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General palaeontology

The earliest remains of a *Citrus* fruit from a western Mediterranean archaeological context? A microtomographic-based re-assessment

Les plus anciens restes d'un fruit de Citrus dans un contexte archéologique de l'Ouest de la Méditerranée ? Une révision basée sur la microtomographie

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ABSTRACT

The discovery of the carbonized remains of a *Citrus*-like fruit in a funerary offering deposit dated back to the beginning of the 6th century BC during archaeological work run at Ischia, the island which hosted one among the earliest Greek colonies in southern Italy, has relaunched the question of the spread of *Citrus* through western Mediterranean during Classical Antiquity. Here we apply microtomography (SR-μCT and μCT) to investigate the inner structure of the archaeological specimen (SDC-mr.1a+b). Our high-resolution comparative analysis, which also considered one carbonized modern *Citrus* and a dried modern *Sorbus domestica* (true service tree) and *Malus* type *sylvestris* (wild apple), does not support the original taxonomic attribution (Coubray, 1996).

Mots clés :
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RÉSUMÉ

La découverte de restes carbonisés d'un fruit de type *Citrus* dans un dépôt votif du début du vi[e] siècle av. J.C. dans un site archéologique d'Ischia, l'île des premières colonies grecques du Sud de l'Italie, a relancé la question de la diffusion de *Citrus* dans l'Ouest de la Méditerranée au cours de l'Antiquité. Nous avons utilisé la microtomographie (SR-μCT et μCT) pour caractériser la structure interne du spécimen archéologique (SDC-mr.1a+b). Notre analyse comparative à haute définition, qui prend en considération un *Citrus* actuel carbonisé, une corme (*Sorbus domestica*) et une pomme sauvage (*Malus* type *sylvestris*) séchées, ne confirme pas l'attribution taxonomique originale (Coubray, 1996).

1. Introduction

Archaeological work performed at the Ischia Island, in the bay of Naples, Tyrrhenian Italy, led to the discovery of the carbonized incomplete remains of a fruit likely repre-

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senting *Citrus* sp. preserved in a funerary offering deposit dated to the beginnings of the 6th century BC (Coubray, 1996). The specimen, potentially representing the first occurrence of a citrus fruit in the western Mediterranean during Classical Antiquity, comes from Stipe dei Cavalli, Pastola locality, a site which has been excavated since 1966 by G. Buchner, of the Archaeological Service of Naples (Buchner, 1996), whose results have been later extended and reported by d'Agostino (1996). The special interest of the archaeological work at Stipe dei Cavalli relates to the fact that the Ischia island hosted one of the earliest Greek colonies in southern Italy (Magna Grecia), presumably founded by the Euboeans (Boardman, 1995).

While some consensus exists about the role of Southeast Asia (notably, of south-western China, north-eastern India, Burma, and the Malay archipelago) in the origin and diversification of the genus *Citrus* (Gmitter and Hu, 1990), the remains reported so far are still too sparse to trace a reliable history of the diffusion pattern of this fruit towards other areas, notably the Mediterranean (Webber et al., 1967). The etymology-based traditional arguments supporting the role played by the Greeks, or the Jews, in the dissemination of the *Citrus* tree across the Mediterranean (Andrews, 1961; Isaac, 1959; Tolkowsky, 1938) are currently considered out of date, but the paucity of the archaeobotanical record (see below), as well as methodological limits in unequivocally assessing the status of specimens from archaeological contexts leave the problem unresolved (for a taxonomic discussion on the Citrinae, see Barkley et al., 2006; Barrett and Rhodes, 1976; Moore, 2001; Scora, 1975). Also, even if still not supported on archaeobotanical ground, the introduction of *Citrus* in Italy is sometimes attributed to the Etruscans (Borgongino, 2006; Forni, 1990), while the presence of this fruit in eastern Mediterranean is testified by a single attribution on remains from the Bronze Age site of Hala Sultan Tekke, Cyprus (Hjelmqvist, 1979). Nonetheless, it should be pointed out that this discovery still deserves confirmation (Zohary and Hopf, 2000).

In *Historia Plantarum* (*Hist. pl.* 4.4.2-3), the Greek writer Theophrastus (c. 372-c. 287) referred to *C. medica* as to a peculiar tree from Media and Persia (Ramón-Laca, 2003). Earlier, according to Democritus (c. 460-c. 370), the citron had reached Europe following the military campaigns led in Persia by Alexander the Great. The *Citrus* fruit, still considered by Virgil as an exotic fruit in the first half of the 1st c. BC (*Georgics* 2.126-7), was firstly appreciated for its medicinal properties (it was supposed to act as a poison antidote), then was used in perfumery.

Before the discovery of the Ischia specimen and the indirect evidence, based on the palynological record from the 8th-6th c. BC Kyme 2 site, in Campania, that *Citrus* was likely cultivated at that time in the area of Cumae, bay of Naples (Bui-Thi, original unpublished data), the archaeobotanical evidence documenting the earliest presence of *Citrus* within the Italian peninsula relies on the discovery of a single mineralized pip from the House of Hercule's Wedding, in Pompeii, dated to the first half of the 2nd c. BC (Ciaraldi, 2007). In the same context, the presence of pollen grains likely belonging to this taxon had been previously reported (Mariotti Lippi, 2000), and six pollen grains of *Citrus* type (*C. medica* or *C. lemon*) had been also identified in the upper part of a core from the lake Averno, near Naples, chronologically corresponding to the Roman period (Grüger et al., 2002).

In this regard, it is noteworthy that, based mainly on aperture number and exine ornamentation, five pollen types have been recognized in the subfamily Aurantioideae, one of the seven subfamilies of the Rutaceae. The pollen grains of Citreae are almost always 4/5 colporate with exines varying from microperforate to coarsely reticulate (Grant et al., 2000). According to the SEM-based classical analytical work performed by Recupero and Russo (1980), the sculpture of the ectoexine represents a diagnostic feature in all *Citrus* taxa.

More recently, pips and carbonized fragments of a fruit resembling hesperidia have been discovered in a cremation deposit related to the 1st c. AD temple of Fortuna, again in Pompeii (Matterne, original unpublished data). In this ancient Roman town, *Citrus* trees bearing fruits are frequently and finely represented on the wall paintings, which points to the fact that *Citrus* was probably extensively cultivated in the surroundings of Naples since at least the 1st c. AD (Jashemski et al., 2002). At approximately the same time, both lemon and citron fruits are represented on a mosaic exhibited at the Terme Museum of Rome (Jashemski et al., 2002). Beside this record, additional archaeobotanical findings of *Citrus* come from the Roman Egypt (Van der Veen, 2001; Van der Veen and Tabinor, 2007).

In summarise, while the cultivation of *Citrus* is fully established in the mid-Tyrrhenian area (notably, in Campania) at the beginning of the Christian era, the problem of the first introduction of this taxon in the western Mediterranean (which likely occurred during the first millennium BC) remains a matter of discussion, thus the interest of the discoveries at the Ischia island.

The original carpological analysis and tentative assessment of the carbonized specimen from Stipe dei Cavalli were mostly based on its outer appearance (Coubray, 1996). Among the various macroscopic morphological features, the lumpy exocarp morphology, details of the partially observable pericarp, and the short peduncle were taken into account. Nonetheless, such criteria do not guarantee a reliable taxonomic assessment as they do not represent unique (autapomorphic) features, and the alternative attribution to taxa of the Maloideae family, namely, to *Malus* and *Sorbus*, cannot be discarded (Rohrer et al., 1991, 1994).

Here we use synchrotron radiation (SR-μCT) and industrial microtomography (μCT) in order to qualitatively and quantitatively characterize the inner structural morphology of the fruit remains from Ischia and to test the value of its original attribution to *Citrus* sp.

2. Material and methods

The incomplete carbonized specimen, of globular shape, consists of two separate but complementary and articulating partial fragments, SDC-mr.1a+b (Fig. 1). Maximum bipolar and equatorial diameters of the reconstructed specimen correspond to 19.6 mm and 20.6 mm, respectively.

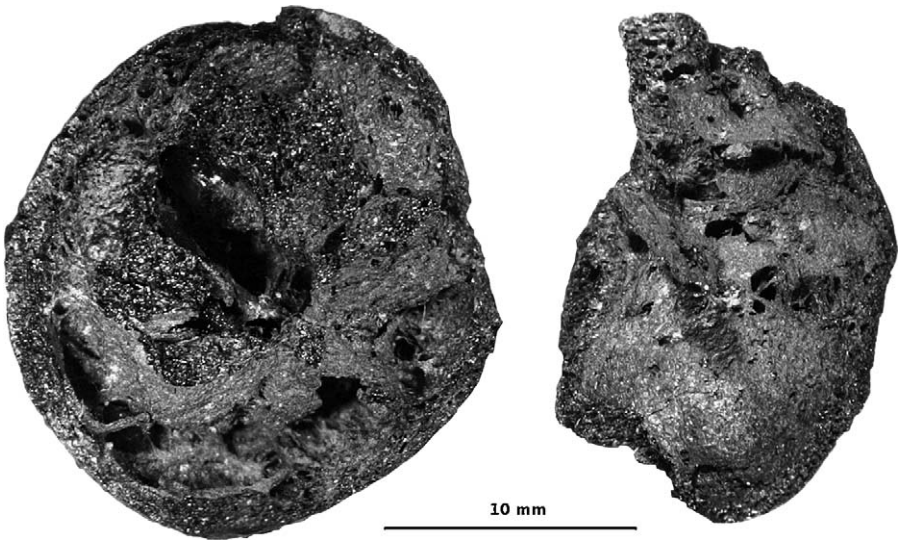

Fig. 1. The carbonized fruit specimen SDC-mr.1a+b from the 6th century BC archaeological site of Stipe dei Cavalli, Ischia island (Tyrrhenian Italy), originally attributed to *Citrus* (Coubray, 1996). The two articulating incomplete original fragments, **a** (left) and **b** (right), are shown separately.
Fig. 1. Restes carbonisés d'un fruit (spécimen SDC-mr.1a+b) du VI^{e} s. av. J.C. retrouvé sur le site archéologique de Stipe dei Cavalli, île d'Ischia (Italie Tyrrhénienne), précédemment attribués au genre *Citrus* (Coubray, 1996). Les deux fragments incomplets originellement retrouvés en connexion, **a** (à gauche) et **b** (à droite), sont ici présentés séparés.

SDC-mr.1a+b has been firstly detailed at a resolution of 45.5 μm × 45.5 μm × 43.64 μm at the beamline ID17 of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Despite some obvious macroscopic alterations of its original size and morphology reflecting heat exposure, its preliminary 2- and 3D virtual rendering showed a rather distinct endostructural signal, suitable for the extraction of a number of diagnostic features.

The analytical and comparative records specifically used in this study have been subsequently realized at the *Centre de Microtomographie* of the University of Poitiers, France. The μCT equipment used, a X8050-16 Viscom AG (a microfocus X ray tube coupled with a 1004 × 1004 camera and a 9 inch triple-field image intensifier), is a "multi-scale" X-ray inspection system which allows the analysis of variable sized objects (for example, see Bayle

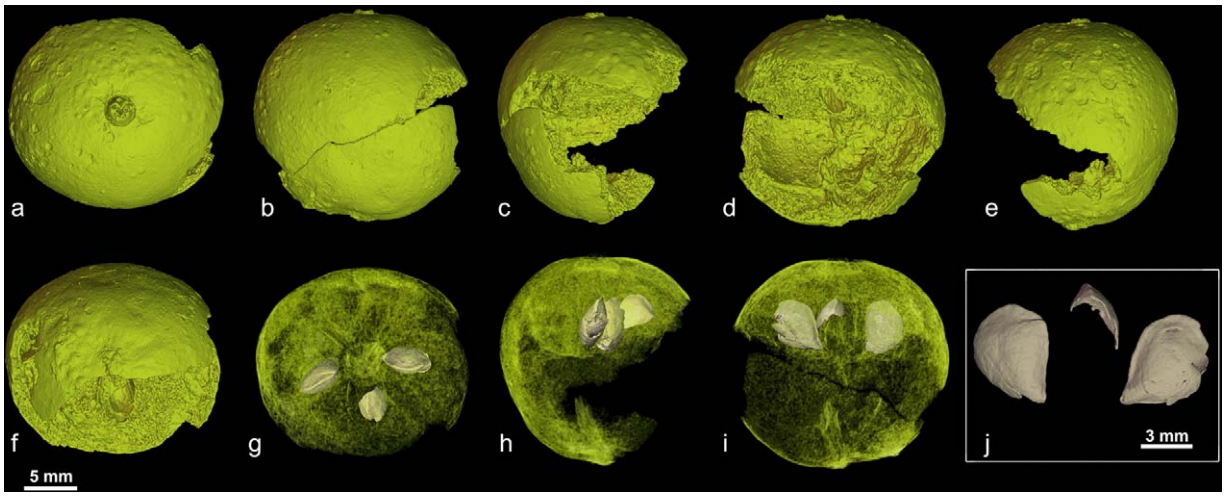

Fig. 2. Microtomographic-based 3D virtual rendering of the specimen SDC-mr.1a+b. The two separate fragments, **a** and **b**, have been virtually articulated. **a**: polar view from the calix-peduncle area; **b**: equatorial view; **c**: equatorial view perpendicular to **b**; **d**: equatorial view opposite to **b**; **e**: equatorial view opposite to **c**; **f**: polar view opposite to **a**; **g**: polar view opposite to **a** (as in **g**) in semi-transparency; **h**: equatorial view perpendicular to **b** (as in **c**) in semi-transparency; **i**: equatorial view opposite to **b** (as in **d**) in semi-transparency; **j**: detail of the three seeds visible in **g**, **h**, and **i**.
Fig. 2. Représentation 3D virtuelle du spécimen SDC-mr.1a+b. Les deux fragments, **a** et **b**, ont été virtuellement remis en connexion. **a**: vue polaire de la région calice-pédoncule; **b**: vue équatoriale; **c**: vue équatoriale perpendiculaire à **b**; **d**: vue équatoriale opposée à **b**; **e**: vue équatoriale opposée à **c**; **f**: vue polaire opposée à **a**; **g**: vue polaire opposée à **a** (comme en **f**) en semi-transparence; **h**: vue équatoriale perpendiculaire à **b** (comme en **c**) en semi-transparence; **i**: vue équatoriale opposée à **b** (comme en **d**) en semi-transparence; **j**: détail des trois graines visibles en **g**, **h**, et **i**.

et al., 2010; Bondioli et al., 2010; Buquet-Marcon et al., 2009; Mazurier et al., 2007). The scans parameters were: 60 to 70 kV, 0.7 to 0.8 mA current, 1500 radiographic views on 360° (each 0.24°), and 32 integrations by view. The final spatial resolution for SDC-mr.1a+b is 22.61 μm.

For comparative purposes, the following three modern specimens (one carbonized under controlled experimental conditions, two dried) have been also detailed and imaged following the same analytical procedures: a carbonized *Citrus x latifolia* (Persian lime) from Egypt (28.11 μm of spatial resolution); a *Sorbus domestica* (true service tree) from

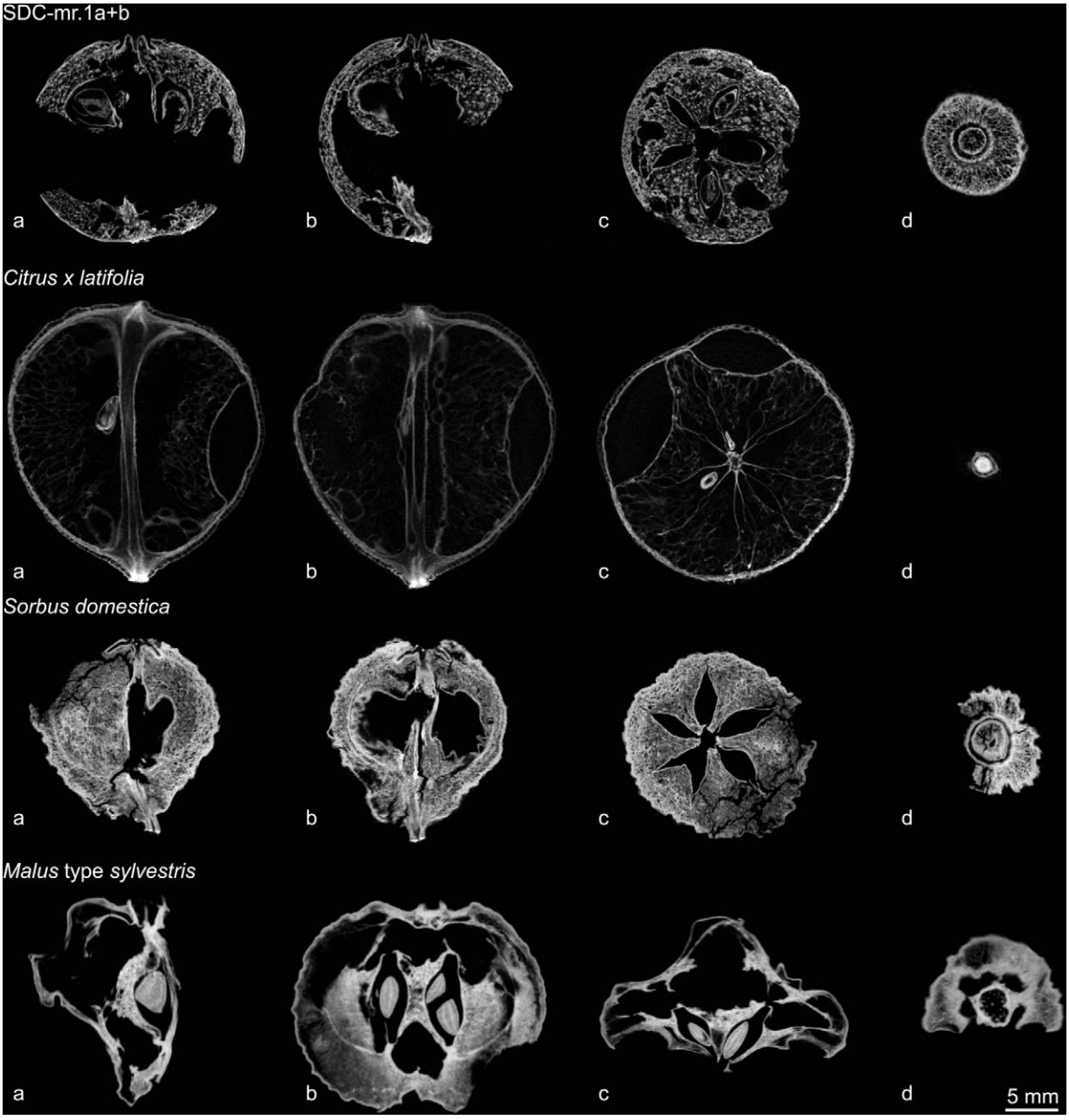

Fig. 3. Microtomographic-based longitudinal (**a**, **b**) and axial (**c**, **d**) virtual cross-sections illustrating the inner structural morphology of: the archaeological specimen SDC-mr.1a+b, a carbonized *Citrus x latifolia* (Persian lime), *Sorbus domestica* (true service tree), *Malus* type *sylvestris* (wild apple). **a**: longitudinal section through the center (bipolar); **b**: bipolar section perpendicular to **a**; **c**: equatorial section; **d**: axial section detailing the calix-peduncle area (corresponding to the upper pole imaged in **a** for SDC-mr.1a+b, *Sorbus* and *Malus*, and to the lower pole in *Citrus*).

Fig. 3. Sections virtuelles longitudinales (**a**, **b**) et axiales (**c**, **d**) illustrant les structures internes: du spécimen SDC-mr.1a+b; d'un spécimen actuel carbonisé de *Citrus x latifolia* (citron perse); d'un spécimen séché de *Sorbus domestica* (corme); et d'un spécimen séché de *Malus* type *sylvestris* (pomme sauvage). **a**: section longitudinale par le centre (bipolaire); **b**: section bipolaire perpendiculaire à **a**; **c**: section équatoriale; **d**: section axiale montrant la région calice-pédoncule (correspondant au pôle supérieur imagé en **a** pour le spécimen SDC-mr.1a+b, *Sorbus* et *Malus*, et au pôle inférieur pour *Citrus*).

France (21.22 μm); and a *Malus* type *sylvestris* (wild apple) from France (34.71 μm).

All reconstructions have been made at the *Études Recherches Matériaux* company (www.erm-poitiers.fr) using the software DigiCT v.2.4.2 (Digisens) 64-bit version running on a 2.5 GHz Quad-Core Intel Xeon processor Windows XP 64 Dell workstation with 32 GB of DDR RAM and two NVIDIA GPUs boards (Quadro FX 5600 and Telsa C870). This workstation allows using the SnapCT Digisens acceleration plug-ins for tomographic reconstruction, which takes advantage of the GPU processing capabilities. The final volumes were reconstructed with isotropic voxels. Virtual sections and 3D rendering were performed by means of Avizo 6.1 (Mercury Computer Systems Inc.) 64-bit version.

The virtual merging of the two originally separate parts of SDC-mr.1 (i.e., a and b), has been realized via Avizo 6.1 by applying a rigid transformation (only global rotation and translation) based on landmarks sets along the two sides of the original facture rim.

3. Results and discussion

The microtomographic-based 3D virtual reconstruction of the archaeological specimen from Ischia is rendered in different perspectives and imaging effects in Fig. 2 (a to j), where the lumpy exocarp morphology is evident. As shown in Fig. 2b and i, the two originally separate fragments almost perfectly fit. In the preserved and measurable parts of the fruit, slight wall thickness variation is locally found, likely reflecting the carbonized nature of the specimen. The region which in *Citrus* corresponds to the calix-peduncle (Fig. 2a) is quite well preserved. The structure, with an erected orientation, presents five lobes and a depression, or cup, in the scar. At its opposite face (Fig. 2f), the shape of the scar of the alleged style is slightly convex and wrinkled. Rendering in semi-transparency reveals the inner presence of a multi-locular core consisting of five carpels (Fig. 2g, h, i), still preserving three seeds, one single per locule. Such structural morphology was previously not accessible for direct observation (Coubray, 1996). Each ovary results in a separate locule composing the core. The ovary walls show a stiffer texture than the surrounding flesh (Fig. 2). The carpels are not fully connate and there is an opening running axially in the center of the fruit.

In our analysis, as imaged in Fig. 2j, we were in the conditions to virtually isolate and tentatively assess the size of two almost complete seeds.

Measures of the seed imaged to the left of Fig. 2j are as follows: height = 5.58 mm (minimal estimate), breadth = 4.63 mm, thickness = 2.41 mm. Values obtained for the seed imaged to the right of the same image are: 5.97 mm, 4.33 mm (minimal), and 2.27 mm (minimal), respectively.

A selected number of longitudinal (a, b) and axial (c, d) virtual cross-sections comparatively imaging at various levels the structural morphology of SDC-mr.1a+b, the carbonized Persian lime (*Citrus x latifolia*), the true service tree (*Sorbus domestica*), and the wild apple (*Malus* type *sylvestris*) are shown in Fig. 3. As a whole, the slices unequivocally demonstrate the substantial differences in structural organization and proportions between the fruit remain from Stipe dei Cavalli and the carbonized *Citrus* specimen used for comparison, as well as the striking resemblance between the former and the true service tree fruit.

Besides the shape differences between the globular SDC-mr.1a+b and the oval Persian lime, which are clearly not related to carbonization, in *Citrus* the pericarp (flavedo), the mesocarp (albedo, relatively thin is this specimen), and the endocarp are distinctly recognizable (Fig. 3a, b, c), and variously sized oil glands, missing in SDC-mr.1a+b, are also visible within its pericarp (Schneider, 1968; Swingle and Reece, 1967). The central axis, derived from the floral axis, is evident with its system of vascular bundles. In the endocarp, the thin partitions between the locules (septa) and the vesicles (juice sacs), both structures missing in the archaeological specimen, are well appreciable. Also, quite differently from SDC-mr.1a+b (Fig. 3d), in *Citrus* the woody tissues of the stem-like calix-peduncle define a significantly denser structure (Fig. 3d).

As imaged by the comparative cross-sections (Fig. 3), the structural morphology of the two representatives of the Maloideae, *Sorbus* and *Malus*, deeply differs from that characterizing *Citrus*, and better fits that of SDC-mr.1a+b, notably in the case of the true service tree, *Sorbus domestica*. In fact, similarly to the carbonized archaeological specimen, *Sorbus* shows a multilocular core composed of five carpels converging into an opening running axially in the center of the fruit, and the region of the calyx (top of the image in slices a and b, and slice d) presents a depression in cup (Rohrer et al., 1991, 1994). Additional similarities between the two specimens are found in the ovary wall structural organization and in the endocarpal morphology as a whole. In this comparative perspective, the *Malus* type *sylvestris* investigated in the present study shows some unique features not found in both SDC-mr.1a+b and *Sorbus domestica*.

4. Conclusions

Based on the present high-resolution microtomographic investigation, which granted access to its previously unexplored inner structural morphology, we can confidently state that our results do not support the original attribution to a *Citrus* fruit of the specimen SDC-mr.1a+b from the 6th century BC site of Stipe dei Cavalli, Ischia island (Coubray, 1996). Rather, the comparative analysis has shown some interesting similarities between the archaeological specimen and a member of the Maloideae, *Sorbus domestica*.

The Maloideae are a subfamily of the Rosaceae characterized by pome fruits composed of one or more carpels surrounded by accessory tissue (Rohrer et al., 1991, 1994). As opposed to the Citrinae, the presence of the Maloideae in the western Mediterranean, notably in the Italian peninsula, is well ascertained since the Neolithic times (Castelletti, 1996; Castelletti et al., 2001) through the Classical Antiquity (Borgongino, 2006; Ciaraldi, 2007). Nonetheless, despite the evident structural affinities with the true service tree fruit revealed by non-invasive virtual imaging, a conclusive taxonomic attribution of the archaeological specimen SDC-mr.1a+b will be likely pos-

sible only when additional MEB-based subtle information about its epidermis, calyx, and seed coat morphology will be taken into account, comparatively.

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