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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 $F_1 = 3^n$, $F_1 = ([3^n - 1] : 1)$, total $\geq F_2$ 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

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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., KU196321.1) against the Philippines (KU196379.1), and Indonesia (KU196348.1) 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. lorelindoensis*), *O. hupensis quadrasi* (*O. quadrasi*), *and O. minima. Oncomelania hupensis* consisted of *O. hupensis chiui, O. hupensis formosana, O. hupenseis hupensis, O. hupensis nosophora, O. hupensis robertsoni* (*O. robertsoni*), and *O. hupensis tangi*. The East to West hypothesis suggests that precursors of *Oncomelania* 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. *Oncomelania hupensis* re-colonized Japan, the Philippines and Sulawesi to replace antecedent form (Attwood *et al*., 2015). There are two species of the genus *Oncomelania: Oncomelania hupensis* and *O. minima. Oncomelania hupensis* consists of five subspecies. *Oncomelania 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 *Oncomelania* (KR002673.1) and 84.40% with the Philippines's *Oncomelania* (DQ112287.1). In addition, the sequence results showed that the Philippines's *Oncomelania* 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 *Oncomelania* 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 McVeigh 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, gene drives for controlling schistosomiasis can be used. For example, anti-*Schistosoma* such as fibrinogen-related proteins, thioster-containing proteins (Maier *et al*., 2019), or cluster of polymorphic transmembrane genes (Tennessen *et al*., 2020) can be inoculated into the ovaries of female *O. lorelindoensis* 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, $F_{n+1} = ([6^n - 1] : 1)$ 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_2 progeny had a ratio of five schistosomiasis-resistant snails to one susceptible snail, for example.

In this study, I report the spread of *S. japonicum*, species and subspecies of the genus *Oncomelania,* and genetic manipulation techniques in the genus *Oncomelania* for the objectives of this study.

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)

KU196398.1 (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)

DQ212796.1 (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 *Oncomelania 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 PTC2 TM1 gene is an obvious candidate to defense against schistosomiasis (Tennessen *et al*., 2020). Either PTC1 TM1 or PTC2 TM1 genes can be coupled to a CRISPR-mediated gene drive and spread through wild-type snails' 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 PTC1 TM1 and / or PTC2 TM1 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 *O. 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 F_1, F_2, and F_3.

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. jaonicum* (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 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).

Table 1: Percentage identities of *Schistosoma japonicum* for

China, Japan, Indonesia, Philippine and Taiwan

Country/region	**Indonesia KU196348.1**	**Philippine KU196379.1**
KU196306.1 Mid-Yangtze-THP	99.89%	99.87%
KU196408.1 West	99.72%	99.71%
KU196377.1 Mid- Yangtze-THP	99.61%	99.60%
KU196358 Japan	99.34%	99.33%
KU196299.1 Mid-Yangtze-PLB	99.31%	99.30%
KU195362.1 West	99.29%	99.28%
KU196398.1 Taiwan	98.00%	98.01%

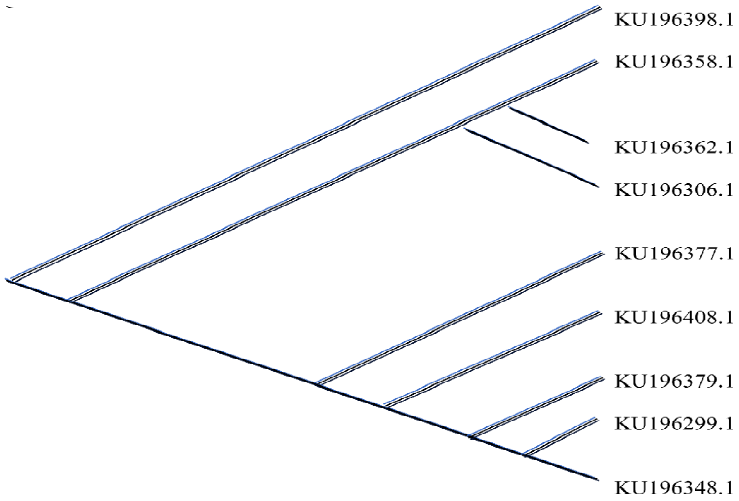


Figure 1: BLAST tree view slanted cladogram of *Schistosoma japonicum*

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. Percentage identities of genus *Oncomelania* of China, Japan, Taiwan and the Philippines

Accession	**Japan AB611791.1**	**Country/region**
KR002675.1	**84.62%**	**West China**
KR002673.1	**82.36%**	**Japan**
KR002674.1	**82.19%**	**China**
DQ112271.1	**82.13%**	**Taiwan**
DQ112287.1	**82.13%**	**Philippine**
GU367391.1	**81.97%**	**China**
DQ212796.1	**81.97%**	**China**
DQ112282.1	**81.97%**	**Taiwan**
Accession	**Japan KR002673.1**	**Country/region**
DQ112271.1	**95.15%**	**Taiwan**
GU367391.1	**94.81%**	**China**
DQ112282.1	**94.48%**	**Taiwan**
DQ212796.1	**94.14%**	**China**
KR002674.1	**93.97%**	**China**
KR002675.1	**88.11%**	**West China**
DQ112287.1	**86.26%**	**Philippine**
Accession	**Philippine DQ112287.1**	**Country/region**
KR002675.1	**87.25%**	**West China**
KR002673.1	**86.26%**	**Japan**
DQ112271.1	**85.58%**	**Taiwan**
DQ212796.1	**85.11%**	**China**
DQ112282.1	**84.95%**	**Taiwan**
GU367391.1	**84.48%**	**China**
KR002674.1	**84.40%**	**China**
AB611791.1	**82.13%**	**Japan**

Table 2 (Cont.)

Accession	West China KR002675.1	Country/region
DQ212796.1	**88.80%**	**China**
DQ112282.1	**88.61%**	**Taiwan**
DQ112271.1	**88.27%**	**Taiwan**
KR002673.1	**88.11%**	**Japan**
KR002674.1	**88.46%**	**China**
DQ112287.1	**87.25%**	**Philippine**
Accession	**China GU367391.1**	**Country/region**
KR002674.1	**98.66%**	**China**
DQ212796.1	**97.96%**	**China**
DQ112282.1	**97.65%**	**Taiwan**
DQ112271.1	**97.49%**	**Taiwan**
KR002673.1	**94.81%**	**Japan**
KR002675.1	**88.29%**	**West China**
DQ112287.1	**84.48%**	**Philippine**

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

nosophora (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).

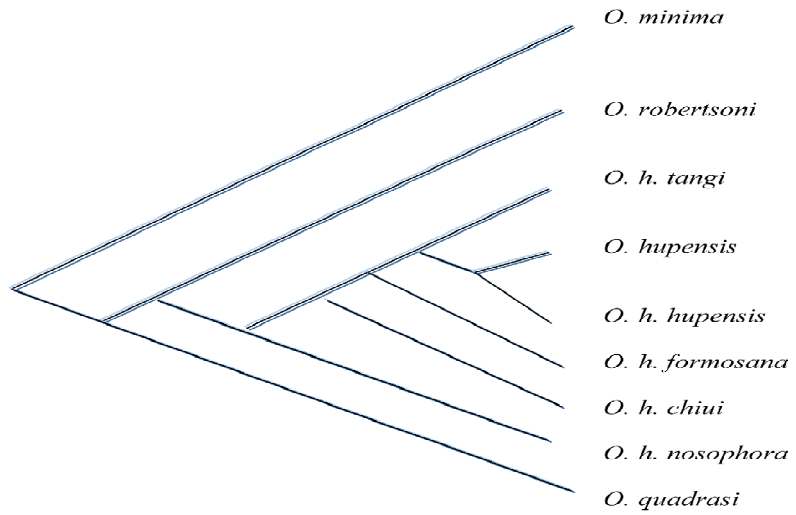


Figure 2: BLAST tree view slanted cladogram of the genus *Oncomelania*

Note: Southeast Asia's *Oncomelania* was close to *Oncomelania 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 3: The genus *Oncomelania*

Species	*O. hupensis*	*O. robersoni*	*O. minima*
O. h. hupensis	**98.66%**	**88.46%**	**82.19%**
O.h. tangi	**97.96%**	**88.80%**	**81.97%**
O. h. formosana	**97.65%**	**88.61%**	**81.97%**
O. h. chiui	**97.49%**	**88.27%**	**82.13%**
O. h. nosophora	**94.81%**	**88.11%**	**82.36%**
O. quadrasi	**84.48%**	**87.25%**	**82.13%**

3.3. Genetic biocontrol of Schistotomiasis 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-

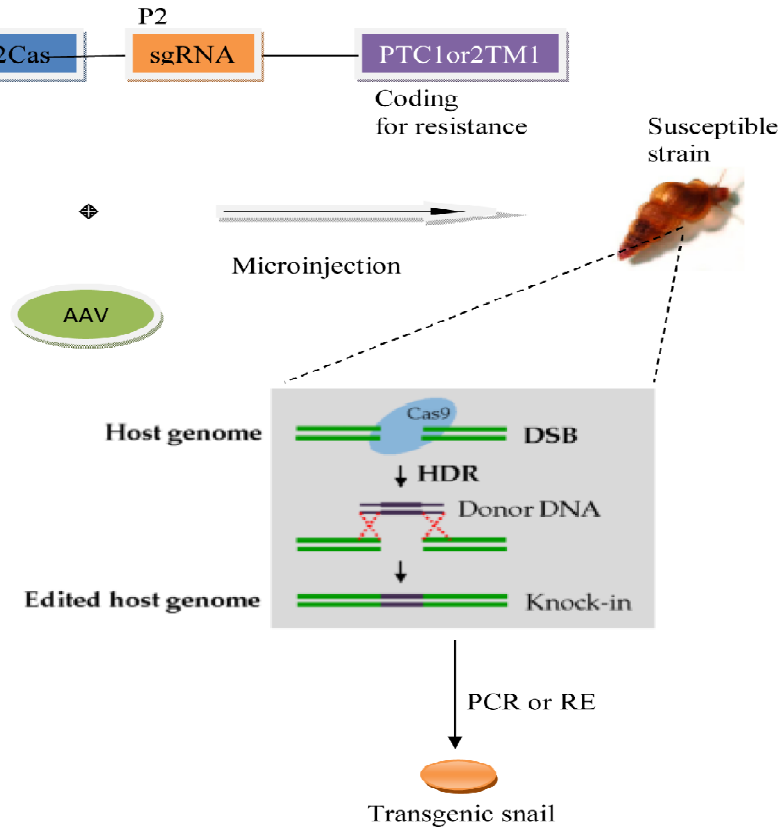


Figure 3: Transgenic snails

Note: Creation transgenic snails via clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system in a snail vector schistosome. DSB, double-strand break; HDR, homology-directed repair; PCR, polymerase chain reaction; RE, restriction enzyme (Modified from Famakinde, 2018). (*Oncomelania* snail image taken from Gordon *et al.*, 2019).

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 shistosomiasis-

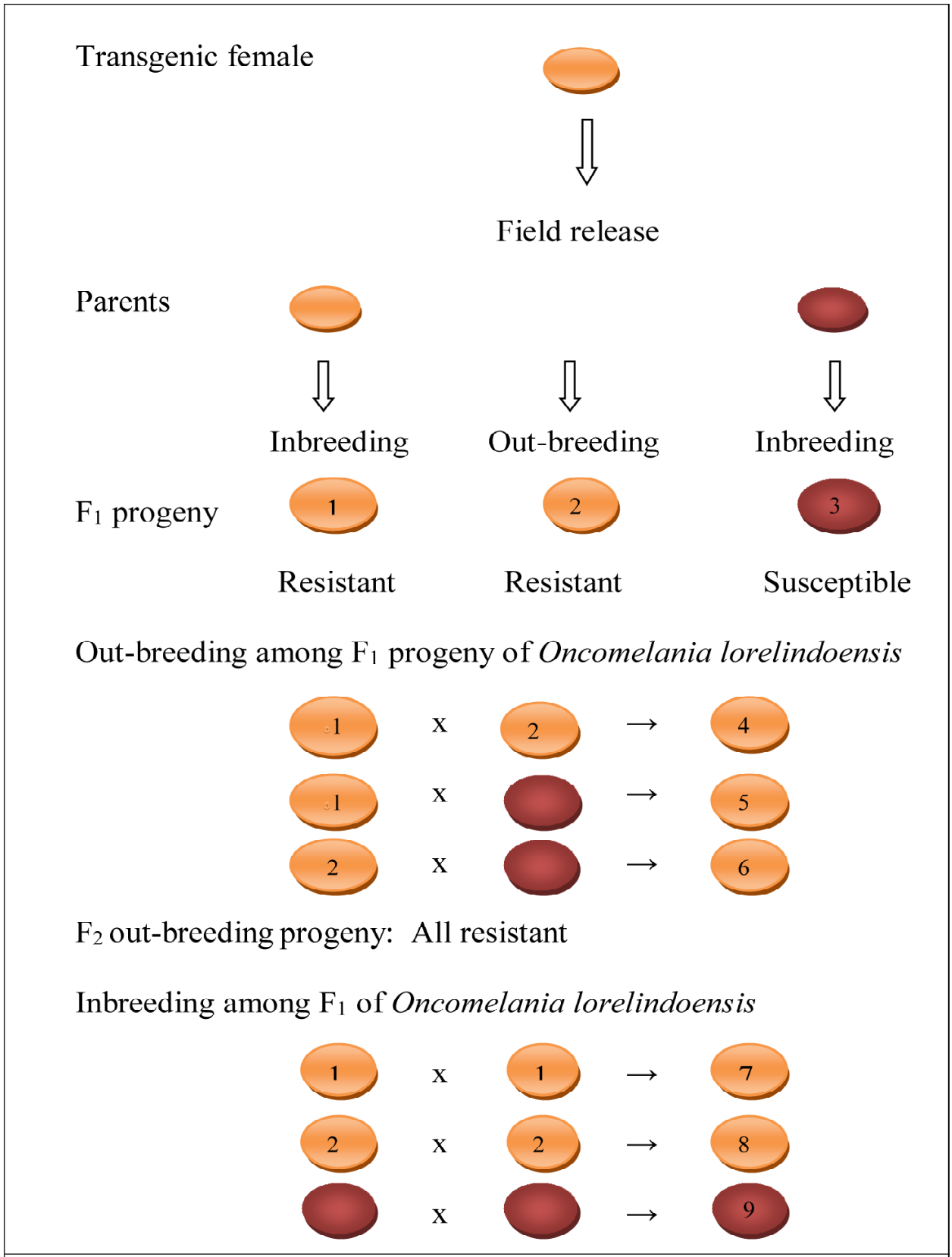


Figure 4: Transgenic snails in the field

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.

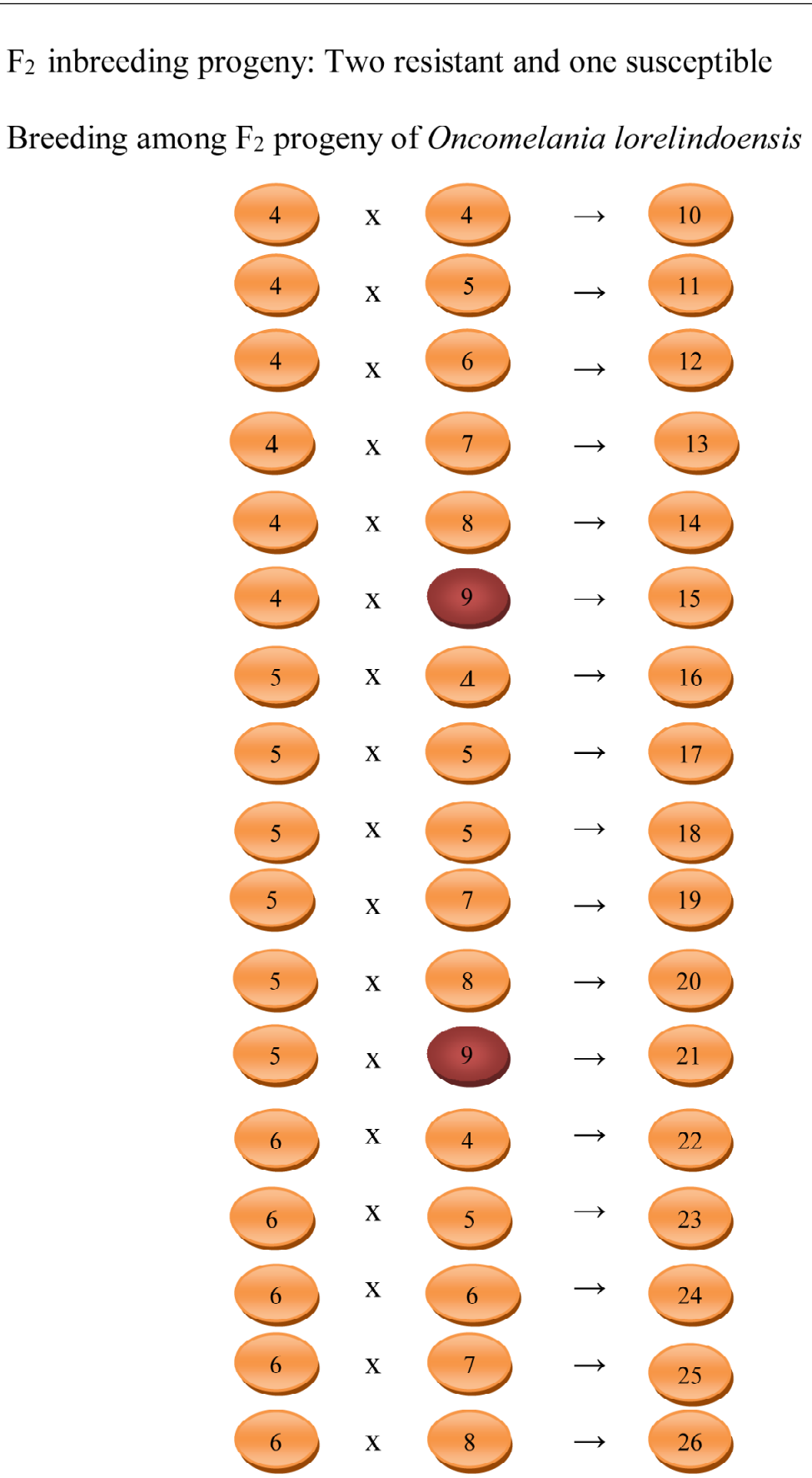
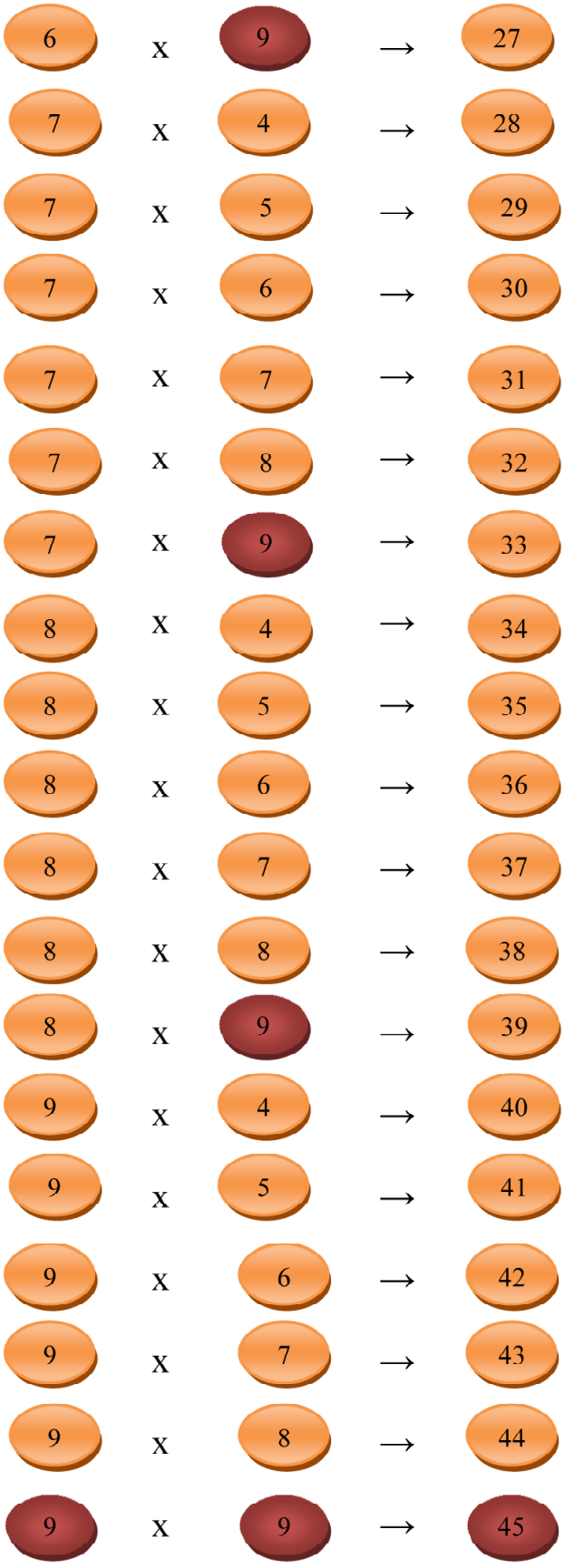


Figure 4 (Cont.)



F_3 progeny: thirty-five resistant and one susceptible.

Figure 4 (Cont.)

susceptible snails resulted in a ratio of two schistosomiasis-resistant snails to one susceptible snail in F_1 progeny and total was $F_1 = 3^1 = 3$ snails. In F_2, it resulted in a ratio of five schistosomiasis-resistant snails to one susceptible snail ($F_{n+1} = ([6^n - 1] : 1)$) and total was $F_2 = 6^1 = 6$ snails. These results resulted in mathematical ideas for F_1 is ($[3^n - 1] : 1$) in which n is 1 and the mathematical idea for $F_{n+1} \geq 2$ is ($[6^n - 1] : 1$) in which n is natural number. Total $F_1 = 3^n$ and total $F_{n+1} = 6^n$.

4. Discussion

For *S. japonicum,* lineages from Southeast Asia were in Haplogroup A. *S. japonicum* from the lake regions of China in Haplogroup A1a 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 parasite 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 Philippine'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 to 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 clade brought out the Fujian coastal plain (China), Japan, Taiwan, and the Philippines populations of *O. hupensis hupensis*. Such a re-colonization of Japan by mainland Chinese *O. hupensis* is consistent with the "East to West" hypothesis. This hypothesis proposes that *Oncomelania* was from Australia and via the Philippines, according to this hypothesis, after reaching Japan; Proto-*Oncomelania* gives rise to *O. hupensis* in mainland China. *Oncomelania hupensis* (KR002674.1) re-colonized Japan (KR002673.1), and spread to West China (KR002675.1). *Oncomelania 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 *Oncomelania 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. Oncomelania 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. hupensis,* 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. Oncomelania hupensis* differs from *O. quadrasi* (Table 3), suggesting *O. quadrasi* did not originate from *O. hupensis* as it is too distance. *Oncomelania 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). *Oncomelania hupensis lindoensis* 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 robersoni* 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. lorelinduensis* in Central Sulawesi.

Table 4: The genus *Oncomelania*

Name	Country/region
O. hupensis	**China**
O. h. chiui	**Taiwan**
O.h. formosana	**Taiwan**
O. h. hupensis	**China**
O. h. nosophora	**Japan**
O. h. tangi	**China**
O. lorelindoensis	**Indonesia**
O. minima	**Japan**
O. robertsoni	**China**
O. quadrasi	**Philippine**

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

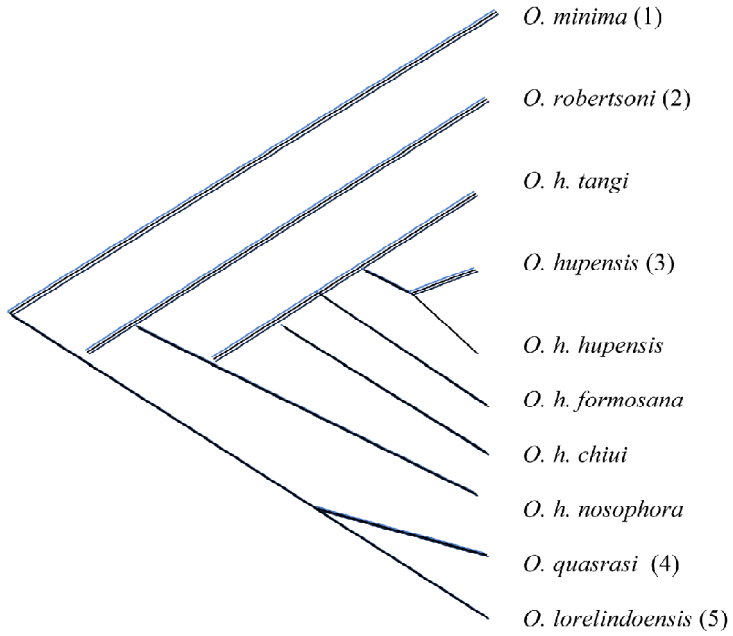


Figure 5: The genus *Oncomelania*

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_3 progeny (F_{2+1} = ([$6^2 - 1$] : 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. Ultasonography can also detect this disease in humans (Figure 6).

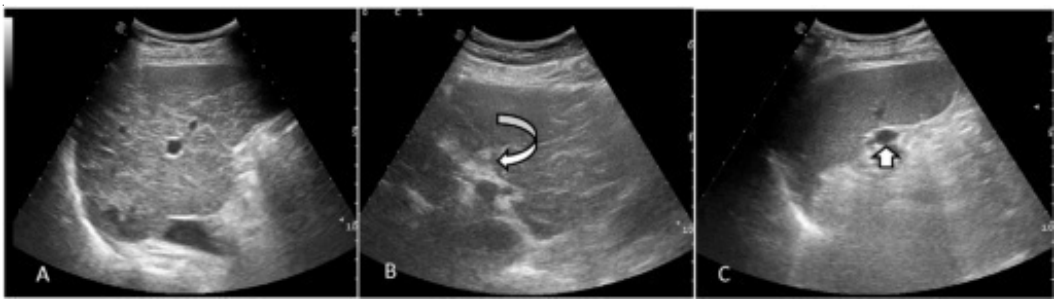


Figure 6: Hepatosplenic schistosomiasis in human

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

5. Conclusion

Sulawesi's *S. japonicum* originated from Japan and China. Its intermediate host is *O. lorelindoensis*. *Oncomelania lorelindoensis* is close to *O. quadrasi.* 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.

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