (15)	-	6.5 (3)	100.0 (46)
GES	2.0 (2)	-	1.0 (1)	17.0 (17)	3.0 (3)	1.0 (1)	1.0 (1)	24.0 (24)	21.0 (21)	14.0 (14)	16.0 (16)	100.0 (100)
JER	1.0 (1)	-	6.0 (6)	39.0 (39)	-	3.0 (3)	2.0 (2)	14.0 (14)	30.0 (30)	-	5.0 (5)	100.0 (100)
SPL	-	1.9 (1)	-	5.8 (3)	-	-	1.9 (1)	26.9 (14)	5.8 (3)	42.3 (22)	15.4 (8)	100.0 (52)
OLN	1.0 (1)	-	3.0 (3)	32.0 (32)	1.0 (1)	1.0 (1)	-	14.0 (14)	12.0 (12)	11.0 (11)	25.0 (25)	100.0 (100)
SO	**32.8 (5)**	6.0 (1)		**49.0 (7)**			6.0 (1)	6.0 (1)				100.0 (15)
SG	-	-	-	**90.6 (48)**	3.8 (2)	-	-	-	3.8 (2)	1.9 (1)	-	100.0 (53)
SS				**70.4 (91)**	1.5 (2)		**25.1 (33)**		1.5 (2)	1.5 (2)		100.0 (130)
Overall												**38.1 % (1,052)**

Tab. 7 Correct classification percentages among modern populations of sloe and archaeological samples of Sa Osa (SO) Santa Giusta (SG) and Sssari (SS). The numbers of fruit stones that were analysed are in brackets.

Discussion

Based on results obtained, the image analysis system was able to correctly classify, with high percentages three batches of archaeological endocarps of *Prunus* belonging to three different periods as *P. spinosa*.

The close relationship highlighted by the comparative analysis between the archaeological and the modern samples of *P. spinosa*, allowed to hypothesize that the endocarps found in Santa Giusta jars and in Sa Osa might have been gathered in the slopes of Monte Arci, located at just 10 Km from the studied archaeological site. This result allows assuming that sloes contained inside the amphorae were collected nearby of the storage site and that they were probably destined for other colonies.

It is not clear why the endocarps of SS are so similar to the modern one of South Sardinia. This aspect deserves more detailed information.

Certainly, the high values rates of identification percentages for the endocarps of Sa Osa and Via Satta with the modern population of Monte Arci, suggest that this location probably was a very productive economic site in the past for trades and for the procurement of food products since prehistoric times. Monte Arci is an isolated massif of basaltic nature that is located in the plain of Campidano Sardinia. It was important in the prehistoric Sardinia for the present of obsidian, very abundant in its slopes. Obsidian was more suitable for manufacture of flint tools and weapons. This volcanic glass has helped to create and grow the first the overseas businesses of Sardinia, who have brought contacts with distant peoples, useful to the formation of a remarkable civilization (Lugliè et al. 2006). It cannot exclude the hypothesis that prehistoric people who came very often to take obsidian in the deposits of Monte Arci had acquired a good knowledge of the surrounding environment for the harvest of wild fruits for sustenance.

Toghether sloe, wild fruits were identified in large quantities in the well N of Sa Osa (Sabato et al. 2014). This confirms that since the Bronze Age Period to the present day, fresh consumption and use in different fields of wild fruits, has been maintained over time proving to be an important WCR. Probably the Nuragic people had knowledge about the properties of this plant suggesting a significant role of *P. spinosa* tree in the past economies. These concentrations may represent fruit storage and preservation.

These results were possible thanks to the exceptional state of preservation of samples. In fact, although common in large parts of Europe, waterlogged macro plant remains are unusual in the Mediterranean area where plant preservation is generally by charring (Pollmann et al. 2005). The advantage of the waterlogged remains is that storage conditions allow endocarps not to be distorted. Otherwise happens in charred seeds when during the process of carbonization, different variables are able to modify and to alter the original morphology of the seeds, such as the temperature, the time exposure, the anoxic condition, the chemical composition and the amount of humidity contained in the seeds (Smith and Jones 1990; Hillman et al. 1993; Mangafa and Kotsakis 1996).

The cases of sites with waterlogged materials are relatively few, especially in Sardinia. However, in recent years, thanks to new recovery of the archaeological excavations in areas potentially rich in plant remains the archaeobotanical informatio of Sardinia landscape were increased. The waterlogged samples analyzed in this chapter represent an important data for the archaeological knowledge of the genus *Prunus* in Sardinia.

Examples of early contexts with waterlogged plant remains in the Peninsula include the Neolithic sites of La Marmotta (Rome) (Fugazzola Delpino et al. 1993).

There are, however, other examples from the Mediterranean such as the Middle Bronze Age pit from San Lorenzo a Greve (Florence) (Mariotti Lippi et al. 2010), Ostia antica (Pepe et al. 2013; Sadori et al. 2014), the classical and Medieval contexts from northern Italy (Bandini Mazzanti et al. 2005; Bosi et al. 2009; Bosi et al. 2011; Rinaldi et al. 2013). These medieval contexts are very similar to the site of Via Satta (SS) here presented.

Regarding the use of *P. spinosa* at present days, its fruits and flowers are used in different ways.
As report by Atzei (2003) and Campanini (2009) numerous uses of *P. spinosa* drupe and flowers in Sardinia ethnobotany literature, are documented.
Finally, Gómez Bellard et al. (1990) documented the use of the sloe for ritual purposes in some Punic tombs where charcoal remains of sloe, maybe used as fuel for the human body cremation or ritual offerings: Also in the Roman cemeteries, the use of fresh fruit of sloe as ritual offerings has been well documented (Preiss et al. 2005; Cooremans 2008; Bouby et al. 2011; Rottoli and Castiglioni 2011).
In Sardinia, there are no similar findings and more studies should be done about it.

Conclusions

The discovery of a large quantity of *P. spinosa* remains preserved in waterlogged contexts at the sites of Sa Osa, Santa Giusta and Via Satta have allowed us to investigate the use of this fruit and consume along the centuries in Sardinia.
Thanks to this study, for the first time, it was possible to investigate through image analysis system about the morphology and morphometry of archaeological *P. spinosa* endocarps from Sardinia. These sites, together with the archaeological site of Duos Nuraghes, are currently the only finds of *P. spinosa* in Sardinia documented by archaeological sources. Thanks to image analysis it was possible to understand the role of wild fruit as WCR in Sardinia through time and study their hypothetical origin.
According to data obtained from the LDA analysis, the endocarps of *P. spinosa* have maintained the typical phenotypic characteristics of the specie in relation to modern populations utilized for the comparison proving to be an important complement to the diet of the local populations since the prehistory.
Finally, the 98 morphometric features measured on the germplasm resulted a valid tool to achieve a clear discrimination among archaeological samples. The obtained results prove, once again, that image analysis techniques can be considered as a useful tool in taxonomic investigations, also in archaeobotany field as demonstrated by many authors for other edible species (Terral et al. 2010; Bouby et al. 2013; Orrù et al. 2013; Sabato et al. 2014; Ucchesu et al. 2014b; Pagnoux et al. 2015).

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General conclusions

The main goals of this research project are to interpret and understand the relation between human communities and the exploitation of plant resources in the past, thus understanding agricultural and alimentary behaviors in the present time.

The study of plant remains, offers the opportunity to explore the various practices related to the use of wild plants and understand how these have been selected for domestication.

The identification of wild and cultivated plants could facilitate the understanding of the role they have in the subsistence economy in Sardinia, and how this is strictly related to dietary habits.

The study of traditional varieties aims to create models of development, recovery and enhancement of ancient fruit. Also sets the basis for proposals for the recovery of cultural traditions and local economies.

In conclusion, the main achievements discussed the present PhD thesis can be summarized in the following points:

1. The application of image analysis technique for an adequate definition of the endocarp morpho-colorimetric and morphometric parameters represents an important diagnostic factor in the plant taxonomy studies and consequently may be of great help for the improvement of the management and the effective *ex situ* conservation in the germplasm banks.
2. For the first time, it was possible to investigate about the morphology and morphometry of *P. domestica* endocarps of traditional local varieties from Sardinia. Endocarp morpho-colorimetric features, EFDs and Haralick's descriptors obtained by image analysis allowed to implement a statistical classifier able to identify and classify the studied varieties of *P. domestica*, identifying plausible synonymy groups and confirming that the endocarp retain some characters directly related to the fruit skin color.
3. The discovery of well-preserved waterlogged endocarps of *P. domestica* from the Phoenician-Punic settlement of Santa Giusta could be evidence that the introduction of primitive cultivated forms of plums in Sardinia have been introduced by the Phoenicians people. Moreover, these endocarps represent the oldest findings and they are the oldest evidence of cultivated plums in the western Mediterranean Basin.
4. Finally, for the first time, it was possible to investigate through image analysis system about the morphology and morphometry of archaeological *P. spinosa* endocarps from Sardinia. These sites are currently the only finds of *P. spinosa* remains in waterlogged conditions documented in Sardinia by archaeological sources. Thanks to image analysis system it was possible to understand the hypothetical origin.

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Annex 1

Activities carried out during the PhD

Publications

- ✓ Lo Bianco M., Grillo O., Cremonini R., Sarigu M., Venora G. (in press) Characterisation of Italian bean landraces (*Phaseolus vulgaris* L.) using seed image analysis and texture descriptors. A. J. Crop Sci. 9: 1022-1034.
- ✓ Marzo A., Herreros R., Zreik Ch. (Eds.). 2015. Guide of Good Restoration Practices for Mediterranean Habitats. Ecoplantmed, ENPI, CBC-MED. (www.ecoplantmed.eu/).
- ✓ Ballesteros D., Meloni F., Bacchetta G. (Eds.). 2015. Manual for the propagation of selected Mediterranean native plant species. Ecoplantmed, ENPI, CBC-MED. (www.ecoplantmed.eu/).

International Conferences

- ✓ 10° Congress of Società Botanica Italiana onlus (SBI) - II International Plant Science Conference (IPSC) "Not only food: sustainable development, agro-biodiversity conservation & human well being"; Pavia, 14 – 17 September 2015.
- ✓ ICEB (International Congress of Ethnobotany), 17-21 November 2014, Cordoba, Spagna.
- ✓ International Conferences Providune Cagliari, 23-24 October 2014.

National Conferences

- ✓ Presentation of the Research Project of Regional Law 7 for basic research, 2012. "Modelli spaziali di accessibilità tra siti nuragici nei paesaggi storici per l'analisi territoriale. 21 may 2014, Alghero.
- ✓ 1° Symposium: "*Vitis vinifera* in Sardegna: dall'età Nuragica all'età Romana", 26 October 2012, Santadi.

Proceedings of the International Congress

- ✓ Sarigu M., Bosi G., Ucchesu M., Loi MC, Bacchetta G. 2015. Image analysis application on waterlogged archaeological *Prunus* remains from a medieval context in Sardinia. II International Plant Science Conference (IPSC) "Not only food: sustainable development, agro-biodiversity conservation & human well being"; Pavia, September 2015. Oral communication.
- ✓ Sarigu M., Ucchesu M., Grillo O., Venora G. Bacchetta G. 2014. *Prunus* L. seeds from two archaeological sites in Sardinia: characterization by image analysis. ICEB (International Congress of Ethnobotany), 17-21 Novembre 2014, Cordoba, Spain. Poster.
- ✓ Sarigu M., Soro L., Tisher S., Randaccio P. Bacchetta G. 2014. Spatial models of accessibility between nuragic sites in the historical landscapes to land planning: the role of Archaeobotanical research. ICEB (International Congress of Ethnobotany), 17-21 Novembre 2014, Cordoba, Spain. Poster.

Summer schools

- ✓ Summer School "Agrobiodiveristy of the Mediterranean area: a heritage to rediscover and conserve", (Gonnosfanadiga and Villacidro,16-21/06/2014)
- ✓ Summer School "La conservazione e gestione del patrimonio forestale della Sardegna" (Bono, 25-28/09/2012).

Research period

- ✓ Period of research for the PhD thesis through the Erasmus program 2012-2013 at Universidad de Castilla la Mancha, Facultad de Ciencias Ambientales y Bioquímica Toledo, Spain from 15/11/2012 to 01/05/2013.