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Exploring Diversity among Grapevines Varieties (*Vitis vinifera* L.) in Ibiza and Formentera (Balearic Islands, Spain) Using Microsatellite Markers, Ampelographic Methods and an Ethnobotanical Approach

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Abstract: *Vitis vinifera* L. has been present in Ibiza and Formentera, two islands of the Balearic Islands (Spain), since the 7th century BC. In the past few years, there have been several studies and investigations on the Balearic Islands. These have focused mainly on Mallorca and Menorca with a small representation of Ibiza and none that take into account Formentera. This research aims to contribute to the knowledge of *Vitis* cultivars cultivated on those islands and to investigate whether there are local cultivars still being grown. To do this, using an ethnobotanical approach, 15 persons were interviewed to gather information about local grapevines, and 36 accessions from 12 plots were characterized using ampelographic descriptors and identified using SSR markers. Relationships of the accessions studied with other cultivars were also assessed. The results show 21 different genotypes profiles, where six were new genotypes: 'Colló de gall', 'Grec', 'Maçanet', 'VIEIV015-Maçanet', 'Morzacà', and 'Vermelleta'. Ten new synonyms and three homonyms have been proposed. Additionally, we suggest three new relationships for the 'Hebén' cultivar, one new relationship for the 'Llora' cultivar and one new relationship for the 'Beba' cultivar. These results show the first reported information for Ibiza and Formentera on *Vitis*.

Keywords: ethnobotany; grapevine; local cultivar; somatic variant; SSR markers; parentage

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1. Introduction

Ibiza (also known by its Catalan name, Eivissa) and Formentera are the two smallest inhabited islands of the Balearic Islands (Spain), which are together known as the Pityusic Islands. These islands have a strong tradition of growing grapevines and winemaking. It is thought that *Vitis vinifera* L. (*Vitaceae*) was brought to Ibiza and Formentera by the Punics in the 7th century BC, who even created structures purposely to cultivate grapevines [1]. During the Middle Ages, other authors, such as Al-Zuhri or Al-Himyari, pointed out the production of sultanas in Ibiza and that the trade with wine continued [2].

In 1851, grape production in the Balearic Islands was affected by powdery mildew (*Uncinula necator*), which substantially reduced production [3]. Then, in 1862, when phylloxera (*Daktulosphaira vitifoliae*) expanded in France, Ibiza and Formentera's production increased to supply the French market [4]. The presence of this disease was detected in

Mallorca in 1891 and later in Menorca in 1892 [5]. Although, at that time, phylloxera had not been detected in Ibiza and Formentera, present day evidence indicates symptoms in various areas of the islands [6].

Today, grapevines continue to be some of the most important plants when considered in socio-economic terms. Spain is the global leader in terms of the area dedicated to this crop cultivation, with 964 kha [7] in the Balearic Islands [8], whose surface is 2336 ha. From this total, Ibiza Island cultivates 58.6 ha and Formentera cultivates 14.3 ha [4]. Despite their relatively small surface, each of these islands has its own wine appellation. Cultivars allowed in these appellations are mainly well-known cultivars and include only a small number of minority cultivars, such as 'Monestrell' or 'Malvasia' [9,10]. This could be one of the main reasons that in Ibiza, 'Monestrell' and 'Garrut' together represent 76.45% of the registered vineyard surface, while in Formentera, 57.5% of the surface comprises 'Monestrell', 'Garrut', and 'Garnatxa tinta' [11].

Grapevines are not only restricted to vineyards for the winemaking industry; they are also a common Mediterranean crop that is present in every farmhouse in the Pityusic Islands as well as other parts of the Balearic Islands [12,13].

Organic agriculture is promoting a revival of the recovery and identification of local germplasm to fight against diseases and climate change in grapevine cultivation [14,15]. In the last 20 years, there has been an interest in the Balearic Islands to study, protect and reintroduce to the market local minor grapevine cultivars in order to differentiate wines produced in this region [16–21]. Most of the investigations undertaken have been in the islands of Mallorca and Menorca, usually on vineyards belonging to wineries. In those works, samples are studied from a molecular point of view in order to assess the enological properties [22–25]. Two other studies have analyzed the local cultivars growing in the Balearic Islands in order to establish their origin and phylogeny [26,27].

Although SSR markers (simple sequence repeats or microsatellites) have been used largely to identify the diversity present in vineyards and to discard any homonyms and synonyms [26–29], in the works concerning the Balearic Islands, Ibiza and Formentera are scarcely represented. Additionally, none of the studies that consider Ibiza samples conducted ampelographic descriptions or ethnobotanical interviews. The only exception is Grup d'Acció Local per al Desenvolupament Rural i Pesquer d'Eivissa i Formentera, who prospected and conducted an ampelographic study between 2012 and 2014 in these territories [13]. That study constitutes the starting point for this research.

Although numerous characterizations, through several molecular markers, have been carried out [30–32], ampelography and other morphological methods [33,34] still remain the first steps when identifying cultivars, and these are useful when distinguishing somaclonal variants or mislabels [35,36].

Despite the fact that classic ampelographic identification is usually difficult, because ampelographic traits can be influenced by cultivar age [37], viruses [38], crop management [39] or even environmental characteristics [35,40], an ampelographic description is needed to inscribe any cultivar in the register of commercial cultivars or the variety catalogue [41,42].

Ethnobotanical interviews have rarely been used in grapevine studies to register information related to the samples considered. Through information given by farmers during the investigation of local cultivars, it is often found that these cultivars have not been registered [43]. These interviews are able to gather important information describing the cultivars, how to grow them and the different particularities that have been transmitted over the generations.

Although there have been some studies in recent years [13,28] that have studied grape samples from the Pityusic Islands, no research work that specifically focuses on grapevine landraces in Ibiza or in Formentera and that takes into account microsatellite identification, ampelographic study and ethnobotanical interviews altogether has been carried out.

Given the above-described current state of the art of *Vitis* research in both of the considered islands, the main goals of this study were to (1) identify vine diversity growing in

the Pityusic Islands using microsatellite markers, ampelography, ethnobotanical interviews and bibliography, (2) characterize the cultivars and landraces found in these islands from genetic and ampelographic points of view, and (3) analyze relationships of the accessions studied with other cultivars and their possible origin.

2. Materials and Methods

2.1. Plant Material and Origin

Between 2012 and 2014, a project was conducted to prospect local grapevine germplasm in the Pityusic Islands on behalf of Grup d'Acció Local d'Eivissa i Formentera [13]. In this work, five farmers had been interviewed, and six plots were prospected. Between 2017 and 2023, adopting an ethnobotanical approach, another 10 persons were interviewed, and another four plots were prospected and added to the study. The plots included in this study involve individuals that allowed us to conduct ampelography and genetic analysis on the cultivars sown, and they also shared valuable knowledge about their vineyard. In total, 12 plots were included in this study, 10 in Ibiza and 2 in the Formentera Islands. The Ibiza samples were mostly located in the Sant Josep de sa Talaia municipality (Figure 1). Most of the plots were orchards in which *Vitis* was one of the products, and as the cultivated extension was less than 1000 m^2, those did not have to be compulsorily registered to an official body [44].

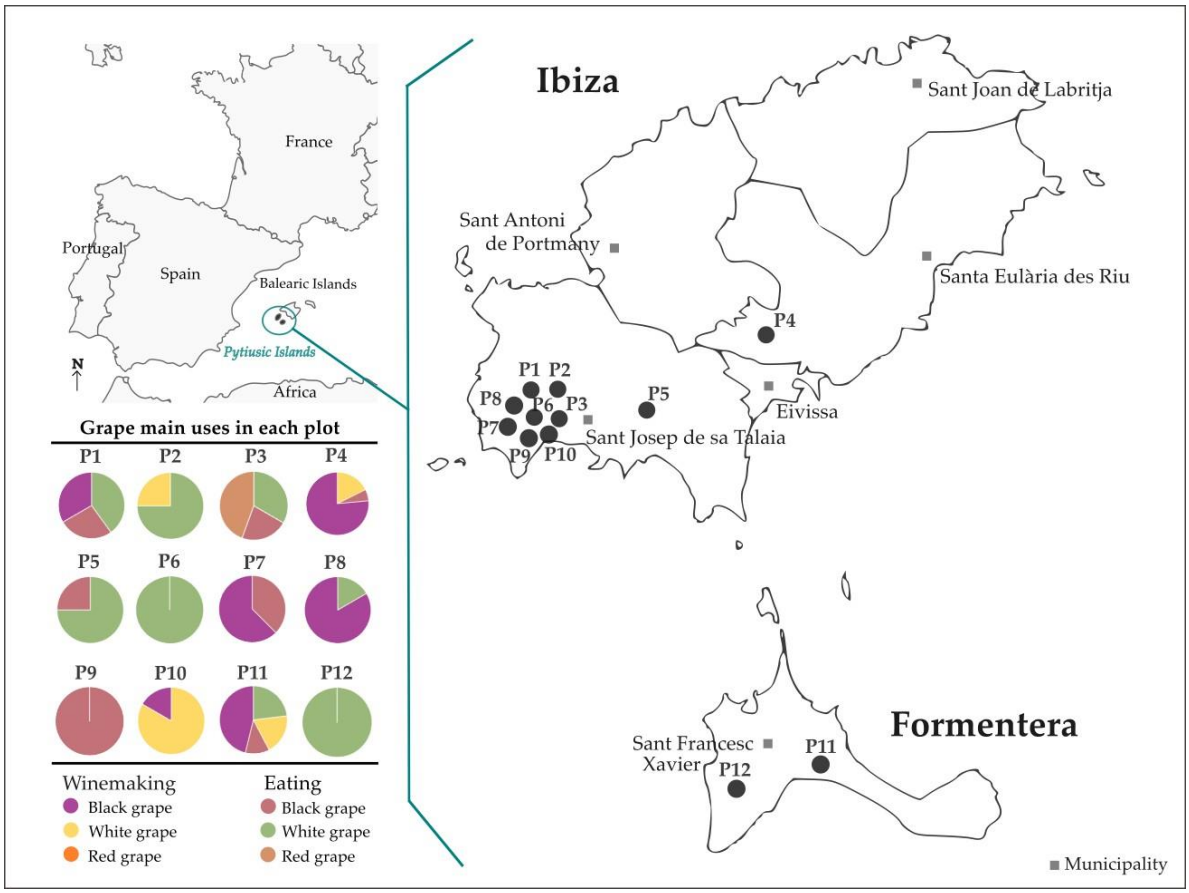

Figure 1. Plot localization and main grape uses in each plot studied in Ibiza and Formentera Islands.

Vineyards were all over 10,000 m^2, and grapes were sold to elaborate the Appellation of Control Origen wines of the islands; in these cases, local cultivars constituted less than 10% of all the grapevines. The plots' characteristics are detailed in Table S1 and summarized in Table 1.

Table 1. Plots and number of plants studied in each plot.

Plot Number	Municipalities, Island	No. of Plants Studied	Rootstock Used	Soil Type	Plant Management	Plant Age	Irrigation	Plot Type
P1	Sant Josep de sa Talaia, Ibiza	15	None	Silt soil	Double cordon	>40 years	No	Plot
P2	Sant Josep de sa Talaia, Ibiza	8	None	Silt soil	Double cordon	>40 years	No	Plot
P3	Sant Josep de sa Talaia, Ibiza	9	American rootstock	Silt soil	Double cordon	>40 years	No	Plot
P4	Santa Eulària des Riu, Ibiza	17	American rootstock	Clay soil	Goblet	>40 years	No	Vineyard
P5	Sant Josep de sa Talaia, Ibiza	4	None	Silt soil	Goblet	<10 years	Yes	Plot
P6	Sant Josep de sa Talaia, Ibiza	4	None	Silt soil	Goblet	>40 years	No	Plot
P7	Sant Josep de sa Talaia, Ibiza	8	None	Silt soil	Goblet	> 40 years	No	Plot
P8	Sant Josep de sa Talaia, Ibiza	6	None	Silt soil	Goblet	>40 years	No	Plot
P9	Sant Josep de sa Talaia, Ibiza	2	None	Silt soil	Goblet	>40 years	No	Vineyard
P10	Sant Josep de sa Talaia, Ibiza	6	None	Silt soil	Goblet	>40 years	No	Plot
P11	Formentera, Formentera	26	None	Silt soil	Double cordon	>40 years	No	Vineyard
P12	Formentera, Formentera	4	None	Silt soil	Goblet	>40 years	No	Vineyard

The accessions selected for this study were those that farmers had identified as local landraces during the prospections, or they had doubts about their origin. The accession named 'Monestrell' was present in all plots, so it was used as a control cultivar to determine the phenology on the other accessions, although samples were not collected on all plots, as it was identified by farmers as the same accession.

The sampling comprised a total of 109 plants grouped into 36 accessions. Those accessions were cultivated in 12 plots, 10 in Ibiza and 2 in Formentera, with different numbers of individuals investigated in each of the *Vitis vinifera* accessions, 27 of which were from Ibiza and 9 of which were from Formentera. Codes VIEIV001 to VIEIV028 corresponded to accessions from Ibiza Island, while code VIEIV004 was removed from this study because it had a doubtful origin and name. Accessions coded VIFOR029 to VIFOR037 were collected from Formentera Island.

The taxon code, local name, number of plants studied, and voucher specimens' number, place and date of collection are shown in Table S2. Voucher specimens for each accession are deposited in the herbarium BC (Botanical Institute of Barcelona).

2.2. Semi-Structured Interviews

A total of 15 semi-structured interviews were performed during the 2012–2014 and 2017–2018 periods. Interviews were conducted with farmers, most of whom were men [45]. Additionally, interviews were held with other farmers following the snowball methodology [46] to gather different information about traditional vines cultivated on the island. In those interviews, the main subjects discussed were cultivars planted or known, plant age, rootstock used, crop management and winemaking processes.

This information is used, together with ampelographic characterization and SSR markers, to identify and clarify synonyms, homonyms and misnaming in the samples here studied.

2.3. Ampelographic Characterization

The morphological characterization has been carried out during two consecutive years, 2017 and 2018, by a single ampelographer, using 35 descriptors from the International

Organization of Vine and Wine (OIV) [42]. For every descriptor, the minimum number of observations established in the OIV protocol [42] has been made. Where this could not be achieved, this has been stated (Table S3). Data, including the registered number for each accession, are available in Table S4.

Metric descriptors such as bunch and grape weight, width and length have been first measured and then converted to category following the descriptors' indications.

The mode of all qualitative descriptors for both years of study has been calculated. If the mode fell between two categories, the most observed one, based on ampelographer experience on field and with those cultivars, has been selected.

2.4. DNA Extraction and Microsatellite Analysis

DNA extractions were carried out from herbarium material using the Qiagen DNeasy 96 Plant Kit (Hilden, Germany) with minor modifications. Twenty-six nuclear microsatellites were simultaneously amplified in two multiplex PCR. The SSR markers included in this study are VVIP60, VVIB01, VVIQ52, VVIH54, VVIN73, VVIP31 [47], VRZAG83, VRZAG79, VRZAG62, VRZAG112 [48], VVMD7, VVMD25, VVMD24 [49,50], VMC1B11 [51] and VVS2 [52]); set B: VRZAG29, VRZAG67 [48], VVMD28, VVMD32, VVMD27, VVMD21, VVMD5 [49,50], VVIV37, VVIV67, VVIN16 and VMC4F3-1 [51]. These 26 microsatellites [53] were chosen because they presented clear profiles that were easy to score, carrying a higher number of alleles, and they are evenly distributed along grapevine chromosomes.

Multiplex PCRs were performed in a final volume of 20 µL containing 1× Multiplex PCR Master Mix (Qiagen, Hilden, Germany) and 5 ng of DNA template. The thermocycler conditions, primers concentration, and primers fluorochrome labeling were those used by Zarouri [54] for Mx01 and Mx02. PCR products were analyzed in an ABI 3130 Genetic Analyzer, and the fragments were sized with GeneMapper 5.0 using GeneScan™-600 LIZ™ Size Standard as the internal marker [54].

2.5. Data Analysis

Standard measures of genetic variation including number of alleles per locus (Na), number of effective alleles per locus (Ne), Shannon information index (I), the observed (*Ho*) and expected (He) heterozygosity, and the probability of identity (PI) were calculated using GenAlEx 6.5 software [55]. The polymorphism information content (*PIC*) was calculated employing Cervus 3.0 software [56]. To determine the genetic uniqueness of each accession, the Excel add-in Microsatellite Toolkit 2001 [57] was used.

The microsatellite genotypes obtained were compared with SSR profiles stored in the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA, hereafter) database [58] with around 8000 unique SSR profiles. These genotypes include 2061 unique profiles that correspond to accessions maintained in the *Vitis* Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). The rest of the genotypes, not present in this collection, came from 209 cultivars included or pending inclusion in the Grapevine Spanish Catalogue [59], 1541 genotypes from information exchange with other European germplasm banks, and 4182 from other IMIDRA investigation works and/or scientific papers. All have been compared after being standardized following a similar method described in [30].

Parentage analysis was performed based on 26 SSR profiles, using Cervus 3.0 software [56]. The analysis was performed with 4208 accessions of the IMIDRA database [58].

Genetic structure was analyzed using a panel diversity comprising 140 cultivars from five different countries, including the studied samples (Table S7). The selection of these cultivars considered the historical connection of Ibiza and Formentera with other countries. Twenty-six non-linked microsatellite mentioned before were used in both analyses. Only unique SSR profiles were used; cultivars with somatic mutations are not represented.

Firstly, the genetic structure was conducted by a Bayesian analysis using the software Structure 2.3.4 [60]. A model, with a putative number between one and ten populations and correlated allele frequencies [61], was assumed. A Monte Carlo Markov Chain run-

length period of 100,000, with 100,000 burn-in steps, and 10 iterations for each number of putative populations were used. The Evanno criterion was used to decide the population number [62]. The membership coefficient threshold defined for individual assignment to a given cluster was Q = 0.80.

Secondly, GeneAlex 6.5 software [55,63] was used to conduct principal coordinate analysis (PCoA), based on standardized covariance of the genetic distances previously calculated for codominant markers in this software.

All statistical analyses were performed with Microsoft Excel [64] and SPSS [65].

3. Results

3.1. Material Prospection, Ampelographic and Molecular Characterization

From this sample group, 285 alleles have been detected for the 26 SSRs studied. The most polymorphic markers for this group of cultivars have been loci VMC4F3-1 and VVMD28, which showed 11 alleles each, whereas VVIQ52, VVIN73, and ZAG29 markers only had three different alleles. The highest informative marker, using the allele effective value for measurement, is the VVMD5 locus with a value of 7.1 that presents 14 different genotypic combinations in this sample group. The 21 different genotypes found in this study can be differentiated using exclusively the genotypes obtained in VMC4F3-1 and VVMD5 loci. A mean value of unbiased expected heterozygosity (He) of 72% and a Shannon's Information Index (I) of 1.5 has been obtained (Table S5).

3.2. Identifications, Synonyms, and Homonyms

One sample from each of the 36 accessions was selected for the microsatellite marker study based on the interviews conducted and considering that all plants in the same accession group had come from the same original plant.

These analyzed samples resulted in 21 different genetic profiles between both islands (Table S6). From the 27 accessions studied in Ibiza Island, there were 21 genotype profiles detected that correspond to 22 cultivars. In Formentera Island, from nine prospected accessions, eight belong to different genotype profiles. Redundant genotypes were due to the existence of somatic mutations such as 'Beba' cultivar (local name 'Palop') that had white and pink berry skin color for accessions VIEIV001, VIEIV007 (local name 'Palop blanc') and VIEIV026 (local name 'Palop vermell'), and on the other hand to misnaming errors and homonyms. Results obtained from comparing genetic profiles with the ones in IMIDRA's database are included in Table 2.

Table 2. Synonyms, homonyms, and misnaming.

Genotype Code	Accession Code	Island	Local Name	Prime Name *	Observation	VIVC Proposed Name
GEN_0074	VIEIV001	Ibiza	Palop blanc	Beba	New synonym	Palop blanc
GEN_0105	VIEIV002	Ibiza	Primerenc	Valencí tinto	New synonym	Primerenc
GEN_0204	VIEIV003	Ibiza	Moscatell	Moscatel de Alejandría	Described synonym	-
MEXT_0504	VIEIV005	Ibiza	Monestrell	Llora	New synonym	Monestrell
GEN_0208	VIEIV006	Ibiza	Mamella de vaca	Ahmeur bou Ahmeur	New synonym/ new homonym	Mamella de vaca
GEN_0074	VIEIV007	Ibiza	Palop blanc	Beba	New synonym	Palop blanc
MEXT_3954	**VIEIV008**	Ibiza	Morzacà	-	New found genotype	Morzacà
MEXT_3940	**VIEIV009**	Ibiza	Grec	-	Homonym/New found genotype	Grec
MEXT_3955	**VIEIV010**	Ibiza	Maçanet	-	New found genotype	Maçanet
GEN_0022	VIEIV011	Ibiza	Sultanita	Sultanina	New synonym	Sultanina
GEN_0881	VIEIV012	Ibiza	Fresa	Agawam	Misnaming; *Vitis* interspecific crossing	-
GEN_0003	VIEIV013	Ibiza	Sant Jaume	Santa Magdalena	New synonym	Sant Jaume

Table 2. *Cont.*

Genotype Code	Accession Code	Island	Local Name	Prime Name *	Observation	VIVC Proposed Name
GEN_0575	VIEIV014	Ibiza	Ferrana	Danugue	New synonym	Ferrana
MEXT_3957	**VIEIV015**	Ibiza	Maçanet	-	New found genotype	VIEIV015-Maçanet
GEN_1259	VIEIV016	Ibiza	Giró	Callet negrella	Misnaming	-
GEN_0104	VIEIV017	Ibiza	Fogoneu	Fogoneu		-
MEXT_3958	**VIEIV018**	Ibiza	Vermelleta	-	New found genotype	Vermelleta
MEXT_3940	**VIEIV019**	Ibiza	Grec	-	Homonym/New found genotype	Grec
GEN_0105	VIEIV020	Ibiza	Palop negre	Valencí tinto	New synonym	Palop negre
GEN_0003	VIEIV021	Ibiza	Santa Margalida	Santa Magdalena	New synonym	Santa Margalida
MEXT_0504	VIEIV022	Ibiza	Monestrell de xingló	Llora	New synonym	Monestrell
MEXT_0504	VIEIV023	Ibiza	Monestrell d'Alger	Llora	New synonym	Monestrell
MEXT_3955	**VIEIV024**	Ibiza	Blanqueta	-	Misnaming/New found genotype	Maçanet
MEXT_3959	**VIEIV025**	Ibiza	Colló de gall	-	New found genotype	Colló de gall
GEN_0074	VIEIV026	Ibiza	Palop vermell	Beba roja	Somatic mutation	-
GEN_0575	VIEIV027	Ibiza	Ferrana	Danugue	New synonym	Ferrana
MEXT_3955	**VIEIV028**	Ibiza	Maçanet	-	New found genotype	Maçanet
MEXT_3955	**VIFOR001**	Formentera	Moscatel	-	Misnaming	Maçanet
GEN_0391	VIFOR002	Formentera	Batista	Mansès de Tibbús	Misnaming	-
GEN_0018	VIFOR003	Formentera	Garnatxa blanca	Garnacha tinta	Somatic mutation	-
GEN_0062	VIFOR004	Formentera	Fogoneu	Tinto velasco	Misnaming	-
MEXT_0504	VIFOR005	Formentera	Monestrell	Llora	New synonym	Monestrell
GEN_0575	VIFOR006	Formentera	Palop negre	Danugue	Misnaming	-
MEXT_3940	**VIFOR007**	Formentera	Palop blanc	-	Misnaming/New found genotype	Grec
MEXT_3955	**VIFOR008**	Formentera	Mancet	-	Misnaming	Maçanet
GEN_0111	VIFOR009	Formentera	Grec	Quigat	Misnaming	-

Bold: new genotypes found in this study. * Name registered in VIVC catalogue.

Those 21 identified cultivars are mostly cultivars used traditionally in the Spanish winemaking industry; only five cultivars came from other countries ('Ahmeur bou Ahmeur', 'Agawam', 'Danugue', 'Moscatel de Alejandría', and 'Sultanina'), which either have double use or are exclusively used as table grapes.

The most interesting result about the genetic profiles is that six genotypes, corresponding to 12 accessions, could not be identified either using the extensive IMIDRA's database [59] or the *Vitis* International Variety Catalogue (VIVC) [66]. Those accessions correspond to 'Colló de gall' (mext_3959), 'Grec' (mext_3940), 'Maçanet' (mext_3955), 'VIEIV015_Maçanet' (mext_3957), 'Morzacà' (mext_3954) and 'Vermelleta' (mext_3958) (Table 2).

On the other hand, all 'Monestrell' accessions studied in this work (VIEIV005, VIEIV022, VIEIV013, VIFOR005) have been identified as 'Llora' (mext_0504). These accessions have the same genetic profile as an accession prospected in 2012 in Menorca Island by Institut de Recerca i Formació Agrària i Pesquera (IRFAP) of a minority cultivar named 'Llora' [67], which has not been published in VIVC but is currently in the process of being included in the Spanish vine cultivar catalogue [68].

In total, 2 somatic mutants, 18 cultivars and an interspecific cross were verified.

Unidentified genotype cultivars were named with the name given by the farmer during the interviews. If more than one name had been given for different accessions resulting in the same genotype, the most common cited name was chosen [46]. Those names were 'Maçanet', 'Grec', 'Morzacà', 'Colló de gall' and 'Vermelleta'. Within those new genotypes, there are three detected homonyms. One is for accession VIEIV015 that was named 'Maçanet' by the farmer, but the genotype is not the same as the other accessions identified as 'Maçanet'. The other homonym would be the 'Grec' cultivar name, as it is

a registered synonym for the 'Alcanon' cultivar registered in VIVC [66] (Table 2). On the other hand, the 'Grec' cultivar found in the Formentera sample has been identified as 'Quigat', which would be considered to be a misnaming for 'Grec' until more research has been made (see Table 2).

Ten accession names have been identified as new synonyms. The four 'Monestrell' accessions, three from Ibiza and one from Formentera Island, have all been identified as the same genotypes for the cultivar 'Llora'. 'Sant Jaume' and 'Santa Margalida' are synonyms for the 'Santa Magdalena' cultivar. 'Primerenc' is a synonym for 'Valencí tinto', 'Palop negre' is a synonym for 'Valencí tinto', 'Palop blanc' is a synonym for 'Beba' and 'Sultanita' is a synonym for 'Sultanina' (Table 2).

Also, two other synonyms have been detected during this study. One is found for accessions VIEIV014 and VIEIV027, which is named 'Ferrana' for 'Danugue'. This would be considered a homonym, as 'Ferrana' is registered in VIVC [66] as a synonym for 'Planta fina', and in this case study, two farmers have cited this cultivar with the same name [45]. The last homonym is the accession VIEIV006, identified as 'Ahmeur bou Ahmeur' and locally named 'Mamella de vaca', which is a name that is registered for another genotype profile in VIVC [66].

Two somatic mutations were detected: one for the 'Beba' cultivar of accessions VIEIV001 and VIEIV007 which are named 'Palop blanc' and accession VIEIV026 named 'Palop vermell'. When contrasted with the morphological characterization (Table S3), it is revealed that VIEIV026 has a red grape color, which could be due to a somatic mutation in the cultivar as García-Muñoz [26] detected in her study, too. The other possible somatic mutation that has been detected in previous works is for the 'Garnacha tinta' cultivar, as the local accession is named 'Garnatxa blanca' [69].

There are eight misnaming cases considered in this study for the following cultivars: 'Batista' for 'Mansès de Tibbús', 'Fogoneu' instead of 'Tinto Velasco', 'Giró' for 'Callet negrella', 'Grec' for 'Quigat', 'Blanqueta', 'Moscatell' and 'Mancet' for 'Maçanet', as 'Mancet' was cited by the farmers as a red grape cultivar, and this one has white grapes [46], and 'Palop blanc' for 'Grec' (Table 2).

3.3. Putative Parentage Relationships

The search for compatible trios (parents and offspring) and duos (parent–offspring) was performed based on 26 SSRs loci. From the analysis of the parentage, we established 4 compatible trios (Table 3) and 71 duos (Table 4). The trio analysis results confirm what García-Muñoz [26] had already established for 'Fogoneu' and 'Callet negrella', syn. 'Mansès de cabdell' (Table 3).

Table 3. Compatible trios in Ibiza and Formentera samples.

Offspring ID	First Candidate ID	Pair LOD Score	Second Candidate ID	Pair LOD Score	Trio Loci Mismatching	Trio LOD Score	Published
Fogoneu	Mansès de cabdell	2.26×10^{15}	Excursac	2.40×10^{15}	0	4.89×10^{15}	[26]
Callet negrella	Beba	2.00×10^{15}	Giró sardo	2.10×10^{15}	0	4.44×10^{15}	[26]
Maçanet	Hebén	2.05×10^{15}	Cigenera	2.33×10^{15}	0	5.34×10^{15}	Not published
Colló de gall	Hebén	2.21×10^{15}	Breval negro	2.19×10^{15}	0	5.10×10^{15}	Not published

From the primary analysis conducted, we have a main group with 'Hebén' being the parent of six cultivars. From this, five relationships had already been reported in Cipriani [70], García-Muñoz [26] and D'Onofrio [71]. Three new first-grade relationships have been found ('Maçanet', 'Morzacà' and 'Colló de gall') and one new second grade relationship with 'Beba', a descendent of 'Hebén' and an unknown cultivar, with another undetermined cultivar obtaining the newfound genotype named 'Grec'. The other five new first-grade relationship have been detected within cultivars: 'Mansès de Tibbús' with 'Mansès de cabdell'; 'Callet negrella' with 'Valencí tinto'; 'Grec' with 'Valencí tinto'; 'VIEIV015-Maçanet' with 'Llora', although it could not be determined which would be the

parent and which would be the offspring in this last one; and 'Danugue' with 'Albaranzeuli blanco' (Table 4).

Table 4. Possible parent–offspring relationships for the accessions studied.

Progeny	Parent ID	Consistent Loci	Pair LOD Score	Published
Santa Magdalena	Planta Fina de Pedralba	26/26	8.42×10^{14}	[71]
Beba	Hebén	26/26	5.44×10^{14}	[70]
Valencí tinto	Beba	26/26	1.17×10^{15}	[71]
Valencí tinto	Callet negrella	26/26	9.93×10^{14}	**Not published**
Quigat	Hebén	26/26	5.60×10^{14}	[71]
Moscatel de Alejandría (Moscatel de Málaga/Moscatel de Setúbal/Moscatel Graúdo)	Moscatel de grano menudo (Moscatel Morisco/ Sárga muskotály)	26/26	2.13×10^{15}	[70]
Mansès de Tibbús	Mansès de cabdell	26/26	1.51×10^{15}	**Not published**
Callet negrella	Valencí tinto/Grumiere	26/26	9.93×10^{14}	**Not published**
Grec *	Beba	26/26	6.02×10^{14}	**Not published**
Grec *	Valencí tinto/Grumiere	26/26	6.01×10^{14}	**Not published**
Morzacà *	Hebén	26/26	7.73×10^{14}	**Not published**
Maçanet *	Hebén	26/26	1.06×10^{15}	**Not published**
VIEIV015-Maçanet *	Llora	26/26	1.68×10^{15}	**Not published**
Colló de gall *	Hebén	26/26	9.51×10^{14}	**Not published**
Danugue	Albaranzeuli bianco	22/22	5.42×10^{14}	**Not published**

Bold: new parentage relations found in the study. * New genotypes found.

3.4. Genetic Structure

To analyze the genetic structure, 140 unique genotypes were considered, including the 20 obtained from the Pityusic Islands prospection and a *Vitis* cultivar diversity panel. The 'Agawam' genetic profile (interspecific cross) has not been considered because it could distort results. Also, two different approaches were used, Structure and PCoA, to infer the population structure and geographical assignment of the accessions studied in this work.

From structural analysis, three main grouping levels were identified: K = 2, K = 3 and K = 5 (Figure S1). The membership coefficient threshold defined for individual assignment to a given cluster was Q = 0.80.

K = 2 showed a group with Spanish-origin cultivars with 52.38% of the studied samples grouped showing a possible relationship with Spanish cultivars and another group that included 30% of the cultivars analyzed whose origin was diverse (Maghreb, Italy, France, Greece, etc.). Cultivars assigned to group 2 represented 30% of the cultivars analyzed from Ibiza and Formentera. There were three cultivars that could not be assigned to a certain group as probabilities were under 0.8 (Table S8), which were then considered to be admixed genotypes. Those were 'Santa Magdalena', 'Quigat' and 'VIEIV015-Maçanet' (Figure S2).

Three groups were observed in K = 3, group 1, which had French-related cultivars, (indicated by red-colored bars); group 2 with Maghrebi, Italian and Greek origin (indicated by green-colored bars); and group 3, which had Spanish-related cultivars (indicated by blue-colored bars) (Figure 2).

In this case, as in the previous K = 2, more than half of the samples (55%) from Ibiza and Formentera were grouped with the cultivars that had Spanish origin (Table S9). Spanish cultivars in this analysis have been organized into two groups: cultivated in Balearic Islands, according to García-Muñoz [26], and the rest were from the other important or ancient Spanish cultivars.

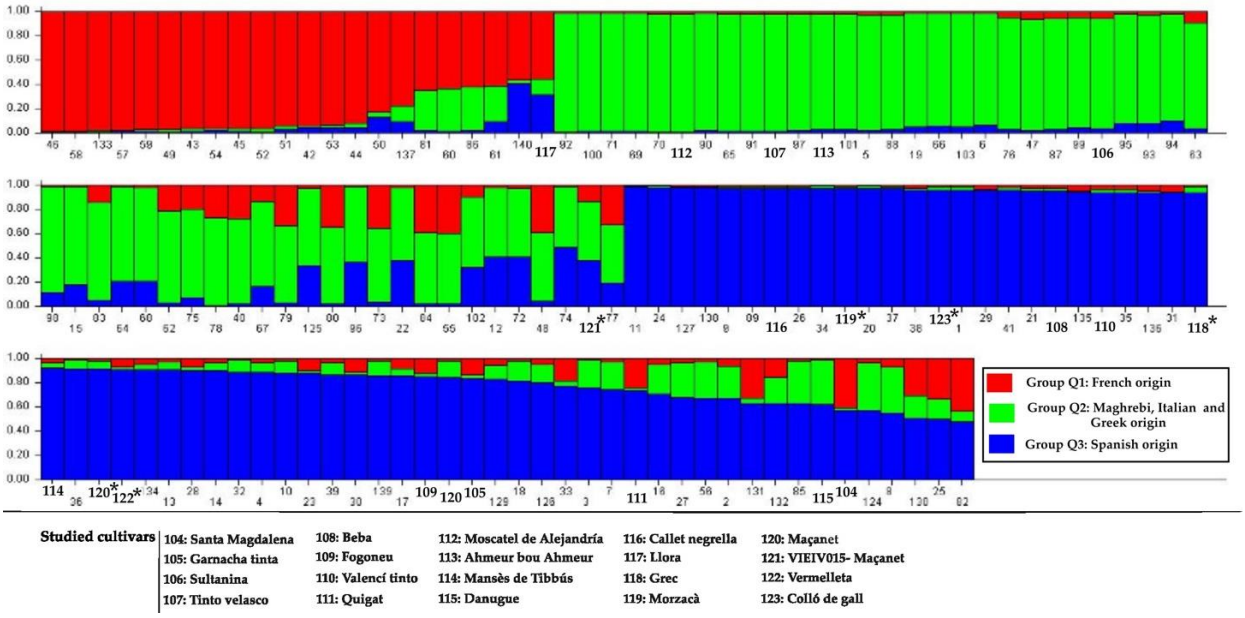

Figure 2. Representation of the 20 genotypes found in Ibiza and Formentera Island using Structure software 2.3.4. version for K = 3. In **bold** are the samples studied, samples with * are new found genotypes [60].

Samples that were grouped into the Spanish-origin group included the following: 'Garnacha tinta' (q = 0.842), 'Fogoneu' (q = 0.857), 'Vermelleta' (q = 0.911), 'Maçanet' (q = 0.917), 'Mansès de Tibbús' (q = 0.923), 'Grec' (q = 0.938), 'Valencí tinto' (q= 0.942), 'Beba' (q = 0.950), 'Colló de gall' (q = 0.960), 'Morzacà' (q = 0.975) and 'Callet negrella' (q = 0.977). This aggregation, except the 'Danugue' cultivar, was the same as that obtained in K = 2.

Overall, 20% of the samples have relations with cultivars that have Maghrebi, Italian and Greek origin. These are 'Ahmeur bou Ahmeur' (q = 0.952), 'Sultanina' (q = 0.906), 'Tinto Velasco' (q = 0.963) and 'Moscatel de Alejandría' (q = 0.970).

Meanwhile, 25% of the cultivars studied that could not be assigned to a certain group.

In K = 5 aggrupation, five groups can be observed, and 55% of the cultivars studied are located in group 3, where Spanish origin cultivars are located. These cultivars are 'Callet negrella' (q = 0.968), 'Morzacà' (q = 0.939), 'Vermelleta' (q = 0.903), 'Beba' (q = 0.893), 'Valencí tinto' (q = 0.869), 'Grec' (q = 0.866), 'Fogoneu' (q = 0.850), 'Colló de gall' (q = 0.837), 'Mansès de Tibbús' (q = 0.837), 'Maçanet' (q = 0.8200) and 'Garnacha tinta' (q = 0.814). In group 2, which has cultivars with Maghrebi origin, 10% of the cultivars are found; those are 'Ahmeur bou Ahmeur' (q = 0.870) and 'Tinto velasco' (q = 0.813). In group 5, there is only the 'Sultanina' cultivar (q = 0.810). The admixed origin accounted for 25% of the studied cultivars (Figure 3) (Table S10).

Genetic analysis by principal coordinates (PCoA, Figure 4) was carried out, and the results obtained are consistent with the results obtained with Structure software 2.3.4 version. In this analysis, prospected cultivars are located in three of the five main origin groups. Group 1 was formed by Spanish origin cultivars, where 'Hebén' is represented at the far end of the group, which means that it is the least mixed cultivar in this group. This matches with the Structure results, where the cultivar has the largest value for Q within its group. In this group, the following cultivars are included: 'Danugue', 'Maçanet', 'Grec', 'Colló de gall', 'Valencí tinto', 'Santa Magdalena', 'Garnacha tinta', 'Quigat', 'Vermelleta', 'Fogoneu', 'Mansès de Tibbús' and 'Callet negrella'. Group 2 is mostly grouping cultivars originated in Maghreb, and 'Ahmeur bou Ahmeur', 'Tinto velasco', 'Sultanina' and 'VIEIV015-Maçanet' appear to be related. The last group, group 3, has cultivars with French origin, and it is where cultivar 'Llora' is: between French, Italian and Greek groups in this analysis.

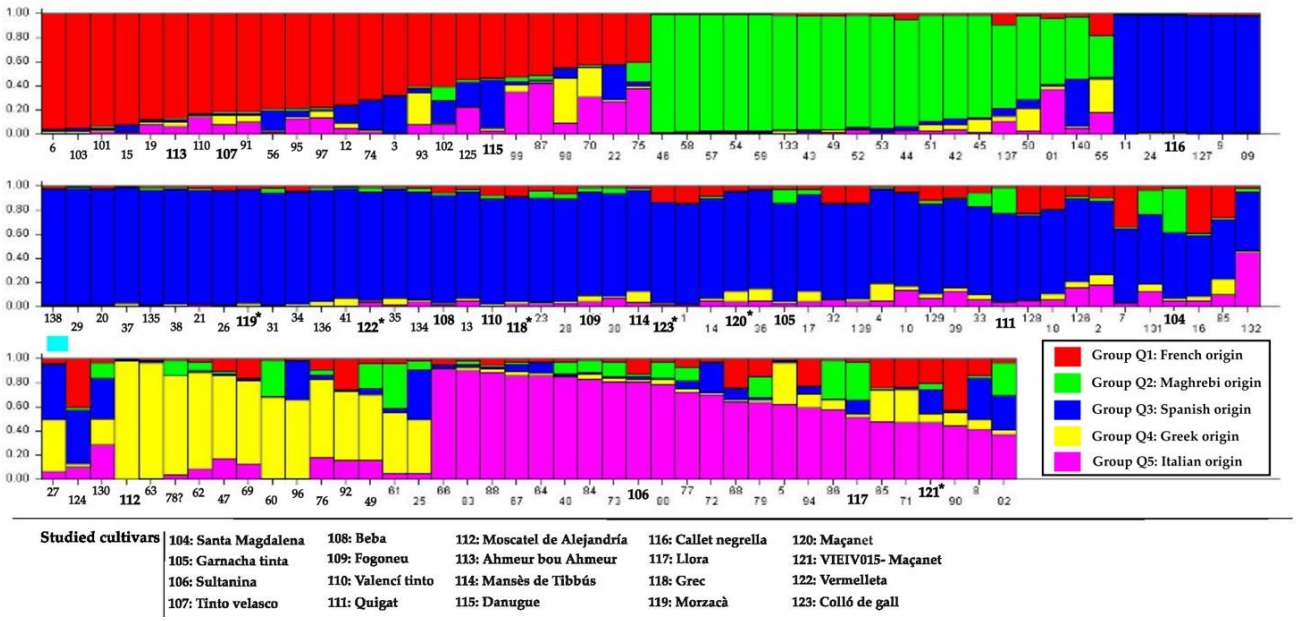

Figure 3. Representation of the 20 genotypes found in Ibiza and Formentera Island using Structure Software 2.3.4 version for K = 5. In **bold** are the samples studied, samples with * are new found genotypes [60].

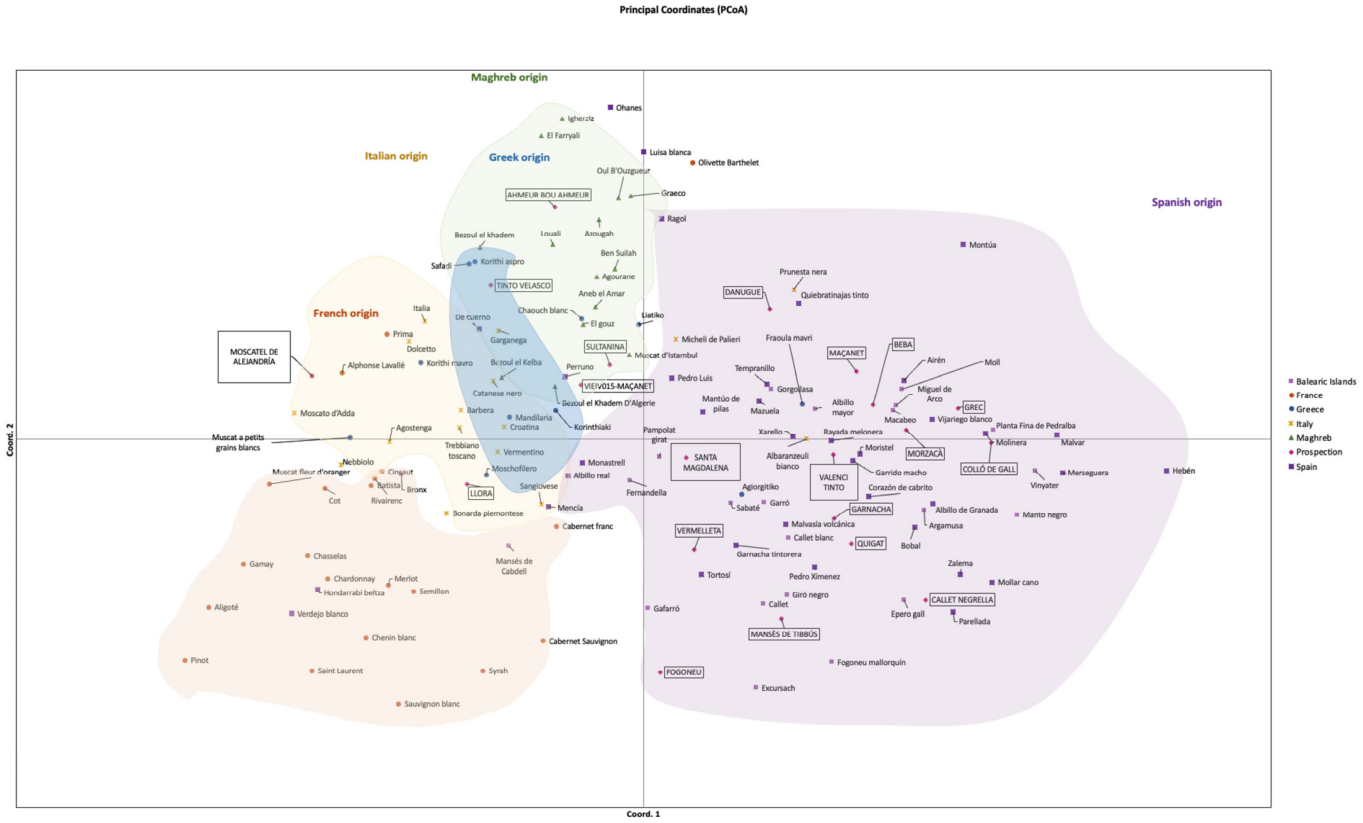

Figure 4. Representation of individuals of the genetic analyses by principal coordinates based on standardized covariance of the genetic distance calculated for 26 codominant markers with GeneAlex software 6.5, using 140 cultivars that include cultivars from five countries as well as the studied samples [55].

Considering only traditional Spanish and Balearic cultivars (Figure 5), some differentiation can be observed between Balearic cultivars, 'Hebén' relatives, and cultivars related to those with Oriental or European origin.

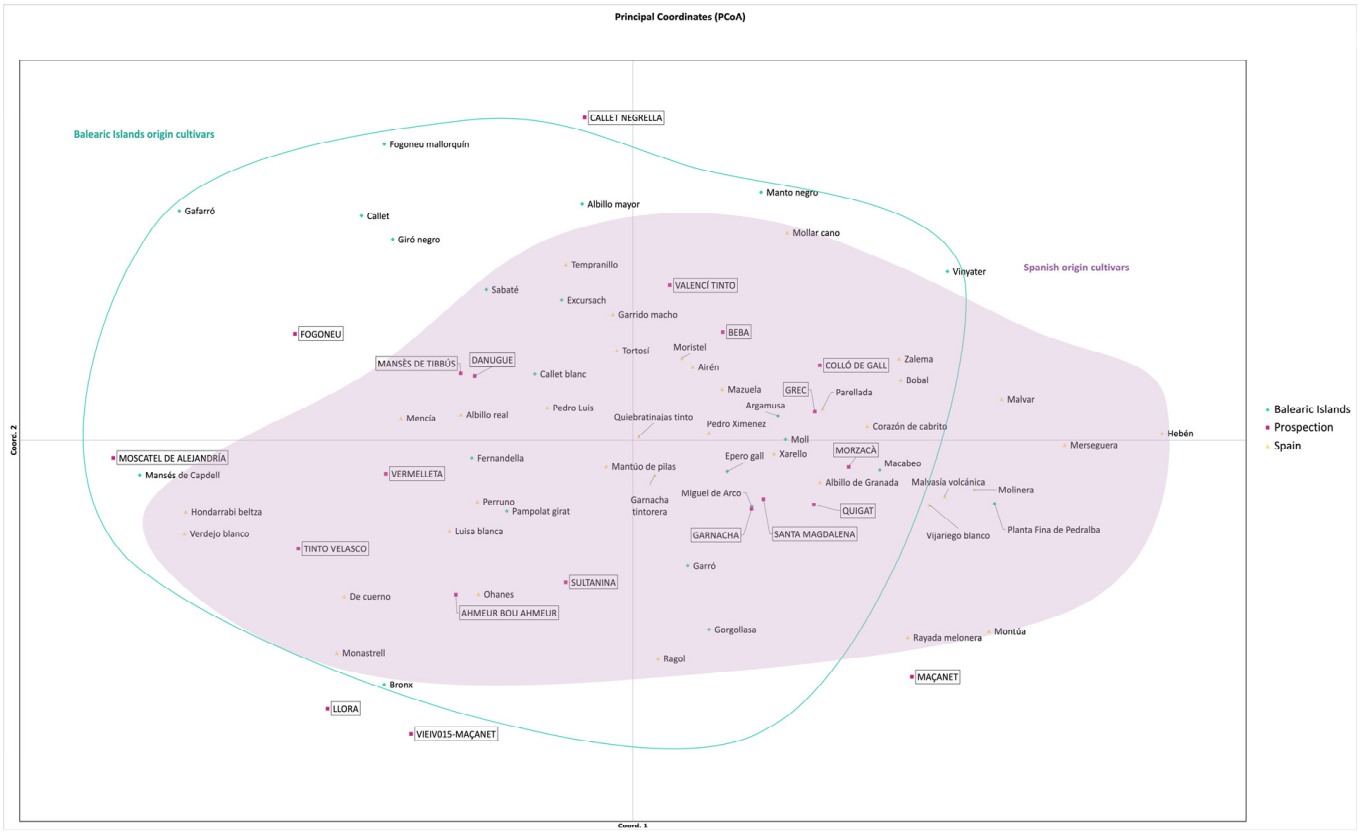

Figure 5. Representation of individuals of the genetic analyses by principal coordinates based on standardized covariance of the genetic distance calculated for 26 codominant markers with GeneAlex software 6.5, considering 78 traditional Spanish and Balearic Islands cultivars, prospection samples included [55].

4. Discussion

4.1. Possible Origin of the Studied Samples

Structure and PCoA results show that most accessions studied here were grouped according to their geographical regions of origin. The highest value of ΔK (Evanno Criterion [62]) was obtained for K = 2 (Figure S1), dividing accessions in a group made up by Spanish cultivars, against another group constituted by cultivars from the rest of Mediterranean origins (Greek, French, Italian and Maghrebi). This clustering is inconsistent with other authors whose results reflect Negrul [72] eco-geographic classification [73,74], since *pontica* and *occidentalis* proles are grouped together, which could indicate a bias in the analysis because of a higher number of Spanish cultivars, many of which are related to 'Hebén'. Regarding K = 3, those previous results are corroborated, since proles *pontica* and *occidentalis* are differentiated, being the third group the Spanish cultivars. Regarding K = 5, the groups correspond to *pontica* (Q5), *occidentalis* (Q3), and *orientalis* subproles *capsica* or Muscat (Q4), according to Emanuelli (2010), who grouped Spanish cultivars with Sultanina descendants in *proles orientalis* subproles *antiasiatica*, using SNPs to differentiate between both groups. A new independent group is obtained in this work shaped by Maghrebi cultivars (Q2) and located in PCoA between *pontica* and the Spanish cultivars group, which in this study, together with Spanish group, constitutes the group proles *orientalis* subproles *antiasiatica*.

According to the results obtained in this work with Structure software 2.3.4 version and PCoA, most of the new or traditional cultivars found in the prospection are related to the Spanish cultivars group, except for 'VIEIV015-Maçanet' and 'Llora', which show a higher component of Greek origin. 'Llora' would be mixed with prole *occidentalis* while 'VIEIV015-Maçanet' would be mixed with prole *orientalis-antiasiatica.* This could correspond with the Phoenician commercial routes around the Mediterranean Sea, where they would have interchanged grapevines between different islands (Cerdà [75] in Piqueras [76]).

Analyzing only Spanish and Balearic cultivars by PCoA, it is observed that new genotypes 'Colló de gall', 'Grec', 'Maçanet' and 'Morzacà' have a strong relationship with traditional Spanish cultivars, as they are all related to the 'Hebén' cultivar, as it is also shown by parentage analysis for some of them. The prospection cultivars 'Fogoneu', 'Danugue', 'Mansès de Tibbús' and 'Callet negrella' could show a stronger relationship with traditional Balearic cultivars as 'Callet', 'Gafarró', 'Fogoneu mallorquí' or 'Giró negre'.

4.2. Cultivar Analysis and Their Possible Origin

Recent works on prospection of grapevine cultivars in the Balearic Islands cite some of the cultivars prospected in this study (Table 5).

Table 5. Prospected cultivars in Ibiza and Formentera with same genotypes as others cited in recent works.

Cultivar	Cited in
Beba	[19–21,26,27,67,77]
Callet negrella	[19,21,26]
Fresa	[19]
Fogoneu	[19–21,26,27,77,78]
Quigat	[19–21,26,27,77]
Llora	[28,67,77]
Moscatel de Alejandría	[19,27,77]
Mansès de Tibbús	[19,21,27]
Santa Magdalena	[21,26–28,77]
Sultanina	[19,27]
Tinto velasco	[27,77]
Valencí tinto	[19,21,27,77]

4.2.1. Traditional Balearic Cultivars

Nowadays, the 'Monestrell' cultivar is authorized in wine appellation that exists in both Ibiza and Formentera, and two other traditional cultivars are authorized in Formentera's wine appellation, which are 'Fogoneu' and 'Premsal' [9,10]. There is another wine appellation that can be used by all the winemakers in the Balearic Islands, Vi de les Illes Balears, that has three Balearic cultivars authorized: 'Moll', 'Callet' and 'Manto negro' [79].

Some of the cultivars, like 'Beba', cited as 'Calops', 'Fogoneu', 'Quigat' quoted as 'Cagat', probably because of its pronunciation in the Catalan spoken in the Balearic Islands has been spelt in two different ways, 'Llora', 'Maçanet', 'Monestrell' or 'Moscatell' have been reported since the end of the 19th century [80,81] in the Balearic Islands, which suggests that these cultivars are traditionally cultivated in this geographic area, although citations about the presence of specific cultivars in Ibiza and Formentera Islands are scarce (Table S11). 'Callet negrella', 'Fogoneu', and 'Quigat' are minority cultivars from the Balearic Islands, as García-Muñoz [26] and Marsal [27] confirm with their work.

'Beba', a well-known eating cultivar distributed in different parts of Spain [82], has been reported in Ibiza by Pérez-Cabrero [83] as 'Palop', which is a synonym registered in VIVC for this cultivar. Ludwig Salvator [81] describes for Ibiza and Formentera a white grape cultivar that could also be found producing red-colored grapes, although he does not cite the name of these grapes: it could be 'Beba roja', a somatic mutation with red grapes that García-Muñoz [26] detected, which we have also found when interviewing farmers cited as 'Palop vermell' and confirmed with the SSR markers results (Table S6). Locally,

farmers use 'Palop blanc' to refer to the cultivar that has white grapes, as *blanc* means white in Catalan. In the work of García-Muñoz [26], the names used are 'Calop blanc' and 'Calop roig' or 'Calop vermell', as *roig* means also red in Catalan. The 'Palop blanc' name for 'Beba' is not registered in VIVC and would be proposed as a synonym for this cultivar.

When comparing ampelographic characterizations [58,84] with ours, it can be observed that leaves from somatic mutations are quite similar, although 'Palop blanc' accessions have shorter teeth in the leaves than 'Palop vermell' (Table S3). Differences in genome size have also been found [6] that are probably caused by somatic mutations when the management and environmental conditions of the crops are the same.

'Beba' is an offspring of 'Hebén' (syn. 'Gibi'), a female Spanish cultivar that is a parent of many cultivars in Spain [85] and also many Balearic cultivars [26]. Our results are coincident with the ones obtained in those previous works.

An offspring of 'Beba' and 'Giró sardo', as García-Muñoz [26] first published, and we confirm with our results (Table 3), is 'Callet negrella', which has been recorded in this study as 'Giró'. This cultivar has been reported for the Balearic Islands in Mestre [86]. It is not registered in the VIVC database [66], but it has been registered in the Spanish variety catalogue [59], and it appears in the Balearic Islands local cultivars catalogue [84]. In this case, the name given by the farmer would be considered a case of misnaming for this cultivar.

Another traditional Balearic cultivar is 'Fogoneu' [26]. Although there are no antique bibliographical references for its presence in Ibiza and Formentera, it has been cited by farmers as a local cultivar and is used in winemaking to improve wine color [45], and it is also authorized in Formentera's wine appellation [10]. During parentage analysis, our results confirm that 'Fogoneu' is an offspring of 'Excursac' and 'Mansès de cabdell' (syn. 'Giro nero'), as García-Muñoz [26] had published.

'Mansès de Tibbús' is also considered to be traditionally grown in the Balearic Islands, as it has been reported by García de los Salmones [87] and García-Muñoz [26]. Although it has not been specifically cited for Ibiza and Formentera, it has been discovered in one of the studied plots. This cultivar that had not been located in any previous prospection on Balearic Islands *Vitis* cultivars and until now was only conserved in IMIDRA's [88] and IRFAP's collections [27].

'Llora' is considered a local minor cultivar cited in Menorca in the end of the 19th century [80,89]. There are also citations [87] for the 'Lloras' cultivar in Menorca and 'Lloreta' in Mallorca that could refer to this cultivar due to the name similarities. In recent times, a sample of this cultivar was detected in Menorca in 2012 [67] but was not located before any other prospection in the Balearic Islands. It also appears in Bota's report [28] (Table 5).

While the 'Llora' cultivar was not specifically mentioned for Ibiza and Formentera, 'Monestrell' was cited [87], which is the name provided by the farmers during the interviews for 'Llora' accessions [45]. 'Monestrell' is also the main black grapevine grown in the Pityusic Islands [11]. It is among the authorized cultivars in Formentera and Ibiza wine appellations [9,10] and was the most cited cultivar for winemaking during the interviews [45]. García de los Salmones [87] also cites 'Mostrell' for Ibiza Island, which phonetically resembles 'Monestrell'; this could either be a misspelling or two cultivars.

The use of 'Monestrell' as a synonym of 'Llora' could be supposed to be widely spread on the Pityusic island as the four 'Monestrell' accessions studied in this work, three samples located in Ibiza and one in Formentera, have all been identified as the 'Llora' cultivar.

Comparing the results on ampelographic descriptors from IMIDRA's database for 'Monestrell' cultivars and ours for 'Llora' cultivars, ampelographic traits are similar, so this might explain the use of 'Monestrell' as a synonym.

The four Monestrell accessions studied have shown different results in the ampelographic descriptors (Table S3). Descriptor OIV065 for leaf blade size is bigger for both 'Monestrell' named accessions, while it is smaller for 'Monestrell d'Alger' and 'Monestrell de xingló' (Figure 6). From the genome size studies, 'Monestrell d'Alger' had a higher 2C DNA content than the other accessions within the same group [6]. There are even differ-

ences in the naming of the accession, where two of them are cited as 'Monestrell d'Alger', because a farmer's relative brought the original plant from Algier [90] and 'Monestrell de xingló' because the plant produces double bunches.

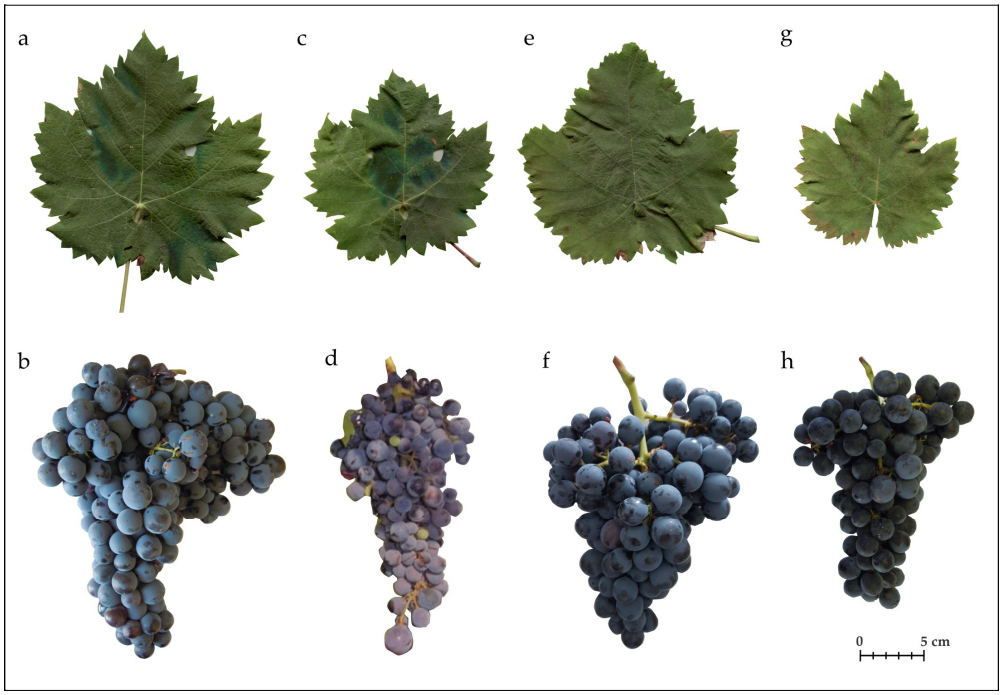

Figure 6. 'Monestrell' cultivars studied in Ibiza and Formentera: (**a**,**b**) 'Monestrell' leaf and bunch accession VIEIV005 (Ibiza); (**c**,**d**) 'Monestrell' leaf and bunch accession VIFOR005 (Formentera); (**e**,**f**) 'Monestrell de xingló' leaf and bunch accession VIEIV022 (Ibiza); (**g**,**h**) 'Monestrell d'Alger' leaf and bunch accession VIEIV023 (Ibiza).

During the interviews and the prospection prior to the ampelographic characterization, farmers cited 'Monestrell fort' and 'Monestrell ros' accessions that could not be located. One of the farmers interviewed had stated that the 'Monestrell' cultivar found in Ibiza Island was different from those that he had seen on the mainland [45]. No references could be found in the bibliography consulted for these different names given by farmers.

From the origin point of view, on one hand, the PCoA results show that this cultivar could have an Italian origin. Italian-origin cultivars in PCoA are also related to Greek and French origin, as these cultivars are located in between them (Figure 4). On the other hand, Structure results showed that this cultivar could be related to French or Greek origin cultivars. From K = 5, 'Llora', although that is an admixed cultivar, it has a stronger component of Greek origin, and the K = 3 result shows that the origin is nearest to the French cultivars, which corroborates the results obtained in PCoA.

A bibliographic reference could confirm this French origin as the 'Llora' cultivar is also cited as 'Marselleres' for Menorca Island [80]. 'Marselleres' could refer to Marseille in France. Piqueras [76] found a citation from the Phoenicians culture about a wine that was named *Lora*, but no other information was provided.

Taking into account the above results and considering that the Phoenician spread vines and viniculture across the Mediterranean territories, such as Marseille in France (Brun [91] in Terral [92]), Ibiza [1] or Maghreb (Rivera [93], Buxó [94] cited in Terral [92]), it could be possible that 'Llora' is a Greek-origin cultivar and is also related to French-origin cultivars because it might be an ancestor of one of them. Further studies should be conducted to confirm this hypothesis and to explore the presence of this cultivar in other places such as Marseille or Algiers.

On the other hand, while reviewing the literature, the names 'Monastrell' and 'Monestrell' have been used to refer to the same cultivar; this could lead to misinterpretation as

two different cultivars. Because of this, we suggest that from now on, the name 'Monestrell' should be written as in this paper, and not *Monastrell*, to be able to trace future works and have a spelling homogeneity criterion. The origin of name is the Latin word *monesteriellu*, diminutive of *monasterium*, abbey (*monestir* in Catalan), as it is thought to be a cultivar sown mostly in abbeys [95].

After the aforementioned findings, 'Monestrell' would be proposed to be included in the VIVC catalogue as a synonym of 'Llora'. Further studies should be made to verify if the other 'Monestrell' names detected as 'Monestrell d'Alger' or 'Monestrell de xingló' are found in other plots and also identified as the 'Llora' cultivar to be able to propose those as synonyms. Also, other plots in the Pityusic Islands should be prospected as there are other citations for 'Monestrell' such as 'Monestrell ros' or 'Monestrell fort' [45] that had not been located yet.

'Quigat', a traditional Balearic cultivar [26], has been misnamed in this study for the newly found genotype 'Grec'. However, more samples should be studied in the islands to find any more use cases of this to better determine if it is a misnaming or a new synonym.

4.2.2. Spanish Cultivars

A traditional Spanish cultivar, such as 'Garnacha tinta', has been detected as a somatic mutation known as 'Garnatxa blanca', which was also cited with this name by the farmer. This somatic mutation is published in VIVC [66]. In García de los Salmones [87], there is only a reference for the 'Garnatxa negra' cultivar in Ibiza but not the white cultivar that has been found in this study.

Another Spanish traditional cultivar found in this study is 'Ahmeur bou Ahmeur', although it has been named locally as 'Mamella de vaca', which means cow's udder in Catalan. This name would considered a synonym for 'Ahmeur bou Ahmeur', as this cultivar has a registered synonym in VIVC, 'Teta de vaca', which is the Spanish translation of 'Mamella de vaca'. There is no reference of this cultivar in the Balearic Islands in the works consulted; only Marsal [27] had a sample with the same name, but this was identified as 'Afus ali', and they proposed 'Mamella de vaca' as a new synonym for that cultivar.

'Santa Magdalena' and 'Valencí tinto' cultivars were also identified in this study. The 'Santa Magdalena' cultivar has been found locally named by farmers as 'Sant Jaume' and 'Santa Margalida' (Table 4). Favà [96] cited 'Sant Jaume' and 'Santa Magdalena' as different cultivars in the Ibiza islands, but he did not conduct SSR makers identification. 'Santa Magdalena' has been cited as a synonym for 'Jaumillo' [77], which is a diminutive for the name Jaume. No references have been found for 'Santa Margalida' apart from the connection between the farmers reporting that this cultivar is an early ripening one [45], and Saint Margaret's day which is in early July in the Spanish catholic calendar, so this could explain the relation with the name. Comparing the ampelographic traits of 'Santa Magdalena' cultivars found (Table S3; [58,84]), the leaves from the Balearic Grapevine Catalogue and ours are very similar, while the ones in IMIDRA's descriptors are slightly different. As a result, for the 'Santa Magdalena' cultivar, 'Sant Jaume' and 'Santa Margalida' would be proposed as new synonyms to be included in VIVC.

The 'Valencí tinto' cultivar has been identified in two samples in this study: one was cited as 'Palop negre', which has also been cited for 'Valencí tinto' in Mallorca [77], and another one was cited as 'Primerenc'. Both names would be proposed to be registered in VIVC as new synonyms for the 'Valencí tinto' cultivar in the Balearic Islands, although further studies are needed to validate these propositions.

4.2.3. French Cultivars

'Danugue' is also a French cultivar that had not been identified until now in any of the recent works (Table 5). It has been identified in three samples, two of which were named 'Ferrana' by two different farmers and one of which was cited as 'Palop negre'. García de los Salmones [87] cites a 'Ferrana negra' cultivar for Ibiza. Nowadays, 'Ferrana' has been used as a synonym for 'Planta fina' and as a homonym for the Moroccan cultivar

'Bourboulenc' [66]. In this case, we propose that 'Ferrana' would also be registered in the VIVC database as a synonym for 'Danugue', and we consider 'Palop negre' a misnaming for the 'Ferrana' cultivar.

In the VIVC database, 'Danugue' has French origin and is related to 'Breval negro' [71,96]. From this study, we have found that 'Danugue' is also related to 'Albarazeuli bianco', which is an Italian cultivar offspring of 'Hebén'. D'Onofrio [71] has cited other local cultivars in the island of Sardinia, which were also related to 'Hebén', showing that there was a cultivar movement from Spain to Italy. Also, the Structure and the PCoA results show that this cultivar seems to have a Spanish origin, so these results with the above mention might suggest that these cultivar was spread through the Mediterranean in the past.

4.2.4. New Identified Genotypes

Six new genotypes have been discovered, which do not appear in any of the databases consulted [58,66]. The proposed names, 'Colló de gall', 'Grec', and 'Maçanet', the latter with its homonyms 'VIEIV05-Mançanet', 'Morzacà and 'Vermelleta', are the ones local farmers reported, as these have not been found for these genotypes.

'Colló de gall' is one of the newfound genotypes. There are no citations of this cultivar for Ibiza Island before Favà's [95] work on *Vitis* names in the Catalan language. In Bota [28], there is a sample cited as a newfound genotype named 'Colló de gall', but no ampelographic description or microsatellite results have been published yet. This cultivar name means rooster's testicle, translated from Catalan (being *gall*, rooster, and *colló,* testicle). He also cites other synonyms for this cultivar, such as 'Botó de gall', 'Colló de gat' or 'Botó de gat (where *gat*, means cat in Catalan). He suggests that *botó* is used as a synonym of *colló* in order not have to use *colló* with its sexual connotation.

Favà's [95] descriptions for the 'Colló de gall' cultivar does not match with our ampelographic characterization (Table S3). In his work, this cultivar is described to have large bunches and to be very productive, while the studied plants in this work are not very productive, and bunches tend to be small to medium. In any case, it must be considered that the plants studied are very old [37], not grafted on American rootstock, and are not watered, and all this could alter the ampelographic descriptions [35].

From the parentage analysis, this cultivar might be an offspring of Spanish cultivar 'Hebén', which makes sense with the results obtained in Structure analyses, where this cultivar is grouped with Spanish-origin cultivars in all groupings obtained.

The new identified genotype locally named 'Grec' has been one of the most reported cultivar names during the interviews [45]. It is cited in García de los Salmones [87] in Ibiza Island as 'Grech' (an old Catalan spelling for *Grec*, meaning Greek), although with red grapes, and with white grapes in Menorca. This last reference might refer to our cultivar as it has white grapes (Table S3). The name *Grec* was also used to refer to a type of wine [76,95]. Favà [95] and the farmers [45] cite also 'Grec' as a synonym of 'Malvasia'. 'Malvasia' is one of the authorized cultivars in winemaking appellation regulations in Ibiza and Formentera [9,10]. Taking this synonym of 'Malvasia' for 'Grec', these cultivars are being used to produce wines within the appellation in Ibiza Island. In Bota [28], there is a 'Grec' accession from Ibiza, but this report does not specify any other information. Also, there is a recent ampelographic description of a 'Grec' cultivar [84] from Ibiza that has similar results to our ampelographic descriptions (Table S3). Consulting the VIVC catalogue, the 'Grec' name appears as a synonym of 'Alcanon' and 'Greco', this last one was considered by Favà [95] as a translation to Italian of the name 'Grec', and it is also registered in the VIVC catalogue as 'Malvasia'. So, in this case, 'Grec' would be proposed as a homonym for the new genotype.

From our Structure analysis results, 'Grec' might have Spanish origin, as in all groupings, it falls within the Spanish-origin cultivars. Parentage analysis suggests this, as 'Grec' is related to 'Beba', which is an offspring of 'Hebén' [26]: an ancient Spanish cultivar that has originated many other cultivars [85]. The results also suggest it could be a sibling of 'Valencí tinto', as this is confirmed to be 'Beba' offspring by VIVC (Table 3).

'Maçanet' cultivar is another of the new genotypes discovered in this study. It has been cited for Menorca Island [80] and for Ibiza [83,87], although García de los Salmones [87] describes this cultivar as a black grape color cultivar, while ours has white grapes (Table S3). In Favà [95], he registered 'Mançaneta' as a synonym of 'Maçanet' as being originally from outside Ibiza island, but all farmers interviewed have cited 'Maçanet' as a traditional cultivar of Ibiza [45]. He proposes that the ancient cultivar 'Masanel' from the south of Spain could be related to 'Maçanet and also to 'Manzanilla' cultivars, as both have similarities to the names *mançana* and *manzana*, meaning apple in Catalan and Spanish, respectively.

The above-mentioned information suggests a Spanish origin for this cultivar, which the Structure results obtained seem to back up, as this cultivar falls in the Spanish origin group either in K = 2 and K = 3. From the parentage results, this cultivar is the offspring of 'Hebén' and 'Cigenera', which are both Spanish-origin cultivars [66] (Table 3). Despite this, the relationship with the 'Manzanilla' cultivar [95] has been checked, but there is a mismatch of 10 loci from the 26 compared, so there is not a significant relation between these two cultivars that might only be related etymologically.

For the 'Maçanet' cultivar, a homonym has been detected, as accession 'VIEIV015-Maçanet' has resulted in a different genotype from the 'Maçanet' cultivar that had not been detected in either IMIDRA's database [58] or VIVC [66]. From the parentage analysis (Table 4), there is a significant relationship between 'VIEIV015-Maçanet' and 'Llora', but it is not possible to determine which is the parent and which is offspring without chloroplast DNA, which has not been analyzed in this study.

One of the accessions that was identified as 'Maçanet' was locally named 'Blanqueta'. The name 'Blanqueta' has been cited as a synonym of 'Merseguera', which is a cultivar that has been cultivated in the Valencian community since 1800 [95]. More samples must be studied to be able to determine if this is a synonym of the 'Maçanet' cultivar, although farmers reported them as different cultivars. One of them also stated that the 'Blanqueta' cultivar was very hard to graft, which he did not state for the 'Maçanet' cultivar [45]. Bota [28] names a 'Maçanet' sample as a non-registered cultivar in her report but does not provide any other information.

Another unregistered genotype found in this study is the 'Morzacà' cultivar. This cultivar has only been found cited by a farmer in Ibiza Island in Favà's work [95]. Etymologically, this name could be derived from the word *morzar*, meaning to have breakfast in Catalan, and *ca*, which means dog in Catalan, suggesting that this cultivar was not good to eat, as it was dog's food [95]. In the interviews conducted in this study, this cultivar was cited for winemaking, and a farmer also explained that it was not a very aromatic grape [45], which could make sense with Favà's [95] etymology of the name. From Structure analysis, it is grouped with the Spanish-origin cultivars, and from parentage analysis, it appears that it could be an offspring of 'Hebén', which aligns with the Structure results. 'Morzacà' would be proposed as a prime name to be included in VIVC for this genotype and as a local cultivar from Ibiza and Formentera.

'Vermelleta' is the last cultivar in this study that has been found to have a unique genotype profile that has not been detected before. The name of this cultivar might be related to the color of the grapes as it has red, small grapes (Table S3) and 'Vermelleta' is a feminine diminutive of red in Catalan. Ludwig Salvator [80] cites the 'D'en Vermell' cultivar in Menorca Island and Bota [28] cites a cultivar named 'Vermellet'. Both are related to the name red in Catalan, although 'D'en Vermell' refers to be property of a man named *Vermell*, and the name cited in Bota seems to refer to the color of the grape as a masculine diminutive. In the case of Bota's study [28], no other information has been published yet, so it cannot be known if they are the same cultivars.

In the case of 'Morzacà' and 'Vermelleta', there is also no earlier reference before 2000s [95] which might confirm that those are old minor cultivars.

5. Conclusions

This study to assess and identify *Vitis* cultivars in the Pityusic islands has been conducted from a different point of view than most similar works conducted in Europe, as it uses ethnobotanical interviews to select samples in plots with the idea to recover cultivars that are not officially documented and also to gather information that could help to identify the cultivars and their origin.

With this approach, it has been possible to have an image of the current state of cultivars maintained in plots and vineyards in parts of Ibiza and Formentera islands, discovering six new genotypes that had not been published before, one that was only maintained in a conservation collection, and ten new synonyms, which have been detected and proposed to be included in the VIVC catalogue. Most of these cultivars belong to local traditions, especially the 'Monestrell' (syn. 'Llora') cultivar, which is present in the 100% of the studied plots.

As the plots in this study were concentrated mostly in the Sant Josep de sa Talaia municipality, in Ibiza, further studies should be made with the approach used in this study to cover all Ibiza and Formentera Islands, as more minor cultivars could still be found.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae9121307/s1, Figure S1. ΔK graphic., Figure S2. Graphical representation of the 20 genotypes found in Ibiza and Formentera Island by Structure software for K = 2 (Stanford University, 2.3.4 version, 2012); Table S1. Plot information; Table S2. Samples codes and vouchers numbers; Table S3. Ampelographic descriptors mode values 2017 and 2018 for the samples accessions studied; Table S4. Observation number for each accession, descriptor and year; Table S5: SSR markers diversity indexes; Table S6. SSR markers accession analyses results; Table S7. Cultivars considered Structure analysis; Table S8. Sample codes and probabilities for K = 2; Table S9. Sample codes and probabilities for K = 3; Table S10. Sample codes and probabilities for K = 5; Table S11. Bibliographic references for research cultivars in the Balearic Islands and Ibiza and Formentera.

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