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▣ MINI-REVIEW

Paragonimus and Paragonimiasis in Vietnam: an Update

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Abstract: Paragonimiasis is a food-borne parasitic zoonosis caused by infection with lung flukes of the genus *Paragonimus*. In Vietnam, research on *Paragonimus* and paragonimiasis has been conducted in northern and central regions of the country. Using a combination of morphological and molecular methods, 7 *Paragonimus* species, namely *P. heterotremus*, *P. westermani*, *P. skrjabini*, *P. vietnamensis*, *P. proliferus*, *P. bangkokenis*, and *P. harinasutai*, have been identified in Vietnam. Of these, the first 3, *P. heterotremus*, *P. westermani*, and *P. skrjabini*, are known to infect humans in other countries. However, in Vietnam, only *P. heterotremus*, found in some northern provinces, has been shown to infect humans. Even nowadays, local people in some northern provinces, such as Lai Chau and Yen Bai, are still suffering from *P. heterotremus* infection. In some provinces of central Vietnam, the prevalence and infection intensity of *P. westermani* metacercariae in freshwater crabs (the second intermediate hosts) are extremely high, but human cases have not been reported. Likewise, although *P. skrjabini* was found in Thanh Hoa Province, its pathogenicity to humans in Vietnam still remains uncertain. The results of molecular phylogenetic analyses of Vietnamese *Paragonimus* species provides new insights on the phylogeny and taxonomy of the genus *Paragonimus*. Comprehensive molecular epidemiological and geobiological studies on the genus in Vietnam and adjacent countries are needed to clarify the biodiversity and public health significance of the lung flukes.

Key words: *Paragonimus*, lungfluke, *Paragonimus heterotremus*, *Paragonimus westermani*, *Paragonimus skrjabini*, paragonimiasis, Vietnam

INTRODUCTION

Paragonimiasis is a food-borne parasitic zoonosis caused by infection with lung flukes of the genus *Paragonimus* and is a serious public health issue in some endemic areas. *Paragonimus* and paragonimiasis, therefore, remain a focus of research in actual or potential endemic areas [1]. New *Paragonimus* species and new endemic foci of paragonimiasis are still being discovered [2-4].

In Vietnam, *Paragonimus* and paragonimiasis have become a public health issue in the last 2 decades [5-7]. Until 2006, only 1 species, *P. heterotremus*, was known to occur in Vietnam and is the only confirmed species to cause human infections [8]. Since 2006, large scale investigations for *Paragonimus* and paragonimiasis have been conducted in northern and central Vietnam as a collaborative research between Japanese and Vietnamese parasitologists. To date, at least 7 *Paragonimus* species have been discovered in the country. This series of discoveries has brought new insights on taxonomic status, genetic variation, and phylogenetic relationships of *Paragonimus* species in Asia, which will be briefly reviewed here.

HISTORICAL BACKGROUND

From 1906 until 1995, *P. westermani*, the widespread Asian lung fluke, was considered to be the only lung fluke present in Vietnam and was assumed to be the cause of human paragonimiasis. However, there was no solid evidence regarding the identity of the species responsible. No morphological investigation of metacercariae or adults had been done [9,10]. In 1994 [5], endemic paragonimiasis was found in Sin Ho district, Lai Chau Province, northern Vietnam [6]. From 1995 to 1997, 2 species, *P. heterotremus* and *P. ohirai*, were identified in this area on the basis of morphology [11,12]. However, subse-

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quent surveys in the northern provinces, including Lai Chau, found only *P. heterotremus* metacercariae in mountain crabs [6,7,13]. Since then, we have been conducting extensive surveys for *Paragonimus* species in northern and central Vietnam using a combination of morphological and molecular analyses for metacercariae from crab hosts and adults obtained by experimental infections. So far, 7 *Paragonimus* species have been found in Vietnam. In order of discovery, these are *P. heterotremus*, *P. vietnamensis*, *P. proliferus*, *P. bangkokensis*, *P. westermani*, *P. harinasutai*, and *P. skrjabini* [2,14-19].

MORPHOLOGICAL FEATURES OF *PARAGONIMUS* SPP. IN VIETNAM

Except for the adults of *P. heterotremus* found in natural hosts [6], adult worms of all other *Paragonimus* species were collected from laboratory animals by experimental infection with metacercariae isolated from the second intermediate host crabs. Since metacercariae of more than 1 species were sometimes found in the same individual crab host in the same sampling area [14-19], the identification of metacercariae requires experience and good eyes. Here are some keys to species identification.

KEY TO IDENTIFICATION OF *PARAGONIMUS* METACERCARIAE IN VIETNAM

1a. Metacercariae usually found excysted (about 2.5×0.7 mm) in crabs ..... *P. proliferus* (Fig. 1g)
b. Metacercariae usually found encysted form in crabs 2
2a. Metacercariae <300 μm in diameter ..... *P. heterotremus* (Fig. 1a)
b. Metacercariae >300 μm in diameter 3
3a. Metacercariae about 400-500 μm in diameter 4
b. Metacercariae about 700-800 μm in diameter 6
4a. Cyst walls thin; metacercariae U-shaped in cyst, not occupying entire cyst cavity ..... *P. bangkokensis* (Fig. 1b)
b. Cyst walls thick; metacercariae fully occupy cyst cavity 5
5a. Metacercariae relatively translucent ..... *P. westermani* (Fig. 1c)
b. Metacercariae relatively turbid ..... *P. skrjabini* (Fig. 1d)
6a. Margins ofxcretory bladder wrinkled ..... *P. vietnamensis* (Fig. 1e)
b. Margins of excretory bladder relatively smooth ..... *P. harinasutai* (Fig. 1f)

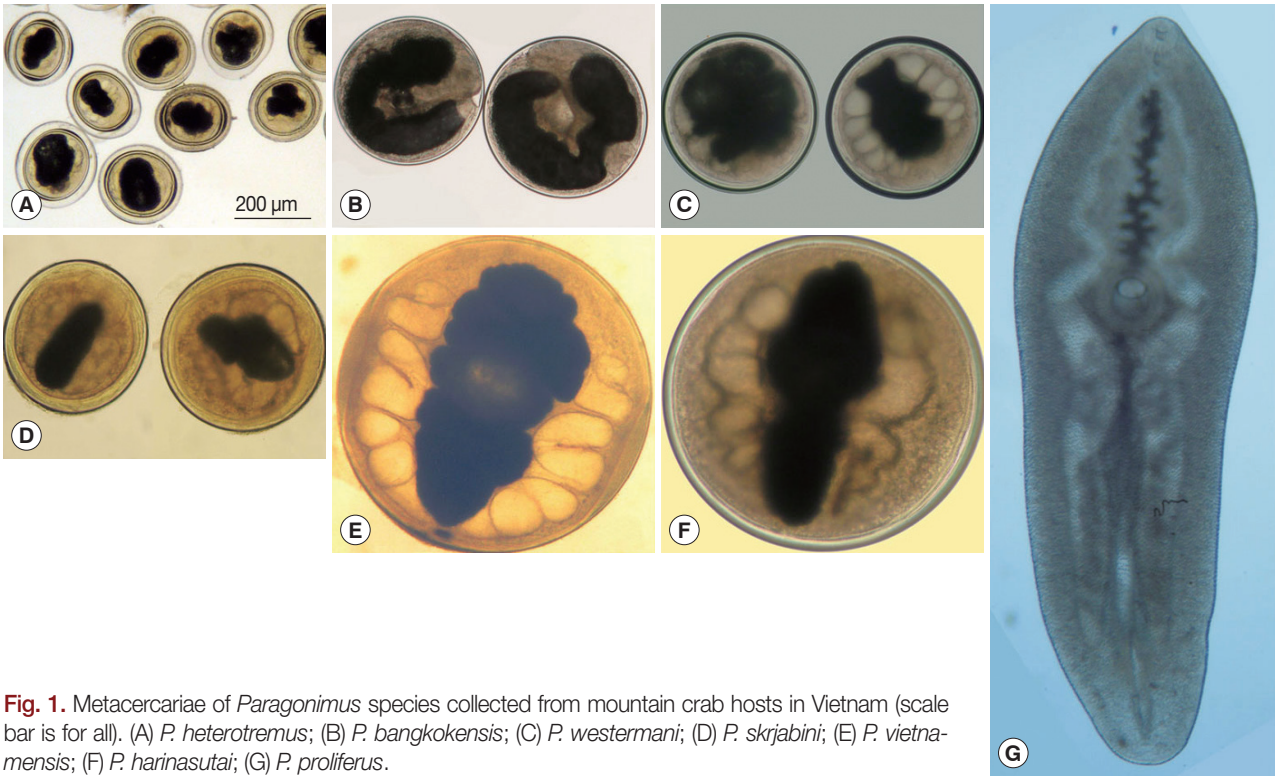

Fig. 1. Metacercariae of *Paragonimus* species collected from mountain crab hosts in Vietnam (scale bar is for all). (A) *P. heterotremus*; (B) *P. bangkokensis*; (C) *P. westermani*; (D) *P. skrjabini*; (E) *P. vietnamensis*; (F) *P. harinasutai*; (G) *P. proliferus*.

KEY TO IDENTIFICATION OF *PARAGONIMUS* ADULTS IN VIETNAM

1a. Body oval; body length:width ratio about 1.4-2.0 ····· 2
b. Body is more elongate; body length:width ratio about 2.5-3.0 ····· 4
2a. Oral sucker nearly twice diameter of ventral sucker ····· *P. heterotremus* (Fig. 2a)
b. Oral sucker almost equal to ventral sucker in diameter ····· 3
3a. Ovary and testes have 5-6 lobes ····· *P. westermani* (Fig. 2b)
b. Ovary and testes finely branched, not lobed ····· *P. vietnamensis* (Fig. 2c)
4a. Spines grouped on body surface ····· 5
b. Spines single on body surface ····· 6
5a. Posterior end of body more tappered; the ventral sucker and the reproductive organs are located more posteriorly ····· *P. bangkokensis* (Fig. 2d)
b. Posterior end of body is relatively pointed; ventral sucker and reproductive organs located more anteriorly ····· *P. proliferus* (Fig. 2e)
6a. Testes not extensive; occupy about 1/10 the body length ····· *P. harinasutai* (Fig. 2g)
b. Testes extensive; occupy about 2/10 the body length ····· *P. skrjabini* (Fig. 2f)

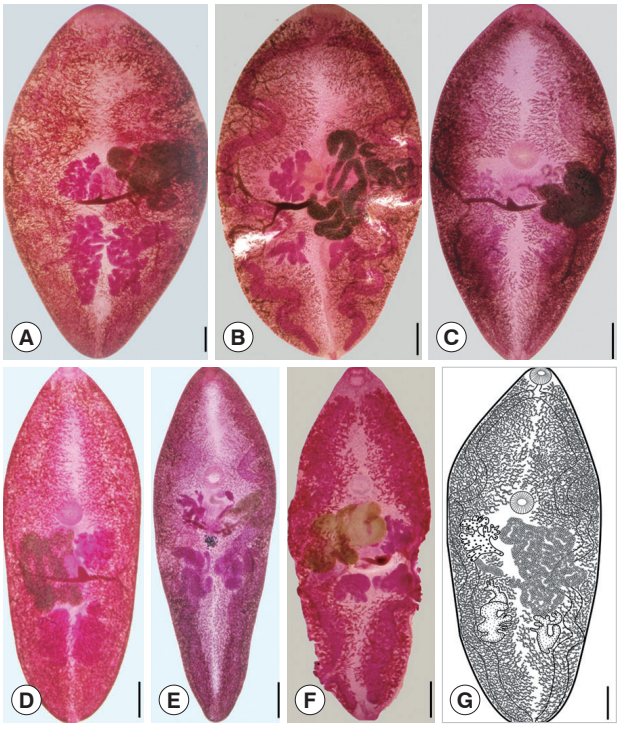

Fig. 2. Adults of *Paragonimus* species in Vietnam. (A) *P. heterotremus*; (B) *P. westermani*; (C) *P. vietnamensis*; (D) *P. bangkokensis*; (E) *P. proliferus*; (F) *P. harinasutai*; (G) *P. skrjabini*.

GEOGRAPHICAL DISTRIBUTION

Geographical distribution of the 7 *Paragonimus* species in Vietnam is summarized in Fig. 3. Metacercariae of *P. heterotremus* have been found in 8 northern provinces where human paragonimiasis cases have also been recorded [7]. In addition, metacercariae of this species have been found in Thanh Hoa Province, in northern central Vietnam, but human cases have not yet been found in this province. In contrast, *P. westermani* was found in 3 central provinces, but no human cases have been detected so far in that region. *P. bangkokensis* and *P. proliferus* were found in both north and central provinces. The remaining species, *P. skrjabini* and *P. harinasutai*, are rare.

As for *P. ohirai* in Vietnam, Le et al. [12] reported adults of this species in 1997 from a pig in Sin Ho district, Lai Chau Province, a mountainous area of northern Vietnam. In that study, *P. ohirai* was identified based on the presence of grouped spines on the worm surface and possession of a ventral sucker

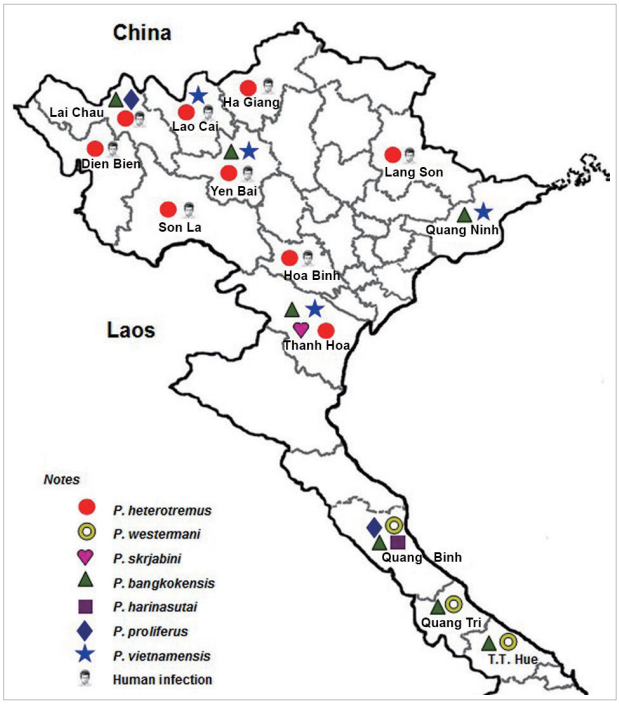

Fig. 3. Geographical distribution of *Paragonimus* species in northern and central Vietnam.

that was larger than the oral sucker. However, these characters are also seen in other species, *P. bangkokensis* and *P. proliferus*, of which metacercariae were also discovered in Sin Ho district, Lai Chau Province [15,16]. Metacercariae of *P. ohirai* have never been found in freshwater crabs in Vietnam. Moreover, *P. ohirai* has been reported only in the coastal regions of Japan, Korea, China, and Taiwan [1]. It is, thus, highly likely that the specimens identified as *P. ohirai* by Le et al. [17] in 1997 were *P. bangkokensis* or *P. proliferus*. Unfortunately, the few specimens collected by Le et al. are not in a good condition for morphological re-examination or molecular study for the accurate re-identification. Whether *P. ohirai* exists in Vietnam or not needs further clarification.

INTER- AND INTRA-SPECIES VARIATION AND MOLECULAR PHYLOGENETIC RELATIONSHIP

The nuclear ribosomal second internal transcribed spacer region (ITS2) is a good marker for inter-species variation, whereas partial mitochondrial cytochrome oxidase subunit 1 gene (CO1) is a good marker for intra-species variation [20]. We have determined ITS2 and CO1 sequences of all *Paragonimus* species in Vietnam and these are now available in GenBank [14-19].

Since it is difficult to identify some closely related *Paragonimus* species based on morphology alone, molecular analyses are necessary and important, especially for the description of a new species. *P. vietnamensis* was described as a new species in 2007 [2]. It is morphologically somewhat similar to several others, such as *P. harinasutai*, *P. microrchis*, and *P. skrjabini*, in either metacercarial or adult stages. However, molecular phylogenetic analyses clearly showed it to be genetically distinct from any other known Paragonimus species [14]. To date, *P. vietnamensis* has been found in crabs collected in 4 provinces and appears to be genetically rather homogeneous.

Likewise, *P. heterotremus* populations from different localities in Vietnam showed high genetic similarities with each other and clustered with those from China and Thailand in phylogenetic trees [8,14]. Based on the ITS2 sequences of eggs collected from patients, human infection with *P. heterotremus* in Vietnam has been confirmed [8,21].

Molecular studies have also provided solid evidence for the presence of *P. westermani* in Vietnam. Attention should be paid to this species because of its wide distribution and its importance as a cause of human paragonimiasis in northeastern Asian countries and the Philippines [1]. *P. westermani* is genetically diverse and has been considered as a species complex including 2 groups: Northeast Asia (Japan, Korea, China, and Taiwan) and Southeast Asia (Malaysia, the Philippines, and Thailand) [22,23]. The recent discovery of *P. westermani* in Vietnam, India, and Sri Lanka provided evidence that much remains to be learned about the *P. westermani* species complex [17,24,25]. *P. westermani* populations found in central Vietnam have high molecular similarities with those of the northeastern Asian group rather than the southeastern Asian group. However, unlike *P. westermani* in northeastern Asia, Vietnamese *P. westermani* seems not to utilize feline or canine final hosts [17], and no human cases due to this species have been found in Vietnam [21]. *P. westermani* recently found in India and Sri Lanka are far distant from other populations [24,25]. Blair et al. [26] first proposed in 2001 an evolutionary scenario, whereby populations of *P. westermani* arose first in Southeast Asia and utilized thiarid snails. Later, by range expansion and the addition of pleurocerid snail hosts, they were able to establish populations in East Asia. More recently, Devi et al. [27] in 2012 considered that the complex arose in southern Asia, perhaps in Sri Lanka, and radiated as populations were established further north and east in Asia, utilizing different snail hosts within the Cerithioidea. However, the presence of 2 distantly related members of the complex in the same place, as in Thailand and Northeast India, is not explained by such a scenario. Moreover, molecular analyses showed that genetic distances between some members of the *P. westermani* group are as great as between very distinct species, indicating that the taxonomy of *P. westermani* complex is more complicated than previously thought. The identities of snail hosts of members of the complex across the range may help to make clear the evolution of this group of lung flukes.

Another species complex, the *P. skrjabini* complex, consists of 2 subspecies, *P. skrjabini skrjabini* and *P. skrjabini miyazakii* [28]. Recently, *P. skrjabini* was found in India [29] and Vietnam [19]. Genetically, the *P. skrjabini* population from Vietnam was almost completely identical with those from Yunnan, China [19]. Remarkably, these Vietnamese and Chinese Yunnan populations, together with the Indian *P. skrjabini* population, form a distinct clade within the *P. skrjabini* complex. In addition, *P. proliferus* should also be placed in the *P. skrjabini* complex [19, 28]. This is a rare species originally reported in China [30]. Recently, we found *P. proliferus* metacercariae in Lai Chau Province in northern Vietnam [15] and Quang Binh Province in

central Vietnam [31]. By molecular phylogenetic analysis, *P. proliferus* in Lai Chau Province was found to have ITS2 and CO1 sequences almost completely identical with those from China, whereas *P. proliferus* found in Quang Binh Province was far distant (5.6% difference in CO1 sequences) from the populations of Lai Chau, Vietnam, and of China. This degree of variation suggests that *P. proliferus* might be widely distributed in Indochina, or even broadly in Southeast Asia. Related to this, *P. hokuoensis*, a species found in a limited area of China and of which metacercariae are much smaller than those of *P. proliferus*, was genetically completely identical with *P. proliferus* and was placed as a synonym of *P. proliferus* [15].

P. bangkokensis and *P. harinasutai* are morphologically distinct from each other at both adult and metacercarial stages. Metacercariae may occur in the same individual crab host [18,32-34]. Recent molecular studies provided strong evidence of their close phylogenetic relationship [16,18]. All *P. harinasutai* and *P. bangkokensis* populations from Thailand, Lao PDR, and Vietnam (and also *P. microrchis* from China as a synonym of *P. harinasutai*) are genetically highly similar to each other and appear as a species complex in both ITS2 and CO1 trees. Two types of indels (AT and ATC) were independently observed in ITS2 sequences among *P. harinasutai*/*P. bangkokensis* populations. These indels are not species-specific, but have some tendencies to associate with particular geographical populations, indicating recent gene flow between *P. harinasutai* and *P. bangkokensis* and recent divergence of these 2 species [16,18]. The recent discovery of naturally hybridized specimen between *P. bangkokensis* and *P. harinasutai* in central Vietnam [35] strongly supports their close relationship in the same species complex.

INTERMEDIATE AND FINAL HOSTS FOR *PARAGONIMUS* SPP. IN VIETNAM

To maintain the life cycle of *Paragonimus* species, at least 3 hosts are required: freshwater snails as the first intermediate host; crustaceans as the second intermediate host; and mammals as the definitive hosts. So far, there have been few reports about the first intermediate host for *Paragonimus* spp. in Vietnam. This may be due partly to the low prevalence of infection typical in snail hosts and to difficulties in identification of snail species. Doanh et al. [13] in 2002 found *Paragonimus* sp. cercariae in 2 snail species, both of which were tentatively identified as *Oncomelania* species, at low infection rates (0.2-0.75%). Almost at the same time, De et al. [7] in 2003 reported *Melanoides* sp. as the first intermediate host of P. heterotremus with the infection rate of 1.4-5.0%. A figure of the cercaria was not provided. However, experimental infection of Melanoides sp. with *P. heterotremus* miracidia has been unsuccessful in several studies [13,36].

All 7 *Paragonimus* species in Vietnam utilize mountain crabs of the family Potamidae as the second intermediate host [13-19]. Prevalences of metacercariae of *P. heterotremus* and *P. westermani* are much higher than those of the other species. The infection rate of *P. heterotremus* metacercariae is the highest (3.0-99.8%); followed by *P. westermani* (2.0-94.2%), *P. proliferus* (3.0-32.0%), *P. bangkokensis* (2.0-31.0%), *P. vietnamensis* (1.0-29.6%), *P. skrjabini* (25.0%), and *P. harinasutai* (4.0%). The coexistence of more than 1 *Paragonimus* species in the same crab host was frequently found [14-19]. These results indicated that there is no competition between *Paragonimus* spp. metacercariae in the second intermediate hosts.

For the definitive hosts, only *P. heterotremus* was found in naturally infected dogs and civets, but adult worms of all other species were obtained by experimental infection [6,7,14-19]. Dogs and cats were suitable hosts for *P. heterotremus*, *P. bangkokensis*, *P. proliferus*, *P. vietnamensis*, *P. skrjabini*, and *P. harinasutai* although the period required for maturation was variable among the species. Although dogs and cats are generally suitable hosts for P. westermani elsewhere in its range, Vietnamese *P. westermani* populations were hardly able to develop into mature adults in these animals. To elucidate the precise life cycle of *Paragonimus* species, more extensive surveys on the intermediate and final hosts of *Paragonimus* spp. in wildlife in Vietnam are necessary.

HUMAN PARAGONIMIASIS

The first case of paragonimiasis in Vietnam was reported in 1906 [9]. However, no much attention was paid to this disease until 1994 when a number of patients were diagnosed in Sin Ho district of Lai Chau Province, northern Vietnam [5,6]. Since then, several surveys revealed that paragonimiasis is endemic in 8 provinces of the northern mountainous areas (Fig. 3). The prevalence ranged from 0.2 to 11.3% [6,7]. The majority (66.7-86.0%) of patients were children under 15 years of age [6,7]. In spite of over 15 years of repeated mass screening, treatment, and education about paragonimiasis, Doanh et al. [21] in 2011 still found patients in some previously reported endemic areas, especially in Sin Ho district of Lai Chau Province, where sero-

prevalence rate was 12.7% (28/220) by multiple dot-ELISA screening test. Continuous efforts on health education are, therefore, required to encourage the local people to avoid *Paragonimus* infection.

CONCLUSION ANF PERSPECTIVES

Paragonimus species in Vietnam are more diverse than previously thought. Using a combination of morphology and molecular studies, at least 7 *Paragonimus* species have been found in northern and central Vietnam. All these species use mountain crabs of the family Potamidae as the second intermediate host. Metacercariae of *P. heterotremus* are the commonest in northern provinces, but this species has not been found in central Vietnam. In contrast, *P. westermani* is dominant in central Vietnam where *P. heterotremus* has not yet been found. Such differences in distribution may depend on the distribution of specific first intermediate hosts, a topic which has not been studied much in Vietnam.

The results of molecular phylogenetic analyses have provided a comprehensive taxonomy of Vietnamese *Paragonimus* and have given new insights on the phylogeny and taxonomy of the genus. The phylogenetic structure of the genus is far more complicated than previously thought, drastically influencing our understanding of the taxonomy of *Paragonimus* species.

To date, only *P. heterotremus* is confirmed to infect humans in Vietnam, and the ability of *P. westermani* and *P. skrjabini* to infect humans in Vietnam is still questionable. Although crab hosts in central provinces are found to be heavily infected with *P. westermani* metacercariae, the final hosts of this species in Vietnam are unknown.

Conclusively, recent discoveries of *Paragonimus* spp. in Vietnam have provided important data related to the epidemiology, taxonomy, and phylogeny of the genus *Paragonimus*. These also raised further questions such as: Are there more *Paragonimus* species and endemic areas in Vietnam, especially in southern Vietnam? Do any *Paragonimus* spp. other than *P. heterotremus* infect humans? How are the life cycles of *Paragonimus* spp. maintained in Vietnam? Much remains to be done.

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