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# STUDY ON THE COMPOSITION AND DISTRIBUTION OF HAWKMOTHS FAMILY (LEPIDOPTERA: SPHINGIDAE) IN VIETNAM

## SUMMARY OF DISSERTATION ON BIOLOGY

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# GENERAL INFORMATION

## The urgency of disertation

The family Sphingidae (Insecta: Lepidoptera: Bombycoidae), commonly known as hawkmoths or sphinx moths, consists of more than 1,750 species worldwide except Antarctica (Kitching, 2024). The hawkmoths are the second largest family in the order Lepidoptera, characterized by the streamlined adults and the horned larvae. Due in part to their large size and great beauty, hawkmoths are also one of the favorite groups for butterfly collectors. Hawkmoths are also used as a focus taxon in many ecological investigations, functional morphology, plant-animal physiology, biogeography because of the good knowledge on their systematics, ecology, morphology, and natural history. There are also records of hawkmoths larvae serving as human food in some countries such as China, Thailand, and Central Africa. Additionally, some hawkmoths have been used as potential biological control agent of its larval host plants, such as *Hyles euphorbiae euphorbiae*, which is a natural enemy of the weeds *Euphorbia cyparissias* and *E. esul*a in the United States (Kitching & Cadiou 2000). Both diural and nocturnal hawkmoths with long proboscides which have long been recognized as major pollinators (Kitching & Cadiou 2000) and some species have a potential to be agricultural pests, examples of which include *Acherontia lachesis*, *Agrius convolvuli* và *Theretra oldenlandiae* (Plant Protection Research Institute, 1976).

Vietnam is a tropical country with the 14th highest level of biodiversity in the world. However, the knowledge of the hawkmoth fauna in Vietnam still remains poor, while general and detailed information on hawkmoths biodiversity is rather good in many world regions especially in temperate area. Based on the above situation, I carry out this project and systematic research hawkmoths fauna focus on National Parks (NP) and Nature Reserves (NR) in Vietnam.

**Objectives of the study**

- Taxonomical achievements: create a comprehensive hawkmoths checklist that adds previous lists and and keys identification for hawkmoths species in Vietnam, using DNA barcoding to identify two homomorphic species of the genus *Cechetra.*
- Ecological data achievements: construct distribution maps of some hawkmoths genaral in Vietnam. Assessment of the distribution and diversity of hawkmoths in natural geographical regions and different habitats in Vietnam.

**Research content**

**Content 1**: Study on classification of hawkmoths in Vietnam

**Content 2**: Study on the distribution and diversity characteristics of hawkmoths according to natural geographical regions and habitats in Vietnam

**Scientific and pratical basic of the thesis**

*Scientific significance*

The study provides important and up-to-date scientific data on the species composition, distribution, and diversity of the hawkmoths fauna in Vietnam.

*Practical significance*

The identification keys will be useful as preliminary data for future studies in hawkmoths of Vietnam. On the basis of the results of the species composition, distribution, and population diversity contribute to the management and conservation of the diversity of hawkmoths in Vietnam. The collection of hawkmoths contributes to the “National Natural Specimen

Collection" of the Vietnam National Museum of Nature, serving to display for tourism, education, and further research.

## CHAPTER 1. OVERVIEW

### 1.1. Reports on hawkmoths in the world

### *1.1.1. Study on taxonomy of hawkmoths in the world*

The hawkmoths were first classified by Linnaeus in 1758 when he described 14 species with sphinx-like morphology and grouped them into a genus named "Sphinx" Later, Drury (1773) and Cramer (1775, 1777, 1779) described and illustrated several new species of the genus *Sphinx*.

Samouelle (1819) adopted the name Sphingidae for the family in the 19th century, and this family is still in use today. The early hawkmoth classification systems did not divide genera into tribes and subfamilies, but instead described species and genera. Boisduval (1875), Butler (1876), and Hampson (1892) were among the first to develop systems for categorising species into more extensive groups. The first global checklist of the hawkmoths was published in the book "Catalogue of Lepidoptera Heterocera" in 1892 by Kirby.

At the beginning of the 20th century, Rothschild & Jordan (1903) developed the first complete classification system for the hawkmoths family, consisting of two major subgroups: Asemanophorae and Semanophorae. In the mid-20th century, Clark was the author of numerous research works on the taxonomy of the hawkmoths. Particularly during the years 1916 to 1938, he described and named over 195 species, subspecies, and a new genus called Barbourion. Jasen (1932) treated two subgroups as subfamilies, Asemanophorinae and Semanophorinae and thus the subfamilies becam tribes in Rothschild & Jordan' classification. Bell & Scott (1937) published "The fauna of British India, including Ceylon and Burma", which provided information on the classification and distribution of the hawkmoths at that time, along with a key for identifying the species. Later, Hodges (1971) renamed the two subfamilies Semanophorinae and Asemanophorinae to Macroglossinae and Sphinginae. The initial concept of the group corresponding to Macroglossinae today was proposed by Nakamura (1970, 1976, 1978). This century, the catalogs and checklists of the hawkmoths on a global scale were published by d'Abrera (1986) and Bridges (1993).

In addition, the hawkmoths faunas in various regions in the world was beginned to studied, particularly the Southeast Asian area (Yang, 1978; Chu & Wang, 1980; Yang & Wu, 1981; Zhu & Wang, 1997 in Chine; Cadiou & Kitching, 1990; Tennent, 1992; Inoue *et al*., 1996;  in Thailand; Kitching & Spitzer, 1995; Kitching & Brechlin, 1996 in Vietnam). At the end of the 20th century, Kitching and Cadiou (2000) published a comprehensive checklist of global hawkmoths in the book "Hawkmoth of the world: An Annotated and Illustrated Revisionary Checklist (Lepidoptera; Sphingidae)." The authors also proposed a classification system based on the results of phylogeny research.

In the 21st century, studies on the taxonomy of the hawkmoths have expanded, combined with the molecular research, contributing to clarifying unresolved issues in taxonomy and evolutionary biology. Regier *et al*. (2001) concluded on the phylogenetic relationship of the three subfamilies, with Smerinthinae grouped with Sphinginae, and Macroglossinae forming a separate branch. In 2003, a method using molecular biology to quickly classify species based on DNA sequences was newly known as DNA barcoding. In animals, the DNA barcode is taken from the gene that encodes the protein on the mitochondrion cytochrome c oxidase subunit 1 (CO1 or COX1). DNA barcoding research in hawkmoths focusses on difficult-to-classify morphological species. DNA barcoding has assisted scientists in discovering many new species hidden within previously published ones due to similar external morphology with only minor differences in wing patterns and genital structures, most notably the works of Melichar T., Kitching I.J., and Haxaire J in this field.

During this period, DNA barcoding has been a powerful tool in the classification research of the hakmoths family. Kawahara *et al*. (2009) conducted a study based on morphological characteristics and the analysis of 5 nuclear genes, which showed that Smerinthinae and Sphinginae form a separate branch from Macroglossinae. Based on the results of Kawahara *et al.* (2009), Kitching *et al*. (2018) provided the phylogenetic relationships of the subfamilies and an updated list of up to 1,750 species with 205 genera from 4 subfamilies within the hawkmoths of the worldwide, which have been recorded to date and are continuously updated on the Sphingidae Taxonomic Inventory website.

In recent years, scientists have focused on revisions of several genera based on comparisons of morphological and DNA barcoding analysis of existing species, such as the genera *Dahira* (Moore, 1888), *Smerinthulus* Huwe, 1895, *Eupanacra* Cadiou & Holloway, 1989; *Acosmeryx* Boisduval, 1875; *Ambulyx* Westwood, 1847; *Marumba* Moore, 1882, and *Xylophanes* Hübner, 1819, studied by Haxaire *et al*. (2021-2023). Jiang *et al*. (2024) revision on the genus *Rhagastis* Rothschild & Jordan, 1903 from China and restored the species status of 5 subspecies.

In summary, the history of research on the classification of the hawkmoths of the world has been and continues to develop strongly with a combination of traditional and modern methods to this day. Current studies focus on combining morphological analysis with molecular data to address the existing issues in the taxonomy of the hawkmoths family, as traditional classification methods face many limitations and must contend with the morphological diversity and variability. The classification system of Kitching *et al.* (2018) is the most complete and accurate system currently available.

### *1.1.2. Study on the distribution and diversity of hawkmoths in the world*

Studies on the distribution of the hawkmoths are limited to species composition in each country, for example, Smith (1993) studied the distribution of 13 hawkmoths species in western North America, and Zolotuhin (1997) provided a distribution map of the species *Proserpinus proserpina* (Pallas, 1772) in Eastern Europe and Central Asia. Beck *et al.* (2006) studied the correlation between species distribution range and species richness in Southeast Asia. Han *et al*. (2015) studied the spatial and temporal variability of hawkmoths in Korea. Recently, data on the distribution often combined in invetory works of hawkmoth in each country, such as the reports by Irungbam & Irungbam (2019), Irungbam & Fric (2021), Koren & Sasic (2023).

The diversity studies focus on assessing the species composition and seasonal abundance of the hawkmoths, including the research by Owen (1969, 1972), Stradling *et al.* (1983), Schulze & Fiedler (1997). In addition, studies on the disturbance habitat of the hawkmoths have also received attention, notably the works of Touroult & Gall (2001), Schulze & Fiedler (2003), Júnior & Schlindwein (2005), Beck *et al*. (2006), Ignatov *et al*. (2011). Recent ecological studies have paid much attention to the biodiversity and conservation of the hawkmoths in a national park or nature reserve. Mohagan *et al*. (2018) studied the diversity of hawkmoths across various vegetation types in nature reserve and ecotourism areas in the Philippines. Smith (2022) studied the diversity and distribution of hawkmoths in Paraguay and conducted a preliminary assessment of their conservation status using the main criteria for evaluation by the IUCN. Recently, Shere-Kharwar *et al*. (2024) conducted a study using DNA barcoding to elucidate the ecological dynamics related to the distribution patterns of species belonging to the genus *Theretra* Hübner, 1819 in India. In general, there are not many studies on the diversity hawkmoths; current research trends focus on factors affecting species diversity to provide basic data contributing to conservation efforts and responses to global climate change.

## 1.2. Reports on hawkmoths in Vietnam

### *1.2.1. Study on taxonomy of hawkmoths in Vietnam*

The first list of hawkmoths in Vietnam was published by Kitching & Spitzer (1995), which recorded 117 species. Later, studies on new species in Vietnam were published by many authors, especially Cadiou (1996), Kitching & Brechlin (1996), Brechlin (1997), Eitschberger (2009), Eitschberger & Melichar (2010), Zolotuhin & Ryabov (2011). So fas, 175 species and 61 genera belonging to 3 subfamilies has been recorded from Vietnam (Zolotuhin & Ryabov, 2012). In the past few years, numerous new species were recorded in Central Vietnam from the revision of genera (Brechlin, 2014-2019; Ivshin *et al.*, 2018; Haxaire *et al.*, 2021; Haxaire & Melichar, 2024).

In general, studies on the classification of hawkmoths are very limited. Using DNA barcoding on the hawkmoths in Vietnam is mostly published by foreign scientists; currently, 371 DNA barcode of 127 species has been recorded on BOLD (The Barcode of Life Data System). Research on the categorisation keys of hawkmoths in Vietnam has not been published until now.

### *1.2.2. Study on the distribution and diversity of hawkmoths in Vietnam*

Study on the distribution and diversity of hawkmoths in Vietnam have only been interested by a few authors and the number of articles is not much (Truong, 2004; Tran Thieu Du *et al.*, 2011; Hoang Vu Tru *et al.*, 2011; Pham Hong Thai *et al.*, 2013; Bui Minh Hong & Phan Hong Nhung, 2015; Pham Thi Nhi *et al.*, 2015; Tran Anh Vu (2016); Pham *et al.*, 2019, 2021).

In conclusion, studies on the distribution and diversity of hawkmoths have been conducted on a limited scale.

# CHAPTER 2. STUDYING OBJECT AND METHODS

## 2.1. Object for studying

Mature stage hawkmoths belonging to order Lepidoptera in Vietnam

## 2.2. Methods for studying

### *2.2.1. Time for studying*

The research was conducted from 2019 to 2024. In addition, specimens collected in some of our national parks and nature reserves previously from 2009 to 2019 were used for the research.

***2.2.2. Sites for studying***: The research was conducted mainly in National Parks and Nature Reserves in Vietnam

### *2.2.3. Method to classification research*

Light traps, insect nets, inheritance methods, method of processing and preservation of hawkmoths samples in the laboratory and method of identification are used in this study.

Hawkmoths species are classified based on morphological characteristics of mature stage. For homologous species, male genitalia features and DNA bardode are used for species identification. Genitalia were dissected accordingly to the standardized techniques used in Lepidopterology. The terminology for naming different parts of genitalia will follow Clarke (1941) and Robinson (1976). In this study, two morphologically similar species of the genus *Cechetra* were identified using DNA barcoding with the support of the VIETBIO project (Innovative approaches to biodiversity discovery and characterization in Vietnam).

The terms of taxonomy are used in this study according to Bell & Scott, 1937 and Hundsdoerfer & Kitching, 2020. The terms of of taxonomy in Vietnamese is based on Vũ Văn Liên & Vũ Quang Côn (2021).

+ Identification of hawkmoth was done by using available taxonomic literature such as Rothschild & Jordan (1903), Bell & Scott (1937), Kitching & Spitzer (1995), Zolotuhin & Ryabov (2012) and website Sphingidae Taxonomic Inventory (Kitching, 2024).

+ The identification key is constructed in the form of a dichotomous while inheriting the classification key of Bell & Scott (1937). Higher classification and nomenclature follow Kitching *et al*. (2018).

### *2.2.4. Method to distribution and diversity research*

Research on distribution characteristics and diversity assessment according to natural geographical regions and habitats in Vietnam.

Shannon-Weiner (H') diversity indexes, species richness Margalef (d), dominance indexes (D) and Sorenxen (SI) similarity index used to analyse the dataset.

The data were processed using Excel 2016 software and Primer V6 software.

# CHAPTER 3. RESULTS

## 3.1. Taxonomy of hawkmoths in Vietnam

### *3.1.1. The species composition of hawkmoths in Vietnam*

Results recorded 181 species and subspecies of 60 genera, 4 subfamilies belonging to Sphingidae family in Vitenam, including Langiinae (1 species, 1 genus), Macroglossinae (102 species, 25 genera), Smerinthinae (63 species, 22 genera), and Sphinginae (15 species, 12 genera), among which 8 species and 5 subspecies are endemic. A total of 2,208 samples from 110 species were collected from this study, and 71 species were recognized from previous research.

***Table 3. 2.*** The species composition of hawkmoth in Vietnam

| **No.** | **Subfamily** | **Genus** | | **Species** | |
|---|---|---|---|---|---|
| | | **Number** | **Percentage** | **Number** | **Percentage** |
| 1 | Langiinae | 1 | 1.6 | 1 | 0.6 |
| 2 | Macroglossinae | 25 | 41.7 | 102 | 56.3 |
| 3 | Smerinthinae | 22 | 36.7 | 63 | 34.8 |
| 4 | Sphinginae | 12 | 20 | 15 | 8.3 |
| **Total** | | **60** | **100** | **181** | **100** |

Out of a total of 60 genera recorded in the study areas of Vietnam, 31 genera have only 1 species (accounting for 51.7%), 10 genera have 2 species (accounting for 16.7%), 5 genera have 3 species (accounting for 8.3%), 3 genera have 4 species (accounting for 5%), 4 genera have 5 species (accounting for 6.7%), 1 genus has 7 species (accounting for 1.7%), 2 genera have 9 species, and 4 genera have the largest number of species, including *Macroglossum* (23 species), *Ambulyx* (13 species), *Theretra* (12 species), and *Eupanacra* (10 species).

Among the 110 species collected in the study, 5 areas have been published with the first list of hawkmoths, specifically 17 species in Ta Lien Trai (Lai Chau), 38 species in Song Thanh NP. (Quang Nam), 47 species in Nam Nung NR. (Dak Nong), 34 species in Bidoup-Nui Ba NP. (Lam Dong), 42 species in Sao La NR. (Thua Thien Hue), and 33 species in Kon Chu Rang NR. (Gia Lai).

### *3.1.2. Identification keys to subfamilies, genera, and species of the hawkmoths in Vietnam*

Based on the morphological characteristics of species which collected samples and original description of species without specimens, identification keys were constructed and inheritited the keys of Bell & Scott (1937).

The identification key to the subfamilies, genera and some the identification key to the species of some genera in Vietnam (including at least two species) were built.

**The identification key to the subfamilies of hawkmoths in Vitenam**

1. First segment of palpus with a patch of short sensory hairs on the inner surface near base ……………………………… Macroglossinae
- First segment of palpus without a patch of short sensory hairs on the inner surface near base ………..…………………….....………….. 2

2. Proboscis as long as or longer than body ……………… Sphinginae
- Proboscis no longer than the body or absent …………….....…….... 3

3. Outer margin of the forewing with light and dark lunules in the

crenulations ......................................................... Langiinae

- Outer margin of the forewing is straight or dentate without with light and dark lunules in the crenulations ............. Smerinthinae

### *3.1.3. Using DNA barcode to indentification homologous species belonging to genus Cechetra in Vietnam*

5 species belonging to genus *Cechetra* were recognized in Vietnam, of which 4 species including C. *lineosa*, C. *scotti*, C. *bryki*, and C. *subangustata continentalis* have very similar external morphology. Two of the four morphologically similar species (*C. lineosa* and *C. subangustata*) were identified by DNA barcode.

Total DNA was extracted from the specimen’s hindleg tissues and analyzed by electrophoresis on a 0.9% agarose gel. A region of CO1 gene was amplified using primers LCO2198 and HCO1490 with 658 bp (Folmer *et al*., 1994), respectively sequence 5’ GGTCAACAAATCATAAAGA TATTGG 3’ and 5’ TAAACTTCAGGGTGACCAAAAAATCA 3’. The images obtained from PCR products of 17 hawkmoths specimens were clear, without auxiliary bands, and suitable for the size of the CO1 genome of about 650 bp. The sequencing reactions were performed at Macrogen Corporation in the Netherlands.

The comparison results of 16 sequences of samples (preliminarily identified is *C. lineosa*) were compared with 3 sequences with accession numbers KY962521, KC182180, KC182176 obtained from Genbank and 2 sequences with accession numbers MT994235, MT994234 previously published by Le *et al.* (2022) shows the sequences of the same species (Figure 3.68). These samples were accurately identified as the species *C. lineosa*.

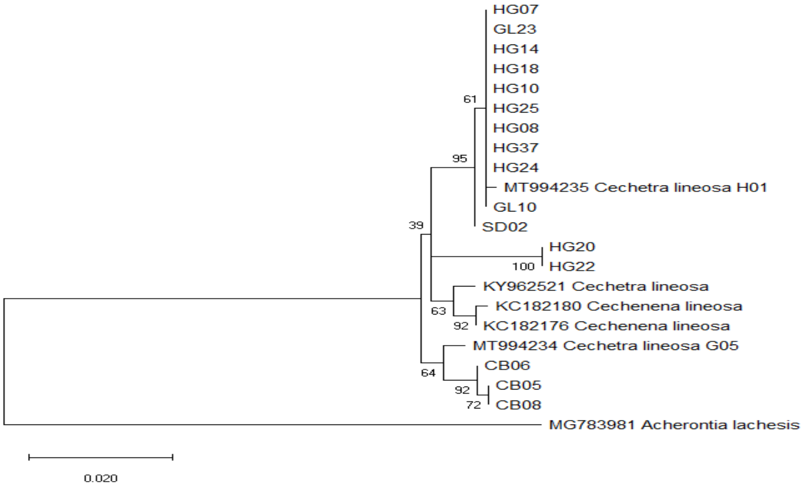


***Figure 3.68***. Relationship of *Cechetra lineosa*

The comparison results of the HG23 sample sequence with the previously published sequence with the code MT994231 by Le *et al*. (2022), and the two sequences with the codes JN677799 and KP720043 obtained from Genbank, show that the sequences of the same species have a bootstrap value of 84% (Figure 3.69). The sample have been identified as the species *Cechetra subangustata*.

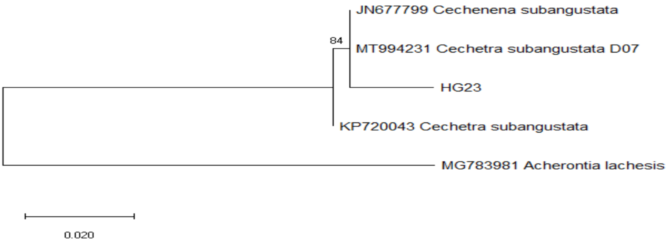


***Figure 3.69***. Relationship of *Cechetra subangustata*

Three species of *Cechetra minor, C. lineosa*, and *C. subangustata* belonging to the genus *Cechetra* in Vietnam have been added to the current database at GenBank (NCBI), including 7 sequences in the previous publication (Le *et al*., 2022) comprising MT994230, MT994231,

MT994232, MT994233, MT994234, MT994235, MT994236, and 17 sequences in this study ranging from PP961897 to PP961913.

## 3.2. Distribution and diversity characteristics of the hawkmoths in Vietnam

### *3.2.1.* Distribution and diversity of the hawkmoth according to natural geographical regions in Vietnam

*3.2.1.1. The species composition of hawkmoths according to natural geographical regions in Vitenam*

A total of 110 species, 47 genera, and 4 subfamilies of hawkmoths are recorded in eight natural geographical zones of Vietnam.

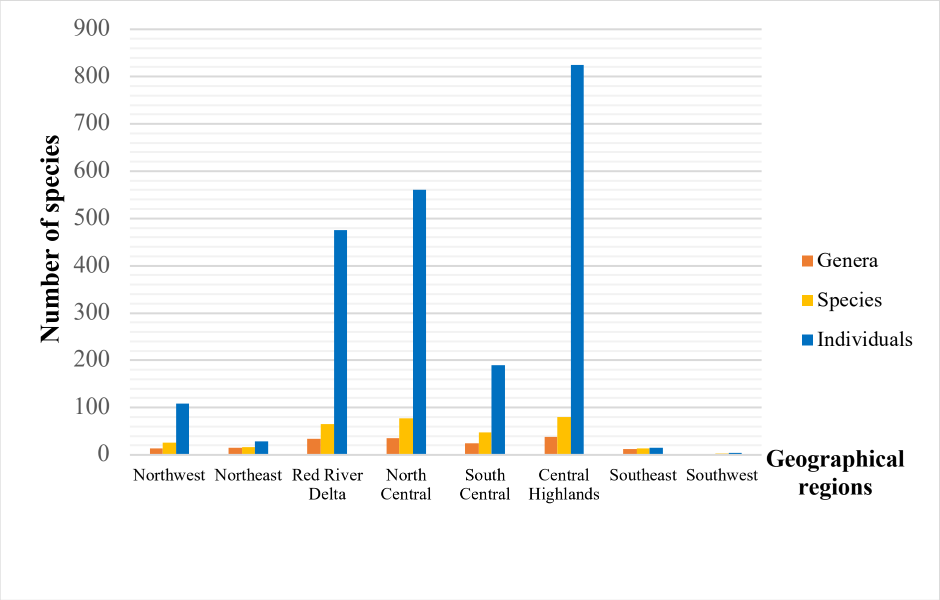


***Figure 3.71***. Geographical distribution chart of the hawkmoths in Vietnam

Regarding the species composition, the Red River Delta, North Central, and Central Highlands are the two regions with the richest species and genus diversity in all three subfamilies: Macroglossinae, Smerinthinae, and Sphinginae. In contrast, the Southeast and Southwest regions are the least diverse.

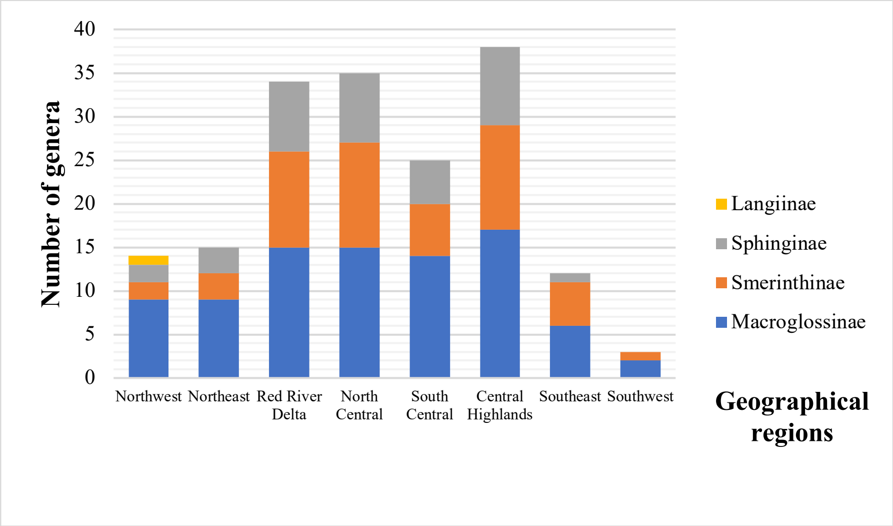


***Figure 3.72***. Number of genera by subfamily according to natural geographical regions of Vietnam

Regarding the composition of the genera of the hawkmoths, the genus *Theretra* of the subfamily Macroglossinae predominates in most of the study areas, while in the South Central region, the genus *Ambulyx* of the subfamily Smerinthinae predominates.

Regarding the species composition, among the 110 recorded species, none are distributed across all 8 geographical regions. Only the species *Daphnis hypthous crameri* is widely distributed in 7 geographical regions, except for the Southeast region. 5 species are distributed across 6 geographical regions, including *Angonyx testacea, Pergesta acteus, Theretra nessus, Ambulyx moorei,* and *Acherontia lachesis*. The number of species distributed across 5 natural geographical regions includes 14 species, 22 species present in 4 geographical regions, 21 species present in 3 natural geographical regions, 19 species appearing in 2 natural geographical regions, and 26 species only distributed in one natural geographical region.

Among the 26 species with limitation distribution area, 4 species were found in the Northwest region (*Langia zenzeroides, Rhagastis gloriosa, Rhagastis lunata, Clanis undulosa*), 2 species were appeared in the Northeast region (*Callambulyx kitchingi* and *Smerinthus szechuanus*), 6 species were recorded in the Red River Delta (*Eupanacra metallica, Griseosphinx marchandi, Macroglossum bombylans, Morwennius decoratus, Polyptychus trilineatus, Apocalypsis velox evjatkini*), 4 species were found in the North Central region (*Hippotion celerio, Macroglossum divergens heliophila, Macroglossum neotroglodytus, Cypa latericia*), 2 species were recorded in the South Central region (*Gnathothlibus erotus, Ambulyx canescens*), and 8 species were found in the Central Highlands region (*Eurypteryx geoffreyi, Macroglossum belis, Ambulyx schauffelbergeri, Callambulyx diehli, Cypoides chinensis, Marumba sperchius, Rhodoprasina callantha callantha, Hyloicus centrovietnama*). No records exist of species with a restricted distribution in the Southeast or Southwest. Restricted species to the Northwest region, inhabiting high mountain ranges over 2000 meters above sea level, can be classified as montane species and serve as indicators of hawkmoths distribution in Vietnam.

### *3.2.1.2. Distribution map of some hawkmoths genera in Vietnam*

Distribution maps were only generated with the species recorded in the research, encompassing 47 distribution maps of 110 species from 47 genera: *Langia, Acosmerycoides, Acosmeryx, Ampelophaga, Angonyx, Cechenena, Cechetra, Dahira, Daphnis, Elibia, Enpinanga, Eupanacra, Eurypteryx, Gnathothlibus, Griseosphinx, Hippotion, Macroglossum, Pergesa, Rhagastis, Theretra, Ambulyx, Amplypterus, Anambulyx, Barbourion, Callambulyx, Clanis, Craspedortha, Cypa, Cypoides,* *Daphnusa, Marumba, Morwennius, Parum, Phyllosphingia, Polyptychus, Rhodoprasina, Smerinthus, Acherontia, Agrius, Apocalypsis, Cerberonoton, Dolbina, Hyloicus, Megacorma, Meganoton, Notonagemia, Psilogramma.*

#### *3.2.1.3. Dominant species and diversity indexes*

The majority of dominant species belong to the subfamily Macroglossinae, with only one species, *Callambulyx rubricosa*, belonging to the subfamily Smerinthinae. The dominance index (DI) among different natural geographical regions shows little variation, ranging from 0.15 to 0.32, with an average value of 0.37±0.39. The Margalef species richness index (d) among natural geographical regions ranges from 4.45 to 12.16, with an average value of 8.84±3.27. Table 3.2 shows that the North Central region has the highest dominant species index (DI = 12.16), followed by the Central Highlands (DI = 11.76), the Red River Delta (DI = 10.38), and the South Central (DI = 8.96). The Northwest region has the lowest species richness (DI = 5.33), followed by the Northeast region (DI = 4.45). The two regions of Southeast and Southwest have very few samples and species collected, so the biodiversity indices and dominant species were not assessed in this study.

***Table 3.10.*** Dominant and diversity indexes in natural geographical regions

| Geographical regions | Dominant species index (DI) | Margalef index (d) | Shannon - Weiner index (H') |
|---|---|---|---|
| Northwest | 0.32 | 5.33 | 2.75 |
| Northeast | 0.27 | 4.45 | 2.62 |
| Red River Delta | 0.16 | 10.38 | 3.59 |
| North Central | 0.16 | 12.16 | 3.81 |
| South Central | 0.17 | 8.96 | 3.47 |
| Central Highlands | 0.15 | 11.76 | 3.90 |
| Southeast | - | - | - |
| Southwest | - | - | - |
| **Average** | **0.37±0.39** | **8.84±3.27** | **3.36±0.54** |

When comparing the Shannon-Weiner biodiversity index (H') among different geographic regions, it shows that the Central Highlands has the highest diversity (H’=3.9), followed by the North Central region (H’=3.81), the Red River Delta (H’=3.59), and the South Central region (H’=3.47). These are areas with good and very good levels of diversity. The two regions with lower diversity levels are the Northwest (H’=2.75) and the Northeast (H’=2.62).

### *3.2.1.4. Similarity in the species composition of the hawkmoths among natural geographical regions in Vietnam*

The results of species composition similarity between natural geographical regions in Vietnam are also clearly shown in the similarity diagram between the regions (Figure 3.73). The degree of similarity in species composition between the regions is approximately 18%, and it branches according to decreasing similarity, forming corresponding clusters where the species composition is closest between the two regions of the Central Highlands and North Central, followed by the Red River Delta region, which has the species composition closest to the Central Highlands and North Central cluster. The remaining locations exhibit species composition similarities of less than 50%.

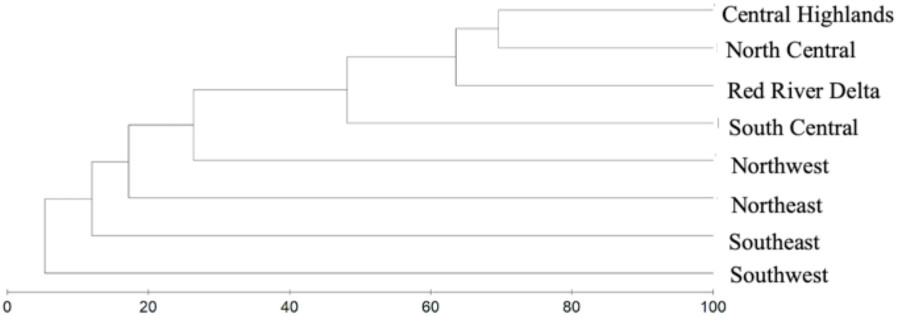


***Figure 3.73***. Similarity in the species composition of the hawkmoths among natural geographical regions in Vietnam

### *3.2.2. Distribution and diversity of the hawkmoth according to habitats*

#### *3.2.2.1. The species composition of hawkmoths according to habitats in Vietnam*

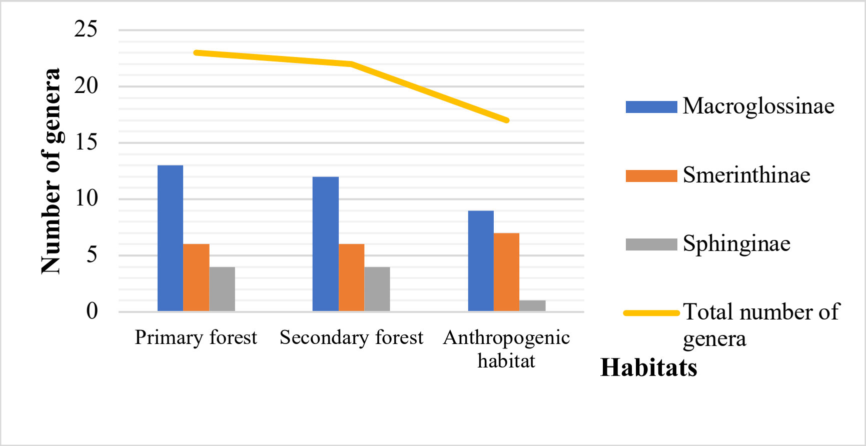
Regarding the species composition structure by different habitats, the highest number and diversity of species belong to the secondary forest habitat, followed by the primary forest habitat, and the least in the anthropogenic habitat (Table 3.13). The conclusion is also compatible with the butterfly research of Vu & Vu (2011) and Vu Van Lien & Vu Quang Con (2020), which show that the butterfly community is more diverse and wealthy in secondary forests than in primary forests.

***Table 3.13.*** The species composition of hawkmoths in the studied habitats

| **Habitat** / **Subfamily** | Primary forest | | Secondary forest | | Anthropogenic habitat | |
|---|---|---|---|---|---|---|
| | N | P | N | P | N | P |
| Macroglossinae | 26 | 61.9 | 31 | 70.45 | 15 | 57.69 |
| Smerinthinae | 12 | 28.57 | 9 | 20.45 | 10 | 38.46 |
| Sphinginae | 4 | 9.53 | 4 | 9.1 | 1 | 3.85 |
| **Total** | **42** | **100** | **44** | **100** | **26** | **100** |

*Note: N is Number of species, P is percentage (%)*

Regarding the number of genera hawkmoth in the three studied habitats, the total number of genera decreases from the primary forest habitat to the anthropogenic habitat. However, when each subfamily is examined,



the Smerinthinae subfamily exhibits the reverse trend, but the difference is not significant.

***Figure 3.74.*** Chart of number of genera of the hawkmoths in the study habitats

In all three studied habitats, the genus *Theretra* of the subfamily Macroglossinae has the richest species diversity. This demonstrates that they are hawkmoths species with a wide distribution and a strong ability to adapt to changes in the ecology.

### *3.2.2.2. Dominant species and diversity indexes*

The analysis results show that in the primary forest habitat, *Acosmeryx sericeus* is the first dominant species, followed by *Theretra nessus* as the second dominant species. In the secondary forest habitat, *Angonyx testacea* is the first dominant species, and *Theretra nessus* is the second dominant species. In the anthropogenic habitat, *Acosmeryx sericeus* is the first dominant species, *Theretra clotho* and *Theretra pallicosta* are the second dominant species. All of the prominent species belong to the Macroglossinae subfamily.

The dominance index (DI) among the three habitats shows little variation, ranging from 0.17 to 0.28, with an average value of 0.24±0.06. The

Margalef species richness index (d) among the three habitats ranges from 5.74 to 8.6, with an average value of 7.34±1.46. As can be observed, the secondary forest and anthropogenic habitats have the highest and approximately equal richness, but the natural forest habitat has the lowest.

When the Shannon-Weiner biodiversity index (H') was compared among the three examined habitats, it was shown that primary forest had the most diversity (3.43), followed by secondary forest (3.25), while the least diversity was found in anthropogenic habitats (2.92). The average value of the Shannon-Weiner biodiversity index (H') in the three studied habitats is 3.2±0.26. Thus, hawkmoths diversity ranges from good to very good in primary and secondary forest habitats, but is only moderate in anthropogenic habitat. Overall, the diversity of hawkmoths in the studied habitats is at a good level. This outcome somewhat matches reality and is compatible with our research findings above.

***Table 3.15.*** Dominant and diversity indexes in the studied habitats

| **TT** | **Sinh cảnh** | Dominant species index (DI) | Margalef index (d) | Shannon - Weiner index (H') |
|---|---|---|---|---|
| 1 | Primary forest | 0.17 | 7.67 | 3.43 |
| 2 | Secondary forest | 0.28 | 8.60 | 3.25 |
| 3 | Anthropogenic habitats | 0.27 | 5.74 | 2.92 |
| **Average** | | **0.24±0.06** | **7.34±1.46** | **3.2±0.26** |

*3.2.2.3. Similarity in the species composition of the hawkmoths in the studied habitats*

The species composition of the primary forest is similar to that of the secondary forest (0.41 < SI = 0.58 and 0.44 < 0.6). The similarity of species compostion among three habitats, the primary forest, the secondary forest, and the anthropogenic habitat is rather low (0.41) (Figure 3.73).

***Figure 3.75.*** Similarity in the species composition of the hawkmoths in the studied habitats

## CONCLUSION AND RECOMMENDATION

### CONCLUSION

1. The hawkmoths fauna in Vietnam is highly diverse and rich. Recording 181 species and subspecies of 60 genera belonging to 4 subfamilies: Langiinae, Macroglossinae, Smerinthinae, and Sphinginae in Vietnam, including 8 species and 5 subspecies endemic to Vietnam. The first checklist of hawkmoths in five regions including Lai Chau, Quang Nam, Đak Nong, Lam Đong, Thua Thien Hue, and Gia Lai were recorded.

2. Identification keys to the subfamily, genus, and species of the hawkmoths family were published for the first time in Vietnam, including one for the subfamily, three for the genus, and 29 for the species. DNA barcoding is used to accurately classify two morphologically similar species belonging *Cechetra* genus in Vietnam. 17 DNA barcodes from three species of the *Cechetra* genus were successfully registered in Genbank.

3. The species in the family Sphingidae are characterized by a wide and diverse distribution according to natural geographical regions and habitats. The genus *Theretra* belonging to the subfamily Macroglossinae, has

a wide distribution and a high adaptability to changes in the ecosystem, dominating most geographical areas as well as research habitats. Four species, *Langia zenzeroides, Rhagastis gloriosa, Rhagastis lunata,* and *Clanis undulosa,* with limited distribution in the high mountains of Northwest Vietnam, can be used as indicators for montane species in Vietnam.

4. 47 distribution maps were created for 110 species and 47 genera of the hawkmoths in Vietnam. Habitat distribution characteristics demonstrate that hawkmoths species can adapt to a variety of habitats, with secondary forest having the most species, followed by primary forest, and anthropogenic habitat having the fewest.

5. In terms of diversity among geographical regions, the three regions of Central Highlands, North Central, and Red River Delta have good to very good levels of diversity, whereas Northwest and Northeast have lower to fair levels of diversity. According to the habitat, the diversity of hawkmoths in primary and secondary forests ranges from fair to very good, whereas it is moderate in restored anthropogenic habitat. Overall, the hawkmoths fauna in Vietnam has an average diversity level ranging from good to very good.

6. Regarding the similarity in species composition among geographical regions, the North Central region and the Central Highlands are the most similar (SI = 0.70), followed by the Red River Delta, which has a species composition closest to the North Central region (SI = 0.68) and the Central Highlands (SI = 0.59). The remaining areas have a species composition similarity of less than 50%. In terms of habitat, the species composition of primary forests is more similar to that of secondary forests than to that of artificially restored forests.

## RECOMMENDATION

1. Continue clarify the taxonomic position of morphologically similar species based on the combination of morphological methods and

DNA barcoding.

2. Further research on the biological and ecological characteristics of endemic species to have a comprehensive database as a scientific basis for biodiversity conservation in Vietnam.

## CONTRIBUTIONS OF THE DISSERTATION

- Classification keys to species of hawkmoths family has been published from Vietnam for the first time, using DNA barcoding to identify two homomorphic species of the genus *Cechetra.*

- Proving a comprehensive hawkmoths checklist that adds previous lists in Vietnam. Adding new data on the distribution characteristics and diversity assessment of hawkmoths species in Vietnam according to natural geographical regions and habitats.

# LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Le Q.T**, Vu V.L., 2024. Checklist of hawkmoths (Lepidoptera: Bombycoidea: Sphingidae) in the Central Highlands of Vietnam, *Journal of Threatened Taxa* 16(1): 24503–24528. https://doi.org/10.11609/jott.8636.16.1.24503–24528
2. **Le Q.T.**, Tran T.V.T, Vu V.L., 2023. First record of *Daphnis nerii* (Linnaeus, 1758) (Lepidoptera: Sphingidae) from Vietnam, *Proceesings of the 11 th National Conference on Entomology*, 11, 196-199
3. **Le Q.T.**, Vu V.L, Tran T.V.T, 2022. DNA barcodes in indentification of some species of hawkmoths (Lepidoptera: Sphingidae), *Academia Journal of Biology*, 44(1): 135–143. https://doi.org/ 10.15625/2615-9023/15742
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6. Tran Thi Viet Thanh, To Van Quang, **Le Quynh Trang**, 2021. Total DNA extraction protocol for butterfly. *Proceedings of the 3 rd National Scientific Conference of Vietnam Natural Museum Sysem*, 3, 351-356
7. **Le Quynh Trang**, Vu Van Lien, 2021. The male genitalia of two homeomorphic species of hawkmoths (Lepidoptera: Sphingidae) in Vietnam. *Journal of Tropical Science and Technology*, 24, 299-304