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Source: Journal of Wildlife Diseases, 38(2) : 463-467

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.2.463

Journal of Wildlife Diseases, 38(2), 2002, pp. 463–467

Basidioboliasis in Anurans in Florida

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ABSTRACT: Members of the genus *Basidiobolus,* a saprophytic fungus, have been associated with the digestive tracts of a wide variety of amphibians and reptiles. To elucidate the relationship of *Basidiobolus* sp. with amphibians in central Florida (USA), we document the occurrence of the fungus in the digestive tracts of *Bufo terrestris, Buffo quercicus, Hyla femoralis, Hyla cinerea, Hyla gratiosa, Hyla squirella, Osteopilus septentrionalis,* and *Rana utricularia.* Species that occupy terrestrial habitats (*B. terrestris, B. quercicus,* and *R. utricularis*) were found to harbor *Basidiobolus* spp. more frequently (83, 78, and 91%, respectively) than those that occupied a more arboreal habitat (*H. cinerea, H. squirella, H. femoralis, H. gratiosa,* and *O. septentrionalis* (50, 56, 55, 56, and 70%, respectively).

Key words: Anurans, *Basidiobolus* spp., saprophytic fungi, survey.

Basidiobolus spp. (Zygomycota, Zygomycetes, Entomophthorales [Eidam, 1886]) are filamentous saprotrophic fungi. This organism has been isolated from the environment of every continent except Antarctica (Nelson et al., 1998). The fungus can be isolated from soil and detritus; however, it is most easily isolated from the intestinal contents of a wide variety of herpetofauna and some mammals (Drechsler, 1956; Hutchison and Nickerson, 1970; Nickerson and Hutchison, 1971; Coremans-Pelseneer, 1973; Tills, 1974; Josue and Quimio, 1976; Gugnani and Okafor, 1980; Miller, 1983; Chaturvedi et al., 1984; Miller and Turnwald, 1984; Okafor et al., 1984; Speare and Thomas, 1985). The relative ease of isolating *Basidiobolus* sp. from the fecal contents of herpetofauna compared to isolating it from soil and detritus has led a number of authors to postulate that the reservoir for this fungus in some environments may be the digestive tracts of animals (Coremans-Pelseneer, 1974; Gugnani and Okafor, 1980; Enweani et al., 1997; Nelson et al., 1998).

The nature of the relationship between the fungus and its animal host is unknown. The presence of *Basidiobolus* sp. in the digestive tracts of amphibians has not been associated with pathology; thus, it does not appear to be a pathogen of the reptilian or amphibian intestinal tract (Levisohn, 1927). *Basidiobolus* sp. elaborates numerous exoenzymes into the surrounding medium including protease, lipase, and chitinase (Coremans-Pelseneer, 1974; Okafor et al., 1987; Okafor and Gugnani, 1990; Okafor, 1994). These enzymes may aid in digestion of insects eaten by the animal hosts. The presence of the fungus in the intestinal tract could also influence the parasite burden of animals harboring the fungus. Therefore, the nature of the association of *Basidiobolus* sp. with its host could be either mutalistic or commensalistic. In order to describe the association and distribution of *Basidiobolus* sp. with anurans in central Florida (USA), we collected 169 animals represented by eight species and four different genera to document the distribution of this fungus with anurans that primarily have an arboreal habitat to those with a primarily terrestrial habitat. We also utilized observations of fecal pellets to see if there was a correlation between presence of the fungus in fecal pellets and state of digestion of the contents.

Animals were collected in the following locations in west central Florida: Pine Flatwoods Wilderness Park, Lutz, Hillsborough County, (28°7′N, 82°20′W); the University of South Florida Botanical Garden, Tampa, Hillsborough County (28°04′N, 82°25′W), and Ona, Hardee County (27°29′N, 81°55′W).

During the breeding season of 1998, animals from the areas studied were cap-

TABLE 1. Anuran digestive tracts colonized by *Basidiobolus* sp. in Florida.

Habitat	Species (*n*)	Positive (%)
Terrestrial	*Bufo quercicus* (9)	7 (78)
	Bufo terrestris (24)	20 (83)
	Rana utricularia (22)	20 (91)
Arboreal	*Hyla cinerea* (32)	16 (50)
	Hyla squrella (32)	18 (56)
	Hyla gratiosa (16)	9 (56)
	Hyla femoralis (11)	6 (55)
	Osteopilus septentronalis (22)	20 (91)

TABLE 2. Composition of fecal pellets of anurans in Florida.

	Major component of fecal pellet (% of pellets)		
Species	Ants	No visible prey	Large insect parts
Bufo quercicus	75	25	0
Bufo terrestris	62	12	27
Hyla cinerea	3	69	28
Hyla squrella	9	44	48
Hyla femoralis	18	55	27
Hyla gratiosa	0	13	87
Osteopilus septentronalis	0	35	65
Rana utricularia	0	9	91

tured by hand during nocturnal searches and deposited into small plastic containers that were surface sterilized by soaking in a 10% bleach solution. The animals were held in these containers until they defecated, after which they were returned to the areas from which they were collected. The fecal pellet was aseptically transferred to a sterile filter in the lid of a petri dish. A plate of Sabouraud dextrose agar (SDA, Difco Laboratories, Detroit, Michigan, USA) was placed over the inverted lid containing a fecal pellet. The conidia of *Basidiobolus* sp. are explosively discharged and collect on the surface of the medium. The plates were inspected daily for signs of fungal development. Fungal colonies growing on the media were aseptically transferred to new media and identified to the generic level according to the key of O'Donnell (1979). The taxonomy of the species that make up this genus is currently uncertain with only two taxa universally recognized. Thus, no attempt was made to identify the isolates to the species level.

Large fecal pellets were teased apart and distributed over the filter paper in order to fit in the space between the lid and the surface of the inverted SDA plate without touching the surface. Small pellets were left intact. Each fecal pellet was examined using a dissecting microscope with 30× magnification. The major constituents of the fecal pellet were noted when nondigested insect parts were present. The pellets also were observed for evidence of other fungal growth.

The test of independence (Chi square test) was performed using the G-statistic with Williams Correction (Sokal and Rohlf, 1981) This statistic was then compared to the Chi square distribution for a single degree of freedom. The test for independence was judged at the probability level of $P \leq 0.05$.

In the areas studied, *Basidiobolus* sp. was a frequent constituent of the gut flora of the common anuran taxa. It was present in 116 of 168 (69%) animals examined (Table 1). The fungus was found in 50–91% of individuals of the species examined. The arborial species (*H. cinerea, H. squirella, H. femoralis, H. gratiosa,* and *O. septentrionalis*) harbored the fungus less frequently than species that have a more terrestrial habitat preference *R. utricularia, B. quercicus,* and *B. terrestris*) (Table 1). The mean frequency of colonization for arborial species was 57% and those with a more terrestrial habitat had a mean frequency of colonization of 86%. This relationship was statistically significant (G-statistic = 14.47, critical value = 10.8, $P >$ 0.001). There was no clear correlation between the completeness of digestion of the prey of the animal and the colonization of the gut with *Basidiobolus* sp. (Table 2).

Our results confirm that, in central Florida, *Basidiobolus* sp. is a common inhabitant of the intestinal tract of anurans. Prey choice appeared to affect the frequency of colonization of the intestinal tract of the animals studied. Animals that had a diet that was rich in ants (*B. terrestris, B. quercicus*) tended to have a higher frequency of colonization than those that did not. Two exceptions were *R. utricularia* and *O. septentrionalis.* These two species had the highest colonization frequency, although ant segments were not observed in any of their fecal pellets. *Bufo terrestris* and *B. quercicus* often were recovered in the same areas of transects as *R. utricularia* indicating that all three species could have overlapping sources of prey. However, this was not the case for *O. septentrionalis. Osteopilus septentrionalis* was exclusively associated with man-made structures and their diet may be considerably different than that of the other tree frogs. This frog was also unusual in that it was often found in tightly packed groups as opposed to the other tree frogs which only occurred as solitary individuals. Thus, it is possible that the colonization frequency with *Basidiobolus* sp. in this species is not truly representative of other tree frogs.

All fecal pellets consisted of well-digested contents and showed no clear correlation to presence or absence of *Basidiobolus* sp. (Table 1 and 2). In some instances larger and/or heavier parts of the insect exoskeletons survived digestion and allowed general identification of the prey. These parts included head and thorax sections of ant prey in *B. terrestris* and *B. quercicus* and leg segments of crickets, grasshoppers, and spiders in larger anuran species.

Capillaconidia of *Basidiobolus* sp. have an adhesive pad and are capable of attachment to exoskeletons of passing insects (Blackwell and Malloch, 1989). Both capilliconida and ballistosporic conidia are produced from fecal pellets containing *Basidiobolus* sp. (Nelson, pers. obs.). Thus, amphibians that occupy more terrestrial habitats may more often eat insects that have had contact with (or fed upon) fecal pellets containing this fungus. This includes ants and other ground foraging insects. These data and previous reports suggest that *Basidiobolus* sp. is frequently associated with amphibians in inter- and intra-generic comparisons. Because *Basidiobolus* sp. has been associated with epidemics in both caged as well as wild populations of amphibians, it can no longer be considered a totally benign constituent of the normal flora of these animals. Recent reports of Taylor et al. (1999a, b, c) present data demonstrating that disturbance of hormonal cycles and stress may predispose amphibians to cutaneous infection with *Basidiobolus* sp. Stress and alterations of natural biological rhythms may be imposed on amphibians by fluctuations in abiotic environmental conditions. If this occurs, animals in otherwise "pristine" environments may become susceptible to infection by *Basidiobolus* sp., leading to an epidemic and decline in population size (Daszak et al., 1999).

The pathogenic potential of *Basidiobolus* sp. in amphibians has not been fully explored. The work of Taylor et al. (1999a) demonstrated that under appropriate conditions this fungus can infect toad skin. However, its association with the toad in a natural setting was not described. For instance, it was not reported whether the Canadian toads used in this study harbored *Basidiobolus* sp. as part of the normal flora of its intestinal tract. The question remains whether all *Basidiobolus* sp. isolates are equally capable of causing disease in these animals. There is a possibility that the design of the experiment from Taylor et al. (1999a) utilized a rare virulent strain of fungus since the source of that inoculum was from previously infected tissue. This would make *Basidiobolus* sp. appear to be a more virulent amphibian pathogen than it is under normal conditions. There has been only one other recorded incidence of *Basidiobolus* sp. causing mass mortality in amphibians (Groff et al., 1991) yet this study, as well as others (Hutchison and Nickerson, 1970; Nickerson and Hutchison, 1971; Coremans-Pelseneer, 1973; Tills, 1974; Porto and Milanez, 1979; Gugnani and Okafor, 1980; Imwidthaya et al., 1982; Okafor et al., 1984; Cochrane et al., 1989; TeStrake et al., 1989), demonstrated that this fungus is often associated with anurans. The data available still suggests that this fungus normally has a limited capacity to cause disease in these animals. Additionally, it is not clear whether a *Basidiobolus* sp. infection caused or contributed to the deaths of the animals in the study by Taylor et al. (1999c) because the chytrid fungus *Batrachochytrium dendrobatidis* also has been linked to animal mortality in the same populations (U.S. Geological Service, 2000).

In the last decade, there have been an increasing number of reports describing amphibian population declines (Barinaga, 1990; Blaustein and Wake, 1990); the definitive causes for these declines remain controversial (Pechmann et al., 1991; McCoy, 1994). Fungal and parasitic infections as well as synergistic effects of biotic and abiotic forces have been implicated (Blaustein and Wake, 1990; Corn, 1998; Carey et al., 1999; Daszak et al., 1999). This underscores the need for additional experimentation to evaluate the pathogenic potential of this fungus in amphibians. Because *Basidiobolus* sp. is commonly found in the amphibian gut flora, understanding the pathogenesis of *Basidiobolus* sp. infection in amphibians has direct implications for management of threatened amphibian populations as well as colonies kept for species preservation.

In this study, we have only looked for the presence of *Basidiobolus* sp. in eight of 16 species of anurans found in west central Florida. Some species in the state have been perceived as undergoing population declines, although mostly as a result of human induced habitat alteration (Delis et al., 1996). Therefore, we encourage expansion of this investigation to include all anuran taxa in the state with special emphasis on species that are threatened by both biological and human forces. In addition, we strongly recommend that further investigations be conducted to elucidate the effect of different stresses on the ability of *Basidiobolus* sp. to cause infections in amphibians and its possible role in global amphibian population declines.

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Received for publication 7 July 2000.