Something old, something new:

Integrated approach to the taxonomy of the rectal endociliates of the globally invasive amphibian *Xenopus laevis.*

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1. Background

Although amphibians act as hosts to a variety of parasite groups, the protozoan gut ciliates are perhaps the most abundant, yet understudied group of parasites that infect amphibians. Previous studies documenting intestinal ciliates of amphibians from South Africa are limited since they employ outdated taxonomic techniques. These studies primarily dealt with the systematics of ciliate groups, rarely identifying ciliates below the genus level. Even in the case of one of the world's most widely distributed amphibian invaders and well-known model animal, the African Clawed Frog *Xenopus laevis* (Daudin, 1802) (Anura: Pipidae), the study of protozoan parasites has fallen behind that of its metazoan counterparts. Consequently, difficulties arise when rectal endociliate assemblages are compared among populations of the host, in their native range as well as elsewhere. Thus, we aim to design an approach that involves both classic and modern techniques for the better identification of rectal endociliates of *Xenopus laevis*.

2. The Integrated Approach with preliminary results of *Xenopus laevis* ciliates

Classic Hematoxylin staining methods

- Classical ciliate taxonomy is mainly based on staining methods which expose distinctive nuclear components and patterns in the cilia on the surface of the cell body.
- Mayer's hematoxylin facilitates internal morphological observations by enhancing the visibility of the taxonomically important nuclear morphology

Scanning Electron Microscopy (SEM)

SEM

- Better external morphological examinations
- Aid in measurement of important morphological characteristics such as ciliary structure

DNA Barcoding

In progress

Live observations

- Advanced microscopy facilitates study of the external and internal morphological characteristics
- Swimming patterns can be observed and possibly be used as an identification characteristic

3. Results and Conclusion

With the help of our integrated approach to taxonomy, we found three morphospecies of rectal ciliates in *Xenopus laevis* thus far. These species are preliminary identified as a) *Protoopalina xenopodus; b) Balantidium xenopodus*; and most probably another *Balantidium* species (Figure 1) . In light of the invasive status of *Xenopus laevis*, taxonomic clarity of this understudied group will form the basis of future studies on the diversity, symbiotic relationship and ecological role of these possible co-invaders. Furthermore, an improved workflow of the identification of the rectal ciliates in *Xenopus laevis* can be applied to other anuran hosts.

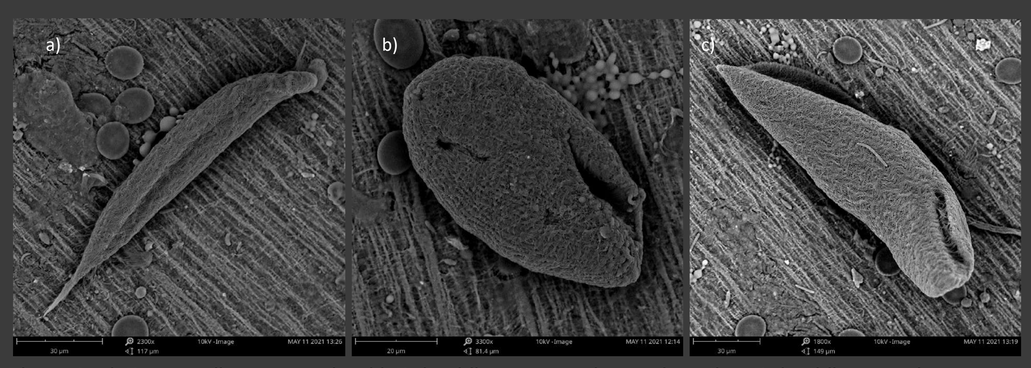

Figure 1: a) *Protoopalina xenopodus*; b) *Balantidium xenopodus*; and another *Balantidium* species (preliminary identified)

Key References

- Metcalf, M. M., 1923. The Opalinid ciliate infusorians – Smithsonian Institute U.S. Natural Museum, 120: 484.
- Thurston, J.P. 1970. Studies on some Protozoa and helminth parasites of Xenopus, the African Clawed toad. Revue de Zoologie et de Botanique Africaines, 82:349-368.

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