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Short Communication

The Origin of Indonesian Guinea Pig (*Cavia porcellus*) Inferred from Mitochondrial Cytochrome-b Gene

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AB and WPBP convinced the research and wrote the initial draft. AR and RH supervised the research project. BAS contributed in sample collection and proof reading the manuscript. All authors approved and contributed equally to the final version of the manuscript for publication

Key words
***Cyt-b* gene, Guinea pigs, Haplotype, Indonesia, Mitochondria, Sequencing**

ABSTRACT

Guinea pig (*Cavia porcellus*) is the one of Rodentidae animals that originated from Andes Mountains of South America. This study was aimed to determine the origin of Indonesian guinea pig based on partial mitochondrial Cytochrome-b (*Cyt-b*) gene sequence. Ten male guinea pigs at seven months of age collected from West Java of Indonesia were used as the experimental animal. Nonetheless, only five animals showed amplified 876 bp product. The sequencing analysis revealed that a transversion mutation of g.688T>A was occured in the *Cyt-b* gene of animals under study. Interestingly, a transtition mutation of g.519T>C in *Caviine Cyt-b* gene can be used to discriminate guinea pigs of Indonesian and South American. However, the UPGMA tree revealed that guinea pigs from Argentina, Peru, Colombia and Europe are grouped into similar cluster with Indonesian guinea pigs. In conclusion, there are two haplotypes of Cyt-b gene in the Indonesian guinea pigs *i.e.* T (4 heads) and A (1 head) haplotypes. In the future, the morphometrical and morphological studies in guinea pigs from Indonesia and South America are important to investigate the geographical effects on separated species.

Guinea pigs (*Cavia porcellus*) are one of the Rodentidae species that originated from Andes Mountains of South America (Dunnum *et al*., 2010). Generally, guinea pigs were used as a laboratory and pet animals in the world (Meredith, 2015; Lossi *et al*., 2016; Depreux *et al*., 2018). As the muslim country, the meat of guinea pig (Cuy meat) is not popular in Indonesia. However, the Cuy meat are consumed by most South American people (Jurado-Gamez *et al*., 2016). A Cuy meat has high protein content (20.3%), and low content fat (7.8%); compared with other meat products, like birds (18.2% protein and 10.2% fat), cattle (18.7% protein and 18.2% fat) and pigs (12.4% protein and 35.8% fat). So, it has high nutritional potential (Rosenfeld, 2008). In Indonesia, guinea pig can possibly be kept for meat production in the future since it can be fed with rabbit feed (Trejo-Sanchez *et al*., 2019). Moreover, guinea pig meat is high in protein and low in fat contents (Moreno and Arteaga-Minano, 2018). Good farming practices must be implemented since it can also be a reservoirs of zoonotic yeasts (Buela *et al*., 2022).

Unfortunately, there are no literature studies that explain the origin of guinea pigs in Indonesia. Early opinion suggested that guinea pigs may have been brought to Indonesia by European people when they came to Molucca, Java and Sumatra islands for spice trade with local kingdoms. Other have suggested that guinea pig might have been imported in Indonesia during Dutch Colonial period (18th century) as pet animal. Presently, the origin of animals can be detected with a mitochondrial sequence information. Thus, about 92.70% of the mitogenome in *C. porcellus* is similar to *C. apera* (Wahedi *et al*., 2020). Nonetheless, a *Cytochrome-b* (*Cyt-b*) gene is one of mitochondrial genes that is used for DNA barcoding in guinea pig (Spotorno *et al*., 2006; Dunnum *et al*., 2010; Diaz *et al*., 2016). This study aimed to reveal the origin of Indonesian guinea pig based on partial mitochondrial *Cyt-b* gene. The results in this study are important to

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initiate the further research that investigating Asian and South American guinea pigs with taxonomic study.

Materials and methods

Ten male guinea pigs at seven months of age with the avearge body weight of 465.90±64.34 g were collected from the farmers at Kedungbadak Village, Bogor, West Java of Indonesia and used for the present expermintal study. The blood samples (3ml) was added EDTA in tube and then stored at -20 °C for the next analysis. The DNA extraction was performed using Genomic DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's protocols.

The amplification of *Caviine Cyt-b* gene was performed in a total reaction of 30 µL consisting 9 µL of DNA template; 9 µL of PCR master mix (Bioline, USA), 4.8 µL of nuclease free water and 0.6 µL of each primer. The primer pairs in this study referring to Spotorno *et al.* (2004) as follows: Forward: 5'- TCC AAT GTA GGA ATT ATG ACC CAC C-3' and Reverse: 5'- TTT CCC ATC TCT GGC TTA CAA GAC -3'. The amplification of *Caviine Cyt-b* gene was performed in a Mastercycler gradient (Eppendorf, Germany) with the PCR program as follows: 95.0 °C for 5 min (initial denaturation) and 35 cycles with 95 °C for 15 sec (denaturation); 50.7 °C for 15 sec (annealing); 72 °C for 30 sec (extension) and 72 °C for 3 min (final extension). The DNA electrophoresis was performed in 1% of agarose gel at 110 V for 30 min and stained with SYBR® Safe DNA gel stain (Invitrogen, USA). The DNA fragments were vizualised using G-Box Documentation System (Syngene, UK). The sequencing analysis was carried out at First Base Laboratories Sdn Bhd (Malaysia) using ABI PRISM® 377 DNA sequencer with with the BigDye® Terminator 3.0 Cycle Sequencing Kit.

Alignment of DNA sequence was performed using BioEdit software (Hall, 2011). Otherwise, a MEGA software (Hall, 2013) was used to construct the phylogenetic tree using UPGMA methods with 1000 × bootstrap replications. Therefore, many *Caviine Cyt-b* gene sequences of Guinea pig from South America such as Argentina (AY228362), Bolivia (AY382790), Chile (AY382793, AY228361), Colombia (AF490405), Paraguay (AY382791) and Peru (AY247008, AY382795, AY247008, AY228363, AY245098, AY245096, AY245097, AY382799) were collected from GenBank database to compare with Indonesian Guinea pigs. Otherwise two Cavidae species of *C. apera* (AY382790) and *C. tschudii* (AY382792) were involved in analysis as the wild ancestor of guinea pig (Rosenfeld, 2014). Otherwise, three Rodentidae species of *Microcavia niata* (AY382788), *Dolichotis patagonum* (AY382787) and *Galea musteloides* (AY466603) were used as the outgroup in phylogenetic tree.

Results and discussion

Five individuals of guinea pigs can be amplified using the universal *Cyt-b* gene primer pairs from Spotorno *et al.* (2004) with the amplicon ranges of 1000 to 1500 bp (Fig. 1). While, five other guinea pigs can not be amplified using thats primer. However, the partial sequences of *Cyt-b* gene of Indonesian guinea pig in this study were submitted in the NCBI database with the GenBank accession number of OR633256-OR633260. Consequently, a specific primer pairs must be designed to amplify the *Cyt-b* gene of Indonesian guinea pigs in the future. According to the *Cyt-b* gene sequence, a transversion mutation of 688T>A were detected in one sample of Indonesian guinea pig (Fig. 2).

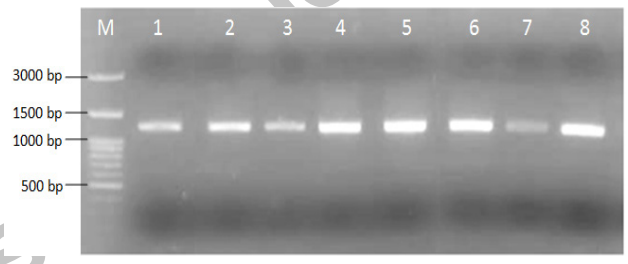

Fig. 1. The amplification of mitochondrial *Cyt-b* gene in Indonesian guinea pig (*Cavia porcellus*) on 1% agarose gel. M: DNA ladder 100 bp; Line 1-8: DNA sample.

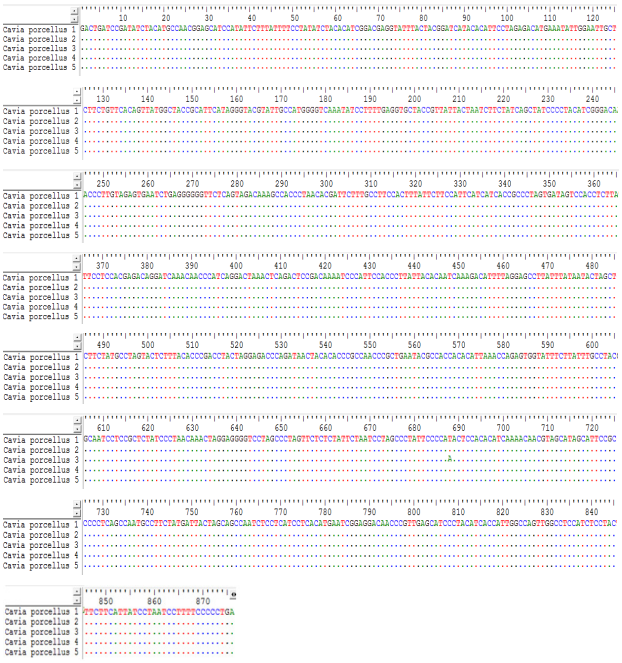

Fig. 2. Alignment of partial *Cyt-b* gene (876 bp) in guinea pigs (*Cavia porcellus*) of Indonesia.

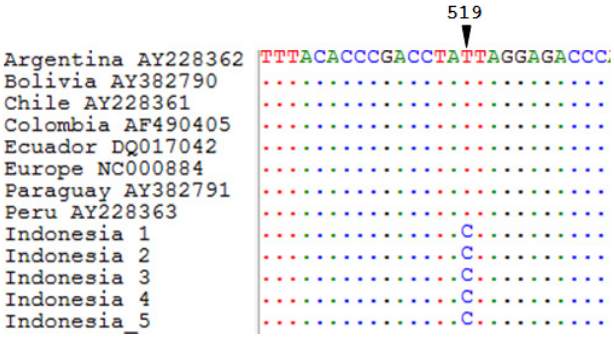

Fig. 3. Detection of a genetic marker at 519th position to discriminate guinea pigs (*Cavia porcellus*) from Indonesia and South America/ Europe.

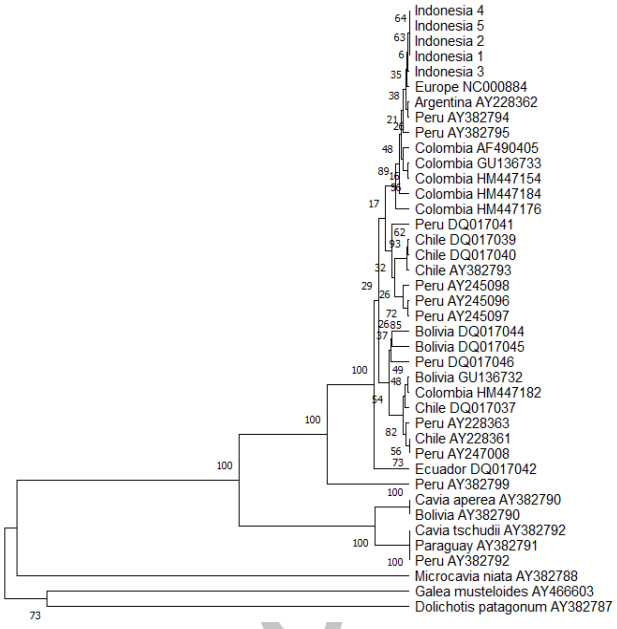

Fig. 4. UPGMA tree (1000 × bootstrap replications) between guinea pig (*Cavia porcellus*) from Indonesia and South American countries based on *Caviine Cyt-b* gene.

Compared with *Cyt-b* gene of Indonesian guinea pigs, a transition mutation of g.135G>A and a transversion mutation of g.576A>T were detected in the South American guinea pigs. Otherwise, an indel mutation of g.578_579. indel. AC were also detected in *Cyt-b* gene of South American guinea pigs (Diaz *et al*., 2016). Interestingly, a transition mutation of g.519T>C were detected in *Cyt-b* gene and used as the genetic marker to discriminate guinea pigs from Indonesia and South American (Fig. 3). However, the UPGMA tree of *Cyt-b* gene revealed that mainly Indonesian guinea pigs are close to European guinea pigs (Fig. 4). Subsequently, the South American guinea pigs can be classified into three common genera lineages of Tschudii, Aperea and Porcellus. In addition, mostly South American and Indonesian guinea pigs are grouped into Porcellus lineage. Moreover, Ecuadorian guinea pigs were also classified into similar cluster with Colombian, Peruvian and Chilean guinea pigs based on *Cyt-b* gene (Diaz *et al*., 2016). In addition, the guenea pigs of Puerto Rico, Carriacou and Colombia are classified into similar cluster based on *Cyt-b* gene (Kimura *et al*., 2016).

Compared with other *Rodentidae* species, *Cavia* sp. are close to *Microcavia niata, Galea musteloides* and *Dolichotis patagonum* based on *Cyt-b* gene (Spotorno *et al*., 2004). In addition, guinea pig was clustered with porcupine based on *Cyt-b* gene (Ma *et al*., 1999). Moreover, guinea pig was not clustered with rat (*Rattus norvegicus*) and mouse (*Mus musculus*) based on mitochondrial *12S rRNA* and *16S rRNA* genes but it was grouped in the same cluster with rabbit (*Oryctolagus cuniculus*). Despite this, *Cavia* was grouped in the separated cluster with *Mus* and *Rattus* genera based on *Cyt-b* gene (Spradling *et al*., 2001). Contrastly, guinea pig, mouse and rat are grouped into similar cluster based on sex determining region on the Y chromosome (SRY) gene (Margarit *et al*., 1998). According to the UDP glucuronosyltransferase 1 family polypeptide A3 (*UGT1A3*) gene, guinea pig is classified into similar cluster with Ma's Night monkey (*Aotus nancymaae*) as reported by Mingsha and Shouzhang (2022).

Spotorno *et al*. (2006) suggested that there were three phases of human interaction with guinea pigs: The initial domestication from *C. tschudii* to *C. porcellus*, which probably occurred in southern Peru/northern Chile, followed by two subsequent modern selection processes outside of South America resulting in the laboratory and pet breeds of Europe and improved breeds for the South American meat market. Lord *et al*. (2020) stated that people transported guinea pigs from the South American mainland at begining arround AD 600 for introducing them to the Caribbean Antilles from a Peruvian-derived population, possibly two times, through existing social networks of interaction and trade routes. According to Lord *et al*. (2020), South American guinea pigs were translocated to Europe (early 17th century) and USA (early 19th century).

Hence, this early investigation suggested that guinea pigs in Indonesia originated from Europe that close with guinea pigs from Argentina, Peru and Colombia. Further study is required for evaluating the genetic diversity of Indonesian guinea pigs with microsatellite markers is important since this method can accurately explain the population structure of guinea pig (Burgos-Paz *et al*., 2011; Aviles *et al*., 2015; Ayagirwe *et al*., 2017; Aviles-Esquivel *et al*., 2018).

Conclusion

The early investigation revealed that two variations of *caviine Cyt-b* gene sequence were observed in Indonesian guinea pigs. A transition mutation (T>C) at 519th position in *caviine Cyt-b* gene of Indonesian guinea pig is identified as the genetic marker to discriminate Indonesian and South American or European guinea pigs. Howewer, the *caviine Cyt-b* gene revealed that guinea pigs of Indonesia, Europe, Argentina, Peru and Colombia were clustered together.

Animal ethics approval

This study was conducted under the animal ethics protocols of Faculty of Agriculture, Djuanda University, Indonesia. (Certificate No: 1082/01/Faperta-A/X/2023).

Statement of conflict of interest

The authors have declared no conflict of interesrt.

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