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1 **Genetic profile of scrapie codons 146, 211 and 222 in the *PRNP* gene locus in**
2 **three breeds of dairy goats**

3

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16

17 **Abstract**

18 Polymorphisms at *PRNP* gene locus have been associated with resistance against
19 classical scrapie in goats. Genetic selection on this gene within appropriate breeding
20 programs may contribute to the control of the disease. The present study characterized
21 the genetic profile of codons 146, 211 and 222 in three dairy goat breeds in Greece. A
22 total of 766 dairy goats from seven farms were used. Animals belonged to two
23 indigenous Greek, Eghoria (n=264) and Skopelos (n=287) and a foreign breed,
24 Damascus (n=215). Genomic DNA was extracted from blood samples from individual
25 animals. Polymorphisms were detected in these codons using Real-Time PCR
26 analysis and four different Custom TaqMan[®] SNP Genotyping Assays. Genotypic,
27 allelic and haplotypic frequencies were calculated based on individual animal
28 genotypes. Chi-square tests were used to examine Hardy-Weinberg equilibrium state
29 and compare genotypic distribution across breeds. Genetic distances among the three
30 breeds, and between these and 30 breeds reared in other countries were estimated
31 based on haplotypic frequencies using fixation index F_{ST} with Arlequin v3.1 software;
32 a Neighbor-Joining tree was created using PHYLIP package v3.695. Level of
33 statistical significance was set at $P=0.01$. All scrapie resistance-associated alleles
34 (146S, 146D, 211Q and 222K) were detected in the studied population. Significant
35 frequency differences were observed between the indigenous Greek and Damascus
36 breeds. Alleles 222K and 146S had the highest frequency in the two indigenous and
37 the Damascus breed, respectively (*ca.* 6.0%). The studied breeds shared similar
38 haplotypic frequencies with most South Italian and Turkish breeds but differed
39 significantly from North-Western European, Far East and some USA goat breeds.
40 Results suggest there is adequate variation in the *PRNP* gene locus to support
41 breeding programs for enhanced scrapie resistance in goats reared in Greece. Genetic

42 comparisons among goat breeds indicate that separate breeding programs should
43 apply to the two indigenous and the imported Damascus breeds.

44

45 **Introduction**

46 Scrapie is an infectious, neurodegenerative and fatal disease of sheep and goats.
47 Along with bovine spongiform encephalopathy, Creutzfeldt-Jacob disease in humans
48 and chronic wasting disease in cervids, scrapie belongs to the group of transmissible
49 spongiform encephalopathies (TSEs), also known as prion diseases. Scrapie is the
50 result of the accumulation of PrP^{Sc} in the central nervous system, which is an
51 abnormal β -sheet rich isoform of the normal α -helix rich PrP^C protein [1].

52 In sheep, susceptibility to classical scrapie is modulated by the *PRNP* gene which
53 encodes PrP^C protein [2]. Polymorphisms at codons 136 (A/V), 154 (Q/R) and 171
54 (Q/R/H) of this gene have been associated with susceptibility (ARQ and VRQ
55 haplotypes) or resistance (ARR haplotypes) to classical scrapie [3,4,5,6]. Based on
56 these findings a five-group risk classification system has been developed [7] and
57 applied by different countries in selective breeding programs to control and eradicate
58 the disease [8].

59 In goats, previous research has revealed more than 30 polymorphic codons in the
60 *PRNP* gene. Amongst these, polymorphisms at codons 142 (I/M), 143 (H/R), 146
61 (N/S/D), 154 (H/R), 211 (R/Q) and 222 (Q/K) have been associated with resistance or
62 susceptibility to clinical manifestation of the disease in field studies [9-16].
63 Experimental studies have shown that alleles 222K, 146S and 211Q confer the
64 strongest degree of resistance after oral and/or intracerebral challenge and, therefore,
65 are considered the most suitable candidates for selective breeding programs in goats
66 [17-22]. This is also supported by the European Food Security Authority (EFSA) in

67 their scientific opinion on the genetic resistance to TSEs in goats [23]. However, there
68 are currently no formal regulations to underpin selective breeding programs in goats,
69 equivalent to those in sheep. The notion is that success of such programs is subject to
70 in-depth knowledge of the frequency and distribution of *PRNP* polymorphisms
71 conferring resistance to classical scrapie [14,23]. Different estimates of allelic
72 frequency have been published for breeds in China [24], Japan [25], USA [26], Italy
73 [27-29], France [13], UK [30], Cyprus [31] and Turkey [32]. Given the observed
74 differences between countries and breeds, EFSA concluded that any selective
75 breeding programs should be developed and managed at national level, and in each
76 case, the frequency of the resistance-associated alleles and haplotypes should be
77 assessed for each breed of interest [23].

78 Dairy goats constitute a significant sector of livestock farming in Greece. The Greek
79 goat population is approximately 3.8 million [33], which makes it the largest in
80 Europe and one of the largest worldwide. This population is composed mainly of the
81 Eghoria and Skopelos indigenous breeds, as well as the imported Damascus breed
82 along with various types of crossbreeds. Scrapie was first reported in Greece in 1986
83 and has since been considered a major health problem in both sheep flocks and goat
84 herds. Studies on the frequencies of classical scrapie resistance-associated alleles in
85 goats of Greece are limited [15,34,35]. These studies either focused on a small
86 number of goats from scrapie-affected herds [34] or animals of unspecified breeds
87 [15,35]. A systematic large-scale population study of the *PRNP* gene polymorphisms
88 in dairy goat breeds reared in Greece is missing.

89 The objective of the present study was to assess the genetic profile of codons 146, 211
90 and 222 in the *PRNP* gene locus in the three key dairy goat breeds in Greece, namely

91 Eghoria, Skopelos and Damascus in order to determine the feasibility of breeding
92 programs aiming at enhancing resistance to classical scrapie.

93

94 **Materials and methods**

95 **Ethics statement**

96 This study followed the European Directive 86/609/EEC and its national
97 implementation in Greece Presidential Decree No 160/1991 (Governmental Gazette
98 No A' 64). Blood sampling of dairy goats was performed in commercial farms within
99 the EU SOLID project (FP7-KBBE-266367). This research was approved by the
100 Research Committee of The Aristotle University of Thessaloniki (26362/03.05.2011).

101

102 **Animals**

103 A total of 766 dairy goats of two indigenous Greek breeds (Eghoria and Skopelos, n =
104 264 and n = 287 goats, respectively) and one foreign breed (Damascus, n = 215 goats)
105 were used. Animals of 1-4 years of age were randomly selected from seven farms
106 (two with Eghoria, two with Skopelos and three with Damascus goats). Goat herds
107 were selected as representatives of the prevailing farming systems in the country
108 based on the *a posteriori* typology scheme described by Gelasakis *et al.* [36]. These
109 were low-input pastoral farming systems characterized by grazing throughout the
110 year, random mating and minor differences in management practices between herds
111 [36]. Based on the latter, the selected number of seven herds was considered sufficient
112 for the study. Relations of the selected animals were unknown since in all cases,
113 random mating was performed. However, considering that the number of bucks and
114 their replacement rate were high, over-representation of lineages is unlikely.

115

116 **Genotyping**

117 Blood samples were collected from the jugular vein in EDTA vacutainers and
118 genomic DNA was extracted using GeneJET Whole Blood Genomic DNA
119 Purification Mini Kit (Thermo Scientific, Waltham, Massachusetts, USA) according
120 to manufacturer's instructions. Polymorphisms at codons 146 (N/S/D), 211 (R/Q) and
121 222 (Q/K) were detected using a novel genotyping approach. Contrary to previous
122 studies, which used PCR amplification of the open reading frame of caprine *PRNP*
123 gene and sequencing of the PCR products, we focused on the most important codons
124 (146, 211 and 222) and developed four separate Real-Time PCR reactions with four
125 different Custom TaqMan[®] Single Nucleotide Polymorphism (SNP) Genotyping
126 Assays (Applied Biosystems, Foster City, California, USA). Real-Time PCR is fast,
127 sensitive and suitable for SNP detection. This method has been also used in sheep for
128 the detection of polymorphisms at codons 136, 154 and 171 in the *PRNP* gene [37].
129 Each SNP Genotyping Assay consisted of a mix of sequence-specific forward and
130 reverse primers to amplify the polymorphic sequence of interest and two TaqMan[®]
131 MGB (minor groove binder) probes, FAM (6-carboxyfluorescein) and VIC (2'-chloro-
132 7-phenyl-1,4-dichloro-6-carboxy-fluorescein) dye-labeled to detect the amplified
133 product (Table 1). Primers and probes were designed by Applied Biosystems using
134 designated software and then individually tested by mass spectroscopy to verify the
135 accuracy of the resulting synthesized oligonucleotide. Validation of the genotyping
136 method was performed using sequenced goat genomic DNA samples with all possible
137 genotypes at codons 146, 211 and 222.

138

139 **Table 1. Primer and probe sequences used in four Custom TaqMan® SNP**
 140 **Genotyping Assays for the detection of polymorphisms 146 (N/S), 146 (N/D), 211**
 141 **(R/Q) and 222 (Q/K).**

SNP	Primer	
	Forward	Reverse
146(N/S)	GCCATGAGCAGGCCTCTTATA	GGGTAACGGTACATGTTTTTCACGAT
146(N/D)	GCCATGAGCAGGCCTCTT	GGGTAACGGTACATGTTTTTCACGAT
211(R/Q)	GAACTTCACCGAAACTGACATCAAG	ACTGGGTGATGCACATTTGCT
222(Q/K)	TGGTGGAGCAAATGTGCATCA	GGGAAGAAAAGAGGATCACACTTG
	Probe [†]	
	FAM	VIC
146(N/S)	TTTTGGCAGTGA CTATG	CATTTTGGCAATGA CTATG
146(N/D)	CATTTTGGCAATGA CT	ATACATTTTGGCGATGA CT
211(R/Q)	AATGGAGCAAGTGGTG	ATAATGGAGCGAGTGGTG
222(Q/K)	CTGGGATTCTCTCTTGTACTG	TGGGATTCTCTCTGGTACTG

142 [†]In all cases except for 146(N/D), FAM dye-labeled probes were used for the
 143 detection of the mutated sequences and VIC dye-labeled probes for the detection of
 144 the wildtype sequences.

145

146 PCR reactions were performed in 12.5 µl mixtures containing 6.25 µl of KAPA
 147 PROBE FAST qPCR Master Mix (2X) Universal (Kapa Biosystems, Wilmington,
 148 Massachusetts, USA), 0.3125 µl of each SNP Genotyping Assay Mix (40X) and 1 µl
 149 (*ca.* 50 ng) of genomic DNA. Thermal cycling included: a) an initial denaturation step
 150 at 95°C for 3 min and b) 45 cycles of denaturation at 95°C for 3 s and primer
 151 annealing/extension at 62°C for 30 s. All Real-Time PCR analyses (n = 3,064) were

152 performed using Applied Biosystems StepOnePlus™ Real-Time PCR System and
153 genotypes were determined through amplification plots with QPCR Applied
154 Biosystems Step One Software v2.3 ([dx.doi.org/10.17504/protocols.io.qeidtce](https://doi.org/10.17504/protocols.io.qeidtce)).

155

156 **Genetic Analyses**

157 Genotypic, allelic and haplotypic frequencies regarding codons 146, 211 and 222
158 were calculated within and across breed, based on counting of the respective
159 genotypes of individual animals. Hardy-Weinberg equilibrium state was examined at
160 each codon and breed using a chi-square test:

$$161 \chi^2 = \sum[(O - E)^2/E]$$

162 Where

163 O is the observed number of each genotype;

164 E is the expected number of each genotype assuming Hardy-Weinberg equilibrium;

165 Summation is over all possible genotypes.

166 Pairwise comparison of genotypic distribution between breeds at each codon was
167 carried out using a chi-square test. In each case, number of observed genotypes in one
168 breed was compared to expected number based on the allelic frequency in the other
169 breed. Moreover, genetic distances among the three studied breeds were estimated on
170 the basis of haplotypic frequencies using fixation index F_{ST} and Arlequin v3.1
171 software [38]. To add substance to these comparisons, genetic distances were also
172 calculated between the three studied breeds and 30 other goat breeds from different
173 countries, for which haplotypic frequencies were available in the existing literature
174 (S1 Table). In each case, the significance of difference from the null value was tested
175 with 1,000 permutations. The matrix of genetic distances between breed pairs was
176 used to create a Neighbor-Joining tree deploying the PHYLIP package v3.695 [39].

177 In all the above analyses, level of statistical significance was set at $P=0.01$.

178

179 **Results**

180 **Frequencies**

181 Genotypic, allelic and haplotypic frequencies in the *PRNP* gene locus are presented in
182 Tables 2 and 3 and S2 Table, respectively. Across all breeds, four out of six possible
183 genotypes at codon 146 (NN, NS, ND and SS), and two out of three at each of codons
184 211 (RR and RQ) and 222 (QQ and QK) were detected. Genotypes carrying
185 resistance-associated alleles, namely 222QK, 146NS, 211RQ, 146ND and 146SS
186 were found at a frequency of 8.74%, 3.39%, 2.87%, 0.13% and 0.13%, respectively
187 (Table 2). All resistance-associated alleles were observed in the three breeds
188 collectively; the most frequent was 222K (4.37%), followed by 146S (1.83%), 211Q
189 (1.44%) and 146D (0.06%, Table 3). Within breed, allele 222K had the highest
190 frequency in the two indigenous Greek breeds (5.87% and 5.92% in Eghoria and
191 Skopelos, respectively), whereas 146S was the most frequent in Damascus (6.05%,
192 Table 3). Frequencies of the resistance-associated polymorphisms dictated the
193 estimation of haplotypic frequencies in the *PRNP* gene locus; haplotypes carrying
194 multiple resistance-associated polymorphisms were not detected (S2 Table).

195 All three codons were found to be in Hardy-Weinberg equilibrium ($P>0.01$) in all
196 breeds, suggesting absence of direct or indirect genetic selection pressure.

197

198 **Table 2. Genotypic frequencies (%) and corresponding number of animals (in**
199 **parenthesis) at codons 146, 211 and 222 of the *PRNP* gene locus.**

		Breed
--	--	--------------

Codon	Genotype	Eghoria ^a	Skopelos ^a	Damascus ^b	Total
146	NN	99.62 (263)	99.65 (286)	87.91 (189)	96.35 (738)
	NS	0.00 (0)	0.35 (1)	11.63 (25)	3.39 (26)
	SS	0.00 (0)	0.00 (0)	0.46 (1)	0.13 (1)
	ND	0.38 (1)	0.00 (0)	0.00 (0)	0.13 (1)
	DD	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	DS	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
211	RR	96.97 (256)	100.00 (287)	93.49 (201)	97.13 (744)
	RQ	3.03 (8)	0.00 (0)	6.51 (14)	2.87 (22)
	QQ	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
222	QQ	88.26 (233)	88.15 (253)	99.07 (213)	91.26 (699)
	QK	11.74 (31)	11.85 (34)	0.93 (2)	8.74 (67)
	KK	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)

200 ^{a,b} Comparison of genotypic distributions between breeds; different superscripts
201 denote significant differences ($P < 0.01$); distributions were compared within codon
202 and outcome was the same in all three codons.

203

204 **Table 3. Allelic frequencies (%) at codons 146, 211 and 222 of the *PRNP* gene**

205 **locus.**

Codon	Allele	Breed			
		Eghoria	Skopelos	Damascus	Total
146	N	99.81	99.83	93.95	98.11
	S*	0.00	0.17	6.05	1.83
	D*	0.19	0.00	0.00	0.06

211	R	98.48	100	96.74	98.56
	Q*	1.52	0.00	3.26	1.44
222	Q	94.13	94.08	99.53	95.63
	K*	5.87	5.92	0.47	4.37

206 *Indicates alleles which are considered to confer resistance to scrapie.

207

208 Genetic comparison among the three goat breeds in Greece

209 Genotypic distribution in Damascus breed differed significantly ($P < 0.01$) from that in
 210 Eghoria and Skopelos breeds for each of the three codons (Table 2). No significant
 211 differences ($P > 0.01$) were observed between Eghoria and Skopelos breeds in any
 212 codon.

213 Genetic distance analysis (Table 4) showed that Eghoria and Skopelos breeds shared
 214 similar haplotypic frequencies implying genetic relatedness ($F_{ST} = 0.000$, $P > 0.01$),
 215 whereas they differed significantly from Damascus breed ($F_{ST} = 0.020$, $P < 0.001$ and
 216 $F_{ST} = 0.028$, $P < 0.001$, respectively), thereby corroborating the genotypic distribution
 217 results.

218

219 **Table 4. Genetic (F_{ST}) distances (above diagonal) and corresponding P-values**
 220 **(below diagonal) among Eghoria, Skopelos and Damascus breeds.**

Breeds	Eghoria	Skopelos	Damascus
Eghoria		0.000	0.020
Skopelos	0.31543		0.028
Damascus	<0.00001*	<0.00001*	

221 *Indicates significant P-values ($P < 0.01$); five decimal points is the maximum number
 222 provided by the Arlequin software.

223

224 **Genetic comparison between the three goat breeds in Greece**
225 **and breeds in other countries**

226 Estimated genetic distances between goat breeds reared in Greece and in other
227 countries are presented in Table 5. Small F_{ST} estimates and high (non-significant) P-
228 values denote high levels of genetic relatedness between respective breeds. The three
229 studied breeds shared similar haplotypic frequencies ($P>0.01$) with most breeds reared
230 in neighboring Mediterranean regions (Southern Italy, Turkey and Cyprus). Notable
231 exceptions were the observed distances ($P<0.01$) from four Southern Italian breeds
232 (Garganica, Girgentana, Derivata di Siria and Pantellaria), and the Damascus breed of
233 Cyprus and Turkey. Significant genetic distances ($P<0.01$) were also observed from
234 goat breeds reared in North-Western Europe (Northern Italy, France and UK) and Far
235 East (Japan and China). Regarding breeds from the USA, Greek goats were
236 genetically distant ($P<0.01$) from LaMancha and Nubian, while a significant
237 difference was also observed between Skopelos and Alpine and Saanen breeds.

238

239 **Table 5. Genetic (F_{ST}) distances and corresponding P-values between goat breeds**
240 **reared in Greece and other countries.**

	Breeds in Greece					
	Eghoria		Skopelos		Damascus	
	F_{ST}	P-value	F_{ST}	P-value	F_{ST}	P-value
Other breeds (Region/Country [†])	F_{ST}	P-value	F_{ST}	P-value	F_{ST}	P-value
Garganica (Southern IT)	0.058	<0.00001*	0.076	<0.00001*	0.082	<0.00001*
Maltese (Southern IT)	0.009	0.19824	0.018	0.09961	0.037	0.01562
Ionica (Southern IT)	0.000	0.90723	0.000	0.78711	0.018	0.07812

Red Mediterranean (Southern IT)	0.000	0.77441	0.000	0.99902	0.016	0.06445
Girgentana (Southern IT)	0.064	<0.00001*	0.079	<0.00001*	0.087	<0.00001*
Derivata Di Siria (Southern IT)	0.034	<0.00001*	0.044	<0.00001*	0.060	<0.00001*
Pantellaria (Southern IT)	0.169	<0.00001*	0.216	<0.00001*	0.119	<0.00001*
Camosciata Delle Alpi (Northern IT)	0.059	<0.00001*	0.087	<0.00001*	0.040	<0.00001*
Saanen (Northern IT)	0.030	0.00098*	0.051	<0.00001*	0.021	0.00879*
Roccoverano (Northern IT)	0.063	<0.00001*	0.093	<0.00001*	0.046	<0.00001*
Valdostana (Northern IT)	0.027	<0.00001*	0.047	<0.00001*	0.017	0.00390*
Damascus (CY)	0.037	<0.00001*	0.046	<0.00001*	0.011	0.00293*
Damascus (TR)	0.263	<0.00001*	0.307	<0.00001*	0.159	<0.00001*
Akkeci (TR)	0.043	0.04980	0.074	0.01465	0.018	0.12793
Saanen (TR)	0.002	0.24805	0.013	0.06250	0.007	0.12109
Kilis (TR)	0.024	0.01171	0.031	0.01170	0.023	0.01855
Alpine (FR)	0.018	<0.00001*	0.032	<0.00001*	0.025	<0.00001*
Saanen (FR)	0.099	<0.00001*	0.127	<0.00001*	0.078	<0.00001*
Dairy breeds (UK)	0.027	<0.00001*	0.025	<0.00001*	0.042	<0.00001*
Boer (CN)	0.612	<0.00001*	0.656	<0.00001*	0.518	<0.00001*
Saanen (JP)	0.053	<0.00001*	0.077	<0.00001*	0.027	<0.00001*
Alpine (USA)	0.030	0.01465	0.052	0.00488*	0.010	0.12109
Oberhasli (USA)	0.012	0.09570	0.024	0.05371	0.007	0.17480
Toggenburg (USA)	0.000	0.86426	0.000	0.35742	0.010	0.08105
LaMancha (USA)	0.176	<0.00001*	0.212	<0.00001*	0.093	<0.00001*
Nubian (USA)	0.317	<0.00001*	0.360	<0.00001*	0.214	<0.00001*
Saanen (USA)	0.013	0.01367	0.019	0.00391*	0.007	0.07227

241 †IT= Italy, CY= Cyprus, TR= Turkey, FR= France, UK= United Kingdom, CN=
242 China, JP= Japan, USA= United States of America

243 *Indicates significant P-values ($P < 0.01$); five decimal points is the maximum number
244 provided by the Arlequin software.

245

246 Results in Table 5 are broadly consistent with the Neighbor-Joining tree presented in
247 Fig 1. The latter revealed two major geographical clusters; one including the three
248 studied breeds and most breeds from other Mediterranean countries, and another
249 including most of the North-Western European and the Far East breeds. Goat breeds
250 from the USA were distributed across both clusters.

251

252 **Fig 1. Unrooted Neighbor-Joining tree based on F_{ST} genetic distances.**

253 Colors indicate two major geographical clusters: Blue cluster includes: E_GR,
254 Eghoria (Greece); S_GR, Skopelos (Greece); I_S_IT, Ionica (Southern Italy);
255 RM_S_IT, Red Mediterranean (Southern Italy); D_GR, Damascus (Greece); D_CY,
256 Damascus (Cyprus); K_TR, Kilis (Turkey); O_USA, Oberhasli (United States of
257 America); S_USA, Saanen (United States of America); DB_UK, Dairy breeds (United
258 Kingdom); T_USA, Toggenburg (United States of America); A_FR, Alpine (France);
259 M_S_IT, Maltese (Southern Italy); DDS_S_IT, Derivata Di Siria (Southern Italy);
260 G_S_IT, Garganica (Southern Italy); GI_S_IT, Girgentana (Southern Italy). Red
261 cluster includes: S_TR, Saanen (Turkey); V_N_IT, Valdostana (Northern Italy);
262 A_USA, Alpine (United States of America); S_IT_N, Saanen (Northern Italy); A_TR,
263 Akkeci (Turkey); S_JP, Saanen (Japan); CDA_N_IT, Camosciata Delle Alpi
264 (Northern Italy); R_N_IT, Roccaverano (Northern Italy); P_S_IT, Pantellaria
265 (Southern Italy); S_FR, Saanen (France); LM_USA, La Mancha (United States of

266 America), D_TR, Damascus (Turkey); N_USA, Nubian (United States of America);
267 B_CN, Boer (China).

268

269 **Discussion**

270 In the present study, the genetic profile of three codons (146, 211 and 222) in the
271 *PRNP* gene locus was characterized for the first time in the three most common dairy
272 goat breeds in Greece (Eghoria, Skopelos and Damascus). Our results demonstrated
273 the presence of polymorphism in this locus which renders it amenable to change via
274 genetic selection. Moreover, differences in *PRNP* haplotypic frequencies were
275 revealed among the studied goat breeds but also between them and breeds reared in
276 other countries, suggesting the importance of breeding programs being custom-
277 designed according to the genetic profile of specific populations. In this regard, our
278 results indicate that separate breeding programs should apply to the two indigenous
279 and the imported Damascus breeds aiming at enhancing scrapie resistance.

280 Although resistance-associated allele frequencies have been previously reported in
281 Greek goats, this is the first large-scale population study involving animals from
282 specific breeds, without clinical signs of scrapie and from farms representative of the
283 major farming systems as described in relevant typologies [36]. Regarding the
284 Eghoria and Skopelos breeds, a previous study reported allele frequencies for 222K
285 (*ca.* 24.0% in Skopelos) and 211Q (*ca.* 7.0% in both breeds) using goats from one
286 highly infected herd [34]. However, these results are not directly comparable with
287 ours as they were based on a different study design and a smaller sample size. Kanata
288 *et al.* [35] reported frequencies in Greek goats higher than our study (3.0%, 0.5%,
289 6.0% and 5.6% for 146S, 146D, 211Q and 222K alleles, respectively) but for fewer

290 animals (436 individuals) of unspecified breeds. Contrary to the latter study, we used
291 purebred goats of the three most common dairy goat breeds reared in Greece.

292 The present study confirms that the dairy goat population in Greece, collectively
293 represented here by the three studied breeds, is one of the very few worldwide where
294 all scrapie resistance-associated alleles in the three codons (146, 211 and 222) have
295 been detected. Previous research in Turkey has also reported presence of all these
296 alleles in the goat population; however, much lower frequencies have been reported
297 for alleles 222K and 211Q [32]. Polymorphic variation in the *PRNP* gene locus
298 reported in our study enables the design and implementation of breeding programs
299 towards enhanced scrapie resistance in goats reared in Greece.

300 The genetic profiles of scrapie codons 146, 211 and 222, revealed certain differences
301 between the studied breeds. The most common resistance-associated allele was 222K
302 in Eghoria and Skopelos (indigenous Greek breeds), whereas 146S in Damascus (*ca.*
303 6.0% in all cases). Such observations were further supported by comparison of
304 genotypic distributions and genetic distance analyses, which showed significant
305 differences between the two indigenous Greek breeds and Damascus. The latter is an
306 imported breed of Middle East origin. Previous studies have suggested that
307 geographical origin may explain a large part of genetic variability among goat breeds
308 [39]. In a microsatellite analysis of European and Middle Eastern goats, Greek breeds
309 were placed in the Central-Mediterranean cluster within which genetic differences
310 among breeds were found to be relatively low [40]. In the same study, breeds of
311 Middle Eastern origin were placed in a separate, East-Mediterranean genetic cluster
312 [40].

313 To further investigate differences in the *PRNP* gene locus among different goat
314 populations, we estimated the genetic distance between the studied breeds and 30

315 other breeds reared in various countries. To the best of our knowledge this is the first
316 time that a large-scale comparison of the *PRNP* gene profile is attempted using
317 collectively all the available data regarding polymorphism frequencies at codons 146,
318 211 and 222 in different dairy goat populations worldwide. In this regard, we derived
319 novel and interesting results about the level of relatedness of these populations. Of
320 course a certain level of caution needs to be exercised when interpreting these results
321 as the relatively small number of polymorphisms and occasionally limited
322 representation in the studied breeds might have influenced the accuracy of the genetic
323 distance estimates. Furthermore, some of the previous studies on breeds reared in
324 Italy, Turkey and USA had been based on just a few animals, which might have
325 affected the representativeness of the calculated haplotypic frequencies.

326 According to previous research, allelic frequencies vary across countries and breeds
327 [13, 24-32]. Results from our comparisons suggest that geographical origin of the
328 breeds combined with population structure, breeding schemes and scrapie incidence
329 seem to be the key determinants of the *PRNP* gene profile. Thus, the studied breeds
330 shared similar haplotypic frequencies with many goat breeds reared in neighboring
331 Mediterranean countries, such as certain breeds in Southern Italy (Maltese, Ionica and
332 Red Mediterranean) [27] and Turkey (Saanen, Akkeci and Kilis) [32]. However,
333 significant genetic distances were also detected in respect to some other breeds reared
334 in neighboring countries. For example, much higher allelic frequencies of 222K were
335 observed in Girgentana (18.7%) and Derivata Di Siria (15.0%), but the allele was
336 absent in Pantellaria [28,29]. Girgentana is an endangered breed of Sicily with a small
337 population size in which significant inbreeding and genetic drift have been recorded
338 [28] potentially leading to this high allelic frequency. Derivata Di Siria goats are
339 reared in a high-scrapie endemic area of Sicily, whereas Pantellaria goats are found in

340 an area where scrapie has never been detected [29], quite possibly explaining the
341 corresponding allelic frequencies in each case. Moreover, Pantellaria goats had allele
342 211Q in high frequency (23.0%) [29]. This could be interpreted as a consequence of
343 the extensive crossbreeding with Saanen and Alpine goats of Northern Italy [29] in
344 which allele 211Q was also found to be very common [27].

345 Furthermore, significant genetic distances were also detected between Greek
346 Damascus and goats of the same breed in Turkey and Cyprus. In both Greek and
347 Cypriot Damascus goats, 146S was the most common allele (*ca.* 6.0% to 7.0%) [31].
348 However, allele 146D, which was not detected in the present study, was also reported
349 in Cyprus. In Damascus goats of Turkey, allele 146D was not detected, but a much
350 higher frequency of 146S (*ca.* 28.0%) was reported [32]. Moreover, alleles 211Q and
351 222K were detected for the first time in Damascus goats of Greece. These results
352 suggest a diverse evolution pattern of the Damascus breed in the three neighboring
353 countries, where different breeding schemes, possibly involving crossbreeding with
354 indigenous breeds, have taken place. Within-breed differences in haplotypic
355 frequencies were also detected between Saanen goats reared in different countries
356 (France, Italy, Turkey and USA) further enhancing the notion of diverse selective
357 breeding in different localities.

358 All goat breeds in the present study were significantly distant from North-Western
359 European and Far East breeds in which the most common alleles were 211Q
360 (Northern Italian, French Alpine, French Saanen and Japanese Saanen breeds) or
361 146S (Chinese Boer), whereas 222K was either not detected or found in very low
362 frequencies [13,24,25,27]. These findings could be explained by the different
363 geographical origin of the breeds and are in agreement with the phylogeographical
364 structure described by Canon *et al.* [40], which placed Northern Italian and French

365 breeds in a different genetic cluster than the Greek ones. Our findings are also in
366 agreement with previous studies of Italian goat breeds [27], where Northern Italian
367 breeds were clustered separately from Southern Italian; the latter shared similar allelic
368 frequencies with the Greek goats.

369 The three breeds of the present study had similar haplotypic frequencies with some
370 breeds from USA. Such results might have been unexpected given that the latter were
371 imported into USA from North-Western Europe. However, the sample size in these
372 breeds on which haplotypic frequencies had been based was relatively small [26],
373 which might have compromised the significance of the comparisons. Furthermore, no
374 genetic scrapie studies of native USA goat breeds were found in the literature. Based
375 on the phylogenetic analysis of goat mitochondrial sequences reported by Amills *et*
376 *al.* [41], significant differences between native American and European populations
377 could be expected.

378 All genetic comparisons among goat breeds discussed above support the scientific
379 opinion of EFSA, which suggests that selective breeding programs towards scrapie
380 resistance should be developed and managed independently within each country and
381 according to established frequencies of resistance-associated alleles for each breed
382 [23]. Therefore, our results suggest that separate breeding programs would be
383 advisable for the Eghoria and Skopelos breeds on the one hand, and Damascus on the
384 other. In all cases, however, caution should be exercised as uni-dimensional selection
385 towards enhancing scrapie resistance could potentially lead to reduction of genetic
386 variability and increased inbreeding, especially given the relatively low population
387 frequencies of resistance-associated alleles [42]. An evidence-based approach to
388 determine the best strategy is necessary. In a previous study in sheep, the impact of
389 various selection strategies on inbreeding was investigated by simulating different

390 frequencies of the resistant ARR haplotype and no negative effect was found when
391 haplotypic frequency was at least 5.0% [43]. Moreover, in Chios Greek sheep, ARR
392 haplotype was found at a frequency of 6.9% [44], which enabled the design of a
393 selective breeding program towards enhancing scrapie resistance. This program is
394 currently being implemented. Furthermore, Cyprus was able to start a breeding
395 program for goat scrapie resistance focused on alleles 146S and 146D with
396 frequencies of *ca.* 6.0% in the goat population [23]. Based on the above, a similar
397 program for goat breeds reared in Greece is feasible. Such a program should aim at
398 increasing the frequency of 222K allele in Eghoria and Skopelos goats, and the
399 frequencies of 146S and, potentially, 211Q alleles in Damascus goats.

400 However, before such breeding programs are implemented, future research should
401 address potentially adverse effects of genetic selection for enhanced scrapie resistance
402 on other important animal traits. There might be a direct impact of *PRNP* gene in the
403 phenotypic expression of such traits and/or genetic linkage between the *PRNP* gene
404 and genes influencing animal performance [42]. Although relevant studies have been
405 published for sheep [44-49], studies on goats are missing.

406 Finally, a critical step towards establishing effective breeding programs is to fully
407 understand the allelic interactions in all codons of interest. A partial dominant effect
408 of allele 222K over the wild-type was experimentally detected by intra-cerebrally
409 inoculating mice with various scrapie isolates; all KK animals were resistant and QK
410 were more resistant than QQ animals showing reduced incidence rates and/or longer
411 incubation periods [19]. To the best of our knowledge, no relevant studies have been
412 published for alleles in other codons. Therefore, experimental goat studies including
413 both heterozygous and homozygous carriers of resistance-associated alleles are
414 warranted.

415

416 **Conclusions**

417 The results of the present study indicate that there is adequate variation in the *PRNP*
418 gene locus to support breeding programs for enhanced scrapie resistance in goats
419 reared in Greece. According to the genetic profile in the three studied codons,
420 separate breeding programs should apply to the indigenous breeds and the imported
421 Damascus breed. Future breeding programs in other countries and breeds should first
422 study the genetic profile of the goat population in question. However, before any
423 measures are taken it is necessary to: i) determine possible adverse effects of selection
424 towards scrapie resistance on other important animal traits and ii) fully understand the
425 allelic interactions in the involved codons.

426

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431

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585

586

587 **Supporting information**

588 **S1 Dataset. Genotypes at codons 146, 211 and 222 of the *PRNP* gene locus of**
589 **individual Eghoria, Skopelos and Damascus goats obtained in the study.**

590

591 **S1 Table. Goat breeds with corresponding number of animals and haplotypic**
592 **frequencies (%) at the *PRNP* gene locus (codon order 146, 211, 222) by**
593 **country and literature reference.**

594

595 **S2 Table. Haplotypic frequencies (%) at the *PRNP* gene locus (codon order**
596 **146, 211, 222).**

597