DNA-typing for Cytochrome Oxidase-1 of *Hystrix indica* (*Rodentia; Hystricidae*) from Kingdom Saudi Arabia

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Abstract: Approximately 600 bp from cytochrome C oxidase subunit 1 gene (CO1) have been sequenced for samples of *Hystrix indica* from different localities in Saudi Arabia. The data were manipulated and aligned by DNA star program and were compared in order to show the base differences among the individuals and there was no base difference among the Saudi samples. By using Blast program inside the package of the NCBI, the sequenced fragment was compared with its counterparts from the same species. The alignment of our data to the Indian haplotypes showed variations at the positions 66, 180, 210, 216, 276, 279, 339, 504 and 510. The variations were synonymous transitions of $G^{66, 210, 504}$ in Indian samples to $A^{66, 210, 504}$ in Saudi samples. Other transition of $A^{276, 279, 510}$ in Indian samples to $G^{276, 279, 510}$ in Saudi samples have also been found. Thymine^{339} in Saudi samples and Indian samples were shown against C^{339} in one Indian haplotype. Among these transitions, only one non- synonymous change was revealed in which isoleucine^{70} in the Saudi samples was substituted with methionine^{70} in Indian haplotypes. We therefore may conclude that the genetic variability within Saudi Arabian *Hystrix indica* supposed to be a small and the species could be threatened or endangered in near future and requires conservational attention. The physiological efficiency of some Indian porcupines may be better than that of the Saudi porcupines.

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1. Introduction

A porcupine contains 29 rodent species belonging to families Erethizontidae or Hystricidae. They are with a coat of sharp spines, or quills, that for defense and camouflage (Vilela *et al.*, 2009).

Porcupines occupy tropical and temperate parts of Asia, Southern Europe, Africa, and North and South America. They live in forests, deserts, rocky outcrops and hillsides. Some New World porcupines live in trees, but Old World porcupines stay on the rocks. Porcupines can be found on rocky areas up to 3,700 m. These animals are generally nocturnal but are occasionally active during daylight. Most porcupines are about 25–36 inch long, with an 8–10 inch tail long.

Weighing between 5.4–16 kg, they are rounded, large and slow. Porcupines' spiny protection resembles that of the unrelated erinaceomorph hedgehogs and monotreme echidnas. Little genetic studies have been conducted for these taxa (Oliveira *et al*., 2011). They are eaten in western culture, but are very popular in Southeast Asia, particularly Vietnam, where the prominent use of them has contributed to significant declines in their populations. Their quills and guard hairs are used for traditional decorative clothing.

The genetic framework of this animal is still controversial and therefore the present study aimed to tackle the genetic variability among the haplotypes of this species which inhabiting Saudi Arabia. The same individuals from the gene bank will also be compared.

In a study on Neotropical porcupines, Voss and Angermann (1997) clarified the taxonomy of some erethizontids.

However, Bonvicino *et al*. (2002) noted that the status of several taxa in this family and their phylogenetic relationships are still poorly understood. *Erethizon* and *Echinoprocta* are recognized as monotypic genera, whereas other species of erethizontids are allocated either to the genera *Coendou* and *Sphiggurus* (e.g [1999, 2005]) or solely to the genus *Coendou* (e.g. [1997,1992]). Bonvicino *et al.* (2002) used the mitochondrial cytochrome *b* gene and karyologic data to clarify the taxonomic status of *Coendou* and *Sphiggurus*. Both kinds of data demonstrated that *Coendou* and *Sphiggurus* represent two evolutionary lineages. Their comparative analyses of the karyotypes showed that species of *Coendou* are karyologically conservative, sharing the same diploid and fundamental numbers. Species of *Sphiggurus*, on the other hand, diverge in diploid number although they share the same fundamental number. There are countless taxonomic issues involving the Erethizontidae, but perhaps no taxon has aroused more controversy than the genus *Chaetomys*, which contains a single species, the thin-spined porcupine *Chaetomys subspinosus*. This species is endemic to the Atlantic

Rainforest in eastern Brazil and, according to Woods and Kilpatrick (2005), it is found from the southern part of the state of Sergipe to the northern part of the state of Rio de Janeiro, including easternmost Minas Gerais. *Chaetomys subspinosus* is considered an endangered species by the U.S. Endangered Species Act, U.S. ESA; a vulnerable species by the International Union for the Conservation of Nature and Natural Resources, IUCN; and a threatened species by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, IBAMA .

Heme-copper oxidases are transmembrane protein complexes in the respiratory chains of prokaryotes and mitochondria which catalyze the reduction of O_2 and simultaneously pump protons across the membrane. The superfamily is diverse in terms of electron donors, subunit composition, and heme types. The number of subunits varies from three to five in bacteria and up to 13 in mammalian mitochondria. Membership in the superfamily is defined by subunit I, which contains a heme-copper binuclear center (the active site where O_2 is reduced to water) formed by a high-spin heme and a copper ion. It also contains a low-spin heme, believed to participate in the transfer of electrons to the binuclear center. Only subunit I is common to the entire superfamily. For every reduction of an O_2 molecule, eight protons are taken from the inside aqueous compartment and four electrons are taken from the electron donor on the opposite side of the membrane. The four electrons and four of the protons are used in the reduction of O_2; the four remaining protons are pumped across the membrane. This charge separation of four charges contributes to the electrochemical gradient used for ATP synthesis. Two proton channels, the D-pathway and K-pathway, leading to the binuclear center have been identified in subunit I of cytochrome c oxidase (CcO) and ubiquinol oxidase. A well-defined pathway for the transfer of pumped protons beyond the binuclear center has not been identified. Electron transfer occurs in two segments: from the electron donor to the low-spin heme, and from the low-spin heme to the binuclear center. The first segment can be a multi-step process and varies among the different families, while the second segment, a direct transfer, is consistent throughout the superfamily (http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=206949).

These new data from sequencing of the cytochrome *b* gene and karyotyping of a female thin-spined porcupine, *Chaetomys subspinosus*, confirm that this species does not belong to the family Echimyidae. Instead, it is related to the Erethizontidae, and belongs to a sister-clade to the other erethizontids. Nevertheless, its basalmost position relative to the Erethizontidae, its high levels of sequence divergence, and its morphological distinctiveness suggest that *Chaetomys* belongs to an early radiation of the Erethizontidae that may have occurred in the Early Miocene, from 23 to 21 million years before the present, and should be allocated to a subfamily of its own, the subfamily Chaetomyinae, sister to the subfamily Erethizontinae, which contains the other erethizontid genera.

2.Material and methods

Animal collection : Different samples were taken from the kingdom of Saudi Arabia covering the central region (Riyadh), the western region (Taif) and southern region (Jazan).

DNA Extraction by Kit : Animals were dissected and 300 µl from blood was add to 100 µl of 5% Chelex-100 and 4ml of proteinase-K (20mg/ml) incubated at least 6 hours at 56ºC followed by 10 minutes at 95ºC (Almeida and Stouthamer, 2003)

PCR of CO1 gene: The PCR has been done in a total volume of 50 µl using a Techne thermocycler. For one reaction, 3 µl of DNA template have been used, plus 25 µl of the PCR mix (5 µl of PCR buffer, 1ml of dNTP's each in a 10mM concentration), 1.25 µl of the forward and the reverse primers located in the corresponding mtDNA genes. 600 base pairs of the mitochondrial COI gene were amplified with primers HyxF:5`- GCATGGGCTGGAATAGTAGGAAC-3` and HyxR:5`-AGCTGGGTCAAAGAAGGTAGTATT-3`. The amplified products were subjected to DNA sequencing through Macrogen Company(Korea).

Bioinformatic analysis was done on the resulted sequences by using DNA Star Inc. software (ver. 1993-2001). Sequences were aligned to published data from gene bank site www.ncbi.nlm.nih.gov.

3.Results and discussion

The extracted DNA has been subjected to PCR experiment and the obtained amplified products have been sequenced. The obtained sequences were aligned by Clustal W and compared to the published COI-sequences of porcupine on the Gene bank (www.ncbi.nlm.nih.gov). The only COI-sequences of porcupine published on the Genbank were Indian haplotypes (accession numbers: JN714177, JN714182, JN714183, and JN714184).

Before comparing our resulted sequences we made an intra-alignment (between KSA-samples of different localities). The intra-alignments indicated to there was no variation in the nucleotides of the studied fragment. However, we obtained (figure 3) convergence of the KSA porcupine to the Indian haplotype- JN714177. Our studied samples showed (figure 3) divergence from other Indian haplotypes- JN714182, JN714183, and JN714184.

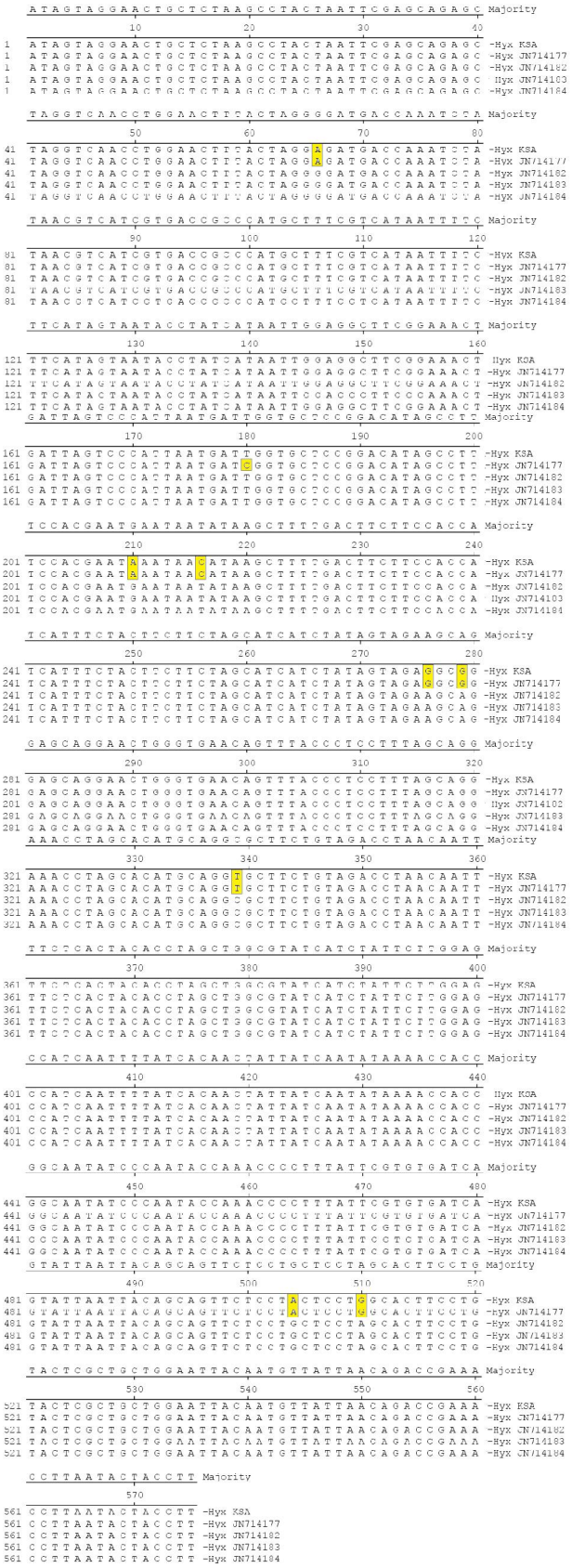
The alignment of our data to the Indian haplotypes (figure 1) resulted in variations at the positions 66, 180, 210, 216, 276, 279, 339, 504, and 510. There was synonymous transitions of $G^{66, 210, 504}$ in Indian samples (JN714182, JN714183, and JN714184) to $A^{66\ 210, 504}$ in Saudi samples (the Indian haplotype- JN714177 showed $A^{66\ 210, 504}$). Other transition of $A^{276, 279, 510}$ in Indian samples (JN714182, JN714183, and JN714184) to $G^{276, 279, 510}$ in Saudi samples (the Indian haplotype- JN714177 showed $A^{276, 279, 510}$) was recorded. Thymine occupies the position 339 in Saudi porcupine samples and Indian samples (JN714182, JN714183, and JN714184), that was substituted with Cytosine in the Indian haplotype- JN714177.

By aligning the translated amino acids (figure 2) we found that, complete identity of amino acid sequences among Saudi samples and Indian ones. However, our study recorded transition of methionine^{70} in Indian samples (JN714182, JN714183, and JN714184) to isoleucine^{70} in both our Saudi samples and the Indian sample haplotype- JN714177. Since, isoleucine (Ile) and methionine (Met) shares hydrophobic characters, we expect no great difference in properties between the two isomers, yet, Met is more polar than Ile due to the presence of Sulphur. We therefore expect that COI of the Indian porcupine samples (JN714182, JN714183, and JN714184) to be more nucleophilic than its counterpart of Saudi samples and in the Indian haplotype- JN714177. The studied domain is considered to be a member in the Heme_Cu_Oxidase_I family of protein complexes (figure 4), according to conserved domain architecture retrieval tool (www.ncbi.nlm.nih.gov/structure/lexington/lexington.cgi). Therefore, we expect COI of the Indian porcupine samples to be physiologically more efficient than its counterpart in Saudi samples and in the Indian haplotype- JN714177. Our explanation is in concordance with that of Richter and Ludwig (2003).

Conclusion

We may conclude that the genetic variability within *Hystrix indica* at Saudi Arabia supposed to be small and the species could be threatened or endangered in near future and required conservational attention. We also reporting a possible difference in the physiological efficiency of COI of Saudi Arabia porcupine than its counterpart of some Indian samples.

Figure 1. The aligned DNA sequence of CO1 gene in the collected samples of *H. Indica* from different Saudi localities (Hyx KSA) with those from India (Hyx-JN714177, JN714182, JN714183, and JN714184). →



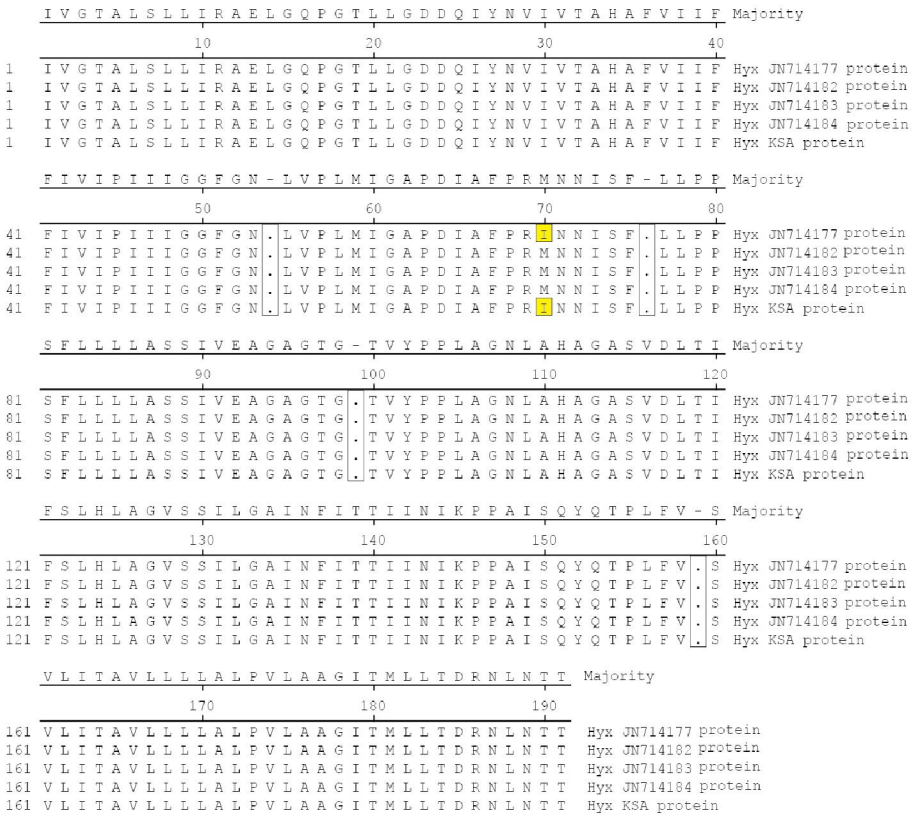


Figure 2. The aligned amino acid sequence of CO1 gene in the collected samples of *H. Indica* from different Saudi localities (Hyx KSA protein) with those from India (Hyx- JN714177 protein, JN714182 protein, JN714183 protein, and JN714184 protein).

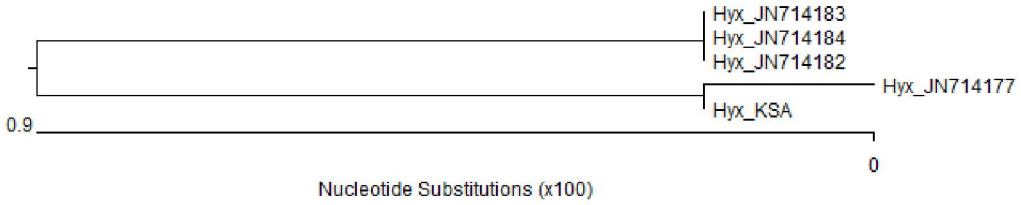


Figure 3. Dendogram showing the Clade for the Hystrix group from the family Hystricidae of rodents.

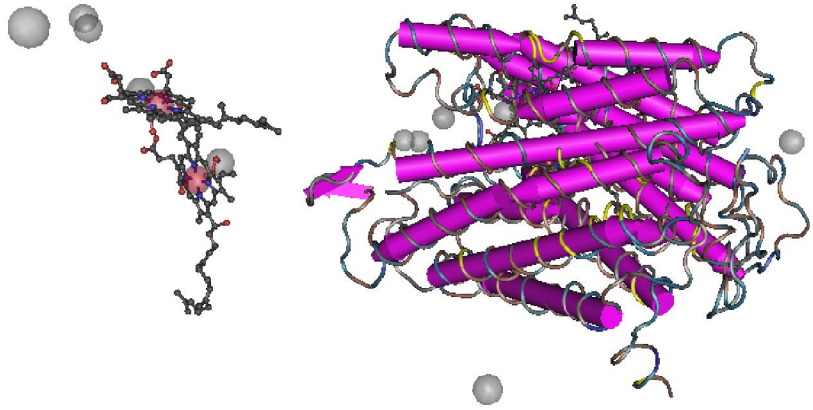


Figure 4. Structure of subunit I mitochondrial cytochrome c oxidase (Coil and tube representation formulated by www.ncbi.nlm.nih.gov/structure/lexington/lexington.cgi)

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