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Occurrence of Blow Fly Species (Diptera: Calliphoridae) in Phitsanulok Province, Northern Thailand

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Abstract. Based on the current forensic importance of blow flies (Diptera: Calliphoridae), their biological aspects have been studied increasingly worldwide. The blow fly fauna in Phitsanulok Province, Northern Thailand was studied from May 2009 to April 2010 in the residential, agricultural, mountainous and forested areas of Muang, Wat Bot, Nakhon Thai and Wang Thong districts, respectively, in order to know the occurrence of blow flies in this province. Collections were carried out monthly using commercial funnel fly traps and sweeping methods, with 1-day tainted pork viscera as bait. Identification of adult blow flies exhibited 14 634 specimens, comprising of 5 subfamilies, 14 genera and 36 species. *Chrysomya megacephala* (Fabricius, 1794) and *Achoetandrus rufifacies* (Macquart, 1843) were the most and second most abundant species trapped, respectively. These two species of carrion flies prevailed in all the types of land investigated. We calculated and compared the diversity indices, species evenness and richness, and similarity coefficients of the blow fly species in various areas. The data from this study may be used to identify the potential of forensically-important fly species within Phitsanulok Province and fulfill the information on blow fly fauna in Thailand.

INTRODUCTION

Blow flies (Diptera: Calliphoridae) belong to the group of insects that are considered medically-important from many points of view. Adult flies not only annoy humans and economically-important animals, but they are also well-known mechanical carriers of numerous pathogens (Sukontason *et al.*, 2007a). The larvae of blow flies are myiasis-producing agents in humans and livestock, resulting in deterioration of welfare for those who have been infested, and thereby creating economic loss. On the other hand, blow fly larvae can be viewed in a positive sense, as specimens associated with human corpses and entomological evidence in global forensics. Investigations into death include estimation of post-mortem interval, determination of toxic substances, ante-mortem trauma and/or determination of relocation if the remains have been moved (Goff & Odom, 1987; Greenberg, 1991; Anderson, 1995; Byrd & Castner, 2001). Researchers have recorded forensic entomology cases progressively from various parts of the world.

From a forensic entomology perspective, relevant biological data on flies (e.g., life history, morphology, insect succession, developmental rate, distribution etc.) are critical, particularly for forensically-important species, in order to increase accuracy of investigation. Therefore, data from research pertaining to the distribution of blow flies is needed. Distribution of indigenous blow fly fauna has been documented previously for many areas such as California in the United States (Brundage *et al.*, 2011), Spain (Baz *et al.*, 2007), Iran (Khoobdel *et al.*, 2008) and Argentina (Patitucci *et al.*, 2011). As for Thailand, the systematic sampling of blow flies has been conducted in Chiang Mai, northern part of the country, where *Chrysomya megacephala* (Fabricius, 1794) was the most abundant species collected by far outnumbering the common house fly, *Musca domestica* Linnaeus, 1758 (Ngoen-klan *et al.*, 2011). For Phitsanulok, a lower Northern Province of Thailand, information on blow fly distribution and abundance is lacking. Therefore, the objective of this study was to determine blow fly species presence in various types of landscape in Phitsanulok Province. The data obtained from this study would support current information on the blow fly fauna of Thailand and be useful for the application as the entomological evidence in forensic investigations.

MATERIALS AND METHODS

The fly survey was conducted in four types of landscape in four districts of Phitsanulok Province, Northern Thailand. These locations were categorized into four kinds of areas, including residential, forest, agricultural and mountainous, according to where different fly species could be expected, due to ecological portioning of habitat. The locality of each study area was geo-referenced by a Garmine Trex Handheld GPS. The information on each study site is illustrated in Table 1 and Figure 1. Commercial funnel fly traps, the Ezi-Trapä (Thailand) and sweeping insect nets were used for collecting the flies. The bait used in this study was 1- day tainted pork viscera which were composed equally of small pieces of pork intestine, heart, liver, and spleen and kept in a closed plastic box for 1 day at room temperature. Two fly traps were deployed individually at each location about 1 km away from each other. Each one contained 200 g of bait, which was placed on the bottom plate of the trap and left for 2 hours (based on our preliminary data; thus revealing that the highest number of flies in the trap was found in a 2-hour period). Furthermore, 1 kg of 1-day tainted pork viscera was used as a bait for collecting flies with a sweeping net. Flies that landed on the bait, and those found around the study area, were collected. After the 2 hour-collection period, all the traps were kept individually in transparent plastic bags and then transported to the laboratory at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand. All the flies were kept in a refrigerator until identification. Only blow flies were identified based on taxonomic identification keys of Tumrasvin *et al.* (1979) and Kurahashi & Bunchu (2011).

Flies were collected in each site monthly, at 30±7 day-intervals between May 2009 and April 2010. The samples of each species were kept at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand. The diversity index (H') was calculated by using the Shannon-Wiener's Index:

$$H' = -\Sigma_{i=1}^{S} (p_i) \ln(p_i),$$

where S is the total number of species and p_i is the frequency of the i^{th} species (the probability that any given individual belongs to the species, hence p).

Species richness and species evenness were also measured by using the following formulas. Species richness is the number of different species in a given area. It is represented in the above equation as S. The species evenness (J') of a community can be represented by Pielou's evenness index:

$$J' = H' / H'max,$$

where H' is the number derived from the Shannon diversity index and $H'max$ is the maximum value of equal to:

$$H'max = \ln S$$

Table 1. Geo-referenced locality of study sites

Landscape type	Latitude	Longitude	Altitude (m)
1. Agricultural area			
(Wat Bot District)			
– Site 1	17°3'18.48°N	100°23'37.14°E	58
– Site 2	17°3'10.91°N	100°23'38.16°E	59
3. Mountainous area			
(Nakhon Thai District)			
– Site 1	17°0'6.33°N	100°59'7.89°E	1089
– Site 2	17°1'3.95°N	100°58'4.04°E	824
3. Forest area			
(Wang Thong District)			
– Site 1	16°51'48.53°N	100°31'13.49°E	269
– Site 2	16°51'48.57°N	100°31'14.45°E	238
4. Residential area			
(Muang District)			
– Site 1	16°43'51.81°N	100°11'42.07°E	45
– Site 2	16°43'38.91°N	100°12'1.99°E	45

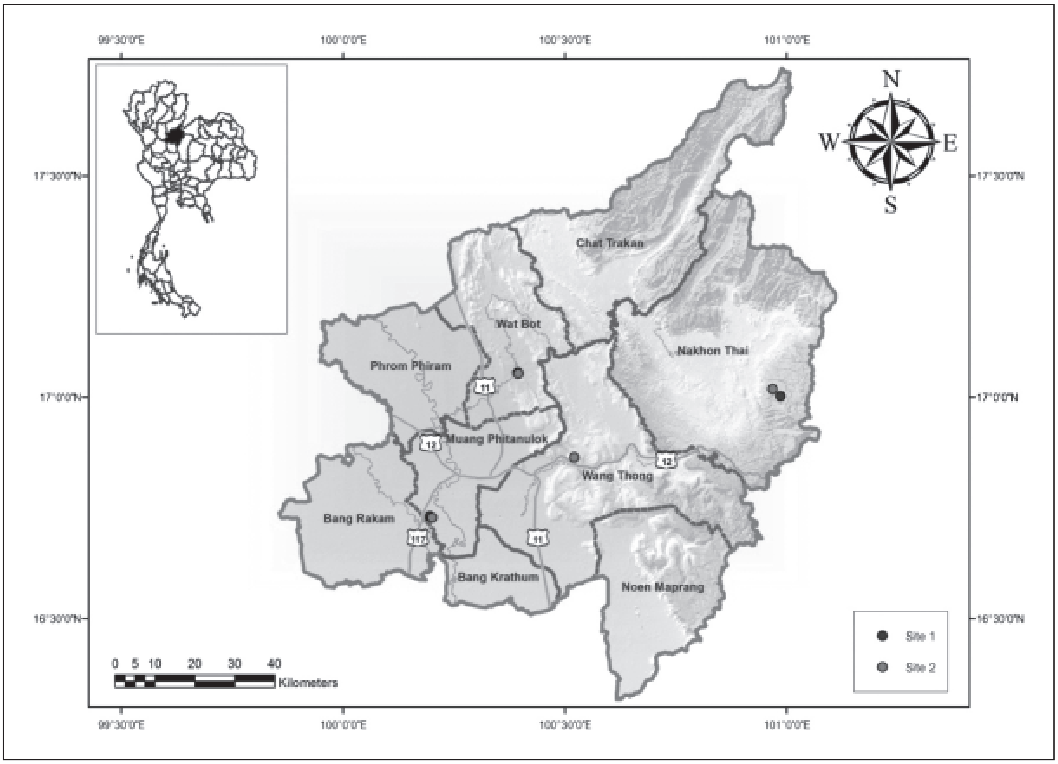

Figure 1. Location of study sites for the collection of blow flies in four districts (Muang, Nakhon Thai, Wang Thong, and Wat Bot of Phitsanulok Province, Thailand)

The similarity of blow fly species was compared by using the Jaccard index (*Cj*), and the similarity coefficients of the blow fly species in the study areas were compared by using the following formula:

$$Cj = a / (a + b + c)$$

where a is the number of species found in both areas, b or c is the number of species found in one specific area, but not in another. The similarity coefficients indicate the co-occurrence of blow fly species in both study areas.

RESULTS

A total of 14 634 blow flies were collected over a one-year period, from which the main climatic parameters recorded at Phitsanulok Meteorological Station are presented in Figure 2. The identified blow flies comprised of 36 species of 5 subfamilies, of which a considerable number of Chrysomyinae was captured, followed by Luciliinae, Rhiniinae, Phumosiinae, and Bengaliinae, consecutively (Table 2). Regarding Chrysomyinae, *C. megacephala* formed the majority of species identified, followed by the hairy maggot fly *Achoetandrus rufifacies* (Macquart, 1843). Other species from this subfamily comprised *Achoetandrus villeneuvi* (Patton, 1922), *Ceylonomyia nigripes* (Aubertin, 1932), *Chrysomya chani* Kurahashi, 1979, *Chrysomya pinguis* (Walker, 1858) and *Chrysomya thanomthini* Kurahashi & Tumrasvin, 1977. As for the second subfamily Luciliinae, *Hemipyrellia ligurriens* (Wiedemann, 1830) showed the peak number in this group, followed by the flies in the genus *Lucilia* such as *Lucilia cuprina* (Wiedemann, 1830), *Lucilia papuensis* Macquart, 1842, *Lucilia porphyrina* (Walker, 1857) and *Lucilia sinensis* Aubertin, 1933. Small numbers of *Hemipyrellia pulchra* (Wiedemann, 1830), *Hypopygiopsis infumata* (Bigot, 1877), and *Hypopygiopsis tumrasvini* Kurahashi, 1977 were also found. Identification in the subfamily Rhiniinae revealed that *Stomorhina discolor* (Fabricius, 1794) was

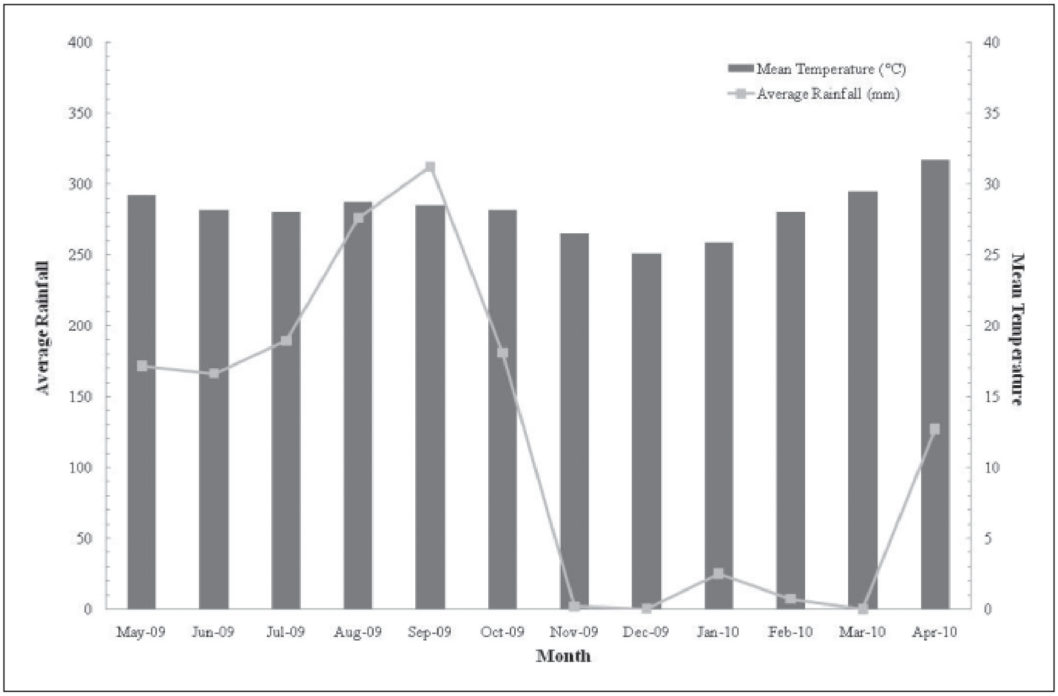

Figure 2. Monthly average rainfall and monthly mean temperature of Phitsanulok Province, Thailand, during fly collections from May 2009 to April 2010

Table 2. Blow fly species in residential, agricultural, mountainous, and forest areas of Phisanulok Province, Thailand (May 2009 – April 2010)

Species	Occurrence of blow fly species				Total
	Residential areas	Agricultural areas	Mountainous areas	Forest areas	
Subfamily Bengaliinae					
Bengalia labiata (Robineau-Desvoidy, 1830)	0	0	0	1	**1**
Subfamily Luciliinae					
Hemipyrellia ligurriens (Wiedemann, 1830)	666	349	3	55	**1 073**
Hemipyrellia pulchra (Wiedemann, 1830)	0	26	0	17	**43**
Hypopygiopsis infumata (Bigot, 1877)	0	0	4	7	**11**
Hypopygiopsis tumrasvini (Kurahashi, 1977)	0	0	1	0	**1**
Lucilia cuprina (Wiedemann 1830)	1	0	0	0	**1**
Lucilia papuensis (Macquart, 1842)	0	6	10	49	**65**
Lucilia porphyrina (Walker, 1857)	0	0	19	1	**20**
Lucilia sinensis (Aubertin, 1933)	0	0	4	17	**21**
Subfamily Phumosiinae					
Phumosia indica (Surcouf, 1914)	0	9	0	11	**20**
Phumosia testacea (Senior-White, 1923)	0	1	0	1	**2**
Subfamily: Chrysomyinae					
Achoetandrus rufifacies (Macquart, 1843)	1 108	656	400	1 196	**3 360**
Achoetandrus villeneuvi (Patton, 1922)	0	11	234	3	**248**
Ceylomonyia nigripes (Aubertin, 1932)	0	36	274	132	**442**
Chrysomya chani (Kurahashi, 1979)	0	6	44	34	**84**
Chrysomya megacephala (Fabricius, 1794)	3 434*	1 639*	1 581*	1 614*	**8 268***
Chrysomya pinguis (Walker, 1858)	0	0	635	0	**635**
Chrysomya thanomthini (Kurahashi & Tumrasvin, 1977)	0	0	24	0	**24**
Subfamily: Rhiniinae					
Cosmina biplumosa (Senior-White, 1924)	0	0	1	0	**1**
Cosmina limbipennis (Macquart, 1848)	0	0	0	2	**2**
Isomyia spp.	0	0	23	3	**26**
Isomyia versicolor (Bigot, 1877)	0	0	2	6	**8**
Isomyia pseudoviridana (Peris, 1952)	0	0	1	1	**2**
Isomyia cupreoviridis (Malloch, 1928)	0	0	1	1	**2**
Isomyia viridaurea (Wiedemann, 1819)	0	0	2	0	**2**
Isomyia oestracea (Se'guy, 1934)	0	0	1	0	**1**
Isomyia singhi (Kurahashi et Thapa, 1994)	0	0	2	0	**2**
Isomyia facialis (James, 1970)	0	0	5	0	**5**
Rhyncomya flavibasis (Senior-White, 1922)	0	0	5	1	**6**
Idiella divisa (Walker, 1861)	0	0	3	0	**3**
Idiella euidielloides (Senior-White, 1923)	0	0	1	0	**1**
Stomorhina spp.	0	2	4	0	**6**
Stomorhina discolor (Fabricius, 1794)	0	35	96	113	**244**
Stomorhina melastoma (Wiedemann, 1830)	0	0	2	0	**2**
Stomorhina siamensis (Kurahashi & Tumrasavin, 1992)	0	0	3	0	**3**
Strongyloneura prolata (Walker, 1860)	0	0	1	0	**1**
Total	**5 209**	**2 776**	**3 386**	**3 265**	**14 636**

* = Predominant species of that area

presented in considerable numbers, when compared to other species in this group - *Idiella divisa* (Walker, 1861), *Idiella euidielloides* Senior-White, 1923, *Cosmina limbipennis* (Macquart, 1848), *Cosmina biplumosa* (Senior-White, 1924), *Isomyia versicolor* (Bigot, 1877), *Isomyia cupreoviridis* (Malloch, 1928), *Isomyia viridaurea* (Wiedemann, 1819), *Isomyia pseudoviridana* (Peris, 1952), *Isomyia oestracea* (Se'guy, 1934), *Isomyia facialis* James, 1970, *Isomyia singhi* Kurahashi & Thapa, 1994, *Isomyia* spp., *Rhycomya flavibasis* (Senior-White, 1922), *Stomorhina siamensis* Kurahashi & Tumrasavin, 1992, *Stomorhina melastoma* (Wiedemann, 1830), *Stomorhina* spp., and *Strongyloneura prolata* (Walker, 1860). For subfamily

Phumosiinae, *Phumosia indica* (Surcouf, 1914) and *Phumosia testacea* (Senior-White, 1923) were found in agricultural and forest areas. Only one specimen of *Bengalia labiata* Robineau-Desvoidy, 1830, belonging to subfamily Bengaliinae, was found in forest areas.

Specific examination of species in each particular landscape indicated that *L. cuprina* was only found in the residential areas, while *B. labiata* and *C. limbipennis* were collected only in forests. Furthermore, 13 species were only captured in the mountainous areas, including *H. tumrasvini*, *C. pinguis*, *C. thanomthini*, *C. biplumosa*, *I. viridaurea*, *I. oestracea*, *I. singhi*, *I. facialis*, *I. divisa*, *I. euidielloides*, *S. siamensis*, *S. melastoma*, and *S. prolata*. Interestingly, infestations of red mites were only observed in some specimens of *S. discolor* from the mountainous areas (Figure 3).

Analysis through the biodiversity indices, species richness and evenness index of blow fly specimens collected in Phitsanulok Province revealed that the greatest richness of species (30) was found in the mountainous areas of Nakhon Thai district (H'=1.69). Fly species collected in the forest, agricultural and residential areas showed a lower biodiversity index, consecutively (Table 3). The highest of species evenness was found in the residential areas (J'=0.63) and lower species evenness was found in mountainous, agricultural, and forest areas, consecutively (Table 3).

Monthly assessments of the four land types, investigated in this study showed that the highest value of species richness indices was observed in August of 2009 (16 in mountainous and 9 in agricultural areas, Table 4). As for forest and residential areas, the highest numbers of species were found in October of 2009 and March of 2010, respectively (Table 4). Comparing median of monthly occurrence of blow fly species found throughout the year, mountainous areas showed the highest number of species, followed by forest, agricultural and residential areas, consecutively.

The degree of similarity of the blow fly species in four study areas is demonstrated in Table 5. The most similarity was found in agricultural and forest areas (Cj = 0.50), while residential and mountainous areas had the least similarity coefficient (Cj = 0.10).

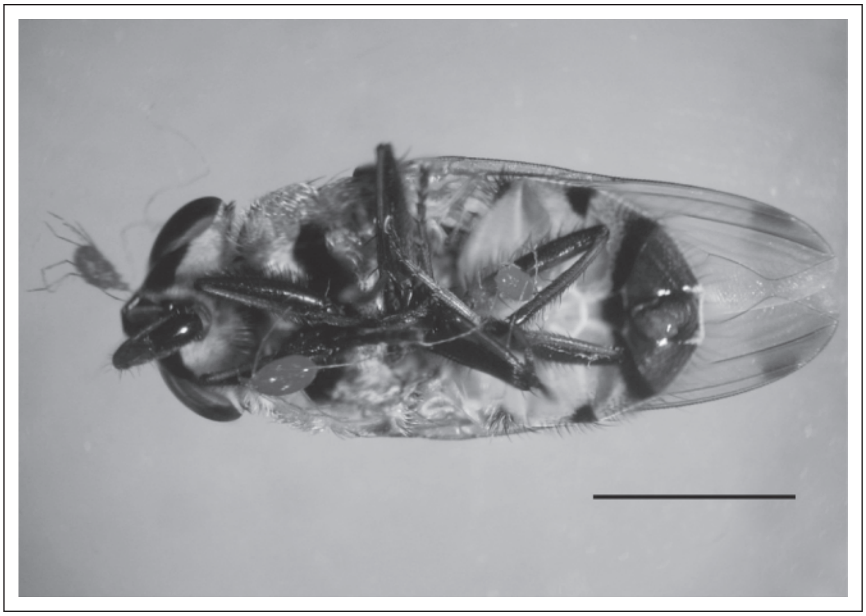

Figure 3. *Stomorhina discolor* infested with red mites (Arachnida: Acari), collected from mountainous areas of Nakhon Thai district, Phitsanulok Province, Thailand. (Bar = 2 mm)

Table 3. Biodiversity indices of blow fly species in residential, agricultural, mountainous, and forest areas of Phisanulok Province, Thailand (May 2009 – April 2010)

Study areas	No. of Genus	No. of Species	Shannon index	Species richness	Evenness index
Residential areas	4	4	0.87	4	0.63
Agricultural areas	7	12	1.14	12	0.46
Mountainous areas	12	30	1.69	30	0.49
Forest areas	12	21	1.27	21	0.42

Table 4. Monthly occurrence of blow fly species in residential, agricultural, mountainous, and forest areas of Phisanulok Province, Thailand (May 2009 – April 2010)

Year	Month	Occurrence of blow fly species			
		Residential areas	Agricultural areas	Mountainous areas	Forest areas
2009	May	3	6	6	8
	June	3	6	11	9
	July	3	8	15	10
	August	3	9	16	10
	September	3	8	11	9
	October	3	8	8	11
	November	3	7	5	7
	December	3	6	8	5
2010	January	3	5	9	8
	February	3	3	10	7
	March	4	3	9	4
	April	2	4	6	5
	Median	**3**	**6**	**9**	**8**

Table 5. Similarity coefficients of blow fly species in residential, agricultural, mountainous, and forest areas of Phisanulok Province, Thailand (May 2009 – April 2010)

Study areas	Similarity coefficient (*Cj*)			
	Residential	Agricultural	Mountainous	Forest
Residential	1	0.23	0.10	0.14
Agricultural	0.23	1	0.28	0.50
Mountainous	0.10	0.28	1	0.47
Forest	0.14	0.50	0.47	1

DISCUSSION

This investigation was the first field research attempting to determine blow fly fauna in Phitsanulok Province, Northern Thailand. Based on the methodology and results obtained from this study, at least 36 calliphorid fly species were recorded in the Phitsanulok area, which represented approximately 38.71% (36/93) of the total blow fly species recently updated in Thailand (Kurahashi & Bunchu, 2011; Bunchu, 2012). New species or new recorded species of Thailand may be found among some specimens of genus *Isomyia* females which could not be identified due to the limitation of morphological taxonomic keys used in this study. Therefore, alternative tools for identification are needed and should be developed further. In this study, *C. megacephala* proved significantly to be the predominant species in the four distinct habitats studied, followed by *A. rufifacies*. Both species had numerous similarities in all land use types investigated, i.e., residential, agricultural, mountainous and forest areas. This information confirmed earlier reports on these two most predominant blow fly species existing in Thailand (Lertthamnongtham *et al.*, 2003; Ngoen-klan *et al.*, 2011), which are also the most forensically important species for this country (Sukontason *et al.*, 2007b). In addition, several other species including *H. ligurriens*, *L. cuprina*, *C. chani*, *C. pinguis*, *A. villeneuvi* and *C. nigripes* were also found to be associated with human corpses in Thailand (Sukontason *et al.*, 2007b) and Malaysia (Lee, 1996; Lee *et al.*, 2004; Syamsa *et al.*, 2010).

Biodiversity indices, monthly occurrence of blow fly species, and similarity coefficients from this study indicated that composition of fly species was different by habitat, and season which is in agreement with several previous reports (Baz *et al.*, 2007; Mulieri *et al.*, 2011; Ngoen-klan *et al.*, 2011). In this study, the least species diversity was observed in the residential areas, which agrees with the data of Mulieri *et al.* (2011). Urbanization is one of the important factors influencing the diversity of blow fly species as found for sarcophagid communities in Buenos Aires, Argentina (Mulieri *et al.*, 2011). On the other hand, the highest evenness index found in residential areas may be due to the successful adaptability of all blow fly species captured in these areas for colonizationin human-modified environment. During the study period of 2009 to 2010, the weather in Phitsanulok Province fluctuated greatly (Figure 2) and the environment of the study sites was subjected to natural disasters such as forest fires and drought. The changing weather in the environment might be due partially to global warming, and this may affect changing species composition in the study areas. Ferraz *et al.* (2010) demonstrated that the climate influenced the abundance and richness of blow fly species in a forest fragment in the Tinguá Biological Reserve, Brazil. Likewise, gradual warming of the climate was important in causing changing arthropod faunal communities rapidly (Turchetto & Vanin, 2004). This phenomenon has been examined rarely in blow flies. Regarding this, the consequence of global warming on the biodiversity of blow fly species, particularly those affecting human involvement, merits further investigations.

Some blow fly species were only found in specific areas: *L. cuprina* was specific to residential areas, *H. tumrasvini*- to mountainous areas, and *B. labiata*- to forest areas. This finding may be due to the biology of each blow fly species which prefers biotic and abiotic factors in the environment differently as found for sarcophagid flies (Mulieri *et al.*, 2011). Moreover, some of these flies, *C. pinguis*, *C. thanomthini*, *C. chani*, *A. villeneuvi* and *C. nigripes*, were collected predominantly in the specific areas - at high altitude in mountainous and forested habitats. This was in line with the previous field surveys in Chiang Mai Province, Northern Thailand (Sukontason, 2010). On the other hand, results in this study showed that *H. ligurriens* occurred predominantly in the low land of residential and agricultural areas at approximately 45 to 60 meters above sea level, instead of the high altitudes of 500 to 2 667 meters previously documented in Chiang Mai (Tumrasvin *et al.*, 1978; 1979). An explanation for the distinction of this fly species among highland areas in two provinces is still unknown; therefore, more studies should be done in the future.

Our results indicated that the biodiversity of blow fly species in all areas was different ($Cj \leq 0.50$). Nevertheless, the blow fly species, found and recorded in particular areas, may be useful in the future for determining corpse relocations by identifying what species could be mostly related to medico-legal cases. The similarity coefficients (*Cj*) of blow fly species presented herein are significant for indicating co-occurrence of species in particular areas. For example, the chance of finding co-occurrence of blow flies in the forest and residential areas (*Cj* = 0.13) is lower than that for the same species in the agricultural and residential areas (*Cj* = 0.50). This information may provide supportive evidence to decision what species could be mostly related to the case and could be used as the evidence for determining corpse relocations further if some alien species were found in these areas. However, the larvae of unproven forensically important species need to be collected from carrions in these study areas and identified carefully in the future to confirm their importance in forensics.

Although only three species (*C. megacephala*, *A. rufifacies*, and *L. cuprina*) of total blow fly species collected in this study have previously been documented as medically-important species, they play a significant role being mechanical carriers of pathogens, parasite eggs, protozoa cysts and arthropods, causing myiasis, and they also can be applied for maggot debridement therapy (Monzon *et al*., 1991; Sukontason *et al*., 2000; Sulaiman *et al*., 2000; Sukontason *et al*., 2005; Fetene & Worku, 2009; Tantawi *et al*., 2010; Ghosh *et al*., 2011; Chaiwong *et al*., 2012; Srivoramas *et al*., 2012). *Chrysomya megacephala*, the most abundant species in all study areas, was considered to have greater epidemiological importance in Pelotas, Brazil (Vianna *et al*., 1998), and to be more potentially dangerous for public health than *M. domestica* in Malaysia and Thailand (Monzon *et al*., 1991; Sukontason *et al*., 2000). Fetene & Worku, (2009) reported that *A. rufifacies* had more potential to transmit helminths than other flies. The larvae of *A. rufifacies* were also shown to be the cause of human myiasis in Thailand (Sukontason *et al*., 2005). *Lucilia cuprina* was only captured in residential area of Muang district which is in agreement with study of Vianna *et al*. (1998) who demonstrated that this species showed the highest synanthropic index (eusynanthropic species). As the population in our study areas was very low, currently *L. cuprina* is not considered to be a epidemiologically important species. However, it was shown to be a mechanical carrier as was found in Ethiopia (Fetene & Worku, 2009). Certainly, the programs to control these filth flies should be planned in all areas, especially in residential areas, to prevent outbreaks of diseases due to their ability in spreading of pathogens. In addition, maggots of *L. cuprina* have already been used for maggot debridement therapy instead of *Lucilia sericata* (Meigen, 1826) in Malaysia and Egypt (Paul *et al*., 2009; Tantawi *et al*., 2010). This application of *L. cuprina* should be implemented further.

In this investigation, a minimal number of blow flies in the subfamily Rhiniinae, was captured only in the mountainous areas in Nakhon Thai district. *Stomorhina discolor* was the predominant species collected in subfamily Rhiniinae, in which most species were rare with limited information about their biology and ecology. Some specimens of *S. discolor* examined showed infestation with red mites (Figure 3). Whether these mites were ectoparasites of this particular species or just use this fly as a means of transport (phoresy) is still questionable, due to limited information on their behaviors. Dear (1977) showed that *S. discolor* adults have been often found on flowers and had a hovering habit, while the larvae have been documented as predaceous maggots (Kurahashi, 1982). The flies of other genera in this subfamily were sometimes observed feeding on flowers. Our careful observation during fly surveys has indicated that *Isomyia* flies like to stay around aromatic flowers of the genus *Lantana* which emits a strong odor. This is in agreement with the report of Kurahashi & Bunchu (2011), who recorded that many blow species of *Isomyia* were found on the blossoms.

In this study, the identification of blow flies was based solely on morphological characteristics, which can be done only by an expert entomologist. Therefore, this kind of identification may be impractical for inexperienced personnel, such as medical or forensic staff. In this regard, a simple pictorial taxonomic key of medically- and forensically-important blow flies of Thailand should be developed in the future. Furthermore, periodical surveillance of blow flies should be continued in view of the fact that fluctuating climatic changes may affect fly communities and their behaviors and eventually involve the human ecosystem.

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