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D-Loop DNA genome sequence of Donggala cattle by using Nanopore analysis

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ABSTRACT

Donggala cattle have been breed for generations, making them a valuable genetic resource for Indonesian livestock species in Central Sulawesi that must be protected and preserved. Donggala cattle which certainly has potential as a regional germplasm resource and study focuses on the analysis in the mitochondrial DNA D-Loop region from liver tissue. This study aimed to determine the complete mtDNA sequencing was performed using WGS mtDNA analysis with nanopore technology by Oxford Nanopore Technologies GridION. Bioinformatics and data analysis were then performed. Results: This study showed that the length of the mtDNA genome is 16,412 bp, consisting of two ribosomal comprises 27 genes with a typical structure. These include 13 protein-coding genes, 22 transfer RNAs, 2 ribosomal RNAs, and noncoding D-loop region, a total of 505 site variations in the D-Loop region The variable site (49%), parsimony (57%) and singleton (43%). Phylogenetic analysis based on the complete mitochondrial genome sequences distinguished into three clades of cattle breed. Including B. javanicus (GenBank data PQ 130485 and PV387265), B. gaurus, B. grunienns and Bali indicus cattle groups showed notable genetic variations. The results of this work offer genomic information capable of supporting the next investigations on the genetic structure and evolutionary background of Donggala cattle.

Introduction

Donggala cattle are one of Indonesia's indigenous breeds, easily recognized by their distinctive grayish white to plain white coat coloration. These phenotypic traits serve as important markers that distinguish them from other local breeds of cattle. Historically, Donggala cattle are thought to have originated from Bos indicus through uncontrolled crossbreeding with Ongole Grade (PO), Madura, and Bali cattle (Yunanto *et al.*, 2020). Despite their long history and cultural value in Central Sulawesi, scientific information on their genetic characteristics and evolutionary background remains scarce.

Molecular studies are crucial for clarifying the genetic identity and phylogenetic relationships of Donggala cattle. Among molecular markers, mitochondrial DNA (mtDNA) has been widely applied because it is maternally inherited, non-recombinant, and evolves rapidly (Jiang *et al.*, 2021). In particular, the mtDNA D-loop region is highly polymorphic and has been extensively used to trace maternal lineages, assess genetic diversity, and investigate the origins of breeds. Its variation provides essential insights into population history and evolutionary dynamics (Teinlek *et al.*, 2018). Considering their status as a valuable local genetic resource, the characterization of Donggala cattle mtDNA is important for supporting conservation and sustainable breeding programs. Therefore, this study aimed to sequence and analyze the mtDNA D-loop region of Donggala cattle using nanopore sequencing technology, providing baseline genomic data for future phylogenetic and conservation research.

Liver tissue was used for DNA extraction from Donggala cattle, as cells with high energy demand typically contain a greater abundance of mitochondrial DNA (mtDNA). The liver samples were sourced from municipal slaughterhouse, with approximately 10 g of tissues collected and stored in Falcon tubes filled with ethanol for preservation. These samples were then processed for genomic DNA (gDNA) extraction following the standard protocol provided by the manufacturer, using the gSYNC DNA Extraction Kit (Geneaid, New Taipei, Taiwan). After extraction, the gDNA underwent quality and quantity assessment before proceeding with genomic mtDNA enrichment, which was carried out using the REPLI-g Mitochondrial DNA Kit (Qiagen, Hilden, Germany). The library preparation process utilized the enhanced mtDNA. The workflow for mtDNA sequencing, and bioinformatics analysis is shown in Fig 1.

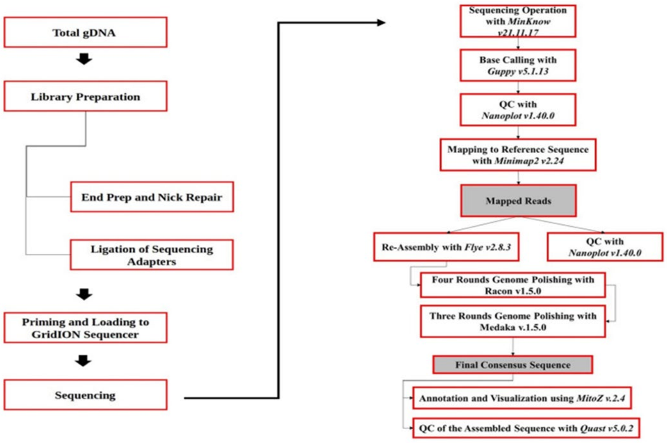

Fig. 1. mtDNA sequencing and bioinformaticsanalysis procedure.

Data analysis was performed using MEGA 12 software. Dloop sequences of Donggala cattle were aligned to identify genetic characters, including gene sequence, position, size, and nucleotide composition. The Dloop sequences of Donggala cattle was also aligned with 25 complete genome sequences of several species of Bos grunienns (GQ4642791, GQ4642981, JQ8460201 and NC0063803), Bos indicus (MK3359201, OR6398481, PP7375331, JN8173021, JN8173051, AY1266971 and NC0059711), Bison bison (NC0123461 and GU9470061), Bos primigenius (NC0139961), Bos taurus (NC0068531), Bos gaurus (MK7702011, MT34589311, MT34589211, MT3606521, MT3606531) and *Bos javanicus* (PQ1304851 and PV3872651) in Genbank as a reference center to identify gene mutations, gene diversity and visualize genetic relationships with phylogenetic tree displays designed based on the rules of the likely hood method.

The analysis revealed (Table 1) a total of 505 site variations in the D-loop region, representing 50.49% of the entire sequence length. Among these, 189 were parsimony sites and 316 were singleton sites. Transition substitutions (A>G, C>T) were more frequent than transversions. Examples of parsimony variations included 19G>A, 21A>G, 39T>C, and 43C>T, while singleton substitutions included 82C>A, 113C>T, 114A>G, and 127A>G. A phylogenetic tree (Fig. 2) was generated using the MEGA program. The Donggala cattle clustered closely with *Bos javanicus*, particularly *Bos javanicus* domesticus (Bali cattle). This clade was clearly distinct from Bos taurus, Bos indicus, Bos grunniens, and Bison bison. These results indicate that Donggala cattle are likely direct descendants of *Bos javanicus* rather than crossbreeds with imported cattle.

The high proportion of variable sites (50.49%) observed in the Donggala cattle D-loop sequence confirms that this region is a mutational hotspot in the mitochondrial genome, consistent with reports for other livestock species (Stoccoro *et al*., 2018; Tolve *et al*., 2023). Such high variability is typical of the D-loop because of its reduced functional constraints compared to protein-coding genes. The predominance of singleton sites suggests the presence of recent mutational events that increase within-population diversity, whereas parsimony sites indicate more stable, recurrent mutations that serve as reliable markers for reconstructing evolutionary relationships (Kolaczkowski and Thornton, 2004). Together, these patterns demonstrate ongoing mutations and historical lineage divergence within the population.

Phylogeographic studies have emphasized that D-loop polymor

Table 1. Polymorphic site of DLoop genes of Donggala cattle.

Genes	Variable site		Parsimony			Singleton		
	n	%	n	%	sites	n	%	
DLoop	505	49	189	57	(19G>A); (21A>G); (39T>C); (43C>T); (44G>A); (45C>T); (53C>T); (55T>C); (56G>A); (57C>T); (72C>T); (73C>T); (80T>C); (81T>C); (85C>T); (87G>A); (97G>A); (99A>C); (103G>A); (104T>C); (107C>T); (120C>T); (121A>G); (139T>C); (140A>G); (141A>T); (142C>A); (145A>T); (147G>A); (148C>T); (151C>A); (152C>T); (153A>C); (154C>A); (156A>G); (159G>C); (161A>C); (162T>C); (164C>T); (181Indel/C); (183G>A); (184T>A); (185A>G); (186Indel/T); (188T>C); (189Indel/C); (190Indel/G); (191A>T); (192A>C); (193T>C); (194T>A); (195A>G); (196A>G); (197T>C); (239A>G); (240A>G); (241Indel/C); (242G>A); (243T>G); (244A>G); (245A>T); (246T>A); (248Indel/C); (257T>C); (272Indel/T); (277A>G); (278A>T); (297Indel/T); (302C>T); (305A>G); (328T>A), (330G>A); (331A>T); (332A>T); (333C>T); (334T>C); (335C>T); (336C>T); (339A>T); (340G>A); (341A>G); (342C>T); (351G>A); (357T>A); (358A>T); (360T>A); (361C>T); (362C>T); (364C>T); (366A>G); (368T>C); (369C>T); (377G>A); (378T>C); (380C>T); (385C>T); (386A>G); (392T>C); (393C>T); (394T>C); (396C>T); (397C>T); (399T>C); (400T>C); (401G>A); (402A>G); (403C>T); (405A>G); (406C>T); (409G>A); (410C>T); (414A>C); (418A>G); (421T>C); (422C>T); (426T>G); (431C>T); (443A>C); (448C>T); (449C>T); (480A>G); (483A>G); (484A>G); (489C>T); (490C>T); (493C>T); (513G>A); (514A>G); (516T>C); (517T>C); (532T>C); (533T>C); (540T>C); (545T>C); (549A>G); (576C>T); (579T>C); (589A>G); (590T>C); (591T>C); (593T>C); (597C>T); (629T>C); (632G>A); (635A>G); (651C>T); (675C>A); (676C>T); (727A>G); (734T>C); (749Indel/A); (773T>C); (794A>G); (797G>A); (800A>G); (803G>C); (804G>A); (809G>A); (810C>T); (821C>T); (833A>G); (835T>C); (837A>G); (844T>C); (860T>C); (863T>c); (864C>T); (873C>T); (874C>T); (889C>T); (890C>T); (896C>T); (897A>G); (909C>T); (913T>C); (926T>A); (928A>G); (930T>C); (932G>A); (936A>G); (939T>A); (943C>T); (947G>A); (948A>C); (962A>T); (972C>T); (974A>T); (976T>C)); (992G>A); (996A>G); (998G>C)	316	43	(70T>C); (82C>A); (113C>T); (114A>G); (127A>G); (134G>A); (135C>A); (161A>C); (163G>A); (183G>A); (192A>C); (199T>G); (200A>G); (271A>G); (282G>T); (283T>A); (286A>G); (289G>A); (294T>G); (300T>A); (308C>T); (309C>A); (313C>T); (314A>G); (320G>A); (321T>C); (323A>C); (324G>A); (325T>C); (327C>A); (337A>G); (353A>G); (388C>G); (391A>C); (395A>T); (419T>A); (420A>G); (423C>T); (427A>T); (428C>A); (429C>A); (432T>G); (433A>C); (434G>A); (436T>A); (437C>G); (439C>G); (440G>C); (441A>C); (442G>T); (444T>G); (445T>C); (446A>C); (451C>T); (452C>T); (454T>A); (455G>A); (456C>T); (457C>T); (458G>T); (459C>A); (460G>C); (461T>C); (462G>A); (466C>A); (467C>T); (468A>T); (469G>T); (474C>A); (479A>C); (481G>A); (487G>A); (491C>T); (492C>G); (494C>A); (495T>C); (496T>C); (499C>A); (500G>A); (504C>A); (505G>A); (506G>A); (507G>T); (508C>A); (509C>T); (511A>C); (512T>C); (518G>C); (519T>C); (520G>A); (521G>C); (522G>T); (523G>A); (525T>C); (526C>T); (527G>A); (528C>A); (531T>A); (534A>C); (536T>C); (538A>C); (539A>C); (541T>C); (542T>C); (543T>C); (546C>T); (548G>C); (550C>G); (552T>A); (553C>T); (554T>G); (555G>C); (556G>A); (557T>C); (558T>C); (561T>C); (562T>C); (563C>A); (564T>A); (565T>G); (566C>T); (567A>G); (570G>A); (571C>T); (572C>A); (574T>G); (575C>T); (584C>T); (588A>T); (592G>A); (594C>A); (595C>A); (596A>G); (598T>A); (600T>A); (602T>A); (603C>A); (604C>T); (605T>A); (606C>T); (608T>A); (610A>T); (611A>G); (615G>T); (617C>A); (618A>G); (620C>A); (623G>A); (624A>T); (625T>A); (626G>A); (627G>T); (641C>A); (644C>T); (645C>A); (649G>A); (656C>T); (659A>G); (660A>T); (661C>A); (663G>A); (665G>A); (666C>G); (668G>A); (669T>C); (670C>A); (671A>T); (678T>A); (679G>C); (680G>A); (683T>C); (684T>C); (685T>C); (686T>C); (687T>A); (690T>C); (693T>A); (695T>A); (696G>A); (698G>C); (699G>A); (700G>A); (701A>G); (703G>A); (708G>A); (713A>C); (714G>A); (715C>T); (716T>A); (717A>G); (718T>A); (719G>C); (720G>A); (721C>G); (722C>T); (723G>A); (724T>C); (725C>A); (726A>T); (728A>G); (729G>T); (730G>A); (732C>A); (733C>T); (735G>A); (737C>T); (740G>A); (743G>A); (745G>T); (747A>G); (750T>C); (753T>A); (757G>C); (758C>A); (760G>T); (761G>A); (762A>C); (763C>A); (765T>C); (768C>A); (770G>C); (771C>T); (775T>C); (777G>T); (778A>G); (779G>A); (782C>A); (791A>T); (792T>C); (793G>C); (796A>T); (798G>C); (799C>A); (802G>A); (805C>T); (806A>C); (807T>A); (808T>C); (811A>G); (812G>A); (814C>T); (817T>C); (818G>C); (819G>A); (820T>C); (823C>T); (824A>G); (825G>C); (826G>C); (827A>G); (829A>G); (836T>C); (845A>G); (846T>C); (849T>A); (850A>G); (851T>C); (853T>A); (854C>A); (855C>G); (856C>G); (857C>A); (865C>T); (866A>C); (868A>C); (869A>G); (870A>C); (871A>T); (872A>C); (875T>G); (876C>G); (880C>A); (882T>G); (885A>T); (887A>G); (891A>G); (892C>G); (893C>G); (894A>T); (898C>T); (899T>A); (905C>T); (906A>G); (907G>A); (914C>T); (924A>G); (929C>T); (935T>C); (937A>G); (938A>G); (940T>C); (941T>C); (942T>A); (945A>C); (949T>C); (951T>A); (952C>A); (956A>T); (957C>G); (960A>C); (964T>C); (965A>T); (966G>T); (967C>T); (968A>C); (973A>T); (977A>T); (979A>C); (985T>C); (988A>C); (990A>C); (991A>G); (993C>T); (995C>G); (999C>G)

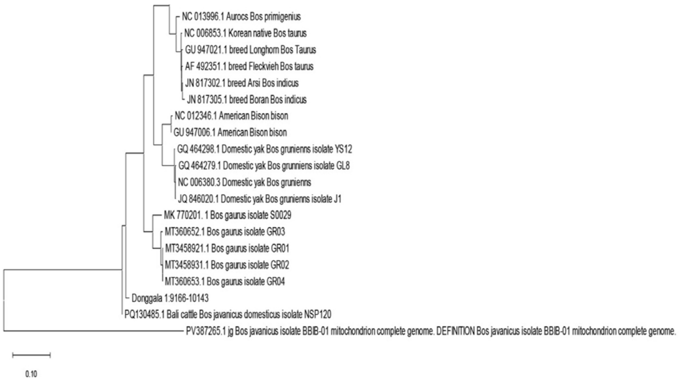

Fig. 2. Molecular Phylogenetic analysis using Dloop sequence of sapi Donggala by Maximum Likelihood method.

phisms provide robust information on maternal ancestry, demographic history, and breed structure (Kumar and Kumar, 2018; Tarekegn *et al.*, 2018). The clustering of Donggala cattle with Bali cattle supports their close genetic affinity with *Bos javanicus*, strengthening the hypothesis that Donggala cattle are local descendants of the domesticated banteng. This finding extends the known distribution of *Bos javanicus* domesticus beyond Bali and Nusa Tenggara to Central Sulawesi (Xia *et al.*, 2018; Zhang *et al.*, 2020). This suggests that banteng domestication may not have been confined to a single region but rather occurred in multiple locations across Indonesia.

From a conservation perspective, recognizing Donggala cattle as *Bos javanicus* domesticus highlights their importance as a unique indigenous genetic resource (Agung *et al.*, 2018; Andersson and Purugganan, 2022). Their close relationship with Bali cattle presents opportunities for selective breeding, genetic conservation, and livestock improvement programs that prioritize local adaptability and enhance biodiversity. Furthermore, genomic characterization provides valuable information for developing national breeding strategies, ensuring the sustainable utilization of Donggala cattle while supporting food security and biodiversity conservation (Hastarina *et al.*, 2025).

Conclusion

This study demonstrates that Donggala cattle possess high genetic variation in the mtDNA D-loop region, confirming their close relationship with *Bos javanicus* (Bali cattle). The identified polymorphisms provide useful genetic markers for phylogenetic studies and highlight the importance of conserving Donggala cattle as a unique Indonesian genetic resource.

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Conflict of interest

The authors have no conflict of interest to declare.

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