ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Critical factors for assembling a high volume of DNA barcodes

by

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APPENDIX 1.

SPECIMENS AND PROTOCOLS USED IN THE EVALUATION OF DNA ISOLATION METHODS AND DIFFERENT POLYMERASES

A. SPECIMENS

A set of 90 samples from moth, bird and fish taxa were chosen for use in the DNA extraction method evaluation. For moths, two sets of samples were selected: a recent set consisting of samples collected 2004 and a set of archive samples that represent year-classes from 2003 to 1932. Previously frozen, ethanol fixed tissue from birds and fish was divided into six equal pieces (approximately 1-2 mm^3). One leg from each moth specimen was divided into eight equal pieces (approximately 2-4 mm). Excess tissue was reserved for additional analyses if required.

Recent Moth Plate

	Process ID	Voucher ID	Collection Year	Identification	Source
1	LCH205-04	04HBL003205	2004	Hyles lineata	Hebert P.D.N.- U.G.
2	LOT001-04	04HBL002001	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
3	LOT002-04	04HBL002002	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
4	LOT003-04	04HBL002003	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
5	LOT004-04	04HBL002004	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
6	LOT005-04	04HBL002005	2004	Darapsa pholus	Hebert P.D.N.- U.G.
7	LOT006-04	04HBL002006	2004	Darapsa pholus	Hebert P.D.N.- U.G.
8	LOT007-04	04HBL002007	2004	Darapsa pholus	Hebert P.D.N.- U.G.
9	LOT009-04	04HBL002009	2004	Lapara bombycoides	Hebert P.D.N.- U.G.
10	LOT010-04	04HBL002010	2004	Lapara bombycoides	Hebert P.D.N.- U.G.
11	LOT011-04	04HBL002011	2004	Lapara bombycoides	Hebert P.D.N.- U.G.
12	LOT012-04	04HBL002012	2004	Lapara bombycoides	Hebert P.D.N.- U.G.
13	LOT013-04	04HBL002013	2004	Eumorpha pandorus	Hebert P.D.N.- U.G.
14	LOT014-04	04HBL002014	2004	Eumorpha pandorus	Hebert P.D.N.- U.G.
15	LOT015-04	04HBL002015	2004	Sphinx chersis	Hebert P.D.N.- U.G.
16	Blank1				
17	LOT016-04	04HBL002016	2004	Cressonia juglandis	Hebert P.D.N.- U.G.
18	LOT017-04	04HBL002017	2004	Cressonia juglandis	Hebert P.D.N.- U.G.
19	LOT018-04	04HBL002018	2004	Cressonia juglandis	Hebert P.D.N.- U.G.
20	LOT020-04	04HBL002020	2004	Cressonia juglandis	Hebert P.D.N.- U.G.
21	LOT025-04	04HBL002025	2004	Paonias excaecatus	Hebert P.D.N.- U.G.
22	LOT026-04	04HBL002026	2004	Paonias excaecatus	Hebert P.D.N.- U.G.
23	LOT028-04	04HBL002028	2004	Paonias excaecatus	Hebert P.D.N.- U.G.
24	LOT029-04	04HBL002029	2004	Paonis astylus	Hebert P.D.N.- U.G.
25	LOT030-04	04HBL002030	2004	Paonis astylus	Hebert P.D.N.- U.G.
26	PHMNB091-04	04HBL00537	2004	Paonias excaecatus	Hebert P.D.N.- U.G.
27	PHMNB092-04	04HBL00538	2004	Sphinx kalmiae	Hebert P.D.N.- U.G.
28	PHMNB093-04	04HBL00539	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
29	PHMNB094-04	04HBL00540	2004	Smerinthus jamaicensis	Hebert P.D.N.- U.G.
30	XAB057-04	04HBL005057	2004	Smerinthus cerisyi	Hebert P.D.N.- U.G.
31	XAB058-04	04HBL005058	2004	Smerinthus jamaicensis	Hebert P.D.N.- U.G.
32	Blank2				
33	XAB062-04	04HBL005062	2004	Ceratomia undulosa	Hebert P.D.N.- U.G.
34	XAB128-04	04HBL005128	2004	Paonias myops	Hebert P.D.N.- U.G.
35	XAB158-04	04HBL005158	2004	Smerinthus jamaicensis	Hebert P.D.N.- U.G.
36	XAB182-04	04HBL005182	2004	Paonias excaecatus	Hebert P.D.N.- U.G.
37	XAB210-04	04HBL005210	2004	Paonias myops	Hebert P.D.N.- U.G.
38	XAB336-04	04HBL005336	2004	Paonias myops	Hebert P.D.N.- U.G.
39	XAB337-04	04HBL005337	2004	Deidamia inscripta	Hebert P.D.N.- U.G.
40	XAB561-04	04HBL005561	2004	Paonias myops	Hebert P.D.N.- U.G.
41	LGSM095-04	DNA-ATBI-0095	2004	Manduca jasminearum	Hebert P.D.N.- U.G.
42	LGSM098-04	DNA-ATBI-0098	2004	Paonias astylus	Hebert P.D.N.- U.G.

43	LGSM337-04	DNA-ATBI-0337	2004	Darapsa myron	Hebert P.D.N.- U.G.
44	LGSM338-04	DNA-ATBI-0338	2004	Manduca jasminearum	Hebert P.D.N.- U.G.
45	LGSM340-04	DNA-ATBI-0340	2004	Dolba hyloeus	Hebert P.D.N.- U.G.
46	LGSM341-04	DNA-ATBI-0341	2004	Sphinx eremitus	Hebert P.D.N.- U.G.
47	LGSM342-04	DNA-ATBI-0342	2004	Darapsa pholus	Hebert P.D.N.- U.G.
48	Blank3				
49	MHASC003-05	04-SRNP-14623	2004	Xylophanes anubus	Janzen D.H. - U.P.
50	MHASC004-05	04-SRNP-14138	2004	Adhemarius gannascus	Janzen D.H. - U.P.
51	MHASC005-05	04-SRNP-14175	2004	Pachylia syces	Janzen D.H. - U.P.
52	MHASC006-05	04-SRNP-22447	2004	Xylophanes pistacina	Janzen D.H. - U.P.
53	MHASC007-05	04-SRNP-15014	2004	Perigonia ilus	Janzen D.H. - U.P.
54	MHASC008-05	04-SRNP-14620	2004	Xylophanes anubus	Janzen D.H. - U.P.
55	MHASC009-05	04-SRNP-14214	2004	Perigonia ilus	Janzen D.H. - U.P.
56	MHASC010-05	04-SRNP-14216	2004	Nyceryx coffaeae	Janzen D.H. - U.P.
57	MHASC011-05	04-SRNP-14436	2004	Nyceryx coffaeae	Janzen D.H. - U.P.
58	MHASC012-05	04-SRNP-35658	2004	Nyceryx exima	Janzen D.H. - U.P.
59	MHASC013-05	04-SRNP-34629	2004	Enyo ocypete	Janzen D.H. - U.P.
60	MHASC014-05	04-SRNP-55066	2004	Callionima denticulata	Janzen D.H. - U.P.
61	MHASC015-05	04-SRNP-47768	2004	Callionima falcifera	Janzen D.H. - U.P.
62	MHASC016-05	04-SRNP-47993	2004	Callionima falcifera	Janzen D.H. - U.P.
63	MHASC017-05	04-SRNP-33613	2004	Xylophanes adalia	Janzen D.H. - U.P.
64	Blank4				
65	MHASC018-05	04-SRNP-33169	2004	Xylophanes porcus	Janzen D.H. - U.P.
66	MHASC019-05	04-SRNP-33097	2004	Xylophanes porcus	Janzen D.H. - U.P.
67	MHASC020-05	04-SRNP-12786	2004	Manduca florestan	Janzen D.H. - U.P.
68	MHASC021-05	04-SRNP-12935	2004	Manduca florestan	Janzen D.H. - U.P.
69	MHASC022-05	04-SRNP-12361	2004	Manduca barnesi	Janzen D.H. - U.P.
70	MHASC023-05	04-SRNP-12358	2004	Manduca barnesi	Janzen D.H. - U.P.
71	MHASC024-05	04-SRNP-12364	2004	Manduca barnesi	Janzen D.H. - U.P.
72	MHASC025-05	04-SRNP-45794	2004	Manduca occulta	Janzen D.H. - U.P.
73	MHASC026-05	04-SRNP-46778	2004	Manduca occulta	Janzen D.H. - U.P.
74	MHASC027-05	04-SRNP-46938	2004	Manduca occulta	Janzen D.H. - U.P.
75	MHASC028-05	04-SRNP-46860	2004	Manduca occulta	Janzen D.H. - U.P.
76	MHASC029-05	04-SRNP-47139	2004	Manduca corallina	Janzen D.H. - U.P.
77	MHASC030-05	04-SRNP-22760	2004	Manduca corallina	Janzen D.H. - U.P.
78	MHASC031-05	04-SRNP-2725	2004	Adhemarius ypsilon	Janzen D.H. - U.P.
79	MHASC032-05	04-SRNP-22185	2004	Xylophanes tyndarus	Janzen D.H. - U.P.
80	Blank5				
81	MHASC033-05	04-SRNP-23883	2004	Erinnyis ello	Janzen D.H. - U.P.
82	MHASC034-05	04-SRNP-22932	2004	Neococytius cluentius	Janzen D.H. - U.P.
83	MHASC035-05	04-SRNP-33124	2004	Oryba kadeni	Janzen D.H. - U.P.
84	MHASC044-05	04-SRNP-30968	2004	Xylophanes zurcheri	Janzen D.H. - U.P.
85	MHASC046-05	04-SRNP-2515	2004	Eumorpha triangulum	Janzen D.H. - U.P.
86	MHASC047-05	04-SRNP-33639	2004	Xylophanes adalia	Janzen D.H. - U.P.
87	MHASC048-05	04-SRNP-22728	2004	Oryba kadeni	Janzen D.H. - U.P.
88	MHACG024-04	04-SRNP-2138	2004	Eumorpha fasciatus	Janzen D.H. - U.P.
89	MHACG056-04	04-SRNP-22910	2004	Pachylia syces	Janzen D.H. - U.P.
90	MHACG064-04	04-SRNP-22911	2004	Pachylia syces	Janzen D.H. - U.P.
91	MHACG072-04	04-SRNP-33098	2004	Xylophanes guianensis	Janzen D.H. - U.P.
92	MHACG080-04	04-SRNP-31296	2004	Cocytius lucifer	Janzen D.H. - U.P.
93	XAA888-04	04-SRNP-2481	2004	Manduca hannibal	Janzen D.H. - U.P.
94	XAA911-04	04-SRNP-1909	2004	Xylophanes zurcheri	Janzen D.H. - U.P.
95	XAA919-04	04-SRNP-2147	2004	Xylophanes chiron	Janzen D.H. - U.P.
96	Blank6				

Archival Moth Plate

	Process ID	Specimen Voucher ID	Collection Year	Identification	Source
1	MHACG001-04	03-SRNP-23540	2003	Eumorpha triangulum	Janzen D.H. - U.P.
2	MHACG002-04	03-SRNP-5921	2003	Cocytius lucifer	Janzen D.H. - U.P.
3	MHACG003-04	03-SRNP-8401	2003	Cocytius duponchel	Janzen D.H. - U.P.
4	MHACG009-04	03-SRNP-23544	2003	Eumorpha triangulum	Janzen D.H. - U.P.
5	MHACG010-04	03-SRNP-16922	2003	Cocytius lucifer	Janzen D.H. - U.P.
6	MHACG017-04	03-SRNP-23543	2003	Eumorpha triangulum	Janzen D.H. - U.P.
7	MHACG025-04	03-SRNP-23545	2003	Eumorpha triangulum	Janzen D.H. - U.P.
8	MHACG033-04	03-SRNP-23787	2003	Erinnyis ello	Janzen D.H. - U.P.
9	MHACG062-04	03-SRNP-23551	2003	Eumorpha triangulum	Janzen D.H. - U.P.

10	MHACG070-04	03-SRNP-23760	2003	Eumorpha triangulum	Janzen D.H. - U.P.
11	MHACG074-04	03-SRNP-23542	2003	Eumorpha triangulum	Janzen D.H. - U.P.
12	MHACG082-04*	03-SRNP-23806	2003	Eumorpha triangulum	Janzen D.H. - U.P.
13	MHACG131-04	03-SRNP-10100	2003	Eumorpha anchemola	Janzen D.H. - U.P.
14	MHACG139-04	03-SRNP-10312	2003	Eumorpha anchemola	Janzen D.H. - U.P.
15	MHACG163-04	03-SRNP-10313	2003	Eumorpha anchemola	Janzen D.H. - U.P.
16	Blank1				
17	MHASC051-05	01-SRNP-11808	2001	Xylophanes libya	Janzen D.H. - U.P.
18	MHASC056-05	01-SRNP-11436	2001	Xylophanes libya	Janzen D.H. - U.P.
19	MHASC057-05	01-SRNP-10540	2001	Xylophanes libya	Janzen D.H. - U.P.
20	MHASC068-05	01-SRNP-9831	2001	Xylophanes pluto	Janzen D.H. - U.P.
21	MHASC069-05	01-SRNP-1136	2001	Xylophanes zurcheri	Janzen D.H. - U.P.
22	MHACG081-04	01-SRNP-25117	2001	Cocytius antaeus	Janzen D.H. - U.P.
23	MHACG060-04	01-SRNP-11692	2001	Cocytius duponchel	Janzen D.H. - U.P.
24	MHACG015-04	01-SRNP-10483	2001	Eumorpha megaeacus	Janzen D.H. - U.P.
25	MHACG079-04	01-SRNP-3718	2001	Eumorpha megaeacus	Janzen D.H. - U.P.
26	MHACG071-04	01-SRNP-22175	2001	Eumorpha megaeacus	Janzen D.H. - U.P.
27	MHACG087-04	01-SRNP-3719	2001	Eumorpha megaeacus	Janzen D.H. - U.P.
28	MHACG180-04*	01-SRNP-10480	2001	Eumorpha fasciatus	Janzen D.H. - U.P.
29	MHACG123-04	01-SRNP-21086	2001	Eumorpha anchemola	Janzen D.H. - U.P.
30	MHACG395-04	01-SRNP-4561	2001	Neococytius cluentius	Janzen D.H. - U.P.
31	MHACG496-04	01-SRNP-14689	2001	Pachylia syces	Janzen D.H. - U.P.
32	Blank2				
33	MHASC063-05	97-SRNP-2411	1997	Xylophanes libya	Janzen D.H. - U.P.
34	JSPC299-03	97-SRNP-1511	1997	Xylophanes chiron	Janzen D.H. - U.P.
35	JSPC310-03	97-SRNP-1872	1997	Xylophanes cyrene	Janzen D.H. - U.P.
36	JSPC370-03*	97-SRNP-2400	1997	Xylophanes tersa	Janzen D.H. - U.P.
37	JSPC599-03	97-SRNP-1122	1997	Xylophanes anubus	Janzen D.H. - U.P.
38	JSPC601-03	97-SRNP-1879	1997	Xylophanes cyrene	Janzen D.H. - U.P.
39	JSPC677-03	97-SRNP-1538	1997	Adhemarius ypsilon	Janzen D.H. - U.P.
40	MHACG183-04	97-SRNP-4422	1997	Eumorpha vitis	Janzen D.H. - U.P.
41	JSPC678-03	97-SRNP-4724	1997	Adhemarius ypsilon	Janzen D.H. - U.P.
42	MHACG075-04	97-SRNP-1630	1997	Cocytius lucifer	Janzen D.H. - U.P.
43	MHACG036-04	97-SRNP-4293	1997	Cocytius duponchel	Janzen D.H. - U.P.
44	MHACG006-04	97-SRNP-9832	1997	Cocytius antaeus	Janzen D.H. - U.P.
45	MHACG188-04	97-SRNP-9753	1997	Eumorpha fasciatus	Janzen D.H. - U.P.
46	MHACG107-04	97-SRNP-9831	1997	Eumorpha fasciatus	Janzen D.H. - U.P.
47	MHACG099-04	97-SRNP-9752	1997	Eumorpha fasciatus	Janzen D.H. - U.P.
48	Blank3				
49	MHASC088-05	89-SRNP-545	1989	Eumorpha satellitia	Janzen D.H. - U.P.
50	MHASC089-05	89-SRNP-380	1989	Eumorpha satellitia	Janzen D.H. - U.P.
51	MHASC092-05	89-SRNP-827	1989	Eumorpha satellitia	Janzen D.H. - U.P.
52	MHACG157-04	89-SRNP-163C	1989	Eumorpha satellitia	Janzen D.H. - U.P.
53	MHACG134-04	89-SRNP-304	1989	Erinnyis oenotrus	Janzen D.H. - U.P.
54	MHACG165-04	89-SRNP-658	1989	Eumorpha satellitia	Janzen D.H. - U.P.
55	MHACG149-04	89-SRNP-528	1989	Eumorpha satellitia	Janzen D.H. - U.P.
56	MHACG141-04	89-SRNP-339	1989	Eumorpha satellitia	Janzen D.H. - U.P.
57	MHACG329-04	89-SRNP-192	1989	Pachylioides resumens	Janzen D.H. - U.P.
58	MHACG337-04	89-SRNP-191	1989	Pachylioides resumens	Janzen D.H. - U.P.
59	MHACG488-04	89-SRNP-345	1989	Pachylia ficus	Janzen D.H. - U.P.
60	JSPC165-03*	89-SRNP-501	1989	Manduca florestan	Janzen D.H. - U.P.
61	MHACG102-04	88-SRNP-63	1988	Erinnyis oenotrus	Janzen D.H. - U.P.
62	MHACG132-04	88-SRNP-101	1988	Erinnyis crameri	Janzen D.H. - U.P.
63	MHACG124-04	88-SRNP-89	1988	Erinnyis crameri	Janzen D.H. - U.P.
64	Blank4				
65	PHPNG1974-1	PHPNG1974-1	1974	Sphingidae sp. 1	Hebert P.D.N.- U.G.
66	PHPNG1974-2	PHPNG1974-2	1974	Sphingidae sp. 1	Hebert P.D.N.- U.G.
67	PHPNG1974-3	PHPNG1974-3	1974	Sphingidae sp. 1	Hebert P.D.N.- U.G.
68	PHPNG1974-4	PHPNG1974-4	1974	Sphingidae sp. 1	Hebert P.D.N.- U.G.
69	PHPNG1974-5	PHPNG1974-5	1974	Sphingidae sp. 2	Hebert P.D.N.- U.G.
70	PHPNG1974-6	PHPNG1974-6	1974	Sphingidae sp. 2	Hebert P.D.N.- U.G.
71	PHPNG1974-7	PHPNG1974-7	1974	Sphingidae sp. 2	Hebert P.D.N.- U.G.
72	PHPNG1974-8	PHPNG1974-8	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
73	PHPNG1974-9	PHPNG1974-9	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
74	PHPNG1974-10	PHPNG1974-10	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
75	PHPNG1974-11	PHPNG1974-11	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
76	PHPNG1974-12*	PHPNG1974-12	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
77	PHPNG1974-13	PHPNG1974-13	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
78	PHPNG1974-14	PHPNG1974-14	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.
79	PHPNG1974-15	PHPNG1974-15	1974	Sphingidae sp. 3	Hebert P.D.N.- U.G.

80	Blank5				
81	Miller1	196189	1932	Cocytius duponchel	Miller S.E. - S.I.
82	Miller2	196188	1933	Cocytius duponchel	Miller S.E. - S.I.
83	Miller3	196187	1933	Cocytius antaeus	Miller S.E. - S.I.
84	Miller4	196203	1932	Pachylia ficus	Miller S.E. - S.I.
85	Miller5	196202	1932	Pachylia syces	Miller S.E. - S.I.
86	Miller6	196205	1932	Pachylia ficus	Miller S.E. - S.I.
87	Miller7	196201	1932	Pachylia syces	Miller S.E. - S.I.
88	Miller197	196197	1933	Aleuron chloroptera	Miller S.E. - S.I.
89	Miller195	196195	1932	Eupyrrhoglossum sagra	Miller S.E. - S.I.
90	Miller10	196196	1933	Aleuron iphis	Miller S.E. - S.I.
91	Miller11	196193	1933	Enyo ocypete	Miller S.E. - S.I.
92	Miller12*	196192	1932	Enyo ocypete	Miller S.E. - S.I.
93	Miller198	196198	1932	Aleuron chloroptera	Miller S.E. - S.I.
94	Miller16	196206	1932	Pachylioides resumens	Miller S.E. - S.I.
95	Miller17	196191	1932	Eumorpha vitis	Miller S.E. - S.I.
96	Blank6				

Bird Plate

	Process ID	Voucher ID	Collection Year	Identification	Source
1	KKBNA307-05	UWBM 57128	1994	Poecile gambeli	Birks S. - B.M.N.H.C.
2	KKBNA363-05	UWBM 69161	1996	Chloroceryle americana	Birks S. - B.M.N.H.C.
3	KKBNA351-05	UWBM 68994	1996	Myiarchus tuberculifer	Birks S. - B.M.N.H.C.
4	KKBNA178-05	USNM 613020	1989	Actitis hypoleucos	Weigt L. - S.I.
5	KKBNA421-05	UWBM 73909	2002	Molothrus aeneus	Birks S. - B.M.N.H.C.
6	KKBNA400-05	UWBM 66147	2000	Dendroica coronata	Birks S. - B.M.N.H.C.
7	KKBNA375-05	UWBM 58525	1997	Sphyrapicus nuchalis	Birks S. - B.M.N.H.C.
8	KKBNA294-05	UWBM 46768	1993	Cinclus mexicanus	Birks S. - B.M.N.H.C.
9	KKBNA318-05	UWBM 54048	1995	Empidonax hammondii	Birks S. - B.M.N.H.C.
10	KKBNA364-05	UWBM 69173	1996	Phalacrocorax brasilianus	Birks S. - B.M.N.H.C.
11	KKBNA352-05	UWBM 68995	1996	Vireo flavoviridis	Birks S. - B.M.N.H.C.
12	KKBNA337-05	UWBM 55986	1996	Sporophila torqueola	Birks S. - B.M.N.H.C.
13	KKBNA422-05	UWBM 73910	2002	Molothrus aeneus	Birks S. - B.M.N.H.C.
14	KKBNA401-05	UWBM 66154	2000	Poecile gambeli	Birks S. - B.M.N.H.C.
15	KKBNA179-05	USNM 621130	1996	Aeronautes saxatalis	Weigt L. - S.I.
16	Blank1				
17	KKBNA295-05	UWBM 46758	1993	Cinclus mexicanus	Birks S. - B.M.N.H.C.
18	KKBNA181-05	PSM 21816	1991	Anous stolidus	Weigt L. - S.I.
19	KKBNA365-05	UWBM 69181	1996	Ceryle torquata	Birks S. - B.M.N.H.C.
20	KKBNA353-05	UWBM 69009	1996	Contopus sordidulus	Birks S. - B.M.N.H.C.
21	KKBNA338-05	UWBM 69977	1996	Protonotaria citrea	Birks S. - B.M.N.H.C.
22	KKBNA180-05	USNM 586114	1996	Anas cyanoptera	Weigt L. - S.I.
23	KKBNA402-05	UWBM 66155	2000	Poecile gambeli	Birks S. - B.M.N.H.C.
24	KKBNA377-05	UWBM 72507	1997	Fulica americana	Birks S. - B.M.N.H.C.
25	KKBNA308-05	UWBM 49891	1994	Geothlypis trichas	Birks S. - B.M.N.H.C.
26	KKBNA183-05	USNM 621306	1991	Campylorhynchus brunneicapillus	Weigt L. - S.I.
27	KKBNA366-05	UWBM 69185	1996	Ceryle torquata	Birks S. - B.M.N.H.C.
28	KKBNA354-05	UWBM 69017	1996	Columbina inca	Birks S. - B.M.N.H.C.
29	KKBNA182-05	USNM 620711	1994	Calonectris diomedea	Weigt L. - S.I.
30	KKBNA339-05	UWBM 69981	1996	Dendroica graciae	Birks S. - B.M.N.H.C.
31	KKBNA372-05	UWBM 68293	n/a	Somateria fischeri	Birks S. - B.M.N.H.C.
32	Blank2				
33	KKBNA378-05	UWBM 58109	1997	Megascops kennicottii	Birks S. - B.M.N.H.C.
34	KKBNA309-05	UWBM 49908	1994	Dendroica occidentalis	Birks S. - B.M.N.H.C.
35	KKBNA321-05	UWBM 54091	1995	Euphagus cyanocephalus	Birks S. - B.M.N.H.C.
36	KKBNA367-05	UWBM 69439	1996	Protonotaria citrea	Birks S. - B.M.N.H.C.
37	KKBNA355-05	UWBM 69277	1996	Camptostoma imberbe	Birks S. - B.M.N.H.C.
38	KKBNA273-05	UWBM 43871	1992	Lagopus mutus	Birks S. - B.M.N.H.C.
39	KKBNA427-05	UWBM 76086	2003	Sitta pygmaea	Birks S. - B.M.N.H.C.
40	KKBNA373-05	UWBM 68294	n/a	Somateria fischeri	Birks S. - B.M.N.H.C.
41	KKBNA184-05	USNM 623291	1995	Cepphus grylle	Weigt L. - S.I.
42	KKBNA317-05	UWBM 51070	1994	Motacilla alba	Birks S. - B.M.N.H.C.
43	KKBNA322-05	UWBM 54092	1995	Euphagus cyanocephalus	Birks S. - B.M.N.H.C.
44	KKBNA368-05	UWBM 69194	1996	Protonotaria citrea	Birks S. - B.M.N.H.C.
45	KKBNA356-05	UWBM 69034	1996	Myiarchus tuberculifer	Birks S. - B.M.N.H.C.
46	KKBNA340-05	UWBM 56045	1996	Quiscalus mexicanus	Birks S. - B.M.N.H.C.

47	KKBNA425-05	UWBM 76075	2001	Polioptila melanura	Birks S. - B.M.N.H.C.
48	Blank3				
49	KKBNA374-05	UWBM 68295	n/a	Somateria fischeri	Birks S. - B.M.N.H.C.
50	KKBNA387-05	UWBM 64438	1999	Stellula calliope	Birks S. - B.M.N.H.C.
51	KKBNA329-05	UWBM 53466	1995	Empidonax difficilis	Birks S. - B.M.N.H.C.
52	KKBNA323-05	UWBM 53997	1995	Poecile rufescens	Birks S. - B.M.N.H.C.
53	KKBNA369-05	UWBM 69443	1996	Camptostoma imberbe	Birks S. - B.M.N.H.C.
54	KKBNA357-05	UWBM 69287	1996	Vireo flavoviridis	Birks S. - B.M.N.H.C.
55	KKBNA341-05	UWBM 56079	1996	Chloroceryle americana	Birks S. - B.M.N.H.C.
56	KKBNA186-05	NRM 946516	2001	Charadrius morinellus	Weigt L. - S.I.
57	KKBNA185-05	USNM 600036	1988	Chamaea fasciata	Weigt L. - S.I.
58	KKBNA388-05	UWBM 64439	1999	Stellula calliope	Birks S. - B.M.N.H.C.
59	KKBNA330-05	UWBM 54879	1995	Synthliboramphus hypoleucus	Birks S. - B.M.N.H.C.
60	KKBNA324-05	UWBM 53998	1995	Poecile rufescens	Birks S. - B.M.N.H.C.
61	KKBNA187-05	USNM 609133	2001	Chloroceryle americana	Weigt L. - S.I.
62	KKBNA358-05	UWBM 69314	1996	Tyrannus melancholicus	Birks S. - B.M.N.H.C.
63	KKBNA342-05	UWBM 56092	1996	Myiodynastes luteiventris	Birks S. - B.M.N.H.C.
64	Blank4				
65	KKBNA243-05	UWBM 48399	1998	Molothrus aeneus	Birks S. - B.M.N.H.C.
66	KKBNA393-05	UWBM 68190	2000	Thryomanes bewickii	Birks S. - B.M.N.H.C.
67	KKBNA383-05	UWBM 65261	1997	Columbina passerina	Birks S. - B.M.N.H.C.
68	KKBNA333-05	UWBM 57001	1995	Gavia adamsii	Birks S. - B.M.N.H.C.
69	KKBNA325-05	UWBM 53832	1995	Dendroica townsendi	Birks S. - B.M.N.H.C.
70	KKBNA371-05	UWBM 69205	1996	Columbina inca	Birks S. - B.M.N.H.C.
71	KKBNA359-05	UWBM 69066	1996	Chloroceryle americana	Birks S. - B.M.N.H.C.
72	KKBNA343-05	UWBM 56153	1996	Quiscalus mexicanus	Birks S. - B.M.N.H.C.
73	KKBNA244-05	UWBM 72455	1988	Quiscalus mexicanus	Birks S. - B.M.N.H.C.
74	KKBNA416-05	UWBM 68270	2001	Chaetura vauxi	Birks S. - B.M.N.H.C.
75	KKBNA188-05	USNM 630613	1994	Cinclus mexicanus	Weigt L. - S.I.
76	KKBNA334-05	UWBM 57002	1995	Gavia adamsii	Birks S. - B.M.N.H.C.
77	KKBNA326-05	UWBM 53834	1995	Dendroica townsendi	Birks S. - B.M.N.H.C.
78	KKBNA304-05	UWBM 51054	1994	Salpinctes obsoletus	Birks S. - B.M.N.H.C.
79	KKBNA360-05	UWBM 69076	1996	Columbina inca	Birks S. - B.M.N.H.C.
80	Blank5				
81	KKBNA344-05	UWBM 56191	1996	Columbina passerina	Birks S. - B.M.N.H.C.
82	KKBNA245-05	UWBM 72456	1988	Quiscalus mexicanus	Birks S. - B.M.N.H.C.
83	KKBNA417-05	UWBM 68269	2001	Chaetura vauxi	Birks S. - B.M.N.H.C.
84	KKBNA189-05	USNM 627152	1994	Coccyzus minor	Weigt L. - S.I.
85	KKBNA335-05	UWBM 57003	1995	Gavia adamsii	Birks S. - B.M.N.H.C.
86	KKBNA327-05	UWBM 54000	1995	Passerella iliaca	Birks S. - B.M.N.H.C.
87	KKBNA305-05	UWBM 51058	1994	Picoides albolarvatus	Birks S. - B.M.N.H.C.
88	KKBNA361-05	UWBM 69078	1996	Vireo flavoviridis	Birks S. - B.M.N.H.C.
89	KKBNA345-05	UWBM 56192	1996	Columbina passerina	Birks S. - B.M.N.H.C.
90	KKBNA301-05	UWBM 57374	1993	Passerina versicolor	Birks S. - B.M.N.H.C.
91	KKBNA418-05	UWBM 68268	2001	Sphyrapicus ruber	Birks S. - B.M.N.H.C.
92	KKBNA190-05	USNM 607905	2001	Coereba flaveola	Weigt L. - S.I.
93	KKBNA336-05	UWBM 57004	1995	Gavia adamsii	Birks S. - B.M.N.H.C.
94	KKBNA192-05	USNM 621178	1990	Contopus sordidulus	Weigt L. - S.I.
95	KKBNA191-05	USNM 626354	2001	Columbina passerina	Weigt L. - S.I.
96	Blank6				

Fish Plate

	Process ID	Voucher ID	Collection Year	Identification	Source
1	FOA034-04	BW-A034	1996	Furgaleus macki	Ward R.D. - CSIRO
2	FOA035-04	BW-A035	1998	Furgaleus macki	Ward R.D. - CSIRO
3	FOA039-04	BW-A039	n/a	Galeorhinus galeus	Ward R.D. - CSIRO
4	FOA041-04	BW-A041	n/a	Galeorhinus galeus	Ward R.D. - CSIRO
5	FOA042-04	BW-A042	1995	Mustelus antarcticus	Ward R.D. - CSIRO
6	FOA043-04	BW-A043	1996	Mustelus antarcticus	Ward R.D. - CSIRO
7	FOA054-04	BW-A054	2001	Mustelus lenticulatus	Ward R.D. - CSIRO
8	FOA055-04	BW-A055	2001	Mustelus lenticulatus	Ward R.D. - CSIRO
9	FOA058-04	BW-A058	1995	Carcharhinus dussumieri	Ward R.D. - CSIRO
10	FOA059-04	BW-A059	1995	Carcharhinus dussumieri	Ward R.D. - CSIRO
11	FOA065-04	BW-A065	1996	Carcharhinus sorrah	Ward R.D. - CSIRO
12	FOA066-04	BW-A066	1996	Carcharhinus sorrah	Ward R.D. - CSIRO

13	FOA073-04	BW-A073	1996	Prionace glauca	Ward R.D. - CSIRO
14	FOA074-04	BW-A074	2000	Prionace glauca	Ward R.D. - CSIRO
15	FOA078-04	BW-A078	1997	Rhizoprionodon acutus	Ward R.D. - CSIRO
16	Blank1				
17	FOA079-04	BW-A079	1997	Rhizoprionodon acutus	Ward R.D. - CSIRO
18	FOA219-04	BW-A219	1996	Himantura uranakoides	Ward R.D. - CSIRO
19	FOA220-04	BW-A220	1996	Himantura uranakoides	Ward R.D. - CSIRO
20	FOA247-04	BW-A247	1994	Urolophus cruciatus	Ward R.D. - CSIRO
21	FOA249-04	BW-A249	1994	Urolophus cruciatus	Ward R.D. - CSIRO
22	FOA257-04	BW-A257	1997	Urolophus westraliensis	Ward R.D. - CSIRO
23	FOA259-04	BW-A259	1997	Urolophus westraliensis	Ward R.D. - CSIRO
24	FOA266-04	BW-A266	1994	Myliobatis australis	Ward R.D. - CSIRO
25	FOA267-04	BW-A267	1994	Myliobatis australis	Ward R.D. - CSIRO
26	FOA306-04	BW-A306	1996	Gephyroberyx darwinii	Ward R.D. - CSIRO
27	FOA307-04	BW-A307	1995	Gephyroberyx darwinii	Ward R.D. - CSIRO
28	FOA310-04	BW-A310	1998	Hoplostethus gigas	Ward R.D. - CSIRO
29	FOA311-04	BW-A311	1998	Hoplostethus gigas	Ward R.D. - CSIRO
30	FOA315-04	BW-A315	n/a	Hoplostethus latus	Ward R.D. - CSIRO
31	FOA317-04	BW-A317	n/a	Hoplostethus latus	Ward R.D. - CSIRO
32	Blank2				
33	FOA331-04	BW-A331	1994	Neocyttus rhomboidalis	Ward R.D. - CSIRO
34	FOA332-04	BW-A332	1994	Neocyttus rhomboidalis	Ward R.D. - CSIRO
35	FOA338-04	BW-A338	1999	Pseudocyttus maculatus	Ward R.D. - CSIRO
36	FOA339-04	BW-A339	1999	Pseudocyttus maculatus	Ward R.D. - CSIRO
37	FOA423-04	BW-A423	n/a	Neosebastes thetidis	Ward R.D. - CSIRO
38	FOA425-04	BW-A425	n/a	Neosebastes thetidis	Ward R.D. - CSIRO
39	FOA451-04	BW-A451	1996	Lepidotrigla mulhalli	Ward R.D. - CSIRO
40	FOA453-04	BW-A453	1996	Lepidotrigla mulhalli	Ward R.D. - CSIRO
41	FOA484-04	BW-A484	1995	Cymbacephalus nematophthalmus	Ward R.D. - CSIRO
42	FOA485-04	BW-A485	1995	Cymbacephalus nematophthalmus	Ward R.D. - CSIRO
43	FOA489-04	BW-A489	1995	Cymbacephalus parilis	Ward R.D. - CSIRO
44	FOA490-04	BW-A490	1997	Cymbacephalus parilis	Ward R.D. - CSIRO
45	FOA493-04	BW-A493	1995	Platycephalus arenarius	Ward R.D. - CSIRO
46	FOA495-04	BW-A495	1995	Platycephalus arenarius	Ward R.D. - CSIRO
47	FOA506-04	BW-A506	1995	Platycephalus endrachtensis	Ward R.D. - CSIRO
48	Blank3				
49	FOA507-04	BW-A507	1995	Platycephalus endrachtensis	Ward R.D. - CSIRO
50	FOA547-04	BW-A547	1995	Macquaria ambigua	Ward R.D. - CSIRO
51	FOA548-04	BW-A548	1995	Macquaria ambigua	Ward R.D. - CSIRO
52	FOA552-04	BW-A552	1995	Macquaria colonorum	Ward R.D. - CSIRO
53	FOA553-04	BW-A553	1995	Macquaria colonorum	Ward R.D. - CSIRO
54	FOA561-04	BW-A561	1998	Maccullochella peelii	Ward R.D. - CSIRO
55	FOA562-04	BW-A562	1998	Maccullochella peelii	Ward R.D. - CSIRO
56	FOA573-04	BW-A573	n/a	Cephalopholis miniata	Ward R.D. - CSIRO
57	FOA574-04	BW-A574	1999	Cephalopholis miniata	Ward R.D. - CSIRO
58	FOA576-04	BW-A576	1995	Cephalopholis sonnerati	Ward R.D. - CSIRO
59	FOA578-04	BW-A578	1995	Cephalopholis sonnerati	Ward R.D. - CSIRO
60	FOA592-04	BW-A592	1995	Plectropomus maculatus	Ward R.D. - CSIRO
61	FOA593-04	BW-A593	1995	Plectropomus maculatus	Ward R.D. - CSIRO
62	FOA597-04	BW-A597	1994	Polyprion oxygeneios	Ward R.D. - CSIRO
63	FOA598-04	BW-A598	1995	Polyprion oxygeneios	Ward R.D. - CSIRO
64	Blank4				
65	FOA602-04	BW-A602	1997	Cromileptes altivelis	Ward R.D. - CSIRO
66	FOA604-04	BW-A604	1998	Cromileptes altivelis	Ward R.D. - CSIRO
67	FOA606-04	BW-A606	1998	Caprodon longimanus	Ward R.D. - CSIRO
68	FOA607-04	BW-A607	1998	Caprodon longimanus	Ward R.D. - CSIRO
69	FOA609-04	BW-A609	1996	Epinephelus morrhua	Ward R.D. - CSIRO
70	FOA610-04	BW-A610	1998	Epinephelus morrhua	Ward R.D. - CSIRO
71	FOA615-04	BW-A615	1994	Epinephelus multinotatus	Ward R.D. - CSIRO
72	FOA617-04	BW-A617	1994	Epinephelus multinotatus	Ward R.D. - CSIRO
73	FOA859-04	BW-A859	1995	Scomberomorus semifasciatus	Ward R.D. - CSIRO
74	FOA860-04	BW-A860	1995	Scomberomorus semifasciatus	Ward R.D. - CSIRO
75	FOA864-04	BW-A864	1996	Thunnus alalunga	Ward R.D. - CSIRO
76	FOA865-04	BW-A865	1996	Thunnus alalunga	Ward R.D. - CSIRO
77	FOA869-04	BW-A869	1996	Thunnus albacares	Ward R.D. - CSIRO
78	FOA870-04	BW-A870	1996	Thunnus albacares	Ward R.D. - CSIRO
79	FOA890-04	BW-A890	1996	Xiphias gladius	Ward R.D. - CSIRO
80	Blank5				
81	FOA892-04	BW-A892	1996	Xiphias gladius	Ward R.D. - CSIRO
82	FOA899-04	BW-A899	1996	Makaira mazara	Ward R.D. - CSIRO

83	FOA900-04	BW-A900	n/a	Makaira mazara	Ward R.D. - CSIRO
84	FOA903-04	BW-A903	1996	Tetrapturus angustirostris	Ward R.D. - CSIRO
85	FOA904-04	BW-A904	1996	Tetrapturus angustirostris	Ward R.D. - CSIRO
86	FOA916-04	BW-A916	1994	Hyperoglyphe antarctica	Ward R.D. - CSIRO
87	FOA917-04	BW-A917	1994	Hyperoglyphe antarctica	Ward R.D. - CSIRO
88	FOA921-04	BW-A921	1999	Tetragonurus cuvieri	Ward R.D. - CSIRO
89	FOA922-04	BW-A922	2000	Tetragonurus cuvieri	Ward R.D. - CSIRO
90	FOA923-04	BW-A923	n/a	Cubiceps squamiceps	Ward R.D. - CSIRO
91	FOA924-04	BW-A924	n/a	Cubiceps squamiceps	Ward R.D. - CSIRO
92	FOA929-04	BW-A929	1995	Ariomma indica	Ward R.D. - CSIRO
93	FOA930-04	BW-A930	1995	Ariomma indica	Ward R.D. - CSIRO
94	FOA933-04	BW-A933	1996	Pampus argenteus	Ward R.D. - CSIRO
95	FOA934-04	BW-A934	2000	Pampus argenteus	Ward R.D. - CSIRO
96	Blank6				

* Additional negative controls for DNA polymerase evaluation; B.M.N.H.C= Burke Museum of Natural History and Culture; S.I.= Smithsonian Institution; U.G.=University of Guelph; U.P.=University of Pennsylvania; CSIRO=Common wealth Scientific and Industrial Research Organisation.

B. DNA ISOLATION

With the exception of homemade methods, DNA extraction protocols were completed following the manufacturers instructions. Protocols for Silitom and DryRealease (Appendix 2) are listed below.

Silitom DNA extraction

This protocol is based on the methods of Elphinstone et al. (2003) and Boom et al. (1990) with modifications to accommodate handling of small specimens.

1. A small amount of tissue (2-3 mm^3 or 2-4 mm of insect leg) was added into each well of a 96-well microplate (maximum capacity 250 µl) containing 60 µl of Lysis buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0, 0.5% SDS and 24 µg proteinase K. The plate was covered with 8-strip caps and incubated overnight at 55ºC.

2. The plate was mixed by rotation and centrifuged at 1500 g for 1 min to precipitate cellular debris.

3. The binding buffer (6 M guanidine thiocyanate, 20 mM EDTA pH 8.0, 10 mM Tris-HCl pH 6.4, 4% Triton X-100) was pre-warmed at 55ºC and mixed with an equal volume of 95% ethanol, and 120 µl of mix was added to each well. The plate was mixed and centrifuged at 1000 g for 1 min to precipitate cellular debris.

4. To prepare diatomaceous earth, 3 ml of water was added to a 15 ml conical tube. Approximately 1.5 g of diatomaceous earth (Sigma, D3877) was added to a tube and diluted with ddH_2O to a total volume of 15 ml. The tube was closed and shaken (to make MUD); particles were allowed to settle for 30 min – 3 hours. The upper liquid layer was removed; tube was refilled with ddH_2O and allowed to settle again for 30 min – 2 hours. After the second settling the liquid layer was aspirated (or poured off) and ddH_2O equal in volume to semisolid MUD was added to the tube.

5. 10 µl of diatomaceous earth suspension in water (MUD) was added to each well of a filtration plate (Multiscreen MAHVN4550, Millipore, MA, USA) and placed on top of a vacuum manifold.

6. Lysates and binding mix were transferred to the Multiscreen HV filtration plate, mixed with MUD by gentle pipetting and allowed 1 min for binding of DNA to MUD.Vacuum was applied to remove the binding buffer.

7. To remove binding buffer from MUD-DNA complex, 250 µl of ice-cold wash buffer (50% EtOH, 50 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, pH 8.0) was added to wells. Vacuum was applied to remove the wash buffer.

8. The wash step was repeated two additional times, to ensure all binding buffer was removed.

9. The plate was tapped on a clean Kimwipe to remove residual wash buffer, and placed back on the manifold under vacuum for 10-15 min to further remove residual wash buffer and dry the diatomaceous earth. Alternatively, the plate could be dried in an incubator preheated to 55ºC. This drying step is critical to avoid cross contamination between wells during the elution step.

10. The filtration plate was secured on top of a 96-well skirted PCR microplate using a Millipore collar, rubber bands and tape. 50 µl sterile ddH_2O water prewarmed to 56ºC, was added to each well and incubated for 5 min.

11. The plate was centrifuged at 3000 g for 10 min to elute the DNA and was sealed and stored at 4ºC.

C. PCR CONDITIONS USED IN THE EVALUATION OF DNA ISOLATION METHODS

Each PCR reaction had a total volume of 12.5 µl and contained 2.0 µl of template DNA, 5% trehalose (D-(+)-Trehalose dehydrate), 1.25 µl of 10x ThermoPol reaction buffer (New England Biolabs, Beverly, MA), 2.5 mM of $MgCl_2$, 1.25 pmol each of forward and reverse primer, 50 µM of dNTP, and 0.3125 U of *Taq*DNA polymerase (New England Biolabs, Beverly, MA). The thermocycle profile for moth samples consisted of 94ºC for 1 min, five cycles of 94ºC for 40 sec, 45ºC for 40 sec, and 72ºC for 1 min, followed by 35 cycles of 94ºC for 40 sec, 51ºC for 40 sec, and 72ºC for 1 min, with a final extension at 72ºC for 5 min. Fish and bird samples were amplified using a thermocycle profile consisting of 94ºC for 2 min, 35 cycles of 94ºC for 30 sec, 54ºC for 30 sec, and 72ºC for 1 min, with a final extension at 72ºC for 10 min.

D. PROTOCOLS FOR DNA POLYMERASE EVALUATION

Four DNA polymerase enzymes were evaluated using fresh and archived moth DNA isolated using the Nucleospin 96 column tissue kit (Machery Nagel Inc., Easton, PA, USA) (Appendix 2). The manufacturers' suggestions for optimal PCR conditions were followed for each enzyme. The amount of DNA used in each reaction was constant (2.0 µl). PCR product yield for each enzyme was evaluated with three primers sets; LepF/LepR and miniLepF1/LepR (see below). Listed below are brief descriptions of the enzymes and the PCR conditions used for each.

TaqDNA Polymerase (New England Biolabs, Beverly, MA, USA)

Each PCR reaction had a total volume of 25 µl and contained 2.0 µl of DNA, 2.5 µl of 10X ThermoPol reaction buffer, 2.5 mM of $MgCl_2$, 2.5 pmol each of forward and reverse primer, 50 µM of dNTP, and 0.625 U of *Taq*DNA polymerase. The thermocycle profile consisted of 94ºC, for 2 min followed by 35 cycles 94ºC for 40 sec, 51ºC for 40 sec, and 72ºC for 1 min, with a final extension at 72ºC for 5 min.

Restorase DNA Polymerase (Sigma-Aldrich, St. Louis, MO)

Each PCR contained 2.0 µl of DNA, 2.5 µl of 10X Restorase reaction buffer, 0.2 mM dNTP mix, and 1.25 U of Restorase enzyme. The cycling profile started with a preincubation period of 5 min at 4ºC, for DNA repair, 5 sec at 94ºC, and a pause at 75 º (10 pmol of forward and reverse primer were added to the PCR reaction at this step). Thermocycling was resumed for amplification for 30 cycles of 94ºC for 5 sec, 51ºC for 20 sec, 68ºC for 1 min, with a 1 min final extension at 68ºC.

AccuTaq LA DNA polymerase mix (Sigma-Aldrich, St. Louis, MO)

Each PCR had a total volume of 25 µl and contained 2.0 µl of template DNA, 2.5 µl of 10X AccuTaq LA buffer 0.5 mM dNTP mix, 10 pmol of forward and reverse primer, and 1.25 U of AccuTaq DNA polymerase. The thermocycle profile was 30 sec at 98ºC, followed by 30 cycles of 94ºC for 5 sec, 51ºC for 20 sec, 68ºC for 1 min, with a 1 min final extension at 68ºC.

Diamond DNA Polymerase (Bioline USA Inc., Randolph, MA)

Each PCR had a total volume of 25 µl and contained 2.0 µl of template DNA, 2.5 µl of 10X NH_4 buffer, 5 mM of $MgCl_2$, 2.5 pmol of forward and reverse primer, and 2.5 U of Diamond DNA polymerase. The cycling conditions were 94ºC for 1 min, followed by 35 cycles of 94ºC for 40 sec, 51ºC for 1 min, 72ºC for 2 min, followed by a 5 min final extension at 72ºC.

E. PRIMERS

Below is a list of primers used for amplification of a fragment of *cox1* gene, located in the 5' region, from moths, birds and fish. For moth samples forward primers were paired with LepR to amplify fragments with different sizes. The size of the PCR product for each primer set is listed.

Target specimens	Primer	Sequence (5' – 3')	PCR product (bp)	Reference
Moths (Lepidoptera)	LepR	TAAACTTCTGGATGTCCAAAAAATCA		Hebert et al. (2004a)
	LepF	ATTCAACCAATCATAAAGATATTGG	658	Hebert et al. (2004a)
	MiniLepF1	GCTTTCCCACGAATAAATAATA	407	Hebert et al. (2004a)
	LepF152-2	ACTATCTTTTGATCRAATACCATTRT	155	Ivanova (pers com)
Birds	BirdF1	TTCTCCAACCACAAAGACATTGGCAC	696	Hebert et al. (2004b)
	BirdR1	ACGTGGGAGATAATTCCAAATCCTGG		Hebert et al. (2004b)
Fish	VertF2	TCAACCAACCACAAAGACATTGGCAC	655	Ward et al.(2005)
	VertR1	TAGACTTCTGGGTGGCCAAAGAATCA		Ward et al. (2005)

F. SCREENING PCR PRODUCTS

PCR products were separated on a 2% agarose E-Gel96 gel (Invitrogen, Carlsbad, CA, USA)**,** visualized under UV light and photographed with a AlphaImager 3400 imaging system (Alpha Inootech, San Leandro, CA, USA). Identical camera settings were used for all photographs. Four independent blind counts were performed and the counts were averaged (images are available upon request).

G. SEQUENCING PROTOCOL

The PCR products from each DNA isolation method and the DNA polymerase evaluation experiments were sequenced. The total volume of the sequencing reaction was 10 µl consisting of 1.875 µl of 5X sequencing buffer (Applied Biosystems, Foster City, CA, USA), 0.25 µl of BigDye terminator v3.1 (Applied Biosystems, Foster City, CA, USA), 5 µl of 10 % trehalose and 10 pmol of the primer LepR, BirdR1 and VertR1 for moths, birds and fish samples, respectively. The sequencing cycle had an initial denaturing step of 96ºC for 2 min, followed by 30 cycles of 96ºC for 30 sec, 55ºC for 15 sec, and 60ºC for 4 min. The sequencing products were purified following the Sephadex clean-up protocol (Appendix 2) and analyzed on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

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APPENDIX 2.

ROUTINE LABORATORY PROTOCOLS FOR HIGH-VOLUME DNA BARCODING

A. DNA ISOLATION

General practices

Clean the bench top with ethanol before and after setting up extractions. Always use clean, acid or flame sterilized forceps between specimens. For acid sterilization briefly soak forceps and pestles in 5N HCl for 30 sec directly before use. Rinse forceps and pestles in two separate ddH_20 washes to remove excess HCl. For flame sterilization, soak forceps in 95-100% ethanol and ignite on propane burner for 1-2 seconds. Repeat if necessary.

DryRelease DNA isolation for recent specimens

This method is a based on Chelex/proteinase K isolation and is suitable for recent specimens (less than one year old). For best results, dried specimens such as insect legs should be soaked in 95 % ethanol prior to extraction.

Extraction buffer (100 ml)

Chelex-100 (Bio-Rad laboratories)	6 g
1% Sodium Azide	10 ml
1M Tris-HCl pH 8.3	1 ml
Ultrapure H_2O	up to 100 ml

Store at 4ºC in 10 ml aliquots.

1. Add 10 µl of proteinase K to 100 µl extraction buffer (for 96 samples, add 1 ml of proteinase K to 10 ml of extraction buffer). Final volume can vary (40-110 µl) to accommodate different sample sizes and should be optimized for each tissue type.

2. Dispense 100 µl DryRelease solution using a multichannel or regular pipette (use wide-bored tips) in each well of a 96-well microplate (maximum capacity 250 µl). Mix solution while dispensing to make sure that the Chelex resin is equally dispersed between wells.

3. Put a small amount of tissue (e.g. 1-2 mm of insect leg or 1-2 mm^3 of ethanol preserved tissue) into each well of the microplate. To prevent cross-contamination, work with one row at a time, closing each row with an 8-strip cap.

4. Incubate at 55ºC for 12-24 hours.

5. Tape over the lids and vortex (only for muscle tissue).

6. Centrifuge the microplate at 1000 g for 5 min.

7. Incubate the microplate in a thermocycler at 95ºC for 20 min to denature the proteinase K.

8. Store the microplate at –20ºC.

9. Prior to adding DNA to a PCR reaction, centrifuge the microplate at 1000 g for 5 min.

10. Use 1-2 µl of DNA sample in a PCR reaction. Use the upper phase of the liquid to prevent Chelex granules from entering the PCR reaction.

NucleoSpin96 Tissue Kit DNA isolation for recent and archived specimens

NucleoSpin96 Tissue Kit (Machery-Nagel, Düren, Germany) is a silica-based DNA extraction kit and is suitable for the isolation of DNA from recent and archived (more than one year old) specimens. The protocol used is according to manufacturer's instruction with minor modifications:

1. Add a small amount of tissue (e.g. 2-4 mm of insect leg or 2-3 mm^3 of ethanol preserved tissue) to each well of a round-well block supplied with the kit.

2. Prepare a working solution of proteinase K by combining 180 µl of buffer T1 with 25 µl of Proteinase K for each sample. Transfer 200 µl of the working solution into each well of the round-well block.

3. Seal wells with cap strips provided and shake vigorously for 10 – 15 sec to mix.

4. Centrifuge at 1500 g for 15 sec to collect samples at the bottom of the wells.

5. Incubate at 56ºC for a minimum of 6 hours or overnight to allow digestion. Tape down cap strips to prevent them from occasionally popping off.

6. Centrifuge at 1500 g for 15 sec to remove any condensate from the cap strips.

7. For each sample, add 200 µl of 95% ethanol and 200 µl of binding buffer BQ1.

8. Shake vigorously for 10 – 15 sec and centrifuge at 1500 g for 10 sec to remove any sample from the cap strips.

9. Remove cap strips and transfer the lysate (about 600 µl) from the wells of the round-well block into the wells of the NucleoSpin tissue binding plate placed on top of a square-well block.

10. Seal plate with self-adhering PE foil supplied with kit.

11. Centrifuge at 5600 – 6000 g for 10 min to bind DNA to the silica membrane.

12. First wash step: Add 500 µl of buffer BW to each well of the binding plate. Seal with a new self-adhering PE foil and centrifuge at 5600 – 6000 for 2 min.

13. To accommodate the volume of flow through, replace the square-well block with a sterile square-well block.

14. Second wash step: Add 700 µl of buffer B5 to each well of the binding plate. Seal with a new self-adhering PE foil and centrifuge at 5600 – 6000 for 4 min.

15. Remove the self-adhering PE foil and place the binding plate on an open rack of MN tube strips. Incubate at 56°C for 30 min (or at 70°C for 10 min) to evaporate residual ethanol.

16. Dispense 30 – 100 µl of ddH_20 (prewarmed to 56°C) directly onto the membrane in each well of the NucleoSpin tissue binding plate and incubate at room temperature for 1 min.

17. To elute DNA, centrifuge at 5600 - 6000 g for 2 min, and centrifuge again at 5600 – 6000 g. Remove the NucleoSpin tissue binding plate and discard. Transfer the eluted DNA to a 96 well microplate and seal.

18. DNA can be temporarily stored at 4ºC or at –20ºC for long-term storage.

19. Use 1-5 µl of the DNA for PCR.

B. PCR AMPLIFICATION

General practices

The amount of DNA used will depend on the concentration of the sample. It is best to keep the volume of template as low as possible to avoid adding enzyme inhibitors that may be present, and to avoid unspecific amplification of excess DNA. Primers should be tested with positive samples prior to first use and, to prevent degradation, primers should not be stored for a long period of time (i.e. more than one year). The addition of trehalose (D-(+)-Trehalose dehydrate; e.g. 10%) can improve PCR success rate and allows for pre-dispensed 96-well plates of sequencing reaction to be prepared in advance and stored at -20ºC for 1-3 months. The use of filter tips is recommended for all PCR reagents to avoid contamination.

PCR reaction mix

Below is the recipe for a typical PCR reaction mix:

Reagent	**Volume (µl)**
10% trehalose	6.25
H_2O + DNA	4
10X PCR buffer	1.25
50 mM $MgCl_2$ (2.5 mM)	0.625
10 mM dNTP	0.0625
10 µM forward primer	0.125
10 µM reverse primer	0.125
Taq polymerase	0.0625
Total	12.5

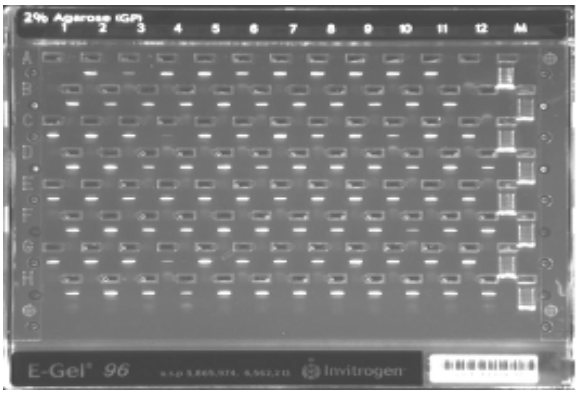
Thermocycler profile

For *cox1* amplification, it is ideal to begin the annealing at a low temperature (45ºC) for a few initial cycles to allow the primers to bind to the template and then raise the temperature (to 51-55ºC; optimized depending on the primers Tm) to avoid excessive non-specific binding of primers. For example, the typical thermocycle profile for the Lepidoptera primers consists of 94ºC for 1 min, five cycles of 94ºC for 40 sec, 45ºC for 40 sec, and 72ºC for 1 min, followed by 35 cycles of 94ºC for 40 sec, 51ºC for 40 sec, and 72ºC for 1 min, with a final extension at 72ºC for 5 min. For *cox1* amplification of vertebrate samples we use a thermocycle profile consisting 94ºC for 2 min, 35 cycles of 94ºC for 30 sec, 54ºC for 30 sec, and 72ºC for 1 min, with a final extension at 72ºC for 10 min.

C. PCR PRODUCT SCREENING

PCR products are separated on a 2% agarose E-Gel96 gel (Invitrogen, Carlsbad, CA, USA). Since this system is bufferless it minimizes the exposure to hazardous ethidium bromide. However, gloves should be worn when handling and loading the gel.
4-5 µl of PCR products are loaded onto the agarose gel with an 8- or 12-multichannel pipettor. The running procedure is straightforward and is done according to the manufacturer's instructions for 6-12 min. The gel is visualized and photographed on a UV transilluminator equipped with digital camera (see the image below). The gel image is aligned and arranged using the E-Editor 2.0 software (Invitrogen, Carlsbad, CA, USA).

A typical E-Gel96 gel image of *cox1* amplification



Note: M=DNA size marker; white bands indicate PCR products; clear slots are the loading wells.

D. SEQUENCING

Sequencing reaction

Sequencing reactions are done using the BigDye (Applied Biosystems, Foster City, CA, USA) cycle sequencing chemistry. Below is the typical sequencing reaction mix based on a 1/16 dilution of the BigDye reagent. The amount of PCR product can be increased up to 2 µl (depending on the intensity of the band on an agarose gel).

Reagent	**Volume (µl)**
BigDye v3.1	0.25
BigDye sequencing buffer (5X)	1.875
10% trehalose	5
10 µM primer	1
ddH_2O	0.875
PCR product	1
Total	10

Note: for BigDye sequencing buffer (5X) either use the 5X reaction buffer produced by Applied Biosystems (Foster City, CA, USA) or 400 mM Tris-HCl pH 9.0, 10 mM $MgCl_2$.

Sequencing reactions are run in a thermocycler with an initial denaturing step of 96ºC for 2 min, followed by 30 cycles of 96ºC for 30 sec, 55ºC for 15 sec, and 60ºC for 4 min. The annealing temperature can be varied according to the primer specificity but 55ºC works well for most *cox1* sequencing reactions.

Sequencing reaction clean-up

Sequencing reaction clean-up is done using a Sephadex column method in a MultiScreen-HV filtration plate (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. The entire sequencing reaction is added to Sephadex columns for purification.