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Article

Microarthropods Living on the Endemic Tree *Zelkova abelicea* (Ulmaceae) with Particular Attention to Collembola Diversity

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Abstract: *Zelkova abelicea* is an endemic tree species growing in several localities in the mountainous regions of Crete, Greece. To date, the microarthropod species associated with this tree species have not been identified. Since *Z. abelicea* populations are isolated and fragmented, it was hypothesized that the characteristics of microarthropod assemblages, particularly in the case of springtails (Collembola), would vary and differ among localities. Moreover, rare microarthropod species that colonize microhabitats not included in previous studies on *Zelkova* trees were expected to be recorded. Samples were collected from the bark and twigs of *Z. abelicea* at eight localities in all main mountain ranges. Among the collected material, Collembola were the most numerous (10,285), followed by Acari (2237) and representatives of Psocoptera (422). The obtained material and statistical analyses showed that the arthropod assemblages differed considerably at each experimental site, with the most distinct assemblage characteristics observed at the Gerakari site on Mt. Kedros in central Crete. The most numerous specimens were species of Collembola: *Xenylla maritima* (3844), *Xenylla* sp. 2 (*maritima* complex) (3364) and *Xenylla* sp. 1 (*maritima* complex) (2631). A total of 33 Collembola species were recorded, of which 19 had not been previously reported in Crete. Among them, 11 species were likely new to science and will be the subject of separate taxonomic studies.

Keywords: Collembola; Arachnida; Insecta; biodiversity; ecology of arthropods; zoogeography

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1. Introduction

Relict tree species were originally widely distributed on Earth thousands or even millions of years ago. As a result of changing climatic and environmental conditions, they are presently found only sporadically in places where they encounter appropriate conditions for their survival [1]. Examples of such relict trees include species belonging to the genera *Aesculus*, *Laurus*, *Liquidambar*, *Juglans*, *Parrotia*, *Pterocarya*, *Rhododendron* and *Zelkova* [2,3].

Relict trees play a tremendous role from a scientific perspective and for effective biodiversity preservation [4,5]. Many relict tree species are relatively rare, and as such, they are under legal protection in some countries. In turn, a considerable number of old that constitute unique microhabitats remain, thus promoting conducive conditions for the preservation of biodiversity [6]. These microhabitats, sometimes referred to in the literature as "tree-related microhabitats" [7,8], shelter a wide range of organisms, from fungi to bryophytes, invertebrates, birds and mammals.

The genus *Zelkova* (Ulmaceae) is a relict genus from the so-called Arcto-Tertiary geoflora [9], whose members were important components of forests in the Northern Hemisphere during the Paleogene. The six extant species are distributed throughout western and eastern Asia (Caucasus: *Z. carpinifolia* (Pall.) Koch), East Asia (*Z. serrata* (Thunb.) Makino) and China (*Z. schneideriana* Hand.-Mazz and *Z. sinica* Schneid.), although two found are on Mediterranean islands (Sicily, Italy: *Z. sicula* Di Pasq., Garfì & Quézel, and Crete, Greece: *Z. abelicea* (Lam.) Boiss.). Habitat loss, logging, increased drought periods and limited reproduction represent major threats for these species. Both Mediterranean species have been assigned a high threat level according to the IUCN Red List of threatened species [10,11].

Some *Zelkova* species have been the subject of entomological studies, with the most spectacular results presented by Barbagallo [12], who described *Zelkovaphis trinacriae*, which is a new Eriosomatine aphid genus and species that lives on *Z. sicula* on Sicily. In turn, Mazzeo et al. [13] presented a list of 23 insect species of Hemiptera, while Campo et al. [14] summed up the knowledge on insect and fungal species associated with this tree species from Sicily. Hsin-Ting et al. [15] inspected insects at monthly intervals and recorded insects that fed on or utilized *Z. serrata* in a 100-hectare investigation plot in Pingtung County (Southern Taiwan). A total of 91 insect species were recorded, including Coleoptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Orthoptera and Psocoptera. With regard to feeding guilds, 32 species were recognized as defoliators, 12 species were recognized as sap suckers, 3 species were recognized as stem borers, 31 species were recognized as dead wood feeders, and 13 species utilized this tree species in ways other than the above categories. Ohsawa [16] conducted investigations to elucidate the life cycle and ecological characteristics of the beetle *Trachys yanoi*, an important pest of *Z. serrata* in Japan. Two new species of eriophyoid mites (*Tegolophus zelkofoliae* and *Rectalox dorsoenodis*) were found on *Z. carpinifolia* in Golestan Province, Iran. Both new species were vagrants on the leaf underside, and no damage was observed on the infested plants [17].

Zelkova abelicea (Lam.) Boiss is an endemic species growing in several localities in all mountainous regions of Crete above 900 m a.s.l. [18]. A majority of specimens show stunted growth and a dwarfed, bushy plant habit primarily due to browsing by goats. Tree specimens, which are much less common, reach 15–20 m in height [19]. They frequently grow in the vicinity of abandoned shepherd shelters and have historically been pollarded to use the leaves for summer forage [20,21].

To date, specific research has not been conducted on invertebrates living on *Z. abelicea*. Only two Phytoseiidae mites (Acari) have been recorded [22], and one Hymenoptera species has been recorded [23]. This research gap encouraged the authors to initiate a series of studies focused on assemblages of invertebrates colonizing this endemic tree species.

Populations of *Z. abelicea* on Crete are situated in mountainous regions. In Crete, the five main mountain ranges are isolated from each other by lowland areas. Thus, trees in one locality have little to no contact with those growing in other sites, which is supported by the limited genetic exchange among *Z. abelicea* trees between mountain ranges and, in some situations, between populations within a mountain [24]. Thus, our research hypothesis was that the characteristics of microarthropod assemblages, especially springtails (Collembola) on *Z. abelicea* trees, will vary and differ from locality to locality. The aim of this study was to determine the assemblage characteristics in each locality. Then, based on these results, we conducted a statistical analysis to identify the diversity of species of each arthropod group for every locality. Considering that some arthropod species are known to be closely associated with specific tree species, we expected to record very rare arthropod species or to potentially find species new to science.

2. Methods

2.1. Field Studies

The material was collected at eight experimental sites distributed over the entire range of *Z. abelicea* on Crete (Figure 1).

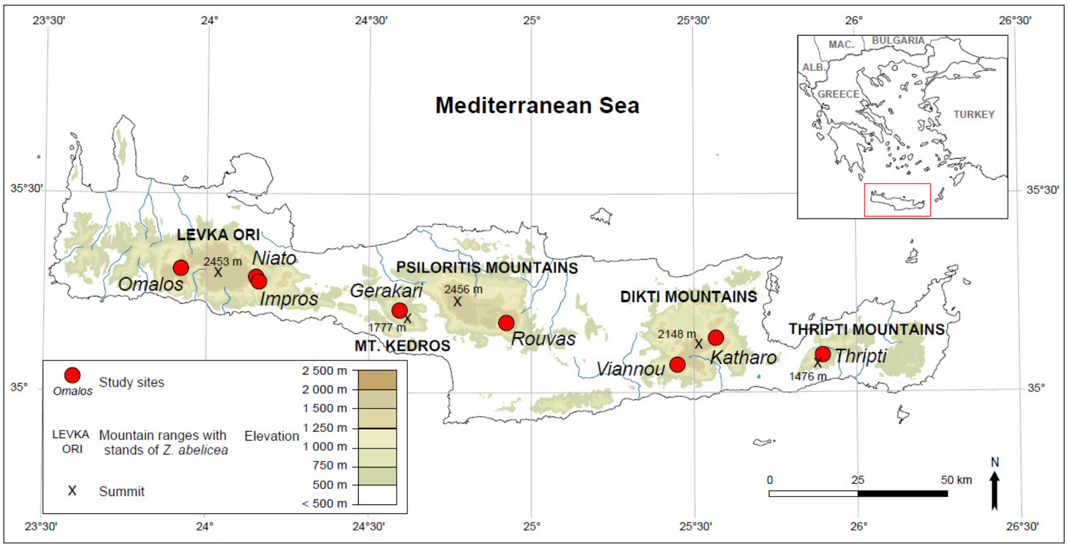

Figure 1. Sampled localities (red dots) on Crete (Greece) with *Zelkova abelicea* trees.

1. Omalos, Levka Ori (Latitude 35, 31901; Longitude 23, 91871), Altitude—1160 m a.s.l., topology: Slope, microhabitat: Bark of arborescent trees, date—21 May 2019, Coll. D. Ghosn;
2. Niato, Levka Ori (35, 287527; 24, 145503), 1215 m a.s.l., doline, branches of dwarfed individuals, 21 May 2019, Coll. D. Ghosn;
3. Impros, Levka Ori (35, 270546; 24, 15315), 1175 m a.s.l., slope, bark of arborescent trees, 21 May 2019, Coll. D. Ghosn;
4. Gerakari, Mt. Kedros (35, 194829; 24, 606713), 1255 m a.s.l., slope, bark of arborescent trees, 11 October 2018, Coll. D.J. Gwiazdowicz;
5. Rouvas, Psiloritis Mountains, (35, 164333; 24, 922794), 1320 m a.s.l., slope, bark of arborescent trees, 10 October 2018, Coll. D.J. Gwiazdowicz;
6. Viannou, Dikti Mountains, (35, 064291; 25, 469778), 1320 m a.s.l., slope, bark of arborescent trees, 9 October 2018, Coll. D.J. Gwiazdowicz;
7. Katharo, Dikti Mountains, (35, 148004; 25, 567558), 1160 m a.s.l., slope, bark of arborescent trees, 9 October 2018, Coll. D.J. Gwiazdowicz;
8. Thripti, Thripti Mountains, (35, 080588; 25, 887408), 1150 m a.s.l., doline, branches of dwarfed individuals, 14 May 2019, Coll. D. Ghosn.

At each sampling site, samples were collected from five trees (one tree—one sample) growing at a distance of a few to tens of meters apart. A sample of the outer trunk bark layer was cut off with a knife from well-developed arborescent trees. In the case of dwarfed specimens, branches were cut off with pruning shears. It was due to the fact that the bark on the trunk of young or dwarf trees is smooth and thin, while on old and large trees it is thick, cracked and frequently colonized by mosses and lichens (Figure 2). The collected material was placed in paper bags. The weight of each sample ranged from 200 to 250 g.

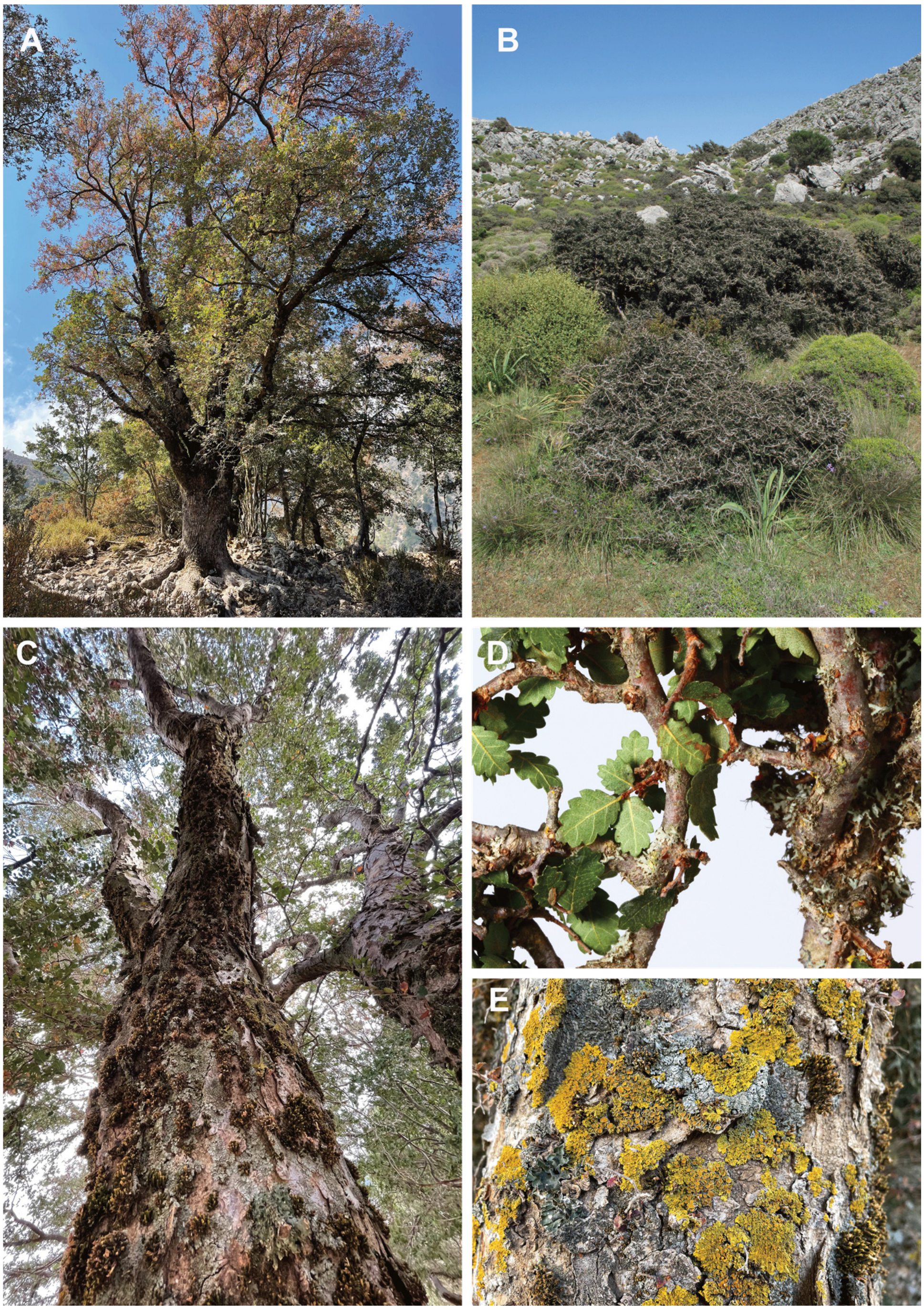

Figure 2. *Zelkova abelicea* trees with microhabitats for invertebrates. (**A**) Large trees (Omalos). (**B**) Dwarfed individuals heavily browsed by goats (Thripti). (**C**) Bark of large trees (Gerakari). (**D**) Bark of browsed individuals with lichens (Thripti). (**E**) Bark of large tree covered by several species of lichens form the genera *Xanthoria*, *Pleurostica* and *Physconia* (Gerakari) (Photos: G. Kozlowski—(**A**–**C**,**E**); Hans-Rüdiger Siegel—(**D**)).

2.2. Laboratory Procedures

The collected samples were placed into Tullgren funnels for 72 h and extracted in 96% ethanol. The extracted arthropods were classified into several groups of arthropods, e.g., spiders, mites, springtails and insects. For this purpose, a Zeiss Stemi 2000 stereoscopic microscope was used.

At this stage of the study, species determinations were limited to the most numerous group, which were springtails (Collembola). A Nikon Eclipse E600 phase contrast microscope was used to identify the Collembola. The extracted specimens of springtails were cleared in Nesbitt's fluid and slide-mounted in a Hoyer medium to prepare semi-permanent microscopic slides necessary for taxonomic analysis. The taxonomic identification of Collembola was carried out based on the following papers: Gisin [25], Stach [26,27], Massoud [28], Ellis [29], Kaprus' & Weiner [30], Jordana et al. [31], Pomorski & Skarżyński [32], Simon Benito & Deharveng [33], Fjellberg [34,35], Pomorski [36], Bretfeld [37], Carapelli et al. [38], Potapov [39], Thibaud et al. [40], Gioia Cipola et al. [41], Skarżyński et al. [42] and Lafooraki et al. [43]. For each site, the number of specimens found for each taxonomic group was counted. In the case of Collembola, the number of recorded species was also provided.

Among the insect material, Acari, Pseudoscorpionida, Myriapoda, and Insecta specimens are stored in the collection of Poznan University of Life Sciences at the Department of Forest Entomology and Pathology, Collembola specimens are stored in the collection of the University of Wrocław at the Department of Invertebrate Biology, Evolution and Conservation, and Araneae are stored in the collection of Adam Mickiewicz University at the Faculty of Biology, Poznań, Poland.

2.3. Statistical Analyses

A cluster analysis [44,45] performed to detect groups of similar sites was run on a Bray-Curtis distances matrix. This method analyzed the Hellinger-transformed the number of specimens using the Manhattan distance matrix and the Ward method. The Hellinger distance is widely used in ecological studies [46]. Cluster analysis is a numerical method that does not consider trends found in community data. Therefore, to compare the species diversity among experimental sites, a principal coordinate analysis (PCoA) [47] based on Bray-Cutris distances was applied. Since the focus of the study was to compare localities in terms of Collembola species, only the ordination method was used to analyze variation throughout arthropod assemblages. Collembola species that preferred a given habitat were identified using multilevel pattern analysis [48]. To describe the Collembola communities, Simpson's diversity index [49], Pielou's evenness index [50] and the dominance index [51] were estimated for each location. To verify whether the number and population size of a species were dependent on the geographical location or altitude of the sampling site, a Mantel test [45] was applied to compare the distance matrix established for geographical coordinates and altitude for the collected samples with the community dissimilarity matrix. All calculations were performed in the R 3.6.1 environment [52] using the vegan [53], indicator species [48], and stats packages.

3. Results

3.1. Diversity of Microarthropod Assemblages in Separate Zelkova abelicea Localities

The class Collembola had by far the highest number of specimens (10,285) in the samples (Table 1). Representatives of 11 orders belonged to the class Insecta, among which the most numerous were Psocoptera (422), Hymenoptera (245) and Thysanoptera (163). Within the class Arachnida, the most numerously represented orders were Acari (2237) and Araneae (212), while the least numerous order was Pseudoscorpiones (20). The highest mean number of specimens per sampling site was reported for Collembola (258), while the lowest number was reported for Lepidoptera (0.05) and Rhaphidioptera (0.03). Representatives of these two orders of insects were detected only sporadically, with a maximum of one specimen per sampling site (Table 1).

Table 1. Total number of specimens in each arthropod group for every sampling site in each of the five mountain ranges. The average number of specimens per tree with the standard error as well as the minimum and maximum are shown.

Systematics/Mountains		**Levka Ori**			**Kedros**	**Psiloritis**	**Dikti**		**Thripti**	
Group of Arthropods/ Localities		**Omalos**	**Niato**	**Impros**	**Gerakari**	**Rouvas**	**Viannou**	**Katharo**	**Thripti**	**Mean ± SE (min, max) Number of Specimens per Tree**
Arachnida	Araneae	109	16	10	15	12	40	2	8	5.3 ± 1.6 (0, 52)
	Pseudoscorpiones	2	0	4	5	0	0	0	9	0.5 ± 0.2 (0, 6)
	Acari	593	44	55	1248	45	61	48	143	55.9 ± 16.9 (0, 427)
Myriapoda		7	0	0	4	0	0	0	0	0.3 ± 0.2 (0, 5)
Collembola		1521	84	951	5325	40	283	355	1726	258.0 ± 76.1 (0, 2279)
Insecta	Coleoptera	2	0	0	22	1	8	1	17	1.3 ± 0.5 (0, 15)
	Dermaptera	0	0	5	0	0	0	0	0	0.1 ± 0.1 (0, 3)
	Diptera	0	0	0	8	0	0	5	1	0.4 ± 0.2 (0, 8)
	Entognatha	12	0	20	25	0	0	3	15	1.9 ± 0.8 (0, 20)
	Hemiptera	4	0	0	4	2	1	1	7	0.5 ± 0.2 (0, 3)
	Heteroptera	0	0	0	3	1	0	1	9	0.4 ± 0.2 (0, 9)
	Hymenoptera	190	0	25	27	0	0	2	1	6.1 ± 3.3 (0, 102)
	Lepidoptera	0	0	0	1	0	0	1	0	0.05 ± 0.03 (0, 1)
	Psocoptera	122	13	18	53	65	37	36	78	10.6 ± 2.1 (0, 51)
	Rhaphidioptera	0	1	0	0	0	0	0	0	0.03 ± 0.03 (0, 1)
	Thysanoptera	13	15	16	20	12	13	54	20	4.1 ± 1.0 (0, 30)
TOTAL		2575	173	1104	6760	178	443	509	2034	345.3 ± 86.8 (0, 2279)
Mean ± SE (min, max) number of specimens per tree		515.0 ± 120.9 (167, 844)	34.6 ± 12.35 (0, 64)	220.8 ± 38.0 (99, 294)	1352.0 ± 402.3 (435, 2501)	35.6 ± 14.7 (0, 64)	88.6 ± 38.1 (0, 189)	107.8 ± 24.3 (47, 183)	408.0 ± 250.4 (29, 1396)	

The variation in the number of arthropod specimens per sampled tree is represented in Figure 3 for every sampling locality. The highest number of specimens was observed at the Gerakari site (per tree average: 1352), where the scatter in the number of specimens in the sampled trees was also the greatest (min: 435, max: 2501). The three sites in the Levka Ori showed strong variations in terms of the numbers of specimens. Indeed, Omalos had the highest number of specimens per sampled tree (average: 515.) and a heterogeneous distribution in the number of specimens per sampled tree (min: 167, max: 844). Impros had an intermediate value (average: 221, min: 99, max: 294), while Niato had the lowest number of specimens (average: 35, min: 0 max: 64). The two sites from Dikti were similar in terms of sample sizes per tree. However, in Katharo, fewer samples predominated (the graph is wider at the bottom), whereas in Viannou, samples with a higher number of specimens predominated. Rouvas was similar to Niato and had small samples. In Thripti, the scatter in the number of specimens in the sampled trees was large because one sample included 1396 specimens while the other four samples had between 29 and 290 specimens. Moreover, certain samples from Niato, Rouvas and Viannou had no microarthropods at all (Table 1).

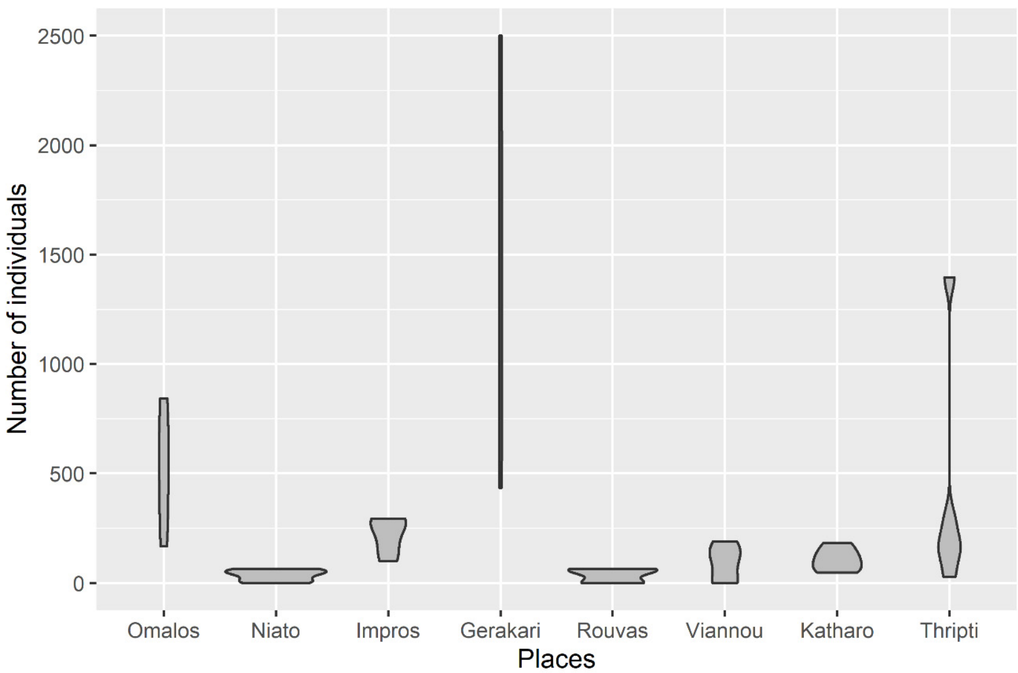

Figure 3. Number of microarthropod specimens per study site. The width of the violin plots is representative of the frequency of data points.

To more accurately illustrate the observed trends in terms of similarity of the microarthropod community, an ordination method based on PCoA distances was applied, with the Hellinger method used to transform data (Figure 4). The centroids and overlapping areas form four groups of sites that are similar in terms of their microarthropod communities: (1) Impros, Niato and Viannou, (2) Thripti and Katharo, (3) Omalos and Rouvas, and (4) Gerakari, which presented a centroid separate from the others (relation to the first axis), although the site had certain commonalities with several other sites. The highest number of specimens by far as well as the highest number of microarthropod groups (14 out of 16) were recorded in Gerakari. Samples from Gerakari were quite close together on the PCoA graph, especially along the first axis, which shows their great similarity. Thripti accounted for a very large area, which is consistent with the results presented in Table 1, with as many as 12 out of 16 microarthropod groups found at this site, although the samples were spaced apart in relation to both axes (they are not very similar). Rouvas accounted for the smallest area and was contained completely within Thripti and Omalos. Thus, all of the microarthropod orders found at Rouvas were also found at the two other sites. At the two sites in the Dikti Mountains, the total number of microarthropod groups was 12 in Katharo and 7 in Viannou. All the orders found in Viannou were also recorded in Katharo. The number of specimens in most microarthropod groups was greater in Viannou than in Katharo except for Collembola and Thysanoptera. For the three sites in the Levka Ori, the lowest number of specimens was recorded in Niato, which also presented the lowest number of microarthropod groups (6), whereas 11 and 8 arthropod groups were found in Omalos and Impros, respectively, and these groups also presented a much higher number of specimens. Samples from Niato and Omalos were spaced apart with regard to the first axis but spaced quite close together with regard to the second axis. Samples from Impros were similar (close to each other) along the first axis and dissimilar (far apart) along the second axis.

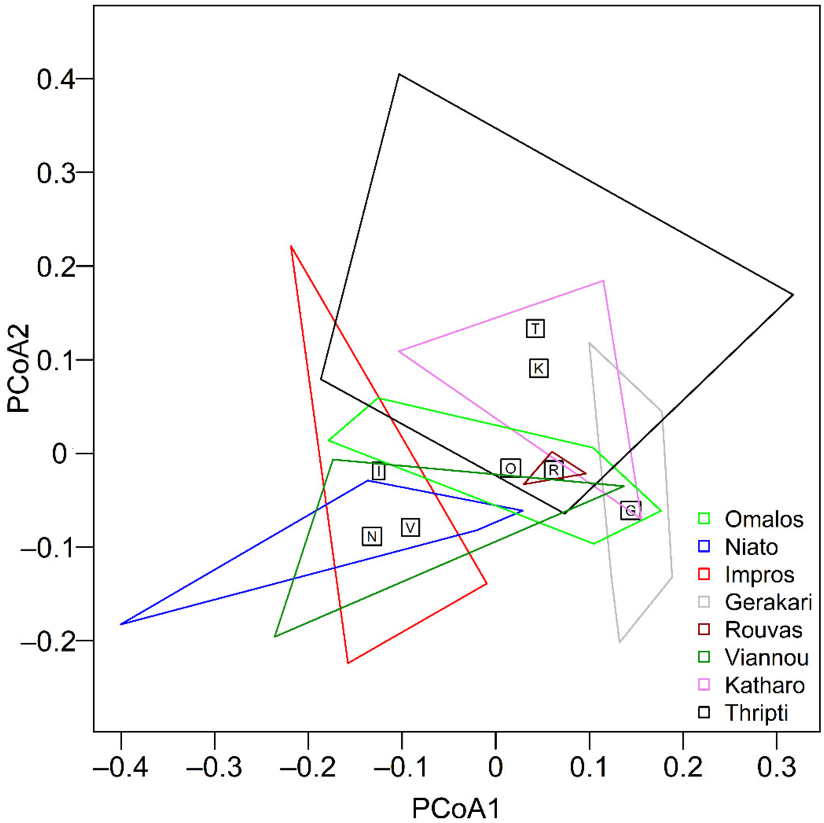

Figure 4. Centroids determined from the PCoA analysis showing the numerical diversity among the samples for each study site (% of total variability: PCoA1—32%, PCoA2—25%). The center of each centroid is indicated by a lettered square representing the study sites (i.e., O: Omalos, N: Niato, I: Impros, G: Gerakari, R: Rouvas, V: Viannou, K: Katharo, T: Thripti).

3.2. Diversity of Collembola Communities on Zelkova abelicea Trees

In the collected material, Collembola was by far the most numerous group of microarthropods. Therefore, this class was analyzed in more detail. A total of 33 species were recorded, among which 19 have not been previously reported from Crete [29,32,43,54–59]. Among these 19 species, 11 are likely new to science and will be the subject of separate taxonomic studies (Table 2). Three species of the genus *Xenylla* were represented in the greatest number. *Xenylla maritima* (3844) was dominant, followed by *Xenylla* sp. 2 (*maritima* complex) (3364) and *Xenylla* sp. 1 (*maritima* complex) (2631) (Table 2). Most species were represented by single specimens. Due to the small population size and the presence of juvenile forms, some specimens were only identified at higher taxonomic units, e.g., Anurophorinae 1 and Anurophorinae 2.

Table 2. List of Collembola species in systematic order with the number of recorded specimens per site. Additionally, the average number of species per tree (±standard error, minimum and maximum), the average number of specimens per tree (±standard error, minimum and maximum), the Simpson index (measure of diversity), the Pielou Index (measure of evenness) and the dominance index are shown. * Species new to the fauna of Crete, ** species likely new to science.

	Mountain		**Levka Ori**		**Kedros**	**Psiloritis**	**Dikti**		**Thripti**
	Site	**Omalos**	**Niato**	**Impros**	**Gerakari**	**Rouvas**	**Viannou**	**Katharo**	**Thripti**
1.	*Hypogastrura* cf. *gisini* **				2				
2.	*Xenylla* sp. 1 (*maritima* complex) **	1436	40	933		25	139		58
3.	*Xenylla* sp. 2 (*maritima* complex) **	38	24	8	3233		6		55
4.	*Xenylla maritima*	1	15		1759		132	354	1583
5.	*Protanura* sp. **				1				
6.	*Deutonura* sp. **				2				
7.	*Endonura* sp. **				1				
8.	*Friesea* cf. *cassagnaui* **				1				
9.	*Friesea* sp. **				4				
10.	*Pseudachorutella* sp. **				2				
11.	*Protaphorura aurantiaca*				4				
12.	*Thalassaphorura franzi* *	2							1
13.	*Metaphorura affinis*				1				
14.	Anurophorinae 1 *			2				1	
15.	Anurophorinae 2 *			1					
16.	*Folsomia ksenemani*				2		1		
17.	*Folsomia quadrioculata* *				36				
18.	*Hemisotoma pontica*				6				
19.	*Isotoma* sp.				2				
20.	*Isotomurus fucicolus* *				3				
21.	*Uzelia* cf. *kuehnelti* **	8	4	7	5	7	1		10
22.	*Vertagopus arboreus* *				6				
23.	*Vertagopus* cf. *persicus* **				72				
24.	*Entomobrya handschini*	25							
25.	*Entomobrya multifasciata*	4	1		77	3			5
26.	*Lepidocyrtus lanuginosus*				1				
27.	*Lepidocyrtus* cf. *lignorum*				3				
28.	*Lepidocyrtus* sp. 1				2				
29.	*Lepidocyrtus* sp. 2				2				
30.	*Orchesella taurica* *	1			96				
31.	*Pseudosinella octopunctata*	6			1				
32.	*Seira ferrarii* *					5	4		14
33.	*Sminthurinus alpinus bisetosus*				1				

Table 2. *Cont.*

Mountain Site	Omalos	Levka Ori Niato	Impros	Kedros Gerakari	Psiloritis Rouvas	Dikti Viannou	Katharo	Thripti Thripti
TOTAL SPECIES	9	5	5	27	4	6	2	7
TOTAL SPECIMENS	1521	84	951	5325	40	283	355	1726
Average number of species	4.00 ± 0.84 (2, 6)	1.80 ± 0.58 (0, 3)	2.00 ± 0.45 (1, 3)	9.60 ± 3.11 (4, 21)	2.00 ± 0.84 (0, 4)	1.4 ± 0.6 (0, 3)	1.20 ± 0.20 (1, 2)	3.60 ± 0.68 (2, 6)
Average number of specimens	304.2 ± 117.4 (24, 661)	16.8 ± 6.04 (0, 33)	190.2 ± 41.38 (57, 273)	1065 ± 392.4 (105, 2279)	8 ± 3.51 (0, 18)	56.6 ± 31.34 (0, 133)	77 ± 24.06 (32, 165)	346.4 ± 256.2 (16, 1359)
Simpson Index	0.18 ± 0.11 (0.01, 0.61)	0.37 ± 0.13 (0.0, 0.53)	0.05 ± 0.02 (0.0, 0.11)	0.47 ± 0.12 (0.07, 0.8)	0.52 ± 0.05 (0.43, 0.57)	0.23 ± 0.21) (0.01, 0.65)	0.0 ± 0.002 (0.0, 0.2)	0.32 ± 0.12 (0.04, 0.77)
Pielou’s Index	0.25 ± 0.13 (0.03, 0.72)	0.82 ± 0.05 (0.75, 0.92)	0.19 ± 0.04 (0.11, 0.23)	0.44 ± 0.09 (0.13, 0.7)	0.77 ± 0.04 (0.69, 0.83)	0.37 ± 0.30 (0.06, 0.98)	0.09 ± NA (0.09, 0.09)	0.48 ± 0.14 (0.11, 0.88)
Dominance Index	0.82 ± 0,11 (0.39, 0.99)	0.63 ± 0,13 (0.47, 1.00))	0.95 ± 0.02 (0.89, 1.00)	0.53 ± 0.12 (0.2, 0.93)	0.48 ± 0.05 (0.43, 0.57)	0.77 ± 0.21 (0.35, 0.99)	1.0 ± 0.004 (0.98, 1.00)	0.68 ± 0.12 (0.23, 0.96)

The highest number of Collembola species (27) and the greatest number of specimens by far (5325) were recorded in Gerakari, followed by Omalos (9 spp., 1521) and Thripti (7 spp., 1726) (Table 2). Katharo had the lowest number of species (2 spp.), while Rouvas had the lowest number of specimens (40). Within the Levka Ori, Omalos stands out as having a higher number of Collembola species compared to the two other sites (9 spp. and 5 spp.). Niato and Impros not only had lower biodiversity than Omalos but also had a lower number of specimens (Table 2, Figure 5). All three aforementioned sites shared three common species, although this species differed significantly in terms of its population size among the sites. The two sites in Dikti had a similar mean number of species per sample but showed differences in the total number of species, with six in Viannou and only two in Katharo. Only one species was shared between these two sites. None of the species was shared among all sampling sites; however, *Uzelia* cf. *kuehnelti* was found at all sites except for Katharo and all three *Xynella* species were found at all sites except two (Table 2). Twenty-two species occurred only once, among which all (except two) were restricted to the trees at Gerakari.

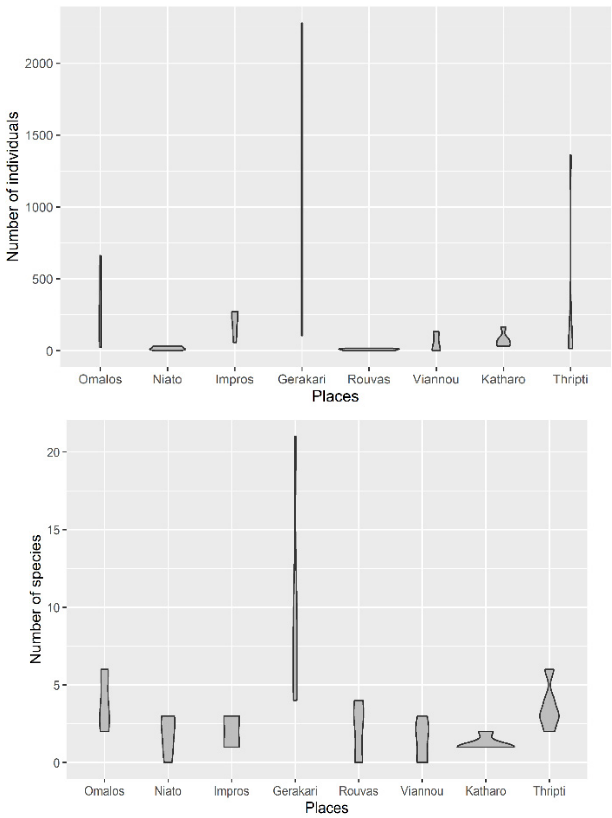

Figure 5. Number of specimens and number of species of Collembola per study site. The width of the violin plots is representative of the frequency of data points.

The cluster analysis of similarities showed that Collembola assemblages in Gerakari differed markedly from all the other assemblages. The sites in Omalos and Impros, which are located in the Levka Ori Mountains, showed considerable similarity, whereas the Niato site, which is geographically very close to Impros, was completely different and positioned closer to Thripti. It may be related to the character of the microhabitat from which the samples were collected. In both Niato and Thripti, twigs were harvested, while bark was harvested from trunks in other sites. In turn, the Collembola assemblages at the two sites located in the Dikti Mountains (Katharo and Viannou) differed slightly (Figure 6).

The PCoA analysis for Collembola showed that Gerakari stands out compared to all other sites due to the presence of many species that are not found elsewhere. In addition, Katharo did not share a common area with any of the other sites because only two species were recorded there, making the site difficult to position. Most of the other sites overlapped. Thripti and Impros were almost fully encompassed in Niato. Impros, Niato and Omalos had a large common area (Figure 7). Moreover, these areas overlapped with those distinguished by samples from Rouvas and Viannou. For these areas, one common indicator species was identified: *Xenylla* sp. 1 (*maritima* complex) (Table 3). Similar to the cluster analysis, samples from Katharo were located closest to the Viannou site, which confirmed their considerable similarity.

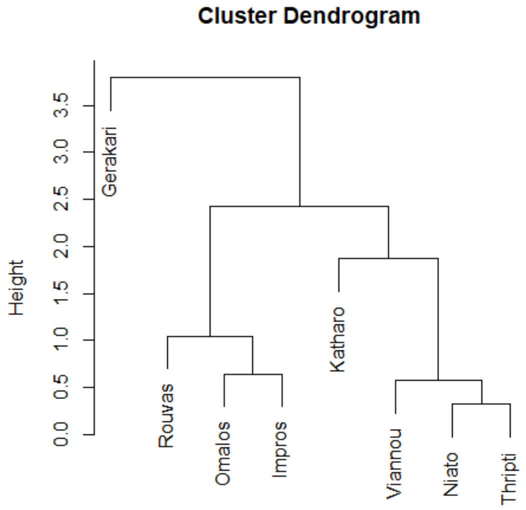

Figure 6. Cluster analysis showing the similarity of localities depending on the Collembola communities.

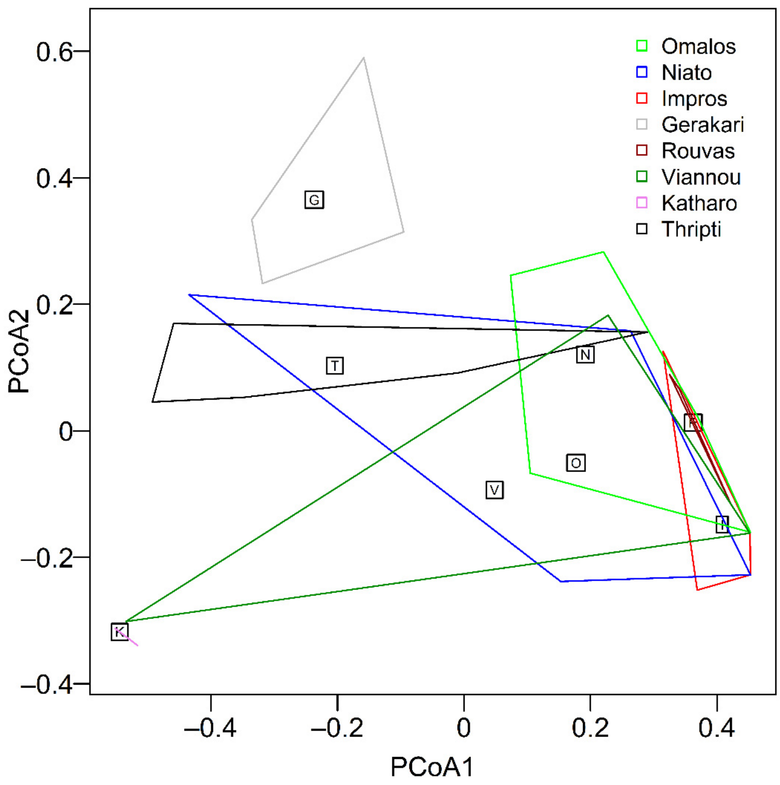

Figure 7. Centroids obtained from the PCoA analysis showing the numerical diversity of Collembola in the study sites (% of total variability: PCoA1—56%, PCoA2—21%). The center of each centroid is indicated by a lettered square representing each study site (i.e., O: Omalos, N: Niato, I: Impros, G: Gerakari, R: Rouvas, V: Viannou, K: Katharo, T: Thripti).

Table 3. Indicator species for localities based on a multilevel pattern analysis.

	Stat	*p* Value
Group Gerakari		
Orchesella taurica Stach, 1960	0.809	0.0020
Lepidocyrtus cf. *lignorum* (Fabricius, 1793)	0.742	0.0120
Vertagopus cf. *persicus* Potapov, Yoosefi & Shayanmehr, 2020	0.717	0.0143
Group Gerakari + Rouvas		
Entomobrya multifasciata (Tullberg, 1871)	0.632	0.0150
Group Katharo + Thripti		
Xenylla maritima Tullberg, 1869	0.682	0.0037
Group Gerakari + Niato + Thripti		
Xenylla sp. 2 (*maritima* complex)	0.621	0.0160
Group Impros + Niato + Omalos + Rouvas + Viannou		
Xenylla sp. 1 (*maritima* complex)	0.743	0.0003

The multilevel pattern analysis identified the indicator species shared by the highest number of sampling sites. These indicator species are the most numerously represented species found in many localities and in many samples (Table 3).

A Mantel test was conducted to compare the distance matrix established for the geographical coordinates and altitude where the samples were collected with the community dissimilarity matrix. The test ($r = 0.2329$, $p = 0.116$) showed no dependence between the community dissimilarity matrix and distances between locations matrix (calculated from the longitude and latitude as well as the elevation of a given site). Thus, the occurrence of Collembola species was not found to be dependent on the location or altitude of a given site.

4. Discussion

Arthropods are known to contribute to a very important fraction of global biodiversity [60]. However, limited research has focused on this group of organisms. Moreover, even fewer studies have investigated microarthropod communities, especially Collembola communities living on trees or associated with tree microhabitats in the Mediterranean, let alone Crete [61,62]. Our study provides a small but significant addition to this field of study, as corroborated by the 19 recorded Collembola species that had never been reported for Crete, which included 11 species, i.e., almost one-third of the recorded species) that are likely new to science.

Several studies have shown that the presence, range or continuity and connection of some species, groups or even populations of organisms distributed on Crete are highly influenced by the strong topographical and/or geological history and structures present on the island [24,63–70]. The character of arthropod assemblages is also influenced by natural conditions, e.g., the host plant, which creates specific microhabitats [71]. The influence of such factors was also observed in our study, at least for Collembola, with each *Z. abelicea* locality presenting a specific assemblage. Indeed, longitudinal, latitudinal or altitudinal trends were not observed for the Collembola species, which suggests that the distribution and diversity of Collembola assemblages growing on *Z. abelicea* trees are not influenced by macroenvironmental conditions (e.g., precipitation, temperature, and drought) or by between-site epiphytic lichen and bryophyte community differences. In fact, the diversity and distribution of epiphytic lichens and bryophytes growing on *Z. abelicea* were found to differ along a longitudinal gradient, which was likely related to differences in regional climatic patterns [72–74].

In addition, the cluster analysis (Figure 6) revealed that sites situated within the same mountain range showed greater similarity than sites situated in other mountain ranges. A striking exception to this statement was observed for Niato, which was dissimilar to all Levka Ori sites despite being geographically very close to Impros but similar to the easternmost site Thripti. A possible explanation is that both Niato and Thripti were the only sites where samples were collected from dwarfed *Z. abelicea* communities and the only sites situated in flat dolines, whereas samples from all other sites were gathered from arborescent trees situated on sloped areas. Therefore, we can conclude that at least in the case of arborescent trees or trees situated on slopes, the absence of a continuous *Z. abelicea* population between mountains associated with the complex topography of Crete seems to be a determining factor for Collembola assemblages. Further and more in-depth analyses should be undertaken to investigate this matter.

Based on the PCoA and cluster analysis, the most different character of assemblages was recorded at the Gerakari site on Mt. Kedros, where the highest number of species (27) and specimens of Collembola (5325) were recorded. The multilevel pattern analysis identified the indicator species for this site, including the xeroresistant species *Orchesella taurica*, which lives in forests and open sites in SE Europe [26,75,76]; the eurytopic species *Lepidocyrtus* cf. *lignorum*, which is widely distributed in the Holarctic [35]; and the species *Vertagopus* cf. *persicus*, which is likely new to science. Indeed, the Gerakari site on Mt. Kedros proved to be the richest in terms of the number of specimens collected as well as the number of arthropod groups and Collembola species found, and it showed striking differences in its arthropod population compared to all other sites. Indeed, from the 27 Collembola species recorded at Gerakari, twenty were found nowhere else, which indicates the uniqueness of the site and its dissimilarity with the other sampling sites. These results can be assessed based on the study by Fazan et al. [77], who found a richer diversity of epiphytic bryophytes on *Z. abelicea* growing at the same study site on Mt. Kedros. Although the reasons behind these differences exceed the scope of the present article, some hypotheses can be proposed. Indeed, arthropod communities, including Collembola, are known to be very sensitive to a multitude of different factors, including microhabitat conditions [78–80], vegetation type and plant richness [81–83], landscape heterogeneity [84] and land-use practices, such as grazing [84–92], which are known to impact arthropod communities. All of these factors, and many more, could explain to some extent the variations found between study sites in our experiment.

Based on the analyses, such as the PCoA, we can state that the most stable assemblage occurred at the Omalos site, which presented large, monumental trees with trunks and branches that were abundantly covered with lichens and bryophytes. At the other sites, the trees were smaller and occasionally grew as low shrubs that were nibbled upon by goats. This obviously determined the richness of the microhabitats and, as a consequence, the species richness of microarthropods and the different characteristics of the assemblage. Moreover, the three other xeroresistant species should be highlighted: *Entomobrya multifasciata*, which was characteristic of the Gerakari and Rouvas sites and is common in forests and open sites in Palearctic [35,76]; *Xenylla maritima*, which distinguished the Katharo and Thripti sites and has been recorded from mosses, lichens and bark in Europe and New Zealand [42]; and finally, *Xenylla* sp. 1 (*maritima* complex), which distinguished Impros, Niato, Omalos, Rouvas and Viannou sites and is probably new to science.

The discovery of nineteen species of Collembola new to the fauna of Crete, including eleven that are likely new to science, showed how much new information can be provided by the study of specific microhabitats, which was the tree *Z. abelicea* in our study case. The research conducted thus far in Crete, although extensive, has focused mainly on soil and litter assemblages [29,55–58]. The results of the current taxonomic research on the abovementioned species that are likely new to science will be published in separate works. These species may provide a basis for a deeper consideration of the specificity of the Collembola assemblages associated with *Z. abelicea*.

5. Conclusions

At each site where *Z. abelicea* trees were found, a different characteristic of microarthropod assemblages was recorded. Among the collected material, the most numerous groups of organisms were Collembola (10,285), Acari (2237) and representatives of Psocoptera (422). The analyses focused on Collembola showed that the site at Gerakari (Mt. Kedros) stood out in terms of the species assemblage. Moreover, relatively rare species, as well as eleven species likely new to science, were recorded, highlighting the specific character and value of Collembola assemblages that colonize endemic *Z. abelicea* trees. The observation of 19 previously unrecorded species on Crete species and 11 species likely new to science and the results of these pilot studies justify the need for further research on the microarthropods colonizing this unique endemic tree.

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