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Short Communication

Molecular Characterization and Genetic Identification of Pakistani Partridges Through DNA-Barcoding

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ARA developed the idea and supervised the research. MZH, AI and AAA and MW prepared material and collected data. Data was analysed by MZH, AI, SF and MT. The gene analysis and submission was done by MZH, AW, AA and AI. RMA and AA has drafted the manuscript.

Key words
***Alectoris chukar, Ammoperdix heyi, COI* gene, DNA barcoding, Molecular characterization**

ABSTRACT

Now a days, DNA barcoding using *COI* gene (mitochondrial cytochrome c oxidase subunit I) has gained more attention, because of these barcodes validity and simplicity for identification of bird species has increased a lot. In present study, 650bp region of *COI* gene of two bird species, Chukar patridge (*Alectoris chukar*) and Sand patridge (*Ammoperdix heyi*) are sequenced. Total 6 birds (03 birds from each species) were selected. Each sample was aligned with its reference sequence of *COI* gene available on NCBI and every nucleotide position which did not align with reference sequence was studied to identify SNPs. Our results demonstrated that *A. chukar* formed a single cluster with *Alectoris philbyi* while *Alectoris rufa* and *Alectoris melanocephala* were a bit distant relatives of *A. chukar.* The study clearly suggesting efficacy and accuracy of DNA barcodes for molecular identification and characterization of species. Further, barcoding technique used for analysis of molecular diversity and genetic identification of partridges provide a valuable information about population structure, phylogenetic history, molecular conservation and species identification.

Being an indispensable piece of biological system, birds are abundant creature that exist on earth. They have shown a regular approach towards plants pollination and in control of bugs. The accumulated excrement of birds is widely used as compost. In addition, meat of few feathered creatures is utilized as a healthy diet worldwide. Through environment, a few winged animals transport assortment of things like seed scattering, dust transportation and microorganisms spread as well (Valentini *et al*., 2008).

The partridges (non-migratory birds) belong to genus *Alectoris* of family Phasianidae and considered as birds of hilly, open and dry terrains. They show huge variations in their morphological characteristics (greyish breast and back, buff belly and reddish legs). Their face is white, usually prominent with dark gorget and are known as rotund birds. There are various reported species of partridges including Philby's patridge (*Alectoris philbyi*), chukar patridge (*Alectoris chukar*), rock patridge (*Alectoris graeca*) and Red- legged patridge (*Alectoris rufa*). The representatives of these birds are found inhibit in northern side of Africa, southern Europe and across Pakistan in Asia. Among all above given species, red-legged partridge and chukar have been introduced to Hawaii and Canada. However, the hybrid between these two species are also common in Great Britain (Khan *et al*., 2010). Although sand partridges (*Ammoperdix heyi*) and chukars (*Alectoris chukar sinaica*) of the Negev Desert are sympatric in some areas, sand partridges are endemic to arid regions, whereas chukars are primarily mesophilous (Kleinhaus *et al*., 1985).

A methodology of DNA barcoding using *COI* sequences (cytochrome oxidase subunit I) has extensive potential in discrimination of species that are closely related across diverse phyla in kingdom Animalia (Herbert *et al*., 2003a, b). A single barcode of DNA act as a rapid tool in discovery of lineages within a huge population that might be regarded as undiscovered species (Tavares and Baker, 2008). In addition, this methodology is useful to differentiate species when it is difficult to match adults with immature specimens like fish larvae (Peg *et al*., 2006) or

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species having polymorphic life cycle (Lane *et al*., 2007). Ilegal trading of animal by products from endanger species can also be monitored via this genetic tool (Valentini *et al*., 2008; Waugh, 2011).

In recent era, barcoding of DNA has been utilized for bird's species identification in different regions of the world. However, barcodes of some species such as chukar patridge and sand patridge have not been established tenaciously (Chaves *et al*., 2008). So, in present work the sequencing of *COI* gene (having 650bp region) of both targeted species chukar patridge and sand patridge were performed and their sequences are matched with previously determined sequences of other species of same genus.

Materials and methods

Six birds from two different species (3 birds from each species) were selected randomly during the year 2012 to 2016 with the participation of the Punjab Wildlife and Parks Department and Pet Center of University of Veterinary and Animal Sciences, Lahore. The bird's identification was made based upon their morphological characters.

Blood samples (100-300 µl) were collected from brachial wing vein of each in a vial having 40µL of EDTA solution (0.5M, pH 8.0) and stored at -20°C before extraction of genomic DNA according to Sambrook and Russel (2001) using Thermo Fisher Scientific Kit, USA. This DNA was used for amplification of *COI* gene using primers as follows (Hebert *et al*., 2004).

F 5' TTCTCCAACCACAAAGACATTGGCAC 3'

R 5' ACGTGGGAGATAATTCCAAATCCTG 3'

The PCR products (700bp) was sequenced on Applied Bimolecular System Instrument ABI 3100 Genetic Analyzer in Center for Applied Molecular Biology, Punjab, Pakistan. Sequences were analyzed by Bio Edit software and for homology and Clustal W2 was used for pairwise alignment.

By making use of Fast Minimum Evolution Method, phylogenetic trees for both the bird species were constructed in which *COI* gene sequences of Pakistani patridges were compared with all available sequences of birds to look over their phylogeny and taxonomy (Desper and Gascuel, 2004; Danish *et al*., 2008). The MEGA 6.0 software was used for further phylogenetic analysis.

Results and discussion

In the present study, the *COI* gene of Pakistani patridges were sequenced. The sequences of gene were submitted to National Centre for Biotechnology Information Genbank and different analysis of *COI* gene sequences were performed including homology analysis. The homology anatomization of the reference sequence and *COI* gene sequences of closest species; chukar patridge and sand patridge disclosed single nucleotide polymorphisms at various sites in *COI* gene sequences. The phylogenetic tree using 650bp nucleotide segment of *COI* gene was created followed by the comparison of *COI* gene sequences of both Chukar patridge and Sand patridge with all available sequences of patridges. The results showed that *A. chukar* formed a single cluster with *Alectoris philbyi*. *A. rufa* and *Alectoris melanocephala* were a bit distant relatives of *A. chukar* (Fig. 1). In addition, *A. heyi* showed a single cluster relationship with *Francolinus* genus. The genus *Francolinus* formed a single cluster indicated that *F. francolinus* and its sister species are sharing a common ancestor. Outer group of *A. chukar*; *F. francolinus and F. pondicerianus* are Chinese *Arborophila bruneopectus* (KC352730.2) bar-backed partridge, showed that Pakistani *A. chukar, F. francolinus* and *F. pondicerianus* had evolved from Chinese bar backed partridge (Fig. 2).

Many investigators also used mitochondrial control region sequences for phylogenetic and molecular diversity analysis of avain species (Lerner *et al*., 2009; Ozaki *et al*., 2010). A study was designed by Huang *et al*. (2007) in which he utilized mitochondrial control regions for analysis of phylogeographical structure of rusty patridges (neck-laced patridges). The control region sequences have demonstrated phylogeographical structure existence among population of rock patridges resulting from genetic divergence in Southern refugia (Lucchini and Randi, 1998).

Further, Fleischer *et al*. (2006) had conducted an experiment for DNA analysis of seven endangered specimens of wood- pecker (*Campephilus principalis)* and showed the documentation of their molecular diversity. The sequence analysis of these wood peckers had provided an essential DNA barcode resource for discernment of these endangered species. Although patridges have not considered threatened, it is likely that many vulnerable local populations exist within the range of species resulting from hunting and habitat loss and may need special attention (Kark *et al*., 1999). Barilani *et al*. (2007) in his study have disclosed introgessive hybridization, suggesting that the released captive-bred patridges have hybridized and reproduced in nature polluting the wild rock patridge population's gene pool in Greece. It means that a fine understanding of evolutionary history and phylogeography provides important information about habitat preference and physical barriers influence on gene flow in birds (Morris-Pocock *et al*., 2010).

Conclusion

In conclusion, the 650bp sequence of mitochondrial cytochrome c oxidase subunit I act as an important

Fig. 1. The phylogenetic tree of the Chukar patridge (*Alectoris chukar*) based on *COI* gene analysis.

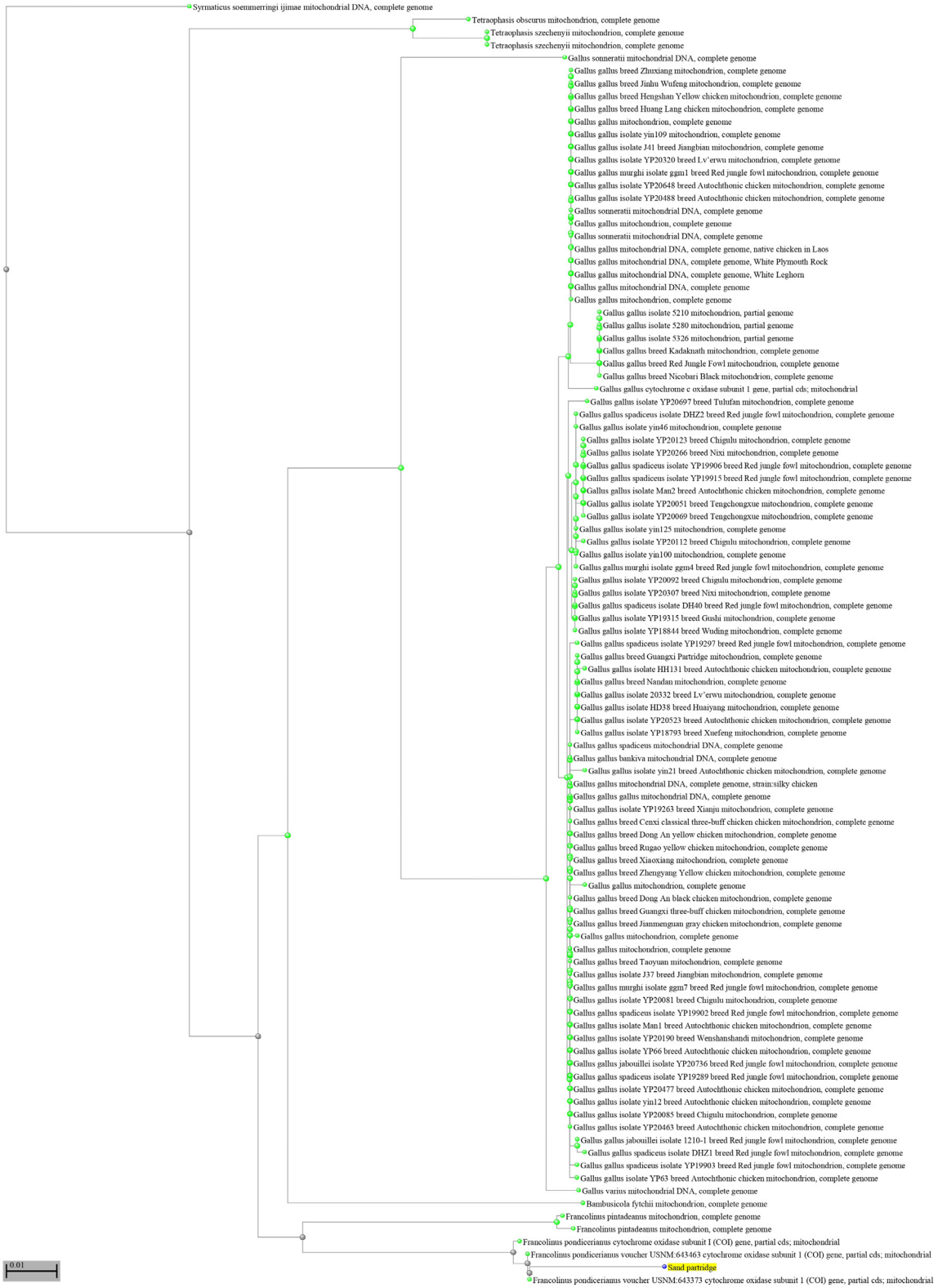

Fig. 2. The phylogenetic tree of the sand patridge (*Ammoperdix heyi*) based on *COI* gene analysis.

phylogenetic marker because trees that are obtained with its data set coincides with pre-established phylogeny of patridges species.

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Conflict of interest statement

The authors have declared no any conflict of interest in this study.

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