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Genetic variations of *Lansium domesticum* Corr. accessions from Java, Sumatra and Ceram based on Random Amplified Polymorphic DNA fingerprints

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ABSTRACT

Yulita KS (2011) Genetic variations of Lansium domesticum *Corr. accessions from Java, Bengkulu and Ceram based on Random Amplified Polymorphic DNA fingerprints. Biodiversitas 12: 125-130.* Duku (*Lansium domesticum* Corr.) is one of popular tropical fruits in SE Asia. The species has three varieties, known as duku, langsat and kokosan; and duku is the most popular one for being the sweeties fruit. Indonesia has several local varieties of duku, such as duku Condet, duku Sumber and duku Palembang. This present study aimed to assess genetic diversity of 47 accessions of duku from Java, Sumatra, and Ceram based on RAPD fingerprints. Ten RAPD's primers were initially screened and five were selected for the analysis. These five primers (OPA 7, 13, 18, OPB 7, and OPN 12) generated 53 scorable bands with an average of 10.6 polymorphic fragments per primer. Percentage of polymorphism ranged from 16.89% (OPA 7 and OPN 12) to 24.54% (OPB 7) with an average of 20.16% polymorphism. OPB 7 at 450 bp was exclusively possessed by accession 20 (Java), OPA 18 at 500 bp was by accession 6 (Java), 550 bp by 3 clones from Bengkulu. While OPN 12 at 300 bp and OPA 13 at 450 bp were shared among the accessions. Clustering analysis was performed based on RAPD profiles using the UPGMA method. The range of genetic similarity value among accessions was 0.02-0.65 suggesting high variation of gene pool existed among accessions.

Key words: *Lansium domesticum,* duku, RAPD, genetic variation.

INTRODUCTION

Duku (*Lansium domesticum* Corr.) belongs to the family of Meliaceae is one of the popular fruits in Indonesia. The main distribution of this species is in Southeast Asia, but the species has also found in Suriname, Puerto Rico and Australia (Othman and Suranant 1995). The species is closely related to *L. membranaceum* Kosterm. (Mabb.) and *L. breviracemosum* Kosterm. (Mabberley et al. 1995). Recent taxonomic treatment of Meliaceae (Mabberley et al. 1995) did not assign infra-specific rank for *L. domesticum*. Nevertheless, there are three varieties of *L. domesticum* that have been widely known namely duku, langsat and kokosan. Hence, for practical purpose, Mabberley et al (1995) suggested to write *L. domesticum* cv kokosan or *L. domesticum* 'kokosan' when referring to kokosan variety. These three varieties are widely known to the local fruit market and can be distinguished mainly on the basis of their fruit morphology. Among of these varieties, duku is the most preferable fruit since it has no latex, few seeds, sweet berry and pleasant aroma. Duku has also medicinal and cosmetic properties from its peel extract (Tilaar et al. 2008)

Some of widely known local cultivars of duku include duku Condet from Jakarta, duku Papongan from Tegal, duku Kalijajar from Purbalingga, duku Karangkajen and duku Klaten from Yogyakarta, duku Woro from Rembang, duku Sumber from Kudus, duku Palembang, duku Padang Batung from South Kalimantan. However, the most famous local cultivar is duku Palembang. This present study has collected 47 accessions of duku from Bengkulu (Sumatra), Kudus (Central Java), Germplasm Garden of Cibinong Science Centre (*Kebun Plasma Nutfah* Cibinong Science Centre; KPN-CSC) and Bogor Botanic Garden (*Kebun Raya Bogor*; KRB) with aimed to evaluate genetic variability among the accessions using Random Amplified Polymorphic DNA (RAPD).

RAPD is a molecular marker that has been widely used for genotyping plant species (Jimenez et al. 2002; Chakrabarti et al. 2006; Dnyaneshwar et al. 2006; Keller-Przyby³kowicz et al. 2006), evaluation of genetic relationship (Upadhyay et al. 2004; Goh et al. 2005) and variation (Martin et al. 2002; Ferriol et al. 2003; Fan et al. 2004; Adetula 2006; Guo et al. 2007; Jain et al. 2007). It is random fragment amplification technique, which based on amplification of DNA fragment randomly using single arbitrary primer. The main advantages of this marker include rapid and cost-efficient in terms of operational aspects.

MATERIALS AND METHODS

Sample

Forty-seven accessions consist of 43 *L. domesticum* 'duku', three *L. domesticum* 'kokosan' and one *Lansium*

sp. that were collected from KRB (10 accessions), KPN-CSC (9 accessions), Central Java (14 accessions) and Bengkulu (14 accessions) were being used in this study (Table 1). The biogeographical coverage of these collections include Java, namely Kudus in Central Java and Jakarta (26 accessions), Sumatra i.e. Bengkulu and Palembang (17 accessions), Ceram (1 accession) and unidentified locations within Malesian region (3 accessions). Samples were collected as dried leaves stored in silica gel.

Extraction of total DNA genome

Total DNA genome was extracted from the dried leaves using modified CTAB (Doyle and Doyle 1990) by addition of RNAse 200 µg/mL. The total DNA genome was electrophorezed on 0.7% agarose gel in 1X TAE buffer at 100 volt for 30 min, followed by ethidium bromide staining before photographed using gel documentation system (Atto Bioinstrument).

Tabel 1. List of samples used in the study.

Accession no	Vernacular/species name	Source of materials
KD 1	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 2	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 3	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 4	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 5	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 6	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 7	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 8	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 9	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 10	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 11	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 12	Duku Sumber	Sumber Hamlet, Hadipolo Village, Jekulo Sub-district, Kudus District, Central Java
KD 13	Duku Sumber	Golan Tepus Village, Mejobo Sub-district, Kudus District, Central Java
KD 14	Duku Sumber	Golan Tepus Village, Mejobo Sub-district, Kudus District, Central Java
CD 1	*L. domesticum*	XIX.F.124, KRB
CD 2	*L. domesticum*	III.C.35, KRB (origin: Malesia)
CD 3	*L. domesticum*	III.C.59, KRB
CD 4	*L. domesticum*	III.B.100A, KRB
CD 5	*L. domesticum*	III.B.100, KRB (origin: Malesia)
CD 6	*L. domesticum*	III.B.6, KRB (origin: Java)
CD 7	*Lansium* sp.	III.C.106, KRB (origin: Ceram, Maluku)
CD 8	*L. domesticum*	III.B.142, KRB
CD 9	*L. domesticum*	XI.B.VII.215, KRB (origin: Malesia)
CD 10	*L. domesticum*	XII.B.VIII.46, KRB
CD 11	Duku lokal	KPN-CSC, tag no. 1, I.B (origin: Jakarta)
CD 12	Duku kokosan	KPN-CSC, tag no. 2, I.B (origin: Jakarta)
CD 13	Duku kokosan	KPN-CSC, tag no. 3, I.B (origin: Jakarta)
CD 14	Duku lokal	KPN-CSC, tag no. 4, I.B (origin: Jakarta)
CD 15	Duku lokal	KPN-CSC, tag no. 5, I.B (origin: Jakarta)
CD 16	Duku kokosan	KPN-CSC, tag no. 8, I.B (origin: Jakarta)
CD 17	Duku Palembang	KPN-CSC, tag no. 35, I.B (origin: Palembang)
CD 18	Duku Palembang	KPN-CSC, tag no. 45, I.B (origin: Palembang)
CD 19	Duku Palembang	KPN-CSC, tag no. 65, I.B (origin: Palembang)
APH 22	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 23	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 24	Duku	Rena Panjang Village, Lubuk Sandi Sub-district, Seluma District, Bengkulu
APH 25	Duku	Gunung Agung Village, Arga Makmur Sub-district, North Bengkulu District, Bengkulu
APH 26	Duku	Gunung Agung Village, Arga Makmur Sub-district, North Bengkulu District, Bengkulu
APH 27	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 28	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 29	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 30	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 31	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 32	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 33	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 34	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu
APH 35	Duku	Padang Pelasan Village, Air Periukan Sub-district, Seluma District, Bengkulu

PCR RAPD amplification

RAPD amplification was performed in Takara thermocycler following Williams et al. (1990) with random decamer primers (OPA 7, OPA 13, OPA 18, OPB 7, OPN 12) obtained from Operon Technologies (Almeda, USA). Amplifications were performed in 15 µl reaction volume containing 1x PCR Green Master Mix (Promega, USA), 2 µM primer (Operon Technology), and ~10 ng of DNA template. Amplified products were separated in 2% agarose gel in 1X TAE buffer at 50 volt for 120 min. The gels were stained with 0.5 µg/mL ethidium bromide solution and visualized and photographed using gel documentation system (Atto Bioinstrument). The PCR reactions were done twice to ensure the reproducibility and consistency of the PCR products.

Data analysis

The RAPD bands were scored manually based on the profiles obtained from gel electrophoresis photos, as present (1) or absent (0), each of which was treated as a putative locus. Data analyses were performed using NTSys-PC (Numerical Taxonomy System, version 2.02i, Rohlf 1998). The SIMQUAL (Similarity for Qualitative Data) program was used to calculate the Jaccard's similarity coefficient, a common estimator of genetic identity. Similarity matrices were utilized to construct the UPGMA (unweighted pair group method with arithmetical average) dendrograms. Finally, a principal coordinate analysis was performed in order to highlight the resolving power of the ordination.

RESULTS AND DISCUSSION

Pattern of RAPD fingerprints

Amplifications of genomic DNA of 47 accessions using five primers yielded 53 fragments that could be scored. The number of amplified fragments ranging from 9 (OPA 7 and OPN 12) to 13 (OPB 7), with an average of 10.6 polymorphic fragment per primer and which varied in size from 300 (OPA 18 and OPN 12) to 1700 pb (OPA 18). Percentage of polymorphism ranged from 16.89% (OPA 7 and OPN 12) to 24.54% (OPB 7) with an average of 20.16% polymorphism (Table 2). The selection of primers and the number of primers used to amplify the DNA template would be crucial to produce polymorphic bands because these selections will determine attachment of primers to their complementary sequences of the DNA templates (Tingey et al. 1994). This present study used only bands that were existed between 300-1700 bp. Generally, bands below 300 bp were inconsistent, while bands above 1700 bp could not be well separated during electrophoresis.

Genetic variations observed were mainly based on differences on RAPD profiles found in all accessions. Generally all the 53 RAPD bands were found in all accessions (Figure 1-3). Common bands that were existed in all accessions was OPN 12 at 300 bp (Figure 3). However, few bands were only existed within certain accessions, i.e. OPB 7 at 450 bp and OPA 18 at 500 bp were recorded at two accessions from Java (no 20 and 6, respectively). Thus, these unique bands may potentially be served as provenance diagnostic character. Nevertheless, in order to enable RAPD be used as diagnostic marker for provenance identification, may need more sampling at populational level because when more samples were included there might be more unique bands found or vice versa, the specific bands observed in certain provenance may not be any longer unique bands.

Estimation of genetic diversity

A dendrogram based on UPGMA analysis grouped the 47 accessions into two clusters (A and B) and five minor clusters (C) located outside the main cluster, with Jaccard's similarity coefficient ranging from 0.02 to 0.65 (Figure 4). This may be implied considerable wide range of genetic variations among accessions. A rather different result was recorded from a study on *Lansium domesticum* in Peninsular Malaysia (Song et al. 2000) that reported high genetic similarity (0.43-0.98). The main cluster (A and B, united by coef. 0.15) mainly comprises duku collected from Java in addition to kokosan, duku Palembang dan six of duku Bengkulu (Figure 4). Meanwhile, eight accessions of duku Bengkulu and Ceram were placed outside the main cluster. Dendrogram did not indicate any clear pattern of clustering to the location in which they were collected because the putative similar bands originating from RAPDs in different individuals are not necessarily homologous although they may share the same size. Similar results were observed in blackgram (Souframanien and Gopalakrishna 2004) and *Xanthomonas axonopodis* pv *dieffenbachiae* strain (Khoodoo and Jaufeerally-Fakim 2004).

Table 2. Percentage of polymorphism in five RAPD primers and their distribution in each bioregion, namely Java, Sumatra, and Ceram.

Primer's name	DNA sequence	Number and percentage of polymorphic loci (PPL)	Size range (bp)	Common bands (bp)	Unique bands observed in each bioregion (bp)		
					Java	Sumatra	Ceram
OPA 7	5′ GAA ACG GGT G 3′	9 (16.89%)	450-1200	-	450	-	-
OPA 13	5′ CAG CAC CCA C 3′	11 (20.75%)	400-1400	450	-	-	-
OPA 18	5′ AGG TGA CCG T 3′	11 (20.75%)	300-1700	-	500	550	-
OPB 7	5′ GGT GAC GCA G 3′	13 (24.54%)	400-1500	-	-	-	-
OPN 12	5′ CAC AGA CAC C 3′	9 (16.89%)	300-1500	300	-	-	-
Average		10.6 (20.16%)					

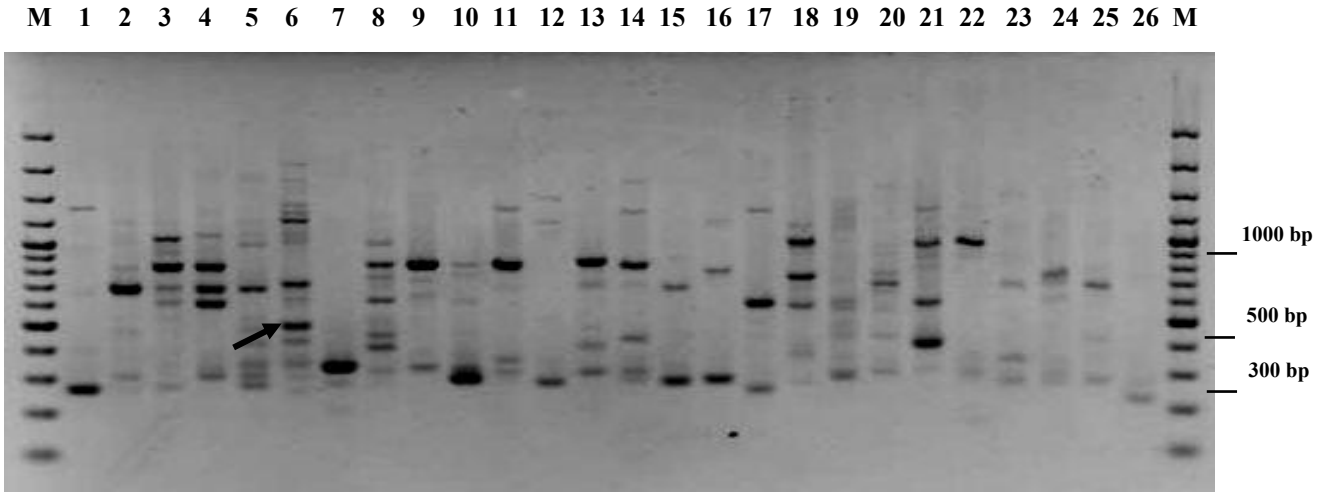

Figure 1. RAPD profiles of 26 accessions of *Lansium domesticum* using OPA 18. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku lokal, Lane 26: Kokosan. Arrow: specific band found in accession 6.

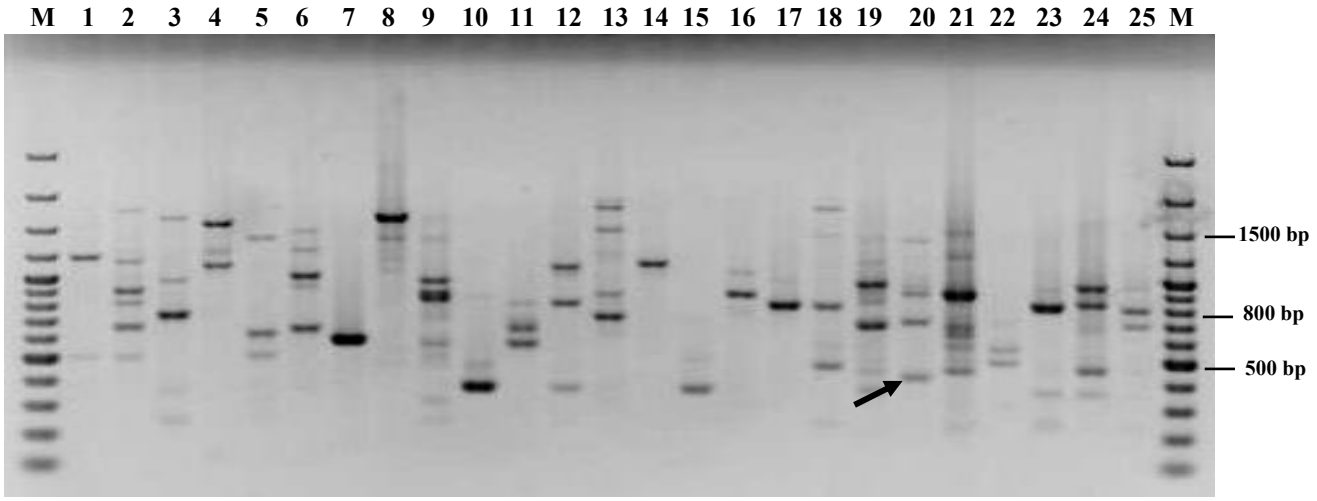

Figure 2. RAPD profiles of 25 accessions of *Lansium domesticum* using OPB 7. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku lokal. Arrow: specific band found in accession 20.

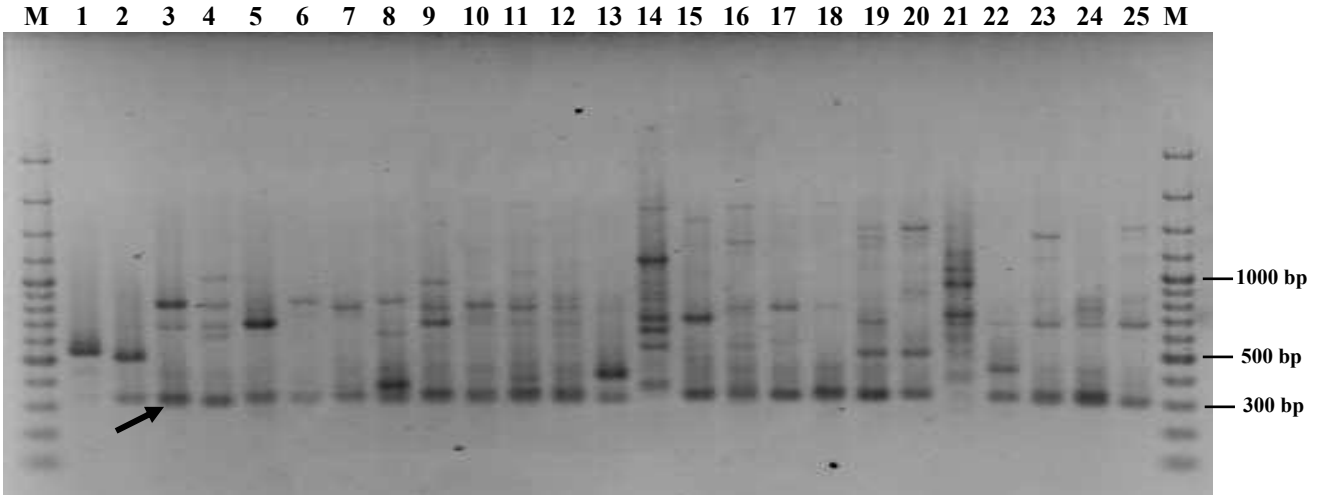

Figure 3. RAPD profiles of 25 accessions of *Lansium domesticum* using OPN 12. M: 100 bp plus DNA marker (Fermentas), Lane 1-14: Duku Sumber, Lane 15-20: *L. domesticum*, Lane 21: *Lansium* sp., Lane 22-24: *L. domesticum*, Lane 25: Duku Lokal. Bold arrow: common band.

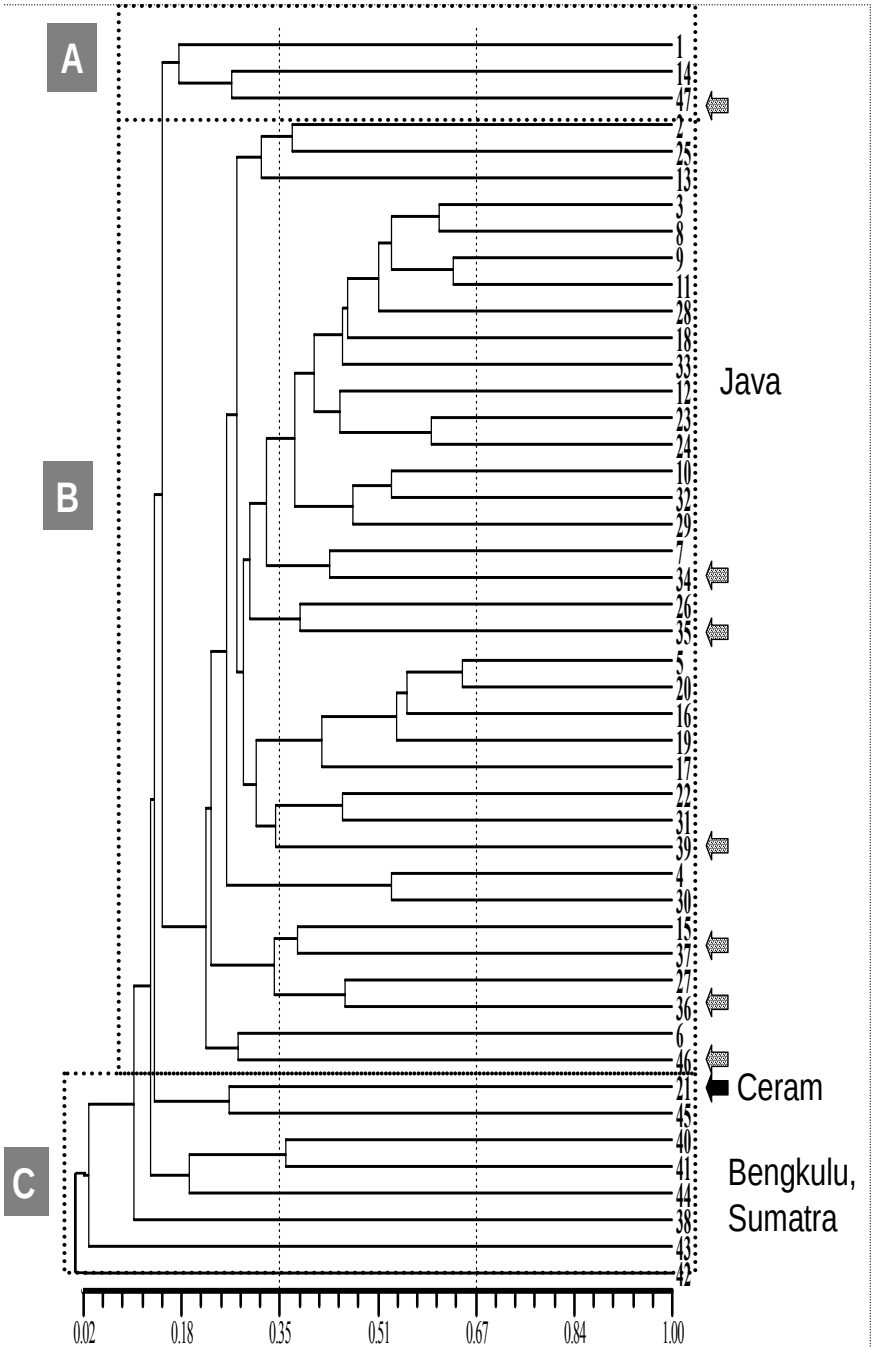

Figure 4. Dendrogram indicating genetic relatedness among 47 accessions of *L. domesticum* 'duku' and *L. domesticum* 'kokosan' based on Jaccard coefficient of similarity and UPGMA algorithm. Reference line indicated by vertical dotted lines. Solid arrow: accession from Ceram. Dotted arrow: accessions from Sumatra. Numbers correspond to sample used in Table 2.

Cluster A (coef. 0.18) and B (coef. 0.22) forming a big cluster, with most of the accessions were grouped in cluster B. Unidentified species (*Lansium* sp., no. 21) collected from KRB that was originated from Ceram has genetic similarity of 0.25 with Duku Bengkulu. The grouping of duku Palembang collected from KPN-CSC as well as kokosan into the main cluster may be implied genetic similarity between them and duku from Java. This may due to domestication process that has taken a very long period of time raising the possibility of outcrossing among local varieties. Kokosan (26, 27 and 30) were not forming a group but were grouped with duku from Central Java (coef. 0.55-0.69). Marsolais et al. (1993) suggested that the range of 0.50 using RAPD may be implied the occurrence of interspecific hybrid, while range between 0.61-0.99 could suggest genetic similarity at the species level in Lilac. While interspecific hybrid in *Mentha spicata* and *M. arvensis* shared 56 and 49% similarity to the parents (Shasany et al. 2005). In addition, Kiew et al. (2003) have observed the existence of hybrid in Duku-langsat (*L. domesticum* var. *domesticum*).

The genetic closeness among accessions can be explained by the high degree of commonness in their pedigree. Accessions 5 and 20 have the highest genetic similarity (coef. 0.65, Figure 4). Accession 5 was a parent tree of duku Sumber, while accession 20 was identified as *Lansium domesticum* from Java collected from KRB. Thus, accession 20 may have been collected from an area of where duku Sumber was mainly distributed. Accession 38, 43 and 42 (from Bengkulu) were placed at the basal dendrogram and not forming a group with any of the accessions. These accessions were seemed to be the most distinct and their DNA profiles are likely to contain the greatest number of novel alleles.

The result of PCA was to some extent not comparable to the cluster analysis (Figure 3) except for duku Bengkulu (Figure 5). Four accessions (39, 30, 4 and 28) appear to be distinct from other accessions in the PCA while these accessions were having considerably high similarity with other accessions from Java in the dendrogram. Otherwise, the remaining components were grouped in PCA may contributed the total variation correspond to the polymorphic locii.

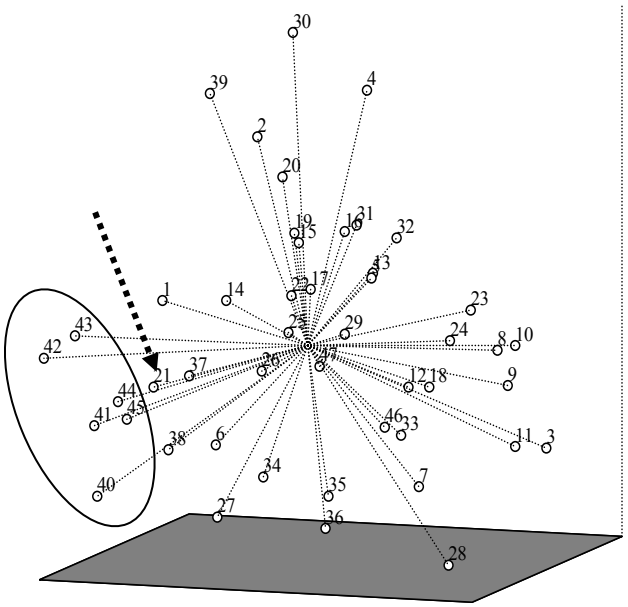

Figure 5. Three-dimensional plot of principal coordinate analysis using 47 accessions of *Lansium domesticum* 'duku' and *L. domesticum* 'kokosan'. The numbers plotted represent individual sample and corresponds to notes in Figure 2. Dotted arrow: *Lansium* sp. from Ceram. Circle correspond to the grouping at Figure 4 as Duku Bengkulu. Vertical line axis Z.

CONCLUSION

Five RAPD primers produced 53 polymorphic bands ranging in size from 300-1700 bp. These bands were used to assess genetic variation among 47 accessions of duku and kokosan. The genetic similarity range between 0.02 and 0.65 indicating wide range of genetic variations among the accessions. Of the 5 selected primers, OPB 7 produced the highest number of bands (13) while OPA 7 and OPN 12 yielded the least number of bands (9) with the average of 10.6 polymorphic bands per primer. Common bands that were existed in all accessions were OPN12 at 300 bp and OPA 13 at 450 bp. While unique bands were recorded from OPB 7 at 450 bp and OPA 18 at 500 bp belong to two accessions from Java (no 20 and 6 respectively); OPA 18 at 550 bp was found in Bengkulu's provenances (39, 42, 44). Results from cluster analysis suggested that all accessions were grouped randomly into some clusters not in accordance to the locations where they were collected. This study indicated the presence of genetic variability among accessions of duku and kokosan that could be detected by RAPD marker.

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A non-invasive identification of hormone metabolites, gonadal event and reproductive status of captive female tigers

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ABSTRACT

Putranto HD (2011) A non-invasive identification of hormone metabolites, gonadal event and reproductive status of captive female tigers. Biodiversitas 12: 131-135. As a non-invasive method, fecal sample provides some advantage for animal and collector. The purpose of the present study were to monitor the reproductive status of female Siberian tigers (*Panthera tigris altaica*) by assessing changes in fecal during natural ovarian activity and pregnancy and to identify whether progesterone (P4) exists and what kinds of P4 metabolites excreted into the feces. Two female tigers were fed a diet consisting of meat. Drinking water was available *ad libitum*. Feces were collected ones to twice a week. The fecal contents of P4 and estradiol-17β (E2) were determined by EIA and P4 metabolites were separated by a modified HPLC. The EIA results shown that during its natural ovarian activity the E2 contents showed cyclic changes at the average of 27.0 d interval, however, no distinct cycles were shown in fecal P4 contents of non-pregnant tiger. In contrary, the fecal P4 contents in pregnant tiger increased remarkably after copulation approximately 2- to 6-fold higher than the mean value. The HPLC results indicated that two peaks were primarily detected fraction 63- 64 min (identified metabolites) and fraction 85 min (not identified metabolite) in feces of pregnant tiger. However, P4 detected only small amount in feces. It is possible to assess non-invasively gonadal events such as luteal or follicular activity or ovulation of Siberian tigers by endocrine monitoring based on fecal P4 and E2 to understand reproductive status.

Key words: EIA, feces, HPLC, reproductive status, female tiger.

INTRODUCTION

Tiger, *Panthera tigris*, is classified into order Carnivora and family Felidae (MacDonald 2001). It is the largest species among Felidae family member. This species is divided into eight subspecies namely Bengal (*Panthera tigris tigris*), Siberian (*P. t. altaica*), Indochinese (*P. t. corbetti*), Sumatran (*P. t. sumatrae*), South China (*P. t. amoyensis*), Javan (*P. t. sondaica*), Bali (*P. t. balica*), and Caspian (*P. t. virgata*) tiger (Mazák 1981). Unfortunately, there are only five living subspecies tigers left on the world recently. The critically endangered subspecies, the Siberian tiger (*P. t. altaica*) is estimated at approximately less than 400 and 400-500 individuals in the wild (Putranto et al. 2006a; 2007a).

Reproduction in tigers remains poor and unclear. With its restricted population, it would be a challenge for scientists to learn and familiarize themselves with the reproductive potential of tigers to improve the breeding potential of captive tigers. There are limited scientific references on the reproductive physiology and endocrinology of tigers (Graham et al. 2006; Putranto et al. 2006a, b; 2007a, b). Gonadal steroid hormone analysis is a major point in reproduction, and assessing its changes could allow the enhancement of captive breeding programs.

Serum hormones are the most accurate reflection of gonadal activity, and it has been used widely to monitor the profile of reproductive physiology and endocrinology status in endangered animals. Serum collection process especially from endangered animal body, induced several injure side effects such as stressful, impractical and difficulties. Non-invasive method by using fecal sample becomes the best preference because fecal sample collection is providing some advantage such as non-stressful for animal and non-risk to the sample collector, and thus easy to collect without any timing or period limitation (Putranto et al. 2006a, b; 2007a, b; 2009). Fecal sampling for fecal hormone analysis has been available for non-invasive method with long-term advantages (Putranto et al. 2007a,b).

The food has been digested and excreted from the body through the digestive tract as waste. Feces contain many important physiological signals such as sex steroid hormones and these metabolites. Generally sex steroid hormones are synthesized from cholesterol and carried in the blood flow bound to specific carrier proteins such as sex hormone binding globulin or corticosteroid binding globulin. These hormones act on target organs and are converted to many metabolites by 5α- or 5β-hydrogenase and 3α- or 3β-OH steroid dehydrogenase, then they are

excreted into urine through kidney and with the bile into feces (Putranto et al. 2009).

The amount of 85-95% of steroid hormones metabolites are excreted in feces of domestic cat (Brown 2006). Specifically, progesterone (P4) metabolites in domestic cat is metabolized and excreted primarily both conjugated and unconjugated metabolites into feces (Brown et al. 1993). In non domestic felids, leopard cat (*Felis bengalensis*) and clouded leopard (*Neofelis nebulosa*), it has been reported that the P4 metabolism may be similar between those two species and were almost one conjugated metabolite (> 90%). However in cheetah (*Acinonyx jubatus*), it has been identified as three immunoreactive metabolites fractionating with percentages of 42, 51 and 7%, respectively (Brown et al. 1993).

Previous reports were described about the combination of two methods (high performance liquid chromatography (HPLC) and enzyme immunoassay (EIA) to confirm the excreted hormone metabolites (Putranto et al. 2009). The HPLC method and the EIA method were applied to separate the specific metabolites and to confirm the presence of that steroid hormone metabolites. The purpose of the present study were to monitor the reproductive status of female Siberian tigers by assessing changes in fecal during natural ovarian activity and pregnancy and to identify whether P4 exists and what kinds of P4 metabolites excrete into the feces of pregnant female Siberian tiger by using the combination of HPLC and EIA methods.

MATERIALS AND METHODS

Animal and fecal sample collection

The animal monitored included two female Siberian tigers. Tiger No.1 (Japanese Studbook No. 179, 14 years of age at the beginning of this study) housed at Yokohama Zoological Gardens Zoorasia, Japan and it was a non-pregnant female. Tiger No.2 (International Studbook No. 4441, 7 years of age at the beginning of this study) housed at Tama Zoological Park, Japan and it was a pregnant female when this study conducted. Generally, they were fed a diet consisting of horse meat, chicken and rabbit meat. Drinking water was available ad libitum. They had free access to the natural photoperiod in an outdoor paddock during the daytime. Female tiger No.1 was kept alone in an individual indoor chamber during nighttime and have a free access to socialize with male in outdoor paddock during daytime, and tiger No.2 lived together with a male in a paddock. .

Feces were collected ones to twice a week during 14 months of collection period for both tigers. There were 190 fecal samples collected from tiger No.1 and 142 samples collected from tiger No. 2. Totally, there were 332 samples collected. Fresh sample collected from sleeping chamber within at least 18 h after excretion. The feces placed into a plastic bag size 200 x 140 x 0.04 mm (Uni Pack Mark Series-G, Seisan Nippon Co., Tokyo, Japan) and stored at -20°C immediately after collection.

Sample extraction and hormones analysis

The fecal extraction procedure was described in previous reports (Kusuda et al. 2006a, b, c; 2007; Putranto et al. 2007a, b, c). The fecal contents of P4 was determined by enzyme immunoassay (Kusuda et al. 2006a, b, c; 2007; Putranto et al. 2007a, b, c). P4 antiserum (LC-28; Teikoku hormone Mfg. Medical, Kanagawa, Japan) mainly cross-reacts with P4 (100%), 5α-pregnanedione (62.2%), pregnenolone (6.3%), 11-deoxycorticosterone (3.9%), 17α-hydroxyprogesterone (2.3%), and 11α-hydroxy-progesterone (1.2%). Estradiol-17β (E2) antiserum (QF-121; Teikoku hormone Mfg. Medical) mainly cross-reacts with E2 (100%), estrone-3-sulfate (8.0%), 16-epiestriol (5.3%), estrone (3.2%), and estriol (1.8%).

HPLC procedure

The HPLC method in this study was a modification of previous report (Schwarzenberger et al. 2000). Prior to HPLC method, pooled extracts randomly chosen from those twenty three samples during early to late pregnancy were cleaned up on a Sep-Pak C-18 cartridge. Progesterone metabolites were separated by a modified HPLC method with a reverse-phase Nova-Pak C-18 column using acetonitrile/water (40/60 v/v) mixture (Putranto et al. 2009). One hundred and twenty fraction samples (1 mL) were collected.

P4 metabolites by EIA procedure

Those 120 fractions, by EIA method were assayed to confirm the presence of P4 metabolites (Kusuda et al. 2006a, b, c; 2007; Putranto et al. 2007a, b, c). The antiserum cross reaction was similar to first EIA method.

Data analysis

All fecal values are presented as the weight in dried feces. Data of first EIA procedure for hormone analyses (P4 and E2) are shown as the mean ± SEM. Beyond the second EIA procedure, the presence of P4 peaks was compared to the eluted position of some standards of P4 metabolites (Table 1, Figure 1).

Table 1. Elution time of steroid hormone and metabolites

Steroid metabolites	Elution time (min)
PdG	3
20α-OHP	31
5β-pa-3β, 20α-diol or 5α-pa-3α, 20α-diol	35
Pd	43
P4	51
5β-pa-3β-ol-20-one or 5β-pa-3α-ol-20-one	63-64
5α-pa-3β-ol-20-one	68
5α-pa-3α-ol-20-one	79
5α-DHP	98

The length of the ovarian cycle was calculated as the number of days of the peak fecal E2 interval for periods not exceeding 50 d (that is, longer than about twice the estimated cycle length) (Putranto et al. 2006a; 2007a). The peak fecal E2 contents were defined as those values that were greater than the mean of all values from an individual tiger (Putranto et al. 2006a; 2007a).

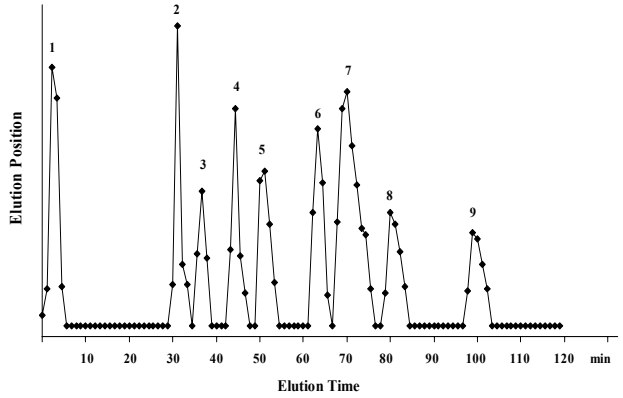

Figure 1. The chromatogram for elution position of some steroid metabolites. Numbers (1-9) indicate steroid hormone and metabolites presence. No.1 = pregnanediol-glucuronide, 2 = 20α-OHP, 3 = 5β-pa-3β, 20α-diol or 5α-pa-3α, 20α-diol, 4 = pregnanediol, 5 = progesterone, 6 = 5β-pa-3β-ol-20-one or 5β-pa-3α-ol-20-one, 7 = 5α-pa-3β-ol-20-one, 8 = 5α-pa-3α-ol-20-one, 9 = 5α-DHP.

RESULTS AND DISCUSSION

Serum hormones are the most accurate reflection of gonadal activity. However, the inconvenient method of serum collection could conduced several injure side effects such as stressful, impractical and difficulties. Lately, some studies have shown similar patterns in serum and fecal hormones, and utilization of fecal samples as a noninvasive tool is widely used to monitor gonadal activity in tigers (Putranto et al. 2006a, b; 2007a, b).

In this study, there were two different reproductive status of female Siberian tigers, non-pregnant and a pregnant female tigers. By using an EIA method, in this study also we can assess the fecal contents of steroid hormones such as P4 and E2 levels and understand about the gonadal events and its reproductive physiological status. Putranto et al. (2009) stated that the combination between EIA and HPLC method would allow us to identify whether P4 exists and what kinds of P4 metabolites excrete into the feces of female Siberian tiger.

Reproductive cyclicity and pregnancy in Siberian tigers

Range of fecal P4 and E2 during non pregnancy in female Siberian tigers is described in Table 2. The value for fecal P4 and E2 varied between 0.27 to 38.19 and 0.09 to 18.52 μg/g (n = 332, Table 2), respectively. In female Siberian tiger No. 1, the fecal P4 contents were an average of 0.78 ± 0.96 μg/g (n = 100, Figure 2). Although only two changes were recorded on February and March, no distinct cycles were shown in fecal P4 contents.

Table 2. The range of fecal P4 and E2 contents during non-pregnancy in female Siberian tigers

Female	Fecal P4	Fecal E2
	(μg /g)	
No. 1	0.27- 9.40	0.15- 3.76
No. 2	0.05- 32.32	0.04- 4.01

During its natural ovarian activity, non-pregnant female Siberian tiger No. 1 had no distinct cycles were shown in her fecal P4 contents. This value is smaller than the fecal P4 contents in female Sumatran tiger (12,17 μg/g) and its fecal P4 contents changed cyclically. The cycle length based on changes in fecal P4 in female Sumatran tiger was 58.3 ± 2.7 d (Putranto et al. 2007b). Furthermore, the value of fecal P4 contents in female Siberian tiger is also smaller than Bengal tiger which could reached 36.05 μg/g level and changed cyclically (Putranto 2008). It seems that female Sumatran and Bengal tigers excreted more fecal P4 into feces than female Siberian tiger.

The fecal E2 contents in female No. 1 and 2 were an average of 0.49 ± 0.61 μg/g (n = 107) with a peak of 1.69 ± 1.04 μg/g (n = 12, Figure 2) and 0.39 ± 0.55 μg/g (n = 100) with a peak of 1.37 ± 0.93 μg/g (n = 14, Figure 3), respectively. The ovarian cycle based on changes in fecal E_2 profiles of female Siberian tiger No. 1 and 2 are described in Table 2.

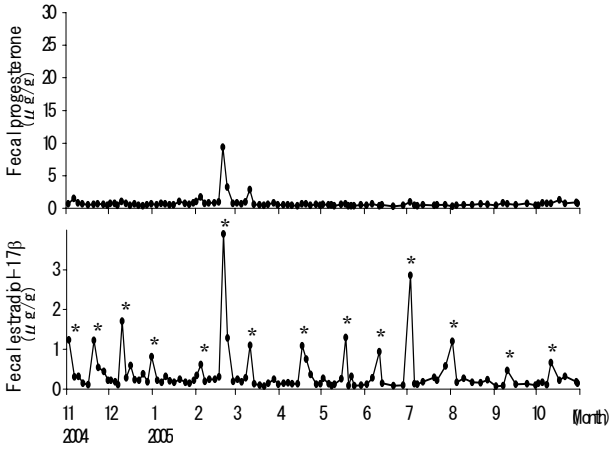

Figure 2. Changes in the fecal contents of P4 (upper) and E2 (lower) in female Siberian tiger (No.1, Yokohama Zoological Gardens). The 14 asterisks indicate the peaks of fecal estradiol-17β. (Putranto et al. 2007a).

The fecal E2 contents in these two female Siberian tigers were reached average of 0.39 to 0.49 μg/g level and changed cyclically. The value of female Siberian tigers fecal E2 contents were similar to female Bengal tiger (0.45 μg/g) (Putranto 2008), however it was smaller than female Sumatran tiger (2.36 μg/g) (Putranto et al. 2007b). The pattern of fecal E2 profiles from three tiger subspecies (Siberian, Bengal and Sumatran) were not similar. The Sumatran and Bengal tigers are shown no cyclical changes, however, Siberian tiger is shown a cyclical changes.

In two female Siberian tigers of this study, the fecal E2 contents showed cyclic changes at the average of 27.0 d interval. A similar finding was made in a previous report, which showed cyclic changes at 29.3 ± 4.4 d intervals in crossbreed Bengal-Siberian tigers (Graham et al. 2006). Our findings suggest that fecal E2 was probably excreted in parallel with follicular growth and that the remarkable changes in the fecal E2 contents indicate a regular ovarian cycle.

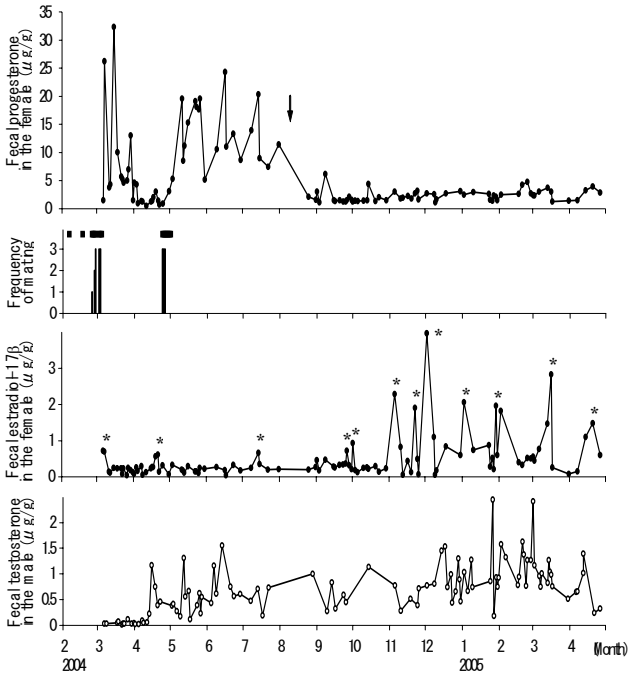

Figure 3. Changes in fecal contents of P4 and E2 in female Siberian tiger (No. 2, , which was kept at Tama Zoological Park). The arrow indicates parturition. A higher score for copulation means higher frequency. Horizontal bars indicate the periods when the female was housed together the male. The 12 asterisks indicate the peaks of E2 (Putranto et al. 2007a).

The fecal P4 contents of female No. 2, which was kept with male, increased remarkably after copulation on March 3 and April 24 (Figure 3). The P4 contents were approximately 2- to 6-fold higher than the mean value (5.20 ± 6.13 μg/g, n = 107) but the first copulation did not result in pregnancy. During pregnancy, the fecal P4 contents varied from 0.94 to 24.29 μg/g. However, the highest level of fecal P4 contents (24.29 μg/g) in this pregnant female is smaller than in pregnant female Bengal tiger (104.17 μg/g) (Putranto 2008). The length of the pregnancy in female Siberian tiger was 106 d, and this number is similar to the length of pregnancy in female Bengal tiger which was approximately 105 d (Putranto 2008).

In the case of pregnancy, the fecal contents of female Siberian tiger increased remarkably after copulation. From the beginning of pregnancy, the fecal P4 content increased and remains high during 106 days of pregnancy; however the fecal P4 contents decreased to baseline after parturition (Figure 3). The duration of the increased P4 contents after copulation differed between non-pregnancy and pregnancy. The P4 steroid hormone has a role function to maintain the pregnancy of female mammals (Brown et al. 2006). Therefore, the differences between non-pregnancy and pregnancy female tiger's fecal P4 contents in this study are strengthen the previous report result.

In leopard cats, clouded leopards, snow leopards, and cheetahs, it has been reported that the duration of increased fecal P4 contents during presumed pseudopregnancy is about half the pregnancy length (Putranto 2008). The main indicator to distinguish between pregnant and pseudopregnant conditions is the duration of increased fecal P4 content. Thus, fecal P4 monitoring can be used to confirm pregnancy in Siberian tigers.

The fecal E2 contents of pregnant female Siberian No. 2 began to change remarkably from November 2004. Female No. 2 and this cub were reared in the outdoor paddock on October 2, 2004. The remarkable changes in fecal E2 contents probably reflect recurrence of estrus, because weaning and natural light stimulation might affect the ovarian cycle.

Major progesterone steroid metabolites excreted in feces

The results were shown that two peaks were primarily detected fraction 63- 64 min (might be 5β-pregnane-3β-ol-20-one or 5β-pregnane-3α-ol-20-one) and fraction 85 min (not identified metabolite) in feces of pregnant female Siberian tiger (Table 3, Figure 4). However, in this study P4 detected only small amount in feces of pregnant Siberian tiger.

Table 3. Major P4 and steroid hormone metabolites excreted in feces of pregnant female Siberian tiger

Steroid metabolites	Elution time (min)
PdG	3
5β-pa-3β-ol-20-one or 5β-pa-3α-ol-20-one	63- 64
Unknown	85

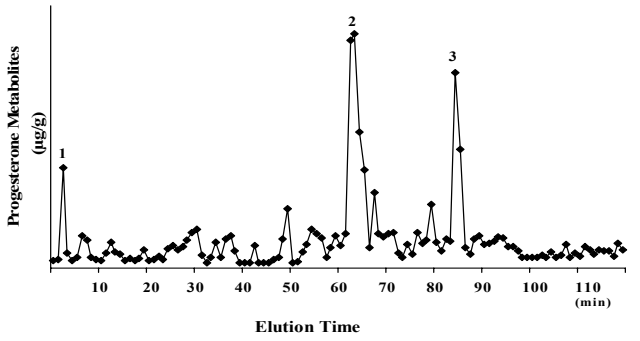

Figure 4. The chromatogram for major P4 metabolites excreted in feces of pregnant female Siberian tiger (No.2, Tama Zoological Park). Numbers indicate P4 metabolites presence in feces consecutively. No.1 = pregnanediol-glucuronide, 2 = 5β-pa-3β-ol-20-one or 5β-pa- 3α-ol-20-one, 3 = unknown.

Previous studies of steroid metabolites composition in feces of several felids has been conducted by international researchers. However, this study would be the first description and preliminary data of major metabolites of progesterone steroid excreted in feces of pregnant female Siberian tiger.

The chromatogram of this study is shown that progesterone (P4) detected only small amount in feces of Siberian tiger. The major P4 steroid metabolites excreted were presence in the form of conjugated progesterone

which was 5β-pregnane-3β-ol-20-one or 5β-pregnane-3α-ol-20-one. Another peak eluting in fraction 3 min could be pregnanediol-glucuronide (PdG). The previous report confirmed that the presence of several progestogens in the feces of felids (Graham et al. 1995). Almost 90% of P4 metabolites in clouded leopard and leopards cat was the form of conjugated progesterone (Brown et al. 1993). In pregnant female Siberian tiger, probably a great portion of circulating P4 are metabolized into some pregnants and excreted into feces. Our result proved that during pregnancy in female Siberian tiger, the reproductive organs produced a great portion of a conjugated P4 and excreted into feces.

The tigers in this study had no seasonal reproductive pattern. Although tigers in the wild can breed all year, they typically breed during the winter or spring (about the end of November to April) (MacDonald 2001). Furthermore MacDonald (2001) stated that Bengal tigers in the wild seem to breed all year; however, the peak of breeding activities is from November to February. Furthermore, tigers are known as induced ovulators and rare individuals are spontaneous ovulators (Brown 2006; Graham et al. 2006). The relationships between successful breeding and hormone patterns should be clarified and the reproductive/physiological mean of these ovulation patterns in tigers should also be understood to improve the captive breeding of tigers in the future.

CONCLUSION

It was concluded that it is possible to assess non-invasively gonadal events such as luteal or follicular activity or ovulation of Siberian tigers by endocrine monitoring based on fecal P4 and E2 contents. The utilization of fecal steroid hormones is also useful to confirm pregnancy in female Siberian tiger by a continuous fecal P4 contents analyses. We can also assess non-invasively the pregnancy in female Siberian tiger by continuous fecal P4 analyses combining HPLC and EIA methods. In pregnant female Siberian tiger, a great portion of circulating P4 is probably metabolized into some pregnants and excreted into feces.

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First record of *Odontanthias unimaculatus* (Tanaka 1917) (Perciformes: Serranidae) from Indonesia

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ABSTRACT

Peristiwady T (2011) First record of Odontanthias unimaculatus *(Tanaka 1917) (Perciformes: Serranidae) from Indonesia. Biodiversitas 12: 136-140.* Seven specimens of *O. unimaculatus* were collected from Bitung, North Sulawesi between 7 January and 18 August 2009. They were caught from depths of about 100 m in association with other deep water fishes as *Epinephelus*, *Pristipomoides* and *Etelis*. *O. unimaculatus* was most similar to *O. grahami*, in sharing the following characters: dorsal fin soft rays 14, anal fin soft rays 7, scales on lateral line 36-37 and gill rakers on upper limb 13-14. If caudal fin shape and coloration were ignored, *O. unimaculatus* would seem most closely related to *O. tapui* and *O. chrysostictus*. Their body proportions were nearly the same. However the later species had longer body width, third dorsal and second anal spine but had shorter longest dorsal ray length, longest anal rays, caudal-fin length and caudal concavity. Other little different characters of *O. unimaculatus* with other seven species were the proportion of upper jaws length and proportion of body depth. Initially this species was described as new species from Tanabe, Wakayama Prefecture, Japan. Other specimens were reported from Suruga Bay, Japan, Keelung and Kaohsiun, Taiwan and Lubang Island, Philippines and now recorded also in Bitung, North Sulawesi, Indonesia.

Key words: *Odontanthias unimaculatus*, Serranidae, Anthiinae, new record, taxonomy, Sulawesi, Indonesia.

INTRODUCTION

Most anthiine fishes are of small size living in hard-bottom habitat at depths of about 100-400 m (Randall and Heemstra 2006) therefore it is rarely taken by scuba diving, gill-netting or trawling. Difficulties of catch, small fish size and little commercial value of the species (Chen and Shao 2002; Randall and Hoese 1995; Randall 1996; Randall and Heemstra 2006, 2007), anthinne fishes are not well represented in museum collections of which some species were described as new species based on a single or several specimens (Anderson 2006, 2008; Anderson and Baldwin 2002; Chen and Shao 2002; Kon et al. 2000; Peristiwady et al. 2010; Randall 1996; Randall and Pyle 2001; Randall and Heemstra 2006, 2007; William 2008; Wu et al. 2011). The anthiine specimens in this studies were taken by hook and line by fishermen who targeting deep water groupers or snappers as *Epinephelus*, *Pristipomoides* and *Etelis*. Although the fish was taken by hook and line but because the fish size was normally small, therefore the fishermen were almost never bring the fish to the market.

The Subfamily Anthiinae of the family Serranidae comprises of about 24 genera and more than 100 species that inhabit coral and deep reef habitats in tropical and warm temperate seas (Akhilesh et al. 2009). The genus *Odontanthias* is characterized as follows: Dorsal fin rays X,12-19; anal fin rays III,7-8; pectoral rays 15-19; lateral line complete or incomplete; vertebrae 26; interorbital space convex; mouth not large; tongue, vomer, palatines and mesopterygoids with a large patches of small villiform teeth; posterior margin of preopercle strongly serrate with a prominent flat spine or enlarge spine at the angle; and body depth 1.9-2.7, head length 2.35-2.85 both in standard length (SL) (Randall and Heemstra 2006).

In Indo-Pacific region, 13 species of *Odontanthias* were reported by Randall and Heemstra (2006), in Indonesian waters three species were reported by Kimura et al. (2003); Kuiter and Debelius (2006); Masuda et al. (1984); Peristiwady (2006); Randall and Heemstra (2006): *Odontanthias borbonius* (Valenciennes, 1823), *O. chrysostictus* (Günther, 1872) and *O. rhodopeplus* (Günther, 1872). Herein, it was found this species bringing the total number of species of this genus known in Indonesian waters to four.

MATERIALS AND METHODS

The specimens of *Odontanthias unimaculatus* were collected from several fish markets at Bitung, North Sulawesi (Figure 1). The specimens were caught by hook and line. Specimens were photographed when fresh and then preserved in 10% formalin for about one week and transferred to 70% ethanol for permanent preservation and further examination.

Methods of counting and measuring followed Randall and Heemstra (2006) with additional measurement as measurements of all spines and rays length of dorsal and

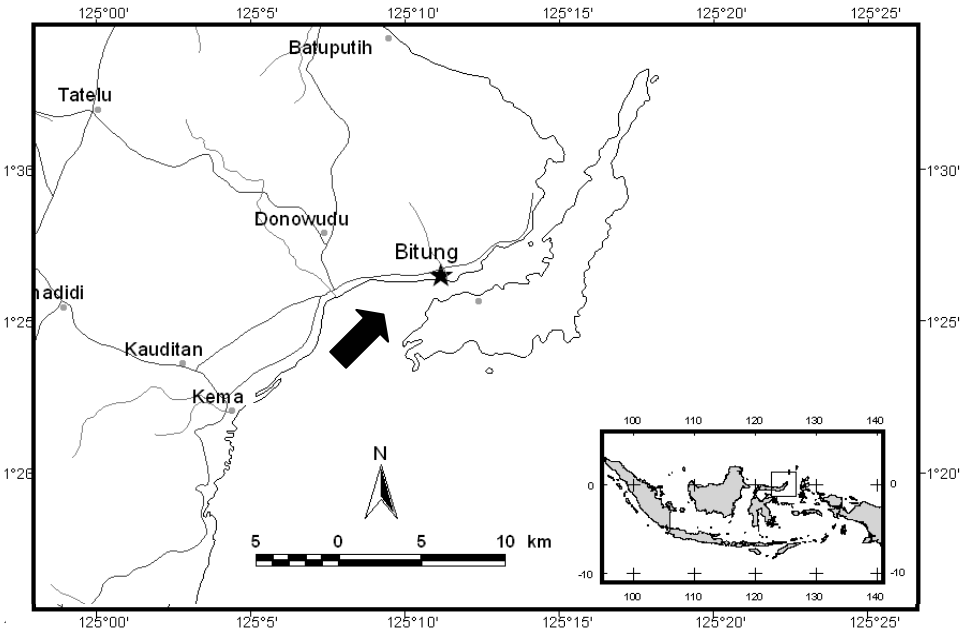

Figure 1. Location of Girian and Winenet, Bitung, North Sulawesi, Indonesia where the specimens were collected (arrow)

anal fins and suborbital width. All measurements were made with digital calipers to the nearest 0.01 mm. Cyanine blue was used to examine and count the scales. All lengths are reported as standard length (SL) and body depth (BD). The specimens are deposited at LBRC-F (References Collection of Technical Implementation Unit for Marine Biota Conservation, Indonesian Institute of Science), Bitung, North Sulawesi, Indonesia.

RESULTS AND DISCUSSION

Odontanthias unimaculatus (Tanaka, 1917) (Figure 2; Table 1).

Holotype: *Anthias unimaculatus* Tanaka 1917: 199 (type locality, Tanabe, Wakayama Prefecture, Japan); Tanaka (1922) *in* Randall and Heemstra (2006): 23, pl. VI B, Tables 1-3, figs. 1 L, 6, 9.

Material examined: Seven specimens, 93.73-116.52 mm SL, all specimens collected in Bitung, North Sulawesi, Indonesia. LBRC-F 1391, LBRC-F 1394, 93.73-101.71 mm SL, Girian Fish Market, 14 December 2009; LBRC-F 1635, 94.21 mm SL, Girian Fish Market, 29 July 2010; LBRC-F 1704, 116.52 mm SL, Girian Fish Market, 17 September 2009; LBRC-F 1715, 110.8 mm SL, Girian Fish Market, 20 September 2009; LBRC-F 1738, 103.44 mm SL, Girian Fish Market, 25 September 2010; LBRC-F 1747, 97.69 mm SL, Girian Fish Market, 27 September 2010;

Description: Measurements and counts are shown in Table l. Dorsal-fin rays X,14; anal rays III, 7; all dorsal and anal rays branched, the last joined to base; pectoral-fin ray 17, uppermost rays unbranched; pelvic rays I, 5, all rays branched, second outer rays forming filament; lateral-line scales 36-37 (37); scales above lateral line to origin of dorsal fin 6-8 (7); scales below lateral line to origin of anal fin 17-20 (19); scales above lateral line to base of middle dorsal fin 3; oblique rows of scales on check 7-8; gill rakers 13-14 + 29 (total rakers (42-43).

Body deep and compressed, the width 2.26-2.54 (2.39) in body depth; head length 2.75-2.95 (2.80) all in SL; eye large, the orbit diameter 2.81-3.31 (3.03) in HL; snout length 4.34-5.07 (4.66) in HL; interorbital space convex, the least bony width 3.41-3.59 (3.52) in HL; least caudal peduncle depth 2.53-2.85 (2.61) in HL; caudal peduncle length 1.43-1.76 (1.61) in HL.

Mouth terminal, oblique and not large, forming an angle of about 45º to horizontal line of head, the lower jaw projecting; maxilla reaching slightly posterior to a vertical through center of pupil, the upper jaw length 2.02-2.30 (2.09) in HL; each anterior of upper jaw with a stout canine teeth, inner upper jaw and lower jaw with rows of minute teeth, outer rows with several teeth longer than inner teeth, half distance of lower jaw with stout and long recurved canines bigger than upper jaw canines.

Nostrils slightly in front of upper horizontal center margin of pupil, the anterior with a flap that almost reaches the anterior of nostril aperture; large sensory pore found in front of anterior nostril, other sensory pores at around lachrymal, infra-orbital and below lower jaw.

Opercle with three flat spines, middle one largest and most posterior, slightly closer to lower than upper spine; upper opercular spine blunt and covered by scale; posterior margin of preopercular edge of preopercle with 15-27, angle of preopercle with a large flat spine about half way to margin of subopercle; serrae on ventral edge of preopercle 1-4; margin of subopercle with 0-3, margin of interopercle with 2-3, opercular flap pointed.

Lateral line complete, broadly arched over pectoral fin closed to dorsal fin base, running parallel to dorsal body contour below dorsal fin; its highest point below base of 6th dorsal spine.

Table 1. Morphometric data and meristic counts for *Odontanthias unimaculatus*. Minimum and maximum measurements are presented as percentages of body depth and mean value between brackets.

Number of specimens	7
Value	**min-max (mean)**
Standard length (mm)	93.73-116.52 (102.48)
Count	
Dorsal-fin rays	14-14 (14)
Anal-fin rays	7-7 (7)
Pectoral-fin rays	18-18 (18)
Pelvic-fin rays	5-5 (5)
Scales on lateral line	36-37 (36.57)
Scales above lateral line	6-8 (7.07)
Scales below lateral line	16.5-20 (18.64)
Gill rakers on upper limb	13-14 (13.50)
Gill rakers on lower limb	29-29 (29.00)
Total gill rakers	42-43 (42.50)
In % BD	
Head length	81.93-89.24 (84.13)
Body width	39.37-44.34 (41.96)
Predorsal length	73.37-80.20 (76.09)
Prepelvic length	83.19-90.69 (86.53)
Preanal length	144.06-158.07 (152.81)
Dorsal-fin base length	150.88-164.54 (155.64)
Anal-fin base length	44.29-47.60 (45.90)
Longest dorsal ray length	59.87-74.77 (70.53)
Pectoral-fin length	70.43-78.76 (73.73)
Pelvic-fin length	75.13-98.70 (85.95)
Caudal-peduncle depth	31.35-33.11 (32.21)
Caudal-peduncle length	50.38-58.54 (52.44)
1th dorsal spine	16.11-20.48 (17.78)
2nd dorsal spine	24.73-30.33 (26.68)
3th dorsal spine	39.99-56.00 (46.64)
Longest dorsal ray length (3th)	59.87-74.77 (70.53)
First anal spine	19.96-24.86 (22.72)
Second anal spine	36.37-43.74 (40.25)
Third anal spine	32.92-42.01 (37.35)
Longest anal ray	45.47-52.08 (48.94)
Caudal-fin length	79.83-95.31 (86.72)
Pelvic spine length	46.43-48.58 (47.75)
Snout length	16.63-19.24 (18.09)
Orbit diameter	25.26-31.77 (27.84)
Interorbital width	23.00-24.86 (23.91)
Upper-jaw length	38.74-41.41 (40.19)
Sub-orbital width	7.76-9.75 (9.05)
Caudal concav	44.43-56.59 (50.69)

Scales ctenoid; predorsal part of head scaled anteriorly reaching base of upper lip; maxilla and mandible completely naked; preorbital from nostrils to below front of orbit naked; small scales on base of all fins; scales on two upper and lower lobes caudal rays almost reaching tip, base of the middle caudal fin with scales to about one third of caudal fin; base of pectoral and anal fins with small scales.

Origin of dorsal fin above the beginning of posttemporal, predorsal length 1.08-1.14 (1.11) in HL; first dorsal spine short 1.35-1.59 (1.50), about half length of second dorsal spine, second dorsal spine 1.32-2.10 (1.76) in third dorsal spine; third dorsal spine longest, 1.49-2.23 (1.83) in HL; third dorsal ray longest forming filament, 1.10-1.42 (1.20) in HL; origin of anal fin beneath third dorsal ray; first anal spine 1.59-2.16 (1.78) in length of second spine; second anal spine slightly shorter than third, 0.82-0.97 (0.93) in length of third spine; second or third anal ray longest, 1.57-1.84 (1.72) in HL; caudal fin deeply emarginated, the upper and lower lobes not forming filament, the fin length 2.38-3.00 (2.72) in SL; caudal concavity 4.09-5.42 (4.69) in SL; pectoral fin not pointed, the ninth ray longest, reaching base of first anal spine, 1.09-1.19 (1.14) in HL; origin of pelvic fin on a vertical through between base of third and fourth dorsal spine; second pelvic ray longest forming filament reaching the first base of anal spine, the length 0.86-1.11 (0.98) in HL.

Color when fresh (Figure 1): body pink dorsally grading to pale pink ventrally. Upper part of body with white bright spot on scale, lower part of body scale pale red. Head pink dorsally, pale pink ventrally, with a yellow blotches running from interorbital space to anterior of dorsal fin base; a second yellow stripe from upper lip, passing suborbital space to above the biggest flat spine at opercle; yellow bright botches at pectoral fin base; spinous portion of dorsal fin pale pink, upper part of dorsal spine portion with a large pale yellow area from the first to anterior part of dorsal rays;, incised portion of dorsal spine bright yellow, basal soft portion of dorsal fin pale pink, the outer part pale yellow; pectoral fin pale pink; inner rays of pelvic fins pale pink; anal fin yellowish; other rays and membranes pale pink; caudal fin pink, each lobe yellow stripe at inner and outer lobe.

Color after preservation: Head and body uniformly pale whitish, some specimens with three brownish line on body, head with a faint dusky blotch dorsally around preopercle;

Distribution and ecological notes: Initially this species was described as new species from Tanabe, Wakayama Prefecture, Japan. Other specimens were reported from Suruga Bay, Japan (Katayama 1960 *in* Randall and Heemstra 2006), Keelung and Kaohsiun, Taiwan (Lee 1990 *in* Randall and Heemstra 2006) and Lubang Island, Philippines (Randall and Heemstra 2006) and now recorded also in Bitung, Sulawesi Island, Indonesia.

Comparison and Remarks: The meristic data of *Odontanthias unimaculatus* was most similar to *O. grahami*, in sharing the following characters: dorsal fin soft rays 14, anal fin soft rays 7, scales on lateral line 36-37 and gill rakers on upper limb 13-14. If caudal fin shape and coloration were ignored, *O. unimaculatus* would seem most closely related to *O. tapui* and *O. chrysostictus*. The body proportions data between *O. unimaculatus* and *O. chrysostictus* are nearly the same, however the later species has longer body width, third dorsal spine and second anal spine but has shorter longest dorsal ray length, longest anal rays, caudal-fin length and caudal concavity. Other little different characters of *O. unimaculatus* with other seven species were the proportion of upper jaws length and proportion of body depth (Figure 3A and B).

The fish were collected using vertical hook and line by fishermen who targeting deep water groupers or snappers as *Epinephelus*, *Pristipomoides* and *Etelis*. The depth of the catch was about 100-150 meter.

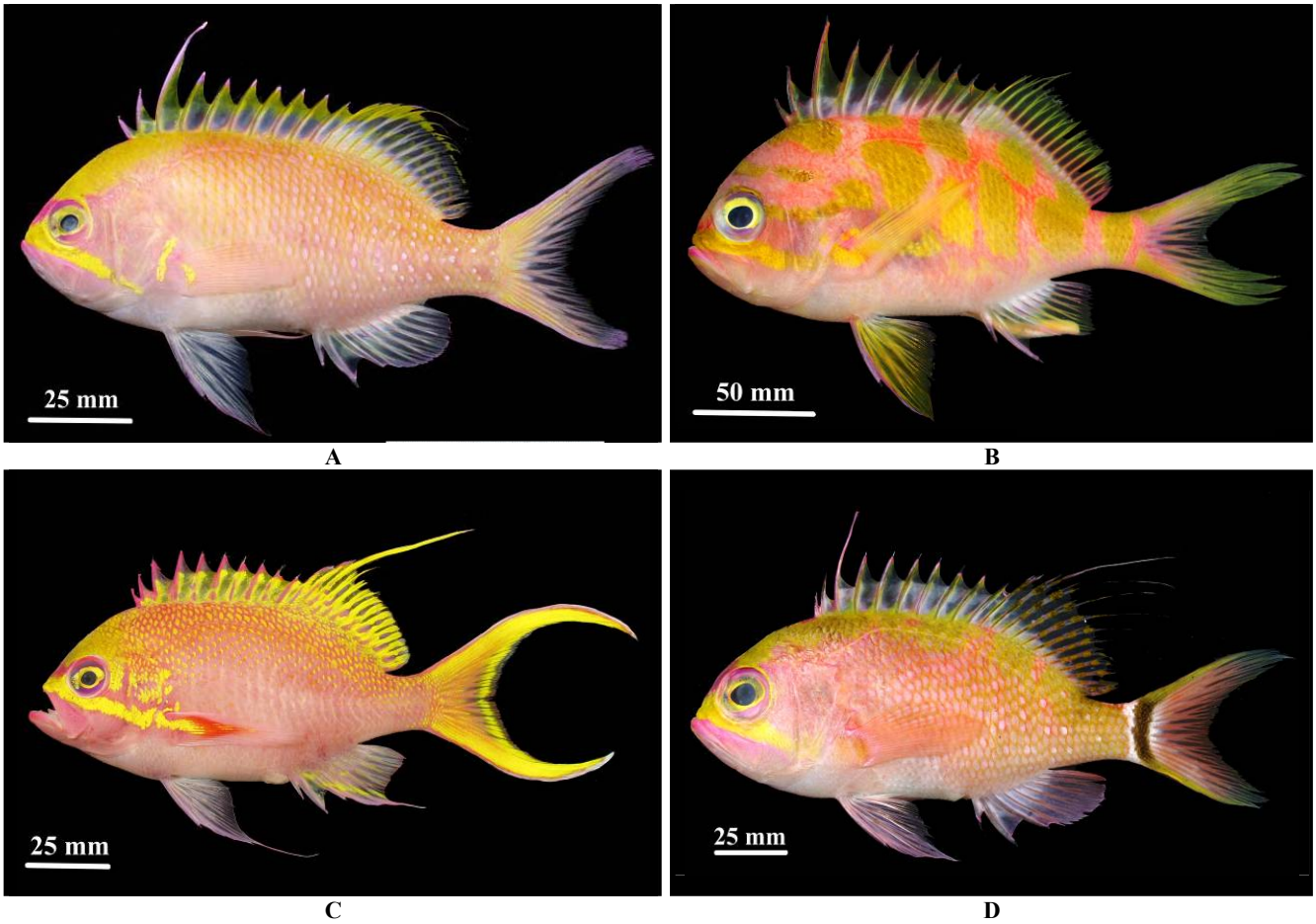

Figure 2. Four species of anthiine fish of the genus *Odontanthias* found from Bitung, North Sulawesi, Indonesia. (A). *O. unimaculatus*; (B). *O. borbonius*; (C). *O. chrysostictus*; (D). *O. rhodopeplus.*

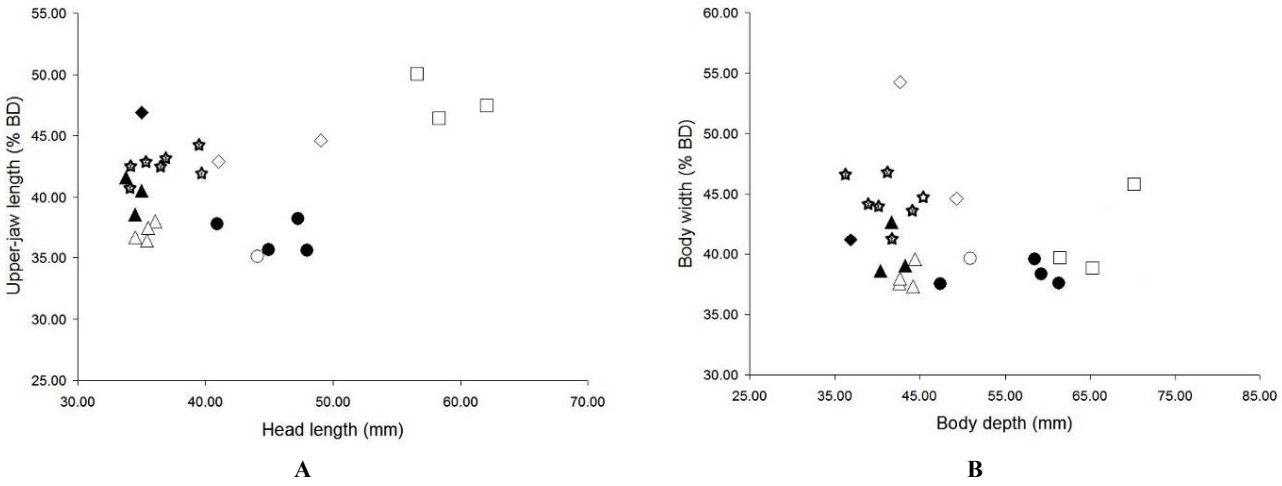

Figure 3. Ratio of upper jaws length (A) and body width (B) of eight species. Note: solid circles (*O. chrysostictus*); open diamond-shapes (*O. dorsomaculatus*); solid diamond-shapes (*O. grahami*); open triangles (*O. katayamai*); open squares (*O. rhodopeplus*); solid triangles (*O. tapui*); star (*O. unimaculatus*); open circles (*O. wassi*).

Comparative materials: *O. chrysostictus*: LBRC-F 001392, 133.3 mm SL, no data on depth, hook and line, Fish market Girian, Bitung, North Sulawesi, Indonesia, 15 December 2009, collected by T. Peristiwady; LBRC-F 001287, 109.66 mm SL, no data on depth, hook and line, Batuputih, Bitung, North Sulawesi, Indonesia, 16 October 2009, collected by T. Peristiwady; FRLM 34846, 134.23 mm SL, no data on depth, hook and line, Bitung, North

Sulawesi, Indonesia, 13 November 2008, collected by S. Kimura, H. Sakakibara and T. Peristiwady; *O. dorsomaculatus*: HUMZ 74194, 134 mm SL, Saya de Malha Bank, 120 m, 3 September 1977; HUMZ 73951, 110 mm SL, Saya de Malha Bank, 120 m, 2 September 1977 (Katayama and Yamamoto 1986); *O. grahami* Randall & Heemstra: AMS I.32142-010, 94 mm SL, Australia, 126-130 m, collected by Ken J. Graham, 16 February 1991 (Randall and Heemstra 2006); *O. katayamai*: BPBM 8527, 163 mm SL, Mariana Islands, Guam, collected by Fish and Wildlife, Government of Guam, 20 June 1968 (Randall et al. 1979); BPBM 5848, 126.5 mm SL, Mariana Islands, 300 m, collected by Fish and Wildlife, Government of Guam, 7 April 1967 (Randall et al. 1979); URB 78-0148, 145 mm SL, Ryukyu Islands, more than 100 m, collected by T. Yoshino, 6 June 1973 (Randall et al. 1979); MNHN 1978/136, 150.7 mm SL, Ryukyu Islands, collected by T. Yoshino, August 1973 (Randall et al. 1979); *O. rhodopeplus*: UMRP 37795, 167.5 mm, Fish market Okinawa, no data on depth, collected by T. Yoshino; UMRP 0492, 160.5 mm, Fish market, Okinawa, no data on depth, collected by T. Yoshino; UMRP 10524, 159 mm, Fish Market Okinawa, no data on depth collected by T. Yoshino; *O. tapui*: MNHM 1978/459, 141 mm, Society Islands, Tahiti, about 300 m, collected by Jean Tapu, April 1975 (Randall et al. 1979); RUSI 4680, 157 mm SL, Cook Islands, collected by Ronald Powell, July 1964; BPBM 17345, 127 mm SL, Society Islands, Tahiti, collected by Anthony Nahacky, 1973 (Randall et al. 1979); *Odontanthias wassi* Randall & Heemstra: BPBM 29373 (holotype), 1 specimen, 121.0 mm SL, American Samoa, Ofu Island, off Ofu Village, about 100 meters, hook and line, collected by Paul Pedro (local fishermen), 2 September 1983.

CONCLUSION

This species, *Odontanthias unimaculatus*, is the first record of anthiine fish genus *Odontanthias* from Indonesia waters. This record was based from seven specimens captured by using vertical hook and line on deep water groupers or snappers fishing grounds from the depth of about 100-150 meter. This first record is bringing the total number of species of this genus known in Indonesian waters become four species (*O. borbonius*, *O. chrysostictus*, *O. rhodopeplus* and *O. unimaculatus*). In the world, this species was also found from Tanabe, Wakayama Prefecture and Suruga Bay, Japan; Keelung and Kaohsiun, Taiwan and Lubang Island, Philippines.

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Morphological divergences among three sympatric populations of Silver Sharkminnow (Cyprinidae: *Osteochilus hasseltii* C.V.) in West Sumatra

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ABSTRACT

Roesma DI, Santoso P. 2011. Morphological divergences among three sympatric populations of Silver Sharkminnow (*Cyprinidae: Osteochilus hasseltii* C.V.) *in West Sumatra. Biodiversitas 12: 141-145.* Silver sharkminnow (*Osteochilus hasseltii* C.V.) named by local people as Asang is one of potential Cyprinid fishes species found in several different ecosystems in West Sumatra. The differences of habitat types and another ecological factor among populations may have significant influences on variation and differentiation of morphological characters of this species. In order to elucidate the pattern of morphological divergence, meristic and morphometric characters of *O. hasseltii* in Singkarak and Dibawah Lake and adjoining river were compared. Phenogram based on cluster analysis showed specific morphological divergence among populations. There were 23 characters significantly different among all compared populations, the highest degree of differentiation was found between Singkarak and Dibawah Lake population (22 characters significantly different) and the most similar population were Singkarak Lake and Ombilin an outlet river of lake (only six characters significantly different).

Key words: fish, *Osteochilus hasselti*, meristic, morphometric.

INTRODUCTION

Silver sharkminnow (*Osteochilus hasseltii* C.V) is known as one of widely distributed cyprinids species in many freshwater ecosystems in Sundaland, Indochina, Burma, and introduced to Sulawesi, inhabitant of lakes, river streams and ponds. They could be distinguished morphologically from the other species of the genus by having 12-18 branched dorsal rays; 6-9 rows of spots along scale rows (not always distinct), and a large round blotch on the caudal peduncle, no black midlateral stripe; sometimes with a spot above a pectoral fin (Kottelat et al. 1993).

In West Sumatra, *O. hasseltii* is common cyprinid species in the lakes and rivers, named by local people as Asang Fish. It is frequently catched by fishermen and traded intensively in the traditional markets as potential comodity of freshwater fisheries. Intensive use of small mesh gill nets, electrofishing and dynamite or poisons for catching fishes has given impacts on stability of the number of natural populations for several decades. At least, it has limited the maximum reached size of remaining fishes.

There are Singkarak and Dibawah lake as the natural habitat of *O.hasseltii* among several aquatic ecosystems in West Sumatra. Singkarak is the second largest lake in Sumatra after Toba lake with 107.8 km^2 of surface area and located at 362 m above sea level. The natural outlet is Ombilin river which flows to the Malacca strait. This lake has been experienced by antropogenic polutions continuosly for long period such as organic and anorgnic polutants, pesticides, detergens and another things from people around or run off from the inlet rivers. Naturally, it is connected to Dibawah lake by Batang Lembang river which flows from Dibawah lake into Singkarak. Dibawah lake has 11.2 km^2 of surface area and located at higher altitude than Singkarak (1462 m above sea level) (Lehmusluoto et al. 1997). Differ from Singkarak, Dibawah lake has better quality of water with limited polutant sources. Surroundings area are dominated by farmlands and underbrushes. The fishermen activities are less in compared with Singkarak, make it possible to state that the natural populations of fishes stay in relatively stable.

Although Singkarak and Dibawah lakes are included to sympatric category, the differerences in altitude, surface area, and some ecological aspects would be possible forces generate the differences in fish species variation and differentiation living within. These external factors usually have a great influences on species divergence morphologically (Naesje et al. 2004) or ecologically (Fraser et al. 2005). From Mauguit et al. (2010), it was concluded that environmental forces are proposed to be significant strength to form fundamental morphological, physiological and physical changes of fish from hatcling to adulthood. Therefore, in term of some stated reasons, it is evident that there will be a specific pattern on morphological differences among fish populations in Singkarak and Dibawah lakes. This study is aimed to observe the degree of intraspecific diversity of *O. hasseltii* among those sympatric populations based on morphological characters. These informations are expected to be one of important baseline data in prepararation of biodiversity conservation policies.

MATERIALS AND METHODS

Sample collection

Samples of fish were collected by following standard procedures according to Cailiet et al. (1996) using nets and backpack electro fishing apparatus (12 volt) depending on the site where samples are collected (Singkarak Lake, Ombilin River as an outlet of Singkarak, and Dibawah Lake). Unstable characters (especially coloration) which might be lost after preservation were noted and photographed. Each of samples were labeled and fixed in 10% of formalin and later preserved in 70% ethanol. The identification key of Cyprinids species from Weber & de Beaufort (1916) and Kottelat et al. (1993) was used to confirm the validity of fish species. All specimens are at present lodged in the Laboratory of Genetics and Cytology Universitas Andalas, Padang, West Sumatra but the majority will be deposited later at the Museum Zoologicum Bogoriense Fish collection, Cibinong, Bogor, Indonesia.

Morphological characters (morphometric and meristic)

Morphometric measurments and meristic counts were conducted by following Cailiet et al. (1996) and Costa et al. (2003). All counts and measurements were made from specimens preserved in 70% ethanol. Morphometric measurements and meristic count were taken point to point from left side of fishes using digital calipers to the nearest 0.1 mm. Small meristic characters were examined under binocular microscope. Of 28 morphometric and meristic characteristics that have been analyzed, the measurements taken are as follows: Total length (TL), Standard length (SL), Depth of caudal peduncle (DCP), Length of caudal peduncle (LCP), Length of predorsal (LPr), Length of dorsal spine (LDS), Length of anal spine (LAS), Body depth (BD), Brachiostegal rays (BR), Length of pectoral (Lpec), Length of pelvic (Lpel), Length of longest dorsal spine (LLDS), Head length (HL), Head width (HDW), Snout length (SnL), Sub-orbital width (SOL), Orbit to preopercle distance (OPD), Eye diameter (ED), Upper jaw length (UJL), Dorsal fin spines (DS), Dorsal soft ray (DSR), Anal spines (AS), Anal soft ray (ASR), Total pectoral rays (TPR), Scales along lateral line (SALL), Scales above lateral line (SabLL), Scales below lateral line (SBLL), Scales before dorsal fin (SBGF). In addition we also measured 21 body proportions (truss) according to Strauss and Bookstein (1982).

Statistical analysis

In order to standardize the different of overall body size among specimens, all morphometric measurements data were divided by standard length (SL) and presented as ratio. Cluster analysis using Unweighted Pair Group Method Arithmetic Average (UPGMA) with NTSYSpc Ver.2.02i obtained from Herbarium ANDA, Padang, West Sumatra, was conducted to examine the relations among characters across all populations. The result of cluster analysis were used to produce a phenogram of which cluster of similar characters could be identified. Taxonomic distance from each population was estimated by Euclidean distance (Rohlf 2001). Non parametric Kruskall-Wallis test was used to identify the morphological variations among populations, and Mann-Whitney *U* test was used to observe the differences between populations. Both of the test were generated by using SPSS statistics software for PC.

RESULTS AND DISCUSSION

A total of 111 specimens have been analyzed morphologically, representing Singkarak Lake (60 ind.) Dibawah Lake (39 ind.) and Ombilin River (12 ind.). The characters of *O. hasseltii* are rather compressed body; subinferior mouth; upper lip continuous with skin of the snout by a groove; but coninuous with the skin of the snout; 33-38 lateral line scales; plaint caudal fin; 5-7 scales in between lateral line and origin dorsal fin; 5 scales in between lateral line and origin pelvic fin; flat abdomen and rounded; lateral line extending onto median caudal ray; simple ray/anal fin spine posterior edge non serrated; anal fin with 5-6 soft rays and 1 spinous;15-18 soft rays on dorsal fin; nostrils with membrane; no tubercles on snout; large round spot on caudal peduncle; large round spot on caudal peduncle.

The phenogram which was constructed base on 49 morphological characters by using UPGMA cluster analysis show the specific pattern of phenetic relationship among three populations of *O. hasseltii* as in Figure 1. Dibawah Lake population separated significantly with Singkarak Lake and Ombilin River by 10.35 euclidean coefficient. Both of Singkarak Lake and Ombilin River populations were the closest sympatric populations phenetically, expressed the higher degree of similarities in their morphological structures.

The divergences of morphological characters among all compared populations were relatively high with 23 characters significantly different based on Kruskall-Wallis test ($p \leq 0.05$) (Table 1). The characters consisted of 18 morphometric characters and five meristic characters. It indicated the significant degree of morphological variations among individual of fishes both of intrapopulation and interpopulation. From Mann-Whitney *U* test (Table not showed) we have the specific information (Table 2) which showed that the degree of morphological divergences were varies and it strongly supported the phenetic pattern found from the previous cluster analysis. The highest degree of morphological differentiations was Singkarak versus Dibawah lake (22 characters significantly different), followed by Dibawah lake versus Ombilin river (13 characters significantly different) and the most similar were Singkarak and Ombilin populations (only six characters significantly different). There was nothing among 49 analyzed characters performance the consistency of differentiation for all comparisons.

The divergences on morphological structures among populations of fishes species are common biological phenomenon, but the pattern of their differentiations are usually unique related to the strength of affecting factors experienced (Keeley et al.2005). Manel et al.(2003) stated that divergences may has genetic based pattern in allopatric populations but it is rare for sympatric populations.

Table 1. Comparison of *O. hasseltii* interpopulation by Kruskall-Wallis test of standard length (SL) and of ratio of measurements. For each sample, mean and standard deviation, minimum and maximum values are given below each character, n = sample size; *significance level p≤0.05

Characters	Population			Kruskall-Wallis test *O. hasseltii*
	O. hasseltii Dibawah Lake	*O. hasseltii* Singkarak Lake	*O. hasseltii* Ombilin River	
	n = 39	n = 60	n = 12	
TL	1.28±0.04	1.29±0.03	1.27±0.04	X²=7.161
	1.46-1.22	1.37-1.21	1.37-1.24	*p*=0.028*
SL	118.79±15.96	127.35±25.17	130.80±26.18	X²=2.574
	178.03-92.99	183.06-96.18	174.79-102.98	*p*=0.276
DCP	0.14±0.01	0.14±0.01	0.14±0.02	X²=8.515
	0.15-0.13	0.15-0.12	0.21-0.13	*p*=0.014*
LPr	0.47±0.01	0.46±0.02	0.46±0.07	X²=17.391
	0.52-0.45	0.53-0.43	0.68-0.42	*p*=0.000*
BD	0.36±0.01	0.35±0.02	0.36±0.05	X²=6.484
	0.39-0.34	0.39-0.32	0.51-0.33	*p*=0.059
BR	0.03±0.02	0.03±0.01	0.03±0.01	X²=1.563
	0.04-0.02	0.04-0.02	0.04-0.01	*p*=0.458
Lpec	0.19±0.01	0.19±0.01	0.20±0.02	X²=3.782
	0.24-0.16	0.22-0.15	0.28-0.19	*p*=0.151
Lpel	0.19±0.01	0.19±0.01	0.20±0.02	X²=4.236
	0.21-0.17	0.21-0.16	0.26-0.18	*p*=0.120
LLDS	0.24±0.02	0.24±0.02	0.23±0.03	X²=2.484
	0.29-0.21	0.28-0.19	0.31-0.21	*p*=0.289
HL	0.22±0.01	0.23±0.01	0.24±0.03	X²=5.809
	0.24-0.20	0.25-0.21	0.32-0.22	*p*=0.055
HDW	0.12±0.01	0.12±0.01	0.13±0.01	X²=2.831
	0.13-0.11	0.14-0.11	0.16-0.11	*p*=0.243
SnL	0.05±0.01	0.05±0.01	0.05±0.01	x²=4.946
	0.06-0.04	0.07-0.02	0.07-0.03	*P*=0.084
SOL	0.09±0.01	0.09±0.01	0.08±0.01	x²=0.608
	0.11-0.07	0.11-0.06	0.11-0.06	*P*=0.738
OPD	0.06±0.01	0.06±0.01	0.07±0.01	X²=7.132
	0.07-0.05	0.08-0.05	0.08-0.05	*p*=0.028*
ED	0.05±0.01	0.05±0.01	0.05±0.01	X²=1.948
	0.06-0.04	0.06-0.04	0.07-0.04	*p*=0.378
UJL	0.06±0.01	0.06±0.01	0.06±0.01	X²=1.188
	0.08-0.04	0.08-0.04	0.08-0.05	*p*=0.552
GW	0.14±0.01	0.14±0.01	0.15±0.02	X²=13.447
	0.18-0.12	0.16-0.13	0.22-0.14	*p*=0.001*
DS	1.00±0.00	1.00±0.00	1.00±0.00	X²=0.000
	1.00-1.00	1.00-1.00	1.00-1.00	*p*=1
DSR	16.35±0.68	15.92±0.70	16.17±0.72	X²=7.384
	18.00-15.00	17.00-15.00	17.00-15.00	*p*=0.025*
AS	1.00±0.00	1.00±0.00	1.00±0.00	X²=0.000
	1.00-1.00	1.00-1.00	1.00-1.00	*p*=1
ASR	5.00±0.00	5.05±0.22	5.00±0.00	X²=3.726
	5.00-5.00	6.00-5.00	5.00-5.00	*p*=0.155
TPR	12.18±0.77	12.67±0.84	12.83±0.83	X²=9.839
	14.00-11.00	15.00-11.00	15.00-12.00	*p*=0.007*
SALL	34.58±0.81	35.26±0.88	35.33±0.98	X²=10.616
	36.00-33.00	38.00-34.00	37.00-34.00	*p*=0.005*
SAbLL	6.00±0.00	5.97±0.28	6.00±0.00	X²=0.627
	6.00-6.00	7.00-500	6.00-6.00	*p*=0.731
SBLL	5.00±0.00	5.00±0.00	5.00±0.00	X²=0.000
	5.00-5.00	5.00-5.00	5.00-5.00	*p*=1
SBDF	11.08±0.56	10.46±0.63	10.67±0.65	X²=12.931
	12.00-11.00	12.00-9.00	12.00-10.00	*p*=0.002*
SASP	6.95±0.22	6.55±0.42	6.67±0.49	X²=13.780
	7.00-7.00	7.00-5.00	7.00-5.00	*p*=0.001*
CFR	16.78±0.56	16.85±0.53	16.83±0.39	X²=0.521
	18.00-15.00	18.00-15.00	17.00-16.00	*p*=0.770
T1	0.18±0.01	0.18±0.01	0.19±0.03	X²=9.998
	0.22-0.16	0.20-0.16	0.29-0.17	*p*=0.670
T2	0.29±0.02	0.28±0.02	0.30±0.04	X²=5.231
	0.32-0.26	0.34-0.25	0.40-0.26	*p*=0.073
T3	0.39±0.02	0.37±0.03	0.39±0.05	X²=19.563
	0.46-0.35	0.41-0.36	0.55-0.37	*p*=0.000*
T4	0.20±0.02	0.22±0.02	0.23±0.03	X²=35.324
	0.24-0.17	0.26-0.18	0.28-0.19	*p*=0.000*
T5	0.14±0.01	0.15±0.01	0.17±0.02	X²=47.872
	0.16-0.12	0.18-0.13	0.24-0.14	*p*=0.000*
T6	0.15±0.02	0.16±0.01	0.16±0.03	X²=6.789
	0.19-0.12	0.18-0.14	0.25-0.14	*p*=0.034*
T7	0.11±0.01	0.10±0.01	0.10±0.01	X²=5.789
	0.11-0.11	0.12-0.09	0.14-0.09	*p*=0.055
T8	0.22±0.01	0.23±0.02	0.25±0.04	X²=10.219
	0.26-0.19	0.28-0.19	0.35-0.22	*p*=0.006*
T9	0.06±0.01	0.06±0.01	0.06±0.01	X²=8.736
	0.08-0.05	0.07-0.04	0.09-0.06	*p*=0.013*
T10	0.24±0.01	0.224±0.02	0.27±0.04	X²=8.258
	0.27-0.20	0.30-0.20	0.37-0.22	*p*=0.016*
T11	0.27±0.02	0.27±0.02	0.27±0.03	X²=2.139
	0.30-0.22	0.30-0.20	0.36-0.25	*p*=0.343
T12	0.23±0.01	0.22±0.01	0.23±0.03	X²=4.697
	0.27-0.21	0.27-0.21	0.33-0.21	*p*=0.096
T13	0.42±0.01	0.42±0.02	0.44±0.04	X²2.817
	0.45-0.39	0.48-0.36	0.59-0.40	*p*=0.244
T14	0.35±0.01	0.34±0.02	0.37±0.05	X²=9.804
	0.38-0.33	0.38-0.31	0.53-0.33	*p*=0.011*
T15	0.35±0.01	0.35±0.02	0.36±0.04	X²=2.500
	0.39-0.33	0.39-0.31	0.49-0.33	*p*=0.286
T16	0.36±0.01	0.35±0.02	0.37±0.04	X²=7.274
	0.40-0.34	0.39-0.31	0.49-0.34	*p*=0.026*
T17	0.39±0.02	0.38±0.02	0.40±0.06	X²=11.061
	0.44-0.37	0.42-0.35	0.57-0.37	*p*=0.004*
T18	0.36±0.02	0.34±0.02	0.36±0.05	X²=16.892
	0.39-0.33	0.40-0.30	0.49-0.33	*p*=0.000*
T19	0.23±0.01	0.21±0.01	0.22±0.03	X²=43,215
	0.26-0.21	0.24-0.19	0.30-0.19	*p*=0.000*
T20	0.18±0.01	0.18±0.01	0.19±0.03	X²=2.835
	0.21-0.14	0.21-0.16	0.27-0.17	*p*=0.242
T21	0.22±0.01	0.23±0.01	0.23±0.03	X²=18.897
	0.25-0.17	0.27-0.20	0.33-0.21	*p*=0.000*

Table 2. Differentiated morphological characters among compared populations of *O. hasseltii* based on Mann-Whitney *U* test statistical analysis

Characters	Compared populations		
	Dibawah Lake vs Singkarak Lake	Dibawah Lake vs Ombilin River	Singkarak Lake vs Ombilin River
TL	-	+	+
DCP	+	-	+
LPr	+	+	-
BD	+	-	-
HL	+	-	-
GW	+	+	-
DSR	+	-	-
TPR	+	+	-
SALL	+	+	-
SBDF	+	+	-
SASP	+	+	-
T2	+	-	-
T3	+	-	-
T4	+	+	-
T5	+	+	-
T6	+	-	-
T7	+	-	-
T8	-	+	-
T9	-	+	+
T10	-	+	+
T14	+	-	+
T16	+	-	+
T17	+	-	-
T18	+	-	-
T19	+	+	-
T21	+	-	-
Total diff. char.	22	13	6

Note: (+) indicated significant differentiation, (-) no differentiation

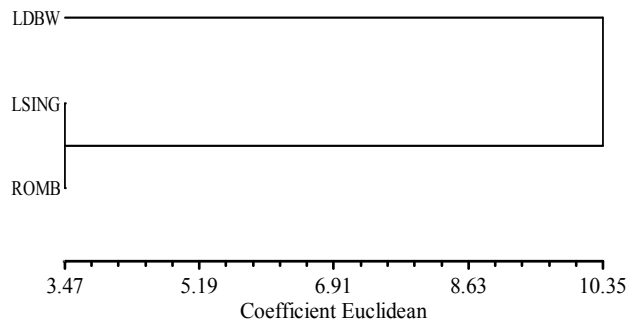

Figure 1. Phenogram of three populations of *O. hasseltii* based on morphological data: LDBW (Dibawah Lake), LSING (Singkarak Lake), ROMB (Ombilin River, outflow of Singkarak Lake).

Taking deduction from of those evidences, the pattern of morphological differentiation of *O. hasseltii* among three sympatric populations confidently related to ecological or environmental forces than the genetic based changes. As populations within each lake and river are not completely isolated by physical barrier to gene flow, other factors must be involved in the variation. At least the selective force might have contributed to the divergences those *O. hasseltii* was selection on ecological traits. Szalai et al. (2003) observed that there were the density-dependent growth regulation generated the morphological divergence of *Coregonus hoyi* in lake Michigan. Hjelm et al. (2003) also found that the feeding performance and the diet shift in fish species related to change in functional morphology over ontogeny. Naesje et al. (2004) stated that the variation among populations of fish characters could be induced by ecological factors interacted with fundamental genetic roles. According to Rutaisire et al. (2005), variations and differentiations among fish populations, notably in morphological characters, should be drived by the differences in environmental conditions during ontogeny, level of food and predators availablity, difference in spawning area, and the level of polutants intensity and antropogenic stresses. According to Nakamura (2003), and Naesje et al. (2004), there were significant differences in the mean number of dorsal fin rays and diameter of pupil and the body of nature fish is determined by food availability and physical environment during ontogeny between rivers system. Langerhans and Makowicz (2009) found the unique morphological variations in live bearing fish (*Gambusia caymanensis*) forced by presence of predators and historical island effects in Cayman islands.

Concerning with *O. hasseltii* in three studied populations, there are some ecological factors proposed as the complex of morphological divergences inducers. Firstly, difference in water temperature may play the significant roles on rate of individual growth. Notably in Dibawah lake, lies in higher altitude than Singkarak lake and Ombilin river, the water temperature is almost extremely lowest among others. According to Kassam et al. (2003), it should be given an impact on fish early from their ontogeny development to adult stage. Samaee et al. (2006) as well as Krabbenhoft et al. (2009) described that other environmental factor also underlying morphological changes such as water clarity, water depth and flow, food availability and physical complexity. Second, polutant intensity of Dibawah lake is distinctly lower in comparing to the others, whereas Singkarak and Ombilin river are experienced with so many kind of polutant sources directly or undirectly produced from dwellers surroundings and pesticides and fertilizer recidues of farmlands. It conducts the differences in cascading effects of environmental stress to comunities and populations. Schaack and Chapman (2003) and Keeley et al. (2005) mentioned that the level of environmental stress to the species including polutions would be acted as one of the main forces toward the species divergences in case of specific adaptive change in characters.

The variations of morphological characters of *O. hasseltii* among sympatric populations also proposed to be induced by the impact of species composition of whole fishes communities. Singkarak lake and Ombilin river consist more diverse of fish species than Dibawah lake, promote the differences of cascading manifestation of intraspecific and interspecific competition among fishes, notably for food sources and space. Salsabila (1987) identified 29 species, members of 11 families in Singkarak lake. Usman and Amir (1995) reported seven species of fishes in Dibawah lake. The complexity of comunities composition of fishes combined with spatial scale of habitat types and landscapes promoted the species divergences (Wang et al. 2003). Layman et al. (2005) also proposed that the combination of fish assemblage composition in combination with commercial netting has been taken an important role on morphological differences.

The effect of current in aquatic ecosystem between lake and river is commonly found to be the significant factor on fishes morphological divergence. Many organisms can modulate their morphology in response to environmental cues. Body morphology of an individual has a great importance for its performance in prevailing environment. Robinson and Parson (2002) stated that morphological plasticity of fishes species may play a role in the notably high rate of divergences. According to McGuigan et al. (2003) there appears a clear relationship between shape and function in adaptations of Rainbow fish (*Melanotaenia* spp.) inhabited rivers and lake. The body form correlates with the swimming performance of an individual. Turan et al. (2004) concluded that the major of morphological characters at the intraspecific levels i.e. phenotypic variation is not directly under genetic control but is subjected to environmental modification. It is the apropriate way to understand the dissimilarities of morphological characters of Singkarak and Dibawah lake compared to Ombilin river's population.

Even though the study on species diversity of *O. hasseltii* was only limited for its morphological aspects, there were some important informations concerning the signs of specific patterns of species adaptation toward the environmental forces. It has significant morphological variations and divergences among sympatric populations and seems to be dominated by ecological aspect as

discussed above. The clear confirmation based on genetic analysis must be conduct on the same species and locations for the future researches.

CONCLUSION

There were 23 characters significantly different among Singkarak and Dibawah Lake and adjoining river populations, the highest degree of differentiation was found between Singkarak and Dibawah Lake population (22 characters significantly different) and the most similar population were Singkarak Lake and Ombilin an outlet river of the lake (only six characters significantly different). Concerning with *O. hasseltii* in three studied populations, there are some ecological factors proposed as the complex of morphological divergence inducers. The genetic analysis must be conducted on the same species and locations for the future researches. The information will define the importance of the lakes and rivers in diversity.

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Diversity and useful products in some Verbenaceous member of Melghat and Amravati regions, Maharashtra, India

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ABSTRACT

Ingole SN. 2011. Diversity and useful products in some Verbenaceous member of Melghat and Amravati regions, Maharashtra, India. Biodiversitas 12: 146-163. Verbenaceae is a large family of very diverse habit. The present study deals with detailed characteristics, distribution and economically important products of some verbenaceous members of Melghat and Amravati regions. During the survey twenty members belonging to fourteen genera of Verbenaceae were collected. Some members occur abundantly either in wild or cultivated state like *Lantana camara* L. var. *aculeata* Mold., *Lantana flava* Medik., *L. nivea* Vent., *Glandularia bipinnatifida* (Schauer) Nutt., *Duranta erecta* L., *Vitex negundo* L., *Volkameria inermis* L., *Clerodendrum phlomidis* L. f., *Clerodendrum splendens* G. Don, *Nyctanthes arbor-tristis* L. etc. while *Petrea volubilis* L., *Gmelina arborea* Roxb., *G. philippensis* Cham., *Stachytarpheta jamaicensis* (L.) Vahl., *S. mutabilis* (Jacq.) Vahl., *Rotheca serrata* (L.) Steane & Mabb., *Holmskioldia sanguinea* Retz. are not much common and occur in limited locations. *Phyla nodiflora* (L.) Greene, a creeping much-branched herb is found typically in wet places. *Tectona grandis* L. f. occurs very variable in size according to its habitat and is common dominant tree in forest of Melghat and also planted in plains. *Clerodendrum infortunatum* L., a gregarious tomentose shrub is exclusively found in shades of forest at limited spots in higher elevations of Melghat. The various members are not only beautiful ornamentals but also the source of important medicinal products useful in a broad range of diseases including skin disorders and snake remedies; they contain alkaloids, sterols, saponin, glucosides, dyes etc. and are economically quite important e.g. as high quality timber. On basis of morphological diversity the generic key is provided.

Key words: Verbenaceae, diversity, Melghat, Amravati, medicinal plants.

INTRODUCTION

Verbenaceae is a comparatively a large family composed of about 35 genera and about 1000 species (Atkins 2004). The name Verbenaceae was given by Persoon (1806) and has been conserved over older name Pyrenaceae (Vent 1799). In India it is represented by nearly 22 genera and more than 110 spp, some of the members being only grown as ornamentals. Verbenaceae is a widely distributed family of very diverse habit. They may be trees, shrubs or herbs, lianas occur fairly commonly, but no general distinctive habit is peculiar to the family as a whole.

Verbenaceae is a family of mainly tropical flowering plants. The plants in Verbenaceae can usually be recognized by combination of traits, containing trees, shrubs and herbs notable for heads, spikes, or clusters of small flowers, many of which have aromatic smell (Stevens 2001), with opposite leaves and flowers with slightly bilateral corolla symmetry. Their fruits are fleshy or dry, generally with two or four seeds, often dividing into two or four segments (Marx et al. 2010).

Herbs, lianas, shrubs or trees, sometimes with prickles or thorns; stems usually square in cross-section; usually with iridoids; often with phenolic glycosides. Hairs simple, gland-headed, with ethereal oils (including terpenoids), and non-glandular, usually unicellular, sometimes calcified or silicified. Leaves opposite or occasionally whorled, simple, sometimes lobed, entire to serrate, with pinnate venation; stipules lacking. Inflorescences indeterminate, the lateral units individual flowers or appearing as individual flowers or appearing as individual flowers, forming racemes, spikes, or heads, terminal or axillary. Flowers bisexual, bilateral. Sepals 5, connate, the calyx tubular to bell shaped, persistent, occasionally enlarged in fruit. Petals 5 (but sometimes seemingly 4 fusion of the upper pair), connate, the corolla weakly 2-lipped, the lobes imbricate. Stamens 4, didynamous; filaments adnate to corolla; pollen grains usually tricolpate, exine thickened near apertures. Carpels 2, connate, ovary superior, unlobed to ±4-lobed, 2-locular but appearingly 4-locular due to development of false septa, but sometimes 1 carpel suppressed and then appearing only 2-locular, with axile placentation; style not apically divided, terminal; stigma usually 2-lobed, conspicuous, with well developed receptive tissue. Ovules 2 per carpel (i.e. usually 1 in each apparent locule), each marginally attached (attached directly to margins of false partitions), with 1 integument and a thin walled megasporangium. Nectar disc usually present. Fruit a drupe with 2 or 4 pits (single and 2-lobed in Lantana), or a schizocarp splitting into 2 or 4 nutlets; endosperm lacking (Judd et al. 2002).

Family Verbenaceae traditionally divided into different tribes such as I. Phrymeae, II. Stilbeae, III. Chloantheae, IV. Verbeneae, V. Viticeae, VI. Caryopterideae, VII. Symphoreae, and VIII. Avicennieae (Bentham and Hooker 1862-1883). According to recent phylogenetic studies (Cantino 1992; Olmstead et al. 1993, 2000, 2001; Wagstaff and Olmstead 1997) Verbenaceae is not the family it used to be and these studies have shown that numerous genera traditionally classified in Verbenaceae belong instead in Lamiaceae. The biggest change involves the wholesale transfer of some 10 tribes and over 50 genera to the Lamiaceae (Cantino et al. 1992). Several smaller groups have been segregated into their own or other families as well. What remains in Verbenaceae s.s. comprises most of Briquet's (1895) subfamily Verbenoideae. Competing morphology-based classifications that rely on different traits conflict in significant ways (Marx et al. 2010). Molecular studies by Marx et al (2010) based on analysis of 7 chloroplast DNA regions for 109 species, representing all genera except one monotypic genus; provide inference into evolutionary relationships in Verbenaceae. The molecular phylogeny shows that name of the traditional classifications reflect phylogenetic relationships very well. Marx et al (2010) presented a new tribal classification, including one new tribe, Neospartoneae trib. nov. Eight clades are recognized as tribes viz. Casselieae, Citharexyleae, Duranteae, Lantaneae, Neospartoneae trib. nov., Petreeae, Priveae and Verbeneae. Lantaneae and Verbeneae together form a derived clade that comprises approximately two thirds of the species in Verbenaceae. The genera like *Clerodendrum*, *Gmelina*, *Holmskioldia*, *Tectona*, *Vitex*, etc. are now moved to Lamiaceae (GRIN 2011). The difficulty that led to confusion in distinguishing Verbenaceae from Lamiaceae had to do with the degree of separation of the locules and the position of the style, used by many treatment and keys (e.g. Cronquist 1981) to distinguish the families. However, the fundamental distinction has to do with where the ovules attach in relation to the false partitions that divide each carpel into two locules. In Verbenaceae, the ovules attach directly to the margins of the false carpel septa, whereas in Lamiaceae, the ovules attach to the sides of the in rolled carpel walls (Marx et al. 2010).

In the present work, 14 traditional genera of Verbenaceae occurring in the Amravati and Melghat region are studied. According to molecular research given by Marx et al (2010), the following tribes are recognized in which the studied genera are classified. Petreeae Briquet i.e. *Petrea volubilis* L., Duranteae Bentham i.e. *Duranta erecta* L., *Stachytarpheta jamaicensis* Vahl., *Stachytarpheta mutabilis* (Jacq.) Vahl., Verbeneae Dumortier i.e. *Glandularia bipinnatifida* (Schauer *Phyla nodiflora* (L.) Greene), Lantaneae Endlicher i.e. *Lantana camara* L.Nutt. Rest of the genera falls in tribe Viticeae of Bentham and Hooker (1862-1883), e.g. *Tectona*, *Gmelina*, *Clerodendrum*, *Holmskioldia*, and *Vitex*, which are excluded from Verbenaceae, once were the part of it. Hence, all are also taken for the present study. Some workers had suggested inclusion of *Nyctanthes arbor-tristis* L. in Verbenaceae. Therefore it has been also studied.

MATERIALS AND METHODS

The plant materials for the present study were collected from different habitats in Amravati district (Maharashtra, India). Regular survey was made in different localities of this district including forest area of Melghat, plane cultivated area and various local gardens for cultivated members. Herbarium specimens of collected plants were made and macrocharacters were studied in the field. Geographical and ecological factors were carefully considered while recording microvariations in the species. The collection was made in accordance with the flowering season to enable the collection of the flowering material and proper diagnosis. The investigatory vegetative and floral critical study of the species was carried out in the laboratory by using dissecting and binocular microscope. The identifications were checked with reference to standard floras. Describing major venation pattern, terminology of Hickey (1973, 1979) is followed. The list of genera and species collected during present investigation is summarized as follows (Table 1).

Table 1. The genera and species of Verbenaceae collected during present investigation in Melghat and Amravati regions, Maharashtra, India.

Tribe	Genus	Species epithet
Duranteae	*Duranta*	*erecta* L.
	Stachytarpheta	*jamaicensis* Vahl.
		mutabilis (Jacq.) Vahl.
Lantaneae	*Lantana*	*camara* L.
		flava Medik.
		nivea Vent.
Petreeae	*Petrea*	*volubilis* L.
Verbeneae	*Glandularia*	*bipinnatifida* (Schauer) Nutt.
Viticeae	*Clerodendrum*	*infortunatum* L.
		phlomidis L. f.
		splendens G. Don
	Gmelina	*arborea* Roxb.
		philippensis Cham.
	Holmskioldia	*sanguinea* Retz.
	Nyctanthes	*arbor-tristis* L.
	Phyla	*nodiflora* (L.) Greene
	Rotheca	*serrata* Steane & Mabb.
	Tectona	*grandis* L. f.
	Vitex	*negundo* L.
	Volkameria	*inermis* L.

RESULTS AND DISCUSSION

Clerodendrum infortunatum L.

Clerodendrum infortunatum L. Sp. Pl. 637.1753, *Clerodendrum viscosum* Vent. Jard. Malm. : t. 25.1803; C. B. Cl in Hook. f. Fl. Brit. India 4: 594.1885; Cooke, Fl. Pres. Bombay 2: 513.1958 (Repr.); Naik Fl. Marathwada 698.1998; Singh et al. Fl. Mah. 2: 691.2001; Yadav and Sardesai, Fl. Kolhapur Dist. 372.2002; Almeida Fl. Mah. 4: 119.2003.

A perennial, gregarious, tomentose shrub 2.5-3 m high, woody, branches yellow, villous, branchlets purplish-green,

bluntly quadrangular, densely pubescent, with yellowish silky hairs. Leaves 15-19x9-10.5 cm, ovate, villous on both sides, more so on nerves and with small obscure glands beneath, acuminate, serrate, cordate at base; petioles 21-22x0.5-0.9 cm, cylindric, pubescent. Flowers 4.1 cm long, pinkish-white in large terminal cymose, pubescent, erect panicles, 15-30x10-20 cm, upper branches and calyces reddening; pedicels 0.5-0.6 cm long, pubescent; bracts 1x0.4-0.5 cm, leafy, deciduous, boat shaped, ovate, hairy, green-purplish, gland dotted at tips, acute, entire. Calyx 1.5x0.8 cm, campanulate, divided up to base into 5 segments, segments equal, large, 1.5x0.7 cm, broadly lanceolate-ovate, silky pubescent on both sides, purplish-red, gland dotted outside, 3-veined, acute, entire, persistent, much enlarged in fruit. Corolla 3.1 cm long, white with pinkish-purple tinge at throat, tube 1.9 cm long, slender, straight; limb 2 cm across, somewhat oblique, lobes 5, spreading, nearly equal, 1.1x0.7-0.8 cm, ovate-oblong, concave, pubescent outside, obtuse, entire. Stamens 4, inserted below the throat, much exserted, longer 3-3.5 cm long, shorter 2.8-3 cm long; filaments slender, filiform, basally purplish, distally white, glabrous; anthers 0.4x0.2 cm, oblong, cells parallel, deep brown, glandular, dorsifixed, introrse. Ovary 0.15x0.1 cm, elongate, glabrous, green, 4-lobular, 4-celled; 1-ovule in each cell; style 3.5 cm long, much exserted, filiform, pinkish-purple, glabrous; stigma purple, glabrous. Drupes 0.6-0.8 cm in diameter, globose, black, enclosed by pinkish-red enlarged calyx (Figure 24).

Field notes. Gregarious, tomentose shrub with long petioled villous ovate, serrate leaves. At limited spots in Melghat in higher elevations. Flowers opening time: 6 to 8 pm. Major venation pattern pinnate brochidodromous. Flowers and Fruits: June to December.

Distribution: In Maharashtra, Kerala, Andamans, Burma, Taiwan and Indonesia.

Habitat and ecology: In shade of forests at limited spots at higher elevation in Chikhalda (Melghat) not common.

Vernacular name: Bhandira (Marathi)

Uses. In Indonesia, is used for gonorrhea, juice as lotion. The leaves and roots are employed externally for tumors and skin disorders (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

Clerodendrum phlomidis L. f.

Clerodendrum phlomidis L. f. Suppl. 292.1781, *Clerodendrum multiflorum* (Burm. f.) O. Ktze. Rev. Gen. Pl. 3: 526.1891. *Volkameria multiflorum* Burm. f. Fl. Ind. 137, t. 45, f. 1.1786. C. B. Cl. in Hook. f. Fl. Brit. India 4: 590.1885; Cooke, Fl. Pres. Bombay 2: 511.1958 (Repr.); Naik Fl. Marathwada 698.1998; Singh et al. Fl. Mah. 2: 690.2001; Yadav and Sardesai Fl. Kolhapur Dist. 371.2002; Almeida Fl. Mah. 4: 116.2003.

A large scrambling, bitterly aromatic shrub or small tree, 4-8 m tall. Bark light-brown; branches pubescent, lenticellate, often drooping. Leaves 4-6x3.5-6 cm, broadly ovate to subrhomboid, pubescent, brittle, acute, crenate-dentate, undulate, subentire or entire, variously toothed in midportion, subcordate at base; petioles 1.8-2.5 cm long, densely pubescent. Flowers 4 cm long, medium sized, white or pink, fragrant in small dichotomous axillary cymes forming a rounded terminal panicle, 9-11 cm long, pedicels 0.3-0.5 cm long, finely pubescent; bracts 0.4-0.7x0.15-0.4 cm, caducous, obovate or lanceolate, leafy, pubescent on both the sides, shortly acuminate, entire, cuneate at base, with distinct median nerve. Calyx 1x0.9 cm, campanulate, 5-toothed, green, segments divided half way down, glabrous, unaltered or very slightly enlarged in fruit, segments equal, ovate, glabrous or very finely hairy, with median nerve, shortly acuminate. Corolla white or pink, 3.5 cm long, limb 2.1 cm across; tube 2.5x0.1 cm, long, slender, straight, greenish-white, with 4-5 obscure stripes, very finely pubescent outside and with shining hairs inside, limb more or less oblique, with 5 spreading 1x0.5 cm, nearly equal, elliptic, finely pubescent on lower side, glabrous above, obtuse, entire lobes. Stamens 4, inserted below the throat, much exserted, logner 2 cm long, shorter 0.8 cm long; filaments filiform, white, purplish distally, pubescent below, anthers 0.2x0.1 cm, oblong, cells parallel, deep purplish-brown, dorsifixed, introrse. Ovary 0.2x0.15cm, globose, green, papillate, 4-partite, 4-celled; 1-ovule in each cell; style 3.5-3.6 cm long, much exserted, filiform, white-purplish at top, glabrous; stigma glabrous, purplish. Drupes 0.6x0.5 cm, broadly obovoid, succulent, 4-lobed with 1-pyrene in each, sometimes lobes suppressed, wrinkled, depressed, enclosed by persistent calyx (Figures 19, 20).

Field notes. A large scrambling bitterly aromatic shrub or small tree with drooping branches and sub-rhomboid, brittle, crenate-dentate to entire leaves. Flowers white or rarely rosy pink. Flowers opening time: 8 to 9 pm. Major venation pattern actinodromous. Flowers and Fruits: October to January.

Distribution: In Kerala, Maharashtra and Himalayan regions of India and few Asian countries.

Habitat and ecology: Common throughout the plains in hedges and along banks of nalas. Also planted as a field-hedge.

Vernacular name: Taikal (Marathi)

Uses. Useful in inflammation, root is used as bitter tonic and given in convalescences and measles (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

Clerodendrum splendens G. Don

Clerodendrum splendens G. Don in Edinb. Phil. J. 9: 349.1824 non A Cheval. 1920; Bailey, Man. Cult. Pl. 845.1949; Naik, Fl. Marathwada 2: 700.1998. Singh et al. Fl. Mah. 2: 703.2001.

Woody, handsome straggling shrub reaching to great height. Leaves 11-14x8-12 cm, wide ovate, glabrous, deep green above, purplish marginally, pubescent on nerves beneath, short acuminate, entire, cordate-rounded at base, petioles 0.4 cm long, glabrous, thick, cylindric, purplish. Flowers 2 cm long, crimson in much branched cymose roundish panicles, 9-10 cm long, peduncles 2.8x0.25 cm, quadrangular, compressed, finely pubescent or nearly glabrous, purplish-green; pedicels 1 cm long, cylindric, finely pubescent, brownish-green; bracts small, lower ones slight larger, in pairs at forks, 0.25-0.3 cm long, less than 0.1 cm across, linear, shorter than calyx, glabrous or finely

pubescent, brownish, very acute, entire. Calyx 0.5-1x0.5-0.6 cm, campanulate, base globose, divided more than half way down into 5 segments, equal, 0.35-0.7x0.2-0.3 cm, lanceolate, very acute, entire, persistent, not much enlarged in fruit, basally green, deep pinkish-red tinged, glabrous, midvein pale-red. Corolla bright red-scarlet, crimson, 0.4 cm long, hypocrateriform, limb 2 cm across; tube 1.5-2x0.15-0.2 cm, slender, straight, slight oblique near limb, bright red, yellow tinged at bottom, with 9-pale yellowish-red stripes, conspicuous inside, glabrous; limb more or less oblique, with 5 spreading, subequal, oblong, concave, obtuse, entire or crenulate lobes, anterior middle lobe 0.15x0.5 cm, rest 1.2x0 6 cm, glabrous. Stamens 4, inserted below the throat, much exserted, longer 2 cm long, shorter 1.6-1.7 cm long; filaments slender, filiform, pale yellowish up to half way, pale red distally, shortly hairy upwards, glabrous below, twined at maturity; anthers 0.3x0.25 cm, oblong, cells parallel, deep brown-grey, dorsifixed, extrorse. Ovary 0.2x0.3 cm, globose, pale green or yellowish, glabrous, glaucous, 4-lobular, 4-celled; 1-ovule in each cell; style 4 cm long, much exserted, filiform, red, glabrous; stigma shortly 2-fid, red, glabrous (Figure 23).

Field notes. Woody handsome scandent shrub with entire cordate-rounded shortly acuminate leaves. Flowers opening time: 1 to 1.30 pm. Major venation pattern pinnate brochidodromous. Flowers and Fruits: December to February.

Distribution: In Kerala Ceylon.

Habitat and ecology: Grown as ornamental, grown along trellis and walls in gardens for its showy blossoms and screen of foliage; propagated from stem cuttings.

Vernacular name: Sankrant Vel (Marathi)

Common name: Flaming Glorybower (English)

Uses. Beautiful ornamental along trellis and walls in gardens. In Malaya, infusion of vegetative parts is drunk as a purgative and applied externally to distended abdomen (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Duranta erecta* L.**

Duranta erecta L. Sp. Pl. 637.1753. *D. repens auct. non* L. 1753. *D. plumieri* Jacq. Select. Strip. Arn. 186.1763; C. B. Cl. in Hook. f. Fl. Brit. India 4: 560.1885; Cooke, Fl. Pres. Bombay 2: 518.1958 (Repr.); Singh et al. Fl. Mah. 2: 704.2001; Yadav and Sardesai Fl. Kolhapur Dist. 372.2002; Almeida Fl. Mah. 4: 120.2003.

An evergreen, perennial, bushy shrub up to 14 m high, with drooping branches, bearing axillary thorns, stem lenticellate, pale brownish, branchlets appressed hairy. Leaves 4-7.5x2-3.5 cm, ovate, softly hairy on both sides, acute, subentire or serrate, cuneate and slight asymmetric at base, petioles 0.6-0.7 cm long, softly pubescent. Flowers 1 cm long, purple or bluish-white, borne in profusion in terminal erect or drooping panicles or loose racemes, 6-8 cm long, daughter axes shorter, softly pubescent, green; pedicels 0.2-0.3 cm long, shortly pubescent, lasting longer along with peduncles, bracts lower 0.5-0.6x0.3 cm, subulate, leafy, acute, green, entire, caducous, shorter than calyx, softly pubescent, upper ones 0.05-0.1x0.2 cm, narrow-ovate. Calyx 0.5x0.2 cm, tubular, 5-toothed, yellowish-green, softly pubescent outside, orange colored in fruits, segments distinct, 0.4x0.1 cm, unequal, 3 slight longer, triangular, acute, Corolla purple or bluish-white, 0.7 cm long, limb 0.4 cm across, slight 2-lipped, tube 0.4x0.15 cm, oblique, softly hairy inside and outside, lobes 0.2x0.1 cm, broad, obtuse, hairy on both sides, yellowish at throat. Stamens 4, included, inserted at middle of tube, longer 0.2 cm long, shorter 0.15 cm long, filaments glandular, anthers 0.5 cm long, yellow, dorsifixed, introrse. Carpels 4, ovary 0.1x0.07 cm, globose, green, glabrous, tetralocular, 2-ovules in each cell, style 0.2-0.3 cm long, glabrous, stigma capitate, papillose. Drupes 1.2x0.7-0.8 cm, fleshy, globose-obovoid, glabrous, glaucous, orange colored at maturity, falling off with persistent orange calyx, and part of pedicel. Seeds 8 (Figures 11, 12).

Field notes. An evergreen bushy shrub. Flowers opening time: 8 to 9 am. Major venation pattern **:** pinnate semicraspedodromous. Flowers and Fruits: throughout the year.

Distribution: Native in South America, Florida-Brazil range and West Indies.

Habitat and ecology: Grown as hedge plant.

Vernacular names: Vilayati mendi (Marathi).

Common name: Golden dew drops (English).

Uses. Grown as hedge. Leaves contain saponin and fruits contain alkaloid analogous to narcotine. Seeds yield oil (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

Glandularia bipinnatifida (Schauer) Nutt.

Glandularia bipinnatifida (Schauer) Nutt. in DC. Prodr. 11: 553.1847; *Verbena bipinnatifida* Bailey, Man. Cult. Pl. 840.1949; Naik Fl. Marathwada 707.1998; Singh et al. Fl. Mah. 2: 706.2001; Yadav and Sardesai Fl. Kolhapur Dist. 375.2002; Almeida Fl. Mah. 4: 133.2003.

A prostrate decumbent, perennial herb; stem hirsute. Leaves 3.5x6.4 cm (0.4 cm across the middle part), deeply pinnatifid, linear-oblong segments 2.5-4.5 cm long, hirsute on both sides, acute, decurrent at base. Flowers 2.5 cm long, medium sized, showy, pink-purplish or white in dense, solitary capitate-corymbose spikes 2-5 cm long, elongating in fruit; bracts 0.7-0.8x0.1 cm, lanceolate, conspicuous, slight shorter than calyx, acute, hairy, entire, green. Calyx 1.3x0.2 cm, tubular, 5-toothed, 5-ribbed, densely pubescent on ribs, setaceous, segments acuminate. Corolla pink-purplish, 2.2 cm long, limb 1.3 cm across, more or less 2-lipped; tube 1.5x0.1 cm, slight longer than calyx, cylindric, slight oblique, hairy inside; limb with 5 spreading lobes, unequal, oblong, retuse or emarginate, posterior lobe largest 0.8x0.4cm, 2-laterals 0.7x0.3 cm, 2-anterior smallest, 0.6x0.3 cm, glabrous. Stamens 4, inserted at middle of corolla tube, included, longer 0.2 cm long, shorter 0.1 cm long, anthers 0.05 cm long, yellow, dorsifixed, introrse. Ovary 0.2x0.07 cm, globose-elongate, glabrous, pale-green, 4-lobed, 4-celled, 1-ovule in each cell; style 1.3 cm long, white, glabrous, stigma minute, green, bifid, sticky. Fruits 0.3x0.1 cm, dry, elongate-oblong, 4-lobular, enclosed in calyx, black on ripening, splitting into 4, 1-seeded pyrenes (Figures 8, 9).

Field notes. A prostrate decumbent herb with pinnatifid leaves. Flowers opening time: 11.30 am to 12 pm. Major venation pattern actinodromous. Flowers and Fruits: throughout the year.

Distribution: Cultivated in many gardens of India.

Habitat and ecology: Grown in gardens as an ornamental.

Common name: Dacota vervain (English)

Uses. Useful for beautifying landscape, but susceptible to dampness (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Gmelina arborea* Roxb.**

Gmelina arborea Roxb. Pl. Cor. 3: 42, t. 246.1815; C. B. Cl in Hook. f. Fl. Brit. India 4: 581.1885; Cooke, Fl. Pres. Bombay 2: 504.1958 (Repr.); Naik Fl. Marathwada 707.1998; Singh et al. Fl. Mah. 2: 692.2001; Yadav and Sardesai Fl. Kolhapur Dist. 372.2002; Almeida Fl. Mah. 4: 121.2003.

A medium-sized, unarmed, deciduous tree, 15 m tall. Bark smooth, whitish-grey, young parts pale-yellow, tomentose, covered with fine white mealy pubescence. Leaves large 17-17.5x10 cm, cordate, pubescent, mature ones glabrous above, fulvous-tomentose beneath, shortly acuminate, entire, young leaves often with few large distant teeth, cordate at base, petioles 5-8 cm long, cylindric, puberulous with shining glands at top. Flowers showy, 2.5-3.5 cm long, brownish-yellow, usually in small, opposite decussately arranged small cymes of about 3 flowers along the axis of a densely fulvous-hairy terminal panicles about 14-20 cm long; buds clavate, angular, pedicels 0.8-0.9 cm long, densely fulvous-hairy, bracts 0.1x0.05 cm, lower ones ovate, upper linear-lanceolate, entire, acute, caducous, leaving scars, densely pubescent with whitish hairs. Calyx 0.4-0 5x0 6 cm, broadly campanulate, 5-toothed, teeth unequal, small, distinct, triangular, acute, anterior two larger, gland dotted with roundish small glands, posterior 3 densely fulvous-hairy, persistent, unaltered in fruit. Corolla 3-5 cm long, limb 2 cm across, brownish-yellow, 2-lipped, infundibuliform, ventricose in upper part, deciduous, tube 1.5x0.8 cm, oblique, above 1.1 cm across, yellow inside, hairy on both sides; limb oblique, spreading, 5-lobed, 2-lipped, upper lip small, deeply divided into 2-oblong, obtuse lobes, 1-1.2x1-1.2 cm, lower lip large, 3-lobed, the middle lobe largest, much longer, broader, 2.1x2 cm, bright yellow, brownish marginally, projecting forward with conspicuous mid nerve, elevating from the lower side, ovate, sub-obtuse, with undulate-crenulate margin; lateral lobes smaller 1.6x1.5-1.6 cm, brownish-yellow, obovate-rounded, with inconspicuous mid nerve, lobes densely hairy outside with whitish, soft hairs. Stamens 4, inserted little below the throat, longer 2 cm. long, shorter 1.5 cm long, bright yellow, shorter than the corolla, with short, fine, yellowish papillate hairs; anthers 0.2x0.1 cm, conspicuous, deep brown-orange, oblong, dorsifixed, introrse. Ovary 0.4-0.5x0.3-0.5 cm, globose, pale-green, whitish below with distinct rim at top around stylar base, glabrous, 4-celled, 1-ovule in each cell, style 2.1-2.3 cm long, slender, yellow, glabrous, stigma yellow, shortly 2-fid with subequal arms. Drupes 2.3-2.5x2 cm, smooth, obovoid, fleshy, green when young, turning orange-yellow on ripening with whitish dots concentrated basally (Figure 14).

Field notes. Natural or cultivated medium sized, unarmed, deciduous tree with grey smooth bark, occasional in Melghat. Flowers opening time: Early hours of morning. Major venation pattern pinnate festooned brochidodromous. Flowers and Fruits: February to May.

Distribution: Scattered in deciduous forests in the greater parts of India and Andamans, up to an altitude of 5000 ft.

Habitat and ecology: Occasional in Melghat forests, common in deciduous forests, planted in plains.

Vernacular name: Shivan (Marathi)

Common name: Grey teak (English).

Uses. Wood is used for furniture, carriages, printing box, musical instruments, picture frames, artificial limbs etc. and employed for bridges. All plant parts useful in medicine; leaf juice is used for ulcers. Flowers are given blood diseases. Root is an ingredient of Ayurvedic preparation "Dasamula". Bark and roots contain traces of alkaloid. Root is bitter tonic. Leaves are used as a feed for silk worms (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Gmelina philippensis* Cham.**

Gmelina philippensis Cham. In Linnaea 7: 109.1832. *G. hystrix* Schult. ex Kurz in J. As. Soc. Bengal 39.81.1870; C. B. Cl in Hook. f. Fl. Brit. India 4: 582.1885; Cooke, Fl. Pres. Bombay 2: 505.1958 (Repr.); Naik Fl. Marathwada 702.1998; Singh et al. Fl. Mah. 2: 704.2001; Yadav and Sardesai Fl. Kolhapur Dist. 372.2002; Almeida Fl. Mah. 4: 122.2003.

A thorny sprawling shrub, branches drooping, spines axillary in pairs, short. Leaves 7-8x3.5-4 cm, elliptic, glabrous, glaucous beneath with scattered round glands, young leaves often trilobed with 3 equidistantly placed teeth; petioles 1x0.05 cm. Flowers large, showy 5-6x2.5 cm, yellow with 0.2 cm long pedicels in pendulous panicles, upto 15-22 cm long, enclosed in large 3.3x2 cm, showy, membranous, purple veined bracts, broadly ovate, acute or shortly acuminate, entire. Calyx 0.6x0.6 cm, broadly campanulate, 5-toothed, teeth short, unequal, anterior two gland dotted with 2 small roundish glands, persistent, unaltered in fruit. Corolla large, bright yellow, 5-6 cm long, limb 2.5 cm across, 2-lipped, infundibuliform, ventricose in upper part, deciduous, tube 1.5x0.3 cm, oblique, with sticky white sap, pubescent outside; lobes 5, upper lip 2-lobed, 2.5x2 cm, anterior laterals 1.6x1.2 cm, middle 1.2x1.4 cm, slight crenulate. Stamens 4, inserted below the throat, shorter than the corolla, longer 2.5 cm long, shorter 1.3 cm long, bright yellow hairy, anthers 0.3x0.2 cm, deep brown, oblong, dorsifixed, introrse. Ovary 0.4x0.3 cm, globose, with buldging outgrowth below, pale-green, with distinct green rim at top, glabrous, 4-celled, 4-lobed, 1-ovule in each cell, style 3.8 cm long, slender, pale-yellow, curved distally, glabrous; stigma very shortly and unequally 2-fid, pale-yellow, longer arm 0.2 cm, glabrous. Drupes 1.5-2.3x1-1.5 cm, succulent,

glabrous, obovoid, green, white dotted throughout, yellow on ripening (Figure 15).

Field notes. A thorny sprawling shrub, with drooping branches. Thorns axillary in pairs. Flowers opening time: 6 to 7 am. Major venation pattern pinnate brochidodromous. Flowers and Fruits: August to February.

Distribution: Grown in gardens of India.

Habitat and ecology: Grown in gardens, not common.

Vernacular name: Shivan (Marathi)

Uses. In Malaya, the plant is pounded with lime and applied with poultice to the throat for relieving cough. Grown as ornamental for beautiful pendulous inflorescence (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Holmskioldia sanguinea* Retz.**

Holmskioldia sanguinea Retz. Obs. 6: 31.1791; C. B. Cl in Hook. f. Fl. Brit. India 4: 590.1885; Cooke, Fl. Pres. Bombay 2: 518.1958 (Repr.); Naik Fl. Marathwada 702.1998; Singh et al. Fl. Mah. 2: 705.2001; Yadav and Sardesai Fl. Kolhapur Dist. 372.2002; Almeida Fl. Mah. 4: 122.2003

A perennial, straggling evergreen shrub, 3-5 m high, branches drooping, puberulous. Leaves 7-8x4-4.5 cm, ovate, pubescent along nerves beneath, acuminate, entire or sometimes finely serrate, cordate at base; petioles 1.3 cm long. Flowers 2.8 cm long, red or yellow in axillary and terminal panicles of cymes, 2.5-5 cm long, puberulous, with saucer shaped petaloid calyx; pedicels 0.6-0.9 cm long, thin, bracts small, 0.2x0.1 cm, ovate, deciduous, shorter than pedicels and calyx; bracteoles 2, small. Calyx upto 2.5 cm in diameter, spreading broadly and uniformly from base upward, obconic, membranous, red to orange, glabrous inside, with fine reticulum of veins, margin entire, obscurely sinuate. Corolla dark red, 2-2.5 cm long, tubular, cylindric, curved, tube 1.4x0.2 cm, widening upward, with 6-8 stripes, hairy outside, limb 0.8x0.5-0.6 cm, oblique, somewhat 2-lipped, with 5 short lobes, hairy outside, convolute outwardly, lower lip 0.3-0.4 cm across, upper smaller, 0.25 cm across. Stamens 4, inserted half way above in tube, shortly exserted, longer 1.1.5 cm long, shorter 0.95 cm long, filaments red, hairy, papillose, glandular; anthers 0.15x0.1 cm, ovate, cells parallel, papillose, glandular, dorsifixed, introrse. Ovary 0.15x0.2 cm, obtuse or obscurely depressed, glandular, green, 4-lobular, imperfectly 4-celled; 1-ovule in each cell, laterally attached; style 1.2 cm long, red, shortly exserted, glabrous; stigma shortly 2-fid. Mature ovary 0.4-0.5 cm long, obovoid, black, included in enlarged calyx (Figures 25, 26, 27).

Field notes. A straggling evergreen shrub with drooping branches and ovate, entire, acuminate leaves and red or yellow flowered forms. Flowers opening time: Midnight to early hours of morning. Major venation pattern pinnate brochidodromous. Flowers and Fruits: October to January.

Distribution: Throughout India including Himalayas, Burma, Madagaskar.

Habitat and ecology: Grown in gardens, not much common.

Common names: Chinese hat (English), Cup saucer (English), Coolies hat (English)

Uses. Plant is eaten by sheep and goats. The wood is light red and moderately hard grown as ornamental (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Lantana camara* L. var. *aculeata* (L.) Mold.**

Lantana camara L. var. *aculeata* (L.) Moldenke in Torreya 34: 9. 1934. *L. aculeata* L. Sp. Pl. 627. 1753; *L. camara auct. non* L. 1753; C. B. Cl. In Hook. f. Fl. Brit. India 4: 562.1885; Cooke, Fl. Pres. Bombay 2: 498. 1958 (Repr.); Naik Fl. Marathwada 703.1998; Singh et al. Fl. Mah. 2: 693. 2001; Yadav and Sardesai Fl. Kolhapur Dist. 374.2002; Almeida Fl. Mah. 4: 123.2003.

A straggling or scandent aromatic shrub, 2-3.5 m tall, stem brown with stout recurved prickles, branchlets opposite. Leaves 5-8x3.5-5 cm, ovate, scabrous, rugose above, hairy, acute, crenate-serrate, rounded at base, petioles 3x0.15-0.2 cm, pubescent. Flowers 1.5x2-2.5 cm, pink, red, yellow-orange turning into pink-orange in axillary, opposite, pedunculate capitate spikes, 1.5x2-2.5 cm, elongating in fruit, peduncles 3-4 cm long, 4-angled, scabrous, hairy; bracts 0.7x0.15 cm, linear, conspicuous, longer than calyx, acuminate or acute, entire, subpersistent, pale-green, pubescent, lower ones 0 8-0.9x0.3 cm, leafy, ovate. Calyx 0.15x0.1 cm, truncate, obscurely toothed, persistent, membranous, hairy, pale-green. Corolla yellow-orange changing to pink, 2 cm long, salver shaped, deciduous; tube 1.5-2x0.1-0.15 cm, oblique, hairy, limb 0.4 cm across, lobes 4-5, spreading, bigger ones 0.5x0.3 cm, smaller 0.2x0.2 cm, rounded, acuminate, glabrous. Stamens 4, inserted about middle of tube, included, longer and shorter filaments less than 0.1 cm long, anthers less than 0.1x0.1 cm, oblong, yellow, basifixed, introrse. Ovary less than 0.1x0.1 cm, glabrous, green, 2-celled, ovule solitary, attached laterally, close to the base of each cell, style 0.15 cm long, thin, glabrous, stigma oblique, bilobular, sub-capitate, glandular, sticky. Drupes 0.4x0.4 cm, globose, shining, green, fleshy, black on ripening, separating into two 1-celled, 1-seeded pyrenes (Figure 1).

Field notes. A straggling or scandent aromatic shrub, with stout recurred prickles. Flowers opening time: about 6.30 to 7.00 pm. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: throughout the year.

Distribution: Native in Tropical America.

Habitat and ecology: Very common throughout in diverse habitats, common in waste places. Gregarious in many parts of Melghat.

Vernacular names: Ghaneri (Marathi), Raimuni (Marathi)

Uses. Leaves contain tannins, sugars, and resin, lantadenes, steroid such as lancamarone. Bark contains quinine with strong antipyretic and antispasmodic properties. The flowers yield volatile oil similar to that of leaf oil. Leaves and twigs for green manure in forest, polishing woods, substitute for tea. Fruits edible. Stems are used as toothbrush. A decoction is given in case of tetanus, rheumatism, malaria, etc (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976)

***Lantana flava* (L.) Medik.**

Lantana flava Medik. in Ait. Acad. Theod. Palat. 3: 225.1775; *L. camara* var. *flava* Mold. Fl. Ceylon 4: 299.1983; Almeida Fl. Mah. 4: 124.2003.

An ornamental small shrub, 60-90 cm high; prickles on branches small, obscure. Leaves 6.5-7x2.8-3 cm, narrowly ovate, acute at base, serrate, teeth smaller, petioles 0.8-1.0 cm long. Flowers sulphur yellow. Calyx 1.5x0.1 cm, teeth somewhat distinct (Figure 2).

Field notes. Ornamental shrub. Flowers opening time: 6 to 7 pm. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: throughout the year.

Distribution: Native in Tropical America.

Habitat and ecology: Planted in gardens

Vernacular names: Ghaneri (Marathi)

Uses. Planted in gardens, in landscaping, beautification of avenues.

***Lantana nivea* Vent.**

Lantana nivea Vent.; *L. camara* var. *nivea* Vent. Bailey Cyclop. Amer. Hort. 884.1900; Gopalswamy Iyeng. Gard. Ind. Ed. 2: 276.1935; Moldenke in Lilloa 4: 290.1939; Bailey Manu. Cult. Pl. ed. 2: 842.1949; Almeida Fl. Mah. 4: 124.2003.

A shrub 2.5 m high. Leaves 4-10x2.3-5 cm, lanceolate-ovate or narrowly ovate, finely serrate, petioles 1-2x0.12 cm. Flowers white. Calyx 0.15x0.1 cm, obscurely toothed. Corolla 1.5x0.6-0.7 cm, white, when young, yellow tinged at mouth (Figure 3).

Field notes. Ornamental shrub. Flowers opening time: 6 to 7.30 pm. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: throughout the year.

Distribution: Native in Tropical America.

Habitat and ecology: Planted commonly in gardens

Vernacular names: Ghaneri (Marathi)

Uses. Popular ornamental, planted in gardens.

***Nyctanthes arbor-tristis* L.**

Nyctanthes arbor-tristis L. Sp. Pl. 6.1753; C. B. Cl in Hook. f. Fl. Brit. India 3: 603.1882; Cooke, Fl. Pres. Bombay 2: 176.1958 (Repr.); Naik Fl. Marathwada 525.1998; Almeida Fl. Mah. 3a: 190.2001. Singh et al. Fl. Mah. 2: 311.2001.

A large shrub or small tree, upto 10 m high, with greyish-green rough bark. Very scabrous all over with short, stiff whitish hairs, branchlets opposite, sharply quadrangular, strigose. Leaves 9-11x3.2-7.5 cm, opposite, ovate, scabrous above, densely pubescent beneath, conspicuously so on nerves, main nerves conspicuous beneath, acuminate, entire or toothed with one or few large distant teeth; petioles 0.5-0.8x0.2 cm, hairy, stout, lower mature leaves with shorter ones. Flowers 2 cm long, delightfully fragrant, white with orange tube, sessile in bracteate fascicle of 3-5 in each pedunculate head, arranged in trichotomous cymes; peduncles sharply 4-angled, slender, pubescent, axillary, solidary and in terminal trichotomous cymes, arranged in pyramidal panicle 15-18 cm long, each fascicle 6-8 cm long, with 4-bracts forming involucre at base, central flower ebracteate, one pair larger, outwards, each one 0.9x0.8 cm, slight smaller inwards, each 0.75-0.9x0.7-0.8 cm, pairs diagonally arranged, bracts herbaceous, ovate or suborbicular with central prominent nerve at which margins folded inwardly, scabrous, finely pubescent and shining inside, glabrous outside, apiculate, mucronate, entire. Calyx 0.6-0.7x0.5 cm, narrowly campanulate, truncate, obscurely 5-toothed, hairy outside, glabrous inside, faint green, persistent, unaltered in fruit; segments less than 0.05-0.1x0.3 cm. Corolla with orange tube and white limb, 1.3 cm long, hypocrateriform, deciduous, opens in evening and falls off in the morning, glabrous, tube 1-1.1x0.4-0.5 cm, cylindric, straight, finely ribbed, orange colored; limb 2.5-3 cm across, white, 4-8 lobed, lobes 1.6-1.8x0.9 cm, unequally divided at apex, oblong-obcordate, margins outwardly rolled, cuneate at base, twisted in bud. Stamens 2, short, equal, 0.6-0.7 cm long, epipetalous, inserted near the top of tube, included; filaments completely adnate to the tube, glabrous, deep orange-yellowish; anthers 0.3x0.1-0.15 cm, pale brown, dorsifixed, introrse. Ovary 0.2cm long, pale green, glabrous, 2-celled; 1-ovule in each cell; style 0.4 cm long, pale green, glabrous; stigma subcapitate, green. Capsules 1.8x1.5 cm, suborbicular or obcordate, much compressed, finely pubescent, chartaceous, reticulately veined, mucronate, 2-celled, green when young, pale-brown at splitting stage, separating into 2-flat, 1-seeded carpels (Figure 28).

Field notes. Small tree with grayish green bark. very scabrous all over. Flowers opening time: 7 to 8 pm. Major venation pattern pinnate festooned brochidodromous. Flowers and Fruits: August to December.

Distribution: Wild in Sub-Himalayan region up to 1500 m from Chenab to Nepal, also in Rajashtan, Madhya Pradesh.

Habitat and ecology: Frequent all along the hilly western borders of Satpuras occurring wild and in natural habitat, growing gregariously covering dry steep hill sides and rocky grounds. It is cultivated in gardens and courtyards of houses for its fragrant flowers.

Vernacular names: Parijatak (Marathi), Khurasli (Marathi)

Common name: Night jasmine (English)

Uses. The bright orange corolla tube contains a coloring matter nyctanthin, a glucoside. The corolla tubes were formerly used for dyeing silk. Sometimes in conjunction with safflower, turmeric, indigo. Grown in gardens and courtyards for fragrant flowers (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Petrea volubilis* L.**

Petrea volubilis L. Sp. Pl. 625.1753; Bailey, Man. Cult. Pl. 843.1949. Cooke, Fl. Pres. Bombay 2: 518.1958 (Pepr.); Naik Fl. Marathwada 705.1998; Singh et al. Fl. Mah. 2: 705.2001; Yadav and Sardesai Fl. Kolhapur Dist. 374.2002; Almeida Fl. Mah. 4: 126.2003.

An extensive perennial liana, up to 10 m high, stem ash-colored, covered with grayish pubescence, lenticellate. Leaves 14-16x6.5-8 cm, elliptic, scabrous above, pubescent

on nerves beneath, acute or shortly acuminate, entire, undulate, lobate at base; petioles 0.7x0.15 cm, cylindric, pubescent. Flowers 2-2.3 cm long, showy, purple in pendulous, 15-19 cm long axillary racemes, remarkable for large persistent calyx, remaining long after fallen corollas, rachis slender, green below, purplish upwards, densely covered with soft whitish pubescence; pedicels slender, 1.8 cm long, cylindric, purplish-green, densely pubescent with shining hairs, bracts 1.5x0.4 cm, caducous, distinct with young flowers, linear, shorter than calyx, acute, entire, softly hairy with shining hairs, older ones leaving a dot like scar, older bracts apparently slight extraxillary. Calyx campanulate, petaloid, purplish-blue, often becoming green, dry in fruits, tube 0.5x0.25 cm, narrow at base, slight expanded above, densely pubescent outside, lobes 5, 2.2-2.5x0.4-0.5 cm, spreading, radiating, star shaped, equidistantly placed, oblong, sub-acute or rounded, posterior two, slight smaller, each 1.8-1.9x0.4 cm, with reticulum of fine veins and prominent midvein 5, very short 0.2x0.05 cm, acute, alternating scally papillae present in throat, densely pubescent outside with whitish shining hairs. Corolla deep violet, often deciduous, funnel shaped, 2.2x2.3 cm; tube 0.6-1.7x0.3 cm, short, cylindric, oblique, glabrous, whitish at base, distally purple with obscure purplish stripes, short shining hairs outside, hairy inside; lobes spreading, somewhat unequally 2-lipped, intensely purple, posterior lobe 0.4x0.3 cm, oval-oblong, sub-acute with distinct white patch, roundish, showing 3 distinct, prominent veins, merging into fine reticulum, posterior pair 0.9-1.2x0.5 cm, anterior one 1.3-1.4x0.7 cm. Stamens 4, equal, inserted at mouth of corolla tube, filaments 0.15 cm long, delicate, white-purplish, finely pubescent, anthers 0.7 cm long, oblong, brownish-purple, basifixed, extrorse. Ovary very small, 0.1x0.15 cm, oblong, obscurely bilobed, ovate acute, green, glabrous with yellowish small dot like gland at a side, obscurely 2-celled, 1-ovule in each cell, style 0.2 cm long, reddish-purple below, white distally, slight bent basally, glabrous; stigma oblique, deep purplish, very shortly 2 fid. Drupes enclosed in calyx, 2 celled, 2 seeded. After corolla falls off, fruiting calyx later on after long time falls off on ground by floating in rotating fashion like a wheel, along with air current, pedicels and peduncle persistent for quite a long time even after calyx and corolla fall off (Figure 10).

Field notes. An extensive liana with ash colored stem. Flowers opening time: 9.30 to 11 am. Major venation pattern pinnate brochidodromous. Flowers and Fruits: December to March.

Distribution: Native in Tropical America.

Habitat and ecology: Grown as ornamental in gardens.

Common names: Purple Wreath (English), Queen's Wreath (English)

Uses. Grown as ornamental in gardens for beautiful purple colored star shaped calyces and deep blue corollas.

***Phyla nodiflora* (L.) Greene**

Phyla nodiflora (L.) Greene in Pittonia 4: 46.1899; Sant. In Rec. Bot. Surv. India 16(1): 211.1967 (3rd Rev. ed.). *Verbena nodiflora* L. Sp. Pl. 20.1753. *Lippia nodiflora* (L.) A. Rich. in Michaux, Fl. Bor. Amer. 2: 15.1803; C.B.Cl. in Hook. f. Fl. Brit. India 4: 563.1885; Cooke, Fl. Pres. Bombay 2: 499.1958 (Repr.); Naik Fl. Marathwada 705.1998; Singh et al. Fl. Mah. 2: 693.2001; Yadav and Sardesai Fl. Kolhapur Dist. 374.2002; Almeida Fl. Mah. 4: 126.2003.

Perennial, much branched, prostrate, creeping herb, rooting at nodes, stem appressed hairy with white medifixed hairs, finely 5-6 ribbed. Leaves 2-2.6x1 cm, obovate to spathulate, upper half serrate, teeth sharp, 3-5, appressed hairy on sides, thick, fleshy, rounded, shortly mucronate, attenuate at base. Flowers very small, 0.35 cm long, whitish-yellowish-pink, usully in 7-9 whorls in axillary cylindrical 0.6-0.9x0.6 cm condensed spikes, elongate and spicate in fruit; peduncles 2-3.5 cm long, born on upright branches from the axil of one only of each pair of leaves, lower bracts larger, 0.2x0.2 cm, elliptic-round, upper smaller, 0.5x0.1-0.2 cm, obovate, purplish at apex, mucronate, glabrous, cuneate at base, shorter than corolla, herbaceous, margins membranous, hairy. Calyx 0.15 cm long, membranous, purplish at tip, deeply 2-lobed, compressed, mitre shaped, pubescent on back, 2-linear, acuminate lobes closely enclosing the fruit, projecting beyond it. Corolla whitish-yellowish changing to pink, mouth deep pink, 0.35 cm long, limb 0.3 cm across, unequally 2-lipped, glabrous, pushed off as a calyptra by ripening fruit, tube cylindric, straight, limb 2-lipped, upper lip bifid, erect, 0.1x0.05 cm, lower lip 3-lobed, central lobe largest, 0.15x0.1 cm, cordate, mucronate, rests narrowly ovate, subacute, 0.1x0.05 cm. Stamens 4, filaments less than 0.1 cm long, included; anthers ovate, less than 0.1x0.1 cm, dorsifixed, introrse. Ovary small, 0.05 cm long, glabrous, green, 2-lobed, 1-2 ovules in each cell, laterally attached near the base of cell, style 0.15 cm long, glabrous, stigma oblique, sub-capitate. Drupes 0.2-.3x0.2 cm, ellipsoid, dry, enclosed in the accrescent calyx, two 1-seeded plano-convex pyrenes (Figures 4, 5).

Field notes. Much branched, prostrate, creeping herb, stem appressed hairy with medifixed hairs. Flowers opening time: 1 to 1.30 pm. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: July to December.

Distribution: throughout India.

Habitat and ecology: In wet places, almost throughout India, ascending 900 m in the hills. Common in marshy places, frequent throughout, along lake margins, drains, near water taps, water margins in streams and rivers.

Vernacular names: Gour Mundi (Marathi), Ratoliva (Marathi)

Uses. Plant is valued for making lawns. The leaves are eaten in Ceylon and an infusion is taken as tea in Philippines. Plant possesses cooling diuretic properties, used to cure pain in knee joints. Fresh plant is applied for maturants for boils, swollen cervical glands and chronic ulcers (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Rotheca serrata* Steane & Mabb.**

Rotheca serrata Steane & Mabb. Novon 8: 206 (1998), *Clerodendrum serratum* (L.) Moon. Cat. 46.382,1824; C. B. Cl in Hook. f. Fl. Brit. India 4: 592.1885; Cooke, Fl.

Pres. Bombay 2: 512.1958 (Repr.). *Volkameria serrata* L. Mant. 90.1767; *Clerodendrum serratum* (L.) Moon.; Naik Fl. Marathwada 699.1998; Singh et al. Fl. Mah. 2: 691.2001; Yadav and Sardesai Fl. Kolhapur Dist. 371-2.2002; Almeida Fl. Mah. 4: 117.2003;

An erect, perennial shrub, 1-2 m tall, branches bluntly quadrangular or hexangular. Leaves 14-18x6.5-7.5 cm, opposite or in whorls of 3, where branches hexangular, elliptic-obovate, mature ones glabrous, mucronate, sharply serrate, cuneate at base; petioles 0.6cm long, stout. Flowers 3x1.3 cm, showy, dull light-blue in pubescent dichotomous cymes, each in axile of a large leafy bract, collectively forming attractive, long lax terminal pyramidal erect villous panicle 15-25 cm long; pedicels 0.4 cm long, shortly hairy, twisted; bracts 1.2-2.5 cm long, smaller 0.6-0.7x0.2 cm, obovate to lanceolate, pubescent, shorter than calyx, acute, entire, medianly nerved, subpersistent, colored. Calyx 0.6 cm long, cup-shaped, pubescent, truncate, unaltered in fruit, segments very small, less than 0.1 cm long, equal, triangular, acute. Corolla pale blue, 2.4 cm long; tube 0.9-1x0.2 cm, cylindric, oblique at the mouth, hairy inside at the staminal insertion, limb 1.3-2 cm across, 2-lipped, with the large lower lobe, often appearing upper in flower, dark bluish-purple, lobes 5, spreading, unequal, 0.9-1x0.6 cm, larger 1.6x0.5 cm, oblong-elliptic, shortly clawed, concave, pubescent on outer side, glabrous inside, with reticulum of veins, obtuse. Stamens 4, inserted below the throat, much exserted, longer 2.7 cm long, shorter 2.1cm long, filaments curved, purplish, densely hairy at base; anthers 0.25x0.15 cm, oblong, cells parallel, brownish, dorsifixed, extrorse. Ovary 0.3x0.2 cm, green, glabrous, globose, 4-lobed, 4-celled; 1-ovule in each cell; style 3.1 cm long, exserted, purplish-white, glabrous; stigma glabrous. Drupes 0.8x0.6-1.2 cm, obovoid, fleshy, purplish-black at maturity, 2-4 lobed (Figure 21, 22).

Field notes. An erect shrub with bluntly quadra or hexangular branches with opposite or whorled sharply serrated leaves. Flowers opening time: 5 to 7 pm. Major venation pattern pinnate eucamptodromous. Flowers and Fruits: September to December.

Distribution: In Kerala, A.P., Maharashtra, Sikkim, Malaya and Cambodia.

Habitat and ecology: Frequent in Melghat valleys and shady slopes. Occasional in hedges in the periphery of hilly tract.

Vernacular name: Bharangi (Marathi)

Uses. Having antiallergic and antihistamine property. Root is pungent and bitter, antihelminthic, useful in bronchitis, asthma, fever, hiccough. Leaves are one of the snake remedies (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Stachytarpheta jamaicensis* (L.) Vahl.**

Stachytarpheta jamaicensis (L.) Vahl., Enum. Pl. 1: 206.1804; Rajendran & Daniel in Bull. Bot. Surv. India 34: 167.(1992) 1997. *Verbena jamaicensis* L. Sp. Pl. 1753. *Stachytarpheta indica auct. non* (L.) Vahl. 1804; C.B.Cl. in Hook. f. Fl. Brit. India 4: 564.1885 p.p.; Cooke, Fl. Pres. Bombay 2: 501.1958 (Repr.) p.p.; Singh et al. Fl. Mah. 2: 696.2001; Yadav and Sardesai Fl. Kolhapur Dist. 375.2002; Almeida Fl. Mah. 4: 130.2003.

A perennial, herbaceous undershrub, 1 m high, dichotomously branched, lower branches woody, young branches hairy. Leaves 8-10x3-4.5 cm, ovate, rugose, rough above, villous on the nerves beneath, acute, sharply serrate, base tapering decurrent into obscure petioles. Flowers 2.9x1.2 cm, bluish-violet, more or less half immersed in rachis, arranged in long slender, softly hairy, terminal spikes 16-45x5 cm, the rachis shallowed beneath each flower; bracts 0.5x0.15-0.2 cm, lanceolate, conspicuous, shorter than calyx, acuminate, green, scarious and hairy marginally, entire. Calyx 0.6x02-0.22 cm, tubular, elongate, persistent, somewhat compressed, 4-toothed, segments less than 0.1 cm long, distinct, acute, membranous, medianly green nerved. Corolla deep blue-violet, with whitish tinge at bottom of tube, 1.4x0.9-1.2 cm; tube 0.9x0.2 cm, slender with abundant colorless sugary sap, cylindric, curved, oblique, hairy inside, limb 1.2 cm across, lobes 5, spreading, unequal, rounded, posterior 2-lobes 0.5-0.6x0.7-0.9 cm, anterior lobes 3, middle one smallest, 0.6x0.3 cm, laterals 0.6x0.5 cm, glabrous. Stamens 2, perfect of lower pair, inserted at middle of tube, included, staminodes 2, minute; filaments less than 0.1 cm long, slender, whitish, thin, hairy below, anthers 0.3x0.1 cm, cells vertical, divaricate, glabrous, pale yellow-reddish, dorsifixed, extrorse. Ovary 0.15x0.05 cm, oblong-elongate, green, glabrous, 2-celled, 1-ovule in each cell, attached laterally near the base of the cell; style 0.7-0.8 cm long, exserted, filiform, white, glabrous; stigma deep green, capitate, shortly 2-fid. Drupes 0.2-0.3x0.1 cm, elongate-oblong, enclosed in calyx, black on ripening, splitting into two 1-seeded pyrenes (Figure 6).

Field notes. Herbaceous undershrub with dichotomous branching. Flowers opening time: Early hours of morning. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: July to December.

Distribution: Native in America, throughout India, Car Nicobar Islands.

Habitat and ecology: It is a native of America, occasionally planted in gardens as an ornamental. Commonly naturalized on Chikhalda plateau.

Common name: Porter coral weed (English)

Uses. Leaves contain glucoside stachytarphine and an alkaloid. In Java, stem tips are eaten as a flavoring. In Brazil leaves are used for adulterating tea and exported to Europe. It is also used for treating intestinal worms and stomach ailments, juice for cataract. An infusion of bark is used against Diarrhea. The leaves are used in cardiac troubles and rubbed on sprains (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

Stachytarpheta mutabilis (Jacq.) Vahl

Stachytarpheta mutabilis (Jacq.) Vahl, Enum. Pl. 1: 209.1804; Bor & Raiz. Some Beautiful Indian Climbers and Shrubs 152. 1990 (Repr.). *Verbena mutabilis* Jacq. Coll. Bot. 2: 334.1758; Singh et al. Fl. Mah. 2: 705.2001; Almeida Fl. Mah. 4: 131.2003

An undershrub, woody at base, up to 1.80 m high; branches pubescent Leaves 5-6x4 cm, ovate, scabrous,

rugose above, pubescent beneath, acute or shortly acuminate, serrate-dentate, teeth not sharp, rounded, base decurrent into obscure petioles. Flowers 2.7x1 cm, crimson-rose, in stout, terminal, 30-60x0.6 cm spikes, bracts 1x0.3 cm, sharply acuminate, projecting into a fine shaft, shorter than calyx, margins scarious, hairy, entire. Calyx 1.3x0.23 cm, 4-toothed, segments acute, membranous, medianly green nerved. Corolla rose, 1.5-2.5 cm long; tube 1.5x0.3 cm, slender, with pale pink stripes, bright rose-red-pink, whitish inside at mouth and bottom, with nectar, cylindric, curved, oblique, hairy inside; limb 1cm across, unequally 5-lobed, lobes spreading, darker above with dark pink tinge, posterior lobes 3, 0.6x0.3 cm, equal, large, cordate, round, emarginate, anterior left lobe deeply unequally bilobed, smallest lobe 0.5x0.2 cm, central; right lobe 0.5x0.4 cm, smaller, cordate, shining, finely pubescent at lower side and marginally. Stamens 2, perfect of lower pair, inserted at middle of corolla tube, included, staminodes 2, indistinct; filaments 0.1 cm long, hairy below, pale-yellowish, anthers 0.3x0.1 cm, cells vertical, divaricate, pale-yellow, reddish, dorsifixed, extrorse. Ovary 0.15x0.5 cm, oblong-elongate, green, glabrous, 2-celled, 1-ovule in each cell, attached laterally near the base of cell; style 0.7-0.8 cm, exserted, filiform, white, glabrous, stigma deep green, capitate, shortly 2-fid. Drupes 0.2-0.3x0.1 cm, elongate-oblong, black on ripening, enclosed in calyx, splitting into two 1-seeded pyrenes (Figure 7).

Field notes. Ornamental undershrub. Flowers opening time: Early hours of morning. Major venation pattern pinnate semicraspedodromous. Flowers and Fruits: July to December.

Distribution: Native in Tropical America.

Habitat and ecology: Cultivated in plains.

Common name: Coral weed (English)

Uses. Leaves used for adulterating tea and are pounded with lime are applied to swollen wounds and sours. Plant is useful for treatment of tumors (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976) besides an ornamental, which became rare in Amravati.

***Tectona grandis* L. f.**

Tectona grandis L. f. Suppl. 151.1781; C. B. Cl. in Hook. f. Fl. Brit. India 4: 570.1885; Cooke, Fl. Pres. Bombay, 2: 503.1958 (Repr.); Naik Fl. Marathwada 707.1998; Singh et al. Fl. Mah. 2: 696.2001; Yadav and Sardesai Fl. Kolhapur Dist. 375.2002; Almeida Fl. Mah. 4: 131.2003.

A large deciduous tree with rounded crown, very variable in size according to its habitat, 20-50 m tall. Dark buff colored bark peeling off in longitudinal flakes; young branches stellately tomentose. Leaves very large, 40-42x21.5 cm, elliptic-ovate or obovate, scabrous, deep green, apparently glabrous above, lower surface stellate gray or tawny tomentose, pale green, with elevating prominent yellow, thick main veins and midrib, acute, entire, slight undulate or very finely serrate, cuneate at base, petioles 3-7x0.7-1 cm or very short, thick, soft stellate grayish-brown tomentose. Flowers small, 0.7x0.5 cm, white, fragrant in erect large terminal cymose panicles, about 30 cm long, bracts in pairs at each fork, lower 1x0.4 cm, grayish-brown, stellately tomentose, uninerved, upper bracts smaller 0.5x0.23 cm, linear-lanceolate, acute; peduncle stellately tomentose; pedicel 0.2 cm long. Calyx 0.3-0.4x0.3 cm, campanulate, persistent, stellately tomentose, lobes 5-6, 0.2-0.3x0.2 cm, spreading, subequal, linear, acute, in fruit enlarged upto 2x1.2 cm or more, inflated, globose, bright-green, enclosing fruit, more or less crumpled, reticulately veined. Corolla white, purplish tinged at throat, 0.5x0.5-0.6 cm, rosaceous, lobes 5-6, equal, 0.3x0.2 cm, undulate, with adaxial central groove, spreading, regular, deciduous, finely stellate hairy on nerves beneath; tube very short, 0.1 cmx0 2 cm, straight. Stamens 5-6, inserted near the base of corolla rim, equal, exserted, alternating with lobes; filaments 0.3 cm long, slender, white, hairy; anthers less than 0.1x0.1 cm, ovate-round, with distinct parallel cells, bright brown-yellowish, papillose, dorsifixed, extrorse. Ovary 0.2x0.2 cm, fleshy, densely tomentose, greenish, base bright orange, 4-celled; ovule solitary in each cell; style 0.3-0.4 cm long, exserted, white, with conspicuous tuft of white hairs basally, stigma very shortly 2-fid, papillose. Drupes 0.5x0.7 cm, sub-globose, more or less 4-lobed, densely stellate hairy, enclosed in enlarged calyx. *Tectona grandis* plant material collected from different places many times show minor variations in some morphological characters as height of plant, length of petiole, margin and base of lamina and in some microcharacters. They found to be correlated as trees with shorter height, showing bushy appearance bearing entire, elliptic leaves with longer petioles and where major venation pattern is Pinnate Brochidodromous and marginal ultimate venation is looped and trees which are taller with ovate-elliptic broader leaves and very short petiole, showing finely serrated margin of lamina where venation is Pinnate Festooned Bronchidodromous in lower and Brochidodromous in upper portion (Figure 13).

Field notes. A large deciduous tree with rounded crown, very variable in size according to its habitat, stellately tomentose. Flowers opening time: 8 to 10.30 am. Major venation pattern pinnate brochidodromous. Flowers and Fruits: June to September.

Distribution: Indigenous to peninsular India and Madhya Pradesh.

Habitat and ecology: Very common and the dominant tree in forests of Melghat, deciduous forests and other hilly tracts. Planted in the plains.

Vernacular names: Sag (Marathi), Sagwan (Marathi)

Common name: Teak (English).

Uses. It enjoys world wide reputation as a quality timber and used in construction of bridges, lorry bodies, carts and carriages, agricultural implements and heavy packing cases, for making musical instruments, used in chemical industries and labs' bench tops. Powder of wood is said to use in ailing skin inflammation. Leaves contain 6% tannin & use for dyeing silk. Bark contains betulinic acid (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Vitex negundo* L.**

Vitex negundo L. Sp. Pl. 638.1753; C. B. Cl in Hook. f. Fl. Brit. India 4: 583.1885; Cooke, Fl. Pres. Bombay 2: 508.1958 (Repr.); *V. trifolia* Grah. Cat. Bombay Pl. 155.1839 non L.; Naik Fl. Marathwada 708.1998; Singh et al. Fl. Mah. 2: 699.2001; Yadav and Sardesai Fl. Kolhapur Dist. 376.2002; Almeida Fl. Mah. 4: 135.2003.

A large, aromatic shrub or a small slender tree, 3.5 m high, branchlets grayish-white, finely tomentose. Leaves 3-5 foliate, leaflets variable in size, terminal 8-10.5x2-2.7 cm, laterals smaller, ovate-lanceolate, nearly glabrous above, white tomentose beneath, attenuate, entire, sometimes finely serrate, acute at base, base slight asymmetric in lateral leaflets. Common petioles 5-6 cm long, petiolules of terminal leaflet 1-1.3 cm long, lateral leaflets with very short petiolules, finely tomentose. Flowers bluish-purple or white, small, 0.7-0.8 cm long, in pedunculate, branched tomentose cymes, about 16 cm long, opposite along the quadrangular tomentose rachis of a large, terminal, compound, pyramidal panicle about 23-30 cm long, bracts less than 0.1 cm long, lanceolate, caducous, shorter than calyx, finely tomentose, entire, pale brownish-green. Calyx 0.4x0.2 cm, campanulate, white tomentose, 5-toothed, teeth triangular, 0.4 cm long, equal, persistent, enlarged in fruit. Corolla 0.7-0.8 cm long, limb 0.3 cm across, white or pale-purple, with dark bluish-purple tinge inside throat, tomentose outside, hairy inside at the insertion of stamens; tube 0.1 cm long, straight, slender, limb 5-lobed, 2-lipped, middle lobe of the lower lip largest 0.4 cm long, upper lip small, 0.1x0.1 cm, divided to the base into 2-obtuse lobes, lower lip large, 0.4x0.5 cm with 2-short oblong obtuse lateral lobes 0.1x0.15 cm and largest central broadly obovate, crenulate lobe 0.4x0.3 cm with distinct median nerve and dark purple shaded curved part. Stamens 4, inserted at the throat, exserted, longer 0.4 cm long, shorter 0.25 cm long, filaments slender, whitish, densely hairy at the base, at insertion with long, whitish-purplish, distinct bunch of hairs, anthers less than 0.05 cm, deep purple-brown, dorsifixed, introrse, cells parallel, pendulous, later divaricate. Ovary minute, less than 0.1x0.1 cm, globose-oblong, pale-green, glabrous, glandular, 4-celled, 1-ovule in each cell; style 0.5 cm long, filiform, exserted, white-purplish, glabrous; stigma whitish, shortly 2-fid, glabrous. Drupes 0.3x0.25-0.3 cm, globose-ovoid, 4-seeded, black on ripening, enclosed at base of slight enlarged calyx (Figure 16).

Field notes. A large aromatic shrub or small tree with grayish-white bark, finely tomentose branchlets and 3 to 5 foliate leaves. Flowers opening time: Early morning to 11 am. Major venation pattern pinnate brochidodromous. Flowers and Fruits: July to February.

Distribution: Throughout India, ascending to altitude of 1500 m in outer Himalayas and other Asian countries.

Habitat and ecology: Common as hedges along fields and waste places, in beds of streams and rivers.

Vernacular name: Nirgudi (Marathi)

Uses. Plant is useful for planting against the soil erosion and afforestation, also the hedge plant. It is one of the common plants of Indian medicine. Leaves as tonic and insect repellent. Its antibacterial activity with presence of steroids, alkaloids, tannins, and phenols is reported. Plant is stomachic, useful in promoting hair growth, eye diseases, and inflammation and is anthelminthic (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).

***Volkameria inermis* L.**

Volkameria inermis L. Sp. Pl. 637.1753, L. *Clerodendrum inerme* (L.) Gaertn. Fruct. Sem. 1: 271, t.75.1788; C. B. Cl in Hook. f. Fl. Brit. India 4: 589.1885; Cooke, Fl. Pres. Bombay 2: 511.1958 (Repr.). Naik Fl. Marathwada 698.1998; Singh et al. Fl. Mah. 2: 690.2001; Yadav and Sardesai Fl. Kolhapur Dist. 371.2002; Almeida Fl. Mah. 4: 115.2003.

A straggling much branched, glabrescent, strongly smelling shrub, 2.5 m high, branchlets purplish-green. Leaves variable, 4-6x2.5-3.5 cm, and elliptic, glabrous, obtuse, entire, cuneate at base, petioles 0.8-1 cm long, finely pubescent. Flowers 3.5 cm long, white, with pink tinge in axillary pedunculate cymes, usually 3-flowered, peduncles 2.5-3 cm long, slender; pedicels 0.9 cm long, bracts 0.2 cm long, minute, shorter than calyx, linear, glabrous, finely acute, entire, green, bracteoles minute. Calyx 0.5x0.4 cm, campanulate, 5-toothed, teeth acute, 0.1x0.15 cm, distinct, triangular, with purplish tips, glabrous, leathery, accrescent. Corolla white, with purple tinge at tips, 4.7 cm long, hypocrateriform, tube 3.3x0.2 cm, straight, slender, glabrous outside, hairy inside; lobes subequal, 1-1.1x0.5-0.6 cm, shortly clawed, narrow-ovate, oblong, sub-acute to obtuse, with crenulate margins, glabrous. Stamens 4, inserted slight below the throat, longer 5 cm long, shorter 3.5 cm long; filaments hairy and whitish at base, deep purple, shining, much exserted, twining at maturity, anthers 0.2x0.1 cm, oblong, cells parallel, deep brown, dorsifixed, extrorse. Ovary 0.2x0.15 cm, slight elongate, green, glabrous, 4-lobed, 4-celled; 1-ovule in each cell; style 5 cm long, much exserted, filiform, basally white, deep purple distally, glabrous, shining; stigma deep purple, acutely 2-fid, arms 0.2 cm, glabrous. Drupes 0.6-1.2 cm long, pyriform, succulent, 4-chambered, black at maturity, encircled by veined, enlarged calyx, separating into 4-woody pyrenes of which 1-3 are sometimes suppressed (Figures 17, 18).

Field notes. Straggling much branched, glabrescent, strongly smelling shrub with entire elliptic leaves. Flowers opening time: 4.45 to 6 pm. Major venation pattern pinnate brochidodromous. Flowers and Fruits: July to December.

Distribution: In Maharashtra, Kerala and throughout India. In Australia, China and Mauritius.

Habitat and ecology: Common in village outskirts, usually grown as hedge plant.

Vernacular names: Baad (Marathi), Koynel (Marathi), Vanjai (Marathi)

Uses. Leaves contain sterols and are employed as febrifuge, antiperiodic and quinine substitute in fever in India and smeared with oil applied to wounds. In pacific area the bark is used in making baskets. Boiled roots useful in curing rheumatism (Wealth of India 1950, 1952, 1956, 1959, 1962, 1966, 1976).


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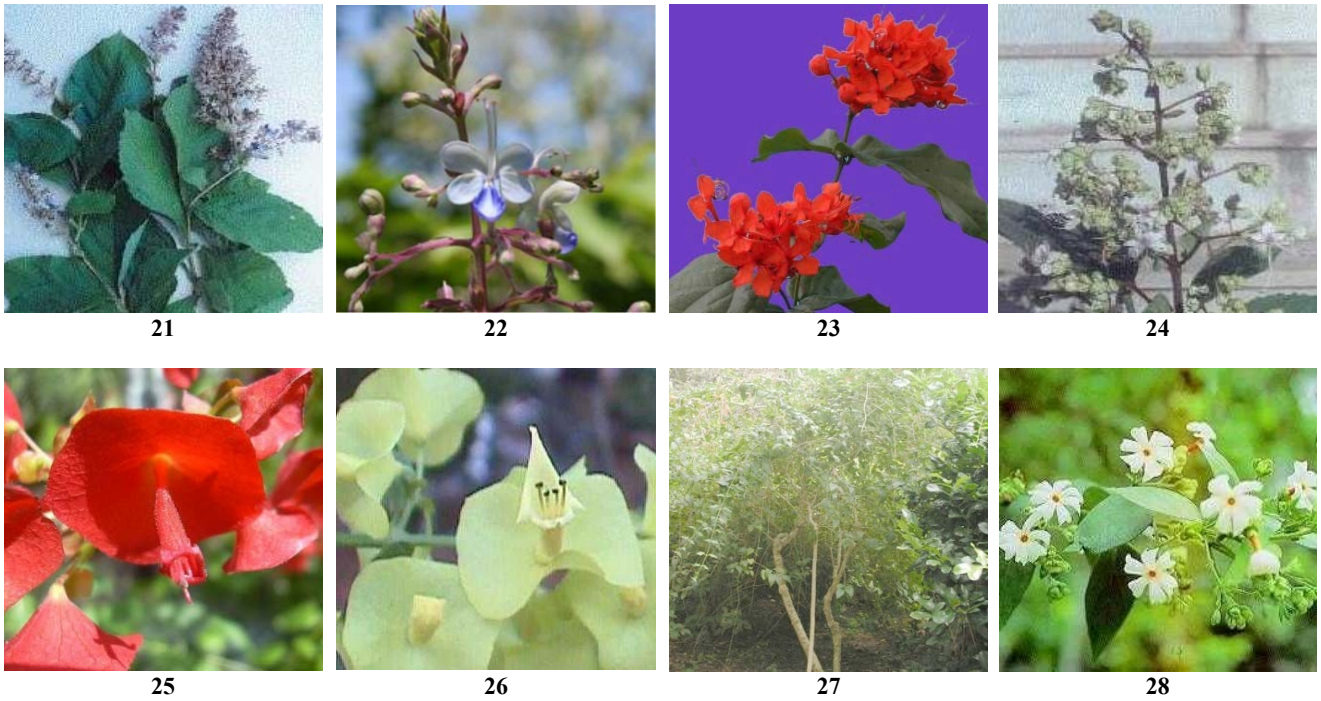

Figures: 1. *Lantana camara* var. *aculeata*, 2. *Lantana flava* (www.wellgrowhorti.com), 3. *Lantana nivea*, 4. *Phyla nodiflora*-spike, 5. *Phyla nodiflora*, 6. *Stachytarpheta jamaicensis*, 7. *Stachytarpheta mutabilis*, 8. *Glandularia bipinnatifida* (http: //src.sfasu.edu), 9. *Verbena bipinnatifida*-leaves, 10. *Petrea volubilis*, 11. *Duranta erecta*, 12. *Duranta erecta*-Fruits, 13. *Tectona grandis*-enlarged inflated calyx enclosing fruit, 14. *Gmelina arborea*, 15. *Gmelina philippensis*, 16. *Vitex negundo*, 17. *Volkameria inermis*, 18. *Volkameria inermis*-fruits, 19. *Clerodendrum phlomidis*-white flowers (www.flickr.com), 20. *Clerodendrum phlomidis*-rosy pink flowers, 21a. *Rotheca serrata*-leaves, 21. *Rotheca serrata*-flower, 22. *Clerodendrum splendens*, 23. *Clerodendrum infortunatum*, 25. *Holmskioldia sanguinea*-red flowers, 26. *Holmskioldia sanguinea*-yellow flowers, 27. *Holmskioldia sanguinea*-Habit, 28. *Nyctanthes arbor-tristis.*

Morphological diversity

Regarding morphological diversity, the studied species share the prominent characteristic features of the family Verbenaceae:

Diverse habits

Verbenaceae has diverse habits, namely (i) Herbs i.e. *Phyla nodiflora* a prostrate creeping and *Glandularia bipinnatifida* prostrate decumbent herbs (ii) Herbaceous undershrubs i.e. *Stachytarpheta jamaicensis* and *S. mutabilis (*iii) Shrubs i.e. *Lantana camara* var. *aculeata*, var. *nivea* are straggling or scandent shrubs with recurved prickles, *L. camara* var. *flava* is small shrub (iv) Liana i.e. *Petrea volubilis (*v) Large shrub or small tree i.e. *Vitex negundo,* (vi) Trees i.e. *Gmelina arborea* a medium sized deciduous and *Tectona grandis* a large deciduous.

Holmskioldia sanguinea is straggling evergreen shrub with drooping branches. *Volkameria inermis* is straggling much branched, *Rotheca serrata* is erect, *Clerodendrum infortunatum* is gregarious, tomentose, *Duranta erecta* a bushy and *Gmelina philippensis* sprawling spinous with drooping branched, *Clerodendrum splendens* is large climbing, shrub. *Nyctanthes arbor-tristis* is large shrub or small scabrous tree. Bark is characteristic for certain species.

Plants with typical strong or bitter aroma

Lantana camara var. *aculeata* with typical strong, *Volkameria inermis, Clerodendrum phlomidis, Vitex negundo* with typical bitter aroma.

Quadrangular stems

In all species. *Nyctanthes arbor-tristis* stem quadrangular, strigose.

Simple or occasionally compound leaves

Simple leaves in all species studied, compound 3-5 foliate in *Vitex negundo.* Leaves exstipulate, petiolate or sessile, entire, sub-entire, serrate, or crenate-serrate, dentate, opposite decussate.

Flowers

Bisexual, mostly zygomorphic, rarely sub-regular, usually without fragrance, sessile or shortly pedicellate. Flowers in *Tectona grandis* as well as in *Nyctanthes arbor-tristis* sub-regular, with delightful fragrance. Flowers are very small, minute in *Phyla nodiflora* and largest, showy in *Gmelina arborea* and *G. philippensis.*

Inflorescence

A spike, raceme, head, cyme or panicle. In *Lantana camara* it is capitate spike, in *Phyla nodiflora* is condensed cylindrical spike (head). In *Glandularia bipinnatifida*

corymbose, terminal spike. In *Duranta erecta, Petrea volubilis* racemes or panicle of racemes, and panicle of cymes in *Tectona grandis, Gmelina arborea, G. philippensis, Vitex negundo, Volkameria inermis, Clerodendrum phlomidis, Rotheca serrata, Clerodendrum infortunatum, C. splendens* and *Holmskioldia sanguinea.* In *Nyctanthes arbor-tristis* it is in bracteate fascicle of 3 to 5, in each pedunculate head, arranged in trichotomous cymes.

Bracts

Verbenaceae have various bracts, namely: (i) Flowers bracteate. Bracts small, caducous, subulate or linear in *Petrea volubilis , Duranta erecta, Gmelina arborea, Vitex negundo, Volkameria inermis, Clerodendrum phlomidis,* subpersistent, colored in *Rotheca serrata,* glabrous brownish in *Clerodendrum splendens,* ovate, green in *Holmskioldia sanguinea (*ii) Conspicuous, green, leafy, linear, longer than calyx-in *Lantana camara* var. *aculeata, L.* flava, *L. nivea (*iii) Acuminate, shorter than calyx-in *Stachytarpheta jamaicensis,* sharply acuminate projecting into a shaft in *S. mutabilis,* slight shorter than calyx in *Glandularia bipinnatifida (*iv) Grayish-brown, longer than calyx, stellately tomentose in pairs at each fork in *Tectona grandis.* Conspicuous, elliptic-round, obovate, mucronate with purplish tip in *Phyla nodiflora.* Large, showy, membranous, purple veined enclosing calyx in *Gmelina philippensis.* Leafy, boat shaped, green-purplish, gland dotted at tips in *Clerodendrum infortunatum.* In all species bracts are entire.

Bracteoles

Generally absent but noted in *Volkameria inermis* and *Holmskioldia sanguinea* and are small, similar to their bracts.

Calyx

Persistent, limb 4 to 5 partite or toothed, aestivation usually valvate, enlarged or unaltered in fruits. It is truncate, obscurely toothed, membranous in L. *camara* var. *aculeata, L. flava, L. nivea*; membranous, deeply 2-lobed, mitre shaped, compressed in *Phyla nodiflora;* tubular, elongate, compressed, membranous, 4-toothed in *Stachytarpheta jamaicensis* and *S. mutabilis;* tubular, 5-toothed, 5-ribbed, setaceous in *Glandularia bipinnatifida;* tubular, unequally 5-toothed, yellowish green, orange colored in fruits in *Duranta erecta;* campanulate, petaloid, purplish-blue, dry and green in fruiting, with star shaped radiating subequal 5-lobes with reticulum, and calyx papillae at throat in *Petrea volubilis.* Their reference is given as large involucriform epicalyx (in System of Botany Descriptive and Analytical by J.D. Hooker, Reprint 1986), campanulate, stellately tomentose, with 5-6 spreading subequal lobes, inflated in fruiting in *Tectona grandis;* broadly campanulate with small 5-unequal teeth, anterior two larger gland dotted in *Gmelina arborea* and *G. philippensis;* campanulate, with tomentose, with 5 equal teeth in *Vitex negundo;* campanulate, glabrous with 5 acute, purplish tipped teeth in *Volkameria inermis;* campanulate, green with 5-segments divided half way down in *Clerodendrum phlomidis;* cup-shaped, truncate with 5 small, equal segments in *Rotheca serrata;* campanulate, with globose base glabrous, divided more than half way down into 5 equal segments, deep pinkish-red tinged in *C. splendens,* campanulate, divided up to base into 5 large, equal, silky pubescent, purplish-red gland dotted in *Clerodendrum infortunatum;* saucer shaped, spreading broadly and uniformly from base upward, obconic, 2.5 cm in diameter, petaloid, red to orange or yellow, with fine reticulum with entire or obscurely sinuate margin in *Holmskioldia sanguinea.* In *Nyctanthes arbor-tristis* it is narrowly campanulate, truncate, obscurely, 5-toothed, pale green, and unaltered in fruits.

In majority of species studied, calyx is pubescent outside or on both sides. It is persistent, unaltered or very slightly altered in fruits in *Lantana camara* var. a*culeata, L. flava, L.* n*ivea, Stachytarpheta jamaicensis, S. mutabilis,* in *Phyla nodiflora* it is closely enclosing the fruit, *Glandularia bipinnatifida, Petrea volubilis, Gmelina arborea, G. philippensis, Clerodendrum phlomidis, Rotheca serrata, C. splendens, Holmskioldia sanguinea.* It is enlarged or much enlarged in *Duranta erecta, Tectona grandis* in which it is enclosing fruit, *Vitex negundo, Volkameria inermis,* much enlarged and reddish in *Clerodendrum infortunatum.*

Corolla

Tubular, limb 4 to 5 fid, usually unequal and labiate, rarely regular. Aestivation imbricate or quincuncial. Form of corolla salver shaped with oblique tube in *Lantana camara* var. *aculeata, L. flava, L. nivea,* tubular, unequally 2-lipped with straight tube in *Phyla nodiflora,* tubular with spreading emarginate or entire lobes in *Stachytarpheta jamaicensis, S. mutabilis,* funnel shaped with oblique tube in *Petrea volubilis,* 2-lipped with slight oblique tube and oblong retuse or emarginate lobes in *Glandularia bipinnatifida,* slight 2-lipped with obtuse lobes and oblique tube in *Duranta erecta,* regular with 5 to 6 spreading lobes in *Tectona grandis,* 2-lipped, infundibuliform, ventricose in upper part with oblique tube in *Gmelina arborea* and *G. philippensis,* limb 2-lipped with straight tube in *Vitex negundo,* hypocrateriform in *Volkameria inermis*, *Rotheca serrata* and *Clerodendrum* (all species), tubular with very short lobes oblique tube in *Holmskioldia sanguined.* In *Nyctanthes arbor-tristis* it is rotate or hypocrateriform and with distinct twisted aestivation. Corolla lobes in majority of species unequal. Corolla tube hairy inside or outside or on both sides or glabrous. In *Vitex negundo* hairy inside at insertion of stamens. In *Duranta erecta* hairy inside, lobes hairy or glabrous. In *Tectona grandis* lobes are finely stellately tomentose on nerves beneath. Floral colour ranging from white, yellow-white, pink, orange-pink, yellow, yellowish-brown, crimson rose, deep bluish purple, white with purplish tinge at throat.

Stamens

4, Didynamous by arrest of the fifth, sometimes 2 by arrest of the 3 upper, very rarely 5 fertile, epipetalous on the corolla tube or throat, filaments hairy, glandular or glabrous, included or exerted; anthers 2-celled sometimes diverging, dehiscence longitudinal. Stamens only 2, by

arrest of 3 in *Stachytarpheta jamaicensis* and *S. mutabilis,* 4 and equal in *Petrea volubilis,* 5-6 in *Tectona grandis,* filaments glandular in *Duranta erecta,* papillate in *Gmelina arborea,* hairy in *G. philippensis,* densely hairy at base in *Vitex negundo* and *Rotheca serrata;* glabrous shining twined at maturity in *Volkameria inermis, C. splendens, Clerodendrum infortunatum,* pubescent below in *Clerodendrum phlomidis,* red, hairy, papillose, glandular in *Holmskioldia sanguinea.*

In *Nyctanthes arbor-tristis* stamens are 2 and they are completely adnate to tube, but their number is 2 by arrest is doubtful; anthers usually described as dorsifixed and extrorse, but in present observation both types dorsifixed and occasionally basifixed and introrse or extrorse conditions are found. Anthers are dorsifixed except in *Lantana camara* in all varieties, *Petrea volubilis* where they are basifixed Extrorse condition is observed in *Stachytarpheta jamaicensis, S. mutabilis, Petrea volubilis, Tectona grandis, Volkameria inermis, Rotheca serrata, Clerodendrum splendens.* In *Nyctanthes arbor-tristis* they are dorsifixed and introrse. Stamens included in *Lantana camara* in all varieties, *Phyla nodiflora, Stachytarpheta jamaicensis, S. mutabilis, Verbena bipinnatifida, Petrea volubilis, Duranta erecta,* much exserted in *Vitex negundo Volkameria inermis, Clerodendrum phlomidis, Rotheca serrata*, *Clerodendrum splendens, C. infortunatum* and little exerted in *Holmskioldia sanguinea,* filaments are white, purplish, yellow, and red in different members. They are twisted in all species of *Clerodendrum*, *Rotheca serrata* and *Volkameria inermis*. In *Nyctanthes arbor-tristis* stamens are included, filaments petaloid and completely adnate to corolla tube.

Carpels

2, Syncarpous, ovary superior, mostly 4-lobular, 2 to 4 partite with mostly 1, occasionally 2-ovules in each locule, appearing in axile placentation. Style terminal. Stigma simple capitate or unequally shortly 2-fid. In *Duranta erecta* carpels 4, syncarpous and 2-ovules per locule.

Fruits

Mostly drupaceous, fleshy or dry separating into 1-seeded pyrenes. It is dry in *Phyla nodiflora, Stachytarpheta jamaicensis, S. mutabilis,* stellately tomentose, fleshy in *Tectona grandis.* It is succulent, fleshy in most species, black at maturity in *Lantana camara, L. flava*, *L. nivea*, *Volkameria inermis, Clerodendrum infortunatum, Rotheca serrata, Clerodendrum phlomidis,* orange in *Duranta erecta,* yellowish-orange in *Gmelina arborea,* yellow in *G. philippensis.* In *Nyctanthes arbor-tristis* it is suborbicular, much compressed, chartaceous 2-celled, green when young and brown at splitting stage, separating into 2-flat 1-seeded carpels. In *Clerodendrum splendens* fruits are undeveloped (not seen).

Key to genera

The morphological generic key for separation of genera studied is given as:

1. Herbs or under shrubs
  2. Leaves divided into linear divisions …….. *Glandularia*
  2. Leaves not divided into linear divisions
    3 Prostrate herbs; flowers white or pale-pink; fertile stamens 4 ……. *Phyla*
    3. Erect undershrubs, flowers blue, fertile
1. Shrubs or trees
  4. Leaves palmately compound ……... *Vitex*
  4. Leaves simple
    5. Inflorescence racemose
      6. Woody climbers; calyx lobes longer than corolla tube, purple colored ……... *Petrea*
      6. Woody straggling shrubs, calyx lobes shorter than corolla tube, green
        7. Flowers in capitate spikes; fruits 2-seeded purplish-black ……. *Lantana*
        7. Flowers in panicled racemes; fruits 8-seeded, yellow ……... *Duranta*
    5. Inflorescence cymose
      8. Corolla lobes contorted; stamens 2; fruits capsular …….. *Nyctanthes*
      8. Corolla lobes imbricate; stamens 4 to 6, fruits drupaceous
        9. Large trees, corolla regular; stamens equal; fruits enclosed in much inflated calyx …….. …….. *Tectona*
        9. Shrubs or medium-sized trees; corolla irregular, stamens didynamous; fruits not as above
          10. Medium sized trees, calyx with 2 to 7 nectary glands …….. *Gmelina*
          10. Scandent shurbs, calyx without nectary glands
            11. Calyx rotate, saucer shaped, entire ……. ……..... *Holmskioldia*
            11. Calyx campanulate, not saucer shaped, 5-toothed or 5-partile …… *Clerodendrum*
              12. Flowers white ……..… *Volkameria*
              12. Flowers purplish …… *Rotheca*

Discussion

The species in present study are traditionally falling into two tribes Verbeneae and Viticeae (Bentham and Hooker, 1862-1883). This division of tribes and their sub-tribes are treated by Bentham as being more natural than any subsequently proposed schemes. The divisions are on main basis of: (i) Tribe-Verbeneae-Inflorescence indefinite (ii) Tribe-Viticeae-Inflorescence cymose, definite.

The studied characters are in accordance with this scheme. The notable aspects are: (i) Though in tribe Verbeneae, inflorescence of indefinite type. The flower numbers are not too large, as that in tribe Viticeae, where they are in many cases numerous. In *Nyctanthes arbor-tristis* flowers sessile in bracteate fascicle of 3-5 in each pedunculate head (ii) Majority of plants are propagated by seeds and vegetative cuttings. Purely vegetatively propagated species is *Clerodendrum splendens.*

As tribal characterization is mainly based on inflorescence nature, occurrence of mixed types of forms found in these two tribes.

Interestingly Pande and Pande (2001) reported hitherto unreported and abnormal epiphytic habit of *Lantana camara* for the first time from the Kumaon Himalaya but

such abnormal epiphytic habit for *L. camara* is yet neither reported nor observed from the local region.

Floral morphology of *Phyla nodiflora* observed with similar characters as noted by Maheshwari (1954).

In *Rotheca serrata* leaves are opposite decussate with quadrangular stems and in whorls of 3 with hexangular stems. This correlation may be on basis of fundamental principles of shoot organization as discussed by Kaplan (2001) in relation to relationship of leaf to stem. According to his discussion, the shoots of higher plants are typically characterized as being differentiated into nodes and internodes. The nodes, by definition, are sites of leaf insertion, whereas the internodes are considered to be the stem units that typically elongate between the points of leaf insertion. Each internode is not just stem, but a compound structure consisting of decurrent leaf bases, that run along the length of the internode below it and hence periphery of stem transection is actually adnated leafbase tissue adnating with it and these components being inseparable, the term "Shoot" is used. if the point of leaf insertion, in fact is not restricted to nodes but runs along the length of what traditionally has been called the internode, then one could predict that the transectional shape of an internode will reflect the pattern of phyllotaxis of its shoot. In shoots with an opposite and decussate phyllotaxis, internodes are square as a consequence of the four diagonal orthostichies of leaf insertion. By contrast, internodes in shoots with a two ranked or distichous phyllotaxis have a bilaterally symmetrical or elliptical shape, reflecting their alternate pattern of leaf insertion. Internodes with a helical or polystichous phyllotaxis exhibit a polygonal outline. Leaf insertions extend along the length of an internode because the leaves are initiated from the periphery of the shoot apex before there is any significant extension of the internodes. Since the incipient internode is such short region, a part of the leaf base, is inevitably included with shoot elongation. However, this relationship is observable in primary body and becomes obscure or lost in secondary one. Hence reason behind ternate leaves in *Rotheca serrata* with hexagonal outline of stem can be understood. It is also can be understood in general that Verbenaceous stem is quadrangular as having opposite decussate phyllotaxy.

In *Clerodendrum phlomidis* rarely pink flowered forms are found but otherwise these variants are similar in major morphological and anatomical details and are forms of same species.

Tectona grandis showing minor but distinct morphological variations in leaf margin, (entire or serrulate), petiole length (very short or longer) and tree form (bushy or tall), which are paralleling the finer anatomical differences, also may be treated as different genetical clones. Variation refers to the observable differences in individuals for a particular trait. These differences may partly be due to genetic factors and partly due to the environmental effects. From the studies, it is inferred that considerable genetic variation in field height and collar diameter exists among clones of *Tectona grandis* (Gera et al. 2001). Large variations were found both within and between the five-tested provenances regarding heartwood percent and content of silica and calcium by Kjar et al (1999). Genetic differentiation between populations of *Tectona grandis* L.f. was examined in 9 quantitative characters and 10 allozyme loci by Kjar et al (1996) and large differences between populations were revealed in the quantitative traits. According to authors the larger differentiation between populations in morphological traits than in allozyme markers is probably a result of adaptation through natural selection and possibly, a higher mutation rate in allozyme loci.

These type of minor variations specially observed in leaf margins in many studied species of the Verbenaceae in which the original leaf margin is entire but in same plant or different some varied leaves are noticed as in *Vitex negundo,* where leaf margin entire but some variants show finely serrulate margin; in *Gmelina philippensis* same plant having younger leaves tridentate with distally placed teeth; in *G. arborea* young leaves are with distantly placed teeth; in *Holmskioldia sanguinea* some leaves may be finely serrulate. In *Nyctanthes arbor-tristis* same plant may posses' majority of leaves as entire but at the same time many are with distantly placed teeth.

Atkins (1996) placed *Holmskioldia sanguinea* Retz. in Labiatae (Lamiaceae). Many floras also retained it under Verbenaceae but according to recent studies it is placed in Lamiaceae.

The family Verbenaceae has been circumscribed much more broadly by most systematics (e.g. Cronquist 1981) and separated from Lamiaceae by the presence of terminal (vs. gynobasic) style. Judd et al (2002) included only the traditional subfamily Verbenoideae (excluding the tribe Monochileae). As traditionally delimited Verbenaceae are paraphyletic, while Lamiaceae are polyphyletic. In order to make Lamiaceae monophyletic, nearly two-thirds of the genera usually included within Verbenaceae (e.g. *Callicarpa*, *Clerodendrum*, *Vitex*, and *Tectona*) are transferred to Lamiaceae (Cantino 1992; Cantino et al. 1992; Thorne 1992). As redefined, Verbenaceae can be distinguished from Lamiaceae by their indeterminate racemes, spikes, or heads (vs. inflorescences with an indeterminate main axis and cymosely branched lateral axes, these often reduced and forming pseudowhorls); ovules attached on the margins of false septa (vs. ovules attached on the sides of false septa); simple style with conscipicuous, 2-lobes stigma (vs. usually apically forked style with inconspicuous stigmatic region at the tip of each style branch); pollen exine thickened near apertures vs. not thickened; and nonglandular hairs exclusively unicellular (vs. multicellular, uniseriate). In addition, the flowers tend to be less strongly two lipped. The style of Verbenaceae is exclusively terminal, while in Lamiace it varies from terminal to gynobasic.

The inclusion of *Petrea* within Verbenaceae is not supported by cpDNA data alone (Olmstead et al. 1993; Wagstaff and Olmstead 1997; Wagstaff et al. 1998), but it is supported by combination of morphology and cpDNA sequences (Cantino 1992).

Emphasis on different traits has resulted in Conflict between taxonomic systems in Verbenaceae, but none of the traditional systems align well with the molecular

phylogeny, which suggests that homoplasy is rampant in all of the traits used in those classifications (Marx et al. 2010).

Briquet's (1895) treatment of the family has been the one most widely accepted. His classification is based on the number of locules in each carpel, the number of ovules in each locule, and inflorescence morphology. This classification was not accepted by Junell (1934), who suggested that abortion of a carpel had occurred independently several times; this is confirmed by study of Marx et al (2010), in which *Baillonia*, *Casselia*, tribe Petreeae, tribe Neospartoneae, a clade in tribe Duranteae, and tribe Lantaneae except Coelocarpum all have unicarpellate ovaries.

Some authors (e.g. Troncoso 1974) have used fruits as a diagnostic trait for tribes; for example, fleshy or dry fruits, further divided, or not, into mericarps; this also according to Marx et al (2010) has proved misleading for defining groups within Verbenaceae. All members of tribes Petreeae, Casselieae, Neospartoneae, and Citharexyleae (except Rehdera), as well as Duranta and Lanta, have fleshy fruits, and the rest have dry fruits, which shows that this character does not support suprageneric groups consistent with these phylogenetic results.

Inflorescence morphology and structure have been misunderstood and not correctly interpreted until recent studies (Martinez et al. 1996; Martinez and Mulgura 1997; Mulgura et al. 1998, 2002; Drewes and Martinez 1999). Consequently, classifications based on racemose or spicate flowering shoots in the terminal or axillary position (Schauer 1847; Briquet 1895) have been unnatural. A compound inflorescence with both terminal and branched lateral flowering shoots, termed a "heterothetic paniculiform pleiobotryum," has been suggested to be the primitive or central form in Verbenaceae, from which other forms were derived through processes such as condensation, reduction and truncation (Martinez et al. 1996). Lantaneae generally have flowering shoots only in axillary positions (homothetic pleiobotrya), a derived condition from the heterothetic pleiobotrya found in most other tribes, except Casselieae.

Traditional tribal classifications based on morphology have been misleading with respect to evolutionary relationships in Verbenaceae (Marx et al. 2010). The results presented by Marx et al (2010) permit the realignment of genera into a new tribal classification, which recognizes a new tribe, Neospartoneae. The phylogeny presented by him can serve as a basis for further work to better understand the evolution of traits, such as fruit and inflorescence architecture, which have misled previous systematists studying Verbenaceae.

CONCLUSION

The verbenaceous members of Melghat and Amravati regions of Maharashtra, India show morphological diversity within the family limits and are important plant species regarding their useful products and ornamental value. There is clear demarcation of morphological characters of Bentham and Hooker's tribes Verbeneae and Viticeae in which studied genera and species are accommodated. According to recent molecular studies and classification, the tribe Verbeneae is retained in Verbenaceae and splitted into different tribes, while genera of Viticeae are transferred into Lamiaceae.

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Nest density as determinants for habitat utilizations of Bornean orangutan (*Pongo pygmaeus wurmbii*) in degraded forests of Gunung Palung National Park, West Kalimantan

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ABSTRACT

*Prasetyo D, Sugardjito J (2011) Nest density as determinants for habitat utilizations of Bornean orangutan (*Pongo pygmaeus wurmbii*) in degraded forests of Gunung Palung National Park, West Kalimantan. Biodiversitas 12: 164-170.* Conversion of forests into non-forests areas particularly for the development of timber industry and oil palm plantation in Ketapang district, West Kalimantan province was drastically increased recently. We have conducted an assessment for the density of Bornean orangutans *Pongo pygmaeus wurmbii* L. in degraded forests of the boundary of Gunung Palung National Park, West Kalimantan in 2004 and 2005. We used line-transects nest-count to survey 39,6 km length at 13 sites including 7 in side the park and the other 6 situated out side the park which holds a difference status of forest management. The differences of nest density between degraded forests habitat was calculated. The old degraded forest which has been logged for 5 years or more, were containing more new nests in a cluster compared to the recently as well as currently logged. The highest number of orangutan's nests could be found in the old degraded forest in side the park, whereas the lowest one was obtained in the currently logged protection forest with the density of 3.70 ind/km^2 and 0.06 ind/km^2 respectively. We compare these results with the survey undertaken previously in the area when the logging and forest conversion activities have just begun in the region in 2001.

Key words: degraded forests, nest-count, orangutan density, *Pongo pygmaeus wurmbii*.

INTRODUCTION

West Kalimantan forests support one of Borneo's few remaining large orangutan populations. Most of these populations, however, are inhabit forests outside protected areas and hence, are threatened by conversion for concession-based timber industry and oil palm plantation. Together with inconsistency of land-use policy, the establishments of palm oil and timber industries have resulted in highly fragmented and degraded forests (EIA 1998; Rijksen and Meijaard 1999; FWI 2002). It has been shown that deforestation rate in the Ketapang district of west Kalimantan was significantly increased between 2000 and 2005. The primary dry lowland and peat swamp forests in Ketapang district were reduced 15% and 28% respectively during this period (Adhikerana and Sugardjito 2010). Unfortunately, those forests provide good habitat for orangutan.

The orangutan is known to inhabit primary and secondary forest and is typically found in lowland dipterocarp, freshwater and peat swamp forests. It has also been recorded from hill forests up to about 1,500 m although it occurs in a lower density than in other forest habitats (MacKinnon 1974; Rijksen 1978; Payne 1988; Rijksen and Meijaard 1999; Yanuar et al. 1996). All of those forest habitats, however, are under pressures. Further, habitat degradation and fragmentation have been identified as the major driver of extinctions of many tropical forest species (Laurance et al. 1998). It has been long recognized that the main factor of deforestation is human population pressures (Brown and Pearce 1994).

In the beginning of implementation of district autonomy, forest encroachments for subsistence agriculture nearly existed in all over area in Indonesia including Kalimantan. Previous results estimated that the total area of orangutan habitat in Kalimantan has decreased 2.8% annually (Wich et al. 2008). Pressures from hunting for bush meat and pet trade have even made the Bornean orangutan population more vulnerable and it leads to the status of the species into endangered (Sugardjito 1995; Ancrenaz et al. 2008).

The population estimate of Bornean orangutan has been made by previous authors who have ever made gross survey in the island. MacKinnon (1985) has estimated the population of orangutan in Kalimantan about 156,000 whereas, Sugardjito and van Schaik (1991) have suggested the population which inhabits protected areas in Kalimantan was ranging between 20,000 and 30,000 individuals. Later on 2004 in the orangutan population and habitat viability analysis, the experts have proposed the total number of orangutan population in Kalimantan was 54,000 (Singleton et al. 2004).

The Gunung Palung National Park which is located in the districts of Ketapang and Kayong Utara is suitable habitat for orangutans. The forest landscape in this region consists of various types of habitats from coastal lowland forest up to hill forest. This type of habitats provides orangutans to move and utilize different habitats in order to exploit food sources in the area (Leighton 1993). With the rapid reduction of forest habitat especially after intensive forest conversion between 2000 and 2005 (Adhikerana and Sugardjito 2010), the assessment on population status of orangutan in the degraded forests of Gunung Palung area is urgently needed. The purpose of this survey was to assess the status of orangutan population in the Gunung Palung National Park, specifically in its boundary areas where human pressures are mostly occur. Further, the principal aims of our field survey were to estimate orangutan population densities in degraded forest habitats with various degrees of disturbances and to identify the determinants of intensity utilization of degraded forests by orangutans.

MATERIALS AND METHODS

Study sites

The sites which are located in boundaries of Gunung Palung National Park either in side or out side the park have been selected. We chose the area surrounding the boundaries of the park which are frequently under pressure due to human activity and it leads to disturbances. The periods of disturbances were different between sites. Some have been disturbed more than 5 years while the others just disturbed between 2 and 5 years before the survey. The followings are 13 sites that have been surveyed during 2004 and 2005 (Figure 1, Table 1).

Lubuk Baji

Located in side the Park with the geographic positioned at S 01º13'05.6" and E 110º00'36.1". The area categorized as old degraded forest consists of primary lowland forest which forms a continuous canopy of trees between 20 and 30 m high. The illegal logging was very few in this site and it was happened in a period between 1998-2003. In the adjacent boundary, there are community's garden occupied by fruit trees such as durian, lansium, rambutan and mangoesteen.

Sungai Benawai

Situated within the Park with geographical positioned at S 01º12'46.9" and E 110º01'48.5". The habitat categorized as current degraded forest consists of peat swamp forest with open canopy trees of less than 20 m with less than 30 cm diameter at breast high. The majority of tree species belongs to Dipterocarpaceae trees that are commonly used as host tree for strangling figs the staple food of orangutan. Land-use conflict occurs in this site. Local people were active to enter the area and open the garden while taken the wood.

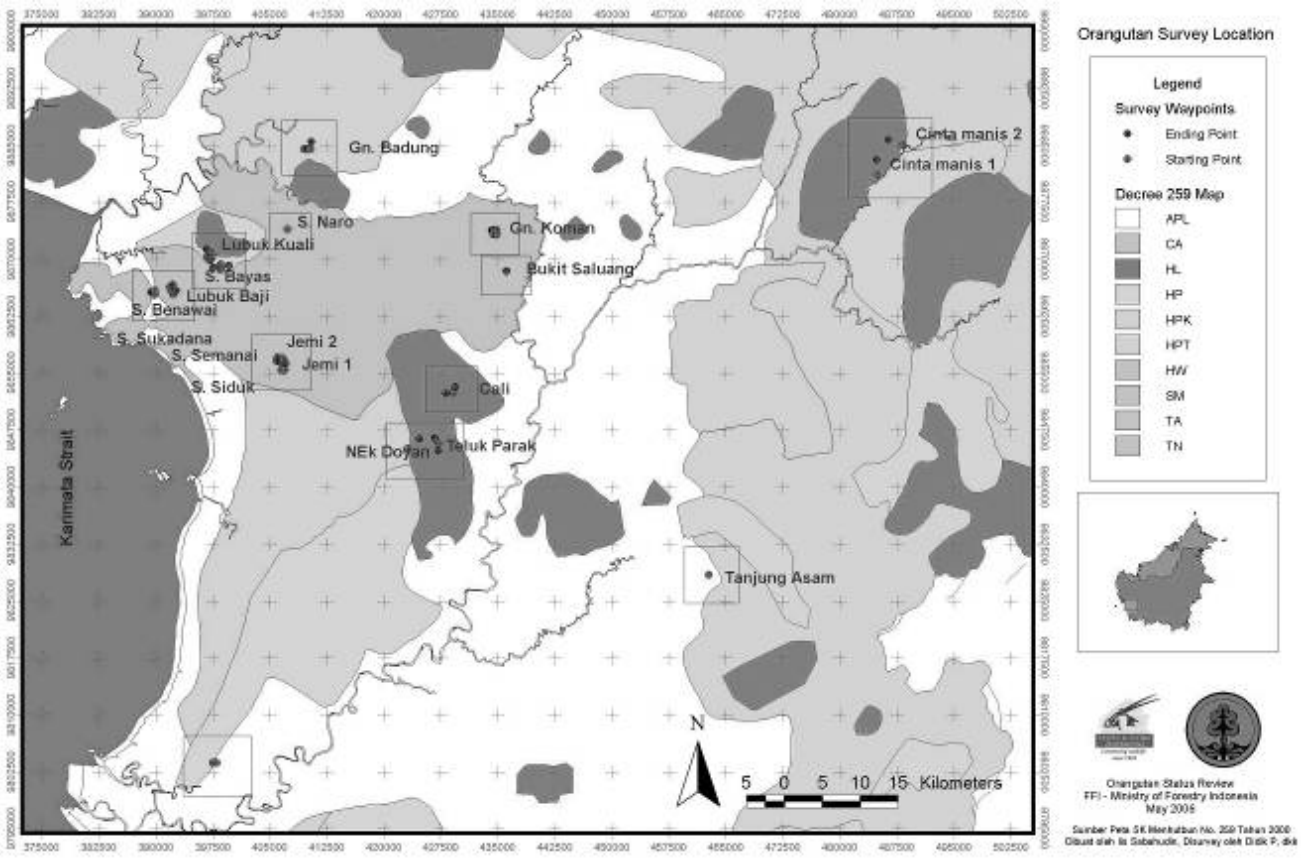

Figure 1. Survey sites inside Gunung Palung National Park and its surroundings.

Table 1. Habitat information in survey sites.

Location	Geographic positioned	Habitat type	Habitat status	Legal status
Lubuk Baji	S 01º13' 05.6"; E 110º 00' 36.1"	Primary lowland forest	Old degraded forest	NP
Sungai Benawai	S 01º 12'46.9"; E 110º 01'48.5"	Secondary peat swamp forest	Current degraded forest	NP
Sungai Bayas	S 01°10’51.6; E 110°05’19.7”	Primary peat swamp forest	Old degraded forest	NP
Jemi	S 7° 4' 6.34" S; E 181° 20' 42.17"	Primary lowland forest	Recent degraded forest	NP
Gunung Koman	S 74° 12' 11.25"; E 103° 51' 11.33"	Secondary lowland forest	Old degraded forest	NP
Bukit Saluang	S 7° 39' 28.254"; E 181° 31' 49.90"	Secondary lowland forest	Recent degraded forest	NP
Sungai Naro	S 7° 10' 33.508"; E 181° 29' 50.99"	Secondary Lowland and peat swamp forest	Recent degraded forest	NP
Nek Doyan	S 01º 24'03.5"; E 110º 18'34.4"	Secondary lowland forest	Current degraded forest	PF
Teluk Parak	S 01º 23'42.3"; E 110º 20'45.2"	Secondary lowland forest	Current degraded forest	PF
Cali	S 01º 20' 08.1"; E 110º 21' 48.5"	Secondary lowland forest	Current degraded forest	PF
Gunung Badung	S 7° 15' 25.404" E 181° 35' 12.19"	Secondary lowland and peat swamp forest	Recent degraded forest	PF
Lubuk Kuali	S 01° 10’23.1”; E 110° 04’11.2”	Secondary peat swamp forest	Old degraded forest	CF
Tanjung Pasar	S 01°46’35.3”; E 110°04’19.9”	Secondary peat swamp forest	Recent degraded forest	CF

Note: NP = National Park, PF = Protection Forest; CP = Community Forest.

Sungai Bayas

Located in side the Park with geo-positioned at S 01°10’51.6” and E 110°05’19.7”, we categorized as old degraded forest. It consists of primary peat swamp forest with very little disturbances and holds many food tree species of orangutan. There are new growths of vegetation in some parts where forest fire occurred in 1997-1998.

Jemi

The site is situated in side the Park with geo-positioned at S 7°4'6.3" S and E 181° 20'42.1". It was logged by people in 1990s and categorized as recent degraded forest. It consists of lowland primary forest with very steep slope. The forest canopy is continuous with trees high more than 25 m and 50 cm dbh. The majority of trees grow especially the timber species such as Dipterocarpaceae and food trees of orangutan like.

Gunung Koman

As the part of National Park area, this site is categorized as old degraded forest with geo-positioned at S 74°12'11.2" and E 103°51'11.3". Surroundings the Gunung Koman forest was planted by commercial fruit trees, but local people still reserve the forest for water resources. Canopy covered was high density, is almost 65% with height of tree between 15-25 meters.

Bukit Saluang

The site is located in side the Park with geo-positioned at S 7°39'28.2" and E 181°31'49.9". It consists of lowland secondary forest and categorized as recent degraded forest. Some big productive trees still occur in this site, such as meranti and bangkirai.

Sungai Naro

In side the Park with the geo-positioned at S 7°10'33.5" and E 181°29'50.9". The access to this site only from river. Illegal selective cutting of trees occurs in small portion by local people, so we categorized as recent degraded forest. It consists of lowland and peat swamp forests habitat. Many big trees still left with the diameter more than 50 cm. Some food trees of orangutan can be found here such as nyatoh, kumpang, langsat whereas the commercial trees are bangkirai, belian, meranti, galam tikus.

Nek Doyan

Located out side the Park but, it is within the protection forest of Gunung Tarak with the geo-positioned at S 01º24'03.5" and E 110º18'34.4". It categorized as current degraded forest consists of lowland secondary forest with open canopy. It is dominated by trees with less than 20 m high at 30 cm dbh. There were only a few trees with 50cm e.g. available in scattered places. The food trees of orangutan (Ficus spp) are very few. Active logging and encroachment are found during survey. Wildlife hunting is also often in this site. Cultivated durian trees are found in this area.

Teluk Parak

It also located in side the protection forest of Gunung Tarak. It consists of lowland secondary forest and categorized as current degraded forest with open canopy and only few big trees are left. The geo-positioned of the site is S 01º23'42.3" and E 110º20'45.2". This site was the remained of forests after big forest fire in 1998. The trees left are mostly small size with diameter at brest height less than 30 cm. Illegal logging was found very active during the survey.

Cali

Situated in side of the protection forest in Gunung Tarak with the geo-positioned at S 01º20' 08.1" and E 110º21'48.5". The habitat is similar to Teluk Parak where illegal logging active and local people were encroaching forest to establish subsistence agriculture land and it is categorized as current degraded forest.

Gunung Badung

Located out side the Park with geo-positioned at S 7°15'25.4" and E 181°35'12.1". It was logged very recent as log trail can be found everywhere and we categorized as recent degraded forest. It consists of secondary peat swamp and lowland forests habitat with steep landscape. Many sungkai trees grow in the peat swamp area whereas in

lowland forest are medang, kubing, belian, gelam tikus and gaharu. Hunting of animal frequently occurs in this site.

Lubuk Kuali

Located at S 01°10'23.1" and E 110°04'11.2" out side the Park. It consists of peat swamp forest with little disturbances and categorized as old degraded forest. Big trees with diameter more than 30 cm and more than 30 m height are still available. The forest relatively in good condition with closed canopy and it situated in between north-east and north-west parts of the park.

Tanjung Pasar

Located closed to Ketapang town with geo-positioned at S 01°46'35.3" and E 110°04'19.9". It consists of secondary peat swamp forest with few food trees of orangutan and categorized as recent degraded forest. This forest is managed by community. Animal hunting frequently occur in this site although the target is not orangutan.

Procedures

The field survey was carried out during September - October 2004 and May – June 2005. Orangutan nests were counted by observers who walked slowly along line transects with minimum length 2 km. The distance between transect lines was at least 3 km apart. We defined logging here is hand logging which was common in the area (Cannon et al. 1994). This technique normally targets only a few commercial tree species and involves the use of hand-held chainsaws and human-powered removal of timber to water-courses (Whitmore 1984). It was not mechanized logging with heavy machinery, and therefore, is considered less destructive to forest structure (Ayres and Johns 1987).

The nature of forest disturbance was recorded qualitatively in three categories: (i) 'current', i.e. areas currently subject to timber extraction, (ii) 'recent', i.e. areas subject to timber extraction within the last 2 - 4 years, and (iii) 'old', i.e. areas in which timber extraction had not taken place for at least 5 years. This classification was modified from Morrogh-Bernard et al. (2003). All nests visible from the transect line were recorded.

Nest count was conducted at each transect line and the perpendicular transect-to-nest distances (m) were measured for every nest observed. The decay stage of nests was categorized in five classes following van Schaik et al. (1995) i.e. (i) fresh, leaves still green; (ii) older nest still in original shape, leaves still attaches but brown; (iii) old, holes appearing in nest; (iv) very old, twig and branches still present, but no longer in original shape; 5) only twigs still present. Orangutans build a nest near to the last food tree they visited during their daily activity (Sugardjito 1983). We measured the distance between nests when we encountered several nests in one spot during nest count survey.

Data analysis

Orangutan nest densities for each habitat type were calculated using the software program called DISTANCE 4.0 release 2 (Buckland et al. 1993). This technique uses data of transect length, number of nest observed, and perpendicular distance of each nest from the transect line. Then nest densities are converted to orangutan densities. Following van Schaik et al. (1995) we used the following basic formula for calculating nest density (Dn) from line transect surveys.

Dn = N / (L x 2w), N = number of nest observed, L = the length of transect covered (km), and w = the effective strip width of habitat type censuses. Then nest density was converted into orangutan density (Dou) through addition of some parameters. Dou = Dn x 1/ (p x r x t), where p, is the proportion of nest makers in the population, r, is the rate at which nests are produced (number per individual per days), t, is the estimated time a nest remains visible. The t value has been obtained from published data, (399 for peat swamps, 259 for dry low land and 380 for hill forests). Johnson et al. (2005). As for r value, we used 1 since the published range of r values for Borneo have been identified between 0.9 and 1.16 nests per individual per day (Lackman-Acrenaz et al. 2001; Johnson et al. 2005).

We measured the distances between nests of the same nest class category in one spot when we detect more than one nest. This is in order to detect the differences of visiting frequency of orangutans in different category of degraded forests. Chi-square test has been used to detect the differences.

RESULTS AND DISCUSSION

Orangutan is a cryptic arboreal animal and difficult to detect in the forest. They build a nest every days for sleeping and sometime it makes a resting nest during the day (Prasetyo et al, 2009). Nest count survey, therefore, provides the only feasible means of obtaining orangutan density estimates in a short period of time. A total of 678 orangutan nests were observed along 39.6 km of transects through peat swamp, lowland and hill forests. The following orangutan densities derived from nest count have been calculated from the data shown in Table 2.

The density of orangutan in Sungai Bayas was the highest. The site was little disturbed due to small illegal logging for more than 5 years ago. However, since 2003 the orangutan patrol and monitoring units or OPMU has regularly patrolled and monitored the illegal activities in this area. Consequently, the disturbances did not expand to a wider range of the habitat and other wildlife species could also be protected from hunting. Various categories of orangutan nests can be observed in this site. Previous authors indicated that orangutan suffer temporary, density declines following reduced impact logging (Felton et al. 2003; Morrogh-Bernard et al. 2003; Johnson et al. 2005; Marshall et al. 2006) but recover to pre-logging densities if forests are allowed to regenerate (Knop et al. 2004). Recovery can be realized by retaining soft-pulp fruit bearing trees and climbers while strictly enforcing patrol system to protect the animal from hunting (Robertson and van Schaik 2001). This would be the case for Sungai Bayas forest habitat.

Table 2. Number of orangutan nests observed in Gunung Palung National Park and surroundings.

Location	Nest	Transect lenght (m)	Density estimate (ind/km^2)	Forest status
Lubuk Baji	40	1800	2.19	NP
Sungai Benawai	69	2400	2.35	NP
Sungai Bayas	148	4000	3.70	NP
Jemi	57	6000	2.08	NP
Gunung Koman	1	1000	0.06	NP
Bukit Saluang	43	3000	0.88	NP
Sungai Naro	53	3500	1.48	NP
Nek Doyan	8	2250	0.09	PF
Teluk Parak	20	2000	0.80	PF
Cali	14	2000	0.16	PF
Gunung Badung	36	4400	2.06	PF
Lubuk Kuali	152	4000	2.30	CF
Tanjung Pasar	37	3250	1.50	CF
Total	678	39600		

Note: NP = National Park, PF = Protection Forest; CP = Community Forest.

As for sites of Lubuk Baji and Sungai Benawai, the density was a bit lower than the previous site because, the logging was occurred when the decentralization just began in 2001 or 3 years before the survey was conducted. Monitoring and patrolling system in these areas should be reinforced continuously in order to enable forest regeneration and to protect orangutan from human pressure, as well as durian plantation surrounded the forest.

Previous report indicated that population density of orangutan in the Gunung Palung National Park was 3 ind/km^2 overall with primary peat swamp forest densities of over 4 ind/km^2 (Johnson et al. 2005). We estimate that in the recently disturbed areas (2-5 years previously) in side the park still holds an orangutan density of 2 ind/km^2. The lowest population density has been found in survey sites of the adjacent protection forests, Gunung Tarak i.e. Nek Doyan, Teluk Parak, and Cali. These areas were logged when we did a field survey and the density of orangutan was found 0.09 ind/km^2 which is low compared to the area where recently disturbed in side the park. The survey team has encountered with some loggers when surveyed in these 3 sites. Although the status are protection forest, but no forest management is undertaken. According to the previous study, the habitat of orangutan particularly outside the park was reduced up to 3.1 km^2 each year due to logging activities either legal or illegally, and forest conversion for agriculture, mining and plantation (Meijaard and Wich 2007). The direct impact for orangutan was loss of their food trees as well as nesting trees. It has been identified that orangutans build their nests in trees near to the last food trees they visited during the day (Sugardjito 1983). Large body size, arboreal, and frugivorous are the characteristics of orangutans. Tree stands are, therefore, very critical to the survival of orangutans. The analysis shows that the cluster of nests with various categories of classes between levels of forest disturbance are different (X^2 = 8,40 and p < 0,01). This result was similar to previous findings that orangutan density was reduced less in peat swamp forests rather than in dry lowland forests after logging (Felton, et al. 2003; Aveling 1982; Rao and van Schaik 1997). The high number of nests in clusters with various categories of classes in the old degraded forests such as Sungai Bayas and Lubuk Kuali have indicated that visiting frequencies of orangutan to these sites were high (Figure 2).

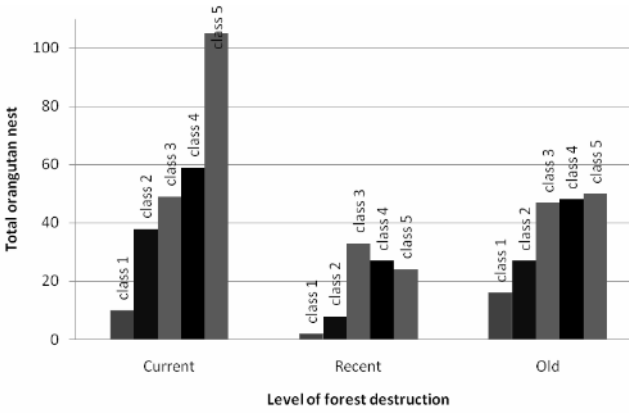

Figure 2. The distributions of orangutan nest classes in different level clusters of forest destruction.

In contrast, the similar phenomenon can not be found in survey sites which are logged during the field survey. Further, in addition to the high visiting frequencies, the old degraded forests have also been visited by a group of orangutans instead of an individual. This can be shown by the number of nests of the same category which are built in a cluster. Due to the forest of Lubuk Kuali is situated between two parts of boundaries of the park i.e. north-west and north-east, it might functions as a corridor area..

When fruit availability is abundant, orangutans may form a social group to harvest fruit sources while moving from one to another block of forests (te Boekhorst et al. 1990; Sugardjito 2009). The forest of Lubuk Kuali may serve as a path way for orangutan when fruiting availability abundant. The existence of this site, therefore, is very crucial to the survival of orangutan population in the area. Despite the importance of providing connection between forest blocks, the status of Lubuk Kuali is a customary forest which is managed by community. There is no legal protection status. Awareness program to socialize the function of this forest in term of wildlife corridor is therefore, critically importance to sustain orangutan population in Gunung Palung National Park. It does not only for the protection of orangutan but it would secures other wildlife species that regularly utilized Lubuk Kuali forest corridor. The importance of forest corridor is to connect fragmented forest in order to provide wildlife species to secure their breeding and seasonal movement to harvest their food sources (MacArthur and Wilson 1967). The main threat to orangutans in the Gunung Palung region has been identified as hand-logging (Meijaard and Neijman 2000), whereas particular threat to the Gunung Tarak forest is the development of the short cut road that connects villages between Naek Doyan to Teluk Parak and Cali.

This road will separate the forest of Gunung Tarak into two parts while providing the way for illegal loggers to carry logs out.

Lubuk Baji and Sungai Bayas are the sites where the nests of orangutan were detected most. These two sites are the boundaries which are situated in side the park and regularly patrolled by Orangutan Protection and Monitoring Unit or it is popularly called OPMU. These sites were destructed 5 years or more before the survey conducted and therefore, it might be recovered quickly due to the active operations of OPMU since 2003 in the area (Adhikerana and Sugardjito 2010). In contrast, the situations are very different in the locations of Gunung Tarak protection forest such as Nek Doyan, Teluk Parak, and Tanjung Pasar where ongoing distruction still occur during the survey. Orangutan almost disappears in all sites of these degraded forests. When hand logged in this forest did not expand to a wider area and quickly to be controlled it is possible that in five years the area could be utilized for the reintroduction of orangutan rehabilitants. Thirty two thousands hectares of Gunung Tarak forest at least could support 0,35 ind/km^2 of orangutan if it is well protected. It is essential that conservation measures are taken to protect orangutan outside protected areas. By implementing conservation oriented management in the production or protection forests where orangutan exists, it would reduce the number of orangutans captured.

Timber or palm oil companies normally capture the orangutan when they encountered in their concessionary areas and transfer it to the nearest rehabilitation centre. This action has taken a lot of resources including the cost of handling and caring of the animal in the centre prior to release in the wild. In the strategies and conservation action plan of orangutan it was recommended that the rehabilitation centers will be closed by 2017 (Suhartono et al. 2007). By then the centre will only focus on the reintroduction program. The aim of this strategy is to protect the population of orangutan in their natural habitat instead of securing the individuals in rehabilitation center. Creating an alternative to protected areas, where more local management is actively participated, would create local support commitment for conservation, which is assumed to be crucial for success. This effort has been initiated by Fauna and Flora International in collaboration with the district government and private sectors through orangutan conservation support program in large multifunction landscapes in the Ketapang district of west Kalimantan.

CONCLUSION

The existence of orangutan's nests in a forest habitat could be used as an indicator of visiting frequency of the species to the respective habitat. This study showed that the highest number of orangutan's nests was found in the old degraded forest inside the park, whereas the lowest one was obtained in the currently logged protection forest with the density of orangutan at 3.70 ind/km^2 and 0.06 ind/km^2 respectively. The high number of nest with difference classes found in a cluster indicates that the forest habitat has been used frequently by the species. The implication for this finding is when the selective hand logged forests are well protected in order to secure the orangutans from hunting then the logged forest habitat would recover and it could be used again by orangutans.

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Conservation of maleo bird (*Macrocephalon maleo*) through egg hatching modification and ex situ management

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ABSTRACT

*Rusiyantono Y, Tanari M, Mumu MI (2011) Conservation of maleo bird (*Macrocephalon maleo*) through egg hatching modification and ex situ management. Biodiversitas 12: 171-176.* Over exploitation of maleo bird eggs has become the main problem. In addition, habitat demolition and fragmentation have also caused decrease in maleo bird population. This research aimed to know the effectiveness of hatching pattern to produce maleo breeding, studying breeding pattern of maleo bird through hatching approaches of feed quality and temperature adjustment, and studying maleo bird respond towards caring pattern adjustment by measuring plasticity value. There were two phases in this research. The first phase was hatching by using modified incubator. The other one was the caring of the breeding from the result of hatching through feed pattern management using protein and energy balancing. The results of the research indicated that the hatching success was 65%; however, life endurance of the birds from birth to one month of age was only 40%. Their growth showed sufficiently high increase after passing critical period in their body-weight based on feeding pattern containing 21% of protein that was 64.93 g and 62.59 g for maleo in Lore Lindu National Park (LLNP) and Bangkiriang Wildlife Reserve (BWR), respectively. Their monthly body-weight increase was 33.06 g in average of feeding pattern containing 13% of protein for LLNP maleo birds and 36.99 g for the maleo in BWR. It was found that feeding pattern containing higher content of protein (21%) promoted significant increase in the body-weight of maleo birds. Feeding such birds with high protein content feed along with sufficient energy triggered their growth speed. Based on the findings, it was concluded that maleo birds could be preserved by way of hatching, while the birds could be fed with feed containing high protein and energy in order to accelerate their growth after hatching.

Key words: maleo, conservation, hatching, daily gain, feed conversion.

INTRODUCTION

Maleo bird (*Macrocephalon maleo* Sal. Muller 1846) is a kind of endemic wild animal of Sulawesi island. Its existence is protected by the government regulation No. 7 Year 1999 dated 27 January 1999 concerning the preservation of Flora and Fauna Species (Noerdjito and Maryanto 2001). In the efforts of Maleo conservation, problems often exist in terns of egg utilization either for individual, ceremonial, or social purposes of the local community which, in fact, ignores the concepts of maleo bird conservation. Over exploitation of maleo bird eggs has become the main problem. In addition, habitat demolition and fragmentation have also caused decrease in maleo bird population. This fact has made that maleo bird has recorded in the *IUCN Red Data Book* (International Union for Conservation of Nature and Natural Resources) categorized as *endangered* (MacKinnon 1981; Argeloo 1991; Gunawan 1995).

Various efforts have been taken for preserving maleo from the extinction, such as breeding them in their original habitat. However this effort does not bring a good result. Another way to preserve maleo is through biotechnological reproduction approach. Until recently there has been very little information on maleo breeding. The incubation period is around 62-85 days, depends on the soil temperature, and with the best temperature of maleo eggs around 32-35°C (Dekker and Brom 1990). The average temperature of maleo eggs to hatch was around 33-35°C with average of soil humidity of 96.5% in the morning, 70.7% in the afternoon, and 89.5% at night (Wiriosoepartho 1980). In the efforts of increasing the reproduction efficiency, the researchers bred maleo eggs in a controlled temperature and humidity incubator. Naturally, the mating of maleo has never been spectated in the hatchery. This means that the mating was done in other place in the forest. It is a monogamy animal. Male and female maleo are never separated more than a few meters when feeding, hatching, and sleeping on big horizontal tree branches (Jones et al. 1995). Thus, in order to increase their mating intensity, they were put in one controlled pen. For the time being, technology of hatching maleo bird eggs and controlled breeding pattern are promising for conservation of this endangered species.

MATERIALS AND METHODS

Materials

This Research has been done in different site and the egg collected from two places namely: (i) Lore Lindu National Park (LLNP) which is located in the center of

Sulawesi Island, (ii) Bangkiriang Wildlife Reserve (BWR), which is located in the coastal area, is passed through by a provincial street connecting Luwuk to an areas surrounding the conservation area in Luwuk District, 600 km to the east of Palu, Central Sulawesi, Indonesia. This research started from July until November 2005

Procedures

Egg collection and selection

The maleo bird eggs were collected from two locations which have different topography, namely: (i) Lore Lindu National Park which is located in the center of Sulawesi Island. The location has little impact of sea breeze where the maleo bird egg collection took place, especially sector of Saluki, Tuwa village that is classified as type A and B according to the climate type of *Smith Ferguson* and was surrounded by steep hills and has an altitude of 235-305 m asl. and steep stream flow. (ii) Bangkiriang Wildlife Reserve, which is located in the coastal area, is passed through by a provincial street connecting Luwuk to an areas surrounding the conservation area in Luwuk District, 600 km to the east of Palu City, Central Sulawesi.

The period collection of Maleo bird eggs is a month. Collected eggs were then selected on the basis of performance and intactness of eggshell. The intact and clean eggshell was put in next selection for morphometrical measurement.

Egg index measurement

In order to get egg index data, it should be done morphometrically by looking on egg shape, egg weight (gr) (measured by using Ohaus capacity scale 2610 g with *triple beam balance*), egg length (mm), and egg width (mm) which measured using caliper with carefulness 0.05 mm.

Hatching method

The selected maleo eggs further hatched using incubator by temperature 33.5-34.4°C or the average 34.061±0.187°C with relative humidity = RH is around 69-74% or the average 70.875±1.431. The model of incubator used is made of wood and play-wood by 130 cm length and 70 cm height with the capacity of 64 eggs. The machine equipped with micro-switch thermostat, thermohygrometer, 4 electric light bulbs (60 watt), egg-telescope, water container for humidity arrangement and a room with the same size as the machine used as the place for the breeding newly hatched maleo.

Incubation period and hatchability

The incubation period was counted from the time when eggs were put into incubator until they hatched. The eggs were given code based on their habitats. To know the affectivity of egg-hatching, it needs to measure the hatchability formula proposed by Effendi (1974) as follows:

$$S\ (\%) = Nt/No \times 100\%$$

S = hatchability
Nt = hatching eggs
No = eggs observed

The breeding pattern of maleo

The hatching maleo was put into a scaffolding individual stall equipped with feed hopper and water container as well as thermostat. The feeding used was commercial bird feed in the growth phase for day old chick with crude protein (CP) content 21%, Metabolic Energy (ME) 3000 kkal kg and CP content 13 %. ME 3000 kkal kg. Feed and water were given according to *ad-libitum*. Water given were added was vitamin "anti-stress" (1mg/100 mL)

Feed intake

Feed intake was counted amount of feed intake in grams per month and the total intake of maleo birds for five months breeding.

Daily gain

Daily gain was counted monthly live weight gain of maleo in five months. To know daily gain through measuring live weight of maleo by formula as follows:

Live weight at 7^{th} of week-live weight at 1^{st}

Feed conversion

Feed intake and its efficient utilization is one of the major concerns in poultry as feed cost is one of the highest components of total cost of production (Rosario et.al. 2007). The value of feed conversion counted by feed intake in gram divides live weight of maleo.

Phenotypic plasticity

The animal performance is influenced by genotype, environment and interaction between genotype and environment has either positive or negative impacts toward animal performance. The number of phenotypic plasticity is calculated base on the characteristics difference of two different environments

RESULTS AND DISCUSSION

Index of eggs

Eggs are available in different shapes. These shapes can be differentiated using a shape index **(**Altuntas and Şekeroğlu 2008). Alkan et al. (2008) stated that egg shape index was predictable with better accuracy from egg weight, egg width and length. In comparison to other poultry species, a study of determining the effect of shape index and egg weight of quail (*Coturnix coturnix japonica*) eggs on the property of hatchability, hatchling weight and weekly body weight gain has been performed by Copur et al. (2010). They concluded that 13 g hatching egg weight group in quail is the appropriate optimal weight in terms of hatchability of fertile eggs and growing period performances, although those properties do not change according to variations in shape index. Based on the collected eggs from the field, only 45% could be proceed to hatching process. The average weight of eggs applied in this research was between 160-226 g while the index of the eggs weight was about54.0-63.0 (average 59.0). The eggs which were out of standard might have been caused by factors such as nature (rain) and transportation.

Table 1. Morphometric of maleo eggs were collected at different two habitats

Morphometric	Habitat	
	LLNP	BWR
	n = 63	n = 42
Weight (g)	208.10[a] ± 12.39	210.39 [a]± 10.74
Length (cm)	10.30[a] ± 0.34	10.28 [a]± 0.25
Wide (cm)	6.11[a] ± 0.18	6.11[a] ± 0.11
Indeks (L/P)	59.36 ± 2.63	59.47 ± 1.83

Note: Same letter in the same row between morphometric measurements of LLNP and BWR showed no significant differences (P>0.05)

The measurement results in LLNP egg morphometric and BWR, no statistically significant difference, so the size of eggs in both places (LLNP and BWR), is no different (Table 1). According to Sumangando (2002) obtain egg weight range 110-250 g, length 9.7 to 10.7 cm long and 5.7 to 6.2 cm wide. This variation is very possible because of differences in location, availability of food, as well as differences in parent maleo season as place for laying eggs. Meanwhile, Farooq et al. (2001) indicated that egg weight was easily predictable from egg length and width. Information on egg weight along with egg width and length will further open the domain for trying out various prediction equations in order to predict eggshell weight and shell thickness (Khurshid et al. 2003). The weight and size of eggs obtained from LLNP and BWR also vary, the weight and size of eggs is likely due to the laying of maleo age varied.

Hatching

Hatchability was obtained at incubator 70% and 65% for eggs collected from TNL and BWR respectively. An average obtained hatchability of 67.5%. The result is relatively high compared with semi-natural hatchery in LLNP and BWR. The humidity below 40% and above 80% will reduce hatchability of chicken eggs (Mansjoer 1985). Embryos that died probably caused by the inverted position of the laying of eggs in an incubator, and eggs which fall when other eggs hatched. Low hatchability may also be influenced by the distance between hatchery process and location of egg collected to far and also the influence of the season, which at the time of egg collection to coincide with the rainy season, so the eggs and water intruding happen decay.

Tanari et al. (2008) suggests that there are two factors that influence the process of embryogenesis and hatching of biological factors and environmental factors. For comparison on turtle nesting, they are also making their nests in sand; incubate their eggs by relying on the sand temperature of 29ºC. If the temperature is too hot, the young turtles can be killed so that turtles have not hatched.

Compared with hatchability obtained in semi-natural hatchery, the hatchery using remote incubator for hatching eggs are promising Maleo. Hatching which is done in situ gives not good results because of several weaknesses, among others, the difficulty of control of temperature and humidity due to seasonal changes frequently. Thus hatching using the incubator is the best solution to further increase egg hatchability maleo. Overall of hatching process can be seen at Figure 1. The result of even this contribution to help solve problems that is less successful hatching in both natural habitats and in semi-natural hatchery.

Observation was done on a daily basis in order to see the egg condition. Duration required to process outside of the Maleo from shell was approximately 3 hours.

Maleo bird breeding

A critical period of maleo to grow is up to one month old and this period could be seen as a high rate of death of young maleo which can reach up to 60%. Such a condition needs further research in order to know the most determining factors of the birds to be survived (Dekker 1990). It was found that monthly live weight gain of maelo using 21% of protein as a feeding pattern was 64.93 g and 62.59 g in average for maleo from Lore Lindu National Park and Bangkiriang Wildlife Reserve respectively. In feeding pattern with protein content of 13%, their monthly live weight gain development was 33.06 g in average for the birds from LLNP and 36.99 from BWR. Based on those results, feeding with higher protein content (21%) can lead the birds gained more weight. Feeding with high protein content along with sufficient energy would optimize maleo growth speed.

Feed intake

The average of feed intake in grams per month and total intake of maleo birds for five months breeding can be found in Table 1. An intake of maleo birds of LLNP and of BWR for five months breeding showed an increase of their intake, either in the level of 21% or 13% protein. The amount of intake in the level of 13% protein was relatively higher than 21%. Low intake of 21% protein might be caused by the sufficient need of protein for the birds, reported that the amount of protein used for growing native chicken and its hybrid with Rhode Island Red is determined by protein content in feed intake.

Table 2. Average of intake (g/bird) per moth for maleo birds within three months breeding

Habitat	Protein intake (%)	Month			Total
		1	2	3	
LLNP	21	540.00	578.33	615.00	577.78
	13	583.33	610.00	635.00	609.44
BWR	21	531.67	561.67	601.67	565.00
	13	580.00	611.67	655.00	615.56

An amount of 21% protein intake was relatively higher than of the level of 13% for the birds to both LLNP and BWR. The amount of protein intake was determined by a quality and sufficiency intakes as well as organ capacity *(intestinum)*. A quality and sufficiency intakes lead to determine a genetic potential optimalization owned by an animal. Behavioral performance depends on genes owned by an animal, but supporting environmental condition is needed to give opportunity for a total characteristic performance (Hardjosubroto 1994). Furthermore, Martojo (1992) stated that environment directly influences the phenotype of an animal through feed, diseases, managements, but not the genotype. It is also described that possible influence to genotype may indirectly happen by way of natural or artificial selection.

Figure 1. Hatching process of maleo bird in captivity

Steps	Activities	Figure
1	Egg which is newly hatched begins cracking its eggshell because of active pressure from both legs of egg pointed shape that is predicted about one and third on the pointed shape of the egg. The process can be indicated with shell cracking where after that it will be followed by producing a dark red liquid and then became clear red. Such active pressure repeatedly appears until forming a big crack on the egg. Such each pressure followed by a breaking phase about 15 minutes.	A,B
2	When the egg hole became bigger until the bottom of the egg, such active pressure became loss so that leg and claw have a space to help for making the hole bigger.	C
3	The breaking phase at each pressure is also same when breaking egg beginning. The phase of breaking became shorter in minutes when a young maleo wants to leave the eggshell.	D
4	The young maleo which is still in the eggshell has already an open eye or it may be called semi artificial.	F
5	A free young maleo from the eggshell is directly out of incubator where physical condition of this bird has already strong with its fully feather as well as strong leg and claw. Unfortunately, this young maelo needs more time to take a rest in order to be recovered and has still no power to avoid attack from surrounded.	G
6	The activities of young maleo until the age of 1 week have shown no powerful movement if at just a hand in distance, but avoiding instinct have been done by the bird in a way of running or jumping with a great distance about 1 m. This condition can lead the young maleo will be threatened by other predators at its habitat especially when the bird out of its natural hatching.	H
7	At age of 2 weeks, the young maleo can do low fly (about 2 m) with about 3 m in distance.	I

Daily gain

The average of monthly live weight gain of maleo in three months can be found in Table 2. The birds gained weight every month either in the level of 21% or 13% of protein. However, the body-weight showed a better result in the intake of 21% protein than that of 13% protein. In terms of gain within five months, the birds of LLNP showed a better result of weight gain than the birds of BWR when fed in the level of 21% protein. Unlike when fed in the level of 13% protein, the birds from BWR showed a better result of weight gain than the ones from LLNP.

Table 2. An average of body-weight (g/bird) of maleo in monthly basis weighing

Habitat	Protein intake (%)	Month			Average
		1	2	3	
LLNP	21	243.59	306.41	377.07	309.02
	13	192.66	225.24	257.06	224.98
BWR	21	209.40	277.31	342.59	276.43
	13	186.40	226.58	265.91	225.40

The average increase of body-weight can be found in Table 3. It showed that when the birds fed 13% of protein, there was a decrease in weight gain of LLNP birds, while there was an increase in weight gain of BWR birds. However, in the level of 21% protein, birds of LLNP showed a better increase in weight gain.

Feed intake does not show linear relation with gain. Every livestock has different capability in feed conversion (Sidadolog and Yuwanta 2000). This thing, from genetic aspect, can be understood that differences happened were caused by extreme environment.

Table 3. Average of body-weight gain (in gram) of maleo in monthly basis

Habitat	Protein intake (%)	Month			Average
		1	2	3	
LLNP	21	61.30	62.82	70.67	64.93
	13	34.77	32.58	31.82	33.06
BWR	21	54.57	67.92	65.28	62.59
	13	31.50	40.13	39.33	36.99

If two or more individuals develop and grow in the same environment and show different phenotype, it can be concluded that the two individuals have different genotype. In contrast, although there are two or more individuals having the same genotype but develop in different environment, their phenotype may be different (Schlichting and Levin 1984). The domesticated maleo are still showing body weight gain so that they can be adapted out of their original habitat.

Feed conversion

A value of feed conversion of 21% protein tended to be fluctuated for both resource places (LLNP and BWR). This situation was caused by the amount of intake which is not always linear with the body-weight gain of maleo.

Table 4. Average of feed conversion for maleo

Habitat	Protein intake (%)	Month			Total
		1	2	3	
LLNP	21	8.81	9.21	8.70	8.91
	13	16.78	18.72	19.96	18.48
BWR	21	9.74	8.27	9.22	9.08
	13	18.41	15.24	16.65	16.77

Feed conversion value of breeding native of 20-22 months old chicken was 11.17 to 18.17. Feed conversion of 1 year old chicken was 16.23 (Olomu and Offiong 1980). By providing 21% protein level, the birds of LLNP were more efficient than the ones of BWR. On the contrary, giving 13% protein level, the birds of BWR were more efficient than the ones of LLNP. This showed that maleo birds of BWR were easier to grow up than the ones of LLNP.

Body-weight plasticity

Phenotypic plasticity means function of phenotypic value shown by livestock in different environment. Kolmodin et al.,(2002) states that phenotypic plasticity does not belong a genotype, but specific for others. Plasticity phenotype value of body-weight of maleo from LLNP was relatively bigger than those of BWR. This showed that the birds from LLNP decreased in gain relatively bigger than those from BWR, 31.87 g and 25.60 g respectively. Gene plasticity of maleo from LLNP was more influential to body-weight for more plasticity, while the gene plasticity of birds from BWR was able to control body-weight decrease.

In one hand, habitat condition of LLNP which provides abundant feed caused maleo from LLNP could not survive in an environment with limited feed stock, on the other hand, maleo from BWR could survive in an environment with bad feedstock. This showed that there was difference in phenotypic plasticity between birds from LLNP and those from BWR.

CONCLUSION

Weight of eggs did not influence egg hatchability, while egg index has a bigger chance to be hatching which was around 54-63. Feeding with high level of protein and energy increased body-weight of maleo. Maleo birds are able to adapt to a new environment or their external habitat.

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Effect of land use change on ecosystem function of dung beetles: experimental evidence from Wallacea Region in Sulawesi, Indonesia

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ABSTRACT

Shahabuddin (2011) Effect of land use change on ecosystem function of dung beetles: experimental evidence from Wallacea Region in Sulawesi, Indonesia. Biodiversitas 12: 177-181. The deforestation of tropical forests and their subsequent conversion to human-dominated land-use systems is one of the most significant causes of biodiversity loss. However clear understanding of the links between ecological functions and biodiversity is needed to evaluate and predict the true environmental consequences of human activities. This study provided experimental evidence comparing ecosystem function of dung beetles across a land use gradient ranging from natural tropical forest and agroforestry systems to open cultivated areas in Central Sulawesi. Therefore, standardized dung pats were exposed at each land-use type to assess dung removal and parasite suppression activity by dung beetles. The results showed that ecosystem function of dung beetles especially dung burial activity was remarkably disrupted by land use changes from natural forest to open agricultural area. Dung beetles presence enhanced about 53% of the total dung removed and reduced about 83% and 63% of fly population and species number respectively, indicating a pronounce contribution of dung beetles in our ecosystem.

Key words: land use change, ecosystem function, dung beetles.

INTRODUCTION

Dung beetles in the sub family Scarabaeinae (Coleoptera: Scarabaeidae) have important ecological roles related to nutrient cycling. Removing and burying dung, either for adult feeding or for oviposition and subsequent feeding of the larvae (Hanski and Cambefort 1991) has important ecological consequences in terms of ecosystem functions such as soil fertilization and aeration (Mittal 1993; Wilson 1998), increased rates and efficiency of nutrient cycling as well as plant nutrient uptake and yield (Wilson 1998; Miranda et al. 2000), and secondary seed dispersal of seeds defecated by frugivorous vertebrates (Andresen 2002, 2003).

Dung burial is the initial step to most of the beneficial functions of tropical dung beetles and such activity, the removal of resources for competitors, therefore is also a mechanism by which dung breeding fly numbers may be reduced (Ridsdill-Smith et al. 1988). Fresh mammal dung is an important resource for a variety of dung-breeding flies as well as dung beetles. Several pestiferous, dung-dwelling fly species (principally *Musca autumnalis, M. vetustissima, Haematobia thirouxi potans, H. irritans exigua and H. irritans irritans*) have followed the introduction of livestock globally. Fly infestations has been reported reduce the livestock productivity (Guglielmone et al. 1999) and represent an enormous financial burden to livestock producers (Byford et al. 1992). Recently, Losey and Vaughan (2006) estimated that the annual value of ecological services provided by native insects in the United States to be more than $ 57 billion including $ 0.38 billion through dung burial activity by dung beetles. A series of ecosystem function of dung beetles has been comprehensively reviewed by Nichols et al. (2008).

Studies on dung beetles have been conducted in Indonesia (Hanski and Krikken 1991; Gillison et al. 1996; Shahabuddin et al. 2005, 2007, 2010; Shahabuddin 2010). However, that study more emphasized on diversity and community structure of dung beetles and not pays much attention on ecological function of dung beetles across a habitat disturbance gradient, including in Sulawesi.

As a key landmass within the Wallacea biogeographic region, one of the world's biodiversity and endemism hotspots, Sulawesi has extremely valuable in terms of conservation (Cannon et al. 2007; Myers et al. 2000). The loss of forest habitat and forest degradation on this equatorial island (Cannon et al. 2007) reflect the situation found in several countries of Southeast Asia: deforestation is still happening, possibly even at increasing rates (Sodhi et al. 2004; Koh 2007), with new forms of land-use gaining ground.

While several studies have been reported that conversion of natural habitats such as tropical forests to land-use systems is responsible for the decline of diversity of most taxonomic groups including insects (Lawton et al. 1998; Schulze et al. 2004; Shahabuddin et al. 2005, 2010), effect of land-use change on ecosystem function of the studied taxa is rarely investigated (but see Andresen 2003; Horgan 2005; Slade et al. 2007). Most of those studies

focused on direct first-order effects i.e. on diversity and abundance of selected taxa but did not emphasized to the second-order effects of land-use change related to the ecological roles of the studied taxa. Additionally, of few comparative field studies from the tropical area recorded ecosystem function of dung beetles (i.e., Klein 1989; Andresen 2003; Slade et al. 2007) those study do not covering arrange of habitat type from natural forest to agricultural area.

The present study, conducted in Lore Lindu National Park, Central Sulawesi, aimed to analyze effects of forest conversion to land-use systems on ecosystem function of dung beetles mainly on dung removal activity and suppression the population of parasitic flies inhabited in herbivore dung.

MATERIALS AND METHODS

Study area

The study area is located on the northern margin of the Lore Lindu National Park (LLNP) in Central Sulawesi, Indonesia. The Lore Lindu National Park, a local biodiversity hotspot is covering an area of 229,000 ha and located southeast of Palu, the province capital of Central Sulawesi. All study sites were selected in Palolo Valley in the vicinity of the Bobo villages (01°07'10.2" S - 119°59'40.2" E) and situated at an altitude between 800 and 1000 m asl.

Fields study was conducted from June to August 2010 in four land-use types: natural forest (NF), selectively logged forest (SF), agroforestry systems (cacao plantations with Gliricidia as shadow trees; CP) and open cultivated area (OC). For each habitat type three site replications were selected. Detailed description of each land-use type was shown in Table 1.

Table 1. Description of each land-use type studied

Land-use type	Land-use type description
Natural Forest (NF)	Lower montane forest; big emergent trees and numerous medium-sized trees form a multi-layered canopy; height of upper canopy layer 20-30m with single big emergent trees up to 40m; well-developed under storey layer of small trees/scrubs, ginger and rattan up to 4-8m high; herb layer dominated by Rubiaceae and ferns.
Selectively logged forest (SF)	Single emergent trees up to 30 m; closed canopy layer 15-20 m high; herb layer 0.5-2m high and dominated by ferns and Rubiaceae. Some selective logging activities took place in all sites, however, the plots are so far just slightly affected.
Cacao agroforestry system (CP)	Ca. 5 yrs old cacao plantations (ca. 1 ha) with *Gliricidia sepium* (Leguminoceae) and *Musa* sp. as shaded trees; cacao trees up to 2-3 m high; *G. sepium* trees 7-9 m high; some sites has herb layer with 20-30 cm high
Open cultivated area (OC)	Two of study sites were maize fields. The rest was a pasture land (ca. 0.5 ha.)

Dung beetles and dung removal activity

Dung removal activity was studied by expose four experimental dung pats from fresh cow dung (fitting in 300 ml plastic containers) with a mean fresh weight of ca. 258 ± 15.3 g at all study sites. Two of the baits were wrapped in 2 mm insect screening that excluded dung beetles to utilize the dung, a further 2 baits were open (unprotected from beetles). Baits were randomly placed on the soil surface at each site and were collected after 18 days where more than 50% of the dung pat has been buried by dung beetles (Shahabuddin 2007). The collected dung pats were stored separately in plastic bags. In the laboratory, they were dried at 100°C for 96 hours and weighed using an analytical balance (Sartorius MC 410 S) (Sanchez et al. 2004). The mean dry weight of 10 fresh dung pats not exposed in the field was used as a control (ca. 55.2 ± 7.1 g). The percentage of dung removed was estimated by the differences between control and remaining dung pats (after exposure).

Dung beetle and fly activity

Fly abundance and richness were monitored using the similar standardized bait used in the dung removal study as described above. Six baits were set out at each site, three of the baits were wrapped in 2 mm insect screening that excluded dung beetles but allowed flies to lay eggs into the dung, a further three baits were open (unprotected from beetles). Baits were randomly placed on the soil surface at each site and one of each bait type (open and beetle-exclusion) was collected each day for 3 days. The baits were placed on dry sand in individual plastic containers covered with muslin and held for 3 weeks in an insectary to allow flies to emerge, after which time the baits were dissected to remove all remaining fly larvae and pupae. Flies were identified to family level by using Borror et al. (1996) and counted.

Data analysis

Data were tested for normal distribution by Shapiro-Wilk's test and apply appropriate transformation before performed data analysis (Zar 1999). The effects of bait type (beetle presence/absence) and habitat type on dung removal and flies suppression activity of dung beetles were analyzed using a two-way ANOVA. Results from both habitat types were pooled since habitat had no significant effect on fly-parameters in the experiment. Most of statistical analysis was performed by using STATISTICA software (Statsoft 2004).

RESULTS AND DISCUSSION

Effect of land-use and bait type on dung decomposition

Percentage of dung decomposed or removed differed significantly between habitat types and bait type (land-use, $F_{3,16} = 5.58$, $p < 0.05$; presence/absence of beetles, $F_{1,16} = 123.15$, $p < 0.001$; interaction, $F_{3,16} = 1.37$, $p > 0.05$). Additionally, protecting bait from dung beetles access significantly reduced the amount of removed dung (Figure 1) and presence of dung beetles could be increased about 53% of the total removed dung.

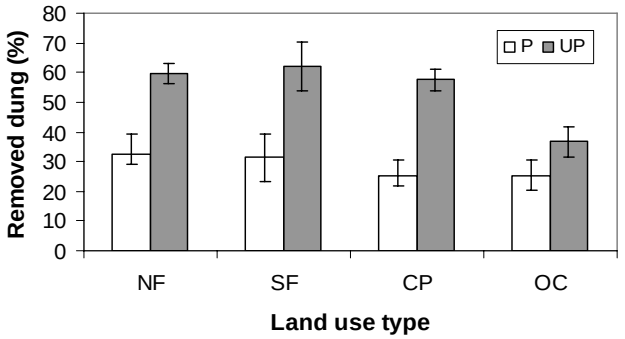

Figure 1. Percentage of removed dung (± SD) from unprotected (UP) and protected (P) bait in relation to land-use type

The present study showed a significant contribution of dung beetles to dung removal. However this ecological function was disrupted by land-use change from natural forest to agricultural area. Although percentage of removed dung was decreased from natural forest to open cultivated area, this study only detected a significant reduction of buried dung on open cultivated area. Percentage of removed dung at the natural forest, disturb forest and cacao agroforestry were nearly similar. This results was in line with previous study conducted at similar study sites found that diversity of dung beetles at forest sites has no significant different with those of cacao agroforestry but remarkably higher than that of open area (Shahabuddin 2010).

The likelihood that the dung pats was still removed in a smaller rate in the absence of dung beetles indicating that other organisms were also involved on dung removed. Termites and earthworms were known has capacity to create tunnels and redistribute soil. Herrick and Lal (1996) found the contribution of termites on dung buried and soil removed. While several studies have demonstrated that some earthworms are efficient dung removers in Europe (Holter 1979), Australia and New Zealand (Baker 1994), their dung related contribution to bioturbation in areas with a higher diversity of Scarabaeinae dung beetles is unknown.

Dung burial activity by dung beetles reported has important roles in increasing soil fertility. Shahabuddin et al. (2008) found that removal dung by dung beetles significantly increase of the total content of N, P and K. Also Omaliko (1984) reported that dung decomposition increased concentrations of nitrogen, potassium, phosphor, magnesium and calcium of soil up to 42-56 days after dung exposure. Furthers, dung burial activity altered environmental conditions, reduce pH of dung, speeds it incorporation into the soil and greatly reducing loss of Nitrogen as ammonia gas (NH_3) (Yokohama et al. 1991).

Many factors including the traits and community structure of dung beetles influenced dung burial activity. Although species diversity has a strong correlation with dung burial rate (Larsen et al. 2005), effect of biomass (Horgan 2005) and functional group diversity (Slade et al. 2007) on dung removal proved to more importance compared with species diversity. A laboratory experiments noted that dung beetles size and biomass were the best predictors for the amount of removed dung, while the number of species involved was just of minor importance (Shahabuddin et al. 2008). While large beetle species are functionally more efficient than smaller ones on dung removal activity (Shahabuddin et al. 2008), large-bodied beetle species tended to be more prone to land-use change from natural forest to human dominated land use type (Shahabuddin et al. 2007). Therefore the loss of those large species due to changes of land use may cause a significant decrease in ecosystem function.

Dung burial activity proved to be not only important for maintaining or increasing soil fertility but also has several other advantages such as enhancing total nitrogen and phosphorus of plants as well as its yield, improving plant regeneration through dung-seed dispersal activity, and increasing plant palatability by reducing plants fouled with dung (see Nichols et al. 2008). Therefore, in natural ecosystems the reduction of dung beetle populations most likely has cascading and long-term effects throughout the ecosystem (Klein 1989; Larsen et al. 2005).

Effect of land-use and bait type on flies population

A total of 438 flies (323 imago and 115 larvas) were collected during study period. The most predominant families were Sphaeroceridae, Tachinidae, and Muscidae. They comprise about 73.1% of the total fly specimens (Table 2).

Table 2. Number of specimens of each fly family emerged from unprotected (UP) and protected (P) bait from dung beetles access.

Family	Bait type		Total
	UP	P	
Sphaeroceridae	0	98	98
Tachinidae	11	62	73
Muscidae	9	56	65
Hippoboscidae	1	34	35
Stratiomyidae	0	21	21
Bombyliidae	0	15	15
Calliphoridae	0	13	13
Scatopsidae	0	3	3
Larvae	17	98	115
Total	38	400	438

Majority of the specimens (82.8%) were emerged from unprotected bait and only three of eight families (37.5%) were collected from colonized bait by dung beetles (Table 2). These results indicated a tremendous effect of dung beetles presence on reducing flies population. However land-use type has no significant effect on the number fly family collected (Number of specimens; land-use type, $F_{3,19} = 0.67$, $P = 0.58$; presence/absence of beetles $F_{1,19} = 19.85$, $P < 0.001$. Number of family; land-use type, $F_{3,19} = 1.86$, $P = 0.17$; presence/absence of beetles $F_{1,19} = 33.01$, $P < 0.001$).

Although, the fly population emerged was reduced with dung age in both bait type, they showed a similar pattern, the number of flies was lower from the protected bait. There is no interaction between dung age and bait type (dung age, $F_{2,18} = 10.41$, $P < 0.001$; presence/absence of beetles $F_{1,18} = 39.27$, $P < 0.001$; interaction, $F_{2,18} = 2.21$, $P = 0.14$). While the number of collected family decreased with dung age, they all were lower at the unprotected bait. Dung age was interact with presence/absence of beetles on determine the number of family collected because the

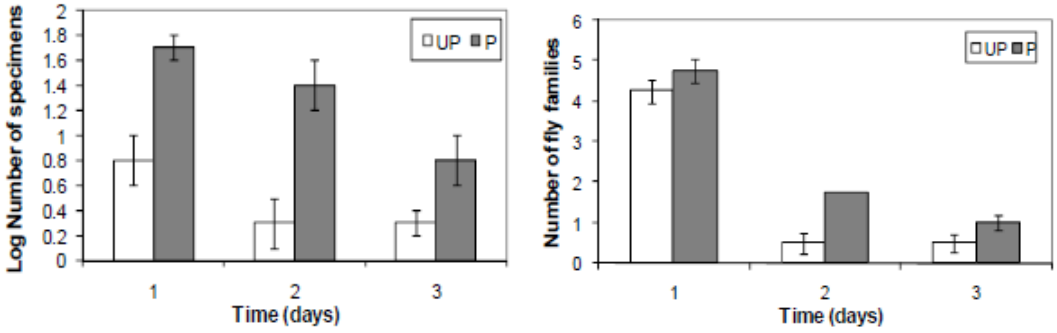

Figure 2. The effects of beetle exclusion on fly numbers and the number of flies family (±SD) emerging from 300 ml cow dung baits. UP=unprotected bait, P=protected bait (beetle exclusion).

collected family were nearly similar on both bait type on first day but significantly decreased at next day (dung age, $F_{2,18} = 8.49$, $P < 0.005$; presence/absence of beetles $F_{1,18} = 39.79$, $P < 0.001$; interaction $F_{2,18} = 8.77$, $P < 0.005$) (Figure 2).

When and where dung beetles and dung flies co-occur, fly survival tends to decline as a consequence of asymmetrical competition for dung resources, mechanical damage of eggs by beetles, and fly predation by mites phoretic on dung beetles. A series of experimental manipulations of dung beetle and fly densities in artificial dung pats report elevated fly mortality in the presence of Scarabaeinae beetles, both in the laboratory and field (Wallace and Tyndale-Biscoe 1983; Ridsdill-Smith and Matthiessen 1988; Ridsdill-Smith and Hayles 1990; Bishop et al. 2005). Shortly, fly mortality caused by dung beetle activity is a combined consequence of (i) direct mechanical damage to fly eggs and early instars caused during adult beetle feeding (ii) unfavorable microclimates for fly eggs and larvae caused by dung disturbance and (iii) resource competition with older larvae, primarily from removal of dung for brood balls (reviewed by Nichols et al. 2008).

This is the first study in Sulawesi, a hearts of Wallacea region and probably in Indonesia known as megadiversity country documented effect of forest modification to human dominated land-use type on ecosystem function of dung beetles. Land use changes from natural forest to agricultural area proved to has detrimental effect on ecosystem function of dung beetles especially dung burial activity.

The likelihood that habitat disturbance due to land-use changes has pronounced effect on both diversity and ecosystem function of dung beetles (and other insect groups such as native bees, Kremen et al. 2004) indicating that effect of forest disturbance and land-use changes should not be only focus on it is direct effect to diversity of taxa studied but to ecological role of those taxa as well. This is particularly relevant with some hypothesis explain the biodiversity-ecosystem function relationships (see Schwartz et al. 2000; Giller and Donovan 2002).

As with most ecosystem services, before dung beetle services can be properly integrated with conservation planning or practice, additional research on dung beetle biodiversity ecosystem function (BEF) relationships and links between ecosystem functions and services will be required. A research agenda suggested by Kremen (2005) provides a near perfect fit to this task, suggesting future work that would identify: (i) the key species or traits providing ecosystem functions, (ii) the relationships between ecosystem function and community assembly and disassembly processes, (iii) the environmental factors influencing the production of ecosystem functions, and (iv) the spatio-temporal scales relevant to both providers and their functions (Kremen 2005). The most recent dung beetle BEF work has begun to advance our understanding of points 1-3, by identifying the specific-specific and community traits responsible for both ecological function (effect of traits) and sensitivity or resistance to environmental change (response traits) (Horgan 2005; Larsen et al. 2005; Slade et al. 2007; Shahabuddin et al. 2005, 2010; Shahabuddin 2008, 2010).

CONCLUSION

Dung beetles has a important role on dung burial activities and suppressing the fly population and these ecosystem function especially dung burial activity were remarkably disrupted by land use changes from natural forest to open agricultural area. Dung beetle presence elevated about 53% of the total dung removed and reduced about 83% and 63% of fly population and richness, respectively.

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Carbon baseline as limiting factor in managing environmental sound activities in peatland for reducing greenhouse gas emission

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ABSTRACT

Saharjo BH (2011) Carbon baseline as limiting factor in managing environmental sound activities in peatland for reducing greenhouse gas emission. Biodiversitas 12: 182-186. The total carbon stock in Indonesia was estimated to be around 44.5 Gt or about 53.1% of the total carbon stock in tropical areas. Over 1990-2002, it was estimated that around 3.5 Gt of carbon was released in Sumatra and about 0.81-2.56 Gt was released in Central Kalimantan due to the 1997 fire alone. It was recognized that deforestation, high exploitation of peat and peat fire were behind the huge emissions of Greenhouse Gases in Indonesia. Results of a research conducted in Central Kalimantan peatland, showed that the total carbon stock at logged over area was estimated around 413.972 t ha^{-1} (0-30 cm depth of peat) and at burnt area was 411.349 t ha^{-1} (0-30 cm depth of peat). Meanwhile it had been well recognized that most of opened peatlands had been occupied by *Acacia crassicarpa* and oil palms. Research carried out in East Kalimantan showed that the carbon stock of 25 years old oil palm planted on mineral soil was about 180 t ha^{-1}, which is less than that of carbon stock produced by peatland clearance. This indicated that although plants occupied peatland, high Greenhouse Gas emissions were still produced, meaning that global climate change would continue and created high risk impacts.

Key words: carbon stock, peat, oil palm, emission, GHG.

INTRODUCTION

The total amount of carbon stored in tropical peatland is about 83.3 Gt where 44.5 Gt or about 53.1% is found in Indonesia across the three main islands, i.e., Sumatra, Kalimantan and Papua (West Papua) with total carbon stored of 18.3 Gt (41.1%), 15.1 Gt (33.8%) and 10.3 Gt (23%) respectively. The majority of Indonesian peatland (16.5 million ha) are deeper than 60 cm with a minimum organic content of 65% (Andriesse 1974). Peat formation is a true carbon sink, the carbon being sequestered out of the system and converted into peat through biological activity. Peat swamps forests originally represent major ecosystems in Indonesia and ranged between 16.5-27 million ha. In their original state, Indonesian peat swamp forests sequestered between 0.01-0.03 Gt of carbon annually. These important ecosystems have however in recent years been reduced through drainage and conversion to agriculture lands and other activities (Sorensen 1993).

Southeast Asia (ADB 2009) has contributed 12% of the world's total greenhouse gas (GHG) emissions in 2000, an increase of 27% over 1990 and twice as fast as the global average rate of increase. Emissions from land use change and forestry (LUCF) sectors were 75%, energy 15% and agriculture 8%. Emissions rose fastest in the energy sector (83% during 1990-2000), while about 59% of the total emissions that came from Indonesia, are largely generated by LUCF. The highest CO_2 emission in Indonesia (PEACE 2007) is due to forestry activities. Deforestation and land conversion cover about 75% followed by 23% of energy used in forestry sectors and 2% from forest industry. Forest fire was the main contributor for deforestation and land conversion which cover about 57%. The 1997 forest fire alone released 3,000 to 9,000 $MtCO_2e$ to atmosphere (Page et al. 2002). Fire alone annually released about 1,400 Mt of carbon supported by dry peatland decomposition of about 600 Mt. If emissions from peatland fires (which are also caused by deforestation and drainage) are included, the total CO_2 emission number is significantly higher. Over 1997-2006, CO_2 emissions from peatland fires in Indonesia were several times higher than those due to peat decomposition in drained peatland areas: 1400 Mt/year to possibly as much as 4300 Mt $year^{-1}$ (Hooijer et al. 2006, 2010).

Peatland management and restoration of organic soil practices have the potential to sequester carbon by 7.33-139.33 t CO_2 ha^{-1} $year^{-1}$ and reduce N_2O emission by 0.05-0.28 t CO_2-eq ha^{-1} $year^{-1}$ (ADB 2009). The sequestration of carbon can be achieved by avoiding the drainage of organic or peaty soils that are known to contain high densities of carbon, or by re-establishing a high water table in the area (Freibauer et al. 2004). Furthermore, emission of GHGs from drained organic soils can be reduced by avoiding the planting of row crops and tubers, avoiding deep ploughing, and maintaining a shallower water table (IPCC 2007). Restoring peatland areas or organic soils can reduce the runoff from agricultural fields and settlements (ADB 2009), which causes eutrophication, algal blooms, and hypoxic dead zones in lakes, estuaries, bays and seas. It can

also reduce flood damages; stabilize shorelines and river deltas; retard saltwater seepage; recharge aquifers; and improve wildlife, waterfowl, and fish habitat. Restoration of organic soils can also improve soil quality as well as aesthetic and amenity values, promote biodiversity and wildlife habitats and support energy conservation.

The research calculates the above ground carbon stock of 25 years old oil palm planted on mineral soil. Such data would provide insights in order to determine whether oil palms should be planted on high or low carbon stock areas. The carbon loss which occurs when tropical forest is converted to oil palm plantation, by far exceeds the carbon sequestration during one cycle of oil palm growth (25 years). The overall carbon loss is further enhanced when the plantation is located on peat. When oil palm plantations replace grasslands, carbon sequestration exceeds carbon loss by the conversion of grasslands. In such case, oil palm plantations act as a net carbon sink (Brinkman 2009). Finally carbon stock might be the limiting factor for environmental sound activities especially for oil palm and industrial forest plantations on peatland to obtain significant reduction of greenhouse gas emission.

MATERIAL AND METHOD

Study site

The research was carried out in August 2009 at oil palm plantation of 25 years old owned by a farmer located in the Kuaro Sub-district, Paser District within East Kalimantan Province, Indonesia. The oil palms were planted on mineral soil with spacing of 9 m x 7 m comprised of 125 trees per ha. Since the beginning of the plantation through to certain periods, the oil palm farmer has used non-organic fertilizers and pesticides to increase land productivity.

Biomass

Above-ground biomass

As many as three 25 years old oil palms trees were selected as samples for calculating the above ground biomass and carbon stock. These trees were selected from the total plants standing on the plantation where each tree must represent the condition of the plantation. In order to measure the above ground biomass of selected oil palms, destructive sampling was used by cutting down each sampled tree using chainsaw, followed by separation of tree parts from the lower part to the top. The stem of each sampled tree was divided into horizons of 1-2 m long. For each horizon, fresh weight (FW_x) of each morphological tree compartments (stem, branch, twig, and leaf) were separately weighed and recorded. To analyze the basic characteristics of the plant, i.e. water content and fixed carbon, a small amount of 200 g were taken from each morphological compartment of the sampled trees.

Understorey biomass

Ten (10) 1 m^2 sub-plots were established in understorey plot for above ground biomass measurement. All understoreys (shrubs, grass and litter) were cleaned, separated according to morphological compartments, weighed and recorded. For each understorey, a 200 g of fresh sample was taken for analysis in the laboratory, to determine the basic characters, similar to the method used for tree morphological compartments.

Moisture content

About 1-2 gram of test sample was weighed ($B0_x$), and oven dried with temperature of 105±3°C for 24 hours to determine the dry weight (DW_x). Water contents of each test sample (WC_x) were calculated following the equation:

$$WC_x = \frac{BO_x - DW_x}{DW_x}$$

Based on the results of water content values, biomass for each tree morphology (W_x) was calculated using the following equation:

$$W_x = \frac{FW_x}{WC_x + 1}$$

Biomass calculation

Biomass of oil palm trees and understorey were calculated following the equation:

$$Bx = (TDW \times 100)/A$$

Bx is the biomass of each morphological compartment (kg), TDW is the total dry weight of each morphological compartment (kg) and A is the plot area (m^2).

Carbon

Fixed carbon

Fixed carbon calculation for each tree morphology was made after determination of (a) volatile matter and (b) dust.

Volatile matter content (VM) was determined by placing test sample inside a tightly closed porcelain dish and placed in an oven of temperature 950°C. The steps were: first, test sample was inserted close to the front door of the oven with temperature of 300°C for 2 minutes, and then transferred to the side of the oven with temperature of 500°C for 3 minutes, and again transferred into the inside of the oven with temperature of 950°C for 6 minutes. The dish containing heated powder was then cooled inside an exicator for 1 hour and weighed. The content of the volatile matter was expressed in percent of weight using the following equation:

$$VM = (A-B)/A \times 100\%$$

A is the oven dry weight at 105°C, B is the test sample weight minus the weight of dish and the remaining test sample at 950°C.

Dust content (DC) determination was done as follows: test sample of 2 g was placed into a porcelain dish and put into an oven starting with a temperature of 0°C up to 600°C for 6 hours. The dish was then taken out from the oven, cooled inside an exicator and weighed. The amount of dust content was calculated based on the following equation:

DC = (Weight of dust/weight of oven dried test sample) x 100%

After the volatile and dust contents were measured, fixed carbon (FC) could then be calculated using the following equation:

FC = 100%-VM-DC

Carbon stock

Carbon stock of oil palm and understorey were calculated following the equation:

Cx = (FC x 100)/A

Cx is the carbon of each morphological compartment (kg), FC = fixed carbon of each morphological compartments (kg) and A is the plot area (m^2).

RESULTS AND DISCUSSION

Biomass

Diameters of the selected oil palm trees varied between 65 cm to 95 cm, while the height varied from 10 m to 12 m. Oil palm stem (trunk) had the highest average dry weight with value of 84.32 t ha^{-1}, followed by leaflet, fruit and petiole with 10.74 t ha^{-1}, 0.78 t ha^{-1} and 0.18 t ha^{-1} respectively, with a total of about 98.91 t ha^{-1} (Table 1).

Table 1. Average dry weight of 25 years old oil palm planted on mineral soil in Paser District, East Kalimantan

Part of oil palm	Average dry weight per plant (ton)	Average dry weight per ha (ton)
Stem (trunk)	0.67	84.32
Leaflet	0.08	10.74
Fruit	0.006	0.78
Petiole	0.00017	0.22
Inflorescence	0.00014	0.18
Weeds in the trunk	0.02	2.67
Total	0.78	98.91

The highest average dry weight of the 25 years old oil palm plantation on mineral soil was dominated by understorey with value of 7.47 t ha^{-1}, followed by grass and dry litter, each with a value of 5.57 t ha^{-1} and 2.14 t ha^{-1} respectively, with a total of approximately 15.18 t ha^{-1} (Table 2).

Table 2. Average dry weight of understorey and litter of 25 years old oil palm planted on mineral soil at Paser District, East Kalimantan

Source	Average dry weight (ton ha^{-1})
Dry litter	2.14
Understorey	7.47
Grass	5.57
Total	15.18

Carbon

The carbon content of each part of oil palm varied from 31.15% at petiole part to 47.79% at inflorescence. The highest carbon stock for the 25 years oil palm planted on mineral soil was dominated by stem (trunk) with values 29.13 t ha^{-1}, followed by leaflet with 3.87 t ha^{-1}, fruits 0.35 t ha^{-1}, inflorescence 0.09 t ha^{-1} and petiole 0.07 t ha^{-1}, giving a total of 34.48 t ha^{-1} (Table 3).

Table 3. Carbon content and carbon stock of 25 years old oil palm planted on mineral soil at Paser District, East Kalimantan

Part of oil palm	Average dry weight (ton ha^{-1})	Carbon content (%)	Carbon stock (ton ha^{-1})
Stem (trunk)	84.32	34.55	29.13
Leaflet	10.74	36.00	3.87
Fruit	0.78	45.38	0.35
Petiole	0.22	31.15	0.07
Inflorescence	0.18	47.79	0.09
Weeds in the trunk	2.67	36.30	0.97
Total	98.91		34.48

The carbon content of average dry weight of understorey varied from 34.05% at dry litter to 40.32% at grass stage. The highest carbon stock was dominated at understorey with a value 2.48 t ha^{-1}, followed by grass with 2.25 t ha^{-1} and dry litter 0.73 t ha^{-1}, giving a total of 5.46 t ha^{-1} (Table 4).

Table 4. Carbon content and carbon stock of understorey of 25 years old oil palm planted on mineral soil at Paser District, East Kalimantan

Source weight	Average dry (t ha^{-1})	Carbon content (%)	Carbon stock (t ha^{-1})
Dry litter	2.14	34.05	0.73
Understorey	7.47	33.17	2.48
Grass	5.57	40.32	2.25
Total	15.18		5.46

Discussion

Based on data in Table 1 and 2, the total above ground biomass for 25 years old oil palm planted on mineral soil was 113.28 t ha^{-1}. The total above ground carbon stock as shown in Table 3 and 4 was 39.94 t/ha, or equal to 146.58 t of CO_2-eq ha^{-1}. If below ground carbon stock was estimated at maximum of 20% (commons used value), hence the total carbon stock at 25 years old oil palm planted on mineral soil was 46.84 t ha^{-1} or equal to 171.32 t of CO_2-eq ha^{-1} or 6.85 t of CO_2-eq ha^{-1} $year^{-1}$. Results of research conducted in the Province of Riau of 19 years old oil palm planted on mineral soil showed that the total biomass of ground and below ground was 108.58 t ha^{-1}, while the total carbon stock of above and below ground was 40.28 t ha^{-1}, or equal to 147.83 t of CO_2-eq ha^{-1} or 7.78 t of CO_2-eq ha^{-1} $year^{-1}$ (Tjitrosemito and Mawardi 2000).

The amount of carbon bound in oil palm plantation biomass is primarily a function of palm growth and the understorey. Published values on the quantity of above

ground biomass on oil palm plantations range from 50 t ha^{-1} to over 100 t ha^{-1} towards the end of the plantations economical live span after 20-25 years (Brinkman 2009).

Biomass from oil palm root increased as the above ground biomass increased, where the maximum volume depend on the soil characteristics and water availability. Germer and Saurborn (2008) calculate the average biomass of roots at 20±5 t ha^{-1}. Biomass from ground vegetation decreases as the oil palm vegetation grows and the sunlight it receives decreases. Germer and Sauerborn (2008) also calculate the average ground vegetation biomass at 2.5±1 t ha^{-1} with the assumption that the maximum development of ground vegetation is 10 t ha^{-1} and the linear decrease of biomass from reduced sunlight of 1 ton ha^{-1} from five years since planting. Based on this description, Germer and Sauerborn (2008) calculate the total above and below ground biomass to be 82.5±26 t ha^{-1} and carbon fixation to be 35.3±11 t ha^{-1} during the economic planting period of the palm oil plantation period or equivalent to 129.3±40.3 t of CO_2-eq ha^{-1}. Henson (2008) has also made a quantification of carbon absorbed by oil palm plantations in Malaysia. The average carbon absorbed by 25 year old oil palm trees is 2.09 t of carbon ha^{-1} $year^{-1}$ (equivalent to 7.66 t of CO_2-eq), where 80% originates from carbon stored within the system (from ground vegetation, coarse litter and 20% from oil palm production). The total accumulated carbon of the oil palms at the end of the cycle is 44 t carbon ha^{-1}. The average carbon stored in areas that have been planted for a period of 30 years is 35.4 t ha^{-1}, equivalent to Germer and Sauerborn's (2008) findings at 35.3 t carbon ha^{-1}. Literature studies conclude that the amount of carbon which may be absorbed by oil palm plantations is 35 t for a period of 25-30 years, or equivalent to 130 t of CO_2-eq ha^{-1} (Brinkman 2009).

The above figures showed that oil palm plantation may absorb as much as 130-180 t of CO_2-eq during the economic planting period of 25-30 years. If the establishment of oil palm plantation was supposed to provide better environmental quality to human life, this indicated that plantation should be conducted on lower carbon stock area. Fargioni et al. (2008) concluded that converting native forests to biofuel production results in large carbon debts like converting lowland tropical rainforest in Indonesia and Malaysia to palm biodiesel would result in a biofuel carbon debt of 610 t ha^{-1} of CO_2-eq that would take approximately 86 years to repay. Converting tropical peatland rainforest to palm production incurs a similar biofuel carbon debt from vegetation, but the required drainage of peatland causes an additional sustained emission of approximately 55 t of CO_2-eq ha^{-1} $year^{-1}$ from oxidative peat decomposition. Peatland of average depth (3 m) could release peat-derived CO_2-eq for about 120 years. Total net carbon released would be approximately 6,000 t ha^{-1} of CO_2-eq, taking 840 years to repay (Fargioni et al. 2008).

The calculation of how much oil palm plantation may absorb CO_2 during its economic cycle should be initiated based on the type of land use.

The GHG emission that resulted from changes of the above and underground biomass depends on the original biomass stock present on the land, as well as the question whether original biomass is removed through decomposition, or through burning (Brinkman 2009). Emissions arising from changes in carbon stocks during the development of a new plantation and during the operations of a plantation are in particular related to changes in above ground and underground biomass, as well as soil organic matter (including peat). Establishing and operating oil palm plantations may have three different impacts upon above ground and below ground carbon stocks, (Brinkman 2009) namely: (i) The establishment of a plantation leads to the removal of originally present above ground and below ground biomass, e.g. forest, grassland; (ii) A palm plantation stores carbon through the growth of oil palms; (iii) Establishing and operating oil palm plantations on peat requires ongoing drainage, thus causing ongoing peat oxidation.

It had been well recognized that most of opened peatlands in Indonesia had been occupied by *Acacia crassicarpa* and oil palm. Above ground biomass of 6 years old *A. crassicarpa* in peatland was 138.32 t ha^{-1} while the total carbon stock was 28.39 t ha^{-1} or equal to 104.18 t of CO_2-eq ha^{-1} (Limbong 2009). Results of the research conducted in Central Kalimantan peatland (Siregar et al. 2004) show that the total carbon stock at logged over area is estimated around 413.972 t ha^{-1} (0-30 cm depth of peat) and at burnt area is 411.349 t ha^{-1} (0-30 cm depth of peat).

Under undisturbed conditions, the primary peat forest could be absorbed carbon through the accumulation of peat and biomass. This ecosystem functions as a carbon sink through the accumulation of peat. Drainage and degradation of the primary peat forest result in carbon emissions through peat decomposition. The conversion of peatlands to oil palm plantations require 60-80 cm drainage below the surface, supported by peat decomposition and GHG emissions (Brinkmann 2009). There are several uncertainties produced by researchers in peatlands regarding the true value of GHG emissions, however literature studies suggest that a realistic value of GHG emissions from drained peatlands is 25-55 t of CO_2-eq ha^{-1} $year^{-1}$. Based on an intensive literature study on CO_2 emissions related to peat drainage on different use of land, it is generally concluded that deeper drainage causes higher emissions of CO_2 ha^{-1}. However, specific reference on CO_2 emissions from oil palm plantations provide the value of 55 t CO2-eq ha^{-1} $year^{-1}$ from drainage at a depth of 60 cm (Melling et al. 2005) and 54 t CO_2-eq ha^{-1} $year^{-1}$ from drainage at a depth of 80 cm (Muruyama and Bakar 1996). Henson (2008) concludes that there is a large uncertainty regarding the magnitude of carbon emissions from peat and its relation to drainage intensity and peat subsidence. He uses the figure of 7.2 t of carbon ha^{-1} $year^{-1}$ (based on Wösten et al. 1997) and 9.17 t of carbon ha^{-1} $year^{-1}$ (Melling et al. 2007). This is equivalent to 25-30 t of CO_2-eq ha^{-1} $year^{-1}$. Furthermore, longer term lifecycle analyses also arrive at clearly negative values for CO_2 emissions from peat degradation, e.g. 1.8 kg of CO_2 $m^{-2}year^{-1}$ (Germer and Sauerborn 2008), 3.7-5.5 kg of CO_2 $m^{-2}year^{-1}$ (Reijnders and Huijbregts, 2008), 3.9 kg of CO_2 $m^{-2}year^{-1}$ (Wicke et al. 2008) and 5.5-7.3 kg of CO_2 $m^{-2}year^{-1}$

(Fargioni et al. 2008). Muruyama and Bakar (1996) have estimated an amount of 54 t CO_2-eq ha^{-1} $year^{-1}$ emitted from oil palm plantations on peat at 80 cm drainage depth.

As mentioned earlier, during the economic planting period of 25-30 years on mineral soil, oil palm plantation stored carbon at about 5.2 to 8 t ha^{-1} $year^{-1}$ which may absorbed about 130-200 t of CO_2-eq. The six (6) years planted *A. crassicarpa* on peatland has above ground biomass value of 138.32 t ha^{-1} which has a total carbon stock of 28.39 t ha^{-1} or equal to 104.18 t of CO_2-eq ha^{-1}. If both carbon stock values were compared, this showed that the ability of oil palm and *A. crassicarpa* to absorb CO_2 were less than the CO_2 emission from opening of 30 cm depth of peatland. This produced a contradictive situation in term of Greenhouse Gas emission reduction efforts, because those plants produce less carbon stocks compared to peatland clearance. In other words, this indicated that even though plants occupied peatland area, however high emissions of Greenhouse Gas were still produced. Thus, if an oil palm or industrial plantation would be established on peatlands, they should have carbon stock or higher ability to absorb CO_2 than during peatland clearance, otherwise there would be no significant emission reductions occurred and high risk impacts would eventually created. Therefore, it can be said that carbon stock could be used as a baseline or might be used as a limiting factor for environmental sound activities especially for oil palm plantation and industrial forest plantation on peatlands in order to achieve significant reductions of greenhouse gas emission.

CONCLUSION

Reduction of Greenhouse Gas emission is the solution in minimizing the negative impacts of global climate change where it needs mitigation and adaptation efforts. Global climate change is actually the product of development, meaning that its reduction must come from the development itself. Reduction of greenhouse gas emission on peatland should start prior to land clearance activity. This means that carbon stock calculation should be carried out prior to plantation activities where the results should be compared to the established commodities of the future existing plantation as a compensation mechanism, which would provide one of the best solutions in reducing greenhouse gas emissions.

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Abstract:

Assaeed AM (2007) Seed production and dispersal of *Rhazya stricta***.** 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS (2000) Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds) Toward mount Lawu national park; proceeding of national seminary and workshop on biodiversity conservation to protect and save germplasm in Java island. Sebelas Maret University, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto (2004) Soil macro-invertebrates diversity and inter-cropping plants productivity in agroforestry system based on sengon. [Dissertation]. Brawijaya University, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L (2008) A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com

Publication manuscript "in press" can be cited and mentioned in reference (bibliography); "personal communications" can be cited, but cannot be mentioned in reference. Research which not be published or "submitted" cannot be cited.

Some annotation. Manuscript typed without sign link (-) (except repeated word in Indonesian). Usage of letter "l" (el) to "1" (one) or "O" (oh) to "0" (null) should be avoided. Symbols of α, β, χ, etc. included through facility of insert, non altering letter type. No space between words and punctuation mark.

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