Detection and characterization of wolbachia endosimbiont in wild bee (*apis cerena*) using the cytochrome C oxidase subunit I gene

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Abstract. *Apis cerena*; is as wild bee or honey bee, widespread in almost all regions of Indonesia, these bee economic value as the honey it produces. Indonesian people, especially in Tanjung Peropa Southeast Sulawesi who use bees as a honey-producing source maintain honey bees with a honeycomb claim system that lives in hives in forested trees or found in people's homes. Until now, there is no breeding or breeding business. Wobachia is endosimbiont which was infected Artrophoda, including insects, which can influence the dynamics of these insects population and can reduce insect perform and viability. Therefore, molecular detection of the presence of Wolbachia in *Apis cerena* is important. The COI gene is a gene proposed as an animal barcode. There is evidence of primer use of the COI gene as a barcode which is often contaminated with Wolbachia COI gene which is not a target. The individual bee was extracted the genome DNA using the CTAB (cetyltrimethyl ammonium bromide )method, and then the cytochrome oxidase gene (COI) was umplified using an animal barcode primer. The amplification results are then sequenced and then characterized. Using Wolbachia COI gene data available on GenBank as a comparison, reconstruction of phylogenetic tree of Wobachian base on COI nucleotide sequences, so the position taxon of Wolbachia shall be determine. Phylogenetic tree reconstruction make with a Neiberjoining method with the Kimura 2-meter models and 1000x boostrapped. The results showed that the Wobachi COI gene was successfully amplified by these primer animal barcode, along 701bp. A proof that wild bees have been infected by Wolbachia. The findings of this study prove that PCR method are very sensitive to be used to detect existenceWolbachia in bees. This also means that the primer used to reveal animal barcodes, specifically vertebrates, can also amplify the Wolbachia gene; a Rickettsia. The gene has special characteristics, namely 633 base pairs (bp) conserved, and 24 bp variable nucleotides. Of these there are 12 nucleotides which unique for Wobachians who infected *A.cerena* from Tanjung Peropa, Kendari Southeast Sulawesi. Base N composition dominated of Timine. The phylogenetic tree showed that Wolbachia from *Apis cerena*, is closely related to Wolbachia endosymbiont Hymenoptera, with 99% of boostrapped value.

1. Introduction

There are 5 species of native honey bees known in Indonesia, namely: *Apis andreniformis, A. dorsata, A. cerana, A. koschevnikovi,* and *A. nigrocincta. A. cerena* is a type of honey bee that is characterized by the smallest body size, with a transverse pattern on their abdomen as deep as 5 band. These bees usually make a nest in a closed place (1). Wolbachia is Ricketteia; intracellular groups of bacteria that infect the reproductive tissue of arthropods, insects and some nematodes. This bacterium was first discovered in mosquito cells; *Culex pipiens* and is described as one species, namely *W.pipientis*
(commonly known as Wolbachia) (2). Wolbachia has a high diversity and is distinguished as a supergroup, each supergroup is distinguished based on variations in its nucleotide sequences. The nucleotide sequence was amplified with several primers, including: 16sRNA, rDNA, FtsZ, and Wsp (3,4). Modification of nucleotide sequences in the FtsZ primer, and wsp was used by (5) to estimate Wolbachia in pseudoscorpion and evaluate the effectiveness of antibiotic use in these hosts.

Initially only 5 Wolbachia supergroups were known, namely the A, B, C, D and E supergroup (6). Furthermore, the F, G, H supergroup using the ftsZ, gltA, and groEL gene genes (protein coding genes) were added (7). A host can be infected by one or more Wolbachia supergroups, While a Wolbachia supergroup can infected more than one host. At arthropods are found Wolbachia supergroup A, B, F, and G (spider), Nematode (C, D, F), Springtail (E) and Termite (H) (2,8). Insect is more dominated by type B of wobachia (6).

Wolbachia is transmitted through the cytoplasm of the egg and affects the host's reproductive system in various ways, including; changing the sex ratio (killing male embryos) in Cordylochernes scorpioides (5), causing feminization of the male terrestrial and locust Isopods (9), Wolbachia infecting Cylisticus convexus (wCc), causing cytoplasmic incompatibility (Cytoplasmic Incompatibility) males (9), an effect commonly found in insects (10). Apart from the negative effects described earlier, an interesting phenomenon proposed by (11), that Wolbachia that infects ladybugs (Cimex lectularius), is in the bacteriome and is a symbiotic mutualis of nutrition. Wolbachia as endosymbiont in the ladybug is transmitted vertically through plasma cells (somatic stem cells) found in oocytes, infecting in the early stages of embryogenesis. If the endosimbion is removed from the host (Cimex lectularius), the host experiences abnormal growth and sterility.

Wolbachia detection in several insects and arthropods has been carried out. The primers used are variable. 16SrDNA primers, ftsZ and wsp to detect Wolbachia in fruit fly (Anastrepha) and showed positive results, despite the sensitivity of these primers, varying; all samples showed that they contained Wolbachia group A when detected using 16S rDNA primers and wsp A and wsp B (3). But failure occurred by 44.9%, if using ftsZ primers. Furthermore Primer16s RNA was used in Apis mellifera cornica, and found that the population in the area was 100% infected with Wolbachia (12). When construction of animal barcodes, Wolbachia contaminants have also been found in the process which are basically not the target gene, however the sequence of contaminants can be distinguished from the target sequence (13). So far, there has never been a molecular detection of Wolbachia in A. mellifera or A. cerena found in Indonesia, especially in Southeast Sulawesi, using any primer. Therefore this research is important to do, in order to preserve the use of wild bees as a source of income for the community, especially in Tanjung Peropa, Southeast Sulawesi. The data obtained are preliminary and original data that can be used as basic data for ecological and biological studies, especially the genetics of wild bees and Wobachians.

Cytochrome C oxidase sub unit I (COI) is one of the genes found in mitochondrial of the eukaryotic cell. Which is encoding the amino acid cytochrome oxidase sub unit 1. This gene has a total length of about 15,000 base pairs; known as Cox A in bacteria (prokaryotic cell). A short end of the 5 COI gene (648 bp), proposed as an animal DNA barcode by researchers from Guelph University in Ontario, Canada since 2003 (14-15). DNA coding varies greatly between individuals within the same species, to a small degree. Normally, small variations in individuals within the same species are smaller than between individuals in a different population / species (16). DNA coding has been successfully applied to how many invertebrates and vertebrates, starting from springstail (17), butterflies (18-21), parasitoids (22), mayflies (23), ants (24), bees (25), birds (26-27), fish (28-30), bats (31), etc.

There is a phenomenon that in making animal barcodes by using COI primers, it is often obtained an unexpected amplicon (PCR product), namely the amplification of the Wolbachia COI gene as endosimbon in barcoded insects (4, 13). From previous studies, it is known that the CoxA gene segment varies with inter-species, There are 57 haplotype of the COX gene in Wolbachia endosimbon Hymenoptera, and several number of haplotyped in other host insects (13). Thus, the genetic markers used so far for Wolbachia detection will increase with the presence of COI genetic markers (CoxA).
2. Material and Method

Sampling location *Apis cerena* in Southeast Sulawesi is naturally preserved, namely that the api is kept in a particular place (usually on the edge of a forest tree, which is periodically moved for honey harvest, by expelling the bee colony using fire. The colony will move to trees or other places, so honey can be harvested in the nest of the colony. The sample of this study was taken from *Apis cerena* that was cultured like that, in the Tanjung Peropa, Wildlife Park Area, Southeast Sulawesi. There are four colonies in those area with coordinate points S:4°13’25.9 - E122°. 44.27.6 to S:4°13’22.9 - E122°. 44.97.6

2.1 DNA extraction

DNA extraction is carried out using conventional methods (32), with some adjustments, namely: head, wings and internal organs (digestive apparatus and viscera) removed, thus separating the chest and abdomen. The chest and abdomen then crushed until smooth, while adding 2x CTAB buffer (100 ml 1 M Tris HCl pH 8.0 + 280 ml 5 M NaCl + 40 ml of 0.5 M EDTA + 20 g of CTAB (cetyltrimethyl ammonium bromide), Bring the total volume to 1 L with ddH$_2$O), as much as 500 µL. After pulverizing, the sample was put into a 5 µL eppendorf tube, and CTAB was added to a volume of 700 µL, to be subsequently incubated at a water bath at 56 °C, for 2 hours. Every 30 minutes, the sample is blocked, so it does not settle. After that, the sample was added to 500 µL chloroform, homogenized, then centrifuged at 450 rpm for 12 minutes. The supernatant is then transferred to a new eppendorf tube. The supernatant was then added 0.08x the volume of 7.5 M cold ammonium acetate and 0.54x the volume of isopropanol or in a ratio of 28 µL: 204.12 µL for a sample volume of 350µL; homogeneous by flipping through the eppendorf tube. Subsequently incubated in a freezer for 40 minutes. After that, the sample was centrifuged at 145 rpm for 10 minutes. The liquid is discarded, and the pelelet is washed with 70% alcohol. After that centrifuged with kec 145 for 3 minutes. 70% alcohol is discarded, and the sample is added with absolute alcohol 3 times in volume, flipped and then centrifuged again for 3 minutes. The alcohol is discarded and the pellet is air dried at room temperature for ± 30 minutes. Pellets are dissolved with the addition of TE 1x, and ready for the next stages.

To visualize DNA, submarine electrophoresis was used with 1% agarose gel, and 2.5 µL EtBr was added. Positive control was used in the form of COI gene DNA samples, insects that had been amplified in previous studies (Suriana and Marwansyah 2017, unpublished). Extracted genomic DNA was used as a template for the amplification of the Wolbachia COI gene that infected bees (*A. cerena*).

2.2 COI gene amplification

COI gene amplification is carried out using polymerase chain reaction (PCR) methods. PCR is done by standard methods, using a Thermo plus PCR machine. The primer used is fordward_sequence: attaacaacatacataagatagg, and reverse_sequence: taaacttctggatgtccaaaaaatca (13). The total volume of the solution is 50 µL that contain: 25 µL, 2x PCR master mix containing SMO-HiFiTM, 10x HiFiTM buffer, 25 mM MgSO4, dNTPs mix and DMSO. Primer F and R, each 10 pico totaling 2.5 µL. DNA template 2 µL and ddH2O 18 µL. PCR machine conditions are adjusted to the company's recommendations (smobio) PCR kit providers, namely predenaturation at temperature 94 for 2 ', 35x cycles: denaturation 94, for 15 '', anneling 53 for 15'', extension 68 for 30 '', followed by post extension at temperature 68 for 1 minute and hold at 10. Samples were then electrophoresed to detect the presence or absence of Wolbachia COI gene amplification products, which indicates that the bee is infected with Wolbachia

The amplified sample is then sent to the sequencing service provider. Results of sequencing on the blast at http://www.ncbi.nlm.nih.gov/. Nucleotide blasts are performed to ensure that the results are the COI of Wolbachia gene. After that the nucleotide sequence analysis is performed.
2.3 Characterization of the COI gene

Multiple alignment of COI gene fragments, Wolbachia using Clustal W (MEGA7 software). Some COI gene data available at bank is included in this alignment as a comparison. Because the gene COI sequences are not all the same length, cutting at both end 3 and end 5 is done, to ensure that the compared sequences are equal. Some comparative sequences are: Wolbachia endosymbiont of Hymenoptera sp. (JN625952.1), Wolbachia endosymbiont of Hymenoptera sp. (JN625951.1), Wolbachia endosymbiont of Hymenoptera sp. (JN625950.1), Wolbachia endosymbiont of Nesomyrmex (JN625826.1), Wolbachia endosymbiont of Cerapachys (JN625785.1), Culex pipiens isolate 2AF (KJ500032.1), Wolbachia endosymbiont of Cerapachys (JN625785.1), Culex pipiens isolate 2AF (KJ500032.1), Wolbachia endosymbiont of Hylaeus (KP1863), Wolbachia endosymbiont of Rhinusa rara (KJ620006.1), Wolbachia endosymbiont of Megastigmus (KJ535725.1), Wolbachia endosymbiont of Diaphorina citri (KT960930.1), Wolbachia endosymbiont of Culex pipiens (KF278668.1), Wolbachia pipientis (MH605294.1), Wolbachia pipientis (MH605291.1), Wolbachia pipientis (MH422527.1), Wolbachia endosymbiont of Andrena sp.2 (KP265896.1), Wolbachia endosymbiont of Lasiosglossum tegulare (KP265886.1), Wolbachia endosymbiont of Augochlorella aurata (KP265881.1), Wolbachia endosymbiont of Halictus ligatus (KP265876.1), Wolbachia endosymbiont of Augochlorella aurata (KP265881.1), Wolbachia endosymbiont of Halictus ligatus (KP265876.1), Wolbachia endosymbiont of Halictus ligatus (KP265876.1), Wolbachia endosymbiont of Halictus ligatus (KP265876.1), Wolbachia endosymbiont of Augochlorella aurata (KP265881.1), Wolbachia endosymbiont of Hymenoptera sp. (JF960240.1), Wolbachia endosymbiont of Hymenoptera sp. (JN625793.1), Wolbachia endosymbiont of Hymenoptera sp. (JN625809.1), Wolbachia endosymbiont of Hymenoptera sp. (JN625807.1), and Wolbachia endosymbiont of Hymenoptera sp. (JN625952.1), Wolbachia endosymbiont of Crematogaster (JN625793.1).

3. Result

Of the 4 bee colonies, only 2 DNA samples were detected by the PCR method containing the Wolbachia COI gene, which is indicated by the presence of COI gene amplification products (Figure 1.)

The amplified Wolbachia COI gene is then sequenced and characterized theirs nucleotides. The COI nucleotide sequence of the Wolbachia gene that infects Apis cerena Tanjung Peropa over 701 base pairs (base pairs = bp), and has been characterized along 658 bp. Using the COI Wolbachia
endosimbion DNA fragment in Hymenoptera (13), which is available on GenBank, the characteristics of the COI gene sequence from Wolbachia endosimbiont *Apis cerena* are presented in Table 1.

**Table 1.** Characteristics of the nucleotide fragments of the Wolbachia endosymbiont *Apis cerena* COI gene

| Property            | Number of bases | (total bases compared) | Total comparison bases | Percentage |
|---------------------|-----------------|------------------------|------------------------|------------|
| Conserved           | 633             | 658                    | 96.2                   |
| Variable            | 24              | 658                    | 3.64                   |
| Parsimony informative | 13              | 658                    | 2                      |
| Singelstone         | 12              | 658                    | 1.8                    |

Table 1 shows that conserved nucleotides are much larger than variable nucleotides. This indicates that nucleotides are maintained from generation to generation at species level. Variable nucleotides, which have several informative parsimony nucleotides, mean that these nucleotides contain at least two types of nucleotides (or amino acids), and at least two of them occur with a minimum frequency of two. And among these variables there are also some that are singleton. Nucleotides such as this have a tendency to be a differentiator of species, or can be used as a marker of species. So far Wolbachia is still only known as 1 species, namely *W. pipiensis*, thus singlestone nucleotides in this species can be used as markers at lower levels, namely 'strains, which in previous researchers were known as‘ supergroups ‘. Different nucleotides and difference sites are presented in Table 2.

**Table 2.** Twenty-four different DNA sites of fragments of the Wolbachia COI gene

| Sites                          | Individuals compared |
|--------------------------------|----------------------|
| Wolbachia_JN625952             | 112222222334444556666 |
| Wolbachia_JN625951             | 7303608172 814       |
| Wolbachia_JN625950             | 090011355 4690077262 |
| Wolbachia_JN625826             | 2233                |
| Wolbachia_JN625785.1           |                     |
| Wolbachia_JN625809.1           |                     |
| Wolbachia_JN625807.1           |                     |
| Wolbachia_JF960240.1           |                     |
| Wolbachia_JN625952             |                     |
| Wolbachia_Cerapachys_JN625783.1|                     |
| Wolbachia_Crematogaster_JN62593.1 |                 |
| Wolbachia_endosymbiont_of_Nesomyrmex_JN625826 |                     |
| Wolbachia_endosymbiont_of_Cerapachys_JN625785.1 |                     |
| Wolbachia_endosymbiont_of_Apis cerena from Tanjung Peropa |                     |

Note: The dot is identical nucleotides. The situs number reading from up to down.

The variation of DNA sequences, is a very common phenomenon, both in chromosomal genes and extra chromosomal genes, the difference is whether the rate / speed of variation occurs, because it is related to the rate of evolution. The variation of DNA sequences can be caused by several things, including the occurrence of a single base substitution in a sequence. This substitution can be a substitution of synonyms and nonsynonyms (33). Substitution from purine to pyrimidine and reverse
pyrimidine to purine (nonsinonym substitution), for example Thymine substitution on the site that should be occupied by Adenine / Guanine and vice versa, while primidine substitution to pyrimidine and purine to purine, (synonym substitution), for example Thymine substitution on the site that should be occupied by Adenine/Guanine and vice versa. In Table 2 it appears that from 24 different sites, different nucleotides are due to synonym substitution (A → G or C → T), as many as 22 sites, while the other two sites, namely sites 478 and 622 are non-synonym substitution (A → C). Variations between single bases in the COI sequence between Wolbachia compared show the diversity of the sequences (table 2). Based on (34), the third position nucleotides of COI show a high incidence of base substitutions in common with other protein-coding genes. It leads to a rapid rate of molecular evolution that is about three times greater than that of 12S or 16S rDNA. This gene has a rapid evolution rate to allow the discrimination of not only closely associated species but also phylogeographic groups within a single species (35,36). This is common and can cause changes in the composition of nucleotides between sequence. The composition of nucleotides between species is presented in Table 3.

Table 3. Composition of nucleotides between fragments of the Wolbachia COI gene

| Wolbachia Number access | Nitrogen bases of nucleotide | Total |
|------------------------|-------------------------------|-------|
|                        | T(U)  | C   | A   | G   |      |
| JN625809.1             | 38.1  | 18.1| 23.4| 20.4| 658  |
| W.Endos JN625952       | 38.1  | 18.1| 23.6| 20.1| 656  |
| W.Endos JN625951       | 38.0  | 18.1| 23.9| 20.1| 658  |
| W.Endos JN625950       | 38.0  | 18.1| 23.8| 20.1| 656  |
| W.Endos JN625826       | 37.7  | 18.5| 23.3| 20.5| 658  |
| W.Endos JN625785.1     | 37.7  | 18.5| 23.4| 20.4| 658  |
| W.Endos JN625809.1     | 38.1  | 18.1| 23.4| 20.4| 658  |
| W.Endos JN625807.1     | 38.3  | 17.9| 23.4| 20.4| 658  |
| W.Endos JF960240.1     | 38.2  | 17.8| 23.7| 20.2| 657  |
| W.Endos JN625952.1     | 38.1  | 18.1| 23.6| 20.1| 656  |
| W.Endos JN625793.1     | 37.5  | 18.5| 24.0| 20.2| 650  |
| W.Endos. A. cerena from Tanjung peropa |        |      |      |      |
| Avg.                   | 38.0  | 18.1| 18.1| 20.3| 656  |

In table 3 it appears that the percentage of bases in the Wolbachia nucleotide sequence ranges from 17.8% (base C) to 38.6% (base T (U)), the largest percentage base is T (U) and the smallest is C and A. In all wolbachia endosimion compared the composition is consistent. Apis cerena, base composition T (U) is the most, then A, then G and finally is C. This composition is relatively the same found in Wolbachia endosimion Hymenoptera (JN625807.1) and (JF960240.1), while the composition of Nitrogen base is on other wolbachia also tend to be the same. Genetic distances between species compared are presented in Table 4.
Table 4. Genetic distance between Wolbachia, based on COI gene fragments.

| SD/Genetics dist | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                  | 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00|
| 1                |     |     |     |     |     |     |     |     |     |     |     |     |
| 2                | 0.01|     |     |     |     |     |     |     |     |     |     |     |
| 3                |     | 0.00|     |     |     |     |     |     |     |     |     |     |
| 4                |     | 0.00| 0.00|     |     |     |     |     |     |     |     |     |
| 5                |     | 0.02| 0.02| 0.01|     |     |     |     |     |     |     |     |
| 6                |     | 0.02| 0.02| 0.02| 0.00|     |     |     |     |     |     |     |
| 7                | -0.00| 0.01| 0.01| 0.01| 0.01|     |     |     |     |     |     |     |
| 8                | 0.00| 0.01| 0.01| 0.01| 0.01| 0.00|     |     |     |     |     |     |
| 9                |     | 0.01| 0.01| 0.01| 0.01| 0.02| 0.01| 0.01|     |     |     |     |
| 10               |     | -0.00| 0.00| 0.00| 0.02| 0.02| 0.01| 0.01| 0.01|     |     |     |
| 11               |     | 0.01| 0.00| 0.00| -0.00| 0.01| 0.02| 0.01| 0.01| 0.01|     |     |
| 12               |     | 0.01| 0.02| 0.02| 0.02| 0.02| 0.02| 0.02| 0.01| 0.02| 0.02| 0.02|

Note: 1. Wol Hymenop ASGLE589-10 (JN625809.1). 2. Wolbachia (JN625952). 3. Wolbachia (JN625951) 4. Wolbachia (JN625950). 5. Wolbachia (JN625826). 6. Wolbachia (JN625785.1). 7. Wolbachia Hymenoptera sp. (JN625809.1). 8. Wolbachia Hymenoptera sp. (JN625807.1). 9. Wolbachia Hymenoptera sp. (JF960240.1). 10. Wolbachia Hymenoptera sp. (JN625952.1). 11. Wolbachia Crematogaster (JN625793.1). 12. Wolbachia endosimbiont of Apis cerana Tanjung Peropa.

In Table 4, it appears that some samples do not have a distance, which indicates that the sample has the same nucleotide base sequence. The biggest distance is 0.02. To determine the relative position of the Wolbachia endosimbion Apis cerena among other Wolbachia endosimbions, a molecular filogeny reconstruction was performed, as shown in Figure 2.
Figure 2. The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 1.28329319 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. The analysis involved 31 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 259 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [4].
4. Discussion
There are currently 4 genus Apis in Indonesia. One of them is *Apis cerena* (1). *Apis cerena* is known as honey bee, based on its economic function as a producer of honey. Apis is also known to have an ecological function, namely as a pollinator. The function basically develops as a symbiosis of mutualism, where bees visit flowers to look for nectars, and indirectly they help pollinate flowering plants. Because of this important role, biological and ecological data about honey bees are important to be equipped for the use and survival of honey bees. Wobachia is Rickettsia endosimbion in most arthropods, including insects. The presence of Wolbachia that infects has many impacts on the dynamics of the host population. Molecular detection of Wolbachia's presence in host insects or in most arthropods has been carried out. Some primers are used, among others, primers for the 12SrRNA gene, rDNA, Wsp (Wolbachia protein) FtsZ and others.

In this study, the primer used was COI (Cytochrome Oxidase C sub unit I) primer, to detect the presence of Wolbachia in *Apis cerena*. COI (called cox or Cox A in prokaryotic organisms), is one of the genes found in mitochondria, which controls the formation of cytochrome amino acids. This amino acid is an amino acid that functions in the cellular respiration chain (37). The 5’end of the COI gene, along 648 bp has been proposed as an animal molecular barcode on the grounds that this gene is better able to distinguish individual variations in populations than variations between populations, which means they have different evolutionary rates. The COI gene has been shown to be a differentiator and even a molecular species identification tool in some invertebrate and vertebrate animals (14,15).

By comparing some of the COI endosymbion sequences available in the genBank, know that Wolbach infected *Apis cerena* has a higher conserved area than the variable region. There are 24 different nucleotide sites between wolbachia that are compared and among those sites there are 5 true sites that can be used as genetic markers. This is in line with what was stated by (38) that Barcodes have also been used for population genetic and phylogeographic analysis, identification of prey in gut contents, detection of invasive species, forensics, and seafood safety. More controversially, barcodes have been used to delimit species boundaries, reveal cryptic species, and discover new species. DNA barcode has also succeeded in separating 10 haplotypes in native Chinese chickens (39). COI essential for local ducks barcoding system (40).Positive results when using the COI and FtsZ genes to detect Wolbachia in the blood to diagnose the syndrome associated with Filaria in cats was done (41).

The results of this study indicate that there is a variation at the species level of 3.64%, table 1. This is in line with what was raised that with COI as a genetic marker, there was variation in inter-population and inter-population variation. These variations are caused by the substitution of nitrogen bases that are both synonymous (non sense), as well as non synonym and sense. Mutations in the COI gene sequence, especially in the third base, are usually mutations that do not change the amino acids controlled by the gene in the COI sequence. Synonyms and meaningless mutations are caused by generative codons, so the third nucleotide may be different, but the amino acids encoded by the codon do not change, for example changing guanine (G) to adenine (A), cytosine (C) and uracil (U), the codon still controls the formation of the amino acid glycine, so glycine is coded by 4 codon series. Filogeny trees reconstructed according to the Kimura 2-parameter model place Wolbachia endosimbion bees from Tanjung Peropa in a clade with Wolbachia that infects Hymenoptera, including Crematogaster (a type of ant), with a bootstrap value of 99%> this indicates the COI gene is robust in determining the taxonomic position of animals compared to Hymenoptera, including Crematogaster (a type of ant), with a bootstrap value of 99%, this indicates the COI gene is robust in determining the taxonomic position of animals compared to Hymenoptera, including Crematogaster (a type of ant), with a bootstrap value of 99%. Genetic distances ranging from 0.01 to 0.02 indicate closeness between species, in other words different sequences of base N with very small percentages.

5. Conclusion
Primer COI, able to detect the presence of Wolbachia endosimbiont in wild bees (*Apis cerena*). From these sequences; 658 bp a long that was characterize and showed that are 96.2% conserved, 3.64% Variable, 2% parsimony informative, and 1.8% singelstone nucleotide. Composition of nucleotide
dominate of Timine (38.0%). Range of genetics distance is 0.01 to 0.02. Wolbachia that infected wild bee (*Apis cerena*), from Tanjung Peropa Southeast Sulawesi lie to Wolbachia that infected Hymenoptera in same clade on phylogenetics tree base on COI sequences gene with 99% boostrapped value.

**Acknowledgments**

We are thank all of our colleagues at the Zoology laboratory of MIPA Faculty, for their enthusiasm, diligence and assistance in this project. We wish to make particular mention and thanks to Miss Asrianingsi, Miss Tri Asria and many member of Macala UHO who have sampling of wild bee *Apis cerena*. We thank the anonymous reviewers whose comments helped to improve this manuscript.

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