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# Georgian Native Grapevine Cultivars ‘Alexandrouli’, ‘Dzveli Alexandrouli’ and ‘Mujuretuli’: Description, Genetic Relationship and Hypotheses About Their Origin

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## Abstract

Three Georgian native grape cultivars, ‘Alexandrouli’, ‘Dzveli Alexandrouli’ and ‘Mujuretuli’ (*Vitis vinifera* L.), widely grown in the Racha province of western Georgia, were investigated by SSR molecular markers and chemotaxonomic, ampelographic and phenological methods. The investigations aimed to clarify genetic and phenetic correlations among the cultivars and to learn their possible genetic relationships. The results showed that ‘Alexandrouli’ and ‘Mujuretuli’ are two phenotypically distinct clones with identical genetic profiles. Probably ‘Mujuretuli’ originated in the Racha province as a mutant of ‘Alexandrouli’. On the other hand, ‘Dzveli Alexandrouli’ is genetically and ampelographically distinct. Hence, ‘Dzveli Alexandrouli’ and ‘Alexandrouli’ can be considered as homonym cultivars. Results emphasize the importance of integrating genotyping and phenotyping methods with ethnographic and written sources in germplasm characterization.

The identification of local germplasm by molecular marker genotyping and morphological phenotyping is an essential aspect of maintaining crop biodiversity. These activities are generally performed in germplasm collections, although they could lead to erroneous conclusions when they are not integrated with historical and ethnographic information. In this study, we aimed to clarify the relationships among three native Georgian grape (*Vitis vinifera* L.) cultivars, which have been under debate for the last century.

At the end of 20<sup>th</sup> century, the conservation of Georgian germplasm became urgent. For the new independent Georgia, economic difficulties during this period were many, and funding for the maintenance of field collections was lacking. The University of Milan saved part of the Georgian germplasm collection in Italy, propagated it and returned it to Georgia under the framework of the International project “Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black sea area” led by Bioversity International (formerly “Inter-

national Plant Genetic Resources Institute” (IPGRI), of Rome, Italy) (16). In Italy, the preserved germplasm was characterized by DNA fingerprinting, ampelographic descriptors and chemotaxonomic methods (17, 27). The results presented in this article are part of these investigations.

Georgian native cultivars ‘Alexandrouli’ (syn. ‘Alexandreuli’) and ‘Mujuretuli’ are used to make the well-known semi-sweet red Georgian wine called “Khvanchkara” and other high-quality table wines in the Racha-Lechkhumi province of western Georgia (Fig. 1). Traditionally, these two cultivars are grown in the same vineyards at different ratios - typically ‘Alexandrouli’ is predominant, while ‘Mujuretuli’ occupies up to 15% of the vineyard area (21). ‘Dzveli Alexandrouli’ is another black grape native to Racha province. At present it has a very limited distribution within this region, and only a few accessions are maintained in national germplasm collections (20, 33).

According to the literature, ‘Alexandrouli’ originated in the Racha region (15, 21, 25, 26,

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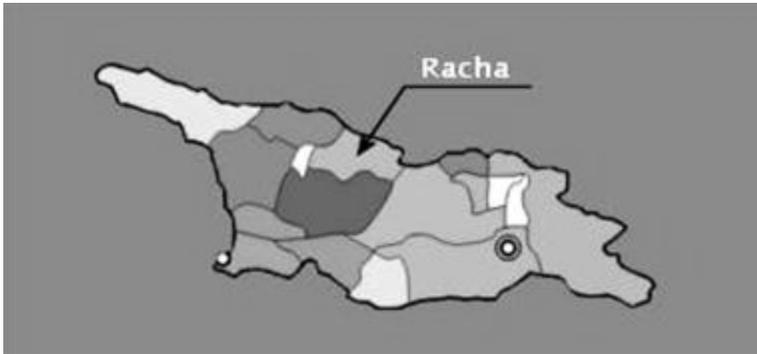


Fig. 1. Map of Racha province in the country of Georgia.

33) and, based on ethnographic information, it was believed to have been derived from a local wild grapevine (25). The origin of ‘Mujuretuli’ is under debate. The historian Javakhishvili (13) mentioned that the name ‘Mujuretuli’ could have been derived from ‘Mujireti’, a village in the Imereti province, which borders on Racha, based on semantics. However, other authors (15, 20, 33) have pointed out that Javakhishvili’s hypothesis is acceptable only in general terms considering the very ancient origin of Georgian native germplasm. For many years, the exchange of cultivars among different regions of Georgia was common. In addition, there are several counterarguments to Javakhishvili’s view:

- i) ‘Mujuretuli’ is not grown in the mentioned village at present and no viticultural references confirming cultivation of ‘Mujuretuli’ in the village “Mujireti” in the past exist;
- ii) according to personal accounts from living grape growers and written accounts (6, 15, 21) ‘Mujuretuli’ has long been cultivated together with ‘Alexandrouli’ in the same vineyards within the Racha-Lechkhumi province;
- iii) the two cultivars have very similar ampelographic and agronomic characteristics.

On these bases, it can be concluded that the cultivar ‘Mujuretuli’ did not originate in Imereti province, but could be native to Racha province.

Because of the close phenotypic resemblance of the two cultivars, Demetradze and Mirotadze (5), Ketskhoveli et al. (15), and Mirotadze and Bregadze (21) hypothesized that ‘Mujuretuli’ may be a bud mutation of ‘Alexandrouli’. Tsertsvadze (33, 34) had an opposite view and wrote, “We do not agree with the above mentioned opinion, we do not think that ‘Mujuretuli’ is a sport of ‘Alexandrouli’ and I supposed that these cultivars have deeper affinity because they could be originated from similar source”.

Regarding a possible relationship between ‘Dzveli Alexandrouli’ and ‘Alexandrouli’, no information is given in references, despite the similarity in their names, and despite the opinion of Tsertsvadze (33) that ‘Dzveli Alexandrouli’ “has no similarity with ‘Alexandrouli’”. This homonymy invites us to verify, by ampelographic and genetic methods, the relationship between the two cultivars. Here, ‘Alexandrouli’, ‘Mujuretuli’ and ‘Dzveli Alexandrouli’ were investigated by morphological, chemical and molecular means, with the support of ethnographic references, to verify their distinctiveness and possible genetic relationships.

### Materials and Methods

*Plant materials and conditions of cultivation.* The Georgian native cultivars ‘Alexandrouli’, ‘Dzveli Alexandrouli’ and ‘Mujuretuli’ were represented by accessions from a germplasm collection established in Gorizia (north-

eastern Italy), with plant material collected from the grapevine collection, established by the Georgian ancient cultivars on the locality Dighomi (Tbilisi, Georgia) in 1967-1968. Each accession was represented by 5 plants. The three cultivars were investigated within a more general survey which included 144 other Georgian native cultivars and 8 reference European cultivars (17). Adopting Koeppen's classification (31), Gorizia has a mesothermal climate with transitional characters between an oceanic type (Cfb) and a Mediterranean one (Csa). It is characterized by hot but not humid summers and cold and rainy winters. Average summer and winter temperature are 21.5-22.5°C and 4°C respectively. Mean annual rainfall is 1350-1400 mm, with a pattern that presents two minima (February and July) and two maxima (November and June). The soil is alluvial, coarse textured, deep and rich in stony material, sufficiently rich in organic matter (*ca.* 2%) and well supplied with mineral nutrients via fertilization. The vineyard was established in 2000. Plant were spaced at 2.4 m (interrow) x 1 m (intra-row) for about 4,160 plants/ha, and trained to the classical Guyot system, leaving after winter pruning a 2 bud spur and a 10-12 bud cane. The interrow soil was kept weed-free by mechanical tillage and two glyphosate herbicide treatments yearly.

*Ampelography and ampelometry.* Harmonized ampelographic descriptors of OIV (23), IPGRI (12) and GENRES 081 (8) were used for the investigation. Each cultivar was characterized by a total of 58 descriptors. According to the protocols, measurements and observations (including phenological records) were taken from 10-12 shoots from at least three plants. Fruit ripening time was determined by weekly fruit chemical analysis to identify the point of maximum sugar content, and minimum acidity before any visible symptoms of berry dehydration. Ampelometric records, including leaf, cluster, berry and seed measurements were analyzed by the ampelographic software "SuperAmpelo" (30). Data were recorded during the four year period 2003-2006.

*Virus testing.* The vines of the three culti-

vars were tested for the possible presence of viruses and virus-like organisms according to Directive 2002/11/CE regulating the commercialization of grapevine propagation material. Specifically, we tested for ArMV (Arabic mosaic virus), GFLV (Grapevine fanleaf virus), GLRaV - 1, 2, 3 (Grapevine ampelovirus type I, II and III), GVA (Grapevine virus A) and GVB (Grapevine virus B). All the tests were negative.

*Anthocyanin analyses.* Anthocyanin analysis was done according to Mattivi et al. (18). The skins from 20 frozen berries were extracted in two phases for 12 and 2 hours, with 100 and 50 ml methanol respectively. The total extract was filtered to remove plant debris, evaporated to dryness in a rotary evaporator at 37°C and dissolved in aqueous 0.3% perchloric acid - methanol (73:27 v/v). Anthocyanin profile was determined at 520 nm using a Shimadzu HPLC LC-10 AD (Shimadzu, Tokyo, Japan) connected to a Shimadzu UV-VIS detector SPD-10 A (18). The extracts were analysed by HPLC under the following conditions: flow rate 0.45 ml/min; temperature 40°C; column Purospher RP18, 5 µm (250 x 4 mm) preceded by a guard column Purospher RP18, 5 µm (4 x 4 mm) (Merck, Darmstadt, Germany); solvent A: methanol; solvent B: aqueous 0.3% perchloric acid - Elution: linear gradient from 27% to 43% A in 32 minutes, from 43% to 68.5% in 13 minutes, from 68.5% to 100% in 2 minutes, then isocratically with 100% A for 3 minutes; re-equilibrating time: 5 minutes; loop: 10 µl. A calibration curve was established using malvidin-3-monoglucoside and results were expressed as "malvidin-3-monoglucoside equivalents".

The anthocyanin profiles were outlined as relative levels of delphinidin-3-monoglucoside, cyanidin-3-monoglucoside, petunidin-3-monoglucoside, peonidin-3-monoglucoside, and malvidin-3-monoglucoside by summation of their acetic and p-coumaric esters.

*DNA extraction and microsatellite analyses.* DNA was extracted from leaflets using the Plant DNeasy Kit (Qiagen, Milano, Italy). Fourteen microsatellite loci - VVS2 (32), VVMD7 (3), VVMD21, VVMD24,

VVMD25, VVMD27, VVMD28 (2), VrZAG21, VrZAG47, VrZAG64, VrZAG67 (29), VrZAG62, VrZAG79 (28) - were assessed (17). Initially 6 microsatellite loci were tested in all three cultivars; afterward, 8 additional loci were tested only for 'Alexandrouli' and 'Mujuretuli'. PCR was performed in a total volume of 20  $\mu$ l containing 20-40 ng of genomic DNA, 1X reaction buffer (200mM Tris-HCl (pH 8.0), 500 mM KCl), 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.1  $\mu$ M of each primer. PCRs were carried out in a PTC 100 thermal cycler (MJ Research Inc. Waltham, Massachusetts, USA, under the following cycling conditions: 2 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 50°C and 60 sec at 72°C; and a final hold of 3 min at 72°C. The forward primer of each pair was labeled with a fluorescent dye (6-FAM, VIC, NED, and PET). Separation of SSR alleles was performed on a ABI 310 Genomic Analyser (Applied Biosystems, Foster City, California, USA) using the POP-4 polymer and a 47 cm-long capillary; allele sizing was performed with the GeneMapper software 3.7 (Applied Biosystems).

*Statistical analyses.* The Nei's (22) genetic distance was calculated using Microsat software (19). To better clarify the identity between 'Alexandrouli' and 'Mujuretuli' the software GIMLET v.1.3.2 (33) was used to define the Probability of Identity (PI) values. These were calculated for each microsatellite locus and over all loci basing on allele frequencies as described by Paetkau and Strobeck (24). This parameter is useful to evaluate the probability that 2 individuals drawn at random from a population will have the same genotype at multiple loci. To verify that our set of SSR markers was powerful enough we also computed the Probability of Identity among full siblings (PIsibs) (6), which is defined as the probability that 2 full-sibs individuals drawn at random from a population have the same multilocus genotype. These parameters were estimated on a sample population of 144 *Vitis vinifera* L. accessions traditionally grown in Georgia. The two accessions under debate were, of course, included in this group.

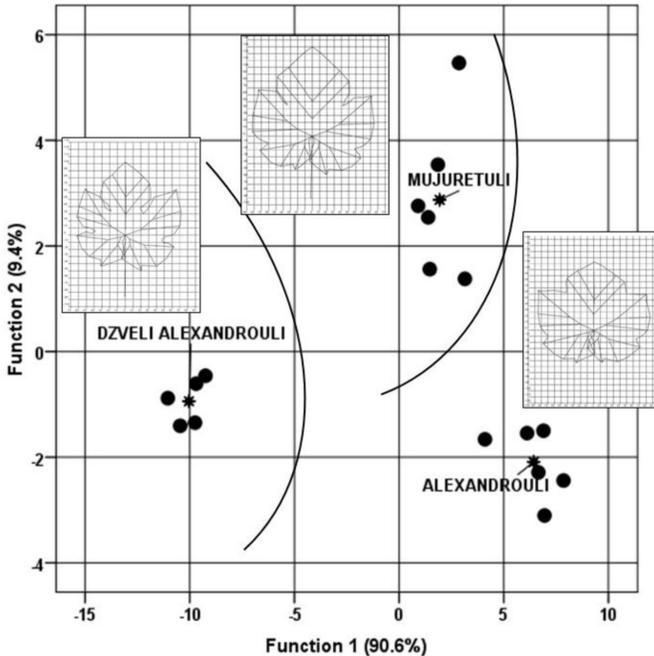
The software ML-RELATE (14) was also used to estimate relatedness among these 144 Georgian cultivars. ML-RELATE is able to calculate the maximum likelihood estimates of relatedness (R) (1, 37). The software was also used to perform a probability calculation to verify the hypothesis that 'Alexandrouli' and 'Mujuretuli' may be considered, basing on our data, full-sibs instead of clones. In fact the program discriminates among 4 common pedigree relationships: unrelated, half-siblings, full siblings and parent-offspring; suggesting which is the most probable among the four, basing on the calculation of the natural logarithm of the relationship with the highest likelihood (LnL(R)).

Chemotaxonomic and morphometric data were processed by uni- and multivariate analyses. Comparisons among accessions were verified by general linear model including accession and year and their interaction as sources of variation. Means were separated according to Tukey's HSD test ( $P < 0.05$ ). Canonical discriminant analyses were used for processing measured leaf parameters. Statistical data were analyzed using the software SPSS 14.0 (Chicago, USA).

## Results and Discussion

The study of relationships among native Georgian grape cultivars is an intriguing subject due to the ancient history of their cultivation. Most of the difficulties presented by this topic derive from the limited written information about Georgian ampelographic assortment, at least before the 19<sup>th</sup> century. This lack of information can be overcome by combining the use of molecular markers with phenotypic characterization based on classic and modern ampelography (17, 36).

Regarding leaf morphology, at the first statistical analysis step, 44 main phyllometric descriptors were involved, determining size and shape of a leaf blade: 9 leaf descriptors were able to significantly separate 'Alexandrouli' and 'Mujuretuli' and 19 descriptors were able to separate 'Dzveli Alexandrouli' and 'Alexandrouli' from each other (data not shown). On the second step, 20 main phyllometric



**Fig. 2.** Scatter plot representing the classification of the three grape cultivars according to the first two discriminant functions built on the basis of 14 phyllometric indices selected out of 20. The percentage of total variability explained by each discriminant function is reported in parentheses in the axis label. The most discriminant variables were: angles between main and lateral veins, size of the central lobe and the degree of opening and overlapping of the petiolar sinus.

descriptors, determining the shape of a leaf blade, were selected according to their ability to describe the leaf shape independently from the leaf size. A canonical discriminant analysis was also done. Two main functions were produced by the discriminant analysis based on 14 traits; the two were able to explain 90.6% and 9.4% of the total variance respectively (Fig. 2). 'Alexandrouli', 'Mujuretuli' and 'Dzveli Alexandrouli' were clearly separated, i.e. they have distinctly different leaf shapes. 'Dzveli Alexandrouli' was more different than the other two. 'Alexandrouli' and 'Mujuretuli' had greater similarities, despite visible differences in lobature and the shape of the petiole sinus (Fig. 2).

*a) 'Alexandrouli' vs. 'Mujuretuli'*

Among the 58 ampelographic descriptors,

'Alexandrouli' and 'Mujuretuli' were similar for 47 and dissimilar for 11. As a result, the level of similarity between 'Alexandrouli' and 'Mujuretuli' was 81.0%. One of the main differences was the shape of the berry, which in 'Mujuretuli' was wider on the top and narrower on the bottom compared to 'Alexandrouli'. Other dissimilar characters were: anthocyanin coloration of the shoot tip, degree of lignification of peduncle, cluster size, leaf shape and size.

Statistical analysis of biometric data on clusters and tendrils (Table 1) indicated that 'Mujuretuli' and 'Alexandrouli' were not different in cluster length and weight, but were different in cluster width. 'Alexandrouli' and 'Mujuretuli' had loose clusters. No differences were found among the two cultivars in length of peduncles or tendrils.

**Table 1.** Comparison among the three grape cultivars in biometric, chemical and phenological parameters.

Cultivar	Alexandrouli	Mujuretuli	Dzveli Alexandrouli
<u>Biometric descriptions of cluster and tendril<sup>z</sup></u>			
Cluster Length (mm)	160.8 a <sup>x</sup>	164.9 a	171.0 a
Cluster Width (mm)	110.8 b	89.1 a	112.2 b
Cluster Weight (g)	104.2 a	88.6 a	291.3b
Berry Weight (g)	1.08 a	1.09 a	1.30 b
Peduncle Length (mm)	26.6 a	27.7 a	29.7 a
Tendril Length (mm)	20.0 a	19.5 a	19.5 a
<u>Juice sugar and acidity levels<sup>z</sup></u>			
°Brix	23.5 b	24.5 b	20.1 a
pH	3.17 a	3.26 a	3.16 a
Titrateable acidity (g/l of tartaric acid)	6.95 b	7.00 b	6.29 a
<u>Anthocyanins<sup>y</sup></u>			
Anthocyanin total (mg/kg grapes)	689.52a	701.96a	209.44b
<u>Anthocyanin forms<sup>y</sup> (% of total anthocyanin)</u>			
1. Delphinidin-3-glucoside	3.67a	4.53a	7.64b
2. Cyanidin-3-glucoside	1.40a	1.52a	4.21b
3. Petunidin-3-glucoside	5.24a	6.09a	6.89a
4. Peonidin-3-glucoside	9.63a	9.01a	8.68a
5. Malvidin-3-glucoside	63.12a	61.86a	46.68b
(1-5) acetates	3.63a	3.85a	10.29b
(1-5) p-coumarates	13.32a	13.15a	15.61b
<u>Phenological comparison<sup>zw</sup></u>			
Bud break	106 a	107 a	108 a
Flowering	156 a	155 a	157 a
Veraison	219 a	219 a	233 b
Harvest	280 a	280 a	286 a

<sup>z</sup> Average data of three years

<sup>y</sup> Harvested in 2006

<sup>x</sup> Means within a row followed by the same letter are not statistically different according to Tukey's HSD test (P< 0.05)

<sup>w</sup> With numbers representing the day of the year for each phenological event

According to the statistical analysis of sugar and acidity data of berry juice at harvest, 'Mujuretuli' and 'Alexandrouli' had a very similar ripening profiles (Table 1). The total content of anthocyanins in the berry skin at maturity in 'Alexandrouli' was similar to 'Mujuretuli' (Table 1) and moreover, their anthocyanin profiles were more or less similar in the proportions of different anthocyanins. 'Mujuretuli' and 'Alexandrouli' did not differ in phenology (Table 1).

The six tested microsatellite loci (VVS2, VrZAG62, VrZAG79, VVMD27, VVMD7

and VVMD28) showed identical allele profiles for 'Alexandrouli' and 'Mujuretuli' on the first step (Table 2). To confirm their identity, we analyzed another 8 loci (Table 2). Results confirmed the molecular identity of the 2 accessions, giving rise to a Nei's distance matrix value of 0.

'Alexandrouli' and 'Mujuretuli' genetic fingerprints at 14 loci were used together with the other 142 accessions described with the same tools to verify, first of all, the discrimination power of the selected SSR loci; for this purpose the PI and PIsibs value were calculated

**Table 2.** Length (bp) of fragments amplified for 14 SSRs in the grape cultivars 'Alexandrouli', 'Mujuretuli' and 'Dzveli Alexandrouli'<sup>2</sup>.

Cultivar	VVS2	VrZAG62	VrZAG79	VVMD27	VVMD7	VVMD28	VVMD5
'Alexandrouli'	139 - 150	188 - 202	234 - 248	177 - 183	245 - 245	234 - 244	224-224
'Mujuretuli'	139 - 150	188 - 202	234 - 248	177 - 183	245 - 245	234 - 244	224-224
'Dzveli Alexandrouli'	133 - 139	189 - 200	252 - 258	177 - 187	247 - 251	236 - 236	224-232

Cultivar	VVMD21	VVMD24	VVMD25	VrZAG21	VrZAG47	VrZAG64	VrZAG67
'Alexandrouli'	251-251	205-205	236-252	188-204	155-161	142-163	129-150
'Mujuretuli'	251-251	205-205	236-252	188-204	155-161	142-16	129-150

<sup>2</sup> NEI's (1978) genetic distance is equal to 0 comparing 'Alexandrouli' versus 'Mujuretuli' and 0.578 comparing 'Dzveli Alexandrouli' versus 'Alexandrouli' / 'Mujuretuli'.

and, with all 14 loci considered, the combined probability of identity (PI) was on the order of  $10^{-8}$  and the combined probability of identity of siblings (PIsibs) was on the order of  $10^{-6}$ , in the 144 accessions considered. This clearly shows that the 14 microsatellite loci selected to characterize 'Alexandrouli' and 'Mujuretuli' have a good discrimination capability. On these bases, 'Alexandrouli' and 'Mujuretuli' can be considered as monophyletic cultivars. Nevertheless, we have also performed an estimation of possible relationships relating 'Alexandrouli' and 'Mujuretuli'. Results of this analysis are shown in Table 3. The results obtained from the software ML-RELATE define that we cannot exclude a different kind of relationship relating the two accessions. So beside the possibility that the two cultivars under discussion are derived one from the other by clonal selection we have to consider also the hypothesis that they could be linked by a full sibling relationship (Table 3).

Anthocyanins, extracted from berry skin, showed a similar content and profile. Most ampelographic and ampelometric characters and leaf shape were similar. These two cultivars had almost the same ripening time and grape must characteristics. The cultivars share a common place of origin, they are spread in the same province and they are usually cultivated together in the same vineyards. All these data allow us to consider these two cultivars as monophyletic.

On the other hand, the identical DNA pro-

files and the similar characteristics described above are unable to explain the ampelographic dissimilarities of these two cultivars, such as berry shape, and some aspects of cluster, shoot tip and leaf morphology. We must stress that SSR markers are not able to resolve small differences such as berry pigmentation or the presence of pubescence in the leaves caused by spontaneous mutations (sports) (7, 11). The presence of dissimilar characters with a common SSR profile suggest that 'Alexandrouli' and 'Mujuretuli' could be considered as mutant cultivars with a common monophyletic origin from the same seedling mother, and thus could be considered as clones (sports) or that they could be full siblings. Here it is interesting to note that sports are a useful tool for studies of the genetic basis of phenotypes and in this work have identified a new clonal pair for study.

To better understand which among these two hypotheses is correct it is interesting to speculate what happened to these two related cultivars and where their history started. One hypothesis is that the two cultivars are mutation products of a third cultivar. This consideration does not seem to have a real foundation, mainly because the molecular characterization (17) done on Georgian germplasm did not highlight any other cultivars with such SSR profiles. Secondly, documents of the 20<sup>th</sup> century postulate that 'Alexandrouli' gave rise to 'Mujuretuli', through a bud mutation event, and that this mutated line was soon

**Table 3.** List of Ln-likelihoods for each of the four following relationships: unrelated, half sibs, full sibs, parent-offspring<sup>2</sup>.

				Delta ln(L)			
Most probable relationship	Ind1	Ind2	LnL(R)	Unrelated	Half-Siblings	Full-Siblings	Parent Offspr.
Full-Siblings	Mujuretuli	Alexandrouli	-77.59	39.79	22.23	-	14.1

<sup>2</sup> The *Most probable relationship column* lists the relationship with the highest likelihood linking Alexandrouli and Mujuretuli; the *LnL(R)* column lists the loglikelihood of this relationship and the *Delta Ln(L)* columns list how much lower the loglikelihood are for the other relationships. This means, for example, that the loglikelihood of the 2 individuals to be *Unrelated* is 39.79 less than the loglikelihood of the *Full Sibling* relationship hypothesis.

separated and called ‘Mujuretuli’ (21, 25). This hypothesis was based on the fact that ‘Alexandrouli’ has an older tradition and has been cultivated more extensively in the Racha-Lechkhumi vineyards than ‘Mujuretuli’; this situation remains true at the present time. For example, according to the inventory of vineyards in 1985 (10), ‘Alexandrouli’ was cultivated on 145 ha and ‘Mujuretuli’ only on 8 ha (5.5%). However, due to the popularity of “Khvanchkara” wine on the Russian market, more recently the amount of land devoted to the cultivation of this cultivar has increased from 5.5% to 39.7%, as reported in the 2004 Census (9). The common place of origin, as stated before, supports the hypothesis of a clonal derivation of one cultivar from the other.

Supposing Racha-Lechkhumi province as the place of origin, we observe that, the word “Mujuro” has two meanings in this region (25). It refers to the bottom of a clay jar “Kvevri” traditionally used in winemaking, but it is also the word for a stick with an iron piece on the bottom used by hunters. “Both these things have more or less a similar shape: both have a prolonged body with a wider top and a narrow bottom, similarly to the berry of ‘Mujuretuli’. Probably the name of the cultivar was originated regarding similarity of shapes”, concludes Pruidze (25).

The ancient cultivation tradition is witnessed by the number of references we can find for the two cultivars. But, how old are these two cultivars? The name ‘Alexandrouli’ used to indicate the grapevine cultivar is first mentioned in a reference in 1874. ‘Mujuretuli’

is mentioned in the work of Bakhtadze and Dandurov at the end of the 19<sup>th</sup> century (cited in Ref. 25). But, due to the limited number of older Georgian manuscript sources, the origin of these cultivars could be even older. According to the ampelographer D. Tabidze (cited in Ref. 25), ‘Mujuretuli’ could have originated from ‘Alexandrouli’ several centuries ago, an opinion also proposed by Javakhishvili (13). Both these authors believed that ‘Alexandrouli’ and ‘Mujuretuli’ were the most ancient Georgian native cultivars, with usage of several centuries.

Pruidze (25) reports a folk story about the discovery of ‘Alexandrouli’ in a forest and its transfer to a vineyard by King Alexandre (Georgian variant of the name “Alexander”). The cultivar was later distributed under the name ‘Alexandrouli’, which in English means “from Alexander”. Unfortunately this history does not clarify the chronology of ‘Alexandrouli’s appearance, because the history of Georgia is full of kings and princes called Alexander. The hypothesis about the introduction of ‘Alexandrouli’ by King Alexandre was also rejected by Ketskhoveli et al. (15).

*b) ‘Alexandrouli’ vs. ‘Dzveli Alexandrouli’*

The same 58 ampelographic descriptors were used to compare ‘Alexandrouli’ and ‘Dzveli Alexandrouli’. Among them, 39 were similar and 19 were dissimilar. As a result, the level of similarity between ‘Alexandrouli’ and ‘Dzveli Alexandrouli’ was 67.2%. ‘Alexandrouli’ and ‘Dzveli Alexandrouli’ had many dissimilar descriptors related to young shoots, young leaves, mature leaves,

inflorescences, clusters, berries and production and the characteristics of the must. Important easily recognized differences were: 'Alexandrouli' had hermaphroditic flowers, loose clusters and lower sugar content than 'Dzveli Alexandrouli', which had female flowers and dense clusters.

Statistical analysis of biometric data on clusters and tendrils (Table 1) indicated that 'Dzveli Alexandrouli' had significantly higher cluster mass, with more than twice the average weight of 'Alexandrouli' as a result of cluster density. 'Dzveli Alexandrouli' had dense clusters. 'Dzveli Alexandrouli' has a significantly heavier berry weight than 'Alexandrouli'. No differences were found among the two cultivars in length of peduncles or tendrils.

According to the statistical analysis of sugar and acidity data of berry juice at harvest, 'Dzveli Alexandrouli' demonstrated lower sugar accumulation and greater titratable acidity than 'Alexandrouli' (Table 1). The total content of anthocyanins in the berry skin at maturity in 'Dzveli Alexandrouli' was less than a third that of 'Alexandrouli' (Table 1). In addition, 'Dzveli Alexandrouli' showed a different anthocyanin profile in comparison to 'Alexandrouli'. In particular 'Dzveli Alexandrouli' had lower malvidin and higher levels of acetic esters than 'Alexandrouli' (Table 1).

In phenology 'Dzveli Alexandrouli' exhibited a later veraison than 'Alexandrouli' (Table 1). Despite a later veraison, the physiological maturity of 'Dzveli Alexandrouli' was not significantly later than the other cultivars. This was due to the shortage of heat units at the end of the growing season which forced us to collect grapes before the real attainment of the physiological ripening.

'Dzveli Alexandrouli' shared only three alleles (at VVS2, VVMD27 and VVMD5 loci) with 'Alexandrouli', and had different allele profiles in all the other tested loci (Table 2). Nei's genetic distance between 'Dzveli Alexandrouli' and 'Alexandrouli'/'Mujuretuli' was 0.578. Based on these results we consider 'Alexandrouli' and 'Dzveli Alexandrouli' to be homonyms.

The DNA fingerprinting performed in this work did not confirm a close relationship between 'Alexandrouli' and 'Dzveli Alexandrouli'. These two cultivars have many different ampelographic, morphological, chemical, and phenological characters, as confirmed by the statistical analysis. As a conclusion we can state that they should be considered as two different, homonym cultivars.

The reason why these two accessions share the same name is still unresolved and only a hypothesis can be made. The word 'Dzveli' in Georgian means 'Old', so we could think the first cultivar under cultivation was 'Dzveli Alexandrouli' and that this cultivar gave rise to the modern 'Alexandrouli' – the female flower, characterizing 'Dzveli Alexandrouli', might have easily been involved in spontaneous crosses or even in the first attempts at breeding. This hypothesis finds a supporter in N. Tsertsvadze (33), who writes that 'Dzveli Alexandrouli' is older than 'Alexandrouli'; once again the female flower could help us in dating these two cultivars due to the fact that female flowers, among cultivated grapevines, could be considered as an ancient character. Supporting this hypothesis we should also mention the position in the same cluster of a dendrogram based on SSR data constructed with 147 Georgian cultivars (17), the common area of cultivation and some morphological common characteristics. Based on the present data and historical information, we can only speculate that 'Dzveli Alexandrouli' is one of the ancestors of 'Alexandrouli', even though a first degree relationship must be rejected.

*Conclusion.* This research work represents a case study of paradigmatic importance in the evaluation of genetic resources of vegetatively propagated crops. Multiple approaches including genotyping, phenotyping, as well as collecting literary and ethnographic information, allowed us to separate two closely related cultivars, 'Alexandrouli' and 'Mujuretuli', which only by molecular fingerprints would have been classified as identical. According to the International Code of Nomenclature for Cultivated Plants – Seventh Edition, Art.

2.2 (4), a cultivar is “an assemblage of plants that has been selected for a particular attribute or combination of attributes and that is clearly distinct, uniform, and stable in these characteristics and that when propagated by appropriate means retains those characteristics”. Our data clearly confirm what is believed by Rachian viticulturists who consider ‘Alexandrouli’ and ‘Mujuretuli’ as distinct cultivars due to their clear distinct phenotype for berry shape and in addition to different leaf shape.

Programs of germplasm conservation and evaluation should carefully consider phenotyping and all available local sources of knowledge / information to maintain the crop biodiversity. Based on molecular, ampelographic, morphological, chemical and bibliographic data, we may conclude that:

1. ‘Alexandrouli’ and ‘Mujuretuli’ are similar but phenotypically distinct cultivars with identical genetic profiles, representing two different clonal lines with a monophyletic origin.
2. Probably ‘Mujuretuli’ originated as a point mutation of ‘Alexandrouli’ in Racha province.
3. ‘Dzveli Alexandrouli’ and ‘Alexandrouli’ are clearly different genotypes but homonym cultivars.
4. The example of ‘Alexandrouli’ and ‘Mujuretuli’ once again confirms the theory of mutant origin of some cultivars.

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