



Exorchis sp. in the catfish *Silurus asotus* and *Oncomelania hupensis* in marshlands of Poyang Lake, China: A potential biological control tool for *Schistosoma japonicum*

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ARTICLE INFO

Keywords:

Oncomelania hupensis

Schistosoma japonicum

Exorchis sp.

Biological control strategy

Field survey

ABSTRACT

Oncomelania hupensis is the obligate intermediate host of *Schistosoma japonicum*, highlighting the medical importance of interrupting this unique and long-standing parasite-host interaction in controlling schistosomiasis transmission. It has been reported that a catfish trematode *Exorchis* sp. could have the potential to function as an effective anti-schistosomal agent in the snail host. However, the feasibility of this eco-friendly biological control strategy should be comprehensively investigated and evaluated in endemic areas for schistosomiasis. In this study, a field survey was conducted from 2012 to 2016 in the marshlands of Poyang Lake, which is one of the highly endemic regions for schistosomiasis in China. Results showed that more than half of *Silurus asotus* (65.79%) were infected with *Exorchis* sp., and the average intensity of infection was 14.21 per fish. And the average infection rate of *Exorchis* sp. in *O. hupensis* is 1.11%. These findings indicated that there are abundant biological resources for the implementation of this biological control strategy in the marshlands of Poyang Lake. The data presented here provide solid evidences for the practical application of this biological control strategy, thereby contributing to achieving the goals of the elimination of schistosomiasis.

1. Introduction

Schistosomiasis, one of the neglected tropical diseases, remains to be a significant public health problem in tropical and sub-tropical areas, especially in poor communities without potable water and adequate sanitation (Colley et al., 2014). Estimates show that almost 240 million people are affected, and more than 700 million people are at risk in 78 countries worldwide (WHO, 2023). In China, schistosomiasis japonica caused by the pathogen *Schistosoma japonicum* was mainly prevalent in regions along the Yangtze River basin including 12 provinces (or autonomous regions and municipalities) (Zhou et al., 2021). In recent years, remarkable progresses have been made in the prevention, control and elimination of schistosomiasis japonica through consecutive and vertical integrated control strategies implemented by the Chinese government (Wang et al., 2021; Zhang et al., 2022). However, several critical problems including the emergence of praziquantel-resistant parasites because of high rates of re-infection (Yu et al., 2021),

environmental pollution and damage caused by the extensive use of chemical molluscicides (Zheng et al., 2021; Shi et al., 2022), increased emerging and re-emerging snail habitats (Li et al., 2021; Zhang et al., 2022) and treatment of patients with advanced *S. japonicum* infection (Zhang et al., 2022) remain to be solved. Therefore, alternative effective anti-schistosome strategies are still urgently needed.

In addition to being the obligate intermediate host of *S. japonicum*, the freshwater snail *Oncomelania hupensis* can also serve as the first intermediate host for *Exorchis* sp., a trematode that infects the edible catfish (*Silurus asotus*), but not humans (Suliman et al., 2014; Li et al., 2020b). Based on the observation that an individual snail can harbor only one trematode species at a time, Tang et al. discovered that the development of all the larval *S. japonicum* could be inhibited and blocked in *O. hupensis* by pre-infection with larval *Exorchis* sp. (Tang et al., 2009, 2012, 2013, 2014), thus providing an alternative opportunity for possible biological control of *S. japonicum* in snail host. This important discovery could have the potential to reduce *S. japonicum*

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<https://doi.org/10.1016/j.ijppaw.2023.04.013>

Received 24 March 2023; Received in revised form 28 April 2023; Accepted 28 April 2023

Available online 29 April 2023

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infection and transmission independent of antihelminthic and molluscicides use, thereby meeting the principles and standards of One Health concept (Hong et al., 2022; Erkyihun and Alemayehu, 2022). However, it needs further investigations including field surveys and underlying mechanisms to certify whether or not this approach can be used for reducing and blocking the prevalence of schistosomiasis in endemic areas.

Currently, schistosomiasis japonica is mainly endemic in areas surrounding the Dongting Lake, Poyang Lake and beaches along the Yangtze River, in where more than 90% of *Oncomelania* snails live (Zheng et al., 2002). Dongting Lake, located in the northern part of Hunan province and on the south bank of the middle reaches of the Yangtze River, provides ideal natural habitats for oncomelamid snails (Li et al., 2020a). Therefore, the Dongting Lake region is recognized as one of the most highly endemic for schistosomiasis in China (Yang et al., 2021). A field survey in regions surrounding the Dongting Lake (Tang et al., 2008) conducted by Tang et al. showed that the infection rate of *Exorchis* sp. in *O. hupensis* snails was 3.3%, while that in *S. asotus* was 99.31%, and the average intensity of infection for each *S. asotus* was 115.4 per fish. These results indicated that in Dongting Lake area, there are sufficient biological resources for implementation of biological control strategy for reducing *S. japonicum* infection and transmission.

Poyang Lake (28°22'–29°45'N, 115°47'–116°45'E), located in the middle and lower reaches of the Yangtze River and in the north of Jiangxi Province, is the largest inland freshwater lake in China (Xue et al., 2021). The ecological, environmental and geographical features of Poyang Lake with a watershed area of 1.62×10^5 km² are suitable for *Oncomelania* snail growth and breeding, thereby making it be another highly endemic region for schistosomiasis (Xue et al., 2021; Xia et al., 2019). In this study, a field survey in marshlands of Poyang Lake from 2012 to 2016 was conducted to investigate and analysis the prevalence of *Exorchis* sp. and *S. japonicum* in *O. hupensis* and *S. asotus*. The results presented here will provide solid evidences to evaluate the feasibility of this eco-friendly biological control strategy for reducing and preventing *S. japonicum* infection and transmission.

2. Materials and methods

2.1. Ethics statement

All the experimental procedures involved were performed strictly in

accordance with the protocol (code 2021 KT-53) approved by the Ethics Committee of School of Basic Medical Science, Central South University, Changsha, China. The protocols of housing, breeding and care of the animals followed the ethical requirements of the government of China.

2.2. Study area

The study was conducted in October from 2012 to 2016 at Shi Li Hu (29° 25' N, 116° 01' E) in Xingzi county, Jiangxi Province, which is located on the western bank of the Poyang Lake in southern China (Fig. 1). The lake usually maintains a higher water level between May and September every year, and is with a lower temperature in the winter season, making it difficult to collect *O. hupensis* snails (Xue et al., 2021; Xia et al., 2019). The ecological and geographical features of Poyang Lake are very suitable for *O. hupensis* snail growth and breeding, and a total of 13 counties (cities, districts) (Xia et al., 2019) including Xingzi county in the areas around the lake are endemic for schistosomiasis japonica.

2.3. Snail sampling and maintenance

Adult *O. hupensis* snails were collected from a predetermined marshland site at Shi Li Hu and were transported to laboratory in paper envelopes. All the field-collected snails were maintained in the laboratory in batches of 100 adults placed in 30 × 20 × 5 cm containers, which were paved with a layer of rough straw paper wetted with dechlorinated water every day and kept under laboratory conditions (25 ± 1 °C, 12-hr light/dark cycle) (Tang et al., 2009; Li et al., 2020b).

2.4. Catfish sampling and maintenance

Adult *S. asotus* fish were collected from the same site at Shi Li Hu and were transported to laboratory in plastic containers supplied with water. All the field-collected fish were maintained in the laboratory in containers containing fresh water and each container was equipped with an aerator, and kept under laboratory conditions (25 ± 1 °C, 12-hr light/dark cycle) (Tang et al., 2008; Sulieman et al., 2014).

2.5. Laboratory examination of *O. hupensis* by shedding method

All the field-collected *O. hupensis* snails were placed individually into

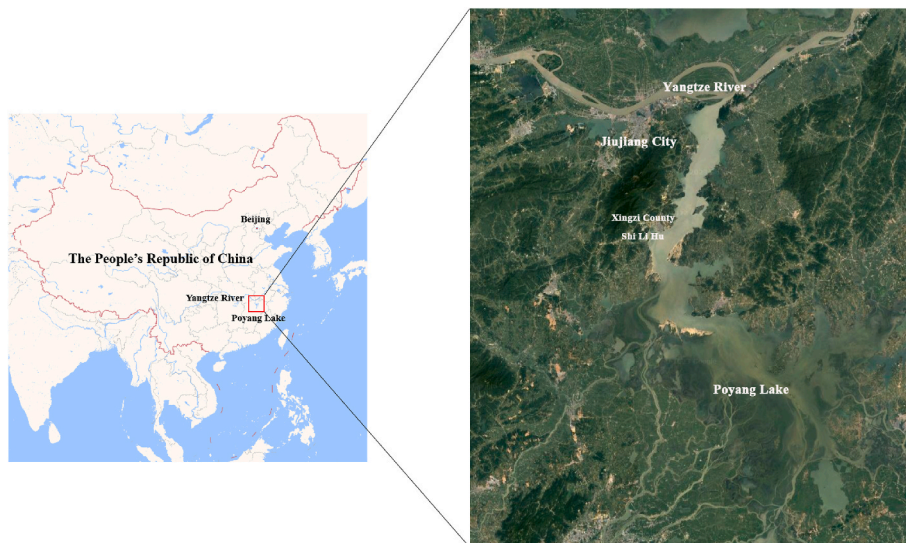


Fig. 1. Geographical location of the study area. Poyang Lake is located in the middle and lower reaches of the Yangtze River and in the north of Jiangxi Province. This study was conducted at Shi Li Hu (29° 25' N, 116° 01' E) in Xingzi county, Jiujiang City, Jiangxi Province, which is located on the western bank of the Poyang Lake in southern China.

a tissue culture plate chamber containing 2 ml of clear de-chlorinated tap water, then covered and left under fluorescent light (Tang et al., 2008; Li et al., 2020b). After 2 h, these snails were examined under a stereo light microscope. The infected snails were carefully isolated and the liberated cercariae were killed by Lugol's iodine solution and identified. All the field-collected snails were screened individually three times at one week interval for any patent trematode infection.

2.6. Laboratory examination of *S. asotus*

All the field-collected *S. asotus* fish were killed and their intestine were dissected under a dissecting microscope by adding 0.75% saline solution (Tang et al., 2008, 2009, 2012, 2013, 2014; Sulieman et al., 2014; Li et al., 2020b). Then the isolated intestinal contents were carefully examined for the presence of *Exorchis* sp. trematode under the stereo light microscope. The collected *Exorchis* sp. trematode were placed into small petri dishes containing 0.75% saline solution and counted under the microscope.

3. Results

The freshwater snail *O. hupensis* is the unique intermediate host of *S. japonicum*, and it also can be as the first intermediate host of *Exorchis* sp., indicating that its central role in the transmission of schistosomiasis japonica (Li et al., 2016, 2020b). In this study, *O. hupensis* were collected from 2012 to 2015 to investigate and analysis the prevalence of *Exorchis* sp. in *O. hupensis* at Poyang Lake (Fig. 2A–C). The results showed that a total number of 19528 *O. hupensis* snails were examined (Table 1). The infection rate of *Exorchis* sp. in *O. hupensis* was 1.87%, 0.51%, 1.06% and 0.14% from 2012 to 2015, respectively (Fig. 2D). The average infection rate of *Exorchis* sp. in *O. hupensis* was 1.11% (Fig. 2E). No *S. japonicum* infected snails were detected.

It has been reported that the trematode *Exorchis* sp. could function as an effective anti-schistosomal agent (Tang et al., 2009, 2012, 2013,

Table 1

Investigation of *Exorchis* sp. in *O. hupensis* at Poyang Lake.

Time	2012	2013	2014	2015
Total number	7113	4128	5476	2811
<i>Exorchis</i> sp.	133	21	58	4

2014). As the definitive host for *Exorchis* sp., *S. asotus* plays a critical role in the implementation of this biology control strategy (Sulieman et al., 2014). Therefore, a field survey was conducted from 2012 to 2016 to investigate and analysis the prevalence of *Exorchis* sp. in *S. asotus* at Poyang Lake (Fig. 3A–C). The results showed that a total number of 494 *S. asotus* were examined (Table 2). The infection rate of *Exorchis* sp. was 56.82%, 75.56%, 67.09%, 63.81% and 72.18%, and the intensity of infection was 14.45, 15.24, 16.87, 14.18 and 12.22 per fish from 2012 to 2016, respectively (Fig. 3D and E). The average infection rate of *Exorchis* sp. was 65.79%, and the average intensity of infection was 14.21 per fish (Fig. 3F and G).

These results indicated that there are abundant biological resources for the implementation of this eco-friendly biological control strategy for reducing schistosomiasis prevalence in the marshlands of Poyang Lake.

4. Discussion and conclusion

Schistosomiasis, caused by blood flukes of the genus *Schistosoma*, is the second most prevalent parasitic disease after malaria in terms of socioeconomic and public health importance, especially in the developing countries (Bergquist and Gray, 2019). In 2021, the WHO has launched a new road map for 2021–2030 that aims to eliminate the suffering from schistosomiasis by 2030 (WHO, 2020; Wang et al., 2022). Therefore, facing the new situation and multiple challenges during the progress towards the elimination of schistosomiasis, alternative effective and environment-friendly schistosomiasis control approaches for

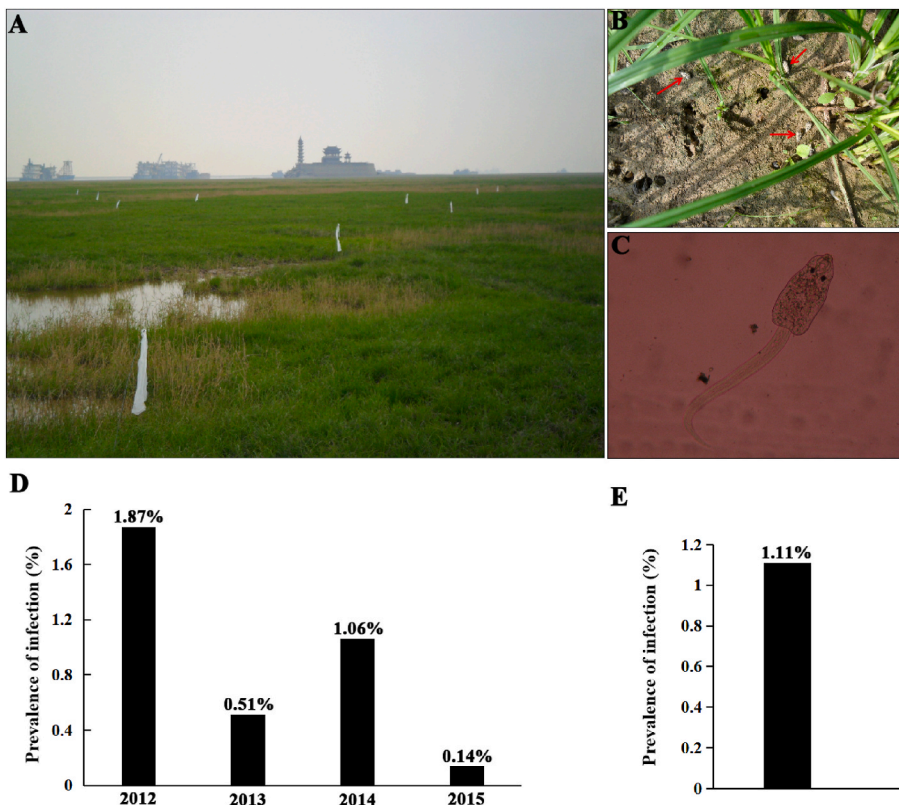


Fig. 2. The infection rate of *Exorchis* sp. in *O. hupensis* collected from the marshland of Poyang Lake from 2012 to 2015. (A) The natural habitat of *O. hupensis*. (B) High grass region inhabited by large numbers of *O. hupensis* are shown, and the snails were marked with red arrows. (C) The cercaria of *Exorchis* sp. collected from *O. hupensis*. (D) The infection rate of *Exorchis* sp. in *O. hupensis* was 1.87%, 0.51%, 1.06% and 0.14% from 2012 to 2015, respectively. (E) The average infection rate of *Exorchis* sp. in *O. hupensis* collected from the marshland of Poyang Lake from 2012 to 2015 was 1.11%. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

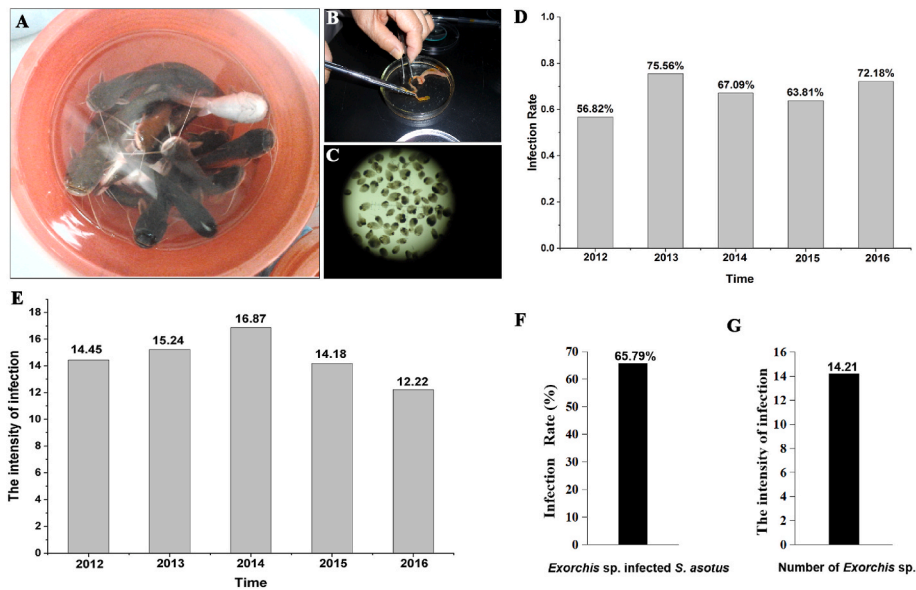


Fig. 3. The prevalence of *Exorchis* sp. in *S. asotus* collected from the marshland of Poyang Lake from 2012 to 2016. (A) The adult *S. asotus* fish collected from Shi Li Hu were kept in plastic containers supplied with water. (B) The intestine of collected *S. asotus* fish were dissected in a glass Petri dish containing 0.75% saline solution. (C) The isolated *Exorchis* sp. adult trematodes isolated from the intestine of infected *S. asotus*. (D) The infection rate of *Exorchis* sp. in *S. asotus* is 56.82%, 75.56%, 67.09%, 63.81% and 72.18% from 2012 to 2016, respectively. (E) The intensity of infection of *Exorchis* sp. in *S. asotus* is 14.45, 15.24, 16.87, 14.18 and 12.22 per fish from 2012 to 2016, respectively. (F) The average infection rate of *Exorchis* sp. in *S. asotus* collected from the marshland of Poyang Lake from 2012 to 2016 was 65.79%. (G) The average intensity of infection of *Exorchis* sp. in *S. asotus* collected from the marshland of Poyang Lake from 2012 to 2016 was 14.21 per fish.

Table 2
Investigation of *S. asotus* infected with *Exorchis* sp. at Poyang Lake.

Time	2012	2013	2014	2015	2016
Infected	75	34	53	67	96
Uninfected	57	11	26	38	37
Total number	132	45	79	105	133
<i>Exorchis</i> sp.	1084	518	894	950	1173

eradicating the overwhelming burden caused by this parasitic disease are urgently needed.

It has been well-demonstrated that the control strategies of schistosomiasis are mainly based on periodic large-scale treatment of at-risk population groups with praziquantel, access to potable water, adequate and improved sanitation, hygiene education and behaviour change, and environmental management and snail control (WHO, 2023). What's more, in addition to these established control strategies, Tang et al. reported that (Tang et al., 2009, 2012, 2013, 2014) all the larval *S. japonicum* would be killed and eliminated in the snail host by pre-infection with a trematode *Exorchis* sp., which infects *S. asotus* but not humans. This important discovery indicates that *Exorchis* sp. could have the potential to function as an effective anti-schistosomal agent, and it can be employed for blocking the development of *S. japonicum* in snail host. However, before being successfully accepted and applied for interrupting the transmission of schistosomiasis, the feasibility of this eco-friendly biological control strategy should be comprehensively investigated and evaluated by conducting a field survey in endemic areas for schistosomiasis.

As one of the highly endemic regions for schistosomiasis in China, the marshlands of Poyang Lake provide suitable habitat for *O. hupensis* growth and breeding (Xue et al., 2021; Xia et al., 2019). In this study, a field survey was conducted in marshlands of Poyang Lake from 2012 to 2016. The results showed that more than half of *S. asotus* (65.79%) were infected with *Exorchis* sp. (Fig. 3F), and the average intensity of infection was 14.21 per fish (Fig. 3G), indicating that there are adequate *Exorchis* sp. which can be used for infecting *O. hupensis* snails. Moreover, according to the survey, the infection rate of *Exorchis* sp. in *S. asotus* was stable with a slight increase during these five years from 2012 to 2016 (Fig. 3D), indicating that the population of *Exorchis* sp. at Poyang Lake keeps growing. That is to say, the trematode has shown great adaptability with the ecological environment of Poyang Lake, reminding that they could coexist with *O. hupensis* for a long time. Therefore, the catfish

trematode *Exorchis* sp. may have the potential to function as an anti-schistosomal agent in the marshlands of Poyang Lake.

The freshwater snail *O. hupensis* is the only intermediate host of *S. japonicum*, indicating that interrupting this unique and long-standing parasite-host interaction contributes to controlling schistosomiasis transmission (Li et al., 2016; Yuan et al., 2022). Additionally, snails become infected with *Exorchis* sp. when they ingest its mature eggs spread in the faeces of infected *S. asotus*, and then several mature cercariae are released from snails about seven months post infection (Suliman et al., 2014; Li et al., 2020b). Our survey data presented here demonstrated that the average infection rate of *Exorchis* sp. in *O. hupensis* is 1.11% (Fig. 2E). These results indicated that there are still enough space to put the biological control strategy into practice in the marshlands of Poyang Lake. Here, no *S. japonicum* infections in snails were observed, indicating that the schistosomiasis control strategy has been effective in this area, but there is still a risk of schistosomiasis transmission (Li et al., 2023). Therefore, it also reminds us to realize the necessity of applying for the alternative effective and environment-friendly schistosomiasis control strategies.

This biological control strategy reported here could be a one health approach for controlling and blocking schistosomiasis transmission in China (Erkyihun and Alemayehu, 2022), and the potential anti-schistosomal tool *Exorchis* sp. plays a central role in this process. Therefore, it will be of great importance to determine the species of *Exorchis* sp. cited in this study. Solodovnik et al. reported that there are currently five valid species of the genus *Exorchis* including *E. oviformis*, *E. marobursae*, *E. multivitellarius*, *E. ovariolobularis* and *E. dongtinghuensis* (Solodovnik et al., 2021). However, it was recently reported that *E. mupingensis* has been identified and described as another new type species of *Exorchis* (Jiang and Guo, 2014). As a result, the genus *Exorchis* actually includes six identified species. Given that *Exorchis* sp. cited here was discovered at Poyang Lake, a habitat for this trematode which is not previously reported, it is not clear whether the species belongs to one of the six reported species of *Exorchis*, which is a limitation of this study. Therefore, we will do more investigations to determine the species in our future research.

In summary, a field survey in the marshlands of Poyang Lake was conducted to investigate and evaluate the feasibility of biological control of *S. japonicum* in *O. hupensis* snail host by using *Exorchis* sp. trematodes, which use the carnivorous fish *S. asotus* rather than humans as its definitive host. The results obtained in the present study provide solid evidences for the practical application of this biological control strategy,

thereby contributing to achieving the goals of the elimination of schistosomiasis.

Funding

This research was funded by the National Natural Science Foundation of China (No. 31270938, 82102428), and the Natural Science Foundation of Hunan Province (No. 2022JJ40663).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The staff at Schistosomiasis Control Station, Xingzi County, Jiangxi Province, China, are sincerely thanked for their continuous support and assistance in the field collection of experimental *O. hupensis* snails and *S. asotus* fish.

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