

Identification of Sex and Fungal Infection Screening of Wyoming Anurans

Part 2: Chytridiomycosis

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Background

The purpose of this document is to report on the screening of Chorus Frogs (*Pseudacris triseriata* complex) and Wyoming Toads (*Anaxyrus baxteri*) for the presence of chytridiomycosis, a fungal infection caused by *Batrachochytrium dendrobatidis*. The disease has been diagnosed in Wyoming Toads (Taylor et al. 1999, Odum and Corn 2005) and in Chorus Frogs in the Rocky Mountains (Rittmann and Muths 2003). It is possible to use routine histological staining to identify skin infected with the fungus (Pessier et al. 1999, Berger et al. 2000), and this approach not only shows that the organism is present at a locality but also is capable of causing disease within a population.

Methods

Fungal identification was conducted using histological preparation of 17 *P. triseriata* and 10 *A. baxteri* collected at two locations (Mortenson Lake and Vedauwoo). Tissue processing was the same as that described in Part 1 of the report. In addition to serial sectioning of the whole body (sagittal sections in frogs and cross sections in toads) as previously described, a single hindlimb was sectioned along its longitudinal axis in most specimens. A total of three sections of the body and one section of the hindlimb were observed in most specimens (hindlimb sections were not performed in three samples). Although the orientation of the body sections was different between frogs and toads, both included skin of the pelvic patch. Combined with the hindlimb sections, the screening therefore included skin that tends to show the highest likelihood of chytrid infection (Pessier et al. 1999, Berger et al. 2000, Puschendorf and Bolanos 2006).

Sections were observed using light microscopy following the protocol described in Berger *et al.* (2000). Screening resulted in three categories of diagnosis: positive, suspect positive, and negative. A positive diagnosis was assigned when fungal zoosporangia were observed and the epidermis showed areas of sloughing. Individuals that were diagnosed as suspect positive demonstrated thickening of the epidermis (hyperkeratosis) with possible zoosporangia in its basal layers. Negatives lacked clear examples of these features.

Results

Chorus Frogs

Of the two sites sampled, likely chytridiomycosis was observed in specimens from Mortenson Lake but not Vedauwoo (Table 1). At Mortenson Lake two specimens were diagnosed as positive (Fig. 1), with an additional three suspected of disease. Both of the positives were from the sagittal sections from the body.

Table 1. Diagnoses of fungal pathogenesis in the skin of Chorus Frogs and Wyoming Toads. The basis of a diagnosis is given in the methods. Note that histological sections were not available from the hindlimb of six Chorus Frogs.

Species	Site	ID	Body	Hindlimb		
Chorus Frog	Vedauwoo	VWCFWB01	Negative	Negative		
		VWCFWB02	Negative	Negative		
		VWCFWB03	Negative	Negative		
		VWCFWB04	Negative	No specimen		
		VWCFWB05	Negative	No specimen		
		VWCFWB06	Negative	Negative		
		VWCFWB07	Negative	Negative		
	Mortenson	MTCFWB01	Suspect positive	No specimen		
		MTCFWB02	Negative	Negative		
		MTCFWB03	Negative	Negative		
		MTCFWB04	Suspect positive	Negative		
		MTCFWB05	Positive	Negative		
		MTCFWB06	Negative	Negative		
		MTCFWB07	Negative	Suspect positive		
		MTCFWB08	Suspect positive	Suspect positive		
		MTCFWB09	Negative	Negative		
		MTCFWB10	Positive	Negative		
		Wyoming Toad	Mortenson	MTWTWB01	Suspect positive	Negative
				MTWTWB02	Suspect positive	Negative
MTWTWB06	Positive			Suspect positive		
MTWTWB07	Negative			Suspect positive		
MTWTWB10	Suspect positive			Negative		
MTWTWB11	Negative			Suspect positive		
MTWTWB22	Suspect positive			Suspect positive		
MTWTWB23	Negative			Negative		
MTWTWB27	Suspect positive			Negative		
MTWTWB28	Positive	Negative				



Figure 1. Histology of skin showing finfection in a *P. triseriata* metamorph (MTCFWB05). The bracketed tissue shows sloughing epidermis (SE) within which are various stages zoosporangia with one containing zoospores (Z). Bar = 50 μ m.

Wyoming Toads

Similar to the prevalence of chytridiomycosis in Chorus Frogs at Mortenson Lake, two Wyoming toads exhibited infection (Table 1, Fig. 2). An additional seven individuals had symptoms associated with disease (Fig. 3).

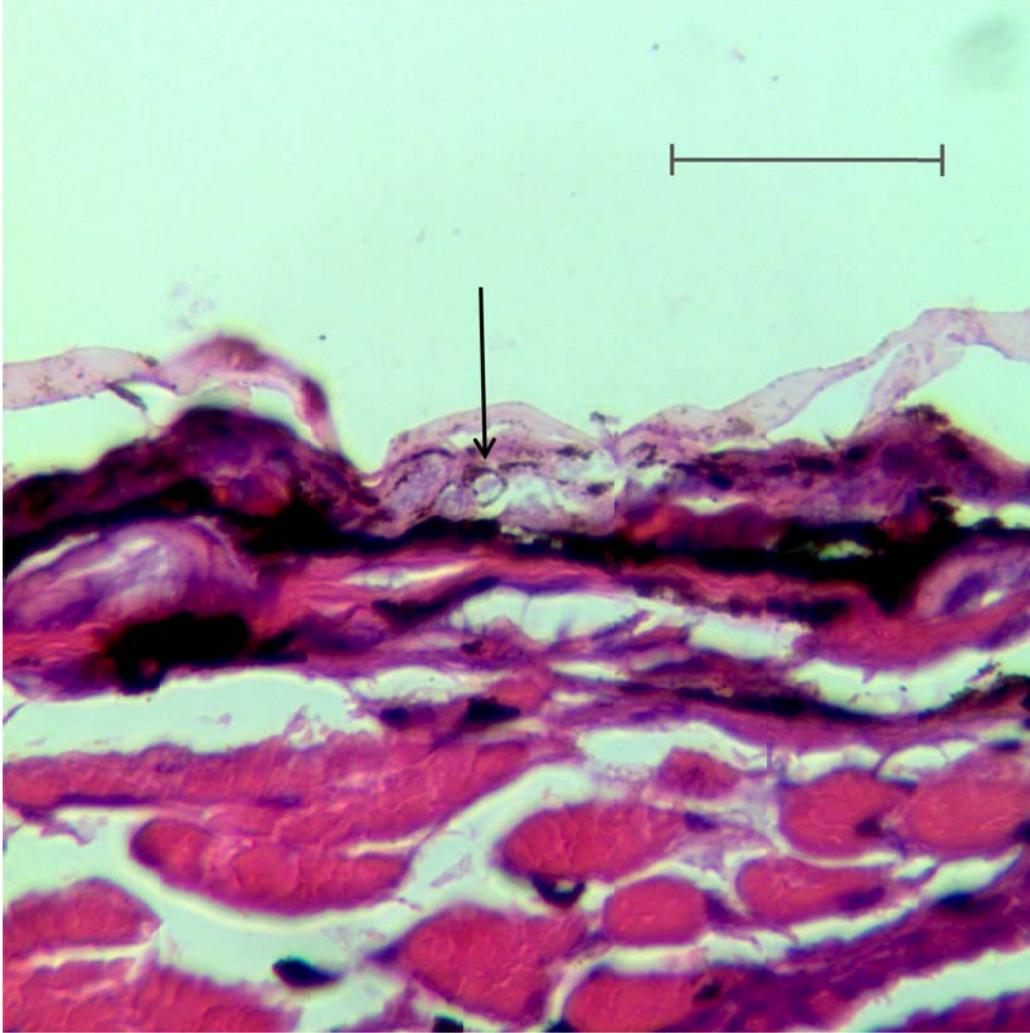


Figure 2. Skin of a Wyoming toad (MTWTWB06) that exhibits hyperkeratosis and a zoosporangium (indicated by arrow). Bar = 50 μ m.



Figure 3. Skin of a Wyoming toad (MTWTWB01) that exhibits focal hyperkeratosis (indicated by arrows) resulting in a 'suspect positive' diagnosis. Bar = 50 μ m.

Conclusions

The observations made here are consistent with the conclusion that both species are susceptible to chytridiomycosis at Mortenson Lake and they follow earlier observations of the presence of the chytrid related mortality in Wyoming Toads at the site (Taylor et al. 1999, Odum and Corn 2005). Metamorphic toads are thought to be particularly susceptible to the disease (Garner et al. 2009). While there are individuals identified as positive in this sample, the areas of hyperkeratosis and ulceration were not widespread within an individual suggesting that the infections observed were mild (Berger et al. 2000). Further study will be required to understand the current relationship between infection and mortality at the site in both species.

There was no evidence of infection at Vedauwoo. Due to the potential for false negatives when relying on histological screening however, this does not necessarily mean that there is no chytrid at

the site. Puschendorf and Bolanos (2006) suggest that up to 17 sections are needed to determine infection status with 95% confidence. This sampling effect can be seen in the comparison between the body and hindlimb sections (Table 1). More positive and suspect positive diagnoses are seen in the body sections and this is probably due to the higher number of sections reviewed in the former. Given the potential for sampling bias, the absence of chytrid at Vedauwoo should be considered preliminary.

References

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