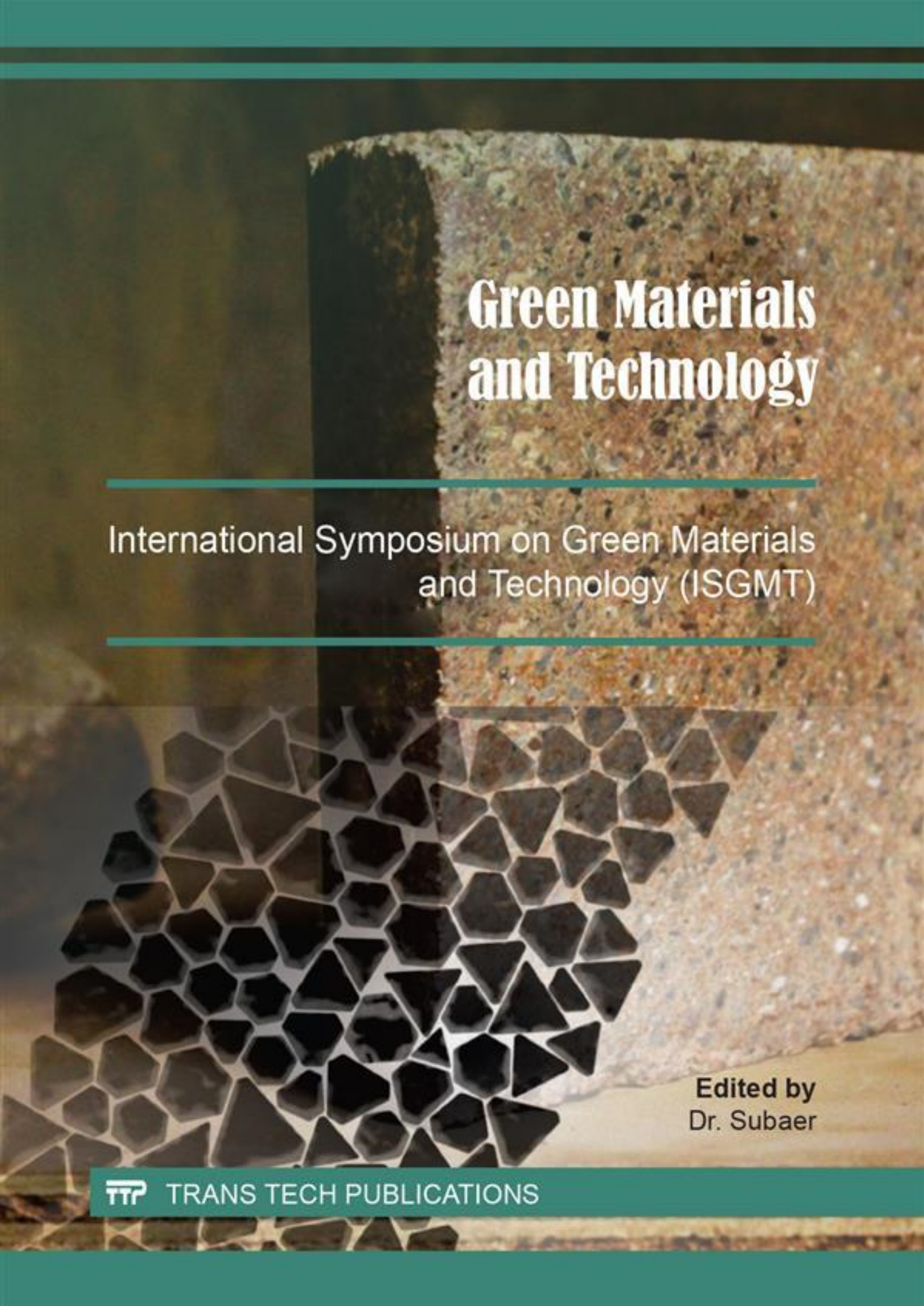
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Preface

Dean's Greeting

Bismillahirrahmanirrahim.
Assalamu alaikum warahmatullahi wabarakatuh
Good Morning Ladies and Gentlemen.

I would like to take this opportunity to express my sincere appreciation to all keynote speakers and invited speakers for accepting our invitation to share their research findings and best practices here, in the 3rd ICMSTEA and 1st ISGMT. We do hope that the best practices in the field of Mathematics, Sciences, Technology, and Education can be well communicated among researchers and academics gathered in this conference. I would also like to take this opportunity to thank the Chair of ICMSTEA committee for organizing this special event to form a platform for networking as well as the exchange of lessons learnt and best practices.

Ladies and Gentlemen,

Implementation of the 3rd ICMSTEA and 1st ISGMT is not only expected to be a forum for disseminating the results of the latest findings in the field of each study, but also expected to be a networking forum for academics and researchers. We hope that the meeting of researchers and academics from various institutions in this place can be a starting point for future collaborations.

Behind the implementation of these activities, there are hard work, commitment and outstanding cooperation of the executive committee. Therefore, to the whole team of the executive committee, I really thank for your hard work and enthusiasm for the success of the 3rd ICMSTEA and 1st ISGMT.

Finally, as the Dean of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, I want to congratulate you all for participating in ICSMTEA and ISGMT 2018. Welcome to the city of Makassar, hopefully this activity can bring benefit for all of us.

Wassalamualaikum Wr. Wb.

Remarks of the Chairman
The 3rd ICMSTEA and the 1st ISGMT 2018

Assalamualaikum warahmatullah wabarakatuh.

Alhamdulillah, all praises belong only to *Allah Subhanahu Wa Ta'ala* which always give His grace and guidance so the 3rd ICMSTEA and the 1st ISGMT 2018 which organized by the Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar can be held. Greetings and blessings aimed to the Messenger of Allah, Prophet Muhammad and the entire family and friends.

As the chair of the committee of the 3rd ICMSTEA and the 1st ISGMT 2018, I would like to deliver my highest appreciation to the *Keynote Speakers,* Dato' Professor Dr. Zul Azhar Zahid Jamal, Dr. Saleh Abdurrahman, M.Sc, Assoc. Prof. Dr. Andriana Surleva and all the invited speakers who have been willing to spend their time to share their knowledge, perspectives, and best practices to all the participants of the 3rd ICMSTEA and the 1st ISGMT 2018.

The 3rd ICMSTEA and the 1st ISGMT 2018 aimed to provide the chances for researchers and academics to share and discuss the latest findings in their field of expertise, thus it will also provide the chance for students to gain more insight on the development of knowledge in the realm of science. Besides keynote speakers and invited speaker session, there will be a parallel session where researchers can share their latest findings. The parallel session will be divided into several groups based on the topic of their study, including Mathematics, Physics, Chemistry, Biology, Education, and related fields. The I do hope that the knowledge and experience of all the speakers who participated in this forum would be beneficial in supporting the dissemination of the latest research findings, thus strengthen the position of the field of study as one of the answers in solving problems in the community.

On behalf of the committee of the 3rd ICMSTEA and the 1st ISGMT 2018, I do apology for any inconvenience you may experience during your time in this event. Hopefully, all participants can enjoy the knowledge sharing of the conference as well as the atmosphere and the friendliness of Makassar.

May Allah bless our activities.
Waalaikumussalam Warahmatullahi Wabarakatuh.

Chair of the committee,

Drs. Subaer, M.Phil., Ph.D.

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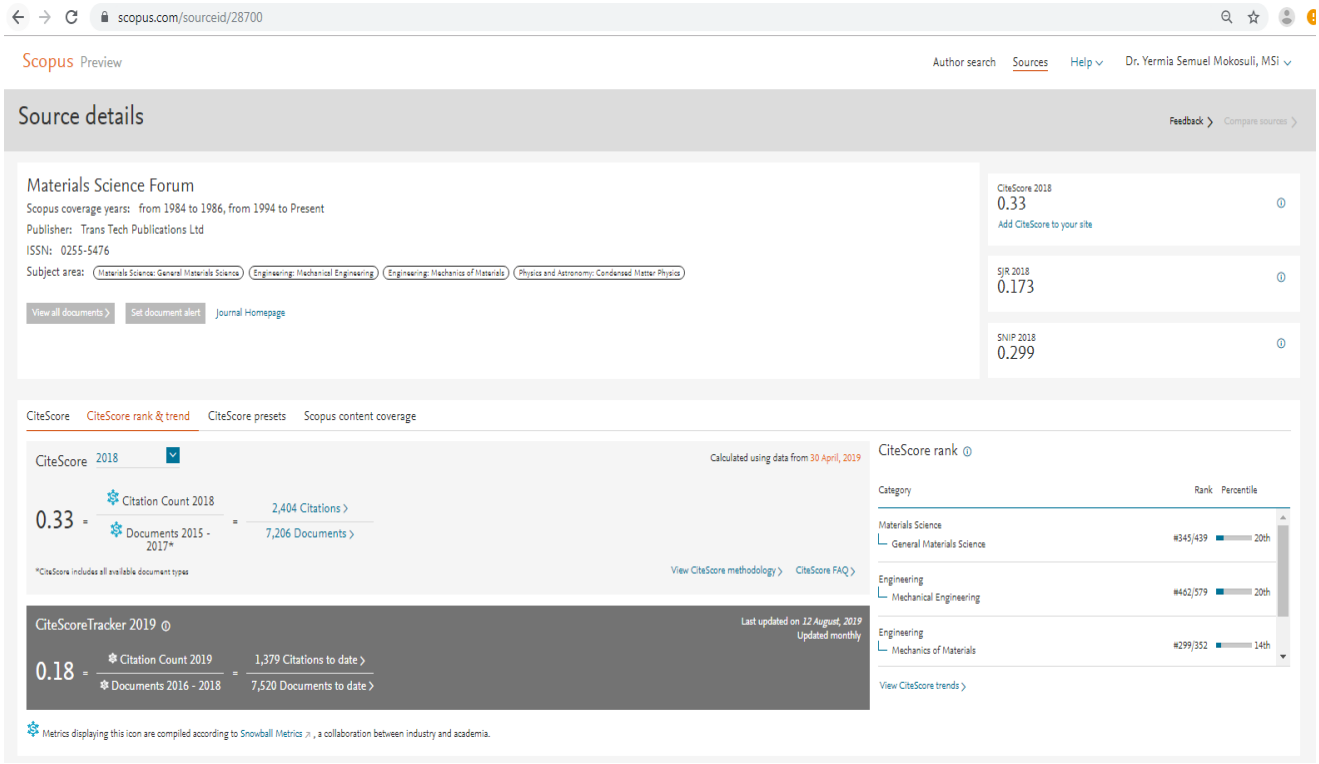
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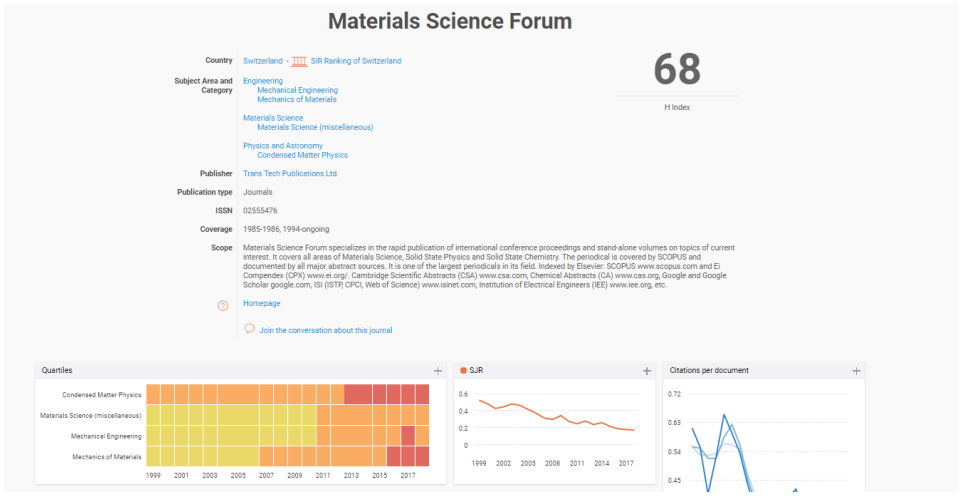
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Philogenic Relationship of Wild Pigs and Local Pig from North Sulawesi Based on the Growth Hormone Gene (GH Gene)

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Keywords : growth hormone gen, local pigs, phylogeny tree, Sulawesi Utara

Abstract. Growth hormone regulates reproduction and growth in mammals. A study was conducted to obtain the characteristics of the GH gene, in local pigs in North Sulawesi. Pig samples were obtained from traditional farmers, from four districts in North Sulawesi. DNA extraction and purification, using pig pituitary tissue. Amplification of GH gene, performed by PCR method. Visualization of CO1 gene amplikon, performed by electrophoresis technique. Sequencing, conducted through the First BASE Singapore sequencing service. The results show that there is a variation of local pigs CO1 gene in North Sulawesi. Variations are also found in the amino acid sequence encoded by the GH gene. Knowledge of the characteristics of local pig gh gene, the basics of selection of local pigs superior to North Sulawesi.

Introduction

Local pigs are wild pigs, which have been domesticated for a long time and have experienced high adaptation to certain environments. Indonesia has five species of local pigs, from eight existing species in the world[1]. Some areas that have long been breeding local pigs are Minahasa, North Tapanuli, Bali, Sumba, Toraja and Papua. Local pig farm, done conventionally. Local pigs are fed agricultural wastes, human food waste, and non-planted crops. Local pig farmers do not use factory-made feed, so the growth of pigs takes place naturally. This makes local pork taste more savory and with a higher unsaturated fatty acid composition than hybrid pigs[2].

Local Indonesian pigs, endangered, among others, due to breeders prefer to breed imported pigs and hybrid pigs, which produce more meat, making it more economically profitable. The population of some local Indonesian pigs is the local pig of Jawa (Sus verrucosus), Local pig of Kalimantan (Sus barbatus), local pig of Sulawesi (Sus celebensis) and Babirusa (Babyroussa babyrusa). The population of all local pigs, already in the vulnerable to endangered stages are almost threatened to perish[3,4,5]

The need for pork in Asia is high, therefore the potential of Indonesia to be a pork exporter country is very large. In 2016, Indonesia's pig production is at 342,346 metric tons [6]. Data from the Central Bureau of Statistics showed that live pig exports to Singapore in July 2016 reached the US $ 4.58 million (Rp61.77 billion), up 11.61%, compared to June's value of U $ 4.10 million (Rp55.3 billion). Pork consumers prefer local pigs compared to hybrid pigs. The productivity of local pigs is relatively smaller compared to imported pigs. However, it is necessary to make a superior local pig selection, based on both phenotypic and genotypical characters.

The productivity of local pigs, influenced by the process of reproduction, hormone production, and feed given[7]. Growth hormone (GH) is a hormone synthesized and secreted by the anterior

pituitary gland section. GH plays a role in regulating metabolism and growth in mammals [8]. GH genes provide the biological effects on individual growth after birth, the rate of growth and milk production[9,10]. GH gene plays an important role in reproduction, embryogenesis, lactation, and growth of pigs [7,11,12]. Injections of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), increase production of growth hormone in [7]. On the other hand, increased expression of growth hormone increases the performance and growth of pigs. The characteristics of the GH gene owned by local pigs are potentially developed into molecular markers, for superior local pig selection. A study was conducted to obtain superior local pigs in North Sulawesi based on the characteristics of the GH gene.

Materials and Methods

Samples

Local pig samples obtained from several districts in North Sulawesi are northern minahasa, minahasa, mongondow and sanger balls (Figure 1). The pituitary gland is used as a tissue source, for DNA extraction. The organs of the pituitary gland are preserved in 70% ethanol, further down to the laboratory.

Figure 1. Location of local pigs sampling in Sulawesi island
(map source : www.google.co.id/maps/place/Sulawesi/.com) .

Figure 2. The Sulawesi local pigs, sampling location Langowan, Minahasa.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from pituaitary gland tissue using Genomic DNA Mini Kit (Tissue) according to the manufacturer's protocol. Amplification of GH gene using PCR method, was applied MyTaq HS Red Mix Bioline (Table 1 and Table 2).

Table 1. The Componets of PCR.

PCR Component	Volume (µL)
2x MyTaq HS Red Mix Bioline	25
Primer Forward : : 5'-TGGTGTTTGGCACCTCAGAC-3' (Miao Zhiguo *et al*. 2012)	1
Primer Reverse : HCO2198 : 5'-CGTCATCACTGCGCAAGTTT-3' (Miao Zhiguo *et al*. 2012)	1
DNA babi lokal	2
ddH_2O	21
Total	**50**

Table 2. The Conditions of PCR.

Cycle	Time (Second)	Temperatur (°C)	Phase
35 x	60	94	Denaturation
	30	50	Annealing
	30	72	Ekstension
	60	72	Final Ekstension

PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp). Purified PCR products were analyzed by electrophoresis in 1% agarose gel. The molecular size of the amplified products was estimated using 1 kbp DNA ladder (Biometra). PCR products were sequenced using AB1 PRISM Dye Terminator Cycle Sequencing Ready Reaction System, version 1.1. (Applied Biosystems) in FIRST BASE Singapura

Sequences Analyses and Phylogeny trees reconstructed

Obtained sequences were aligned using BioEdit, Geneious v11.0.3 and MEGA 6.0 software. Sequences were subjected to Basic Local Alignment Search Tool (BLAST) in order to perform sequence similarity searches (www.ncbi.nih.gov.com). Nucleotide frequencies were calculated using BioEdit software. Phylogenetic trees were reconstructed using MEGA 6,0 software.

Results and Discussion

Amplification of GH gene areas, local pigs from five districts in North Sulawesi were successful. PrimaryGH gene with forward:: 5'-TGGTGTTTGGCACCTCAGAC-3 'and reverse 5'-CGTCATCACTGCGCAAGTTT-3' [11], show clearly visible band patterns in the electrogram, electrophoresis results. The thickness of the band formed, indicating the number of GH amplicon genes, which were successfully amplified by the PCR method. Based on a 1 kb ladder DNA size as a comparison, it shows the length of the GH gene, from each local pig sample being in the range 300 bp - 400 bp (Figure 2). It can be concluded that extraction using local pig pituitary gland, successfully isolated total DNA well.

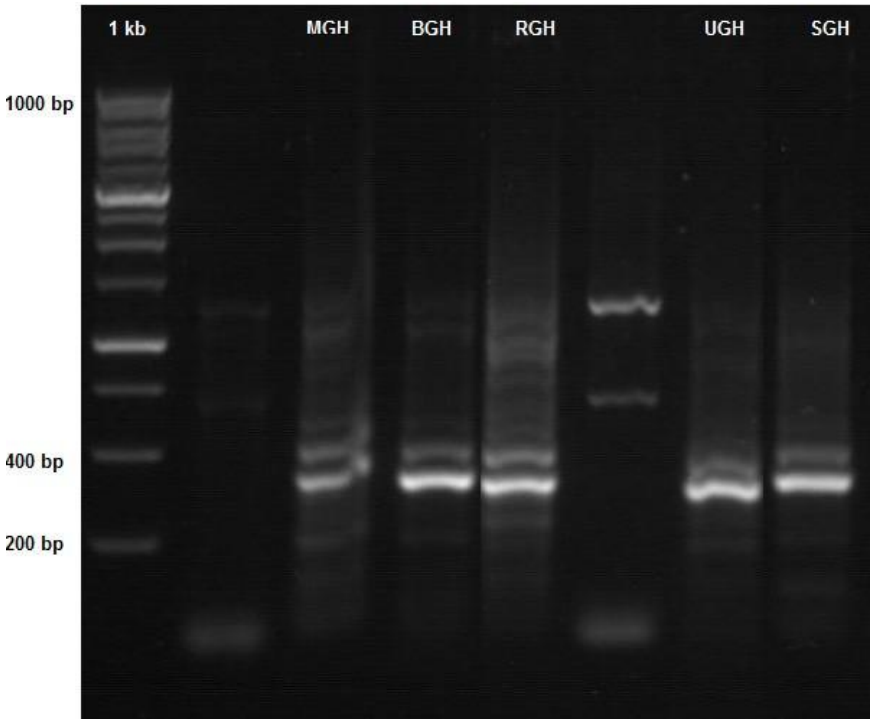


Figure 3. Visualization of amplicons of GH gene fragments, local pigs from Sulawesi.

GH gene sequencing results, visualized in a chromatogram using the Geneious v11.0.3 program. The mean HQ score was 89.3%, indicating the ouput quality of the GH gene sequence, local pigs from Sulawesi were excellent. GH forward and reverse sequence genes, aligned to obtain a consensus area. Alignment is done by Bioedit program in order to obtain an accurate sequences, to reduce the risk of errors in the results BLAST, and fault tree phylogeny reconstruction. Characteristics of the GH gene, local pigs of North Sulawesi, have been obtained based on sequence analysis. The length of the GH gene, local pigs are 351 bp (MGH), 438 bp (BGH), 356 bp (RGH), 432 bp (UGH) and 379 bp (SGH). The difference in the length of the GH consensus gene area has shown variations in the GH gene, local pigs in North Sulawesi (Table 3).

Table 3. Characteristics of GH gene sequences, local pigs of North Sulawesi.

No	Sample	Sequences characteristics
1	Babi Lokal Minahasa (MGH)	DNA molecule: MGH Length = 351 base pairs Molecular Weight = 107819,00 Daltons, single stranded Molecular Weight = 214318,00 Daltons, double stranded G+C content = 65,24% A+T content = 34,76% Nucleotide Number Mol% A 69 19,66 C 126 35,90 G 103 29,34 T 53 15,10
2	Babi Lokal Bolaang Mongondow Utara (BGH)	DNA molecule: BGH Length = 438 base pairs Molecular Weight = 133110,00 Daltons, single stranded Molecular Weight = 267180,00 Daltons, double stranded G+C content = 61,64% A+T content = 37,90% Nucleotide Number Mol% A 79 18,04 C 125 28,54 G 145 33,11 T 87 19,86 R 1 0,23 K 1 0,23
3	Babi Lokal Bolaang Mongondow (RGH)	DNA molecule: RGH Length = 356 base pairs Molecular Weight = 109258,00 Daltons, single stranded Molecular Weight = 217366,00 Daltons, double stranded G+C content = 65,17% A+T content = 34,83% Nucleotide Number Mol% A 69 19,38 C 126 35,39 G 106 29,78 T 55 15,45
4	Babi Lokal Minahasa Utara (UGH)	DNA molecule: UGH Length = 432 base pairs Molecular Weight = 132068,00 Daltons, single stranded Molecular Weight = 263439,00 Daltons, double stranded G+C content = 59,72% A+T content = 38,66% Nucleotide Number Mol% A 85 19,68 C 137 31,71 G 121 28,01 T 82 18,98
5	Babi Lokal Sulawesi Tengah (SGH)	DNA molecule: SGH Length = 379 base pairs Molecular Weight = 115950,00 Daltons, single stranded Molecular Weight = 231042,00 Daltons, double stranded G+C content = 58,05% A+T content = 40,11% Nucleotide Number Mol% A 73 19,26 C 123 32,45 G 97 25,59 T 79 20,84

Alignment of the GH gene, local pig North Sulawesi, has shown many sites where there are differences in nucleotides. Alignment with the Multalin program, obtained the consensus of the GH gene, from five regions in Sulawesi (Figure 4).

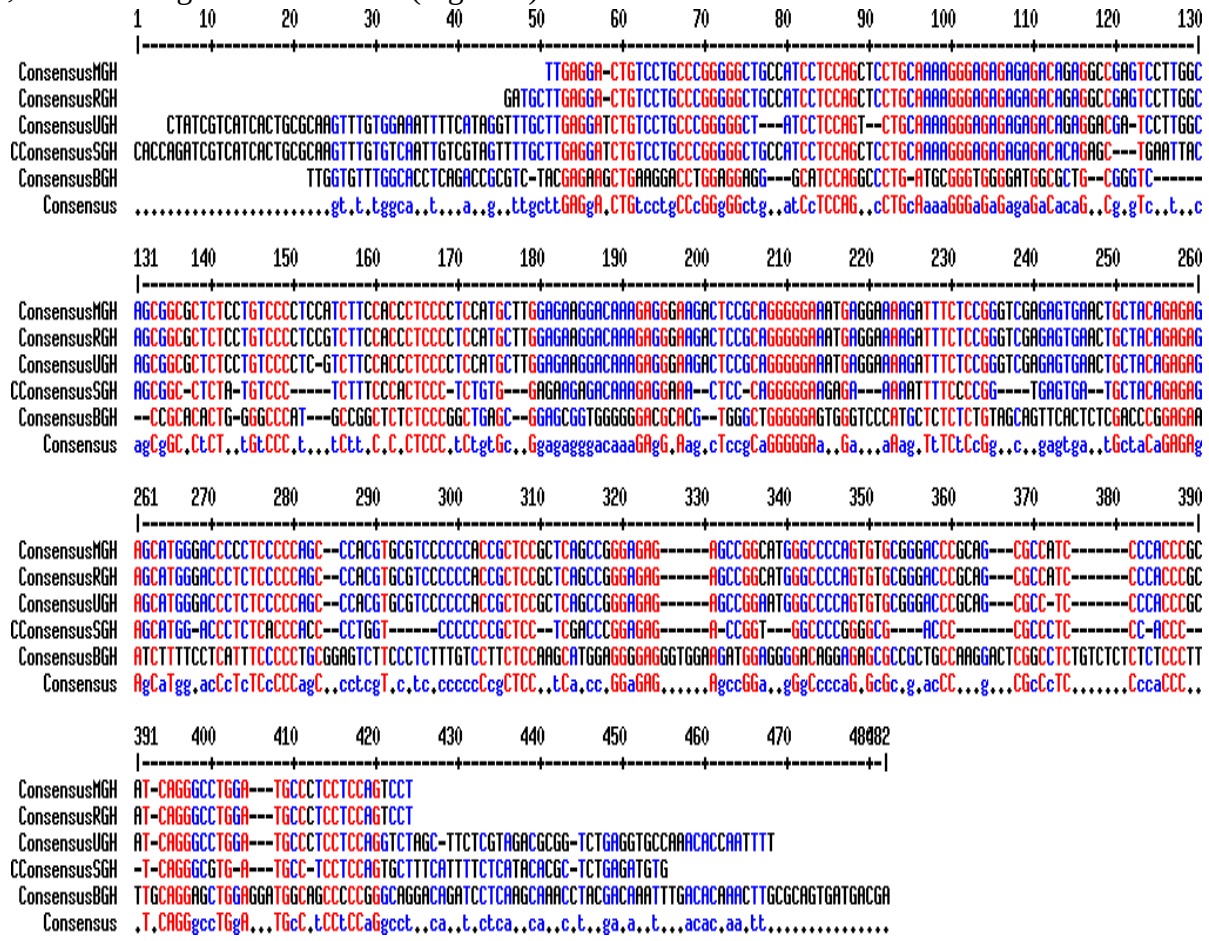


Figure 4. Align the GH gene with the Multalin program.
(http://multalin.toulouse.inra.fr/multalin/cgi-bin/multalin.pl).

The dots indicate the same amino acid encoded by all the GH gene from Sulawesi. If any sequence has a difference in amino acids, then the amino acid name symbol is written. Based on translations, amino acids encoded by the GH gene, have shown many differences. Sites where transition and transversion mutations occur in the GH gene sequence (Figure 5) have affected the type of amino acid translation. Thus, there has been a variety of amino acids encoded by the GH gene, local pigs in Sulawesi.

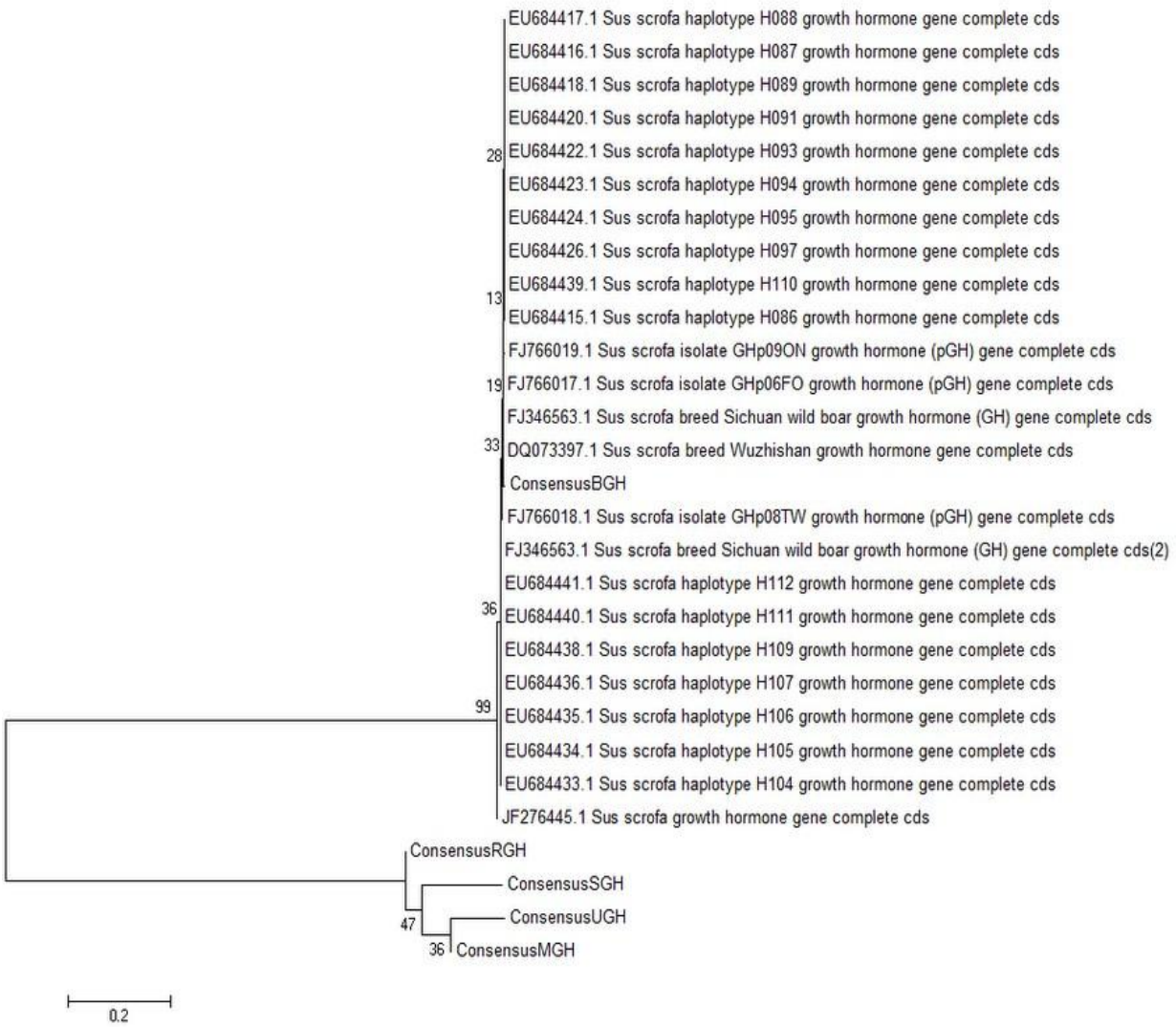
Figure 5. Comparison of amino acid composition translated by GH gene, local pigs in North Sulawesi

The results of basic local alignment searching tools, on the NCBI website, showed the GH gene, local pigs from Minahasa, Bolaang Mongondow Utara, and Sulawesi Tengah, have the closest similarity, with *Sus scrofa* [FJ766018.1], (99%). On the other hand, the GH gene, local pig Bolaang Mongondow and Minahasa Utara, has the closest similarity to *Sus scrofa* [EU684439.1], (99%). Compared with previous research, that local pigs of Sulawesi, have species names, *Sus celebensis*[4,5], it was confirmed BLAST results on the NCBI website, July 4, 2018, no data was found on the gene bank NCBI, similarity of GH gene with *Sus celebensis* (Table 4).

Table 4. GH gene sequence, which has the closest similarity, BLAST results on the NCBI website.

No	Samples	Similarity Sequence	% Similarity	Location
1	MGH	*Sus scrofa* isolate GHp08TW growth hormone (pGH) gene, complete cds GenBank: FJ766018.1	99 %	Sichuan 625014, China
2	BGH	*Sus scrofa* isolate GHp06FO growth hormone (pGH) gene, complete cds GenBank: FJ766017.1	99 %	Sichuan 625014, China
3	SGH	*Sus scrofa* isolate GHp06FO growth hormone (pGH) gene, complete cds GenBank: FJ766017.1	99 %	Sichuan 625014, China
4	RGH	*Sus scrofa* haplotype H110 growth hormone gene, complete cds GenBank: EU684439.1	99%	East Asia
5	UGH	*Sus scrofa* haplotype H110 growth hormone gene, complete cds GenBank: EU684439.1	99%	East Asia

The reconstruction of the phylogeny tree, based on the GH gene sequence, has been done to obtain the status of species, and the evolutionary relationships, local pigs in North Sulawesi. Phylogeny construction is carried out with 24 sequences of the bank gene, BLAST results, which have a similarity of 99%. Phylogeny tree construction is done with two models, namely: Neighbor-Joining and Minimum Evolution. In both models, the same phylogenetic tree topography is produced. This reinforces the construction of phylogeny trees that have been formed. The phylogenetic tree forms two monophyletic groups. The first group is the GH gene sequence, local Sulawesi pigs namely: samples from Bolaang Mongondow, Sulawesi Tengah, Minahasa Utara and Minahasa. The second monophyletic group was 24 BLAST-related sequences and samples from Bolaang Mongondow Utara (Figure 6).


EU684417.1 Sus scrofa haplotype H088 growth hormone gene complete cds
EU684416.1 Sus scrofa haplotype H087 growth hormone gene complete cds
EU684418.1 Sus scrofa haplotype H089 growth hormone gene complete cds
EU684420.1 Sus scrofa haplotype H091 growth hormone gene complete cds
28
EU684422.1 Sus scrofa haplotype H093 growth hormone gene complete cds
EU684423.1 Sus scrofa haplotype H094 growth hormone gene complete cds
EU684424.1 Sus scrofa haplotype H095 growth hormone gene complete cds
EU684426.1 Sus scrofa haplotype H097 growth hormone gene complete cds
EU684439.1 Sus scrofa haplotype H110 growth hormone gene complete cds
13
EU684415.1 Sus scrofa haplotype H086 growth hormone gene complete cds
FJ766019.1 Sus scrofa isolate GHp09ON growth hormone (pGH) gene complete cds
19
FJ766017.1 Sus scrofa isolate GHp06FO growth hormone (pGH) gene complete cds
FJ346563.1 Sus scrofa breed Sichuan wild boar growth hormone (GH) gene complete cds
33
DQ073397.1 Sus scrofa breed Wuzhishan growth hormone gene complete cds
ConsensusBGH
FJ766018.1 Sus scrofa isolate GHp08TW growth hormone (pGH) gene complete cds
FJ346563.1 Sus scrofa breed Sichuan wild boar growth hormone (GH) gene complete cds(2)
EU684441.1 Sus scrofa haplotype H112 growth hormone gene complete cds
36
EU684440.1 Sus scrofa haplotype H111 growth hormone gene complete cds
EU684438.1 Sus scrofa haplotype H109 growth hormone gene complete cds
EU684436.1 Sus scrofa haplotype H107 growth hormone gene complete cds
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EU684435.1 Sus scrofa haplotype H106 growth hormone gene complete cds
EU684434.1 Sus scrofa haplotype H105 growth hormone gene complete cds
EU684433.1 Sus scrofa haplotype H104 growth hormone gene complete cds
JF276445.1 Sus scrofa growth hormone gene complete cds
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ConsensusUGH
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ConsensusMGH
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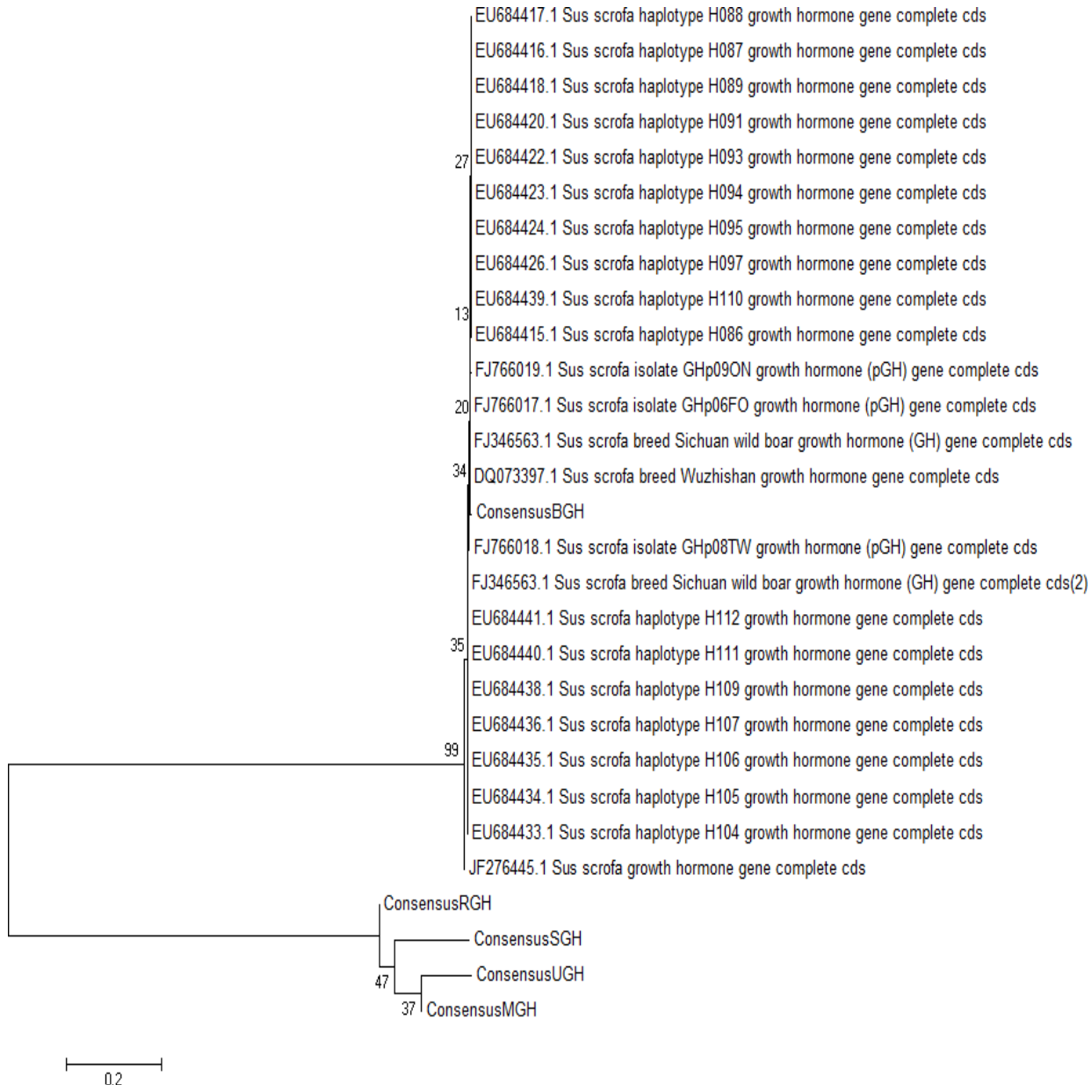

b.

Figure 6. The result of reconstruction of phylogeny tree, local Sulawesi pig, based on GH gene, with 24 sequences of BLAST results. The phylogeny tree is built with MEGA 6.0; bootstrap 1000 x. (a). Neighbor Joining Model (b). Minimum Evolution Model.

This phylogenetic tree explains that based on the GH gene, local Sulawesi pigs originate from a common ancestor, except for local pigs from Bolaang Mongondow Utara. However, they form the same monophyletic group, but no one forms a node. Therefore, confirmed the variation of the GH gene, local pigs in North Sulawesi. From the phylogenetic tree formed, local pigs from Bolaang Mongondow are the oldest local pig, based on the evolutionary history of the GH gene. Molecular barcoding using the CO1 gene, also cannot confirm the status of local pig species in Minahasa, North Sulawesi[13].

GH genes play a role in the growth and health of mammals [14.] Growth hormone plays a role in postnatal growth regulation, stimulation of anabolic processes such as bone growth and protein synthesis[15]. The GH gene affects milk production, fat content and cow's milk protein[16]. With the vital role of the GH gene, the molecular characteristics of the GH gene greatly affect the growth and development of mammal animals. GH gene research, as a molecular marker for superior livestock selection, has been widely practiced on Cattle. GH gene is recommended by many researchers, as a

molecular marker in the selection of superior cows [17, 18,19]. Variations in the GH gene have been used as the basis for genetic selection of Balinese cattle [20]. Based on the results of this study, the proposed GH gene, potentially developed as a molecular marker for superior local pig selection.

Conclusion

GH gen variation has been found in local pigs of North Sulawesi. GH gene variation, affect the amino acid composition encoded. The GH gene, potentially developed as a molecular marker, for superior local pig selection.

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