Ramkhamhaeng International Journal of Science and Technology (2018) 1(2): 33-40

ORIGINAL PAPER

Molecular-taxonomic investigation of the wedge clam *Donax scortum* (Linnaeus, 1758) from the Andaman Sea Coast of Thailand

Siriluck Rongprakhon[a], Thamasak Yeemin[a], Charernmee Chamchoy[a], Laongdow Jungrak [a], Sittiporn Pengsakun [a], Wanlaya Klinthong [a], Manoch Wongsuryrat [b], Makamas Sutthacheep [a,*]

[a]Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamark, Bangkok, Thailand
[b]Hat Chao Mai National Park, Department of National Parks, Wildlife and Plant Conservation, Sikao, Trang Province, Thailand

*Corresponding author: *smakamas@hotmail.com*

Received: 28 June 2018 / Revised: 24 July 2018 / Accepted: 17 August 2018

Abstract. The wedge clam *Donax scortum* (Linnaeus, 1758) is an economically important bivalve in Trang Province and its vicinity in the Andaman Sea. Based on morphological taxonomy, the dominant wedge clam along the Andaman Sea coast of Thailand was identified as *D. scortum*. The aim of this study was to develop specific COI primers for *D. scortum* as a molecular approach for reliable taxonomic identification. The wedge clam samples were collected from four localities within fishing grounds, including Hat Chao Mai and Hat Pakmeng in Trang Province, Hat Thung Talay in Krabi Province and Bo Jed Luk in Satun Province. DNA barcoding testing was conducted by sequencing the partial mitochondrial cytochrome c oxidase subunit I (COI) gene. Phylogenetic studies showed that specimens of *D. scortum* from all study sites were same species in the phylogenetic tree. However, possible cryptic species should be investigated by further studies using morphological and molecular genetic data to clarify their evolutionary history. The COI gene-based investigation may not be sufficient for identification of this clam species. Future investigation on complete mitochondrial genomes may show a different explanation for the evolutionary history of *D. scortum*. This study provides important data for phylogenetic study of *D. scortum*.

Keywords: *Donax scortum*, molecular genetics, phylogenetic tree, DNA barcoding, Andaman Sea

1. Introduction

The wedge clam *Donax scortum* (Linnaeus, 1758) is an infaunal bivalve in the intertidal zone of the Indian Ocean and is found abundantly in Trang Province and its vicinity. It is one of the famous unique cuisine of tourists in Thailand. Wedge clams of the genus Donax are an essential component of the shallow-bottom macrofauna of exposed sandy beaches (Ansell 1983). They are typical inhabitants of sandy and muddy beaches in tropical and temperate zones. In Thailand, it is one of the economically essential clams harvested along the Andaman Sea coast (Tanyaros 2010). In the past several years, the number of wedge clams in natural populations has decreased, due mainly to overfishing, so implementation of an effective management strategy is necessary (Pengsakun et al. 2017). The wedge clam is found along the coast of the Andaman Sea, from Satun Province to Krabi Province, a distance of about 500 km (Jitpukdee et al. 2015; Rongprakhon et al. 2018). This area is the main fishing ground for local fishers.

Based on morphological taxonomy, the dominant wedge clam along the Andaman Sea coast of Thailand was identified as the Indian Ocean distributed common wedge clam *Donax scortum* (Linnaeus, 1758) (Bivalvia: Donacidae). This clam is triangular-shaped, having its external surface colors of yellowish brown or brownish black (Tanyaros 2010; Singh et al. 2012; Klinthong et al. 2017; Singh 2017). High densities of *D. scortum* were reported on the Hat Pak Meng beach, at 70 – 300 m from the shoreline with a median grain size 0.0922 mm while its lower population densities were found on Hat Chao Mai beach, at 170 – 410 m from the shoreline with a median grain size 0.0914 mm.

These wedge clam populations may be also observed in subtidal zones of Hat Chao Mai National Park, Trang Province (Pengsakun et al. 2017). The populations of *D. scortum* in Trang Province had one reproductive cycle a year with several stages of gamete development. The small sizes (3-4 cm length) of this clam showed immature stages while the bigger sizes, 4-7 cm length, developed gametes from February to June. The small sizes (<4 cm) of *D. scortum* should not be harvested and establishing protected areas for this clam in the Andaman Sea is needed (Chamchoy et al. 2018).

DNA barcoding has been used as an approach to molecular taxonomic identification of animals for two decades (Hebert et al., 2003). This method is a reliable, standardized, cost-effective, and widely-applied tool for biodiversity examinations on different developmental stages (Hebert et al., 2003; Mikkelsen et al. 2007; Guarniero et al. 2010; Radulovici et al., 2010; Weigelt et al. 2016). It is based on sequences from the 5′ region of the mitochondrial cytochrome c oxidase subunit I (COI) gene as marker for species-level identification (Abbadi et al., 2016; Weigelt et al. 2016). In the present study, we aimed to develop specific COI primers for *D. scortum* as a molecular approach for reliable taxonomic identification of this species from the Andaman Sea coast of Thailand.

2. Materials and Methods

2.1 Sample collection

The wedge clam samples were collected from four localities within fishing grounds in Thai waters along the Andaman coast, including Hat Chao Mai and Hat Pakmeng in Trang Province, Hat Thung Talay in Krabi Province and Bo Jed Luk in Satun Province (Figures 1 and 2). The collected samples were preserved in 97% ethanol, transferred to the laboratory, until the DNA extraction.

2.2 DNA extraction and PCR amplification

Total genomic DNA was extracted from mantle tissue using the Genomic DNA PureLink®Genomic DNA Kit (Invitrogen, CA, USA) following the manufacturer's protocol. All extraction products were stored at -20ºC. The mitochondrial COI gene was amplified by using the primers COIDonax-F(5′GAGTDATAATRCGKACTGARYTRATAC-3′), and COIDonax-R (5′-GTGAATRAATAAHACNGGRTCYCC-3′) were designed using Primer3 (Nantón, A. et al. 2015.) PCR was performed in a final volume of 25 µL genomic containing 2.5 µL of 10X Taq buffer, 1.5 µL of 25 mM MgCl2, 2 µL of 2.5 mM dNTPs, 2 µL of 10 mM each primer, 0.5 µL of 5 unit Taq DNA polymerase, 11.5 µL of ddH2O and 5 µL of total genomic DNA (50-100 ng). The amplification process consisted of an initialization step of 3 min at 94 °C, followed by 34 amplification cycles of; denaturation at 45 sec at 94 °C, annealing 45 sec at 41 °C, extension 45 sec at 72 °C, and a final extension step at 72 °C for 3 min. PCR products were migrated on a 1 % agarose gel electrophoresis. The gels were stained with Novel juice, visualized and recorded on a transilluminator Gel Documentation (Bio Rad). The amplification product was purified using a DNA product purification kit (PCR Clean-Up & Gel Extraction Kit (PureDirex) and sequenced by Gibthai Co., Ltd. (Macrogen Inc. Seoul, Korea).

2.3 DNA Sequencing

The purified PCR products were directly sequenced. DNA sequence data sets were refined using CAP3 software (Huang & Madan, 1999), and consensus sequences were extracted from forward and reverse sequences. The consensus sequences were deposited at GenBank (LC516669, LC516670, LC516671, LC516672, LC516673, LC516674, LC516675, LC516676, LC516677, LC516678, LC516679, LC516680, LC516681, LC516682, LC516683 and LC516684). To investigate phylogenetic relationships, entire COI coding sequences of *D. scortum* were obtained from GenBank. Additionally, records from different Donax species were retrieved and *Strombus canarium* was also retrieved for the outgroup.

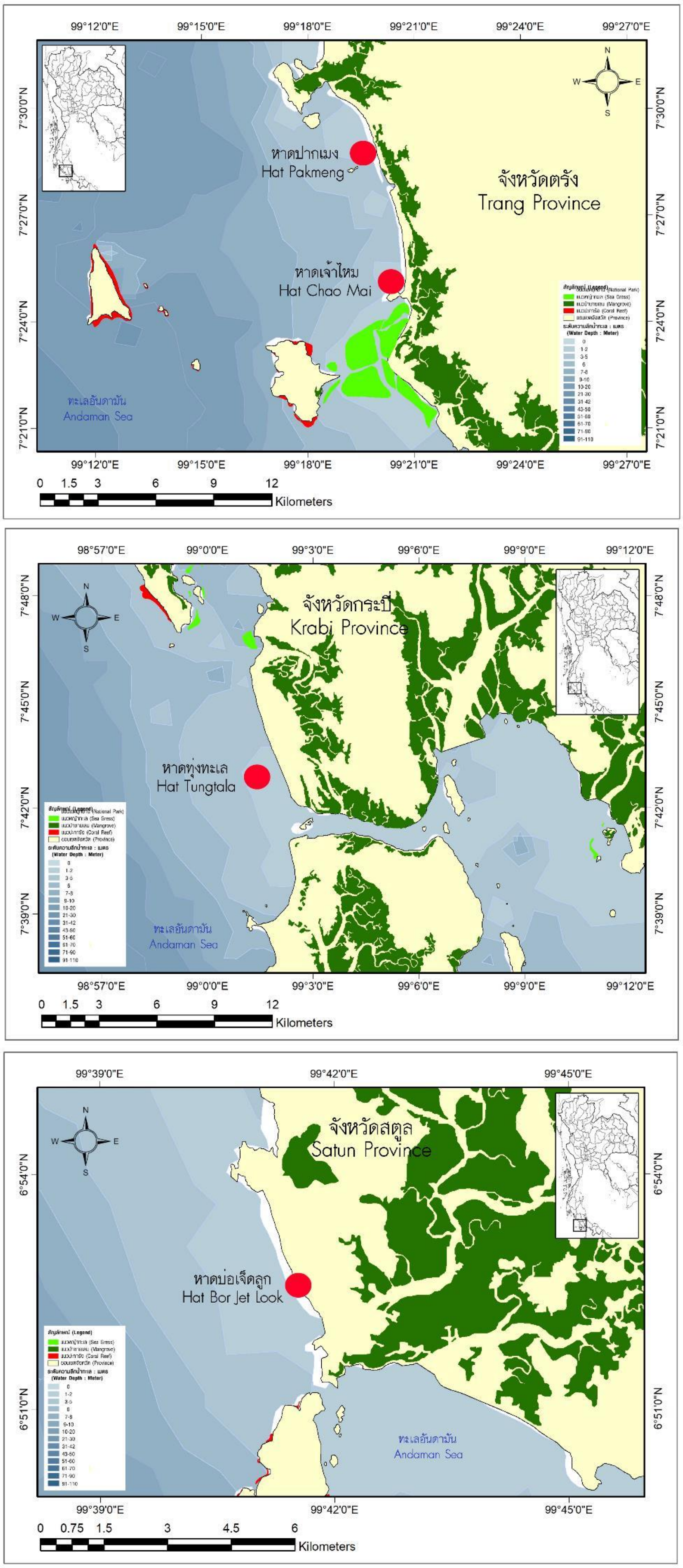
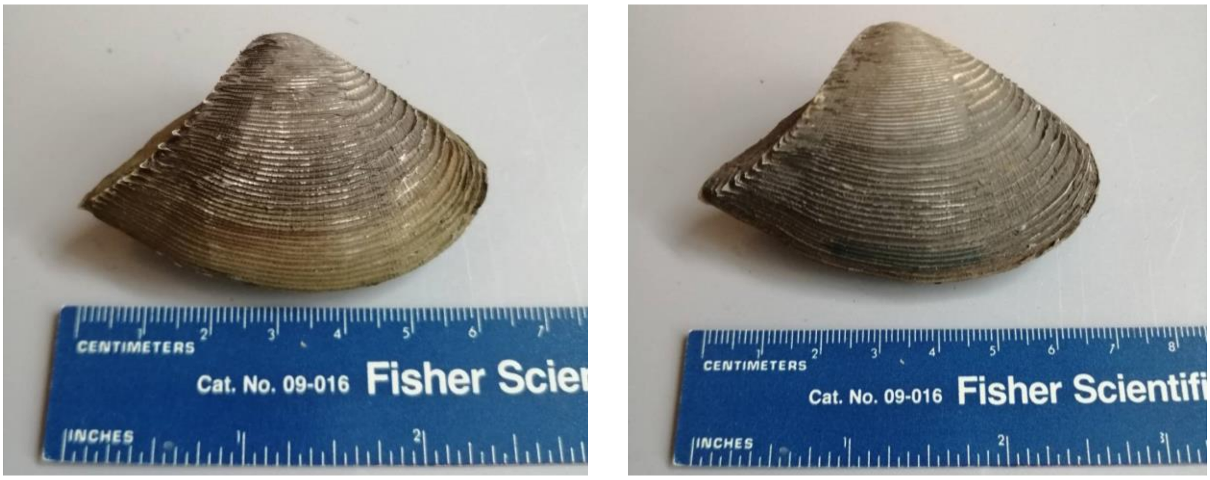


Figure 1. Maps showing study sites in Trang, Krabi and Satun Provinces, the Andaman Sea

2.4 Data analysis

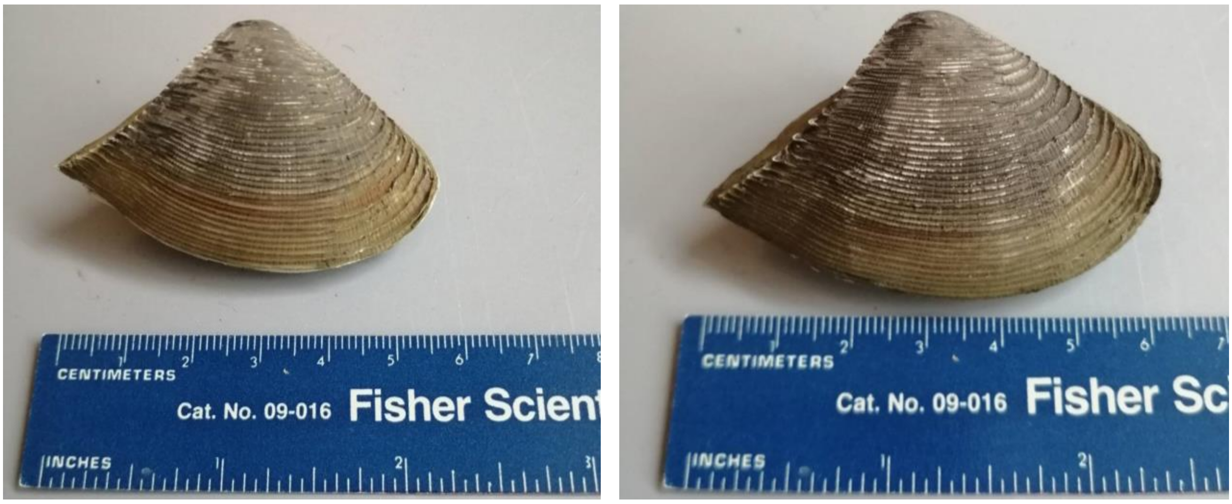
DNA sequences were checked against entries archived in the National Center for Biotechnology Information database (NCBI) and then edited. Furthermore, retrieved sequences were aligned using ClustalW ver. 1.83 (Thompson et al., 1994) for phylogenetic analysis.

The phylogenetic analysis was conducted to examine the relationships of the wedge clam. The maximum likelihood method was used with the Kimura two-parameter model, and the bootstrap method with 1,000 replicates was used to determine the statistical support. Phylogenetic and evolutionary analyses were conducted using MEGA 7.0 (Kumar et al. 2016).



Hat Chao Mai in Trang Province

Hat Pakmeng in Trang Province



Hat Thung Talay in Krabi Province

Bo Jed Luk in Satun Province

Figure 2. Specimens of *D. scortum* from the study sites in Trang, Krabi and Satun Provinces

3. Results and Discussion

For the analysis of nucleotide sequences, DNA barcoding of the clams retrieved from Genebank was used in this study, including *Donax cuneatus*, *D. variabilis*, *D. faba*, *D. variegatus* and *D. trunculus* (Table 1). The obtained sequences, which are 596 bp of the COI gene, were analyzed in terms of nucleotide composition. In the phylogenetic tree, the separations between *D. scortum* and *Donax* spp. can be clearly observed, and *D. scortum* was grouped monophyletic. Phylogenetic studies showed that specimens from Hat Chao Mai and Hat Pakmeng in Trang Province, Hat Thung Talay in Krabi Province and Bo Jed Luk in Satun Province were same species in the phylogenetic tree (Figure 3). The wedge clam inhabits along the Andaman Sea coast of Thailand without any apparent geographic barriers and ocean circulation flows northward in the spawning season of the wedge clam (Aungtonya et al. 2000; Singh 2017). These environmental factors promote gene flow and maintain genetic homogeneity among marine populations at the study sites. Molecular genomic characterization of mtDNA in this study supports previous reports for several marine invertebrates in the Andaman Sea, particularly violet vinegar crab *Episesarma versicolor* and oceanic paddle crab *Varuna litterata* (Supmee et al. 2012; Suppapan et al. 2017)

Our findings indicate that the specimens from Hat Chao Mai and Hat Pakmeng in Trang Province, Hat Thung Talay in Krabi Province and Bo Jed Luk in Satun Province belong to *D. scortum*. Future studies should investigate possible cryptic species using both morphological characters and molecular genetic data to clarify the evolutionary history of *D. scortum*. Some specimens from Satun Province are morphologically different with those from Trang and Krabi Provinces. Therefore more comprehensive taxonomic and anatomical examinations of collected specimens from different locations are required.

Several environmental factors, particularly temperature, salinity, and irradiation stress, have influenced to mitochondrial sequence variation and mutation rate. Phylogenetic examinations revealed that specimens from geographically closer animals are observed as more relative species in the phylogenetic tree. (Wallace and Chalkia 2013; Luo et al. 2015; Kim et al. 2017). In this study, collection of specimens from Satun Province which inhabit in the subtidal zone should be concentrated to investigate their differences from those in the intertidal areas (Rongprakhon et al. 2018). There is a hypothesis concerning correlation between geographical distance and genetic distance (Yang et al. 2013). However, further morphological and molecular genetic studies are needed to compare this clam species from other locations in the Indo-Pacific region.

DNA barcoding using COI gene-based investigation is helpful for species identification and explaining the evolutionary history of animals, however this method may not be sufficient for some case studies (Endo et al 2005; Kim et al. 2017). Complete mitochondrial genomes studies provide more promising for species identification. Future examination on complete mitochondrial genomes may show a different explanation for the evolutionary history of *D. scortum*.

.

Table.1 Data retrieved from GenBank for the present study.

Species	Country	Accession Number	Reference
Strombus canarium	United States of America	DQ525210	Latiolais et al. (2006)
Donax cuneatus	Japan	AB691952	Ujino & Matsukuma (2013)
D. cuneatus	Japan	AB040842	Okazaki, M. (2000)
D. faba	Japan	AB040844	Okazaki, M. (2000)
D. faba	Japan	AB040845	Okazaki, M. (2000)
D. vittatus	Spain	MF668323	Fernández-Pérez, J. et al. (2017).
D. vittatus	Spain	MF668322	Fernández-Pérez, J. et al. (2017).
D. vittatus	Spain	MF668321	Fernández-Pérez, J. et al. (2017).
D. vittatus	Spain	MF668320	Fernández-Pérez, J. et al. (2017).
D. vittatus	Spain	MF668319	Fernández-Pérez, J. et al. (2017).
D. vittatus	Spain	MF668318	Fernández-Pérez, J. et al. (2017).

Species	Country	Accession Number	Reference
D. variegatus	United States of America	KX713460	Combosch D. J. et al. (2016)
D. variegatus	Spain	MF668378	Fernández-Pérez, J. et al. (2017).
D. variegatus	Spain	MF668379	Fernández-Pérez, J. et al. (2017).
D. variegatus	Spain	MF668380	Fernández-Pérez, J. et al. (2017).
D. variegatus	Spain	MF668381	Fernández-Pérez, J. et al. (2017).
D. variegatus	Spain	MF668382	Fernández-Pérez, J. et al. (2017).
D. trunculus	Spain	KY951441	Pasantes, J. J. (2017)
D. trunculus	Spain	KY951442	Pasantes, J. J. (2017)
D. trunculus	Spain	KY951443	Pasantes, J. J. (2017)
D. trunculus	Spain	KY951444	Pasantes, J. J. (2017)
D. trunculus	Spain	KY951445	Pasantes, J. J. (2017)
D. trunculus	Spain	KY951446	Pasantes, J. J. (2017)

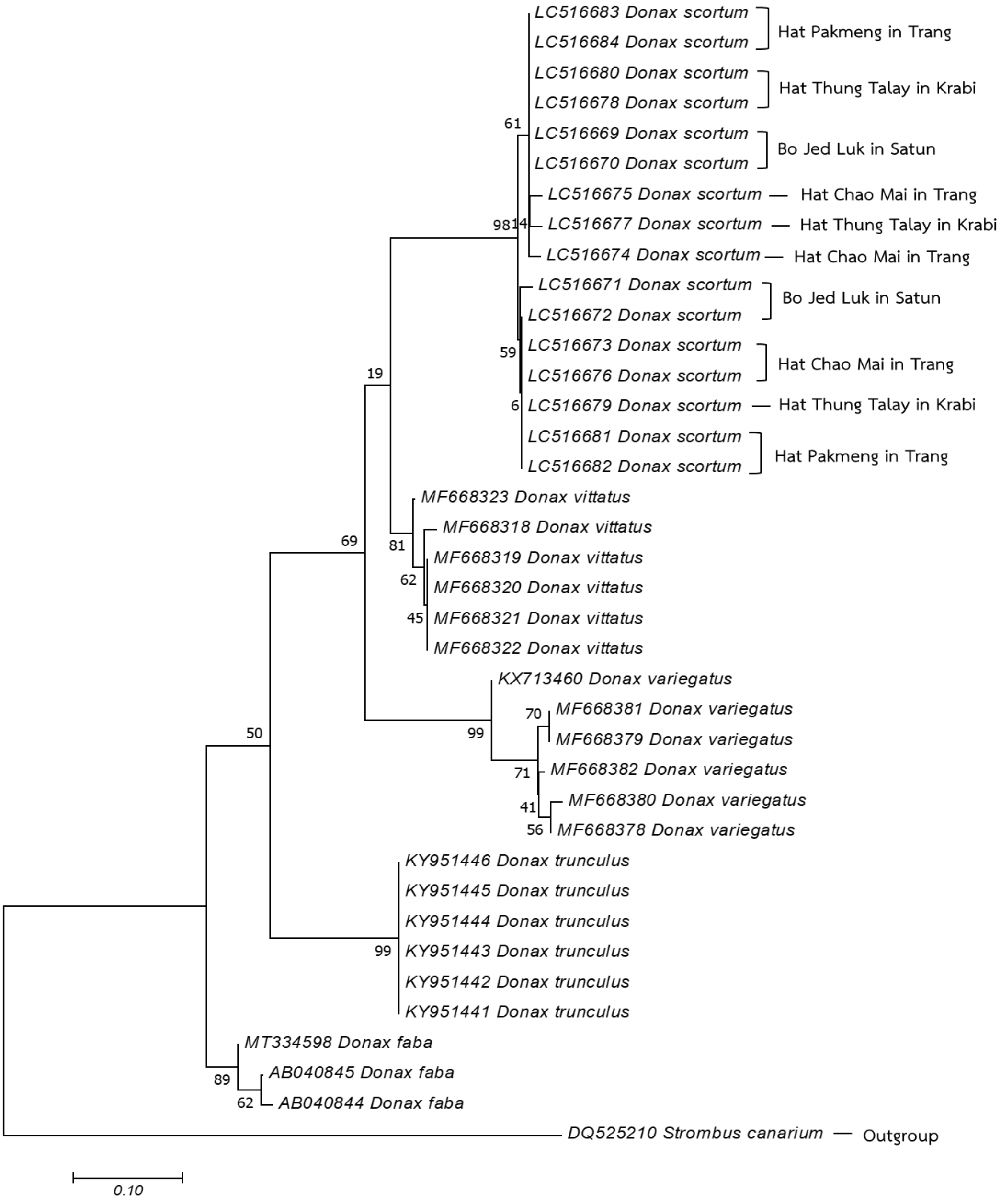


Figure 3. Maximum-likelihood phylogenetic tree constructed under the Kimura 2-parameter model based on 16 mtDNA COI nucleotide sequences of wedge clam, with *Strombus canarium* as the outgroup. Bootstrap values are shown to the left of nodes

Acknowledgments

The authors would like to thank the staffs of Hat Chao Mai National Park, Department of National Parks, Wildlife and Plant Conservation and the Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University for any supports during field surveys. This research was funded by the National Science and Technology Development Agency (NSTDA).

References

Abbadi M, Marciano S, Tosi F, De Battisti C, Panzarin V, Arcangeli G, Cattoli G (2017) Species identification of bivalve molluscs by pyrosequencing. J Sci Food Agric 97(2): 512–519

Ansell AD (1983) The biology of the genus *Donax*. In McLachlan A. and Erasmus T. (eds) Sandy beaches as ecosystems. The Hague: Dr. W. Junk Publishers, pp. 607–635

Endo K, Noguchi Y, Ueshima R, Jacobs TH (2005) Novel repetitive structures, deviant protein-encoding sequences and unidentified ORFs in the mitochondrial genome of the brachiopod *Lingula anatina*. Mol Evol 61:36e53

Guarniero I, Plazzi F, Bonfitto A, Rinaldi A, Trentini M, Passamonti M (2010) The bivalve mollusc *Mactra corallina*: genetic evidence of existing sibling species. J Mar Biol Assoc UK 90(3):633–644

Huang X, Madan A (1999) CAP3: A DNA sequence assembly program. Genome Res 9(9):868–877

Hebert PDN, Cywinska AA, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. P Roy Soc Lond B Bio 270:313–321

Jitpukdee S, Tantikamton K, Thanee N, Tantipanatip W (2015) Species diversity of benthic macrofauna on the intertidal zone of seacoasts in Krabi, Trang and Satun Provinces, Thailand. IJAT 11(8):1767–1780

Kim SG, Karagozlu MZ, Kim CB (2017) Phylogenetic investigations of *Lingula anatina* among some northwestern Pacific populations, based on mitochondrial DNA cytochrome c oxidase subunit I gene. J Asia-Pac biodivers 10(2):162–166

Klinthong W, Yeemin T, Sutthacheep M, Wongsuryrat M, Prickchoopon T, Aunkhongthong W, Niyomthai P (2017) Population structure of wedge clam on sandy beaches in Trang Province. Proceedings of the 43rd Congress on Science and Technology of Thailand (STT 43) 2017:300–303

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874

Luo YJ, Satoh N, Endo K. 2015. Mitochondrial gene order variation in the brachiopod *Lingula anatina* and its implications for mitochondrial evolution in lophotrochozoans. Mar. Genom 1:31e40

Mikkelsen NT, Schander C, Willassen E (2007) Local scale DNA barcoding of bivalves (Mollusca): a case study. Zoologica Scripta 36(5):455–463

Nantón A, Freire R, Arias-Pérez A, Gaspar MB, Méndez J (2015) Identification of four Donax species by PCR–RFLP analysis of cytochrome c oxidase subunit I (COI). Eur Food Res Technol 240(6):1129–1133

Pengsakun S, Yeemin T, Sutthacheep M, Wongsuryrat M, Printrakoon C, Prickchoopon T, Aunkhongthong W (2017) Temporal and spatial distribution of *Donax scortum* (Bivalvia: Donacidae) from Hat Chao Mai National Park. Proceedings of the 43rd Congress on Science and Technology of Thailand (STT 43) 2017:278–283

Radulovici A, Archambault P, Dufresne F (2010) DNA barcodes for marine biodiversity:moving fast forward. Diversity 2:450–472

Rongprakhon S, Yeemin T, Sutthacheep M, Chamchoy C, Aunkhongthong W, Niyomthai P, Mue-suea O (2018) Abundance of the wedge clam, Donax scortum from small scale fisheries in Krabi, Trang and Satun Provinces. Proceedings of the 44th Congress on Science and Technology of Thailand (STT 44) pp. 326–331

Singh YT, Krishnamoorthy M, Thippeswamy S (2012) Seasonal changes in the biochemical composition of wedge clam, *Donax scortum* from the Padukere beach, Karnataka. Recent Res Sci Technol 4(12)

Singh YT (2017) Status of population dynamics of the Asian wedge clam, *Donax scortum* (Bivalvia: Donacidae): a first report from Asia. J Mar Biol Assoc UK 97(8):1635–1642

Tanyaros S (2010) Sand elimination by *Donax scortum* (Dance, 1982) (Bivalvia: Donacidae). Molluscan Res 30:138–142

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680

Wallace DC, Chalkia D (2013) Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb Perspect Biol 5:a021220.http://dx.doi.org/10.1101/cshperspect.a021220Singh YT, Krishnamoorthy M, Thippeswamy S (2012) Seasonal changes in the biochemical composition of wedge clam, Donax scortum from the Padukere beach, Karnataka. Recent Res Sci Technol 4(12)

Weigelt R, Lippert H, Borges LM, Appelqvist C, Karsten U, Bastrop R (2016) First time DNA barcoding of the common shipworm *Teredo navalis* Linnaeus 1758 (Mollusca: Bivalvia: Teredinidae): Molecular-taxonomic investigation and identification of a widespread wood-borer. J Exp Mar Biol Ecol 475:154–162

Yang S, Lai X, Sheng G, Wang S (2013) Deep genetic divergence within a “living fossil” brachiopod *Lingula anatina*. J Paleontol 87:902e908