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ORIGINAL ARTICLE

White-nose disease causative agent *Pseudogymnoascus destructans* present in Romania and Moldova

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ABSTRACT

While the presence of *Pseudogymnoascus destructans* (the causative agent of white-nose disease) is widely known in European locations, no detailed study has been carried out in Romania and the Republic of Moldova. In the period of 2014-2018, we sampled 21 locations (caves and mine galleries) in the two countries. These are important hibernacula and maternity sites for several bat species, mostly for *Myotis myotis* and *M. blythii*. Analyses identified seven sites positive for *P. destructans*, six in Romania and one in the Republic of Moldova, with no associated mass mortality, as is generally the case in Europe. Highest prevalence of positive samples comes from the Leșului cave (68% out of 16 samples), which is consistent with the cave's complex structure and movement of individuals during arousal periods in winter. All positive samples from bats come from *M. myotis*, with one exception, a *M. blythii* specimen from the Republic of Moldova. Given the limited number of samples and sites analysed, the detection of *P. destructans* in at least seven sites suggests that the fungus is rather common in several parts of Romania and the Republic of Moldova.

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INTRODUCTION

White-nose disease (WND), the disease associated with white-nose syndrome (WNS) is caused by *Pseudogymnoascus destructans* (Minnis & Lindner 2013, formerly *Geomyces destructans*, Gargas et al. 2009), a psychrophilic fungus first observed on hibernating bats in 2006 in Howes Cave (New York state, USA, Blehert et al. 2009). Since the mid-2000s, the presence of WND and *P. destructans* has resulted in mass mortalities of several North American bat species, for example *Myotis lucifugus* (Frick et al. 2010) and *M. septentrionalis* (Frick et al. 2015). Millions of individuals have died so far, leading to declines over 95% in several species, and population extirpations (Frick et al. 2015). While the presence of *P. destructans* in Europe has been studied extensively (Puechmaille et al. 2011), some regions and countries have limited or no records (ex. Romania, Spain), in part due to lack of extensive sampling.

Pseudogymnoascus destructans infects bats during the critical period of hibernation, growing initially on skin surfaces (pinnae, muzzle, patagium), and often presenting externally as a white fungal coating (hence the name "white-nose" disease/syndrome). The fungus proceeds to invade the epidermal tissues, forming cup-like lesions and ulcerations of the patagium, and also extending deeper into the connective tissues, resulting in inflammations and necrosis (Meteyer et al. 2009). Although the pathology of the disease is similar in Europe and North America, the symptoms differ (Wibbelt et al. 2013). In North America, depletion of fat reserves, altered torpor patterns and aberrant winter behaviour are common outcomes (Reeder & Turner 2008, Veilleux 2008, Turner & Reeder 2009), while these symptoms have not been observed in bats in Eurasia (e.g. Puechmaille et al. 2011, Zukal et al. 2016, Fritze & Puechmaille 2018, Fritze et al. 2021). This results in a contrasting situation with regards to the impact on bat populations with mass mortality observed in North America, but not in Europe (Puechmaille et al. 2011, Fritze & Puechmaille 2018). This pattern can be explained by the fact that *P. destructans* has been present in Europe for millennia (Puechmaille et al. 2011, Leopardi et al. 2015, Drees et al. 2017), while it has only recently been introduced to North America (Leopardi et al. 2015).

European overview

The earliest record of *P. destructans* in Europe is from a museum specimen of *M. bechsteinii*, collected in 1918 (Forêt de Russy, France), and from which Campana et al. (2017) amplified a fragment of its characteristic IGS region. In addition, several bats from Germany in the early 1980s (Feldmann 1984) and from the Czech Republic and Slovakia in the mid-1990s (Martínkova et al. 2010) were reported to have white fungal growth on their muzzle, similar to that caused by *P. destructans* (Fritze et al. 2021). The first actual European *P. destructans* identification after the 2006-2007 outbreak in the United States came from France (Puechmaille et al. 2010), with Wibbelt et al. (2010) adding Germany, Switzerland and Hungary. Along with the first report of *P. destructans* from Europe, Puechmaille et al. (2010) hypothesised that (1) the fungus was widespread in Europe and that (2) bats in Europe may be tolerant/resistant to the disease rather than susceptible. Both hypotheses were confirmed, with the addition of clear (culture and genetic analysis) or suspected (photographic or visual) *P. destructans* presence from eleven countries (Austria, Belgium, Czech Republic, Denmark, Estonia, the Netherlands, Poland, Romania, Slovakia, Turkey and Ukraine) in Puechmaille et al. (2011) and Martínkova et al. (2010). In 2013 a regular hibernaculum survey in Uviraljka cave (continental Croatia, Papuk Nature Park) identified 18 dead *M. myotis*, later confirmed to be infected with *P. destructans* (Pavlinić et al. 2015) but no clear causal link could be confirmed between the deaths and presence of the fungus. Subsequently, Luxembourg (Mestdagh et al. 2012), Portugal (Paiva-Cardoso et al. 2014), Bulgaria (Zhelyazkova et al. 2020), England (Barlow et al. 2015), Latvia, Russia and Slovenia (Zukal et al. 2016), and Italy (Garzoli et al. 2019, 2021) were confirmed to have *P. destructans*. The fungus has yet to be confirmed from the Scandinavian Peninsula despite the fact that specific surveys were carried out to search for it (e.g. Nilsson 2012).

Current study

To date, the presence of *P. destructans* and associated lesions were identified in at least 62 bat species (listed in the supplementary information from Hoyt et al. 2021), from across 28 countries in Europe and Asia. This indicates that the fungus is a generalist species, and all bat species hibernating underground within the range of *P. destructans* are at risk of infection (Zukal et al. 2014). The list includes several species of the Romanian and Moldovan bat fauna that form hibernation clusters of tens and hundreds of specimens, like *M. myotis, M. blythii, Miniopterus schreibersii, M. capaccinii* or *Rhinolophus ferrumequinum*. The presence of *P. destructans* in Romanian hibernacula was so far covered by Puechmaille et al. (2011), involving only photo documentation or visual reports on *M. myotis* and *M. blythii* in three locations. Besides this work, there is currently no published data on the presence of *P. destructans* in the Republic of Moldova. Here we provide the first detailed evidence of the fungus's presence in the two countries and investigate whether, like in other European countries, its presence is not associated with mass mortality.

MATERIAL AND METHODS

Sampling

In 2014-2018 we sampled a total of 21 sites in the two countries: 19 in Romania, and two in the Republic of Moldova, respectively at 15 caves and six abandoned mines (Table 1, Fig. 1B). Sampling occurred during late hibernation in March-April, except for one site, which was sampled in January. Using sterile polyester dry swabs (COPAN, 164KS01) and without handling the bats (Puechmaille et al. 2010, 2011), 76 individual samples were collected from bats exhibiting fungal growth (Fig. 2) at 19 sites. Species sampled were predominantly *M. myotis* and *M. blythii* ($N_{SAMPLES}$=72), but also *R. ferrumequinum* ($N_{SAMPLES}$=3) and *M. schreibersii* ($N_{SAMPLES}$=1). In addition, environmental samples near hibernating bats were collected during the same time of year by swabbing hibernacula walls ($N_{SAMPLES}$ = 41, N_{SITES} = 5) and collecting sediment material ($N_{SAMPLES}$ = 1, N_{SITES} = 1). For environmental swabbing, we used the same material as described above for swabbing bats (see Fischer et. al. 2022). The sediment sampling consisted of collecting 5 sub-samples at different locations within one hibernaculum. These 5 sub-samples were then pooled in equal proportions to represent the sediment of the hibernaculum. Alongside the sampling, the presence of dead bats was investigated and recorded. After collection, all samples were frozen at -20°C until culture/analysis. Collection of samples in Romania was done under permits nr. 454/2014, nr. 54/2018 and nr. 68/2018, issued by the Speleological Heritage Commission. Collection of samples in the Republic of Moldova was done in the frame of the national bat monitoring programme.

Site description

Eighteen sampling locations are important hibernacula, of which eight are also important maternity sites (Pocora & Pocora 2011, Bücs et al. 2012, Pocora et al. 2012, Bücs et al. 2015, Nistreanu et al. 2016, Bücs et al. 2021, Caldari 2021, Nistreanu et al. 2021, Bücs et al. 2022a, 2022b, Bücs & Cornel 2022, Table 2). Colony size ranges from several thousand individuals in Meziad, Leșului or Rarău caves, several hundred in Bătrânului or Gura Dobrogei caves, to a few isolated bats in short mine galleries (Table 1 & 2). Overall, the predominant species in sampled locations are *M. myotis* and *M. blythii*, followed by *R. ferrumequinum* and *R. hipposideros*. In terms of complexity (Table 1), sites range from multi-level cave systems exceeding lengths of 1 km (for example Meziad, Mare de la Merești and Leșului caves), to short and straight tunnels, with no secondary structures (for example Crăciunești and Agighiol mines). For the present study, we defined levels of complexity from 1 to 5, with the following categories: 1. short (under 100 m) structures, with no secondary galleries; 2. 100-200 m long structures, with short secondary galleries; 3. 200-500 m long structures with various secondary galleries and diverse microclimate; 4. 500-1.000 m long structures, with diverse secondary galleries, halls, etc.; 5. complex, multi-level and/or longer (over 1 km) structures. Complex and/or long underground structures present a wide temperature gradient (from min. 2,5 to max. 12,7°C in studied sites), while short tunnels are usually prone to low winter temperatures as they are more

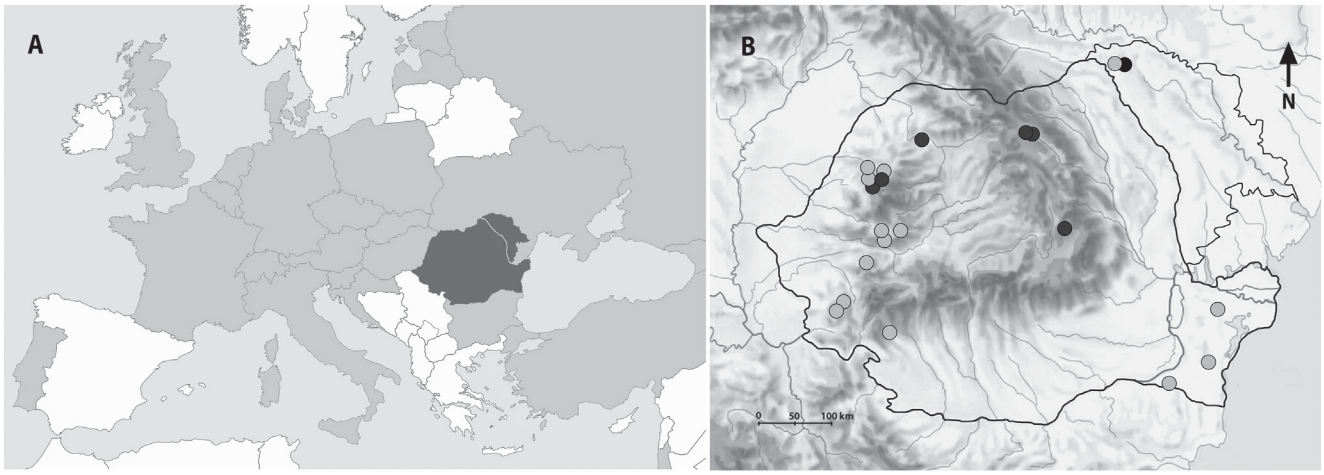

Fig. 1 - A. Current knowledge on *P. destructans* occurrence (light grey) in Europe (references in the main text). Note that the presented distribution does not differentiate for exact locations or regions within countries, but indicates *P. destructans* presence for countries in general. Dark grey: countries investigated in the present study. **B**. Locations of the present study in Romania and the Republic of Moldova, with positive *P. destructans* sites indicated in black (see also Table 2).

Table 1 - Locations of the present study with country, location type, altitude (m), length (km), and level of complexity.

Location	Country	Type	Altitude	Length	Level of complexity
Agighiol	RO	Mine	125	0.20	2
Albioara	RO	Mine	426	2.50	5
Bătrânului	RO	Cave	596	1.63	5
Bisericuța	RO	Cave	1233	0.31	3
Canaraua Fetii	RO	Mine	97	0.40	3
Comarnic	RO	Cave	417	6.20	5
Crăciunești	RO	Mine	317	0.10	1
Cupcini	MD	Mine	183	4.50	5
Fusteica	RO	Cave	225	1.27	5
Gordinești	MD	Mine	173	3.00	5
Gura Dobrogei	RO	Cave	79	0.65	4
Întorsuri	RO	Cave	596	0.22	3
Leșului	RO	Cave	688	1.29	5
Măgurici	RO	Cave	293	0.54	3
Mare de la Merești	RO	Cave	652	1.57	5
Meziad	RO	Cave	466	6.29	5
Ponorul Uscat	RO	Cave	664	0.15	2
Rarău	RO	Cave	1487	0.34	3
Rarău	RO	Mine	1563	0.60	3
Românesti	RO	Cave	335	0.94	4
Sălișteˇ	RO	Mine	435	0.50	3

influenced by outside conditions (for example the main gallery of the Rarău mine, around 0°C).

qPCR

Samples from hibernacula environments usually contained several non-*P. destructans* fungi and/or bacteria, making it challenging to detect and isolate pure cultures of *P. destructans*. As we could not isolate any *P. destructans* from an initial 22 wall swabs plated (see results & culture protocol below), we subsequently used a two-step approach whereby only samples that were positive for *P. destructans* DNA were plated. To prepare swab samples collected from the walls of hibernacula for DNA extraction we first transferred each of the wall swabs to a tube containing 500 µL sterile Milli-Q water, covering the cotton material of the swab. The soaked swab was vortexed for 30 seconds, left to incubate for 30 minutes at 10°C, and then vortexed again for another 30 seconds. To obtain the maximum amount of fungal material out of the cotton swab, the swab was turned around in the tube using sterile tweezers (so that the cotton side of the swab was at the top of the tube) and the tube was then centrifuged for 5 minutes at 7.000 rpm. The swab was removed from the tube and the remaining liquid containing the sampled material was used for DNA extraction. To prepare the sediment sample for DNA extraction, 0.1 g of the sample was thoroughly mixed into 500 µL sterile Milli-Q water and then briefly centrifuged so that the supernatant contained fungal material without any larger pieces of sediment and could be used further. A rapid DNA extraction was performed by using the PrepMan® Ultra Sample Preparation Reagent (Life Technologies), as described in Zhelyazkova et al. (2019). Real-time PCR targeting the intergenic spacer region was performed as described in Zhelyazkova et al. (2019). With each tested sample batch, a negative (i.e., water) and positive (morphologically confirmed *P. destructans*) control were run to confirm that no contaminations or pipetting errors occurred.

Culturing

Samples (excluding qPCR-negative wall samples as well as the sediment sample) were cultured on dextrose-peptone-yeast agar (DPYA) following Vanderwolf et al. (2016). Bat swabs were cultured directly by lightly touching the cotton swab onto several places of the culture medium and spreading the material across the culture. Wall samples were cultured by transferring some of the sample liquid (see preparation for qPCR above) onto the culture plate (initially 20 µL and 100 µL of the liquid were used from each sample, which was adjusted for further cultures of the same sample depending on the growth intensity of *P. destructans* and other microbiota). Upon germination of spores (visible under the microscope within 2-4 days after plating), a subset of spores was physically separated and moved to fresh petri dishes containing culture medium as described in Fischer et al. (2022). All isolates were sealed in petri dishes and stored upside down at 10°C for at least 6 weeks.

Confirmation of *P. destructans* presence at hibernacula

Isolates presenting a morphology consistent with *P. destructans* (54 isolates from bats) were amplified using a panel of 18 microsatellites and two mating-type markers (Dool et al. 2020, Drees et al. 2017). Successful amplification at more than 10 loci identified isolates as being *P. destructans* (Fischer et al. 2022).

RESULTS

From the 21 locations sampled (Fig. 1B, Table 2), seven (six in Romania and one in the Republic of Moldova) contain 22 bat or wall/sediment samples that were positive for *P. destructans* (successfully cultured fungus or PCR amplified fungus DNA). The highest occurrence of positive samples comes from the Leșului cave (68% of 16 samples), the remaining locations having only 1-3 positive samples, despite higher sampling intensity (ex. Rarău Cave, only 1/13 positive samples). Positive samples concerning bats all came from *M. myotis* or *M. blythii*, and samples collected from walls or sediments that are positive for *P. destructans* also come from environmental swabs collected near *M. myotis* and *M. blythii* hibernation clusters. Altitudinal range for *P. destructans* is between 183 - 1563 m, with the lowest point in Cupcini Mine (Republic of Moldova), and the highest in the Rarău Mine (Romania). All *P. destructans* positive sites are over 300 m long, with diverse secondary structures and consequently diverse microclimate (complexity levels 3-5).

Fig. 2 - *Myotis myotis* and *Myotis blythii* clusters (**A**, **C**) and individual bat (**B**) in Leșului Cave (Romania), showing fungal growth during the hibernation census of 2017, and sampling for this study (photos: Bücs Sz.L.).

Table 2 - Locations of the present study, with predominant use (hibernation and/or maternity colonies) and species, number of samples and number of *P. destructans* positive samples. Numbers for predominant species represent the maximum for the 2010-2022 period (references in the main text). Abbreviations: Rfer - *Rhinolophus ferrumequinum*, Rhip - *Rhinolophus hipposideros*, Rmid - *Rhinolophus euryale* and/or *Rhinolophus blasii*, Mmyo/bly - *Myotis myotis* and/or *Myotis blythii*, Mcap - *Myotis capaccinii*, Mdas - *Myotis dasycneme*, Mdau - *Myotis daubentonii*, Msch - *Miniopterus schreibersii*, Bbar - *Barbastella barbastellus*, Paur - *Plecotus auritus*, Ppip - *Pipistrellus pipistrellus*, Nnoc - *Nyctalus noctula*, Enil - *Eptesicus nilssonii*.

Location	Hibernation	Maternity colony	Nr. of samples	Pd positive
Agighiol	Rfer (7)	-	1	-
Albioara	Mmyo/bly (24), Rhip (9), Rfer (8), Bbar (8), Mdau (8)	-	11	3
Bătrânului	Rfer (594), Mmyo/bly (258), Rhip (44)	-	4	-
Bisericuța	Mmyo/bly (107)	-	5	-
Canaraua Fetii	Rfer (40), Reur (40)	Msch (386), Mmyo/bly (180)	1	-
Comarnic	Rfer (1.333), Mmyo/bly (59), Rhip (31)	-	3	-
Crăciunești	Rhip (10)	-	2	-
Cupcini	Mbly (397), Mdau (116)	-	6	1
Fusteica	Mmyo/bly (690), Msch (234), Rfer (232), Rmid (109), Mcap (75)	Mmyo/bly (1.300), Msch (600), Mcap (300)	5	-
Gordinești	Paur (220), Mdau (220), Mbly (31)	Mbly (800)	9	-
Gura Dobrogei	Rfer (40), Mmyo/bly (60)	Mmyo/bly (200), Msch (250)	2	-
Întorsuri	Mmyo/bly (26)	-	3	-
Leșului	Mmyo/bly (3.003), Rfer (817), Reur (40), Rhip (26), Mdas (23)	-	16	11
Măgurici	Msch (100), Mmyo/bly (45), Rfer (36)	Mmyo/bly (750), Msch (500)	5	3
Mare de la Merești	Rhip (363), Mmyo/bly (1906)	Mmyo/bly (1.200), Msch (800)	18	2
Meziad	Ppip (2.266), Msch (1.694), Rfer (422), Rhip (169), Mmyo/bly (140), Nnoc (134)	Msch (4.000), Mmyo/bly (2.000)	4	-
Ponorul Uscat	Mmyo/bly (152)	-	1	-
Rarău (Cave)	Mmyo/bly (3.758)	-	13	1
Rarău (Mine)	Mmyo/bly (53), Bbar (47), Enil (15)	-	6	1
Românesti	Msch (3.710), Rfer (124), Rhip (94), Mmyo/bly (71)	Msch (2.500), Mmyo/bly (937)	1	-
Săliște	Rhip (72), Rfer (14)	-	2	-

DISCUSSION

Despite *P. destructans* presence in several hibernacula from Romania and one from the Republic of Moldova, as in the case of the European bat fauna (Puechmaille et al. 2011), no associated mass-mortality has been observed, and no dead specimens were observed during sample's collection. The pan-European results of Puechmaille et al. (2011) describe *M. myotis* as the most common bat species with *P. destructans* (see also Blomberg et al. 2023), a finding corroborated by our study. The only positive *P. destructans* sample from the Republic of Moldova (Cupcini Mine) was from a *M. blythii* specimen, however the species occurs more frequently in the country than *M. myotis* (Caldari 2022). Among the studied sites found positive for *P. destructans*, several caves (e.g. Leșului and Măgurici caves) are frequently visited by research groups, and/or are attractive tourist destinations (e.g. Mare de la Merești Cave, with thousands of tourists each year). Therefore as illustrated by the likely human-mediated jump of *P. destructans* from the central part of the United States to Washington state, covering a distance of 2100 km (Lorch et al. 2016), the human role in transporting *P. destructans* should not be underestimated (Zhelyazkova et al. 2020).

The highest prevalence of *P. destructans* in our samples was from Leșului Cave (68% of samples, six positive samples from bats, five positive samples from the cave wall), suggesting the fungus is widespread at this site. Leșului cave is a hibernaculum with complex structure and multiple levels, dominated by *M. myotis* and *M. blythii*, forming clusters of 10-200 bats along most of the cave (Bücs et al. 2012) and which could enable the fungus to be transported to all sections of the cave during periodic winter arousals associated with movement within the site (Blažek et al. 2019, Zhelyazkova et al. 2023).

In contrast, while having the highest number of samples of the study ($N_{SAMPLES}$=18), Mare de la Merești Cave only presents two *P. destructans* positive samples (1 wall sample and 1 sediment mix), but the vast majority of hibernating *M. myotis* and *M. blythii* clusters are high in the cave ceiling, impossible to reach. The single positive wall sample out of 15, combined with no positive among two sampled bats (*M. myotis* and *M. blythii*), suggests that not all the cave environment has yet been contaminated. Distance from the entrance, and hence, effects of outside temperature could play a role in *P. destructans* occurrence, especially in case of complex cave/mine systems. For example, all positive *P. destructans* samples in our study come from over 300 m long locations, with diverse secondary structures and consequently diverse microclimate (complexity levels 3-5). In turn, short, simple structures, with limited microclimate pockets, seem to be more prone to external influence such as temperature and sunlight, potentially reducing *P. destructans* survival on the hibernaculum walls. Although our sampling was too limited to investigate if *P. destructans* spatially varies in abundance within a hibernaculum, data collected over larger scales indeed confirm that environmental conditions (e.g. temperature and humidity) are important factors along with species composition (Blomberg et al. 2023). Given that the walls act as environmental reservoir for *P. destructans* and that bats replenish this environmental reservoir (Fischer et al. 2022), it is likely that *P. destructans* spores are 'deposited' over large sections of bat hibernacula, even in sections where bats are not hibernating or where the fungus cannot survive (e.g. walls exposed to direct sunlight near entrances). Altogether, there is no doubt that the fungus is more widespread that we could detect at any of the positive sites herein investigated. Regarding *P. destructans* negative sites, especially those with complex structure/length (ex. Comarnic Cave, Meziad Cave), more sampling would be needed to increase the probability of sampling *P. destructans* if it is present. As often in biology, absence of evidence is not evidence of absence, an aphorism that is particularly true with pathogens in general. Though given the large distribution of the fungus across Europe (Puechmaille et al. 2011) and the increasing number of reported sites with the fungus, it seems probable that most sites in Europe have been exposed to *P. destructans* in the recent past. In this context, it would be enlightening to better understand in which circumstances (biotic and abiotic) the fungus thrives on bats. Blomberg et al. (2023) significantly contributed to understanding disease presence patterns by identifying large-scale relationships with temperature, rainfall, and bat species composition. Their findings suggest that most of Romania and the Republic of Moldova are suitable habitats for the fungus, with the highest suitability in the Romanian mountains and the northern lowlands of both countries. Therefore, sites in these regions should be preferentially targeted when searching for *P. destructans*.

Given that many bat species move between their winter and summer roosts, it is possible that they transport the fungus to other hibernacula in the region (though see Fischer et al. 2021, 2022). As demonstrated by the ringing of bats in North-Western Romania (Bücs et al. 2013), *M. myotis* can move at least 23 km between swarming sites and hibernacula, data that is consistent with the seasonal movements of the species across Europe (47 km on average, Hutterer et al. 2005). While not studied with ringing, the two sites of the present study that are closest to each other, the Rarău Cave and the Rarău Mine (600 m distance), are both positive for *P. destructans*, suggesting the movement of individual bats. While only one of two studied Moldovan sites (the Cupcini Mine) was positive for *P. destructans*, there is only 15 km between Cupcini and the Gordinești Mine, which is within the seasonal movement distance of *M. blythii* (around 15 km, Hutterer et al. 2005).

Almost all positive *P. destructans* samples come from locations in the northern regions of Romania and Moldova (Fig. 1B), with southern regions (especially Dobrogea and Banat in Romania) being underrepresented in the sample pool (12% of samples, with zero positive). This is particularly striking in the Banat region (South-Western Romania), where there are several hibernacula with significant *M. myotis* and *M. blythii* populations, only four being sampled for the present study (10 samples, 8%). Many of these hibernacula are complex structures with diverse microclimates, for example Buhui Cave, with 1.300+ *M. myotis* and *M. blythii* in hibernation, at 4-9°C (Bücs et al. 2022a), favouring *P. destructans* presence (Blomberg et al. 2023).

Several caves present challenges for adequate sampling (especially directly from bats) with hibernation clusters located at varying heights, often above 5-10 m. In contrast, 50% of positive *P. destructans* samples come from a single location, the Leșului cave (North-Western Romania), which has a maximum height of 2-2,5 m for most of its length, meaning that *M. myotis* and *M. blythii* clusters are within arms' reach and easy to observed and sample. In addition, not identifying *P. destructans* in potential locations could also be due to sampling too early in the season (for example Bătrânului cave sampled in January), samples storage methods (freezing and thawing cycles likely significantly decrease *P. destructans* viability), and/or the presence of a high number of other microorganisms in samples. This latter aspect could also explain why we detected *P. destructans* DNA (via qPCR) from wall samples but did not obtain pure cultures. This aspect highlights the relatively low quantities of *P. destructans* spores in the environment in relation to other microbiota (Fischer et al. 2020, 2022). Given that tens of *M. myotis* and *M. blythii* hibernacula are known throughout Romania (Bücs et al. 2022b) and the Republic of Moldova (Caldari 2022), we expect that several additional sites will test positive for *P. destructans* in the future. Most probably, the vast majority of hibernacula in these countries have a historical presence of *P. destructans*, with differences only in the abundance of the fungus.

CONCLUSIONS

The presence of the WND causative agent *P. destructans* in several hibernacula from Romania and one from the Republic of Moldova is herein demonstrated, but without associated mass-mortality. Overall, tens of hibernacula remain unchecked in both countries, especially in the South-Western Romanian Banat region. However, as fast and reliable methods are developed for *P. destructans* identification in the field (e.g. the visual *Pd*-score of Fritze et al. 2021, the LAMP assay of Niessen et al. 2022), the survey and monitoring of WND will become easier and even less invasive for bats. Along with other pathogens such as viruses or bacteria (Mühldorfer et al. 2011, Kemenesi et al. 2018), *P. destructans* is one of many species that can have a significant negative impact on bat populations. Although its impact on European bat populations seems limited at present, the situation could change in the near future. Indeed, changes in virulence or host switches are widely documented in pathogenic fungi (e.g. Brasier 2000, Giraud et al. 2010, Stukenbrock 2016), and this deserves attention. Additionally, climate change will undoubtedly modify the host-pathogen interaction (see Blomberg et al. 2023), but the outcome of this interaction remains to be investigated. It therefore becomes urgent to develop and coordinate national and international programs to monitor key diseases and mortality along with existing bat population monitoring schemes (Van der Meij et al. 2015, Fritze & Puechmaille 2018). While discovering yet undescribed pathogens is becoming easier via technological breakthroughs, we need to move beyond simple pathogen cataloguing and advance our understanding and characterization of the relationship between bats and their pathogens.

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