

Spatial and temporal variation of floral visitors and their visitation pattern on *Acanthus ilicifolius* L.: A case study from the Indian Sundarbans

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Abstract

Acanthus ilicifolius is a spiny semi-woody evergreen shrub which is one of the most dominant mangrove associate species. Although its floral biology is well documented, there is not enough systematic study of floral visitors of this species. Successful plant reproduction is highly dependent on plant-pollinator interaction. The present study aims to understand the range of floral visitors of *A. ilicifolius* and their spatio-temporal variation in visitation pattern from the Indian Sundarbans. This study was conducted in five islands of Sundarbans Biosphere Reserve (SBR), namely, Bali, Gosaba, Satjelia, Gangasagar and Bakkhali during 2015 and 2016. Fifteen flower visitors under five taxonomic orders, i.e., Hymenoptera, Coleoptera, Diptera, Lepidoptera and Passeriformes were identified. Seven out of these fifteen species were reported for the first time as floral visitor of *A. ilicifolius*. Order Hymenoptera was the most frequent insect group, whereas *Apis mellifera* of this order was the predominant flower visitor. Although Gangasagar and Bakkhali being tourist spots, face greater anthropogenic interference, they had the highest abundance of floral visitors. A significant variation in floral visitor abundance was observed across different time of the day throughout the study period. Floral visitors were most abundant in the morning between 8 a.m. to 12 p.m. This study provides the first comprehensive report of diversity and abundance of the floral visitors and their visitation pattern on *A. ilicifolius*.

Implications for insect conservation: Proper documentation of the ecology of insect pollinators would automatically help to conserve the plants along with the insects, and thus preserve the entire mangrove ecosystem of the Sundarbans as well as mangroves around the world.

Introduction

Three groups of plants species occurring in the mangrove regions are generally identified. These include: (i) major mangrove species; (ii) minor mangrove species; and, (iii) mangrove associates (Shanker et al. 2013; Tomlinson 2016). Major mangroves are those that exclusively occur in the mangrove community and possess morphological and physiological adaptations for survival in a saline environment. They can mainly contribute to community structuring. Minor mangroves on the other hand rarely constitute a major component of the mangrove community (Tomlinson 2016). *Acanthus ilicifolius* (Sea Holly) was classified as a mangrove associated plant (Tomlinson 2016) but is now considered as minor mangrove species (Danda et al. 2017). *Acanthus* is the only genus of the family Acanthaceae, which occupies mangrove habitats (Saranya et al. 2015). This is native to Asiatic countries and Pacific islands and is commonly found in moderately saline inter-tidal areas, riverbanks, sides of canal and roadsides of most of the mangrove zones in India (Akter et al. 2020).

A. ilicifolius is a spiny semi-woody, bushy, evergreen shrub with a height of 0.6 to 1.5 meters on average. It has spines on the margins on its leaves and at stem nodes (Santhiavalli and Jayanthi 2016). This plant has a very dense growth, bears swallow tap roots, but stilt roots also can be seen occasionally (Verma and Shah 2020). The stem has clear nodes and internodes with simple leaves, elliptic-oblong and wavy. This plant has large gullet shaped flowers with green calyx and violet corolla. Flowers are about 50 mm long and 32 mm in diameter [Supplementary Information Fig. S11]. They have raceme type of inflorescence with large quantity of nectar secretion from base of the flower with powdery yellow pollen. Flowers are usually sessile, 2-lipped, hairy outside. Stamens have thick filaments and anthers are densely bearded (Naskar and Mandal 1999). The flowering season of this plant in Sundarbans is from May to August (Chowdhury et al. 2016; Chakrabarty et al. 2022).

This plant plays a critical role in protecting mangrove ecosystem. Several studies have focused on this species due to its production of a wide range of secondary metabolites (Singh and Aeri 2013). Different parts of the plant such as root, leaf and other aerial parts are used for various medicinal applications. In the pharmacological aspects, this plant is used for its anti-inflammatory, anti-leishmanial, osteoblastic, hepatoprotective, and anti-cancer activity. In traditional

and ethnobotanical aspects, this plant has a popular usage in Chinese and Indian medicine. Different parts of *A. ilicifolius* are used for curing dyspepsia, paralysis, and asthma (Singh and Aeri 2013). The leaves of this plant are also used as antidote in snake bite (Velmani et al. 2016).

There have been several studies on floral biology of this economically important plant species all over the world. Floral morphology and biology of *A. ilicifolius* have been studied from Australia (Primack et al. 1981) and the Godavary estuaries in Southern India (Aluri 1990). Along with floral biology, Aluri (1990) had mentioned *Xylocopa latipes*, *Xylocopa pubescens*, *Rhynchium metallicum*, *Nectarinia asiatica* and *Nectarinia zeylonica* as nectar feeder of this species. A documentation of 2 ant species, *Camponotus compressus*, and *Polyrhachis rastellata* were recorded from this species from East Midnapore, West Bengal (Roy et al. 2018). Besides, there have been some studies reporting flower visitors by their common names in various mangrove communities across different parts of India (Remadevi et al. 2008; Therattil and Olakkengil 2011; Arjitha et al. 2014; Therattil 2017). The role of anthropogenic interferences on the insect species composition of *A. ilicifolius* from Bangladesh Sundarbans was also reported by (Akter et al. 2020). However, these studies were all qualitative and there has been no systematic quantification of floral visitors or their visitation pattern on this important mangrove associate species.

Given the gross lack of understanding of the floral visitation ecology of *Acanthus ilicifolius*, this study aimed at the temporal and spatial variation in floral visitors and their visitation pattern. This is the first report of floral visitors of *Acanthus ilicifolius* from the Indian Sundarbans. In this context, the present study addressed three primary questions:

- a. What are the floral visitors of this species, and what is their functional role in interaction with the plant?
- b. What is the spatial variation in floral visitation composition across five different sites with varying anthropogenic interferences?
- c. What is the diurnal pattern and interannual variation in the faunal visitor composition?

Materials And Methods

Study area

Sundarbans, a UNESCO World Heritage Site, is one of the largest mangrove ecosystem in the world, which spreads over two countries India and Bangladesh. The Indian part of Sundarbans lies between the coordinates 21.533° to 22.667° N and 88.083° to 89.850° E (Giri et al. 2014). Indian Sundarbans includes almost 9600 sq. km. area (4200 sq. km. of reserve forest and 5400 sq. km. of non-forest inhabited region) constitute the biosphere region which is known as Sundarbans Biosphere Reserve (SBR) (Mitra et al. 2016). The study was conducted in across 5 islands in the SBR, namely Bali (22.072° N, 88.703° E), Gosaba (22.133° N, 88.826° E), Satjelia (22.102° N, 88.889° E), Bakkhali (21.562° N, 88.272° E), and Gangasagar (21.644° N, 88.062° E) (Fig. 1). The study site at Bali was most distant from human settlement and therefore was the least disturbed site. Gosaba is situated nearby protected area and also with lower degree of disturbance. The site at Satjelia was situated by the side of local villages with moderate to low anthropogenic interferences. Also, apiculture has been a common practice of the villagers there. Therefore, the abundance of *Apis mellifera* was comparatively high in this area. While Bakkhali is a tourist spot, Gangasagar is a pilgrimage area. These two sites are subjected to a very high degree of disturbances. All of these sites experience 3 pronounced seasons- summer season (March-May); monsoon season (June-October) and winter season (November-February). The flowering timing of *A. ilicifolius*, was in late summer and early rainy season with mean temperature lying between 28–30°C. The total monthly precipitation increased from around 150mm in May to 260mm in June, with July experiencing heavy rainfall of over 700mm (NASA 2022).

Data Collection and preservation of insect species

The study was conducted for two years, 2015 and 2016, in Sundarbans Biosphere Reserve, during the flowering period of *Acanthus ilicifolius*. At each site, five quadrates of 5×5 square meters were randomly selected. The observation was done in these quadrats at different times of the day across two years. The quadrates were surveyed between 6 a.m. to 6 p.m. with 2-hour intervals like 6 to 8 a.m. (T1), 8 to 10 a.m. (T2), 10 a.m. to 12 p.m. (T3), 12 to 2 p.m. (T4), 2 to 4 p.m. (T5) and 4 to 6 p.m. (T6). During each interval, a quadrate was observed by multiple observers for a continuous period of 10 minutes (Kato and Kawakita 2004). Thus, a total of 5 quadrates were observed in multiple cycles for 2 hours during each time interval. This sampling led to a total of 1898 floral visitors observed across the five sites in two years. For the common insects, identification was done in the field by using standard literature without capturing or killing the insect, and photographs were taken wherever possible. However, for those species which could not be identified visually, they were collected from the flowers by insect net and identified with the help of various experts of Zoological Survey of India, Kolkata.

Categorizations of interaction of fauna with the plant

The interaction of floral visitors with *A. ilicifolius* observed throughout the study period were classified in a hierarchical manner from flower visitor to nectar collector to pollen and nectar collector. This classification was based on repeated observation of interaction pattern initially starting with floral visitor and upgrading to pollen and nectar collector as per observation. The following criteria were used for classification:

Flower visitor- Visitors that touched the accessory reproductive parts, calyx, and corolla, but neither touched any part of androecium and gynoecium. Thus, they neither collected pollen nor nectar.

Nectar collector- Flower visitors which only collected nectar from the flower without touching any part of androecium and gynoecium.

Pollen and nectar collector- Flower visitors which collected both pollen and nectar from this plant.

The flower visitors identified as nectar collector were not observed to carry pollen at the time of observation. However, this is solely based on our observation and may differ from that reported in literature. None of flowers visited by these observed floral visitors were bagged and followed till fruit set. Thus, these were only termed as visitors but not confirmed to be pollinators.

Data Analysis

Among all of the floral visitors, *Atherigona oryzae*, of the family Muscidae was observed for two time points in only one year, and at only one site, Bakkhali, only in 2015. Here, paddy fields were situated in close proximity and this species had been reported as pest of rice (Senapati and Satpathy 1983). Besides, at the time of visitation, these did not exhibit any foraging behavioural activity, typical of floral visitors. Therefore, this dipteran species was considered as an accidental flower visitor in the study and was excluded from further statistical analysis. For all qualitative analysis, relative abundance was calculated in percentage of total individuals observed. Floral visitation pattern was qualitatively expressed as percentage of species across sites, years and by type of interaction with *A. ilicifolius* as described in the section above. The diurnal pattern of floral visitation was expressed as the percentage of maximum observed for each species throughout the day, when summed across all years and across all sites. On this normalised data, a threshold of 30 percent was used to determine the time of onset and offset in a day. Onset was defined as the earliest when the insect abundance was more than the threshold and offset was the earliest when abundance was less

than the threshold. If these conditions were met more than once a day, multiple onset and offset (bouts) were noted. For each bout, mean, median and modal timing weighted by abundance was calculated. For quantitative analysis, the visitation data was checked for normality. Since the data was heavily positively skewed, none of the transformation gave a distribution similar to normal. Hence Generalized Linear Mixed Model (GLMM) with negative binomial fitting was used to understand the effect of site, time of the day and year on visitor species composition. Similarity and dissimilarity between sites and time of the day with regards to visitor diversity and abundance was calculated using Bray-Curtis dissimilarity index.

Results

Diversity and association status of floral visitors

A total of 16 species from 5 orders were observed on *Acanthus ilicifolius* from 5 sites across all time points in 2 years of observation. Among these, the order Hymenoptera (8 species) was most dominant in terms of abundance, comprising 48.96% of all observations. Among Hymenoptera, *Apis mellifera* followed by *Apis dorsata* were the most dominant flower visitors of *A. ilicifolius*. This was followed by the order Coleoptera (1 species) comprising 32.49%, Diptera (2 species) comprising 8.40%, and Lepidoptera (3 species) comprising 7.39%. Sunbirds comprised of only 2.75% of the total observation (Table 1, [Supplementary Information Fig. SI2-SI3]). When considered across sites, Hymenoptera and Coleoptera were consistently dominant across all the sites. While Lepidoptera were consistently present across all the sites, no Diptera was observed in Bakkhali, and Sunbird was only observed in Bakkhali and Gangasagar (Fig. 2).

Table 1
List of observed floral visitors of *Acanthus ilicifolius* recorded from Sundarbans Biosphere Reserve.

Abbreviation	Species	Family	Order	Status	Rel Freq
AD	<i>Apis dorsata</i>	Apidae	Hymenoptera	P & N	7.87
AM*	<i>Apis mellifera</i>	Apidae	Hymenoptera	P & N	22.66
AG*	<i>Amegilla</i> sp.	Apidae	Hymenoptera	P & N	1.53
XP	<i>Xylocopa pubescens</i>	Apidae	Hymenoptera	P & N	1.90
XL	<i>Xylocopa latipes</i>	Apidae	Hymenoptera	Nectar	0.69
OS	<i>Oecophylla smaragdina</i>	Formicidae	Hymenoptera	P & N	7.34
TR*	<i>Tetraponera rufonigra</i>	Formicidae	Hymenoptera	Nectar	4.65
RH	<i>Rhynchium</i> sp.	Vespidae	Hymenoptera	Visitor	2.32
MD*	<i>Micraspis discolor</i>	Coccinellidae	Coleoptera	Visitor	32.49
AO	<i>Atherigona oryzae</i>	Muscidae	Diptera	Accidental	N/A
BC*	<i>Bactrocera cucurbitae</i>	Tephritidae	Diptera	Visitor	2.32
SS*	<i>Sepedon spehegea</i>	Sciomyzidae	Diptera	Nectar	6.08
TL*	<i>Tirumala limniace</i>	Nymphalidae	Lepidoptera	P & N	6.44
OTU-L1	Lepidopteran species 1		Lepidoptera	Visitor	0.58
OTU-L2	Lepidopteran species 2		Lepidoptera	P & N	0.37
OTU-SU	Sunbird (<i>Nectarina</i> sp.)	Nectariniidae	Passeriformes	P & N	2.75

In the table headers, 'Abbreviation' refers to 2 letter code used to refer to a species (these abbreviations will be used in subsequent figures); 'Species' is corresponding valid scientific name, with 'Family' and 'Order' the species belong to. 'Status' is the type of interaction of floral visitor with *A. ilicifolius* as mentioned in methodology. 'P & N' refers to both pollen and nectar feeder; 'Nectar' is only nectar feeder; 'Visitor' is only floral visitor and 'Accidental' is accidental floral visitor. Species abbreviations marked by '*' are first report of floral visitor *A. ilicifolius* and not seen elsewhere. 'Rel Freq' refers to the relative frequency of the species among all observation, expressed as percentage.

The type of interaction of the visitors with the plant during the study was quantified. Among all the visits from the 8 species of order Hymenoptera, more than 81% of visits were from pollen and nectar collector, about 14% were only nectar collector and 5% were just flower visitors (Fig. 3). Four species from Apidae, namely *Apis mellifera*, *Apis dorsata*, *Amegilla* sp. and *Xylocopa pubescens* were both pollen and nectar feeder, while *Xylocopa latipes* was only nectar feeder. From class Formicidae, *Oecophylla smaragdina* was both pollen and nectar feeder while *Tetraponera rufonigra* was observed to be a nectar-robber. *Rhynchium* sp. was just a flower visitor. *Micraspis discolor* was the only coleopteran reported in this study which was a flower visitor. In the order Diptera, only 28% of the visits came from *Sepedon spehegea* who is only nectar feeder, while 72% of visits from *Bactrocera cucurbitae* were flower visitor. More than 92% of the Lepidopteran visits were from both pollen and nectar collector, while 8% were just flower visitor. *Tirumala limniace* was the most abundant Lepidopteran species and was both pollen and nectar feeder. An unidentified sunbird was the only bird species that was observed as both pollen and nectar collector from *A. ilicifolius*.

Interannual variation in diversity and abundance of visitors

Although there were slight differences in the abundance of each species across the two years of observation, the differences were not significant ($\chi^2 = 1.07$, $p = 0.30$). Species like *Micraspis discolor* and *Apis mellifera* were consistently high in both the years. Most species were observed in both the years some species like *Xylocopa latipes* and the unidentified Sunbird were observed only in 2015 but not in 2016 (Fig. 4).

Spatial variation in floral visitors

Similar to interannual variation in visitor abundance, there was no significant difference in abundance between the five sites ($\chi^2 = 2.19$, $p = 0.70$). Among the sites, Bali had the highest diversity with 12 of the 16 species while Gosaba had the second highest diversity with 11 species. Bakkhali had the lowest species diversity with only 7 out of the 16 species observed. While *Micraspis discolor* was consistently abundant across all the sites, *Apis mellifera* was most abundant in Bakkhali, Gangasagar, and Satjelia and *Apis dorsata* was most abundant in Bali and Gosaba. Bray-Curtis dissimilarity index was used to show similarity between sites. Bakkhali and Gangasagar shared lowest dissimilarity (0.14) (Fig. 5), implying that these two sites were most similar in terms of species diversity and abundance. Satjelia showed moderate dissimilarity with both Gangasagar and Bakkhali (0.31). In the other clade, Bali and Gosaba shared the second lowest dissimilarity of 0.28. The highest dissimilarity in visitor species composition were shown between Gosaba and Bakkhali (0.70), followed by Gosaba and Gangasagar (0.61).

Diurnal variation in floral visitors

There was significant variation in visitor diversity and abundance across the time of the day ($\chi^2 = 112.53$, $p < 0.001$), although this did not differ by site ($\chi^2 = 15.33$, $p = 0.76$). All of the species was observed from 8 to 10 a.m. (T2) Similarly, timeframes of 6 to 8 a.m. (T1) and 10 a.m. to 12 p.m. (T3) had 15 out of the total 16 species observed. There was a general reduction in visitor abundance from 12 to 4 p.m. (T4 and T5), but again increased in the late afternoon from 4 to 6 p.m. (T6). Among the 16 visitor species, 5 had a single foraging bout in the morning, 10 species had 2 bouts- once in morning and once in late afternoon, while only one species, *Micraspis discolor*, was available throughout the day (Fig. 6). A cluster analysis using Bray-Curtis dissimilarity index showed that, among these six timeframes T4 and T5 showed the least dissimilarity (0.20) followed by T2 and T3 (0.22) (Fig. 7). There is also low dissimilarity between T4 and T6 (0.25). The highest dissimilarity in visitor species composition were shown by three different time frames, that was between T2 and T5 (0.65), followed by T2 and T4 (0.57).

Discussion

Diversity and association status of fauna with *A. ilicifolius* from the Indian Sundarbans

A total of 13 species was observed from 7 studies on floral visitors of *Acanthus ilicifolius* from various mangrove forests of western India, Bangladesh Sundarbans, and Australia (Table 2). Among these 13 species, 9 species had been exclusively reported in other studies while 4 species reported in common. These 4 species included *Apis dorsata* (Akter et al. 2020), *Xylocopa latipes* (Aluri et al. 2017), *Xylocopa pubescens* (Aluri et al. 2017), and *Oecophylla smaragdina* (Roy et al. 2018). In addition to these, the current study reported 7 identified unique floral visitors, and 5 other unidentified species not reported elsewhere (Table 2). *Micraspis discolor* followed by *Apis mellifera* were the most

dominant floral visitors in the study site. *M. discolor* have been known to be a predator of aphids (Shanker et al. 2013) as well as low pollen carrying capacity (Chakraborti et al. 2019). While *A. mellifera* is a crucial pollinator of most ecosystems (Hung et al. 2018). *Apis dorsata* was another important flower visitor in the study site. Thus overall, most of the floral visitors might be potential pollinators.

Table 2
Complete checklist of floral visitors of *Acanthus ilicifolius* from global literature and the current study.

Sl No	Species	Order	Family	Status	Reference
1	<i>Amegilla</i> sp.	Hymenoptera	Apidae	Visitor	#
2	<i>Amegilla cingulata</i> ** (Fabricius, 1775)	Hymenoptera	Apidae	Pollen and Nectar feeder	[1]
3	<i>Apis dorsata</i> Fabricius, 1793	Hymenoptera	Apidae	Visitor	[2], #
4	<i>Apis mellifera</i> Linnaeus, 1758	Hymenoptera	Apidae	Visitor	#
5	<i>Apis cerena</i> Fabricius, 1793	Hymenoptera	Apidae	Pollinator	[2]
6	<i>Apis florea</i> Fabricius, 1787	Hymenoptera	Apidae	Pollinator	[2]
7	<i>Xylocopa latipes</i> (Drury, 1773)	Hymenoptera	Apidae	Pollinator	[3], [4], [1], #
8	<i>Xylocopa pubescens</i> Spinola, 1838	Hymenoptera	Apidae	Nectar feeder	[3], [4], [1], #
9	<i>Oecophylla smaragdina</i> Fabricius, 1775	Hymenoptera	Formicidae	Visitor	[5], #
10	<i>Tetraponera rufonigra</i> (Jerdon, 1851)	Hymenoptera	Formicidae	Nectar feeder	#
11	<i>Allorhynchium metallicum</i> (Saussure, 1853)***	Hymenoptera	Vespidae	Pollinator	[3]
12	<i>Rhynchium</i> sp.	Hymenoptera	Vespidae	Visitor	#
13	<i>Odynerus</i> sp.	Hymenoptera	Vespidae	Pollen feeder	[1]
14	<i>Micraspis discolor</i> (Fabricius, 1798)	Coleoptera	Coccinellidae	Visitor	#
15	Unidentified lepidoptera 1	Lepidoptera		Visitor	#

The 'Species' column represents the valid scientific name of species, along with their 'Order' and 'Family' they belong to. 'Status' is the type of interaction of the floral visitor species with *A. ilicifolius*, with interaction categorized as Visitor, Pollen feeder, Nectar feeder, Pollen and Nectar feeder, and Pollinator. In the 'Reference' column, those marked with # indicates our report and that with the numbers correspond to the actual references mentioned below the table.

** Valid synonym for *Anthophora cingulate* of the family Anthophoridae, which was originally reported by Aluri et al. (2017).

*** Valid synonym for *Rhynchium metallicum* of the family Vespidae, which was originally reported by Aluri (1990).

**** Valid synonym for *Nectarinia jungularis* of the family Passeriformes, which was originally reported by Primack et al. (1981).

#. Current study, 1. Aluri et al. (2017), 2. Akter et al. (2020), 3. Aluri (1990), 4. Reddi and Aluri (1997), 5. Roy et al. (2018), 6. Primack et al. (1981)

SI No	Species	Order	Family	Status	Reference
16	Unidentified lepidoptera 2	Lepidoptera		Pollen and Nectar feeder	#
17	<i>Borbo cinnara</i> (Wallace, 1866)	Lepidoptera	Hesperiidae	Nectar feeder	[1]
18	<i>Tirumala limniace</i> (Cramer, 1775)	Lepidoptera	Nymphalidae	Pollen and Nectar feeder	#
19	<i>Atherigona oryzae</i> Malloch, 1925	Diptera	Muscidae	Visitor	#
20	<i>Bactrocera cucurbitae</i> (Coquillett, 1899)	Diptera	Tephritidae	Visitor	#
21	<i>Sepedon spegea</i> (Fabricius, 1775)	Diptera	Sciomyzidae	Visitor	#
22	Unidentified sunbird	Aves	Passeriformes	Pollen and Nectar feeder	#
23	<i>Nectarinia asiatica</i> (Latham, 1790)	Aves	Passeriformes	Pollinator	[3]
24	<i>Nectarinia zeylonica</i> (Linnaeus, 1766)	Aves	Passeriformes	Pollinator	[3]
25	<i>Cinnyris jugularis</i> (Linnaeus, 1766)****	Aves	Passeriformes	Pollinator	[6]
The 'Species' column represents the valid scientific name of species, along with their 'Order' and 'Family' they belong to. 'Status' is the type of interaction of the floral visitor species with <i>A. ilicifolius</i> , with interaction categorized as Visitor, Pollen feeder, Nectar feeder, Pollen and Nectar feeder, and Pollinator. In the 'Reference' column, those marked with # indicates our report and that with the numbers correspond to the actual references mentioned below the table.					
** Valid synonym for <i>Anthophora cingulate</i> of the family Anthophoridae, which was originally reported by Aluri et al. (2017).					
*** Valid synonym for <i>Rhynchium metallicum</i> of the family Vespidae, which was originally reported by Aluri (1990).					
****Valid synonym for <i>Nectarinia jugularis</i> of the family Passeriformes, which was originally reported by Primack et al. (1981).					
#. Current study, 1. Aluri et al. (2017), 2. Akter et al. (2020), 3. Aluri (1990), 4. Reddi and Aluri (1997), 5. Roy et al. (2018), 6. Primack et al. (1981)					

46 percent of the species in the study site were pollen and nectar feeder, while nectar feeder and floral visitor were present in equal proportions of about 27 percent. In contrast, about 67 percent of species in global literature were reported to be pollinator, while 11 percent were pollen, nectar, and both pollen and nectar feeder. Although it was not tested for the floral visitors to be exclusively pollinators, about a total of 73 percent of floral visitors could be potential pollinators. This was comparable to that observed in global literature. *Tetraponera rufonigra* was observed to be a nectar-robber. They were observed to take nectar from outside, at the base of the flower instead of the conventional opening without touching the androecium or gynoecium (Newman and Thomson 2005).

Temporal and spatial variation in visitor diversity and abundance

Variation in abiotic conditions like temperature and rainfall can be expected to result in differences in floral visitor abundance. However, there was no variation in temperature and rainfall observed across the two years. Additionally, field observation suggested that there was no variation in flowering phenology and intensity across the two years. These might explain the lack of interannual variation in floral visitor abundance. Besides temporal variation, spatial variation in floral visitor abundance was also not observed. This might be because the sites were not distant enough to result in variation in floral visitor diversity. Although the sites, Gangasagar and Bakkhali, experience highest anthropogenic interference as compared to the other sites, this did not result in differences in floral visitor abundance. Empirical observation and previous work done at these sites (Chakraborti 2019) suggested that, tree species composition were more or less similar across the five sites. Although other environmental parameters like soil and water salinity and pH were not measured, they could be assumed to be similar across sites due to their close proximity. Similarity in all these factors might be responsible for the lack of differences on floral visitor diversity and abundance across the five sites (González et al. 2014). Although there was no significant variation in floral visitor abundance, *Apis dorsata* was more abundant in Bali and Gosaba, while *Apis mellifera* was more abundant in Bakkhali, Gangasagar, and Satjelia. This was because the later sites were around local villages where box of *Apis mellifera* were set up by local villagers for Apiculture.

Diurnal pattern of floral visitors on *Acanthus ilicifolius*

Diurnal pattern of floral visitors showed that the timeframe between 8 to 10 a.m. showed the highest visitor diversity and abundance. This time is generally most preferred for foraging by floral visitors (Knop et al. 2018). Thus, species visiting during this time might potentially play a major role as potential pollinators of *A. ilicifolius*. Besides this primary bout in the morning, many of the floral visitors had a secondary bout in the afternoon. This event is also common among foragers (Herrera 1990) and is beneficial for both the plant and the floral visitors. In terms of similarity in visitor diversity and abundance between timeframes, results showed the period between 12 to 4 p.m. (T4 and T5) to be most similar to each other, than 8 a.m. to 12 p.m. (T2 and T3). This might be misleading due to the fact that abundance of floral visitors was lowest in the afternoon and highest in the morning. This highest similarity might have resulted from the inherently low abundance as opposed to the true similarity between T2 and T3 when abundance was actually high. Besides, the highest dissimilarity between the morning and the afternoon timeframes was actually due to lack of abundance of floral visitors at those afternoon timeframes (Azmi et al. 2015; Abdel-Galel et al. 2021).

Conclusion

The current study is the first report of floral visitors of *Acanthus ilicifolius* from Indian Sundarbans, not only describing the floral visitors but their temporal and spatial variation in visitation pattern. The results highlighted the diurnal activity pattern of floral visitors with most of their visitation occurring in the morning to noon. It also revealed that the abundance of family Apidae is significantly higher during the peak visitation time, implying their role as most important potential pollinators of *A. ilicifolius* of the study sites. In spite of the sites experiencing different degree of anthropogenic interferences, the study did not find significant variation in visitor diversity and abundance. This study provides the baseline data that can be used for further identification of true pollinators of this economically important mangrove species. Identification and preservation of the ecology of pollinators is crucial for conserving mangroves. Thus, this would help in future work on conservation of the threatened mangrove habitat of Sundarbans and mangroves around the world.

Declarations

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Author contributions

Tania Chatterjee: Conceptualization, Methodology, Supervision, Investigation, Formal analysis, Visualization, Validation, Writing - original draft, Writing - review & editing; Souparna Chakrabarty (Equal contribution): Investigation, Data curation, Visualization, Software, Writing - original draft, Writing - review & editing; Sankarshan Roy: Investigation, Methodology, Data curation; Olive Biswas: Investigation, Data curation, Methodology; Bulganin Mitra: Conceptualization, Supervision, Project administration, Resources, Punarbasu Chaudhuri: Conceptualization, Supervision.

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Conflict of Interests

The authors have no competing interests to declare that are relevant to the content of this article. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. The authors have no financial or proprietary interests in any material discussed in this article.

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Figures

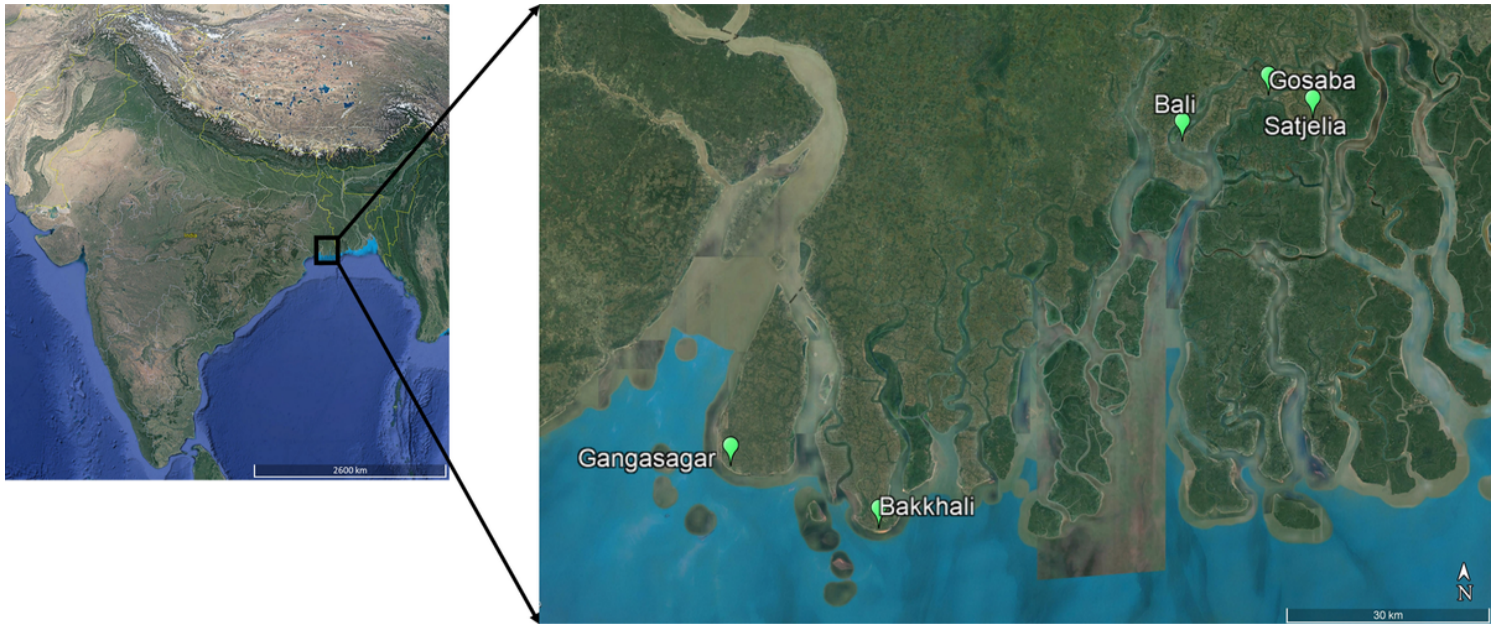


Figure 1

Location of the study sites in Sundarbans Biosphere Reserve. Study sites are marked by green pins: Bali (22.072° N, 88.703° E), Gosaba (22.133° N, 88.826° E), Satjelia (22.102° N, 88.889° E), Bakkhali (21.562° N, 88.272° E), and Gangasagar (21.644° N, 88.062° E)

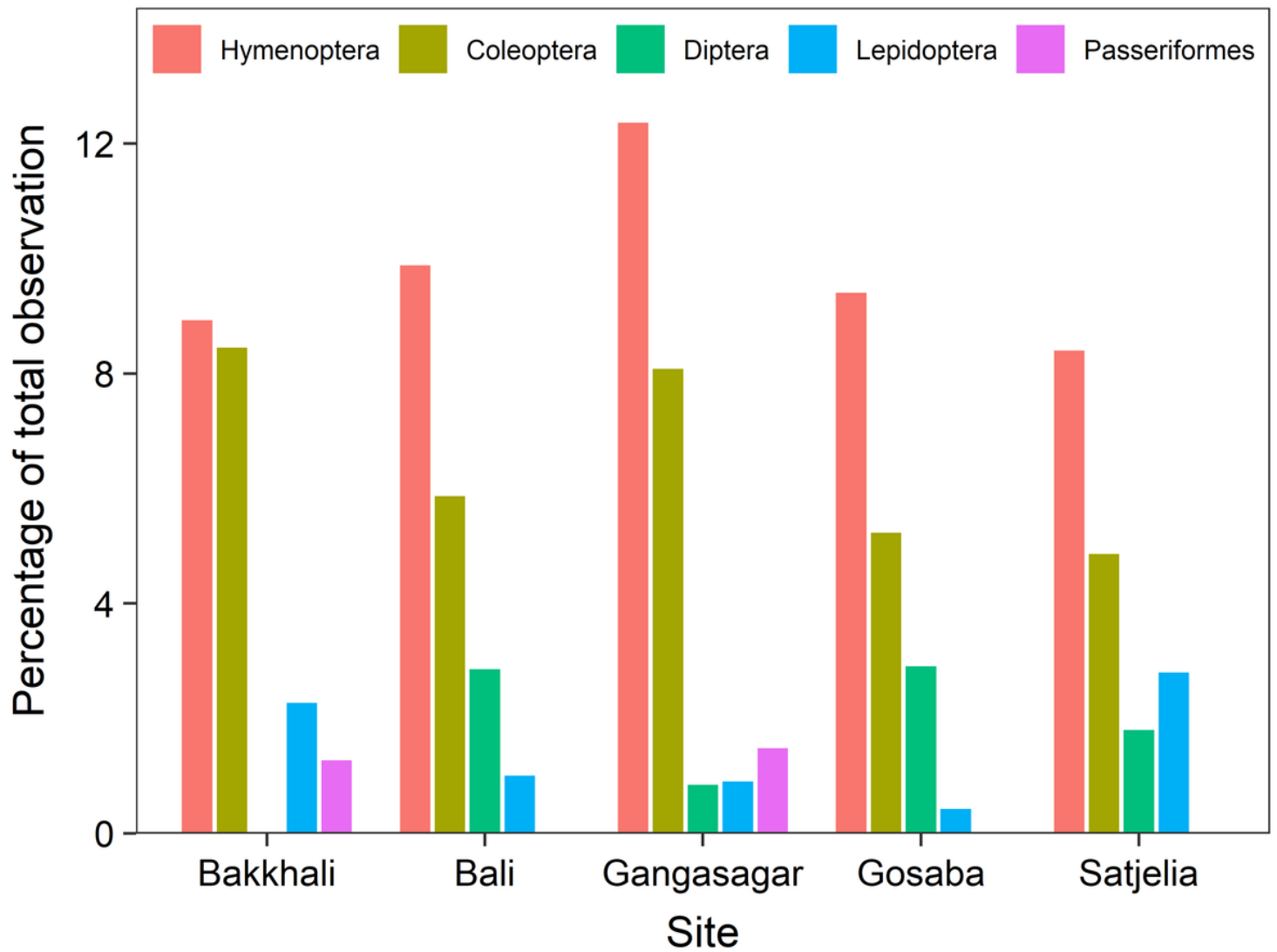


Figure 2

Differences in floral visitor Order across the five study sites. Numbers on the y-axis represent the visitor order in each site as a percentage of total observed visitation

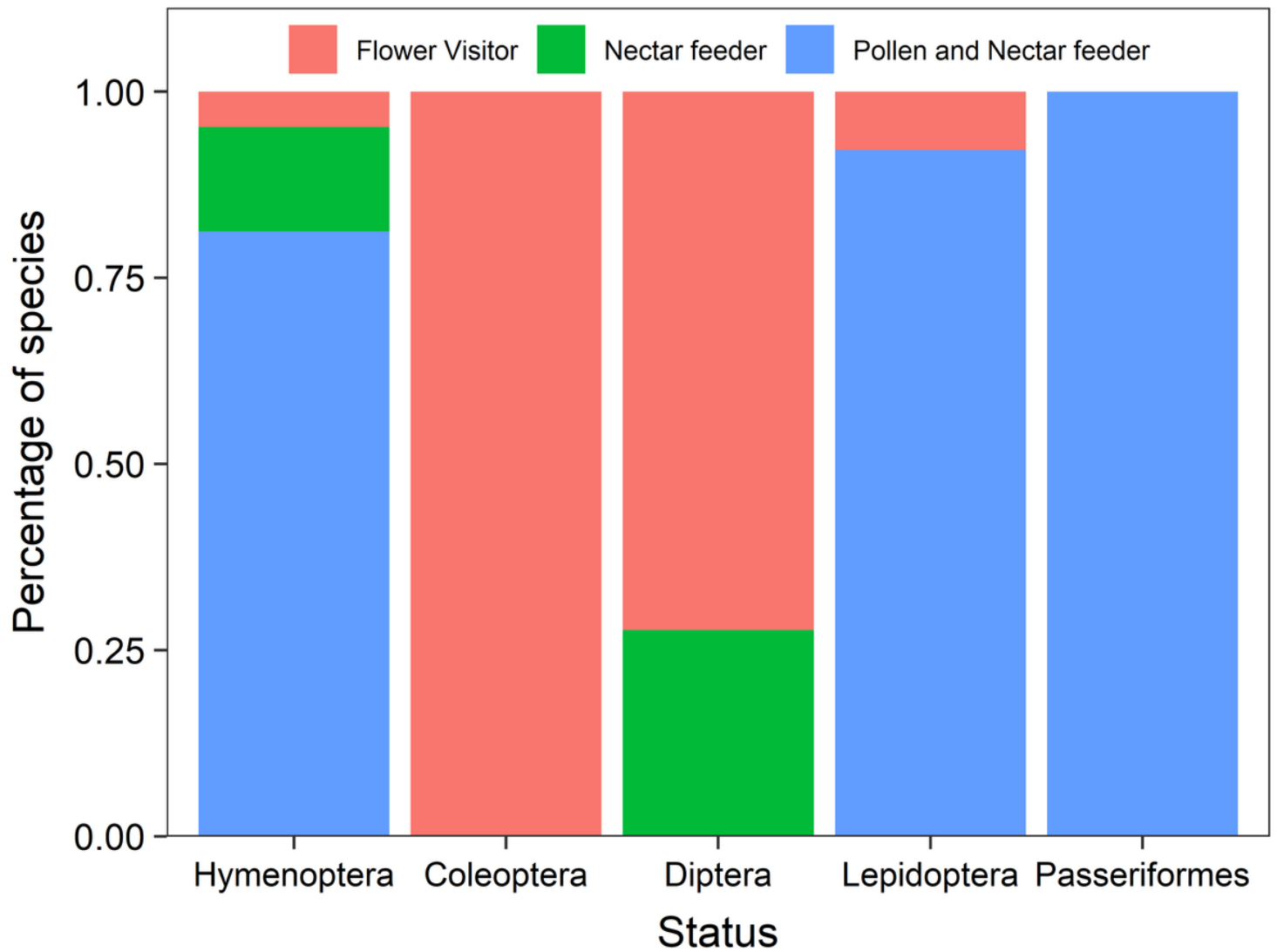


Figure 3

Stacked column representing the proportion of species with different types of interactions with *A. ilicifolius* in the study site. Numbers on the y-axis represent the proportion of species with different interaction type for each Order of floral visitor

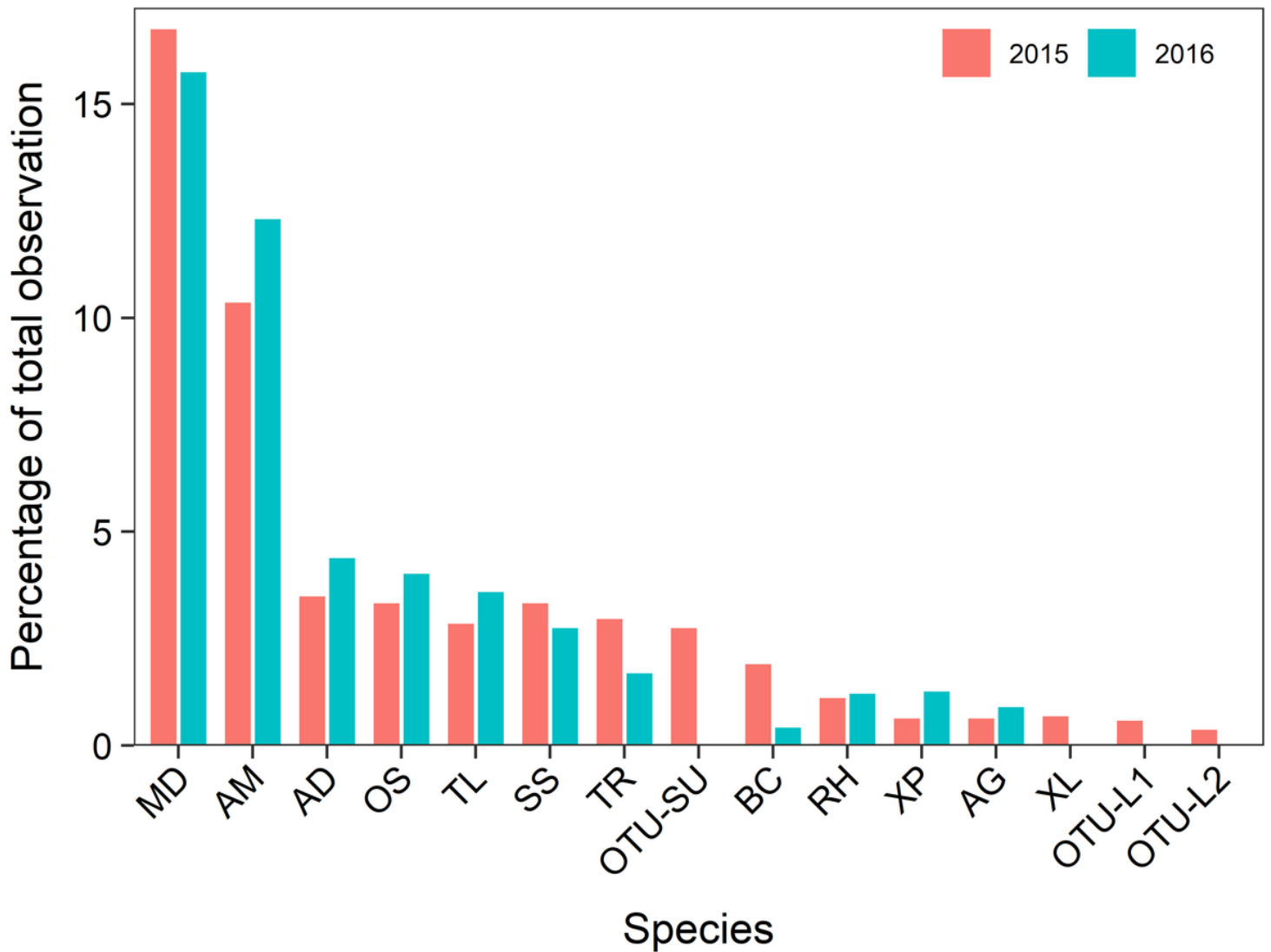


Figure 4

Interannual variation in each species of floral visitors of *A. ilicifolius* across the two years. Numbers on the y-axis represent the percentage of each species out of the total observed visitation

The species abbreviation are as follows: MD- *Micraspis discolor*, AM- *Apis mellifera*, AD- *Apis dorsata*, OS- *Oecophylla smaragdina*, TL- *Tirumala limniace*, SS- *Sepedon sphegea*, TR- *Tetraponera rufonigra*, OTU-SU- Sunbird (*Nectarina* sp.), BC- *Bactrocera cucurbitae*, RH- *Rhynchium* sp., XP- *Xylocopa pubescens*, AG- *Amegilla* sp., XL- *Xylocopa latipes*, OTU-L1- Lepidopteran species 1, OTU-L2- Lepidopteran species 2

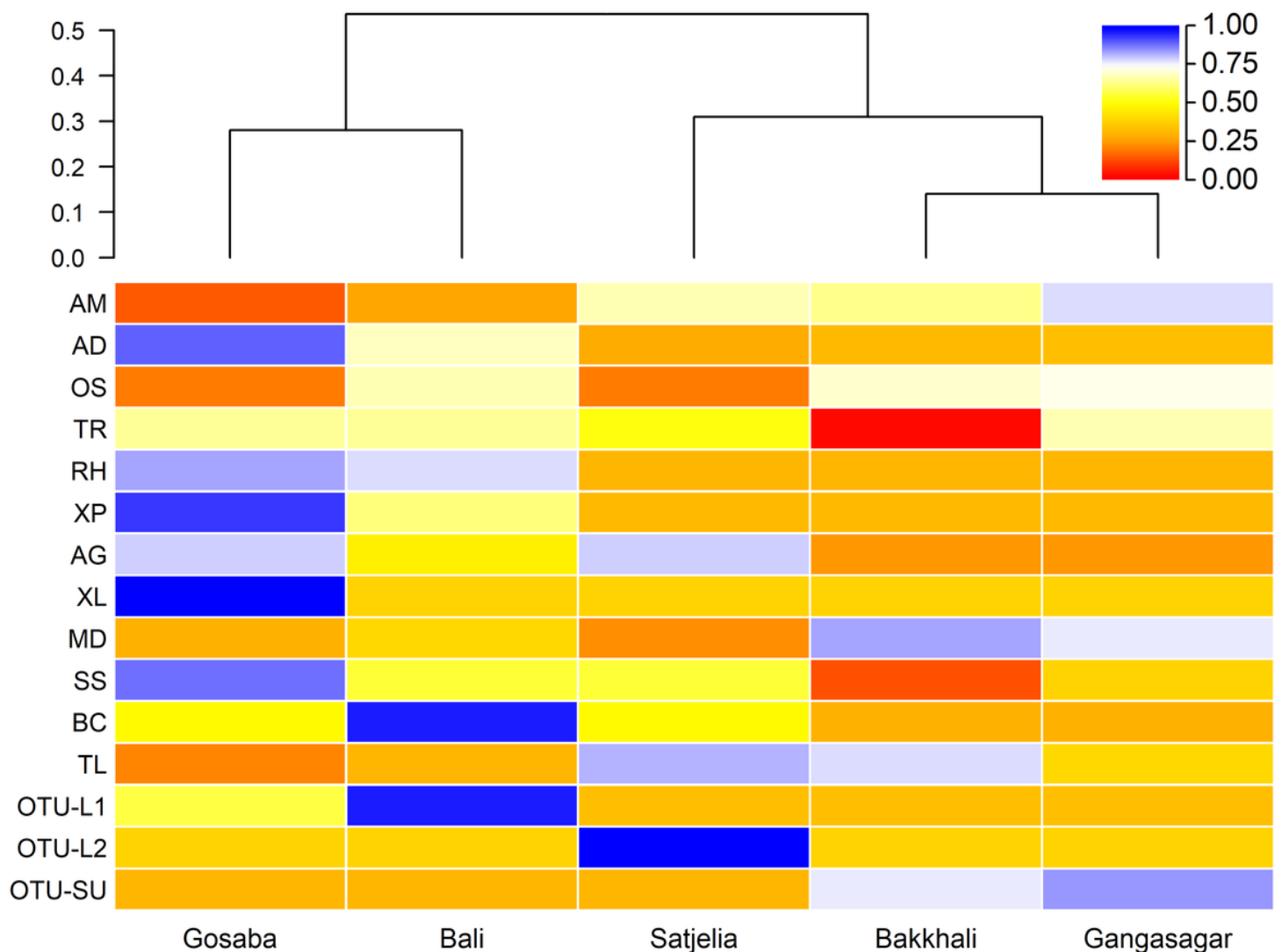


Figure 5

Similarity and dissimilarity between the sites in terms of floral visitor diversity and abundance. The top dendrogram represent the hierarchical clustering of the sites along with the Bray-Curtis dissimilarity distance on the left y-axis. The bottom heatmap represent the relative proportion of each species across the five sites. The heatmap scale in the top right corner represent heatmap colours, with the lowest proportion represented by red and highest proportion represented by blue colour

The species abbreviation are as follows: AM- *Apis mellifera*, AD- *Apis dorsata*, OS- *Oecophylla smaragdina*, TR- *Tetraponera rufonigra*, RH- *Rhynchium* sp., XP- *Xylocopa pubescens*, AG- *Amegilla* sp., XL- *Xylocopa latipes*, MD- *Microspis discolor*, SS- *Sepedon sphegea*, BC- *Bactrocera cucurbitae*, TL- *Tirumala limniace*, OTU-L1- Lepidopteran species 1, OTU-L2- Lepidopteran species 2, OTU-SU- Sunbird (*Nectarina* sp.)

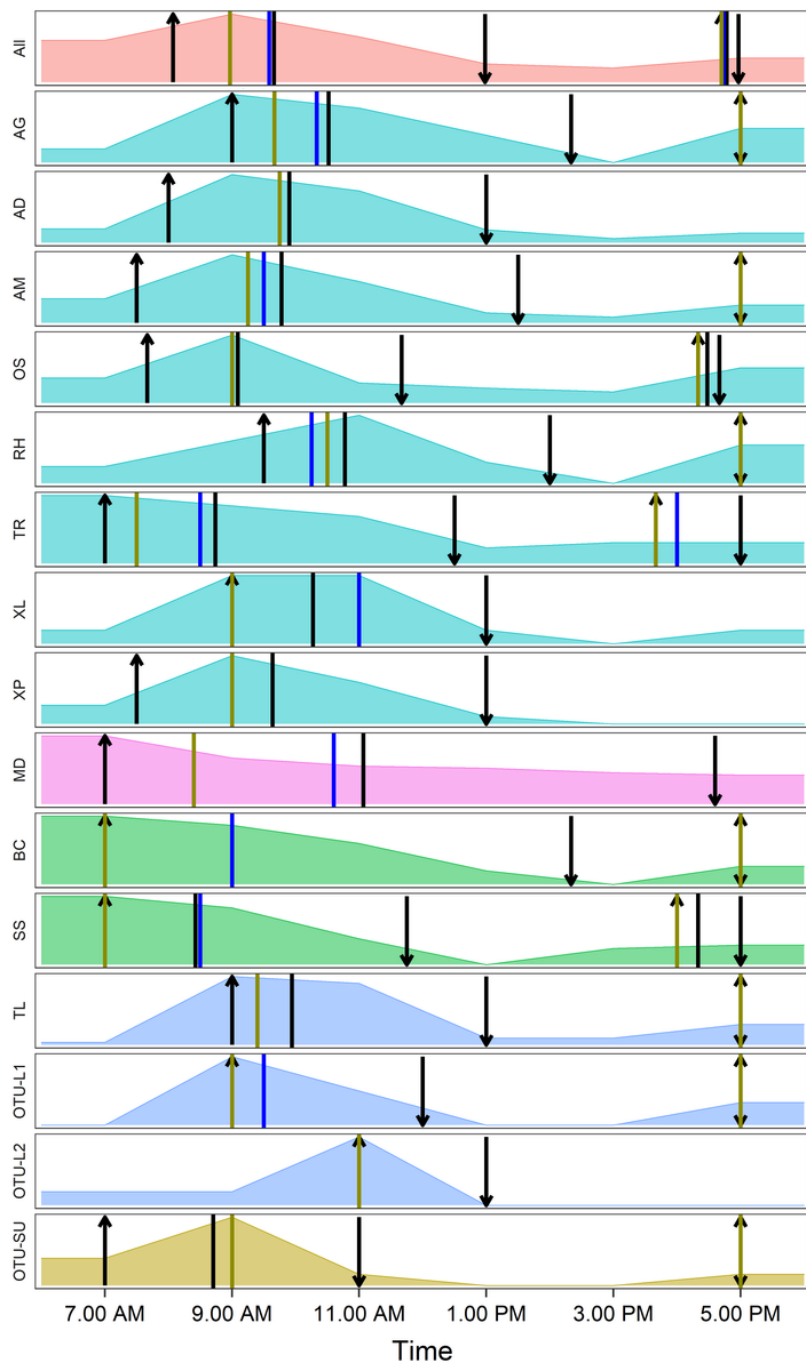


Figure 6

Variation in diurnal timing of floral visitors. The up arrow indicates mean onset timing for each species while the down arrow indicates offset timing. The black vertical line is the mean timing, blue is median while yellow is the modal timing. The species are grouped by Order they belong to. For each species, the shaded area indicates the variation in percentage of individuals across the day on a scale between 0 to 100. For species with more than one prominent bout of activity, arrows indicating the timing of each bout are provided

The species abbreviations are as follows: All- all species together, AG- *Amegilla* sp., AD- *Apis dorsata*, AM- *Apis mellifera*, OS- *Oecophylla smaragdina*, RH- *Rhynchium* sp., TR- *Tetraponera rufonigra*, XL- *Xylocopa latipes*, XP- *Xylocopa pubescens*, MD- *Micraspis discolor*, BC- *Bactrocera cucurbitae*, SS- *Sepedon speghea*, TL- *Tirumala limniace*, OTU-L1- Lepidopteran species 1, OTU-L2- Lepidopteran species 2, OTU-SU- Sunbird (*Nectarina* sp.)

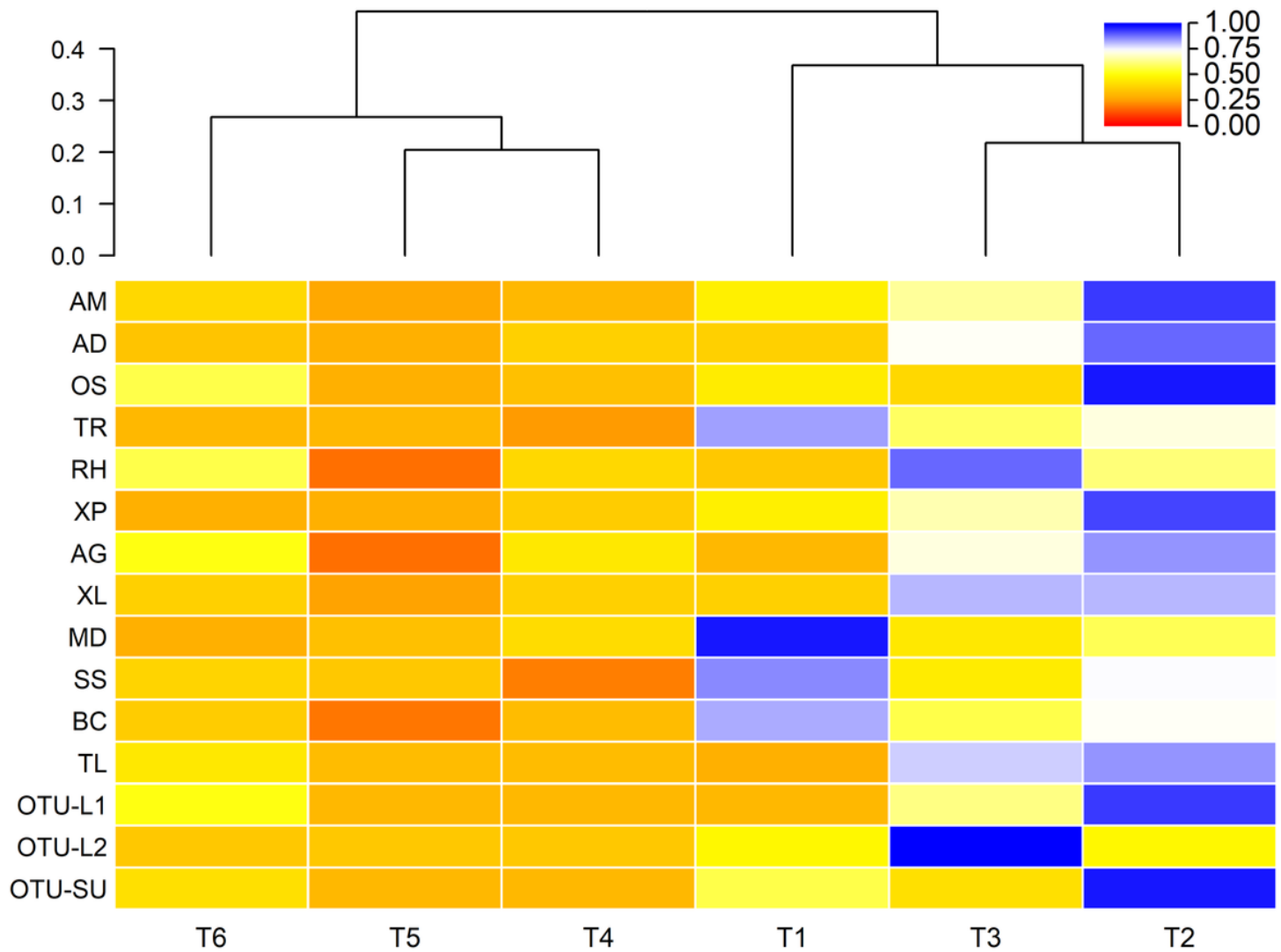


Figure 7

Similarity and dissimilarity between different timeframe across the day in terms of floral visitor diversity and abundance. The top dendrogram represent the hierarchical clustering of the timeframes along with the Bray-Curtis dissimilarity distance on the left y-axis. The bottom heatmap represent the relative proportion of each species across the five time of day. The heatmap scale in the top right corner represent heatmap colours, with the lowest proportion represented by red and highest proportion represented by blue colour

The species abbreviation are as follows: AM- *Apis mellifera*, AD- *Apis dorsata*, OS- *Oecophylla smaragdina*, TR- *Tetraponera rufonigra*, RH- *Rhynchium* sp., XP- *Xylocopa pubescens*, AG- *Amegilla* sp., XL- *Xylocopa latipes*, MD- *Micraspis discolor*, SS- *Sepedon spegea*, BC- *Bactrocera cucurbitae*, TL- *Tirumala limniace*, OTU-L1- Lepidopteran species 1, OTU-L2- Lepidopteran species 2, OTU-SU- Sunbird (*Nectarina* sp.)

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