Supplementary S3: Results of preliminary study (older samples from 2008-2011)

From the preliminary study, we recovered correct barcoding sequences from 37/138 Salting-Out [1] and 26/137 Phenol-Chloroform extracted samples. Identification success was dependent on both the species and extraction method (GLM quasibinomial: $F_{6,239}$=2.69, P=0.015) and also between species and the sampling solution (GLM quasibinomial: $F_{18,245}$=3.60, P<0.001). One species (*Anobium costatum*) produced no positive identifications from the sequencing data, whereas another (*Taphrorychus bicolor*) produced 29/40 correct identifications, with the success in this species dependent on the sampling solution. In general, using Ethanol as the sampling solution and extracted samples using the Salting-Out method recovered greater DNA yield and produced more positive sequence identifications (Fig A, Table A). Yield was measured on a Nanodrop 100 in the preliminary study, as opposed to a Quantus Fluorometer used in the main study.

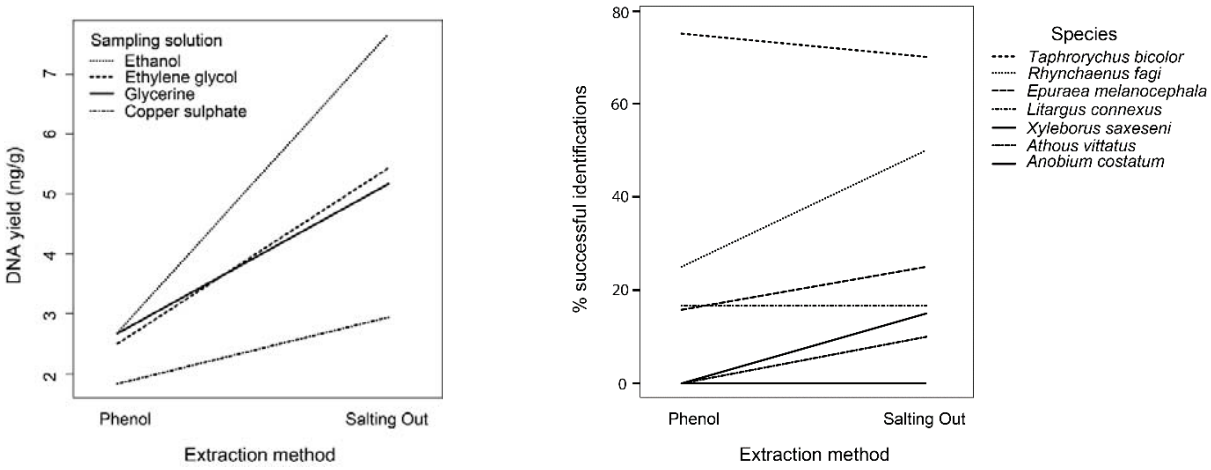

Figure A: Mean DNA yield (ng/g) across the different sampling solutions and extraction methods, and percentage of successfully identified individuals across species and extraction method.

Table A: Summary of data from the preliminary study comparing DNA and barcoding success from seven species, using two extraction methods from four sampling solutions.

Species	Solution	Extraction method	Mean DNA yield (ng/g)	ID success	No. samples
Anobium costatum	Copper sulphate	Salting Out	7.51	0	5
Anobium costatum	Copper sulphate	Phenol	2.51	0	5
Anobium costatum	Ethanol	Salting Out	7.04	0	5
Anobium costatum	Ethanol	Phenol	2.26	0	5
Anobium costatum	Ethylene glycol	Salting Out	5.38	0	5
Anobium costatum	Ethylene glycol	Phenol	3.39	0	5
Anobium costatum	Glycerine	Salting Out	1.65	0	5
Anobium costatum	Glycerine	Phenol	1.58	0	5
Athous vittatus	Copper sulphate	Salting Out	0.41	0	5
Athous vittatus	Copper sulphate	Phenol	1.70	0	5
Athous vittatus	Ethanol	Salting Out	1.60	2	5
Athous vittatus	Ethanol	Phenol	0.35	0	5
Athous vittatus	Ethylene glycol	Salting Out	0.45	0	5
Athous vittatus	Ethylene glycol	Phenol	1.23	0	5
Athous vittatus	Glycerine	Salting Out	3.13	0	5
Athous vittatus	Glycerine	Phenol	0.93	0	5
Epuraea melanocephala	Copper sulphate	Salting Out	2.62	0	5
Epuraea melanocephala	Copper sulphate	Phenol	0.58	0	5
Epuraea melanocephala	Ethanol	Salting Out	10.49	3	5
Epuraea melanocephala	Ethanol	Phenol	2.72	2	5
Epuraea melanocephala	Ethylene glycol	Salting Out	2.74	2	5
Epuraea melanocephala	Ethylene glycol	Phenol	1.69	1	5
Epuraea melanocephala	Glycerine	Salting Out	3.56	0	5
Epuraea melanocephala	Glycerine	Phenol	1.06	0	5
Rhynchaenus fagi	Copper sulphate	Salting Out	4.63	2	5
Rhynchaenus fagi	Copper sulphate	Phenol	1.69	1	5
Rhynchaenus fagi	Ethanol	Salting Out	2.40	1	5
Rhynchaenus fagi	Ethanol	Phenol	0.82	1	5
Rhynchaenus fagi	Ethylene glycol	Salting Out	9.90	3	5
Rhynchaenus fagi	Ethylene glycol	Phenol	4.17	2	5
Rhynchaenus fagi	Glycerine	Salting Out	6.95	4	5
Rhynchaenus fagi	Glycerine	Phenol	1.50	1	5
Taphrorychus bicolor	Copper sulphate	Salting Out	0.57	0	5
Taphrorychus bicolor	Copper sulphate	Phenol	2.68	2	5
Taphrorychus bicolor	Ethanol	Salting Out	13.41	5	5
Taphrorychus bicolor	Ethanol	Phenol	4.20	5	5
Taphrorychus bicolor	Ethylene glycol	Salting Out	11.63	4	5
Taphrorychus bicolor	Ethylene glycol	Phenol	2.58	3	5
Taphrorychus bicolor	Glycerine	Salting Out	13.49	5	5
Taphrorychus bicolor	Glycerine	Phenol	5.35	5	5
Xyleborus saxeseni	Copper sulphate	Salting Out	3.49	0	5
Xyleborus saxeseni	Copper sulphate	Phenol	2.88	0	5
Xyleborus saxeseni	Ethanol	Salting Out	6.89	3	5
Xyleborus saxeseni	Ethanol	Phenol	2.77	0	5
Xyleborus saxeseni	Ethylene glycol	Salting Out	7.36	0	5
Xyleborus saxeseni	Ethylene glycol	Phenol	2.54	0	5
Xyleborus saxeseni	Glycerine	Salting Out	5.53	0	5
Xyleborus saxeseni	Glycerine	Phenol	2.71	0	5
Litargus connexus	Copper sulphate	Salting Out	1.00	1	4
Litargus connexus	Copper sulphate	Phenol	0.55	0	4
Litargus connexus	Ethanol	Salting Out	11.88	2	5
Litargus connexus	Ethanol	Phenol	5.65	2	5
Litargus connexus	Ethylene glycol	Salting Out	0.60	0	5
Litargus connexus	Ethylene glycol	Phenol	1.91	0	5
Litargus connexus	Glycerine	Salting Out	1.06	0	4
Litargus connexus	Glycerine	Phenol	5.92	1	4

References

1. Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution 13: 510-524.