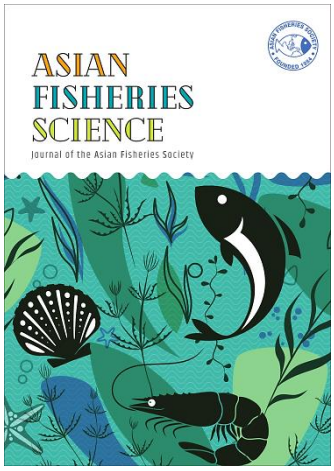

Identification of *Phalacronotus bleekeri* (Günther, 1864) (Siluridae) From the Mekong Delta, Vietnam and Use of Morphological Analysis to Separate Populations

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Abstract

This study identified *Phalacronotus* to species level and examined its morphometric variation among three collection localities, and between sexes, in the Mekong Delta, Vietnam. Specimens were collected from local markets and fishers, including An Giang (n = 36) and Can Tho (n = 36) in the Hau River and Tien Giang (n = 30) in the Tien River for morphological measurements and DNA barcoding analysis. Eight mitochondrial cytochrome oxidase subunit I (*COI*) sequences of *Phalacronotus* sp. shared 99.6 ± 0.23 % similarity with five GenBank (GB) *P. bleekeri* sequences and differed by 7.6 % to 13.3 % from seven GB *P. apogon* sequences. The Vietnamese specimens of *Phalacronotus* were similar to *P. bleekeri* in number of anal fin rays (75–80) and the shape of the upper jaw teeth. Thus, the *COI* sequences and these morphological characteristics indicate that *Phalacronotus* sp. samples collected in the Mekong Delta are *Phalacronotus bleekeri* (Günther, 1864). Morphometric characteristics differed between populations in 15 of 19 indices ($P < 0.05$) and between females and males in three characteristics relating to the belly. Head characteristics, body depth, and caudal peduncle depth were the main parameters contributing to the inter-population variation. Based on size-adjusted data, 96.7 % of specimens were correctly classified from the Tien Giang population, while 27.8 % and 33.3 % were misclassified between the Can Tho and An Giang populations, respectively. These results indicate that morphometric parameters of *P. bleekeri* varied mainly between populations in the two Mekong River tributaries.

Keywords: catfish, DNA barcode, morphology, *Phalacronotus*, species identification

Introduction

The catfish family Siluridae is widely distributed throughout the Eurasian continent, mainly in freshwater environments (Haig, 1950; Bornbusch, 1995; Ng and Ng, 1998; Unlu et al., 2012; Roberts, 2014). This family currently contains 13 valid genera and 104 species (Fricke et al., 2021). The silurid genus *Phalacronotus* is native to South and Southeast Asia (Ng, 2003; Elvyra et al., 2020) with four valid species: *Phalacronotus apogon* (Bleeker, 1851), *Phalacronotus bleekeri* (Günther, 1864), *Phalacronotus micronemus* (Bleeker, 1846), and *Phalacronotus parvanalis* (Inger and Chin, 1959) (Ferraris, 2007; Kottelat, 2013). Members of the genus are characterised by the absence of a dorsal fin, a short maxillary barbel, and the presence of vomerine teeth (Bleeker, 1858; Haig, 1950). According to Rainboth (1996), some key characteristics to distinguish these species are the presence of a dark spot at the caudal fin base of *P. micronemus*, the absence of a pelvic fin in *P. parvanalis*, vomerine teeth in a smoothly curved band in *P. bleekeri*, and vomerine teeth in an angular band in *P. apogon*. These latter two species have similar body colouration, making them especially difficult to differentiate. *Phalacronotus parvanalis* is distributed within northeastern Borneo, and *P. micronemus* within Thailand and Indonesia, while *P. bleekeri* and *P. apogon* have overlapping distributions in the Mekong River (Ferraris, 2007; Kottelat, 2013).

In the Mekong basin (in Lao, Thailand, Cambodia, and Vietnam) and the Malay Peninsula, the occurrence of three species of *Phalacronotus* has been reported, but their scientific names have not been determined due to a lack of taxonomic information (Nagao Natural

Environment Foundation, 2021). In the Mekong Delta, Tran et al. (2013) found only one species of the genus *Phalacronotus* but did not provide a specific identification. It is unclear whether one of two species of *Phalacronotus* occurs in the Mekong Delta: *P. bleekeri* and/or *P. apogon*. To answer this question, an effective approach is to utilise a combination of morphology and DNA barcoding tools.

The morphological tool used for fish species identification is based on the external morphological description (Omer, 2017) and/or the morphometric analyses of body shape and size correlations (Ko et al., 2013). Morphometrics can vary between populations and sexes (Paknejad et al., 2014) and thus fish population identification can be based on morphological variation (Istead et al., 2015; Lazzarotto et al., 2017). However, variation in morphological characteristics due to the environment (Jonsson and Jonsson, 2019; Hamel et al., 2020) can make species identification challenging. Morphological information alone would be inadequate to distinguish the samples of *Phalacronotus* collected during this study from other congeneric species. Hence, molecular tools, particularly DNA barcoding, can provide additional support for species identification. Conventional DNA barcoding based on cytochrome oxidase subunit 1 (*COI*) has been used to monitor the biological diversity of fish (Ivanova et al., 2007; Ferri et al., 2009; Turanov et al., 2019), identify new or cryptic species (Hebert et al., 2004; Sriwattanarothai et al., 2010), and to study species evolution (Xu et al., 2019). However, when the *COI* sequences are aligned in GenBank (GB) or the Barcode of Life Data (BOLD) system, the contribution of DNA barcoding to species identification can be limited, as there are a great many voucher specimens for species assigned to *Phalacronotus* that resemble the specimens; hence correct identification of the species can be difficult (Ward et al., 2009; Keat-Chuan Ng et al., 2017). Therefore, a combination of DNA barcoding and morphological methods can be used to overcome the limitations of each method to resolve species identity and discover species plasticity.

Minimal information about intraspecific morphological variation in body shape and size has been reported for any species of *Phalacronotus* (Bornbusch, 1995). Specimens of *Phalacronotus* collected from two-branch rivers of the Mekong Delta can show high morphological variation due to environmental conditions, fishing pressure, and genetic components (Schlichting and Wund, 2014; Tams et al., 2018; Hamel et al., 2020). This study aimed to (i) provide correct species identification for samples of *Phalacronotus* collected from three localities in the Mekong River Delta by combining morphological and DNA barcoding methods, and ii) examine these samples for morphological variation between sexes and collection locality. Data from this study can improve understanding of the diversity and intraspecific phenotypic plasticity of *Phalacronotus* in the Mekong Delta region, which could help to improve fish stock identification (Azad et al., 2020).

Materials and Methods

Sampling site and sample collection

Samples of *Phalacronotus* were collected from August 2020 to February 2021 from fishers and local sellers at three different locations in the Mekong River Delta, Vietnam, at An Giang (AG) and Can Tho (CT) in the Hau River, and at Tien Giang (TG) in the Tien River, two branches of the Mekong River (Fig. 1). A total of 102 (36 AG, 36 CT, and 30 TG) specimens were collected for morphological measurements, while eight specimens (TG = 3, CT = 2, and AG = 3) were used for DNA barcoding.

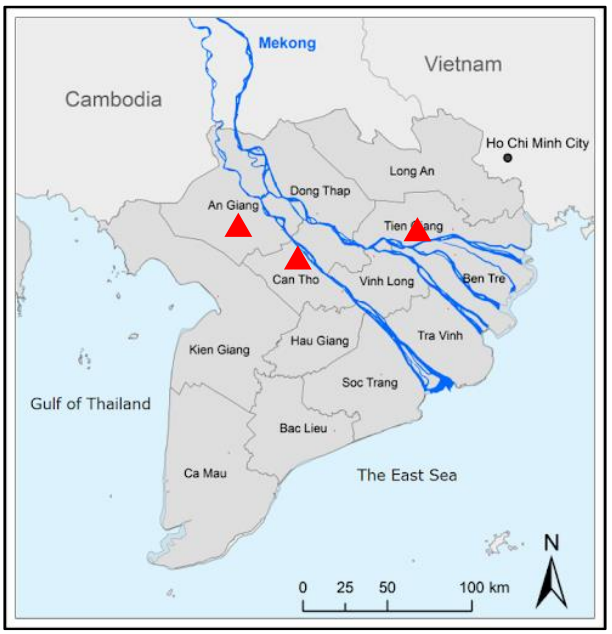

Fig. 1. Map illustrating the three sites where *Phalacronotus* was collected in the Hau (An Giang and Can Tho) and Tien (Tien Giang) rivers.

Morphological measurements

The specimens were examined for the shape of their teeth, and meristic characteristics were counted, including the number of anal fin rays (AFR), ventral fin rays (VFR), and pectoral fin rays (PFR). The sex was then determined by external observation of adults and by dissecting the gonads of immature specimens. In mature specimens, females typically had larger bellies as compared to males.

For morphometric parameters, specimens were weighed with an electronic scale (to 0.01 g) and body length, fin lengths, distance from mouth to fin (or distance before fins), and head morphology were measured using ImageJ (https://imagej.nih.gov/ij/). Individual fish were then photographed beside a ruler, which was used for calibration during image analysis.

Each image was scaled by setting one centimetre in the ruler to equal a selected range of pixels within the image. Morphometric parameters were measured by drawing straight lines (Fig. 2) and exporting their values to a Microsoft Excel and SPSS files for further analyses.

DNA barcoding analysis

DNA was extracted from fish tissues or fin clips using the ammonium acetate protocol (Saporito-Irwin et al., 1997). The quality of DNA extractions was checked through 0.8 % agarose electrophoresis. The *COI* gene of eight samples was amplified using a universal primer pair of FishF2_t1 and FishR2_t1 (Ward et al., 2005; Ivanova et al., 2007). The sequences of the forward and reverse primers are as follows:

FishF2_t1: 5′-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3′

FishR2_t1: 5′-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3′

The PCR reactions had a total of 25 µL, including 12.5 µL master mix (1X), 1 µL MgCl2 (25 mM), 0.3125 µL each primer (10 µM), 2.5 µL DNA (25 ng.µL^{-1}), and 8.375 µL nuclease-free water (Promega Corporation, USA). The temperature cycles of reactions (following the previous study by Ward et al., 2005) were 95 °C initial denaturation for 2 min, 35 cycles of amplification of 94 °C denaturation for 30 sec, 52 °C annealing for 40 sec, and 72 °C extension for 90 sec, and one final extension cycle of 72 °C for 10 min.

The purified PCR products were sent to a sequencing service (Nam Khoa company, Ho Chi Minh City, Vietnam), where sequencing was performed using the Sanger method.

Morphometric data analyses

Before statistical analysis, the raw data were processed in two ways. First, length measurements were calculated for ratios of standard length or head length. The parameters reflecting the body form and fin length were converted into percentages of the standard length. Measurements of the head were calculated as ratios of the head length. Second, as body sizes of fish differed between collection localities, the original data were size-adjusted based on the approach of Elliott et al. (1995) using the following formula:

$$M_{adj} = M\,(Ls / Lo)^{b}$$

where M: the original size, M_{adj}: size-adjusted measurement, Lo: standard length of each fish, Ls: average value of the standard length of all samples of three collections. Parameter b was estimated for each character as the slope of the regression of log M to log Lo, using the logarithm of all samples.

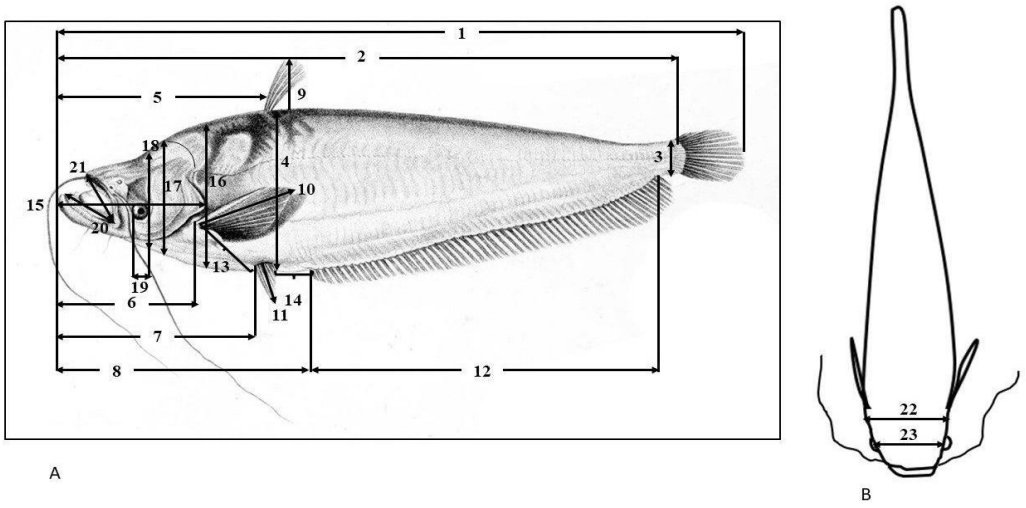

Fig. 2. Morphometric measurements, (A) taken on the body of *Ompok pabda* (Hamilton, 1822) by Chaklader et al. (2016) and (B) head width and interorbital distance applied in the current study for *Phalacronotus*.

Note: 1 = TL: total length, 2 = SL: standard length, 3 = BD: body depth, 4 = CPD: caudal peduncle depth, 5 = PDD: pre-dorsal fin distance, 6 = PPD: pre-pectoral fin distance,7 = PVD: pre-ventral fin distance, 8 = PAD: pre-anal fin distance, 9 = DFL: dorsal fin length,10 = PFL: pectoral fin length, 11 = VFL: ventral fin length, 12 = ABL: anal base length, 13 = DPV: distance pectoral to ventral, 14 = DVA: distance ventral to anal, 15 = HL: head length, 16 = HD1: head (body) depth measured at the end of operculum, 17 = HD2: head (body) depth measured at end of the supraoccipital crest, 18 = HD3: head (body) depth measured at the beginning of postorbital, 19 = ED: eye diameter, 20 = UJL: upper jaw length, 21 = LJL: lower jaw length, 22 = HW: head width, 23 = IOD: interorbital distance.

Two data sets (ratios and adjusted measurements) were analysed using two-way ANOVA to test the effects of collection locality, sex, and interaction. Differences among fish the three localities were examined by Duncan's multiple range test. Principal component analysis (PCA) was used to identify the key characteristics that lead to overall differences and variance estimation among the collections. Discriminant analysis (DA) was used to visualise the population differentiation and measured the validated correction of individual assignments to their original population. These statistical analyses were performed using SPSS 22.0.

DNA sequence analysis

The quality of the *COI* sequences was first checked using the FinchTV program (http://www.geospiza.com). All sequences were then aligned and trimmed manually in MEGA 7.0 software (Kumar et al., 2016). For species identification, the investigated sequences were compared with the *COI* databases in the BOLD system (www.Boldsystems.org), using the species identification tool (Ratnasingham and Hebert, 2007), and in GB, using BLAST. Genetic distances between highly matched species and the investigated sequences were estimated using the Kimura two-parameter (K2P) model (Kimura, 1980) with 1000 bootstrap replications. The neighbour-joining phylogenetic tree in MEGA 7.0 was generated with 1000 bootstrap replications to show the species relationship between the sequences in this study and published sequences.

Results

Species identification

Morphological identification

Samples of *Phalacronotus* were examined for key morphological characteristics, including meristic traits and the shape of the teeth. Meristic traits were similar among fish from the three collection localities (Table 1). All samples had the same number of ventral fin rays (9) and a similar number of pectoral fin rays (14–15). The anal fin rays in the three collections had different mode values but overlapping ranges.

The external appearance (Fig. 3A) and the tooth shape (Fig. 3B) of sample TG02 are shown as an example for the samples of *Phalacronotus*. Its upper jaw teeth are arranged in a thin, vomerine curved band that runs parallel to the connected premaxillary teeth. This tooth shape differs from that of *P. apogon*, as reported by Bleeker (1858) (Fig. 3C).

Results of DNA barcoding

COI sequences (664 base pairs) of *Phalacronotus* were submitted to GB with the accession numbers from OM345063 to OM345070 (n = 8). The eight sequences had 5 variable sites, generating 3 haplotypes with an average K2P genetic distance of 0.2 ± 0.1 %. When these sequences were compared to the *COI* database in BOLD, it was revealed that all tested specimens (100 %) belonged two possible species in the genus

Table 1. Meristic indices of countable characters of *Phalacronotus* in three populations.

Collection locality	n	Factors	AFR	PFR	VFR
Tien Giang (TG)	10	min-max	75-79	14-15	9
		mode	79	15	9
An Giang (AG)	10	min-max	76-80	14-15	9
		mode	78	15	9
Can Tho (CT)	10	min-max	76-80	14-15	9
		mode	77	15	9

n: sample size, AFR: anal fin ray; PER: pectoral fin ray; VFR: ventral fin ray.

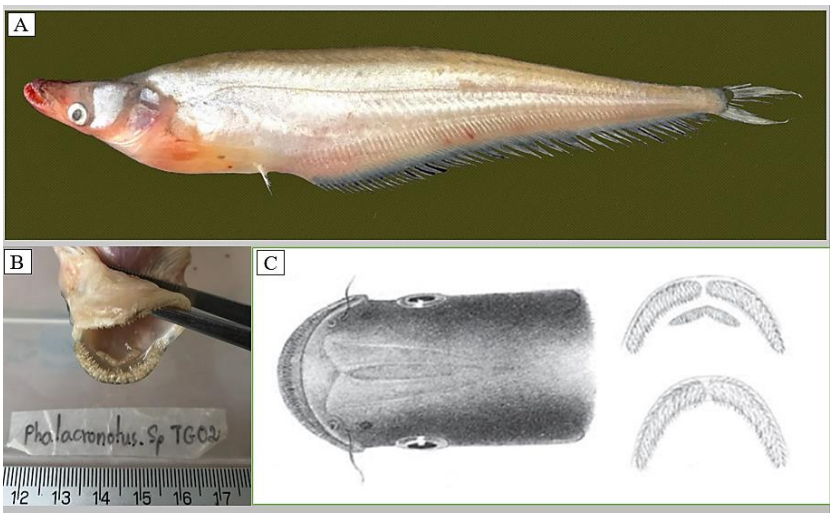

Fig. 3. The different shapes of premaxillary teeth used to distinguish between two species of *Phalacronotus*.

Note: specimen (A) and (B): *Phalacronotus TG02*, female (standard length = 19.61 cm; weight = 42.72 g), (C): the teeth of *P. apogon*.

Phalacronotus, *P. apogon* and/or *P. bleekeri*. The 15 *COI* sequences that were available for these two species in both BOLD and GB (1st September 2021) shared different degrees of similarity with the samples of *Phalacronotus* analysed in this study (Table 2), from 86.5 % to 99.9 % similarity for *P. apogon* (n = 10) and 99.6 % identity for *P. bleekeri* (n = 5). The phylogenetic tree showed that the *Phalacronotus* sp. sequences in this study clustered with the five GB sequences of *P. bleekeri* (KF805376 to KF805380) and three sequences of *P. apogon* (MK448159, MK448160, and EF609377), which was divergent from the other cluster of seven sequences of *P. apogon* (MH732892 to MH732897, and MK448087) (Fig. 4).

Morphometric variations among samples of Phalacronotus *from different collection localities and between sexes*

Two-way ANOVA analysis showed no interaction effects between sex and collection locality on morphometric indices of the *Phalacronotus* specimens ($P > 0.05$). The results showed that 15 of 19 morphometric indices were significantly different between the three localities ($P < 0.05$; Table 3), whereas BD, PPD, DPV, and VFL showed no differences between localities ($P > 0.05$). The adjusted measurements yielded results that were similar to those of the morphometric indices (data not shown).

Comparing the two sexes showed that females (n = 47) had a larger body size than males (n = 55), and three of the 19 adjusted measurements were significantly larger in females ($P < 0.05$): body depth (BD), distance from ventral to anal fins (DVA), and head depth measured at the end of the operculum (HD1).

The canonical function 1 (CF1) and canonical function 2 (CF2) were plotted to allow visual examination of the distribution of all samples based on morphometric indices (Fig. 5A) and adjusted data (Fig. 5B). In the discriminant plot of morphometric indices, all collections were separated from each other. However, the plot based on adjusted data showed that the two collections in the Hau River (CT and AG) became compounded, while the collection from the Tien River (TG) was more divergent.

The first two principal components (PC1 and PC2) explained 27.3 % and 17.0 % of the variation in morphometric indices. When the effect of body size was considered, the variation explained by PC1 decreased to 19.6 %, with important parameters including head depth measured at the end of the operculum, body depth, caudal peduncle depth, and head length. PC2 increased to 17.4 % of variation explained with important characteristics being the distance of pectoral to ventral fins, pre-anal fin distance, anal base length, and head depth measured at the end of the supraoccipital crest (Fig. 2). Although morphometric indices and adjusted data provided different outputs, both data sets revealed a similar separation between populations.

Table 2. Mean (below diagonal) and standard error (above diagonal) of *COI* sequence identity (%) between samples of *Phalacronotus* and GenBank sequences of *P. apogon* and *P. bleekeri*. GenBank accession numbers in parentheses.

No.	Specimens	1 (n = 8)	2 (n = 3)	3 (n = 6)	4 (n = 1)	5 (n = 5)
1	*Phalacronotus* sp. (this study)	-	0.05	1.07	1.54	0.23
2	*Phalacronotus apogon* (MK448159, MK448160, and EF609377)	99. 9	-	1.08	1.55	0.26
3	*Phalacronotus apogon* (MH732892 to MH732897)	92.3	92.3	-	1.40	1.08
4	*Phalacronotus apogon* (MK448087)	86.5	86.5	87.1	-	1.57
5	*Phalacronotus bleekeri* (KF805376 to KF805380)	99.6	99.5	92.2	86.2	-

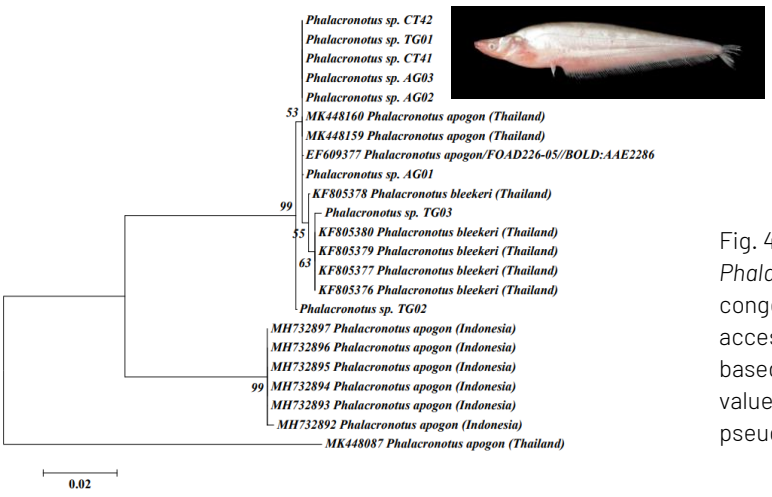

Fig. 4. K2P distance neighbour-joining tree of *Phalacronotus* sp. in the Mekong Delta and the congeneric species published in GenBank (the accession number before the species name), based on mtDNA *COI* gene sequences. Bootstrap values greater than 50 % were shown after 1,000 pseudoreplications.

Table 3. Morphometric indices (% ± SD) of body parameters compared to standard length and head length of *Phalacronotus* collected at three different localities in the Mekong River Delta.

Parameters				*P*-values for two-way ANOVA		
	AG (36)	CT (36)	TG (30)	Population (P)	Sex (S)	P*S
Wt	19.7 ± 5.8^{a}	134.2 ± 94.6^{c}	79.4 ± 38.6^{b}	<0.01	<0.01	<0.01
TL	16.5 ± 1.5^{a}	29.2 ± 6.6^{c}	25.6 ± 3.4^{b}	<0.01	<0.01	<0.01
Ratio to standard length						
BD	18.3 ± 1.5^{a}	18.3 ± 1.4^{a}	18.5 ± 1.3^{a}	0.865	0.025	0.827
CPD	3.63 ± 0.22^{a}	3.93 ± 0.27^{b}	3.88 ± 0.25^{b}	<0.01	0.670	0.216
PPD	21.2 ± 1.2^{a}	21.6 ± 1.4^{a}	22.0 ± 1.7^{a}	0.119	0.791	0.500
PVD	31.1 ± 1.2^{a}	32.0 ± 1.8^{b}	32.2 ± 1.4^{b}	<0.01	0.207	0.394
PAD	36.5 ± 1.1^{a}	37.5 ± 1.8^{b}	37.4 ± 1.5^{b}	0.016	0.016	0.471
PFL	14.2 ± 1.2^{a}	14.5 ± 0.9^{a}	13.8 ± 1.3^{a}	0.042	0.311	0.681
VFL	4.98 ± 0.70^{a}	5.00 ± 0.64^{a}	5.36 ± 0.53^{b}	0.234	0.035	0.126
ABL	62.3 ± 1.3^{b}	60.6 ± 1.7^{a}	61.6 ± 1.5^{b}	<0.01	0.097	0.342
DPV	12.5 ± 1.3^{ab}	12.8 ± 1.2^{b}	12.1 ± 1.2^{a}	0.086	0.052	0.562
DVA	3.67 ± 0.70^{a}	3.45 ± 0.55^{a}	3.64 ± 0.49^{a}	0.033	0.318	0.325
HL	23.0 ± 0.8^{a}	23.3 ± 1.5^{a}	25.7 ± 1.0^{b}	<0.01	0.136	0.454
Ratio to head length						
HD1	75.3 ± 6.3^{b}	73.8 ± 5.1^{b}	69.3 ± 3.8^{a}	<0.01	0.050	0.604
HD2	53.8 ± 6.4^{c}	47.7 ± 6.2^{b}	41.0 ± 1.9^{a}	<0.01	0.815	0.289
HD3	39.5 ± 2.9^{c}	37.9 ± 2.5^{b}	34.8 ± 1.3^{a}	<0.01	0.756	0.493
ED	12.6 ± 1.0^{b}	11.8 ± 1.0^{a}	11.7 ± 1.9^{a}	<0.01	0.110	0.548
UJL	26.9 ± 2.5^{b}	18.4 ± 4.4^{a}	19.9 ± 1.7^{a}	<0.01	0.043	0.080
LJL	31.9 ± 3.4^{b}	22.1 ± 4.8^{a}	22.7 ± 1.9^{a}	<0.01	0.011	0.374
HW	52.5 ± 4.0^{b}	53.1 ± 3.9^{b}	48.3 ± 3.0^{a}	<0.01	0.370	0.763
IOD	44.7 ± 3.8^{b}	46.2 ± 3.8^{b}	40.9 ± 2.3^{a}	<0.01	0.372	0.853

Note: An Giang (AG); Can Tho (CT); Tien Giang (TG); total weight (Wt); total length (TL); body depth (BD); caudal peduncle depth (CPD); pre-pectoral distance (PPD); pre-ventral distance (PVD); pre-anal distance (PAD); pectoral fin length (PFL); ventral fin length (VFL); anal base length (ABL); distance pectoral to ventral (DPV); distance ventral to anal (DVA); head length (HL); head depth measured at the end of operculum (HD1); head depth measured at end of the supraoccipital crest (HD2); head depth measured at the beginning of postorbital (HD3); eye diameter (ED); lower jaw length (LJL); upper jaw length (UJL); head width (HW); interorbital distance (IOD). Values in the same row with the same superscript are not significantly different ($P > 0.05$).

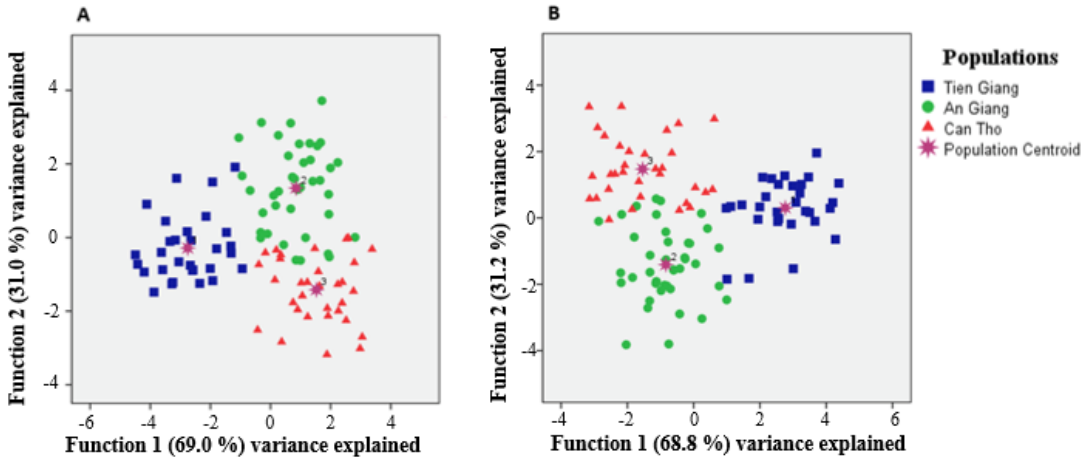

Fig. 5. Canonical discriminant analysis based on morphometric indices (A) and adjusted measurements (B) of *Phalacronotus* collected at three localities in the Mekong River Delta.

The overall random assignment of individuals to their correct collection locality was high (94.1 % for indices and 76.5 % for adjusted data) (Table 4). The proportion of correct assignments based on ratio data was highest for fish collected at TG (100 %) and AG (100 %). Based on the adjusted data, the percentage of correct individual assignments for the TG fish remained the highest (96.7 %), while correct assignments for fish from AG and CT decreased to 69.4 % and 66.7 %, respectively.

Discussion

Species identification

DNA barcoding with the support of morphological analyses can solve ambiguity in species identification (Pereira et al., 2008; Imtiaz et al., 2017). The species of *Phalacronotus* occurring in the Mekong Delta has not been previously identified, due to insufficient information and inconsistent morphological descriptions in the literature (Tran et al., 2013; So et al., 2019; Praxaysombath et al., 2020). Although the key characteristics of species belonging to the genus *Phalacronotus* were reported by Rainboth (1996), morphological similarities and disconcordance in species' COI databases make species identification challenging. In this study, DNA barcoding and morphological analyses were combined to identify samples of *Phalacronotus* collected from the Mekong Delta.

Species identification using BOLD confirmed with 100 % probability that all sampled fish belonged to the genus *Phalacronotus*, and that the species involved was either *P. apogon* and/or *P. bleekeri*. Published *COI* sequences, genetic distance (Table 2) and phylogenetic relationships (Fig. 4), indicate that the two species are highly divergent. The *P. bleekeri* group (KF805376 to KF805380, n = 5) varied from the *P. apogon* group, which included six sequences from Indonesia (MH732892 to MH732897) and one, MK448087, from Thailand, with 7.6 % and 13.3 % sequence difference, respectively. These levels were within the range of interspecies genetic distances reported in previous studies (Ward et al., 2005; Abdullah et al., 2017; Zainal-Abidin et al., 2021). The other three *P. apogon* sequences (MK448159 and MK448160 uploaded in 2019 from Thailand, and EF609377 uploaded in 2007 from Asia without country information (Ward and Holmes, 2007)) seen in the *P. bleekeri* cluster could be misidentified. *Phalacronotus* specimens in this study had high sequence similarities (99.6 ± 0.23 %) with all five *P. bleekeri* sequences, indicating that these samples were more likely to be from this species.

Morphological evidence, including the upper jaw teeth and the number of anal fin rays support this interpretation. The shape of the upper jaw teeth is a key characteristic in differentiating between *P. apogon* and *P. bleekeri* (Rainboth, 1996). *Phalacronotus apogon* has the vomero-palatine teeth in a thinner, vomerine angular band parallel to the unconnected band of the premaxillary teeth (Fig. 3C), whereas *P. bleekeri* has the upper jaw teeth placed in a thin, vomerine crescent-shaped (curved) band, parallel to connected premaxillary teeth (Rainboth, 1996). The *Phalacronotus* samples collected from the Mekong Delta had upper jaw teeth identical to those described for *P. bleekeri* (Fig. 3B). Additionally, the number of anal fin rays was reported to differ between the two species, ranging from 78 to 91 for *P. apogon* and from 77 to 85 for *P. bleekeri* (Rainboth, 1996). The present samples had 75 to 80 anal fin rays, corresponding more closely to the range for *P. bleekeri*.

In summary, combining evidence from DNA barcoding and morphometric differences in the upper jaw teeth form and the range of anal fin rays indicate that the samples of *Phalacronotus* sp. examined in the present study from the Mekong Delta should be classified as *P. bleekeri*. This species name is thus used for the following discussion.

Morphometric variation of P. bleekeri *in the Mekong Delta*

Morphometric variation within the species *P. bleekeri* has not been previously reported. The present study shows that its morphometric parameters varies by population (15/19 indices) rather than by sex (3/19), with no interaction effects. Females were larger than males in three parameters (based on adjusted data), including distance from ventral to anal fins, head depth, and body depth, which are related to the larger size of the female's belly. Similarly, Jacquemin and Pyron (2016) found that females of cyprinid fish have

Table 4. The percentage of individual assignments of samples of *Phalacronotus* into three collection localities based on morphometric indices and adjusted measurements.

Collection locality	Morphometric indices			Adjusted measurement			Total (n)
	TG	AG	CT	TG	AG	CT	
TG	100	0	0	96.7	3.3	0	100 (30)
AG	0	100	0	2.8	69.4	27.8	100 (36)
CT	5.6	11.1	83.3	0	33.3	66.7	100 (36)

Note: 94.1 % (Ratio) and 76.5 % (Adjusted) of original grouped cases correctly classified. An Giang (AG); Can Tho (CT); Tien Giang (TG).

deeper bodies as compared with males. Among the three locations, the AG and CT populations in the Hau River were more similar to the TG population when the size effects were removed. In general, *P. bleekeri* is sensitive to environmental changes during water discharge (Baran, 2006; Cunico and Agostinho, 2006), and the fish can migrate for feeding and spawning (Schmutz and Mielach, 2015). As the Hau and Tien rivers differ in flow patterns, river depth, and temperature (Nguyen and Tanaka, 2007; Tran et al., 2014), these differences in the environment are the likely cause of morphometric variations in populations of *P. bleekeri* distributed in the two rivers.

Consequently, discriminant analysis based on adjusted data (removed size-effects) revealed that individuals from the TG population were highly classified (96.7 %), whereas 27.8 % to 33.3 % of individuals from the CT and AG populations were incorrectly assigned. Similarly, Hakim et al., (2020) found two distinct populations of Indian mackerel (*Rastrelliger kanagurta* (Cuvier, 1816)) among three different populations (Lancang Island, Cirebon, and Madura Island). Sen et al. (2011) also found a high rate of misidentification (28 %) in four populations of Indian scad (*Decapterus russelli* (Rüppell, 1830)) from the east and west coasts of India.

In this study, the principal component analysis revealed that head measurements, body depth, and caudal peduncle depth were important parameters for distinguishing between populations of *P. bleekeri*. Similarly, Duong et al. (2019) stated that the head size and caudal peduncle depth were the main characteristics to differentiate between wild and cultured populations of snakehead (*Channa striata* (Bloch, 1793)).

Conclusion

Using morphological and DNA barcoding data, this study has shown that the species of *Phalacronotus* collected from the Mekong Delta is *P. bleekeri*, and that morphometric parameters vary between populations in the two branches of the Mekong River. The important characteristics that can be used to differentiate populations of *P. bleekeri* include head measurements, body depth, and caudal peduncle depth. The species exhibits little difference between males and females. The findings of this study are useful for species identification within the genus *Phalacronotus* and the identification of populations of *P. bleekeri* in the Mekong River branches.

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