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Identification, genetic diversity, and comparative evolution of the striped snakehead *Channa striata* (Bloch, 1793) in Wallacea, Indonesia

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Abstract. *Irmawati, Meimulya, Tassakka ACMAR, Nadiarti, Rukminasari N, Kadriah IAK, Nasrullah H, Alimuddin. 2022. Identification, genetic diversity, and comparative evolution of the striped snakehead* Channa striata *(Bloch, 1793) in Wallacea, Indonesia. Biodiversitas 23: 3327-3337.* Striped snakehead, *Channa striata* populations have declined in some areas of Wallacea. This study aimed to identify the species, analyze the genetic diversity, and trace the evolutionary relationships of snakeheads in the Wallacea region with snakeheads in other geographic areas. This study was identified all specimens in this study as *C. striata*. The Wallacean *C. striata* sequences comprised three haplotypes with five polymorphic sites (single nucleotide polymorphisms, SNPs). No mutations were detected in the COI nucleotides of *C. striata* in the Tempe Lake complex, Patampanua River, and Bojo River; therefore, they belonged to the same haplotype. The *C. striata* from the Tangkoli tertiary drainage had unique haplotypes that differed from other *C. striata* haplotypes. The intraspecific genetic distance within *C. striata* was low while the interspecific genetic distance within the genus *Channa* was quite large, indicating that mutation rates are quite high in the genus *Channa*. Haplotype evolution showed that *C. striata* in Wallacea shared a common ancestral pathway with *C. striata* in Kalimantan, Java, and Bali and had a different ancestral pathway with *C. striata* in Lampung (Sumatra). The nucleotide composition of the *C. striata* COI gene followed the general pattern of the Teleostei and the Pisces superclass (T>C>A>G) with the G+C base percentage lower than the A+T percentage. The data from this study will be useful as a basis for designing germplasm conservation strategies and designing strategies to maintain *C. striata* populations and develop strains to meet future needs.

Keywords: Channidae, Cytochrome Oxidase Subunit I, haplotype, mutation, polymorphism, SNP

INTRODUCTION

The striped snakehead, *Channa striata* (Bloch 1793), family Channidae, is an economically important freshwater fish in Southeast Asia with a large and growing market demand especially in Indonesia (Muntaziana et al. 2013; Hien et al. 2015; Alam et al. 2022). In certain regions in Indonesia, the striped snakehead was sold at a high price. In South Sulawesi, snakeheads sell for around IDR 100,000/kg (around US$ 6.8), and IDR 54,000/kg in South Kalimantan (BPBAT Mandiangin 2014). This fish is very popular due to its white flesh, pleasant flavor, and relatively low number of intramuscular spines compared to many other fishes (Dayal et al. 2013; Rahman and Awal 2016). It also has high nutritional content, health benefits, and pharmaceutical properties (Sahu et al. 2012). Snakeheads currently being widely farmed in Southeast Asia (Duong et al. 2019; Hien et al. 2021; Alam et al. 2022) as well as in India (Kumari et al. 2018) and Bangladesh (Alam et al. 2022). However, snakehead farming is constrained by limited seed availability, and poor recovery or recruitment of young fish (juveniles) to natural stocks/populations (IUCN 2015; Alam et al. 2022), the low captive breeding survival rate in the initial period due to huge size variation and cannibalism (Rahman and Awal 2016), and the lack of appropriate formulated diets (Hien et al. 2015).

Snakehead populations are increasingly threatened by the combined effects of climate change, overexploitation, anthropogenic factors, invasive species, and habitat degradation (Arthington et al. 2016; Duong et al. 2019; Hubert et al. 2012). Overexploitation of snakehead populations is caused by high market demand and has resulted in a sharp decline in the quality and abundance of snakehead stocks (Duong et al. 2019; Alam et al. 2022; Kumar et al. 2022). The increasingly intensive exploitation of snakeheads has promoted destructive fishing practices using non-selective and environmentally unfriendly fishing gear (Harianti 2013). Furthermore, the freshwater ecosystems which serve as natural snakehead habitats are increasingly fragmented and often subject to high anthropogenic pressures, potentially leading to reproductive isolation and reduced genetic diversity (Pavlova et al. 2017). For example, the snakehead habitats in the Lake Tempe complex, Bajo River, and Tangkoli tertiary drainage, are under high anthropogenic stress: heavy fishing pressure, agriculture and plantation expansion, and high sedimentation rates (Harianti 2013). Ovotestes and early gonad maturation were also detected in snakeheads from the Bojo River and were thought to be due to anthropogenic factors (Irmawati et al. 2019). These

studies highlight the need for accurate data to support the sustainable management of snakehead populations, especially in the Wallace area.

The provision of identification and genetic diversity is one strategy to promote the sustainable use and management of fish populations. Accurate species identification is vital for the management of aquatic biological resources, and genealogical data are especially critical for fish breeding programs (Irmawati 2017a). Such information is important *inter alia* because mistaken identification can compromise ecological estimation (Gorleri and Areta 2021) leading to continued exploitation of endangered species. The genetic variation could also be associated with adaptation to fluctuating environmental conditions, reproduction, and the invasion of different fish species or strains (Barrett and Schluter 2008; Funk et al. 2012).

Fish species can be identified using morphological methods; however, morphological identification is often difficult because there can be many similarities between species, and identification is costly and time-consuming (Erdozain et al. 2019). Moreover, the characteristics that are important for diagnosis can disappear as a result of adaptation to the environment (Prehadi et al. 2015). Within the genus *Channa,* there is considerable taxonomic uncertainty, with recent studies indicating widespread historical misidentification (Serrao et al. 2014; Conte-grand et al. 2017). Current approaches use molecular identification methods and analyze the genetic variation of the fish. This study applied the random amplified polymorphic DNA (RAPD) method, which examines the entire genome, as well as methods that focus on the structural and functional aspects of specific genes in the delineation of species, such as cytochrome b (Kim et al. 2012; Duong et al. 2019), mitochondrial Cytochrome Oxidase Subunit I (COI) (Lakra et al. 2010; Irmawati et al. 2017b; Alam et al. 2022), mitochondrial ND2 and tRNA (Li et al. 2006), the mitochondrial D-loop (Baisvar et al. 2019; Duong et al. 2019); mitochondrial 16S RNA (Lakra et al. 2010), and metabarcoding that represents a technological advance (Erdozain et al. 2019). In particular, the use of the Cytochrome Oxidase Subunit I (COI) gene as a barcode for molecular species identification and the analysis of genetic variation has opened new perspectives in collecting and analyzing genomic resources from a wide variety of taxa (Xu et al. 2015; Pappalardo et al. 2018; Zhou et al. 2019). The COI gene is an efficient molecular marker for unambiguous species identification because the nucleotide sequences are highly conserved within species (Boonkusol and Tongbai 2016; Zhou et al. 2019). Based on the background outlined above, this study was designed to identify snakeheads in the Wallacea region, analyzing their genetic diversity and comparing their evolutionary patterns with snakeheads from other geographic areas. The results will be useful for evaluating (and potentially eventually managing) the evolutionary patterns of snakehead for improved function, in particular with respect to their adaptive ability in fluctuating environments. The data produced can support the development of a species conservation strategy to prevent future loss of wild snakehead populations and genetic diversity. The data will also provide a basis for identifying potential broodstock and designing breeding programs as a solution to overcome the scarcity of snakehead seed for aquaculture.

MATERIALS AND METHODS

Sample collection

Snakehead specimens used in this study were caught using gillnets. Two specimens were collected from each of the four sampling sites in Wallacea. These were: the Tempe Lake complex (DT, representing overexploitation area), the Tangkoli tertiary drainage (T) and the Bojo River (BJ) (representing agricultural watershed, and the Patampanua River (PL, representing brackish habitat around pond aculture) (Figure 1). Muscle tissues clippings were taken from each specimen and were preserved in ethanol 96% for genetic (DNA) analysis.

DNA extraction

DNA was extracted through the isopropanol-ethanol extraction method using the Pure Gene Core Kit A (Qiagen, USA) following the manufacturer's instructions. The extracted DNA was quantified through spectrophotometry (wavelengths 260/280 nm).

Target sequence amplification and PCR product visualization

The COI gen of each snakehead specimen was amplified through polymerase chain reaction (PCR) using the universal primer pair FishF2 (5'- TCA ACC AAC CAC AAA GAC ATT GGC AC -3') and FishR2 (5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3'). The PCR reaction volume was 50 μL, comprised of 25 μL HS red-mix MyTaq enzyme (Bioline, UK); 20 μL nuclease-free water; 2 μL each primer, and 1 μL DNA template. The PCR profile comprised an initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s, with final elongation at 72°C for 1 min and then storage for an indefinite time at 4°C. The amplified PCR product was visualized through electrophoresis on 1% agarose gel.

Sequencing

The amplified PCR products were sent to the 1st Base laboratory (Malaysia) for Sanger sequencing on an ABI3500 Genetic Analyzer (Applied Biosystems, USA) following the manufacturer's protocols. For accuracy, the DNA strands amplified from each specimen were sequenced in both directions using the FishF2 and FishR2 primers.

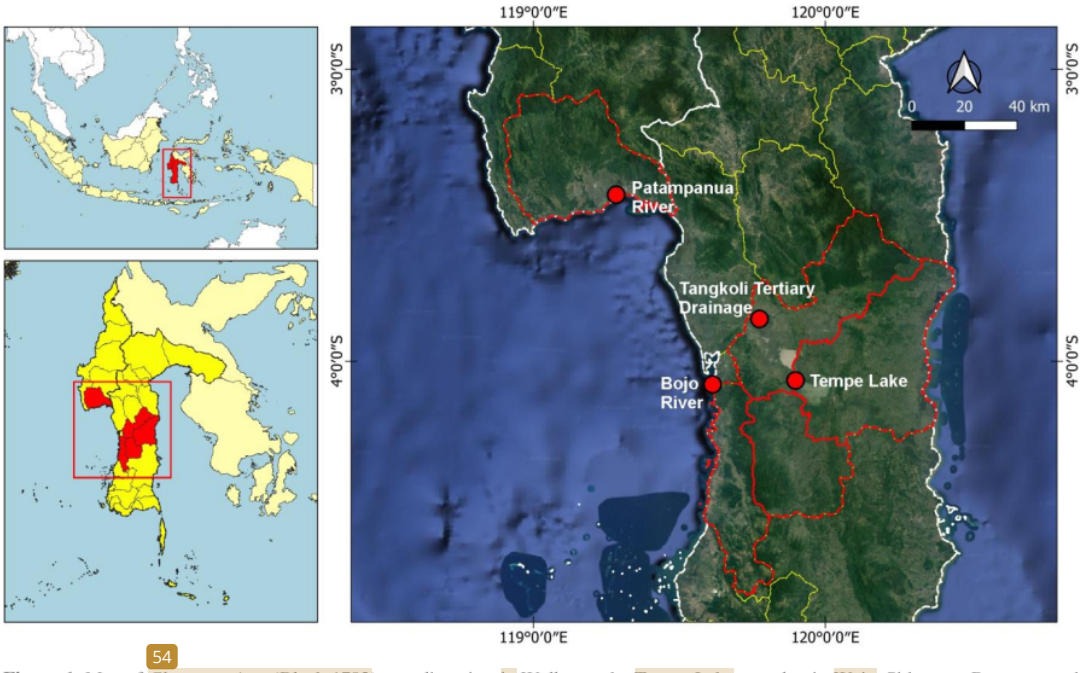

Figure 1. Map of *Channa striata* (Bloch 1793) sampling sites in Wallacea: the Tempe Lake complex in Wajo, Sidenreng Rappang, and Soppeng Regencies; the Patampanua River in Polewali Mandar Regency; the Bojo River in Barru Regency; and the Tangkoli tertiary drainage in Sidenreng Rappang Regency

Data analysis

The partial COI mitochondrial gene sequences obtained were edited in GeneStudio™ Professional to check for and edit or trim any ambiguous nucleotides. The online BLASTn (Basic Local Alignment Search Tool, nucleotide) was used to identify the snakehead specimens through alignment with COI gene sequence accessions deposited in the NCBI GenBank repository (www.ncbi.nlm.nih.gov). The BLASTn alignment results, including the % query cover, % identity, and e-value were tabulated. The sample, BLASTn output, and outgroup sequences were aligned using the ClustalW routine (Thompson et al. 1997) in MEGA (Molecular Evolutionary Genetics Analysis) ver. 10.1.1 (Kumari et al. 2018). Phylogenetic reconstruction was performed using the maximum likelihood Kimura 2-parameter model (Kimura 1980) with 1000 bootstrap replicates (Ren et al. 2013; Wang et al. 2016). Genetic variation, unique haplotype, and evolutionary analyses were performed in DNAsp ver.6.12.03 (Librado and Rozas 2009) and Network ver. 5.0.1.1 (Bandelt et al. 1999).

RESULTS AND DISCUSSION

Molecular identification

This study successfully isolated, amplified, and sequenced partial COI gene sequences from eight snakehead specimens collected from four populations in Wallacea. NCBI GenBank accessions for *Channa striata* had 99%-100% coverage with the sequences from Wallacea which had ≥98% identity with *C. striata* accessions from Central Kalimantan (MF496960.1), West Java (KU692418.1), Central Java (KU692421.1), and Lampung (KJ937421.1) in Indonesia; and Vietnam (MK368524.1). The e-value of 0.00 means that all specimens in this study were identified as *Channa striata* with a very high level of confidence (Table 1).

Genetic variation and haplotype

Analysis of 647 bp partial COI gene sequences from the eight *C. striata* specimens in this study and 11 partial *C. striata* COI gene sequences from the NCBI GenBank showed that 619 bp (95.67% nucleotide sites) were conserved across all 29 sequences while 28 bp (4.33% nucleotide sites) were polymorphic. The latter comprised 10 (1.54% nucleotide sites) parsimony sites and 18 (2.78% nucleotide sites) singletons. Partial COI gene sequences of the *C. striata* from Wallacea had five (0.77% nucleotide sites) polymorphic sites, comprising two (0.31% nucleotide sites) parsimony sites and three (0.46% nucleotide sites) singletons (Table 2).

Table 1. BLASTn query cover, identity, and e-value of snakehead sample sequences with *Channa striata* nucleotide sequences deposited in the NCBI GenBank

Specimen codes	Query cover (%)	E-value	Identity (%)	Accession number and country	Reference
DT9, DT10,	100	0.00	100	MF496960.1- *C. striata,* Central Kalimantan, Indonesia	Conte-Grand et al. (2017)
PL4, PL8,	100	0.00	100	KU692418.1- *C. striata,* West Java, Indonesia	Dahruddin et al. (2016)
BJ27, BJ26	100	0.00	99.85	KU692421.1- *C. striata,* Central Java, Indonesia	Dahruddin et al. (2016)
	100	0.00	98.61	KJ937421.1- *C. striata,* Lampung, Indonesia	Serrao et al. (2014)
	99	0.00	98.76	MK368524.1- *C. striata*, Vietnam	Quang and Van (2019)
T1	100	0.00	99.69	MF496960.1- *C. striata*, Central Kalimantan, Indonesia	Conte-Grand et al. (2017)
	100	0.00	99,69	KU692418.1- *C. striata,* West Java, Indonesia	Dahruddin et al. (2016)
	100	0.00	99,54	KU692421.1- *C. striata,* Central Java, Indonesia	Dahruddin et al. (2016)
	100	0.00	98.76	KJ937421.1- *C. striata,* Lampung, Indonesia	Serrao et al. (2014)
	99	0.00	98.45	MK368524.1- *C. striata*, Vietnam	Quang and Van (2019)
T2	99	0.00	99.23	MF496960.1- *C. striata,* Central Kalimantan, Indonesia	Conte-Grand et al. (2017)
	100	0.00	99.23	KU692418.1- *C. striata,* West Java, Indonesia	Dahruddin et al. (2016)
	100	0.00	99.07	KU692421.1- *C. striata,* Central Java, Indonesia	Dahruddin et al. (2016)
	100	0.00	98.30	KJ937421.1- *C. striata,* Lampung, Indonesia	Serrao et al. (2014)
	99	0.00	97.98	MK368524.1- *C. striata*, Vietnam	Quang and Van (2019

Notes: DT9, DT10: snakeheads from the Tempe Lake complex; PL4, PL8: snakeheads from the Patampanua River, Polewali Mandar Regency; BJ27, BJ26: snakeheads from the Bojo River, Barru Regency; T1, T2: snakeheads from the Tangkoli tertiary drainage, Sidenreng Rappang Regency. Nucleotide sequences of DT9, DT10, PL4, PL8, BJ27, and BJ26 were 100% equal

Table 2. Genetic variation of *Channa striata* within the Wallacea group (Tempe Lake complex, Patampanua River, Bojo River, Tangkoli tertiary drainage) and between groups (*C. striata* from Wallacea and other regions)

Variation	n	Base pairs	Haplotypes	Conserved sites	Variable sites	Parsimony sites	Singleton sites
Within-group							
Nucleotide	8	647	3	642	5	2	3
Amino acids	8	215	3	210	1	1	0
Between groups							
Nucleotide	19	647	12	619	28	10	18
Amino acids	19	215	12	208	3	1	2

Channa striata from the Tempe Lake complex, Patampanua River and Bojo River sites all had the same haplotype (H_1). Meanwhile, *C. striata* from the Tangkoli tertiary drainage had two different and unique haplotypes (H_2 and H_3), which were not found in specimens from other sites in Wallacea or GenBank accessions from other regions. The polymorphic sites in *C. striata* from Wallacea comprised two guanine-adenine (G⇋A) transition substitutions at nucleotide sites 74 and 507; two cytosine-thymine (C⇋T) transition substitutions at nucleotide sites 267 and 432; and one adenine-thymine (A⇋T) transverse substitution at site 42. The lowest variation between *C. striata* haplotypes (one polymorphic site) was between the H-1 from Wallacea and *C. striata* haplotypes from Kalimantan (H_5) and Central Java (H_6). Meanwhile, the Wallacean H_1 haplotype differed most (eight polymorphic sites) with *C. striata* haplotypes H_10 (Philippines) and H_11 (Vietnam). In general, the composition of the 647 bp *C. striata* partial COI gene sequences, including all sequences from Wallacea, followed the pattern T>C>A>G; the exceptions were *C. Striata* from China and Malaysia (C>T>A>G). The COI *C. striata* sequences tended to have a higher percentage of A+T than G+C. The GC percentage was >56% for the first codon position, and <44% for the second and third codons (Table 3).

A 16 haplotypes median vector joining network was constructed for 647 bp COI partial gene sequences from the eight Wallacean *C. striata* specimens (haplotypes H_1-3), 11 *C. striata* GenBank accessions from other regions (haplotypes H_4-12), and four congeneric species in the genus *Channa* (haplotypes H_13-16) (Figure 2). There are three main sub-branches in the *C. striata* branch of this network: *C. striata* from Wallacea, Kalimantan, Java, and Bali; *C. striata* from Southeast Asia and China; and *C. striata* from Lampung (Sumatra). The evolution of *C. striata* from Lampung (Sumatra, H_7) appears to have separated from that of *C. striata* in other regions of Southeast Asia and China but is still grouped within the *C. striata* branch relative to congeneric species within the evolutionary trajectory of the genus *Channa*.

Table 3. Nucleotide base variation, GC and AT composition (%), and GC composition (%) for each codon position in partial *Channa striata* (Bloch, 1793) COI gene haplotype sequences

Haplotype	Variation nucleotide base sites	Quantity (%)		G+C% and codon position		
		G+C	A+T	1st	2nd	3rd
	1111222222233444445556666					
	2457957801266778823599013224					
	1254991670817094792358747472					
H_1	TATGCTAAGTCGCACACGCTCAGTTAGT	299 (46.21)	348 (53.79)	56.48	43.06	39.07
H_2	...A.....A.....	297 (45.90)	350 (54.10)	56.48	42.59	38.60
H_3	.T.A.....T.....T...A.....	295 (45.60)	352 (54.40)	56.48	42.59	37.67
H_4	.....G.....A.C.....	300 (46.37)	347 (53.63)	56.48	43.06	39.53
H_5	.....C.....	300 (46.37)	347 (53.63)	56.48	43.06	39.53
H_6	.....G.....	300 (46.37)	348 (53.79)	56.48	43.06	39.53
H_7	..C.T...A.....T.....A.A.	297 (45.90)	350 (54.10)	56.94	43.06	37.67
H_8	.....TA.....G..	298 (46.06)	349 (53.94)	56.48	43.06	38.60
H_9	C.C.....C...G..G.....A..C	304 (46.99)	343 (53.01)	56.94	43.06	40.93
H_10	C.C.....C...GG.G...A...A...	302 (46.68)	345 (53.32)	56.94	43.06	40.00
H_11	C.C.....C....G.G...AG..A...	302 (46.68)	345 (53.32)	56.94	43.06	40.00
H_12	C.C.....C...G..G.....CA...	304 (46.99)	343 (53.01)	57.41	43.06	40.47

Note: H_1: DT9, DT10, PL4, PL8, BJ26, BJ27, *C. striata* from Central Kalimantan (MF496960.1) and West Java (KU692418.1); H_2: T1; H_3: T2; H_4: *C. striata* from West Kalimantan (KJ937425.1); H_5: *C. striata* from Kalimantan (MF496962.1), H_6: *C. striata* from Central Java (KU692421.1); H_7: *C. striata* from Lampung (KJ937421.1); H_8: *C. striata* from Bali (KU692423.1); H_9: *C. striata* from China (KU852443.1); H_10: *C. striata* from the Philippines (KC789522.1); H_11: *C. striata* from Vietnam (MK368524.1), H_12: *C. striata* from Malaysia (MF496953.1)

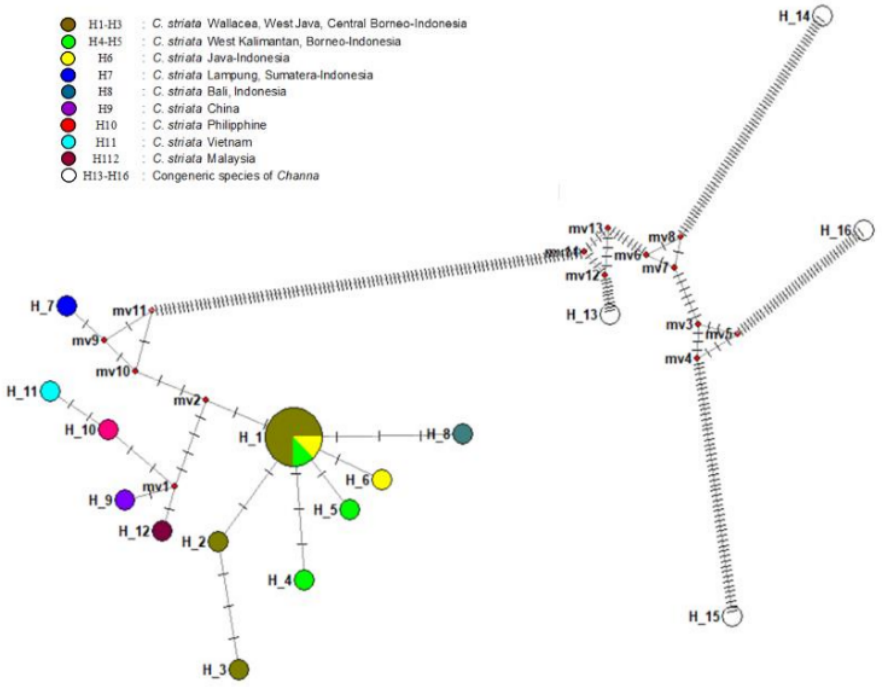

Figure 2. Evolution of *Channa striata* mtDNA COI haplotypes in Wallacea relative to *C. striata* from other regions and congeneric species in the genus *Channa*. The haplotype network was constructed based on 647 bp sequences from eight *C. striata* specimens collected from four sites in Wallacea (haplotypes H_1-H_3), NCBI GenBank accessions representing eleven *C. striata* from other regions (haplotypes H_4-H_12) and four congeneric species: *C. pardalis* (India, H_13); *C. andrao* (India, H_14); *C. gachua* (Bogor, Indonesia, H_15); and *C. bleheri* (India, H_16). The bars on the lines connecting the haplotypes each represent a single nucleotide difference between the nucleotide sequences

Phylogenetic tree and population differentiation (F_{ST})

The phylogenetic and genetic distance analyses included the eight Wallacean *C. striata* partial COI gene sequences generated in this study as well as NCBI GenBank partial COI gene accessions for 11 *C. striata* and four congeneric species: *C. andrao* (MF496662.1), *C. gachua* (MF496735.1), *C. pardalis* (MG825618.1), and *C. bleheri* (MF496702.1). The specimens from the Tempe Lake complex, Patampanua River, Bojo River and Tangkoli tertiary drainage resolved into the *C. striata* clade together with *C. striata* from West Kalimantan (KJ937425.1), Central Kalimantan (MF496960.1), Kalimantan (MF496962.1), West Java (KU692418.1), Central Java (KU692421.1), Bali (KU692423.1), Lampung (KJ937421.1), Malaysia (MF496953.1), China (KU852443.1), the Philippines (KC789522.1) and Vietnam (MK368524.1). This clade was well separated from the clades containing the congeneric species *C. andrao*, *C. gachua*, *C. pardalis*, and *C. bleheri* (Figure 3).

Intraspecific variation and genetic distance varied from 0 to 5 and 0.0000 to 0.0077, respectively, while interspecific variation and genetic distance varied from 112 to 138 and 0.1267 to 0.2133, respectively (Table 4). Between population, pairwise genetic differentiation in *C. striata* based on the COI gene sequences was low between populations within Wallacea, between populations from various regions within Indonesia, and between populations in Indonesia and other countries (F_{ST}: 0.0015 to 0.0201). In contrast, the F_{ST} between *C. striata* and congeneric species ranged from 0.1731 (*C. striata* with *C. pardalis* and *C. andrao*) and 0.2133 (*C. striata* and *C. bleheri*).

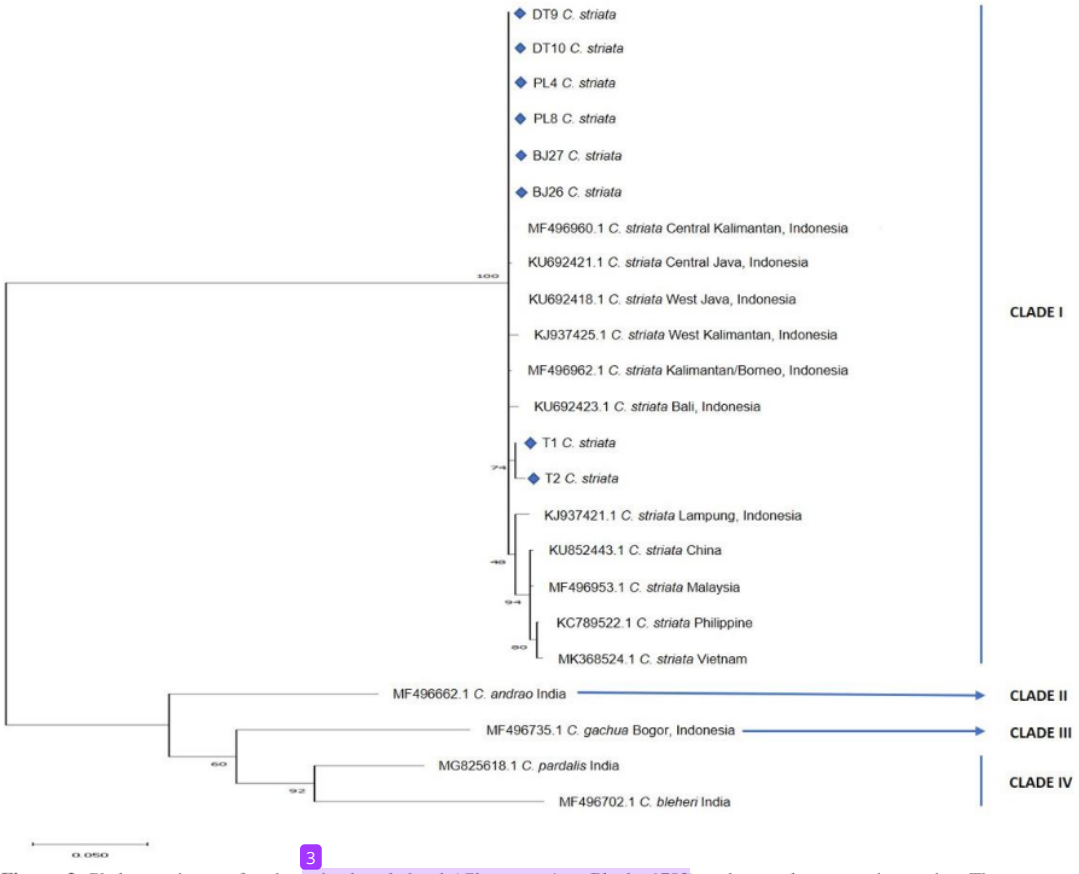

Figure 3. Phylogenetic tree for the striped snakehead (*Channa striata* Bloch, 1793) and several congeneric species. The tree was constructed based on 647 bp mitochondrial partial COI gene sequences. The diamond symbol indicates specimen *C. striata* specimens from Wallacea (Tempe Lake complex; Patampanua River, Polewali Mandar Regency; Bojo River, Barru Regency, and the Tangkoli tertiary drainage, Sidenreng Rappang Regency); other *C. striata* sequences and congeneric species sequences are NCBI GenBank accessions

Table 4. Intraspecific and interspecific nucleotide variations (below diagonal) and genetic distance (above diagonal) between *C. striata* in Wallacea, *Channa striata* from other regions, and congeneric species (*C. pardalis, C. andrao, C. gachua, C. bleheri*)

	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_10	H_11	H_12	H_13	H_14	H_15	H_16
H_1	0	0.0031	0.0077	0.0046	0.0015	0.0015	0.0093	0.0046	0.0108	0.0124	0.0124	0.0108	0.1762	0.1731	0.1808	0.2102
H_2	2	0	0.0046	0.0077	0.0046	0.0046	0.0124	0.0077	0.0139	0.0155	0.0155	0.0139	0.1793	0.1731	0.1808	0.2102
H_3	5	3	0	0.0124	0.0093	0.0093	0.0170	0.0124	0.0185	0.0201	0.0201	0.0185	0.1824	0.1747	0.1808	0.2133
H_4	3	5	8	0	0.0062	0.0062	0.0139	0.0093	0.0155	0.0170	0.0170	0.0155	0.1793	0.1777	0.1839	0.2071
H_5	1	3	6	4	0	0.0031	0.0108	0.0062	0.0124	0.0139	0.0139	0.0124	0.1777	0.1747	0.1824	0.2087
H_6	1	3	6	4	2	0	0.0108	0.0062	0.0124	0.0139	0.0139	0.0124	0.1777	0.1747	0.1824	0.2117
H_7	6	8	11	9	7	7	0	0.0139	0.0139	0.0155	0.0155	0.0139	0.1731	0.1777	0.1824	0.2071
H_8	3	5	8	6	4	4	9	0	0.0155	0.0170	0.0170	0.0155	0.1793	0.1762	0.1839	0.2102
H_9	7	9	12	10	8	8	9	10	0	0.0046	0.0077	0.0031	0.1808	0.1793	0.1839	0.2087
H_10	8	10	13	11	9	9	10	11	3	0	0.0031	0.0046	0.1808	0.1824	0.1870	0.2102
H_11	8	10	13	11	9	9	9	11	5	2	0	0.0077	0.1793	0.1808	0.1886	0.2117
H_12	7	9	12	10	8	8	9	10	2	3	5	0	0.1777	0.1793	0.1839	0.2087
H_13	114	116	118	116	115	115	112	116	117	117	116	115	0	0.1298	0.1267	0.1097
H_14	112	112	113	115	113	113	115	114	116	118	117	116	84	0	0.1329	0.1499
H_15	117	117	117	119	118	118	118	119	119	121	122	119	82	86	0	0.1422
H_16	136	136	138	134	135	137	134	136	135	136	137	135	71	97	92	0

Note: H_1: DT9, DT10, PL4, PL8, BJ26, BJ27, *C. striata* from Central Kalimantan (MF496960.1), *C. striata* from West Java (KU692418.1); H_2: T1; H_3: T2; H_4: *C. striata* from West Kalimantan (KJ937425.1); H_5: *C. striata* from Kalimantan (MF496962.1); H_6: *C. striata* from Central Java (KU692421.1); H_7: *C. striata* from Lampung (KJ937421.1); H_8: *C. striata* from Bali (KU692423.1); H_9: *C. striata* from China (KU852443.1); H_10: *C. striata* from the Philippines (KC789522.1); H_11: *C. striata* from Vietnam (MK368524.1); H_12: *C. striata* from Malaysia (MF496953.1); H_13: *C. pardalis* (MG825618.1); H_14: *C. andrao* (MF496662.1); H_15: *C. gachua* (MF496735.1); H_16: *C. bleheri* (MF496702.1)

Discussion

The striped snakehead or snakehead murrel *C. striata* is categorized as a species of least concern in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Chaudhry et al. 2019), despite widespread threats including habitat degradation and heavy fishing pressure (Baisvar et al. 2019), and reports of substantial declines in *C. striata* abundance in some areas of Wallacea (Ndobe et al. 2013). This study succeeded in isolating 647 bp *C. striata* partial COI gene sequences and showed that genetic differentiation of *C. striata* was relatively low compared to some other fish species, globally and in the Wallacea region. Low genetic differentiation has also been reported between *C. striata* populations in the Philippines, Thailand, and Brunei (0.0000) with an F_{ST} value between *C. striata* in South Sumatra and several other geographic areas of 0.006-0.019 (Syaifudin et al. 2020). In contrast, Jamaluddin et al (2011) report a wider range of COI gene F_{ST} values (0.0000-0.8526) in *C. striata* from Penang, Malaysia. Using microsatellite markers, Robert et al (2018) obtained the F_{ST} values of 0.0024-0.4629 for *C. striata* in Sabah, Malaysia.

This study identified three haplotypes and five polymorphic SNPs in *C. striata* from four unconnected freshwater habitat sites in Wallacea. At three of these sites (Tempe Lake complex, Patampanua River, and Bojo River) all specimens (DT9, DT10, PL4, PL8, BJ26, BJ27) had the same haplotype (H_1). The two *C. striata* specimens collected from the Tangkoli tertiary drainage (T1 and T2) each had a different haplotype (H_2 and H_3), neither of which had yet been deposited in GenBank. The H_1 haplotype has also been reported from Central Kalimantan (GenBank accession MF496960.1) and West Java (accession KU692418.1. The H_2 and H_3 haplotypes differed at three SNP sites (0.5%); two SNPs (0.3%) differed between H_2 and H_1; and five SNPs (0.8%) differed between H_3 and H_1 (Table 3). The genetic variation in the COI gene found in *C. striata* collected from the Tangkoli tertiary drainage could be due to adaptation in a habitat highly modified by human activity and other anthropogenic factors or could represent mutations possibly induced by pollution with agricultural pesticides used in the surrounding area, especially for irrigated rice farming. Genotoxicity (DNA damage) has been reported in the spotted snakehead *C. punctata* exposed to organophosphate pesticides such as profenofos (PFF) (Pandey et al. 2018) and chlorpyrifos (CPF).

Combining *C. striata* from Wallacea (this study) and other regions (GenBank accessions), this study identified 12 haplotypes and 28 SNPs. Fewer *C. striata* haplotypes were identified compared to studies in Vietnam and Bangladesh using the primer pair FishF1-FishR1 (Alam et al. 2022) which found 15 haplotypes but with just 18 SNPs, far fewer than the 28 haplotypes in this study. Low genetic differentiation is reflected in the low between site F_{ST} values, indicating high nucleotide base sequence stability in the COI gene, probably due to the high GC codon usage (>56% at the 1st position, 42-44% at 2nd position, and around 37-41% at the 3rd position).

The *C. striata* from the Tangkoli tertiary drainage (T1 and T2) had different haplotypes but had the same amino-acid composition because the transverse substitutions and base transitions between the two haplotypes occurred in the 3rd codon position (AC**A** – AC**T** at site 42; CC**C** – CC**T** at site 267; and GC**C** – GC**T** at site 243) and do not change the translated amino-acid. In contrast, the G⇋A transition (A**G**C – A**A**C) distinguishing H_1 from H_2 and H_3 at site 74 changes the translated amino acid from serine in H_1 to asparagine in H_2 and H_3; this is, therefore, a missense mutation. Base variations/changes between H_1, H_2, and H_3 were predominantly singletons, but with a ratio that is not too different from parsimony sites (Table 3). Intraspecific variation in the COI gene sequence has been used as a marker in ecological studies. Kitanishi et al (2018), developed a single nucleotide polymorphism (SNP) genotyping method for *Oxyurichthys platypus* to assess exotic haplotype invasion in Japan while Verspoor et al (2012) used mtDNA SNPs as a comparatively simple and cost-effective marker method to determine the population origin (natal river) of European salmon, *Salmo salar*.

The transition/transversion ratio (ti/tv) for haplotypes H_1 and H_2 is zero (0) with a genetic distance of 0.0031 while for haplotypes H_1 and H_3 ti/tv = 4 with a genetic distance of 0.0077. The ratio ti/tv up to 4 indicated that the COI gene could be used as an effective molecular marker to identify different Channidae species. Almost all analyzed genomic DNA nucleotide sequences show that the rate of transitions (T⇋C, A⇋G) is higher than that of transversions (T⇋A, T⇋G, C⇋A, C⇋G (Saha et al. 2021; Zhou et al. 2011), and bias in the transition/transversion ratio (ti/tv) is a general characteristic of DNA sequence evolution. Such bias is more common in animal mitochondrial DNA (mtDNA) than in nuclear DNA or chloroplasts. The estimation of the ti/tv bias rate is important for understanding the evolutionary patterns recorded in DNA sequences as well as for making reliable estimates of genetic distance and accurate phylogenetic reconstruction (Chakraborty et al. 2018). The maximum genetic distance was 0.0077 indicate that the snakehead *C. striata* in Wallacea have not yet reached the level of subspecies differentiation. However, these cryptic species could be turned into subspecies when they are forced to adapt to dramatically altered environments. When humans turn the environment, it is becoming important to understand how rapidly populations can adapt to a new environment. Populations adapt to their new environments in two distinct ways i.e. selection of pre-existing genetic variation and selection of new mutations (Barrett and Schluter 2008).

Analysis of the nucleotide composition of mitochondrial COI gene sequence is important, because the mitochondrial cytochrome oxidase (CO) gene is involved in complex IV of the electron transport system, and dysfunction of the CO gene can cause a variety of diseases (Uddin et al. 2018). This study showed that the *Channa striata* COI nucleotide composition pattern is T>C>A>G, a pattern typical within the Teleostei and the Pisces superclass in general (Chen et al. 2016; Osho et al. 2021), but contrast with base composition of the *C. striata* from

Thailand (Boonkusol and Tongbai 2016), and different from the nucleotide composition of some fishes such as the Asian seabass (*Lates calcarifer*) (Irmawati et al. 2020). The same COI nucleotide pattern was also reported by Kombong and Arisuryanti (2018) in *C. striata* in Lake Sentani Jayapura Papua and in African snakehead *Parachanna obscura* in Nigeria's freshwater (Osho et al. 2021). The guanine and cytosine (GC) content of *C. striata* in this study was less than 50% ranging from 45.60% to 46.21%. This is typical of fish, birds, and mammals COI, COII, and COIII genes which tend to be rich in AT (Uddin et al. 2018) as can be seen in Table 3. Transitions can determine the efficiency of translation of a gene or the frequency of codon usage, which in turn can affect GC bias. The universal phenomenon of unequal frequency of synonymous codons is known as codon usage bias (Liu et al. 2022). Variations in the frequency of synonymous codons can occur due to natural selection as a form of adaptation and can be related to the lifestyle of an organism and/or mutational pressure (Barbhuiya et al. 2020; Lamolle et al. 2022). This study revealed that the most frequently occurring codons in the *C. striata* COI gene were CUU and CUC, both of which encode for leucine; AUC, encoding for isoleucine; and GCU which encodes for alanine. In amphibians, natural selection has resulted in a high frequency of the codons CGA, TGA, and AAA in all genes and orders are (Barbhuiya et al. 2020), while the codons UUU, AUU, UUA, UUG, and CUA occur in very high proportions in the COI gene of *Herdmania momus* Savigny, 1816 (Nariyampet and Hajamohideen 2019). According to Chakraborty et al (2018), natural selection is a factor that plays a role in the codon usage bias of the ATP6, COI, COIII, CYB, ND4, and ND4L mitochondrial genes.

The F_{ST} values of *C. striata* from Wallacea ranged from 0.0000 to 0.0077, which means that the genetic differentiation among the *C. striata* populations studied can be classified as very low, even though the four *C. striata* populations live in well-separated habitats. The number of variable sites (28) in this study is lower compared to the 39 sites reported in 647 bp mitochondrial COI gene sequences of indigenous and exotic *C. striata* from Bangladesh (Alam et al. 2022) and the 66 variable sites found in 396 bp *C. striata* sequences from Thailand (Boonkusol and Tongbai 2016), but higher than the number of variable sites (10) observed in 368 bp sequences from Malaysian *C. striata* populations (Jamaluddin et al. 2011). Lower between population genetic distances indicate a greater similarity; this low genetic distance may indicate the existence of genetic flow between these populations, with past and/or current reproductive and hence genetic interaction (Gustiano et al. 2013). The low between population genetic distances in this study also indicate that these populations may have low genetic diversity which could be related to low abundance and hence non-random mating patterns (Westbury et al. 2019; Muhajirah et al. 2021).

The low genetic variation between *C. striata* from the island of Sulawesi in Wallacea and *C. striata* from three nearby regions (Kalimantan, Java, and Bali) indicates common descent from a relatively recent shared ancestor. The *C. striata* from these four regions appear to have had a much more distant relationship with the *C. striata* from Lampung (Sumatra) which appears to have had a more recent common ancestor with congeneric species *C. andrao*, *C. gachua*, *C. pardali*, and *C. Bleheri*. Meanwhile, *C. striata* from the Philippines and Vietnam seem to have diverged from a comparatively recent common ancestor and are more distantly related to *C. striata* from Wallacea, Sumatra, Malaysia, and China.

In conclusion snakeheads collected from four sites in the Wallacea region (Tempe Lake complex, Patampanua River, Bojo River, and the Tangkoli tertiary drainage) were identified as the species *Channa striata*. Three haplotypes were found; *C. striata* from the Tempe Lake complex, Patampanua River, and Bojo River had the same haplotype, while *C. striata* from the Tangkoli tertiary drainage had two different unique haplotypes with 2-3 nucleotide base mutations which may have been caused by anthropogenic pressure originating from irrigated rice farming. The *C. striata* from the Wallacea region share a recent ancestral pathway with *C. striata* from Kalimantan, Java, and Bali and have a divergent ancestral pathway with *C. striata* from Lampung (Sumatra) and other geographic regions.

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