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Morphological and molecular insights into diversity of *Brevitrygon* (Elasmobranchii, Myliobatiformes, Dasyatidae) in Thai waters

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Abstract

Stingrays of the genus *Brevitrygon* in Thailand are captured as bycatch in fisheries, and a proportion of the catch is used for local consumption, but information on their biodiversity remains limited. This study examines the diversity of *Brevitrygon* species collected from the Gulf of Thailand and the Andaman Sea. A total of 148 specimens were morphologically identified as either *Brevitrygon heterura* (Bleeker, 1852) or *Brevitrygon* cf. *imbricata*, based on diagnostic characters. Morphometric analysis revealed that all specimens more closely resembled *B. heterura*, with no significant differences between morphotypes or sexes. Genetic analysis based on *COI* sequences from 39 samples further supported these results. All samples grouped within the *B. heterura* clade with strong bootstrap support and formed two distinct genetic subgroups corresponding to the Gulf of Thailand and the Andaman Sea. The mean intraspecific divergence (1.08%) fell within the expected range for population-level variation in elasmobranchs, with interspecific divergence from other *Brevitrygon* species exceeding 9%. These results indicate that *B. heterura* in Thai waters likely represents a single species. While both morphometric and genetic data suggest regional differentiation, the observed variation falls within the normal range for a single species, providing no evidence of cryptic diversity. This study underscores the value of integrating morphological and molecular data to better understand population structure and taxonomy in stingrays.

Keywords

Andaman Sea, Gulf of Thailand, intraspecific variation, stingrays, taxonomy

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Introduction

The genus *Brevitrygon* (Family: Dasyatidae) includes small to medium-sized benthic stingrays found in shallow coastal waters of the Indo-West Pacific (Last et al. 2016a). These stingrays are characterized by a small body size, a strongly depressed and suboval disc, an elongate and angular snout, reduced or rudimentary tail folds, and a well-defined secondary denticle band on the dorsal disc. The tail is relatively slender and exhibits marked sexual dimorphism; adult females develop a distinct bulbous expansion on the distal portion of the tail. Collectively, these morphological characters distinguish *Brevitrygon* from other members of the family Dasyatidae. However, species-level identification within *Brevitrygon* remains challenging due to subtle morphological variations, limited diagnostic features, and overlapping geographic distributions (Last et al. 2023).

Currently, five species within the genus *Brevitrygon* are recognized, with the distributions of most species proposed (Last et al. 2023). However, the distribution of *Brevitrygon imbricata* (Bloch et Schneider, 1801) in the Indian Ocean is unclear. This species has been listed in Thai waters along with *Brevitrygon heterura* (Bleeker, 1852) based on morphological characteristic (Krajangdara et al. 2019). Although these species share many morphological similarities, several key differences have been identified. *B. heterura* typically exhibits an oval-shaped disc with length slightly longer than width, whereas *B. imbricata* displays a more rounded disc with nearly equal width and length (Last et al. 2016b). The snout of *B. heterura* is pointed and projected forward, in contrast to the more rounded snout of *B. imbricata*. In adult female *B. heterura*, the tip of the tail shows a structure called a bulbous, which is absent in both male *B. heterura* and *B. imbricata*.

Although it is likely that the distribution of *B. imbricata* does not extend to the Andaman Sea of Thailand, recent observations in Thailand have reported specimens resembling *B. imbricata*. Such ambiguous individuals have been provisionally referred to as *B.* cf. *imbricata* (see Krajangdara et al. 2022). This morphotype and *B. heterura* represent diversity of the genus *Brevitrygon* in Thailand and worth examining. While traditional morphological assessments, especially external morphometric measurements, remain foundational in elasmobranch taxonomy, the integration of molecular techniques, particularly mitochondrial DNA barcoding using the cytochrome c oxidase I (*COI*) gene, has significantly enhanced species-level discrimination and has been widely applied in cartilaginous fishes (Ward et al. 2005; Cerutti-Pereyra et al. 2012; Pavan-Kumar et al. 2015; Loh et al. 2023).

This study aims to assess the species diversity of the genus *Brevitrygon* in Thailand using both morphometric and molecular examinations, with a particular focus on *B. heterura*, which has been identified as highly vulnerable to bottom trawl fisheries in the Gulf of Thailand (Noranarttragoon et al. 2023), thereby highlighting the importance of accurate species identification for informing biodiversity assessments and supporting effective conservation strategies.

Materials and methods

Sample collections and morphological identification. Specimens of stingrays identified as members of the genus *Brevitrygon* were collected from the fishing ports and vessels between May 2022 and January 2024 in the Gulf of Thailand, and the Andaman Sea (Fig. 1). A total of 148 specimens were collected (Table 1). Specimens were weighed and sexed on site. Tissue samples of right pelvic fins were preserved in 95% ethanol for genetic analysis. The specimens were kept on ice and transported to the Department of Animal Production Technology and Fisheries, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), and stored in a –20°C freezer for further analysis. The survey and collection procedures were approved by the Institutional Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang, Thailand (Approval No. CC-KMITL/2025/001).

Table 1. Number of specimens (*n*), sex ratio, and disc width range (minimum–maximum) of *Brevitrygon* collected from each sampling location.

Collection site		Disc width Max–Min [cm]	Male (*n*)	Female (*n*)	Sex-ratio
Gulf of Thailand	Chon Buri	18.8–19.1	1	2	0.5:1
	Rayong	16.4–18.5	1	2	0.5:1
	Chanthaburi	15.8–24.9	11	10	1.1:1
	Nakhon Si Thammarat	14.9–16.7	3	2	1.5:1
	Songkhla	15.5–19.0	5	4	1.3:1
	Prachuap Khiri Khan	12.9–14.6	4	4	1:1
	Total	**12.9–24.9**	**25**	**24**	**1:1**
Andaman sea	Phuket	9.9–24.9	53	41	1.3:1
	Trang	16.7	1	0	—
	Satun	17.3–21.7	3	1	3:1
	Total	**9.9–26.5**	**57**	**42**	**1.4:1**

Specimens were initially identified as *Brevitrygon heterura* or *B.* cf. *imbricata* based on key morphological characteristics (Fig. 2) and referred to as two different morphotypes (Last et al. 2016a, 2023; Krajangdara et al. 2022). Morphometric measurements were taken using calipers and rulers to the nearest 0.1 cm. In addition, as gender may influence morphological traits, this factor was also tested for morphological variations.

Morphological characteristics were recorded following the methods described in Last et al. (2016a). Two additional measurements were included in this study: mid-shoulder denticle length (MSD-L) and mid-shoulder denticle width (MSD-W). A total of 41 morphological characteristics were examined (Fig. 3), with 40 of them expressed as morphometric ratios relative to disc width (Suppl. material 1) Principal Component Analysis (PCA) was performed using R software to visualize morphological variation between two morphotypes*: B. heterura* and *B.* cf. *imbricata.* The PCA was conducted using size-normalized variables representing relative body proportions. Variables that contained too few distinct values were

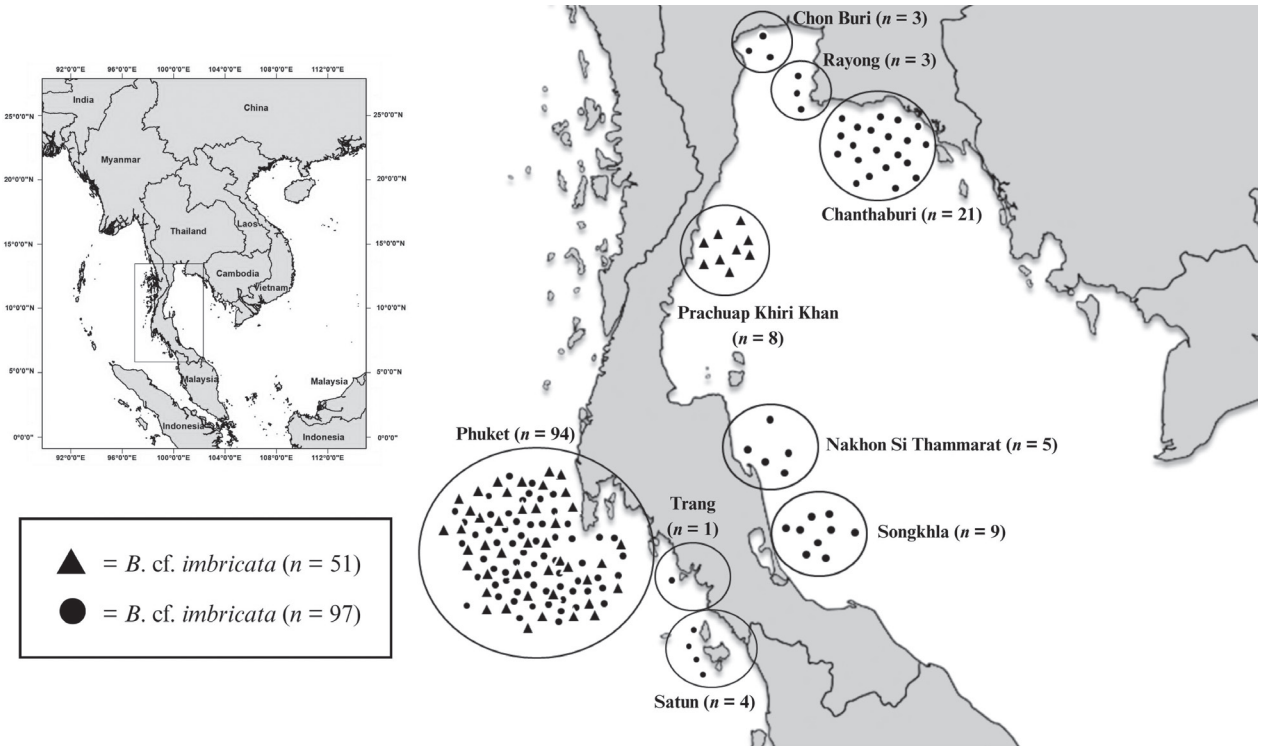

Figure 1. Sampling stations in the Gulf of Thailand and Andaman Sea of Thailand.

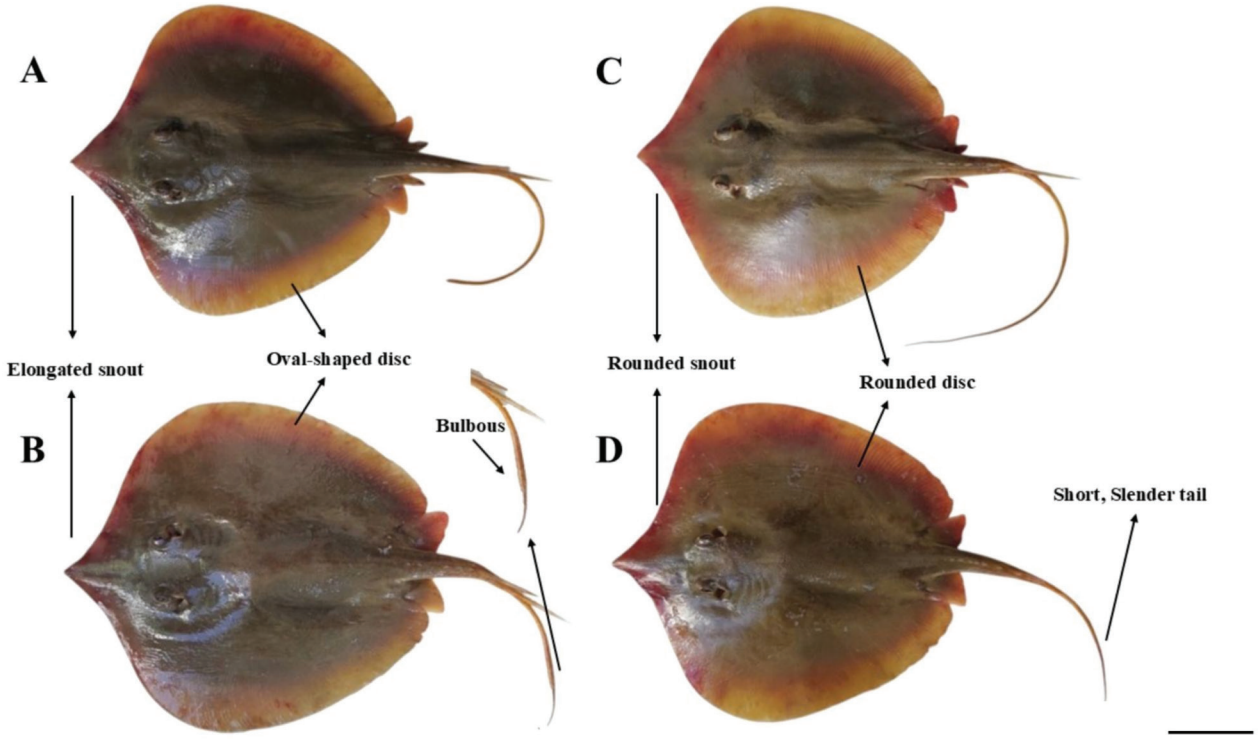

Figure 2. Specimens of the genus *Brevitrygon* collected in this study. **A**. *Brevitrygon heterura* male sample; **B**. *Brevitrygon heterura* female sample; **C**. *Brevitrygon* cf. *imbricata* male sample; **D**. *Brevitrygon* cf. *imbricata* female sample. The scale bars represent 5 cm.

removed (Suppl. material 2). MANOVA was used to test factors influencing morphological variation of specimens including morphotypes, geographic locations, and sex. Fourteen morphometric traits of the specimens were analyzed in comparison with reference data for *B. heterura* and *B. imbricata* (Last et al. 2016b, 2023).

Molecular analysis of *Brevitrygon* specimens. The availability of budget restricted the sample size in this study. There were 39 tissue samples of clear species identification and ambiguous identification selected for a genetic study including 12 samples from the Gulf of Thailand and 27 samples from the Andaman Sea.

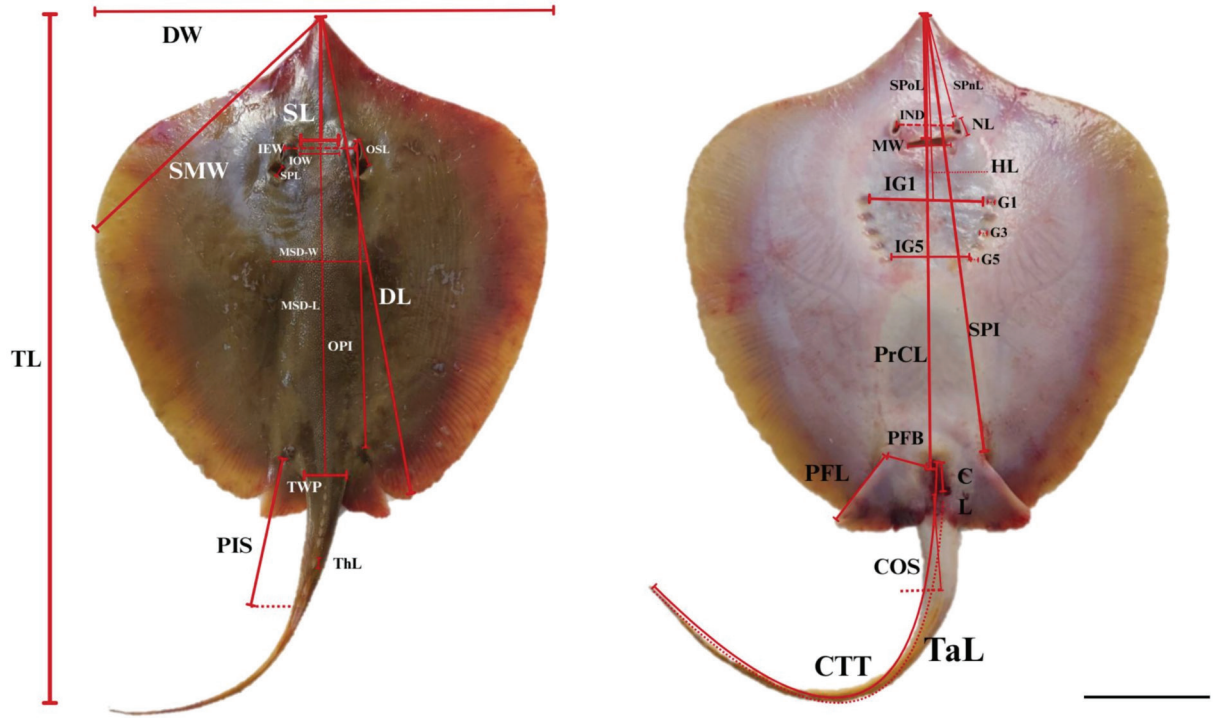

Figure 3. Morphometric measurements of *Brevitrygon* specimens (Character abbreviations explained in Table 2). The scale bars represent 5 cm.

The selection was randomly drawn from two morphotypes from both the Gulf of Thailand and the Andaman Sea. DNA was extracted from 1 mg of pelvic fin tissue using Qiagen DNeasy® Blood & Tissue Kits (Qiagen, California, USA) following the manufacturer's instructions. Partial sequence of the *COI* gene was amplified by polymerase chain reaction (PCR) with a pair of primers, FishF1 (5′–TCAACCAACCACAAAGACATTGGCAC–3′) and FishR1 (5′– TAGACTTCTGGGTGGCCAAAGAATCA–3′) (Ward et al. 2005). DNA amplifications were performed in 20 µL volumes containing of PCR buffer (10×) 2 µL, $MgCl_2$ (2.5 mmol· μL^{-1}) 2 µL, dNTPs (2.5 mmol· μL^{-1}) 2 µL, i-Taq™ DNA Polymerase (5U· μL^{-1}) 0.2 µL (Intron, Korea), forward and reverse primer (10 pmol· μL^{-1}) 0.5–1.0 µL, and DNA template 1–2 µL. Optimized cycling conditions were 95°C for 2 min, 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and final extension of 72°C for 10 min. Successfully amplified PCR products were visualized, purified, and sequenced. The obtained sequences were edited and aligned using MEGA12 (Kumar et al. 2024). Final alignments were 512 base pairs (bp). All sequences were deposited in NCBI (Suppl. material 3). The Nucleotide Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1997) was used to search for the similarity of sample sequences based on the nucleotide collection database in NCBI. Megablast algorithm, optimized for highly similar sequences, was employed. Species identification was based on the top BLAST hits, prioritizing results with the highest query coverage and percent identity. Phylogenetic relationships were inferred from aligned *COI* gene sequences using the Maximum Likelihood (ML) method in MEGA12, applying the HKY+G substitution model with 1000 bootstraps. A maximum likelihood tree was constructed to examine the genetic divergence between *B. heterura* and *B.* cf. *imbricata*. Reference sequences of *Brevitrygon* spp. from NCBI were included in the analysis with *Fluvitrygon signifer* (Compagno et Roberts, 1982) as an outgroup. Kimura-2-Parameter (K2P) method was used to calculate inter- and intraspecific genetic variation between *B. heterura* and *B.* cf. *imbricata* via MEGA12.

Results

Morphometric analysis. Morphometric measurements of the two morphotypes more closely resembled those of *Brevitrygon heterura* than of *B. imbricata* reported in previous studies (Table 2), although some specific ratios differed. Notably, our specimens exhibited a relatively greater interorbital width to orbit length compared to both *B. heterura* and *B. imbricata* from previous studies. Based on these measurements, *B.* cf. *imbricata* aligned more closely with *B. heterura*. Both morphotypes were found in both the Gulf of Thailand and the Andaman Sea. To further explore morphological variation, principal component analysis (PCA) was performed on 14 morphometric ratios. The first two components accounted for nearly all morphological variations, with PC1 explaining 66.63% and PC2 16.95% of the variance (Fig. 4). The PCA ordination did not show clear separation or distinct clustering between the two morphotypes or across geographic locations, indicating substantial overlap in morphometric traits. However, MANOVA indicated that

Table 2. Morphometric ratios of *B. heterura*, *B. imbricata*, and two morphotypes of *Brevitrygon* in this study.

Morphometric ratios	Abbreviation*	*B. heterura*	*B. imbricata*	*B.* cf. *imbricata* (this study)	*B. heterura* (this study)	Reference
Disc length to disc width	DL/DW	1–1.1 times	1.1 times	1–1.1 times	1–1.1 times	Last et al. 2016b
Orbit and spiracle length to snout length	OSL/SL	2.9–3.1 times	2.9–3.6 times	2.4–4.3 times	2.5–4.2 times	Last et al. 2016b
Interorbital width to orbit length	IOW/OD	1.2–2.1 times	2 times	1.9–2.7 times	2.0–2.7 times	Last et al. 2016b
Tail length to disc width	TaL/DW	1–1.7 times	1.5–1.7 times	0.7–1.8 times	0.6–1.7 times	Last et al. 2016b
Thorns in median on tail	–	4–6 spines	>6 spines	3–8 spines	3–6 spines	Last et al. 2016b
Snout to pectoral-fin insertion to snout to maximum width	SPI/SMW	1.8–2.4 times	1.9–2.3 times	1.2–2.6 times	1.2–2.0 times	Last et al. 2023
Snout preoral to Mouth width	SPoL/MW	2.8–4.4 times	2.7–3.3 times	2.5–5.1 times	2.4–5.0 times	Last et al. 2023
Snout preoral to distance between 1st gill slits	SPoL/IG1	1.1–1.6 times	1.2–1.4 times	1.1–1.6 times	1.1–1.6 times	Last et al. 2023
Eye length to spiracle length	EL/SPL	1.1–1.9 times	1.2–1.7 times	0.8–2.6 times	0.8–2.5 times	Last et al. 2023
Orbit diameter to spiracle length	OD/SPL	0.7–1.2 times	0.8–1.2 times	0.6–1.1 times	0.6–1.4 times	Last et al. 2023
Interorbital width to orbit diameter	IOW/OD	1.2–2.1 times	1.6–2.0 times	1.3–1.8 times	1.4–2.0 times	Last et al. 2023
Nostril length to distance between nostrils	NL/IND	2.1–3.5 times	2.4–3.0 times	2.2–3.0 times	2.3–4.3 times	Last et al. 2023
Distance between 1st gill slits to distance between the 5th gill slits	IG1/IG5	1.0–2.3 times	2.5–3.6 times	1.0 –1.9 times	1.0–1.8 times	Last et al. 2023
Distance between 1st gill slits to distance between nostrils	IG1/IND	1.8–2.2 times	1.2–1.3 in male 1.3–1.4 in female	1.8–2.3 in male 1.9–2.8 in female	1.4–2.3 times	Last et al. 2023

*Abbreviation refers to measurements shown in Fig 3.

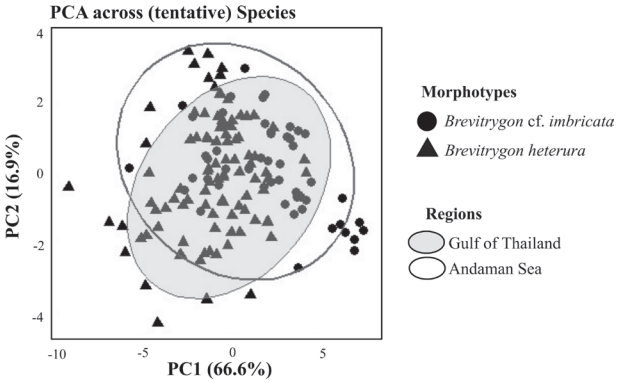

Figure 4. PCA analysis of morphometric measurements of two morphotypes of *Brevitrygon* specimens from Thai waters.

geographic location significantly influenced morphological variation (F = 2.528, df = 14, P = 0.003), whereas morphotype and sex had no significant effect (F = 1.006, df = 14, P = 0.452). Individuals of both sexes were more similar within the same location than across locations, indicating that geographic separation had a stronger effect on morphology than sex.

Phylogenetic relationships and genetic distance. All analyzed *COI* sequences were subjected to BLASTn searches against the NCBI nucleotide database. Results showed that all 39 specimens matched *B. heterura* with high identity values (>98%), supporting their species assignment. No specimen matched *B. imbricata* or other congeneric taxa more closely, further confirming the morphological identification (Table 3). The phylogenetic tree reconstructed using the Maximum Likelihood method revealed that all specimens clustered with *B. heterura* with strong bootstrap support, and were clearly distinct from other *Brevitrygon* species, particularly *B. imbricata*. Within the *B. heterura* clade, two well-supported sub-groups were observed. One consisted of specimens from the Gulf of Thailand and the eastern side of the Malay Peninsula, while the other included those from the Andaman Sea (Fig. 5). Genetic distance analysis using K2P model showed intraspecific divergences within the *B. heterura* clade ranging from 0.0% to 2.88% (mean 1.08%). Each regional subgroup exhibited divergences of approximately 0.0%–1.40% in the Gulf of Thailand and 0%–1.19% in the Andaman Sea. Interspecific divergences between *B. heterura* and other congeners ranged from 9.28% to 10.66% (Table 4).

Discussion

Examination of external morphology of *Brevitrygon* specimens collected from the Gulf of Thailand and Andaman Sea revealed two morphotypes: *B. heterura* and *B.* cf. *imbricata*. However, morphometric analysis of these specimens revealed a close resemblance to *B. heterura* rather than *B. imbricata*. PCA showed overlapping morphometric characteristics between these morphotypes, indicating a degree of similarity in body shape. Although sexual dimorphism has been documented in other stingray species, such as *Dasyatis pastinaca* and *D. marmorata* in the northeastern Levantine Basin (Yeldan and Gundogdu 2018; Yigin and Ismen 2013), sex was found to have no significant effect on morphometric variation in this study aligning with previous observations in many stingray species, including *Brevitrygon* (Last et al. 2016a). These differences suggest that the role of sex in morphological variation is likely species-specific and may be linked to differing reproductive strategies.

Geographic differences in morphology were subtle but statistically detectable. While the PCA ordination showed substantial overlap and no clear geographic clustering, MANOVA results indicated that morphometric traits differed significantly between specimens from the Gulf of Thailand and the Andaman Sea, suggesting that geographic variation is present but not strongly expressed along the major axes of variation. This pattern mirrors findings in other stingray genera, such as *Neotrygon*, where morphologically similar populations exhibit distinct morphometric and genetic differences across coastal regions (Borsa et al. 2018). Geography not only shapes morphological

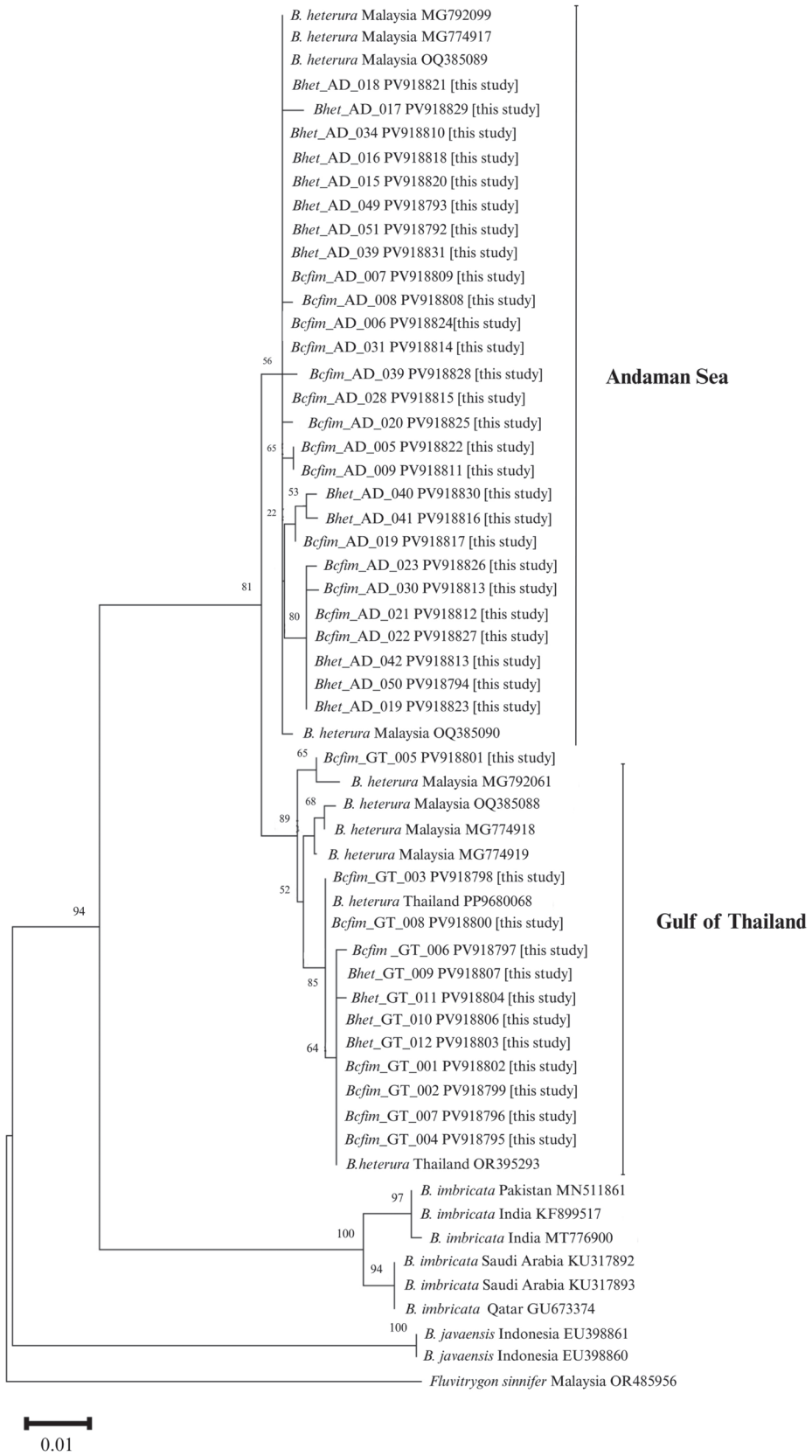

Figure 5. Phylogenetic tree of two morphotypes of *Brevitrygon* specimens from Thai waters (*B. heterura*: Bhet; *B*. cf. *imbricata*: Bcfim; GT: Gulf of Thailand; AD: Andaman Sea).

variation but also influences genetic structure (Lim et al. 2021; Then et al. 2025). Molecular analysis showed that all specimens formed a single clade, consistent with *B. heterura*, but were divided into two genetically distinct subgroups, with one from the Andaman Sea, located in the eastern Indian Ocean, and the other from the Gulf of Thailand, representing the Pacific Ocean. The mean intra-specific genetic divergence between these subgroups was

Table 3. Comparison of species identification based on morphology and genetics of two *Brevitrygon* morphotypes.

Samples	Morphotype identification	Genetic identification based on *COI*	% identity with NCBI database
Andaman Sea			
Bcfim_AD_039	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	100.00%
Bcfim_AD_028	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.00%
Bcfim_AD_030	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.64%
Bcfim_AD_031	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	100.00%
Bcfim_AD_005	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.84%
Bcfim_AD_006	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	100.00%
Bcfim_AD_007	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.39%
Bcfim_AD_008	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.69%
Bcfim_AD_009	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.69%
Bcfim_AD_019	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.85%
Bcfim_AD_020	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.85%
Bcfim_AD_021	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.39%
Bcfim_AD_022	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	98.60%
Bcfim_AD_023	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	98.29%
Bhet_AD_039	*Brevitrygon heterura*	*Brevitrygon heterura*	99.84%
Bhet_AD_040	*Brevitrygon heterura*	*Brevitrygon heterura*	99.61%
Bhet_AD_041	*Brevitrygon heterura*	*Brevitrygon heterura*	99.10%
Bhet_AD_042	*Brevitrygon heterura*	*Brevitrygon heterura*	99.54%
Bhet_AD_015	*Brevitrygon heterura*	*Brevitrygon heterura*	99.69%
Bhet_AD_016	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_AD_034	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_AD_018	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_AD_019	*Brevitrygon heterura*	*Brevitrygon heterura*	98.16%
Bhet_AD_051	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_AD_049	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_AD_050	*Brevitrygon heterura*	*Brevitrygon heterura*	99.69%
Gulf of Thailand			
Bcfim_GT_008	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.68%
Bcfim_GT_002	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.68%
Bcfim_GT_003	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.36%
Bcfim_GT_006	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.84%
Bcfim_GT_007	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	100.00%
Bcfim_GT_004	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.84%
Bcfim_GT_001	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	100.00%
Bcfim_GT_005	*Brevitrygon* cf. *imbricata*	*Brevitrygon heterura*	99.84%
Bhet_GT_009	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_GT_011	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%
Bhet_GT_010	*Brevitrygon heterura*	*Brevitrygon heterura*	99.67%
Bhet_GT_013	*Brevitrygon heterura*	*Brevitrygon heterura*	88.84%
Bhet_GT_012	*Brevitrygon heterura*	*Brevitrygon heterura*	100.00%

approximately 1.08%, which falls within the expected range for population-level differences in elasmobranchs (Ward 2009; Pavan-Kumar et al. 2015; Loh et al. 2023; Appleyard et al. 2025). This suggests population structuring rather than cryptic diversity. Similar patterns have been observed in other stingray groups. For instance, *Rhinoptera* species in the western Atlantic show genetically distinct populations across regions, despite minimal morphological differences and no evidence of hidden species (Carney et al. 2017; Palacios-Barreto et al. 2024). Such genetic structuring has been attributed to factors including benthic temperature, current patterns, philopatry, and site fidelity, which may limit gene flow and reinforce regional differentiation. Ray behavioral traits such as low mobility, demersal habitat preference, and natal homing likely contribute to the observed structure in *Brevitrygon* populations as well (Li et al. 2014).

Several stingray species exhibiting benthic and ovoviviparous life histories show similar patterns of strong site fidelity and limited dispersal. For instance, *Neotrygon kuhlii* (Müller et Henle, 1841) from the Indo-West Pacific displays sharp genetic differentiation attributed to its sedentary benthic habits (Borsa et al. 2018), and *B. imbricata* and *Brevitrygon walga* (Müller et Henle, 1841) are recorded as ovoviviparous benthic species with potential site fidelity (Last et al. 2016b). Several coastal stingray species exhibit seasonal site fidelity and spatially restricted movements, contributing to localized population structuring. For instance, *Bathytoshia brevicaudata* (Hutton, 1875) demonstrates strong seasonal residency in a marine protected area in New Zealand, supporting the role of behavioral traits in shaping stingray population dynamics (Le Port et al. 2012). These behavioral characteristics likely contribute to the genetic differentiation

Table 4. Pairwise K2P genetic distances between *Brevitrygon heterura* and *B.* cf. *imbricata.*

Groups	*B. heterura* Gulf of Thailand	*B.* cf. *imbricata* Gulf of Thailand	*B. heterura* Andaman	*B.* cf. *imbricata* Andaman
heterura Gulf of Thailand				
B. cf. *imbricata* Gulf of Thailand	0.0%–1.40%			
B. heterura Andaman	1.82%–2.46%	1.40%–2.46%		
B. cf. *imbricata* Andaman	1.82%–2.88%	1.40%–2.88%	0.0%–1.19%	
B. heterura (NCBI)	0.0%–2.24%	0.0%–2.24%	0.0%–2.24%	0.0%–2.66%
B. imbricata (NCBI)	9.55%–10.38%	9.52%–10.66%	9.28%–10.34%	9.28%–10.34%

observed between *B. heterura* populations from the Gulf of Thailand and the Andaman Sea.

All specimens examined are likely members of a single species, *B. heterura* despite the presence of two morphotypes, consistent with the distribution range proposed by Last et al. (2023). This supports the view that previously identified *B.* cf. *imbricata* specimens fall within the morphological variation of *B. heterura* rather than representing a separate species. Recognizing geographically structured populations is essential for effective conservation and fisheries management, particularly in regions where environmental heterogeneity and limited gene flow influence biodiversity (Cabral et al. 2003; Mahe et al. 2014; Rocamontes-Morales et al. 2021). The present findings, when considered alongside recent taxonomic revisions, suggest that current species boundaries within *Brevitrygon* may be more complex than previously recognized (Last et al. 2023; Ehemann et al. 2024). This underscores the need for integrative approaches incorporating wider geographic coverage and additional genetic markers to robustly resolve species boundaries and evolutionary relationships within *B. heterura* and closely related taxa. Given the conservation status of *B. heterura* as globally vulnerable based on IUCN Red List, comprehensive studies on population structure and genetic diversity are crucial for maintaining populations of this species.

This study sheds light on morphological and genetic variation in *B. heterura* from Thai waters, yet several limitations remain. The sample size and geographic scope, while representative of two key regions, do not encompass the species' full distribution. Broader sampling across Southeast Asia and the eastern Indian Ocean is needed to assess whether the observed genetic structuring holds across its range. Additionally, reliance on a single mitochondrial marker (*COI*) may underestimate genetic complexity, as it reflects only maternal inheritance (Paz-Vinas et al. 2021; Clark and Pinsky 2024). Future studies should incorporate nuclear markers such as microsatellites or SNPs to better resolve population structure and gene flow.

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Supplementary material 1

Morphometric characters of *Brevitrygon* spp.

Authors: Phiphat Wongmanee, Jenjit Khudamrongsawat, Tassapon Krajangdara, Wansuk Senanan, Thadsin Panithanarak, Ratima Karuwancharoen, Wanlada Klangnurak

Data type: docx

Explanation note: (41 variables).

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Link: https://doi.org/10.3897/aiep.56.177135.suppl1

Supplementary material 2

Raw morphometric measurements of specimens

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Data type: docx

Explanation note: Variables used in PCA and MANOVA are indicated by an asterisk (*).

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Link: https://doi.org/10.3897/aiep.56.177135.suppl2

Supplementary material 3

Accession numbers of *Brevitrygon* and outgroup taxa from NCBI

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Data type: docx

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