

Identification and Distribution of Wedge Clams (Donacidae: Bivalvia) in Thailand by Geometric Morphometric and Molecular Analysis

Cheewarat Printrakoon^{1,*} , Sasimanas Unajak² , and Javier H. Signorelli³ 

¹Animal Systematics and Ecology Specialty Unit (ASESRU), Department of Zoology, Faculty of Science, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand. *Correspondence: E-mail: cheewarat.p@ku.th, fscicrp@ku.ac.th (Printrakoon)

²Department of Biochemistry, Faculty of Science, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand. E-mail: fscissmn@ku.ac.th (Unajak)

³Instituto de Biología de Organismos Marinos, IBIOMAR (CCT CONICET-CENPAT), Bvd. Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina. E-mail: jsignorelli@cenpat-conicet.gob.ar (Signorelli)

Received 16 November 2021 / Accepted 7 October 2022 / Published 29 December 2022
Communicated by Benny K.K. Chan

The phylogenetic relationship of living Thai Donacidae was herein studied. Two methodologies, geometric morphometrics (GM) and genetic analysis of *COI* sequences, were combined and applied to identify the valid taxa and explain biodiversity and the distribution pattern in this family. A total of 587 living specimens were tested to analyze the shape and size patterns by Elliptic Fourier Analysis (EFA). Shell identification and GenBank sequences were added to construct the phylogenetic relationship and haplotype network. Centroid size was used to identify the specimens to the subgenus level. *Donax (Hecuba) scortum*, was easily distinguished from other species by Principal Component analysis (PCA) of shell size and shape. *Donax (Dentilaton) incarnatus* and *Donax (Deltachion) semisulcatus semisulcatus* were identified using Canonical Variates Analysis (CVA). Pairwise comparison of EFA was used for species level recognition, particularly shape overlap was observed for medium and small shell size. Based on genetic distance and haplotype network of *COI* sequences, *Donax (Latona) faba* and *D. (Latona) solidus* could be grouped in the same clade. Intraspecific and interspecific genetic data variation of some common species in different geographical localities of Thailand was observed. Three distribution patterns of *Donax* species were observed along the two-marine system of Thailand.

Key words: Thailand, Bivalve, *Donax*, Elliptic Fourier shape analysis, *COI*.

BACKGROUND

Thailand is surrounded by the tropical seas of the Indo-West Pacific. It comprises two marine ecosystems. The eastern coastline outlines the Gulf of Thailand, which is located in the Sunda shelf of the Pacific Ocean. Thailand's western coastline borders the Andaman Sea in the Indian Ocean (Spalding et al. 2007; Pochai et al. 2017; Chan et al. 2022). Each system is influenced by marine currents and different environmental conditions that affect the biodiversity of marine organisms, such as seaweed (Pongparadon et al. 2015 2017), sea

urchins (Coppard et al. 2021) and barnacles (Tsang et al. 2012; Chan et al. 2022). Phylogeographical studies of molluscs are scarce, and most existing studies focus on gastropods. For example, Reid et al. (2006) studied five species of *Echinolittorina* snail and Oskars and Malaquias (2019), analysing the geographical distribution, genetic diversity, species boundaries and ecological speciation of Haminoeidae gastropods in the Indian Ocean and the western Pacific. A previous study of the class Bivalvia examined the phylogeny of members of the family Pectinidae living in Thai waters (Mahidol et al. 2007).

The family Donacidae (Bivalvia) includes more than 100 species and 14 living subgenera (e.g., Dall 1892; Lyngø 1909; Lamy 1914; Wade 1967; Coan 1973 1983; Subba Rao and Dey 1986; Paredes and Cardoso 2001; Huber 2010 2015). The members of this family are distributed in warm waters around the world (Abbott 1974; Ansell 1985; Huber 2010) and vary in shell shape and colour (Ansell 1985; Donn 1990; Soares et al. 1998; Huber 2010; Tan and Low 2013; Ambarwati and Faizah 2017; Signorelli and Printrakoon 2020).

Almost 30 Donacidae species have been registered as living in Thai waters (Lyngø 1909; Suvatti 1937 1938; Nielsen 1976; Tantanasiwong 1979; McCoy and Chongpeepien 1988; Carpenter and Niem 1998; Kilburn and Hylleberg 1998; Sanpanich 1998 2011; Aungtonya et al. 1999; Swennen et al. 2001; Robba et al. 2002). However, eight species have recently been confirmed as valid (Signorelli and Printrakoon 2020). They are: *Donax (Deltachion) spinosus* Gmelin 1791, *Donax (Deltachion) semigranulosus* (Dunker 1877), *Donax (Dentilaton) incarnatus* Gmelin 1791, *Donax (Hecuba) scortum* (Linnaeus 1758), *Donax (Latona) cuneatus* Linnaeus 1758, *Donax (Latona) faba* Gmelin 1791, *Donax (Latona) solidus* Dunker 1798 and *Donax (Paraserrula) introradiatus* Reeve 1855. Although the present work follows the classification developed by Signorelli and Printrakoon (2020), *Donax (Deltachion) bruneirufi* Huber, 2012 and *Donax (Deltachion) semisulcatus semisulcatus* Hanley, 1843 must replace *D. (Paraserrula) introradiatus* and *D. (Deltachion) semigranulosus* (Dunker 1877), respectively. Previous classifications have also overlooked Huber (2012). Recently, Raven (2021) has suggested that the specimen assigned by Signorelli and Printrakoon (2020) to *Donax (Latona) cuneatus* should be assigned to *Donax (Latona) bicolor* Gmelin, 1791. However, the classifications reported by Signorelli and Printrakoon (2020) will be followed in the present study until new data become available.

Crampton and Maxwell (2000) suggest that shape outline analysis can be used to test history, function, habits and phylogenetic relationships of the bivalve Crassatellidae. Signorelli and Printrakoon (2020) further suggest that the GM method may be useful for identifying intra- and interspecific variation in Thai Donacidae.

Donacidae have trigonal shells that are medium-sized to small, solid and laterally compressed. The external shell varies; it may be smooth or radially lined, while the internal shell has a deep pallial sinus. The umbo is opisthogyrate and hinged; two cardinals and well-developed laterals and a pallial sinus are normally present. The intra- and interspecific shape variation observed within this family could impact identification

(Signorelli and Printrakoon 2020).

The main function of bivalve shells is protection (Akberali and Trueman 1985; Alyakrinskaya 2005). The morphology of bivalve shells is influenced by a combination of genotype, phenotype value, and environmental interactions (Wada 1986; Roopnarine et al. 2008). Phenotypic plasticity and genetic differentiation also contribute to intra- and interspecific variations in shell morphology (e.g., Gould 1971; Ackerly 1992; Manuel and Dadswell 1993; Gaspar et al. 2002; Ubukata 2003; Márquez et al. 2011; Signorelli et al. 2012).

Geometric morphometric (GM) methods are an excellent tool for quantifying and comparing variations in the size and shape of living organisms (Rohlf 1990; Wheeler 2008; Webster and Sheets 2010). GM methods have also been used to explore how an organism's morphology covaries with other correlated factors (Bookstein 1978; Blackith and Rayman 1971; Rayment 1980; Wheeler 2008). This methodology has been applied in studies of systematic and evolutionary biology, of functional anatomy, of fisheries management and of the aquaculture of molluscs (e.g., Innes and Bates 1999; Roopnarine et al. 2008; Costa et al. 2010; Serb et al. 2011; Márquez et al. 2010a b 2011; Signorelli et al. 2012; Rufino et al. 2013). GM can also be used to reveal similarities or differences in shape outlines via elliptic Fourier analysis (Ferson et al. 1985; Palmer et al. 2004; Zieritz and Aldridge 2009; Godefroy et al. 2012; Rufino et al. 2013; Dapar and Tabugo 2018). When homologous landmarks are difficult to define, outline-based GM is recommended (Rohlf 1990). Outline analysis examines variations in shape due to changes in open or closed curves (perimeters), defined by non-fixed landmarks (Webster and Sheets 2010). EFA can be used to test morphometric variations in shell outlines (Ferson et al. 1985; Palmer et al. 2004; Godefroy et al. 2012). According to Kuhl and Giardina (1982), elliptic Fourier descriptors quantitatively and mathematically describe the overall shape of something. This technique is used to transform coordinate information related to contours into Fourier coefficients. EFA has previously been used to explore shell shape variation in several species in order to better understand stratigraphic, geographic and habitat variations (Ferson et al. 1985; Zieritz and Aldridge 2009; Rufino et al. 2013; Dapar and Tabugo 2018).

Genetic data can also be used for species-level identification of marine organisms (Wheeler 2008; Feng et al. 2010 2011; Nantón et al. 2015; Souza et al. 2016; Fernández-Pérez et al. 2017). Mitochondrial *COI* sequences are one of the most popular markers used in genetic analyses. This approach has been proven to be suitable for bivalve identification (Matsumoto 2003;

Feng et al. 2010 2011; Mao et al. 2011; Layton et al. 2014; Nantón et al. 2015; Fernández-Pérez et al. 2017; Wang et al. 2017).

Several DNA studies on *Donax* species have been conducted. Laudien et al. (2003) studied genetic and morphological variations in four populations of the surf clam *Donax serra* (Roding) from South African waters. Yokogawa and Kondo (2006) examined the phylogeny of the Japanese *Donax*. *COI* sequences have also been used to estimate inter- and intraspecific similarities between populations living in geographically distant regions (Carstensen et al. 2009; Nantón et al. 2015; Fernández-Pérez et al. 2017).

The main objective of the present study is to outline a possible approach for identifying and exploring the phylogenetic relationships of eight valid species of the genus *Donax* that live in Thai waters; our approach uses GM and genetic data.

MATERIALS AND METHODS

Study area and shell collection

The sampled localities, distributed along the Gulf of Thailand and Andaman Sea, are listed in table 1. The relative distribution (%) of eight valid species along the coastline are illustrated in figure 1. All sampled specimens were collected by hand from the intertidal zone during low tide in February 2017 to May 2018. Soft parts were removed for molecular analyses and left valves were cleaned and air-dried. In addition, dead shells from the museum collections of the Phuket Marine Biological Centre and Institute of Marine Science, Burapha University, Chonburi Province were included in the GM analysis.

Geometric Morphometric and statistical analysis

A total of 587 specimens were randomly selected. Digital images were taken with a Canon EOS 1500D digital camera. 60 points over the internal part of the left valve outline were digitized with the tps Dig2 software (Rohlf 1996) (Fig. 2) by one observer (C.P.). Raw data (x, y coordinates) were transformed to Elliptic Fourier analysis (EFA, Crampton 1995) that was carried out using PAST program version 2.17 (Hammer et al. 2001; Hammer and Harper 2006). Using a mathematical approach, this methodology reveals the size and shell shape variation by the transformation of coordinates data related to contours into centroid size score and Fourier coefficients, respectively. The Elliptic Fourier analysis performed in this study avoided the homologous

points of internal shell such as pallial lines or adductor muscle scar that were difficult to recognized. 120 coefficients were represented in Cosine and Sine so that Fourier harmonics were generated to approximate shell shape as a complete ellipse for each outline following Crampton (1995). Fourier coefficients were used as morphometric variables in a multivariate analysis, summarized by a Principal Component analysis (PCA), to visualize shell shape variation. Canonical Variates analysis (CVA) of Fourier coefficients was computed to check outline discrimination along discriminant axis of overlooking and within size classified group. Fourier coefficients were imported to statistical analysis. One-way Multivariate Analysis of Variance (MANOVA) was carried out to demonstrate the distinction between groups. Fourier coefficients of multiple variates were found after pairwise comparisons analysis (post-hoc), whereas the discrimination of two sets of multivariate data was calculated with the Hotelling's T^2 test and presented by the discriminant scores. Statistical analyses were performed with the SPSS version 24 for the Kruskal-Wallis test and for the analysis of centroid size between eight species.

Molecular analysis of cytochrome c oxidase subunit I (*COI*)

DNA extraction and amplification of *COI* gene

In this study, the DNA was extracted from 95% ethanol preserved adductor muscle tissue using the phenol-chloroform extraction method that was described by Li et al. (2002). About 100 ng of genomic DNA was subjected to polymerase chain reaction (PCR) of cytochrome c oxidase subunit I (*COI*) gene. Briefly, the amplification of *COI* gene (710 bp) was carried out using *COI* gene specific primers (Folmer et al. 1994). Reaction mixture contained: genomic DNA, 10 mM pmol of each primer (Bioscience Resource Project, Pathumthani, Thailand), 2 µl of 10x *ExTaq* polymerase (Takara, Japan), 200 mM of deoxynucleoside triphosphates (dNTPs) (Thermo Fisher Scientific), and 0.1 U of *ExTaq* polymerase (Takara, Japan). The amplification conditions were conducted as follows: (i) 95°C, 5 min; (ii) 95°C, 30 s; 50°C, 30 s; and 72°C, 30 s for 35 cycles; (iii) extension step at 72°C, 10 min. The amplified PCR products were visualized on 2% agarose gel by gel documentation system (Las500, GE). Approximately 700 bp amplicon of the *COI* gene was purified using a gel extraction kit (Favorgen Biotech Corporation, Ping-Tung, Taiwan), according to the manufacturer's instruction, and subjected for sequencing (Macrogen, Seoul, Korea). The molecular identification of *COI* gene was assessed by nucleotide sequencing (Macrogen Inc.

Table 1. List of material from 28 localities tested in the geometric morphometric analysis. Gulf of Thailand (GT), Andaman Sea (AN)

Species	ID	Locality name	Province	Region	Locality, No.
<i>Donax (Latona) cuneatus</i>	0 to 17	Manao Bay	Prachubkirikhun	GT	16
	18 to 21	Suan Luong Beach	Prachubkirikhun	GT	20
	25 to 42	Manao Bay	Prachubkirikhun	GT	16
	43 to 44	Don Samlan	Prachubkirikhun	GT	18
	23 to 24	Kung Wiman Bay	Chantaburi	GT	4
	45 to 46	Kung Wiman Bay	Chantaburi	GT	5
	47 to 50	Mae Rumpheung Beach	Rayong	GT	7
	51	Ban Phae	Rayong	GT	6
	52 to 53	Patong Beach	Phuket	AN	23
	22	Nai Yang Beach	Phuket	AN	22
	59 to 64	Sam Phaya Beach	Prachubkirikhun	GT	13
	56 to 57	Sam Phaya Beach	Prachubkirikhun	GT	14
<i>Donax (Latona) solidus</i>	55	Manao Bay	Prachubkirikhun	GT	16
	58	Patong beach	Phuket	AN	23
	54	Bak Meug, Sikoa	Trang	AN	27
	95 to 154	Manao Bay	Prachubkirikhun	AN	16
	155 to 162	Sai Khao Beach	Prachubkirikhun	GT	19
<i>Donax (Latona) faba</i>	179 to 188	Sai Khao Beach	Prachubkirikhun	GT	20
	213 to 321	Beok Tein Beach	Petchaburi	GT	11
	234 to 235	Ban Kood, Kood Is.	Trat	GT	1
	238 to 239	Chao Lao Beach	Chantaburi	GT	3
	240 to 241	Kung Wiman Bay	Chantaburi	GT	4
	243 to 247	Leam Sing	Chantaburi	GT	2
	242	Leam Maephim	Rayong	GT	5
	237	Mae Rumpheung Beach	Rayong	GT	7
	198 to 212	Pataya	Chonburi	GT	9
	236	Ban Amphur	Chonburi	GT	8
	65 to 94	Tungken Bay	Phuket	AN	25
	163 to 178	Sapha Hin Beach	Phuket	AN	26
	189 to 197	Chalong Bay	Phuket	AN	24
	232 to 233	Patong Beach	Phuket	AN	23
	248 to 296	Patong Beach	Phuket	AN	23
	297 to 305	Manao Bay	Prachubkirikhun	GT	16
	306 to 365	Kho Chongkrachok	Prachubkirikhun	GT	15
	453	Bo Bang Beach	Songkha	GT	21
<i>Donax (Deltachion) spinosus</i> <i>Donax (Dentilaton) incarnatus</i>	366 to 371	Leam Sing	Chantaburi	GT	2
	454 to 474	Mae Rumpheung Beach	Rayong	GT	7
	372 to 402	Bak Meung, Sikoa	Trang	AN	27
	423 to 452	Bak Meung, Sikoa	Trang	AN	28
	403 to 422	Patong Beach	Phuket	AN	23
	480 to 507	Chao Samran Beach	Petchaburi	GT	10
	508 to 527	Beok Tein Beach	Petchaburi	GT	11
	475 to 479	Mae Rumpheung Beach	Rayong	GT	7
	528 to 532	Kho Chongkrachok	Prachubkirikhun	GT	15
	533 to 534	Manao Bay	Prachubkirikhun	GT	16
<i>Donax (Deltachion) semisulcatus semisulcatus</i>	546 to 547	Manao Bay	Prachubkirikhun	GT	17
	535 to 538	Kho Chongkrachok	Prachubkirikhun	GT	15
	539 to 545	Kho Takeab Bay	Prachubkirikhun	GT	12
	548	Moeng Prachub	Prachubkirikhun	GT	14
	549	Vanakorn Beach	Prachubkirikhun	GT	17
	554 to 557	Chao Lao Beach	Chantaburi	GT	3
	562 to 572	Leam Sing	Chantaburi	GT	2
	550 to 553	Mae Rumpheung Beach	Rayong	GT	7
	558 to 561	Mae Rumpheung Beach	Rayong	GT	8
	573 to 578	Bak Meng, Sikoa	Trang	AN	27
	579 to 585	Chao Mai National Park	Trang	AN	28

Korea) and BLAST search analysis.

Sequence analysis

The data set includes 13 sequences from the two sides of Thailand's coastal area, the Andaman Sea and the Gulf of Thailand. On the Andaman side, five sequences of *COI* gene were obtained from Phuket Province: Patong Beach (PTP1, PTP2, PTP3), Ao

Ao Tung Khen Bay (ATP1) and Trang Province, Bak Meng, (BMT1). At the Gulf of Thailand, there were eight *COI* sequences from wedge clam collected from Prachubkirikhan Province at Sam Phaya Beach (SPP1, SPP2, SPP3) and Kho Takeab Beach (TKP3), from Rayong Province at Mae Rumpheung Beach (MRR1) and Ban Phae (PAR3), and from Chantaburi Province at Chao Lao Beach (CLC1) and Kung Wiman Bay (KWC1) (Table 2). To identify the species of

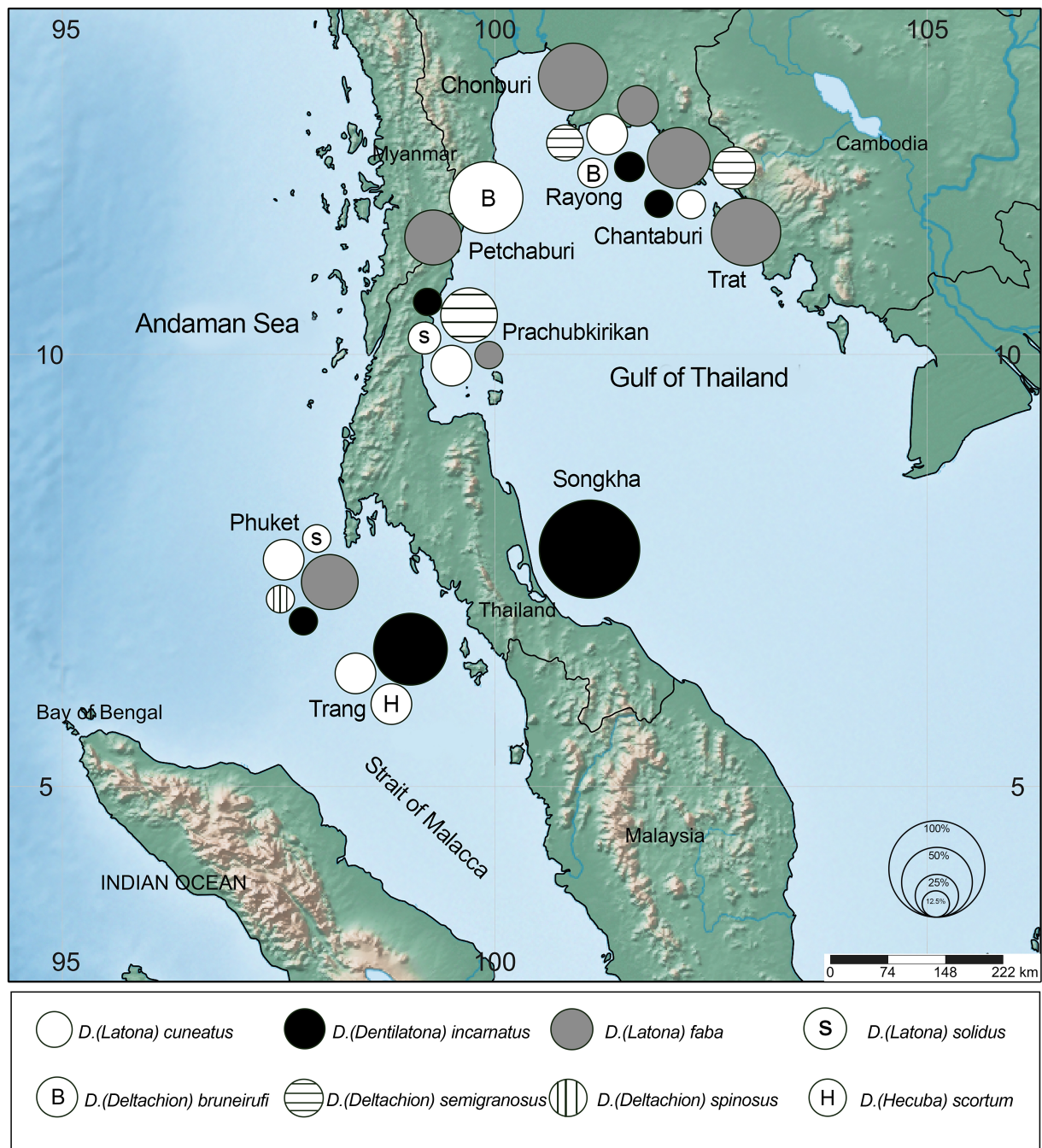


Fig. 1. The relative distribution (%) of eight *Donax* species along the coastline show the sampling location and the distribution pattern.

Donax spp. collected from the beaches of Thailand bordering the Andaman Sea and Gulf of Thailand, homology identification of *COI* gene (approximately 750 bp) was performed using BLAST search analysis against the nucleotide database (nr/nt). The 13 sample sequences and available nucleotide sequence of *COI* genes of different Thai *Donax* spp. were demonstrated. Accessible databases were used to estimate the multiple sequence alignment analysis (Table 2) using ClustalW (Thompson et al. 1994). The sequence of *COI* genes was internally trimmed for phylogenetic tree analysis using the Maximum Likelihood method and Kimura 2-parameter (K2P, Kimura 1980) model with 1,000 replicates in MEGA 10.0 (Kumar et al. 2018). To investigate the phylogenetic relationships of *Donax* species within the Superfamily Tellinoidea and between other species in different Superfamily, available in GenBank. Moreover, genetic distances were calculated to quantify sequence divergences among individuals using Kimura's K2P models (Kimura 1980). Then pairwise (uncorrected-p) sequence distances of each of the eight species of Thai *Donax* were also implemented in MEGA 10.0. Interspecific K2P distances were calculated for those species with at least two sequences, and intraspecific K2P distances were calculated between species in the entire data set to reconstruct a matrix the genetic distances among populations (Kumar et al. 2018). Nucleotide sequences obtained in this study have been deposited into the GenBank database (MT334588-MT334600). In addition, the haplotype network was constructed and edited with PopART software (Clement et al. 2002) and used a randomized minimum spanning network (Leigh and Bryant 2015). The output

of PopART evaluated the geographic distribution of genetic diversity of Thai *Donax* (Fig. 14B). *Donax* (*Latona*) *faba* (MT334600), *D. (Latona) cuneatus* (MT334594), *D. (Latona) solidus* (MT334597), *D. (Dentilatona) incarnatus* (MT334591), *D. (Hecuba) scortum* (MT334588) and *D. (Deltachion) semisulcatus* (MT334589) were the reference sequences.

RESULTS

Centroid Size

The size distribution is defined as centroid size mean values. Centroid sizes of Thai *Donax* were highly variable. From the lowest to the highest value, *Donax* (*Deltachion*) *spinosus*: 5.18 ± 0.58 ; *D. (Deltachion) bruneirufi* 5.86 ± 0.45 ; *D. (Deltachion) semisulcatus semisulcatus* 5.92 ± 0.71 ; *D. (Dentilatona) incarnatus* 7.45 ± 0.97 ; *D. (Latona) faba* 9.19 ± 1.39 ; *D. (Latona) solidus* 10.52 ± 0.74 ; *D. (Latona) cuneatus* 11.21 ± 2.02 and *D. (Hecuba) scortum* 25.09 ± 1.80 . Box-plot mean and standard error (SE) of centroid size frequency were tested with a non-parametric Kruskal-Wallis test. The result of centroid size classification showed four groups, based on the subgenus level (Fig. 3).

The specimens belonging to *Donax* (*Hecuba*) *scortum* (Linnaeus, 1758) were significantly larger than others, with a chi-squared value of 435.3 and a *p*-value of 6.43×10^{-90} for Mann-Whitney pairwise comparisons of centroid size among the eight species (Fig. 3). The centroid size of species in the subgenus *Latona* (*solidus*, *cuneatus* and *faba*) did not differ significantly, with *p*-values of 0.6178 and 0.0569, respectively, that shared the same square area. The centroid sizes of those in the subgenus *Deltachion* (*spinosus*, *bruneirufi* and *semisulcatus semisulcatus*) also did not differ significantly, with *p*-values of 0.077 and 0.6178, respectively, that showed in the same square area (Fig. 3).

Shape Outlines

The contours of the shell outlines were reconstructed using EFA coefficients and decomposed into basic harmonic waves. The first ten harmonics in the EFA, which define the main aspects of shell shape for each species, are illustrated using a mean outline (Fig. 4). The shell shape variation observed in Thai *Donax* species indicates that the shells are usually longer than their height ($SL > SH$). Exceptions are *D. (Latona) solidus* (Fig. 4B) and *D. (Dentilatona) incarnatus* (Fig. 4E); in these species, shell height and length are very close. However, the shell of *D. (Dentilatona) incarnatus*

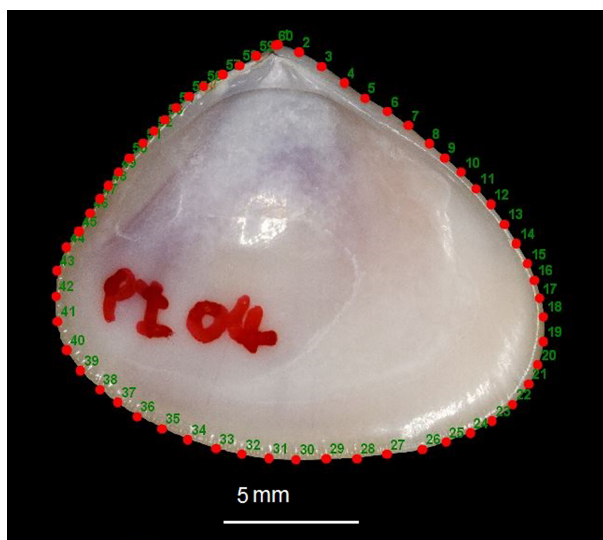


Fig. 2. 60 Outline digitized landmarks along the inner edge of left valve. Scale bar = 5 mm.

can be distinguished by its blunter posterior end, higher slope, and curved ventral margin compared to *D. (Latona) solidus*. *Donax (Hecuba) scortum* can easily be distinguished from other species based on shell shape due to its concave posterior dorsal margin and the location of the umbos at the midpoint (Fig. 4H).

Principal Component Analysis (PCA)

In the shape test of *Donax* outlines, the first three principal components explained 95% of the total

variation. PC I explains 65.692% of variance, PC II explains 23.992% and PC III explains 4.912%. The first two PCAs show high variation in ellipses (95% confidence) (Fig. 5). The smaller cluster group indicates the shape of *Donax (Hecuba) scortum*. This species is totally differentiated from the others, with a positive score for PC I (0.2, 0.4) and a negative score for PC II (-0.2, -0.1). The scatter plot for PC I (y-axis) vs centroid size (x-axis) also separates *D. (Hecuba) scortum* from other Thai Donacidae. *D. (Hecuba) scortum* has the largest centroid size (range = 20 to 30) and PC I (range

Table 2. List of wedge species used in phylogenetic tree and haplotype network analysis

Sample Name	Sampling site	Province/Country	Code	Accession NO.	Reference
<i>Donax (Dentilaton) incarnatus</i>	Chao Lao Beach	Chantaburi	CLC1	MT334591	This study
<i>Donax (Latona) cuneatus</i>	Kung Wiman Bay	Chantaburi	KWC1	MT334594	This study
<i>Donax (Latona) faba</i>	Tungken Bay	Phuket	ATP1	MT334596	This study
<i>Donax (Dentilaton) incarnatus</i>	Patong Beach	Phuket	PTP1	MT334590	This study
<i>Donax (Latona) cuneatus</i>	Patong Beach	Phuket	PTP2	MT334595	This study
<i>Donax (Latona) solidus</i>	Patong Beach	Phuket	PTP3	MT334597	This study
<i>Donax (Dentilaton) incarnatus</i>	Sam Phaya Beach	Prachubkirikhun	SPP1	MT334593	This study
<i>Donax (Latona) solidus</i>	Sam Phaya Beach	Prachubkirikhun	SPP2	MT334598	This study
<i>Donax (Latona) faba</i>	Sam Phaya Beach	Prachubkirikhun	SPP3	MT334599	This study
<i>Donax (Deltachion) semisulcatus semisulcatus</i>	Kho Takeab Bay	Prachubkirikhun	TKP1	MT334589	This study
<i>Donax (Dentilaton) incarnatus</i>	Mae Rumpheung	Rayong	MRR1	MT334592	This study
<i>Donax (Latona) faba</i>	Mae Rumpheung	Rayong	PAR3	MT334600	This study
<i>Donax (Hecuba) scortum</i>	Bak Meng, Sikoa	Trang	BMT1	MT334588	This study
<i>Donax (Latona) cuneatus</i>		Japan		AB040842.1	Okazaki unpublished
<i>Donax (Latona) faba</i>		Japan		AB040845.1	Okazaki unpublished
<i>Donax (Hecuba) scortum</i>		Thailand		MW177945.1	Supmee et al. 2021
<i>Donax dysoni</i>		India		MH817001.1	Bhaskar et al. unpublished
		China		JN859982.1	Yu et al. unpublished
<i>Donax semistriatus</i>		Spain		MF668317.1	Fernández-Pérez et al. 2017
<i>Donax trunculus</i>		Spain		KY951446.1	García-Souto et al. 2017
<i>Donax variegatus</i>		Spain		MF668378.1	Fernández-Pérez et al. 2017
<i>Donax asper</i>		Germany		GQ868449.1	Carstensen et al. 2009
<i>Donax fossor</i>		USA		MW628287.1	Hill-Spanik et al. 2021
<i>Donax hanleyanus</i>		Germany		GQ868444.1	Carstensen et al. 2009
<i>Donax obesulus</i>		Peru		MH194549.1	Marin et al. 2018
<i>Donax variabilis</i>		USA		MW628290.1	McElroy et al. unpublished
<i>Donax deltoides</i>		USA		JN133779.1	Kappner et al. unpublished
<i>Macoma balthica</i>		Canada		KF643962.1	Layton et al. 2014
<i>Moerella iridescens</i>		China		JN859967.1	Yu et al. unpublished
<i>Solecurtus divaricatus</i>		China		JN859983.1	Yu et al. unpublished
<i>Soletellina diphos</i>		China		MN176050.1	Wang et al. unpublished
<i>Semele scabra</i>		China		JN859974.1	Yu et al. unpublished
<i>Nuttallia olivacea</i>		China		MG517170.1	Jiang et al. unpublished
<i>Acanthocardia tuberculata</i>		Canada		EU733168.1	Kirkendale 2009
<i>Tridacna squamosa</i>		China		MF969181.1	Liu et al. unpublished
<i>Mactra chinensis</i>		China		KC205926.1	Ni et al. unpublished
<i>Meretrix</i>		China		KP976276.1	Shen et al. unpublished
<i>Meretrix lyrata</i>		China		KP976267.1	Shen et al. unpublished
<i>Paphia amabilis</i>		China		JN898945.1	Cheng et al. unpublished
<i>Calyptogena magnifica</i>		China		KT345581.1	Liu et al. unpublished
<i>Arctica islandica</i>		USA		KX713445.1	Combosch et al. 2017

= 0.2 to 0.4); the other species are grouped in a large uniform cluster (Fig. 6). The PC II axis separates *D. (Dentilatona) incarnatus* from the other species, but this species overlaps with the others on the PC I axis (Fig. 5).

Morphological Shell Outline Differences at the Family Level

Significant differences in the shell shape (Figs. 5 and 6) of *Donax (Hecuba) scortum* exclude it from a Canonical Variates Analysis (CVA). In this analysis, the

first and second CVs explained 59.69% and 34.81% of variance, respectively.

The shapes of seven species of Donacidae differed significantly based on a multivariate analysis of variance (MANOVA) (Wilks' lambda = 0.06716; $p = 0$; $F = 145.6$; $d.f. = 36, 2466$) (Fig. 7). *Donax (Dentilatona) incarnatus* (CV I = 0.8106, 6.5422) (Fig. 7) can clearly be distinguished from the other species. *Donax (Latona) solidus*, *D. (Latona) cuneatus* and *D. (Latona) faba*, all included within subgenus *Latona*, are grouped in the same cluster (CV I = -6.6174 to 1.2327). Finally, a third

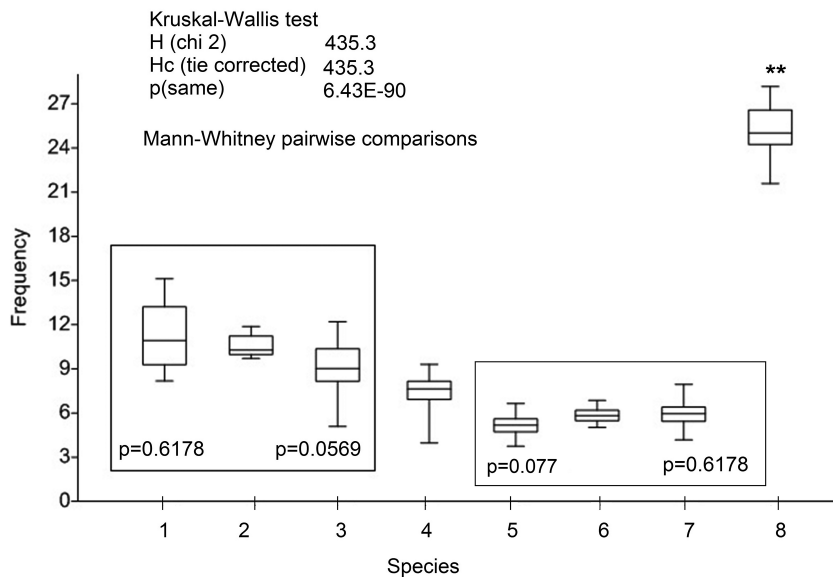


Fig. 3. Box-plot Mean and SE of Size Frequency, Kruskal-Wallis test, non-parametric ANOVA, of eight Donacidae valid species. 1: *Donax (Latona) cuneatus*; 2: *D. (Latona) solidus*; 3: *D. (Latona) faba*; 4: *D. (Dentilatona) incarnatus*; 5: *D. (Deltachion) spinosus*; 6: *D. (Deltachion) bruneirufi*; 7: *D. (Deltachion) semisulcatus semisulcatus*; 8: *D. (Hecuba) scortum*. Double asterisks (**) highly significant at $p \leq 0.01$. In square box means centroid size in group is not significantly different at $p > 0.05$ under Mann-Whitney pairwise comparisons.

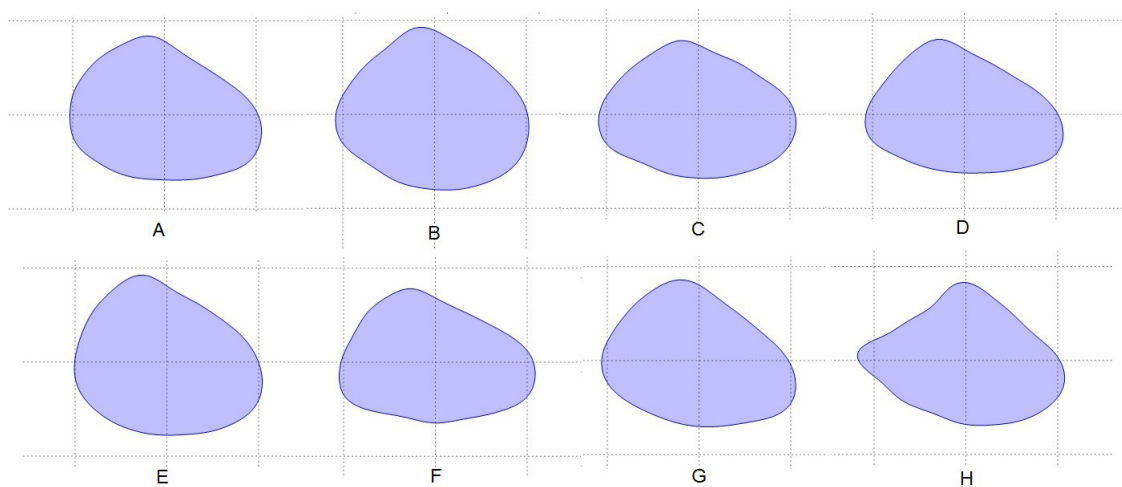


Fig. 4. Mean outline shape constructed from 10 harmonics of elliptic Fourier analysis of eight *Donax* species from Thai waters: *Donax (Latona) cuneatus* (A), *D. (Latona) solidus* (B), *D. (Latona) faba* (C), *D. (Deltachion) spinosus* (D), *D. (Dentilatona) incarnatus* (E), *D. (Deltachion) bruneirufi* (F), *D. (Deltachion) semisulcatus semisulcatus* (G) and *D. (Hecuba) scortum* (H).

cluster group, *Deltachion*, includes *Donax (Deltachion) semisulcatus semisulcatus*, *D. (Deltachion) spinosus* and *D. (Deltachion) bruneirufi* (CV II = -6.1614, -0.08356) (Fig. 7).

Morphological Shape Discrimination Among Overlapping Species

A separate CVA was conducted for species in the subgenus *Latona*, which are grouped in a single

cluster (Fig. 7). In this analysis, CV I and CV II explain 95.06% and 5.945% of variance, respectively (Fig. 8). A MANOVA showed significant differences between *Latona*'s groups (Wilks' lambda = 0.2288; $p = 5.813\text{E-}63$; $F = 25.74$; $d.f. = 20, 472$). *Donax (Latona) faba* partly overlaps with *D. (Latona) solidus* and with *D. (Latona) cuneatus* (Fig. 8). Discriminate analyses of overlapping species were conducted using pairwise comparison (Fig. 9). In this analysis, *D. (Latona) faba* had a non-significant separation of 86.6% from *D.*

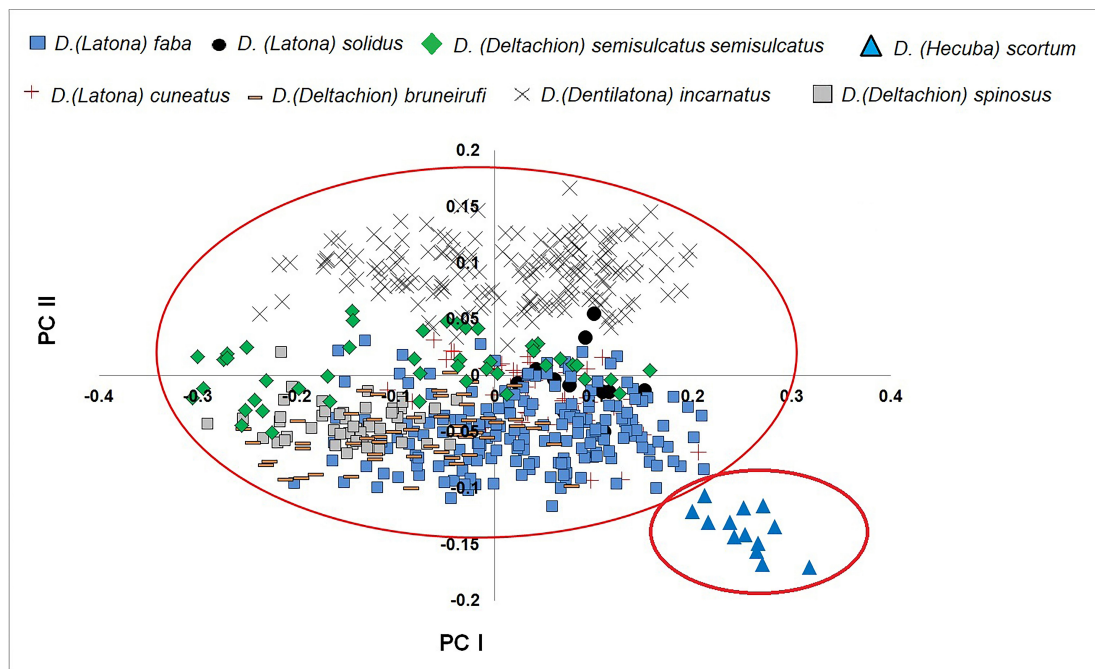


Fig. 5. Plot graph of PCA scores of EF coefficient, circle represent 95 percent confidence ellipses.

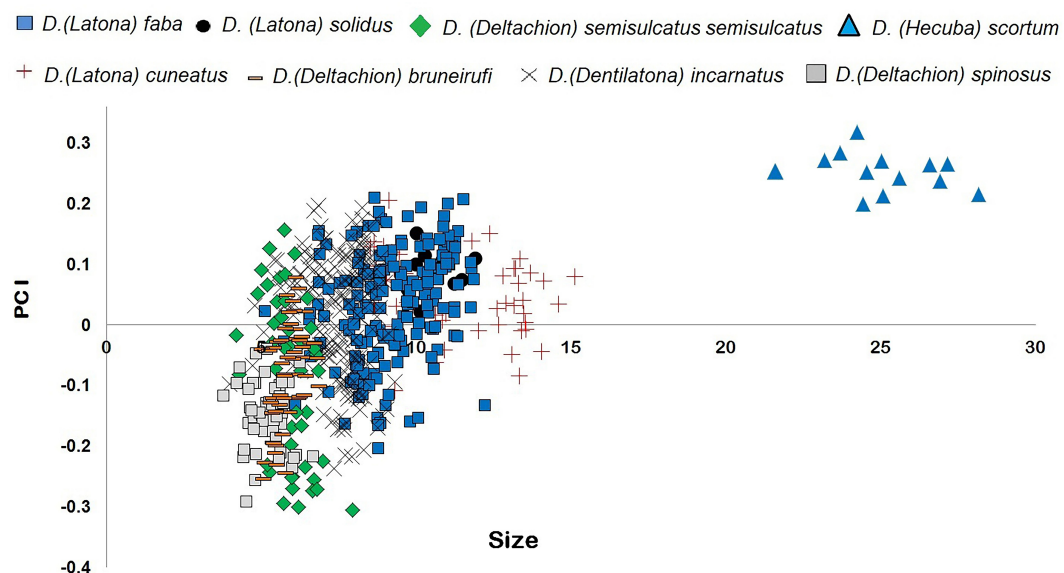


Fig. 6. Scatterplot of shape, represent by PC I score (y-axis) per centroid size (x-axis) of eight *Donax* species.

(Latona) solidus (Hotelling's $t^2 = 46.958$; $p = 2.479\text{E-}07$; $F = 7.6226$) (Fig. 9A). The outline of *D. (Latona) solidus* is more extended at dorsal and ventral margins than that of *D. (Latona) faba*, but the anterior and posterior ends of the outlines of these species are similar

(Fig. 12 B). *D. (Latona) faba* also had a separation of 98.39% from *D. (Latona) cuneatus* (Hotelling's $t^2 = 132.06$; $p = 5.477\text{E-}13$; $F = 21.184$) (Fig. 9B).

The mean shape outline of *D. (Latona) cuneatus* was clearly broader than that of *D. (Latona) faba* at

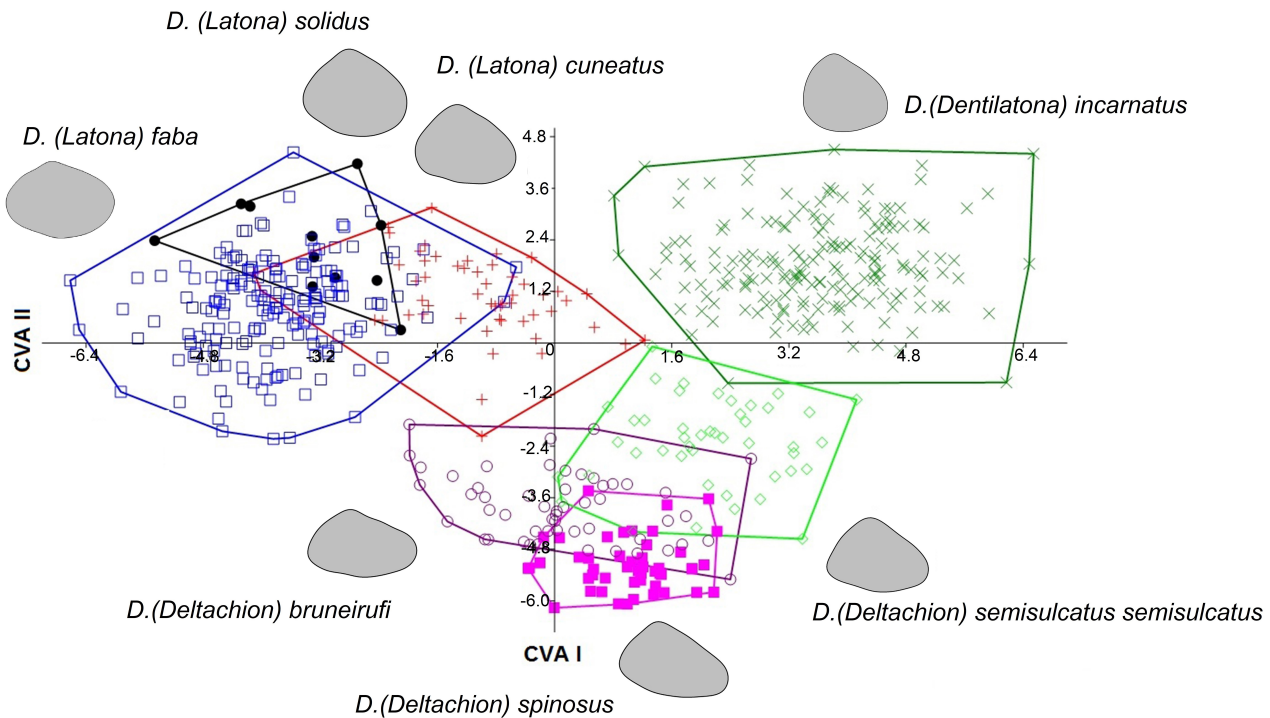


Fig. 7. CVA of EFA coefficients and mean shape outline from seven species under same group. Within each species, the specimen is enclosed by a convex hull polygon. Separation on the first two canonical analysis axes.

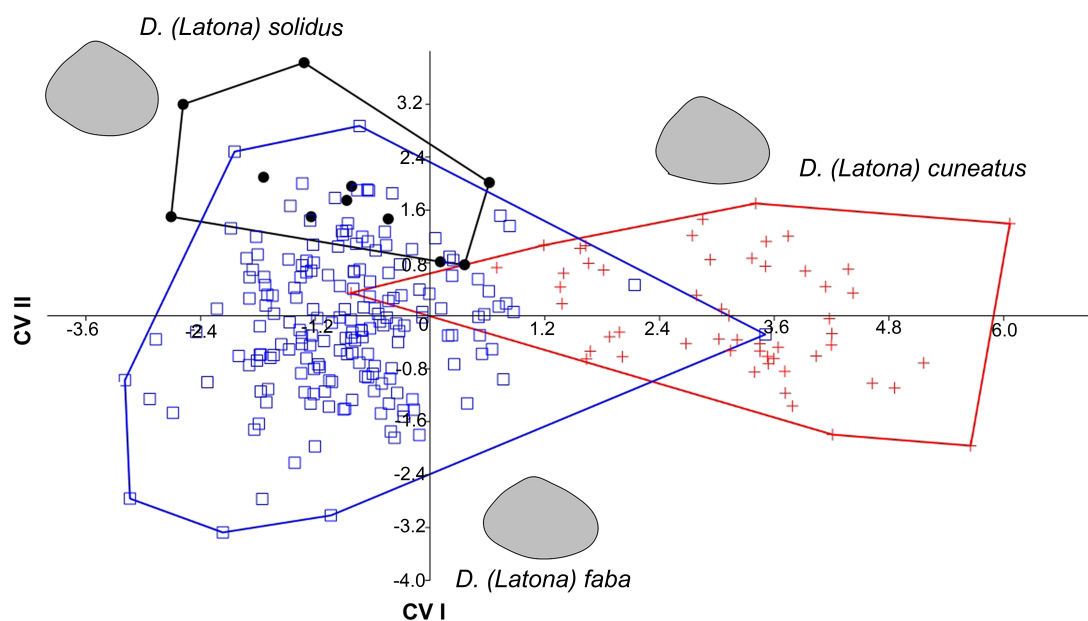


Fig. 8. CVA of EFA coefficients and mean shape outline from three overlapping species from subgenus *Latona*, large size species group. Within each species, the specimens are enclosed by a convex hull polygon.

three points—the dorsal and ventral posterior and anterior end region—in a plot graph of outline overlins (Fig. 12C). The outline shape pairwise comparison of *Donax (Latona) solidus* and *D. (Latona) cuneatus* revealed significant differences with 95.38% identity (Hotelling's $t^2 = 132.06$; $p = 5.477\text{E-}13$; $F = 21.184$) (Fig. 9C). *D. (Latona) solidus* is more enlarged along the umbo, in the antero-dorsal margin and on the antero-ventral and posterior ends than that of *D. (Latona) cuneatus* (Fig. 12A).

The subgenus *Deltachion*, which includes *Donax (Deltachion) semisulcatus semisulcatus*, *D. (Deltachion) spinosus* and *D. (Deltachion) bruneirufi* (Fig. 7), was

analysed using CVA. CV I and CV II explained 82.78% and 17.22% of variance in this group, respectively (Fig. 10). The MANOVA indicated significant differences (Wilks' lambda = 0.0774; $p = 5.773\text{E-}70$; $F = 60.09$; $d.f. = 12, 278$). *Donax (Deltachion) semisulcatus semisulcatus* differed significantly from the other species (CV I = -6.2947, -1.3327) (Fig. 10). The outline of *D. (Deltachion) semisulcatus semisulcatus* is rounded, and the shell is taller and has a more extended ventral margin and antero-dorsal area (Fig. 4G) than the other two species.

Although the CV II of *D. (Deltachion) spinosus* overlaps with that of *D. (Deltachion) bruneirufi*

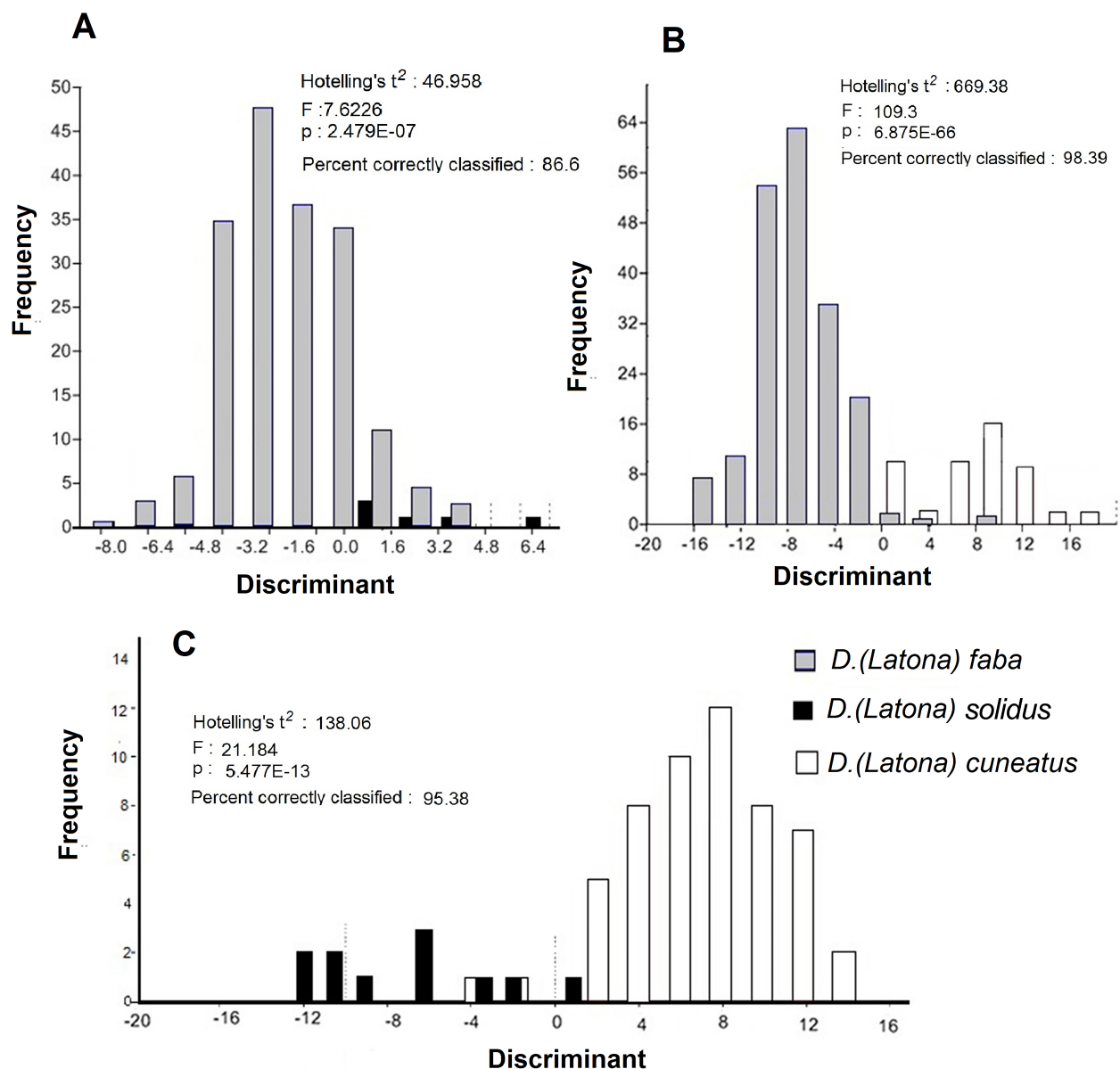


Fig. 9. Frequency distribution of Discriminant analysis of subgenus *Latona* by two population comparison. *Donax (Latona) faba* and *D. (Latona) solidus* (A); *D. (Latona) faba* and *D. (Latona) cuneatus* (B); *D. (Latona) solidus* and *D. (Latona) cuneatus* (C).

(Fig. 10), these species also differ significantly based on a discrimination test (Hotelling's $t^2 = 204.78$; $p = 5.666\text{E-}21$; $F = 32.424$; 92.16% correctly classified) (Fig. 11). A plot graph with the outlines overlaid shows that *D. (Deltachion) bruneirufi* is characterised by a more elongated shell at the posterior end and some expansion at the antero-dorsal and antero-ventral

margins; the umbos is also closer to the anterior than in *D. (Deltachion) spinosus* (Fig. 12D).

Molecular Analysis of Cytochrome c Oxidase Subunit I (COI) Genes and Haplotype Network

Of the eight valid species living in Thai waters

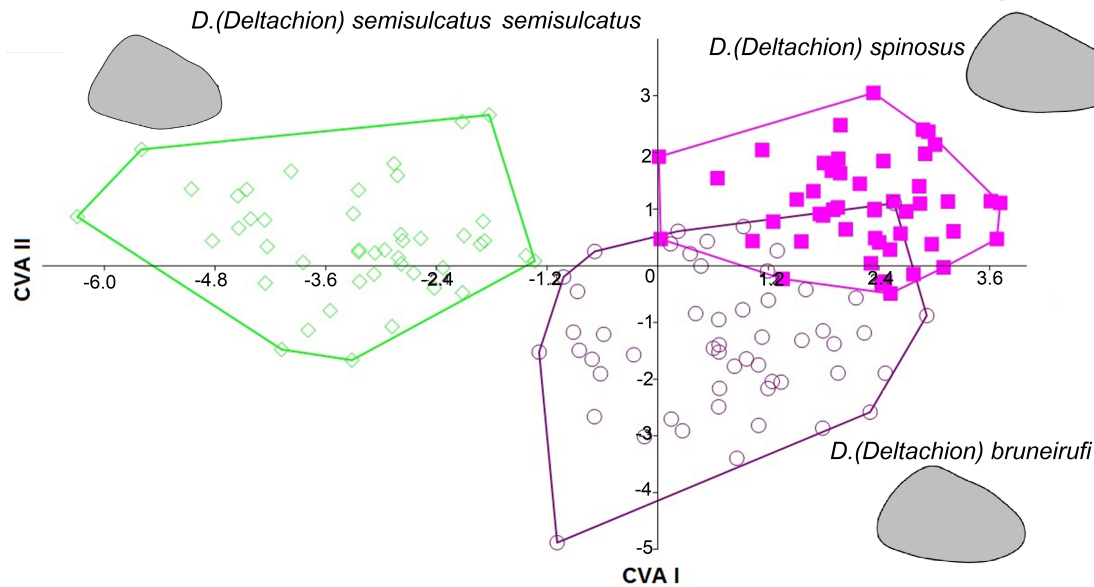


Fig. 10. CVA of EFA coefficients and mean shape outline from three overlapping species of subgenus *Deltachion*, small size group. Within each species, the specimen is enclosed by a convex hull polygon.

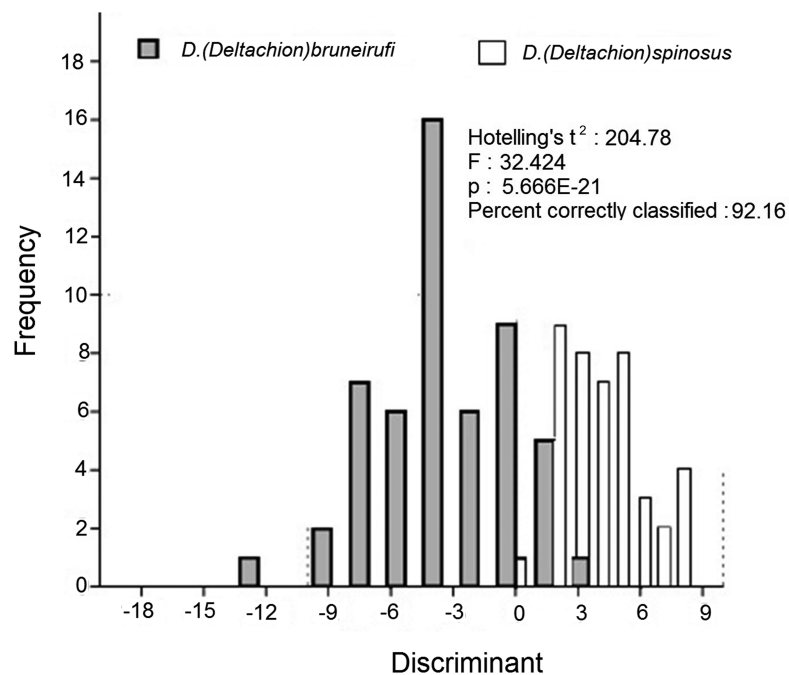


Fig. 11. Frequency distribution of Discriminant analysis of two overlapping populations, *Donax (Deltachion) bruneirufi* (grey) and *D. (Deltachion) spinosus* (white).

examined in this study, only six were included in the molecular analysis due to the availability of DNA. All sequences of *COI* genes obtained from wedge clams in this study were deposited in GenBank (Table 2).

A phylogenetic analysis of Thai *Donax* was conducted using *COI* genes from other Tellinoidea species and from outgroups belonging to different superfamilies, such as Arcticoidea, Cardioidea, Mactroidea, Pliocardiinae and Veroidea (Table 2).

The results confirm that the Thai wedge clams in the present study belong to the superfamily Tellinoidea. Our results also show that the family Donacidae is closely related to the family Psamobiidae, as indicated by *Nuttallia olivacea* in the *COI* sequence.

Moreover, the *COI* sequences of Thai *Donax* are grouped in an Asian cluster and are more closely related to *Donax (Latona) faba* and *D. (Latona) cuneatus* from Japan and to *D. dysoni* from China and India than to *D. (Hecuba) scortum* found in the Andaman Sea or to *D. deltoidea* from the United States (Fig. 13, Table 2).

Five clades of Thai *Donax* have been reported that can be clearly distinguished based on *COI* gene sequences. The first cluster was reported in BMT1 (MT334588) and has 100% identity with *D. (Hecuba) scortum* (LC516684) from the same locality, the Andaman Sea (Supmee et al. 2021) (Fig. 13).

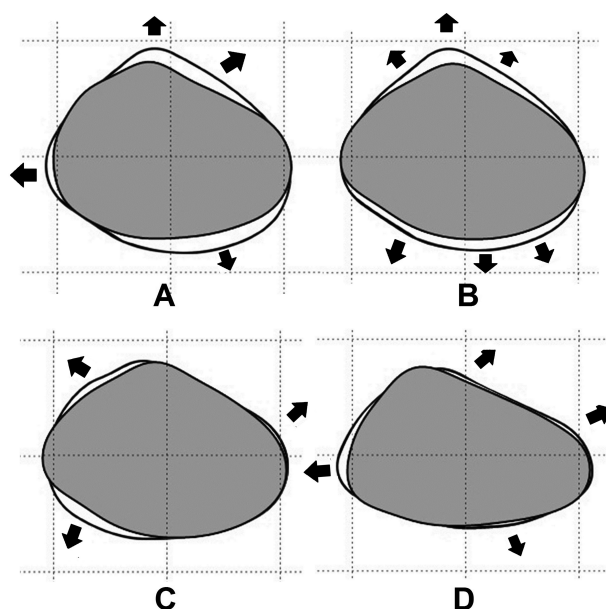


Fig. 12. Outline overlay, size-normalized average outline of each two species in discriminant analysis; *Donax (Latona) solidus* (white) and *D. (Latona) cuneatus* (grey) (A); *D. (Latona) solidus* (white) and *D. (Latona) faba* (grey area) (B); *D. (Latona) cuneatus* (white area) and *Donax (Latona) faba* (grey area) (C); *D. (Deltachion) bruneirufi* (white) and *D. (Deltachion) spinosus* (grey) (D). Arrows highlight distinct position from overlay shape of white area species extend from grey area species.

The largest cluster has 100% identity with *D. (Latona) faba* (AB040845.1) from Japan. In the *D. (Latona) faba* cluster, two subclades have been reported. SPP2, SPP3 and PAR3 from the Gulf of Thailand are included in one subclade (73% identity); the other subclade includes PTP3 and ATP1 from the Andaman Sea (79% identity).

Although morphological identification of ATP1, SPP3 and PAR3 suggested the presence of *D. (Latona) faba* and of PTP3 and SPP2, the presence of *COI* sequences from *D. (Latona) solidus* (Table 2) suggest that PTP3 and SPP2 belong to the *D. (Latona) faba*, with 100% identity (Fig. 13).

The clade including KWC1 and PTP2 from Japan was assigned to *D. (Latona) cuneatus* (AB 040842.1), with 98% and 89% identity, respectively. This clade is close to the *D. (Latona) faba* cluster, branching at 66% identity.

The uppermost clade, with the four sequences MRR1, SPP1, CLC1 and PTP1, refer to *D. (Dentilaton) incarnatus*, which had 99% identity with *Donax dysoni* from the GenBank records. In this group, two subclades were identified based on geographic area. The first subclade (MRR1, SPP1 and CLC1) is found in the Gulf of Thailand and has 98% identity with *D. dysoni* from China (JN859982.1). This subclade has sequence separation from the second subclade (PTP 1), which is found in the Andaman Sea and has 90% identity with *D. dysoni* from India (MH817001.1) (Fig. 13).

Novel phylogenetic names are based on morphology. TKP1 was identified as *Donax (Deltachion) semisulcatus semisulcatus* and separated out. However, the *COI* sequence for TKP1 is similar to that of the clade of *D. (Dentilaton) incarnatus*, with 86% identity (Fig. 13, Table 2).

An analysis of K2P resulted in an estimation of genetic distance that was similar to the results of the phylogenetic analysis. Genetic distances are matrices that summarise the differences between pairs of populations (Table 3).

For Thai *Donax*, the interspecific genetic distance ranged from 0.14% to 25.83%; the distances were particularly large for species in subgenus *Latona* (0.14% to 20.06%). Within this context, the lowest genetic distances were observed between *D. (Latona) faba* and *D. (Latona) solidus* (0.14 to 1.43%), whereas the highest were observed between *D. (Latona) faba* and *D. (Deltachion) semisulcatus semisulcatus* (25.37 to 25.83 %). *Donax (Hecuba) scortum* showed the highest genetic distances (more than 20%) in comparison with the other species (Table 3).

The intraspecific genetic distances amongst *Donax (Latona) faba*, *D. (Dentilaton) incarnatus* and *D. (Latona) cuneatus* were less than 5% for species

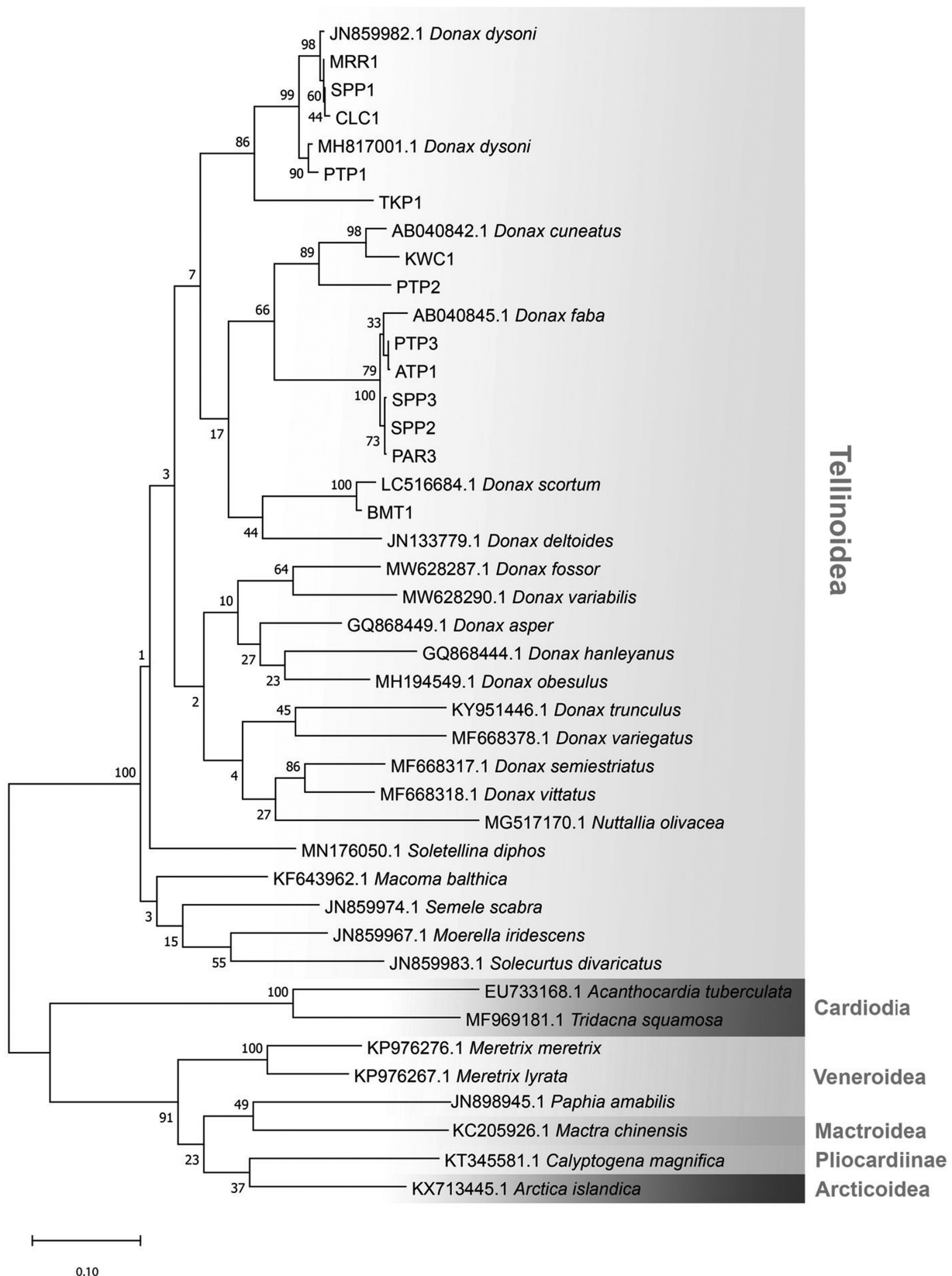


Fig. 13. Phylogenetic analysis based on genetic sequence derived from cytochrome oxidase I (COI) gene of *Donax* spp. and other related species using the maximum likelihood (ML) method. The bootstrap values were calculated with 1000 replicates.

found in the Gulf of Thailand and the Andaman Sea. The genetic distance between *D. (Latona) faba* and *D. (Dentilaton) incarnatus* was 0.14% to 4.25%. The genetic distance between *D. (Latona) cuneatus* and the other studied species exceeds 13.92% (Table 3).

The connection values in the haplotype network are 100%, except where noted (Fig. 14A). The calculated nucleotide diversity was 0.117006, there were 215 segregating sites, and a single divergence in the subgenus *Latona* was observed.

The length of each branch corresponds to one nucleotide substitution between haplotypes, except where indicated by the number of black bars. These results suggest a close relationship between *D. (Latona) solidus* and *D. (Latona) faba*, illustrated by the lower number of black bars, indicating 10 base substitution differences, and by these species' close relationships across co-distributed localities with other species. In the other branch, *D. (Latona) cuneatus* is directly connected to *D. (Dentilaton) incarnatus*, indicated by 117 base substitution differences. *D. (Deltachion) semisulcatus* and *Donax (Hecuba) scortum* differ from *D. (Dentilaton) incarnatus* by 102 and 120 base substitutions, respectively.

DISCUSSION

Geometric Morphometrics

The present study used a combination of geometric morphometric methods and molecular analysis to precisely identify wedge clam species found in Thai waters (Table 4). This study of Thai *Donax* supports the use of shape outline analysis to discriminate bivalve

species, in line with previous studies (Ferson et al. 1985; Palmer et al. 2004; Zieritz and Aldridge 2009; Godefroy et al. 2012; Rufino et al. 2013; Dapar and Tabugo 2018). In the present study, size allometry was successfully used to classify individuals at the subgenus level. Species in the subgenus *Latona* had similar centroid size distribution (range = 9.19–11.21), as did smaller species in the subgenus *Deltachion* (range = 5.15–5.92). In addition, size allometry was particularly useful for identifying at species level at *Donax (Hecuba) scortum*, which could be clearly distinguished from other species.

For the eight valid species found in Thai waters that were included in our geometric morphometry outline analysis, shape similarity is enclosed in a 95% confidence ellipse. Only *Donax (Hecuba) scortum* clearly differs from other Thai donacids in a PCA analysis. In addition, shell shape covaries with size allometry in *D. (Hecuba) scortum*. The shell shape differences between *D. (Hecuba) scortum* and other species include a more inflated shell, a keeled form and an acute posterior end with an elongated rostrate (Subba Rao et al. 1992; Hubber 2010; Ramakrishna and Dey 2003; Signorelli and Printragoon 2020). The elongated posterior end of *Donax (Hecuba) scortum* could be related to an adaptation to the relatively high energy in its habitat (Crampton and Maxwell 2000). Stanley (1970) suggests that posterior elongation in bivalve shells minimises the siphon length of thick valves in bivalves that dig shallow burrows in specific types of soft sediment. The main habitat for this species in Thailand is mud or muddy sand substrates; other species live in sand or coarse sand (Signorelli and Printragoon 2020).

Our study demonstrated that GM based on an

Table 3. Matrix genetic distance of 13 *COI* genes sequences of from *Donax* in Thailand

	*PAR3	*SPP3	ATP1	PTP3	*SPP2	PTP2	*KWC1	*SPP1	*MRR1	*CLC1	PTP1	*TKP1	BMT1
*PAR3 <i>Donax (Latona) faba</i>	0												
*SPP3 <i>Donax (Latona) faba</i>	0.0028	0											
ATP1 <i>Donax (Latona) faba</i>	0.0157	0.0158	0										
PTP3 <i>Donax (Latona) solidus</i>	0.0143	0.0143	0.0014	0									
*SPP <i>Donax (Latona) solidus</i>	0.0014	0.0014	0.0143	0.0128	0								
PTP2 <i>Donax (Latona) cuneatus</i>	0.1964	0.2006	0.1902	0.1921	0.1933	0							
*KWC1 <i>Donax (Latona) cuneatus</i>	0.1915	0.1956	0.1914	0.1933	0.1934	0.1392	0						
*SPP1 <i>Donax (Dentilaton) incarnatus</i>	0.21923	0.2236	0.2230	0.2210	0.2212	0.1951	0.2050	0					
*MRR1 <i>Donax (Dentilaton) incarnatus</i>	0.2171	0.2214	0.2209	0.2189	0.219	0.1971	0.2029	0.0014	0				
*CLC1 <i>Donax (Dentilaton) incarnatus</i>	0.2255	0.2300	0.2294	0.2274	0.2275	0.1947	0.2046	0.0042	0.0056	0			
PTP1 <i>Donax (Dentilaton) incarnatus</i>	0.2164	0.2207	0.2196	0.2176	0.2183	0.2068	0.2020	0.0379	0.0394	0.0425	0		
*TKP1 <i>Donax (Deltachion) semisulcatus</i>	0.25377	0.2583	0.2537	0.2558	0.2558	0.2427	0.2340	0.1700	0.1720	0.1721	0.1684	0	
BMT1 <i>Donax (Hecuba) scortum</i>	0.2238	0.2282	0.2259	0.2279	0.2257	0.2173	0.2313	0.2066	0.2086	0.2107	0.2110	0.2474	0

Locality: meaning *: Gulf of Thailand.

EFA of shell outline can be used to identify groups of bivalve species. In particular, *Donax (Dentilatona) incarnatus* provides a good example of clear separation based on a shape outline CVA. This species is

characterised by an elongated antero-dorsal margin, a straight postero-dorsal margin with a rounded anterior end, and a convex ventral margin. In contrast, *Donax (Deltachion) semisulcatus semisulcatus* has a trigonal

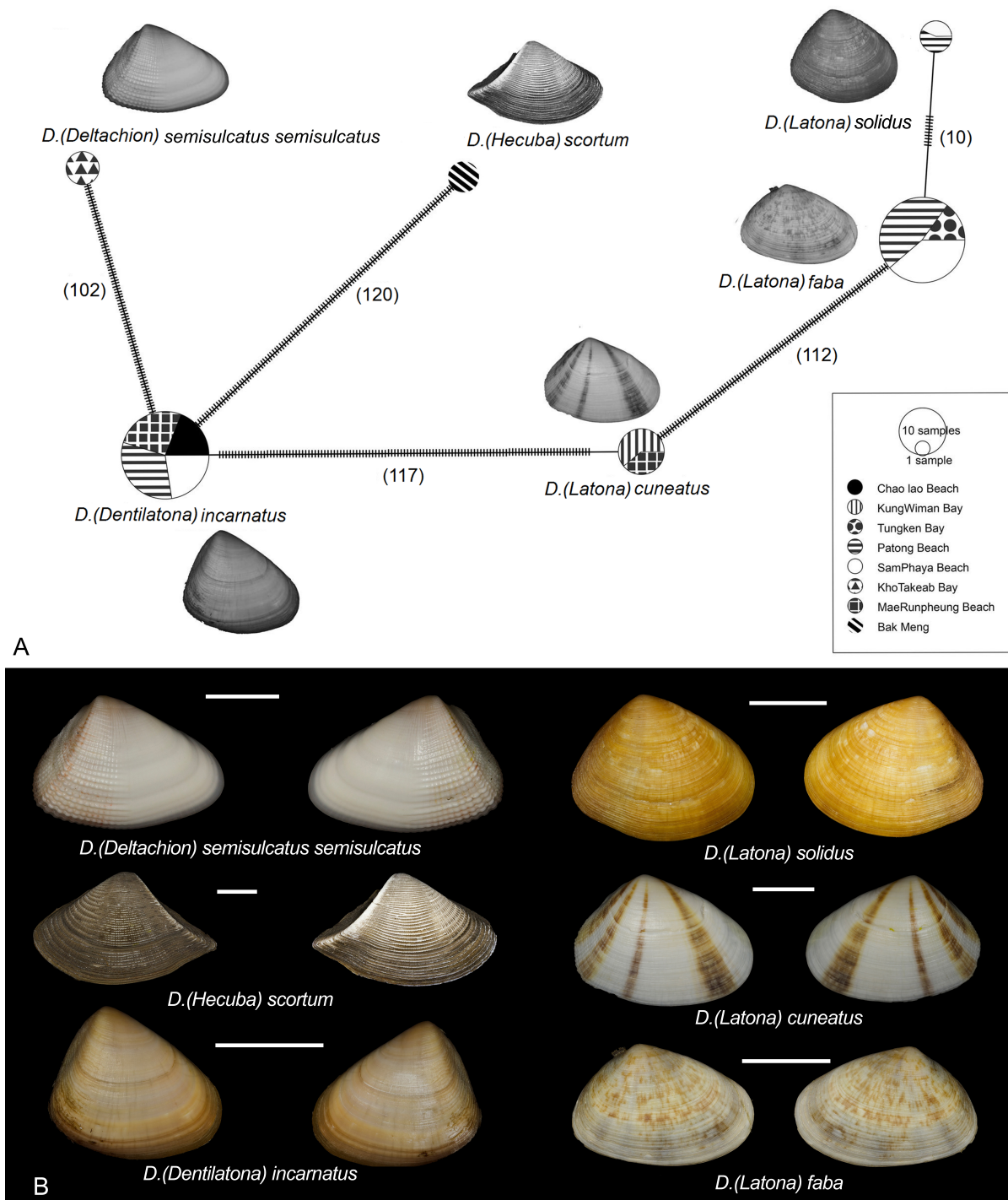


Fig. 14. The haplotype network analysis of six species based on *COI* sequences were constructed in shell morphology picture. Scale in 1 to 10 samples of available localities. The nucleotide diversity was calculated at 0.117006 and Number of segregating sites at 215. Number in parenthesis represents nucleotide substitution difference (A); Colour illustrations of collected Thai *Donax* species for molecular analysis of cytochrome *c* oxidase subunit I. Scale bar: B = 1 cm.

shape with a curve at the midpoint, a narrowly rounded anterior end, a short posterior end, and a slightly convex, obliquely truncated ventral margin (Sinorelli and Printragoon 2020). These smaller-sized species in the subgenus *Deltachion* can be clearly identified via CVA. Pairwise comparison via EFA resulted in 87.5% discrimination, demonstrating the effectiveness of GM for distinguishing species. This method is particularly useful when valid species have overlapping shapes, such as the species in the subgenus *Deltachion* (*semisulcatus semisulcatus*, *spinosus* and *bruneirufi*). This combined approach results in 92.16% discrimination of *Donax* (*Deltachion*) *spinosus* from *D. (Deltachion) bruneirufi* at the species level. These results confirm the usefulness of EFA for revising similar taxa. GM can be used to

identify species with similar shell shapes and sizes, such as *D. (Deltachion) semisulcatus semisulcatus* and *D. (Deltachion) bruneirufi*, which also belong to similar taxa (Raven 2021). CVA of species in the subgenus *Latona* demonstrated similarities in centroid size distribution and shell outline. Pairwise comparison distinguishes *Donax* (*Latona*) *cuneatus* from *D. (Latona) faba* and from *D. (Latona) solidus* with more than 95% confidence. However, *Donax* (*Latona*) *solidus* overlaps with *D. (Latona) faba* by 86.6%, resulting in less than 90% discrimination.

According to Hammer and Harper (2006), the degree of correct assignment necessary for splitting taxonomic species is debatable, but it should definitely be greater than 90%. This overlapping is supported by

Table 4. Summary of precision identification of the wedge clam in Thailand

Species identification		Species analysis	
(Signorelli & Printrakoon 2020)		Morphometric analysis	
	Centrioud size	Shell outline	% Discrimination
<i>Donax (Dentilatona) incarnatus</i>	<i>Dentilatona</i> , 7.45	<i>D. (Dentilatona) incarnatus</i>	
<i>Donax (Deltachion) semisulcatus semisulcatus</i>	<i>Deltachion</i> , 5.92	<i>D. (Deltachion) semisulcatus semisulcatus</i>	
<i>Donax (Deltachion) spinosus</i>	<i>Deltachion</i> , 5.15	<i>D. (Deltachion) spinosus</i>	<i>D. (Deltachion) spinosus</i> , and 92.16% from <i>D. (Deltachion) bruneirufi</i>
<i>Donax (Deltachion) bruneirufi</i>	<i>Deltachion</i> , 5.86	<i>D. (Deltachion) bruneirufi</i>	<i>D. (Deltachion) bruneirufi</i> , and 92.16% from <i>D. (Deltachion) spinosus</i>
<i>Donax (Latona) cuneatus</i>	<i>Latona</i> , 11.21	<i>D. (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i> , and 98.39% from <i>D. (Latona) faba</i> , and 95.38% from <i>D. (Latona) solidus</i>
<i>Donax (Latona) faba</i>	<i>Latona</i> , 9.19	<i>D. (Latona) faba</i>	<i>D. (Latona) faba</i> , and 98.39% from <i>D. (Latona) cuneatus</i> , and 86.6% from <i>D. (Latona) solidus</i>
<i>Donax (Latona) solidus</i>	<i>Latona</i> , 10.52	<i>D. (Latona) solidus</i> / <i>D. (Latona) faba</i>	<i>D. (Latona) solidus</i> / <i>D. (Latona) faba</i> , and 86.6% from <i>D. (Latona) faba</i> , and 95.38% from <i>D. (Latona) cuneatus</i>
<i>Donax (Hecuba) scortum</i>	<i>D. (Hecuba) scortum</i> , 25	<i>D. (Hecuba) scortum</i>	

Species identification		Molecular data		Locality	Code
(Signorelli & Printrakoon 2020)		Haplotype Network analysis		COI gene analysis	
<i>Donax (Dentilatona) incarnatus</i>	<i>D. (Dentilatona) incarnatus</i>	<i>D. (Dentilatona) incarnatus</i>	<i>D. (Dentilatona) incarnatus</i>	Thailand gulf	CLC1, SPP1
				Andaman sea	MRR1
				Thailand gulf	PTP1
<i>Donax (Deltachion) semisulcatus semisulcatus</i>	<i>D. (Deltachion) semisulcatus semisulcatus</i>	<i>D. (Deltachion) semisulcatus semisulcatus</i>	<i>D. (Deltachion) semisulcatus semisulcatus</i>	Thailand gulf	TKP1
<i>Donax (Deltachion) spinosus</i>	N/A	N/A	N/A	Andaman sea	N/A
<i>Donax (Deltachion) bruneirufi</i>	N/A	N/A	N/A	Thailand gulf	N/A
<i>Donax (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i>	Thailand gulf	KWC1
	<i>D. (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i>	<i>D. (Latona) cuneatus</i>	Andaman sea	PTP2
<i>Donax (Latona) faba</i>	<i>D. (Latona) faba</i>	<i>D. (Latona) faba</i>	<i>D. (Latona) faba</i>	Andaman sea	ATP1, SPP3
				Thailand gulf	PAR3
<i>Donax (Latona) solidus</i>	<i>D. (Latona) solidus</i> ≤ <i>D. (Latona) faba</i>	<i>D. (Latona) solidus</i> = <i>D. (Latona) faba</i>	<i>D. (Latona) solidus</i> = <i>D. (Latona) faba</i>	Andaman sea	PTP3
				Thailand gulf	SPP2
<i>Donax (Hecuba) scortum</i>	<i>D. (Hecuba) scortum</i>	<i>D. (Hecuba) scortum</i>	<i>D. (Hecuba) scortum</i>	Andaman sea	BMT1

the similarities of these taxa. They are similar in shell length and outline, but the present study demonstrates that they can be distinguished based on the dorsal area, umbo and ventral margin.

Raven (2021) also observes that *D. (Latona) solidus* and *D. (Latona) faba* have similar shapes and colour patterns; these two species have been found syntopically in several localities, and many records of *D. (Latona) faba* from the South China Sea region concern specimens of *D. solidus*. In addition, these two taxa have been reported as living mostly in the same habitat (Signorelli and Printragoon 2020), suggesting similar life adaptations and burrowing dynamics. Stanley (1970) suggests a connection between burrowing behaviours and geometric shell form in *Donax*. Donacidae typically burrow in a vertical orientation with the pedal emergence below and the siphons above (Stanley 1970). Carstensen et al. (2009) also demonstrated that shape variation in sympatric clams, *D. marincovichii* and *D. obesulus*, is affected by beach morphodynamics. However, in our outline analysis of species in the subgenus *Latona* living in Thai waters, this was not so evident. Rufino et al. (2007) suggest that outline analysis results in approximately 6.3% misidentification; this percentage may also apply to identification based on shell variation. Thus, classical morphological analysis still plays a role in an integrative approach that combines GM, molecular and traditional morphological approaches to correctly identify taxa.

Molecular Analyses

The findings of the molecular analyses in the present study align with the GM results and provide species-level discrimination of over 92%. Five clades in Indo-Pacific Donacidae, including Thai wedge clams, had close molecular relationships.

The first clade includes *Donax (Hecuba) scortum*, which is easily identified via GM or by genetic data with 100% identity. A total of 115 specimens from the Andaman coast supported this result, following the partial-sequences technique for the mitochondrial DNA cytochrome oxidase subunit I gene (mtDNA *COI*) with fewer than 426 bases (Supme et al. 2021). Supme et al. (2021) found 32 haplotypes and observed haplotype diversity and nucleotide diversity of 0.887 and 0.00541, respectively.

The largest clade observed in the present study includes the species in the subgenus *Latona*, which has two recognized subclades. The first subclade includes *Donax (Latona) cuneatus* from different geographic areas; our samples matched specimens from East Asia (98%) more closely than those found in the Andaman Sea. The second subclade includes other members of

the subgenus *Latona*. Five specimens were matched to *Donax (Latona) faba* based on *COI* gene data from the Gene Bank. The specimens identified as *Donax (Latona) solidus* from the Gulf of Thailand (SPP2) and from the Andaman Sea (PTP3) via GM in the present study were genetically close to *D. (Latona) faba*. Less than 1.5% and only ten base substitutions differentiated these two taxa. According to Kalinowski (2002), populations with similar alleles have shorter genetic distances. Thus, our genetic results align with the low rate of correct discrimination via CVA that we found between *Donax (Latona) faba* and *D. (Latona) solidus*. It is also possible that phenotypic plasticity in *Donax (Latona) solidus* could be related to our results, as indicated by sympatric polymorphism.

Phenotypic plasticity is a common feature in many bivalves, as indicated by variations in shape, size range, sculpture and colour. Carstensen et al. (2009) report similar results for the sympatric species *Donax marinconvivhi* Coan 1983 and *D. obesulus* Reeve 1854. These taxa were distinguished via GM, but no significant genetic differences were observed (567 base pairs). The authors conclude that the two morphotypes do not represent separate species and should both be classified under *D. obesulus* (Carstensen et al. 2009). The case of *Donax (Latona) solidus* and *D. (Latona) faba* may be similar to the study of Carstensen et al. (2009). Carstensen et al. (2009) demonstrated that genetic tools can help clarify the taxonomic status of two sympatric surf clams with ambiguous identification based on morphology. This example of two species that are morphologically distinct but genetically similar is explained by phenotypic plasticity due to local environmental conditions. Carstensen et al. (2009) suggest that the shape differences between these two populations of *D. obesulus* could be affected by beach hydrodynamics. Phenotypic plasticity could also explain species that are widely distributed and morphologically distinct but not genetically separated (Proćkòw et al. 2017). For example, Proćkòw et al. (2013) observed that the small air-breathing land snails *Trochulus hispidus* and *T. sericeus/plebeius* from the same geographical region did not differ anatomically or genetically. However, broadly distributed species that were better adapted to different environments differed in shell shape (Proćkòw et al. 2018).

Phenotypic plasticity has also been observed in *D. serra* populations living in different habitats (Soares et al. 1998). The same likely occurs with *Donax (Latona) faba*, which have recently been reported to live in different types of substrates in coastal habitats, including mud sand, fine sand, medium sand and fine sand mixed with gravel (Signorelli and Printragoon 2020). Phenotypic plasticity suggests high genetic

variability, and *Donax (Latona) faba* have been shown to have more genetic variability than four species of Japanese Donacidae (Yokogawa and Kondo 2006; Fujio et al. 1983). Moreover, Smith (1975) demonstrated high degrees of polymorphism in *D. (Latona) faba*, identifying 14 morphs for shell colour. Similar findings for this species have been reported from other locations in Asia (Tan and Low 2013; Ambarwati and Faizah 2017).

The *COI* sequence identified as *Donax dysoni* based on GenBank data was assigned to *Donax (Dentilatona) incarnatus* with 98% identity. Carstensen et al. (2009) suggest that taxonomic misidentifications of *Donacidae* contribute to numerous synonymies. This result suggests that genetic data must be assigned carefully after type material comparisons to avoid misidentification. It is possible that, in previous biological studies in the South China Sea and East Asia, *D. dysoni* (Deshayes 1854) has been identified without considering types or malacological collections (Raven 2021).

In our study, intraspecific phylogeographic patterns of genetic distance were observed in *Donax (Latona) faba*, *D. (Dentilatona) incarnatus* and *D. (Latona) cuneatus*. Although the genetic distance values were low, *D. (Latona) faba* had the lowest percentage (less than 1.6%) of the species living in Thai waters. Kalinowski (2002) suggests that short genetic distances between populations might indicate relatively common gene flow, conserved gene flow or a large population. However, *D. (Latona) cuneatus* showed high intraspecific genetic variation, supported by a high percentage of genetic distance.

COI sequences are considered standard markers; in our study, *COI* sequences confirmed the assignment of *D. (Latona) cuneatus* and *D. (Latona) faba* to the subgenus *Latona*. The evolutionary relationship between these taxa is demonstrated by a short phylogenetic distance; this aligns with previous findings by Yokogawa and Kondo (2006). The genetic distance between *D. (Latona) cuneatus* and *D. (Latona) faba* was also shorter than the distances observed in other subgenera such as that between *D. (Deltachion) semigranosus* and *Tentidonax kiusiuensis* (Yokogawa and Kondo 2006). On the other hand, the results of our molecular analysis of genetic distance at the subgenus level correlated with our findings based on shell morphology. Hubber (2010) finds that *Dentilatona* is morphologically similar to the species in *Latona*, suggesting a close evolutionary history. Phenotypic variation is partially affected by the genetic variability in wedge shell populations. In our molecular analysis, the haplotype networks and genetic distances support a close relationship between species in this region, such as *D. (Latona) faba* and *D. (Latona)*

solidus, in line with Raven (2021).

This study highlights the first report of *COI* sequence in GenBank of *Donax (Deltachion) semisulcatus semisulcatus* and *D. (Dentilatona) incarnatus* from Thailand. However, no sequences of *D. (Deltachion) spinosus* or *D. (Deltachion) bruneirufi* could be analysed due to a lack of available DNA.

Biogeography and Distribution Patterns in Thai Waters

The tropical Indo-Pacific region hosts the highest rate of marine biodiversity in the world (Bowen et al. 2016). Intra- and interspecific biogeographic patterns are frequently related for species found in this region (Dawson 2001). Donacidae is one of the largest families worldwide; these species are found in temperate and warm waters (Huber 2010). Raven (2021) showed that the South China Sea and East Asia host relatively high numbers of Donacidae (13 valid species). Thai waters are also home to many types of Donacidae (Signorelli and Printrakoon 2020; Raven 2021). However, fewer than four species have been identified in the Southeast Asian region: two species in the Philippines (Poppe 2011), three in Malaysia and Singapore (Morris and Purchon 1981; Tan and Low 2013) and four in Indonesia (Prashad 1932; Dharma 2005). Nine or ten species have been reported in Vietnam (Raven 2021; Hylleberg and Kilburn 2003). Finally, there are six species in each Thai water system. The high biodiversity of Donacidae in Thai waters may be explained by the movement of donacid larvae, which undergo planktonic dispersion through ocean currents that contribute to the biogeographical differences of these two water systems.

The present study demonstrates three distribution patterns of Donacidae living in Thai waters. (i) The first pattern includes three species, *D. (Latona) cuneatus*, *D. (Latona) faba* and *D. (Dentilatona) incarnatus*, which are distributed throughout Thai waters in both the Andaman Sea and the Gulf of Thailand. (ii) Two species were registered only in the Andaman Sea: *D. (Deltachion) spinosus* and *D. (Hecuba) scortum*. (iii) Two species were recorded only in the Gulf of Thailand: *D. (Deltachion) semisulcatus semisulcatus* and *D. (Deltachion) bruneirufi* (Fig. 1).

The distribution of species from the Gulf of Thailand (Thailand's eastern coast) is similar to that of the update reported in the South China Sea (Raven 2021), whereas *Donax* species from the Andaman Sea (Thailand's western coast) is similar to that of the previous report in the Indian Ocean (Subba and Dey 1986; Ramakrishna and Dey 2003).

The three *Donax* species *D. (Latona) cuneatus*, *D. (Latona) faba* and *D. (Dentilatona) incarnatus* are the

most common species in both of these water systems; they are also widely distributed across the Indo-West Pacific. This may be explained by the high dispersal abilities of molluscs, which can follow glaciation across two or more ecoregions (Reid et al. 2006). Distribution patterns (ii) and (iii) above, indicating different compositions of species in the Gulf of Thailand and the Andaman ecoregion, could be affected by present-day ocean currents and Pleistocene glaciation. This explanation is also supported by previous research on barnacles in both Thai water systems (Sojisuporn et al. 2010; Pochai et al. 2017; Sukparangsi et al. 2019; Chan et al. 2022).

The strongest explanation for the distribution of Thai Donacids is provided by Raven (2021), who studies this family in the South China Sea and East Asia. Raven suggests that some endemic species in the South China Sea may have originated during one or more of the Pleistocene glaciations, which caused significant drops in sea level, leading to the subaerial emergence of a large part of the Sunda Shelf and consequently the closure of the straits connecting the South China Sea with the Indian Ocean and the Java and Sulu Seas. During such periods of isolation, the regions with beaches decreased substantially, leading to increased competition amongst species (Raven 2021). This could explain the distributions of different species in Thailand's two water systems.

CONCLUSIONS

Our results illustrate that a combination of GM methods and genetic analysis is useful for biodiversity and distribution studies. Molecular data play a key role in specific differentiation in systematic surveys. The genetic variability of wedge clam populations in Thailand's two water systems illustrates adaptation under biogeographic distribution patterns. Our study is the first to report *COI* sequences for *D. (Deltachion) semisulcatus semisulcatus* and *D. (Dentilatona) incarnatus* found in Thai waters to the GenBank.

Acknowledgments: The authors thank the Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand for research funding. International SciKu Branding (ISB) funding was received from the Faculty of Science, Kasetsart University. CP would like to acknowledge Dr. Kitithorn Sanpanich from Institute of Marine Science, Burapha University and Charatsee Aungtonya from Phuket Marine Biological Center (PMBC) for kindly helping with the Reference Collection of Thailand. JHS would like to acknowledge CONICET, which is a member of

the “Carrera del Investigador Científico”. International SciKu Branding (ISB) funding, Faculty of Science, Kasetsart University. This work was partially supported by the PICT-2019-03433.

Authors' contributions: Cheewarat Printrakoon: sampling design, field sampling, Geometric Morphometric analysis. Sasimanas Unajak: Genetic analysis. Javier H. Signorelli: editing of paper, and analysis of results.

Availability of data and materials: All the studied material shells were deposited into the museums of Department of Zoology, Faculty of Science, Kasetsart University, Thailand. DNA sequences generated in this study have been deposited into GenBank database.

Consent for publication: All the authors consent to the publication of this manuscript.

Ethics approval consent to participate: This research followed the guidelines the research animal care and use for scientific research from Kasetsart University, Thailand. Animal use personal ID (CP): UT-01987-2558 and Project approval ID: ACKU65-SCI-005.

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