FAMILY-SPECIFIC COX1 PRIMERS FOR IDENTIFYING APHIDS FROM DEGRADED SAMPLES

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INTRODUCTION & AIM

Aphids (Hemiptera: Aphididae) are significant agricultural pests and efficient vectors of plant viruses. Accurate species identification is critical for ecological studies and integrated pest management. However, molecular identification of preserved specimens is often hampered by DNA degradation [1]. This study aimed to develop family-specific COX1 primers that target short overlapping fragments suitable for amplifying degraded DNA, enabling species identification from old aphid samples collected in South Africa.

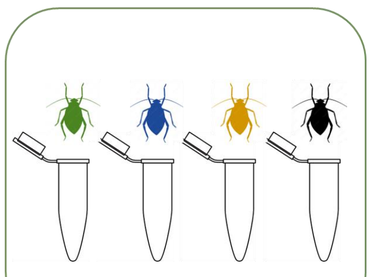
MATERIALS & METHOD

OVERVIEW OF COX1 BARCODING WORKFLOW

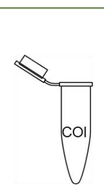
BUCKET TRAP

APHID COLLECTION

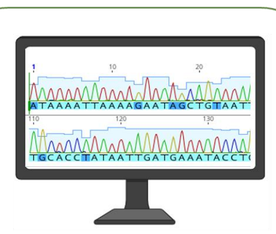
PRE-SORTING
AND MORPHOLOGICAL
IDENTIFICATION



DNA EXTRACTION



AMPLIFICATION OF *COX1*
+
SEQUENCING



MOLECULAR
IDENTIFICATION

STUDY AREA AND SAMPLE COLLECTION

Aphid samples were collected using bucket traps between 2006 and 2008 and preserved in ethanol for long-term storage. Morphological identification was performed using available taxonomic keys[2].

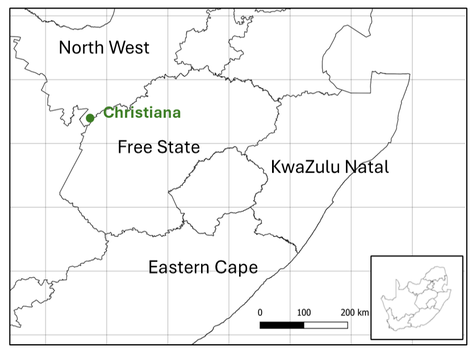


FIGURE 1. LOCATION OF CHRISTIANA, SOUTH AFRICA

PRIMER DESIGN

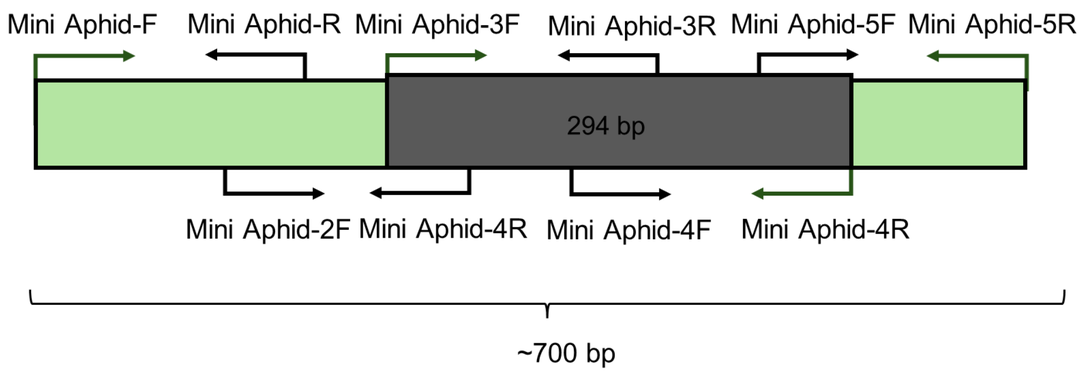


FIGURE 2. PRIMER SCHEMATIC FOR COX1 MINI-BARCODING.

Schematic representation of the COX1 barcode region (~700 bp), showing the locations of family-specific primers designed in Geneious Prime v2025.1 (www.geneious.com). Mitochondrial genomes from 33 aphid species were aligned to identify conserved regions for primer design. Short overlapping amplicons (162–178 bp) were used to amplify degraded DNA in fragments. A 294 bp mini-barcode region (grey block) was selected based on robust amplification and taxonomic resolution, and was used for species identification in moderately degraded samples. Primer binding sites are indicated by arrows

RESULTS AND DISCUSSION

TABLE 1: SPECIES IDENTIFICATION USING MINI-BARCODE AND COX1 ASSEMBLY

Sample ID	Morphological ID	Mini-barcode Success	Assembled COX1	Species Confirmed (BLAST)
01	*Acyrthosiphon kondoi*	✓	✓	*Acyrthosiphon kondoi*
02	*Acyrthosiphon pisum*	✓	✓	*Acyrthosiphon pisum*
03	*Macrosiphum euphorbiae*	✓	✓	*Macrosiphum euphorbiae*
04	*Metopolophium dirhodum*	✓	✓	*Metopolophium dirhodum*
05	*Tetraneura fusiformis*	✓	✓	*Tetraneura fusiformis*
06–31	26 other species	✗	✗	Not identified (DNA too degraded)

Of the 31 species, 5 were identified using the 294 bp mini-barcode after COX1 fragments were amplified, assembled, and compared to reference sequences, while this technique was ineffective for the remaining 26 species due to severe DNA degradation.

CONCLUSION

Using family-specific primers and a gene-walking approach, we successfully amplified degraded aphid DNA in short overlapping fragments, enabling assembly of full-length COX1 barcodes. The 294 bp mini-barcode provided comparable species-level resolution to the full-length COX1 sequence — even when direct amplification of standard barcodes failed. This strategy is effective for moderately degraded, field-collected samples and supports broader use of short, targeted barcoding regions for biodiversity monitoring.

REFERENCES

1. Dedryver, C.A.; Le Ralec, A.; Fabre, F. The Conflicting Relationships between Aphids and Men: A Review of Aphid Damage and Control Strategies. C. R. Biol. 2010, 333, 539–553.
2. Millar, I.M. The Aphids (Homoptera: Aphidoidea) of South Africa. Entomol. Mem. S. Afr. Dep. Agric. Dev. 1990, 78, 1–105.