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Phylogeographic structure and historical dispersal of Schistosoma japonicum across East and Southeast Asia

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Article

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Abstract

The evolutionary relationships and geographic differentiation of *Schistosoma japonicum* populations across East and Southeast Asia remain incompletely resolved. This study investigated phylogenetic structure and regional dispersal patterns using mitochondrial genomes and ND1, ND4, and COX3 gene sequences from 84 isolates. Phylogenetic reconstruction revealed clear divergence between *S. japonicum* and *S. sinensium*, supporting their distinct evolutionary positions within the Asian schistosome lineage. Comparative analyses further identified pronounced genetic differentiation between Chinese populations and isolates form Taiwan. ND1 sequence comparations demonstrated extremely high genetic identity exceeding 99% among strains from China, Japan, Indonesia, and the Philippines. These results indicate strong genetic continuity among geographically distant populations despite wide regional separation. Phylogeographic patterns support a mainland Chinese origin followed by historical dispersal toward Japan and Southeast Asian archipelagos. These findings provide new insights into the evolutionary history and regional dissemination pathways of *S. japonicum* across East and Southeast Asia.

1. Introduction

Schistosoma japonicum causes schistosomiasis japonica in both humans and animals [1]. In definitive hosts, it resides in the portal and mesenteric veins [2], causing intestinal schistosomiasis in humans [3–5]. Schistosomiasis japonica is a waterborne disease of ancient origin, with evidence suggesting its existence for over 2,100 years. Symptoms resembling ascites and splenomegaly have been traced back to as far as 2,697 B.C., clinical symptoms similar to those of Katayama syndrome have been known since 400 B.C [6]. The disease is found in East Asia and Southeast Asia [7]. In East Asia, *S. japonicum* occurs in China and Taiwan [8–12], though it is no longer relevant in Japan. In Southeast Asia, the parasite is found in Indonesia and the Philippines [12–14]. In terms of recent statistics, *S. japonicum* infected 37,600 people in China in 2017 [15], 216 people in Indonesia in 2022 [16], and 2.7 million people in the Philippines [17]. Approximately 70 million people are at risk of *S. japonicum* infection in Asia [18–20].

S. japonicum split from *S.* sinensium [21]. Phylogenetic analyses have presented conflicting findings regarding its origin. Some studies suggest *S. japonicum* is derived from Sichuan and Yunnan Provinces, China, and then radiated eastward along the Yangtze River, establishing itself in central and eastern China [22]. In contrast, a phylogenetic reconstruction by Yin *et al.*, which used the *S. japonicum* mitochondrial DNA (mtDNA) genome, revealed a different pattern: the species originated the lake regions and the eastern Yangtze River before radiating to western China, Japan, and Southeast Asia [23]..

The life cycle of *S. japonicum* requires both snails and vertebrate hosts. The intermediate host is the amphibious snail *Oncomelania* spp. [11], while definitive hosts include diverse mammals [14]. Although schistosomes are known to infect birds [24–26], the role of avian species in *S. japonicum* transmission

remains uncertain. However, evidence shows that zoonotic species can infect wild birds [27], suggesting that birds may contribute to transmission of *S. japonicum* parasites. Dunbar et al. (2024) emphasized that mobile avian hosts may facilitate local spread through coprophagy and fecal contamination [28], while Diaz et al. (2023) suggested that the adaptive nature of schistosomes may allow future host shifts to new host [29], including birds as dfinitive host of *S. japonicum*. Further research is needed to clarify their role.

A single *S. japonicum* female can release approximately 3,500 eggs per day [30]. In freshwater, eggs hatch into miracidia, which then seek the intermediate host [3], *Oncomelania* snails. *Oncomelania* can release approximately 15 to 160 cercariae per day. These cercariae then seek and infect definitive hosts [7, 31]. The transmission and spread of schistosomiasis japonica lead to genetic diversity in the parasite. Various tools, such as population genetic analyses and sequencing technologies, can be used to predict these diversities.

Molecular markers, such as the ND1, ND4, and COX3 genes, as well as those within the mitochondria, are available to assess *S. japonicum* genetic diversity. For example, Zhao *et al.* used the ND1 and the ND4 genes in a study that suggested the Taiwanese *S. japonicum* population was genetically distinct and completely diverged from the others [9, 32]. Endemic populations of *S. japonicum* in China, Indonesia, and the Philippines show different levels of diversity, and the overall genetic diversity of the species is high [9, 32, 33].

Several bioinformatic tools can be used to align DNA sequences and reconstruct phylogenetic relationships, including ClustalW, NCBI BLAST and ETE. For example, NCBI BLAST compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches [34]. The E value from BLAST, which is an expectation value, can be used to infer homology. An E value < 0.01 or E < 0.05 suggests a statiscally significant match. Although a low E value and a low P value can be roughly equivalent when the E value is very small, they are distinct statistical concepts [35]. BLAST can also be used to infer functional and evolutionary relationships and help identify members of a gene family [34]. The ETE toolkit can provide phylogenetic outputs from inference methods such as PhyML and RaxML.

This study examines phylogeographic patterns of *S. japonicum* across East Asia and Southeast Asia, considering its evolutionary relationships with *S. sinensium*, using 84 mitochondrial sequences.

2. Materials and methods

Sources for this research included academic platforms such as NCBI, PubMed, ScienceDirect, UC Berkeley, UNESCO, and WHO platforms. All content from the NCBI, UC Berkeley, UNESCO, and WHO platforms was open access, with rights for free reuse, though one ScienceDirect article was not.

Nucleotide NCBI BLAST was performed to obtain E values and identity percentages, which were then used to construct slanted cladograms. This method allows reproducible data (nucleotide sequences)

deposited in GenBank. A threshold of E ≤ 0.05 was used to identify significant matches, corresponding to default algorithm parameters (e.g., match/mismatch scores and filter of low complexity regions) [35]. In this research, sequences were included if they represented verified closely related within *S. sinensim* and *S. japonicum* or within *S. japonicum* itself for mitochondrial genes [21]. These include the ND1 partial coding regions, ND1 complete coding regions, ND4 complete coding regions, COX3 partial coding regions, or complete mitochondrial genome. The resulting cladograms illustrated close evolutionary relationships among organisms, with the highest percentage match within each group indicating the inferred relationship. These data was used to determine the phylogenetic relationships of *S. japonicum* from the Yangtze River Basin (Yunnan, Jiangxi, and Jiangsu) and to compare with isolates from other regions, including Japan, Indonesia, Taiwan, and the Philippines. In certain case, PhyML were used to support NCBI BLAST results.

In this study, sequences were selected to reveal the evolutionary relationships of *S. sinensium* (Sichuan, China) with *S. japonicum* (China and Taiwan), using *S. indicum* (Bangladesh) as an outgroup. These sequences were also used to investigate the evolutionary relationships of *S. japonicum* from Jiangxi, China, by comparing them with isolates from other regions of China, Japan, Taiwan, the Philippines, and Indonesia. Sequences with less than 90% identity were identified as different species (*S. indicum, S. japonicum, S. malayensis, S. mekongi, S. ovuncatum*, and *S. sinensium*).

All sequence data for *S. sinensium* and *S. japonicum* were sourced from the NCBI database. A tiered BLAST approach was used, where a standard megablast search was first performed, followed by a more sensitive BLASTn search if the results were nonsignificant [9]. Pairwise dot plots and the coding sequence (CDS) feature were employed to identify sequence differences. The analysis focused on determining the number of base and amino acid variations, which provides valuable insight into the genetic diversity *S. japonicum* strains. Note that formal analyses for base substitutions and amino acid replacements were not conducted. A total of 84 mitochondrial sequences of *S. japonicum* and *S. sinensium* were included in the present study.

2.1. Nucleotide BLAST for relationship between S. sinensium *and* S. japonicum

For this nucleotide BLAST analysis, reference sequences were obtained from the GenBank database at the NCBI. Sequences of the ND1 gene (including partial and CDS) and other mitochondrial DNA were used to investigate the relationships between *S. sinensium* and *S. japonicum* populations within the Yangtze River Basin, China. The accession numbers for the sequences are provided in Table S1.

The relatioonships between *S. sinensium* and *S. japonicum* were analyzed using nucleotide BLAST. The resulting percentage identities, E values, and slanted cladograms were used to compare sequences from provincies within the Yangtze River Basin (Yunnan, Jiangxi, and Jiangsu) and Taiwan. These data were analyzed to identify the *S. japonicum* lineage most closely related to *S. sinensium.* The branches of the slanted cladogram illustrate the rlationships among *S. japonicum* poulations and their infection areas. *S.*

indicum was used as an outgroup to root the phylogenetic tree and infer evoultonary relationships. A total of 84 mitochondrial sequences of *S. japonicum* and *S. sinensium* were ultimately selected from GenBank and used for subsequent phylogenetic analyses.

2.2. Relationships between the ND1 of S. japonicum

For nucleotide BLAST analysis of *S. japonicum*, reference sequences were obtained from the GenBank database (NCBI). Specifically, the complete mitochondrial ND1 CDS was retrieved. The GenBank data were sourced from the study by Yin et al. (2015) [32], and the accession numbers are detailed in Table S2.

Nucleotide BLAST results were used to determine the phylogenetic relationships of *S. japonicum* in the study regions. These results provided percentage identity, E value, and a slanted cladogram to visualize relationships among the isolates.

2.3. Relationships between the ND4 gene of S. japonicum

For nucleotide BLAST analysis, the complete mitochondrial ND4 CDS of *S. japonicum* was obtained as a reference from GenBank (NCBI). The sequence data were sourced from the study by Yin et al. (2015) [32] and included sequences from China, Japan, Indonesia, the Philippines, and Taiwan. The accession numbers for the sequences used in this analysis are listed in Table S3.

The percentage identity, E values, and a slanted cladogram derived from nucleotide BLAST were used to analyzed the phylogenetic relationships and infer the origin of *S. japonicum* populations in China, Japan, Indonesia, the Philippines, and Taiwan.

2.4. Relationships between the COX3 gene and mitochondria in S. japonicum

For nucleotide BLAST analysis, reference sequences were accessed from the GenBank database at the NCBI. This dataset included both the partial COX3 CDS and complete mitochondrial genomes from various regions. Data were sourced from China, Indonesia, Japan, Taiwan and the Philippines. The accession numbers for all sequences used are detailed in Table S4. .

The phylogenetic relationships of *S. japonicum* populations from China, Japan, Indonesia, Taiwan, and the Philippines were analyzed using nucleotide BLAST. Percentage identities and E values were obtained, and the resulting data were used to construct a slanted cladogram that visualizes the relationships between parasites from these regions. ClustalW and RaxML were used to obtain cladogram to be compared to cladogram of NCBI BLAST.

2.5. Relationships among the complete mitochondrial genome of S. japonicum

Reference sequences for the complete mitochondrial genome of *S.* japonicum were obtained from the GenBank database at NCBI for nucleotide BLAST analysis. These data were originally published by Yin *et al.* (2015) [23] and include sequences from China, Japan, Indonesia, the Philippines, and Taiwan. The accession numbers for the sequences used in this study are provided in Table 5.

Nucleotide BLAST results from the complete mitochondrial genome were used to determine the percentage identity, E value, and phylogenetic relationships of *S. japonicum* populations across China, Japan, Indonesia, the Philippines, and Taiwan. The branches of the resulting cladogram illustrate the relationships among parasite populations in these regions.

3. Results

3.1 Sequence analysis of S. sinensium *and* S. japonicum

Phylogenetic analyses were performed using 84 mitochondrial sequences of *S. japonicum* and *S.* sinensium across East and Southeast Asia. A BLASTn of the ND1 gene revealed that Sichuan *S. sinensium* shares 73.23% identity with Taiwan *S. japonicum*. Expanding on this, more sequences revealed that Taiwan *S. japonicum* shares 96.87% identity with *S. japonicum* fromYunnan, which represents the highest percentage found in mainland China. The analysis further detailed the close relationships between mainland China strains: Yunnan *S. japonicum* shares 99.87% identity with the Sichuan strain, which, in turn, shares 99.61% identity with the Jiangxi and Anhui Guichi strains and 99.35% identity with the Hunan Changde strain. In contrast, Sichuan *S. sinensium* displayed lower identity percentages with other strains: 72.29% with Anhui Guichi and Jiangxi *S. japonicum*, 72.04% with Anhui Tongling *S. japonicum*, and 71.79% with strains from Jiangsu, Sichuan, and Yunnan. The identity values for Hubei, Hunan Yueyang, and Hunan Changde *S. japonicum* were 71.54%, 71.46%, and 71.28%, respectively, while the identity with Bangladesh *S. indicum* was 75.00% (Table 1, Fig. 1, and Figure S1). In addition, Sichuan *S. sinensium* exhibited 106 base substitutions and 54 amino acid replacements compared to Jiangxi and Anhui Guichi *S. japonicum*, and 107 base substitutions and 54 amino acid replacements compared to Anhui Tongling *S. japonicum*.

Table 1
Accession, E-value and percentage of Schistosoma sinensium and

***Schistosoma japonicum* relationships for the ND1 gene**			
Species	**Accession**	**E-value**	**S. sinensium**
			AF465913.1
S. japonicum	FJ852203.1	3e-53	72.29%
S. japonicum	KP793743.1	3e-53	72.29%
S. japonicum	FJ852232.1	4e-52	72.04%
S. japonicum	FJ852250.1	2e-50	71.79%
S. japonicum	FJ852220.1	2e-50	71.79%
S. japonicum	FJ852216.1	2e-50	71.79%
S. japonicum	FJ852248.1	2e-49	71.54%
S. japonicum	KP793758.1	4e-51	71.46%
S. japonicum	FJ852245.1	8e-48	71.28%
S. japonicum	KP793776.1	6e-62	73.23%
S. indicum	NC_047240	2e-16	75.00%
S.: Schistosoma			

The cladogram (Fig. 1) illustrates three main branches. The first branch, positioned separately from the others, includes *S. indicum* from Bangladesh, *S. sinensium* from Sichuan, *S. japonicum* from Taiwan, and *S. japonicum* from Hunan Yueyang. A second branch comprises *S. japonicum* from Anhui Guichi, Jiangxi, Anhui Tongling, Jiangsu, Hubei, and Hunan Changde. The third branch is composed of *S. japonicum* from Yunnan and Sichuan.

3.2 Sequence analysis of the ND1 gene in S. japonicum

Megablast sequencing results for the mitochondrial ND1 gene (complete CDS) revealed a 100% identity between Jiangxi *S. japonicum* and Hunan Changde *S. japonicum*. A 99.89% identity was observed with *S. japonicum* strains from Anhui Guichi, Anhui Tongling, Hunan Yueyang, Indonesia, the Philippines, and Yunnan. A comparison also showed that Jiangxi *S. japonicum* shares 99.78% identity with Indonesian and Sichuan *S. japonicum*, a 99.66% identity with Japanese *S. japonicum*, a 99.55% identity with Hunan Yueyang *S. japonicum*, a 99.44% identity with Hubei *S. japonicum*, a 99.33% identity with another Indonesian *S. japonicum*, and a 96.75% identity with Taiwan *S. japonicum*. All resulting E values were 0.0 (Table 2 and Fig. 2).

Table 2
Accession, E value and percentage of Schistosoma japonicum

relationships for the ND1 gene, complete CDS, mitochondrial			
Species	**Accession**	**E-value**	**S. japonicum**
			KP793763.1
S. japonicum	KP793756.1	0.0	100%
S. japonicum	KP793743.1	0.0	99.89%
S. japonicum	KP793747.1	0.0	99.89%
S. japonicum	KP793759.1	0.0	99.89%
S. japonicum	KP793779.1	0.0	99.89%
S. japonicum	KP793769.1	0.0	99.89%
S. japonicum	KP793783.1	0.0	99.89%
S. japonicum	KP793777.1	0.0	99.78%
S. japonicum	KP793765.1	0.0	99.78%
S. japonicum	KP793780.1	0.0	99.66%
S. japonicum	KP793758.1	0.0	99.55%
S. japonicum	KP793749.1	0.0	99.44%
S. japonicum	KP793778.1	0.0	99.33%
S. japonicum	KP793776.1	0.0	96.75%
S. indicum	NC_047240.1	4e-50	66.06%
S.: Schistosoma			

The cladogram (Fig. 2) shows that Yunnan and Taiwan *S. japonicum* form a separate branch. Strains from Anhui Tongling and Sichuan are clustered together, as are *S. japonicum* from Japan, Hunan, and Hubei. A comparison between Japanese *S. japonicum* and Hunan Yueyang *S. japonicum* showed 99.66% identity, with different three base substitutions but no amino acid replacements. When compared with Hubei *S. japonicum*, it showed a 99.55% identity, with four base substituions and one amino acid replacement. In addition, Indonesian and Philippine *S. japonicum* each had one base substitution relative to Jiangxi *S. japonicum*.

3.3 Sequence analysis of the ND4 gene in S. japonicum

The megablast analysis for the complete mitochondrial CDS, specifically the ND4 gene, revealed that Jiangxi *S. japonicum* shares 99.92% identity with *S. japonicum* from Sichuan and Yunnan. The identity

with Anhui Guichi and Hunan Changde was 99.84%, and with Anhui Tongling *S. japonicum* was 99.76%. Jiangxi *S. japonicum* also showed 99.69% identity with Hunan Yueyang *S. japonicum*, all Indonesian strains, and the Philippine strain. Further comparisons yielded an identity of 99.61% with Japan *S. japonicum*; 99.45% with Hunan Yueyang *S. japonicum*, 99.44% with Hubei *S. japonicum*, and 97.73% with Taiwan *S. japonicum*. All E values were 0.0 (Table 3 and Fig. 3).

Table 3
Accession, E value and percentage of Schistosoma japonicum

relationships for the ND4 gene, complete CDS, mitochondrial			
Species	**Accession**	**E-value**	**S. japonicum**
			KP793805.1
S. japonicum	KP793807.1	0.0	99.92%
S. japonicum	KP793811.1	0.0	99.92%
S. japonicum	KP793785.1	0.0	99.84%
S. japonicum	KP793798.1	0.0	99.84%
S. japonicum	KP793789.1	).0	99.76%
S. japonicum	KP793801.1	0.0	99.69%
S. japonicum	KP793819.1	0.0	99.69%
S. japonicum	KP793820.1	0.0	99.69%
S. japonicum	KP793821.1	0.0	99.69%
S. japonicum	KP793825.1	0.0	99.69%
S. japonicum	KP793822.1	0.0	99.61%
S. japonicum	KP793800.1	0.0	99.45%
S. japonicum	KP793791.1	0.0	99.37%
S. japonicum	KP793818.1	0.0	97.37%
S. indicum	NC_047240.1	9e-42	66.05%
S.: Schistosoma			

Based on the cladogram and sequence analysis (Fig. 3), Taiwanese *S. japonicum* forms a distinct, stand-alone branch. Japan *S. japonicum* is clustered with Hunan Yueyang and Hubei *S. japonicum.* Within this group, Japan *S. japonicum* shares 99.69% identity with the Hunan Yueyang strain, differing by four base substitutions and two amino acid replacements. It shares 99.61% identity with the Hubei strain, a difference that includes five base substitutions and two amino acid replacements. In addition, Hunan Changde and Hunan Yueyang form a separate, distinct branch. Anhui Tongling *S. japonicum* clusters with

the Philippine and Indonesian strains, sharing 99.76% identity with both. Compared to all Indonesian *S. japonicum*, it has three base substitutions and two amino acid replacements, while it has three base substitutions and one amino acid replacement when compared to the Philippine strain. Finally, Jiangxi *S. japonicum* is grouped with the strains from Anhui Guichi, Sichuan, and Yunnan.

3.4 Sequence analysis of the COX3 gene and complete mitochondrial genome in S. japonicum

Megablast results for the COX3 gene, partial mitochondrial CDS, and complete mitochondrial genome revealed that Jiangxi *S. japonicum* shared 100% identity with strains from Sichuan, Anhui, and Jiangsu. It showed 99.83% identity with strains from Indonesia, Japan, the Philippines, Taiwan, Hunan, and Hubei, but only 96.65% identity with the Yunnan strain. All analyses yielded an E value of 0.0 (Table 4, Fig. 4, and Figure S1).

Table 4
Accession, E-value and percentage of Schistosoma japonicum relationships for the COX3 gene

Species	Accession	E-value	S. japonicum EU927628.1
S. japonicum	EU927627.1	0.0	100%
S. japonicum	EU927612.1	0.0	100%
S. japonicum	EU927635.1	0.0	100%
S. japonicum	JQ781214.1	0.0	99.83%
S. japonicum	JQ781215.1	0.0	99.83%
S. japonicum	KU196357.1	0.0	99.83%
S. japonicum	KF279410.1	0.0	99.83%
S. japonicum	EU927625.1	0.0	99.83%
S. japonicum	EU927619.1	0.0	99.83%
S. japonicum	EU927638.1	0.0	99.65%

S.: Schistosoma

Analysis of the slanted cladogram revealed that the Jiangsu and Yunnan *S. japonicum* sequences clustered on distinct branch. The Anhui Guichi and Taiwan strainss were grouped together, sharing 99.83% identity. The Jiangxi *S. japonicum* strain clustered with the Japan, the Philippines, and Indonesia strains. A comparison showed that while the Jiangxi strain had one base pair substitution, it exhibited no corresponding amino acid replacement when compared to other three strains.

3.5 Sequence analysis of the complete mitochondrial genome of S. japonicum

Megablast results for the complete mitochondrial genome revealed that Jiangxi *S. japonicum* had varying degrees of identity with other strains (Table 5 and Fig. 5). The highest identity was observed with Anhui Tongling(99.87%), followed by Hunan Yueyang (99.86%), Anhui Guichi, Indonesia, and the Philippines (99.82%); and Yunnan (99.81%). Lower identities were found with Sichuan (99.75%), Hubei (99.69%), Japan (99.40%), Hunan Yueyang (99.32%) and Taiwan (98.02%). All the E values were 0.0.

In the slanted cladogram, *S. japonicum* from Taiwan formed, a distinct, stand-alone branch. The Japan strain was in the same group as the Hunan Yueyang strain, differing by 40 base substitutions and six amino acid replacements. A separate group included strains from Indonesia and te Philippines, Hunan Yueyang and Anhui Guichi. The Indonesian strain shared 99.89% identity with the Anhui Guichi strain, with 16 base substitutions and three amino acid replacements. The Indonesian strain also showed 99.80% identity with the Hunan Yueyang strain. Finally, the Philippine strain shared 99.87% identity with the Anhui Guuichi strain and 99.29% identity with the Hunan Yueyang strain, with 18 base substitutions and three amino acid replacements when compared to the Anhui Guichi strain.

Table 5. Accession, E-value and percentage of *Schistosoma japonicum* relationshipps for the mitochondrial comple genome

Species	**Accession**	**E-value**	***S. japonicum***
			KU196367.1
S. japonicum	KU196313.1	0.0	99.87%
S. japonicum	KU196317.1	0.0	99.87%
S. japonicum	KU196347.1	0.0	99.86%
S. japonicum	KU196305.1	0.0	99.82%
S. japonicum	KU196307.1	0.0	99.82%
S. japonicum	KU196348.1	0.0	99.82%
S. japonicum	KU196379.1	0.0	99.82%
S. japonicum	KU196408.1	0.0	99.81%
S. japonicum	KU196389.1	0.0	99.75%
S. japonicum	KU196318.1	0.0	99.69%
S. japonicum	KU196328.1	0.0	99.69%
S. japonicum	KU196359.1	0.0	99.40%
S. japonicum	KU196338.1	0.0	99.32%
S. japonicum	KU196398.1	0.0	98.02%
S. indicum	NC_047240.1	5e-150	75.14%

S.: Schistosoma

4. Discussion

S. sinensium diverged from *S.* ovuncatum [21]. Both *S. ovuncatum* and *S. sinensium* occur across China, India, and Thailand, reflecting their broad distribution within Asian schistosome lineages [37]. Subsequently, *S. japonicum* diverged from *S. sinensium*, followed by divergence of *S. mekongi* and *S. malayensis* from *S. japonicum* [21]. *S. japonicum* represents one of five species within this lineage, alongside *S. ovuncatum, S. sinensium, S. mekongi*, and *S. malayensis* [21, 38]. Unlike its intermediate host genus *Oncomelania*, believed to originate from eastern Indonesia, *S. japonicum* spread from Chinese lake regions to East and Southeast Asia [21, 23]. Consistent phylogenetic evidence indicates that *S. japonicum* remains closely related to *S. sinensium* within the broader Asian schistosome evolutionary lineage [21, 37–38].

Previous study suggested that *S. japonicum* populations form four primary clades located in mainland Chinese, Indonesia, the Philippines, and Taiwan [39]. Taiwanese *S. japonicum* genetically distinct from other regional populations and diverged approximately forty-five thousand years ago [32, 39]. Later divergence events occurred more recently, with Philippine populations separating from Chinese lake regions approximately seven thousand five hundred years ago. Subsequently, Sichuan and Yunnan populations diverged roughly five thousand four hundred years ago from earlier mainland Chinese ancestral lineages [39]. Indonesia and Japanese *S. japonicum* populations diverged from Chinese populations approximately five thousand years ago, reflecting later regional dispersal events. Some researchers therefore proposed that Taiwanese and Southeast Asian populations could represent subspecies within the broader *S. japonicum* lineage [39]. Notably, Taiwanese *S. japonicum* exhibits zoophilic behavior but lacks zoonotic transmission potential, distinguishing it from other regional populations [40].

Sequence analyses conducted in this study indicate that *S. japonicum* diverged from *S. sinensium* in Taiwan, followed by additional divergences events in Anhui Guichi and Jiangxi populations. Taiwanese *S. japonicum* exhibits greater genetically similarity to Yunnan strains than to several other mainland Chinese populations examined here. The Taiwanese strain shares 96.87% sequence identity with Yunnan *S. japonicum*, suggesting potential historical evolutionary connections between these regions. Yunnan strains display extremely high identity, approximately 99.87%, with *S. japonicum* populations from Sichuan, Anhui Guichi, and Jiangxi lake regions. High identity values between Yunnan and lake-region strains suggest eastward parasite dispersal from southwestern China toward central and eastern regions. This pattern agrees with Young *et al.* (2015) [22], who reported eastward spread forming central and eastern Chinese population group [21, 22]. *S. sinensium* shares approximately 72% identity with Anhui Guichi and Jiangxi *S. japonicum* populations examined in this study. Approximately 73% identity was also observed between *S. sinensium* and Taiwanese *S. japonicum*, supporting their deeper evolutionary divergence (Table 1, Fig. 1, and Figure S1). Previous research suggested that *S. japonicum* spread from Anhui Guichi and Jiangxi lake regions toward mainland China, Japan, Indonesia, and the Philippines [23].

Sequence comparisons indicate that Sichuan *S. sinensium* shows closer genetic affinity to Anhui Guichi and Jiangxi *S. japonicum* than to Anhui Tongling populations. Sichuan *S. japonicum* displays 54 amino acid replacements relative to Anhui Guichi, Anhui Tongling, and Jiangxi populations. These patterns suggest strong genetic relatedness among Sichuan, Anhui Guichi, and Jiangxi *S. japonicum* strains. Evidence further indicates that Anhui Guichi and Jiangxi populations subsequently dispersed toward Anhui Tongling, Hubei, Jiangsu, Hunan, Yunnan, and Sichuan.

The present findings also suggest increased prevalence of *S. japonicum* specifically within Anhui Guichi and Jiagxi lake regions. Earlier research did not clearly identify this geographic concentration of parasite populations within mainland China [23, 39]. Phylogenetic evidence further suggests that Taiwanese *S. japonicum* populations represent older lineages relative to mainland Chinese populations. These results

also support earlier conclusions that divergence of *S. japonicum* from *S. sinensium* occurred relatively early in Asian schistosome evolution [12, 37].

ND1 gene sequences reveal complete identity between Jiangxi and Hunan Changde *S. japonicum* populations examined in this study (Table 2). Other mainland Chinese, Japanese, Indonesian, and Philippine populations show slightly lower identities ranging from 99.55% to 99.89%. These findings indicate that Southeast Asian and Japanese populations retain extremely close genetic relationships with mainland Chinese *S. japonicum*. Hunan Changde and Jiangxi strains share approximately 97% identity with Taiwanese *S. japonicum*, representing the lowest similarity among Asian strains examined.

The ND1 cladogram further shows that Taiwanese and Yunnan *S. japonicum* form a distinct phylogenetic branch (Fig. 2). Anhui Tongling and Sichuan populations cluster within the same group, indicating strong genetic similarity between these regional populations. Japanese *S. japonicum* clusters with Hunan Yueyang and Hubei strains within the same phylogentic group. Japanese and Hunan Yueyang sequences display no amino acid replacements, whereas comparisons with Hubei reveal a single replacement. These findings suggest that Japanese *S. japonicum* likely originated from populations related to Hunan Yueyang lineage.

All Indonesian and Philippine *S. japonicum* strains cluster together within the same phylogenetic group based on the ND1 gene sequences (Table 2 and Fig. 2). Indonesian populations share extermely high identity values between 99.44% and 99.89% with Jiangxi strains from mainland China. These results indicate that Southeast Asian populations likely originated from either Hunan Changde or Jiangxi ancestrl lineages. Sequence alignments show only one base substitution and amino acid replacements relative to Hunan Changde or Jiangxi strains. Previous reports suggested that Philippine lineages diverged first from Chinese lake populations before iving rise to Indonesian and Japanese lineages [39].

ND4 gene analyses indicate relatively low genetic divergence among mainland Chinese *S. japonicum* populations examined in this study. Jiangxi populations share greater than 99% identity with most mainland Chinese, Japanese, Indonesian, and Philippine strains (Table 3). These findings contrast with previous reports suggesting higher genetic divergence among Chinese *S. japonicum* populations [32, 39]. Taiwanese populations show approximately 98% identity with Jinagxi populations, confirming deeper divergence from mainland Chinese strains.

ND4 cladogram analyses further indicate that Japanese *S. japonicum* populations likely derived from Hunan Yueyang populations in central China. Japanese strains display similar amino acid replacements to those observed in Hunan Yueyang and Hubei populations (Fig. 3). However, Japanese strains show fewer base substitutions relative to Hunan Yueyang than to Hubei strains. These results further support a potential Hunan Yueyang origin for Japanese *S. japonicum* populations.

Anhui Tongling populations share approximately 99.76% identity with Southeast Asian strains from Indonesia and the Philippines. Philippines strains shows one amino acid replacement relative to Anhui Tongling, whereas Indonesia strains exhibit two replacements. These findings suggests that Southeast

Asian populations likely originated from Anhui Tongling ancestral lineages in mainland China. Identity values further suggest that Philippine populations diverged first, followed by Indonesian and Japanese populations.

Analyses of COX3 gene sequences and complete mitochondrial genomes further clarify phylogenetic relationships among regional populations. Taiwanese populations shares 99.83% identity with Anhui Guichi and Jiangxi strains (Table 4, Fig. 4, and Figure S1). These findings suggest that Taiwanese populations may have originated from mainland Chinese populations. Jiangxi, Japanese, Indonesian, and Philippine populations cluster within the same phylogenetic group in mitochondrial analyses. Extremely high identity values and minimal amino acid replacements indicate recent divergence among these regional populations.

Complete mitochondrial genome analyses further support relationships observed in gene-specific phylogenetic reconstructions. Japanese populations cluster closely with Hunan Yueyang strains, supporting previous interpretations of their evolutionary origin (Fig. 5). Indonesian populations cluster with Hunan Yueyang, Anhui Guichi, and Philippine populations in the same phylogenetic group. Lower substitution numbers relative to Anhui Guichi strains suggest ancestral relationships linking Southeast Asian populations with Chinese lake-region lineages.

Mitochondrial analyses overall indicate that Japanese populations likely originated from central Chinese provinces including Hunan and Hubei. Southeast Asian populations were compared with strains from Anhui Guichi, Anhui Tongling, Hunan Changde, and Jiangxi. Results demonstrate extremely close genetic relationships among Chinese, Japanese, and Southeast Asian populations. However, Taiwanese populations form a distinct lineage that diverged earlier from other regional populations.

Genetic studies also reveal that several Indonesian ethnic groups possess ancestral connections with populations from China or Taiwan [41]. These migrations occurred approximately three to four thousand years ago and may have facilitated parasite dispersal [23]. Archaeological evidence from megalithic cultural traditions in Central Sulawesi further supports ancient migration patterns within this region [41]. These patterns suggest that *S. japonicum* in Lore Lindu National Park may have been introduced from southern China approximately four thousand years ago [23].

Analyses of mitochondrial genome sequences, including ND1, ND4, COX3, and complete mitochondrial genomes, indicate that Japanase *S.* japonicum likely originated from Hunan and Hubei provinces. Comparative analyses of Southeast Asia and Chinese isolates further demonstrate close genetic relationships, with the exception of the distinct Taiwanese lineage. These findings provide new insights into the phylogeographic structure and historical patterns of *S. japonicum* across East and Southeast Asia. Although this study analyzed 84 mitochondrial sequences using robust phylogenetic approaches, the absence of nuclear genetic markers may limit resolution of deeper evolutionary relationships. Overall, the results contribute to a better understanding of parasite evolution and regional transmission dynamics.

5. Conclusions

Studies on *Schistosoma* species reveal several key genetic relationships and dispersal patterns. The Sichuan *S. sinensium* lineage was the first to diverge into what is the Taiwanese *S. japonicum*. The Taiwanese population itself is a mix of the original strain and lineage from mainland China. In mainland China, the Yunnan *S. japonicum* population diversified, giving rise to the Sichuan, Anhui Guichi, and Jiangxi strains, which subsequently spread through the Far East, including Japan, the Philippines, Indonesia, and Taiwan. Specifically the Japanese *S. japonicum* strain originated from the Anhui Guichi, Hunan Yueyang, and Jiangxi. Similarly, the Southeast Asian *S. japonicum* strains are descendants of Chinese populations from Anhui Tongling, Anhui Guichi, Hunan Changde, and Jiangxi. Overall, the genetic evidence confirms a close relations between *S. japonicum* strains found in Japan and Southeast Asia and those in China.

Declarations

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Author contributions: Conceptualization, M.N; methodology, M.N; validation, M.N.; formal analysis, M.N.; investigation, M.N.; data curation, M.N.; writing—original draft preparation, M.N.; writing—review and editing, M.N.; visualization, M.N.; supervision, M.N.

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Data availability: All the data generated or analyzed durig this study are included in this published article [and its supplementary information file].

Conflict of interest: The author declares no conflict of interest.

Informed consent statement: Not applicable.

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Figures

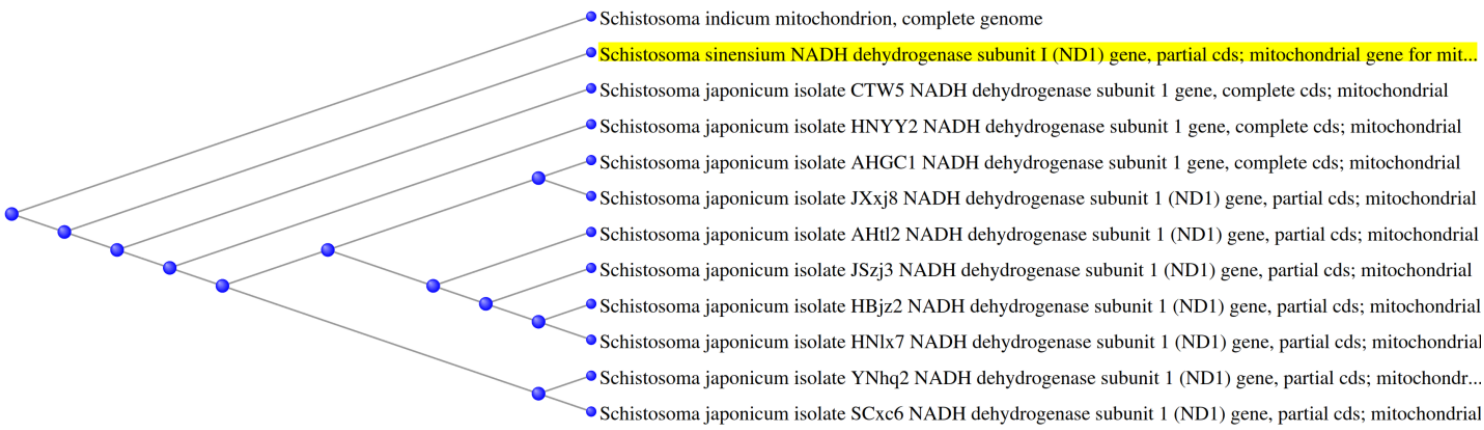


Figure 1

Nodes 21 (0 selected) and view port at (0,0) of 948x336. Cladogram illustrating phylogenetic relationships between *S. japonicum* and *S. sinensium*using ND1 gene and the complete mitochondrial genome data(NCBI). Note the distinct branching of *S. indicum* and the Taiwan (CTW%) lineage. The cladogram shows genetic divergence within *S. japonicum*, with the Taiwan (73.23%), Jiangxi (72.29%), and Anhui Guichi (72.29%) isolates forming distinct clades. The yellow highlighting part in the figure body is Query Sequence and others are Subject Sequence.

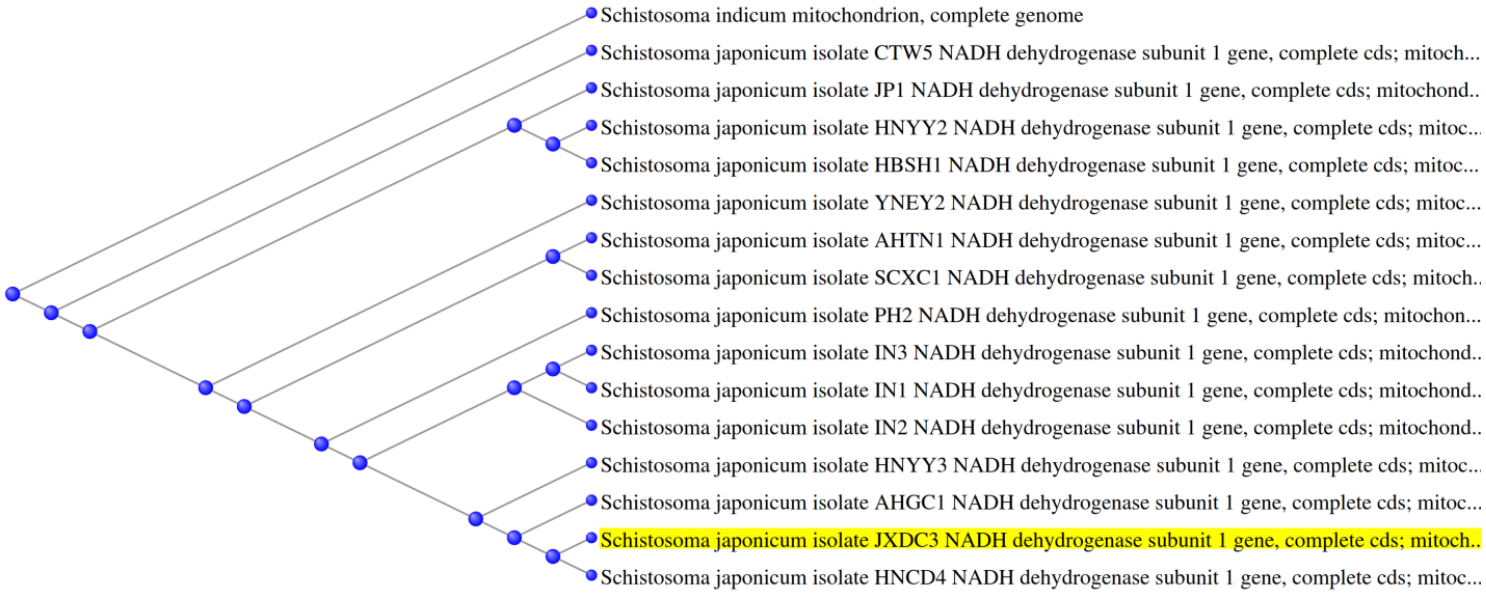


Figure 2

Nodes 31 (0 selected) and view port at (0,0) of 948x336. Tree view of *Schistosoma japonicum* relationships for the ND1 gene, complete CDS; mitochondrial (taken from NCBI). For example, Yunnan *Schistosoma japonicum* (YNEY2) forms a stand-alone branch. The yellow highlighting part in the figure body is Query Sequence and others are Subject Sequence.

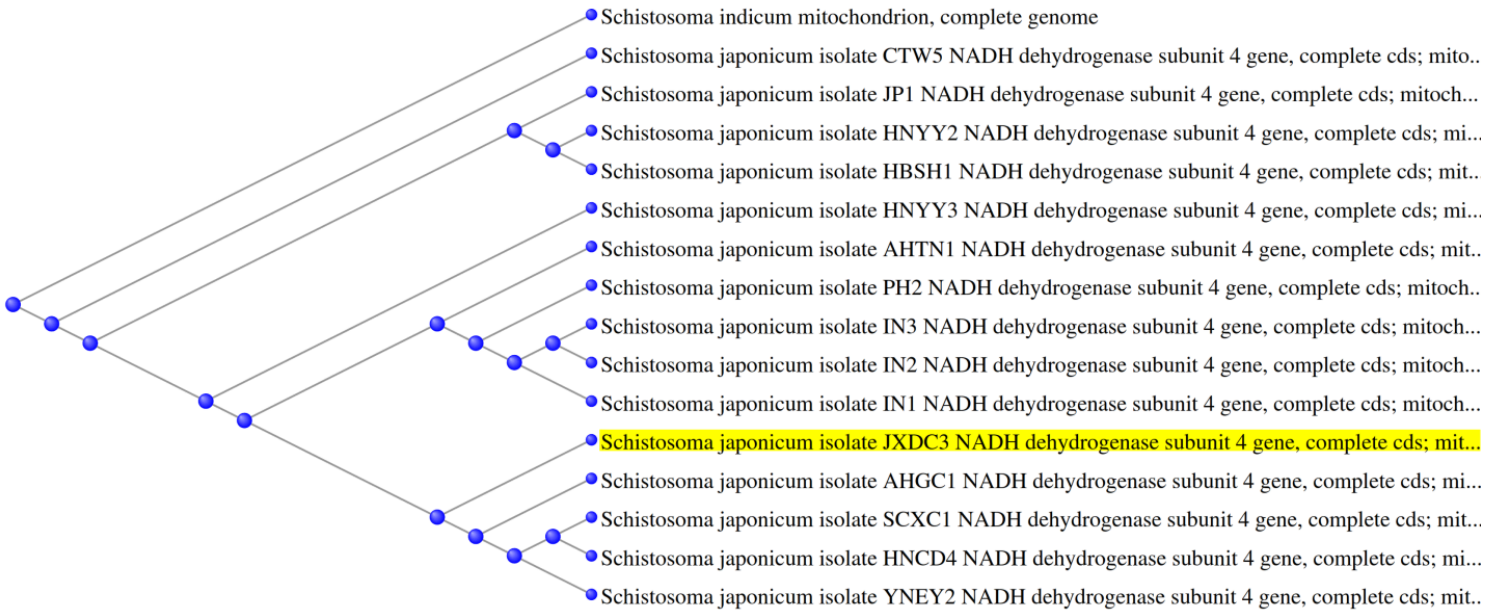


Figure 3

Nodes 31 (0 selected) and view port at (0,0) of 948x336. Tree-view of *Schistosoma japonicum* relationships for the ND4 gene, complete CDS, and mitochondria. For example, Japan (JP1), Hunan Yueyang (HNYY2) and Hubei (HBSH1) are within the same group. The yellow highlighting part in the figure body is Query Sequence and others are Subject Sequence

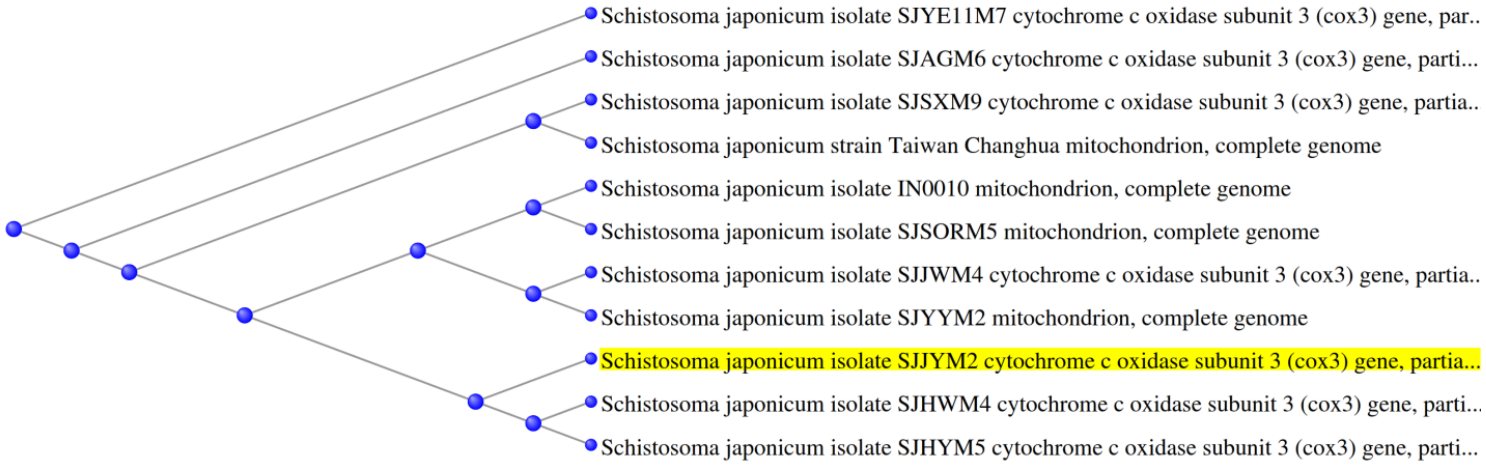


Figure 4

Nodes 21 (0 selected) and view port at (0,0) of 948x336. Tree view of *Schistosoma japonicum* relationships for the COX3gene mitochondrion, complete genome. For example, Sichuan *Schistosoma japonicum* (SJSXM9) and Taiwan Changhua are within the same group. The yellow highlighting part in the figure body is Query Sequence and others are Subject Sequence.

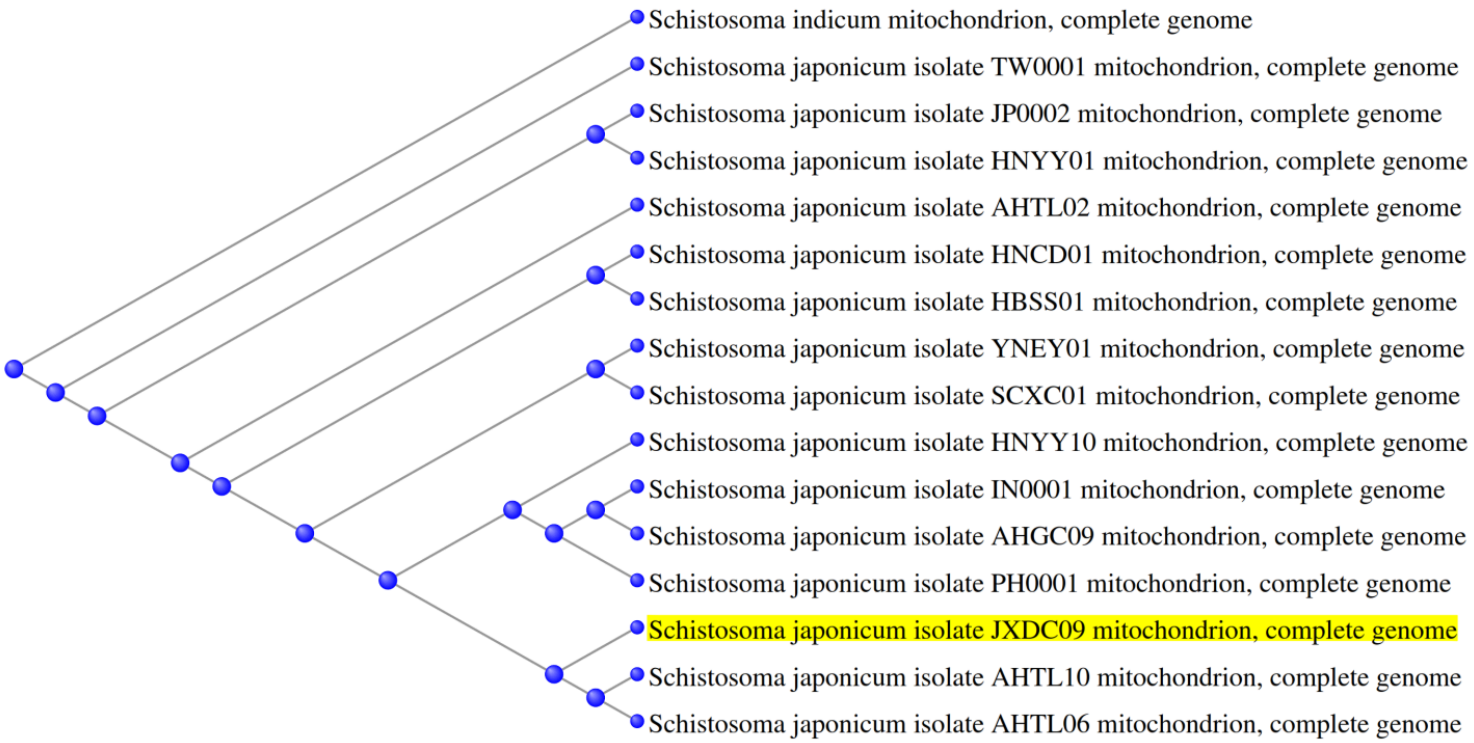


Figure 5

Nodes (0 selected) and view at (0,0) of 948x336.. Tree view of the complete mitochondrial genome of *Schistosoma japonicum*. For example, Taiwan TW0001 (KU196398.1) forms a stand-alone branch, and JP0002 (KU196359.1) and HNYY01 (KU196338.1) are within the same group. HNCD01 (KU196328.1), HBSS01 (KU196318.1), YNEY01 (KU196408.1), SCXC01 (KU196389.1), HNYY10 (KU196347.1), IN0001 (KU196348.1), AHGC09 (KU196307.1), AHGC07 (KU196305.1), PH0001 (KU196379.1), JXDC09 (KU196367.1), AHTL10 (KU196317.1), and AHTL06 (KU196313.1). The yellow highlighting part in the figure body is Query Sequence and others are Subject Sequence.

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