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ABSTRACT

Durian (*Durio zibethinus*) brings in princely revenue for the fruit economy in Southeast Asia, ushering the current trend of clearing forests for durian plantations. Despite the thorny fruit's popularity and increasing bat-durian papers, not many associate their vital plant-pollinator relationship. This unfamiliarity has led to the persisting negative connotations of bats as agricultural pests and worse, a disease carrier amplified by the Covid-19 pandemic. This review focuses on the bat-durian relationship comprising botanical insights and pollination ecology in relevance to the wider pteropodid-plant interactions. The majority of the studies compiled have concluded that bats are the most effective pollinator for durian than insects. Six fruit bat species (Chiroptera: Pteropodidae) have been recorded pollinating durian flowers, with several other pteropodid species speculated to pollinate durian, including in non-native countries. Lastly, we address the research gaps for the bat-durian relationship, which can also be applied to other chiropterophilous plants.

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1. Introduction

Pollination is a vital process for flowering plants to produce seeds, creating offspring for the next generation. Animals play a major role in pollination as approximately 87.5% of the global flowering plants are pollinated by animals (Ollerton et al. 2011). Insects like bees are generally etched in the media attention and academics as pollinators of flowers, but vertebrates like bats (mammals) and birds also serve the same role but are not extensively perceived (Triplett et al. 2012; Ratto et al. 2018). Nectarivorous bats of the family Pteropodidae in the Paleotropics and Phyllostomidae in the Neotropics pollinate about 528 angiosperm species globally (Fleming et al. 2009). Flowers pollinated by bats usually conform to the chiropterophilous syndrome, which includes drab or white coloration, nocturnal anthesis, unpleasant odor and bell or tuft shape (Marshall 1983; Fleming et al. 2009). These bats not only pollinate ecologically significant plants like *Sonneratia* spp. (Paleotropic) and bromeliad plants (Neotropics) but also contribute to economically important crops such as *Durio zibethinus*, *Parkia* spp., *Musa* spp., *Mangifera indica*, *Stenocereus queretaroensis*, *Hylocereus* spp., *Agave* spp., *Ceiba pentandra* and *Coffee arabica* (Fujita and Tuttle 1991; Mickleburgh et al. 1992; Garibaldi et al. 2011; Raghuram et al. 2011 & Bumrungsri et al. 2013; Göttinger et al. 2019). Despite providing the essential pollination services to commercial plants at night,

bats are often perceived as agricultural pests, while other more destructive animals such as *Ratufa* spp. (Asian giant squirrels), *Macaca nemestrina* (Southern pig-tailed macaque), *Arctitis binturong* (Binturong) and *Sus barbatus* (Bearded pig) receive less attention for such actions (Fujita and Tuttle 1991; Aziz et al. 2017b).

One bat pollinated plant gaining popularity and revenue in Southeast Asia is the durian tree (*Durio zibethinus*), also known as the King of Fruits. The durian is a thorny fruit with a pungent odor juxtaposed for its creamy texture and unique taste (Brown 1997). According to Safari et al. (2018), the exported durian fruits brought in revenue of USD 500 million (Thailand), USD 17 million (Malaysia) and USD 21,000 (Indonesia). Its economic success can also be attributed to about 200 varieties, including Monthong, Kanyou, Chanee, Musang King, and D24. The genus *Durio* has various degrees of self-incompatibility among species, and many require cross-pollination from animals, mainly bats (Brown 1997; Bumrungsri et al. 2013; Ng et al. 2020). Common bat pollinators for durian in the region include *Eonycteris spelaea* (Cave nectar bat), *Pteropus hypomelanus* (Island flying fox), *Pteropus alecto* (Black flying fox), *Pteropus vampyrus* (Large flying fox), *Acerodon celebensis* (Celebes flying fox) and *Macroglossus minimus* (Lesser long-tongued nectar bat) (Brown 1997; Bumrungsri et al. 2013; Thavry et al. 2017; Aziz et al. 2017a & Sheherazade et al.

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2019). A recent study from Stewart and Dudash (2017b) added *Rousettus leschenaulti* (Leschenault's rousette bat) as an opportunistic nectar feeder during mass durian flowering supplementarily pollinate the durian flowers.

Aside from bats, birds and bees also visit durian flowers and may act as supplementary pollinators. The giant honey bee (*Apis dorsata*) is a more frequent visitor to durian flowers than *E. spelaea* but has a less pollination success record (Bumrungsri et al. 2009). However, *Apis dorsata* filled the pollination niche in areas where the bats are scarce and have a higher pollination success record with the Monthong durian cultivar (Wayo et al. 2018). Meanwhile, in Borneo, spiderhunters (*Arachnothera robusta* and *A. chrysogenys*) and orange-bellied flowerpecker (*Dicaeum trigonostigma*) were dusted with pollen after feeding on the nectar of *Durio grandiflorus* and *D. kutejensis* respectively, in which the latter is one of the more popular native durian species in Borneo. These *Durio* plants have flowers with both chiropterophily and ornithophily characteristics (Yumoto 2000). Although birds and bees can pollinate chiropterophilous plants, it is not as effective as the bats themselves due to floral adaptations that functions to attract and reward bats (Garibaldi et al. 2011).

This review intends to discuss the plant-pollinator interactions between bats and the durian tree. In this review, the durian tree refers to *Durio zibethinus* because it is the main species from its genus to be cultivated for large-scale agriculture. Meanwhile, the fruit bats mentioned in this review refer to frugi-nectarivorous and nectarivorous bats in general. Frugi-nectarivorous bats are fruit bats with a general plant diet, including fruit and nectar while nectarivorous bats are specialists feeding exclusively on nectar (Stewart and Dudash 2017b). In addition, we also define flying foxes as large fruit bats (*sensu stricto* genus *Pteropus* and *Acerodon*, forearm length > 120 mm, (Tsang and Wiantoro 2019); small to medium fruit bats with a forearm length < 90 mm, comprising multiple genera such as *Eonycteris*, *Rousettus* and *Cynopterus* (Kingston et al. 2009; Francis 2019).

There is a slight disconnect in research involving both organisms, as one focused only on the botanical aspects of the durian tree while the other focused on the ecological aspects of the bats. To address this gap, we prompt the following questions: (1) what is the diet of frugi-nectarivorous and nectarivorous bats throughout the year, especially during the non-flowering season of durians? (2) How is the quality of durian fruit production with and without bat pollination? We also briefly discuss DNA metabarcoding (Next-Generation Sequencing) as a tool to study the diet and pollination of fruit bats (frugi-nectarivore and nectarivore). Finally, we discuss the factors that influence the low efficiency of pollination success by insect pollinators on the durian tree.

2. Assessment on durian plant-bat pollinator interactions

Recently, the contributions of vertebrate pollinators in wild and crop plant production have been highlighted globally. Ratto et al. (2018) concluded that their meta-analysis showed that chiropterophilous plants are more dependent on bats than any other vertebrate pollinators; chiropterophilous plants that are pollinated by other vertebrates showed an average of 83% reduction in fruit/seed production. Durian-

bat interactions were not explored in detail previously, as most studies were either focused on the pollinator or the plant itself. The majority of past studies on this topic either focused on the foraging ecology, bat diet and bat pollination process or durian flowering, pollination (natural & artificial) and fruit production (Table 1). Pollination exclusion experiments provided the link to both durian and bat relationship, elucidating the significance of bat pollination in durian production, but there is the labor cost versus ecological benefit point debated upon as manual pollination seems to produce the highest fruit yield than open pollination (Bumrungsri et al. 2009; Chaiyarat et al. 2020).

Henceforth, there are bound to be some research gaps in truly understanding the durian plant-bat pollinator interactions. A total of 28 bat pollination and durian articles were selected for this paper by keyword search such as 'Durian pollination', 'Bat pollination' and 'Durian + Bats' in many research search engines, including Google Scholar, Web of Knowledge and ScienceDirect.com. These past studies are classified based on the focus of their work which are either; (1) Bats; (2) Durian; or (3) Durian-Bat. All these past studies feature both bats and durian either in the results or discussion, even in a minor capacity in which the smallest connection in a study might provide insight into the bat-durian interactions. Next, a comparison of methodologies was highlighted to provide an overview based on (1) Sampling techniques, (2) Botanical aspects, and (3) Pollination exclusion. We also include three closely related studies (honorable mentions) since we believe these studies can be replicated the most for the durian tree. Finally, we compiled the major findings of each research in Table 1.

3. Review of bats as a pollinator of durians

3.1. Methods for studying nectarivorous bats

Common knowledge of the past dictates flying foxes is attracted to the nectar of durian flowers, as stated in the 1910 Federal Malay States tour guide (Harrison 1910). The earliest written records about bats as a pollinator of durians were in 1889 by Beccari and 1929 by Boedjin and Danser, which detailed their observations of bats visiting durian flowers, one species identified as *Macroglossus* sp. (Brown 1997). The sampling techniques in past studies involve bat trapping (10 studies), pollen collection (6 studies), fecal collection (5 studies), camera trapping (4 studies), direct observation (4 studies) or DNA extraction and sequencing (4 studies) in assessing the diet and ecology of the bats (Table 1). Radio-tracking (2 studies) was used to track the movement of bats in assessing their foraging ecology and tree visitation (Funakoshi and Zubaid 1997; Acharya et al. 2015). Insect trapping (2 studies) was also implemented to identify insect floral visitors to chiropterophilous plants.

Bat trapping involves using mist nets to capture pteropodid bats for pollen collection and precise species identification to compensate for the uncertainty during camera trapping and direct observations (Bumrungsri et al. 2013 & Aziz et al. 2017a). Bat feces were collected either from underneath the bat roosting colony or from the captured bats, in which bats usually will defecate after being kept in cloth bags for a few hours (Bumrungsri et al. 2013 & Thavry et al. 2017). However, it is worth noting that fecal collection can be susceptible to cross-contamination if the roosting

Table 1. Summary of selected durian plant-bat pollinator interaction past studies.

	Reference	Sampling Technique	Botanical Aspect	Pollination Exclusion	Major Findings
Bats	Funakoshi and Zubaid 1997	BT; FC; RT			- <i>C. brachyotis</i> was reported to consume durian flowers.
	Bumrungsri et al. 2013	BT; PC; FC			- <i>E. spelaea</i> pollinate economically important plants in Thailand, including durian, petai and wild banana
	Acharya et al. 2015	BT; DO; RT			- Distance range (1-17.9 km) from roosting cave to foraging site for <i>E. spelaea</i> with a mean home range size (460.8-564.5ha)
	Stewart and Dudash 2016b	BT; PC;			- nectar specialist bats are more important pollinators among fruit bats - Pollinator Importance (PI) depends on pollen transfer effectiveness rather than flower visitation rate
	Thavry et al. 2017	BT; FC			- diet of <i>E. spelaea</i> in Cambodia at least 13 plant taxa, including durian - 3 significant colonies (> 1000) known in Kampot, Cambodia
	Aziz et al. 2017c	FC; DNA			-NGS can add value to conventional micro histology in identifying plant material but is unable to determine which plant parts were ingested
	Lim et al. 2018a	FC; DNA			- 55 plant species were detected, including <i>Durio</i> sp. - <i>E. spelaea</i> exploit many food resources even in highly disturbed areas - Raises question on the 'nectar specialist' category as ferns and figs were also detected.
Durian	Lim et al. 2018b	FC; DNA			- <i>C. cf. brachyotis</i> 'Sunda' exploit novel food resources at urban and agricultural areas - <i>C. brachyotis</i> possibly disperse native pioneer plant seeds
	Chan et al. 2021	FC; DNA			- 33 plant species were detected in <i>C. brachyotis</i> fecal samples - Urbanisation is not negatively associated with reliance on native plants or dietary breadth
	Stroo 2000		HS		- Bat pollinated plants have larger pollen in general - Pollen features are not significantly associated with bat pollination
	Honsho et al. 2004a		MFS	OP; AP; SP	-Durian is self-incompatible - Recommend Kradum Thong cultivar as polliniser (pollen source)
	Honsho et al. 2004b		FP		- Clarify durian flowering process - Anthesis (1600-1900hrs); Pollen release occurs at the end of anthesis
	Honsho et al. 2007			AP	- Effective pollination period of durian only lasts one night
	Ogawa et al. 2005		FP; MFS		- Flower budding: 34 days; Flowering: 19 days; Fruiting: 28 days
	Honsho et al. 2009		MFS	AP; SP	- Strong positive correlation between fruit characteristics and both seed number and weight - Kradum Thong has a large genetic distance from other cultivars, making it suitable for cross-pollination
	Kozai et al. 2014		MFS	AP; SP	- Kradum Thong cultivar is self-compatible
	Stewart and Dudash 2016a	BT	PT	OP	- <i>E. spelaea</i> transfers more pollen to conspecific flowers - There are different patterns of pollen deposition on bats
	Stewart and Dudash 2017a	BT; PC			- Field evidence supports experimental work - Plants place pollen on precise areas of the bats' body - Diverse floral morphologies limit interspecific pollen transfer
	Hau and Hieu 2017		FP		- Recommend treatments to induce durian flowering year-round for off-season durian production in Vietnam
	Wayo et al. 2018		FP; MFS	OP; IP; AP;	- OP produce a higher fruit set than IP - Nocturnal insects are important pollinators to the Monthong cultivar as it has similar stamen and pistil length.
	Ng et al. 2020	IT; DO	FP	OP; AP; SP	- Flowering process of <i>Durio graveolens</i> , <i>D. griffithii</i> , <i>D. kutejensis</i> & <i>D. zibethinus</i> - Fruit bats as a primary pollinator for <i>D. graveolens</i> , <i>D. kutejensis</i> & <i>D. zibethinus</i> - Spiderhunter as primary pollinator for <i>D. kutejensis</i> - Insects observed as secondary pollinators of <i>Durio</i> spp.
Durian-Bat	Gould 1978	DO			- Nectar production in durian flowers (0.36 ml); Bat visitation patterns
	Bumrungsri et al. 2009	CT	FP; MFS	OP; IP; AP; SP	- Pollen release at 1930-2000hrs - Fruit bats are major pollinators of durian; No mature fruit set in IP
	Sritongchuay et al. 2016	BT; CT; DO	FS		- Dominant visitors to durian flowers are nectarivorous bats - Durian fruit set is negatively affected by distance to the nearest cave
	Aziz et al. 2017a	BT; CT; DO	FS		- Flying foxes had a positive, mature fruit set - Clear vertical stratification in feeding niches of <i>E. spelaea</i> and <i>P. hypomelanus</i>
	Stewart and Dudash 2017b	BT; PC	FP		- Generalist nectarivore utilize both big-bang and steady-state floral resources - Specialist nectarivore only stick to steady-state plants

(Continued)

Table 1. Continued.

Reference	Sampling Technique	Botanical Aspect	Pollination Exclusion	Major Findings
Sheherazade et al. 2019	CT	FP; MFS	OP; IP	- Higher fruit set from bat-pollinated flowers - Bat pollination services ~\$117/ha /fruiting season - AP produce the highest fruit set, followed by bat pollination
Chaiyarat et al. 2020	BT; PC	MFS	OP; IP; AP	- Non-intensive orchards rely on bats for pollination service - OP record highest fruit set
Zalipah and Adzemi 2017		MFS	OP; IP; AP; SP	- Bats are a more efficient pollinator for <i>Sonneratia alba</i> than moths - Quantify the quality of pitaya fruits pollinated by bats and insects
Tremlett et al. 2020	CT	MFS	OP; IP;	- Fruit yield and quality decrease significantly without bats
de Araujo et al. 2020	IT; PC	FP	OP; IP	- Nocturnal bees visits do not result in fruit set - The bees collect pollen for their nest, not for pollination

Keys: BT: Bat Trapping, PC: Pollen Collection, FC: Fecal Collection, CT: Camera Trap, DO: Direct Observation, RT: Radio-tracking, IT: Insect Trapping, FP: Flowering Phenology, PT: Pollen Transfer, HS: Herbarium specimen, MFS: Mature Fruit Set, OP: Open Pollination (usually bats), IP: Insect Pollination (includes manual hand-cross and emasculum), SP: Self-pollination (also known as facilitated autogamy), DNA: DNA extraction and sequencing.

colony is shared between different species or the same cloth bag was used for many individuals. Both pollen and fecal collection serve to obtain pollen used to identify the plant species adhered on the body or ingested by the bats respectively. DNA extraction and sequencing are also used to determine the diet of frugi-nectarivorous and nectarivorous bats by detecting and identifying DNA from plant materials available in bat feces. The differences in methods reflect the research objectives of the authors as pollen collection, fecal collection, plus DNA extraction and sequencing provide dietary data of bats while camera trapping and direct observation provide evidence of interactions between bats and durian flowers plus bat visitation rates to the flowers.

3.2. Bat species known to pollinate durian

We compile a list of bat species that are confirmed to pollinate durian flowers in Table 2. One common bat species associated with durian pollination is *Eonycteris spelaea* (Start and Marshall 1976). The distribution of *E. spelaea* spreads widely across South Asia and Southeast Asia, with colonies ranging from dozens in small limestone crevices and urban buildings to thousands roosting in massive caves (Kruttsch 2005). One such large colony in Palawan consist of 50,000 individuals (Taylor and Tuttle 2019). This vast distribution makes *E. spelaea* a common subject in understanding and comparing bat-durian interactions across Southeast Asia along with flying foxes (*Acerodon celebensis*, *Pteropus alecto*, *P. hypomelanus* & *P. vampyrus*). In addition to its vast distribution, *E. spelaea* can adapt and exploit food resources in modified habitats like agricultural areas, which are often patchy and isolated aside from natural forests (Acharya et al. 2015).

While *E. spelaea* frequently visits and pollinates durian trees compared to other bat species, *Rousettus leschenaulti*, a frugi-nectarivorous bat, carries a significantly larger durian pollen load (Stewart and Dudash 2016b). *R. leschenaulti* occur in localities such as Songkhla (Thailand), Phnom Sila cave (Cambodia), Wang Kelian State Park (Malaysia), Sumatra, Bali, and Lombok (Indonesia) (Maryanto and Maharadantunkamsi Achmadi 2002; Matveev 2005; Stewart and Dudash 2016b; Jayaraj et al. 2013). *R. leschenaulti* will likely forage on favorable floral resources (durian flowers) when these resources are plentiful to supplement their diet with nectar aside from regularly feeding on fruits (Stewart and Dudash 2017b). The difference of durian pollen load between *Eonycteris spelaea* and *Rousettus leschenaulti* needs further clarification on how bat foraging behavior affects pollen displacement and how significant body size difference influences pollen load.

Table 2. List of confirmed bat species that pollinated durian flowers.

Species	References
<i>Acerodon celebensis</i>	Sheherazade et al. 2019
<i>Eonycteris spelaea</i>	Brown 1997; Bumrungsri et al. 2013; Bumrungsri et al. 2013; Sritongchuay et al. 2016; Lim et al. 2018a; Acharya et al. 2015; Thavry et al. 2017; Aziz et al. 2017a & Stewart and Dudash 2016b
<i>Pteropus alecto</i>	Sheherazade et al. 2019
<i>Pteropus hypomelanus</i>	Aziz et al. 2017a
<i>Pteropus vampyrus</i>	Brown 1997; Gould 1978; Francis 2019; Kunz and Jones 2000
<i>Rousettus leschenaulti</i>	Stewart and Dudash 2016b & Stewart and Dudash 2017b

On the other hand, the two larger counterparts to *E. spelaea* are *Eonycteris major* and *Eonycteris robusta*, which are endemic to Borneo and the Philippines respectively. *Eonycteris major* is associated with primary forest, occurring sympatrically with *E. spelaea* distributes across Borneo such as Mount Penrisen in Sarawak, Crocker Range Biosphere Reserve in Sabah, Batu Apoi National Park in Brunei and East Kalimantan (Struebig et al. 2010; Jayaraj et al. 2006; Yoh et al. 2020; Kofron 2002). *Eonycteris robusta* distributes on most large Philippine islands, including the Greater Luzon, Greater Mindanao and Greater Negros-Panay, found in caves and lowland forest (Heaney et al. 2006; Heaney and Roberts 2009). The foraging ecology of both species are poorly studied due to the overlapping roosts with *E. spelaea* and lower numbers in general (Heaney et al. 2006; Tababa et al. 2012). Thus, we have little information on the resource partitioning between the *Eonycteris* bat species, particularly on whether the endemic members pollinate durian. It is also worth noting that durian is not native to the Philippines and is cultivated as an economic crop, with the largest producer coming from Mindanao (Arellano 2018). Even so, there is a very high possibility that native bats can be attracted to non-native trees, as shown in Honduras and Darwin, Australia where bats have been observed pollinating durian trees (Baker 1970; Lim and Luders 2009).

Although flying foxes are generally regarded as frugivores, their contribution to pollination particularly durian, should not be discounted as shown in Pulau Tioman, Malaysia (*Pteropus hypomelanus*) and Sulawesi, Indonesia (*P. alecto* & *Acerodon celebensis*), where the durian flowers there primarily depended on bats as pollination agents (Aziz et al. 2017a; Sheherazade et al. 2019). Other distribution records for *Pteropus hypomelanus* include Malaysia (Pulau Langkawi, Pulau Pangkor & Pulau Perhentian), Vietnam (Soc Trang), the Philippines (Northern Sierra Madre Mountains) and Indonesia (Sumatra, Java & Maluku Islands) (Maryanto and Maharadatunkamsi Achmadi 2002; Rahman et al. 2013; Thong et al. 2015; Mohd-Yusof et al. 2020; Duya et al. 2020). *P. alecto* occurs in eastern Indonesia including the Lesser Sundas, Maluku Islands and Papua (Maryanto and Maharadatunkamsi Achmadi 2002). Another underrated flying fox species that pollinates durian and flowers of many forest trees (ex: *Erythrina fusca*, *Octomeles sumatrana* & *Pterocymbium tinctorium*) is *Pteropus vampyrus* which displays territorial behavior, leading other bats to forage in other Durio trees, which promotes cross-pollination (Gould 1978; Kunz and Jones 2000; Stier and Mildenstein 2005; Francis 2019). *P. vampyrus* has a widespread range across Southeast Asia with multinational home-range with localities such as Benut, Lenggong & Similajau (Malaysia), Surat Thani (Thailand), Northern Sierra Madre Mountains (Philippines) and Sumatra, Jawa & Lesser Sundas (Indonesia) (Gumal et al. 1997; Maryanto and Maharadatunkamsi Achmadi 2002; Wacharapluesadee 2005; Epstein et al. 2009; Duya et al. 2020). Contrary to negative local perception as pests and often blamed for damages caused by other animals, these flying foxes (*P. hypomelanus*) do not damage the durian flowers as shown in camera trap videos that they are largely non-destructive, only feeding on the flower's nectar (Fujita and Tuttle 1991; Aziz et al. 2017a; Sheherazade et al. 2019). Sheherazade et al. (2019) states that the larger size of flying foxes possibly allows them to carry larger pollen loads and

transport the pollen over longer distances than smaller bats. However, further research is needed to quantify the pollen transfer effectiveness as more pollen adhered on the flying foxes are likely displaced during long flights. There is also merit to smaller bats like *Eonycteris spelaea* as they have a higher flower visitation frequency per night compared to the territorial behavior of *Pteropus hypomelanus* and *Pteropus vampyrus* (Gould 1978; Acharya et al. 2015; Aziz et al. 2017a).

As discussed above, the reputation of flying foxes as pests is wildly perceived, possibly due to their large size that can cause flowers to drop during feeding which on occasion the bat culling costs more than the actual crop damage (Fujita and Tuttle 1991; Aziz et al. 2016). The irony is that farmers usually conduct thinning to only 10% of the flowers left on a tree to increase fruit size and uniformity, allowing the highest fruit yield at harvest (Ketsa et al. 2020). Standard practice for durian farmers has already been carried out in nature by bats (*P. hypomelanus* & *E. spelaea*) which cause minimal damage and loss of flower parts (Aziz et al. 2017a). Only one paper reported on *Cynopterus brachyotis* feeding upon durian flowers opportunistically with *D. zibethinus* detected in fecal samples in Lim et al. 2018b; however, there is no concrete evidence that these bats damage the durian flowers as DNA sequencing of the fecal samples are unable to determine which plant part was consumed (Funakoshi and Zubaid 1997). This doubt is further added upon when three *Cynopterus* species (*Cynopterus brachyotis*, *Cynopterus horsfieldii* & *Cynopterus sphinx*) did not regularly carry the pollen of chiropterophilous plants (Stewart and Dudash 2017b).

To sum up, bats play an important role as pollinators to the durian tree. Since durian is a seasonal tree, its flowers are only available at certain times in the year, and thereby one has to wonder what these bats feed upon during durian off-season. Bumrungsri et al. (2013) and Thavry et al. (2017) have provided an all-year-round diet for *E. spelaea* for Thailand and Cambodia, respectively, which aside from durian entails plants like *Parkia* spp., *Musa* spp., *Oroxylum indicum* and *Bombax anceps*. The temporal variation of the diet between these two countries reflects differences in local floral availability and flowering phenology (Bumrungsri et al. 2013; Thavry et al. 2017; Table 3). It is worth mentioning that an eight-month dietary data for *P. hypomelanus* is also available including plants such as *Ficus* spp., *Syzygium* sp. and *Terminalia catappa*, though the parts of the plants consumed cannot be identified by Next-Gen Sequencing (NGS) in which it requires further clarification on which plants did *P. hypomelanus* pollinate (Aziz et al. 2017c). All in all, their diet and foraging ecology significantly shaped the pollination biology of durians; thus, it is paramount to study this aspect of bats in bat-durian interactions, not only for bat conservation efforts but also for sustainable durian agriculture production (Table 3).

3.3. Taxonomy of nectarivorous bats

The taxonomy of Old World fruit bats (Family: Pteropodidae) has always contained points of uncertainties and contradictions between morphology and molecular data. Case in point is the pairwise genetic distance of *Eonycteris spelaea* and *Macroglossus minimus* at about 15.7%, a large difference for two species that supposedly belongs in the same

Table 3. Diet of confirmed bat species that pollinate durian in one year.

Bat species	Plant species (with parts consumed)	Country	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Eonycteris spelaea</i>	<i>Durio zibethinus</i>	N&P	Cambodia											
		N&P	Thailand											
	<i>Durio</i> sp.	DS	Malaysia				NA							
	<i>Bombax anceps</i>	N&P	Cambodia											
	<i>Bombax</i> spp.	N&P	Thailand											
	<i>Ceiba pentandra</i>	N&P	Cambodia											
		N&P	Thailand											
		DS	Malaysia											
	<i>Musa acuminata</i>	N&P	Thailand											
		DS	Malaysia											
	<i>Musa balbisiana</i>	DS	Malaysia				NA							
	<i>Musa</i> spp.	N&P	Cambodia											
		N&P	Thailand											
		DS	Malaysia				NA							
	<i>Oroxylum indicum</i>	N&P	Cambodia											
N&P		Thailand												
DS		Malaysia				NA								
Bat species	Plant species (with parts consumed)	Country	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Eonycteris spelaea</i>	<i>Parkia</i> spp.	N&P	Cambodia											
		N&P	Thailand											
	<i>Sonneratia</i> spp.	N&P	Cambodia											
		N&P	Thailand											
	Arecaceae	DS	Malaysia				NA							
	<i>Arenga</i> spp.	N&P	Thailand											
	<i>Artocarpus heterophyllus</i>	DS	Malaysia				NA							
	<i>Cocos nucifera</i>	N&P	Thailand											
	Cyatheaceae	DS				NA								
	<i>Dicranopteris</i> sp.	DS	Malaysia				NA							
	<i>Duabanga grandiflora</i>	DS				NA								
	<i>Eucalyptus</i> spp.	N&P	Cambodia											
	<i>Eugenia</i> sp.	N&P	Thailand											
	<i>Macaranga</i> sp.	DS				NA								
	<i>Mangifera indica</i>	DS				NA								
<i>Manilkara zapota</i>	DS	Malaysia				NA								
<i>Syzygium samarangense</i>	DS				NA									
Thelypteridaceae	DS				NA									

subfamily, Macroglossinae (Rovie-Ryan et al. 2008). A very recent study proposes a new classification based on an extensive > 8000 bp matrix which results in new subfamilies (Notopterisinae & Macroglossusinae), new tribes (Melonycterini, Pteralopini & Harpyioncterini) and species reallocations (Almeida et al. 2020). Notopterisinae elevated the genus *Notopteris* to subfamily level, a rare nectar bat group in New Caledonia (Taylor et al. 2020; Almeida et al. 2020). Next, the new subfamily Macroglossusinae is now restricted to two genera consisting of five species in total (*Macroglossus* & *Syconycteris*), in which they are considered as true nectar bats. Meanwhile, the genus *Eonycteris* is relocated to the subfamily Rousettinae verifying the position of *Eonycteris spelaea* and *Rousettus amplexicaudatus* in one clade at the neighbour-joining (NJ) tree (Rovie-Ryan et al. 2008; Almeida et al. 2020). This taxonomic revision means that *Eonycteris* can no longer be categorized as nectar bats in which

suspicions have been raised before that this group may not feed exclusively on nectar (Start and Marshall 1976; Lim et al. 2018a). However, in this review, we still categorize *Eonycteris* as nectar-feeding bats due to the fact there is no concrete confirmation on the matter.

Other Old World nectar-feeding bats include the new tribe Melonycterini (genera: *Melonycteris* & *Nesonycteris*) of Australasia plus the Central African genera *Megaloglossus* and *Plerotes* (Rovie-Ryan et al. 2008; Almeida et al. 2020; Taylor et al. 2020; Nesi et al. 2021). The taxonomy of the other frugi-nectarivorous bat genera remains unchanged with *Rousettus* in the subfamily Rousettinae, while *Pteropus* and *Acerodon* are classified in the subfamily Pteropodinae, though *Pteropus* still have subgenera classification unresolved (Almeida et al. 2014; Almeida et al. 2020).

Easily overlooked, the family Mystacinidae endemic to New Zealand represents the third nectarivorous family in

Bat species	Plant species (with parts consumed)	Country	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
<i>Eonycteris spelaea</i>	<i>Xanthostemon chrysanthus</i>	DS Malaysia				NA									
<i>Pteropus hypomelanus</i>	Durio sp.	N&P	Malaysia	NA											
	Ficus	DS													
	Anacardiaceae	DS													
	Diospyros	DS													
	Lamiales	DS													
	Moraceae	DS													
	Rubiaceae	DS													
	Terminalia	DS													
<i>Rousettus leschenaulti</i>	Durio zibethinus	N&P	Thailand												
	<i>Artocarpus chama</i>	F	India*												
	<i>Artocarpus heterophyllus</i>	F													
	<i>Artocarpus lacucha</i>	F													
	<i>Averrhoa carambola</i>	F													
	<i>Bauhinia variegata</i>	N&P													
	<i>Bischofia javanica</i>	F													
	<i>Bombax ceiba</i>	FL													
	<i>Bridelia retusa</i>	F													
<i>Rousettus leschenaulti</i>	<i>Calophyllum polyanthum</i>	F		India*											
	<i>Carallia brachiata</i>	F													
	<i>Carica papaya</i>	F													
	<i>Erythrina stricta</i>	FL, N													
	<i>Erythrina variegata</i>	FL, N													
	<i>Ficus spp.</i>	F													
	<i>Flacourtia jangomas</i>	F													
	<i>Litchi chinensis</i>	F													
	<i>Mangifera indica</i>	F													
	<i>Meyna spinosa</i>	F													
	<i>Myrica esculenta</i>	F													
	<i>Psidium guajava</i>	F													
	<i>Rubus ellipticus</i>	F													
	<i>Syzygium claviflorum</i>	F													
	<i>Syzygium cumini</i>	F													
	<i>Syzygium grande</i>	F													
	<i>Willughbeia edulis</i>	F													
	<i>Ziziphus jujuba</i>	F													

Chiroptera (Arkins et al. 1999). Although *Mystacina tuberculata* has an omnivorous diet, it consumes a substantial amount of nectar and is currently known to be an important pollinator comparable to birds on the island country (McCartney et al. 2007). The flowers visited by *Mystacina tuberculata* like *Metrosideros excelsa*, *Dactylanthus taylorii* and *Eucalyptus sp.* have some of the chiropterophilous flower characteristics in which one common trait is the abundance of nectar produced (Arkins et al. 1999; McCartney et al. 2007; Pattemore and Wilcove 2012; Bylsma et al. 2014).

On the other side of the world, Phyllostomidae makes up for the New World nectar bats, which are represented by the subfamily Glossophaginae and Lonchophyllinae (Datzmann et al. 2010; Bolzan et al. 2015). Examples of bats include *Lep-tonycteris yerbabuena* that pollinates pitaya fruits in Mexico

and *Platalina govenensium* pollinating columnar cacti respectively (Tremlett et al. 2020; Fleming and Holland 2018). The longest tongue of any mammal, relative to body length, hails from a phyllostomid bat, *Anoura fistulata*, at about 84.9 mm which is 150% of its total body length (Muchhala 2006). Unlike Paleotropical nectar bats, neotropical nectar-feeding bats do not strictly feed on nectar but also preys upon insects and fruits depending on the degree of specialization (Coelho and Marinho-Filho 2002; Clare et al. 2014a; Taylor et al. 2020). Other frugi-nectarivorous (e.g. *Uroderma*) and omnivorous (e.g. *Phyllostomus*) phyllostomid bats also exploit floral resources opportunistically (Fleming and Muchhala 2008; Giannini and Brenes 2001; Taylor et al. 2020). One insectivorous bat species (*Antrozous pallidus*) seasonally consume nectar when flowers of Mexican giant cacti (*Pachycereus pringlei*) are abundant, representing

Bat species	Plant species (with parts consumed)	Country	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Pteropus alecto</i>	<i>Acacia auriculiformis</i>	NA												
	<i>Buchanania arborescens</i>	F												
	<i>Carallia brachiata</i>	NA												
	<i>Exocarpos latifolius</i>	NA												
	<i>Ficus opposita</i>	F												
	<i>Ficus racemosa</i>	NA												
	<i>Nuclea orientalis</i>	F												
	<i>Syzygium nervosum</i>	F												
	<i>Terminalia microcarpa</i>	F												
	<i>Timonius timon</i>	F												

Keys; NA: Not Assessed, F: Fruit, FL: Flower, N&P: Nectar & Pollen, DS: DNA Sequencing (unable to identify which plant parts was consumed), *: Plant species in this country can also be found either native or introduced in Southeast Asia.

Sources; Cambodia: [Thavry et al., 2017](#), Thailand: [Bumrungsri et al., 2013](#); [Stewart & Dudash, 2017b](#), Malaysia: [Lim et al., 2018a](#); [Aziz et al., 2017c](#), India: [Vanlalnghaka, 2015](#), Australia: [Palmer et al., 2000](#).

Notes for [Lim et al., 2018a](#): Only the frequently and moderately detected plant species data are taken due to the sheer number of total species.

one of the few plant-pollinator interactions by insect bats (Frick et al. 2014).

4. Review of durian as a chiropterophilous crop plant (Malvaceae: *Durio zibethinus*)

4.1. Overall descriptions of durian

The genus *Durio* is native in Southeast Asia, comprising about 30 known species. Only *Durio zibethinus* is cultivated for agriculture on a large scale. Wild durian trees can reach heights of 30–40 m, typical of a rainforest tree, while cultivated durian trees average about 10–12 m (Brown 1997). The durian tree is a big-bang species that undergoes mass flowering, averaging about 1000 flowers per night seasonally (Bumrungsri et al. 2013; Stewart and Dudash 2016a). The durian flowering season slightly varies across regions, even within the same country, as two to four weeks of drought period is required to induce the flowering process (Safari et al. 2018; Ketsa et al. 2020). Depending on the climate, the flowering season could also arrive early or late every year, added with the regional time variations, creating the illusion of durian availability all year round (Table 4; Brown 1997; Pascua and Cantila 1992).

Durian flowers are usually ramiflorous (Brown 1997) or cauliflorous depending on the cultivar (Honscho et al. 2004b). The flower generally has five petals but varies

among cultivar between four to six petals and is grouped into clusters of 5–30 flowers (Brown 1997). The average volume of nectar in this flower is 0.36 ml (Gould 1978). Flowering phenology observations were conducted by both Honscho et al. (2004b) and Ogawa et al. (2005) to record the timing and process of durian flowering. Durian flowers open in mid-afternoon (Brown 1997) or around 16:00hrs (Honscho et al. 2004b) till nighttime but only release pollen around 19:00 hrs. This behavior is likely due to adaption for bat pollination. Individual durian flowers have a very short lifespan and effective pollination period (EPP), ranging from only one night (Honscho et al. 2007) to a few days (Ogawa et al. 2005). The latter had concluded that the flowering period of individual durian trees is 19 days. Based on the durian past studies shown in Table 1, each study focuses on different parts of the durian plant involved in pollination, which are the flowers (2 studies), pollen (3 studies) and formation of fruit set (2 studies). The authors also highlighted artificial pollination experiments as a means to increase fruit yield and reduce unpredictability in fruit sets.

4.2. Pollen morphology and pollination mechanism for durian

Pollen morphology and differential pollen placement were studied by Stroo (2000), Stewart and Dudash (2016a) and Stewart and Dudash (2017a) respectively to assess the extent of bat pollination influencing pollens of bat pollinated plants including durian. Durian pollen has an oblate shape with psilate exine (smooth surface) and a size range of 20–80µm with a mean of 55–67µm (Brown 1997; Stroo 2000). Pollen shape and exine ornamentation are not significantly associated with bat pollination; only pollen size is correlated as bat pollinated plants have larger pollen than non-chiropterophilous plants (Stroo 2000). Figure 1 shows the different pollen morphology of bat pollinated plants and *Durio* plants, in which some of them share the same bat pollinator (*Eonycteris spelaea*). Variation in pollen characteristics is very large; for example, *Bauhinia macrostachya* has an oblate shape with a perforate surface on one end, while *Irlbachia alata* has a tetrad shape with a coarsely verrucate surface on the other end of the spectrum. Meanwhile, the pollens of *Ceiba pentandra* and *Oroxylum indicum* are quite different compared

Table 4. Durian flowering seasons in Southeast Asia.

Country/place	Flowering season
Malaysia	
Merapoh, Pahang	Mar-May (pers. obs.)
Tioman	Apr-May, Jul–Aug & Oct–Nov (Aziz et al. 2017a)
Sarawak	Apr: Start flowering, Jul-Oct: Peak flowering (Ng et al. 2020)
Thailand	
Songkhla	Mar-Apr (Stewart and Dudash 2016a; Bumrungsri et al. 2009)
Indonesia	
Sulawesi	June-Sep (Brown 1997)
	Oct-Nov (Sheherazade et al. 2019)
Cambodia	Jan (Brown 1997)
Vietnam	Dec-Jan (Hau and Hieu 2017)
Philippines	
Davao City	Jan-May (Brown 1997)
Los Banos	Feb (Brown 1997)
Mindanao & Sulu	May-June (Brown 1997)

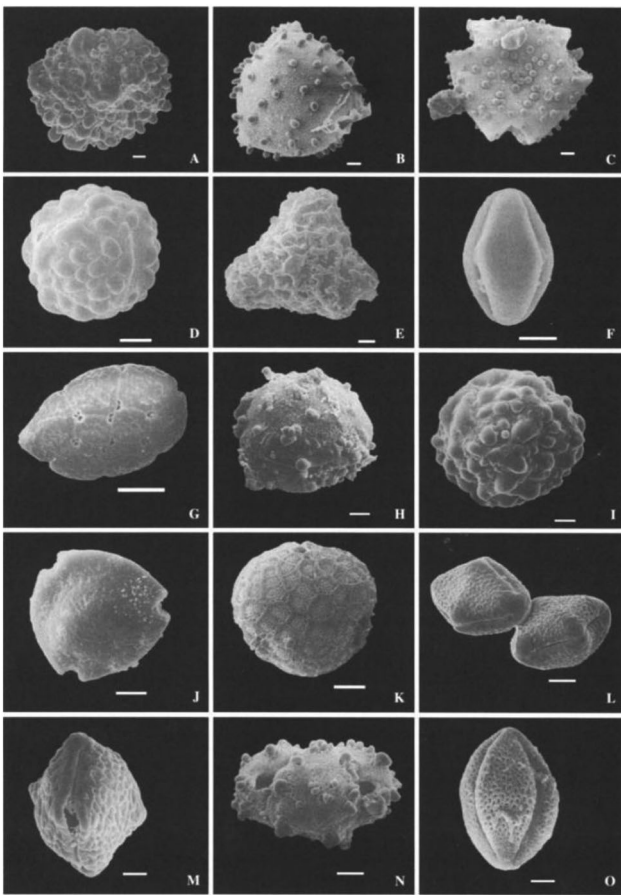


Figure 1. Pollen grains of *Durio* and other bat pollinated plants (10 μ m). (A) *Bauhinia macrostachya*; (B) *Bauhinia megalandra*; (C) *Bauhinia rufa*; (D) *Elizabetia paraensis*; (E) *Eperua falcata*; (F) *Hymenaea courbaril*; (G) *Calliandra confusa* (polyad) (H) *Durio kutejensis*; (I) *Durio oblongus*; (J) ***Durio zibethinus***; (K) *Ceiba pentandra*; (L) *Caryocar pallidum*; (M) *Caryocar villosum*; (N) *Irlbachia alata* (tetrad); (O) *Oroxylum indicum* (Stroo 2000).

to durian pollen, in which the former pollen has an oblate shape but with a coarsely reticulate surface while the latter pollen has a prolate shape with a reticulate surface.

On the other hand, differential pollen placement is the mechanism of plants that share the same pollinator placing pollen on different parts of the pollinator's body to increase pollination efficiency (Stewart and Dudash 2016a). In our context of durian, the pollinators in question are the *Eonycteris spelaea* and *Rousettus leschenaulti*. Field evidence reveals differential pollen placement on *E. spelaea* and *R. leschenaulti*, in which durian pollen is most abundant on the wings of bats (Stewart and Dudash 2017a). However, the pollen placement by big-bang plant species like *Durio zibethinus* is less accurate compared to steady-state plants, possibly due to their flowers occurring in large clusters and the foraging behavior of bats that crawl all over the flower clusters. Meanwhile, steady-state plants rely greatly on differential pollen placement to reduce interspecific pollen transfer; for instance, *M. acuminata* pollen is concentrated on the face of *E. spelaea*, *Macroglossus sobrinus* and *Macroglossus minimus* in both field and experimental study (Stewart and Dudash 2017a). In general, both the pollen transfer experiment in flight cage and field evidence are consistent, revealing that diverse flower morphologies apply differential pollen placement mechanisms and thus limiting interspecific pollen transfer (Stewart and Dudash 2016a & Stewart and Dudash 2017a).

Artificial pollination involves manual labor to facilitate pollination in the absence of natural pollinators. Farmers conduct artificial pollination to increase fruit yield and manipulate the seasonality of durian fruit production (Brown 1997). Durian farmers will thin flower clusters to 2–5 flowers per cluster to increase the fruit size and consistency. Anthers will be removed (emasculatation) in the afternoon and bagged to prevent undesirable pollen. Pollination is done manually at anthesis around 19:00hrs by touching the anthers containing pollen with the exposed stigma of a durian flower (Honsho et al. 2004a; Lim and Luders 2009; Ketsa et al. 2020). Cross-pollination (depending on the cultivar) produce a higher fruit rate ranging from 20% to 60% compared to self-pollination which has a fruit rate < 5% (Lim and Luders 2009; Ketsa et al. 2020). Self-pollinated fruit is undesirable due to the distorted shape, uneven husk thickness and less desirable durian flesh quality (Lim and Luders 2009). Honsho et al. (2009) conducted artificial self- and cross-pollination experiments to observe the effect of different pollen sources from distinct durian cultivars on fruit sets. Self-pollinated fruit had a smaller yield and fruit size compared to the cross-pollinated fruit. They concluded Kradum Thong durian cultivar had the greatest pollination success with other durian cultivars due to its large genetic distance, increasing the probability of successful seed formation leading to higher fruit yield. This large genetic distance is apparent in its different morphology (oval with distinct symmetrical lobes) and phenological traits (early fruiting) compared to other Thailand durian cultivars (Lim and Luders 2009). This experiment simulated the role of bats as pollinators in maintaining the genetic diversity of durian by transporting the pollens, especially in long distances.

There are over 100 Malaysian durian cultivars and 200 Thai durian cultivars registered, but only about 13 Malaysian cultivars and 4 Thai cultivars grown on a large commercial scale (Brown 1997; Jabatan Pertanian Malaysia, n.d.). Each cultivar has a slightly different morphology which may arise into different traits. These differences may influence the pollination process in which the similar stamen and pistil lengths in Monthong durian flowers enable bees to pollinate them as effectively as bats, despite the fact bees are much smaller than bats (Wayo et al. 2018). Meanwhile, ovule development in the cross- and self-pollinated flowers of Thailand durian cultivars revealed that the Kradum Thong cultivar does not exhibit true self-incompatibility. Self-pollinated flowers from the Kradum Thong cultivar can still produce an acceptable fruit set, unlike other durian cultivars (Kozai et al. 2014). Therefore, the research gap on durian cultivar pollination differences requires a future assessment not only to increase pollination efficiency but can also be applied to natural pollinators like bats and bees.

4.3. Factors that influence the low efficiency of pollination success by insect pollinators on chiropterophilous plants (i.e. the durian tree).

Flower visitation does not essentially lead to pollination as some animals exploit floral resources without transferring pollen, generally regarded as nectar thieves (Souza et al. 2016). In the case of the durian tree, the majority of past studies have shown that bats mainly pollinate their flowers with accounts of insects like *Apis dorsata* and stingless bees

visiting the durian flowers (Table 1). However, most studies have shown consistent results in which insect pollination yielded a lower fruit set than bat pollination (Bumrungsri et al. 2009; Aziz et al. 2017a; Sheherazade et al. 2019 & Chaiyarat et al. 2020). There is also a need to investigate the quality of durian production with or without bats to provide a clearer and convincing picture to the durian farmers and the public, as past studies only accounted for the mature fruit set count. One such study provides evidence that pitaya fruits (*Stenocereus queretaroensis*) pollinated by other animals (birds and insects) are lighter and have less sugar content, with an overall decrease in quality compared to bat-pollinated pitaya fruits (Tremlett et al. 2020). Whether durian follows the same quality pattern needs corroboration, but we can postulate the results to be similar as artificial pollination demonstrate the durian cultivar cross-pollinated with another durian cultivar with larger genetic distance (different morphology & phenology) will produce fruit with heavier flesh (Honsho et al. 2009). To reiterate, this artificial pollination study simulates the role of bats pollinating durian over long distances, which have a higher probability of possessing wider genetic diversity such as specific genes that are resistant to fungal infections or genes that makes the fruit much sweeter (Husin et al. 2018). Even without the fruit quality aspect, there is no denying that insects are less efficient pollinators for durian. Therefore, we will attempt a discussion on why insect pollinators yield lower fruit sets compared to bats.

4.3.1. Floral traits

Floral traits that reflect adaptations to the pollination of specific animal groups are defined as pollination syndrome (Fenster et al. 2004). The pollination syndrome hypothesis has been debated upon as the plethora of flower species are visited by different pollinator groups and thus cannot be reliably used to predict the pollinators of many flower species as with *Sonneratia alba*, which has bats and nocturnal moths as its pollinator (Ollerton et al. 2009; Zalipah and Adzemi 2017). For *Durio* spp., five insect taxa were observed acting as secondary pollinators: honey bees, stingless bees, pollen beetles, thrips, and nocturnal wasps (Ng et al. 2020). Nevertheless, floral traits that match its hypothesized pollinator consistently come up as the most efficient pollinator, even in the presence of supplementary pollinator groups (Ashworth et al. 2015). This pattern is especially true for the durian tree as bats are its most efficient pollinator.

As mentioned previously, the floral traits of durian coincide with the nocturnal behavior of bats, discouraging insects from fully utilizing their floral resources (pollen and nectar). The classical bat flower syndrome also seen in *Sonneratia griffithii* (nocturnal anthesis, flower lasts for one night, dull or whitish coloration, strong or fermented odor, a large quantity of pollen and nectar & flowers in exposed positions, away from foliage for easy bat access) is adapted for bats (Figure 2) contrasting greatly with the insect pollination syndrome (Heithaus 1982; Proctor et al. 1996; Nuevo-Diego et al. 2020).

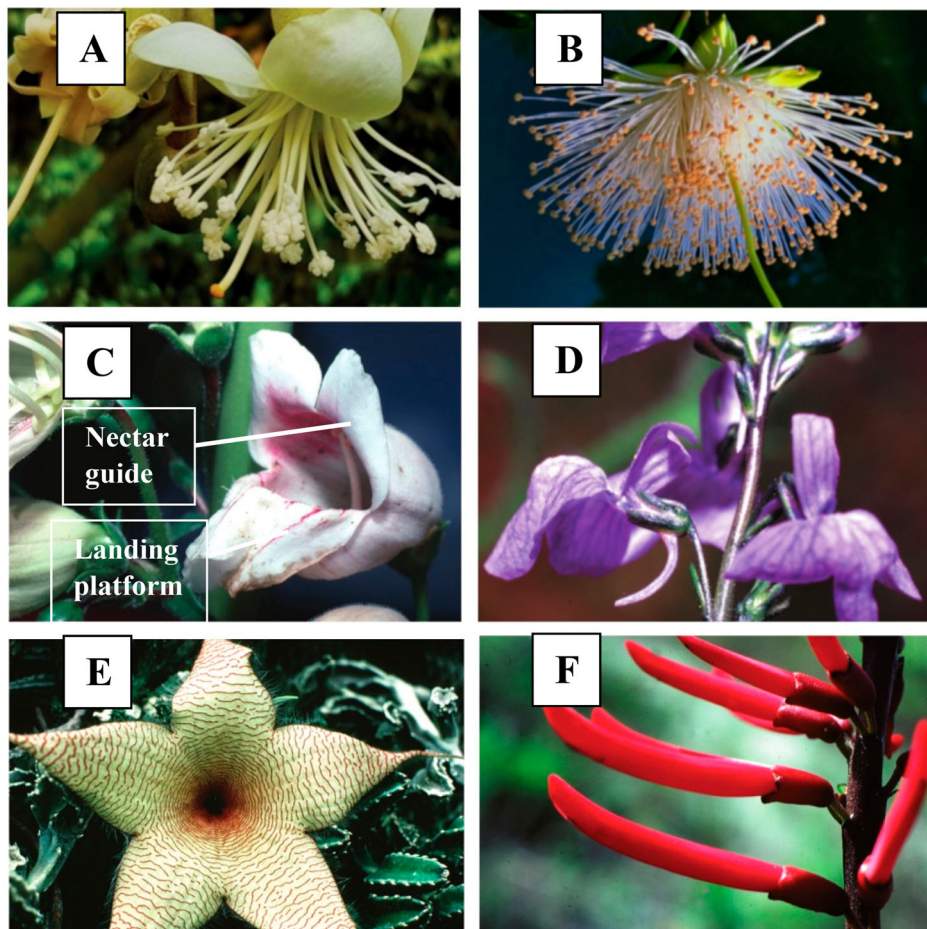


Figure 2. Floral traits associated with pollination syndrome. A *Durio zibethinus*, bat-pollinated B. *Sonneratia griffithii* bat-pollinated C. *Penstemon eximius*, bee-pollinated D. *Nuttallanthus texanus*, butterfly-pollinated E. *Stapelia gigantea*, fly-pollinated F. *Erythrina caffra*, bird-pollinated.

Insect pollination is indisputably the most common and varied with not only just bees but also ants, beetles, butterflies, moths and flies (Proctor et al. 1996; Simpson 2019). Flowers correlated with bees and butterflies tend to be colorful, have a striking appearance and produce a fragrant scent. Bee flowers have nectar guides which are specialized patterns to orient the bee for maximum effective pollination, while butterfly flowers have long nectar-filled spurs that limit nectar acquisition to the insect with a long proboscis. Flies correspond to flowers with brown or maroon coloration, which emit a fetid or rotting odor, while bird flowers are brightly colored, tend to be red, relatively large and tubular (Simpson 2019; Figure 2).

The comparisons are disparate when comparing durian with three other entomophilous Malvaceae plant species (*Malachra capitata*, *Gossypium tomentosum* & *Hibiscus tiliaceus*). All three Malvaceae flowers have a bright yellow color, diurnal anthesis followed by pollen release compared to the dull or whitish flowers and late afternoon anthesis with nocturnal pollen release of the durian (Pleasants and Wendel 2010; Raju and Raju 2013; P Aluri et al. 2020). *Malachra capitata* & *Hibiscus tiliaceus* also produce lesser nectar volume than durian, which are 0.3 and 1.8 μ l respectively. By contrast, nocturnal insect pollinators (bees, moths, hawkmoths & wasps) do pollinate chiropterophilous plants but are not as efficient as bats (Lovig 2013; Pequeno et al. 2016; Zalipah and Adzemi 2017; Wayo et al. 2018). To reiterate, in areas where bats are scarce either naturally or by habitat fragmentation, these nocturnal insect pollinators step up from supplementary to primary pollinators (Pequeno et al. 2016; Wayo et al. 2018; Taylor et al. 2020).

Another important aspect that attracts pollinators is floral scents. The floral scent of durian has yet to be fully discerning, with many assuming the flower has the same strong odor as its fruit counterpart, though few including one study claim the scent to be aromatic (Honsho et al. 2004b). Typical chiropterophilous flowers produce a strong odor containing sulfur compounds that attract nectarivorous bats (von Helversen et al. 2000; Paiva et al. 2019). However, Old World bat flowers in West Africa contain no sulfur compounds as their New World counterparts as olfactory cues have a diminished role in open areas (Pettersson et al. 2004; Paiva et al. 2019). One such piece of evidence supports this pattern with *Ceiba pentandra* in West Africa has no sulfur compounds in their floral scents compared to the same species in Costa Rica (Pettersson et al. 2004). Another preference test shows that *Eonycteris spelaea* is not innately attracted to dimethyl disulfide (DMDS), a powerful floral bat lure in many neotropical chiropterophilous plants like neotropical nectar bats do (Carter and Stewart 2015). Conversely, fruit bats in Malaysia do react strongly to ripe fig fruit odor and possibly use olfactory cues when foraging (Hodgkison et al. 2007). The frugi-nectarivorous bat, *Cynopterus sphinx* can discriminate different odors from multiple sources (fruit & flowers) during foraging like limonene from *Parkia* sp. flowers and DMDS in flowers of *Bauhinia* sp. (Elangovan et al. 2006). Whether durian floral scent contains sulfur or any chemical compounds that play a larger role in attracting nectarivorous bats is unclear, hence, requiring further research like gas chromatography and mass spectrometry (Hodgkison et al. 2007).

In general, pollination syndrome helps researchers understand the mechanism of floral diversification, particularly

regarding plant-pollinator relationships (Fenster et al. 2004). The floral traits of pollinator-dependent species, especially those with self-incompatibility have higher predictability in pollination syndrome (Rosas-Guerrero et al. 2014). While insect pollinators have low efficiency of pollination success on the durian (*Durio zibethinus*), more research needs to be conducted for the other durian cultivars and other members of the *Durio* genus that may be more insect-friendly for a comprehensive outlook on its pollination ecology.

4.3.2. Pollinator size

The small size of insects clearly cannot be compared to the pollen load carried by bats which is obviously larger. Small insects also have a lesser chance to receive stigmatic contact for pollination. *Apis dorsata*, the giant honey bee can carry an average of 11.5 pollen load while *Eonycteris spelaea* and *Rousettus leschenaulti* can carry a higher average of 47 and 100 pollen load respectively (Acharya et al. 2011; Stewart and Dudash 2016b; Wayo et al. 2018). Even with the smallest nectarivorous bat species (*Macroglossus minimus*), the landing behavior of bats ensures sufficient contact receiving a large amount of pollen per surface area. When a bat lands on the durian flower, its momentum shakes the inflorescence, causing anthers and stigmas to rub against the bat's body, face, and wings (Bumrungsri et al. 2009). In addition, bats can transport the higher pollen load over long distances (more than 10 km) compared to insects like stingless bees, which forage up to 2.1 km and *A. dorsata* that normally forage about 400 m even though capable for long-distance migration (Punchihewa et al. 1985; Kuhn-Neto et al. 2009; Acharya et al. 2015). *E. spelaea* travels from their roosting cave to the foraging sites with a range of 1-17.9 km, in which 15 of the identified foraging sites are fruit orchards and house yards (Acharya et al. 2015). Long-distance pollination by bats promotes cross-pollination and high outcrossing rates in fragmented forests and isolated durian orchards. This service is conducted more efficiently by bats maintaining genetic diversity and genetic continuity of durian meta-populations (Fleming et al. 2009; Ashworth et al. 2015).

4.3.3. Nectar theft

Nectar theft is the act of flower visitors removing nectar foregoing the transfer of pollens (Maloof and Inouye 2000). Accounts from previous studies have shown that bees avoid stigmatic contact while extracting nectar from the calyx of the durian flower (Acharya 2014; Aziz et al. 2017a). According to Wayo et al. (2018), *Apis cerana*, the Asian honey bee occasionally visits durian flowers by landing in the corolla to extract nectar. This behavior might result from nocturnal anthesis as the bees visit the flowers during the late afternoon and early evening, gaining no pollen during foraging. Specialized floral traits of the durian make the nectar inaccessible to other pollinators may incentivize nectar theft as it is the only way to access floral nectar (Souza et al. 2016; Irwin et al. 2010). Even nocturnal bees are not exempt from nectar theft as *Ptiloglossa latealcarata* exploit floral resources (nectar & pollen) from a bat-pollinated plant, *Caryocar brasiliense* but do not pollinate them, resulting in no fruit set (de Araujo et al. 2020). Another instance of nectar theft to durian flower comes from the bird group in which a crimson sunbird, *Aethopyga siparaja* visited closed flower buds and punctured the base with

their beak to collect nectar (Sheherazade et al. 2019). Other flower visitors may opt to rob nectar due to floral nectar competition with one another (Irwin et al. 2010). The argument that nectar thieves may have a positive impact and not entirely negative was brought up by Maloof and Inouye (2000). The bees might have a more subtle effect on changing the behavior of bats by increasing flight distance, visiting more flowers and reducing time spent on a flower when faced with lesser amounts of nectar in robbed flowers. These behavioral changes will likely increase the pollen flow rate and distance (Maloof and Inouye 2000). Whether or not the insects that have been documented to visit durian flowers such as *Apis dorsata*, *Apis cerana*, stingless bees and pollen beetles exhibit clear nectar robbing behaviors and such behavior provide a subtle positive impact to durian and other chiropterophilous plants need further clarification.

5. Review of durian plant-bat pollinator interactions

5.1. Co-evolutionary adaptations of nectarivorous bats and chiropterophilous durian

The interactions between bats and the durian tree are mutualistic in which each organism influences and adapts to one another to reap the benefits. Morphologically speaking, nectarivorous bats have evolved elongated, narrow rostrum and an elongated, protrusible, brush-like tongue with the lateral and tip areas covered with long tip filiform papillae which maximize the surface area of the tongue via capillarity to lap up nectar extensively (Howell and Hodgkin 1976; Hollar and Springer 1997). Frugi-nectarivorous bats have fewer tip

filiform papillae on the surface area of the tongue as they only feed on nectar opportunistically, while in contrast, insectivorous bats have no tip filiform papillae and generally less filiform papillae around the tongue (Howell and Hodgkin 1976; Massoud and Abumandour 2020) (Figure 3). Nectarivorous bats also possess hairs with scales that project away from the shaft, collecting and retaining pollen grains. In comparison, insectivorous bats have hairs with smooth scaling (Howell and Hodgkin 1976).

Likewise, the durian developed flowers with chiropterophilous syndrome traits like dull or white coloration, nocturnal anther dehiscence (pollen release) and cup-shaped with elongated stamen and pistil filaments (Honscho et al. 2004b; Fleming et al. 2009). The dull or whitish coloration of durian flowers function as camouflage from other visitors and may be used as visual cues for bats (Fleming et al. 2009). The floral shape of durian (cup-shaped with elongated stamens and pistils) might inhibit insects taking its nectar as bees have been observed to mainly hovering at the end of anthers, and stingless bees have to force the anthers open to obtain nectar stored inside the calyx (Brown 1997; Aziz et al. 2017a). Durian flowers bud from trunk (cauliflory) and major branches (ramiflory) help large flying foxes to move between flower clusters by hanging from the branch and smaller fruit bats to land easily on the flowers as they are not obstructed by thick foliage (Pettersson et al. 2004; Aziz et al. 2017a; Sheherazade et al. 2019). This flower placement of durians is a paramount feature not only for the plant to bear the upcoming weight of a mature fruit but also supporting the weight of Paleotropical fruit bats in contrast to Neotropical nectar bats, which typically hover while visiting flowers (Baker 1961; Fleming and Muchhala 2008).

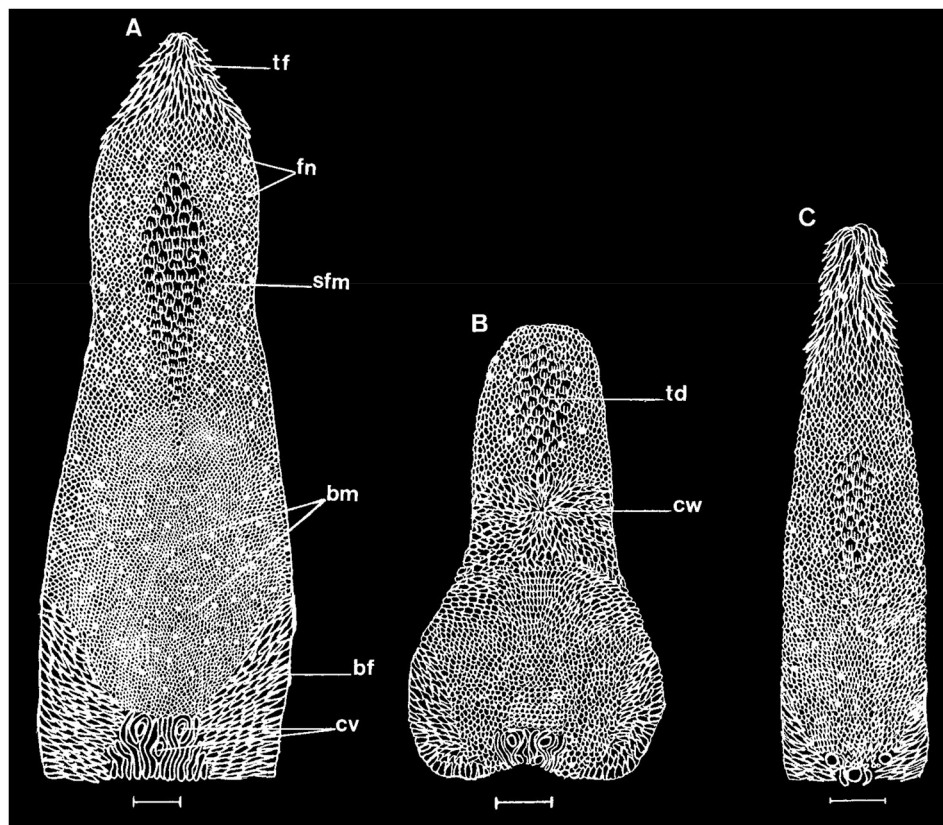


Figure 3. Dorsal surface of tongues displaying papillae (2 mm scale). A *Pteropus scapulatus* (Frugi-nectarivore); B *Nyctimene robinsoni* (Frugivore); C *Syconycteris australis* (nectarivore). td: tridentate; fn: fungiform; bf: base filiform; tf: tip filiform; sfm: simple fringed mechanical; bm: basket-like mechanical; cv: circumvallate; cw: central whorl (Birt et al. 1997).

According to Fleming and Muchhala 2008, Paleotropical fruit bats are more strongly associated with tree flowers than their neotropical counterparts, which feed upon flowers from a larger number of plant genera, including vines and epiphytes. In general, the different floral traits, particularly the flower shape of durian and other bat-pollinated flowers influence the feeding behavior of nectarivorous bats. Figure 4 shows *Glossophaga soricina* hovers near the flowers while the other three bat species lands on the flower.

Durian nectar contains fructose, sucrose and glucose with a 2:2:1 ratio (Lim and Luders 2009). Nectar secretion starts during anthesis, peaking around the evening till just after midnight, with sucrose concentration following the same trend (Bumrungsri et al. 2009; Ng et al. 2020). The timing of the durian flowers coincides with the nocturnal behavior of bats as pollen release occurs at night even though the flowers open in the late afternoon; thus, diurnal insects do not assist in pollination (Aziz et al. 2017a; Tremlett et al. 2020). Sucrose concentration in durian nectar ranges from 9.95% to 21.9%, providing the bats sufficient energy to transfer pollen over long distances and complete the pollination process (Bumrungsri et al. 2009; Sheherazade et al. 2019). In Bromeliaceae, the bat-pollinated plant species rewards bats by producing large amounts of nectar which contain 25 times more sugar than in insect-pollinated flower equivalents; whether the durian provides similar nectar content is yet to be quantified (Göttlinger et al. 2019).

Nonetheless, the plentiful amount of nectar with rich sugar concentration produced by the durian may undergo fermentation as in bertam palms (*Eugeissona tristis*), in

which the nectarivorous bats presume to have considerable ethanol tolerance (Wiens et al. 2008; Orbach et al. 2010). Yeasts involved in fruit fermentation and decay have been found in the gut mycobiota of *Eonycteris spelaea*, providing evidence of the ethanol tolerance in Old World fruit bats (Li et al. 2018). One study pointed out that the calcium content of nectar in chiropterophilous plants is higher than non-chiropterophilous plants, which raises the question of whether nectar is the primary source of calcium for nectarivorous bats, an important mineral especially for lactating females (Barclay 2002). Thus, there is a considerable research gap regarding durian nectar that is worth looking at.

5.2. Factors influencing bat pollination on durian flowers

Bat pollination is greatly influenced by spatial and temporal variation of food availability. Spatial variation refers to habitat and land use, while temporal mainly refers to flowering phenologies. Spatial variation is increasingly striking as habitat loss and forest fragmentation take place in the name of development, threatening bat populations globally. Fragmented forest landscape, not only destroys current and potential bat roosting sites but also unintentionally isolates caves and karst outcrops in which increases the distance between the bats' habitat and their foraging grounds (Furey and Racey 2016; Lim et al. 2017). The increasing distance may not greatly affect long-distance foragers like *E. spelaea* (17.9km) and *P. vampyrus* (130km) but could lead to other ripple effects



Figure 4. Nectar-feeding bats visiting flowers. A *Glossophaga soricina* at flowers of *Mabea occidentalis* (Euphorbiaceae); B *Eonycteris spelaea* on *Durio zibethinus* flowers (Malvaceae); C *Artibeus jamaicensis* on a flower of *Ochroma pyramidale* (Malvaceae); D *Pteropus conspicillatus* at flowers of *Castanospermum australe* (Fabaceae) (Fleming et al. 2009).

as the bats have to spend more energy to forage, decreasing flower visitation frequency and pollination rate in which influence chiropterophilous plants like durian (Epstein et al. 2009; Struebig et al. 2009; Acharya et al. 2015). This situation is evident where the orchard is in close proximity with the caves, the durian fruit set is high, and vice versa (Sritongchuay et al. 2016). Thus, caves serve as an indirect role of pollinator sources to durian trees in the vicinity.

In terms of temporal variation, bat specialization is higher during the low flowering season (fewer flowers available) than peak flowering season (Sritongchuay et al. 2016). Specialization is defined as the tendency of a species to take up a narrower niche breadth (Villalobos et al. 2019). This finding is supplemented by Stewart and Dudash (2017b) that showed specialist nectarivorous bats such as *Macroglossus sobrinus* and *M. minimus* mainly forage on steady-state plant species (low number of flowers all year). For generalist nectarivores, these bats will probably stick to one or two predictable floral resources at various times throughout the year, as one such example with *E. spelaea*, which feed upon *Musa acuminata*, *Parkia* sp. and *Sonneratia* sp. (Stewart and Dudash 2017b). Unlike temperate regions that follow the optimal foraging theory, tropical regions revealed the opposite in which the low floral resources result in a higher degree of specialization, possibly caused by increasing competition (Souza et al. 2018).

It is no wonder that most of the bat species that pollinate durian are generalist nectarivores like *E. spelaea* and frugineectarivores like *P. hypomelanus* and *Rousettus leschenaulti* due to durian being a big-bang plant species when in season (high number of flowers for a short period). These bats would most likely switch between steady-state plants to big-bang plants, consuming both steady-state and big-bang plants when preferred floral resources are locally abundant (Stewart and Dudash 2017b). However, the author only focused on six bat-pollinated plant species: big-bangs (*Ceiba pentandra* & *Durio zibethinus*) and steady states (*Musa acuminata*, *Oroxylum indicum* *Parkia* spp. & *Sonneratia* spp.), though on a smaller scale, raises the notion of competition among nectarivorous bats. As mentioned in the previous paragraph, the increasing competition during the low flowering season as a result of dominating over preferred floral resources may reduce niche overlap (Tinoco et al. 2017). Body size is one factor influencing niche partitioning among nectarivorous bats as the larger *E. spelaea* enable them to commute long distances to gain a more rewarding floral resource compared to the small *Macroglossus sobrinus* and *M. minimus* which live near their foraging sites (Stewart and Dudash 2017b). The strong site fidelity demonstrated by *E. spelaea* possibly lead to the bats avoiding other bat species' foraging sites, focusing along their commuting paths. This site fidelity behavior is akin to being laser-focused results that on the bats occasionally ignore new durian flowering patches (Acharya et al. 2015). Vertical stratification has also been observed in which *P. hypomelanus* feed on flowers higher (> 20 m) in the trees compared to *E. spelaea* (~10 m) (Aziz et al. 2017a).

With regards to intraspecific competition among foraging bats, one study demonstrated that *Leptonycteris yerbabuenae*, the lesser long-nosed bats in the Sonoran Desert, Mexico use reinforcement learning strategy to learn which flower contains more nectar and returns to the same flower later in

which at the same time discourage other bats to feed on the same flower that has decreased in nectar. When every bat in the flock uses the same learning strategy, this incidentally allows for resource partitioning and reduces conflict (Goldstein et al. 2020). However, this behavior has yet to be documented in Old World fruit bats and could be seen with nectarivorous and frugi-nectarivorous bat flocks pollinating durian trees.

Pollination exclusion experiment enables researchers to determine the main pollinator of a flower species and its pollination effectiveness. Based on all durian-bat past studies mentioned in this review, most researchers use similar exclusion parameters (open pollination & insect pollination) with differences in regards to artificial pollination methods (manual, emasculation or facilitated autogamy) (Table 1). Manual hand-crossed pollination is an industry standard in Thailand, with the addition of planting more than one durian cultivars on a farm to promote outcrossing (O'Gara et al. 2004; Honsho et al. 2009). In other durian producing Southeast Asian countries, planting different cultivars is sufficient and are advised to be supplemented with manual pollination if the yield is too low (O'Gara et al. 2004; Universiti Putra Malaysia 2012). Bumrungsri et al. (2009), Sheherazade et al. (2019) and Chaiyarat et al. (2020) revealed that bat pollinated durian flowers produced substantially higher fruit sets than insect-pollinated durian flowers. However, note that in these studies, manual hand-crossed pollination consistently shown to yield the highest fruit set compared to open pollination by bats and insects, although the difference in yield was not statistically significant (Bumrungsri et al. 2009; Wayo et al. 2018; Chaiyarat et al. 2020). This yield pattern is possibly due to the random nature of open pollination depending upon the availability of bats in the area and its population, which have been decreasing in recent years (Kingston 2013; Aziz et al. 2016). Another factor is that the bats highly likely avoid the small number of flowers in the experiment, pursuing more abundant floral resources elsewhere (Bumrungsri et al. 2009; Chaiyarat et al. 2020). Despite that, bats played a paramount role in durian pollination in non-intensively managed or small orchards plus maintaining the genetic diversity of durians; hence its contribution should not be fully discounted (Fleming et al. 2009; Govindaraj et al. 2015; Chaiyarat et al. 2020). These pollination exclusion studies not only clarify and quantify the effectiveness of bat pollination on durian but also provide a safer and less labor alternative to growers, especially to those who hand pollinate at night as it is more time consuming and has a higher hazard risk (Ketsa et al. 2020).

To sum up, there is still much to clarify on bat pollination even in our small context of bat-durian interactions. While this review mainly discusses the cultivated *Durio zibethinus*, the other wild *Durio* species should not be forgotten as these plant species may provide insight into the evolution of bat pollination in the region. There are possibly other bat species that pollinate these *Durio* species that may be able to pollinate the cultivated durian just like *Rousettus leschenaulti* which deserve further research. Nevertheless, the comprehensive all-year-round *Eonycteris spelaea* (Table 3) not only provide data for the conservation management of the species but also for sustaining pollinator sources for the durian plantation to increase fruit yield with less labor (Bumrungsri et al. 2013; Thavry et al. 2017).

6. DNA metabarcoding as a tool to study pollination and diet of fruit bats.

DNA Metabarcoding (hereafter ‘metabarcoding’, also known as next-generation sequencing) is a viable tool to determine plant-pollinator interactions and determine the diet of a species by utilizing high throughput sequencing for taxa identification from multiple-species samples (Dormontt et al. 2018). Meanwhile, DNA barcoding (hereafter ‘barcoding’) is a molecular technique that relies on amplification of specific DNA regions called barcodes by Polymerase Chain Reaction (PCR) to profile the unique DNA sequences of species that are present in single-species samples, ideally non-contaminated samples (Cristescu 2014). When barcoding was first introduced, most studies focused on sequencing of a specific region for as many species as possible to build up a database that is readily available in public databases for future reference; this includes Barcode of Life Database (BOLD) and the National Center for Biotechnology Information (NCBI) (Ratnasingham and Hebert 2007; Benson et al. 2015). The advancement of DNA sequencing with metabarcoding allows DNA from multiple samples and multiple species to be sequenced in parallel in a single run. This method provides a robust, accurate, fast and cost-effective option to study both plant-pollinator interactions and an animal’s diet. As the cost of sequencing reduces and sequencing efficiency improves, more data can be obtained from a single run of multiple samples. In the context of bat studies, samples could be collected from pollen that attaches to bats that could be sampled by swabbing the face and body of captured bats and stored in a buffer or ethanol for preservation (Edwards et al. 2019). For dietary or gut metagenomic analysis, faeces could be collected and stored inside ethanol for later DNA extraction and DNA sequencing (Frantzen et al. 1998; Murphy et al. 2002).

One of the key aspects of metabarcoding is the barcodes that are chosen for a specific study. Once a barcode is selected, primers were designed to amplify the barcode regions. The barcodes *rbcL* and *matK* are a standard for plant identification, though several other loci, including ITS, *psbA-trnH*, cpDNA and *ycf1* are used to improve resolution in targeted taxons of interest (Hollingsworth and Forrest 2009; Dormontt et al. 2018; Li et al. 2021). Primer design to amplify the barcodes should amplify regions that are highly distinct to allow for species discrimination. However, the primer itself should be in a highly conserved region to prevent primer mismatching causing false negatives when present DNA materials are not amplified due to primer mismatch (Aziz et al. 2017c).

For identification of plant material, the region that codes for ribulose biphosphate carboxylase large chain (*rbcL*) were usually selected and may be paired with other plant barcodes such as ITS2 and *trnL* (Lim et al. 2018a; Edwards et al. 2019; Chan et al. 2021). There is no single barcode universally for plants as each candidate barcodes have trade-offs and imperfections; as such *rbcL* has inadequate performance for seed plants and ITS for incomplete concerted evolution problems (Dong W and Li C 2015). Hence, pairing barcodes are always recommended, some experts even suggest that a single plant DNA barcode is unrealistic (Crautlein et al. 2011). For identifying species of plant visiting bats through sheet collection of faecal pellets, a combination of COI barcodes and plant barcodes could be

utilized to detect both host organism and their food items (Tournayre et al. 2020).

Before barcoding, scientists relied on visual observation to study both the pollination and diet of a species (Orly et al. 2011). The norms used to be a visual observation of the foraging behavior of target species as well as morphological analysis of pollen and feces. Field observation is difficult when the target species is hard to find and observe, especially for volant and nocturnal species such as bats. Faeces were usually dried and observed for undigested tissues for food item identification. For smaller food items and pollen, microscopy will be used for species identification. This method, however, is laborious and requires experienced experts for the accurate identification of food items and pollen. In addition, the identification of tissue items will be limited to undigested hard tissues that remain in the feces.

Barcoding studies involving bats were initially more on the genetic diversity of bats to create a library of bat barcodes that could be used for species identification for further work (Hernández-Dávila et al. 2012). Most dietary analysis for bats were mostly conducted for insectivorous bats due to the agriculture significance of insectivorous bats as a natural predator to pests in agricultural areas (Aizpurua et al. 2018; Russo et al. 2018; Weier et al. 2019; Kemp et al. 2019; Cohen et al. 2020; Kolkert et al. 2020). DNA metabarcoding has been used to study diet composition (Clare et al. 2009); diet specialization (Garin et al. 2019; Orly et al. 2011; Wray AK and Banik 2018; Vesterinen et al. 2016); resource partitioning and dietary overlap (Burgar et al. 2014; Salinas-Ramos et al. 2015; Arrizabalaga-Escudero et al. 2018; Roswag et al. 2018; Vesterinen et al. 2018; Aldasoro et al. 2019); spatial variation of diet (Deagle et al. 2019; Hemprich-Bennett et al. 2020); diet seasonality (Clare et al. 2014b); and sexual dietary bias for insectivorous bats (Mata et al. 2016). Metabarcoding has also been utilized to study parasite diversity in Brazilian bats by using 18S ribosomal RNA as a barcode (Dario et al. 2017).

Furthermore, DNA metabarcoding studies in plant visiting bats have been limited as pollination studies are more popular among bee studies. This trend is due to the nature of honey helping preserve DNA, allowing for more effective sequencing. Bees are also more well known for their pollination contribution, easier to study and are not regarded as a pest. Metabarcoding has been used to detect and quantify plant-pollinator interactions in insects (Pornon et al. 2016); long-distance migration of moths and butterflies (Chang et al. 2018; Suchan et al. 2019); the structure of pollen transfer network of hoverflies (Lucas et al. 2018); quantitative network and honey biodiversity in honeybees (Valentini et al. 2010; Hawkins et al. 2015; Bell et al. 2017).

Metabarcoding studies for dietary preference for plant visiting bats remain limited. DNA metabarcoding has been used to identify the diet of frugivorous Jamaican fruit bats (Hayward 2013), frugi-nectarivorous *Pteropus hypomelanus* (Aziz et al. 2017c), nectarivorous *Eonycteris spelaea* (Lim et al. 2018a), *Leptonycteris yerbabuena* and *Choeronycteris mexicana* (Edwards et al. 2019). Besides that, barcoding was also used to study the impact of urbanization on the diet of fruit bats and nectar bats in tropical countries (Lim et al. 2018b; Chan et al. 2021). The *rbcL* barcode is the most frequently used barcode due to its universal appeal in all five studies either independently (Hayward 2013; Aziz et al. 2017c) or paired with another primer such as ITS2,

trnL and trnH-psbA (Lim et al. 2018a; Edwards et al. 2019; Chan et al. 2021).

Moreover, metabarcoding is more straightforward, faster, requires less expertise and is more accurate, especially in identifying similar-looking insects or pollen (Aziz et al. 2017c; Lim et al. 2018b). An advantage of metabarcoding in bat studies is that it can be completely non-invasive as sample collection does not necessarily require bat trapping either by harp trapping or mist-netting (Swift et al. 2018). Fecal samples can be collected under bat roost and allow fruit pulp identification, which are often overlooked in morphological analysis of feces as they are usually digested and hard to identify (Hayward 2013).

While placing sheets under a roost is a good non-invasive sampling strategy, problems may arise if more than one species of bats occupy a roost. Bat fecal pellets are usually pooled together to determine the overall diet of the colony (Mata et al. 2019). Thus, pellets from different species of bats might be mixed, causing it difficult to assign food items to either species. This predicament could be avoided if each pellet is sequenced separately, but it would be more laborious, time-consuming, costly and would limit amplification of food items with low DNA quantity leading to false negatives as at least seven to twelve fecal pellets are needed to obtain 80% of the total diet (Mata et al. 2019). This inaccuracy is why a more invasive capture and fecal collection directly from individual bats is still favorable for some studies as it allows physical identification of species and also allows the collection of pollen that attaches to the face and body of the bats by swabbing (Aldasoro et al. 2019; Garin et al. 2019).

Another limitation of the metabarcoding approach is the inability to identify parts of the plants that have been consumed (Aziz et al. 2017c; Lim et al. 2018b). Data provided by metabarcoding is usually represented as either a qualitative Frequency of Occurrence (FOO), which measures the absence/presence of a food item in a sample and a quantitative Relative Read Abundance (RRA) (Deagle et al. 2019). It is hard to measure consumption for plant food items as the amount of chloroplast is different in different parts of the plant (Aziz et al. 2017c). Furthermore, it has also been shown that the sequence reads only have a weak correlation with the relative abundance of pollen grains due to chloroplast copy number bias as it will vary depending on the plant tissue type (Bell et al. 2019). Discriminating closely related species may be problematic, causing assignments to be done at the genus level (Aziz et al. 2017c; Edwards et al. 2019).

The Illumina MiSeq is currently the most popular NGS platform for DNA metabarcoding studies across various fields due to their affordable cost coupled with low sequencing error rates and optimal sequencing depth (Liu et al. 2020; Kulski 2016). Other NGS platforms include Ion Torrent, Supported Oligonucleotide Ligation and Detection (SOLiD), DNA nanoball sequencing and the discontinued 454 pyrosequencing (Kulski 2016; Slatko et al. 2018; Xu et al. 2019). Each platform has its machines with varying throughput levels with its advantages and trade-offs; for example, Illumina has the MiniSeq, MiSeq, NextSeq, NovaSeq and HiSeq models (Liu et al. 2020; Kulski 2016; Slatko et al. 2018). Further advancement in NGS technologies gives rise to the third generation sequencers: Long-read sequencing (PacBio & Oxford Nanopore), which directly

sequence single DNA molecules skipping the amplification procedure in real-time unlike the short-read DNA fragments of NGS (PHG Foundation 2018). Long-read sequencing has been used to map the genomes of bat species (*Eonycteris spelaea* & *Pteropus medius*), providing new insights and becoming a valuable resource for future bat research (Wen et al. 2018; Fouret et al. 2020). Oxford Nanopore has also been used in metabarcoding, utilizing the potential of long-read sequences for higher phylogenetic resolution and taxonomic level, which may be lost with short-read sequences of the previous generation (Santos et al. 2020; Baloglu et al. 2021; Davidov et al. 2020). While long-read sequencing overcomes the limitation of NGS by sequencing long stretches of DNA, the technology cannot fully replace NGS due to their higher error rates and the scalability in data analysis (Adewale 2020; Pearman et al. 2020; Amarasinghe et al. 2020). Depending on the taxonomic group, there is a trade-off between higher recall at long read lengths and reads per run (Pearman et al. 2020). The low scalability not only necessitates data processing speed when it takes longer with larger genomes but also impacts data generation, leading to a sizeable IT cost (Adewale 2020; Amarasinghe et al. 2020).

Lastly, DNA metabarcoding is fast becoming a key instrument for ecological studies requiring species identification, be it from direct sampling or environmental samples. The recent decade has been an important period for the development of DNA metabarcoding for both pollination and dietary studies in bats, as multiple studies have been done to compare traditional methods with DNA metabarcoding. Currently, due to the limitations of DNA metabarcoding most studies still suggest a combination of metabarcoding and morphological analysis for dietary analysis (Aziz et al. 2017c; Lim et al. 2018a; Chan et al. 2021). As metabarcoding becomes ubiquitous, more improvements will be added to overcome the current limitations of metabarcoding.

7. Conclusion

To summarize, durian plant-bat pollinator interaction is an integral part of the evolution and pollination ecology of both organisms. The durian flower has adapted and conformed to the bat pollination syndrome, while bats have been proven many times as the most efficient durian pollinator compared to other vertebrate and invertebrate pollinators. While researchers from a zoological and botanical background focused primarily on the bats and the durian tree respectively, these studies complement each other and are further bridged by bat-durian interaction studies. Pollination exclusion experiment gives us an insight into durian-bat interactions and how fruit set count differs amid different pollination treatments. Future studies can shed light on the pollination ecology of the rest of the wild durian species (genus: *Durio*), providing a comprehensive insight into the evolutionary relationship of this plant group and its pollinators; in our context, its relation to fruit bats (Pteropodidae). On a side note, studies can be conducted for plants that are not fully chiropterophilous but may have bats as secondary pollinators like jackfruit (Start and Marshall 1976; Lim et al. 2018a) to understand the significance of bat pollination at the community level, as suggested by Aziz et al. 2021.

On the contrary, with many academic papers detailing the benefits of bat pollination on the durian tree, the durian industry has yet to pick up on the matter, likely due to their reluctance to change their standards; the prime example is the clearing of forest reserve to plant durian. Therefore, there is a need to conduct research added with an economic aspect such as cost–benefit analysis and other economic valuation methods that will incentivize durian farmers to adopt sustainable practices, which will help bats in the long run. As stated in this review, comprehensive all-year bat diet studies for some bat species are a good start, which can be the basis for sustainable, green agroforestry plantations not just for durian but other economically important plants as means to keep the bats all-year near the plantations. It is equally paramount to protect and conserve the roosting sites (caves and forests) of these fruit bats to ensure their survival, allowing them to provide necessary ecosystem services for us.

Last but not least, the study on the quality of durian production with/without bat pollination could bring out a much-needed discussion to the durian industry stakeholders along with the study of pollination differences among durian cultivars. While we have discussed the factors that lower insect pollination efficiency, a clearer verification study can be conducted in future, especially on the local level; our suggestion is to conduct a community experiment in which one local farmer follow our bat-friendly recommendations while the other continue using the industry standard. Hopefully, this experiment and other equivalent solutions provide enough relevant context for durian farmers and policy-makers to adopt a more sustainable and environmentally friendly agriculture practice.

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