DETERMINATION OF OSTRACODA (CRUSTACEA) FAUNA OF SOME FRESHWATER CAVES IN THE WESTERN BLACK SEA REGION OF TURKEY

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MAY 2011

DETERMINATION OF OSTRACODA (CRUSTACEA) FAUNA OF SOME FRESHWATER CAVES IN THE WESTERN BLACK SEA REGION OF TURKEY

by

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THESIS SUBMITTED TO

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES OF

THE ABANT İZZET BAYSAL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

IN

THE DEPARTMENT OF BIOLOGY

MAY 2011

Approval of the Graduate School of Natural and Applied Sciences.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

DETERMINATION OF OSTRACODA (CRUSTACEA) FAUNA OF SOME FRESHWATER CAVES IN THE WESTERN BLACK SEA REGION OF TURKEY

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May 2011, 63 pages

In this study nine ostracod taxa belonging to superfamily Cypridoidea were determined from 17 sampling stations of 11 caves in The Western Black Sea region. Among the taxa, eight of them (*Ilyocypris bradyi*, *I. inermis*, *Candona neglecta*, *Ilyocypris* sp., *Candona* sp., *Psychrodromus* sp. *Pseudocandona* sp. and *Heterocypris* sp.) were detected from inside and entrance of caves, but *Psychrodromus olivaceus* was recorded from outside of Çayırköyü cave. Unweighted Pair Group Mean Averages (UPGMA) distinguished the sites into 3 main clusters based on physical and ecological characteristics. Accordingly, about 85% of similarity were found among the sites. The species (*I. bradyi, I. inermis* and *C. neglecta*) reported in this present study shows cosmopolitan distribution. Thus, they have a chance of adaptation to cryptic conditions and can tolerate the ecological changes caused possibly by flooding events. Among all the nine taxa, living adult individuals of *I. bradyi, I. nermisi and C. neglecta* were reported herein for the first time from the cave environments in the literature. In addition to them, the other four taxa (*Ilyocypris* sp., *Candona* sp., *Heterocypris* sp., and *Pseudocandona* sp.) are also the new records for cave Ostracoda fauna of Turkey.

Keywords: Ostracoda, cave, cosmopolitan, distribution, ecology

ÖZET

TÜRKİYENİN BATI KARADENİZ BÖLGESİNDE BULUNAN BAZI TATLI SU MAĞARALARININ OSTRAKOD (CRUSTACEA) FAUNASININ BELİRLENMESİ

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Mayıs 2011, 63 sayfa

Bu çalışmada Cypridoidea üst familyasına ait 9 taksa, Batı Karadeniz Bölgesindeki 11 mağarada örneklenen 17 istasyondan tespit edilmiştir. Bu taksalar arasında 8 tanesi (*Ilyocypris bradyi*, *I. inermis*, *Candona neglecta*, *Ilyocypris* sp., *Candona* sp., *Psychrodromus* sp. *Pseudocandona* sp., ve *Heterocypris* sp.) mağara içlerinde ve girişlerinde, bir takson ise (*Psychrodromus olivaceus)* sadece Çayırköyü mağarasının dışında kaydedilmiştir. Ağırlıksız Çiftli Grup Ortalama Analizi, örneklenen istasyonları fiziksel ve ekolojik karakterlerine göre ve % 85 benzerlik oranı ile üç ana gruba ayırmıştır. Buna göre birbirine bölgesel olarak yakın olan istasyonların benzerliği görülmüştür. Bu çalışmada rapor edilen türler (*I. bradyi, I. inermis* ve *C. neglecta*) kozmopolitan dağılım göstermektedir. Bu nedenle, bu türler kapalı ortamlara ve sel gibi dış olaylarla değişen ekolojik değişmelere adapte olma ve bu değişimleri tolere etme şanslarına sahiptirler. Bu dokuz taksa arasında *I. bradyi*

I. inermis ve C. neglecta türlerinin canlı ve ergin bireyleri mağara ve ortamlarından ilk kez bu çalışmada bildirilmiştir. Bunlara ek olarak, diğer dört takson (*Ilyocypris* sp., *Candona* sp., *Heterocypris* sp., ve *Pseudocandona* sp.)'da Türkiye'nin mağara Ostrakoda faunası için yeni kayıttır.

Kelimeler: Ostrakoda, mağara, kozmopolitan, dağılım, ekoloji

In remembrance of my mother

Zahide YAVUZATMACA

ACKNOWLEDGEMENTS

I am extremely grateful and indebted to my advisor Prof. Dr. Okan KÜLKÖYLÜOĞLU for his support, encouragement, inspiration, guidance and critical discussions at all stages of my master study. I would also like to thank Geologist Hamdi MENGİ for his guidance and help on selection the caves and supplying the additional information about the caves. I express my thanks to my friends Elif BAŞAK and Necmettin SARI for their help during sampling time. I want to thank to Assoc. Prof. Dr. Mustafa ÜNAL (Abant İzzet Baysal University) for diagnosis of grasshopper. I also want to thank to Assoc. Prof. Dr. Murat ÖZBEK (Ege University) and Assist. Prof. Dr. Cem AYGEN (Ege University) for diagnosis of gammarus and zooplankton, respectively. My thanks also goes to Dr. Marie Jose OLIVIER (Lyon University) for providing information on cave ostracods in France. This study was supported by Scientific Research Project Agency of Abant İzzet Baysal University (Project No. 2010.03.02.354).

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CHAPTER I

1. INTRODUCTION

The class Ostracoda (Latreille, 1806) are the most diverse group of suphylum Crustacea in phylum Arthropoda. The name "ostracod" is derived from Greek word "ostrakon" which meaning as "shell" (Meisch, 2000). They are widely distributed small bivalved aquatic invertebrates, usually 0.3-5 mm long but, some marine species exceed 30 mm in length (Dolermo, 1991; Meisch, 2000). The number of both marine and non-marine ostracod species have been detected approximately 65,000 and in which about 20,000 living species may be exist (Ikeya et al., 2005).

Ostracoda are divided into two sub-classes (Myodocopa and the Podocopa) and five orders (Myodocopida, Halocyprida, Paleocopida, Platycopida and Podocopida) (Horne et al., 2002). Subclass Myodocopa consists of Myodocopida and Halocyprida orders and the other three orders Paleocopida, Platycopida and Podocopida represent the Subclass Podocopa (Horne, 2003). The three of these orders (Myodocopida, Platycopida and Podocopida) are termed as the living lineages of Ostracoda. Platycopida and Myodocopida are exclusively marine and the order Podocopida which includes brackish, marine and freshwater or all non-marine ostracods. The non-marine Ostracoda are comprised of three superfamilies that are Cypridoidea, Cytheroidea and Darwinuloidea. The main distinctive features of Ostracods are their calcified carapace (Meisch, 2000). They are also known as "oldest microfauna" (Dolermo, 1991) because the carapace is easily fossilized and preserved in sediments (Holmes and Horne, 1999). Therefore, these calcified shells are used for paleoclimatic and paleolimnological studies. Over the last few decades, ostracods have been used to obtain information about past history of environmental conditions, to reconstruct paleoclimatic changes, and to provide information about ecological conditions of aquatic systems (Forester, 1991; Smith, 1993; Holmes et al., 1998, Külköylüoğlu, 1998; Mischke et al., 2005; Mischke and Wünnemann, 2006; Wünneman et al., 2006; Jiang et al., 2008).

1.1. Morphology of Ostracoda

Ostracoda are bivalved crustaceans. Their most distinctive feature is the carapace (calcium carbonated) consisting of two dorsally articulated valves for protection of their soft body parts and these valves connected to each other by a dorsal hinge. Therefore, carapace completely enclosed the appandages of organisms which include pair of limbs. The valves are mainly closed by central adductor muscles whose traces on each valve called "muscle scars" can be seen from outside view (Meisch, 2000). The eye structure is different between orders of Ostracoda, the order Myodocopida have compound eyes but the orders Podocopida, Platycopida, Palaeocopida and Halocyprida have "nauplius" eyes like a dark spot on the dorsal part of the carapace (Ikeya et al., 2005). In adult form of Ostracoda, there are eight paired appendages present as antennule (A1), antennae (A2), mandible (Md), maxillule (Mx1), first thoracopod (T1), second thoracopod (T2), third thoracopod (T3), and copulatory organ/uropods (Meisch, 2000; Martens, 2003). Like other crustaceans, ostracods grow by moulting and after eight moulting stages they become adult.

1.2. Ecology of Ostracoda

Ostracods are widely distributed over a range of marine and non-marine aquatic habitats and sometimes in semi-terrestrial habitats including, temporary or permanent ponds, lakes, springs, streams, trough, ditches, irrigation canals, caves, in most organic mats and in cups of certain plants (Dolermo, 1991; Meisch, 2000). This wide distribution rate depends mostly on their active or passive transportation. Moreover, the active transport is by species themselves but the passive transport is by human, birds, fishes (Rossi et al., 2003) and their resting eggs are distributed by wind (Horne and Martens, 1998). They are distributed over a range of area. Which makes Ostracoda to be sensitive to the physical, chemical and ecological attributes of different aquatic habitats (Benson, 1990; Külköylüoğlu and Vinyard, 2000) and show different tolerance levels to biotic and/or abiotic environmental variables. Besides, their distribution is correlated to water quality and ecological preference of species (Mezquita et al., 2001; Külköylüoğlu, 2003; Viehberg, 2005; Mischke et al., 2007). The different response to environmental changes show that ostracods can be used as an indicator species to determine water quality and temporal changes in environment caused by anthropogenic, seasonal and climatic events (Külköylüoğlu and Vinyard, 2000; Mezquita et al., 2001; Külköylüoğlu, 2004; Külköylüoğlu and Dügel, 2004; Ruiz et al., 2004). Since their occurence is related to several factors, it is important to understand the value of them (Külköylüoğlu, 2004). In addition the ecological requirements of individual species is important (Külköylüoğlu, 2003) for estimation of past, current and future habitat conditions (Dolermo, 1991; Külköylüoğlu, 2005b). Hence, using ostracods as bioindicator with inadequate knowledge about their ecological preferences may not provide sufficient results. Therefore, the relationship between ostracods and environmental variables should be adequately investigated by measuring physical and chemical variables in aquatic habitats (Külköylüoğlu, 2003; Li et al., 2010) along with correct taxonomic description.

In the concept of indicator species, this question may be considered "Why the indicator species is important?" This is because, "any changes in environmental or habitats are detected by sensitive biotic integrator of condition over time" (Sarı and Külköylüoğlu, 2010). The antropogenic events may be important negative factors on the species diversity. The consequences of such events leads to changes in physical and chemical variables of aquatic habitats, thus reducing species diversity (Lake and Bermuta, 1986; Death and Winterbourn, 1995; Verschuren et al., 1999). In such a case, competitor species may have advantages due to their relatively high tolerances to such changes, so they are widely distributed in these habitats. In this view the phenomenon of "pseudorichness" proposed by Külköylüoğlu (2004) can be used to describe the levels of deterioration. The concepts agrees that the ratio of specialist or native species to cosmopolitan species tends to be decrease. This is an indication of reduction in water quality. Therefore, the bioindicator species can provide useful information concerning the habitat quality.

1.3. Cave Science

Speology has been defined as the science of investigation the caves. In the same sense, the biospeology is termed as the biology of caves. Therefore, the biospeologist studies the cave organisms,their habitats and cave ecosystems (Taylor, 2006).

1.4. Formation of Caves

Cave is the naturally formed underground cavity which comprise of an opening is large enough for human pass and enter a distance where sunlight cannot reach. In addition, there are some formations present in caves, which are stalagmites and stalactites as a result of deposition of minerals from dissolved rocks by dripping waters (Ozansoy and Mengi, 2006).

Different rock types form the caves. Most caves especially found in Karst areas are carbonate rocks (dolomite or limestone) in other words in soluble rocks. The draining water underground collects CO_2 and become acidic and so the carbonate rock dissolve and then form caves. Some other cave types are Lava caves, Sea caves and Sandstone caves (Garstang et al., 2008).

1.5. Cave Zonation

Caves are divided into four distinct zones that are Entrence Zone, Twilight Zone, Transition Zone and Deep Zone (Mohr and Poulson, 1966).

1.5.1. Entrence Zone: The zone, there is the connection of underground with surface area and receive sunlight so show variable temperature, they may contain green plants.

1.5.2. Twilight Zone: This is a region, where light diminishes to zero or absent, so they do not have plants.

1.5.3. Transition Zone: This is a zone of partial darkness or slight and increase or decrease in temperature and moisture may be felt.

1.5.4. Deep Zone: This is a region of total darkness. No traces of light and is the deepest zone. The air temperature of that part remains constant over all of year and humidity is high because of the low rate of evaporation.

1.6. Cave Ecosystems

Caves have different habitat types that are divided into terrestrial (land area) or aquatic (water area) and another division is based on the light zone (photic zone). When you compare the cave habitats with surface habitats, the underground habitats have scarce food because the food supply to cave inhabitants comes from some region of the surface area and which implies that the green plants cannot grow in cave, except some plants like mosses and liverworts may be encountered at the entrance of caves (Ozansoy and Mengi, 2006) The food transportation into the caves can be by flowing water, and others are by air dispersal , e.g. spores of pollen, organic nutrients, eggs, guano (feces of bats), some insects and animal also get as indirect agents of food dispersal. The organic materials in eggs, guano and in washed plants are available for fungus and bacteria which break them down into simple nutrients for other organisms (Gibert and Veng, 2002). According to Humphrey (2009) groundwater ecosystems are complex structure because they contain a lot of ecological niches due to water cathment. Therefore, the adaptation depends on time, distance along with a flow path or distance from surface. In addition to that, the amount of dissolved organic matter used by animals is decreased due to reduction in downstream flow. However, such reduction in flow eventually play crucial role on delimiting energy sources for groundwater ecosystems. This is also important because such energy in this ecosystem depend solely on those imported organic matter from outside as the only source of food for the organisms.

Changes occurring in the fluctution of organic matter in the groundwater system can cause spatio-temporal heterogeneity inside the caves. The platform or base of every food chain are decomposer like fungus and bacteria, whish supports the herbivores and the carnivores. (Gibert and Veng, 2002). Due to, this sequences the nutrients in caves are mostly originated from outside (allochthonous materials). Therefore, if connection of cave to surface water is weak, the rate of water current into the caves is reduced. Thus, the food transportation and sedimentation rate is negatively effected by such reduction, which is a negative effect on the troglobitic species composition (Maddocks and Iliffe, 1986).

1.7. The Life in Caves

The most important group of organisms that live in caves are originated from the surface species by thousands or even millions of years ago. Danielopol et al. (1994) stated that ecological flexibility, width of ecological tolerance, type of preadaptation and the capacity of species to perceive and invade new habitats can effect ostracod species to migrate or be dispersed through subterranean and epigean aquatic habitats. This moving of animals from surface to cave is explained by

regressive evolution, which indicates that species evolved from animals with pre-adaptation or traits well suited for cave and where the effect of biological factors as predation and competition that is much reduced than when compared to open aquatic environments (Vermeij, 1987) or by progressive adaptive changes especially based on morphology and habitat selection (Tabuki and Hanai, 1999). Also Rouch and Danielopol (1987) suggested this movement by "active migration model" and "the refuge under constrains paradigm" that are especially generalist species (e.g., some (if not all) cosmopolitan species) move subterranean enviroments when the surface water is not suitable and the second way is for protection from predators, respectively. The cave dwelling species have troglomorphic characters (lack of eyesight, un-pigmented) and small bodied (Maguire, 1960; Christiansen, 1962) but, developing the other features for example, longer antennal aesthetascs (chemo-sensorial setae), elongated feeler and appendages when compared to their epigean relatives (Danielopol et al., 2000). Moreover, dwelling caves show low reproductive rates, slower metabolisms or slow growing rate and are good bioaccumulator because of poor amount of nutrients as a result of that the species have a long ontogenic periods (Culver, 1982; Humphreys, 2009). Therefore, the distribution of stygobiotic species is low because nutrients availability constraints their dispersion (Ferreira et al., 2007).

Biologists classify the cave animals due to their adaptive degrees and so the terrestrial cave dwellings have prefix **Troglo** but aquatic dwellings have prefix **Stygo**. According to this classification, there are three types of organisms;

1.7.1. Trogloxenes or Stygoxenes; organisms spend much of their time above ground for food, mate or part-time shelter the caves for nesting or hibernation (eg; bats and rodents).

1.7.2. Troglophiles or Stygophiles; complete their life cycles above or underground and leave from the cave only for food and if suitable habitats is existed above ground they can survive on there (earthworms, spiders, beetles, etc…).

1.7.3. Troglobites or Stygobites; they survive only underground and cannot survive above ground. They adapted to darkness and complete their life cycles in there. They have long antennae for vibrational sensory function, a good sense of smell, long fins or legs, smaller bodies than surface species and long life span (crustaceans, insects, arachnids, etc…) (Gibert et al., 1994; Gibert and Veng, 2002; Taylor, 2006).

1.8. Previous Works on Caves in Turkey

In Turkey, besides some other taxonomic groups (e.g., Arachnida, Crustacea, Insecta, etc…), there is a few records of cave Ostracoda. Since the high number of caves present in Turkey such reports and studies are not enough (Nazik, 1985). In other words, the present biospeologic studies are negligible due to the abundance of caves.

The first faunistic study of caves in Turkey to be done by Mr. Miralay Dr. Abdullah in Yarımburgaz cave (İstanbul) in 1865. During the 20th century Professor Curt Kosswig collected some specimens from the caves of Anatolia and published articles about them (Kunt et al., 2010). Some of them are about Isopoda (Verhoeff, 1936), Diplopoda (Verhoeff, 1943), Insecta (Coleoptera) (Jeannel, 1947). Moreover, then the Knuth Lindberg firstly studied the Ilıksu cave in Zonguldak in 1952 (Kunt et al., 2010) and detected the 150 m of the stream passing into that cave and collect specimens and than continue studied the speologic area of Turkey especially the research topic was Copepoda (Crustacea). Hartmann (1964) reported some Ostracoda (Crustacea) species from cave environments in Zonguldak (Table 1). Çağlar (1965) also worked on the grundwater fauna of bats (Chiroptera) in Turkey. In the study of Pesce (1992) the Cyclopids (Crustacea, Copepoda) and description of the *Diacyclops languidoides anatolicus* n. ssp based on 1987 collection from different groundwaters of Turkey were emphesized.

In addition, during the 21st century, there were some master thesis about biospeology (Erkan, 2002, and Paksuz, 2004). Balık et al. (2002) reported the 2 taxa of Chordata, 2 of Mollusca and 8 of Arthropoda from Yelköprü Cave (Dikili, İzmir). Additionally, the two new cave dwelling species of beetles; Laomostenus (Antisphodrus) from western Anatolia (Coleoptera, Carabidae) were reported by Casale et al. (2003). Topçu and Kunt (2005) published a list of cave Insecta of Turkey. Özbek and Güloğlu (2005), reported a new species of Amphipod from Peynirlikönü cave, Anamur, Turkey. Demir et al. (2008), record a new species of spider, Palliduphantes (Araneae: Linyphiidae) from Manaspoli Cave. Kunt et al. (2008a) record the three species of Nesticidae from different caves of Turkey and also Kunt et al. (2008b) published the speleofauna of Dim cave for finding 25 taxa of Arthropoda. Topçu et al. (2008) recorded the *Troglohyphantes karolianus* (Araneae: Linyphiidae) which is a new species from İnögü cave in Konya.

1.9. Studies on Groundwater Ostracoda out of Turkey

Maguire (1960) studied the cavernicolous ostracods of two different caves and lethal effect of light on them. Danielopol (1982) reported three groundwater Candoninae (Ostracoda, Crustacea) from different parts of Romania. Maddocks and Iliffe (1986) described the 33 ostracod species from 24 inland marine caves of Bermuda. Angel and Iliffe (1987) described an halocyprid ostracod belonging to erected primitive subfamily Deeveyinae in eight anchialine caves on Bermuda. *Cypria cavernae* was reported from caves of Trieste (Italy) by Wagenleitner (1990). Maddocks and Iliffe (1991) reported 12 species of freshwater podocopid ostracods from caves of Australia and New Zealand. The two new stygobiont species of Candoninae from Montenegro described by Karanovic (1999). Tabuki and Hanai (1999) reported a troglobitic sigillid genus, Klasella, from submarine caves of Ryukyu Islands. *Danielopolina kornickeri* sp. n. (Ostracoda, Thaumatocypridoidea) were described from an anchialine cave in Western Australia by Danielopol et al. (2000a). The 21 genera and around 110 groundwater ostracod species recorded in Pilbara, Australia by Reeves et al. (2007). Gido et al. (2007) described the stygobiotic *Dolekielle europaea nov. sp. nov.* (Ostracoda, Limnocytheridae) from Southern France. Ferreira et al. (2007) reported 23 species of underground water of France. Petkovski et al. (2009) reported some freshwater ostracods in Herzegovina that collected from three caves and in one riverine interstital site. The two specimens belongs to genus Shellecandona were determined by Pieri, et al. (2009) from undergroundwater in Friuli Venezia Giulia (Northeast Italy). Also, some of the Ostracoda (Crustacea) species/taxa reported previously from cave environments are listed in Table1. In this table the species especially from freshwater cave and cave connection with freshwater are preferred. The present study is on freshwater cave ostracods, therefore, we did not give the species marked from marine caves on the table. In the table, some species are written more than one because of showing their distribution (Table 1). Besides, there are many previously reported species from marine caves. However, the number of species or taxa in Table 1 is not absolute, that may changeable because of overlooked literature.

By having about 40.000 caves, Turkey has an important richness. The 1300 of them were certified by General Directorate of Mineral Research and Exploration (MTA) of Turkey (Ozansoy and Mengi, 2006). Due to these abundance of cave formations, the researches about cave dwelling organisms are not enough. Most of the present studies are not programmed research and depend on chance of sampling (Kunt et al., 2010). Therefore, we choose this topic for investigation. This is the first study in that perspective about Cave Ostracoda in Turkey. The aim of this study includes 1) to determine the ostracod fauna of 11 caves selected in the Western Black Sea region, 2) to contribute knowledge on Ostracoda diversity and ecology, 3) and to emphasize the similarity between caves and their species compositions.

Table 1: The species or taxa previously reported from different cave environments (abbreviations; a: freshwater cave, b: anchialine cave, c: inland marine cave and d: unknown cave)

Name of species	**a**	**b**	**c**	**d**	**Location**	**Reference**
Pseudocandona trigonella				+	Slovenia	Klie, 1931
Pseudocandona cavicola				+	Slovenia	Klie, 1935
Pseudocandona dispar	+				Zonguldak, Turkey	Hartmann, 1964
Ilyocypris gibba	+				Zonguldak, Turkey	Hartmann, 1964
Ilyocypris decipiens	+				Zonguldak, Turkey	Hartmann, 1964
Psychrodromus olivaceus	+				Zonguldak, Turkey	Hartmann, 1964
Potamocypris fallax				+	Britain	by Fox (1967) in Proudlove et. al., 2003
Candona sp.	+				Texas	by Charles D. Wise in Maguire, 1960
Batucyprattinae new subfamily	+				West Malaysia	Victor and Fernando, 1981
Batucypretta paradoxa n. gen.n.sp.	+				West Malaysia	Victor and Fernando, 1981
Mixtacandona botosaneanui	+				Romania	Danielopol, 1982
Deeveya spiralis		+			Turks and Caicos Islands	Kornicker and Iliffe, 1985
Spelaeoecia barri		+			Bahama	Kornicker and Iliffe, 1985
Deeveya sp.		+			Bahama	Kornicker and Iliffe, 1985
Deeveya styrax		+			Bahama	Kornicker and Iliffe, 1985
Deeveya hirpex		+			Bahama	Kornicker and Iliffe, 1985
Spelaeoecia parkeri		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina bahamensis		+			Bahama	Kornicker and Iliffe, 1985
Deeveya jillae		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina exuma		+			Bahama	Kornicker and Iliffe, 1985
Spelaeoecia capax		+			Bahama	Kornicker and Iliffe, 1985
Spelaeoecia styx		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina exuma		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina kakuki		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina sp.		+			Bahama	Kornicker and Iliffe, 1985
Danielopolina exleyi		+			Bahama	Kornicker and Iliffe, 1985
Cytherella bermudensis			+		Bermuda	Maddocks and Iliffe, 1986
Cytherella kornickeri			+		Bermuda	Maddocks and Iliffe, 1986
Cytherelloidea irregularis			+		Bermuda	Maddocks and Iliffe, 1986
Neonesidea omnivaga			+		Bermuda	Maddocks and Iliffe, 1986

Table 1 continued

Paranesidea bensoni			+		Bermuda	Maddocks and Iliffe, 1986
Aponesidea iliffei			+		Bermuda	Maddocks and Iliffe, 1986
Havanardia keiji			+		Bermuda	Maddocks and Iliffe, 1986
Glyptobairdia cororata			+		Bermuda	Maddocks and Iliffe, 1986
Anchistracheles hartmanni			+		Bermuda	Maddocks and Iliffe, 1986
Propontocypris minacis			+		Bermuda	Maddocks and Iliffe, 1986
Propontocypris sp.			+		Bermuda	Maddocks and Iliffe, 1986
Propontocypris (Ekpontocypris) lurida			+		Bermuda	Maddocks and Iliffe, 1986
Argilloecia sp.			+		Bermuda	Maddocks and Iliffe, 1986
Pontocyprididae n. gen. n. sp.			+		Bermuda	Maddocks and Iliffe, 1986
Heterocypris punctata			+		Bermuda	Maddocks and Iliffe, 1986
Candona sp.			+		Bermuda	Maddocks and Iliffe, 1986
Dolerocypria bifurca			+		Bermuda	Maddocks and Iliffe, 1986
Paracypris crispa			+		Bermuda	Maddocks and Iliffe, 1986
Paracypridinae spp.			+		Bermuda	Maddocks and Iliffe, 1986
Cyprideis edentata			+		Bermuda	Maddocks and Iliffe, 1986
Hemicytherura bradyi			+		Bermuda	Maddocks and Iliffe, 1986
Microcytherura sp.			+		Bermuda	Maddocks and Iliffe, 1986
Jugysocythereis pannosa			+		Bermuda	Maddocks and Iliffe, 1986
Neocaudites navianii			+		Bermuda	Maddocks and Iliffe, 1986
Occultocythereis angusta			+		Bermuda	Maddocks and Iliffe, 1986
Callistocythere sp.			+		Bermuda	Maddocks and Iliffe, 1986
Loxoconcha oculocrista			+		Bermuda	Maddocks and Iliffe, 1986
Cobanocythere sp.			+		Bermuda	Maddocks and Iliffe, 1986
Paradoxostoma sp.			+		Bermuda	Maddocks and Iliffe, 1986
Xestoloberis spp.			+		Bermuda	Maddocks and Iliffe, 1986
Polycope spp.			+		Bermuda	Maddocks and Iliffe, 1986
Myodocopina spp.			+		Bermuda	Maddocks and Iliffe, 1986
Kennethia major		+			New Caledonia	Maddocks et al., 1991
Mungava sp.		+			New Caledonia	Maddocks et al., 1991
Paracypria n. sp.		+			New Caledonia	Maddocks et al., 1991

Table 1 continued

Candona sp.		+			New Caledonia	Maddocks et al., 1991
Darwinula sp.		+			New Caledonia	Maddocks et al., 1991
Dolerocypria n. sp.		+			New Caledonia	Maddocks et al., 1991
Cypretta sp.		+			New Caledonia	Maddocks et al., 1991
Heterocypris sp.		+			New Caledonia	Maddocks et al., 1991
Cypretta minna	+				Australia	Maddocks and Iliffe, 1991
Cypretta viridis	+				Australia	Maddocks and Iliffe, 1991
Candonocypris incosta	+				Australia	Maddocks and Iliffe, 1991
Cypridopsis sp.	+				Australia	Maddocks and Iliffe, 1991
Gomphodella maia	+				Australia	Maddocks and Iliffe, 1991
Heterocypris incongruens	+				Australia	Maddocks and Iliffe, 1991
Sarscypridopsis cf. aculeata	+				Australia	Maddocks and Iliffe, 1991
Newhamia fenestrata	+				Australia	Maddocks and Iliffe, 1991
Candona sp.	+				Tasmania, New Zealand	Maddocks and Iliffe, 1991
Scottia sp.	+				New Zealand	Maddocks and Iliffe, 1991
Danielopolina elizabethae		+			Jamaica	Kornicker and Iliffe, 1992
Spelaeoecia jamaicensis		+			Jamaica	Kornicker and Iliffe, 1992
Pontopolycope mylax		+			Jamaica	Kornicker and Iliffe, 1992
Spelaeoecia styx		+			Bahama	Kornicker and Iliffe, 1998
Danielopolina exuma		+			Bahama	Kornicker and Iliffe, 1998
Spelaeoecia capax		+			Bahama	Kornicker and Iliffe, 1998
Deeveya exleyi		+			Bahama	Kornicker and Iliffe, 1998
Danielopolina sp.		+			Bahama	Kornicker and Iliffe, 1998
Spelaeoecia bermudensis		+			Bermuda, Baham	Kornicker and Iliffe, 1998
Trajancandona natura	+				Montenegro	Karanovic, 1999
Trajancandona particula	+				Montenegro	Karanovic, 1999
Genus Pseudocandona				+	United States	Culver et al., 2000
Danielopolina kornickeri		+			Australia	Danielopol et al., 2000b
Polycope sp.				+	Lecce (Italy)	Karanovic and Pesce, 2001
Pseudolimnocythere hypogea				+	Lecce (Italy)	Karanovic and Pesce, 2001
Plesiocypridopsis newtoni				+	Lecce (Italy)	Karanovic and Pesce, 2001
Cryptocandona brehmi				+	Japan	Namiotko and Danielopol, 2001
Candona candida				+	Britain	Proudlove et al., 2003
Pseudocandona sywulai	+				Croatia	Namiotko et al., 2004

Table 1 continued

Pseudocandona jeanneli				+	Indiana	Indiana Naturel Data Center, 2005
Pseudocandona marengoensis				+	Indiana	Indiana Naturel Data Center, 2005
Sagittocythere barri				+	Indiana	Indiana Naturel Data Center, 2005
Pseudocandona jeanneli				+	?	Lewis and Lewis, 2005
Pseudocandona sp.				+	?	Lewis and Lewis, 2005
Dolerocypria iliffei		+			New Caledonia	Maddocks, 2005
Paracypria ubaris		+			New Caledonia	Maddocks, 2005
Mungava woutersi		+			New Caledonia	Maddocks, 2005
Mungava xariessa		+			New Caledonia	Maddocks, 2005
Pseudocandona zschokkei				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Cavernocypris subterranea				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Psychrodromus betharrami				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Sphaeromicola cebennica cebennica				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Sphaeromicola hamigera				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Sphaeromicola topsenti				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Fabaeformiscandona breuili				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Schellencandona sp. *schellenbergi*				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Schellencandona sp. *insueta*				+	France	Ferreira et al., 2007 by mail from M.J. OLIVIER
Candona sp.	+				Herzegovina	Petkovski et al., 2009
Ilyocypris sp.	+				Herzegovina	Petkovski et al., 2009
Cypridois vidua (1 valve of instar)	+				Herzegovina	Petkovski et al., 2009
Pseudocypridopsis sywulai n. sp.	+				Herzegovina	Petkovski et al., 2009
Cypria sketi (carapace)	+				Herzegovina	Petkovski et al., 2009
Candona cf. lindneri	+				Herzegovina	Petkovski et al., 2009
Ilyocypris inermis	+				Herzegovina	Petkovski et al., 2009
Heterocypris sp.	+				Herzegovina	Petkovski et al., 2009
Shellecandona cf. aemonae	+				Herzegovina	Petkovski et al., 2009
Cypria ophtalmica	+				Herzegovina	Petkovski et al., 2009
Cyclocypris cf. globosa	+				Herzegovina	Petkovski et al., 2009
Pseudocypridopsis hartmanni n. sp.	+				Herzegovina	Petkovski et al., 2009
Candona lactea	+				Richland Country, Wisconsin	Anonymous, 2010
Candona crogmaniana	+				Richland Country, Wisconsin	Anonymous, 2010
Cypridopsis sp.	+				Richland Country, Wisconsin	Anonymous, 2010

CHAPTER II

2. MATERIAL AND METHODS

2.1. Site description

During this study, 11 caves (Söğütlü Cave, Kızılcık Cave, Sarıkaya Cave, Aksu Cave, Gökçeağaç Cave, Ilıksu Cave, Gökgöl Cave, Cumayanı Cave, Çayırköy Cave, İnağzı Cave, and Sofular Cave) from three locations (Zonguldak, Düzce and Adapazarı) in the Western Black Sea region of Turkey (Figure 1) were chosen. Because of the proximity to Bolu and to be registrated by MTA, these caves are selected. The following informations about caves visited are provided from Hamdi Mengi, unless otherwise indicated.

2.1.1. Söğütlü Cave: The cave is located at the northeast of the Adapazarı province and near Mağara village which is 12 km east of the Söğütlü county. Söğütlü cave is 470 m long and developed in the middle-upper Devonian, lower Carboniferous aged and snake formation in limestone landscape according to melt. It is spring positioning (water flowing from inside to outside of the cave), multi storey, fossil, active (water present permenantly) or half active (water present temporarily) cave with ponds produced by groundwater flow (Figure 2).

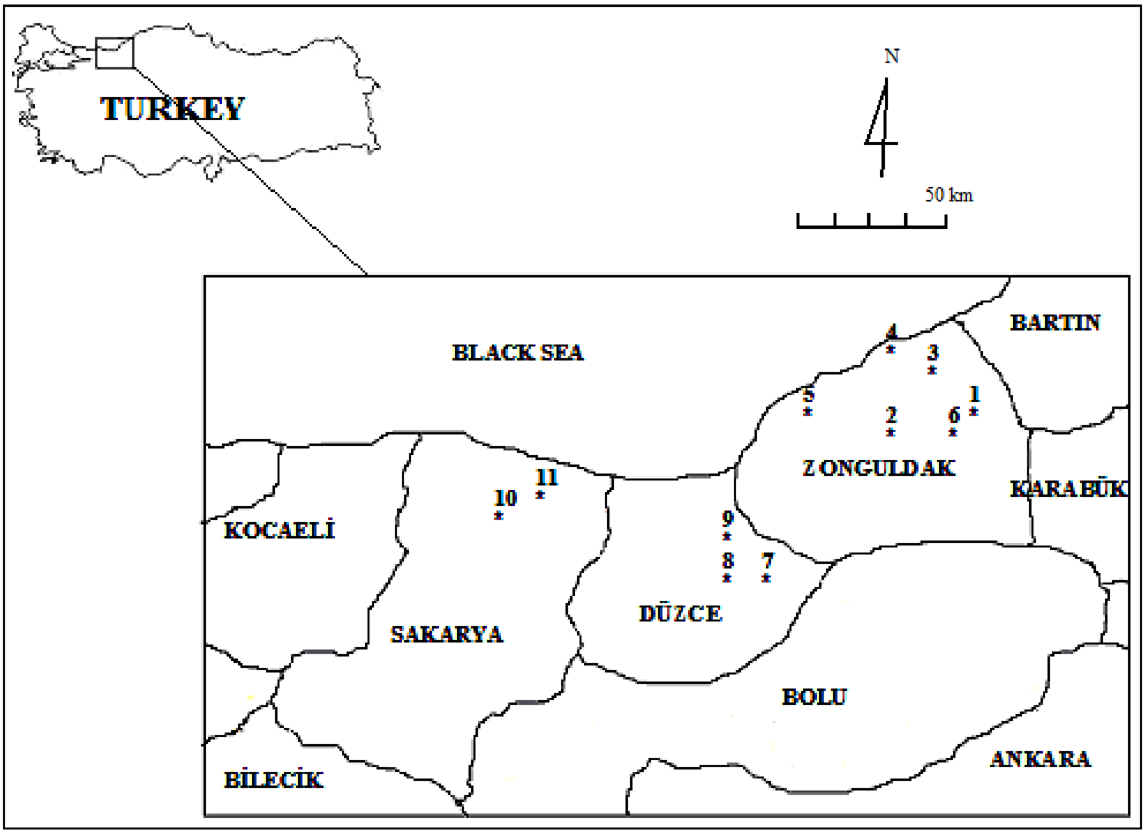

Figure 1: The figure above shows the location of eleven caves sampled from Sakarya, Düzce and Zonguldak cities in Western Black Sea region of Turkey. (abbreviations; 1: Çayırköy Cave; 2: Gökgöl Cave; 3: Cumayanı Cave; 4: İnağzı Cave; 5:Ilıksu Cave; 6: Sofular Cave; 7: Aksu Cave; 8:Gökçeağaç Cave; 9: Sarıkaya Cave,; 10: Söğütlü Cave; 11: Kızılcık Cave).

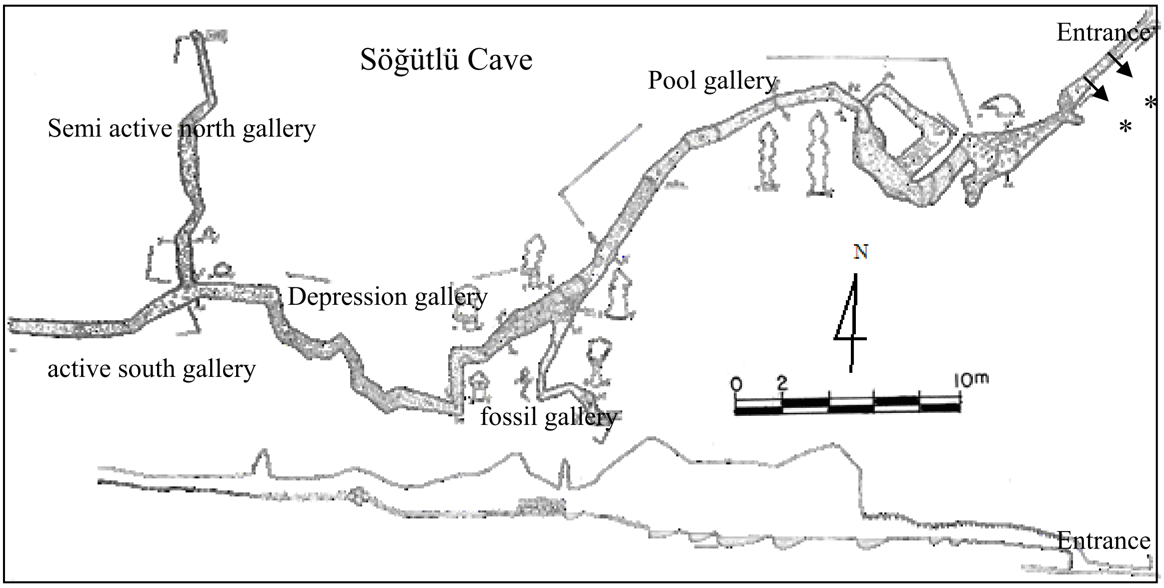

Figure 2: The map of Söğütlü Cave (* represent sampling sites) (modified from Hamdi MENGİ).

2.1.2. Kızılcık Cave: Kızılcık cave is situated in Kızılcık village located at the 7 km southest of the Karasu county in Adapazarı city. This cave is 619 m long, and the upper Creteceaus-lower Eocene aged, horizontally developed due to Akveren formation with half-active, spring positioning and contains one pond produced by groundwater flow.

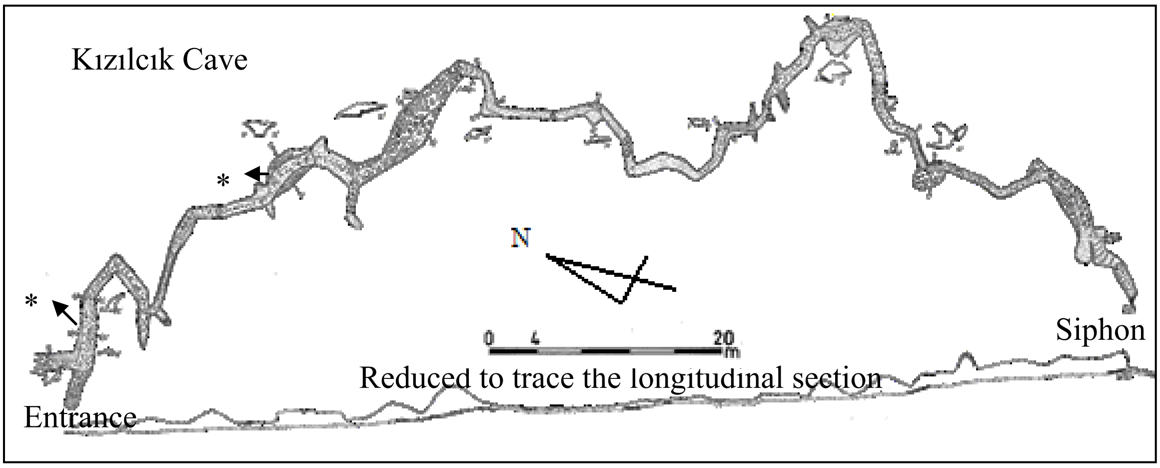

Figure 3: The map of Kızılcık Cave (* represent sampling sites) (modified from Hamdi MENGİ).

2.1.3. Sarıkaya Cave: The cave is located nearest the Sarıkaya village which to be 5 km South-west of the Yığılca county and 30 km northeast of the Düzce province. The cave (Figure 4) is 717 m long and horizontally developed in the limestone according to melt and which is a doline positioning and half-active fossil cave. There are small lakes and a waterfalls created ground creek.

2.1.4. Aksu Cave: It is the continium of the Sarıkaya cave. The cave is 896 m long, horizontally or slim slope developed in the melting limestone and an active spring positioning cave. There are small lakes, waterfalls and huge vessel created ground creek is exist (Figure 5).

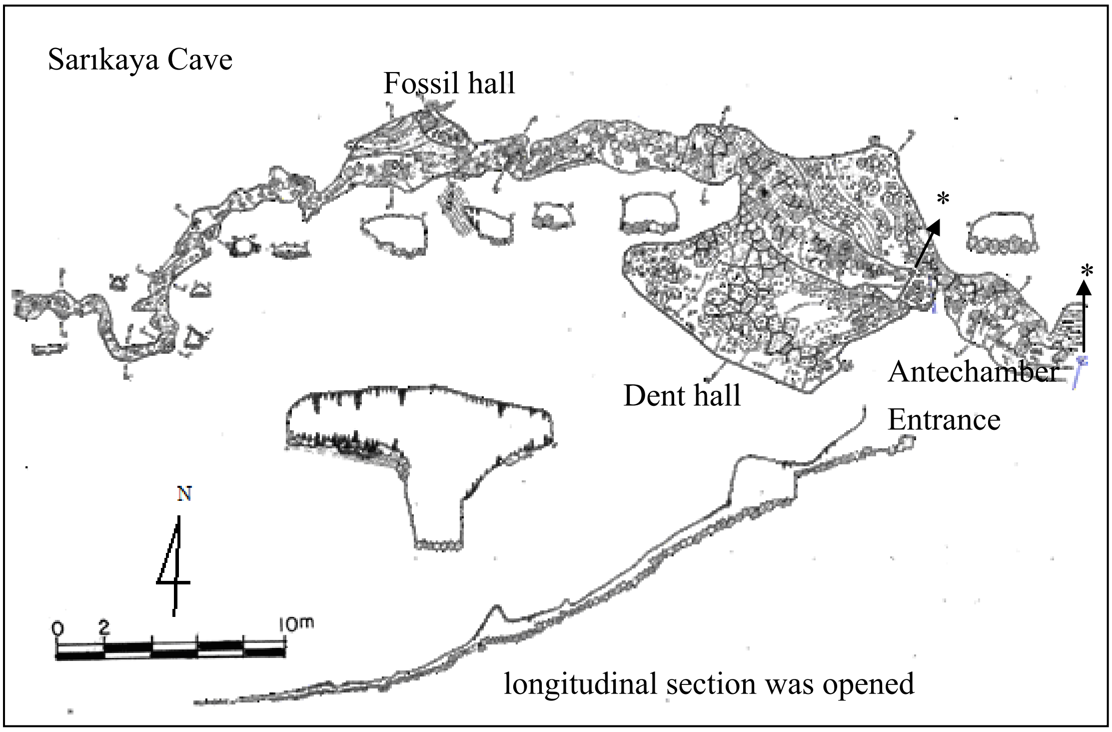

Figure 4: The map of Sarıkaya Cave (* represent sampling sites), (modified from Hamdi MENGİ).

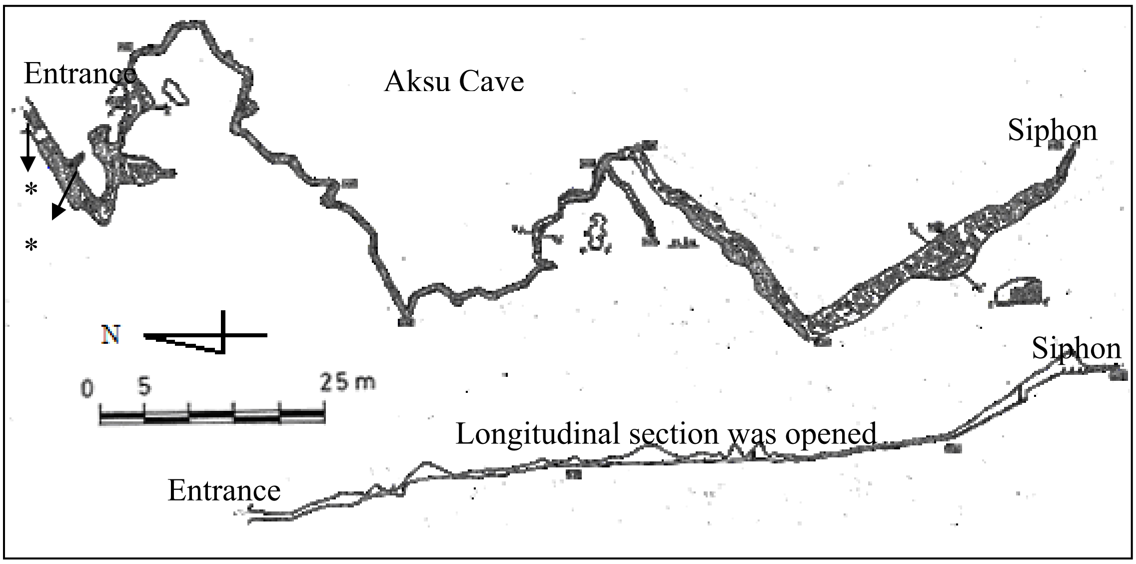

Figure 5: The map of Aksu Cave (* represent sampling sites); (modified from Hamdi MENGİ).

2.1.5. Gökçeağaç Cave: The cave is exist in the Gökçeağaç village located at about 3 km South-west of the Yığılca county which to be at the northeast of Düzce province. This cave is developed from melting limestone landscape, doline positioning (water flowing from outside into the cave), half-active and contains a small lakes produced creek.

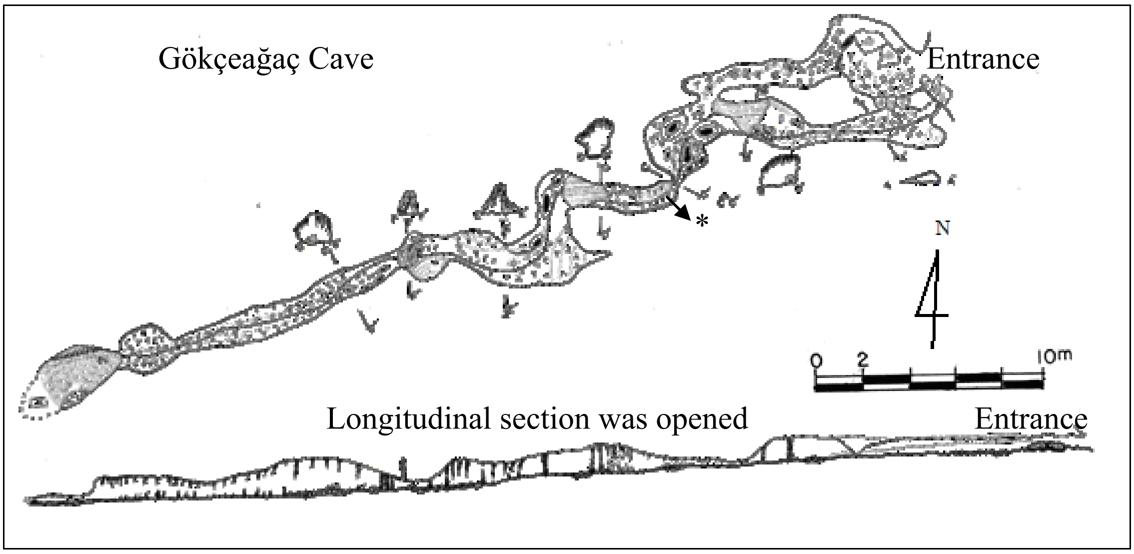

Figure 6: The map of Gökçeağaç Cave (* represents sampling site), (modified from Hamdi MENGİ).

2.1.6. Ilıksu Cave: The cave is at the Ilıksu district of the Zonguldak-Ereğli highway 12th kilometreside and which is 606 m long, developed in the limestone landscape and a spring positioning cave (Figure 7). In the cave a continiously flowing ground creek is found (Mengi, 1995).

2.1.7. Gökgöl Cave: It is located fifth km of at the Zonguldak-Ankara highway. Gökgöl cave is 3500 m long, middle-upper Devonian lower Carboniferous aged, snaked formation developed from limestone landscape and a spring positioning cave (Figure 8). Inside of the cave a continiously flowing ground creek exists (Mengi, 1995).

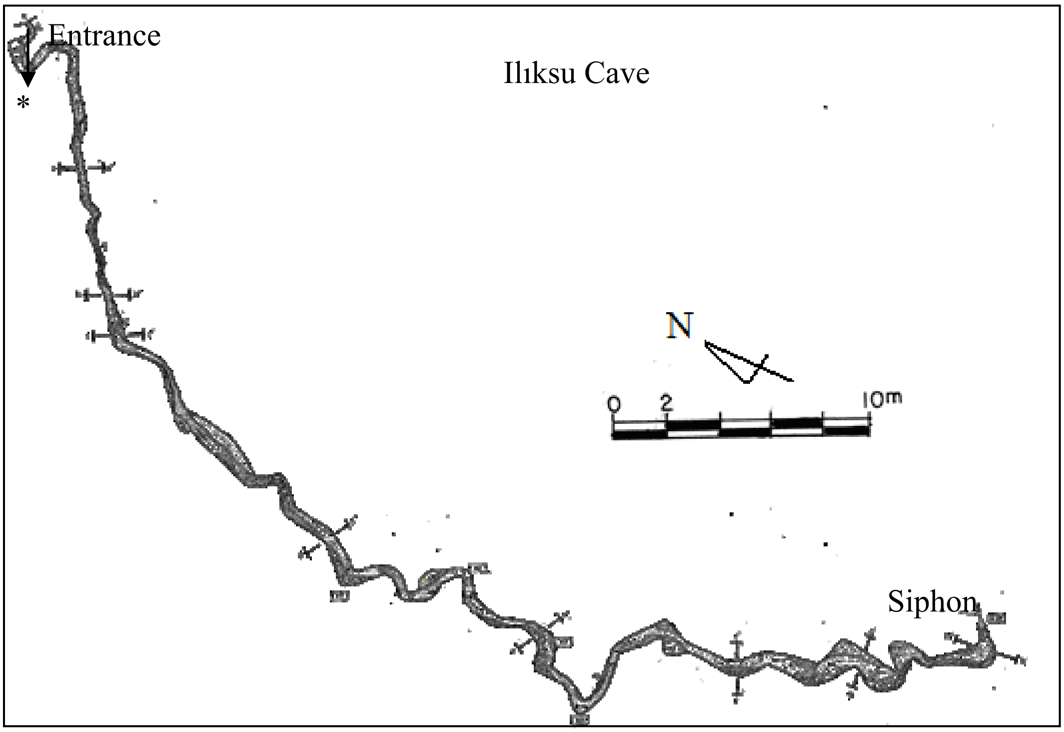

Figure 7: The map of Ilıksu Cave (* represents sampling site), (modified from Mengi, 1995).

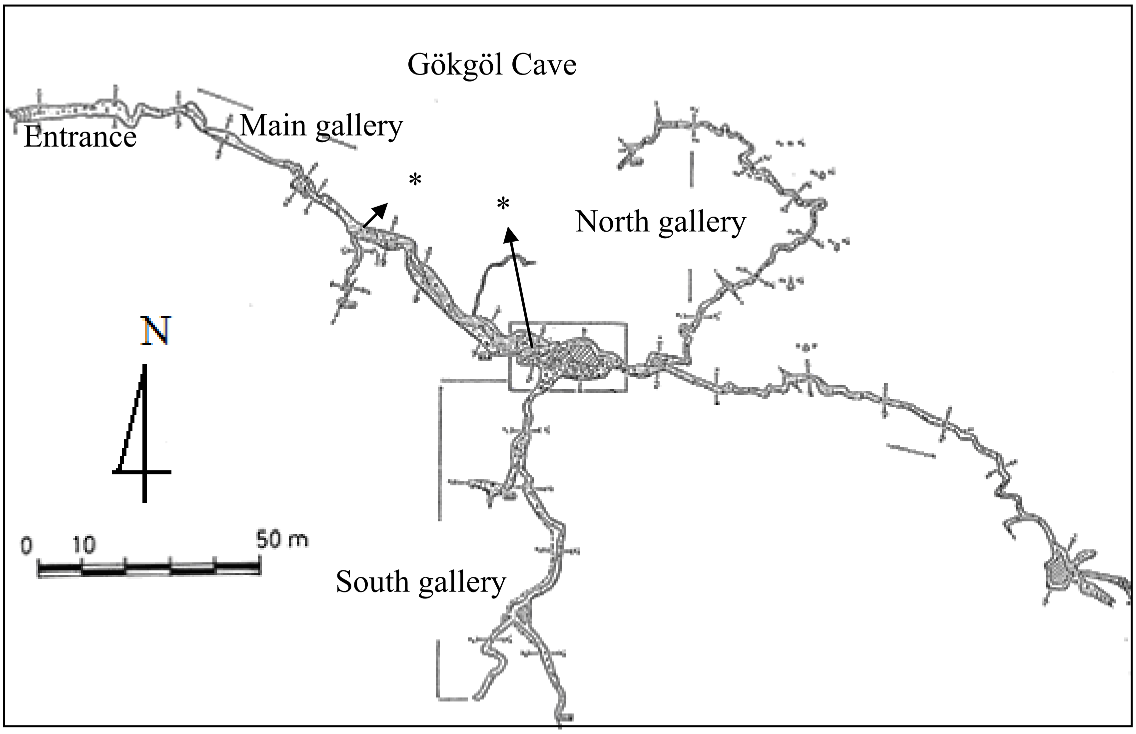

Figure 8: The map of Gökgöl Cave (* represent sampling sites) (modified from Mengi, 1995).

2.1.8. Cumayanı Cave: The cave is found present at Cumayanı village (Çatalağzı, Zonguldak). Cumayanı is about 1110 m long, continium of Kızılelma cave and part of about 10 km cave systems in this areas. It is Apsiyen aged, Kapuz formation, horizontally developed and a spring positioning active cave. There is a flowing ground water inside the cave (Mengi, 1995).

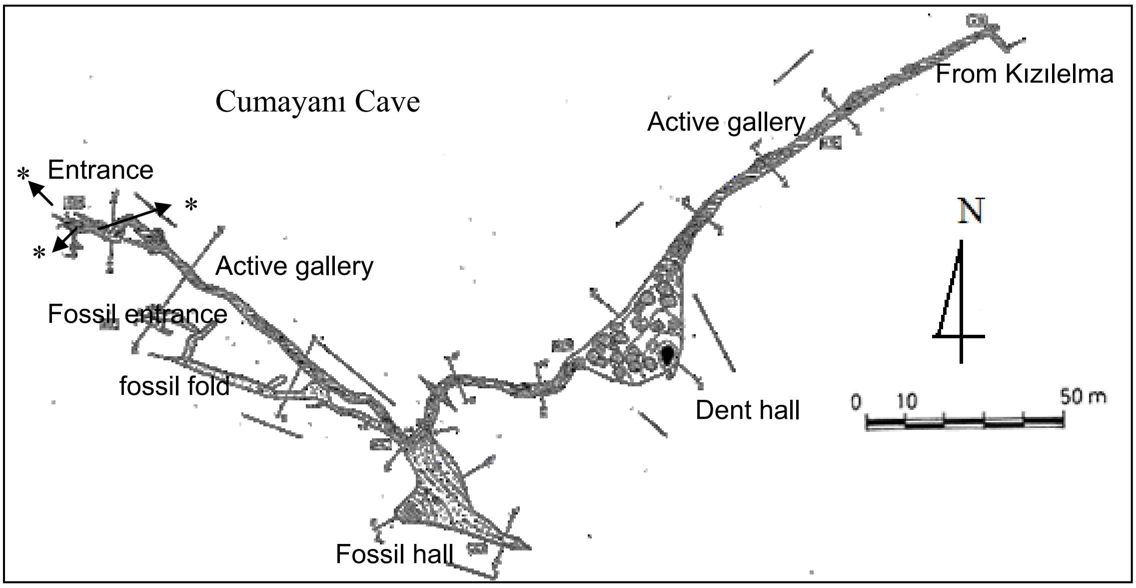

Figure 9: The map of Cumayanı Cave (* represent sampling sites) (modified from Mengi, 1995).

2.1.9. Çayırköy Cave: The cave is located at about 25 km southeast of Zonguldak province. It is 1150 m long, Cenomonian aged, Tasmaca formation, developed into limestone landscape and a spring positioning active cave (Figure 10) (Mengi, 1995).

2.1.10. İnağzı Cave: Cave is situated at the İnağzı district from Kilimli county, Zonguldak. İnağzı cave (Figure 11) is about 793 m long, Albiyen aged, Kırımsa formation, developed into the limestone landscape according to melt and a spring positioning semi-active cave. During rainy seasons the water level can reach to ceiling of the cave (Mengi, 1995).

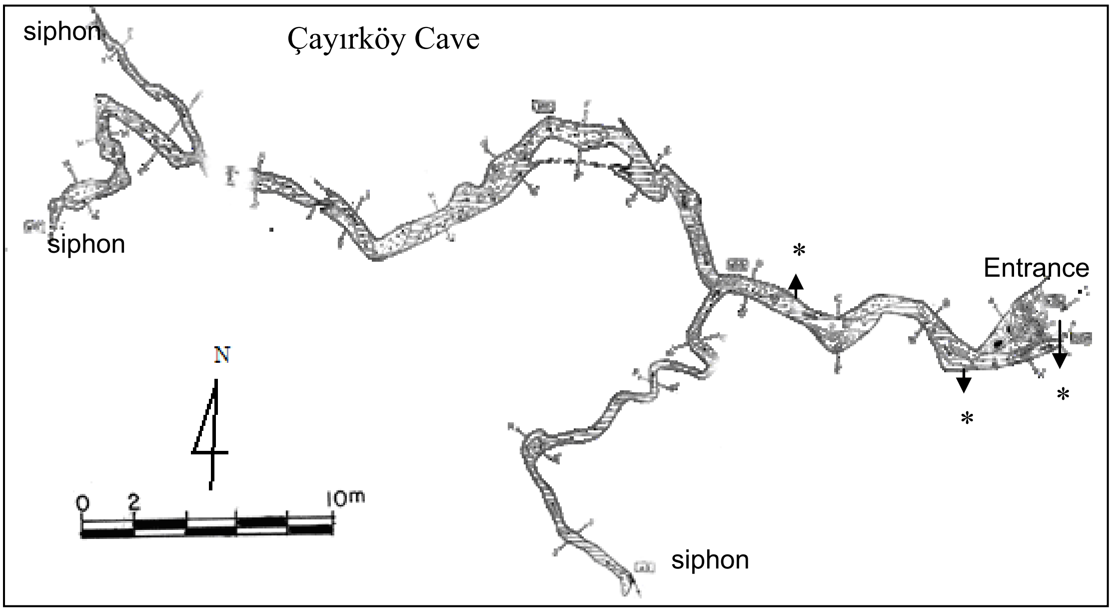

Figure 10: The map of Çayırköy Cave (* represent sampling sites) (modified from Mengi, 1995).

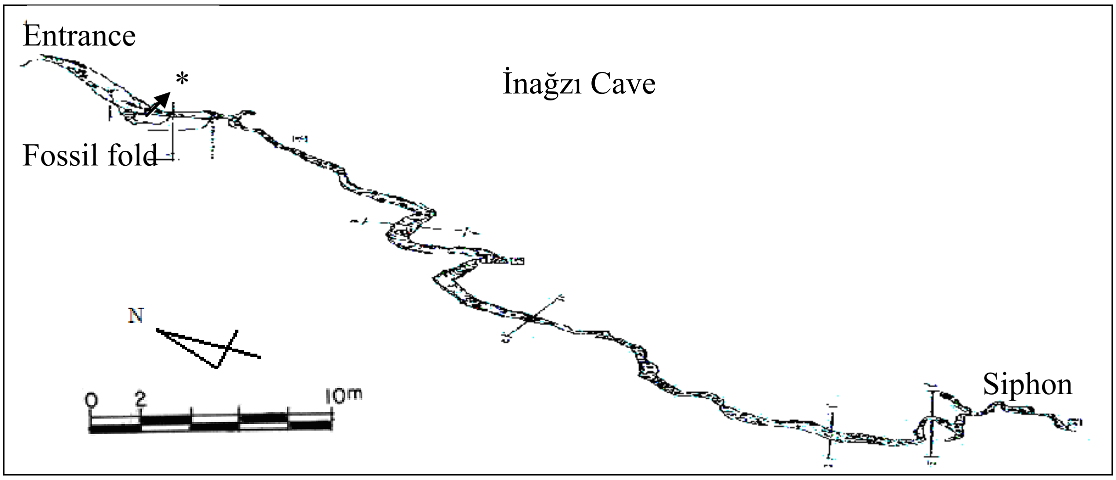

Figure 11: The map of İnağzı Cave (* represents the sampling site) (modified from Mengi, 1995).

2.1.11. Sofular Cave: The cave which is about 490 m long, is located near the Sofular village in Zonguldak. The development of cave is half-landscape and half-vertical and is a fossil cave with 3 of storied connection to each other. Additionally, the cave is Viziyon aged and happened from lower layer of snake formation (Mengi, 1995).

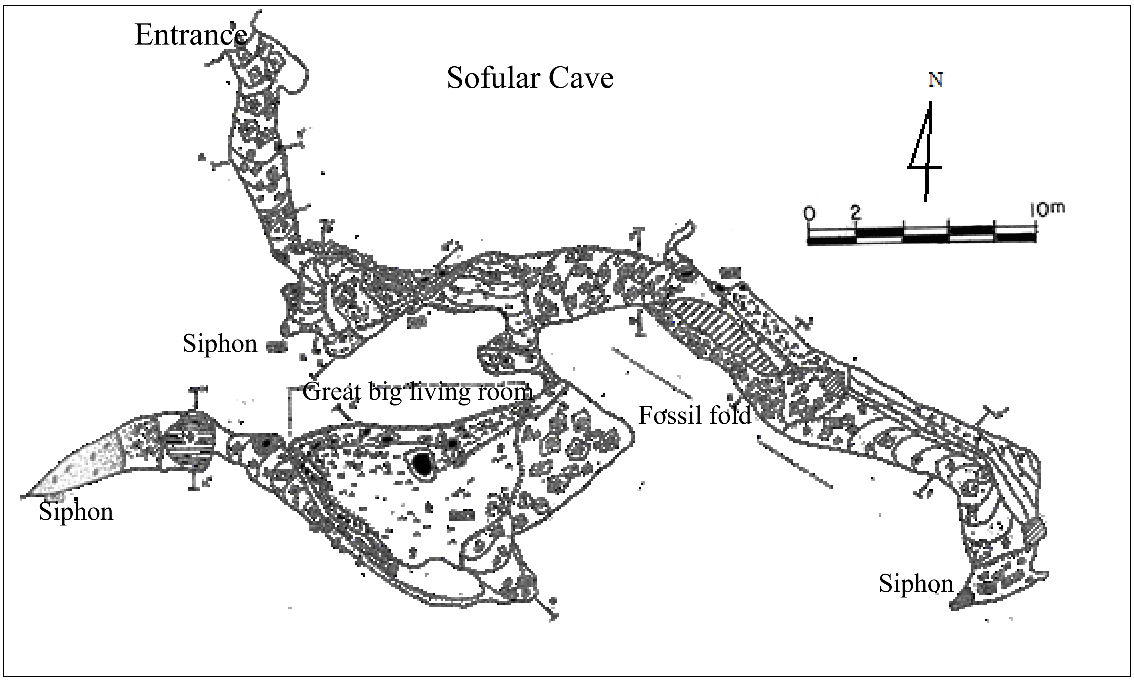

Figure 12: The map of Sofular Cave, (modified from Mengi, 1995).

2.2. Sampling and Measurements

The samples were collected from different habitats of the chosen caves between September and October of 2010 with a hand-net (150 µm mesh size) where the water available. The material was fixed in 70% of ethanol in plastic jars (500 ml). In order to reduce possible consequences of "Pseudoreplication" (Hurlbert, 1984), the ecological variables (pH, redox potential, salinity, electrical conductivity, temperature, dissolved oxygen and percent oxygen saturation) were measured before sampling. pH/ORP meter (Hanna model HI-98150) was used for the first two variables and the rest were measured by an oxygen-temperature meter (YSI model 85). The Standart Hydrogen Electrode (SHE) values were calculated from redox potential measured at sampling site. A global positioning system (Garmin GPS 12 XL) was used to obtain geographical data (altitude, latitude and longitude). The air temperature and moisture were measured by an anemometer (Testo 410-2 model). In laboratory, samples were washed under pressurized tap water and filtered through 3 standart sized sieves (0.25, 1.00 and 1.5 mm in size) and stored in 70% ethanol. The ostracods were seperated from sediment under stereomicroscope (Olympus ACH 1X) and fixed again with 70% ethanol. Each specimen was dissected in lactophenol and mounted on a permanent slide. Taxonomic assignment was done under Olympus BX-51 microscope by using systematic keys of Meisch (2000) based on soft body parts and carapace structures. Damaged individuals, single (subfossil) valves, empty carapace were not identified at species level and were not used in the analyses.

2.3. Statistical Analysis

For the statistical analyses the raw data was log (e) transformed for obtaining normal distribution of variables. Unweighted Pair Group Mean Averages (UPGMA) was used for understanding relativeness among the caves. The dendrogram of UPGMA was constructed by application of parametric Pearson coefficient test that was used for correlation between caves by using the ecological variables. The association between ecological parameters was also showed by UPGMA. The relationship among these ecological variables was evaluated with SPSS 11.01 Version. The Pearson correlation analyses was used to test correlations among 8 environmental variables on UPGMA dendrogram. The UPGMA analyses were performed after using Multi- Variate Statistical Package (MVSP) version 3.1. (Kovach, 1998).

CHAPTER III

3. RESULTS

In all, nine taxa (*Candona neglecta*, *Ilyocypris bradyi*, *I. inermis*, *Psychrodromus olivaceus*, *Candona* sp., *Ilyocypris* sp., *Pseudocandona* sp., *Psychrodromus* sp., and *Heterocypris* sp.) were reported from the present study. Distribution of species per site is given in Table 3.

3.1. Systematic Descriptions

The recorded nine taxa are belonging to superfamily Cypridoidea.

PHYLUM: ARTHROPODA

SUBPHYLUM: CRUSTACEA Pennant, 1777

CLASS: OSTRACODA Latreille, 1806

SUBCLASS: PODOCOPA Müller, 1894

ORDER: PODOCOPIDA Sars, 1866

Suborder Podocopina Sars, 1866

Infraorder Cypridacopina Jones, 1901

Superfamily Cypridoidea Baird, 1845

Family Candonidae Kaufmann, 1900

Subfamily Candoninae Kaufmann, 1900

Genus Candona Baird, 1845

Candona neglecta (Sars, 1887)

Candona sp.

Genus Pseudocandona Kaufmann, 1900

Pseudocandona sp.

Family Ilyocyprididae Kaufmann, 1900

Subfamily Ilyocypridinae Kaufmann, 1900

Genus Ilyocypris Brady and Norman, 1889

Ilyocypris bradyi (Sars, 1890)

Ilyocypris inermis (Kaufmann, 1900)

Ilyocypris sp.

Subfamily Herpetocypridinae Kaufmann, 1900

Genus Psychrodromus Danielopol and Mc Kenzie, 1977

Psychrodromus olivaceus (Brady and Norman, 1889)

Psychrodromus sp.

Subfamily Cyprinotinae Bronshtein, 1947

Genus Heterocypris Claus, 1892

Heterocypris sp.

3.2. Data Analyses

The ecological variables measured from 17 of 21 stations (Table 2). Some data of physico-chemical variables are not available for four sites because;

- We were not able to reach the water in deeper parts of Sofular cave due to physical obstracles.
- İnağzı cave there was no enough water for measurements but only percolating water was present.
- Sarıkaya cave where at the outer site only creepage water was present.
- The grinder located at the outside of the Çayırköy cave there was seepage water so we could not obtain physico-chemical measurements.

Table 2: The ecological measurements from 17 stations. (the abbreviations; air T°C: air temperature; T°C: water temperature; EC (µS/cm): electrical conductivity; Sal (‰): salinity; pH; DO (mg/l): dissolved oxygen; DO %: oxygen saturation; SHE (mV): redox potential).

Name of city	Station name	elevation	air T°C	moisture %	T°C	EC	Sal	pH	DO	DO %	SHE	Coordinate	Date
Zonguldak	Çayırköyü (inside)	147			12,7	392	0,19	7,57	10,56	95,1	194,08	N41°27'110", E31°59'240"	24.09.2010
Zonguldak	Çayırköyü (entrance)	147	18,9	67,4	12,3	401	0,19	7,71	11,8	120	207,97	N41°27'110", E31°59'240"	24.09.2010
Zonguldak	Çayırköyü (outside)	147	20,5	73,5	13,3	265	0,16	7,93	10,78	95,1	209,02	N41°27'110", E31°59'240"	24.09.2010
Zonguldak	Çayırköyü (near the grinder)	147										N41°27'110", E31°59'240"	24.09.2010
Zonguldak	Gökgöl (inside 1)	147	17,3	87,6	13	502	0,24	7,64	11,11	105,6	205,51	N41°26'448", E31°49'926"	25.09.2010
Zonguldak	Gökgöl (inside 2)	147			13	419	0,2	7,65	11,11	103	207,22	N41°26'448", E31°49'926"	25.09.2010
Zonguldak	Cumayanı (inside)	147	20	78,8	12,7	1003	0,5	7,51	2,09	19,1	190,98	N41°29'492", E31°53'806"	25.09.2010
Zonguldak	Cumayanı (entrance)	147	21,2	74,2	12,2	825	0,41	7,58	9,85	91,4	204,82	N.41°29'492", E31°53'806"	25.09.2010
Zonguldak	İnağzı (inside)	36	22,1	68,9							223,25	N41°28'750", E31°49'335"	25.09.2010
Zonguldak	Ilıksu (outside)	15	24,5	68,2	12,5	361	0,17	7,6	11,55	107	205,3	N41°24'362", E31°41'168"	25.09.2010
Zonguldak	Sofular	462	24,3	56.9							223,25	N41°25'649", E31°57'130"	25.09.2010
Düzce	Aksu (inside)	467	17,2	86	12,5	234	0,11	8,28	11,9	115	199,75	N40°56'452", E31°23'586"	26.09.2010
Düzce	Aksu (outside)	467	19,5	79	14,2	403	0,19	8,13	8,2	80,7	203,25	N40°56'452", E31°23'586"	26.09.2010
Düzce	Gökçeağaç (inside)	462	20,3	68,9	12,1	427	0,21	7,72	5,7	52,6	204,18	N40°55'827", E31°25'551"	26.09.2010
Düzce	Sarıkaya (inside)	621	20,9	64,7	14,7	535	0,26	8,09	9,46	92,7	203,07	N40°56'071", E31°23'921"	26.09.2010
Düzce	Sarıkaya (entrance)	621			15,6	440	0,21	8,35	8,55	85,1	204,34	N40°57'305", E31°26'214"	26.09.2010
Düzce	Sarıkaya (outside)	621	19,2	85,1							223,25	N40°57'305", E31°26'214"	26.09.2010
Adapazarı	Söğütlü (entrance)	138	18,9	74,7	13	676	0,33	8,17	10,8	106,5	199,98	N40°53'454", E30°28'426"	23.10.2010
Adapazarı	Söğütlü (inside)	138	18,4	85,9	13,1	675	0,33	8,3	11,13	107,4	199,26	N40°53'454", E30°28'426"	23.10.2010
Adapazarı	Kızılcık (entrance) Right	34	18,2	88,2	12,9	431	0,21	7,6	9,12	87,2	201,87	N41°03'145", E30°41'581"	23.10.2010
Adapazarı	Kızılcık (inside)	34	19,2	84,4	13,2	459	0,22	7,65	11,22	111,5	201,2	N41°03'145", E30°41'581"	23.10.2010

SHE (mV) or Standart Hydrogen Electrode calculated from Eh (mV) or redox potential: SHE= Eh+207+0.65*(25-T°C).

Table 3: The detected species/taxa and number of them from outside, entrance and inside of caves in Zonguldak, Düzce and Adapazarı (Sakarya) (abbreviations; Cn: *Candona neglecta,* Cns: *Candona* sp., Hsp: *Heterocypris* sp., Ib: *Ilyocypris bradyi,* Ii: *Ilyocypris inermis,* Isp: *Ilyocypris sp.,* Psp: *Pseudocandona* sp., Po: *Psychrodromus olivaceus,* Pos: *Psychrodromus* sp., ca: carapace, lv: left valve, rv: right valve).

Name of city	Name of cave	Code	Cn	Cns	Hsp	Ib	Ii	Isp	Psp	Po	Pos
Zonguldak	Çayırköyü (inside)	Zçi			1 lv						
Zonguldak	Çayırköyü (entrance)	Zçe									
Zonguldak	Çayırköyü (outside)	Zço			2 lv			2 lv, 3 rv			
Zonguldak	Çayırköyü (near the grinder)	Zçng				22				37	
Zonguldak	Gökgöl (inside 1)	Zgi									
Zonguldak	Gökgöl (inside 2)	Zgi2									
Zonguldak	Cumayanı (inside)	Zci									
Zonguldak	Cumayanı (entrance)	Zce									
Zonguldak	İnağzı (inside)	Zii							2 ca		
Zonguldak	Ilıksu (outside)	Zılo									
Zonguldak	Sofular	Zso									
Düzce	Aksu (inside)	Dai									
Düzce	Aksu (entrance)	Dao		1 rv				2 lv, 4 rv	1 lv, 1 rv		4 lv, 5 rv
Düzce	Gökçeağaç (inside)	Dgi		1 ca, 1 lv, 1rv			2	4 ca, 3 lv, 5 rv	1 ca, 1 lv, 1 rv		
Düzce	Sarıkaya (inside)	Dsi	3	6 lv, 12 rv		2		4 ca, 2 lv, 4 rv	2 ca		
Düzce	Sarıkaya (entrance)	Dse	21					5 ca, 2 lv, 4 rv			2 ca, 2 lv, 3 rv
Düzce	Sarıkaya (outside)	Dso									
Adapazarı	Söğütlü (entrance)	Ase						1 rv			
Adapazarı	Söğütlü (inside)	Asi									
Adapazarı	Kızılcık (entrance)	Ake									
Adapazarı	Kızılcık (inside)	Aki									

Based on environmental variables, Unweighted Pair Group Mean Averages (UPGMA) showed three main clusters (Figure 13) among 17 sites with about 85% similarity. The first group included Aki, Ake and Zılo when the second cluster consists of eleven sites (Dsi, Dgi, Dao, Dçi, Asi, Ase, Zcui, Zcue, Zço, Zgi, and Zçe. The third also obtains 3 sites (Dse, Zgi2 and Zçi).

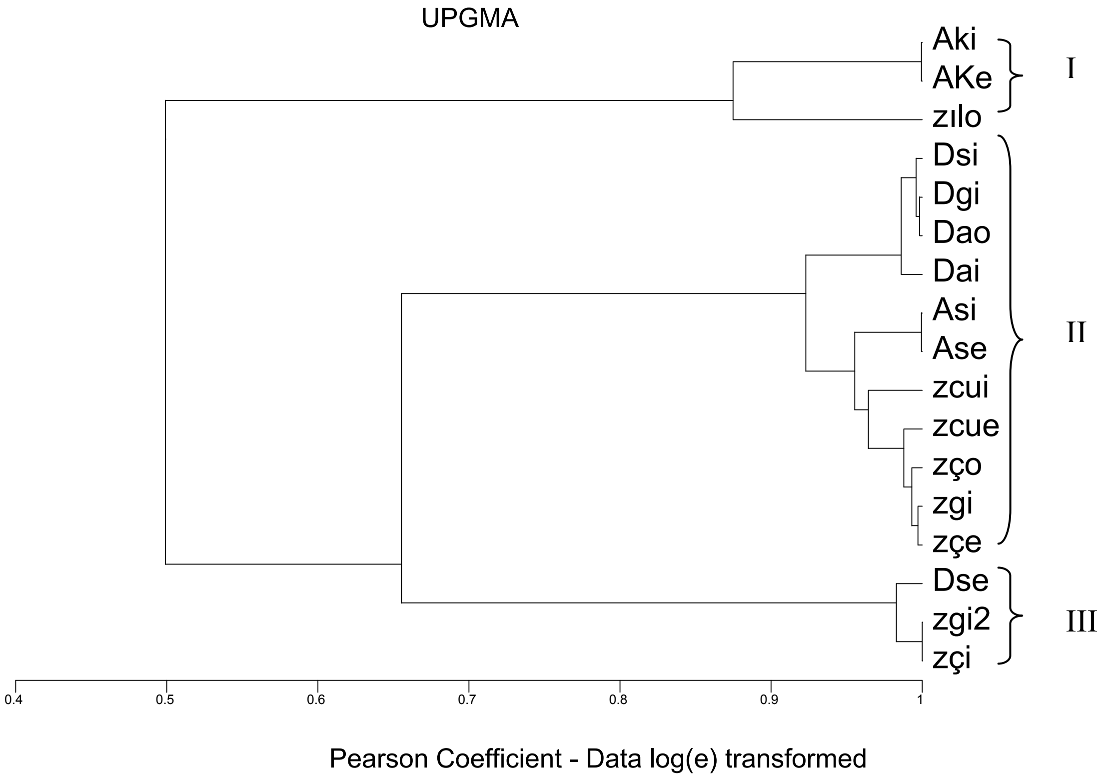

Figure 13: Dendrogram of Unweighted Pair Group Mean Averages (UPGMA) showing clustering relationship among 17 sampling sites (abbreviations same in Table 3).

Unweighted Pair Group Mean Averages (UPGMA) was used for relationship between eight ecological variables. Of which, 80% of similarity was shown for three main groups (Figure 14). First group contains 3 variables (pH, dissolved oxygen and water temperature) and the second groups represents moisture and air temperature, when the third group includes redox potential, electrical conductivity and elevation. Such relationships among these groups were found as expected.

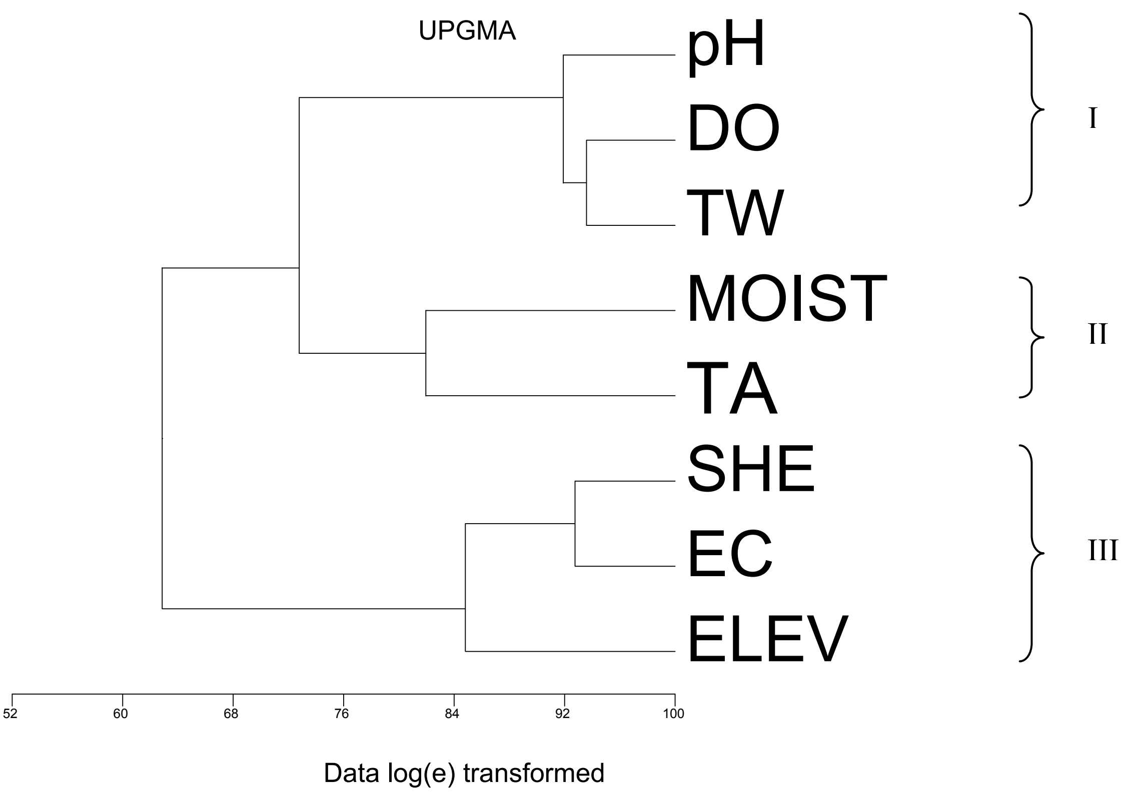

Figure 14: Unweighted Pair Group Mean Averages (UPGMA) shows clustering among 8 environmental variables (abbreviations: ELEV (m): elevation; TA (ºC): air temperature; MOIST (%): moisture; TW (ºC): water temperature; EC (µS/cm): elctrical conductivity; pH: redox potential; DO (mg/l): dissolved oxygen, SHE (mV): redox potential).

Pearson correlation analysis emphasize the relationship between eight environmental variables (Table 4) and the groups showed on Figure 14 supported by that results. Accordingly, there was a negative significant correlation (P<0.01) between air temperature and moisture (r = -0.724). There is also a negatively significant (P<0.05) correlation among electrical conductivity and redox potential (r = -0.502) but water temperature had a positively significant correlation (P<0.05) to pH (r = 0.584).

Table 4: Correlation between eight ecological variables (abbreviations same with Figure14).

Correlations

		ELEV	TA	MOIST	TW	EC	pH	DO	SHE
ELEV	Pearson Correlation	1	-.151	-.206	.166	-.191	.309	-.208	-.146
	Sig. (2-tailed)	.	.591	.462	.524	.463	.227	.423	.564
	N	18	15	15	17	17	17	17	18
TA	Pearson Correlation	-.151	1	-.724**	-.001	.059	-.333	-.120	.387
	Sig. (2-tailed)	.591	.	.002	.997	.841	.245	.684	.154
	N	15	15	15	14	14	14	14	15
MOIST	Pearson Correlation	-.206	-.724**	1	-.057	.027	.089	.096	-.434
	Sig. (2-tailed)	.462	.002	.	.847	.927	.763	.745	.106
	N	15	15	15	14	14	14	14	15
TW	Pearson Correlation	.166	-.001	-.057	1	-.094	.584*	-.072	.091
	Sig. (2-tailed)	.524	.997	.847	.	.719	.014	.784	.729
	N	17	14	14	17	17	17	17	17
EC	Pearson Correlation	-.191	.059	.027	-.094	1	-.187	-.549*	-.502*
	Sig. (2-tailed)	.463	.841	.927	.719	.	.473	.022	.040
	N	17	14	14	17	17	17	17	17
pH	Pearson Correlation	.309	-.333	.089	.584*	-.187	1	.191	.051
	Sig. (2-tailed)	.227	.245	.763	.014	.473	.	.463	.846
	N	17	14	14	17	17	17	17	17
DO	Pearson Correlation	-.208	-.120	.096	-.072	-.549*	.191	1	.475
	Sig. (2-tailed)	.423	.684	.745	.784	.022	.463	.	.054
	N	17	14	14	17	17	17	17	17
SHE	Pearson Correlation	-.146	.387	-.434	.091	-.502*	.051	.475	1
	Sig. (2-tailed)	.564	.154	.106	.729	.040	.846	.054	.
	N	18	15	15	17	17	17	17	18

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

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

CHAPTER IV

4. DISCUSSION AND CONCLUSION

In this study seven ostracod taxa (*Ilyocypris inermis, I. bradyi, Candona neglecta, Ilyocypris* sp., *Candona* sp., *Pseudocandona* sp., and *Heterocypris* sp.) were detected from entrance and inside of the caves studied (Table 3). The other two taxa, *P. olivaceus* and *Psychrodromus* sp. were collected from the outside of Çayırköyü Cave and entrance of Aksu and Sarıkaya caves, respectively.

Among the species, living adult individuals of *I. inermis, I. bradyi and C. neglecta* were reported herein for the first time from the cave environments in the literature.

4.1. *Candona neglecta*

This species generally present in shallow muddy ditches, lakes, brackish coastal water (Bhaita and Singh, 1971), in organically rich water (Roca and Baltanas, 1993; Mischke, 2001). Besides, the species is always encountered not only in lakes, springs, brooks and slow flowing habitats (Külköylüoğlu et al., 2007) but also in underground waters (Meisch 2000), who stated that species occur in a wide range of aquatic habitats and also prefer cold waters but tolerate a temperature up to 20 ºC.

Viehberg (2006) found species from freshwater habitats of Norhest Germany at 6 ºC water with a tolerance to temperature at 2.2 ºC and Külköylüoğlu et al. (2007) detected species from eutrophic lake at 28 ºC water temperature. Rieradeval and Roca (1995) suggested that *C. neglecta* shows low diversity when the temperature is low. The species were reported all around year from Yumrukaya Reedbeds, Bolu (Külköylüoğlu, 2005a) and high chance of survive all of year because of cosmopolitan characteristics (Karakaş Sarı and Külköylüoğlu, 2008). Thus, it is called euryecious meaning high tolerances and wide distribution of the species (Külköylüoğlu and Yılmaz, 2006; Karakaş Sarı and Külköylüoğlu, 2008). In this study the species was detected from two sites located at the entrance and inside of the Sarıkaya cave with a pH of water 8.09 - 8.35. Roca and Baltanas (1993) found species in range of pH (6.8 – 9.4), dissolved oxygen (9.46 – 8.55 mg/l) as well as low dissolved oxygen 1.83 mg/l (Karakaş Sarı and Külköylüoğlu, 2008) to 15.08 mg/l by Külköylüoğlu (2005b). The other ecological variables (Table 2) measured in this study showed similar range with air temperatures (29.3 – 7.5 ºC), electrical conductivity (314.5 – 924 µS/cm) and redox potential (218.57 - 145.97 mV) stated by Külköylüoğlu (2005b). Li et al. (2010) showed wide range of the species to salinity (12.3-0.92‰) and chemical variables (Na^+, K, Ca^{+2}, Mg^{+2}, Cl^-, SO_4^{-2}, CO_3^{-2}, HCO_3^{-2}). In our study, we detected species at 621 m above sea level (a.s.l.) but Mezquita et al. (1999) stated that *C. neglecta* related to high altitudes. However, most recent studies (Külköylüoğlu et al. in review) showed that altitudinal changes are not primary factor on species distribution. Accordingly, it is suggested that species can be found in different altitudinal ranges as long as the conditions of aquatic habitats are available. Considering its cosmopolitan characteristics, the occurrence of *C. neglecta* within the caves could be expected because of the wide tolerance ranges to physico-chemical variables. This wide tolerance allows the species easily adapted to new habitats. The transportation of species to cryptic condition may probably be happened by water flow from outside to inside of caves.

4.2. *Ilyocypris bradyi*

The species prefer cooler water of springs and streams, slow flowing waters, rivers, lakes, brooks, pools, ponds, swamps, wetlands (Mezquita et al., 1999; Meisch, 2000; Mischke et al., 2003) and interstitial habitas (Marmonier and Creuze des Chatelliers, 1992). The species was also found in slightly saline inland waters (Meisch, 2000). Like *C. neglecta, I. bradyi* is mostly present in habitats with wide range of ecological variables and show stygophilic characters (Mezquita et al., 1999). Due to its wide geographical distribution, it considered as cosmopolitan (Külköylüoğlu et al., 2007). Meisch (2000) stated that the species is euryhaline and polythermophilic because it tolerates to wide range of salinity and temperature. Li et al. (2010) pinpointed its tolerance to salinity (0.32- 13.98‰) and several chemicals detected in river, spring, reservoirs and swamp puddles, supporting the euryhaline characteristic of species stated by Meisch (2000). Species was also found in relatively less saline alkaline water (Külköylüoğlu and Vinyard, 2000). The other ecological variables, the optimum and tolerance of species to redox potential (147.41 – 296.24mV SHE) respectively (Külköylüoğlu and Dügel, 2004), dissolved oxygen (0.48 – 13.46 mg/l), pH (6.1-8.5), electrical conductivity (272.9 – 864 µS/cm), water temperature (9 – 24.4 ºC) and air temperature (13,2 – 23.1ºC) (Mezquita et al., 1996; Külköylüoğlu, 2005b; Karakaş Sarı and Külköylüoğlu, 2008; Li et al., 2010). In the present study, the species with environmental variables (Table 2) within the ranges reported by previous researches and detected at elevation 147 – 621a.s.l. Additionally, it was recently reported from southern part of the Friuli Venezia Giulia, Italy below 240 m (Pieri et al., 2009), at 907 m in Bolu by Külköylüoğlu (2004) and with different elevations (950, 1000 and 1150 m) in Spain by Mezquita et al. (1996). Salinity and elevations may not be primary factor for distribution of species. The wide tolerance of species to environmental variables, interstitial habitat characteristic and stygophilic characters may support the presence of it within cave habitats. Like *C. neglecta*, the moving of species into cave may passively be transported by flowing water.

4.3. *Ilyocypris inermis*

Ilyocypris inermis is characterized as cold stenothermal and mesorheophilic (Meisch, 2000). The species was reported from two wells in Burgerland, Austria (Löffler 1964, in Meisch 2000). Like *I. bradyi, I. inermis* have wide tolerance to environmental variables (Mezquita et al., 1999). The species is bottom dependent and occurs not only in cold waters but also in warm water. The species recorded in a range of water temperature (11.44 – 18 ºC) (Mezquita et al., 1996; Külköylüoğlu et al., 2010). It shows wide tolerance to ecological variables, such as dissolved oxygen (2.98- 9.62 mg/l), electrical conductivity (61.10 – 749.8 µS/cm), pH (7.04-8.55), redox potential (139.58 – 211.78mV) and air temperature (13.0 – 18.2 ºC) (Mezquita et al., 1996; Külköylüoğlu and Yılmaz, 2006; Külköylüoğlu et al., 2010). Also Külköylüoğlu and Yılmaz (2006) stated that species tolerate low oxygen content, moderate pH and wide range of water temperature from a limnocrene spring. Mezquita et al. (1999) suggested *I. inermis* is generalist and one of the most succesful species occuring in Eastern Iberian streams and springs. Moreover, Mezquita et al. (1996) recorded species from a spring and a small river in Spain at elevation 1000 m and it reported by Pieri et al. (2009) from Italy below 240 m. In this study species recorded only from a pond inside of the Gökçeağaç cave, with an air temperature 20.3 ºC and elevation 462 a.s.l., and the other ecological variables (Table 2) show similar pattern like previous researches. According to these studies, it is most likely that *I. inermis* may have a cosmopolitan distribution but more ecological studies including measurements of physico-chemical variables should be done for generalization of this view. Table 3 shows the numbers of living adult specimens from inside of Gökçeağaç cave where the existence of the species may be related to cosmopolitan characteristics. Most recently, Petkovski et al. (2009) reported one juvenile carapace of species from a freshwater cave in Herzegovina, as *I. inermis*. However, such description based on one specimen's carapace even the specimen belongs to juvenile form is not easy since the difficulties on taxonomic problems occur among the adult Ilyocypris species (Van Harten 1979; Meisch 1988, 2000; Martens, 1991).

4.4. *Psychrodromus olivaceus*

Psychrodromus olivaceus, another living species found in this study, has been reported from ponds, lakes and brooks fed by springs (Meisch, 2000) and in interstitial habitats (Marmonier and Creuze des Chatelliers, 1992). The species shows wide tolerance to ecological variables within cosmopolitan distribution so as called "euryecious" (Külköylüoğlu and Yılmaz, 2006; Dügel et al., 2008; Sarı and Külköylüoğlu, 2010). The species was found to be tolerant to wide range of pH (6.46

– 8.27), electrical conductivity (61.10 – 697µS/cm), water temperature (1.68 – 24.4 ºC), dissolved oxygen (1.74 – 20 mg/l) and redox potential (155.98 – 211.78 mV) (Külköylüoğlu and Yılmaz, 2006; Dügel et al., 2008; Karakaş Sarı and Külköylüoğlu, 2008). This species can be oligothermophilic because of its preference for cold fresh waters with low salinity and dissolved oxygen (Külköylüoğlu, 2009).

All of these four species are living but the other five taxa are also determined in the subfossil form. The four of them encountered inside of the caves that are *Candona* sp., *Pseudocandona* sp., *Ilyocypris* sp., and *Heterocypris* sp. (Table 3). The presence of genus Candona was supported by Danielopol et al. (1994) who stated that it was a dominant group in Ostracoda colonizing the world subterranean freshwater. Reeves et al. (2007) supported this idea the most abundant group of groundwater of Arid Pilbara region, Australia. Besides, *Candona* sp. was previously reported from cave environments by Maddocks and Iliffe (1986), Maddocks et al. (1991) and Petkovski et al. (2009) (Table 1). The genus Pseudocandona especially lives in stagnant temporary or permanent waters, and also underground habitats such as, wells, cave waters and interstitial underground water of streams (Meisch, 2000). Hartmann (1964) reported one species (*Pseudocandona dispar*) belongs to this genus from Erikli Cave in Zonguldak, Turkey (Table 1). Culver et al. (2000) called the species as stygophilic after reporting from cave in the United States. Also *Ilyocypris* sp. was marked from cave by Petkovski et al. (2009). The one valve of *Heterocypris* sp. reported inside of the one cave and it was previously recorded from cave environments by Maddocks et al. (1991) and Petkovski et al. (2009). The other two taxa, *Psychrodromus* sp. and *P. olivaceus* were encountered from entrance and outside of the caves Aksu and Sarıkaya, and Çayırköy, respectively (Table 3). The species (*P. olivaceus*) was previously reported from Kapız Cave in Zonguldak by

Hartmann (1964) (Table 1). Also, one species (*Psychrodromus betharrami*) belongs to genus Psychrodromus (Table 1) was reported from cave environments in France by Ferreira et al. (2007). According to this result, this genus may be encountered within caves by increasing sampling sites at different cave environments.

Gunn (2004) stated that the podocopid ostracods have related lineages in caves and karst especially the species belongs to Cypridoidea, Cytheroidea because these groups have ability to adapt to subterranean habitats. This may be an answer of why taxa reported in present study belong to only Cypridoidea? It is possible that increasing numbers of sampling stations at different caves can elevate the numbers of species in different genera. However, considering the wide ecological tolerances to variety of environmental variables of individual species, it is possible to find species with cosmopolitan characteristics. Once species are adapted to cave conditions, evolutionary process continuous and also Gibert and Veng (2002) stated that physical fragmentation of subterranean environments foster this process and speciation. Such adaptation may however, be partially (if not all) depending on species tolerance levels.

4.5. Evaluation of Data

As a result of UPGMA, the habitats located in Düzce clustered a sub-group (Sarıkaya inside, Gökçeağaç inside, Aksu outside and Aksu inside) within second main group but the Sarıkaya entrance located in a different cluster with two locations of Zonguldak (Gökgöl inside 2 and Çayırköy inside). The clustering of Düzce caves (Figure 13) was based on the similarities of ecological variables used; however, occurrence of three taxa (*Candona* sp., *Ilyocypris* sp., and *Pseudocandona* sp.) might be as important factor on cave clustering (Table 3). Implication of this approach is to use biotic data (e.g., species numbers or binary data) and/or abiotic as well. Besides, the other sampled areas of Zonguldak form a cluster with Söğütlü inside and Söğütlü entrance that are located at the Adapazarı. The Kızılcık inside and Kızılcık entrance form a different group with Ilıksu outside (Figure 13). The presence of caves sampled from Adapazarı within the one main and one subgroup of Zonguldak may be explained by spring positioning and by similar the environmental variables. The clustering of caves in Zonguldak and Adapazarı may not be related to the distance between the sites, as supported by Sarı and Külköylüoğlu (2010) who stated that similarity and dissimilarity between habitats was most likely based on species composition, but distance. The formation of cluster by Sarıkaya entrance with Gökgöl inside 2, and Çayırköy inside may due to geomorphologic similarity and also similarity of ecological variables may be an effective factor (Figure 13). Also, the presence of small flowing creek in all of them and the presence of small lakes, waterfalls within Sarıkaya entrance and Gökgöl inside 2 is an another important factor. The occurrence of Ilıksu outside, like Sarıkaya entrance in a different cluster is interesting, because the spring positioning of these two caves is different. The water flowing of the cave obtain Ilıksu outside from inside to outside but it is opposite in Sarıkaya entrance. The cluster obtain Ilıksu outside with Kızılcık inside and Kızılcık entrance may interpreted because the altitude of them have smaller value to compare other habitat types and the close similarity of pH and water temperature support the formation of this group.

The results of Pearson Correlation Analysis (Table 4) supported the formation of clusters among eight ecological variables (Figure 14). The variables in each cluster correlated to each other. The relationship between air temperature and moisture is already known; the air have a capacity to hold water vapour if increase the air temperature so increase water evaporation but when the water vapour pass the saturation state of air these water vapour close to each other in the air than turn backs as liquid so decrease humidity. The water evaporation holding state of closed or opened areas different because of temperature. If air temperature increses, the moisture of air decrease. Our finding support that the air temperature and moisture had a significantly ($P<0.01$) negative correlation (-0.724). The water temperature and pH had a strong relationship (0.584) but electrical conductivity showed negatively significant correlation ($P<0.05$) to dissolved oxygen and redox potential. In general, when temperature increase the salinity of water increases and the dissolved oxygen decrease. Külköylüoğlu and Dügel (2004) also stated that water temperature and pH negatively correlated with DO and EH and so the DO increased in cold water. These results support our findings.

The interstitial habitat characteristic is an important factor for the presence of ostracods in caves. Because the animals dwelling in these habitats look like morphologically preadapted and have high tolerance to environmental variables so they actively or passively dispersed to caves or subterranean habitats (Danielopol and Rouch, 2004). The dispersion to these cryptic conditions especially depend on surface water catchment or aquifer (Reeves et al., 2007) and habitat characteristics (Creuze des Chatelliers and Marmonier, 1993). The water cathment or connection to surface water mostly effect the species composition and distribution because of sediments moving by that flowing water so the presence of detritus and organic materials or food supply depend on it. The effect of sediment types and detritus on ostracod distribution has already been proposed (Danielopol, 1976; Maddocks and Iliffe, 1986). Accordinly, Maddocks and Iliffe (1986) pinpointed that ostracods were detected from 15 Bermudian caves with soil and silt sediments but less from rock and gravel. These authors mentioned that ostracods were especially found on algae, coralline detritus and sediments carried from surface waters. Consequently, if the cave have poorest connection to surface water, the species composition can be low. Similarly, we found more ostracods especially from ponds and slow flowing waters with muddy and silty sediment types than flowing waters.

The situation explained above is also related to the climatic conditions, in which any changes and seasonal variations negatively effect species diversity. Higuti et al. (2010) underlined that especially flooding effect on homogenization of habitats can be critical for biodiversity because of its consequences on sedimentation. Because of the absence of primary producers in these cryptic conditions (Stoch and Galassi, 2010), food transported from outside may located in small microhabitats and each microhabitat may be used as a small niche for different groups of organisms. Our data supports that the water of ostracods in caves studied obviously flowing from outside to inside.

Malmqvist (1997) stated that the habitat characteristics important for dispersion of species and the low altitude and high conductivity correlated with hypogean habitats. Therefore, the changes in altitude change the air temperature, depth of water, dissolved oxygen, redox potential and generally change all ecological variables that effect the morphology and taxonomy of ostracods (Reeves et al., 2007). Also Humphrey (2009) proposed that spatio-temporal distribution of Stygobitic species primarily effected by dissolved oxygen. The caves are special habitats and inside conditions are generally stable. However, these habitats have their special ecological variables if compared to surface habitats. The ecological conditions are not mostly changed if the outer events are not effective. Therefore, the species adapted to these extreme conditions develop specific metabolism because the energy source is very low that is necessary for survive. Thus, the question may be answered why the species in caves different from their congener in surface habitats? This is because species develop stygophilic characters to tolerate these extreme conditions and especially morphological changes observed. These previous statements support our results because the species reported in present study show cosmopolitan distribution. Also, they have translucent colour. These changes support their adaptation and describe the importance of environments on organisms.

The most hypogean dwellers are Crustacean that proposed by Stoch and Galassi (2010) suggested that Crustacea comprise ca. 70% of groundwater species that are mostly Copepoda, Amphipoda to less Ostracoda. Also Ferreira et al. (2007) showed that distribution of 380 species among crustaceans, molluscs and annelids were 83.5 %, 12%, and 2.5% respectively. The wide distribution of Crustacean due to other groups in the hypogean habitats explained by Danielopol (2000b) and due to lack of competitors. Reeves et al. (2007) reported the percentage presence of ostracods from groundwater that 56 % of site and 47 % of 751 samples. In our study, the percentage presence of ostracods are 54.5 % of site, 42.8 % of samples of all site (outside, entrance and inside of caves) but 54.5 % of samples of only inside and entrance of caves. Besides, Amphipoda were noted with a 52.4 % of all sampled stations. Our results show similarities with the previous marks.

4.6. Conclusion

These previous research and our finding suggested that the subterranean habitats very sensitive to outer events that is because of the food dependence of hypogean habitats to surface or absence of primary producer. The aquatic species in caves or other subterranean habitats mostly prefer stagnant, unconsolidated sediments and areas connected to surface water. The species detected in caves are generally cosmopolitan characteristic with wide tolerance level to ecological variables. Therefore, this characters allow them easily distributed over a range of area or even into this cryptic conditions. Because of hard environmental conditions, the species diversity of caves are lower than surface habitats. In addition, the distribution of organisms in caves or subterranean habitats shows that mostly Amphipoda but less Ostracoda stated by previous researches and in our study. The *Gammarus* sp. (Gökgöl inside2, Çayırköy outside, Cumayanı inside, Çayırköy near the grinder, Aksu entrance, Sarıkaya inside and entrance), *Gammarus arduus* G. Karaman, 1975 (Söğütlü inside), *Gammarus komareki* Schaferna, 1922 (Çayırköy entrance and inside), and *Gammarus cf. balcanicus* Schaferna, 1922 (Ilıksu outside) were noted from different sampled stations. Besides, one salamander (*Ommatotriton ophryticus* Steinitsz, 1965) from Gökçeağaç Cave and one grasshoppers (*Gryllmorpha dalmatina* Ocskay, 1832) from Sofular Cave was reported. Additionally, one copepod and a few copepod with one nauplius larvae were reported from inside of Aksu and Söğütlü caves, respectively. Also, two species of zooplankton (*Diacyclops bisetasus* Rehberg, 1880 and *Tropocyclops prasinus* Fischer, 1860) were noted at the entrance of Cumayanı cave. The similarity of our data with previous studies may allow us the make a conclusion; generally the distribution of especially Amphipoda grater than Ostracoda. The presence of these organisms in cave environments may help to explain the presence of ostracods within caves. Because of feeding behaviour of ostracods on dead animal body, skin mucus of Amphibians (Meisch, 2000) and feeding pattern between salamanders and ostracods stated by Ottonello and Romano (2011) may describe the occurrence of ostracods in cryptic conditions.

Sum of all,

- The ecological reguirements of species, water flowing, preadaptation to hypogean life, and sediment types are effective on the ostracods distribution within the caves.
- The flowing water has a negative effect on stygobitic species especially events like flooding.
- The connection of caves to surface water is very important for organic material and species transportation.
- The reported taxa in our study are generally widespread through the surface water.
- *Ilyocypris inermis, I. bradyi, Candona neglecta, , Ilyocypris* sp., *Candona* sp., *Pseudocandona* sp., and *Heterocypris* sp. are the first record for freshwater cave Ostracoda (Crustacea) fauna of Turkey.

4.7. Suggestions

- The distribution of ostracods in caves may be well understand by further research over a wide range of areas.
- For better understanding of moving of ostracods from surface to inside or vice versa, especially the genetic researches should be done.
- Considering the possibilities of finding new results, cave studies like this one should be increased in future.

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PHOTOS

Photo 1: Çayırköyü inside

Photo 2: Çayırköyü outside

Photo 3: Gökgöl inside


Photo 4: Cumayanı inside


Photo 5: İnağzı inside


Photo 6: Sofular inside

Photo 7: Sarıkaya inside

Photo 8: Aksu outside

Photo 9: Aksu inside

Photo 10: Söğütlü inside

Photo 11: Kızılcık inside

Photo 12: Gökçeağaç inside