

CANADIAN CENTRE FOR DNA BARCODING
DNA Testing Laboratory Report

Date of issue: October 3, 2017

Page 1 of 8

FORENSIC CASE INFORMATION

File Number: BIO-17-126_Kiewa-Catchment-Landscape-Groups_2017-09-06
Accession Number: BIO-17-126
Client Name: Belinda Pearce
Client Address: Kiewa Catchment Landcare Groups
387 Hellhole Creek Rd.
Kergunyah, Vic 3691
Australia
Contact Name: opundo1@optusnet.com.au

ITEMS

Description: 6 earthworm samples: samples 1 to 6 (CCDBST-02820 to CCDBST-02825)
Dates Received: September 7, 2017
Received From: Brianne St.Jacques, Administrative Assistant
Dates of Analysis: September 7, 2017 to September 29, 2017
Collector/Collection Site: Janet Topan / Canadian Centre for DNA Barcoding, Biodiversity of Ontario,
University of Guelph, 50 Stone Road East, Guelph

METHODS

To ascertain the identity of the species, a piece of each earthworm was subsampled using sterile techniques, total genomic DNA was extracted using a validated spin column DNA extraction protocol. The target genetic marker (barcode region of the mitochondrial DNA) was amplified using the Polymerase Chain Reaction (PCR) using full length primer cocktails: *C_LepFolF/C_LepFolR*; followed by cycle sequencing with a standardized commercially available BigDye Terminator v3.1 kit. Sequencing reactions were analyzed by high-voltage capillary electrophoresis on an automated ABI 3730xL DNA Analyzer. DNA sequences recovered from the unknown samples were compared against the species sequence reference library in the Barcode of Life Data System (BOLD) accessible at <http://www.boldsystems.org/>

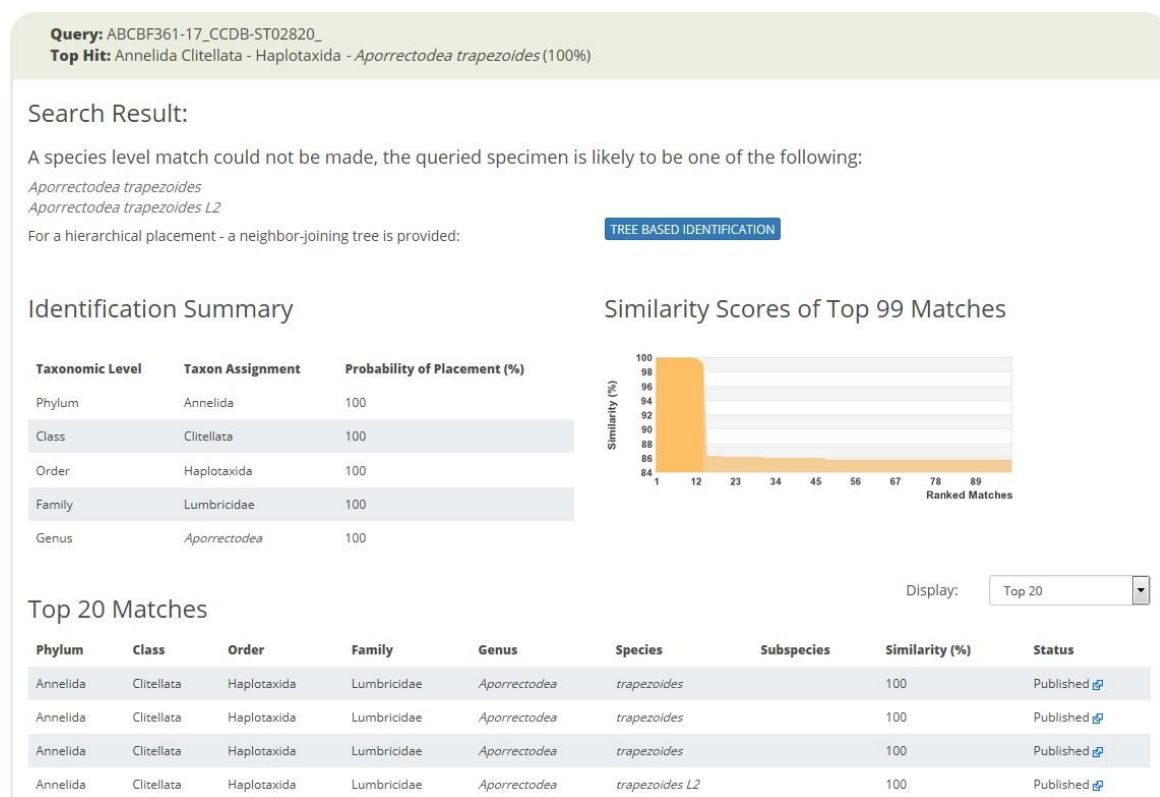
IMAGING

All items were photographed in the Photography Lab Area by Janet Topan, using a Canon ELPH 300 HS, 12.1 megapixels. Pictures were uploaded to the BOLD website into the secure project called "CCDB forensic sampling [ABCBF]". See Appendix 1 for item images.

DNA SEQUENCING RESULTS



DNA SPECIES IDENTIFICATION MATCH PERCENTAGE on BOLD



Query: ABCBF362-17_CCDB-ST02821_

Top Hit: Annelida Clitellata - Haplotaxida - *Aporrectodea trapezoides* (100%)

Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

Aporrectodea trapezoides

Aporrectodea trapezoides L2

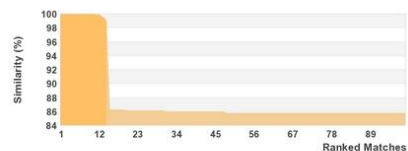
For a hierarchical placement - a neighbor-joining tree is provided:

TREE BASED IDENTIFICATION

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Annelida	100
Class	Clitellata	100
Order	Haplotaxida	100
Family	Lumbricidae	100
Genus	<i>Aporrectodea</i>	100

Similarity Scores of Top 99 Matches



Top 20 Matches

Display:

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i> L2		100	Published 🔗

Query: ABCBF363-17_CCDB-ST02822_

Top Hit: Annelida Clitellata - Haplotaxida - *Aporrectodea trapezoides* (100%)

Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

Aporrectodea trapezoides

Aporrectodea trapezoides L2

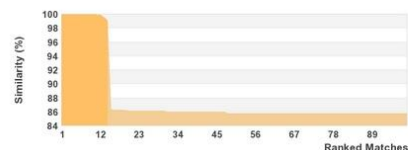
For a hierarchical placement - a neighbor-joining tree is provided:

TREE BASED IDENTIFICATION

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Annelida	100
Class	Clitellata	100
Order	Haplotaxida	100
Family	Lumbricidae	100
Genus	<i>Aporrectodea</i>	100

Similarity Scores of Top 99 Matches



Top 20 Matches

Display:

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i>		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i> L2		100	Published 🔗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>trapezoides</i> L2		100	Published 🔗

Query: ABCBF364-17_CCDB-ST02823_

Top Hit: Annelida Clitellata - Haplotaxida - *Aporrectodea longa* (100%)

Search Result:

The submitted sequence has been matched to *Aporrectodea longa*. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.

A species page is available for this taxon:

Closest matching BIN (within 3%):

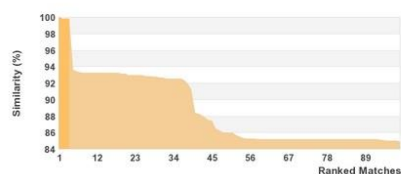
For a hierarchical placement - a neighbor-joining tree is provided:

[SPECIES PAGE](#)[BIN PAGE](#)[TREE BASED IDENTIFICATION](#)

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Annelida	100
Class	Clitellata	100
Order	Haplotaxida	100
Family	Lumbricidae	100
Genus	<i>Aporrectodea</i>	100
Species	<i>Aporrectodea longa</i>	100

Similarity Scores of Top 99 Matches



Top 20 Matches

Display:

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>longa</i>		100	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>longa</i>		99.85	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>longa</i>		99.85	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>longa</i>		99.85	Published ↗

Query: ABCBF366-17_CCDB-ST02825_

Top Hit: Annelida Clitellata - Haplotaxida - *Aporrectodea caliginosa* (99.69%)

Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

Aporrectodea caliginosa
Aporrectodea caliginosa L3
Aporrectodea handlirschi
Aporrectodea jassyensis
Aporrectodea trapezoides

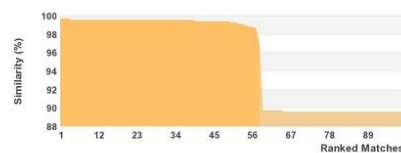
For a hierarchical placement - a neighbor-joining tree is provided:

[TREE BASED IDENTIFICATION](#)

Identification Summary

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Annelida	100
Class	Clitellata	100
Order	Haplotaxida	100
Family	Lumbricidae	100
Genus	<i>Aporrectodea</i>	100

Similarity Scores of Top 99 Matches



Top 20 Matches

Display:

Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>caliginosa</i>		99.69	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>caliginosa</i>		99.69	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>caliginosa</i>		99.69	Published ↗
Annelida	Clitellata	Haplotaxida	Lumbricidae	<i>Aporrectodea</i>	<i>caliginosa</i>		99.54	Published ↗

INTERPRETATION

Bidirectional forward and reverse sequences were generated from the earthworms. Resulting trace files were assembled into contigs and consensus sequences were then manually edited in CodonCode Aligner (version 4.1.1.) software. Sequences of the mitochondrial DNA COI gene were compared against the Full Length Record Barcode Database available in BOLD, the Barcode of Life Data. Based on a percentage of nucleotide sequence divergence (number of nucleotide substitutions) between a sequence from test sample and a reference DNA barcode, the closest match was used to infer species identity of the DNA contributor in the corresponding test sample. Images, primers, sequences and their associated trace files with quality scores were uploaded to the secure BOLD project called "CCDB forensic sampling [ABCBF]".

SUMMARY

Full length DNA barcodes were generated from 5 of the 6 earthworms.

Sample 1, CCDB-ST02820, is a 100% BOLD match to *Aporrectodea trapezoids* and *Aporrectodea trapezoides* L2.

Sample 2, CCDB-ST02821, is a 100% BOLD match to *Aporrectodea trapezoids* and *Aporrectodea trapezoides* L2.

Sample 3, CCDB-ST02822, is a 100% BOLD match to *Aporrectodea trapezoids* and *Aporrectodea trapezoides* L2.

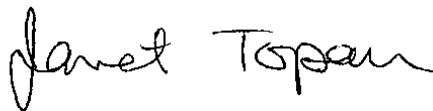
Sample 4, CCDB-ST02823 is a 100% BOLD match to *Aporrectodea longa*.

Sample 6, CCDB-ST02825 is a 99.69% BOLD match to *Aporrectodea caliginosa*, and a 99.54% BOLD match to *Aporrectodea caliginosa* L3, and a 99.03% BOLD match to *Aporrectodea handlirschi*, and a 98.78% BOLD match to *Aporrectodea jassyensis*, and a 98.62% BOLD match to *Aporrectodea trapezoids*.

CONCLUSIONS

The present testing has provided species-level taxonomic identifications of 5 of the 6 earthworms submitted by Belinda Pearce from Kiewa Catchment Landcare Groups. Based on appropriate statistical BOLD match calculations and a reasonable degree of scientific certainty of the BOLD reference library, taxonomic identity of detected DNA source in these samples is considered practically proven.

RESULTS REPORTED BY:



Janet Topan, Research Analyst

RESULTS REVIEWED BY:



Dr. Evgeny V. Zakharov, Director, CCDB

All inquiries pertaining to this report should be directed to Janet Topan (jtopan@uoguelph.ca) and Evgeny V. Zakharov (zakharov@uoguelph.ca). This report should not be reproduced, except in full, without written approval of the CCDB.

Disclaimer: "THIS REPORT AND ALL ATTACHMENTS HERETO ARE CONFIDENTIAL AND SUBJECT TO SOLICITOR-CLIENT PRIVILEGE. DO NOT FORWARD, CIRCULATE, DISTRIBUTE, COPY OR DUPLICATE THIS REPORT OR ANY ATTACHMENT HERETO WITHOUT WRITTEN AUTHORIZATION."

Appendix 1. Image Inventory



CCDBST-02820 (Sample 1)



CCDBST-02821 (Sample 2)





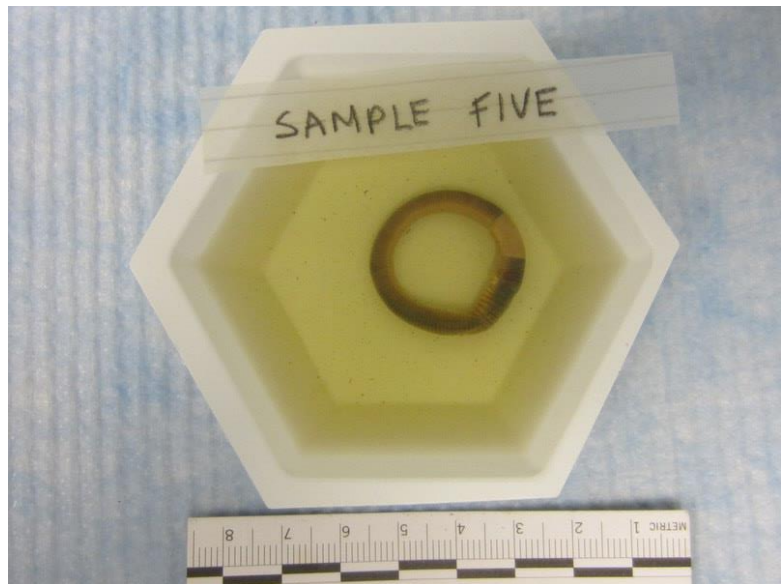
CCDBST-02822 (Sample 3)



CCDBST-02823 (Sample 4)



CCDBST-02824 (Sample 5)



CCDBST-02825 (Sample 6)

