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Short Communication

# Amphibian chytrid fungus *Batrachochytrium dendrobatidis* in terrestrial field frog *Rana arvalis* from northeastern Poland

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## ABSTRACT

Live specimens of the adult field frog *Rana arvalis* were collected from northeastern Poland during their reproductive season. Histological diagnosis was positive in two of the seven specimens for chytridiomycosis caused by *Batrachochytrium dendrobatidis*. Different stages of pathogen–immature, mature with zoospores, empty and collapsed–were identified. Large numbers of sporangia were found in the toes and forepart of the abdomen of the frog. This is the second report of chytridiomycosis in *R. arvalis* in Europe and the first in Poland.

**KEYWORDS:** chytridiomycosis, *Rana arvalis*, field frog, Poland

## INTRODUCTION

Amphibian chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *B. dendrobatidis*) has been reported in six continents [1]. This pathogen was detected for the first time in some amphibian species from Australia and America [2]. The infection is often associated with mass mortalities of juvenile and adult amphibian specimens. In tadpoles, it causes deformation of the mouth apparatus [3]. However, infections have also been detected in newts, toads and frogs. This chytrid pathogen has infected about 300 species of amphibian already, especially in Australia and in the Americas [1]. Reports on the presence of *B. dendrobatidis* in imported amphibian species in Europe [4] and in wild autochthonous population of *Alytes obstetricans* in Spain [5] were published in 2000 and 2001, respectively. The first record of the fungus in Polish wild populations of green frogs (*Rana* kl. *esculenta* and *Rana lessonae*) was published in 2009 [6]. Later, in northeastern Poland, this fungus was reported in wild populations of frog *Rana temporaria* [7] and in toad *Bufo bufo* [8].

In this context, we decided to investigate the presence of *B. dendrobatidis* in the terrestrial frog *Rana arvalis* Nilsson, 1842, which is a commonly occurring species in Poland.

## MATERIALS AND METHODS

Seven live adult field frogs *R. arvalis* were collected from an agricultural area of the Suwałki Lake District (53°57.6'N - 23°04'E), during their reproductive season. The ecological characterization of this environment was described in our previous paper [7]. The specimens were euthanised and preserved in 10% formalin solution. After 24 h of rinsing in water, the specimens were transferred to 75% ethanol. Strips of skin (approximately 5 x 10 mm) from five sites on the ventral part of the body (three from abdomen, one from thigh and one from toe) were prepared for histological examination. A calibrated eyepiece micrometer was used to make a variety of measurements on 25-mm long sections at the centre of each strip, including estimation of the number of sporangia [9]. Standard histological techniques for light microscopy were applied. The samples were sectioned into 6 μm fragments, then stained with haematoxylin and eosin, and examined at 60x and 100x magnification [10]. For chytrid

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fungus identification, the procedures described by Berger *et al.* [11] and Pessier *et al.* [12] were used.

## RESULTS

Two of the seven field frog species were positive for the presence of *B. dendrobatidis* in the ventral part of the skin (Table 1). In these samples, different stages of the sporangia (immature, mature with zoospores, empty and collapsed) were observed in the stratum corneum and stratum granulosum. In addition, immature zoosporangia containing granular basophilic and eosinophilic material were found. We observed that the mature zoosporangia ranging from 5.8 to 9.8 μm in diameter had thin walls, and the zoospores with diameters ranging from 1.2 to 2 μm had a single flagellum (19–20 μm length). It is worth noting that the empty post-discharge sporangia tubes and sporangia with characteristic septa were observed most frequently in the stratum corneum. Similar zoosporangial morphology of *B. dendrobatidis* was observed by other investigators [9, 10]. In our investigation, the mean number of sporangia in the cells of the field frog ranged from 20.7 (terminal part of abdomen) to 42.5 per mm skin section (toes).

## DISCUSSION

The field frog is a common amphibian species occurring in Poland [13] and also in the Suwałki Lake District [14, 15]. Its life-cycle, except for the mating period, takes place on land. In northeastern Poland, the nuptial period of *R. arvalis* runs its course in the rushes of ponds and lakes around reeds, mace and other aquatic plants. This period depends on the weather conditions and most frequently takes place in April.

This is the first report on the chytrid pathogen found in a field frog in Poland. In this investigation, we obtained the *B. dendrobatidis*-infected native amphibian species from the northeastern part of the country.

Mutschmann [16] observed mass mortality of specimens of field frogs in Brandenburg (Germany) for the first time; only a few years later, the term ‘chytridiomycosis’ was introduced in a paper by Federici *et al.* [17]. In our work, we did not observe any dead frogs. Moreover, the morphological forms of *B. dendrobatidis*, such as sporangia, zoosporangia and zoospores were of the same size as described by others [2, 11, 12]. The only difference was the average number of sporangia of this pathogen observed in the ventral amphibian skin.

In the results of our previously published papers [7, 8], the average number of sporangia in the ventral skin of land living *Rana temporaria* [7] and *Bufo bufo* [8] from the Suwałki Lake District were similar to the average number of sporangia in *B. bufo* specimens’ skin (41.2 $mm^{-1}$). However, in the ventral skin of the green tree frog *Litoria caerula* from Queensland or northern New South Wales (Australia), there were twice as many sporangia per mm of the skin surface [9].

The infection of *B. dendrobatidis* usually appears in the keratinized amphibian epidermis [2, 18, 19]. However, it has also been observed in the skin of metamorphic and adult amphibian individuals and in the tooth rows and jaw sheaths of amphibian larvae. The oral discs of most tadpoles (larvae) contain keratinized jaw sheaths and tooth rows that are normally pigmented [20] and infected by *B. dendrobatidis* while the skin

**Table 1.** *Batrachochytrium dendrobatidis* infection in *Rana arvalis*.

| Stage | Sex | (+) positive (-) negative | Average no. sporangia $mm^{-1}$ ventral skin | | | | |
|---|---|---|---|---|---|---|---|
| | | | Forepart | Middle | Terminal | Thighs | Toes |
| Adult | Male | - | | | | | |
| Adult | Female | - | | | | | |
| Adult | Female | + | 35.6 | 28.2 | 22.4 | 32.8 | 42.5 |
| Juvenile | Unknown | - | | | | | |
| Adult | Female | - | | | | | |
| Adult | Female | + | 32.2 | 30.6 | 20.7 | 29.5 | 32.2 |
| Adult | Male | - | | | | | |

remains unaffected [2, 21]. These tadpoles were not one of the mass mortality specimens [22].

According to Daszak *et al.* [23] the tadpoles act as reservoir hosts for *B. dendrobatidis* and as vectors that transmit the pathogen to other hosts [24]. Rachowicz and Vredenburg [25] demonstrated that tadpoles infected by chytrid fungal zoospores could transmit the infection amongst each other and to postmetamorphic animals. They also revealed that infected tadpoles remained clinically healthy, while infected postmetamorphic forms died. This study indicated that the transmission of the pathogen can occur between tadpoles or between tadpoles and postmetamorphic animals of one species. This raises the possibility that an accumulation of tadpoles might provide extremely large reservoirs for the fungus, as they spread the disease to postmetamorphic animals [25]. Wood *et al.* [26] revealed that this chytrid fungal pathogen can survive, and remain viable, away from a host amphibian for up to seven weeks in lake water [27] and up to three hours out of water [28]. Di Rosa *et al.* [29] observed encysted zoospores with a thick wall on the skin surface of water amphibians, which embodies a resting spore, a saprobe or a parasitic form that conditionally are/were non-pathogenic. Therefore, this fungal pathogen can inhabit areas without causing disease [30, 31].

Relatively little is known about *B. dendrobatidis* ecology, and its mode of transmission is still not fully understood [26]. The long distance transmission occurs principally via infected amphibians in the global amphibian trade for the pet, science and food industries [2, 32, 33]. It is assumed that the incidence of chytridiomycosis started with South African clawly frogs (*Xenopus laevis*) that were exported to all countries of the world and were widely used in pregnancy tests since the 1930's [33, 34]. There are many possible vectors for the chytrid pathogen locally, which may spread from one amphibian specimen to another by close or direct contact during mating, schoding larvae or other aggregative behaviour [35]. This pathogen may be transmitted from one waterbody to another on amphibian limbs, bird feathers and human vectors, such as vehicles, hiking boots [23, 28] and walking shoes [36]. The Suwałki Lake District is on the autumnal and springtime migration route of the waterfowl, which are one of the many possible vectors of *B. dendrobatidis* [32]. During this period, all the amphibian species are active in these areas [6-8, 14, 15, 37, 38].

## CONCLUSION

The investigated specimens of field frog *R. arvalis* were collected from northeastern Poland (53°57.6'N - 23°04'E). Standard histological techniques and light microscopy were used. Two of the seven specimens of the field frog species were positive for the presence of *B. dendrobatidis* in the samples of the ventral parts of the skin. The average number of sporangia of the chytrid fungus ranged from 20.7 (terminal skin) to 42.5/mm skin section (toe clips). This is the second report of chytridiomycosis in *R. arvalis* in Europe and the first in Poland.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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