eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Philogenetic Analysis of S. *Scabiei* Var. *Hominis*, Var. *Cuniculi*, and Var. *Marmoota*

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ARTICLE INFO

Keywords:
Phylogenetic
CO 1
ITS 2
Scabies

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i10.352

ABSTRACT

Background: Based on data from the 2017 Global Burden of Disease, it is known that Indonesia is the country with the highest number of scabies incidences out of 195 countries in the world. In addition to humans, scabies is also an infectious disease in mammals with a prevalence of 300 million infestations every year, even in animals the mortality rate from scabies is very high. Until now, it is still unclear whether there is a cross-infestation between human and animal scabies. Morphologically, S. scabiei between variants cannot be distinguished, while research on genetic variation is still not much done. Therefore, it is necessary to conduct studies to enrich genetic information and phylogenetic analysis. **Methods :** The samples tested were positive PCR results for ITS 2 and CO 1 genes, each on five amplicons, namely three S. scabiei var. hominis, one var. cuniculi, and one var. marmoota. Phylogenetics were arranged based on the closeness of the base arrangement in variants and geographically available in NCBI. **Results** : Of the five sample isolates studied, the ITS 2 gene amplicon is located in the 417bp band and the phylogenetic analysis of its sequencing has two branches that are unable to distinguish var. hominis and var. animalia. The CO1 gene has electrophoretic results at 317 bp as well as phylogenetic analysis results that specifically divide branches between var. hominis and var. animalia. **Conclusion** : S. scabiei var. hominis and var. marmoota, and var. cuniculi are distinct species and are host monospecific.

1. Introduction

Sarcoptes scabiei is an ectoparasite of the order Acarina that causes scabies in humans and mammals. (1) Globally, S. scabiei infects 300 million mammals annually and causes serious but neglected health problems in both humans and animals. (2) Southeast Asia ranks second with the highest prevalence of scabies after East Asia, and Indonesia ranks first out of 195 countries in the world. (3)

Previous studies have shown that S. scabiei has different taxonomic varieties depending on the specificity of the host, although they cannot be distinguished morphologically. While the specificity of the host is still a controversy whether in fact S scabiei isolated from various species of hosts have similarities so that they can or cannot live in other hosts. (4–6)

In this study, S. scabiei var. hominis, var. cuniculi, and var. marmoota from Indonesia, identified their genetic and phylogenetic composition using the ITS 2 and CO1 genes. Phylogenetics were arranged based on the proximity of variants and geographic. By knowing the closeness of this composition and phylogenetic structure, it can be seen the possibility of scabies infestation in humans by these two animals which will also play a role in the prevalence, transmission and

prevention of scabies transmission.

2. Methods

The study was conducted from October 2018 to June 2019. Sampling of S. scabiei var. hominis conducted at Al Ittifaqiah Islamic Boarding School, South Sumatra. While the sample of S. scabiei var. cuniculi and var. marmoota were taken from rabbits and guinea pigs in Palembang, South Sumatra. DNA extraction was carried out at the Biomolecular Laboratory, Faculty of Medicine, Sriwijaya University using the Qiagen Extraction DNA Kit according to the manufacturer's procedure. The primer of ITS 2 gene used in this study is the accession number KJ739615.1. The first CO1 primer was nested PCR with EU256388.1 and the second with LN874269.1. The PCR results were electrophoresed with 2% agarose gel at 75 volts for 50 minutes. Positive isolates (showing base length 417bp and 317bp) were included as samples for sequencing and phylogenetic analysis.

Quality control of nucleotide bases is done by trimming (Chromas program). The alignment process with BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) is carried out to identify the similarity of the input organism (query) with the subject sequence in the database. The phylogenetic tree was formed using the MEGA X program with the alignment of Clustal W construct Maximum Likehood Kimura-2 model with bootstrap 1000. Meanwhile, genetic distance analysis used pairwise distances with the kimura-2 model.

3. Results

In Figures 1A and 2A are isolates of the same sample of S. scabiei, which was found in 5 human hosts. Based on electrophoresis PCR results showed a good specific band on var. hominis and var. cuniculi and var. marmoota. PCR results showed that the isolates of S. scabiei var. hominis, var. cuniculi and var. marmoota in the ITS 2 gene has a base length of 417bp, while the CO1 gene produces a band at 317bp, the same as in the previous study. (5) Sequencing was only performed on samples that had clear bands on both ITS 2 and CO 1 genes, namely in the third, fourth and fifth samples (Figure 1A on isolates samples 13, 4, and 7).

Phylogenetic analysis of the ITS 2 gene in the sample isolates of S. scabiei produced two branches with values of 60% and 42% which can be seen in Figure 3. While the phylogenetic analysis of the CO 1 gene produced three branches and showed greater values, namely 99 and 98%. (Figure 4). In both phylogenetic analyzes, an outgroup was used, namely Psoroptes cuniculi where the outgroup proved to be located outside the branch of S. scabiei.

Figure 4 shows that samples of S. scabiei var hominis (34208040A, 3420842B, and 3420844C) have a genetic relationship with S. scabiei var hominis from other locations, namely from China Xi'an KJ48523 and KR05191.

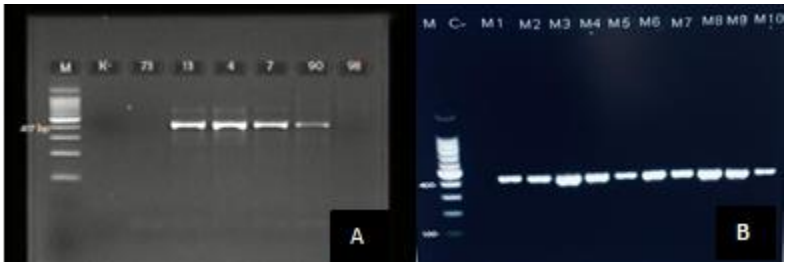

Figure 1. ITS 2 gene PCR results on sample isolates of S. scabiei showed a positive value in the 417bp band; A. S. scabiei var. hominis, K- is negative control; B. C- is a negative control, M1 – M5 S. scabiei var. cuniculi from the same rabbit host, B. M6 – M7 S. scabiei var. marmoota from the same guinea pig host.

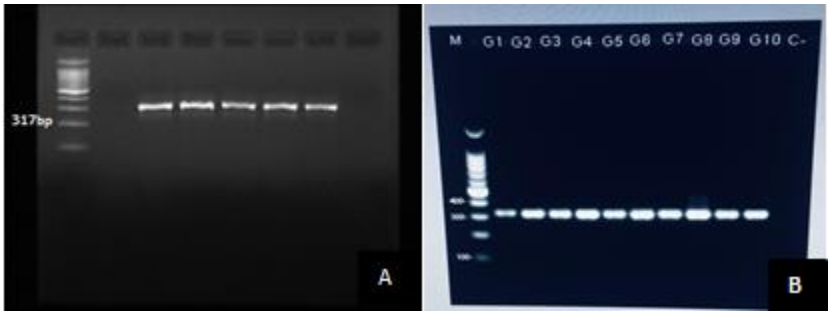

Figure 2. PCR results for the COX 1 gene in the sample isolates of S. scabiei showed a positive value in the 317bp band. A S. scabiei var. hominis; B. C- is negative dick. G1 – G5 S. scabiei var. cuniculi and G6 – G7 S. scabiei var. marmoota.

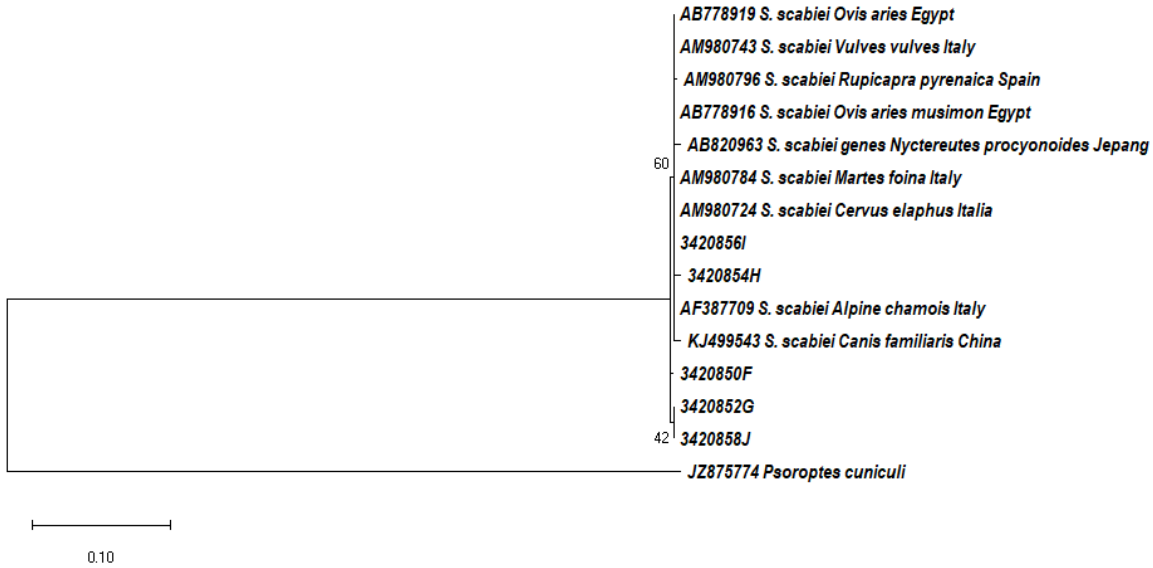

Figure 3. Results of ITS2 phylogenetic analysis of S. scabiei isolates using the Maximum Likehood Kimura-2 (MEGA X) method and validation with Bootstrap 1000 times (minimum value of 30%).

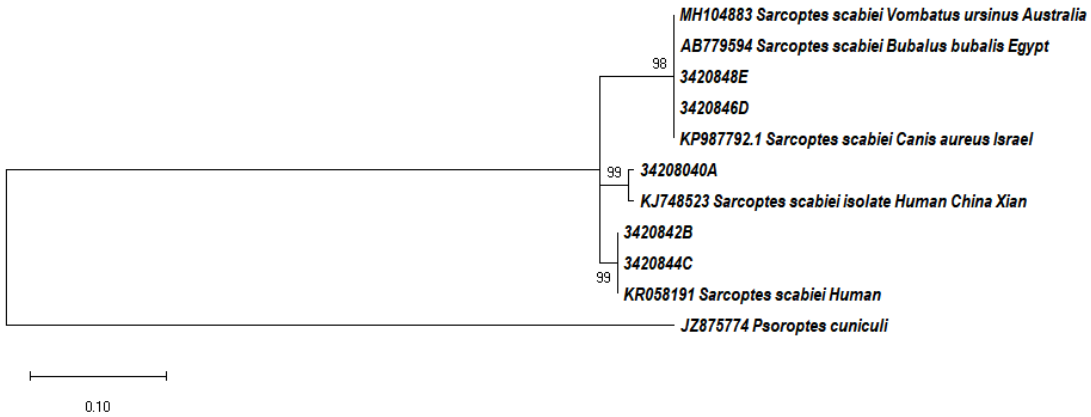

Figure 4. Phylogenetic analysis results of COX 1 gene isolates of S. scabiei samples using the Maximum Likehood Kimura-2 (MEGA X) method and 1000 times Bootstrap validation (minimum value of 30%).

4. Discussion

Previous studies have attempted to answer the question of whether S. scabiei that infests multiple hosts is the same species that can cause cross-infestation or is a species with monospecificity. (4,5,7). Through sequencing characterization of S. scabiei and comparing sequencing databases from around the world, it is possible to determine the genetic variation and possible cross-infestation.(8) However, the characterization of the sample isolates var. hominis is still limited because of the difficulty of obtaining samples of ordinary human scabies. Until now the molecular research of S. scabiei var. more developed animals. (8–10)

Phylogenetic analysis of the ITS 2 gene resulted in 2 branches, where var. hominis is in the same branch as var. marmoota, while var. other hominis have branches with var. cuniculi. ITS 2 gene still clusters var. hominis and var. animalia in the same branch. So it can be seen that the ITS 2 gene is not able to identify S. scabiei properly, in variants or geographically as in previous studies. (5) In addition, the low bootstrap value indicates a limited level of polymorphism variability. The ITS 2 gene in other studies even stated that S. scabiei from various locations was one species with heterogeneous hosts. (11)

In the next phylogenetic analysis, the CO1 gene, resulted in three more specific branches. Three sample isolates var. hominis, one of which has phylogenetic affinity with isolate var. hominis from China Xi'an, while the remaining two have phylogenetic closeness with isolates of var. hominis from other locations (5,4). The same results were also obtained in another study which stated that the CO1 gene was able to provide specific results in clustering variants, especially var. hominis, even geographically. In this study, the CO1 gene was able to cluster up to 5 var branches. hominis from various geographies. var. marmoota and var cuniculi in the phylogenetic tree have different branches from var. hominis and has close affinity with isolate var. other animalia. Even in this study, the bootstrap validation value is up to 98 – 99%. This indicates that the CO1 gene is suitable for identifying and analyzing the phylogenetic characteristics of the S scabiei variant compared to the ITS 2 gene (4,5).

The phylogenetic tree formed in this study, both in the CO 1 and ITS 2 genes, was able to show that S. scabiei var. hominis and var. animal, in genetic variation, indeed comes from different species and is monospecific in the host but has not been able to answer the possibility of cross-infestation between hosts. It is necessary to conduct research with a larger number of samples both host and geographically, so that more complete and in-depth results are obtained.

5. Conclusion

S. scabiei var. hominis and var. marmoota, and var. cuniculi are distinct species and are host monospecific.

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