



Research Article

Morphometric Analysis and Host Range of the Genus *Pentalonia* Coquerel (Hemiptera: Aphididae) Infesting Banana in Java

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ABSTRACT

Banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is known as vector of *Banana bunchy top virus* (BBTV) that threatening production of banana worldwide. It was reported recently that *P. nigronervosa* and *P. caladii* is “cryptic species”. A good and proper identification is necessary to verify the correct species and its status. Research was conducted to identify and to find the host range of banana aphids in Java. Aphid collection was conducted in several locations in West Java, Central Java, and East Java. Eleven morphometric characters were analyzed to assess the morphometric variations among banana aphids. Morphological identification and principle component analysis (PCA) approach were conducted for accurate identification of banana aphids. Two species of aphids were found during the survey in Java, i.e. *P. nigronervosa* and *P. caladii*. *P. nigronervosa* mostly infested bananas (*Musa* spp.), and a few was found on heliconia (*Heliconia* sp.) and banana traveler (*Ravenala madagascariensis*). In contrast, *P. caladii* generally infested taro (*Colocasia esculenta*), turmeric (*Curcuma longa*), costus (*Costus* sp.), dumbcane (*Dieffenbachia* sp.), but rarely on bananas.

Keywords: Banana bunchy top virus, morphology, *Pentalonia caladii*, *Pentalonia nigronervosa*

INTRODUCTION

Pentalonia nigronervosa is the most important pest on banana and known as vector of Banana bunchy top virus (BBTV). This species plays an important role on the spread of banana bunchy top disease (Dale, 1987; Hu *et al.*, 1996; Watanabe *et al.*, 2013). Infection of BBTV has been reported on various banana genotypes in the world and resulted in 100% yield losses (Dale, 1987). Incidence of BBTV reached 100% and 96% on banana cv. Cavendish Williams and plantain hybrid variety PITA 23, respectively in Southern region of Cameroon, Africa (Ngatat *et al.*, 2017). In contrast, incidence of BBTV in Indonesia is low, i.e. ranged from 0 % to 38.6% (Nurhayati, 2003). Since Indonesia and other countries in Southeast Asia are considered having the highest genetic diversity of wild banana, it is important to be prepared for an appropriate management strategy of BBTV and its vector.

In genus *Pentalonia*, *P. nigronervosa* and *P. caladii* are known as “cryptic species”. Previously, Hardy (1931) placed *P. caladii* in synonymy with *P.*

nigronervosa but recently Foottit *et al.* (2010) re-established the identity of *P. nigronervosa* and *P. caladii* each as full species based on morphometric and molecular analysis. Furthermore, the host range of the two species is slightly different. *P. nigronervosa* mostly feeds on banana and occasionally feeds on Heliconia species (Nordam, 2004; Bhadra & Agarwala, 2010; Foottit *et al.*, 2010; Suparman *et al.*, 2011; Miller *et al.*, 2014), whereas *P. caladii* feeds on *Alocasia* sp., *Caladium* sp., *Elettaria* sp., *Hedychium* sp., *Xanthosoma* sp., and *Zingiber* sp. (Nordam, 2004; Miller *et al.*, 2014).

Identification of aphid infesting banana in Java has been done a long time ago by Nordam (2004). He reported *P. nigronervosa* as the single species of aphid infesting banana in Java based on the specimens collected by van der Goot in 1916 and D. Nordam in 1976–1977. Due to the most recent taxonomy of banana aphids, it is necessary to update the status of banana aphids in Indonesia, including in Java. The two cryptic species, *P. nigronervosa* and *P. caladii*, are only distinguished by the ultimate

rostrum segment (URS) in which the URS of *P. caladii* is shorter than *P. nigronervosa* (Nordam, 2004; Footitt *et al.*, 2010; Miller *et al.*, 2014). Multivariate analysis approach on morphometric of aphids has been used by some authors to investigate morphological variation within and between populations of aphids (Blackman, 1987; Footitt *et al.*, 2010).

In this study, there were some morphometric characters of aphids infesting bananas were studied using multivariate analysis and t-test to get accurate identification to confirm morphological identification keys by Blackman & Eastop (2006) and Miller *et al.* (2014). In addition, we also conducted the host plants survey of aphids infesting banana to collect appropriate data for the purpose of developing a strategy of controlling banana aphids and BBTV.

MATERIALS AND METHODS

Collection of Aphids

Aphids were collected by purposive sampling method from different plants belong to the Family Musaceae, Araceae, Zingiberaceae, Heliconiaceae, Costaceae, and Strelitziaceae. Aphid collection was conducted in several locations in West Java, Central Java and East Java in August 2017 to January 2018 (Table 1). Aphids were collected from the plants using a soft brush and then stored in eppendorf tube containing 70% alcohol. Information of location, altitude, collection date, and host species were recorded.

Preparation of Aphid Slide Specimens

Aphids were mounted with modified procedure of Blackman & Eastop (2000) in Laboratory of Insect Biosystematics, Department of Plant Protection,

Table 1. Distribution of aphids infesting banana in Java

No	Location (village, district, regency/city)	Altitude (m asl)	Coordinate	Banana cultivar/ species	Banana genome	n	Aphid species
1	Tanah Baru, Bogor Utara, Bogor	257	S=006°35.430' E=106°49.145'	Lampung	AA	9	<i>P. nigronervosa</i>
2	Cihideungudik, Ciampea, Bogor	216	S=006°34.277' E=106°42.730'	Siem	ABB	7	<i>P. nigronervosa</i>
3	Sukaharja, Ciomas, Bogor	264	S=006°36.231' E=106°44.982'	Ambon	AAA	6	<i>P. nigronervosa</i>
4	Bogor Botanical Garden, Bogor	260	S=006°35.893' E=106°48.136'	<i>Musa veluntina</i>	-	7	<i>P. nigronervosa</i> & <i>P. caladii</i>
5	Cisarua, Cisarua, Bogor	819	S=006°40.292' E=106°56.314'	Mas Kepok	AA ABB	9 8	<i>P. nigronervosa</i> <i>P. nigronervosa</i>
6	Sukakarya, Megamendung, Bogor	747	S=006°40.060' E=106°54.126'	Ambon Tanduk	AAA AAB	10 10	<i>P. nigronervosa</i> <i>P. nigronervosa</i>
7	PTPN VIII, Parakan Salak, Sukabumi	496	S=006°49.000' E=106°44.375'	Barangan Cavendish Raja Buluh	AAA AAA AAB	9 10 5	<i>P. nigronervosa</i> & <i>P. caladii</i> <i>P. nigronervosa</i> <i>P. nigronervosa</i>
8	Pohgading, Gembong, Pati	252	S=006°41.739' E=110°57.054'	Raja	AAB	7	<i>P. caladii</i>
9	Balesari, Windusari, Magelang	588	S=007°25.004' E=110°10.976'	Raja Nangka Mas	AAA AA	9 6	<i>P. nigronervosa</i> <i>P. nigronervosa</i>
10	Kembang Kuning, Windusari, Magelang	580	S=007°25.412' E=110°10.842'	Patilan	ABB	6	<i>P. nigronervosa</i>
11	Babadan, Ngajum, Malang	791	S=008°01.385' E=112°31.366'	Sobo	ABB	11	<i>P. nigronervosa</i>
12	Kebobang, Wonosari, Malang	710	S=008°02.691' E=112°30.143'	Raja Sajen	AAB	9	<i>P. nigronervosa</i>
13	Kalistail, Genteng, Banyuwangi	209	S=008°21.230' E=114°08.612'	Kepok	ABB	11	<i>P. nigronervosa</i>
14	Genteng Weten, Genteng, Banyuwangi	203	S=008°21.284' E=114°09.725'	Awak	ABB	2	<i>P. nigronervosa</i>

IPB University, Bogor. Aphids from the field were placed on test tube containing 95% alcohol and heated on hot plate at 80 to 100°C for 3 minutes. The aphids were then transferred to the syracuse watch glasses and the ventral side of the abdomens was punctured using a small needle. The aphids were transferred to test tube containing 10% KOH solution and heated until the aphid body became transparent, then the aphids were rinsed with aquadest for 2 times. In order to remove the water content from the aphid body, subsequent immersion in alcohol solution with concentration of 50%, 70%, 80%, 95% and 100% was proceeded for 5–10 minutes on each concentration. Finally, the aphids were immersed in clove oil for 10 minutes to remove the alcohol from the aphid body and then aphids were mounted using Canada balsam for permanent slide purpose. The specimens were then dried on warmer slider at 35 to 40°C for 2 to 3 weeks.

Identification and Morphometric Measurements

Identification of aphid was conducted morphologically following key identification in “Aphids on the World’s Herbaceous Plants and Shrubs” by Blackman & Eastop (2006) and “Review and Key to Aphid (Hemiptera: Aphididae) in Micronesia” by Miller *et al.* (2014). Eleven morphometric measurements were performed according to Footitt *et al.* (2010), i.e. length of body (BL), head width (HW), antennal length (AL), length of antennal segment I–II (A1–2), length of antennal segment III–V (A3–5), length of antennal segment VI (A6), length of ultimate rostrum segment (URS), length of siphunculus (Sph), length of hind femur (Fem), length of hind tibia (Tib) and length of caudal (Cau). The morphometric measurement was only conducted on apterous samples. Morphometric characters measurement was derived from image-measurement using Stereo Microscope Leica M 205C and Leica Application Suit Software Version 4.4.0.

Data Analysis

Data of morphometric characters of aphids were analyzed using principle component analysis (PCA) followed by analysis of significant difference of morphometric characters using t-test. Data analysis was performed using PAST version 3.18 (Hummer *et al.*, 2001) and no data transformation was done before analysis.

RESULTS AND DISCUSSION

Morphometry of Aphid Species Infesting Banana

A total of 151 specimens were examined for morphological characters. Two species of aphids were identified, i.e. *P. nigronervosa* and *P. caladii* (Table 1). The two species have closely similar characters: length of the antennae is longer than the body, abdomen I and VII without marginal tubercles, apterous frequently having rhinaria at the third antennal segment, parallel or divergent frontal antennal tubercles, siphunculus elongate or swollen with 0–2 rows apical reticulation, femur pale basally and dark distally. The two species were only distinguished by the length of ultimate rostrum segment (URS), in which URS of *P. caladii* is shorter than those of *P. nigronervosa* (Figure 1). Footitt *et al.* (2010) and Miller *et al.* (2014) recorded that the URS length of *P. caladii* is generally less than 0.13 mm whereas those of *P. nigronervosa* is more than 0.13 mm; the mean length of URS length of *P. nigronervosa* and *P. caladii* was 0.152 ± 0.005 mm and 0.123 ± 0.003 mm, respectively.

Principle component analysis (PCA) was performed to observe morphological variations between *P. nigronervosa* and *P. caladii*. Of the eleven principle components, only the first two principle components were suitable for further analysis due to its eigenvalue, i.e. more than 1 (Figure 2). The first two principle components explained 75.1% of morphology variation in both species (Table 2). The first and second principle component (PC1 and PC2) contributed 66.4% and 9.7% of variance data, respectively. Component loading of the eleven morphological traits showed evenly contributed to PC1, but loading of URS length showed strongly contributed to PC2 with score 0.797.

Principle component analysis (PCA) showed morphometric differences of *P. nigronervosa* and *P. caladii*. The morphometric scatter plot of *P. nigronervosa* and *P. caladii* exhibited separation projection (Figure 3). The character of URS length was the most contributed in the differentiation of both species. This result confirmed previous study by Footitt *et al.* (2010) that examined *P. nigronervosa* collected from banana and *P. caladii* collected from non-banana host. We also demonstrated that *P. caladii* collected from banana and non-banana host had no differences on URS length.

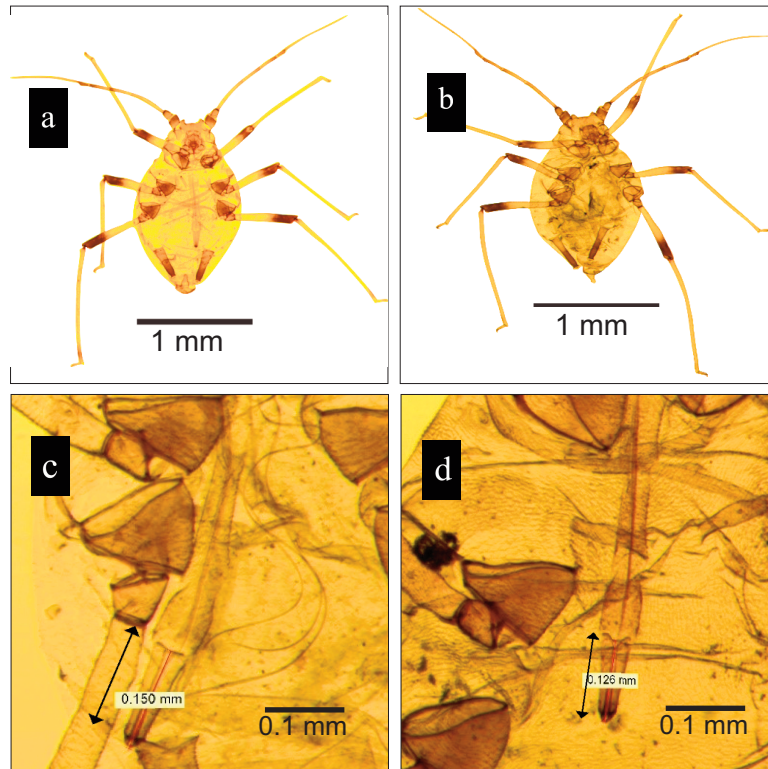


Figure 1. Morphology of adult banana aphids: ventral side of *Pentalonía nigronervosa* (a) and *Pentalonía caladii* (b); ultimate rostrum segment (URS) of *Pentalonía nigronervosa* (c) and of *Pentalonía caladii* (d)

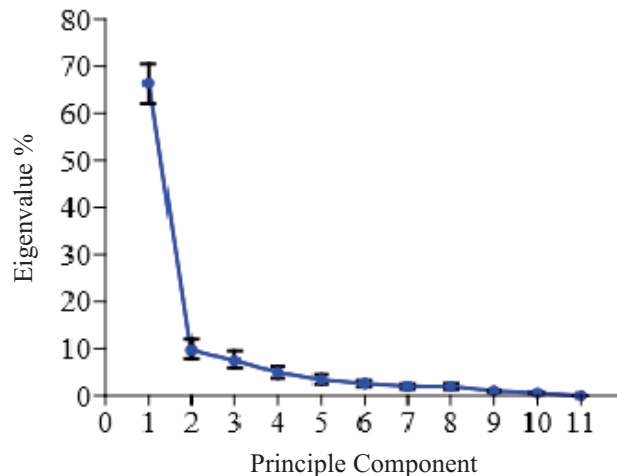


Figure 2. Eigenvalue of eleven principle components of morphometric characters of aphids infesting banana

T-test analysis showed there were some morphometric characters that significantly different between *P. nigronervosa* and *P. caladii*, i.e. URS length (t-test=28.5; P-value <0.01), head width (t-test=5.72; P-value<0.01), antennal length (t-test =2.12; P-value<0.05), length of antennal segment I–II (t-test=2.97; P-value<0.01), and length of antennal segment VI (t-test=6.60; P-value<0.01) (Table 3). However, there was no significant

differences of the body length (t-test=1.03; P-value>0.05), length of antennal III–V (t-test=0.97; P-value>0.05), length of hind femur (t-test=1.76; P-value>0.05), length of hind tibia (t-test=0.50; P-value>0.05), length of siphunculus (t-test=0.62; P-value>0.05) and length of cauda (t-test=0.41; P-value>0.05) between *P. nigronervosa* and *P. caladii*. Of the characters showing significant differences between the two species, only URS length had no

Table 2. Loading score of the first two principle components (PC1 and PC2) of aphids infesting banana

Variable	PC1	PC2
Body length (BL)	0.280	-0.079
Head width (HW)	0.291	0.355
Antennal length (AL)	0.344	0.005
Length of antennal segment I-II (A1+2)	0.317	0.050
Length of antennal segment III-V (A3-5)	0.332	-0.104
Length of antennal segment VI (A6)	0.282	0.171
Length of URS (URS)	0.171	0
Length of hind femur (Fem)	0.347	-0.079
Length of hind tibia (Tib)	0.340	-0.207
Length of siphunculus (Sph)	0.312	-0.305
Length of cauda (Cau)	0.257	-0.217
Eigenvalue	7.30	1.06
Proportion of variation (%)	66.4	9.7
Cumulative proportion (%)	66.4	75.1

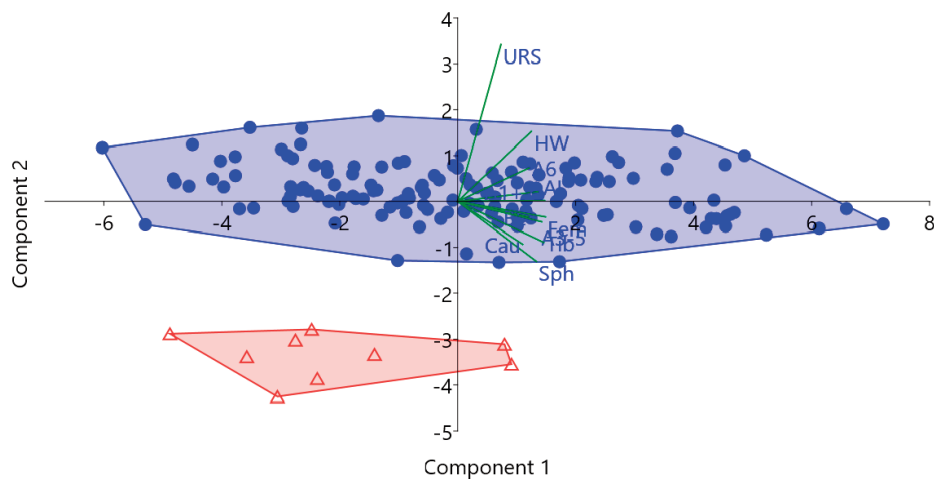


Figure 3. Scatter plot of the first two principle components of morphometric characters of aphids infesting banana: *Pentalonia nigronevosa* (●), *P. caladii* (Δ); morphometric characters observed (URS, HW, BL, AL, AL1–2, AL3–5, AL6, Fem, Tib, Sph, Cau) were shown on Table 3

overlapping measurement character. Both PCA and t-test indicated that URS length is the only one character that clearly showed differentiation of *P. nigronevosa* and *P. caladii* though the two species infesting different host plants.

The aphid infesting different host plants frequently exhibits variation in morphometric and fitness characters. Aphids reared on poor nutrient quality of host plants are smaller than those reared on rich nutrient quality (Dixon & Kindlmann, 1994). The nutrient quality of a host plant and aphid nutritional biology affects the fitness of aphid (Awmack & Leather, 2002; Powel *et al.* 2006). The previous study showed that *P. nigronevosa* reared on banana plant had the longest longevity than taro and red

ginger flower (Robson *et al.*, 2007). Badhra & Agarwala (2010) also reported that two congeneric species of banana aphid, *P. nigronevosa* and *P. caladii* showed differences in biological character when host plant transfer was performed.

Host Range of *P. nigronevosa* and *P. caladii*

Two banana aphid species, *P. nigronevosa* and *P. caladii*, were found on 3 and 6 host plants, respectively (Table 4). *P. nigronevosa* was mostly found on banana (*Musa* spp.), and frequently found on *Heliconia* sp. and *Ravenala madagascariensis*. Laboratory experiment conducted previously indicated that banana aphid *P. nigronevosa* can feed and live on some species of zingiberaceous and araceous (Suparman *et al.*, 2017). However, *P. nigronevosa*

Table 3. Mean value of eleven morphometric characters of *Pentalonia nigronervosa* and *P. caladii* on banana

Variable	<i>P. nigronervosa</i>		<i>P. caladii</i>		t-test
	n	(mean ± SD)	n	(mean ± SD)	
Body length (BL)	142	1.474 ± 0.158	9	1.418 ± 0.154	1.03 ^{NS}
Head width (HW)	142	0.429 ± 0.023	9	0.383 ± 0.030	5.72**
Antennal length (AL)	142	1.628 ± 0.107	9	1.551 ± 0.061	2.12*
Length of antennal I-II (A1+2)	142	0.171 ± 0.009	9	0.162 ± 0.007	2.97**
Length of antennal III-V (A3-5)	142	0.752 ± 0.068	9	0.730 ± 0.045	0.97 ^{NS}
Length of antennal VI (A6)	142	0.704 ± 0.040	9	0.659 ± 0.018	6.60**
Length of URS (URS)	142	0.152 ± 0.005	9	0.123 ± 0.003	28.5**
Length of hind femur (Fem)	142	0.558 ± 0.044	9	0.532 ± 0.037	1.76 ^{NS}
Length of hind tibia (Tib)	142	1.056 ± 0.076	9	1.070 ± 0.081	0.50 ^{NS}
Length of siphunculus (Sph)	142	0.316 ± 0.022	9	0.321 ± 0.012	0.62 ^{NS}
Length of cauda (Cau)	142	0.106 ± 0.009	9	0.107 ± 0.008	0.41 ^{NS}

Remarks: NS indicates no significant difference ($P > 0.05$), * indicates significant difference at $P < 0.05$ and ** indicates significant difference at $P < 0.01$

Table 4. Population of *Pentalonia nigronervosa* and *P. caladii* on different host plants

Aphid species	Host plant		Aphid population (aphid/plant)
	Species	Family	
<i>P. nigronervosa</i>	Banana (<i>Musa</i> spp.)	Musaceae	0–614
	Heliconia (<i>Heliconia</i> spp.)	Heliconiaceae	0–17
	Banana traveler (<i>Ravenala madagascariensis</i>)	Strelitziaceae	0–54
<i>P. caladii</i>	Taro (<i>Colocasia esculenta</i>)	Araceae	0–61
	Dumbcane (<i>Dieffenbachia</i> sp.)	Araceae	0–78
	Turmeric (<i>Curcuma longa</i>)	Zingiberaceae	0–10
	Costus (<i>Costus</i> sp.)	Costaceae	0–12
	Banana (<i>Musa</i> spp.)	Musaceae	0–17
	Banana traveler (<i>Ravenala madagascariensis</i>)	Strelitziaceae	0–9

was rarely found on those alternative plants in the present survey. On the other hand, *P. caladii* was observed more on *Colocasia esculenta* (Araceae), *Dieffenbachia* sp. (Araceae), *Curcuma longa* (Zingiberaceae), *Costus* sp. (Costaceae), and rarely on banana and *R. madagascariensis* (Figure 4). Furthermore, the highest population of *P. caladii* was observed on *C. esculenta* and *Dieffenbachia* sp. Other host plants of *P. caladii* that was reported previously including *Alocasia* sp., *Caladium* sp., *Elettaria* sp., *Hedychium* sp., *Xanthosoma* sp., and *Zingiber* sp. (Nordam, 2004; Duay *et al.*, 2014; Miller *et al.*, 2014). This finding suggested that the host range of *P. caladii* is wider than those of *P. nigronervosa*.

Survey conducted across Java confirmed that banana was the main host plant of *P. nigronervosa* and alternative host plant of *P. caladii*. Out of 151 specimens collected from banana plants, 142 specimens

(94% of samples) were *P. nigronervosa* and 9 specimens (6% of samples) were *P. caladii*. The specimens of *P. caladii* were collected from cv. Raja in Pati, Central Java (seven specimens), cv. Barangan in Sukabumi, West Java and *Musa velutina* in Bogor, West Java (each one specimen). Although the occurrence of *P. caladii* is low in banana, its ability to transmit viruses should be considered. *P. caladii* was reported to transmit *Cardamom bushy dwarf virus* (CBDV), a new Babuvirus within the family Nanoviridae (Venugopal, 1995; Mandal *et al.*, 2004).

Understanding the host range of banana aphids is important to determine the potency of plant species, especially those adjacent to banana plants, as the reservoir of insect vector of BBTB. Banana aphids were typically found in large number only on suckers, rather than on mature plant parts (Young & Wright, 2005). Aphids prefer feeding on phloem and less commonly on xylem of herbaceous shrubs, trees,

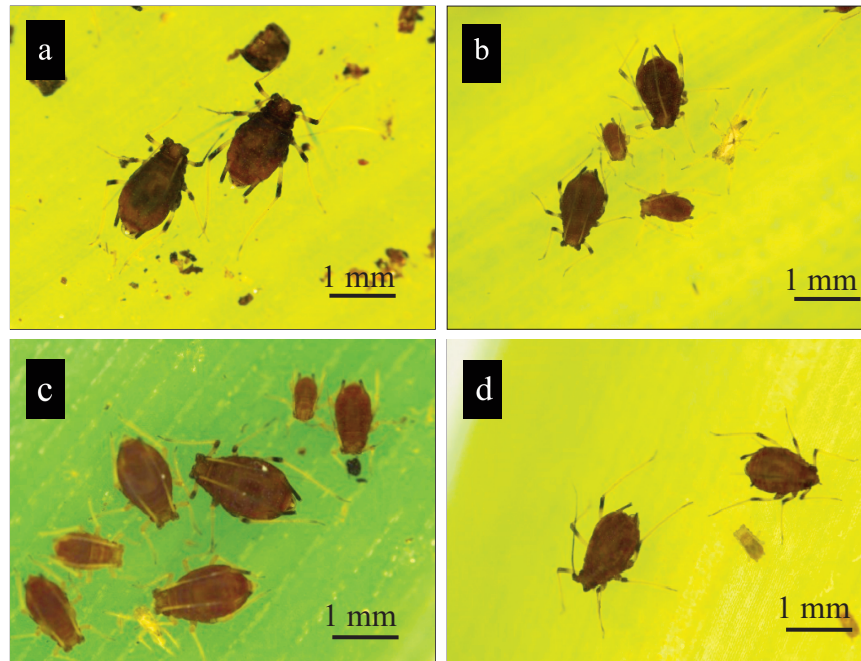


Figure 4. Cryptic species of “banana aphids” on different host plants: *Pentalonia nigronervosa* on *Musa* sp. (a), *Pentalonia nigronervosa* on *Ravenala madagascariensis* (b), *Pentalonia caladii* on *Dieffenbachia* sp. (c), *Pentalonia caladii* on *Colocasia esculenta* (d)

weeds and cultivated plants (Blackman & Eastop, 2000). On the large colonies, the aphids dispersed to the upper leaf, and winged adults (alatae) can also be found although in small number. The alatae play more important role in transmission and spread of BBTv due to its mobility.

CONCLUSION

Two species of aphids infesting banana in Java were identified, i.e. *P. nigronervosa* and *P. caladii*. The two species could be differentiated using morphometric characters and its host range. The host plants of *P. nigronervosa* were mostly Musaceae and rarely on Heliconiaceae and Strelitziaceae, while *P. caladii* were found on Araceae, Zingiberaceae, Costaceae, and rarely on Musaceae and Strelitziaceae. The alternative host plants will provide sustainable food for the aphids, therefore the existence of this alternative host plants must be considered seriously in the controlling strategy of the banana aphids and BBTv.

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