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Current situation and characterization of the Eurasian wild grapevine in Asturias region (Northwest of the Iberian Peninsula)

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Summary

This work shows the location and study of relic populations of the Eurasian wild grapevine *Vitis vinifera* ssp. *sylvestris* (Gmelin) Hegi in the Asturias region (NW of the Iberian Peninsula). The comprehensive study includes the description of their habitats, of the main botanical supporters of these lianas and of the parasitic species causing them biological stress, the ampelographic description of the populations, wine elaboration from bunches of female vines and a genetic study based on 240 nuclear SNP data. The results obtained have allowed to verify the abundance of this genetic resource in the eastern and central areas of the region. The species that support this liana were different depending on the position (alluvial, colluvial or coastal) of the populations. Several ampelographic differences have been confirmed between male and female individuals. The erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eryophidae) and mildews were the most frequent parasitic species found in the populations. Berries presented a low sugar content, and wines a low alcoholic content and a high total polyphenol index and colour intensity. Feral Vitaceae were found accompanying some of the *sylvestris* populations mainly in riverbanks and colluvial positions. The genetic study showed a clear distinction of the *sylvestris* vines from the most common grape varieties in the region, even though one of the *sylvestris* has a compatible parent-offspring relationship with the variety 'Camaraou Noir'. The progressive reduction of this subspecies as a consequence of the human activities, plagues and diseases highlights the importance of regulating its conservation in order to avoid its extinction.

Keywords

ampelography, ecology, microvinifications, parasitic species, SNP, *Vitis vinifera* ssp. *sylvestris* (Gmelin) Hegi.

Introduction

The four glacial periods along the Quaternary age have marked the distribution worldwide of the habitats of the botanical communities and the appearance of new species belonging to the same genus. In the case of the *Vitis* L. genus, in the current territory of the United States of America this Vitaceae could emigrate towards more southern latitudes because in this geographical area the mountain ranges have a North–South orientation. The same happened in the most oriental part of China, a geographical area where the relief is slightly pronounced, and another proliferation of *Vitis* species took also place. So, at present time, in both geographical areas, some tens of *Vitis* species can be found (Zohary and Spiegel-Roy, 1975).

In the rest of the Eurasian continent, the mountain ranges have an East-West orientation. So, the migration capacity towards the South was restricted in the glacial periods (Lehmann and Böhm, 2011). Nowadays, from the Iberian Peninsula up to Afghanistan (Hindu Kush mountain-range) there is only one wild native grapevine, *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi (Arnold, 2002), which sheltered mainly in Transcaucasia and in the South of the Mediterranean basin (Huglin, 1986). Some populations can also be found in the African Maghreb. According to Ocete *et al.* (2007), this subspecies can be found between the parallels 30–31°N (Ourika river, Morocco) and the 49–50°N in the Rhine river (Ludwigshafen, Germany).



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The Eurasian wild subspecies is dioecious and constitutes the wild parental of cultivars belonging to the taxon *Vitis vinifera* L. subspecies *sativa* (DC.) Hegi, which are generally hermaphrodite (Levadoux, 1956). Only in very few cases hermaphrodite wild vines with tricolporated pollen appear, due to mutation of male specimens (Ocete *et al.*, 2007). Recent data suggest that dioecy was lost during domestication through a rare recombination event between male and female haplotypes (Massonnet *et al.* 2020). These hermaphrodite vines were selected by humans, due to their power of self-pollination and, in consequence, of a higher level of fecundation success and fruitset to produce a higher harvest than the female ones. Then they were chosen to establish plantations outside natural habitats from the Neolithic period (McGovern, 2003).

The Eurasian wild grapevine is a hydrophilic liana, climbing thanks to tendrils that use accompanying vegetation as supporters, mainly trees and shrubs, in order to access to an adequate light intensity, required for flowering. It needs soils submitted to a constant renewal of substratum and variable periods of flood (Ocete *et al.*, 2004). Its main habitats are azonal alluvial formations, mainly riverbank ripisilvas composed of deciduous species developed along rivers and creeks. In Central Europe and in the South of the Caucasian region, some populations develop on floodplains, usually of the big rivers (Terpó, 1969; Schumann, 1974). Other populations can be found in the mouth of some rivers, growing on arenosols, as well as in the coastal areas or in the shores of lakes and lagoons. Some populations are situated in colluvial position, on the slopes of mountains and hills or growing on cliffs of coastal areas.

The wild grape bunches were the material of the first vinifications carried out by human beings. Their berries have been reported in Spain as human food in diverse epochs, from the Paleolithic (Rivera and Walker, 1989) up to the last post-war (mid 20th century), and also for the production of home-made vinegar, e.g. in Grazalema Mountain range (Cadiz, Spain) till the end of past century. This vinegar was, with halite, the main preservative of food for centuries. The pharmacopeia is full of medicinal remedies that have its origin in the wild vine, principally from the sap, leaves and must (Ocete *et al.*, 2020). The vine shoots have been used for the manufacture of ropes, used even by the Spanish Navy fleet, and hoops for the construction of fishing traps (Ocete *et al.*, 2011). These wild vines have been also used as native rootstocks very well adapted to the Balkan region (Zimmermann, 1958). Finally, it is necessary to remark the presence of wild or cultivated seeds in burials of diverse ages, because grapevine bunches were part of funeral offerings from the Bronze age up to the Paleo-Christian one (Torres-Vila and Mosquera, 2004).

Before of the arrival of the powdery and downy mildews, the wild grapevine was very common. But these diseases were responsible for the death of the most sensitive vines of every population (Ocete *et al.*, 2007) and, together with the human impact, drastically reduced the presence of the wild grapevines in natural ecosystems (Arnold, 2002). The agricultural use of the fertile soils of the riverbanks, the transformation of the water courses and dams construction, the cleanliness of the

banks, the modernization of the road network, the construction of recreational free time areas and car parking lots near beaches (Ocete *et al.*, 2007) and the use of weeding machines and herbicides to keep clean the ditches of the roads and highways (Martínez de Toda, 1991) have caused that currently its geographical distribution is usually highly fragmented in small isolated relic populations (Scali *et al.*, 2018). Another problem that hinders the renewal of the wild grapevines happens in areas with high cattle growing and hunting, where herbivorous massively eat the new seedlings (Ocete *et al.*, 2007).

The massive use of American rootstocks and *Vitis* interspecific crossings used for grape production (frequently called direct producer hybrids or French hybrids) to reconstitute the vineyards destroyed by phylloxera brought another pernicious consequence: These Vitaceae, when escaped from vineyards, are invasive plants which have displaced the autochthonous wild grapevine of their natural habitats (Arrigo and Arnold, 2007; Iriarte-Chiapusso *et al.*, 2013).

To try to stop this destructive process for the taxon, some European countries, like France, Austria, Germany and Hungary, have a specific legal agenda for the wild grapevine. The reality is that its implementation is stalled, as it was observed in the French Basque Country (Rodríguez-Miranda *et al.*, 2016).

The aim of this work was to locate and characterize the wild grapevine populations in the territory of Asturias, North-western Spain. The study of wild grapevine populations *in situ* is a very complicated task, which in many cases lasts for several years, during which the disappearance of individuals is common, in many cases due to human action. For this reason, many aspects of this study have been addressed in a global manner, providing descriptive data rather than quantitative data, on specific populations or as a whole. Thus, the populations have been georeferenced, studied genetically, and their habitats and male and female individuals have been described. Information about the local traditional uses of this subspecies, parasitic species, the main supporters from the accompanying vegetation and the anthropogenic negative impacts on these populations have been collected. The final objective is to underline the need to conserve these populations and to safeguard them through a legal figure of specific protection for this taxon.

Material and Methods

1. Plant material

Surveys were carried out in the different ecosystems of Asturias: river-bank forests, colluvial positions and coastline (cliffs and arenosols) during the vegetative season between 1998-2019. Habitats and GPS coordinates of the vines at the ends of the populations were noted (Supplemental Table 1). The main supporters from the accompanying vegetation were identified using botanical keys and confirmed with publications on the regional flora (Díaz *et al.*, 1994; Fernández *et al.*, 2014). Information obtained asking local people about traditional uses of this subspecies and the presence of feral *Vitaceae* were also noted.

2. Ampelographic descriptions

They were carried out *in situ* along the annual development of the vines in river bank forests and coastal areas, more specifically in a sample of 30 individuals of each sex from the Deva river (Pop 2), Cares river (Pop 7) and España beach (Pop 33) populations. Descriptions were performed by two ampelographers for a minimum of two seasons, following the methodology of the «OIV descriptor list for grape varieties and *Vitis* species» (OIV, 2009). At flowering time (June) the sex of the individuals was noted. In total, 95 ampelographic descriptors of young shoot, shoot, young leaf, mature leaf, woody shoot, flower, inflorescence, bunch, berry, phenology and vigor were recorded.

3. Parasitic species

In parallel with the ampelographic descriptions, the determination of parasitic species was carried out on aerial organs: leaves, sarments and bunches. In the case of roots, they were unearthed up to a maximum deep of 40 cm to evaluate the presence of *Daktulosphaira vitifoliae* (Fitch), *Armillaria mellea* (Vahl.) and *Rosellinia necatrix* (Hartig).

4. Wine elaboration and analysis

Harvests were carried out in the second fortnight of October 2017 on 2-6 exemplars of three populations (Deva and Cares rivers and España beach: Pop 2, 7 and 33 respectively) with a yield enough to elaborate wine. Bunches were destemmed by hand, discarding visually immature berries. They were squeezed by a manual press, without breaking the seeds. Each must sample obtained after pressing was weighted, and later the corresponding seeds and skins from each sample were added to the must to start the fermentation. Fermentations were conducted at 20°C with native yeasts, grape pomace being punched down manually twice a day for 15-18 days, until the alcoholic fermentation ended. No sulphites were added to the musts or wines.

Musts and wines were analysed following the procedures described by OIV (2015) and detailed in Maghradze *et al.* (2020). Brix degree in the musts was determined by refractometry. In the wines, the following parameters were determined: alcoholic degree (Near-Infrared spectroscopy, SpectraAlyzer Wine), pH and total acidity (automatic potentiometry, CDR WineLab Analyzer), tartaric and L-malic acids (enzymatic determination, Cetlab 600), total polyphenol index (TPI), anthocyanins and colour intensity (spectrometric determination, LAMBDA 265 PDA UV/Visible Spectrophotometer).

5. DNA analysis

Genomic DNA was extracted from leaf tissue of 36 vines from different populations (Supplemental Table 1) using Qiagen Plant DNeasy kit as recommended by the manufacturer. DNAs were profiled for 240 nuclear SNP markers, through the genotyping services provided by the Centro Nacional de Genotipado (CEGEN) using Veracode or iSelect technology. This set includes a core set of 48 SNPs used for cultivar identification (Cabezas *et al.*, 2011) and an extended

set of 192 SNPs for parentage and diversity analyses (Cunha *et al.*, 2020).

Genetic profiles were compared with the about 3000 genotypes available in the ICVV-SNP database for the core set of 48 SNPs. Two genetic structure analyses were done, one including all the non-redundant genotypes obtained for the wild vines in this study and another including additionally the genotypes of nine grape cultivars common in Asturias region and nearby: 'Albarín Blanco' (variety number at the *Vitis* International Variety Catalogue-VIVC (www.vivc.de): 22838), 'Alfrocheiro' (= 'Bruñal', VIVC 277), 'Alvarinho' (= 'Albariño', VIVC 15689), 'Carrasquín' (VIVC 2123), 'Mencia' (VIVC 7623), 'Prieto Picudo Tinto' (VIVC 9694), 'Savagnin' (= 'Traminer' (VIVC 17636), 'Trousseau Noir' (= 'Merenzao', (VIVC 12668), and 'Verdelho Branco' (= 'Verdejo Blanco', VIVC 22304), taken from the ICVV-SNP database. For these analyses, 206 SNPs with less than 15 % of missing data in the genotypes of the wild samples were used. First, a dissimilarity matrix was calculated using the DARwin software package v. 6.0.21 (Perrier and Jacquemoud-Collet, 2006). This matrix was used for a factorial analysis using Principal Coordinate analysis (PCoA) and to construct an Unweighted Neighbour Joining (UwNJ) distance tree based on 10,000 bootstrap replicates.

A parentage analysis was done using the non-redundant 240-SNPs genotypes obtained in this work and those stored in the ICVV-SNP database to detect possible first-order kinship relationships (trios and duos or parent-offspring pairs), using the likelihood-based method implemented in Cervus v.3.0 (Kalinowski *et al.*, 2007) as previously detailed (Cunha *et al.*, 2020). The likelihood of each detected trio and duo was evaluated considering the natural logarithm of the overall likelihood ratio (LOD) score.

Results

1. Plant material

The situation of the populations studied is compiled in Supplemental Table 1 and shown in Fig. 1, being most of them situated between the eastern and central areas of the region.

Wild grapevine populations are relic in the majority of the nuclei found. They were located in different positions and used different trees and shrubs as supporters to climb. In alluvial positions, along river-bank forests, the main supporters found are: *Acer campestre*, *Acer monspessulanum*, *Alnus glutinosa*, *Crataegus monogyna*, *Cornus sanguinea*, *Corylus avellana*, *Fraxinus excelsior*, *Ficus carica*, *Genista occidentalis*, *Laurus nobilis*, *Populus nigra*, *Rosa sempervirens*, *Rubus ulmifolius*, *Salix atrocinerea*, *Salix eleagnos*, *Salix spinosa*, *Tilia platyphyllos*, *Ulex europaeus*, *Ulmus glabra* (Fig. 2).

In colluvial position in Cares valley, the main supporter is *Prunus spinosa* (Fig. 3). On the other hand, in scattered remains of wooded areas, surrounded usually by artificial grasslands, used for the exploitation of cattle in many cases and

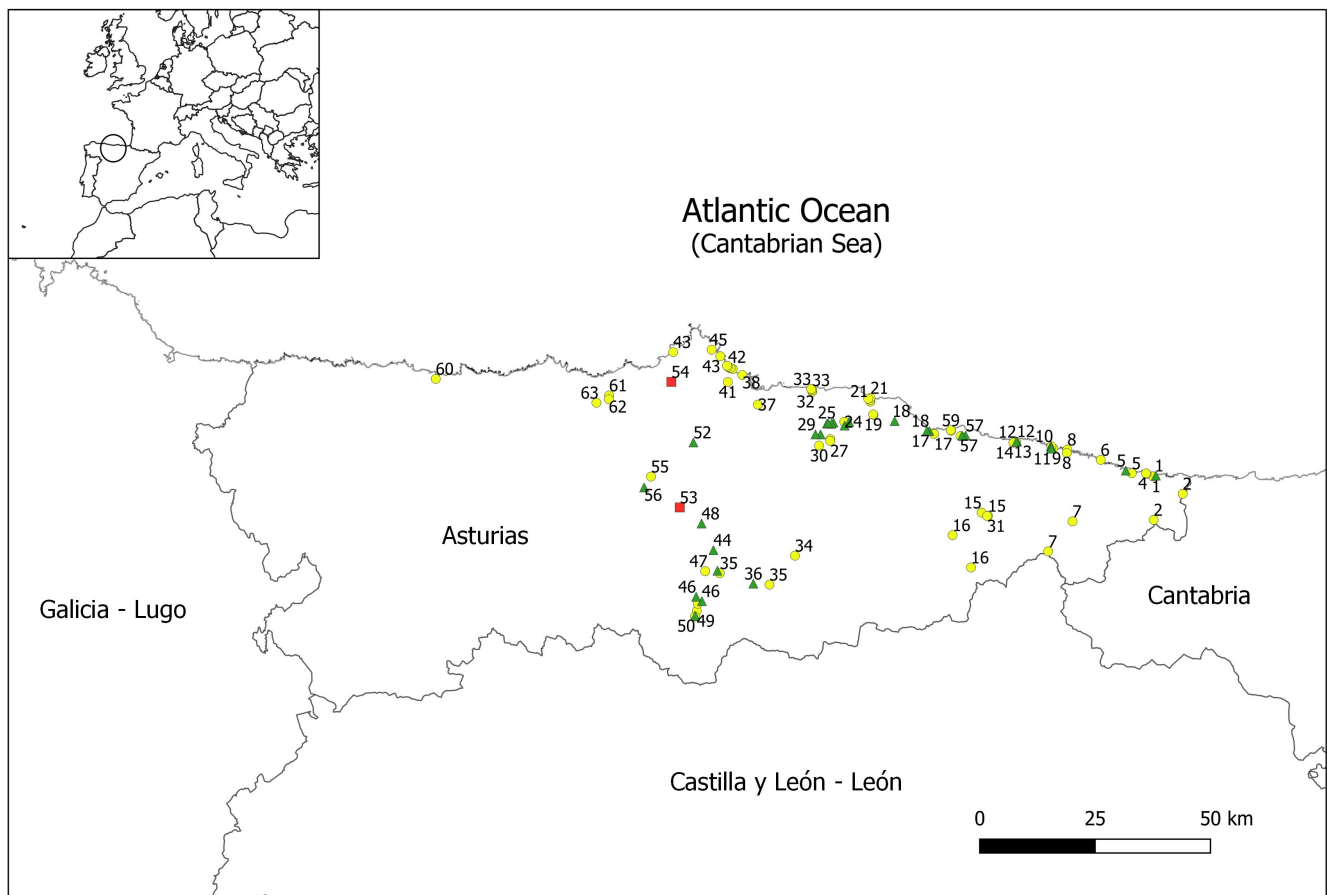


Fig. 1. Localization of Asturias and distribution of the wild vines found. Yellow circles: Wild vines; Green triangles: wild vines analysed for SNP; Red squares: feral Vitaceae. Numbers correspond to wild vine populations (Supplemental Table 1).



Fig. 2: Exemplar of wild grapevine climbing on its botanical supporter in the Deva river bank forest.

also in very residual places conserving small rests of natural vegetation within urban areas, the main supporters are: *Ficus carica*, *Fraxinus excelsior*, *Laurus nobilis*, *Prunus spinosa*, *Rosa canina*, *Rosa sempervirens*, *Rubus ulmifolius*, *Salix alba*, *Sambucus nigra* and *Ulex europaeus*.

In coastal positions, mainly growing on cliffs or sandy soils, the main vine supporters are: *Calluna vulgaris*, *Erica mackayana*, *Erica tetralix*, *Erica vagans*, *Ficus carica*, *Genista anglica*, *Genista occidentalis*, *Olea europea* var. *sylvestris*, *Ulex europaeus* and *Ulex minor* (Fig. 4).

Accompanying the vines, other species of lianas are very frequent in different habitats, such as *Clematis vitalba*, *Hedera helix*, *Humulus lupulus*, *Lonicera periclymenum*, *Rubia perigrina* and *Smilax aspera*.

Concerning feral Vitaceae, the presence of rootstocks and French hybrids was frequent in several populations, mainly in riverbanks and some colluvial positions. The biggest group of them with a total of 21 individuals is situated close to Caudal river (43°18'28.2"N, 5°52'28.1"W). Among them, exemplars of Riparia Gloire de Montpellier, Rupestris du Lot and Jacquez were the most numerous. Also, near the locality of Avilés (43°33'6.6"N, 5°54'31.3"W), six vines of Rupestris du Lot and two of Jacquez were found among wild grapevine liana (see red squares in Fig. 1).



Fig. 3: Wild grapevines using mainly *Prunus spinosa* bushes as supporters in a colluvial position (Cares valley).



Fig. 4: The karstic landscape of Gulpiyuri beach, where the grapevines grow on the calcareous wall above the high tide level.

About the traditional uses of wild grapevine in Asturias, the only testimony found was the construction of hoops of fishing traps for lobster around Toranda beach.

2. Ampelographic descriptions

The main characteristics of female and male specimens are described below on a sample of 30 individuals of each sex (Table 1) from Pop 2 (Deva river), Pop 7 (Cares river) and Pop 33 (España Beach).

The tip of the young shoot is fully open in exemplars of individuals of both genders, while its intensity of anthocyanin pigmentation is none or low with edged appearance. The size of the mature leaf usually is medium in female and small, sometimes very small in male exemplars. The number of the lobes is smaller in the case of the females. The petiolar sinus is less opened in the case of females. In both cases the colouration becomes reddish in autumn.

Table 1: Ampelographic characteristics of wild grapevine plants (female and male) located in Asturias studied in situ in the Deva river (Pop 2), Cares river (Pop 7) and España beach (Pop 33) populations. For each descriptor, the table shows the range of variation observed in 30 individuals of each sex.

OIV Descriptor	Females	Males
Young shoot: Opening of the shoot tip (OIV 001)	5 (fully open)	5 (fully open)
Young shoot: distribution of anthocyanin coloration on prostrate hairs of the shoot tip (OIV 002)	1 (absent)-2 (piping)	1 (absent)-2 (piping)
Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip (OIV 003)	1 (none or very low)- 3 (low)	3 (low)
Young shoot: density of prostrate hairs on the shoot tip (OIV 004)	3 (low)-5 (medium)	3 (low)
Young shoot: density of erect hairs on the shoot tip (OIV 005)	1 (none or very low)- 3 (low)	1 (none or very low)
Shoot: attitude (before tying) (OIV 006)	3 (semi-erect)	3 (semi-erect)
Shoot: colour of dorsal side of internodes (OIV 007)	2 (green and red)	2 (green and red)
Shoot: colour of ventral side of internodes (OIV 008)	2 (green and red)	1 (green)-2 (green and red)
Shoot: colour of dorsal side of nodes (OIV 009)	2 (green and red)-3 (red)	2 (green and red)
Shoot: colour of ventral side of nodes (OIV 010)	2 (green and red)-3 (red)	1 (green)-2 (green and red)
Shoot: density of erect hairs on nodes (OIV 011)	1 (none or very low)	1 (none or very low)
Shoot: density of erect hairs on internodes (OIV 012)	1 (none or very low)	1 (none or very low)
Shoot: density of prostrate hairs on nodes (OIV 013)	1 (none or very low)	1 (none or very low)
Shoot: density of prostrate hairs on internodes (OIV 014)	1 (none or very low)-3 (low)	1 (none or very low)-3 (low)
Shoot: intensity of anthocyanin coloration on bud scales (OIV 015-2)	1 (none or very weak)-3 (weak)	1 (none or very weak)-3 (weak)
Shoot: number of consecutive tendrils (OIV 016)	1 (2 or less)	1 (2 or less)
Shoot: length of tendrils (OIV 017)	1 (very short)	1 (very short)-3 (short)
Young leaf: colour of the upper side of blade (4th leaf) (OIV 051)	4 (copper-reddish)	1 (green)-3 (bronze)
Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf) (OIV 053)	3 (low)	1 (none or very low)- 3 (low)
Young leaf: density of erect hairs between main veins on lower side of blade (4th leaf) (OIV 054)	1 (none or very low)- 3 (low)	1 (none or very low)
Young leaf: density of prostrate hairs on main veins on lower side of blade (4th leaf) (OIV 055)	1 (none or very low)-3 (low)	1 (none or very low)- 3 (low)
Young leaf: density of erect hairs on main veins on lower side of blade (4th leaf) (OIV 056)	1 (none or very low)	1 (none or very low)
Mature leaf: size of blade (OIV 065)	3 (small) – 5 (medium)	1 (very small)- 3 (small)
Mature leaf: shape of blade (OIV 067)	2 (wedge-shaped) – 3 (pentagonal)	3 (pentagonal)
Mature leaf: number of lobes (OIV 068)	1 (entire leaf)- 2 (three)	2 (three)-3 (five)
Mature leaf: colour of the upper side of blade (OIV 069)	5 (medium green)	5 (medium green)
Mature leaf: area of anthocyanin coloration of main veins on upper side of blade (OIV 070)	1 (absent)	1 (absent)
Mature leaf: area of anthocyanin coloration of main veins on lower side of blade (OIV 071)	1 (absent)	1 (absent)
Mature leaf: goffering of blade (OIV 072)	1 (absent or very weak)- 3 (weak)	1 (absent or very weak)- 3 (weak)
Mature leaf: undulation of blade between main or lateral veins (OIV 073)	1 (absent)	1 (absent)
Mature leaf: profile of blade in cross section (OIV 074)	1 (flat)- 3 (involute)- 4 (revolute)	1 (flat)- 3 (involute)- 4 (revolute)
Mature leaf: blistering of upper side of blade (OIV 075)	3 (weak)-5 (medium)	3 (weak)
Mature leaf: shape of teeth (OIV 076)	2 (both sides straight)- 3 (both sides convex)	2 (both sides straight)- 3 (both sides convex)
Mature leaf: size of teeth in relation to blade size (OIV 077)	3 (small)	3 (small)- 5 (medium)
Mature leaf: length of teeth compared with their width (OIV 078)	3 (short)	3 (short)
Mature leaf: degree of opening/overlapping of petiole sinus (OIV 079)	3 (open)	1 (very wide open)
Mature leaf: shape of base of petiole sinus (OIV 080)	1 (U-shaped)-2 (brace-shaped) -3 (V-shaped)	1 (U-shaped)- 2 (brace-shaped)

Table 1: Continued

OIV Descriptor	Females	Males
Mature leaf: teeth in the petiole sinus (OIV 081-1)	1 (none)	1 (none)
Mature leaf: petiole sinus base limited by veins (OIV 081-2)	1 (not limited)	1 (not limited)
Mature leaf: degree of opening/overlapping of upper lateral sinuses (OIV 082)	1 (open)	1 (open)
Mature leaf: teeth in the upper lateral sinuses (OIV 083-2)	1 (none)	1 (none)
Mature leaf: density of prostrate hairs between the main veins on lower side of blade (OIV 084)	1 (none or very low)	1 (none or very low)-3 (low)
Mature leaf: density of erect hairs between the main veins on lower side of blade (OIV 085)	1 (none or very low)	1 (none or very low)
Mature leaf: density of prostrate hairs on main veins on lower side of blade (OIV 086)	1 (none or very low)-3 (low)- 5 (medium)	3 (low)
Mature leaf: density of erect hairs on main veins on lower side of blade (OIV 087)	1 (none or very low)	1 (none or very low)
Mature leaf: prostrate hairs on main veins on upper side of blade (OIV 088)	1 (absent)	1 (absent)
Mature leaf: erect hairs on main veins on upper side of blade (OIV 089)	1 (absent)	1 (absent)
Mature leaf: density of prostrate hairs on petiole (OIV 090)	1 (none or very low)	1 (none or very low)
Mature leaf: density of erect hairs on petiole (OIV 091)	1 (none or very low)	1 (none or very low)
Mature leaf: length of petiole compared to length of middle vein (OIV 093)	3 (slightly shorter)-5 (equal)	3 (slightly shorter)-5 (equal)
Mature leaf: depth of upper lateral sinuses (OIV 094)	1 (absent or very shallow)-3 (shallow)	3 (shallow)-5 (medium)
Woody shoot: cross section (OIV 101)	2 (elliptic)	2 (elliptic)
Woody shoot: structure of surface (OIV 102)	2 (ribbed)-3 (striate)	2 (ribbed)- 3 (striate)
Woody shoot: main colour (OIV 103)	2 (brownish)	2 (brownish)
Woody shoot: lenticels (OIV 104)	1 (absent)	1 (absent)
Woody shoot: erect hairs on nodes (OIV 105)	1 (absent)	1 (absent)
Woody shoot: erect hairs on internodes (OIV 106)	1 (absent)	1 (absent)
Flower: sexual organs (OIV 151)	4 (reflexed stamens and fully developed gynoecium)	1 (fully developed stamens and no gynoecium)
Inflorescence: number of inflorescences per shoot (OIV 153)	2 (1.1 to 2 inflorescences)	3 (2.1 to 3 inflorescences)
Bunch: length (peduncle excluded) (OIV 202)	1 (very short)-3 (short)	
Bunch: width (OIV 203)	1 (very narrow)	
Bunch: density (OIV 204)	3 (loose)	
Bunch: length of peduncle of primary bunch (OIV 206)	1 (very short)-3 (short)	
Bunch: lignification of peduncle (OIV 207)	1 (at the base only)-5 (up to about the middle)	
Bunch: shape (OIV 208)	2 (conical)	
Bunch: number of wings of the primary bunch (OIV 209)	2 (1-2 wings)	
Berry: length (OIV 220)	1 (very short)	
Berry: width (OIV 221)	1 (very narrow)	
Berry: uniformity of size (OIV 222)	1 (not uniform)	
Berry: shape (OIV 223)	2 (globose) -3 (broad ellipsoid)	
Berry: colour of skin (OIV 225)	6 (blue black)	
Berry: uniformity of colour of skin (OIV 226)	1 (not uniform)	
Berry: thickness of skin (OIV 228)	7 (thick)	
Berry: hilum (OIV 229)	2 (visible)	
Berry: intensity of flesh anthocyanin coloration (OIV 231)	1 (none or very weak)	
Berry: juiciness of flesh (OIV 232)	1 (slightly juicy)-2 (medium juicy)	

| Table 1: Continued

OIV Descriptor	Females	Males
Berry: must yield (OIV 233)	3 (little)	
Berry: firmness of flesh (OIV 235)	2 (slightly firm)	
Berry: particular flavor (OIV 236)	1 (none)- 4 (herbaceous)	
Berry: length of pedicel (OIV 238)	1 (very short)	
Berry: ease of detachment from pedicel (OIV 240)	2 (easy)	
Berry: formation of seeds (OIV 241)	3 (complete)	
Berry: length of seeds (OIV 242)	1 (very short)	
Berry: weight of seeds (OIV 243)	1 (very low)-3 (low)	
Berry: transversal ridges on dorsal side of seeds (OIV 244)	1 (absent)	
Time of bud burst (OIV 301)	5 (medium)	5 (medium)
Time of full bloom (OIV 302)	5 (medium)	5 (medium)
Time of beginning of berry ripening (veraison) (OIV 303)	5 (medium)	
Time of full physiological maturity of the berry (OIV 304)	7 (late)	
Time of beginning of wood maturity (OIV 305)	7 (late)	7 (late)
Autumn colouration of leaves (OIV 306)	1 (yellow)-2 (reddish)	1 (yellow)-2 (reddish)
Vigour of shoot growth (OIV 351)	5 (medium)	3 (weak)-5 (medium)
Growth of lateral shoots (OIV 352)	3 (weak)-5 (medium)	7 (strong)
Length of internodes (OIV 353)	3 (short)- 5 (medium)	5 (medium)
Diameter of internodes (OIV 354)	1 (very small)- 3 (small)	3 (small)



| Fig. 5: Male vine at flowering time (June).

Male individuals show a higher number of inflorescences per shoot (Fig. 5), with flowers that generally present fully developed stamens and no gynoecium (flower Type 1, according to OIV 2009). On the other hand, female flowers show reflexed

stamens and fully developed gynoecium. The berries are sub-spherical and their diameter is lower than one centimetre. The pulp thickness is very low due to the presence of seeds well developed. The colour of the skin is blue black in most of the berries. In general, seeds of wild grapevines are morphologically different from cultivated ones with exceptions such as Listan Prieto (Cervantes *et al.* 2021). They show a more rounded outline with smaller size and a shorter peak. The global ampelographic description is compiled in Table 1, according to OIV (2009) Descriptor list.

3. Parasitic species

The most common species of arthropods on vine leaves was *Colomerus vitis* (Pagenstecher) (Acari, Eryophidae). Its eri-neum strain caused symptoms in the practical totality of the vines sampled, producing false galls, which constitute the nests of the mite. In seven vines situated along the river-bank forest belonging to the Deva river (Pop 7), symptoms caused by the curl leaf strain of this species were also found. No presence of the bud strain was detected in any examined vine. The infestation caused by another eriophid, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae) had a lower incidence. In the case of insects, *Empoasca vitis* (Goëthe) (Homoptera, Cicadellidae) and *Thrips angusticeps* Uzel (Thripidae, Thripinae) constituted the most frequent species, found mainly on the coast populations. Occasionally, some gnawed areas of young leaves could be observed. They were caused by larvae of Lepidoptera, generally of sphingids and noctuids, mainly on coastal population nuclei, where adults of *Hippotion celerio* L. (Lepidoptera, Sphingidae) were captured.

The main sanitary problems found were those caused by powdery mildew, *Erysiphe necator* (Burr.) and downy mildew, *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni. No symptoms caused by Grapevine fanleaf virus (GFLV), phylloxera *Daktulosphaira vitifoliae* (Fitch) (Hemiptera, Phylloxeridae), *Armillaria mellea* (Vahl.) or *Rosellinia necatrix* (Hartig) were observed.

4. Wine elaboration and analysis

All the female vines produced small and very scattered bunches of red berries, with very scarce yield. Berry size was always less than 1 cm in diameter in all the samples. Only three populations had enough amount of healthy ripe berries to produce a minimum volume of must suitable for wine elaboration and analysis. They belong to the Deva and Cares rivers and the coastal line, España beach (Pop 2, 7 and 33).

The must yield and the sugar content of the berries were very low. Concerning wines, they presented low ethanol content and high TPI and colour intensity (Table 2).

5. DNA analysis

A total of 36 wild samples from 21 populations were genotyped for 240 SNPs. Thirty-three different genotypes were found (Supplemental Table 2). Matchings were found between samples taken close in the same population or very close: three from populations 10-11 and two from population 12. The remaining genotypes were unique among the about 3000 genotypes existing in the ICVV-SNP database.

Fig. 6a shows the projections of the wild and cultivated vine genotypes on the two first axes obtained in the PCoA. Cultivars are grouped separately of the wild vines through the axis 1, which explained 32.89 % of the total variation. The UWNJ tree also shows that separation, while most wild plants from the same population cluster together (Fig. 6b). The UWNJ tree obtained from the analysis of wild plants alone shows that most plants from the same population are close, with some

exceptions like one vine from Pop 46 (Fig. 6c). No clear grouping can be observed among wild populations.

Parentage analyses show none full trio (father-mother-offspring) where any of the wild vines were involved. Four duos were found, two of them for the same vine, 17386 from Pop 25. It shares one allele per locus with the cultivar 'Camaraou Noir' and with another wild vine of a very close population (Pop 26). Besides, two vines of the Pop 46 also showed a compatible parent-offspring relationship. Interestingly, one wild vine of the Pop 57 formed a duo with a sylvestris from Asturias present in the ICVV database from a previous study, Vivoli O 1-4 (Table 3; de Andrés et al. 2012).

Discussion

Asturias is an Eurosiberian region with a humid climate, situated in the Northwest of the Iberian Peninsula, between the Cantabrian sea and the Cantabrian mountain range with an area of 10,604 km². Most of the Asturian wild grapevine populations found in these surveys are concentrated in the eastern and central zones of this territory. They are distributed in alluvial and colluvial positions, also in the mouth of rivers and along the coast on cliffs and beaches. Populations in alluvial positions have been also found in other regions, for example on floodplains of big rivers as the Rhine or the Danube, between others (Schumann, 1974); in the mouth of the Guadalquivir (Spain) and the Danube (Romania) rivers; or in the coastal area of the Uccelina Park (Italy) (Ocete, personal communication). Concerning colluvial positions, wild grapevine populations have been described on the slopes of mountains and hills of the Cantabrian Mountain range (Spain), Jura (France), Piedmont (Italy) or the Alps (Switzerland) (Ocete et al., 2000, 2018). Also, on cliffs of coastal areas, as in the Cantabrian seaside, from Lapurdi (French Basque Country) (Rodríguez-Miranda et al., 2016) up to Western Asturias (Spain) (Álvarez Arbesu and Cabello, 1999).

The plants used as supporters to climb by the wild grapes differed in function of the position of the populations, the al-

Table 2: Must and wine parameters from bunches collected from vines in the wild

Wine parameters	Pop 2 Deva river	Pop 7 Cares river	Pop 33 España beach
Weight of mature berries collected (g)	743	831	578
Weight of must obtained (g)	121	128	92
Must yield (%)	16.3	15.4	15.9
Brix degree in must	13.4	12.7	13.9
Alcoholic degree (% v/v)	6.7	6.2	7.1
pH	3.2	3.0	3.5
Total acidity (g/L Tartaric acid)	10.3	11.4	9.8
Tartaric acid (g/l)	5.3	5.8	5.0
L- Malic acid (g/l)	2.2	2.6	2.0
Total polyphenol index (TPI)	99	92	112
Anthocyanins (mg/l)	503	478	517
Colour intensity	19	17	20

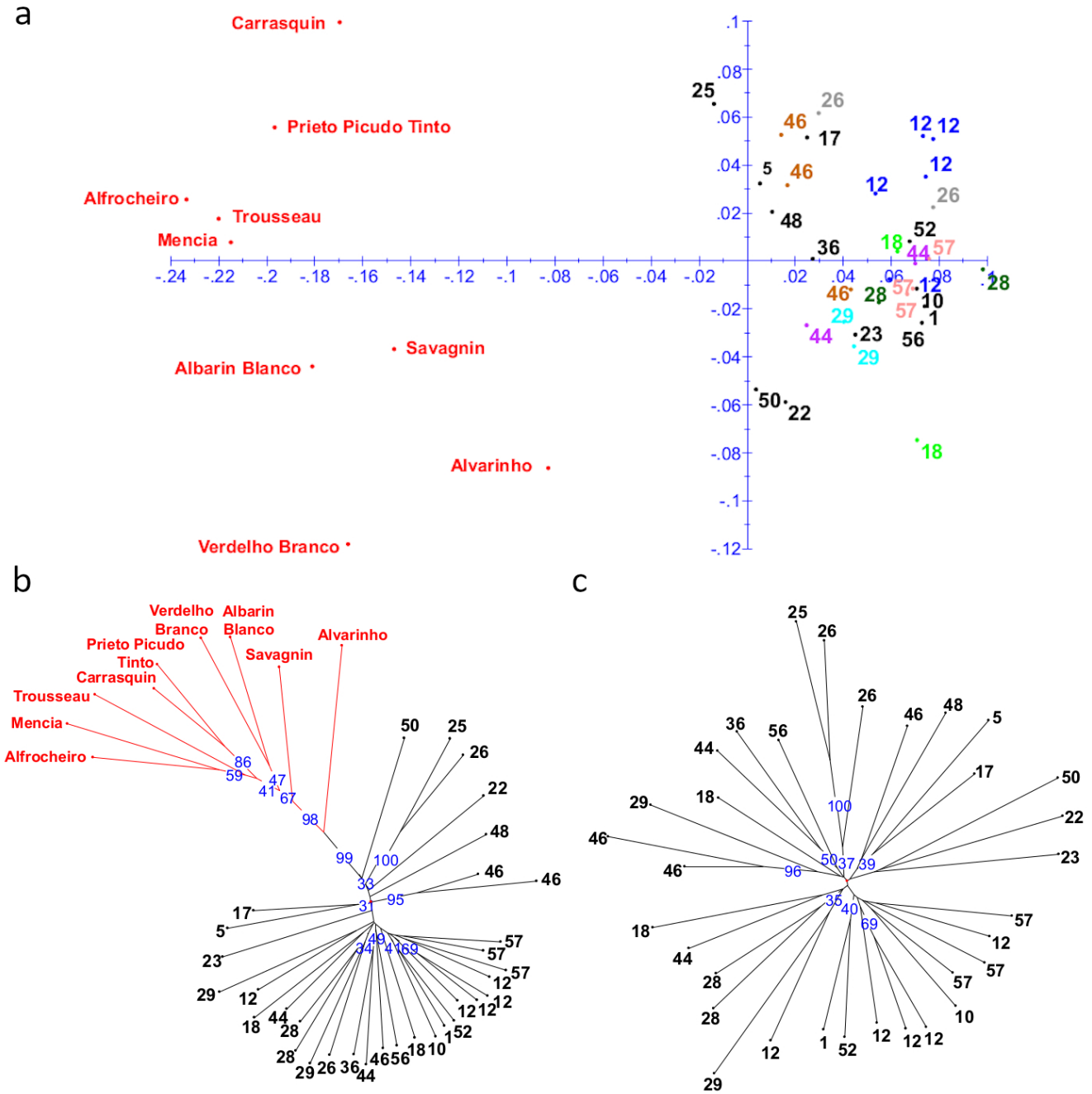


Fig. 6: Representation of genetic distances based on SNP data. a: PCoA of wild vines and nine grape cultivars common in Asturias region; b: UwNJ tree of the same samples, and c: UwNJ tree of only the wild samples. Numbers in black font correspond to wild vine populations (Supplemental Table 1). Numbers in blue font (b and c) correspond to bootstrap values in percent.

Table 3: Duos (putative parent-offspring relationships) found for the wild vines from Asturias

Vine 1	Pop vine 1	Vine 2	Pop vine 2	SNPs compared	Mismatching SNPs	LOD score
17386	25	17384	26	221	0	59.72
17386	25	‘Camaraou Noir’	-	217	0	30.59
17482	46	17483	46	216	1	60.21
17476	57	Sylvestris Vivoli O 1.4	-	212	1	44.05

livial positions along the riverbank forests having a greater diversity of species.

Concerning the ampelographic description of male and female individuals, the most remarkable difference between both sexes in the wild grapevine is, obviously, the type of flower, and its direct consequence: only the female individuals develop berries. Also, little differences have been observed in several parameters such as the colour of shoot nodes and internodes, and the size, shape and number of lobes of the leaf blade, between others (Ocete *et al.*, 2007).

Wild grapevine has traditionally had a wide application as food, in pharmacopoeia or in the naval industry for the manufacture of ropes and fishing traps. However, the only testimony found about traditional uses in Asturias was the construction of hoops of fishing traps for lobster around Toranda beach, like in other maritime Spanish regions, as in the case of Barbate in Andalusia (Cadiz province) (Ocete *et al.*, 2011).

The number of species of pests and diseases which cause symptoms on the aerial zone of the vines are very scarce in the various populations surveyed from the Iberian Peninsula to the Southern Caucasus (Ocete *et al.*, 2020), as in our case. The level of infestation and infection varies from one specimen to another, within the same population and with the climatic conditions of each year. This difference in degrees of sensitivity shown by different specimens of the same population, which are sometimes in physical contact, constitutes another exponent of the genetic diversity of this subspecies that may be worth preserving and studying.

Concerning the parasitic species found in Asturias, *Colomerus vitis* has been widely reported in wild grapevine populations of Spain (Extremadura, Andalucía, Castilla-La Mancha) and from other regions of Europe such as Portugal, France, Switzerland, Germany or Hungary (Ocete *et al.*, 2000). *Empoasca vitis* and *Thrips angusticeps* were the most frequent insect species. Both species are also found in practically the rest of wild grapevine populations situated along the Cantabrian Coast; they are also very frequent in vineyards producing Chacolí wine in Guipúzcoa and Vizcaya (Ocete *et al.*, 2004).

Powdery and downy mildews were the main sanitary problems as it was indicated in other different Spanish populations by Ocete *et al.* (2007). Both American species prevent the development of the scarce seedlings found in all kinds of habitats, limiting the renewal of each population. At root level, it is remarkable the absence of symptoms caused by phylloxera on absorbent hairs. The absence of symptoms caused by this insect is due to edaphic conditions, such as the terra rossa soil formed by the weathering of marls and the washing or leaching of calcium carbonate of the calcareous rocks situated along the coast, and/or sandy soil texture. Another striking fact is the absence of mycelia of fungi that cause root rot, *Armillaria mellea* and *Rosellinia necatrix*, although some of their botanical supporters, mainly poplars, usually present this health problem.

Elaboration of wine from *sylvestris* vines growing wild is a very difficult task for many reasons: bunches are difficult to reach, cut and pick, low yield, uneven ripening... Besides, the vegetative growing without any control of the source ×

sink balance is not compatible with accurate comparisons of the potential production of the different genotypes and their fruit composition (with the exception may be of the polyphenolic index). Thus, these results have to be taken cautiously but still, they provide interesting clues on wines from wild vines and their potential usefulness. Analytical data of wine samples are far from those shown by wines from cultivated varieties. It is due to the mentioned reasons and to the fact that wild grapevines have not undergone large human selection, a great difference with respect to the cultivars. The alcoholic degree varied between 6.2 % and 7.1 %, greater than those registered on microvinifications of wild grapevines carried out in Southern Caucasus region, the cradle of viticulture (Maghradze *et al.*, 2020), but less than half of some values found in samples obtained from populations situated in Extremadura (Spain) (Ayala *et al.*, 2012). The acid content of the wines obtained is interestingly high, and the proportion of tartaric and malic acids present in the wine was striking. Thus, the concentration of tartaric acid varies between 5 and 5.8 g·L⁻¹, while the corresponding to malic acid is situated within the interval 2 and 2.6 g·L⁻¹.

Due to the small size of wild berries, even when cultivated *ex situ* as varieties (Benito *et al.* 2017), they are very interesting because of their large surface: volume (skin to pulp) ratio, compared to the cultivated varieties, which is in line with the high values found in these samples of wines for the total polyphenols index, situated between the interval 92 and 112. Thus, they showed intense colour, with values in the range 17–20. Meanwhile, the concentration of anthocyanins oscillated between 478 and 517 mg·L⁻¹. These values are quite inferior to those reported from a sample from the Ega river (Spanish Basque country) (Meléndez *et al.*, 2016). Microvinifications of wild grapevine produce red wines with good total acidity and colour intensity, two very important characteristics that could provide to the *sylvestris* vines an added value for breeding of red cultivars in Mediterranean areas, in addition to other traits such of salt tolerance (Carrasco *et al.* 2022; Daldoul *et al.* 2022).

Genetic analyses allowed to identify 33 unique genotypes among the 36 samples analyzed. Matching samples were found close and would correspond to the same vine or to a natural asexual propagation. Genetic structure analyses clearly showed that the wild vines are genetically different from the most cultivated varieties in that region, indicating the absence of local introgression events of *V. sylvestris* into *V. sativa* subspecies. Structure analyses, together with the rest of data collected about them, indicate that these wild plants do belong to the subspecies *sylvestris*. The only exception could be the vine 17386 from Pop 25, which form a compatible duo with the cultivar ‘Camaraou Noir’. Precisely, this vine 17386 was, among the wild plants analysed in this work in Asturias region, the one with the lowest ancestry coefficient (0.7431) for the *V. sylvestris* population in a wider genetic structure study with about 2000 genotypes, including more than 400 *V. sylvestris* (data not shown); in that study ‘Camaraou Noir’ showed an ancestry coefficient to the *V. sylvestris* population of 0.3532, indicating that vine 17386 could be an offspring of a *V. sylvestris* plant and ‘Camaraou Noir’. This finding high-

lights the situation of risk of the subspecies *V. sylvestris* by introgression of the *V. sativa* subspecies.

The main causes of wild grapevine loss in the region are similar to those from other geographical areas: public works, mainly widening and layout of new communication routes, cleaning of their ditches, creation and expansion of industrial states and parking areas. As an example, in 2018 in the left part of the karstic wall of Gulpiyuri, the smallest beach in the Iberian Peninsula, some part of the natural vegetation, including two vines were destructed by human action. Another human cause of the loss of wild vines is the adaptation of the land to new areas of cultivation and meadows to graze livestock. The presence of North American fungal diseases is the most serious and impacting sanitary problem able to destroy vines. Another cause, with a much lower effect, is the presence of feral Vitaceae, such as rootstocks and *Vitis* hybrid producers of grape, which act as invasive plants. They escaped from vineyards and farms, where they were sometimes used as ornamental plants. Occasionally, as in the case of some riverbank forests belonging to water courses, the continuous cleaning of vegetation to practice salmon fishing every year between May and August also contributes to eliminate wild vines.

According to the information provided above, it is necessary to safeguard *in situ* and *ex situ* the maximum biodiversity of the wild populations, which lodge an important genetic pool (Zdunic et al., 2017), because they grow unaffected from human selection and their reproduction is mainly sexual. *In situ* conservation is needed to keep them naturally evolving, what requires laws for their legal protection. Conservation in germplasm collections it is also necessary like with ancient traditional cultivars, in order to safeguard the gene pool of this subspecies for the future, where it might be needed for the sustainability of the grape and wine industry.

Conclusion

Vitis vinifera subsp. *sylvestris* (Gmelin) Hegi is disseminated in the central and eastern part of the Asturias region, occupying a wide diversity of habitats and growing over a great number of species of supporters. Studied vines are genetically different from the most common cultivars of the region, and grouped mostly accordingly to their population of origin. The potential of this subspecies for the improvement of the cultivated vine, as well as the high levels of degradation of its habitats and the consequent loss of this genetic resource make a legal figure of protection for this taxon necessary, which is not included in the Regional Catalogue of Endangered Species of the Flora of the Principality of Asturias (Decree 65/95 of 27th April, Official Bulletin of the Principality of Asturias).

Acknowledgements

Part of this work was supported by the INIA RF2008-00019-CO2-00 Project ("Prospección, localización y caracterización del recurso fitogenético vid silvestre (*Vitis vinifera* L., subsp. *sylvestris* (Gmelin) Hegi) en el Principado de Asturias", 2008-2011). On the other hand, we want to highlight the help provided in the analysis of the wine samples by Dr. Carmelo

García Barroso (Centro Andaluz de Investigaciones Vitivinícolas), recently deceased and by M^a Cruz Ayala (Instituto La Laboral de Lardero, La Rioja).

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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