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Review Article

Taxonomic Confusion Regarding the Intermediate Host of *Schistosoma mansoni* in China: A Review of Studying on *Biomphalaria straminea* Complex Classification

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Abstract

The Biomphalaria populations that distribute in Guang Dong province, China, include Biomphalaria straminea and Biomphalaria kuhniana. These two species, together with Biomphalaria intermedia, have been clustered into one group named Biomphalaria straminea complex, for their morphological similarities. However, the different species differ in their compatibility with S. mansoni. B. straminea is susceptible to the fluke, but B. kuhniana and B. intermedia are apparently refractory to infection. The correct identification of this species complex is important, because it is the basis of schistosomiasis transmission. Currently, there are more and more cases that infected with S. mansoni that returning from Africa have been reported in China. Therefore, it's necessary to summary the studying on B. straminea complex taxonomy, which is helpful to propose intervention and research priorities to tackle the risk of transmission of S. mansoni.

INTRODUCTION

Schistosomiasis is a major neglected tropical disease (NTD) in the tropics and subtropicS. Since 1851, Theodor Bilharz first described the Schistosoma in human, the study of genus Schistosoma (Trematoda: Digenea) and its intermediate host is always a hotspot in the field of public health. In China, schistosomiasis is caused by Schistosoma japonicum, with Oncomelania hupensis acting as the only intermediate host. Whereas there are several species of Planorbis caenosus of Biomphalaria genus (Gastropoda: Planorbidae) serve as obligatory molluscan hosts of S. mansoni (Preston, 1910) that epidemic in Africa, South America, the middle east and the Caribbean islandS. The mollusks of Biomphalaria genus play an important role in the transmission of schistosomiasis, because the distribution of *S. mansoni* is close tied to these freshwater snailS. There are 34 species of Biomphalaria widely distributed in many part of Africa, South America and the Caribbean islands, 12 African [1,2] and 22 Neotropical [3-5]. In Africa, all of the 12 African species of *Biomphalaria* are susceptible to infection [2]. In South America and the Caribbean islands, only B. glabrata, B. tenagophila and B. straminea have been found naturally infected by S. mansoni. Other three species, B. amazonica, B. peregrinates and B. cousini were infected experimentally with this parasite, being considered hosts of the trematode in potential [6-9].

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In recent 40 years, *Biomphalaria* gradually spread in Southern China. It was first reported in 1974 that one kind of the intermediate host of *S. mansoni*, *B. straminea* (Dunker, 1848), were collected at Hong Kong by a malacologist [10]. In 1982, the specie was found in many aquafarm near the Chinese border at New Territories [11]. Meanwhile, it was found in Guangdong province China across the northern border of Hong kong [12]. Then this population was neglected until its reported that highdensity *B. straminea* habitats have been identified in many rivers of Shenzhen city recently [13].

With the accelerating pace of globalization, China has invested heavily in Africa and exported a large number of experts and workers to there. It was reported that some of these persons infected with *S. haematobium* or *S. mansoni* when they returned to China [14,15]. Once these patients, who infected by *S. mansoni*, are imported to regions where the *B. straminea* are present, there is a high possibility of transmission of *S. mansoni* in China.

But it has been a recognized problem that the jumble situation about the classification of *B. straminea* [16,17]. *B. straminea* is commonly shares many morphological similarities with *B. kuhniana* (Clessin, 1883) and *B. intermedia* [18], causing taxonomic confusion. For this reason, these three species were

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clustered into one group by Paraense named *B. straminea* species complex [16]. Through the sequencing of both mitochondrial 16S and ITS1 and ITS2 region of Biomphalaria species, confirmed the close relationship between these three species [19]. Notably, *B. straminea* has an epidemiological importance in the transmission of *S. mansoni*, but *B. kuhniana* and *B. intermedia* are resistant to *S. mansoni* infection. Actually, the classification of freshwater snails plays an important role in the epidemiological control of *schistosomiasi S.*

In China, health technicians have not thoroughly discussed the accurate species of *Biompalaria* that distribute in southern China [13,20,21]. But [22] suggested that the populations in Guangdong province may involve not only *B. straminea*, as originally reported from Hong Kong in the 1970s, but also *B. kuhniana* that is apparently refractory to infection. Therefore, it's necessary to summary the studying on *B. straminea* complex taxonomy, which is helpful to propose intervention and research priorities to tackle the risk of transmission of *S. mansoni*.

MORPHOLOGICAL STUDIES

There are two kinds of morphological identification methods of *Biomphalaria*. One is the shell morphology another is the anatomical descriptionS. Shell morphology, namely conchology, is one aspect of malacology. It's the study of mollusc shells including the description of shell, columellar, aperture, operculum, even aimo acid composition and so on [23]. The first comprehensive conchological text dates back to Martin Lister's *Historia Conchyliorum* in 1685-1692, which have over

1000 engraved plates. In nineteen century, conchology became the standard method for describing new species [9].

In Biomphalaria species, the shell morphological criteria for specific identification incuding: (a) shell height and diameter taken at their largest size (Mandahl-Barth 1957); (b) whorl number; and (c) shape of whorls and aperture [9]. Since B. straminea and B. kuhniana were described by Dunker (1848) and Clessin (1883) respectively, they have been identified exclusively by shell characters [24,25]. The shell characters for Biomphalaria straminea complex is presented in Table (1). As we can see, there is only a bit differently in shell size between these three snailS. However, this character is too one-sided to identify, because relying on only one aspect of an organism's morphology can be misguided. Conchology has only limited value in molluscan taxonomy, because it's difficult to recognize fullgrown shells in small-sized species [16]. And on the other hand, conchologist can be easily confused by ecophenotypic variation [4]. It's so ambiguous that *B. kuniahna* has been misidentified as B. straminea several times [8,26].

some level, shell morphology But. on has a good potential of popularization in China. As we all know, Planorbis is a genus of flat shelled gastropods, which has a worldwide distribution. Chinese medical workers seems to be very familiar with local genus of Planorbis, for example, genera Gyraulus, Hippeutisand, Polypylis and Indoplanorbis [27,28]. But, Biomphalaria is an invasive species that people don't know very much about it. By shell morphological criteria, B. straminea complex can be identified and all other genera of air-breathing freshwater snails that distribute in China widely, such as genera Gyraulus and Hippeutis and can be rule out easily [29,30]. So shell morphology can help Chinese medical workers who are not specialized in malacology in field application.

In order to looking for a more reliable character, the anatomical descriptions of the soft parts of *Biomphalaria* genera have been reported in mid-twentieth century [1,31]. But the most credible taxonomical tool in *Biomphalaria* is relying on the reproductive system description. Some structures include the ovotestis (form and number of diverticulae), sperm duct (shape), penial complex (relative size of the preputium and penis sheath), vas deferens (shape and size relative to penis sheath), prostate (form and number of diverticulae), and vagina (size, presence/ absence of a pouch and corrugations) can serve as taxonomic characters because they exhibit variation in presence, size, and form [9]. These reproductive tracts of snails have a complex morphology and seem quite different from each other [32,33]. Along with these characters, description of new *Biomphalaria* species has been carried out [34-37].

Some key characters of the reproductive tracts used to distinguish among *B. straminea* complex is presented in Table (2). Their reproductive system shares some similaritieS.

Table 1: Shell characters of B. straminea complex.								
Species	Shell size of adult snails	Width at the aperture	Numbers of whorls	References				
B. staminea	8.5-16.5mm	2.3-3.0mm	4-4.75	Paraense, 1988				
B. Kuhniana	6.3-10mm	2.3-2.8mm	4-4.5	Paraense, 1988				
B. Intermedia	7.4-12mm	2.3-2.8mm	4-4.5	Paraense & Deslandes, 1962				

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But there are significant variances in penial complex and prostate diverticulae between these three specieS. What this means in practice has yet to be spelled out [16,22]. So the anatomy of the reproductive tracts is a reliable tool for identifying *B. straminea* complex. However, the application of this method requires a veteran researcher and the use of sophisticated instruments so that are not available in many parts of China. So a more simple and direct method is needed to deal with the problem.

BIOCHEMICAL AND MOLECULAR STUDY

The extensive intraspecific variation in anatomical and morphological characters due to the identification of freshwater snails become complicated [34]. On the other hand, Chinese researchers have extensive experience in biochemical and molecular study of *Oncomelania hupensis* [38]. Morphological studies of *B. straminea* complex differentiation have been used widely in South America, but molecular-based tools maybe give more support to morphological identification in China.

Biochemical studies, including allozyme and isozyme analysis, are used to gauge evolutionary histories and relationships between different species of organisms as well as identify the Biomphalaria. B. glabrata was compared with seven other species of Biomphalaria using 20 allozyme loci and found that it is phenetically and phylogenetically more like the African species [39]. Earlier studies based on several isozymes analysis has been used for the differentiation of some of Biomphalaria species [40-42]. Two Biomphalaria species which are indistinguishable from the shell, B. tenagophila and B. occidentalis, were identified using isozyme patterns obtained by horizontal gel electrophoresis successfully [43]. This technology also can be used to detect the genotoxicity in Biomphalaria [44]. After the B. stramenia were introduced into Hongkong in the early 1970s, Woodruff and Mulvey studied the electrophoretically detectable genetic variation in 19 enzymes in four population samples and suggested that that two southern populations were derived from the original colonists by dispersal but that a northern population represents a second introduction in about 1982 [45]. However, this technique had certain limitations, for example, the requirement for large quantities of biological material, and the small number of loci examined consequently providing relatively little information at the intra-specific level [46].

With the development of molecular biology, more and more available methodologies, based on DNA sequences analysis, such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and simple sequence repeat anchored PCR (SSR-PCR), have been applied to explore the genetic variability of *B. straminea* complex [47] analyzed the sequences of ITS region of *B. straminea* by PCR-RFLP technique firstly. Then the ITS region of rRNA of *B. straminea* complex examined by [48] using PCR-RFLP, which showed the close relationship between B. straminea and B. kuhniana. Using the same methodology [26,49] identified the Biompalaria in Colombia as B. kuhniana which were misidentified as B. straminea previously. In the meantime, through analyzed the ITS 2 region of rRNA of B. straminea complex, indicated that *B. kuhniana* is more closely related with B. straminea than with *B. intermedia* [50]. Similar results were achieved, through SSR-PCR, using *B. straminea* complex [51]. Recently, [17] characterized 10 variable microsatellite loci in B. kuhniana and the cross-species amplification provided some variability among B. straminea species complex. But the PCR-RFLP was more suitable than the SSR-PCR for interspecific analysis, for the latter clustered populations of different species and did not present reproducibility in their trees [48].

[19] detected 23 species *Biompalaria*, including *B. straminea* complex, using the DNA sequences of both mitochondrial 16S and ITS1 and ITS2 region of rRNA. This result suggested that when the region 16S was separately analyzed, the population from northern Brazil (*B. straminea* PA) clustered with *B. kuhniana*, but the population from southern Brazil (*B. straminea SP*) clustered with *B. intermedia*. But all these populations cluster into one group after the analysis of the three DNA regionS. This may be a useful method, together with conchology, to support field survey in China, for lack of anything better.

FUTURE TRENDS AND CONCLUSION

Considering the complex composition of Biompalaria species, the task of eliminate the risk of transmission of *Schistosomiasis mansoni* in China has a far to go. The following research priorities are proposed to enhance the capacity of disease prevention.

- 1. A confirmation of the current distribution and identification of *B. straminea* complex is absolutely necessary. This systematic survey should be performed in the pearl river delta region near Shenzhen city, which covering all bodies of inland water, such as rivers, streams, ponds, farmlands and aquafarmS. And the species of freshwater snails can be identified based on both shell morphology and DNA sequence data.
- 2. Determination the susceptibility of *B. straminea* and *B. kuhniana* respectively are required. B. straminea plays an important role in transmission of *S. mansoni* in Brazil, even though it is not highly susceptible [7]. *B. kuhniana* is

Table 2: Reproductive system characters of E	iomphalaria straminea	complex L= length;	; W= width; <i>ps</i> = pe	enis sheath; pp	= preputium;	DS= distal
section, Wps = width of penis sheath; VP+ = vag	inal pouch present.					
	(

Species	Ovotestis diverticulae	Penial complex	Vas deferens	Prostate diverticulae	Vagina		
B. Intermedia	60, Mostly simple	L: <i>ps</i> = <i>pp</i> W: <i>ps</i> < <i>pp</i>	DS≈Wps	7-15,Mainly branched	Regular, no VP +		
B. staminea	60, Mostly simple	L: <i>ps > pp</i> W: <i>ps < pp</i>	DS≈Wps	10-20, Mainly branched	Regular, no VP +		
B. Kuhniana	60, Mostly simple	$L:ps \ge pp$ W: ps < pp	DS≈Wps	4-7, Mainly branched	Regular, no VP +		
Source: Adapted from published source (Toledo and Fried, 2010).							

reported that apparently refractory to infection [4], but the naturally infected mollusks in Venezuela identified as *B. straminea* are perhaps *B. kuhniana* [8], leading to the susceptibility status of *B. kuhniana* is uncertain.

- 3. The geographic distribution of *B. straminea* and *B. kuhniana* are closely associated with environmental events, such as temperature, biodiversity and water body. So it is needed to establish habitat model base on recording the data of *B. straminea* growth environment to describe the spatio-temporal distribution dynamics of *B. straminea* populationS.
- 4. In view of the spread of potential intermediate hosts of *S. mansoni* in the southern China, snail control strategies are considered a priority for the prevention of *schistosomiasis* transmission. Both environmental management and biological control, such as elimination of natural water bodies and introduction of snail competitors, are considered as effective methods to control target snail populations [52].

The *Biompalaria* populations distribute in China involve not only *B. straminea*, but also *B. kuhniana*. Regarding the *B. straminea* as an intermediate host of *S. mansoni*, it is commonly confused with *B. kuhniana* which is resistant to *S. mansoni* infection. In view of this, shell morphology and molecular biology are useful tools to be applied in field survey.

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