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Second internal transcribed spacer (ITS-2) as genetic marker for molecular characterization of *Sarcoptes scabiei* in rabbits from several areas of East Java, Indonesia

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Abstract

Objectives: The purpose of this study is to use the second internal transcribed spacer (ITS-2) to determine the molecular characteristics of *Sarcoptes scabiei* in rabbits from several areas of East Java.

Methods: Collecting *S. scabiei* mites from rabbits with clinical signs of scabies; DNA extraction with minikit QIAamp DNA; polymerase chain reaction amplification; nucleotide sequence analysis; homology and phylogenetic tree using the Neighbor-Joining method in the program molecular evolutionary genetics analysis-7 (MEGA-7).

Results: Sequence analysis of ITS-2 *S. scabiei* from five regions in East Java showed an identity >91.23% with isolates from China (KX695125.1). The phylogenetic analysis of ITS-2 *S. scabiei* from Mojokerto rabbits has a close relationship with AB82977.1; Surabaya and Nganjuk rabbits are closely related to KX695125.1; while Sidoarjo and Pasuruan rabbits are closely related to EF514469.2. and AB369384.1.

Conclusions: The homology analysis of all samples showed identity of more than 91.23% with isolate China (KX695125.1). The sequences of ITS-2 gen of *S. scabiei* from rabbits in several areas were relatively close to *S. scabiei* obtain various hosts from National Centre for Biotechnology Information (NCBI) data.

Keywords: ITS-2; rabbit; *Sarcoptes scabiei*.

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Introduction

Scabies is a highly contagious skin disease caused by *Sarcoptes scabiei* (*S. scabiei*) is one of the most important human and animal diseases. It has been reported that around 100 million people in the world are infected with *S. scabiei* with a prevalence between 0.2 and 71.4% [1]. Currently, scabies is an emerging or re-emerging parasitic skin disease and could threaten the health of humans and animals in the world [2, 3]. Scabies in humans is a public health problem, characterized by intense itching, inflammation, manifests as skin allergies (hypersensitivity type IV), and associated with the mites burrowing into the stratum granulosum of the epidermis [4]. Scabies diagnosis was carried out by using the skin scraping method but it was difficult in mild infections. The development of serological diagnosis and vaccine sub-units in humans and animals is still needed through molecular research with various gene loci [2, 5]. For the molecular typing and molecular characterization of *S. scabiei* were used various genetic markers such as ITS-2, COX-1, 12S rRNA, and 16S rRNA [3, 6, 7, 8]. The ITS-2 gene locus has advantages compared to other molecular regions, it has a high level of sensitivity, 100 genome replication, and also the ITS-2 has a high rate of evolution so it can be used as a genetic marker for detection of genetic mutations due to differences in geographic location [9]. The purpose of this study is to use the second internal transcribed spacer (ITS-2) to determine the molecular characteristics/identity of *S. scabiei* in rabbits from several areas of East Java. This research is a preliminary study that can be further developed for the study of serological diagnostic kits and subunit vaccines in animals, and in humans using a sample of *S. scabiei* var. *hominis*.

Materials and methods

This research according to standard operating procedures and approved by the Ethic committee of Veterinary Medicine Faculty, Airlangga University, certificate No. 630-KE. In this study, skin

scrapings of 22 rabbits with clinical symptoms of scabies were collected from rabbit farms in Sidoarjo, Pasuruan, Mojokerto, Nganjuk, and Surabaya. The character of the selection of the five-area samples is as follows: (1). the largest number of rabbit farms in the area, (2) high cases of scabies and a severe degree of infection, (3) dirty cage conditions, especially the dry season. The scraped mites are collected for molecular examination processing [8, 10].

DNA extraction and polymerase chain reaction (PCR) assay

DNA extraction was carried out using a QIAamp DNA Minikit (Qiagen, Hilden, Germany) according to the factory protocol. Amplification of the 304 bp ITS-2 *S. scabiei* fragment was carried out using the forward primer (5′ CGG TTT CGT CAC ACT TCG ATG 3′) and reverse (5′ CGG GTA TTC TCG CTT GAT CTG 3′). Subsequently, the PCR test of the ITS-2 encoding gene segment thermocycling in an automatic thermocycler (Biorad) with an initial denaturation at 94 °C for 5 min; followed by 35 cycles of template denaturation, 94 °C for 30 s, primer annealing at 54 °C for 30 s, DNA extension at 72 °C for 30 s, and final extension at 72 °C for 5 min (Qiagen, Hilden, Germany). Analysis of the PCR product was carried out by electrophoresis using 2% agarose gel [7, 11]. PCR products were purified using the QIAquick, PCR purification kit (Qiagen).

DNA sequencing and phylogenetic tree analysis

DNA sequencing was carried out in an automatic DNA sequencer (ABI 3730XL, Solgent Co. Ltd., South Korea). The nucleotide sequence was read using molecular evolutionary genetics analysis-7 (MEGA-7) software and the DNA sequence analysis was carried out using the Basic Local Alignment Search Tool (BLAST) on the Gene Bank ("http://www.ncbi.nlm.nih.gov/BLAST"). The phylogenetic tree was analyzed using the MEGA-7 software with the Construct/Test Neighbor-joining tree and Bootstrap method and multiscale Bootstrap analyses with 1,000 Replications were conducted.

Results

The Results of the PCR test were read on 2% agarose gel electrophoresis showing that the PCR product with high specifications was a single band at the 304 bp position in accordance with the amplification target. The DNA sequencing analysis results of *S. scabiei* from five regions in East Java showed an identity 91.23–98.68% for *S. scabiei* Chinese isolates (Accession number on Gene Bank: KX695125.1).

Sequencing result of *S. scabiei* nucleotide

Multiple alignment results from the nucleotide sequences showed differences in nucleotide arrangements of deletion and substitution mutations from the ITS-2 *S. scabiei* encoding gene that infected rabbits from the Surabaya, Sidoarjo, Mojokerto, Pasuruan, and Nganjuk isolates if aligned with Chinese isolate (KX695125.1) (Figure 1).

Result of phylogenetic tree analysis

The phylogenetic tree analysis results of *S. scabiei* from several regions of East Java isolates with data on the Gene Bank showed that *S. scabiei* from rabbit isolates of Mojokerto and *S. scabiei* on *Capricornus scispus* Japan isolate with accession number AB82977.1 have a close relationship, *S. scabiei* Surabaya and Nganjuk isolate have a close relationship with the reference sequence that used for primer design of Chinese isolate with accession number KX695125.1, and *S. scabiei* Sidoarjo and Pasuruan isolate have a close relationship with Chinese isolate (accession number EF514469.2) and *S. scabiei* of *feral raccoon* isolate from Japan with accession number AB369384.1. *S.scabiei* isolates from Surabaya, Sidoarjo, Pasuruan, Nganjuk, and Mojokerto also showed close kinship with *S.scabiei* from several hosts in several countries with a pairwise distance showing at 0.000 (Figure 2).

Discussion

The nucleotide composition change that occurred in *S. scabiei* isolates from Surabaya, Nganjuk, Pasuruan, Sidoarjo, and Mojokerto cities showed deletions and substitutions (Figure 1). The only changes in the partial nucleotide sequence of *S. scabiei* DNA were silent mutations because genetic mutations that occurred have a percentage of less than 9%, and the identity level was more than 91.23%. In this study, *S. scabiei* isolates came from five regions in East Java with different geographical locations. The degree of polymorphism can be influenced by the diversity of the host and geographic location and by different genetic markers [4, 5, 12]. Deletion and substitution of a nucleotide may be caused by the mite's activity to adapt of host cells including made of tunnels in stratum granulosum during their life cycle to obtained nutrients from host cells [13, 14]. Phylogenetic tree analysis showed that *S. scabiei* isolate from Mojokerto was in the same branch as the Japanese isolate *C. crispus* (AB820977.1). The Surabaya and Nganjuk isolates are in the same branch as the Chinese rabbit isolates (KX695125.1) and these isolates are also used as reference sequences on primer design to PCR tests. Sidoarjo and Pasuruan isolates are in the same branch as

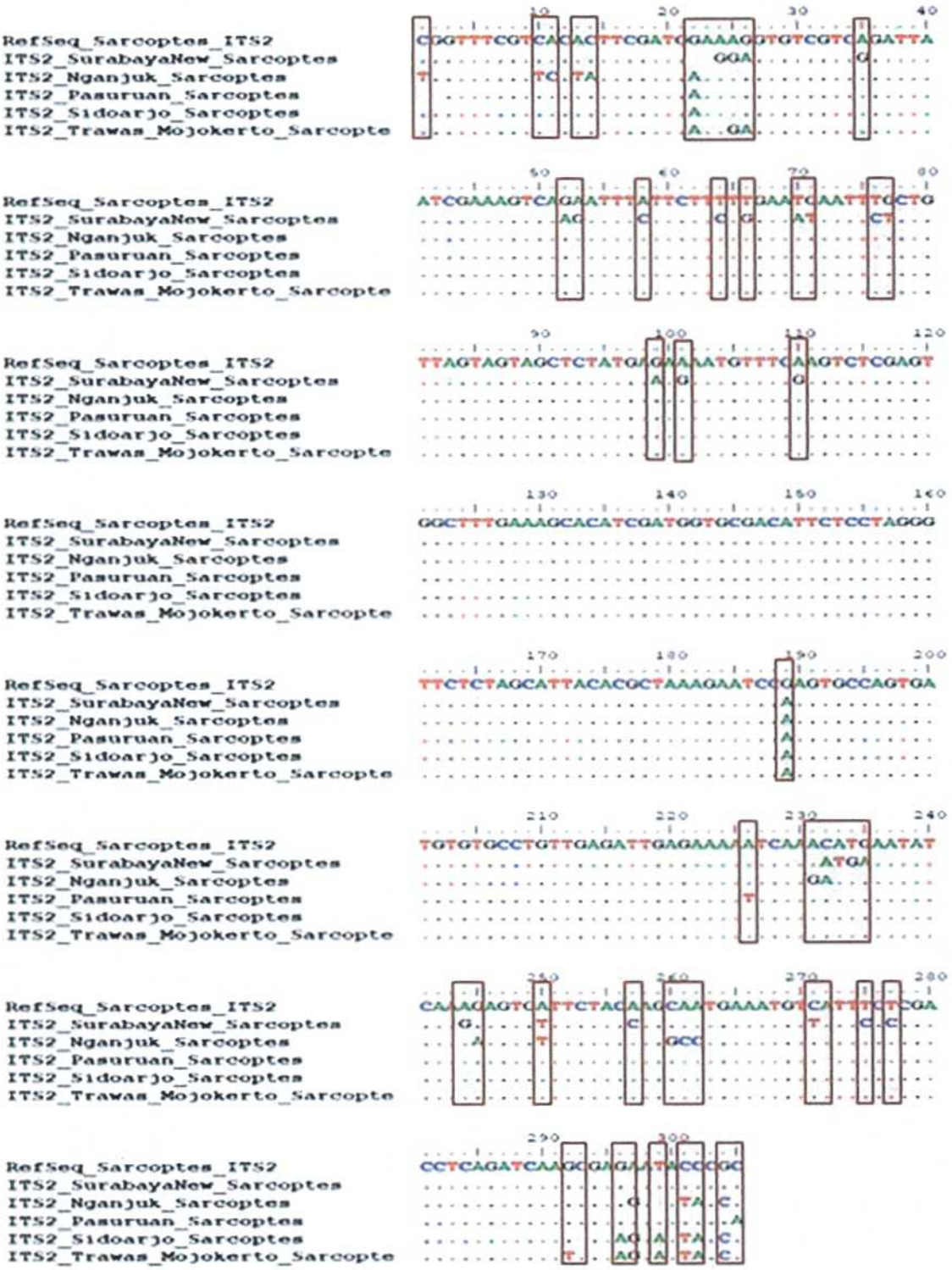

Figure 1: Multiple alignments of *S. scabiei* nucleotide sequences isolate from Surabaya, Nganjuk, Pasuruan, Sidoarjo, and Mojokerto with Chinese isolate (KX695125.1).

the Chinese isolate from rabbits (EF514469.2) as seen in Figure 2. The kinship that occurs can be caused by *S. scabiei* migration through the host of rabbits or other animals and humans [2, 5].

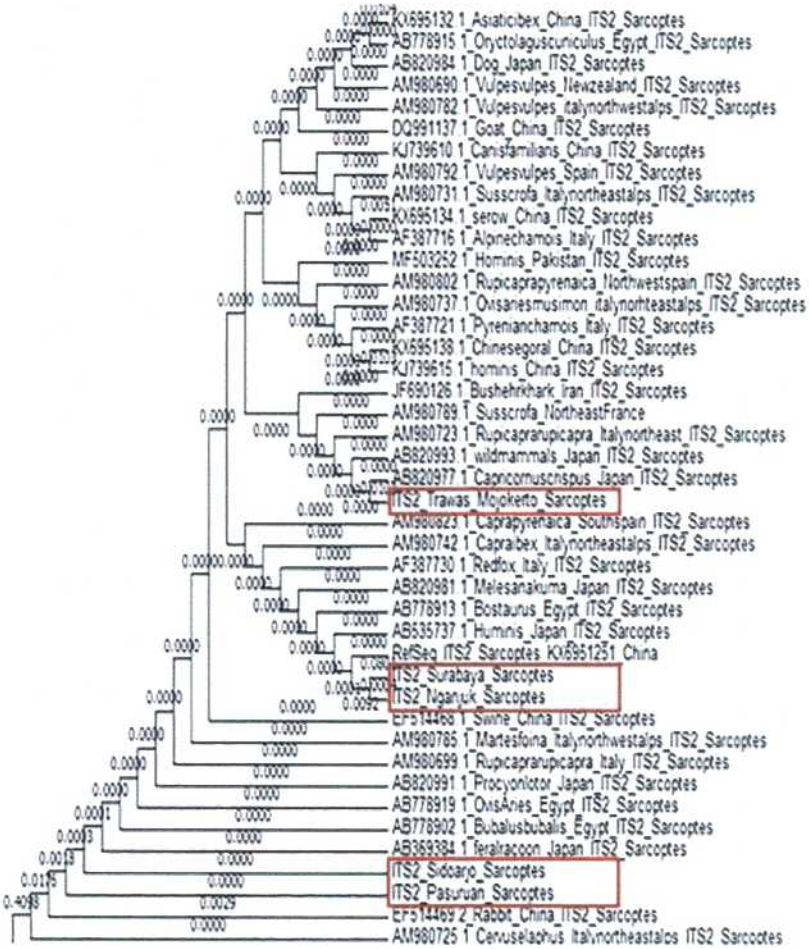

Figure 2: Phylogenetic tree analysis of the ITS-2 encoding gene of *S. scabiei* from Surabaya, Nganjuk, Pasuruan, Sidoarjo, and Mojokerto rabbit isolates on Gene Bank *S. scabiei* data.

Conclusions

The homology analysis of all samples showed an identity of more than 91.23% with isolate China (KX695125.1). The sequences of the ITS-2 region of *S. scabiei* from rabbits in several areas were relatively close to *S. scabiei* obtain various hosts from National Centre for Biotechnology Information (NCBI) data.

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References

1. Romani L, Steer AC, Whitfeld MJ, Kaldor JM. Prevalence of scabies and impetigo worldwide: a systematic review. Lancet Infect Dis 2015;15:960–7.
2. Andriantsoanirina V, Ariey F, Izri A, Bernigaud C, Fang F, Charrel R, et al. *Sarcoptes scabiei* mites in humans are distributed into three genetically distinct clades. Clin Microbiol Infect 2015;21:1107–14.
3. Arlian LG, Morgan MS. A review of *Sarcoptes scabiei*: past, present and future. Parasites Vectors 2017;10:297.
4. Walton SF, Pizzutto S, Slender A, Viberg L, Holt D, Hales BJ, et al. Increased allergic immune response to *Sarcoptes scabiei* antigens in crusted versus ordinary scabies. Clin Vaccine Immunol 2010;17:1428–38.
5. Amer S, Wahab TAE, Metwaly AEN, Ye J, Roellig D, Feng Y. Preliminary molecular characterizations of *Sarcoptes scabiei* (Acari: Sarcoptidae) from farm animals in Egypt. PLoS ONE 2014;9:e94705.

6. Makouloutou P, Suzuki K, Yokoyama M, Takeuchi M, Yanagida T, Sato H. Involvement of two genetic lineages of *Sarcoptes scabiei* mites in a local mange epizootic of wild mammals in Japan. J Wildl Dis 2015;5:69–78.
7. Fraser TA, Carver S, Martin AM, Mounsey K, Polkinghiorne A, Jelocnik M. A *Sarcoptes scabiei* specific isothermal amplification assay for detection of this important ectoparasite of wombats and other animals. PeerJ 2018;6:e5291.
8. Lastuti NDR, Rohman A, Handiyatno D, Chrismanto D, Desiandura K. Sequence analysis of the cytochrome C oxidase subunit 1 gene of *Sarcoptes scabiei* isolate from goats and rabbits in East Java, Indonesia. Vet World 2019;12: 959–64.
9. Li CY, Sun Y, Xie Y, Zhou X, Gu XB, Lai WM, et al. Genetic variability of wildlife-derived by the ribosomal ITS-2 and mitochondrial 16S genes. Exp Appl Acarol 2018;76:53–70.
10. Lastuti NDR, Hastutiek P, Suwanti LT, Chrismanto D. Exploration *Sarcoptes scabiei* antigenic protein which play roles in Scabies pathogenesis in goats and rabbits. Iran J Parasitol 2018;13:446–72.
11. Wong SS, Poon RW, Chau S, Wong SC, To KK, Cheng VC, et al. Development of conventional and real time quantitative polymerase chain reaction assay in the diagnosis and monitoring of scabies. J Clin Microbiol 2015;53:2095–102.
12. Naz S, Chaundhry FR, Rizvi DA, Ismail M. Genetic characterization of Sarcoptes scabiei var. Hominis from scabies patients in Pakistan. Trop Biomed;35:796–803.
13. He R, Gu X, Lai W, Peng X, Yang G. Transcriptome-microRNA analysis of *Sarcoptes scabiei* and host immune response. PLos One 2017;12:e0177733.
14. Bhat SA, Mounsey KE, Liu X, Walton SF. Host immune responses to the itch mite, *Sarcoptes scabiei*, in humans. Parasit Vectors 2017; 10:385.