Livestock and Animal Research
Accredited by Directorate General of Strengthening for Research and Development No. 10/E/KPT/2019

Open Access
Livest. Anim. Res., November 2023, 21(3): 181-188
p-ISSN 2721-5326 e-ISSN 2721-7086
https://doi.org/10.20961/lar.v21i3.71611

Original Article

Genetic characterization and lineage analysis of Pasundan (Rancah) cattle in West Java, Indonesia: Insights from ZFY gene sequencing

Johar Arifin[1], Widya Pintaka Bayu Putra[2,*], Syahruddin Said[2], and Thobela Louis Tyasi[3]

[1]Faculty of Animal Science, Padjadjaran University, Jatinangor, West Java, 45363, Indonesia
[2]Research Center for Applied Zoology, National Research and Innovation Agency, Bogor, West Java, 16911, Indonesia
[3]Department of Agricultural Economics and Animal Production, School of Agriculture and Enviromental Sciences, University of Limpopo, Sovenga, 0727, South Africa
*Correspondence: widy008@brin.go.id

Received: February 17th, 2023; Accepted: November 24th, 2023; Published online: November 30th, 2023

Abstract

Objective: This study was carried out to characterize Pasundan (Rancah) cattle from West Java of Indonesia based on a partial Zinc Finger Y-linked (ZFY) gene sequence.
Methods: Total of fourteen (14) DNA samples of unrelated Pasundan bulls at the breeding station (BPPIBT-SP Ciamis, West Java) were used for sequencing analysis. The blood samples were collected from jugular vein of each bull for the DNA extraction analysis. Along 979 bp of ZFY gene was amplified using primer pairs of forward: 5′- GGT GAG GGC ACA TGA GTT C -3′and reverse: 5′- CTC TGC AGG TGG TTG TGT AA -3′. Therefore, a forward sequencing method was performed in the present study to obtain the ZFY gene sequences. Along 714 bp of *bovine* ZFY gene sequences were used for determining the Neighbor-Joining (NJ) tree for Pasundan bulls with 1000 × bootstrap replications using a MEGA computer program. The genetic diversity parameters of haplotype diversity (H_d), nucleotide diversity (p_i), Tajima's D test and Fu's Fs statistics were calculated using a DNAsp computer program.
Results: Total of fourteen (14) haplotypes of ZFY gene and seventy (70) mutation sites were detected in the Pasundan bulls. The haplotype diversity (H_d) and nucleotide diversity (p_i) in Pasundan's ZFY gene diversity were 1.00 (high) and 0.01 (low), respectively. The neutrality test of Tajima's D and Fu's Fs statistic values were -1.42 and -6.47, respectively. *Bovine* ZFY gene can be classified into two clades of ZFY-1 and ZFY-2. Therefore, the Neighbor-joining (NJ) tree (1000×bootstrap) revealed that the Pasundan cattle are classified into clade ZFY-1 together with *Bos taurus* (AF241273, AF465179). Meanwhile, a clade ZFY-2 consisted of *Bos taurus* (AF465181, DQ336536), *Bos indicus* (DQ336537), and *Bos javanicus* (DQ336578).
Conclusion: The ZFY gene of Pasundan bulls were polymorphic with seventy mutation sites. Therefore, Pasundan bulls were characterized into ZFY-1 clade or *Taurine* lineage based on ZFY gene sequence variaton. The crossbreeding program with *Bos taurus* straw to increase meat production of cattle may affecting the genetic structure of Pasundan bulls since this program is widely applied in West Java through many years ago.

Keywords: Haplotype; Pasundan bulls; Sequencing; West Java; ZFY gene

INTRODUCTION

Pasundan (Rancah) cattle is one of Indonesian native cattle that originated from West Java Province of Indonesia. Pasundan cattle have been decided as the Indonesian native cattle through the decision of the Indonesian Ministry of Agriculture No: 1051/Kpts/RI/SR.10/2014 [1]. Generally, Pasundan cattle are kept as beef cattle with an adult weight of 309.50±57.37 kg for males and 148.15±14.45 kg for females [2]. Pasundan bulls are able to reach 216.00±26.3 kg of slaughter weight; 110.75±16.5 kg of carcass weight and 53.42±2.08% of carcass percentage [3]. According to the mitochondrial DNA (mtDNA) of Cytochrome-b (Cyt-b) and D-loop genes, Pasundan cattle were closed to *Bos javanicus* [4,5]. In addition, Pasundan cattle was closed to Madura cattle (*Bos indicus*) based on the mitochondrial COI gene [6]. Unfortunately, a study to characterize Pasundan cattle based on Y-chromosomal diversity is not reported before. The Zinc Finger Y-linked (ZFY) gene is one of Y-chromosomal genes that used for characterization in Bovidae family species [7,8]. In the cattle, ZFY gene has the length of 20,780 bp with seven (7) exonic regions (GenBank: AF465181). Despite detecting paternal lineage, the ZFY gene can be used to identify the typical sex of buffalo [9]. In addition, the ZFY gene can be used to detect the presence of Y chromosome in the cow that affecting infertility [10].

Genetic characterization of livestock animals is important to describe their genetic composition. Therefore, genetic diversity is usefull to characterize the breeds of livestock based on molecular information. Moreover, Y-chromosome diversity is important to elucidate patterns, timing of speciation and phylogeography and hybridization events [7]. Previous studies have been determined the paternal origin of Lakor goat [11], Nigerian cattle [12], Bharal sheep [13] and Cinta Senese pig [14] based on ZFY gene sequence. This study aimed to characterize Pasundan cattle based on partial ZFY gene sequence. Despite this, the results in the present study had the potency for determining the genetic marker of reproductive traits in Pasundan bulls in the future.

MATERIALS AND METHODS

DNA samples

A total fourteen (14) blood samples from different adult Pasundan bulls were collected for the analysis. All Pasundan bulls in this study were kept at the breeding station (BPPIBT-SP Ciamis) in West Java, Indonesia. The blood samples (about 5 mL) were taken from jugular vein using venoject and vacutainer tube containing EDTA. The DNA extraction was assessed with Genomic DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's protocol.

Amplification of ZFY gene and sequencing

Amplification of ZFY gene was performed in a total volume of 30 μL containing 9 μL of DNA template; 0.6 μL of each primer; 15 μL of PCR mix (Mytaq HS Red Mix, Bioline, UK) and 4.8 water nuclease free. Thermocycling conditions for ZFY gene were as follows: pre-denaturation at 95°C for 5 minutes, followed by 35 amplification cycles of denaturation at 95°C for 30 seconds; annealing at 55.4°C at 1 minutes 30 seconds; extension at 72°C at 30 seconds and final extension at 72°C at 5 minutes.Therefore, the PCR products (5 μL) were electrophoresed through 1% agarose gels (1 g of agarose powder and 100 mL of 1 x Tris Buffer EDTA) and stained with SYBR™ Green (1 μL per 10

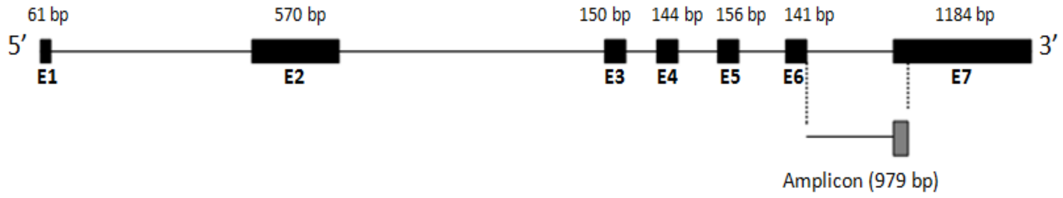

Figure 1. Scheme of *bovine* ZFY gene (GenBank: NC_016145.1) with the seven exons (black box). Along 979 bp of *bovine* ZFY gene (GenBank: AF465181) was amplified comprising 196 bp of partial exon 7 (grey box).

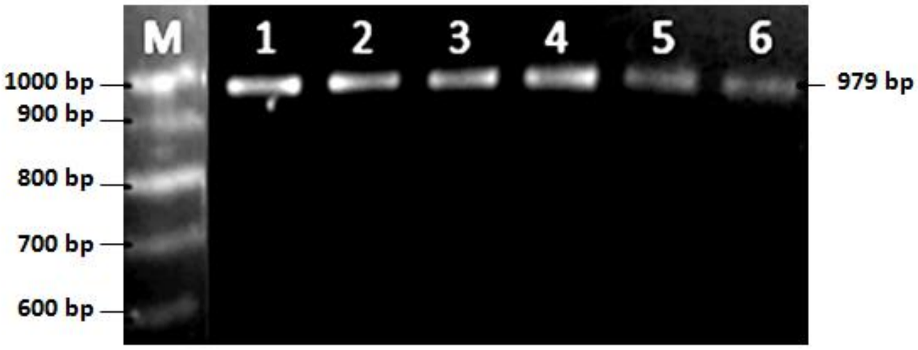

Figure 2. The amplification of ZFY gene in Pasundan bulls along along 979 bp at 1% of agarose gel. M: DNA marker 100 bp; Line 1 - 6: DNA sample.

mL agarose liquid). The DNA visualization was performed in 1% agarose gel with G-Box Documentation System (Syngene, UK). A primer pair of Forward: 5′- GGT GAG GGC ACA TGA GTT C -3′ and Reverse: 5′- CTC TGC AGG TGG TTG TGT AA -3′ [15] was used in this study to amplify *bovine* ZFY gene (GenBank: AF465181) along 979 bp. Thus, the primer pairs in this study are able to amplify the ZFY gene from intron 6 to exon 7 regions (Figure 1). In addition, the forward sequencing analysis was performed in the present study by the 1st Base Laboratory Service of Malaysia.

Sequence analysis

The sequence analysis was performed with three (3) molecular softwares of BioEdit [16], MEGA11 [17] and DNAsp [18]. BioEdit software was used for sequence alignment and contig analysis. MEGA-X software was used to obtain the phylogenetic tree using Neighbor-joining (NJ) method (1000×bootstrap) with the sequence references of *Bos taurus* (AF241273, AF465179, AF465181, DQ556336), *Bos indicus* (DQ336537) and *Bos javanicus* (DQ336538). A DNAsp software was used to obtain number of mutation site, Haplotype diversity (H_d), nucleotide diversity (p_i), neutrality test of Tajima’s D and Fu’s Fs statistics tests.

RESULTS

Along 979 bp of ZFY gene amplicons was successfully amplified in the 1% of agarose gel as shown in Figure 2. However, 778 bp of partial ZFY gene sequence of Pasundan bulls is able to evaluate based on the chromatogram graphics. In addition, total 48 mutation sites (excluding indel mutation) were detected in the 714 bp of Pasundan ZFY gene as shown in Figure 3. A total of seventeen (17) mutation sites were detected in the exon 7 region of *bovine* ZFY gene. Therefore, total 52 amino acids were detected in the partial exon 7 region of *bovine* ZFY gene with 12 amino acid changes (23%) as shown in Figure 4. Hence, these mutation sites can classify the *bovine* ZFY gene into ZFY-1 and ZFY-2 clades. Moreover, the Neighbor-joining tree revealed that Pasundan cattle are classified into ZFY-1 cluster together with *Bos taurus* (AF241273, AF465179) as shown in Figure 5. Therefore, *Bos javanicus* ,*Bos indicus* and the other *Bos taurus* (DQ336536, AF465181) are classified into ZFY-2 cluster. The genetic diversity parameters of partial ZFY gene in Pasundan bulls were presented in Table 1. Therefore, a total of fourteen (14) haplotypes were detected in the animals under study based on the mutation sites as shown in Figure 3. The H_d and p_i values in this study were 1.00 (high) and 0.01 (low), respectively. Thus, the neutrality tests of Tajima’s D and Fu’s Fs statistics were -1.42 and -6.47, respectively.

Table 1. The genetic diversity parameters for ZFY gene of Pasundan cattle

Parameter	Value
Number of observed sequence	14
Number of observed site	714
Number of polymorphic site	48
Number of haplotype	14
Haplotype diversity (H_d)	1.00
Nucleotide diversity (p_i)	0.01
Tajima’s D	-1.42
Fu’s Fs statistic	-6.47

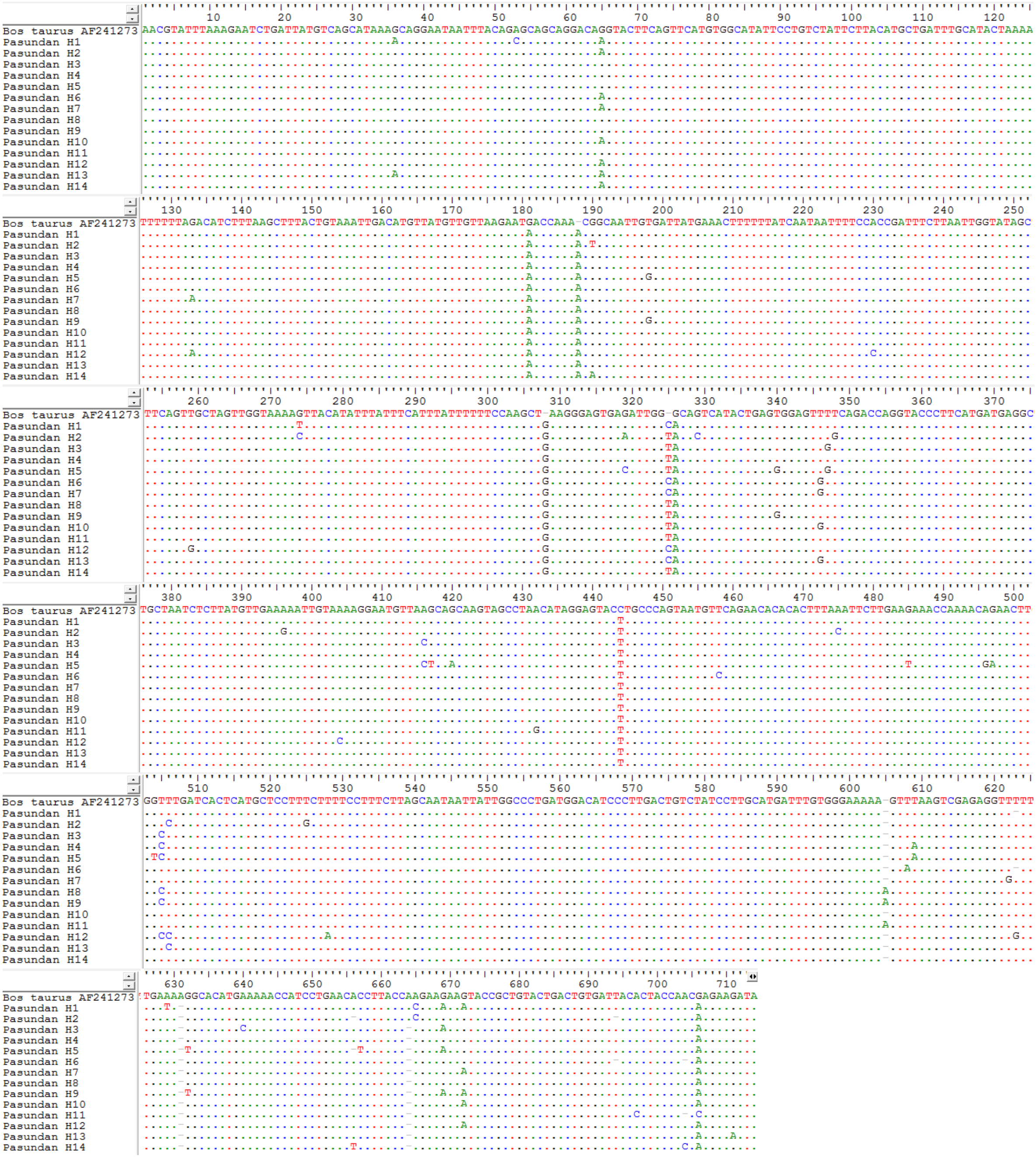

Figure 3. Partial sequence of ZFY gene region (714 bp) in Pasundan cattle belonging to exon 7 region (559th - 714th nucleotide).

DISCUSSION

The genetic diversity in ZFY gene of Pasundan cattle are high with 48 polymorphic sites and H_d value of 1.00. Nei and Kumar [19] stated that H_d is classified into low (<0.50) and high (>0.50) categories. The H_d value can be influenced by outbreeding with the same breeds from different geographical regions. Currently, the government of West Java was distributed frozen sperm (straws) from many Pasundan bulls for purebreeding program in West Java province with artificial insemination (AI). Consequently, the H_d value in Pasundan bulls can be increased because of straws distribution. Despite this, hybridization between different sub-species also influences the genetic diversity of species [7]. Actually, Pasundan is an indigenous cattle with two genetic introgresions of *Bos indicus* and *Bos javanicus* based on mitochondrial D-loop [5] and COI [20] genes. Nonetheless,

```
Species/Abbrv            * * * * * * * * * * * * * * *     * * * *   *     * *   * * * *   * *   * * * * * * * * *     *     * *
1. Bos_taurus_AF241273   M D I P * L S I L A * F V G K ? F K S R G F L K R H M K N H P E H L T K K K Y R C T D C D Y T T N E K I
2. Pasundan_H1           M D I P * L S I L A * F V G K ? F K S R G ? L I ? H M K N H P E H L T T K K Y R C T D C D Y T T N K K I
3. Pasundan_H2           M D I P * L S I L A * F V G K ? F K S R G F L K ? H M K N H P E ? L T T K K Y R C T D C D ? T T N K K I
4. Pasundan_H3           M D I P * L S I L A * F V G K ? F K S R G F L K ? H M Q N H P E H L T ? K K Y R C T D C D Y T T N K K I
5. Pasundan_H4           M D I P * L S I L A * F V G K ? L K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
6. Pasundan_H5           M D I P * L S I L A * F V G K ? L K S R G F L K ? H M K N H P E ? L T ? K K Y R C T D C D Y T T N K K I
7. Pasundan_H6           M D I P * L S I L A * F V G K ? Y K S R G ? L K ? H M K N H P E H L T ? K K Y R C T D C D ? T T ? K K I
8. Pasundan_H7           M D I P * L S I L A * F V G K ? F K S R G V L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
9. Pasundan_H8           M D I P * L S I L A * F V G K K F K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
10. Pasundan_H9          M D I P * L S I L A * F V G K K F K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
11. Pasundan_H10         M D I P * L S I L A * F V G K ? F K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
12. Pasundan_H11         M D I P * L S I L A * F V G K K F K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y P T ? Q K I
13. Pasundan_H12         M D I P * L S I L A * F V G K ? F K S R G C L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
14. Pasundan_H13         M D I P * L S I L A * F V G K ? F K S R G F L K ? H M K N H P E H L T ? K K Y R C T D C D Y T T N K K I
15. Pasundan_H14         M D I P * L S I L A * F V G K ? F K S R G F L K ? H M K N H P E L L T ? K K Y R C T D C D Y T T T K K I
```

Figure 4. Amino acid changes in the partial exon 7 gene of *bovine* ZFY gene region

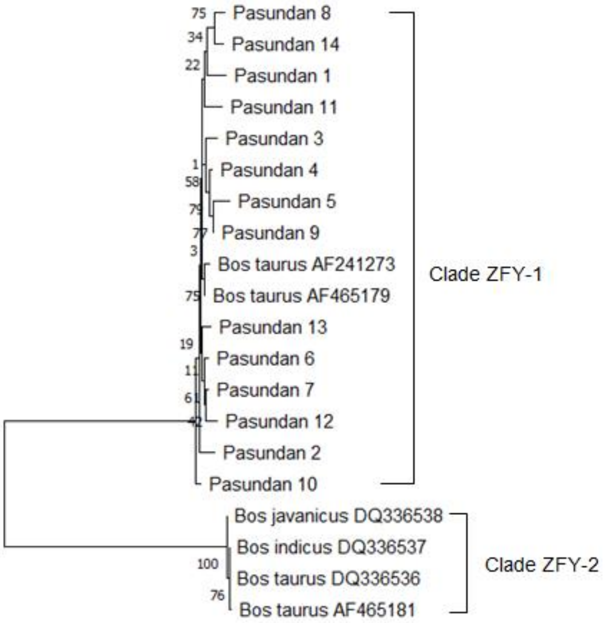

Figure 5. Neighbor-joining (NJ) tree (1000×bootstrap replications) between haplotype ZFY gene (714 bp) of Pasundan cattle and three (3) sub-species of cattle in Indonesia *i.e. Bos indicus*, *Bos taurus* and *Bos javanicus*.

Pasundan bulls are grouped into ZFY-1 clade and different with *Bos indicus* and *Bos javanicus* that grouped into ZFY-2 clade.

The p_i value in this study was 0.02 and classified into the low category. The p_i value can be classified into low (0.01 - 0.04), medium (0.05 - 0.07) and high (0.08 - 0.10) categories [19]. In this study, the neutrality test (Tajima's D and Fu's) showed in a negative value and indicating that low nucleotide variation in Pasundan cattle was caused by species expansion [19]. Arifin *et al*. [21] reported that the migration of Pasundan cattle from Majalengka to Sumedang and Indramayu was increased the allelic frequency of bloods albumin protein. Contrastly, Chaichanatong *et al*. [8] found a low genetic diversity in ZFY gene of Banteng (*Bos javanicus*) at two different geographical regions of Thailand. In addition, Clawson *et al*. [15] found a polymorphic site in ZFY gene of US beef cattle (*Bos taurus*) *i.e.* g.610C>T (GenBank: AF465181) but its was not found in Pasundan cattle (data not shown).

Previous studies reported that the H_d value in *bovine* Y-chromosome was showed high in Indian Zebu cattle (0.63±0.03) [22], Ethiophian cattle (0.75±0.01) [23], Creoles (0.78±0.02), Iberian breeds (0.71±0.02) and cattle of Atlantic island (0.68±0.04) [24]. Meanwhile, low H_d value in *bovine* Y-chromosome was showed in Polish cattle (0.04±0.02) [25], Lidia cattle breed (0.42) [26], continental Europe cattle (0.54±0.03) and Zebu cattle (0.39±0.08) [24].

According to ZFY gene, the Pasundan cattle are closed to *Taurine* lineage. The crossbreeding program through with *Taurine* straws through artificial insemination (AI) technique may be caused the presence of *Taurine* introgression in Pasundan cattle inferred from paternal lineage. Hence, many Pasundan bulls at West Java have are similar to *Bos taurus* with the characteristics of humpless (34.77%) and presence of white colour in muzzle (3.91%), horn (4.80%) and switch of tail (2.73%) [1]. However, Agung *et al*. [27] reported that Pasundan and Madura (*Bos indicus*) cattle have a similar genetic admixture based on twelve (12) microsatellite markers.

CONCLUSION

There are two (2) clades of *bovine* ZFY gene *i.e.* ZFY-1 and ZFY-2 based on partial exon 7 diversity. All Pasundan bulls under study were classified into ZFY-1 cluster together with *Bos taurus*. Hence, the exon 7 region of ZFY gene can be used as the genetic marker to discriminate Pasundan cattle with *Bos javanicus* and *Bos indicus* cattle breeds.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENT

The authors are gratefull to BP3IPTEK Jawa Barat for supporting on blood samples collection in the farmers. The authors also thank to Noval Alfalah and Ryan Adi Saputro (B.Sc. students of Faculty of Animal and Agricultural Sciences, Diponegoro University) for their help on the laboratory analysis.

REFERENCES

1. Said, S., W. P. B. Putra, P. P. Agung, and H. Yuhani. 2017. Phenotypic, morphometric characterization and population structure of Pasundan cattle at West Java, Indonesia. Biodiversitas. 18:1638-1645. Doi: 10.13057/biodiv/d180444
2. Iqbal, M., D. Rahmat, and N. Hilmia. 2017. Evaluation of estimated body weight based on Winter formula and actual body weight deviation of Pasundan cattle. e-Jurnal Mahasiswa. 16:1. [cited Feb 23, 2023]. Available from: http://www.jurnal.unpad.ac.id/ejournal/article/view/10938/4898.
3. Hilmia, N., D. Rahmat, P. Edianingsih, and Y. Faisal. 2022. Comparison of slaughter weight, carcass weight and carcass percentage of Rancah and Ongole grade cattle based on mutations in Leptin gene. Zira'ah. 47:137-141.
4. Hartatik, T., D. N. H. Hariyono, and Y. Adinata. 2019. Genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on Cytochrome b gene sequences. Biodiversitas. 20:17-22. Doi: 10.13057/biodiv/d200103
5. Salimah, I., P. P. Agung, S. Said, A. Farajallah, and D. Perwitasari. 2022. Origin and phylogenetic analysis of Pasundan cattle based on D-loop of mitochondrial genome. IOP Conf. Ser. Earth. Env. Sci. 1001:012041. Doi: 10.1088/1755-1315/1001/1/012041
6. Wulandari, A., V. M. A. Nurgiartiningsih, Kaswati, and T. E. Susilorini. 2019. Kinship of several Indonesian local cattle by using DNA mitochondrial COI (Cytochrome Oxidase Sub-unit I). Int. Res. J. Adv. Engin. Sci. 4:165-167.
7. Nijman, I. J, D. C. J. van Boxtel, L. M. van Cann, Y. Marnoch, E. Cuppen, and J. A. Lenstra. 2008. Phylogeny of Y chromosomes from Bovine species. Cladistics. 24:723-726. Doi: 10.1111/j.1096-0031.2008.00201.x
8. Chaichanathong, S., W. Klinsawat, M. Sukmak, A. Sakulthai, W. Wajjwalku, S. Sripiboon S., N. Kaolim, S. Nakbhun, B. Tunpradit, T. Nipanunt, W. Tipkantha, M. Yindee, and N. Thongtip. 2021. Genetic characterization of Banteng (*Bos javanicus*) populations in Thailand for conservation. Thai J. Vet. Med. 51:647-654. Doi: 10.14456/tjvm.2021.78

9. Pande, A. and S. M. Totey. 1998. ZFX and ZFY loci in water buffalo (*Bubalus bubalis*): Potential for sex identification. Genet. Analysis: Biomol. Engin. 14:85-88. Doi: 10.1016/s1050-3862(97)10004-3.
10. Shamsuddin, S. B. 1996. The use of zinc finger protein gene to detect the presence of Y chromosome in the cows. [Thesis]. Universiti Putra Malaysia, Selangor. [cited Feb 23, 2023]. Available from: http://www.-psasir.upm.edu.my/id/eprint/8615/1/FSAS_1996_16_A.pdf
11. Kunda, R. M., P. Kakisina, M. Rumanta, M. Moniharapon, and S. D. Volkandari. 2022. Paternal phylogenetics relationships based on zinc finger protein-Y (ZFY) gene in the Lakor goat breed. UPJOZ. 43:16-22. Doi: 10.56557/UPJOZ/2022/v43i213206
12. Mauki, D. H., A. C. Adeola, S. I. Ng'ang'a, A. Tijjani, I. M. Akanbi, O. J. Sanke, A. M. Abdussamad, S. C. Olaogun, J. Ibrahim, P. M. Dawuda, G. F. Mangbon, P. S. Gwakisa, T. T. Yin, M. S. Peng, and Y. P. Zhang. 2021. Genetic variation of Nigerian cattle inferred from maternal and paternal genetic markers. Peer J. 9:e10607. Doi: 10.7717/peerj.10607
13. Feng, J., C. Lajia, D. J. Taylor, and M. S. Webster. 2001. Genetic distinctiveness of endangered dwarf blue sheep (Pseudois nayaur schaeferi): evidence from mitochondrial control region and Y-linked ZFY intron sequences. J. Hered. 92:9-15. Doi: 10.1093/jhered/92.1.9
14. Gabbianelli, F., F. Alhaique, G. Romagnoli, L. Brancazi, L. Piermartini, C.Ottoni, A. Valentini, and G. Chillemi. 2020. Was the Cinta Senese pig already a luxury food in the late middle ages? Ancient DNA and archaeozoological evidence from Central Italy. Genes. 11:85. Doi: 10.3390/genes-11010085
15. Clawson, M. L., M. P. Heaton, J. M. Fox, C. G. Chitko-McKown, T. P. L. Smith, and W. W. Laegreid. 2004. Male-specific SRY and ZFY haplotypes in US beef cattle. Anim. Genet. 35:245-249. Doi: 10.1111/j.1365-2052.2004.01122.x
16. Hall, T. 2011. BioEdit: An important software for molecular biology. GERF Bullet. Biosci. 2:60-61.
17. Hall, B. G. 2013. Building phylogenetic trees from molecular data with MEGA. Mol. Biol. Evol. 30:1229-1235. Doi: 10.1093/molbev/mst012
18. Librado, P. and J. Rozas. 2009. DnaSP v5. A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451-1452. Doi: 10.1093/bioinformatics/-btp187
19. Nei, M. and S. Kumar. 2000. Molecular evolution and phylogenetic. Oxford University Press, New York.
20. Wulandari, A., V. M. A. Nurgiartiningsih, Kuswati, T. E. Susilorini, P. A. Partogi. 2019. Kinship of several Indonesian local cattle by using DNA mitochondrial COI (Cytochrome Oxidase Sub-unit I). Int. Res. J. Adv. Eng. Sci. 4:165-167.
21. Arifin, J., S. B. Komar, E. Y. Setyowati, U. Yunasaf, Indrijani, and Sulasmi. 2015. The gene distribution, equilibrium law, and effective population size post migration of Pasundan at Majalengka Regency. JIT 15:1-7. Doi: 10.24198/jit.v15i2.9518
22. Ganguly, I., C. J. S. Singh, S. P. Dixit, M. Sodhi, A. Ranjan, S. Kumar, and A. Sharma. 2020. Y-chromosome genetic diversity of *Bos indicus* cattle in close proximity to the centre domestication, Sci. Reports. 10:9992. Doi: 10.1038/s41598-020-66133-3
23. Li, M. H., M. Zerabruk, O. Vangen, I. Olsaker, and J. Kantanen. 2007. Reduced genetic structure of north Ethiophian cattle revealed by Y-chromosome analysis. Heredity. 98:214-221.
24. Ginja, C., M. C. T. Penedo, L. Melucci, J. Quiroz, O. R. Martinez-Lopez, M.A. Revidatti, A. Martinez-Marinez, J. V. Delgado, and L. T. Gama. 2010. Origins and genetic diversity of new world Creole cattle: inferences from mitochondrial and Y chromosome polymorphisms. Anim. Genet. 41:128-141. Doi: 10.1111/j.1365-2052.2009.01976.x
25. Prusak, B., W. Sawicka-Zugaj, A. Korwin-Kossakowska, and T. Grzybowski. 2015. Y chromosome genetic diversity and breed relationships in native Polish cattle assessed by microsatellite markers. Turk. J. biol. 39:611-617. Doi: 10.3906/biy-1502-9

26. Cortez, O., I. Tupac-Yupanqui, S. Dunner, J. Fernandez, and J. Canon. 2011. Y chromosome genetic diversity in the Lidia bovine breed: a highly fragmented population. J. Anim. Breed. Genet. 128:491-496. Doi: 10.1111/j.1439-0388.2011.00951.x

27. Agung, P. P., F. Saputra, M. S. A. Zein, A. S. Wulandari, W. P. B. Putra, S. Said, and Jakaria. 2019. Genetic diversity of Indonesian cattle breeds based on microsatellite markers. Asian-Australas. J. Anim. Sci. 32:467-476. Doi: 10.5713/ajas.18.0283