

## Original Article

# Morphological and molecular characterisation of Asian Clam, *Corbicula fluminea* Megerle von Mühlfeld, 1811 (Venerida, Cyrenidae) elucidates geographical variation in Thailand

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**Abstract**

The genus *Corbicula* is taxonomically equivocal due to being described with vast morphological dispersion, but narrow genetic variation. This study aimed to characterise the taxonomy and distribution of the *Corbicula* in Thailand. The phenotype of *Corbicula* was characterised based on morphometric characteristics. Then, mtDNA cytochrome b (cytb) was used to evaluate the genotype. The study indicates that the morphometric characteristics, namely shell height (SH), shell width (SW), anterior length (AL), posterior length (PL), and length posterior-anterior shell (LPAS), significantly differed between the evaluated samples. Canonical discriminant analysis revealed a significant difference between the morphometric characters by location. The genotype characterisation revealed a single species, *Corbicula fluminea* that inhabited the Thailand freshwaters, regardless of the geographical range. Phylogenetic analysis using Maximum Likelihood (ML), Neighbour Joining (NJ), and Maximum Parsimony (MP) methods demonstrated haplotype polymorphism. Therefore, phenotype variation of *C. fluminea* resulted from haplotype polymorphisms across the geographical range.

**Keywords:** Thailand, *Corbicula fluminea*, morphology, genetics, taxonomy

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**1. Introduction**

The Asian clams from the family Corbiculidae are found widely distributed in the freshwater ecosystem. This occurrence is believed to stem from the Chinese migration from the mainland to other places, where the *Corbicula* was brought as food (Wang, Zhang, Zhang, Li, & Xiao, 2014). Meanwhile, the *Corbicula* was found natively inhabiting East and Southeast Asia (Yusof, Sow, Ramli, Rak, & Wei, 2020). *Corbicula* was recorded inhabiting the Malay archipelago of Malaysia, Indonesia, and Thailand, with significant role as

food (Ramli, Ayyapan, Yusoff, Eh Rak, & Lee, 2020). The human activities combined with its opportunistic life-strategy induced a quick expansion and appearance of the *Corbicula* genus in their distribution, so these became the dominant species in the benthic communities (Sousa, Antunes, & Guilhermino, 2008). On the other hand, the *Corbicula* species inhabit a wide geographical range and can be found in freshwaters, estuaries, and mangrove environments (Macintosh, Ashton, & Havanon, 2002). This geographical range of environmental factors has contributed to the variations in *Corbicula* phenotypes, which complicates taxonomical identification.

Shell characteristics have been a phenotypic determinant in taxonomy of bivalves, although the shell variations may occur due to plasticity (Hamli, Idris, Rajae, &

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Kamal, 2015; Renard, Bachmann, Cariou, & Moreteau, 2000). Generally, two recognised shell colors (yellow and dark-purple) have been identified along with significant dimension ratios (Wang *et al.*, 2014). According to Schmidlin (2011), morphometric characterisation is reliable in interspecific, but intricate in intraspecific levels, since the different morphologies may still have similar genotypes. Previously, *Corbicula* was assigned more than 200 names based on morphology evaluation due to their shell variability (Gomes *et al.*, 2016; Pigneur, Marescaux, Roland, Etoundi, & Descy, 2011). However, this number has reduced recently, as two eminent species of *Corbicula* namely *Corbicula fluminea* and *Corbicula fluminalis* (Müller, 1774) were recognised in East Asia after incorporation of phenotypic variation, demographic data, ecology, physiology, and reproductive behaviour (Renard *et al.*, 2000). In recent years, many genetic studies of the genus *Corbicula* have been published. The genetic diversity of *C. fluminea* in Hongze Lake was assessed based on mitochondrial cytochrome b gene (mtDNA cytb) and the polymorphisms were varied among the ten populations (Zhu *et al.*, 2017). Previously, Gomes *et al.*, (2016) incorporated mtDNA cytb in an evaluation of *C. fluminea* genetic diversity in Portugal while the genetic relation of *C. leana* and *C. fluminea* in Japan was effectively elucidated and identified based on the mtDNA cytb sequence (Yamada, Ishibashi, Kawamura & Komaru, 2010).

In Thailand, the *Corbicula* taxonomy is still uncertain since the earliest description of *Corbicula* was made in 1864. For example, the *Corbicula* was named *C. gustaviana*, *C. hydiginia*, *C. moltkiana*, and *C. lamarkiana*, as deposited in Mollusk Museum, Mahidol University, Thailand. Formerly, the researchers emphasised the morphology characteristics in defining the *Corbicula* species without consideration of demography, ontogenetic variation, and other biological attributes (Kijviriyaya, Upatham, Viyanant & Woodruff, 1991). Then, *Corbicula* in Thailand was speculated as *C. fluminea* after examination of the allozyme variation from 40 sites throughout the country (Kijviriyaya *et al.*, 1991). The *Corbicula* in Thailand was genetically recognised as *C. fluminea* (AF468018) (Siripattawan, Park, & Foighil, 2000). This equivocal identification would complicate the future conservation works and resource management of this species. The taxonomy and systematic status of the genus *Corbicula* in its native range remain not resolved (Park & Kim, 2003). Hence, a combination of phenotypic methods and genetic analysis (based on mitochondrial cytochrome b) were used to discover their systematic relationship. In addition, the cytb sequences were compared with pre-existing cytb sequences sampled in Asia. Therefore, the current study aimed to characterise the morphology and genetics of the genus *Corbicula* and elucidate their variations across the geographical range.

## 2. Materials and Methods

Collections of *Corbicula* were sampled from seven localities namely Buriram (15°31'60"N, 103°28'25"E), Maha Sarakham (16°22'59"N, 103°26'83"E), Lopburi (14°99'37"N, 101°02'22"E), Surin (14°64'05"N, 103°39'30"E), Pattani (6°71'87"N, 101°28'20"E), Saiburi, Narathiwat (6°58'91"N, 101°49'66"E) and Sungai Golok, Narathiwat (5°96'03"N, 101°94'21"E), all in Thailand (Figure 1). These locations



Figure 1. Map of Thailand with yellow text at the sampling locations

were selected based on tributaries of major rivers located in the Northeast (Chao Phraya River, Mun River and Chi River) and in the Southern part (Saiburi River and Sungai Golok River). *Corbicula* was collected by using a scoop and sieve, and placed into plastic containers supplemented with wet tissue to ensure they stayed alive until reaching the laboratory (Eh Rak *et al.*, 2021). Then, 210 samples were fixed in 96% ethanol for DNA sequence analysis. The shell variations of *Corbicula* species were examined in a morphometric study of shell characters. A total of 210 individuals were measured to the nearest 0.01 mm with a digital caliper.

### 2.1 Morphometric and statistical analysis

For the morphometric study, 30 individuals were randomly selected from each sample site. The viscera were removed, and the empty shells of *Corbicula* were dried overnight in an oven at 60°C. Next, six morphometric characters were measured: shell length (SL) (the most significant anteroposterior dimension), shell height (SH) (the maximal dorsal-ventral dimension), shell width (SW) (left-right dimension with both valves), anterior length (AL), posterior length (PL) and length from anterior adductor muscle scar to posterior adductor muscle scar (LPAS) (Figure 2) (Ramli *et al.*, 2020). Additionally, the morphological forms of *Corbicula* were observed and determined based on the descriptions of the European forms: round dark-coloured (R), round light-coloured (Rlc), and saddle (S) (Peñarrubia *et al.*, 2017; Pigneur *et al.*, 2011). The morphometric allometry was determined for five characters subjected to SL as the predictor. The variations in the morphometric characters were calculated with a one-way analysis of variance (ANOVA) where the Tukey HSD *post*

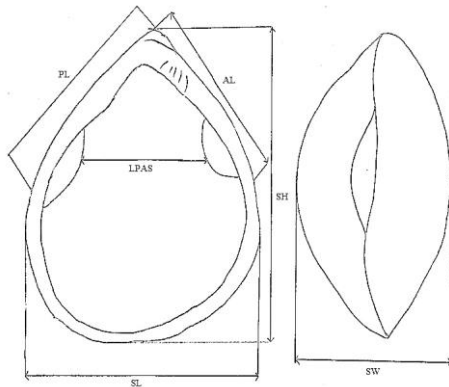


Figure 2. Morphometric characteristic of *Corbicula fluminea*. SL: standard length, SW: shell width, SH: shell height, AL: anterior length, PL: posterior length and LPAS: Length from anterior adductor muscle scar to posterior adductor muscle scar



Figure 3. Two morphotypes of *Corbicula fluminea* found in a recent study. (1) Saddle (s) morphotype and (2) Round (R) morphotype

*hoc* test determined the significant differences at probability level  $P < 0.05$ . Then, canonical discriminant analysis was carried out using the allometric data to determine the most important factors in classifying the *Corbicula* populations. The statistical analyses were performed using SPSS (version 23; SPSS Inc.).

## 2.2 Genetic analysis

The total genomes of 210 *Corbicula* from seven localities were extracted following a standard protocol (Sambrook & Russell, 2001). The isolated DNA was quantified using NanoDrop 2000 UV-Vis Spectrophotometer, and the quality was evaluated in 1% agarose gel electrophoresis. For PCR amplification sequencing, a primer pair of cytochrome b (cytb) was applied: (F:5'-GAAGGGTTCATGCTAACGGAG-3'; R:5'-GTGGTGTACTTATAGGGTCGGC-3') (Genbank accession number: ABB551548). Amplification was executed in a total volume of 50  $\mu$ L containing 5  $\mu$ L of 10 x reaction buffer, 0.08 U/ $\mu$ L exTEN DNA polymerase, 1.6  $\mu$ L of dNTP (2.5 mmol/L), 2.5  $\mu$ L of forward and reverse primer (10  $\mu$ M), 1  $\mu$ L of template DNA, and 36.6  $\mu$ L of nucleus-free water. PCR amplifications were carried out with the following program: denaturing at 94°C for 5 min, followed by 38 cycles of 94°C for 45 s, annealing at 60°C for 45 s and 72°C for 1 min; with a final extension at 72°C for 20 min. PCR amplicons were separated on 1% agarose gel electrophoresis. Then, the PCR products were sent to the 1<sup>st</sup> Base Laboratory for purification and

sequencing. The mtDNA cytb sequences were aligned in consensus using Multiple Sequence Alignment CLUSTALW. Then, Maximum likelihood (ML), Neighbour-Joining (NJ), and Maximum Parsimony (MP) were used in constructing phylogenetic trees based on the mtDNA cytb sequences. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The haplotypes were inferred using DnaSP 6.0 (Gomes *et al.*, 2016).

## 3. Results and Discussion

### 3.1 Morphometric analysis

Many studies have reported the use of shell morphology characteristics for identification in molluscs (Hamli, Idris, Abu Hena, & Wong, 2012; Sousa *et al.*, 2009). The uses of morphometric characterisations in taxonomic and systematic studies are cheaper, easier, and faster than molecular genetic analysis (Hamli *et al.*, 2012). However, in some cases, there is a need for support and complementary information from a genetic analysis to assess the molluscs (Echave, Spielman, & Wilke, 2016). Therefore, an evaluation was carried out by combining morphometric and genetic analysis in determining the existing species of *Corbicula* in seven locations in Thailand. Furthermore, this study sampled the *Corbicula* from seven locations in Northeast Thailand (Lopburi, Maha Sarakham, Buriram, and Surin) and Southern Thailand (Saiburi, Pattani, and Sungai Golok) (Figure 1).

Similar morphometric characters (SH/SL and SW/SL) were observed in *Corbicula* species collected from Lopburi, Buriram, and Surin. This finding indicates that the samples collected were from identical species with similar morphometric characteristics (Table 2). A similar finding was reported in a study conducted by Hamli *et al.*, (2012) on *Polymesoda erosa*, *Polymesoda expansa*, and *Polymesoda bengalensis* from Sarawak, Malaysia. Other than that, there are abiotic factors, such as a shallow habitat producing a higher ratio of shell-length (SL) to shell-width (SW) compared to a deeper habitat (Hamli *et al.*, 2012). However, this statement does not suffice to explain the shell variation that was contradictory to the recent findings where the *Corbicula* from the lake in Lopburi and Surin have similar characteristics with those that are collected from Buriram. The morphometric characters for these *Corbicula* populations in Lopburi, Buriram and Surin are significantly different in the ratios SH/SL, SW/SL, and AL/SL from Maha Sarakham. Geographically, Mun and Chi Rivers are important rivers in Thailand, extending from the main river, the Mekong River to the north and south, particularly in the northeast provinces in Thailand. Hence, the morphometric character analysis showed significant divergence between Mun and Chi tributaries such as in *Corbicula* from Buriram and Maha Sarakham. Meanwhile, the *Corbicula* population from Lopburi sampled from Pa Sak Dam was found significantly similar in four morphometric characters with Surin (Table 2). This finding may demonstrate the shell shape of *Corbicula* inhabiting lentic water systems, such as lakes and ponds. Additionally, polluted and non-polluted environments influence the condition of the clam, in addition to the contrast of lentic and lotic environment (Ramli *et al.*, 2020).

On the other hand, *Corbicula* populations from Southern Thailand significantly differed in the ratios SH/SL, SW/SL, AL/SL, and PL/SL compared with those from Northern Thailand (Table 2). Furthermore, the habitats of the *Corbicula* in these three locations (Sungai Golok, Pattani, and Saiburi Rivers) are generally sandy loam to loamy sand and shallow, which is slightly similar to Mun and Chi Rivers (Najjah *et al.*, 2017) Hence, the morphology variations could be explained as a result from the microhabitat and macrohabitat factors that determine the plasticity of *Corbicula* morphology. In general, *Corbicula* in all sampled areas has a rounded shape with yellow to greenish-brown colour, which may be similar to the *C. fluminea* shell morphology. This finding agreed with Ishibashi *et al.*, (2003) in description of the *C. fluminea* shell morphology in Japan, as mentioned above. Although a few samples were found to be of rounded shape and purplish-black in colour, they were classified as *C. fluminea* as well. For instance, the inner colour is strongly associated with the response to microhabitat, where white form is largely found in sandy silt while purple dark is abundant in rocky or muddy habitats (Hillis & Patton, 1982; Ramli *et al.*, 2020).

The canonical discriminant analysis determined the significant morphometric characters that contributed to the segregation of the populations. The distribution shows the separation of *Corbicula* populations from Southern Thailand (Saiburi, Sungai Golok, and Pattani) with Northern Thailand (Lopburi, Surin, Maha Sarakham, and Buriram) (Figure 4). Consequently, the morphological analysis could not distinctly distinguish (instead leaving overlap in distributions) the *Corbicula* populations, but still demonstrated the geographical range distribution. Hence, molecular evaluation in conjunction with morphological evidence could perhaps anchor the species identification and distinguish closely related species due to environmental factors (Ramesha & Sophia, 2015; Wang *et al.*, 2014). Moreover, freshwater bivalve species possess the ability to adapt to different niches (Hedtke, Stanger-Hall, Baker, & Hillis, 2008).

On the other hand, three morphotypes were classified as round dark-coloured (R), round light-coloured (Rlc), and saddle (S) among the *Corbicula* populations (Table 1). Morphologically, these forms are described as round and broad forms with deep ridges (R), narrow forms with closely spaced ridges (S) (Figure 3), and Rlc refer to round form with a lighter shell whose interior is white-yellow (Pfenninger, Reinhardt, & Streit, 2002) According to Pigneur *et al.*, (2011), forms R and S corresponded to *C. fluminea* and *C. fluminalis*, respectively, while Rlc also referred to *C. fluminea* with a different form. On the other hand, these forms may be found

inhabiting the same habitat, as in a recent study that explains the sympatric evolution. For instance, two shell colours and forms were found sympatrically inhabiting Datong Lake, China (Wang *et al.*, 2014): green and pink type (Ishibashi *et al.*, 2003); and green and yellow type (Yamada *et al.*, 2010) in Japan. Still, further analysis showed they were not distinctly different species. Hence, the variation of these forms could provide discrimination of species, but needs to be backed with genetic evidence.

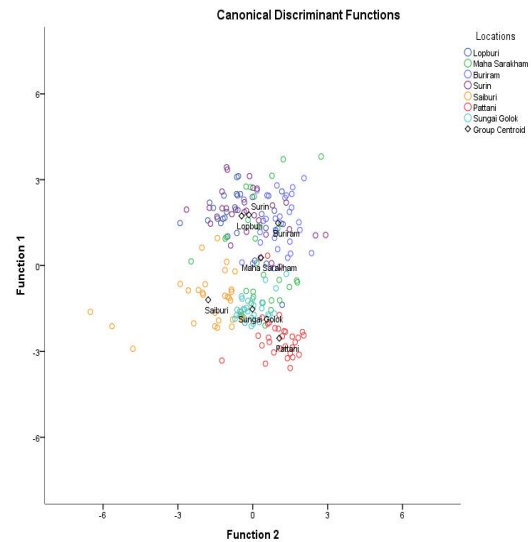


Figure 4. Morphometric analysis using a discriminant analysis-group graph for all locations

Table 1. Sampling sites of *Corbicula fluminea*. in Thailand and morphotype

Location	River/Lake	Morphotype (R, Rlc and S)
Buriram	Mun River	R
Maha Sarakham	Chi River,	Rlc
Lopburi	Pa Sak Chonlasit	S
Surin	Huai Saneng,	S
Pattani	Pattani River	R
Saiburi	Saiburi River	Rlc
Sungai Golok	Golok River	Rlc

\*R=Dark-colored round, Rlc=Light-colored round, S=Saddle

Table 2. Morphometric characteristic proportion with standard length (SL) of *Corbicula fluminea*

Morphometric ratio	Lopburi	Maha Sarakham	Buriram	Surin	Pattani	Saiburi	Sungai Golok
SH/SL	0.980±0.03 <sup>a</sup>	0.924±0.08 <sup>b</sup>	0.971±0.06 <sup>a</sup>	0.982±0.04 <sup>a</sup>	0.845±0.03 <sup>d</sup>	0.931±0.05 <sup>b</sup>	0.891±0.02 <sup>c</sup>
SW/SL	0.701±0.04 <sup>a</sup>	0.656±0.08 <sup>b</sup>	0.734±0.06 <sup>a</sup>	0.706±0.04 <sup>a</sup>	0.578±0.03 <sup>c</sup>	0.575±0.06 <sup>c</sup>	0.611±0.03 <sup>c</sup>
AL/SL	0.535±0.12 <sup>d</sup>	0.580±0.10 <sup>c</sup>	0.653±0.07 <sup>b</sup>	0.522±0.10 <sup>d</sup>	0.752±0.04 <sup>a</sup>	0.654±0.06 <sup>b</sup>	0.649±0.02 <sup>b</sup>
PL/SL	0.504±0.07 <sup>b</sup>	0.458±0.06 <sup>c</sup>	0.545±0.07 <sup>a</sup>	0.462±0.04 <sup>c</sup>	0.496±0.03 <sup>b</sup>	0.559±0.07 <sup>a</sup>	0.501±0.03 <sup>b</sup>
LPAS/SL	0.675±0.03 <sup>a</sup>	0.649±0.08 <sup>a</sup>	0.681±0.03 <sup>a</sup>	0.682±0.03 <sup>a</sup>	0.585±0.04 <sup>b</sup>	0.598±0.05 <sup>b</sup>	0.569±0.02 <sup>b</sup>

Remark: SH: Shell height; SL: Shell length; SW: Shell width; AL: Anterior length; PL: Posterior length; LPAS: Length of posterior adductor scar to anterior adductor scar. Different superscripts in a row indicate significant differences at level P<0.05



### 3.2 Genetic analysis

This study found that the partial mtDNA cytb gene sequence of *Corbicula* populations in Thailand is closely related to the *C. fluminea*. This genetic finding coincides with previous allozymes analysis, which demonstrated only *C. fluminea* existed in Thailand (Kijviriyaya *et al.*, 1991). Although the phylogenetic tree formed distinct branches of *Corbicula* populations, the genetic variation based on the bootstrap value indicates low genetic variation. Maximum likelihood (ML) (Figure 5), Neighbour-Joining (NJ) and Maximum Parsimony (MP) (Figure 6) were used in analysing the genetic distance of *Corbicula* populations using mtDNA cytb gene fragments. As a result, these phylogenetic trees showed similar topologies which explained the mtDNA cytb gene showing only low-level sequence divergences within species. Intraspecific divergences in *Corbicula* were found lower in Buriram and Surin which is elucidated by ancestral history. Possibly they were sourced from the Mun River population but the responses to environmental factors diversified the phenotypes. Reproductive behaviours of hermaphrodites and androgenesis lessen genetic diversity. Furthermore, a previous report mentioned the hermaphrodite behaviour of *Corbicula* in Thailand lowering the population variability and individual heterozygosity (Kijviriyaya *et al.*, 1991). Hence, a recent study provides evidence of the genetic diversity in *C. fluminea* which is probably relevant to other sites.

Referring to the phylogenetic trees, the *Corbicula* from the Chi River (Maha Sarakham) branched earlier than others. The sequence alignment with the BLAST tool showed the resemblance to the *C. leana* for this *Corbicula* sample, whereas other *Corbicula* populations evaluated were highly similar to *C. fluminea*. In Japan, mtDNA cytb sequences analysis was able to discriminate *C. fluminea* and *C. leana* (Okawa, Kurita, Kanno, Koyama, & Onikura, 2016). Nevertheless, the genetic distance between these two populations is insignificant, since male *C. leana* was synonymised to *C. fluminea*. The genetic divergence may occur due to speciation where the incompatibility of gene combinations leads to isolation or adaptation to different niche requirements (Shapiro, Leducq, & Mallet, 2016). Moreover, the taxonomy relationship between *C. leana* and *C. fluminea* is equivocal since the *Corbicula* exhibited high polymorphism in shell morphology. This polymorphism in phenotypes suggested that *C. leana* and *C. fluminea* coexisted in Datong Lake, China (Wang *et al.*, 2014), although previous work identified both species as *C. fluminea* (Qiu, Meng, Yu, Liu, & Wang, 2010). Then, previous work had reported the genetic differences between these species (Park & Kim, 2003; Siripattawan *et al.*, 2000). However, a more recent genetic analysis was unable to distinctly distinguish *C. fluminea* and *C. leana* since they were similar in mtDNA sequences even though some variation was observed (Komaru *et al.*, 2013; Yamada *et al.*, 2010). Hence, the previous findings declare *C. leana* as *C. fluminea* in the recent studies.

Unfortunately, in this study *Corbicula* from another main river such as the Chao Phraya River could not be found, but it is believed that *C. fluminea* inhabits this river. A previous record found that *C. fluminea* inhabited this river and the specimen was deposited in Mollusk Museum, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (Kijviriyaya *et al.*, 1991). Besides, a recent study evaluated the

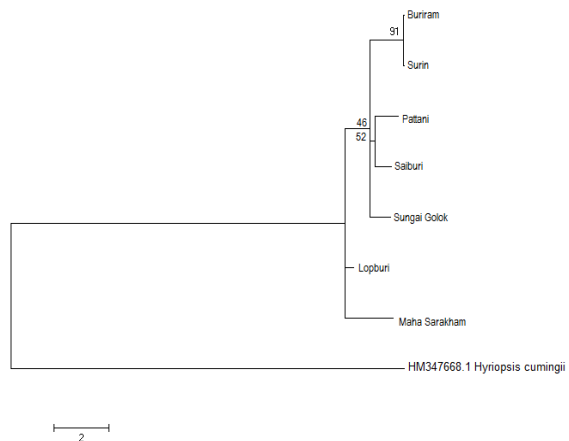


Figure 5. Phylogenetic tree constructed by the Maximum Likelihood (ML) method using a 481-bp partial fragment of the mitochondrial gene cytochrome b (mtDNA cytb) dataset for *Corbicula fluminea*. The construction of the tree incorporated *Hyriopsis cumingii* (HM347668) sequence as an out-group and used the Tamura-Nei substitution model. The bootstrap was run for 1000 replicates.

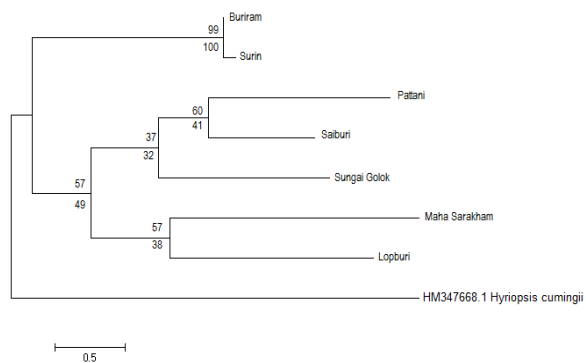


Figure 6. Phylogenetic tree constructed by the Neighbor-Joining (NJ) method using a 481-bp partial fragment of the mitochondrial gene cytochrome b (mtDNA cytb) dataset of *Corbicula fluminea*. The construction of the tree used the sequence for *Hyriopsis cumingii* (HM347668) as an out-group and was based on Kimura 2-parameter genetic distance. Similar topology resulted from Maximum Parsimony (MP). The bootstrap values for NJ are above the branches and for MP they are below branches.

*Corbicula* from the Pa Sak Dam, Lopburi, identified as *C. fluminea*. Pa Sak Dam is sourced from the Pa Sak River, where this river is directed into Chao Phraya River at Ayutthaya. Therefore, similar ancestry to *C. fluminea* could have been inhabiting Chao Phraya River, which agrees with the previous finding based on this geographical factor. On the other hand, a similar inference could be deduced since the genetic variation of *C. fluminea* in Saiburi and Pattani was not much separated compared with Sungai Golok. Furthermore, these rivers have connected with many small tributaries where the Saiburi River stretched into the Pattani district. Hence, these distributions of *C. fluminea* have closely related to its dispersal mechanism, whether it is passively downstream or upstream transported (Hubenov, Trichkova, Kenderov, & Kozuharov, 2013).

In comparison with deposited mtDNA cytb data of Asian *Corbicula* in NCBI, the evaluated *C. fluminea* from Thailand were grouped together with *C. fluminea* from China (Figure 7). Hence, recent findings revealed that the *C. fluminea* has low genetic variation regardless of the geographical range. On the other hand, the *C. japonica* were in a separate group (Figure 7) since this clam was found living in brackish waters in Japan (Yamada, Ishibashi, Tayoda, Kawamura, & Komaru, 2014). The topology explains the divergence between freshwater and brackish water *Corbicula*. An earlier study mentions that the *C. japonica* was synonymised to eminent species such as *C. fluminalis* which is also known as brackish *Corbicula* (Korniusshin, 2004). Meanwhile, the *Corbicula* sp. from Dongting Lake, China was found separated from other samples, possibly due to undistinguished taxa. The *Corbicula* sp. is currently synonym to *C. fluminea* since it has similar phenotype, reproductive behaviour and lives in freshwater habitat (Ramli *et al.*, 2020).

#### 4. Conclusions

In a conclusion, all results in the present study showed that *C. fluminea* is the only species that inhabits Thailand freshwaters regardless of their phenotype variation and geographical ranges. Analyses of morphology and molecular biology are appropriate to identify and discriminate against the *Corbicula* population. Certainly, *Corbicula* taxonomy is complicated to describe, since the phenotype variation is contributed by many factors such as genetic materials, geographical and environmental factors, or interactions between these types of factors that need to be accounted for. A comprehensive taxonomy work such as analysis of nuclear genetic markers instead of mitochondrial DNA, proteomic, reproductive behaviours, and geographical range may elucidate and systematic status of the *Corbicula* taxonomy.

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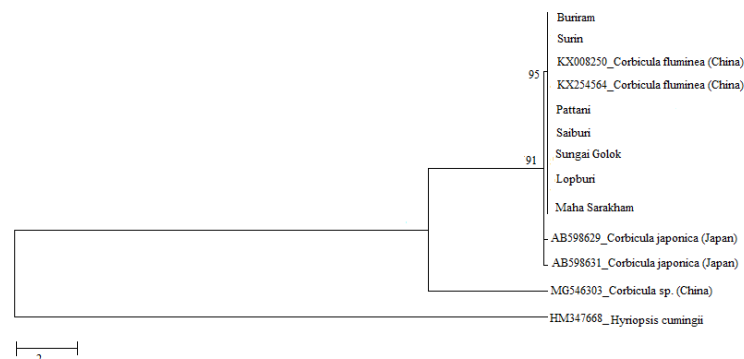


Figure 7. Phylogenetic tree constructed by the Maximum Likelihood (ML) method using a 481-bp partial fragment of the mitochondrial gene cytochrome b (mtDNA cytb) dataset for Asian *Corbicula* sp. (NCBI) and *Corbicula fluminea* in recent study. The construction of the tree used the sequence for *Hyriopsis cumingii* (HM347668) as an out-group and used the Tamura-Nei substitution model.

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