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New Records for Tardigrada Species from the High Mountain Region in Turkey (Mount Verçenik, Rize)

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ABSTRACT

Phylum Tardigrada, which has been quite a little studied both around the world and Turkey, belongs to members of a metazoan. In this study eight moss and lichen samples were collected and investigated from a variety of elevations of the Verçenik Mountain in Rize (Turkey). In total 62 specimens, 11 exivium and 10 eggs of tardigrades were found. Five species were identified, and they belong to genera: *Acanthechiniscus*, *Pseudechiniscus*, *Macrobiotus*, *Paramacrobiotus* and, *Ramazzottius*. Three of them are new records for Turkish fauna i.e. *Acanthechiniscus victor* (Ehrenberg, 1853), *Pseudechiniscus ramazzottii ramazzottii* Maucci, 1952 and *Macrobiotus spectabilis* Thulin, 1928. Including these three species, the tardigrade fauna of Turkey increased to 54. In this paper, we present the previous study a full list of the known high mountain and alpine tardigrade species with their localities. All specimens are deposited in the Aquatic Animals Research Laboratory at Ankara University.

Keywords: *Acanthechiniscus victor*, Alpine, fauna, *Macrobiotus spectabilis*, *Pseudechiniscus ramazzottii ramazzottii*

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Türkiye'nin Yüksek Dağ Bölgesinden Tardigrada Türleri için Yeni Kayıtlar (Verçenik Dağı, Rize)

Öz: Dünyada ve Türkiye'de çok az çalışılmış olan Tardigrada filumu, metazoa üyelerine aittir. Bu çalışmada Rize'deki Verçenik Dağı'nın farklı yükseltilerinden elde edilen sekiz adet yosun ve 1 adet liken örneği toplanmış ve araştırılmıştır. Toplamda 62 birey, 11 exivium ve 10 tardigrat yumurtası bulunmuştur. Beş tür tespit edilmiş ve bunlar *Acanthechiniscus*, *Pseudechiniscus*, *Macrobiotus*, *Paramacrobiotus* ve *Ramazzottius* cinslerine aittir. Bunlardan üçü, Türkiye faunası için yeni kayıtlardır. Bunlar: *Acanthechiniscus victor* (Ehrenberg, 1853), *Pseudechiniscus ramazzottii ramazzottii* Maucci, 1952 ve *Macrobiotus spectabilis* Thulin, 1928. Bu üç türün de dahil edilmesiyle, Türkiye'nin tardigrat faunası 54'e yükselmiştir. Bu çalışmada daha önceden elde edilen yüksek dağ ve alpin türlerinin tam listesini sunulmuştur. Tüm örnekler Ankara Üniversitesi Sucul Canlıları Araştırma Laboratuvarında saklanmaktadır.

Anahtar kelimeler: *Acanthechiniscus victor*, Alpin, fauna, *Macrobiotus spectabilis*, *Pseudechiniscus ramazzottii ramazzottii*

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Introduction

Tardigrades were first observed by German scientist Johann A. E. Goeze in 1773. Three years later Italian scientist Lazzaro Spallanzani (1776), gave them a name Tardigrada (meaning "slow stepper"). From the first discovery to date 1246 tardigrade species have been reported around the world (Degma and Guidetti 2007; Vicente and Bertolani 2013; Degma et al. 2018).

Very limited papers have been published on Turkish Tardigrada up to now (Kaczmarek et al. 2012). However, Maucci conducted quite intensive studies on Turkish tardigrade in 1973, 1975, 1978, 1979, 1980, 1981 and 1985. Later, only Morgan (1977), Binda (1988), Kaczmarek et al. (2012) and Ürkmez et al. (2018) reported a few more tardigrade species from this region and up to now only 51 species which are belonging to 18 genera have been recorded from Turkey.

Lichens and mosses resistant to drought, which melt under the snow, grow under the snow, or in rock cracks, are encountered in Alpine zone. These plants which are durable to the drought may be exposed to the fundamental physical variances at short ranges because of the interaction between temperature and ground and also soil moisture which changes according to the effect of sun and wind (Atay et al. 2009). Tardigrades are one of the invertebrates adapting to these short-term physical changes and conformance to adverse environmental conditions.

There are very limited studies on the distribution and diversity of alpine tardigrades both in the world and in Turkey (Rodríguez-Roda 1951; Nelson 1975; Dastych 1980, 1985, 1987; Beasley 1988; Kathman and Cross 1991; Utsugi 1997; Collins and Bateman 2001; Nichols et al. 2001; Herrera-Vásquez and Vargas 2003; Guil et al. 2009; Kaczmarek et al. 2011). Up to now, only four species i.e. *Hypsibius microps* Thulin 1928, *Echiniscus testudo* (Doyère 1840), *Echiniscus granulatus* (Doyère 1840) and *Richtersius coronifer* (Richters, 1903), have been found in high mountain localities in Turkey (Maucci 1975, 1978, 1980). Some of the tardigrade species obtained from the alpine or subalpine zones are endemic only at certain altitudes, while others are cosmopolitan (Ramazzotti and Maucci 1983; Dastych 1988).

Our present study is the first high-altitude study to date on Turkish Tardigrada. Verçenik Mountain is located in the northern part of Turkey in Kaçkar Mountain Regions and originated from Alpide orogeny (Okay 2008). Up to now, zoogeographic and taxonomic information of tardigrades has been presented which is identified from alpine and subalpine zones in Turkey.

Materials and Methods

In August 2016, eight moss and lichen samples were collected on various altitudes of the Verçenik Mountain in Rize (Figure 1). But the tardigrades were found only in five of them (list of samples are presented in Table 1). All fresh samples were put into the small paper envelopes and allowed to dry slowly.

In the laboratory rehydrated samples were filtrated by the sieve of 25 and 400 μm, and the remaining residual was taken to a petri dish (Nelson 2002; Convey and McInnes 2005). Then tardigrades and their eggs were placed in a separate petri dish for identification on a stereomicroscope. All specimens were mounted on microscopic slides in Hoyer's medium, four adults and five eggs were prepared for Scanning Electron Microscopy (SEM) (JEOLJSM–6060 LV) analysis, following the protocols by Guidetti et al. (2000). The identification of specimens was carried out by analyzing morphological characters on the Phase-contrast microscope (Zeiss Axio ImagerM1) and SEM.

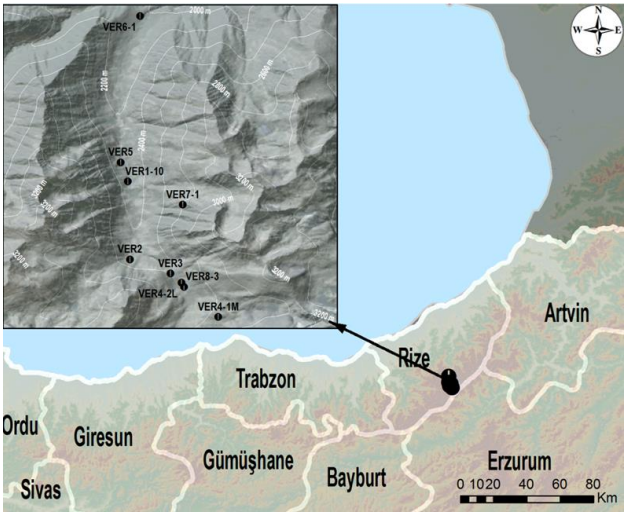

Figure 1. The geographic position of sampling sites.

Species were identified using a key to the world fauna of Tardigrada (Ramazzotti and Maucci 1983) and several original papers (Dastych 1988; Bertolani and Rebecchi 1993; Stec et al. 2018). All materials are deposited at the Aquatic Animals Research Laboratory at Ankara University.

Table 1. Distribution of the species found in the moss and lichen samples collected in Verçenik Mountain/Rize.

Sample Code	Coordinates latitude, longitude	Date of collecting	Above sea level (a.s.l)	Habitat	Species composition
VER1-10	N40°45'44" – E40°54'20"	25/08/2016	2308 m	Epilithic moss	1,2,3
VER2	N40°44'47" – E40°54'21"	25/08/2016	2247 m	Epilithic moss	-
VER3	N40°44'36" – E40°54'51"	25/08/2016	2131 m	Epilithic moss	5
VER4-1M	N40°44'05" – E40°55'26"	26/08/2016	2606 m	Epilithic moss	4
VER4-2L	N40°44'30" – E40°54'59"	26/08/2016	2606 m	Lichen	1
VER5	N40°45'58" – E40°54'14"	26/08/2016	2145 m	Epilithic moss	-
VER6-1	N40°47'45" – E40°54'28"	26/08/2016	2214 m	Epilithic moss	1,3
VER7-1	N40°45'27" – E40°55'00"	26/08/2016	2610 m	Epilithic moss	-
VER8-3	N40°44'27" – E40°55'01"	26/08/2016	2611 m	Epilithic moss	1,5

Acanthechiniscus victor (1), *Pseudechiniscus ramazzottii ramazzottii* (2), *Macrobiotus spectabilis* (3), *Paramacrobiotus cf. richtersi* (4) and *Ramazzottius oberhaeuseri* (5)

Results

A total of 62 specimens, 10 eggs, and 11 exivium were isolated and 5 species were identified. Three of them are new records for the Turkish Tardigrade fauna. A list of the identified Turkish high mountain and alpine tardigrade, with their localities and zoogeographical and taxonomic comments, are in Table 2.

Table 2. List of all obtained tardigrade species from Turkish high mountains and alpine regions, with their localities, and zoogeographical and taxonomic comments.

Taxa	asl**	Localities	Remarks
Cornechiniscus cornutus (Richters, 1907)	2400 m	Europe; Turkey, Bulgaria, Romania, Italy, Germany, Greece, Switzerland, Poland, Austria, Ireland, France, Russia, Africa; Algeria (McInnes et al. 2017), Libya, Asia; Iran, Afghanistan, India, Mongolia, Pakistan, China, N. America; Canada (6).	It was found in Erzurum/Pasinler (3,4). But according to Dastych (1988), it classified as submontane and distributed in Holarctic (6).
Echiniscus granulatus (Doyère, 1840)	2400 m	Europe; Italy, Turkey, France Greece Norway, Croatia, Austria, England, Ireland, Spain, Poland, Hungary, Germany, Portugal, Bulgaria, Africa; Morocco, Algeria, Russia, Georgia, Asia; Iran, Pakistan, Mongolia, Indonesia, N. America (6).	It was observed in Erzurum/Pasinler from Turkey (3). It is submontane and distributed in Holarctic (Dastych 1988).
Echiniscus merokensis suecicus Thulin, 1911	2200 m	Europe; Turkey, Italy, Norway, Sweden, Scotland, England; Iceland, Alps, Czechoslovakia, France, Yugoslavia, Switzerland, Africa; Angola, N. America; Greenland (6).	It was reported in Bursa/Uludağ Mountain at 2200 m (2,3) but it was found between 0 and 400 m in the Faroe Islands by Trygvadóttir and Kristensen (2013).
Echiniscus testudo (Doyère, 1840)	2000-2400 m	It is a cosmopolitan species and Holarctic. Its distribution is most of the Palearctic biogeographic region (6, Jørgensen et al. 2007, McInnes et al. 2017).	It was only observed in Erzurum and Ağrı from Turkey (2,3). In contrast, it was found between 0 and 200 m in the Faroe Islands by Trygvadóttir and Kristensen. (2013).
Ramazzottius oberhaeuseri (Doyére, 1840)	2000 m	It is a cosmopolitan, known from numerous locations in Europe from Greenland, the Arctic and Antarctic, North and South America, Africa, Afghanistan and New Zealand (Ramazzotti and Maucci 1983, McInnes et al. 2017).	It was observed in many cities in Turkey (1,2,3,4,5) and reported at an altitude of 4.300 m in Mount Blancgroup/Europe (Ramazzotti and Maucci 1983).
Acanthechiniscus victor (Ehrenberg, 1853) *	2606 m (7)	Europe; Turkey (7), Italy, Romania, Switzerland, Poland, Alps, Norway, Iceland), Russia. North America, Canada; Axel Heiburg Island, Greenland (6).	In this study, it was found from rock mosses and lichen in Rize/Verçenik Mountain. According to Ramazzotti (1956), it is classified as an alpine.
Pseudechiniscus facettalis Petersen, 1951	1200-1600 m	Europe; Turkey, Italy, Austria, Greece, Alps, Portugal, Spain, Africa; Kenya (McInnes et al. 2017), New Zealand; South Island, North America; Canada; Axel Heiburg Island, Greenland, South America; Brazil, Venezuela, Tierra del Fuego (6).	Although these species have been reported at low altitude, it is still classified as alpine species by Ramazzotti (1956). It was found Bozdağ/İzmir in Turkey (1,2,3).
Pseudechiniscus ramazzottii ramazzottii Maucci, 1952*	2308 m (7)	Europe; Turkey, Italy, Hungary, Alps, Russia, North and South America (6,7).	This species is a new record for Turkey. It was collected in moss samples from rock (7).
Hypsibius microps Thulin, 1928	1.800 m	Europe; Turkey, Italy, Istria, Turkey, Sweden, Finland, Norway, Greece, Poland, Hungary, Austria, Czech Republic, Portugal, Spain, Bulgaria, Russia, Africa; Algeria, Asia; India, Mongolia, Korea, N. America; Greenland; Disko Island, S. America; Brazil, Argentina (6).	Known only from two localities in Turkey (2,3). Cosmopolitan (6), however records of this species need to be verified (Kaczmarek and Michalczyk 2009).
Isohypsibius duranteae (Maucci, 1978)	2200-2400 m	It is endemic for Turkey (3,6).	It was only collected in Erzurum/Pasinler, Ağrı/ Tahir and originally described from Turkey (3).
Isohypsibius macrodactylus (Maucci, 1978)	2400 m	Europe; Turkey, Cyprus, Italy, Russia, Africa; Algeria (6; Tekatlı and Altındağ 2017).	It was reported in Ağrı and originally described from Turkey (3). On the other hand, it occurred at lowland altitudes (13 m asl) in Cyprus (Tekatlı and Altındağ 2017).
Mesobiotus harmsworthi (Murray, 1907)	1450 m	Cosmopolitan species, known from many localities throughout the world (6), but the species' geographic range is unknown (Kaczmarek et al. 2012).	It was observed in Ağrı, Antalya, Bolu, Burdur, Bursa, Izmir and Kastamonu from Turkey (1,2,3).

Table 2. Continued

Taxa	asl**	Localities	Remarks
Macrobiotus pallarii Maucci, 1954	2000 m	Europe; Turkey, Italy, Poland, Yugoslavia, Greece, Norway, Turkey, Hungary, Russia, Asia; North Korea, N. America (6).	It was reported in Bolu, Kastamonu, and Tunceli from Turkey (1,2,3).
Macrobiotus persimilis Binda and Pilato, 1972	2000 m	Europe; Turkey, Italy, Greece, Spain, Portugal, Africa; Tunisia, Libya, Australia; Sydney, N. America; Greenland (6).	It was found in Balıkesir, Bolu, Diyarbakır, Erzincan, Sakarya, Uşak and Van from Turkey (2, 4).
Macrobiotus spectabilis Thulin, 1928*	2308 m (7)	Europe; Turkey, Poland, Norway, Sweden, Italy, France, Russia, North America, South America (6, 7).	The present study, it was collected from rock moss in Rize/Verçenik Mountain from Turkey.
Tenuibiotus hystricogenitus (Maucci, 1978)	1350-2400 m	Europe; Turkey, Germany, Greece (6).	It was found in Erzurum and Çorum from Turkey (3).
Richtersius coronifer (Richters, 1903)	1800 m	Europe; Turkey, Norway, Sweden, Italy Greece, Scotland, Spain, Poland, Austria, France, Switzerland, Bulgaria, Hungary, Romania, Germany, Arctic, Africa; Tunisia, Algeria, Congo, Asia; Nepal, Mongolia, North America and South America (6, McInnes et al. 2017).	It was found in Ağrı, Çorum, Izmir, Van from Turkey (2,3,4). *R. coronifer* is considered as an alpine species by Ramazzotti (1956).

Source codes: 1- Maucci (1973), 2- Maucci (1975), 3- Maucci (1978), 4- Maucci (1979), 5- Maucci (1980), 6- McInnes (1994), 7- present study. Asterisks (*) are symbolized as a new record and (**) obtained from Turkish territory altitude. The list of species ordered according to Degma et al. (2018).

Taxonomic accounts

New records for Turkey were marked by an asterisk (*).

Phylum: Tardigrada Spallanzani, 1777

Class: Heterotardigrada Marcus, 1927

Order: Echiniscoidea Marcus, 1927

Family: Echiniscidae Thulin, 1928

Genus: *Acanthechiniscus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi and Guidetti, 2016

1. *Acanthechiniscus victor* (Ehrenberg 1853)*

Material examined: 25 specimens and 6 exuviae collected from two rock mosses and lichen.

Remarks: Species new for Turkey and most abundant in studied samples (Figure 2). It is considered an alpine species by Ramazzotti (1956). The specimens obtained correspond perfectly to the characterization of this species by Ramazzotti and Maucci (1983) and Dastych (1988). It has been recorded in many countries (Italy, Romania, Switzerland, Poland, Alps, Spitsbergen, Iceland, USSR, USA, Canada, Greenland) (McInnes 1994).

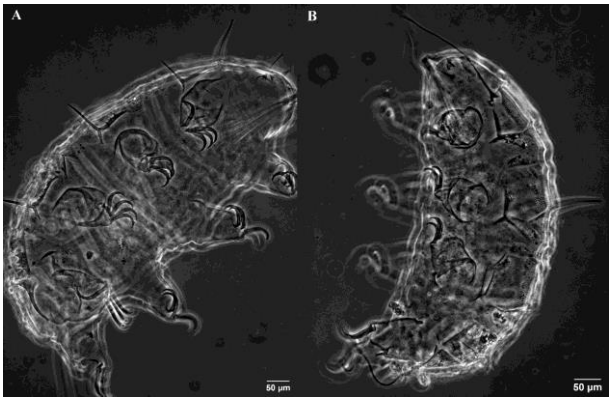

Figure 2. *Acanthechiniscus victor.* A) View of the ventral side B) Dorsal view.

Genus: *Pseudechiniscus* Thulin, 1911

2. *Pseudechiniscus ramazzottii ramazzottii* Maucci, 1952*

Material examined: 12 specimens and 2 exivium collected from two rock moss.

Remarks: Up to now it has been recorded sixth times (Italy, Hungary, Alps, Russia, North and South America), Ramazzotti (1956), Ramazzotti and Maucci (1983) and Iharos (1985). In this study, it was collected from moss samples at 2308 m (Figure 3).

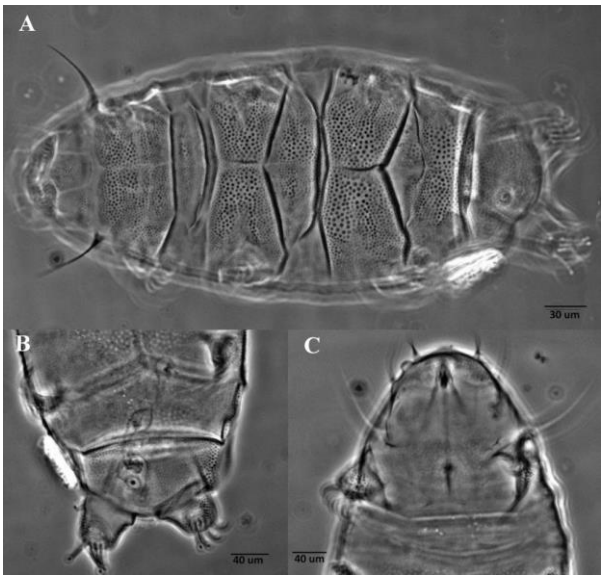

Figure 3. *Pseudechiniscus ramazzottii ramazzottii* A) Dorsal view B) Caudal margin of the pseudosegmental plate C) View of cephalic zone.

Class: Eutardigrada Richters, 1926

Order: Parachela Schuster, Nelson, Grigarick and Christenberry, 1980

Family: Macrobiotidae Thulin, 1928

Genus: *Macrobiotus* C.A.S. Schultze, 1834

3. *Macrobiotus spectabilis* Thulin, 1928*

Material examined: 6 specimens and 3 eggs collected from two rock moss.

Remarks: It is a new record for Turkey. It was observed from rock mosses at an altitude of 2308 meters in Rize/Verçenik Mountain (Figure 4). According to Dastych (1988), it is classified as a mountain (mesoalpine) species. It is known from few localities from Siberia, Poland, Italy, France, Finland, Russia, USA, Argentina, China and Norway (Ramazzotti and Maucci 1983; McInnes 1994; Zhang and Sun 2014). The found specimens correspond perfectly to the characterization of this species presented by Dastych (1973) and Maucci and Pilato (1974).

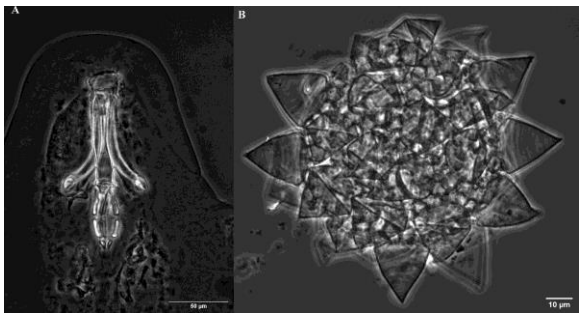

Figure 4. *Macrobiotus spectabilis* A) Buccal apparatus B) Egg.

Genus: *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009

Subgenus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009

4. *Paramacrobiotus cf. richtersi* (Murray, 1911)

Material examined: 5 specimens and 3 eggs collected from rock moss.

Remarks: It is one of the species of more common tardigrades and widespread everywhere, and present in different habitats, including terrestrial moss (Ramazzotti and Maucci 1983). In the study of Dastych (1988), on polish tardigrades, this species found in an area up to 2000 meters high. In this study, it was obtained from rock mosses at an altitude of 2131 m (Figure 5). In Turkey, it was collected between 250-1140 m altitude in Ankara, Antalya, Burdur, Çanakkale, İzmir and Van (Maucci 1973, 1975, 1980; Kaczmarek et al. 2012). But this genus has been revised by some researchers Kaczmarek et al. 2017; Marley et al. 2018).

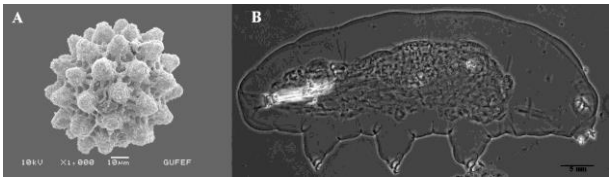

Figure 5. *Paramacrobiotus cf. richtersi* A) Egg B) View of the ventro-dorsal side.

Family: Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey, and Linse, 2008

Genus: *Ramazzottius* Binda and Pilato, 1986

5. *Ramazzottius oberhaeuseri* (Doyère 1840)

Material examined: 11 specimens and 3 eggs collected from two rock moss samples.

Remarks: According to Ramazotti (1956), this species is not alpine but can be encountered high altitude in Poland (Dastych 1988). It is an extremely wide-spread and very common species (Ramazzotti and Maucci 1983). It was found in Afyonkarahisar, Ağrı, Ankara, Antalya, Bolu, Çorum, Erzincan, Erzurum, Hakkari, Gaziantep, Kastamonu, Kırkareli, Uşak, Konya, Tunceli, Van between 250-2000 m from Turkey (Maucci 1973, 1975, 1978; Morgan 1977; Kaczmarek et al. 2012). All records of *Ramazzottius oberhaeuseri* prior to its redescription (Stec et al. 2018) should be verified (Figure 6).

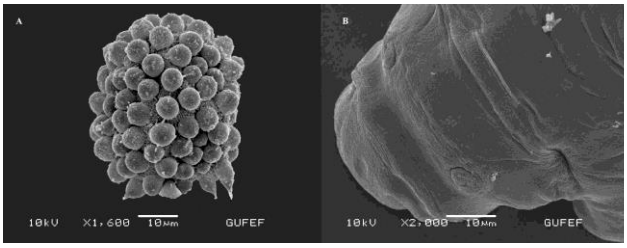

Figure 6. *Ramazzottius oberhaeuseri* A) Egg B) Eliptical organ.

Discussion

Alpine, subalpine, boreo-alpine, and nival regions are one of the few studied regions, as access to these regions is both difficult and land conditions are appropriate only during a certain period of summer. So far, the number of Tardigrada obtained in studies on these regions in the world is quite limited.

The alpine regions are located above the last tree line (Pechlaner 1971). Some researchers have splitthe tardigrade species into groups according to altitudinal ranges and localities (Ramazzotti and Maucci 1983; Dastych 1987). Ramazzotti and Maucci (1983), defined tardigrade species obtained from 500 m and above areas as alpine species, while Dastych (1988), defined the lower limit as 1000 m. In addition, studies are showing that the alpine region starts at 400 m and above (Trygvadottir and Kristensen 2013). The alpine zone varies according to the region in Turkey. Subalpine regions begin at about 1800 m. After approximately 2000 m, it continues as typical alpine regions (Atay et al. 2009). The alpine zone for Verçenik Mountain starts at 2000 m (Gürbüzer 2018). Therefore, our samples can be classified as alpine species. Previously obtained alpine species

from Turkey were compared with this study (Table 2).

In the studies conducted so far, not only the distribution, abundance and diversity of the elevation according to species, but also the species obtained from different regions in the same alpine zone have been studied (Collins and Bateman 2001; Trygvadottir and Kristensen 2013). According to Dastych (1980); increasing the number of individuals together with the height decreased; In his research in 1987, he stated that species diversity increased as the height increased. This result was supported by Rodríguez-Roda (1951). Additionally, Guil et al. (2009) cited that the abundance of a tardigrade is the highest at 1000-2000 m asl.; the lowest is 0-500 m asl. But other researchers have found that the abundance is not more than 0-500 m. asl (Utsugi 1997; Collins and Bateman 2001). Furthermore, Nichols (1999) stated that there is no relationship between height and tardigrade distribution in the study of the distribution of tardigrade on Dugger Mountain in Alabama. In addition, Kathman and Cross (1991) found in their research that there is no effect of height on tardigrades in the Mountains of Vancouver in Canada. Kaczmarek et al. (2011) cited that, even not linear, increasing of height revealed the abundance of a tardigrade. It is possible to reach tardigrade which is not very common in algae samples obtained at 2000- and 4000-meters altitude (Ramazzotti and Maucci 1983).

It is known that temperature, humidity, food availability, competition, predation, and parasitism are effective on tardigrade populations (Dastych 1982; Ramazzotti and Maucci 1983; Collins and Bateman 2001). Although there are limited studies on the effect of biotic and abiotic factors on the animal, some researchers have tried to understand whether the altitude has a role in this living organism. There have been contradictory studies on this subject, but some researchers have found a correlation between height and diversity (Kaczmarek et al. 2011).

Most of the Tardigrade species obtained in Turkey at 2000 meters and above are observed to be tychoalpine species (an organism that is found at every altitude) compared to other areas. There is no study available on these creatures obtained associated with altitude. Until now, the relationship between the species obtained up to 2000 meters and above and altitude has not been determined when compared with other studies. The reason for this difference is thought to be due to the environmental conditions and microhabitat where the samples are exposed to, rather than the effect of the altitude on the habitat where the samples are taken.

Many studies on the Phylum Tardigrada have focused on limnoterrestrial tardigrades, which are easy to access and have a lot of diversity. Since transportation and weather conditions are difficult in Alpine regions, very few studies have been done so far. The data obtained is not enough to illuminate the distribution and diversity of the tardigrades in these regions. In the future, studies in high-altitude regions will give us more information about the distribution and characteristics of these animals.

Acknowledgments

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