THE PROTISTAN PATHOGEN PERKINSEA (A.K.A. DERMOMYCOIDES, PERKINSUS-LIKE ORGANISM, AND ALVEOLATE PATHOGEN) AND ITS IMPACT ON SOUTHEASTERN AMPHIBIANS.

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Amphibian Die-offs:
The protist pathogen primarily referred to as Perkinsea (a.k.a. Dermomycoides, Perkinsus-like organism and alveolate pathogen) has been implicated in the mass mortality events of tadpoles since 1998 (Green, Converse, & Schrader, 2002). This pathogen has since been directly tied to mortality events in 43 states and has been shown to be present and actively infecting frogs globally (Atkinson, 2016; Chambouvet et al., 2015; Cook, 2008; Davis, Yabsley, Kevin Keel, & Maerz, 2007; Green et al., 2002; Isidoro-Ayza et al., 2017; Karwacki, Atkinson, Ossiboff, & Savage, 2018). This pathogen is currently considered the third most concerning amphibian pathogen in the United States and also has been found co-infecting tadpoles along with Ranavirus (Frog virus3) during large-scale mortality events (Isidoro-Ayza et al., 2017; Landsberg et al., 2013). The mortalities caused by this pathogen occur most often in Ranid frogs, particularly *Rana sphenocephala*, *R. sevosa*, *R. capito*, *R. catesbeiana*, and *R. sylvatica*, and Perkinsea has been tied to the poor recruitment and recovery of *R. sevosa*, a federally protected species with only ~200 adults left in the wild (Atkinson, 2016; Cook, 2008; Davis et al., 2007; Isidoro-Ayza et al., 2017). The species of Perkinsea present throughout most of the globe may be less likely to cause mortality compared to the pathogen species present in North America, but the amount of research conducted on Perkinsea outside of the US is relatively little (Chambouvet et al., 2015). Additionally, this pathogen does not always exhibit mass mortality events but has been shown to negatively impact populations of *R. sevosa* through slower, individual mortality (Atkinson, 2016). Specifically, in caged tadpoles naturally infected with Perkinsea, individuals died in small numbers over the course of a month resulting in complete loss of all individuals in the cage without a mass mortality event (Atkinson, 2016). Given the overall lack of information on the occurrence of and factors leading to Perkinsea-related mortality events, substantially more research is needed on this pathogen.

General Information about the pathogen:
The taxonomy for Perkinsea is highly disputed, and this has resulted in the organism not being formally described in the literature. Given the amount of published literature on this pathogen, there is little known about the general life cycle or the ecology of this organism and what may lead this pathogen to cause mass mortality events. Experimental challenges have begun to
answer these questions, but the results are only present in grey literature (Atkinson, 2016; Cook, 2008). Work conducted in wild populations identified that the tadpole life stage and members of the family Ranidae are more likely to have high level of infections, and that there may be a strong seasonal component to when the pathogen is causing mortality (Karwacki et al., 2018). Additionally, environmental factors such as pH also may influence the infection rate of Perkinsea, but more research is needed to conclusively determine this (Atkinson, 2016).

Diagnostics and signs of disease:
Similar to many diseases, there are no pathognomonic lesions for disease caused by Perkinsea infection, resulting in many mortality events being misdiagnosed (Isidoro-Ayza et al., 2017). However, there are some common (yet not pathogen specific) external signs noted during a Perkinsea-related mortality event. Infected tadpoles and juvenile frogs often appear lethargic, and hemorrhages can be seen on the ventral surface of the body (Green et al., 2002). The liver is often enlarged and fluorescent orange or white, resulting in visualization of the liver through the skin (Isidoro-Ayza et al., 2017). The pathogen infects multiple tissues, but infections seem to be more common in the liver, often with total effacement of the organ (Karwacki et al., 2018). The presence and number of Perkinsea spores can be determined diagnostically using a variety of genetic and histopathologic methods. The intensity of Perkinsea spores in a given tissue can be readily determined using sectioned and stained tissues (Green et al., 2002). The methods described in Isidoro-Ayza et al. for sectioning and staining tissues can be used to both determine the likely cause of mortality and quantify the pathogen burden present in a particular tissue (Isidoro-Ayza, Grear, & Chambouvet, 2018). Additionally spores can be readily separated from intestinal or liver tissue by immersing the tissue in amphibian saline solution and emulsifying the it (Cook, 2008). This method will indicate the presence of spores, and you can quantify the number of spores present in solution but is not as accurately as histology-based or genetic-based methods. The spores themselves are relatively circular with a thick cellular membrane and range in size from 4-6 μm in diameter (Cook, 2008). Primers and protocols for both conventional and quantitative PCR (qPCR) have proven effective at determining the presence and quantity of Perkinsea spores within a specific sample (Chambouvet et al., 2015; Karwacki et al., 2018). The qPCR protocol has been specifically shown to closely match the results of the histology-based methods within the same individuals (Karwacki et al., 2018).
Perkinsea appears to be causing numerous die-offs of amphibian populations in the southeastern portion of the United States and may be influencing the conservation for some species (Atkinson, 2016; Davis et al., 2007; Isidoro-Ayza et al., 2017; Landsberg et al., 2013). More work is needed to understand the fundamental disease ecology of this pathogen, and what impact it may be having on anuran populations in the United States. Currently nothing is known on the origin of this pathogen, or what can be done to mitigate the presence of this pathogen. Additionally we do not know much regarding effective sterilization techniques, and at this time the method perscribed to remove the pathogen is to use a solution of 10% bleach and water left to sit for at least five minutes prior.
Figure 1: Perkinsea spores separated from tadpole liver tissue after the tissue was placed into amphibian saline solution and emulsified. The resulting solution was then pipetted onto a hemocytometer slide and viewed at 1000X magnification. One of the spores in this image was highlighted via a red arrow.

Figure 2: Gross lesions of a heavily infected southern leopard frog (*Rana sphenocephala*) tadpole. The liver shows clear signs of being heavily infected with Perkinsea with most of the liver tissue being replaced by the protist (photo credit: Veronica Urgiles).
Figure 3: From Karwacki et al., 2018. Comparison of liver (A-C), kidney (mesonephros; D-F), and skeletal muscle (G-I) histopathology in larval leopard frogs (*R. sphenoecephala*). Uninfected control tissues (A, D, G) are compared to individuals with mild to moderate (B, E, H) and severe (C, F, I) histologic evidence of perkinsiosis. 500X Magnification.

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References:
Atkinson, M. S. (2016). The effects of the protist parasite Dermomycoides sp., on the dusky gopher frog (Rana sevosa) and the southern leopard frog (Rana sphenocephala).