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Schistosoma japonicum complex: COI-sequences variations of parasites and their intermediate hosts analyzed using BLAST

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In this research, I report on the relationship between *S. ovuncatum, S. sinensium, S. japonicum, S. mekongi,* and *S. malayensis.* In addition, I also report on the relationship between five species of *Oncomelania* and *Neotricula aperta, Robertsiella* spp, and *Tricula* spp. Furthermore, I describe the formation of the genus *Oncomelania* from its predecessor forms. The results of nucleotide BLAST showed that *S. japonicum* shares 99.45% identity with *S. malayensis*, 98.77% identity with *S. mekongi*, 98.07% identity with *S. sinensium*, and 97.85% identity with *S. ovuncatum*. In addition, the sequence of *S. malayensis* shares 100% identity with *S. mekongi.* The E value is less than 0.01. There was no amino acid replacement in the alignment results of the *S. japonicum* complex. There are no base substition in *S. malayensis* and *S. mekongi, 37* base substitutions in *S. malayensis and S*. *sinensium*, ten base substitutions in *S. malayensis* and *S. japonicum*, five base substitutions in *S. malayensis* and *S. ovuncatum*. The tree-view slanted cladogram showed that *S. sinensium* is a sister to *S. ovuncatum.* It split into *S. japonicum. Schistosoma japonicum* splits into *S. mekongi* and *S. malayensis.* The results also showed that *Oncomelania robertsoni* shares 86.12% identity with *Neotricula aperta*, 85.88% identity with *Robertsiella* spp., and 85.28% identity with *Tricula bollingi*. The E value is less than 0.01. There are two amino acid replacements in *O. robertsoni* and *N. aperta* alignments, four amino acid replacements and 84 base substitutions in *O. robertsoni* and *Robertsiella,* and two amino aciid replacements and 88 base substitutions in *O. robertsoni* and *T. bolingi*. The tree-view slanted cladogram showed that *Tricula* spp. split into *N. aperta* and *Robertsiella* spp. This study showed that *Oncomelania* rose from its predecessor forms. Nucleotide BLAST results showed that *S. ovuncatum* is close to *S. sinensium. Schistosoma sinensium* split into *S. japonicum, S. mekongi,* and *S. malayensis. Oncomelania robertsoni* was close to *N. aperta* than to *Robertsiella* spp., and then *Tricula* spp. (*Tricula bollingi*)*. Oncomelania* species emerged from their predecessor forms.

Key words*: Schistosoma japonicum complex, Neotricula aperta, Oncomelania, Oncomelania lorelindoensis, schistosomiasis, Robertsiella spp., Tricula spp.*

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Introduction

Blood flukes of the genus *Schistosoma* cause schistosomiasis infections in animals and humans. The genus *Schistosoma* infects approximately 220 to 230 million people (Sanchez et al., 2021). Schistosomiasis causes 24,072 to 200,000 deaths annually, and 779 million people are at risk. In addition, it causes a world burden of 3.3 million disability-adjusted life years (Nelwan, 2022). It is one of the main parasitic diseases that are important for public health in tropical and sub tropical countries (Orpin et al., 2022). The disease can spread through water-based development projects, immigration, and travel or tourism (Nelwan, 2019). It occurs in Africa, Asia, South America, and several Caribbean islands (Haggag et al., 2019; Neves et al., 2015). It can also occur in non developing countries such as France (Rothe et al., 2021).

Five *Schistosoma* species occur in the Far East: *Schistosoma japonicum, S. malayensis, S. mekongi, S. ovuncatum,* and *S. sinensium* (Wang et al., 2021)*.* Three of them can cause human schistosomiasis: *Schistosoma japonicum, S. malayensis,* and *S. mekongi* (Al-Olayan et al., 2016; Gordon et al., 2019). *Schistosoma japonicum* occurs in East Asia and Southeast Asia, i.e., China, Indonesia, and the Philippines (Budiono et al., 2020). *Schistosoma malayensis* occurs in Malaysia, and *S. mekongi* occurs in Cambodia and Laos (Gordon et al., 2019).

Latif et al. (2013) showed that *S. malayensis* is approximately 10% different from *S. mekongi* and approximately 25% different from *S. japonicum* genetically (Latif et al., 2013), suggesting that *S. malayensis* has a closer family relationship with *S. mekongi* than with *S. japonicum. Schistosoma ovuncatum* occurs in Thailand. *Schistosoma sinensium* occurs in China and Thailand. *Schistosoma sinensium* can infect rodents, rabbits, and tree shrews (Wang et al., 2021). *Schistosoma ovuncatum* can infect rodents, rats, and *Tricula bollingi* (Inceboz, 2022).

The *Schistosoma japonicum* complex life cycle requires two hosts: a definitive host and an intermediate host. Sexual or asexual reproduction occurs in these parasites. Sexual reproduction occurs in mammals (Nelwan, 2022) or nonmammal hosts (Brant and Loker, 2005; Walker, 2011; Budiono et al., 2019). Mammals can include cattle, dogs, horses, and humans (Budiono et al., 2019). Schistosomes can infect birds (Brant and Loker, 2005; Walker, 2011), suggesting that schistosomes may infect mammals and nonmammals. Mouahid et al. (2018) stated that cercariae enter specific host skin, forming schistosomula, grow into schistosomes and adult worms, mate, and produce eggs. Definitive hosts release eggs into the external environment through feces. In fresh water, these eggs form miracidia, hatch, and infect an intermediate host, the *Oncomelania* snail. Inside the snail, this develops into a mother sporocyst and then produces daughter sporocysts. Daughter sporocysts produce either cercariae or more daughter sporocysts (Mouahid et al., 2018). Asexual reproduction occurs in intermediate hosts (Nelwan, 2019).

For intermediate hosts, *Schistosoma malayensis* infects *Robersiella* spp. snails (Gordon et al., 2019). *Schistosoma mekongi* infects *Neotriculla aperta* snails (Attwood et al., 2019). *Schistosoma ovuncatum* and *S. sinensium* infect *Tricula* spp. snails (Wang et al., 2021). *Schistosoma japonicum* infects the genus *Oncomelania*: *Oncomelania hupensis* (Fang et al., 2022)*, O. lorelindoensis, O. robertsoni,* and *O. quadrasi* (Nelwan, 2022; ZooBank, 2022). My sequence results showed that *Oncomelania hupensis* differs from *Robertsiella* spp. by approximately 16% and from *Neotriculla aperta* by approximately 15%, suggesting that *O. hupensis* has a closer relationship to *Neotricula aperta* than to *Robertsiella* spp.

The study of the evolution of the genus *Schistosoma* (Lawton et al., 2011) and the evolution of their intermediate hosts, especially the genus *Oncomelania* (Nelwan, 2022), has been an area of major debate and research (Lawton et al., 2011). Latif et al. (2013) showed the relatedness between *S. japonicum, S. malayensis,* and *S. mekongi* (Latif et al., 2013). However, this explanation still requires further information regarding the relatedness. In addition, the relatedness of the five *Oncomelania* species isolates and *Neotricula aperta, Robertsiella* sp. and *Tricula* sp. does not yet have a detailed explanation. More information is needed regarding, for example, the percentage of the relationship between *Oncomelania* and *N. aperta*. In addition, Carney et al. discovered the intermediate host of Sulawesi *S. japonicum* in Lindu Valley in 1971 and named it *Oncomelania hupensis lindoensis* (Sudomo, 1983). This means that both of them admit that Sulawesi *Oncomelania* originates from China. My sequences show that Sulawesi *Oncomelania* originated from its antecedent form, i.e., proto-*Oncomelania lorelindoensis* in Sulawesi, the eastern part of Indonesia. It is beyond the *O. hupensis* group.

In this paper, I report on the relatedness between *S. japonicum, S. malayensis, S. mekongi, S. ovuncatum,* and *S. sinensium.* I also report on the relationship between five *Oncomelania* species, *Neotricula aperta, Robertsiella* spp, and *Tricula* spp. Moreover, I describe the development of the genus *Oncomelania* from its antecedent form.

Materials and Methods

In this study, I used the nucleotide NCBI BLAST to assess similar identity using two or more alignments and tree views of Asian *Schistosoma* and its intermediate hosts. The NCBI BLAST sequences are reliable for carrying out analyses of the relatedness of Asian *Schistosoma* isolates and the relatedness of its intermediate host isolates.

I used nucleotide BLAST for two or more alignments to obtain accession numbers, E values, identity percentages, and query cover percentages. The sequences used in this study were all from GenBank for the *S. japonicum* complex and its intermediate hosts. The nucleotide BLASTs were performed with the nucleotide NCBI BLAST. For query cover, if the first BLAST (megablast) was nonsignificant, I performed BLAST for somewhat similar sequences (blastn) (Nelwan, 2022). These data were used for a tree-view slanted cladogram of Asian *Schistosoma* spp. and their intermediate hosts. These intermediate hosts include *Neotricula aperta, Oncomelania*, and *Robertsiella* sp.

To see how the sequences might differ, I used pairwise dots for identities and the coding sequence (CDS) feature from the NCBI. For the sequence differences, from the nucleotide blast results, go to the Alignments tab, and in the Alignment view drop-down menu, select Pairwise with dots for identities, and then click the checkbox next to the CDS feature. In the results, there are four lines. The top line is a query sequence containing amino acid translation. The second line comprises bases where the subject sequence is identical to the query sequence and is replaced by dots. Bases where the subject sequence differs from the query sequence appear in the third line as A, C, G, or T. The fourth line shows the amino acid translation for the subject sequence. I used the alignment results to count total base substitutions in the third line and total amino acid replacements in the fourth line. The CDS feature is good for comparing the relatedness of taxa.

Creation of the tree-view slanted cladogran was as follows: First, from the nucleotide NCBI BLAST results go to the Description tab and click the Distance Tree of the Results links. Second, in the rectangle tree, go to the menu Tools > Layout and select slanted cladogram (Nelwan, 2022; Smith, 2023). Rectangle cladogram from NCBI is comparable to rectangle cladogram from PhyML (Nelwan, 2023).

Nucleotide BLAST on the *Schistosoma japonicum* complex

For the nucleotide BLAST on the *S. japonicum* complex, I accessed the reference sequence from GenBank at the National Center for Biotechnology Information (NCBI). For the *S. japonicum* complex, I used 18S for analysis. I performed a megablast analysis of these data. Data include accession numbers and locations (Table 1).

Table 1. Data used in locus 18S of *Schistosoma japonicum* complex

Species	accession	location
S. japonicum	AY157226.1	Philippines, Southeast Asia
S. malayensis	AY157227.1	Malaysia, Southeast Asia
S. mekongi	AF465928.1	Laos, Southeast Asia
S. ovuncatum	AF465929.1	Thailand, Southeast Asia
S. sinensium	AY157225.1	China, East Asia

S.: Schistosoma

Nucleotide BLAST results were used to show relatedness between species in the *Schistosoma japonicum* complex. The results were also used to show identity similarity percentage, difference of amino acids if any, difference of genetic code (bases), and tree relationships in evolution.

Nucleotide BLAST on intermediate hosts of the *Schistosoma japonicum* complex

For nucleotide BLAST on intermediate hosts, I accessed the reference sequence from GenBank at NCBI that originated from sources such as Attwood et al. (2015) and Kameda and Kato (2011). The source is the cox1 locus (CO1) and the megablast. Data included accession numbers and their locations (Table 2).

Table 2. Data used in locus COI for intermediate hosts of *Schistosoma japonicum* complex

Species	accession	location
Oncomelania minima	AB611791.1	Japan, East Asia
Neotricula aperta	AF531541.1	Laos, Southeast Asia
Robertsiella spp.	AF531550.1	Malaysia, Southeast Asia
Oncomelania robertsoni	KR002675.1	China, East Asia
Tricula bollingi	AF531553.1	Thailand, Southeast Asia
Oncomelania hupensis	GU367391.1	China, East Asia
Oncomelania quadrasi	DQ112287.1	Philippines, Southeast Asia

Nucleotide BLAST results were used to show a relationship between the intermediate hosts of the *Schistosoma japonicum* complex. The results were also used to show identity percentage, difference of amino acids if any, difference of bases, and tree relationships in evolution.

Megablast on *Oncomelania hupensis*

For the megablast on *O. hupensis,* I accessed the reference sequence from GenBank at NCBI. Data included accession numbers (Table 3).

Megablast results showed a relationship between *O. hupensis, O. hupensis hupensis, O. hupensis nosophora, O. robertsoni,* and *O. quadrasi.* The results were also used to compare different amino acids, different bases, and tree relationships in evolution. These data were obtained by performing a megablast of *O. hupensis* NC_013073.

Table 3. Data used on *Oncomelania hupensis* megaclast

Species	accession	location
Oncomelania hupensis	NC_013073.1	China, East Asia
Oncomelania h. hupensis	JF284688.1	China, East Asia
Oncomelania h. hupensis	JF284690.1	China, East Asia
Oncomelania h. hupensis	JF284692.1	China, East Asia
Oncomelania robertsoni	JF284697.1	China, East Asia
Oncomelania quadrasi	JF284698.1**	Philippines, Southeast Asia
Oncomelania h. nosophora	LC276225.1	Japan, East Asia
Oncomelania h. nosophora	LC2766.1	Japan, East Asia
Oncomelania robertsoni	EU079378.1	China, East Asia
Oncomelania robertsoni	LC276228.1	China, East Asia
Oncomelania quadrasi	LC276227.1	Philippines, Southeast Asia

h.: hupensis

Results

Percent sequence identity of the *Schistosoma japonicum* complex

Nucleotide BLAST, which is a megablast, showed that Philippines *S. japonicum* shares an identity of 99.45% with Malaysia *S. malayensis*, 98.77% identity with Laos *S. mekongi*, 98.07% identity similarity with China *S. sinensium*, and 97.85 identities with Thailand *S. ovuncatum*. E values are 0.0 for Malaysia *S. malayensis* and China *S. sinesnsium*, 1e-167 for Laos *S. mekongi*, and 1e-162 for Thailand *S. ovuncatum* (Table 4 and Figure 1). Moreover, the sequence of Malaysia *S. malayensis* shares 100% identity with Laos *S. mekongi*.

There was no amino acid replacement in the alignment results. In addition, there were no base substitutions in the alignments of Malaysia *S. malayensis,* Laos *S. mekongi,* Philippines *S. japonicum,* China *S. sinensium,* and Thailand *S. ovuncatum.* In Malaysia *S. malayensis* and Laos *S. mekongi*, there are no base substitutions (247/247 = 100%), 37 base substitutions (242/247 = 98%) in Malaysia *S. malayensis* and China *S. sinensium,* and ten base substitutions (1792/1802 = 99%) in Malaysia *S. malayensis* and Philippines *S. japonicum*, five base substitutions (242/247 = 98%) in Malaysia *S. malayensis* AY157227.1 and Thailand *S. ovuncatum*.

Percent sequence identity of intermediate hosts of the *Schitosoma japonicum* complex

The megablast results showed that Japan *O. minima* shares 82-85% identity with all the listed species. In addition, China *O. hupensis* shares 82-84% with all the listed species. All the E values are < 0.01 (Table 5, Figure 2, and Figure S).

There were amino acid replacements and base substitutions in the alignments of *O. robertsoni, Neotricula aperta, Robertsiella,* and *Tricula bollingi.* In the China *O. robertsoni* and Laos *N. aperta* alignments, there are two amino acid replacements and 83 base substitutions (515/598 = 86%), four amino acid replacements and 84 base substitutions (511/595 = 86%) in China *O. robertsoni* and Malaysia *Robertsiella,* and two amino acid replacements and 88 base substitutions (510/598 = 85%) in China *O. robertsoni* and Thailand *T. bollingi.*

Percent sequence identity of *Oncomelania hupensis*, *O. robertsoni*, and *O. quadrasi*

The megablast results showed that China *O. hupensis* shares 95-98% identity with all China *O. hupensis hupensis* and all Japan *O. hupensis nosophora*, indicating both are subspecies of China *O. hupensis*. The percentage is more than 95%. It also shares 89-91% identity with all Philippines *O. quadrasi* and all China *O. robertsoni*), indicating both are beyond *O. hupensis* group. All the E-values equal to 0.0 (Table 6 and Figure 3).

In this study, it was taken alignment results between China *O. hupensis* and Philippines *O. quadrasi*, China *O. robertsoni* and China *O. hupensis*, and China *O. robertsoni* and Philippines *O. quadrasi*. In the China *O. hupensis* and Philippines *O. quadrasi* alignments, there are 153 amino acid replacements and 1596 base substitutions (13599/15195 = 89%). In the China *O. robertsoni* and China *O. hupensis* alignments, there are 173 amino acid replacements and 1569 base substitutions (13636/15205 = 90%). In the China *O. robertsoni* and Philippines *O. quadrasi* alignments, there are 170 amino acid replacements and 1675 base substitutions (13527/15202 = 89%).

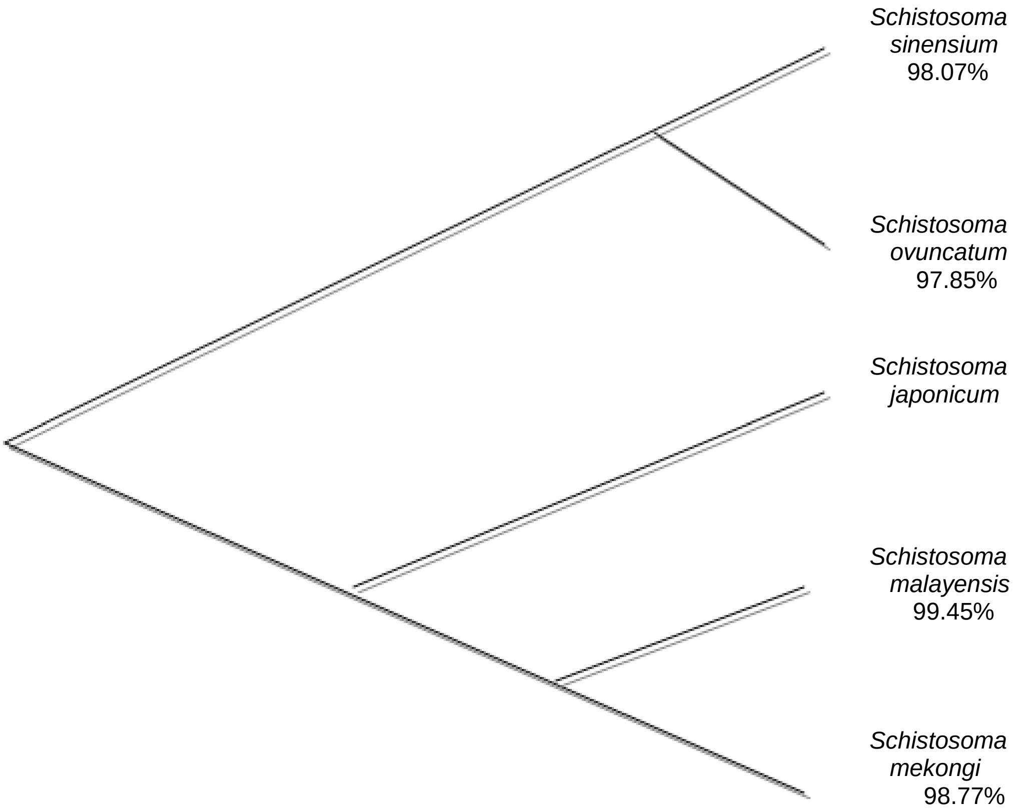

Figure 1. BLAST tree views slanted cladogram of Asian *Schistosoma* spp. Relatedness of Asian *Schistosoma: S. ovuncatum, S sinensium*, *S. japonicum, S. japonicum, S. malayensis,* and *S. mekongi* with their percentages, respectively*. Schistosoma ovuncatum* and *S. sinensium* form their own branches in one group. *Schistosoma japonicum, S. mekongi,* and *S. malayensis* form branches in another group. Modified from NCBI BLAST pairwise alignment.

Table 4. Sequences analysis in locus 18S of *Schistosoma japonicum* complex

Species			***S. japonicum***
	Accession	**E value**	**AY157226.1**
S. malayensis	AY157227.1	0.0	99.45%
S. mekongi	AF465928.1	1e-167	98.77%
S. sinensium	AY157225.1	0.0	98.07%
S. ovuncatum	AF465929.1	1e-162	97.85%
Species			***S. malayensis***
	Accession	**E value**	**AY157227.1**
S. mekongi	AF465928.1	2e-131	100%
S. japonicum	AY157226.1	0.0	99.45%
S. ovuncatum	AF465929.1	1e-122	97.98%
S. sinensium	AY157225.1	0.0	97.92%

S.: Schistosoma

Table 5. Intermediate hosts of *Schistosoma japonicum* complex

Species			***Oncomelania minima***
	Accession	**E value**	**AB611791.1**
Neotricula aperta	AF531541.1	0.0	85.43%
Robertsiella spp.	AF531550.1	3e-169	84.64%
Oncomelania robertsoni	KR002675.1	3e-169	84.62%
Tricula bollingi	AF531553.1	3e-164	83.75%
Oncomelania hupensis	GU367391.1	9e-160	82.44%
Oncomelania quadrasi	DQ112287.1	3e-169	82.23%
Species			***Oncomelania hupensis***
	Accession	**E value**	**GU367391.1**
Neotricula aperta	AF531541.1	0. 0	84.62%
Robertsiella spp.	AF531550.1	0. 0	84.08%
Tricula bollingi	AF531553.1	1e-179	82.78%
Species			***Oncomelania robertsoni***
	Accession	**E value**	**KR002675.1**
Neotricula aperta	AF531541.1	0. 0	86.12%
Robertsiella spp.	AF531550.1	0. 0	85.88%
Tricula bollingi	AF531553.1	1e-180	85.28%
Species			***Oncomelania quadrasi***
	Accession	**E value**	**DQ112287.1**
Neotricula aperta	AF531541.1	0. 0	83.39%
Robertsiella spp.	AF531550.1	0. 0	83.56%
Tricula bollingi	AF531553.1	8e-176	82.38%

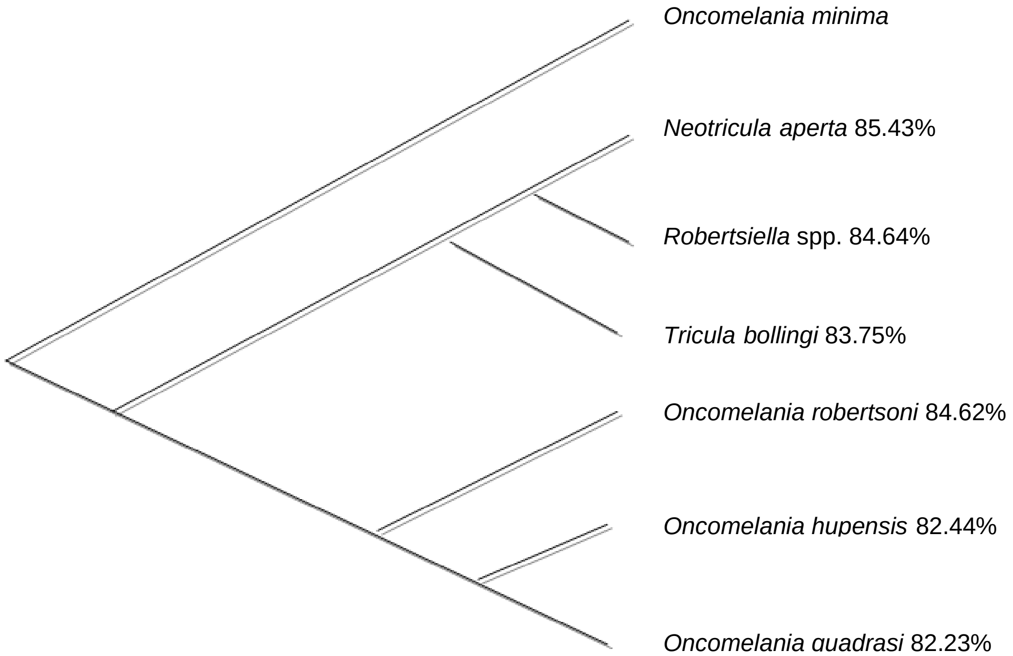

Figure 2. BLAST tree view slanted cladogram of Asian snails (modified from NCBI BLAST pairwise alignment). Asian schistosomiasis intermediate hosts: *O. hupensis, O. lorelindoensis, O. robertsoni, O. quadrasi, N. aperta, Robertsiella* spp, and *Tricula* spp. *Oncomelania hupensis, O. minima, O. robertsoni,* and *O. quadrasi* (*Pomatiopsinae*) form branches in a group, respectively. *Neotricula aperta, Robertsiella* spp. and *T. bollingi* (*Triculinae*) form their branches in another group. *Pomatiopsinae* and *Triculinae* were derived from the common ancestor (*Pomatiopsidae*).

Table 6. Megablast results on *Oncomelania hupensis*

Species			***O. hupensis***
	Accession	**E value**	**NC_013073.1**
O. h. hupensis	JF284692.1	0.0	98.00%
O. h. hupensis	JF284688.1	0.0	97.80%
O. h. hupensis	JF284690.1	0.0	97.68%
O. h. nosophora	LC276225.1	0.0	95.44%
O. h. nosophora	LC276226.1	0.0	95.37%
O. h. quadrasi	LC276227.1	0.0	91.06%
O. robertsoni	LC276228.1	0.0	90.13%
O. robertsoni	JF284697.1*	0.0	90.06%
O. robertsoni	EU079378.1	0.0	89.68%
O. quadrasi	JF284698.1	0.0	89.50%
Species			***O. robertsoni***
	Accession	**E value**	**EU079378.1**
O. robertsoni	LC276228.1	0.0	94.04%
O. robertsoni	JF284697.1*	0.0	93.38%
O. hupensis	NC_013073.1	0.0	89.68%
O. quadrasi	LC276227.1	0.0	88.98%
O. quadrasi	JF284698.1**	0.0	88.50%

O.: *Oncomelania; h.*: *hupensis;*

* Attwood et al. (2015)

** Incorrectly listed as *Oncomelania hupensis hupensis* on GenBank (Attwood et al., 2015)

Discussion

Many theories about the origin of the genus *Schistosoma* of African or Asian origin have been stated and developed with their respective arguments (Lawton et al., 2011). Davis (1992) introduced the idea that the genus *Schistosoma* arose before the split of Gondwanaland more than 150 million years ago (Ma) and had begun exploiting pulmonate and pomatiopsid snails, whose fossil records suggest a Gondwanan origin (Sady et al., 2015) This theory implies that the dispersal of the genus *Schistosoma* was due to continental drift and that the Asian ancestor of the schistosomes was carried across to Asia when India separated from Africa and moved forward in Asia 70-148 Ma, generating the *S. indicum* and *S. japonicum* complex (Lawton et al., 2011).

The hypothesis “out of Asia” is the most commonly accepted hypothesis for the spread of schistosomiasis. This hypothesis suggests a migration followed by the dispersion of schistosomiasis from Asia to Africa (Nahum et al., 2012). The Asian schistosome ancestor may have originally had a pomatiopsid or a pulmonate snail host. Snyder and Loker (2000) suggest that the schistosome colonized Africa approximately 15-20 Ma (Au et al., 2023). Following the invasion of the African continent, the parasites evolved to exploit pulmonate snails exclusively, thereby developing a more specialized host range (Lawton et al., 2011).

Modern genetic analysis shows that the genus *Schistosoma* originates from Asia (Lawton et al., 2011). This parasite has at least two descendants, invading the African continent independently. In Africa, these descendants are easily radiated, parasitizing exclusively planorbid snails. Back in Asia, the parasites diversified into a group of species characterized by the absence of an egg spoke (Nahum et al., 2012). The genus *Schistosoma* is split into six clades, which correlate with the different geographical distributions of the parasites. The genus forms the *S. japonicum* complex. It comprises *S. japonicum, S. malayensis, S. mekongi, S. ovuncatum,* and *S. sinensium* (Lawton et al., 2011). *Schistosoma japonicum* and subsequently *S. mekongi* diverged from antecedents resembling *S. sinensium*. *Schistosoma sinensium* is a relative of *S. ovuncatum.* It occurred in northern India, northwest Thailand, and southern China (Wang et al., 2021). In this study, it is revealed that *S. ovuncatum* diverged into *S. sinensium* and Asian human *Schistosoma.* Percent sequence identity and E values support this finding (Table 4 and Figure 1).

Schistosoma japonicum spreads eastward and establishes distinct groups in the central and eastern provinces of China along the Yangtze River (Young et al., 2015). However, Yin et al. (2015) suggested that *S. japonicum* spread from the middle and lower reaches of the Yangtze River to the mountainous regions of China, to Japan, and then to Southeast Asia (Yin et al., 2015). Schistosomiasis japonica occurs in China, Indonesia, and the Philippines (Maezawa et al., 2018). Its intermediate hosts include *O. lorelindoensis* (formerly *O. lindoensis*) and *O. robertsoni* (formerly *O. hupensis robertsoni*) (Hauswald et al., 2011).

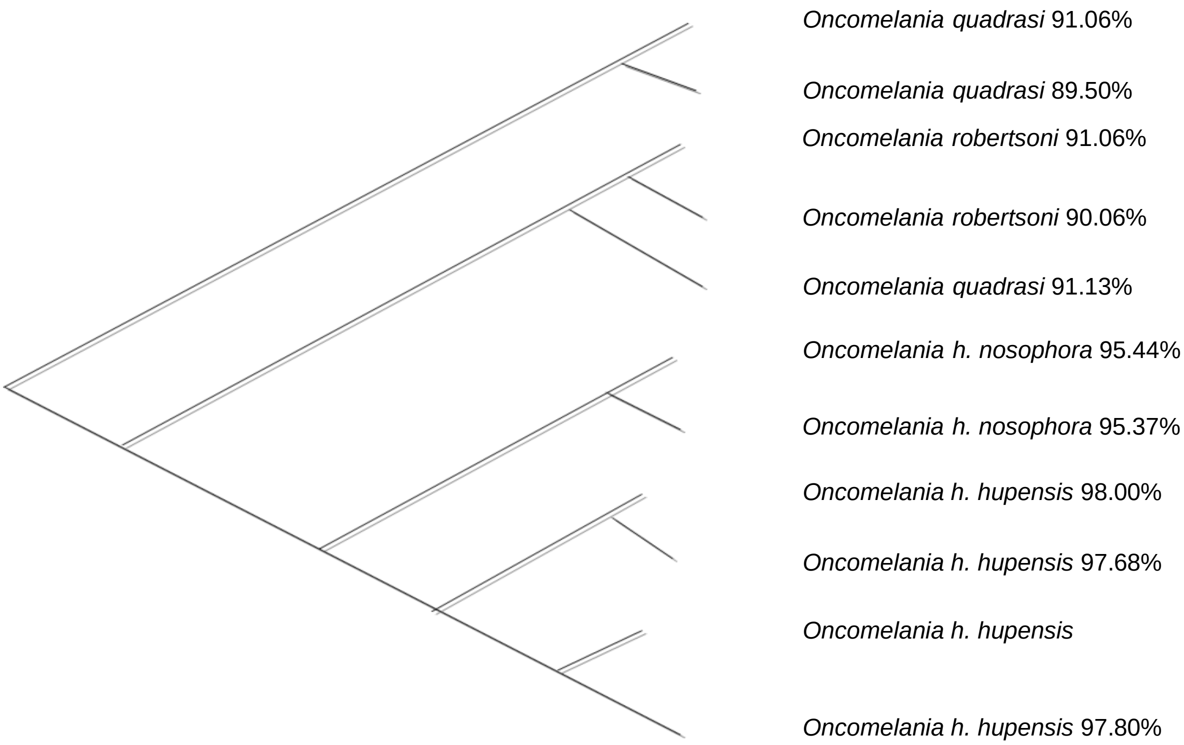

Figure 3. *Oncomelania* China, Japan, and Southeast Asia. Relatedness of *O. hupensis, O. h. hupensis, O. h. nosophora, O. robertsoni,* and *O. quadrasi*. *h.: hupensis.* Modified from NCBI BLAST pairwise alignment.

Phylogenetic tracking showed that *S. malayensis* originated from *S. mekongi* radiation in Cambodia at 2.5 Ma and approximately 3.8 Ma for *S. japonicum* (Attwood et al., 2019). The divergence of the *S. sinensium* group in Central Asia is dated to 4.6 Ma (Attwood et al., 2008). Phylogeography tracking shows that proto-*S. malayensis* entered Vietnam from Hunan via the Red River Valley (Attwood et al., 2019). The aothors also stated that the *Schistosoma malayensis* clade entered Southeast Asia using a Vietnam-to-Cambodia route. Molecular dating suggests a radiation of *S. mekongi* into Cambodia and Laos at approximately 1.3 Ma. In addition, *S. malayensis-mekongi* diverged from *S. sinensium* during their migration from Hunan (Attwood et al., 2019). Furthermore, Lawton et al., (2011) suggested that *S. sinensium* is a sibling of *S. japonicum* (Lawron et al., 2011). It is also a sister group to other species: *Schistosoma ovuncatum, S. mekongi, S. malayensis,* and *S. japonicum. Schistosoma ovuncatum* is relatively close to *S. sinensium* (Wang et al., 2021). In Asia, three *Schistosoma* species cause human schistosomiasis: *Schistosoma japonicum, S. malayensis,* and *S. mekongi* (Leshem et al., 2009). *Schistosoma malayensis* and *S. mekongi* differ only in biogeography, life cycle parameters, and intermediate hosts (Attwood et al., 2008).

According to the obtained in this research sequencing results, I found that Philippines *S. japonicum* shares 99.45% identity with Malaysia *S. malayensis* and E value equals to 0.0. It also shares 98.77% identity with Laos *S. mekongi* and E value equals to 1e-167, suggesting that Philippines *S. japonicum* is closer to Malaysia *S. malayensis* than Laos *S. mekongi*. However, the sequence of Malaysia *S. malayensis* resulted in 100% identity with Laos *S. mekongi* and E value equals to 2e-131. It also resulted in 99.45% identity with Philippines *S. japonicum* and E value equals to 0.0 (Table 4), indicating Philippines *S. japonicum* first diverged into Laos *S. mekongi* then to Malaysia *S. malayensis.* All E values are close to 0.0. This means that the evolutionary relationships among these species have very robust sequence matches. In addition, according to pairwise alignments with dots for identities and CDS, Malaysia *S. malayensis* has no different genetic codes (bases) from Laos *S. mekongi*. However, it has ten different bases (99%) from

Philippines *S. japonicum*, 37 different bases from China *S. sinensium*, and five different bases from Thailand *S. ovuncatum* and China *S. sinensium*. This suggests that Malaysia *S. malayensis* is close to Laos *S. mekongi* than to Philippines *S. japonicum.* Philippines *S. japonicum* is 98.07% close to China *S. sinensium* with E value equals to 0.0 and 97.85% to Thailand *S. ovuncatum* with E value equals to 1e-162, meaning that China *S. sinensium* diverged from Thailand *S. ovuncatum* and then to Philippines *S. japonicum* as described in slanted cladogram. Moreover, Laos *S. mekongi* diverged from Philippines *S. japonicum* and Malaysia *S. malayensis* diverged from Laos *S. mekongi.* It is according to the branching patterns and percentage identities in slanted cladogram (Table 4 and Figure 1).

Two subfamilies of Asian schistosomes intermediate hosts comprise *Pomatiopsinae* and *Triculinae* (Kameda and Kato, 2011). *Triculinae* radiate as aquatic snails in freshwater habitats in Southeast Asia and Southern China, namely Yunnan Province, China. It also occurs in Tibet (Wang et al., 2021). *Pomatiopsinae* spread throughout the world and have a variety of lifestyles ranging from aquatic to amphibian. *Triculinae* can include *Neotricula aperta, Robertsiella* spp., and *Tricula* spp. such as *Tricula bollingi* and *Tricula hortensis* (Liu et al., 2014). *Pomatiopsinae* belongs to the genus *Oncomelania,* which comprises *O. hupensis* (Liu et al., 2014) *O. lorelindoensis, O. robertsoni,* and *O. quadrasi* (Nelwan, 2022). *Oncomelania* occurs in Sulawesi, the Philippines, Taiwan, Japan, and China. It is an intermediate host of *S. japonicum. Neotricula aperta* is the intermediate host of *S. mekongi.* It is the only intermediate host for *S. mekongi*. Attwood and Upatham (2012) suggested that there are three strains of *N. aperta: α, β,* and *γ.* Only the *γ*-strain is epidemiologically significant (Attwood and Uphatam, 2012). *Robertsiella* spp. is an intermediate host of *S. malayensis. Tricula bollingi* and *Tricula hortensis* are intermediate hosts of *S. sinensium* (Wang et al., 2021). In addition, *Tricula* spp. can also be an intermediate host of *S. ovuncatum*.

According to the obtained in this work sequence results of the COI gene, China *O. robertsoni*, which belongs to *Pomatiopsinae,* shares 86.12% identity with Cambodia/Laos *N. aperta* and E value equals to 0.0. It has the highest percentage among *Triculinae*. The second place is *Robertsiella* sp. Malaysia, with an identity similarity of 85.88% and E value equals to 0.0. The third most common species was Thailand *Tricula bollingi,* with an identity similarity of 85.28% and E value equals to 1e-180 (Table 5). This means that all *Onomelania, Neotricula aperta* and *Robertsiella* spp. were derived from the same ancestor. The genus *Oncomelania* belongs to *Pomatiopsinae*, while the genera *Neotricula, Robertsiella* and *Tricula* belong to *Triculinae*. All were derived from *Pomatiopsidae* as the same ancestor. According to alignments pairwise with dots for identities and CDS, China *O. robertsoni* has 83 different bases (86%) from Laos *N. aperta*, 84 different bases (86%) from Malaysia *Robertsiella* sp., and 88 different bases (85%) from Thailand *T. bollingi*. Furthermore, the sequence results show that Laos's *N. aperta* shares approximately 84-86% identity with China *O. hupensis,* China *O. robertsoni*, China *O. hupensis hupensis*, and China *O. hupensis tangi*. These findings suggest that China *O. robertsoni* is close to Laos's *N. aperta* (86.12%) than to Malaysia *Robersiella* sp. (85.88%) and Thailand *Tricula bollingi* (85.28%). According to the branching patterns and percentage identities, it seems that China *O. robertsoni* (*Pomatiopsinae*) came from the same ancestor (*Pomatiopsidae*) as Laos's *N. aperta,* Malaysia *Robertsiella* sp., and Thailand *Tricula bollingi* (*Triculinae*).

Nelwan (2022) suggests that the genus *Oncomelania* comprises five species: China *Oncomelania hupensis,* Sulawesi *O. lorelindoensis,* Japan *O. minima,* China *O. robertsoni,* and Philippines *O. quadrasi*. However, the author also points out that GenBank data for Sulawesi *O. lorelindoensis* (formerly *O. hupensis lindoensis*) are not yet available (Nelwan, 2022). *Oncomelania hupensis lindoensis* (Sulawesi *O. lorelindoensis*) has a distant relationship as a subspecies of China *O. hupensis.* It shares 86.10% identity with China *O. hupensis hupensis* (Sutrisnawati et al., 2022). In addition, Sulawesi *O. lorelindoensis* and Philippines *O. quadrasi* have a 6.2 pairwise difference in the percentage of the 12S rRNA gene (Okamoto et al., 2003). This suggests that Sulawesi *O. lorelindoensis* and Philippines *O. quadrasi* have distant relatedness. If the percentage of identity is high, it should be Philippines *O. quadrasi,* a subspecies of Sulawesi *O. lorelindoensis.* Liu et al. (2014) suggested that proto-*Oncomelania* originated from Northwest Australia, which today forms parts of Borneo and eastern Indonesia (Liu et al., 2014), including Sulawesi. This suggests that Sulawesi proto-*Oncomelania,* i.e., proto-*Oncomelania lorelindoensis,* is older than the Philippines proto-*Oncomelania,* i.e., proto-*Oncomelania quadrasi.* Other proto-*Oncomelania* comprises China proto-*Oncomelania hupensis,* Japan proto-*Oncomelania minima,* and China proto-*Oncomelania robertsoni*, due to each species of *Oncomelania* rising from its antecedent form.

In China, there are two species of *Oncomelania*: China *O. hupensis* and China *O. robertsoni.* China *O. hupensis* has subspecies such as China *O. hupensis hupensis* and China *O. hupensis tangi* (Nelwan, 2022). To make sure of this, I performed NCBI BLAST on China *O. hupensis*. I took eleven *Oncomelania* from the sequence results. These results show that China *O. hupensis* shares 97.68-98.00% identity with all China *O. hupensis hupensis*. In addition, the sequence of China *O. hupensis* shares approximately 95% identity with all Japan *O. hupensis nosophora*. All E values are 0.0, indicating very strong robust sequence matches (Table 6). This suggests that all China *O. hupensis hupensis* and all Japan *O. hupensis nosophora* are closely related to China *O. hupensis*. This confirms that they are all subspecies of China *O. hupensis,* as suggested by Nelwan (2022). Moreover, the sequence results of China *O. hupensis* shares 90.06% identity with China *O. robertsoni* and 89.50% identity with Philippines *O. quadrasi*. According to Pairwise with dots for identities and CDS, China *O. hupensis* has 130 different amino acids from Philippines *O. quadrasi*. It has 1322 different bases from Philippines *O. quadrasi* (89%). China *O. robertsoni* has 173 different amino acids from China *O. hupensis*. It has 1569 different bases from China *O. hupensis* (13636/15205 = 89.63%). China *O. robertsoni* has 170 different amino acids from Philippines *O. quadrasi*. It has 1675 different bases from Philippines *O. quadrasi* (89%). These findings suggest that China *O. robertsoni* and Philippines *O. quadrasi* are distantly related to China *O. hupensis.*

Nelwan (2022) suggested that China *O. robertsoni* and Philippines *O. quadrasi* are full species in the genus *Oncomelania*. In addition, Sulawesi *O. lorelindoensis* is more closely related to China *O. robertsoni* than China *O. hupensis.* In the current study, I found that China *O. robertsoni* EU079378.1 shares 94.04% identity with all China *O. robertsoni*, *Oncomelania hupensis* has two species: China *O. hupensis hupensis* and Japan *O. hupensis nosophora* (Table 6 and Figure 3). Moreover, China *O. hupensis,* China *O. robertsoni,* and Philippines *O. quadrasi* are in different groups in a tree-view slanted cladogram (Figure 3). This finding supports the fact that China *O. hupensis,* China *O. robertsoni,* and Philippines *O. quadrasi* are separate species. This supports the fact that the genus *Oncomelania* comprises five species: China *O. hupensis,* Sulawesi *O. lorelindoensis,* Japan *O. minima,* China *O. robertsoni,* and Philippines *O. quadrasi*. In addition, these findings also support that China *O. hupensis* comprises five subspecies: China *O. hupensis hupensis,* Taiwan *O. hupensis chiui,* Taiwan *O. hupensis formosana,* Japan *O. hupensis nosophora,* and China *O. hupensis tangi* (Nelwan, 2022).

Sequences of China *O. hupensis* COI and Philippines *O. quadrasi* COI against all China *O. robertsoni* of the Sichuan Plain (SCB) (Attwood et al., 2015) confirm that Philippines *O. quadrasi* and China *O. robertsoni* are distinct from China *O. hupensis*. For example, the sequence of Philippines *O. quadrasi* shares 87.77% identity with China *O. robertsoni* and E value equals to 0.0 (Table S1), and the sequence of China *O. hupensis* shares 88.99% similarity with China *O. robertsoni* and E value equals to 0.0 (Table S2). The sequence of China *O. hupenis* shares an identity of 84.48% with Philippines *O. quadrasi* (Nelwan, 2022). This supports the fact that Philippines *O. quadrasi* and China *O. robertsoni* did not drive from China *O. hupensis* because they have a distant relationship. In addition, it also supports the fact that China *O. hupensis,* Sulawesi *O. lorelindoensis,* Japan *O. minima,* China *O. robertsoni,* and Philippines *O. quadrasi* are derived from their antecedent forms. Thus, the genus *Oncomelania* is derived from its antecedent forms. Moreover, these findings support the fact that, except for China *O. hupensis, Oncomelania* developed in its own territory. China *O. robertsoni* was developed in China. Sulawesi *O. lorelindoensis* was developed in Sulawesi (Indonesia). Japan *O. minima* were developed in Japan. Philippines *O. quadrasi* developed in the Philippines. China *O. hupensis* gave rise to Taiwan *O. hupensis chiui,* Taiwan *O. hupensis formosona,* China *O. hupensis hupensis*, Japan *O. hupensis nosophora,* and China *O. hupensis tangi.*

Sequence results did not support the idea that China *O. robertsoni* has subspecies. China *O. robertsoni* of the Sichuan Plain (SCB) (Attwood et al., 2015) shares 91.20% identity with China *O. robertsoni* and E value equals to 0.0 (Table S3). The sequence of China *O. robertsoni* from the Sichuan Anning River Valley (SAV) (Attwood et al., 2015) showed that China *O. robertsoni*, for example, shares 91-92% identity with all China *O. robertsoni* and E value equals to 0.0 (Table S4). The sequence of China *O. robertsoni* in the SCB shares 97.63% identity with China *O. robertsoni* and E value equals to 0.0 (SAV), shares 95.55% identity with China *O. robertsoni* and E value equals to 0.0 (YEB) (Table S5). These results show that there are four China *O. robertsoni* clades in China. *Oncomelania robertsoni* SAV, SCB, and YEB is a novel clade of *O. robertsoni.* All E values in Table S1 through Table S5 are 0.0, suggesting very robust sequence matches.

This study has advantages and limitations. Advantages can include the easy use and reliability of the BLAST technique. For example, the sequence of China *O. hupensis* NC_013073 using NCBI BLAST can produce other data, such as Philippines *O. quadrasi* LC276227.1 and China *O. robertsoni* LC276228.1. Another example, China *O. robertsoni* KR002675.1 in Table 1 is new data. In the NCBI BLAST results, the *O. robertsoni* group would occupy a separate group from other species in the slanted cladogram (see Figure 3). Thus, it does not require new data collection. NCBI BLAST is as reliable as ClustalW, MUSCLE, PhyML and RaxML. The limitations are that, in some cases, GenBank accession numbers are not accurate. For example, China *O. hupensis hupensis* JF284697 should be China *O. robertsoni* JF284697. China *O. hupensis hupensis* JF284698 should be Philippines *O. quadrasi* JF284698. There were no samples of parasites and intermediate hosts taken to obtain partial DNA sequences using the popular Former and Palumbi primers to compare data from previous studies, for example, although it does not affect the results of this study. In addition, data on *O. hupensis lindoensis* (*O. lorelindoensis*) are not yet available at NCBI. In this study, I used only GenBank data from Philippines *O. quadrasi* to assess Southeast Asian *Oncomelania* as an intact species. However, this does not mean that Sulawesi *O. lorelindoensis* is not a full species of the genus *Oncomelania.* This is due to the proto-*Oncomelania* coming from eastern Indonesia, namely Sulawesi. Then, off to the Philippines, Japan, and China.

Conclusions

The *Schistosoma japonicum* complex comprises *S. japonicum, S. malayensis, S. mekongi, S. ovuncatum,* and *S. sinensium. Schistosoma japonicum, S. malayensis,* and *S. mekongi* cause human schistosomiasis in the Far East. The sequence results showed that in Southeast Asia, *Schistosoma ovuncatum* diverged to *S. sinensium, S. japonicum, S. mekongi,* and then to *S. malayensis.* The sequence results reveal that *S. japonicum* is close to *S. sinensium* than that of *S. ovuncatum,* meaning that *S. ovuncatum* diverged to *S. sinensium* then to *S. japonicum. Schistosoma malayensis* is 100% close to *S. mekongi* than that of *S. japonicum* with 99.45%*,* meaning that *S. japonicum* diverged to *S. mekongi* then to *S. malayensis. Schistosoma ovuncatum* and *S. sinensium* are not human schistosomiasis. Intermediate hosts of Far East schistosomiasis can include *Neotricula aperta, Oncomelania* snails, *Robertsiella* sp., and *Tricula* sp. All *Oncomelania* are *Pomatiopsinae*, while *Neotricula, Robertsiella* and *Tricula* are *Triculinae. Neotricula aperta, Robertsiella* sp, and *Tricula* sp. occur in Southeast Asia. In addition, *Tricula* sp. can also be found in China and India. Southeast Asian *Oncomelania* includes *O. lorelindoensis* and *O. quadrasi. Oncomelania hupensis* and *O. robertsoni* occur in China. All *Oncomelania* have 82 to 86% close to *N. aperta, Robertsiella* sp., and *Tricula bollingi.* For example, *O. robertsoni* is 86.12% close to *N. aperta,* followed by *Robertsiella* sp. with 85.88% and *Tricula bollingi* with 85.28%*,* suggesting that they derived from the same ancestor (*Pomatiopsidae*)*.* This study is the first to show that *Pomatiopsinae* and *Triculanae* have relatedness through *O. robertsoni* and *N. aperta. Oncomelania minima* occurs in Japan. *Oncomelania lorelindoensis* occurs in Indonesia, and *O. quadrasi* occurs in the Philippines. Proto-*Oncomelania* is derived from eastern Indonesia, i.e., Sulawesi. In addition, *Oncomelania* was derived from its antecedent forms at its respective locations. This is the first study explaining that the genus *Oncomelania* rose from its antecedent forms. In addition, this study confirms that this genus consists of five species: *O. hupensis, O. lorelindoenis, O. minima, O. robertsoni,* and *O. quadrasi. Oncomelania robertsoni* comprises four clades, and one of them is a novel clade of *O. robertsoni.*

Novel findings of this study include relatedness of *Schistosoma japonicum* complex, relatedness of *Pomatiopsinae* and *Triculinae*, and the new status of *Oncomelania.* This study reveals clearly the relatedness of *Schistosoma japonicum* complex. It was derived from *S. ovuncatum*, diverged to *S. sinensium, S. japonicum, S. mekong,* and *S. malayensis.* Intermediate hosts of Asian human schistosomiasis were derived from *Pomatiopsidae* as common ancestor. These include the genera *Oncomelania, Neotricula, Robertsiella,* and *Tricula.* The genus *Oncomelania* belongs to *Pomatiopsinae*. The genera *Neotricula, Robertsiella* and *Tricula* belong to *Triculinae*. The close relationships between these two subfamilies are in *O. robertsoni* and *N. aperta.* This relatedness reveals that *O. robertsoni* and *N. aperta* were derived from the common ancestor. *Oncomelania robertsoni* represents *Pomatiopsinae* and *N. aperta* represents *Triculinae*. *Pomatiopsinae* and *Triculinae* were derived from *Pomatiopsidae*. This study also reveals the genus *Oncomelania* was derived from its predecessor forms in Sulawesi. It is proto-*Oncomelania lorelindoensis*. This predecessor form spread to Philippines, Japan, and China. In its own habitats, each predecessor forms gave rise to *Oncomelania* snails: China *Oncomelania hupensis,* Sulawesi *O. lorelindoensis,* Japan *O. minima,* China *O. robertsoni,* and Philippines *O. quadrasi. Oncomelania hupensis* formed its subspecies: *Oncomelania h. hupensis, O. h. tangi, O. h. chiui, O. h. formosana,* and *O. h. nosophora.* Others do not have subspecies. In addition, this study also reveals that *O. robertsoni* has four clades. Although this study did not support that *O. robertsoni* has a subspecies, further investigation is still needed.

Additional information

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References

Al-Olayan, E.M. et al. (2019). *Ceratonia siliqua* pod extract ameliorates *Schistosoma mansoni*-induced liver fibrosis and oxidative sress. *BMC Complementary and Alternative* Medicine, 16**,** 434. https://doi.org/10.1186/s12906-016-1389-1.
Attwood, S.W, Fatih, F.A, Upatham, .E.S. (2008). DNA-sequence variation among *Schistosoma mekongi* populations and related taxa; phylogeography and the current distribution of Asian schistosomiasis. *PloS Negl Trop Dis*, 2(3), e200. https://doi.org/10.1371/journal.pntd.000200.
Attwood, S.W., Upatham E.S. (2012). Observations on *Neotricula aperta* (Gastropoda: Pomatiopsidae) population densities in Thailand and Central Laos: implications for the spread of Mekong schistosomiasis. *Parasites & Vectors*, 5, 126. https://doi.org/doi:10.1186/1756-3305-5-126.
Attwood, S.W, et al. (2015) Comparative phylogenitic studies on *Schistosoma japonicum* and its snail intermediate host *Oncomelania hupensis:* origins, dispersal and coevolution. *PloS Negl Trop Dis*, 9(7), e0003935. https://doi.org/10.1371/journal.pntd.0003935.
Attwood, S.W., Liu, L., Huo, G-N. (2019). Population genetic structure and geographical variation in *Neotricula aperta* (Gastropoda: Pomatiopsidae), the snail intermediate host of *Schistosoma mekongi* (Digenea: Schistosomatidae). *PloS Negl Trop Dis*, 13(1), e0007061. https://doi.org/10.1371/journal.pntd.0007061.
Au, F.F.A. et al. (2023). Status quo and future perspectives of molecular and genomic studies on the genus *Biomphalaria*—the intermediate snail host of *Schistosoma mansoni*. *Molecular Sciences*, 24, 4895. https://doi.org/10.3390/ijms24054895.
Brant, S.V., Loker, E.S. (2005). Can specialized pathogens colonize distantly related hosts? Schistosome evolution as a case study. *PloS Pathog*, 1(3), e38. https://doi.org/10.1371/journal.ppat.0010038.
Budiono, N.G. et al. (2019). The contribution of domestic animals to the transmission of schistosomiasis japonica in the Lindu Subdistrict of the Central Sulawesi Province, Indonesia. *Veterinary World*, 12(10), 1591-1598. https://doi.org/10.14202/vetworld.2019.1591-1598.
Budiono, N.G. et al. (2020) Humoral responses to *Schistosoma japonicum* soluble egg antigens in domestic animals in Lindu Subdistrict, Central Sulawesi Province, Indonesia. *International Journal of One Health*, 6(2), 99-108. https://doi.org/10.14202/IJOH.2019.99-108.
Davis, G.M. (1992) Evolution of prosobranch snails transmitting Asian *Schistosoma;* coevolution with *Schistosoma*: a review. *Prog Clin Parasitol*, 3, 145-204.
Fang, J. et al. (2022) Single-target detection of *Oncomelania hupensis* based on improved YOLOv5s. *Front. in Bioeng. Biotechnol,* 10, 861079. https://doiorg/10.3389/fbioe.2022.861079.
Gordon, C.A et al. (2019). Asian schistosomiasis: current status and prospects for control leading to elimination. *Trop. Med. Infect.*, 4: 40. https://doi.org/10.3390/tropicalmed4010040.
Inceboz, T. (2022) One health concept against schistosomiasis: an overview. New Horizon for schistosomiasis research, intechopen, London. https://doi.org/10.5772/intechopen.106912.
Haggag, A.A. et al. (2019). Thirty-day Daily Comparisons of Kato-Katz and CCA Assays of 45 Egyptian Children in Areas with Very Low Prevalence of *Schistosoma mansoni*. *Am J Trop Med*, 100(3), 578-583. https://doi.orh10.4269/ajtmh.18-0829.
Hauswald, A.K. et al. (2011). Stirred, not shaken: genetic structure of the intermediate snail host *Oncomelania hupensis robertsoni* in an historically endemic schistosomiasis area. *Parasites & Vectors*, 4, 206. https://doi.org10.1186/1756-3305-4-206.
Kameda, Y., Kato, M. (2011) Terrestrial invasion of pamatiopsid gastropods in the heavy-snow region of the Japanese archipelago. *BMC Evolutionary Biology*, 11, 118. https://doi.org/10.1186/1471-2148-11-118.
Latif, B. et al. (2013). Autochthhonous human schistosomiasis, Malaysia. *Emerging Infectious Diseases*, 19(8), 1340-1341. https://doi.org/10.3201/eid1908.121710.
Lawton, S.P. et al. (2011). Genomes and geography: genomic insights into the evolution and phylogeography of the genus *Schistosoma. Parasites and Vectors*, 4, 131. https://doi.org/10.1186/1756-3305-4-131.
Leshem, E., Marva, E., Schwartz, E. (2009) Travel-related schistosomiasis acquired in Laos. *Emerging Infectious Diseases*, 15(11), 1823-1825. https://doi.org/10.3201/eid1511.090611.
Liu, L. et al. (2014). A phylogeny for the pomatiopsidae (Gastropoda: Rissooidea): A resource for taxonomic, parasitological, biodiversity studies. *BMC Evolutionary Biology*, 14, 29. https://doi.org/10.1186/1471-2148-14-29.
Maezawa, K. et al. (2018). Real-time observation of pathophysiological processes during murine experimental *Schistosoma japonicum* infection using high-resolution ultrasound imaging. *Tropical Medicine and Health*, 46: 1. https://doi.org/10.1186/s41182-017-0082-5.
Mouahid, G. et al. (2018). Transplantation of schistosome sporocysts between host snails: A video guide. *Wellcome Open Research*, 3(3). https://doi.org/10.12688/wellcomeopenres.

Nahum, L.A. et al. (2012). New frontiers in *Schistosoma* genomics and transcriptomics. *International of Parasitology Research*, 2012(849132), 1-11. https://doi.org/10.1155/2012/849132.

Nelwan, M.L. (2019). Schistosomiasis: life cycle, diagnosis, and control. *Current Therapeutic Research*, 91, 5-9. https://doi.org/10.1016/j.curtheres.2019.06.001.

Nelwan, M.L. (2023) *Oncomelania lorelindoensis:* the intermediate host of Sulawesi's *Schistosoma japonicum.* Preprint at https://doi.org/10.2103/rs.3.rs-3471885/v1

Nelwan, M.L. (2022). Indonesia *Schistosoma japonicum*: Origin, genus *Oncomelania,* and elimination of the parasite with cluster genes inoculated into female *Oncomelania lorelindoensis* via CRISPR/Cas9 system. *Afr. J. Bio. Sc,* 4(4), 23-38. https://doi.org/10.33472/AFJBS.4.4.2022.23-38.

Neves, B.J. et al. (2015). Natural Products as Leads in Schistosome Drug Discovery. *Molecules,* 2, 1872-903. https://doi.org/10.3390/molecules20021872.

Okamoto, M. et al. (2003) Pgylogenetic relationships of snails of the genera *Oncomelania* and *Tricula* inferred from the mitochondrial 12S rRNA gene. *Jpn. J. Trop. Med. Hyg.*, 31(1), 5-10.

Orpin, J.B., Mzungu, I., Usman-Sani, H. (2022). Prevalence of schistosomiasis among primary school pupils in Guma LGA of Benue state. *Afr. J. Bio. Sc.*, 4(4), 48-55. https://doi.org/10.3347/AFJBS.4.4.02.48-55.

Rothe, C. et al. (2021). Developing Epidemicity of Schistosomiasis, Corsica, France. *Emerging Infectious Diseases*, 27(1),319-321. https://doi.org/10.3201/eid2701.204391.

Sady, H., et al. (2015) New insights into the genetic diversity of *Schistosoma mansoni* and *S. haematobium* in Yemen. *Parasites & Vectors,* 8, 544. https://doi.org/10.1186/s13071-015-1168-8.

Sanchez, R.C.O. et al. (2021) Immunoinformatics design of multiepitope peptide based vaccines against *Schistosoma mansoni* using transmembrane proteins a target. *Front. Immunol,* 12, 021706. https://doi.org/10.3389/fimmu.2021.021706.

Smith, E. (2023). BLAST compares & identifies sequences. *Berkeley Library*, https://guides.lib.berkeley.edu/ncbi/blast

Sudomo, M. (1983). Cross infectivity study of four subspecies of *Oncomelania hupensis* in four geographical strains of *Schistosoma japonicum*. *Proceedings ITB*, 16(2): 41-46.

Sutrisnawati, Ramadhan, A., Trianto, M. (2022) Molecular identification of *Oncomelania hupensis lindoensis*, snail intermediate hosts of *Schistosoma japonicum* from Central Sulawesi, Indonesia. *Biodiversitas*, 23(11), 5989-5994. https://doi.org/10.13057/biodiv/d231153.

Walker, J.A. (2011). Insight into the functional biology of schistosomes. *Parasites & Vector*, 4, 203. https://doi.org/10.1186/1756-3305-4-203.

Wang, X. et al. (2021). First report of *Schistosoma sinensium* infecting *Tupaia belangeri* and *Tricula* sp. LF. *International Journal for Parasitology: Parasites and Wildlife*, 14(2021), 84-90. https://doi.org/10.1016/j.ijppaw.2021.01.005.

Yin, M. et al. (2015). Codispersal of the blood fluke *Schistosoma japonicum* and *Homo sapiens* in the Neolithic age. *Scientific Reports*, 5, 18058. https://doi.org/10.1038/srep18058.

Young, N.D. et al. (2015). Exploring molecular variation in *Schistosoma japonicum* in China. *Scientific Reports*, 5, 17345. https://doi.org/10.1038/srep17345.

ZooBank, https://zoobank.org/References/d71b8509-ec6e-492b-8e81-8910957a7b6a

Supplementary files

Table S1. Percentage identities of *Oncomelania quadrasi* and *Oncomelania robertsoni* CO1

Species			***O. quadrasi***
	Accession	**E value**	**DQ112287.1**
O. robertsoni	DQ212800.1	0.0	87.77%
O. robertsoni	DQ212803.1	0.0	87.62%
O. robertsoni	DQ212805.1	0.0	87.62%
O. robertsoni	KR002675.1	0.0	87.25%
O. robertsoni	DQ212797.1	0.0	86.83%
O. robertsoni	DQ212798.1	0.0	86.83%
O. robertsoni	DQ212813.1	0.0	86.83%
O. robertsoni	DQ212799.1	0.0	86.68%
O. robertsoni	DQ212801.1	0.0	86.68%
O. robertsoni	DQ212802.1	0.0	86.68%
O. robertsoni	DQ212806.1	0.0	86.68%
O. robertsoni	DQ212807.1	0.0	86.68%
O. robertsoni	DQ212808.1	0.0	86.68%
O. robertsoni	DQ212809.1	0.0	86.68%
O. robertsoni	DQ212810.1	0.0	86.68%

Species			*O. quadrasi*
	Accession	**E value**	**DQ112287.1**
O. robertsoni	DQ212812.1	0.0	86.68%
O. robertsoni	DQ212811.1	0.0	86.36%
O. robertsoni	JF284697.1	0.0	86.36%
O. robertsoni	AF531547.1	0.0	85.91%

O.: Oncomelania; S1: Supplementary 1

Table S2. Percentage identities of *Oncomelania hupensis* and *Omcomelania robertsoni* CO1

Species			*O. hupensis*
	Accession	**E value**	**GU367391.1**
O. robertsoni	DQ212802.1	0.0	88.99%
O. robertsoni	DQ212797.1	0.0	88.77%
O. robertsoni	DQ212798.1	0.0	88.77%
O. robertsoni	DQ212803.1	0.0	88.71%
O. robertsoni	DQ212805.1	0.0	88.71%
O. robertsoni	DQ212800.1	0.0	88.67%
O. robertsoni	KR002675.1	0.0	88.29%
O. robertsoni	AF531547.1	0.0	87.84%
O. robertsoni	DQ212813.1	0.0	87.77%
O. robertsoni	DQ212808.1	0.0	87.62%
O. robertsoni	DQ212809.1	0.0	87.62%
O. robertsoni	DQ212810.1	0.0	87.62%
O. robertsoni	DQ212812.1	0.0	87.62%
O. robertsoni	JF284697.1	0.0	87.30%
O. robertsoni	DQ212799.1	0.0	86.99%
O. robertsoni	DQ212801.1	0.0	86.99%
O. robertsoni	DQ212804.1	0.0	86.99%
O. robertsoni	DQ212806.1	0.0	86.99%
O. robertsoni	DQ212807.1	0.0	86.99%
O. robertsoni	DQ212811.1	0.0	86.68%

O.: Oncomelania; S2: Supplementary 2

Table S3. Megablast results on *Oncomelania robertsoni* Sichuan Plain SCB

Species			*O. robertsoni*
	accession	**E value**	**KR002675.1**
O. robertsoni	DQ212808.1	0.0	99.67%
O. robertsoni	DQ212809.1	0.0	99.67%
O. robertsoni	DQ212810.1	0.0	99.67%
O. robertsoni	DQ212812.1	0.0	99.67%
O. robertsoni	DQ212797.1	0.0	99.50%
O. robertsoni	DQ212798.1	0.0	99.50%
O. robertsoni	DQ212813.1	0.0	99.50%
O. robertsoni	JF284697.1	0.0	98.49%
O. robertsoni	DQ212799.1	0.0	97.83%
O. robertsoni	DQ212801.1	0.0	97.83%
O. robertsoni	DQ212802.1	0.0	97.83%
O. robertsoni	DQ212804.1	0.0	97.83%
O. robertsoni	DQ212806.1	0.0	97.83%
O. robertsoni	DQ212807.1	0.0	97.83%
O. robertsoni	DQ212811.1	0.0	97.49%
O. robertsoni	DQ212800.1	0.0	95.48%
O. robertsoni	DQ212803.1	0.0	95.32%
O. robertsoni	DQ212805.1	0.0	95.32%
O. robertsoni	AF531547.1	0.0	91.20%

O.: Oncomelania; S3: Supplementary 3

Table S4. Megablast results on Oncomelania robertsoni Sichuan Anning River Valley (China) – SAV

Species	accession	E value	*O. robertsoni* AF213339.1
O. robertsoni	DQ212814.1	0.0	100%
O. robertsoni	DQ212815.1	0.0	100%
O. robertsoni	DQ212816.1	0.0	100%
O. robertsoni	DQ212831.1	0.0	98.90%
O. robertsoni	DQ212834.1	0.0	98.90%
O. robertsoni	DQ212835.1	0.0	98.90%
O. robertsoni	DQ212836.1	0.0	98.90%
O. robertsoni	DQ212822.1	0.0	98.75%
O. robertsoni	DQ212825.1	0.0	98.75%
O. robertsoni	DQ212826.1	0.0	98.75%
O. robertsoni	DQ212827.1	0.0	98.75%
O. robertsoni	DQ212828.1	0.0	98.75%
O. robertsoni	DQ212830.1	0.0	98.75%
O. robertsoni	DQ212832.1	0.0	98.75%
O. robertsoni	DQ212833.1	0.0	98.75%
O. robertsoni	DQ212817.1	0.0	98.59%
O. robertsoni	DQ212823.1	0.0	98.59%
O. robertsoni	DQ212824.1	0.0	98.59%
O. robertsoni	DQ212818.1	0.0	98.43%
O. robertsoni	DQ212819.1	0.0	98.43%
O. robertsoni	DQ212820.1	0.0	98.43%
O. robertsoni	DQ212828.1	0.0	98.43%
O. robertsoni	DQ212821.1	0.0	98.28%
O. robertsoni	DQ212837.1	0.0	98.28%
O. robertsoni	DQ212838.1	0.0	98.28%
O. robertsoni	DQ212839.1	0.0	98.28%
O. robertsoni	DQ212840.1	0.0	98.28%
O. robertsoni	DQ212841.1	0.0	95.77%
O. robertsoni	DQ212842.1	0.0	95.77%
O. robertsoni	DQ212843.1	0.0	95.77%
O. robertsoni	DQ212845.1	0.0	95.77%
O. robertsoni	DQ212847.1	0.0	95.77%
O. robertsoni	DQ212849.1	0.0	95.77%
O. robertsoni	DQ212851.1	0.0	95.77%
O. robertsoni	DQ212848.1	0.0	95.61%
O. robertsoni	EU079378.1	0.0	92.16%
O. robertsoni	JF284697.1	0.0	92.16%
O. robertsoni	DQ112250.1	0.0	91.69%
O. robertsoni	DQ212844.1	0.0	91.07%
O. robertsoni	DQ212846.1	0.0	91.07%

O.: Oncomelania; S4: Supplementary 4

Table S5. Megablast results of *Oncomelania robertsoni*

Species	accession	E value	*O. robertsoni* AF531547.1	Location SCB
O. robertsoni	DQ212846.1	0.0	97.63%	SAV
O. robertsoni	DQ212844.1	0.0	97.46%	SAV
O. robertsoni	DQ112252.1	0.0	96.79%	YEB
O. robertsoni	DQ112250.1	0.0	96.79%	SAV
O. robertsoni	JF284691.1	0.0	96.15%	SAV
O. robertsoni	EU079378.1	0.0	96.15%	SAV
O. robertsoni	AF253075.1	0.0	95.89%	YEB
O. robertsoni	DQ212852.1	0.0	95.72%	YEB

Table S5. Megablast results of *Oncomelania robertsoni*

Species	accession	E value	*O. robertsoni* AF531547.1	Location SCB
O. robertsoni	DQ212850.1	0.0	95.40%	SAV
O. robertsoni	AF253074.1	0.0	95.55%	YEB
O. robertsoni	AF213339.1	0.0	91.88%	SAV
O. robertsoni	DQ212836.1	0.0	91.54%	SAV
O. robertsoni	DQ212812.1	0.0	91.20%	SCB
O. robertsoni	KR002675.1	0.0	91.20%	SCB
O. robertsoni	DQ212851.1	0.0	90.52%	SAV
O. robertsoni	DQ212803.1	0.0	90.19%	SCB

*O.: Oncomelania,*Sichuan Anning River Valley (China)-SAV, Sichuan Plain (China)-SCB, Yunnan: Erhai Basin-YEB; S5: Supplementary 5

Комплекс *Schistosoma japonicum*: варіації COI-послідовностей паразитів та їх проміжних господарів, проаналізованих за допомогою BLAST

М.Л. Нельван

У цьому дослідженні я розглядаю взаємозв'язок між *S. ovuncatum, S. sinensium, S. japonicum, S. mekongi* та *S. malayensis*. Крім того, я аналізую взаємозв'язок між п'ятьма видами роду *Oncomelania* та *Neotricula aperta*, *Robertsiella* spp. і *Tricula* spp. Також я описую формування роду *Oncomelania* з його попередніх форм. Результати нуклеотидного аналізу за допомогою BLAST показали, що *S. japonicum* має 99,45% ідентичності з *S. malayensis*, 98,77% з *S. mekongi*, 98,07% з *S. sinensium* і 97,85% з *S. ovuncatum*. Послідовність *S. malayensis* виявила 100% ідентичності з *S. mekongi*. Значення Е було меншим за 0,01. У результатах вирівнювання амінокислотних послідовностей у комплексі *S. japonicum* не було виявлено замін. У *S. malayensis* і *S. mekongi* не зафіксовано замін основ, між *S. malayensis* і *S. sinensium* виявлено 37 замін основ, між *S. malayensis* і *S. japonicum* — 10 замін основ, між *S. malayensis* і *S. ovuncatum* — 5 замін основ. На кладограмі було показано, що *S. sinensium* є сестринським видом до *S. ovuncatum*. Від них відокремився *S. japonicum. Schistosoma japonicum* розділився на *S. mekongi* та S. *malayensis*. Результати також показали, що *Oncomelania robertsoni* має 86,12% ідентичності з *Neotricula aperta*, 85,88% з *Robertsiella* spp. і 85,28% з *Tricula bollingi*. Значення Е було меншим за 0,01. У вирівнюванні *O. robertsoni* та *N. aperta* виявлено дві заміни амінокислот, чотири заміни амінокислот і 84 заміни основ між *O. robertsoni* та *Robertsiella* spp., а також дві заміни амінокислот і 88 замін основ між *O. robertsoni* та *T. bollingi*. На нахиленому кладограмі дерево показало, що *Tricula* spp. розділився на *N. aperta* та *Robertsiella* spp. Це дослідження показало, що *Oncomelania* виник з попередніх форм. Результати нуклеотидного BLAST підтвердили, що *S. ovuncatum* близький до *S. sinensium*. *Schistosoma sinensium* розділився на *S. japonicum, S. mekongi і S. malayensis*. *Oncomelania robertsoni* був ближчий до *N. aperta*, ніж до *Robertsiella* spp., і до *Tricula* spp. (*Tricula bollingi*). Види роду *Oncomelania* виникли з їхніх попередників.

Ключові слова: *Schistosoma japonicum complex, Neotricula aperta, Oncomelania, Oncomelania lorelindoensis, schistosomiasis, Robertsiella spp., Tricula spp.*

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