BIODIVERSITAS
Volume 25, Number 12, December 2024
Pages: 4894-4899

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251226

Phylogeny of *Wolbachia* in *Drosophila* mutant from Bandung, Indonesia

ENDANG SRIMURNI KUSMINTARSIH[1,♥], PESONA GEMILANG[1], AGUS NURYANTO[1], HANAN HASSAN ALSHEIKH MAHMOUD[1,2], TRISNOWATI BUDI AMBARNINGRUM[1]

[1]Faculty of Biology, Universitas Jenderal Soedirman. Jl. Suparno 63, Banyumas 53122, Central Java, Indonesia.
Tel.: +62-281-638794, Fax.: +62-281-631700, ♥email: endangsk2402@gmail.com
[2]Department of Fisheries Science, College of Natural Resources and Environmental Studies, University of Bahri. Bahri El-Ingaz Street, Khartoum North (Bahri) 11111, Sudan

Manuscript received: 31 July 2024. Revision accepted: 14 December 2024.

Abstract. *Kusmintarsih ES, Gemilang P, Nuryanto A, Mahmoud HHA, Ambarningrum TB. 2024. Phylogeny of* Wolbachia *in* Drosophila *mutant from Bandung Indonesia. Biodiversitas 25: 4894-4899. Wolbachia* is a microorganism known as an intracellular endosymbiont in arthropods that causes reproductive changes and most commonly cytoplasmic incompatibility. *Wolbachia pipientis* A has been divided into two major subgroups (A and B) and 17 subgroups. Therefore, the present study aims to determine the presence of *Wolbachia* subgroup in *Drosophila* mutants and to analyze the phylogeny, of *Wolbachia* in *Drosophila* mutants using *Wolbachia* surface protein (*wsp*) primer genes. The presence of *Wolbachia* was determined based on the DNA band pattern on the agarose gel. The phylogenetic relationship among *Wolbachia* in *Drosophila* mutants was inferred from a phylogenetic tree. Based on the neighbor-joining method, the phylogenetic tree reconstruction was done in MEGA X software. The result shows that *Wolbachia* from 7 types of *Drosophila* mutant samples (taxi, ebony, dumpy, miniature, sepia, white, eye missing, in one subgroup as *Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (wsp) gene, partial cds., while between samples and out-group is closely related to *Wolbachia* sp. in *Diabrotica cristata* 16S rRNA sub-group A. This result is the first systematic survey of *Wolbachia* in *Drosophila* mutants and the first survey classifying *Wolbachia* infections by subgroup. The research future is to explore *Wolbachia* in many more insects.

Keywords: Bandung phylogeny, sequencing, sub-group, *Wolbachia pipientis*

INTRODUCTION

Wolbachia is an obligate intracellular endosymbiont that belongs to the group of alpha-proteobacteria. This bacterium was found on arthropods and filarial nematodes of the family Onchocercidae (Scola et al. 2015). *Wolbachia* was initially found in the ovaries of *Culex pipiens* Linnaeus, 1758. The previous studies by Kittayapong et al. (2000), Bennet et al. (2012), Uday et al. (2015), and Ma et al. (2017) stated that so far *Wolbachia* is separated into 17 subgroups, from A-Q. Those subgroups are found in arthropods (A, B, E, G, H, I, K, M, N, O, P, and Q (Lefoulon et al. 2016), and nematodes (C, D, J)). Subgroup F is unique as it contains both nematode and arthropod-infecting strains (Lefoulon et al. 2016). However, there are no previous studies that explain *Wolbachia* which is found in *Drosophila* mutant, Due to that, it is important to show the similarity of *Wolbachia* infected in *Drosophila* mutant. Constructing phylogenetic trees is also important to determine the subgroup of *Wolbachia* carried by *Drosophila* mutants. Based on the description above, this research aims to identify a subgroup and build the phylogenetic relationship of *Wolbachia* in *Drosophila* mutant which is related to *Wolbachia* which is isolated from other organisms, such as insects, mites, crustacea, and the genetic distance.

Mutations can affect both genotype and phenotype. It is known that there are four types of *Drosophila melanogaster* Meigen, 1830 mutants, namely body part mutants, eye color mutants, wing shape mutants, and body color mutants. Of the four types of mutants, there are 10 types of mutants, namely eye missing, yellow-white, sepia, vestigial, ebony, black, taxi, curly, white, and miniature. In terms of eye color, there are white, scarlet, or dark red, and blackish brown (sepia). Mutations in body color consist of black, yellow, or dark brown (ebony) mutants. Mutations that occur in wings are divided into curly wings (curve upwards), taxi (length stretched away from the body), miniature (wings stretched along the body), and dumpy (split wings). Mutations that occur in an eye shape include oval and large shapes (roughoid), reduced eyes (lobe), having no eyes (eye missing), and narrow eyes (barr) (Weasner BM et al. 2016; Xiao et al. 2017; Hartwell 2018; Kandasamy et al. 2021; Varte et al. 2022). However, research by Oktarianti et al. 2021 indicates that there are no differences in phylogeny between wild and mutant *Drosophila* as mentioned that *D. melanogaster* wild type and all mutant strains have high similarity to *D. melanogaster* 28S rRNA gene partial sequence (NCBI GenBank Accession number GU597379) (identity score up to 99%). The phylogenetic tree showed that *D. melanogaster* plum mutant was closely related to *D. melanogaster* 28S rRNA gene partial sequence (NCBI GenBank Accession number GU597379), they also belonged to one cluster. Next to this cluster are sepia and clot mutants, in addition to the wild type and black mutant in one cluster. All the mutants and wild types belonged to one clade, while the vestigial mutant stayed in a different

clade. Research by O'Grady and DeSalle (2018) about the phylogeny of the genus *Drosophila* shows that the first visible mutants discovered in Morgan's fly laboratory to the many whole genomes that are being generated today, these flies have made important contributions to our understanding of nearly every aspect of modern biology.

The genus *Drosophila*, contains over 1600 species, for over a century, one species in this group, *D. melanogaster*, has been key to studies of animal development and genetics, genome organization and evolution, and human disease (O'Grady and DeSalle 2018). Due to the many interests in *Drosophila*, researchers are working with it, and the result shows twelve drosophilids have been awarded Nobel Prizes. One reading the historical account above might wonder why, despite the progress made over many years, so much remains unknown concerning the phylogenetic relationships within this important model system. However, much like *D. melanogaster* serves as a model for human genetics Drosophilidae is also a powerful model for future systematic research. This system, and how *Drosophila* biologists work toward a complete understanding of their study organism, will help set the tenor of integrative systematic research across the tree of life. Apart from being interesting to study in terms of mutation and genetics, *Drosophila* has even won a Nobel Prize, it is also interesting to study the endosymbionts in it, including *Wolbachia* and other endosymbionts which have not been studied much. Therefore, research on endosymbionts other than Wolbachia is still wide open.

MATERIALS AND METHODS

Sampling

Sample *Drosophila* mutants were taken from the culture at the Universitas Padjadjaran, Bandung. There are seven types of *Drosophila* mutants i.e., taxi/Tx, ebony/€, sepia/Se. white/w, eye missing/eym, miniature/m), and dumpy/dp. This research has been conducted based on ethical clearance no. 1521/EC/KEPK/VII/2024.

DNA isolation

Genomic DNA of *Wolbachia* was isolated from flies using the QIAprep-Spin Miniprep kit (QIAGEN). Adult flies were inserted 1.5 mL into a sterile microtube. A total of 180 µL of tissue lysis buffer (ATL buffer) was added to the sample, and the sample was smashed using the microsmash tool (pestle). Then, 20 µL proteinase K and 200 µL lysis buffer (AL buffer) were added to the sample and homogenized with vortex, and incubated at 70°C for 10 min. A total of 200 µL of 100% cold ethanol was sequentially added to the sample and homogenized again to denature the proteinase K. The supernatant was pipetted and transferred to DNeasy ion exchange spin columns which are above 2 mL of the collection tube and centrifuged at 8,000 rpm for 1 min. DNeasy spin columns were moved to new collection tubes of 2 mL, and 500 µL of wash buffer (AW 1 buffer) was added to each sample to perform unwanted ethanol washing from the sample and to conduct the centrifugation. The collected supernatant and collection tubes were discarded. The DNeasy spin columns were replaced with new ones, as before into 2 mL collection tubes and as much as 500 µL of AW 2 Kandasamy V Kandasamy for 1 min, and dried on the DNeasy membrane. The collected supernatant and collection tubes were discarded. The DNeasy columns were placed above 1.5 mL microtubes, and 75 µL elution buffer (AE buffers) were pipet directly above the membrane. Samples were centrifuged for 1 min at 8000 rpm to remove DNA. DNeasy spin columns were removed, and DNA samples were stored at -20°C for future use. Calculation of concentration and purity of DNA isolation products were carried out using the NanoDrop 1000 spectrophotometer (Table 1).

Wsp gene amplification and sequencing

The *wsp* gene was amplified with a polymerase chain reaction. Amplification was performed using specific primers for *Wolbachia* outer surface protein (*w*sp) gene of *Wolbachia*, forward primer *wsp*81F (5′-TGGTCCAATAAGTGATGAAGAAAC-3′), and reverse primer *wsp*691R (5′-AAAAATTAAACGCTACTCCA-3′) yield a 0.6-kb segment of wsp. A master mix reaction for PCR was made consisting of 13.5 µL of ultrapure water, 2 µL of 10× polymerase buffer, 2 µL of 25 mM $MgCl_2$, 0.5 µL of 20 µM primer wsp81F, 0.5 µL of 20 µM primer *wsp*691R, 0.5 µL of 10 mM dNTP (deoxynucleotide) where each 0.2 mL microtube was added the DNA template of sample (1 µL) and master mix (19 µL). PCR process was conducted under an initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, followed by 72°C for 10 min. The fragments were separated by 1% agarose gel electrophoresis and visualized by staining with ethidium bromide (Borst 2005). A 40 µL of PCR unpurified product from each sample was sent to 1st base DNA sequencing service.

Phylogenetic analysis

The sequence of *wsp* genes obtained in this study was assembled using Bio-Edit, and multiple sequence alignments were conducted with ClustalW using MEGA X (Kumar et al. 2018).

Table 1. DNA concentration and purity of *Drosophila* mutant

Sample Code	Individual Number	DNA Concentration (ng/µL)	DNA Purity (260/280 nm)
tx	31	268.76	2.20
e	31	403.13	2.08
se	42	497.30	2.13
w	17	716.66	2.06
eym	15	396.19	2.00
m	15	412.27	2.07
dp	10	557.30	1.96

The sequences' similarity for fragments generated in this study was compared in the GenBank database using a Blastn search (https://www.ncbi.nlm.nih.gov). Accession numbers were obtained after all sequences generated in this study were submitted to NCBI GenBank (Table 2). Partial sequences obtained in this study were used to build a phylogenetic tree using the Neighbor-Joining (NJ) method in Mega X software (Kumar et al. 2018). The length of the branch was estimated based on Kimura's two-parameter evolution model. Bootstrap testing was carried out as many as 1000 repetitions. Trees were inferred with the Tamura3-parameters model by 1000 replicates bootstrapping. To estimate the level of phylogenetic relationship with *Wolbachia* in *Tribolium confusum* (Subgroup B) X65674.1, *Wolbachia* endosymbiont of *Litomosoides sigmodontis* (Subgroup D), AF0690.1, *Wolbachia* endosymbiont of *Rhinocyllus conicus* (Subgroup F) 85267.1, *Wolbachia* endosymbiont of *Diabrotica cristata* (Subgroup A) AY007550.1, and *Wolbachia* endosymbiont of *Culex pipiens* (Subgroup E) AF179630.1 were retrieved from GenBank and used to construct a phylogenetic tree.

RESULTS AND DISCUSSION

Measurement of DNA concentration of *Drosophila* mutant

DNA isolation is carried out from seven types of *Drosophila* mutant i.e., taxi (tx); ebony €; sepia (se); white (w); eye missing eym); miniature (m); and dumpy (dp). The results of DNA isolation of the mutant species (10-42 individuals) showed a high DNA concentration, which ranged between 268.76 and 716.66 ng/µL. The purity of isolated DNA (260/280 nm) was ranged from 1.96 to 2.20 (Table 1).

Detection of *wsp* gene of *Wolbachia* on *Drosophila* mutant samples using the PCR

The result of gel electrophoresis on samples tx; eym; m; dp showed that there was only one thick DNA band that is on 600 bp. However, in e; se; w showed there were five DNA bands consisting of two DNA bands (measuring 600 bp and 1500 bp thinner band, and three very thin DNA bands (measuring 750 bp; between 1000 and 1500 and between 1500 and 2000). There should be only one band in 600 bp, but it appears five bands caused by dimer (Figure 1). For PCR cycle-sequencing reactions in four samples (tx; eym; m; dp) DNA bands with size 600 bp were obtained as a DNA template. After that, sequencing was conducted by FIRSTBASE.

The results of PCR showed that in three types of *Drosophila* mutants (e, se, w), multiple bands appear. This can be predicted as dimers, proven after the process of sequencing, and the results show only one size with a length of 600 bp is fine with a good sequence, and another size could not be found as a good result of them, so then we did not continue these sequences on the discussion and only used 600 bp.

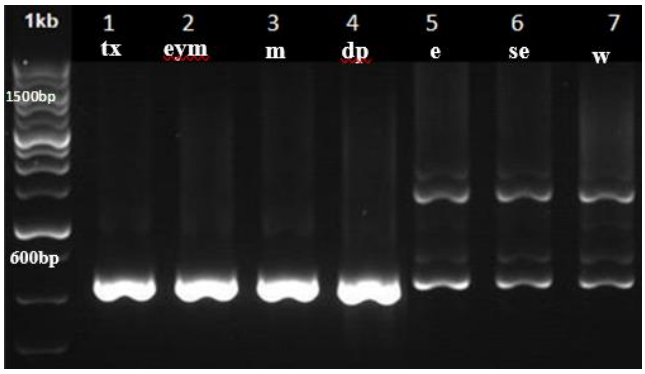

Figure 1. The results of electrophoresis of *Wolbachia wsp* gene in *Drosophila* mutant samples. One band in wells 1-5 (tx; eym, m, dp), and five bands in wells 6-8 (e; se; w). Band size based on DNA marker 1 kb from bottom to top: tape 1, size 600 bp; 2nd band 1500 bp

Table 2. The results for sequence similarity of *Wolbachia wsp* gene sequence data in *Drosophila* mutant samples

No.	Same codes	Close related BLASTn result	Size (bp)	Identity (%)	Query coverage (%)	Sequence references
1	dp	*Wolbachia* sp. *w*Mel isolates Yunnan outer surface protein precursor (*wsp*) gene, partial cds	651	97.69	99	FJ403332
2	e	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	656	97.39	99	FJ403332
3	se	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	659	96.94	99	FJ403332
4	w	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	654	97.69	99	FJ403332
5	tx	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	654	97.55	99	FJ403332
6	eym	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	653	97.38	99	FJ403332
7	m	*Wolbachia* sp. *w*Mel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds	662	96.20	99	FJ403332

Drosophila mutant samples in this study that are infected by *Wolbachia* were obtained from stocks (they were not taken from nature), and were already maintained ten years ago. Vertical transmission is one of the features that occurs by *Wolbachia* to be the reason why *Drosophila* mutant can still be infected by these bacteria, although only maintained in the bottles. It can be said that the infection that occurs in these samples is a result of maternal transmission from previous hosts or vertically. There are at least two important consequences of this transmission behavior. First, *Wolbachia* transmission success relies on the reproductive success of its host. The second important constraint associated with vertical transmission is that a host offspring's cytoplasm is uniparentally inherited from its mother. Thus, only females provide the physical means by which their offspring can be directly infected, with males effectively being a dead end for vertical transmission. The PCR band resulted from a *Drosophila* mutant with codes (dp, tx, eym, m, e, se, and sw) referred to as single strain infection of *Wolbachia*. The single infection phenomenon was supported by the fact that Cytoplasmic Incompatibility (CI) is the most common occurrence in *Wolbachia* infection. Evidence of single infection by *Wolbachia* species on *Drosophila* stocks has been widely discussed by many scientists. One of its discussions is from Clark et al. (2005), who reported that *D. melanogaster* was taken from 609 stocks from The Bloomington *Drosophila* Stock Center (BDSC) at Indiana University, and 28.9% of this stock was positively affected by a single strain infection from *Wolbachia*. *Drosophila* stock consisted of wild-type and several P-element mutagenesis screens that had been deposited in BDSC for 15 years.

Taxonomic status of *Wolbachia*

To obtain sequence similarity from sequence data of the PCR product of *Wolbachia wsp* gene that is stored in the international DNA databank, the sequencing of *Wolbachia wsp* gene in *Drosophila* mutant was sent as query sequences in the Basic Logarithmic Search Tool Nucleotide program (BLASTn) (Table 2).

The blast of each DNA sequence showed that the wMel Yunnan is similar to all types of *Drosophila* mutant isolates. This strain is isolated from *D. melanogaster* so it is called *w*Mel. Moreover, *w*Mel strains can usually cause weak Cytoplasmic Incompatibility (CI) (Ilinsky and Zakharov 2011; Shropshire et al. 2021). This statement is proven by the age of the culture which is decades old and *Wolbachia* is still living in its colonies because if the CI is strong, a few offspring will survive.

Phylogenetic analysis of *Wolbachia* from *Drosophila* mutant based on *wsp* genes, the result of phylogenetic analysis with MEGA X shows that there are two branches, one branch consists of *Wolbachia* in *Drosophila* mutant sample and *Wolbachia* sp. wMel isolate Yunnan outer surface protein precursor (*wsp*) gene, partial cds, and the other branch consists of *Wolbachia* from out-group. This indicates that the phenetics of *Drosophila* mutant is not caused by *Wolbachia*, a very common endosymbiont that infects insects, including *Drosophila* mutants. Subsequently, Oktarianti et al. (2021) indicate that there are no differences in phylogeny between wild and mutant *Drosophila* as mentioned that *D. melanogaster* wild type and all mutant strains have high similarity to *D. melanogaster* 28S rRNA gene partial sequence (GenBank Accession number GU597379) (identity score up to 99%). The phylogenetic tree showed that *D. melanogaster* plum mutant was closely related to *D. melanogaster* 28S rRNA gene partial sequence (GenBank Accession number GU 597379.1) (Figure 2).

The distance between samples and *Wolbachia* sp. wMel isolate Yunan is the same, namely 0.0, while the distance between samples and out-group is closely related to *Wolbachia* sp. in *Diabrotica cristata* 16S rRNA is 0.01, then *Wolbachia* from *Drosophila* mutant is closely related to subgroup A (Table 3). The determination of these subgroups is based on single-gene phylogeny and supported by multi-locus sequence typing (Ellegaard et al. 2013). In this case, the classification of *Wolbachia* subgroups does not represent all of the genomes because we use the *w*sp gene which is a single locus gene. The *wsp* gene is the favorite gene to be used in classify *Wolbachia*. The *wsp* gene has faster development than the 16S rRNA and *fts*Z gene.

Table 3. Analysis of the genetic distance

X656754.1 Wolbachia sp from T.confosum 16S rDNA Sub Group B											
White	1										
Taxi	1,48	0									
Sepia	1,45	0	0								
Miniatur	1,48	0	0	0							
M85267.1 Endosymbiont Rynocyllus conicus 16S rRNA complate Sub Group F	0,04	1,63	1,63	1,58	1,63						
FJ4033302.1Wolbachis Sp wMel isolate Yunan outor surface protein precusor (wsp) gene partial cds	1,48	0	0	0	0	1,63					
Eyemissing	1,48	0	0	0	0	1,62	0				
Ebony	1,48	0	0	0	0	1,63	0	0			
dampy	1,48	0	0	0	0	1,63	0	0	0		
AY007550.1 Wolbachia sp Dcris 16S wolbachia 16S ribosomal rRNA gene partial sequence Sub Group A	0,01	1,59	1,58	1,58	1,59	0,03	1,59	1,59	1,59		
AF179630.1*Wolbachia pipientis* 16S ribosomal RNA gene partial sequence Sub Group E	0,03	1,59	1,59	1,59	1,59	0,03	1,59	1,59	1,59	0,01	
AF069068.1. Endosymbiont of Litomosoides sigmodontis 16S ribosomal RNA gene partial sequence Sub Group D	0,05	1,63	1,63	1,63	1,63	0,04	1,63	1,59	1,63	0,04	0,05

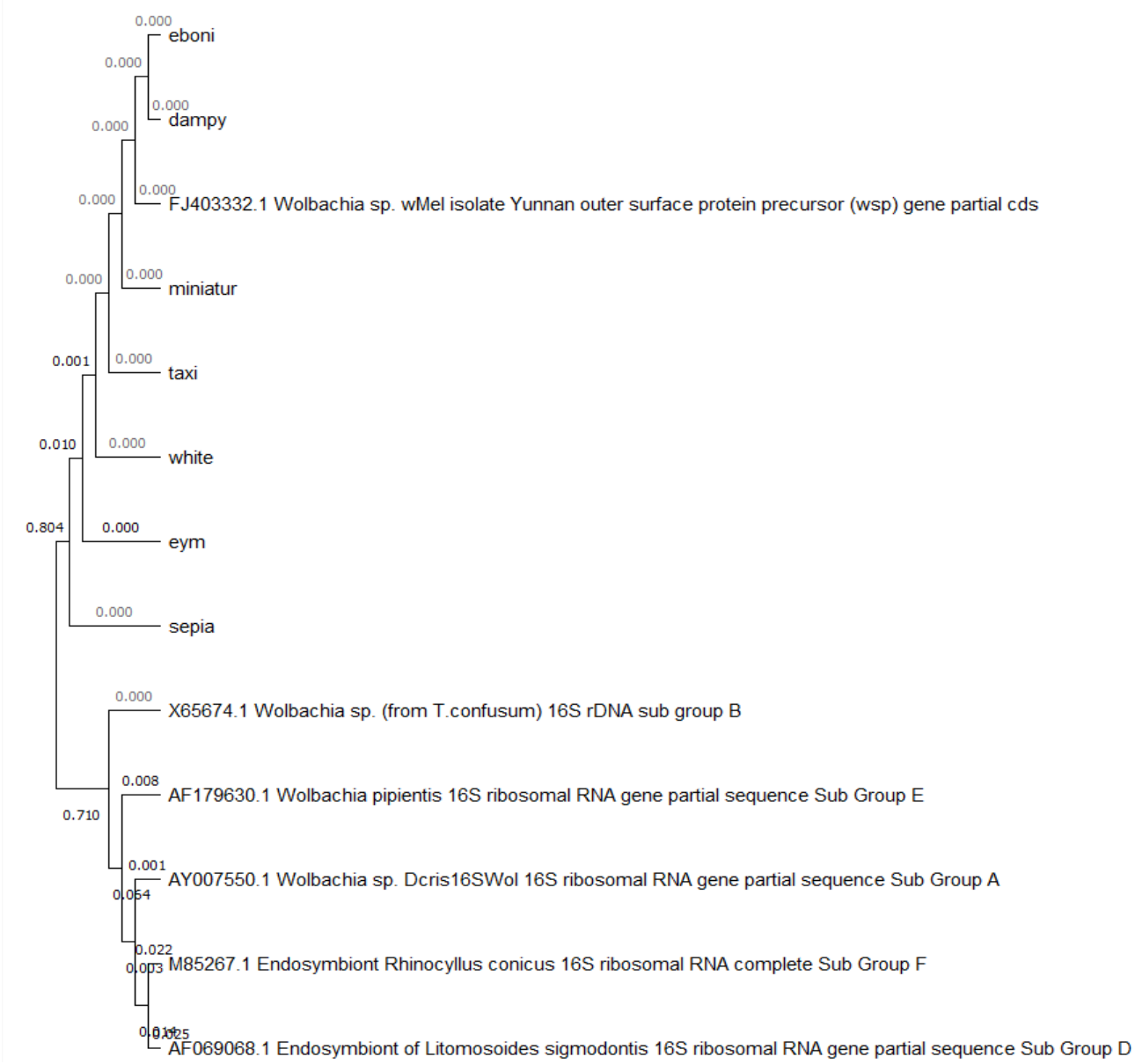

Figure 2. Phylogenetic tree of *Wolbachia* samples based on *wsp* genes *Wolbachia* and *Wolbachia* from Subgroup *Wolbachia* in *Tribolium confusum* (Subgroup B) X65674.1, *Wolbachia* endosymbiont of *Litomosoides sigmodontis* (Subgroup D), AF069068.1, *Wolbachia* endosymbiont of *Rhinocyllus conicus* (Subgroup F) M85267.1, *Wolbachia* endosymbiont of *Diabrotica cristata* (Subgroup A) AY007550.1, and *Wolbachia* endosymbiont of *Culex pipiens* (Subgroup E) AF179630.1

Based on the result of distance analysis, it shows that *Wolbachia* on *Drosophila* mutant has the same group as to *wMel* isolate from Yunan 1.48 (0.0), same as all samples. While the genetic distance relationship between each sample in *Drosophila* mutants to other subgroups is close to *Wolbachia* endosymbiont of *Diabrotica cristata* (Subgroup A) (0.01). This indicates that the phenetics of *Drosophila* mutant is not caused by *Wolbachia* which is a very common endosymbiont that infects insects. The genome of *Wolbachia* is very diverse (Ros et al. 2009). This genomic and phenotypic diversity makes *Wolbachia* increase its abundance as intracellular bacteria. Genomic diversity and phenotype in *Wolbachia* are caused by its ability to make changes to the genomic exchange. The genomic exchange that occurs in *Wolbachia* is caused by the reason that *Wolbachia* is a cosmopolitan bacterium and acts as a facultative endosymbiont. Since the discovery of many eye color mutants, the eye color pigments of *D. melanogaster* have been the subject of numerous investigations. Beadle and Tatum (1930) discovered the concept of one gene-one enzyme (in Beadle and Tatum 1941), but with the development of science, this concept changed to one gene-one polypeptide (Berg and Singer 2003). There are two pathways for eye pigment synthesis in *Drosophila*, namely: rosin, and ommochrome. The results of protein synthesis, molecular biologists began to think about the concept of one gene-one protein. However, many proteins are composed of two or more different polypeptide

chains, and each polypeptide is specified by its respective gene. Therefore, two classes of pigments, the brown "ommochromes and the red drosopterins, contribute to the typical eye color phenotype of *Drosophila* and serve as light-screening pigments. The biosynthetic pathways of these two pigments are distinct and do not share enzymes; ommochromes are synthesized from tryptophan, whereas drosopterins are synthesized from guanosine-5′-triphosphate (GTP).

In conclusion, *Wolbachia* infects *Drosophila* mutants and does not play a role in the occurrence of mutations. Mutations are changes that occur in genetic material, there are two types of mutations, namely point mutations and chromosomal mutations. Mutations can affect both genotype and phenotype.

ACKNOWLEDGEMENTS

We would like to thank the Universitas Padjajaran, Bandung, Indonesia for supplying the *Drosophila* mutants. This project was funded by the Universitas Jenderal Soedirman, Banyumas.

REFERENCES

Beadle GW, Tatum EL. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc Natl Acad Sci USA 27: 499-506. DOI: 10.1073/pnas.27.11.499.

Bennett GM, Pantoja NA, O'Grady PM. 2012. Diversity and phylogenetic relationships of *Wolbachia* in *Drosophila* and other native Hawaiian insects. Fly (Austin) 6 (4): 273-283. DOI: 10.4161/fly.21161.

Berg P, Singer M. 2003. George Beadle, an uncommon farmer: The emergence of genetics in the 20th century. CSHL Press, NY.

Borst P. 2005. Ethidium DNA agarose gel electrophoresis: How it started. IUBMB Life 57 (11): 745-747. DOI: 10.1080/15216540500380855.

Clark ME, Anderson CL, Cande J, Karr TL. 2005. Widespread prevalence of *Wolbachia* in laboratory stocks and the implications for *Drosophila* research. Genetics 170 (4): 1667-1675. DOI: 10.1534/genetics.104.038901.

Ellegaard KM, Klasson L, Näslund K, Bourtzis K, Andersson SG. 2013. Comparative genomics of *Wolbachia* and the bacterial species concept. PloS Genet 9 (4): e1003381. DOI: 10.1371/journal.pgen.1003381.

Hartwell L, Goldberg ML, Fischer JA, Hood LE, Aquadro CF. 2018. From genes to genomes (p. 960). Genetics. McGraw-Hill Education, New York.

Ilinsky YY, Zakharov IK. 2021. Cytoplasmic incompatibility in *Drosophila melanogaster* is caused by different *Wolbachia* genotypes. Russ J Genet Appl Res 1: 458-462. DOI: 10.1134/S2079059711020031.

Kandasamy S, Couto K, Thackeray J. 2021. A docked mutation phenocopies dumpy oblique alleles via altered vesicle trafficking. Peer J 9: e12175. DOI: 10.7717/peerj.12175.

Kittayapong P, Baisley KJ, Baimai V, O'Neill SL. 2000. Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). J Med Entomol 37 (3): 340-345. DOI: 10.1093/jmedent/37.3.340.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.

Lefoulon E, Bain O, Makepeace BL, d'Haese C, Uni S, Martin C, Gavotte L. 2016. Breakdown of coevolution between symbiotic bacteria *Wolbachia* and their filarial hosts. PeerJ 4: e1840. DOI: 10.7717/peerj.1840.

Ma Y, Chen WJ, Li ZH, Zhang F, Gao Y, Luan YX. 2017. Revisiting the phylogeny of *Wolbachia* in Collembola. Ecol Evol 7 (7): 2009-2017. DOI: 10.1002/ece3.2738.

O'Grady PM, DeSalle R. 2018. Phylogeny of the Genus *Drosophila*. Genetics 209 (1): 1-25. DOI: 10.1534/genetics.117.300583.

Oktarianti R, Sholihah A, Masruroh D, Wathon S, Senjarini K. 2021. Identification and phylogenetic analysis of *Drosophila melanogaster* based on ITS 2 rDNA sequences. IOP Conf Ser: Earth Environ Sci 747: 012038. DOI: 10.1088/1755-1315/747/1/012038.

Ros VI, Fleming VM, Feil EJ, Breeuwer JA. 2009. How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). Appl Environ Microbiol 75 (4): 1036-1043. DOI: 10.1128/AEM.01109-08.

Scola BL, Bandi C, Raoult D. 2015. *Wolbachia*. Bergey's Manual of Systematics of Archaea and Bacteria, 1-12. DOI: 10.1002/9781118960608.gbm00906.

Shropshire JD, Rosenberg R, Bordenstein SR. 2021. The impacts of cytoplasmic incompatibility factor (cifA and cifB) genetic variation on phenotypes. Genetics 217 (1): 1-13. DOI: 10.1093/genetics/iyaa007.

Uday J, Kumar S, Puttaraju HP. 2015. Detection and phylogenetic affiliation of *Wolbachia endosymbiont* from *Drosophila melanogaster*. Entomol News 124 (4): 270-276. DOI: 10.3157/021.124.0404.

Varte V, Kairamkonda S, Gupta U, Manjila SB, Mishra A, Salzberg A, Nongthomba U. 2022. Neuronal role of taxi is imperative for flight in *Drosophila melanogaster*. Gene 833: 14693. DOI: 10.1016/j.gene.2022.146593.

Weasner BM, Weasner BP, Neuman SD, Bashirullah A, Kumar JP. 2016. Retinal expression of the *Drosophila* eyes absent gene is controlled by several cooperatively acting cis-regulatory elements. PLoS Genet 12 (12): e1006462. DOI: 10.1371/journal.pgen.1006462.

Xiao C, Qiu S, Robertson RM. 2017. The white gene controls copulation success in *Drosophila melanogaster*. Sci Rep 7 (1): 7712. DOI: 10.1038/s41598-017-08155-y.