



Presence of important agro-economic fungi in common frugivorous bats from southcentral Mindanao, Philippines

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Abstract

Bat association to microorganisms such as fungi has become a concern to many wildlife ecologists. This study aimed to identify fungal species present from common frugivorous bats in southcentral Mindanao, Philippines. Agro-economically important fungal species were sampled from different body parts of common fruitbats. The fungal samples were grown and purified on Sabouroud Dextrose Agar medium. A total of 58 fungal isolates under three fungal genera (*Aspergillus*, *Penicillium*, and *Fusarium*) were morphologically identified. Among fruitbats, *Cynopterus brachyotis* has the highest number of isolates. While *Aspergillus* was the most common fungal group recorded among fruitbats. Consequently, the result of this study will open the investigation on the roles of agro-economic fungal groups in the ecology and conservation of frugivorous bats.

Key words – Chiroptera – Fungal Diversity – *Fusarium* – Orchard

Introduction

The role played by microorganisms in the environment is crucial in maintaining the balance and integrity of the process in different ecosystems (Zak et al. 2003, Baird et al. 2004). The high adaptive capacity of fungi to thrive in a wide range of suitable ecological niche for microbes enable them to survive and spread in almost all habitats in the biosphere, these include animal bodies, as symbionts, parasites, or vectors (Dunne 2001, Antia et al. 2003). As a consequence, these microbial associates may pose either beneficial or detrimental impact to the behavior of its host and the ecosystem where they live (Glick 1995, Beard et al. 2002, Partida-Martinez & Hertweck 2005, Rosenberg et al. 2007).

Among these microorganisms, fungi are one of the most successful symbionts (Alberti et al. 2001, Fisher et al. 2012). In the tropics, the majority of crop infection are primarily caused by the fungi (Drenth & Guest 2016). The degree of contamination is enhanced by anthropogenic

activities and the presence of animal hosts and vectors in the environment (Burdon & Chivers 1982, Feng et al. 1990, Anderson et al. 2004). Furthermore, the high tolerance of fungi to wide climatic range allow them to infect crops in different stages and potentially produce toxins (Aziz et al. 1998, Polišenská et al. 2008, Pessu et al. 2011, Anjorin et al. 2013, Atanda et al. 2013).

Bats have established a well-known relationship with the ecosystem and human communities (Jones et al. 2009, Schneider et al. 2009, Tanalgo et al. 2016). In the tropics, bats provide a diverse ecological service such as pollination, seed dispersal, and insect pest control which directly or indirectly benefits humans (Jones et al. 1999, Kunz et al. 2011). Though bats provide various essential ecosystem services their potential as a reservoir of pathogenic microorganisms are important to be elucidated (Calisher et al. 2008, Leendertz et al. 2015). The ability of bats of true flight allows them to exploit wide distances making them a potential 'driver' of microbial transmission (Calisher et al. 2006, Calisher et al. 2008).

The association of fruitbats with microorganisms and pathogens is highly linked to its high immunity towards infection and developed resistance against pathogens (Calisher et al. 2006, Calisher et al. 2008, Leendertz et al. 2015). This makes fruitbats as a good candidate reservoir of diverse microbes including fungi (Seelan et al. 2008, Voyron et al. 2011). This highlights the importance of fungal detection on animals especially bats, which are capable of traversing different habitat types and can possibly transfer microbes in wider areas. The impact of an animal-borne disease could be easily mitigated if the source of the disease is known, contact to animal vectors is lessened, and the ecology of the association is understood.

In the Philippines, studies on bat diversity and distribution are well-documented (Heaney et al. 1998, Sedlock et al. 2014). Information on bat-fungi association is however, understudied in the country. Hence, in this study, we explored the association of agro-economic fungi (*Aspergillus*, *Penicillium*, and *Fusarium*) from frugivorous bats (fruitbats) commonly occurring from agricultural ecosystems. Fruitbats belonging to the family Pteropodidae is a diverse group comprising more than 25 species in the Philippines (Heaney et al. 1998). In southcentral Mindanao, there are approximately ten species of fruitbats known to occur in different ecosystem types such as forests, caves including agricultural (Tanalgo et al. 2012, Achondo et al. 2014, Tanalgo & Tabora 2015, Tanalgo et al. 2017). Consequently, we aim to provide baseline information on the presence of agro-economic fungi associated in fruitbats from southcentral Mindanao, Philippines hoping to further pave the way in understanding the ecology of fruitbats and their symbionts.

Materials & Methods

Bat sampling and collection

Bats were collected from a patch surrounded by orchard and fruit bearing trees such as *Mangifera indica* (Mango), *Nephellium lappaceum* (rambutan), *Garcinia mangostana* (mangosteen), *Durio zibethenus* (Durian) and *Psidium guajava* (guava) from Kabacan, North Cotabato, Philippines (southcentral Mindanao).

Bats were sampled using a monofilament mist nets (12 × 6 m) established from 1800H and regularly checked every 20 minutes until 0100 H. The collected fruitbats samples were identified morphologically using the keys of Ingle & Heaney (1992). Samples were marked to avoid recapture and field standard measurements were taken (Seelan et al. 2008). Insectivorous bats captured were excluded from the measurements and identification. To minimize the physiological and physical stress on bats, only a minimal number of fruit bat individuals were sampled. After swabbing and measurements, captured fruit bats were released at the study site.

Fungal isolation and identification

Fungi were sampled from the anus, ear, wing and mouth through swabbing. The sterile cotton swabs were inoculated into screw-capped tubes containing 900 µl of Buffered Peptone Solution (BPS) to prevent samples from drying. An aliquot of 0.1 mL was spread on Potato Dextrose Agar

(PDA) and were incubated at 25°C to 37°C for five to seven days. The fungal mycelia of the isolates were transferred to the same medium to obtain a pure culture of the desired organisms.

Macromorphological characteristics of fungi were described. For the identification, a small tuft of mycelia was lifted from a section of the colony and placed on the glass slide. A drop of lactophenol blue was added as a stain. Prepared slides were observed under the microscope and micromorphological characteristics were described (Seelan et al. 2008). Photographs were taken using a digital camera for documentation. The strains were initially identified using keys of Raper & Fennell (1965). The identification of fungal isolates was confirmed by the resident mycologist.

Data analysis

The fungal groups were classified according to morphospecies and the number of isolates (n) represents the abundance of the morphospecies (%). The abundance presented are based on morphospecies and per fungal group. A Single-link Bray-Curtis similarity analysis using Biodiversity Pro 2.0 software (McAleece et al. 1997) was used to compare the occurrence of fungal morphospecies similarity per species and body parts.

Results

A total of 58 isolates representing 29 morphospecies of three genera were isolated from four species of fruitbats: *Cynopterus brachyotis* (Lesser Dog-Faced Fruit Bat), *Macroglossus minimus* (Tooth-Daggered Fruit Bat), *Ptenochirus jagori* (Greater-Musky Fruit Bat), and *Rousettus amplexicaudatus* (Geoffroy's Rousette). These fruitbats are known to be highly common and abundant in agricultural areas, orchards, and observed to forage from fruit crops (Achondo et al. 2014, Tanalgo & Tabora 2015). We isolated four fungal groups from different body parts of fruitbats (Tables 1, 2). In terms of the fungal groups isolated, the genus *Aspergillus* spp. is the most common group with 37 isolates under 18 morphospecies followed by *Penicillium* spp. with 13 isolates under eight morphospecies and the least from *Fusarium* spp. with four isolates belonging to a single morphospecies. In Addition, there are three undetermined isolates.

Table 1 Source of agro-economic fungal isolates from fruitbats from southcentral Mindanao, Philippines

Fungal Groups	<i>C. brachyotis</i>	<i>M. minimus</i>	<i>P. jagori</i>	<i>R. amplexicaudatus</i>
<i>Aspergillus</i> spp.	Anus, Ear, Mouth, Wings	Anus, Ear, Mouth, Wings	Anus, Ear, Mouth, Wings	Anus, Ears, Mouth
<i>Fusarium</i> spp.	Wings	Anus, Mouth	Negative	Negative
<i>Penicillium</i> spp.	Anus, Ear, Mouth, Wings	Ears, Mouth, Wings	Ears	Anus, Mouth
Undetermined groups	Mouth	Wings	Negative	Wings
Total Number	20	14	17	7

In terms of bat fungal associates (Fig. 1), *C. brachyotis* has the highest number identified with 20 morphospecies [*Aspergillus* spp. (n=8), *Penicillium* spp. (n=4), *Fusarium* spp. (n=1), and undetermined species (n=1), while *P. jagori* has 17 isolates (*Aspergillus* spp. (n=15) and *Penicillium* spp. (n=2)]. There are 12 fungal morphospecies isolated from *M. minimus* (*Aspergillus* spp. (n=6), *Penicillium* spp. (n=2), *Fusarium* spp. (n=1), and single undermined) isolate while, *R. amplexicaudatus* has the lowest fungal isolate with only six morphospecies [*Aspergillus* spp. (n=3), *Penicillium* spp. (n=2), and one undetermined (n=1)]. Noteworthy, among fungal isolates, *Fusarium* sp. is the least recorded fungal species recorded on from *C. brachyotis* (wings) and *M. minimus* (anus and mouth).

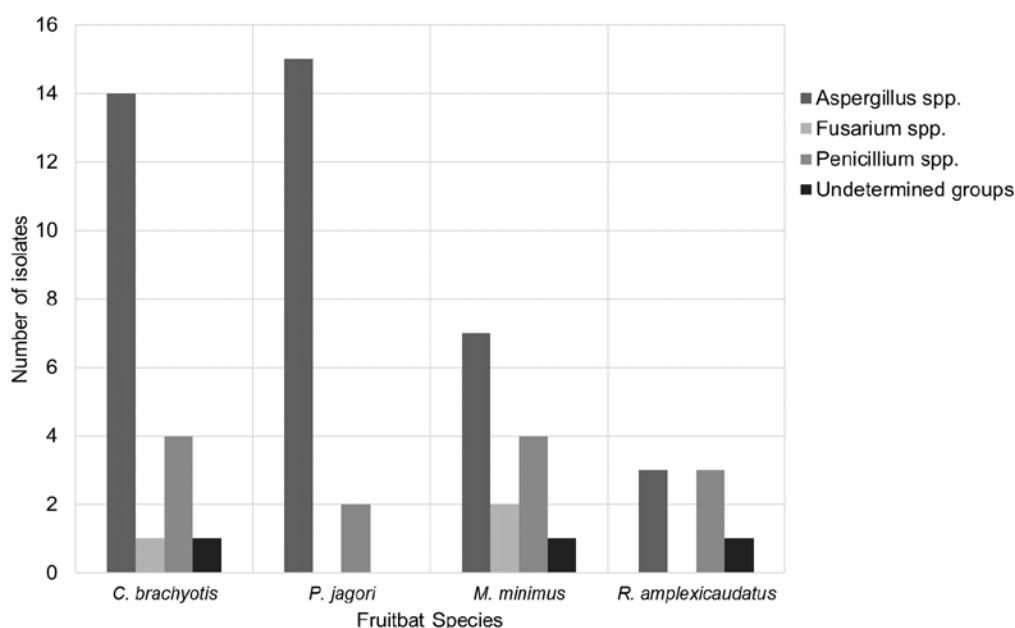


Fig. 1 – The abundance of fungal isolates among fruitbats species.

Table 2 Number of fungal morphospecies isolated from four fruitbat species southcentral Mindanao, Philippines

Morphospecies	<i>C. brachyotis</i>	<i>M. minimus</i>	<i>P. jagori</i>	<i>R. amplexicaudatus</i>
<i>Aspergillus</i> sp. 1	0	0	1	0
<i>Aspergillus</i> sp. 2	1	0	0	0
<i>Aspergillus</i> sp. 3	1	0	0	0
<i>Aspergillus</i> sp. 4	2	2	0	1
<i>Aspergillus</i> sp. 5	0	0	2	0
<i>Aspergillus</i> sp. 6	0	0	1	0
<i>Aspergillus</i> sp. 7	0	1	1	0
<i>Aspergillus</i> sp. 8	4	1	4	0
<i>Aspergillus</i> sp. 9	0	1	2	0
<i>Aspergillus</i> sp. 10	0	0	1	0
<i>Aspergillus</i> sp. 11	0	0	1	0
<i>Aspergillus</i> sp. 12	2	0	0	1
<i>Aspergillus</i> sp. 13	1	1	1	0
<i>Aspergillus</i> sp. 14	2	0	0	0
<i>Aspergillus</i> sp. 15	0	1	0	0
<i>Aspergillus</i> sp. 16	0	0	1	0
<i>Aspergillus</i> sp. 17	0	0	0	1
<i>Aspergillus</i> sp. 18	1	0	0	0
<i>Penicillium</i> sp. 1	0	0	1	0
<i>Penicillium</i> sp. 2	0	0	1	2
<i>Penicillium</i> sp. 3	1	0	0	0
<i>Penicillium</i> sp. 4	1	1	0	1
<i>Penicillium</i> sp. 5	1	0	0	0
<i>Penicillium</i> sp. 6	1	1	0	0
<i>Penicillium</i> sp. 7	0	1	0	0
<i>Penicillium</i> sp. 8	0	1	0	0
<i>Fusarium</i> sp. 1	1	2	0	0
Undetermined sp. 1	1	0	0	1
Undetermined sp. 2	0	1	0	0

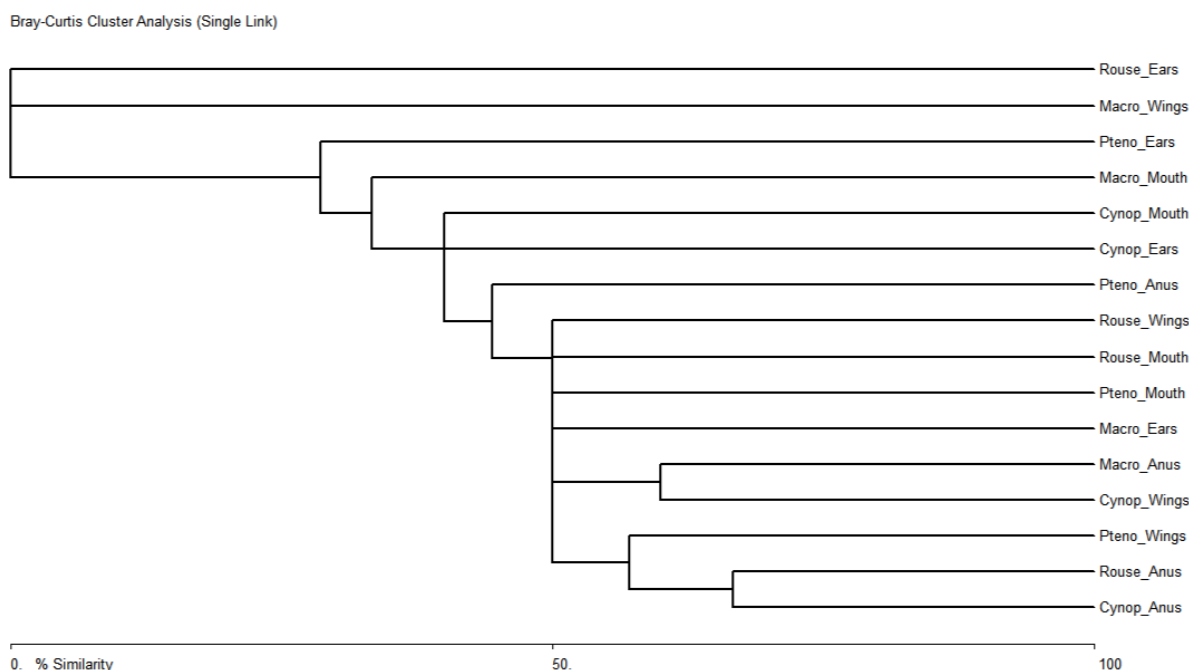


Fig. 2 – Bray-Curtis distance-based species similarity analysis of fungal groups among different body parts of fruitbats species.

Similarity analysis using single-link relationship revealed that fungal records among different species of fruitbats are generally low (less than 50%). Two species of fruitbats, *M. minimus* and *C. brachyotis* were very similar at 41.17% compared to *P. jagori* and *R. amplexicaudatus* with 27.03% and 29.63% distance similarity respectively (Table 3). In terms of similarity fungal isolates strictly based on different body parts, a higher similarity on the fungal isolates was found in the anus of both *C. brachyotis* and *R. amplexicaudatus* (66% similarity). While, there are unique isolates only found from the wings of *M. minimus* (*Aspergillus* sp. 15, *Penicillium* sp. 7 and 8) and ears of *R. amplexicaudatus* (*Aspergillus* sp. 17) (Figs 2).

Table 3 Similarity index (%) among species based on the abundance of morphospecies.

	<i>C. brachyotis</i>	<i>M. minimus</i>	<i>P. jagori</i>	<i>R. amplexicaudatus</i>
<i>C. brachyotis</i>	*	41.1765	27.027	29.6296
<i>M. minimus</i>	*	*	25.8064	19.0476
<i>P. jagori</i>	*	*	*	8.3333
<i>R. amplexicaudatus</i>	*	*	*	*

Discussion

In this study, the fungal group from genera *Aspergillus* and *Penicillium* were two of the most frequently occurring fungal genera associated to fruitbats (Fig. 1). These genera are naturally found in the air and ambient farm environment (Karwowska 2005), a similar ambient ecosystem preferred by the sampled fruitbats (Heaney et al. 1998). Additionally, the spore size of *Aspergillus* and *Penicillium* ranges at about 2.5 to 3 μm , making them easily carried and dispersed in the environment. Whilst, the genus *Fusarium* has relatively larger spore size ranging from about 5 to 13 μm and its survival depends on a specific substrate of dispersing successfully (Joffe 1986). In comparison, *Aspergillus* and *Penicillium* can thrive in a moisture reduced environment, unlike *Fusarium* that requires a high amount of higher water activity (Gibson et al. 1994, Ploetz, 2015). Hence, *Aspergillus* and *Penicillium* groups were very common from all the fruitbat species captured at the sampling site.

This study is emphasized to detect the presence of fungal groups commonly associated with crops. The growing concerns about crop contamination have become a major problem in the agricultural sector up to the present. Among all contaminants, fungi were identified as the major causative agent of crop infection. Four (4) fungal genera including *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. were grouped as ‘field fungi’ due to their high occurrence and abundance in wide agricultural areas and different crop species. In crops, the contamination is chiefly during vegetative stage up to harvest (storage) and post-harvest process. Also, two of these fungal genera, *Aspergillus* spp. and *Penicillium* spp. could further contaminate stored dried crops considering them as ‘storage fungi’ (Pit et al. 1993, Kendra 2009). In addition, there are more than 30 species of *Aspergillus* causing spoilage to crops and reducing yields (Pitt & Hocking 2009, Pitt et al. 2013). In a similar investigation on Malaysian bats, a total of six *Aspergillus* species were recorded including an opportunistic strain of *Aspergillus fumigatus*. Additionally, six *Aspergillus* species were isolated from three species of fruitbats (*Cynopterus brachyotis*, *Penthetor lucasi* and *Balionycteris maculata*) (Seelan et al. 2008, Seelan & Anwarali 2009). The isolated fungal species are considered highly important due to their capacity to produce mycotoxins and as a disease agent (Carlson & Ensley 2003, Ismaiel & Papenbrock 2015).

The genus *Fusarium* was the least isolated fungal group from fruitbats. This fungus is known to cause the wilt disease in many banana plantations in the Philippines and neighbouring Southeast Asian countries up to other tropical countries (Ploetz 2015), which was isolated in the mouth and wings of *Macroglossus minimus* and *Cynopterus brachyotis* respectively, both are known as pollinators and visitors of wild banana (Stewart & Dudash 2017). However, most of the cultivated bananas in the country are cloned and does not require pollinators, but *M. minimus* is still observed to visit naturally grown banana in orchards. Recently in the Philippines, banana industry was highly impacted by an infestation of the Banana wilt (Ordenez et al. 2015).

In addition, previous studies in Italy recorded 15 fungal species including *Aspergillus*, *Fusarium*, and *Penicillium* from insectivorous bats (Voyron et al. 2011). The habitat of bats may be another factor on how bats harbor these fungal species. For example, in Poland, 34 species of filamentous fungi were isolated from a bat cave reserve including species from *Aspergillus*, *Penicillium*, and *Fusarium* (Kokurewicz et al. 2016). Ogorek et al. (2016) also isolated *Aspergillus* and *Penicillium* from both guano and air from caves occupied by bats in Slovakia. It is also important to take note that the diet of fruitbats, which mainly consist of ripe fruits makes them possible good candidate for either fungal contamination or conduits of fungal infection from one crop to another (Sepiah 1985). These fruitbats are known visitors and forage from many fruit crops in the country including durian, rambutan, lanzones, and figs (Tanalgo & Tabora 2015).

This is the first attempt to assess the presence of important fungal groups in common fruitbats in south-central Mindanao, Philippines. The current study is only limited to the detection of specific fungal groups from different body parts and therefore, it cannot conclude the pathogenicity of these fungal groups and its effects to fruitbats as well as to the crops. The results may serve as an indicator of the impacts of different land-use changes to bat habitats. The structure of the habitat plays an important role in disease transfer and spread making it important to maintain the intactness and connectivity of agricultural lands from known bat habitats (ie primary forests). Additionally, the changing environmental conditions such as increasing relative temperature and precipitation, coupled with rapid growth of human populations and urbanization may increase the risk of disease outbreaks in the future as it alters the resistance and susceptibility of an organism towards pathogens (McKee et al. 2004, Gallana et al. 2013, Mann et al. 2015, Newbold et al. 2015). Lastly, the role played by bats in sustaining diverse ecosystem services within agricultural lands should be importantly considered. Particularly, fruitbats are important pollinators of many orchard species such as Durian (Bumrungsri et al. 2013), aside from effective seed dispersers (Kunz et al. 2011).

The assessment and analysis suggest that fruitbats harbor agro-economic fungal groups known to have important roles to different crops in the Philippines. Therefore, this study provides evidentiary support that fruitbats may be important conduits of agro-economic fungal transfer in

respect to their dietary requirements and their capacity to traverse from one habitat to another especially from forest to orchard ecosystems. Nevertheless, the study is limited and further quantitative investigations are necessary to fully understand the roles of fungal symbionts in the ecology and conservation of frugivorous bats.

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