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Phylogenetic Analysis of *Schistosoma japonicum* Based on 16S rRNA Gene

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Abstract

Schistosomiasis is a neglected tropical disease caused by trematode worms of the genus *Schistosoma* and remains a public health concern in several endemic areas of Central Sulawesi, Indonesia. DNA-based molecular approaches have been increasingly applied to support species identification and the analysis of genetic relationships in *Schistosoma japonicum*. This study aimed to investigate the phylogenetic relationships and genetic variation of *S. japonicum* based on the mitochondrial 16S rRNA gene. Samples of *Oncomelania hupensis lindoensis* were collected from endemic areas surrounding Lake Lindu, Central Sulawesi, Indonesia. Detection of *S. japonicum* was performed using the crushing method, followed by DNA extraction with the GS 100gSYNCTM DNA Extraction Kit. Amplification of the 16S rRNA gene was carried out using the Polymerase Chain Reaction (PCR) technique with the primers LR13107-F and LR12647-R. Sequence data were analyzed using BLAST, MEGA 11, MESQUITE, DNASTAR, GeneStudio, and DnaSP software. Phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods with 10,000 bootstrap replicates. PCR amplification produced clear and high-quality DNA bands. BLAST analysis revealed query cover values ranging from 99.90% to 100% and sequence identity of 100% with *Schistosoma japonicum* sequences available in GenBank. Genetic variation analysis indicated low genetic diversity, characterized by two haplotypes, one variable site, haplotype diversity of 0.500 ± 0.170, and nucleotide diversity of 0.00055 ± 0.00015. Nucleotide composition analysis showed that thymine (T) was the most abundant nucleotide (43.53%), and the A+T content (72.14%) was higher than the G+C content (27.87%), a characteristic feature of mitochondrial genomes. Phylogenetic reconstruction using both NJ and ML methods consistently clustered the Central Sulawesi samples with *S. japonicum* populations from Shanghai and Guangdong, China, with strong bootstrap support values ranging from 99% to 100%. Genetic distance analysis based on the Kimura 2-Parameter model revealed genetic divergence values of 0.00–0.00% among Central Sulawesi samples and between the Indonesian and Chinese populations, indicating very close genetic relationships and high genetic homogeneity. These findings demonstrate that *S. japonicum* populations in Central Sulawesi exhibit low genetic variation and share close phylogenetic relationships with other Asian populations, providing valuable molecular information to support the development of schistosomiasis surveillance and control strategies in Indonesia.

Keywords: *Schistosoma japonicum*; 16S rRNA gene; phylogenetic analysis; genetic variation; Central Sulawesi.

INTRODUCTION

Schistosomiasis is one of the neglected tropical diseases that continues to pose a significant global public health challenge, affecting more than 200 million people across tropical and subtropical regions and causing substantial socioeconomic impacts (Nelwan, 2024). The disease is caused by trematode worms of the genus *Schistosoma*, among which *Schistosoma japonicum* is recognized as the most pathogenic species due to its ability to infect a wide range of mammalian hosts and its high reproductive capacity (Yin et al., 2015). In Asia, *S. japonicum* is distributed in several countries, including China, the Philippines, and Indonesia, with Central Sulawesi representing one of the major endemic regions, particularly in the Lindu, Napu, and Bada areas (Budiono et al., 2019). Transmission of *S. japonicum* involves a complex life cycle comprising two hosts: the freshwater snail *Oncomelania hupensis* as the intermediate host and humans as well as other mammals as definitive hosts (Attwood et al., 2015). Recent surveillance data from Central Sulawesi indicate that although the prevalence of schistosomiasis remains relatively low, it increased from 0.47% in 2024 to 0.60% in 2025. Furthermore, transmission persists with an uneven distribution of cases among endemic areas, suggesting that the disease has not yet been fully eliminated and remains at a low endemic level (Central Sulawesi Provincial Health Office, 2024; 2025).

Various schistosomiasis control measures have been implemented, including mass drug administration with praziquantel, control of intermediate host snail populations, and environmental modification aimed at reducing snail habitats. However, the effectiveness of

these strategies continues to face several challenges, particularly due to the complexity of ecological factors and the persistence of the parasite's life cycle in endemic regions (Rahman et al., 2021). In addition, conventional diagnostic methods such as the Kato–Katz technique have limited sensitivity for detecting low-intensity infections, thereby increasing the risk of underdiagnosis among infected populations (Macalanda et al., 2024). Consequently, DNA-based molecular approaches have emerged as more sensitive and accurate alternatives for detecting infections and investigating the genetic variation and phylogenetic relationships of *Schistosoma japonicum* (Kumara et al., 2024).

One of the molecular approaches widely employed in genetic studies is mitochondrial DNA (mtDNA) analysis, which offers several advantages, including a high copy number, maternal inheritance, and sensitivity to genetic variation (Wallace, 2018; Elyasigorji et al., 2023). Mitochondrial DNA is particularly valuable for genetic diversity studies because it occurs in much higher copy numbers within cells than nuclear DNA and exhibits a relatively higher mutation rate (Hendiari et al., 2020). Among mitochondrial markers, the 16S rRNA gene is extensively used in phylogenetic studies due to the presence of both conserved and variable regions, enabling the identification of evolutionary relationships among organisms (Mandal et al., 2014; Church et al., 2020). Phylogenetic analyses based on this gene can provide important information regarding genetic variation, dispersal patterns, and evolutionary relationships among *S. japonicum* populations (Subari et al., 2021). Nevertheless, studies investigating the genetic variation of *S. japonicum* in Indonesia, particularly in Central Sulawesi, remain limited, highlighting the need for further research to fill this knowledge gap (Zhao et al., 2012).

In this context, the application of DNA-based molecular approaches offers significant advantages by generating more precise and reliable data for schistosomiasis surveillance and control programs (Kumara et al., 2024). One of the most commonly used molecular markers in population genetics and phylogenetic studies is the mitochondrial 16S rRNA gene, owing to its effectiveness in supporting the development of strategies for the management and control of target organisms (Mandal et al., 2014). Furthermore, integrating phylogenetic analyses with epidemiological information enables the identification of high-risk areas, monitoring of parasite dispersal patterns, and formulation of more effective and targeted intervention strategies (Macalanda et al., 2024). The application of this approach to *Schistosoma japonicum* populations in Central Sulawesi is expected to enrich scientific knowledge regarding their genetic variation and distribution dynamics, thereby supporting sustainable, evidence-based control efforts tailored to the environmental characteristics of Indonesia (Rahman et al., 2021). However, to date, no phylogenetic study of *Schistosoma japonicum* from Central Sulawesi based on the mitochondrial 16S rRNA gene has been reported. Therefore, this study aimed to characterize the phylogenetic relationships of *Schistosoma japonicum* using a molecular marker approach in order to support the development of more effective schistosomiasis control strategies.

MATERIALS AND METHODS

Study Area

This study was conducted in March 2026 in the Lake Lindu region, Lindu District, Sigi Regency, Central Sulawesi Province, Indonesia. The study area is recognized as one of the schistosomiasis-endemic regions in Indonesia and is characterized by a landscape consisting of rice fields, wetlands, and irrigation channels adjacent to forested areas. The region exhibits high humidity and a tropical climate, providing favorable environmental conditions for the survival and proliferation of *Oncomelania hupensis lindoensis*, the intermediate host of *Schistosoma japonicum* (Sutrisnawati & Ramadhan, 2025). These ecological characteristics contribute to the persistence of the parasite's transmission cycle and make the area an important location for studies on the genetic diversity and phylogenetic relationships of *S. japonicum*.

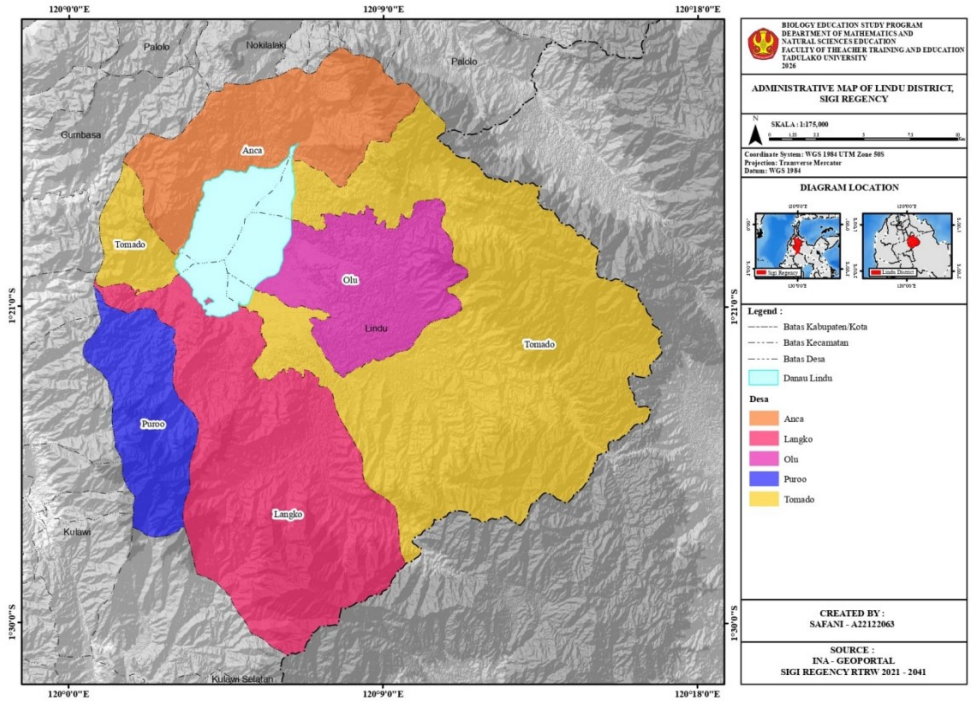

Figure 1. Map of the research location in Lindu, Sigi Regency, Central Sulawesi, Indonesia.

Procedures

Sample Collection

Samples of *Oncomelania hupensis lindoensis* were collected using an exploratory malacological survey in potential habitats, including rice fields, riverbanks, irrigation channels, and muddy areas. Snails were collected manually using forceps to preserve their morphological integrity. The geographic coordinates of each sampling site were recorded using a Global Positioning System (GPS) device to ensure accurate spatial data. Specimens were labeled according to the sampling location and collection date before being transported to the laboratory for further analysis. Subsequently, the snails were examined using the crushing method by placing the specimen on a microscope slide and adding three drops of water to facilitate observation of internal tissues and detection of cercariae as an indicator of *Schistosoma japonicum* infection.

DNA Extraction

Genomic DNA was extracted using the GS 100gSYNCTM DNA Extraction Kit (Geneaid) following the manufacturer's standard protocol. Tissue samples were incubated with lysis buffer and Proteinase K at 60°C for 2.5 h until complete lysis was achieved. The resulting lysate supernatant was then processed with GSB buffer, absolute ethanol, and a GS column to facilitate DNA binding. This was followed by washing and drying steps to remove contaminants and impurities. Finally, DNA was eluted using the provided elution buffer and stored at −20°C until further use in PCR amplification.

PCR Amplification and DNA Sequencing

DNA amplification was performed using the Polymerase Chain Reaction (PCR) technique targeting the mitochondrial 16S rRNA gene with the primers LR13107-F and LR12647-R in a total reaction volume of 25 µL. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. The amplified PCR products were subsequently submitted to the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, for DNA sequencing using an Applied Biosystems Genetic Analyzer 3500.

Gel Electrophoresis

Verification of PCR amplification products was performed using 1% agarose gel electrophoresis prepared by dissolving 0.2 g agarose in 20 mL of 1× TAE buffer and staining with FluoroSafe. A volume of 2 µL of each PCR product was loaded into the gel wells together with a DNA ladder serving as a molecular size marker. Electrophoresis was conducted at 50 V for 17–20 min until distinct DNA bands were clearly visible. The gels were then visualized under a UV transilluminator and photographed to confirm successful amplification of the target DNA fragments.

Data Analysis

The sequencing data obtained in the form of forward and reverse .ab1 files were edited and assembled using GeneStudio and DNASTAR software to generate

consensus sequences. The resulting sequences were subsequently analyzed using Nucleotide BLAST at the National Center for Biotechnology Information (NCBI) to verify species identity based on sequence similarity with reference sequences available in the GenBank database. The verified sequences were then aligned using MESQUITE version 3.51 (Maddison & Maddison, 2018) and converted into FASTA format for further analyses in MEGA 11. Genetic distances among sequences were estimated using the Kimura 2-Parameter (K2P) model, while phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods with 10,000 bootstrap replications to assess the statistical support for each phylogenetic branch. Furthermore, genetic variation analyses, including haplotype diversity and nucleotide diversity, were performed using DnaSP version 6. These analyses were conducted to evaluate the level of genetic diversity and evolutionary relationships among Schistosoma japonicum populations from Central Sulawesi and to compare them with reference populations available in GenBank.

RESULTS AND DISCUSSION

Amplification and Sequence Similarity of *Schistosoma japonicum*

The amplification of the mitochondrial 16S rRNA gene of *Schistosoma japonicum* from samples collected in endemic areas of Central Sulawesi was successfully performed using PCR with the forward primer LR13107-F and the reverse primer LR12647-R (Figure 2). Electrophoretic analysis of the PCR products revealed high-quality DNA bands characterized by clear and well-defined patterns. The presence of distinct DNA bands indicates the successful amplification of the target gene fragment and confirms the effectiveness of the PCR protocol used in this study. The high quality of the amplified DNA fragments provides a reliable basis for subsequent molecular analyses, including DNA sequencing, sequence alignment, genetic distance estimation, and phylogenetic reconstruction. Well-defined PCR bands also suggest that the extracted DNA possessed sufficient purity and concentration for amplification, with minimal contamination or degradation. Therefore, the amplification results demonstrate that the selected primer pair was effective in targeting and amplifying the mitochondrial 16S rRNA region of *S. japonicum*, enabling further investigation of its genetic variation and phylogenetic relationships.

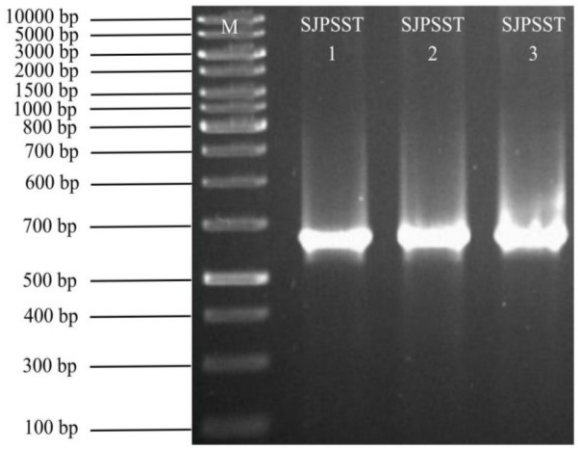

Figure 2. The amplification results of the mitochondrial 16S rRNA gene showed that the sample labeled PBPSST represented *Schistosoma japonicum* collected from the area surrounding Lindu District, Sigi Regency, Central Sulawesi Province.

The DNA sequences obtained were analyzed using the Nucleotide BLAST tool available at the National Center for Biotechnology Information (NCBI) to evaluate the query coverage and percentage similarity of the three DNA sequences generated in this study. This analysis was conducted to confirm species identity by comparing the obtained sequences with reference sequences available in the GenBank database (Table 1).

Table 1. BLAST analysis of mitochondrial 16S rRNA gen sequences of S. japonicum Central Sulawesi.

Code	BLAST			Verification	Location
	% Identity	% *Query Cover*	Accession Number *GenBank*		
ST.1.1	100	99.90	KR855668.1	*Schistosoma japonucim*	Central Sulawesi
ST.1.2	100	99.90	KR855668.1	*Schistosoma japonucim*	Central Sulawesi
ST.1.3	100	100	KR855668.1	*Schistosoma japonucim*	Central Sulawesi

Genetic Variation of *Schistosoma japonicum*

Genetic variation analysis of *Schistosoma japonicum* sequences based on the mitochondrial 16S rRNA gene, with a sequence length of 648 bp, revealed relatively low genetic diversity among the samples examined. Among the nine individuals analyzed, two haplotypes were identified, with one variable site and one parsimony-informative site detected. The haplotype diversity was recorded at Hd = 0.500 ± 0.170, indicating a moderate level of haplotype variation, whereas the nucleotide diversity of π = 0.00055 ± 0.00015 reflected a very low level of nucleotide differences among the sequences, as presented in Table 2.

Table 2. Intraspecific genetic variation of *S. japonicum* based on mitochondrial 16S rRNA gene sequences compared with *S. japonicum* from GenBank.

Sample Code	bp	Number of Individual	Number of Haplotype	Variable Site	Parsimony Site	Haplotype Diversity (Hd)	Nucleotide Diversity (π)
ST.1.1 ST.1.2 ST.1.3 KP793868.1 KP793867.1 KP793866.1 KP783865.1 KP793864.1 GQ403728.1	648	9	2	1	1	0,500 ± 0,170	0,00055 ± 0,00015

Nucleotide Composition of *Schistosoma japonicum*

DNA consists of four nitrogenous bases, namely thymine (T), cytosine (C), adenine (A), and guanine (G). Adenine (A) pairs with thymine (T) through two hydrogen bonds, whereas cytosine (C) pairs with guanine (G) through three hydrogen bonds; consequently, C–G base pairs exhibit greater stability than A–T base pairs. The analysis of the nucleotide composition of *Schistosoma japonicum* based on the mitochondrial 16S rRNA gene revealed that thymine (T) was the most abundant nucleotide, accounting for 43.53%, followed by adenine (A) (28.61%), guanine (G) (18.91%), and cytosine (C) (8.96%). The nucleotide composition showed that the A+T content reached 72.14%, which was considerably higher than the G+C content of 27.87%, reflecting a characteristic feature of mitochondrial genomes, as presented in Table 3.

Table 3. Average nucleotide composition of *S. japonicum.*

Code	T (U)	C	A	G	A+T	G+C	Location	References
ST.1.1	43,53	8,96	28,61	18,91	72,14	27,87	Central Sulawesi	Research Data
ST.1.2	43,53	8,96	28,61	18,91	72,14	27,87	Central Sulawesi	Research Data
ST.1.3	43,53	8,96	28,61	18,91	72,14	27,87	Central Sulawesi	Research Data
KP793868.1	43,53	8,96	28,61	18,91	72,14	27,87	Shanghai China	Yin *et al*. (2015)
KP793867.1	43,53	8,96	28,61	18,91	72,14	27,87	Shanghai China	Yin *et al*. (2015)
KP793866.1	43,53	8,96	28,61	18,91	72,14	27,87	Shanghai China	Yin *et al*. (2015)
KP783865.1	43,53	8,96	28,61	18,91	72,14	27,87	Shanghai China	Yin *et al*. (2015)
KP793864.1	43,53	8,96	28,61	18,91	72,14	27,87	Shanghai China	Yin *et al*. (2015)
GQ403728.1	43,53	8,96	28,61	18,91	72,14	27,87	Guangdong China	Li *et al*. (2009)
Average	43,53	8,96	28,61	18,91	72,14	27,87		

Phylogenetic Tree and Genetic Distance

Phylogenetic trees were reconstructed using MEGA11 software based on the Neighbor-Joining (NJ) and Maximum Likelihood (ML) approaches, employing the P-Distance model for the NJ analysis and the Kimura 2-Parameter (K2P) model for the ML analysis, with 10,000 bootstrap replications. The analysis included 16S rRNA gene sequences comprising three *Schistosoma japonicum* sequences from Central Sulawesi, several *Schistosoma* reference sequences retrieved from GenBank, and *Bacillus cereus* used as the outgroup.

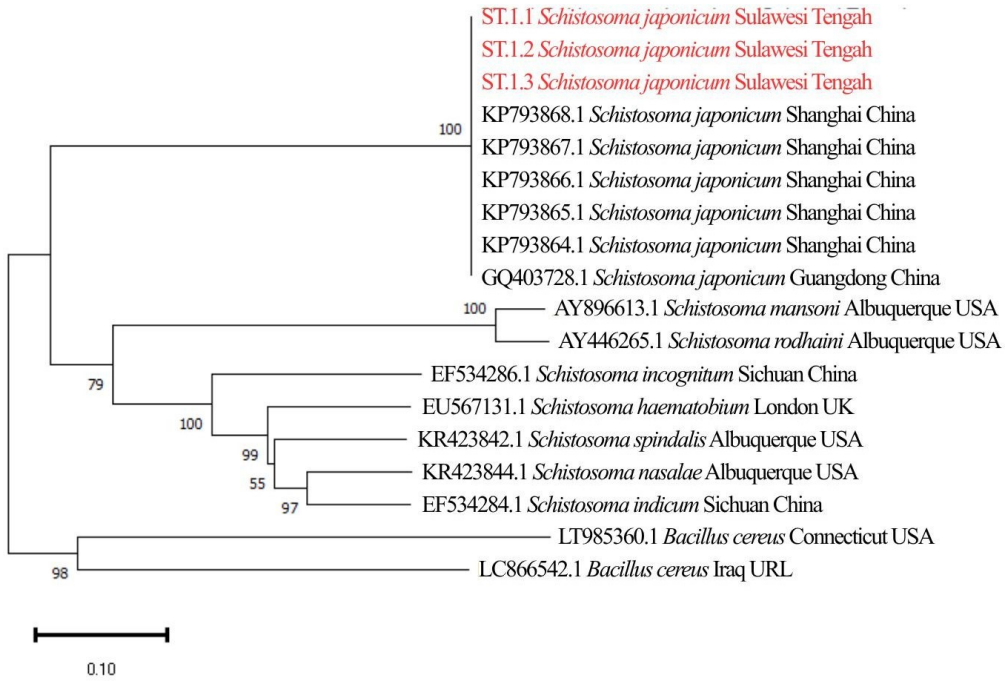

Figure 3. Phylogenetic tree reconstructed using the Neighbor-Joining (NJ) method based on the p-distance model with 10,000 bootstrap replications.

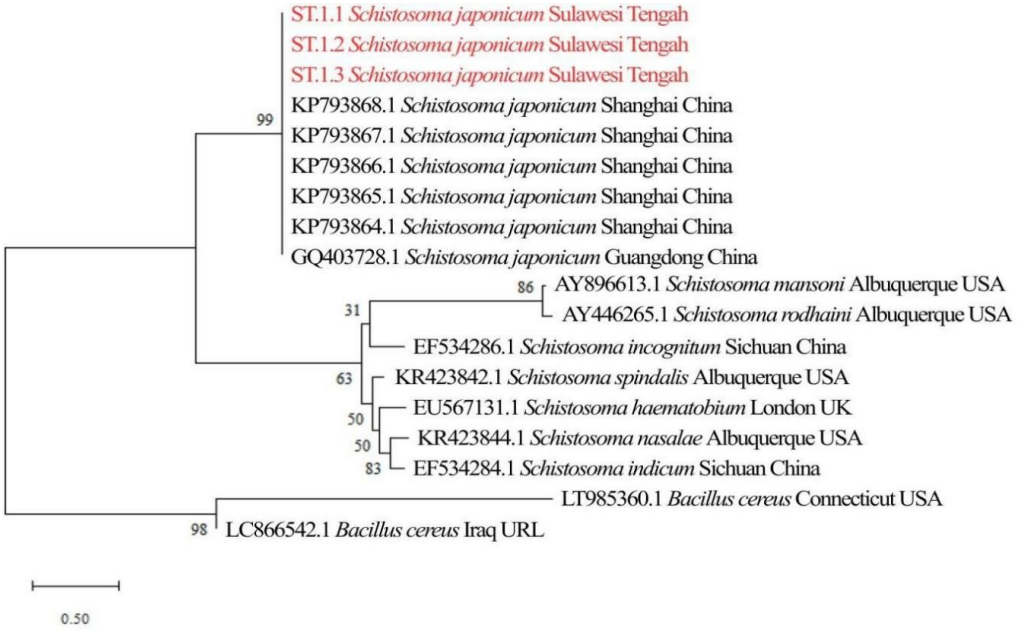

Figure 4. Phylogenetic tree reconstructed using the Maximum Likelihood (ML) method based on the Kimura 2-Parameter (K2P) model with 10,000 bootstrap replications.

Phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods implemented in MEGA 11 (Tamura et al., 2021) based on mitochondrial 16S rRNA gene sequences. The resulting phylogenetic analyses generated several distinct clades, with each species consistently grouped within its respective lineage. The three *Schistosoma japonicum* sequences from Central Sulawesi showed the closest genetic relationship to *S. japonicum* sequences from Shanghai and Guangdong, China, supported by high bootstrap values ranging from 99 to 100, indicating strong confidence in the inferred tree topology. Other *Schistosoma* species, including *S. mansoni*, *S. rodhaini*, *S. haematobium*, *S. indicum*, *S. nasale*, *S. spindale*, and *S. incognitum*, formed separate branches according to their respective phylogenetic affiliations. *Bacillus cereus*, used as the outgroup, formed an external branch distinct from all *Schistosoma* species, thereby helping to clarify the direction of evolutionary relationships and establish the root of the phylogenetic tree.

Table 4. Genetic distance of the mitochondrial 16S rRNA gene of *Schistosoma japonicu.*

	ST.1.1_Schistosoma_japonicum_Sulawesi_Tengah	ST.1.2_Schistosoma_japonicum_Sulawesi_Tengah	ST.1.3_Schistosoma_japonicum_Sulawesi_Tengah	KP793868.1_Schistosoma_japonicum_Shanghai_China	KP793867.1_Schistosoma_japonicum_Shanghai_China	KP793866.1_Schistosoma_japonicum_Shanghai_China	KP793865.1_Schistosoma_japonicum_Shanghai_China	KP793864.1_Schistosoma_japonicum_Shanghai_China	GQ403728.1_Schistosoma_japonicum_Guangdong_China	KR423844.1_Schistosoma_nasalae_Albuquerque_USA	KR423842.1_Schistosoma_spindalis_Albuquerque_USA	AY896613.1_Schistosoma_mansoni_Albuquerque_USA	AY446265.1_Schistosoma_rodhaini_Albuquerque_USA	EU567131.1_Schistosoma_haematobium_London_UK	EF534284.1_Schistosoma_indicum_Sichuan_China	EF534286.1_Schistosoma_incognitum_Sichuan_China	LT985360.1_Bacillus_cereus_Connecticut_USA	LC866542.1_Bacillus_cereus_Iraq_URL
ST.1.1_Schistosoma_japonicum_Sulawesi_Tengah																		
ST.1.2_Schistosoma_japonicum_Sulawesi_Tengah	0,00%																	
ST.1.3_Schistosoma_japonicum_Sulawesi_Tengah	0,00%	0,00%																
KP793868.1_Schistosoma_japonicum_Shanghai_China	0,00%	0,00%	0,00%															
KP793867.1_Schistosoma_japonicum_Shanghai_China	0,00%	0,00%	0,00%	0,00%														
KP793866.1_Schistosoma_japonicum_Shanghai_China	0,00%	0,00%	0,00%	0,00%	0,00%													
KP793865.1_Schistosoma_japonicum_Shanghai_China	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%												
KP793864.1_Schistosoma_japonicum_Shanghai_China	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%											
GQ403728.1_Schistosoma_japonicum_Guangdong_China	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%										
KR423844.1_Schistosoma_nasalae_Albuquerque_USA	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%									
KR423842.1_Schistosoma_spindalis_Albuquerque_USA	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	59,03%	21,15%								
AY896613.1_Schistosoma_mansoni_Albuquerque_USA	70,17%	70,17%	70,17%	70,17%	70,17%	70,17%	70,17%	70,17%	70,17%	56,68%	54,09%							
AY446265.1_Schistosoma_rodhaini_Albuquerque_USA	70,36%	70,36%	70,36%	70,36%	70,36%	70,36%	70,36%	70,36%	70,36%	57,07%	54,73%	7,89%						
EU567131.1_Schistosoma_haematobium_London_UK	58,45%	58,45%	58,45%	58,45%	58,45%	58,45%	58,45%	58,45%	58,45%	23,60%	21,08%	55,56%	55,70%					
EF534284.1_Schistosoma_indicum_Sichuan_China	59,56%	59,56%	59,56%	59,56%	59,56%	59,56%	59,56%	59,56%	59,56%	16,02%	19,95%	56,20%	55,58%	21,23%				
EF534286.1_Schistosoma_incognitum_Sichuan_China	59,27%	59,27%	59,27%	59,27%	59,27%	59,27%	59,27%	59,27%	59,27%	31,67%	27,90%	56,60%	56,49%	32,58%	30,71%			
LT985360.1_Bacillus_cereus_Connecticut_USA	76,94%	76,94%	76,94%	76,94%	76,94%	76,94%	76,94%	76,94%	76,94%	72,47%	73,57%	72,90%	74,28%	75,00%	72,33%	76,11%		
LC866542.1_Bacillus_cereus_Iraq_URL	69,51%	69,51%	69,51%	69,51%	69,51%	69,51%	69,51%	69,51%	69,51%	71,84%	72,88%	72,15%	74,51%	74,51%	70,52%	75,50%	65,61%	

Genetic distance analysis using the Kimura 2-Parameter (K2P) model revealed genetic distances of 0.00%–0.00% among *Schistosoma japonicum* samples from Central Sulawesi, indicating the absence of genetic divergence among these samples. Similarly, the genetic distances between the Central Sulawesi *S. japonicum* samples and *S. japonicum* populations from Shanghai and Guangdong, China, were also 0.00%–0.00%. These findings reflect a very high level of genetic homogeneity among the examined *S. japonicum* populations. In contrast, the genetic distances between *S. japonicum* and other *Schistosoma* species were considerably higher, ranging from 58% to 70%. This substantial divergence indicates clear evolutionary separation among these species, as presented in Table 4. A commonly accepted threshold for species delimitation based on genetic divergence is approximately 3%; sequences showing divergence values greater than 3% are generally considered to represent different species, whereas divergence values equal to or less than 3% indicate conspecific populations (Zhang & Bu, 2022).

Discussion

DNA amplification is one of the most important laboratory techniques in molecular biology, functioning to increase the number of DNA copies obtained from extracted samples so that sufficient genetic material is available for accurate and comprehensive downstream analyses. Through this process, molecular investigations can be performed with a higher level of precision and reliability. In the present study, the mitochondrial 16S rRNA gene of *Schistosoma japonicum* was successfully amplified using the Polymerase Chain Reaction (PCR) method with the specific primers LR13107-F and LR12647-R. The PCR process consisted of repeated cycles of denaturation, annealing, and extension, which systematically generated the target DNA fragment in a specific and sufficient quantity for subsequent analyses. The success of PCR amplification is strongly influenced by appropriate primer design that matches the target sequence, as well as optimized reaction conditions, including temperature profiles and reagent composition, to minimize the formation of non-specific products. The amplified PCR products were subsequently visualized by agarose gel electrophoresis to evaluate the size and quality of the resulting DNA fragments and to verify sample integrity prior to sequencing and phylogenetic analyses. This step plays a crucial role in ensuring the validity of molecular data, particularly in taxonomic studies, parasite diagnostics, and investigations of population genetic variation, as the genetic information obtained can be used to identify species and elucidate patterns of genetic diversity within target populations (Trianto & Purwanto, 2022).

The amplification results of the 16S rRNA gene of *Schistosoma japonicum* from Central Sulawesi produced distinct and well-defined DNA bands corresponding to the expected fragment size, indicating that the PCR process was successfully performed without evidence of DNA degradation or sample contamination. The absence of additional bands or smearing on the electrophoretic gel further demonstrates the high specificity of the primers used for the target gene region. These findings confirm that the PCR protocol applied in this study was capable of detecting *S. japonicum* DNA accurately and sensitively. Similar results were reported by Sutrisnawati and Ramadhan (2025), who demonstrated that amplification of mitochondrial genes in *S. japonicum*

generated specific and high-quality DNA fragments suitable for molecular analyses. Furthermore, Nucleotide BLAST analysis against the GenBank database revealed 100% sequence identity with a query coverage ranging from 99.90% to 100%, confirming that the obtained sequences exhibited extremely high homology with reference sequences of *S. japonicum* deposited in GenBank. This finding strongly supports the identification of the analyzed samples as *S. japonicum*. Moreover, the exceptionally high sequence similarity indicates that the 16S rRNA gene is highly conserved, making it a valuable molecular marker for species identification and phylogenetic studies (Sutrisnawati & Ramadhan, 2025).

Genetic variation refers to differences in genetic material ranging from nucleotide-level changes to genome-wide alterations that may influence an organism's ability to adapt to its environment. In general, higher levels of genetic variation increase the likelihood of population survival because individuals possess diverse responses to environmental changes (Li et al., 2017). In this study, genetic variation was assessed using mitochondrial 16S rRNA gene sequences by examining the number of haplotypes, variable sites, parsimony-informative sites, haplotype diversity (Hd), and nucleotide diversity (π). Based on a 648 bp sequence dataset obtained from nine individuals, two haplotypes, one variable site, and one parsimony-informative site were identified. Genetic variation analysis of *Schistosoma japonicum* from Central Sulawesi revealed a haplotype diversity value of Hd = 0.500 ± 0.170, indicating a moderate level of haplotype variation. In contrast, the nucleotide diversity value of π = 0.00055 ± 0.00015 indicated extremely low nucleotide variation. These findings suggest that the population possesses a very close genetic relationship, exhibits high genetic homogeneity, and shows only minor sequence differences among individuals. Such results also indicate that the 16S rRNA gene is relatively conserved, making it an effective molecular marker for species identification and phylogenetic analysis (Zhao et al., 2012).

The nucleotide composition analysis of *Schistosoma japonicum* showed that the mitochondrial 16S rRNA gene consists of four nitrogenous bases: thymine (T), cytosine (C), adenine (A), and guanine (G). Among these nucleotides, thymine (T) was the most abundant, accounting for 43.53% of the total sequence composition, followed by adenine (A) (28.61%), guanine (G) (18.91%), and cytosine (C) (8.96%). Consequently, the A+T content reached 72.14%, substantially exceeding the G+C content of 27.87%. The predominance of A+T is a characteristic feature of mitochondrial genomes and may influence the molecular properties of the analyzed sequences. A relatively low GC content can reduce primer-binding efficiency because GC base pairs provide greater stability through stronger hydrogen bonding interactions (Wallace, 2018; Nova et al., 2023). Furthermore, variation in nucleotide composition can be used to identify genetic similarities and differences among individuals for species identification purposes (Triandiza et al., 2021). The uniform nucleotide composition observed in this study further supports the conserved nature of the mitochondrial 16S rRNA gene, highlighting its usefulness for comparing genetic similarities among populations and species. Previous studies have demonstrated that genetic variation in *Schistosoma japonicum* differs among geographic regions and can therefore provide valuable information regarding population structure and evolutionary history (Li et al., 2017). The conserved nature of the 16S rRNA gene also explains its widespread application as a molecular marker in phylogenetic reconstruction and molecular identification of trematodes, including *Schistosoma japonicum*. This marker has been extensively utilized in molecular studies because of its effectiveness and reliability in resolving phylogenetic relationships at the species level (Aris et al., 2013).

Phylogenetic analysis is an important approach in parasitological studies because it can reveal genetic relationships among parasite populations and identify patterns of dispersal, evolution, and adaptation to specific environments. This approach is generally performed by analyzing genetic variation using molecular markers and visualizing the results through haplotype networks or phylogenetic trees. A study by Li et al. (2017) demonstrated that genetic variation within parasite populations can provide valuable information regarding population structure and evolutionary history. In the present study, phylogenetic analysis of the mitochondrial 16S rRNA gene was conducted using MEGA11 software with the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods, applying the p-distance and Kimura 2-Parameter (K2P) models, respectively, and supported by 10,000 bootstrap replicates. Both methods generated highly similar tree topologies and consistently grouped samples according to their evolutionary relationships. The three *Schistosoma japonicum* samples from Central Sulawesi clustered together with *S. japonicum* populations from Shanghai and Guangdong, China, with strong bootstrap support values ranging from 99–100%, indicating a high level of confidence in the inferred phylogenetic relationships. Other *Schistosoma* species formed distinct clades according to their evolutionary lineages, whereas *Bacillus cereus* was used as an outgroup to clarify the direction of evolution and establish the root of the phylogenetic tree.

Genetic distance analysis based on the K2P model revealed genetic distances of 0.00%–0.00% among the *Schistosoma japonicum* samples from Central Sulawesi, indicating the absence of detectable genetic divergence among the analyzed specimens. Similarly, no genetic divergence was observed between the Central Sulawesi population and populations from Shanghai, Guangdong, and other regions of China, reflecting an exceptionally

high degree of genetic homogeneity. In contrast, the genetic distances between *S. japonicum* and other *Schistosoma* species ranged from 58% to 70%, demonstrating substantial evolutionary divergence and clear species differentiation. In general, smaller genetic distance values indicate closer evolutionary relationships among populations or species. Therefore, the results suggest that the Central Sulawesi population shares a very close genetic affinity with reference populations from China. According to Zhang and Bu (2022), a genetic divergence threshold of approximately 3% is commonly used for species delimitation; values exceeding 3% indicate different species, whereas values of 3% or lower suggest conspecific populations. The complete absence of genetic divergence observed in this study further confirms that the Central Sulawesi samples belong to the same species, *S. japonicum*, and exhibit a highly conserved mitochondrial 16S rRNA sequence across geographically separated populations.

CONCLUSIONS

Phylogenetic analysis of *Schistosoma japonicum* using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods demonstrated that the analyzed samples have a very close evolutionary relationship with the reference sequences available in GenBank. This close relationship is supported by high bootstrap values, query coverage reaching 100%, and a high level of sequence similarity, indicating a strong and consistent phylogenetic association among the samples. Furthermore, the genetic variation analysis revealed a low number of haplotypes and low nucleotide diversity values, further confirming that the *S. japonicum* population examined in this study possesses a relatively low level of genetic variation.

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