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RESEARCH ARTICLE

DNA BARCODES OF THE PLECO (LORICARIIDAE, PTERYGOPLICHTHYS) IN THE CILIWUNG RIVER.

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Abstract

Pleco (*Pterygoplichthys sp.*) is an invasive freshwater species belong to LoricariidaeFamily and comes from Costa Rica, Panama, South America. Distribution of pleco spread throughout the freshwater of the world. One of the habitats in Indonesia is the Ciliwungriver and along the river with high abundance. Until now there has been no research results related to molecular identification of the fish especially from Ciliwung river. Therefore, it is necessary to know the molecular analysis of DNA to identify pleco from Ciliwungriver by using the technique of DNA barcoding gene CO1. The methodology consisted samples preparation, DNA extraction, DNA quantification, gene CO1 amplification, and DNA base sequence reading. Results identification using gene CO1 barcodes with a length of 650bp fragment showed that pleco from Ciliwung river has a 100% similarity with *Pterygoplichthyspardalis*species.There are transversion substitution nucleotide at nucleotide in positions 306 (C→T), 339 (G→A), 387 (C→T), and 471 (T→C), but this should not affect the amino acid sequence changes in pleco from Ciliwungriver.

Introduction:-

Background of the Study:-

Pleco*(Pterygoplichthys sp.)* is an invasive freshwater species including Family Loricariidae and comes from Costa Rica, Panama, and South America. Distribution of Plecospread from the tropic district until to Indo-Pacific (Yu &Quailing 2014). The research result from Wu et al. (2011) states that there are three species of Plecoare most abundant in the world that is *Pterygoplichthyspardalis, P. disjunctivus, P. multiradiatus*. Two of these species are found in Indonesia, namely the species *P.pardalis* and*P. disjunctivus.*

The originate habitat Plecoare rivers, lakes and creeks (Nice et al. 2012). One river in Indonesia which becomes habitat PlecoisCiliwung river. According toRatmini (2009) Plecofound along the Ciliwung river with high abundance.

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The abundance of PlecoinCiliwung river for Plecohave a high level of adaptation in a polluted environment and the body of Plecocovered with plates of hard scales (Rachmatika&Wahyudewantoro 2006). Therefore, Plecodidn'thave predators and be a competitor in obtaining food on the river ecosystem (Haiti 2007), so the species is considered to have contributed to decline in fish endemic ecosystem species Ciliwung river ecosystem (Kusumah 2011).

Results of Hadiaty research (2011) shows the data rate of loss the species endemicfromCiliwung river in2009 reached 92.5% of the initial number of about 187 species and decreased to 20 species, including five species of which is the introduction of fish species. This type of that fish is lost in the waters of the Ciliwungriver is betutu fish and Balidafish (Wowor 2010)

Morphological diversity and genetic information of an organism is very useful for the characterization of the type, the development, distribution by thetime and space. Characterization, development and distribution of populations are needed to determine the step of conservation, management and sustainable utilization. The level of diversity among the population, especially genetic diversity can be used as a step to estimate the level of risk of extinction and the abundance of an organism (Singkam et al., 2011). One of themethod to analyze the genetic diversity is molecular detection.

Molecular detection method was developed to analyze the genetic diversity of a species. *Polymerase chain reaction* (PCR) is a molecular detection method *of in vitro* without the use of living organisms to amplify a specific territory of a strand DNA. The reaction is limited by the primer pair (short oligonucleotide) using DNA polymerase enzyme and dNTPs as a number (Widowati 2013). The result of PCR is used to stage a reading strand bases in analyzing phylogenetic kinship.

One of the methods in tracing the phylogenetic relationships of a species commonly did is see the resemblance of mitochondrial DNA (mtDNA). Many studies related phylogenetic studies both invertebrate and vertebrate animals used mtDNA CO1 as the gene sign or genetic barcode (Maramis&Warouw 2014). DNA barcoding is a molecular technique to identify species using differences in nucleotide sequence from standardized gene regions (Hubert et al. 2003). DNA *barcoding* is based on fragments of mtDNA gene *Cytochrome Oxidase I* (COI), which serves as a *'barcode'* for identifying species (Ward et al., 2005).

CO1 genes of the mitochondrial genome are a gene that is often used as standard sign genes in animal identification. The superiority of CO1 gene is to have a universal primer solid, so as to identify the 5 'end from the most groups of animals. CO1 gene also has the highest molecular evolutionary compared with other genes in the mitochondria, thus having a low intraspecific variation and interspecific in height between adjacent taxa (Hajibabaei et al. 2006)
Until now there has been no research results related molecular identification of the originate from of PlecoCiliwung river. Therefore it is necessary for the molecular analysis of DNA PlecofromCiliwungriveroriginate using DNA *barcoding* techniques.

Objectives and Benefits Research:-
This study was conducted to identify Pleco*(Pterygoplichthys sp.)* As a molecular marker gene CO1, knowing the genetic diversity, and the identification of changes in the nucleotide sequence Plecoin theCiliwung river Section of South Jakarta. The results are expected to be a source of information and databases for the genetic diversity of Pleco*(Pterygoplichthys sp.)* in the Ciliwungriver.

Methodology:-

The time and place of study:-
The study was conducted in August 2015-June 2016 include sampling of Plecoand molecular analysis. Sampling of Pleco conducted in August-November 2015 around Ciliwung of Rindam Jaya to Bidaracina (Figure 1). Furthermore, molecular analysis performed in March-June 2016 in the Laboratory of Genetics and Development Research Institute of Ornamental Fish Aquaculture, Ministry of Maritime Affairs and Fisheries, Depok.

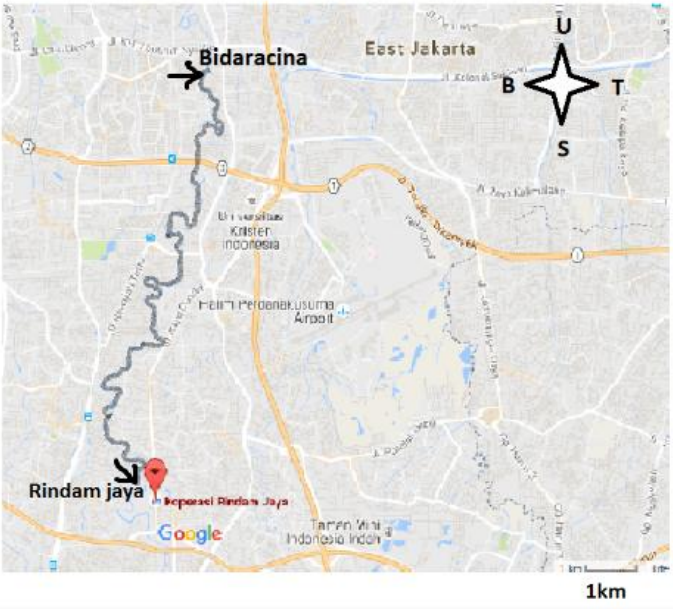


Figure 1. Location of sampling of Rindam Jaya - Bidaracina
(Source: Google Maps)

Sample Preparation:-
Decision fin muscle tissue as a source of DNA is done in Laboratory of Biology, University of Al Azhar Indonesia (UAI). The fins are used is the tip of the dorsal fin, pectoral and pelvic fins. Samples were cut using scissors and weighed 5-10 mg, then put into a 1.5 µL micro-tubes containing 70% alcohol. Samples were then stored for 1-2 days before being moved into micro-tubes containing 1.5 µL of absolute alcohol. Molecular analysis includes extraction, quantification, amplification, visualization, and the reading strand of DNA bases performed in the Laboratory of Genetics and Development Research Institute of Ornamental Fish Aquaculture, Ministry of Maritime Affairs and Fisheries, Depok.

Extraction:-
DNA extraction performed on 28 samples of fish using DNA *GsyncGeneaideExraction kit*. The extraction method followed the procedures issued by the company, involves the separation of the network, the destruction of cells, DNA binding, washing, and removal. Phase separation of the network is done by taking about 5-10 mg samples were cut into 1.5 µL micro tube and then added 200 µL GST Buffer and 20 µLproteins K. Further samples of homogenized by vortex and incubated for 24 hours at a temperature of 60^0C.

Phase cell destruction is done after incubation of samples with the addition of 200 µL GSB back Buffer and incubated for 20 minutes at a temperature of 200C. Further samples were centrifuged for 2 min at 15000 g to separate the supernatant with pellets. Supernatant is taken and separated into a new micro tube.

The next stage is the binding of DNA by adding 200 µL of absolute alcohol in the supernatant and homogenized. Samples were then transferred on *columntube*2 mL and the *collecting tube* which then centrifuged at 15 000 g for 1 minute. The liquidsolution*column tube*disposedand then *collecting tube* was transferred to a new tube.

Washing stage by adding 400 µL of *Wash Buffer*in*1 coloumn tube* and centrifuged at 15,000 g for 30 seconds. The solution liquid to the *collecting tube* was removed and reassembled. For the drying process is done centrifugation at 15,000 g for 3 min. Coloumn phase of displacement by moving the tube into a new micro tube and added 100 µL*Nuclease Free Water* (NFW) that has been heated at 60 ° C after that silenced for the last 3 minutes and centrifuged at 15,000 g for 30 seconds to get the DNA.

Quantification of DNA:-
DNA quantification is done using a spectrophotometer G*ene Quant* with standard volume of 80 µL NFW containing 2 µL of sample DNA extraction results.

DNA amplification:-
Fish amplification using the primers F1 (51- TCA - ACC - AAC - CAC - AAA - GAC - ATT - GGC - AC -31) and R1 (51- TAG - ACT - TCT - GGG - TGG - CCA - AAG - AAA - TCA -31) (Ward et al., 2005).

Table 1:- The PCR program

Step	**Temperature (^{0}C)**	**Time**
Inisiasi	94	5 second
Denaturasi	94	30 second
Anneling	52	30 second
Elongasi	72	30 second
Post-elongasi	72	5 second

The PCR process is carried out as many as 35 cycles. Components PCR 50 µL consists of nuclease free water (NFW) as much as 11 µL, *forward* primer F1 and *revers* R1 respectively of 2 µL, master mix 25 µL (containing dNTP, buffer and taq polymerase), and the DNA samples of 10 µL ,

Visualization:-
Visualization of DNA using agarose gel (1.5%) with *peq green dyes* DNA and *RNA dye*. The result of extraction with the addition of *loading dye* was electrophoreses in 1 µL and a DNA sample as much 5 µL. Electrophoresis was performed on the current strength of 100 volts for 30 minutes and then photographed using a gel doc with UV rays of a wavelength of 302 nm.

Readings strand bases|:-
Readings strand bases conducted on 13 samples from 28 samples are amplified namely with sample numbers 6, 7, 9, 13, 15, 17, 20, 21, 22, 24, 25, 26, and 28. Selection of sequencing refers to the pattern of the abdomen. The pattern of abdominal Pleco fish in the study were classified into three (A = black spots, B = lines are not interrupted, and C = a combination of the pattern of black spots and black lines).
Readings base strand done using *ABI'S Sequens Scanner*. Sequencing the form of DNA sequences which are then read and analyzed using MEGA 7.0.Further aligned with the access number listed in the NCBI *GenBank* by BLAST to obtain the identification of the species of the sample (Maramis&Warouw 2014).

Results And Discussion:-

The concentration and purity of DNA Fish Pleco fishoriginate fromCiliwung River:-
The results of the measurement of DNA concentration of fish samples ranged between 36-484 ng / µL and purity index value (A260 / A280) on average is worth 1.96 (Appendix 1). DNA concentration is the number of DNA (pg) in the sample (µL). The number of DNA concentration in a sample is determined by the activity of organ samples and expressing certain genes in an organ (Tiara et al. 2014). DNA purity is a purity level of DNA samples obtained from the A260 / A280 (Sambrook and Russell 2001).

Measurement of DNA concentration using a *Gene-Quant* has a principle wavelength spectrophotometer with 260λ. A beam of UV light passed through a sample of a specific wavelength to see the purity and concentration of DNA. This is done to measure the purity, DNA concentration, protein concentration, and absorbance. Absorbance value is the value measured using a wavelength of 260, so that the absorbance value is the reference number of the DNA concentration (Tiara et al. 2014).

Extraction of DNA can be obtained from muscle tissue and blood. Muscles on the fins are a system of organs that have a central role in the movement of fish. Striated muscle groups are named according to the place of attachment, such as enforcement muscle dorsal fin and pectoral fins towing muscle (Rahardjo *et al.* 2010). The use of fin muscle tissue as a source of the DNA showed that the muscle tissue of fish fins Pleco fish can be a good source of DNA for molecular analysis.

Based on *one way* ANOVA statistical test showed that the source of the DNA of the dorsal fin, pectoral and pelvic fins have DNA purity values were not significantly different (Figure 2). Significance probability value of 0541 which means> 0.05 showed no significant difference between types of fins are used as a source of DNA (Appendix 2). According to Newson (2013) if the significance value of <0.05 means that there are significant differences among the treatments.

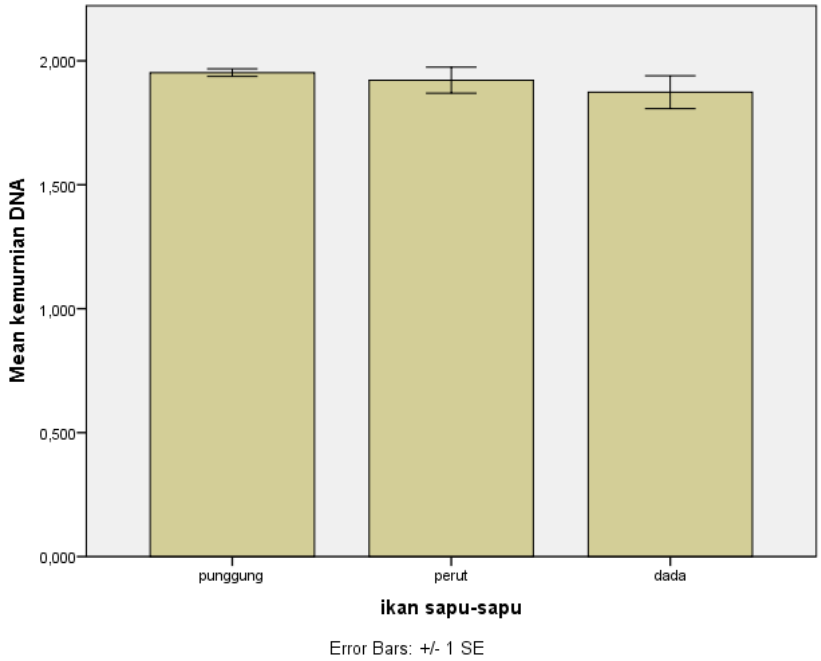


Figure 2:- The average value of purity DNA from several types of fins

DNA purity Pleco fishoriginate from Ciliwung river has a value of purity (A260 / A280) an average of 1.9 so that it can be expressed as pure DNA. DNA is said to be pure if it has a purity index of 1.8 to 2.2 (Aryahiyyah 2014; Wardani& Sari 2015). DNA which has a purity value of less than 1.8 indicate the presence of protein contamination, and if the DNA has a purity value of more than 2.0 indicate contamination of RNA. This can occur because of the absence of additional RNase enzyme that works to degrade RNA. Protein contamination can be caused by lack of protein degradation in the washing step (Aryahiyyah 2014). In the process of isolation of DNA, protein is a contaminant that must be degraded (Sambrook&Russel 2001).

Visualization the Results of DNA ExtractionPlecooriginate fromCiliwungriver:-

Visualization of the extracted DNA Pleco fish on a 1.5% agarose gel shows that DNA can be clearly seen (Figure 3). This is consistent with the concentration and purity of DNA was good so the quality of the electrophoresis results is clearly visible on a 1.5% agarose gel. Electrophoresis using a marker size of 100 bp.

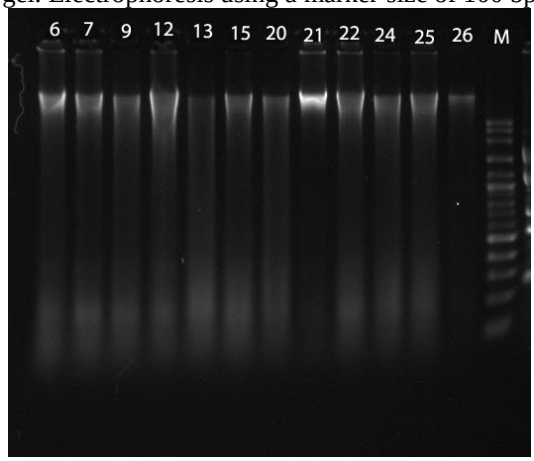


Figure 3:- Results of DNA extraction Pleco fish(M = Marker; 6,7,9,12,13,15,20,21,22,24,25,26 = No. Sample fish)
Electrophoresis is the movement of electrically charged substances due to the influence of an electric field (Wardani& Sari 2015). DNA molecules including negatively charged compounds so that the process of electrophoresis, DNA migrate toward the positive pole. DNA molecule migration velocity depends on the concentration of the gel used, the size of the molecules being analyzed, as well as the power supply voltage is supplied. Agarose gel used to separate, identify, and purify DNA fragments. The movement of DNA fragments in agarose gels is strongly influenced by the composition and solubility of the ion electrophoresis buffer. If the concentration of ions is very little the electrical conductivity is very small and DNA migration becomes slow. Excessive ion concentration will result in the gel melts and denatured DNA. DNA electrophoresis technique also requires *loading buffer*. This *buffer* works to increase the sample density that is at the bottom well and gives color to the DNA fragment to facilitate observation of the process of electrophoresis (Sambrook&Russel 2001).

CO1 gene amplification Fish Pleco fishoriginate fromCiliwungRiver:-
CO1 gene amplification product Pleco fishoriginate fromCiliwungRiver is clearly visible on a 1.5% agarose gel. This indicates that the Primer F1 and R1 successfully amplify the COI gene Pleco fishfishoriginate fromCiliwungRiver in fragment length of 650 bp (Figure 4).

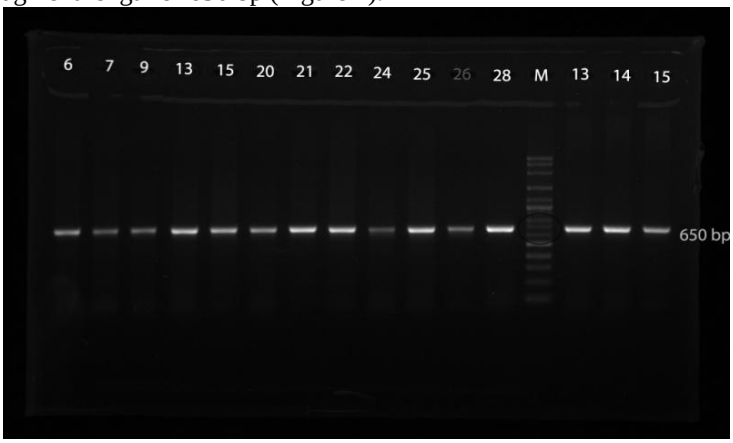
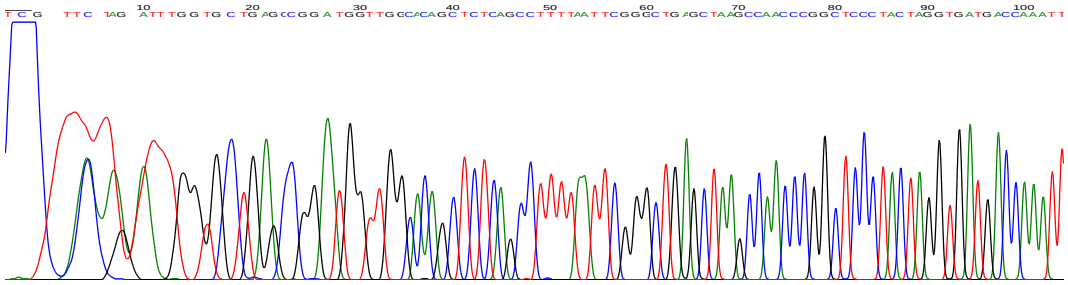


Figure 4. Results of CO1 gene amplification Pleco fish(M = Marker; 13,14,15 = positive control; 6,7,9,13,15,20,21,22,24,25,26,28 = No . Samples of Pleco fish fromCiliwungriveroriginate)

F1 primer and R1 have managed to amplify the gene CO1 Pleco fish with long 615 bp fragment (Yu &Quilang 2014). In research Jumawan et al. (2011) F1 primer and R1 succeeded in amplifying the gene CO1 Pleco fish with long fragment of 650 bp. Research Bijukumar et al. (2015) using the primers F1 and R1 CO1 gene amplification produces fish with long Pleco fish 565 bp fragment. Hajibabaei& McKenna (2012) states that the gene CO1 barcodes can be done with a length of 454-650 bp fragment of 650 bp and a total length of CO1 gene fragment for DNA barcoding.

CO1 gene readout strand BasaPleco fishoriginate fromCiliwungRiver:-
Based on the reading strand of the COI gene base 13 fish samples Pleco fishCiliwungriveroriginate from obtained sequences with a length of 680 bp fragment (Figure 5). According Hajibabaei et al. (2007) Long sequences are still within the limits of the standard barcode for animals.



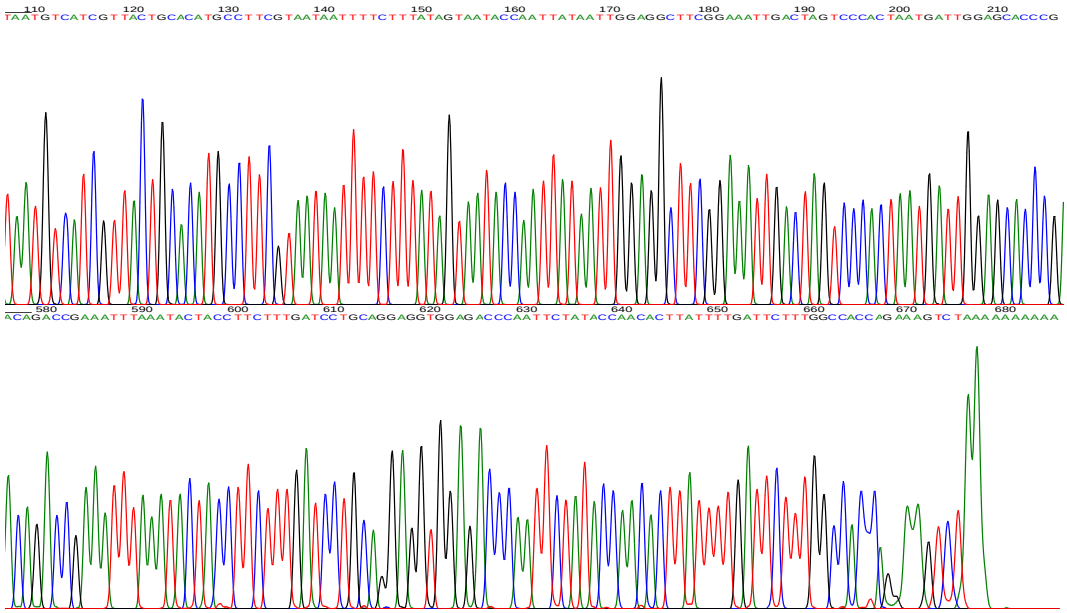


Figure 5:- Ektogram reading strand of DNA bases Pleco fishoriginate fromCiliwungriver before cutting (680 bp)

The nucleotide sequences of 13 samples of Pleco fishoriginate fromCiliwungRiver then compared with the nucleotide sequences in *GenBank* NCBI (*National Center for Biotechnology Information*). It aims to determine the similarity of the reading strand fragments to the data base GenBank using BLAST (*Basic Local Alignment Search Tool)* on *megablast-high similarity* (Table 3).

Table 2. Comparison of similarity between the base strand 13 fish samples Pleco fishoriginate fromCiliwungriver with NCBI GenBank Data

No.	Code Pleco	Identities (%)	Data Base genbank	Code genbank
1	CIL 017-A	100%	*Pterygoplichthys pardalis*	JF769358.1
2	CIL 007-C	100%	*Pterygoplichthys pardalis*	JF769358.1
3	CIL 013-C	100%	*Pterygoplichthys pardalis*	JF769358.1
4	CIL 026 –C	100%	*Pterygoplichthys pardalis*	JF769358.1
5	CIL 028-B	100%	*Pterygoplichthys pardalis*	JF769357.1
6	CIL 025-C	100%	*Pterygoplichthys pardalis*	JF769357.1
7	CIL 024-C	100%	*Pterygoplichthys pardalis*	JF769357.1
8	CIL 022-A	100%	*Pterygoplichthys pardalis*	JF769357.1
9	CIL 021-C	100%	*Pterygoplichthys pardalis*	JF769357.1
10	CIL 020-C	100%	*Pterygoplichthys pardalis*	JF769357.1
11	CIL 015-A	100%	*Pterygoplichthys pardalis*	JF769357.1
12	CIL 009-B	100%	*Pterygoplichthys pardalis*	JF769357.1
13	CIL 006-C	100%	*Pterygoplichthys pardalis*	JF769357.1

Results blast on NCBI *genbank* indicates that nucleotide sequence Pleco fishoriginate fromCiliwungriver showed an average value of 100% identification accuracy with *Pterygoplichthys pardalis* species (Table 3). This suggests that the originate from of the Pleco fish from Ciliwung river is one species. Hubert et al. (2003) similarity of identity with an average of 97% shows the same species.

Research Bijukumar et al. (2015), Yu &Quilang (2014) and Jumawan et al. (2011) phylogenetic similarity identity Pleco fish showed the same up to 100%, so that the CO1 gene can produce a high resolution for identification of the species as a standard barcode. According to Yu and Quilang (2014) the species *P. Pardalis* and *P. disjunctivus* is probably the same species or synonyms and have a low genetic diversity. Distinguishing morphological characters are dark lines continuously on *P.disjunctivus*, whereas in *P. pardalis*have dark spots (Chavez et al. 2006). Research Bijukumar et al. (2015) shows the similarity of 100% and 0% genetic distance between species *P. pardalis*, *P. disjunctivus* and *P.ambrosetti*. These results suggest that differences in the pattern of the abdomen is not the main character to identify fish species Pleco fish.

Variations Nucleotide and Amino Acid Fish Pleco fishoriginate fromCiliwungRiver:-

The composition of the nucleotide Pleco fishoriginate fromCiliwung river is A = 26.23%, T / U = 30.60%, C = 26.18%, and G = 16.99%. For the process of translation into amino acids ururan cutting 11 bp fragment or to find the start codon AUG (ATG). After cutting with a nucleotide variation obtained nucleotide fragment length 653 bp. Furthermore nucleotide positions can be analyzed to determine the location of the transition and tranversi substitution. There transversion nucleotide substitution in fish samples Pleco fishoriginate fromCiliwung river as much as 4 points namely to nucleotide position 306 (C→T), 339 (G→A), 387 (C→T), and 471 (T→C) (Figure 6).

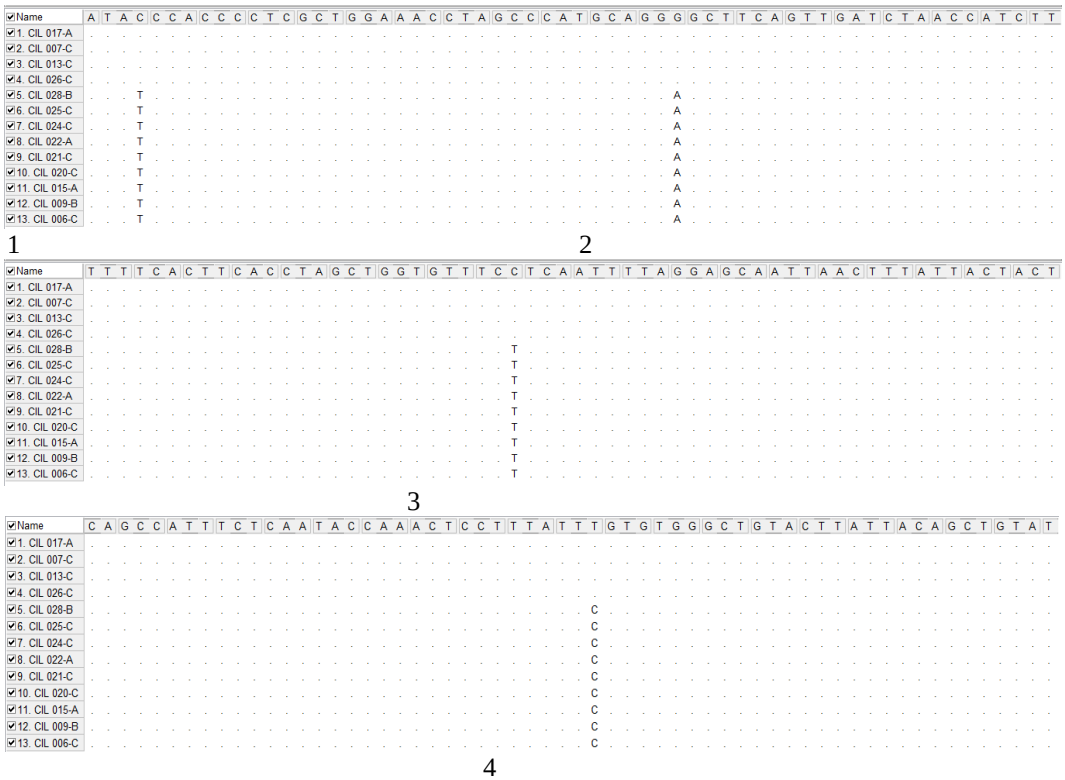


Figure 6:- Variations in the nucleotide composition and changes in nucleotide variations Pleco fish originate fromCiliwung river (1 = position information to the nucleotide-306, 2 = position of nucleotides to 339, 3 = to the nucleotide position 387, 4 = all nucleotide position 471)

The analysis shows that there are different variations of the arrangement of nucleotides in Pleco fishoriginate fromCiliwungriver. Differences in the nucleotide arrangement causes the Pleco fishoriginate fromCiliwung river splits into two on the construction of phylogenetic clade (Figure 7). Therefore, the fourth point of nucleotides serves as the main characteristics of each *clade* and distinguishing nucleotide sequences between individuals. Ubaidilah and Sutrisno (2009) states that if the DNA sequence emerges from a common ancestor sequence, the sequence will gradually separate offspring through nucleotide differences due to mutations or point mutations.

Nucleotide sequence variation along the 653 bpPlecofishoriginate fromCiliwung river ported 217 amino acid sequences. Changes nucleotide variation on four nucleotide positions (306, 339, 387, and 471) did not affect the changes of amino acid variation. Nucleotide variation occurs in nucleotide position to 306 (TAC→TAT) which translations the amino acid position 102 (Y) that is Tyrosine, all nucleotide position 339 (GGG→GGA) translates to the amino acid position 113 (G) is Glycine, to nucleotide position 387 (TCC→TCT) translates to the amino acid position 129 (S) is Serine, and all nucleotide position 471 (TTT→TTC) translations the amino acid position 157 (F) is *Phenylalanine* (Appendix 3).

Changes in the nucleotide variation four positions do not change the amino acid sequence translated. According to Lynch and Jaryl (1993) amino acid sequence changes occur more slowly in CO1 gene so that accuracy in phylogenetic. The substitution of base pairs (*base-pair substitution*) is the turn of one nucleotide and partner with

another pair of nucleotides. Some substitution called silent mutations (*silent mutation*) from an excess of genetic code. Thats not influental the amino acid sequence translated. In other words, nucleotide mutations do not change the amino acid translation of the same. Some codons can translate the same amino acids if there is a difference in the third base of the triplet codon (Campbell et al., 2008), as in the TTT and TTC codon that translates the same amino acid that is *Phenilalanine* (F).

Construction Phylogenetic Fish Sweep broom:-

The results of phylogenetic construction Pleco fishoriginate fromCiliwungriver and *out group* genus *Synodontis (Synodontisdecoratus, S.euterus, and S.leopard)* showed genetic distance apart. Plecofishoriginate fromCiliwung river is divided into two *clade* this is due to changes in the four nucleotide variations (Figure 7).

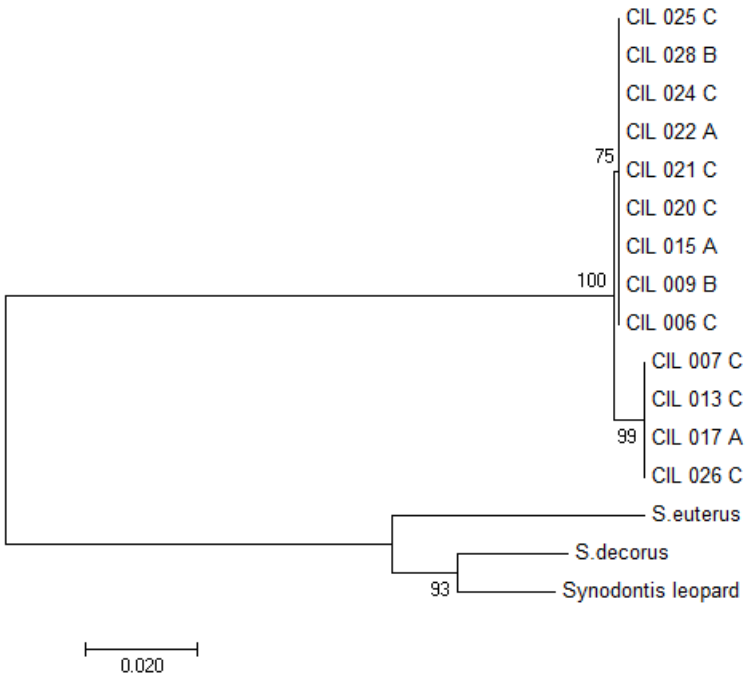
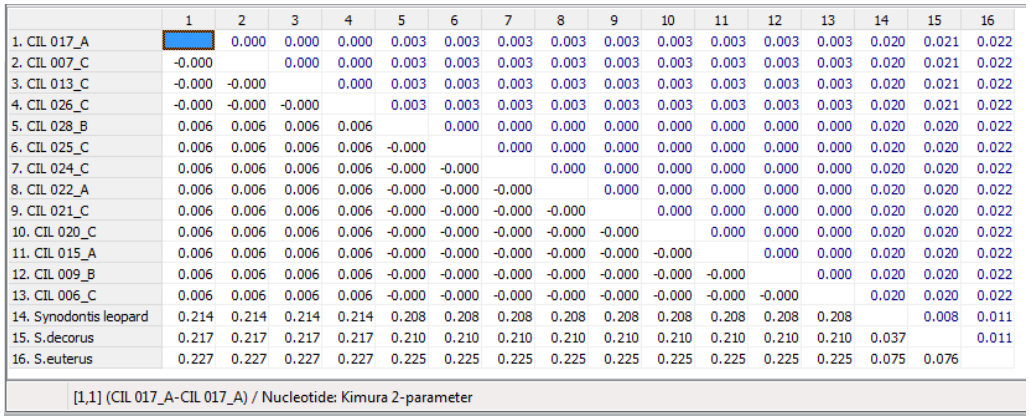


Figure 7. Construction of phylogenetic Pleco fish originate fromCiliwungriver with NJ-boostrap 1000x (649 bp)

According Mahardika and Parede (2008) the method most commonly used method is the *Neighbor-Joining* (NJ). The branching pattern of phylogenetic tree based on the distance matrix is formed between the pair populations. Long branches of the phylogenetic tree describes the number of nucleotide substitutions in the form of DNA polymorphism. Skala is located under a phylogenetic tree showing the size of the distance between sequences. Numbers located on the branches of the phylogenetic tree shows the value *boostrap* (Mahardika&Parede 2008). *Bootstrap* value in fish samples Pleco fish showed a value of 100%. Bootstrap analysis was conducted to test the validity of the construction of phylogenetic trees. Phylogenetic trees giving information about the classification of the population based on evolutionary relationships. In the reconstruction of phylogenetic trees, the molecular data more widely used because it is considered more stable in the process of evolution compared with the morphological data (Dharmayanti 2011).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. CIL 017_A		0.000	0.000	0.000	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.020	0.021	0.022
2. CIL 007_C	-0.000		0.000	0.000	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.020	0.021	0.022
3. CIL 013_C	-0.000	-0.000		0.000	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.020	0.021	0.022
4. CIL 026_C	-0.000	-0.000	-0.000		0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.020	0.021	0.022
5. CIL 028_B	0.006	0.006	0.006	0.006		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.022
6. CIL 025_C	0.006	0.006	0.006	0.006	-0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.022
7. CIL 024_C	0.006	0.006	0.006	0.006	-0.000	-0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.022
8. CIL 022_A	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000		0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.022
9. CIL 021_C	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000	-0.000		0.000	0.000	0.000	0.000	0.020	0.020	0.022
10. CIL 020_C	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000	-0.000	-0.000		0.000	0.000	0.000	0.020	0.020	0.022
11. CIL 015_A	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000		0.000	0.000	0.020	0.020	0.022
12. CIL 009_B	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000		0.000	0.020	0.020	0.022
13. CIL 006_C	0.006	0.006	0.006	0.006	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000		0.020	0.020	0.022
14. Synodontis leopard	0.214	0.214	0.214	0.214	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208		0.008	0.011
15. S.decorus	0.217	0.217	0.217	0.217	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.037		0.011
16. S.euterus	0.227	0.227	0.227	0.227	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.075	0.076	

[1,1] (CIL 017_A-CIL 017_A) / Nucleotide: Kimura 2-parameter

Figure 8. Construction of fish genetic distance Pleco fishoriginate fromCiliwungriver

Fish genetic distance Pleco fishoriginate fromCiliwung river is 0.0-0.03 (Figure 8). This suggests that the genetic distance were lower in Pleco fishoriginate fromCiliwungriver, so the Pleco fishoriginate fromCiliwung river is the same species. According to Hebert et al. (2004) and Ward et al. (2009) said that the genetic distance of more than 0:03 can show different types. This is evident with the genetic distance Pleco fishoriginate fromCiliwung river with *out group* genus *Synodontis* has a genetic distance of 0:18 to 0:20 (Figure 10).

Conclusion:-

Pleco fishoriginate fromCiliwung river had been identified using DNA *barcodes* CO1 on a fragment length of 650 bp. The nucleotide sequences Pleco fish aligned on NCBI genbank showed an average value of 100% identification accuracy with *Pterygoplichthyspardalis* species. There transversion nucleotide substitution at nucleotide position to 306 (C→T), 339 (G→A), 387 (C→T), and 471 (T→C), but this should not affect the amino acid sequence changes in Pleco-baby fish originate fromCiliwung river.

Appendix 1. The concentration and purity of the fish DNA Pleco fishoriginate fromCiliwung River.
Sample Concentration of DNA purity (A260 / A280)

Sample	**Contretation of DNA**	**Purity (A260/A280)**
1	54	1,929
2	274	2,015
3	480	1,9
4	246	1,952
5	398	1,932
6	168	2,049
7	228	1,966
8	484	1,906
9	164	2,05
10	160	1,633
11	268	1,971
12	320	2
13	228	2
14	374	1,928
15	190	1,979
16	190	1,9
17	308	1,901
18	170	1,809
19	210	1,842
20	154	2,026
21	114	1,9
22	236	2
23	68	2

24	152	2
25	210	1,981
26	36	2,25
27	206	1,981
28	176	2,095
Rata-rata		**1,960535714**

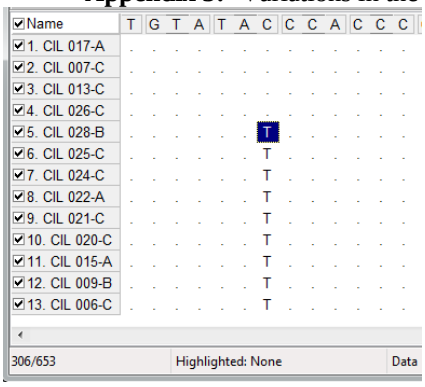
Appendix 2:- The results of the analysis of DNA purity Plecofishoriginate fromCiliwung River

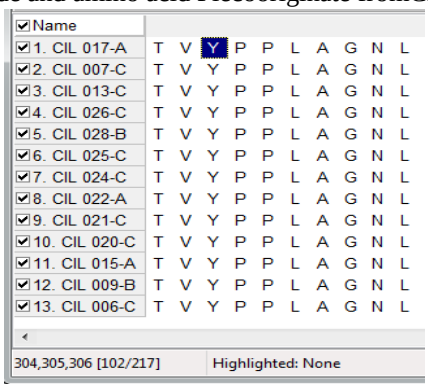
ANOVA

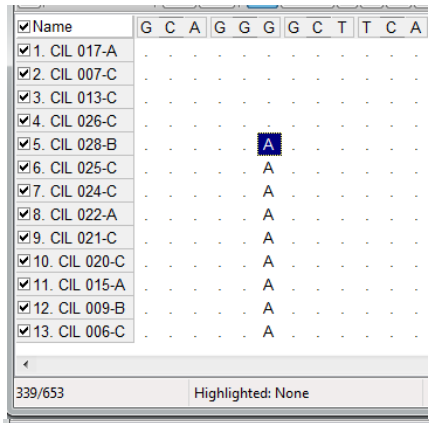
kemurnian DNA

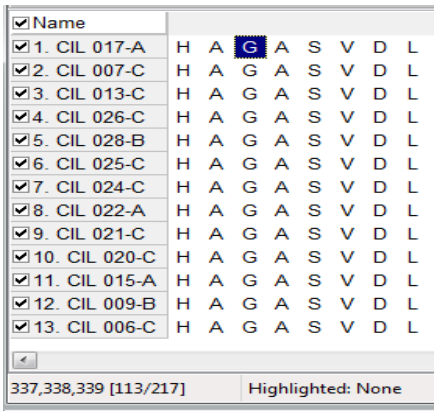
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,022	2	,011	,636	,541
Within Groups	,311	18	,017		
Total	,333	20			

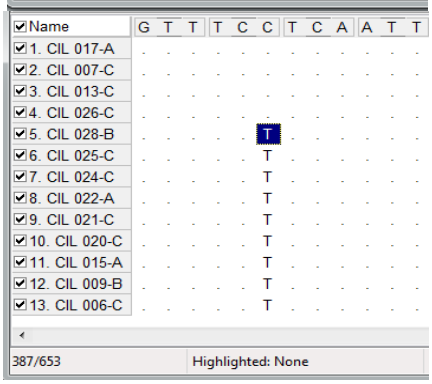
Appendix 3:- Variations in the nucleotide and amino acid Plecooriginate fromCiliwung

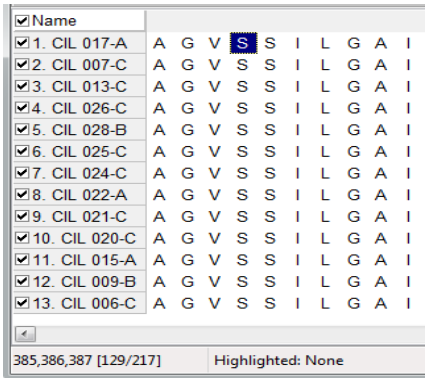


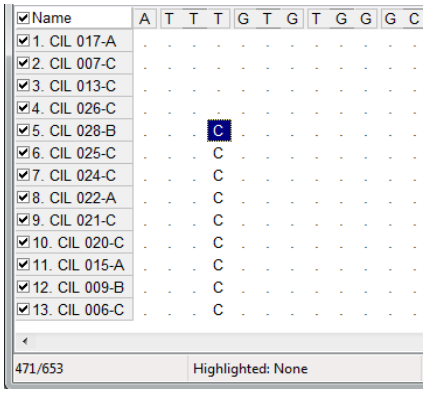


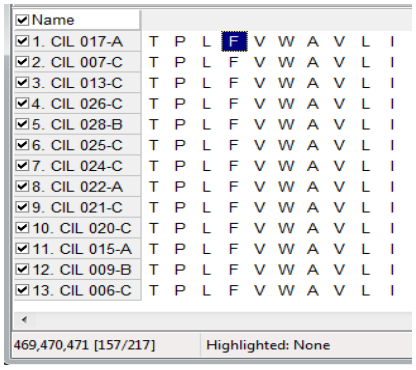












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