

DNA barcoding correctly identifies genera and species in a large shorebird clade

Rebecca Elbourne and Alan J. Baker
 Department of Natural History, Royal Ontario Museum, Toronto, ON
 and Department of Ecology and Evolutionary Biology University of Toronto, Toronto, ON

To test the effectiveness of DNA barcoding in a large globally distributed clade of birds we sequenced the standardized fragment of ~650bp from 87 genera, 228 species and 685 individuals in the Order Charadriiformes. To reduce the risk of amplifying pseudogenes we first amplified a ~1 Kb fragment and used internal sequencing primers on this template. The great majority of taxa could be reliably identified by their barcodes. Here we present the barcodes obtained for the family Scolopacidae since it is the most extensively barcoded of the larger shorebird families. With just 15 species out of 89 not yet barcoded and those missing concentrated in just 4 of the 22 genera, this data set provides an excellent opportunity to test the performance of barcodes in a large group of closely related taxa. Substantial within-species splits were found in Dunlin (*Calidris alpina*, 1.1% divergence), Black-tailed Godwit (*Limosa limosa*, 1.5% divergence), Redshank (*Tringa totanus*, 0.9%) and Whimbrel (*Numenius phaeopus*, 3.7%), though in no case were species clusters ambiguous as a result of intraspecific divergence. Barcoding confirms multigene evidence that Wilson's Snipe is not a separate species (*Gallinago delicata*) as listed in Clements (2005), but instead is better treated as a subspecies of the Common Snipe (*Gallinago g. Delicata*).

Introduction

Order Charadriiformes consists of 355 species of shorebirds in 19 families and > 80 genera (ABBI Species Checklist, 2007). A few examples include sandpipers, auklets, gulls, terns, plovers, jacana and snipes.

Many species are widely dispersed with populations on multiple continents. A good estimate of barcode variance must therefore include samples from disparate populations.

Here we report on the progress made in barcoding a large clade of shorebirds (the Scolopacidae) at the ROM



Methods

DNA extraction performed using 96-well glass fibre extraction plates following the non-automated version of an extraction protocol developed at the Biodiversity Institute of Ontario (Ivanova et al., 2006).

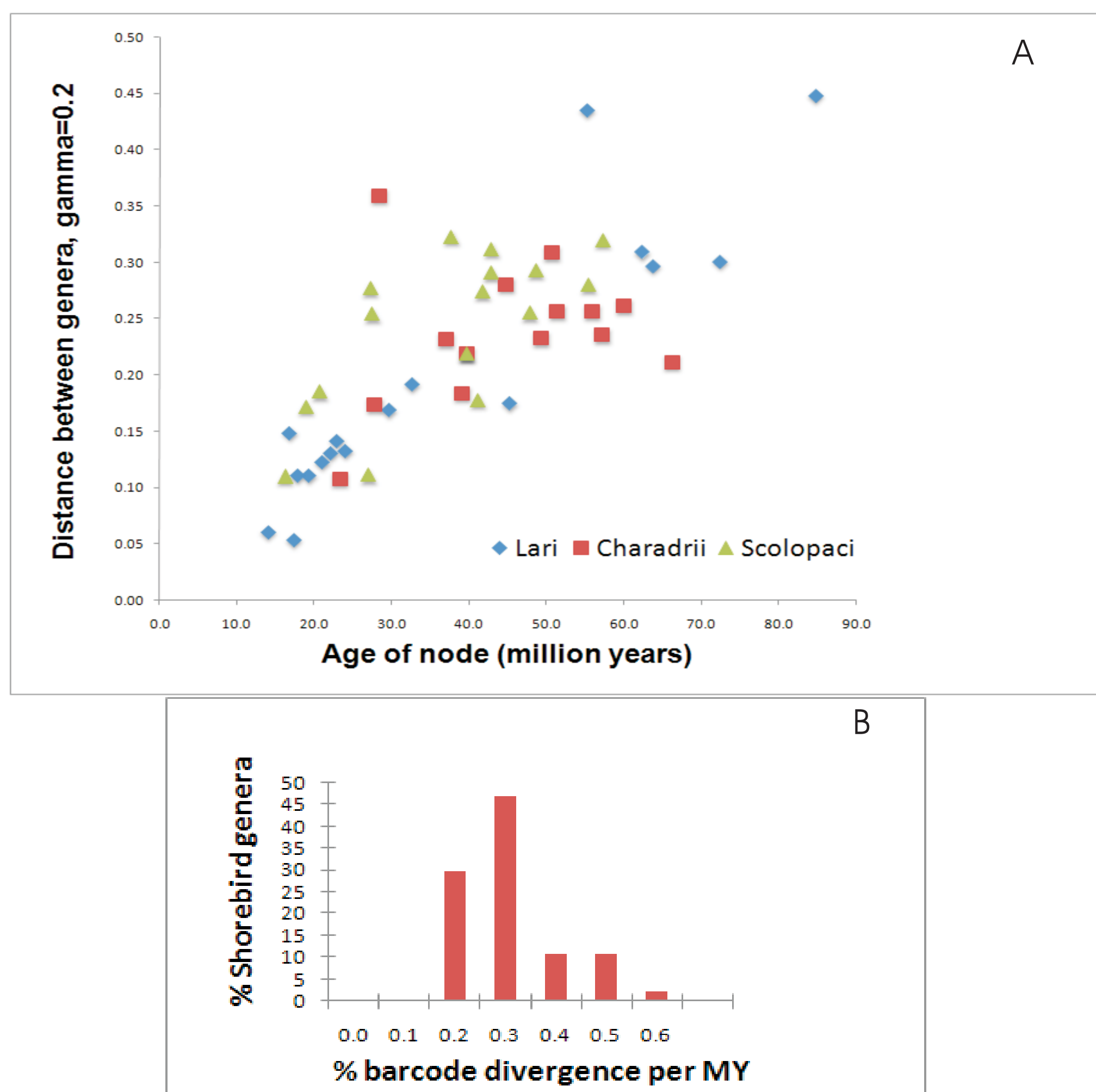
PCR amplification of 1Kb DNA sequence that begins in a highly conserved portion of the upstream tRNA gene to avoid pseudogenes. Internal primers are used in bi-directional sequencing on an ABI3100 automated sequencer.

Distance calculations performed in MEGA3.1 using the K2P. Rates of barcode divergence were estimated using node dates calculated in a Bayesian, relaxed-clock framework with multiple fossil calibration points (Baker AJ et al., 2007).

Results

1. Rates of COI divergence in Shorebird genera

Fig.1. Genetic distance between genera plotted vs. Divergence time.



2. Shorebird Species

Table 1. Summary of ROM and published barcodes (Kerr et al., 2007; Hebert et al., 2004) of shorebirds. Families in green are >75% Complete.

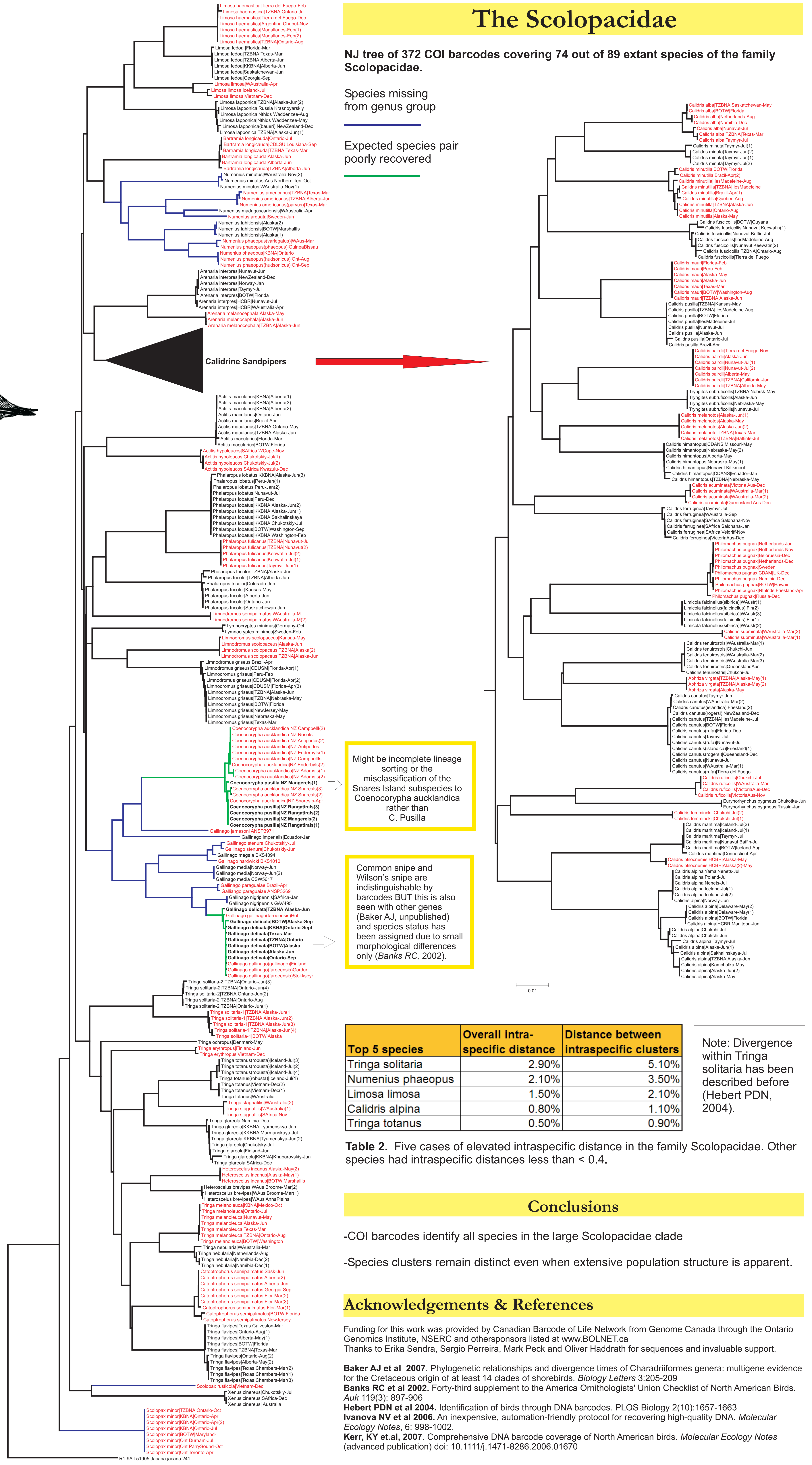
Family	Number of species (ABBI, 2007)	Species barcoded	Barcodes by ROM	Total barcodes
ALCIDAE	23	22	45	91
BURININUS	9	4	6	6
CHARADRIIDAE	66	45	95	123
CHIONIDAE	2	2	2	2
DROMADIDAE	1	1	2	2
GLAREOLIDAE	17	7	10	10
HAEMATOPODIDAE	11	11	75	78
IBIDORHYNCHIDAE	1	0	0	0
JACANIDAE	8	4	7	9
LARIDAE	56	30	20	99
STERNIDAE	44	35	91	130
PEDIONOMIDAE	1	1	1	1
PLUVIINELLIDAE	1	1	1	1
RECURVROSTRIDAE	10	9	21	24
ROSTRATULIDAE	2	2	9	9
RYNCHOPIDAE	1	1	3	3
SCOLOPACIDAE	89	74	278	375
STERCORIDAE	7	5	10	25
THINOCORIDAE	4	3	11	11
Total	353	257	685	1001

The Scolopacidae

NJ tree of 372 COI barcodes covering 74 out of 89 extant species of the family Scolopacidae.

Species missing from genus group

Expected species pair poorly recovered



Might be incomplete lineage sorting or the misclassification of the Snares Island subspecies to *Coenocorypha auklandica* rather than *C. pusilla*

Common snipe and Wilson's snipe are indistinguishable by barcodes BUT this is also seen with other genes (Baker AJ, unpublished) and species status has been assigned due to small morphological differences only (Banks RC, 2002).

Top 5 species	Overall intra-specific distance	Distance between intraspecific clusters
<i>Tringa solitaria</i>	2.90%	5.10%
<i>Numenius phaeopus</i>	2.10%	3.50%
<i>Limosa limosa</i>	1.50%	2.10%
<i>Calidris alpina</i>	0.80%	1.10%
<i>Tringa totanus</i>	0.50%	0.90%

Table 2. Five cases of elevated intraspecific distance in the family Scolopacidae. Other species had intraspecific distances less than < 0.4.

Conclusions

- COI barcodes identify all species in the large Scolopacidae clade
- Species clusters remain distinct even when extensive population structure is apparent.

Acknowledgements & References

Funding for this work was provided by Canadian Barcode of Life Network from Genome Canada through the Ontario Genomics Institute, NSERC and others persons listed at www.BOLNET.ca
 Thanks to Erika Sendra, Sergio Perreira, Mark Peck and Oliver Hadrath for sequences and invaluable support.
Baker AJ et al 2007. Phylogenetic relationships and divergence times of Charadriiformes genera: multigene evidence for the Cretaceous origin of at least 14 clades of shorebirds. *Biology Letters* 3:205-209
Banks RC et al 2002. Forty-third supplement to the American Ornithologists' Union Checklist of North American Birds. *Auk* 119(3): 897-906
Hebert PDN et al 2004. Identification of birds through DNA barcodes. *PLOS Biology* 2(10):1657-1663
Ivanova NV et al 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6: 998-1002.
Kerr, KY et al, 2007. Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes* (advanced publication) doi: 10.1111/j.1471-8286.2006.01670