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Molecular detection and phylogenetic analysis of *Schistosoma japonicum* in Central Sulawesi, Indonesia using COI gene

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Abstract. *Sutrisnawati, Ramadhan A. 2025. Molecular detection and phylogenetic analysis of* Schistosoma japonicum *in Central Sulawesi, Indonesia using COI gene. Biodiversitas 26: 4042-4047.* Schistosomiasis, an endemic disease in several tropical regions, including Indonesia, is caused by *Schistosoma japonicum*, making its early detection crucial for controlling the spread. Molecular detection using the Cytochrome Oxidase 1 (COI) gene not only aids in diagnosing infections but also contributes to environmental DNA (eDNA) studies by detecting parasite DNA from environmental samples such as water or soil. This study aimed to detect *Schistosoma japonicum* in Central Sulawesi, Indonesia, using the COI gene as the molecular target. Fecal samples from individuals suspected of infection were collected from various endemic regions in the area. The Polymerase Chain Reaction (PCR) technique successfully amplified a 585 bp fragment of the COI gene, considered both sensitive and specific for *S. japonicum* detection. Out of the five samples analyzed, a number tested positive, and the resulting amplicons showed 99.21-99.52% sequence similarity with *S. japonicum* sequences in the GenBank database. Phylogenetic analysis placed these isolates within a well-supported clade together with *S. japonicum* from other parts of Indonesia and neighboring countries, confirming their close evolutionary relationship. Minor nucleotide variations were observed, suggesting potential local adaptations that may have epidemiological relevance. These results confirm the presence of *S. japonicum* in Central Sulawesi and demonstrate the utility of the COI gene in both detection and genetic characterization. The findings also highlight the value of incorporating molecular data into schistosomiasis surveillance to improve risk mapping, guide intervention priorities, and monitor possible drug resistance emergence. This approach complements traditional diagnostic methods, which often have reduced sensitivity in light infections. In conclusion, the COI gene provides a reliable tool for identifying and understanding the genetic diversity of *S. japonicum* in Indonesia. Future research should expand sample sizes and geographic coverage to further elucidate the population structure of *S. japonicum* and support more targeted, sustainable schistosomiasis control strategies locally and globally.

Keywords: Cytochrome oxidase I, molecular detection, *Oncomelania hupensis, Schistosoma japonicum,* Sulawesi

INTRODUCTION

Schistosoma japonicum (Katsurada, 1904) is a species responsible for causing schistosomiasis, a neglected tropical disease with significant public health and economic impacts in endemic regions (Hamid 2019). Individuals suffering from schistosomiasis are at risk of chronic infection, which may lead to severe complications, including liver fibrosis, intestinal damage, and even death (Verjee 2019). Central Sulawesi in Indonesia is one of the endemic areas reported for *S. japonicum*, especially in the regions surrounding Lake Lindu and other rural areas (Budiono et al. 2019). The pathogenic lifecycle passes through two hosts: the intermediate host, freshwater snails (*Oncomelania hupensis* (Gredler, 1881)), and the definitive host, mammals, including humans. Understanding its prevalence, distribution, and genetic diversity is important for developing effective control and prevention strategies (Wu et al. 2021). In many endemic communities, persistent transmission is influenced by ecological factors, agricultural activities, and close contact between humans and livestock.

The government has made significant efforts to control this endemic disease, including the administration of Niridazole and Stibophen (Nelwan 2019), the eradication of intermediate host snails, and improvements to irrigation systems (Olkeba et al. 2020). These measures have reduced disease incidence, albeit only temporarily. However, Faust et al. (2020) reported the emergence of drug resistance in schistosomiasis treatment, associated with increasing patient ages. Humans and other mammals serve as the definitive hosts of this parasite (Hugot et al. 2022). The worm eggs, excreted with feces, hatch and release miracidia, which actively swim to find snail hosts, *Oncomelania hupensis* subsp. *lindoensis* (G.M.Davis & Carney, 1973) (Van Beest et al. 2022; Chai and Jung 2024). This complex cycle, combined with the persistence of suitable snail habitats, makes eradication challenging in rural landscapes.

For *S. japonicum* detection, traditional methods such as the Kato-Katz technique a microscopic approach to identify eggs in stool samples are still widely used (Chen et al. 2021). Although practical for field use, these methods have limitations, such as low sensitivity in light infections. Environmental factors and a shortage of trained personnel further reduce their suitability for remote areas (Rahman et al. 2021). This may result in underestimated prevalence and delayed intervention. The advancement of molecular tools, particularly DNA-based diagnostics, has transformed parasite detection (Kumar et al. 2024). Among these, mitochondrial genes such as Cytochrome Oxidase 1 (COI) are notable for

their reliability in species identification and genetic characterization (Elyasigorji et al. 2023).

The COI gene is a mitochondrial marker widely applied in molecular studies of various organisms, including parasites (Selcuk et al. 2022). This gene is highly conserved within a species but varies considerably between taxa, making it ideal for DNA barcoding and phylogenetic studies. While commonly used in other taxa, COI is crucial for studying *S. japonicum*, especially in assessing genetic diversity, population structure, and evolutionary history (Kifle et al. 2020). Its high copy number in mitochondrial DNA also enhances sensitivity, enabling detection of low concentrations of parasite DNA in biological or environmental samples (Noreikiene et al. 2020). These attributes make COI-based methods valuable for early detection and environmental DNA (eDNA) monitoring.

Central Sulawesi, Indonesia provides a unique setting for studying *S. japonicum* due to its confirmed endemicity and ecological diversity (Budiono et al. 2019). Previous studies have documented significant infection rates in both humans and animals, along with the presence of *Oncomelania* snails as intermediate hosts. However, few molecular studies focus on *S. japonicum* in this area. This knowledge gap limits understanding of genetic variation and its potential effects on transmission and control (Elyasigorji et al. 2023). Without molecular data, it is difficult to determine if local parasite populations exhibit unique traits influencing pathogenicity, treatment response, or environmental resilience.

In this context, COI-based molecular detection offers a robust solution, providing accurate and reliable data for surveillance and control (Wu et al. 2021; Kumar et al. 2024). Integrating phylogenetic analysis with epidemiological mapping can help identify high-risk zones, trace parasite movement, and inform targeted intervention strategies. Applying COI analysis to *S. japonicum* in Central Sulawesi will fill critical gaps in scientific knowledge and contribute to sustainable, evidence-based control programs suited to Indonesia's diverse environments.

MATERIALS AND METHODS

Study design and malacological survey

In the present study, malacological surveys were conducted randomly by manually searching for *O. hupensis* snails. The surveys were conducted in identified and potential snail habitats in Central Sulawesi, Indonesia. Each survey lasted at least one hour, during which the snails were collected using tweezers and plastic containers. After collection, the snails were sorted, counted, and then crushed. To determine the infection rate, each snail was crushed and analyzed microscopically. Three equally sized drops of distilled water were placed on each slide. A single snail was placed in each droplet using tweezers. The snails were gently crushed, just enough to break the shell, by placing a clean slide on top of the slide containing the snails. The tissues were pulled apart with tweezers or a dissecting needle to facilitate the release of the sporocysts or the characteristic forked cercariae of *S. japonicum*, which serve as indicators of snail infection.

DNA extraction, amplification, electrophoresis, and sequencing

Genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol with minor modifications. Filter paper samples were processed using Salivette tubes (Sarstedt, Germany), followed by lysis, incubation, and centrifugation steps optimized for fecal material. Detailed reagent volumes, incubation times, and centrifugation parameters are provided in the Supplementary Materials. Extracted DNA was stored at -20°C until amplification. Mitochondrial DNA was amplified targeting the Cytochrome Oxidase 1 (COI) gene using the primer pair Sj_COI_F (5′-TTTGATAACTAATCACGGTATAGCAA-3′) and Sj_COI_R (5′-CGAGGCAAAGCTAAATCACTC-3′) as described by Fornillos et al. (2019). PCR reactions were conducted using 2× DreamTaq Green PCR Master Mix under standard thermocycling conditions, including 50 cycles of amplification with annealing at 60°C. PCR products were visualized via electrophoresis on 1% agarose gels stained with FluoroSafe and viewed under UV illumination. Amplicons were sent to First Base Sdn Bhd (Malaysia) via P.T. Genetika Science (Jakarta, Indonesia) for bidirectional sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI 3730xl Genetic Analyzer (Applied Biosystems).

Data analysis

Information obtained from DNA sequencing results was processed using the GeneStudio program and validated with SeqMan and EditSeq in the DNASTAR program (DNASTAR Inc., Madison, USA). Sequencing reactions were performed on each individual using both forward and reverse primers. Chromatograms were manually examined to identify ambiguous bases and stop codons. The *S. japonicum* CO1 sequences were then aligned using ClustalW in the MEGAX program. The structure of the COI nucleotides was determined using the MEGAX program. Genetic distances were analyzed using the MEGAX program with the Kimura-2 Parameter (K2P) model and summarized in a Neighbor-Joining (NJ) tree, which is the standard procedure used in barcoding studies with 1000 bootstrap replicates. The reproduction of the phylogeny tree was examined utilizing the Neighbor Joining and Maximum Likelihood strategies with 1000 bootstraps utilizing the MEGAX program and Bayesian Inference utilizing the BEAST program (Suchard et al. 2018). The Bayesian Information Criterion (BIC) executed in jModelTest 2.1.10 (Darriba et al. 2012) was utilized to decide the best-fit transformative model. According to the Bayesian Information Criterion (BIC), the optimal substitution model for this analysis is HKY with invariant sites (HKY+I). The Markov Chain Monte Carlo (MCMC) was run for 106 cycles ages to appraise the back probabilities conveyance with an inspecting frequency set to 1000. The agreement trees were envisioned in FigTree 1.4.4 (Rambaut 2019).

RESULTS AND DISCUSSION

PCR amplification and sequence identification

The results showed that the amplification of the mitochondrial COI gene from five *Schistosoma japonicum* samples collected in Central Sulawesi produced a fragment length of approximately 585 bp (Figure 1). The sequence alignment results from the chromatogram editing process ranged between 589 to 597 bp. Based on the outcomes, all five samples collected from Central Sulawesi were identified as belonging to the genus *Schistosoma* and consisted of a single species, *S. japonicum*. The similarity of the sequences compared to the data in GenBank was 99.21 to 99.52% (Table 1).

Sequence alignment

The alignment of five *S. japonicum* arrangement tests from Central Sulawesi yielded a spotless grouping (a succession that came about after the arrangement and cutting course) of 585 bp. The high-quality COI sequences of each species were then used for intraspecies analysis. The COI groupings arrangement of *S. japonicum* from Central Sulawesi and other countries kept in GenBank brought about a section length of 580 bp. This outcome was then exposed to the intraspecies investigation (nucleotide creation and genetic distance). For phylogenetic tree examination, the clean COI groupings (580 bp) of 10 examples addressed 1 genus and 10 species, in particular *Schistosoma mansoni* (Sambon, 1907), *S. japonicum, S. haematobium* ((Bilharz, 1852) Weinland, 1858), *S. mekongi* (Voge, Bruckner & Bruce, 1978), *S. intercalatum* (Fisher, 1934), *S. bovis* ((Looss, 1876) Blanchard, 1895), *S. mattheei* (Veglia & Le Roux, 1929), *S. spindalis* (Montgomery, 1906), *S. indicum* (Montgomery, 1906), and *S. nasale* (Rao, 1933) (Agniwo et al. 2024), recorded on GenBank, were utilized. Two COI sequences of *O. hupensis* (Accession numbers: HQ851138.1 and HQ851130.1) were used as an outgroup.

Phylogenetic analysis and genetic distance

The tree reconstruction analysis yielded identical tree topologies, as presented in Figure 2. The jModelTest2 analysis identified the HKY model with invariant sites (HKY+I) as the most appropriate substitution model, based on the Bayesian Information Criterion (BIC) (Abadi et al. 2019). The reconstructed phylogenetic tree of *S. japonicum* from Central Sulawesi, along with *Schistosoma* species from various regions available in GenBank, formed distinct and well-supported clades.

The formation of these clades was strongly supported by bootstrap values ranging from 95-100% in both the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods. Additionally, the Bayesian Inference (BI) analysis yielded a posterior probability of 1.00, further confirming the robustness of the clade structures. These consistently high bootstrap values and posterior probabilities indicate that the formation of the observed clades is stable and statistically reliable, demonstrating clear phylogenetic relationships among the analyzed *Schistosoma* species.

Our phylogenetic investigation in view of effectively enhanced COI qualities likewise showed *S. japonicum* from Central Sulawesi is settled within a similar clade of another *S. japonicum* from various regions (Accession number: EU340357.1 and EU340358.1) (Figure 2) with an all-around supported bootstrap and on the hubs. The bootstrap and back likelihood estimates demonstrate the consistency of information appearing in rates. Low qualities imply that the arrangement gives a different tree geography on each test. The considered all-around upheld bootstrap incentive for the greatest probability examination is at >75%. However, based on our sequence analysis, variations were observed in several regions, but they were insufficient to distinguish the samples. In the paper presented by Sutrisnawati et al. (2022), the authors demonstrated that the COI quality could recognize the taxa up to species level on account of the exceptionally restricted varieties of the locale. These varieties of nucleotides can be utilized by a person who recognizes the species. The high variety of nucleotides among a grouping of tests is a successful device for distinguishing *Schistosoma* spp. (Zhang and Hanner 2012).

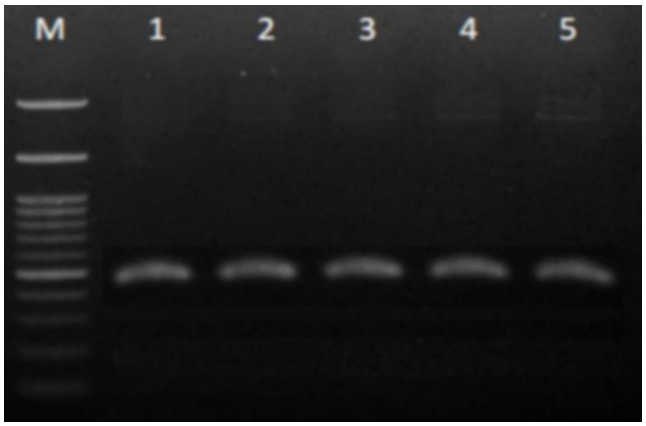

Figure 1. PCR amplification result of the COI mitochondrial gene of *Schistosoma japonicum* from Central Sulawesi, migrated in 1% agarose electrophoresis. 1 to 5: The sample code, M: Marker visualized from the DNA ladder 1 kb (GENEAID)

Table 1. Species identification based on the GenBank database using BLAST

Sample code	Identified species from GenBank	Similarity (%)	Query cover (%)	Accession number	References
SJ-01	*Schistosoma japonicum*	99.32	100	EU340357.1	Unpublished
SJ-02	*Schistosoma japonicum*	99.30	100	EU340357.1	Unpublished
SJ-03	*Schistosoma japonicum*	99.52	100	EU340357.1	Unpublished
SJ-04	*Schistosoma japonicum*	99.36	100	EU340357.1	Unpublished
SJ-05	*Schistosoma japonicum*	99.21	100	EU340357.1	Unpublished

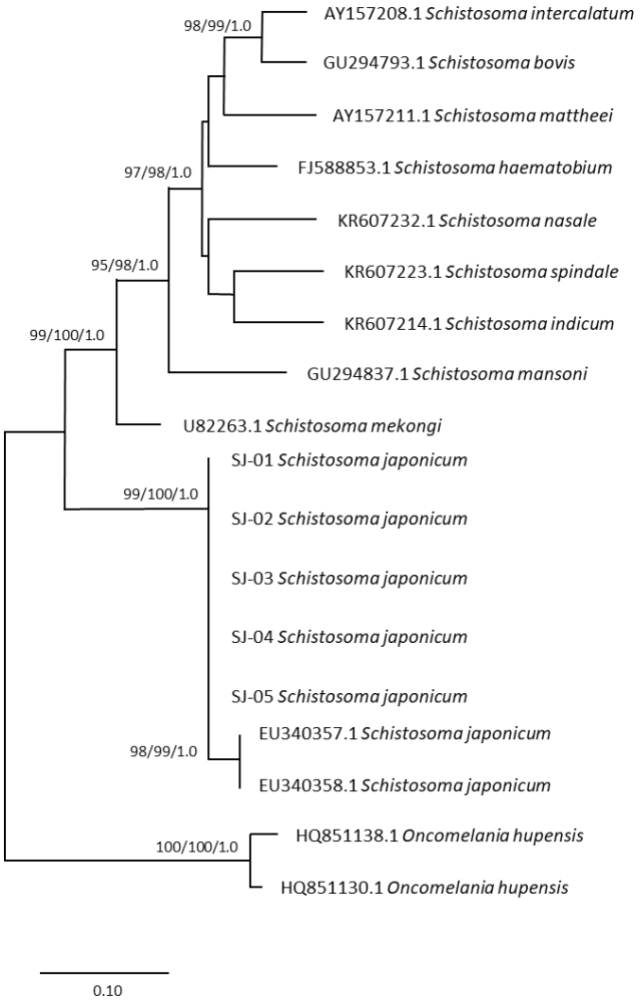

Figure 2. Phylogenetic tree reconstruction based on Neighbor-Joining (NJ), Maximum-Likelihood (ML), and Bayesian Inference (BI) topology of *Schistosoma* spp. and outgroup based on the COI gene sequence (585 bp). The node represented the number bootstrap (NJ and ML) and Bayesian Posterior Probability (Bayesian Inference)

In view of our genetic distance investigations of five examples (SJ-01 to SJ-05) of COI mtDNA qualities of *S. japonicum* from Central Sulawesi, and nine examples of *Schistosoma* spp. various regions recorded on GenBank, it was shown that our examples have a high hereditary distance (at the level of 4%) to the *S. mekongi, S. mansoni, S. indicum, S. spindalis, S. nasale, S. bovis, S. intercalatum, S. mattheei,* and *S. haematobium*. It was viewed that there was hereditary disengagement that happened, and hereditary design is all around kept up with to shape the single species (Brraich and Akhter 2015). As per Zemlak et al. (2009), the threshold for intraspecies hereditary distance in species is 3.5%. Assuming that it surpasses 3.5%, it is viewed as an alternate animal group.

Discussion

Schistosoma japonicum, a parasitic trematode, is a major public health concern, particularly in tropical regions such as Indonesia. This species is the main cause of schistosomiasis, a neglected tropical disease that affects millions of people worldwide (Hamid 2019). Its complex life cycle involves two hosts: Freshwater snails such as *O. hupensis* as the intermediate host, and humans or other mammals, including water buffalo, as the definitive hosts. The cycle begins when eggs are excreted in the feces or urine of an infected host and enter freshwater. Free-swimming miracidia hatch and infect suitable snails, developing into sporocysts that produce cercariae. After several weeks, cercariae are released into the water, where they penetrate the skin of a definitive host, transform into schistosomula, and migrate through the bloodstream to mature in the mesenteric veins. Mated adult worms then produce eggs, some of which are excreted to continue the cycle, while others may lodge in tissues, causing granulomas and disease symptoms (Verjee 2019; Chen et al. 2021; Wu et al. 2021; Elyasigorji et al. 2023). Distinctive features of *S. japonicum* include its zoonotic potential, high egg production, and reliance on freshwater environments, making water management, snail control, sanitation, and drug treatment key components in schistosomiasis control. Understanding its genetic characteristics is also crucial for accurate diagnosis, monitoring, and intervention strategies (Budiono et al. 2019).

In this study, the detection of *S. japonicum* was conducted using the Cytochrome Oxidase 1 (COI) gene, a mitochondrial marker known for its effectiveness in species identification due to its conserved yet variable nucleotide regions. This research focuses on samples collected from Central Sulawesi, Indonesia, a region where the epidemiology of *S. japonicum* is not yet fully understood. The COI gene has been widely used as a molecular marker in phylogenetic studies due to its high resolution in distinguishing closely related species (Selcuk et al. 2022). The gene provides valuable insights into the genetic diversity and evolutionary relationships of parasitic organisms. For this study, *S. japonicum* specimens were collected from infected hosts in Central Sulawesi. Genomic DNA was extracted from the samples, and the COI gene was amplified using Polymerase Chain Reaction (PCR) (Kifle et al. 2020). The amplified sequences were then subjected to phylogenetic analysis to determine the genetic relationship of the Central Sulawesi *S. japonicum* isolates compared to those from other regions.

The phylogenetic tree showed that the *S. japonicum* isolates found in Central Sulawesi formed a clade with *S. japonicum* isolates from the rest of Indonesia and other neighbouring countries like the Philippines and China. It was strongly supported by high bootstrap values (>95%) and posterior probabilities, which strongly affirm the similarity between these isolates (Rambaut 2019). The findings confirm that *S. japonicum* from Central Sulawesi shares a common evolutionary lineage with isolates from other endemic regions. However, variations in specific nucleotide regions within the COI gene were observed, suggesting potential local adaptations or genetic drift (Konorov et al. 2021). The use of the COI gene for the detection of *S. japonicum* has significant implications for schistosomiasis research and control. The high discriminatory power of this marker enables accurate species identification, which is crucial for epidemiological studies and the development of targeted interventions (Elyasigorji et al.

2023). By understanding the genetic structure of *S. japonicum* populations, researchers can better predict transmission dynamics, track parasite migration, and identify potential drug resistance patterns. Furthermore, the study highlights the importance of molecular tools in complementing traditional diagnostic methods, which often rely on morphological characteristics of eggs or adult worms. Molecular approaches, such as COI gene analysis, provide a faster, more accurate, and non-invasive means of detecting and identifying *S. japonicum* (Sengupta et al. 2022). This is particularly valuable in regions like Central Sulawesi, where limited data on the parasite's distribution and genetic diversity exist.

These phylogenetic patterns, when interpreted from an epidemiological perspective, emphasize that the genetic data obtained can serve as a foundation for applied disease control strategies. Genetic data such as these can be integrated into risk mapping to identify high-transmission zones, guide prioritization of intervention areas, and monitor potential drug resistance emergence, thereby supporting more targeted and sustainable control programs. The high discriminatory power of the COI marker enables accurate species identification, which is essential for epidemiological surveillance and the design of region-specific intervention strategies (Elyasigorji et al. 2023). In addition, incorporating molecular evidence into national schistosomiasis surveillance systems will enhance the precision of control measures, allow early detection of potential outbreaks, and strengthen long-term elimination efforts. By understanding the genetic structure of *S. japonicum* populations, it becomes possible to better predict transmission dynamics, track parasite migration, and improve control efforts in both human and animal reservoirs.

Overall, the integration of molecular detection, phylogenetic analysis, and epidemiological interpretation provides a more comprehensive framework for addressing schistosomiasis in endemic areas such as Central Sulawesi. These combined approaches not only improve diagnostic accuracy but also inform more effective, evidence-based control measures that are adaptable to local ecological and socio-economic contexts. Strengthening such integrated surveillance systems is essential to reduce transmission, prevent re-emergence, and ultimately progress towards the elimination of schistosomiasis in Indonesia.

In conclusion, this research demonstrates the utility of the COI gene in detecting and characterizing *S. japonicum* from Central Sulawesi, Indonesia. These findings contribute to a deeper understanding of the genetic diversity and phylogenetic relationships of this important parasite. The need for future studies to expand the sample size and geographic scope is clear, and we would like to invite the scientific community to join us in this endeavor to elucidate further the population structure of *S. japonicum* and its implications for schistosomiasis control efforts in Indonesia and beyond.

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