

ORIGINAL ARTICLE

Phylogenetic Analysis of Astigmatid Mites *Sarcoptes scabiei* and *Dermatophagoides farinae* using ITS-2 as a Genetic MarkerShumaila Naz¹, Farhana Riaz Chaudhry², Dilawar Abbas Rizvi³, Muhammad Ismail⁴**ABSTRACT**

Objective: The coding of astigmatid mites based on their morphological and developmental characteristics often leads to uncertainty in the results. The ribosomal internal transcribed spacer (ITS-2) region, being highly conserved in eukaryotes is commonly employed as a barcode for identification of mite species. The present study was an attempt to characterize the gene sequences of astigmatid mites i.e. *Sarcoptes scabiei* (*S. scabiei*), *Dermatophagoides farinae* (*D. farinae*) using ITS-2 as a genetic marker.

Study Design: Descriptive study.

Place and Duration of Study: The study was conducted at Department of Dermatology, Military Hospital (MH), Rawalpindi from September 2012 to October 2013.

Materials and Methods: In order to characterize relationship of astigmatid mites, the ITS-2 marker was successfully amplified and sequenced. The resulting ITS-2 gene sequences were aligned using Clustal W. MEGA 7 was used to construct phylogenetic tree of the aligned sequence.

Results: The phylogenetic tree showed an overall genetic distance of 0.53 indicating close genetic relationship among astigmatid mite species. Pairwise distance was calculated for the ITS-2 gene and low genetic diversity values were observed within *S. scabiei* and *D. farinae* that range from 0.003-0.008 and 0.006-0.038 respectively.

Conclusion: The study supports the view that the ITS-2 region can be used to identify morphologically difficult astigmatid mites but is not useful in characterization of different species based on the geographical distribution. This study has important implication in our understanding of the epidemiology of *S. scabiei* and *D. farinae* and development of control strategies in human transmission.

Key Words: *Dermatophagoides Farina (Derf)*, *House Dust Mite (HDM)*, *ITS-2 region*, *Pakistan*, *Sarcoptes scabiei var. hominis*.

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Introduction

Due to the ubiquitous nature of parasitic mites, they are known to infect invertebrates, vertebrates and

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plants. The parasitic infections are responsible for massive disease burden to humans and animals which in turn leads to higher levels of morbidity and mortality than any other infection.^{1,2,3} The morphological identification of different developmental stages of mites often leads to uncertainty in the results. In the adult stage, mites are difficult to identify with high level of accuracy; complexity and similarity in taxonomy and morphology has made it challenging to differentiate between them with great precision.^{4,5,6}

The limited availability of genomic data of astigmatid mites has implications related to genetic structure, epidemiology and monitoring protocols of the mites.^{7,8} Advances in molecular studies have allowed

the exploration of the whole genomic sequence of mites⁹ which has enabled the recognition of taxonomically complex species and to comprehend associations between mite taxa.^{10,11}

Phylogenetic relationships among different mite families are based on their morphological characteristics, allergen characterization, geographical distribution and physiological responses of mites to the environment and association with their hosts.^{12,13} Currently, molecular tools used to evaluate genetic diversification of species and to study phylogenetic interaction and relatedness among individual species are employing mitochondrial genes, ribosomal genes and internal transcribed spacers.^{14,15}

In acarological research, the internal transcribed spacer ITS-2 gene has been used to study organization and kinship of a population, and to identify taxonomically challenging species.¹⁶ Previous literature showed that the ITS-2 region has been used as a genetic marker for scrutinizing the diversity and exploring phylogenetic relationships among astigmatid mites.¹⁷ ITS-2 marker has been used to successfully verify the taxonomy of closely related species and to differentiate and identify different species of astigmatid mites.^{18,19} In this study, we used ITS-2 region to characterize astigmatid mites i.e. *S. scabiei* and house dust mites (HDMs) and then apply it as a phylogenetic marker to infer the genetic relationships among these two group of species.

Materials and Methods

Sample Collection

The ethical approval for the collection of mites was granted by the Institutional Review Board (IRB) of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan (letter No. PMAS-AAUR/ZOOL 362 approved on 22-3-2012). The *S. scabiei* mites were isolated from scabies infested patients visiting Military Hospital (MH), Rawalpindi after obtaining written informed consent from 20 individuals with ordinary scabies between September 2012 and October 2013. The cases of ordinary scabies were confirmed through clinical presentation, positive identification of mites and mite parts under the microscope.

For the collection of HDM, dust samples were collected from mattresses of beds and couches in the randomly selected houses and other places of

human dwelling using vacuum cleaner.²⁰ The saturated sodium chloride floatation method was used to isolate collected HDM from dust with supernatant filtered through 45µm filter paper.²¹ The astigmatid mites were then identified under the microscope. Both *S. scabiei* and HDM mites were then fixed in 70% ethanol and stored at 4°C until further processing.

Extraction of Genomic DNA

The genomic DNA of 20 individual astigmatid mites i.e. 10 of each *Sarcoptes* and HDM mites were extracted individually using the hotshot plus thermal shock technique.²² According to this method, 25 µl of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA) was added in thermal-cycler tubes followed by three cycles of thermal shocks. Then 25 µl neutralizing reagent (40 mM Tris-HCl) was added to disrupt the exoskeleton and to adjust the pH. The concentration of DNA was determined by nanodrop 2000 Spectrometer (Thermo Scientific, USA) at 260 and 280 nm wavelength.

PCR Amplification and Sequencing

The DNA of mites was amplified by using ITS-2 forward and reverse primers of sequence 5' CGACTTTCGAACGCATATTGC 3' and 5' GCTTAAATTCAGGGGGTAATCTGC3' respectively.^{23,24}

PCR was carried out in a final volume of 20 µl with the following steps: pre-denaturation for 2 min at 95 °C, followed by 30 cycles of the 30 s at 94 °C, 30 sec at 56 °C, 50 sec at 72 °C. 8 µl PCR product of HDM was digested individually with restriction enzymes Hinf I (10 U) and Taq I (20 U). Hinf I was incubated at 37°C and Taq I at 65°C overnight. The amplicons were visualized on 3% agarose gel, stained with ethidium bromide. The Big Dye® Terminator v3.1 cycle sequencing kit was used to directly sequence 10 purified PCR products in both directions by ABI 310 genetic analyzer (Applied Biosystems, Foster City, USA).

Sequence Analysis

ChromasPro (version 1.5) software (<https://chromaspro.software.informer.com/1.5/>) was used to edit and assemble the sequences. To verify results of sequenced samples, BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) was performed. The resulting top hit nucleotide sequences of ITS-2 were aligned using ClustalW (<http://www.genome.jp/tools->

bin/clustalw). The aligned sequences were further used to study and construct phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA 7) based on Kimura-2-parameter (K2P) distance.²⁵

Results

Genomic DNAs of *S. scabiei* and HDM mites were isolated from a total of twenty specimens and digested PCR products with both Hinf I and Taq I confirmed the morphologically identified *D. farinae* and no unexpected bands of PCR products or restriction fragments were observed.

The sequences of ITS-2 gene from *S. scabiei* and *D. farinae* showed 99% similarity respectively with whole genome of astigmatid mite (Accession no. AB778919.1 and GQ205600.1). The sequences obtained as a result of this study were submitted in GenBank under accession numbers KR010367, KJ409446, KT724354 and KT724355 for two *S. scabiei* samples (Sspak1, Sspak2) and two *D. farinae* samples (DerfPak1 and DerfPak2) respectively as shown in Table 1.

Table 1: Astigmatid mite sequences length followed by GenBank accession numbers for the ITS-2 gene

| Mite Species | Code | Sequence Length (bp) | GenBank Accession No |
|-------------------|----------|----------------------|----------------------|
| <i>S. scabiei</i> | Sspak1 | 376 | KR010367 |
| | Sspak2 | 359 | KJ409446 |
| <i>D. farinae</i> | DerfPak1 | 338 | KT724354 |
| | DerfPak2 | 335 | KT724355 |

The length of the ITS-2 sequences was between 335bp and 376bp for *S. scabiei* var. *hominis* and *D. farinae* respectively. ITS-2 sequences of astigmatid mites showed differences in terms of their length because of insertions and deletion at several sites. Three variable sites were determined for both astigmatid mites i.e. at 68, 235 and 272 positions in ITS-2 sequence of *S. scabiei* and 97, 174 and 281 nucleotide positions in the ITS-2 sequences of *D. farinae* when compared with reported ITS-2 gene sequences in NCBI nucleotide database.

Table 2: Pair wise distances for *Dermatophagoides farinae* on the basis of ITS-2 genetic variation

| | 1 | 2 | 3 | 4 | 5 | 6 |
|----------------------|-------|-------|-------|-------|-------|-------|
| <i>Der.f (Japan)</i> | 0.000 | | | | | |
| <i>Df-4</i> | 0.006 | | | | | |
| <i>DerfPak1</i> | 0.009 | 0.009 | | | | |
| <i>DerfPak2</i> | 0.012 | 0.013 | 0.003 | | | |
| <i>Df-3</i> | 0.025 | 0.025 | 0.016 | 0.019 | | |
| <i>Df-1</i> | 0.032 | 0.032 | 0.035 | 0.038 | 0.028 | |
| <i>Df-2</i> | 0.032 | 0.032 | 0.035 | 0.038 | 0.028 | 0.000 |

Tandem repeats of nucleotides (AAA)_n, (TTT)_n and (GATT)_n were observed in the ITS-2 gene of both astigmatid mites are useful in determining

Table 3: Pair wise distances for *Sarcoptes scabiei* on the basis of ITS-2 genetic variation

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---------------|-------|-------|-------|-------|-------|-------|
| <i>SsPak1</i> | 0.000 | | | | | |
| <i>SsPak2</i> | 0.000 | | | | | |
| <i>Ss-1</i> | 0.003 | 0.003 | | | | |
| <i>Ss-2</i> | 0.005 | 0.005 | 0.008 | | | |
| <i>Ss-4</i> | 0.005 | 0.005 | 0.008 | 0.000 | | |
| <i>Ss-5</i> | 0.005 | 0.005 | 0.008 | 0.000 | 0.000 | |
| <i>Ss-3</i> | 0.005 | 0.005 | 0.008 | 0.000 | 0.000 | 0.000 |

evolutionary percentage and for certain genealogical tests.

Phylogenetic Analysis

The phylogram constructed by using MEGA 7 software revealed two distinct clusters denoted by cluster I and cluster II (Fig 1) separating *S. scabiei* from the HDMS.



Fig 1: Phylogenetic relationship of *S. scabiei* and HDM mites based on ITS-2 sequences

The phylogenetic tree Cluster I includes *D. farinae* samples indicating close genetic similarity with bootstrap value of 100. The Pakistani *D. farinae* (DerfPak1 and DerfPak2) lie within the HDM cluster, showing closer genetic relationship to each other than to the other HDMS included in the study (bootstrap value = 73). Cluster II includes the Pakistani *S. scabiei* samples (SsPak1 and SsPak2) and other *S. scabiei* sequences with bootstrap value of 93. The phylogram showed an overall genetic

distance of 0.1 indicating close genetic distance between HDM and *Sarcoptes* mites, indicating close genetic correlation.

Pairwise Distance Calculation

Pairwise distance was calculated on the basis of ITS-2 gene sequences of *D. farinae* and *S. scabiei* mites (Table 2 and Table 3). The values for genetic diversity were calculated within species for both *S. scabiei* and *D. farinae* separately. The values of genetic diversity for *D. farinae* range from 0.006-0.038 with an average of 0.022. Similarly, the values of genetic diversity for *S. scabiei* range from 0.003-0.008 with an average of 0.004. These values indicate that genes were genetically related with each other and there was little genetic diversity among them. The overall genetic distance between *S. scabiei* and *D. farinae* was calculated to be 0.53.

Discussion

In the present study we characterized *S. scabiei* var. *hominis* sequences and compared them with other reported ITS-2 (mostly animals as a host) as previous studies on astigmatid mites did not include *Sarcoptes* mite from human host.^{23,26} Results of the studies carried out in Pakistan showed that ITS-2 sequences of *D. farinae* is characterized by 3 transitions i.e. Y, R and W and of *S. scabiei* var. *hominis* sequences is characterized by two transitions and one transversion.

The comparison of ITS-2 sequences of astigmatid mites with sequences obtained from GenBank revealed that the sequences could not be used to differentiate geographical populations implying that the ITS-2 region was highly conserved in mites from different areas.^{26,27} The lower level of intraspecific variation and the relatively higher level of interspecific variation in ITS-2 region show that these genes may be suitable for studying the phylogeny of astigmatid mites. These species of mites were positioned in the phylogenetic trees according to their morphological characteristics.²⁸

As reported by Yang et al²⁷ the genetic distances between the species were higher as compared to distances within the species.²⁶ The study revealed that ITS-2 region is suitable for phylogenetic studies of astigmatid mites as this region shows relatively lower variation within the species and higher variation between the different species of mites. These astigmatid mites were placed in the

phylogenetic tree based on their genetic characteristics.

Our study with ITS-2 region is also supported by Suarez-Martinez et al. in which it has been established that ITS-2 region is not able to discriminate geographically isolated mite species. The mitochondrial 12S rDNA is also not able to reflect differences between the families Pyroglyphidae and Acaridae²⁹ because of sequence conservation the use of this marker is limited. In comparison, ITS-2 region is advantageous in studying population structure of astigmatid mites and can help us study low-level phylogenetic relationships between astigmatid mites.^{23,26}

Conclusion

The present study is the first report from Pakistan using ITS-2 marker for the phylogenetic analysis of astigmatid mites. Although it is challenging to distinguishing the astigmatid mites based on their morphological characteristics, the ITS-2 region can be for this purpose. However, ITS-2 region is not convenient for geographical discrimination of different species³⁰ but it can be used to study low-level phylogenetic relationships between astigmatid mites.

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