

Supplementary Material For:

# Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol

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## Specimen collection and handling

Forty-five plant specimens representing 45 different species were collected at the University of Guelph. A 0.5-mm leaf tissue sample from each plant was placed in a 2-mL Eppendorf tube (Cat. no. RN2005-GMT; DOT Scientific Inc., Burton, MI, USA) containing 95% ethanol. Twenty-five insect specimens representing 12 species of Trichoptera (caddisflies) and Ephemeroptera (mayflies) were collected by UV-light trapping from Elora, Ontario, Canada. Each individual insect was placed in a 2-mL Eppendorf tube containing 95% ethanol. Seven older ethanol preserved specimens were selected from the archival material at the Biodiversity Institute of Ontario.

## PCR and sequencing conditions

Twenty-five-microliter PCR reactions were composed of 0.5  $\mu$ L dNTPs (10mM), 2.5  $\mu$ L 10 $\times$  Invitrogen PCR Buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 1  $\mu$ L 50 mM MgCl<sub>2</sub>, 0.5  $\mu$ L 10  $\mu$ M each primer (Supplementary Table 2), 0.5  $\mu$ L Invitrogen's Platinum *Taq* DNA polymerase (5 U/ $\mu$ L) and 2  $\mu$ L DNA. Amplification was performed for 35 cycles using Eppendorf's Mastercycler eppgradient S thermal-cycler (Supplementary Table 3). Amplicons were visualized electrophoretically on an agarose gel. Two microliters of each amplicon were subsequently used directly for Sanger sequencing using Applied Biosystems's BigDye Terminator chemistry V3.1 (Foster City, CA, USA). Sequencing reactions were cleaned using EdgeBio's AutoDTR96 (Gaithersburg, MD, USA) and visualized on an ABI 3730xl sequencer (Applied Biosystems).

**Supplementary Table S1. Freshly collected and old specimens of plant and animal species used for direct PCR and subsequent sequencing of different target genes from 95% ethanol preservative. The age of the older animal specimens is indicated in parentheses.**

Fresh plant specimens	Specimen #	PCR & sequencing
<i>Acer negundo</i>	ANG09-09	<i>rbcL</i>
<i>Acer saccharinum</i>	ANG26-09	<i>rbcL</i>
<i>Agrimonia striata</i>	ANG16-09	<i>rbcL</i>
<i>Allium</i> Spp	ANG12-09	<i>rbcL</i>
<i>Alliaria petiolata</i>	ANG01-09	<i>rbcL</i>
<i>Buxus sempervirens</i>	ANG19-09	<i>rbcL</i>
<i>Caragana pygmaea</i>	ANG32-09	<i>rbcL</i>
<i>Cardamine hirsuta</i>	ANG45-09	<i>rbcL</i>
<i>Cerastium vulgatum</i>	ANG24-09	<i>rbcL</i>
<i>Chaenomeles japonica</i>	ANG43-09	<i>rbcL</i>
<i>Chelidonium majus</i>	ANG11-09	<i>rbcL</i>
<i>Cornus stolonifera</i>	ANG17-09	<i>rbcL</i>
<i>Daucus carota</i>	ANG08-09	<i>rbcL</i>
<i>Deutzia gracilis</i>	ANG42-09	<i>rbcL</i>
<i>Erythronium americanum</i>	ANG13-09	<i>rbcL</i>
<i>Euonimus europaea</i>	ANG35-09	<i>rbcL</i>
<i>Euonimus sachalinensis</i>	ANG34-09	<i>rbcL</i>
<i>Forzicija europaea</i>	ANG37-09	<i>rbcL</i>
<i>Genista tinctoria</i>	ANG31-09	<i>rbcL</i>
<i>Geum urbanum</i>	ANG10-09	<i>rbcL</i>
<i>Iris versicolor</i>	ANG39-09	<i>rbcL</i>
<i>Lamium purpureum</i>	ANG02-09	<i>rbcL</i>
<i>Ligustrum vulgare</i>	ANG38-09	<i>rbcL</i>
<i>Linaria vulgaris</i>	ANG22-09	<i>rbcL</i>
<i>Liriodendron tulipifera</i>	ANG29-09	<i>rbcL</i>
<i>Magnolia cobus</i>	ANG28-09	<i>rbcL</i>
<i>Malva neglecta</i>	ANG21-09	<i>rbcL</i>
<i>Plantago major</i>	ANG04-09	<i>rbcL</i>
<i>Potentilla simplex</i>	ANG03-09	<i>rbcL</i>
<i>Potentilla sterilis</i>	ANG20-09	<i>rbcL</i>
<i>Prunella vulgaris</i>	ANG07-09	<i>rbcL</i>
<i>Prunus serotina</i>	ANG40-09	<i>rbcL</i>
<i>Prunus virginiana</i>	ANG30-09	<i>rbcL</i>
<i>Ranunculus repens</i>	ANG25-09	<i>rbcL</i>
<i>Rhus aromatica</i>	ANG41-09	<i>rbcL</i>
<i>Ribes uva-crispa</i>	ANG14-09	<i>rbcL</i>
<i>Salix purpurea</i>	ANG18-09	<i>rbcL</i>
<i>Stellaria media</i>	ANG44-09	<i>rbcL</i>
<i>Syringa pekinensis</i>	ANG33-09	<i>rbcL</i>
<i>Taraxacum officinale</i>	ANG06-09	<i>rbcL</i>
<i>Trifolium repens</i>	ANG05-09	<i>rbcL</i>
<i>Trillium grandiflorum</i>	ANG15-09	<i>rbcL</i>
<i>Verbascum thapsus</i>	ANG23-09	<i>rbcL</i>
<i>Vinca major</i>	ANG27-09	<i>rbcL</i>
<i>Xanthocarpus sorbifolium</i>	ANG36-09	<i>rbcL</i>

*rbcL*: large subunit of the ribulose-bisphosphate carboxylase; *COI*: cytochrome c oxidase 1; 28S rDNA: large subunit of nuclear ribosomal DNA

**Supplementary Table S1 (continued). Freshly collected and old specimens of plant and animal species used for direct PCR and subsequent sequencing of different target genes from 95% ethanol preservative. The age of the older animal specimens are indicated in the parentheses.**

<b>Fresh animal specimens</b>		
<b>Ephemeroptera (mayflies)</b>		
<i>Leucrocuta jewetti</i>	EPH10-09	CO1, 28S rDNA
<i>Leucrocuta jewetti</i>	EPH17-09	CO1, 28S rDNA
<i>Maccaffertium modestum</i>	EPH05-09	CO1, 28S rDNA
<i>Maccaffertium modestum</i>	EPH14-09	CO1, 28S rDNA
<b>Trichoptera (caddisflies)</b>		
<i>Ceratopsyche bronta</i>	TRI13-09	CO1, 28S rDNA
<i>Ceratopsyche bronta</i>	TRI19-09	CO1, 28S rDNA
<i>Ceratopsyche sparna</i>	TRI04-09	CO1, 28S rDNA
<i>Ceratopsyche sparna</i>	TRI23-09	CO1, 28S rDNA
<i>Ceratopsyche</i> Spp	TRI08-09	CO1
<i>Ceratopsyche</i> Spp	TRI20-09	CO1, 28S rDNA
<i>Cheumatopsyche campyla</i>	TRI12-09	CO1, 28S rDNA
<i>Cheumatopsyche campyla</i>	TRI24-09	CO1, 28S rDNA
<i>Cheumatopsyche</i> Spp	TRI01-09	CO1, 28S rDNA
<i>Cheumatopsyche</i> Spp	TRI09-09	CO1, 28S rDNA
<i>Cheumatopsyche</i> Spp	TRI16-09	CO1, 28S rDNA
<i>Cheumatopsyche</i> Spp	TRI18-09	CO1, 28S rDNA
<i>Chimarra obscura</i>	TRI07-09	CO1, 28S rDNA
<i>Chimarra obscura</i>	TRI11-09	CO1, 28S rDNA
<i>Chimarra obscura</i>	TRI21-09	CO1, 28S rDNA
<i>Chimarra</i> Spp	TRI03-09	CO1, 28S rDNA
<i>Chimarra</i> Spp	TRI25-09	CO1, 28S rDNA
<i>Polycentropus cinereus</i>	TRI15-09	CO1, 28S rDNA
<i>Protophila maculata</i>	TRI06-09	CO1, 28S rDNA
<i>Psychomyia flavida</i>	TRI02-09	CO1, 28S rDNA
<i>Psychomyia flavida</i>	TRI22-09	CO1, 28S rDNA
<b>Older animal specimens</b>		
<i>Glassiphonia</i> spp. (10 years)	PAAN3	CO1
<i>Onisimus litoralis</i> (10 years)	PLAM17	CO1
<i>Cicada centipede</i> (7 years)	MARTH7	CO1
<i>Lepeophtheirus salmanis</i> (8 years)	PACO2	CO1
Buthidae spp. (7 years)	MARTH3	CO1
<i>Onisimus litoralis</i> (9 years)	PLAM10	CO1
<i>Sylon hippolytes</i> (7 years)	PABA2	CO1
<i>rbCL</i> : large subunit of the ribulose-bisphosphate carboxylase; <i>CO1</i> : cytochrome c oxidase 1; 28S rDNA: large subunit of nuclear ribosomal DNA		

**Supplementary Table S2. Primers used for PCR amplification of various genes from different specimens**

Sample	Gene	Primer code and sequence (5'-3')	Reference
Mescal liquid	<i>COI</i> (130-base, mini-barcode)	LepF-ATTCAACCAATCATAAAGATATTGG UnivR-GAAAATCATAATGAAGGCATGAGC	5, 7
Plants	<i>rbcL</i>	rbcLaF-ATGTCACCACAAACAGAGACTAAAGC rbcLaR-GTAAAATCAAGTCCACCRG	9
Caterpillar in mescal and other animals	<i>COI</i> (658 base, barcode)	LC01490-GGTCAACAAATCATAAAGATATTGG HCO2198-TAAACTTCAGGGTGACCAAAAATCA	6
	28S rDNA	Mo6-CCCCCTGAATTTAAGCATAT D3B-TCGGAAGGAACCAGCTACTA	8

**Supplementary Table S3. PCR amplification regime used for each gene**

Gene	Initial denaturation	Repeat 35 cycles			Final extension
		Denaturation	Annealing	Extension	
<i>COI</i>	95°C / 2 min	94°C / 30 s	46°C / 60 s	72°C / 30 s	72°C / 5 min
28S rDNA			58°C / 45 s	72°C / 90 s	
<i>rbcL</i>			55°C / 30 s	72°C / 60 s	