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Genetic diversity of genus *Filopaludina* in the upper northeastern Mekong Basin of Thailand revealed by mitochondrial DNA sequences

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Abstract

This research was applied DNA barcoding tool for the molecular identification of genus *Filupaludina* in Mekong basin northeastern Thailand using mitochondrial-cytochrome c oxidase I (mt-COI). Ninety snails were collected from 9 locations along the river in four provinces (Loei, Nong Khai, Beung Kan, and Nakhon Phanom Provinces,). DNA extraction of individual snail was amplified using COI primers. The PCR products were selected and examined nucleotide sequence before being genetic analysis. The phylogeny analysis suggested that *Filupaludina* species showed monophyletic with disseminated clades and they were genetically similar to F. *martensi*. The haplotype diversity observed in *Filupaludina* species showed 10 patterns of distribution. The genetic distance within subspecies F. *martensi martensi* and *Filupaludina martensi cambodjensis* showed mean values equal to 0.1739 and 0.1203, respectively. In addition, the genetic distance within *Filupaludina sumatensi polygramma* showed 0.3431. Finally, unique haplotypes were commonly found in all locations. These findings revealed that the gene flow of *Filupaludina* between different locations is limited, leading to the genetic differences that were detected. This study is the first report of the genetic sequence of *Filupaludina martensi cambodjensis*, and *Filupaludina sumatensi polygramma* in the upper northeastern of Thailand.

Keywords: Genetic diversity, Viviparidae, COI gene

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1. Introduction

The freshwater gastropod family Viviparidae Gray, 1847 is widely distributed in the world but absent from America and South America [1]. In Thailand Viviparidae were reported of all 8 genera are *Filopaludina, Trocotaia, Eyriesia, Idiopoma, Makongia, Sinotaia, Anulotaia* and *Cipangopaludina*. The pond snail *Filopaludina martensi* is previously knowing as *Siamopaludina*. This species is widely distributed and able to adapt to many habitats (lakes, ponds, canals, rivers and mountain streams with strong currents) often divided into many races and forms, more than 12 species, most of which are rassenkreis, one part synonyms, and the other part are subspecies spread in different region in Thailand, Laos, Cambodia, Myanmar, Malaysia, Indonesia along with Tonkin and south of china.[2]

Filopaludina sp. are well known as food for local people and also the only one genus that has been reported to the first or secondary snail intermediate hosts of parasite [2]. Three species (*F. martensi martensi, F. sumatrensis dorliaris* and *F. sumatrensis polygramm*) were reported to the parasitological importance of *Eechinostoma metacercaria* [3 – 5] which is the cause of Human echinostomiasis.[6] The four echinistome metacercaria, *Echinostoma malayanum, E. revolutum , E. ilocanum,* and *Hypoderaeum conoideum* have been reported for infecting human in Thailand[5] In these cause, the information of identifying the snail taxonomic status of hosts is important for health ecology and public health.

The taxonomy of viviparids is primarily based on shell morphology and the characters use only the shell shape, size, and sculpture. Whereas the color is limited use. By the reason of the pond snail is an animal with high shell variation.[8] In Addition too the generic names of viviparid snails *Vivipara, Viviparus, Bellamya,* and *Filopaludina* were called in the Philippines, Indonesia, Thailand and Malaysia, respectively. The genera and species of snail identifying are extremely confused in Southeast Asian countries.

2. Objectives

The objective of this study was to determine genetic diversity of *genus Filopaludina* in the Mekong basin of Thailand, based on molecular analysis.

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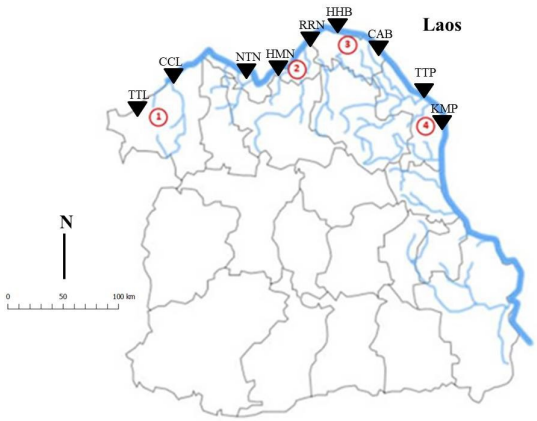

Figure 1: Map showing the sampling sites in four provinces in Mekong basin of northeastern Thailand (1) Loei (2) Nong khai (3) Beung kan (4) Nakhon Phanom. Table 1 for detail.

Table 1. List of snail sample collection localities.

Code	Coordinates	District	Province	Habitat	Number
TTP	17°34'34.0284"N, 104°35'27.3372" E	Tha Utan	Nakhon Phanom	canal	10
CCL1	17°52'5.0016" N, 101°39'23.5512" E	Chaing Kan	Loei	Rice paddy	10
TTL	17°32'21.6564" N, 101°25'48" E	Thali	Loei	Rice paddy	10
NTN	17°50'31.3332" N, 102°35'4.2864" E	Thabor	Nong khai,	Pond	10
HMN	17°55'4.7064" N, 102°48'12.8556" E	Meuang Nongkhai	Nong khai,	Canals	10
RRN	18°13'44.7672" N, 103°10'30.792" E	Ratanawapee	Nong khai,	Swamp	10
PSN	18°2'46.1616"N, 102°18' 23.5656"E	Sang Kom	Nong khai,	pond	10
HHB	18°25'55.416" N, 103°26'12.9804" E	Horkam, Meang	Beung kan	Swamp	10
CAB	18°16'18.9156 " N, 103°50'11.7744 " E	Chaiyaporn, Meang	Beung kan	pond	10

3. Material and Methods

3.1 Populations sample

One hundred and fifty two were collected from 28 locations in the Mekong basin of northeastern Thailand (Loei, Nong Khai, Beung Kan and Nakhon Phanom Provinces) (Fig. 1) (Table 1). All living samples were anesthetized by immersing in clove oil solution (clove oil: water 55:500 ml) and cleaned with water. The individual foot tissue of snail was cut and frozen in -20 °C for further genetic analysis. The sample shells were washed with water, air dry and collected for external morphology for classification according to the method of Brandt R.A.M. (1974) [2 based on shell morphology and characteristics (size, height, shape, skin texture, shell color, stripe pattern on the ridge, ridge, composition of the operculum, operculum thickness and operculum color).

3.2 DNA amplification and sequencing

The genomic DNA was extracted from snail foot tissue using GF-1 tissue DNA extraction kit (Vivantis Technologies Sdn. Bhd, Malasia) according to the manufacturer's instruction with some modifications. The PCR amplification of mitochondrial gene was done with the set of primers cytochrome oxidase subunit I (COI) (5'GGTCAACAAATCATAAAGATATT GG-3') and performed in a final volume of 50 ml. The reaction mixture contained 10x buffer with 1nM M_gCl_2 10 μM of each primer, 1U of *Taq* DNA polymerase and 50 nM of DNA sample. The PCR protocol comprised of initial denaturation at 94 °C for 3 min, followed by 10 cycles of denaturation at 94 °C for 40 s, 50 °C for 45 min, 72 °C 30 min. followed by 36 cycles of annealing at 94 °C for 30 min 50 °C for 45 s, extension at 72 °C for 45 min and final extension at 72 °C for 5 min. The amplified products were checked using 1% agarose gel electrophoresis and sent for gel purification and sequencing by the Apical Scientific Sdn Bhd Company.

Table 2. Genetic distance calculated base on COI sequence comparing the 10 sample. The number of base substitutions between sequences are shown. Analyses were conducted using the Kimura 2-parameter model.

		F. m. muensis					*F. m. cambodjensis*			*F.s. polygramma*	
		TTP1	**HMN2**	**RRN2**	**TTL1**	**RRN3**	**CCL12**	**HHB1**	**CAB1**	**PSN2**	**NTN2**
	TTP1										
	HMN2	0.06294									
F. m. muensis	RRN2	**0.01134**	0.06505								
	TTL1	0.32167	0.31587	0.34377							
	RRN3	0.11955	0.10119	0.12656	0.36216						
	CCL12	0.11584	0.10214	0.12934	0.348069	0.04479					
F. m. cambodjensis	HHB1	0.16391	0.16290	0.17237	0.361028	0.07906	0.09941				
	CAB1	0.14000	0.15312	0.13793	0.401702	0.16811	0.16492	0.22637			
F.s. polygramma	PSN2	0.15455	0.13586	0.15455	0.360771	0.14641	0.14416	0.19257	0.18402		
	NTN2	0.29372	0.29587	0.28801	**0.431801**	0.30476	0.29062	0.34319	0.31848	0.34313	

Figure 2: Specimen of Pond snail subspecies of the genus *Filopaludina*.

4. DNA sequences analysis

The snail from 9 populations were selected 10 specimens for genetic study. The mitochondrial DNA sequences were alignment with BioEdit version 7.2.6 to comparison between freshwater gastropod DNA (550 bp COI gene) and Genbank DNA sequence. Phylogenetic analyses were conducted for the combined data set using inferred the Maximum-likelihood method. The evolutionary distances were computed using Kimura 2-parameter model were performed in MEGA-X.[6, 7]

5. Result and Discussion

The genetic distance from pair-wise comparison of COI sequence represents in (Table 5). Two species and ten sequences were comprised that provided. The value of genetic distance between subspecies among four *F.martensi martensi* is (0.01134-0.12656) among the F. *martensi cambodjensis* is (0.09941-0.40170) and among the F. *sumatrensis* is 0.34313. The Intraspecific variation shows highly different score. In the case of *F. martensi martensi* which collected from Nakorn Phamom and Nong Kai shown very closely relationship (0.01134). But the *F. martensi martensi* which collected from Leoi is diverging from other members (TTP, HMN and RRN) 0.32167, 0.31587 and 0.34377 respectively. Infer that Loei Province geography is highlands and high mountains scattered relate to high differentiation of same species. [12] While only two of *F. martensi cambodjensis* give a high genetic distance between HHB and CAB (0.226371) which collected from same province but HHB was closer with RRN (0.172369) than CAB1. Found that the unique haplotypes were commonly dependent on different distance and locations with significant genetic differences between all populations. According to previous study genetic structure and geographical variation of *Bithynia siamensis goniomphalossensu lato* (Gastropoda: Bithyniidae) in the Lower Mekong basin revealed by mitochondrial DNA sequences and Phylogeny of freshwater viviparid snails in Japan by molecular phylogeny and taxonomy.[11, 13] These studies also show the population of *B.s. goniomphalosin* in the Lower Mekong in different catchments represented the gene flow between different collecting location. The highest genetic distance is 0.431801 of *F.sumatensis polygramma* and *F. martensi martensi* explain the extremely unique haplotypes. For the result of interspecific variation of three species show the minimum value is 0.01134-0.34313 and the maximum value is 0.34313 – 0.431801 confirm the snail variation is very high and rapidly variable on genetics.[13 – 15]

The phylogenetic tree is divided the monophyletic into six clades: clade A, clade B, clade C, clade D clade E and clade F. This study shows as clade A. The Taxa1 (RRN3, HHB1 and CCL12 sequences) was classified similarly to *F. martensi martensi* (MN997958- MN997960). They are similar of external morphology as well. It is possible to confuse in the genus name of viviparid snails in Southeast Asian countries and difficultly to use the key of external morphology to identify the snail in this family as mentioned above.[4] Whereas these snail might be invasive by human activities [16, 17] The clade E CAB1 was close with *F. sumatrensis* (MN997972, MN997978). Infer that CAB1 which was classified to specie *F. martensi* Beung kan, Thailand and *F. sumatrensis* might be immigrated by human[15]. The

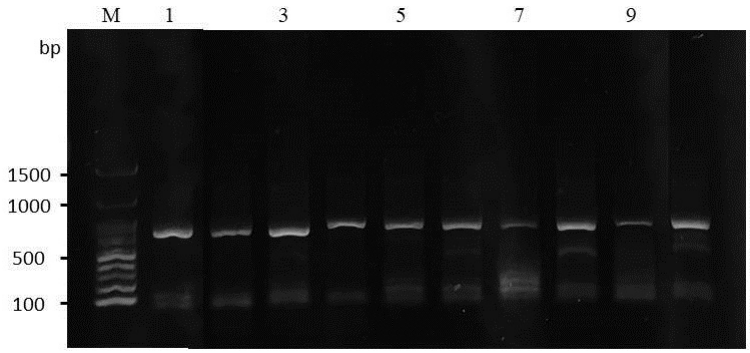

Figure 3: Gel electrophoresis of PCR products of snail using COI primer. Lane M = 100bp DNA ladder, lanes 1-9 = *Filopaludina* sp.

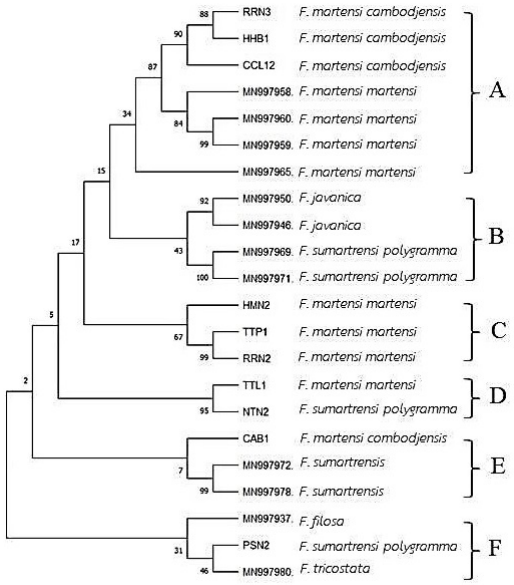

Figure 4: Phylogenetic tree of gastropod based on combined sequences from the COI genes. Each label represents a species/subspecies name followed by the specimen ID.GenBank; MN997937- MN997980.

clade F PSN1 was close with *F. filose* and *F. tricotata* (MN997937, MN997980) possibly that PSN1 which was classified to specie *F. sumatrensis polygramma* from Nong Kai was misclassified. And the specimen TTP1and RRN2 are very close relationship with 99% bootstrap replication

6. Conclusions

The PCR amplification and DNA sequencing were successful. The phylogenetic tree provided four clades and all samples of snail *Filopaludina* sp. from Mekong basin were the same clade. It can be said that the genetic diversity of *Filopaludina* sp. in Mekong basin of northeast Thailand is closer than other clades. This study is the first report of the genetic sequence of *Filupaludina martensi cambodjensis* and *Filupaludina sumatensi polygramma* in the upper northeastern Mekong basin of Thailand.

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