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MORPHOLOGICAL AND MOLECULAR CHARACTERS OF *Mimegralla* spp. (DIPTERA: MICROPEZIDAE) ON ZINGIBERACEAE IN CENTRAL JAVA

KARAKTER MORFOLOGI DAN MOLEKULER Mimegralla *spp. (DIPTERA: MICROPEZIDAE) PADA PERTANAMAN ZINGIBERACEAE DI JAWA TENGAH*

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ABSTRACT

Rhizome fly, *Mimegralla* sp. (Diptera: Micropezidae) is a major pest on Zingiberaceae plants. Some of those fly species have been reported attacking some of Zingiberaceae plants, but in Indonesia, only one species, *Mimegralla coeruleifrons* has been reported as being a pest. Since Indonesia has many species of Zingiberaceae plants, it may raise a prediction that more than one species of Mimegralla was found on this plants. Therefore, a taxonomic research on the species of rhizome flies to find the species other than *M. coerulifrons* on Zingiberaceae plants is urgently required. This study was conducted by using hand-picking method on Mimegralla adult inhabiting Zingiberaceae plants (ginger, turmeric, javanese ginger, and aromatic ginger), and was then identified by using morphological characters and through molecular technique by using mtCO1 gene. The results showed that *M. albimana* and *M. coeruleifrons* found at four zingiberaceae plants were the member of Mimegralla. As a conclusion, these two species have high values of phylogenic relationship (88%) and bootstrap (92).

Keywords: *Mimegralla* spp., molecular, morphology

INTISARI

*Lalat rimpang (*Mimegralla *spp.) adalah hama utama pada beberapa tanaman anggota Family Zingiberaceae. Beberapa spesies lalat rimpang dilaporkan menyerang tanaman Zingiberaceae, di antaranya jahe, kunyit, temulawak, dan kencur. Di Indonesia hanya satu spesies yang pernah dilaporkan menyerang tanaman jahe yaitu* Mimegralla coeruleifrons. *Sementara itu, jenis tanaman Zingiberaceae yang ditanam di Indonesia sangat beragam, sehingga muncul dugaan bahwa Mimegralla yang menyerang tanaman Zingiberaceae di Indonesia tidak hanya* M. coeruleifrons *saja. Oleh karena itu, penelitian untuk mengidentifikasi spesies-spesies lalat rimpang yang menyerang tanaman Zingiberaceae di Indonesia perlu dilakukan. Penelitian ini dilakukan menggunakan metode pemungutan/penangkapan (*hand-picking*) imago Mimegralla pada tanaman keempat tanaman Zingiberaceae. Imago yang diperoleh diidentifikasi secara morfologi dan molekuler menggunakan gen* mtCO1. *Hasil penelitian ini menunjukkan bahwa* M. albimana *dan* M. coeruleifrons *yang ditemukan pada pertanaman Zingiberaceae merupakan satu genus yang sama yaitu Mimegralla. Hasil identifikasi molekuler menunjukkan bahwa kedua lalat rimpang tersebut mempunyai derajat kekerabatan dengan nilai homologi sebesar 88%, dan nilai* bootstrap *pada pohon filogeni sebesar 92*.

Kata kunci: Mimegralla *spp., molekuler, morfologi*

INTRODUCTION

Zingiberaceae plants, including genus Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria, and Costus (Joy *et al*., 1998), are the main materials in producing herbal medicines, which is containing volatile oils and oleoresins as tonic and stimulant for human body. Rhizome fly is noted as a major pest in ginger plants (Steyskal, 1964), turmeric (Nair, 1980), and aromatic ginger (Balfas *et al*., 2000) in several countries, including Indonesia.

Two species of rhizome fly are reported attacking Zingiberacea in India (Maxwell-Lefroy & Howlett, 1909), those are *Mimegrallla coeruleifrons* Macq. (Ghorpade *et al*., 1983) and *M. albimana* Doleschall (Ghorpade *et al*., 1988). But only one species, *M. coeruleifrons* Macq., was reported attacking Zingiberaceae in Indonesia (Balfas, 2002). However, the diverse species of Zingiberaceae planted in Indonesia raises asumption that the number of Mimegralla attacking rhizomes might be more than one species. Therefore, a thorough study should be done to clarify this issue. This study was aimed to know the species of Mimegralla attacking Zingiberaceae in Central Java by using morphological and molecular characters. In addition, the molecular identification by using

mtCO1 characters on rhizome fly (*Mimegralla* spp.) has never been done, and even the information of base nucleotide was never found in NCBI GenBank. So identification using mtCO1 need to be done to understand the phylogenic relationship.

MATERIALS AND METHODS

Sampling and Preservation

Mimegralla spp. adults were collected by hand-picking from ginger (*Zingiber officinale*), turmeric (*Curcuma domestica*), Javanese ginger (temulawak, *Curcuma xanthorrhiza*), and aromatic ginger (kencur, *Kaempferia galanga*) plants in Karanganyar District (latitude of 7°37'53,674", longitude 110°56'58,957" and altitude of 204.7 m above sea level) and Purworejo District (latitude 7°42'15,660", longitude 110°1'47,537" LE and latitude of 81.5 m above the sea level). Sampling was conducted in area, where symptomatic of rhizome fly, i.e. rich of organic material rot (Hennig, 1935) and wilt (Karmawati *et al*., 1990) were found. Sampling were conducted in October 2016, when the rainy season started and Zingiberaceae thrives at this season. Heavy rainfall is the optimum condition of the microorganisms to grow well.

Adult of rhizome flies were used for morphological and molecular identifications, and insect collection as well. Adults used for morphology identification were pinned using insect needles no. 00 and put into 96% alcohol for molecular identification.

Identification

Morphological and molecular identification were conducted at Laboratory of Basic Entomology and Laboratory of Virology, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

Morphological identification of fly was done by using Leica MZ16 and Leica KL1500 LCD Microscopes and Optilab advance on the whole part of body to describe shape, size, color, and genitalia parts as specific characters of species. Magnification of microscopes showed in Table 1.

DNA extraction was done by using Genomic Mini Kit from Geneaid, and the extraction product was then amplified by PCR using LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) as forward primer and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) as reverse primer (Folmer *et al*., 1994). The kit used in PCR reaction was Go Taq Green Master Mix kit: Go Taq Green Master Mix 12.5 µL; primer forward 1 µL; reverse primer 1 µL; DNA template 2 µL; and Nuclease Free Water 8.5 µL. Stages of PCR cycles were shown in Table 2. The results of subsequent DNA amplification were visualized by agarose gel electrophoresis (SIGMA) and observed using BIO-RAD UV transluminator 2000. Sequencing was carried out by sending samples to 1st Base DNA Sequencing (Selangor, Malaysia). Finally, to analyze the degree of neighborhood joining values, Bioedit software was used, and followed by Mega 5.2. and BLAST software. The Bioedit was

Table 1. Magnification for morphology identification

Component identification	Magnification	
	Mimegralla coeruleifrons	*Mimegralla albimana*
All body	11.4 ×	20.0 ×
Caput	51.2 ×	64.0 ×
Thorax	40.0 ×	51.2 ×
Abdomen	25.6 ×	40.0 ×
Wings	25.6 ×	32.0 ×
Antenna	128.0 ×	128.0 ×
Copulatory fork	100.8 ×	100.8 ×
Ovipositor	32.0 ×	80.0 ×

Table 2. PCR stages cycle

No.	Reaction	Temperature	Time	Cycle
1	Predenaturasi	95ºC	2 minutes	
2	Denaturasi	95ºC	30 seconds	30 cycles
3	Annealing	55ºC	30 seconds	
4	Extention	72ºC	1 minute	
5	Final extention	72ºC	5 minutes	
6	Delay	4ºC	5 minutes	

used to make base paired alignment of forward and reverse nucleotide bases, and to get the percent of clustal consensus. We used MEGA 5.2. for making phylogeny tree and used BLAST for getting homology percentage.

RESULTS AND DISCUSSION

In this study 78 samples of suspected Mimegralla was found in Zingiberaceae plants (ginger, turmeric, javanese ginger, and aromatic ginger). The result of morphological identification showed that the samples was consisted of two different Mimegralla species, i.e. *Mimegralla albimana* and *M. coeruleifrons* (Figure 1). To complete the observation on the differensiation between these two species, three individual samples of each species were selected randomly, and were examined by using molecular technique.

Morphology Identification

Stilt-legged fly (*Mimegralla* spp.) is a member of family Micropezidae (Diptera: Acalyptratae), and there are 583 spesies of Micropezidae reported, which are divided into 52 genera and 5 subfamilies (Pape *et al*., 1758). Most of Micropezidae species were found in tropic and subtropic areas. This fly has unique characteristics by their long and slender feet.

Aczel (1959) mentioned that a specific character of Mimegralla is the occurence of ocellar plate on the posterior of upper superior orbital or very close to the fore ocellus placed in a line. Frontal stripe are more than a half of total width of frons. Upper anterior orbital stand in tomentosa frontal or form a boundary line between the frontal stripe and genovertical plate. Postvertical and genal bristle always absen.

1. Forceps in apex of copulatory fork tapered at the ends with intersect bristles (in males). Shape of ovipositor oval with membraneus region at the end the shorter (Figure 2). Body color is brownish red and yellowish ..... *albimana*

2. Forceps in apex of copulatory fork show widened with a short bristle does not intersect (in males). Shape of ovipositor conical with an elongated tip at membraneus region (Figure 3). Overall body color is dark blue and black ..... *coeruleifrons*

M. albimana and *M. coeruleifrons* could be distinguished by their color. *M. albimana* has brownish red and yellow on its body. Facet are maroon, antenna are reddish yellow, abdomen black and brownish yellow, legs bright yellow with black stripes on coxa, more dark to the apex. Whereas *M. coeruleifrons* has blackish-dark-blue body. Facet are dark blue and metallic when exposed to the light, antennae are dark brown, abdomen is black and gray, and legs are brownish with black stripes on coxa. Hennig (1935) also reported, *M. albimana* legs are continuosly dark whereas *M. coeruleifrons* legs are dark.

However, when the body of *M. albimana* and *M. coeruleifrons* were observed closer by using higher magnification, some differences were found. These differences were observed on preabdomen, ovipositor, wings, and copulatory fork. Body of *M. albimana* was stout and looks more convex than long, length of wings were 3.5 to 3.7 times than width, and lenght of body was 4.3 to 4.5 times than width (Figure 1). Whereas *M. coeruleifrons* was more slender and elongated and wing length was 3.8 to 4.5 times than width. Body lenght 4.6 to 4.9 times than width.

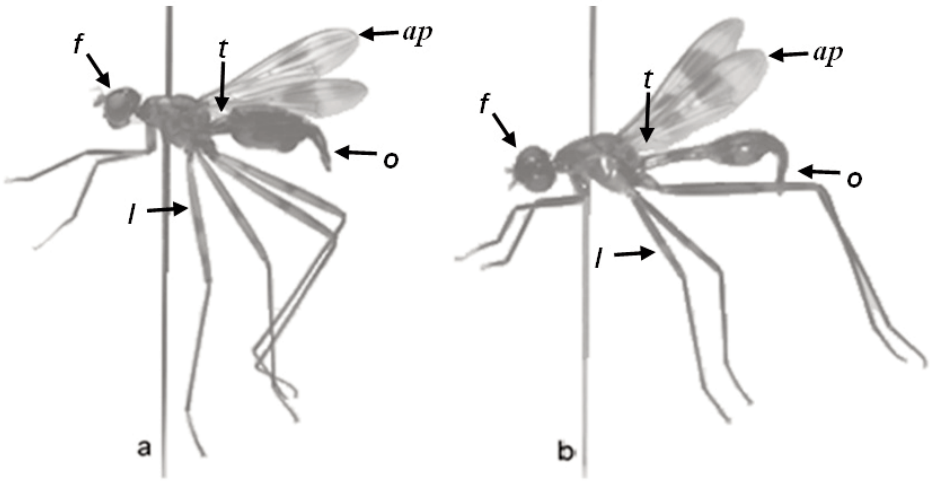

Figure 1. Female adult of *Mimegralla albimana* (a); adult of *Mimegralla coeruleifrons* (b): frons (f), tergite 1−3 (t), apex of wing (ap), ovipositor (o), legs (l)

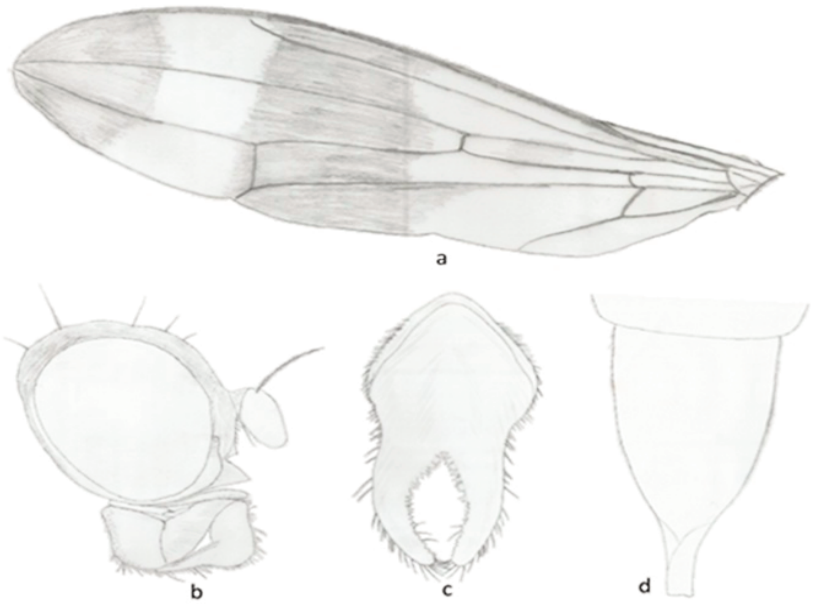

Figure 2. Wing (a), head (b), copulatory fork (c), and ovipositor (d) of *Mimegralla albimana*

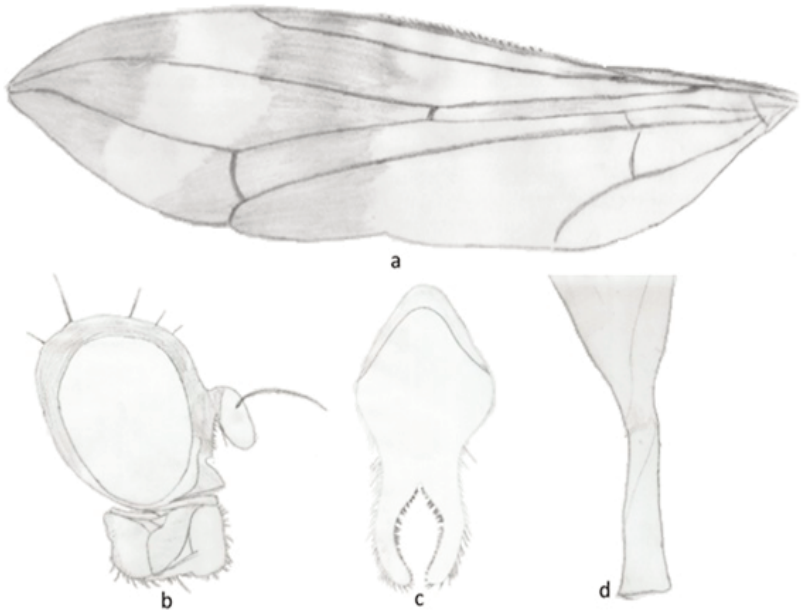

Figure 3. Wing (a), head (b), copulatory fork (c), and ovipositor (d) of *Mimegralla coeruleifrons*

Molecular Identification

DNA amplification on adult was done by using cytochrome c oxidase subunit 1 in mitochondrial (mtCO1) part and was performed by universal primers LCO1490 and HCO2198, which are mostly used for invertebrates (Folmer *et al*., 1994).

DNA amplification was visualized using BIO-RAD UV transluminator 2000, and a single band at 600 bp. This product then was sent to 1st Base DNA Sequencing at Selangor, Malaysia. The result of DNA sequencing of *M. albimana* showed, that it has 681 bp (base pairs) of DNA nucleotide bases, whereas *M. coeruleifrons* has 678 bp. These results were consistent with results of PCR visualization as described above. The nucleotide bases of *M. albimana* was compared with *M. coeruleifrons* and three other species (Table 3), the member of Micropezidae Family, to determine the value of homology between the first two species and the other three. The nucleotide bases of the three other species was taken from GenBank database as shown at Figure 4.

The nucleotide bases of five species were compared to determine the level of homology in percent. The closeness of phylogenic relationship in clustal consensus

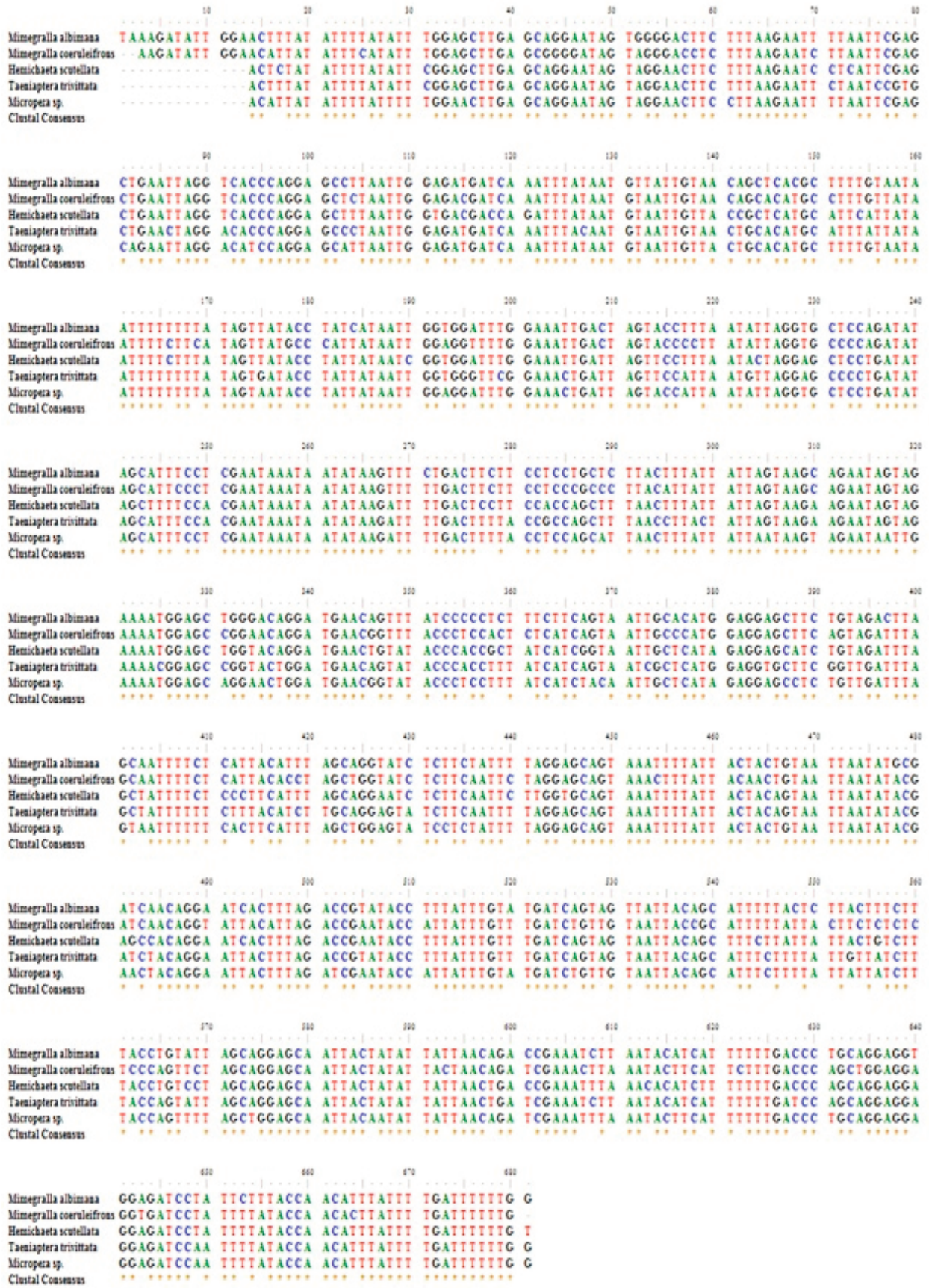

Figure 4. Clustal consensus between *Mimegralla* spp. and three others species of Micropezidae Family from GenBank database

Table 3. Origin of *Mimegralla* spp. and three other species of Micropezidae Family from GenBank database were used for phylogenetic comparison

Species	Total of nucleotide base (bp)	Origin	Source
Mimegralla albimana	681	Karanganyar	This research
Mimegralla coeruleifrons	678	Karanganyar	This research
Hemichaeta scutellata	1493	CR: Dominical Hacienda Baru	Jackson *et al*. (2014) KM28700.1
Taeniaptera trivittata	1376	USA: SC: Georgetown Co.	Jackson *et al.* (2014) KM287302.1
Micropeza sp.	1405	Argentina	Jackson *et al.* (2014) KM287330.1

Table 4. Percentage of homology from nucleotide base of *Mimegralla albimana* and three others species of Micropezidae Family from GenBank database

No.	Species	1	2	3	4	5
1	*Mimegralla albimana*	ID				
2	*Mimegralla coeruleifrons*	88%	ID			
3	*Hemichaeta scutellata*	88%	85%	ID		
4	*Taeniaptera trivittata*	87%	84%	88%	ID	
5	*Micropeza* sp.	87%	84%	86%	89%	ID

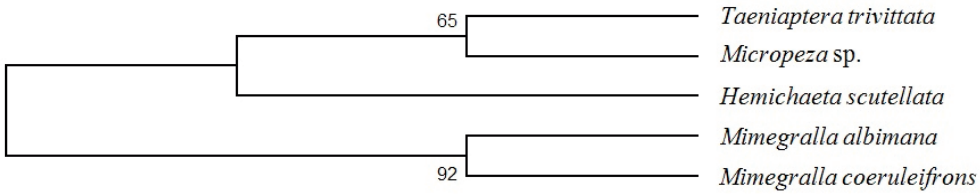

Figure 5. Phylogeny tree of *Mimegralla* spp. and others species of Micropezidae Family

could be determined by counting nucleotide base without an asterisk (*). The result indicated that *M. coeruleifrons* has closest relationship with *M. albimana* (88%) than with the other three species (*Hemichaeta scutellata*, *Taeniaptera trivittata*, and *Micropeza* sp.) on homology (Table 4) and clustal consensus (Figure 4). Sokal and Sneath (1963) stated that the closeness of phylogenic relationship between organisms are indicated by the similarity of common characters. Figure 5 showed that *M. coeruleifrons* and *M. albimana* has a bootstrap value at 92, means that these species are member of Mimegralla Genus. Bootstrap analysis is used to test the validity and reliability of data (Hedges, 1992). Thus, the value of 92 means that the data in phylogeny analysis has high validity and reliability.

CONCLUSION

M. coeruleifrons and *M. albimana* found in Zingiberaceae plants (ginger, turmeric, javanese ginger, and aromatic ginger) were member of the same genus, Mimegralla, by the facts that these two species have high values of phylogenic relationship (88%) and bootstrap (92).

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