



First record of Western Black-Bridged Leaf Turtle, *Cyclemys atripons* Iverson & McCord, 1997 (Testudines, Geoemydidae), in Vietnam

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Abstract

We report the distribution of the Western Black-bridged Leaf Turtle, *Cyclemys atripons* Iverson & McCord, 1997, from Vietnam for the first time. This species was recorded on Phu Quoc Island, Kien Giang Province, southern Vietnam based on morphological and molecular evidence. This record extends the distribution of this species approximately 100 km east of the previously recorded location in Kok Kong, Cambodia. This discovery raises the recorded number of species of genus *Cyclemys* in Vietnam to three: *C. atripons*, *C. oldhamii* Gray, 1863, and *C. pulchristriata* Fritz, Gaulke & Lehr, 1997.

Keywords

Freshwater turtle, Indochina, Mekong delta, Phu Quoc Island, range extension.

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Introduction

Leaf turtles of the genus *Cyclemys* Bell, 1834 comprise seven recognized species occurring throughout South and Southeast Asia (Fritz et al. 2008; Turtle Taxonomy Working Group 2017). Vietnam has two confirmed species; namely, Southeast Asian Leaf Turtle, *C. oldhamii* Gray, 1863, and Eastern Black-bridged Leaf Turtle, *C. pulchristriata* Fritz, Gaulke & Lehr, 1997 (Turtle Taxonomy Working Group 2017). *Cyclemys oldhamii* resides in northern Vietnam (Thua Thien Hue Province northwards), Cambodia, Laos, Myanmar, and Thailand, while

C. pulchristriata has been recorded in southern Vietnam (from Da Nang southwards to Ba Ria-Vung Tau Province) and easternmost Cambodia (Fritz et al. 1997; Fritz et al. 2008; Nguyen et al. 2009; Nguyen et al. 2018) (Fig. 1). In a recent survey on Phu Quoc Island, Phu Quoc District, Kien Giang Province, Vietnam, we observed several turtles, in local houses, resembling two morphologically similar species, *C. atripons* Iverson & McCord, 1997 and *C. pulchristriata* which can only be distinguished by molecular analysis (Fritz et al. 2008). Molecular analysis of samples collected from Phu Quoc Island confirms that this population belongs to *C. atripons*, a

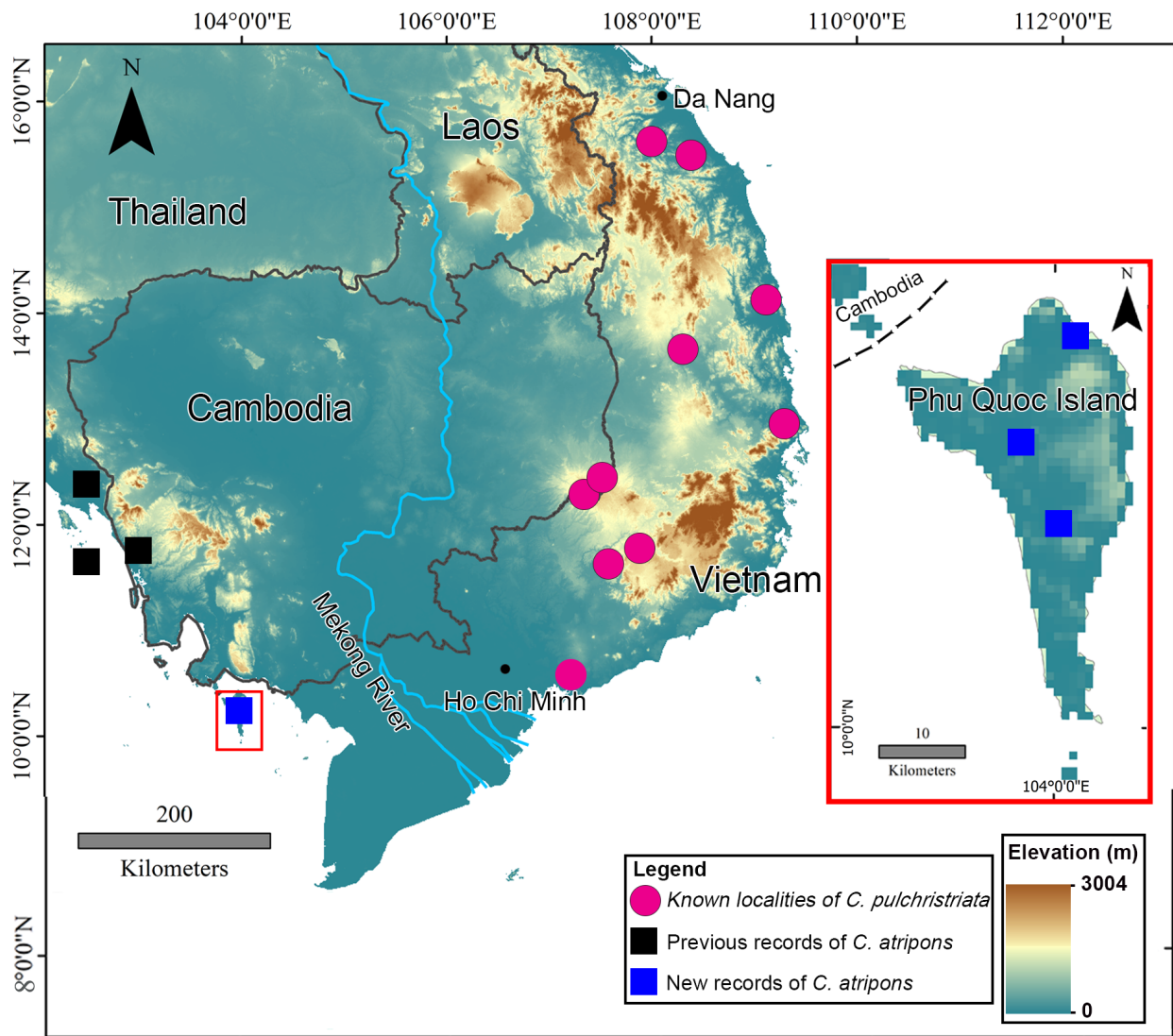


Figure 1. Map showing the distribution of *Cycllemys atripons* (squares) and *C. pulchristriata* (circles). The former was previously recorded within southeastern Thailand and southwestern Cambodia, while the latter is known from southern Vietnam and eastern Cambodia. The new records of *C. atripons* (blue squares) extend its distribution to Phu Quoc Island, Vietnam.

species that has only been previously recorded in southeastern Thailand and southwestern Cambodia (Fritz et al. 2008) (Fig. 1). Thus, we provide the first record of this species in Vietnam.

Methods

Surveys were conducted from 17–24 November 2019 in Phu Quoc National Park, Phu Quoc Island, Phu Quoc District, Kien Giang Province, southern Vietnam. Geographic coordinates were obtained using a Garmin GPS-MAP 60CSx GPS receiver (Garmin Ltd, Olathe, Kansas, USA) and recorded using the WGS84 datum. A total of 14 turtles observed in local households were documented, photographed, and measured. Tissue samples for molecular analysis were taken from either tail tips or oral swabs, stored in absolute alcohol, and subsequently deposited in the Institute of Tropical Biology Zoological Collection (ITBCZ), Ho Chi Minh City, Vietnam. Data from each observed individual including location

details, measurements, and photographs were taken and deposited in the database of the Asian Turtle Program (ATP) of Indo-Myanmar Conservation (IMC) under field record numbers IMC 3409–3418 and IMC 3420–3426.

DNA extraction, amplification, and sequencing of the mitochondrial *cyt b* gene followed Fritz et al. (2008). The GenBank accession numbers of the new sequences are MT366589–MT366592 (Table 1). The dataset used for phylogenetic analyses of the mitochondrial *cyt b* gene (984 bp) included selected sequences from Fritz et al. (2008), Praschag et al. (2009), and Nguyen et al. (2018), and our four new sequences, reaching a total of 32 sequences. The dataset included all nine available sequences of *Cycllemys atripons*, nine sequences of *C. pulchristriata*, two sequences of each remaining species of *Cycllemys*, and the four new sequences from Phu Quoc Island. One sequence of *Heosemys spinosa* Gray, 1831 was chosen as an outgroup taxon based on Fritz et al. (2008) (Table 1).

The raw nucleotide sequence generated was

Table 1. The Cyt *b* gene sequences for *Cyclemys* spp. used in this study. Data were obtained from Fritz et al. (2008), Praschag et al. (2009), Nguyen et al. (2018), and our new sequences; see Fritz et al. (2008) for museum abbreviations.

Species	Locality	Sample	GenBank No.	Remarks/Reference
<i>C. atripons</i>	Thailand: Trat Province	USNM 81865	DQ444271	Holotype of <i>C. atripons</i>
	Thailand: Trat Province	USNM 53423	DQ444270	Paratype of <i>C. atripons</i>
	Cambodia: Koh Kong Province	FMNH 259051	AM931624	Obtained from local turtle-trader
	Cambodia: Koh Kong Province	FMNH 259052	AM931625	Obtained from local turtle-trader
	Cambodia: Koh Kong Province	MTD T 4491	AM931626	Obtained from local fisherman
	Cambodia: Koh Kong Province	MTD T 4492	AM931627	Obtained from local fisherman
	Cambodia: Koh Kong Province	MTD T 4490	AM931628	Obtained from local fisherman
	Cambodia: Koh Kong Province	MTD T 4487	AM931629	Obtained from local hunter
	Unknown	MTD D 42516	AM931630	Obtained at Orasey market in Phnom Penh
	Unknown	MTD T 17	AM931631	Obtained at Cau-Mong market, Saigon
	Vietnam: Kien Giang Province: Phu Quoc	ITBCZ 5083	MT366589	Obtained from local people
	Vietnam: Kien Giang Province: Phu Quoc	ITBCZ 5085	MT366590	Obtained from local people
	Vietnam: Kien Giang Province: Phu Quoc	ITBCZ 5091	MT366591	Obtained from local people
Vietnam: Kien Giang Province: Phu Quoc	ITBCZ 5095	MT366592	Obtained from local people	
<i>C. dentata</i>	Indonesia	MTD T 4210	AM931635	International pet trade
	Indonesia: Java	BMNH 1946.1.22.62	DQ444272	Lectotype of <i>C. dentata</i>
<i>C. enigmatica</i>	Indonesia: Sumatra: Padang	NHMH 9811	AM931644	Holotype of <i>C. enigmatica</i> Fritz, Guicking, Auer, Sommer, Wink & Hundsdoerfer, 2008
	Malaysia: Penang	MTD T 4226	AM931645	Obtained from local turtle-trader
<i>C. fusca</i>	Myanmar: Kachin State	MTD D 42578	AM931651	Holotype of <i>C. fusca</i> Fritz, Guicking, Auer, Sommer, Wink & Hundsdoerfer, 2008
	Myanmar: Sagaing Division	CAS 210070	AM931654	Collected in the field
<i>C. gemeli</i>	India: Assam: Jia Bhoroli River Region	NHMH 37153	AM931656	Holotype of <i>C. gemeli</i>
	India: Nameri National Park	MTD:TD 5058	FM877770	Collected in the field
<i>C. oldhamii</i>	Laotian/Vietnamese border region: vicinity of Lao Bao.	MTD D 42537	AM931666	Obtained from local turtle-trader
	Myanmar: Tanintharyi: Mergui	BMNH 1947.3.5.63	DQ444274	Lectotype of <i>C. oldhamii</i>
<i>C. pulchriata</i>	Cambodia: Mondolkiri	FMNH 259050	AM931681	Collected in the field
	Unknown	MTD D 42550	AM931683	Cambodian turtle trade
	Unknown	MTD D 42552	AM931685	Cambodian turtle trade
	Unknown	MTD D 43785	AM931686	Obtained at Cau-Mong market, Saigon
	Unknown	MTD T 20	AM931691	Obtained at Cau-Mong market, Saigon
	Unknown	MTD T 4206	AM931692	Obtained at Cau-Mong market, Saigon
	Unknown	–	AY434617	Pet trade
	Vietnam: Ba Ria–Vung Tau Province	Unvouchered	MK014303	Collected in the field

initially verified by eye using SEQMAN PRO™ (DNA-STAR Lasergene 7, Madison, WI). Selected sequences were aligned using the CLUSTALW (Thompson et al. 1997) option in MEGA v. 7.0 (Kumar et al. 2016) with default parameters. Mean uncorrected genetic distances (*p*-distances) between sequences and species were calculated using MEGA 7.0.

The matrilineal genealogy was inferred using Bayesian inference (BI) and Maximum Likelihood (ML). MODELTEST v.3.06 (Posada and Crandall 1998) was used to estimate the optimal evolutionary models for the dataset. The best-fit model for BI was the GTR+I model of DNA evolution as suggested by the Akaike Information Criterion. BI was conducted in MRBAYES v. 3.2 (Ronquist et al. 2012); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with 1 cold chain and 3 heated chains for 2 million generations and sampled every 1000 generations. Five independent MCMCMC runs were performed and the first 25% trees were discarded as burn-in. Confidence in topology was assessed by posterior probability (BPP, Huelsenbeck and Ronquist 2001). The ML analysis was performed using IQ-TREE v. 1.6.7.1 (Nguyen et al. 2015) with 1000 ultrafast bootstrap

replications (Minh et al. 2013) and confidence in node topology was tested by non-parametric bootstrapping with 1000 replicates (BS, Felsenstein 1985). ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE was used to estimate the optimal evolutionary model, and the best-fit model was the K3Pu+F+I as suggested by the Bayesian Information Criterion.

Six measurements were taken using a dial calliper (SHAN™ 1–300 mm, model 186-314S, China) to the nearest 0.1 mm: maximum carapace length, maximum carapace width, maximum carapace height, maximum plastron length, mid-seam plastron length, and tail length. Body mass was recorded to the nearest 0.1 g using 2500 g Pesola® spring scales (Pesola AG, Schindellegi, Switzerland). We note that not all measurements could be taken from every individual due to constraints of working in the local community.

Results

Cyclemys atripons Iverson & McCord, 1997

New record. VIETNAM • 2 ♂, 4 ♀; Kien Giang Province, Phu Quoc District, Bai Thom Commune; 10°24.52'N;

104°01.74'E; alt. 30 m; 17 Nov. 2019; Q.N. Lam, L.T. Nguyen leg.; field record numbers IMC 3409–3414; DNA collection numbers ITBCZ 5083–ITBCZ 5088 • 1 ♂; Kien Giang Province, Phu Quoc District, Cua Duong Commune; 10°17.76'N; 103°58.11'E; alt. 16 m; 21 Nov. 2019; Q.N. Lam, L.T. Nguyen leg.; field record number IMC 3418; DNA collection number ITBCZ 5089 • 4 ♀; Kien Giang Province, Phu Quoc District, Duong To Commune; 10°12.79'N; 104°00.57'E; alt. 12 m; 22 Nov. 2019; Q.N. Lam, L.T. Nguyen leg.; field record numbers IMC 3420–3423; DNA collection numbers ITBCZ 5090–ITBCZ 5093 • 1 juvenile; Kien Giang Province, Phu Quoc District, Duong To Commune; 10°12.79'N; 104°00.57'E; alt. 12 m; 22 Nov. 2019; Q.N. Lam, L.T. Nguyen leg.; field record number IMC 3424; DNA collection number ITBCZ 5094 • 2 ♀; Kien Giang Province, Phu Quoc District, Bai Bon Commune; 10°15.72'N; 104°04.66'E; alt. 4 m; 24 Nov. 2019; Q.N. Lam, L.T. Nguyen leg.; field record numbers IMC 3425–3426; DNA collection numbers ITBCZ 5095–ITBCZ 5096.

Identification. Genetically, both BI and ML analyses in our study are similar to those of Fritz et al. (2008) and Nguyen et al. (2018); the trees show that our samples from Phu Quoc Island are nested within the branch of *C. atripons* with high supports (BPP and BS = 1.0 and 99%, respectively; Fig. 2). The uncorrected *p*-distance between our samples from Phu Quoc Island and all available sequences of *C. atripons* range from 0.00% to 0.28% and intraspecific uncorrected *p*-distances of *C. atripons* sequences including our samples is 0.28%. This result confirms that our samples are *C. atripons*. The *p*-distance between *C. atripons* and the most similar

species, *C. pulchriata*, is 6.95% and between *C. atripons* and other species of *Cyclemys* ranges from 3.26% (*C. dentata* Gray, 1831) to 14.03% (*C. gemeli* Fritz et al., 2008).

Morphological characteristics of our specimens match the description of *C. atripons* (Iverson and McCord 1997; Fritz et al. 2008). Adult maximum carapace length ranges 149.5–159.9 mm in three males; 172.4–207.3 mm in 10 females; 117.8 mm in one juvenile. The carapace is ovoid or elongate-shaped, with three keels of which the middle one is the most developed; the posterior edge of the carapace is strongly denticulate. The shell is relatively flat (carapace height 50.5–50.6 mm in two males; 53.1–70.4 in seven females; 40.9 mm in one juvenile). The plastron is oval and shorter than the carapace (maximum plastron length 153.0–162.6 mm in three males, 169.3–198.3 mm in 10 females, 110.7 mm in one juvenile); there is a hyo-hypoplastral hinge; the anal notch is small with an acute-angled or obtuse-angled shape. The tail is somewhat thick and short, 34 mm in one male, 27.4–38.5 mm in five females, other samples have uncompleted or missing tail tips.

Carapace colour ranges from olive to light brown with a radiating pattern visible on all observed individuals. The plastron is cream, yellow-brown, or bright yellow in colour, with short black flecks (lines radiating from scute corners). Radiations on the seams of the plastral scutes are present in some individuals but not in others (five and six specimens, respectively); the juvenile has more radiations on the seams of the plastral scutes than other adults (Fig. 3I). There are small dark specks on the top of the head; yellow to salmon-coloured stripes

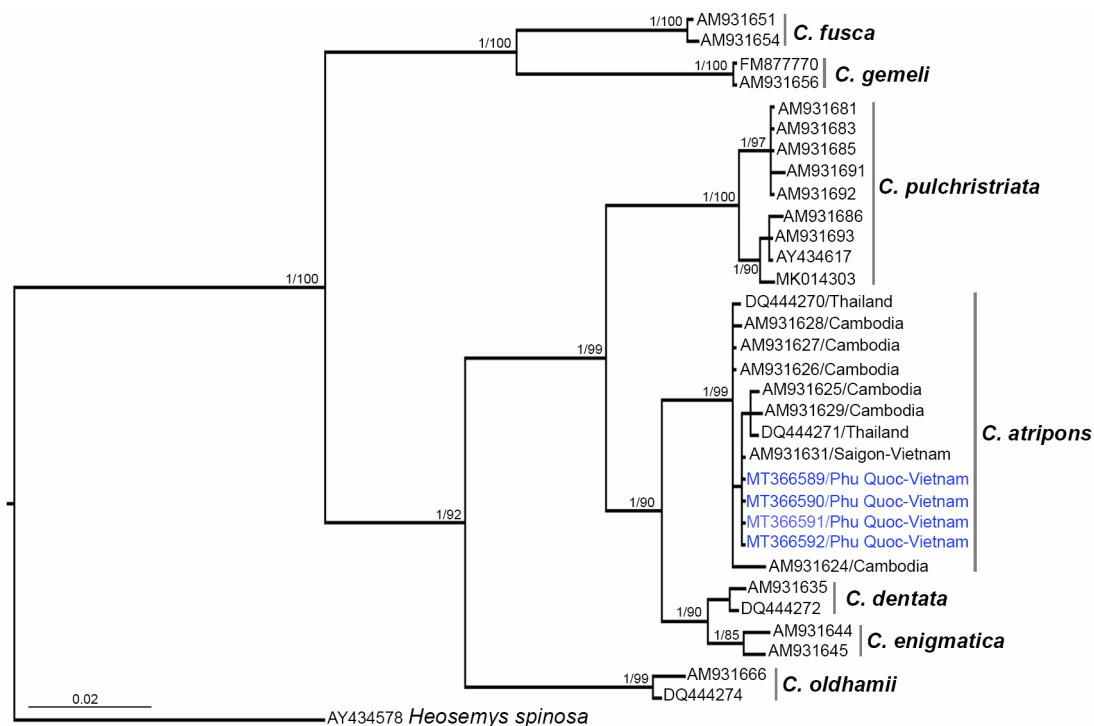


Figure 2. The Bayesian inference (BI) phylogeny for all seven species of *Cyclemys*. Values at nodes are BPP/BS, respectively. New samples from Phu Quoc Island are marked by blue.

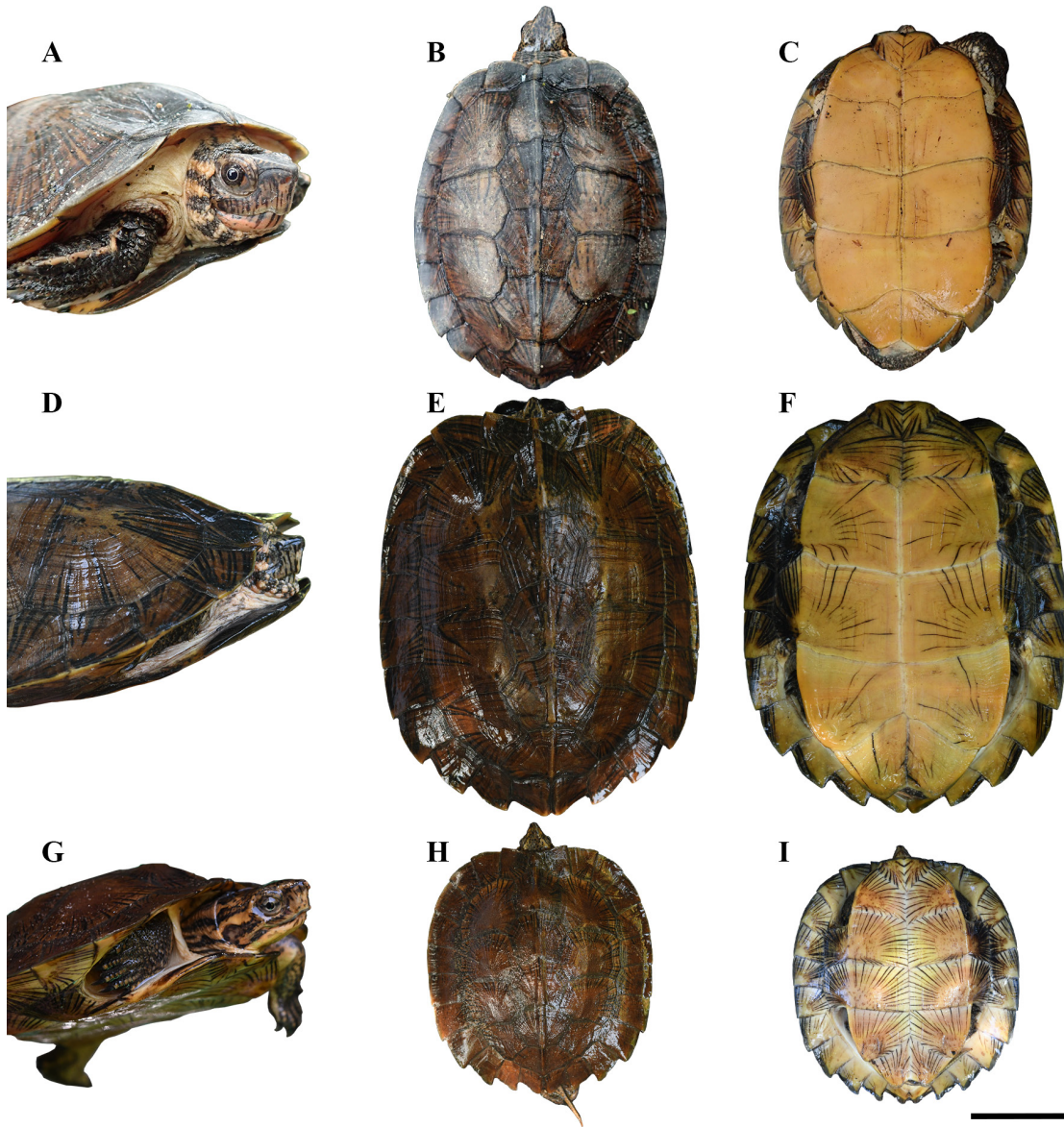


Figure 3. *Cyclemys atripons* from Phu Quoc Island, Kien Giang Province, Vietnam. **A–C.** Adult male IMC 3413/ITBCZ 5087. **D–F.** Adult female IMC 3423/ITBCZ 5093. **G–I.** Juvenile IMC 3424/ITBCZ 5094. From left to right in each row: lateral view of head and body, view of carapace, and view of plastron. Scale bar = 5 cm except A, D, and G not to scale.

on the temporal region, neck, and underside of the neck (Fig. 3). Males were lighter than females in weight (the average weight of two males is 530 g and 955 g in eight females), while the juvenile weighed 200 g.

Discussion

Uetz et al. (2020) followed Nguyen et al. (2009) in recording *Cyclemys dentata* in Vietnam; this record was based on a specimen reported by Bourret (1939) in Hon Gai (Quang Ninh Province, northeast Vietnam). However, Fritz and Ziegler (1999) suggested that this specimen probably belonged to *C. tcheponensis* Bourret, 1939 (now *C. oldhamii*) based on the size of plastron and the fact that it is the only *Cyclemys* species naturally occurring in this region, this was confirmed by the DNA analysis by Guicking et al. (2002). As a result, both Fritz

et al. (2008) and the Turtle Taxonomy Working Group (2017) list Malaysia, Philippines, and Indonesia, but not Vietnam, as the distribution of *C. dentata*. Unfortunately, Uetz et al. (2020) did not cite the checklist of Turtle Taxonomy Working Group (2017). Herein, we follow the comprehensive data on turtles of the world of Turtle Taxonomy Working Group (2017) to exclude *C. dentata* from the fauna of Vietnam.

Uetz et al. (2020) also recorded *C. atripons* in Annam (now central Vietnam), but this location is actually the type locality of *C. pulchristriata*, a morphologically similar species (Fritz et al. 1997). In addition, a sample of *C. atripons* has been reported from Cau Mong market, Saigon (now Ho Chi Minh city, southern Vietnam) but the origins of this turtle are unknown (Fritz et al. 2008). Thus, the distribution of this species in Vietnam has not yet been confirmed and was not placed in

Vietnam chelonian checklists (Nguyen et al. 2009; Turtle Taxonomy Working Group 2017). This study, therefore, records the occurrence of *C. atripons* in Vietnam for the first time.

According to the local people who were keeping the turtles, these animals had been caught by hand or using traps at low elevation, mostly in fast-moving streams within Phu Quoc National Park with no animals being reported to have come from the trade. Most of the turtles were juveniles or subadults when they were caught. We recorded the longest captivity time of *C. atripons* in one local residential area as nearly 10 years in three individuals while there was an average of 2–5 years in other individuals.

After visiting numerous local pagodas, animal (both domestic and wildlife) farms, and main markets in the area, no individuals of this species were observed in these locations. We also interviewed approximately 15 wild-animal traders in markets in Phu Quoc, but none of them claimed to have traded or sold this species. However, this could be due to the cautious nature of some wildlife traders and the illegality of hunting and trading tortoises and freshwater turtles in Vietnam. Due to the geographic location of Phu Quoc, off the southeastern coast of Cambodia, and the lack of other trade related species observed during interviews, we believe that these animals have originated from the wild.

The presence of *C. atripons* on Phu Quoc Island is an interesting but not surprising finding, as the distance from Phu Quoc to mainland Cambodia is less than 40 km and approximately 100 km to a recently identified location of this species (Kok Kong Province, Cambodia). This makes the mainland population of *C. atripons* the geographically closest species to Phu Quoc Island. The distance from Phu Quoc to this species' type locality in Trat Province, Thailand, is approximately 300 km. Individuals of some other species of reptiles from Phu Quoc are genetically and morphologically similar to individuals of these same species in southwest Cambodia and Thailand (western Mekong) (e.g. *Acanthosaura cardamomensis* Wood et al., 2010 and *Sphenomorphus maculatus* (Blyth, 1853); Stuart et al. 2006; Nguyen et al. 2009; Bobrov 2013).

This result is an additional contribution to the distribution knowledge of *Cyclemys* in Vietnam and increases the number of *Cyclemys* species in Vietnam to three: *C. atripons*, *C. oldhamii*, and *C. pulchriata* (Nguyen et al. 2018; this study). Further research is needed to fully understand the distribution of this genus in Vietnam and Indochina and to document *C. atripons* in the wild.

With regards to conservation implications for this species, it is unlikely to have much effect due to the small size of Phu Quoc, and to its *C. atripons* population compared to its range in Cambodia and Thailand. However, Phu Quoc National Park is well-protected and occupies much of the island, which suggests that this newly recorded population is in a healthy state. Enforcement should be used where necessary to prevent further

hunting of the species on the island. While no specific conservation actions are proposed at present, the protection of an island population provides some advantages when considering the reduction of risk to genetic pollution or disease introduction. As such, the trade of non-native or mainland tortoises or freshwater turtles to Phu Quoc islands should be prohibited where escapes or releases could affect this population in the long term. Additionally, the species' presence within a new country, Vietnam, should draw more attention to the species. *Cyclemys atripons*, like all of its congeners except one (*C. dentata*—Near Threatened), has not been evaluated by the IUCN Red List as of 2019 (IUCN 2020) but this will change in the future as more information is elucidated on the species.

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Authors' Contributions

LTN collected material in the field, analysed data, and wrote the manuscript; NQL collected material in the field and assisted with data analysis; JC assisted with manuscript writing; HVH, TEMM, and SNN reviewed the manuscript.

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