SHORT COMMUNICATION

DNA barcoding of the White-Collared Kingfisher *Todiramphus chloris* (Boddaert 1783) (Alcedinidae) using the mitochondrial cytochrome c oxidase subunit I gene

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The White-Collared Kingfisher (*Todiramphus chloris*) is a resident Philippine bird species. In accordance with the objective of the All Birds Barcoding Initiative (ABBI) to barcode all bird species in the world, this study reports the first barcodes of *T. chloris* using the mitochondrial gene cytochrome c oxidase subunit I (COI). COI sequences from this species as well as from other members of the family Alcedinidae available in Genbank and the Barcodes of Life Data (BoLD) Systems were compared in order to test for the utility of COI to delineate species. Monophyly of the species was established, supporting the use of barcodes for species discovery. Sequences between *T. chloris* and *T. sanctus*, however, revealed a close association between the two species which warrants further taxonomic review.

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INTRODUCTION

The White-Collared Kingfisher, *Todiramphus chloris* (Boddaert 1783), is a medium-sized kingfisher belonging to the family Alcedinidae, subfamily Daceloninae, sometimes under the alternative family Halcyonidae (Moyle 2006, Christidis and Boles 2008). The genus *Todiramphus* is composed of 22 species (Dickinson 2003). *Todiramphus* was for a time placed under *Halcyon*, with *T. chloris* previously named as *H. chloris* in light of DNA hybridization experiments (Sibley and Monroe 1990) but was then 'unlumped' when it became clear that *Halcyon* was polyphyletic and is most likely composed of at least two lineages, an Afro-Asian *Halcyon* and Australasian *Todiramphus* (Schodde and Mason 1997, Woodall 2001). *T. chloris* is the most conspicuous kingfisher in the Philippines (Kennedy et al. 2000). It exhibits a wide geographical range extending from Northeast Africa, east and southeast China, the Ryukyus, south and southeast Asia, New Guinea, Australia, and the southwest Pacific Ocean (Kennedy et al. 2000).

Figure 1. Representative specimen (UPD10) of *Todiramphus chloris* from UP Diliman

Species identification is a crucial step in all biological studies. Without proper species identification, appropriate action regarding biological issues would be ineffective (Armstrong and Ball 2005). However, traditional taxonomy may sometimes fail in its role in effective species identification, or there may not be enough qualified people to do proper identification. Scientists are therefore looking for more rapid and accurate methods of species delineation that rely less on taxonomic expertise. DNA barcoding can address this problem (Hebert et al. 2003, Tautz et al. 2003). Molecular techniques provide far more accurate and quantitative means of species identification than morphology-based methods. So far, DNA barcoding performs well in unraveling a whole new wealth of information that it is expected to provide more insights on taxonomy and systematics (Hebert et al. 2003, Hajibabaei et al. 2007). Hebert et al. (2004) proposed that a 650-bp fragment from the 5' end of the cytochrome c oxidase subunit I (COI) gene be used as a barcode marker for animals.

The All Birds Barcoding Initiative (ABBI, http://www.barcodingbirds.org), a flagship project under the Consortium for the Barcode of Life (CBOL, http://barcoding.si.edu) aims to barcode at least five individuals per species of bird. As part of this initiative, this study aimed to report for the first time the COI barcode of *Todiramphus chloris* as well as to test the utility of COI barcodes to delineate species within the Alcedinidae. Further, a COI gene tree was constructed to confirm previous assertions that the Australasian *Todiramphus* be separated from the Afroasian genus *Halcyon*.

MATERIALS AND METHODS

Five individuals from the University of the Philippines Diliman campus, Quezon City, Philippines were caught using mist nets. Morphometric data such as bill length, bill depth, tarsus length, wing length, tail length and weight were taken, as well as photographs in lieu of actual voucher specimens, all of which were submitted to Barcode of Life Data (BoLD) Systems. Figure 1 shows a specimen (UPD10) of *T. chloris* caught in the campus. The ends of some of the tail feathers were clipped in order to mark the captured bird before release and to prevent recapture. DNA was extracted from blood feathers and a 650bp fragment of the COI was amplified using PCR with primers BirdF1 and BirdR1 of Hebert et al. (2004). The purified PCR products were sent to Macrogen in Korea for sequencing. A gene tree for the family Alcedinidae was constructed using 30 unique COI sequences from this study, Genbank and the Barcode of Life Data (BoLD) Systems. Uncorrected p-distances were used to

calculate for the intraspecific and interspecific divergences for utility testing of COI barcodes within the Alcedinidae.

RESULTS AND DISCUSSION

In line with the ABBI effort of barcoding all of the world's bird species, this study presents the first barcodes for the *T. chloris* from five individuals. From the five sequences obtained (GenBank Accession HM62277-81), two unique haplotypes were found. BLAST results revealed that both haplotypes were 98.1% and 98.3% similar, respectively, with *Todiramphus sanctus vagans* (GenBank Accession EU410489).

The gene tree (Figure 2) agrees with the currently established phylogenetic relationships using molecular data (Moyle 2006) with respect to monophyly and lineage sorting. Monophyly of the traditional subfamilies Alcedininae, Daceloninae and Cerylinae as well as monophyly of species were clearly resolved. In this tree, three distinct lineages are formed, the first containing *Halcyon coromanda*, which is basal to a splitting between a *Dacelo* clade and a *Todiramphus* clade. What is clear from the COI tree, which also mirrors Moyle's (2006) findings, is that there is clustering of *T. chloris* with *T. sanctus* to form a monophyletic group divergent from *Halcyon*, which supports the establishment of *Todiramphus* as distinct from *Halcyon* (Woodall 2001).

Barcoding has been promoted as useful in delineating species by using a species threshold concept in which sequences differing by more than ten times the minimum intraspecific variation or beyond an empirically determined divergence limit (e.g., 2.7%) are said to be of different species (Hebert et al. 2004). Though most of the taxa from the Alcedinidae follow this rule, there are peculiar exceptions. Sequence for *T. chloris* differs only by 1.38% from *T. sanctus,* which is less than the empirical 2.7% or from the calculated 10x rule value (1.5% from 0.15% minimum intraspecific variation). Based on the species threshold concept, *T. chloris* and *T. sanctus* are most likely of the same species and should be flagged for taxonomic reevaluation. However, consideration of other

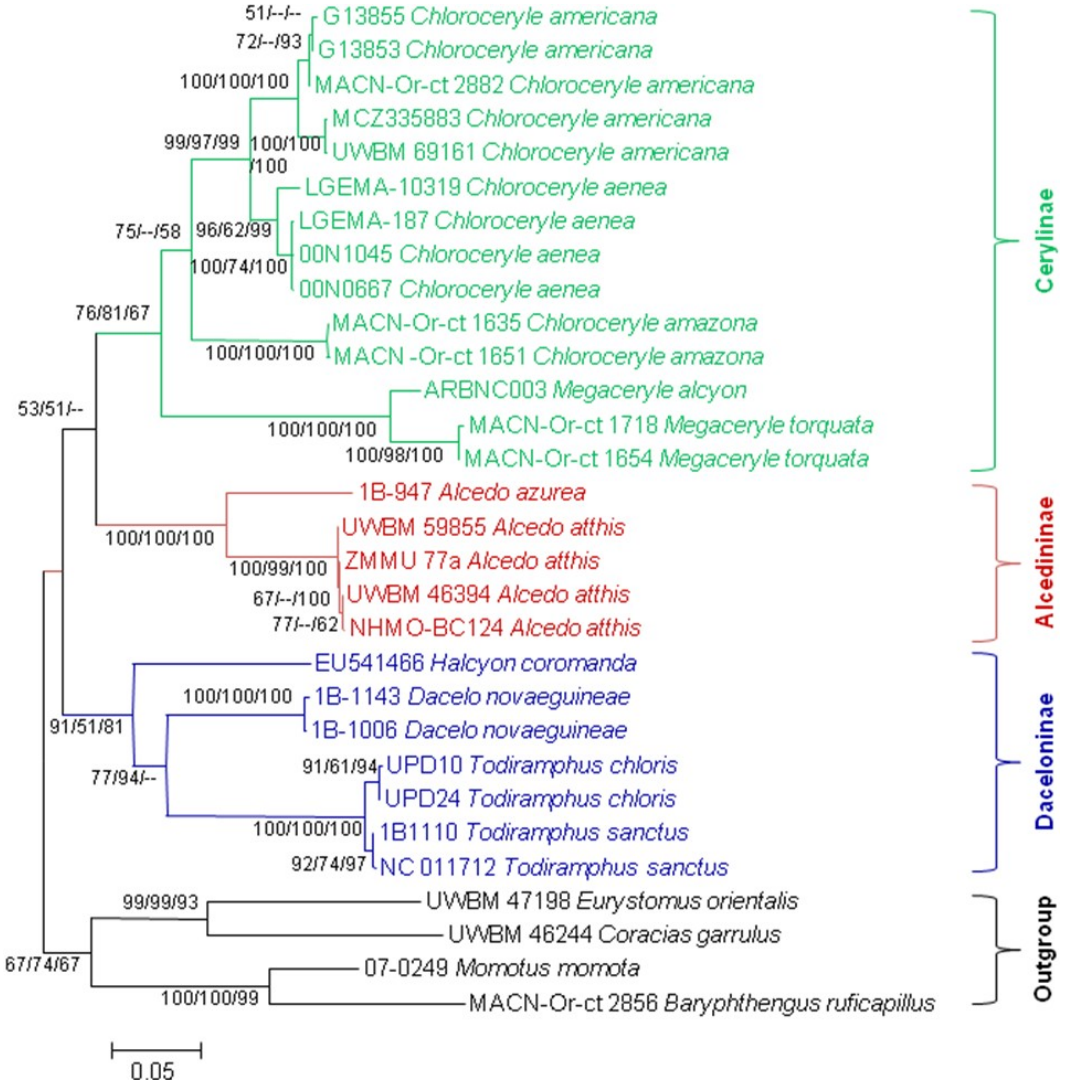

Figure 2. Maximum likelihood tree of the Alcedinidae based on 651 nucleotides of the COI gene and using the GTR+Γ as optimal model. The tree is rooted on motmots (Momotidae) and rollers (Coraciidae) as outgroup taxa. Values on nodes indicate percentage bootstrap support based on ML/NJ/MP analyses; values less than 50% are not shown. Scale bar represents five nucleotide changes for every 100 nucleotides.

taxonomic characters such as morphology and ecology should still be taken into account to indicate if they are indeed really the same species. Furthermore, samples from different biogeographic regions should also be taken into consideration for the possibility of the presence of other haplotypes.

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CONTRIBUTION OF INDIVIDUAL AUTHORS

Adrian U. Luczon – contributed in sampling, sample processing, manuscript writing, analysis of data, final manuscript revisions and submission for publication.
Abdel Hadi M. Mohammad Isa – contributed in sampling, analysis of data, and manuscript writing.
Jonas P. Quilang, PhD – contributed in writing the proposal and application for funding.
Perry S. Ong, PhD – contributed in developing the concept of DNA barcoding in the Philippines, writing the proposal, application for funding and inputs in the manuscript.
Ian Kendrich C. Fontanilla, PhD – contributed in writing the proposal, sampling, application for funding, analysis of data, manuscript writing, and manuscript revisions.

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