Taxonomic re-evaluation of African anuran trypanosomes with the morphological, morphometric, and molecular diagnosis of *Trypanosoma nelspruitense* Laveran, 1904

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Background

- *Trypanosoma* spp. are extracellular blood parasites, infecting all vertebrate classes globally.
- Several species can cause dangerous diseases in humans and livestock.
- Aquatic trypanosomes' ecological interactions and phylogeny are not well-understood, due to their complex life cycles and a lack of molecular data.
- All 13 species of the African anuran trypanosomes were described in the 20th century.
- Only one species has currently been described from South Africa, *T. nelspruitense* Laveran, 1904.
- The aim of this study was to provide a basis for future taxonomic work on amphibian trypanosomes.

Methods

Specimen Collection

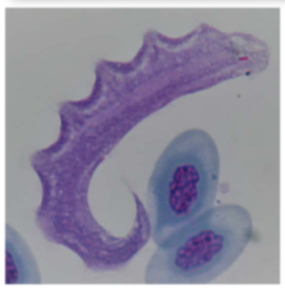
- Blood samples from the type host, *Amietia delalandii* (Common River Frog), were collected from various sites in South Africa, including the type locality.

Morphological characterisation

- Blood smear slides were stained with a Giemsa solution and microscopically screened.
- Trypanosome specimens were morphologically measured.

Molecular analysis

- Two overlapping fragments of the 18S rRNA gene were targeted for amplification, using a nested PCR method.
- A maximum likelihood (ML) phylogeny was constructed using sequences from current literature and GenBank for comparison.



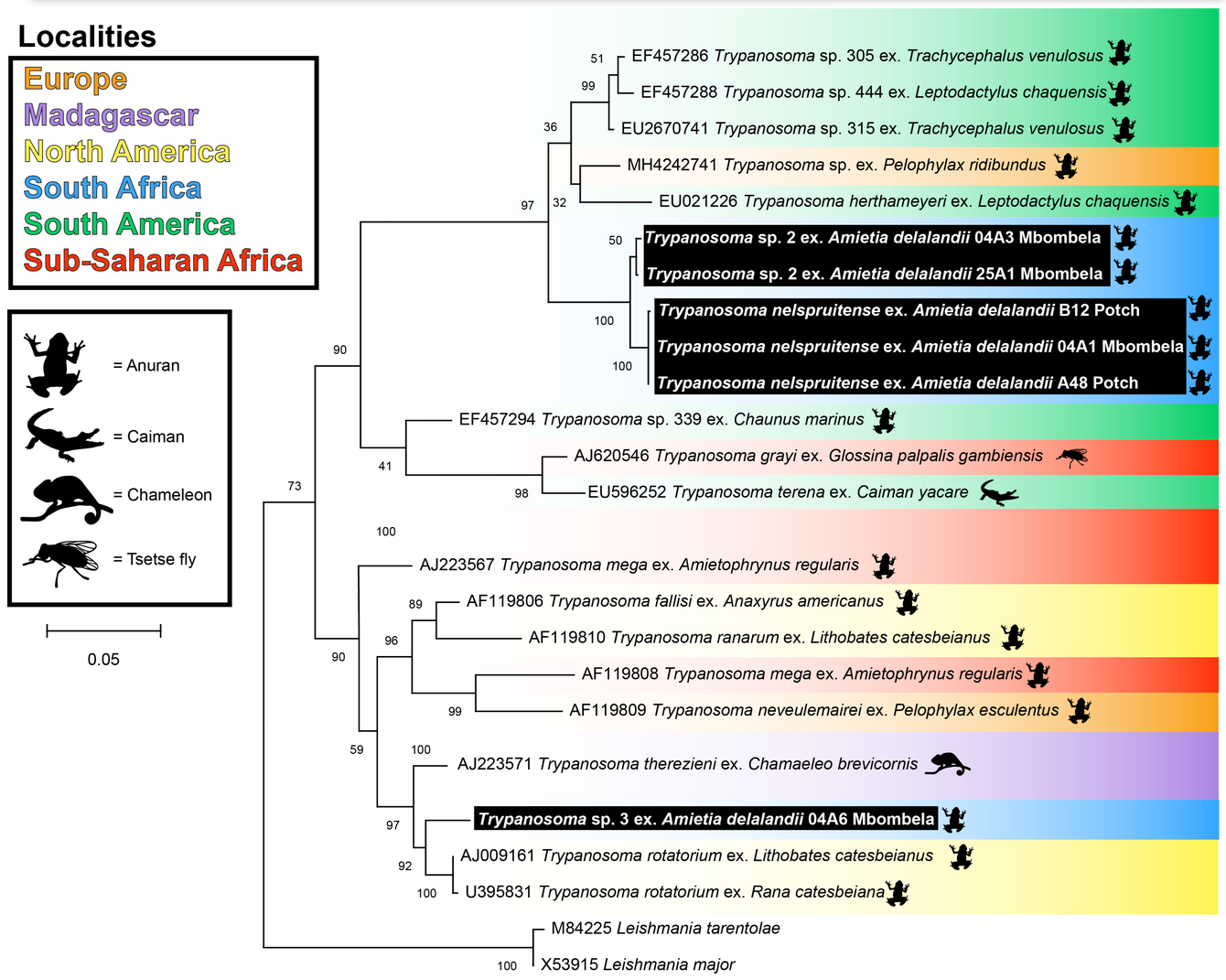
T. nelspruitense

***Trypanosoma* sp. 2**

***Trypanosoma* sp. 3**

Results & Discussion

- Six of the seven frogs collected in Mbombela were found to be infected with *Trypanosoma* spp. None of the 13 tadpole samples were infected. The samples from a previous study in Potchefstroom were known to be infected with trypanosomes.
- Morphological analysis is notoriously unreliable with *Trypanosoma* specimens, therefore it is used in combination with molecular analyses.
- The morphological and morphometric analyses showed three distinct species, which are supported with the phylogeny.
- Possible different forms of the same species were also observed.
- No morphological measurements of closely related species are currently available for comparison.
- *T. nelspruitense, Trypanosoma* sp. 2 and sp. 3 from this study were placed in new subclades within the aquatic trypanosome phylogeny.
- Academic trypanosomatid phylogenies can often be misleading due to the multi-host life cycles causing overlap of the clades.



Nodal bootstrap values are indicated as a percentage. Scale shown is nucleotide substitutions per site.

Conclusion

- Morphological, morphometric and molecular data of three species of *Trypanosoma* (including *T. nelspruitense*) is presented in this study.
- This is the first study to provide molecular data for species of *Trypanosoma* from South African anurans, setting a platform for future research.

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NRF National Research Foundation