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RESEARCH ARTICLE

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The forgotten, ancient olive trees of the Spanish northwest: A first molecular and botanical analysis

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Abstract

No country has a larger area under olive (*Olea europaea* subs. *europaea* var. *europaea*) cultivation than Spain. In the Spanish northwest, however, this crop has largely been forgotten, even though olive oil was once an important product of the area. Sadly, apart from a few scraps of information handed down orally, little information exists regarding the genotypes grown, or from where they may have originally come. Many centuries-old olive trees, however, can still be found in the area, some even forming groves now part of open woodland but which may harbour an important genetic reservoir. The present work describes a botanical and molecular analysis of these ancient trees, following a survey of allegedly native genotypes surviving in different locations in Galicia. Comparison of their molecular profiles with those in the World Olive Germplasm Bank of Cordoba, and those in the database compiled by the Agronomy Department of the University of Cordoba, revealed two known Galician genotypes, ´Brava Gallega´ and ´Mansa Gallega´, and the Portuguese genotype ´Cobrancoça´. Six genotypes present in neither database were also detected. In addition, some misidentifications of the ´Mansa´ genotype in recent studies were clarified. Botanical analysis confirmed the molecular results in all cases. The findings suggest a larger survey should be performed so that the full olive genetic diversity of this region can be recorded and preserved.

Additional keywords: *Olea europaea* L; ´Brava Gallega´; ´Mansa Gallega´; unknown genotypes; Galicia; morphological descriptors; SSRs.

Authors' contributions: Conception and design of the experiments: MCM, JLS. Surveying for plant material and data analysis: MCM, JLS, SB, PG. Botanical analysis and drafting of the manuscript: MCM, PG. Microsatellite analysis: PG. Fund raising and overall supervision: MCM.

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Introduction

Olives (*Olea europaea* subs. *europaea* var. *europaea*), wheat and grapes are some of the oldest of all crops (Zohary & Hopf, 1994). Olives are normally cultivated between 30º and 45º N and S, and in other areas where the climate is Mediterranean (Barranco *et al*., 2000). Spain has 2,554,829 ha under olive cultivation, and is the world's foremost producer of olive oil (Ministerio de Agricultura, Pesca y Alimentación-MAPA, 2018); its output accounts for 60% of all the EU's olive oil and 45% of that produced worldwide (International Olive Oil Council, 2015[1]). These data provide an idea of the economic and environmental importance of olives in Spain.

Many olive genotypes are grown around the world, and many of those growing in the most important olive oil-producing countries have been described (Barranco *et al*., 2000; Belaj *et al*., 2002; Bartolini *et al*., 2005; Rallo *et al*., 2005; Fendri *et al*., 2010 and 2014; Haouane *et al*., 2011; Lazovic *et al*., 2016; Sakar *et al*., 2016). In Spain, over 250 are reported in use (Barranco *et al*., 2005; Vargas-Gómez & Talavera-Lozano, 2012), but the current number used in the main commercial plantations is small (Rallo *et al*., 2005). The variation in Spanish olive germplasm has been studied in certain areas (Viñuales, 2007; Díez *et al*., 2011; Gómez *et al*., 2012; Trujillo *et al*., 2014; Martí *et al*., 2015). In marginal areas, however, much less work of this kind has been done, and in some places no surveys or

[1] http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures?lang=en_US

characterisations have been undertaken at all. Such is the case of Galicia in the NW Peninsular.

The Atlantic-influenced climate of this region is not currently associated with olive cultivation, yet old references cite olives trees being grown here (Alonso de Herrera, 1513; Contreras, 1798; Hidalgo-Tablada, 1870). The importance of olive production in the past is evident in archaeological finds such as primitive oil mills dating from the 1st-2nd centuries BCE (Fernández de la Cigoña & Martínez, 2003), and numerous references to olive trees and olive oil in the region's toponymy. A strong oral tradition also exists among the region's inhabitants that testify to families having produced their own olive oil for generations. This residual cultivation of olive trees has persisted in the area until the present day, but in the last 10 years there have been several initiatives that have attempted to recover olive production as part of the regional economy. Indeed, between 2008 and 2017, the area under olive cultivation increased from just 10 ha to 272 ha (MAPA, 2018).

While a number of recent studies have examined the olive oils produced in Galicia (Espinosa-Sánchez, 2010; Reboredo-Rodríguez *et al*., 2014a, 2014b and 2015), most of these oils were not produced by native trees (Reboredo-Rodríguez *et al*., 2015) but by recently planted and commonly cultivated genotypes from Andalusia, such as ´Picual´ and ´Arbequina´. Indeed, while the agricultural biodiversity of Galicia's woody-plant crops-grapes (Gago *et al*., 2009; Martínez *et al*., 2018), apples (Pereira-Lorenzo *et al*., 2007), pears (dos Santos *et al*., 2011; Pereira-Lorenzo *et al*., 2012) and chestnuts (Pereira-Lorenzo *et al*., 1997) has been studied, that of the region's olive trees is almost unknown. Localising, characterising and conserving the genotypes that may still be found in this geographical area is vital to avoid the genetic erosion of the species and to save their traits for use in olive improvement programmes. A recent article by Reboredo-Rodríguez *et al*. (2018), and the doctoral thesis of Reboredo-Rodríguez (2015), identified a number of olive genotypes from this region. However, these contributions covered only a very small part of the territory and some of the molecular results were contradictory. Wider and more rigorous and systematic surveying is required to catalogue the area's olive tree biodiversity and to allow their inclusion in the Spanish list of olive varieties of commercial interest.

The present work reports the localisation of ancient olive trees in Galicia, their characterisation using botanical and molecular markers, and examines whether or not these trees represent unknown native genotypes. Back in the 19th century, Hidalgo-Tablada (1870) suggested that olive genotypes might be characterised via certain leaf, fruit and endocarp variables, the shape of the tree, and other features. Nowadays the International Olive Council (IOC) uses the genotype classification system of Barranco *et al*. (2005), which employs botanical and agronomic markers. The present work, however, introduces a further morphometric inspection of the leaf. Martínez & Grenan (1999) developed a graphic method for visualizing the differences that appeared in biometric studies of the grapevine leaf. This method provides a highly realistic representation of the foliar morphology and has been used to compare genotypes (Martínez & Pérez, 2000; Santiago *et al*., 2005; Martínez, 2007; Gago *et al*., 2009; Martínez *et al*., 2018) and clones (Martínez *et al*., 2005). Martínez & Grenan´s (1999) method has been adapted in the present work, in order to be used in the study of olive average leaves. Finally, simple sequence repeats (SSRs) markers were also used in genotype identifications. Many genetic characterisation studies have used different sets of SSRs, and the results have greatly increased our knowledge of olive genetic heritage in different areas (Cipriani *et al*., 2002; Belaj *et al*., 2004 and 2011; Gil *et al*., 2006; Sarri *et al*., 2006; Baldoni *et al*., 2009; Muzzalupo *et al*., 2010; Fendri *et al*., 2010; Diez *et al*., 2011; Martí *et al*., 2015; Lazovic *et al*., 2016; Sakar *et al*., 2016). Together, all these techniques provide a glimpse of the possibly notable olive diversity of the Spanish Northwest.

Material and methods

Plant material

A literature review was performed on olive cultivation in Galicia in order to determine the priority areas to be surveyed. Orally transmitted information was then collected from growers in the chosen areas to record people's recollections of olive trees, and to make note of any locally used genotype names. An initial survey was then undertaken to find old trees. Some of these were clearly centuries old, as manifested by the size of their trunks and the references made to them by different generations of the owning families. Some were no longer used in an agricultural sense, although a number of these retired trees had taken on an ornamental role. A total of 18 trees were sampled for the present work. Each tree was given a code number (Table 1 and Fig. 1).

Molecular characterisation

Genomic DNA was extracted from fresh young leaves of all 18 trees located, using the cetyltrimethylammonium bromide (CTAB) protocol method origi-

Table 1. List of the olive samples included in the study.

Sample code	Collection site (Province)	Cultivation status
1	Ourense	Abandoned cultivation
2	Ourense	Fruit production
3	Ourense	Fruit production
4	Ourense	Ornamental
5	Ourense	Ornamental
6	Lugo	Fruit production
7	Lugo	Abandoned cultivation
8	Lugo	Abandoned cultivation
9	Lugo	Abandoned cultivation
10	Ourense	Fruit production
11	Pontevedra	Ornamental
12	A Coruña	Ornamental
13	A Coruña	Abandoned cultivation
14	Pontevedra	Ornamental
15	Pontevedra	Ornamental
16	A Coruña	Ornamental
17	A Coruña	Ornamental
18	Pontevedra	Ornamental

nally developed by Murray & Thompson (1980) and modified by De la Rosa *et al*. (2002).

A set of 13 SSRs were analysed: ssrOeUA-DCA03, ssrOeUA-DCA09, ssrOeUA-DCA11, ssrOeUA-DCA15, ssrOeUA-DCA16, ssrOeUA-DCA18 (Sefc *et al*., 2000) GAPU59, GAPU71B, GAPU101, GAPU103 (Carriero *et al*., 2002); UDO99–019, UDO99–024 and UDO99-043 (Cipriani *et al*., 2002). These markers were selected for their high efficiency and resolving power in previous olive genotype characterisation studies (Baldoni *et al*., 2009; Trujillo *et al*., 2014).

Polymerase chain reactions (PCR), performed in 20 µL volumes, involved 2 ng of genomic DNA, 1X supplied PCR buffer (Biotools, Spain), 200 µM of each dNTP (Roche), 1.5 mM MgCl, 0.25 units of Taq DNA polymerase (Biotools, Spain) and 0.2 µM of forward (fluorescently labelled) and reverse primers. All reactions were performed in a Perkin-Elmer 9600 thermocycler as follows: denaturation at 94ºC for 5 min, 35 cycles of 94ºC for 20 s, 50-59ºC for 30 s, 72ºC for 30 s, and a final extension at 72ºC for 8 min. Amplicons were detected using an ABI 3130 Genetic Analyzer (Applied 181 Biosystems/HITACHI) using the GeneScan 400 HD-Rox internal standard. The genotypes ´Frantoio´ and ´Arbequina´ were used as controls in all runs.

The allele profiles were sized in base pairs (bp) and characterized using Genescan 3.7 software (Applied Biosystems). A code number was assigned to the different SSR profiles defined.

Additionally, for each SSR marker, the total number of alleles at each locus (Na), and the observed (Ho) and expected (He) heterozygosity, were determined using GenAlex v.6.503 software (Peakall & Smouse, 2006 and 2012). The probability of identity index (PI) and the polymorphism information content (PIC) were calculated using Power Marker v.3.25 software (Liu & Muse, 2005). Genotypes showing only one fragment amplified by a pair of primers at a particular locus were deemed homozygous at that locus.

Botanical characterisation

The qualitative botanical characteristics examined were those described by Barranco *et al*. (2005) and adopted by the International Union for the Protection of New Varieties of Plants (UPOV Code: OLEAA_EUR) for the description and identification of olive cultivated genotypes. These characteristics include:

— Leaf: shape, width, and longitudinal curvature of the leaf blade (40 leaves were taken from the mid area of 8-10 of the year's shoots, chosen from among the most representative of each tree, and always from the south-facing side).

— Drupe: weight, shape, symmetry, maximum transverse diameter, apex and base shape, and presence/absence of a tip (40 drupes were examined).

— Endocarp: weight, shape, symmetry position A, symmetry position B, position of the maximum transverse diameter, shape of the apex, shape of the base, roughness of the surface, number of vascular bundles, distribution of vascular bundles, and presence of mucron (40 endocarps were examined).

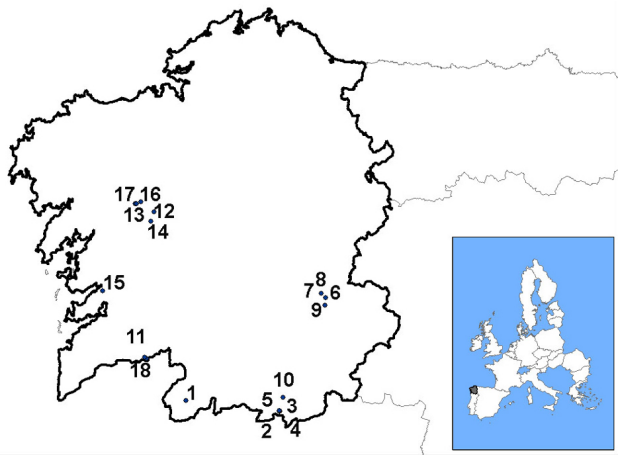

Figure 1. Map of Galicia, a region in northwestern Spain, showing the location of the 18 trees examined (see Table 1).

The characterisation of the leaf was complemented using an adapted version of the method of Martínez & Grenan (1999) used to construct 'mean leaves' of grapevine genotypes. Forty young leaves were taken from shoots of the present year in the crown of each tree. These were then herborized, photographed, and the images used to determine the lengths and angles shown in Fig. 2 (performed using AnaliSIS FIVE® software). The mean values were then used to construct a mean leaf for each tree. This method provides a recognisable image that can be compared against others.

Principal components analysis (PCA) was also performed to group the trees depending upon their morphology using the measured leaf variables, and upon certain quantitative variables recorded for the drupes and endocarps (drupe length, drupe width, drupe weight, endocarp length, endocarp width, endocarp weight, and pulp weight). Since the different trees were found growing under different soil, climatic and cultivation conditions, the raw values for these variables were not used in this analysis, but rather the relationships between them (Table 2), which reflect the resulting morphology. All statistical calculations were performed using SAS software v.9.3 (SAS Inst. Inc., Cary, NC, USA).

Genotype identification

The criteria used in genotype identification were those described by Trujillo *et al*. (2014), *i.e*., the pairwise comparison of SSR and morphological profiles with those in databases (the World Olive Germplasm Bank of Cordoba [WOGBC] and the Agronomy Department of the University of Cordoba [UCO] databases).

Results

Molecular characterisation

SSR variability

A total of 57 alleles were detected for the 13 SSR loci examined. The number of alleles per locus ranged from two (UDO99-19 and GAPU59) to seven (ssrOeUA-DCA09 and UDO99-43) with an average of 4.38 alleles per locus (Table 3).

The He value ranged from 0.180 (UDO99-019) to 0.810 (ssrOeUA-DCA09 and UDO99-43), with a mean value of 0.654. The PIC values were always over 0.5 (Table 3), except for UDO99-019 (0.164), UDO99-024 (0.442), GAPU59 (0.375) and ssrOeUA-DCA15 (0.495).

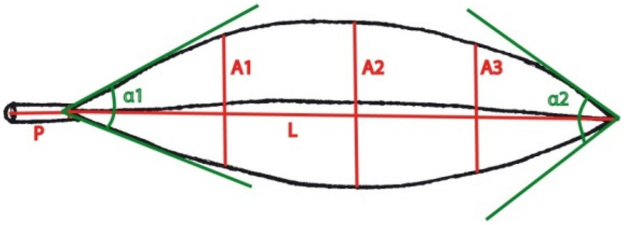

Figure 2. Lengths and angles measured for the preparation of the mean leaf of each tree. Lengths: L, A2, A1, A3 and P; Angles: α1 and α2.

Ten different molecular profiles or genotypes were recorded among the 18 trees examined (Table 4) which were grouped as follow: 7 trees gave rise to unique SSR profiles (not duplicated in any other tree) and 1 trees had SSR profiles in common with other trees resulting in the identification of three SSR profiles among them.

Genotype identification

When the molecular profiles were compared (in 2015) with those in the WOGBC and UCO databases (performed by the person responsible for molecular identifications), three matches were returned. Tree 11 was identified as belonging to the genotype ´Mansa Gallega´, trees 6 and 7 as belonging to ´Brava Gallega´, and trees 1, 2, 4, 5 and 10 to the Portuguese genotype ´Cobrancoça´ (Table 4).

The literature search and conversations with growers returned only two cultivated genotypes names, ´Brava´ and ´Mansa´, which were used generically to describe ostensibly native Galician olive trees. Interestingly, both names are recorded by the WOGBC as referring to material introduced elsewhere from Galicia.

Table 2. Relationships between different leaf, drupe and endocarp variables.

Leaf relationships[a]
Rel 1 = A2/L
Rel 2 = A1/L
Rel 3 = A3/L
Rel 4 = A1/A2
Rel 5 = A3/A2
Drupe & endocarp relationships
Rel A = length/drupe width at position A[b]
Rel B = length/width of endocarp at position A[b]
Rel C = pulp weight/drupe weight
Rel D = endocarp weight/drupe weight
Rel E = pulp weight/endocarp weight
Rel F = drupe width/endocarp width
Rel G = drupe length/endocarp length

[a]See Fig. 2. [b]Position A, according to the UPOV code.

Table 3. Size range (base pairs), number of alleles (Na), observed (Ho) and expected (He) heterozygosity, probability of identity (PI) and polymorphism information content (PIC) for each SSR locus.

SSR locus	Size range	Na	H_o	H_e	PI	PIC
ssrOeUA-DCA03	227-253	6	1.000	0.800	0.070	0.770
ssrOeUA-DCA09	160-206	7	1.000	0.810	0.061	0.785
ssrOeUA-DCA11	130-178	4	0.900	0.745	0.113	0.697
ssrOeUA-DCA15	243-263	3	0.500	0.585	0.262	0.495
ssrOeUA-DCA16	122-171	6	1.000	0.795	0.072	0.765
ssrOeUA-DCA18	166-183	5	1.000	0.720	0.126	0.672
UDO99-019	97-129	2	0.200	0.180	0.689	0.164
UDO99-024	164-185	3	0.600	0.505	0.308	0.442
UDO99-043	170-216	7	1.000	0.810	0.063	0.784
GAPU59	210-220	2	0.800	0.500	0.375	0.375
GAPU71B	121-141	4	0.900	0.655	0.171	0.603
GAPU101	189-217	4	1.000	0.685	0.161	0.623
GAPU103	133-184	4	0.100	0.715	0.135	0.661
All loci		57				
Mean		4.38	0.769	0.654	0.200	0.603

Botanical characterisation

Leaf qualitative botanical variables (Table 5) were noted. The three types of leaf blade shape cited by Barranco *et al*. (2005) were found among the trees studied although only tree 9 (Unknown Genotype 5) showed the lanceolate shape. Most of the trees have a medium width and flat leaf blade.

Leaf lengths and angles were measured (Table S1 [suppl.]), and the relationships between them were calculated and used for drawing mean leaves (Fig. 3). Drupe and endocarp qualitative botanical variables were recorded following the method of Barranco *et al*. (2005) (Tables 6 and 7). Only fruits from trees identified as belonging to genotype 'Cobrancoça' showed a high weight (Table 6). Tree number 3 (Unknown Genotype 3) presented fruits with spherical shape and with the maximum transverse diameter toward the base, the rest of the studied fruits were ovoid or elongated with the maximum diameter centred (Table 6). Finally, none of the fruits studied presented an evident nipple (Table 6). Regarding the endocarp qualitative botanical variables (Table 7), only genotype ´Mansa Gallega´ (tree 11) presented endocarps with a low weight and, again, endocarps from tree 3 (Unknown Genotype 3) differed from the rest in shape (ovoid), position of maximum diameter (toward the base) and shape of the base (round).

Quantitative drupe and endocarp variables measured, and the relationships between them were calculated (Table S2 [Suppl.]).

The results of the PCA on the leaf variables (Table 1 and Fig. 4) show the two first axes account for 85.68% of the variance (Prin 1 accounted for 51.37% of the variance, and Prin 2 for 34.31%).With respect to axis 1 (Prin 1), the variables with the greatest weight were Rel 1 (A2/L) and Rel 3 (A3/L). Both relationships provide information regarding leaf shape (elliptical, elliptic-lanceolate, or lanceolate). With respect to axis 1 (Prin 2), the variables with the greatest weight were Rel 4 (A1/A2) and Rel 5 (A3/A2), which provide information on the longitudinal profile of the leaf, *i.e*., the proportional distance over which the two sides of the leaf remain parallel (*e.g*., note the difference between mean leaves 5, 12 and 18 in Fig. 3).

With respect to Prin 1 (Fig. 4), the trees with elliptical leaves (12, 13 and 17) are distributed more to the right, and those with more lanceolate leaves (5, 9 and 18) towards the left. The majority, *i.e*., trees with elliptic-lanceolate leaves (as shown in Table 5), are situated between these other positions. With respect to Prin 2 (Fig. 4), the leaves of trees 9 and 5 were clearly separated from the rest, indicating their morphology to be different too, with the leaves of tree 9 wider and those of tree 5 narrower than all others. In addition, the reduction in width at the apex and peduncle was less in the leaves of tree 5 than in all others. Finally, trees 9 and 5 also differed from all others in terms of the pattern of change in leaf width along the length of the blade.

The results of PCA (Fig. 5) on the calculated drupe and endocarp variables from Table 1, show the two first axes to account for 95.51% of the variance (Prin 1 accounted for 73.67% of the variance, and Prin 2 for 20.84%).

For Prin 1, the variable with the most positive weight was Rel C (pulp weight/drupe weight), and that with most negative weight was Rel D (endocarp weight/drupe weight). For Prin 2, the variable with the most positive weight was Rel B (endocarp length/endocarp width), followed by Rel A (drupe length/drupe width); these provide information on the shape of the endocarp and drupe respectively.

With respect to Prin 1, those trees with drupes with a more meaty pulp (*i.e*., less endocarp) fall to the right of the diagram (Fig. 5); these correspond to the genotypes ´Cobrancoça´, ´Brava Gallega´ and Unknown Genotype 5. Those trees with drupes

Table 4. Allelic profiles (bp) of the 18 olive trees with respect to the 13 microsatellite loci examined.

Sample	ssrOeUA-DCA03		ssrOeUA-DCA09		ssrOeUA-DCA11		ssrOeUA-DCA15		ssrOeUA-DCA16		ssrOeUA-DCA18	
6	237	251	182	192	140	178	243	254	124	152	166	176
7	237	251	182	192	140	178	243	254	124	152	166	176
1	237	251	160	204	140	178	243	254	122	124	166	176
2	237	251	160	204	140	178	243	254	122	124	166	176
4	237	251	160	204	140	178	243	254	122	124	166	176
5	237	251	160	204	140	178	243	254	122	124	166	176
10	237	251	160	204	140	178	243	254	122	124	166	176
11	227	243	180	182	130	140	254	254	144	152	166	183
13	227	251	170	182	130	160	243	254	144	159	166	176
15	227	251	170	182	130	160	243	254	144	159	166	176
16	227	251	170	182	130	160	243	254	144	159	166	176
17	227	251	170	182	130	160	243	254	144	159	166	176
18	237	243	180	204	140	140	254	254	122	152	166	183
3	237	251	160	206	160	178	243	243	124	152	168	172
8	237	247	182	204	140	178	243	243	124	152	168	176
9	243	247	160	204	160	178	263	263	152	171	168	176
14	227	251	170	182	130	160	243	254	144	159	166	176
12	227	253	170	182	130	160	243	254	144	159	166	176

Table 4. Continued.

Sample	UDO99-019		UDO99-024		UDO99-043		GAPU59		GAPU71B		GAPU101		GAPU103		Identification[a]
6	129	129	164	185	172	204	210	220	127	141	191	217	133	133	Brava Gallega
7	129	129	164	185	172	204	210	220	127	141	191	217	133	133	Brava Gallega
1	129	129	185	185	208	216	210	220	121	141	191	217	133	133	Cobrancoça
2	129	129	185	185	208	216	210	220	121	141	191	217	133	133	Cobrancoça
4	129	129	185	185	208	216	210	220	121	141	191	217	133	133	Cobrancoça
5	129	129	185	185	208	216	210	220	121	141	191	217	133	133	Cobrancoça
10	129	129	185	185	208	216	210	220	121	141	191	217	133	133	Cobrancoça
11	97	129	164	177	170	216	220	220	124	127	189	191	159	159	Mansa Gallega
13	129	129	177	185	170	212	210	220	124	141	189	191	159	159	Unknown 1
15	129	129	177	185	170	212	210	220	124	141	189	191	159	159	Unknown 1
16	129	129	177	185	170	212	210	220	124	141	189	191	159	159	Unknown 1
17	129	129	177	185	170	212	210	220	124	141	189	191	159	159	Unknown 1
18	97	129	177	185	170	216	210	220	124	141	189	217	133	159	Unknown 2
3	129	129	185	185	172	216	210	210	121	141	197	217	184	184	Unknown 3
8	129	129	185	185	172	204	210	220	141	141	191	217	184	184	Unknown 4
9	129	129	185	185	172	216	210	220	127	141	191	217	184	184	Unknown 5
14	129	129	177	185	170	212	210	220	124	141	189	191	161	161	Unknown 6
12	129	129	177	185	170	214	210	220	124	141	189	191	159	159	Unknown 7

[a]Identified by comparison with molecular profiles held in the WOGBC (World Olive Germplasm Bank of Cordoba) and UCO (University of Cordoba) databases.

possessing heavier endocarps and less pulp (´Mansa Gallega´ and Unknown Genotype 1) fall towards the left (Fig. 5). With respect to Prin 2, Unknown Genotype 3 remains clearly separated from the rest. This was represented by the only tree with spherical-to-oval drupes and oval endocarps (see Tables 6 and 7). All the other trees had fruits with an elliptical endocarp.

Table 5. Qualitative leaf characteristics of the analysed trees, showing the mode values for 40 leaves.

Sample code	Genotype name	Leaf blade: shape[a]	Leaf blade: width[b]	Leaf blade: curvature of longitudinal axis[c]
		CPVO 6	*CPVO 5*	*CPVO 7*
		UPOV 7	*UPOV 6*	*UPOV 9*
6	Brava Gallega	EP	M	FL
7	Brava Gallega	EP-LA	N	FL
1	Cobrancoça	EP-LA	M	FL
2	Cobrancoça	EP-LA	M	FL
4	Cobrancoça	EP-LA	M	FL
5	Cobrancoça	EP-LA	M	FL
10	Cobrancoça	EP-LA	M	FL
11	Mansa Gallega	EP-LA	M	FL
13	Unknown 1	EP	M	FL
15	Unknown 1	EP-LA	M	FL
16	Unknown 1	EP-LA	M/W	EP
17	Unknown 1	EP	W	FL
18	Unknown 2	EP-LA	M	FL
3	Unknown 3	EP-LA	M	FL
8	Unknown 4	EP-LA	M	FL
9	Unknown 5	LA	N	FL
14	Unknown 6	EP-LA	W	FL
12	Unknown 7	EP	W	EP

CPVO: Community Plant Variety Office code characteristic number; UPOV: International Union for the Protection of New Varieties of Plants code characteristic number. [a]elliptic = EP; elliptic-lanceolate = EP-LA; lanceolate = LA. [b]narrow = N; medium = M; wide = W. [c]Flat = FL; Epinasty = EP.

Discussion

This study on the almost forgotten olive trees of northwestern Spain aims to provide their first botanical and molecular characterisation, and to compare this local germplasm with that conserved in databases. The results provide a glimpse of the olive diversity that the region may still hold.

The molecular profiles of the 18 examined trees grouped them into nine genotypes, of which three could be identified: ´Brava Gallega´, ´Mansa Gallega´ and ´Cobrancoça´. For now, the identity of the other six genotypes remains unknown. These results agree with those of other studies that have genetically or morphologically characterised centuries-old olive trees in peripheral growing areas; where only a small proportion of those examined represented genotypes with a commercial use (Díez *et al*., 2011; Salimonti *et al*., 2013; Martí *et al*., 2015; Lazovic *et al*., 2016; Sakar *et al*., 2016). Similar results have been reported also for centuries-old grapevines (Martínez & Pérez, 2000; Santiago *et al*., 2005; Gago *et al*., 2009).

SSRs are widely used as markers in the identification of olive genotypes (Cipriani *et al*., 2002; Baldoni *et al*., 2009; Díez *et al*., 2012; Jakše *et al*., 2013; Reboredo *et al*., 2018). In the present work, the loci GAPU059 and UDO99-019 showed low-level polymorphism, and were therefore little informative in identifying the genotypes of the examined trees. Reboredo *et al*. (2018) reported the same for these two loci. Loci UDO043 and ssrOeUA-DCA9 showed the greatest discriminatory power, in agreement with the results of other authors who examined olive material from different areas (Baldoni *et al*., 2009; Salimonti *et al*., 2013; Trujillo *et al*., 2014).

The morphological characteristics of the endocarp, which are considered very stable, are also widely used in olive genotype identification (Barranco *et al*., 2000; Fendri *et al*., 2010). It is also usual to make use of the characteristics of the leaves or drupes. Certainly, the size of the leaves and drupes may differ depending upon the edaphoclimatic conditions, but it should be remembered that in grapevine the effect of 'genotype' dominates that of 'edaphoclimatic conditions' (Martínez & Grenan, 1999). In other words, although the size of the leaves and drupes may be different, their shape is constant. Further, the use of relationships between measurements of different variables eliminates the effect of

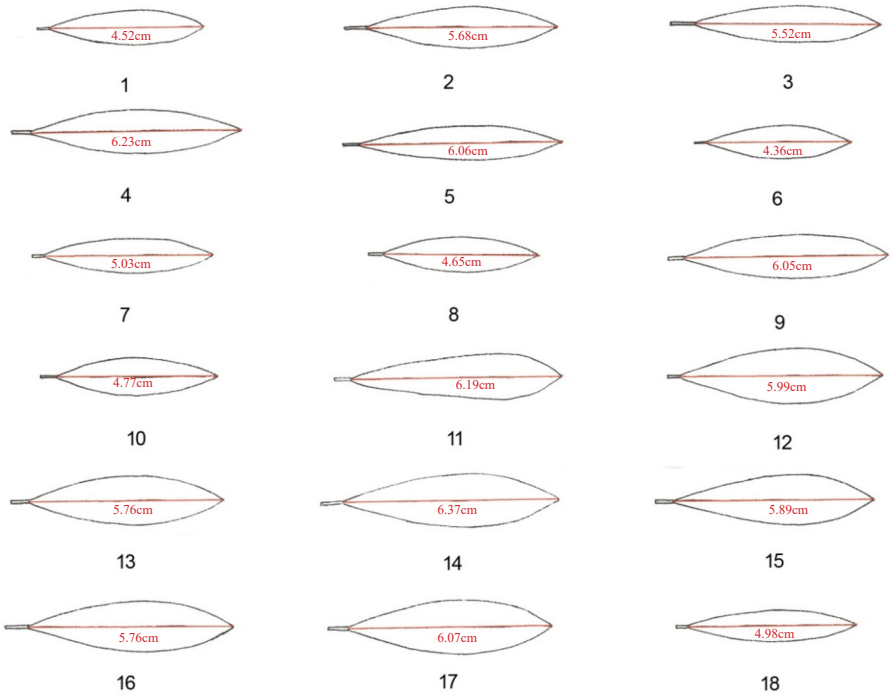

Figure 3. Mean leaf for each of the 18 examined trees, produced according to the adapted method of Martínez & Grenan (1999). Leaves 1, 2, 4, 5 and 10 = Cobrancoça; leaf 3 = Unknown Genotype 3; leaves 6 and 7 = Brava Gallega; leaf 8 = Unknown Genotype 4; leaf 9 = Unknown Genotype 5; leaf 11 = Mansa Gallega; leaf 12 = Unknown Genotype 7; leaves 13, 15, 16 and 17 = Unknown Genotype 1; leaf 14 = Unknown Genotype 6; and leaf 18 = Unknown Genotype 2.

growing conditions, and the mean leaves constructed from them provide an excellent identification tool.

In the present work, the trees with the same molecular profiles fell into the same PCA-determined groups based on their endocarp characteristics. This did not always happen, however, with respect to the leaves; indeed, large qualitative and quantitative differences were seen between trees with identical molecular profiles. Such was the case for the ´Cobrancoça´ trees; these grouped together in terms of their leaf qualitative variables (leaf blade shape, leaf blade width and longitudinal curvature of the leaf), but not in terms of their quantitative variables (leaf lengths and angles). In contrast, the Unknown Genotype 1 trees (13, 15, 16 and 17) grouped together in terms of their qualitative but not their quantitative variables. The same was true for the ´Brava Gallega´ trees (trees 6 and 7). With respect to drupe qualitative characteristics, Unknown Genotype 1 was also heterogeneous, especially in terms of fruit colour (Table 6). This might be explained in that although all fruits were collected on the same day (by different teams), the trees grew in different areas and their fruit may not have been of equal ripeness. Salimonti *et al*. (2013) suggests that many of the differences seen within genotypes could be the result of the existence of different clones, as reported for grapevine (Boso *et al*., 2004; Martínez *et al*., 2005).

It is possible that a larger number of SSR markers might have led to different genotype identifications, though this is unlikely given that 13 were examined. This has been reported in grapevine, although a reduced number (just six) of highly discriminatory SSRs are now recognised that can identify nearly all genotypes (OIV, 2009).

Recently, Reboredo *et al*. (2018) published an article in which cultivated olive material from the same region was examined, and three different genotypes were found among a 32-olive-tree sample; also, using a set of 14 SSRs loci, a total of 37 alleles were reported in the cited work. In the present work, nine different SSRs profiles were found in an 18-olive-tree sample, and a total of 55 alleles detected with a set of 13 SSRs loci. This might be explained in that the present survey covered a much wider sampling area where locations with different numbers of olive trees are present. Historical records for these locations confirmed their past association with active olive cultivation. In addition, the present work selected centuries-old olive trees; these were documented as such in some cases, and at least referred to as such by oral tradition in others.

Table 6. Drupe qualitative characteristics (as set out in the method of Barranco *et al*., 2005) for the studied trees. Results represent the mode for 40 examined drupes; trees 8, 10 and 18 were not included since they produced no fruit.

Sample code	Genotype name	Weight[a]	Shape[b]	Symmetry[c]	Maximum transverse diameter[d]	Apex[e]	Base[f]	Nipple[g]	Over colour at full maturity[h]
		CPVO 8	*CPVO 9*	*CPVO 11*		*CPVO 12*	*CPVO 14*	*CPVO 13*	*CPVO 10*
		UPOV 16	*UPOV 18*	*UPOV 23*		*UPOV 24*	*UPOV 26*	*UPOV 25*	*UPOV 22*
6	Brava Gallega	M	O	S	C	R	T	A	B
7	Brava Gallega	M	O	S	C	R	R	A	B/RW
1	Cobrancoça	M	EL	S	C	P	T	S	V
2	Cobrancoça	H	O	AS	C	R	T	S	B
4	Cobrancoça	H	O	SA	C	R	T	S	B
5	Cobrancoça	H	O	SA	C	R	T	S	B
10	Cobrancoça	-	-	-	-	-	-	-	-
11	Mansa Gallega	L	O	SA	C	R	T	A	B
13	Unknown 1	L	EL	AS	C	P	T	S	V
15	Unknown 1	M	O	AS	C	P	T	S	B
16	Unknown 1	L	EL	AS	C	R	R	S	V
17	Unknown 1	L	EL	AS	C	P	T	S	RW
18	Unknown 2	-	-	-	-	-	-	-	-
3	Unknown 3	M	S	S	B	R	T	S	RW
8	Unknown 4	-	-	-	-	-	-	-	-
9	Unknown 5	M	O	S	C	R	T	A	B/RW
14	Unknown 6	M	EL	AS	C	R	T	S	V
12	Unknown 7	M	O	SA	C	P	T	A	B

CPVO: Community Plant Variety Office code characteristic number; UPOV: International Union for the Protection of New Varieties of Plants code characteristic number. [a]low = L; medium = M; high = H. [b]spherical = S; ovoid = O; elongated = EL. [c]Symmetry of position A: symmetric = S; slightly asymmetric = SA; asymmetric = AS. [d]toward the base = B; centred = C. [e]Form of the apex in position A: pointed = P; rounded = R. [f]Form of the base in position A: truncated = T; rounded = R. [g]Tip or Nipple: absent = A; slight= S; present = P. [h]violet = V; red wine = RW; black = B.

The molecular profile assigned to the genotype ´Brava´ by Reboredo-Rodríguez *et al*. (2018) matches that of ´Brava Gallega´ in the present work (in both cases the SSR profiles were compared to those held in the WOGBC and UCO databases). However, the molecular and morphological (which included only endocarp information) profiles assigned by Reboredo-Rodríguez *et al*. (2018) to the genotype ´Mansa´ (reported as ´Unknown´ by Reboredo-Rodríguez, 2015) did not match those of ´Mansa Gallega´ as determined in the present work and in the consulted WOGBC and UCO databases. It is important to note that the molecular profile and botanical characterisation reported here as identifying the genotype ´Mansa Gallega´ correspond exactly to those recognized by the Spanish Department of Agriculture (MAPAMA, 2017).

The correct molecular characterization of genotypes is important to prevent confusion with other genotypes with similar morphological characteristics and also to use this plant material in breeding programs and in commercial propagation. SSR analysis is a powerful tool for genotype characterization. In olive, intra-genotype genetic diversity has been reported using SSR markers (Muzzalupo *et al*., 2010; Caruso *et al*., 2014; Trujillo *et al*., 2014), for these authors, SSR profiles that are differentiated by one or several dissimilar alleles are classified into the same genotype. These are classified as ´molecular variants´ and are treated as ´clones´ within the main variety due to somaclonal mutations. But in other woody species SSR markers are not considered as an effective approach to detect genetic differences among clones (Imazio *et al*., 2002; Bouhadida *et al*., 2007; Pereira-Lorenzo *et al*., 2007).

The ´Mansa Gallega´ identified in the present work was located in the south of the Province of Pontevedra − a long way from the sampling area studied by Reboredo-Rodríguez *et al*. (2018). However, the trees studied that were identified as belonging to ´Brava Gallega´ were located in the same area studied by the latter authors. Finally, the molecular profile assigned to the genotype ´Picuda´ by Reboredo-Rodríguez *et al*. (2018) was not found among those detected in the

present work. Indeed, neither ´Picual´ nor ´Arbequina´, nor indeed any other genotype cultivated in Spain's most important olive-producing regions, was represented by the examined trees. The olive-growing area closest to Galicia is in northern Portugal; the detection of the Portuguese genotype ´Cobrancoça´ (trees 1, 2, 4, 5 and 10) is therefore not very surprising. Fig. 1 shows all these ´Cobrancoça´ trees to be located within a few kilometres of the Portuguese border. It is rather more surprising that no other specimen of this genotype was found away from this area. It is also of note that no specimens of a genotype extensively grown in Portugal, known as ´Galega´ (Cordeiro *et al*., 2008) - a name that suggests it originated in Galicia - were found in the present study.

Trees 1-10, all known locally under the name of ´Brava´, were found in areas where olive growing has more of a tradition. However, only trees 6 and 7 had a molecular profile that matched with the profile recorded for the genotype ´Brava Gallega´ in the WOGBC and UCO databases. Trees 1, 2, 4, 5 and 10 were found to belong to the genotype ´Cobrancoça´ (Cordeiro *et al*., 2008), and others belonged to unknown genotypes (both in terms of their molecular profile and botanical characteristics). The name ´Brava´ appears to be used locally to refer to many different genotypes; only one of them, of course, is the ´Brava Gallega´ genotype. The term *´brava´* in fruticulture is used to refer to plant grown from a seed and normally used as a seedling rootstock, but in this particular case the olive growers in this area use this term to refer to a number of genotypes with a high agronomic quality and clearly distinct from a wild olive or a rootstock and that they propagate using cuttings.

The second most locally used genotype name was ´Mansa´, but only one tree (tree 11) actually had a molecular profile that matched that deposited in the WOGBC and UCO databases.

The problems of homonyms and synonyms affecting Galicia's olive trees is not the same as that which affects grapevine genotypes (Martínez *et al*., 2018). While grapevine genotypes may have synonyms, they always identify the same genotype. For example, the genotype that goes by the name ´Tempranillo´ in the Rioja winemaking region, is called Tinta Fina in the Ribera del Duero region, and has different names in other areas. However, even though viticulturists may use these different names, they all identify the same genotype through association with the same leaf and cluster characteristics. ´Brava´ and ´Mansa´, in

Table 7. Endocarp qualitative characteristics (as set out in the method of Barranco *et al.*, 2005) of olives from the studied trees. Results represent the mode for 40 examined endocarps; trees 8, 10 and 18 were not included since they produced no fruit.

Sample code	Genotype name	Weight[a]	Shape[b]	Symmetry position A[c]	Symmetry position B[c]	Position of the maximum transverse diam[d]	Shape of the apex[e]
		CPVO16	*CPVO15*	*CPVO17*	*CPVO18*	*CPVO*	*CPVO21*
		UPOV32	*UPOV31*	*UPOV33*	*UPOV34*	*UPOV*	*UPOV37*
6	Brava Gallega	H	EP	SA	S	C	P
7	Brava Gallega	H	EP	SA	S	C	P
1	Cobrancoça	H	EL	A	S	C	P
2	Cobrancoça	VH	EP	SA	S	C	P
4	Cobrancoça	H	EL	SA	S	C	P
5	Cobrancoça	VH	EP	SA	S	C	P
10	Cobrancoça	-	-	-	-	-	-
11	Mansa Gallega	L	EP	SA	S	C	P
13	Unknown 1	M	EP	SA	S	C	P
15	Unknown 1	M	EP	SA	S	C	P
16	Unknown 1	M	EP	A/SA	S	C	P
17	Unknown 1	M	EP	A	S	A	P
18	Unknown 2	-	-	-	-	-	-
3	Unknown 3	M	O	S	S	B	R
8	Unknown 4	-	-	-	-	-	-
9	Unknown 5	M	EP	A/SA	S	A	R
14	Unknown 6	M	EP	SA	S	C	P
12	Unknown 7	H	EP	SA	S	C	P

Table 7. Continued.

Sample code	Genotype name	Shape of the base[e]	Roughness of the surface[f]	Number of vascular bundles[g]	Distribution of vascular bundles[h]	Presence of mucron[i]
		CPVO23	*CPVO24*		*CPVO20*	*CPVO22*
		UPOV39	*UPOV40*		*UPOV36*	*UPOV38*
6	Brava Gallega	P	S	M	R	P
7	Brava Gallega	P	R	M	R	P
1	Cobrancoça	P	S	L	R	P
2	Cobrancoça	P	R	M	R	P
4	Cobrancoça	P	R	L/M	R	P
5	Cobrancoça	P	R	M	R	P
10	Cobrancoça	-	-	-	-	-
11	Mansa Gallega	P	S	L	R	A
13	Unknown 1	P	S	L	R	P
15	Unknown 1	P	S	L/M	R	P
16	Unknown 1	P	S	L	R	P
17	Unknown 1	P	S	L	R	P
18	Unknown 2	-	-	-	-	-
3	Unknown 3	R	R	M	R	A
8	Unknown 4	-	-	-	-	-
9	Unknown 5	P	R	M	R	P
14	Unknown 6	P	S	L	R	P
12	Unknown 7	P	S	L	R	P

CPVO: Community Plant Variety Office code characteristic number; UPOV: International Union for the Protection of New Varieties of Plants. code characteristic number. [a]low = L; medium = M; high = H; very high = VH. [b]ovoid = O; elliptic = EP; elongated = EL. [c]symmetric = S; slightly asymmetric = SA; asymmetric = A. [d]toward the base = B; centred = C; toward the apex = A. [e]pointed = P; rounded = R. [f]smooth = S; rough = R. [g]low = L (less than 7); medium = M (7 to 10). [h]regular = R. [i]present = P; absent = A.

contrast, are not terms that identify respective olive genotypes in Galicia. In conversations with growers in the present work, it was noted that they used the terms with entirely different genotypes. The affirmation by Reboredo-Rodríguez *et al*. (2018) that Galicia ´Mansa´ is a homonym of the genotypes ´Brava´ and ´Mansa´, and that ´Mansa´ is a synonym of the genotype ´Brava´, seems not to hold up.

Trees 3, 8 and 9, which were located very close to one another, each represented an unknown genotype (Unknown Genotypes 3, 4 and 5 respectively), with each showing different molecular and botanical differences. The presence of different unknown genotypes in such a small area hints at the diversity yet to be discovered. Also, tree 18, which was located close to tree 11, was of another unknown genotype (Unknown Genotype 2).

Trees 13, 15, 16 and17 all belonged to Unknown Genotype 1. The age of these trees, plus their being found over a wide area, suggests that the vegetative propagation of olive trees has long been performed in the region. Tree 14 (Unknown Genotype 6) was found in the same cultivation area that trees 13, 16 and 17 (Unknown Genotype 1) but it has a molecular profile that differs in one SSR locus from this genotype (trees 13, 16 and 17). Tree 12 was also found in the same area but its molecular profile differs in one allele for two loci from the Unknown Genotype 1; in addition, this tree also differs from trees of Unknown Genotype 1 in some morphological characteristics, as the absence of nipple in the fruit or the high weight in the endocarp.

The results suggest that Galicia may be a reservoir of olive diversity. This agrees with the thinking of other authors (Trujillo *et al*., 1990; Zohary & Hopf, 1994; Claros *et al*., 2000; Cordeiro *et al*., 2008) who suggest the majority of the region's olive genotypes to be native and to have spread little to other areas. Apart from providing new genetic material, such native genotypes could provide information of use in other scientific studies. For example, studies on the domestication and parentage of olive trees (Trujillo *et al*., 2014; Diez *et al*., 2015) have normally examined genotypes native to more Mediterranean areas. Galicia's native genotypes could add new variability and molecular heterogeneity to be considered in such studies.

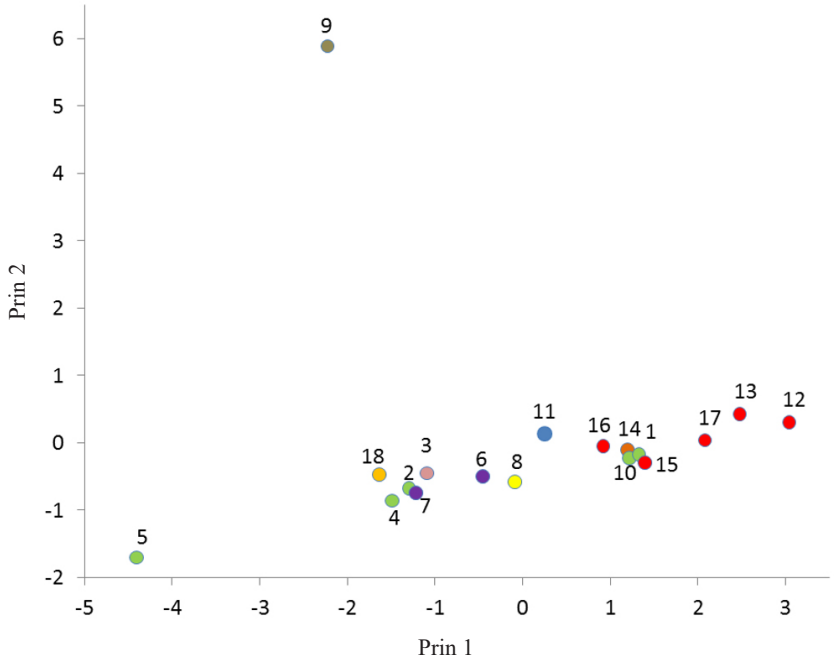

Figure 4. Results of PCA analysis for the leaf relationships Rel 1, Rel 2, Rel 3, Rel 4 and Rel 5, and leaf angle measurements α1 and α2. The different colours identify the trees shown to be identical in the SSR analysis (name of genotypes explained in Fig. 3).

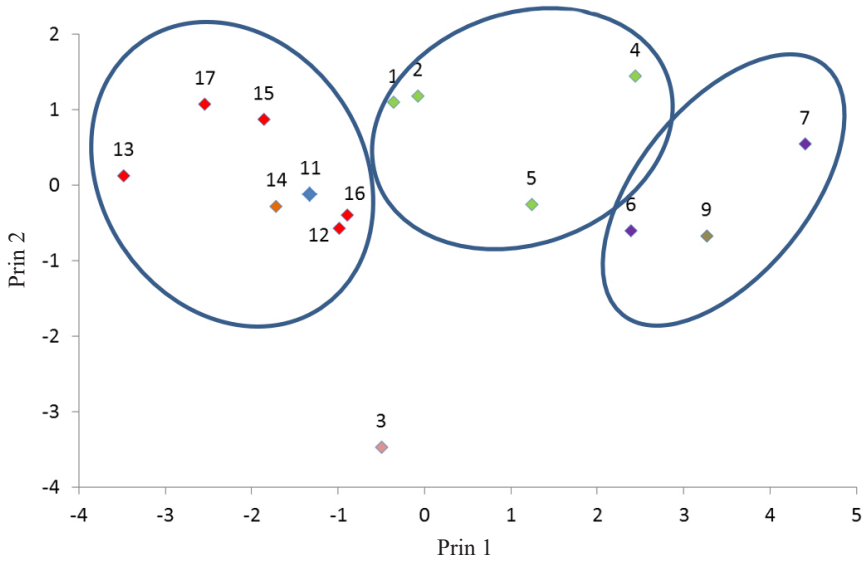

Figure 5. Results of PCA analysis for the drupe and endocarp relationships Rel A, Rel B, Rel C, Rel D, Rel E, Rel F and Rel G. No values were available for trees 8, 10 or 18. The different colours identify the trees shown to be identical in the SSR analysis (name of genotypes explained in Fig. 3).

The present work provides the molecular profiles and complete botanical descriptions of some unreported, local olive genotypes surviving in Galicia. The results identified two potentially native genotypes ´Brava Gallega´ and ´Mansa Gallega´, and clarified certain misidentifications of the latter by other authors. Six unknown genotypes were also detected, as well as the Portuguese genotype ´Cobrancoça´. The evidence suggests that olive trees have been cultivated in the region for centuries, and that the diversity of native genotypes is high. This diversity should be preserved as part of Europe's agricultural heritage, but also because it may offer scientific and commercial opportunities. A larger survey should be performed to determine the

full range of Galicia's olive tree diversity, followed by agricultural studies that might indicate the potential of the region's rediscovered genotypes.

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References

Alonso de Herrera G, 1513. Agricultura general. Imprenta Real, Madrid, Spain.

Baldoni L, Cultrera NG, Mariotti R, Ricciolini C, Arcioni S, Vendramin GG, Buonamici A, Porceddu A, Sarri V, Ojeda MA, *et al*., 2009. A consensus list of microsatellite markers for olive genotyping. Mol Breeding 24 (3): 213-231. https://doi.org/10.1007/s11032-009-9285-8

Barranco D, Cimato A, Fiorino P, Rallo L, Touzani A, Castañeda C, Serafini F, Trujillo I, 2000. World olive catalogue of olive varieties. International Olive Oil Council, Madrid, Spain.

Barranco D, Trujillo I, Rallo L, 2005. Variedades de olivo en España. Mundi-Prensa, Madrid, Spain.

Bartolini G, Prevost G, Messeri C, Carignani C, 2005. Olive germplasm: genotypes and world-wide collections. FAO SaPGRSo (ed), FAO, Rome.

Belaj A, Satovic Z, Rallo L, Trujillo I, 2002. Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor Appl Genet 105: 638-644. https://doi.org/10.1007/s00122-002-0981-6

Belaj A, Cipriani G, Testolin R, Rallo L, Trujillo I, 2004. Characterization and identification of the main Spanish and Italian accessions by simple sequence repeat markers. Hort-Science 39: 1557-1561. https://doi.org/10.21273/HORTSCI.39.7.1557

Belaj A, Leon L, Satovic Z, de la Rosa R, 2011. Variability of wild olives (*Olea europaea* subsp *europaea* var. *sylvestris*) analyzed by agro-morphological traits and SSR markers. Sci Hortic 129 (4): 561-569. https://doi.org/10.1016/j.scienta.2011.04.025

Boso S, Santiago JL, Martínez MC, 2004. Intravarietal agronomic variability in the cv. Albariño (*Vitis vinifera* L.). Am J Enol Vitic 55: 279-282.

Bouhadida M, Casas AM, Moreno MA, Gogorcena Y, 2007. Molecular characterization of Miraflores peach variety and relatives using SSRs. Sci Hort 111: 140-145. https://doi.org/10.1016/j.scienta.2006.10.018

Carriero F, Fontanazza G, Cellini F, Giorio G, 2002. Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theor Appl Genet 104: 301-307. https://doi.org/10.1007/s001220100691

Caruso T, Marra FP, Costa F, Campisi G, Macaluso L, Marchese A, 2014. Genetic diversity and clonal variation within the main Sicilian olive cultivars based on morphological traits and microsatellite markers. Sci Hortic 180: 130-138. https://doi.org/10.1016/j.scienta.2014.10.019

Cipriani G, Marrazzo MT, Marconi R, Cimato A, Testolin R, 2002. Microsatellite markers isolated in olive (*Olea europaea* L) are suitable for individual fingerprinting and reveal polymorphism within ancient genotypes. Theor Appl Genet 104: 223-228. https://doi.org/10.1007/s001220100685

Claros MG, Crespillos R, Aguilar ML, Canovas FM, 2000. DNA fingerprinting and classification of geographically related genotypes of olive-tree (*Olea europaea* L.) Euphytica 116: 131-142. https://doi.org/10.1023/A:1004011829274

Contreras JM, 1798. Historia del célebre santuario de Nuestra Señora de Las Hermitas, situada en las montañas que baña el rio Bibey en tierra del Bollo, Reyno de Galicia, y Obispado de Astorga. Ed. Francisco de Toxar, Salamanca, Spain.

Cordeiro AI, Sanchez-Sevilla JF, Alvarez-Tinaut MC, Gomez-Jimenez MC, 2008. Genetic diversity assessment in Portugal accessions of *Olea europaea* by RAPD markers. Biol Plantarum 52 (4): 642-647. https://doi.org/10.1007/s10535-008-0125-1

De La Rosa R, James CM, Tobutt KR, 2002. Isolation and characterization of polymorphic microsatellites in olive (*Olea europaea* L.) and their transferability to other genera in the Oleaceae. Mol Ecol Notes 2 (3): 265-267. https://doi.org/10.1046/j.1471-8286.2002.00217.x

Diez CM, Trujillo I, Barrio E, Belaj A, Barranco D, Rallo L, 2011. Centennial olive trees as a reservoir of genetic diversity. Ann Bot 108: 797-807. https://doi.org/10.1093/aob/mcr194

Diez CM, Imperato A, Rallo L, Barranco D, Trujillo I, 2012. Worldwide core collection of olive genotypes based on simple sequence repeat and morphological markers. Crop Sci 52: 211-221. https://doi.org/10.2135/cropsci2011.02.0110

Diez CM, Trujillo I, Martínez-Urdiroz N, Barranco D, Rallo L, Marfil P, Gaut BS, 2015. Olive domestication and diversification in the Mediterranean Basin. New Phytol 206: 436-447. https://doi.org/10.1111/nph.13181

Dos Santos ARF, Ramos-Cabrer AM, Diaz-Hernandez MB, Pereira-Lorenzo S, 2011. Genetic variability and diversification process in local pear genotypes from northwestern Spain using microsatellites. Tree Genet

Genomes 7 (5): 1041-1056. https://doi.org/10.1007/s11295-011-0393-3

Espinosa-Sánchez J, 2010. Mito y realidad del aceite de Quiroga. GDR Ribeira Sacra, Lugo, Spain.

Fendri M, Trujillo I, Trigui A, Rodriguez-Garcia MI, Ramirez JDA, 2010. Simple sequence repeat identification and endocarp characterization of olive tree accessions in a Tunisian germplasm collection. Hortscience 45: 1429-1436. https://doi.org/10.21273/HORTSCI.45.10.1429

Fendri M, Ferreira E, Calado L, Abreu I, Rodríguez-García MI, Amorim MI, Alche JD, 2014. Discrimination of Portuguese and Spanish olive genotypes using microsatellite markers. Acad J Agric Res 2: 58-61.

Fernández de la Cigoña E, Martínez X, 2003. O aceite en Galicia guía das lagaretas castrexo-romanas, medievais e modernas. Ed. Asociación Galega para a Cultura e a Ecoloxía, Pontevedra, Spain.

Gago P, Santiago JL, Boso S, Alonso-Villaverde V, Grando S, Martínez MC, 2009. Biodiversity and characterization of twenty-two *Vitis vinifera* L. genotypes in the Northwestern Iberian Peninsula. Am J Enol Vit 60 (3): 293-301.

Gil FS, Busconi M, Machado AD, Fogher C, 2006. Development and characterization of microsatellite loci from *Olea europaea*. Mol Ecol Notes 6 (4): 1275-1277. https://doi.org/10.1111/j.1471-8286.2006.01513.x

Gómez MC, Delgado FJ, Parra MC, 2012. Identificación de variedades de olivo cultivadas en Extremadura mediante marcadores morfológicos y moleculares. Universidad de Extremadura Servicio de Publicaciones, Badajoz, Spain.

Haouane H, El Bakkali A, Moukhli A, Tollon C, Santoni S, Oukabli A, El Modafar C, Khadari B, 2011. Genetic structure and core collection of the World Olive Germplasm Bank of Marrakech: towards the optimised management and use of Mediterranean olive genetic resources. Genetica 139 (9): 1083-1094. https://doi.org/10.1007/s10709-011-9608-7

Hidalgo-Tablada J, 1870. Tratado del cultivo de la vid en España y modo de mejorarlo. Librería de la Señora Viuda e Hijos de Don José Cuesta, Madrid.

Imazio S, Labra M, Grassi F, Winfield M, Bardini M, Scienza A, 2002. Molecular tools for clone identification: The case of the grapevine cultivar 'Traminer'. Plant Breed 121 (6): 531-535. https://doi.org/10.1046/j.1439-0523.2002.00762.x

Jakše J, Štajner N, Tomić L, Javornik B, 2013. Application of microsatellite markers in grapevine and olives. In: The Mediterranean genetic code - Grapevine and olive; Sladonja B & Poljuha D (eds.). pp: 25-50. IntechOpen. https://doi.org/10.5772/53411

Lazovic B, Adakalic M, Pucci C, Perovic T, Bandelj D, Belaj A, Mariotti R, Baldoni L, 2016. Characterizing ancient and local olive germplasm from Montenegro. Sci Hortic 209: 117-123. https://doi.org/10.1016/j.scienta.2016.06.022

Liu K, Muse SV, 2005. Power marker: Integrated analysis environment for genetic marker data. Bioinformatics 21 (9): 2128-2129. https://doi.org/10.1093/bioinformatics/bti282

MAPA, 2018. Anuario de estadística agraria 2017. Ministerio de Agricultura, Pesca y Alimentación. https://www.mapa.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica [11/04/2019].

MAPAMA, 2017. Resolución de la Dirección General de Producciones y Mercados Agrarios de 23 de Octubre de 2017, por la que se reconocen oficialmente una serie de variedades de especies frutales, propuestas por la Dirección General de Ganadería, Agricultura e Industrias Alimentarias de la Xunta de Galicia. Dirección de Validación: https://sede.administracion.gob.es/pagSede-Front/servicios/consultaCSV.htm (Código Seguro de Verificación: CSV: GEN-68e0-ec35-2596-15ab-d855-e52f-2900-2339). [11/04/2017].

Martí AFI, Forcada CFI, Company RSI, Rubio-Cabetas, MJ, 2015. Genetic relationships and population structure of local olive tree accessions from Northeastern Spain revealed by SSR markers. Acta Physiol Plant 37: 1726. https://doi.org/10.1007/s11738-014-1726-2

Martínez MC, 2007. La colección de variedades de vid del Consejo Superior de Investigaciones Científicas (Misión Biológica de Galicia). La Semana Vitivinícola 3198: 3926-3929.

Martínez MC, Genan S, 1999. A graphic reconstruction method of an average leaf of vine. Agronomie 19: 491-507. https://doi.org/10.1051/agro:19990607

Martínez MC, Pérez JE, 2000. The forgotten vineyard of the Asturias Princedom (north of Spain) and ampelographic description of its genotypes (*Vitis vinifera* L). Am J Enol Vit 51 (4): 370-378.

Martínez MC, Boso S, Santiago JL, 2005. Los clones de Albariño (*Vitis vinifera* L) seleccionados en el Consejo Superior de Investigaciones científicas. Editorial CSIC, Madrid, Spain.

Martínez MC, Boso S, Gago P, Muñoz-Organero G, De Andrés M, Gaforio L, Cabello F, Santiago JL, 2018. Value of two Spanish live grapevine collections in the resolution of synonyms, homonyms and naming errors. Aus J Grape Wine Res 24 (4): 430-438. https://doi.org/10.1111/ajgw.12348

Murray MG, Thompson WF, 1980. Rapid isolation of high molecular weight plant DNA. Nucl Acid Res 8: 4321-4325. https://doi.org/10.1093/nar/8.19.4321

Muzzalupo I, Chiappetta A, Benincasa C, Perri E, 2010. Intra-cultivar variability of three major olive cultivars grown in different areas of central-southern Italy and studied using microsatellite markers. Sci Hortic 126: 324-329. https://doi.org/10.1016/j.scienta.2010.07.014

OIV, 2009. OIV descriptor list for grape varieties and Vitis species (2nd ed). International Organisation of Vine and

Wine, http://www.oiv.int/oiv/info/enplubicationoiv#grape. [28/04/2017].

Peakall R, Smouse PE, 2006. GENALEX 6: Genetic analysis in Excel Population genetic software for teaching and research. Mol Ecol Notes 6: 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE, 2012. GenAlEx 65: Genetic analysis in Excel Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Pereira-Lorenzo S, Fernandez-Lopez J, 1997. Description of 80 genotypes and 36 clonal selections of chestnut (*Castanea sativa* Mill) from Northwestern Spain. Fruit Var J 51 (1): 13-27.

Pereira-Lorenzo S, Ramos-Cabrer AM, Díaz-Hernández, MB, 2007. Evaluation of genetic identity and variation of local apple cultivars (*Malus* x *domestica* Borkh.) from Spain using microsatellite markers. Genet Resour Crop Evol 54: 405-420. https://doi.org/10.1007/s10722-006-0003-7

Pereira-Lorenzo S, dos Santos ARF, Ramos-Cabrer AM, Sau F, Diaz-Hernandez MB, 2012. Morphological variation in local pears from north-western Spain. Sci Hortic 138: 176-182. https://doi.org/10.1016/j.scienta.2012.02.007

Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I, 2005. Variedades de olivo en España. Coed. Junta de Andalucía, MAPA & Ed Mundi-Prensa, Madrid.

Reboredo-Rodríguez P, 2015. Caracterización aromática y fenólica de aceitunas y aceites de oliva producidos en Galicia. Doctoral thesis. Univ. Vigo, Ourense, Spain.

Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B, Simal-Gándara J, 2014a. Improvements in the malaxation process to enhance the aroma quality of extra virgin olive oils. Food Chem 158: 534-545. https://doi.org/10.1016/j.foodchem.2014.02.140

Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B, Simal- Gándara J, 2014b. Quality of extra virgin olive oils produced in an emerging olive growing area in north-western Spain. Food Chem 164 (1): 418-426. https://doi.org/10.1016/j.foodchem.2014.05.043

Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B, Fregapane G, Salvador MD, Simal-Gándara J, 2015. Characterisation of extra virgin olive oils from Galician autochthonous varieties and their co-crushings with Arbequina and Picual cv. Food Chem 176: 493-503. https://doi.org/10.1016/j.foodchem.2014.12.078

Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B, Simal-Gándara J, Trujillo I, 2018. Genotypic and phenotypic identification of olive genotypes from north-western Spain and characterization of their extra virgin olive oils in terms of fatty acid composition and minor compounds. Sci Hort 232: 269-279. https://doi.org/10.1016/j.scienta.2018.01.015

Sakar E, Unver H, Ercisli S, 2016. Genetic diversity among historical olive (*Olea europaea* L) genotypes from southern Anatolia based on SSR markers. Biochem Genet 54 (6): 842-853. https://doi.org/10.1007/s10528-016-9761-x

Salimonti A, Simeone V, Cesari G, Lamaj F, Cattivelli L, Perri E, Desiderio F, Fanizzi FP, Del Coco L, Zelasco S, 2013. A first molecular investigation of monumental olive trees in Apulia region. Sci Hortic 162: 204-212. https://doi.org/10.1016/j.scienta.2013.08.005

Santiago JL, Boso S, Martín JP, Ortiz, JM, Martínez, MC, 2005. Characterization and identification of grapevine genotypes (*Vitis vinifera* L) from northwestern Spain using microsatellite markers and ampelometric methods. Vitis 44 (2): 67-72.

Sarri V, Baldoni L, Porceddu A, Cultrera NGM, Contento A, Frediani M, Belaj A, Trujillo I, Cionini PG, 2006. Microsatellite markers are powerful tools for discriminating among olive genotypes and assigning them to geographically defined populations. Genome 49 (12): 1606-1615. https://doi.org/10.1139/g06-126

Sefc KM, Lopes S, Mendonca D, Dos Santos MR, Machado MLD, Machado AD, 2000. Identification of microsatellite loci in olive (*Olea europaea* L) and their characterization in Italian and Iberian olive trees. Mol Ecol 9: 1171-1173. https://doi.org/10.1046/j.1365-294x.2000.00954.x

Trujillo I, Ojeda MA, Urdiroz NM, Potter D, Barranco D, Rallo L, Diez CM, 2014. Identification of the Worldwide Olive Germplasm Bank of Córdoba (Spain) using SSR and morphological markers. Tree Genet Genomes 10: 141-155. https://doi.org/10.1007/s11295-013-0671-3

Vargas-Gómez P, Talavera-Lozano S, 2012. *Olea*. In: Flora Ibérica Plantas vasculares de la Península Ibérica e Islas Baleares; Andrés C, Talavera S, Quintanar A (eds.). Vol 11, pp: 136-139. Real Jardín Botánico, CSIC, Madrid. http://www.floraiberica.org/ [29/04/2017].

Viñuales J, 2007. Variedades de olivo del Somontano. Área de Desarrollo de la Diputación de Huesca, Instituto de Estudios Altoaragoneses, Huesca, Spain.

Zohary D, Hopf M, Weiss E, 1994. Olive: *Olea europaea*. In: Domestication of plants in the Old World, pp: 137-143. Clarendon Press, Oxford, England.