Sains Malaysiana 45(7)(2016): 1089–1095

Phylogenetic Relationships of Waders (Charadriiformes: Scolopacidae) in Sarawak Inferred from Cytochrome Oxidase I and Recombinant Activating Gene 1

(Hubungan Filogenetik Burung Laut (Charadriiformers: Scolopacidae) di Sarawak yang Tersimpul daripada Sitokrom Oksidase I dan Rekombinan Gen Pengaktif 1)

NURUL ASHIKEEN AB RAZAK*, MUSTAFA ABDUL RAHMAN & ANDREW ALEK TUEN

ABSTRACT

Family Scolopacidae includes the sandpipers, shanks, snipes, godwits and curlews. Systematic classifications of shorebirds at the higher level have been successfully resolved. Nevertheless, the phylogeny of shorebirds in the familial level is still poorly understood. Thus, this phylogenetic study on Scolopacidae was conducted upon the framework provided by the first sequence-based species-level phylogeny within the shorebirds to determine the phylogenetic relationships among family members of Scolopacidae in West Borneo, Sarawak using combined gene markers, mtDNA Cytochrome Oxidise I (COI) and nucDNA Recombinant Activating Gene 1 (RAG1). A total of 1,342 base pair (bp) were inferred from both COI and RAG1 gene from 45 sequences constituted of 15 species Scolopacidae sampled from Sarawak namely Xenus cinereus, Actitis hypoleucos, Tringa totanus, Tringa glareola, Tringa stagnatilis, Heteroscelus brevipes, Calidris alba, Calidris ruficollis, Calidris ferruginea, Calidris tenuirostris, Calidris alpina, Gallinago stenura, Gallinago megala, Numenius arquata, *and* Numenius phaeopus. *The phylogenetic tree was constructed with* Charadrius mongulus *derived as an outgroup. The Bayesian Inference (BI) tree constructed supported grouping of species into several lineages of Numeniinae, Calidrinae, Scolopacinae and Tringinae. The groupings of species into several lineages correlate with morphological features that contribute to their adaptation and ability of the species to fit to their ecosystems.*

Keywords: Cytochrome Oxidase I; phylogenetic; Recombinant Activating Gene 1; waders

ABSTRAK

Famili Scolopacidae merangkumi burung kedidi biasa, burung kedidi kaki merah, burung berkek dan burung kedidi kendi. Pengelasan sistematik burung laut pada peringkat lebih tinggi telah berjaya diselesaikan. Namun, filogeni burung laut pada peringkat famili masih belum difahami. Sehubungan itu, kajian filogenetik ke atas Scolopacidae telah dijalankan mengikut rangka kerja yang diberikan oleh filogeni berasaskan-urutan-pertama aras-spesies dalam kalangan burung laut untuk mengenal pasti hubungan filogenetik dalam kalangan family Scolopacidae di barat Borneo, Sarawak, menggunakan penanda molekul berbeza; mtDNA Siktokrom Oksidase I (COI) dan nucDNA Recombinan Gen Pengaktif 1 (RAG1). Sejumlah 1,342 pasangan asas (bp) diperoleh daripada kedua-dua jenis gen COI dan RAG1 daripada 45 jujukan merangkumi 15 spesies Scolopacidae yang disampel dari Sarawak iaitu Xenus cinereus, Actitis hypoleucos, Tringa totanus, Tringa glareola, Tringa stagnatilis, Heteroscelus brevipes, Calidris alba, Calidris ruficollis, Calidris ferruginea, Calidris tenuirostris, Calidris alpina, Gallinago stenura, Gallinago megala, Numenius arquata *dan* Numenius phaeopus. *Pokok filogenetik telah dibina menggunakan* Charadrius mongulus *sebagai kumpulan luar. Pokok Bayesian Inference (BI) yang dibina menyokong perkumpulan spesies mengikut keturunan masing-masing iaitu Numeniinae, Calidrinae, Scolopacinae dan Tringinae. Perkumpulan spesies kepada beberapa keturunan berkait rapat dengan ciri morfologi yang telah menyumbang kepada adaptasi dan kebolehan spesies ini menyesuaikan diri dalam ekosistem mereka.*

Kata kunci: Burung laut; filogenetik; Rekombinan Gen Pengaktif 1; Sitokrom Oksidase I

INTRODUCTION

Shorebirds are embedded in order Charadriiformes consisting more than 350 species and 19 families (Clements 2007). This falls into three sub-clades: Scolopaci (waders), Charadrii (plovers) and Lari (Baker et al. 2007; Mayr 2011; Paton & Baker 2006; Paton et al. 2003). Sub-clades Scolopaci consist of several families including Scolopacidae, Jacanidae, Rostratulidae, Thinocoridae and Pedionomidae. The largest of these families is the Scolopacidae with 90 species (Clements et al. 2010) including the sandpipers, shanks, snipes, godwits and curlews. In family Scolopacidae, 65 species of waders were recorded worldwide (Clements et al. 2010), while 35 species were recorded in Borneo alone (Smythies 1999). Variation in life-histories, behavioral ecology and morphological traits makes this order a popular subject to study.

The study of order Charadriiformes were well established based on nuclear and mitochondrial DNA sequence by Ericson et al. (2003), Pereira and Baker

(2005) and Pereira et al. (2002). In relation to this, Thomas et al. (2004) presented the supertree method to described phylogenetic relationship within shorebirds. The study was more focused on the relationship of higher level of order Charadriiformes including the family level. The supertree method lacks measures of nodal support and was known to be highly prone to biases depending on which source trees are included in the analysis and by treating well-supported and poorly supported source trees as equally likely. Additionally, the supertree presented in Thomas et al. (2004) fell short in providing good resolution for many congeneric species because there was not enough supporting trees or completely absent for some groups.

The first species-level /interspecific study of Scolopaci showed that Bayesian is an excellent tool to study evolution traits in the enormous group of Scolopacidae (Gibson 2010). On a general level, the family was considerably well-resolved but relationship within *Tringa* (shanks) and *Calidris* (small sandpipers) were still poorly understood. The shanks are characterised by medium-length bill and relatively long legs with bright yellow to orange. In a study conducted by Pereira and Baker (2005), both the morphological evolution and DNA data were mapped on to main phylogeny trees (ML/BAYES) with inferences on their intraspecific relationship. The result showed a parallel relationship of species at the basal lineages. However, the placement of common redshank (*Tringa totanus*), wood sandpiper (*Tringa glareola*) and grey-tailed tattler (*Heteroscelus brevipes*) in both trees were incongruent with each other. Although the phylogenetic relationships within shorebirds are well established at the family level based on nucDNA and mtDNA sequences, they are still some phylogenetic uncertainties within many groups of species and genera for some subfamilies. Thus, this study was aimed to determine and resolve the phylogenetic relationship of species in the family Scolopacidae. Additionally, pairwise-distance matrix was calculated in order to determine the interspecific relationship percentage within the family.

Materials and Methods

A total of 15 species consisting of 53 individuals were successfully collected from six selected sampling sites in Sarawak, Borneo based on their distributional record. The sampling sites were Buntal (N 01° 41'40.84'' E 110°22'23.01''), Sambir (N 01°34'30.04'' E 110°32'51.79'' and N 01°41'56.6'' E 110°22'09.1''), Sungai Aur (N 01°67'39.90'' E 110 ° 24'45.40''), Pulau Bruit (N 02 °79'37.20'' E111° 34'69.40'' and N 02°45'45.41'' E 111°21'40.35''), Lundu (N 01°40'30.93'' E 109°51'47.14'') and Kampung Chupak (N 01°16'51.74'' E 110°24'26.55''). Blood samples were taken from the individuals captured. The samples were then extracted using modified CTAB protocol with 30.0 µL of final product (Grewe et al. 1993). A pair of mtDNA Cytochrome Oxidase I (COI) oligonucleotide primers and a pair of nucDNA Recombinant Activating Gene 1 (RAG1) were used to amplify the targeted sequence of the extraction product; forward primer for COI gene (5'-CCT GCA GGA GGA GGA GAY CC-3') and reverse primer for COI gene (5'-CCA GAG ATT AGA GGG AAT CAG TG-3') (Palumbi et al. 1991); and forward primer for RAG1 gene (5'-TCTGAATCGGAAATTCAAGCTGTT-3') and reverse primer for RAG1 gene (5'-GATGCTGCCTCGGTCGGCCACCTTT-3') (Groth & Barrowclough 1999). The amplification products of targeted COI region are approximately 700 bp whereas the targeted region for RAG1 approximately 1300 bp. For the amplification, 25 µL of final PCR reaction mixture was prepared using the following PCR reaction mixture: 1.0 µL of DNA template was added to the initial mixture of 5 µL 10× buffer (Promega), 2.0 µL $MgCl_2$, 0.5 µL dNTPs (10 µM) and 1.5 µL each of both COI and RAG1 (25 pmol/mL) primers. Lastly, 13.7 µL of ddH_20 was stock up to complete the final mixture and added later with 0.3 µL of 5 unit/mL *Taq* Polymerase (Promega).

PCR configurations for COI gene were operated using Thermacycler Personal machine for a 30 total cycles with an initial denaturation at 94°C for 2 min, followed by denaturation step at 94°C for 1 min, annealing at 48.5 to 55.5°C for 1 min, extension at 72°C for 2 min and a final extension of 72°C for 5 min. For RAG1 gene, amplification were done with 36 total cycles with an initial denaturation at 94°C for 5 min, followed by denaturation step at 94°C for 40 s, annealing at 55°C for 40 s, extension at 72°C for 1 min and a final extension of 72°C for 7 min.

Finally, 1.0 µL of the PCR product stained with 1.0 µL of 6× loading dye were run on 1% agarose gel on 90 Kilowatts (KW). Product size was indicated by GeneRuler™ 100 bp DNA ladder plus (Fermentas, Canada). Purification of PCR products were conducted using Wizard® SV Gel and PCR Clean-up System (Promega, USA). Approximately 490 bp of the nucleotide sequences of the mtDNA COI region and 880 bp of nucDNA RAG1 region were successfully sequenced. The complete 45 sequences were chosen out of the 53 products based on the quality and total base pair lengths of the samples. Two sequences of mtDNA COI and RAG1 of Lesser sand plover (*Charadrius mongulus*) (SBL06, SBL07) were derived as outgroup.

Data Analysis

The Sequence Scanner version 1.0 program was used to visualize DNA sequence analysis result. Multiple alignments of the sequences were combined and edited manually using the CLUSTAL X program (version 1.81; Thompson et al. 1997). After alignment, the sequences were translated into amino acids by using Molecular Evolutionary Genetic Analysis (MEGA) 5 (Tamura et al. 2011) and the occurrences of stop codons were removed. Nucleotide variation and pairwise genetic distances (the number of nucleotide substitutions per site) were computed using MEGA version 5 (Tamura et al. 2011). The sequence divergence (Saitou & Nei 1987) analysis was estimated using Kimura 2-parameter (Kimura 1980) model.

Combined dataset of two genes (COI and RAG1 genes) were performed to improve resolution of nodes and bootstrap support and to see clear separation of groups within family Scolopacidae. The partition homogeneity test (Farris et al. 1995) was implemented in PAUP version 4.0 beta (Swofford 2002) to determine if the two gene datasets could be combined for phylogenetic analyses.

Neighbour-joining methods utilising distance sequences by Kimura 2-parameter model (K2P) (Kimura 1980) with 1000 replicates were conducted in PAUP. The distance method using K2P was also performed to estimate nucleotide divergence.

Model test 3.6 (Posada & Crandall 1998) was used in order to determine the best model of evolution for ML. The model evolution was determined based on Akaike information criterion (AIC). The goodness of fit of a model to the data was generalized time reversible+proportion, invariant+gamma (GTR+I+G) for combined gene. Bayesian inference was applied on four independent analyses with each analysis consists of four chains, random starting trees and uniform prior distribution parameter. The Metropolis-coupled Markov chain Monte Carlo (MCMC) were processed under best selected model by AIC (GTR+G+I) for 10 million generations, sampling trees every 100 generations and the first 200,000 generations were discarded as burn-in which was determined by using the Average Standard Deviation of Split Frequencies that is reported in MrBayes 2.0 (Heulsenbeck & Ronquist 2001). BI analysis produced trees containing Bayesian posterior probabilities (BPP) which were considered strongly supported if 95% were present in the trees.

Results and Discussion

A total of 1342 bp were sequenced with 965 were conserved, 102 variable characters were parsimony uninformative and 297 were parsimony informative sites. The average value of nucleotide composition based on 1342 bp of RAG1 and COI gene showed that 'A' was relatively high compared to others, with mean value of 30% followed by T: 25%, C: 24% and G: 21%. Similarly, in the first codon and third codon, again 'A' was relatively high compared to others (A: 31.4%, T: 19.4%, C: 23.5%, G: 25.7%) and; the third position (A: 28.3%, T: 26.3%, C: 28%, G: 17.4%). In the second codon position, 'T' is the highest compared to others (T: 29.7%, C: 22.7%, A: 29.3%, G: 18.9%).

The genetic distances among interspecific group were low ranged from 0.66% to 10.71%. All sequences were separated by outgroup which ranged from 10.58% to 12.38%. In genus *Calidris* the range was between 0.40% and 5.35%, while in genus *Tringa*, it was 0.99% to 9.34%. The genetic distance of individuals within the same species were very low between 0.30% and 2.81%.

A total of 1342 bp of partial combined gene was retrieved from 45 samples of 15 species namely Terek sandpiper (*Xenus cinereus*), common sandpiper (*Actitis hypoleucos*), whimbrel (*Numenius phaeopus*), Eurasian curlew (*Numenius arquata*), common redshank (*Tringa totanus*), wood sandpiper (*Tringa glareola*), grey-tailed tattler (*Tringa brevipes*), marsh sandpiper (*Heteroscelus stagnatilis*), red-necked stint (*Calidris ruficollis*), sanderling (*Calidris alba*), dunlin (*Calidris alpina*), curlew sandpiper (*Calidris ferruginea*), great knot (*Calidris tenuirostris*), pintail snipe (*Gallinago stenura*), Swinhoe's snipe (*Gallinago megala*). Lesser sand plover (*Charadrius mongulus*) was derived as outgroup for the phylogenetic tree.

Numenius group remains as their basal group to the tree in Figure 1. The major monophyletic groups were split into two major clades of *Calidris* and *Gallinago* with other Scolopacids (BI: 1.00BPP). *Gallinago* are sisters to *Tringa* cladded to *Actitis* and *Xenus* supported with BI: 0.79BPP.

Genetic Associations Among and Within Scolopacidae Species

Based on traditional classification, Scolopacidae was split into five subfamilies (Jehl 1968). However, Livezey (2010) and Smythies (1999) have recognized four subfamilies which are Arenariinae, Calidrinae, Tringinae and Scolopacinae and maintained phalaropes as separate family. They embedded curlews within Tringinae and separate turnstones and small sandpipers into different subfamilies which are Arenariinae and Calidrinae. In another study, Gibson and Baker (2012) utilized three mtDNA plus a nuclear gene and identified eight monophyletic subfamilies in family Scolopacidae. The subfamilies were described using broad English group names, sandpipers, shanks, phalaropes, snipes, woodcocks, dowitchers, godwits and curlews. The general branching patterns in the study were well-resolved except for internal taxonomic confusions within Calidrinae (small sandpipers).

In this study, the tree constructed supported grouping of species into several lineages by Gibson (2010) except for Limosinae and Arenariinae where no representative samples were acquired from any of the sampling areas. All species were grouped into their respective subfamilies which were Numeniinae, Calidrinae, Scolopacinae and Tringinae. The Numeniinae served as the basal lineage to others followed by Calidrinae, Scolopacinae and the recent lineage of Tringinae.

The genetic variation usually correlates with morphological features that contribute to their adaptation and ability of the species to fit to their ecosystems (Barrett & Schluter 2008). In the case of Scolopacidae family, different species of waders displayed variation of bill structure and shape (Myers 2009; Smythies 1999) that enable them to group together under specified genus and subfamilies. The *Numenius* sp. was characterized by decurved long bills with blunt tip that were mostly confined to coastal areas during winter.

On the other hand, *Calidris* sp. can be described by short and small blunt to sharp decurved and slightly

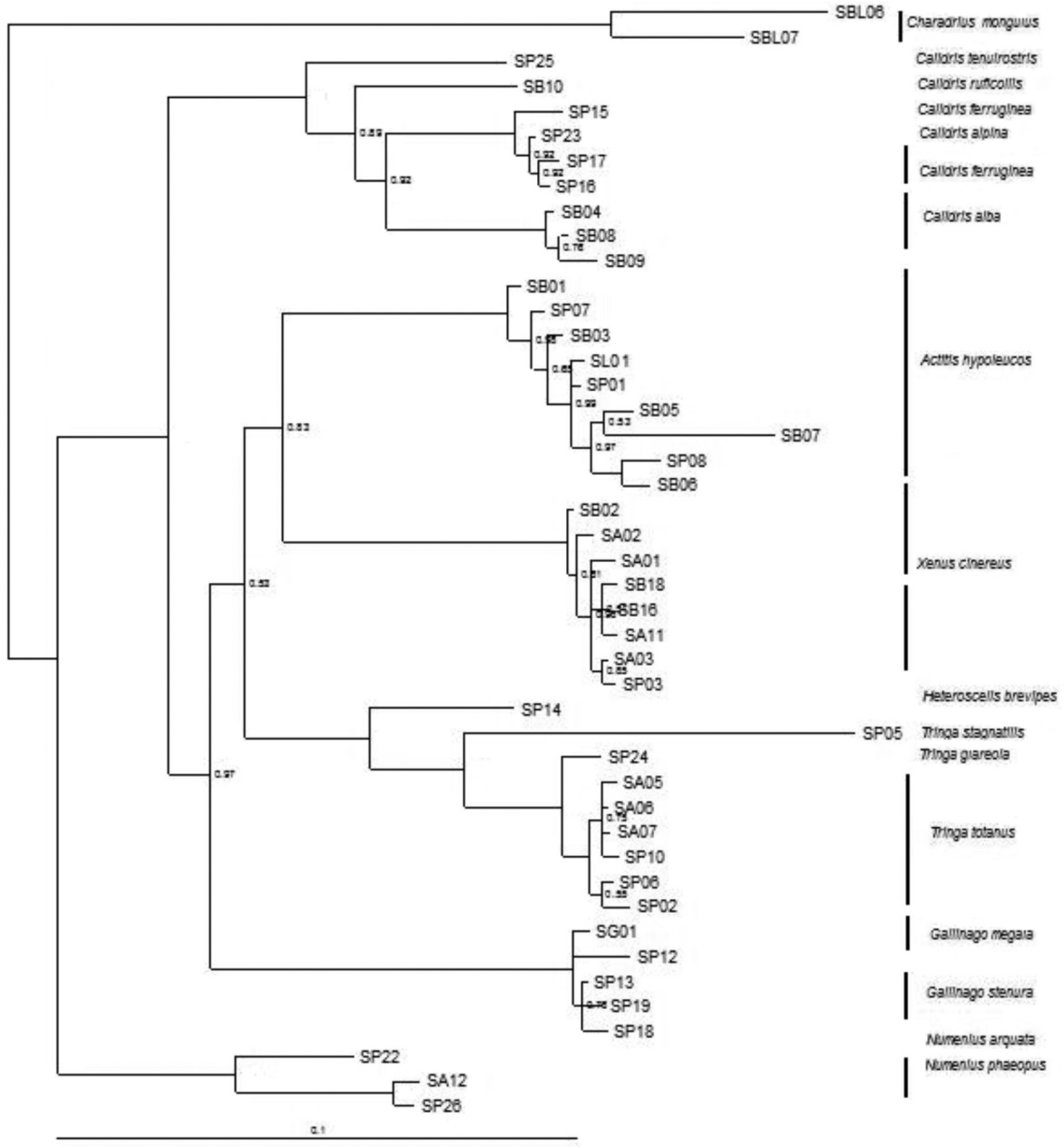

FIGURE 1. Bayesian tree of Scolopacidae for combined gene based on GTR+G+I model for the last 250,000 trees after 3 million generations. Values on the branches represent Bayesian posterior probabilities (BPP). All nodes received a posterior probability of 1.00 unless otherwise labeled

decurved bill that prefer the same habitat as *Numenius*. Scolopacinae includes *Gallinago* sp. illustrated with medium length straight bill with crown stripe at the base of the bill. The *Gallinago* sp. is generally known for having cryptically colored plumage that inhabits inland areas. The last recent diverged species based on the tree provided is genus *Tringa*. *Tringa* is characterized by straight and upturned medium length bill shape with both blunt and sharp end. This species inhabits both inland and coastal area during winter.

PHYLOGENY USING COI AND RAG1 GENE

Out of all phylogenetic trees, the use of combined gene provided the best estimation of phylogenetic relationship among members in family Scolopacidae with most of the branches were supported by high Bayesian Posterior Probability (BPP) values. These sequences complement each other in providing stronger support for nodes at various depths in with respect to the monophyletic species grouping. The COI region is considered as a faster-evolving gene which supported the more shallow nodes of the

Scolopacids tree while for the deeper splits of the tree, it was supported by the slower-evolving gene such as the RAG1 gene (Braun & Kimball 2002). Several studies showed that phylogenies based on single genes (or random shorter sequences) have less resolution and statistical power than using longer sequences (Braun & Kimball 2002; Weibel & Moore 2002). Furthermore, the use of a single gene, especially mtDNA alone, may not reflect the 'true tree' of phylogenetic reconstruction due to a number of reasons, including past hybridization, gene duplication or incomplete lineage sorting (Brown 1983).

In combined gene tree, the first genus diverged after the basal lineage was the monophyletic of *Calidris* sandpipers or the subfamily Calidrinae. Species within this genus can be seen having grayish upperparts with broad darker streak during non-breeding season. Within the subfamily Calidrinae, *Calidris tenuirostris* served as the basal lineage to other species followed by *C. ruficollis* which was clustered to *C. ferruginea* together with *C. alpina* and a monophyly of *C. alba* supported with high BPP value ranges from 0.79 to 1.00. The cladding of species however contradicts with Gibson (2010) except for the basal lineage of Calidrinae. In Gibson's study, *C. alpina* and *C. alba* were claded together and formed sister lineage to *C. ruficollis* and *C. ferruginea*. Different character in mtDNA COI and nucDNA RAG1 gene used resulted in unresolved cladding of species within Calidrinae. At this point, my combined gene trees have yet to resolve the genetic relationship between *C. alpina* and *C. ferruginea*.

The subfamily Scolopacinae consisting of *G. stenura* and *G. megala* can be considered as cryptic species. In the BI analysis of combined genes, the tree was unresolved as shown by polytomy branches. In the tree *Gallinago sternura* formed a monophyletic group to sister clade of *Gallinago megala*, yet the taxa branches showed polytomy nodes.

The cladding of genus *Tringa* in the combined gene tree was coincided with the phylogenetic tree produced by Pereira and Baker (2005) and Gibson (2010), which placed *Xenus* and *Actitis* outside the remaining Tringinae. In Gibson (2010), *Xenus* represented the basal lineage to *Actitis*, *Tringa* and *Heteroscelus* in the monophyletic group, whereas in this phylogenetic tree, *Tringa+Heteroscelus* were sister clade to both *Xenus+Actitis*. Pereira and Baker (2005) have examined affinities of *Tringa* species using three independent datasets which include mtDNA, nucDNA and set of 70 osteological and mycological characters (Strauch 1978). They found out that *Xenus* and *Actitis* were two basal lineages for the group and distinct from other shanks (*Tringa*) lineage. Based on their findings, they also suggested that the tattlers (*Heteroscelus*) should be changed to *Tringa* as tattlers were embedded within the Tringinae. According to Bank et al. (2006), The American Ornithologist's Union supported the findings and accepted *Heteroscelus* changed to *Tringa*. Recently, the changes of the genera were still relatively vague since *Heteroscelus* were preferred to be described as tattlers instead of *Tringa*. In this study, the cladding of *Tringa glareola, T. totanus* and *T. stagnatilis* were compatible with the matching trees that of Pereira and Baker (2005). The tree depicted the association of *T. glareola* as sister to *T. totanus* compared to *T. stagnatilis*. However, in Gibson (2010), *T. glareola* was more closely related to *T. stagnatilis* compared to *T. totanus*. By looking at their morphological characters, most of the Tringinae shared the derived characters of having bright yellow and red legs. The coloration retained as ancestral stated for the group from bright yellow legs of *Heteroscelus brevipes* to dull yellow legs of *T. stagnatilis* and *T. glareola*, diverged to red legs of *T. totanus* (Pereira & Baker 2005).

Using longer mitochondrial sequences with different rates of evolution (Paton & Baker 2006) would be much more informative to infer or reconstruct the phylogeny whereas utilizing a single, relatively short gene did not contain enough phylogenetic-informative sites and therefore will most likely to provide inconsistency of cladding within the studied taxa (Cummings et al. 1995; Rosenberg & Feldman 2001). Paton and Baker (2006) had examined the substitution patterns among selected mitochondrial genes. The result showed specific genes (especially ND5, ND4, ND2 and COI) that are better suited for phylogenetic analyses among shorebird families because of their relatively homogeneous nucleotide composition among lineages and slower accumulation of substitutions at third codon positions. Those genes also offered phylogenetic utility in both closely and distantly related lineages. Furthermore, among mtDNA they also revealed that the slower-evolving genes support the deeper splits of the shorebird tree, (12S, 16S, COI, COII but also ND2) and faster-evolving genes resolve the more shallow nodes (ATP6, ND4, ND1, ND5 and Cyt *b*). Apart from that, more representative for each species are needed to improve the resolution of phylogenetic relationship in family Scolopacidae.

CONCLUSION AND RECOMMENDATION

Taken collectively, most of the species were grouped accordingly into monophyletic groups. Close-related species were more likely to be similar in their biology as compared to more distant related species. This is parallel to the morphological evolution among the family members which is distinctive by their bill and leg (tarsus) characters. The varying bill shapes and leg dimensions have shown the most adaptive functions with regards to their respective foraging behaviors (usage of bill to probe for food) and habitat preferences or geographical regions. On another note, this study was still unable to resolve the intra-specific relationship of the Scolopacidae family to a high degree of confidence. The usage of COI marker was deemed suitable only to resolve the phylogenetic relationship up to the genus level. Therefore, more data sets are required to enhance the evolutionary information on this particular family which includes additional sequences of representative locales and data

from different regions of potentially useful DNA markers. Regardless of the drawback mentioned, the result of this study was consistent with previous study by Gibson (2010).

ACKNOWLEDGEMENTS

The authors thank the Shell Chair Research Grant Scheme (SRC/02/2007(02)) for the funding; Universiti Malaysia Sarawak, Sarawak Forest Department and Sarawak Forestry Corporation for supporting the research work.

REFERENCES

Avise, J.C. 2004. *Molecular Markers, Natural History, and Evolution*. 2nd ed. Sunderland, Massachusetts: Sinauer.

Baker, A.J., Pereira, S.L. & Paton, T.A. 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, J., van Buren, A., Cherel, Y. & Whitfield, J.B. 2006. Genetic evidence for three species of Rockhopper Penguins *Eudyptes chrysocome*. *Polar Biol*. DOI 10.1007/s00300-006-0160-3.

Barrett, D. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends in Ecology & Evolution* 23(1): 38-44.

Braun, E.L. & Kimball, R.T. 2002. Examining basal avian divergences with mitochondrial sequences: model complexity, taxon sampling and sequence length. *Syst. Biol*. 51: 614-625.

Brown, W.M. 1983. Evolution of animal mitochondrial DNA. In *Evolution of Genes and Proteins,* edited by Nei, M. & Koehn, R.K. Sunderland, Massachussets: Sinauer Associates.

Clements, J.F., Schulenberg, T.S., Iliff, M.J., Sullivan, B.L. & Wood, C.L. 2010. *The Clements Checklist of Birds of the World: Version 6.5*. New York: Cornell University.

Cummings, M.P., Otto S.P. & Wakeley, J. 1995. Sampling properties of DNA sequence data in phylogenetic analysis. *Mol. Biol. Evol*. 12: 814-822.

Ericson, P.G.P., Envall, I., Irestedt, M. & Norman, J.A. 2003. Inter-familial relationships of the shorebirds (Aves: Charadriiformes) based on nuclear DNA sequence data. *BMC Evol. Biol*. 3: 16.

Farris, J.S., KaÈ llersjoÈ, M., Kluge, A.G. & Bult, C. 1995. Constructing a significance test for incongruence. *Syst. Biol*. 44: 570-572.

Gibson, R. & Baker, A. 2012. Multiple gene sequences resolve phylogenetic relationships in shorebird suborder Scolopaci (Aves: Charadriiformes). *Molecular Phylogenetics and Evolution* 64: 66-72.

Gibson, R. 2010. Phylogenetic relationships among the Scolopaci (Aves: Charadriiformes): Implications for the study of behavioral evolution. M.Sc. Thesis. University of Toronto (Unpublished).

Grewe, P.M., Krueger, C.C., Aquadro, C.F., Bermingham, E., Kincaid, H.L. & May, B. 1993. Mitochondrial variation among lake trout (*Salvenilus namaycush*) strains stocked into Lake Ontario. *Can. J. Fish. Aquat. Sci*. 50: 2397-2403.

Groth, J.G. & Barrowclough, G.F. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Mol. Phylogenet. Evol*. 12: 115-123.

Huelsenbeck, J.P. & Ronquist, F. 2001. Mrbayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Jehl Jr., J.R. 1968. Relationships in the Charadrii (shorebirds): a taxonomic study based on color patterns of the downy young. *Mem. San Diego Soc. Nat. Hist*. 3: 1-54.

Kimura, M. 1980. A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.

Livezey, B.C. 2010. Progress and obstacles in the phylogenetics of modern birds. In *Evolution of Modern Birds*, edited by Dyke, G. & Chiappe, L. Berkeley: University of California Press. pp. 117-145.

Mayr, G. 2011. The phylogeny of Charadriii form birds (shorebirds and allies) - reassessing the conflict between morphology and molecules. *Zool. J. Linn. Soc*. 161: 916-934.

Myers, S. 2009. *A Field Guide to the Birds of Borneo*. UK: New Holland Publication.

Palumbi, S.A., Martin, S., Romano, W.O., McMillan, L., Stice, L. & Grabowski, G. 1991. *The Simple Fool's Guide to PCR*. Honolulu, HI: Department of Zoology and Kewalo Marine Laboratory, Univ. of Hawaii.

Paton, T.A. & Baker, A.J. 2006. Sequences from 14 mitochondrial genes provide a well-supported phylogeny of the charadriiform birds congruent with the nuclear RAG-1 tree. *Molecular Phylogenetics and Evolution* 39: 657-667.

Paton, T.A., Baker, A.J., Groth, J.G. & Barrowclough, G.F. 2003. RAG-1 sequences resolve phylogenetic relationships within Charadriiform birds. *Mol. Phylogenet Evol*. 29(2): 268-278.

Pereira, S.L. & Baker, A.J. 2005. Multiple gene evidence for parallel evolution and retention of ancestral morphological states in the shanks (Charadriiformes: Scolopacidae). *Condor* 107: 514-526.

Pereira, S.L., Baker, A.J. & Wajntal, A. 2002. Combined nuclear and mitochondrial DNA sequences resolve generic relationships within the Cracidae (Galliformes Aves). *Syst. Biol*. 51: 946-958.

Rosenberg, N.A. & Feldman, M.W. 2001. *The Relationship between Coalescence Times and Population Divergence Times. Modern Developments in Theoretical Population Genetics*. Oxford: Oxford University Press.

Saitou, N. & Nei, M. 1987. The neighbor-joining method - a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4: 406-425.

Sibley, C. & Ahlquist, J. 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution*. New Haven: Yale University Press.

Smythies, B. 1999. *The Birds of Borneo*. 4th ed. Kota Kinabalu: Natural History Publications Borneo.

Swofford, D.L. 2002. PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Massachusetts: Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.

Thomas, G.H., Wills, M.A. & Székely, T. 2004. A supertree approach to shorebird phylogeny. *BioMed Central Evolutionary Biology* 4: 1-18.

Thompson, J.D., Gibson, T.J. & Plewniak, F. 1997. The clustal X windows interface: Flexible strategies for multiple sequence alignment aided by the quality analysis tools. *Nucleic Acids Res*. 24: 4876-4882.

Weibel, A.C. & Moore, W.S. 2002. Molecular phylogeny of a cosmopolitan group of woodpeckers (genus *Picoides*) gased on COI and cyt *b* mitochondrial gene sequences. *Mol. Phylogenet. Evol*. 22: 65-75.

Nurul Ashikeen Ab Razak* & Andrew Alek Tuen
Institute of Biodiversity and Environmental Conservation
Universiti Malaysia Sarawak
94300Kota Samarahan, Sarawak
Malaysia

Mustafa Abdul Rahman
University College Sabah Foundation
Jalan Sanzac, 88100 Sembulan
Kota Kinabalu, Sabah Negeri di Bawah Bayu
Malaysia

*Corresponding author; email: ekinrazak@gmail.com

Received: 25 November 2014
Accepted: 30 January 2016