

# Molecular analysis of individually laser-dissected microfilariae reveals *Acanthocheilonema reconditum* disguised as *Dirofilaria immitis*

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## Introduction

Canine filarial parasites, including *Dirofilaria immitis* and *Acanthocheilonema reconditum*, can cause zoonotic infections in humans. *D. immitis* and *A. reconditum*, however, respond differently to drug treatments and, therefore, it is necessary to discriminate between these two parasites during diagnosis.

## Methods

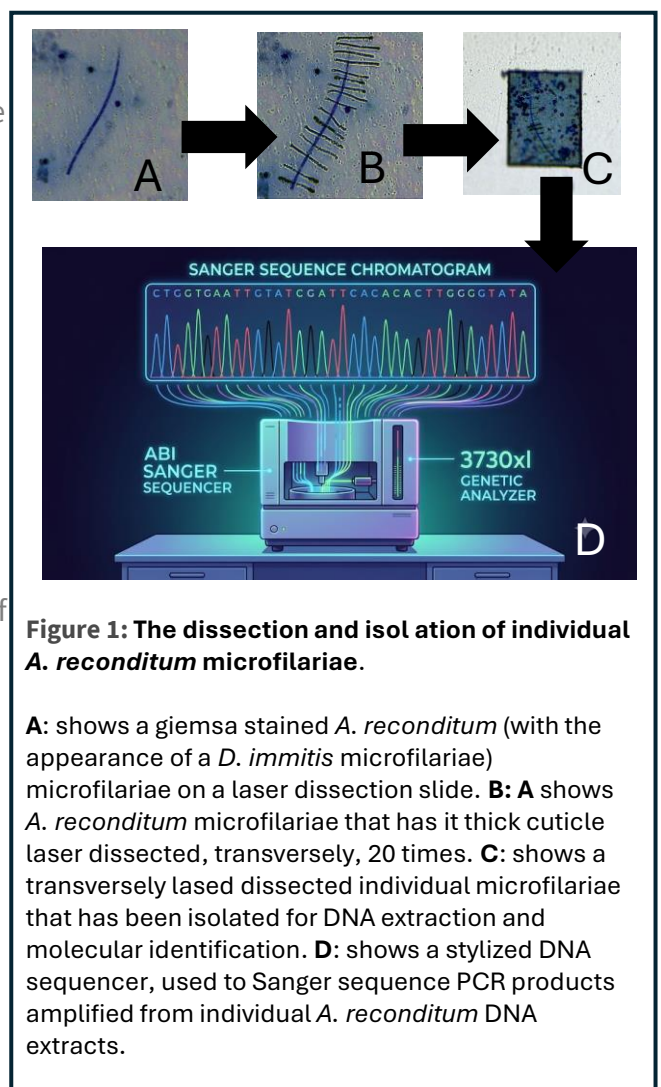
Light microscope blood surveys of canines were used to identify the presence of *D. immitis* and *A. reconditum* infections and co-infections in dogs that were life-long residents of Manaus (Amazonas, Brazil). The ITS-1 and 5S nuclear ribosomal DNA sequences and the mitochondrial CO1 and 12S gene sequences of filarial parasites were amplified by PCR and then Sanger sequenced for the molecular taxonomic classification of 20 individual microfilariae. These 20 individual microfilariae were isolated by laser dissection from a single canine blood sample, which morphological analysis of microfilariae suggested contained both *D. immitis* and *A. reconditum* parasites (See Figure 1).

## Results

A total of 20 individual microfilariae, which all morphologically appeared as *D. immitis*, had two or more taxonomically informative PCR fragments amplified and Sanger sequenced. More than five ITS-1, 5S, 12S and CO1 DNA sequences were recovered from the twenty individual microfilariae. All recovered sequences showed 100% identity to database reference sequences matching *A. reconditum*.

## References

- (1) Barbosa et al., (2023). *Revista Brasileira de Parasitologia Veterinária*, 32(2), e000223.
- (2) Post et al., (2009). Laser-assisted microdissection for the study of the ecology of parasites in their hosts. *Molecular ecology resources*, 9(2), 480-486.
- (3) Portela et al., (2024). *Current Research in Parasitology & Vector-Borne Diseases*, 5, 100168.



**Figure 1: The dissection and isolation of individual *A. reconditum* microfilariae.**

**A:** shows a giemsa stained *A. reconditum* (with the appearance of a *D. immitis* microfilariae) microfilariae on a laser dissection slide. **B:** A shows *A. reconditum* microfilariae that has its thick cuticle laser-dissected, transversely, 20 times. **C:** shows a transversely laser-dissected individual microfilariae that has been isolated for DNA extraction and molecular identification. **D:** shows a stylized DNA sequencer, used to Sanger sequence PCR products amplified from individual *A. reconditum* DNA extracts.

## Conclusions

*A. reconditum* is present in the state of Amazonas and indeed the city of Manaus. *A. reconditum* microfilariae are more difficult to distinguish from *D. immitis* than has previously been reported and it may, in fact, not be possible to reliably discriminate between these two species by the morphological features of their microfilariae alone. Although the possibility that *A. reconditum* and *D. immitis* form hybrids cannot be ruled out by this work, no evidence of hybrids or gene flow between these parasites was detected in our study.