

The First Record of Adult Spotted Sea Bass (*Lateolabrax maculatus*, McClelland, 1844) from the Coastal Waters of Quang Ninh Province, Vietnam

Nguyen Van Giang¹, Phan Thanh Nghi² & Nguyen Bich Hanh³

¹Research Institute for Marine Fisheries, Haiphong 182180, Vietnam

²Bai Tu Long National Park, Quang Ninh 205350, Vietnam

³Quang Ninh Department of Science and Technology, Quang Ninh 200000, Vietnam

Abstract

Spotted sea bass (*Lateolabrax maculatus*, McClelland, 1844) is a species belonging to the genus *Lateolabrax* (Lateolabracidae), and is characterized by the many distinct black spots on the bodies of mature fish. For the first time, six specimens have been recorded in Quang Ninh province. The specimens were collected from Luon Cave in Bai Tu Long National Park in May-June 2019. The new specimens collected from Quang Ninh, Vietnam are different from the recorded specimens from Japan, Korea, Taiwan, and China in terms of lateral line scales (LLS) as the samples collected in Quang Ninh, Vietnam have 94-97 scales and the samples collected from the other countries have 66-82 scales. Phylogenetic analysis of the 16S rDNA sequence confirmed that all of the samples collected in Quang Ninh, Vietnam belonged to the species of spotted sea bass.

Keywords

Bai Tu Long, *Lateolabracidae*, morphology, national park, phylogenetic

Introduction

The family Lateolabracidae, order Perciformes, is comprised of eight species and three genera worldwide, including the genus *Lateolabrax*, which consists of three species: sea bass (SB), *Lateolabrax japonicus* (Cuvier, 1828); spotted sea bass (SSB), *Lateolabrax maculatus* (McClelland, 1844); and blackfin sea bass, *Lateolabrax latus* (Katayama, 1957) (Bae *et al.*, 2016). The sea basses of the genus *Lateolabrax* are common East Asian coastal marine fishes (occasionally also occurring in freshwater) (Yokogawa, 2019). Species of the *Lateolabrax* complex (including *L. japonicus* and *L. maculatus*) are morphologically similar, which

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Correspondence to
nvgiang@rimf.org.vn

has led to considerable confusion regarding their taxonomic status. Generally, *L. maculatus* (widely considered to be a color variant of *L. japonicus*) is characterized by many clear black dots on the dorsolateral body, whereas on *L. japonicus*, the black dots are absent or faded in adult fish (Katayama, 1984; Kang, 2000). Thus, SSB and SB are generally distinguished by the presence or absence, respectively, of black spots. However, because some individuals of *L. japonicus* have black spots on the dorsolateral body, even in the adult stages, distinguishing between the two species solely based on the presence of black spots is difficult (Bae *et al.*, 2016).

In terms of external morphology and anatomical structure, many previous publications have pointed out the different characteristics of species in the *Lateolabrax* genus, including Chyung (1977), Yokogawa & Seki (1995), Park *et al.* (1996), Kim & Jun (1997), Yokogawa *et al.* (1997), Kang (2000), and Yokogawa (2019). In addition to morphology studies, DNA molecular genetics studies have also been conducted by Park *et al.* (1996), Han *et al.* (2015), and Bae *et al.* (2016), indicating differences among species in the genus *Lateolabrax*.

In Vietnam, *Lateolabrax* fish have been identified as *L. japonicus* by Nguyen Nhat Thi (1991), Kottelat (2001), Nguyen Nhat Thi & Nguyen Van Hao (2005), and Nguyen Huu Duc *et al.* (2014). Recently, *L. maculatus* also has been identified in the estuary area of northern Vietnam. However, in addition to this new insight into the distribution of *Lateolabrax maculatus*, the authors also said that SSB is distributed along coasts from the Yellow to East Seas (South China Seas) (Katayama, 1984; Yamada *et al.*, 2007; Yokogawa, 2013), of which the northern Vietnamese coasts facing the Gulf of Tonkin are probably the most southern distribution for the genus *Lateolabrax* (Nguyen Nhat Thi, 1991; Kottelat, 2001; Nguyen Nhat Thi & Nguyen Van Hao, 2005; Tran *et al.*, 2017; Nguyen *et al.*, 2019).

During our recent field trip in the Bai Tu Long National Park of Quang Ninh Province in northern Vietnam, we collected six specimens of spotted sea bass which agreed with the previously published descriptions of *L.*

maculatus. They could be assigned to *L. maculatus* based on morphological characters and molecular data, and we reported spotted sea bass adults from Vietnam for the first time. Hence, this study aimed to examine the morphological characteristics of *L. maculatus* in adulthood and its distribution pattern in relation to the cave environment, which are fundamental to understanding the essential habitat and conservation needs of the species.

Materials and Methods

Field surveys were conducted in May and June 2019 in Bai Tu Long National Park, Quang Ninh Province along the northern coast of Vietnam (**Figure 1**). We used handline fishing to collect the fish samples living in the cave, including fishing rods, single hooks, and live shrimp bait. Samples of muscle tissue were preserved separately in 95% ethanol, and voucher specimens were fixed in approximately 80% ethanol and later transferred to 70% ethanol for permanent storage. The specimens were deposited at the Research Institute for Marine Fisheries (RIMF), Hai Phong, Vietnam.

Morphological characters. Dimensions of the fish were taken with digital calipers to the nearest 0.1 mm, and the methods of measurements and counts followed Yokogawa (2019). The following abbreviations were used for the measurable characteristics: **AFL**, anal fin (longest ray) length; **BD**, body depth; **BWT**, body width; **CFL**, caudal fin length; **CFND**, caudal fin notch depth; **CPAD**, caudal peduncle anterior depth; **CPD**, caudal peduncle depth; **CPL**, caudal peduncle length; **FDL**, first dorsal fin (longest spine) length; **HL**, head length; **IOW**, inter-orbital width; **LJL**, lower jaw length; **OD**, orbital diameter; **PAL**, pre-anus length; **PDL**, pre-dorsal length; **POL**, post-orbital width; **POPW**, post-orbital preopercular width; **P1FL**, pectoral fin length; **P2FL**, pelvic fin length; **SDFL**, second dorsal fin (longest ray) length; **SL**, standard length; **SNL**, snout length; **SOW**, sub-orbital width; and **UJL**, upper jaw length. The following abbreviations were used for the counting characteristics: **AFR**, anal-fin ray; **AFS**, anal fin spine-ray; **DFR**, dorsal-fin

The first record of adult spotted sea bass from the coastal waters of Quang Ninh province, Vietnam

soft ray; **DFS**, dorsal-fin spine; **LGR**, lower-limb gill raker; **LLS**, pored scale on lateral line; **P1FR**, pectoral fin ray; **P2FS**, pelvic fin spine-ray; **P2FR**, penvic-fin ray; **SAL**, scale above lateral line; **SBL**, scale below lateral line; **TGR**, total gill raker; and **URG**, upper-limb gill raker.

Molecular data and phylogenetic analyses. In order to support the generic placement of the species, we obtained muscle samples from the species. Total DNA was extracted from 30mg of fish muscle tissue using tissue extraction kits from Wizard® Genomic DNA Purification (Promega, USA) following the manufacturer's instructions. The study conducted PCA to amplify the 16S rDNA gene fragment using the primer pair 16Sar 5'-CGCCTGTTTATCAAAAACAT-3' and 16Sbr

5'-CCGGTCTGAACTCAGATCACGT-3' (Palumbi, 1996). The PCR reaction was conducted with a total volume of 50 μ L, including 5 μ L DNA, 10 μ L Green Gotaq® Flexi Buffer 5X, 3 μ L MgCl₂ 25mM, 2 μ L dNTPs, 2 μ L of each primer (10mM), 0.25 μ L Taq polymerase (5 unit μ L⁻¹), and enough water to fill the volume. The reactions were run on iCycler thermal cyclers (Bio-Rad) according to the temperature cycle: initial denaturation at 94°C for 3 minutes, then 38 cycles of 94°C for 30 seconds, 48°C for 45 seconds, and 72°C for 30 seconds, and the final extension step at 72°C for 5 minutes. The PCR products were electrophoresis tested for results on 1.5% agarose gel containing ethidium bromide for DNA staining.

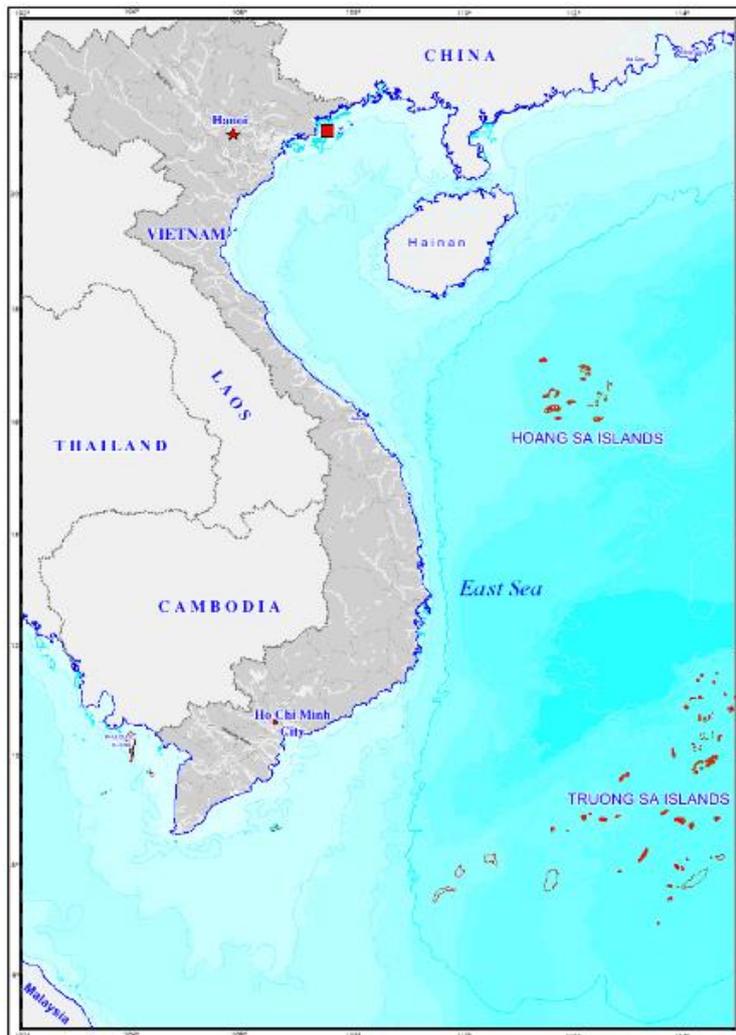


Figure 1. Map showing the survey site (red square) in Bai Tu Long National Park, Quang Ninh Province, northern coast of Vietnam

The PCR products were purified by Promega's Wizard Gel and PCR Clean-up System kits according to the manufacturer's instructions and used as direct templates for the pre-sequential reactions according to the dye-labeled dideoxy terminator principle (Big Dye Terminator v.3.1, Applied Biosystems) with similar primers to those used in the PCR reaction under the following thermal cycler program: 96°C for 20 seconds, 50°C for 20 seconds, and finally 60°C for 4 minutes. The products were then analyzed using an ABI Prism 3.700 DNA Analyzer (Applied Biosystems).

The sequences were connected using Geneious Pro 5.5.7 software (Kearse *et al.*, 2012) and verified by the Nucleotide BLAST program on Gen Bank's website (ncbi.nlm.nih.gov/Blast.cgi). The sequences were aligned with BioEdit v.7.0.1 (Hall, 1999) using the Clustax feature, in order to conduct checks and truncate a number of positions to achieve a common length for all the sequences. Analysis of the phylogenetic relationships was conducted based on the maximum likelihood algorithm using MEGA 6 software (Tamura *et al.*, 2013) with the repeatability of 1000 times. The bootstrap (BT) value was calculated to determine the accuracy of the algorithm.

Results

Description of the specimen from Vietnam

Specimens examined

Six specimens, RIMF0001 (SL 419.9mm), RIMF0002 (SL 363.3mm), RIMF0003 (SL 362.2mm), RIMF0004 (SL 358.8mm), RIMF0005 (SL 543.3mm), and RIMF0006 (SL 542.2mm), were collected by Phan Thanh Nghi, Tran Hoai Nam, and Nguyen Van Giang in May and June 2019, within Cave Luon-Ang Cai De in Bai Tu Long National Park (21°5'27" N 107°33'42" E), Van Don district, Quang Ninh province. The morphological characters of the Vietnam specimens agreed well with the previously reported description of *Lateolabrax maculatus*.

Description

The general appearance of the specimens is

shown in **Figure 2**, and the meristic and morphometric data of the six specimens are given in **Table 1**. The head was slightly longer and pointed [31.2 (30.4-32.5) % of SL], while the dorsal and ventral profiles were straight. The snout was shorter than a head length behind the eye, and longer than the eye diameter [21.7 (21.1-23) % of HL]. The eyes were near the dorsal region of the head, and shorter than the inter-orbital width [15.1 (14.2-16) % of HL]. The inter-orbital area was slightly convex. The mouth had a lower jaw longer than the upper jaw, and was without barbels.

The body height was compressed [24 (22.6-25.7) % of SL], about 1.8-2.0 times the body width, and the caudal peduncle was slender, about 2.0-2.5 times longer than deep [20.1 (18.4-22.1) % of SL]. The highest body point was at the origin of the dorsal fin, and the lowest at the caudal peduncle. The dorsal body profile was convex, while the ventral profile was straight or slightly rounded. The lateral line was complete with 94-97 scales, and there were 14-16 scales above the lateral line and 16-17 scales below the lateral line.

There were two dorsal fins, positioned high, interconnected with 12-13 spines and with 12-14 soft rays [13.8 (12.6-14.4) % of SL]. The first spine was the lowest and highest was the 5th ray. The dorsal-fin origin was inserted slightly in front of vertical with the pelvic-fin origin. The distal edge of first dorsal fin was concave and the second dorsal fin was convex. The pectoral fin was rounded with one simple and 15 branched rays. The pelvic fin was rounded, with one spine ray and 5 branched rays, and the fin origin scale was present. The anus was immediately in front of the anal fin. The anal fin had three spine rays and was short [14.2 (12.1-15.6) % of SL]. The caudal fin was deeply forked with 9+8 being branched.

Color in life

The head was colored dark turquoise on the back, dark gray around the orbital and on the side, dark gray on the upper jaw, lower jaw, and maxilla, and silver on the posterior maxilla and preopercle. The body was dark brown on the back with many clear black spots on and above

The first record of adult spotted sea bass from the coastal waters of Quang Ninh province, Vietnam

Table 1. Morphometric and meristic characters of *L. maculatus*, mean, and range

Character	Abbreviation	N	Mean \pm SD	Range
Standard length (mm)	SL	6	431.6 \pm 89	358.8 - 543.3
Morphometrics (% SL)				
Pre-anus length	PAL	6	65 \pm 1.7	63.5 - 67.2
Body depth	BD	6	24 \pm 1.1	22.6 - 25.7
Body width	BWT	6	12.8 \pm 0.7	11.9 - 13.7
Caudal peduncle depth	CPD	6	9.2 \pm 0.6	8.4 - 9.9
Caudal peduncle anterior depth	CPAD	6	10 \pm 4.9	11.3 - 13
Caudal peduncle length	CPL	6	20.1 \pm 1.2	18.4 - 22.1
Pre-dorsal length	PDL	6	33.3 \pm 0.9	32.3 - 34.4
First dorsal fin (longest spine) length	DFDL	6	13.8 \pm 0.7	12.6 - 14.4
Second dorsal fin (longest ray) length	SDFL	6	12.5 \pm 1.1	11.2 - 14.1
Caudal fin length	CFL	6	19.9 \pm 1.7	18.2 - 22.6
Caudal fin notch depth	CFND	6	6.4 \pm 1	4.9 - 7.4
Anal fin (longest ray) length	AFL	6	14.2 \pm 1.4	12.1 - 15.6
Pectoral fin length	P1FL	6	16.6 \pm 1.5	15.1 - 18.7
Pelvic fin length	P2FL	6	17.1 \pm 1.3	15.1 - 19.3
Head length	HL	6	31.2 \pm 0.8	30.4 - 32.5
Snout length	SNL	6	6.8 \pm 0.2	6.6 - 7
Post-orbital preopercular width	POPW	6	7.2 \pm 0.4	6.8 - 7.7
% HL				
Snout length	SNL	6	21.7 \pm 0.7	21.1 - 23
Orbital diameter	OD	6	15.1 \pm 0.8	14.2 - 16
Inter-orbital width	IOW	6	18.7 \pm 0.9	17.4 - 20
Sub-orbital width	SOW	6	13.3 \pm 0.7	12.7 - 14.5
Post-orbital width	POL	6	63.4 \pm 1.3	61.7 - 65
Post-orbital preopercular width	POPW	6	23.2 \pm 0.9	21.4 - 24
Upper jaw length	UJL	6	44.2 \pm 1.4	42.6 - 46
Lower jaw length	LJL	6	49 \pm 1.7	46.6 - 51.1
Counts				
Dorsal fin spine and soft ray	DFS, DFR	6	XII-XIII, 12-14	XII-XIII, 12-14
Anal fin spine-ray and fin ray	AFS, AFR	6	III, 8	III, 8
Pectoral fin ray	P1FR	6	15 \pm 0	15
Pelvic fin spine-ray	P2FS, P2FR	6	I, 5	I, 5
Pored scale on lateral line	LLS	6	95.3 \pm 1.2	94 - 97
Scale above lateral line	SAL	6	15 \pm 0.9	14 - 16
Scale below lateral line	SBL	6	16.8 \pm 0.4	16 - 17
Upper-limb gill raker	UGR	6	8 \pm 0	8
Lower-limb gill raker	LGR	6	16.2 \pm 0.4	16 - 17
Total gill raker	TGR	6	24.2 \pm 0.4	24 - 25



Figure 2. Lateral view of *Lateolabrax maculatus* specimen (RIMF0002, 363.3 mm SL)

the lateral line and a few spots below. The side of the body was light turquoise and silver white on the ventral surface. The dorsal fin was dark turquoise at the origin and bold on the rays. In between the rays, there were black dots on the interradiation membranes. The pectoral and pelvic fins were yellowish green in the first rays or covered the whole fins. The anal fin was plain greyish while the caudal fin was dark gray.

Ecological notes

All six specimens of *L. maculatus* were found in the Luon Cave of Bai Tu Long National Park, Quang Ninh Province, Vietnam (**Figure 3**). Spotted sea bass live in caves, surrounded by layered limestone, at a depth range of 2.2-2.5m. The water conditions were 25.5-27.9°C, pH 6.82-8.06, DO 4.72-5.43 mg L⁻¹, and salinity 5-30.5‰.

Phylogenetic analysis

Studying the genetic relationships of the three species in the *Lateolabrax* genus was based on 16S rDNA fragments. Data used for the genetic analysis were retrieved from Gen Bank with the three accession codes KT852999.1, KR780682.1, and KR780681.1, and our two gene sequences labeled RIMF0001 and RIMF0002. The phylogenetic tree, as seen in **Figure 4**, shows a clear grouping of genes of the

same species into a branch with a high bootstrap value (99%), which is distinguished from species in different branches. This confirmed that the recently collected specimens from Bai Tu Long National Park, Quang Ninh, Vietnam belong to *Lateolabrax maculatus* and differ from the two species *L. japonicus* and *L. latus*.

Discussion

Only minor morphological differences were identified between the populations collected in Vietnam's Quang Ninh waters and those previously described and disclosed in the waters of Japan, Korea, Taiwan, and China (e.g., the pored scale on the lateral line - in the present study 94-97 LLS vs. 66-82 LLS of *L. maculatus*). In particular, as only six individuals of this species are known, we consider the new findings from Vietnam as conspecific with *Lateolabrax maculatus*, which is supported by strong genetic compliance.

Numerous studies on morphological characteristics have pointed out the differences between species in the genus *Lateolabrax*. Yokogawa (2019) pointed out that *Lateolabrax maculatus* is easily distinguished from other seabass species. It differs from *L. latus* by



Figure 3. Habitat of *Lateolabrax maculatus*, in Luon Cave of Bai Tu Long National Park, Quang Ninh Province, Vietnam

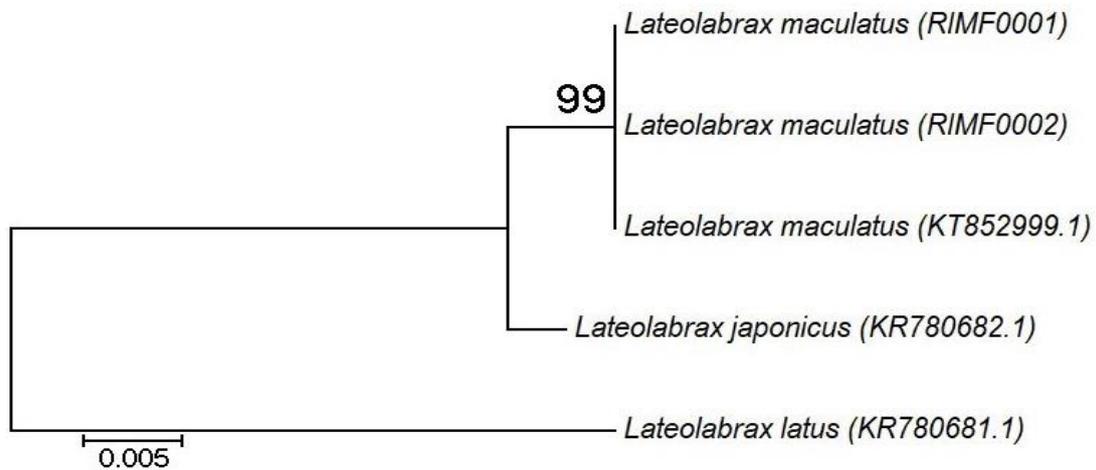


Figure 4. Maximum likelihood tree of *Lateolabrax* based on mitochondrial 16S rRNA sequence data (603 aligned sites). The number above the branches is the bootstrap value (> 95%).

having a caudal peduncle anterior depth [% of standard length (SL)] $\leq 15\%$ (vs. $> 15\%$), snout length (% of SL) $\leq 9\%$ (vs. $> 9\%$), upper and lower jaw lengths [% of head length (HL)] $\leq 45\%$ and 49% (vs. $> 45\%$ and 49% , respectively), 14 or fewer dorsal fin rays (vs. 15 or 16, rarely 14), and anal fin ray counts of eight or fewer (vs. 9-11 anal fin rays). *Lateolabrax maculatus* differs from *L. japonicus* by the following features: a post-orbital preopercular width

(POPW) [% of snout length (SNL)] $\geq 90\%$ (vs. $< 90\%$) [POPW (% of SL) $\geq 7.5\%$ in specimens ≤ 200 mm SL (vs. $< 7.5\%$), SNL (% of SL) $\leq 7.7\%$ in specimens > 200 mm SL (vs. $\geq 7.7\%$)], 18 or 19 caudal vertebrae (usually) [rarely 20 (9.2%)] (vs. 20 caudal vertebrae (usually) or 21) [rarely 19 (13.5%)], 34 or 35 total vertebrae (usually) [rarely 36 (6.6%)] (vs. 36 total vertebrae (usually) or 37 [rarely 35 (13.5%)]), straight first anal pterygiophore (vs. modestly arched first

anal pterygiophore in specimens ≥ 90 mm SL), and usually many clear black spots on the lateral and dorsal body regions (usually on or partly below the lateral line) (vs. spots/dots absent on body in specimens > 260 mm SL (although some specimens ≤ 260 mm SL have some dots restricted to the upper part above the lateral line).

Chyung (1977) suggested that SSB could be distinguished from SB by body depth and the number of dorsal fin rays. Based on isozyme analysis, Park *et al.* (1996) revealed that SSB might be either a distinct species or subspecies. Kim and Jun (1997) indicated that SSB could be distinguished from SB by the number of gill rakers, body depth, and eye diameter. Furthermore, Kang (2000) reported that SSB was a distinct species (*L. maculatus*), different from *L. japonicus* in the number of vertebrae and gill rakers. On the other hand, Yokogawa *et al.* (1997) suggested that SB inhabiting the Ariake Sea in Japan might be SB-SSB hybrids, because the numbers of lateral line pores, gill rakers, and vertebrae were intermediate between those of SB (*L. japonicus*) and SSB or intermediate sea bass (ISB) (*Lateolabrax* sp.), and because SB and SSB (or ISB) share diverse haplotypes (Yokogawa & Seki, 1995; Yokogawa *et al.*, 1997).

Many studies have used nuclear DNA sequences to determine the differences among species in *Lateolabrax* such as the genetic divergence and genetic structure of the two closely related fish species *L. maculatus* and *L. japonicus* in the Northwestern Pacific were inferred from AFLP markers (Han *et al.*, 2015); the genetic differences between wild and hatchery populations of Korean spotted sea bass (*L. maculatus*) were inferred from microsatellite markers (An *et al.*, 2013); the population genetic structure of sea bass (*L. japonicus*) in Korea was based on multiplex PCR assays with 12 polymorphic microsatellite markers (An *et al.*, 2014); the genetic divergence of fish in the genus *Lateolabrax* (Perciformes: Percichthyidae) (Yokogawa, 1998); and the complete mitochondrial genomes of three sea bass *Lateolabrax* (Perciformes, Lateolabracidae) species (Shan *et al.*, 2016). All the genetic studies have shown them to be independent species.

The first discovery of a member of the genus *Lateolabrax* inside Vietnam once again confirms *L. maculatus* has been distributed in Vietnam's northern coast.

Conclusions

This was the first record of adult spotted sea bass (*Lateolabrax maculatus*, McClelland, 1844) from the coastal waters of Quang Ninh Province, Vietnam. The samples collected from Quang Ninh, Vietnam are different from the recorded specimens from Japan, Korea, Taiwan, and China in terms of lateral line scales as the samples collected in Quang Ninh, Vietnam have 94-97 scales and the samples collected from the other countries have 66-82 scales. Phylogenetic analysis of the 16S rDNA sequence confirmed that all of the samples collected belonged to the species of spotted sea bass *L. maculatus*, a species of high economic value; but its growth, reproduction, nutrition, behavior, population, and habitat in the natural environment have not been studied. Therefore, the above issues are in need of continued research in order to conserve populations and breed breeding of the spotted sea bass *L. maculatus* in the future.

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