Characterization of mitochondrial COX-1 gene of *Sarcoptes scabiei* from rabbits in East Java, Indonesia

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**ABSTRACT**

**Objective:** The purpose of this study was to characterize the mitochondrial COX-1 gene of *Sarcoptes scabiei* in rabbits from three districts of Malang, Nganjuk, and Kediri, East Java, Indonesia. The gene was aligned with a DNA isolated from *S. scabiei* of Chong’qing rabbit (accession number: EU256388.1) to construct a molecular analysis of phylogenetic in *S. scabiei* COX-1 gene.

**Materials and Methods:** This study has been verified by the Committee Ethics (Faculty of Veterinary Medicine, Universitas Airlangga). The mites were collected and identified from rabbits that have an indication of scabies infection. DNA was extracted with QIAamp DNA mini kit and polymerase chain reaction (PCR) analysis was done. The PCR products were purified with the protocol of the BigDye XTerminator™ Purification Kit (Thermo Scientific) and were double-sequenced with the forward and reverse PCR primers of ABI PRISM 310 Genetic Analyzer. The sequence product was confirmed with Clone Manager Professional 9 (Sci-Ed Software) and the Neighbor-Joining method was done with MEGA6 to build a phylogenetic tree.

**Results:** The target product of DNA amplification in this PCR was around 290-bp. The amplicon was visualized in 2% of agarose gel electrophoresis. The homology analysis of these sequences showed that it had more than 99% similarity.

**Conclusion:** COX-1 gene sequences of *S. scabiei* from rabbits in Malang, Nganjuk, and Kediri were very similar to COX-1 gene sequences in *S. scabiei* acquired from several hosts according to NCBI data.

**Introduction**

*Sarcoptes scabiei* causes an infectious skin disease called scabies, and every year more than 300 million people are infected. Sarcoptes mites manifestation was reported to infect more than 100 mammal species, including human, domestic animal, and wild animal [1–3]. Scabies caused economic losses because it inhibits growth, decreases feed conversion rate, high in morbidity and mortality rate. Scabies is a very contagious disease, described by dermatitis, hyperkeratosis, alopecia, pruritic, and crust formation. Scabies pathogenesis was related to hypersensitivity reaction [4–6]. Many countries and international organization realize how important scabies is, that is included as one of the most common diseases and treated as “neglected tropical disease,” and scabies charges as an emerging/re-emerging infectious disease [7,8].

Mites protein is known as antigen; when antigen enters the body, it will activate lymphocyte B cells to produce immunoglobulin. Several studies have shown that *S. scabiei*’s protein of goat and rabbit isolates is immunogenic which can be developed for diagnostic kits and vaccine sub-units [6,9]. Vaccination is a good ecological alternative for effective parasite control. Moreover, anti-parasite vaccine that is effective against scabies is not yet developed; it is because of some factors like the intricacy of interaction between the host immune system and the parasite, also the mechanism...
of the host immune system, and large number of protein that coded by parasite is not yet known therefore, it is very hard to find the protein that has the capacity to give protective immunity [10]. There is no immunodiagnostic test or commercial subunit vaccine available for scabies. Some research has focused on producing a recombinant protein from *S. scabiei* to detect the host’s immune response, to develop a subunit vaccine, and serodiagnostic [11–13]. Study of genetic characterization of *S. scabiei* using a marker of subunit 1 cytochrome c oxidase (COX-1) and the second internal transcribed spacer (ITS-2) are well developed to achieve the aims of molecular epidemiology investigation and the provision of subunit vaccines for scabies in animals [14–17].

COX-1 gene is the most informative for investigating molecular epidemiology and often used by researchers for a marker of genetic characterization of *S. scabiei* from animals and human [14,18,19]. Based on field observations, the number of scabies cases in rabbits in Indonesia is increasing elevate. However, there have been no case reports because the condition has been treated with acaricide [9]. Vaccination is a good ecological alternative for effective parasite control. Anti-parasite vaccine that is effective against scabies is not yet developed because there are lacks of the genetic characterization data on *S. scabiei*. The purpose of this study was to characterize the mitochondrial COX-1 gene of *S. scabiei* in rabbits from three districts of Malang, Nganjuk, and Kediri, East Java, Indonesia. The gene was aligned with a DNA isolated from *S. scabiei* of Chongqing rabbit (accession number: EU256388.1) to construct a molecular analysis of phylogenetic in *S. scabiei* COX-1 gene.

**Materials and Methods**

**Ethical approval**

This research has been approved by the Ethics Commission, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia with certificate number 630-KE.

**Identification and Collecting mites of *S. scabiei* from rabbits**

The mites were collected and identified from rabbits that have an indication of scabies infection.

Identification of *S. Scabiei* was carried out at Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga. The selected Sarcoptes were centrifuged at 3,000 rpm for 10 min and this step was performed at least three times to get the result free of dirty materials that still carried in the scraping process. The deposit result kept in a freezer at −20°C, to be processed into DNA extraction [9,14].

**DNA extraction and amplification**

DNA extraction was carried out and followed the extraction protocols of the QIAamp DNA mini kit (Qiagen, Hilden, Germany) [20]. The amplification target of mitochondrial COX-1 gene in *S. scabiei* was 290-bp of polymerase chain reaction (PCR) products. DNA templates were obtained from the samples after extraction procedures. The Primers were designed, which refer to the GenBank Accession Number EU256388.1, the mitochondrial COX-1 gene of *S. scabiei* was isolated from Chongqing rabbit. The forward primer is 5’-TCT TAG GGG CTG GAT TTA GTA TG-3’ and the reverse primer is 5’-AGT TCC TCT ACC AGT TCC AC-3’. PCR amplification was constructed in Biorad iCycles IQ. PCR steps were done for 35 cycles in the following temperatures, initial denaturation (95°C 5 min), denaturation (95°C 30 sec), annealing (50°C 60 sec), extension (72°C 60 sec), and final extension (72°C 5 min). The amplicon was visualized in 2% of agarose gel electrophoresis under UV illuminator [14].

The PCR products were purified according to the protocol of the BigDye X Terminator™ Purification Kit (Thermo Scientific) and were double-sequenced with the forward and reverse PCR primers of ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

**Bioinformatics analysis**

The sequence was analyzed with program Clone Manager Professional 9 (Sci-Ed Software) where the reverse primer was inverted to make complete alignments. The inverted result then aligns to compare with DNA from Chongqing rabbit (Accession Number EU256388.1).

For comparison with other related DNA sequences, the COX-1 DNA fragment sequence of *S. scabiei* isolated from Malang, Nganjuk, and Kediri rabbits was used as a query search with the program Nucleotide BLAST. All hits that have nucleotide identity of 80% or higher; along with all sequences resulted from this investigation, were aligned using the ClustalW2 program [22]. The sequence product was confirmed with Clone Manager Professional 9 (Sci-Ed Software) and the Neighbor-Joining method was done with MEGA6 to build a phylogenetic tree [23], with a COX-1 sequence from *Megaselia* sp. (GenBank accession No. KT103510.1) as an outer group.

**Results and Discussion**

*Sarcoptes scabiei* mites were isolated from Malang, Nganjuk, and Kediri rabbits, with clinical symptoms such as skin thickening, crust formation, hair loss on the areas around the eyes, ears, mouths, and legs. The amplicon products of PCR were visualized in 2% agarose gel electrophoresis and under the UV illuminator it showed a correct band 290 bp that is at the position between 200 and 300 bp of the markers (Fig. 1).

This study showed that the sequencing of COX-1 gene (Fig. 2) DNA *S. scabiei* rabbits originated from Kediri, Malang, and Nganjuk, after analyzed with Clone Manager Professional 9 program (Sci-Ed Software) using DNA
Figure 1. PCR product of *S. scabiei* from rabbit of Kediri (S1), Malang (S2), Nganjuk (S3), Negative Control (K), and Marker (M).

![PCR Product Image]

| EU256388 | 1   | TCTAGGGGCTGAGTATAGTGTATTCTGATATCAATTATCTCAACCAATAGGAATTT |
|---------|-----|----------------------------------------------------------|
| Kediri  | 1   | TCTAGGGGCTGAGTATAGTGTATTCTGATATCAATTATCTCAACCAATAGGAATTT |
| Malang  | 1   | TCTAGGGGCTGAGTATAGTGTATTCTGATATCAATTATCTCAACCAATAGGAATTT |
| Nganjuk | 1   | TCTAGGGGCTGAGTATAGTGTATTCTGATATCAATTATCTCAACCAATAGGAATTT |
| EU256388 | 61  | CTATAAATTCTATATTATATTAAATTACGGTATACCGCCCATGCTTTTATTATATTTTT |
| Kediri  | 61  | CTATAAATTCTATATTATATTAAATTACGGTATACCGCCCATGCTTTTATTATATTTTT |
| Malang  | 61  | CTATAAATTCTATATTATATTAAATTACGGTATACCGCCCATGCTTTTATTATATTTTT |
| Nganjuk | 61  | CTATAAATTCTATATTATATTAAATTACGGTATACCGCCCATGCTTTTATTATATTTTT |
| EU256388 | 121 | TTATAGTAATACCTATTATATAATAGGGATTTGGAAATTTATATACCTCTATATATAG |
| Kediri  | 121 | TTATAGTAATACCTATTATATAATAGGGATTTGGAAATTTATATACCTCTATATATAG |
| Malang  | 121 | TTATAGTAATACCTATTATATAATAGGGATTTGGAAATTTATATACCTCTATATATAG |
| Nganjuk | 121 | TTATAGTAATACCTATTATATAATAGGGATTTGGAAATTTATATACCTCTATATATAG |
| EU256388 | 181 | GCCTCGCTGATAGCTACCTCGATTGATAAATTATAGTTGGTTATATACCTCTACCAT |
| Kediri  | 181 | GCCTCGCTGATAGCTACCTCGATTGATAAATTATAGTTGGTTATATACCTCTACCAT |
| Malang  | 181 | GCCTCGCTGATAGCTACCTCGATTGATAAATTATAGTTGGTTATATACCTCTACCAT |
| Nganjuk | 181 | GCCTCGCTGATAGCTACCTCGATTGATAAATTATAGTTGGTTATATACCTCTACCAT |
| EU256388 | 241 | CTTTTAACATTATACAAATTCTTTATGTTGGAACGTTAGAGAAACT |
| Kediri  | 241 | CTTTTAACATTATACAAATTCTTTATGTTGGAACGTTAGAGAAACT |
| Malang  | 241 | CTTTTAACATTATACAAATTCTTTATGTTGGAACGTTAGAGAAACT |
| Nganjuk | 241 | CTTTTAACATTATACAAATTCTTTATGTTGGAACGTTAGAGAAACT |

Figure 2. Sequence alignment of COX-1 partial gene mitochondrial DNA of *S. scabiei* from rabbits of Kediri, Malang, and Nganjuk East Java, Indonesia.
sequence with the GenBank accession No. EU256388.1 showed more than 99% similarity. The phylogenetic tree of mitochondrial COX-1 gene of *S. scabiei* from Kediri, Malang, and Nganjuk was relatively close to 19 *S. scabiei* isolates obtained from the NCBI nucleotide database with their Accession Numbers (Fig. 3).

The sequence products showed that Nganjuk scabies infected rabbits had one base pair different. In the nucleotide number 30, Cytosine (C) was replaced by Guanine (G). The sequence from Kediri infected rabbits showed a different result. There were two base pair differences and the two base pairs of Thymine and Adenine (TA) were replaced by guanine (G) in the base pair number 215–216. There was no base pair difference in Malang scabies infected rabbits.

Research by Lastuti et al. [21] demonstrated that the partial CDS of mitochondrial COX-1 gene of *S. scabiei* from Lamongan goats was very similar to Mojokerto rabbits, with a homology identity of 99%. That previous study confirmed that out of 290 bp in Lamongan scabies-infected goats, there was one base pair different. In the nucleotide number 26, Guanine was replaced by Adenine.

The guanine residue is conserved in other *S. scabiei* COX-1 gene. Based on homology analyses with marker ITS-2 by Gu et al. [24], it showed high homology of more than 96.6% among six isolates of *S. scabiei* (De Geer). Thus, high homogeneity results mean that isolates from China and other locations belonged to a single and heterogeneous species.

According to the literature, guanine found in Guanosine Triphosphate plays a role in cellular processes such as cell growth regulation, signal transduction, and protein transport [25], possibly related to the pathogenesis of scabies. Mites of *S. scabiei* originated from rabbits that showed clinical symptoms of severe scabies, with histopathological changes such as parakeratosis, acanthosis, inflammatory cell infiltration, degeneration, and congestion [6,26]. *Sarcoptes scabiei* antigen induces cytokine expression in fibroblast and keratinocytes cells. The secretion of cytokine will stimulate eosinophils to secrete granules which cause allergic reactions such as edema, mucous secretion, and leukocyte infiltration [27].

The *S. scabiei* mites that were genetically characterized were from rabbits infected with severe scabies from rabbit

![Figure 3](http://bdvets.org/javar/)

**Figure 3.** Phylogenetic analysis of partial CDS of COX-1 gene of *S. scabiei* isolated from several different species. All sequences were aligned by using ClustalW2 and the cladogram was built using the Neighbor-Joining method.
farms from Kediri, Malang, and Nganjuk. Rabbits originating from the area in addition to meeting their region needs, also sold to other areas, especially East Java to meet the needs of rabbit meat, animal experiment, and kept as a pet. Most of the rabbits were traditionally maintained with humid cage conditions and lack of sanitation, thus causing transmission among rabbits, especially in one population, making it possible that *S. scabiei* mites have adapted to their hosts and have grown long enough [9]. The incidence of scabies in America and Australia is an emerging infectious disease. Transmission occurs through direct contact between individual or contaminated materials with mites. The life cycle of *S. scabiei* mites takes about 3 weeks [16].

The study of genetic detection of *S. scabiei* by several researchers showed a change in the nucleotide of mitochondrial DNA COX-1 gene sequencing between animal species and geographical differences. Whereas sequencing ITS-2 gene did not show geographical differences and host adaptation [10,17]. Detection of *S. scabiei* genetic with COX-1 gene as a marker can be used as epidemiology study of *S. scabiei* mites from various animal species and geography to control scabies infection [10,16].

*Sarcoptes scabiei* mite was a single species but has many variants according to its host (rabbit, goat, and dog) [15,24,28]. Mitochondrial DNA of *S. scabiei* isolated from rabbits originated from Kediri, Malang, and Nganjuk, East Java, Indonesia, all show COX-1 partial gene sequences that are highly similar (>99% identity) to those found in GenBank. Molecular analysis of phylogenetic in COX-1 sequences confirmed that it has three branches of *S. scabiei*: (1) large, unresolved branch, including sequences from Kediri, Malang, and Nganjuk rabbits, and sequences obtained from the GenBank of *S. scabiei* from different host and regions; (2) branch of *S. scabiei* from Australia wombats and *S. scabiei* isolate B1; and (3) Branch of *S. scabiei* type *hominis* from Australia and *Megaselia* sp. from Canadian insects, used as an outer group in this analysis.

The haplotypes of *S. scabiei* between koala and wombat have very high similarity (99.1%–100%). Close to a full-length phylogenetic analysis of mitochondrial genomes confirmed three branches of *S. scabiei* (in human and two marsupial), no apparent difference in geographic or host species [16].

This research could be further developed to explore the genetic diversity of *S. scabiei* from animals species in Indonesia to produce subunit vaccines for scabies infection [29,30].

**Conclusion**

COX-1 gene sequences of *S. scabiei* from rabbits in Malang, Nganjuk, and Kediri were very similar (>99%) with COX-1 gene sequences of *S. scabiei* acquired from several hosts according to NCBI data.

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**Conflict of interest**

The authors declared that they have no conflict of interest related to this research.

**Authors’ contribution**

NDRL, as a research coordinator, designed the plan research work. AM and WMY did the laboratory works and analyzed the results. All authors read and approved the final manuscript.

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