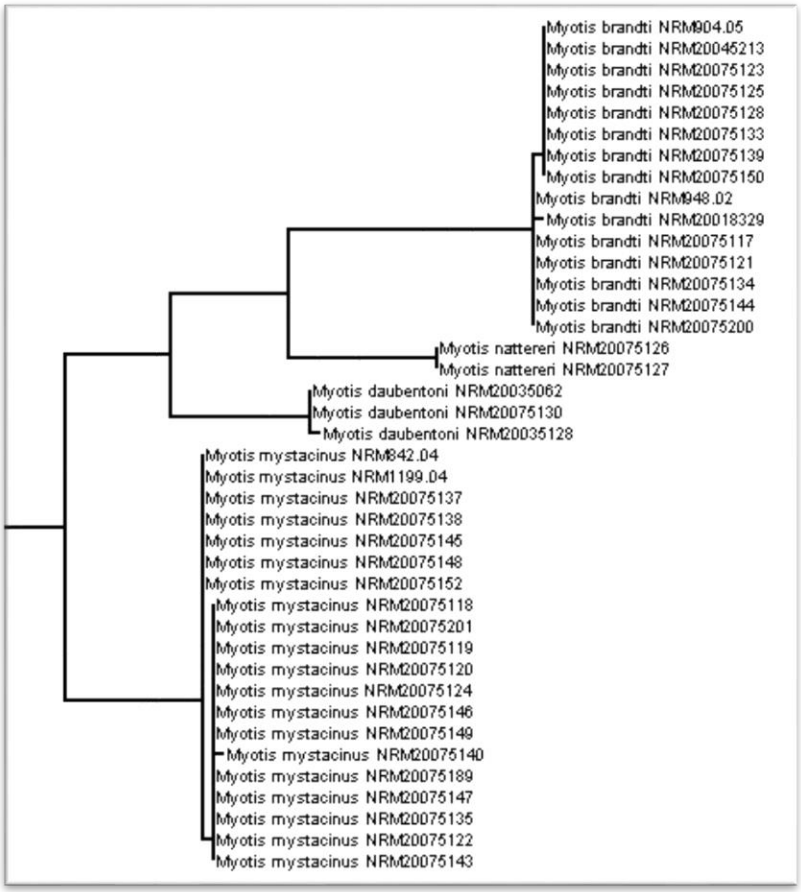
A DNA key to Swedish vertebrates – final report



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A DNA key to Swedish vertebrates – final report

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Background

The project "*A DNA key to Swedish vertebrates*" was carried out between 2006 and 2011 at the Molecular Systematics Laboratory (Swedish Museum of Natural History), NRM dnr 2006-00674. It focused on all vertebrates occurring regularly within the Swedish territory, i.e. mainland and adjacent waters. In accordance with the barcoding standards set out by the Consortium for the Barcode of Life (http://www.barcoding.si.edu), the COI gene was selected as the principal barcoding marker. In addition, a portion of the 16S rRNA gene was used as a secondary marker. The aim with the project was to demonstrate the power and usefulness of DNA barcoding by producing a DNA identification key for all Swedish vertebrates that can serve as tool for identifying animal and plant products subject to trade (both legal and illegal), fisheries management, nutrient control, etc.

A main report was written in April 2012 by Dario Zuccon who also conducted most of the laboratory work in the project. In the summer 2012 Veronica Nyström completed the report in several important aspects. Among other things, it included results from several species that had not been available to Zuccon and some taxonomic changes were also made in the tables. Nyström also describe some new primers and primer combinations that allowed the sequencing of species for which the amplification previously had failed, especially some bird species.

The texts in Zuccon's and Nyström's reports are partly overlapping and the current report combines them. The tables of number of sequenced species and individuals are from Nyström's report. Also the detailed descriptions of laboratory methods and samples in section 6 are from Nyström's report.

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Project "A DNA key to Swedish Vertebrates"

1. Introduction

All species can be identified by unique DNA sequences. DNA barcoding is a global initiative that aims to produce a catalogue of unique sequences for all the species of the world. DNA barcodes will allow non-specialists to accurately identify species, and since barcodes work on all life stages (eggs, sperm, seed, larvae), as well as on incomplete specimens or small fragments (food ingredients, forensic evidence, stomach contents), it can be used for a wide range of applications. Potential applications include food control, fisheries management and identification of products of animals and plants subject to restrictions in national and international trade (e.g., CITES).

The project "Barcoding the Swedish Vertebrates" has been carried out between 2006 and 2011 at the Molecular Systematics Laboratory (Swedish Museum of Natural History). It focused on all vertebrates occurring regularly within the Swedish territory, i.e. mainland and adjacent waters. In accordance with the barcoding standards set out by the Consortium for the Barcode of Life (http://www.barcoding.si.edu), the COI gene has been selected as the principal barcoding marker. In addition, a portion of the 16S rRNA gene has been used as a secondary marker.

We have demonstrated the power and usefulness of DNA barcoding by producing a DNA identification key for 75% of all Swedish vertebrates (491 out of 657 species). We have also established a model for continued barcoding activities in Sweden, by developing guidelines for collection and for long-term storage of vouchers and material intended for DNA studies. The DNA sequences are being published on the NRM website where it is also possible to compare unknown sequences with this new library of sequences from Swedish vertebrates. We will also make available at the website a guide, directed to collectors and end-users and adapted for Swedish conditions, produced to specify conditions for obtaining high quality tissue and appropriate procedures for PCR, amplification, sequencing, alignment and storage of sequencer output, tissue and vouchers.

2. Samples

Species inclusion criteria. The species included in the checklist of the terrestrial Vertebrates are those considered of regular occurrence within the Swedish territory and nearby waters, including alloctonous species with regular populations in Sweden. Vagrants, escapes or any other category of non-regular occurrence has been excluded. We used standard field guides to define the status of Swedish species for mammals, amphibians and reptiles. The bird checklist follows strictly the SFO checklist. The fish checklist has been provided by Sven Kullander.

Taxonomy. The bird taxonomy follows the Clement Checklist, in agreement with the ABBI recommendations. The fish taxonomy agrees with the FishBase checklist. For mammals, amphibians and reptiles we followed the traditional nomenclature. Before a publication/release of the barcoding data for these three classes a specialist should be consulted for an updated taxonomy, especially for bats, shrews and rodents.

Sample coverage. The sample coverage is good for mammals and birds only, rather good for fishes and poor for amphibians and reptiles (Table 1). In particular, for mammals, birds and fishes, all common or rather common species have been included in the barcoding project. The missing birds missing are either rare, occurring in low number as migrants, winter or summer visitors. For mammals, only few bats and shrews, and one dolphin are missing.

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Table 1: Samples of Swedish vertebrates sequenced in the project until June 2012.

	Mammals	Birds	Herps	Fishes
Swedish species	77	307	23	253
Species obtained	70 (88.6%)	275 (89.6%)	10 (43.5%)	159 (62.8%)
Species with 1 sample only	7 (8.9%)	38 (12.4%)	4 (17.4%)	34 (13.4%)
Species with 2+ samples	63 (79.7%)	238 (77.5%)	6 (26.1%)	125 (49.4%)
Missing species	9 (11.4%)	32 (10.4%)	13 (56.5%)	94 (37.2%)
Extracted samples	181	534	17	286
Sequences: 16S (completed)	177 (97.8%)	529 (99.1%)	16 (94.1%)	280 (97.9%)
Sequences: COI (completed)	175 (96.7%)	530 (99.3%)	14 (82.4%)	273 (95.5%)

Amphibians and reptiles are the least sampled groups, reflecting the lack of a specific tissue collection in the museum. All obtained samples were either animal found dead by staff members during the last two years, or provided thanks to the contact of Stefan Lundberg with an external research group. A few mammals and birds samples were obtained from Ajtte, Svenskt fjäll- och samemuseum (6 samples) and Göteborgs naturhistoriska museum (7 samples). The tissue samples are currently stored within the NRM tissue collections, while the vouchers remain in the respective original museum.

When multiple samples were available for a species, we selected the samples using three criteria: 1) presence of a voucher; 2) collection during the breeding season (birds only); 3) geographic origin.

The majority of terrestrial vertebrate samples have an associated voucher, and almost all fishes. The exception is represented by about 40 bat samples. They belong to two species difficult to identify morphologically, *Myotis brandti* and *M. mystacinus*. They were used as a test of the reliability of 16S as a barcoding marker (see below).

3. Sequencing COI

The selected barcoding region is a portion of the COI (cytochrome oxydase I) gene of 648bp, starting at position 58 of the mouse genome.

Fishes. The sequencing of COI in fishes proved to be easy and the published protocol by Ward et al. (2005) worked well. Ward et al. (2005) suggested four primers, a primary couple (FishF1 and FishR1), plus two alternative primers (FishF2 and FishR2).

With very few exceptions (<10 samples), the primers amplified a single, strong band. With the primary primer couple we amplified 80% of samples, and an additional 15% with one of the other primer combinations. Only 5% of samples did not produce any amplification product, the same proportion observed by Ward et al. (2005) for Australian fishes.

Amphibians and Reptiles. For amphibians and reptiles we followed the protocol of Smith et al. (2008). The protocol seems to be reliable, although the number of species tested is limited, only 10. We obtained good amplification products for all species but one. Both individuals of *Rana arvalis* did not produce any product, and it is possibly due to a primer mismatch.

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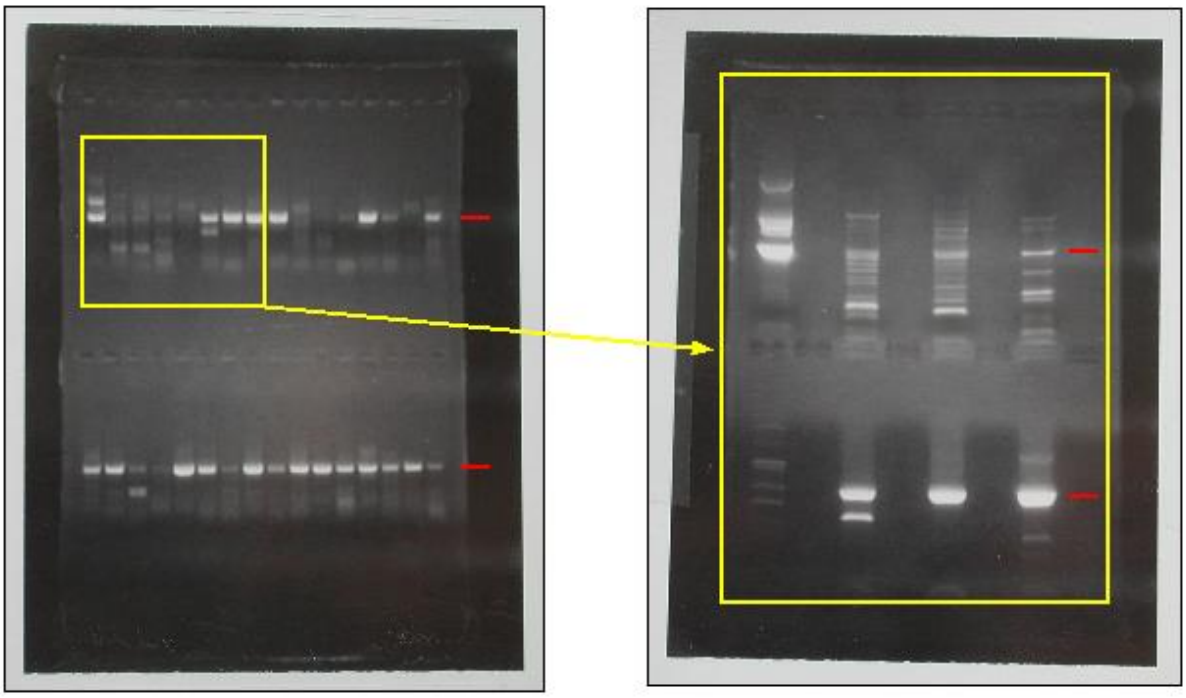

Figure 1. Left: results of the amplification of the COI gene for some birds species using the Hebert et al.'s (2004) protocol, visualised on a 0.8% agarose gel. Only a minority of samples produce a single bands. In many cases extra bands and primer dimmers are clearly visible. Right: the first eight samples from the left image are run on a 1.5% agarose gel to improve the band separation. Only one out of eight samples produces a single bands. Red tags indicate the size of the expected amplification products. The species in the left image are (left to right, top to bottom): *Corvus corax*, *Hirundo rustica*, *Loxia curvirostra*, *Motacilla alba*, *Anser anser*, *Buteo buteo*, *Strix nebulosa*, *Muscicapa striata*.

Birds. Contrary to several publishes statements, the sequencing of COI for birds proved to be extremely difficult, the published protocols unreliable and with poor repeatability. At first, we followed the indication of Hebert et al. (2004), but only a minority of samples generated a single, clean band of expected size. In most cases, the amplification according to the published protocol generated multiple bands (see Fig. 1). Changes in the amplification profile, either number of cycles, denaturation/annealing/ extension time, annealing temperature, did not produce any significant improvement. The amplification problems were taxonomically widespread, affecting all bird groups, but Anatidae appeared especially difficult to amplify.

Given the substantial failure of the published protocol, we developed a different strategy that proved to be efficient and reliable. We designed bird specific primers annealing on the two flanking tRNA and used these to amplify the entire COI gene. The amplification product of this PCR was used as template for a nested PCR using the standard primers suggested by Hebert et al. (2004).

With the new protocol we obtained sequences from all bird species. In several cases the nested PCR produces concatamers (Fig. 2), but these do not affect the quality of the sequences. However, we obtained pseudogenes for two species. One sample of *Glaucidium passerinum* produced a pseudogene, but the correct sequence was obtained with a different primer combination. Instead both samples of *Bucephala clangula* generated a sequence that is rather similar to those obtained from North American and Norwegian samples, but in a phylogenetic tree it falls well outside the species clade. Using alternative primer combinations we obtained the same pseudogene sequence or no amplification at all.

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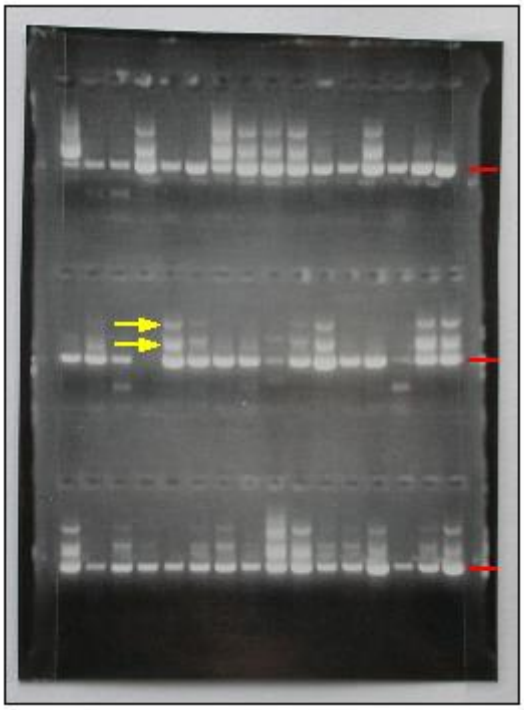

Figure 2. Results of the amplification of the COI gene for some birds species using the nested amplification protocol. The PCR products of the nested amplification are visualised on a 0.8% agarose gel. Almost all species tested generate the expected product. The concatamers are indicated with yellow arrows. Red tags indicate the size of the expected amplification products.

The entire dataset of the COI bird sequences has been submitted to the BOLD home page (www.barcodinglife.org) as the barcoding project "Birds of Sweden".

Mammals. Currently no primers suitable for the amplification of COI in all mammals have been proposed. Few studies were dedicated to barcoding mammals, but in all case these studies were focusing on specific taxa (e.g. pikas Lissovsky et al. 2007, bats Clare et al. 2007).

Due to the lack of robust protocols, no sequencing was attempted for mammals. All attempts to use on mammals the published protocols for birds and fishes failed to produce good quality amplification products.

4. Sequencing 16S rRNA

The mitochondrial gene for the 16S rRNA proved to be an excellent marker. The amplification protocol is exceptionally robust, and worked well with almost all samples. The region amplified is a portion of the third domain spanning about 550 bp.

I had problems in only two cases, one mammal and one bird. In birds the primer combination apparently does not produce any amplificate in *Limosa limosa*. However, only one sample is available for this species and a second sample should be tested. In mammals we obtained an aberrant sequence in all four samples of *Sicista betulina*. The last third of the sequence is matching well the sequences of closely related rodents, but the first portion is unreadable due to double peaks along the whole

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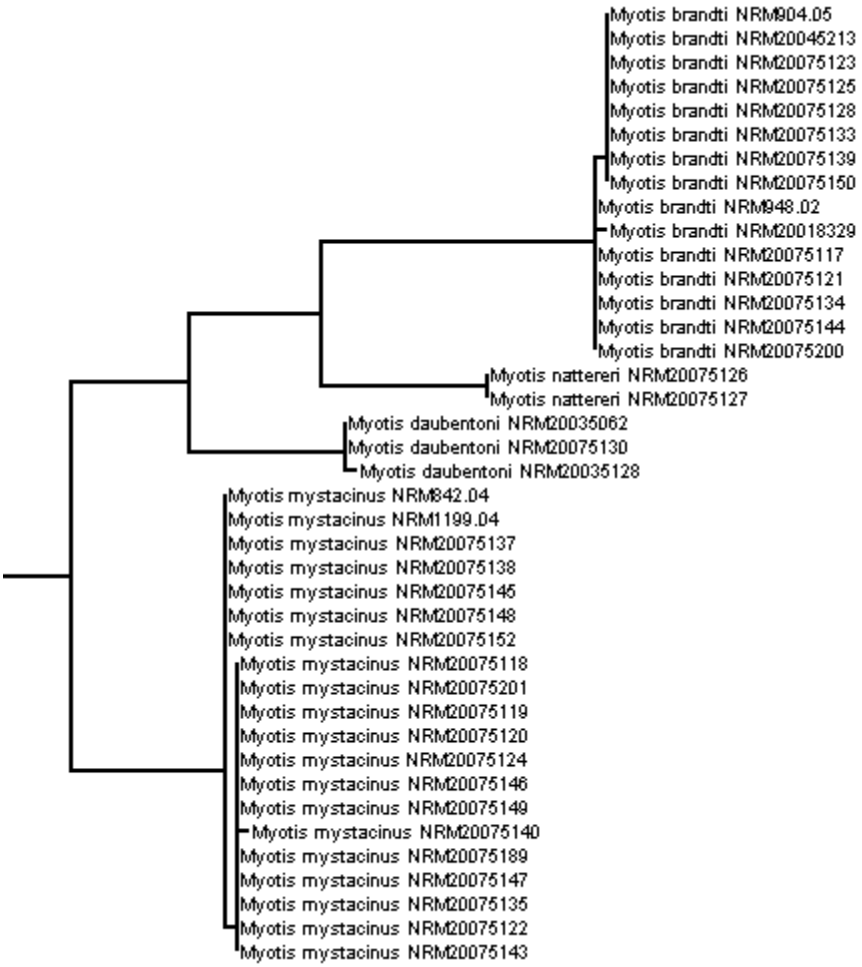

Figure 3. Neighbour-Joining tree of the *Myotis* bats obtained from the 16S rRNA sequences.

chromopherogram. It is likely that in this species the primer combination co-amplifies the target sequence plus a nuclear copy.

In all intraspecific comparisons, the genetic distances within species are null or very, very low. In general, almost all species can be identified using the 16S sequences alone. Few exceptions exist, but these involve species groups that proved to be difficult to separate genetically with any marker (e.g. *Carduelis hornemanni*/*C. flammea*), or groups showing extensive hybridization and gene introgression (e.g. large *Larus* gulls, *Anser* geese).

The reliability of the 16S rRNA sequences as an identification tool has been tested with a group of bat samples belonging to two species difficult to identify morphologically, *Myotis brandti* and *M. mystacinus*. Some specimens were in poor condition or too young to be confidently identified. The sequencing proved that the 16S sequences are suitable to identify these bat species, with a minimal intraspecific variability (Fig. 3).

The 16S sequences present alternated highly conserved and very variable regions that correspond to the loop and stems of the secondary structure of the 16S rRNA unit. This organization makes the sequences difficult to align in the loop regions, even among closely related species. On the contrary, the stem regions are so conserved that few mutations exist among the Vertebrates (Fig. 4). Thanks to this property of the 16S, we designed a primer couple that can amplify a very short fragment of 120-140bp in all

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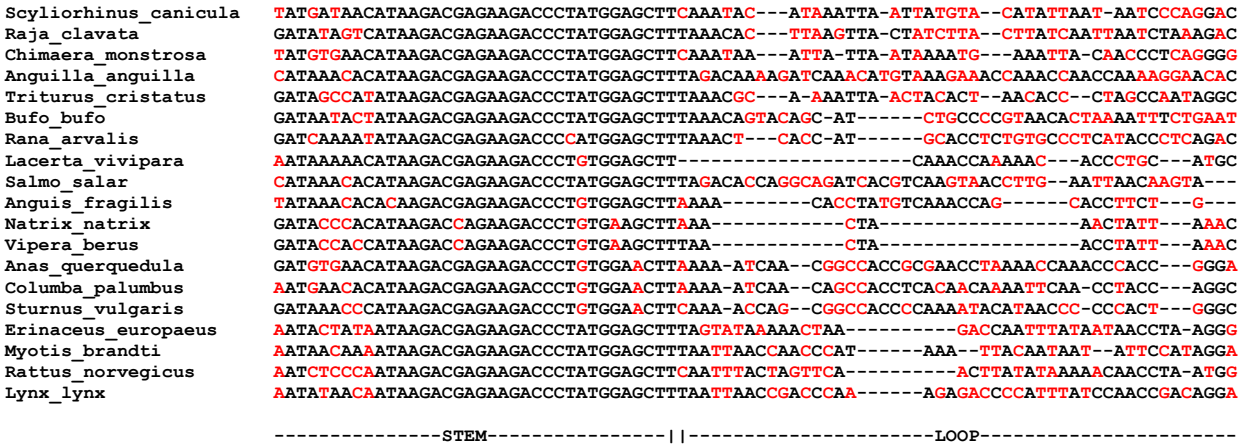

Figure 4. Alignment of a portion of the 16S rRNA sequences of selected vertebrates showing the conserved stem and hypervariable loop regions.

vertebrates. It is especially useful when only short fragment can be amplified via PCR due to degraded DNA. The region covered by this fragment includes a long hypervariable loop and it is suitable for identifying the majority of Vertebrate species at the species level. The primer sequences are:

16SintF: 5'-ACA TAA GAC GAG AAG ACC CTG TGG A-3'
16SintR: 5'-CAA GGT CGC CCC AAC CrA A-3'

5. Amplification protocols

Amplification protocol for COI in Fishes

Primers:

FishF1: 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3'
FishF2: 5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3'
FishR1: 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'
FishR2: 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3'

Key reference: Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Series B, Biological Science, 360: 1847-1857.

Amplification profile:

initial denaturation 5' at 95°C
35 cycles of denaturation 30" at 95°C, annealing 30" at 55°C, extension 60" at 72°C
final extension 8' at 72°C

The primer couple FishF1 and FishR1 is the best primer combination and it works for about 80% of samples. Alternative combinations may be used in case of amplification failure.

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Amplification protocol for COI in Amphibians and Reptiles

Primers:

LepF1: 5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3'
LepRI: 5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3'

Key reference: Smith MA, Poyarkov NA Jr & Hebert PDN. 2008. CO1 DNA barcoding amphibians: take the chance, meet the challenge. Molecular Ecology Resources 8: 235-246.

Amplification profile:

initial denaturation 5' at 95°C
35 cycles of denaturation 30" at 95°C, annealing 30" at 52°C, extension 60" at 72°C
final extension 8' at 72°C

Notes: the sample tested is small, just 10 species, but it the protocol seems to work well. In only one species, *Rana arvalis*, I never obtained any products (two individual tested).

Amplification protocol for COI in Birds

Published primers:

BirdF1: 5'-TTC TCC AAC CAC AAA GAC ATT GGC AC-3'
BirdR1: 5'-ACG TGG GAG ATA ATT CCA AAT CCT G-3'
BirdR2: 5'-ACT ACA TGT GAG ATG ATT CCG AAT CCA G-3'
BirdR3: 5'-AGG AGT TTG CTA GTA CGA TGC C-3'

Key reference: Hebert PDN, Stoeckle MY, Zemlak TS & Francis CM. 2004. Identification of birds through DNA barcodes. PLoS Biol. 2: 1657-1663.

Unpublished primers:

CO1-ExtF: 5'-ACG CTT TAA CAC TCA GCC ATC TTA CC-3'
CO1-ExtR: 5'-AAC CAG CAT ATG AGG GTT CGA TTC CT-3'

Amplification profile: first PCR using CO1-ExtF and CO1-ExtR

initial denaturation 5' at 95°C
2 cycles of denaturation 30" at 95°C, annealing 30" at 66°C, extension 50" at 72°C
2 cycles of denaturation 30" at 95°C, annealing 30" at 64°C, extension 50" at 72°C
16 cycles of denaturation 30" at 95°C, annealing 30" at 62°C, extension 50" at 72°C
final extension 8' at 72°C

Amplification profile: nested PCR using internal primers

initial denaturation 5' at 95°C
4 cycles of denaturation 30" at 95°C, annealing 30" at 64°C, extension 10" at 72°C
4 cycles of denaturation 30" at 95°C, annealing 30" at 62°C, extension 10" at 72°C
22 cycles of denaturation 30" at 95°C, annealing 30" at 60°C, extension 10" at 72°C
final extension 8' at 72°C

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Notes: CO1-ExtF and BirdR1 are the best primer combination for the nested PCR. In case of failure, it is possible to use BirdR2. If even the second combination fails, one of the other primer combinations should work.

Amplification protocol for 16S rRNA

Primers:

16SARL: 5'-CGC CTG TTT ATC AAA AAC AT-3'
16SBRH: 5'-CCG GTC TGA ACT CAG ATC ACG T-3'

Key reference: Palumb SR. 1996. Nucleic acids II: The polymerase chain reaction. Pp. 205-247. In: Hillis DM, Moritz C & Mable BK. (Eds.) Molecular Systematics, second edition. Massachusetts: Sinauer.

Amplification profile:

initial denaturation 5' at 95°C
35 cycles of denaturation 30" at 95°C, annealing 30" at 55°C, extension 50" at 72°C
final extension 8' at 72°C

Notes: it worked well in almost all samples tested. Neither secondary bands nor primer dimmers have been observed.

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6. Supplementary information

Samples

To complement the DNA-analyses performed within the project "A DNA key to Swedish vertebrates", 26 new bird samples, of which one individual had been sampled before (GNM2008-21.562:12), and 18 new mammal samples were analysed for both COI and 16S. The samples were extracted using Molestrips DNA tissue kit (GeneMole) and amplified according to the PCR-profiles below. In addition, all previously extracted mammal samples were analysed for COI, and samples that had failed to amplify for 16S were re-run. Bird samples that had failed to amplify for either COI or 16S were also re-run. No samples of herps or fishes were analysed.

During the process, it turned out that some of the samples seem to have been mislabeled. The species names for the following individuals were changed:

- NRM20018289: This individual has been changed from *Sorex isodon* to *S. araneus* (matches the other *S. araneus* samples for both markers).
- NRM20035128: This individual has been changed from *Myotis mystacinus* to *M. daubentonii* (matches the other *M. daubentonii* samples for both markers).
- NRM20085064: This individual has been changed from *Myodes/Clethrionomys rufocanus* to *Myopus schisticolor* (matches the other *Myopus schisticolor* samples for both markers)
- NRM20065003 and NRM20065004: These individuals have been changed from *Apodemus sylvaticus* to *A. flavicollis* (matches the other *A. flavicollis* samples for both markers).
- NRM996492: This individual has been changed from *Strix uralensis* to *S. aluco* (matches the other *S. aluco* for both markers).

Some of the accession numbers and specimen details were also found to be incorrect and were changed:

- GNM21562-2 has been changed to GNM2005-21.562:2
- GNM21754-1 has been changed to GNM2006-21.742:1. This individual is a juvenile and not an adult.
- GNM21754-7 has been changed to GNM2006-21.742:7
- NRM.A197310192 has been changed to NRM.A197300192. This individual is a male.
- GNM20600-3 has been changed to GNM2000-20.600:3. This individual is a female.
- NRM20006268: The sex and collection date is unknown for this individual.

Specimen details and sequences for the new bird samples have been submitted to the barcoding project "SWEBI" in the Barcode of Life Data System (http://www.barcodinglife.org). Thirty-one of the old bird specimens that were reported as "submitted" (submission dates 2008-11-21, 2007-08-15 and 2008-06-04) could not be found in the SWEBI-project on BOLD (probably not moved/copied from the BISE-project). Of these, four samples did not seem to have worked for COI and one individual was not from Sweden (see excel file "Bird SpecimenData VN120223"). Six of these 31 samples were re-run since the existing sequences were of poor quality.

Sequencing of COI

Mammals. Four new primers (MamF1, MamF2, MamR1, MamR2) were designed for the amplification of COI in mammals. Using the PCR-protocol below, the primer pair MamF1 and MamR1 worked well for the majority of samples and produced single, strong bands. One of the two *Mustela vison* samples (NRM20025118) and the only sample of *Globicephala melas* (NRM20055004) failed to amplify for COI with all primer combinations (but worked for 16S). The four samples of *Microtus agrestis* produced non-matching sequences. Sequences from three of the samples (NRM20075243, NRM20075106,

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NRM20085001) resembled previously published sequences of *Microtus agrestis*. However, when one of these samples (NRM20075243) was re-run it produced a non-matching sequence (ca 80 bp mismatch). This sequence resembled the sequence obtained from the forth sample (NRM20025173) but did not match any sequence on BOLD or GenBank.

Birds. The primer combination COI-ExtF (Johnsen *et al.* 2010) and VR1 (also called FishR1, Ward *et al.* 2005) worked well for the majority of bird samples and produced single, strong bands. This primer pair was successfully used for most of the new samples and also for six samples that had failed to amplify with other primer combinations (NRM986236 *Luscinia luscinia*, NRM976163 *Sitta europaea*, NRM20006689 *Pluvialis squatarola*, NRM986593 *Dendrocopos minor*, NRM996289 *Streptopelia decaocto*, NRM996391 *Anser erythropus*).

Sequencing of 16S rRNA

Amplification and sequencing of 16S was performed according to the protocol on page 8 in the original report. All new samples, including a second *Limosa limosa* sample, worked well and produced single, strong bands. The first *Limosa limosa* sample (NRM20016233) was re-run but failed to amplify. One of the three *Sterna caspia* samples (NRM.A1970/10274) and the *Balaenoptera acutorostrata* sample (NRM20035136) that had not worked before were however successfully re-run. The four samples of *Sicista betulina* were also re-run but still produced aberrant sequences, possibly caused by co-amplification of a numt.

Amplification protocol for COI in Mammals

Primers:

MamF1: 5’-TCA GCC ATT TTA CCT ATG TTC AT-3’
MamF2: 5’-TTC TCA ACT AAC CAC AAA GAT ATC G-3’
MamR1: 5’-ACT TCA GGG TGT CCG AAG AAT CA-3’
MamR2: 5’-ACT TCA GGG TGA CCA AAR AAT CA-3’

Amplification profile:

Initial denaturation 5’ at 95°C
3 cycles of denaturation 30’’ at 95°C, annealing 30’’ at 58°C, extension 50’’ at 72°C
32 cycles of denaturation 30’’ at 95°C, annealing 30’’ at 56°C, extension 50’’ at 72°C
Final extension 8’ at 72°C

The primer pair MamF1 and MamR1was the best primer combination and worked well for most of the samples. Alternative combinations can be used in case of amplification failure.

Amplification protocol for COI in Birds

Primers:

COI-ExtF: 5’-ACG CTT TAA CAC TCA GCC ATC TTA CC-3’
VR1 (FishR1): 5’-TAG ACT TCT GGG TGG CCA AAG AAT CA-3’

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References: Johnsen A, Rindal E, Ericson PGP, Zuccon D, Kerr KCR, Stoeckle MY & Lifjeld JT. 2010. DNA barcoding of Scandinavian birds reveals divergent lineages in trans-Atlantic species. Journal of Ornithology 151(3): 565-578.

Ward RD, Zemlak TS, Innes BH, Last PR, Herbert PDN. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 360: 1847-1857.

Amplification profile:

Initial denaturation 5' at 95°C
3 cycles of denaturation 30'' at 95°C, annealing 30'' at 64°C, extension 50'' at 72°C
32 cycles of denaturation 30'' at 95°C, annealing 30'' at 62°C, extension 50'' at 72°C
Final extension 8' at 72°C

The primer pair COI-ExtF and VR1 worked well for most of the samples. In case of amplification failure, alternative primers and amplification profiles can be found in the original report.

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7. Updated list of barcodes of Swedish Vertebrates obtained in the project

The number of samples extracted and the number of COI and 16S sequences obtained for each species. The species for which no samples were obtained are underlined.

Table 2: Samples of Swedish fishes sequenced in the project until June 2012.

Higher taxon	Species	Extracted samples	COI sequences	16S sequences
AMPHIOXIFORMES				
Branchiostomatidae	*Branchiostoma lanceolatum*	2	1	2
MYXINIFORMES				
Myxinidae	*Myxine glutinosa*	2	1	1
PETROMYZONTIFORMES				
Petromyzontidae	*Lampetra fluviatilis*	2	2	2
	Lampetra planeri	2	2	2
	Petromyzon marinus	2	2	2
CHIMAERIFORMES				
Chimaeridae	*Chimaera monstrosa*	2	2	2
LAMNIFORMES				
Alopiidae	*Alopias vulpinus*			
	Cetorhinus maximus			
	Lamna nasus			
CARCHARHINIFORMES				
Triakidae	*Galeorhinus galeus*	2	2	2
	Mustelus asterias	2	2	2
Carcharhinidae	*Carcharhinus longimanus*			
	Prionace glauca			
Pentanchidae	*Galeus melastomus*			
Scyliorhinidae	*Scyliorhinus canicula*	2	2	2
	Scyliorhinus stellaris			
SQUALIFORMES				
Etmopteridae	*Etmopterus spinax*	2	2	2
Somniosidae	*Somniosus microcephalus*			
Squalidae	*Squalus acanthias*	2	2	2
SQUATINIFORMES				
Squatinidae	*Squatina squatina*			
RAJIFORMES				
Rajidae	*Amblyraja radiata*	2	2	2
	Dipturus batis			
	Dipturus linteus			
	Dipturus nidarosiensis			
	Dipturus oxyrinchus			
	Leucoraja fullonica			
	Leucoraja naevus			
	Raja clavata	2	2	2

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MYLIOBATIFORMES				
Dasyatididae	*Dasyatis pastinaca*			
Myliobatidae	*Myliobatis aquila*			
ACIPENSERIFORMES				
Acipenseridae	*Acipenser baeri*			
	Acipenser gueldenstaedti			
	Acipenser ruthenus			
	Acipenser oxyrinchus			
	Huso huso			
ANGUILLIFORMES				
Anguillidae	*Anguilla anguilla*	2	2	2
Congridae	*Conger conger*			
Nemichthyidae	*Nemichthys scolopaceus*	1	1	1
CLUPEIFORMES				
Clupeidae	*Alosa alosa*	2	2	2
	Alosa fallax	2	2	2
	Clupea harengus	2	2	2
	Sardina pilchardus			
	Sprattus sprattus	2	2	2
Engraulididae	*Engraulis encrasicolus*	1	1	1
CYPRINIFORMES				
Cyprinidae	*Abramis ballerus*	2	2	2
	Abramis brama	2	2	2
	Abramis bjoerkna	2	2	2
	Abramis vimba	1	1	1
	Alburnus alburnus	2	2	2
	Aspius aspius	2	2	2
	Carassius carassius	2	2	2
	Ctenopharyngodon idella			
	Cyprinus carpio	1	1	1
	Gobio gobio	2	2	2
	Hypophthalmichthys molitrix			
	Hypophthalmichthys nobilis			
	Leucaspius delineatus	2	2	2
	Leuciscus idus	2	2	2
	Leuciscus leuciscus	2	2	2
	Pelecus cultratus	2	2	2
	Phoxinus phoxinus	2	2	2
	Rutilus rutilus	2	2	2
	Scardinius erythrophthalmus	2	2	2
	Squalius cephalus	1	1	1
	Tinca tinca	2	2	2
Cobitidae	*Cobitis taenia*	2	2	2
Balitoridae	*Barbatula barbatula*	2	2	2

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SILURIFORMES				
Siluridae	*Silurus glanis*	2	2	2
ARGENTINIFORMES				
Argentinidae	*Argentina silus*	2	2	2
	Argentina sphyraena	2	2	2
OSMERIFORMES				
Osmeridae	*Mallotus villosus*			
	Osmerus eperlanus	2	2	2
SALMONIFORMES				
Coregonidae	*Coregonus albula*	2	2	2
	Coregonus maraena			
	Coregonus maxillaris			
	Coregonus megalops			
	Coregonus nilssoni	1	1	1
	Coregonus pallasii			
	Coregonus peled	1	1	1
	Coregonus trybomi	2	2	2
	Coregonus widegreni			
Salmonidae	*Hucho hucho*			
	Oncorhynchus clarki			
	Oncorhynchus gorbuscha			
	Oncorhynchus kisutch			
	Oncorhynchus mykiss			
	Oncorhynchus nerka			
	Salmo salar	2	2	2
	Salmo trutta	2	2	2
	Salvelinus alpinus	2	2	2
	Salvelinus fontinalis	2	2	2
	Salvelinus namaycush			
	Salvelinus umbla			
	Thymallus thymallus	2	2	2
ESOCIFORMES				
Esocidae	*Esox lucius*	2	2	2
STOMIIFORMES				
Sternoptychidae	*Argyropelecus olfersii*			
	Maurolicus muelleri			
Paralepididae	*Arctozenus risso*			
	Magnisudis atlantica	1	1	1
Myctophidae	*Notoscopelus kroyeri*	1	1	1
LAMPRIDIFORMES				
Lampridae	*Lampris guttatus*	1	1	1
Regalecidae	*Regalecus glesne*			
Trachipteridae	*Trachipterus arcticus*	1	1	1
GADIFORMES				
Gadidae	*Gadiculus argenteus*	1	1	1
	Gadus morhua	2	2	2
	Melanogrammus aeglefinus	2	2	2

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	Merlangius merlangus	2	2	2
	Micromesistius poutassou	2	2	2
	Pollachius pollachius			
	Pollachius virens	2	2	2
	Trisopterus esmarkii	3	3	3
	Trisopterus luscus	1	0	1
	Trisopterus minutus	2	2	2
Lotidae	*Brosme brosme*	2	2	2
	Ciliata mustela	2	2	2
	Ciliata septentrionalis			
	Enchelyopus cimbrius	2	2	2
	Gaidropsarus vulgaris	2	2	2
	Lota lota	2	2	2
	Molva dypterygia			
	Molva molva	2	2	2
Macrouridae	*Coryphaenoides rupestris*	2	2	2
	Malacocephalus laevis			
Merlucciidae	*Merluccius merluccius*	2	2	2
Phycidae	*Phycis blennoides*			
Ranicipitidae	*Raniceps raninus*	2	2	2
OPHIIDIFORMES				
Carapidae	*Echiodon drummondi*	1	0	1
LOPHIIFORMES				
Lophiidae	*Lophius piscatorius*	2	2	2
ZEIFORMES				
Zeidae	*Zeus faber*	2	2	2
BERYCIFORMES				
Berycidae	*Beryx decadactylus*			
BELONIFORMES				
Belonidae	*Belone belone*	2	2	2
Scomberesocidae	*Scomberesox saurus*			
GASTEROSTEIFORMES				
Macroramphosidae	*Macroramphosus scolopax*			
Gasterosteidae	*Gasterosteus aculeatus*	2	2	2
	Pungitius pungitius	2	2	2
	Spinachia spinachia	2	2	2
Syngnathidae	*Entelurus aequoraeus*	1	1	1
	Nerophis lumbriciformis			
	Nerophis ophidion	1	1	1
	Syngnathus acus	1	1	1
	Syngnathus rostellatus	1	1	1
	Syngnathus typhle	2	2	2
MUGILIFORMES				
Mugilidae	*Chelon labrosus*	2	2	2
	Liza aurata			
	Liza ramada			

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SCORPAENIFORMES				
Sebastidae	*Helicolenus dactylopterus*	3	3	3
	Sebastes norvegicus			
	Sebastes viviparus	2	2	2
Triglidae	*Chelidonichthys cuculus*	1	1	1
	Chelidonichthys gurnardus	2	2	2
	Chelidonichthys lastoviza	1	1	1
	Chelidonichthys lucerna	2	2	2
	Trigla lyra			
Cottidae	*Artediellus atlanticus*			
	Cottus gobio	2	2	2
Cottidae	*Cottus koshewnikowi*			
	Cottus poecilopus	1	1	1
	Cottus poecilopus			
	Icelus bicornis			
	Micrenophrys lilljeborgii			
	Myoxocephalus scorpius	2	2	2
	Taurulus bubalis	2	2	2
	Triglops murrayi	2	2	1
	Triglopsis quadricornis	2	2	2
Agonidae	*Agonus cataphractus*	2	2	2
Cyclopteridae	*Cyclopterus lumpus*	2	2	2
Liparidae	*Liparis liparis*			
	Liparis montagui	1	1	1
PERCIFORMES				
Moronidae	*Dicentrarchus labrax*	2	2	2
Polyprionidae	*Polyprion americanus*			
Centrarchidae	*Micropterus dolomieu*			
	Micropterus salmoides			
Percidae	*Gymnocephalus cernua*	2	2	2
	Perca fluviatilis	2	2	2
	Sander lucioperca	2	2	2
Carangidae	*Trachurus trachurus*	2	2	2
	Naucrates ductor			
	Trachinotus ovatus			
Bramidae	*Brama brama*	1	1	1
	Pterycombus brama	1	1	1
Sparidae	*Boops boops*	1	1	1
	Oblada melanurus			
	Pagellus acarne			
	Pagellus bogaraveo			
	Pagellus erythrinus			
	Sparus aurata			
	Spondyliosoma cantharus			

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Sciaenidae	*Argyrosomus regius*			
Mullidae	*Mullus surmuletus*	2	2	2
Labridae	*Acantholabrus palloni*	2	2	2
	Centrolabrus exoletus	2	2	2
	Centrolabrus exoletus			
	Ctenolabrus rupestris	2	2	2
	Labrus bergylta	2	2	2
	Labrus mixtus	2	2	2
	Symphodus melops	2	2	2
Zoarcidae	*Lycenchelys sarsii*			
	Lycodes gracilis	2	2	2
	Zoarces viviparus	2	2	2
Stichaeidae	*Chirolophis ascanii*			
	Leptoclinus maculatus			
	Lumpenus lampretaeformis			
Pholididae	*Pholis gunnellus*	2	2	2
Anarhichadidae	*Anarhichas denticulatus*			
	Anarhichas lupus	2	2	2
	Anarhichas minor	1	1	1
Ammodytidae	*Ammodytes marinus*	2	2	2
	Ammodytes tobianus	2	2	2
	Hyperoplus lanceolatus	2	2	2
Trachinidae	*Trachinus draco*	2	2	2
Gobiesocidae	*Diplecogaster bimaculata*			
Callionymidae	*Callionymus lyra*	2	2	2
	Callionymus maculatus	1	0	1
Gobiidae	*Aphia minuta*	1	1	1
	Crystallogobius linearis	1	1	1
	Gobius niger	2	2	2
	Gobiusculus flavescens	2	2	2
	Lebetus scorpioides			
	Lesueurigobius friesii	2	1	2
Gobiidae	*Pomatoschistus microps*	2	2	2
	Pomatoschistus minutus	2	2	2
	Pomatoschistus norvegicus	1	1	1
	Pomatoschistus pictus	1	1	1
	Thorogobius ephippiatus			
Gempylidae	*Lepidopus caudatus*			
	Nesiarchus nasutus	2	2	1
Scombridae	*Auxis rochei*			
	Euthynnus alletteratus			
	Katsuwonus pelamis			
	Orcynopsis unicolor			
	Sarda sarda			
	Scomber scombrus	2	2	2
	Thunnus thynnus			
Xiphiidae	*Xiphias gladius*	2	2	2
Centrolophidae	*Centrolophus niger*	2	1	1

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	Hyperoglyphe perciformis			
	Schedophilus medusophagus			
Caproidae	*Capros aper*	1	1	1
PLEURONECTIFORMES				
Bothidae	*Arnoglossus laterna*	1	1	1
Scophthalmidae	*Lepidorhombus whiffiagonis*			
	Phrynorhombus norvegicus	2	1	1
	Phrynorhombus norvegicus	1	0	0
	Psetta maxima	2	0	2
	Scophthalmus rhombus	2	0	2
	Zeugopterus punctatus	2	2	2
Pleuronectidae	*Glyptocephalus cynoglossus*	2	2	2
	Hippoglossoides platessoides	2	2	2
	Hippoglossus hippoglossus	1	1	1
	Microstomus kitt	2	2	2
	Platichthys flesus	2	2	2
	Pleuronectes limanda	2	2	2
	Pleuronectes platessa	2	2	2
Soleidae	*Buglossidium luteum*	1	1	1
	Solea solea	2	2	2
TETRAODONTIFORMES				
Balistidae	*Balistes capriscus*			
	Canthidermis maculata			
Molidae	*Mola mola*	2	2	2

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Table 3: Samples of Swedish amphibians and reptiles sequenced in the project until June 2012.

Higher taxon	Species	Extracted samples	COI sequences	16S sequences
AMPHIBIA				
URODELA				
Salamandridae	*Triturus cristatus*	2	2	2
	Triturus vulgaris	2	2	2
	Salamandra salamandra			
ANURA				
Bufonidae	*Bufo bufo*	1	1	1
	Bufo calamita	1	1	1
	Bufo viridis			
Discoglossidae	*Bombina bombina*			
Hylidae	*Hyla arborea*			
Pelobatidae	*Pelobates fuscus*			
Ranidae	*Rana agilis*			
	Rana arvalis	2	0	1
	Rana catesbeiana			
	Rana dalmatina			
	Rana esculenta			
	Rana lessonae			
	Rana temporaria	3	3	3
REPTILIA				
SQUAMATA				
Anguidae	*Anguis fragilis*	1	1	1
Lacertidae	*Lacerta agilis*			
	Lacerta vivipara	2	1	1
Colubridae	*Coronella austriaca*			
	Natrix natrix	2	2	2
Viperidae	*Vipera berus*	1	1	1

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Table 4: Samples of Swedish birds sequenced in the project until June 2012.

Higher taxon	Species	Extracted samples	COI sequences	16S sequences
GAVIIFORMES				
Gaviidae	*Gavia stellata*	2	2	2
	Gavia arctica	2	2	2
	Gavia immer			
	Gavia adamsii	1	1	1
PODICIPEDIFORMES				
Podicipedidae	*Tachybaptus ruficollis*	1	1	1
	Podiceps grisegena	2	2	2
	Podiceps cristatus	2	2	2
	Podiceps auritus	2	2	2
	Podiceps nigricollis			
PROCELLARIIFORMES				
Procellariidae	*Fulmarus glacialis*	2	2	2
	Puffinus puffinus	1	1	1
Hydrobatidae	*Oceanodroma leucorhoa*			
PELECANIFORMES				
Sulidae	*Morus bassanus*	2	2	2
Phalacrocoracidae	*Phalacrocorax carbo*	2	2	2
CICONIIFORMES				
Ardeidae	*Ardea cinerea*	2	2	2
	Ardea alba	1	1	1
	Botaurus stellaris	2	2	2
Ciconiidae	*Ciconia nigra*	1	1	1
	Ciconia ciconia	2	2	2
Threskiornithidae	*Plegadis falcinellus*	1	1	1
ANSERIFORMES				
Anatidae	*Cygnus olor*	2	2	2
	Cygnus cygnus	2	2	2
	Cygnus columbianus	1	1	1
	Anser fabalis	2	2	2
	Anser brachyrhynchus			
	Anser albifrons			
	Anser erythropus	3	3	3
	Anser anser	2	2	2
	Branta bernicla	2	2	2
	Branta leucopsis	2	2	2
	Branta canadensis	2	2	2
	Branta ruficollis			
	Tadorna tadorna	2	2	2
	Anas penelope	2	2	2
	Anas strepera	2	2	2
	Anas crecca	2	2	2
	Anas platyrhynchos	2	2	2
	Anas acuta	2	2	2
	Anas querquedula	2	2	2
	Anas clypeata	2	2	2
	Aythya ferina	2	2	2
	Aythya fuligula	2	2	2

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	Aythya marila	2	2	2
	Netta rufina	1	1	1
	Somateria mollissima	2	2	2
	Somateria spectabilis			
	Polysticta stelleri			
	Clangula hyemalis	2	2	2
	Melanitta nigra	2	2	2
	Melanitta fusca	1	1	1
	Bucephala clangula	2	2	2
	Mergellus albellus	2	2	2
	Mergus serrator	2	2	2
	Mergus merganser	2	2	2
FALCONIFORMES				
Pandionidae	*Pandion haliaetus*	2	2	2
Accipitridae	*Pernis apivorus*	2	2	2
	Milvus milvus	2	2	2
	Milvus migrans			
Accipitridae	*Haliaeetus albicilla*	2	2	2
	Circus aeruginosus	2	2	2
	Circus cyaneus	2	2	2
	Circus macrourus	2	2	2
	Circus pygargus	2	2	2
	Accipiter nisus	2	2	2
	Accipiter gentilis	2	2	2
	Buteo buteo	2	2	2
	Buteo lagopus	2	2	2
	Aquila pomarina			
	Aquila clanga	1	1	1
	Aquila chrysaetos	2	2	2
Falconidae	*Falco tinnunculus*	2	2	2
	Falco vespertinus	1	1	1
	Falco columbarius	2	2	2
	Falco subbuteo	2	2	2
	Falco rusticolus	2	2	2
	Falco peregrinus	2	2	2
GALLIFORMES				
Tetraonidae	*Lagopus lagopus*	2	2	2
	Lagopus muta	2	2	2
	Tetrao urogallus	2	2	2
	Tetrao tetrix	2	2	2
	Bonasa bonasia	2	2	2
Phasianidae	*Perdix perdix*	2	2	2
	Coturnix coturnix	2	2	2
	Phasianus colchicus	2	2	2
GRUIFORMES				
Gruidae	*Grus grus*	2	2	2
Rallidae	*Rallus aquaticus*	2	2	2
	Crex crex	2	2	2
	Porzana parva			
	Porzana porzana	1	1	1
	Gallinula chloropus	2	2	2
	Fulica atra	2	2	2

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CHARADRIIFORMES				
Haematopodidae	*Haematopus ostralegus*	2	2	2
Recurvirostridae	*Recurvirostra avosetta*	2	2	2
Charadriidae	*Vanellus vanellus*	3	3	3
	Pluvialis apricaria	3	3	3
	Pluvialis squatarola	2	2	2
	Charadrius hiaticula	2	2	2
	Charadrius dubius	3	3	3
	Charadrius alexandrinus			
	Charadrius morinellus	2	2	2
Scolopacidae	*Scolopax rusticola*	2	2	2
	Lymnocryptes minimus	2	2	2
	Gallinago media			
	Gallinago gallinago	2	2	2
	Limosa limosa	2	2	1
	Limosa lapponica	2	2	2
	Numenius phaeopus			
	Numenius arquata	2	2	2
	Tringa erythropus	2	2	2
	Tringa totanus	2	2	2
	Tringa nebularia	2	2	2
	Tringa ochropus	2	2	2
	Tringa glareola	3	3	3
	Xenus cinereus			
	Actitis hypoleucos	2	2	2
	Arenaria interpres	3	3	3
	Calidris canutus	2	2	2
	Calidris alba	1	1	1
	Calidris minuta	2	2	2
	Calidris temminckii	2	2	2
Scolopacidae	*Calidris ferruginea*	2	2	2
	Calidris alpina	2	2	2
	Calidris maritima	1	1	1
	Limicola falcinellus	2	2	2
	Philomachus pugnax	2	2	2
	Phalaropus lobatus	2	2	2
	Phalaropus fulicarius	1	1	1
Stercorariidae	*Stercorarius skua*	3	2	2
	Stercorarius pomarinus			
	Stercorarius parasiticus	1	1	1
	Stercorarius longicaudus	3	2	2
Laridae	*Larus canus*	2	2	2
	Larus marinus	2	2	2
	Larus hyperboreus			
	Larus argentatus	2	2	2
	Larus fuscus	2	2	2
	Larus cachinnans			
	Larus michahellis			
	Larus ridibundus	2	2	2
	Larus melanocephalus	1	1	1
	Larus minutus	2	2	2
	Xema sabini	2	2	2

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	Rissa tridactyla	2	2	2
	Rhodostethia rosea	2	2	2
Sternidae	*Sterna caspia*	3	3	3
	Thalasseus sandvicensis	2	2	2
	Sterna hirundo	2	2	2
	Sterna paradisaea	2	2	2
	Sternula albifrons	2	2	2
	Chlidonias leucopterus			
	Chlidonias niger	1	1	1
Alcidae	*Alle alle*	2	2	2
	Uria aalge	2	2	2
	Alca torda	2	2	2
	Cepphus grylle	3	3	3
	Fratercula arctica	2	2	2
COLUMBIFORMES				
Columbidae	*Columba livia*	2	2	2
	Columba oenas	2	2	2
	Columba palumbus	2	2	2
	Streptopelia turtur	1	1	1
	Streptopelia decaocto	2	2	2
CUCULIFORMES				
Cuculidae	*Cuculus canorus*	2	2	2
STRIGIFORMES				
Tytonidae	*Tyto alba*	2	2	2
Strigidae	*Bubo bubo*	2	2	2
	Bubo scandiacus	2	2	2
	Strix aluco	3	3	3
	Strix uralensis	2	2	2
	Strix nebulosa	2	2	2
	Surnia ulula	2	2	2
	Glaucidium passerinum	2	1	1
	Aegolius funereus	2	2	2
	Asio otus	2	2	2
	Asio flammeus	2	2	2
CAPRIMULGIFORMES				
Caprimulgidae	*Caprimulgus europaeus*	2	2	2
APODIFORMES				
Apodidae	*Apus apus*	2	2	2
CORACIIFORMES				
Alcedinidae	*Alcedo atthis*	2	2	2
Meropidae	*Merops apiaster*			
Coraciidae	*Coracias garrulus*	2	2	2
Upupidae	*Upupa epops*	2	2	2
PICIFORMES				
Picidae	*Jynx torquilla*	2	2	2
	Dendrocopos minor	2	2	2
Picidae	*Dendrocopos leucotos*	2	2	2
	Dendrocopos major	3	3	3
	Picoides tridactylus	2	2	2
	Dryocopus martius	2	2	2
	Picus viridis	2	2	2
	Picus canus	2	2	2

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PASSERIFORMES				
Alaudidae	*Lullula arborea*	1	1	1
	Alauda arvensis	2	2	2
	Eremophila alpestris			
Hirundinidae	*Riparia riparia*	2	2	2
	Hirundo rustica	2	2	2
	Delichon urbica	2	2	2
Motacillidae	*Motacilla alba*	2	2	2
	Motacilla citreola			
	Motacilla flava	2	2	2
	Motacilla cinerea	1	1	1
	Anthus campestris	1	1	1
	Anthus trivialis	2	2	2
	Anthus pratensis	2	2	2
	Anthus cervinus			
	Anthus petrosus			
	Anthus godlewskii	1	1	1
Regulidae	*Regulus regulus*	2	2	2
	Regulus ignicapillus	2	2	2
Bombycillidae	*Bombycilla garrulus*	2	2	2
Cinclidae	*Cinclus cinclus*	2	2	2
Troglodytidae	*Troglodytes troglodytes*	2	2	2
Prunellidae	*Prunella modularis*	2	2	2
Turdidae	*Turdus torquatus*	1	1	1
	Turdus merula	2	2	2
	Turdus pilaris	2	2	2
	Turdus iliacus	2	2	2
	Turdus philomelos	2	2	2
	Turdus viscivorus	2	2	2
Sylviidae	*Locustella naevia*	2	2	2
	Locustella fluviatilis			
	Locustella luscinioides	1	1	1
	Locustella lanceolata	2	2	2
	Acrocephalus schoenobaenus	2	2	2
	Acrocephalus scirpaceus	2	2	2
	Acrocephalus dumetorum			
	Acrocephalus palustris	3	3	3
	Acrocephalus arundinaceus	1	1	1
	Hippolais icterina	2	2	2
	Phylloscopus trochilus	2	2	2
	Phylloscopus collybita	2	2	2
	Phylloscopus fuscatus	1	1	1
	Phylloscopus humei	1	1	1
	Phylloscopus sibilatrix	2	2	2
	Phylloscopus proregulus	2	2	2
	Phylloscopus inornatus	2	2	2
	Phylloscopus borealis			
	Phylloscopus trochiloides	2	2	2

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	Sylvia atricapilla	2	2	2
	Sylvia borin	2	2	2
	Sylvia communis	2	2	2
	Sylvia curruca	2	2	2
	Sylvia nisoria	2	2	2
Muscicapidae	*Muscicapa striata*	2	2	2
	Ficedula hypoleuca	2	2	2
	Ficedula albicollis	2	2	2
Muscicapidae	*Ficedula parva*	1	1	1
	Erithacus rubecula	2	2	2
	Luscinia luscinia	2	2	2
	Luscinia svecica	2	2	2
	Phoenicurus ochruros	2	2	2
	Phoenicurus phoenicurus	2	2	2
	Saxicola rubetra	2	2	2
	Saxicola rubicola			
	Saxicola torquata	1	1	1
	Oenanthe oenanthe	2	2	2
	Oenanthe pleschanka	1	1	1
Paradoxornithidae	*Panurus biarmicus*	2	2	2
Aegithalidae	*Aegithalos caudatus*	2	2	2
Paridae	*Poecile palustris*	2	2	2
	Poecile montana	3	3	3
	Poecile cincta	1	1	1
	Periparus ater	2	2	2
	Lophophanes cristatus	2	2	2
	Parus major	2	2	2
	Cyanistes caeruleus	2	2	2
	Cyanistes cyanus	1	1	1
Sittidae	*Sitta europaea*	2	2	2
Certhiidae	*Certhia familiaris*	3	3	3
Remizidae	*Remiz pendulinus*	2	2	2
Oriolidae	*Oriolus oriolus*	2	2	2
Laniidae	*Lanius collurio*	2	2	2
	Lanius excubitor	2	2	2
	Lanius minor			
Corvidae	*Perisoreus infaustus*	2	2	2
	Garrulus glandarius	2	2	2
	Pica pica	2	2	2
	Nucifraga caryocatactes	3	3	3
	Corvus monedula	2	2	2
	Corvus frugilegus	2	2	2
	Corvus corone			
	Corvus cornix	2	2	2
	Corvus corax	2	2	2
Sturnidae	*Sturnus vulgaris*	2	2	2
Emberizidae	*Emberiza citrinella*	3	3	3
	Emberiza hortulana			
	Emberiza pusilla	2	2	2
	Emberiza rustica	2	2	2
	Emberiza schoeniclus	3	3	3
	Emberiza calandra	1	1	1

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	Calcarius lapponicus	2	2	2
	Plectrophenax nivalis	2	2	2
Fringillidae	*Fringilla coelebs*	2	2	2
	Fringilla montifringilla	2	2	2
	Pinicola enucleator	2	2	2
	Carpodacus erythrinus	3	3	3
	Loxia pytyopsittacus	2	2	2
	Loxia curvirostra	2	2	2
	Loxia leucoptera	2	2	2
	Carduelis chloris	2	2	2
	Carduelis flammea	2	2	2
	Carduelis hornemanni	3	3	3
	Carduelis spinus	2	2	2
	Carduelis carduelis	2	2	2
	Carduelis flavirostris	1	1	1
	Carduelis cannabina	1	1	1
	Serinus serinus	1	1	1
	Pyrrhula pyrrhula	2	2	2
	Coccothraustes coccothraustes	2	2	2
Passeridae	*Passer domesticus*	2	2	2
	Passer montanus	2	2	2

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Table 4: Samples of Swedish birds sequenced in the project until June 2012.

Higher taxon	Species	Extracted samples	COI sequences	16S sequences
INSECTIVORA				
Erinaceidae	*Erinaceus europaeus*	2	2	2
Soricidae	*Sorex minutissimus*	3	3	3
	Sorex minutus	2	2	2
	Sorex caecutiens			
	Sorex araneus	3	3	3
	Sorex sinalis			
	Sorex isodon			
	Neomys fodiens	2	2	2
Talpidae	*Talpa europaeus*	2	2	2
CHIROPTERA				
Myotidae	*Myotis mystacinus*	20	20	20
	Myotis brandtii	15	15	15
	Myotis nattereri	2	2	2
	Myotis bechsteini			
	Myotis daubentonii	3	3	3
	Myotis dasycneme			
Pipistrellidae	*Pipistrellus pipistrellus*			
	Pipistrellus pygmaeus	3	3	3
	Pipistrellus nathusii	2	2	2
Vespertilionidae	*Nyctalus noctula*	2	2	2
	Eptesicus nilssoni	2	2	2
	Eptesicus serotinus			
	Vespertilio murinus	2	2	2
	Plecotus auritus	2	2	2
	Barbastella barbastellus			
CARNIVORA				
Canidae	*Canis lupus*	2	2	2
	Vulpes vulpes	2	2	2
	Alopex lagopus	2	2	2
	Nyctereutes procyonides	2	2	2
Ursidae	*Ursus arctos*	2	2	2
Procyonidae	*Procion lotor*	1	1	1
Mustelidae	*Mustela erminea*	3	3	3
	Mustela nivalis	2	2	2
	Mustela vison	2	1	2
	Mustela putorius	2	2	2
	Martes martes	2	2	2
	Gulo gulo	2	2	2
	Meles meles	2	2	2
	Lutra lutra	2	2	2
Felidae	*Lynx lynx*	2	2	2
Phocidae	*Phoca vitulina*	2	2	2
	Phoca hispida	2	2	2
	Halichoerus grypus	2	2	2

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CETACEA				
Delphinidae	*Tursiops truncatus*			
	Lagenorhynchus albirostris	2	2	2
	Lagenorhynchus acutus	2	2	2
Phocoenidae	*Phocoena phocoena*	2	2	2
Balenopteridae	*Balaenoptera acutorostrata*	3	3	3
	Balaenoptera physalus	1	1	1
Delphinidae	*Delphinus delphis*	1	1	1
	Globicephala melas	1		1
ARTIODACTYLA				
Suidae	*Sus scrofa*	2	2	2
Cervidae	*Dama dama*	2	2	2
	Cervus elaphus	2	2	2
	Alces alces	2	2	2
	Rengifer tarandus	2	2	2
	Capreolus capreolus	2	2	2
Bovidae	*Ovibos moschatus*	2	2	2
RODENTIA				
Sciuridae	*Sciurus vulgaris*	2	2	2
Castoridae	*Castor fiber*	1	1	1
Cricetidae	*Lemmus lemmus*	2	2	2
	Myopus schisticolor	4	4	4
	Myodes rutilus	1	1	1
	Myodes rufocanus	3	3	3
	Myodes glareolus	2	2	2
	Arvicola terrestris	2	2	2
	Ondatra zibethicus	2	2	2
	Microtus agrestis	4		4
	Microtus oeconomus	1	1	1
Muridae	*Apodemus flavicollis*	4	4	4
	Apodemus sylvaticus	2	2	2
	Apodemus agrarius	2	2	2
	Rattus norvegicus	2	2	2
	Mus musculus	3	3	3
	Micromys minutus	3	3	3
Gliridae	*Muscardinus avellanarius*	2	2	2
Dipodidae	*Sicista betulina*	4	4	
LAGOMORPHA				
Leporidae	*Lepus europaeus*	2	2	2
	Lepus timidus	2	2	2
	Oryctolagus cuniculus	2	2	2

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