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Reproductive Biology of *Fopius vandenboschi* (Fullaway) (Hymenoptera: Braconidae) in the Laboratory

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Abstract

Fopius vandenboschi (Fullaway) is one of the natural enemies of oriental fruit flies in genus *Bactrocera*. It is a koinobiont solitary endoparasitoid that parasitizes first and second instars of oriental fruit flies. The advantages of this parasitoid are the ability to parasitize several host species and the length of female longevity. Reproductive biology of *F. vandenboschi* in this research showed that it produced average 21.0±13.4 offspring per female, equivalent to net reproductive rate of 8.4 female offspring per generation. The sex ratio is equal to 0.6 : 0.4 ($\male$: $\female$) and the mean generation time of is 22.5 days. These results can be applied for mass-rearing production of *F. vandenboschi* for inoculative and inundative release to enhance management populations of *Bactrocera* fruit fly pest in Thailand.

Keywords: *Fopius vandenboschi* (Fullaway), *Bactrocera*, Reproductive biology, Natural enemies, Mass-rearing

1. Introduction

Parasitoid is an insect whose larva living, developing, feeding on its host tissue and finally kills the host at eclosion. Although larva of parasitoid is a parasite, adult parasitoid is free-living feeds on honeydew, nectar and pollen (Koul & Dhaliwal, 2003). The categorization of parasitoids can be done in several ways such as classified according to stage of host as egg, larval, pupal or adult parasitoid; classified according to the number of its egg in host as solitary or gregarious parasitoid or may be classified according to its larval feeding behavior as endoparasitoid or ectoparasitoid. Parasitoid is one of the natural enemies that frequently use in biological control program which has been successively researched and developed for more than 100 years. This concept can be used for control or reduce pest populations, in addition it is safe for agriculturists, consumers and environment (Clausen, 1978; Clausen, Clancy, & Chock, 1965).

In Thailand, tephritid fruit flies in the genus *Bactrocera* are considered as important pests because they can cause serious damage to economic fruits (Department of Agriculture Thailand, 2003a; Waterhouse, 1993). Moreover, some species such as the oriental fruit fly, *Bactrocera dorsalis* (Hendel) has resistance to pesticides and wide host range (Vontas et al., 2011) that makes it difficult to control. Therefore, the biological control program using parasitoids has been widely used to manage this fruit fly species. The important parasitoid species in Thailand are

Diachasmimorpha longicaudata (Ashmead), *Fopius arisanus* (Sonan) and *Fopius vandenboschi* (Fullaway) (Hymenoptera: Braconidae) (Vargas et al., 2012). *F. vandenboschi* is a solitary endoparasitic wasp that parasitizes first and second instars (Van Den Bosch & Haramoto, 1953) of several fruit fly pests (Diptera : Tephritidae) such as, *Ceratitis capitata* (Wiedemann), *Bactrocera cacuminatus* (Héring), *Bactrocera dorsalis* complex, *Bactrocera correcta* (Bezzi)*, Bactrocera latifrons* (Hendel)*, Bactrocera pedestris* (Bezzi)*, Bactrocera tryoni* (Froggatt) and *Carpomya vesuviana* Costa (Chinajariyawong et al., 2000; Wharton & Gilstrap, 1983). It distributes in Indo-Pacific region from Pakistan through Taiwan including Thailand (Wharton & Gilstrap, 1983). The advantages of *F. vandenboschi* are the ability to parasitize various tephritid fruit fly pest species and the longer female lifespan than any other related parasitoids. Combined with the high capability of female parasitoid in host searching and the ability to survive under unsuitable environmental conditions and high competition with other parasitoids (Ramadan, 2004; Ramadan, Wong, & Messing, 1995), *F. vandenboschi* is a remarkable parasitoid for application in biological pest control programs.

The augmentation is one of the key techniques in the processes of biological control, by increasing in the numbers of natural enemies in agricultural area (Department of Agriculture Thailand, 2003b). This strategy enhances the efficacy and achievement of pest control. Augmentation requires understanding

The biology of that particular natural enemy especially reproductive biology. Thus, this study was aimed to provide the useful information about the reproductive biology of *F.vandenboschi* in laboratory for mass-rearing production and used in inoculative or inundative release of this parasitoid species which will allow for effective pest control of *Bactrocera* fruit flies in Thailand.

1. Materials and Methods

2.1 Tephritid fruit flies and parasitoids

B. dorsalis were obtained from Plant Pest Management Research Group, Department of Agriculture Thailand and maintained in laboratory at Department of Biology, Faculty of Science, Burapha University, Chon Buri Campus. The adult fruit flies were reared in ventilated plastic container with 26.5 cm diameter and 27 cm high, provided a 10% honey and yeast extract powder as food source.

F. vandenboschi was collected from ripening java apple (*Syzygium samarangense*) infested with tephritid fruit fly larvae from Bangkok, Thailand in 2013. Fruits were placed in containers until larvae developed into puparia and emerged. Adult parasitoids were identified using keys published by Wharton and Gilstrap (1983) and reared in ventilated plastic container with 26.5 cm diameter and 27 cm high provided a 10% honey as food source. Both parasitoid and host fly (*B. dorsalis*) were maintained under laboratory condition at 27 ± 2 °C, with 70 ± 10% RH and a photoperiod of 12L:12D. The voucher specimens of parasitoid and host fly were kept at Department of Biology, Faculty of Science, Burapha University, Chon Buri Campus.

2.2 Reproductive biology of *F. vandenboschi*

In order to obtain virgin *F. vandenboschi* of the same age, parasitized *B. dorsalis* puparia were isolated individually in 1.5 ml microtube and examined under stereo microscope few days before parasitoid emergence when parasitoid pupa can be seen inside the host puparium. Paired three days old sexually mature male *F. vandenboschi* (Hagen, 1953) with one day old female in ventilated plastic box (11x11x6.5 cm), allowed to mate for 24 h. The experiments were conducted for 40 pairs. The reproductive biology of *F. vandenboschi* was tested on the first instar *B. dorsalis*. Each pair of mated *F. vandenboschi* was provided with 100 larvae in mashed ripe banana packed in a modified oviposition unit (4.5x5.5x1 cm plastic box with nylon lid). The oviposition unit was exposed to parasitoid for oviposition for three days until the larvae turn into third instar, and then take the oviposition unit out. Replaced with the new unit

twice which will be correspond to age at peak oviposition of *F. vandenboschi* (8.4 ± 1.3 days) (Ramadan et al., 1995). The exposed larvae were reared until adult fruit flies and parasitoids emerged, counted the number and calculated as the average number of offspring and net reproductive rate. The date that first female offspring emerged were averaged as the mean generation time of *F. vandenboschi*.
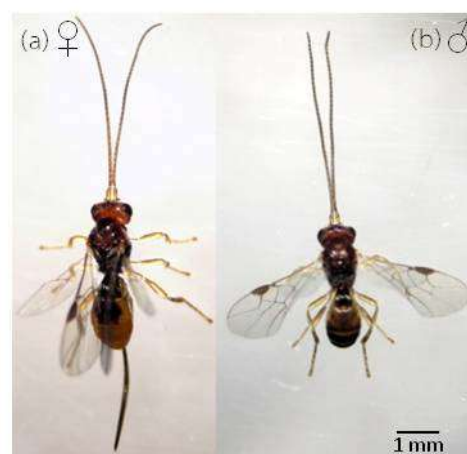
Figure 1. *Fopius vandenboschi* (Fullaway); (a) female, (b) male.

2. Results

The reproductive biology of *F. vandenboschi* in laboratory condition examined from 40 pairs of parasitoid was shown in Table 1. The average number of both male and female offspring that female parasitoid produced was 20.1±13.4 offspring. The progeny sex ratio was more biased toward male (502 offspring) than female (337 offspring). The net reproductive rate was 8.4 female offspring per generation and this parasitoid species required 22.5 days for new female offspring production.

Table 1. Reproductive parameters of *Fopius vandenboschi*.

Average number of offspring produced per female (±SD)	Sex ratio (♂ : ♀)	Mean generation time (days)*	Net reproductive rate (per generation)*
21.0±13.4	0.6 : 0.4	22.5	8.4

* according to Vargas et al. (2002)

3. Discussion and Conclusions

F. vandenboschi is a parasitoid of important tephritid fruit fly species in tropical and subtropical areas (Chinajariyawong et al., 2000; Wharton &

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Gilstrap, 1983). It reproduces by arrhenotokous parthenogenesis (Heimpel & De Boer, 2008; Shaw & Huddleston, 1991), which unfertilized eggs (n) develop to males and fertilized eggs (2n) develop to females. Consequently, female parasitoid can specify sex of its offspring as male or female based on the host size at time of oviposition (Godfray, 1994). So, female parasitoid plays a major role in biological control as the parasitized agent. In addition, it also has roles in increasing and maintaining parasitoid population in the level that can effectively control the pest fly (Stouthamer, Luck, & Werren, 1992). In this study, *F. vandenboschi* had average parasitization rate of 21.0% similar to the study on mass-rearing of Ramadan (2004) that the parasitization rate was in the range of 14.9-33.6% and occurred at peak rate when female parasitoid was 11 to 15-day-old. Ramadan et al. (1995) and Vargas et al. (2002) also reported that the average total number of eggs laid by female *F. vandenboschi* during lifetime were 33.3-34.2 eggs/female. Therefore, the number of *F. vandenboschi* offspring in this study was lower than the number of its eggs. This may due to the difference in population size between this and those experiments which influence mating behavior of *F. vandenboschi*. In general, insect is a social animal that need social stimulation to increase breeding success which involve population growth (Bompard, Amat, Fauvergue, & Spataro, 2013). Accordingly, the mass production of parasitoid should be reared in large population to increase breeding rate and increase chance to obtain female offspring.

The net reproductive rate of *F. vandenboschi* in this study, which determined from number of female offspring, was consistent with Vargas et al. (2002). Although the reproductive rate of *F. vandenboschi* was the lowest compared to other related fruit fly parasitoids (Vargas et al., 2002), it was found that female longevity of *F. vandenboschi* was the longest (26-30 days) (Ramadan, 2004). In addition, its mean generation time (22.48 days) was shorter than its lifespan. These turned into the advantages of this parasitoid species because the presence of female parasitoid in environment in long period of time will increase chance of parasitism. However, *F. vandenboschi* has a unique reproductive characteristic that is a newly emerged male required a premating period to reach sexual maturity before being ready to mate (Hagen, 1953). Thus, to ensure that male parasitoid will emerge first and mature before female emergence, female *F. vandenboschi* usually laid unfertilized eggs in the early phase of its lifespan and switched to lay more fertilized eggs when it was older (Ramadan, Wong, & Wong, 1991). In this experiment, *F. vandenboschi* was allowed to lay egg

until it was 11-day-old which Ramadan (2004) reported that it was in the period of the highest oviposition and the highest number of female offspring production. The result in this study then showed the bias sex ratio of offspring toward male (60%) whereas Ramadan (2004) and Vargas et al. (2002) presented the sex ratio in their experiment was bias toward female (56-57%). This indicates the significance of the age interval after the 11-day-old of this parasitoid species that has high impact on the increasing number of female offspring. Consequently, the mass-rearing production of *F. vandenboschi* should be used mated female aged over 11 days (Ramadan, 2004) which will produce more female offspring to use as effective agent in inoculation and inundation biological control.

The research on reproductive biology of *F. vandenboschi* can be further used in mass-rearing production for augmentation in tephritid fruit fly control. In conclusion, selecting appropriate age of the breeders should be done to obtain large number of female offspring. In addition, the application of *F. vandenboschi* with other related parasitoid species such as *D. longicaudata* that attack late instars tephritid fruit fly will establish complex interaction among parasitoids. It will help facilitate the effective and success biological control.

4. Acknowledgements

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Species Diversity of Aquatic Fauna in Seagrass at Rockgarden Village, Rayong Province

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Abstract
The diversity of aquatic fauna such as fish, shrimp and crab behind Rockgarden Village in Kleang District, Rayong Province by surveying and collecting samples were in two stations of different habitat types i.e. inside and outide seagrass. The collection sampling of aquatic fauna using small beach embankment carried out 3 times in November 2016, January and March 2017 and three replications in each time. The samples were collected by dragging net with 2 mm mesh size at 1-1.2 m of a depth level. The total faunal species comprised 24 species from 20 families, 5 orders and 2 classes as Actinopterygii in fish group with 4 orders i.e. Perciformes (13 families, 17 genera and 16 species), Syngnathiformes (1 family, 1 genera and 1 species), Atheriniformes (1 family, 1 genera and 1 species) and Tetraodontiformes (2 families, 2 genera and 2 species) and Malacostraca in crab and shrimp with 1 order as Decapoda (3 families, 4 genera and 4 species). The 22 species (18 fish species, 3 crab species and 1 shrimp species) were found inside seagrass while 15 species (11 fish species, 3 crab species and 1 shrimp species) were found outide seagrass. The fish group was the most abundance and then was the crab and shrimp group. Family of fish which is the most abundance in this area was Siganidae. The diversity index of aquatic fauna was inside seagrass as 1.042 and outside seagrass as 0.188. This survey indicated that the inside seagrass area was more abundant aquatic fauna than the outside seagrass area and was important in habitat, nursery ground and food source of aquatic fauna.

Keywords: Species diversity, Aquatic fauna, Seagrass, Rockgarden Village

1. Introduction

Seagrasses are plants that spread along the coast and can grow well in shallow water with sunlight. Scientists believed that the indigenous plants which originate from single-celled organisms called diatoms that live in the sea. The evolution of land plants such as mosses, ferns and cycads, which are very high in the evolution of dinosaurs decreased. Land plants developed the most advanced angiosperms (flowering plants) several million years ago. A group of flowering plants, seagrass special group only developed down to the sea. Seagrass species widely distributed in shallow, coastal waters worldwide in tropical and subtropical region. They can reproduce by rhizome and sexual reproduction with flowers. When the female flowers were fertilized, they will be developed as fruits which are within the seeds and spread on other area (Department of Marine and Coastal Resources [DMCR], 2017).

Rockgarden Village in Kleang District, Rayong Province is a village with seagrass area behind the village is a large 3 in Rayong. The total area is about 1,029,375.874 square meters or approximately 643 acres (DMCR, 2017). Seagrass ecosystems are vital to the coast because there are so many living creatures, including the grass itself. The creatures have adapted well in water that has been up and down all the time (Intharasook, 1999; Vongpanich, Sinanan, & Chanasakulniyom, 2008). They are a role as a source of refuge and feeding habitat for aquatic species to spawning and larvae of fish to strength before heading out to sea or coral reef, especially fish, shrimp, crab and shell, including rare sea creatures like turtles and dugongs (Dolar, 1991; Upanoi, 2005; Wanna & Daungdee, 2017). Moreover, seagrass also helps to filter water and improves water quality. The root system of seagrass allows the adhesive to prevent the soil erosion, so they are the important

ecosystem. The seagrass in the area tends to deteriorate and declines by human activity, in particular, the construction of the jetty, fishing in the seagrass, business travel and littering or wastewater discharge into the sea lead to sedimentation causes damage to seagrass, so seagrass ecosystems were imbalance. In addition, impacts to the bay from increasing population and industrial development of the Tampa Bay area resulted in large seagrass reduction (Johansson & Greening, 2000).

From the above mentioned problems, causing pollution of the environment affects to changes in coastal seagrass beds impact on diversity and abundance of aquatic life. This research studied in seagrass area around Rockgarden Village in Rayong Province for the study of aquatic biodiversity and abundance to know the status of aquatic fauna in the seagrass and impact to make everyone aware of the importance of seagrasses in terms of the habitats of aquatic larvae, hidden sources of aquatic fauna which lead to the restoration plan and sustainable solutions in the future.

2. Materials and Methods
2.1 The survey and sampling of aquatic fauna

Survey samples were collected from the second station is inside and outside of seagrasses behind Rockgarden Village in Kleang District, Rayong Province, including all three times in November 2016, January and March 2017 because this time period changes the season beteween winter to summer, three replications for 10 min in each replication at 1-1.2 m of a depth level by dragging net over a small embankment. Each net size of 5 m width, 1.5 m height, 7 m length and 2 mm mesh size (modified from Vongpanich, Sinanan, & Chanasakulniyom, 2008). The Kuicheai seagrass (*Haludule uninervis*) is the dominant seagrass species in the survey area. Using satellite maps compared to the actual location. Then explore the area on a map as well as Geocoding and maintain a living example by freezing to be identified in the laboratory.

2.2 Identification of aquatic fauna species

Samples were stored under the classification taxonomy according to the manual classification of aquatic fauna species in the Andaman seagrass (Vongpanich, 2006), Invertebrates in the Songkhla Lake Basin (Mardnui & Plathong, 2009b), Fish in the Songkhla Lake Basin (Mardnui & Plathong, 2009a) and www.marinespecies.org (World Register of Marine Species, 2014). This is based on external morphological characteristics of shape, pattern classification by fish and other landmarks. The group classified by the shape of a crab carapace and shapes and classified shrimp with green shrimp, a sort of articulate and rudders.

Figure 1. Aquatic fauna surveying area behind Rockgarden Village in Kleang District, Rayong Province; green pin is inside seagrass area and black pin is outside seagrass area. (Google Earth, 2016).

2.3 Data analysis
2.3.1 Species diversity index

Used to calculate the Shannon-Weiner Diversity Index according to Washington (1984), calculated using the following formula

$$H = -\sum_{i=1}^{s} [p_i * (\ln p_i)]$$

H = Species diversity index of Shannon-Weiner Diversity Index

p_i = The proportion of individual and total samples

S = Species number found in each station

2.3.2 Species evenness index

A value indicating the spread of the species and season each station if the survey station is high shows that season and explore it includes species that are in line and a similar distribution by way of Pielou's index (Clarke & Warwick, 2001), using the following formula

$$E = H/\ln S$$

E = Species evenness index of Pielou's index
S = Species number found in each station

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H = Species diversity index

2.3.3 Species richness index

A value that indicates the diversity of aquatic fauna in each station, and if the season is very valuable survey shows that there are more species diversity, based on the calculation of the number of species found according to the method of Margalef index (Clarke & Warwick, 2001), using the following formula

$$R = (S-1)/\ln N$$

R = Species richness index of Margalef index
N = Total individual number found
S = Species number found in each station

2.3.4 Relative abundance (RA)

Shows the frequency of aquatic fauna found during the study which describes the distribution of marine spatial (Clarke & Warwick, 2001), using the following formula

$$RA = \frac{\text{Abundance of each species}}{\text{Total of all species}} \times 100$$

3. Results

3.1 Taxonomy of aquatic fauna

The survey of species diversity of aquatic fauna inside and outside of seagrass behind Rockgarden Village in Kleang District, Rayong Province found that 15 species inside seagrass and 3 species outside seagrass in November, 16 species inside seagrass and 13 species outside seagrass in January and 15 species inside seagrass and 11 species outside seagrass. All 20 families, 24 species are divided into groups of fish 1 class 4 orders 17 families 18 genus and 20 species, the crab 1 class 1 order 2 families 3 genus and 3 species and shrimp 1 class 1 family 1 genus and 1 species as shown in Table 1 of appendix.

3.2 Biological diversity of aquatic fauna inside and outside seagrass

When analyzing a variety of animals in each survey area that are all aquatic fauna 22 and 15 species, 10,378 and 460 individuals, species diversity of 1.042 and 0.188, species evenness of 0.377 and 0.069 and species richness of 2.260 and 1.506 inside and outside seagrass, respectively

(Table 2) indicating that inside seagrass has a variety of aquatic fauna more than outside seagrass.

Table 2. The Ecological index of aquatic fauna in each survey area.

Ecological index	Survey area	
	Inside seagrass	Outside seagrass
Total species	22	15
Total individuals	10,378	460
Species diversity (H)	1.042	0.188
Species evenness (E)	0.337	0.069
Species richness (R)	2.260	1.506

3.3 Evaluation of aquatic fauna relative abundance inside and outside seagrass

The species diversity of aquatic fauna behind Rockgarden Village in Kleang District, Rayong Province, including all three times in November 2016, January and March 2017 found 18 families, 22 species and 10,378 individuals inside seagrass. The aquatic fauna which was high relative abundance found 5 species such as *Siganus javus* 72.56%, *Terapon puta* 13.75%, *Siganus canaliculatus* 5.12%, *Lethrinus lentjun* 1.64% and *Penaeus semisulcatus* 1.83% and was low relative abundance found 3 species such as *Chelonodon patoca* 0.019%, *Scatrophagus argus* 0.009% and *Matuta victor* 0.009%. The aquatic fauna outside seagrass found 13 families, 14 species and 460 individuals which was high relative abundance such as *Siganus canaliculatus* 54.78%, *Lethrinus lantjun* 14.57%, *Aerherinomorus duodecimalis* 10.87%, *Sillago Aeolus* 3.70% and *Terapon puta* 3.04% and was low relative abundance such as *Monacanthus chinensis* 0.43%, *Thalamita crenata* 0.22% and *Matuta victor* 0.22% shown in Table 3 of appendix. Assessing the relative abundance of aquatic fauna status in this study was different in surveyed frequency of each species between inside and outside seagrass to serve about the abundance and conservation status of aquatic fauna outside seagrass.

4. Discussion

Siganidae are fish group which is the most abundance inside seagrass area around Rockgarden such as *Siganus javus* and *Siganus canaliculatus* corresponds to Vongpanich et al. (2008) studied aquatic fauna inside seagrass at Phuket Island that Signidae are the most abundant species include the marine fish found Siganidae by the white supremacy. We found that different fish *Siganus javus* over *Siganus canaliculatus*, which shows that a group of
fish species are abundant inside seagrass both the Andaman Sea and the Gulf of Thailand. Coast island also has an abundance of crab (*Portunus pelagicus*) the highest, followed by *Portunus*

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sanguniolentus and *Thalamita* spp., respectively, to explore inside seagrass around Rockgarden area found abundance of *Portunus pelagicus* and *Thalamita crenata*, indicating that abundance of aquatic fauna inside seagrass between Rockgarden and Phuket Island nearby. But the seagrass of Rockgarden didn't find *Portunus sanguniolentus* due to the environment of different species of seagrass which is used as a food source and habitat of *Portunus sanguniolentus*.

Aongsara (2014) reported that the Talet Bay, Nakhonsithammarat Province found that there are *Siganus javus* very abundant of 44.66%, followed by *Secutor ruconius* of 10.57%, *Leiognathus splendens* 9.72%, *Leiognathus decorus* of 6.79%, *Ambassis kopsi* of 3.59%, *Metapenaeus lysianassa* of 2.31% and *Terapon puta* of 1.77% which *Siganus javus* as the fish were abundant only local resources seagrass are consistent this survey. It shows that *Siganus javus* found in seagrass pointed out that the seagrass habitat and food sources for *Siganus javus*. Pinto and Punchihewa (1996) studied the useful of fish inside seagrass around Nagumbo Estuary Sri Lanka found that Gobies and Siganid take advantage of seagrass in finding food and refuge fish include a lot of *Siganus javus* in seagrass. Jeewarongkakul (2004) conducted a survey of aquqtic fauna inside Baan Tha-Lane seagrass, Krabi Province collected total 4,543 individuals of fish which the family Siganidae is the most abundant include *Siganus canaliculatus* of 66.15% and *Siganus javus* of 9.44% which is different from this survey found *Siganus javus* of 72.55% and *Siganus canaliculatus* of 5.11%. Satapoomin, & Satapoomin (2005) were examined the stomach contents of fish in the Eastern side of seagrass, Phuket Province found that 84% of gastric banding *Siganus javus* as seagrass and algae. This indicates that *Siganus javus* into the seagrass along the food supply for itself. This shows that the prevalence Siganidae in the seagrass, whether it is the Gulf of Thailand or the Andaman Sea.

Baan Tha-Lane has species diversity index of aquatic fauna as 1.51, species evenness of 0.39 which is consistent with a variety of fish over space exploration in the grass near the Rock Garden Village. The Baan Laem Som, Phangnga Province found a total of 4,937 fish were the highest abundance in the area such as *Siganus canalicutus* of 41.72%, *Lesthrinus lentjun* of 4.23% and *Siganus javus* of 1.96% and species diversity index equal to 2.04 and species evenness of 0.54, which is very high compared to seagrass beds of Rockgarden Village.

Because Baan Lane seagrass found all eight species of seagrass such as *Enhalus acoroides*, *Thalassia hemprichii*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Halophila minor*, *Halophila ovalis*, *Haludule pinifolia* and *Haludule uninervis*. Dominant seagrass species is *Halophila ovalis* which covers about 80% of the total area. Baan Laem Som seagrass, Phangnga Province with an area of approximately 0.8 square kilometers of sea grass found 8 species of seagrass similar to Baan Lane seagrass, Krabi Province. The dominant seagrass species, including grass-covered area along the seagrass *Enhalus acoroides* 70% unlike Rockgarden seagrass which was the dominant seagrass *Haludule uninervis* and probably found only one seagrass in this area as a result, the diversity of aquatic fauna in Rockgarden seagrass.

Upanoi (2005) surveyed crustaceans group in the mangroves and seagrass beds, Phuket Province found *Penaeus semisulcatus* in small amounts and only seagrass and its abundance of shrimp difference with Rockgarden seagrass in Rayong, which surveyed shrimp dominant. Additionally, animals in seagrass ecosystem will have to share the use of space by time. During the daytime meet the most diverse group of fish and during the night to find the most shrimp (Boonphienphol, 2007), including the appearance of seagrass in the area will affect the amount and type of aquatic faunas found. The phylum arthropoda to find the maximum area of short leaves seagrass and phylum chordata in the core area, typically long leaves seagrass. It shows that the survey found the type and abundance of aquatic fauna are specific to the type and characteristics of seagrass in various areas (Pengchumrus et al., 2008).

5. Conclusions

A survey of aquatic fauna using small beach seine inside and outside of seagrass behind Rockgarden Village in Kleang District, Rayong Province found that the fish group are the most diversity of 20 species, followed by the crab 3 species and shrimp 1 species. The station which was the most diversity has 22 species of inside seagrass with species diversity of 1.042, species richness of 2.260 and species evenness of 0.337. The high abundance inside seagrass found 5 species such as *Siganus javus*, *Terapon puta*, *Siganus canaliculatus*, *Penaeus semisulcatus* and *Gerres oyena*. The outside seagrass found 14 species has species diversity of 0.188, species richness of 1.506 and species evenness of 0.069 and high abundance 5 species such as *Siganus canaliculatus*, *Lethrinus lentjun*, *Aerihinomorus duodecimalis* and *Sillago aeolus*. These indicated that the inside seagrass area has species diversity index higher

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than outside seagrass. The database of the aquatic fauna diversity is the necessary information to be studied further and a guideline for the sustainable conservation of aquatic fauna.

6. Acknowledgements

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Appendix

Table 1. Taxonomy of aquatic fauna.

Phylum	Class	Order	Family	Genus	Species
Fish group Chordata	Actinopterygii	Perciformes	Gerreidea	*Gerres*	*Gerres oyena*
			Terapontidae	*Terapon*	*Terapon puta*
				Pelates	*Pelates quadrineatus*
			Siganidae	*Siganus*	*Siganus javus*
					Siganus canaliculatus
			Lujanidae	*Lutjanus*	*Lutjanus russelli*
					Lutjanus fulviflamma
			Mulidae	*Upeneus*	*Upeneus tragula*
			Lethrinidae	*Lethrinus*	*Lethrinus lentjun*
			Gobidae	*Favonigobius*	*Favonigobius reichei*
			Chealotodontidae	*Parachealotodon*	*Parachealotodon ocellatus*
			Scatophagidae	*Scatophagus*	*Scatophagus argus*
			Labridae	*Halichoeres*	*Halichoeres bicolor*
			Sphyraenidae	*Sphyraena*	*Sphyraena barracuda*
			Leiognathidae	*Leiognathus*	*Leiognathus* spp.
			Sillaginidae	*Sillago*	*Sillago aeolus*
		Syngnathiformes	Syngnathidae	*Hippichthys*	*Hippichthys cyanospilos*
		Atheriniformes	Atherinidae	*Aerherimomorus*	*Aerherimomorus duodecimalis*
		Tetraodontiformes	Monacanthidae	*Monacanthus*	*Monacanthus chinensis*
			Tetraodontidae	*Chelonodon*	*Chelonodon patoca*
Shrimp and crab group Arthropoda	Malacostraca	Decapoda	Portunidae	*Portunus*	*Portunus pelagicus*
				Thalamita	*Thalamita crenata*
			Matutidae	*Matuta*	*Matuta victor*
			Penaeidae	*Peneaus*	*Peneaus semisulcatus*

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Table 3. The abundance of aquatic fauna inside and outside seagrasses.

aquatic fauna species inside seagrass	F	N	A	aquatic fauna species outside seagrass	F	N	A
1. *Gerres oyena*	1	148	1.426	1. *Terapon puta*	2	14	3.043
2. *Terapon puta*	3	1427	13.750	2. *Siganus javus*	2	32	6.956
3. *Pelates quadrilineatus*	2	44	0.423	3. *Siganus canaliculatus*	2	252	54.782
4. *Siganus javus*	3	7530	72.557	4. *Lethrinus lentjun*	2	67	14.565
5. *Siganus canaliculatus*	2	531	5.116	5. *Favonigobius reichei*	3	4	0.869
6. *Lutjanus russelli*	3	24	0.231	6. *Paracheatodon ocellatus*	2	9	1.956
7. *Lutjanus fulviflama*	1	5	0.048	7. *Leiognathus* spp.	1	5	1.086
8. *Upeneus tragula*	1	4	0.038	8. *Sillago aeolus*	1	17	3.695
9. *Lethrinus lentjun*	3	170	1.638	9. *Hippchthys cyanospilos*	1	3	0.652
10. *Favonigobius reichei*	3	32	0.308	10. *Aerherimomorus duodecimalis*	3	50	10.869
11. *Paracheatodon ocellatus*	3	101	0.973				
12. *Scatophagus argus*	1	1	0.009	11. *Monacanthus chinensis*	1	2	0.434
13. *Halichoeres bicolor*	2	3	0.028	12. *Thalamita crenata*	1	1	0.217
14. *Sphyraena barracuda*	1	6	0.057	13. *Matuta victor*	1	1	0.217
15. *Hippchthys cyanospilos*	3	50	0.481	14. *Penaeus semisulcatus*	2	3	0.652
16. *Aerherimomorus duodecimalis*	1	5	0.048				
17. *Monacanthus chinensis*	2	5	0.048				
18. *Chelonodon patoca*	1	2	0.019				
19. *Thalamita crenata*	3	42	0.404				
20. *Portunus pelagicus*	3	57	0.549				
21. *Matuta victor*	1	1	0.009				
22. *Penaeus semisulcatus*	3	190	1.830				

Remark: F = Surveyed frequency   N = Total individual number found   A = Relative abundance

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A New Mixture Lomax Distribution and Its Application

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Abstract

In this paper, we propose a new mixture Lomax distribution for positive continuous random variable. The new mixture Lomax distribution has two component distributions which are Lomax distribution and length biased Lomax distribution. We have derived and studies in probability properties which include the probability density function, cumulative distribution function, survival function, hazard function, moment about origin, mean, variance, coefficient of skewness and coefficient of kurtosis. Next, we study the estimation parameter of new mixture Lomax distribution by using maximum likelihood estimation. Finally, application of new mixture Lomax distribution is illustrated by real data set which is analyzed using Akaike's information criterion (AIC). It is shown that the proposed distribution fits much better than some other existing Lomax distributions.

Keywords: Mixture Lomax distribution, Length biased, Lomax distribution

1. Introduction

Lomax distribution was introduced by Lomax (1954). Lomax distribution is Pareto distribution Type II which is heavy tailed distributions for positive continuous random variable. It used in business, economics, queuing theory and Internet traffic modeling (Chen, Addie, Zukerman, & Neame, 2015). In the literature, some extension of Lomax distribution are available such as Gamma-Lomax distribution by Cordeiro, Ortega, and Popović (2013), Transmuted exponentiated Lomax distribution by Ashour and Eltehiwy (2013), Poisson-Lomax distribution by Al-Zahrani and Sagor (2014), Power Lomax distribution by Rady, Hassanein, and Elhaddad (2016) and Length-biased weighted Lomax distribution by Afaq, Ahmad, and Ahmed (2016).

Mixture distributions are popular tools for generating flexible distribution with good statistical properties. Specifically, let $0 < p < 1$ and $f_{X_1}(x)$ and $f_{X_2}(x)$ are the probability density function of the random variables X_1 and X_2, respectively, then the probability density function of the X (being a mixture of X_1 and X_2) is expressed as

$$f_X(x) = p f_{X_1}(x) + (1-p) f_{X_2}(x), \quad x > 0.$$

In the literature, some mixture distribution of several distributions are available such as mixture inverse Gaussian distribution (Balakrishnan, Leiva, Sanhueza, & Cabrera. 2009; Jorgensen, Seshadri, &

Whitmore, 1991), Lindley distribution (Ghitany, Atieh, & Nadarajah, 2008), three parameter crack distribution (Bowonrattanaset, 2011) and two parameter crack distribution (Saengthong, & Bodhisuwan, 2014). The mixture distribution with weight parameter (p) has many desirable properties in some applications, parameter estimation may still have problems. Jorgensen, Seshadri, and Whitmore (1 9 9 1) and Gupta and Akman (1 9 9 5) have mentioned that solving nonlinear simultaneous equations are non-trivial issues. Then, Bowonrattanaset (2011) showed the estimates of p are out of the closed interval [0,1] .

Mixture Lomax distribution has two component distributions which are Lomax distribution and length biased Lomax distribution. The probability density function of Lomax distribution (Aryal & Tsokos, 2011) and Length-biased Lomax distribution (Subba Rao, Naga Durgamamba, & Kantam, 2014), respectively, are given by

$$f_{X_1}(x) = \frac{q}{b\left[1 + x/b\right]^{1+q}} \quad \text{and}$$

$$f_{X_2}(x) = \frac{q(q-1)x}{b^2\left[1 + x/b\right]^{1+q}}, \quad x > 0, b, q > 0.$$

Mixture Lomax distribution has two component distributions which are Lomax distribution and

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length biased Lomax distribution with probabilities p and $1-p$, respectively, as follows:

$$f(x) = p\frac{q}{b\left[1+x/b\right]^{1+q}} + (1-p)\frac{q(q-1)x}{b^2\left[1+x/b\right]^{1+q}}$$

where $x > 0, 0 < p < 1, b, q > 0$.

Therefore, in order to solve such problems, a new weight parameter is considered. We introduce a new mixture Lomax distribution which is obtained by adding a new weight parameter. In section 2, we present probability density function, cumulative distribution function, survival function, hazard function, moment about origin, mean, variance, coefficient of skewness and coefficient of kurtosis. In section 3, we estimate parameter of new mixture Lomax distribution by using maximum likelihood estimation method. In section 4, we present an illustrative example with estimated parameters. Finally, we conclude in section 5.

2. New Mixture Lomax Distribution

In this section, we develop the new mixture Lomax distribution. For this new distribution, we present probability density function, cumulative distribution function, survival function, hazard function, moment about origin are given, respectively, in Theorem 1 – 5.

2.1 Probability density function of new mixture Lomax distribution

Definition 1. Let X_1 and X_2 random variables are independent and identically distributed (i.i.d.). X_1 is a random variable of Lomax distribution and X_2 is a random variable of length biased Lomax distribution. The probability density function of new mixture Lomax distribution (X) by the mixture between X_1 and X_2 with parameter $b > 0$ and $q > 0$ is defined as

$$f_X(x;b,q) = \left(\frac{b}{1+b}\right)f_{X_1}(x;b,q) + \left(\frac{1}{1+b}\right)f_{X_2}(x;b,q).$$

Theorem 1. Let X is a random variable of new mixture Lomax distribution with $b > 0$ and $q > 0$. The probability density function of new mixture Lomax distribution is

$$f(x) = \frac{q(b^2 + qx - x)}{\left(b^3 + b^2\right)\left(1+x/b\right)^{1+q}}$$

where $x > 0$, $b > 0$ and $q > 0$.

Proof of Theorem 1. In Definition 1, The probability density function of new mixture Lomax distribution is given as

$$f_X(x;b,q) = \frac{b}{1+b}\cdot f_{X_1}(x;b,q) + \frac{1}{1+b}\cdot f_{X_2}(x;b,q)$$

$$f(x) = \frac{b}{1+b}\cdot\left(\frac{q}{b[1+(x/b)]^{1+q}}\right)$$

$$+ \frac{1}{1+b}\cdot\left(\frac{q(q-1)x}{b^2\left[1+x/b\right]^{1+q}}\right).$$

Therefore, the probability density function of new mixture Lomax distribution is given by the expression

$$f(x) = \frac{q(b^2 + qx - x)}{\left(b^3 + b^2\right)\left(1+x/b\right)^{1+q}}.$$

The shape of probability density new mixture Lomax function is shown in Figure 1.
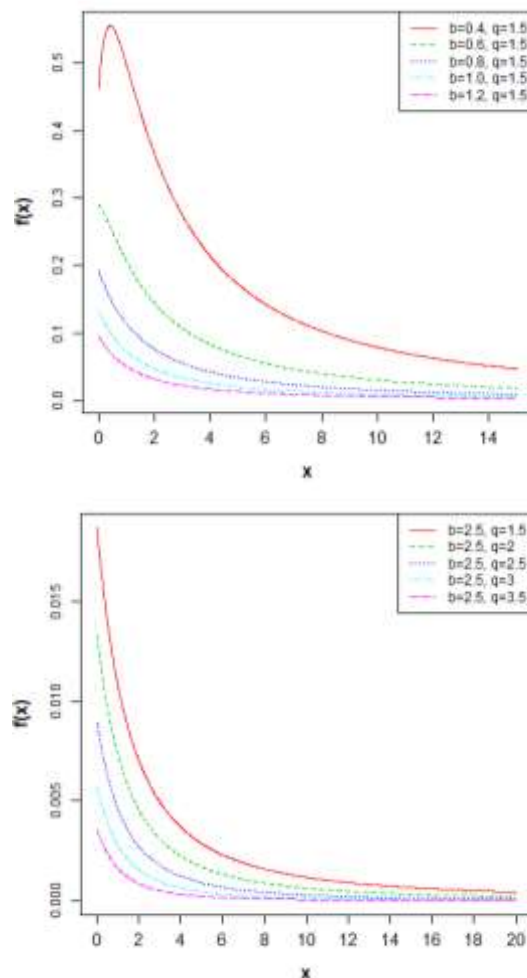
Figure 1. Plot of the probability density new Lomax function: versus x for selected b and q.

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2.2 Cumulative distribution function of new mixture Lomax distribution

Theorem 2 . Let X is a random variable of new mixture Lomax distribution with $b > 0$ and $q > 0$. The cumulative distribution function of new mixture Lomax distribution is given as

$$F(x) = \frac{1}{1+b} \cdot \left(1+b - \left(1+b+\frac{qx}{b}\right)\left(1+\frac{x}{b}\right)^{-q} \right)$$

where $x > 0$, $b > 0$ and $q > 0$.

Proof of Theorem 2. If X is a random variable of new mixture Lomax distribution, then cumulative distribution function of new mixture Lomax distribution can be given as

$$F(x) = \int_0^x f(t;b,q)\, dt$$

$$F(x) = \frac{b}{1+b} \int_0^x f_{X_1}(t;b,q)\, dt + \frac{1}{1+b} \int_0^x f_{X_2}(t;b,q)\, dt$$

$$F(x) = \frac{b}{1+b} \cdot F_{X_1}(x;b,q) + \frac{1}{1+b} \cdot F_{X_2}(x;b,q)$$

The cumulative distribution function of (Aryal & Tsokos, 2011) and length biased Lomax distribution (Subba Rao et al., 2014), respectively, are as follows:

$$F(x_1) = 1 - \left(1+\frac{x_1}{b}\right)^{-q}$$

and $F(x_2) = 1 - \left(1+\frac{qx_2}{b}\right)\left(1+\frac{x_2}{b}\right)^{-q}$.

The following result has obtained

$$F(x) = \frac{1}{1+b} \cdot \left(1+b - \left(1+b+\frac{qx}{b}\right)\left(1+\frac{x}{b}\right)^{-q} \right).$$

The shape of cumulative distribution function of new mixture Lomax distribution is shown in Figure 2.

2.3 Survival function of new mixture Lomax distribution

Theorem 3 . Let X is a random variable of new mixture Lomax distribution with $b > 0$ and $q > 0$. The survival function of new mixture Lomax distribution is given as

$$S(x) = 1 - \frac{1}{1+b} \cdot \left(1+b - \left(1+b+\frac{qx}{b}\right)\left(1+\frac{x}{b}\right)^{-q} \right).$$

Proof of Theorem 3. The cumulative distribution function of new mixture Lomax distribution is

$$F(x) = \frac{1}{1+b} \cdot \left(1+b - \left(1+b+\frac{qx}{b}\right)\left(1+\frac{x}{b}\right)^{-q} \right).$$

The survival function is obtained by solving

$$S(x) = \int_x^\infty f(t)\, dt = 1 - F(x).$$

Then, survival function of new mixture Lomax distribution is

$$S(x) = 1 - \left[\frac{1}{1+b} \cdot \left(1+b - \left(1+b+\frac{qx}{b}\right)\left(1+\frac{x}{b}\right)^{-q} \right) \right].$$
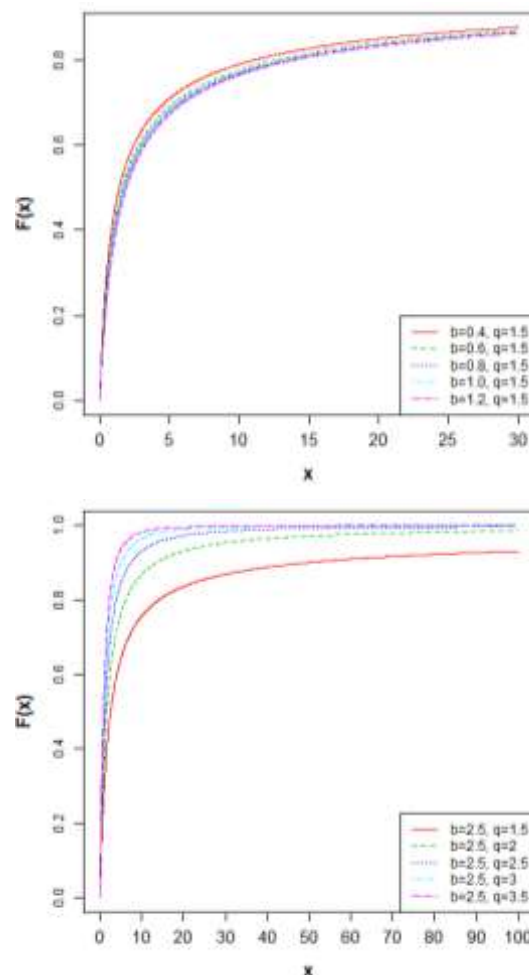
Figure 2 . Plot of cumulative distribution function of new mixture Lomax distribution plots function: versus x for selected b and q.

Next, the shape of survival new mixture Lomax function is shown in Figure 3.

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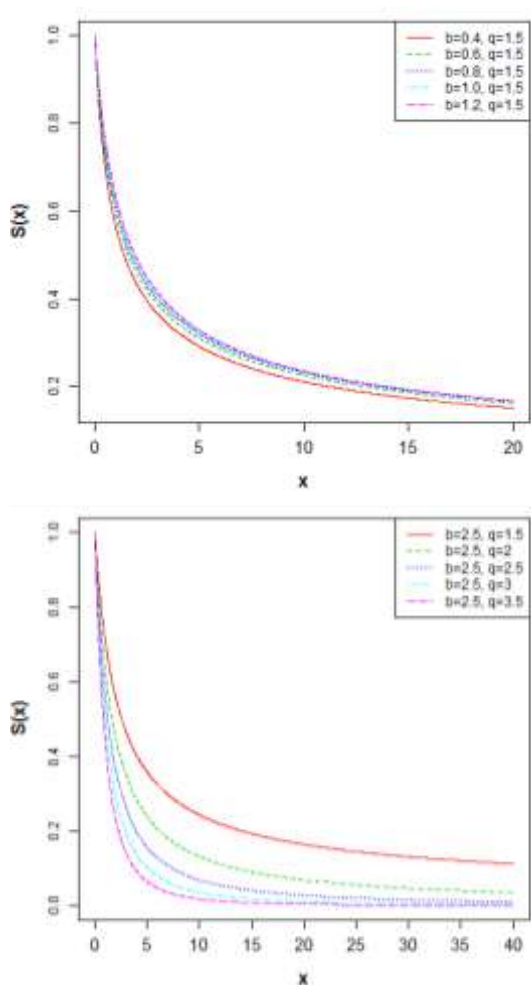
Figure 3 . Plot of the survival new mixture Lomax function: versus x for selected b and q .

2.4 Hazard function of new mixture Lomax distribution

Theorem 4 . Let X is a random variable of new mixture Lomax distribution with $b > 0$ and $q > 0$. The hazard function of new mixture Lomax distribution is given as

$$h(x) = \left[q(b^2 + qx - x) \right] / \left[\left(b^3 + b^2 \right)\left(1 + x/b \right)^{1+q} \right.$$

$$\times \left(1 - \frac{1}{1+b} \cdot \left(1 + b - \left(1 + b + \frac{qx}{b} \right)\left(1 + \frac{x}{b} \right)^{-q} \right) \right) \right].$$

Proof of Theorem 4. The probability density function and survival function of new mixture Lomax distribution are

$$f(x) = \frac{q(b^2 + qx - x)}{\left(b^3 + b^2 \right)\left(1 + x/b \right)^{1+q}} \quad \text{and}$$

$$S(x) = 1 - \left[\frac{1}{1+b} \cdot \left(1 + b - \left(1 + b + \frac{qx}{b} \right)\left(1 + \frac{x}{b} \right)^{-q} \right) \right]$$

.

The hazard function is obtained by solving
$$h(x) = \frac{f(x)}{S(x)} .$$
Then, we get

$$h(x) = \left[q(b^2 + qx - x) \right] / \left[\left(b^3 + b^2 \right)\left(1 + x/b \right)^{1+q} \right.$$

$$\times \left(1 - \frac{1}{1+b} \cdot \left(1 + b - \left(1 + b + \frac{qx}{b} \right)\left(1 + \frac{x}{b} \right)^{-q} \right) \right) \right].$$
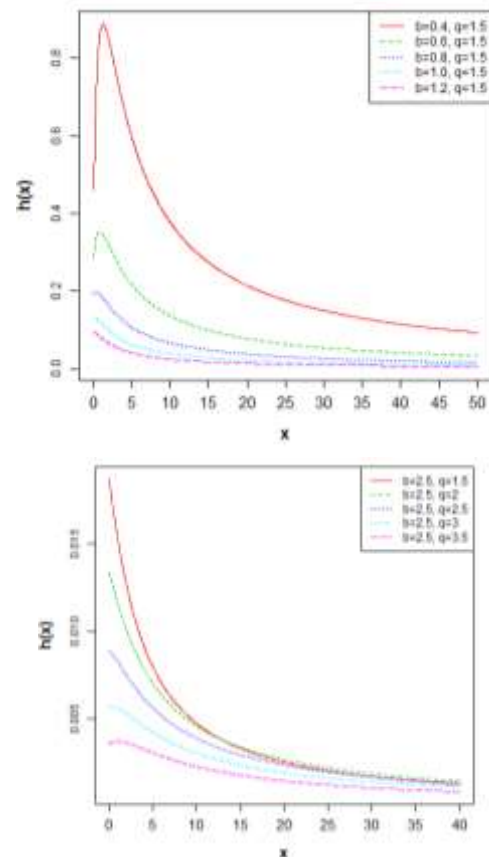
The shape of hazard new mixture Lomax function is shown in Figure 4.

Figure 4 . Plot of the hazard new mixture Lomax function: versus x for selected b and q .

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Theorem 5. The k th moment about the origin of a random variable X, where $X \sim NML(b,q)$ is given by the following:

$$E(X^k) = \frac{qb^{k+1}}{1+b}\sum_{r=0}^{k}\binom{k}{r}(-1)^{r+1}\left(\frac{1}{k-r-q}\right)$$
$$+ \frac{q(q-1)b^k}{1+b}\sum_{r=0}^{k+1}\binom{k+1}{r}(-1)^{r+1}\left(\frac{1}{k-r-q+1}\right).$$

Proof of Theorem 5. The k th moment about origin of X can be determined by direct integration using the probability density function, we have

$$E(X^k) = \int_0^\infty x^k f(x)dx = \int_0^\infty x^k \frac{q(b^2+qx-x)}{(b^3+b^2)(1+x/b)^{1+q}}dx$$

$$E(X^k) = \frac{q}{1+b}\int_0^\infty \left(\frac{x^k}{[1+(x/b)]^{1+q}}\right)dx$$
$$+ \frac{q(q-1)}{(1+b)}\int_0^\infty \left(\frac{x^{k+1}}{b^2[1+x/b]^{1+q}}\right)dx$$

$$= \frac{q}{1+b}\int_1^\infty b^k(t-1)^k t^{-(1+q)}b\, dt$$

$$+ \frac{q(q-1)}{(1+b)\,b^2}\int_1^\infty b^{k+1}(t-1)^{k+1}t^{-(1+q)}b\, dt$$

$$= \frac{qb^{k+1}}{1+b}\int_1^\infty (t-1)^k t^{-(1+q)}\, dt$$

$$+ \frac{q(q-1)b^k}{1+b}\int_1^\infty (t-1)^{k+1}t^{-(1+q)}\, dt$$

$$= \frac{qb^{k+1}}{1+b}\int_1^\infty \sum_{r=0}^{k}\binom{k}{r}t^{k-r}(-1)^r t^{-(1+q)}\, dt$$

$$+ \frac{q(q-1)b^k}{1+b}\int_1^\infty \sum_{r=0}^{k+1}\binom{k+1}{r}t^{k+1-r}(-1)^r t^{-(1+q)}\, dt$$

$$= \frac{qb^{k+1}}{1+b}\sum_{r=0}^{k}\binom{k}{r}(-1)^r \int_1^\infty t^{k-r-q-1}\, dt$$

$$+ \frac{q(q-1)b^k}{1+b}\sum_{r=0}^{k+1}\binom{k+1}{r}(-1)^r \int_1^\infty t^{k-r-q}\, dt$$

$$E(X^k) = \frac{qb^{k+1}}{1+b}\sum_{r=0}^{k}\binom{k}{r}(-1)^{r+1}\left(\frac{1}{k-r-q}\right)$$
$$+ \frac{q(q-1)b^k}{1+b}\sum_{r=0}^{k+1}\binom{k+1}{r}(-1)^{r+1}\left(\frac{1}{k-r-q+1}\right)$$

Corollary 1. Let X is random variable of new mixture Lomax distribution. Then the first - four moments of X are given, respectively, as follows:

$$E(X) = \frac{b^2}{(1+b)(q-1)} + \frac{2b}{(1+b)(q-2)}, where\ q>2$$

$$E(X^2) = \frac{2b^3}{(1+b)(q-1)(q-2)} + \frac{6b^2}{(1+b)(q-2)(q-3)}\ where$$
$$q>3$$

$$E(X^3) = \frac{6b^4}{(1+b)(q-1)(q-2)(q-3)}$$
$$+ \frac{24b^3}{(1+b)(q-2)(q-3)(q-4)}, where\ q>4$$

$$E(X^4) = \frac{24b^5}{(1+b)(q-1)(q-2)(q-3)(q-4)}$$
$$+ \frac{120b^4}{(1+b)(q-2)(q-3)(q-4)(q-5)}, where\ q>5.$$

Proof of Corollary 1. Applying k th moment about the origin of random variable of new mixture Lomax distribution for $k=1,2,3$ and $k=4$ yields the desired results.

Based on the results given in relations of k th moment about the origin of random variable of new mixture Lomax distribution for $k=1,2,3$ and $k=4$, the mean, variance, coefficient of skewness and coefficient of kurtosis are given, respectively, as follows:

1) Mean

$$E(X) = \frac{b^2}{(1+b)(q-1)} + \frac{2b}{(1+b)(q-2)}$$
where $q>2$

2) Variance

$$Var(X) = E(X^2) - [E(X)]^2$$
$$= \frac{2b^3}{(1+b)(q-1)(q-2)} + \frac{6b^2}{(1+b)(q-2)(q-3)}$$
$$- \left[\frac{b^2}{(1+b)(q-1)} + \frac{2b}{(1+b)(q-2)}\right]^2$$
where $q>3$

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3) Coefficient of Skewness

$$Skewness(X) = \frac{E(X^3) - 3E(X)Var(X) - \left(E(X)\right)^3}{\left(Var(X)\right)^{\frac{3}{2}}}$$

4) Coefficient of Kurtosis

$$Kurtosis(X) = \left[E(X^4) - 4E(X^3)E(X) + 6E(X^2)\left(E(X)\right)^2 \right.$$
$$\left. -3\left(E(X)\right)^4 \right] / \left(Var(X)\right)^2$$

The mean, variance, coefficient of skewness and coefficient of kurtosis for new mixture Lomax distribution for different values of the parameters b and q in Figure 5.
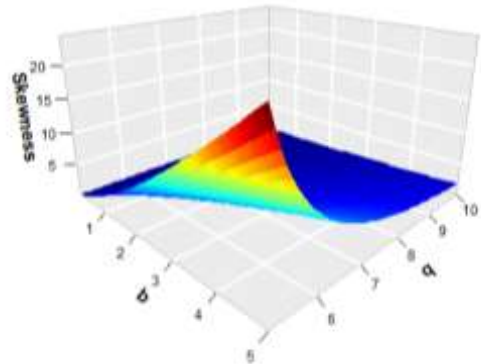
(C) Coefficient of Skewness
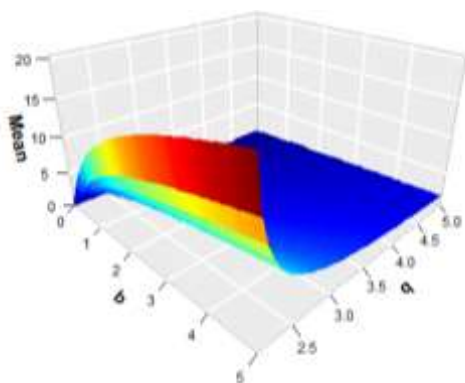
(A) Mean
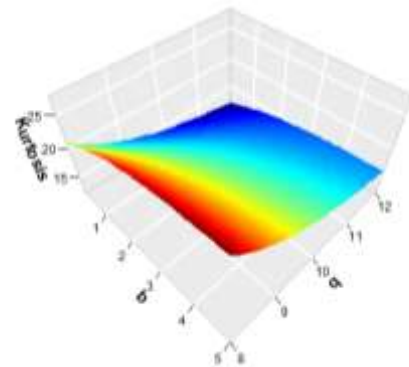
(D) Coefficient of Kurtosis
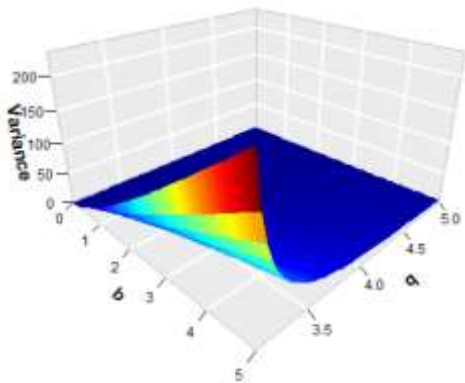
Figure 5 . Plot of the mean (A), variance (B) , coefficient of skewness (C) and coefficient of kurtosis (D) for new mixture Lomax distribution : versus b and q .

3. Parameters Estimation

In this section, we consider maximum likelihood estimation to estimate the involved parameters of new mixture Lomax distribution. The likelihood function of new mixture Lomax distribution with b and q parameter is defined in Definition 2.

Definition 2 . Suppose that a random sample $X_1, X_2, ..., X_n$ is collected from new mixture Lomax distribution, Then the likelihood function of the observed sample is given by

$$L(b,q;x_1,x_2,x_3,...,x_n) = \prod_{i=1}^{n} \left[\frac{q(b^2 + qx_i - x_i)}{\left(b^3 + b^2\right)\left(1 + x_i/b\right)^{1+q}} \right].$$

(B) Variance

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The maximum likelihood estimates of the parameters are obtained by direct maximization of the log-likelihood function is produced as follows;

1) The likelihood function and log-like likelihood of new mixture Lomax distribution are, respectively, given by

$$L(b,q) = L(b,q;x_1,x_2,x_3,...,x_n)$$

$$= \prod_{i=1}^{n} \left[\frac{q(b^2+qx_i-x_i)}{(b^3+b^2)(1+x_i/b)^{1+q}} \right]$$

$$\log L(b,q) = n \log q + \sum_{i=1}^{n} \log(b^2+qx_i-x_i)$$

$$- n\log(b^3+b^2) - (1+q)\sum_{i=1}^{n}\log(1+x_i/b).$$

2) Finding the optimal values of the parameters is obtained by differentiating in $log\ L(b,q)$ with b and q. Then, it gives rise to following equations:

$$\frac{d}{db}\log L(b,q) = \sum_{i=1}^{n}\frac{2b}{(b^2+qx_i-x_i)} - \frac{n(3b^2+2b)}{(b^3+b^2)}$$

$$+ (1+q)\sum_{i=1}^{n}\frac{b^{-2}}{(1+x_i/b)} \qquad (1)$$

$$\frac{d}{dq}\log L(b,q) = \frac{n}{q} + \sum_{i=1}^{n}\frac{x_i}{(b^2+qx_i-x_i)} - \sum_{i=1}^{n}\log(1+x_i/b) \quad (2)$$

3) The MLE solutions of $\hat{b},\hat{q}$ can be obtained by equating the (1) and (2) to zero and solving the resulting equations simultaneously using a numerical procedure with the Newton-Raphson method.

4. Numerical Result

In this section, we have considered a dataset corresponding to remission times (in months) of a random sample of 1 2 8 bladder cancer patients given in Lee and Wang (2003). We have fitted the new mixture Lomax distribution to the dataset using MLE, and compared new mixture Lomax distribution with Lomax, old mixture Lomax and length biased Lomax distributions which are showed in Table 1. The model selection is carried out using AIC (Akaike information criterion).

Table 1. Maximum likelihood Estimate and AIC.

Distribution	Estimate parameters			AIC
	$\hat{p}$	$\hat{b}$	$\hat{q}$	
Lomax	-	11.09	94.47	799.13
Length biased Lomax	-	4.33	10.97	793.84
Old mixture Lomax	0.21	15.75	75.11	785.34
New mixture Lomax	-	12.35	45.12	772.21

We fitted the Lomax, length biased Lomax, old mixture Lomax and new mixture Lomax distributions to this data set. The maximum likelihood estimation was used. We obtained the estimates parameters and AIC statistic for all distributions are shown in Table 1. The AIC statistic is shown that the new mixture Lomax distributions distribution is the best fit for this data.

5. Conclusion

This paper provides a new mixture Lomax distribution for lifetime data. Various interesting mathematical statistics properties of new mixture Lomax distribution such as its hazard rate function survival function, moment about origin and expressions for mean, variance, coefficient of skewness and coefficient of kurtosis which have been discussed. We estimate parameters of new mixture Lomax distribution by using maximum likelihood estimation method. In application, we compare the fit of the new mixture Lomax distribution with Lomax, length biased Lomax and old mixture Lomax distributions by real data. The AIC statistics indicates that the new mixture Lomax distribution is best fit for real data.

6. Acknowledgement

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Separation of Blue Ballpoint Pen Inks- A Comparison of Solvent Systems on Thin Layer Chromatography Techniques

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Abstract
This article is chromatographic analysis of inks for forensic science. Thin layer chromatographic technique (TLC) is often used in separation of writing inks because it is rapid and requires no sophisticated instrumentation. The repeatability and reproducibility of TLC analyses of inks depend on several factors. However, the critical importance is the use of solvent for the extraction process and for mobile phase. The purpose of the present study is to compare the effectiveness of blue ballpoint pen ink separation by TLC between different solvent extraction and variant mobile phase systems. Thirty blue ballpoint pens commonly used in Thailand were extracted from document then three solvents (ethanol, acetone or dichloromethane) and five solvent systems as mobile phase were used to separate pigment compounds in each sample. The R_f values were calculated for discrimination analysis of all blue pens via two-way ANOVA. The results showed that the most important factor affecting ink classification was solvent extraction. The ethanol was the best solvent for extraction and the optimal mobile phase was n-butanol: ethanol: water (50:15:10 v/v/v) having statistically significant level of 0.05. This mobile phase system could be used to classify all 30 inks into 12 different groups with the discrimination power (DP) of 89.20%. In the future, the qualitative data from TLC plates will more reliable by multivariate statistical techniques can also be applied on effectively interpretation.

Keywords: Forensic, Ballpoint pen inks, Thin-layer chromatography, Ink analysis

1. Introduction

The complexity and globalization of today's technological society makes it necessary for people to relate or convey important documents. As such, document forgery is a common problem, especially using pen or ink in writing to forge or edit documents and signatures. Most questionable documents consist of forged bank checks, bills, handwritten correspondence, contracts and others, which require analysis of ballpoint pen inks. Ink analysis is an important forensic procedure because it can reveal useful information for an investigation. Modern inks contain mixtures of various substances that are meant to improve ink characteristics (Roux, Novotny, Evans, & Lennard, 1999; Vila, Ferrer, & Garcia, 2007; Vogt, Vogt Becker, & Rohde, 1997). The most important component of coloring material comes in the form of dyes, pigments, and various combinations. Dyes are soluble in the vehicle that is a mixture of solvents, oils and resins. This carrier is an important component of the ink, which affects its flowing and drying characteristics. The solvent mixes a variety of types of inorganic materials such as glycol, glycol ether or allopathic alcohol, which have a boiling point higher than 180 °C so they can be stable at room temperature. The evaporation of the solvent and prevention of fouling at the tip of the pen results in self-locking by default. This process does not affect the flow of ink when it is written on paper. Pigments are solid, opaque particles that have molecules linked together in crystalline structures, which provide color for the ink. The color depends on the raw materials used in production. Mostly, blue can be obtained with substituted triphenylmethane pigment. Other substances used to improve certain properties will have different characteristics in accordance with the particular purpose of the pen (Thanasoulias, Parisis, & Evmiridis, 2003). The aim of most analyses is comparison of different writing inks on document, which is the primary goal of

investigations (Djozan, Baheri, Karimian, & Shahidi, 2008).

Forensic document examination, especially the analysis of inks, can be divided into two approaches including non-destructive document and destructive document. Non-destructive analytical methods will choose specific characteristics of ink to serve as parameters, such as its colors, luminescence and radiation absorption. Questionable documents may be differentiated by properties of transmission, reflection and fluorescence spectra obtained for inks deposited on the paper surface. However, the methods of physic-chemical analysis can determine the type and composition of ink, leading to ink identification (Feraru & Meghea, 2014). Destructive document analysis starts by removing a small section from the ink line with extraction solvent to open up more avenues of analysis. In particular, the chromatographic separation of colored pigments from component dyes can be useful. Even though a blue ballpoint pen can only write in one color, the ink is actually made from a mixture of different colored pigments. This method has proven highly productive for the comparison and matching of ink with the database of chromatograms (Ismail, Austad, & Mat Desa, 2014; Lewis, 1996; Samanidou, Nikolaidou, & Papadoyannis, 2004; Zlotnick & Smith, 1999).

Thin layer chromatography (TLC) is a solid-liquid from chromatography, which can separate the composition of the sample for the components distributed between two phases. The stationary phase is normally a polar adsorbent and a single or combination of solvents that is a mobile phase to dissolve the substance from the stationary phase. Different substances have different adsorption and movement properties. TLC is a simple approach to use, as well as being rapid, inexpensive, and minimally destructive to the document because it requires only a small amount of sample for examination. At the same time, it can show a high degree of characterized selectivity and repeatability for results. Accordingly, this study focuses on destructive document analysis using the chemometrics approach for data analyses that use both mathematical and statistical methods to improve the accuracy of identification and the discrimination of pen inks (Lee, Shandu, Razi, Ishak, & Osman, 2015; Senior, Hamed, Masoud, & Shehata, 2012).

The TLC plate gaining popularity is alumina adsorbent. This method is often performed using the retention factor (R_f) values for the qualitative evaluation of chromatograms. These values are used to compare the TLC results from all study inks. These comparisons help to find the characteristics of inks, resulting in estimating the source of questionable ink. Ink analysis by TLC is a powerful forensic tool and has provided important evidence that had a significant impact on the outcome of several high profile cases (Barker, Ramotowski, & Nwokoye, 2016). This is an important part of creating a reference ink library by using the same type of TLC plate for searching and comparing. Minor changes in the mobile phase system could generate more significant changes in the colorants R_f values and expand to potential false negative. One of several factors that especially critical regard to the repeatability and reproducibility of the results that is the use substance suitable for isolation of sample from the substrate of document (i.e. paper) and chose appropriate mobile phase system. The purpose of this research focused on investigating the effects of ink separation by TLC comparison of different solvents for extraction in conjunction with different mobile phase systems.

2. Materials and Methods

2.1 Equipment and chemicals

Random sampling a number of blue ballpoint pen inks from different 30 from 90 blue ballpoint pen inks have been purchased from local markets in Bangkok, Thailand (at the time of study) show in Table 1.

Table 1. The list of studied blue ballpoint pens.

No.	Commercial characteristics
1	GRIP X P5 FABER-CASTELL (0.5 mm)
2	Pentel ENERGEL BL107 (0.7 mm)
3	XF STAEDTLER LUNA Ball
4	REBNOK Hi SPIRIT
5	Semi Gel WIN pen (0.7 mm)
6	QuanTumGeloPlus⁺Power 1248 (0.7 mm)
7	Uni-ball Signo DX (0.38 mm) MITSUBISHI UM-151
8	M&G 0.5 mm Gel Pen
9	REBNOK Ultra Grip
10	PAPER:MATE REYNOLDS 045 (0.8 mm)
11	LANCER Wave 0.5mm. 825 W
12	QuanTumGeloPlus⁺ Curve 125
13	Orange FOR MEN
14	Pentel ENERGEL Loquid Gel Ink Needle Tip 0.5 mm
15	YAYA HANS&JANE@2006 BIN's
16	FABER-CASTELL TRUE GELL (0.7 mm)
17	PAPER:MATE InkJoy 100 XF
18	g'soft SUPER GRIP 0.28
19	Java e-office ball (0.7 mm)
20	UCAN 0.5 GP-007
21	BIC Xtra EZ+ 0.7 BLU
22	Horse Hand-Cuptal N500

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23	UD Intense Gel 0.5 mm
24	g'soft GS007 0.38-BLUE
25	QuanTum SKATE 114 CANDLE
26	Uni JETATREM 101 0.7
27	PAPER:MATE InkJoy 500 RT XF
28	Uni JETATREM SX-210
29	M&G AGP12371 0.7 mm
30	FABER-CASTELL BALL PEN 1423 0.7

Purchased from local markets (May-July 2017)

All pens were allocated reference number during this study. Each pen was used to write the author's name two times on a piece of A4 white paper (Double A, 80 gram.) to be consistent with the documents at crime scene. After that, the inks entry was punch a size circle 5 mm diameter 5 holes from each sample that was used as substrate for depositing inks.

The following chemicals were used in this study: ethanol, acetone, dichloromethane, *n*-butanol, water, ethyl acetate, cyclohexane, methanol, ammonia and toluene. All chemicals were used without any further modifications.

2.2 Extraction of ink from papers

In this experiment, three different solution including ethanol, acetone and dichloromethane were extract ink from paper. Take 5 pieces of one sample per 1 hole, put into spot plate porcelain and write the number on the hole. Add 0.2 ml extract solution into the hole waiting about 20 seconds. Observe the color and concentration of the solution. The extracts were used for further study.

2.3 Thin-layer chromatography

TLC was carried out using TLC-cards with layer thickness 0.2 mm and 2×5 cm aluminum cards. Each extracts was placed in the origins point at 1 cm from the bases of the plates which was marked with pencil. The distance between samples was 3 mm. Five solvent systems were used as mobile phase as shown in Table 2. The distance of each appearance spot in each sample as well as solvent front were recorded for R_f values calculation.

Table 2. Mobile phase systems used in TLC method.

No.	Mobile phase system	Ratio
1	*n*-butanol: ethanol: H_2O	50:15:10
2	ethyl acetate: cyclohexane: methanol: NH_3	70:15:10:5
3	ethyl acetate: *n*-butanol: NH_3	60:35:30
4	ethyl acetate: ethanol: H_2O	70:35:30
5	Toluene: acetone: ethanol: NH_3	30:60:7:2

*The chosen solvent system from Djozan et al. (2008)

2.4 Data analysis

All the statistical analysis was carried out using statistical package for the social sciences (SPSS). Discrimination analysis was conducted based on the data obtained from 3 extractions and 5 mobile phase systems. Two-way ANOVA was conducted to determine the effect from three solution (ethanol, acetone and dichloromethane) for ink extraction and five different mobile phase systems for inks classification. The number of pair was achieved as follows (Zlotnick & Smith, 1999).

$$Number\ of\ pairs = n\ (n-1)/2$$

An observational study was designed so that 30 varieties of ballpoint pens, there were 435 possible pen pairs. Any pair would be labeled as distinguish by discriminating power (DP) which was defined as a ratio of number of differentiated pairs of samples with respect to the total number of all calculated (Smalldon & Moffat, 1973), using the following equation:

$$DP = \frac{Number\ of\ discriminated\ pairs}{Number\ of\ possible\ pairs}$$

3. Results and Discussion
3.1 Thin-layer chromatography analysis

Retention factor (R_f) and color tones of the bands separated by different mobile phase system were used to discriminate inks in blue ballpoint pen. The technique described in this paper was effective in identifying type of the ink used in commercial pen. Figure 1 and 2 show the results of TLC that using ethanol as extract solvent with different mobile phase systems.
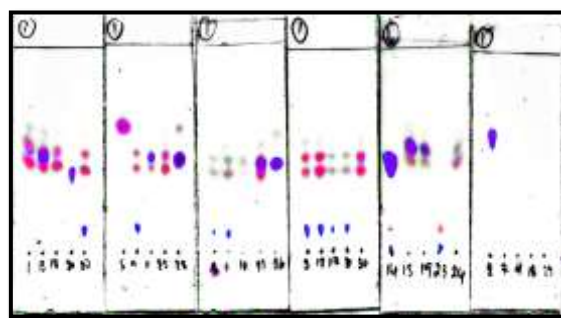
Figure 1. TLC plate containing 30 blue inks extracted by ethanol using system 1 (n-butanol: ethanol: H_2O) as mobile phase.
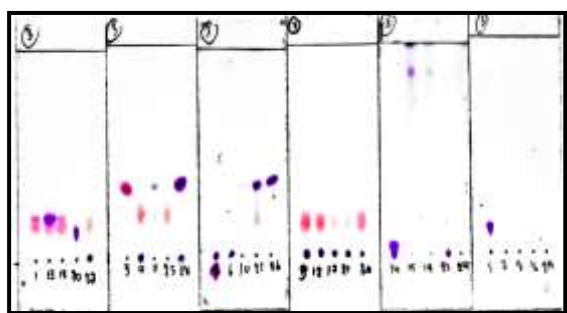
Figure 2. TLC plate containing 30 blue inks extracted by ethanol using system 3 (ethyl acetate: n-butanol: NH$_3$) as mobile phase.

Descriptive statistics are commonly used for summarizing data frequency or measures of central tendency (mean and median). Frequency analysis is a descriptive statistical method that shows the number of occurrences groups of each different extraction and mobile phase systems in TLC techniques. Based on the statistical analysis, each experiment can classify 30 inks into several groups as shown in Table 3.

Table 3. Classification result from each experiment, with different extract solvent and mobile phase system.

Mobile phase system	Extract solvent	Number of groups
1	Ethanol	12
	Acetone	9
	Dichloromethane	8
2	Ethanol	6
	Acetone	9
	Dichloromethane	8
3	Ethanol	10
	Acetone	9
	Dichloromethane	7
4	Ethanol	6
	Acetone	6
	Dichloromethane	5
5	Ethanol	7
	Acetone	5
	Dichloromethane	6

From Table 3, it is obvious that the TLC method using ethanol as the extract solvent and developed with mobile phase system 1 got the most separated pattern from thirty different individual pens. The collected data was able to classify 30 blue ballpoint

pens into 12 different groups as shown in Table 4.

Table 4. TLC results of blue inks, with ethanol extracts and mobile phase system 1.

Group	Color	R$_f$	Pen
1	Pale Blue	0.11	3,4,6,9,12,
	Purple	0.40	17,21,27
	Purple	0.46	
	Pale Purple	0.53	
2	Blue	0.43	26,28
	Purple	0.57	
3	Purple	0.43	15,19,24
	Blue	0.49	
	Purple	0.53	
	Pale Purple	0.60	
4	Purple	0.43	1
	Purple	0.53	
	Pale Purple	0.64	
5	Pink	0.21	5
	Blue	0.57	
6	Pale Pink	0.23	20
	Blue	0.57	
7	Pale Blue	0.03	14
	Pink	0.14	
	Blue	0.46	
8	Pale Blue	0.03	23
	Pink	0.14	
9	Pale Purple	0.43	9
	Pale Purple	0.49	
	Purple	0.60	
10	None	0.00	7,8,16,29
11	Pale Purple	0.37	10,1,25,30
	Pale Blue	0.46	
	Pale Purple	0.53	
12	Purple	0.37	11,13,22
	Blue	0.41	
	Pale Purple	0.46	
	Pale Purple	0.53	

3.2 Evaluation results of analysis

In statistical tests, the two-way analysis of variance (ANOVA) was used to compare the effect of TLC results between different extract solvent and variant mobile phase systems. In this study, the independent variable was extract solvent and mobile phase system and dependent variable was the TLC results. Each R$_f$ value from 30 blue ballpoint pen inks of each experiment was calculated to determine whether the interaction effect between three solvents and five mobile phase systems

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was statistically significant at a level of 0.05. In addition, this study also concludes the following, based on the *p*-values:

• The *p*-value for extract solvent was .224, which indicates that different of extract solvent were not associated with TLC results.

• The *p*-value for mobile phase systems was .000, which indicates that variant solvent were associated with TLC results.

• The *p*-value for the interaction between extract solvent and mobile phase systems was .000, which indicates that the relationship between extract solvent and mobile phase system depends on the variant of mobile phase system.

From profile plots (Figure 3), except the mobile phase system 1, there are no intersection between graphs. It indicates that there was a difference in mean of TLC results in all groups of extraction and no interaction between extraction and mobile phase systems. However, further subgroup testing is required.
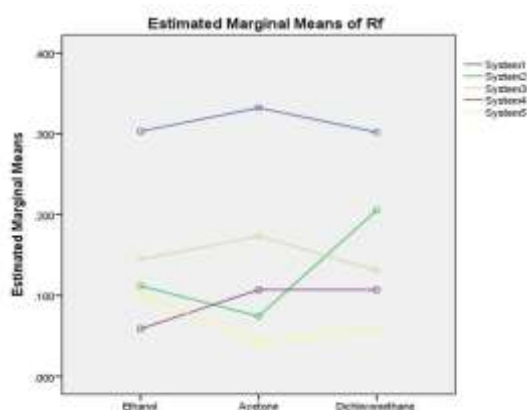

Figure 3. Estimated marginal means of sum R_f .

The results before subgroup testing provided information that the difference mobile phase systems influenced the effect of ink separation. The effect between groups of mobile phase systems was determined by one-way ANOVA (*p<.05*). First, subgroups were created by isolation of the extract solvent (ethanol, acetone and dichloromethane) then ANOVA analysis for each mobile phase system was done.

Mobile phase system 3 and 5 showed Sig. value of .372 and .073, respectively. The *p*-value in there groups was more than .05, conclude that there were not statistically significant difference between subgroups of extract solvent. In the other hands, mobile phase system 1, 2 and 4 shows Sig. value were .000, .000 and .002. The *p*-value in there groups were less than .05; indicates that there was

a statistically significant difference between subgroups of extract solvent. The effect of ink separation in mobile phase system 1 was a statistically significant difference between ethanol /acetone extraction, and ethanol /dichloromethane extraction. The mobile phase system 2 was a statistically significant difference between ethanol / dichloromethane extraction, and acetone /dichloromethane extraction. And the mobile phase system 4 was a statistically significant difference between ethanol / acetone extraction, and acetone /dichloromethane extraction. Next, subgroups were created by isolation of the mobile phase systems then ANOVA analysis for each extract solvent was done. The results were a statistically significant difference in all groups. Descriptive by Post Hoc multiple comparisons were concluding as follows:

• A group of ethanol extracts, the mobile phase system 3 was difference from system 4, and system 1 was difference from other systems at statistically significant level of 0.05.

• A group of acetone extracts, no statistically significant difference between mobile phase system 2 and 5, but the mobile phase system 1 difference from other systems.

• A group of dichloromethane extract, the mobile phase system 1 difference from other systems but no statistically significant difference between mobile phase system 3, 4 and 5.

In addition, the standard deviation plot was checked for shifts in scale by using mean plots, showing mean varies between different groups of data. In the sample plot below, different extraction groups in all mobile phase systems were shown.
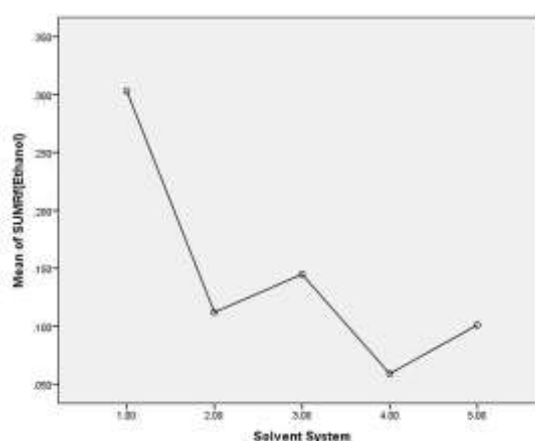
Figure 4. Mean plot of results from ethanol extract all mobile phase systems.
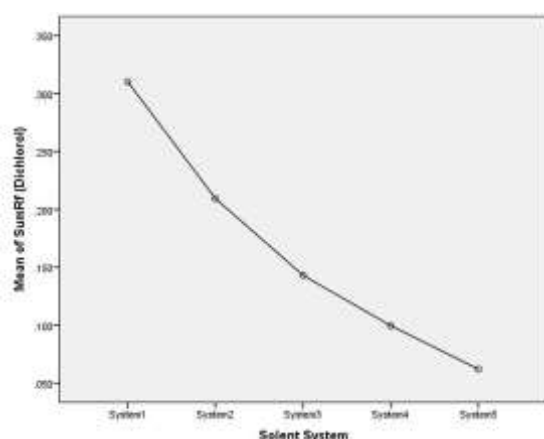
Experiments of ethanol extract with 5 mobile phase systems found difference pattern of ink

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separation as shown in Figure 4. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 3, 2, 5 and 4, respectively.
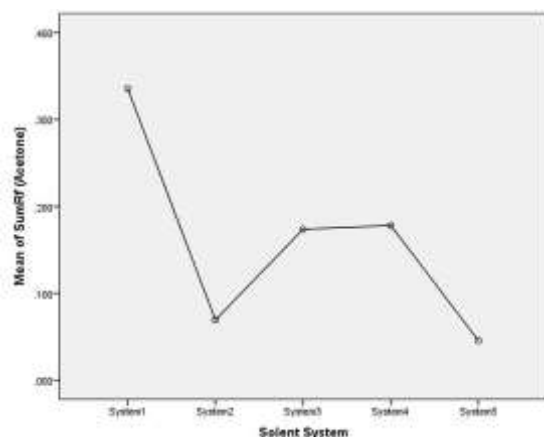
Figure 5. Mean plot of results from acetone extract all mobile phase systems.

Experiments of acetone extract with 5 mobile phase systems found difference pattern of ink separation as shown in Figure 5. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 3, 4, 2 and 5, respectively.

Figure 6. Mean plot of results from dichloromethane extract all mobile phase systems.

Experiments of dichloromethane extract with 5 mobile phase systems found difference pattern of ink separation as shown in Figure 6. The sequence difference in ink separation indicates that system 1 was the most distinguishable, followed by system 2, 3, 4 and 5, respectively.

3.3 Definite discrimination power (DP)

The other way to differentiate various blue ballpoint pens was evaluated by comparing the couple of different inks. The comparison between all possible binary combinations of 30 studied inks, for 450 cases, was done. As a result for ethanol extract using mobile phase system 1, 2, 3, 4 and 5, 388, 324, 381, 316 and 349 pairs were differentiated (Figure 7). The results for acetone extract using mobile phase system 1, 2, 3, 4 and 5, 385, 383, 355, 294 and 320 pairs were differentiated. The last group was a result for dichloromethane extract using mobile phase system 1, 2, 3, 4 and 5, 371, 359, 328, 318 and 353 pairs were differentiated.

In order to show the possibility to differentiate the examined inks by this method the discriminating power (DP) was calculated according to Eq. (2). In this method, the DP was achieved as follows in Table 5.

Table 5. DP of groups in each different extract solvents and mobile phase system (unit: percent).

Extract	Mobile phase system				
	1	2	3	4	5
Ethanol	89.20	74.48	87.59	72.64	80.23
Acetone	88.51	88.05	81.61	67.59	73.56
Dichloro-methane	85.29	82.53	75.40	73.10	81.15

The result DP showed that ethanol extraction with mobile phase system 1 successfully differentiated 47 pen-pair formed from 30 varieties of blue ballpoint pen. The approach proposed here is the effective tool for ink separation and discrimination, consistent with Lee et al. (2015).
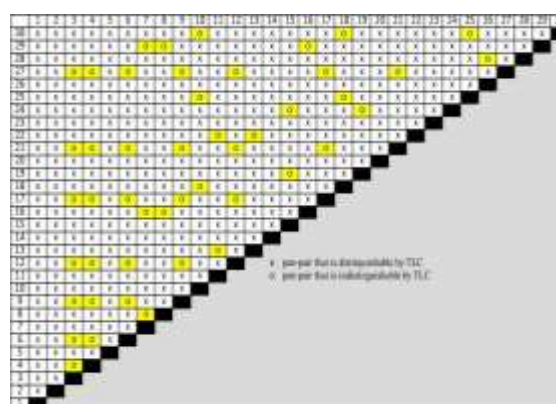
Figure 7. All possible combination of comparing inks with TLC using ethanol extract with mobile phase system 1 as a mobile phase.

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4. Conclusions

The aim of this study is to compare the effectiveness of blue ballpoint pen ink separation by TLC between different solvent extraction and variant mobile phase systems. Two-way ANOVA analysis found that both extract solvent and mobile phase systems were influence to TLC results. The relationship between extract solvents and mobile phase systems depends on the variant of mobile phase systems. The estimated marginal means graph, one-way ANOVA analysis and mean plot graphs, proved that the most distinguishable TLC result of blue ballpoint inks was that using ethanol as the extract solvent and using mobile phase system 1, which consist of n-butanol: ethanol: water (50:15:10 v/v/v), as the developing agent. This combination could classify 30 blue ballpoint pens into 12 different groups with the discrimination power (DP) of 89.20%. This experiment was found to be useful in classification and individualization of a questioned ink from a database through calculating R_f value. In the future, the qualitative data from TLC plates will be converted into quantitative data by using certain analysis software. Therefore, higher DP could be achieved while multivariate statistical techniques can also be applied on data interpretation and lead to development of blue ballpoint pen ink test kit for used in the crime scene.

5. Acknowledgement

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Anthropogenic Impacts on Cave-roosting Bats: A Call for Conservation Policy Implementation in Bukidnon, Philippines

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Abstract

Many caves in the Philippines are amongst the most popular natural ecotourism sites, even though most of them are poorly regulated and understudied. This study investigates the anthropogenic impacts of unsustainable eco-tourism and exploitation on cave-roosting bats in Sumalsag Cave, Bukidnon, Mindanao Island, Philippines. The species richness of the cave-roosting bat fauna was determined using the standard mist-netting method and capture-mark and release technique. The conservation status was assessed based on the International Union for Conservation of Nature (IUCN) Red List. Ecological evaluation and assessment of the cave's speleological characteristics and ecological condition was carried out using the Philippines' standard cave assessment protocol. After completing 15 net-nights field sampling with a capture effort of 180 net/hours, results revealed a total of six (6) species of cave-roosting bats which belongs to three (3) families. Two Philippine endemic species, Ptenochirus jagori and Ptenochirus minor were documented including Miniopterus schreibersii, a species classified under near threatened category. Evidence of human activities were considered for identifying the indirect and direct threats on the bat fauna. Destroyed speleothems and speleogens, excavations, modifications of the cave's features as well as graffiti in the cave walls were recorded. This study recommends regulating eco-tourism activities, protecting the endemic and threatened species and promoting natural restoration of the cave by implementing the existing environmental laws.

Keywords: Threatened species, Chiroptera, Ecotourism, Conservation, Mindanao

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1. Introduction

Over 1,500 caves have been recorded in the Philippines by the Department of Environment and Natural Resources (DENR) and approximately 37% of the caves are found in the second largest island in the archipelago, Mindanao (Department of Environment and Natural Resources, Protected Areas and Wildlife Bureau [DENR-PAWB], 2008). Despite being used in the country's ecotourism, only 42 new caves were assessed in Mindanao based on the DENR Memorandum Circular No. 2007-04 (Department of Environment and Natural Resources, 2013), the remaining 92% remains poorly known and understudied. Caves serves as important habitat for diverse and unique fauna and home for some of critically endangered species of bats such as the Philippine bare-backed fruit bat, *Dobsonia chapmani,* the country's largest cave-roosting and the first mammal declared to be extinct in the Philippines in 1996, but was rediscovered in 2003 by Paguntalan, Pedregosa, and Gadiana (2004). Out of 79 bat species, 49 species of Philippine bats are roosting in caves

(Heaney et al., 2010). Regardless of their ecological and economic importance, many caves in the Philippines are exploited resulting to damages and degradation in the caves' physical characteristics (DENR-PAWB, 2008). Anthropogenic activities like ecotourism and guano mining have resulted to destruction of speleothem, presence of garbage, vandalism, manmade holes and existence of some religious structure in the caves (Tanalgo, Teves, Salvaña, Baleva, & Tabora, 2016). The impact on the resident cave fauna however, is not yet clearly elucidated. Caves fulfill an important role for the survival of bats. Many of which have been widely considered as keystone species. The cave's extremely specific temperatures, patterns of air circulation, physical structures and feeding sites are relatively rare which makes suitable roosting sites for many bats populations (McCracken, 1988). The largest cave in province of Bukidnon and longest cave in Northern Mindanao is the Sumalsag cave system. It is a karst type of cave located in Mt. Palaopao situated between the municipalities Manolo Fortich and

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Sumilao in the province of Bukidnon. The cave is home to some species of cave-roosting bats which are currently being exploited by some local residents as a source of protein. Aside from the unregulated harvesting of guano, illegal mining, treasure hunting, and unauthorized wildlife poaching, the cave is also promoted as one of the province's eco-tourism site. Despite the cave's economic importance, its bio-speleological aspects remains unreported in scientific literature and the various anthropogenic impacts to the cave-roosting bats population badly needs assessment for conservation measures. Hence, this research was conducted to provide the first report on the chiropteran fauna of the Sumalsag Cave which can be utilized by the local government units for implementing environmental laws and policies.

2. Materials and Methods

2.1 The study site

Cave exploration and field investigations were conducted from January 2014 to October 2014 in Sumalsag Cave system situated between Barangay Vista Villa, Sumilao and Dalirig, Manolo Fortich, Bukidnon, Mindanao Island, Philippines (Figure 1).

Figure 1. Location map of study site.

It is geographically situated at 08°21'18"N longitude and 124°55'04"E latitude with 642 meters above the sea level elevation. The air temperature in the cave ranges from 23ºC-24ºC while the relative humidity was recorded ranging

from 76.5% to 84.6%. It is a limestone or karst cave with a distance of approximately 1,859 meters from the cave's main entrance to its exit (Figure 2).

Figure 2. Entrance of Sumalsag Cave.

The cave is surrounded by secondary forest and agricultural vegetation like corn fields and pineapple plantations (Figure 3).

Figure 3. Cleared vegetation and agricultural areas near the cave.

2.2 Sampling method

Five mesh nylon mist nets measuring 12m x 4m x 36mm (Figure 4) were used for capturing the bats during the sampling for three consecutive nights for a total of 15 net-nights.

Figure 4. Mist nets used in capturing the bats.

Five mist nets were placed inside the main cave; the first mist net was placed in the entrance of the main cave and the four others were positioned inside in every chamber of the cave. Exit counts were used in counting the species of bats that left the

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cave for 2 consecutive nights, starting at 1700 h up to 2200 h. A total of 15 net-nights were considered for the whole duration of field sampling using the formula of Sedlock, Ingle, and Balete (2011):

- Number of net-nights = number of nets left open X number of nights in operation

Capture effort was likewise computed using the formula of Medellin, Equihua, and Amin (2000):

- Capture effort $== \sum nets \ x \ \sum hours$

Small pieces of thread were tied to the tarsus of the captured bats. Morphometric data were taken including the sex, age class and different body parameters. After which, they were fed with sugar solution before being released. The key to the bats of Mindanao Island by Ingle and Heaney (1992) was used for the taxonomic identification. Female bats were determined based on the presence of nipples and the examination for a single pair of mammary glands in sub-axillary position, while the males were identified by the presence of a conspicuous penis (Heaney et al., 2010). Age class classification of the bats was based on the ossification of the joints of the wing. The juveniles were identified with swollen joints that were not ossified, sub-adult if partially ossified and lastly fully grow adult if the joints were knobby and fully ossified (Anthony, 1988). Representative voucher bat were euthanized with lidocaine. A gratuitous permit was secured from the Protected Areas and Wildlife Bureau of Department of Environment and Natural Resources in Region 10 for legal collection of voucher specimens. One to two specimens per species were collected for taxonomic confirmations. All collected specimens were labeled properly and deposited in the Zoology Section of the University Museum of Central Mindanao University. Conservation status of identified bats species was based on International Union for Conservation of Nature (2017).

3. Results and Discussions
3.1 Species composition of cave-roosting bats

After completing 15 net-nights with a capture effort of 180 net/hours during the sampling period, a total of 38 individuals from two suborders, three families, five genera and six species of bats were documented in the study site (Table 1). These include members of the family Pteropodidae represented by four species namely, *Ptenochirus*

jagori, *Ptenochirus minjor*, *Eonycteris spelaea* and *Rousettus amplexicaudatus.* Both Rhinolophidae

and Vespertilionodae were represented by a single species. The only rhinolophid bat recorded was *Rhinolophus arcuatus-s.* On the other hand *Miniopterus schreibersii* was the only vespertilionid bat documented in this study.

Table 1. Species composition of cave-roosting bats in Sumalsag Cave System.

Suborder	Family	Species
Megachiroptera	Pteropodidae	*Eonycteris spelaea*
		Ptenochirus jagori
		Ptenochirus minor
		Rousettus amplexicaudatus
Microchiroptera	Rhinolophidae	*Rhinolophus arcuatus-s*
	Vespertilionidae	*Miniopterus schreibersii*

3.2 Annotated taxonomic account of species composition with description;
Suborder: Megachiroptera
Family Pteropodidae

- *Eonycteris spelaea* Dobson, 1871
(Figure 5 A)
Common name: Common Dawn Bat
Morphometric measurements: head and tail length, 121-126 mm; head and body length, 106-111 mm; forearm length, 71-80 mm; ear length, 18-21 mm; tail vent length, 12-16 mm; and hind foot length 13-16 mm. This bat has 4 upper incisors. A pair of kidney-shaped glands can be found lateral to the anus.

- *Ptenochirus jagori* Peters, 1861 (Figure 5 B)
Common name: Greater Musky Fruit Bat
Morphometric measurements: head and tail length, 124-127 mm; head and body length, 110 mm; forearm length, 82-84 mm; ear length, 18-20 mm; tail vent length, 14 mm; and hind foot length, 16-17 mm. The *P. jagori* has a short muzzle, two lower incisors and four upper incisors. This species have a dark yellow fur on its upper back.

- *Ptenochirus minor* Yoshiyuki, 1979
(Figure 5 C)
Common name: Lesser Musky Fruit Bat
Morphometric measurements: head and tail length, 102mm; head and body length, 94 mm; forearm length, 69 mm; ear length, 13 mm; tail vent length, 8 mm; and hind foot length 12 mm. This species of bat has a short muzzle, have 4 upper incisors and 2 lower incisors with light yellow fur on its upper back.

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- *Rousettus amplexicaudatus* Geoffroy, 1810 (Figure 5 D)

Common name: Geoffroy's Rousette

Morphometric measurements: head and tail length, 128-133 mm; head and body length, 113-119 mm; forearm length, 80-87 mm; ear length, 18-21 mm; tail vent length, 12-16 mm; and hind foot length, 16-19 mm. This bat has a moderately long and tapered muzzle, having 4 upper and 4 lower incisors.

Suborder: Microchiroptera

Family Rhinolophidae

- *Rhinolophus arcuatus-s* Peters, 1871 (Figure 5 E)

Common name: Arcuate Horseshoe

Morphometric measurements: head to tail length, 68-69 mm; head and body length, 52-55 mm; forearm length, 42-43 mm; ear length, 19-20 mm; tail vent length, 16-17 mm; and hind foot length, 9-10 mm. The characteristic feature of this bat is a posterior pointed nose-leaf.

Family Vespertilionidae

- *Miniopterus schreibersii* Kuhl, 1817 (Figure 5 F)

Common name: Common Bent-wing

Morphometric measurements: head to tail length, 103-106 mm; head and body length, 55-59 mm; forearm length, 42-44 mm; ear length, 11-12 mm; tail vent length, 46-48 mm; and hind foot length; 9-10 mm. Its feet and wrist of has no pads.
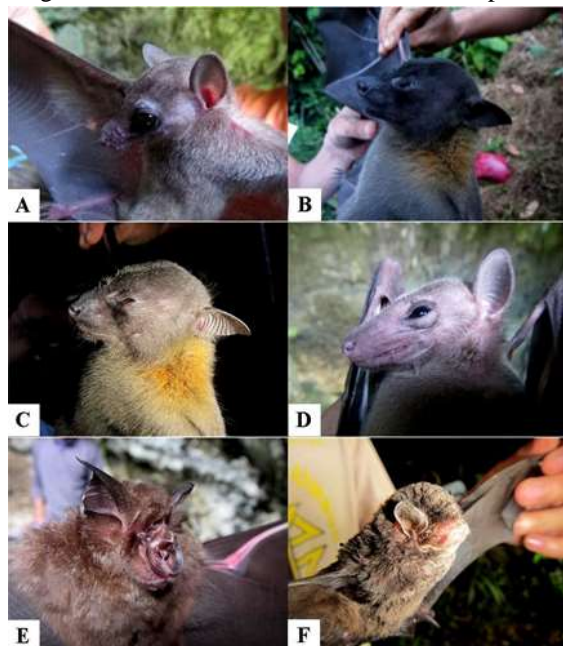

Figure 5. Species composition of bats: **A**. *Eonycteris spelaea* Dobson, 1871; **B**. *Ptenochirus jagori* Peters, 1861; **C**. *Ptenochirus minor* Yoshiyuki, 1979; **D**. *Rousettus amplexicaudatus* Geoffroy, 1810; **E**. *Rhinolophus arcuatus-s* Peters, 1871; **F**. *Miniopterus schreibersi* Kuhl, 1817.

In terms of conservation status, five (5) cave-roosting bat species were categorized as least concerned, these includes *E. spelaea*, *P. jagori*, *P. minor*, *R. amplexicaudatus*, and *R. arcuatus-s*. The verpertilionid bat, *M. schreibersii* is the only species under near threatened category. Two species were considered endemic namely, *P. jagori* which is Philippine endemic, and *P. minor* a Mindanao Island endemic species.

3.4 Assessment of threats in the cave

Based on the assessment, indirect and direct threats to the cave-roosting bats were evident in the area. Indirectly, the cave-roosting bat population faces decline due to negative impacts of anthropogenic activities (Figure 6) such as mining (Figure 7), vandalism (Figure 8) and destruction of cave's physical characteristics (Figure 9) brought about by unregulated ecotourism activities.

The relatively low species composition of cave-roosting bats could be attributed to the presence of various anthropogenic threats and ecological pressures such as changes and disturbances in habitats (Kasso & Balakrishnan, 2013). There is evidence of these activities on the roost sites being linked to the rapid decline of several species of cave roosting bats population (Elliott, 2000).
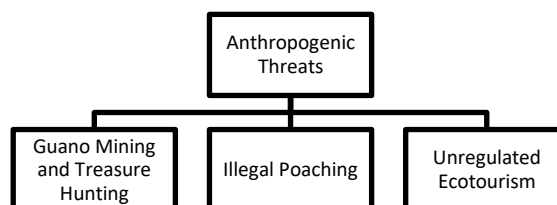
Figure 6. Anthropogenic threats**.**

3.4.1 Guano mining and treasure hunting

Unlawful human activities appeared to be the main threats and cause of destructions in the cave. The excavation inside the cave by treasure hunter and the over collection of guano resulted in many hazardous holes and destruction of the cave's natural structure. Alteration of the cave natural features results in changes in the natural structure of the roost sites as well as affects the flow of the internal climate condition of the cave thereby threatening the cave roosting bats (International Union for Conservation of Nature, 2014). Incidental disturbance and disruptive guano

harvesting may also contribute to the declining bat population (Furey & Racey, 2016).

Figure 7. Excavations inside the cave.

3.4.2 Bats poaching

Resident cave bats are directly threatened by illegal poaching by the locals using improvised fish nets and guns for bat meat as source of protein. Illegal poaching inside the cave by locals for meat using fishing net, sticks and guns may also exacerbate the rapid decline of bat population (Jenkins & Racey, 2008; Kingston, 2010) and is considered as one of the main threats induced by locals to the roosting bats (Tanalgo et al., 2016). These activities eventually blocked every possible flyways of bats that resulted in massive mortalities. In fact, decaying bodies of bats were discovered hanging in the fishing net placed by the local poachers in the main entrance and exit of the cave. This may partly explain why only adult bats were captured and bat emergence was not observed. Declining of bats population could affect the balance in the ecosystems since they act as prey and predator, pollinators, seed dispersers of economically important plants and plays an important role as natural pest control (Fujita & Tuttle, 1991; Hodgkison, Balding, Zubaid, & Kunz, 2003; Kunz, De Torrez, Bauer, Lobova, & Fleming, 2011).

3.4.3 Unregulated ecotourism

Welcome signage and rules and regulations sign were found in the cave. However, the presence of garbage, destroyed speleothems, graffiti and soil trail were observed indicating signs of negative visitor impact. Infrastructures like religious grottos were found in the entrance of the cave as a further result of habitat modification. These habitat modifications and human activities can negatively impact both bats population and the cave natural features (Tanalgo et al., 2016). Influx of unguided tourist in the area resulted in vandalism in the cave walls (Figure 8). Based on interviews with local people in the community, destruction of the cave's

stalagmites and columns was done on purpose to create easy access to the narrow chamber inside the cave.

Figure 8. Vandalism on the cave walls.

One of the primary reason for the decrease of cave-roosting bats population have been reported to be frequent human activities inside the cave (Martin, Leslie, Payton, Puckette, & Hensley, 2003). Aside from the destruction in the cave's natural features, the bright lights produced by tourists also disturb the resident cave-roosting bats. It forbids the bats to roost inside the cave (Agosta, 2002; Aul, Bates, Harrison, & Marimuthu, 2014) and may cause higher death rates in young bats (McCracken, 1989). Such disturbances may cause the bats species to leave the cave resulting in deep effects to ecological services the bats offer like natural pest control, forest reforestation and greatly affects the agriculture sectors (Hodgkison et al., 2003; Jones, Jacobs, Kunz, Willig, & Racey, 2009; Pennisi, Holland, & Stein, 2004). It is unfortunate that the economic importance of bats in providing ecosystem services vital for natural forest succession remains unappreciated.

4. Conclusion

The unregulated ecotourism activities in the cave have many negative impacts to the cave and the cave-roosting bats. Poor management and implementation of the environmental laws resulted in destruction of various natural features of the cave. This may explain the relatively low species richness of cave-roosting bats which is exacerbated by illegal hunting for meat by some locals. This study recommends the proper implementation of the existing environmental laws and local guidelines to protect and conserve all wildlife especially the endemic and threatened species.

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