Indonesia Schistosoma japonicum: Origin, genus oncomelania, and elimination of the parasite with cluster genes inoculated into female Oncomelania lorelindoensis via CRISPR/Cas9 system

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Abstract

Introduction: In this study, I report the progress of Schistosomiasis japonica that focused on dispersion of Schistosoma japonicum, genus Oncomelania, and use of genetic manipulations on Oncomelania lorelindoensis for eliminating schistosomiasis japonica in Central Sulawesi as objectives of this study. Results: Results of Nucleotide BLAST showed that Sulawesi’s S. japonicum originated from Japan and China. Results also showed that Southeast Asian Oncomelania is closer to O. hupensis robertsoni (Oncomelania robertsoni) than to O. hupensis or O. minima. Elimination of S. japonicum parasite in O. lorelindoensis can occur in the field using anti-Schistosoma inoculated into female ovary O. lorelindoensis with CRISPR/Cas9 system. The progeny of transgenic snails in each generation can be calculated by using the mathematical ideas. Mathematical ideas include total $F_1=3^n$, $F_i=([3^n-1]:1)$, total $F_j$ is $F_{n+1}=6^n$, and $F_{n+1}=([6^n-1]:1)$. Conclusion: Nucleotide BLAST results showed that ancestors of Sulawesi’s S. japonicum originated from Japan and China. Oncomelania lorelindoensis is the intermediate host of Sulawesi’s S. japonicum. Any transgenic snail crossed with wild-type O. lorelindoensis can result in S. japonicum-resistant snails.

Keywords: CRISPR/Cas9, Oncomelania, Oncomelania lorelindoensis, Schistosoma japonicum, Schistosomiasis

1. Introduction

Blood flukes of the genus Schistosoma cause schistosomiasis infection. Genus Schistosoma that can infect humans include several species. These include Schistosoma haematobium, S. intercalatum, Schistosoma japonicum, S. malayensis, S. mansoni, and S. mekongi (Butrous, 2019; El Ridi et al., 2012; and Neves et al., 2015). Schistosoma infects about 220 to 230 million people (Ferrari et al., 2021; Nelwan, 2019; Sanches et al., 2021; and Zhou et al., 2020). Schistosomiasis causes 24,072 to 200,000 deaths annually (World Health Organization, 2021), and 779 million people are at risk. In addition, schistosomiasis causes a worldwide burden of 3.3 million disability-adjusted life years. Schistosomiasis occurs in Africa, Asia, South America, and several Caribbean islands. It
can also occur in non-developing countries. Schistosomiasis can spread through water-based development projects, immigration (Nelwan, 2019), and travelling.

Genome sequences on phylogenic showed that S. japonicum originated from China. S. japonicum originated from the Middle and Lower (ML) reaches of the Yangtze River. From the Yangtze River, S. japonicum spread out to Japan, the mountainous areas of China, and then to the Philippines and Indonesia (Yin et al., 2015). S. japonicum has at least 46 species of mammalian definitive hosts (Gordon et al., 2019). Mammalian definitive hosts can include human, mice and wild pigs. In Indonesia, endemic areas of S. japonicum are in the Bada Valley, the Lindu Valley, and the Napu Valley of Central Sulawesi (Budiono et al., 2019; Nelwan, 2019; and Nurwidayati et al., 2018).

Nucleotide BLAST can provide evidences for the spread of S. japonicum and the genus Oncomelania. For example, I found that identity percentages of S. japonicum parasites in Yangtze River (i.e., KU 196321.1) against the Philippines (KU 196379.1), and Indonesia (KU 196348.1) were 99.61% and 99.63%. It suggested that S. japonicum first reached Indonesia, then the Philippines.

The genus Oncomelania is the intermediate host of S. japonicum. It consists of Oncomelania hupensis, O. hupensis lindoensis (O. lori lindoensis), O. hupensis quadra (O. quadra), and O. minima. O. ncomelania hupensis consisted of O. hupensis chuii, O. hupensis formosana, O. hupensis hupensis, O. hupensis nosophora, O. hupensis robertsoni (O. robertsoni), and O. hupensis tangi. The East to West hypothesis suggests that precursors of O. ncomelania originated from Australia (Attwood et al., 2015) and Borneo-Philippine Island (Liu et al., 2014) spread to Japan. After reaching Japan, it gave rise to O. hupensis in China. O. ncomelania hupensis re-colonized Japan, the Philippines and Sulawesi to replace antecedent form (Attwood et al., 2015). There are two species of the genus O. ncomelania: O. ncomelania hupensis and O. minima. O. ncomelania hupensis consists of five subspecies. O. ncomelania minima (Kameda and Kato, 2011) does not have any subspecies.

Nucleotide BLAST of ML Yangtze’s O. hupensis hupensis (KR002674.1) had identity percentages of 93.97% with Japan’s O. ncomelania (KR002673.1) and 84.40% with the Philippines’ O. ncomelania (DQ112287.1). In addition, the sequence results showed that the Philippines’ O. ncomelania was closer to West China’s O. robertsoni (87.25%; KR002675.1) than to China’s O. hupensis (84.48%; GU367391.1). This suggests that the taxonomy of the genus O. ncomelania should be reconsidered.

Praziquantel is the only effective drug for treating all Schistosoma species (El-Nour and Fadladdin, 2021; and Nelwan, 2019). It has been available in the market since 1988. Praziquantel only kills adult worms, and cannot kill schistosomula and juvenile worms. In addition, treatment of schistosomiasis with only one drug for more than thirty years can result in resistant to that drug (El-Nour and Fadladdin, 2021; and Tekwu et al., 2017). It seems that it is important to find a new method for controlling schistosomiasis. To anticipate resistance to praziquantel, genetic manipulation techniques can be used. Genetic manipulation techniques can include such as the adeno-associated virus (AAV) and clustered regularly interspaced short palindromic repeats (CRISPR/ Cas9) system (Nelwan, 2021a). The use of CRISPR/ Cas9 system and AAV vectors has become common in genetic manipulations (Nelwan, 2020; and Nelwan, 2021b). The CRISPR/ Cas9 can deactivate the gene for omega-1 ribonuclease S. mansoni and create parasites not produce omega-1 ribonuclease, or very little of it (Ittiprasert et al., 2019; and McViegh and Maule, 2019). It suggests that CRISPR/ Cas9 can also deactivate omega-1 ribonuclease S. japonicum. Wu et al. suggested that S. japonicum eggs produce omega-1 ribonuclease (Wu et al., 2014). Moreover, genes drive for controlling schistosomiasis can be used. For example, anti-Schistosoma such as fibrinogen-related proteins, thioster-containing proteins (Maer et al., 2019), or cluster of polymorphic transmembrane genes (Tennessee et al., 2020) can be inoculated into the ovaries of female O. lori lindoensis to produce schistosomiasis-resistant snails. As a result, snails will not produce parasites that can infect definite hosts such as humans and mice. In addition, this technique will not kill intermediate host. To find out the number of transgenic snails after being released in the field, mathematical ideas can be used. For example, \( F_{n+1} = (F_n – 1) : 1 \) is useful to find out the number of schistosomiasis-resistant snails and susceptible snails in the field. With this mathematical idea, I found that \( F_n \) progeny had a ratio of five schistosomiasis-resistant snails to one susceptible snail, for example.
2. Materials and methods

I used the nucleotide BLAST and systems of mating approaches in this study. I got a study guide from the University of California Berkeley (UC Berkeley Library) at https://guides.lib.berkeley.edu/ncbi. I used nucleotide BLAST for two or more alignments to have such as accession numbers, identity percentage, and query cover percentage. For query cover, if the first BLAST showed insignificant, I would do BLAST for somewhat similar sequences (blastn). I do not show E-values and query covers in tables. Data from nucleotide sequences results were used to draw tree view slanted cladogram of *S. japonicum*, and the genus *Oncomelania* in evolution. In addition, nucleotide BLAST was used to determine the distribution of *S. japonicum* from China and Japan to Southeast Asia, and the origin of the genus *Oncomelania*. The nucleotide BLAST was performed with the NCBI nucleotide BLAST.

Generation of tree view slanted cladogram was done as follows: First, from the nucleotide NCBI BLAST results go to Description tab and click the Distance tree of the results links. Second, when the rectangle cladogram displays: go to the menu Tools > Layout and select Slanted Cladogram.

The CRISPR/Cas9 system and systems of mating were used to find progeny that resistant to *S. japonicum*. The CRISPR/Cas9 and anti-*Schistosoma* was inoculated into female *O. lorelindoensis*. Systems of mating were intended to predict total progeny in each generation after transgenic snails are introduced in the field.

2.1. Nucleotide BLAST on *S. japonicum*

For the nucleotide BLAST approach in *S. japonicum*, I accessed the reference sequence from GenBank at the National Center for Biotechnology Information (NCBI). These include the accession numbers:

- KU196306.1 (China)
- KU196408.1 (West China)
- KU196358.1 (Japan)
- KU196377.1 (China)
- KU196299.1 (China)
- KU196362.1 (West China)
- KU196388.1 (China)
- KU196384.1 (Taiwan)
- KU196379.1 (Philippine)
- KU196348.1 (Indonesia)

Except the identity percentages, nucleotide BLAST results were also used to determine the spread of *S. japonicum* from China to such as Japan and Indonesia. Tree for relationship in evolution was also shown.

2.2. Nucleotide BLAST on the genus *Oncomelania*

For the nucleotide BLAST approach in the genus *Oncomelania*, I accessed the reference sequence from GenBank at NCBI. These included accession numbers (Attwood et al., 2015; Kameda and Kato, 2011):

- AB611791.1 (Japan)
- KR002675.1 (West China)
- GU367391.1 (China)
- KR002674.1 (China)
- KR002673.1 (Japan)
- DQ112271.1 (Taiwan)
- DQ112276.1 (China)
- DQ112282.1 (Taiwan)
- DQ112287.1 (Philippine)

Except the identity percentage, nucleotide BLAST results were also used to determine the development of the genus *Oncomelania*. Tree for relationship of evolutionary was also shown. In this study, I did not find any information regarding *O. lorelindoensis* for GenBank accessions.
2.3. Creation of transgenic O. lorelindoensis

S. japonicum can be eliminated by genetic and mathematical approaches. Guadalupe Resistance Complex (GRC = PTC1) contains resistance to alleles Biomphalaria glabrata. The PTC1 contains a dominant allele, which confers an 8-fold decrease in infectivity. Both PTC1 and PTC2 suggest a model of interaction via molecular recognition mediated by TM1 gene polymorphism. The TM1 genes include B and T-Cell receptors, Toll-like receptors, major histocompatibility complex genes, and similar host defense genes. The TM1 gene often plays a role in immunological recognition (Maier et al., 2019). Tennessen et al. suggested that polymorphic transmembrane cluster 2 PTC2TM1 genes is an obvious candidate to defense against schistosomiasis (Tennessen et al., 2020). Either PTC1TM1 or PTC2TM1 genes can be coupled to a CRISPR-mediated gene drive and spread through wild-typesnails' population to confer resistance to S. mansoni infection (Maier et al., 2019; and Tennessen et al., 2020). In this study, I used the clusters of polymorphic transmembrane genes PTC1TM1 and/or PTC2TM1 as anti-Schistosomal assuming that these genes exist in the genus Oncomelania.

Elimination of the freshwater snails is not always effective in the long-term. Use of genetic manipulations would be very helpful for the control of S. japonicum, especially through an intermediate host as the genus Oncomelania. These techniques can eliminate parasite without killing the snails. However, uses of genetic manipulations require further investigations before it can work in the field. In this study, I introduced AAV vector and CRISPR/Cas9 for creation of S. japonicum-resistant transgenic female O. lorelindoensis (Nelwan, 2021a). Although I have not had snails that are resistant to this parasite, even in the laboratory, it is likely to eliminate this parasite through genetic manipulations. Based on mathematical calculations, the elimination of schistosomiasis can be done through genetic manipulation in snails. Systems of mating can mathematically produce schistosomiasis-resistant snails.

The three fundamental requirements in editing with CRISPR/Cas9 include Cas9 endonuclease, single-guide RNA (sgRNA), and repair template DNA (donor) (Famakinde, 2018). The Cas9 homolog consists of such as Nme1Cas9 and Nme2Cas9 (Ibraheim et al., 2021). It combs through the genome of the organism and acts as molecular scissor that cuts a specific DNA sequence at a genomic locus. The sgRNA is designed to match and target the desired DNA sequence to be deleted. The donor DNA provides a template for genomic repair of the cleaved locus (Famakinde, 2018). The CRISPR/Cas9 system can tightly hold the anti-schistosomal donor DNA. Delivery vectors such as AAV and lentivirus can be used as delivery tools in genetic manipulations. Delivery vectors are packaged into the same virion (Ibraheim et al., 2021).

For a study need, it could be designed a virion package as AAV:Nme2Cas9:sgRNA:PTC1or2TM1. Then, the package is co-injected into the blastocyst stage embryos of the 0. lorelindoensis. Site for the injection of the entire cassette is in the ovary of the female (Famakinde, 2018; and Nelwan, 2021a) O. lorelindoensis (Nelwan, 2021a). This technique will produce S. japonicum-resistant transgenic snails. If these transgenic snails are released in the field, snails can produce progeny of schistosomiasis-resistant snails in the next generations genetically and mathematically.

2.4. Systems of mating for transgenic snails in the field

Systems of mating in the Schistosoma’s intermediate hosts are not the same. The genus Bulinus and the genus Biomphalaria are hermaphrodites. The genus Bulinus is the intermediate host of S. haematobium. The genus Biomphalaria is the intermediate host of S. mansoni. As the intermediate host of S. japonicum, the genus Oncomelania has separate sexes. Therefore, progeny in each generation in the genus Bulinus/Biomphalaria should be not the same as the genus Oncomelania.

Transgenic snails in the field will follow systems of mating patterns. These systems of mating will include out-breeding and inbreeding. These are crosses between transgenic S. japonicum-resistant snails and S. japonicum-susceptibility wild-type snail. These crosses will be in O. lorelindoensis, including crosses between transgenic, hybrid, and wild type snails. Results will be progeny such as F₁, F₂, and F₃.

3. Results

3.1. Identity percentages of S. japonicum

The nucleotide BLAST results showed that China’s S. japonicum (KU196306.1) shared a similar identity of 99.89% with Indonesia’s S. japonicum (KU196348.1) and 99.87% with the Philippine’s S. japonicum (KU196379.1). The sequence of KU196408.1 shared 99.72% identity with KU196348.1 and 99.71% with KU196379.1. The
sequence of KU196377.1 shared 99.61% identity with KU196348.1 and 99.60% with KU196379.1. Sequence of KU196358.1 shared 99.34% identity with KU196348.1 and 99.33% with KU196379.1. Sequence of KU196299.1 shared 99.31% identity with KU196348.1 and 99.30% with KU196379.1. Sequence of KU196362.1 shared 99.29% identity with KU196348.1 and 99.28% with KU196379.1. Sequence of KU196398.1 shared 98.00% identity with KU196348.1 and 98.01% with KU196379.1 (Table 1 and Figure 1).

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<th>Philippine KU196379.1</th>
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</tr>
<tr>
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<td></td>
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<tr>
<td>KU196377.1</td>
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<td>99.60%</td>
</tr>
<tr>
<td>Mid-Yangtze-THP</td>
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<tr>
<td>KU196398.1</td>
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<tr>
<td>Taiwan</td>
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</table>

**Table 1: Percentage identities of Schistosoma japonicum for China, Japan, Indonesia, Philippine and Taiwan**

**Figure 1: BLAST tree view slanted cladogram of Schistosoma japonicum**

*Note:* Indonesia's Schistosoma japonicum (KU196348.1) and the Philippines's S. japonicum (KU196379.1) flank China's S. japonicum (KU196299.1). The KU196399.1 is close to KU196306.1 that also close to Japan S. japonicum (KU196358.1). Modified from NCBI BLAST pairwise alignment.
The nucleotide BLAST of KU196348.1 (Indonesia) shared 99.87% sequence identity with KU196379.1 (Philippines). All query covers were 100%. These identity percentages suggest that Indonesia’s *S. japonicum* derived from China (e.g., 99.89% and 99.72%), Japan (99.34%), or even Taiwan (98%).

### 3.2. Identity percentages of the genus *Oncomelania*

Nucleotide BLAST results showed that West China’s *O. robertsoni* (KR002675.1) shared 87.25% identity with the Philippines’s *O. quadrasi* (DQ112287.1), query cover is 93% (not shown in Table 2). Japan’s *O. hupensis*...
Table 2 (Cont.)

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<td>DQ112271.1</td>
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<td>KR002673.1</td>
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<td>Japan</td>
</tr>
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<td>KR002674.1</td>
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</tr>
<tr>
<td>DQ112287.1</td>
<td>87.25%</td>
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<table>
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<th>Accession</th>
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<td>Japan</td>
</tr>
<tr>
<td>KR002675.1</td>
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<td>West China</td>
</tr>
<tr>
<td>DQ112287.1</td>
<td>84.48%</td>
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</tr>
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</table>

Note: PLB: Poyang Lake Basin, China; THP: Mid-Yangtze to Lower Yangtze Taihu, Plain, China.

Note: Oncomelania nasophora (KR002673.1) shared 86.26% identity with DQ112287.1. Taiwan’s O. hupensis chiui (DQ112271.1) shared 85.58% identity with DQ112287.1. Taiwan’s O. hupensis formosana (DQ112282.1) shared 84.95% identity with DQ112287.1. China’s O. hupensis hupensis (KR002674.1) shared 84.40% identity with DQ112287.1 (Table 2). Finally, DQ112287.1 shared 82.13% identity with Japan’s O. minima (AB611791.1) (Table 2 and Figure 2).

Figure 2: BLAST tree view slanted cladogram of the genus Oncomelania

Note: Southeast Asia’s O. nesomelania was close to O. robertsoni. Modified from NCBI BLAST pairwise alignment.
I did not find O. lorelindoensis in GenBank for accession. As a result, the nucleotide sequence could not be done.

The nucleotide sequence results showed that O. hupensis developed O. hupensis hupensis, O. hupensis tangi, O. hupensis formosana, O. hupensis nosophora, and O. hupensis chiui. In addition, sequence results showed that O. quadrasi was closer to O. robertsoni than to O. hupensis (Table 3).

<table>
<thead>
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<th>Table 3: The genus Oncomelania</th>
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<tr>
<td>Species</td>
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<td>O. h. hupensis</td>
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<tr>
<td>O. h. tangi</td>
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<td>O. h. formosana</td>
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<td>O. h. chiui</td>
</tr>
<tr>
<td>O. h. nosophora</td>
</tr>
<tr>
<td>O. quadrasi</td>
</tr>
</tbody>
</table>

3.3. Genetic biocontrol of Schistosomiasis japonica

The creation of transgenic female O. lorelindoensis resulted in transgenic female O. lorelindoensis (Figure 3). Then, transgenic female O. lorelindoensis snails were released into the field containing schistosomiasis-
susceptible *O. lorelindoensis* snails. Crossing results between transgenic snails and schistosomiasis-susceptible wild-type snails resulted in as described in Figure 4. For example, transgenic snails crossed with schistosomiasis-

**Figure 4: Transgenic snails in the field**

**Note:** Transgenic snails for field control of schistosomiasis japonica transmission. Transgenic snails released in the field resulted in a ratio of two schistosomiasis-resistant snails to one susceptible snail in $F_1$, a ratio of five schistosomiasis-resistant snails to one susceptible snail in $F_2$, and a ratio of 35 resistant-resistant snails to one susceptible snail in $F_3$. 

Transgenic female

Field release

Parents

Inbreeding

Out-breeding

Inbreeding

$F_1$ progeny

Resistant

Resistant

Susceptible

Out-breeding among $F_1$ progeny of *Oncomelania lorelindoensis*

$F_2$ out-breeding progeny: All resistant

Inbreeding among $F_1$ of *Oncomelania lorelindoensis*
F₂ inbreeding progeny: Two resistant and one susceptible

Breeding among F₂ progeny of *Oncomelania lorelindoensis*

![Breeding Diagram]

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Figure 4 (Cont.)
F₃ progeny: thirty-five resistant and one susceptible.
susceptible snails resulted in a ratio of two schistosomiasis-resistant snails to one susceptible snail in F1 progeny and total was F1 = 3 : 3 snails. In F2, it resulted in a ratio of five schistosomiasis-resistant snails to one susceptible snail (F1 = 1 (6n-1) : 1) and total was F2 = 6n-1 = 6 snails. These results resulted in mathematical ideas for F1 is (3n − 1) : 1 in which n is 1 and the mathematical idea for F2 n+1 2 is (6n − 1) : 1 in which n is natural number. Total F1 = 3 and total F2 n+1 = 6.

4. Discussion

For S. japonicum, lineages from Southeast Asia were in Haplogroup A. S. japonicum from the lake regions of China in Haplogroup A migrated to Southeast Asia at ~3,000-4,000 years ago. It migrated to mountainous regions at ~5,000 years ago. The data suggest that S. japonicum originated from the lake area of China, with the parasites spreading to Japan around 7,000 years ago. It radiated into the mountains of China about 5,000 years ago, and to the Philippines and Indonesia about 4,000 years ago (Yin et al., 2015).

According to sequence results, I found that Sulawesi’s S. japonicum and the Philippines’s S. japonicum shared identity percentages almost the same as China’s S. japonicum (99.89% and 99.87%), West China (99.72% and 99.71%), Japan (99.34% and 99.33%), and West China (99.29% and 99.28%) (Table 1). In addition, Sulawesi’s S. japonicum was close to the Philippines’s S. japonicum (KU196379.1, China (KU196306.1), Japan (KU196358.10, China (KU19299.1), and West China (KU196408.1) (Figure 1). This sequence suggests that Southeast Asia’s S. japonicum originated from Japan and China. Interestingly, Sulawesi’s S. japonicum had a higher identity percentage than with the Philippines’s S. japonicum (Table 1). This suggests that the spread of S. japonicum from either Japan or China to Southeast Asia first reached Sulawesi and then the Philippines. However, if Sulawesi’s S. japonicum derived from Taiwan, it first reached the Philippines and then Sulawesi, Indonesia. These results show that Sulawesi’s S. japonicum and the Philippines’s S. japonicum could originate from China’s ML of the Yangtze River, West China, Japan, or even Taiwan. It did not originate from the ML of the Yangtze River only, indicating that my finding is novel.

Using a molecular clock, the introduction of O. hupensis across mainland China has been dated to the early Miocene (ca 22 million years ago), with a high rate of cladogenesis 8-2 million years ago. It was due to the unusually warm and humid climate of the region at that time and the tectonic turmoil in Japan. The divergence of the S. japonicum clade has been dated at 4.6 million years ago. This implies that the radiation of O. hupensis occurred before that of S. japonicum. If the radiation of the snails and worms is heterochronous, there is no opportunity for coevolution. The implication is also that the ancestral intermediate host differed from those of the present, which again makes coevolution unlikely. For the genus Oncomelania dispersal, the ML reaches of the Yangtze River clad brought out the Fujian coastal plain (China), Japan, Taiwan, and the Philippines populations of O. hupensis. Such a re-colonization of Japan by mainland Chinese O. hupensis is consistent with the “East to West” hypothesis. This hypothesis proposes that O. nomenclation was from Australia and via the Philippines, according to this hypothesis, after reaching Japan; Proto-O. nomenclation gives rise to O. hupensis in mainland China. O. nomenclation hupensis (KR002674.1) re-colonized Japan (KR002673.1), and spread to West China (KR002675.1). O. nomenclation hupensis then radiated to Taiwan, the Philippines, and Sulawesi (Attwood et al., 2015).

According to the sequence results using the NCBI BLAST tool, I found that the Philippines’s O. quadrasi (Garcia et al., 1980) (DQ112287.1) shared an identity of 87.25% with West China’s O. robertsoni (KR002675.1). It is the highest percentage. The second rank was Japan’s O. hupensis nosophora (KR002673.1) with an identity of 86.26%. The third rank was Taiwan’s O. hupensis chiui (DQ112271.1) with an identity of 85.58%. China’s O. nomenclation hupensis (GU367391.1) shared an identity of 84.48% with O. quadrasi (Table 2). These results suggest that O. quadrasi is close to O. robertsoni, O. hupensis nosophora, and O. hupensis chiui, especially O. robertsoni. O. nomenclation hupensis nosophora did not originate from O. minima. It is closer to O. hupensis with an identity of 94.81% compared to O. minima with an identity of 82.36% (Table 2). Thus, O. hupensis nosophora originated from O. minima, not from O. minima. Sequences results confirmed that O. hupensis gave rise to O. hupensis tangi, O. hupensis chiui, O. hupensis formosana, and O. hupensis nosophora. O. nomenclation hupensis differs from O. quadrasi (Table 3), suggesting O. quadrasi did not originate from O. hupensis as it is too distance. O. nomenclation quadrasi is closer to O. robertsoni than to O. hupensis (Table 2 and Table 3). Therefore, O. robertsoni must be a complete species, along with O. lorelindoensis and O. quadrasi. These three species are beyond O. hupensis group and O. minima (Figure 2). O. nomenclation hupensis lorelindoensis should be O. lorelindoensis as it drives from the Lindu Valley and the Lore sub districts (the Bada Valley and the Napu Valley). The Lindu Valley is
within the Lore Lindu National Park. *Oncomelania hupensis quadrasi* must be *O. quadrasi* as suggested by Woodruff et al. (Garcia et al., 1980) and beyond the *O. hupensis* group. Finally, *O. hupensis robertsoni* must be *O. robertsoni* (Figures 2 and 5).

In Central Sulawesi, the endemic areas of *S. japonicum* include the Bada Valley, the Lindu Valley, and the Napu Valley (Budiono et al., 2019; and Samarang et al., 2018). Carney et al. found the intermediate host of Sulawesi's *S. japonicum* in 1971 in the Lindu Valley (Nelwan, 2021c). Based on sequence results and added with *O. lorelindoensis*, I found that the genus *Oncomelania* consist of five species and five subspecies (Table 4 and Figure 5). It is new findings in the genus *Oncomelania*, especially *O. lorelindoensis* in Central Sulawesi.

<table>
<thead>
<tr>
<th>Name</th>
<th>Country/region</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. hupensis</em></td>
<td>China</td>
</tr>
<tr>
<td><em>O. h. chiui</em></td>
<td>Taiwan</td>
</tr>
<tr>
<td><em>O. h. formosana</em></td>
<td>Taiwan</td>
</tr>
<tr>
<td><em>O. h. hupensis</em></td>
<td>China</td>
</tr>
<tr>
<td><em>O. h. nosophora</em></td>
<td>Japan</td>
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<tr>
<td><em>O. h. tangi</em></td>
<td>China</td>
</tr>
<tr>
<td><em>O. lorelindoensis</em></td>
<td>Indonesia</td>
</tr>
<tr>
<td><em>O. minima</em></td>
<td>Japan</td>
</tr>
<tr>
<td><em>O. robertsoni</em></td>
<td>China</td>
</tr>
<tr>
<td><em>O. quadrasi</em></td>
<td>Philippine</td>
</tr>
</tbody>
</table>

**Note:** *O*: *Oncomelania* and *O.h*: *Oncomelania, Hupensis*

![Figure 5: The genus Oncomelania](image)

**Figure 5: The genus Oncomelania**

**Note:** Five species of the genus *Oncomelania*: *O. minima* (1), *O. robertsoni* (2), *O. hupensis* (3), *O. quadrasi* (4), and *O. lorelindoensis* (5).
It could be likely that Sulawesi's *S. japonicum* and *O. lorelindoensis* has spread to other areas of the region. Control of this parasite in Sulawesi can help prevent the spread of the parasite in the region. It can be through genetic manipulation in the intermediate host (Nelwan, 2021b).

Genetic manipulation techniques can help control schistosomiasis, especially schistosomiasis japonica in Central Sulawesi, Indonesia (Nelwan, 2021a). Indonesia has made great efforts to eliminate this parasite (Gordon et al., 2019). However, schistosomiasis still exists and has even expanded to new areas around endemic areas of the parasite (Nurwidayati et al., 2018; and Samarang et al., 2018). To support the elimination of this parasite, genetic manipulations in intermediate host *O. lorelindoensis* can be done. Concepts of creation for transgenic female snails and systems of mating have been available. Based on those concepts, I found that resistant snails has a ratio of 35 schistosomiasis-resistant snails to one susceptible snail in *F*<sub>3</sub> progeny (*F*<sub>2+1</sub> = ([6<sup>2</sup> – 1] : 1) (Figure 4). Unfortunately, genetic manipulation in snails has ethical challenges, and these must be discussed among such scientists, politicians, and relevant communities (Nelwan, 2021a; and Maier et al., 2019).

The National Academies of Science, Engineering, and Medicine (NASEM) have emphasized the importance of an interdisciplinary perspective on gene drive research. It explicitly attends to complex human values and the necessity of the community, stakeholders, and public engagement to accompany technical research and development. Although decision-making involves risk assessment, the prevailing uncertainties of genome engineering technology in snails, other organisms, and its behavior in the wild prevent appropriate risk/benefit analysis. Therefore, some have emphasized the need to allow sufficient time to develop amendments to current regulatory framework (Maier et al., 2019).

There are two limitations of the study. First, critic says that it is likely that sequences with BLASTN techniques have a lower level confidence than with phylogenetic tree analysis techniques. However, it should be known that both BLASTN and phylogenetic tree analysis use nucleotide of DNA/RNA to have tree views in evolutionary. Thus, it should be no different between BLASTN and phylogenetic techniques I think. Second, there is no information in details about CRISPR/Cas9 editing. For example, total snails that would be used in the study were not mentioned.

Detection of schistosomiasis plays an important role in elimination efforts of schistosomiasis. Several detection tools are available: microscopic, serological, molecular, and imagine techniques. For example, ultrasonography technique can detect *S. japonicum* worms in mice (Maezawa et al., 2018). Therefore, this tool can help detect a status of schistosomiasis in an endemic area before or after treatment with genetic biocontrol of schistosomiasis. Ultrasoundography can also detect this disease in humans (Figure 6).

**Figure 6: Hepatosplenic schistosomiasis in human**

*Note:* A and B images of liver showing hepatic schistosomiasis. C shows enlarge spleen with dilated splenic vein (Taken from Sah et al., 2015).

### 5. Conclusion

*S. japonicum* originated from Japan and China. Its intermediate host is *O. lorelindoensis*. *Oncomelania lorelindoensis* is close to *O. quadrasii*. The genus *Oncomelania* consists of five species and five subspecies. Genetic manipulation techniques can help for eliminating schistosomiasis, especially schistosomiasis japonica. These techniques can include AAV vectors and CRISPR/Cas9 system. Based on systems of mating patterns, the number of progeny in each generation can be counted by mathematical ideas. Genetic manipulation technique does not kill snails. However, before use of this technique, ethical issues must carefully be considered.
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Conflicts of interest
I declare that no conflicts of interest exist.

References


