KARYOTYPES OF FRUIT BATS (CHIROPTERA, PTEROPODIDAE) AND RATS (RODENTIA, MURIDAE) FROM Mt. BAWAKARAENG, SOUTH SULAWESI, INDONESIA

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ABSTRACT. *This study aims to analyze karyotypes of Sulawesi's small mammals with Giemsa banding technique. Five fruit bats species (Boneia bidens, Dobsonia viridis, Styloctenium wallacei, Thoopterus nigrescens, Thoopterus suhaniahae) and eight rat species (Bunomys andrewsi, Bunomys chrysocomus, Bunomys heinrichi, Paruromys sp., Rattus dommermani, Rattus exulans, Rattus hoffmanni, Taeromys celebensis) from Mt. Bawakaraeng, South Sulawesi were analyzed. Karyotypes of three species in this study were described for the first time. There were two species of bats and one species of rats showed the best of karyotypes. B. bidens has 2n = 30, FN = 53, FNa = 50, sub metacentric and telocentric for X and Y chromosomes respectively. T. suhaniahae has 2n = 38, FN = 64, FNa = 60 and metacentric X chromosomes. R. hoffmanni has 2n = 44, FN = 61, FNa = 59 and telosentric X chromosomes.*

KEYWORD. Karyotypes, 2n, FN, Metacentric, Telocentric

INTRODUCTION

Rats and bats constitute aproximately 55% of 720 mammal species that have been distributed in Indonesia (LIPI - Bappenas - KLH 2014). Indonesian rats (Muridae) show highest endemism in Sulawesi among all islands. Over 75.7% of island species are endemic. Fruit bats also show high endemism in Sulawesi, of which 10.7% (28 species) is endemic to the island (Maryanto and Higashi, 2011). Moreover, new endemic species of rats and fruit bats are recently discovered in Sulawesi, such as *Margaretamys christinae* from Mekongga mountains (Mortelliti *et al.*, 2012); *Paucidentomys vermidax* from Latimojong mountains (Esselstyn *et al.*, 2012); *Rousettus linduensis* from Lore Lindu National Park (Maryanto and Yani, 2003) and *T. suhaniahae* that widely distributed in Sulawesi and adjacent islands (Maryanto *et al.*, 2012).

Chiroptera and rodentia have been estimated to have a high rate of karyotype evolution (Wilson *et al*.,1975). Karyotype is a complete description of the chromosomes on cells metaphase cells arranged properly (Eldridge *et al*., 1985). Baker and Bickham (1980) applied the G-banding and C-banding technique toward 78 bat species that represent four families (Mormoopidae, Noctilionidae, Phyllostomatidae, and Vespertilionidae) to explain the pattern of karyotype mega-evolution.

Karyotype analysis of rats (Rodentia, Muridae) and fruit bats (Chiroptera, Pteropodidae) in Southeast Asia has been performed on several species. Harada and Kobayashi (1980) conducted a study of *Mus musculus* (2n = 40, FN = 38), *Rattus rattus* chromosomes (2n = 42, FN = 58) (Rodentia, Muridae) and *Aethalops Alecto* (2n = 34), *Cynopterus bachyotis* (2n = 34), *Pteropus vampyrus* (2n = 38, FN = 62) (Chiroptera, Pteropodidae) in Sabah, Malaysia. Li *et al*. (2008) study three rat species (Muridae) from Hainan Island, China, including *Niviventer fulvescens*, *N. lotipes* and *Rattus nitidus*. The results showed *N. fulvescens* (2n = 46, FN = 64) has the same karyotype with *N. fulvescens* in Southeast Asia. While, *N. lotipes* (2n = 52, FN = 66) has distinct karyotypes of other *Niviventer* species. Hood *et al*. (1988) described karyotypes of *Cynopterus sphinx*, *Eonycteris spelaea*, *Macroglossus sobrinus*, *Megaerops niphanae*, *Pteropus lylei*, and *Rousettus amplexicaudatus* in Thailand. The results revealed that an undescribed diploid and fundamental number in *P. lylei* (2n = 26; FN = 72) which is new to the genus *Pteropus*. Futhermore, karyotype of *M. niphanae* (2n = 26; FN = 42) has different diploid number with *M. niphanae* from peninsular Malaysia (2n = 24) also to be identic to some population of closely related species *M. ecaudatus*, which indicates there are chromosome variation among *Megaerops* species because of Robertonian translocation.

Both study of chromosome and karyotype data for fruit bats (Chiroptera, Pteropodidae) and rats (Rodentia, Muridae) in Sulawesi are poorly known. Only several karyotype data of Indonesian fruits bats and rats that have been studied, including *Cynopterus brachyotis javanicus*, *Cynopterus sphinx titthaecheilus*, *Eonycteris spelaea spelaea*, *Macroglossus minimus minimus* from Java (Ando *et al*., 1980); *Bunomys chrysocomus*, *Maxomys hellwaldi*, *Rattus argentiventer*, *Rattus hoffmanni linduensis*, *Rattus marmosurus facetus*; *Margaretamys beccarii* and *Margaretamys elegans* from Sulawesi (Duncan, 1976; Musser, 1981). While, karyotype data of some other taxa such as *Boneia*, *Dobsonia*, *Nyctimene*, *Styloctenium*, *Thoopterus*, *Taeromys* or *Paruromys* genera has not been described. This study aims to provide karyotypes of fruit bats and rats in Sulawesi.

MATERIAL AND METHODS

Sample Collections

The field work was conducted at Mt. Bawakaraeng of South Sulawesi, Indonesia, from September to December 2013. The elevation of the study area is between 1453 to 2165 m asl. Four sites of different altitudes and habitats were set up. Habitats were divided into: (1)

mixed garden (1453 m asl.), (2) pine forest (1545 m asl.), (3) secondary to primary forest (transition forest) (1835 m asl.) and (4) primary forest (2165 m asl.) (Figure 1). Bats and rats were traped by mist nets (9x2.5 m and 12x3 m) and Kasmin cage traps (28x12x12 cm) respectively. Mist net were set up in bats flying coridor such as around fruit trees and river flows, two until three meters above the ground. Cage traps baited with roasted coconut. Traps were placed during four nights continously. All of living specimens were acclimated to cage for karyotype analysis in the field. All individuals were sacrificed as voucher specimens and keep in Museum Zoologicum Bogoriense (MZB).

Karyotyping

Karyotyping was made based on Baker *et al.,* (1982) and Dobigny and Xuereb (2011) with some modifications in incubation and yeast solution. Specimens were injected subcutaneously with 0.1 ml/ 10 g body weight yeast solution (3 g yeast : 2 g dextrose : 12 ml H_2O) for 12 to 24 hours before the next step. The specimens were intraperitoneally injected with mitotic inhibitors Colchicine 0.005% (0.1 ml/ 10 g body weight) for 2 to 3 hours before sacrificed. Bone marrow of humerus in fruit bats and femur in rat were treated to hipotonic KCl 0.075 M solution for 45 minutes at room temperature, fixed in Carnoy solution (3 methanol : 1 acetat acid glacial) for 15 minutes and air-drying. Performing centrifugation and resuspention were carried out in 1200 rpm for 10 minutes. Staining using 10% Giemsa solution for 30 minutes. Observation was done on the microscope with 400 to 1000 magnification. Metaphase images were captured by OptiLab Profesional Microscope camera (Micron Technology, Inc.).

Data Analysis

Chromosomes were arranged by the position of the centromere and chromosome size (Levan *et al*., 1964). Metaphases image were edited by Adobe Photoshop CS3 version 10.0 (Adobe System, Inc.) within 35% magnification. Long arm length (p), short arm length (q), total length and arm ratio (AR) of chromosome were measured using ImageJ version 1.46r software (Abramoff *et al.*, 2004; free software downloaded in http://rsbweb.nih.gov/ij/download.html). All measurements were conducted with five repeatations then averaged. Chromosomes type were classified automatically by ImageJ Levan Plugin (Sakamoto and Zacaro, 2009; free downloaded in http://rsbweb.nih.gov/ij/plugins/index.html). Diploid number (2n), centromer index (CI), fundamental number (FN) and fundamental number autosom (FNa) were calculated as well. CI is the precentage ratio of long arm and total arm length of the chromosome (Loganathan *et al.*, 2012). FN is the total number of chromosome arms either autosomal or sex chromosomes. Whereas, FNA is the total number of body chromosome arm (autosomes) without the sex chromosomes (Baker and Patton, 1967).

RESULT AND DISCUSSION

A total five species (15 individuals) of fruit bats and eight species (24 individuals) of rats were examined for karyotyping, which are mostly endemic species of Sulawesi including *Boneia bidens*, *Dobsonia viridis*, *Styloctenium wallacei*, *Thoopterus nigrescens*, *Thoopterus suhaniahae* (Pteropodidae) and *Bunomys andrewsi*, *Bunomys chrysocomus*, *Bunomys heinrichi*, *Paruromys* sp., *Rattus dommermani*, *Rattus exulans*, *Rattus hoffmanni*, *Taeromys celebensis* (Muridae).

Three species (two species of fruit bats and a species of rat) showed the best karyotype results, those are *B. bidens, T. Suhaniahae* and *R. hoffmanni.* Karyotype of *D. viridis* and *T. nigrescens* were not showed the good result; only diploid number (2n) that were observed. The chromosome of other species (both fruit bats and rats) were not show good results, because the chromosomes were being in the cell (Table 1).

The number and types of chromosomes differ among each species which showed the best karyotype. Specimens *B. bidens* and *T. suhaniahae* have four types of chromosomes i.e metacentric, sub metacentric, sub telocentric and telocentric. *R. hoffmani* have three types of chromosomes, that are sub metacentric, sub telocentric and telocentric. The number of sub metacentric tends to be more and most widely in *B. bidens*. There are more types of telocentric chromosomes in *R. hoffmani* compared to *B. bidens* (Figure 2).

***Boneia bidens* (Jentink, 1879).** Only one adult male (MZB37354) has shown the best karyotype and most of chromosome preparation were not demonstrated in a good condition. The chromosome number *B. bidens* is 2n = 30, consisting of 19 pairs of somatic chromosomes (autosomes) and a pair of sex chromosomes. FNA = 53 and FN = 50 (Figure 3). This species has three types of chromosomes, i.e four pairs of metacentric (M; p = 2.43 to 1.15; q = 2.74 to 1.34; pq = 5.17 to 2.49; CI = 47.9 to 45.23%; AR = 1.22 to 1.10); seven pairs of sub metacentric (SM; p = 1.90 to 0.82; q = 3.83 to 1.39; pq = 5.73 to 2.21; CI = 39.36 to 29.05%; AR = 2.41 to 1.60) and three pairs of telocentric (T; p = 0; q and pq = 3.85 to 1.67; CI = 0; AR = α); The metacentric X chromosome (p = 0.94; q = 2.57; pq = 3.51; CI = 26.77%; AR = 2.74) and the telocentric Y chromosome (q and pq = 1.58 respectively) (Table 2). There is one additional chromosome between the two normal chromosomes (Figure 3, arrowed). The relative length of the short arm (p), long arm (q), total length (pq) and arm ratio (AR) chromosome tend to be stable to the whole of chromosome pairs (both the autosom and sex chromosomes).

Thoopterus suhaniahae (Maryanto *et al.*, 2012). An adult female (MZB 37355) showed the best karyotype. The chromosome number of this species is 2n = 38, FN = 64 and FNa = 60 (Figure 5). This species has 18 autosom and a pair of sex chromosome i.e two pairs of metacentric (p = 1.30 to 1.24; q = 2.18 to1.56; pq = 3.48 to 2.80; CI = 44.25 to 37.41%; AR = 1.34 to 1.70); six pairs of sub metacentric (p = 1.04 to 0.45; q = 2.14 to 1.13; pq = 3.18 to 1.58; CI = 34.45 to 25.84%; AR = 2.93 to 1.98); four pairs of sub telocentric (p = 0.10 to 0.38; q =

3.91 to 1.49; pq = 4.91 to 1.87; CI = 24.60 to 18.06%; AR = 4.56 to 3.13); six pairs of telocentric (q and pq 2.630 to1.156%, respectively) and metacentric for both the X chromosome (p = 1.67 and 1.59; q = 2.38 and 2.00. CI = 41.29% and 44.23%; AR = 1.43 and 1.27, respectively) (Table 3). The relative lengths of the short arm (p), long arm length (q), total (pq) and arm ratio (AR) tend to be stable on the whole chromosome pairs.

***Thoopterus nigrescens* (Gray, 1870).** *T. nigrescens* chromosome number is 2n = 38; the same number of chromosomes with *T. suhaniahae*. Data of FN, FNa and the chromosome type were not available due the image of metaphase were hardly described.

***Dobsonia viridis* (Heude, 1897).** Chromosome number of *D. viridis* is 2n = 36. Data of FN, FNa and chromosome type were not determined because the image of metaphase were not describable.

***Rattus hoffmani* (Matschie, 1901).** Chromosome number of *R. hoffmani* is 2n = 44. FN = 61 and Fna = 59 (Figure 7). This species has four pairs of sub metacentric (p = 1.01 to 0.65; q = 2.54 to 1.25; pq = 3.55 to 1.90; CI = 35.33 to 26.97%; AR = 2.74 to 1.85), three pairs of sub telocentric (p = 0.72 to 0.48; q = 2.20 to 1.61; pq = 2.92 to 2.09; CI = 24.71 to 23.02%; AR = 3.35 to 3.06) and 12 pairs of telocentric (q and pq = 2.73 to 1.21, respectively). Both X chromosomes have telocentric (both q and pq = 2.33 to 2.36) (Table 4). Only one adult female (MZB 37043) that showed the best karyotype. The relative length of short arm (p), long arm (q), total length (pq) and arm ratio (AR) chromosome were stable.

DISCUSSION

Genus *Rousettus* has been resemble to *B. bidens* (Bergmans and Rozendaal 1988). In this study, we found a lower diploid number of *B. bidens* (2n = 30) comparing to several *Rousettus* species from Africa and India (2n = 36) (O'brien *et al.,* 2006). The difference between the diploid numbers of *B. bidens* and *Rousettus* suggested that at least three more pairs of chromosomes lost in this species. In contrast, the chromosome types of *B. bidens* showed the same form as both the autosomes and sex chromosomes of *Rousettus*. The form of sex chromosome XY on *B. bidens* has similar sex chromosomes XY on *R. lanosus*. *B. bidens* additional chromosome is suspected to be supernumery chromosomes as found in *Rattus rattus* (Harada and Kobayashi, 1980). This additional chromosome was also reported from another fruit bat species *Dobsonia praedatrix* (Haiduk, 1983). It is probably due to chromosome aberration in the form of chromatid breaks that has separate fragments from the unknown origin (Bakare *et al.,* 2011).

T. suhaniahae is sympatric with *T. nigrescens* that widely distributed in Sulawesi, Talaud and Wowoni islands (Maryanto *et al.,* 2012). Karyotypes showed no variation in diploid number between *T. suhaniahae* and *T. nigrescens*. The consistence of diploid number between species in a genus were also found in *Rousettus*

(*R. aegyptiacus*, *R. leschenaulti*, *R. lanosus*, *R. angolensis*; 2n = 36) and *Pteropus* (*P. rodricensis*, *P.giganteus*, *P.vampyrus*; 2n = 38) (O'brien *et al.*, 2006; Harada and Kobayashi, 1980). Diploid number and FN were remarkably constant in Pteropodidae family (Hood *et al*., 1988). We are not able to compare chromosome morphology between *T. suhaniahae* and *T. nigrescens* since the chromosome preparation for *T. nigrescens* was less optimal.

Thoopterus are closely related to *Cynopterus* (Andersen, 1912). Some karyotype data of *Cynopterus* was recorded. Diploid number of *C. brachyotis* from Sabah is 2n = 34 which consist of 12 pairs of metasentric and sub metacentric, two pairs of sub telocentric and three pairs of acrocentric. In addition, the sex chromosomes and FN were not determined (Harada and Kobayashi, 1980). Moreover, both karyotypes of *Cynopterus brachyotis javanicus* and *Cynopterus sphinx titthaecheilus* from Java were identic in diploid number, FN and chromosome types (2n = 34, FN = 58, M-SM = 11); which differ in one pair M-SM in *C. brachyotis* from Sabah (Ando *et al*., 1980). Based on our study, *T. suhaniahae* and *T. nigrescens* lose at least two more chromosome pairs than *Cynopterus*. Metacentric and sub metacentric number in *Cynopterus* tends to be many than *Thoopterus* which have many sub metacentric and telocentric. The consistence of diploid number in *Thoopterus* also revealed in *Cynopterus*; showing no variation.

D. viridis is one of *Dobsonia* species that has been distributed in Sulawesi and Mollucas (Corbet and Hill, 1992). The chromosome of some *Dobsonia* species are already studied, those are *D. praedatrix* and *D. molluccensis* from New Guinea (2n = 38, FN = 66 and 2n = 38, FN = 68, respectively) with the chromosome X identical to *Rousettus* (Haiduk, 1983). The chromosomes type of *D. praedatrix* are mostly metacentric and sub metacenric. Based on the research results of *D. viridis* chromosome number (2n = 36) showed *D. viridis* has lost one pair of chromosomes of *D. praedatrix* and *D. molluccensis*. The chromosome types of *D. praedatrix* are mostly metacentric and sub-metacentric (Haiduk, 1983); whereas the chromosome type of *D. viridis* was not determined in this study.

Most Pteropodidae showed diploid numbers trend of 34, 36 and 38 (Haiduk *et al*., 1980) and some species were out of the trend such as *M. niphanae* and *Balionycteris* that has diploid number ranged from 24 to 28 (Hood *et al*., 1988). Based on our study, *B. bidens* has departed to this trend. While *T. suhaniahae*, *T. nigriscens* and *D. viridis* are included in this trend.

R. hoffmanni is one of endemic *Rattus* in Sulawesi. The five subspecies are *R.h linduensis*, *subditivus*, *mengkoka*, *palelae* and *mollicomus* (Laurie and Hill, 1954). In this study, it was difficult to describe sex chromosomes in *R. hoffmanni*. We suspected the pairs of chromosome number 13 are the X chromosomes seen from the chromosome shape and type. The chromosome types of *Rattus* and other rats tend to have many telocentric and X chromosomes has great telocentric (O'Brien *et al*., 2006). *R. hoffmanni* has more telocentric of 13 pairs.

Opposite to chromosome subspecies *R.h linduensis* (2n = 42, FN = 58), *R. hoffmanni* in this study has addition of single pair chromosome. We suspected that *R. hoffmanni* chromosome occur Robertsonian translocation (fission or fusion event) to subspecies *R.h linduensis*. This event also occur among *M. niphanae* species from Thailand and peninsular Malaysia (Hood *et al*., 1988; Yong, 1984; Harada *et al*., 1982). Referring to Atlas of Mammalian Chromosomes, some species of genus *Rattus* have 2n ranged from 38 to 46 (O'Brien *et al.,* 2006; Li *et al.,* 2008). If the *R. hoffmanni* chromosome were compared with *R. Rattus* and *R. norvegicus*, would occur addition of each chromosome 6 and 2 chromosomes.

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Table 1: Chromosome status of four fruit bat species and one rat species from Mt. Bawakaraeng, South Sulawesi

Species	Number of specimens studied		Locality	2n	FN	FNa	X	X	Y
	Male	Female							
Pteropodidae									
B. bidens	3	-	(1)	30	53	50	SM	-	T
T. suhaniahae	6	2	(1), (2)	38	64	60	M	M	-
T. nigrescens	-	1	(1)	38	-	-	-	-	-
D. viridis	1	1	(1)	36	-	-	-	-	-
Muridae									
R. hoffmanni	5	4	(1), (2)	44	61	59	T	T	-

2n : Diploid cell; Fna: Fundamental number autosom; FN: Fundamental number; X : Chromosome X; Y: Chromosome Y; M : Metacentric; SM: Sub Metacentric; ST: Sub Telocentric; T: Telocentric.

Table 2: Morphometric of male *B. bidens* (MZB37354) chromosomes

Chromosome Pair		Relative Length						Total	CI (%)	AR	Morphology
		p (Mean±SD)	Min	Max	q (Mean±SD)	Min	Max				
1	a	2.43±0.28	2.13	2.77	2.74±0.13	2.62	2.92	5.17	46.93	1.14	M
	b	2.26±0.11	2.06	2.33	2.48±0.15	2.23	2.64	4.73	47.66	1.10	M
2	a	2.07±0.13	1.93	2.20	2.44±0.05	2.38	2.49	4.51	45.85	1.18	M
	b	2.16±0.22	1.80	2.38	2.34±0.12	2.24	2.55	4.50	47.93	1.09	M
3	a	1.57±0.07	1.48	1.66	1.82±0.17	1.64	2.04	3.40	46.32	1.16	M
	b	1.43±0.12	1.27	1.59	1.73±0.18	1.49	1.98	3.17	45.23	1.22	M
4	a	1.35±0.10	1.24	1.50	1.54±0.09	1.46	1.66	2.90	46.75	1.14	M
	b	1.15±0.13	1.05	1.36	1.34±0.08	1.2	1.41	2.49	46.23	1.17	M
5	a	1.90±0.24	1.68	2.29	3.83±0.28	3.59	4.28	5.73	33.11	2.05	SM
	b	1.53±0.12	1.39	1.68	3.68±0.14	3.53	3.84	5.21	29.41	2.41	SM
6	a	1.59±0.22	1.30	1.92	3.13±0.07	3.01	3.20	4.71	33.70	2.00	SM
	b	1.76±0.07	1.67	1.84	2.80±0.27	2.52	3.21	4.56	38.62	1.60	M/ SM
7	a	1.22±0.17	0.98	1.42	2.12±0.20	1.84	2.36	3.33	36.47	1.78	SM
	b	1.17±0.10	1.05	1.26	2.01±0.37	1.51	2.50	3.17	36.74	1.73	SM
8	a	1.11±0.10	1.02	1.28	1.94±0.13	1.74	2.08	3.05	36.41	1.76	SM
	b	1.16±0.12	0.96	1.24	1.78±0.16	1.6	1.98	2.94	39.36	1.56	M/ SM
9	a	1.04±0.17	0.86	1.31	1.74±0.17	1.58	1.96	2.78	37.41	1.70	M/SM
	b	0.95±0.12	0.88	1.16	1.79±0.22	1.59	2.12	2.74	34.80	1.88	SM
10	a	0.87±0.09	0.74	0.98	1.80±0.09	1.65	1.90	2.67	32.56	2.09	SM
	b	0.87±0.16	0.68	1.11	1.68±0.03	1.65	1.71	2.55	34.14	1.98	SM
11	a	0.85±0.08	0.73	0.94	1.60±0.05	1.53	1.68	2.45	34.72	1.89	SM
	b	0.82±0.16	0.65	1.08	1.39±0.17	1.14	1.56	2.21	37.07	1.76	SM
12	a	0.00	0.00	0.00	3.85±0.12	3.74	4.04	3.85	0.00	α	T
	b	0.00	0.00	0.00	3.28±0.12	3.17	3.43	3.28	0.00	α	T
13	a	0.00	0.00	0.00	2.54±0.15	2.37	2.78	2.54	0.00	α	T
	b	0.00	0.00	0.00	2.74±0.22	2.56	3.02	2.74	0.00	α	T
14	a	0.00	0.00	0.00	1.86±0.09	1.71	1.93	1.86	0.00	α	T
	b	0.00	0.00	0.00	1.67±0.05	1.63	1.76	1.67	0.00	α	T
X		0.94±0.08	0.84	1.00	2.57±0.10	2.45	2.69	3.51	26.77	2.74	SM
Y		0.00	0.00	0.00	1.58±0.17	1.34	1.80	1.58	0.00	α	T

p: short arm length; q: long arm length; CI: centromer index; AR: arm ratio; a,b : chromosome pair; M : metacentric; SM: sub metacentric; ST: sub telocentric ;T: telocentric

Table 3: Morphometric of female *T. suhaniahae* (MZB 37355) chromosomes

Chromosome Pair		Relative Length						Total	CI (%)	AR	Morphology
		p (Mean±SD)	Min	Max	q (Mean±SD)	Min	Max				
1	a	1.30±0.17	1.03	1.49	2.18±0.16	1.98	2.38	3.48	37.42	1.70	M
	b	1.28±0.12	1.15	1.43	2.02±0.18	1.81	2.23	3.30	38.80	1.57	M
2	a	1.27±0.08	1.19	1.40	1.70±0.215	1.38	1.92	2.97	42.82	1.34	M
	b	1.24±0.09	1.13	1.33	1.56±0.11	1.42	1.68	2.80	44.25	1.27	M
3	a	1.04±0.15	0.80	1.16	2.14±0.07	2.05	2.21	3.18	32.58	2.10	SM
	b	0.88±0.15	0.71	1.04	2.16±0.10	2.02	2.28	3.04	28.97	2.52	SM
4	a	0.86±0.05	0.78	0.91	2.12±0.15	1.91	2.25	2.98	28.77	2.48	SM
	b	0.82±0.08	0.69	0.88	2.10±0.09	1.99	2.20	2.91	28.04	2.59	SM
5	a	0.86±0.18	0.62	1.07	1.88±0.30	1.51	2.24	2.75	31.47	2.31	SM
	b	0.70±0.08	0.61	0.83	2.02±0.06	1.96	2.11	2.71	25.65	2.92	SM
6	a	0.81±0.14	0.67	1.00	1.72±0.13	1.60	1.87	2.54	31.94	2.18	SM
	b	0.68±0.08	0.59	0.78	1.49±0.09	1.38	1.59	2.17	31.37	2.20	SM
7	a	0.68±0.10	0.60	0.85	1.46±0.10	1.29	1.55	2.14	31.84	2.18	SM
	b	0.66±0.12	0.56	0.83	1.26±0.19	0.94	1.40	1.92	34.45	1.98	SM
8	a	0.49±0.06	0.41	0.56	1.41±0.09	1.34	1.55	1.90	25.84	2.93	SM
	b	0.45±0.05	0.39	0.50	1.13±0.01	1.12	1.15	1.58	28.73	2.53	SM
9	a	1.00±0.11	0.88	1.17	3.91±0.15	3.78	4.08	4.91	20.30	3.97	ST
	b	0.73±0.05	0.67	0.78	3.29±0.16	3.13	3.51	4.02	18.06	4.56	ST
10	a	0.93±0.12	0.74	1.03	2.84±0.19	2.62	3.00	3.77	24.60	3.13	ST
	b	0.74±0.08	0.62	0.83	2.78±0.13	2.65	2.97	3.52	21.02	3.80	ST
11	a	0.82±0.16	0.58	1.02	2.52±0.11	2.40	2.61	3.34	24.43	3.20	ST
	b	0.62±0.09	0.49	0.73	2.23±0.20	1.95	2.44	2.85	21.72	3.69	ST
12	a	0.61±0.06	0.54	0.68	2.10±0.08	1.97	2.19	2.71	22.47	3.47	ST
	b	0.38±0.06	0.33	0.48	1.49±0.05	1.44	1.55	1.87	20.49	3.94	ST
13	a	0.00	0.00	0.00	2.63±0.06	2.55	2.71	2.63	0.00	α	T
	b	0.00	0.00	0.00	2.58±0.13	2.43	2.78	2.58	0.00	α	T
14	a	0.00	0.00	0.00	2.37±0.05	2.32	2.43	2.37	0.00	α	T
	b	0.00	0.00	0.00	2.26±0.07	2.17	2.37	2.26	0.00	α	T
15	a	0.00	0.00	0.00	2.15±0.15	1.95	2.38	2.15	0.00	α	T
	b	0.00	0.00	0.00	2.13±0.12	2.02	2.29	2.13	0.00	α	T
16	a	0.00	0.00	0.00	1.10±0.11	1.88	2.16	1.10	0.00	α	T
	b	0.00	0.00	0.00	1.56±0.03	1.53	1.60	1.56	0.00	α	T
17	a	0.00	0.00	0.00	1.44±0.10	1.36	1.60	1.44	0.00	α	T
	b	0.00	0.00	0.00	1.42±0.09	1.34	1.55	1.42	0.00	α	T
18	a	0.00	0.00	0.00	1.30±0.06	1.24	1.38	1.30	0.00	α	T
	b	0.00	0.00	0.00	1.17±0.14	0.95	1.34	1.16	0.00	α	T
X		1.67±0.12	1.55	1.84	2.38±0.12	2.17	2.46	4.05	41.29	1.43	M
X		1.59±0.09	1.46	1.67	2.00±0.16	1.84	2.24	3.59	44.23	1.27	M

p: short arm length; q: long arm length; CI: centromer index; AR: arm ratio; a,b : chromosome pair; M : metacentric; SM: sub metacentric; ST: sub telocentric ;T: telocentric

Table 4: Morphometric of female *R. hoffmanni* (MZB 37043) chromosomes

Chromosome Pair		Relative Length						Total	CI (%)	AR	Morphology
		p (Mean±SD)	Min	Max	q (Mean±SD)	Min	Max				
1	a	1.01±0.07	0.91	1.08	2.54±0.16	2.27	2.68	3.55	28.36	2.54	SM
	b	1.09±0.07	0.98	1.17	2.17±0.08	2.04	2.25	3.26	33.42	2.00	SM
2	a	1.13±0.06	1.07	1.21	2.09±0.09	2.00	2.22	3.22	34.99	1.86	SM
	b	1.01±0.08	0.92	1.09	2.08±0.09	1.95	2.18	3.10	32.75	2.06	SM
3	a	0.95±0.09	0.80	1.03	2.06±0.12	1.94	2.22	3.02	31.43	2.19	SM
	b	0.81±0.13	0.68	0.99	2.11±0.09	2.01	2.24	2.92	27.71	2.66	SM
4	a	0.75±0.04	0.69	0.77	1.93±0.13	1.72	2.09	2.68	27.96	2.57	SM
	b	0.88±0.11	0.72	1.00	1.62±0.13	1.49	1.79	2.50	35.33	1.85	SM
5	a	0.68±0.13	0.56	0.87	1.78±0.16	1.66	2.04	2.46	27.81	2.65	SM
	b	0.80±0.06	0.77	0.90	1.64±0.12	1.53	1.82	2.44	32.82	2.05	SM
6	a	0.55±0.06	0.49	0.62	1.49±0.16	1.31	1.64	2.03	26.97	2.74	SM
	b	0.65±0.16	0.50	0.92	1.25±0.19	0.95	1.47	1.90	34.25	2.05	SM
7	a	0.72±0.05	0.67	0.79	2.20±0.09	2.09	2.28	2.92	24.71	3.06	ST
	b	0.57±0.08	0.51	0.68	1.85±0.11	1.71	1.95	2.42	23.64	3.28	ST
8	a	0.54±0.13	0.43	0.69	1.68±0.05	1.62	1.75	2.22	24.46	3.20	ST
	b	0.51±0.07	0.41	0.61	1.64±0.16	1.45	1.83	2.15	23.79	3.22	ST
9	a	0.48±0.02	0.44	0.50	1.61±0.11	1.44	1.72	2.09	23.02	3.35	ST
	b	0.00	0.00	0.00	2.73±0.13	2.64	2.96	2.73	0.00	∝	T
10	a	0.00	0.00	0.00	2.60±0.13	2.42	2.78	2.60	0.00	∝	T
	b	0.00	0.00	0.00	2.57±0.07	2.48	2.67	2.57	0.00	∝	T
11	a	0.00	0.00	0.00	2.56±0.10	2.46	2.70	2.56	0.00	∝	T
	b	0.00	0.00	0.00	2.50±0.08	2.41	2.61	2.50	0.00	∝	T
12	a	0.00	0.00	0.00	2.43±0.15	2.29	2.68	2.43	0.00	∝	T
	b	0.00	0.00	0.00	2.38±0.08	2.25	2.45	2.38	0.00	∝	T
13	a	0.00	0.00	0.00	2.36±0.13	2.17	2.47	2.36	0.00	∝	T
	b	0.00	0.00	0.00	2.33±0.08	2.25	2.45	2.33	0.00	∝	T
14	a	0.00	0.00	0.00	2.16±0.05	2.11	2.22	2.16	0.00	∝	T
	b	0.00	0.00	0.00	2.08±0.04	2.05	2.14	2.08	0.00	∝	T
15	a	0.00	0.00	0.00	2.04±0.11	1.91	2.21	2.04	0.00	∝	T
	b	0.00	0.00	0.00	2.04±0.05	1.98	2.11	2.04	0.00	∝	T
16	a	0.00	0.00	0.00	2.01±0.08	1.91	2.11	2.01	0.00	∝	T
	b	0.00	0.00	0.00	1.98±0.07	1.90	2.06	1.98	0.00	∝	T
17	a	0.00	0.00	0.00	1.95±0.09	1.85	2.04	1.95	0.00	∝	T
	b	0.00	0.00	0.00	1.94±0.07	1.83	2.03	1.94	0.00	∝	T
18	a	0.00	0.00	0.00	1.89±0.09	1.78	1.98	1.89	0.00	∝	T
	b	0.00	0.00	0.00	1.84±0.08	1.72	1.93	1.84	0.00	∝	T
19	a	0.00	0.00	0.00	1.84±0.13	1.66	1.98	1.84	0.00	∝	T
	b	0.00	0.00	0.00	1.84±0.06	1.74	1.91	1.84	0.00	∝	T
20	a	0.00	0.00	0.00	1.67±0.05	1.61	1.73	1.67	0.00	∝	T
	b	0.00	0.00	0.00	1.62±0.09	1.53	1.74	1.62	0.00	∝	T
21	a	0.00	0.00	0.00	1.59±0.07	1.50	1.67	1.59	0.00	∝	T
	b	0.00	0.00	0.00	1.58±0.07	1.50	1.68	1.58	0.00	∝	T
22	a	0.00	0.00	0.00	1.31±0.05	1.23	1.38	1.31	0.00	∝	T
	b	0.00	0.00	0.00	1.21±0.12	1.07	1.35	1.21	0.00	∝	T

p: short arm length; q: long arm length; CI: centromer index; AR: arm ratio; a,b : chromosome pair; M : metacentric; SM: sub metacentric; ST: sub telocentric ;T: telocentric.

FIGURES

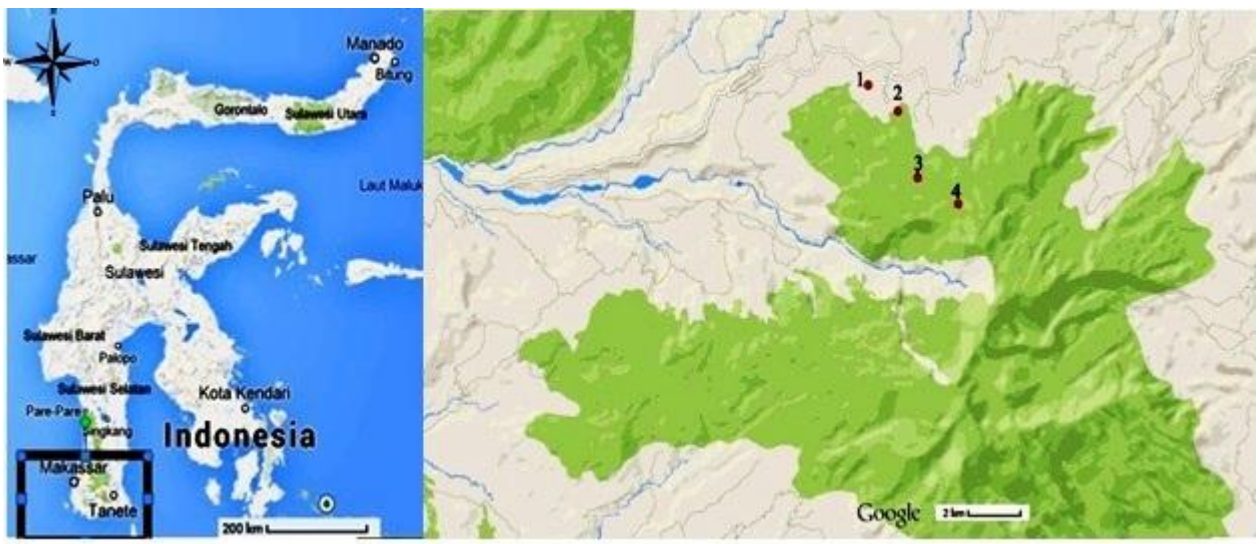

Figure 1: Collecting specimens sites. (**1**) Mixed garden (1453m asl; (S05°15′029″ E119°53′56.0″); (**2**) Pine forest (1545m asl; S05°15′29.3″ E119°54′31.8″); (**3**) Secondary to primary forest (transition forest) (1835m asl; S05°16′42.5″ E119°54′58.5″); (**4**) Primary forest (2165m asl; S05°17′11.4″ E119°55′47.6″) (Source: GoogleMap 2014)

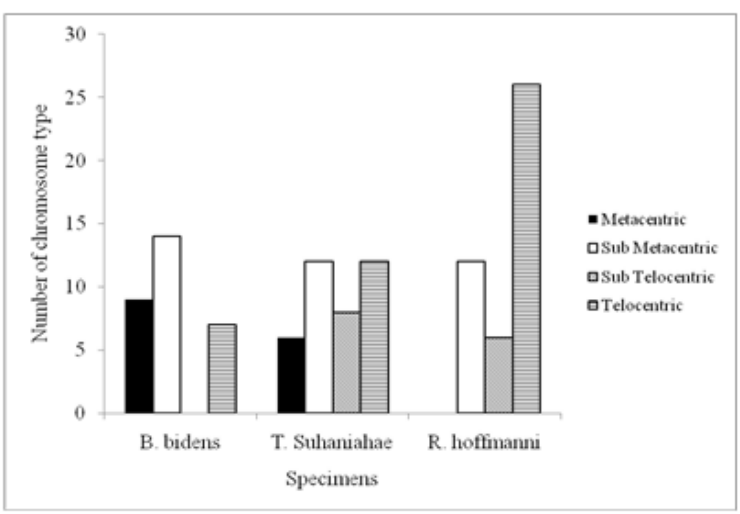

Figure 2: Number of chromosome types of *B. bidens*, *T. suhaniahae* and *R. Hoffmanni*

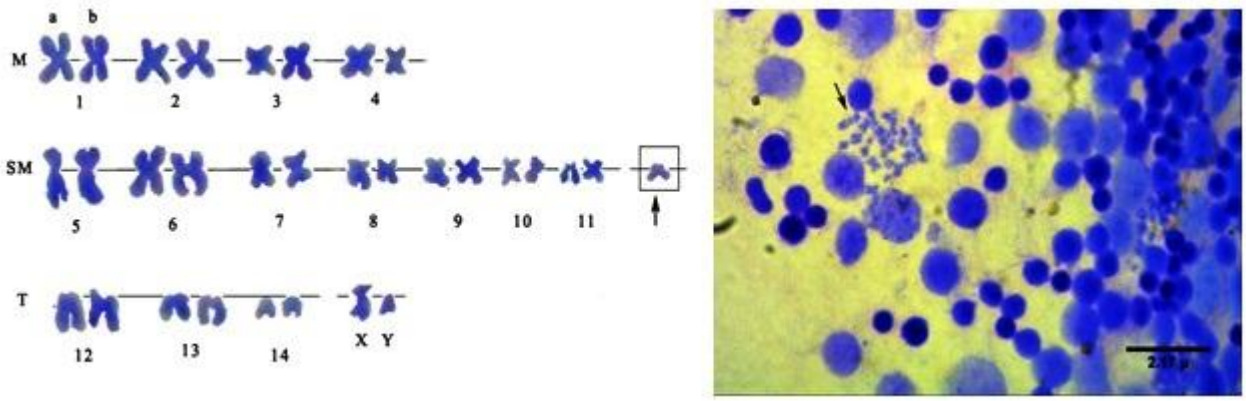

Figure 3: Karyotype of male *B. bidens* (MZB 3734), magnification-1000x, scala bar 2.17 µm. Arrow showed additional chromosome

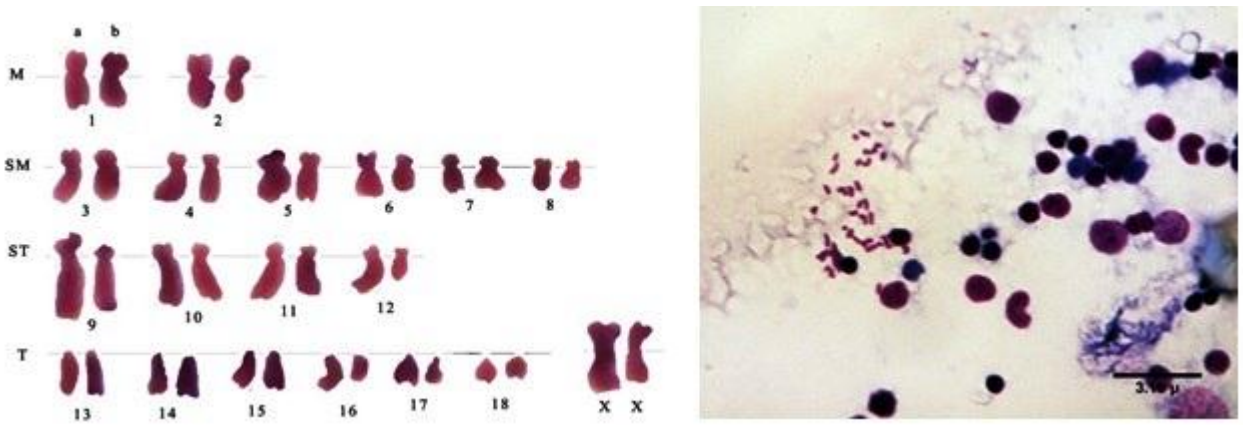

Figure 4: Karyotype of female *T. suhaniahae* (MZB 3735) magnification-1000x, scala bar 3.16 µm

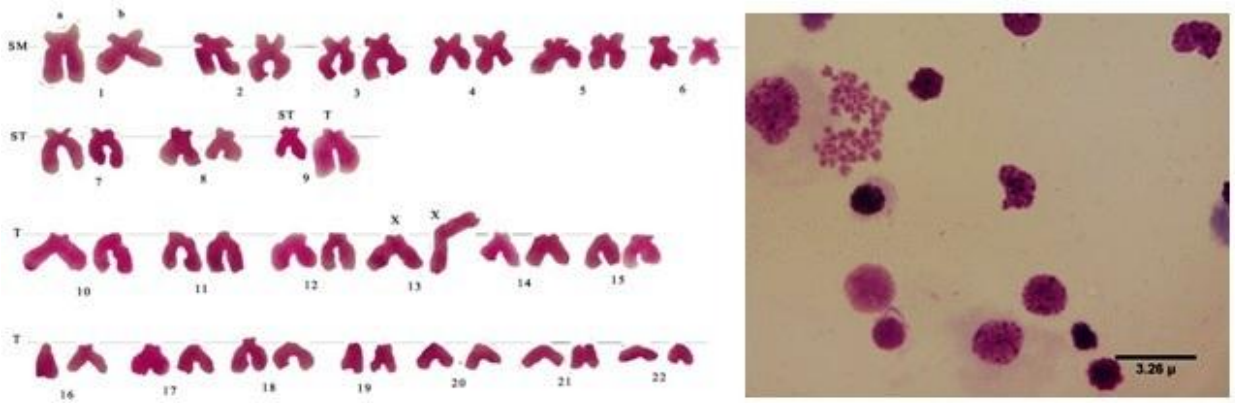

Figure 5: Karyotype of female *R. hoffmanni* (MZB 37043) magnification-1000x, scala bar 3.26 µm