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Fsculty ofAnimal Husbadry, Hasanuddin University, Makassar, South Sulawtsi, 90245, Indoncsia Tel: +62-813439851 14 Fax +62411587211 E-mail pt)enpie@tshoo.con Abstrtctr Morica goat is an endemic species that can be found only in South Sulawesi, Indonesia. lt has b€en reported by the FAO as enclargered species becaus€ its number of population has been significantly decreased.

Domestication by local people has been done by cultivating them with (acaltg goat that leads to an interbreeding process. Consenation of the goats must be done immediately due to its raprdly decreasing population. However, accurate identification and quanlification ofthe goat by DNA analysis is highly important.

The objective ofthis study is to determine the differences amongst marica- kacmg and Capra hircus. C. caucasicd and C- lalcohei from lhe G€nBank. The sequence of the mitochondrial DNA (mtDNA) at d-loop region of J0 Marica goats and five K4carrg goats from three districts in South Sula$esi were investigated. Their nucleotide sequences were comparcd with the sequence ofthe CenBank's Capla sp afld were analyzed using Dendogram neighbor joining tr€e.

The results showed tha! lhere were a few nucleotide difrerences between some Maica and Kacang goat that were located at 20,840 and 980bp ln addition. both nucleotides sequence have shon genetic dislance compared to C ,i/crrj. However, comparing with other Caprd sp, the distance was significantly far. Meanwhile, according to the dendogram, it \ras found that all Goats and aapra sp came liom the sam€ ancestral

lineage.

It can b€ concluded that Marica and r(acant goats could be very closely related with C ,7ircrr but they were drfferent from the C caucastca and C. Jolconert KcyYor.,R: Marica goat', Kacahg goal- Capra hrrc&rl South Sularvesi, mtDNA l. htmductior One of lndonesian's germplasm commodities are goals. Goats spread in different regions with different climates and s€parated in a long time.

Various selections ofenvironmental factors and treatment cause genetic changes in goat's population (Roul et al.,2008\. Marica g@t is a type of local goat that can be found only in South Sulawesi Province, especially in the disrrct ofMaros, Jeneponto, Soppeng and Makassar (Fitra e, a/., 2009). The goats are genetically potential to adapt in the dry land with a very low annual r36I - Lnlernolionol lournol of Agricullure syslems (uAS) minfall.

Manca Soats can survive in the dry season although they only eat dry gass in the rock)_ ground. Unfortunately. according to Food and Agricuhure Organlzalion (2000) Report, its population is very low and is calegorized as endangercd sp€cies. Traditionally, local communities raise various kinds of goats in the same field and cages. [t also happened on Maica Eoat thal was reared togelher wrth (dcarg goar.

consequently interbreedinS and genetic mixing among Soat are inevitable. According to previous observation and inteNiewwith a local traditional breeder, Marica Soats have stong characteristic even they resulted fiom the combinations of Manca urd Kacatg goats. Thus.

even the goats have strong chamctedslic of Marica goat, it does not 8larantee that they are otiginal Marica Coals Molecular genetic studies within and across breeds are essential for the population management (Hall and Bradley, 1995, Ruane, 2000; Simianer, 2005). Performing such purpose, it is believed that using mitochondria DNA (mlDNA) is simply enough than using circular DNA.

Mamalian mtDNA shows several special features such as absences of inron, material inheritance, lack of recombination ev€nts ard a high mutation rate (lrwin eral. l99l) Furthermore, it brings enough information to figure out goat ancestries \Chen et al-. 2005). Complete sequences of bovine mtDNA were published by Anderson e, a/.

(1982), so the mtDNA have widely used for genetic diversity and phylogenetic analysis among different cattle breeds (Bradley e, al , 1996 Troy et a1.,2001). Specifically, D-loop area at mtDNA, non coding area, has nucleotide sequence that is useful to determine

phylogenetic relationship (Hou e, al., 2006). Meanwhile. the preservation of Ma ca Eoats should be done immediately considering the nurnber of its population has declined sharply.

However, before conducting conse ation effort, d€termining the original Marica Eoal is essential. This study is thercfore conducted to determine the kinship between Marica ar,d Kacang goab and the oriBinal Marica from KacdrS goats 2. M.t.ri.ls.ndM.thodr 1.1 Animol Sample DNA was obtained from \I'lrole blood sample because the DNA have specific genelrc chamctenstic and $e size is smaller than nucleus DNA (Chen et ol.,20051.

lt was impossibl€ to find a nnbet of Marica goats that have the same charact€ristic due to the lack of its numbet All Matica Boals comprising of seven goats ftom Maros (T85, TB4. S l. Sl. 52. T86 and TB8). three goats from Makassar (MK3, MK4 and MK5) and 20 goa$ from Jencponto (JNP induk, JNP anak, MCJ2, MCJ3, I, Btgl. Btg2, Btg3. Brg4, TB VIII, Bl, 82, 83, Kl, K2, Kl, Mks7, Mks8. Mkslo. MksTJantan) were analysed. Almost all the goats were female except MksTJantan which was male.

On the othef hand, five Kacang goats were ta.ken from Makassar. 1.2 DNA isolation A tolal of I0 ml of hlrxrd were taken aseptically from the jugular vein of each Marica aid Kacang goats and placed in vacuum glass tub€s containing anticoagulant ACD (acid c itrate dextrose). All samples were stored in a cooler and brought to laboratory for further analysis (Yadav and Yadav,2008).

lr 37 volume I Issue 2 December20l3 - ln this study, only mitochondria DNA was used and purified following the method of Sambrook eral(1989). 2.3 PCR Amplifmtion The primers that were used in this study were desigled to ampliry D-loop region (Table 1). Primer for the DJoop $€s designed based on data Capro hircus sequence (GenBank Accesston number: cU295658 l) Program of Primary 3 output (http://uww-genome wi.mit edu/cgi bin/ primr3.cgiftesults_from-primer3) was used to select primers which are predlcted to give good results. PCR Reaction co[sisted of 2 5 mM MgCl,.

10 mM dNTPs, 100-300 ng template DNA, lopmol each primer and 2U Taq Polwerase (B\o Lab) and its buffer Table L Base sequences and its melting tempemtures ofprimers for ampliffingD-loop region ofmtDNA ofthe goats. PRIMER Base sequenc€ Numb€r ofBase Melring Temperahre Cc) 59,0 The thermal profile included an initial denatumtion at 94qC for 2 min followin8 denaturatlon at 94"C for 2 minutes, annealing at 12C fot 2 mrnules and J5 cycles of elongation and extension at 72qC for I mrn.

using PCR amphficatron Infigen PCR products were migated at 1.270 agarose gel

migmted using IxTBE buffer Submaflne Electrophoresrs [Hoefer. USAJ. The gel was stained with ethidium bromide and observed under UV Tlansluminator (l = 300nm) exposure, and the 1000 bp of standard molecular us€d as a ladder 21 Pwirtcation of PCR product uxing Phylogenetic anabsis Products of PCR amplification \xas purified using GFX purification kit (Amersham, USA) Alignment of sequences was obtained using the Clustal W program (Thompson e, al, 1994). The result was compared with DNA sequence of Capla lrrrrx-r (GenBank Accessron numbel GU295658.1), Capra hir s breed lnner Mongolia White (GenBank: Accession number GU06804S.1). Copra lakonert (GenBanl Accesion number: FJ207525.l) a\d Capra caucasica (Genllank Accession number: JN632609.1) 3, Rasults rtrd Discussion Figure I shows the profile of PCR arnphficatron products of mtDNA gene Some ofthe DNA bands show a smear and even no bands at all.

These might be due to the degradation process during DNA purification or the DNA chains were cut shorter during purification proc€ss. Some DNA do not form visible band that could be caused by the small amount ofsample or aflrcalirE temperature As resLrlts, only ll band are clearll seen which are from B2F, r33l Target Name R/F Dloop CHF F 5' CTCACATTA,AACCTGAGTC,C 3' 20 tr Lnlernoliono Journo!of AgricullLrre Syslems IUAS) Figure I The results ofPCR amplification ofD-loop region ofmtDNA of marica and kacang goats (Ladder: 1000 bp). Table 2.

Sequences comparison ofthe nucleotides polymorphisms ofD-Lopp DNA region lc_Hror3 tB_2_F ,8TG-4-F #_Bellnr_F a<_3_F *Gcanll-F $TK-3.F]fB_7_F tTB_8-o-F ts3_F AATCCTACGA TCAATTCCCA ACAAACTAGG AGG\GTCCTA 401 401 401 401 401 401 401 401 401 401 401 c.. BTG4F, I-B€tina-F, K3-F, JNP Induk_F, S3_F, Mk 3_l 1B_7_F and TB_8_o_F for marica goats, kacang I F for kacang goat.

Table 2 illustrated some pertinent information about nucleotides sequence in the range 40bp. Alrnost all Marica and Kacant goat showed the safie nucleotide sequences as C ,i/c6 except for the parent JNP which is Manaa toat sample from Jeneponlo. This goat has nucleotide c,,1osine at 20bp while others have nucleotide adenine.

In addition, there are similar trend occured in the range 960 and l,000bp (Table 3) cxcluding TB_7 _F atd Kacang _l_F, respectrvely. Sample TB_F_7 which is Marica goat from Jeneponto has nucleotide cytosine while lhe othe$ Marica, Kaca g ar,d C. hircus goals have thymine. On the other hand. at posrtron 980bp. Kacang_l _F (facaat goat) has guanine while others goat have adenine. Meanwhile, in the region of 840bp.two Marica Eoats (S3-F and MK_3F) show a differcnce where at t07bp, both of them have adenine nucleotide.

while the others have guanine Tabl€ 4 described the difrerences of nucleotide sequence between the researched Soats and the goat from CenBank which are C. causica, C .lalconeri a\d C. hitcus inner Mongoli. white cashrnirc. It can be clearly seen that the genetic distance have no difference between Marica Eoat utd lr39 volume I lssLre 2 Decenroer 2013 E Table 3 Sequences comparison of the nucleotides polymorphisms of Dloop region of mtDNA, range E40 bp, 960 bp and I,000 bp. #C_hircus dBj2,F #BTG_4-r #I_Bctm_F #K_3_F #JNP_hduk F *K...nLl_F #MK-3-F #TB-7-F #TB_E_o_F #s3 F 8401 8401 8401 8{01 E40l 8401 8401 E40l #C_hircus tB_2_F #BTG-4.F #l_Betn|a_F #K_3_F #JNP_induk_F #Ka.!nLl_F #MK-]-F #TB-7-F #TB_8_o_F #s3_F ATAAAGACAT AATATGTATATCGTACATTAAACCATCICC [%O] 960l 9601 9601 9601 9601 9601 9601 e60l 9601 9601 f€_hircts lB-?_F #BTG_4.F #I_B.tim_r #K-3_F #JNP_induk F #r'r.rnll_F }MK-3-F #TB-1-F #TB_t_o_F ,s3 f CCCATGCATA TAAGCACGTA CAATGTCCTTATTACCAGTA IIOOO} tr000l lr000l Ir000l ltoool ll000l Ir0001 Ilmol tr 0001 I r0001 Ir000l C.

,rircrj Ttis trend is similar to KacarS goat and C /rirc,rj- Thrs condition can be explained by showing the dendogram Neighbor Joining Tree (Bootstrapping 1000 replications) as seen in Figure 2. It can be seen clearly that based on dendogram Neighbor Joining Tree, all Marica and Kacang goats have a far genetjc distance compare lo C /alconen and C.

catcqsica, bvl their distance is closely related to species of C ,lircl/J. Meanwhile. a clos€r study on this dendogram shows that all goats sample has srmilar genelrc drslance. They are few subsritution mutalDns fotmd n Kacang ud Manca goats. ll might luppen as a resuli of adaptation proaess to environmenEl condition such as the lack of feed resources and selectron by breeders.

In Jeneponto, for cxample, the dry season duration is over seven months per year. lrol ATTTIATGAT CTACTTCACG TGTACGTACA TAATATTAAT C , 'G - nlernoliono Journolof Agriculllre Syslems (UAS) Table 4. Matrix index ofnucleotide sequence's difference between Malica and Kacahg goats compare to the sequence liom the GenBank's goats.

t 2 3 4 5 6 7 t 9 l0 lt 12 13 14 2 B2F] BTG.4-F 00 000 5 K_l_F 0000 ttlt12 E MX-3-F trlrt22 9 TB_7_t tllllt222 I 53F 00000ltlt tttlt2202t 102 t02 102 r02 102 103 rot t03 t03 t02 t03 19 19 79 79 79 E0 E0 E0 80 79 m 106 l1 Cn|ll*E 3E 3t 38 3t 18 39 39 19 39 lE l9 Et Figure 2.

Dendrogmm neighborjoining tree displaying the relationship behveen the sarnples and goats'panicular references b€longing to the Capra sp as determined by D.loop region of mtDNA gene analyses. The numbers at the node arc bootstrap values based on 1,000 re-samplings The bar represents the number of hutations per sequence position Thus, the feeding gass is less available and dry. Elrod dan Stansfield (2007) explained that the

total gene pool may change when phenotype characterisltc is sultable with the environment.

Furthermore, the genes will be rnherilcd to the offsprmg Meanwhlle. a research conducted by Agha ?t al (2008) c lrru rEMdrcd[wn c-ltlft showed that the mdied Med(erranean breeds sampled from A{iican and European populalions seem to have differentiated from each olher wrth onl) litlle genettc exchange b€tween the Seogmphically isolated populatrons Therefore, rhe muratlon of population is onen caused by genettc dnft or 04r l0'IBEoF I Vcl,._r.e I ssJe : Decernce. 2013 !- selection (Nei and Kumar,2000). 4.

CoDclusion It can be conclude that even though there are some difference nucleotides in same Maica and](acart goats, all of the nucleotrdes sequence aLgrunents are simrlar ro the nucleolide of C ,/rcrr (AF5l344l). However. it is different fiom C. caucasica and C. falconeri Future work slrongly recomm€nd€d to p€rform the same analysrs on the other area of Dloop mtDNA oulside the arca of40,960 and l,000bp to figure out other similarities and dissimilarities betwecn Kacang and Moica goats. Rcfaratrcas I Agha S H.F Pilla-S Cald.l Shaa!. M D'Andrea" S. Reale, A.Z.A. Abdelsalam and M H.

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