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Full Length Article

Molecular Classification of Pakistani Rose-Ringed Parakeet using Mitochondrial *ND2* Gene

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Abstract

The present study aimed to genetically identify the indigenous Rose-ringed parakeet of Pakistan using NADH dehydrogenase subunit 2 (ND2) gene polymorphism. Blood samples of 24 unrelated Pakistani rose-ringed parakeets were utilized for isolation of genomic DNA followed by amplification and sequencing of ND2 gene. The analysis of genetic phylogeny of the ND2 gene indicated that the Pakistani rose-ringed parakeet was cladded with *Psittacula krameri* with DNA and amino acid sequence similarity of 97.6 and 98.27%, respectively. Further, comparative analysis indicated 25 changes in nucleotide and six changes in amino acid sequences in ND2 gene of Pakistani rose-ringed parakeet suggestive of an independent evolution of this avian species. On the basis of unique genotype and distinguishing phenotypic characteristics, Pakistani rose-ringed Parakeet should be classified as *Psittacula krameri*. The present report is the first documentation on molecular classification of Pakistani Rose-ringed parakeet on the basis of ND2 gene polymorphism. © 2014 Friends Science Publishers

Keywords: Pakistani rose-ringed parakeet; ND2 gene polymorphism; Mitochondrial DNA; *Psittacula krameri*; Phylogenetic analysis

Introduction

Pakistan is endowed with a majestic array of wild avian fauna. Rose-ringed parakeet commonly known as Kathy parrot belongs to family *Psittacidae.* On the basis of geographical distribution, Rose-ringed parakeets (*Psittacula krameri*) have been classified into four subspecies viz; *P. k. krameri, P. k. parvirostris, P. k. manillensis* and *P. k. borealis* but they have not been genetically characterized as yet (Ahmad *et al.,* 2012a, b). Genetic based investigations are needed for identification of reproducible molecular markers which could be employed for accurate species identification and differentiation. Among the genetic approaches, mitochondrial DNA (mtDNA) markers are potentially authentic tools for species characterization and molecular classification (Lane, 2005; Awan *et al*., 2013).

Mitochondrial genome is considered very important for evolutionary analysis and has potential for identification and delineation of species (Galtier *et al*., 2009). Furthermore, the mutations in mtDNA occur in a chronological manner that enable researchers to investigate these changes and can be associated with geographical distribution and origin of populations. Mitochondrial DNA is an excellent milestone to predict the ancestry of the individuals and leads to accurate conclusions regarding taxonomic relationships (Barrowclough, 1983; Zink, 1991). It harbors greater importance than nuclear DNA and phenotypic traits in order to determine the evolutionary pattern (Irwin *et al*., 1991). Mitochondrial ND2 (mtND2) gene evolves comparatively at a high rate (Otto *et al*., 1996) and has reportedly been an effective mean in discerning the relationships among closely related species (Macey *et al*., 1998). In the present study, we utilized ND2 gene sequences analysis to define the phylography of Pakistani rose-ringed parakeet.

Materials and Methods

A total of 24 Pakistani Rose-ringed parakeets were selected on the basis of their unique morphological characteristics. Blood samples (200 µL) were collected and utilized for DNA isolation using organic DNA extraction method, followed by ND2 gene amplification. Amplicons were visualized on 1.2% agarose gel and were purified using DNA extraction kit (GeneAll, General Biotechnology, Seoul, Korea). Then the amplicons were sequenced using BigDye terminator cycle sequencing kit (Applied Biosystems, USA) on ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA). Chromatograms were analyzed using Chromas Ver. 1.45, (http://www.technelysium.com.au/chromas.html). The sequence alignments were done using BioEdit version 5.0.9 (Hall, 1999). Aligned

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ND2 sequences were imported to MEGA 5.1 (Kumar *et al*., 2004) and Expasy Bioinformatics Resource Portal (http://web.expasy.org.translate) for phylogenetic and amino acid sequence analysis, respectively.

Results

We amplified and analyzed full length ND2 gene of Pakistani rose-ringed parakeets to determine their molecular phylogeny. The nucleotide sequences of ND2 gene from 24 birds were submitted to NCBI GenBank (Accession No. KC823233 to KC823256).

ND2 gene based comparative analysis indicated that Pakistani Rose-ringed parakeets contain 97.6% similarity with Asian and African rose-ringed parakeet (*P. krameri*), 92.22% with Indian Blue-winged parakeet (*P. columboides*), 91.83% with Indian malabar parakeet (*Psittinus cyanurus*), 91.45% with Blue-naped parrot (*Tanygnathus lucionensis*), 90.20% with Song parrot (*Geoffroyus heteroclitus*), 86.45% with Australian and Indonesian Rainbow Lorikeet (*Trichoglossus haematodus*) and 86.16% with Indonesian Red-and-blue lory (*Eos histrio*).

Comparison of ND2 gene sequence of Pakistani rose-ringed parakeet with its closest homologue, *P. krameri* indicated 25 variations in nucleotide sequence (Fig. 1A). Deduced amino acid sequences were also utilized for homology analysis (Fig. 1B). Amino acid sequence based comparative analysis revealed similarity of 98.27% with *P. krameri*, 93.64% with *P. columboides*, 93.35% with *P. cyanurus*, 92.77% with *T. lucionensis* and *G. heteroclitus,* 85.83% with *T. haematodus* and 85.83% with *T. haematodus.*

The phylogenetic analysis demonstrated that Pakistani rose-ringed parakeet clustered in Clade B with *P. krameri*, *P. columboides*, *P. roseate*, *G. heteroclitus*, *P. cyanurus*, *T. lucionensis*, *Eclectus roratus* and *Prioniturus montanus* (Fig. 2). Members of Clade B (*P. krameri*, *P. columboides*, *P. roseate* and *P. cyanurus*) belonging to Asia shared an identity of 90 to 97.97% with Pakistani rose-ringed parakeets. Clade A consisting of *T. haematodus, P. elegans*, *M. undulates*, *P. fulgidus* and *E. histrio* members and belonging to Australia and Indonesia shared a homology of 80 to 92% with Pakistani parakeets. Clade C consisted of two members, *P. picta* belong to Indonesia while *Anodorhynchus leari* was from Brazil.

Discussion

In the present study, mtND2 gene was utilized for characterization of Pakistani rose-ringed parakeet. Previously, the same gene was utilized for characterization of various avian species like *Otus megalotis* and *Mimizuku gurneyi* (Zink *et al*., 1991; Gonzalez *et al.,* 2009; Bradman *et al*., 2011). Comparative analysis revealed that the use of ND2 gene was an alternative of *cytochrome b* (*Cyt b*) gene

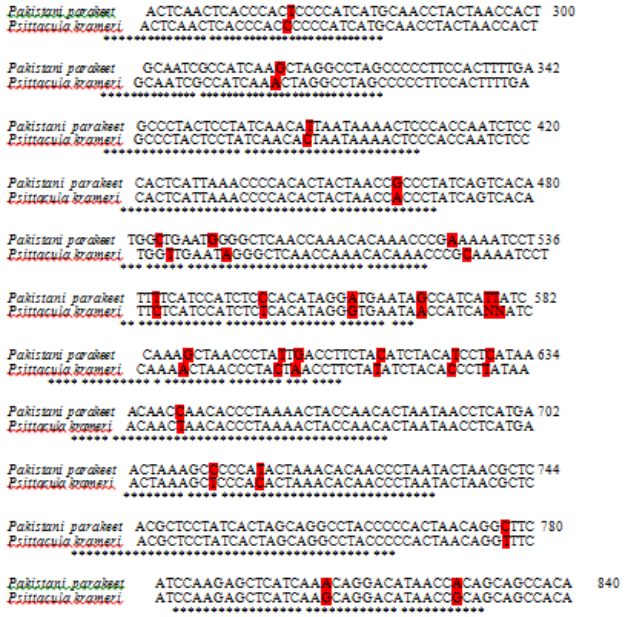

Fig. 1A: Nucleotide alignment of ND2 gene of *Psittacula krameri* and Pakistani rose- ringed parakeet

Fig. 1B: Protein alignment of ND2 gene of *Psittacula krameri* and Pakistani rose- ringed parakeet

Fig. 1: Comparison of ND2 gene sequence from Pakistani rose-ringed parakeet (*Pakistani parakeet*) with its closest homologue *Psittacula krameri.* Fig. 1A and 1B shows the comparison of nucleotide and amino acid sequences, respectively. Asterisks, at the bottom of sequence indicate sequence identity while the highlighted nucleotides are the variation among the two species

for the identification of various avian species of *Phasianidae*, *Anatidae*, *Gruidae*, *Scolopacidae*, *Accipitridae*, *Falconidae*, *Struthionidae* and *Psittacidae* families (Boonseub *et al*., 2009). We have also studied the *Cytb* gene of Pakistani rose-ringed parakeet (Unpublished data) and the results confirmed the findings of present study.

ND2 gene based homology analysis showed 22 transitions and 1 transversion, but not a single indel. The other two nucleotides could not be declared as transition or transversion due to ambiguity in the reference sequence. Quantification of the transitions/ transversions is primarily important for coding genes based characterization and phylogenetic analysis. Transitions are mostly down-weighted as compared to the nucleotide transversion in phylogenetic investigations. Reconstruction of accurate phylogenetic relationship is fairly data dependent, because it plays integral role in numerous genetic indices (Broughton *et al*., 2000). Previous studies of Fitch (1967) and Li *et al*., (1984) also were in favor of high transitional frequency in nuclear and mitochondrial genomes. The elevated rate of nucleotide transition has been reported to show multiple vibrational events in specific nucleotide sites and increase the frequency of homoplasmy (Meyer, 1994; Simons *et al*., 1994). Furthermore, the nucleotide transitional accumulation exhibits the "plateau" effect. That means beyond a specific level, increase in nucleotide transitions becomes neutral even with the increase of overall divergence (Irwin *et al*., 1991). mtDNA variations are pivotal tools in evolutionary, molecular ecology and population genetics. They paved the way for modeling the population history of individuals. Coalescent events in mitochondrial genome (Schierup and Hein, 2000), including ways of population expansion and divergence time (Kuhner *et al*., 1998; Beerli and Felsenstein, 1999) greatly impact on biogeographical distributions of individuals.

It was observed that most of the changes in nucleotide sequence were at third base of codon, so, the coded amino acids were found same-sense. The comparison of amino acids sequences from Pakistani rose-ringed parakeet with *P. krameri* indicated 6 variations; P92S, T156A, T191A, X193I, T239M and A277T (Fig. 1B). The replacement of P92S and A277T were conversion of non-polar to polar amino acids whereas the change of T156A, T191A and T239M were from polar to nonpolar amino acids. The presence of X at position 193 in the reference sequence of *P. krameri* indicates the presence of I or replacement with other amino acid. X-ray crystallographic studies are required to explain the structural and functional relationship due to these replacements.

Phylogenetic analysis further suggested that the Pakistani Rose-ringed parakeet is monophyletic with Asian *P. krameri*, *P. columboides*, *P. roseate*, *G. heteroclitus*, *P. cyanurus*, *T. lucionensis*, *Eclectus roratus* and *Prioniturus montanus*. In view of the preliminary nature of the present study, similar additional studies on the Rose-ringed parakeets (including those endemic in other provinces of Pakistan) are clearly warranted.

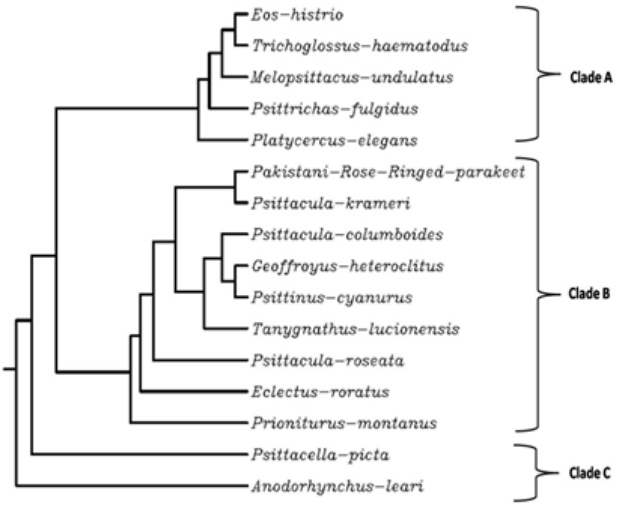

Fig. 2: Phylogenetic tree constructed using the deduced amino acid sequence. The DNA sequences were taken from present study or from NCBI databank; Phylogram is based on ND2 gene sequences of Psittaciformes indicating the molecular classification of the Pakistani rose-ringed parakeet

This is the first study on molecular classification of Pakistani rose-ringed parakeet at sub-species level using nucleotide and amino acid sequences of ND2 gene. These novel polymorphisms might act as marker for the molecular classification of Pakistani rose-ringed parakeet.

References

Ahmad, S., H.A. Khan, M. Javed and K.U. Rehman, 2012a. An estimation of rose-ringed parakeet (*Psittacula krameri*) depredations on citrus, guava and mango in orchard fruit farm. *Int. J. Agric. Biol.*, 14: 149–152

Ahmad, S., H.A. Khan, M. Javed and K.U. Rehman, 2012b. Management of maize and sunflower against the depredations of rose-ringed parakeet (*Psittacula krameri*) using mechanical repellents in an agro-ecosystem. *Int. J. Agric. Biol.,* 14: 286–290

Awan, A.R., E. Umar, M.Z. ul Haq and S. Firyal, 2013. Molecular classification of Pakistani collared dove through DNA barcoding. *Mol. Biol. Reports*, 40: 6329–6331

Barrowclough, G.F., 1983. Biochemical studies of microevolutionary processes. In: *Perspectives in Ornithology*. A.H. Brush, and G.A. Clark Jr., (eds). pp: 223–261. Cambridge University Press, Cambridge UK

Beerli, P. and J. Felsenstein, 1999. Maximum–likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics,* 152: 763–773

Boonseub, S., S.S. Tobe and A.M. Linacre, 2009. The use of mitochondrial DNA genes to identify closely related avian species. *Forensic Sci Int. Genet. Suppl*., S2: 275–277

Bradman, H., P. Grewe and B. Appleton, 2011. Direct comparison of mitochondrial markers for the analysis of swordfish population structure. *Fish Res.,* 109: 95–99

Broughton, R.E., S.E. Stanley and R.T. Durrett, 2000. Quantification of homoplasy for nucleotide transitions and transversions and a reexamination of assumptions in weighted phylogenetic analysis. *Syst. Biol.,* 49: 617–627

Fitch, W.M., 1967. Evidence suggesting a non–random character to nucleotide replacements in naturally occurring mutations. *J. Mol. Biol.*, 26: 499–507

Galtier, N., B. Nabholz, S. Glémin and G. Hurst, 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol*., 18: 4541–4550

Gonzalez, J., G.D. Castro, E. Garcia–del–Rey, C. Berger and M. Wink, 2009. Use of mitochondrial and nuclear genes to infer the origin of two endemic pigeons from the Canary Islands. *J. Ornithol*., 150: 357–367

Hall, T.A., 1999. BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucliec acid Symposium*, 41: 95–98

Irwin, D.M., T.D. Kocher and A.C. Wilson, 1991. Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.,* 32: 128–144

Kumar, S., K. Tamura and M. Nei, 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.*, 5: 150–163

Kuhner, M.K., J. Yamato and J. Felsenstein, 1998. Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, 149: 429–434

Lane, N., 2005. *Power, sex, suicide: mitochondria and the meaning of life*. pp: 368. Oxford University Press, New York, USA

Li, W.H., C.I. Wu and C.C. Luo, 1984. Nonrandomness of point mutation as rejected in nucleotide substitutions in pseudogenes and its evolutionary implications. *J. Mol. Evol*., 21: 58–71

Macey, J.R., J.A. Schulte, A. Larson, Z. Fang, Y. Wang, B.S. Tuniyev and T.J. Papenfuss, 1998. Phylogenetic relationships of toads in the Bufobufo species group from the eastern escarpment of the Tibetan Plateau: A case of vicariance and dispersal. *Mol. Phylogenet. Evol*., 9: 80–87

Meyer, A., 1994. Shortcomings of the cytochrome b gene as a molecular marker. *Trends Ecol. Evol.*, 9: 278–280

Otto, S.P., M.P. Cummings and J. Wakeley, 1996. Inferring phylogenies from DNA sequence data: The effects of sampling. *In: New uses for new phylogenies*. pp. 103–115. Oxford Univ. Press, New York, USA

Schierup, M.H. and J. Hein, 2000. Consequences of recombination on traditional phylogenetic analysis. *Genetics,* 156: 879–891.

Simons, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu and P. Floors, 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87: 651–701

Zink, R.M., 1991. The geography of mitochondrial DNA variation in two sympatric sparrows. *Evolution,* 45: 329–339

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