

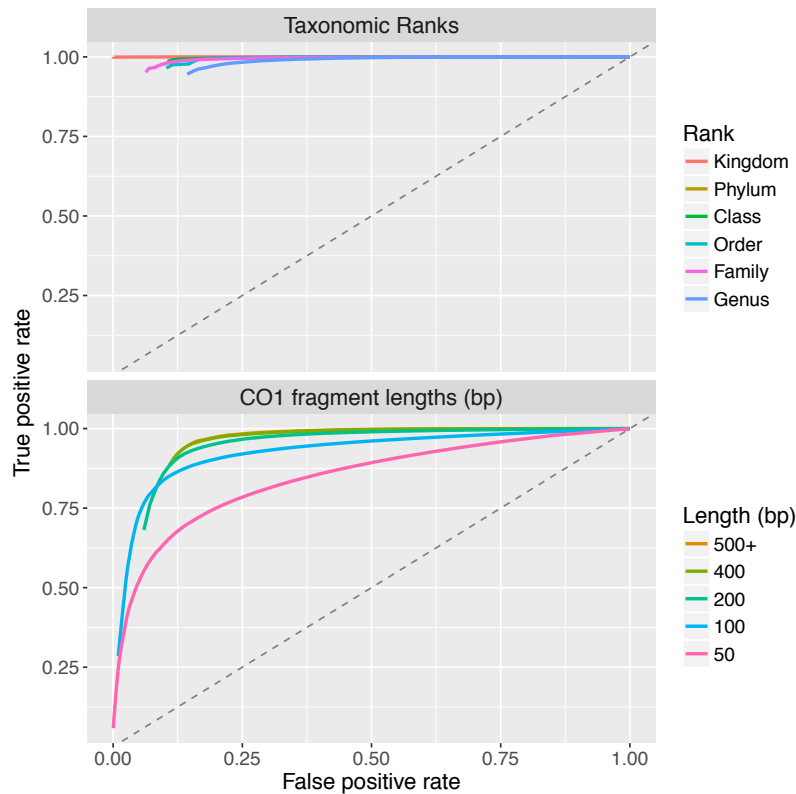
# Automated high throughput animal DNA metabarcode classification

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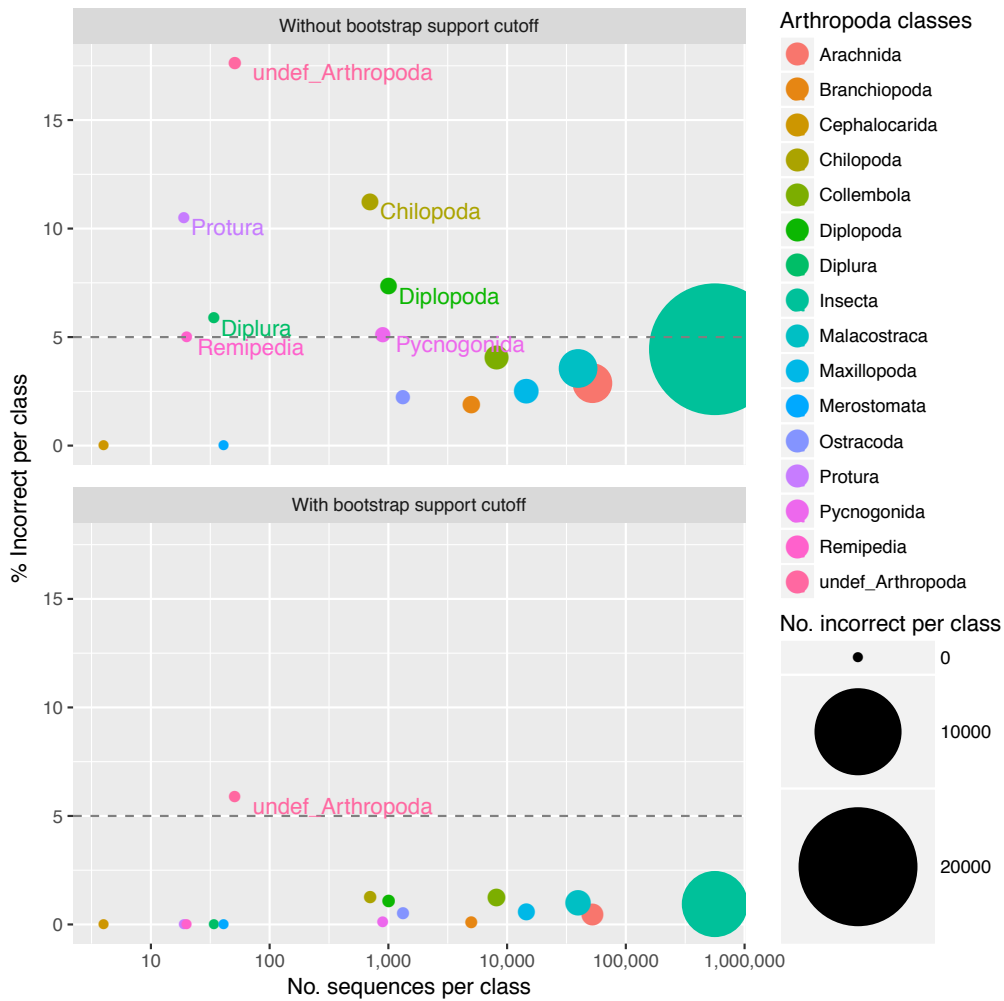
## Supplementary Information

### Supplementary Figures

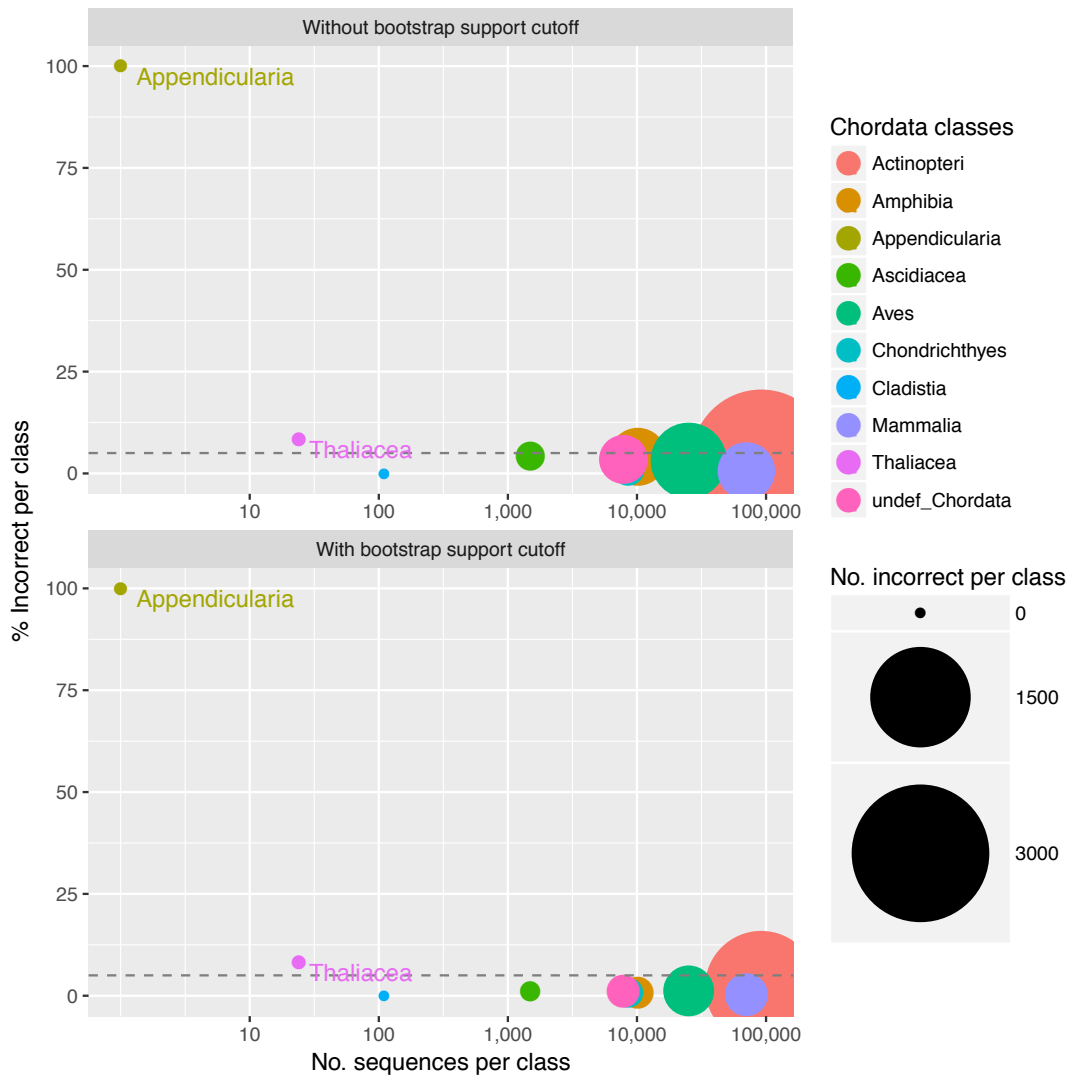
Figure S1: Receiver operator characteristic curves show good classifier performance across a range of taxonomic ranks for the longest CO1 sequences. Top panel: Comparison of false positive rates (FPR) and true positive rates (TPR) among full length (500 bp+) CO1 fragments at various taxonomic ranks. Bottom panel: Comparison among genus rank assignments for a variety of CO1 sequence lengths. A 50% chance line is shown as a grey dashed line. Area under the curve (AUC) values are shown in the legend.



17 Figure S2: Using bootstrap cutoffs as a filter reduces the proportion of incorrect  
 18 taxonomic assignments for full length (500 bp+) CO1 Arthropoda sequences. Top panel:  
 19 The proportion of incorrect assignments from leave-one-out testing is shown. Bottom  
 20 panel: The proportion of incorrect assignments is shown when a bootstrap support cutoff  
 21 of 70% at the genus rank is used as a filter. The grey dashed line shows the 5% incorrect  
 22 assignment cover line. The size of the circles shows the total number of misclassified  
 23 assignments for each Arthropoda class. Results were summarized from leave-one-out  
 24 testing including singletons. The category ‘undef\_Arthropoda’ includes sequences from  
 25 the orders Pauropoda and Symphyla as well as the genus *Prionodiptomus*.  
 26



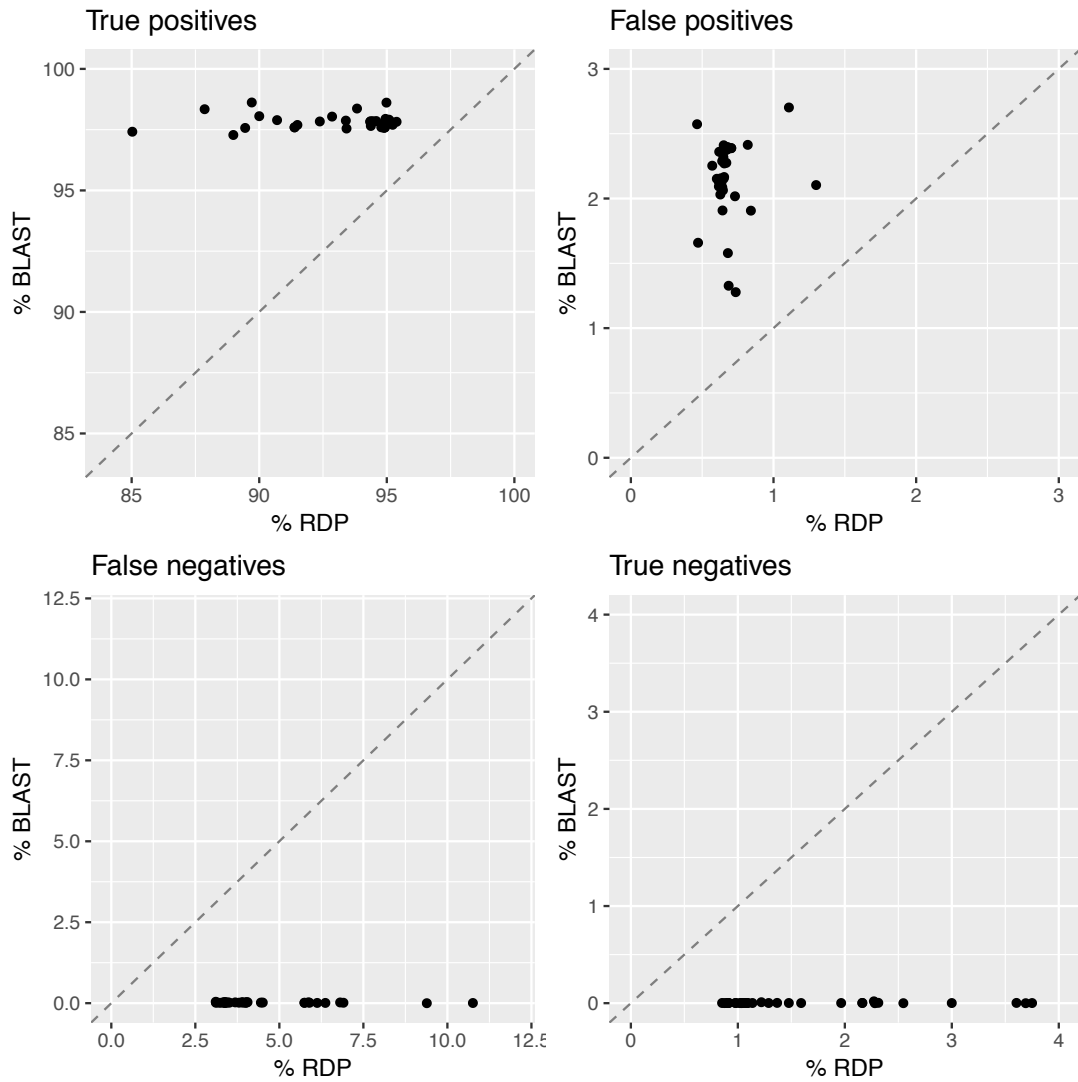
28 Figure S3: Applying a bootstrap support cutoff does not reduce the proportion of  
 29 incorrect taxonomic assignments for under-sampled classes in the Chordata. Top panel:  
 30 Proportion of incorrect taxonomic assignments for all full length CO1 Chordata  
 31 sequences during leave-one-out testing including singletons. Bottom panel: Proportion of  
 32 incorrect taxonomic assignments when a bootstrap support cutoff of 70% at the genus  
 33 assignment rank is used as a filter. The grey dashed line shows the 5% incorrect taxonomic  
 34 assignment cover line. The size of the points shows the total number of misclassified  
 35 assignments per Chordata class. Class Appendicularia is represented by one sequence  
 36 and Thaliaceae is represented by two sequences in the Eukaryote CO1 v1 training set.  
 37 The taxon 'undef\_Chordata' contains sequences from the orders Ceratodontiformes,  
 38 Coelacanthiformes, Crocodylia, Lepidosireniformes, Myxiniiformes, Petromyzontiformes,  
 39 Sphenodontia, Squamata, Testudines, and the family Branchiostomidae.  
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43 Figure S4: Summary of taxonomic assignment outcomes from primer-anchored 200 bp  
44 CO1 sequences using the CO1 Eukaryote v1 training set. A 1:1 line is shown as a grey  
45 dashed line. Note the differing limits on the axes for each outcome. Results summarized  
46 from leave-one-out testing including singletons.  
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52 Figure S5: A matrix that summarizes taxonomic assignment outcomes, the terms used in  
 53 this study, and the relationships between them. Abbreviations are as follows: True  
 54 positive (TP), false positive (FP), false negative (FN), true negative (TN), true positive  
 55 rate (TPR), false positive rate (FPR), false negative rate (FNR), true negative rate (TNR).  
 56

		TRUTH	
		Correct assignment TP + FN	Incorrect assignment FP + TN
PREDICTION	High confidence*	True positive (TP) True positive rate (TPR) TP / (TP + FN)	False positive (FP) False positive rate (FPR) FP / (FP + TN)
	Low confidence	False negative (FN) False negative rate (FNR) FN / (FN + TP)	True negative (TN) True negative rate (TNR) TN / (TN + FP)

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}}$$

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60 **Supplementary Tables**

61

62 Table S1: Taxonomic breakdown of CO1 Eukaryote v1 training set.

Whole training set		Arthropoda		Chordata	
Phylum	No. sequences	Class	No. sequences	Class	No. sequences
Arthropoda	685,651	Insecta	561,841	Actinopteri	91,744
Chordata	215,530	Arachnida	52,328	Mammalia	70,397
Unassigned phylum*	3,079	Malacostraca	39,622	Aves	25,150
Mollusca	3,017	Maxillopoda	14,548	Amphibia	10,154
Echinodermata	2,504	Collembola	8,186	Chondrichthyes	8,594
Annelida	1,551	Branchiopoda	5,016	Unassigned class***	7,862
Ascomycota	330	Ostracoda	1,332	Ascidiacea	1,494
Phaeophyceae	246	Diplopoda	1,008	Cladistia	110
Cnidaria	101	Pycnogonida	898	Thaliacea	24
Nematoda	69	Chilopoda	703	Appendicularia	1
Chaetognatha	55	Unassigned class**	51		
Platyhelminthes	53	Merostomata	41		
Bacillariophyta	30	Diplura	34		
Nemertea	12	Remipedia	20		
Brachiopoda	10	Protura	19		
Porifera	9	Cephalocarida	4		
Bryozoa	4				
Xenacoelomorpha	2				
Total sequences	912,253		685,651		215,530

63 \*Rhodophyta, Alveolata, Oomycetes

64 \*\*Prionodiptomus

65 \*\*\*Ceratodontiformes, Coelacanthiformes, Crocodylia, Lepidosireniformes, Myxiniiformes,

66 Petromyzontiformes, Sphenodontia, Squamata, Testuines, Branchiostomidae

67

68 Table S2: CO1 Eukaryote v2 set summary.

<b>Training set</b>	<b>Number of taxa (all ranks)</b>	<b>Number of sequences</b>
Whole training set	123,145	912,621
Arthropoda	92,525	685,805
Chordata	27,475	215,727
Outgroup taxa	3,143	11,089

69

70

71 Table S3: Singletons in the CO1 Eukaryote v1 and the CO1 Eukaryote v2 training set.

<b>CO1 Eukaryote v1 training set</b>			
<b>Rank</b>	<b>No. unique taxa</b>	<b>No. singletons</b>	<b>% Unique taxa that are singletons</b>
Superkingdom	1	-	-
Kingdom	4	0	-
Phylum	18	0	-
Class	62	4	6.5
Order	389	27	6.9
Family	2,972	319	10.7
Genus	26,553	6,120	23.0
<b>CO1 Eukaryote v2 training set</b>			
<b>Rank</b>	<b>No. unique taxa</b>	<b>No. singletons</b>	<b>% Unique taxa that are singletons</b>
Superkingdom	1	-	-
Kingdom	4	-	-
Phylum	19	1	5.3
Class	63	5	7.9
Order	390	28	7.2
Family	2977	319	10.7
Genus	26,593	6,130	23.1
Species	93,112	30,958	33.2

72



73 Table S4: Suggested minimum bootstrap support cutoff values to recover 99% correct  
 74 assignments with the CO1 Eukaryote v2 training set. This analysis is based on leave-  
 75 one-out testing where singletons were excluded from the analysis. These cutoffs are  
 76 based on 66.8% of the sequences in the original training set. 'N/A', not applicable, refers  
 77 to the inability to observe 99% correct taxonomic assignments.  
 78

<b>Rank</b>	<b>500bp+</b>	<b>400bp</b>	<b>200bp</b>	<b>100bp</b>	<b>50bp</b>
	<b>Minimum bootstrap support cutoff (%)</b>				
Superkingdom	0	0	0	0	0
Kingdom	0	0	0	0	0
Phylum	0	0	0	0	0
Class	0	0	0	0	30
Order	0	0	0	10	70
Family	0	0	20	20	70
Genus	30	30	30	30	80
Species	N/A	N/A	N/A	N/A	N/A
	<b>Reduction of sequences classified after applying minimum bootstrap support cutoff (%)</b>				
Superkingdom	0.0	0.0	0.0	0.0	0.0
Kingdom	0.0	0.0	0.0	0.0	0.0
Phylum	0.0	0.0	0.0	0.0	0.0
Class	0.0	0.0	0.0	0.0	2.4
Order	0.0	0.0	0.0	0.4	37.5
Family	0.0	0.4	1.4	3.5	59.2
Genus	1.1	1.7	2.5	7.1	72.7
Species	N/A	N/A	N/A	N/A	N/A

79

80 Table S5: Proportion of incorrect taxonomic assignments for Eukaryota phyla using the  
 81 RDP classifier with the CO1 Eukaryote v1 training set. Calculated from leave-one-out  
 82 testing results where singletons were included. We used a bootstrap support cutoff value  
 83 of 70% for full length (500 bp+) CO1 sequences at the genus rank where indicated.  
 84

<b>Eukaryote Phyla</b>	<b>No. misclassified sequences</b>	<b>Total sequences</b>	<b>% Misclassified (No cutoff)</b>	<b>% Misclassified (Cutoff)</b>
Annelida	41	1,551	2.6	0.5
Arthropoda	28,895	685,651	4.2	0.9
Ascomycota	5	330	1.5	0.3
Bacillariophyta	2	30	6.7	0.0
Brachiopoda	0	10	0.0	0.0
Bryozoa	1	4	25.0	0.0
Chaetognatha	4	55	7.3	5.5
Chordata	5,159	215,530	2.4	1.2
Cnidaria	17	101	16.8	7.9
Echinodermata	97	2,504	3.9	2.1
Mollusca	114	3,017	3.8	0.4
Nematoda	16	69	23.2	7.2
Nemertea	1	12	8.3	0.0
Phaeophyceae	10	246	4.1	0.4
Platyhelminthes	5	53	9.4	0.0
Porifera	1	9	11.1	0.0
undef_undef_Eukaryota*	105	3,079	3.4	1.3
Xenacoelomorpha	0	2	0.0	0.0
Across all phyla	34,473	912,253	3.8	1.0

85 \*undef\_undef\_Eukaryota: Bangiophyceae, Dinophyceae, Florideophyceae, Oligohymenophorea, and  
 86 Oomycetes.  
 87

88 Table S6: Proportion of incorrect taxonomic assignments for all Arthropoda classes using  
 89 the RDP classifier with the CO1 Eukaryote v1 training set. Calculated from leave-one-  
 90 out testing results where singletons were included. We used a bootstrap support cutoff  
 91 value of 70% for full length (500 bp+) CO1 sequences at the genus rank where indicated.  
 92

<b>Arthropoda Classes</b>	<b>No. misclassified sequences</b>	<b>Total sequences</b>	<b>% Misclassified (No cutoff)</b>	<b>% Misclassified (Cutoff)</b>
Arachnida	1,506	52,328	2.9	0.5
Branchiopoda	94	5,016	1.9	0.1
Cephalocarida	0	4	0.0	0.0
Chilopoda	79	703	11.2	1.3
Collembola	333	8,186	4.1	1.2
Diplopoda	74	1,008	7.3	1.1
Diplura	2	34	5.9	0.0
Insecta	24,945	561,841	4.4	0.9
Malacostraca	1,409	39,622	3.6	1.0
Maxillopoda	365	14,548	2.5	0.6
Merostomata	0	41	0.0	0.0
Ostracoda	30	1,332	2.3	0.5
Protura	2	19	10.5	0.0
Pycnogonida	46	898	5.1	0.1
Remipedia	1	20	5.0	0.0
undef_Arthropoda*	9	51	17.6	5.9
Across all classes	28,895	685,651	4.2	0.9

93 \*undef\_Arthropoda: Pauropoda, Symphyla  
 94

95 Table S7: Proportion of incorrect taxonomic assignments for all Chordata classes using  
 96 the RDP classifier with the CO1 Eukaryote v1 training set. Calculated from leave-one-  
 97 out testing results where singletons were included. We used a bootstrap support cutoff  
 98 value of 70% for full length (500 bp+) CO1 sequences at the genus rank where indicated.  
 99

<b>Chordata Classes</b>	<b>No. misclassified sequences</b>	<b>Total sequences</b>	<b>% Misclassified (No cutoff)</b>	<b>% Misclassified (Cutoff)</b>
Actinopteri	3,115	91,744	3.4	2.1
Amphibia	415	10,154	4.1	0.8
Appendicularia	1	1	100.0	100.0
Ascidiacea	63	1,494	4.2	1.1
Aves	791	25,150	3.1	1.2
Chondrichthyes	93	8,594	1.1	0.8
Cladistia	0	110	0.0	0.0
Mammalia	408	70,397	0.6	0.3
Thaliacea	2	24	8.3	8.3
undef_Chordata*	271	7,862	3.4	1.2
Across all classes	5,159	215,530	2.4	1.2

100 \*undef\_Chordata: Ceratodontiformes, Coelacanthiformes, Crocodylia, Lepidosireniformes, Myxiniformes,  
 101 Petromyzontiformes, Sphenodontia, Squamata, Testudines, Branchiostomidae  
 102

103 Table S8: Proportion of incorrect taxonomic assignments for all Insecta orders using the  
 104 RDP classifier with the CO1 Eukaryote v1 training set. Calculated from leave-one-out  
 105 testing results where singletons were included. We used a bootstrap support cutoff value  
 106 of 70% for full length (500 bp+) CO1 sequences at the genus rank where indicated.  
 107

<b>Insecta Orders</b>	<b>No. misclassified sequences</b>	<b>Total sequences</b>	<b>% Misclassified (No cutoff)</b>	<b>% Misclassified (Cutoff)</b>
Archaeognatha	5	578	0.9	0.1
Blattodea	52	574	9.1	1.4
Coleoptera	6,672	89,484	7.5	1.1
Dermaptera	6	37	16.2	0.0
Diptera	4,503	118,896	3.8	0.8
Embioptera	24	64	37.5	4.7
Ephemeroptera	185	6,722	2.8	0.3
Grylloblattodea	1	1	100.0	0.0
Hemiptera	0	50,064	0.0	1.7
Hymenoptera	1,768	42,154	4.2	1.2
Isoptera	64	1,055	6.1	1.8
Lepidoptera	7,540	211,318	3.6	0.8
Mantodea	62	401	15.5	2.5
Mantophasmatodea	0	30	0.0	0.0
Mecoptera	17	285	6.0	1.8
Megaloptera	17	469	3.6	1.7
Neuroptera	125	1,545	8.1	2.3
Odonata	244	3,553	6.9	1.2
Orthoptera	338	6,885	4.9	1.9
Phasmatodea	87	786	11.1	1.0
Phthiraptera	15	1,103	1.4	0.0
Plecoptera_Insecta	72	2,679	2.7	0.1
Psocoptera	25	1,267	2.0	0.1
Raphidioptera	6	44	13.6	2.3
Siphonaptera	52	346	15.0	2.3
Strepsiptera	15	149	10.1	1.3
Thysanoptera	105	4,065	2.6	0.7
Trichoptera	528	17,276	3.1	0.3
undef_Insecta*	2	9	22.2	11.1
Zoraptera	0	2	0.0	0.0
Across all orders	22,530	561,841	4.0	0.9

108 \*undef\_Insecta: Lepidotrichidae, Lepismatidae, Nicoletiidae

109 Table S9: Proportion of incorrect taxonomic assignments for all Actinopteri orders using  
 110 the RDP classifier with the CO1 Eukaryote v1 training set. Calculated from leave-one-  
 111 out testing results where singletons were included. We used a bootstrap support cutoff  
 112 value of 70% for full length (500 bp+) CO1 sequences at the genus rank where indicated.  
 113

<b>Actinopteri Orders</b>	<b>No. misclassified sequences</b>	<b>Total sequences</b>	<b>% Misclassified (No cutoff)</b>	<b>% Misclassified (Cutoff)</b>
Acanthuriformes	11	827	1.3	0.6
Acipenseriformes	12	348	3.4	2.9
Albuliformes	3	70	4.3	1.4
Alepocephaliformes	15	161	9.3	3.7
Amiiformes	0	6	0.0	0.0
Anabantiformes	12	1,325	0.9	0.0
Anguilliformes	58	1,341	4.3	2.6
Argentiniformes	16	155	10.3	7.1
Ateleopodiformes	1	12	8.3	0.0
Atheriniformes	25	789	3.2	1.6
Aulopiformes	20	534	3.7	1.5
Batrachoidiformes	5	68	7.4	0.0
Beloniformes	21	517	4.1	1.7
Beryciformes	16	364	4.4	3.3
Blenniiformes	87	1,357	6.4	1.1
Carangiformes	44	2,041	2.2	1.6
Centrarchiformes	23	1,376	1.7	1.4
Chaetodontiformes	36	975	3.7	2.6
Characiformes	201	3,544	5.7	3.7
Cichliformes	292	2,007	14.5	11.5
Clupeiformes	70	1,767	4.0	2.9
Cypriniformes	593	13,285	4.5	3.4
Cyprinodontiformes	60	1,810	3.3	0.4
Elopiformes	0	90	0.0	0.0
Ephippiformes	0	98	0.0	0.0
Esociformes	1	348	0.3	0.0
Gadiformes	37	1,883	2.0	1.3
Galaxiiformes	3	258	1.2	0.0
Gobiiformes	169	4,371	3.9	2.0
Gonorynchiformes	0	58	0.0	0.0
Gymnotiformes	10	481	2.1	0.8
Hiodontiformes	0	24	0.0	0.0
Holocentriformes	8	376	2.1	1.9
Istiophoriformes	3	473	0.6	0.6
Kurtiformes	69	1,070	6.4	3.6

Labriformes	38	2,403	1.6	0.7
Lampriformes	7	53	13.2	7.5
Lepidogalaxiiformes	0	2	0.0	0.0
Lobotiformes	0	75	0.0	0.0
Lophiiformes	34	411	8.3	3.2
Mugiliformes	58	1,125	5.2	2.9
Myctophiformes	46	802	5.7	3.1
Notacanthiformes	3	119	2.5	0.8
Ophidiiformes	17	180	9.4	5.0
Osmeriformes	4	458	0.9	0.4
Osteoglossiformes	20	505	4.0	2.8
Pempheriformes	8	349	2.3	1.4
Perciformes	208	12,065	1.7	0.9
Percopsiformes	2	28	7.1	7.1
Pholidichthyiformes	1	1	100.0	0.0
Pleuronectiformes	78	2,475	3.2	1.6
Polymixiiformes	0	17	0.0	0.0
Salmoniformes	3	2,437	0.1	0.1
Scombriformes	56	3,290	1.7	1.3
Semionotiformes	0	68	0.0	0.0
Siluriformes	244	5,637	4.3	2.7
Spariformes	19	2,198	0.9	0.7
Stomiiformes	32	301	10.6	6.0
Stylephoriformes	0	5	0.0	0.0
Synbranchiformes	21	439	4.8	4.3
Syngnathiformes	65	1,587	4.1	1.4
Tetraodontiformes	37	1,531	2.4	1.8
undef_Actinopteri*	181	8,283	2.2	1.3
Uranoscopiformes	9	513	1.8	0.8
Zeiformes	3	178	1.7	0.6
Across all orders	3,115	91,744	3.4	2.1

114 \*undef\_Actinopteri: Ambassidae, Bathyclupeidae, Callanthiidae, Caproidae, Centropomidae, Cepolidae,  
115 Champsodontidae, Congrogadidae, Dichistiidae, Dinopercidae, Embiotocidae, Emmelichthyidae, Gerreidae,  
116 Grammatidae, Haemulidae, Hapalogenyidae, Lactariidae, Leptobramidae, Lutjanidae, Malacanthidae,  
117 Menidae, Monodactylidae, Moronidae, Opistognathidae, Parascorpididae, Plesiopidae, Polynemidae,  
118 Pomacanthidae, Pomacentridae, Priacanthidae, Pseudochromidae, Scatophagidae, Sciaenidae, Siganidae,  
119 Sillaginidae, Sphraenidae, Symphysanodontidae, Toxotidae, Trichonotidae  
120

121 Table S10: Number of class Insecta CO1 reference sequences in the 2013 GenBank-  
 122 Genus Insecta training set (Porter et al., 2014) and the current 2016 CO1 Eukaryote v1  
 123 training set.  
 124

<b>Insecta Orders</b>	<b>2013</b>	<b>2016</b>
Archaeognatha	87	578
Blattodea	173	574
Coleoptera	27,325	89,484
Dermaptera	31	37
Diptera	21,838	118,896
Embioptera	30	64
Ephemeroptera	3,294	6,722
Grylloblattodea	1	1
Hemiptera	10,600	50,064
Hymenoptera	15,084	42,154
Isoptera	403	1,055
Lepidoptera	95,360	211,318
Mantodea	74	401
Mantophasmatodea	30	30
Mecoptera	34	285
Megaloptera	360	469
Neuroptera	434	1,545
Odonata	1,305	3,553
Orthoptera	3,292	6,885
Phasmatodea	681	786
Phthiraptera	179	1,103
Plecoptera_Insecta	1,016	2,679
Psocoptera	7	1,267
Raphidioptera	5	44
Siphonaptera	9	346
Strepsiptera	9	149
Thysanoptera	406	4,065
Trichoptera	8,257	17,276
undef_Insecta*	9	9
Zoraptera	-	2
Across all orders	190,333	561,841

125 \*undef\_Insecta: Lepidotrichidae, Lepismatidae, Nicoletiidae



126 Table S11: Top five Insecta orders with the most CO1 reference sequences 500 bp+  
127 identified to the species rank in the GenBank nucleotide database.  
128

<b>2013</b>	<b>No. Seqs</b>	<b>2016</b>	<b>No. Seqs</b>
Lepidoptera	95,360	Lepidoptera	211,318
Coleoptera	27,325	Diptera	118,896
Diptera	21,838	Coleoptera	89,484
Hymenoptera	15,084	Hemiptera	50,064
Hemiptera	10,600	Hymenoptera	42,154

129

130

131 Table S12: Bottom five Insecta orders with the least reference sequences 500 bp+  
132 identified to the species rank in the GenBank nucleotide database.  
133

<b>2013</b>	<b>No. Seqs</b>	<b>2016</b>	<b>No. Seqs</b>
Grylloblattodea	1	Grylloblattodea	1
Raphidioptera	5	Zoraptera	2
Psocoptera	7	undef_Insecta	9
Strepsiptera	9	Mantophasmatodea	30
undef_Insecta*	9	Dermaptera	37

134 \*undef\_Insecta: Lepidotrichidae, Lepismatidae, and Nicoletiidae  
135

136 Table S13: Proportion of incorrect genus rank taxonomic assignments from the top 5  
 137 best-represented Insecta orders from two training sets has reduced from 2013 to 2016  
 138 (Porter et al., 2014, Current study). Results were summarized from leave-one-out testing  
 139 of full length CO1 sequences (500 bp +), singletons included. No bootstrap support  
 140 cutoff was used.  
 141

<b>Top Insecta Orders</b>	<b>2013</b>		<b>2016</b>	
	<b>No. sequences</b>	<b>% Incorrect</b>	<b>No. sequences</b>	<b>% Incorrect</b>
Hemiptera	10,600	11.2	50,064	0
Coleoptera	27,325	9.1	89,484	7.5
Diptera	21,838	7.9	118,896	3.8
Hymenoptera	15,084	6.3	42,154	4.2
Lepidoptera	95,360	3.9	211,318	3.6

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143  
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Table S14: CO1 primers from the literature included in this study. Primers are listed in the order they would be found when reading from the 5'→3' direction of the plus strand.

Primer Name	Orientation	Published Primer Sequence (5'→3' orientation)*	References
LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	(Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994)
Lep-F1	Forward	ATTCAACCAATCATAAAGATAT	(P. D. Hebert, Penton, Burns, Janzen, & Hallwachs, 2004)
Uni-MinibarF1	Forward	TCCACTAATCACAARGATATTGGTAC	(Meusnier et al., 2008)
Fish_miniE_F	Forward	ACYAAICAYAAAGAYATIGGCAC	(Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015)
Uni-MinibarR1	Reverse	GAAAATCATAATGAAGGCATGAGC	(Meusnier et al., 2008)
EPT-long-univR	Reverse	AARAAAATYATAAYAAAIGCGTGIAIIGT	(Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011)
A	Forward	GGIGGITTTGGIAATTGAYTIGTICC	(Hajibabaei, Spall, Shokralla, & van Konynenburg, 2012)
ArF1	Forward	GCICCWGAYATRCITTYCCICG	(J. Gibson et al., 2014)
ArF2	Forward	GCICCI GAYATRCITTYCCICG	(J. Gibson et al., 2014)
ArF3	Forward	GCICCRGAYATRCITTYCCACG	(J. Gibson et al., 2014)
ArF4	Forward	GCICCCGATATRCITTYCCYCG	(J. Gibson et al., 2014)
ArF5	Forward	GCICCI GAYATRCITTYCCICG	(J. Gibson et al., 2014)
ArF10	Forward	CCWGATATAKCITWYCCICG	(J. Gibson et al., 2014)
B	Forward	CCIGAYATRCITTYCCICG	(Hajibabaei et al., 2012)
MF1	Forward	GCTTCCCACGAATAAATAATA	(Hajibabaei et al., 2006)
MLepF1-Rev	Reverse	CGTGGA AAWGCTATATCWGGTG	(Brandon-Mong et al., 2015)
230_R	Reverse	CTTATRTRTTTATICGIGGRAAIGC	(J. F. Gibson et al., 2015)
Fish_miniE_R	Reverse	CTTATRTRTTTATICGIGGRAAIGC	(Shokralla, Hellberg, et al., 2015)
mlCOIintF	Forward	GGWACWGGWGAACWGTWTAYCCYCC	(Leray et al., 2013)
Fish_miniF_F	Forward	GGIACIGGITGRACIGTITAYCCYCC	(Shokralla, Hellberg, et al., 2015)
C	Forward	GITGAACIGTITAYCCICC	(Hajibabaei et al., 2012)
MH-MR1	Reverse	CCTGTTCCAGCTCCATTTTC	(Hajibabaei et al., 2006)
C_R	Reverse	GGIGGRTAIACIGTTCAICC	(Shokralla, Porter, et al., 2015)
D	Reverse	CCTARIATIGAIGARAYICCIGC	(Hajibabaei et al., 2012)
ArR2**	Reverse	GTRATIGCICCI GCIARWACWGG	(J. Gibson et al., 2014)
ArR3**	Reverse	GTRATWGCICCI GCTARWACWGG	(J. Gibson et al., 2014)
ArR5**	Reverse	GTRATIGCICCI GCIARIACIGG	(J. Gibson et al., 2014)
ArR6**	Reverse	GTRATIGCICCI GYAAIACIGG	(J. Gibson et al., 2014)

ArR7**	Reverse	GTRATTGCYCCIGCIARIACIGG	(J. Gibson et al., 2014)
ArR9**	Reverse	GTRATIGCICCIGCWARIACWGG	(J. Gibson et al., 2014)
E	Reverse	GTRATIGCICCIGCIARIAC	(Hajibabaei et al., 2012)
F	Reverse	CCIGCIGGRTCIAARAAIGAIGT	(Hajibabaei et al., 2012)
Fish_miniF_R	Reverse	CTTCAGGGTGICCGAARAATC	(Shokralla, Hellberg, et al., 2015)
HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	(Folmer et al., 1994)
Lep-R1	Reverse	TAAACTTCTGGATGTCCAAAAA	(P. D. Hebert et al., 2004)

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\*5' -> 3' orientation as forward primers would align to the plus strand and for reverse primers as they would align to the minus strand  
\*\*These reverse primer sequences were published in the 5' -> 3' orientation as they would align to the plus strand. Presented here in the 5' -> 3' orientation as they would align to the minus strand for consistency.