BIODIVERSITAS
Volume 11, Number 1, January 2010
Pages: 24-28

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)
DOI: 10.13057/biodiv/d110106

Butterfly diversity as a data base for the development plan of Butterfly Garden at Bosscha Observatory, Lembang, West Java

TATI SURYATI SYAMSUDIN SUBAHAR♥, ANNISA YULIANA
Ecology and Biosystematics Research Group, School of Life Sciences and Technology, Bandung Institute of Technology, Jl. Ganesa No 10, Bandung 40132, West Java, Indonesia, Tel./fax.: +62-22-2534107, +62-22-2511575, ♥email: tati@sith.itb.ac.id, tatibio76@yahoo.com

Manuscript received: 6 July 2009. Revision accepted: 12 November 2009.

ABSTRACT

Subahar TSS, Yuliana A (2010) Butterfly diversity as a data base for the development plan of Butterfly Garden at Bosscha Observatory, Lembang, West Java. Biodiversitas 11: 24-28. Change of land use and the increasing number of visitors to Bosscha area was one factor for the development plan of butterfly garden in the area. The objectives of this research were to examine butterfly diversity and its potential for development plan of butterfly garden. Butterfly diversity and its richness conducted by standard walk methods. Host plant and larval food plant was recorded during butterfly survey. Public perception on the development plan of butterfly garden was examined by questionnaire. The results showed that 26 species of butterfly was found in Bosscha area and *Delias belisama belisama* was the most dominant species. Public perceptions consider that the development plan of butterfly garden will give benefit to the community; not only providing new insight (40.41%), additional tourism object (23.97%) and will gave aesthetical value (17.12%). Twelve local species should be considered for development plan of butterfly garden: *Papilio agamemnon, P. demoleus, P. memnon, P. sarpedon, Delias belisama, Eurema hecabe, Danaus chrysippus, Argynnis hyperbius, Cethosia penthesilea, Hypolimnas misippus, Melanitis phedima* and *Euthalia adonia.* Host plant*: Bougainvillea spectabilis, Citrus aurantium, Lantana camara, Macaranga tanarius* and food plants*: Citrus aurantium, Cosmos caudatus, Eupatorium inulifolium, Gomphrena globosa, Hibiscus rosa-sinensis, Lantana camara,* and *Tithonia diversifolia.*

Key words: host plant, butterfly diversity, Bosscha Observatory, standard walk.

INTRODUCTION

Urbanization and agriculture are some of the biggest threats to the world biodiversity that led to local species extinction due to habitat fragmentation and decreasing of open space (Connor et al. 2003; Hedblom 2007). Land use change and reducing open space for human settlement and agriculture are the general issue in Northern area of Bandung, West Java, including Bosscha Observatory area. So far, Bosscha has a unique role; as the only major Observatory in Indonesia, even in South East Asia (Anonymous 2007) has made Bosscha as the only one astronomical tourism objects in Indonesia. Bosscha Observatory visited by various groups of people: students, researchers etc and the number of visitor always increase every year. The number of tourist visits on the year 2006 was 55,419 people with an average of 4618 persons per month. In 2007, the number of visitors increased dramatically that was 72,037 people with an average per month of 4735 people (Anonymous 2007) and in the year of 2008, the number of visitors was 63,480 people (not published). Increasing number of visitors from time to time has led Bosscha Observatory as an educational heritage. The landscape of Bosscha was an agricultural area, however with the increasing number of local residents and visitors to the area has made the area as an urban resident with its all supporting activities. As consequences, open space reduced and in the night time, light intensity from the residential area became one of the factors that disturb the activity of observatory which required minimal light condition (Ernanto 2006). Environmental conditions at Bosscha area with light pollution, increase number of visitors (who are interested to understand natural phenomena through star observation) and the limited capacity of the observatory require diversification of function of the area.

Environmental changes can affect the biological diversity. Sundufu and Dumbuya (2008) study the habitat preferences of butterflies in the Bumbuna forest, Northern Sierra Leone showed that accumulated species richness and diversity indices in the disturbed habitats were lower than the forest. Learning from the San Francisco Bay, the impact of urbanization has caused 43% loss of butterfly species as a result of habitat fragmentation and loss of habitat for insects (Connor et al. 2003). Butterflies are good indicator species to monitor ecological changes in a habitat (Sreekumar and Balakrishnan 2001). Information on butterfly diversity in the region is very scarce. Tati-Subahar et al (2007) described butterfly stratification in Tangkuban Parahu (a mountainous area, Northern part of Bandung) from 1400-2000m above sea level (asl). Up till now there has been no report on butterfly diversity in Bosscha area and its vicinity. Therefore the objective of this research was to examine butterfly diversity and its potential for

development plan of butterfly garden. Hope this result can contribute to the biodiversity database on development plan of butterfly garden as tourist object and also as natural laboratory for environmental education and conservation.

MATERIALS AND METHODS

This research was conducted in Bosscha and Cihideung which was a hilly area about 15 km to the north of Bandung City, West Java, Indonesia. Bosscha observatory (107°36 'BT and 6°49' LS) was located at 1310 m asl (above sea level). The study site in Bosscha was an area of 6 ha consisted of 3 plots: The first plot was front park of the observatory: the park was covered by grass. In the middle of the area found *Bougainvillea* and *Lantana camara*. The second plot was a center park of the observatory which was covered by grass. During observations found some flowering plants at the road edge. The third plot was a back yard; there were shrubs, grasses and bamboo.

The second location was Cihideung, known as a center for cultivation and trading of ornamental plants and fruit trees. This area lies along the road from south to the north of Bandung City. The study site (1032-1203 m asl) was 3 km in long covered by different type of flowering plants which consist of 4 plots: The first plot was the southern part, the flowering plants found only in one side of the road. At second plot, the flowering plant was located on both sides of the road. At plot 3, flowering plants found only in one side of the road and plot 4 was the middle of the flower garden which was closed to Bosscha.

Survey on butterfly diversity conducted by *standard walk* methods (Pollard and Yates 1995; Tati-Subahar et al. 2007), i.e. walking along the plot while counting and recording the number of butterflies seen or encountered. The observation width was limited to about 5m. The presence and the number of known butterflies in each plot were directly recorded. After being examined and recorded morphologically by photograph, the butterfly released into natural habitat. No individual marking was conducted. Unidentified butterfly were collected using an insect net for later identification. The specific locations in which butterfly were observed, e.g. on flowers, other plant parts were also recorded. Surveyed was conducted in two periods, first period was on 8-24 May 2008 and the second was on 24-30 June 2008. Both periods of surveyed were the end of the rainy season and early dry season. Observation started from 07.00 am and finished around 12.00 am every day. Microclimate condition during the surveyed varied, temperature ranges from 21^{o}C-26^{o}C and relative humidity ranges from 72%-83 %. Species identification was conducted at Ecology and Biosystematic Laboratory of SITH-ITB Bandung follows Roepke (1932), Smart (1975), Preston and Mafham (2000), and Noerdjito and Aswari (2003). Identification of host and food plants conducted in Herbarium Bandungense-SITH ITB Bandung. Information on host and food plants for each species of butterfly was completed based on observations and information from different locations (Nurcahya 2003; Soekardi 2002; Tati-Subahar et al. 2007). Host plants are the female butterfly lays their eggs on. The newly emerged (caterpillars) only eat the plant that they are hatched on. Food plants are the plants that adult butterfly forage on. Sorensen Index of Similarity (Odum 1971; Mueller-Dumbois and Ellenberg 1974) was used to compare butterfly community between Bosscha and Cihideung area.

Potential utilization of butterfly as tourism object for environmental education and conservation were evaluated by distributing questioners to 86 students of Lembang district (about one km from Bosscha area). Evaluation on ecosystem services was analyzed following methods of Boyer and Polasky (2004). Community perception on the benefit of development plan of butterfly garden was evaluated by distributing 75 questioners randomly to Bosscha Observatory visitors every day during one week.

RESULTS AND DISCUSSION

Diversity and abundance of butterfly

During this research, 848 individuals of butterfly were recorded in Bosscha (26 species of 5 families), which were consisted of 4 species of Papilionidae, 4 species of Pieridae, 16 species of Nymphalidae, one species of Lycaenidae and one species of Hesperiidae. Butterfly survey in Cihideung found 41 species from 5 families which consisted 7 species of Papilionidae, 5 species of Pieridae, 26 species of Nymphalidae, two species of Hesperiidae and one species of Lycaenidae. These species were a new record of butterfly species from Bosscha and Cihideung, since there was not any previous documentation. Butterfly communities in Bosscha and Cihideung were almost similar (Sorensen Index of Similarity = 60.87%). Although Cihideung as a central of ornament plants with highest diversity of flowering plants, but individual number of butterfly observed during observation in Cihideung was lower than Bosscha (Table 1). As consequence, the total number of host plants and food plant recorded during periods of observation was lower.

The highest relative abundance of butterfly species in Bosscha was *Delias belisama belisama* (32.19%) followed by *Ypthima philomela philomela* (20.64%), *Papilio sarpedon* (11.08%) and the rest of 14.50% were distributed to 19 species of butterfly that have a relative abundance about 0.76% per species (Figure 1a). In Cihideung, *D. belisama belisama* was also dominant with its relative abundance was 53, 21%, followed by *Cethosia penthesilea* (10.35%), and the rest of 34 species have a relative abundance about 0.3% per species (Figure 1b). Domination of *D. belisama belisama* in the two study site related to its characteristic as a polyfagous insect which has a wide range of host and food plants and could found in different habitats (Soekardi 2002). In determining the pattern of butterfly community, relative abundance of butterfly and plants resources was an important aspect that characterizes butterfly community (Yamamoto et al. 2007).

Table 1. Butterfly occurrence at Bosscha and Cihideung in May-June 2008.

Family		Species	Occurrence	
			Bosscha	Cihideung
Papilionidae	1	*Papilio agamemnon agamemnon*	49	16
	2	*P. demoleus sthenelinus*	1	1
	3	*P. demolion*	0	1
	4	*P. helenus enganius*	0	2
	5	*P. memnon memnon*	36	5
	6	*P. polytes javanus*	0	1
	7	*P. sarpedon sarpedon*	94	25
Pieridae	8	*Appias lyncida*	0	1
	9	*Delias belisama belisama*	273	333
	10	*D. hyparete*	7	1
	11	*Eurema alitha*	2	0
	12	*E. hecabe*	21	11
	13	*Leptosia nina*	0	2
Nymphalidae	14	*Faunis canens*	0	1
	15	*Neptis hylas matuta*	14	7
	16	*N. nandina nandina*	0	1
	17	*Pantoporia nefte nefte*	0	1
	18	*Danaus chrysippus*	10	2
	19	*Danaida eryx furius*	0	3
	20	*Euploea mulciber*	0	5
	21	*Argynnis hyperbius javanica*	25	6
	22	*Cethosia penthesilea penthesilea*	1	65
	23	*Hypolimnas bolina iphigenia*	1	0
	24	*H. misippus misippus*	6	3
	25	*Junonia almana javana*	0	2
	26	*Junonia atlites atlites*	0	11
	27	*J. iphita horsfieldi*	65	1
	28	*J. orithya minagara*	0	1
	29	*Elymnias hypermnestra hypermnestra*	11	47
	30	*E. nesaea*	1	2
	31	*Lethe manthara*	1	0
	32	*L. rohria godana*	11	2
	33	*Melanitis leda simesa*	0	4
	34	*M. phedima phedima*	1	0
	35	*Mycalesis mineus*	0	1
	36	*M. sudra sudra*	33	2
	37	*Ypthima philomela philomela*	175	1
	38	*Pareba vesta vestoides*	1	1
	39	*Euthalia adonia adonia*	2	0
	40	*Pyrameis cardui cardui*	0	2
	41	*P. dejeani*	0	1
	42	*Cyrestis lutea lutea*	0	2
	43	*C. nivea nivea*	0	1
Lycaenidae	44	*Anthene lycaenina*	4	49
Hesperiidae	45	*Notocrypta paralysos*	3	2
	46	*Discophora celinde celinde*	0	1

Some butterfly species were common in both locations, i.e. *D. belisama*, *P. agamemnon*, *P. memnon*, *P. sarpedon*, *Eurema hecabe*, *Neptis hylas matuta*, *Argynnis hyperbius* and *Elymnias hypermnestra*. The other species found only in one location, such as *Hypolimnas bolina*, *Lethe manthara*, *Melanitis phedima*, and *Euthalia adonia* which only found in Bosscha. During the observation, some species found only one individual, for example seven species (*P. demoleus*, *Cethosia penthesilea*, *Hypolimnas bolina*, *Elymnias nesaea*, *Lethe manthara*, *Melanitis phedima and Pareba vesta vestoides*) found only one individual in Bosscha. While in Cihideung, 16 species (*Papilio demoleus*, *P. demolion*, *P. polytes*, *Appias lyncida*, *Delias hyparete*, *Faunis canens*, *Neptis nandina*, *Pantoporia nefte*, *Junonia iphita*, *Junonia orithya*, *Mycalesis mineus*, *Ypthima philomela*, *Pareba vesta vestoides*, *Pyrameis dejeani*, *Cyrestis nivea* and *Discophora celinde*) found only one individual during the observations. Those species were in a high risk condition when the disturbance occurred in the habitat as consequences the species would be very vulnerable or even extinct.

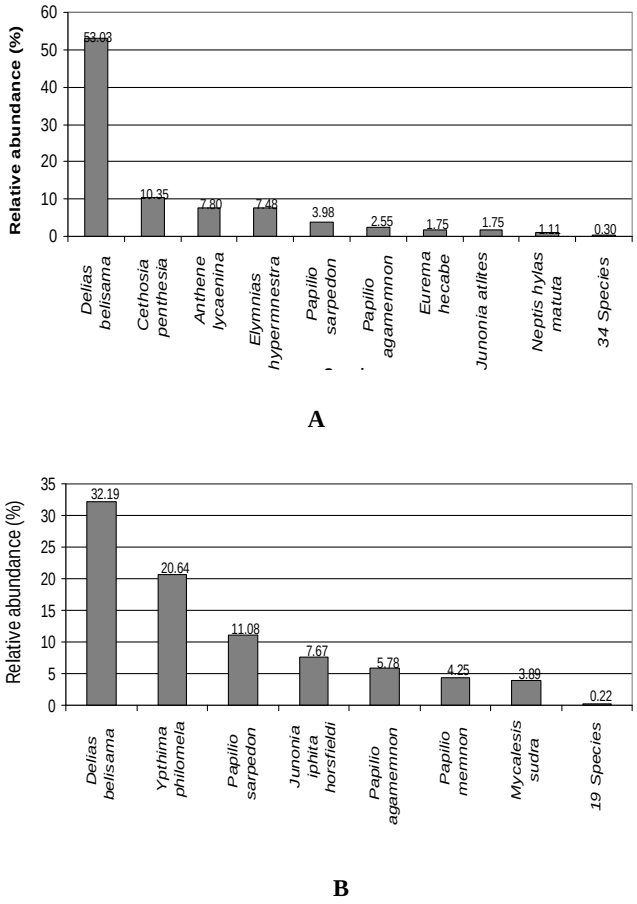

Figure 1. The relative abundance of butterfly in Bosscha (a) and in Cihideung (b).

Host plants and food plants of butterfly

Studies on plant species in the same locations found at least seven species of flowering plants as food sources for butterfly, i.e. *Citrus aurantium*, *Cosmos caudatus*, *Eupatorium inulifolium*, *Gomphrena globosa*, *Hibiscus rosa-sinensis*, *Lantana camara* and *Tithonia diversifolia* (Table 2). As food plant, *L camara* visited by several species of butterfly such as *D. belisama belisama*, *Elymnias hypermnestra*, and *Papilio sarpedon*. In Cihideung, *Gomphrena globosa* the purple flower was not only as food plants for *Cethosia penthesilea*, but also as host plants for *Argynnis hyperbius javanica* and *Junonia iphita*. This phenomenon was also observed by Nurcahya (2003), where the butterfly choose plants and flowers with an attractive light color, such as red, orange, yellow, and purple flowers as their host plants.

During observations, *Bougainvillea spectabilis*, *Citrus aurantium* and *Macaranga tanarius* found as host plants for several butterflies (Table 2). For example *Citrus sp.* was a host plant of Papilionidae, such as *Papilio memnon*, *P. helenus*, and *P. demoleus*. There was evidence that related groups of butterfly species associated with particular plants (as larva host plants or imago food plants) and that phenomenon was also found by Queiroz (2002).

Potential utilization of butterfly

As an ecosystem, Bosscha area provide many benefits to humans not only as tourism object but also as butterfly habitat, which was also provide a number of important services. The open-access to Bosscha ecosystem and the characteristic of ecosystem itself as public good often neglected for its value of the ecosystem services.

The presence of an open space with flowering plant, as habitat and food for butterfly, will give an additional value for environmental education. Students perception (40.41%) consider that the presence of butterfly garden will increase their insights on the butterfly science; 23.97% of the students thought butterfly garden can be an additional object besides the observatory (stars observation); 17.12% of the students thought butterfly garden will embellish the observatory landscape (Figure 2). Most of the students (40.91%) was curious on butterfly life cycle (Figure 3), they were interested to observed (i) how butterfly eggs attached to host plant; (ii) how developmental phase or metamorphoses of butterfly happened, from egg to the caterpillar or larvae; and (iii) how the pupae emergence and become a young butterfly with its beautiful color. From economic aspect, about 58% of observatory visitors or domestic tourists are willing to pay the additional cost Rp. 3000 or more to visit butterfly garden in Bosscha (Figure 4). Based on these results, the potential of butterfly diversity in the area can be managed as a tourist object for conservation and environmental education.

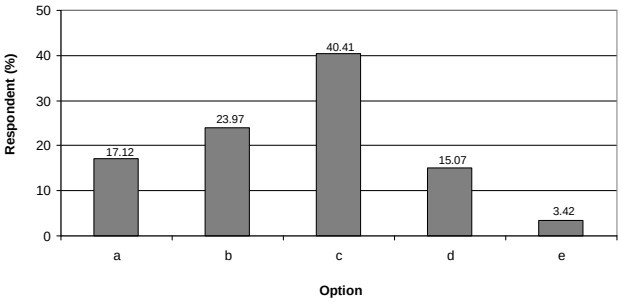

Figure 2. Respondent perception (n=86) on the advantage of butterfly garden (a. to embellish the observatory landscape; b. media to get information out of astronomy; c. to add another perspective field of science; d. media to study human interaction with the environment; e. media to learn more about the butterfly).

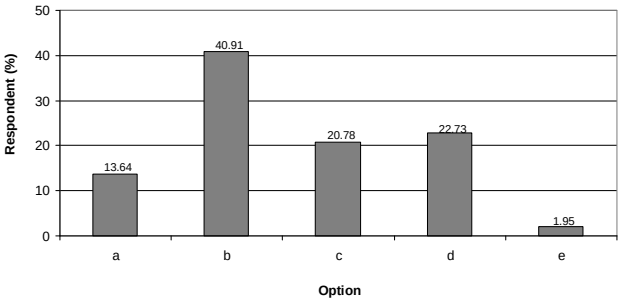

Figure 3. Public expectation (n=86) on the availability of information in butterfly garden (a. color and pattern of butterfly; b. butterfly life cycle; c. interaction among butterfly population; d. butterflies in their habitat; e. the origin of butterfly species).

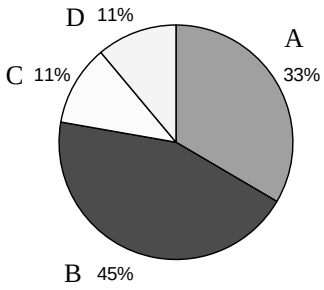

Figure 4. Respondent willingness (n=75) to pay for future butterfly garden facilities in Bosscha. A < Rp. 5000, B = Rp. 5000, C Rp. 10000, D > Rp. 10000.

Table 2. Host and food plants use by several butterfly in Bosscha and Cihideung

Plants species		Butterfly species		Role
Family	**Species**	**Family**	**Species**	
Amaranthaceae	*Gomphrena globosa*	Nymphalidae	*Argynnis hyperbius*	HP
			Cethosia penthesilea	HP
			Junonia iphita	HP
Asteraceae	*Cosmos caudatus*	Nymphalidae	-	HP
	Eupatorium inulifolium	Nymphalidae	*Ypthima philomela*	HP
		Lycaenidae	*Anthene lycaenina*	HP
	Tithonia diversifolia	Pieridae	*Delias belisama*	HP
		Nymphalidae	*Ypthima philomela*	HP
		Lycaenidae	*Anthene lycaenina*	HP
Euphorbiaceae	*Macaranga tanarius*	Pieridae	*Delias belisama*	FP
Malvaceae	*Hibiscus rosa-sinensis*	Nymphalidae	-	HP
		Papilionidae	-	HP
Nyctaginaceae	*Bougainvillea spectabilis*	Nymphalidae	*Pareba vesta vestoides*	FP
Rutaceae	*Citrus aurantium*	Papilionidae	*Papilio demoleus*	HP & FP
			Papilio memnon	HP & HP
			Papilio polytes	HP & FP
Verbenaceae	*Lantana camara*	Pieridae	*Delias belisama*	HP
		Nymphalidae	*Ypthima philomela*	HP
		Papilionidae		HP

Note: HP = Host plants; FP = Food plants

Development plan of butterfly garden

Learning from the experience of several places such as "The San Francisco Bay Area" whose use the garden as a habitat for insect conservation area, one of the insect groups is butterfly, it seems that recreational activities could harmonize with conservation of beneficial insects (Connor et al.

2003). In Indonesia, the experience of "Gita Persada" butterfly garden through ecological engineering by habitat modification has been successful in bringing back the butterfly which was lost from the forest area of Mount Betung, Lampung–Sumatra Island (Soekardi 2002). Another experience in Bantimurung-Bulusaraung National Park (South Sulawesi), a sustainable community-based on butterfly rearing had been successfully maintaining butterfly diversity in natural habitat (Harlina 2006). Experiences and success stories in some places where habitat rehabilitation and management of open space through butterfly garden can give an added-value of the area as educational tourism object. The implementation of development plan conducted in several activities such as creating a semi-natural rearing facility for butterfly by using native species. Based on the relative abundance and butterfly characteristics, there were 12 species to be considered, i.e., *P agamemnon, P. demoleus, P. memnon, P. sarpedon, Delias belisama, Eurema hecabe, Danaus chrysippus, Argynnis hyperbius, Cethosia penthesilea, Hypolimnas misippus, Melanitis phedima* and *Euthalia adonia*. Habitat modification will be the most important steps in order to prepare host and food plant for butterfly. These activities consist of planting and preparing for rearing facilities. At least seven species could be prepared as host and food plants such as *Citrus aurantium, Cosmos caudatus, Eupatorium inulifolium, Gomphrena globosa, Hibiscus rosa-sinensis, Lantana camara and Tithonia diversifolia* (Detail technical implementation will be described in separate article).

The development plan of butterfly garden in Bosscha area is one of the optimization of ecosystem services. As an ecosystem, Bosscha area and its vicinity has a higher value than astronomical tourist object. Referring to Daily et al. (2002), a variety of ecosystem services provided by Bosscha was: the existence value of the area not only as a habitat for butterfly and other biotic components but can be extended to the whole open space as water catchments area and flooding controller for Bandung area (as an indirect value). Butterfly diversity could be used as a media to provide knowledge for the local and regional communities on the importance of maintaining biodiversity and improving the quality of environment.

CONCLUSIONS

Twenty six species of butterfly has been found in Bosscha area and 41 species in Cihideung. These results indicated that Cihideung area could be considered as a source for butterfly diversity in Bosscha area. *Delias belisama belisama* was the dominant species, but for development plan of butterfly garden there were 12 species to be considered i.e. *Papilio agamemnon, P. demoleus, P. memnon, P. sarpedon, Delias belisama, Eurema hecabe, Danaus chrysippus, Argynnis hyperbius, Cethosia penthesilea, Hypolimnas misippus, Melanitis phedima* and *Euthalia adonia. Bougainvillea spectabilis, Citrus aurantium, Lantana camara, Macaranga tanarius, Cosmos caudatus, Eupatorium inulifolium, Gomphrena globosa, Hibiscus rosa-sinensis* and *Tithonia diversifolia* could be considered as plants that can guarantee the presence of butterfly in Bosscha. The advantages of the presence of butterfly garden in Bosscha area not only giving additional insight for the visitors of Bosscha Observatory but also educating the community on preserving biodiversity and the environment.

ACKNOWLEDGEMENT

We thank to Bosscha Observatory Management (Dr. Taufik Hidayat) for his permission in using the facility. To DGHE (Directorate General for Higher Education), National Education Department for its support to SITH-ITB Bandung.

REFERENCES

Anonymous (2007) Bul Observ Bosscha 4: 1-30.
Boyer T, Polasky S (2004) Valuing urban wetland: a review of non-market valuation studies. Wetland 24: 744-755.
Connor EF, Hafernik J, Levy J, Moore VL, Rickman JK (2003) Insect conservation in an urban biodiversity hotspot: the San Francisco Bay area. J Insect Cons 6: 247-259.
Daily C, Alexander S, Ehrlich PR, Goulder L, Lubchenco J, Matson PA, Mooney HA, Postel S, Schneider SH, Tilman D, Woodwell GM (2002) Ecosystem services: benefit supplied to human societies by natural ecosystems. Issues Ecol 6: 1-18.
Ernanto D (2006) Sinyal SOS bagi Observatorium Bosscha. Harian Umum Sore Sinar Harapan. 26 April 2006.
Harlina (2006) Butterfly management as tourist object in Bantimurung-Bulusaraung National Park (TNB), Kabupaten Maros, Sulawesi Selatan [Thesis]. Bandung Institute of Technology, Bandung. [Indonesian]
Hedblom M (2007) Birds and butterflies in Swedish urban and peri-urban habitats: a landscape perspective [Dissertation]. Swedish University of Agricultural Sciences, Uppsala.
Mueller-Dombois DM, Ellenberg H (1974) Aims and method of vegetation ecology. John Willey and Sons, New York.
Noerdjito WA, Aswari P (2003) Survey method and fauna monitoring: 4th seri butterfly Papilionidae. Division of Zoology, Research Centre for Biology, Indonesian Institute of Science, Bogor. [Indonesia]
Nurcahya A (2003) Plant communiy and its potential in supporting butterfly survival (sub-ordo Rhopalocera) at Tanjung Putus and Pulau Tegal, Teluk Lampung [Undergraduate Thesis]. Bandung Institute of Technology, Bandung. [Indonesian]
Odum EP (1971) Fundamental of ecology. 3rd ed. W.B. Saunders, London.
Preston K, Mafham (2007) 500 butterflies: butterflies from arround the world. Grange Books, Rochester.
Queiroz JM (2002) Host plant use among closely related *Anaea* butterfly species (Lepidoptera, Nymphalidae, Charaxinae). Braz J Biol 62: 657-663.
Roepke W (1932) De vlinders van Java. E. Dunlop & Co, Batavia.
Smart P (1975) The illustrated encyclopedia of the butterfly world. The Hamlyn Publishing Group, London.
Soekardi H (2002) Diversity of Papilionidae in Gunung Betung forest, Lampung, Sumatra: rearing and habitat engineering as baseline for conservation [Dissertation]. Bandung Institute of Technology, Bandung. [Bahasa Indonesia]
Sreekumar PG, Balakrishnan M (2001) Habitat and altitude preferences of butterflies in Aralam Wildlife Sanctuary, Kerala. Trop Ecol 42: 277-281.
Sundufu A, Dumbuya R (2008) Habitat preferences of butterflies in the Bumbuna forest, Northern Sierra Leone. J Insect Sci 8: 64.
Tati-Subahar SS, Amasya AF, Choesin DN (2007) Butterfly (Lepidoptera: Rhopalocera) distribution along an altitudinal gradient on Mount Tangkuban Parahu, West Java, Indonesia. Raff Bull Zool 55: 175-178
Yamamoto N, Yokoyama J, Kawata M (2007) Relative resource abundance explains butterfly biodiversity in island communities. Proc Natl Acad Sci USA 104: 10524-10529.