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RESEARCH ARTICLE

Multivariate analysis of the effects of site factors on the distributions of raphignathoid mites (Acari: Raphignathoidea)

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ASBTRACT: This study was carried out to examine the relationships between the distributions of raphignathoid mite (Acari: Raphignathoidea) species and their temporal and environmental site factors (i.e. season, habitat type, and altitude) in Pülümür Valley and its immediate environs, Turkey. Data was collected from 306 samples from the various locations in the study area. 70 raphignathoid mite species were identified. The most common raphignathoid species, genus, and family are *Neognathus terrestris*, *Eustigmaeus,* and Stigmaeidae, respectively. It has been also found that the greatest number of raphignathoid mite specimens has been encountered in autumn, followed by spring, summer, and winter, respectively.

Before applying the statistical analysis, rare species (i.e., the species having less than 5% frequency value) were omitted from the data matrix. Thus, the first (original) matrix size was reduced from 70×306 to 20×242. Elevation is a continuous variable whereas seasons and habitat types are nominal data. Therefore, each of the seasons (winter, spring, summer, and autumn), and each of habitat types [i.e., litter, soil, soil and organic components, mixed material consisting of moss and liken, and the others (manure and ant nest)] were inserted as binary data [present (1), absent (0)] in the data matrix.

Canonical Correspondence Analysis (CANOCA) was applied to define the species-site relationships. Findings indicate that *Caligonella haddadi*, *Neognathus terrestris*, *Cryptognathus lagena*, *Raphignathus gracilis,* and *Stigmaeus devlethanensis* are significantly associated with moss and lichen habitat whereas litter, grassy and mossy soil are more suitable habitat types for *Favognathus amygdalus*, *F. cucurbita*, *Raphignathus kuznetzovi*, *Eustigmaeus dogani*, *E. pinnatus*, *E. segnis*, *Ledermuelleriopsis aminiae*, *L. ayyildizi,* and *Storchia robusta*. In addition to this, three species *Favognathus cucurbita*, *Eustigmaeus segnis,* and *Ledermuelleriopsis plumosus* are positively and six species *Caligonella humilis*, *Neognathus terrestris*, *Favognathus amygdalus*, *Raphignathus gracilis*, *Raphignathus kuznetzovi* and *Ledermuelleriopsis aminiae* are negatively related to elevation.

Keywords: Mite, multivariate methods, ordination techniques, habitat, environmental factors.
Zoobank: https://zoobank.org/2ECC595A-6AB1-40FC-BD21-EB47B207EB57

INTRODUCTION

The mites classified in the subclass Acari are members of the small arthropods. Raphignathoidea Kramer is a superfamily belonging to the mite suborder Prostigmata in the order Trombidiformes. This group currently consists of 1087 species in 69 genera within 12 families. Presently, this superfamily is represented in Turkey by 217 species in 26 genera of 8 families (Doğan, 2019; Beron, 2020).

The superfamily Raphignathoidea contains many free-living predators, but a few are herbivores feeding on moss and pollen, and others parasites of insects (Fan and Zhang, 2005; Beron, 2020). Some of the predatory forms are suitable biological control agents of spider mites (Tetranychidae), eriophyid mites (Eriophyidae), and scale insects (Coccoidea) in agriculture and forestry (Fan and Zhang, 2005; Beron, 2020). Like other organisms, raphignathoid mites have important relationships with their environment, but these relationships are not yet known due to a lack of research. Although there are many taxonomic and faunistic studies on raphignathoid mites, the researches on ecology and distribution of the mites are limited (Doğan, 2019). Only Somuncu and Koç (2012), Akyol and Koç (2016), Koç and Poyraz Tınartaş (2017) have surveyed seasonal distributions of raphignathoid mites.

Multivariate analysis is concerned with the simultaneous statistical analysis of multiple variables. Those methods can be divided into two general groups as classification (clustering) methods and ordination techniques. The goal of classification methods is to establish a set of meaningful groups of similar objects by investigating relationships between objects. Ordination techniques developed on the basis of Gradient analysis consist of two types, direct type and indirect type (Whittaker, 1962). In the indirect type, changes in living organisms are studied apart from environmental factors while environmental factors are investigated only in the data interpretation step. In direct type, changes in species are studied directly through study environmental factors (Khansari et al., 2016). One of the most commonly used direct methods of species ordination is Canonical Correspondence Analysis (CANOCA) (Özkan et al., 2009). Thus, in this present study, we used CANOCA for exploring the between the distributions of raphignathoid mites and their temporal and environmental site factors.

MATERIALS AND METHODS

Site description

The study area (39°35'26.0"N 39°52'19.5"E – 39°06'04.4"N 39°33'18.7"E) is on the route of the road connecting the province of Tunceli to Erzincan, and also covers the Pülümür Valley which is situated among mountains with an altitude of 3,000 meters. With its rich biodiversity and untouched nature, it exhibits a different beauty in every season of the year (Işık, 2012; Köksal and Ulaşoğlu, 2012; Babacan et al., 2017). The study area has a continental climate with hot, dry summers and cold, snowy winters. Majority of the area covers by travertine deposits, coarse clastic deposits, and gypsiferous clastic deposits. Basaltic extrusive rocks, diabase and the intrusive igneous rocks are also present (Afshar, 1965). It is the intersection point of the Euro-Siberian, Iran-Turanian, and Mediterranean plant geographies. Oak forests commonly cover in the study area and its surroundings, and there are sparse plants in the area such as Scots Pine, juniper, rosehip, hawthorn, elm, willow, and tamarisk (Babacan et al., 2017; Armağan, 2020).

Field survey and extraction of mites

Sampling studies were carried out after obtaining legal permissions from the General Directorate of Agricultural Research and Policies (50411936-604.02-E.2200901) and the General Directorate of Nature Conservation and National Parks (72784983-488.04-44455), two units of TR Ministry of Agriculture and Forestry. The samplings were performed monthly in the research area between October 2018 and September 2019. Totally 306 samplings were made in the area and a Global Position System (GPS) was used for taking coordinates and altitudes of the sampling sites. All collected samples were carried to the laboratory in a plastic bag. Mite specimens were extracted from the samples collected for 7 days with the aid of Berlese-Tullgren funnels. Raphignathoid mites were picked using a micropipette under a Leica EZ4 stereo microscope, then cleared in 60% lactic acid and mounted on microscopic slides in Hoyer's medium. The specimens were examined and counted by using a Leica DM 4000B phase-contrast microscope. It was then identified to species level using the published paper (i.e., Summers and Schlinger, 1955; Summers, 1962; Summers and Chaudhri, 1965; Luxton, 1973; Wood, 1973; Kuznetsov, 1978; Meyer and Ueckermann, 1989; Fan, 2000, 2004; Fan et al., 2003a,b, 2016, 2019; Fan and Zhang, 2004, 2005; Doğan, 2008).

Data set

In total 70 mite species were determined after field survey and laboratory works (Table 1). The frequency and the abundance values of the species, genus, and families are given in Figure 1.

Many of the species have low frequency values. Rare species (i.e., the species less than 5% frequency value throughout the data set) were removed to reduce bias in the analysis. The remaining 20 mite species were taken for the analysis. Thus, the first (original) matrix size was reduced from 70×306 to 20×242. In the study, the used explanatory variables are elevation, seasons, and habitat types.

Elevation is a continuous variable whereas the others are nominal variables. Therefore, each season and each habitat type were inserted as binary data [present (1), absent (0)] in the data matrix. Site factors and species were coded and given in Tables 1-2.

Table 1. The species list of the study area and their codes.

Species	Family	Codes
Barbutia anguineus (Berlese)	Barbutiidae	S1
Caligonella haddadi Bagheri & Maleki	Caligonellidae	S2
Caligonella humilis (Koch)		S3
Molothrognathus bahariensis Khanjani & Ueckermann		S4
Molothrognathus crusis Summers & Schlinger		S5
Molothrognathus kamili Doğan		S6
Molothrognathus phytocolus Meyer & Ueckermann		S7
Molothrognathus terrulentus Meyer & Ueckermann		S8
Neognathus eupalopus Meyer & Ueckermann		S9
Neognathus pusillus Doğan & Doğan		S10
Neognathus spectabilis (Summers & Schlinger)		S11
Neognathus terrestris (Summers & Schlinger)		S12
Neognathus ueckermanni Bagheri, Doğan & Haddad		S13
Cryptognathus ayyildizi Akyol & Koç	Cryptognathidae	S14
Cryptognathus lagena Kramer		S15
Cryptognathus summersi Robaux		S16
Favognathus amygdalus Doğan & Ayyıldız		S17
Favognathus bafranus Doğan		S18

Table 1 (continued).

Favognathus cucurbita (Berlese)	Cryptognathidae	S19
Favognathus dakotaensis (McDaniel & Bolen)		S20
Favognathus kamili Dönel & Doğan		S21
Favognathus rosulatus Doğan & Doğan		S22
Saniosulus deliquus Doğan, Bingül & Doğan	Eupalopsellidae	S23
Raphignathus collegiatus Atyeo, Baker & Crossley	Raphignathidae	S24
Raphignathus gracilis (Rack)		S25
Raphignathus hecmatanaensis Khanjani & Ueckermann		S26
Raphignathus kuznetzovi Doğan & Ayyıldız		S27
Raphignathus ueckermanni Koç & Kara		S28
Raphignathus zhaoi Hu, Jing & Liang		S29
Cheylostigmaeus tarae Khanjani	Stigmaeidae	S30
Cheylostigmaeus urhani Dönel & Doğan		S31
Cheylostigmaeus n. sp.		S32
Eustigmaeus anauniensis (Canestrini)		S33
Eustigmaeus capitatus Stathakis, Kapaxidi ve Papadoulis		S34
Eustigmaeus collarti (Cooreman)		S35
Eustigmaeus dogani Khanjani, Fayaz, Mirmoayedi & Ghaedi		S36
Eustigmaeus erzincanensis Doğan		S37
Eustigmaeus jiangxiensis Hu, Chen & Huang		S38
Eustigmaeus nahidae Gheblealivand & Bagheri		S39
Eustigmaeus pinnatus (Kuznetsov)		S40
Eustigmaeus rhodomela (Koch)		S41
Eustigmaeus sculptus Doğan, Ayyıldız & Fan		S42
Eustigmaeus segnis (Koch)		S43
Eustigmaeus setiferus Bagheri, Saber, Ueckermann, Ghorbani & Bonab		S44
Eustigmaeus turcicus Doğan & Ayyıldız		S45
Ledermuelleriopsis aminiae Nazari & Khanjani		S46
Ledermuelleriopsis ayyildizi Doğan		S47
Ledermuelleriopsis plumosus Willmann		S48
Ledermuelleriopsis toleratus Kuznetsov		S49
Mediolata aegyptiaca (Zaher & Soliman)		S50
Prostigmaeus amplius Doğan, Doğan & Bingül Türk		S51
Stigmaeus bifurcus Bingül, Doğan & Dilkaraoğlu		S52
Stigmaeus creber Barilo		S53
Stigmaeus devlethanensis Akyol & Koç		S54
Stigmaeus erzincanus Doğan, Bingül, Dilkaraoğlu & Fan		S55
Stigmaeus fidelis Kuznetsov		S56
Stigmaeus furcatus Dönel & Doğan		S57
Stigmaeus glabrisetus Summers		S58
Stigmaeus livschitzi Kuznetsov		S59
Stigmaeus longipilis (Canestrini)		S60
Stigmaeus mitrofanovi Khaustov		S61
Stigmaeus pilatus Kuznetsov		S62
Stigmaeus pulumurensis Doğan & Doğan		S63
Stigmaeus siculus (Berlese)		S64
Stigmaeus tolstikovi Khaustov		S65
Storchia ardabiliensis Safasadati, Khanjani, Razmjou & Doğan		S66
Storchia hendersonae Fan & Zhang		S67
Storchia robusta (Berlese)		S68
Villersia sudetica Willmann		S69
Zetzellia mali (Ewing)		S70

Data analysis

Multivariate methods have been widely used for community data to detect the community pattern and explore the species-site relationships (Ter Braak, 1987; Martin and Bouchard, 1993; Jeglum and He, 1995; Pinto et al., 2006; Fontaine et al., 2007). The effects of environmental characteristics on community patterns are directly quantified by CANOCA. In this way, sampling sites and community members are directly ordinated under the constraint of the site or environmental variables (Legendre and Legendre, 1998; Makerenkov and Legendre, 2002).

In the present paper, canonical correspondence analysis based on linear regression (CANOCA) was applied (Legendre and Legendre, 1998). Site scores obtained from CANOCA were related to the site factors and the species using Pearson correlation and Spearman rank correlation. All the analyses were conducted using Paleontological Statistics (PAST) software version 1.89 (Hammer et al., 2001).

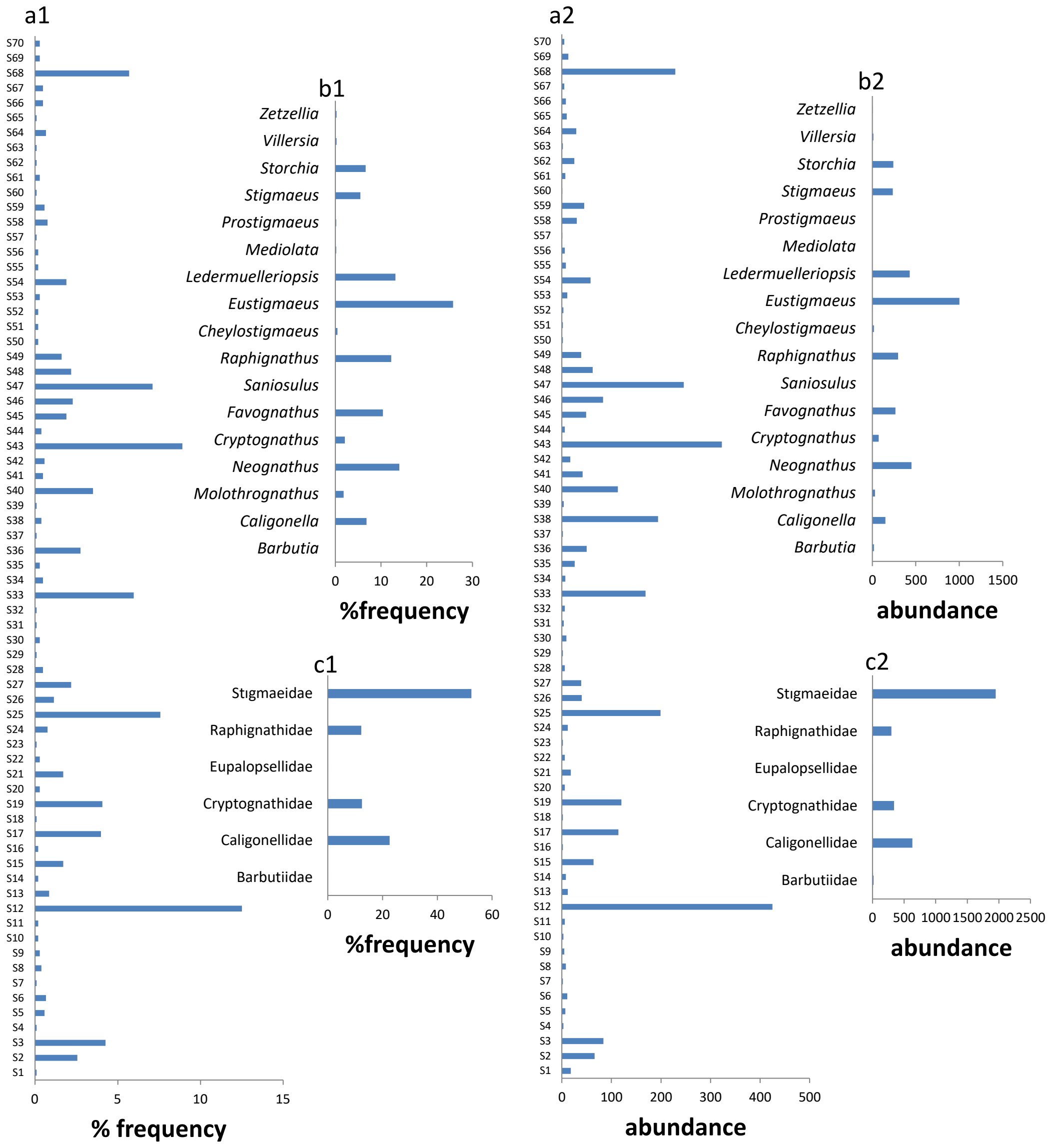

Figure 1. The frequency (1) and abundance (2) values of the species (a), genus (b), and families (c).

Table 2. The codes of site factors.

Habitat types	**Codes**
Litter	HT1
Soil	HT2
Litter, grassy and mossy soil	HT3
Moss and lichen	HT4
Others (manure, ant nest)	HT5
Seasons	**Codes**
Winter	WTN
Spring	SPR
Summer	SMR
Autumn	ATM
Elevation (meter)	**ELEV**

RESULTS

In total 3241 raphignathoid mite specimens within 70 species belonging to six families were determined (Fig. 1). The frequency value of the rarest species (i.e., S1, S4, S7, S18, S23, S29--31, S37, S39, S57, S60, S62-63, and S65) corresponds to a value of 0.09%. The most common species are S12 (12.50%), S43 (8.90%), S25 (7.58%) and S47 (7.10%). In addition to, the rarest genera are *Barbutia* Oudemans (0.09%), *Saniosulus* Summers (0.09%), *Mediolata* Canestrini (0.18%), and *Prostigmaeus* Kuznetzov (0.18%), and the rarest families are Barbutiidae Robaux (0.09%) and Eupalopsellidae Willmann (0.09%). The most common genus and family are *Eustigmaeus* Berlese (25.75%) and Stigmaeidae Oudemans (52.46%), respectively (Fig. 1). Looking at the abundance values in Figure 2, the species S12, S33, S43, S47, and S68 are mostly found in the habitat HT3. The most abundant species in HT3 is S43 with 233 individuals. S43 is also the most abundant species in HT1 with 41 individuals. Similarly, S12 is also the most abundant species in HT4 with 184 individuals. HT2 and HTP5 are the least preferred habitats, with the highest number of individuals not exceeding 17 individuals (S46) in HT2 and 10 individuals (S68) in HT5 (Fig. 2). According to the abundance values (Fig. 2), the species S12, S43, and S47 are mostly found in the season ATM. The most abundant species in ATM is S12 with 175 individuals. S12 and S43 are also the most abundant species in the season SPR with 108 and 125 individuals, respectively. Also, S12 is the most abundant species in the season WNT with 93 individuals. S43 is also the most abundant species in the season SMR with 67 individuals (Fig. 2).

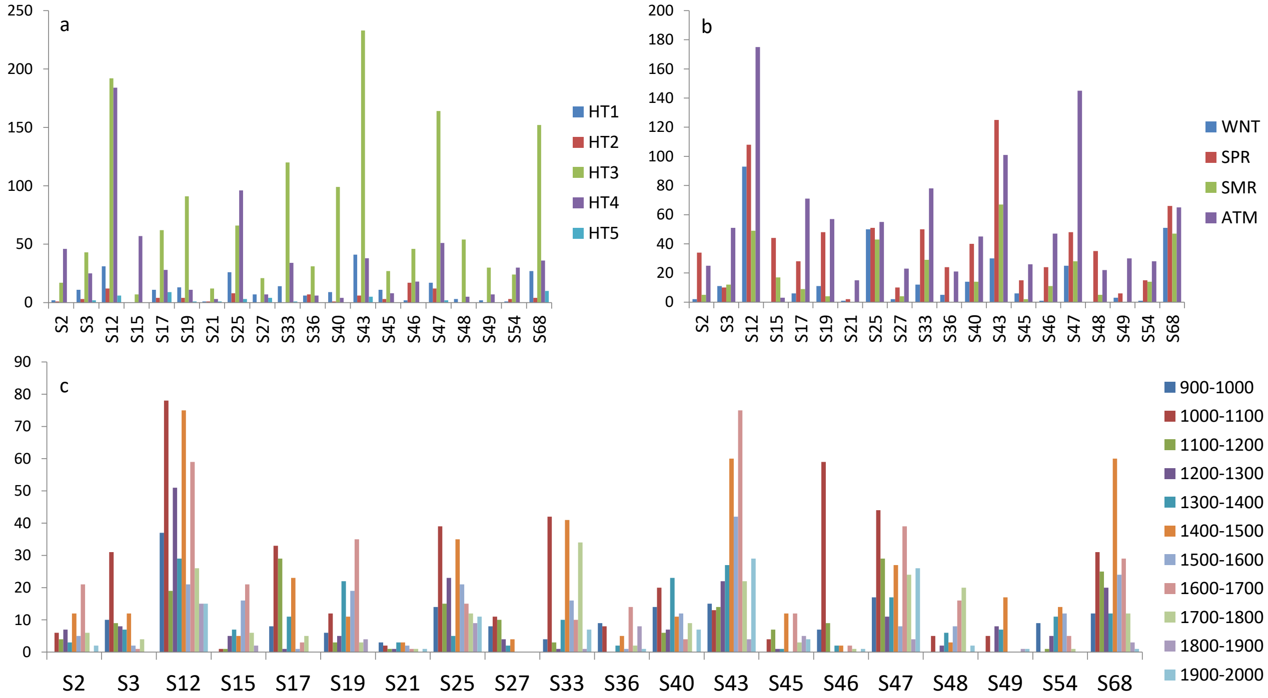

Figure 2. Total abundance values of the species according to habitat types (a), seasons (b) and elevation belts (c).

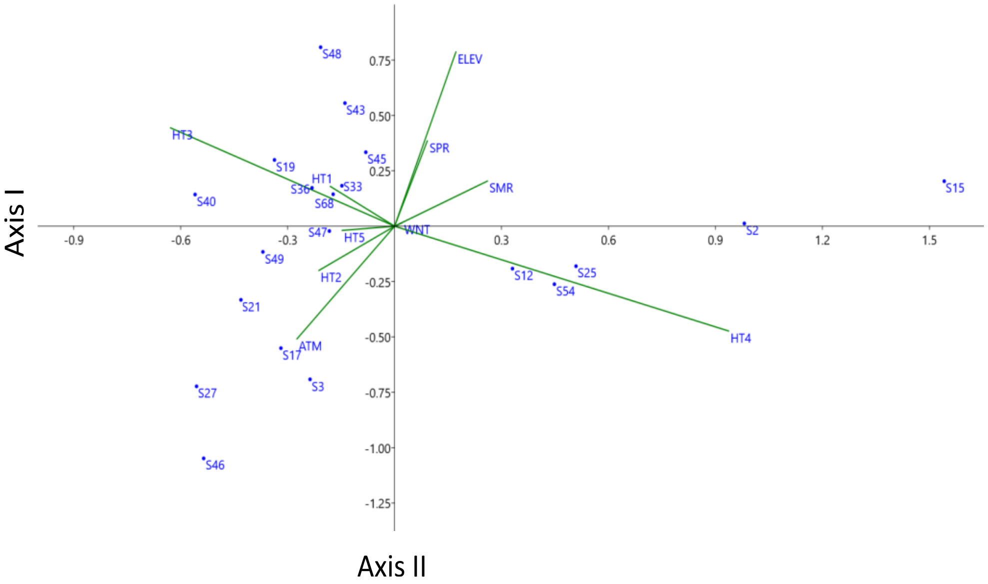

Figure 3. CANOCA results.

The p-values (calculated probabilities) of the first five axes are less than 0.01 after 999 permutations. Eigenvalues of those axes are 0.1826, 0.1523, 0.07099, 0.04716 and 0.03631 respectively. The cumulative percent eigenvalue of the first two axes is 62.97%.

A two-dimensional CANOCA ordinate of the site factors and the species is shown in Figure 3. The correlation results of the site factors and the species with CANOCA axes are given in Tables 3-4. HT3 and HT4 have very strong correlations with CANOCA axes, in particular, with Axis 1 at the level of 0.001.The relationship of SMR with Axis 1 is statistically significant, but this relationship is considerably weaker than the relationship between HT3 and HT4 with the first axis. We, therefore, interpreted Axis 1 as habitat type. The second axis can be interpreted as climatic tolerance because elevation has the greatest correlation coefficient of Axis 2. Besides, ATM and SPR are strongly associated with the Axis 2 at the level of 0.001 and 0.01, respectively (Table 3). The species being positively correlations with Axis 1 are S2, S12, S15, S25, and S54. From the remanding species, negatively correlated species with this Axis are S17, S19, S27, S36, S40, S43, S46, S47, and S68. As can be also seen from the ordination diagram of CANOCA (Fig. 3), it is clear that habitat preferences of S2, S12, S15, S25, and S54 correspond to HT4 whereas HT3 is more suitable habitat types for S17, S19, S27, S36, S40, S43, S46, S47, and S68. Three species S19, S43, and S48 are positively and six species S3, S12, S17, S25, S27, and S46 are negatively associated with Axis 2 at the varied significant levels less than 0.05 (Fig. 1 and Table 4). According to these results and total abundance values of the species shown in Fig. 2c, it can be said that S3, S12, S17, S25, S27, and S46 survive in the warmer sites, especially between 1000 and 1100 meters of the study area. On the contrary, S19, S43, and S48 are likely to resist to cold climatic conditions and refrain from the warmer sites because those species are more abundant between 1600 and 1700 meters.

Table 3. Spearman rank correlation coefficients among nominal site variables and CANOCA axes scores and, Pearson correlation coefficient of elevation with CANOCA axes scores.

	Axis 1		Axis 2	
	c	p	c	p
WNT	0.087	0.179	-0.005	0.944
SPR	-0.059	0.361	0.187	0.003
SMR	0.136*	0.034	0.107	0.096
ATM	-0.086	0.182	-0.246	0.000
HT1	-0.062	0.336	0.089	0.166
HT2	-0.109	0.091	-0.054	0.402
HT3	-0.263	0.000	0.237	0.000
HT4	0.396	0.000	-0.275	0.000
HT5	-0.068	0.294	-0.011	0.866
ELEV	0.086	0.183	0.393	0.000

Table 4. Pearson correlation coefficients between the species and CANOCA axes.

	Axis 1		Axis 2	
	c	p	c	p
S2	0.288	0.000	-0,022	0,736
S3	-0.059	0.360	-0.345	0.000
S12	0.226	0.000	-0.252	0.000
S15	0.625	0.000	0.051	0.429
S17	-0.130	0.043	-0.225	0.000
S19	-0.148	0.022	0.141	0.028
S21	-0.108	0.093	-0.078	0.228
S25	0.206	0.001	-0.195	0.002
S27	-0.127	0.049	-0.173	0.007
S33	-0.121	0.060	0.114	0.077
S36	-0.151	0.019	-0.031	0.635
S40	-0.273	0.000	0.079	0.221
S43	-0.174	0.007	0.503	0.000
S45	0.016	0.802	0.105	0.104
S46	-0.168	0.009	-0.391	0.000
S47	-0.152	0.018	-0.074	0.251
S48	-0.080	0.218	0.165	0.010
S49	-0.107	0.096	-0.042	0.512
S54	0.183	0.004	-0.054	0.404
S68	-0.152	0.018	0.052	0.421

DISCUSSION

According to the frequency values given in Figure 1, the most common raphignathoid species, genus, and family are *Neognathus terrestris*, *Eustigmaeus,* and Stigmaeidae, respectively, in the research area. These results are not surprising. Although Caligonellidae is a small group of raphignathoid mites, *N. terrestris* which is a member of this family, is very common. It is also known that Stigmaeidae is the most abundant and most diverse family in Raphignathoidea, and that *Eustigmaeus* is one of the most common genera in this family (Fan et al., 2016). Seasonal distributions of some mites have been investigated in different countries by several researchers (i.e., Stamou and Sgardelis, 1989; Lee et al., 1993; Gergócs et al., 2011; Önen and Koç, 2011; Wehner et al., 2018). In this context, two studies generated in Turkey have been received attention. In the first study performed by Somuncu and Koç (2012) in Seferihisar, İzmir, 598 individuals have been detected in the sampling area, and it has been revealed that the greatest number (%44.6) of raphignathoid mites were collected in spring, following by winter (%35.7), autumn (%16.2) and summer (%3.3), respectively (Somuncu and Koç, 2012). The second study was conducted by Koç and Poyraz Tınartaş (2017) in Gölmarmara, Manisa province. In that study, it has been determined that the greatest number of raphignathoid mite specimens has been encountered in autumn (%49), followed by summer (19%), winter (17%), and spring (15%), respectively. In our study, 324 (12.7%), 360 (14.1%), 783 (30.7%), and 1083 (42.5%) individuals of the raphignathoid mites were collected from the season winter, summer, spring, and autumn, respectively and, the highest numbers of individuals are found in autumn and spring. The seasonal distribution of the mite individuals in our study is in agreement with those of previous works. The variability in other seasons may have been originated from regional differences and/or temporal differences of sampling.

Members of the superfamily Raphignathoidea are collected generally from edaphic habitats, especially moss- and grass-covered substrates, litters, barks, and lichens (Fan and Zhang, 2005). According to our findings obtained from CANOCA, moss, and lichen are suitable habitats for *Caligonella haddadi*, *Neognathus terrestris*, *Cryptognathus lagena*, *Raphignathus gracilis,* and *Stigmaeus devlethanensis*; however, litter, grassy and mossy soil are more suitable for *Favognathus amygdalus*, *F. cucurbita*, *Raphignathus kuznetzovi*, *Eustigmaeus dogani*, *E. pinnatus*, *E. segnis*, *Ledermuelleriopsis aminiae*, *L. ayyildizi,* and *Storchia robusta*. Although habitat preferences of some mites have been known (i.e., Barendse et al., 2002; Salmane and Brumelis, 2010; Wehner et al., 2016; Manu et al., 2018), as far as we know, those of raphignathoid mites has never been investigated statistically in detail until now.

The other significant findings obtained from CANOCA indicate that *Caligonella humilis*, *Favognathus amygdalus*, *F. kamili*, *R. kuznetzovi,* and *Ledermuelleriopsis aminiae* prefer lower altitudes corresponding to the warmer sites of the study area. On the other hand, *Eustigmaeus segnis, E. turcicus* and *Ledermuelleriopsis plumosus* are likely to resist to cold climatic conditions since they can present relatively high altitudes.

Authors' contributions

Mervenur Ceylan: Creating datasets, data curation, formal analysis. **Salih Doğan:** Supervision, project administration, resources investigation, methodology, writing - review & editing. **Kürşad Özkan:** Conceptualization, visualization, verification, data curation, methodology, formal analysis, writing - original draft, writing - review & editing.

Statement of ethics approval

Not applicable.

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Conflict of interest

We declare that there is no conflict of interest.

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