Parasite X: Understanding the Ecological and Ethical Implications of a Liver Parasite in Pet-Trade Acquired *Smilisca baudinii*

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INTRODUCTION

1. Identifying a Covert Parasitic Liver Infection

- A *Smilisca phaeota* tree frog was purchased from the pet trade to expand a transcriptomic reference library [1]
- These frogs, native to Central and South America, are known for their adaptability and coloration (Fig. 1)
- Dissection revealed a severe liver infection caused by an uncharacterized nematode-like organism (*Parasite X*) despite looking superficially healthy and varying in symptoms of known amphibian parasites (Table 1)

Fig. 1

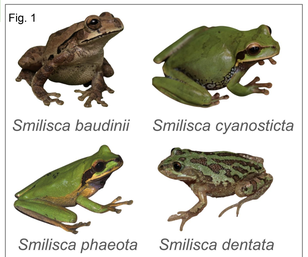


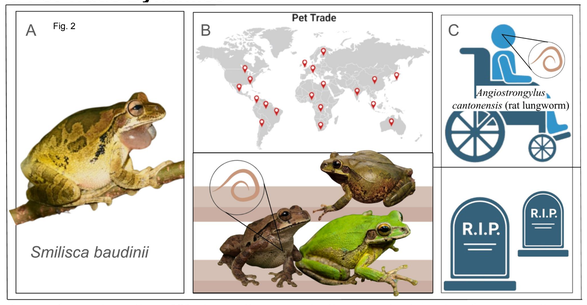
Table 1

Feature	Typical *Rhabdias* Infection	*Parasite X* Infection	Reference
Organ affected	Lungs primarily	Liver	Shine *et al.* 2015
Mucus presence	Excessive	Not visible	Navarre, DVM, 2008
Fungal presence	Co-occurs	Not visible	Gu *et al.* 2024
Lethargy	Common (less movement)	Slightly visible	Merck Vet, 2023
Weight loss	Common (poor appetite)	Slightly visible	Lock, DVM, 2017
Respiratory	Common (open mouth)	Not visible	Shine *et al.* 2015

2. *Smilisca baudinii* and Pet Trade Dynamics

- Genetic analysis confirmed the frog was actually *S. baudinii*
- *S. baudinii* lives in Central America [2], and is the largest *Smilisca* species [3] with notable color variation, and rarely bred or seen in the pet trade [5]
- The U.S. imports many frogs for education [6], breeding, and display, but lack of quarantine enables parasite spread [7], including zoonotic risks like *A. cantonensis* [12] (Fig. 2)

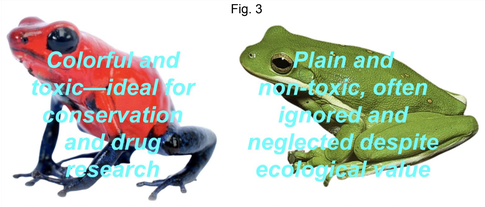
Fig. 2



3. Gaps in Amphibian Parasite Research Due to Neglected Biodiversity

- Neotropical amphibian parasites remain understudied despite known risks [14], and morphological identification is limited by a lack of comparative data and poor preservation [14][16]

Fig. 3



- While researchers and museum collections focus on charismatic species, they fall into excluding many amphibians [15], like species with dull coloration or low appeal, are often left out of conservation efforts [16] (Fig. 3), leading to data gaps due to neglected biodiversity

OBJECTIVES & QUESTIONS

Our objectives are: i) to develop a transcriptomic and mitogenomic pipeline for host and parasite identification, ii) to compare infected and uninfected host frog tissues, iii) to characterize *Parasite X* morphologically, and iv) to determine the parasite and host's mitogenomics and phylogenetics based on barcode gene *COX1*. We ask, what is the identity and biological impact of *Parasite X* in the host, and what does it reveal about parasite diversity in tropical and pet-traded amphibians?

METHODOLOGY

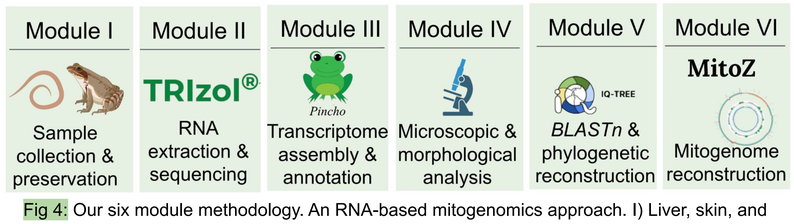


Fig 4: Our six module methodology. An RNA-based mitogenomics approach. I) Liver, skin, and parasite samples were collected under sterile conditions and stored at -80°C, II) RNA was extracted using *TRIzol* [17], and sequenced, III) transcriptomes were assembled with the *Pincho* [18] pipeline, IV) microscopic assessments were done, V) barcode gene *COX1* was used to run *BLASTn* [21, 22] and phylogenetics, and VI) mitochondrial genomes were reconstructed [19-20]

RESULTS

PARASITE X RESULTS

Fig. 5

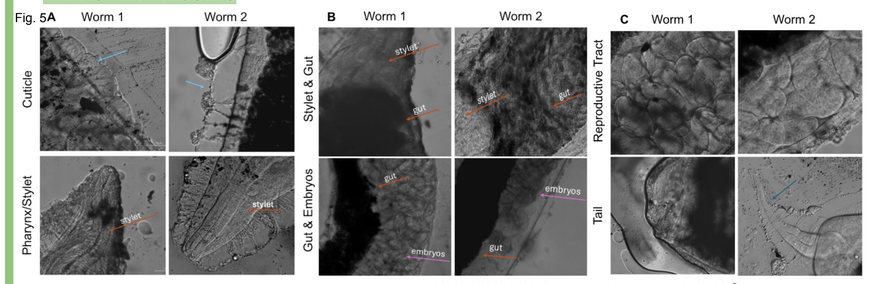
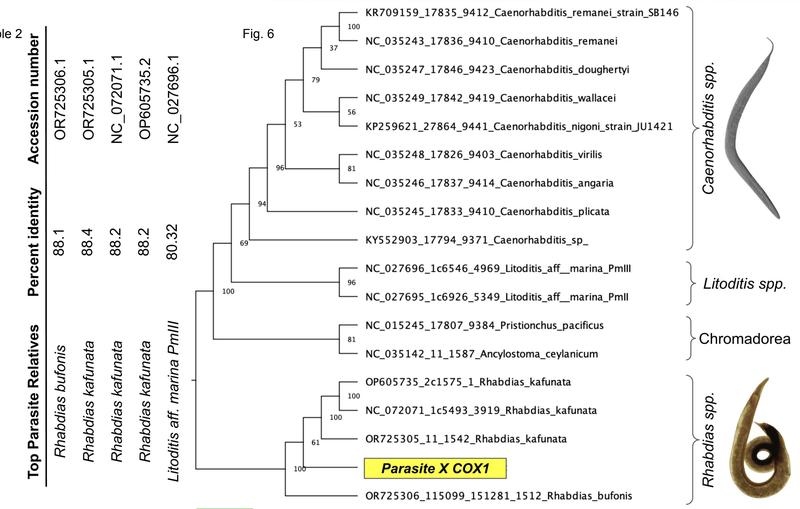


Table 2

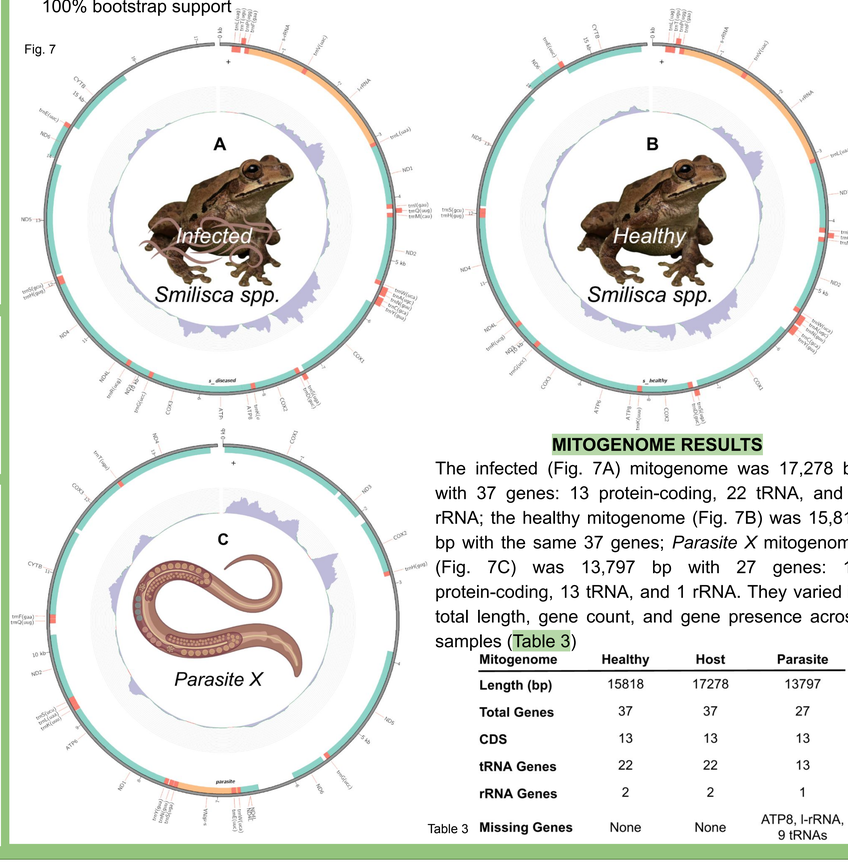
Top Parasite Relatives	Percent identity	Accession number
Rhabdias bufonis	88.1	OR725306.1
Rhabdias kafunata	88.4	OR725305.1
Rhabdias kafunata	88.2	NC_072071.1
Rhabdias kafunata	88.2	OP605735.2
Litoditis aff. marina PmIII	80.32	NC_027696.1

Fig. 6



- Microscopic morphology (Fig. 5) of *Parasite X*: a ruffled cuticle, a single-bulbed stylet, a tubular gut, developing embryos, and a distinct tail appendage in one worm, (consistent with parasitic nematodes)
- *BLASTn* (Table 2) shows that *Parasite X* matched most closely to *Rhabdias spp.* with ~88% identity
- Phylogenetic reconstruction (Fig. 6) confirms that, *Parasite X is* grouped within the *Rhabdias* clade with 100% bootstrap support

Fig. 7



MITOGENOME RESULTS

The infected (Fig. 7A) mitogenome was 17,278 bp with 37 genes: 13 protein-coding, 22 tRNA, and 2 rRNA; the healthy mitogenome (Fig. 7B) was 15,818 bp with the same 37 genes; *Parasite X* mitogenome (Fig. 7C) was 13,797 bp with 27 genes: 13 protein-coding, 13 tRNA, and 1 rRNA. They varied in total length, gene count, and gene presence across samples (Table 3)

Table 3

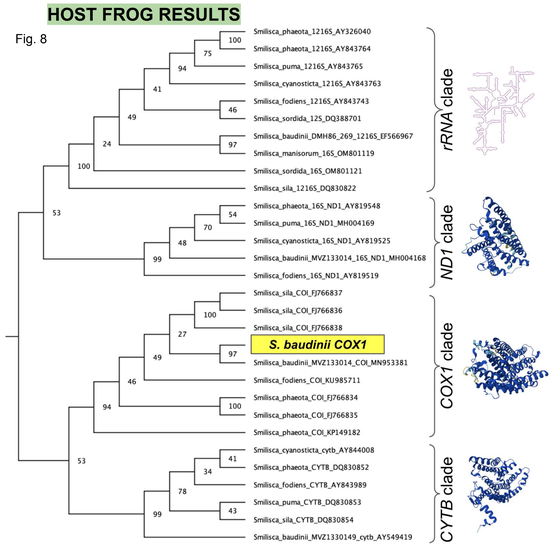
Mitogenome	Healthy	Host	Parasite
Length (bp)	15818	17278	13797
Total Genes	37	37	27
CDS	13	13	13
tRNA Genes	22	22	13
rRNA Genes	2	2	1
Missing Genes	None	None	ATP8, I-rRNA, 9 tRNAs

HOST FROG RESULTS

Table 4

Host & Healthy Frog Relatives	Percent identity	Accession number
Smilisca baudinii	95.7	AY549366.1
Smilisca baudinii	95.59	EF566967.1
Smilisca fodiens	92.85	AY843743.1
Smilisca phaeota	91.67	AY843764.1
Smilisca puma	91.58	AY843765.1
Smilisca cyanosticta	91.38	AY843763.1

Fig. 8



- Based on *BLASTn*, frog mitochondrial sequences matched *S. baudinii* (AY549366.1) with 95.7% identity, 15% query coverage, and a score of 3886; other *S. baudinii* hits showed >94% identity, while related species ranged from ~87–93% with lower scores (Table 4)
- Based on phylogenetic reconstruction, S. baudinii *COX1* clustered with another *S. baudinii* at 97% bootstrap support; sequences formed four clades by gene: rRNA, ND1, COX1, and CYTB (Fig. 8)

DISCUSSION

- **Morphology** implies that Parasite X is a reproductive *Rhabdias* nematode, potentially adapted for liver infection, due to the presence of advanced reproductive structures and variation in developmental features across individuals of the same progeny
- ***BLASTn*** showed that our host and healthy frog are *S. baudinii*, while the parasite is possibly a cryptic or undescribed *Rhabdias spp.*, due to low sequence identity and absence of exact matches in barcode reference databases
- **Mitogenomics** showed that parasitic infection may alter host mitochondrial architecture, due to the observed genome length changes in the infected host, while the genome reduction of the parasite, which is typical of parasitic genomes, is likely due to insufficient data on Neotropical amphibian parasites (highlighting neglected biodiversity)
- **Phylogenetics** confirms the genera our samples belong to and helps resolve relationships not evident through morphology alone
- Overall, using sheer transcriptomics, we were able to show the power of RNA-based mitogenomics to reveal undocumented parasitic diversity, as it was able to simultaneously reconstruct host and parasite genomes, detect misidentifications, and capture potential molecular responses to infection

FUTURE STEPS

- Expand sample sizes and types (e.g. pet trade vs nature) by collecting tissues from diverse frog populations, hosts, and regions to improve genetic diversity [42] and gaps in knowledge
- Compare mitogenomes in healthy vs infected frogs to see if there are mitogenome expansions, especially those with secondary infections like fungal (*Bd*)
- Develop parasite-specific reference databases: Expand nematode mitogenomic and transcriptomic references to improve taxonomic resolution, gene annotation, and *BLASTn* matching in underrepresented taxa like *Rhabdias*

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