Phylogenetic analysis of Gayo and Toraya buffalo breed of Indonesian through mitochondrial D-loop region

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Abstract. The objective of this research was to find the basic data on genetic diversity of DNA D-Loop on Gayo and Toraya buffalo breed and its association with 6 local buffalo (Aceh, Sumut, Riau, Banten, Jateng, NTB, Sulsel). To the best of our knowledge this is the first published data on the complete mitochondrial D-Loop sequence of Gayo and Toraya buffalo. There were 83 samples of DNA which had been sequence; i.e. 45 from Gayo, 5 from Simeulue, 3 from Aceh Besar, Sumut and Riau, 4 from Banten, Jateng, NTB and Sulsel, 8 from Toraya. Results show that Toraya buffalo have a specific haplotype, while gayo buffalo have a closer relationship to Sumut buffalo. The total nucleotide sequenced of Gayo and Toraya buffalo and 6 local buffalo was 317 bp. The genetic distance ranges among Gayo buffalo, Riau, Jateng, Sulsel, NTB (0.010), Gayo buffalo-Sumut buffalo (0.016), and Gayo buffalo-Toraya buffalo (0.023). D-Loop mtDNA analyses showed that most of Gayo buffalo form a haplotype together with six other local buffalo populations, while the Toraya buffalo (striped buffalo) population forms one haplotype that is separate from the entire local Indonesian buffalo population. These finding could be assumed that the Toraya buffalo (striped buffalo) originating from Enrekang forms a specific haplotype which identifies that the Toraya buffalo (striped buffalo) originating from Enrekang is animal genetic resource of native livestock that must be preserved.

1. Introduction

Buffalo, compared to cattle, have not received much attention in improving productivity through the introduction of appropriate technology, so that their production and reproductive performance are still low. Maintaining local livestock resources is important for achieving sustainable food security. The basic capital for the development of the livestock sub-sector is largely determined by ownership, preservation and utilization of livestock biological resources. Therefore, in order to reduce the rate of extinction, especially in local Indonesian livestock, one thing that must be done is efforts to characterize genetic diversity in local livestock. Warwick et al (1995) have stated that the main unit of livestock genetic resources can be a breed, type and line (variety) [1]. The main unit of genetic resources can also be geographically isolated herds. Each unit of the genetic resource has certain morphological characteristics in common that can be distinguished from other groups, related to the natural distribution process of livestock and livestock evolution. Population distribution is a form of ecological and evolutionary processes that have long-term consequences [2]. Topographical separation such as rivers or mountains and governmental jurisdictions such as provincial (country) boundaries can cause actual
morphological diversity, therefore it is necessary to study genetic information such as levels of genetic variation and genetic kinship relationships within and between livestock populations [3].

The Toraya buffalo is a local livestock and one of the animal genetic resources of Enrekang and Toraja Regencies, South Sulawesi Province, whereas buffalo in Gayo are livestock cultivated by Gayo residents who live in the lowlands, such as Linge sub-district in Central Aceh, Uber-Uber and Samarkilang areas in Bener Meriah, and in Gayo Lues. Long before Gayo was known as the largest Arabica coffee producing area in Asia, Gayo was already known as a buffalo livestock (Bubalus bubalis). The way of maintaining it is closely related to the culture and local wisdom in Gayo, so that the maintenance of the Gayo buffalo has its own uniqueness. The existence of the Gayo buffalo and the Toraya buffalo must be preserved. Research and publications on the genetic resources of Gayo buffalo and Toraya buffalo are still limited, both qualitatively and quantitatively. The origin and kinship of the Gayo buffalo. Toraya buffalo and other local Indonesian swamp buffalo have also not been reported.

Several studies have been conducted to determine the genetic distance of buffalo, which identified that the Simeulue buffalo had a close maternal genetic relationship with the Riau buffalo, compared to the buffalo from Aceh Besar [4]. In fact, Aceh Besar and Simeulue districts are in the jurisdiction of one Aceh province. Gayo buffalo is a buffalo in Aceh Province, but there is no data and research that explains the kinship relationship and genetic distance between Toraya buffalo and Gayo buffalo. The purpose of this study was to identify the genetic diversity of the Mitochondrial DNA D-Loop area in Toraya buffalo, Gayo buffalo and six local swamp buffalo subpopulations and to obtain information on genetic distances between local Indonesian swamp buffalo populations. The specific target to be achieved is to be able to identify phylogenetic relationships between species (Toraya buffalo and Gayo buffalo). The desired long-term goal is the prevention of erosion of the local swamp buffalo genetic resources, which requires the direction, programming and design of action of local buffalo conservation strategies on a national scale.

So that it can define the distribution of various local swamp buffalo populations between various provinces on the basis of the consistency of their genetic uniqueness, so that the suitability of the conservation area scale and management of the use of genetic resources from our local swamp buffalo can be determined. The results of this study can be useful and are expected to be used as basic information for consideration in determining national buffalo breeding policies.

2. Research method
This study consisted of two stages, namely taking blood samples and tail feathers in the field followed by molecular analysis in the laboratory. Research locations in the field in Enrekang and Kab. Sidrap, Kab.Aceh Tengah and Kab. Gayo Lues. DNA extraction was carried out at the Laboratory of Genetics and Animal Breeding, Faculty of Animal Husbandry, IPB, and sequencing was carried out at the 1st Base Selangor Laboratory. The research material was the population of the Toraya buffalo and the Gayo buffalo and the DNA of six Indonesian local buffalo. Gayo buffalo blood sampling from the jugular vein, using a 5 ml venoject tube, then stored in an ice box (ice box) to be brought to the Lab. Science of Breeding and Reproduction, Department of Animal Husbandry FP Unsyiah, then the implementation of DNA extraction at the Laboratory of Genetics and Animal Breeding, Faculty of Animal Husbandry IPB. Several stages were carried out, namely: collection of blood samples and tail feathers samples, DNA isolation, PCR, sequencing. Blood was drawn from the jugular vein about 5 ml using a vacutainer tube that was filled with EDTA. The procedure for the isolation of DNA from blood is based on the standard phenolchloroform method [5]. After the isolated DNA is purified and the concentration is known, it is prepared as a template for the Polymerase Chain Reaction (PCR) reaction. DNA isolation carried out at the Laboratory of Genetics and Animal Breeding, Faculty of Animal Husbandry, IPB. PCR product electrophoresis using Alpha Imager machine (alpha innotech) using Alphalmager EP software. PCR products are sent to the Selangor 1st Base Laboratory for sequencing. Sequence results from 1st Base Selangor laboratory will be analyzed using MEGA version 6 [6].
3. Results and discussion
According to Marsan (2015), the swamp buffalo was first domesticated as domesticated livestock in Thailand. Then migrated to the north (China) and south (Sumatra) [7]. From China, the spread of livestock spreads to the Philippines, while from the Indonesian island of Sumatra, livestock migration is widespread to the islands of Java, Sulawesi and Nusa Tenggara. The Sumatran buffalo has a genetic quality that is relatively superior to buffalo from other islands in Indonesia, due to its genetics directly from Thailand. Barker et al (1991) and Tulloh et al (1992) stated that there is very little information on the history of the distribution of mud buffalo in Asia [8,9]. However, it is believed that buffalo have spread throughout the region hundreds of years ago to occupy the Indonesian archipelago. Buffalo farming can adapt to the island environment where they live and are isolated from others, so that they reproduce naturally and even the possibility of deep cross-breeding. In addition, it is stated that there is very little migration between populations and marriage unless it is carried out by humans so that locally there is not much difference. Barker et al (1991) are in line with the results of research conducted by Sari et al (2014) who concluded from the electrophoresis test and DNA sequencing of the Gayo Buffalo that genetically the Gayo buffalo is a type of mud buffalo and since its inception in the Gayo Highlands has never been contaminated by other buffalo genes [4,8].

Molecular determination of the Gayo buffalo has been carried out through mtDNA analysis in the D-Loop area [10], and used buffalo outgroups (Aceh Besar, Simeulue, North Sumatera, Riau, Banten, Central Java, NTB, and South Sulawesi). In figure 1, two different clusters can be identified, where the populations of the Simeulue buffalo and the Riau buffalo form a cluster that is separate from the population of the Gayo buffalo and other local buffalo. The Gayo buffalo population has a close maternal genetic relationship with buffalo populations in NTB and North Sumatra, compared to other populations. Likewise, the buffalo population originating from Aceh Besar has a close genetic relationship with the buffalo population from NTB.

![Figure 1. Phylogenetic population.](image)

![Figure 2. Philogeny of Gayo Buffalo, Toraya Buffalo and six Indonesian local buffalo populations.](image)
One of the animal germplasm that needs to be maintained is the Belang buffalo (*Bubalus bubalis*) a type of mud buffalo with black and white striped skin (Caucasians). The original habitat of this buffalo is in Tana Toraja, South Sulawesi Province, so this buffalo is often called the Tana Toraja buffalo. Based on D-Loop analysis, sequencing of Gayo buffalo, Toraya buffalo and six other buffalo populations illustrates the genetic variation within and between populations. In the NJ-tree, genetic correlation between mtDNA sequences illustrates that samples originating from various regions form the same cluster/group (figure 2).

**Figure 2.** Three different clusters can be identified, where the population of Aceh buffalo and buffalo from North Sumatra form one cluster, while buffalo from Banten, Central Java, NTB, Riau and South Sulawesi form a separate cluster, while the Belang buffalo (Toraya) forms one separate cluster.

**Figure 3.** NJ dendogram based on the 2 parameter method Kimura (317 bp) Gayo buffalo, Toraya buffalo and six Indonesian local buffalo populations.

In figure 2, three different clusters can be identified, where the population of Aceh buffalo and buffalo from North Sumatra form one cluster, while buffalo from Banten, Central Java, NTB, Riau and South Sulawesi form a separate cluster, while the Belang buffalo (Toraya) forms one separate cluster.
separate from the population of the Gayo buffalo (Aceh) and other local buffaloes. The Gayo buffalo population (Aceh) has a close maternal genetic relationship with the buffalo population of North Sumatra, compared to other populations. It can be concluded that the Toraya (striped) buffalo is a unique population because it forms a separate cluster and is separate from other local buffalo populations in Indonesia. On the dendogram (figure 3) it is clear that most of the Gayo buffalo form a haplotype together with six other local buffalo populations, while the Toraya buffalo population (striped buffalo) forms one haplotype separate from the entire local Indonesian buffalo population. This phenomenon illustrates that the Toraya buffalo (striped buffalo) originating from Enrekang forms a specific haplotype which identifies that the Toraya buffalo (striped buffalo) originating from Enrekang is a genetic resource of native livestock that must be preserved.

Analysis of pairwise distance calculation with the 2-parameter Kimura model was used to analyze genetic distance or the proximity of the genetic relationship of Gayo, Toraya buffalo, and six other local buffalo populations. The genetic distance of Gayo buffalo, Toraya buffalo and other local buffalo ranges from 0.000 to 0.025. The largest genetic distance between the Gayo buffalo and the Toraya buffalo is 0.023. The genetic distance between buffalo populations can be seen in Table 1.

|          | Gayo | Banten | Sumut | Jateng | NTB  | Sulsel | Riau | Toraya |
|----------|------|--------|-------|--------|------|--------|------|--------|
| Gayo     |      | 0.010  |       |        |      |        |      |        |
| Banten   |      |        | 0.016 | 0.012  |      |        |      |        |
| Sumut    |      |        | 0.010 | 0.000  | 0.012|        |      |        |
| Jateng   |      |        | 0.010 | 0.000  | 0.012| 0.000  |      |        |
| NTB      |      |        | 0.010 | 0.000  | 0.012| 0.000  |      |        |
| Sulsel   |      |        | 0.010 | 0.000  | 0.012| 0.000  | 0.000| 0.000  |
| Riau     |      |        | 0.010 | 0.000  | 0.012| 0.000  | 0.000| 0.000  |
| Toraya   | 0.023| 0.013  | 0.025 | 0.013  | 0.013| 0.013  | 0.013| 0.013  |

The sequence of close genetic relationships of all buffalo samples used from the closest to the farthest is the Gayo buffalo - the buffalo in Riau, Central Java, South Sulawesi, NTB (0.010), the Gayo buffalo - the North Sumatra buffalo (0.016), and the Gayo buffalo-the Toraya buffalo (0.023). The genetic distance value of some buffalo samples used was less than 0.5, meaning that in general the buffaloes were genetically closely related.

4. Conclusion
Based on the analysis of the mtDNA D-Loop area, the results obtained have proven that the Gayo buffalo and the Toraya buffalo are specific populations compared to other local buffalo populations in Indonesia, having a far genetic distance compared to other local buffalo. This phenomenon illustrates that Toraya buffalo form a specific haplotype which identifies that Toraya buffalo originating from Enrekang Regency is a genetic source of native buffalo livestock that must be preserved.

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[10] Sari E M, Abdullah M A N, Wahab A, Manan and Azim 2016 Pengkajian akademisi pemurnian plasma nutfah kerbau gayo 2016 Laporan Kerja sama Penelitian Jurusan Peternakan Fakultas Pertanian Universitas Syiah Kuala dengan Dinas Kesehatan Hewan dan Peternakan Provinsi Aceh dan Dinas Peternakan dan Perikanan Kabupaten Aceh Tengah (Aceh: Universitas Syiah Kuala)