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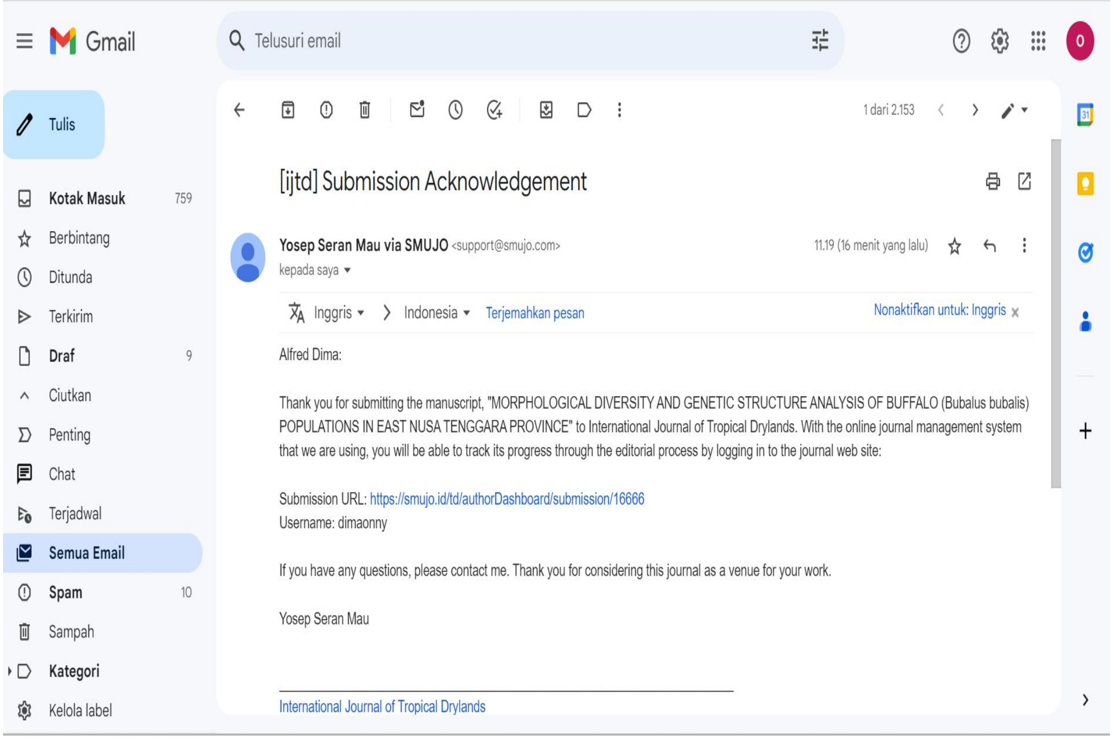

[ijtd] Submission Acknowledgement

Yosep Seran Mau via SMUJO <support@smujo.com>
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Alfred Dima:

Thank you for submitting the manuscript, "MORPHOLOGICAL DIVERSITY AND GENETIC STRUCTURE ANALYSIS OF BUFFALO (Bubalus bubalis) POPULATIONS IN EAST NUSA TENGGARA PROVINCE" to International Journal of Tropical Drylands. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Appendix

MORPHOLOGICAL DIVERSITY AND GENETIC STRUCTURE ANALYSIS OF BUFFALO (*Bubalus bubalis*) POPULATIONS IN EAST NUSA TENGGARA PROVINCE

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Abstract. This research, conducted in 2023, aims to elucidate morphometric diversity and population structure among indigenous buffaloes in East Nusa Tenggara (NTT), Indonesia. The investigation involves 500 buffaloes from four different islands in NTT, with analysis based on 9 morphometric characters. Using Principal Component Analysis, we evaluate morphometric diversity, while the genetic structure of the buffalo population is estimated through the high and low genetic diversity, both in the number and diversity of haplotypes (h) and nucleotides (π). Indigenous buffaloes on the four islands show significant morphometric diversity, where the nine morphometric characters of the mud buffalo can be used as differentiators between populations on the islands of Sarai, Rote Ndao, Flores, and Sumba. The results of Principal Component Analysis state that body length has the highest weighting score as a characteristic of the size of the mud buffalo population in NTT. The analysis of genetic diversity in the mud buffalo population in NTT shows the existence of 2 haplotypes with a nucleotide diversity value (π) of 0.12%, which is considered low.

Keywords: Indigenous buffaloes of NTT, morphometric diversity, Principal Component Analysis, haplotypes.

Buffalo (*Bubalus bubalis*) population in East Nusa Tenggara (NTT) was distributed across 18 regencies, totaling 175,007 individuals in 2019. This population increased to 179,708 individuals in 2020 but decreased to 172,850 individuals by 2023. A similar pattern is observed in the amount of buffalo meat slaughtered for animal protein needs in NTT, with an increase from 1,624,505 kg in 2019 to 2,148,277 kg in 2021. However, in 2022, buffalo meat production declined to 1,232,632.48 kg (BPS Province of NTT 2023). The reduction in the buffalo population in various areas in NTT is attributed to a higher slaughter rate compared to the limited reproductive performance and productivity of buffalo parents (Anggraeni and Triwulaningsih, 2007).

The decrease in the NTT buffalo population is considered to jeopardize the genetic reservoir of buffaloes (Islam *et al.,* 2020; Qiptiyah *et al.,* 2019). Consequently, precautionary measures are imperative, including the examination of morphometric diversity and analysis of phenotypic relationships among indigenous buffaloes. Morphometric characterization offers a through depiction of buffalo genetic diversity (Sari *et al.,* 2023; Ali *et al.*,2023). Morphometric traits are widely employed as standards in livestock selection (Ilham *et al.*,2023; Yilmaz *et al.*, 2013) and can function as criteria demonstrating the potential of livestock (Ramos-Zapata *et al.,*2023).

This study is an initial step in determining the appropriate selection criteria for the implementation of a sustainable selection program or quality improvement. Research on these buffaloes is still limited to a small population and has not yet covered a wide geographic distribution of the larger population. Therefore, information on morphometric diversity combined with molecular data, such as the genetic distance between mud buffaloes in various districts in East Nusa Tenggara (NTT), is still very scarce.

Research on these buffaloes is still limited to a small population and has not yet spread geographically to a larger population. Therefore, information on the genetic distance between local swamp buffaloes in various provinces in Indonesia is still very scarce. This study aims to: (1) study morphometric diversity and identify variables that can distinguish buffalo populations from 10 districts (Sumba mainland, Ngada, Rote-Ndao, West Manggarai, Manggarai, Kupang, and Sabu Raijua). (2) estimate the genetic distance between the 10 buffalo populations. The results of this research are expected to serve as fundamental information for consideration in determining national buffalo breeding policies.

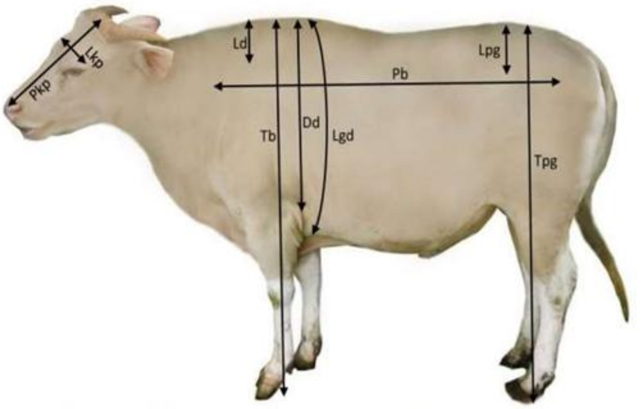
MATERIALS AND METHODS

Study area

This research was conducted from May to September 2023 in 10 districts of the East Nusa Tenggara (NTT) Province, which have the highest population of buffaloes, for morphometric measurements and blood sample collection from buffaloes. The data collection method involved a survey and purposive sampling for determining locations based on the groups of livestock with the highest populations in each district. The selected locations represented subpopulations and breeding sources, with livestock sampling done randomly. The determination of research locations in each district (10 in total) was based on various considerations, such as areas with concentrated buffalo populations and guidance from local government authorities. In some districts, such as Southwest Sumba, East Sumba, West Sumba, Rote-Ndao, Ngada, and Sabu-Raijua, the research locations were spread across several sub-districts. Livestock management at all research locations involved grazing during the day and penning in the evening. The selection of these ten locations was based on the highest concentration of buffalo populations. In other areas, buffaloes were sporadically found and kept by local communities in small numbers. The chosen sample for the study comprised adult male and female buffaloes aged ≥ 3 years (Fahimuddin, 1975).

Morphometric characteristics and data collection

The samples were randomly selected from those available in each sub-district. Wirartha (2006) stated that for research utilizing statistical data, a sample size of at least 30% is sufficient to represent the population. Variables measured included phenotypic characteristics related to quantitative traits. A total of 9 quantitative traits were measured, covering linear measurements such as morphometric variations in head width, head length, chest width, body height, body length, hip width, hip height, and chest circumference, using measurement methods as outlined by Sarbaini (2004) and Abdullah (2008).



Gambar 1. Ukuran-ukuran tubuh sapi (Lkp = lebar kepala, Pkp = panjang kepala, Ld = lebar dada, Tb = tinggi badan, Dd = dalam dada, Lgd = lingkar dada, Pb = panjang badan, Lpg = lebar panggul, Tpg = tinggi panggul).

Sampling and DNA extraction

Blood samples from the jugular vein of unrelated male and female buffaloes were collected for mitochondrial DNA D-Loop analysis from various villages in the buffalo breeding area of Diara. DNA isolation followed the standard SDS-Proteinase-K protocol as described by Sambrook and Russel (2001). Primers used to amplify the mitochondrial D-loop segment were designed based on the complete mitochondrial sequence of buffaloes available in the GenBank database (Accession No. AF547270). The primer set used was F: 5’ AGTCCAAGCATCCCCCAAAATAAA 3’; R: 5’ CGGCCAGCATAATCGAAA 3’. The polymerase chain reaction (PCR) cycle conditions included initial denaturation at 95 °C for 3 minutes followed by 32 cycles of 94°C for 30 seconds; 58°C for 30 seconds; 72°C for 1 minute with a final extension at 72°C for 10 minutes. The amplified products were analyzed on a 1% TAE-agarose gel. After PCR product purification, sequencing was performed using the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA, USA) on an automated DNA sequencer ABI 3130xl.

Statistical analysis

The research data analysis encompassed the comparison of phenotypic and genetic morphological variations in the body, as well as the determination of genetic relationships among local mud buffaloes across the ten populations in the ten research districts. Morphometric data were utilized to compute averages, standard deviations, and coefficients of morphological diversity. The Duncan Multiple Range Test was applied to compare mean values among buffalo populations. Additionally, Principal Component Analysis (PCA) was employed to identify features related to body size and shape in both male and female buffaloes, derived from the covariance matrix (Gasperz, 1992).

The alignment of multiple edited nucleotide sequences was conducted using the MEGA Version 7 software. Analysis of the nucleotide sequences of the mtDNA D-loop haplotype in mud buffaloes involved comparing them with reported populations of mud buffaloes in Indonesia from the GenBank database. These populations included Chinese buffalo, Carabao, and Mediterranean buffalo, with Bos taurus and Bos indicus serving as out-groups (Lei *et al.,* 2007; Kierstein *et al.*, 2004; Kataria *et al.,* 2009; Mishra *et al*., 2010). The Neighbour-Joining method was applied to infer the evolutionary history ([Saitou N, Nei M, 1987). Phylogenetic analysis was performed using MEGA 7 (Kumar S, Stecher G, Tamura K, 2016). Various genetic diversity parameters were analyzed using DNAsp Version 5.0 (Librado and Rozas, 2009).

Results and Discussion

The Morphometric Diversity of the Buffalo (*Bubalus bubalis*) Population in East Nusa Tenggara (NTT)

Descriptive statistical analysis of the linear surface measurements of buffalo body in the population of East Nusa Tenggara (NTT) includes various dimensions, such as head width, head length, chest width, body height, body length, hip width, hip height, and chest circumference. Data on morphometric diversity for each observed variable are presented in Table 1.

Table 1. Morphometric Diversity of Buffaloes (*Bubalus bubalis*) in Four Populations in East Nusa Tenggara (NTT)

Parameters Measurements		**Buffalo in the Mainland of NTT**			
		Sumba	**Rote Ndao**	**Flores**	**Sarai**
Head Width	cm	19.74±3.21^{a}	17.89±4.01^{b}	19.89±3.40^{a}	17.47±3.89^{b}
Head Length	cm	51.36±0.50^{a}	48.98±2.04^{b}	52.05±2.06^{a}	49.01±1.89^{b}
Chest Width	cm	42.82±3.91^{a}	38.80±5.61^{b}	41.57±4.13^{b}	38.60±3.02^{b}
Body Height	cm	135.13±5.22^{a}	132.08±5.78^{a}	134.30±4.26^{a}	129±3.20^{b}
Chest depth	cm	80.59±3.54^{a}	79.30±5.51^{a}	84.86±6.67^{a}	76.05±3.12^{b}
Body Length	cm	143.00±2.47^{a}	136.67±4.97^{b}	145.57±7.32^{a}	135.06±4.20^{b}
Hip Width	cm	39.99±2.21^{a}	39.78±1.35^{a}	40.03±2.01^{a}	37.89±0.35^{b}
Hip Height	cm	137.95±5.11^{a}	130.80±5.76^{b}	139.63±4.50^{a}	132.45±4.20^{b}
Chest Circumference	cm	163.28±5.11^{a}	163.32±9.45^{a}	166.69±10.40^{a}	160.45±6.43^{b}

Note: Morphometric measurement data are presented as mean ± SD; different superscripts (a, b) in the same row for the 4 populations indicate significant differences ($p \leq 0.05$).

The results of the statistical analysis of mud buffalo body size on four islands, namely, Sabu Raijua, Rote Ndao, Flores, and Sumba, show significant differences in 9 morphometric characteristics ($p<0.05$). This indicates that various factors, including relatively diverse environmental variables such as age, management practices, and the quantity and types of feed, contribute to the differences in body size and weight among these subpopulations. Hardjosubroto (1994) stated that diversity can be caused by genetic and environmental influences, or even their interaction. Suitable habitat conditions and year-round feed availability on the islands of Flores and Sumba, characterized by high rainfall and humidity, become crucial factors significantly influencing the morphometric size diversity of mud buffaloes. Conversely, dry climate conditions with low rainfall and limited feed availability, primarily between January and May, will significantly impact the growth of mud buffaloes, especially during their growth period.

Principal Component Analysis (PCA) is used to gather data on the diversity of quantitative traits and determine which traits significantly influence that diversity (Soeroso, 2004). The results of the PCA analysis indicate that body length has the highest weighting score (0.99), followed by body height (0.60), head length (0.55), head width (0.50), chest width (0.40), chest circumference (0.28), hip circumference (0.08), and hip height (0.06). A study conducted by Harmayanti (2008) on male and female mud buffaloes found that body weight has the highest weighting score. The variable with the highest weighting score indicates that the variability process in that variable is faster compared to other variables.

Genetic Diversity of the Buffalo Population in East Nusa Tenggara (NTT)

Amplification results of the control region (d-loop) of local water buffalo (Bubalus bubalis) mtDNA spanned 1145 base pairs (bp). The mtDNA size of the swamp buffalo is approximately 16359 bp (QIAN et al., 2004). The amplification results were matched with the primer annealing sites on the mtDNA gene sequence of Bubalus bubalis (GenBank No. Acc. AY702618). Using the employed primer pairs, the amplification product covered from the cyt-b region to the d-loop region. The amplified target mtDNA control region is highly diverse and evolves more rapidly than other parts of the mitochondrial genome. The extent of genetic diversity, whether high or low, can be indicated by both the quantity and diversity of haplotypes (h) and nucleotides (π). The results obtained in this study identified two types of composite haplotypes, as presented in Table 2.

Table 2. Haplotype Diversity of mtDNA *Bubalus bubalis* Population on Sabu Raijua Island, Rote-Ndao, Flores, and Sumba

Population	h Value	Number of Samples	Number of Haplotypes
Sabu Raijua	0,0	3	1
Rote Ndao	0,1	5	1
Flores	0,2	15	2
Sumba	0,2	12	2

The number of composite haplotypes in a specific buffalo population is generally one, except for on Flores and Sumba Islands, which have two composite haplotypes. The distribution of composite haplotypes indicates the distinct characteristics of swamp buffaloes in each location. Overall, haplotype diversity (h) on Rote-Ndao and Sabu Raijua Islands is 0.0 (uniform), while on the mainland of Flores and Sumba, the haplotype diversity (h) value reaches 0.2. Despite having a higher number of haplotypes compared to the mainland of Rote-Ndao and Sabu Raijua, the haplotype diversity values on the mainland of Flores and Sumba are relatively low, indicating limited genetic diversity in the samples of buffaloes.

Based on the presence or absence of restriction sites in the two haplotypes, it was found that the nucleotide diversity (π) values in all four observed populations of mud buffaloes were 0.12%. Although this figure is slightly low compared to the study conducted by Tanaka *et al.* (1995), who investigated mitochondrial DNA cleavage patterns in river buffaloes and swamp buffaloes using fifteen restriction endonucleases. They found three types of mitochondrial DNA in swamp buffaloes and two types in river buffaloes, with nucleotide diversity ranging from 0.2% to 0.6% for swamp buffaloes and river buffaloes, and diversity of 1.9% to 2.4% for both types of buffaloes. The low nucleotide diversity in the buffalo population in East Nusa Tenggara is suspected to be due to excessive exploitation of mud buffalo livestock, both for trade between islands and the high social value of buffaloes, especially on Flores and Sumba Islands.

In conclusion, that all nine morphometric characteristics of mud buffaloes can be used as distinguishing factors between populations on Sarai Island, Rote-Ndao, Flores, and Sumba. The Principal Component Analysis (PCA) results state that the highest weighting score is body length, distinguishing the size of mud buffaloes in the East Nusa Tenggara population. Furthermore, the results of genetic diversity analysis of the mud buffalo population in East Nusa Tenggara show the presence of 2 haplotypes with a low nucleotide diversity value (π) of 0.12%.

Acknowledgments

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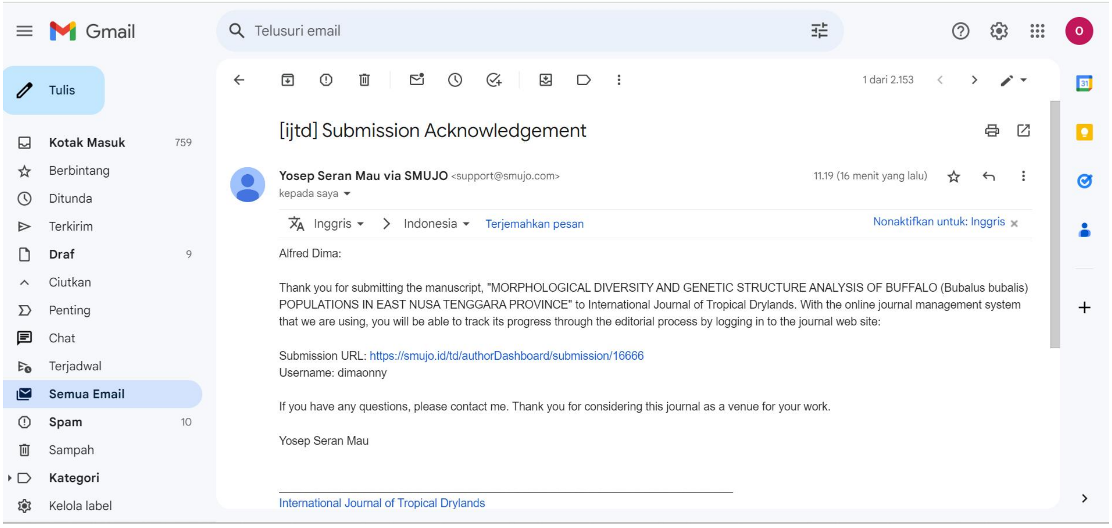

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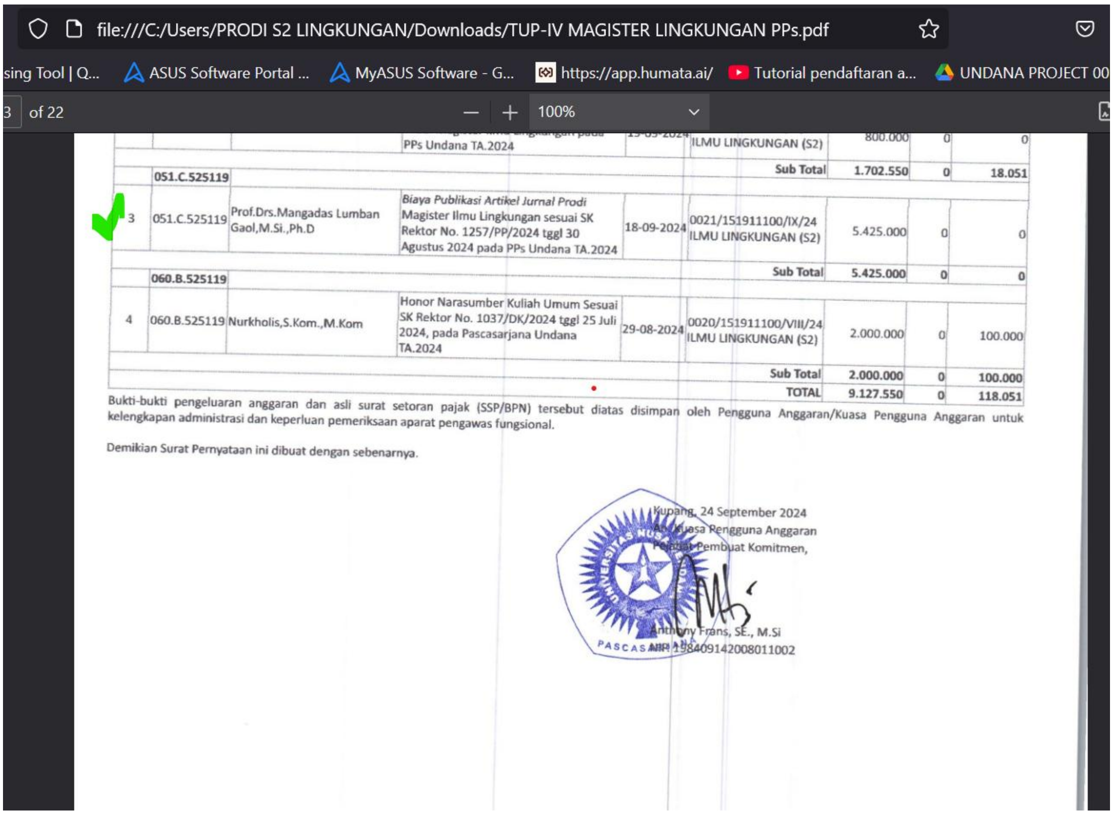

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