

Article

A Primer on Spider Assemblages in Levantine Caves: The Neglected Subterranean Habitats of the Levant—A Biodiversity Mine

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Abstract: Caves share unique conditions that have led to convergent adaptations of cave-dwelling animals. In addition, local factors act as filters on regional species-pools to shape the assemblage composition of local caves. Surveys of 35 Levantine caves, distributed along a climate gradient from the mesic in the north of Israel to hyper-arid areas in the south of Israel, were conducted to test the effect of cave characteristics, location, climate, bat presence, and guano level on the spider assemblage. We found 62 spider species and assigned four species as troglobites, 28 as troglaphiles, and 30 as accidentals. Precipitation, elevation, latitude, minimum temperature, and guano levels significantly affected the composition of cave-dwelling spider assemblages. Caves situated in the Mediterranean region had higher species richness and abundance, as well as more troglobite and troglaphile arachnids. These discoveries contribute to the knowledge of the local arachnofauna and are important for the conservation of cave ecosystems. By comparing spider assemblages of Levantine caves to European caves, we identified gaps in the taxonomic research, focusing our efforts on spider families that may have additional cryptic or yet to be described cave-dwelling spider species. Our faunistic surveys are crucial stages for understanding the evolutionary and ecological mechanisms of arachnid speciation in Levantine caves.

Keywords: accidental cave visitors; Arachnida; Araneae; arid; hypogean; levant; Mediterranean; species diversity; troglobite; troglaphile



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1. Introduction

Different caves, similar patterns. Subterranean habitats (caves and other hypogean habitats) around the world can be found in different climates, rock formations, and biogeographical regions. Additionally, they can be formed by various means such as volcanic, glacial, mechanical, and erosion/solution processes. However, the majority of these subterranean habitats share unique abiotic conditions such as a limitation of light, stable and narrow range of temperature, and high relative humidity [1–3]. The peculiar abiotic conditions found in subterranean habitats, together with specific regional and local factors, determine species richness and assemblage composition of a particular cave. Processes at the regional scale include geological and climatic events together with historical biogeography, dispersal, extinction, and speciation, which shape the regional species pool [4–6]. At the local scale, ecological interactions and local abiotic conditions act as filters on the regional species pool to shape each cave assemblage composition [7–10].

Depending on the number of cave openings, their size and location, as well as the size and depth of the cave, a gradient of light intensity, climatic conditions and nutrients can be found, creating up to five defined zones: entrance, twilight, transition, deep, and air-stagnant zones [1,11–13]. The abiotic conditions at the deep and air-stagnant zones of caves are less affected by seasonality and daily cycles and therefore are more stable environments in comparison to the cave entrance, twilight, and transition zones [14–16]. There is some evidence that different cave zones are inhabited by species with different environmental requirements and constitute discrete assemblages [14].

Connectivity of epigean and hypogean habitats also affect assemblage composition inside the cave via colonization and dispersal within and between epigean and hypogean systems [17]. While it is suggested that species richness of troglobites (organisms obligated to life in caves) is explained by historical biogeography, species richness of troglaphiles (organisms with strong affinity to caves) and accidental or occasional visitors in caves may be explained by local ecological factors [18]. Regions of the world that have rich regional species pools coupled with diverse cave formations and a range of abiotic conditions are expected to have rich assemblages of cave-dwelling visitors and resident species (both troglaphiles and troglobites).

1.1. The Levant and Its Caves

The Levant is a diverse biogeographic unit formed by the northeastern African and northwestern Arabian plates and the eastern Mediterranean Levantine basin [19]. Its geographical location at the junction of three continents (Europe, Asia, and Africa), and diversity of habitats and climate zones (Mediterranean, steppe, and arid) lay the foundation for a diverse regional species pool.

The Levant has a diversity of caves that differ in microclimate, age and the type of their rock substrate [20]. The different rock substrates, with intensive karstification in the north of Israel [21], and a climatic gradient from a mesic alpine climate at the north of Israel (Mt. Hermon) to the arid Negev desert in the south of Israel, resulted in a gradient of Karst features with very few or no karst features in the southern Negev desert and Arava. Furthermore, both epigenic and hypogenic caves (formed above or below the water table, respectively) [22,23] can be found in the Levant in basaltic bedrock [24], sandstone, limestone, dolostone, marl, chalk, chert [25–28], and salt rocks [29]. While many of the caves in the Levant are natural, some are manmade, such as burial caves, and some chambers of natural caves were formed or enlarged as part of a secondary use by man [30].

1.2. Spider and Other Arachnid Assemblages in Caves

It is very common to find troglaphile arachnids in the entrance of Levantine caves, as in caves of other regions of the world. Arachnids (and among them spiders) are often numerically dominant in caves and are considered dominant predators in many cave foodwebs [31–34]. Of the 11 extant terrestrial arachnid orders, five orders (i.e., Araneae, Opiliones, Palpigradi, Pseudoscorpiones, and Scorpiones) and the polyphyletic sub-class Acari were reported to have troglobite species around the world [35–38], while troglobite species of three orders (i.e., Amblypygi [39], Ricinulei, and Schizomida) were reported only from subtropical and tropical regions [35].

The arachnofauna of caves in the Levant is poorly known in comparison with European caves. In the present study, we investigated the spider assemblages, and specifically the presence of accidentals, troglaphile, and troglobite species in eastern Levantine caves (Israel and Palestine) in relation to geographic location, physical characteristics, and ecological zonation (entrance, twilight, and deep) of the caves, as well as the presence of bats and guano in the caves. The data were collected in field surveys between 2013 to 2015. Several new family records for Israel and species new to science discovered in the course of this field survey were reported elsewhere [38–41], including a distribution model for a common Levantine troglaphile pholcid spider: *Artema nephilit* Aharon, Huber, and Gavish-Regev (2017) [17], while some are reported here for the first time. Here, we used the data collected

in the same ecological field survey to describe the spider assemblages in 35 caves along a rainfall gradient and analyzed the environmental factors shaping these assemblages.

2. Materials and Methods

2.1. Field Surveys

Arachnids were collected from 35 caves located in Israel and Palestine (West Bank) (Figure 1) from three cave ecological zones: the cave entrance (inside the cave in the vicinity of the entrance); the twilight zone (in the intermediate part of the cave when it was applicable); and the inner dark zone (when it was applicable), as well as outside each cave (near the cave entrance). The caves are distributed along the climatic gradient from the Mediterranean (mesic) climate in the north and center of Israel (12 caves for each region, among them 6 caves in Palestine, Figures 1 and 2A–F), to the arid and hyper-arid climate in the south of Israel (11 caves, Figures 1 and 2G–I). Data on the caves is summarized in Appendix A.

In each cave ecological zone, temperature and illumination were measured. The temperature was measured using PicoLite 16-K and a single-trip USB temperature logger (FOURTEC), with measurements taken once an hour for 74–77 days. The illumination was recorded at the time of each sampling using an ExTech 401025 Lux Light Meter. The light meter was positioned on the ground until the reading stabilized for a minimum of 1 min. Measurements inside caves ranged between 0 to 420 lux, while measurements outside caves ranged between 60 to 70,000 lux (Supplementary Table S1). Temperature and illuminance were also measured outside the cave. Caves were assigned to three cave size categories: large (13 caves, more than 50 m with twilight and dark zones), medium (12 caves, 11 to 50 m with twilight zone, lacking a dark zone), and small (10 caves, 10 m or shorter, lacking twilight and dark zones) (Figure 1). Cave size and length were estimated from cave maps when available (Israel Cave Research Center) or in the field, and representing the distance from the cave opening toward the darkest region of the cave we could reach. Elevation and geological data were provided by the GIS (geographic information system) center at the Hebrew University of Jerusalem. Annual average precipitation data was taken from the Israel Meteorological Service (<https://ims.gov.il/en/ClimateAtlas>, accessed on 1 February 2021) from 1980–2010, which came from the closest meteorological station to each cave (Appendix A).

Each of the 35 caves was sampled according to a specific protocol twice during 2014 (6 March–6 April 2014 and 22 May–22 June 2014). Due to differences in cave morphology (including microhabitat, fractal shape of the substrates, size, and volume) we standardized our sampling effort by time. Therefore, our protocol included a 20-min thorough visual search by one of three experienced arachnologists in 3 to 10 m long sectors using headlamps and UV lights in each cave ecological zones. In the first visit to each cave, most of the arachnid observed were collected by hand for further identification in the lab. In further visits to each cave, we thought some species populations were more sensitive or common, thus finding it possible to identify a species level *in situ*. Such data were recorded and not collected.

We visited several additional caves outside of the formal 2014 cave survey and collected additional arachnids. On these occasions, only the locality and date were documented for the arachnids. Therefore, we refer to the arachnid species collected in these caves only in the general paragraph of the results and discussion, but not in the analysis of the survey data.

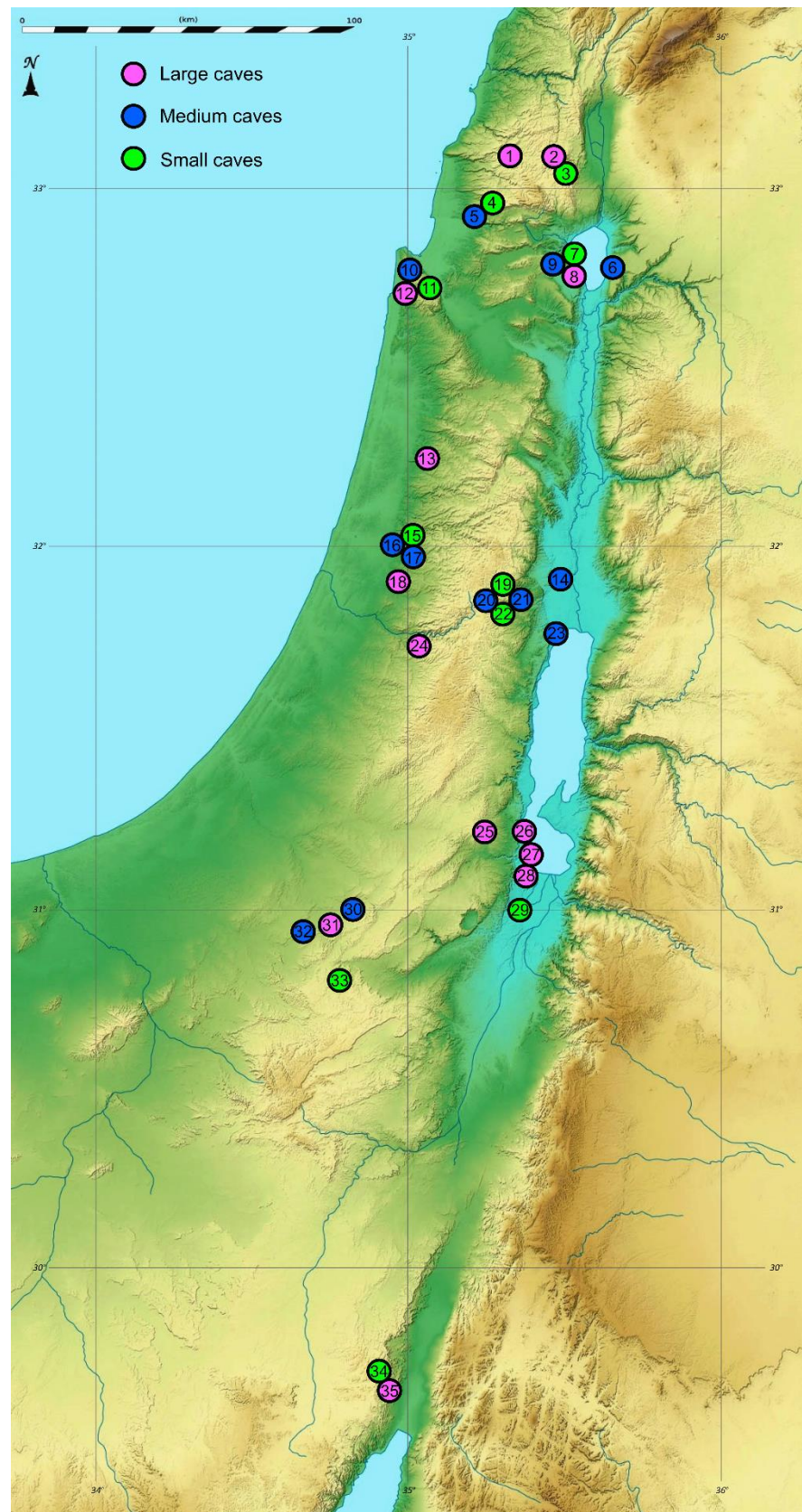


Figure 1. Distribution of the 35 caves. Large caves are represented by pink dots, medium caves by blue dots, and small caves by green dots. Numbers denote a specific cave (see Appendix A).



Figure 2. Caves and habitats from the survey (cave numbers in parentheses). (A–C): Northern Israel, Mediterranean climate. (A): The area near the opening of Yonim cave (upper Galilee, 5). (B): The north-facing slope of Oren wadi where Ezba’ cave (12) is located (taken from Oren cave, Karmel). (C): The south-facing slope of Oren wadi where Oren cave (Karmel, 10) is located. (D–F): Central Israel and Palestine, Mediterranean and semi-arid climates. (D): The area near the opening of Teomim cave (Judean Mountains, 24). (E): The area near the opening of Haruva cave (HaShfela, 18). (F): The north-facing slope of Perat wadi where ‘Perat Inbal cave (cave 1, northern Judean desert, Palestine, 20) is located. (G–I): Southern Israel, arid climates. (G): The east-facing slope where Zavoa cave is located (southern Judean desert, 25). (H): The east-facing slope where Arubotayim cave is located (southern Dead-sea region, 26). (I): The area where Ammude Amram caves are located (Arava valley, 34–35). Pictures: (A–C,F–I) Shlomi Aharon, (D,E) Igor Armiach Steinpress.

2.2. Statistical Analysis

Generalized linear models (GLMs) were used to test the effect of the geographical region (north, center, and south of Israel) on spider richness (Poisson distribution and log link) and abundance (normal distribution and log link) using the entire dataset of troglobite, troglophile, and accidental spider species.

Using a set of GLMs with binomial distribution (logit link), we tested separately for each spider guild [42] the probability that a spider species observed in a cave belongs to the particular spider guild, and whether the probability changes in relation to the chosen environmental variables. Geographic region can encompass different environmental variables. In Israel, there is a strong north to south gradient of several correlated climatic variables such as precipitation, air humidity, evaporation, and air temperature. We added the presence of bats in caves as an additional explanatory variable as bat activity changes the habitat for other cave dwellers, such as spiders. To further test for such effects, we included an alternative model in which, instead of bat presence, we used an estimation of guano level on the cave floor from our field assessment, i.e., none, low (when less than 15% of the cave floor had guano deposition), medium (when 15% to 40% of the cave floor had guano deposition), or high (when more than 40% of the cave floor had guano deposition).

Two multivariate analyses methods were performed in order to better understand the factors affecting the assemblage composition of troglobite and troglophile species: hierarchical clustering, using R v.4.0.3. [43] and a direct CCA ordination (canonical correspondence analysis), using Canoco [44,45]. For the cluster analysis, pairwise Pearson correlation between the cave communities was determined using the 'cor' function with the 'pairwise.complete.obs' method, and the output hierarchically clustered as a Euclidean distance matrix with an 'average' linkage using the 'hclust' function. The resultant clustering was plotted using the 'heatmap.2' function of the 'gplots' package [46], with scaling by row. For the CCA ordination, we used the unrestricted Monte Carlo permutation tests (4999 runs) and forward selection, testing 9 explanatory environmental variables: geographic location (latitude and longitude, continuous), elevation (continuous), cave estimated length (continuous), minimum temperature in the cave during the survey (continuous), average annual precipitation (continuous), rock category of the cave (6 categories: basalt, carbonate rocks, chalk, marlstone, salt, and sandstone), minimum age of the rock (continuous), bat inhabitation (three categories: no bats, insectivorous, frugivorous), and guano level (four categories: no guano, low level, medium level, and high level of guano).

For both multivariate analyses, only 34 of the 35 caves were used, as 'Ammude 'Amram (a small cave, 34) had two Solifugae individuals, but no spiders were found in our two visits. We restricted the statistical analyses to troglobite and troglophile species ($N_{\text{species}} = 32$), omitting the accidental species ($N_{\text{species}} = 30$). The latter were epigeic species that occurred in low abundance and were scattered among the different caves.

3. Results

3.1. Cave Arachnofauna from Field Surveys and Additional Visits to Caves

A total of 1132 arachnids were found during the surveys, comprised mainly of species of the order Araneae (1054 individuals). However, representatives of additional arachnids were also found (Figure 3), i.e., Acari (65 individuals, among them two soft-tick species (Argasidae) *Argas vespertilionis* (Latreille, 1796) (Figure 3A) and *Ornithodoros tholozani* Laboulbène and Mégnin, 1882 (Figure 3B), as well as hard-ticks (Ixodidae)); Pseudoscorpiones (four individuals, awaiting identification, Figure 3C,D); Amblypygi (although many individuals were observed, only three were collected: two Charinidae species *Charinus ioanniticus* (Kritscher, 1959) and *Charinus israelensis* Miranda, Aharon, Gavish-Regev, Giupponi, and Wizen, 2016, Figure 3E) [39]; Scorpiones (although more individuals were observed, we were able to catch only three juveniles (Buthidae)); Solifugae (two juveniles; identification was not possible) and one Opiliones of the species *Mediostoma haasi* (Roewer, 1953) (Nemastomatidae).

In other visits to several additional caves outside of the 2014 survey, we found one additional Opiliones species, the troglolithic eyeless *Haasus naasane* Aharon et al., 2019 (Pyramidopidae, Figure 3F) [38], an Opilioacaridae species (in preparation, Figure 3G, [47]), Palpigradi species (in preparation, Figure 3H, [47]), and one spider family that was not reported before from Israel: Nesticidae (the invasive species *Eidmannella pallida* (Emerton, 1875) and an additional unidentified species, both reported here for the first time).

The 1054 spiders found during the 2014 survey in 34 of the 35 caves were identified to 62 species and morphospecies in 38 genera and 22 families (Supplementary Table S2). As in many faunistic surveys, the Levantine cave spider rank-abundance curve was skewed, with nine species that have 33–152 individuals each and account for 72% of the overall abundance (762 individuals out of 1054), 29 singleton, and doubleton species (42 individuals out of 1054). Moreover, 24 species have 3–28 individuals each (249 individuals out of 1054) (Figure 4). Of the 62 species, 46 are either species with valid taxonomic status (26 species), are in the process of description (six species), or need further taxonomic study (14 species: eight linyphiids, two theridiids (*Steatoda* Sundevall, 1833), two pholcids (*Pholcus* Walckenaer, 1805 and *Spermophorides* Wunderlich, 1992), one theraphosid (*Chaetopelma* Ausserer, 1871), and one oonopid (*Megaonops* Saaristo, 2007)). The rest (16 morphospecies) were

not adults and could be identified only to the genus or family level based on morphology (Supplementary Table S2).

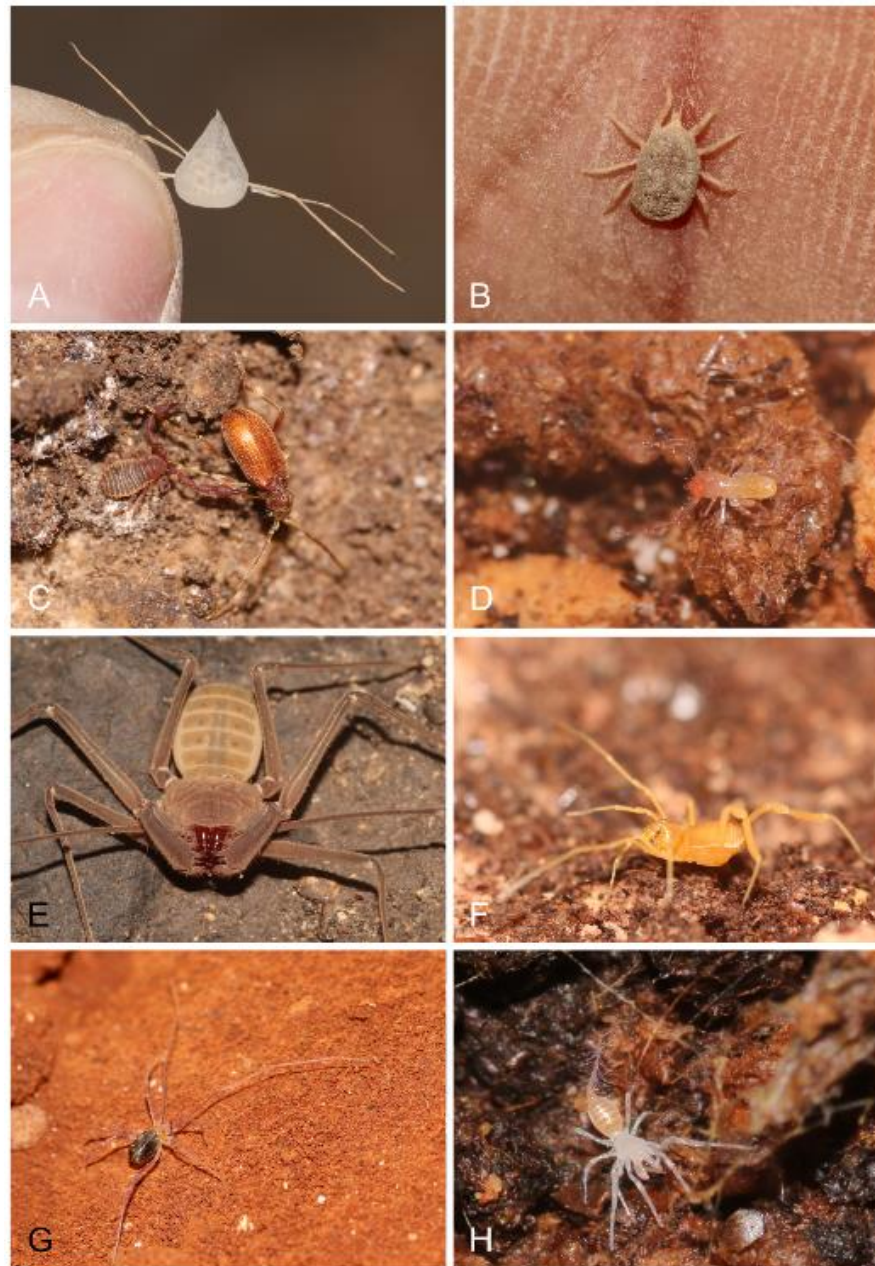


Figure 3. Arachnids found during cave surveys. (A): *Argas vespertilionis* (Latreille, 1796), (Acari, Argasidae); (B): *Ornithodoros tholozani* Laboulbène and Mégnin, 1882, (Acari, Argasidae); (C,D): Pseudoscorpiones; (E): *Charinus israelensis* Miranda, Aharon, Gavish-Regev, Giupponi, and Wizen, 2016, (Amblypygi, Charinidae); (F): *Haasus naasane* Aharon et al., 2019 (Opiliones, Pyramidopidae); (G): Opilioacaridae (Acari); (H): *Eukoenenia* Börner, 1901 (Palpigradi, Eukoeneniidae). Pictures: Shlomi Aharon.

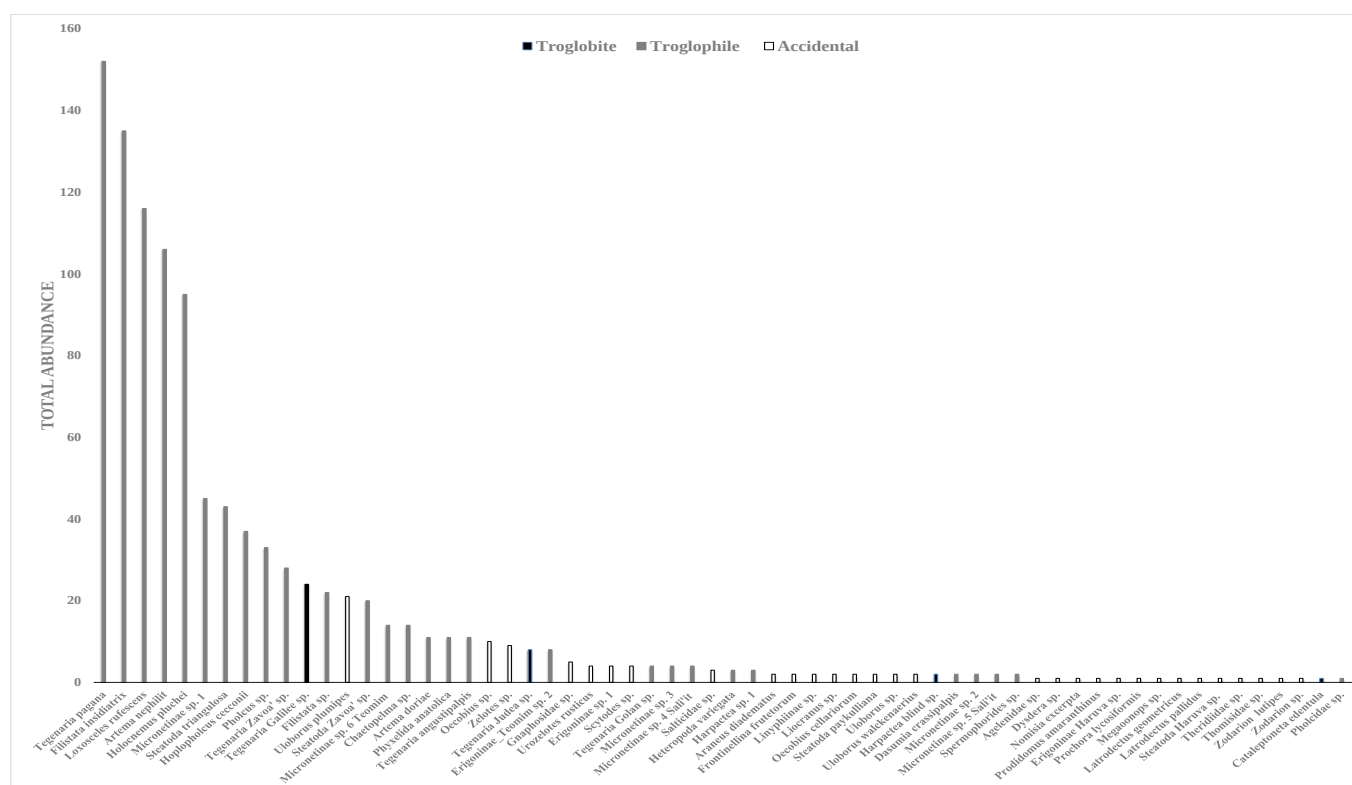


Figure 4. Rank-abundance graph of 62 spider species from 34 Levantine caves ('Ammude 'Amram small cave (34) had no observed spiders during our two visits).

3.2. Cave Geographic Region, Bat Presence and Spider Richness and Abundance

The geographical region of the caves significantly affected spider richness ($N_{\text{species}} = 62$; $N_{\text{individuals}} = 1054$, $\chi^2_2 = 27.41$, $p < 0.001$). Specifically, spider richness in the southern region was significantly lower than in the other geographical regions monitored (Figure 5A, Table 1). Bat presence did not significantly affect spider richness ($\chi^2_1 = 0.002$, $p = 0.968$). Similarly, a non-significant effect was found when the estimate of guano level was used (guano: $\chi^2_2 = 3.845$, $p = 0.279$). Spider abundance followed a similar trend as richness, showing a significant effect of geographic region on abundance ($\chi^2_2 = 7.127$, $p = 0.028$), with fewer individuals detected in southern caves (Figure 5B, Table 1), and no significant effect of bat presence ($\chi^2_1 = 1.884$, $p = 0.170$). The similar model with bat guano level was also not significant (geographical region: $\chi^2_2 = 6.328$, $p = 0.487$; guano level: $\chi^2_2 = 2.438$, $p = 0.487$).

Table 1. Spider species richness and abundance (troglobite, troglophile, and accidentals) in caves by region (35 caves). Numbers denoted a specific cave (see Appendix A).

	South (11 Caves)	Center (12 Caves)	North (12 Caves)
Spider species richness (minimum, median, maximum; mean)	0 ¹ , 2, 8 ² ; 2.27	4 ³ , 7, 10 ⁴ ; 7.08	5 ⁵ , 6, 14 ⁶ ; 6.91
Spider abundance (minimum, median, maximum; mean)	0 ¹ , 9, 56 ² ; 14.73	17 ⁷ , 34, 51 ⁸ ; 33.75	8 ⁵ , 40, 86 ⁹ ; 40.58

¹ 'Ammude 'Amram (small cave, 34); ² Zavoa' (25); ³ Qumeran (23); ⁴ Andartat HaBiq'a' (14); ⁵ Raqqit (11); ⁶ Yonim (5); ⁷ Perat cave # 4 (19);

⁸ Tinsmet (17); ⁹ Berniki (medium cave, 9).

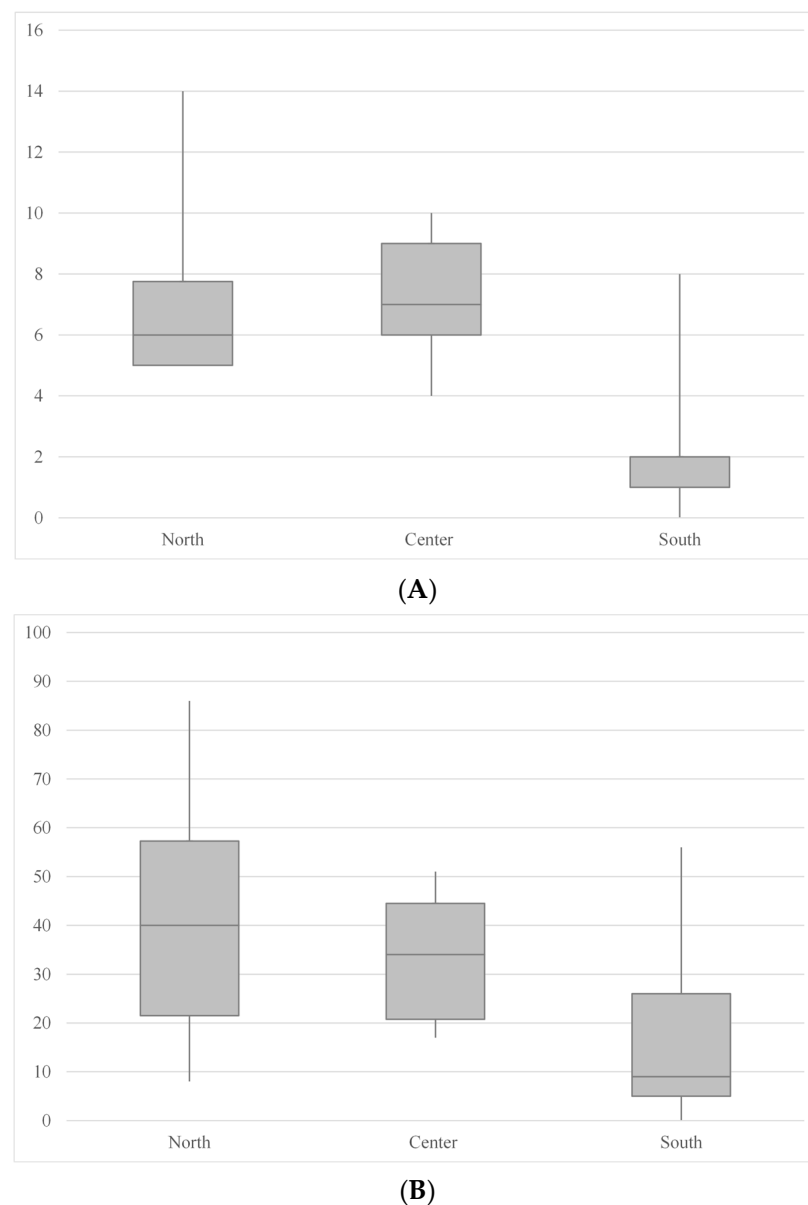


Figure 5. Box plot of (A) species richness and (B) abundance of troglobite, troglophile, and accidental spiders in 35 caves at the north, center, and south of Israel (minimum, first quartile, median, third quartile, and maximum).

3.3. Troglobites and Troglophiles

We assigned each of the 62 spider species in our survey to one of three categories: troglobite, troglophile, and accidental, according to their distribution and the known use of caves as a habitat in Israel and Europe [33]. We also categorized them based on troglomorphic phenotype. This resulted in a list of 32 troglophile and troglobite spider species inhabiting caves in our region and 30 accidental spider species (Supplementary Table S2). Only three spider families included troglobite species: Agelenidae, with two eyeless species found during visits to several additional caves outside of the 2014 survey, and two eye-reduced species in the genus *Tegenaria* Latreille, 1804; Dysderidae with one eyeless species in the genus *Harpactea* Bristowe, 1939, which may be a species complex based on our preliminary morphological study; and Leptonetidae, with one species *Cataleptoneta edentula* Denis, 1955 (Figures 6 and 7) [41].



Figure 6. Troglobite spiders found during cave surveys (cave numbers in parentheses). (A,B): *Tegenaria* Latreille, 1804 (Agelenidae). (A): *Tegenaria* from Yir'on large cave (upper Galilee, 2); (B): *Tegenaria* from Teomim large cave (Judean mountains, 24); (C) *Harpactea* Bristowe, 1939 (Dysderidae) eyeless species from Shetula large cave (upper Galilee, 1); (D): *Cataleptoneta edentula* Denis, 1955 (Leptonetidae) from Ezba' large cave (Karmel ridge, 12). Pictures: Shlomi Aharon.

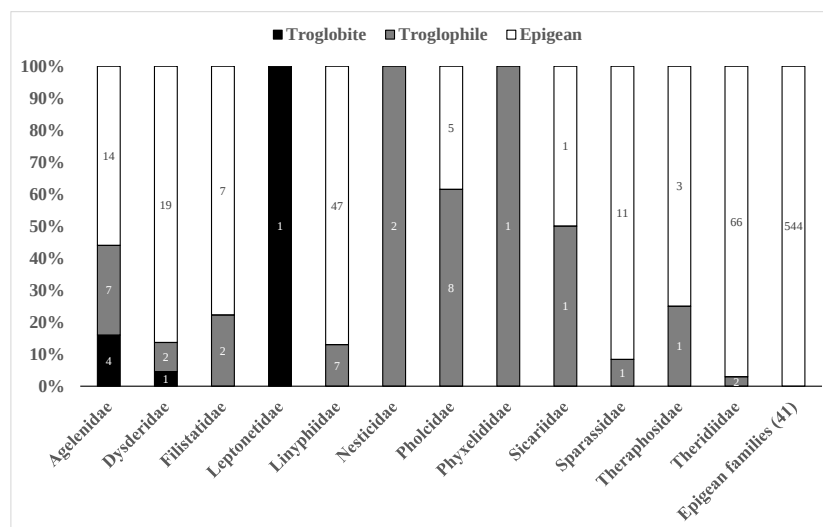


Figure 7. Proportion and number of troglobite, troglophile, and epigean spider species per family in Israel and Palestine (53 families and 758 species including undescribed species, description in progress). Families that have only epigean species were pooled in one column.

In total, 11 spider families in Israel and Palestine included troglophile species (Figures 8 and 9). Pholcidae has 8 troglophile species and is family with the largest number of troglophile species in Israel and Palestine (Figure 7). It includes some abundant species found in caves along the north–south climatic gradient (Figure 8): *Artema nephilit* Aharon, Huber and Gavish-Regev 2017 (Figure 8A) [9,40], *Hoplopholcus cecconii* Kulczyński, 1908 (Figure 8B), *Holocnemus pluchei* Scopoli, 1763 (Figure 8C), and a species in the genus *Pholcus* Walckenaer, 1805 (Figure 8D). However, the three most abundant troglophile species are *Tegenaria pagana* C. L. Koch, 1840 (Agelenidae, Figure 8E), *Filistata insidiatrix* (Forskål, 1775) (Filistatidae, Figure 8F), and *Loxosceles rufescens* (Dufour, 1820) (Sicaridae, Figure 8G), respectively (Figures 4 and 8). We found additional troglophile species (Figures 4 and 7–9)

in the families Theridiidae (*Steatoda triangulosa* (Walckenaer, 1802), Figure 8H), Linyphiidae (six or seven species of the sub-family Micronetinae, Figure 9A–D), Theraphosidae (*Chaetopelma* Ausserer, 1871 species, Figure 9E), Phyxelididae (*Phyxelida anatolica* Griswold, 1990, Figure 9F, [41]), Sparassidae (*Heteropoda variegata* (Simon, 1874), Figure 9G), Dysderidae (*Harpactea* Bristowe, 1939, Figure 9H), and Nesticidae. Supplementary Table S2 includes the full list of the spider species with their localities and distribution.

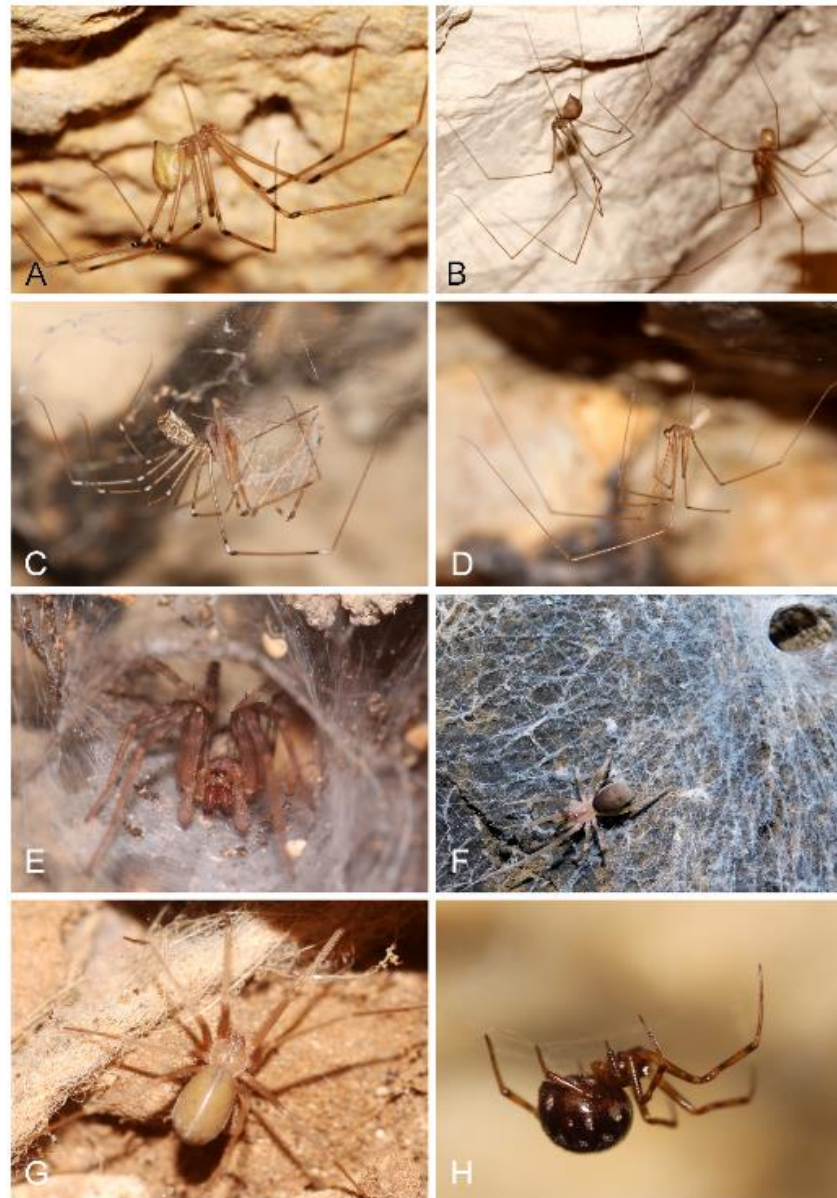


Figure 8. Eight of the nine most abundant troglomorphic spiders found during cave surveys (cave numbers in parentheses). (A–D): Pholcidae: (A): *Artima nephilit* Aharon, Huber and Gavish-Regev 2017 from Oren medium cave (Karmel ridge, 10); (B): *Hoplopholcus cecconii* Kulczyński, 1908 from Yir'on large cave (upper Galilee, 2); (C): *Holocnemus pluchei* Scopoli, 1763 from a cave in Perat wadi (northern Judean desert, 19–22); (D): *Pholcus* Walckenaer, 1805 from 'Inbal cave 1 in Perat wadi (northern Judean desert); (E): *Tegenaria pagana* C. L. Koch, 1840 (Agelenidae) from Teomim cave (Judean mountains); (F): *Filistata insidiatrix* (Forskål, 1775) from 'Inbal cave 1 in Perat wadi (northern Judean desert, 20); (G): *Loxosceles rufescens* (Dufour, 1820) (Sicariidae) from Berniki caves (lower Galilee, 7–9); (H): *Steatoda triangulosa* (Walckenaer, 1802) (Theridiidae) from Tinsihmet cave (western Samaria, 17). Pictures: (A–E,G,H): Shlomi Aharon, (F): Igor Armiach Steinptress.

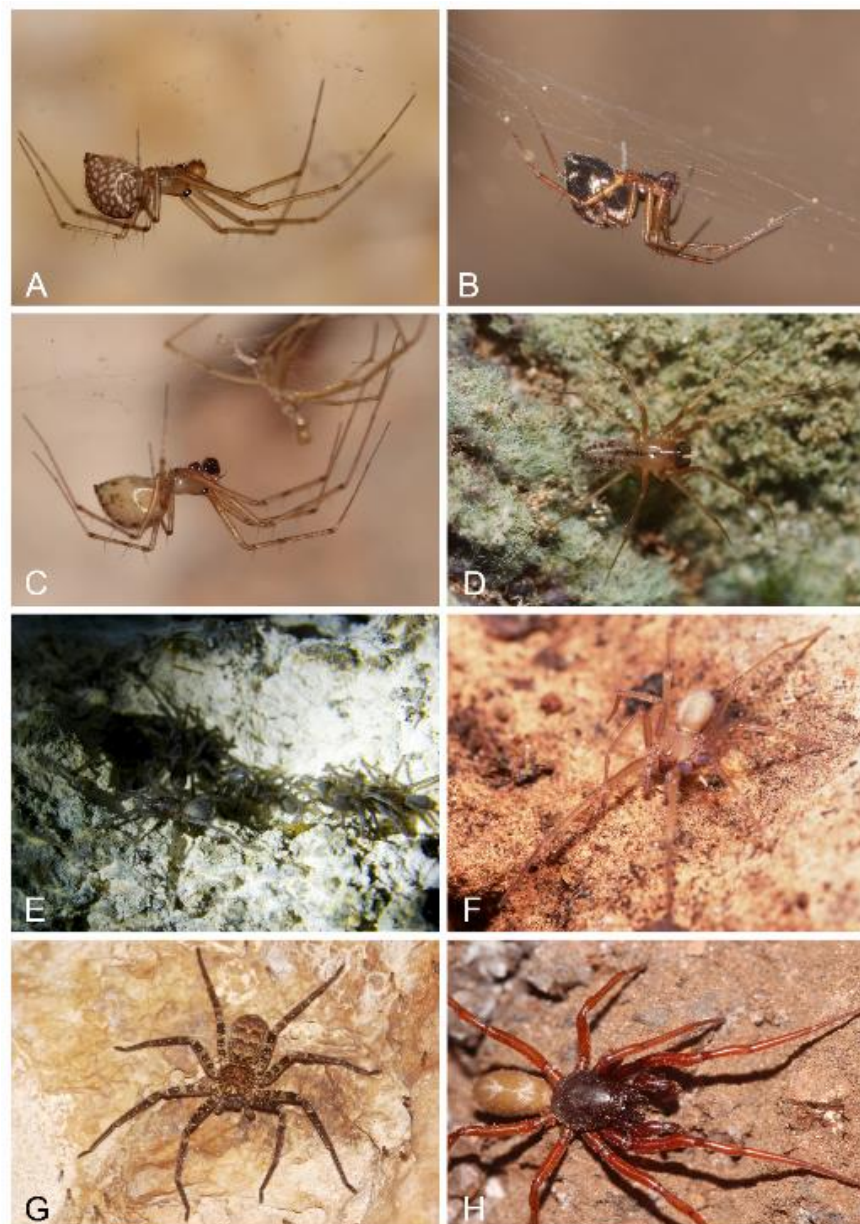


Figure 9. Additional troglophile spiders found during cave surveys. (A–D): Linyphiidae, Micronetinae: (A): Male micronetinae sp. 1 from Besor medium cave (Negev desert, 32); (B): Male micronetinae sp. 6 from Teomim large cave (Judean mountains, 24); (C): Male micronetinae sp. 1? from Bet ‘Arif medium cave (western Samaria, 16); (D): Male micronetinae from ‘Avedat cave (Negev desert, 33); (E): *Chaetopelma* Ausserer, 1871 (Theraphosidae) from Modi’in cave (Judea, not part of the survey); (F): *Phyxelida anatolica* Griswold, 1990 (Phyxelididae) from Haruva cave (HaShfela, 18); (G): *Heteropoda variegata* (Simon, 1874) (Sparassidae) from Yir’on large cave (upper Galilee, 2); (H): *Harpactea* Bristowe, 1939 (Dysderidae) from Ezba’ large cave (Karmel ridge, 12). Pictures: (A–D,F–H): Shlomi Aharon, (E): Igor Armiach Steintress.

3.4. Spider Foraging Guilds

The spiders found in the cave survey represent six foraging guilds [42]: sheet-web weavers, sensing-web weavers, space-web weavers, orb-web weavers, ambush hunters, and other hunters (Supplementary Table S2). Three out of four troglobite species and 24 out of the 28 troglophile species are web-builders (sheet-web: Agelenidae, Linyphiidae, Phyxelididae; space-web: Leptonetidae, Pholcidae, Theridiidae; sensing-web: Filistatidae, Theraphosidae), and only one troglobite and four troglophiles are hunters (Dysderidae, Sicariidae, Sparassidae).

GLM analyses of the geographic region and bat presence or guano level showed that neither factor in the two alternative models was significant (Table 2).

Table 2. GLM analyses of the effect of environmental variables on the probability to detect specific spider guilds in the caves. Wald χ^2 and p values are shown for the two models. Neither geographic region nor bat presence (or guano level) was a significant explanatory variable for any of the guilds.

		Df	Ambush Hunters		Specialists and Other Hunters		Sensing Web		Sheet or Space Web		Orb Web	
			Wald χ^2	p -Value	Wald χ^2	p -Value	Wald χ^2	p -Value	Wald χ^2	p -Value	Wald χ^2	p -Value
Model 1	Geographic region	2	2.144	0.342	2.963	0.227	0.706	0.703	4.440	0.109	1.733	0.420
	Bat presence	1	0.024	0.877	0.394	0.530	0.018	0.892	0.065	0.798	0.001	0.979
Model 2	Geographic region	2	2.047	0.359	4.146	0.126	0.314	0.855	3.776	0.151	1.729	0.421
	Guano amount	3	0.586	0.900	3.021	0.388	0.609	0.894	0.277	0.964	0.339	0.953

3.5. Levantine Cave Spider Assemblages: Similarities and Environmental Variables from Field Survey

We used only the 32 troglobite and troglophile spider species to further analyze the spider assemblages in this cave survey (34 caves). We identified five groups of caves that cluster together based on the correlation between their species assemblages (Figure 10). The most noticeable clusters are based each on one of five dominant (*T. pagana*, *F. insidiatrix*, and *A. nephilit*) and sub-dominant (*S. triangulosa* and *H. ceconii*) troglophile species. One cluster was distinctive from the others by most sharing a single species, *A. nephilit*. This cluster includes caves in arid climates, all of the Dead Sea caves (caves 23, 26–29), one cave from the Negev desert (cave 33), and one cave from the Arava (cave 35). Another cluster was based on *F. insidiatrix* (with either *L. rufescens* or *H. pluchei*), with three upper Galilee caves (caves 3, 5, 8) and four caves from the Judean desert, Galilee, and Karmel (caves 9–10, 20, 22), respectively. All caves in another cluster (which could be divided to three sub-clusters) had *T. pagana* in them (caves 7, 12–13, 15–18, 21). The fourth cluster was based on *S. triangulosa* and includes two caves from the Negev desert (caves 30–31) and a cave from the Karmel (cave 11). Two caves from the upper Galilee (caves 1–2) form the fifth cluster based on *H. ceconii*.

A CCA ordination showed five significant variables explaining 91.4% cumulative percentage variance of the species–environment relation: precipitation (F-ratio = 2.52, $p = 0.0002$), elevation (F-ratio = 2.37, $p = 0.0002$), latitude (F-ratio = 2.03, $p = 0.0032$), minimum temperature (F-ratio = 1.79, $p = 0.0302$), and high guano level (F-ratio = 1.87, $p = 0.0266$) (Figure 11, Table 3). Additional CCA ordination analyses were done using all 62 species (including the accidental species): (1) with 34 caves and the three different ecological zones within the caves (67 samples all together), and (2) with 34 caves and without cave ecological zones, and found similar results. In analysis (1), precipitation, elevation, latitude, minimum temperature bats, and guano level were significant, as were some of the rock categories. Moreover, in analysis (2), precipitation, latitude, and guano level were significant, but not elevation and minimum temperature (see supporting information Figure S1, Tables S3 and S4).

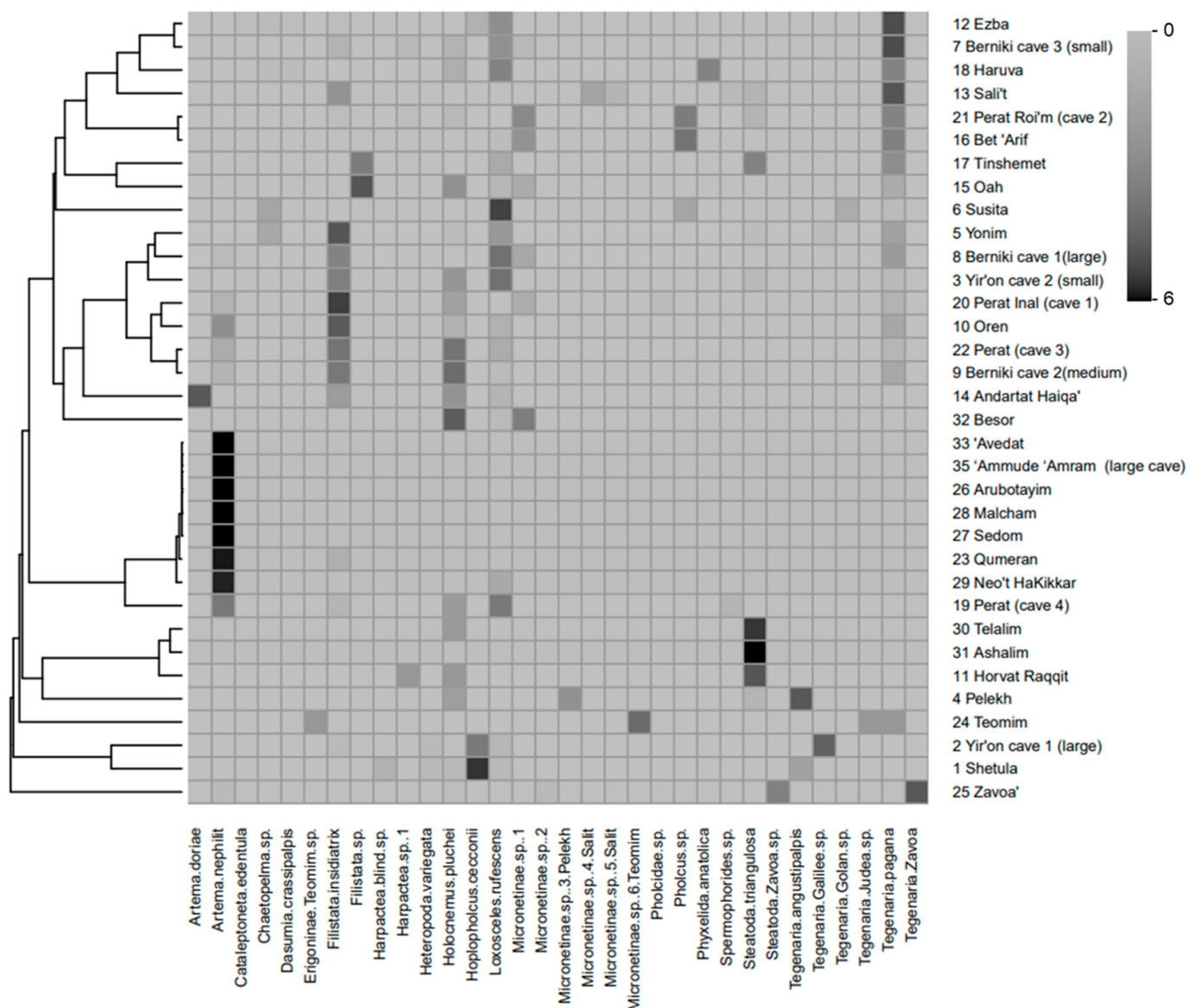


Figure 10. Cluster heatmap for 34 caves (rows) and 32 troglobite and troglophile species (columns) ('Ammude' Amram (cave 34) had no observed spiders during our two visits). Caves are clustered using hierarchical clustering from pairwise Pearson correlation. Darker colors signify higher correlation. Numbers before cave names as in Figure 1 and Appendix A.

Table 3. CCA ordination results.

	Eigenvalues	Species-Environment Correlations	Cumulative Percentage Variance of Species Data	Cumulative Percentage Variance of Species—Environment Relation	Sum of All Eigenvalues	Sum of All Canonical Eigenvalues
Axis 1	0.642	0.917	8.9	31	7.195	2.070
Axis 2	0.589	0.831	17.1	59.5		
Axis 3	0.448	0.839	23.3	81.1		
Axis 4	0.214	0.725	26.3	91.4		

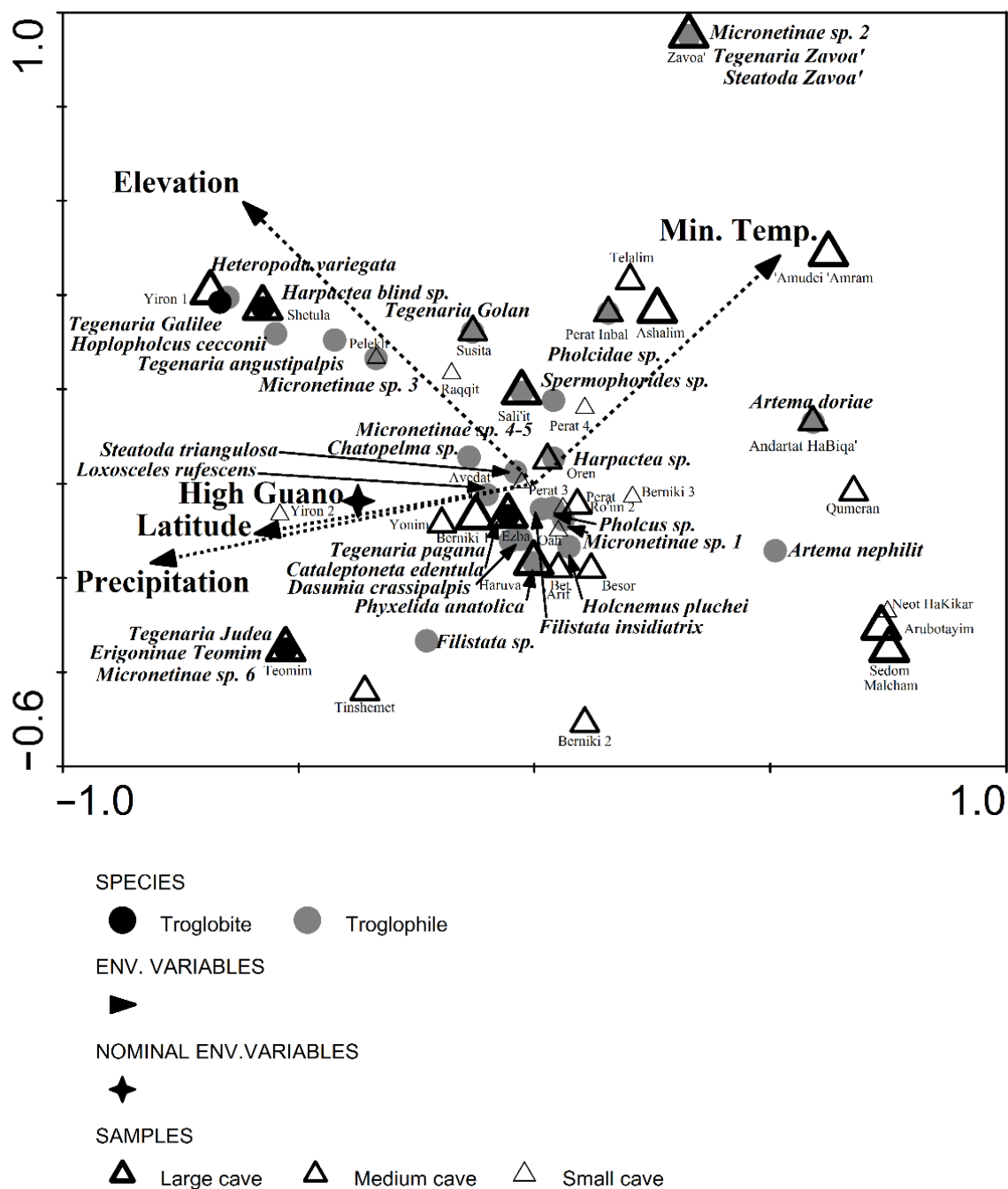


Figure 11. CCA ordination graph of the first and second axes testing 34 caves and 32 troglobite/troglophile species. The significant explanatory variables (precipitation, elevation, latitude, minimum temperature, and high guano level) are plotted on the graph, as well as the cave and species names. Cave sizes are represented by large, medium, and small triangles, respectively, troglobite species by black points, troglophile species by grey points, nominal explanatory variable by a black star, continuous explanatory variables by arrows with the dashed line. The non-dashed line arrows are used to connect between species name and its centroid.

4. Discussion

4.1. Levantine Cave Arachnofauna

We discovered many unique arthropods, including troglobite [38,39] and troglophile [40,41] arachnids and species endemic to our region. Representatives of the orders Amblypygi, Palpigradi, and Pseudoscorpiones were found only in mesic caves in the Mediterranean region, while Opilioacaridae were found in caves in the semi-desert region. Spiders were the most abundant and diverse arachnid order in the caves studied. Currently, 53 spider families, 297 genera, and 758 species are known from Israel ([48], unpublished data). Of them six troglobite species in three families (Agelenidae (two from the survey and two from caves that were not included in the 2014 survey), Dysderidae, and Leptonetidae), and 34 troglophile species in 11 spider families (Figure 7).

4.2. Troglobites and Troglophiles

Based on our results, 20% of the families known from Israel and Palestine include troglobite and/or troglophile species, compared to 34% in Europe (22 out of 64 families reported from Europe [33,49]). From our current data, only about 5% of the spider species known from Israel and Palestine are cave-dwellers (40 species) with approximately 2% endemics. In 2018, Mammola et al. reported a total of 486 cave-dwelling spider species in Europe (195 troglobite and 291 troglophile species) [33], with 90% of them considered endemics of single countries. In the well-studied cave arachnofauna of Slovenia [50,51], for example, 30% of families (out of 43 families) include troglobite or troglophile species, representing about 11% of species known in Slovenia (N = 753). Some of the families with troglobite and troglophile species in Slovenia [33,50] also occurred in our cave survey in Israel and Palestine (Ageleidae, Dysderidae, Leptonetidae, Linyphiidae, Nesticiidae, Pholcidae, and Theridiidae). Linyphiidae had the greatest number of cave-dwelling spider species in Slovenia, with 48 cave-dwelling species out of 221 linyphiid species in total, followed by Dysderidae with seven cave-dwelling species (22 species in total), Agelenidae with seven cave-dwelling species (25 species in total), and Pholcidae with five cave-dwelling species (five species in total). Our survey uncovered a potentially high species richness of troglophiles and troglobites in the same four families as in Slovenia, but with a different order of richness: Agelenidae, Pholcidae, Linyphiidae, and Dysderidae.

Preference of shaded habitats is a common feature of the four most abundant troglophile spider species in our caves. Placing those four species on a cave-affinity continuum provides a better understanding of their distribution in caves. *Loxosceles rufescens* is an opportunistic synanthropic species common in the Mediterranean basin in houses and other manmade habitats (and introduced to other areas around the world) [52]. Although it can be found in shaded natural habitats such as caves and under stones [33], and is very common in caves as reported above, it is not a cave specialist. *Artema nephilit* and *F. insidiatrix* have a higher affinity to caves than to other habitats (Supplementary Table S2, personal observation). Although *A. nephilit* can be found also under boulders, in crevices, and in basements, *F. insidiatrix* can be found in other natural shaded habitats and they each have preference for cave entrances [9,33,49]. Of these four troglophile species, *T. pagana* has the highest affinity to caves and can be found in cave entrance and twilight zones in large numbers. Similar to some other troglophiles, *T. pagana* could also be found in other suitable natural shaded habitats, but it is much more common in caves than in any other shaded habitat in Israel and Palestine (Supplementary Table S2, personal observation, Aharon et al., in preparation).

4.3. Foraging Guilds

Among the cave-dwelling spiders we found, web-builders are more species-rich than hunters (27 vs. five species, respectively; Supplementary Table S2). The four most abundant troglobite spider species in our caves represent four out of the six foraging guilds found: sheet-web weavers (*T. pagana*), sensing-web weavers (*F. insidiatrix*), ambush hunters (*L. rufescens*), and space-web weavers (*A. nephilit*). Higher species richness of web-builders vs. hunters was also found in caves in the Iberian peninsula [53], while ambush hunters and sensing-web weavers were absent from these caves [32,53]. A single species of each of these guilds was abundant in the Levantine caves (*L. rufescens* and *F. insidiatrix*, respectively). The troglobite species of the Levantine caves are represented mainly by sheet-web weavers (Agelenidae), one space-web weaver (Leptonetidae), and one hunter species (or species complex) (Dysderidae). Although the spider family Dysderidae has species that specialize on isopods [42], the diet preferences of the dysderids in caves is unknown [53]. Specifically, we lack diet information on the eyeless and eye-bearing *Harpactea*, eye-bearing *Dysdera*, and *Dasumia crassipalpis* (Simon, 1882) found in the Levantine caves, and therefore we assigned them to the guild of ‘other hunters’.

4.4. Species-Pool, Regions, and Bat Inhabitation

The cave-dwelling spiders of the Levant represent a subset of the regional species-pool, with a maximum of 58 species (the 62 that were found in caves without the four troglobite species) that are known or potentially can be found in epigean habitats. Only two spider families, Leptonetidae and Nesticidae, were found only in hypogean habitats in Israel, while 29 spider families were found only in epigean habitats in Israel, and 22 spider families were found both in hypogean and epigean habitats in Israel (including troglobites, troglophiles and accidentals). This distribution is similar to in agreement with findings in other places of the world [32,53].

Caves in the southern region of Israel had relatively low species richness, with no troglobite arachnids and few troglophile spider species. This desert region, covering about 55% of Israel, harbors many epigean spider species that are adapted to the desert climate (at least 246 according to Zonstein and Marusik [48]) and are rarely found in caves. By contrast, all troglobite arachnids and most troglophile spiders were found in caves located in the Mediterranean mesic climate zone, mainly in the north of Israel but also in central Israel and Palestine (Figures 1, 2 and 7A,B). This could be explained by both the higher precipitation (see results and Figure 11) and intensive karstification in the north of Israel [21], i.e., more caves with regional mesic climate may support higher pre-adapted cave-dwelling species. Our analysis found that geographic region is a significant factor effecting both species richness and abundance in caves, and that precipitation and latitude correlated and had a significant effect on the assemblages (Figure 11).

Our preliminary taxonomic study revealed phenotypic variation between populations of different caves in *Tegenaria*, *Harpactea*, and the micronetine spiders (Linyphiidae). For some of these spiders, morphological variation was found to be lower within cave populations in comparison to between different caves, and these unique morphological characters are sufficient for describing new species. For other spiders, molecular methods were used to assess cryptic species and possible species complexes. Therefore, our future taxonomic efforts will focus on describing the troglobitic and troglophilic species new to science that have mainly been found in caves in the Mediterranean climate region.

One cave in the arid region, Zavoa cave, hosted an exceptional number of spider species (eight species) and individuals (56 individuals) (Figures 1, 2G and 11, Appendix A). Zavoa cave is situated in the southern Judean desert, with an average of 100 mm rainfall a year, and harbors several cave-dwelling species that may be endemics. One additional cave that was not part of our cave survey, A'arak Na'asane, in the northern Judean desert, is home to several troglobite and troglophile endemic arachnids [38,54]. Both Zavoa and A'arak Na'asane are large caves located in a region that experienced aridification during the last glacial interval [55]. Both caves are inhabited by bat colonies. A'arak Na'asane has a high level of guano, while the Zavoa cave has less guano due to the currently lower numbers of bats. We visited several additional caves in the Judean desert, outside the 2014 survey, and found opilioacridiformes and other troglophile arachnids as well as evidence of former use of the caves by bats (dry old guano), but it seems that the bats no longer occupy these caves (unpublished data).

Trajano and de Carvalho (2017) suggested that troglophile species richness could be explained by local ecological factors [18]. Guano is probably an important energy source for cave arthropods and may have a bottom-up effect on the cave food-web, as was shown in caves in Brazil [56,57]. One of our hypotheses was that the level of guano in the cave will positively affect spider richness and abundance, but we did not find this to be significant, but we did find a significant effect of the guano level on the spider assemblage composition in caves (Figure 11, Figure S1, Tables S2 and S3). To test the effect of guano on the Levantine cave spider assemblages in more depth, more information is needed on the ecology of spiders in these caves.

4.5. Levantine Cave Spider Assemblages

We showed that Levantine cave spider assemblages are diverse and are affected by the specific geographic location and its climatic characteristics, as well as by the presence of bats and guano level. While dry caves in the desert climate are dominated by pholcid and theridiid spiders, humid (but not wet) caves in the mesic Mediterranean climate region are dominated by agelenid, filistatid, sicariid, and linyphiid spiders. Dysderid, leptonetid, nesticid, and theraphosid spiders were found only in the mesic Mediterranean climate caves. We could not find effects of the estimated age of the cave, its estimated size, nor the cave ecological zone on spider assemblages. In one analysis (see supporting information), we found a significant effect of the surrounding rock type on the spider assemblage, but most of our caves are located in carbonate rocks (27 of 35) and more samples from basaltic, chalk, marlstone, salt, and sandstone caves are needed in order to have a more balanced analysis (see Appendix A). Additionally, we cannot separate between the geographic location of the cave and its surrounding rock, as some rocks are found solely in one region.

5. Conclusions

To conclude, Levantine cave spider assemblages are diverse, with higher species richness and abundance in caves located in the north of Israel. They tend to have high levels of bat guano and high humidity. We suggest that future research on Levantine cave arachnids focus on caves in the mesic Mediterranean climate region, caves with bats, and caves in arid climate regions that have chambers with high humidity. Our faunistic cave survey is the first and crucial stage in understanding the evolutionary and ecological mechanisms of speciation of arachnids in Levantine caves. Our discoveries contribute to the knowledge of the local arachnofauna in general and are important for conservation of these cave ecosystems. By comparing spider assemblages of Levantine caves to European caves, we identified gaps in the taxonomic research, and expect to focus our efforts on spider families that may have additional cryptic or undescribed cave-dwelling spider species: Agelenidae, Dysderidae, Linyphiidae, and Pholcidae.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13050179/s1>, Figure S1: CCA ordination graph of the first and second axes testing 34 caves with ecological zones (total 67 samples), and 62 spider species. The significant explanatory variables (precipitation, elevation, latitude, no bats, frugivorous bats and insectivorous bats, low, medium and high guano levels, carbonate and salt rocks) are plotted on the graph, as well as the cave, ecological zone, and species names. Cave sizes are represented by large, medium, and small triangles, respectively; troglobite species by black points; troglophile species by grey points; accidental by empty points; nominal explanatory variable by a black star; continuous explanatory variables by arrows with dashed line, while non-dashed line arrows are used to connect between species name and its centroid; Table S1: LUX values for caves and caves ecological sectors (first visit; second visit). “No” denote that the ecological sector was missing due to cave size, “Missing” denote a measurement that was not taken. * measurement was taken just before sunset; Table S2: Full list of the spider species with their localities, distribution and guilds; Table S3: CCA ordination results (67 samples), and 62 spider species); Table S4: CCA values for significant environmental variables

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

District	Geographic Region	Cave	N	E	Elevation	Climate Type	Precipitation (mm)	Cave Minimum Temp. (E, T, D)	Lithology Category	Lithology Geological Age	Cave Size	Length Estimate	Bat	Guano Level	Spider Abundance, Richness
North	Upper Galilee	1 Shetula (P)	33.0873	35.3169	690	Mediterranean	840.4	11.5, 14.5, 15.5	Carbonate rocks	Cenomanian	Large	150	Insectivorous	Low	21, 6
		2 Yir'on (cave 1) (P)	33.0679	35.4665	528		716.4	16.5, 13, 20		Eocene	Large	150	Insectivorous	High	49, 5
		3 Yir'on (cave 2) (P)	33.0672	35.4672	541		716.4	8			Small	10	without	Zero	26, 8
		4 Pelekh (P)	32.9324	35.238	488		648.5	12.5		Turonian	Small	5	without	Zero	16, 5
		5 Yonim (P)	32.9236	35.2168	216		648.5	12.5, 12.5			Medium	50	without	Zero	61, 14
	Golan	6 Susita (P)	32.7793	35.6577	70		382.5	21, 21	Basalt	Pliocene	Medium	50	Insectivorous	Low	31, 5
	Lower Galilee	7 Berniki (cave 3) (P)	32.7775	35.5401	−102		455	17	Carbonate rocks	Turonian	Small	5	without	Zero	23, 6
		8 Berniki (cave 1) (P)	32.7775	35.5401	−102		455	19.5, 17, 20.5			Large	480	Insectivorous	High	53, 6
		9 Berniki (cave 2) (P)	32.7768	35.5413	−166		455	12.5, 12.5			Medium	15	without	Zero	86, 11
	Karmel	10 Oren (P)	32.7144	34.9749	73		611.2	16.5, 15.5			Medium	36	without	Zero	55, 5
		11 Horvat Raqqit (P)	32.7128	35.0123	355		611.2	14.5		Cenomanian	Small	3	without	Zero	8, 5
		12 Ezba' (P)	32.7118	34.9747	120		611.2	14.5, 15.5, 18		Turonian	Large	52	Frugivorous	High	58, 7
Central Israel and Palestine	Northern Samaria	13 Sal'it (P)	32.2454	35.0456	254	Hyper-arid	620.4	17, 18, 19.5		Cenomanian	Large	108	without	Zero	26, 6
	Central Jordan Valley	14 Andartat HaBiq'a' * (E)	32.0524	35.4589	−184		19.9	19.5, 25.5	Carbonate rocks	Turonian	Medium	23.5	Insectivorous	Medium	31, 10
	Western Samaria	15 Oah (P)	32.0053	34.9722	123	Mediterranean	569	16			Small	10	without	Zero	19, 7
		16 Bet 'A'rif (P)	32.0026	34.9642	95		569	15.5, 18, 18			Medium	45	without	Zero	42, 7
		17 Tinshemet (P)	31.9994	34.9681	100		569	15.5			Medium	43	Frugivorous	High	51, 6
	HaShfela	18 Haruva (P)	31.9133	34.9607	180		537.8	14.5, 16, 18	Chalk	ConiacianCampanian	Large	100	without	Zero	43, 9

North	Northern Judean Desert	19 Perat Southern Slope (cave 4) * (PE)	31.8334	35.3054	295	Semi-arid	250	15	Carbonate rocks	Turonian	Small	10	without	Zero	17, 6
		20 Perat 'Inbal (cave 1) * (PE)	31.8332	35.3019	314		250	17, 16.5			Medium	40	without	Zero	37, 9
		21 Perat Ro'im (cave 2) * (PE)	31.8325	35.313	238		250	13.5, 16.5			Medium	25	Insectivorous	Low	45, 9
		22 Perat (cave 3) * (PE)	31.8321	35.3083	268		250	13			Small	10	without	Zero	19, 7
	Northern Dead-Sea Area	23 Qumeran * (E)	31.7556	35.459	−308	Arid	95.8	21, 21		Cenomanian	Medium	20	without	Zero	30, 4
	Judean Mountain	24 Te'omim (P)	31.7262	35.0217	375	Mediterranean	509	13, 13.5, 14.5			Large	134	Frugivorous	High	45, 5
	South	Southern Judean Desert	25 Zavoa' (PE)	31.2086	35.2311	495	Arid	100		22, 23, 23.5	Carbonate rocks	Turonian	Large	600	Unknown
Southern Dead-Sea Area		26 Arubotayim (E)	31.1016	35.39	−348	Hyper-arid	41.1	20, 19.5, 19.5	Salt	Pliocene	Large	60	without	Zero	5, 1
		27 Sedom (E)	31.0872	35.3958	−381		41.1	20, 19.5, 19.5			Large	1800	without	Zero	28, 1
		28 Malcham (E)	31.0765	35.3971	−380		41.1	20, 19.5, 19.5			Large	10,000	without	Zero	11, 1
	29 Ne'ot HaKikkar (Nezirim Burial cave) (E)	30.9911	35.3465	−333	41.1		20.5	Marlstone	Quaternary	Small	4	without	Zero	5, 2	

Negev Desert	30 Telalim (PE)	30.9734	34.7929	482	Arid	91.4	17, 15	Carbonate rocks	Turonian	Medium	27	without	Zero	7, 2
	31 Ashalim (PE)	30.9434	34.7391	404		91.4	17.5, 19.5, 17			Large	540	Insectivorous	Low	13, 1
	32 Besor (PE)	30.9415	34.6961	356		91.4	12, 16.5			Medium	35	without	Zero	26, 5
	33 'Avedat (Nezirim cave) (PE)	30.7941	34.772	601		93.3	11		Eocene	Small	5	without	Zero	9, 2
'Arava Desert	34 'Ammude 'Amram (cave 2) (PE)	29.6518	34.9337	288	Hyper-arid	22.5	21	Sandstone	Cambrian	Small	5	without	Zero	0, 0
	35 'Ammude 'Amram (cave 1) (PE)	29.6515	34.9336	293		22.5	21, 24, 24			Large	80	Insectivorous	Low	2, 2

List of the 35 caves sampled in this study (north to south) and their environmental variables. Localities in Israel and Palestine (West Bank) and transliterated names of the localities follow the “Israel Touring Map” (1:250,000) and “List of Settlements”, published by the Israel Survey, Ministry of Labor. Geographic coordinates are given in WGS84 (decimal degrees). Cave size estimates were corrected from Mammola et al., 2019 [9]: Bet ‘Arif was changed from large to medium category, Arubotayim was changed from medium to large category, Tinsmet was changed from small to medium, and Andartat HaBiqā’ was added to medium in the current analysis.

- (1) Localities in Palestine (West Bank) are marked by asterisk (*). Letters in parentheses after cave name indicate the zoogeographical region: (P)—Palaeartic; (E)—Ethiopian; (PE)—Palaeoeremic (after Por, 1975).
- (2) Precipitation data is taken from Israel Meteorological Service (<https://ims.gov.il/en/ClimateAtlas>, 1 February 2021) for the average annual mean for 1980–2010, from the closest meteorological station for each cave.
- (3) Minimum Temperature is the average of the minimum temperature measured by us for each cave for two months (between the first and second visit to each cave) during the survey.
- (4) Length was estimated from cave maps when available, or in field, and represent the distance from the cave opening toward the darker region of the cave that we could reach.

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