Tropical Biomedicine 35(3): 796–803 (2018)

Genetic characterization of *Sarcoptes scabiei* var. *hominis* from scabies patients in Pakistan

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Received 5 January 2018; received in revised form 24 May 2018; accepted 5 June 2018

Abstract. Scabies occurs in human due to the burrowing ectoparasite *Sarcoptes scabiei* var. *hominis* resulting in intense itching and inflammation, and manifesting as a skin allergy. Limited information is available about the genetic diversity of *S. scabiei* in human. In this study, we characterized *S. scabiei* var. *hominis* using nuclear marker ITS-2, mitochondrial marker 16S and microsatellite markers. To examine the extent of the genetic variation, individual mite gDNA was first amplified using ITS-2, 16S and microsatellite primers by PCR, later amplicons were sequenced directly and analysed by MEGA 7. Sequence analysis of ITS-2 showed no host segregation and geographical isolation, whereas 16S indicated presence of both hosts adapted and geographically segregated populations of *S. scabiei*. Results of microsatellites revealed polymorphism as allelic variability between and within the populations studied. The different varieties of *Sarcoptes* mites belong to different host species and geographic regions recommended that *Sarcoptes* mites are genetically isolated. This is the first report on the molecular characterization of *S. scabiei* var. *hominis* from Pakistan. Additionally, genetic studies including a greater number of specimens are required to comprehend the molecular epidemiology of *Sarcoptes* mite in Pakistan.

INTRODUCTION

Sarcoptes scabiei var. *hominis* causes a common human skin infestation known as scabies (Mellanby, 1944; Orkin and Maibach, 1985; Pence and Ueckermann, 2002; Walton and Currie, 2007). Scabies is identified as a neglected tropical infectious disease, occurs worldwide among all ages, races, genders and socioeconomic strata (Hengge *et al.*, 2006; Payne and Fitchett, 2010). It has been estimated that about 1–10% of the global population is infected with scabies and its infection rate is as high as 50–80% in certain populations (Andrews *et al.*, 2009). More than 15 diverse varieties have been identified which are morphologically similar but have obvious physiological and genetic variations in various hosts (Arlian *et al.*, 1984; Berrilli *et al.*, 2002; Alasaad *et al.*, 2013).

Molecular markers viz. the second internal transcribed spacer (ITS-2) of the nuclear rRNA and the mitochondrial 16S rRNA have applicability in study of diversity in acarology by elucidating population structure and phylogenetic relationships (Navajas and Fenton, 2000; Walton *et al.*, 2007). However, till date most molecular studies on the genetic diversity of *Sarcoptes* mites has concentrated on farm and wild animals from different regions of the world (Berrilli *et al.*, 2002; Zahler *et al.*, 1999; Alassad *et al.*, 2009; Amer *et al.*, 2014; Makouloutou *et al.*, 2015). Previous studies conducted on human subjects in Australia verified the mono-specificity of genus

Sarcoptes among different mite populations using 16S and 12S mitochondrial markers (Skerratt *et al.*, 2002; Walton *et al.*, 2004). Microsatellite analysis supports *S. scabiei* as a distinct extremely variable species with diverse verities showing physiological host-specificity (Walton *et al.*, 1999; Alassaad *et al.*, 2008; Alassad *et al.*, 2011; Alassad *et al.*, 2012). Recently, results of a study by Andriantsoanirina *et al.*, 2015 suggested that *S. scabiei* mites in humans constitute a heterogeneous population.

In Pakistan, scabies is quite common with a higher prevalence rate of 45% in some areas. However, there is no data available on the genetic diversity of *S. scabiei* from Pakistani human population (Javed and Jairamani, 2006; Naz *et al.*, 2013). Therefore, pilot study has been carried out for genetic characterization of *S. scabiei* derived from human hosts to verify the manifestation of host-adapted and geographically segregated *S. scabiei* populations in Pakistan.

METHODS

Ethical approval

This study was reviewed and approved 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) and informed consent was obtained from all patients.

Collection of *Sarcoptes* mites

Sarcoptes mites were collected from scabies infested individuals consulting at four government hospitals i.e. Military Hospital (MH), Rawalpindi, Social Security Hospital, Rawalpindi, Railway General Hospital, Rawalpindi and Federal Government Poly Clinic, Islamabad between September 2012 and October 2013. The cases of ordinary scabies were confirmed by clinical observation, positive identification of mites and mite parts under the microscope. Samples were fixed in 70% ethanol and stored at 4°C until further processing.

DNA extraction

In total, genomic DNAs of 20 mites were extracted individually using the hotshot plus thermal shock technique (Alassad *et al.*, 2008). According to this method, 25 µl of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA) was added followed by three cycles of thermal shocks to disrupt the exoskeleton and 25 µl neutralizing reagent (40 mM Tris-HCl) was added afterwards 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

Primers used in present study are described in Table 1. With ITS-2 primer, 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. For the amplification of 16S gene and microsatellite primers, amplifications conditions were as follows: pre-denaturation for 2 min at 95°C, followed by 30 cycles of 30s at 94°C, 30 sec at 57°C, 50 sec at 72°C. The amplicons were visualized on 3% agarose gel, stained with ethidium bromide. The purified PCR products were sequenced directly using Big Dye® Terminator v3.1 cycle sequencing kit in an ABI 310 genetic analyzer (Applied Biosystems, Foster City, USA) in both directions.

Molecular analysis

ChromasPro (version 1.5) software (http://technelysium.com.au/wp/chromaspro/) was used to assemble the sequences. To verify results of sequenced samples, BlastN (https://blast.ncbi.nlm.nih.gov/ /Blast.cgi/) was performed. The resulting top hit nucleotide sequences of ITS-2 and 16S were aligned using ClustalW (http://www.genome.jp/tools-bin/clustalw) and aligned sequences were further used for phylogenetic tree construction using the Molecular Evolutionary Genetics Analysis (MEGA 7) software. The nucleotide sequences resulted from this study were deposited in GenBank under accession numbers KR010367,

Table 1. Primers used for sequencing and amplification of *Sarcoptes* mite

Primer Name	Primer Sequences 5' to 3'	Primer Size (bp)	Size (bp)	Annealing Temperature (°C)	References
ITS-2_F	CGACTTTCGAACGCATATTGC	21	400	56	Noge *et al.*, 2004
ITS-2_R	GCTTAAATTCAGGGGGTAATC	21			
16S_F	CTAGGGTCTTTTTGTTCTTGG	21	400	57	Walton *et al.*, 2004
16S_R	GTAAGTATACGTTGTTATAAC	21			
Sarms 1_F	GAATTCAAAACAAACACCTAGT	22	120-200	57	Walton *et al.*, 2004
Sarms 1_R	GAATTCATGAATCTGATGAAAATG	24			
Sarms 15_F	ATTAAATCATTGCACAATAGAGCG	24	120-200	57	Walton *et al.*, 2004
Sarms 15_R	CTACCATTAATTTTTTCCACCCTC	24			
Sarms 20_F	GATGAAAACGAGTAGGTGGATAG	23	120-200	57	Walton *et al.*, 2004
Sarms 20_R	CGTCTCTAGACCTATTGCTGGAAC	24			

KJ409446 and MF503255 to MF503259 for ITS-2, KT020826, KT020827 and MF503250 to MF503254 for the mitochondrial 16S gene.

RESULTS

Genomic DNAs of *S. scabiei* var. *hominis* were isolated from a total of twenty specimens. The sequences of ITS-2 and 16S of individual mites were 490 bp and 445 bp in length respectively without detection in size variation from all regions.

ITS-2 sequence analysis

Three novel polymorphic sites were identified in sequence analysis of *S. scabiei* var. *hominis* ITS-2 sequences from different regions of Pakistan compared with reference sequence from GenBank. These sequences were characterized by two transitions and one transversion. The represented sequences with three polymorphic sites have 98% intra-isolate identity. Represented sequences formed a cluster on the tree collectively with reference sequences from GenBank representing the wide host range and geographical dissemination of *S. scabiei* population (Fig. 1). All sequences resulting from mites in human formed a cluster on the tree together with reference sequences which includes isolates from Italy, China, Switzerland, and Egypt. The estimate of average evolutionary divergence of ITS-2 sequence pairs is 0.0010 M. The low approximation of genetic divergence may represent that sequences do not show independent genetic changes. As a result, having no significant impact on the genetic structure of mite population and exhibiting a low polymorphic rate of 0.35%.

16S sequence analysis

Greater variability was observed in the sequences of the mitochondrial 16S rRNA gene as compared to ITS-2 sequences in the form of nucleotide substitutions, insertions or deletions, with a 99% intra-isolate identity. Sequences of the mitochondrial 16S rRNA gene divided the *S. scabiei* isolates into two major groups, one having human isolates and other having animal isolates (Fig. 2). The NJ tree placed sequences from all animal isolates in the basal divergence. The basal cluster for *Sarcoptes* mites also subdivided into different clusters of animal isolates i.e. rabbit, chimp, sheep, vulpes and canis from Japan, Australia, Italy, Panama and Egypt. The estimate of average evolutionary divergence for 16S sequence pairs was 0.424 M. The host and geographical isolation could be seen at 16S loci with comparatively low bootstrap values at most branches in the phylogenetic tree. This may be due to low polymorphic rate 0.4% as there are only few nucleotide substitutions at 16S loci.

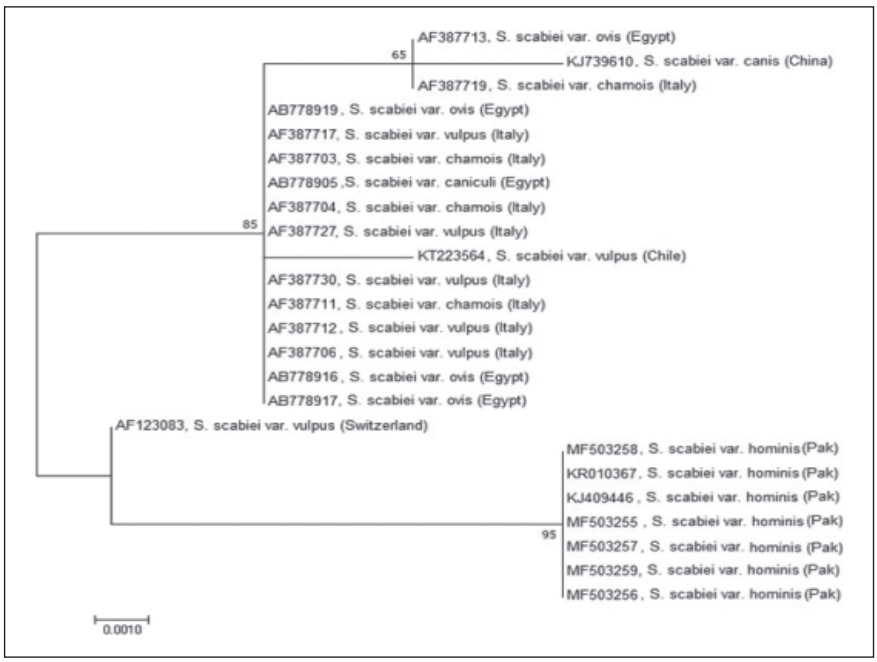

Figure 1. Relationship of identified Pakistani-ITS-2 sequences with selected ITS-2 sequences. The nucleotide sequences of the identified molecules were aligned using ClustalW and phylogenetic tree was constructed using MEGA 7.

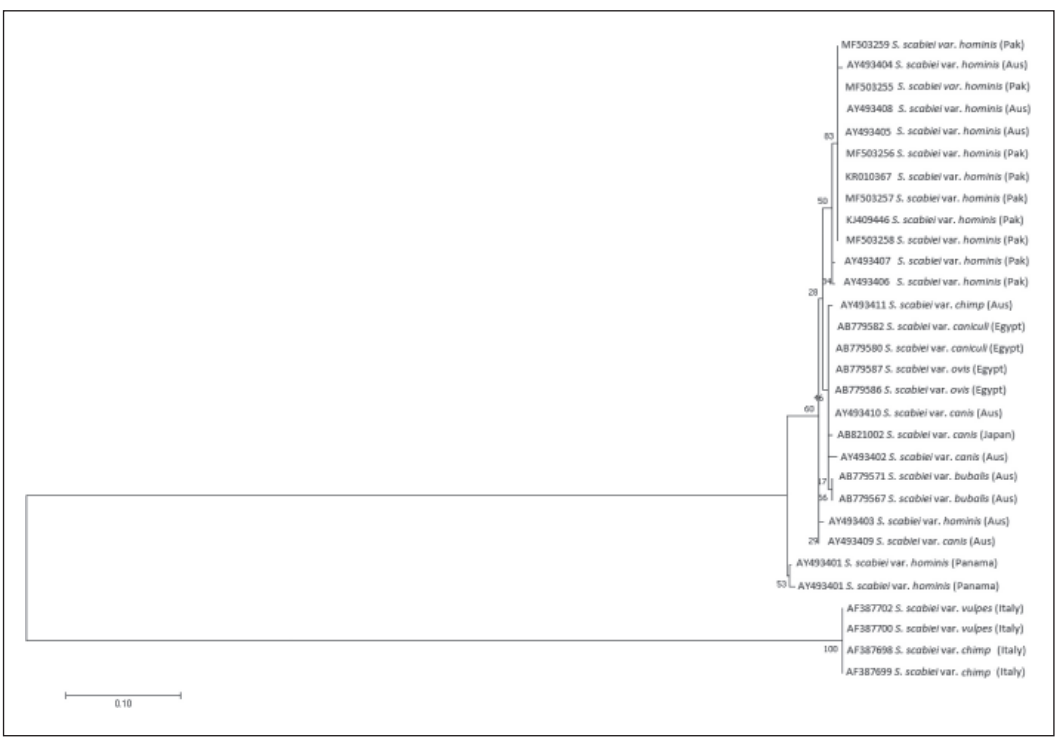

Figure 2. Relationship of identified Pakistani-16S sequences with selected 16S sequences. The nucleotide sequences of the identified molecules were aligned using ClustalW and phylogenetic tree was constructed using MEGA 7.

Table 2. Characteristics of *S. scabiei* microsatellites

Locus	Total alleles observed	Length of smallest allele (bp)	Length of largest allele (bp)
Sarms 1	4	150	200
Sarms 15	2	100	130
Sarms 20	3	140	375

Analysis of microsatellite markers

In total 9 alleles were detected from three microsatellite markers with some of the mites showing the existence of null allele. The Sarms 1 was heterozygous for two alleles (150 bp and 200 bp). The frequency of 170 bp alleles was 0.80 while 200 bp was 0.20 obtained from two different populations with Sarms 1 primer. Sarms 15 was heterozygous for two alleles (100 bp and 130 bp). The frequency of 100 bp was 0.67 while 130 bp was 0.33. Sarm 20 was heterozygous for two alleles (140 bp and 375 bp) with the allelic frequencies of 140 bp and 375 bp were 0.75 and 0.25 respectively (Table 2).

DISCUSSION

In the present study we characterized *S. scabiei* var. *hominis* sequences and compared them with reported worldwide ITS-2 and 16S sequences (mostly animals as a host). The estimated annual incidence of scabies has been reported 1–10% in humans (Andrews *et al.*, 2009). There is limited research literature available on human scabies due to the difficulties in isolation of mites from the human body. Therefore, till date mostly genetic characterization has been done on animal mites rather than human mites.

Results of the studies carried out in Pakistan showed that ITS-2 sequences from *Sarcoptes* mites had low intra-sequence variability with three polymorphic sites. These results are concomitant with those of Zahler *et al.* (1999) who demonstrated the limited degree of genetic polymorphism in *Sarcoptes* mites from different host and geographic locations. Similarly using ITS-2 marker, Gu and Yang, (2008) reported *Sarcoptes* as a single heterogeneous species. Although a high degree of genetic polymorphism has been reported in different studies in individual mite isolates there was no distinctive clustering arising due to different hosts and geographical localities (Berrilli *et al.*, 2000; Alassad *et al.*, 2009). ITS-2 and 16S genes used by Makouloutou *et al.* (2015) also suggested mono-specificity of heterologous populations of *S. scabiei* of wild mammals in Japan.

On the basis of the analysis of partial 16S sequences, the phylogenetic tree obtained has shown low polymorphic rate and represented two distinct clusters due to both geographical locations and hosts. The results were in accordance with the reported findings that both hosts adapted and geographically segregated populations of *S. scabiei* were characterized using 16S DNA (Amer *et al.*, 2014). The results of the present study suggested that mites are genetically isolated as reported by Andriantsoanirina *et al.*, 2015 and Zhao *et al.*, 2014. It has been reported that *S. scabiei* mites were grouped by the host using microsatellites marker, showed that *S. scabiei* mite on human and dog are genetically distinctive (Walton *et al.*, 1999). However, another study validated that grouping of *S. scabiei* mites were affected by both host species and geographical locations (Walton *et al.*, 2002) while no association between host specificity and the geographical location was shown using 12S rRNA (Skerratt *et al.*, 2002).

The three microsatellite markers i.e. Sarms1, Sarms15, and Samrs20 showed polymorphism as allelic variability between and within the populations as determined by Walton *et al.* (1997). Polymorphic markers help to observe differences even in relatively homozygous populations with variability between populations recognized by assessing their respective alleles (Walton *et al.*, 1997) as well as additional geographic separation between host-related populations (Walton *et al.*, 1999). The multi allelic population between and within the population may be

result of multiple infective events. The multi allelic population may also indicate that the *Sarcoptes* mites within the human population were heterozygous as reported by Zahler *et al.* (1999) which confirmed the view that genus *Sarcoptes* consists of single, heterogeneous species.

CONCLUSION

The result of these multi-locus studies maintains the view that different varieties of *Sarcoptes* mites belong to different host species and geographic regions and recommended a common gene pool of *S. scabiei* which represents the existence of single species. Our study is also in agreement with Fain (1978; 1994), who represented the ecological variability of *S. scabiei* due to frequent interbreeding of different varieties which contribute to the genetic discrimination between populations i.e. heterogeneous *S. scabiei*.

Limited number of characterized samples is a major constraint in the current study. The clear depiction needs to characterize multilocus genes of parasites from a variety of hosts, especially humans and companion animals. Advanced molecular biological tools i.e. comparative genomics population and genetics are also needed for better understanding of genetic mechanism responsible for host adaptation and geographic segregation in *S. scabiei*.

Acknowledgement. The authors are thankful to Higher Education Commission (HEC), Islamabad, Pakistan for financial support under 5000 Indigenous Fellowship Program to Shumaila Naz.

Conflict of Interest

The authors declare no conflicts of interest.

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