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Batrachochytrium dendrobatidis in Hungary: an overview of recent and historical occurrence

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Abstract. *Batrachochytrium dendrobatidis* (*Bd*) is a fungal pathogen which causes the emerging infectious disease chytridiomycosis. *Bd* presents low host specificity and threatens amphibians worldwide, thus systematic inventory is the key in order to detect and mitigate the effects of the disease. Extensive data collection was conducted in Hungary in 2009-2015 from 14 different areas. Combined data – recent field sampling on 16 taxa and the examination of archived *Bombina* spp. specimens – from 1360 individuals were analysed with qPCR. Two sentinel taxa, *Bombina variegata* and the members of the *Pelophylax esculentus* complex were marked to monitor the occurrence of *Bd* in two core areas (Bakony Mts and Hortobágy National Park, respectively) of sampling. Climatic variables were also examined in core areas to test their effect on prevalence and infection intensity. Among the sixteen sampled amphibian taxa seven tested positive for *Bd* and the overall prevalence in Hungary was 7.46%. Among the ethanol-fixed *Bombina* spp. individuals *Bd* was not detected. In the first core area (Bakony Mts) the overall prevalence in *B. variegata* was 10.32% and juvenile individuals showed significantly higher prevalence than adults. On the other hand there was a significant negative relationship between infection prevalence and monthly mean air temperature. Finally, in the other core area (Hortobágy National Park) the overall prevalence in *P. esculentus* complex was 13.00%, and no differences were found in prevalence or infection intensity between sexes, sampling years or age classes.

Key words. chytridiomycosis, emerging infectious diseases, *Pelophylax esculentus* complex, *Bombina variegata*, inventory, Central-Europe

Running title: Occurrence of *Bd* in Hungary

INTRODUCTION

Over the past decades several epidemics – caused by emerging infectious diseases – resulted in the large-scale decline of numerous animal species globally (Dobson and Foufopoulos, 2001). One such emerging disease is chytridiomycosis in amphibians caused by the fungal pathogen *Batrachochytrium dendrobatidis* [hereafter, *Bd* (Longcore et al., 1999)]. *Bd* is a highly generalist, waterborne pathogen which is primarily transmitted through direct contact with aquatic zoospores or infected individuals (Fisher et al., 2009). *Bd* is responsible for population declines, mass mortalities and even extinction of species, and presents one of the greatest threats to amphibians worldwide (Berger et al., 1998; Skerratt et al., 2007; Fisher et al., 2009).

Bd is widespread on all continents where amphibians occur (Olson et al., 2013), but the heaviest disease outbreaks were observed in the American Neotropics, Australia, North-America and Western Europe (Fisher et al., 2009). In Europe, the first detection of *Bd* related mass mortalities dates back to 1997 when the first recorded population decline as a result of mass die-off after the emergence of chytridiomycosis was observed in Central Spain, in the Guadarrama Mountain National Park, and targeted the Common midwife toad, *Alytes obstetricans* (Bosch et al., 2001). Though, as a result of the increased attention in the subsequent years, studies performed in the same region revealed that other species are highly susceptible to the disease as well (e.g. *Salamandra salamandra*, *Bufo spinosus*; Bosch and Martínez-Solano, 2006; Bosch et al., 2007). Moreover, the evidenced strong population declines of *A. obstetricans, A. muletensis* and *A. dickhilleni* in the Iberian Peninsula (Bosch et al., 2001; Walker et al., 2010; Bosch et al., 2013; Doddington et al., 2013; Rosa et al., 2013), and the high susceptibility of these species made the midwife toads the "flagship" species of European chytridiomycosis threat.

Central Europe harbours several amphibian species that might be susceptible to chytridiomycosis, such as *S. salamandra, B. bufo, Bombina bombina* or *Bombina variegata* (Baláž et al., 2014a,b). In the recent years *Bd* infection was detected in various areas of the Czech Republic, as a result of a systematic inventory (Civiš et al., 2012). Furthermore, the presence of the fungus was recently reported in low prevalence from Luxembourg (Wood et al., 2009), Poland (Sura et al., 2010; Kolenda et al., 2017), Germany (Ohst et al., 2013), Austria (Sztatecsny and Glaser, 2011), Slovakia (Baláž et al., 2014b) and Italy (Federici et al., 2008; Tessa et al., 2013). New data indicates that the fungus is present also in the Balkans, e.g. in Serbia (Mali et al., 2017), Albania, Montenegro and Macedonia (Vojar et al., 2017). Though, interestingly, no negative effects or *Bd*-linked population declines have been detected from Central-Eastern-Europe so far (Vörös et al., 2014).

Some aspects of chytridiomycosis epizootics show environmental correlates (Olson et al., 2013). *Bd* presents a reasonably wide environmental tolerance under a variety of temperature and precipitation regimes (Ron, 2005), but previous studies postulated that climate (Berger et al., 2004; Bosch et al., 2007; Murray et al., 2009; Blaustein et al., 2010; Rohr et al., 2010; Rödder et al., 2010) and elevation (Lips et al., 2008; Walker et al., 2010; Becker and Zamudio, 2011) can significantly influence *Bd* outbreaks. Furthermore, large intra- and interspecific variations exist, especially in the prevalence (Gründler et al., 2012; Böll et al., 2014; Spitzen-Van Der Sluijs et al., 2014), but also in the intensity of infection (Van Sluys and Hero, 2009; Baláž et al., 2014a; Spitzen-Van Der Sluijs et al., 2014). In addition, behavioural differences influence the susceptibility to *Bd* which is further affected by the intraspecific variability related to sex and life stage (Blaustein et al., 2005, Garcia et al., 2006, Williams and Groves, 2014).

Hungary is situated in the Carpathian Basin, a region with high amphibian diversity due to different climatic and zoogeographical influences (Vörös et al., 2014). Previous findings about the occurrence of *Bd* in Hungary are restricted to a few areas and species where the presence was initially detected (Gál et al., 2012; Baláž et al., 2014b, Vörös et al., 2014, Drexler et al., 2017). Therefore, no large-scale distribution data on *Bd* presence is available to date from the country.

Our study displays multiple goals. First, we present a general overview on the occurrence of *Bd* in Hungary summarising data collected between the years 2009-2015. The data set includes the general occurrence of *Bd* on sixteen amphibian taxa with a special focus on the yellow-bellied toad *Bombina variegata* and water frogs belonging to the *Pelophylax esculentus* complex. We selected these two target taxa because these species may present high levels of infection intensity in Europe and so they may also act as sentinel taxa (Baláž et al., 2014b); in addition, they can play a role in the spread and the persistence of the disease (Baláž et al., 2014a).

Second, by studying *B. variegata* populations in Hungary we assessed whether distinct phylogenetic lineages – Alpine (West of the Danube) and Carpathian, occurring in the North Hungarian Range East of the Danube (Vörös et al., 2006) – express differences in prevalence and infection intensity. Moreover, to explore the historical distribution of *Bd* in Hungary field surveys were complemented with available archived samples of *Bombina* spp. from museum collections which comprise a dataset covering a 70 years' time frame (1936-2005) prior to our field sampling.

Third, in order to further monitor *Bd* infection levels of amphibians in Hungary, we selected one population of two of the most susceptible taxa in Central-Eastern Europe, *B. variegata* and the *P. esculentus* complex (Baláž et al., 2014b), and extensively sampled these populations for three consecutive years in two core areas. Finally, we aimed to use climatic data (monthly mean precipitation and monthly mean air temperature) in these core areas to test if there is any correlation between the previously mentioned climatic variables and the occurrence of *Bd*.

MATERIALS AND METHODS

Data collection

Altogether 1233 specimens belonging to sixteen amphibian taxa were studied in the field between 2009-2015. Sampling was conducted in fourteen different regions in 45 distinct sampling points throughout Hungary, covering a great variety of wetland habitats (i.e. irrigation canals, streams, marshlands, ponds, fishponds, water reservoirs and temporary wetland habitats) and elevations ranging between 84 and 734 m a.s.l. (Fig. 1, Table 1). *Bombina variegata* was surveyed in five regions from Transdanubia (Region 1, 2, 3, 5 and 8 in Table 1 and Fig. 1) representing the Alpine (Western) genetic lineage, and in three regions from the North Hungarian Mountains (Region 10, 12 and 13 in Table 1 and Fig. 1) representing the Carpathian (Eastern) genetic lineage, covering the distribution of the species in Hungary (Vörös et al., 2006). Identification of the two *Bombina* species and their hybrids was performed considering morphological characters plus genetic information provided by previous researches in Hungary (Vörös et al., 2006, 2007). Members of the *Pelophylax esculentus* complex were sampled in eight regions (Region 1, 3, 4, 7, 8, 9, 10 and 14 in Table

1 and Fig. 1). Age classes were characterized as tadpoles, juveniles and adults based on the external features of each species examined in the field. In those cases when we couldn't distinguish between age and sex of an individual we discarded the sample for further analysis. Additionally, 127 ethanol-fixed specimens of *Bombina* spp., deposited in the Hungarian Natural History Museum (Budapest, Hungary) and Savaria Museum (Szombathely, Hungary), collected between 1936 and 2005 from regions matching the current distribution of the species were swabbed (Appendix 1).

Systematic sampling of sentinel taxa in two core areas

Core areas were selected based on the prevalence found previously or in the first year of sampling (Gál et al., 2012; Baláž et al., 2014b). In Bakony Mts, *B. variegata* was systematically sampled in 2010-2012. Data of 2010 were published previously (Gál et al., 2012), thus our analyses includes a comparison of data from 2010 and new data from 2011 and 2012. Surveys were completed between March and September in 2010, April and September in 2011, May and July in 2012. The assigned locality, Iharkút (see asterisk on Fig. 1), is an old open bauxite mine, where human activities are common due to being a famous paleontological research site (Ősi et al., 2012). In Iharkút we were able to locate only two water bodies: a small lake and a nearby stream. Because of the close proximity (ca. 50 meters) and the presumed connection of the two habitats, all the toads belonged to the same population.

Members of the *P. esculentus* complex were screened for *Bd* in the Hortobágy National Park (HNP; see asterisk on Fig. 1). HNP is the largest continuous alkaline steppe in Europe covering 80.000 hectares. This natural reserve is abundant in wetland habitats like alkaline marshes, fishponds, wet grasslands and wet meadows (Ecsedi, 2004). *Pelophylax* species were sampled in three sites at HNP – Nádudvar-Kösély canal near the city Nádudvar, a fish pond system located eastwards to Hortobágy and a marshland system at Egyek-Pusztakócs – between April and October during three consecutive years (2012-2014).

Taxonomic identification of Pelophylax esculentus complex

Water frog taxon identification was determined using the technique described by Hauswaldt et al. (2012), and is based on allele-size polymorphism in intron-1 of the serum albumin gene (SAI–1; Plötner et al., 2009), with a slight modification in PCR protocol (Herczeg et al., 2017). To verify SAI–1 fragments we sequenced representative alleles on a Hitachi 3130 Genetic Analyzer (Applied Biosystems, UK). Consensus sequences were compiled using BioEdit version 7.0.9.0 (Hall, 1999) and aligned manually. If genetic samples were not available we referred to the individuals as *Pelophylax* sp.

Sampling protocol

We collected *Bd* samples following Hyatt et al. (2007) by either swabbing the skin of the individuals or clipping one of the toes. According to Hyatt et al. (2007) skin swabbing and toe clipping show similar performances in detectability of *Bd*. Skin swabbing was performed using two types of sterile swabs (SWA90006; Biolab, Budapest, Hungary, 5 mm diameter; and MW100-100; Medical Wire and Equipment, Wiltshire, England, 3 mm diameter). We collected each sample in a standardized way with three strokes on each side of the abdominal midline, the inner thighs, hands and feet. Toe clipping was performed using sterilized scissors and toe clips were stored in 70% EtOH in a freezer at -80 ˚C. Skin swabs were stored dry in individually labelled vials and transferred to a freezer for longer storage throughout the field season. For both sampling procedures we used a new pair of disposable gloves per individual, and after each sampling event we sterilized all the used sampling equipment in order to avoid cross-contamination. Mouthpart (oral disc) of larvae were swabbed following Hyatt et al. (2007). Ethanol-fixed specimens of *Bombina* spp. were screened by skin swabbing following methodology presented above.

Genetic analysis of Bd samples

DNA was extracted using PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, Massachussetts, USA) following the recommendations of Boyle et al. (2004). Because of size differences between swabs (i.e. 3 mm vs. 5 mm; see above), only the top 3 mm of the larger swabs was used in all cases. Extracted DNA was analysed using real-time quantitative polymerase chain reaction (qPCR) following the amplification methodology of Boyle et al. (2004) and Hyatt et al. (2007) targeting the partial ITS-1 – 5.8S rRNA regions. Samples were run in triplicate and an internal positive control was included (TaqMan exogenous internal positive control reagents; 4308323; Thermo Fisher Scientific, Waltham, Massachussetts, USA) to detect potential inhibitors present in the DNA extractions. We considered evidence of infection if genomic equivalents (GE) were ≥ 0.1 and we considered a sample positive if all three wells returned a positive reaction. When a sample returned an equivocal result, it was re-run. If it again returned an equivocal result, it was considered negative (N = 17, 1.3% of total samples). The templates were run on a Rotor-Gene 6000 real-time rotary analyser (Corbett Life Science, Sydney, Australia). GE were estimated from standard curves based on positive controls of 100, 10, 1, 0.1 developed from the *Bd* isolate IA 2011, from Acherito Lake, Spain. Finally, GE values of the three positive replicates were averaged.

In order to identify lineages of *Bd* found on amphibians in Hungary, 2 µl of DNA extract from three individuals (one juvenile *P. ridibundus* plus one juvenile *B. variegata* from Bakony Mts, and one adult *B. variegata* from Őrség) were selected as template for amplification of a partial fragment of ITS1 rRNA. Nested PCR approach described by Gaertner et al. (2009) was performed. The amplified fragments were sequenced on an Applied Biosystems/Hitachi 3130 Genetic Analyser (Thermo Fisher Scientific, Waltham, Massachussets, USA). Sequences were aligned manually using BioEdit version 7.0.9.0. (Hall, 1999) and were blasted against available sequences from GenBank for identification.

Climatic data

Climatic data were provided by the Hungarian Meteorological Service (OMSZ). For the core areas of *B. variegata* and *P. esculentus* complex climatic data were obtained from the closest meteorological station of each sampling site: Pápa city (47.29, 17.37), 135.5 m a.s.l, 21.5 km distance from Iharkút (Bakony Mts), and Kunmadaras village (47.46, 20.89), 88.8 m a.s.l. 12.5 km distance from Egyek-Pusztakócs (HNP), which is the closest sampling point to the station. We used monthly mean precipitation and monthly mean air temperature data for the period 2010-2014 to test if any relationship between climate and prevalence or infection intensity exists.

Statistical analyses

Statistical analyses were performed in R (version 3.4.4; R Core Team, 2018). Prevalence was expressed as a discrete binomial variable (uninfected vs. infected). Infection intensity was expressed through GE value. First, we calculated infection prevalence (%) of different amphibian species together with their 95% Clopper-Pearson confidence intervals (95% CI) as follows. Prevalence values were obtained by dividing the cumulative number of positive samples with the total number of samples per species and multiplied with 100 to obtain percentile values, while 95% CI values were calculated using the R package 'PropCIs' (function 'exactci'; Scherer, 2018). In *Bd* infected species we calculated the mean, median, SD and range of GE values as well. The same statistics were run to compare the two phylogenetic lineages of *B. variegata*, and in the two sentinel taxa (i.e. *B. variegata* and *P. esculentus* complex) we also tested for differences between study years, sexes and age classes. Prevalence values were compared with Chi-square tests, while infection intensities were compared using Mood's median test, as implemented in the R package 'RVAideMemoire' (function 'mood medtest'; Hervé, 2018).

Finally, in the two sentinel taxa we tested the relationship between climatic variables and prevalence and infection intensity. We note here that the data set of the *P. esculentus* complex was restrained only on *P. ridibundus*, as the *Bd* infection of *P. esculentus* was very low (i.e. two infected individuals in total) and the sample size of *P. lessonae* was also not representative (N = 1). The relationship between the climatic factors and infection prevalence was tested using generalized linear mixed models (GLMMs) with binomial error distribution term and the relationship between the climatic factors and infection intensity was analysed using linear mixed models with Gaussian distribution (LMMs). Prevalence and infection intensity, respectively, were entered as dependent variables in the models, while the focal climatic variable (i.e. air temperature or precipitation) was set as continuous predictor. In all models sampling year was entered as a random effect to control for the interannual variations in infection prevalence or intensity. Additionally, in the case of *P. ridibundus*, collection site ID within the HNP was entered also as a random factor to account for the variations in prevalence and intensity between collection sites. To assure the adequate distribution of model residuals, for the LMMs GE values were log(x+1)-transformed. Prior entering into the models, log(x+1)-transformed GE values and the continuous predictor were scaled to mean = 0 and SD = 1 to improve model convergence (see also Schielzeth 2010). Model fits were checked visually by plot diagnosis. In all cases for the statistical comparison of infection intensities only infected species/individuals were used. Mixed models were constructed using the 'lme4' package for R (Bates et al., 2015), and P-values for the linear mixed models were obtained using the function 'Anova' (type III) from the R package 'car' (Fox and Weisberg, 2011). We used a significance level of $P \leq 0.05$ throughout.

RESULTS

Bd occurrence in Hungary

In Hungary, nine regions were infected with *Bd* and the overall prevalence was 7.46% (95% CI: 6.05–9.07), indicating a low presence of the fungus in the country (Table 1). Among the sixteen sampled amphibian taxa seven were found infected with *Bd,* including one unidentified *Pelophylax* individual (Table 2). Details on prevalence and summary statistics of GE values are presented in Table 2; while the geographic distribution of the sampling sites with the site-specific prevalence is shown in Fig. 1.

Bd occurrence in Bombina variegata

In *B. variegata* the overall prevalence was 12.69% (95% CI: 9.91–15.92). Details on prevalence and summary statistics of GE values for the different regions are presented in Table 3. We found no significant difference between the two lineages of *B. variegata* in infection prevalence ($N_{Alpine} = 422$, $N_{Carpathian} = 82$; $\chi^2 = 0.155$, df = 1, P = 0.693) and intensity ($N_{Alpine} = 52$, $N_{Carpathian} = 12$, P = 0.750). *Bd* was not detected among the ethanol-fixed *B. variegata* specimens.

In Bakony Mts between 2010 and 2012 we sampled 310 individuals of *B. variegata*, among which 32 individuals were found to be infected with *Bd*. Here the overall prevalence was 10.32 % (95% CI: 7.16–14.25), and the mean, median, SD and range of GE values were 15.92, 5.09, 38.60 and 0.159–210.3, respectively. There was no significant difference in infection prevalence ($N_{2010} = 80$, $N_{2011} = 144$, $N_{2012} = 86$; $\chi^2 = 4.980$, df = 2, P = 0.082) nor in intensity between the three study years ($N_{2010} = 13$, $N_{2011} = 14$, $N_{2012} = 5$, P = 0.201), and we found no significant difference in prevalence ($N_{males} = 113$, $N_{females} = 90$; $\chi^2 = 0.241$, df = 1, P = 0.623) and infection intensity between sexes ($N_{males} = 8$, $N_{females} = 2$, P = 0.545). However, there was a significant difference in prevalence between the two age classes ($N_{juveniles} = 105$, $N_{adults} = 204$; $\chi^2 = 11.563$, df = 1, P < 0.001), with juveniles being more infected than adults (proportion of individuals infected: 19.04% versus 5.88%). Differences in infection intensity between the two age classes were not significant ($N_{juveniles} = 20$, $N_{adults} = 12$, P = 0.273). There was significant negative relationship between infection prevalence and monthly mean air temperature ($\chi^2 = 4.482$ df = 1, P = 0.034), and a marginally significant positive relationship between prevalence and monthly mean precipitation ($\chi^2 = 3.611$, df = 1, P = 0.057). There was no significant relationship between infection intensity and monthly mean air temperature

($\chi^2 = 0.180$, df = 1, P = 0.671). However, there was a significant positive relationship between infection intensity and monthly mean precipitation ($\chi^2 = 4.227$, df = 1, P = 0.039); though, this significant relationship disappeared after removing one outlier GE value from the data set ($\chi^2 = 1.510$, df = 1, P = 0.219).

All the three sequences (i.e. sequences obtained from juvenile *P. ridibundus* and *B. variegata* from Bakony Mts, and one adult *B. variegata* from Őrség) were identified as ITS1 rRNA of *Bd*, belonging to the globally dispersed *Bd*-GPL lineage (GenBank accession numbers: MH745069-71). One sequence showed 100% identity with *Bd* from Cape Cod (GenBank accession number: FQ176489.1, FQ176492.1), South Africa (JQ582903-4, 15, 37), and Italy (FJ010547). The second sequence was 100% identical with a sequence of *Bd* from Equador (FJ232009.1), and the third sequence represented a unique haplotype. Genetic distance (p-distance) among sequences ranged between 0.005–0.035.

Bd occurrence in Pelophylax ridibundus

In Hortobágy between 2012 and 2014 we sampled 100 individuals of *P. ridibundus*, among which thirteen were found to be infected with *Bd*. Here the overall prevalence was 13.00% (7.10–21.20), and the mean, median, SD and range of GE values were 11.52, 1.59, 19.63 and 0.635–57.905, respectively. We found a significant difference in infection prevalence between years ($N_{2012} = 35$, $N_{2013} = 48$, $N_{2014} = 17$; $\chi^2 = 27.750$, df = 2, P < 0.001); all the infected individuals being captured in 2012 (prevalence: 37.14%), while no infected individuals being found in 2013-2014. We found no significant difference in prevalence ($N_{males} = 42$, $N_{females} = 30$; $\chi^2 = 0.002$, df = 1, P = 0.958) and infection intensity between sexes ($N_{males} = 7$, $N_{females} = 6$, P = 1.000). Age classes did not differ in infection prevalence ($N_{juveniles}$

= 9, N_{adults} = 72; χ^2 = 0.827, df = 1, P = 0.363). Infection intensities of the different age classes cannot be compared because no infected juveniles were captured. We found no significant relationship between infection prevalence and monthly mean air temperature (χ^2 = 2.375, df = 1, P = 0.123), and between prevalence and monthly mean precipitation (χ^2 = 0.010, df = 1, P = 0.920). Since infection prevalence was relatively low in the *P. esculentus* complex and infected individuals were captured in the same month and year, the relationship between climatic variables and infection intensity could not be tested in this taxa.

DISCUSSION

Low *Batrachochytrium dendrobatidis* prevalence was experienced throughout the country (Table 1, Table 2), with similar or slightly lower values than in neighbouring countries e.g. Czech Republic (Baláž et al., 2014a; 19% average at country level), Austria (Sztatecsny and Glaser, 2011; 5.9-45% at country level) or Poland (Kolenda et al., 2017; 18% average at country level). Overall, seven taxa carried the infection: *Bombina bombina*, *Bombina variegata*, *Bufo viridis*, *Pelophylax ridibundus*, *Pelophylax esculentus*, *Pelophylax* sp. and *Ichthyosaura alpestris*. In accordance with previous studies in Central Europe (Ohst et al., 2013; Baláž et al., 2014a,b; Kolenda et al., 2017), *B. variegata* and the members of the *P. esculentus* complex showed the highest prevalence and *Bd* infection intensity in Hungary. On the other hand, there was no difference in prevalence and infection intensity was detected between the two ancient phylogenetic lineages of *B. variegata*. *Bd* was present in eight of the fourteen studied regions. The highest prevalence was experienced in the Alpine foothills at Őrség (Region 1), Soproni Mts (Region 2), and in the Zemplén Mts (Region 13). These three regions represent the margins of the Alps and Carpathians (respectively) hosting populations with continuous distribution towards the higher regions. On the other hand, the remnant mountain regions, where prevalence was much lower (Regions 3, 10 and 11), are geographically isolated from other higher elevations. In contrast, amphibians from five regions (Regions 5, 6, 8, 9 and 12) seemed to not carry *Bd*. This either indicates that *Bd* has not reached these parts of the country yet, or more comprehensive sampling would be needed to locate its presence.

The Carpathian Basin combines the characteristics of the neighbouring regions. Despite the relatively small extent of Hungary, the climatic elements have distinct temporal and spatial characters (Mezősi, 2017). Although the majority of the country has an elevation of less than 300 m a.s.l., Hungary has several moderately high ranges of mountains and the highest peak located in the Mátra Mts at 1014 m a.s.l. (Table 1, Region 10). Overall, our results rather supporting the relationship between the measured climatic variables and prevalence or infection intensity. We found significant relationship regarding *B. variegata* individuals in the Bakony Mts core area, where prevalence was negatively affected by monthly mean temperature. Furthermore, the monthly mean precipitation positively affected the *Bd* infection intensity. Nonetheless, the robustness of the latter result is questionable, since the relationship disappeared when we excluded an outlier value from the analysis. This substantial effect of one outlier value could have on the outcomes of this analysis suggests the need for an extensive sampling in order to test whether this result is a statistical artefact or a real biological phenomenon.

To determine the time and location of the emergence or introduction of *Bd* in different regions worldwide, it is important to study archived specimens deposited to museum collections. To examine the historical presence of the fungus in Hungary we screened archived specimens of *Bombina* spp. collected in the regions 1, 2, 3, 8, 10, 12, 13 and the Kőszeg Mts (archived data only) between 1936 and 2005. In total 127 specimens were analysed and all of the samples were *Bd* negative. Both for field and for museum samples we used the same detection methodology, following Hyatt et al. (2007). The detection probability with qPCR is more sensitive and accurate compared to conventional PCR or histology (Annis et al., 2004; Boyle et al., 2004; Kriger et al., 2006). There is no difference in regard of *Bd* detectability between sample collection techniques (i.e. skin swabbing, brushing or scraping). Nonetheless, preservation methodology and storage history may have influence on the results (Soto-Azat et al., 2009). The Amphibian Collection of the Hungarian Natural History Museum is stored in ethanol, but no record is available about the mode of initial preparation. As formaldehyde is known to inhibit PCR reaction, there is therefore a slight chance that qPCR reactions failed to detect *Bd* in our archived samples; however, this may be an unlikely possibility.

Although with testing archived specimens we did not find evidence on when *Bd* might have been introduced into the country, our genetic analyses showed that the fungus found on amphibians in Hungary is a member of the *Bd*-GPL lineage. This was confirmed by a recent study tracking the origin of *Bd* using a full genome approach, which detected *Bd*-GPL lineage in Hungary (from Iharkút, Bakony Mts; O'Hanlon et al., 2018) and is in line with previous findings reporting that this lineage has a widespread distribution in Europe (Farrer et al., 2007).

During the surveys in the core area of Bakony Mts (Region 3, Table 1) juvenile *B. variegata* individuals showed a significantly higher prevalence compared to adults. The same pattern was observed for two *B. variegata* populations in a seven-year period study in the

Netherlands, which the authors explained by the less developed immune responses, or immunsupression, following the stress of metamorphosis (Spitzen-van der Sluijs et al., 2017). Quite surprisingly, during our study, two juveniles changed infection state once (recovered from *Bd positive*). It is a relatively common phenomenon in the field, when infected adult frogs lose and regain the infection which may be caused by overwintering tadpoles or larvae acting as reservoirs (Briggs et al., 2010, Spitzen-van der Sluijs et al., 2017). In contrast, it is less frequent with juvenile individuals as it was experienced in our study. Similar pattern was observed for *Epidalea calamita* in Spain, where juveniles changed infection state towards the end of metamorphosis, possibly mediated by the increasing water temperature in permanent ponds (Bosch et al., pers. comm.).

In Iharkút (Bakony Mts), during our study period the environmental conditions changed unexpectedly. The lake which hosted most of the amphibian species – including *B. variegata* – dried out after the first season of sample collection. In the second year only four individuals of *B. variegata* were captured around this locality, however the rest of the specimens (N = 181) found shelter in a nearby stream unsuitable for breeding. During the third year the lake kept dry and only seven out of 87 individuals were found in or around the lake. Even though there was no difference in prevalence between the three years, they showed a downward trend towards significance. Already low prevalence (23%) dropped down to 11% in the second and to 5% in the third year. This trend could be associated with the differences in habitat type, as it was observed for *Salamandra salamandra* in the Guadarrama National Park, Spain (Medina et al, 2015). Here, *Bd* infection was greater in salamander larvae from permanent ponds, while it was absent or weak in temporary water bodies and permanent streams. Also, infection intensity in larval cohorts was reduced when water was flowing rather than standing. Same authors suggested that increased water flow rate reduce the likelihood of successful pathogen transmission.

Chytridiomycosis is limited to the keratinized tissues of the host individual, therefore tadpoles and post-metamorphic amphibians are mostly affected by the disease (Rachowicz and Vredenburg, 2004). Our dataset covered all life stages of amphibians and the presence of the infection was not detected in tadpoles of *B. bufo* and *R. dalmatina* (N = 39). On the other hand, post-metamorphic and juvenile individuals were found infected in the regions 1, 3, 10 and 13 of *B. variegata* and the members of the *P. esculentus* complex, even though all sampled individuals apparently didn't display any clinical sign of chytridiomycosis.

In Central Europe the *P. esculentus* complex is formed by two sexual species, the *P. ridibundus* and the *P. lessonae* and their interspecific mating produces the hybridogenetic *P. esculentus*. Overall, our results in the core area of Hortobágy National Park showed higher *Bd* prevalence in *P. ridibundus* compared to the hybrid *P. esculentus* (Table 2) which is related to the fact that the hybrids have more effective peptide defence system against *Bd* and have a richer peptide repertoire than both parental species (Daum et al., 2012). Further, contrary to what was observed in *B. variegata* in the Bakony Mts core area, we did not find differences in *Bd* infection between life stages and sexes in *P. ridibundus* individuals.

Our results fit into the general pattern showing significant variability in the effects of chytridiomycosis across Europe. The marked difference in species susceptibility between amphibian species/communities of Western and Central-Eastern Europe might be determined by multiple linked factors, e.g. virulence of different *Bd* strains (Farrer et al., 2007), genotype (Savage and Zamudio, 2011), behaviour (Williams and Groves, 2014), microbial skin community compound of host species (Bletz et al., 2013), or structure of amphibian communities (Becker et al., 2014). In the Iberian Peninsula – that received the most attention due to mass amphibian mortalities caused by chytridiomycosis – infection was clustered within high-altitude areas, where environmental conditions are the most optimal for growth of *Bd* (Piotrowski et al., 2004). In contrast, Hungary harbours only low-elevation Mountains, where environmental conditions might be less favourable for *Bd*-linked epidemics. Differences in elevation might explain the relatively lower impact and infection values of amphibians in Hungary, than it was reported for surrounding countries in Central and Eastern Europe (e.g. Austria, Sztatecsny and Glaser, 2011; Czech Republic, Baláž et al., 2014a or Poland, Kolenda et al., 2017).

Since *Bd*-related disease outbreak have been proven to be climate-driven (Bosch et al., 2007), amphibians of Central-Eastern Europe might be heavily impacted in the future due to global climate change. Changes in the climate might alter *Bd* diffusion and make it's spreading less predictable, thus areas not yet affected by epidemics require particular attention and constant monitoring.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at < http://www.unipv.it/webshi/appendix > Manuscript number 22611: Appendix 1.

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Table 1. Summary of regions, sampling locations, coordinates and sampled species in our inventory. mtDNA lineages were indicated as Alpine (Alp) or Carpathian (Carp) in the case of *B. variegata*. Lat = Latitude; Long = Longitude N = Number of individuals sampled; Prev = Prevalence; GE = Genomic equivalents; NA = not applicable

Nr. of region	Alt	Lat	Long	Species	mtDNA lineage *B. variegata*	N	Positive/Sampled	Prev (%)	Prev 95% CI (%)	GE mean	GE median	GE SD	GE range
1-Őrség	315.0	46.87	16.13	*Bombina variegata*	Alp	2	16 / 68	23.53	14.09 – 35.38	34.45	5.01	58.32	0.20 – 182.78
	264.0	46.87	16.45	*Bombina variegata*	Alp	7							
	253.0	46.89	16.43	*Hyla arborea*		1							
	253.0	46.89	16.43	*Lissotriton vulgaris*		1							
	253.0	46.89	16.43	*Rana arvalis*		1							
	253.0	46.89	16.43	*Rana dalmatina*		4							
	315.0	46.90	16.24	*Bombina variegata*	Alp	48							
	315.0	46.90	16.24	*Ichthyosaura alpestris*		1							
	267.0	46.91	16.23	*Pelophylax esculentus*		1							
	315.0	46.90	16.24	*Rana temporaria*		2							
2-Soproni	493.0	47.65	16.48	*Bombina*	Alp	14	4 / 14	28.57	8.38 –	2.05	2.40	1.13	0.48 –

Mts				*variegata*					58.10				2.90
3-Bakony Mts	455.0	47.06	17.67	*Bombina bombina*		2	37 / 606	6.11	4.33 – 8.32	21.15	5.19	45.58	0.16 – 210.30
	316.0	47.23	17.74	*Bombina variegata*	Alp	3							
	327.0	47.27	17.69	*Bombina variegata*	Alp	15							
	327.0	47.27	17.69	*Bufo bufo*		2							
	327.0	47.27	17.69	*Ichthyosaura alpestris*		12							
	327.0	47.27	17.69	*Lissotriton vulgaris*		19							
	327.0	47.27	17.69	*Rana dalmatina*		25							
	348.0	47.23	17.64	*Bombina bombina*		2							
	348.0	47.23	17.64	*Bombina variegata*	Alp	310							
	356.0	47.23	17.65	*Bufo bufo*		61							
	356.0	47.23	17.65	*Bufo viridis*		39							
	348.0	47.23	17.64	*Lissotriton vulgaris*		5							
	356.0	47.23	17.65	*Pelophylax ridibundus*		24							
	348.0	47.23	17.64	*Pelophylax* sp.		4							
	348.0	47.23	17.64	*Rana dalmatina*		83							
4-Hanság	113.0	47.66	16.74	*Bombina*		4	3 / 33	9.09	1.92 –	0.56	0.16	0.70	0.15 –

				bombina					24.33				1.37
	116.0	47.63	17.08	*Pelophylax ridibundus*		29							
5-Mecsek Mts	381.0	46.22	18.33	*Bombina variegata*	Alp	12	0 / 23	0.00	0.00 – 14.82	NA	NA	NA	NA
	232.0	46.16	18.24	*Bombina variegata*	Alp	8							
	415.0	46.20	18.33	*Bombina variegata*	Alp	3							
6-Kiskunság	89.0	46.61	19.12	*Triturus dobrogicus*		13	0 / 13	0.00	0.00 – 24.71	NA	NA	NA	NA
7-Budapest	100.0	47.18	18.53	*Bombina bombina*		4	2 / 18	11.11	1.38 – 34.71	36.77	36.77	50.11	1.34 – 72.20
	111.0	47.42	19.14	*Bufo viridis*		4							
	156.0	47.53	19.22	*Pelophylax ridibundus*		10							
8-Pilis-Visegrádi Mts	168.0	47.78	19.04	*Bombina bombina*		1	0 / 78	0.00	0.00 – 4.62	NA	NA	NA	NA
	418.0	47.78	19.00	*Rana dalmatina*		5							
	261.0	47.57	18.94	*Bufo bufo*		1							
	261.0	47.57	18.94	*Salamandra salamandra*		35							
	216.0	47.64	18.78	*Bombina bombina*		2							
	329.0	47.76	18.85	*Rana temporaria*		2							

	183.0	47.76	18.91	*Salamandra salamandra*		7							
	234.0	47.61	18.88	*Hyla arborea*		1							
	234.0	47.61	18.88	*Pelophylax* sp.		3							
	208.0	47.85	19.12	*Rana temporaria*		1							
	209.0	47.85	19.11	*Salamandra salamandra*		1							
	107.0	47.77	19.09	*Hyla arborea*		2							
	107.0	47.77	19.09	*Pelophylax* sp.		4							
	358.0	47.72	19.06	*Bombina bombina* x *variegata*		1							
	358.0	47.72	19.06	*Bombina variegata*	Alp	2							
	301.0	47.78	18.99	*Pelophylax ridibundus*		8							
	301.0	47.78	18.99	*Rana temporaria*		2							
9-Gödöllő Hills	224.0	47.63	19.38	*Lissotriton vulgaris*		20	0 / 56	0.00	0.00 – 6.38	NA	NA	NA	NA
	156.0	47.53	19.22	*Pelophylax ridibundus*		1							
	111.0	47.76	17.34	*Rana arvalis*		1							
	96.0	47.26	19.23	*Rana arvalis*		17							

	96.0	47.26	19.23	*Rana dalmatina*		3							
	96.0	47.26	19.23	*Triturus dobrogicus*		14							
10-Mátra Mts	492.0	47.90	19.98	*Bombina variegata*	Carp	2	7 / 103	6.80	2.78 – 13.50	6.93	2.13	9.19	0.61 – 23.55
	648.0	47.93	19.89	*Bombina variegata*	Carp	2							
	648.0	47.93	19.89	*Salamandra salamandra*		6							
	598.0	47.90	19.97	*Bombina bombina*		2							
	587.0	47.85	19.96	*Bombina variegata*	Carp	3							
	316.0	47.97	19.52	*Salamandra salamandra*		1							
	720.0	47.90	19.93	*Bombina variegata*	Carp	4							
	403.0	47.92	19.97	*Bombina bombina*		2							
	304.0	47.93	19.98	*Bombina bombina* x *variegata*		1							
	636.0	47.87	19.97	*Bombina variegata*	Carp	32							
	727.0	47.88	20.01	*Bufo bufo*		1							
	727.0	47.88	20.01	*Ichthyosaura alpestris*		11							

	411.0	47.93	19.96	*Pelophylax esculentus*		1							
	727.0	47.88	20.01	*Rana temporaria*		1							
	727.0	47.88	20.01	*Salamandra salamandra*		3							
	364.0	47.90	19.74	*Bombina bombina*		3							
	362.0	47.93	19.76	*Bombina variegata*	Carp	1							
	522.0	47.89	20.10	*Bombina bombina*		6							
	274.0	47.91	20.14	*Bombina bombina* x *variegata*		1							
	633.0	47.89	20.11	*Bombina variegata*	Carp	12							
	636.0	47.93	19.93	*Bombina bombina* x *variegata*		5							
	411.0	47.93	19.96	*Bombina variegata*	Carp	2							
	411.0	47.93	19.96	*Pelophylax esculentus*		1							
11-Bükk Mts	249.0	48.12	20.24	*Bufo bufo*		1	1 / 9	11.11	0.28 – 48.25	8.10	8.10	NA	NA
	320.0	48.15	20.10	*Rana temporaria*		1							

	443.0	48.04	20.56	*Ichthyosaura alpestris*		6							
	330.0	48.15	20.08	*Rana temporaria*		1							
12-Aggtelek Karst	286.0	48.54	20.66	*Bombina variegata*	Carp	6	0 / 12	0.00	0.00 – 26.46	NA	NA	NA	NA
	238.0	48.53	20.64	*Salamandra salamandra*		6							
13-Zemplén Mts	468.0	48.27	21.29	*Bombina variegata*	Carp	10	6 / 22	27.27	10.73 – 50.22	244.00	101.15	328.43	13.03 – 882.54
	281.0	48.48	21.33	*Bombina variegata*	Carp	6							
	341.0	48.48	21.32	*Rana temporaria*		1							
	341.0	48.48	21.32	*Salamandra salamandra*		4							
	449.0	48.40	21.45	*Bombina variegata*	Carp	1							
14-Hortobágy	86.0	47.57	20.94	*Pelophylax esculentus*		18	16 / 178	8.99	5.23 – 14.19	10.48	1.48	17.98	0.64 – 57.91
	84.0	47.60	20.88	*Pelophylax lessonae*		1							
	86.0	47.57	20.94	*Pelophylax ridibundus*		2							
	85.0	47.62	21.08	*Pelophylax esculentus*		25							

	86.0	47.61	21.07	*Pelophylax ridibundus*	56
	86.0	47.63	21.08	*Pelophylax* sp.	12
	85.0	47.44	21.14	*Pelophylax esculentus*	20
	85.0	47.44	21.14	*Pelophylax ridibundus*	42
	84.0	47.45	21.17	*Pelophylax* sp.	2
Total					1233

Table 2. *Batrachochytrium dendrobatidis* (*Bd*) infection in amphibian species sampled in Hungary between the years 2009 and 2015. Prev = prevalence; GE = genomic equivalents of zoospores; NA = not applicable

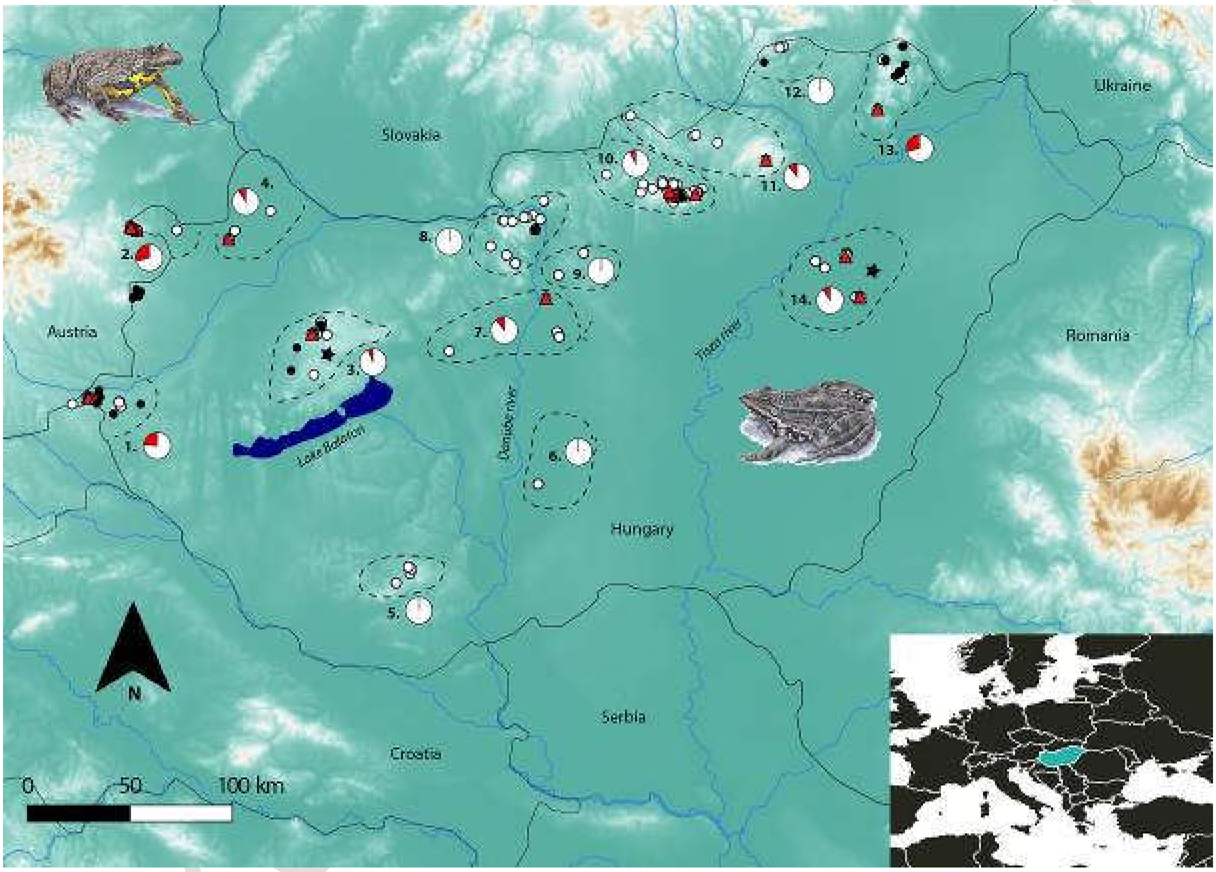
Species	**Positive/Sampled**	**Prev (%)**	**Prev 95% CI (%)**	**GE mean**	**GE median**	**GE SD**	**GE range**
Order Anura							
Family Bombinatoridae							
Bombina bombina	1 / 29	3.45	0.09 – 17.76	16.41	16.41	NA	NA
Bombina variegata	64 / 504	12.70	9.92 – 15.92	40.08	4.96	120.76	0.16 – 882.54
Bombina bombina x *variegata*	0 / 8	NA	0.00 – 36.94	NA	NA	NA	NA
Family Bufonidae							
Bufo bufo	0 / 66	NA	0.00 – 5.44	NA	NA	NA	NA
Bufo viridis	2 / 43	4.65	0.57 – 15.81	36.77	36.77	50.11	1.34 – 72.20
Family Hylidae							
Hyla arborea	0 / 4	NA	0.00 – 60.24	NA	NA	NA	NA
Family Ranidae							
Pelophylax esculentus	2 / 66	3.03	0.37 – 10.52	1.07	1.07	0.41	0.78 – 1.36
Pelophylax lessonae	0 / 1	NA	0.00 – 97.5	NA	NA	NA	NA
Pelophylax ridibundus	21 / 164	12.80	8.10 – 18.91	20.21	1.59	41.72	0.15 – 164.30
Pelophylax sp.	1 / 33	3.03	0.08 – 15.76	15.75	15.75	NA	NA
Rana dalmatina	0 / 120	NA	0.00 – 3.03	NA	NA	NA	NA
Rana arvalis	0 / 19	NA	0.00 – 17.65	NA	NA	NA	NA
Rana temporaria	0 / 11	NA	0.00 – 28.49	NA	NA	NA	NA
Order Caudata							
Family Salamandridae							
Salamandra salamandra	0 / 63	NA	0.00 – 5.69	NA	NA	NA	NA
Triturus dobrogicus	0 / 27	NA	0.00 – 12.77	NA	NA	NA	NA

Lissotriton vulgaris	0 / 45	NA	0.00 – 7.87	NA	NA	NA	NA
Ichthyosaura alpestris	1 / 30	3.33	0.08 – 17.22	8.10	8.10	NA	NA
Total	92 / 1233	7.46	6.05 – 9.07				

Table 3. *Batrachochytrium dendrobatidis* (*Bd*) detection in regions representing the surveyed local populations of *B. variegata* in Hungary. GE = genomic equivalents of zoospores; NA = not applicable

Genetic lineage	**Region**	**Positive/Sampled**	**Prev (%)**	**Prev 95% CI (%)**	**GE mean**	**GE median**	**GE SD**	**GE range**
Alpine	Őrség	16 / 57	28.07	16.97 – 41.54	34.45	5.01	58.32	0.20 – 182.78
	Soproni Mts	4 / 14	28.57	8.39 – 58.10	2.05	2.40	1.13	0.48 – 2.90
	Bakony Mts	32 / 328	9.76	6.77 – 13.49	15.93	5.09	38.61	0.16 – 210.30
	Mecsek Mts	0 / 23	0.00	0.00 – 14.82	0.00	0.00	0.00	NA
	Pilis-Visegrádi Mts	0 / 2	0.00	0.00 – 84.19	0.00	0.00	0.00	NA
Carpathian	Mátra Mts	6 / 58	10.34	3.89 – 21.17	5.36	1.86	8.97	0.61 – 23.55
	Aggtelek Karst	0 / 6	0.00	0.00 – 45.93	0.00	0.00	0.00	NA
	Zemplén Mts	6 / 16	37.50	15.20 – 64.57	244.00	101.15	328.43	13.03 – 882.54
Total		64 / 508	12.59	9.83 – 15.80				

Fig.1. Map of Hungary showing sampling locations of *Bd* negative (black filled circles), *Bd* positive (red/grey triangles) and archived (white circles) samples. Pie charts indicate *Bd* prevalence of the 14 studied geographic regions. Numbers of regions correspond to Table 1. The two core areas are marked with asterisk (Region 3 and 14). Drawing of *Bombina variegata* and *Pelophylax ridibundus* courtesy of Márton Zsoldos.



Appendix 1 List of archived and analysed samples of *Bombina* spp. in this study

Region	**Locality**	**Code**	**Species**	**Date of collection**	**Catalogue number**	**WGSX**	**WGSY**
Aggtelek karts	Aggtelek, Vörös lake	MA1	*B. bombina x B. variegata*	1990.04.19	HNHMHER 2002.680.1	48.47	20.54
Aggtelek karts	Aggtelek, Vörös lake	MA2	*B. bombina x B. variegata*	1990.04.19	HNHMHER 2002.680.2	48.47	20.54
Aggtelek karts	Aggtelek, Vörös lake	MA3	*B. bombina x B. variegata*	1990.04.19	HNHMHER 2002.680.3	48.47	20.54
Aggtelek karts	Aggtelek, Vörös lake	MA4	*B. bombina x B. variegata*	1990.04.19	HNHMHER 90.18.1	48.47	20.54
Bakony Mts	Bakonybél, Vörös János stream	MB1	*B. bombina*	1959.05.20-21.	HNHMHER 2002.419.1	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB2	*B. variegata*	1959.05.20-21.	HNHMHER 2002.609.1	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB3	*B. variegata*	1959.05.20-21.	HNHMHER 2002.609.2	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB4	*B. variegata*	1959.05.20-21.	HNHMHER 2002.609.3	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB5	*B. variegata*	1959.05.20-21.	HNHMHER 2002.609.4	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB6	*B. variegata*	1959.05.20-21.	HNHMHER 2002.609.5	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB7	*B. variegata*	1959.05.20-21.	HNHMHER 2002.610.1	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB8	*B. variegata*	1959.05.20-21.	HNHMHER 2002.610.2	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB13	*B. variegata*	1959.05.20-21.	HNHMHER 60.27.1	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB14	*B. bombina*	1959.05.20-21.	HNHMHER 60.28.1	47.27	17.70
Bakony Mts	Bakonybél, Vörös János stream	MB17	*B. variegata*	1959.05.20-21.	HNHMHER 76.166.1	47.27	17.70
Bakony Mts	Németbánya	MB9	*B. variegata*	1964.06.12-13.	HNHMHER 2002.615.1	47.17	17.55
Bakony Mts	Németbánya	MB10	*B. bombina x B. variegata*	1964.06.12-13.	HNHMHER 2002.678.1	47.17	17.55
Bakony Mts	Németbánya	MB11	*B. bombina x B. variegata*	1964.06.12-13.	HNHMHER 2002.678.2	47.17	17.55
Bakony Mts	Németbánya	MB12	*B. bombina x B. variegata*	1964.06.12-13.	HNHMHER 2002.678.3	47.17	17.55

Bakony Mts	Németbánya	MB15	*B. bombina x B. variegata*	1964.06.12-13.	HNHMHER 64.47.1	47.17	17.55
Bakony Mts	Németbánya	MB16	*B. variegata*	1964.06.12-13.	HNHMHER 64.49.1	47.17	17.55
Kőszegi Mts	Cák, rock mine	SM7	*B. variegata*	1976.07.05	SAMU 87.150.1.4.	47.36	16.52
Kőszegi Mts	Cák, rock mine	SM8	*B. variegata*	1976.07.05	SAMU 87.150.1.4.	47.36	16.52
Kőszegi Mts	Kőszeg	SM6	*B. variegata*	1936.07.15	SAMU 2002.36.1	47.38	16.53
Kőszegi Mts	Kőszeg	SM11	*B. variegata*	1981.07.25	SAMU 87.75.1.2.	47.36	16.49
Kőszegi Mts	Kőszeg	SM12	*B. variegata*	1981.07.25	SAMU 87.75.1.2.	47.36	16.49
Mátra Mts	Padrag	MM11	*B. bombina*	1957.06.20	HNHMHER 2006.49.1	47.07	17.52
Mátra Mts	Padrag	MM12	*B. bombina*	1957.06.20	HNHMHER 2006.49.10	47.07	17.52
Mátra Mts	Padrag	MM13	*B. bombina*	1957.06.20	HNHMHER 2006.49.2	47.07	17.52
Mátra Mts	Padrag	MM14	*B. bombina*	1957.06.20	HNHMHER 2006.49.3	47.07	17.52
Mátra Mts	Padrag	MM15	*B. bombina*	1957.06.20	HNHMHER 2006.49.4	47.07	17.52
Mátra Mts	Padrag	MM16	*B. bombina*	1957.06.20	HNHMHER 2006.49.5	47.07	17.52
Mátra Mts	Padrag	MM17	*B. bombina*	1957.06.20	HNHMHER 2006.49.6	47.07	17.52
Mátra Mts	Padrag	MM18	*B. bombina*	1957.06.20	HNHMHER 2006.49.7	47.07	17.52
Mátra Mts	Padrag	MM19	*B. bombina*	1957.06.20	HNHMHER 2006.49.8	47.07	17.52
Mátra Mts	Padrag	MM20	*B. bombina*	1957.06.20	HNHMHER 2006.49.9	47.07	17.52
Mátra Mts	Padrag	MM21	*B. bombina*	1957.06.20	HNHMHER 57.241.1	47.07	17.52
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM1	*B. variegata*	1969.07.07	HNHMHER 2002.618.1	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM2	*B. variegata*	1969.07.07	HNHMHER 2002.618.2	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM3	*B. variegata*	1969.07.07	HNHMHER 2002.618.3	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM4	*B. bombina x B. variegata*	1969.07.07	HNHMHER 2002.677.1	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM5	*B. bombina x B. variegata*	1969.07.07	HNHMHER 2002.677.2	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM6	*B. bombina x B. variegata*	1969.07.07	HNHMHER 2002.677.3	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM7	*B. bombina x B. variegata*	1967.05.12	HNHMHER 2002.682.1	47.88	20.01

Mátra Mts	Parádfürdő, Pisztrángos-lake	MM8	*B. bombina x B. variegata*	1967.05.12	HNHMHER 2002.682.2	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM9	*B. bombina x B. variegata*	1967.05.12	HNHMHER 2002.682.3	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM10	*B. bombina x B. variegata*	1967.05.12	HNHMHER 2002.682.4	47.88	20.01
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM22	*B. bombina x B. variegata*	1967.05.12	HNHMHER 67.18.1	48.54	21.45
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM23	*B. variegata*	1969.07.07	HNHMHER 69.8.1	48.54	21.45
Mátra Mts	Parádfürdő, Pisztrángos-lake	MM24	*B. bombina x B. variegata*	1969.07.07	HNHMHER 69.9.1	48.43	21.43
Őrség	Cák	SM13	*B. variegata*	1976.04.05	SAMU 87.143.1.4.	47.36	16.51
Őrség	Cák	SM14	*B. variegata*	1976.04.05	SAMU 87.143.1.4.	47.36	16.51
Őrség	Cák	SM15	*B. variegata*	1976.04.05	SAMU 87.143.1.4.	47.36	16.51
Őrség	Cák	SM16	*B. variegata*	1976.04.05	SAMU 87.143.1.4.	47.36	16.51
Őrség	Farkasfa	SM4	*B. variegata*	1983.05.27	SAMU 87.158.1.1.	46.91	16.31
Őrség	Orfalu	SM3	*B. variegata*	1977.07.13	SAMU 87.156.1.2.	46.88	16.29
Őrség	Őriszentpéter, Disznós stream	MO1	*B. variegata*	1970.08.05-08.	HNHMHER 2002.614.1	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO2	*B. variegata*	1970.08.05-08.	HNHMHER 2002.614.2	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO3	*B. variegata*	1970.08.05-08.	HNHMHER 2002.614.3	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO4	*B. variegata*	1970.08.05-08.	HNHMHER 2002.614.4	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO5	*B. variegata*	1970.08.05-08.	HNHMHER 2002.614.5	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO6	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.1	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO7	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.2	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO8	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.3	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO9	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.4	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO10	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.5	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO11	*B. bombina*	1970.08.05-08.	HNHMHER	46.84	16.40

					2002.644.6		
Őrség	Őriszentpéter, Disznós stream	MO12	*B. bombina*	1970.08.05-08.	HNHMHER 2002.644.7	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO17	*B. bombina*	1970.08.05-08.	HNHMHER 70.88.1	46.84	16.40
Őrség	Őriszentpéter, Disznós stream	MO18	*B. variegata*	1970.08.05-08.	HNHMHER 70.89.1	46.84	16.40
Őrség	Sopron	SM5	*B. variegata*	1971.06.10	SAMU 87.147.1.1.	47.39	16.50
Őrség	Szentgotthárd	SM9	*B. variegata*	1977.07.13	SAMU 87.205.1.2.	46.94	16.30
Őrség	Szentgotthárd	SM10	*B. variegata*	1977.07.13	SAMU 87.205.1.2.	46.94	16.30
Őrség	Szőce	MO13	*B. bombina x B. variegata*	1990.08.13	HNHMHER 2002.673.1	46.89	16.57
Őrség	Szőce	MO14	*B. bombina x B. variegata*	1990.08.13	HNHMHER 2002.673.2	46.89	16.57
Őrség	Szőce	MO15	*B. bombina x B. variegata*	1990.08.13	HNHMHER 2002.673.3	46.89	16.57
Őrség	Szőce	MO16	*B. bombina x B. variegata*	1990.08.13	HNHMHER 2002.673.4	46.89	16.57
Őrség	Szőce	MO19	*B. bombina x B. variegata*	1990.08.13	HNHMHER 90.53.1	46.89	16.57
Őrség	Velem	SM17	*B. variegata*	1982.05.13	SAMU 87.124.1.3	47.35	16.48
Őrség	Velem	SM18	*B. variegata*	1982.05.13	SAMU 87.124.1.3	47.35	16.48
Őrség	Velem	SM19	*B. variegata*	1982.05.13	SAMU 87.124.1.3	47.35	16.48
Visegrádi Mts	Leányfalu, Csíkos lake	MP8	*B. bombina*	1952.05.20	HNHMHER 57.16.1	47.72	19.06
Visegrádi Mts	Leányfalu, Rekettyés lake	MP1	*B. bombina*	1960.04.04	HNHMHER 2002.554.1	47.72	19.06
Visegrádi Mts	Leányfalu, Rekettyés lake	MP2	*B. bombina*	1960.04.04	HNHMHER 2002.554.2	47.72	19.06
Visegrádi Mts	Leányfalu, Rekettyés lake	MP9	*B. bombina*	1960.04.04	HNHMHER 60.77.1	47.72	19.06
Visegrádi Mts	Leányfalu, Rekettyés lake	MP10	*B. bombina*	1960.04.04	HNHMHER 60.91.1	47.72	19.06
Visegrádi Mts	Leányfalu, Sztradovavalley	MP4	*B. variegata*	2005.06.09	HNHMHER 2006.136.1	47.72	19.06
Visegrádi Mts	Leányfalu, Sztradovavalley	MP5	*B. variegata*	2005.06.09	HNHMHER 2006.136.2	47.72	19.06
Visegrádi Mts	Leányfalu, Sztradovavalley	MP6	*B. variegata*	2005.06.01	HNHMHER 2007.109.1	47.72	19.06
Visegrádi Mts	Leányfalu, Sztradovavalley	MP7	*B. variegata*	2005.06.01	HNHMHER 2007.109.2	47.72	19.06

Zemplén Mts	Füzér	MZ36	*B. variegata*	1977.04.03-08.	HNHMHER 76.138.1	48.54	21.46
Zemplén Mts	Füzér	MZ5	*B. variegata*	1969.05.10	HNHMHER 2002.612.1	48.54	21.46
Zemplén Mts	Füzér, belowthecastle	MZ1	*B. variegata*	1959.06.12	HNHMHER 2002.611.1	48.54	21.46
Zemplén Mts	Füzér, belowthecastle	MZ2	*B. variegata*	1959.06.12	HNHMHER 2002.611.2	48.54	21.46
Zemplén Mts	Füzér, belowthecastle	MZ3	*B. variegata*	1959.06.12	HNHMHER 2002.611.3	48.54	21.46
Zemplén Mts	Füzér, belowthecastle	MZ4	*B. variegata*	1959.06.12	HNHMHER 2002.611.5	48.54	21.46
Zemplén Mts	Füzér, belowthecastle	MZ32	*B. variegata*	1960.05.16-21.	HNHMHER 59.228.1	48.54	21.46
Zemplén Mts	Füzér, Great Milic	MZ12	*B. variegata*	1960.07.12-14.	HNHMHER 2002.631.10	48.43	21.44
Zemplén Mts	Füzér, Great Milic	MZ13	*B. variegata*	1960.07.12-14.	HNHMHER 2002.631.12	48.43	21.43
Zemplén Mts	Füzér, Great Milic	MZ14	*B. variegata*	1960.07.12-14.	HNHMHER 2002.631.4	48.43	21.43
Zemplén Mts	Füzér, Great Milic	MZ15	*B. variegata*	1960.07.12-14.	HNHMHER 2002.631.7	48.41	21.40
Zemplén Mts	Füzér, Great Milic	MZ34	*B. variegata*	1960.07.13-15.	HNHMHER 60.172.1	48.88	21.46
Zemplén Mts	Istvánkút, Pálháza	MZ7	*B. variegata*	1960.05.16-21.	HNHMHER 2002.626.8	48.46	21.47
Zemplén Mts	Istvánkút, Pálháza	MZ8	*B. variegata*	1957.05.30	HNHMHER 2002.627.1	48.46	21.47
Zemplén Mts	Kőkapu	MZ9	*B. variegata*	1957.05.30	HNHMHER 2002.629.4	48.43	21.44
Zemplén Mts	Kőkapu	MZ10	*B. variegata*	1957.05.30	HNHMHER 2002.629.9	48.43	21.44
Zemplén Mts	Kőkapu	MZ11	*B. variegata*	1957.05.31	HNHMHER 2002.630.1	48.43	21.44
Zemplén Mts	Kőkapu	MZ29	*B. variegata*	1958.06.12	HNHMHER 57.186.1	48.43	21.44
Zemplén Mts	Pálháza, Istvánkút, Istvánkútispring	MZ28	*B. variegata*	1957.05.31	HNHMHER 57.182.1	48.46	21.47
Zemplén Mts	Pálháza, Istvánkút, Istvánkútispring	MZ33	*B. variegata*	1960.07.12-14.	HNHMHER 60.112.1	48.46	21.47
Zemplén Mts	Rostalló	MZ24	*B. variegata*	1977.04.03-08.	HNHMHER 2002.638.1	48.42	21.43
Zemplén Mts	Rostalló	MZ25	*B. variegata*	1977.04.03-08.	HNHMHER	48.42	21.43

					2002.638.5		
Zemplén Mts	Rostalló	MZ26	*B. variegata*	1977.04.03-08.	HNHMHER 2002.638.7	48.42	21.43
Zemplén Mts	Rostalló	MZ27	*B. variegata*	1957.05.30	HNHMHER 2002.638.8	48.42	21.43
Zemplén Mts	Rostalló, Pálháza	MZ37	*B. variegata*	1977.04.03-08.	HNHMHER 77.43.1	48.42	21.43
Zemplén Mts	Suslya Hill	MZ16	*B. variegata*	1960.07.13-15.	HNHMHER 2002.634.1	48.41	21.40
Zemplén Mts	Suslya Hill	MZ17	*B. variegata*	1960.07.13-15.	HNHMHER 2002.634.3	48.43	21.43
Zemplén Mts	Suslya Hill	MZ18	*B. variegata*	1960.07.13-15.	HNHMHER 2002.634.5	48.43	21.43
Zemplén Mts	Suslya Hill	MZ19	*B. variegata*	1959.06.12	HNHMHER 2002.634.6	48.43	21.43
Zemplén Mts	Suslya Hill	MZ35	*B. variegata*	1969.05.10	HNHMHER 60.181.1	48.43	21.43
Zemplén Mts	Telkibánya, Ósvavalley	MZ20	*B. variegata*	1959.06.12	HNHMHER 2002.637.1	48.48	21.34
Zemplén Mts	Telkibánya, Ósvavalley	MZ21	*B. variegata*	1959.06.12	HNHMHER 2002.637.2	48.48	21.34
Zemplén Mts	Telkibánya, Ósvavalley	MZ22	*B. variegata*	1959.06.12	HNHMHER 2002.637.4	48.48	21.34
Zemplén Mts	Telkibánya, Ósvavalley	MZ23	*B. variegata*	1977.04.03-08.	HNHMHER 2002.637.5	48.48	21.34
Zemplén Mts	Telkibánya, Ósvavalley	MZ31	*B. variegata*	1959.06.12	HNHMHER 59.227.1	48.48	21.34
Zemplén Mts	Vadásztető	MZ6	*B. variegata*	1958.06.12	HNHMHER 2002.625.5	48.46	21.47
Zemplén Mts	Vadásztető	MZ30	*B. variegata*	1959.06.12	HNHMHER 58.686.1	48.46	21.47