

Article

Genetic Diversity of the Critically Endangered Big-Headed Turtle (*Platysternon megacephalum*) Based on Wild and Traded Samples: Implications for Conservation

Hanh Thi Ngo^{1,2,3} , Timothy E. M. McCormack⁴, Ha Van Hoang⁴, Thuy Thu Nguyen⁴, Benjamin Tapley⁵ , Mai Huyen Le⁶, Dat Trong Le⁷, Tham Thi Nguyen^{1,8}, Ha Le Thi Trinh⁹, Thomas Ziegler^{2,3} , Truong Quang Nguyen^{10,11} and Minh Duc Le^{1,8,12,*} 

- ¹ Central Institute for Natural Resources and Environmental Studies, Vietnam National University, Hanoi, 19 Le Thanh Tong, Hoan Kiem District, Hanoi 11000, Vietnam; ngohanhhus@gmail.com (H.T.N.); thamnguyentlhp@gmail.com (T.T.N.)
- ² Institute of Zoology, University of Cologne, Zùlpicher Strasse 47B, 50674 Cologne, Germany; ziegler@koelnerzoo.de
- ³ Cologne Zoo, Riehler Straße 173, 50735 Cologne, Germany
- ⁴ Asian Turtle Program—Indo-Myanmar Conservation, Room 1806, C14 Bac Ha Building, To Huu Road, Nam Tu Liem District, Hanoi 12011, Vietnam; tmccormack@asianturtleprogram.org (T.E.M.M.); hvhauwa@gmail.com (H.V.H.); ntthuy@asianturtleprogram.org (T.T.N.)
- ⁵ Zoological Society of London, Regent's Park, London NW1 4RY, UK; ben.tapley@zsl.org
- ⁶ Department of Genetics, Faculty of Biology, University of Science, Vietnam National University, Hanoi, 334 Nguyen Trai Road, Thanh Xuan District, Hanoi 11416, Vietnam; huyenmai.238@gmail.com
- ⁷ Cuc Phuong National Park, Nho Quan District, Ninh Binh 08400, Vietnam; letrongdat@gmail.com
- ⁸ Department of Environmental Ecology, Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi, 334 Nguyen Trai Road, Thanh Xuan District, Hanoi 11416, Vietnam
- ⁹ Department of Oceanic Science and Technology, Faculty of Hydrology, Meteorology, and Oceanography, University of Science, Vietnam National University, Hanoi, 334 Nguyen Trai Road, Thanh Xuan District, Hanoi 11416, Vietnam; lehattrinh112010@gmail.com
- ¹⁰ Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi 10072, Vietnam; nqt2@yahoo.com
- ¹¹ Vietnam Academy of Science and Technology, Graduate University of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi 10072, Vietnam
- ¹² Department of Herpetology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA
- * Correspondence: le.duc.minh@hus.edu.vn



Citation: Ngo, H.T.; McCormack, T.E.M.; Hoang, H.V.; Nguyen, T.T.; Tapley, B.; Le, M.H.; Le, D.T.; Nguyen, T.T.; Trinh, H.L.T.; Ziegler, T.; et al. Genetic Diversity of the Critically Endangered Big-Headed Turtle (*Platysternon megacephalum*) Based on Wild and Traded Samples: Implications for Conservation.

Diversity **2023**, *15*, 958. <https://doi.org/10.3390/d15090958>

Academic Editors: José Luis García-Marín, Rosane Garcia Collevatti and João Pedro Barreiros

Received: 30 June 2023

Revised: 8 August 2023

Accepted: 14 August 2023

Published: 24 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Overexploitation has driven catastrophic declines in most turtle species in Asia. Many turtles are seized annually by law enforcement officers; unfortunately, seized turtles often lack associated information on their provenance. There is also taxonomic confusion regarding the clear delineation of turtle species and subspecies in Asia. This lack of provenance data and taxonomic uncertainty is problematic as it may preclude release, make appropriate release sites hard to select, or result in genetic pollution when animals are released. In this study, we amassed and analyzed the largest number of genetic samples of the critically endangered big-headed turtle (*Platysternon megacephalum*) to date. Our phylogenetic and network results based on nearly 500 sequences of a mitochondrial ND4 fragment corroborate the earlier hypothesis that only two of the three currently recognized subspecies have strong molecular support and that there is greater genetic structuring within one subspecies than has been previously reported. The vast majority of *P. megacephalum* confiscated from the trade in Vietnam belong to Subclade 3 of *P. m. peguense*, and this subclade is the most broadly distributed taxon of the big-headed turtle in Vietnam based on samples with known localities. Nonetheless, for the other two subclades, all their samples originate from the trade and could not be assigned to any geographic provenance because of the lack of wild-caught samples. In addition, our results reveal two trade centers of the species, Quang Ninh Province on the border between China and Vietnam and Kon Tum Province in the Central Highlands. However, smaller animal seizures occur across the range of the species in Vietnam. While the data presented here have facilitated the selection of appropriate release sites for confiscated *P. megacephalum*, the significant costs of genetic screening

and the shortage of samples from wild animals with good localities hinder the rewilding efforts, especially for conservation practitioners working in countries where conservation activities are not well funded and research on poorly studied species is still limited. Nevertheless, we urge that wildlife translocations include robust pre-translocation genetic screening to ensure that all individual animals are reintroduced at appropriate sites.

Keywords: illegal wildlife trade; ND4; Platysternidae; reptile; translocation; Vietnam

1. Introduction

In Asia, many turtle population declines have been driven by unsustainable harvesting to supply Chinese markets [1–3]. A substantial proportion of the trade in turtles is illegal, and animals are often confiscated and housed in rescue centers and some may be rehabilitated or bred for release to supplement depleted populations [4–6]. Wildlife translocations should follow IUCN guidelines for reintroductions and other conservation translocations [7], particularly regarding genetic provenance as wildlife translocations that are not informed by genetics may inadvertently result in outbreeding and introgression [8–10], which may have dire consequences for species [11–13]. Following the IUCN guidelines is often difficult for animals seized from the illegal wildlife trade as their origin is often unknown [14] and animals are often brought together from a large geographic area and shipped through centralized illegal trade routes [15,16]. This is compounded by taxonomic confusion regarding the clear delineation of turtle species and subspecies in Southeast Asia [12,17]. Taxonomic uncertainty has already undermined existing turtle translocation programs [4,18].

A relevant case study involves the big-headed turtle (*Platysternon megacephalum*) in Vietnam. This critically endangered species [19] is the sole representative of the monotypic family Platysternidae. As such, it is recognized as a global priority for conservation due to its global endangerment and evolutionary distinctiveness [20]. This territorial species exhibits site fidelity and is thought to only move short distances, making it a good candidate for translocation [21]. However, its restriction to linear habitats makes the species inherently vulnerable to overexploitation, and it is collected in enormous volumes for consumption and traditional medicine [22–29]. Populations of the turtle have severely declined across their ranges due to overhunting to supply the high demand in China [29–32]. To reduce the market pressure on the species, it has been listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Morphologically, there are three recognized *P. megacephalum* subspecies, including *P. m. megacephalum*, *P. m. peguense*, and *P. m. shiui* [33], all of which occur in Vietnam [18]. The different subspecies of *P. megacephalum* cannot be reliably differentiated from one another based on morphology alone due to geographic variation in both morphology and color pattern [33]. Genetic analyses have also provided support for the existence of the three taxonomic units [34–36]. Nonetheless, previous studies only employed a limited number of samples, especially from Vietnam. As a result, it is still unclear if any other genetically distinct populations have gone unnoticed by the previous molecular analyses. Other turtle species in Southeast Asia with broad distributions, such as *P. megacephalum*, have shown a great deal of genetic differentiation between populations occurring in specific geographic regions, e.g., species in genera *Cuora*, *Pelodiscus*, and *Sacalia* [37–42].

Detailed knowledge of phylogeographic patterns of threatened species is essential when developing ex situ conservation programs in order to maintain the genetic integrity of captive populations and to minimize the risks of genetic pollution to wild populations when the animals are released [12,43]. Between 2015 and 2021, the Turtle Conservation Centre (TCC) of Cuc Phuong National Park received nearly 600 confiscated *P. megacephalum*, which might have been collected from Vietnam's protected areas (Table 1). The aim of this study was to assess the genetic diversity of *P. megacephalum* in Vietnam using both traded

samples and those with reliable locality data in order to ensure the appropriate selection of release sites for seized animals. We also recommend measures to better manage confiscated animals in the face of uncertain origin and lack of resources to perform genetic screening and other necessary tests.

Table 1. Summary of the big-headed turtle confiscations from different sites in Vietnam. FPD: Forest Protection Department.

Date	Case	#Number of Confiscated BHT Individuals	Age Class			Sex Ratio (Female: Male:Unknown)
			Adult	Subadult	Juvenile	
10 June 2015	Ninh Binh FPD, Ninh Binh Province	27	15	4	8	6:8:13
24 August 2015	Ninh Binh FPD, Ninh Binh Province	7	1	1	5	1:2:4
29 September 2015	Hanoi Wildlife Rescue Centre (transferred from Ha Tinh Province)	23	13	5	5	18:5:0
18 May 2016	Ky Son Police, Nghe An Province	10	3	4	3	6:4:0
24 May 2016	Ha Nam FPD, Ha Nam Province	7	3	1	3	2:3:2
7 September 2016	Hanoi Pet Owner	1	0	0	1	0:0:1
4 October 2016	Thai Binh Police, Thai Binh Province	39	15	15	09	16:14:9
9 November 2016	Quang Ninh Province	77	35	29	13	14:27:36
4 April 2017	Bac Giang FPD, Bac Giang Province	4	1	2	1	0:3:1
19 October 2017	Gia Vien FPD, Ninh Binh Province	68	41	18	9	24:36:8
24 May 2018	Tien Yen FPD, Quang Ninh Province	3	3	0	0	1:1:1
17 August 2018	Quang Nam Police	1	0	1	0	0:0:1
6 October 2018	Quang Xuong, Thanh Hoa Province	46	28	2	16	16:15:15
18 November 2018	Pu Mat National Park, Nghe An Province	9	4	4	1	5:4:0
24 April 2019	Pu Mat National Park, Nghe An Province	14	6	5	3	5:4:5
24 April 2019	Huong Son Police, Ha Tinh Province	4	1	1	2	1:1:2
10 June 2019	Quan Son, Thanh Hoa Province	5	1	1	3	0:1:4
16 July 2019	Huong Son, Ha Tinh Province	1	0	1	0	0:0:1
6 August 2019	Quy Chau, Nghe An Province	25	0	0	25	0:0:25

Table 1. Cont.

Date	Case	#Number of Confiscated BHT Individuals	Age Class			Sex Ratio (Female: Male:Unknown)
			Adult	Subadult	Juvenile	
12 September 2019	Hanoi Wildlife Rescue Centre (transferred from Quang Ninh Province)	4	3	1	0	0:4:0
16 October 2020	Que Phong Police, Nghe An Province	6	0	6	0	0:0:6
20 March 2021	Hanoi Wildlife Rescue Centre	2	0	1	1	0:1:1
8 April 2021	Sop Cop Police, Son La Province	20	4	7	9	7:1:12
22 April 2021	Sop Cop Police, Son La Province	2	0	2	0	1:0:1
10 May 2021	Hoa Binh Police	7	1	2	4	2:1:4
29 June 2021	Quang Ninh Province	74	35	26	13	22:22:30
16 Jul 2021	Dak Glei Police, Chu Mom Ray Rescue Centre, Kon Tum Province	52	14	13	25	19:6:27
10 September 2021	Quang Ninh Province	33	30	15	1	15:17:14
16 September 2021	Quang Ninh Province	13				
18 October 2021	Quang Nam Province	2	0	1	1	0:0:2
	Total	586	257	168	161	181:180:225

2. Material and Methods

2.1. Taxonomic Sampling

In total, 465 new samples of *Platysternon megacephalum* were incorporated into the analysis. Of these, 15 were collected from the wild, four were from within the distribution range of *Platysternon megacephalum shiui*, and 11 were from that of *P. megacephalum peguense* based on Rhodin et al. [18]. The rest of the samples were from individuals confiscated from the trade (N = 450) (Figure 1). In addition, we obtained two sequences of the mitochondrial ND4 from GenBank for *P. m. megacephalum* and *P. megacephalum shiui* based on Luo et al. [35] and Rhodin et al. [18] (DQ256377 and DQ016387 instead of *P. megacephalum* as labeled on GenBank), one for *P. megacephalum peguense* (MH822616), and one for the closely related taxon, *Chrysemys picta* (NC002073), to be used as an outgroup species [18,35,44] (Supplementary Materials, Table S1).

2.2. Molecular Data

Total genomic DNA was extracted from blood and tail tissue samples using the DNeasy Blood and Tissue Kit (Qiagen—Hilden, Germany) following the manufacturer's instructions for blood and animal tissue. Samples were collected in a way to cause the least harm to the animals. The genomic extraction was checked by electrophoresis. A negative control was used for every extraction. We amplified a fragment of mitochondrial NADH dehydrogenase subunit 4 for all samples using HotStar Taq Mastermix (Qiagen—Hilden, Germany) and Dream Taq PCR Mastermix (ThermoFisher Scientific—Vilnius, Lithuania). The standard PCR conditions were 95 °C for 15 min with HotStar Taq Mastermix (Qiagen—Hilden, Germany) or 95 °C for 5 min with Dream Taq PCR Mastermix (ThermoFisher Scientific—Vilnius, Lithuania), 35 cycles at 95 °C for 30 s, 45 °C for 45 s, 72 °C for 60 s, and final elongation at 72 °C for 6 min. The PCR volume consisted of 2 µL of each primer at 10 pmol/µL, 5 µL water, 10 µL of Mastermix, and 1–4 µL DNA template depending on the quantity of

DNA. Two new primers were designed to amplify a 689bp fragment of the mitochondrial NADH dehydrogenase 4 gene (ND4) (PlaND4F1—GCTAAAAATCATACTTCCAACCAT and PlaND4R1—GGATTATTGACCCTGCGATTGGAGCTT). ND4 was selected in this study because it has been shown to be an informative marker in resolving issues related to turtle population genetics and taxonomy [17,45–47]. A negative and positive control were used for every PCR reaction. To confirm if the correct size was amplified, 5 μ L of PCR product was run on a 1% agarose gel with GeneRuler 100bp DNA ladder (SM0241, Thermo FisherScientific—Vilnius, Lithuania), 1X TBE buffer, stained with 2 pg/ μ L bromide, and photographed under UV light. Successful PCR products were cleaned using GeneJet PCR Purification Kit (Thermo FisherScientific—Vilnius, Lithuania) following the manufacturer's instructions. Cleaned PCR products were sent to 1st Base (Malaysia) for sequencing.

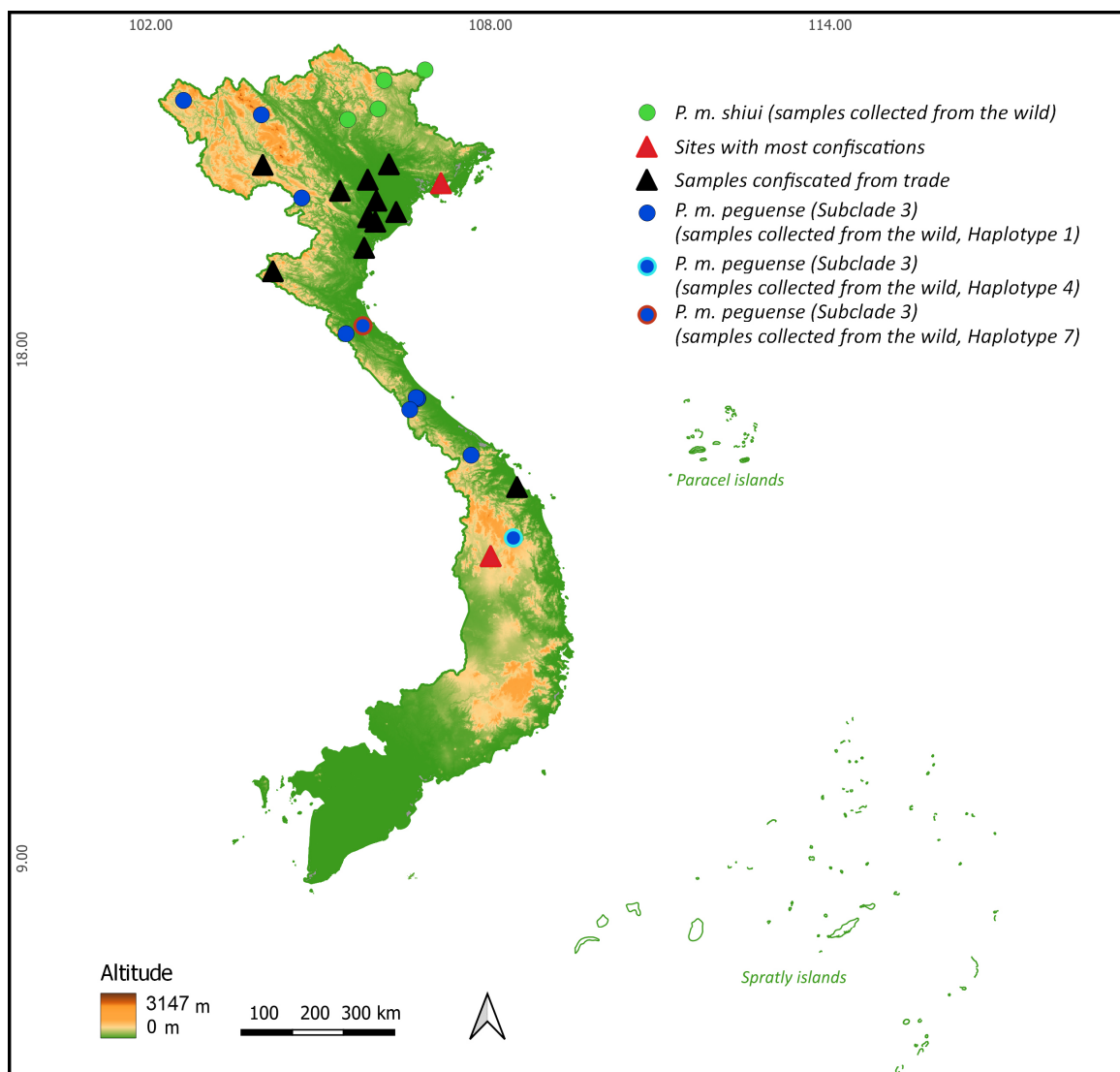


Figure 1. Origin of *Platysternon megacephalum* samples used in this study. Blue and green colors denote samples collected from the wild. Red and black represent confiscation sites.

2.3. Genetic, Phylogenetic, and Network Analyses

The sequences were edited using Sequencher v4.1.4 (Gene Codes Corp, Ann Arbor, MI, USA) by eye and then aligned using Bioedit v7.1.3 with default settings [48]. Data were first evaluated by DnaSP v6.12.03 to determine sequence variation and haplotype diversity [49]. Sequences were then analyzed using two phylogenetic methods, Bayesian inference (BI) in MrBayes v3.2.7 and maximum likelihood (ML) in IQ-TREE v1.6.8 [50,51].

Intraspecific relationships among populations of the *P. megacephalum* were also inferred using the NeighborNet algorithm in SplitsTree v4.14.2 [52,53]. For BI, we performed both single and multiple models by codon partitions to examine the robustness of the tree topology [54,55]. Analyses were conducted with a random starting tree and run for 1×10^7 generations, four Markov chains (one cold, three heated) with default settings. Values of sample points were plotted against the number of generations to detect the stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. Two independent analyses were performed simultaneously. The cutoff point for the burn-in function was set to 60 as $-\ln L$ scores reached stationarity after 60,000 generations. The posterior probability (PP) values for all clades in the final majority rule consensus tree were provided. Nodal support was also evaluated using bootstrap replication (BP) as calculated in SplitsTree (1000 replications) and employing ultrafast BP (10,000 replications) in IQ-TREE.

We regarded UBP or PP value $\geq 95\%$ as strong support for a clade [48,56]. For ML and BI analyses, we used the optimal model calculated by jModelTest v.2.1.10 [57]. The optimal model for nucleotide evolution was set to GTR + G or TVM + G for BI and ML analyses. The network analysis was performed in SplitsTree with the following settings: edge fitting as ordinary least squares, equal angle as chosen splits transformation, least squares to modify weights, and four maximum dimensions as the filtering option. The generated split graph showed a visual representation of conflicting signals in the data by presenting them as a series of parallel edges. The program computed the least squares fit (LSfit) between the pairwise distances from the graph and the distances from the matrix to produce a distance-based unrooted tree diagram using the neighbor-joining algorithm [58]. Uncorrected pairwise genetic distance was calculated in PAUP 4.0 because it has been a preferable way to present data in previous taxonomic studies [59].

3. Results

The final matrix consisted of 470 terminals and 666 aligned characters without internal gaps. The haplotype diversity of *Platysternon megacephalum peguense* was 0.44 and of *P. m. megacephalum/shiui* 0.35, while the nucleotide diversity of *P. m. peguense* and *P. m. megacephalum/shiui* was 0.0019 and 0.0037, respectively. The number of unique haplotypes of *P. m. peguense* and *P. m. megacephalum/shiui* was 22 and 3, respectively. Within *P. m. peguense*, Subclade 1 has three unique haplotypes (20, 21, 22), Subclade 2 possesses two (18 and 19), and the remaining seventeen haplotypes belong to Subclade 3 (1–17) (Supplementary Materials, Matrix S1). All known-locality samples in Subclade 3 were divided into three unique haplotypes: haplotype 1 (337 samples), haplotype 4 (five samples), and haplotype 7 (24 samples). While haplotype 1 has the broadest distribution, each of the other two is restricted to a single location (Figure 1, Supplementary Materials, Matrix S1).

Both ML and BI analyses recovered similar topologies with two major clades with only significant nodal values coming from BI. The first one consisted of *Platysternon m. megacephalum* and *P. megacephalum shiui* and the other comprised only *P. megacephalum peguense* (Figure 2). The sequences regarded as *P. m. megacephalum* from GenBank were paraphyletic with one being more closely related to *P. megacephalum shiui* than the other. The set of relationships received strong support from both ML and BI (Figure 2A). *P. m. peguense* was divided into at least three monophyletic subclades. In fact, Subclade 1 included two lineages, one comprising samples Pm327 and Pm547, and the other comprising samples Pm449, Pm450, Pm453, Pm456, Pm463, Pm465, and Pm466; both lineages were strongly or reasonably corroborated by BI and ML (UFB and PP $\geq 94\%$) (Supplementary Materials, Figure S1). The network analysis produced the same results as those supported by phylogenetic assignments in recovering three subclades within *P. megacephalum. peguense* and two more distantly related clades consisting of *P. megacephalum peguense* and *P. megacephalum shiui* + *P. m. megacephalum* (Figure 2B).

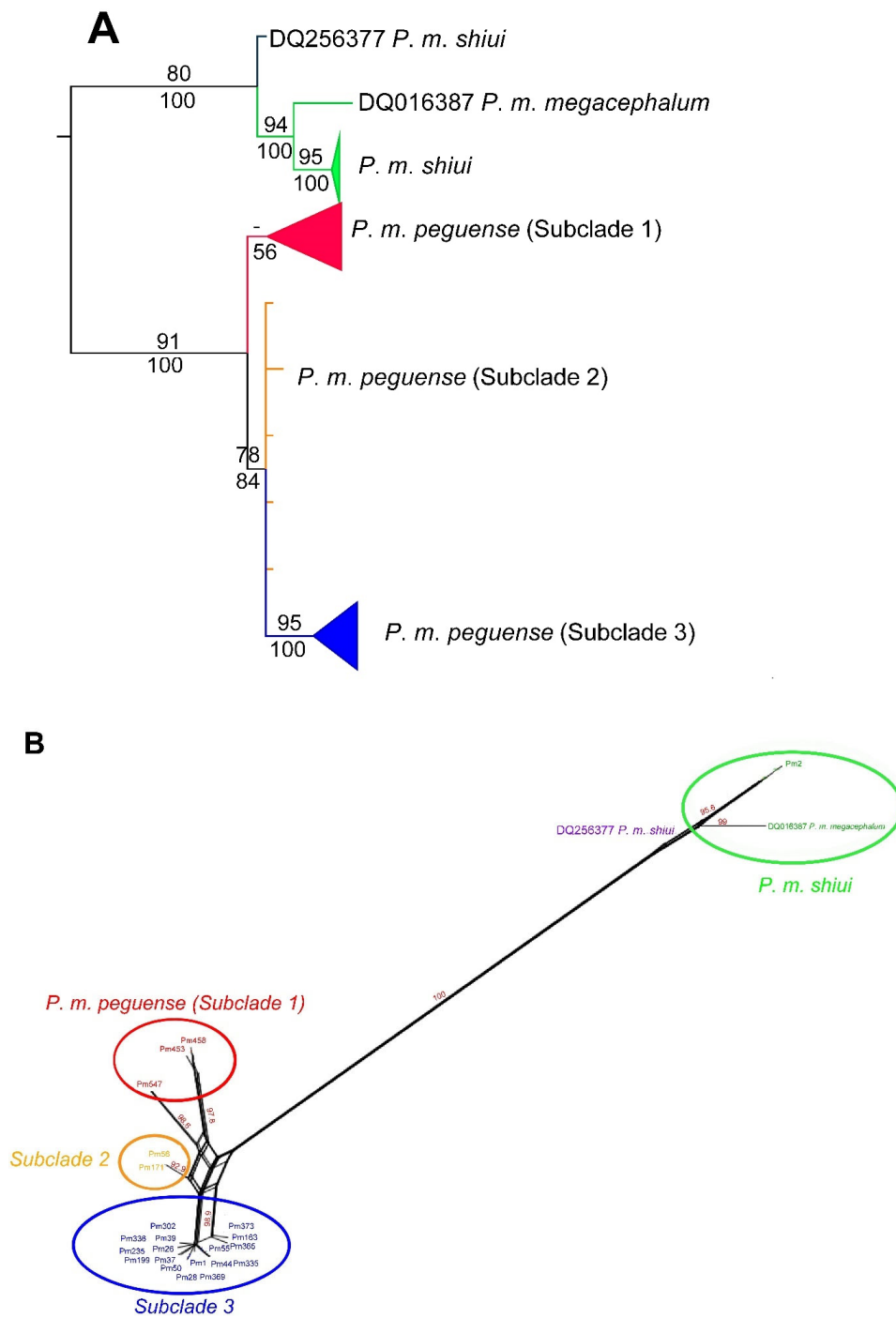


Figure 2. (A). Bayesian cladogram based on a fragment of the mitochondrial ND4 gene. The numbers above and below branches are Bayesian posterior probabilities and maximum likelihood ultrafast bootstrap values. (B). Split tree network based on a fragment of the ND4 gene. Samples presented in each clade are representatives of distinct haplotypes as identified by DnaSP. Red numbers at nodes are representative bootstrap values. Note: Sample with GenBank accession number MH822616 clusters in Subclade 3 in both analyses. A full cladogram of Figure 2A is available in the Supplementary Materials (Figure S1).

In terms of genetic distance, *P. megacephalum shiui* was separated from *P. m. megacephalum* by approximately 1%, comparable to the level of variation within Subclade 1 and 2 of *P. megacephalum peguense*. Similarly, two samples of *P. m. megacephalum* were also differentiated by only 1%. The divergence between *P. megacephalum shiui* + *P. m. megacephalum* and

P. megacephalum peguense is significant, ranging around 5–6.73 %, while the divergence between the three subclades of *P. megacephalum peguense* is about 0.6–1.8% (Table 2).

Table 2. Percentage of uncorrected pairwise distances between and within three clades of *Platysternon megacephalum peguense* and *P. megacephalum shiui*. The highest pairwise distance within each clade is shown in parenthesis. Numbers in parentheses next to clade names correspond to the number of samples assigned to the clades.

Big-Headed Turtle Clades		1	2	3	4	5	6
1	DQ256377 <i>P. m. shiui</i> (1)	(–)					
2	DQ016387 <i>P. m. megacephalum</i> (1)	1.18	(–)				
3	<i>P. m. shiui</i> (9)	0.90	1.19	(0.00)			
4	<i>P. m. peguense</i> (Subclade 1) (9)	5.43–5.58	6.34–6.49	6.35–6.50	(1.51)		
5	<i>P. m. peguense</i> (Subclade 2) (5)	5.02–5.54	5.97–6.48	5.98–6.49	0.91–1.10	(0.17)	
6	<i>P. m. peguense</i> (Subclade 3) (443)	5.24–5.82	6.14–6.72	6.11–6.73	1.36–1.82	0.61–1.26	(1.18)

Only a few of the confiscated samples (N = 5) are clustered with the *Platysternon megacephalum shiui* reference. Most of them (approximately 96%) are clustered in Subclade 3 of *P. megacephalum peguense*. As expected, the distribution of the origin for samples identified as *P. megacephalum shiui* in the cladogram is limited to a small area of northeastern Vietnam. However, the distribution of Subclade 3 (N = 11 samples from the wild and N = 432 samples confiscated from trade) covers a broad geographic region extending from the northwestern part of the country to the Central Highlands (Figure 1). Locations for samples in Subclades 1 (N = 9) and 2 (N = 5) are currently unknown because none had reliable localities information.

4. Discussion

In this study, we amassed and analyzed the largest number of genetic samples of *Platysternon megacephalum* to date. Our phylogenetic results based on nearly 500 sequences corroborate the earlier hypothesis that *Platysternon megacephalum megacephalum* and *P. megacephalum shiui* are more closely related to each other than they are to *P. megacephalum peguense* [35]. However, Luo et al. [35] only included three samples, one collected from Huangshan Mountain, Anhui Province, China (GenBank accession number DQ016387), one from Yunnan Province (GenBank accession number MH822616), and the third without any information on its origin (GenBank accession number DQ256377) [35,60,61]. Luo et al. [35] considered DQ016387 *P. megacephalum megacephalum*, MH822616 *P. megacephalum peguense*, and DQ256377 *P. megacephalum shiui*. While the localities of the first two samples fall within the distributions of the respective subspecies [18], it is unclear why Luo et al. [35] regarded the last sample as *P. megacephalum shiui* as locality data were lacking. In contrast to their identification, this study shows that DQ016387 is more closely related to *P. megacephalum shiui* than DQ256377 (Figure 2).

The subspecies *P. megacephalum shiui* was described in 1987 based on two specimens, USNM 266160 and USNM 266161, acquired from the trade in the vicinity of Lang Son City, Lang Son Province, Vietnam. Its main morphological diagnostic characteristics were stated to be the presence of bright yellow, orange, or pink spots on the head, shell, limbs, sockets, and the ventral surface of the tail [62]. Our observations of *P. megacephalum shiui* contradict this diagnosis because its coloration varied substantially, and the speckles of some animals were brownish rather than bright orange-pink. The results supported by this study reveal that *P. m. megacephalum* is paraphyletic to *P. megacephalum shiui*, and the genetic distance between them is low and falls within the divergence range observed between the different subclades of *P. megacephalum peguense*. On the other hand, *P. m. megacephalum* and *P. megacephalum peguense* exhibit a level of genetic differentiation (5–6.7%) equivalent to independent species when compared with analogous estimates in other turtle species [42,63]. The same conclusion has been recommended by Gong et al. [36].

The genetic analyses presented in this study clearly show that the vast majority of individuals confiscated from the trade in Vietnam belong to Subclade 3 of *P. megacephalum peguense*. The subclade is broadly distributed from Anhui Province, China, to Kon Tum Province, Vietnam, based on samples included in this study. Most of the confiscations took place in Quang Ninh Province, a major export port between Vietnam and China (Figure 2, Supplementary Materials—Table S1). Wildlife and their products seized here often do not have any information about origins. It is, therefore, very challenging to determine the exact geographic provenance of confiscated turtles in Quang Ninh. Our haplotype analysis shows that many samples of haplotype 1 and all of haplotype 4 and haplotype 7, except one of haplotype 7 seized in Ha Nam Province, northern Vietnam, come from this port, although they are naturally distributed far from this location (Figure 1, Supplementary Materials, Matrix S1). Many samples assigned to Subclades 1 and 2 as well as to *P. megacephalum shiui* also originate from animals confiscated at this port (Supplementary Materials, Table S1).

The second most common confiscation site was in Kon Tum Province. As Kon Tum is in the Central Highlands, south-central Vietnam, and far away from the border region with China, it is reasonable to suggest that either the shipments originated from nearby localities or neighboring countries, such as Cambodia or Thailand. Unfortunately, genetic data from *P. megacephalum* populations from these countries are not currently available; therefore, we are unable to draw more conclusions about the origins of animals confiscated in Kon Tum Province. All animals confiscated at this location cluster in Subclade 3 and many in haplotype 1, except for one in Subclade 1 (Supplementary Materials, Table S1). It is intriguing that even though haplotype 4 naturally occurs nearby this confiscation site, all the confiscated samples belonging to this haplotype were found in Quang Ninh Province, northern Vietnam. Similarly, samples of haplotype 7 were all seized in northern Vietnam, probably on their way to China. On the other hand, many samples of haplotype 1 found their way to Kon Tum Province, although the location falls outside of its natural range (Figure 1). Since the major confiscation site in Kon Tum Province is located south of the species distribution, it is apparent that the turtle trade pattern in Vietnam is much more complicated than just the general south-north direction as proposed in previous studies [15,16].

Compared with *P. megacephalum. peguense*, *P. megacephalum shiui* has been recorded in the trade in a much smaller number in Vietnam. We suspect that as *P. megacephalum shiui* inhabit the area near the border between Vietnam and China, they can be conveniently transported to China without going through major highways; therefore, they are less likely to be intercepted by law enforcement officers. It is also possible that the population of *P. megacephalum shiui* has been severely depleted by long-term unsustainable hunting activities with very few individuals left in the wild. Further study is warranted to test these hypotheses and better understand the implications of the trade on different *P. megacephalum* turtle populations.

The inclusion of more samples with robust provenance data in this study confirms the hypothesis that the Red River, a well-known zoological border [64], is the natural barrier separating the two lineages, with *P. megacephalum peguense* occupying the western side (and *P. megacephalum* occurring on the eastern side of the river [36]. Other previous studies suggest that the *P. megacephalum shiui* is probably more broadly distributed with its range extending further west [18]. Nonetheless, our georeferenced samples are still very limited and could not be used to locate the natural ranges of Subclade 1 and 2 of *P. megacephalum peguense*. It is therefore essential to sample and analyze additional individuals from the species range as the uncertainty regarding the distribution ranges of the different *P. megacephalum* lineages has impeded our ability to release confiscated turtles back into the wild because doing so without any knowledge of their distribution can result in genetic pollution of local gene pools [12,65]. Keeping turtles in rescue centers in the long term incurs expense and considerable effort. Furthermore, the aggressive behavior of *P. megacephalum* makes it difficult to keep them for a long time, and the cost of maintaining them in captivity is relatively high because they need to be maintained in individual

enclosures [66,67]. Finally, big-headed turtles require clean water and seasonality [66] if they are to thrive and breed, which makes catering for large numbers of them in rescue centers problematic.

Although genetic screening has been demonstrated to be a useful tool in assisting the appropriate release to the wild of vertebrates that lack provenance information and this approach has been strongly recommended by IUCN [7,12,66], it has rarely been used in practice. One of the barriers is the cost of screening and the number of turtles being seized, especially if more detailed genetic information based on nuclear markers, e.g., microsatellites or single nucleotide polymorphism, is needed to identify genetically distinct populations [39,68–70]. Unfortunately, as most seizures to date have occurred in developing countries and involved their native species, the access to funding sources is restricted or not timely enough to facilitate proper release, which is necessary if the considerable expense of managing a large number of individuals is to be avoided. Furthermore, if exit strategies do not exist for animals that have been confiscated, the resources required to manage animals in rescue centers may become increasingly stretched, and this may have implications for the welfare of individual animals. Sustainable funding solutions need to be developed if translocations in developing countries are to follow IUCN guidelines for reintroductions and other conservation translocations. Finally, expertise in molecular analysis is limited in many Southeast Asian countries, making it difficult to ensure that all wildlife translocations are informed by genetic data when provenance information is lacking. However, with the critical and long-term negative impacts of genetic admixture on native populations, genetic screening should be applied to all individuals whenever possible to prevent impending large-scale biodiversity catastrophes associated with wildlife trade and subsequent conservation translocations. This is also in line with the One Plan Approach to Conservation, developed by IUCN's Conservation Planning Specialist Group (CPSG), which combines in situ and ex situ efforts and various expertise for the optimum and timely protection of species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15090958/s1>, Figure S1: Bayesian cladogram based on a fragment of the mitochondrial ND4 gene. The numbers above and below branches are Bayesian posterior probabilities and maximum likelihood ultrafast bootstrap values. Table S1: Samples of *Platysternon* used in this study. Colors match to those employed in Figure 2A,B.

Author Contributions: Conceptualization, M.D.L., T.E.M.M. and B.T.; methodology, M.D.L., H.T.N. and M.H.L.; validation, H.T.N., M.D.L., T.T.N. (Tham Thi Nguyen), M.H.L. and H.V.H.; formal analysis, H.T.N. and M.D.L.; investigation, H.T.N., T.T.N. (Tham Thi Nguyen) and M.H.L.; resources, T.E.M.M., B.T., M.D.L., H.L.T.T., H.V.H., T.Q.N., D.T.L. and T.T.N. (Thuy Thu Nguyen); data curation, T.T.N. (Thuy Thu Nguyen), H.V.H., M.D.L., H.T.N., D.T.L., T.Z., T.Q.N. and M.H.L.; writing—original draft preparation, M.D.L., B.T. and H.T.N.; writing—review and editing, all authors; visualization, H.T.N. and M.D.L.; supervision, M.D.L., T.E.M.M. and B.T.; project administration, B.T., T.E.M.M. and M.D.L.; funding acquisition, T.E.M.M., B.T., T.Q.N., T.Z. and H.V.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fondation Segré Conservation Fund, Cleveland Metroparks Zoo, US Fish and Wildlife Service, Institute of Ecology and Biological Resources—VAST (Project code IEBR NV.01-23) and by the ZSL EDGE Fellowship for Ha Van Hoang. Research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Sequences of all samples were uploaded to NCBI GenBank with accession numbers OR477227-OR477264. Accession numbers of the ND4 sequences taken from GenBank are provided in Table S1 (data retrieved from the NCBI in February 2023).

Acknowledgments: We thank Hanoi Rescue Center for facilitating sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Van Dijk, P.P.; Palasuwan, T. Conservation status, trade and management of tortoises and freshwater turtles in Thailand. In *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia*; van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J., Eds.; Chelonian Research Foundation: Lunenburg, MA, USA, 2000; pp. 137–144.
2. IUCN/SSC Tortoise and Freshwater Turtle Specialist Group; Turtle Conservation Fund; Turtle Survival Alliance; Turtle Conservancy; Chelonian Research Foundation; Conservation International; Wildlife Conservation Society; San Diego Zoo Global. *Turtles in Trouble: The World's 25+ Most Endangered Tortoises and Freshwater Turtles-2011*; Turtle Conservation Coalition: Lunenburg, MA, USA, 2011.
3. Stanford, C.B.; Iverson, J.B.; Rhodin, A.G.; van Dijk, P.P.; Mittermeier, R.A.; Kuchling, G.; Berry, K.H.; Bertolero, A.; Bjorndal, K.A.; Blanck, T.E.G.; et al. Turtle and Tortoises are in Trouble. *Curr. Biol.* **2020**, *30*, 2211–2224. [[CrossRef](#)] [[PubMed](#)]
4. Fong, J.J.; Parham, J.F.; Shi, H.; Stuart, B.L.; Carter, R.L. A genetic survey of heavily exploited, endangered turtles: Caveats on the conservation value of trade animals. *Anim. Conserv.* **2007**, *10*, 452–460. [[CrossRef](#)]
5. Ziegler, T.; Rauhaus, A.; Mutschmann, F.; Dang, P.H.; Pham, C.T.; Nguyen, T.Q. Building up of keeping facilities and breeding projects for frogs, newts and lizards at the Me Linh Station for Biodiversity in northern Vietnam, including improvement of housing conditions for confiscated reptiles and primates. *Der Zool. Gart.* **2016**, *85*, 91–120. [[CrossRef](#)]
6. Nguyen, H.D.; Pham, T.T.; Nguyen, H.M.; Nguyen, H.X.; Dinh, Q.H.; Pham, N.V.; Tran, T.V.; Hoang, Y.H.; Nguyen, T.T.T.; Nguyen, A.T.L. *Review Report on Wildlife Trade Case Handling at People's Procuracies*; Governance for Inclusive Growth Project: Hanoi, Vietnam, 2017; p. 69.
7. IUCN/SSC. *Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0*; IUCN/SSC: Gland, Switzerland, 2013.
8. Frankham, R.; Ballou, J.; Eldridge, M.D.B.; Lacy, R.C.; Ralls, K.; Dudash, M.R.; Fenster, C.B. Pre-dicting the probability of outbreeding depression. *Conserv. Biol.* **2011**, *25*, 465–475. [[CrossRef](#)]
9. Weeks, A.R.; Sgro, C.M.; Young, A.G.; Frankham, R.; Mitchell, N.J.; Miller, K.A.; Byrne, M.; Coates, D.J.; Eldridge, M.D.B.; Sunnucks, P.; et al. Assessing the benefits and risks of translocations in changing environments: A genetic perspective. *Evol. Appl.* **2011**, *4*, 709–725. [[CrossRef](#)]
10. Banes, G.L.; Galdikas, B.M.; Vigilant, L. Reintroduction of confiscated and displaced mammals risks outbreeding and introgression in natural populations, as evidenced by orang-utans of divergent subspecies. *Sci. Rep.* **2016**, *6*, 22026. [[CrossRef](#)] [[PubMed](#)]
11. Yan, F.; Lü, J.; Zhang, B.; Yuan, Z.; Zhao, H.; Huang, S.; Wei, G.; Mi, X.; Zou, D.; Xu, W.; et al. The Chinese giant salamander exemplifies the hidden extinction of cryptic species. *Curr. Biol.* **2018**, *28*, R590–R592. [[CrossRef](#)] [[PubMed](#)]
12. Le, M.D.; McCormack, T.E.; Van Hoang, H.; Duong, H.T.; Nguyen, T.Q.; Ziegler, T.; Nguyen, H.D.; Ngo, H.T. Threats from wildlife trade: The importance of genetic data in safeguarding the endangered Four-eyed Turtle (*Sacalia quadriocellata*). *Nat. Conserv.* **2020**, *41*, 91–111. [[CrossRef](#)]
13. Shu, G.; Liu, P.; Zhao, T.; Li, C.; Hou, Y.; Zhao, C.; Wang, J.; Shu, X.; Chang, J.; Jiang, J.; et al. Disordered translocation is hastening local extinction of the Chinese giant salamander. *Asian Herpetol. Res.* **2021**, *12*, 271–279.
14. Velo-Antón, G.; Pereira, P.; Gonçalves, D.V. Genetic assignment of captive European pond turtles (*Emys orbicularis*) increases conservation value of recovery centres. *J. Nat. Conserv.* **2021**, *59*, 125953. [[CrossRef](#)]
15. Sterling, E.J.; Hurley, M.M.; Le, M.D. *Vietnam: A Natural History*; Yale University Press: New Haven, CT, USA, 2006; p. 334.
16. Nguyen, V.S.; Vu, N.H.; Dinh, V.T.; Thai, V.H.; Nguyen, T.M.P.; Thai, T.K.O.; Vuong, T.K.H.; Nguyen, T.T. Vietnam, The big market and cross bridges of illegal Wildlife Trade in Asia: Causes and Solutions. *AgBioForum* **2019**, *21*, 35–47.
17. Spinks, P.Q.; Thomson, R.C.; Zhang, Y.; Che, J.; Wu, Y.; Shaffer, H.B. Species boundaries and phylogenetic relationships in the Critically Endangered Asian box turtle genus *Cuora*. *Mol. Phylogenetics Evol.* **2012**, *63*, 656–667. [[CrossRef](#)] [[PubMed](#)]
18. Turtle Taxonomy Working Group. A.G.J. Rhodin, J.B. Iverson, R. Bour, U. Fritz, A. Georges, H.B. Shaffer, P.P. van Dijk. *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*, 9th ed.; Rhodin, A.G.J., Iverson, J.B., van Dijk, P.P., Stanford, C.B., Goode, E.V., Buhlmann, K.A., Mittermeier, R.A., Eds.; Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group; Chelonian Research Monographs 8; Chelonian Research Foundation and Turtle Conservancy: Arlington, VT, USA, 2021; 472. [[CrossRef](#)]
19. Fong, J.J.; Hoang, H.; Li, P.; McCormack, T.; Rao, D.-Q.; Timmins, R.J.; Wang, L.; van Dijk, P.P. The IUCN Red List of Threatened Species 2021:E.T17585A1423706. Available online: <https://www.iucnredlist.org/species/17585/1423706> (accessed on 15 October 2021).
20. Gumbs, R.; Gray, C.L.; Wearn, O.R.; Owen, N.R. Tetrapods on the EDGE: Overcoming data limitations to identify phylogenetic conservation priorities. *PLoS ONE* **2018**, *13*, e0194680. [[CrossRef](#)] [[PubMed](#)]
21. Shen, J.; Meng, F.; Zhang, Y.; Du, W. Field body temperature and thermal preference of the big-headed turtle *Platysternon megacephalum*. *Curr. Zool.* **2013**, *59*, 626–632. [[CrossRef](#)]
22. De Bruin, R.W.F.; Artner, H.G. On the turtles of Hainan Island, southern China. *Chelonian Conserv. Biol.* **1999**, *3*, 479–486.
23. Lau, M.; Chan, B.; Crow, P.; Ades, G. Trade and conservation of turtles and tortoises in the Hong Kong Special Administrative Region, People's Republic of China. In *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia*; Van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J., Eds.; Chelonian Research Monographs 2: Lunenburg, MA, USA, 2000; pp. 39–44.

24. Stuart, B.L.; Timmins, R.J. Conservation status and trade of turtles in Laos. In *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia*; van Dijk, P.P., Stuart, B.L., Rhodin, A.G.J., Eds.; Chelonian Research Monographs 2: Lunenburg, MA, USA, 2000; pp. 58–62.
25. Shi, H.; Parham, J.F.; Lau, M.; Chen, T.H. Farming Endangered turtles to extinction in China. *Conserv. Biol.* **2007**, *21*, 5–6.
26. Gong, S.; Fu, Y.; Wang, J.; Shi, H.; Xu, R. Freshwater turtle trade in Hainan and suggestions for effective management. *Biodivers. Sci.* **2005**, *13*, 239–247. [[CrossRef](#)]
27. Gong, S.P.; Wang, J.C.; Shi, H.T.; Song, R.H.; Xu, R.M. Illegal trade and conservation requirements of freshwater turtles in Nanmao, Hainan Province, China. *Oryx* **2006**, *40*, 331–336.
28. Zhou, Z.H.; Jiang, Z.G. Characteristics and risk assessment of international trade in tortoises and freshwater turtles in China. *Chelonian Conserv. Biol.* **2008**, *7*, 28–36. [[CrossRef](#)]
29. Hoang, H.; McCormack, T.E.M.; Lo, H.; Nguyen, M.; Tapley, B. Hunting and trade of big-headed turtles (*Platysternon megacephalum* Gray 1831) in two protected areas in northern Vietnam. *Herpetol. Notes* **2021**, *14*, 1077–1085.
30. Sung, Y.H.; Karraker, N.E.; Hau, B.C.H. Demographic evidence of illegal harvesting of an endangered Asian turtle. *Conserv. Biol.* **2013**, *27*, 1421–1428. [[CrossRef](#)] [[PubMed](#)]
31. Gong, S.P.; Shi, H.T.; Jiang, A.W.; Fong, J.J.; Gaillard, D.; Wang, J.C. Disappearance of endangered turtles within China's nature reserves. *Curr. Biol.* **2017**, *27*, 170–171. [[CrossRef](#)] [[PubMed](#)]
32. Wu, J.; Wu, Y.; Rao, D.; Zhou, T.; Gong, S. China's wild turtles at risk of extinction. *Science* **2020**, *368*, 838. [[CrossRef](#)] [[PubMed](#)]
33. Ernst, C.H.; Laemmerzahl, A.F. Geographic variation in the Asian big-headed turtle, *Platysternon megacephalum* (Reptilia: Testudines: Platysternidae). *Proc. Biol. Soc. Wash.* **2002**, *115*, 18–24.
34. Zheng, C.; Nie, L.; Wang, J.; Zhou, H.; Hou, H.; Wang, H.; Liu, J. Recombination and evolution of duplicate control regions in the mitochondrial genome of the Asian big-headed Turtle, *Platysternon megacephalum*. *PLoS ONE* **2013**, *8*, e82854. [[CrossRef](#)]
35. Luo, H.; Li, H.; Huang, A.; Ni, Q.; Yao, Y.; Xu, H.; Zeng, B.; Li, Y.; Wei, Z.; Yu, G.; et al. The complete mitochondrial genome of *Platysternon megacephalum peguense* and molecular phylogenetic analysis. *Genes* **2019**, *10*, 487. [[CrossRef](#)]
36. Gong, S.; Suwannapoom, C.; Le, M.; Nguyen, T.Q.; Ge, Y.; Wei, Y.; Gao, Y. Genomic analyses reveal three phylogenetic and their evolutionary histories in the big-headed turtle. *iScience* **2023**, *26*, 107343. [[CrossRef](#)]
37. Stuart, B.L.; Parham, J.F. Molecular phylogeny of the critically endangered Indochinese box turtle (*Cuora galbinifrons*). *Mol. Phylogenet. Evol.* **2004**, *31*, 164–177. [[CrossRef](#)]
38. Spinks, P.Q.; Shaffer, H.B. Conservation phylogenetics of the Asian box turtles (Geoemydidae, Cuora): Mitochondrial introgression, numts, and inferences from multiple nuclear loci. *Conserv. Genet.* **2007**, *8*, 641–657. [[CrossRef](#)]
39. Tiedemann, R.; Schneider, A.R.; Havenstein, K.; Blanck, T.; Meier, E.; Raffel, M.; Zwartepoorte, H.; Plath, M. New microsatellite markers allow high-resolution taxon delimitation in critically endangered Asian box turtles, genus *Cuora*. *Salamandra* **2014**, *50*, 139–146.
40. Farkas, B.; Ziegler, T.; Pham, C.T.; Ong, A.V.; Fritz, U. A new species of *Pelodiscus* from northeastern Indochina (Testudines, Trionychidae). *ZooKeys* **2019**, *824*, 71–86. [[CrossRef](#)] [[PubMed](#)]
41. Gong, Y.-A.; Peng, L.-F.; Huang, S.; Lin, Y.-F.; Huang, R.-Y.; Xu, Y.-H.; Yang, D.-C.; Nie, L.-W. A new species of the Genus *Pelodiscus* Fitzinger, 1835 (Testudines: Trionychidae) from Huangshan, Anhui, China. *Zootaxa* **2021**, *5060*, 137–145. [[CrossRef](#)] [[PubMed](#)]
42. Gong, S.; Fritz, U.; Vamberger, M.; Gao, Y.; Farkas, B. Disentangling the *Pelodiscus axenaria* complex, with the description of a new Chinese species and neotype designation for *P. axenaria* (Zhou, Zhang & Fang, 1991). *Zootaxa* **2022**, *5125*, 131–143. [[PubMed](#)]
43. Ngo, H.T.; Nguyen, T.T.; Le, M.D.; van Schingen, M.; Nguyen, Q.T.; Rauhaus, A.; Vences, M.; Ziegler, T. Genetic screening of captive crocodile lizards (*Shinisaurus crocodilurus*) in Europe. *Der Zool. Gart.* **2020**, *88*, 17–30.
44. Thomson, R.C.; Spinks, P.Q.; Shaffer, H.B. A global phylogeny of turtles reveals a burst of climate-associated diversification on continental margins. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2012215118. [[CrossRef](#)]
45. Fong, J.J.; Chen, T.-H. DANN evidence for hybridization of wild turtles in Taiwan: Possible genetic pollution from trade animals. *Conserv. Genet.* **2010**, *11*, 2061–2066. [[CrossRef](#)]
46. Le, M.D.; Duong, H.T.; Dinh, L.D.; Nguyen, T.Q.; Pritchard, P.C.H.; McCormack, T. A phylogeny of softshell turtles (Testudines: Trionychidae) with reference to the taxonomic status of the critically endangered, giant softshell turtle, *Rafetus swinhoei*. *Org. Divers. Evol.* **2014**, *14*, 279–293. [[CrossRef](#)]
47. Spinks, P.Q.; Thomson, R.C.; Shaffer, H.B. The advantages of going large: Genome-wide SNPs clarify the complex population history and systematics of the threatened western pond turtle. *Mol. Ecol.* **2014**, *23*, 2228–2241. [[CrossRef](#)]
48. Hall, T.A. Bioedit. A user-friendly biological sequences alignment and analysis program for windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
49. Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sánchez-Gracia, A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. [[CrossRef](#)]
50. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic. *Syst. Biol.* **2012**, *61*, 539–542. [[PubMed](#)]
51. Nguyen, L.T.; Schmidt, H.A.; von Haeseler, A.; Bui, M.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [[CrossRef](#)] [[PubMed](#)]

52. Bryant, D.; Moulton, V. Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. *Mol. Biol. Evol.* **2004**, *21*, 255–265. [[CrossRef](#)] [[PubMed](#)]
53. Huson, H.D.; Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **2006**, *23*, 254–267. [[CrossRef](#)]
54. Nylander, J.A.A.; Ronquist, F.; Huelsenback, J.P.; Nieves-Aldrey, J.L. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* **2004**, *53*, 47–67. [[CrossRef](#)]
55. Brandley, M.C.; Schmitz, A.; Reeder, T.W. Partitioned Bayesian analyses partition choice, and the phylogenetic relationship of scincid lizards. *Syst. Biol.* **2005**, *54*, 373–390. [[CrossRef](#)]
56. Hillis, D.M.; Bull, J.J. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **1993**, *42*, 182–192. [[CrossRef](#)]
57. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. JModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [[CrossRef](#)]
58. Saitou, N.; Nei, M. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
59. Swofford, D.L. *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4*; Sinauer Associates: Sunderland, MA, USA, 2001.
60. Peng, Q.-L.; Nie, L.-W.; Pu, Y.-G. Complete mitochondrial genome of Chinese big-headed turtle, *Platysternon megacephalum*, with a novel gene organization in vertebrate mtDNA. *Gene* **2006**, *380*, 14–20. [[CrossRef](#)]
61. Parham, J.F.; Feldman, C.R.; Boore, J.L. The complete mitochondrial genome of the enigmatic bigheaded turtle (*Platysternon*): Description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitochondrial and nuclear DNA. *BMC Evol. Biol.* **2006**, *6*, 11. [[CrossRef](#)] [[PubMed](#)]
62. Ernst, C.H.; McCord, W.P. Two new turtles from Southeast Asia. *Proc. Biol. Soc. Wash.* **1987**, *100*, 624–628.
63. Petzold, A.; Vargas-Ramírez, M.; Kehlmaier, C.; Vamberger, M.; Branch, W.R.; du Preez, L.; Hofmeyr, M.D.; Meyer, L.; Schleicher, A.; Široký, P.; et al. A revision of African helmeted terrapins (Testudines: Pelomedusidae: Pelomedusa), with descriptions of six new species. *Zootaxa* **2014**, *3795*, 523–548. [[CrossRef](#)] [[PubMed](#)]
64. Bain, R.H.; Hurley, M.M. *A Biogeographic Synthesis of the Amphibians and Reptiles of Indochina*; Bulletin of the American Museum of Natural History: New York, NY, USA, 2011; pp. 1–138.
65. Kundu, S.; Mukherjee, T.; Kim, A.R.; Lee, S.R.; Mukherjee, A.; Jung, W.K.; Kim, H.W. Mitochondrial DNA and distribution modelling evidenced the lost genetic diversity and wild-residence of star tortoise, *Geochelone elegans* (Testudines: Testudinidae) in India. *Animals* **2022**, *13*, 150. [[CrossRef](#)] [[PubMed](#)]
66. Shelmidine, N.; Murphy, B.; Massarone, K. Husbandry and propagation of the Chinese big-headed turtle (*Platysternon megacephalum*) at the Wildlife Conservation Society’s Prospect Park Zoo. *Zoo Biol.* **2016**, *35*, 174–179. [[CrossRef](#)] [[PubMed](#)]
67. Gong, S.; Hua, L.; Ge, Y.; Cao, D. Unique multiple paternity in the endangered big-headed turtle (*Platysternon megacephalum*) in an ex-situ population in South China. *Ecol. Evol.* **2019**, *9*, 9869–9877. [[CrossRef](#)]
68. Gong, S.P.; Peng, Y.H.; Hua, L.H.; Tao, J.; Chen, Y.; Deng, J.M. A preliminary observation of the territorial behavior of the big-headed turtle (*Platysternon megacephalum*). In Proceedings of the Herpetological Society of China, Harbin, China, December 2013; p. 67.
69. Huang, H.; Wang, H.; Linmiao, L.; Wu, Z.; Chen, J. Genetic diversity and population demography of the Chinese crocodile lizard (*Shinisaurus crocodilurus*) in China. *PLoS ONE* **2014**, *9*, e91570. [[CrossRef](#)]
70. Xie, H.-X.; Liang, X.-X.; Chen, Z.-Q.; Li, W.-M.; Mi, C.-R.; Li, M.; Wu, Z.-J.; Chu, X.-M.; Du, W.-G. Ancient demographics determine the effectiveness of genetic purging in endangered lizards. *Mol. Biol. Evol.* **2022**, *39*, msab359. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.