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Cover: Mugger Crocodile basking on the banks of Savitri River at Mahad in Maharashtra, India. © Utkarsha M. Chavan.

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COMMUNICATION

Diversity patterns and seasonality of hawkmoths (Lepidoptera: Sphingidae) from northern Western Ghats of Maharashtra, India

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Abstract: As most of the biodiversity studies report the abundance and enlist the species, there is severe data deficiency in understanding the diversity patterns. The present study was designed to carry out periodic diversity assessments to understand the trends in diversity patterns of hawk moths. The study was carried out in the northern Western Ghats in Nashik district. Seven sampling stations were identified and periodic visits to these places were carried out over the span of five years (2011–2015). A total of 463 moths were recorded belonging to 18 species, represented by 10 genera. A new record from Western Ghat, *Theretra sumatrensis* (Joicey & Kaye 1917) is reported for the first time along with its DNA barcode. Six diversity indices (four alpha diversity indices and two beta diversity indices) were employed to understand the diversity dynamics. Whittaker's plot was generated using the rank abundance suggesting high species evenness for all sampling stations. Maximum diversity was observed during Monsoon. Wani was the most diverse sampling station throughout the study period (Shannon's Index = 2.7132±0.060; Simpson's Index = 0.9273±0.006; Brillouin's Index = 2.252±0.089; Fisher's alpha = 10.9472±1.685). Beta diversity was assessed with the help of Dice's coefficient and Jaccard's similarity index. Hence, we recommend rigorous periodic diversity assessments to generate adequate information about diversity that expedites conservational strategies' pace.

Keywords: DNA barcode, moth diversity, new report, range extension, species abundance, Sphingidae.

Abbreviations: CH—Chandwad; IG—Igatpuri; KL—Kalwan; NC—Nashik City; PT—Peint; TM—Triambakeshwar; WN—Wani.

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Author contributions: ASK and SM were involved in project design. Data collection and analysis were performed by ASK, GDK and SM. ASK and SSG were involved in manuscript preparation.

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INTRODUCTION

Insecta, being the most diverse class of Kingdom Animalia, rules the planet with their existence in all habitats. The adaptive features of this group of organisms allowed their natural selection in due course of evolution. Insects have existed since the Silurian period, approximately 420 mya (Misof et al. 2014). Currently, insects account for almost half of the known species on the earth (Chapman 2009). The tropical climate of India provides a conducive environment for a variety of insects. According to Murugan, 80% of insects from India are endemic (Murugan 2006).

Lepidoptera is one of the four super radiations of the class Insecta and includes butterflies and moths. Moths account for 85% of the lepidopteran population, and the remaining 15 % are butterflies and skippers. Moths serve as food for a variety of animals such as birds, bats, and praying mantis (Macgregor et al. 2015). The moth caterpillars are plant feeding, while the adult forms of the moths may be nectar feeding or fruit piercing (Reddy et al. 2005). Thus, forming a major pest clade (Cho et al. 2008). Moreover, their association with the plants makes them an integral part of the ecosystem. As a result, their numbers and availability are a good indicator of ecosystem's health (Thomas 2005).

Diversity studies of sphingid moths from India have been done and reported by many taxonomists (Bell & Scott 1937; Roonwal & Thapa 1963; Subalakmi 2008; Smetacek & Kitching 2012; Chandra et al. 2013, 2014; Kitching et al. 2014; Pathania et al. 2014; Sondhi et al. 2017; Melichar et al. 2018; Iyer & Kitching 2019; Singh et al. 2021). E.C. Cotes and C. Swinhoe conducted preliminary pioneering studies on Sphingid moths (Cotes & Swinhoe 1889). Further substantial work was done by Hampson (Hampson 1892). He reported 121 species of sphingid moths across India. Later, Bell & Scott (1937) documented sphingid from the Indian sub-continent. Almost three decades later, Roonwal & Thapa (1963) enlisted sphingids from peninsular India. Sambath (2011) described documented sphingid fauna from Dalma Wildlife Sanctuary, Jharkhand. Shubhalaxmi et al. (2011) described 45 hawk moths from northern Western Ghats near Mumbai. Sphingid moths from peninsular India were listed by Patil et al. (2013). Chandra et al. (2013) reported sphingid diversity from Veerangana Durgavati Wildlife Sanctuary, Damoh, Madhya Pradesh. The sphingid fauna from Ladakh, Jammu & Kashmir was reported by Smetacek & Kitching (Kitching et al. 2014). Sondhi et al. (2017) described a new species of *Theretra* Hubner from southern Western Ghats. Even though there are many studies of moth diversity, there has been a meagre number of inventories undertaken to focus primarily on the family Sphingidae. Further, there has been one report of sphingid diversity from the northern Western Ghats (Gurule & Nikam 2013). However, this study did not focus entirely on the diversity and dynamics of sphingid moths.

The novelty of the present study lay in the exclusivity and thoroughness attained to understand and analyze the hawkmoth moth diversity. Hawkmoths account for a very low number (~1,400 global species) than other giant moth families such as Erebidae and Geometridae (van Nieukerken et al. 2011); thus, get insufficient attention to the inventories. However, sphingids are known to be major pests but also good pollinators (Madden 1944; Eisikowitch & Galil 1971; Nilsson et al. 1985; Danaher et al. 2019). Hence, their presence creates a unique balance in the ecological niche. Also, these are some of the best flyers amongst the moth community.

MATERIAL & METHODS

Study Area

The present study aimed to analyze the diversity of sphingid moths from the northern Western Ghats (Nashik district), Maharashtra, India. Nashik district is located between 18.33–20.53 °N & 73.16–75.16 °E (Image 1). It covers approximately 15,582 km^2 and lies on the western edge of the Deccan plateau. The Sahyadri Mountains lie in the western part of the district, while Wani and Chandwad hill ranges cover the central part of the district. There are no ranges in the eastern part of the district. Forest coverage is approximately 3,400 km^2. The forests are of mixed type, with Teak and Sissoo being the significant trees. The forests are tropical moist deciduous, tropical dry deciduous and tropical hill forest types. As per Koppen's climate classification, the study area is a tropical wet and tropical dry climate with peak rains in July (McKnight 2017). The Western Ghats divides the district into two parts: The western part lies in the rainfed region, while the eastern part lies in the rain shadow region.

Collection & Identification

From 2011 to 2015, extensive observation and collection were carried out. The study area was thoroughly studied to finalize the sampling stations (Table 1). After the initial survey, seven sampling stations were finalized. Table 2 describes the details of the sampling stations and the collection events. Light traps

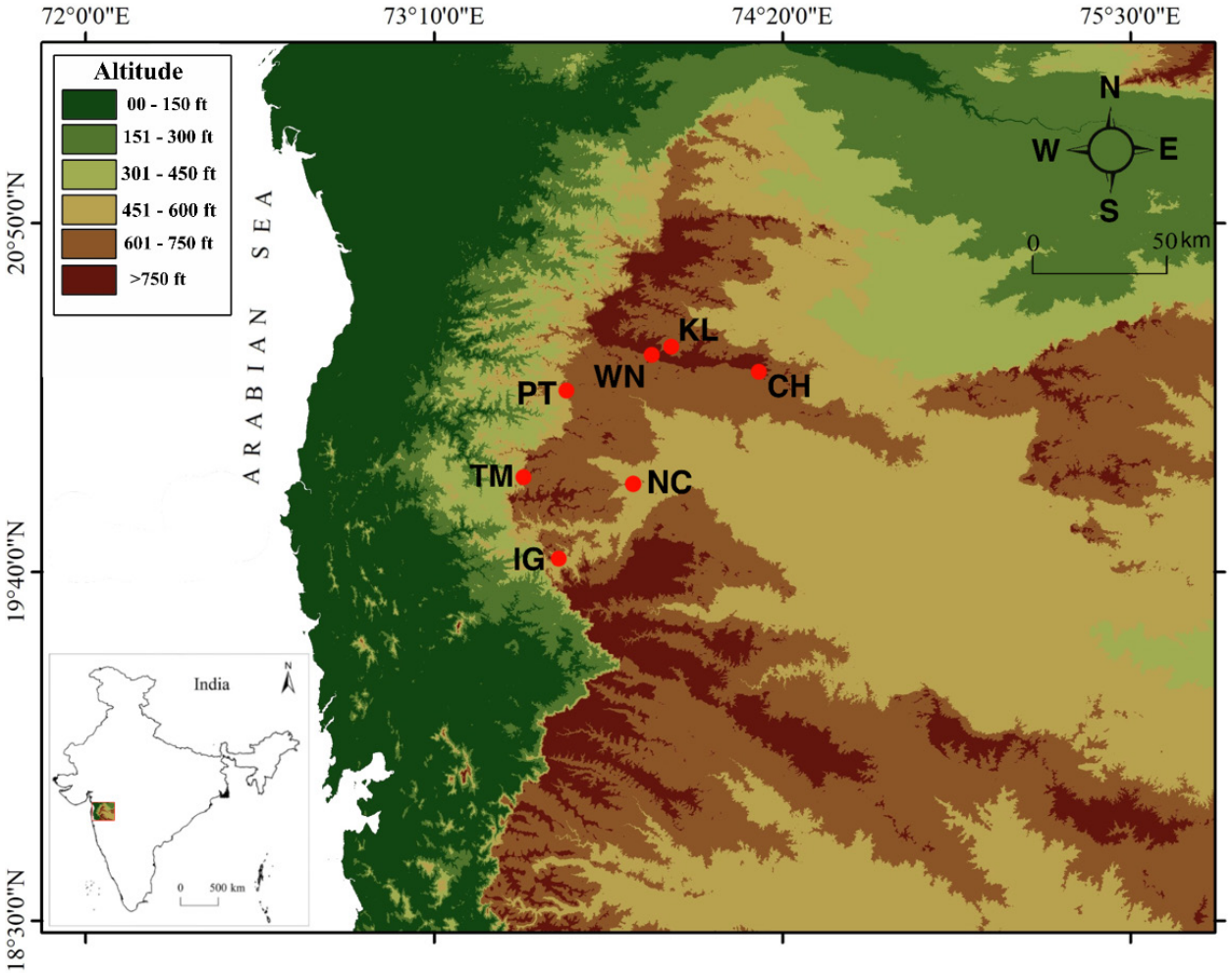

Image 1. Geographical location of the sampling sites.

Table 1. Sampling stations with their co-ordinates.

Name of sampling station	Abbreviation	Latitude	Longitude
Chandwad	CH	20°20'26.42"N	74°15'10.18"E
Igatpuri	IG	19°42'37.21"N	73°34'42.56"E
Kalwan	KL	20°25'12.49"N	73°57'8.99"E
Nashik City	NC	19°57'31.44"N	73°49'47.01"E
Peint	PT	20°15'33.42"N	73°36'40.43"E
Triambakeshwar	TM	19°59'6.19"N	73°28'1.21"E
Wani	WN	20°23'26.49"N	73°54'25.79"E

or a simple spreadsheet operated by fluorescent light (Compton 35 W; Philips tornado 27 W 130–320 V) were used. Collection set up was run from around 1900 h to 0500 h. Inverter batteries were used for operating the lights.

According to Brehm & Axmacher (2006), adaptability and flexibility in collection methods result in better collection and observations. Therefore, the collection assembly type varied initially, and the best-suited method was followed based on the collection conditions. However, hawkmoths vibrate their wings, causing loss of wing scales in the light trap. Moreover, all the observed specimens were not collected, and documentation of moths was easier by spreadsheet setup. Hence, the spreadsheet method was preferred over light traps. The sampling sites with large capture rates are challenging to handle, which is a common experience shared by many lepidopterists. In such situations, collecting, relaxing, and spreading of specimens is arduous (Abang & Ak Karim 2002; Gurule & Nikam 2013). Therefore, moths were observed, and the species abundance was calculated during the collection visits. Unique moth specimens which could not be identified were collected and brought back to the laboratory to investigate further. A small sample size of moths was collected from

each sampling station. Collected moth specimens were spread on spreading boards and were oven-dried for 3–5 days depending upon the size of the moth. The digital documentation was carried out using Nikon 3200 DSLR. Photographs were edited with the help of the software GIMP.

All the specimens were identified with the help of reference manuscripts (Barlow 1982; Holloway 1987; Haruta 1992, 1994, 1995; Inoue et al. 1997; Kendrick 2002; Srivastava 2002; Gurule & Nikam 2013; Sondhi et al. 2017). Unique samples were processed for molecular identification.

Along with the observation and collection of moths, other metadata was also collected, such as time of collection, altitude, the topography of the collection site, overall vegetation, season, and overall rainfall. These metadata are known to affect the moth availability and helps in understanding the ecological dynamics and intricate patterns of moth abundance (Hardwick 1972; McGeachie 1989; Yela & Holyoak 1997; Mittelbach et al. 2007; Chen et al. 2009).

Molecular Identification

Total genomic DNA was isolated using the modified phenol chloroform extraction method (Sperling et al. 1994). The isolated DNA was amplified using readily available markers LCO 1490–HCO 2198. A total reaction mixture of 25 µl was prepared, comprising of 12.5 µl trehalose, 2.5 µl 10X reaction buffer, 1 µl of $MgCl_2$, 2 µl of dNTP, 1 µl each of forward and reverse primer, 1.88 µl of water and 3 µl of DNA template. The thermal cycle included initial heating for DNA denaturation at 94°C for 2 minutes. The next five cycles were at 94 °C for 30 seconds followed by 45 °C for 1 minute and 30 seconds and final extension at 72 °C for 1 minute. The next 35 cycles were for 94 °C for 1 minute 30 seconds, 51 °C for 1 minute and 30 seconds, and extension at 72 °C for 1 minute. PCR product was cycle sequenced and sequencing was carried out in ABI 3130 sequencer. The sequencer files were aligned and edited using Bioedit and were converted to FASTA format. The FASTA files were uploaded on BOLD (Barcode of life Data systems, https://boldsystems.org) (Ratnasingham & Hebert 2007).

Figure 1. Recorded geographical expanse of *Theretra sumatrensis*. (© https://mol.org/species/Theretra_sumatrensis)

Diversity analysis

Four alpha diversity indices were employed: Shannon's, Simpson's, Brillouin's, and Fisher's alpha. Beta diversity was assessed with the help of two indices: Dice's Coefficient and Jaccard's similarity index. These diversity indices were calculated using cumulative abundance data, and all the calculations were performed using the software PAST ver. 4.03.

RESULTS

A total of 463 hawkmoths were recorded over five years (2011–2015). These moths were identified into 18 species represented by three subfamilies and ten genera (Image 2). The most diverse subfamily was Macroglossinae, represented by 12 species, and *Theretra* Hübner, 1819, was the most diverse genus comprising of seven species. Table 3 depicts the systematic position, distribution, and status of all the moths recorded.

T. sumatrensis (Joicey & Kaye 1917), the Southern Spotted Hunter Hawkmoth, was a unique record from this region and has not been previously reported. As described by Joicey & Kaye, 1917, *Theretra sumatrensis* has dull greyish-brown forewing. Black marks are present at the base. The post medial line is faint and marked on veins as dashes. Diffused darker clouds are present across the middle of the forewing. The hindwing is black with paler margins. There is the presence of a distally pointed yellowish patch at the anal angle. Sondhi et al. 2017 have compared the habitus of other species of the genus *Theretra* and have confirmed the similarity between *T. boisduvalii* and *T. sumatrensis*. However, the two can be differentiated by the pattern of colouration where *T. boisduvalii* is more greenish in colour with uniform suffusion while the latter is paler and brownish in colour.

The species identification was confirmed by DNA barcoding, and there is no published article describing its presence in the entire Western Ghats. This species has been reported only from the Himalayan region (Sondhi et al. 2017). Ballesteros-Mejia et al. (2017) have created global distribution maps for sphingid moths. Figure 1 shows the distribution of *T. sumatrensis* according to Ballesteros-Mejia et al. 2017 (https://mol.org/species/Theretra_sumatrensis). Thus, we further extend the range of *T. sumatrensis* to the Western Ghats, the specimens recorded at Wani and Kalwan from Nashik District, Maharashtra, India. Table 4 describes the details of sequences mined from BOLD and GenBank to generate the neighbour-joining tree based on the K_2P model using mitochondrial COI gene. The NJ tree based on K_2P model indicated approximate species relationship within genus *Theretra* (Figure 2).

Table 2. Overview of inventory visits and moth recorded.

Sampling station	Symbol	Inventory visits made between 2011–2015			Number of moths secorded
		Summer	Monsoon	Winter	
Chandwad	CH	5	14	5	59
Igatpuri	IG	5	11	5	60
Kalwan	KL	5	11	5	43
Nashik City	NC	5	6	5	21
Peint	PT	5	6	5	15
Triambakeshwar	TM	5	9	5	24
Wani	WN	5	14	5	241
Grand Total					463

Although the geographical expanse of the present study was small, it exhibited great diversity (Image 1). The seven sampling stations showed varied diversity patterns. The most diverse sampling community was WN, where all the 18 species of moths were recorded, followed by IG and CH, having seven species. Figure 3 describes the relative species abundance at all sampling stations over five years (2011–2015). Figure 4 represents Whittaker's plot for abundance. It is clear from Figure 4A that the species richness and evenness for sampling stations vary drastically. KL, TM, and PT show precisely the same richness, while TM and PT have the same evenness. KL has the least evenness amongst all the sampling stations. On the other hand, WN has maximum richness and evenness in the entire study. Further, Whittaker's plot is originally used to describe species richness and evenness. In contrast, we have also utilized it to compare our observations annually (Figure 4B). This figure helps clarify that collection over five years showed nearly the same trend confirming that there was no bias or error and the collection events were carried out randomly. We want to support this further because the rigorous collection and increased number of samples helped eliminate the errors. Figure 5 illustrates variations in the four alpha diversity indices (Shannon's, Simpson's, Brillouin's, and Fisher's alpha) at all the sampling stations over five years (2011–2015). Figures 6 & 7 are heatmaps elucidating the beta diversity amongst the seven sampling stations.

Table 3. Taxonomic position, distribution, and status of hawkmoths from northern Western Ghats.

Family	Sub-family	Species	Distribution	Status
Sphingidae	Macroglossinae	*Daphnis nerii* (Linnaeus, 1758)	CH, WN	U
		Hippotion celerio (Linnaeus, 1758)	CH, IG, KL, WN	C
		Hippotion rosetta (Swinhoe, 1892)	CH, IG, KL, NC, PT, TM, WN	C
		Hyles livornica (Esper, 1780)	IG, KL, WN	C
		Nephele hespera (Fabricius, 1775)	IG, KL, NC, WN	C
		Theretra alecto (Linnaeus, 1758)	CH, IG, PT, TM, WN	C
		Theretra castanea (Moore, 1872)	WN	R
		Theretra clotho (Drury, 1773)	WN	R
		Theretra gnoma (Fabricius, 1775)	WN	U
		Theretra nessus (Drury, 1773)	WN	U
		Theretra oldenlandiae (Fabricius, 1775)	WN	R
		Theretra sumatrensis (Joicey & Kaye, 1917)	KL, WN	U
	Smerinthinae	*Marumba dyras* (Walker, 1856)	WN	U
		Polyptychus dentatus (Cramer, 1777)	WN	U
	Sphinginae	*Acherontia lachesis* (Fabricius, 1775)	WN	U
		Acherontia styx (Westwood, 1847)	IG, WN	U
		Agrius convolvuli (Linnaeus, 1758)	CH, IG, KL, NC, PT, TM, WN	C
		Psiligramma vates (Butler, 1875)	CH, WN	C

DISCUSSION

India shares 8.1% of global diversity on only 2.4% of the global land area resulting in diversity richness (Balasubramanian 2017). There are 12 mega biodiverse countries in the entire world, and India is one of them. The present study area lies in the Western Ghats, which have been declared World Heritage Site as it harbours humungous diversity. Continuous inventories have proved to generate valuable information and increased taxonomic knowledge (Janzen et al. 2009). Hence, we strongly support and recommend continuous periodical assessments to understand the diversity and its dynamics. The incorporation of molecular tools has also encouraged young taxonomists to indulge more. Moreover, diversity studies are necessary to understand the species distribution and unfold the ecological dynamics. It is frequently observed that diversity studies are reported with the species diversities, which may or may not be combined with the diversity analysis. However, there exist complex dynamics between the species and its environmental and ecological surroundings. Understanding this can help to predict diversity for unassessed areas and develop better conservation strategies. Thus, understanding diversity becomes indispensable.

When diversity indices are applied, interpreting species distribution becomes easier. The present study calculated alpha and beta diversity indices for each sampling station to understand the species distribution. Four indices were used to assess alpha diversity (Shannon's, Simpson's, Brillouin's, and Fisher's alpha) and two to study beta diversity (Dice's Coefficient and Jaccard's similarity index). According to Barrantes & Sandoval (2009), using multiple indices helps eliminate drawbacks of the individual index. Further, the indices chosen in the present study focus on varied aspects of diversity. Shannon's Index describes species diversity. Maximum species diversity was observed for WN (2.7132±0.060) and minimum diversity for TM (0.9683±0.185) Figure 5A. When Shannon's index is calculated, a weighted geometric mean of the proportional abundances is employed. Thus, it reflects the logarithm of actual diversity observed and is used frequently.

Simpson's index uses the weighted arithmetic mean or proportional abundances and describes species richness and evenness. Thus, a high Simpson's index

Table 4. Sequences used to generate Neighbour Joining Tree based on K2P model for species from genus *Theretra*.

Species	Sequence ID	BIN	Genbank Accession	Source
Theretra sumatrensis	MOTH011-12	BOLD:ADG0374	MG783973	Present Study
	MOTH044-14	BOLD:ADG0374	MG783956	Present Study
	MOTH061-14	BOLD:ADG0374	MG783950	Present Study
	MOTH063-14	BOLD:ADG0374	MG783977	Present Study
	SPTVA370-07	BOLD:ABX5127	JN678600	Wilson et al. 2011
Theretra clotho	SPHAP046-06	BOLD:AAB2442	-	https://boldsystems.org
Theretra gnoma	SPTMA241-07	BOLD:AAE7656	JN678605	Wilson et al. 2011
Theretra alecto	SPRBA164-08	BOLD:AAC6760	-	https://boldsystems.org
	SOWC887-06	BOLD:AAC6760	-	https://boldsystems.org
	SOWC888-06	BOLD:AAC6760	-	https://boldsystems.org
Theretra castanea	SPHAP054-06	BOLD:AAW6578	JN678603	Wilson et al. 2011
Theretra oldenlandiae	SPHAP068-06	BOLD:AAA4630	KJ168195	Rougerie et al. 2014
Theretra nessus	SPTMB038-09	BOLD:AAB3024	GU704539	https://www.ncbi.nlm.nih.gov/nuccore/GU704539
	SPHAP055-06	BOLD:AAB3024	KJ168203	Rougerie et al. 2014
Agrius convolvuli (out group)	MOTH177-14	BOLD:AAA2393	MG783948	Shere 2018

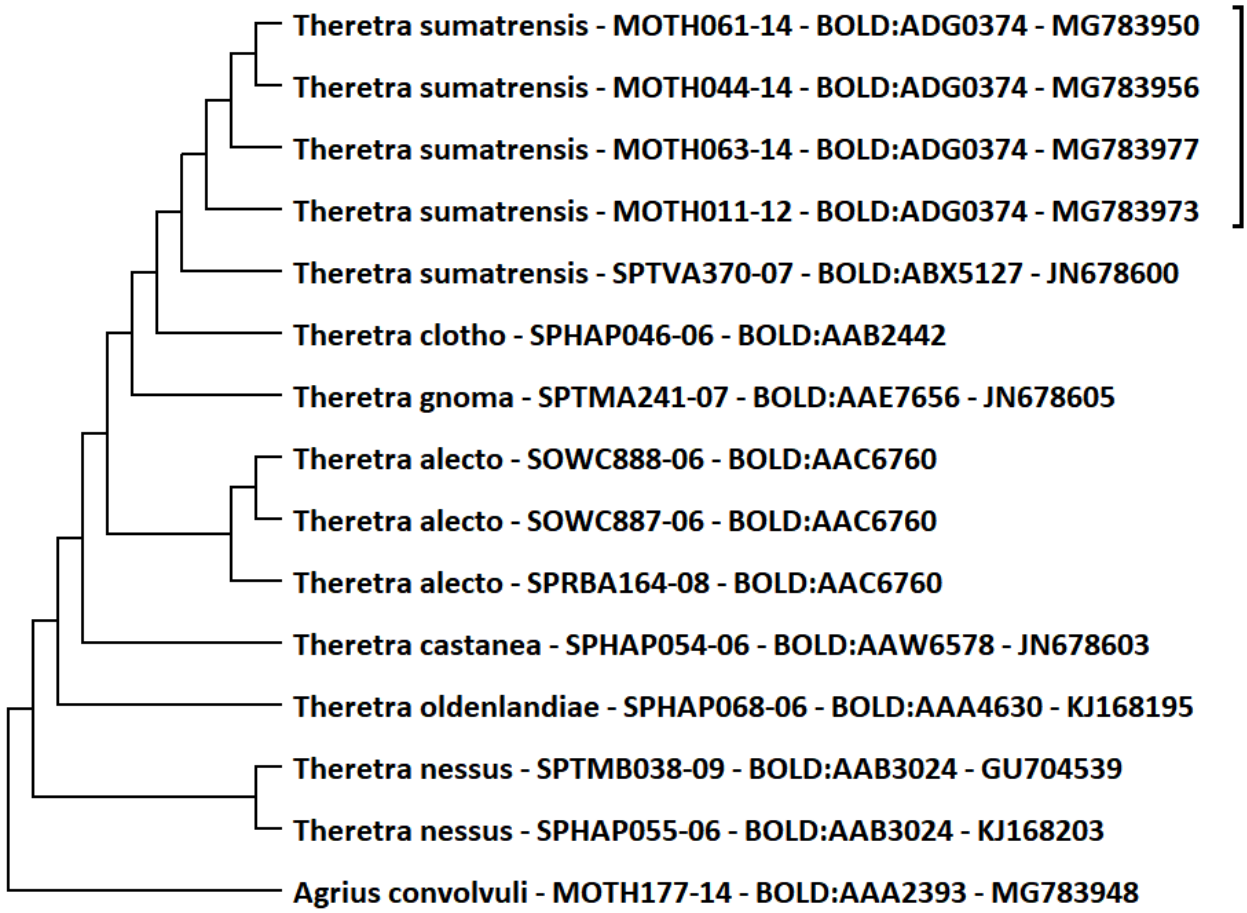

Figure 2. NJ tree based on K2P model of genus *Theretra* using mitochondrial COI gene sequence.

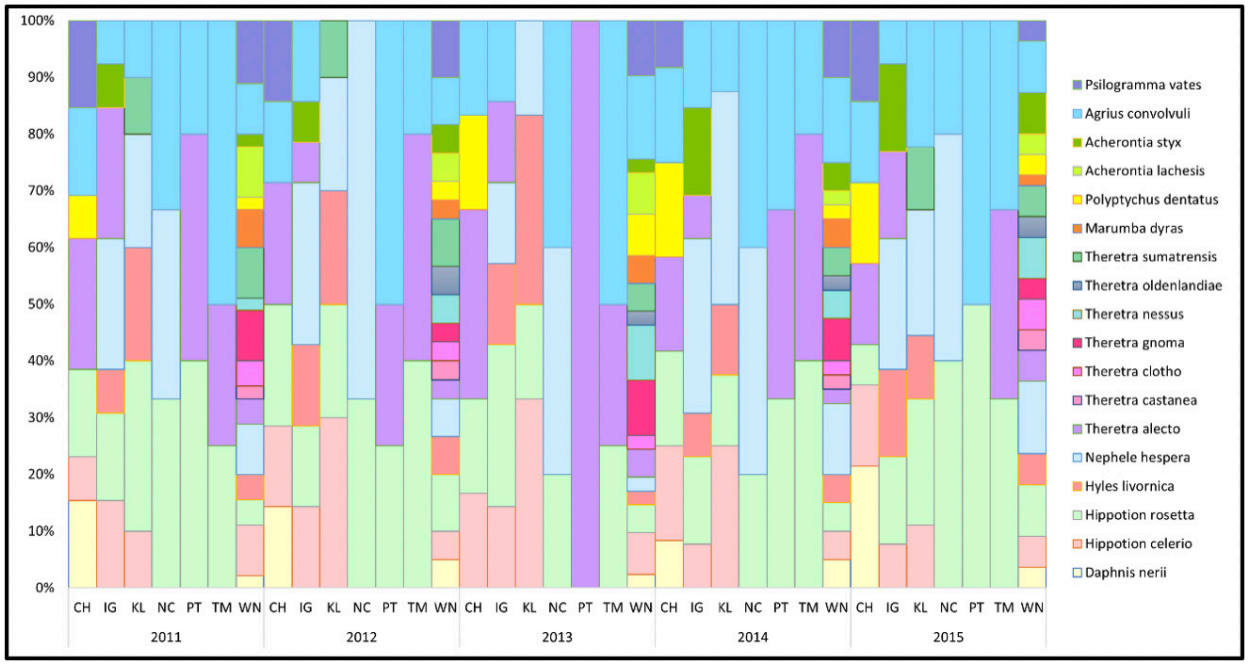

Figure 3. Relative species abundance at all sampling station over five years (2011–2015).

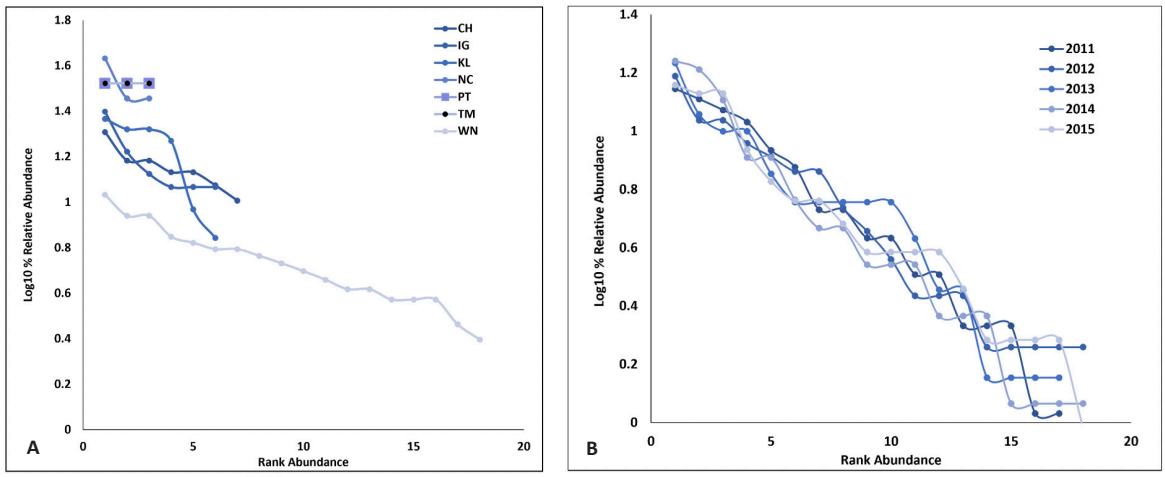

Figure 4. Whittaker's plot: A—Sampling station-wise | B—Sampling year-wise.

suggests higher species richness and evenness. The maximum value for Simpson's index was calculated for WN (0.9273±0.006) and minimum for TM (0.5978±0.086) (Figure 5B). this suggests that the species richness at WN is definitely high and there is an evenness to the species distribution too.

The following index calculated was Brillouin's index. According to Magurran, this diversity index serves better when there is no surety for the randomness of the sample (Magurran 1988). Thus, to eliminate any biases raised unknowingly, we employed this index. The maximum value for this index was calculated again for WN (2.252±0.089) and minimum for again Triambakeshwar TM (0.6056±0.136) (Figure 5C).

Lastly, Fisher's alpha was the fourth alpha diversity index employed. Fisher's alpha has a good discriminating capability in cases where sample sizes vary a lot. As the sample size for all the sampling stations varied (Table 2), this index was used. Maximum value for this index was calculated for WN (10.9472±1.685) and minimum for TM (3.5152±1.108) (Figure 5D). Thus, all the four alpha diversity indices confirm that maximum alpha diversity

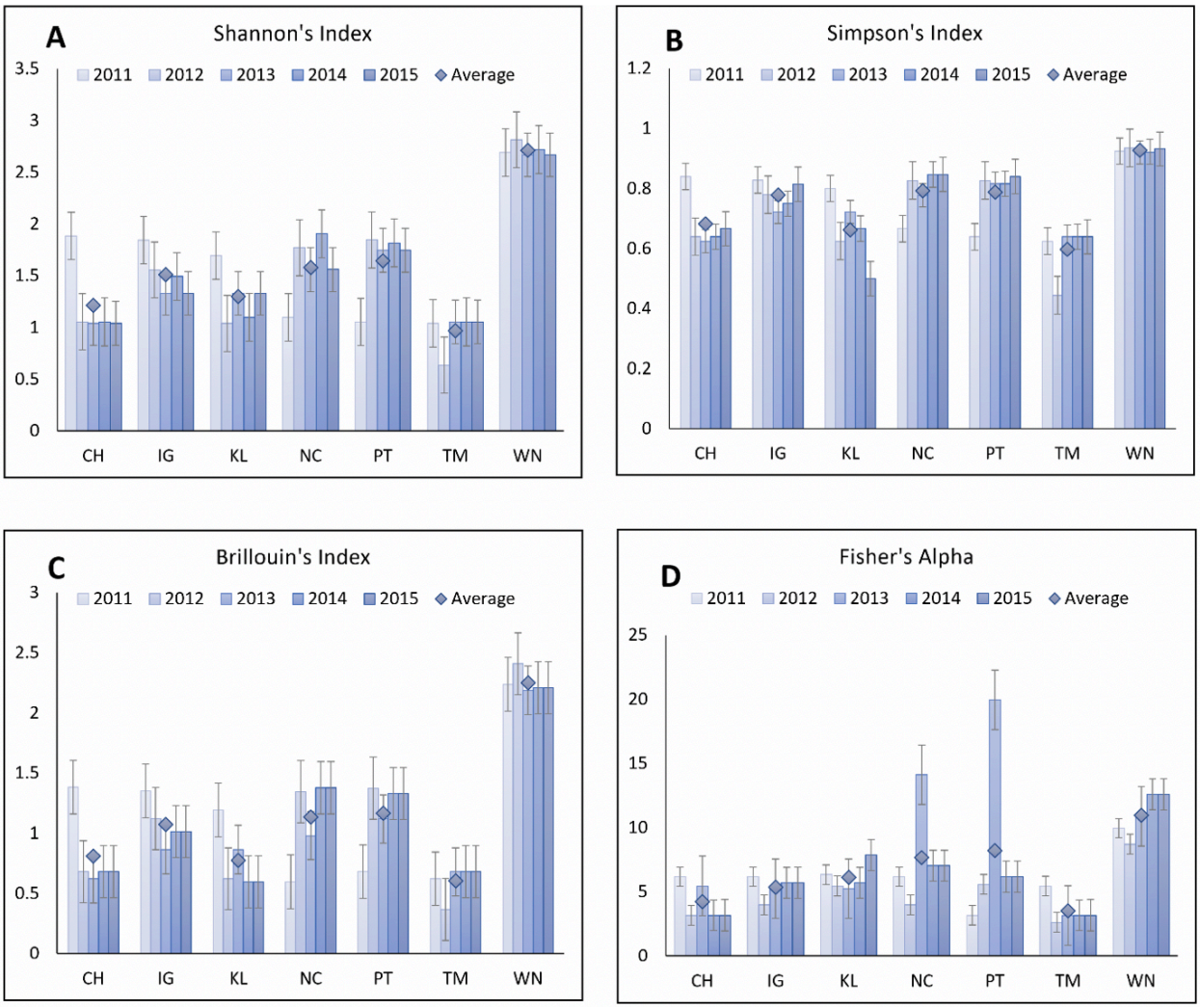

Figure 5. Alpha diversity indices calculated for seven sampling stations over five years (2011–2015).

was observed at WN and minimum at TM.

Dice's coefficient and Jaccard's Similarity index were used to assess beta diversity indices. Beta diversity is used to compare the similarity between two sampling points. The value for beta diversity always lies between 0 and 1, and the level of similarity increases with an increase in value. The sampling points where the value is 0 indicate no common species between those sampling points. On the other hand, when the value is 1, all the species were shared by both sampling points. The maximum value was calculated for TM and PT (1) as both the sampling stations shared all the species. Further, minimum values for Dice's coefficient and Jaccard's similarity index were between WN & TM, PT & NC (Dice's coefficient = 0.2857; Jaccard's Similarity Index = 1.666) (Figure 6, 7). It is also clear from the figure that there was no difference in the pattern; only the values of the indices varied slightly.

In the present study, the seasonality of moths was also observed. It is quite conspicuous from Figure 8 that moth abundance is maximum during monsoon. During summers, almost the majority of species are not found except *Nephele hespera* (Fabricius, 1775), *Agrius convolvuli* (Linnaeus, 1758), and *Psilogramma vates* (Butler, 1875). However, all these individuals were observed at almost the summer's end and the monsoon's beginning. In winters, moth abundance was observed but not as much as during the monsoon. The probable reason for high moth abundance during monsoon could be the conducive environment created at the time. The temperatures decrease with an increase in relative humidity, which favours moths.

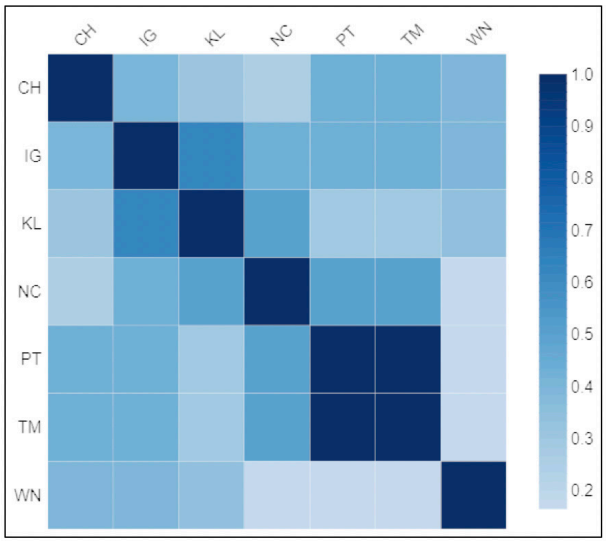

Figure 6. Heatmap for Jaccard's similarity index.

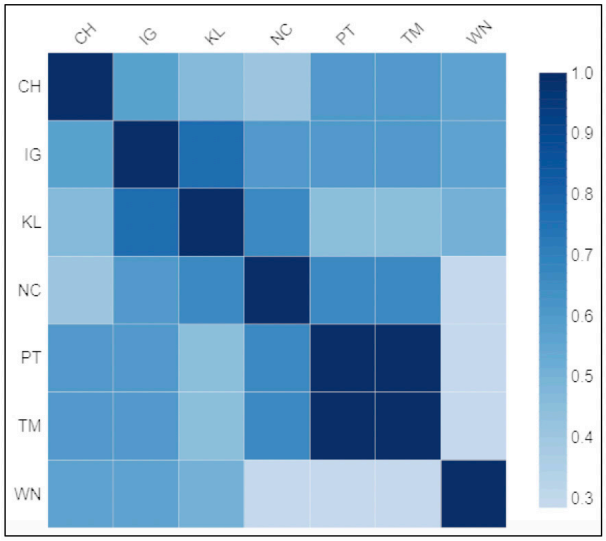

Figure 7. Heatmap for Dice's coefficient.

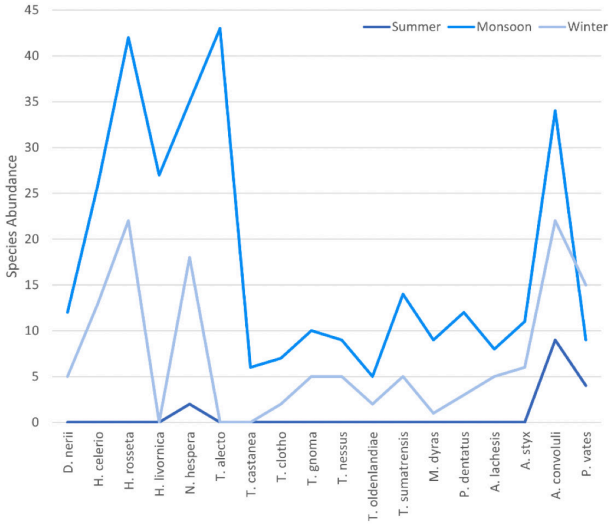

Figure 8. Seasonality in moth distribution.

CONCLUSION

Alpha diversity indices strongly support that high hawkmoth diversity is observed in the northern Western Ghats. However, there is variation in the moth availability at different sampling stations, reinforcing the knowledge that these organisms are sensitive to changes and can be used as flagships. It was conspicuous that the moth availability showed seasonal variations, and maximum diversity was observed during monsoon.

We strongly recommend monitoring the moth diversity throughout the year for a prolonged duration. These data inputs would elaborate the knowledge of natural history information of the moths and reinforce the need for further research on ecological and taxonomic consequences of differences in the seasonal activity. Complete knowledge of the distribution patterns of the individual species helps expound the reasons for species' availability in a peculiar area. When such results are combined with other ecological parameters, a comprehensive database is created. Such complex knowledge further helps in devising conservational strategies. Comprehensive knowledge and stepwise incorporations of the natural history information would lead to a deeper understanding of the complex dynamics of any ecological niche. Moreover, we cannot conserve what we do not know. Thus, we suggest extensive taxonomic studies involving periodic assessments and statistical analysis to monitor the diversity patterns, which would help devise customized conservational strategies for different localities.

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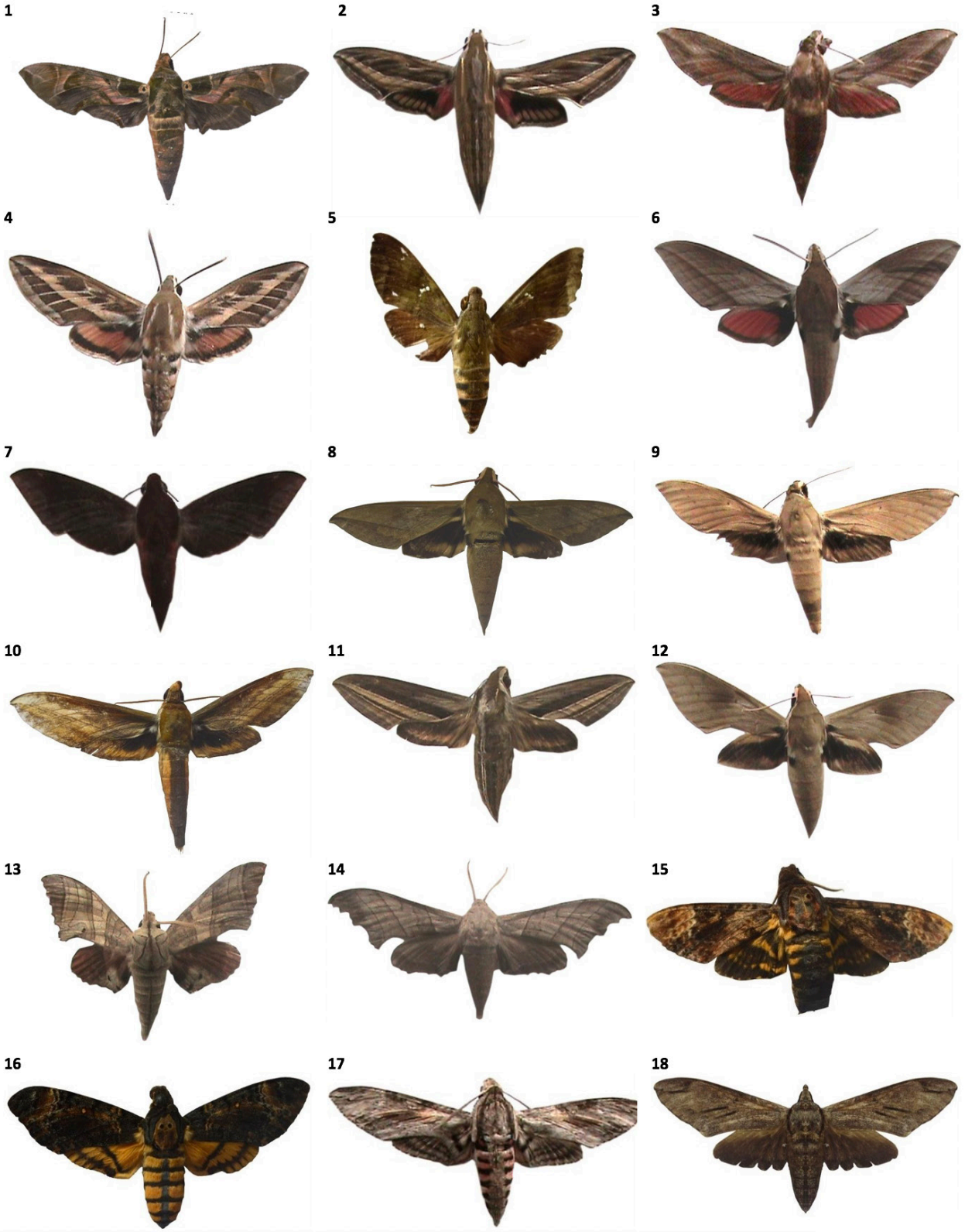

Image 2. 1—*Daphnis* nerii | 2—*Hippotion celerio* | 3—*Hippotion rosseta* | 4—*Hyles livornica* | 5—*Nephele hespera* | 6—*Theretra alecto* | 7—*Theretra castanea* | 8—*Theretra clotho* | 9—*Therertra gnoma* | 10—*Theretra nessus* | 11—*Theretra oldenlandiae* | 12—*Theretra sumatrensis* | 13—*Marumba dyras* | 14—*Polyptychus dentatus* | 15—*Acherontia Lachesis* | 16—*Acherontia styx* | 17—*Agrius convolvuli* | 18—*Psilogramma vates.*

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