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Haplotype Diversity of Swamp Buffalo and River Buffalo Based on Cytochrome B Gene: A Study of Meta-Analysis

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ABSTRACT

Buffalo (*Bubalus bubalis*) is well known as a domesticated buffalo in Asia. The genetic diversity of buffaloes in Asia needs to be studied to ensure a proper breeding program. A meta-analysis study on the cytochrome b gene of the mitochondrial genome from various published data was conducted to evaluate genetic variation and haplo-geography of Asian buffaloes. A meta-analysis is used to provide a comprehensive view of the data. A total of 1369 swamp buffaloes Cytochrome B sequences (from Indonesia (79), Bangladesh (98), China (909), India (4), Laos (96), Taiwan (29), Thailand (54), and Vietnam (100)) and 91 river buffaloes (from China (42), Nepal (42), and Pakistan (7)) were used in this study. Cytochrome B sequences (678 bp) of *Syncerus caffer, Bubalus arnee*, *Bubalus depressicornis, Bubalus quarlesi, Bubalus mindorensis,* swamp buffalo, and river buffalo were determined for their haplotypes using DnaSP v6 program. Haplotypes were analyzed by Principal Coordinate Analysis (PCoA) using Adegenet Package and Hierarchical Clustering on Principal Components (HCPC) methods using Factoextra and FactoMineR Package in R-4.0.0 program. Bayesian analysis of genetic differentiation was implemented in BAPS 6.0. Furthermore, we found 56 haplotypes for swamp buffaloes in eight Asian countries and 5 haplotypes for river buffaloes in Pakistan. We also found 5 haplotypes for outgroup (*B. arnee*, *S. caffer*, *B. depressicornis*, *B. quarlesi*, *B. mindorensis*). Therefore, we found 66 haplotypes in total with outgroup sequences. Based on the PCoA results, three clusters were found. However, the HCPC found eight clusters. Based on HCPC, countries in East and South Asia have four maternal lineages. This is evidence that buffalo domestication has first occurred in East-South Asia. In conclusion, we found four maternal lineages of swamp buffalo and two maternal lineages of river buffalo from ten countries. We also found one maternal lineage for *Syncerus caffer* and one maternal lineage for *B. depressicornis*, *B. quarlesi*, and *B. mindorensis*.

Keywords: haplotype diversity; swamp buffalo; cytochrome b; meta-analysis

INTRODUCTION

Buffaloes are commonly used as livestock for producing meat and milk. However, buffaloes are still used as draught animals and have not been kept intensively like cattle and chickens. In Indonesia, swamp and river buffaloes get less concern than cows and chickens, which are strengthened by better research funding. Genetic diversity studies are needed to determine a good breeding program for buffaloes in the future.

Evaluation of genetic variability and genetic association in populations is essential to control the loss of genetic diversity for selective breeding (Mastrochirico-Filho *et al*., 2019). Breeding programs can be implemented if genetic diversity is observed and maintained. In the same way, genomic tools will allow breeding strategies to ensure the improvement of performance and preserve genetic diversity (Taberlet *et al*., 2011).

Genetic diversity in livestock is also useful for distinguishing between populations. To find out genetic differences between populations, phenotypic traits are generally used or based on gene diversity. Sequence data have been widely known to observe relationships between organisms (Konishi *et al*., 2019). To identify variations in the maternal lineage, the mitochondrial genomes are usually explored. In the mitochondrial genome, there are D-loop, Cytochrome b gene (Cyt B), and Cytochrome Oxidase I (COI) gene markers that are often used to evaluate kinship (Kumar *et al*., 2007; Saputra *et al*. 2013; Paraguas *et al*., 2018). Research conducted by Tobe *et al*. (2010) has shown that the diversity of Cyt B is higher than that of COI. Meta-analysis might contribute

to a better understanding of the domestication history of animals (Guangxin *et al*., 2016). A recent study of cytochrome b in swamp buffalo by Sun *et al*. (2020) showed 5 maternal lineages of swamp buffalo using neighbor-joining in six countries (China, Thailand, Vietnam, Laos, India, and Bangladesh). Kumar *et al.* (2007) suggested that swamp and river buffaloes were domesticated independently and the classification of swamp and river buffaloes as two related subspecies is more suitable. The evaluation of the genetic diversity of cytochrome B in swamp and river buffaloes has been widely carried out. However, a meta-analysis study has never been carried out to analyze the genetic diversity of swamp buffalo in Asia. Therefore, we identified cytochrome B variation in swamp and river buffaloes from various published data in this meta-analysis study.

MATERIALS AND METHODS

A total of 1369 swamp buffalo sequences (originating from Bangladesh, China, India, Indonesia, Laos, Taiwan, Thailand, Vietnam), 91 river buffalo sequences (originating from China, Nepal, and Pakistan), and five outgroup sequences (*Bubalus arnee*, *Bubalus depressicornis*, *Bubalus quarlesi*, *Bubalus mindorensis*, and *Syncerus caffer*) were used in this study. The data used in this study are published data (Table 1). The data used were base positions 70 to 747 (678 bp) of Cytochrome B gene based on GenBank reference (D82894). We used those base positions to accommodate the length of cytochrome B gene sequence of swamp buffalo from Indonesia based on the result reported by Rusdin *et al*. (2020). Sequences data were analyzed using the DnaSP v6 program to determine the haplotypes and haplotype diversity (Rozas *et al*., 2017). The haplotype data were further analyzed using the Principal Coordinate Analysis (PCoA) using the Adegenet Package (Jombart, 2008) in the R-4.0.0 program (R Core, 2020). Furthermore, Hierarchical Clustering on Principal Components (HCPC) was drawn using Factoextra (Kassambara & Mundt, 2020) and FactoMineR (Husson *et al*., 2015) packages from p-distance data in MEGA 7.0.26 (Kumar *et al*., 2016). Bayesian analysis of genetic differentiation was implemented in BAPS 6.0 (Cheng *et al*., 2013).

RESULTS

Based on sequences used in this study, Indonesian buffaloes with 80 sequences have a diversity of haplotypes of 0.6503 (Table 2). In 10 countries, we found

Table 1. Number of sequences from public database

Accession number	Organism	Country	Total (n)	References
D32193	*Bubalus arnee bubalis*	-	1	(Chikuni *et al*., 1995)
D82888	*Syncerus caffer*		1	(Tanaka *et al*., 1996)
D82890	*Bubalus depressicornis*	Indonesia	1	(Tanaka *et al*., 1996)
D82891	*Bubalus quarlesi*	Indonesia	1	(Tanaka *et al*., 1996)
D82895	*Bubalus mindorensis*	Philippines	1	(Tanaka *et al*., 1996)
D82894	*Bubalus bubalis*	Indonesia	1	(Tanaka *et al*., 1996)
JF946519, JF946520, JF946521, JF946522, JF946523, JF946524, JF946525	*Bubalus bubalis (River buffalo)*	Pakistan	7	(Saif *et al*., 2012)
FJ467648 - FJ467917	*Bubalus bubalis*	China	270	(Lei *et al*., 2011)
EF409939, EF409940, EF409941, EF409942	*Bubalus bubalis*	India	4	(Kumar *et al*., 2007)
KR010069-KR010168	*Bubalus bubalis*	Vietnam	100	(Zhang *et al*., 2016)
KR010040-KR010068	*Bubalus bubalis*	Taiwan	29	(Zhang *et al*., 2016)
KR009986- KR010039	*Bubalus bubalis*	Thailand	54	(Zhang *et al*., 2016)
KR009944-KR009985	*Bubalus bubalis (River buffalo)*	Nepal	42	(Zhang *et al*., 2016)
KR009848-KR009943	*Bubalus bubalis*	Laos	96	(Zhang *et al*., 2016)
KR009167-KR009644; KR009687-KR009847	*Bubalus bubalis*	China	639	(Zhang *et al*., 2016)
KR009645-KR009666	*Bubalus bubalis (River buffalo) (Murrah)*	China	22	(Zhang *et al*., 2016)
KR009667-KR009686	*Bubalus bubalis (River buffalo) (Nili Ravi)*	China	20	(Zhang *et al*., 2016)
KR009069-KR009166	*Bubalus bubalis*	Bangladesh	98	(Zhang *et al*., 2016)
BK6, BK7, BK8, BK9, BK12, BK14, BK15, BK18, BK21, BK24, BK28, BD1, BD4, BD7, BD9, BD11, BD13, BD14, BD15, BD19, BD25, KL1, KL2, KL3 KL4, KL5, KL6, KL7, KL8, KL9, KL10, KL11, KLB1, KLB2, KN1, KN2, KN3, KN4, KN5, KN6, KN7, KN8, KN9, KN10, KN11, KN12, KN13, KN14, TRB1, TRB2, TRB3, TRB4, TRB5, TRP6, TRP8, TRT13, TRT14, TRT15, NTB1, NTB2, NTB3, NTB5, NTB6, NTB7, NTB8, NTB9, NTB10, NTB11, BTN1, BTN2, BTN3, BTN4, BTN5, NAD1, NAD2, NAD3, NAD9, NAD10	*Bubalus bubalis*	Indonesia	78	(Rusdin *et al*., 2020)

61 haplotypes, and the haplotype diversity was 0.7020 with 62 polymorphic sites. The smallest haplotype diversity was found in Taiwan (0.1970), and the highest was found in India (1.0000). The highest number of haplotypes was located in China (36 haplotypes), and the lowest number of haplotypes was found in Nepal (3 haplotypes) and Taiwan (3 haplotypes). By using data of *S. caffer*, *B. arnee*, *B. quarlesi*, *B. mindorensis,* and *B. depressicornis,* we found 66 haplotypes (Table 3). Haplotype 16 was the most common with large samples, i.e., 73.50% in China, 7.31% in Vietnam, 5.85% in Laos, 5.05% in Indonesia, 3.47% in Taiwan, 3.47% in Thailand, and 1.45% in Bangladesh (Table 4). We also found unique haplotypes in Bangladesh (6), China (24), India (1), Indonesia (8), Laos (1), Thailand (3), Pakistan (5), and Vietnam (1). Based on PCoA results, we found three clusters (Figure 1). The first cluster consisted of buffaloes from *B. depressicornis,* Bangladesh, China, India, Nepal, and Pakistan. The second cluster included buffaloes from *B. arnee*, *B. quarlesi*, *B. mindorensis*, Bangladesh, China, Indonesia, Laos, Nepal, Taiwan, Thailand, and Vietnam. On the other hand, *S. caffer* was very far from the other clusters.

HCPC showed eight clusters (Figure 2), i.e., cluster I: consisted of buffaloes from *B. arnee*, Bangladesh China, Indonesia, Laos, Nepal, Taiwan, Thailand, and Vietnam; cluster II: consisted of buffaloes from Bangladesh, China, India, Indonesia, Laos, Nepal, Taiwan, Thailand, and Vietnam; cluster III: consisted of buffaloes from Bangladesh, China, India, Nepal, and Pakistan; cluster IV: consisted of buffaloes from Bangladesh, China, India, Nepal, and Pakistan; cluster V: consisted of buffaloes from Pakistan; cluster VI: consisted of buffaloes from Pakistan; cluster VII: consisted of buffaloes from *B. mindorensis*, *B. quarlesi*, and *B. depressicornis*; cluster VIII: consisted of buffaloes from *S. caffer*. According to HCPC results, swamp buffaloes in Asia have four maternal lineages. Interestingly, haplotypes from Pakistan form separate clusters (cluster V and VI) for river buffalo. Indonesia, Laos, Taiwan, Thailand, and Vietnam only have two maternal lineages. Indian buffalo has three maternal lineages. On the other hand, Bangladesh, China, Nepal, and Pakistan have four maternal lineages. What is interesting is that the four maternal lineages are found in East and South Asian countries. Bayesian analysis showed 3 clusters (Figure 3) that were very similar to the results shown by the principal coordinate analysis. Interestingly, we found sequences of river buffaloes from Nepal and China in haplotypes 22 and 23 along with sequences from swamp buffaloes. This result is possible because we only took 678 bp to observe the genetic diversity of the buffalo in this study.

DISCUSSION

Haplotype diversity based on the mtDNA of Egyptian and Indian buffaloes ranged from 0.8236 ± 0.0488 to 0.9428 ± 0.0088 (Nagarajan *et al*., 2015). Lei *et al*. (2011) found that the haplotype diversity of Chinese buffaloes ranged from 0.469 ± 0.039 to 0.815 ± 0.033. Based on this haplotype diversity, Taiwan buffalo has lower haplotype diversity than buffaloes in the other countries. However, the gene diversity based on Cytochrome B in Indonesian buffalo is more diverse than the other studies on GH, GHR, GHRH, Pit1, COI, and microsatellite (Andreas *et al*., 2010; Misrianti *et al*., 2010; Saputra *et al*., 2013, 2020; Sumantri *et al*., 2010). The results we found with the HCPC showed that the haplotypes of *B. depressicornis* (Lowland Anoa), *B. quarlesi* (Mountain Anoa), and *B. mindorensis* (Tamaraw) were self-clustered, and our findings are different from result reported by Tanaka *et al*. (1996) that *B. mindorensis* is genetically closer to swamp buffalo than to anoa.

Data of *B. arnee* (Wild water buffalo) were obtained from Chikuni *et al*. (1995), showing a genetic closeness with swamp buffalo. Zhang *et al*. (2020) suggest the swamp and river buffaloes are descended from distinct populations of wild water buffalo (*B. arnee*). The river buffaloes originating from Pakistan have different haplotypes from the river buffaloes originating from China. Of the seven haplotypes of the Pakistani river buffalo (Saif *et al*., 2012), only five haplotypes are unique in this meta-analysis. Most likely, the domestication of river buffalo occurs in South Asia. Youssef *et al*. (2021) found three maternal lineages for river buffaloes in five countries (Bangladesh, China, Egypt, India, and Italy) based on the mitochondrial genome. The study also found that Indian river buffaloes had more maternal lineage. Nagarajan *et al*. (2015) suggested that the river buffalo was first domesticated in the Northwestern region of India and spread to other parts of the world. In other

Table 2. Haplotype diversity of buffaloes based on country

Population	N	Number of haplotypes	Variable sites	Haplotype diversity
Pakistan	7	6	5	0.9524
Indonesia	79	10	8	0.6537
Bangladesh	98	12	24	0.6714
China	951	36	40	0.6336
India	4	4	2	1.0000
Laos	96	7	8	0.6583
Nepal	42	3	2	0.3821
Taiwan	29	3	6	0.1970
Thailand	54	9	10	0.7121
Vietnam	100	8	9	0.6541
All	1460	61	62	0.7020

Table 3. Haplotype information based on sequence

Haplotype	Sequence
Haplotype 1	*Syncerus caffer* (D82888)
Haplotype 2	*Bubalus arnee* (D32193)
Haplotype 3	*Bubalus mindorensis* (D82895)
Haplotype 4	*Bubalus quarlesi* (D82891)
Haplotype 5	*Bubalus depressicornis* (D82890)
Haplotype 6	JF946525, JF946524, EF409940, KR009082, KR009085-KR009088, KR009091-KR009093, KR009119, KR009125, KR009130, KR009133, KR009135, KR009148, KR009155, KR009157, KR009159, KR009161, KR009162, KR009195, KR009207, KR009208, KR009233, KR009235, KR009238, KR009947, KR009948, KR009954, KR009965, KR009966, KR009972-KR009974, KR009985
Haplotype 7	JF946523
Haplotype 8	JF946522
Haplotype 9	JF946521
Haplotype 10	JF946520
Haplotype 11	JF946519
Haplotype 12	D82894, FJ467720, KR009887, KR009899, KR009900
Haplotype 13	BK6, BK7, BK8, BK9, BK14, BK15, BK21, BK24, BK28, BD1, BD4, BD7, BD9, BD11, BD13, BD14, BD15, BD19, BD25, KL4, KL5, KL6, KL9, KL10, KL11, KN2, KN11
Haplotype 14	BK12
Haplotype 15	BK18
Haplotype 16	KL1, KL2, KL7, KL8, KLB1, KLB2, KN8, KN9, KN10, KN13, TRB1, TRB2, TRB3, TRB4, TRB5, TRP6, TRP8, TRT13, TRT14, TRT15, NTB1, NTB2, NTB3, NTB5, NTB6, NTB7, NTB8, NTB9, NTB10, NTB11, BTN1, BTN2, BTN3, BTN4, BTN5, NAD1, NAD2, NAD3, FJ467715-FJ467719, FJ467721, FJ467725, FJ467727, FJ467729, FJ467761-FJ467779, FJ467781-FJ467917, KR009079, KR009081, KR009090, KR009094, KR009096, KR009099, KR009101, KR009103, KR009105, KR009106, KR009111, KR009167-KR009169, KR009172, KR009174, KR009176-KR009178, KR009181-KR009186, KR009192, KR009193, KR009202, KR009205, KR009214, KR009219, KR009223, KR009225, KR009226, KR009228, KR009229, KR009234, KR009243-KR009246, KR009249, KR009251-KR009254, KR009259, KR009262-KR009265, KR009267, KR009272-KR009274, KR009276, KR009277, KR009279, KR009280, KR009282, KR009285, KR009288, KR009290-KR009292, KR009295, KR009298, KR009303, KR009304, KR009307-KR009310, KR009314, KR009316, KR009318-KR009320, KR009324, KR009328-KR009331, KR009333, KR009341-KR009346, KR009348, KR009350-KR009352, KR009356-KR009364, KR009366, KR009367, KR009369, KR009370-KR009372, KR009375, KR009376, KR009379, KR009381, KR009383-KR009387, KR009389, KR009391-KR009393, KR009396-KR009408, KR009410, KR009411, KR009416, KR009421, KR009424, KR009428-KR009431, KR009433, KR009435, KR009437-KR009440, KR009442-KR009455, KR009457, KR009459-KR009461, KR009463, KR009465-KR009480, KR009483-KR009486, KR009488, KR009491-KR009498, KR009500-KR009502, KR009504-KR009507, KR009509-KR009511, KR009513, KR009515-KR009518, KR009520-KR009522, KR009524-KR009526, KR009528-KR009530, KR009532-KR009537, KR009539, KR009541-KR009550, KR009552, KR009554, KR009556-KR009563, KR009565, KR009568-KR009571, KR009575, KR009579-KR009581, KR009583, KR009585-KR009587, KR009589-KR009594, KR009596-KR009599, KR009603-KR009605, KR009607, KR009609, KR009611, KR009612, KR009615-KR009618, KR009620, KR009622-KR009624, KR009629, KR009632, KR009633, KR009636-KR009644, KR009688, KR009689, KR009692-KR009696, KR009698-KR009700, KR009702-KR009707, KR009710, KR009712, KR009714-KR009717, KR009719, KR009720, KR009723, KR009725, KR009726, KR009729, KR009730, KR009733, KR009736-KR009738, KR009740-KR009743, KR009745-KR009748, KR009750, KR009752, KR009754, KR009757, KR009758, KR009761, KR009762, KR009764, KR009765, KR009768-KR009770, KR009772, KR009773, KR009777, KR009780-KR009784, KR009786-KR009791, KR009793, KR009795-KR009798, KR009800, KR009801, KR009803, KR009804, KR009806, KR009807, KR009810, KR009812, KR009815, KR009818-KR009824, KR009826, KR009829-KR009831, KR009833, KR009834, KR009836, KR009838, KR009839, KR009842-KR009844, KR009847, KR009848, KR009850, KR009851, KR009856, KR009860, KR009861, KR009867, KR009868, KR009870, KR009871, KR009872, KR009874, KR009881, KR009884, KR009888-KR009890, KR009893, KR009897, KR009901-KR009904, KR009907, KR009911-KR009913, KR009922, KR009924-KR009929, KR009932-KR009937, KR009939-KR009941, KR009943, KR009986, KR009987, KR009990, KR009994-KR009996, KR009998, KR010000, KR010001, KR010004, KR010005, KR010007, KR010008, KR010012-KR010014, KR010018, KR010019, KR010021, KR010022, KR010027-KR010029, KR010030, KR010034, KR010039-KR010044, KR010046-KR010058, KR010060-KR010071, KR010073-KR010075, KR010079, KR010081, KR010086, KR010088, KR010090, KR010091, KR010094-KR010096, KR010098-KR010100, KR010102, KR010106, KR010108, KR010109, KR010112, KR010114-KR010116, KR010118, KR010121, KR010124-KR010126, KR010128, KR010129, KR010133-KR010136, KR010139, KR010144-KR010149, KR010152-KR010154, KR010156-KR010159, KR010161, KR010163, KR010165, KR010168
Haplotype 17	KL3
Haplotype 18	KN1, KN4, KN6
Haplotype 19	KN3, KN5, KN7, KN12, KN14
Haplotype 20	NAD9
Haplotype 21	NAD10
Haplotype 22	FJ467648, EF409942, KR009200, KR009231, KR009650, KR009653, KR009667, KR009671, KR009955
Haplotype 23	FJ467649-FJ467657, EF409941, KR009069-KR009077, KR009080, KR009083, KR009084, KR009089, KR009102, KR009104, KR009107, KR009108, KR009112, KR009117, KR009118, KR009121-KR009124, KR009126, KR009127, KR009129-KR009132, KR009134, KR009136-KR009139, KR009141-KR009144, KR009147, KR009149-KR009154, KR009156, KR009158, KR009160, KR009163, KR009164-KR009166, KR009194, KR009196, KR009198, KR009199, KR009201, KR009203, KR009204, KR009209, KR009210, KR009212, KR009215, KR009227, KR009230, KR009236, KR009239-KR009241, KR009645-KR009649, KR009651, KR009652, KR009654-KR009666, KR009668-KR009670, KR009672-KR009686, KR009944-KR009946, KR009949-KR009953, KR009956-KR009964, KR009967-KR009971, KR009975-KR009984
Haplotype 24	FJ467658, FJ467659, FJ467687-FJ467699, FJ467703-FJ467707, KR009170, KR009171, KR009175, KR009179, KR009180, KR009187-KR009191, KR009206, KR009232, KR009296, KR009299 KR009301, KR009321, KR009353, KR009355, KR009365, KR009378, KR009380, KR009382, KR009388, KR009394, KR009412, KR009413, KR009478, KR009503, KR009508, KR009512, KR009514, KR009527, KR009538, KR009551, KR009555, KR009564,KR009566, KR009573, KR009606, KR009608, KR009621, KR009626, KR009627, KR009630, KR009635, KR009697, KR009728, KR009731, KR009732, KR009749, KR009771, KR009817, KR009823, KR009905, KR010032, KR010072, KR010101, KR010110, KR010117, KR010120, KR010123, KR010150

Haplotype	Sequence
Haplotype 25	FJ467660-FJ467685, KR009188, KR009216, KR009248, KR009255, KR009258, KR009268, KR009270, KR009275, KR009281, KR009283, KR009284, KR009287, KR009293, KR009294, KR009300, KR009374, KR009377, KR009414, KR009417, KR009418, KR009422, KR009456, KR009458, KR009462, KR009464, KR009479, KR009482, KR009487, KR009489, KR009519, KR009523, KR009576, KR009578, KR009582, KR009584, KR009588, KR009595, KR009610, KR009625, KR009628, KR009687, KR009691, KR009708, KR009711, KR009718, KR009744, KR009759, KR009792, KR009816, KR009837, KR009840, KR009841, KR009845, KR010057, KR010083, KR010087, KR010093, KR010119, KR010127, KR010164
Haplotype 26	FJ467678-FJ467680, KR009213, KR009217, KR009306, KR009349, KR009373, KR009409, KR009499, KR009577, KR009613, KR009614, KR009619, KR009701, KR009709, KR009785, KR009794, KR010085, KR010122
Haplotype 27	FJ467686, KR009930, KR009931, KR009938, KR009992, KR010002, KR010009, KR010020, KR010023, KR010037, KR010078
Haplotype 28	FJ467700, FJ467708, KR009097, KR009113, KR009221, KR009222, KR009224, KR009312, KR009315, KR009332, KR009334, KR009347, KR009390, KR009481, KR009567, KR009805, KR009811, KR009858, KR009859, KR009865, KR009878, KR009879, KR009906, KR009909, KR009910, KR009914, KR009915, KR010003, KR010006, KR010031, KR010076, KR010077, KR010082, KR010089, KR010113, KR010131, KR010132, KR010137, KR010160
Haplotype 29	FJ467701, FJ467702
Haplotype 30	FJ467709
Haplotype 31	FJ467710, FJ467711
Haplotype 32	FJ467712
Haplotype 33	FJ467713
Haplotype 34	FJ467714
Haplotype 35	FJ467722, FJ467723
Haplotype 36	FJ467724
Haplotype 37	FJ467726
Haplotype 38	FJ467728, FJ467731-FJ467760, KR009116, KR009173, KR009197, KR009218, KR009220, KR009237, KR009242, KR009247, KR009250, KR009256, KR009257, KR009260, KR009261, KR009266, KR009269, KR009271, KR009278, KR009286, KR009289, KR009302, KR009311, KR009313, KR009317, KR009322, KR009323, KR009325-KR009327, KR009336-KR009338, KR009340, KR009354, KR009368, KR009395, KR009415, KR009419, KR009420, KR009423, KR009425-KR009427, KR009432, KR009434, KR009436, KR009441, KR009531, KR009540, KR009559, KR009572, KR009574, KR009600-KR009602, KR009631, KR009713, KR009727, KR009734, KR009735, KR009739, KR009751, KR009753, KR009767, KR009774-KR009776, KR009778, KR009799, KR009802, KR009808, KR009809, KR009813, KR009814, KR009825, KR009849, KR009852-KR009855, KR009857, KR009862-KR009864, KR009866, KR009869, KR009873, KR009875-KR009877, KR009880, KR009882, KR009883, KR009885, KR009886, KR009891, KR009892, KR009895, KR009896, KR009898, KR009908, KR009916-KR009923, KR009942, KR009988, KR009989, KR009991, KR009993, KR009997, KR010010, KR010011, KR010016, KR010024-KR010026, KR010033, KR010045, KR010059, KR010080, KR010084, KR010092, KR010103, KR010105, KR010107, KR010111, KR010130, KR010138, KR010140-KR010143, KR010151, KR010155, KR010162, KR010166, KR010167
Haplotype 39	FJ467730
Haplotype 40	EF409939
Haplotype 41	KR009078
Haplotype 42	KR009095, KR009098, KR009114, KR009335, KR010015
Haplotype 43	KR009100, KR009110, KR009115
Haplotype 44	KR009109
Haplotype 45	KR009120, KR009140, KR009146
Haplotype 46	KR009128
Haplotype 47	KR009145
Haplotype 48	KR009211
Haplotype 49	KR009297
Haplotype 50	KR009305
Haplotype 51	KR009339
Haplotype 52	KR009490, KR009634, KR009724, KR009779
Haplotype 53	KR009553
Haplotype 54	KR009690
Haplotype 55	KR009755
Haplotype 56	KR009756
Haplotype 57	KR009760
Haplotype 58	KR009763
Haplotype 59	KR009766
Haplotype 60	KR009827, KR009828, KR009832, KR009835
Haplotype 61	KR009846
Haplotype 62	KR009894
Haplotype 63	KR009999
Haplotype 64	KR010017
Haplotype 65	KR010035
Haplotype 66	KR010097, KR010104

Table 4. Frequency of haplotype in ten countries

Haplotype	Countries									
	Pakistan	Indonesia	Bangladesh	China	India	Laos	Nepal	Taiwan	Thailand	Vietnam
6	5.41% (2)		54.06% (20)	13.51% (5)	2.70% (1)		24.32% (9)			
7	100% (1)									
8	100 % (1)									
9	100% (1)									
10	100% (1)									
11	100% (1)									
12		20% (1)		20% (1)		60% (3)				
13		100% (27)								
14		100% (1)								
15		100% (1)								
16		5.05% (38)	1.45% (11)	73.40% (552)		5.85% (44)		3.47% (26)	3.47% (26)	7.31% (55)
17		100 % (1)								
18		100% (3)								
19		100% (5)								
20		100% (1)								
21		100% (1)								
22				87.5% (7)	12.5% (1)					
23				61.18% (52)	1.18% (1)		37.64% (32)			
24				88.89% (72)		1.23% (1)			1.23% (1)	8.65% (7)
25				91.57% (76)		1.2% (1)				7.23% (6)
26				90% (18)						10% (2)
27				9.09% (1)		27.27% (3)			54.55% (6)	9.09% (1)
28			5.13% (2)	38.46% (15)		25.64% (10)			7.69% (3)	23.08% (9)
29				100% (2)						
30				100% (1)						
31				100% (2)						
32				100% (1)						
33				100% (1)						
34				100% (1)						
35				100% (2)						
36				100% (1)						
37				100% (1)						
38			0.58 (1)	60.82% (104)		19.88% (34)		1.17% (2)	7.02% (12)	10.53% (18)
39				100% (1)						
40					100% (1)					
41			100% (1)							
42			60% (3)	20% (1)					20% (1)	
43			100% (3)							
44			100% (1)							
45			100% (3)							
46			100% (1)							
47			100% (1)							
48				100% (1)						
49				100% (1)						
50				100% (1)						
51				100% (1)						
52				100% (4)						
53				100% (1)						
54				100% (1)						
55				100% (1)						
56				100% (1)						
57				100% (1)						
58				100% (1)						
59				100% (1)						
60				100% (4)						
61				100% (1)						
62						100% (1)				
63									100% (1)	
64									100% (1)	
65									100% (1)	
66										100% (2)

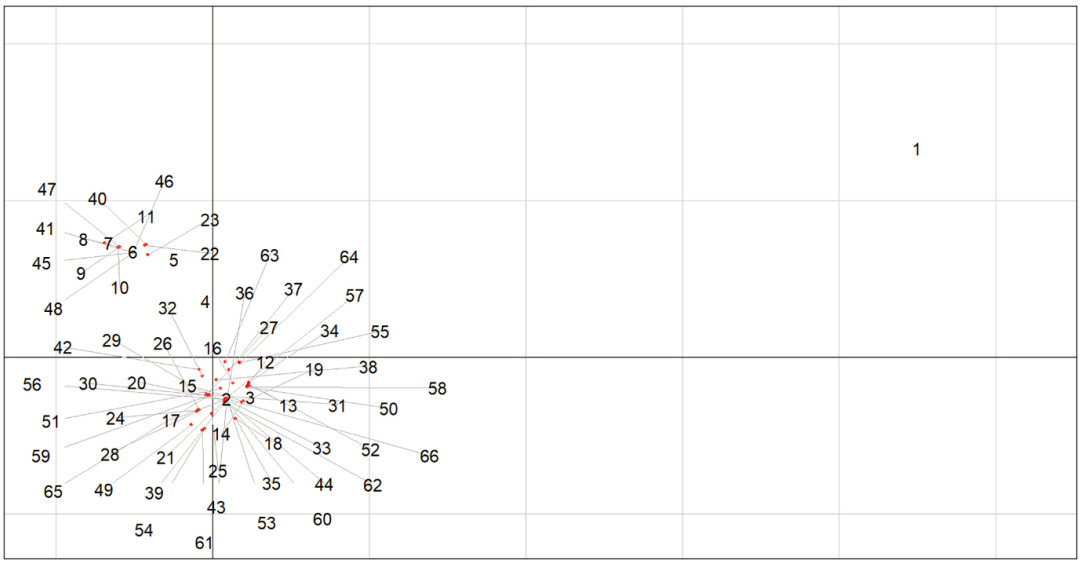

Figure 1. Principal coordinate analysis of haplotype based on cytochrome B

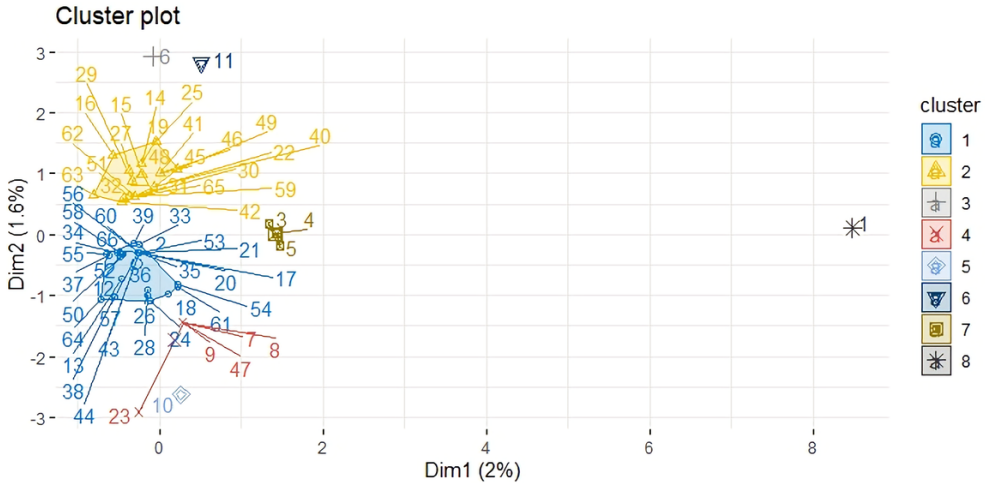

Figure 2. Hierarchical clustering on principal components of haplotype based on cytochrome B

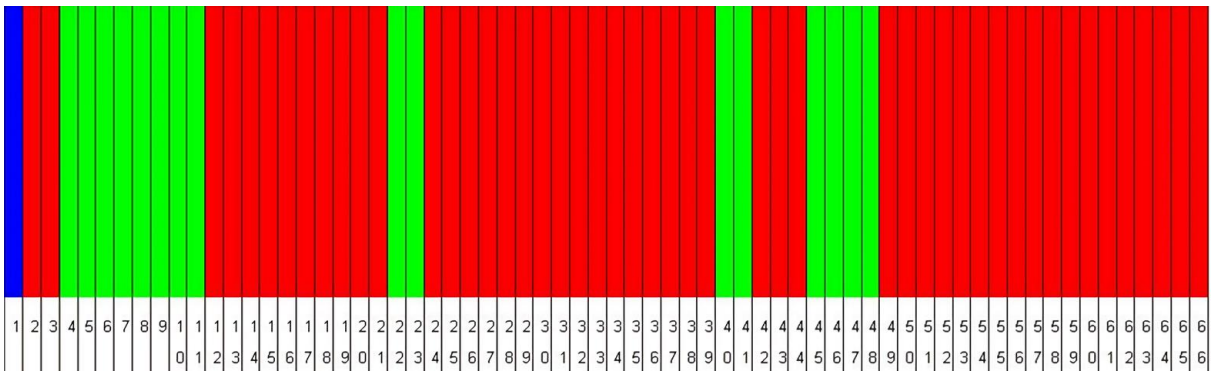

Figure 3. Bayesian analysis of cytochrome B gene haplotypes by BAPS software. Number represent haplotype and color represent cluster (blue= cluster 1, red= cluster 2, and green= cluster 3).

words, the domestication of wild water buffalo occurred on the border of South and East Asia. Rusdin *et al*. (2020) found 10 haplotypes and 9 polymorphic sites for swamp buffalo in Indonesia. Based on this meta-analysis study, we found 8 unique haplotypes of Indonesian swamp buffalo.

Lei *et al*. (2007) found two maternal lineages in Chinese Native Swamp Buffaloes. We got different results because we added sequences carried out by Zhang *et al*. (2016) so that we found four maternal lineages of swamp buffalo in China and Bangladesh. Based on the complete mitochondrial genome of swamp buffalo revealed eight maternal lineages and this evidence support initial major domestication of swamp buffalo, probably between southern China and Vietnam (Wang *et al*., 2017). River and swamp buffaloes were domesticated independently based on these findings. Using the whole genome sequencing, Luo *et al*. (2020) found swamp and river buffaloes shared a common ancestor 1.1-3.5 million years ago. Based on the results of this meta-analysis, it is possible that the domestication of swamp buffalo occurred in East-South Asia.

CONCLUSION

Based on this study, we found four maternal lineages and unique haplotypes for swamp buffaloes, also two maternal lineages for river buffaloes as an outgroup in this study. Based on this research, we suggested that domestication of swamp buffalo occurred in East-South Asia, and domestication of river buffalo occurred in South Asia. Further research with a large sample size in Asian countries is needed to determine the exact number of maternal lineages. Meta-analysis can contribute to a good understanding of the overall data that has been studied to provide comprehensive conclusions.

CONFLICT OF INTEREST

Cece Sumantri serves as an editor of the Tropical Animal Science Journal, but has no role in the decision to publish this article. The authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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