Journal of Limnology and Freshwater Fisheries Research
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Potential Distribution of the Amphibian Pathogen, *Batrachochytrium dendrobatidis* in the Eastern Black Sea Region of Turkey

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ABSTRACT

Although *Batrachochytrium dendrobatidis*, pathogen for amphibians, has been reported in Anatolia, its geographical distribution, as well as its impact on the amphibians in Turkey, remained obscure. In this study, 62 adult individuals belonging to ten different species (*Pelodytes caucasicus*, *Rana dalmatina*, *Rana macrocnemis*, *Bufo bufo*, *Bufo verrucosissimus*, *Bufotes variabilis*, *Hyla savignyi*, *Pelophylax ridibundus*, *Ommatotriton ophryticus*, *and Mertensiella caucasicus*) were collected from five wetland habitats in Eastern Black Sea Region of Turkey. The prevalence and the intensity of *B. dendrobatidis* infections in all the individuals were investigated by using quantitative Real-time-PCR technique and the presence of *B. dendrobatidis* infection was reported for the first time in 13 of the 62 individuals collected from 10 amphibian species from Eastern Black Sea Region of Turkey. The intensity of *B. dendrobatidis* infection ranged from 403.520 to 534.280 genomic equivalents (*GE*) was detected. The highest *GE* between amphibian species were determined in *P. caucasicus* (534.280 *GE*) in Uzungöl (Çaykara-Trabzon) and *B. bufo* (504.00 *GE*) in Lake Karagöl (Şavşat-Artvin).

Keywords: Chytridiomycosis, *Batrachochytrium dendrobatidis*, Anatolia, Amphibia

ARTICLE INFO

RESEARCH ARTICLE

Received : 05.10.2018
Revised : 10.12.2018
Accepted : 15.01.2018
Published : 25.04.2019

DOI:10.17216/LimnoFish.467527

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Doğu Karadeniz Bölgesinde Amfibi Patojeni *Batrachochytrium dendrobatidis*'in Potansiyel Dağılımı

Öz: Amfibi patojeni *Batrachochytrium dendrobatidis* Anadolu'da rapor edilmesine rağmen, hem coğrafik dağılımı hem de Türkiye amfibileri üzerindeki etkisi hala belirsizdir. Bu çalışmada, Doğu Karadeniz Bölgesindeki beş sulak alandan on farklı amfibi türlerine (*Pelodytes caucasicus*, *Rana dalmatina*, *Rana macrocnemis*, *Bufo bufo*, *Bufo verrucosissimus*, *Bufotes variabilis*, *Hyla savignyi Pelophylax ridibundus*, *Ommatotriton ophryticus*, *Mertensiella caucasicus*) ait 62 ergin birey toplandı. Kantitatif Real-time PCR tekniği ile tüm bireylerde *B. dendrobatidis* enfeksiyonlarının prevalansı ve yoğunluğu ile araştırıldı ve Doğu Karadeniz Bölgesi'ndeki 10 amfibi türünden toplanan 62 bireyin 13' ünde *B. dendrobatidis* enfeksiyonu varlığı ilk kez rapor edildi. Enfeksiyon yoğunluğu 403,520-534,340 genomik eşdeğerler arasında değiştiği belirlendi. Amfibi türler arasında en yüksek genomik eşdeğer Uzungöl' deki *P. caucasicus* (534,280) ve Karagöl'de *B. bufo* (504,00) saptandı.

Anahtar kelimeler: Chytridiomycosis, *Batrachochytrium dendrobatidis*, Anadolu, Amphibia

How to Cite
Erişmiş UC, 2019. Potential Distribution of the Amphibian Pathogen, *Batrachochytrium dendrobatidis* in the Eastern Black Sea Region of Turkey. LimnoFish. 5(1): 27-33. doi: 10.17216/LimnoFish.467527

Introduction

Infectious diseases are one of the factors implicated in the declines and extinctions of amphibians in worldwide. *Batrachochytrium dendrobatidis* (*Bd*) is a fungus that colonizes amphibian skin and the associated disease, chytridiomycosis, can disturb cutaneous respiration and osmoregulation and result in the death of the host (Carver et al. 2010). Differences in ecological factors such as host population density, habitat, and age structure may influence the rate at which chytrid spreads through the environment (Daszak et al. 1999). According to Ron (2005), *Bd* was predicted to spread in Anatolia, but the geographic distribution of *Bd* and its effect on Turkish amphibians is poorly understood (Farrer et al. 2011). Though 26 Turkish amphibian species are listed in the International Union for Conservation of Nature (IUCN) Red List,

investigation of the decrease in frog population has become mandatory (Başkale et al. 2013). Only two works were carried out on the distribution of *Bd* in Turkey. Previously, Göçmen et al. (2013) reported that one of two *P. bedriagae* specimens from Göynük Canyon (Antalya) was found as positive for *Bd*. Erişmiş et al. (2014) reported *Bd* infecting wild *P. ridibundus*, *H. orientalis*, *B. variabilis* as well as endemic Beysehir frogs *P. caralitanus* in West Anatolian Region and the District Lakes of South Western Turkey.

Thirty-six amphibian species were recorded in Turkey. Due to Turkey's geographical position, different species spread in different regions and they are exposed to a great number of threats (Şekercioğlu et al. 2011). This includes a number of restricted and rare amphibian species such as *Rana tavasensis* (Franzen et al. 2008), *R. holtzi* (Yildiz and Göçmen 2012), *R. macrocnemis* (Veith et al. 2003), *P. caralitanus* (Bülbül et al. 2011). If such species were susceptible to the fungal infection, the local and isolated populations might easily become extinct. Hence, the location of pathogens and susceptible to species are needed to be determined in Turkey. Management strategies for the containment of *Bd* spreading include the detection of wild and captive populations infected with chytrid disease, the identification of infected geographical areas, and the control of infected animal's movement from one location to another.

Therefore, the main objective of the present study was to determine *Bd* infected amphibian species through quantitative polymerase chain reactions (*qPCR*) (Kriger et al. 2006; Hyatt et al. 2007) in the Eastern Black Sea Region of Turkey.

Materials and Methods

The study was carried out in 6 different areas [Uzungöl (Çaykara-Trabzon, *UZL*), Karagöl (Şavşat-Artvin, *KRL*), Sahara Natural Park (Şavsat-Artvin, *SNP*), Ardeşen (Rize, *ARD*), Lake Şavsat (Şavşat-Artvin, *SVT*)] at 39 to1876 m elevation (*E*) in the eastern Black Sea region (*EBS*) of Turkey (Figure 1).

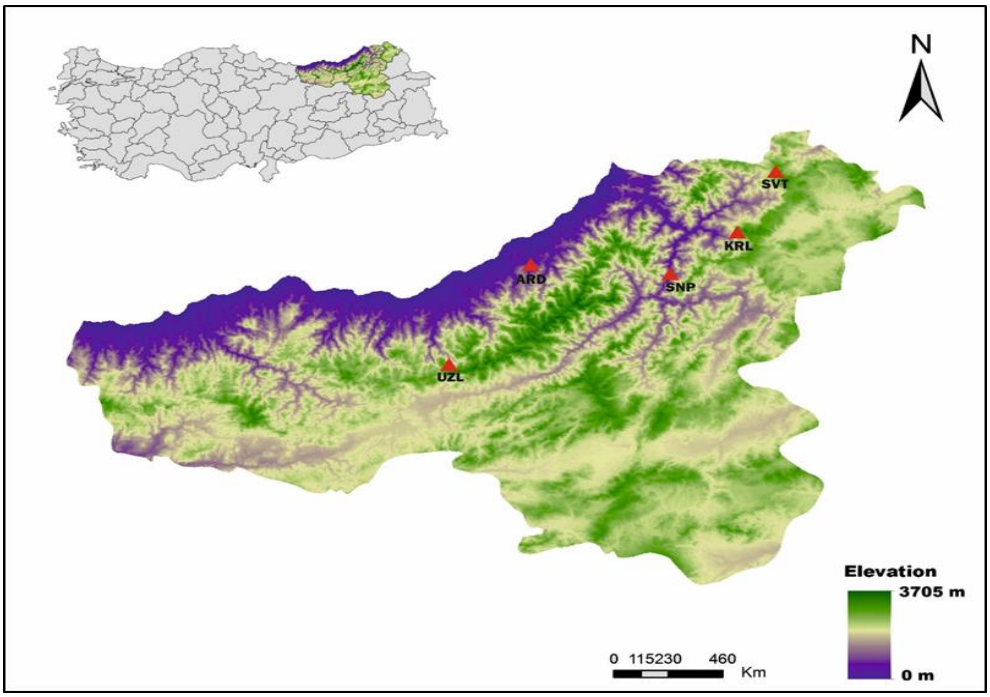

Figure 1. Map showing the collecting sites.

The specimens were collected during the summer season of 2014 (June through August). Air temperature, water pH, and humidity (H) were recorded during the fieldwork (Table 1). For each captured animal, surveyors recorded its GPS coordinates. To prevent the transfer of infected materials among sites, we rinsed all equipment with 5% bleach before entering each location. All of the frogs were handled with latex gloves and gloves were discarded after examination of each animal (Bai et al. 2010). The distribution of zoosporangia of *Bd* was studied in collected 10 species [*P. caucasicus* (*Pc*), *R. dalmatina* (*Rd*), *R. macrocnemis* (*Rm*), *B. bufo* (*Bb*), *B. verrucosissimus* (*Bv*), *B. variabilis* (*Bvs*), *H. savignyi* (*Hs*) *P. ridibundus* (*Pr*), *O. ophryticus* (*Oo*), *M. caucasicus* (*Mc*)].

To determine whether the animal was infected with *Bd* or not by *PCR* analysis, tissue samples were collected using swab method with a sterile cotton tip swab to take the keratinized tissues where *Bd* zoospores were highly concentrated (Marantelli et al. 2004). During *Bd* sampling process, each individual

was swabbed 30 times. We followed the standardized sampling protocol detailed by Hyatt et al. (2007). Samples were stored in 95% ethanol and were kept on ambient temperature (≥10°C) in the field and transported back to the laboratory and stored in a −80 °C freezer (Hyatt et al. 2007). The intensity of infection in all samples was determined by using qPCR (Boyle et al. 2004; Hyatt et al. 2007), with the modifications of methods described by Boyle et al. (2004). It was extracted nucleic acids using 50 µl PrepMan Ultra (Applied Biosystems), and the tip of the swab was used instead of a toe. To ensure the integrity of our results, a negative control (dH_2O) was run in triplicate on every 96-well *PCR* plate (Kriger et al. 2006). We constructed a standard curve to determine the zoospore load. A standard curve was constructed from the control reactions containing 100, 10, 1 and 0.1 *Bd* zoospores and the concentration determined for the test samples expressed as the number of zoospore equivalents. The intensity of infection was measured as the number of genome equivalents (*GE*) per swab, calculated by multiplying the *GE* values generated during the *qPCR* by the dilution factor of the template *DNA*. Swabs were categorized as *Bd* positive at ≥1 *GE* and as *Bd*-negative at <1 *GE* (Kriger et al. 2006; Hyatt et al. 2007; Anna et al. 2011; Erismis et al. 2014). All analyses were performed in triplicate. The percentages of infected individuals and *GE* were not compared among within sites or the species due to the low statistical power of small sample sizes for each species at a site. In the localities with positive *Bd*, we used the zonal statistic routine to extract from the digital maps the environmental variables values from each point (ArcView 3.2, Spatial Analyst). These values were also used to run Principal Component Analysis (*PCA*; implemented in XLSTAT v.3.0) to visualize the degree of clustering in environmental space among *EBS* region of Turkey where *Bd* was found.

Results

We swabbed 62 individuals from the 8 genera, including 10 species that occur in *EBS* region of Turkey. The prevalence and the intensity of *Bd* infections in all the individuals were investigated by using quantitative real-time-*PCR* technique and the presence of *Bd* infection was reported for the first time in 13 of the 62 individuals collected from 10 amphibian species from *EBS* Region of Turkey (Figure 2, Table1).

Bd was not detected only in *ARD* region. We determined the presence of *Bd* infection in 13 out of 62 (20.9%) samples comprising six species: *Pelobates caucasicus* (*Caucasian type-specific), Bufo bufo, B. verrucosissimus, Bufotes variabilis, Pelophylax. ridibundus, Ommatotriton ophryticus* (Table 1).

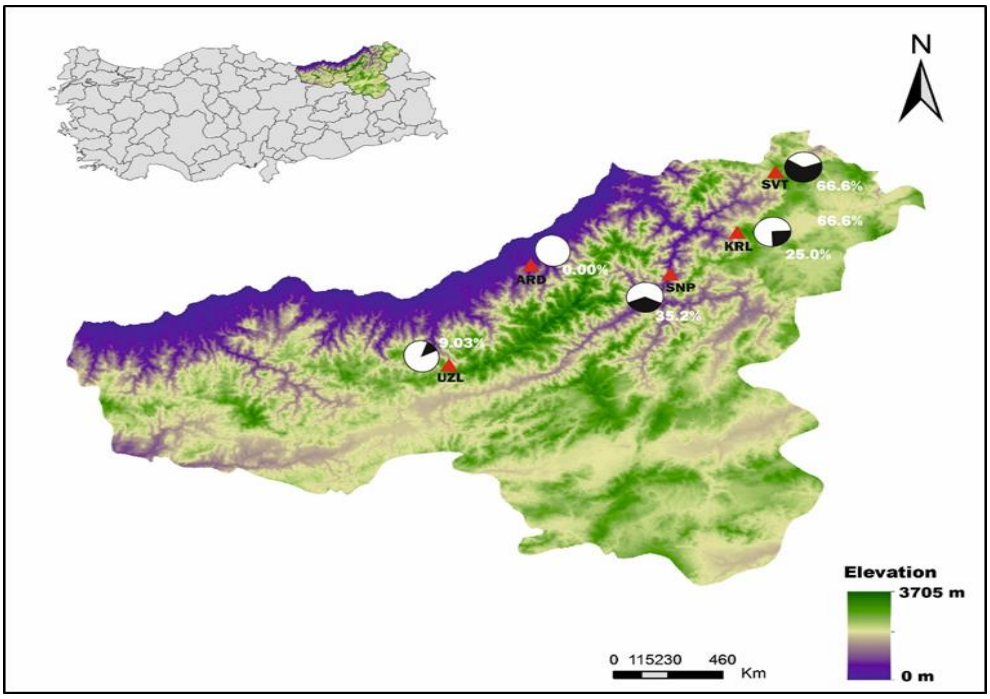

Figure 2. Map of *Bd* prevalence on EBS of Turkey. EBS from all states sampled tested positive and negative for *Bd*, Positive (black) and negative (white) proportions by the state were indicated by pie charts.

Table 1. Prevalence (*Prev*) and Average Genomic Equivalents (*GE*) of *Bd* in Eastern Black Sea Region of Turkey

Species	Locality	Coordinates	*Air* ˚*C*	*H* %	*E* *(a.s.l)*	*N* *(+ve)*	*Prev.* *(%)*	*GEs*
Pc	*UZL*	40˚37′23″N - 40˚16′50″E	26.0	56.0	1164	12(2)	16.0	527±16
Rd	*UZL*	40˚36′24″N - 40˚18′48″E	19.5	78.0	1271	1(0)	-	-
Rm.	*UZL*	40˚35′18″N - 40˚21′19″E	18.5	82.0	1701	2(0)	-	-
Bb	*UZL*	40˚37′07″N - 40˚18′45″E	19.5	78.0	1272	4(0)	-	-
	KRL	40˚56′20″N - 43˚23′30″E	27.0	65.0	1600	4(1)	25.0	504.00
Bv	*UZL*	40˚37′11″N - 40˚17′36″E	19.5	78.0	1278	10(1)	10.0	436.00
Mc	*UZL*	40˚35′27″N - 40˚20′45″E	27.5	72.3	1702	3(0)	-	-
Bvs	*SNP*	41˚14′24″N - 41˚13′12″E	23.0	77.0	1876	2(1)	0.50	463.00
Hs	*ARD*	41˚11′13″N - 40˚59′19″E	28.0	68.0	39.0	2(0)	-	-
Pr	*SNP*	41˚14′24″N - 41˚13′12″E	23.0	77.0	1876	15(5)	33.3	490.00
	KRL	41˚18′32″N - 42˚28′57″E	26.0	65.0	1600	4(1)	25.0	480.00
Oo	*SVT*	41˚17′47″N - 42˚28′37″E	30.5	66.0	1409	3(2)	66.6	405±9.0
Total						62(13)	20.9	473.66±18.44

Sampling localities and examined frog species abbreviations used*; UZL:* Uzungöl/Trabzon, *KRL:* Karagöl/Artvin, *SNP:* Sahara Natural Park/Artvin, *ARD:* Ardeşen/Artvin, *SVT:* Savsat Lake/Artvin*; Pc: Pelodytes caucasicus, Rd: Rana dalmatina, Rm: Rana macrocnemis, Bb: B. bufo, Bv: Bufo verrucosissimus, Mc: Mertensiella caucasicus; Bv: B. variabilis, Hs: Hyla savignyi, Pr: Pelophylax ridibundus, Oo: O. ophryticus respectively*, and *H:* Humidity, *E:* Elevation. *GE* genomic equivalent (including positive samples and negative samples, *GE* represents the burden of infection with *Bd).*

Bd was detected at 4 of 5 sites in *EBS* Region of Turkey were not being specifying *Bd* may be due to the small number of samples (Figure 1). *B. bufo* were sampled at two locations (*UZL* and *KRL*), only one of these locations tested positive for *Bd.* Although *P. ridibundus* were sampled at two regions (*SNP* and *KRL*) but 6 tested positive for *Bd*. The population of both *P. ridibundus* and *B. bufo* (at *KRL*) with a prevalence of 25%. However, we detected *Bd* in only one specimen of *B. verrucosissimus* (n=10). Furthermore, we did not detect any *Bd* on *R. dalmatina, R. macrocnemis, B. bufo,* and *M. caucasicus* (at *UZL*), *H. savignyi* (at *ARD*). In addition, Northern banded newts (*O. ophryticus)* were notable for their highest *Bd* infection rate at *SVT* with the prevalence of 66.6 % than other frog species (Table 1). Therefore, the prevalence of *Bd* infection on the populations of 10 frogs species among *EBS* regions (*UZL, KRL, SNP, SVT*) did differ significantly ($\chi 2$ = 8.43, *df* = 3, $P_{0.05}$> 0.03).

We also detected the rate of *Bd* infection as the mean number of *GE* per sample in 3 replicates. The mean number of *GE* for individual positive samples ranged from 405±9.0 (for O. ophryticus at *SVT* region) to 527±16.0 (for *P. caucasicus* at *UZL* region). The highest intensity of zoospores was found at *P. caucasicus* (527±16.00) at *UZL* region followed by *B. bufo* (504.00) at *KRL* region (Table 1). However, the average GEs among the four regions (*UZL, KRL, SNP, SVT*) individuals of frogs infected by *Bd* were analyzed through multiple comparisons based on a Tukey-HSD post-hoc test, which indicated a not significant difference among them (F = 3.27, *df* = 3;9, p = 0.08). Increasing suitability for both prevalence and *GE* of *Bd* was widely distributed on *EBS* regions of Turkey but was detected lowest in the *ARD* region (Tablo 1).

Final map resulted with areas highly suitable for the presence of *Bd* (Niche Overlap Index (*NOI*) > 0.70) dispersed irregularly overall *EBS* Region of Turkey (Figure 3). There are localities with highly suitable for the fungus in north regions of phytogeographic provinces of Trabzon and Artvin (Northeast of Turkey). *NOI* was varied among regions and was represented by different percentages of covered surface. Areas with 0.70 > OI < 1 (highest suitability for chytrid development) covered only 32.72 % of the total surface while areas with 0.50 > OI < 0.70 covered 56.86 %. Areas with 0.00 > OI < 0.50 only 10.42 of the total surface of *EBS* region of Turkey (Figure 3).

With eigenvalues >1, Principal Component I was positively correlated with (1) Elevation, (2) mean annual temperature, (2) precipitation of wettest explaining 89.6% of the variance of the system. Principal Component II explained 29.0% and was

highly positively correlated only with seasonal temperature. The environmental variables in the localities where *Bd*. was found show that suitable locations for the fungus are possible across a wide range of habitats (Table 1). In the localities with known presence of the fungus, the annual mean temperature ranged from 5℃ (*UZL* and *SVT*) to 23.5℃ (*SNP* and *SVT*), moreover in areas where the OI = 0.89. In addition, our analysis shows that the presence of the fungus in *EBS* region of Turkey is related to precipitations between 68 mm (*UZL* and *SVT*) and 235 mm (*UZL* and *SVT*).

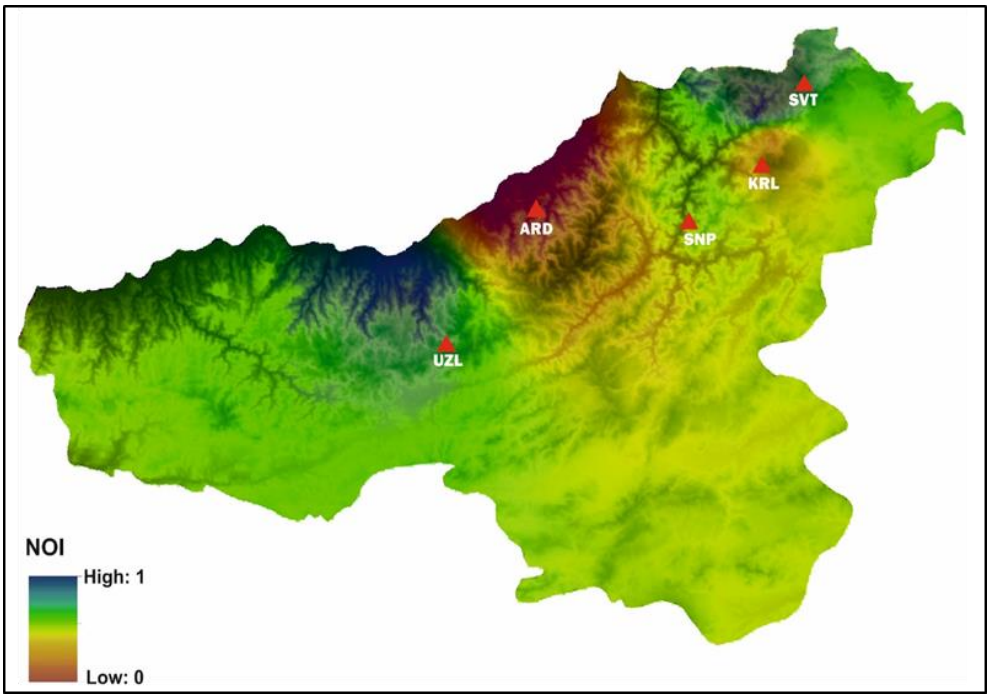

Figure 3. Niche Overlap Index (*NOI*) map of the potential distribution of *Bd* in *EBS* region of Turkey

Discussion

Distribution models showed that *Bd* has likely already spread to most climatically suitable regions (Fisher et al. 2009). Turkey's diverse regions have different climates because of irregular topography. Black Sea region has an oceanic climate (Köppen climate classification: *Cfb*), wet and humid (summer 23°C, winter 7°C) (Sensoy et al. 2008). Our previous study suggests that the Anatolian climate is indeed favorable for the spread of chytridiomycosis (Erismis et al. 2014). Intercalarily, *Bd* has been found in five mainlands including North and South America, Europe, Oceana, Africa, and Asia. However, there have only been two published studies up to date describing the presence of *Bd* in Anatolia at the crossroads of three continents. Therefore, it is not surprising that the *Bd* present in Anatolia.

In this study, a total of 62 specimens from 8 genera, including 10 species was sampled. We detected the presence of *Bd* infection in 13 of 62 (20.09%) samples comprising four species: *P. caucasicus* (*Caucasian type-specific), B. bufo, B. verrucosissimus, P. ridibundus, O. ophryticus* (Table 1). *Bd* was detected at 4 of 5 sites. We detected *Bd* in only one from ten swamp samples of *B. verrucosissimus*. We did not detect any *Bd* on *R. dalmatina, R. macrocnemis, B. bufo* (at *UZL*), *B. variabilis, H. savignyi* and *M. caucasicus*. In addition, Northern banded newts (*O. ophryticus)* were notable for their higher *Bd* infection rate at *SVT* prevalence of 75% than other frogs species. *P. ridibundus* were sampled at two locations (*SNP* and *KRL*) but 6 tested positive for *Bd.* We found a moderate *Bd* rate (20.09 %, *N* = 62) and low zoospore loads (473.66±18.44). The number of zoospores increases during infection. Low numbers may represent an earlier stage of infection, but the shedding of the skin may also contribute to low counts. We observed a widespread prevalence of *Bd* zoospore in apparently healthy adult amphibians in the study areas. Recent work suggests that *Bd* may produce tiny, non-pathogenic resting spores that attach to the amphibian skin surface but without causing disease (Di Rosa et al. 2007). The competing hypothesis contends that chytrid is endemic to many regions and that climate or other factors have altered the host-pathogen relationship, resulting in the recent outbreaks of chytridiomycosis (Morehouse et al. 2003; Weldon et al. 2004). As is also known adults may be protected by acquired immunity (Richmond et al. 2009) and thus may clear or prevent infections more efficiently than juveniles that are naive to *Bd,* infects some amphibian species with little negative effects on the host and do not die therefore may serve

as reservoirs of the disease (Mazzoni et al. 2003). Many amphibian species such as *Xenopus spp*, *R. catesbeiana*, and *B. marinus* carry this disease, also terrestrial species of anurans have been observed with *Bd*, suggesting frog to frog transmission is possible (Kriger et al. 2007).

Differences in morbidity and mortality in experimentally infected amphibians indicate that *Bd* virulence can vary between strains of the same and different lineages. Increased *Bd* growth rate, zoospore production, and sporangial size in pure culture, have been linked with increased host mortality and immunosuppressive activity (Fisher et al. 2009). Our studies suggested that amphibians can evolve resistance to *Bd* and may have the ability to coexist with the disease. The Eastern Black Sea region has unique reptile fauna. These regions are the corridors of the species coming from the Caucasus and the south. The high Anatolian diagonal mountains are a barrier to colonization (Ansell et al. 2011). This study showed that Anatolian diagonal mountains are not a barrier for the colonization of *Bd* in Anatolia. If amphibians can evolve resistance to *Bd* and may have the ability to coexist with the disease, testing for the presence of *Bd* should be mandatory other regions of Turkey.

In conclusion, the uncertain distribution and potential impact of *Bd* presence in Turkey require additional investigation before accurate evaluations can be made. Standardized field surveillance methods and laboratory diagnostic techniques are needed to more carefully investigate. The presence, distribution, virulence to native species and clade membership of *Bd* in Turkey must be verified before its potential impact on Anatolian amphibians can be accurately predicted.

Acknowledgements

This research was supported by Project no. Tubitak 113Z139. Ethical endorsement was ratified by the Ethical Committee of Afyon Kocatepe University and the Turkish Department of Nature Conservation (Permit number, DKMP-51039719).

References

Anna ES, Grismer LL, Anuar S, Onn CK, Grismer JL, Quah E, Muin MA, Ahmad N, Lenker M, Zamudio KR. 2011. First record of *Batrachochytrium dendrobatidis* infecting four frog families from Peninsular Malaysia. Ecohealth. 8(1):121-128. doi: 10.1007/s10393-011-0685-y

Ansell SW, Stenøien HK, Grundmann M, Russell SJ, Koch MA, Schneider H, Vogel JC. 2011. The importance of Anatolian mountains as the cradle of global diversity in *Arabis alpina*, a key arctic–alpine species. Annals of Botany. 108(2):241-252. doi: 10.1093/aob/mcr134

Bai C, Garner TWJ, Garner, LiY. 2010. First evidence of *Batrachochytrium dendrobatidis* in China: discovery of chytridiomycosis in introduced American bullfrogs and native amphibians in the Yunnan Province, China. EcoHealth. 7(1):127-134. doi: 10.1007/s10393-010-0307-0

Başkale E, Yildirim E,Çevik IE, Kaya U. 2013. Population size and age structure of metamorphic and pedomorphic forms of *Ommatotriton ophryticus* (Berthold, 1846) in the Northwestern Black Sea Region of Turkey. J Herpetol. 47(2):270-276. doi: 10.1670/11-116

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis *Batrachochytrium dendrobatidis* in amphibian samples using real-time taqman PCR assay. Dis Aquat Organ. 60(2):141-148. doi: 10.3354/dao060141

Bülbül U, Matsui M, Kutrup B, Eto K. 2011. Taxonomic relationships among Turkish water frogs as revealed by phylo-genetic analyses using mtDNA gene sequences. Zool Sci. 28(12):930-936. doi: 10.2108/zsj.28.930

Carver S, Bell BD, Waldman B. 2010. Does chytridiomycosis disrupt amphibian skin function? Copeia. 2010(3):487-495. doi: 10.1643/CH-09-128

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. 1999. Emerging infectious diseases and amphibian population declines. Emerg Infect Dis. 5(6):735-748. doi : 10.3201/eid0506.990601

Di Rosa I, Simoncelli F, Fagotti A, Pascolini R. 2007. Ecology: the proximate cause of frog declines? Nature. 447(7144):4-5. doi: 10.1038/nature05941

Erismis UC, Konuk M, Yoldas T, Agyar P, Yumuk D, Korcan SE. 2014. Survey of Turkey's endemic frogs for amphibian chytrid fungus *Batrachochytrium dendrobatidis.* Dis Aquat Organ. 111(2):153-157. doi: 10.3354/dao02742

Farrer RA, Weinert LA, Bielby J, Garner TW, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, du Preez LH, Anderson L, Pond SL, Shahar-Golan R, Henk DA, Fisher MC. 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. P Natl Acad Scı Usa. 108(46):18732-18736. doi: 10.1073/pnas.1111915108

Fisher MC, Garner TW, Walker SF. 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu Rev Microbiol. 63:291-310. doi: 10.1146/annurev.micro.091208.073435

Franzen M, Bussmann M, Kordges T, Thiesmeier B. 2008. Die Amphibien und Reptilien der Südwest-Türkei. Bielefeld: Laurenti Verlag 329 p.

Göçmen B, Veith M, Iğci N, Akman B, Godmann O, Wagner N. 2013. No detection of the amphibian pathogen *Batrachochytrium dendrobatidis* in terrestrial Turkish salamanders (Lyciasalamandra)

despite its occurrence in syntopic frogs (*Pelophylax bedriagae*). Salamandra. 49(1):51-55.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Organ. 73(3):175-192. doi: 10.3354/dao073175

Kriger KM, Hines HB, Hyatt AD, Boyle DG, Hero JM. 2006. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time taqman PCR. Dis Aquat Organ. 71(2):141-148. doi: 10.3354/dao071141

Kriger KM, Pereoglou F, Hero JM. 2007. Latitudinal variation in the prevalence and intensity of chytrid *Batrachochytrium dendrobatidis* infection in eastern Australia. Conserv Biol. 21(5):1280-1290. doi: 10.1111/j.1523-1739.2007.00777.x

Marantelli G, Berger L, Speare R, Keegan L 2004. Distribution of the amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. Pacific Conservation Biology. 10(2):173-179. doi: 10.1071/PC040173

Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, Speranza G. 2003. Emerging pathogen of wild amphibians in frogs.*Rana catesbeiana* farmed for international trade. Emerg Infect Dis. 9(8):995-998. doi: 10.3201/eid0908.030030

Morehouse EA, James TY, GanleyA RD, Vilgalys R, Berger L, Murphy PJ, Longcore J E. 2003. Multilocus sequence typing suggests that the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol. 12(2):395-403. doi: 10.1046/j.1365-294X.2003.01732.x

Richmond JQ, Savage AE, Zamudio KR, Rosenblum EB. 2009. Toward immunogenic studies of amphibian chytridiomycosis: linking innate and acquired immunity. Bioscience 59(4):311-32. doi: 10.1525/bio.2009.59.4.9

Ron SR. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis i*n the new world. Biotropica. 37(2):209-221. doi: 10.1111/j.1744-7429.2005.00028.x

Sensoy S, Demircan M, Ulupınar Y, Balta İ. 2008. Türkiye İklimi; [cited 18 Feb 2018]. Available from https://www.mgm.gov.tr/FILES/genel/makale/13_turkiye_iklimi.pdf

Şekercioğlu CH, Anderson S, Akçay E, Bilgin R, Emre Can Ö, Semiz G, Tavşanoğlu C, Baki Yokeş M, Soyumert A, İpekdal K, Sağlam İK, Yücel M, Dalfes HN. 2011. Turkey's globally important biodiversity in crisis. Biol Conserv. 144(12):2752-2769. doi:10.1016/j.biocon.2011.06.025

Veith M, Schmidtler F, Kosuch J, Baran I, Seitz A. 2003. Paleoclimatic changes explain Anatolian mountain frog evolution: a test for alternating vicariance and dispersal event. Mol Ecol. 12(1):185-189. doi: 10.1046/j.1365-294X.2003.01714.x

Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R. 2004. Origin of the amphibian chytrid fungus. Emerg Infect Dis. 10(12):2100-2105. doi: 10.3201/eid1012.030804

Yildiz MZ, Göcmen B. 2012. Population dynamics, reproduction, and life history traits of Taurus Frog, *Rana holtzi* Werner, 1898 (Anura: Ranidae) in Karagöl (Ulukışla, Niğde), Turkey. Herpetologica Romanica.6:1-40.