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Phytochemical Evaluation of Wild-Grown Rosehips from Native Greek *Rosa canina* Genotypes

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Abstract

Rosehip (the "fruit" of *Rosa canina* L, commonly known as dog rose) is an emerging functional food, yet native Greek populations remain under-explored. This study screened 76 wild genotypes from Northern Greece for radical scavenging activity (% RSA), total phenolic content (TPC), and ascorbic acid (AsA). The results showed remarkable antioxidant potential (RSA > 70%), with TPC ranging from 1.02 to 35.96 mg g^{-1} DW, and AsA between 0.72 and 3.57 mg g^{-1} FW. Stepwise multiple regression analysis identified altitude as the primary predictor for RSA (adjusted R^2 = 0.139, p = 0.001) and latitude as a significant modulator for TPC (p = 0.034), reflecting subtle environmental adaptations over a robust genetic baseline. HPLC-PDA-MS characterization revealed a complex profile dominated by procyanidins, catechin derivatives, flavanonols (eriodictyol conjugates), and flavonol 3-*O*-glycosides (mainly quercetin hexosides and pentosides). Exploratory multivariate analysis (PCA) visualized high phenotypic plasticity and identified elite chemotypes (e.g., RPK-5, RCZ-12). Notably, the Rhodopi population exhibited the most extensive multidimensional dispersion despite its geographically restricted collection radius, suggesting a diverse local genetic reservoir. These findings establish a phytochemical map of Greek dog rose germplasm, providing essential criteria for selecting high-quality genotypes for future domestication and exploitation in the nutraceutical and pharmaceutical sectors.

Keywords: *Rosa canina*; rosehips; ascorbic acid; antioxidant activity; polyphenols

Received: 17 March 2026
Revised: 6 May 2026
Accepted: 14 May 2026
Published: 22 May 2026

1. Introduction

Rosa L. genus, a member of the Rosaceae family, consists of more than 300 species, distributed mainly across the temperate and subtropical climate zones of the northern hemisphere. Some species, such as *R. multiflora* or *R. gallica*, have also been introduced to the southern hemisphere (https://www.worldfloraonline.org/taxon/wfo-4000033476#children, accessed on 7 February 2026). In Greece, the local flora includes 23 distinct *Rosa* species (https://portal.cybertaxonomy.org/flora-greece, accessed on 7 February 2026), with *Rosa canina* L. (dog rose)—a hardy, perennial shrub—being the most prominent among them, distributed widely in diverse ecotopes [1]. While the whole plant holds significant horticultural, medicinal, and nutritional value, scientific and economic interest is centered

on its pseudo-fruits, commonly known as "rosehips". Botanically, they are an accessory fruit, developing from late summer to mid-autumn from the fleshy hypanthium that encloses an aggregate of true fruits, the achenes [2,3].

Dog rose has been utilized for therapeutic purposes since antiquity; as a matter of fact, the very name of the species is thought to originate from the ancient Roman belief that the root possessed healing properties against rabid dog bites [4]. Flowers and hips (mainly) have been a part of multiple cultures' folk medicine for centuries, with many of these recipes remaining in use to this day. In Greece, for instance, rosehip decoction has been traditionally employed for the prevention and treatment of flu and common cold [5], a common practice widely documented also across the Balkans, Central and Western Europe, Turkey, and the Middle East [6–9]. Other uses of rosehips include treatments of a wide array of ailments, such as various skin problems, inflammatory conditions (mainly arthritis and rheumatism), diabetes and other metabolic diseases, as well as cardiovascular, genitourinary, and gastrointestinal disorders [5–8,10].

Aside from their (ethno)medicinal applications, rosehips also have various culinary uses, mainly in desserts, such as jams, syrups, beverages, and cakes [7]. Additionally, their phytochemical profile suggests a strong potential for application as a nutraceutical, as numerous studies confirm their remarkably high ascorbic acid (vitamin C) content [1,11–14], along with other components, such as vitamins A, E, and B-complex [7,13,15], minerals (especially Ca and Mg) [3,7,13], carotenoids [14,16], galactolipids [7], and fatty acids [7,15,17]. Polyphenols represent perhaps the most extensively studied class of compounds in rose hips, as they are considered to be a major contributor to the outstanding antioxidant activity, as well as other health-promoting properties of *R. canina* extracts [18]. The main groups of phenolic compounds found in rosehips are hydrolyzable tannins, proanthocyanidins, flavonoids (mainly glycosylated flavonols and anthocyanins, together with flavan-3-ols), and phenolic acids (mostly hydroxycinnamates, even though gallic acid is also abundant, as a component of gallotannins) [6,7,9,13,19–21].

Despite their nutritional value and beneficial properties, rosehips remain a rather unconventional dietary choice, regarded more as a delicacy than a commodity consumed regularly. However, this notion seems to change in recent years, and rosehips are beginning to gain their well-deserved place in the spotlight as a promising functional food. Nevertheless, research on native Greek *R. canina* populations—which represent the southern limits of the species' European distribution—remains rather underreported [1,3,11,22–25].

Given this scarcity of comprehensive phytochemical data, the aim of the study herein was to establish a bioactive baseline for this germplasm. By evaluating a robust set of 76 distinct genotypes, we sought to test the hypothesis that individual spatial coordinates (altitude and latitude) act as metabolic modulators, driving subtle phytochemical shifts within a stable genetic core. Ultimately, this work aimed to identify elite chemotypes with superior antioxidant and phenolic profiles, providing a scientific basis for future breeding and commercial valorization of Greek dog rose.

2. Materials and Methods

2.1. Plant Material

Rosehips were collected from wild-growing shrubs across three distinct regions in Northern Greece between mid-October and mid-November 2019. Selection was limited to fruits at full physiological maturity, as defined by uniform red coloration and specific fruit firmness. Samples of approximately 15 g each were collected from a total of 76 genotypes: 29 from Zagorochoria (Ioannina, Epirus, northwestern Greece), 26 from Rhodopi (Thrace, northeastern Greece), and 21 from Chalkidiki (Central Macedonia, northern Greece) (Figures 1 and S1, Table A1). Taxonomic identification was performed based on

morphological characteristics by Dr. Stefanos Kostas (Laboratory of Floriculture, School of Agriculture, Aristotle University of Thessaloniki), using standard botanical keys. The nomenclature and taxonomic status were verified using the Plants of the World Online database (https://powo.science.kew.org/). Voucher specimens have been deposited in the Herbarium of the Laboratory of Floriculture, School of Agriculture, Aristotle University of Thessaloniki (AUTH), under accession numbers BRC0100–BRC0175. For analytical purposes, samples were assigned internal codes (e.g., RCZ-8), which are cross-referenced with their respective herbarium voucher numbers (e.g., BRC0100) in Table A1.

Figure 1. Sampling localities of *R. canina* in Greece. Different colored shapes represent the sampling sites for each of the 3 distinct populations; Zagorochoria (purple squares), Chalkidiki (yellow circles) and Rhodopi (green triangles).

After the removal of achenes, pericarps were immediately submerged in liquid nitrogen to halt all metabolic processes and were promptly transported to the laboratory, where they were stored at −80 °C. The frozen pericarps were subsequently ground into a fine powder using a pestle and mortar, while liquid nitrogen was continuously added during the procedure. Ground samples were kept at −80 °C until processing.

2.2. Determination of Ascorbic Acid (AsA) Content

The ascorbate content of the frozen rosehips was determined via the ascorbate oxidase (AO) enzymatic assay, using an optimized protocol [26]; 100 mg of plant tissue from each sample was extracted with 2.5 mL of a 5% (*w*/*v*) aqueous metaphosphoric acid solution—prepared by dissolving metaphosphoric acid (metaphosphoric acid 65% p., CAS Nr: 37267-86-0, CHEM-LAB Analytical, Zedelgem, Belgium) in deionized water—followed by centrifugation (12,000× *g*, 4 °C). The supernatant was collected, and after adjusting its pH to 5.6 with a trisodium citrate buffer 10% (Fluka Chemie GmbH, Buchs, Switzerland), it was used immediately for the determination of AsA content. Rosehip extracts were mixed with 0.1 M sodium phosphate dibasic hydrate buffer (pH 6.3) (Riedel-de Haen) in a 1:20 *v*/*v* ratio, and the mixture's absorption was measured spectrophotometrically at λ = 265 nm, before and after the addition of 1 U ascorbate oxidase (A0157-1KU; Sigma-Aldrich, Steinheim, Germany). Ascorbate content was expressed as mg of L-ascorbic acid per g of fresh weight (mg AsA g^{-1} FW) by means of an ascorbic acid standard curve (0–100 μmol L^{-1} AsA, R^2 = 0.997). Each sample was assessed in triplicate, and the mean value was used for further statistical analysis.

2.3. Extraction of Phenolic Compounds, Determination of Total Phenolic Content (TPC), and Evaluation of Radical Scavenging Activity (RSA)

To perform the in vitro assays, a portion of each frozen ground sample was dried in a laboratory oven (35 °C, 24 h). 300 mg of dried plant tissue from each sample was extracted with aqueous methanol ($MeOH_{aq}$) 70% v/v (2 × 30 mL) in a sonicator bath (10 min at room temperature). The extracts were filtered through a paper filter, and the filtrates were combined and dried by means of a rotary evaporator (Rotavapor® R-210, Büchi, Labortechnik, Flawil, Switzerland) at 40 °C. The extracts were further purified by means of an Amberlite XAD-7HP column (9.5 × 1.3 cm), eluted consecutively with acidified water (CH_3COOH 0.3%), deionized water, and MeOH. The methanolic fractions were collected and dried in vacuo (40 °C). For the determination of TPC and evaluation of RSA, extracts were redissolved in dimethylsulfoxide (DMSO, Honeywell Riedel–de Haën, Seelze, Germany) at a concentration of 10 mg mL^{-1}.

An optimized Folin–Ciocalteu protocol was employed for the determination of the extracts' phenolic content [27]; 20 µL of rosehip extract was incubated (8 min, room temperature) with 2.500 µL of deionized water and 400 µL of Folin–Ciocalteu reagent (F9252; Sigma-Aldrich, Darmstadt, Germany). Subsequently, 500 µL of Na_2CO_3 7% (w/v) was added, followed by a second incubation in the dark at 40 °C. The samples' absorbance was read at 750 nm in a UV-vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan), and a gallic acid standard curve (0–1.5 mg mL^{-1}, R^2 = 0.947) was used to calculate their TPC. The results were expressed as mg of gallic acid equivalents per g of dried weight (mg GAE g^{-1} DW), calculated as the mean of three replications.

The extracts' radical scavenging activity was evaluated by means of the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, according to the protocol of Risaliti et al. [28]. In brief, 20 µL of extract was added to a methanolic solution of 0.01 mM DPPH (D211400, Sigma-Aldrich, Darmstadt, Germany), and the reaction mixture's absorbance was measured after 20 min at 517 nm using a UV-vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan). The results were expressed as % radical scavenging activity (% RSA), calculated using the following formula:

$$\% \text{ RSA} = [(A_0 - A_s)/A_0] \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A_s is the absorbance of the assessed sample. The assay was performed in triplicate, and the results represent the mean of three replications. Trolox was used as a reference compound (% RSA = 97.00%).

2.4. HPLC-PDA-MS Analysis of Phenolic Profile

Dried extracts were redissolved in HPLC-grade MeOH at a concentration of 5 mg mL^{-1}, centrifuged and analyzed within the same day (injection volume: 5 µL). For the qualitative analysis of the extracts' phenolic profile, an LC-PDA-MS Thermo Finnigan system (LC Pump Plus, Autosampler, Surveyor PDA Plus Detector, Thermo Scientific, Palo Alto, CA, USA) interfaced with an ESI MSQ Plus (Thermo Scientific, Waltham, MA, USA) and equipped with Xcalibur 2.1 software (Thermo Scientific, Waltham, MA, USA) was used. The separation was performed on a Phenomenex Synergi Hydro-RP 80 Å LC column (150 × 4 mm, 4 µm) maintained at 30 °C, using a mixture of acidified water (0.05% HCOOH) (solvent A) and acetonitrile (solvent B) as the mobile phase, with a flow rate of 1 mL min^{-1}. Gradient elution was carried out as follows: 0 min, 95% A, 5% B; 40 min, 55% A, 45% B; 45 min, 55% A, 45% B (isocratic elution); 45–60 min, 95% A, 5% B (re-equilibration to initial conditions). The PDA scan range was set between 200–800 nm, and UV chromatograms were recorded at 330 and 350 nm. As far as the conditions in the mass spectrometer are concerned, the gas temperature was 350 °C, the nitrogen flow rate

was 10 L min^{-1}, and the capillary voltage was set at 3000 V. The cone voltage was set at 80 and 100 V. The mass spectrometer operated in negative ionization mode in the range from m/z 100 to 800. Spectral data were processed using the Xcalibur 2.1 QualBrowser. Compounds were tentatively identified by their retention times, UV spectra, and fragmentation patterns, in comparison to the literature.

2.5. Statistical Analysis

All results were expressed as mean values ± standard error (SE). To evaluate differences among the three geographical regions, one-way analysis of variance (ANOVA) was performed, followed by Tukey's HSD post hoc test for multiple comparisons ($p < 0.05$). Before ANOVA, the homogeneity of variances was verified using Levene's test.

The relationships between biochemical parameters and geographical coordinates (altitude, latitude, and longitude) were assessed using Pearson's correlation coefficient (r). To identify the primary environmental drivers of phytochemical variability, stepwise multiple linear regression models were constructed for RSA and TPC. The assumptions of the regression models—including linearity, normality of residuals, and homoscedasticity—were verified through residual plots and histograms. Independence of errors was confirmed by the Durbin–Watson statistic (values ranging from 1.69 to 1.79), and potential multicollinearity was monitored via the Variance Inflation Factor (VIF < 3.1). Influential observations were evaluated using Cook's distance (all values < 1.0).

Principal Component Analysis (PCA) was conducted as an exploratory ordination tool to map the multivariate data structure. The suitability of the dataset for PCA was assessed using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity. To further resolve the influence of environmental gradients, genotypes were stratified into six distinct eco-geographical categories based on combined altitudinal and latitudinal ranges.

Statistical processing, correlation, and regression analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA). Multivariate ordination (PCA biplots and 3D plots) and additional data visualization were conducted using PAST (Paleontological Statistics), Version 4.17c.

3. Results

3.1. Ascorbic Acid Content of Assessed Genotypes

The results are presented in Figures 2 and S2a, and Table S1. Ascorbate content among all 76 genotypes fluctuated between 0.72 ± 0.05 mg g^{-1} FW (ROD-61, Rhodopi) and 3.57 ± 0.16 mg g^{-1} FW (RPK-20, Chalkidiki), with an average of 1.70 ± 0.07 mg g^{-1} FW. Regarding the individual regions, rosehips collected from Chalkidiki showed the highest average AsA content (1.79 ± 0.16 mg g^{-1} FW), followed by those from Rhodopi (1.70 ± 0.10 mg g^{-1} FW), while the Zagorochoria region had the lowest average content (1.63 ± 0.10 mg g^{-1} FW). When the means of these three regions were compared, no statistically significant differences occurred ($p > 0.05$; Table S2, Figure 2); as a result, all regions shared the same grouping letter (a). This suggests that environmental or genetic factors affecting the average ascorbic acid biosynthesis are relatively stable across these geographical zones, even if individual genotypic variation (as seen in Chalkidiki) is present.

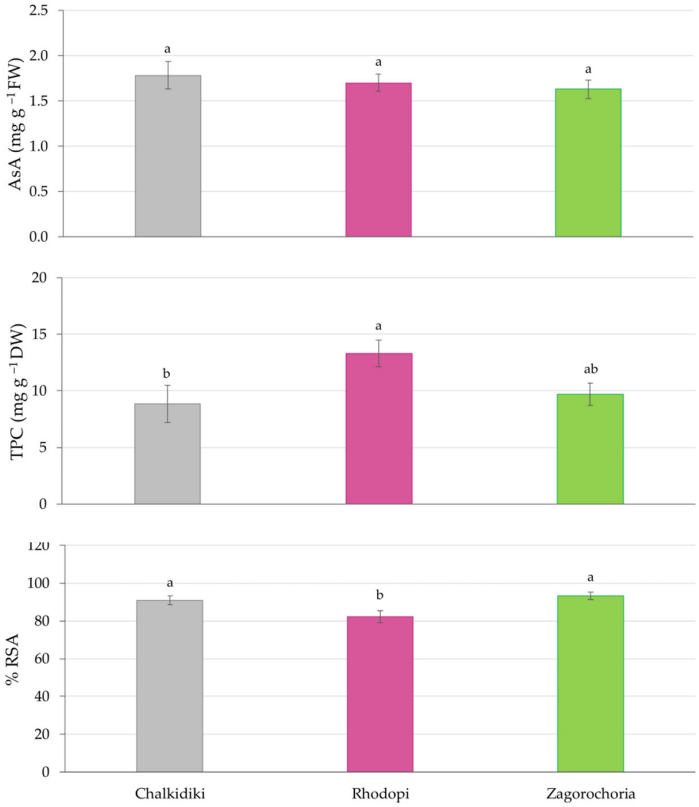

Figure 2. Regional variability of phytochemical parameters in *R. canina* fruits. Mean values (±standard error) for ascorbic acid content (**top chart**), total phenolic content (**middle chart**), and radical scavenging activity (**bottom chart**) across the three studied regions. Different lowercase letters above the bars indicate statistically significant differences according to one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Samples from Zagorochoria, Rhodopi, and Chalkidiki are represented by green, pink, and gray bars, respectively.

As far as distribution patterns within each region are concerned, boxplot analysis (Figure S3) revealed a clear difference. Both Zagorochoria and Rhodopi populations showed moderate variability with symmetric distributions; however, the Chalkidiki population was characterized by a broad distribution range and a marked positive skewness, indicating that while the core of the Chalkidiki population has moderate AsA levels, there are specific genotypes within this region capable of accumulating exceptionally high concentrations of vitamin C (outliers > 3.0 mg g^{-1} FW).

3.2. Total Phenolic Content

The distribution of total phenolic content (TPC) revealed distinct population structures across the three regions (Figures 2, S2b and S3). The Chalkidiki population displayed the widest overall variation (spanning from 1.02 to 35.96 mg g^{-1} DW), a pattern driven significantly by the presence of specific outlier genotypes with exceptionally high phenolic content, despite the main body of the population having the lowest median value. In contrast, the Rhodopi population exhibited a distribution clearly shifted towards higher values; while its absolute range was narrower than that of Chalkidiki, it was characterized by the highest median and a robust interquartile range, indicating that the majority of the Rhodopi genotypes consistently accumulate higher levels of phenolic compounds. The Zagorochoria population occupied an intermediate position regarding both its distribution spread and median values.

In terms of mean content comparison (Figure 2, Table S2), these structural differences translated into significant statistical variations ($p < 0.05$). The Rhodopi region yielded the

highest average TPC (13.31 ± 1.18 mg g^{-1} DW) and was significantly different from the Chalkidiki region, which presented the lowest mean value (8.85 ± 1.85 mg g^{-1} DW). The Zagorochoria population (9.71 ± 0.96 mg g^{-1} DW) showed no statistical difference from either the high-phenolic Rhodopi nor the low-phenolic Chalkidiki populations. Regarding the individual genotypes across all three regions, the Chalkidiki genotype RPK-5 demonstrated the highest TPC value among them (35.96 ± 0.07 mg g^{-1} DW), and RPK-18 (from the same region as well) displayed the lowest one (1.02 ± 0.00 mg g^{-1} DW) (Table S1).

3.3. Radical Scavenging Activity

Virtually all the assessed genotypes exhibited radical scavenging activity above 70% (Figures 2 and S2c, Table S1), except three (ROD-67, ROD-48, and ZAR-6). By contrast, more than a quarter of them (21 out of 76) showed 100% RSA. Variability among genotypes from the three regions is illustrated in Figure S3. The Zagorochoria population exhibited the highest phenotypic homogeneity, characterized by a compact distribution and a high median value, indicating that most genotypes in this region maintain a consistently high antioxidant potential. In contrast, the Rhodopi population displayed a wider dispersion with a notable inter-genotypic variability, suggesting a higher plasticity or genetic diversity regarding antioxidant capacity.

When comparing the mean values among regions (Figure 2), the statistical analysis revealed significant differences ($p < 0.05$; Table S2). More specifically, Zagorochoria and Chalkidiki demonstrated the highest antioxidant activity (93.28 ± 1.90% and 91.01 ± 2.20%, respectively) with no statistically significant difference between them. Conversely, the Rhodopi region showed a significantly lower mean antioxidant capacity (82.42 ± 3.17%) in comparison to the other two locations.

3.4. Correlation and Regression Analysis

To further investigate the relationships between the phytochemical profile and the geographical factors, a Pearson correlation analysis was performed (Table 1). Interestingly, no significant correlations were observed among the three biochemical parameters (RSA, AsA, and TPC), confirming their independent metabolic nature.

Table 1. Pearson correlation matrix between biochemical parameters (RSA, AsA, TPC) and geographical coordinates (altitude, latitude, longitude) of *R. canina* genotypes.

	RSA	AsA	TPC	Altitude	Latitude	Longitude
RSA	1					
AsA	−0.100	1				
TPC	0.034	0.021	1			
Altitude	0.388 **	−0.050	−0.109	1		
Latitude	−0.343 **	0.057	0.244 *	−0.821 **	1	
Longitude	−0.337 **	0.051	0.218	−0.788 **	0.994 **	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Regarding geographical influences, while several relationships reached statistical significance, the correlation coefficients remained low to moderate, indicating that geographical gradients explain only a portion of the total variability. Specifically, radical scavenging activity (RSA) showed a significant positive, moderate correlation with altitude (RSA, r = 0.388, $p < 0.01$), indicating a trend towards stronger radical scavenging potential at higher elevations. Conversely, RSA exhibited significant negative correlations of comparable magnitude with both latitude (r = −0.343, $p < 0.01$) and longitude (r = −0.337, $p < 0.01$), reflecting the geographical gradient of the sampled sites. Furthermore, TPC exhibited a

weak but significant positive correlation with latitude (r = 0.244, $p < 0.05$), implying a subtle gradient of phenolic accumulation in northern populations.

To identify the primary environmental drivers, these relationships were further modeled using stepwise multiple regression analysis (Table S3). Altitude emerged as the sole significant predictor for RSA (adjusted $R^2 = 0.139$, $p = 0.001$), while latitude was the primary predictor for TPC (adjusted $R^2 = 0.047$, $p = 0.034$), with redundant variables such as longitude being excluded from the final models (Table S4).

3.5. Multivariate Analysis and Environmental Stratification

To provide a comprehensive multivariate overview, PCA was performed (Table S5), with data suitability assessed via KMO (0.49) and Bartlett's tests. Consistent with the Pearson analysis, the three principal components explained 100% of the variance (PC1 and PC2 accounting for 70.47%), highlighting the independent nature of RSA, AsA, and TPC.

Initial regional classification (Figure 3a) revealed a dense central "cloud" near the origin, indicating a shared metabolic baseline and substantial overlap among populations. However, the Rhodopi population exhibited the widest multidimensional dispersion, reflecting high phenotypic plasticity, while Zagorochoria showed a subtle horizontal shift towards enhanced antioxidant potential (positive PC1). Moreover, Chalkidiki (red circles) is underrepresented in the upper-right quadrant (simultaneous high RSA and TPC).

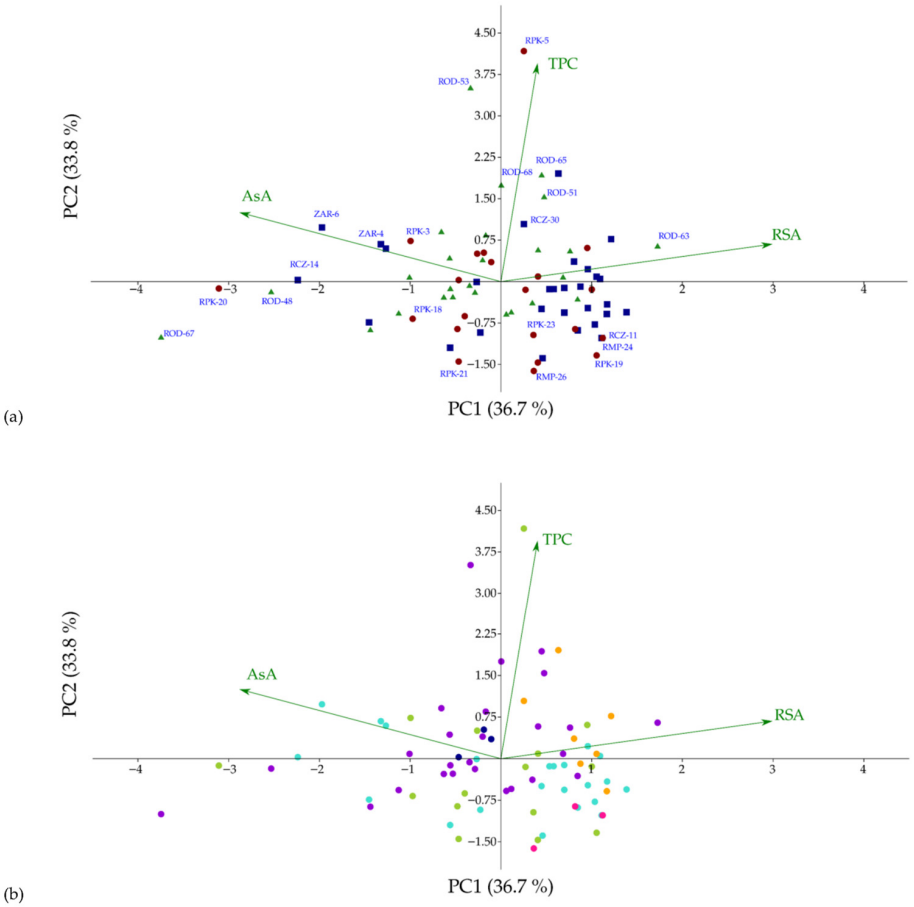

Figure 3. Principal Component Analysis (PCA) biplots illustrating the multivariate phytochemical profile of *R. canina* genotypes. Both panels represent the same PCA space, where green vectors indicate the loadings of biochemical variables: radical scavenging activity (% RSA), ascorbic acid content (AsA), and total phenolic content (TPC). (**a**) Symbols denote individual genotypes categorized by their geographical origin: Zagorochoria (blue squares), Rhodopi (green triangles), and Chalkidiki (red circles). Names of mostly marginal genotypes are included. (**b**) Genotypes are

color-coded according to exploratory eco-geographical zones defined by a combination of altitude and latitude (refer to Table S4 for detailed specifications).

The stratification into six eco-geographical groups (Table S6, Figure 3b) clarified this internal heterogeneity. The high-altitude orange group (Zagorochoria) demonstrated a clearer displacement towards the positive PC1 axis, confirming the altitude-driven enhancement of RSA. In contrast, the northern populations displayed more complex patterns. The northernmost purple group (Rhodopi) exhibited an extensive vertical spread along the PC2 axis, with several genotypes reaching the highest phenolic scores, although the group remained highly scattered. The yellow-green group (Chalkidiki), while also from northern latitudes, was predominantly situated in the lower quadrants, indicating a lower phenolic baseline for these mid-altitude genotypes compared to the northernmost Rhodopi samples. Notably, high-altitude northern genotypes (dark blue) favored PC2 over PC1, suggesting a different metabolic prioritization compared to southern counterparts.

These multivariate trends were further corroborated by the six-group boxplot analysis (Figure 4), which identified altitude as a stabilizer for antioxidant activity (Figure 4c) and highlighted the bimodal influence of latitude and elevation on the phenolic baseline (Figure 4b). In contrast, AsA content (Figure 4a) displayed the highest inter-genotypic plasticity, lacking a clear linear geographical trend.

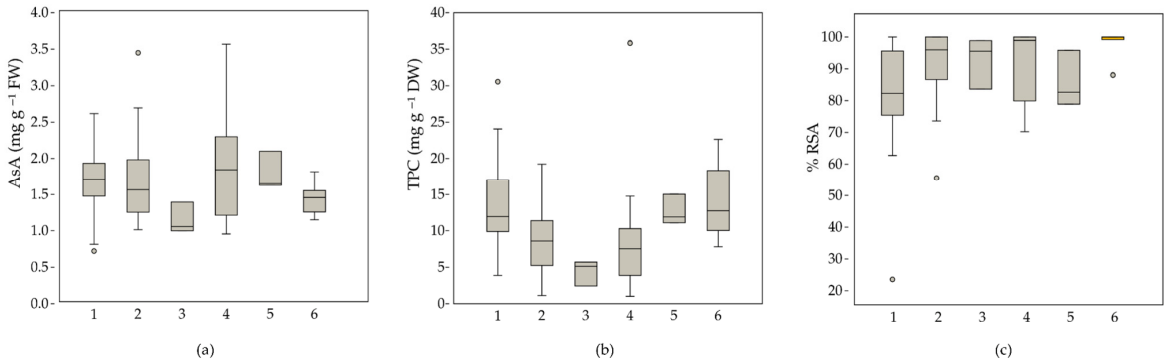

Figure 4. Box-and-whisker plots illustrating the variability of (**a**) ascorbic acid content (AsA), (**b**) total phenolic content (TPC), and (**c**) radical scavenging activity (% RSA) across six eco-geographical categories of *R. canina*. Groups on the X-axis are ordered by increasing altitudinal range: Group 1 (0–300 m); Groups 2–4 (300–600 m); and Groups 5–6 (600–900 m). The horizontal line within each box represents the median value, while the box boundaries denote the 25th and 75th percentiles (interquartile range). Whiskers represent the minimum and maximum values excluding outliers, and individual circles indicate outlier genotypes. For detailed specifications regarding the altitudinal and latitudinal ranges of each group, refer to Table S6.

3.6. Investigation of Phenolic Profile

HPLC-PDA-MS chromatograms of rosehip extracts revealed a highly complex phenolic profile, dominated by the presence of flavan-3-ol (catechin) derivatives and proanthocyanidins, which complicated the analysis (Figure 5), due to the occurrence of a chromatographically non-separable "hump". As a result, it was possible to identify 12 flavonoids that were present in all extracts: two flavanonols (eriodictyol derivatives) and ten flavonol (kaempferol, quercetin, and methylquercetin) glycosides (Table 2).

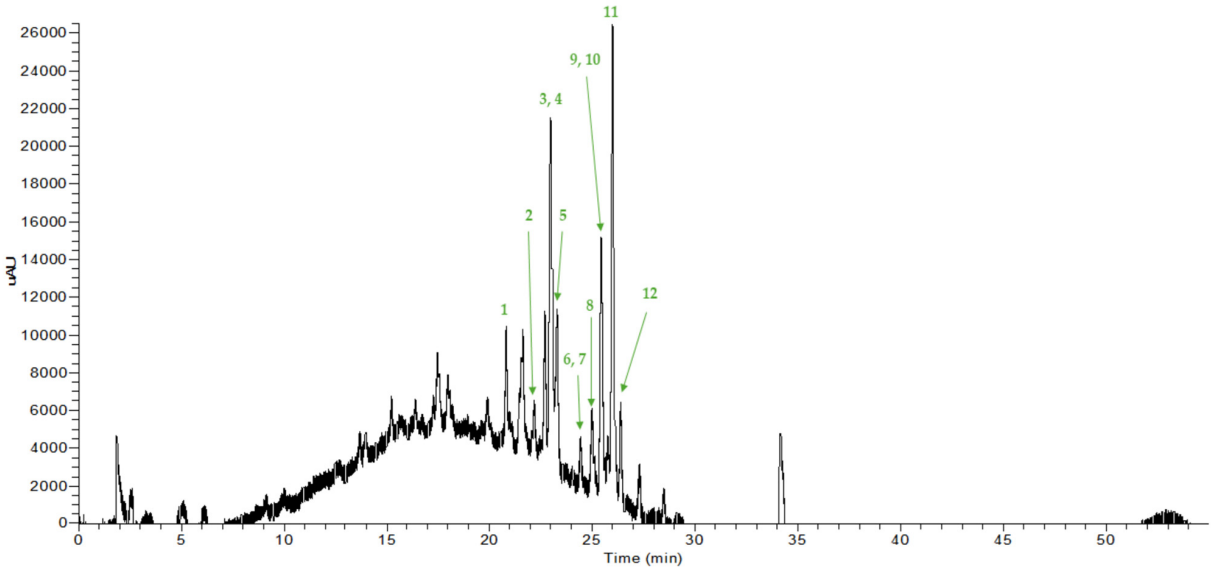

Figure 5. Representative HPLC-PDA chromatogram of rose hip extract at 350 nm. The green-colored numbers in the chromatogram represent the identified compounds, by elution order.

Table 2. Tentative identification of phenolic compounds.

No (Elution Order)	Rt (min)	UV (nm)	(−) *m*/*z*	Identification
1	20.90	267, 330 sh	287, 300, 433, 551	Eriodictyol derivative
2	22.26	273, 336 sh	287, 449, 609	Eriodictyol dihexoside
3	23.01	265, 352	300, 463	Quercetin 3-O-hexoside 1
4	23.01	265, 352	300, 477	Quercetin 3-O-hexuronide [1]
5	23.38	266, 352	300, 463	Quercetin 3-O-hexoside 2
6	24.51	267, 350	285, 417	Kaempferol 3-O-pentoside
7	24.51	267, 350	300, 433	Quercetin 3-O-pentoside 1 [2]
8	25.05	267, 352	300, 433	Quercetin 3-O-pentoside 2
9	25.50	265, 351	300, 433	Quercetin 3-O-pentoside 3
10	25.50	265, 351	315, 447	Methylquercetin 3-O-pentoside 1 [3]
11	26.04	255, 350	300, 447	Quercetin 3-O-rhamnoside
12	26.43	262, 350	315, 447	Methylquercetin pentoside 2

[1] Admixture with **3**. [2] Admixture with **6**. [3] Admixture with **9**.

Compounds **1** and **2** were recognized as eriodictyol derivatives due to the occurrence of a shoulder at ~330 nm in the UV spectrum (which is a characteristic of flavane derivatives), in addition to the presence of a fragment with a m/z ratio of 287. Furthermore, for compound **2,** the fragments at m/z = 449 (−160 amu) and 287 (−162 amu) signified the loss of two hexose residues, leading to its tentative identification as a dihexoside of eriodictyol. According to UV spectra, the rest of the detected compounds (**3**–**12**) were identified as flavonols with a 3-OH substitution pattern, as Band I was observed at ~350 nm [29]. More specifically, compounds **3** and **5** exhibited a common fragment at m/z = 300, which corresponds to a quercetin aglycone, alongside a pseudomolecular ion at m/z = 463, which indicates glycosylation by a hexose moiety (glucose or galactose). Therefore, they were identified as quercetin 3-O-hexoside isomers, while compound **4**—which was co-eluted with compound **3**—was assigned as quercetin 3-O-hexuronide due to a loss of 177 amu. Compounds **6** and **7** were also eluted together at Rt = 24.51 min. The fragments observed at m/z = 417 and 433 were assigned to pseudomolecular ions $[M\text{-}H]^-$, whereas the two ion peaks detected at m/z = 285 and 300 correspond to the aglycones of kaempferol and quercetin, respectively, after the cleavage of a pentose residue (−133 amu). It was, therefore, deduced that said constituents were kaempferol 3-O-pentoside and quercetin 3-

O-pentoside, respectively. Two more quercetin 3-O-pentosides were detected (compounds **8** and **9**), since they generated the same ion peaks at m/z = 433 and 300. According to the literature, xylosides are typically eluted first among pentosides in reverse-phase chromatography, followed by arabinopyranosides and, finally, arabinofuranosides [30,31]; hence, we speculated that the three isomers, **7**, **8,** and **9,** corresponded to quercetin 3-O-xyloside, quercetin 3-O-arabinopyranoside, and quercetin 3-O-arabinofuranoside, respectively. Compound **10** was eluted as an admixture with compound **9** (Rt = 25.50 min) and, based on the fragments it generated (m/z = 315, 447), was tentatively identified as methylquercetin 3-O-pentoside. Quercetin 3-O-rhamnoside (compound **11**) was identified by the ion peaks at m/z = 447 and 300, which signified the cleavage of rhamnose residue (−147 amu), resulting in a deprotonated quercetin aglycone fragment. Finally, a second methylquercetin 3-O-pentoside (compound **12**) was detected at 26.04 min.

4. Discussion

Current trends in consumer behavior indicate a clear shift toward a more health-conscious way of living, with natural products being at the forefront, as the Hippocratic principle of "food as medicine" remains relevant to this day [7,32]. An abundance of studies substantiates the nutritional value and the multifaceted pharmacological activity of rosehips, corroborating traditional medicinal practices while highlighting their safety and efficacy as a functional food [6–9,13,14,33–37].

Assessment of ascorbate content is a key aspect of research on rosehips, as their vitamin C levels exceed those of citrus fruits and other edible berries [7] and humans lack the capacity to biosynthesize it [38]. Ascorbate content of *R. canina* pseudofruits can vastly vary, from less than 0.5 to approximately 20 mg g^{-1} [18,39,40]. The observed intra-species disparity can be attributed to both intrinsic and external factors, as some genotypes have an innate capability of increased ascorbate biosynthesis, which can be modulated as a response to climate conditions (mainly temperature, oxygen content, and sun exposure) and soil composition [18,39,41,42]. Additionally, different methods employed for the determination of AsA may also influence the final result. Aside from ascorbic acid, rosehips tend to accumulate a high content of polyphenols, which also seems to be influenced by complex interactions between genetic makeup and habitat, as well as the experimental protocol followed [11,16,18,39,42–44]. Studies on *R. canina* genotypes from the Balkans and Turkey report average TPC values of a wide range—even exceeding 10 mg GAE g^{-1} FW [9,16,18,39,41,45]. Given their rich and diverse phenolic profile, it comes as no surprise that rosehips exhibit impressive antioxidant activity—which is further amplified by the contribution of other constituents, such as ascorbate and tocopherols [1,9,13,16,39].

The nutraceutical potential of native Greek dogrose has only recently started garnering scientific interest [1,3,11,22–25]. In the present study, wild-grown rosehips from three different regions of Northern Greece were evaluated for their ascorbic acid (AsA) content, phenolic profile, and antioxidant (radical scavenging) activity, revealing distinct distributional patterns and varying degrees of inter-genotypic variability across the three studied regions. AsA content remained relatively stable across the geographical zones, suggesting that average vitamin C biosynthesis is relatively conserved across these environments, despite individual genotypic fluctuations. The observed statistical independence of AsA from RSA and TPC is likely due to the different analytical states of the matrices. AsA was determined in fresh tissue (FW) to preserve its labile nature, whereas RSA and TPC were measured in dried tissue (DW), where ascorbic acid is significantly degraded. The results reported herein concerning the average AsA content of Greek *R. canina* genotypes were in the same order of magnitude as those by Grigoriadou et al. [11] for fully

ripe rosehips (<0.5—approx. 2.0 mg g^{-1} FW) but lower than the ones by Maloupa et al. (3.545 ± 1.282 mg g^{-1} FW) [1].

Regarding radical scavenging potential, the population from Zagorochoria demonstrated both the highest average % RSA and the highest phenotypic homogeneity. This suggests an environmental stabilization of traits at higher altitudes, where elevation-specific stressors—such as increased UV-B radiation and temperature fluctuations—likely trigger the up-regulation of protective secondary metabolites [18,39]. In contrast, the Rhodopi population exhibited significantly higher phenotypic heterogeneity in its antioxidant response and the lowest average; values spanned almost the entire measurement scale, reflecting a remarkably plastic antioxidant profile within this region. A different trend was observed for TPC; here, the Rhodopi and Zagorochoria populations exhibited comparable internal heterogeneity, while presenting a wide metabolic spectrum, with the former maintaining a higher median and a more pronounced positive skewness towards high phenolic levels. This high plasticity in the aforementioned populations contrasts with that of Chalkidiki, which presented the lowest median phenolic baseline, yet it was characterized by the most extreme variability, harboring outliers that spanned from the study's absolute minimum to its maximum. The significant, yet relatively weak, influence of geographical factors ($R^2 < 0.14$) suggests that the hips' chemical composition is governed by a robust intrinsic genetic control, with environmental factors acting as subtle modulators rather than primary determinants. The dense central "cloud" observed in our PCA biplots confirms this metabolic stability, representing a shared "phytochemical baseline" for the species across the Greek territory. In quantitative terms, our results align with those reported for other Greek *R. canina* hips [1,11,22,24,25], despite being on the lower end of the values' range.

The transition from a broad regional classification to an eco-geographical stratification into six categories proved essential for deconstructing the perceived uniformity of the populations. The significant, yet relatively weak, influence of geographical factors ($R^2 < 0.14$) suggests that while the hips' chemical composition is governed by a robust intrinsic genetic control, environmental factors—effectively translated via altitude and latitude coordinates—act as subtle modulators rather than primary determinants. Exploratory multivariate analysis (PCA) was employed as a visualization tool to map this phytochemical scattering, representing a shared "phytochemical baseline" for the species across the Greek territory. Notably, the high plasticity observed in Rhodopi—despite its highly restricted sampling radius—contrasts with the environmental stabilization at higher altitudes, further validating the interplay between robust genetic control and localized environmental modulation.

As far as the qualitative characterization of the extracts is concerned, all HPLC chromatograms were characterized by a "hump", observed between 10 and 25 min, approximately. UV and MS spectra of this area revealed the presence of various flavan-3-ol derivatives, such as catechins and proanthocyanidins. According to Salminen et al. [46], the occurrence of such humps is not a rare phenomenon when analyzing plant extracts containing high levels of proanthocyanidins (oligomers and polymers). In a similar manner to the aforementioned study, ion peaks with unusual m/z values were detected in the (−) MS spectra (in addition to the characteristic fragment at m/z = 289), suggesting the existence of structurally complex condensed tannins, which require further analysis. These phenolic constituents seem to provide a notable contribution to the extracts' radical scavenging—and overall antioxidant—activity, complementing the identified flavonoids [47]. Despite the impediment posed by the proanthocyanidin dominance, 12 flavonoid derivatives were identified across all extracts, the majority of which were quercetin 3-Oglycosides (pentosides, hexosides, a rhamnoside, and a hexuronide). The literature corroborates our findings,

as a wide variety of structurally distinct flavonoid derivatives have been detected from *R. canina* hip extract, with quercetin and kaempferol ones being the most common [11,13,48].

5. Limitations and Prospects

While the present study offers a comprehensive screening of Greek *R. canina* germplasm, certain limitations inherent to wild population studies should be acknowledged. Firstly, the observed phytochemical variability reflects a complex interplay between the genetic background of individual shrubs and their environment. Without the use of clonal replicates grown under standardized conditions, a precise partition of the variance between genotype and environment (G × E) remains tentative. This inherent genetic diversity likely contributes to the metabolic scattering observed in our multivariate models.

Future prospects should involve the establishment of experimental fields using the identified "elite" genotypes (e.g., RPK5 and RCZ12) to evaluate the stability of their bioactive traits. Additionally, more advanced analytical techniques, such as high-resolution mass spectrometry (HRMS), tandem mass spectrometry (MS^n)—even thiolysis coupled with HPLC—could be employed to further elucidate the degree of polymerization and the specific subunit composition of the proanthocyanidin fraction. Such insights will be crucial for the full valorisation of Greek dog rose as a standardized source for the pharmaceutical and functional food industries.

6. Conclusions

In conclusion, our research highlights the nutraceutical significance of native Greek *Rosa canina*, providing a more comprehensive profile through the inclusion of previously unstudied genotypes from Eastern Greece. This broader geographical perspective reveals a robust profile of bioactive compounds, with the results largely aligning with prior literature regarding AsA levels, total phenolic content, and antioxidant activity. Such consistency across diverse regions underscores the reliability of Greek dog rose as a premium raw material for the nutraceutical industry, advocating for its strategic commercialization as a standardized functional food ingredient.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nutraceuticals6020034/s1, Table S1: Overview of AsA content, TPC, and in vitro antioxidant (radical scavenging) capacity values measured; Table S2: One-way ANOVA of the collection region on the biochemical parameters of *R. canina* genotypes; Table S3: Summary of stepwise multiple regression models for antioxidant capacity (RSA) and total phenolic content (TPC) in *R. canina*; Table S4: Variables excluded from the stepwise regression models; Table S5: PCA Eigenvalues and Factor Loadings; Table S6: Classification of *R. canina* genotypes into six eco-geographical categories based on altitudinal and latitudinal gradients. Figure S1: Satellite imagery illustrating the geographical distribution of the 76 *Rosa canina* genotypes sampled across Northern Greece. Figure S2: Detailed individual distribution profiles of phytochemical parameters across 76 *R. canina* genotypes. (a) Ascorbic acid content (AsA; mg g^{-1} FW); (b) total phenolic content (TPC; mg g^{-1} DW); and (c) radical scavenging activity (% RSA). Figure S3: Box-and-whisker plots illustrating the population structure and phenotypic variability of *R. canina* genotypes from three different regions regarding: (a) Ascorbic Acid content (AsA), (b) Total Phenolic Content (TPC), and (c) Radical Scavenging Activity (% RSA).

Author Contributions: Conceptualization, A.K.K. and D.L.; methodology, T.P., E.P. and D.L.; validation, T.P. and E.P.; formal analysis, E.P.; investigation, T.P.; resources, T.P., C.P., A.K.K., S.K. and D.L.; data curation, T.P., E.P. and C.P.; writing—original draft preparation, T.P. and E.P.; writing—review and editing, T.P., E.P., S.K., A.K.K. and D.L.; visualization, E.P. and T.P.; supervision, D.L. and A.K.K.; project administration, A.K.K., S.K. and D.L.; funding acquisition, A.K.K. and S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call "RESEARCH-CREATE-INNOVATE" (project code T1EDK-03919).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings of this study are presented in the manuscript and Supplementary Materials.

Acknowledgments: Konstantinos Papazoglou (Grizo & Prasino Organic Herbal Experience) is acknowledged for his participation in the collection of plant material. The authors would also like to thank Anastasia Karioti (Department of Pharmacy, University of Pisa, Italy, formerly in the Laboratory of Pharmacognosy, Faculty of Health Sciences, School of Pharmacy, Aristotle University of Thessaloniki) for her contribution to LC-PDA-MS analysis of extracts.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

AsA	Ascorbic acid
TPC	Total Phenolic Content
RSA	Radical Scavenging Activity
PCA	Principal Components Analysis
HPLC-PDA-MS	High-Performance Liquid Chromatography coupled with Photodiode Array Detector and a Mass Spectrometer

Appendix A

Table A1. Collection data on assessed genotypes.

Genotype	Region	Latitude	Longitude	Altitude (m)	Collection Date	Voucher Specimen
RCZ-8	Zagorochoria (Ioannina, Epirus)	39.867500	20.649167	539.0	22 October 2019	BRC0100
RCZ-9		39.867500	20.648889	537.0		BRC0101
RCZ-10		39.881667	20.634167	441.0		BRC0102
RCZ-11		39.879722	20.635000	454.0		BRC0103
RCZ-12		39.898333	20.622778	429.0		BRC0104
RCZ-13		39.89778	20.62306	428.0		BRC0105
RCZ-14		39.897778	20.623056	428.0		BRC0106
RCZ-15		39.902778	20.623333	461.0		BRC0107
RCZ-16		39.905400	20.621200	479.8		BRC0108
RCZ-17		39.906111	20.620000	493.0		BRC0109
RCZ-18		39.906389	20.619722	496.0		BRC0110
RCZ-19		39.916389	20.621944	492.0		BRC0111
RCZ-20		39.916389	20.621944	492.0		BRC0112
RCZ-21		39.925833	20.618889	492.0		BRC0113
RCZ-22		39.916944	20.621944	491.0		BRC0114
RCZ-23		39.925556	20.618611	492.0		BRC0115
RCZ-24		39.91694	20.62194	491.0		BRC0116
RCZ-25		39.955833	20.655833	714.0		BRC0117
RCZ-26		39.899722	20.670556	900.0		BRC0118

Table A1. *Cont.*

Genotype	Region	Latitude	Longitude	Altitude (m)	Collection Date	Voucher Specimen
RCZ-27	Zagorochoria (Ioannina, Epirus)	39.955833	20.655556	719.0	22 October 2019	BRC0119
RCZ-29		39.953056	20.658333	692.0		BRC0120
RCZ-30		39.887222	20.672778	848.0		BRC0121
RCZ-31		39.941111	20.680833	483.0		BRC0122
RCZ-33		39.883740	20.674793	852.0		BRC0123
RCZ-34		39.930556	20.671667	690.0		BRC0124
ZAR-4		39.881215	20.634587	440.3		BRC0125
ZAR-6		39.879358	20.635167	451.7		BRC0126
ZAR-7		39.898746	20.622538	427.7		BRC0127
ZAR-10		39.897932	20.623614	432.7		BRC0128
ROD-48	Rhodopi (Thrace)	41.169463	25.438749	110.5	17 October 2019	BRC0129
ROD-49		41.169548	25.439023	113.6		BRC0130
ROD-50		41.169586	25.438855	111.6		BRC0131
ROD-51		41.169808	25.438821	111.0		BRC0132
ROD-52		41.169611	25.439200	115.9		BRC0133
ROD-53		41.169628	25.439536	118.5		BRC0134
ROD-54		41.168880	25.438696	110.6		BRC0135
ROD-55		41.172448	25.444375	143.8		BRC0136
ROD-57		41.171332	25.445770	151.8		BRC0137
ROD-58		41.171884	25.446169	152.0		BRC0138
ROD-59		41.171878	25.447312	157.7		BRC0139
ROD-60		41.172486	25.448125	165.7		BRC0140
ROD-61		41.173636	25.447448	167.5		BRC0141
ROD-62		41.171212	25.448063	166.1		BRC0142
ROD-63		41.171437	25.450395	174.8		BRC0143
ROD-64		41.169884	25.452183	170.8		BRC0144
ROD-65		41.168752	25.452517	168.5		BRC0145
ROD-66		41.168337	25.452044	166.7		BRC0146
ROD-67		41.168477	25.451811	166.8		BRC0147
ROD-68		41.167734	25.452021	161.3		BRC0148
TRI-2	Rhodopi (Thrace)	41.168394	25.452313	166.1	14 November 2019	BRC0149
TRI-3		41.167827	25.452201	161.6		BRC0150
TRI-4		41.167361	25.452234	157.0		BRC0151
TRI-5		41.165631	25.451782	146.4		BRC0152
TRI-6		41.165151	25.451568	143.4		BRC0153
TRI-7		41.164453	25.451202	137.5		BRC0154
RPK-3	Petrokerasa, Megali Panagia (Chalkidiki)	40.525112	23.269444	557.6	6 November 2019	BRC0155
RPK-5		40.523251	23.275331	558.9		BRC0156
RPK-6		40.523107	23.275447	560.4		BRC0157
RPK-8		40.516400	23.292479	514.1		BRC0158
RPK-9		40.516369	23.292674	512.9		BRC0159
RPK-11		40.516284	23.294307	501.4		BRC0160
RPK-12		40.516105	23.294859	498.9		BRC0161
RPK-13		40.517328	23.271439	621.7		BRC0162
RPK-14		40.518470	23.273755	597		BRC0163
RPK-15		40.518585	23.273785	596.7		BRC0164
RPK-16		40.516406	23.292461	514.2		BRC0165
RPK-17		40.516406	23.292461	514.2		BRC0166
RPK-18		40.517048	23.291667	510.3		BRC0167
RPK-19		40.515557	23.295099	492		BRC0168
RPK-20		40.515595	23.295194	492.7		BRC0169
RPK-21		40.511655	23.318246	432.7		BRC0170

Table A1. *Cont.*

Genotype	Region	Latitude	Longitude	Altitude (m)	Collection Date	Voucher Specimen
RPK-22	Petrokerasa, Megali Panagia (Chalkidiki)	40.511750	23.318380	432.9	6 November 2019	BRC0171
RPK-23		40.511853	23.318500	432.8		BRC0172
RMP-24		40.444922	23.672343	497.7		BRC0173
RMP-25		40.444761	23.672479	496.2		BRC0174
RMP-26		40.444786	23.672460	496.4		BRC0175

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