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No detection of the amphibian pathogen *Batrachochytrium dendrobatidis* in terrestrial Turkish salamanders (*Lyciasalamandra*) despite its occurrence in syntopic frogs (*Pelophylax bedriagae*)

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The panzootic fungal amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) is known to cause the lethal skin disease chytridiomycosis (HILLMAN et al. 2009). It irritates the amphibian skin in a way that its crucial physiological functions are disturbed, causing electrolyte depletion and osmotic imbalance (BERGER et al. 1998, VOYLES et al. 2007, 2009, ROSENBLUM et al. 2010).

The worldwide spread of *Bd* has been facilitated by human-mediated vectors, such as African clawed frogs (*Xenopus* spp.). Further, secondary vectors may be man-mediated introductions of alien amphibian populations (e.g., *Lithobates cancrivorus*; FISHER & GARNER 2007, SCHLOEGEL et al. 2012), birds (GARMYN et al. 2012), reptiles (KILBURN et al. 2011), crayfish (MCMAHON et al. 2013), or even the scientists themselves (MORGAN et al. 2007). This is proposed to help the pathogen to spread into major parts of the world where it rose to a major threat to amphibian biodiversity (FISHER et al. 2009). Interestingly, only one expansive and virulent lineage seems to be responsible for the decline of amphibian populations on large geographical scales, while some geographically restricted lineages seem to be less harmful to local amphibian species (FARRER et al. 2011). This led scientists to hypothesise that the virulent lineage may have evolved in Africa, where it occurs with high prevalence and infection rates on a range of local species without causing any noticeable decline (e.g., KIELGAST et al. 2010). When distributed to other continents, it harmed native populations of non-adapted species, with all the known and often fatal consequences (e.g., FISHER et al. 2009).

Interestingly, not all non-African amphibian species are affected to the same degree. BIELBY et al. (2008) discussed a possible link between an anuran species' life history traits and its risk for infection with *Bd*. In addition, some species seem to have a reduced susceptibility to *Bd* due to the kind and composition of skin peptides or their hosted microbial communities (e.g., HARRIS et al. 2006, WOODHAMS et al. 2007a,b, BECKER & HARRIS 2010, SAVAGE & ZAMUDIO 2011, SEARLE et al. 2011).

Recently, several studies highlighted the potential absence of *Bd* in European terrestrial salamanders. Despite reasonable sampling, no infection with *Bd* was observed in Alpine salamanders (*Salamandra atra*; LÖTTTERS et al. 2012) and the genus *Hydromantes* (CHIARI et al. 2013, PASMANS et al. 2013), although the pathogen was known to infect sympatrically occurring amphibian species. Their terrestrial lifestyle was discussed as a reason for the absence of *Bd* in these species (CHIARI et al. 2013), although “a strictly terrestrial life history does not per se exclude or reduce the likelihood of infection by *Bd*” (LÖTTTERS et al. 2012). Reduced mobility, as is known from many terrestrial salamanders, could even reinforce this effect through a low intraspecific transmission rate (LÖTTTERS et al. 2012, CHIARI et al. 2013). In contrast, like in some other amphibians, specific skin peptides may protect some of the species, as was shown for the genus *Hydromantes* by PASMANS et al. (2013).

We here add information on the presence/absence of *Bd* in *Lyciasalamandra* VEITH & STEINFARTZ, 2004 (the former *Mertensiella luschani*), the remaining genus of full terrestrial European salamanders that has not yet been

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Table 1. Turkish *Lyciasalamandra* populations and single anuran specimens tested for *Bd* presence; the last column indicates the Bayesian 95% credible intervals.

Species	Coordinates	Locality	Sample size	Prevalence (Bayesian 95% credible interval)
<i>L. atifi</i>	36°48'27.01"N, 31°45'54.51"E	Fersin	30	0.00 (0.00, 0.11)
<i>L. antalyana</i>	36°51'5.64"N, 30°35'12.52"E	Hurma Köyü	30	0.00 (0.00, 0.11)
<i>L. billae</i>	36°47'8.77"N, 30°34'7.62"E	Kale Tepe	30	0.00 (0.00, 0.11)
<i>L. irfani</i>	36°42'7.00"N, 30°31'20.00"E	Göynük canyon	14	0.00 (0.00, 0.20)
<i>L. yehudahi</i>	36°32'22.00"N, 30°29'9.00"E	Tahtalı Dağı	9	0.00 (0.00, 0.30)
<i>L. yehudahi</i>	36°36'0.00"N, 30°29'11.00"E	Gedelme	10	0.00 (0.00, 0.31)
<i>L. yehudahi</i>	36°35'28.00"N, 30°30'34.00"E	Kuzdere	2	0.00 (0.00, 0.70)
<i>L. yehudahi</i>		total	21	0.00 (0.00, 0.16)
<i>L. arikani</i>	36°28'33.00"N, 30°25'2.00"E	Dağdibi Mevki	10	0.00 (0.00, 0.31)
<i>L. arikani</i>	36°24'43.00"N, 30°25'8.00"E	Erentepe Dağı	22	0.00 (0.00, 0.15)
<i>L. arikani</i>		total	32	0.00 (0.00, 0.11)
<i>L. l. finikensis</i>	36°17'16.00"N, 30°6'7.00"E	Boldağ	30	0.00 (0.00, 0.11)
<i>L. l. basoglui</i>	36°28'29.09"N, 29°24'13.41"E	Saklikent	30	0.00 (0.00, 0.11)
<i>L. l. basoglui</i>	36°12'36.80"N, 29°50'23.38"E	Cevreli	31	0.00 (0.00, 0.11)
<i>L. l. basoglui</i>		total	61	0.00 (0.00, 0.06)
<i>L. l. luschani</i>	36°22'0.06"N, 29°12'18.20"E	Karadere	30	0.00 (0.00, 0.11)
<i>L. fazilae</i>	36°46'38.47"N, 28°39'30.28"E	Gökbel	30	0.00 (0.00, 0.11)
<i>L. flavimembris</i>	36°55'32.05"N, 28°16'42.84"E	Marmaris	30	0.00 (0.00, 0.11)
Total			338	0.00 (0.00, 0.01)
<i>Pelophylax bedriagae</i>	36°42'7.00"N, 30°31'20.00"E	Göynük canyon	2	0.50 (0.09, 0.88)
<i>Pseudepidalea variabilis</i>	36°12'36.80"N, 29°50'23.38"E	Cevreli	1	0.00

checked for the presence of *Bd*. Nine monotypic and one polytypic species of this genus have been described from southern Turkey and some adjacent Greek islands, three of which have only recently been discovered in single mountain chains (GÖÇMEN et al. 2011, GÖÇMEN & AKMAN 2012). All species are viviparous, giving birth to fully developed juveniles (STEINFARTZ & MUTZ 1998) and restricted to outcrops of karst limestone (VEITH et al. 2001).

We collected 330 *Lyciasalamandra* specimens from 12 Turkish populations (Table 1), covering all known Turkish species. Salamanders were opportunistically searched by turning stones during daytime. We intended to collect 30 individuals per population as recommended by DIGIACOMO & KOEPEL (1986) to reliably infer the absence of the pathogen when none of the specimens was tested positive. At two localities, we also collected specimens of other

amphibian species (two juvenile *Pelophylax bedriagae*, one juvenile *Pseudepidalea variabilis*; see Table 1) to test, if *Bd* could be detected from sympatric amphibian species.

Sampling was carried out by swabbing with ordinary cotton cosmetic swabs the ventral surfaces of body as well as fore and hind feet of salamanders. Swabs were kept at ambient temperature (which was never above 10°C) during fieldwork and frozen at -80°C upon return from the field (HYATT et al. 2007).

We used quantitative real-time PCR of the ITS-1/5.8S ribosomal DNA region of *Bd* following the protocol of BOYLE et al. (2004) for *Bd* screening, running a duplicate for each sample. We used a *Bd* standard from ecogenics (Zurich, Switzerland) of concentrations 0.1, 1.0, 10.0 and 100.0 according to HYATT et al. (2007). For each run, we used a positive control (sample with a known high *Bd* concentration) to rule out that PCR inhibition may have caused negative results. For real-time PCR, samples were diluted 1:10. Reactions yielding one genomic equivalent (GE) or above were considered *Bd*-positive (BÖLL et al. 2012). To obtain a Bayesian 95% credible interval for prevalence, we used the R2WinBUGS package and WinBUGS (<http://cran.r-project.org/web/packages/R2WinBUGS/>) to estimate the posterior distribution of prevalence (KÉRY 2010, BÖLL et al. 2012, LÖTTERS et al. 2012). We used a uniform prior for prevalence (e.g., prevalence $\sim U(0,1)$). Three parallel Markov chains with 20,000 iterations each were run, discarding the first 5,000 iterations as burn-in. Chains were not thinned.

None of the salamanders tested positive for *Bd*. Based on our sample sizes, there is a 95% probability of a *Bd* prevalence < 10% in most populations (Table 1). On genus level, the upper threshold of the respective 95% credible interval is even as low as 1%. This renders it likely that *Bd* does in fact not occur on *Lyciasalamandra* in Turkey. One of the two specimens of *Pelophylax bedriagae* swabbed at Göynük canyon tested positive for *Bd*, with an infection rate of 3.5 GE. Repeated tests confirmed this result.

The detection of *Bd* on one of the two tested *Pelophylax* specimens unambiguously shows that *Bd* occurs in Turkey. This is, to the best of our knowledge, the first record of this pathogen from Anatolia, even in the same habitat of *Lyciasalamandra* (Göynük canyon). Firstly, this assures us that the absence of *Bd* on the salamanders was not an artefact, but instead reflects its true absence from these terrestrial species. We will not enter the discussion of what accounts for the absence of *Bd* here again, either their terrestrial life style or the potential presence of immunizing skin compounds (see above). However, and secondly, the detection of an infected water frog in close proximity to several uninfected salamander specimens at least shows that there is a certain probability for the salamanders to come in contact with the pathogen, although direct interaction between frogs and salamanders seems unlikely.

According to the *Bd* mapping project, the fungus has not yet been detected on Turkish amphibians (<http://www.bd-maps.net/maps/>; latest inquiry by 26 February 2013),

and we are not aware of any other published evidence in this part of the world either. However, according to the *Bd* distribution model by RÖDDER et al. (2009), southern Turkey is climatically suitable to the fungus, and it could therefore have been expected to find it in this area. The first detection of *Bd* in Turkey has far-reaching implications for amphibian conservation in an area that is rich in endemic species. Fortunately, the nine endemic species of *Lyciasalamandra* seem to be at least less susceptible to the pathogen. However, the detection of the fungus in one out of three other examined amphibian specimens indicates that a countrywide screening survey is urgently needed. Turkey harbours a highly diverse fauna that is exposed to a multitude of threats (ŞEKERCIOĞLU et al. 2011). It includes a number of restricted and rare amphibian species such as *Rana tavasensis* (known only from its type locality and two other places; FRANZEN et al. 2008), *Rana holtzi* (which, according to BARAN et al. (2007) and YILDIZ & GÖÇMEN (2012), is only known from three lakes in the Bolkar Dağı in the Taurus Mountains; note, however, that according to VEITH et al. (2003) it is conspecific with *R. macrocnemis*), *Pelophylax caralitanus* (endemic to the Lakes District in south-western Anatolia; BÜLBÜL et al. 2011), and the enigmatic newt genus *Neurergus* (for an overview see KAYA et al. 2012). If such species were susceptible to the fungus and became infected, local and isolated populations might easily become extinct, hence driving entire species close and rapidly to extinction.

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