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Population Genetics Analysis of a *Pomacea* Snail (Gastropoda: Ampullariidae) in Thailand and its Low Infection by *Angiostrongylus cantonensis*

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Pomacea is a freshwater snail in family Ampullariidae that is native to South and Central America. This snail is among the more important intermediate hosts for *Angiostrongylus cantonensis* and agricultural pests. Herein, we investigated the prevalence of *A. cantonensis* larvae and the genetic diversity of *Pomacea* samples collected across Thailand based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene sequences. The larval-infection rate was 1.7% in *Pomacea canaliculata* specimens collected from the Uttaradit Province of northern Thailand. We randomly selected specimens of *P. canaliculata* and *P. maculata* for genetic analysis. We analyzed 244 *COI* sequences, including 49 sequences from samples collected from Thailand and a publicly accessible database of snails in their native and non-native ranges. A maximum-likelihood tree of *P. canaliculata* and *P. maculata* revealed two main clades. The genetic diversity analysis identified seven *P. canaliculata* haplotypes and six *P. maculata* haplotypes, and showed genetic differences between the populations of *P. canaliculata* and *P. maculata*. The haplotype networks of *P. canaliculata* and *P. maculata* populations in Thailand are similar to those of populations in multiple countries, indicating that this species spread widely to many parts of the world.

Key words: *Angiostrongylus*, Genetic diversity, Phylogeny, Haplotype, *Pomacea*.

BACKGROUND

Pomacea spp. are freshwater snails in the family Ampullariidae (*i.e.*, apple snails), which are native to South and Central America (Hayes et al. 2015). *Pomacea* spp. are distributed throughout the humid tropics and subtropics. Several species in this genus have been introduced and become widespread in many parts of the world, including Asia, Europe, North America, and the Pacific Islands (Rawlings et al. 2007; Hayes et al. 2008). *Pomacea* was first introduced into the Asia-Pacific region in 1980 as part of the pet trade and as a source of food for human consumption (Cowie et al. 2006; Rawlings et al. 2007; Hayes et al. 2008).

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It quickly spread to many countries in Southeast and East Asia via transportation or was further introduced from its native areas (Halwart 1994). Four species of *Pomacea*—*P. canaliculata*, *P. maculata*, *P. diffusa*, and *P. scalaris*—have been found in Southeast and East Asia (Hayes et al. 2008; Xu et al. 2012). Among them, *P. canaliculata* and *P. maculata* are highly invasive species and the most widely distributed (Hayes et al. 2012). *Pomacea canaliculata* was listed as one the world's 100 most invasive species (Lowe et al. 2000). In addition, The United States Aquatic Nuisance Species Task Force has expressed concerns regarding members of Ampullariidae, including *Pomacea* (Burks et al. 2017).

Pomacea snails have important roles in agriculture and medicine. The snails have a voracious appetite (Qiu et al. 2011), are resistant to desiccation during dry periods (Havel et al. 2014), and reproduce rapidly (Barnes et al. 2008). They are serious agricultural pests (Mochida 1991) that were shown to have effects on the environment (Hayes et al. 2015) and human health (Lv et al. 2009a). Over the past couple of decades, *Pomacea* snails were designated as pests for wetland rice and other crops and shown to cause massive annual economic losses of more than US $40 million and US $18.9 billion in the Philippines and China, respectively (Cowie 2002; Xu et al. 2012). In addition, *Pomacea* snails are natural intermediate hosts of *Angiostrongylus cantonensis* (Lv et al. 2009b), which causes eosinophilic meningitis and meningoencephalitis in humans (Pipitgool et al. 1997). The genus was shown to promote the endemicity and transmission of *A. cantonensis* in China and the United States (Song et al. 2016; Kim et al. 2019). The invasion of these snails facilitates the establishment of the parasite's life cycle, thus increasing the chance that native snails are exposed to *A. cantonensis* in endemic areas (Lv et al. 2006 2009b).

Pomacea canaliculata was illegally introduced into Thailand in 1984. It was used to clean fish aquaria because it consumes several aquatic plants and algae (Keawjam and Upatham 1990). *Pomacea* snails have been reported to cause serious damage to rice crops in Thailand. Three species of *Pomacea* snails are common in Thailand: *P. canaliculata*, *P. maculata*, and *Pomacea* sp. (Keawjam and Upatham 1990). Many other non-native *Pomacea* species are difficult to differentiate from *P. canaliculata* and *P. maculata*, and are frequently misidentified as members of one of these two species (Cowie et al. 2006; Hayes et al. 2008). Thus, morphological variation in *Pomacea* spp. is low, which has led to the use of alternative methods for identification.

Select nucleotide regions have been sequenced to identify several species, including gastropods. Nucleotide sequences of the mitochondrial *12S*, *16S*, and cytochrome c oxidase subunit I (*COI*) regions successfully revealed the presence of three species in the United States: *P. canaliculata*, *P. maculata*, and *P. haustrum* (Rawlings et al. 2007). Phylogenetic analysis of *COI* sequences revealed the presence of two major groups in Japan: *P. maculata* and *P. canaliculata* (Matsukura et al. 2008). In addition, *COI* sequences were used to study the diversity of *Pomacea* species in China, and confirmed the presence of *P. maculata* and *P. canaliculata* (Song et al. 2010; Yang et al. 2018a). No studies of multiple areas have been conducted on the genetic variation of *Pomacea* in Thailand. In the present study, a partial sequence of the *COI* gene was used to confirm the identification of *Pomacea* species. Moreover, genetic variation, phylogeny, and genetic-structure analysis were performed to evaluate the molecular diversity of *Pomacea* in Thailand. In addition, the infection rate of *A. cantonensis* larvae in *Pomacea* was investigated.

MATERIALS AND METHODS

Collection and identification of *Pomacea* snails

Pomacea specimens were randomly collected from 25 provinces across Thailand between May 2017 and July 2018 (Fig. 1, Table S1). Snails were collected by hand in several habitats—*e.g.*, paddy fields, canals, and rivers—and placed in a plastic box with water and air ventilation. They were transported to the Department of Microbiology and Parasitology (Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand). The shell morphologies of the *Pomacea* specimens were primarily identified according to previous studies (Hayes et al. 2012; Arfan et al. 2014; Yang and Yu 2019). *Pomacea canaliculata* was primarily identified based on external shell morphology with unpigmented inner pallial lip, rounded shoulder, smooth shell surface, deep suture, thin shell, and wide umbilical. *Pomacea maculata* was primarily identified based on external shell morphology with red or orange inner pallial lip pigmentation, angulated shoulder, rough shell surface, shallow suture, thick shell, and narrow umbilical.

Isolation of *Angiostrongylus* larvae from *Pomacea*

The bodies of *P. canaliculata* and *P. maculata* snails were removed from the shells. A piece of snail foot tissue was taken from each individual (approximately 8 mm^3) and stored at -20°C until DNA extraction. Most remaining snail tissue was artificially

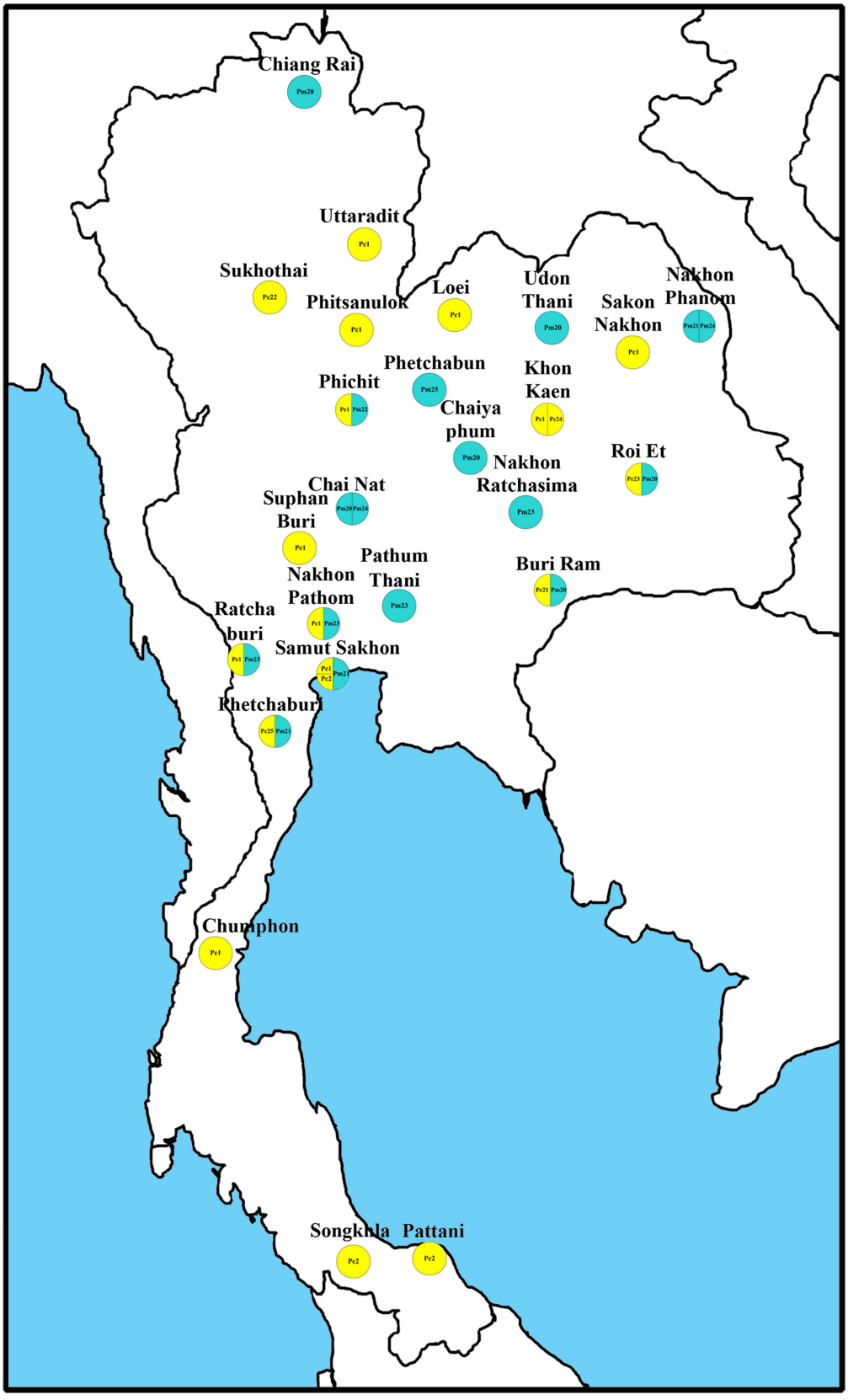

Fig. 1. Geographical locations of the 25 collection sites for *P. canaliculata* and *P. maculata* in Thailand. Details on the collection sites are in table S1.

digested with 0.7% pepsin solution (Acros Organics, Geel, Belgium) according to a previous study (Vitta et al. 2016a). The larva of *Angiostrongylus* species was identified using a light microscope. Morphological descriptions with cylindrical shape, thin and delicate body, two chitinous rods at the anterior end, genital primordium at the middle of the body, cone-shaped tail, moderately curved and pointed tail, and the anus near the rear end of the tail were used as characters for identification (Eamsobhana 2006).

DNA extraction from *Pomacea* snails

Genomic DNA was extracted from individual snails using the NucleoSpin® Tissue Kit (Macherey-Nagel, Duren, Germany) following the manufacturer's instructions. Snail tissue was transferred to a 1.5-mL microcentrifuge tube containing 180 µl of tissue lysis (T1) buffer, then 25 µl of 30 mg/mL proteinase K solution was added. The tissue was ground using a sterile pipette tip for several minutes. The tube was then incubated overnight in a water bath at 56°C and vortexed occasionally during the incubation. On the next day, 200 µl of sample lysis (B3) buffer was added. The tube was vortexed vigorously and incubated in a water bath at 70°C for 10 min. The tube was vortexed again, and 210 µl of 100% ethanol was added to optimize the DNA-binding conditions. The solution was transferred to a NucleoSpin® Tissue Column in a collection tube and centrifuged for 1 min at 11,000 × *g*. The flow-through was discarded, and the column was placed in a new collection tube. Subsequently, two sequential washes through the silica membrane were performed by adding 500 µl of BW buffer and then 600 µl of B5 buffer. The tube was centrifuged for 1 min at 11,000 × *g*. The flow-through was discarded, and the column was placed back into the collection tube. The column was centrifuged again for 1 min at 11,000 × *g* to dry the silica membrane. The NucleoSpin® Tissue Column was placed into a 1.5-mL microcentrifuge tube, and 80 µl of Buffer BE was added. The tube was then incubated at room temperature for 1 min and centrifuged for 1 min at 11,000 × *g*. The genomic DNA was dissolved in Buffer BE. The DNA solution was checked by running it on a 0.8% agarose gel in 1× TBE buffer at 100 V. The gel was stained with ethidium bromide, followed by destaining with distilled water and photographing under ultraviolet light. The DNA solution was stored at -20°C until further use.

Polymerase chain reaction (PCR) amplification and sequencing

Polymerase chain reaction was performed to amplify partial regions of *COI* from *P. canaliculata* and *P. maculata*. A pair of primers was designed based on a deposited GenBank sequence (accession number AB433758) using the Primer-BLAST program. The primers Pc*COI*_forward (5'-ATGATCAGGCCTAGTTGGGG-3') and Pc*COI*_reverse (5'-TTCATCCAGTTCCAGCACCA-3') were obtained and used to amplify a 308-base pair (bp) fragment. PCR was carried out in a total volume of 30 µl, containing 3 µl 10× buffer, 2.1 µl 25 mM $MgCl_2$, 0.6 µl 200 mM dNTPs, 1.2 µl each primer (5 µM; final concentration of 0.2 µM), 0.3 µl 5 U/mL Taq DNA polymerase, 18.6 µl of distilled water, and 3 µl of DNA template (20–200 ng). The PCR temperature profile included an initial denaturation at 95°C for 1 min; followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 40 s, and extension at 72°C for 1 min; with a final extension at 72°C for 5 min. PCR was performed in a Biometra TOne Thermal Cycler (Analytik Jena AG, Jena, Germany). The PCR products were checked by 1.2% agarose gel electrophoresis. Purification of the PCR products was performed using a NucleoSpin® Gel and PCR Clean-Up Kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions. The purified PCR product were checked on a 1.2% agarose gel run at 100 V in 1× TBE buffer. Nucleotide sequencing was performed at Macrogen Inc., Seoul, Korea in both the forward and reverse directions.

Sequencing and phylogenetic analysis

Snails (*P. canaliculata* and *P. maculata*) were identified by running BLASTN searches to find similarities to sequences deposited in the NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide sequences were aligned using ClustalW. The maximum-likelihood (ML) method with HKY model and neighbor-joining (NJ) method with Kimura two-parameter (K2) based on 1,000 bootstrap replicates were performed for the phylogenetic analyses with MEGA 7 (Kumar et al. 2016). Bayesian analysis was performed with MrBayes v3.2. using a four-chain run for 2,000,000 generations of Markov-chain Monte Carlo algorithm (MCMC) simulations (Ronquist et al. 2012). We added 195 *COI* sequences *P. maculata* (n = 100) and *P. canaliculata* (n = 95) from GenBank to the 49 *COI* sequences generated in this study. Genetic distances of *P. canaliculata* and *P. maculata* based on *COI* sequences were calculated according to the Kimura two-parameter method (Kimura 1980).

Haplotype and genetic analysis

Haplotype diversity and nucleotide diversity were

calculated in ARLEQUIN, version 3.5.1.2 (Excoffier and Lischer 2010). Relationships between haplotypes were estimated using the median-joining (MJ) network (Bandelt et al. 1999). MJ-network analysis was performed in NETWORK (version 5.0.1.1) based on the *COI* sequences. In addition, the analysis of molecular variance (AMOVA) performed in ARLEQUIN was used to test the genetic difference among groups.

RESULTS

Infection rate of *A. cantonensis* in *Pomacea*

We collected 386 *P. canaliculata* and 242 *P. maculata* samples from 25 provinces in five regions of Thailand. We found only one *P. canaliculata* sample from Uttaradit Province (in northern Thailand) that was infected with *A. cantonensis* larva (Fig. S1). Only one *A. cantonensis* larva was isolated from that snail. The prevalence of *A. cantonensis* larvae was as low as 1.7% (1/59).

Molecular identification of *Pomacea*

To identify *P. canaliculata* and *P. maculata*, PCR-based analysis and sequencing of the *COI* gene were performed together with a BLASTN search of the sequences. Twenty-seven samples of *Pomacea* (GenBank accession numbers MK858525–MK858551) were identified as *P. canaliculata* with 99–100% identity after a BLASTN search using 282 bp of the *COI* gene. During the analysis of the *COI* sequences (283 bp) of *P. maculata* (GenBank accession numbers MK858552–MK858573), 22 sequences showed the highest similarity (99%) with known sequences of *P. maculata* (GenBank accession numbers AB728579 and GU236491).

Phylogenetic analyses

The phylogenetic trees of *P. canaliculata* and *P. maculata* were reconstructed using the ML, NJ, and BI methods. Based on the 27 *COI* sequences in the present study together with 95 sequences from GenBank results for *P. canaliculata*, the ML tree revealed two main clades (Fig. 2). Clade 1 contained 21 sequences from this study and 39 known sequences downloaded from GenBank; bootstrap support was highest (88%) for the NJ tree. Clade 2 contained six sequences identified from this study and 45 from GenBank; bootstrap support was highest (79%) for the ML tree.

The maximum likelihood tree of 122 *COI* sequences of *P. maculata* (22 sequences from the

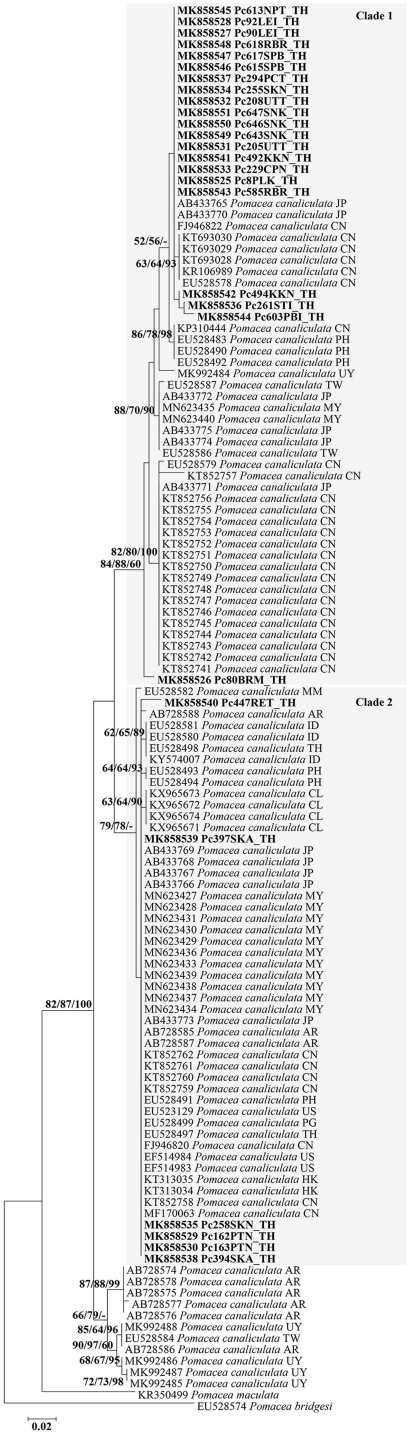

Fig. 2. ML phylogenetic tree generated from 122 sequences of a partial *COI* sequence (282 bp) of *P. canaliculata* (27 sequences from Thailand and 95 sequences from other geographical regions). Support values (ML bootstrap/NJ bootstrap/Bayesian posterior probabilities) are shown above the branches. Bold letters indicate the sequences obtained in this study. *Pomacea maculata* and *P. bridgesi* were used as the outgroups. Abbreviations: TH, Thailand; AR, Argentina; CL, Chile; CN, China; HK, Hong Kong; ID, Indonesia; JP, Japan; MY, Malaysia; MM, Myanmar; PG, Papua New Guinea; PH, Philippines; TW, Taiwan; US, United States; UY, Uruguay.

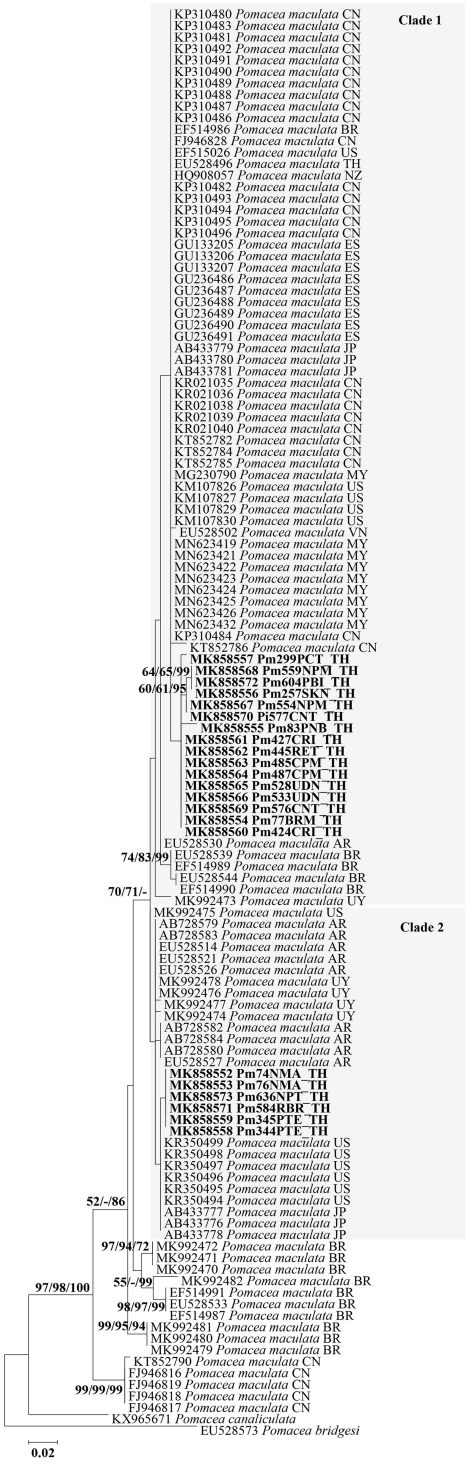

Fig. 3. ML phylogenetic tree generated from 122 sequences of a partial *COI* sequence (283 bp) of *P. maculata* (22 sequences from Thailand and 100 sequences from other geographical regions). Support values (ML bootstrap/NJ bootstrap/Bayesian posterior probabilities) are shown above the branches. Bold letters indicate the sequences obtained in this study. *Pomacea canaliculata* and *P. bridgesi* were used as the outgroups. TH, Thailand; AR, Argentina; BR, Brazil; CN, China; JP, Japan; MY, Malaysia; NZ, New Zealand; ES, Spain; US, United States; UY, Uruguay; VN, Vietnam.

present study and 100 downloaded from GenBank) revealed two main clades (Fig. 3). Clade 1 contained 16 sequences from this study and 62 from GenBank. Clade 2 contained six sequences identified in this study and 23 sequences of reference species; bootstrap support values were highest (71%) for the NJ tree.

Genetic distances between populations of *P. canaliculata* and *P. maculata*

The genetic distances between the populations of *P. canaliculata* and *P. maculata* were calculated to compare the populations in Thailand with those of the native and non-native ranges using *COI* sequences. The genetic distances between the *P. canaliculata* population of Thailand and Argentina (native) was 0.055, while the distance between Thailand and Uruguay (native) was 0.060. The genetic distances between *P. canaliculata* in Thailand and its non-native range—including Chile, China, Hong Kong, Indonesia, Japan, Malaysia, Myanmar, Papua New Guinea, the Philippines, Taiwan, and the United States—was 0.033–0.047 (Table S2). Meanwhile, the genetic distance between *P. canaliculata* and *P. maculata* was 0.135.

The genetic distance between *P. maculata* populations in Thailand and Argentina (native) was 0.015, Thailand and Brazil (native) was 0.033, and Thailand and Uruguay (native) was 0.020. The genetic distances between *P. maculata* in Thailand and its non-native range—including China, Japan, Malaysia, New Zealand, Spain, the United States, and Vietnam—was 0.013–0.020 (Table S3).

Genetic variations between *P. canaliculata* and *P. maculate*

The mitochondrial *COI* sequences (282 bp) were obtained from 27 individuals of *P. canaliculata* from Thailand and 95 sequences from the native and introduced range. Twenty-five haplotypes (Pc1–Pc25) were identified with 45 nucleotide variation sites **(**Fig. 4, Table S4). Of these, 18 haplotypes were unique and seven (Pc1–4, Pc9, Pc13, and Pc15) were shared by at least two populations. Haplotype Pc2 was the most shared among the populations found in Argentina, the United States, Papua New Guinea, China, Hong Kong, Japan, Malaysia, the Philippines, and Thailand **(**Table S5).

Seven haplotypes (Pc1, Pc2, Pc21–25) were identified from 27 Thailand sequences. Among the *P. canaliculata* haplotypes in Thailand, Pc1 was the most widely distributed—in 11 provinces. The second most widely distributed haplotype, Pc2, was found in three provinces of Thailand. The other haplotypes, Pc21–25,

were found in Buri Ram, Sukhothai, Roi Et, Khon Kaen, and Phetchaburi provinces, respectively (Fig. 1, Table S1). The haplotype diversity in each population ranged from 0 in Chile, Hong Kong, Indonesia, Myanmar, Papua New Guinea, and the United States to 1.0000 in Taiwan, with a mean of 0.8533. The nucleotide diversity in each population ranged from 0 in Chile, Hong Kong, Indonesia, Myanmar, Papua New Guinea, and the United States to 0.0401 in Taiwan, with a mean of 0.0361 (Table 1).

Twenty-five haplotypes (Pm1–Pm25) of *P. maculata* from 22 sequences in Thailand and 100 sequences from the native and introduced ranges were identified based on the *COI* gene sequence (283 bp) with nucleotide variations at 39 sites (Fig. 5, Table S6). Of these, 22 haplotypes were unique, and three (Pm1, Pm2, and Pm5) were shared by at least two populations. Haplotype Pm1 was the most shared among the populations found in Brazil, the United States, New Zealand, Spain, China, Japan, Malaysia, and Thailand (Table S7).

Six haplotypes from 27 *COI* sequences of *P. maculata* were identified (Pm20–25). Among the *P. maculata* haplotypes in Thailand, Pm20 was the most widely distributed in six provinces. The second most widely distributed haplotype was Pm23, which occurred in four provinces of Thailand. The remaining haplotypes—Pm21 and Pm24—were less widely distributed, and found in three provinces (Samut Sakhon, Nakhon Phanom, and Phetchaburi) and two provinces (Nakhon Phanom and Chai Nat), respectively. Two haplotypes (Pm22 and Pm25) were found in Phichit and Phetchabun, respectively (Fig. 1, Table S1). The haplotype diversity in each population ranged from 0 in Spain, New Zealand, Malaysia, and Vietnam to 0.9000 in Uruguay, with a mean of 0.7878. Nucleotide diversity in each population ranged from 0 in Spain, New Zealand, Malaysia, and Vietnam to 0.0260 in Brazil, with a mean of 0.0181 (Table 2).

DISCUSSION

The data generated in this study showed that *Pomacea canaliculata* samples collected from the Uttaradit Province in northern Thailand were positive for *A. cantonensis* larva. A previous study reported the prevalences of *A. cantonensis* in snails from several provinces in northeastern Thailand: 20% for *Hemiplecta distincta*, 7.6% for *Achatina fulica*, 5% for *Pila polita*, and 2.4% for *P. pesmei* (Tesana et al. 2009). A recent survey showed that the prevalences of *A. cantonensis* larvae in *A. fulica*, *Cryptozona siamensis*, and *Megaustenia siamensis* snails from several provinces in Thailand were 1.08, 0.07, and 0.01%, respectively (Vitta et al. 2016b). We now report that *A. cantonensis* larva naturally infected *P. canaliculata* snails in Thailand. An experimental study previously noted a low infection

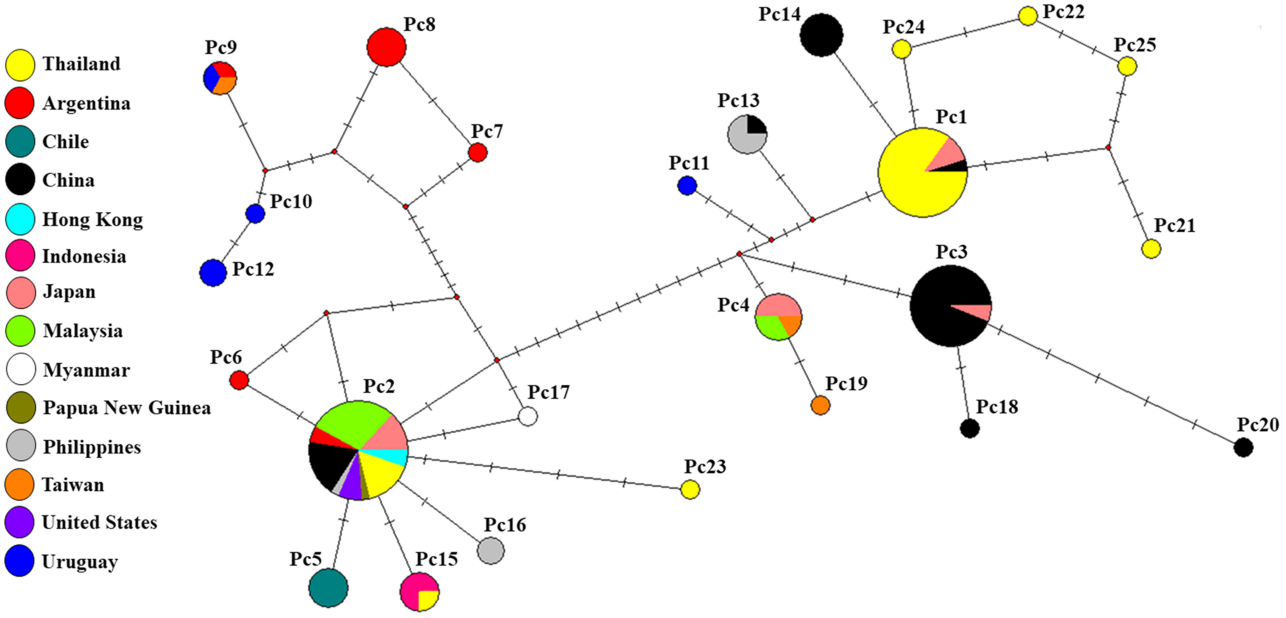

Fig. 4. Mitochondrial DNA genealogy for 122 *COI* sequences (27 sequences from Thailand and 95 sequences from other geographical regions) of *P. canaliculata* constructed by median-joining network method. Each haplotype is represented by a circle. The sizes of the circles are relative to the number of individuals that share a specific haplotype. Each mutation between the haplotypes is represented with a bar. The red dot vertices represent unsampled putative or missing haplotypes.

rate of *A. cantonensis* in *P. canaliculata* (Tesana et al. 2008). A previous study found that *Pila* snails are native to Southeast Asia (including Thailand) (Brandt 1974). These snails play a key role in the transmission of *A. cantonensis* in humans (Eamsobhana 2014).

The rapid spread of *Pomacea* snails in Thailand led it to replace the native snails (Tesana et al. 2008), because the *Pomacea* have a striking ecological

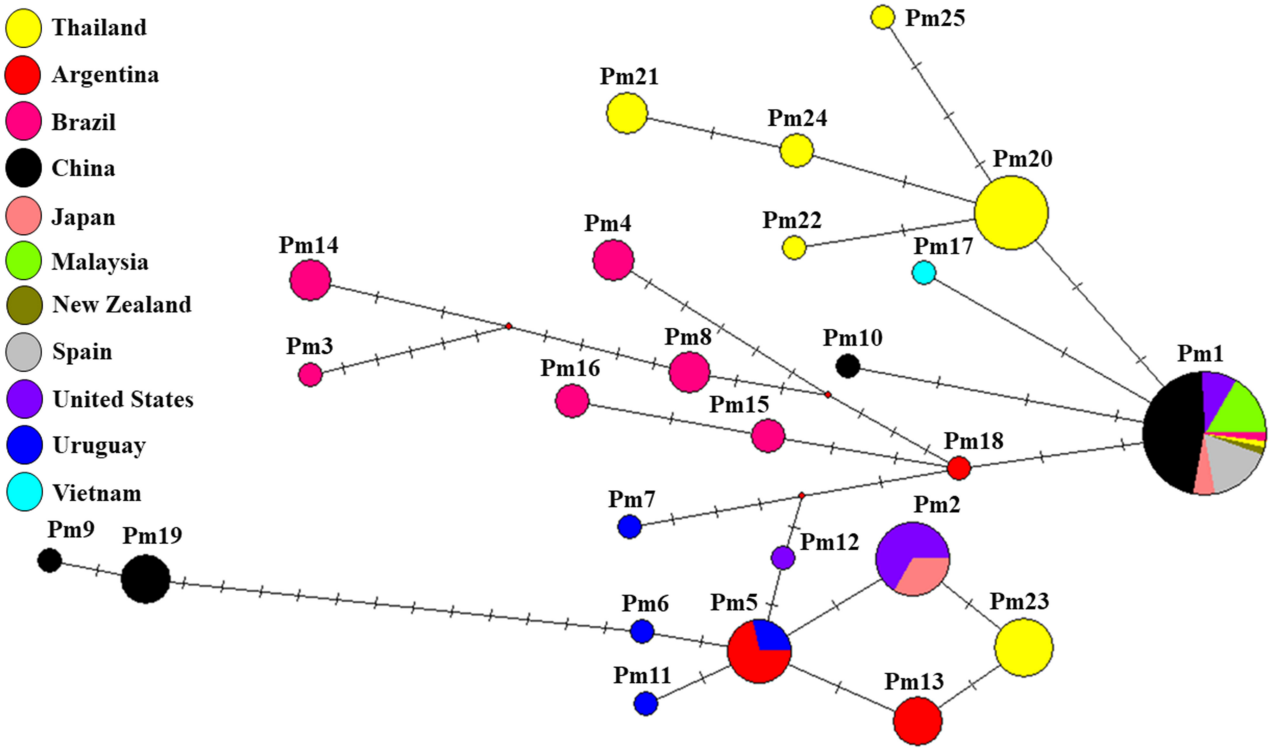

Fig. 5. Mitochondrial DNA genealogy for 32 *COI* sequences (22 sequences from Thailand and 100 sequences from other geographical regions) of *P. maculata* constructed by the median-joining network method. Each haplotype is represented by a circle. The sizes of the circles are relative to the number of individuals sharing a specific haplotype. Each mutation between haplotypes is represented with a bar.

Table 1. Genetic diversity of *P. canaliculata* populations from Thailand and other geographical regions based on mitochondrial cytochrome *c* oxidase I gene sequences

Populations	Number of samples	Haplotypes			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
		Number of haplotypes	Shared haplotypes	Unique haplotype		
Thailand	29	8	3	5	0.6281 ± 0.0894	0.0257 ± 0.0138
Argentina	9	5	2	3	0.8056 ± 0.1196	0.0263 ± 0.0154
Chile	4	1	-	1	0	0
China	32	7	4	3	0.6956 ± 0.0666	0.0268 ± 0.0142
Hong Kong	2	1	1	-	0	0
Indonesia	3	1	1	-	0	0
Japan	11	4	4	-	0.7455 ± 0.0978	0.0310 ± 0.0175
Malaysia	13	2	2	-	0.2821 ± 0.1417	0.0130 ± 0.0079
Myanmar	1	1	-	1	0	0
Papua New Guinea	1	1	1	-	0	0
Philippines	6	3	2	1	0.7333 ± 0.1552	0.0359 ± 0.0221
Taiwan	3	3	2	1	1.0000 ± 0.2722	0.0401 ± 0.0315
United States	3	1	1	-	0	0
Uruguay	5	4	1	3	0.9000 ± 0.1610	0.0312 ± 0.0203
Total	122	42	24	18	0.8533 ± 0.0218	0.0361 ± 0.0183

adaptability (Chaichana and Sumpan 2015). Therefore, *Pomacea* snails were found to be of increasing importance as the intermediate host for *A. cantonensis* transmission in this region. Previous data showed that, in other countries, the prevalence of *A. cantonensis* larvae in *P. canaliculata* was 20.4, 6.1, and 2% in Taiwan, China, and the Philippines, respectively (Wang et al. 2011; Tujan et al. 2016; Hu et al. 2018). Expansive epidemiological data indicate that *P. canaliculata* is becoming the most important intermediate host for *A. cantonensis* in China, Laos, Cambodia, and Vietnam because of its wide environmental tolerance and its high susceptibility to the parasite (Yang et al. 2013; Lv et al. 2018).

The patterns of epidemiological of eosinophilic meningitis caused by *A. cantonensis* in Southeast Asia, Japan, and China are changing. For instance, slugs were widely used in Traditional Chinese Medicine in China (Li et al. 2006), but this usage is declining. In addition, snails were important for animal rearing in Taiwan in the 1970s (Yii 1976), but this practice, too, is diminishing. In contrast, the spread of invasive snails, growth of tourism, and consumption of foods that use snails, which have become popular among tourists, are all important factors driving the spread of *A. cantonensis* (Lv et al. 2009b). Between 1997 and 2006, 116 cases of human angiostrongyliasis in China were traced back to infection via *P. canaliculata* (Zheng et al. 2001; Lin et al. 2003; Yang et al. 2004; Han et al. 2005; Wei et al. 2005). In addition, eating raw *P. canaliculata* or *A. fulica* was confirmed to be the route of *A. cantonensis* infection in the outbreak of angiostrongyliasis in Beijing in 2006 (Lv et al. 2008). Even though *A. cantonensis* infection was low in *P. canaliculata*, the host might be a vector that transmits the parasite to humans who eat "koi hoi," a traditional snail dish in Thailand (Eamsobhana et al. 2010).

In this study, identification of the *Pomacea* snail was confirmed based on 99–100% sequence identity after BLASTN searching. Previously, investigators have used the shell morphology, soft tissue morphology, and egg mass to identify *Pomacea* (Thiengo et al. 1993; Cowie et al. 2006). Using these criteria, it is difficult to distinguish all species of *Pomacea* due to intraspecific variation and ecological environments, especially with *P. canaliculata* and *P. maculata* (Matsukura et al. 2008; Hayes et al. 2012). Differentiating between *P. canaliculata* and *P. maculata* is difficult due to the low variation in shell morphologies (Hayes et al. 2012). To assist in the identification, the *COI* sequence can be used to differentiate between *P. canaliculata* and *P. maculata* (Matsukura et al. 2008; Yang et al. 2018a). These findings agreed with our *COI* sequencing results. Therefore, sequencing a partial region of the *COI* gene may be used to differentiate between *P. canaliculata* and *P. maculata*, due to nucleotide variations in the mitochondrial gene.

Phylogenetic analysis of *Pomacea* revealed two main clades for each species (*P. canaliculata* and *P. maculata*), which was closely related to *Pomacea* from several countries. These data indicate that the *P. canaliculata* and *P. maculata* Thai strains are most closely related to multiple countries. *Pomacea* species—including *P. canaliculata*, *P. maculata*, *P. diffusa*, and *P. scalaris*—have been detected in several countries in Asia (Hayes et al. 2008). *Pomacea canaliculata* and *P. maculata* may have been introduced into Asian countries from multiple origins (Argentina, Brazil, and

Table 2. Genetic diversity of *P. maculata* populations from Thailand and other geographical regions based on mitochondrial cytochrome *c* oxidase I gene sequences

Populations	Number of samples	Haplotypes			Haplotype diversity (h), mean ± SD	Nucleotide diversity (π), mean ± SD
		Number of haplotypes	Shared haplotypes	Unique haplotype		
Thailand	23	7	1	6	0.7826 ± 0.0605	0.0108 ± 0.0065
Argentina	10	3	1	2	0.6444 ± 0.1012	0.0040 ± 0.0032
Brazil	15	7	1	6	0.8952 ± 0.0433	0.0260 ± 0.0144
China	31	4	1	3	0.3419 ± 0.1016	0.0146 ± 0.0083
Japan	6	2	2	-	0.6000 ± 0.1291	0.0127 ± 0.0086
Malaysia	9	1	1	-	0	0
New Zealand	1	1	1	-	0	0
Spain	9	1	1	-	0	0
United States	12	3	2	1	0.6212 ± 0.0867	0.0113 ± 0.0070
Uruguay	5	4	1	3	0.9000 ± 0.1610	0.0098 ± 0.0073
Vietnam	1	1	-	1	0	0
Total	122	34	12	22	0.7878 ± 0.0365	0.0181 ± 0.0098

Central America) (Mochida 1991; Hayes et al. 2008).

Haplotype network analyses of *P. canaliculata* and *P. maculata* from Thailand included sequences from the native and introduced range. Haplotype Pc2 and Pm1 were the most widely distributed in the country, and were shared among snail populations in native and non-native countries. The presence of shared haplotypes among different populations from different countries indicates that the species were widely distributed to many countries from their native range. Although this particular snail has a low dispersal ability, man-made ecological transformation and climate change have driven the spread of exotic species like these and their establishment in new areas (Stachowicz et al. 2002; Bardsley and Edwards-Jones 2007). In addition, the frequent agriculture contacts among neighboring countries or regions further facilitates the introduction of *Pomacea* snails and speeds up their spread into new areas (Cowie 2002; Yusa et al. 2006).

In Thailand, seven haplotypes of *P. canaliculata* were identified, of which Pc1 was the most widely distributed (11 provinces). For *P. maculata*, six haplotypes were identified, and Pm20 was the most widely distributed in six provinces. Our findings are similar to those of Yang et al. (2018b), who identified several haplotypes of *P. canaliculata* from several countries (Yang et al. 2018b). This genetic variation indicates that haplotype diversity is found globally. The genetic variation in *P. canaliculata* and *P. maculata* may result from founder effects or bottlenecks (Matsukura et al. 2013). Founder effects may occur when invading propagules contain only a small number of individuals from a successful invasive population. Both effects may result in a low population diversity in invasive populations (Lombaert et al. 2010). In addition, climate change (Byers et al. 2013) and environment factors such as calcium carbonate (White et al. 2007; Perlman 2016), pH (Byers et al. 2013), salinity (Martin and Valentine 2014), and dissolved oxygen (Seuffert and Martín 2009) may affect *Pomacea* populations in Thailand.

CONCLUSIONS

In conclusion, *Angiostrongylus cantonensis* had a low infection rate in *Pomacea canaliculata* in Thailand. The populations of *P. canaliculata* and *P. maculata* in this country may have undergone recent expansions. Seven and six haplotypes based on the *COI* gene were identified from populations of *P. canaliculata* and *P. maculata*, respectively. Genetic differentiation in some populations of *P. canaliculata* may result from genetic drift. The *Pomacea* population in Thailand may help maintain the *A. cantonensis* life cycle and may be a potential source of transmission among humans. Our finding answers basic ecological questions about *A. cantonensis* and its *Pomacea* host and maps out the genetic diversity of *Pomacea* in Thailand.

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Supplementary materials

Fig. S1. Third stage larvae of *A. cantonensis* isolated from *Pomacea canaliculata* collected from Uttaradit Province of Thailand. It is characterized by two chitinous rods at the anterior end (arrow) being triangular-shaped and half-opened, tail tip slightly curved (arrow-head) (40x magnification under light microscope). (download)

Table S1. *Pomacea canaliculata* and *P. maculata* sampling locations used in this study. (download)

Table S2. Genetic distance between populations of *P. canaliculata* from Thailand with the native and non-native populations based on mitochondrial cytochrome *c* oxidase I gene sequences. (download)

Table S3. Genetic distance between populations of *P. maculata* from Thailand with the native and non-native populations based on mitochondrial cytochrome *c* oxidase I gene sequences. (download)

Table S4. Forty-five variable sites across the 25 haplotypes of *P. canaliculata* based on the 282-bp *COI* sequences. (download)

Table S5. The *P. canaliculata COI* sequences from GenBank used in this study. (download)

Table S6. Thirty-nine variable sites across the 25 haplotypes of *P. maculata* based on the 283-bp *COI* sequences. (download)

Table S7. Available *COI* sequences of *P. maculata* in GenBank used in this study. (download)