

Phylogenetic analysis of pathogenic amphibian *Perkinsea* reveals rapid expansion of its geographic range

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Background

Amphibians are one of the most threatened animal groups. Many factors contribute to their global decline, including infectious disease (Fig 1).

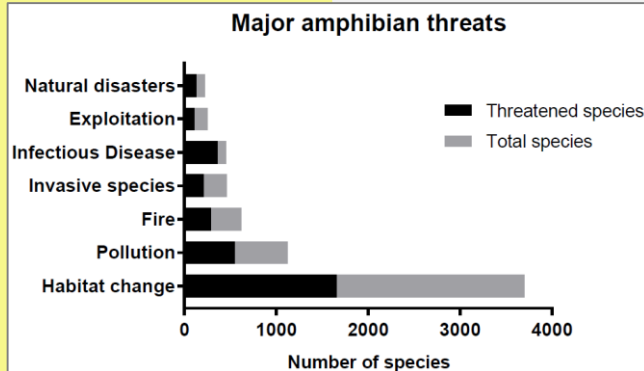


Fig 1: Total and threatened numbers of amphibian species affected by major environmental risks (Chambouvet et al., PLoS Pathog, in press).

An emerging infectious disease has been linked to a protozoan parasite known as Pathogenic *Perkinsea* Clade (PPC), which targets the liver of tadpoles (Fig 2). Previously, PPC was only found in North America, but recent sampling efforts reveal that it also occurs in Central America and Europe. We compared sequence data from these three regions to evaluate the global diversity of PPC.

Fig 2:
PPC-infected *L. sylvaticus* tadpole (A) and metamorph (B)
A. Chambouvet & T. Richards, pers. com.



Methods

- Tadpoles were collected from three sites in Panama (N=81) and one site (N=10) in the UK (Fig 3).
- Livers were screened for PPC using PCR amplification of 18S rDNA. Positives (PA: n=10-15*, UK: n=1-5*) were cloned and Sanger sequenced.
- Sequence data were aligned using Muscle in SeaView, and a phylogenetic tree was constructed using IQTree + ModelFinder (Fig 3).

*Exact numbers are unknown due to pooling of samples.

Results

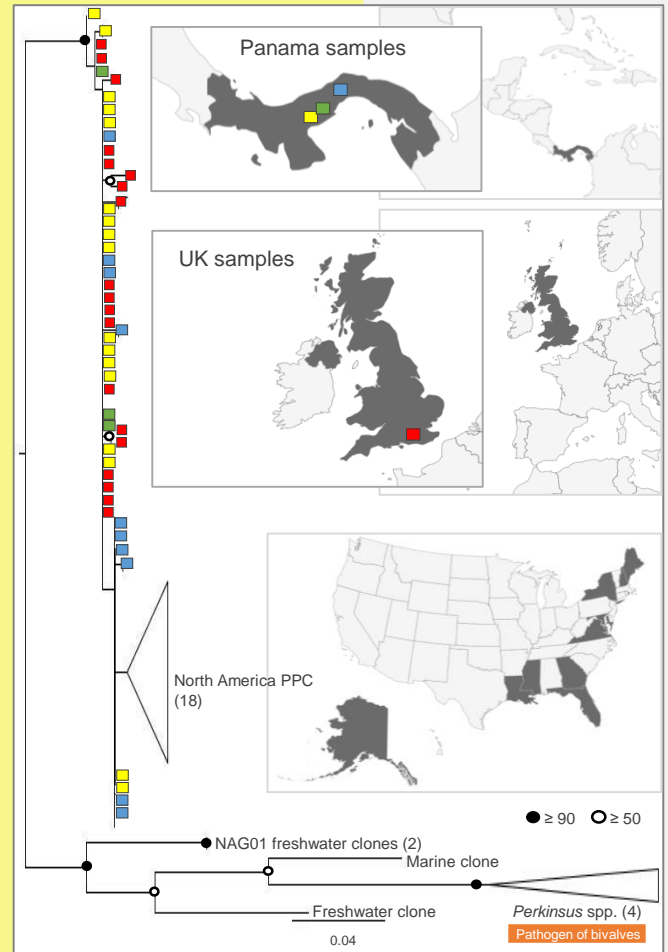


Fig 3: Maximum likelihood tree (100 bootstraps) of the PPC group of *Perkinsea*, based on a portion of the 18S rRNA encoding gene including 293 characters. Bootstrap values are shown at the nodes when the support values were above 90 (closed circle) and 50 (open circle). Each square represents an individual clone sequence. The geographic distribution of sequences are highlighted in dark grey.

Conclusion

Our phylogeny of PPC sequences from North America, Panama, and the UK reveals that they are virtually indistinct. These results are indicative of the rapid expansion of the pathogen's geographic range. Sequencing of tadpole 16S rRNA indicated PPC's host range has concomitantly expanded to include a larger number of hylid species (not shown). These findings should be considered in the development of a conservation strategy.

