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Analysis of 'Marrakesh limetta' (*Citrus* × *limon* var. *limetta* (Risso) Ollitrault, Curk & R.Krueger) horticultural history and relationships with limes and lemons

Diego Rivera [a,*], Antonio Bermúdez [b], Concepción Obón [c], Francisco Alcaraz [a], Segundo Ríos [d], Jorge Sánchez-Balibrea [e], P. Pablo Ferrer-Gallego [f], Robert Krueger [g]

[a] Dep. Biología Vegetal, Fac. Biología, Universidad de Murcia, 30100 Murcia, Spain
[b] Colegio Español de Rabat, 7, Rue Khenifra, Rabat, C.P.: 10000, Morocco
[c] Universidad Miguel Hernández, Campus de Beniel. Escuela Politécnica Superior de Orihuela. Depto. De Biología Aplicada. Ctra. Beniel, Km 3,2. 03312 Orihuela, Alicante, Spain
[d] CIBIO, Universidad de Alicante, Alicante, Spain
[e] Asociación de Naturalistas del Sureste, Pza. Pintor José María Párraga, n° 11 bajo, 30002 Murcia, Spain
[f] Servicio de Vida Silvestre y Red Natura 2000-CIEF (Centro para la Investigación y la Experimentación Forestal) Generalitat Valenciana / VAERSA Avda. Comarques del País Valencià, 114 46930 Quart de Poblet (Valencia), Spain
[g] USDA-ARS National Clonal Germplasm Repository for Citrus & Dates, 1060 Martin Luther King Blvd, Riverside, CA 92507-5437 USA

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ABSTRACT

Citrus (Rutaceae) species have a relevant role in the culture of the Mediterranean countries, not only for their gastronomic or medicinal value, but especially for their ornamental and symbolic importance. The interest on Citrus diversity led to collect and cultivate numerous cultivars around the Mediterranean Sea, at least since the Roman period. Their image is documented in mosaics, paintings, sculptures and coins since the first millennium BC. Among these, sweet and sour limettas *Citrus* × *limon* var. *limetta* (Risso) Ollitrault, Curk & R.Krueger stand for their history and conservation status. Presently sour limetta is cultivated in Morocco, especially in Marrakesh, and in the Valle de Ricote (Murcia, Spain). Our work has three main objectives: to characterize sour limetta on the basis of Spanish and Moroccan populations, as well as its cultural relevance throughout history, especially in painting, gastronomy, agriculture and gardening, to determine their relationships with limes and lemons and to evaluate the availability and state of conservation of that legacy.

We analyze and illustrate the morphology of the plant, flowers and fruits of sour limetta and their differences with other related citrus species and cultivars: sweet limetta, lemon, common lime, Persian lime, bergamot, mellarosa, Meyer lemon, rough lemon, *Pursha Romana* lime, Palestine sweet lime, *Pomum Adami* lemon and Rangpur lime. The above species and cultivars were selected on the base of their known ancestry and pomological characters. The distinctive characters are useful for the identification of sour limetta in the field but also for the identifying images of this plant in paintings. We analyzed the ancestry of the different taxa involved and compared the classification based on phenotypes with the classification based on the proportion of ancestors' genomes in each individual taxa. The image of sour limetta appears in different paintings, from the van Eyck's Ghent Polyptych (early 15th century), and numerous still life works by Italian and Spanish authors of the 16th to 18th centuries. A peculiar type of evidence is provided by presence of limetta in the pictorial catalogues of the Medici's fruit collections displayed in a series of paintings by Bartolomeo Bimbi.

This distinctly sour limetta is sold in the markets of Rabat (and other cities of Morocco), either fresh, candied or brined and canned. It is an important ingredient of traditional Moroccan cuisine. Although sour lime was a widely used ingredient in Italian cuisine during the 17th century, its state of preservation is deplorable outside of Morocco, and even there it is gradually replaced by lemons. We recommend its cultivation as a resource both for obtaining rootstocks as well as for its fruits, both for Moroccan cuisine and that of other parts of the world for the excellent aromas and flavors that they contribute to the dishes. In this way, by promoting its crop, we will prevent it from disappearing. It is also important to preserve sour lime in germplasm banks.

* Corresponding author.
E-mail address: drivera@um.es (D. Rivera).

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1. Introduction

Genus *Citrus* belongs to the Aurantioideae, a monophyletic subfamily of Rutaceae (Morton et al., 2003). *Citrus* include fruit crop trees and shrubs highly appreciated worldwide, not only for their edible fruits but also for the essential oil of their leaves, flowers, and fruit rinds (Bora et al., 2020)

There is a broad consensus among researchers on the relevant role that hybridization and clonal propagation mainly via apomixis (the asexual formation of a seed from the maternal tissues of the ovule), associated with the phenomenon of polyembryony, that is, the formation of several nucellar embryos in the same seed, have played in the genesis and preservation of citrus diversity (Wu et al., 2014, 2018). The identification of a set of "pure" true citrus species: *C. medica* L. -citrons, *C. maxima* (Burm.) Merr. (pummelos), *C. reticulata* Blanco (mandarins), *Citrus hystrix* DC. (Kaffir lime), *C. cavaleriei* H. Lév. ex Cavalerie (Ichang papeda), *C. mangshanensis* S. W. He & G. F. Liu (manghsan mandarin), along with five from other genera (*Fortunella margarita* (Lour.) Swingle (Nagami kumquat), *Eremocitrus glauca* (Lindl.) Swingle (Australian desert lime), *Microcitrus australis* Swingle (Australian round lime), and *M. australasica* Swingle (Australian finger lime), principally, has provided solid insights into the phylogeny of citrus, and their origins, evolution, and dispersal (Wu et al., 2014, 2018; Talón et al., 2020).

The role of hybridization and polyembryony is particularly significant within the Mediterranean secondary center of citrus diversity, where the set of hybrid taxa characteristic of the area (lemons, sweet oranges, limes, lumias, limettas, Mediterranean mandarins, etc.), can be explained as a result of various hybridization events involving different combinations of four pure ancestral species (*C. medica, C. maxima, C. reticulata* and *C. hystrix*) (Curk et al., 2015, 2016; Talon et al., 2020). Acceptance of these premises implies drastic changes in the concept of species within genus *Citrus*, and in the ascription of the different varieties to the recognized taxa and their nomenclature (Curk, 2014). The Tanaka (1961), Swingle and Reece (1967), Hodgson (1967) or Mabberley (1997, 2004) proposals for *Citrus* nomenclature should be systematically reviewed according to the available evidence, taking into account the recent phylogeny-ancestry studies and the phenotypes of individual cultivars (Ollitrault et al., 2020).

Here we must recall that *Citrus cavaleriei* is the valid name for *C. ichangensis* Swingle and *C. hystrix* for *C. micrantha* Wester (GRIN, 2021).

In our present work we focus on the case of Mediterranean sweet and sour limettas. Risso (1813a, reprinted in 1813b) validly published the name *Citrus limetta*, covering seven citrus cultivars including sweet limetta and sour limetta, but also bergamot, and *mellarosa*.

Organisms, and particularly domesticated organisms, constitute in themselves an extremely fragile biological and cultural heritage since they depend on the positive activity of humans for their conservation, evolution and adaptation: they conform the horticultural heritage. The knowledge and preservation of horticultural diversity is a fundamental commitment with the horticultural heritage. The various cultivars of plants are characteristic of different populations, cultures and civilizations, arise from them, contribute to their livelihoods and depend on them for their survival (Rivera and Obón, 1992). Humans are connected by bonds of kinship with particular plant and animal species, and with the "natural" environment more generally. This explains why, for them, it is impossible to disentangle the "cultural" from the "natural" (Harrison, 2015).

These intense relationships are exceptionally reflected by the presence of plant species in the imaginary of different cultures expressed in the form of images, from abstract to extremely realistic, in the plastic arts (painting, sculpture, engraving, etc.) (Kumbaric et al., 2013). These images are part of the cultural heritage, but also constitute a fundamental source of biological information (Rivera et al., 2019, 2020).

In the Islamic culture the use of acidic condiments based on citrus species is essential. Currently the most used are lime (Citrus × aurantiifolia (Christm.) Swingle), lemon (Citrus × limon (L.) Osbeck var. *limon*), and sour limetta (*Citrus × limon* var. *limetta* (Risso) Ollitrault, Curk & R. Krueger). Actually, lime is prevalent in the eastern Mediterranean as an ingredient in medicinal formulas and as a condiment, and lemon in the western Mediterranean especially in Spain, France and Italy. Sour limetta use is almost restricted to the Maghreb, especially in the Moroccan gastronomy, under the name of الليمون بلدي (*allayumun baladi*) (my lemon), where is rapidly being replaced by cheaper common lemon (*C. × limon* var. *limon*). In the past, *C. ×* limon var. limetta was essential as the citric acid supplying agent for food and medicine in the western Mediterranean area. The limetta was a renowned plant, widely used, during the Middle Ages and the Renaissance. But from the eighteenth century, its use and therefore its cultivation decreased, being progressively replaced by the lemon (*C. × limon* var. *limon*). Presently it is only cultivated in some regions of Morocco and in the Ricote Valley (Murcia, Spain). The Marrakesh limetta receives other names such as Moroccan limetta.

Limettas are relatively variable fruits in their shape and degree of acidity and sweetness. They are well recognized by the globose, flattened shape and by the presence of a very deep apical groove surrounding a nipple (Rivera et al., 1997). Among the limettas Rivera et al. (1997) distinguish those with a sweet taste (as *Citrus × limetta* subsp. *limetta*), "limero ordinario" (Fig. 1a) and those with an acidic taste (as *Citrus × limetta* subsp. *murcica*), "*lima ácida*" (Fig. 1b) (Tamaro 1987). This last was named Limonette de Marrakesh by Chapot (1962) and Butelli et al. (2019). Morphologically the fruits are similar and can hardly be distinguished, both have a very pleasant scented rind, the main difference being the degree of acid content of the pulp.

The cultural importance of this plant is not only reflected in the numerous descriptions and citations in the literature but especially in its repeated presence in the painting of, notably, the 17th and 18th centuries.

The citrus taxonomy and nomenclature was recently reviewed by Ollitrault et al. (2020) presenting a proposal consistent with the available data, although the new combinations proposed there are marked as "ined." and they cannot be used until they are validly published in an effective manner in accordance with the provisions of the International Code of Nomenclature for algae, fungi, and plants (Turland et al., 2018). This is the case for "*C. × limon* var. *limetta* ined", which is not yet validly published, although Gargominy et al. (2020) and GBIF (2021) erroneously accept it as such. In order to use this combination we proceed here to validate it:

Citrus* × *limon* var. *limetta (Risso) Ollitrault, Curk & R.Krueger, **comb & stat. nov.** Basionym: ***Citrus limetta* Risso** in *Ann. Mus. Hist. Nat. (Paris)* 20: 195, 1813. **Lectotype (designated here):** [Fig. 2 (in Plate 2 of the article, Plate 4 of the entire volume, before page 213)] in Risso (Ann. Mus. Hist. Nat. (Paris) 20: 169–212 + 2 plates, 1813), which represents one fruiting branchlet with one whole fruit and three flowers (Fig. 2). This new combination was not formally published by Ollitrault et al. (2020) in the page 70 where they clearly specified "ined." Gargominy et al. (2020) did not validated this combination because they gave not a full and direct reference for the basionym including its author and place of valid publication, with page or plate reference and date as required by the Art. 41.5 of the International Code of Nomenclature for algae, fungi, and plants (Turland et al., 2018).

During our study on citrus landraces in the western Mediterranean Region we found in Ojós (the Valle de Ricote, Murcia, Spain) this distinctly sour limetta (Rivera et al., 1997). The same is sold in the markets of Rabat (Morocco), both fresh and candied. It is also commercialized brined and canned through supermarkets (in Morocco, France and Spain). The Ojós' sour limetta, thus, appears to be identical, or very similar, to Marrakesh Limonette, known as "*Limun Beldi*" (Chapot 1962).

Ignorance about this taxon has also led to confusion in numerous cases, especially in South America, where it has been incorrectly named lima mexicana, lima, limon and others. The sour limetta is becoming

culturally a rarity preceding its extinction due to the confusion with lime and lemon. Other names such as "*Limun Buserra*", "*Limun Boussera*" (*allaymun bisraa*) (Bosra lemon) (Chapot, 1962) are incorrectly applied to this Moroccan sour limetta, since these correspond to the smoked *Citrus* × *aurantiifolia* fruits widely used in the Near East and the Mediterranean. In France it has also been sold under the name bergamot because of the aroma but it is a great mistake (Chapot, 1962).

Curk et al. (2016) in their study of the phylogenetic origin of limes and lemons using cytoplasmic and nuclear markers, placed limettas close to lemons, Palestinian sweet limes, bergamots and Meyer lemons that share as common ancestors *Citrus medica* and sour orange (*Citrus* × *aurantium*).

Our work has four main objectives: to morphologically characterize acid limetta on the basis of Spanish and Moroccan populations, and to analyze the relationships between limettas and other close citrus taxa based on morphometric and ancestry evidences. We also intend to analyze its uses in agriculture and gardening, and its cultural relevance throughout history, especially in painting and gastronomy, and, finally, evaluate the state of conservation of that legacy.

2. Materials and methods

2.1. Field work, plant material and morphometry

Ethnobotanical field work was carried out in Sale and Rabat (Morocco), Ricote Valley, Huerta de Murcia and Orihuela (Spain).

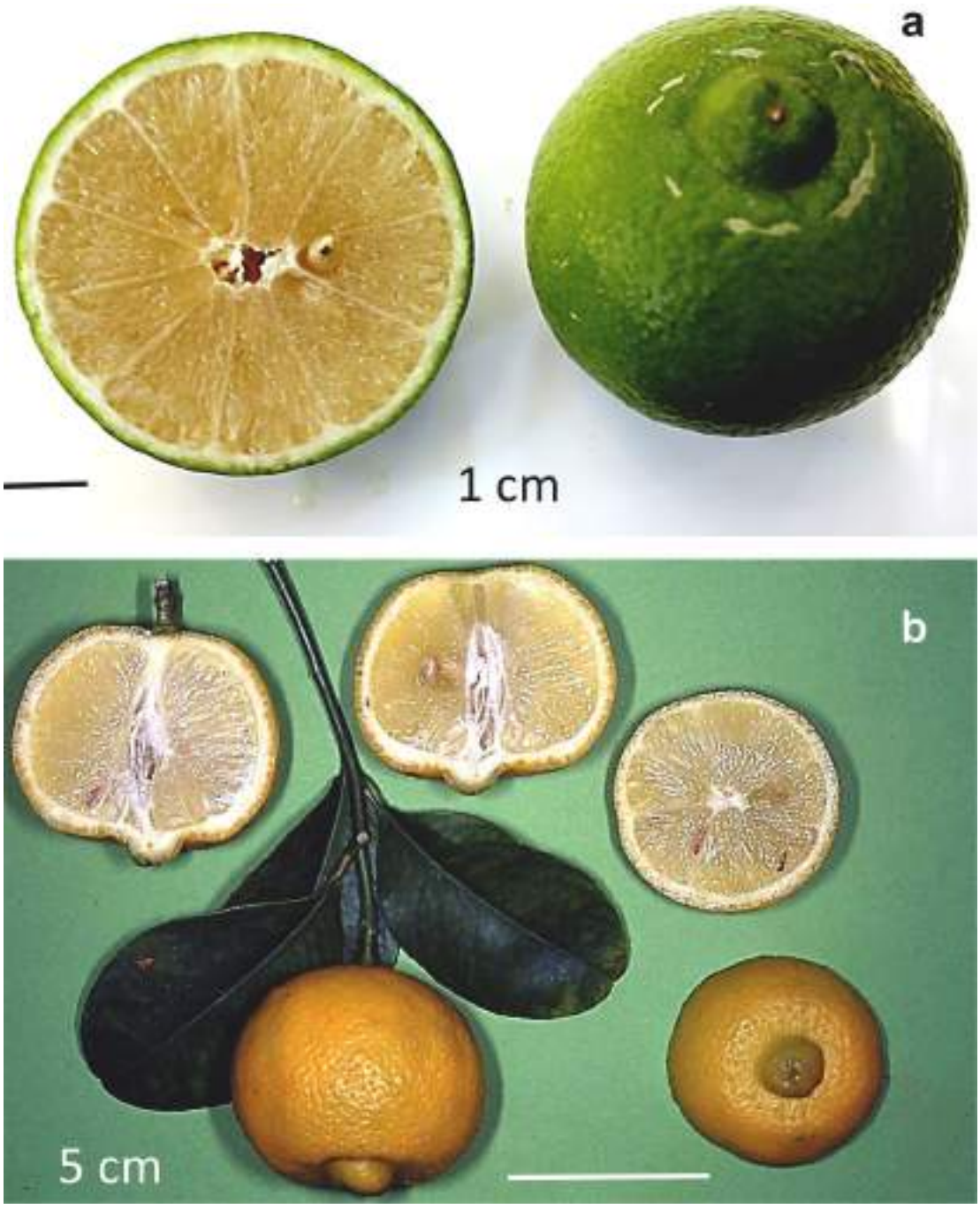

Fig. 1. Fruits of sweet and sour limetta
a, Sweet limetta from Baja California Sur (Mexico); b, Sour limetta from Ojós (Murcia, Spain). Photos: a, Robert Krueger. b, Diego Rivera.

Samples of sour limetta were collected in Murcia in 1996 and 2012 and in the markets of Rabat and Sale (Morocco) in December 2011 and 2012. Trees of sour and sweet limetta were raised from seeds at Murcia and Cartagena (Spain).

We included in the morphometric analysis the following limetta varieties: Ojós' sour limetta, Marrakesh limonette, and Sweet limetta, we also included common lime and Persian lime, and other taxa that according to Curk et al. (2016) present common ancestors with limettas: Bergamot, lemon, mellarosa, Meyer lemon, Palestine sweet lime, *Pomum Adami* lemon, *Pursha Romana* lime, Rangpur lime, and Rough lemon (Table 1).

In order to determine how phenotypically different the 14 analyzed varieties are, we used descriptors for *Citrus* as defined by Hodgson (1967) and IPGRI (1999), reduced to 17 qualitative characters (with 2 to 8 mutually excluding states each) and 10 quantitative (stratified from 3 to 5 mutually excluding levels), which resulted in 97 character-states (Table 1). In this analysis, a specific variety may present more than one of the possible states for each character.

The qualitative characters studied are: shoot tip color, spine density, absence/presence of petiole wings, flower bud color, fruit shape, depressed fruits presence, shape of fruit base, apical alveolar groove, apical nipple, fruit surface texture, fruit skin (epicarp) color, fruit axis consistence, pulp(flesh) color, juice acidity, seed embryony, cotyledon color, and chalazal spot color; and the quantitative characters: spine length on adult tree, leaf lamina length, leaf lamina width, petiole length, number of stamens, fruit length, fruit diameter, epicarp + mesocarp thickness measured at equatorial area, number of segments per fruit, and diameter of fruit axis.

Measurements were taken on fresh material, with at least 15 shoots, leaves, flowers and fruits of each variety. Samples were collected from individuals grown in the "*Jardín de las Hespérides*" citrus collection of the Universidad de Murcia, experimental fields of the Universidad Miguel Hernández at the Escuela Politécnica Superior de Orihuela (Alicante, Spain), and the ANSE nursery at Cartagena (Murcia, Spain).

2.2. Analysis of ancestry and parentage

We used the data by Curk et al. (2016). They calculated the means and confidence interval for the relative contribution of the four basic taxa (*Citrus medica, C. reticulata, C. maxima* and *C. hystrix* (= *C. micrantha* cf. GRIN, 2021) in 90 different citrus varieties (from 10 permuted and aligned independent Structure (2020) run cluster outputs). We took as four variables the mean values of the ancestors' relative contribution for each of the 90 varieties. We defined and calculated two indexes of

Fig. 2. *Citrus limetta* Risso
Lectotype of *Citrus limetta* Risso with leaves flowers and one fruit published by Risso (1813a) as Fig 2 in Plate 2 of the paper, numbered Plate 4 within the whole journal. Image: Risso (1813a).

Table 1
Descriptive characters.

Taxa	Ojós' sour limetta	Marrakech limonette	Sweet limetta	Lemon	Pomum Adami	Rangpur Lime	Palestine sweet lime	Pursha Romana Lime	Rough lemon	Common lime	Persian lime	Meyer lemon	Bergamot	Mellarosa
Scientific Names	*Citrus* × *limon* var. *limetta* (Risso) Ollitrault, Curk & R.Krueger			*Citrus* × *limon* (L.) Osbeck var. *limon*		*Citrus* × *limonia* Osbeck	*Citrus* × *limettioides* Yu. Tanaka	*Citrus* × *limonia* Osbeck		*Citrus* × *aurantiifolia* (Christm.) Swingle	*Citrus* × *latifolia* (Yu. Tanaka) Yu. Tanaka	*Citrus* × *meyerii* Yu.Tanaka	*Citrus* × *bergamia* (Risso) Risso & Poit.	*Citrus* × *mellarosa* Risso
Shoot tip color	Purple	Purple	Green	Faintly purple-tinted	Purple	Purple	Green	Green	Faintly purple-tinted	Faintly purple-tinted	Faintly purple-tinted	Faintly purple-tinted	Green or Faintly purple-tinted	Green or Faintly purple-tinted
Spine density on adult tree	Medium	Medium	Medium	Low or Medium	Low or Medium	Low or Medium	Low	Absent or Low	Low	High	Absent or Low	Absent or Low	Low	Absent
Spine length on adult tree	15–40 mm	15–40 mm	16–40 mm	6–20 mm	16–40 mm	>40 mm	6–18 mm	6–15 mm	6–15 mm	6–25 mm	2 mm	2 mm	6–15 mm	0 mm
Leaf lamina length	10–12 cm	(6.5) 11–15.5 cm	9–13.5 cm	7–14 cm	9–13.5 cm	7–9 cm	8–13.5 cm	7–11 cm	9–11 cm	6–9 cm	8–9.5 cm	9–11 cm	8–15 cm	9–11 cm
Leaf lamina width	6–7 cm	5.5–9 cm	(4)6–7.5 cm	6–7.5 cm	(4)6–7 cm	3–3.5 cm	4.5–8 cm	2.5–5 cm	4.5–5 cm	3–4 cm	5–6 cm	5–6 cm	5–7 cm	3.8–5.5 cm
Absence/ presence of petiole wings	Absent	Absent	Absent	Absent or Present	Present	Absent	Absent or Present	Absent or Present	Absent	Present	Absent or Present	Absent	Absent or Present	Absent or Present
Petiole length	7–10 mm	5–10 mm	5–14 mm	10–14 mm	8–10 mm	8–10 mm	9–14 mm	8–11 mm	8–10 mm	6–10 mm	7–10 mm	10–17 mm	7–20 mm	3–8 mm
Flower bud color	Purple-stained	Purple-stained	White	Deeply purple-stained	Purple-stained	Purple-stained	White	White	Deeply purple-stained	Faintly purple-tinted	Faintly purple-tinted	Faintly purple-tinted	Purple-stained	White
Number of stamens	25–40	20–41	25–40	20–40	20–41	35–45	20–30	20–25	20–25	20–25	20–25	25–40	25–40	20–25
Fruit shape	Spheroid	Spheroid	Spheroid	Ovoid	Spheroid or Ellipsoid	Spheroid or Obovate	Spheroid or Elliptic	Spheroid	Spheroid or Elliptic	Spheroid or Elliptic	Ovoid or Obovate	Ovoid	Spheroid	Spheroid or Pear shaped
Fruit flattening	Depressed	Depressed	Depressed	Not depressed	Slightly depressed	Depressed	Not depressed	Depressed	Slightly depressed	Not depressed	Not depressed	Not depressed	Not depressed	Depressed
Fruit length	5–6.5 cm	4.5–6 cm	5–7 cm	6–12 cm	6–8 cm	6–7 cm	7–10 cm	5–7 cm	6–6.5 cm	5–6.5 cm	6–7 cm	7–9 cm	8–12 cm	4.5–5.5 cm
Fruit diameter	6.5–7.5 cm	4.7–7 cm	5.5–8 cm	5–8 cm	7–9 cm	5–6 cm	7–10 cm	5.5–8 cm	6.5–7.5 cm	3.5–4.5 cm	4–5 cm	5–6 cm	7–10.5 cm	5–7 cm
Shape of fruit base	Convex or Flattened	Convex or Flattened	Convex or Flattened	Long-necked or Convex	Collared	Collared or Low necked	Convex	Flattened or Collared and Long necked	Collared and Long necked	Convex or Low necked	Convex, or Low necked and Collared	Convex	Convex, or Low necked	Flattened
Apex furrow	Deep areolar furrow	Deep areolar furrow	Deep areolar furrow	Areolar furrow deep or shallow	Areolar furrow deep or shallow	Deep areolar furrow	Areolar furrow shallow or Absent	Areolar furrow shallow or Absent	Deep areolar furrow	Areolar furrow Absent	Areolar furrow Absent	Areolar furrow Absent	Areolar furrow Absent	Areolar furrow Absent
Apex nipple	Prominent	Prominent	Prominent	Prominent	Broad	Low	Low	Broad or Absent	Broad	Low	Low	Low	Prominent or Low	Low
Rind surface	Pitted	Pitted	Pitted or Rough	Papillate	Rough	Pitted or Smooth	Smooth	Rough	Rough	Smooth	Smooth	Pitted	Pitted or Papillate	Rough
Thickness of epicarp + mesocarp at equatorial area	2.5–3 mm	2.5–3 mm	2.5–4.5 mm	3.5–10 mm	5–15 mm	2.5–3 mm	1–4.5 mm	2–4.5 mm	3–4.5 mm	1–2.5 mm	2.5–3 mm	3–4 mm	4–8 mm	4–6 mm
Fruit skin (epicarp) color	Light orange	Light orange	Light orange	Green yellow	Yellow	Light orange, Orange or Red-orange	Green yellow or Light orange	Green yellow or Light orange	Green yellow, Light orange, Orange or Red-orange	Green yellow	Green yellow	Orange	Light orange	Light orange
Number of segments per fruit	9–12	9–13	9–11	8–10	10–12	8–10	10	10	10	10–12	10	10	12–15	10–22

(*continued on next page*)

Table 1 (*continued*)

Taxa	Ojós' sour limetta	Marrakech limonette	Sweet limetta	Lemon	Pomum Adami	Rangpur Lime	Palestine sweet lime	Pursha Romana Lime	Rough lemon	Common lime	Persian lime	Meyer lemon	Bergamot	Mellarosa
Scientific Names	*Citrus* × *limon* var. *limetta* (Risso) Ollitrault, Curk & R.Krueger			*Citrus* × *limon* (L.) Osbeck var. *limon*		*Citrus* × *limonia* Osbeck	*Citrus* × *limettioides* Yu. Tanaka	*Citrus* × *limonia* Osbeck		*Citrus* × *aurantiifolia* (Christm.) Swingle	*Citrus* × *latifolia* (Yu. Tanaka) Yu. Tanaka	*Citrus* × *meyerii* Yu.Tanaka	*Citrus* × *bergamia* (Risso) Risso & Poit.	*Citrus* × *mellarosa* Risso
Diameter of fruit axis	6–13 mm	6–13 mm	6–18 mm	2–5 mm	6–13 mm	>14 mm	4.5–13 mm	3–6 mm	>14 mm	<2 mm	2–5 mm	3–4 mm	8–14 mm	0–2 mm
Fruit axis	Hollow	Hollow	Hollow	Solid	Hollow	Hollow	Semi-hollow	Hollow	Hollow	Solid	Solid	Solid	Hollow	Solid
Pulp (flesh) color	Paleyellow	Pale yellow	Pale yellow	Yellow	Green	Orange	Pale yellow	Pale yellow	Pale yellow, Yellow, Orange	Green	Green	Orange	Pale yellow	Yellow
Juice acidity	Strongly acid	Strongly acid	Sweetnot acid	Strongly acid	Strongly acid	Strongly acid	Sweet not acid	Moderately acid	Moderately acid	Strongly acid	Strongly acid	Strongly acid	Moderately acid	Moderately acid
Seeds polyembryony	Mixture of mono and polyembrionic	Mixture of both	Mixture of both	Mixture of both	Mixture of both	Polyembryonic	Polyembryonic	Polyembryonic	Polyembryonic	Polyembryonic	Monoembryonic	Monoembryonic	Monoembryonic	Monoembryonic
Cotyledon color	White	White	White-greenish	White	White	Faintly green	White-greenish	White	Faintly green	White	White	Faintly green	White or Faintly green	White or Faintly green
Chalazal spot color	Purple or cream	Purple	Cream	Purple	Purple	Purple	Light brown (beige)	Cream	Purple	Cream	Cream	Purple	Cream	Cream
Hypothetical ancestry	(*Citrus medica* × *C.* × *aurantium*) × (*Citrus medica* × *C.* × *deliciosa*)				*C.* × *lumia* × *C.* × *limon*	*C. medica* × *C. reticulata*	*Citrus medica* × *C.* × *aurantium*	*C. medica* × *C. reticulata*	*C. medica* × *C. reticulata*	*Citrus medica* × *C. hystrix*	*C.* × *aurantiifolia* × *C.* × *limon*	*Citrus medica* × *C.* × *aurantium*	*Citrus* × *aurantium* L. × *Citrus* × *limon*	*Citrus* × *aurantium* L. × *C.* × *limon*

relative ancestry for each variety: MaRe [1] and MaMe [2]

$$MaRe_i = \ \% \ Citrus \ maxima_i \ / \% \ Citrus \ reticulata_i \quad (1)$$

$$MaMe_i = \ \% \ Citrus \ maxima_i \ / \% \ Citrus \ medica_i \quad (2)$$

Their values are summarized in Supplementary Table 1 and Supplementary Fig. 1, and accordingly the varieties are ascribed to the different citrus taxa.

2.3. Data analysis

2.3.1. Morphometrics

We calculated the pairwise differences between varieties in form of a dissimilarity matrix. Thus the crude matrix consisted in 14 varieties and 97 character-states.

The crude matrix of presence/absence of character-states was used to compute a dissimilarity matrix using Darwin 6 V.6.0.9 (2015–04–15) (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006). The Sokal-Sneath dissimilarity index (un2) was calculated by the following equation [3]:

$$d_{ij} = 2(b+c)/(a+2(b+c)) \quad (3)$$

Where d_{ij} is the dissimilarity between taxa i and j, a: number of variables where x_i = presence and x_j = presence, b: number of variables where x_i = presence and x_j = absence and c: number of variables where x_i = absence and x_j = absence. Dissimilarities are even and are Euclidean distances. The dissimilarity is =0 for two taxa sharing the 97 character-states and =1 for two taxa which present 0 character-state shared. This index concerns 'presence/absence' data where only 'presence' modality is informative, modality 'absence' expressing mainly an absence of information. These two modalities are not symmetrical and their exchange leads to a completely different dissimilarity value. This index considers that a common absence for two units is uninformative to measure their dissimilarity (Perrier and Jacquemoud-Collet, 2006). Therefore, similarity here reflects the number of coinciding character-states and dissimilarity is inversely proportional to this.

2.3.2. Ancestry

With the purpose of determining main citrus taxa, especially those related with *Citrus* × *limon* var. *limetta*, based on common ancestry we used the average values, calculated by Curk et al. (2016) for 90 citrus varieties, of the contribution of the four ancestral taxa in percentage to calculate a dissimilarity matrix. In this matrix the individuals are 90 varieties and the 4 variables are the contribution of each ancestral taxon. Therefore the matrix is presented in terms of frequencies (counts). The chi square dissimilarity index was calculated (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006). This measure expresses a value x_{ik} as its contribution to the sum x_i on all variables and is a comparison of unit profiles [4].

$$d_{ij}^2 = \sum_{k=1}^{K} \left(\frac{x_{ik}}{x_{i.}} - \frac{x_{jk}}{x_{j.}}\right)^2 \left(\frac{x_{..}}{x_{.k}}\right) \quad (4)$$

for $j \neq i$.

Where d_{ij}: dissimilarity between units i and j; $i,j = 1, 2, \ldots\ldots, N$ (varieties, rows), $N = 90$; $k = 1, 2, \ldots\ldots, K$ (variables, columns).

Where $d_{ij} = 1$ means varieties i, j differ in all variables, and $d_{ij} = 0$ means varieties i, j are identical.

These pairwise dissimilarities can be represented in a multidimensional space, but, in order to obtain meaningful graphic representation of these relationships in a two-dimensional plane, we used cluster analysis.

Cluster analysis is a term used to name a set of numerical techniques in which the main purpose is to divide the objects of study into discrete groups. These groups are based on the characteristics of the objects (Kovach, 2007). We used the minimum variance clustering (Ward's

method) that focuses on determining how much variation is within each cluster. In this way, the clusters will tend to be as distinct as possible, since the criterion for clustering is to have the least amount of variation (Kovach, 2007). Ward's method produces a single tree, however, in order to further reducing the uncertainty in the structure of the tree, we used a bootstrapped matrix (200 bootstraps) and a tree construction method (weighted neighbor joining) (Saitou and Nei, 1987) that uses the trees inferred from these bootstrapped dissimilarities to assess the uncertainty of the tree structure.

For the graphic representation, we opted for the software Figtree version 1.4.4. (Rambaut, 2018).

2.4. Historical data and images

Flemish, Spanish and Italian paintings with images of fruits and other foods were studied, paying special attention to the still life subjects, which could represent limettas, which were identified based on the morphological aspects with greater diagnostic value, such as type, shape, size and color of fruits and also flowers, if present. Acidless and acidic fruits are morphologically indistinguishable (Butelli et al., 2019). In the paintings that represent still life with fruits morphologically ascribed to limettas, we identify sweet limetta when the contexts in the image suggest their use as dessert, and acid limetta when they appear together with fish, meat or game, assuming that they are used to flavor this type of food due to their acidity. Online databases consulted include Le Gallerie degli Uffizi (Uffizzi, 2020c), Museo del Prado (Prado, 2020d), Louvre (2020); Rijksmuseum (2020), Tate Gallery (Tate, 2020), The National Gallery (National, 2020).

A bibliographic review was carried out focusing on literature on visual arts, gastronomy and horticulture to study the history and uses of limetta.

3. Results and discussion

3.1. History of sour limetta cultivation

Citrus × *limon* var. *limetta* seems to have been known in the western Mediterranean early in the first millennium AD. The mosaic pavement of the Roman Villa called "*La Voliere*" at Carthage (2nd- 3rd cent AD) (Musee du Bardo, Tunis) "shows branches from citron and lemon trees, the latter with fruit of the almost spherical variety depicted some 1500 years later by the Spanish painter Luis de Melendez" (Tolkowsky, 1938, Plate XXIII; Kammoun, 2020).

Ferrari (1646) described the "*Lima acris*" and compared it with "*Lima dulcis*" (Fig. 3a and b): "Acid [limetta] being distinct from the sweet limetta, in flesh acidity, color lighter, as well as seed slender, and somewhat longer".

Ferrari (1646) mentions in page 317 a "*Lumia Divi Dominici sive salis*" (Fig. 4a) and a "*Lumia salis sive Spatafora notha*" (Fig. 4b), which preserved in salt were used as a condiment, in a similar way as is still done with sour limetta. However their rind (peel) is clearly thicker than that of limetta and their dimensions are larger with respect to the leaves (see Fig. 3a), what prevent us of considering these as true limetta fruits.

Volkamer (1713) in the "*Hesperidium Norimbergesium",* a Latin translation of its original work "*Nurnbergische Hesperides"* (Volkamer, 1708), Chapter 34 of sweet lime, mentions an acid lime type: "We have, another more common lime, the same [but] acid, the use of which is to say, that by macerated in salt, and profusely diffused by the Italians, is a condiment of various meats; and excites the taste of them, is to encourage those who demand a variety of a lot of different tastes".

Later, Volkamer (1714) wrote in Chapter 41: "As far as the Lima di Spagna acida is concerned, I remain connected to the world-famous Padua because of its transmission. Here it is known under the name of "Limon Popino", but it does in some parts agree with the above fruit; and it would not have been wrong to call it the "Spanish sour limon", especially since it is canned and consumed in the same way as above". This Lima di Spagna acida appears illustrated in Fig. 5, see also Volkamer (2020: 294 and 295).

In Italy the sour limetta was known as "*Lima di Spagna acre*" (Clarici, 1726), and "*Limoncello di Spagna*", and in France as "*Citronier Limettier d'Espagne*" (Loiseleur and Michel, 1819; Tamaro 1987). These varieties of sour limes display characteristics identical to those persisting in Murcia and Marrakesh. However, the exhaustive collection of citrus images at MUSA (2011) does not contains any image of this "*Limoncello di Spagna*". Risso and Poiteau (1818) mention the "*Limettier d'Espagne*" which is there described and receives the name *Citrus limetta* var. *hispanica* Risso and Poit., with leaves and fruits similar to those of our "Sour limetta", but having only a slightly sour juice. Butelli et al. (2019) analyzed two accessions that presented intermediate levels of acidity in their fruits, between those of the sour and sweet limetta.

There is no evidence for the early cultivation of this sour limetta in the Americas. Father José de Acosta, late 16th cent AD, mentions in his *Historia Natural y Moral de las Indias* the citrus trees introduction and their sudden proliferation in South America, and listed among these "*limas, cidra y fruta de este linage*" (Hilgert et al., 2014). Gil (1895) mentions that citrus species were cultivated in the islands of the Paraná River and proposed the introduction of other economically interesting citrus cultivars. The list of proposed introduction included "*El limero de España. - Citrus limetta Hispánica (Risso)*" with sour juice and perfumed thin rind. Therefore it is conceivable that someone followed these recommendations.

This lime was frequently cultivated between 1920 and 1960 in the "*Riads à l'Andalouse*" i.e. gardens of Spanish or Spanish-Moorish style, in Morocco (Guillaumin, 1921; Chapot, 1962; Hodgson, 1967).

Valorisation des Produits de Terroir Marocain (2015) mentions the names of this plant in Morocco and the area of cultivation. Local names are: "*Limonette de Marrakesh"* (French), "*Allaymoune"* (Classic Arabic), and "*L'hamed el Beldi"* (Dialectal Moroccan Arabic). The limonette orchards of Marrakesh are generally located in Ouled Hassoun and Alouidane. Limonette, seems to have been introduced more than a century ago and has been conserved and multiplied by farmers in the area in about 150 ha, in plots of less than one ha. The plants come from cuttings from old plantations. Annual production is estimated at 3000 tons, much of which is destined for canning.

3.2. Morphology of sour limetta fruits and trees

This tree, which comprises the "Ojós' sour limetta" and the "Marrakesh limonette" (Table 1), is lightly thorny and fairly robust, vigorous, fast growing, branches erect or spreading (Fig. 6a), forming an open cup. Twigs intensely purple stained.

Leaves ovate, 6.5–15 × 5–9 cm, slightly longer at the edges and more or less toothed (Fig. 6a), less sharp-pointed than those of lemon and somewhat bent-cupped toward the apex, emerald green above, and yellow-green below, glands containing an essential oil with a characteristic aroma. Petiole strong and thick, not winged, 0.7–1 cm. At the base can be a sharp thorn variable in size slender and often deciduous (Fig. 7b) although some branches are unarmed (Fig. 7a). The tree suffers a considerable loss of leaves in late winter, and re-leafs in spring.

Flowers intensely purple stained in the bud stage, arranged in few-flowered axillary clusters with up to 3 flowers (Fig. 6b). They are purplish-white at blooming, with 5 petals (rarely 4 or 6), 10–15 × 7–9 mm, almost straight and slightly acute at their apices, 20–41 stamens. It blooms all year round, depending on water availability, but is especially floriferous in spring. Presence of anthocyanins in flowers and proanthocyanidins in seed chalaza is associated to higher acidity in fruits via a single gene, *Noemi*, encoding a basic helix-loop-helix (bHLH) transcription factor (Butelli et al., 2019).

Fruits medium-sized, 4.5–6.5 cm long and 4.7–7.5 cm in diameter, similar to lemons, but much shorter, round and flattened like tangerine, with a broad and deep alveolar groove around the apex, surrounding a prominent nipple (Fig. 1b). Rind thin or moderately thick, 2–4 mm, light

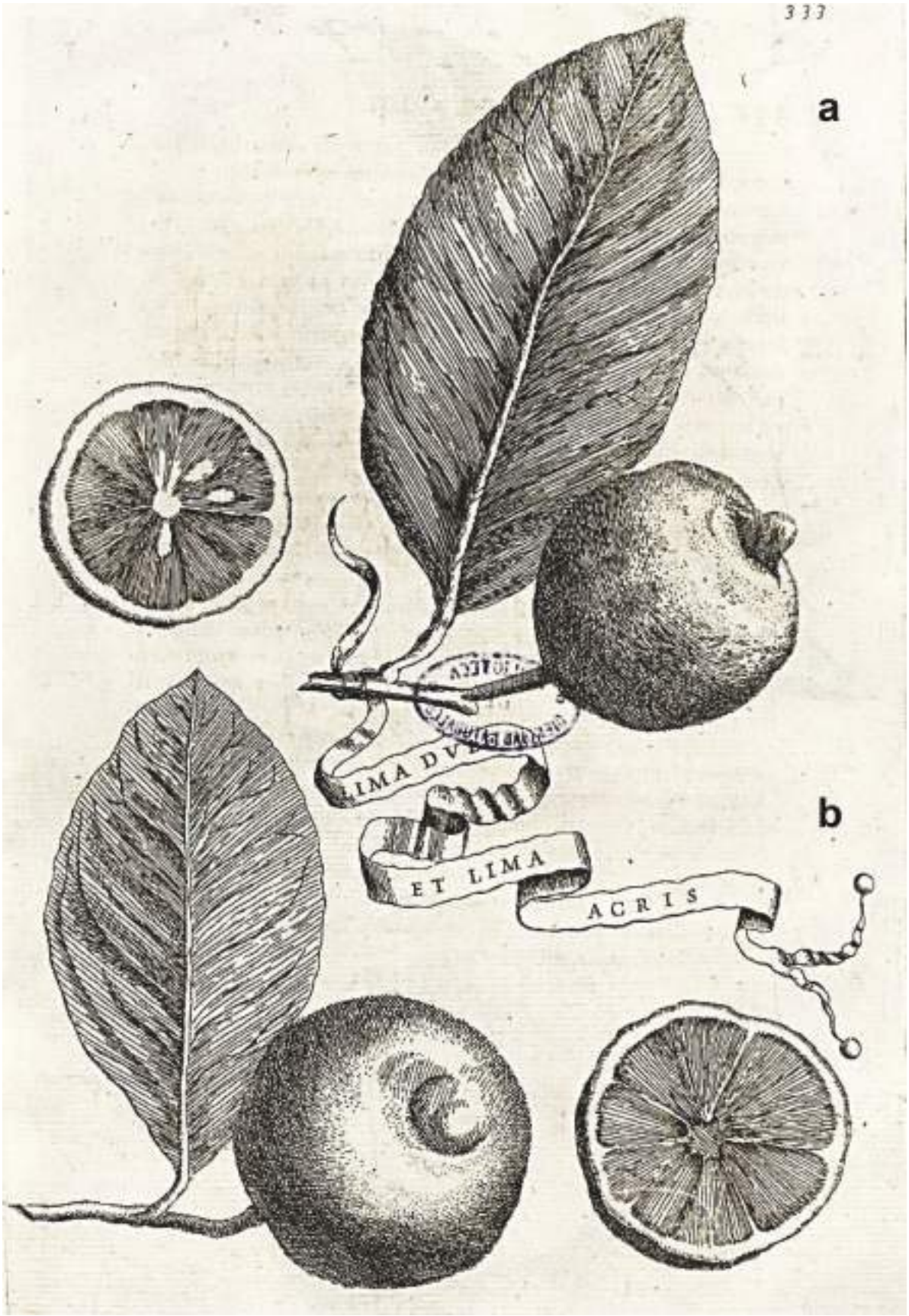

Fig. 3. The "*Lima*" by Ferrari
a, leaf, fruit and fruit section of the *Lima dulcis*; b, leaf, fruit and fruit section of the *Lima acris*. Image: Ferrari (1646).

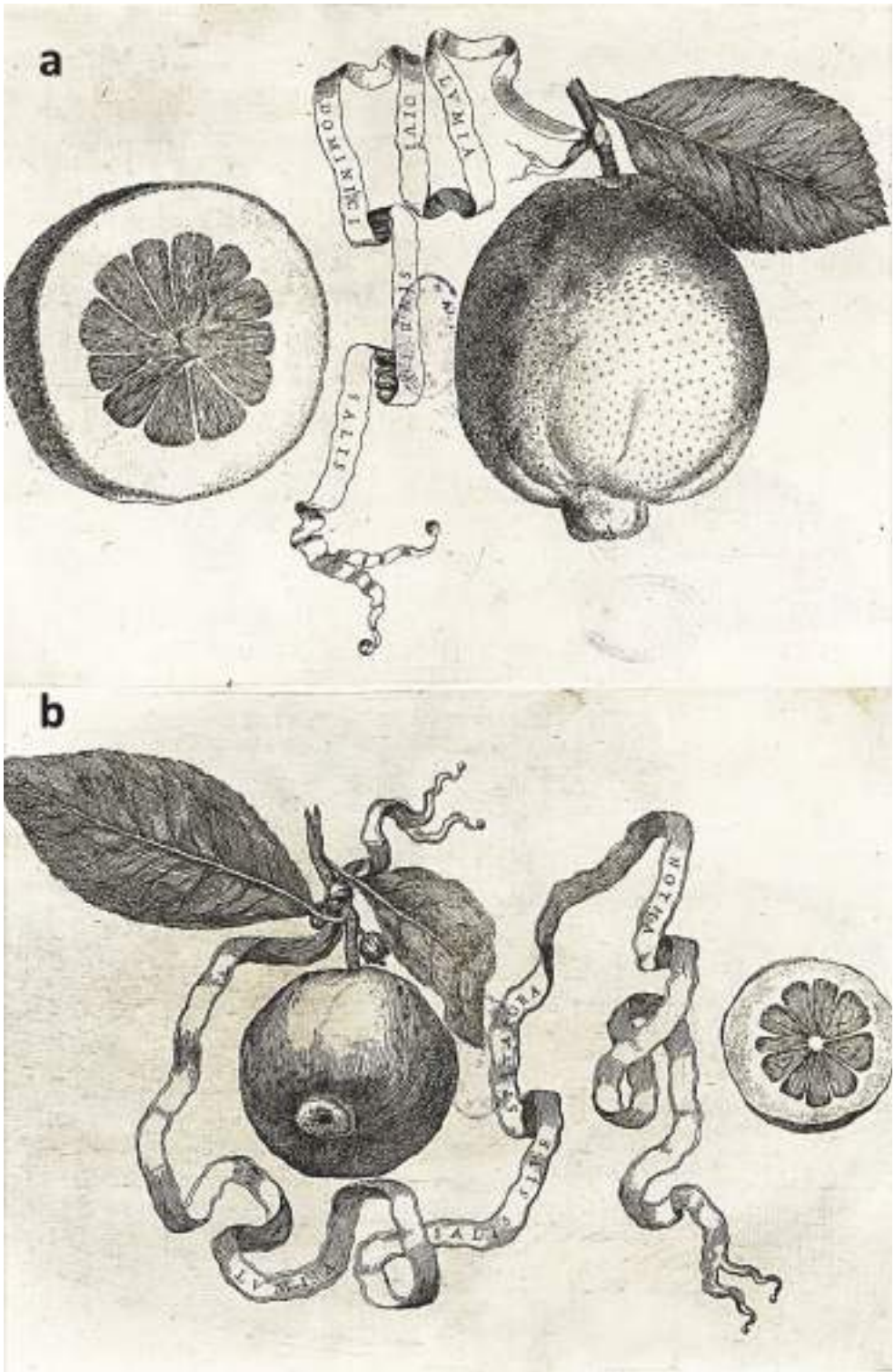

Fig. 4. Similar but unrelated "*Lumia*" fruits by Ferrari
a, leaf, fruit and fruit section of the "*Lumia Divi Dominici sive salis*"; b, leaves, fruit and fruit section of the "*Lumia salis sive spataphora notha*". Images: Ferrari (1646).

yellowish-orange, moderately pitted with sunken oil glands, and somewhat bumpy, has an aroma reminiscent of bergamot. Flesh color pale yellow, distributed in 9–13 segments, juicy and strongly acidic, erroneously, reported as sweet by Curk (2014), with a fine taste and typical aroma that gives off a strong lemon scent. Axis hollow at maturity. Seeds relatively numerous (10–12), 11 mm long, moderately polyembryonic (1–2 embryos each seed). Chalazal spot purple, cotyledons white or somewhat green. The tree produces abundant fruit (Table 1) (cf. Koskinen, 2019).

3.3. Origin and relationships (phenetic and genetic)

Until recently the origin of limetta was unknown but it was postulated that it comes from a cross between the lemon and some form of mandarin and links the Marrakesh limetta to the "Rangpur lime" (*Citrus* × *limonia* Osbeck) (Gmitter and Hu 1990).

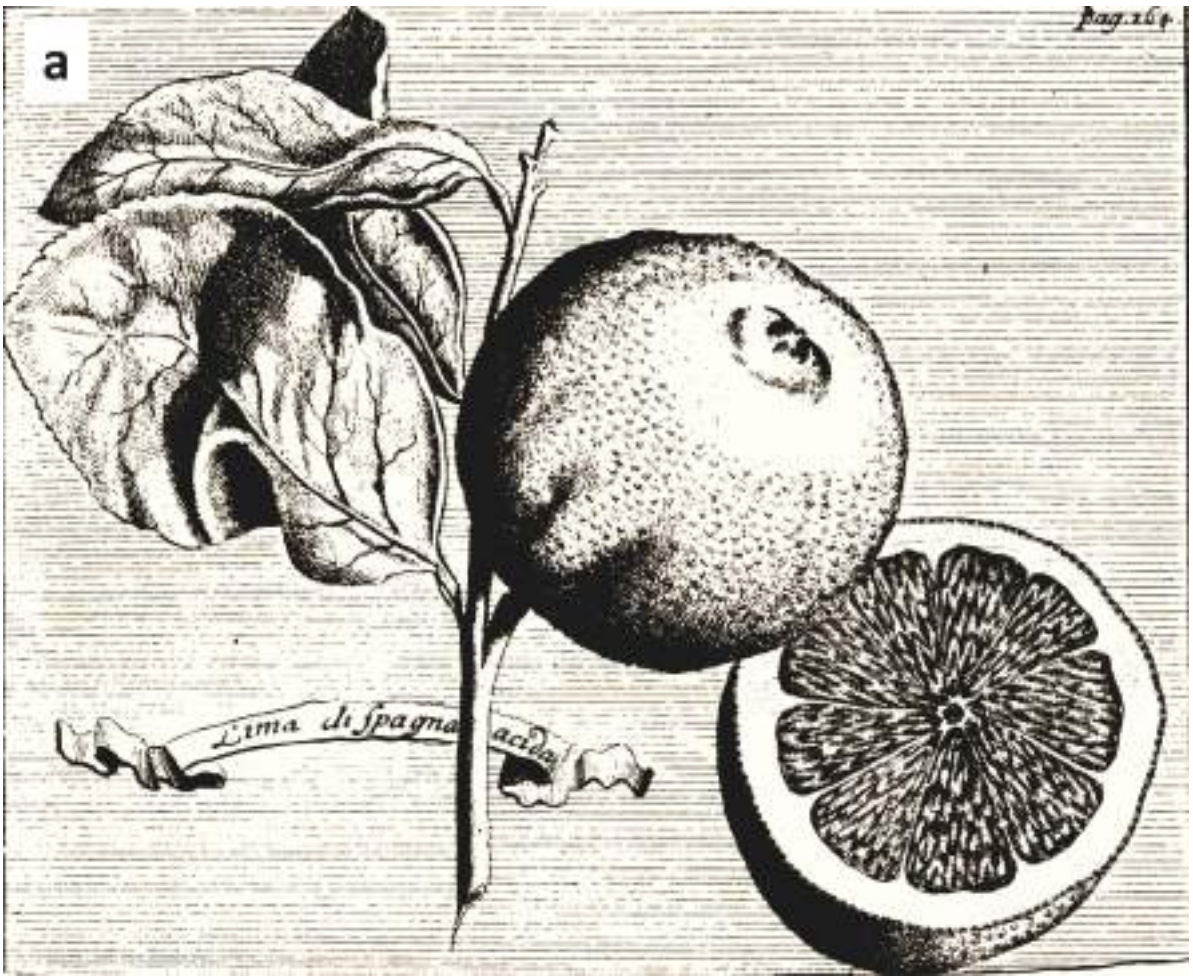

Fig. 5. The "Lima" by Volkamer
a, leaves, fruit and fruit section *"Lima di Spagna acida"*. Image: Volkamer (1714).

The morphometric study based on the selected standard descriptors of *Citrus* (vegetative and reproductive characters) (Table 1), allows to clearly distinguish sweet and acidic limettas from lemons and limes particularly by their flattened fruits, wider than long, and from common and Persian limes, Meyer lemons and Palestinian limes, due to the presence of a deep areolar groove and a prominent apical nipple. The presence / absence of purple tint in shoots, flower buds and chalaza, in parallel with the acidity of the fruits, is governed by the Noemi gene (Butelli et al., 2019). The purple-tinged forms with acidic juices and the undyed, acid-free forms are found in limettas and lemons, suggesting their close relationship. The Weighted Neighbor Joining Tree clusters limettas closer to lemons and especially to "*Pomum Adami*" than to the rest of taxa (Fig. 8a). This supports the taxonomic scheme proposed by Ollitrault et al. (2020) where limettas are a variety of *Citrus* × *limon*.

Curk et al. (2016) analyzed in 133 citrus accessions (ascribed to 90 varieties) three mitochondrial indels and five chloroplastic simple sequence repeats (SSRs) and included the sour limetta within cytoplasmic type 5 along sour oranges and lemons. Similar results were presented by Curk et al. (2015). Ours analysis of ancestry based on the data of Curk et al. (2016) refined their former classification into a cluster with lemons, sweet and sour limetta (limonette), Palestine sweet lime, Rangpur lime and other varieties (Fig. 8b). The estimated citrus basic taxa contributors to the genotype of the Marrakesh (acid) limetta are citron (50%), mandarin (35%), and pummelo (13%). Similar proportions were found for the sweet limetta (Curk, 2014; Curk et al., 2016). Therefore, they are probably direct hybrids between *C. medica* and varieties with admixture structure between *C. maxima* and *C. reticulata*.

We summarized based on Curk et al. (2016) (Supplementary Table 1) the ancestry of hybrid citrus taxa. This shows that *Citrus* × *limon* var. *limetta, C.* × *limon* var. *limon, C.* × *limettioides*, and *C.* × *meyeri*, present *C. medica* as their main ancestor (c. 50%) and with the relative *C. maxima* and *C. reticulata* contributions variable, summing c. 50%. The ratio% *C. maxima* /% *C. reticulata* (MaRe index) (Table 2 and Supplementary Table 1), can explain in part the phenotypic differences between limettas (MaRe values 0.41±0.02), and lemons (MaRe values 0.65±0.35) where pummelo contribution is higher and mandarin lower. *Citrus* × *limettioides* present intermediate values (MaRe = 0.47), which is consistent with the position reflected in the ancestry-based classification (Fig. 8b). Finally, *C.* × *meyeri* varieties present a higher proportion of *C. maxima* (MaRe = 0.98±0.34) associated to a relatively low proportion of *C. medica* (c. 43%). Lower MaRe index values are associated with flattened fruits.

Curk (2014) tested the hypothesis of being the Marrakesh (acid) limetta: (*C. maxima* × *C. reticulata*) × *C. medica*, considering the bitter orange (*C. aurantium*) as a direct interspecific hybrid *C. maxima* × *C. reticulata*. This combination is consistent for 98.3% of the 123 nuclear markers studied. It is supposed that the first individuals resulting from this hybridization originated in the Mediterranean and produced sour fruits, later appearing the sweet mutants via mutations in a single gene, *Noemi*, encoding a transcription factor (Curk, 2014; Butelli et al., 2019). This would be consistent with the fact that true limettas, whether sour or sweet are only known from the Mediterranean (Hodgson, 1967). In order to clearly determine whether their center of origin is in the

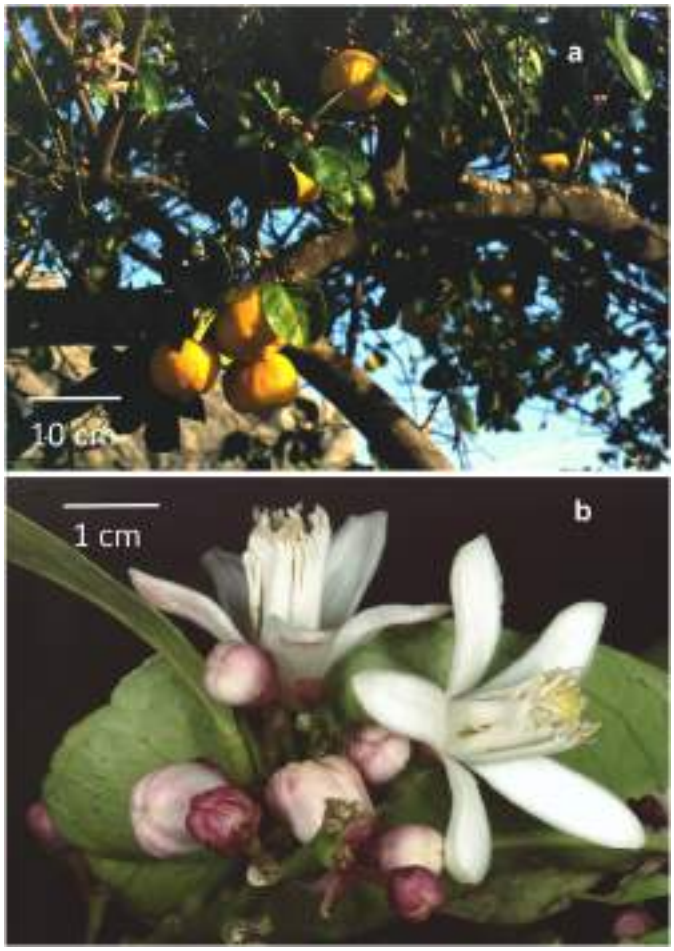

Fig. 6. Flowers and fruits of sour limetta
a, Sour limetta tree with fruits and flowers, b, sour limetta flowers. From Ojós (Murcia, Spain). Photo: a & b. D. Rivera.

Mediterranean or somewhere in Asia, it is important to previously assess the taxonomic status of Indian citrus varieties, such as *mosambi*, which some authors, although unlikely, consider limettas. It is worth to mention here that according to Hodgson (1967) *mosambi* name suggests that this variety was introduced in India by the Portuguese directly from Mozambique in East Africa.

Based on the relative proportion of the four ancestor species and the MaRe and MaMe indexes values, we evaluated three alternative hypotheses (Table 2) for explaining the hybrid origin of lemons and limettas. Hypothetically hybrids would present intermediate values between those of their parents (Table 2).

Hypothesis A of a hybrid *C. medica* × *C.* × *aurantium* presents higher proportion of *C. maxima* and lower of *C. reticulata* than in lemons and limettas. On the contrary, the hypothesis B of a hybrid *C. medica* × *C.* × *deliciosa* implies an extremely low proportion of *C. maxima* ancestry and a higher contribution of *C. reticulata*, which is inconsistent with the values calculated for lemons and limettas. It should be noted here that under *C.* × *deliciosa* we include Type 3 mandarins of Wu et al. (2018) that are determined by their relatively high pummelo admixture pattern

Nevertheless the hypothesis C that assumes the origin of limettas via multiple successive hybridization events approximates better the relative proportions of the four ancestor species and indexes values of lemons and limettas (Table 2). Thus, seemingly the best explanation for the proportions detected of the four ancestral species in limes and lemons (Table 2) is to accept that they originate over at less three hybridization events. In the first place, the hybrids *C. medica* × *C.* × *aurantium* and *C. medica* × *C.* × *deliciosa* would originate, neither necessarily simultaneously nor in the same place or region. Then in a subsequent event, or events, the crossing of these hybrids would have given rise to *Citrus* × *limon* and its variability including limettas.

Regarding the identity of these intermediate hybrids, *C. medica* × *C.* × *aurantium* could be close to one of the varieties with monoembryonic seeds of *C.* × *meyeri* such as "Big fruit lime (Supp. Table 1). On the contrary, we have not found so far a variety that presents values similar to those of the intermediate hybrid *C. medica* × *C.* × *deliciosa*, which is our missing link, although the closest one is the Rangpur lime (*C.* × *limonia*) but presenting an excessively low proportion of the ancestor *C. maxima*. Hybrids of Type 3 mandarin varieties in the sense of Wu et al. (2018) with a high pummelo proportion should be further investigated. With the available evidence above discussed we should focus on Asia for placing the origin of the primary hybrids involved in the origin of lemons and limettas and cannot discard that finally lemons and limettas originate somewhere between Iran and the Eastern Himalayas.

Sweet limetta plants are relatively frequent in Southern Spain (*lima dulce*) (Rivera et al., 1997), Naples and Calabria (Italy) (Neapolitan *limmo* and *lemoncetta Locrese* ethnovarieties) (Cautela et al., 2020) and Central America *(lima de Chichi*) (Mexico). The "*Limon Balotin*" (Risso and Poiteau, 1872) with fruits 7–8 × 8–12 cm is somewhat larger and was grown at the orangery in Versailles. Both "*Lumia*" and "*Limon balotin*" present rinds thicker than those of the sour limetta. The name bergamot (or *bergamotto*) is applied in Sicily and Tunisia, to a lime with sweet fruits and white flowers (which is the sweet limetta or *Arancio di Spagna*). The confusion is most likely due to the fact that the zest essence of this lime has a very marked bergamot odor. It is for the same reason that, in Morocco, also calls bergamot the limonette of Marrakesh or *Limoun Beldi* (Chapot, 1962).

A sweetish to slightly acidic almost globose limetta is cultivated under the name "*Mitha*" in the plains of Punjab, Peshawar Valley and Sind (Pakistan), where is used as a coolant for malarial fever and jaundice (Ud-Din and Ghazanfar, 2014). However, it seems that "*Mitha Nimboo*" is rather the Palestinian sweet lime (*C.* × *limettioides*) and is not directly related to Marrakesh limetta. Dianxiang and Mabberley (2018) did not report such a species.

Sour limes (under the names of "*lima ácida*", and "*lima*") persist in north Argentina but these are not yet fully identified (Hilgert et al., 2014; Stampella 2018). Krueger and cols. found that none of the Baja California Sur accessions resemble our "Sour limetta" (De Grenade et al. 2014). The closest is a sweet lime (Fig. 1a). However, it is sweet, rounder, and has light orange flesh. That was the only lemon- or lime-like fruit collected in Baja California Sur (Mexico).

Indian *mosambi* (also *mousambi* or *musambi*) citrus is reported by several authors as a variety of limetta (Deng et al., 2020). However their entirely globose fruits, without neither nipple nor alveolar groove, neatly distinguish *mosambi* from sour and sweet limettas. Unfortunately we had not accessions available from this citrus for our morphometric analyses. Given the numerous existing studies on the properties and uses of Indian *mosambi* fruits in medicine (Khan et al., 2016), bioremediation (Mondal et al., 2019) and food industry (Younis et al., 2016), it is important that this citrus be taken into consideration in further studies and that its morphometric characteristics and phylogenetic relationships are clarified.

3.4. Horticultural interest of sour limetta

3.4.1. Gardening

It is used as an ornamental small tree in traditional gardens of the "*Andalusian*" style (interior gardens or courtyards associated with house and palace architecture known as "*riads*" or "*riyads*") in Marrakesh and other cities of Morocco (Chapot, 1962): "Marrakesh limonette trees feature in virtually all the Arab gardens (Andalusian *riads*) and contribute to their coloring. The extraordinary profusion of fruit that this tree bears is one of the main factors in its use in ornamentation, where this variety is almost unmatched".

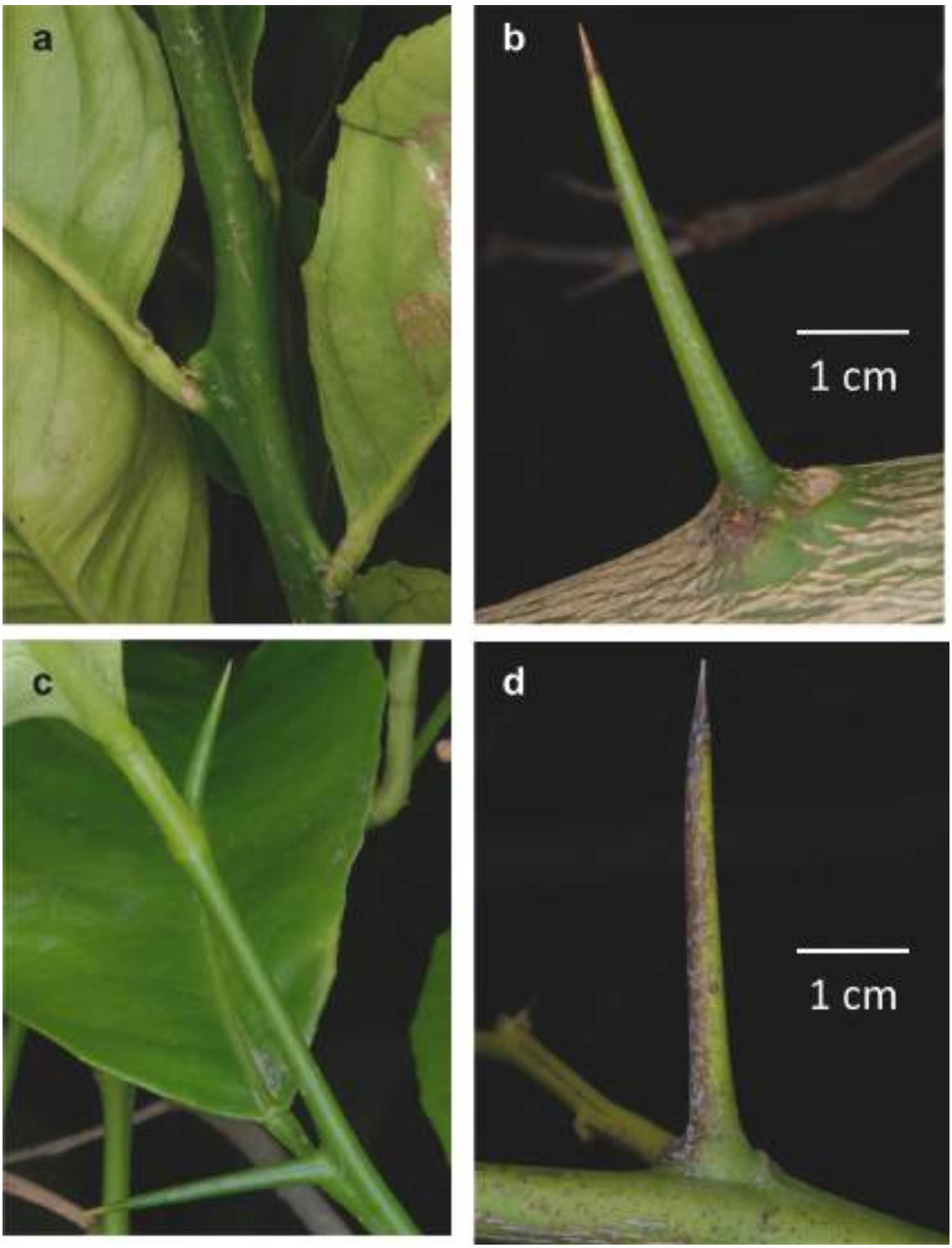

Fig. 7. Spines of sour and sweet limetta
a, unarmed shoot and b, spine, sour limetta cultivated in Molina de Segura (Murcia, Spain), raised from seeds; c, spiny twig and d spine, sweet limetta from Molina de Segura (Murcia, Spain), also raised from seeds. Photos: D. Rivera.

Sour limetta was present in the historical citrus collections of the Medici in Boboli, Castello, Petraia and other Medicean gardens and villas around Florence (Italy). The Medici collection of citrus fruits in the Boboli Gardens was created at the behest of Cosimo I de Medici in the second half of the 1500s (Uffizi, 2020b).

Their presence is attested in the citrus gardens of San Ginés de la Jara monastery (Cartagena, Spain) in the late 16th century by Francisco Cascales (1565–1642). Cascales (1775): "This House has an orchard, which is one of the most famous in Spain. At the beginning there are two copious fountains … Here there are streets of orange trees, lemons, limettas, pummelo, *ponciles* with their very fragrant flowers, without them very beautiful, always green, always pleasant …"

3.4.2. Agriculture

The sour limetta, has been used throughout the Spanish Levant, as one of the oldest rootstocks for sweet orange cultivation and for some

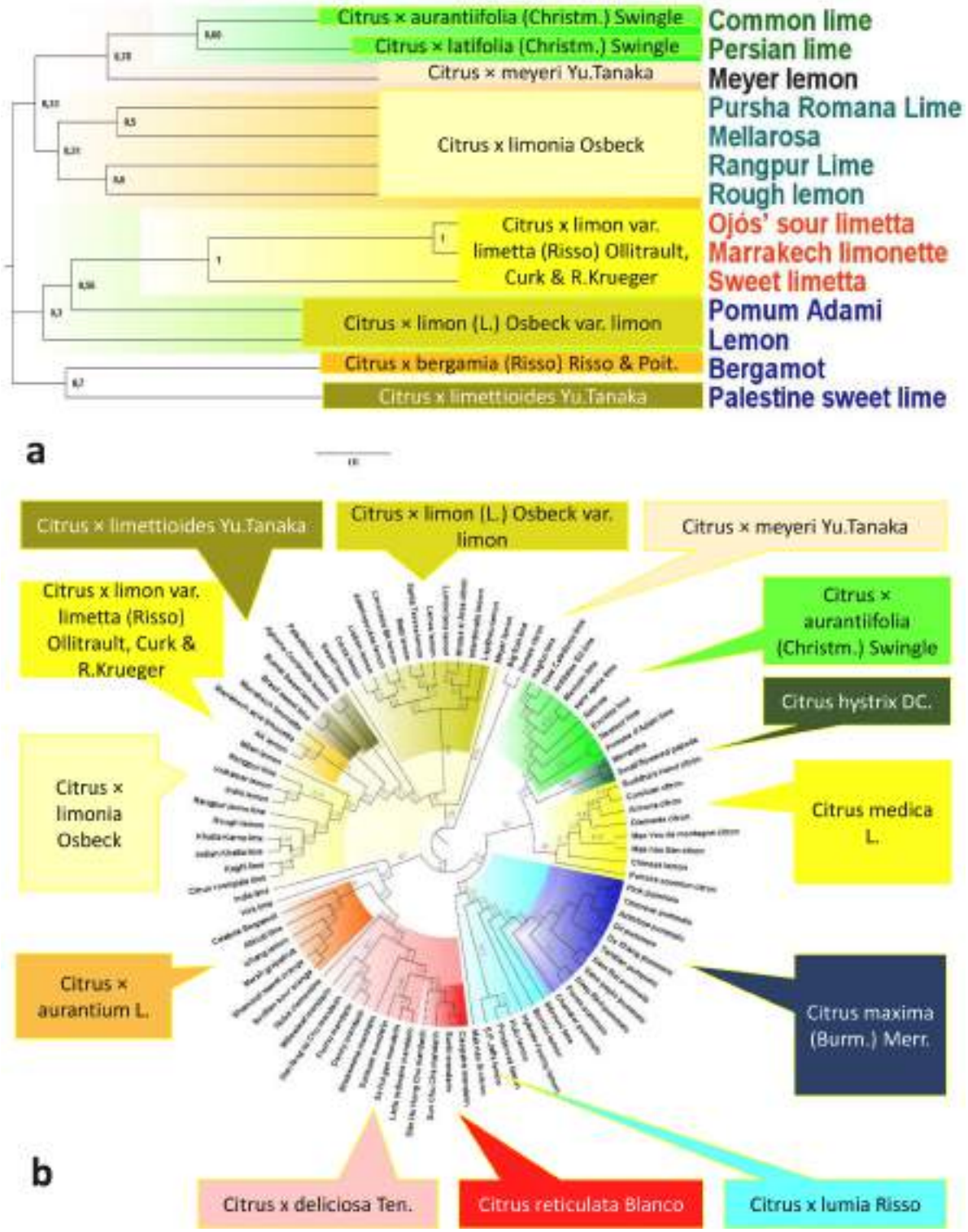

Fig. 8. Sour and sweet limetta within genus *Citrus*
a, phenetic classification; b, classification based on the analysis of common ancestry.

varieties of lemon. The seedlings have traditionally been used as rootstocks for other citrus species. It has good affinity with lemon and sweet orange, especially with blood orange. Its cultivation was relatively common in the Ricote Valley (Murcia, Spain) by 1880 (Escribano, 1884), where it was widely cultivated primarily as a rootstock for grafting orange, lemon, grapefruit, and sweet limetta, but it has almost disappeared today. Chapot (1962) described his resistance against gum disease caused by *Phytophthora*. As a rootstock it provides precocity, and sweeter flesh to plants grafted on to it (Ortuño et al., 1977; Rivera et al., 1997). In Morocco it is propagated by seeds or cuttings and the tree is highly fruit producer (Chapot, 1962).

The Marrakesh limetta gives a very good yield in an essence much sweeter than that of bergamot, and very easy to extract due to the shape of the fruit (Chapot 1962).

Table 2
Main *Citrus* taxa and their ancestry
Values in percentage: average and 95% confidence interval. References: Supplementary Data Table 1, processed from Curk et al., (2016, in their Additional Table 5). Abbreviations: MaRe index values =% of *C. maxima* /% of *C. reticulata*); MaMe index values =% of *C. maxima* /% of *C. medica*).

Taxa	*C. medica*%	*C. reticulata* %	*C. maxima* %	*C. hystrix*%	No. varieties	MaRe index values	MaMe index values
Citrus hystrix DC.	0.70±0	0.70±0	0.70±0	97.90±0	2	1	1
Citrus maxima (Burm.) Merr.	0.70±0	0.75±0.03	97.76±0.05	0.79±0.05	11	131±19	139.7 ± 0.3
Citrus reticulata Blanco	1.25±1.05	95.14±1.9	2.83±1.28	0.79±0.08	8	0.03±0.02	3.75±2.95
Citrus medica L.	96.46±1.03	1.01±0.25	1.56±0.64	0.96±0.31	8	1.55±0.55	0.02±0.01
Citrus × *aurantium* L.	0.83±0.07	47.03±7.54	51.40±7.56	0.80±0.11	3	1.16±0.31	64.55±8.45
Citrus × *deliciosa* Ten.	0.74±0.08	84.10±8.38	14.42±8.38	0.76±0.08	5	0.28±0.19	27.05±17.85
Hypothesis A *Citrus medica* L. × *C.* × *aurantium* L. (average)	48.65	24.02	26.48	0.88	–	1.10	0.54
Hypothesis B *Citrus medica* L. × *C.* × *deliciosa* Ten. (average)	48.60	42.56	7.99	0.86	–	0.19	0.16
Hypothesis C (*Citrus medica* L. × *C.* × *deliciosa* Ten.) × (*C. medica* L. × *C.* × *aurantium* L.) (average)	**48.62**	**33.29**	**17.24**	**0.87**	**–**	**0.52**	**0.35**
Citrus × *limon* (L.) Osbeck var. *limon*	**49.14±2.25**	**30.41±3.09**	**19.61±1.93**	**0.85±0.07**	**15**	**0.65±0.35**	**0.40±0.18**
Citrus × *limon* var. *limetta* (Risso) Ollitrault, Curk & R. Krueger	**49.50±1.13**	**35.20±0.68**	**14.50±0.98**	**0.83±0.17**	**3**	**0.41±0.02**	**0.29±0.02**
Citrus × *limettioides* Yu.Tanaka	47.90±1.67	35.07±0.92	16.27±0.73	0.80±0.00	3	0.47	0.35±0.2
Citrus × *meyeri* Yu.Tanaka	43.43±1.92	27.53±4.13	21.60±4.56	2.78±3.81	4	0.98±0.34	0.62±0.13
Citrus × *bergamia* (Risso) Risso & Poit.	27.5	30.0	41.7	0.8	1	1.39	1.52
Citrus × *limonia* Osbeck	48.64±1.63	49.66±1.06	0.89±0.05	0.86±0.03	9	0.02	0.02
Citrus × *aurantiifolia* (Christm.) Swingle	49.36±1.62	0.78±0.04	0.79±0.04	49.12±1.63	9	1.02±0.12	0.02±0.01
Citrus × *lumia* Risso	49.92±10.30	1.52±1.15	45.87±9.12	2.75±3.63	6	40±32	1.23±0.73

3.4.3. Gastronomy

In Morocco, Marrakesh limetta lemons are known as *citron beldi, limun beldi* or *l'hamd beldi*, which means "traditional lemon". Juice of Sour limetta fruits is used to dress salads in Morocco, hence raw mature fruits are sold during winter at the markets of Rabat and Sale (Morocco) (Fig. 9a and c). In Marrakesh, limettas are sold in the markets from April at a price twofold that of lemons (Chapot, 1962). Another important culinary item is sour limettas candied in sugar syrup (Fig. 9d) or brined as olives (Fig. 9b).

The local population has developed a typical know-how in the processing (conservation) of Marrakesh limonette (Valorisation des Produits de Terroir Marocain, 2015). The product obtained is known under the name of "*Lamsayyer*". It is a specialty mastered by women and the process is simple:

1- Two incomplete cuts (drawing of a cross) to obtain an open limonette then filled with salt;
2- Arrangement of limonettes filled with salt in a container with pressure to reduce the vacuum as much as possible;
3- Over time, the volume occupied by "*lamsayyer*" decreases following the expulsion of water induced by the salt and the container is gradually filled with other salted limonettes

Traditionally, to preserve Marrakesh limetta at home, the fruit is prepared in brine in which 200 g of salt are used per kilo of fruit (Specialty, 2020). Longitudinally slitting the limetta fruits is not mandatory because the rind is thin in contrast with ordinary lemons, which require to be slit and filled with salt. The fruit are then placed in a container that is filled with boiling water and let marinate for at least three weeks before use. An alternative formulation uses only salt, no water, and adds a small amount of olive oil or fills the container with limetta juice.

Brined sour limetta fruits are used after washing in preparing many dishes such as "*tajins*" or "*tahine*" and salads. They are present in the majority of dishes cooked in the region being essential for preparations made with chicken (Chapot, 1962; Anonymous, 2014).

Fruits are also industrially brined and sold canned in Moroccan and European supermarkets, on the shelves of products for Maghrebian cuisine (Fig. 10e and f). Marrakesh sour limetta furnishes the genuine "preserved lemons" called "*L'hamd marakad*" ("sleeping lemons") or "*mssiyar*" ("guided lemons") which are the chief secret ingredient in North African cuisine. However, when this limetta is not available, North African immigrants in Europe use ordinary lemons instead. The "*Qares mraqqed*" is prepared in Algeria following a similar procedure using lemons or sour limetta (Bouayed, 1983).

3.5. The cultural value of sour limetta

3.5.1. The image of the sour limetta in the painting

Although citrus fruits are relatively frequent in paintings of, especially, Flemish, Italian and Spanish authors in the consulted museums, recognizable limettas are relatively rare.

The first identifiable image of limetta appears in the painting of Hubert van Eyck (c. 1366 – 1426) and Jan van Eyck (1390 - 1441). Ghent Polyptych (The Polyptych of the Adoration of the Mystic Lamb). 1420–1432. Oil on oak, Height: 3.4 m x Width: 5.2 m. St. Bavo's Cathedral, Ghent (Wikipedia 2020). On the right margin of the polyptych, the figure of Eva appears holding in her right hand a citrus fruit, with rough, flattened and yellow skin, which would be about 5 cm in diameter (Fig. 10a). It is a figuration of the "apple of Eva". Sneyder (1976), Huylebrouk & Mecsi (2011) and Van der Meer (2017) identified this fruit in the sense of a small Adam's apple or "*lumia*" discerning from other alternatives: *C. medica* "Etrog" or *C. histrix*. However they did not analyzed the possibility of being the "Eva's apple" a limetta fruit. Given the dimensions of the fruit represented it is most likely a limetta (Rivera et al., 1997; Egea-Fernández et al., 2015).

Also within a religious theme Joachim Bueckelaer (1533–1574) painted limetta in The Well-stocked Kitchen, with Jesus in the House of Martha and Mary in the Background. 1566. Oil on panel, Height: 1.7 m x Width: 2.5 m. Rijksmuseum, Amsterdam. Among a profusion of vegetables, fruit, meat, poultry and kitchenware displayed here appears a dish with olives and two limetta fruits (Chapot, 1962).

Cooking was one of the most treated themes in still life, although with different characteristics depending on the geographical areas. In Spain there is a particular variant of the theme of cooking with the *bodegón*, whose most important author was Juan Sánchez Cotán. Limettas appear represented in some of these *bodegones*.

Juan Sánchez Cotán (1560 – 1627). Still life of game, vegetables and fruits. 1602 (Fig. 10b). Oil on canvas, 68 × 88.2 cm. Museo del Prado. Room 008[a] (Prado, 2020a). The limettas appear three on a branch, with leaves, in the upper left corner of the painting, in a context of a still life of small game, with partridges and birds, accompanied by some apples hanging by strings, on the right, and compensated in the lower right

Fig. 9. Marrakesh sour limetta
a, Fresh Marrakesh sour limetta in a street market of Rabat; b, brined sour limetta sold along marinated olives; c, group of fresh sour limetta fruits; d, the same candied; e and f, brined Marrakesh sour limetta sold under the name "*limón en salmuera*" in a supermarket of Murcia (Spain). Photos: A. Bermudez, D. Rivera.

corner by a handful of cardoon leaf stalks. Red and white carrots and long white radishes appear at the base of the foreground. Given the context, it is possible to think that limettas were the sour ones used to flavor meat dishes.

Giovanni Battista Ruoppolo (1629 – 1693). Still life with fruits. Second half of the 17th century (Fig. 10c). Oil on canvas, 87 × 116 cm. Private collection (Wikimedia, 2020a). Still life with a variegated mix of grapes, melons, watermelons, peaches, plums and other fruits. At the base, and in the foreground, three sour limettas appear next to a snail and a cardoon with the leaf stalks.

Giuseppe Ruoppolo (1631 – 1710). Still life with citrus fruits, copper refresher, parrot and cut flowers. Second half of the 17th century (Fig. 10d). Oil on canvas, 40 × 30 cm, copy from the original. (Isaproject, 2020). In a still life, animated by a red parrot and a goldfinch, citrus fruits such as oranges, lemons and citrus stand out. Limettas also appear next to the lemons and in the lower right corner. Existing two separate groups it is possible that the author represented both sweet and sour limettas.

Circle of Francisco de Zurbarán (Fuente de Cantos, Badajoz, 1598 – Madrid, 1664). Still life with glass, fruits, and jar. 1650 approximately (Fig. 11f). Oil on canvas, 39.4 × 62.2 cm. North Carolina Museum of Art, G.52.9.171, it was purchased with funds from the North Carolina State Art Society (Robert F. Phifer Bequest) (NCMA, 2020; Wikimedia, 2020f). Among the fruits a relatively larger limetta type fruit appears to the left

Fig. 10. Limetta in the paintings 15th – 17th cent
a, Eve, from Hubert van Eyck (c. 1366 – 1426), Ghent Polyptych of the Adoration of the Mystic Lamb. 1432; b, Juan Sánchez Cotán (1560 – 1627), Still life of game, vegetables and fruits. 1602; c, Giovanni Battista Ruoppolo (1629 – 1693). Still life with fruits. Second half of the 17th century; d, Giuseppe Ruoppolo (1631 – 1710). Still life with citrus fruits, copper refresher, parrot and cut flowers. Second half of the 17th century. Images: a, Wikipedia (2020); b, Prado (2020a); c, Wikimedia (2020a); d, Isaproject (2020).

of the group. The clearly white flowers associated to it suggests this is a sweet limetta.

Cristoforo Munari (Reggio Emilia 1667 – Pisa 1720). Still life. 1709 (Fig. 11e). Oil on canvas, 74 × 128.5 cm. Le Gallerie degli Uffizi. 1890, n° 4859 (Uffizi, 2020a). A mix of themes includes fruits on a silver plate, citrus fruits conforming to the Medici plant-collector favorites, ultra-fine crystals, polished porcelain and books. A trompe l'oeil effect can be seen in the peeled lemon, which falls down the side of the table. Only the citrus fruit and branch by the lower left corner of the table can be identified as a limetta.

Luis Egidio Meléndez (Naples, 1716 - Madrid, 1780) included limetta fruits in two of his famous still life paintings (Prado 2020b,c). However, there is no evidence whether these limetta fruits were sweet, or sour. The first painting is: Still life with limes, jelly box, butterfly and containers. Third quarter of the 18th century (Fig. 11c). Oil on canvas, 35 × 48 cm. Museo del Prado. Room 088. In the foreground some limettas, arranged in disorder, occupy practically half of the canvas; behind them a container with honey, a popular type with green glaze, such as those from Biar or Lucena (Prado, 2020b).

The second painting is: Still life with limes, oranges, acerolas and watermelon. 1760 (Fig. 11a). Oil on canvas, 47 × 33 cm. Museo del Prado. Room 088 (Prado, 2020c). A group of seven limettas arranged in two rows (4 + 3) appears in the foreground, along with some acerolas. Given the context of sweet fruits (oranges, acerolas and melon) it is

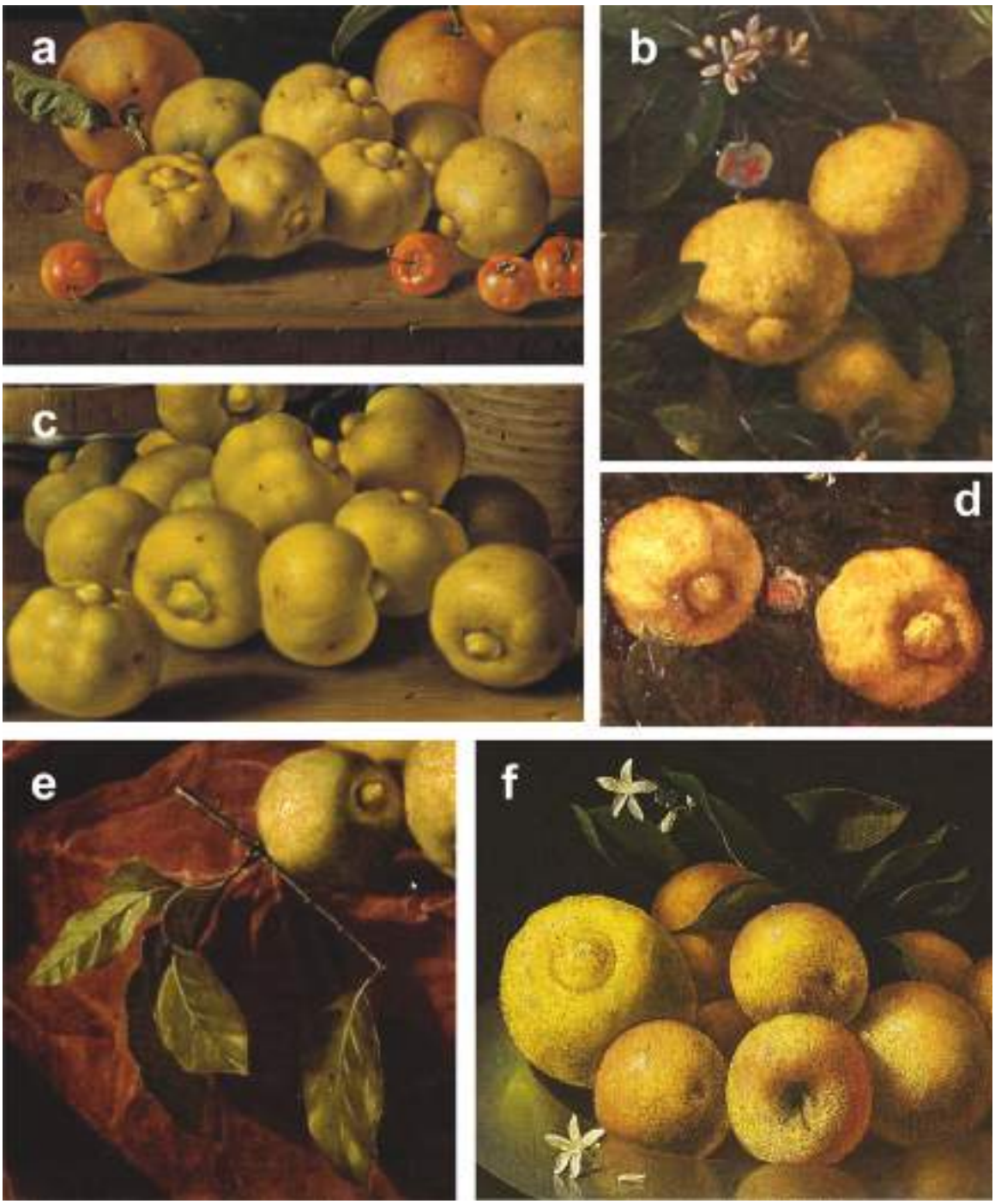

Fig. 11. Limetta in the paintings of 17th-18th cent
a. Luis Melendez, Still life with limes, oranges, acerolas and watermelon, c. 1760; b, Bartolomeo Bimbi, "*Melangolo, cedri e limoni*", 1715; c, Luis Melendez, Still life with limes, jelly box, butterfly and containers, 1770; d, Bartolomeo Bimbi, *"Arance, cedri, lime, limoni e lumie"*. 1715; e, Cristoforo Munari. Still life. 1709; f, Circle of Francisco de Zurbarán. Still life with glass, fruits, and jar. 1650 approximately. Images: a, Prado (2020d); b, Wikimedia (2020b); c, Prado (2020b); d, Wikimedia (2020c); e, Uffizi (2020a); f, Wikimedia (2020f).

possible to think that they are sweet limettas, but the irregular shape of the surface of limettas is more typical of sour ones.

A peculiar type of evidence is provided by the pictorial catalogues of the Medici's fruit collections displayed in a series of paintings by Bartolomeo Bimbi (1648 – 1725). Four among these are devoted to list the citrus collection (Baldini et al., 1982): "*Arance, lime, limoni e lumie*", *"Melangolo, cedri e limoni"*, *"Arance, cedri, lime, limoni e lumie"*, and *"Arance, bergamotti, cedri, limoni e lumie"*. Among the above only two contain images of fruits similar to the acid limetta.

- "*Arance, cedri, lime, limoni e lumie*". 1715. Oil on canvas, 1.74 *m* × 2.33 m. Museo della Natura Morta (Poggio a Caiano, Invent. Castello 612) (Baldini et al., 1982; HomecitrusGrowers, 2020a; Wikimedia, 2020c). Number 10 (*lumia grossa di Spagna*) (Fig. 11d), 11 (*lumia trasparente e bella*) and 29 (*lima nuova di S. Remo*) show some resemblances with limettas.
- "*Melangolo, cedri e limoni*". 1715. Oil on canvas, 1.74 *m* × 2.33 m. Museo della Natura Morta (Poggio a Caiano, Invent. Castello 597) (Baldini et al., 1982; HomecitrusGrowers, 2020c; Wikimedia,

2020b). Number 14 (*melangola*) (Fig. 11b) and 15 (*mela rosa appiolina*) show some resemblances with limettas but may correspond to other flattened citrus types.

The other two paintings of citrus in the Medicean collection, by Bimbi do not represented fruits similar to limettas:

- "*Arance, bergamotti, cedri, limoni e lumie*". 1715. Oil on canvas, 1.74 m x 2.33 m. Museo della Natura Morta (Poggio a Caiano, Invent. Castello 616) (Baldini et al., 1982; HomecitrusGrowers 2020b; Wikimedia 2020d) and "*Arance, lime, limoni e lumie*". 1715. Oil on canvas, 1.30 *m* × 1.60 m, copy from the original. Museo della Natura Morta (Poggio a Caiano, Invent. Castello 594) (Baldini et al., 1982; HomecitrusGrowers, 2020d; Wikimedia, 2020e)

3.6. Availability and conservation status

Several accessions are grown by ANSE in Cartagena (Murcia, Spain). A single accession is kept in the Riverside Citrus Collection (USA) as "Marrakesh Limonette" (CRC 3989, PI 539280), received as bud-wood from *Station Centrale de Recherches sur les Agrumes*, El Menzeh, Kenitra, Morocco, in 1987 (Citrusvariety, 2019; GRIN, 2019). Other accession is at the Station INRA-CIRAD (San Giulianu, Corse, France) as "*Lime de Marrakesh*" (Curk, 2014; INRA, 2014a). Another is at Vivaio Oscar Tintori (Pescia, Italy) as "*limonetta di Marrakesh*" SRA 974. The IVIA (2014) (Valencia, Spain) reports one single accession under "*Limonette de Marrakesh*" IVIA number 484.

Concerning Morocco two repositories grow lime accessions: 54 at El Menzeh (Gharb) and 16 at Melk Zhar (Souss). However, there is no information whether the "Marrakesh Limetta" is in the collections or not (INRA, 2014b). This genotype does not appear to be in the Moroccan collections according to the *Mécanisme National d'Échange d'Information sur les resources phytogénétiques* (Saidi, 2014).

A few nurseries grow this plant: Oscar Tintori (2020) (Italy) offers the plant under "*Limonetta di Marrakesh SRA 974*", Pépinières Bachès (2021) (France) as *Citrus limetta* 'de Marrakesh', and Sapiama (2020) (Morocco) as "*Limonette de Marrakesh*".

4. Conclusions

The sour limetta from Marrakesh (*Citrus* × *limon* var. *limetta*) is a citrus fruit of horticultural, economic, historic and ethnobotanical interest, traditionally used as a condiment in the cuisine of different Mediterranean countries, although its use is currently mainly restricted to Morocco.

The morphological characterization and detailed description of this citrus taxon has made it possible to show the differences with other nearby varieties, with which it has been confused, so that its recognition, cultivation and use is easier.

Thanks to the morphological study and the analysis of its genealogy, its relationships with limes and lemons are clarified, being clearly closer to the latter. The analysis of the relative proportions of ancestors suggests that limettas originated in parallel with lemons through the hybridization of (*Citrus medica* × *C.* × *aurantium*) × (*Citrus medica* × *C.* × *deliciosa*) somewhere between western Mediterranean and the Himalayas.

Despite its current relevance in traditional Moroccan cuisine, the conservation status of sour lime is threatened, as it is being replaced by the less expensive common lemon.

The image of the lime in art has been evidenced between the 15th and 18th centuries, having a descriptive and symbolic value in still life and religious themes. The high quality of the works and their realism made identification easy. This is associated with the wider use and cultivation of sour limettas, both in orchards and in gardens and botanical collections, during the 16th to 18th centuries in Europe.

Given its historical importance within the Mediterranean culture, we propose activate the conservation of the plant and of the associated traditional knowledge especially management and uses, so that future generations can continue to enjoy this hybrid citrus, and its ornamental, gastronomic and cultural value.

Author's contribution

D.R. and C.O. contributed to the study conception and design. A.B. D. R., C.O., S.R., J.S-B., ethnobotany field work and collected the samples. D.R. and C.O. analyzed the data and drafted the manuscript. P.F.-G taxonomy and botanical nomenclature, D.R. and all authors commented on different versions of the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

Authors declare the inexistence of potential conflicts of interest.

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Supplementary materials

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