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2 **Occurrence of *Batrachochytrium dendrobatidis* in Sweden: higher infection prevalence in**  
3 **southern species**

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21 **Running page head: *Bd* in Sweden**

## 1 **Abstract**

2 The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has caused worldwide declines in  
3 amphibian populations. While *Bd* is widespread in southern and central Europe, its occurrence  
4 and distribution in northernmost Europe is mostly unknown. We surveyed for *Bd* in breeding  
5 anurans in Sweden by sampling 1917 amphibians from 101 localities and three regions in  
6 Sweden (Southern, Northern and Central). We found that *Bd* was widespread in southern and  
7 central Sweden, occurring in all nine investigated species and in 45.5 % of the 101 localities  
8 with an overall prevalence of 13.8%. No infected individuals were found in the four northern  
9 sites sampled. The records from central Sweden represent the northernmost records of *Bd* in  
10 Europe. While the proportion of sites positive for *Bd* was similar between the southern and  
11 central regions, prevalence was much higher in the southern region. This was due to southern  
12 species with a distribution mainly restricted to southernmost Sweden having higher prevalence  
13 than widespread generalist species. The nationally red-listed green toad *Bufo viridis* and  
14 the fire bellied toad *Bombina orientalis* had the highest prevalence (61.4% and 48.9%  
15 respectively). Across species, *Bd* prevalence was strongly positively correlated with water  
16 temperature at the start of egg-laying. However, no individuals showing visual signs of  
17 chytridiomycosis were found in the field. These results indicate that *Bd* is widespread and  
18 common in southern and central Sweden with southern species breeding in higher temperatures  
19 and with longer breeding periods having higher prevalence. However, the impact of *Bd* on  
20 amphibian populations in northernmost Europe remains unknown.

21

## 1 **1. Introduction**

2 Emerging infectious disease is an important cause of wildlife population declines and loss of  
3 biological diversity (Daszak et al. 2000, Fisher et al. 2012, Scheele et al. 2019). The chytrid  
4 fungus *Batrachochytrium dendrobatidis* (*Bd*) originating from eastern Asia infects amphibians  
5 and has during the past few decades caused severe population declines in all continents where  
6 amphibians occur (Berger et al. 1998, Skerratt et al. 2007, Lips 2016, O’Hanlon et al. 2018,  
7 Scheele et al. 2019). Currently, 90 species are presumably extinct and 500 species have declined  
8 due to *Bd* infection (Scheele et al. 2019). Australia and the Americas are most severely affected  
9 (Skerratt et al. 2007, Lips 2016, Scheele et al. 2019), with some areas in Central America having  
10 lost 40 % of their amphibian diversity after the emergence of *Bd* (Crawford et al. 2010).

11 *Bd* infects amphibian skin and causes the disease chytridiomycosis, which disrupts normal  
12 epidermal function, ultimately leading to loss of skin homeostatic functions and death (Voyles  
13 et al. 2009). However, the response to *Bd* infection varies significantly between species and  
14 populations, with some species being able to tolerate heavy infection loads without developing  
15 the disease, possibly serving as a reservoir source of infection (Fisher et al. 2009, Gahl et al.  
16 2012, Scheele et al. 2017). *Bd* comprises of several distinct genetic lineages which differ in  
17 their virulence (Farrer et al. 2011, Rosenblum et al. 2013). Of these, the global pandemic lineage  
18 (*Bd*-GPL) is responsible for most of the amphibian population declines worldwide (O’Hanlon  
19 et al. 2018).

20 While *Bd* is widespread in temperate latitudes in North America and Europe, its occurrence at  
21 high latitudes has received little attention. In North America, amphibians infected with *Bd* have  
22 been found up to 62 and 53 °N in northwestern and northeastern Canada, respectively (Schock  
23 et al. 2010, D’Aoust-Messier et al. 2015). In northern Europe, environmental niche models for  
24 *Bd* predict low or intermediate probability of occurrence in most of Scandinavia (Lotters et al.

1 2009, Olson et al. 2013, Li et al. 2013, Xie et al. 2016), however, some models predict high  
2 probability especially at higher altitudes (Xie et al. 2016). Empirical studies on the distribution  
3 of the fungus in northern Europe have been few. Positive observations were made in Denmark  
4 (in 2007, Scalera et al. 2008) and Russia (2011, Reshetnikov et al. 2014) but samples collected  
5 in northernmost Finland (Patrelle et al. 2012) were negative. A study of 197 pool frog  
6 (*Pelophylax lessonae*) samples from central Sweden from late 1990s and early 2000s did not  
7 detect any *Bd* (Garner et al. 2005). Similarly, Sainsbury et al. (2017) did not detect *Bd* in a  
8 sample of 367 amphibians from the same area in central Sweden between 2006 and 2008.  
9 However, in 2010 anurans infected with *Bd* were found at several sites in southernmost Sweden  
10 (Hallengren 2012, see also Kärverno et al. 2018, Kärverno et al. 2019). These observations may  
11 indicate a recent invasion of *Bd* in Sweden and highlight the need for a large-scale survey of  
12 Swedish amphibians.

13 The Swedish anuran fauna can be divided into two groups: cold-adapted widespread generalist  
14 species with a distribution that ranges from southernmost Sweden up to at least the Arctic Circle  
15 in the north, and warm-adapted southern species with their main distribution in Central Europe  
16 and a limited distribution restricted to southern and central Sweden (Sillero et al. 2014). Most  
17 of the rare species also have a population history with periods of low population numbers and,  
18 in some cases, translocations and reintroductions, a history not shared with the widespread  
19 species. Importantly, populations at the edge of their range have been shown to have lower  
20 genetic variation, which may render them more vulnerable to disease (e.g., Savage & Zamudio  
21 2011, Zeisset & Beebee 2014, Dufresnes & Perrin 2015, Cortázar-Chinarro et al. 2017).

22 Here, we investigated the occurrence and prevalence of *Bd* in amphibians from southern, central  
23 and northern Sweden. To investigate if biogeography was associated with *Bd* infection, we  
24 classified the study species as 1) widespread generalist species adapted to low temperatures, or  
25 2) southern species adapted to warmer climates and higher spawning temperatures with

1 restricted distribution in Sweden. Our sampling covered nine of the eleven anuran species  
2 occurring in Sweden, including four species that are currently included in the Swedish red list  
3 (SLU Artdatabanken 2020). Our objectives were to assess i) How common is *Bd* in Sweden? ii)  
4 Do the regions differ in *Bd* occurrence pattern? iii) Do the species differ in *Bd* prevalence, and  
5 more specifically iv) Are there differences between the regionally rare southern species and the  
6 widespread generalist species?

## 7 **2. Methods**

### 8 **2.1 Field sampling**

9 The study was conducted in three regions of Sweden, in the counties of Scania, Blekinge and  
10 Kalmar in the south where *Bd* was found in 2010 (Hallengren 2012), in the counties of  
11 Stockholm and Uppsala in central Sweden, and close to Luleå in Norrbotten county in northern  
12 Sweden (Fig. 1). There were no previous records of *Bd* from central or northern Sweden (but  
13 see Kärverno et al. 2018, 2019, 2020). Here, southernmost Sweden is of special importance  
14 since it harbors all Swedish amphibians (13 species). In Scania, sampling sites were chosen by  
15 using the three earlier known sites of *Bd* occurrence (Hallengren 2012) as starting points and  
16 radiating outwards. In all three regions, ponds with previously known amphibian populations  
17 were sampled and when needed additional ponds were located from satellite maps. In addition,  
18 Scania, Kalmar and Blekinge county boards provided us with samples from sites 30-32 and 75-  
19 80 (Table S1).

20 *Bd* samples were collected between March and June in 2015-2018 (Fig. 1) following the  
21 swabbing method described in Hyatt et al. (2007). Forty-one sites were visited in southern, 56  
22 in central and four in northern Sweden. Samples were collected from nine anuran species of  
23 which three, *Rana arvalis*, *R. temporaria* and *Bufo bufo*, are widespread generalists with  
24 distributions reaching up to (and in the case of *R. temporaria* beyond) the Arctic Circle (Sillero

1 et al. 2014). The remaining six species; *Bufo variabilis*, *Epidalea calamita*, *Bombina*  
2 *bombina*, *Pelobates fuscus*, *Pelophylax esculentus* and *P. lessonae*, are regionally rare species  
3 with distribution restricted to southernmost Sweden, with the exception of *P. lessonae*, which  
4 has an isolated population in central Sweden (Sillero et al. 2014). The number of individuals  
5 caught at each site varied from one to 103, and the number of species sampled from one to five  
6 (Table S1). In total, we sampled 1917 individuals. Anurans were mainly caught at breeding  
7 sites and kept in separate containers until sampling of all individuals had been completed, after  
8 which they were released at the same time to avoid resampling. Individuals were swabbed with  
9 either cotton swabs (2015) or fine-tip rayon swabs (Dryswab™, MWE MW110; 2016-2018).  
10 Cotton swabs were stored in 70% ethanol until DNA extraction. Rayon swabs were kept dry  
11 and stored at 4 °C. To avoid contamination, nitril gloves were used and all equipment was  
12 sterilized with Virkon ® S (1% solution, DuPont) between each individual.

13 To avoid possible effects of differences in sampling effort and swabbing technique, we  
14 excluded sites 30-32, 71-74, 93, 94 and the northern sites (89-92) from all statistical models.  
15 These sites were only used for calculating presence and prevalence of *Bd. P. lessonae*  
16 individuals from sites 71-74 and 93-94 were caught using the methods described in Kärvemo  
17 et al. (2020) in order to investigate temporal changes in infection patterns. Only the results from  
18 the first sampling occasion for each individual were used in the present study. Samples from  
19 sites 30-32 and 75-80 were sent to us by the County Administrative Boards in Skåne, Blekinge  
20 and Kalmar and were thus swabbed by other field workers than the rest of the samples.

## 21 **2.2 DNA extraction and qPCR analysis**

22 DNA was extracted from swabs using the Qiagen DNeasy Blood and Tissue kit (Qiagen, ref.  
23 69506) following the standard protocol with modifications suggested in Kosch and Summers  
24 (2013). Presence of *Bd* was assessed by amplifying the internal transcribed spacer (ITS)-5.8S

1 rRNA region (Boyle et al 2004). 25µl reactions containing 12.5µl 2X Taqman Master Mix  
2 (Applied Biosystem, ref. 4318157), 2.25µl 10µM each of forward and reverse primers, 0.625  
3 µl 10µM MGB probe and 5µl of DNA (diluted x10 in water) were run. Each sample was run in  
4 triplicate. To avoid false negatives because of inhibitors an exogenous internal positive control  
5 (IPC; Hyatt et al. 2007) was added to one well in each triplicate (1µl 10XExo IPC master mix  
6 and 0.5µl 50XExo IPC DNA to each sample; VICTIM dye, Applied Biosystems ref. 4304662).  
7 The qPCR assays were run on a Biorad CFX96 Real Time System machine using amplification  
8 conditions described in Boyle et al. (2004) with standards of 0.1, 1, 10 and 100 genomic  
9 equivalents (GE) (2016-2018 samples) or a positive control of approximately 1-10 GE (2015  
10 samples). An individual was recorded as positive if at least one of the triplicate samples  
11 exhibited a positive signal (i.e. an exponential amplification curve). If the IPC showed signs of  
12 inhibition, negative samples were rerun once before the sample was assigned as not scoreable  
13 (NA) and removed from the data set.

### 14 **2.3 Data analyses**

15 All analyses were conducted in R 3.5.2 (R Core Team 2018). Occurrence, the proportion of  
16 positive sites, was calculated for each region. A site was recorded as *Bd* positive if at least one  
17 individual from the site showed a positive signal for *Bd*. Prevalence, expressed as the proportion  
18 of individuals positive for *Bd*, was calculated for each region, species, site and species-site  
19 combination. The difference in occurrence and prevalence between the two regions was  
20 compared using  $\chi^2$ -tests. Infection intensity was calculated as the mean number of genomic  
21 equivalents (GE) among triplicates. This was only possible for a smaller subset of individuals  
22 (Table 3).

23 We used generalized, binomial mixed-effects linear models (glmer function in the lme4  
24 package, Bates et al. 2014) to test for the effect of region (Southern or Central Sweden) and

1 species group (widespread generalist or restricted southern species) on the presence of *Bd* at  
2 individual level. Region and group were set as fixed effects and site was included as a random  
3 effect. We used the data on *B. bufo*, as this species has a distribution covering both regions and  
4 we had a large enough sample from both southern and central Sweden, to analyse differences  
5 in *Bd* prevalence between years. This was done for the years 2015 and 2016 when a large  
6 enough number of individuals was sampled in both regions. The difference between years was  
7 compared using a  $\chi^2$ -test.

8 We tested for the effect of spawning temperature on prevalence of *Bd* among the species with  
9 linear regression using spawning temperature as the explanatory variable (lm function). We  
10 used temperatures at start of breeding found in the literature (Obert 1975, Banks and Beebee  
11 1987, Höglund and Robertson 1987, Sjögren et al. 1988, Elmberg 1990, Fog et al. 1997, Hettyey  
12 et al. 2009, Kinne et al. 2004, Sicilia et al. 2006).

13

### 14 **3. Results**

15 *Bd* was widespread in two of the three study regions with individuals showing a positive signal  
16 for the chytrid at 46 out of 101 sites (45.5%; Fig 1). Eighteen of 41 (43.9%) and 28 of 56 (50%)  
17 sites were positive for *Bd* in southern and central Sweden, respectively. There was no significant  
18 difference in occurrence between the two regions ( $\chi^2 = 0.15$ ,  $df = 1$ ,  $p = 0.7$ ). No infected  
19 animals were detected in the northern sites. Overall prevalence for all sites and all species was  
20 13.8% with the highest site-specific prevalence at positive sites reaching 100% (Vikhög,  $n = 2$ ,  
21 *Pelobates fuscus*) and 95.5% (Norra Hamnen,  $n = 22$ , *Bufo variabilis*) (Table S1). Prevalence  
22 in the southern region was 19% and in the central region 8.1% (Table 1). There was a significant  
23 difference in prevalence between southern and the central region when all individuals and  
24 species were considered ( $\chi^2 = 44.3$ ,  $df = 1$ ,  $p < 0.001$ ). When including only *B. bufo*, region had



1 no significant effect on infection at individual level ( $\chi^2 = 3.04$ ,  $df = 1$ ,  $p = 0.081$ , Table 2),  
2 although prevalence tended to be higher in central Sweden. There was no significant difference  
3 in prevalence between the years 2015 and 2016 ( $\chi^2 = 1.18$ ,  $df = 1$ ,  $p = 1$ , Table 2).  
4 *Bd* was found in all species with a prevalence varying from 1.9% in *R. temporaria* to 61.4% in  
5 *B. variabilis* (Table 1). The prevalence in the three widespread common species was lower  
6 ( $6.20 \pm 6.19\%$ ) than in the six southern species ( $40.47 \pm 14.75\%$ ; Fig. 2). In general, the rare  
7 southern species, except *P. fuscus*, had prevalence higher than 30%, whereas all common  
8 generalist species had prevalence lower than 15%. Species group had a significant effect on  
9 infection ( $\chi^2 = 74.63$ ,  $df = 1$ ,  $p = 0.001$ ). Spawning temperature was strongly positively  
10 correlated with *Bd* prevalence ( $F_{1,7} = 52.18$ ,  $p < 0.001$ ,  $R^2 = 0.88$ ), all species with prevalence  
11 higher than 30% breeding when water temperature raises over 12 °C (Fig.2). The available data  
12 on infection intensities suggested that infection intensity, estimated as GEs, was very low in the  
13 two common species (*R. arvalis* and *B. bufo*), whereas the southern species, and especially *B.*  
14 *variabilis*, showed significantly higher infection intensity ( $F_{1,60} = 25.94$ ,  $p < 0.001$ ; Table 3).

15

#### 16 **4. Discussion**

17 We found *Bd* at 45.5% of the sites sampled, indicating that the fungus is widespread in southern  
18 Scandinavia, and providing the northernmost observations of this emerging pathogen in Europe  
19 (site 89, Lillträsket, Table S1). This result contrasts with studies in the turn of the millennium  
20 using material from central Sweden, where no *Bd* positive individuals were found in the 197  
21 (Garner et al. 2005) and 367 (Sainsbury et al. 2017) mainly *P. lessonae* samples. In 2017-2018,  
22 prevalence in *P. lessonae* within the same area was 31 % (Table S1). We found no differences  
23 in *Bd* occurrence between the southern and central Swedish sites when looking at the common  
24 species occurring at both regions. Similarly, region had no significant effect on prevalence

1 when data were analyzed on *B. bufo*, for which we had large enough sample sizes at both  
2 regions.. As the earlier studies only cover one region, we cannot with any certainty say when  
3 *Bd* has colonized Sweden, and the fungus can have gone unnoticed for a long time. However,  
4 considering records from other European countries (Bosch et al. 2001, Garner et al. 2005,  
5 Federici et al. 2008, Ohst et al. 2013, O’Hanlon et al. 2018), *Bd* becoming more common in  
6 central Sweden during the early 2000s seems a feasible scenario, broadly agreeing with studies  
7 elsewhere in Europe.

8 We found a strong difference in prevalence between the southern and central regions when  
9 comparing all anuran species. This difference can be explained by the fact that most of the  
10 species with high prevalence were only found in the southern region. There was no difference  
11 in prevalence between years when looking at the data on *B. bufo* only, suggesting that the  
12 difference between the regions was not explained by yearly fluctuations in *Bd* prevalence.  
13 However, we note that *Bd* prevalence has been shown to vary at the same site between years  
14 (e.g., McMillan et al. 2020). For logistic reasons we were not able to sample the same ponds  
15 during consecutive years and more data on inter-annual variation in *Bd* prevalence are needed  
16 to fully explore this topic.

17 While we found infected individuals in all nine investigated species, prevalence differed  
18 strongly among the species, with the regionally rare southern species showing significantly  
19 higher prevalence than the common widespread species. Several ecological and behavioural  
20 characters differ between these two groups. The widespread generalist species aggregate in the  
21 breeding ponds at lower water temperatures (below 10 °C), whereas the rare southern species  
22 require higher temperatures and typically start breeding activities when water temperature  
23 reaches 15 °C or more (Obert 1975, Banks and Beebee 1987, Höglund and G.M. Robertson  
24 1987, Sjögren et al. 1988, Elmberg 1990, Fog et al. 1997, Kinne et al. 2004, Sicilia et al. 2006,  
25 Hettyey et al. 2009). This means that the regionally rare southern species gather for breeding at

1 temperatures closer to the optimum for the fungus (17-25 °C; James et al. 2015), whereas the  
2 widespread common species start breeding at temperatures that are at the lower end of chytrid  
3 temperature tolerance (Piotrowski et al. 2004, Woodhams et al. 2008, Fernández-Beaskoetxea  
4 et al. 2015). In addition, the southern species occur at the northern edge of their range in  
5 southern Sweden and encounter temperatures that are likely at the lower end of their thermal  
6 tolerance (Gutierrez-Pesquera et al. 2016), while summer temperatures in these ponds largely  
7 fall within the optimum of *Bd* (Piotrowski et al. 2004). This thermal mismatch between  
8 pathogen and host tolerances (Nowakowski et al. 2016, Cohen et al. 2017) may make the  
9 southern species more susceptible to *Bd* and explain the high prevalence observed in these  
10 species.

11 The widespread common species group also consists of explosive breeders which spend only a  
12 few days in the breeding ponds, while the rare southern species group consists of prolonged  
13 breeders which often spend several weeks at the breeding sites (Wells 1977). This suggests that  
14 these regionally rare species may run a higher accumulated risk of interactions with infected  
15 individuals, as well as a higher risk of obtaining the infection from the water. Clearly, additional  
16 studies are needed to disentangle the reasons behind the high prevalence in the locally rare  
17 southern species.

18 *Bd*-GPL, the pandemic *Bd* strain which emerged in the mid-20<sup>th</sup> century and caused the  
19 worldwide amphibian decline, was isolated from Swedish *B. variabilis* in 2016 (O’Hanlon et al.  
20 2018). However, while we found high prevalence in many of our study sites, we did not find  
21 signs of chytridiomycosis in any of the individuals and where we had intensity data, such were  
22 low. This can be because of properties of the host immune system being able to keep the  
23 infection at constant low levels or even clear the infection (Kärverno et al. 2020), or the  
24 environment being inhospitable to the fungus. The generally low infection intensities found in  
25 the present study support this notion, however, we note that our data set on infection intensity

1 was very limited. While the infection intensities were generally low, those in *B. variabilis* were  
2 significantly higher, in accordance with the high prevalence found in this species.

3 So far, no cases of chytridiomycosis or unexplained population declines have been reported in  
4 Sweden. The lack of population declines seems to follow the European-wide pattern where  
5 strong *Bd*-linked population declines are rare and biased towards certain taxonomic groups  
6 (Tobler & Schmidt 2010, Balaz et al. 2014, Lips 2016), but we note that some of the species  
7 included in this study show high mortality in experimental infection studies (*B. bufo*: Garner et  
8 al. 2009, Meurling 2019, *B. variabilis*: Bengtsson 2018). Several studies suggest that in cool  
9 climates *Bd* can persist in a host community at relatively low prevalence affecting a subset of  
10 species when environmental conditions are - or become suitable for - the fungus (Spitzen-van  
11 der Sluijs et al. 2017, Mosher et al. 2018). Alternatively, individuals might be able to clear the  
12 infection and therefore not develop the disease (Briggs et al. 2010, Kärvemo et al. 2020). Such  
13 recovery has been reported in several other species (Murray et al. 2009, Kinney et al. 2011,  
14 Sapsford et al. 2015, Daversa et al. 2018). However, long-term data on population trends of  
15 Swedish amphibians are limited and mostly focused on *Bd*-tolerant Ranid species (Woodhams  
16 et al. 2012, Bielby et al. 2015). The available studies show no clear pattern, as increasing, stable  
17 or decreasing population trends have been found (Loman and Andersson 2007, Nyström et al.  
18 2007, Almkvist and Söderman 2016).

19 Importantly, the changing climate is likely to improve the conditions for *Bd* in northern Europe.  
20 For example, niche modelling (Xie et al. 2016) predicts that *Bd* range will shift towards higher  
21 latitudes and altitudes in the northern hemisphere leading to colonization of new regions. Higher  
22 temperatures may also change the relative patterns of host and pathogen optima in relation to  
23 the environment and alter the vulnerability of the host species (Nowakowski et al. 2016, Cohen  
24 et al. 2017). In the face of this emerging pathogen, conservation of Scandinavian amphibian  
25 populations requires special attention. Due to the limited amount of genetic variation at range

1 edges (Hewitt 2000), populations of both the regionally rare species in southern Sweden and  
2 the edge populations of the widespread species in northern Sweden may be especially  
3 vulnerable to disease, as reflected in the limited genetic variation in immune genes in these  
4 populations (Zeisset and Beebee 2014, Cortazar-Chinarro et al. 2017, Meurling 2019; see also  
5 Savage & Zamudio 2011). This calls for an increased awareness for conservationists managing  
6 high-latitude anuran populations, especially in the face of a changing climate. We propose  
7 surveillance and monitoring system for conservation and management of populations of the rare  
8 species so that enhanced awareness could catch any change in the current presence, prevalence  
9 and distribution of *Bd* as well as any declines in population numbers. Furthermore, urgent data  
10 on the occurrence of *Bd* in northern Scandinavia is needed to assess threats on boreal and  
11 subarctic amphibian populations.

12

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7

1

2 **Text Figures**

3 **Fig. 1.**

4 Map of Sweden showing the sites where breeding anurans were sampled for

5 *Batrachochytrium dendrobatidis* (*Bd*) in 2015-2018. The white part of each circle indicates

6 the *Bd*- negative and black the *Bd*-positive proportion of individuals at each site.

7 **Fig. 2.**

8 Relationship between water temperatures at spawning in the studied anuran species and the

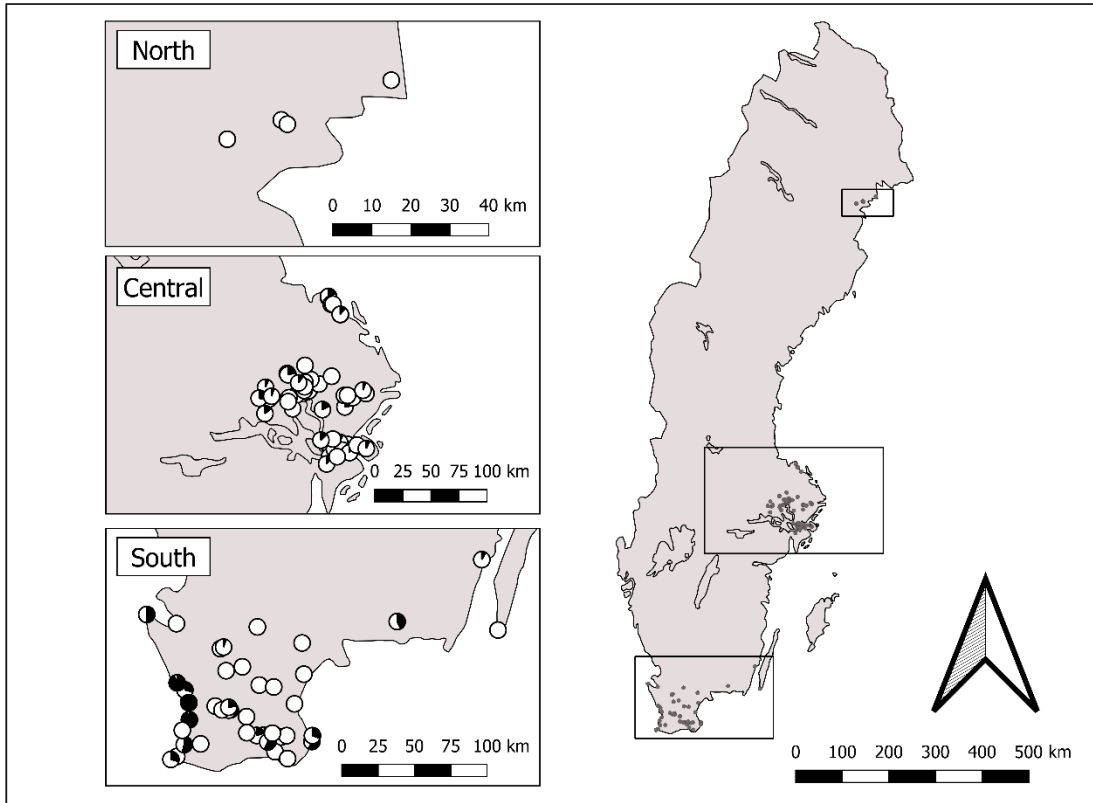
9 species level *Bd* prevalence

10

11



1 Fig. 1



2

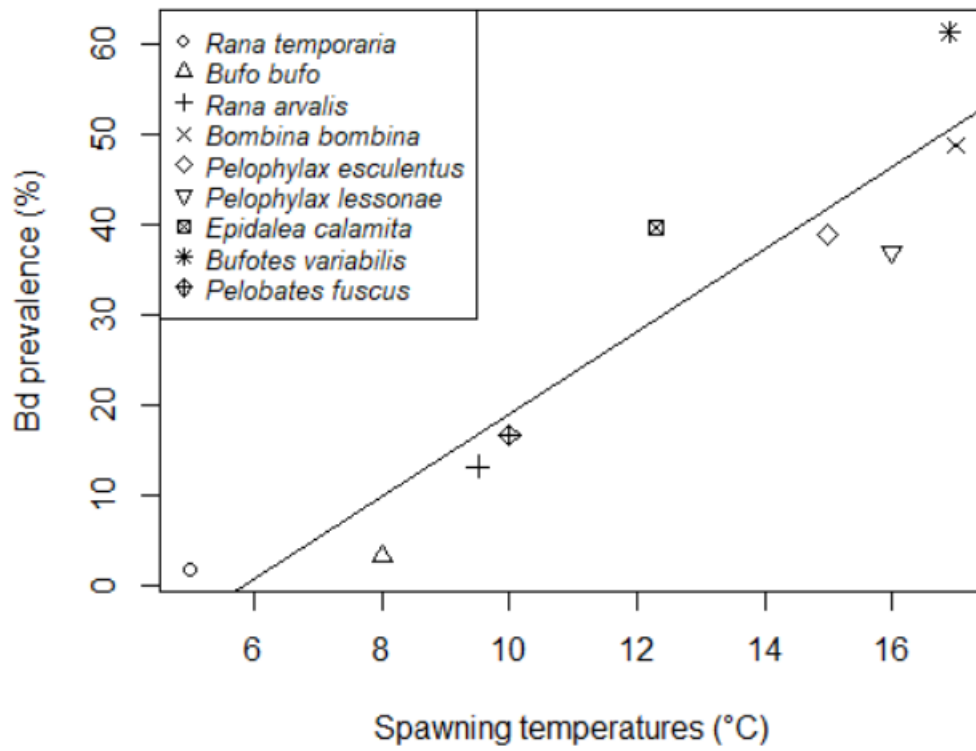
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1 Fig. 2

2



3

1 **Table 1.** *Bd* prevalence for each species, region and the total prevalence for all sampled  
 2 individuals expressed in percent. Numbers in parenthesis describes the number of positive  
 3 individuals out of the total number sampled. If no individuals of a certain species were  
 4 sampled in a region, this is marked with a dash.

5

Species	Regional prevalence (%)			Total prevalence (%)	95% CI (Wilson)
	South	Central	North		
<i>Rana arvalis</i>	13.9 (23/165)	17.1 (21/123)	0 (0/42)	13.3 (44/330)	10.1-17.4
<i>Rana temporaria</i>	1.5 (3/201)	3.4 (2/58)	-	1.9 (5/259)	0.8-4.4
<i>Bufo bufo</i>	1.5 (5/341)	4.5 (27/600)	-	3.4 (32/941)	2.4-4.8
<i>Pelophylax lessonae</i>	63.6 (14/22)	28.4 (19/67)	-	37.1 (33/89)	27.8-47.5
<i>Pelophylax esculentus</i>	38.9 (7/18)	-	-	38.9 (7/18)	20.3-61.4
<i>Bombina bombina</i>	48.9 (22/45)	-	-	48.9 (22/45)	35-63
<i>Bufo variabilis</i>	61.4 (86/140)	-	-	61.4 (86/140)	53.2-69.0
<i>Epidalea calamita</i>	39.8 (33/83)	-	-	39.8 (33/83)	29.9-50.5
<i>Pelobates fuscus</i>	16.7 (2/12)	-	-	16.7 (2/12)	5-45
All species per region	19.0 (195/1027)	8.1 (69/848)	0.0 (0/42)		
95% CI (Wilson)	16.7-21.5	6.5-10.2	0-6.1		
<b>Total prevalence</b>				<b>13.8 (264/1917)</b>	<b>12.3-15.4</b>

6

7

1 **Table 2.** *Bd* prevalence for the *Bufo bufo* subset for the south and central regions and for the  
2 years 2015 and 2016. Prevalence expressed in percent and the numbers in parenthesis  
3 describes the number of positive individuals out of the total number sampled.

4

		<b>Prevalence (%)</b>	<b>95% CI (Wilson)</b>
<b>Region</b>	South	1.5 (5/341)	0.6-3.4
	Central	4.5 (27/600)	3.1-6.5
<b>Year</b>	2015	3.3 (8/244)	1.7-6.3
	2016	3.5 (10/284)	1.9-6.4

5

6

1 **Table 3.** Infection intensity expressed in mean Genomic Equivalents for the subset of all  
2 sampled individuals.

<b>Species</b>	<b>n</b>	<b>Mean GE</b>	<b>95% CI</b>
<i>Rana arvalis</i>	21	0.274	0.0922-0.931
<i>Bufo bufo</i>	2	0.002	0.0004-0.002
<i>Epidalea calamita</i>	12	0.87	0.229-3.01
<i>Bufootes variabilis</i>	21	17.6	6.03-42.8
<i>Pelophylax esculentus</i>	6	2.31	0.562-7.27

3

4