New Distribution Record of *Cechenena transpacifica* (Clark, 1923) and *Theretra acuta* (Vaglia & Liyous, 2010) of Family Sphingidae in Mindanao, Philippines: Identification based on Morphology and CO1 DNA Barcode

Michelle S. Suelo[1]*, Glenda Z. Doblas[1], Alma B. Mohagan[1], Joliesa Mae S. Toledo[1], Olga M. Nuñeza[2], Aimee AbduL[3], Reggie Y. Dela Cruz[1]

[1] Institute of Biological Sciences, Central Mindanao University, University Town, Musuan, Maramag, Bukidnon 8714 Philippines
[2] Department of Biological Sciences, Mindanao State University- Iligan Institute of Technology, Andres Bonifacio Avenue, Iligan City 9200, Philippines
[3] Mapua Malayan Colleges Mindanao, MacArthur Highway, Davao City 8000, Philippines

DOI: https://doi.org/10.56293/IJASR.2024.6018

IJASR 2024
VOLUME 7
ISSUE 4 JULY – AUGUST **ISSN: 2581-7876**

Abstract: Hawkmoths belonging to Family Sphingidae are distinguished by their narrow wings, streamlined bodies and varying length of proboscis making them one of the most important insects in our ecosystem. Two species collected in Mt. Balatukan and Mt. Melibengoy were identified based on morphological description and DNA barcoding (LepF and LepR primers) as *Cechenena transpacifica* (Clark, 1923) and *Theretra acuta* (Vaglia & Liyous, 2010). This species were only recorded to exist in Luzon and Visayas. In this study, we provide detailed morphological description and identification based on DNA barcoding of the two species as reported to be found in Mindanao for the first time. The phylogenetic tree inferred from Maximum-Likelihood Method showing monophyletic clade with Family Sphingidae for the two species.

Keywords: Sphingidae; DNA Barcoding; monophyletic; Maximum-Likelihood

INTRODUCTION

Hawkmoths are one of the ecologically important pollinators in our ecosystem. They play an important role in the stability of terrestrial ecosystem [4]. The Philippines, one of the world's top 17 megadiversity nations, is geographically situated in Southeast Asia and is home to a variety of natural resources [5]. Class Insecta is one of the most prevalent group of living organisms in the planet, claims [20]. There could be up to 5.5 million species in the world. Only 1 million species have been named, and 80% of them are yet undiscovered. Approximately 157,000 different species of lepidopteran are known to exist [20, 3, 19, 22].

The distribution of hawkmoth species is influenced by geographical boundaries and climatic variables such as maximum and lowest temperatures, rainfall, wind, and season length [17]. In tropical ecosystems, the transition between the dry and wet seasons has an effect on the cycles of host plant leaf availability to hawkmoth larvae, which in turn affects the seasonal occurrence of adults [18]. With 122 species of Sphingidae occurring in the Philippines [16, 23] 62 species of these occurs in Mindanao Island [16]. There are approximately 1,700 species of hawkmoths recorded worldwide [15]. The island of Palawan has the most species (73), followed by Luzon (72), Mindanao, Leyte, and Negros, each with 62 species. There are 24 endemic species in the Philippines, and Luzon has the highest percentage of them (71%), followed by Mindanao (58%), Negros (50%) and Leyte (46%). Due to its strong ties to Bornean fauna, Palawan has a low percentage of endemic species (8%) while having the highest species count in the country [7]. The present research findings provide morphological description supported by molecular data for the two newly recorded species distribution in Mindanao, Philippines.

MATERIALS AND METHODS

Study Sites

The study was conducted in two mountain ecosystems in Mindanao namely: Mt. Melibengoy located in T'boli, South Cotabato, 1116.3 masl - 1602.5 masl, 6°5'48.2239" N 124°52'55.14301" E, and Mt. Balatukan in Sitio Civoleg, Gingoog City, Misamis Oriental, 1268.1 masl - 1484.2 masl, 8°43'50.69104" N 125°0'14.37782" E (Fig. 1). Gratuitous permit in Mt. Balatukan (No. R10 2022-34) and Mt. Melibengoy (No. RXII 2022-11) was secured prior to the conduct of the study. The sampling was done on 18-22 December 2022 for Mt. Balatukan, while for Mt. Melibengoy was 16-19 February 2023.

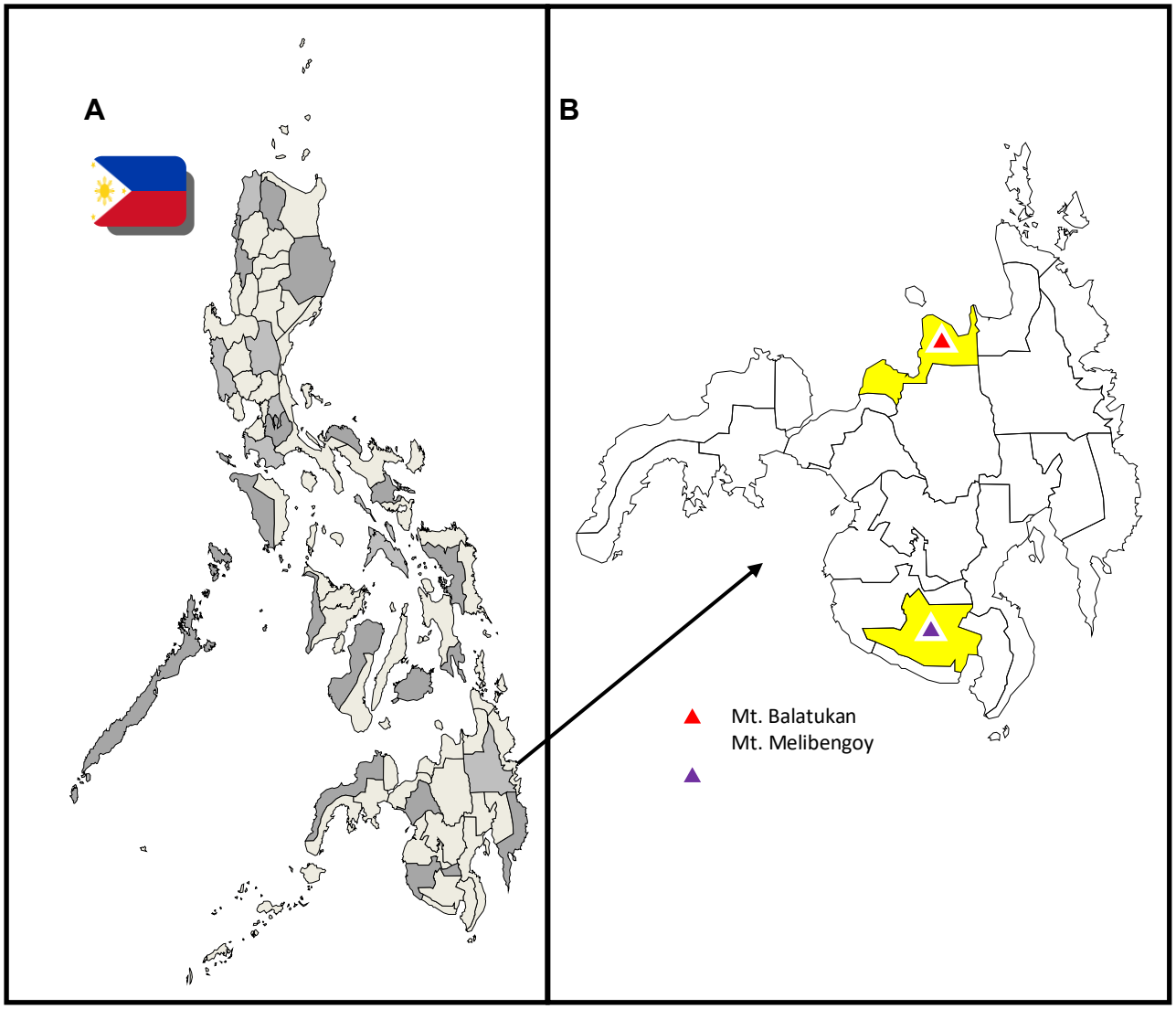

Figure 1. A) Map of the Philippines, B) Map of Mindanao showing Mt. Balatukan, Sitio Civoleg, Gingoog City, Misamis Oriental and Mt. Melibengoy, T'boli, South Cotabato

Sampling procedures, preservation

Light trapping technique was employed in order to collect night flying insect (Fig. 2). The light trap was used for 10 hours from 6pm to 4am, using 500 watts, 12 voltage tungsten bulbs with a source power of 22AC. White sheets were then set up to trap insects. The collected adult hawkmoth species during light trap was euthanized by pressing slightly its thorax [13]. The legs of newly euthanized hawkmoth were collected and placed inside a cryovial tube with absolute ethyl alcohol for preservation. The remaining body were pinned and deposited at the University Museum Zoological Section of Central Mindanao University. Morphological description and measurement reported in cm were examined and noted.

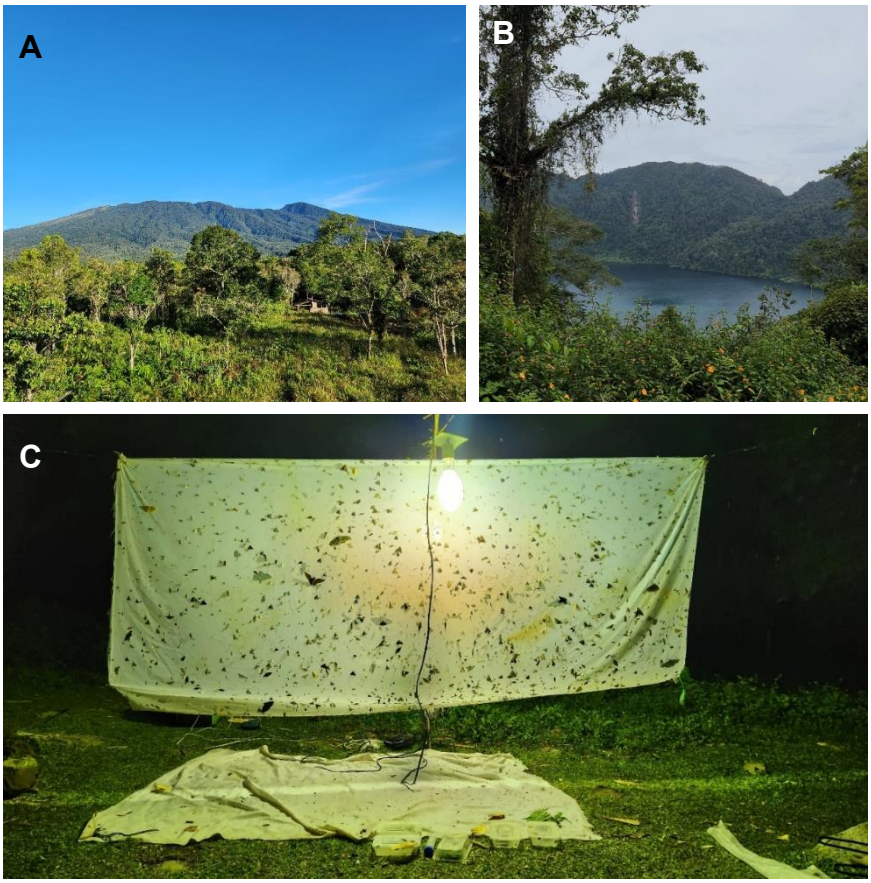

Figure 2. Sampling sites A) Mt. Balatukan, B) Mt. Melibengoy C. Light trapping Technique

DNA Barcoding

Legs of the preserved adult hawkmoth species were used for the extraction of genomic DNA and placed in a sterile 2 ml cryovial tube with proper labels. DNA extraction followed the manufacturer's protocol of the Qiagen DNeasy Blood and Tissue Kit. PCR amplification was carried out using LepF1 and LepR1 primers. Amplicons were then visualized in 1% agarose stained with GelRed (Biotium Inc., USA). Amplicons were sent to Macrogen, South Korea for sequencing. Sequences were then aligned and cleaned using BioEdit Software. MEGA X was used to construct phylogenetic tree. The cleaned sequences were then compared to an existing database using two available tools: Barcode of Life Database (BOLD) System and Basic Local Alignment Search Tool (BLASTS).

RESULTS AND DISCUSSION

The specimens collected from two mountain ecosystems in Mindanao were identified based on DNA Barcoding as *Cechenena transpacifica* and *Theretra acuta* with 99.07% and 97.77% identity based on BLASTS, respectively. While identification also reveals 99.47% for *C. transpacifica* and 100% *T. acuta* using BOLD system. DNA barcoding is a popular genetic method for identifying animal species [10, 14]. As long as library data is credible and well-selected [11] it can be a valuable supplement to taxonomic research that focuses primarily on morphological criteria [6]. A DNA barcode is a short standardized DNA sequence that can be used as a genetic marker for species identification [8]. Herbert and colleagues proposed using a ~658 base pair (bp) section of the COI gene as a universal marker for animal barcoding [9, 1]. Figure 3 reveals that this two species were group together supported with a strong bootstrap value inferred using Maximum Likelihood Method.

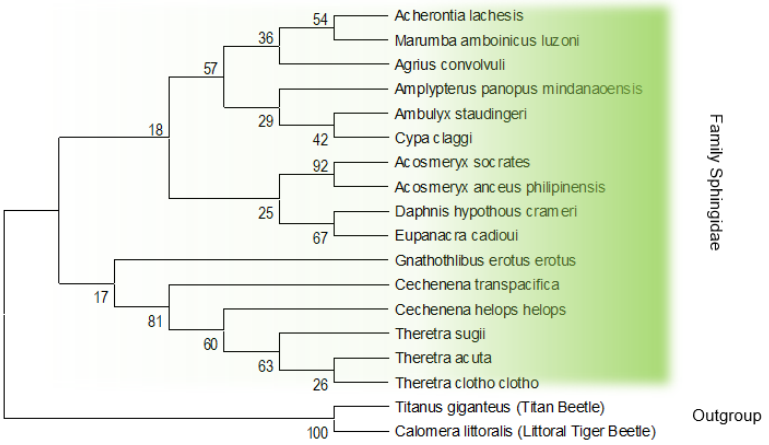

Figure 3. Evolutionary analysis by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method (Fig. 3) and Tamura-Nei model [21]. The tree with the highest log likelihood (-5750.98) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 18 nucleotide sequences. There was a total of 912 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [12].

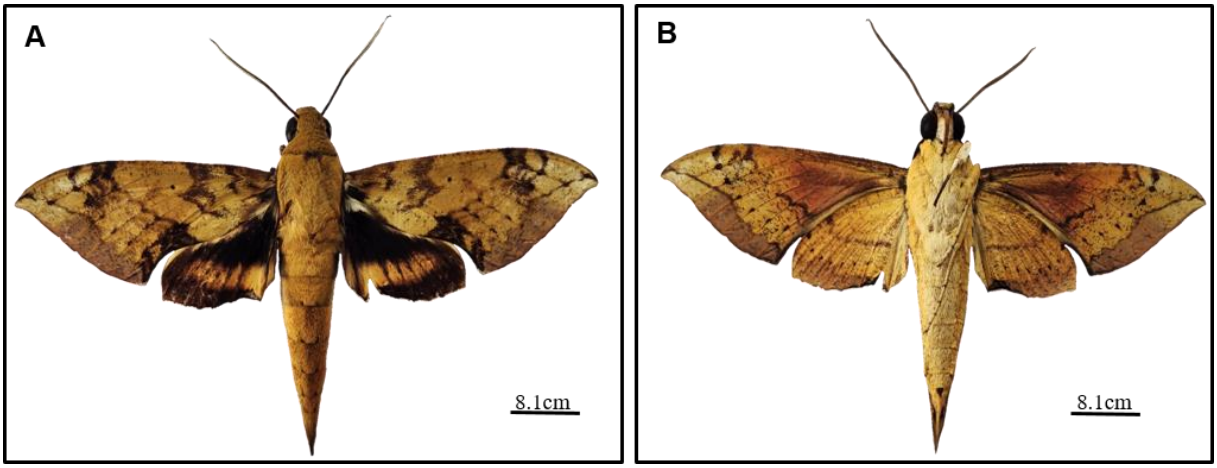

Figure 4. *Cechenena transpacifica* (Clark 1923) A) Dorsal view B) Ventral view

Cechenena transpacifica (Clark 1923) Fig. 4

Measurements (cm): Head: antenna: 1.9cm-2.3cm, proboscis: 4.6cm-6.5cm, eyes: 0.4cm-0.5cm, Thorax: **prothorax:** 0.6cm-0.7cm, mesothorax: 0.4cm, metathorax: 0.3cm-0.4cm, **Abdomen:** eight segment: 2.5cm-3.0cm, setae: 0.4cm-0.7cm, **Legs:** foreleg: 1.7cm-2.8cm, middle leg: 2.4cm-3.0cm, hind leg: 2.5cm-3.6cm. **Forewing:** base to apex: 4.0cm-4.9cm, apex to tornus: 2.3cm-2.9cm, tornus to base: 2.2cm-2.6cm, base: 0.5cm-0.6cm. **Hindwing:** base to apex: 2.2cm-2.6cm, apex to tornus: 1.8cm-2.1cm, tornus to base: 0.9cm-1.5cm, base: 0.3cm-0.4cm.

Morphological Description

The head is dominantly covered with heavy yellow at dorsal part, while ventral part is covered with white hue with black eyes. Dorsal part of the thorax is continuous to the coloration of the head which is heavy yellow while ventral

part is dominantly covered with lighter yellow to white color. Dorsal part of the abdomen is the same coloration with the dorsal part of the thorax with visible black thin horizontal line on each abdominal segment while ventral area is light yellow to white hue with several black spots. Dorsal forewing is dominantly covered with heavy yellow with several black patches. Costal margin is straight then slightly become curved towards its pointed apex, outer margin slightly undulate with dark fringe. Inner margin is curve near the tornus (coated with black), then coated with white as it approaches the base. Basal area to the antemedial area is heavy yellow with dark transverse patches. While median area is covered with heavy yellow, black orbicular spot. Terminal area has several dark wavy discontinuous patches. Black spots present all over the area. While ventral area is dominantly covered heavy yellow to light brown at median area to terminal area with visible transverse small black circle present on each veins and with several black wavy patches as well as black spots. Antemedial area to basal area is covered with yellow orange. Dorsal portion of the hindwing has costal margin coated with yellow from the base until 3/4th towards its pointed apex. Outer margin is undulate with black fringe. Inner margin is coated with yellow-black shade. Basal area to antemedial area is covered with black. Yellow transverse band at median area, but the band is only 3/4th and does not reached the costal margin. Terminal area is black. Its ventral portion is dominantly covered with light yellow. Basal area to antemedial area is covered with yellow, with dark transverse median line. Yellow hue is continuous until half of the terminal area. Small visible black dot present on every segment. Terminal area near outer margin is covered with light brown. Black spots present all over the hindwing.

Diagnosis: *C. transpacifica* replaces *C. aegrota* in the Philippines. The males of the species resemble similar to those of *C. aegrota* and Seitz considered both conspecific. In the female of *C. transpacifica*, the distal half of the forewing has almost fully darkened to brown, with only a few yellow dots remaining in the disc and apex of the wings. The proximal half of the wing is almost uniformly yellowish red with minimal traces of darker maculation, while the upperside of the rearwings lacks the male's prominent darker edge. The impression is of a yellowish-red moth with large brown and black spots. It appears to take two forms in male species. Most specimens, including *C. aegrota*, have a huge black blotch at the base of the hindwing. . In the male genitals of *C. transpacifica*, it has a larger harpe than *C. aegrota*. Without dissection, the species can be differentiated by the red basal region of the forewing underside *C. transpacifica* and black in *C. aegrota* [7].

Distribution: *C. transpacifica* is endemic to the Philippines where it is uncommon and widely distributed in Mindoro, Negros, Leyte, Luzon, Panay and Samar [7].

Materials examined: PHILIPPINES • (3/3) 3♂ Adult. Mount Balatukan, Sitio Civoleg, Gingoog City, Misamis Oriental, Philippines, 1268.1 masl - 1484.2 masl, 8°43'50.69104" N 125°0'14.37782" E, 18-22 December 2022, 6 PM - 4 AM.

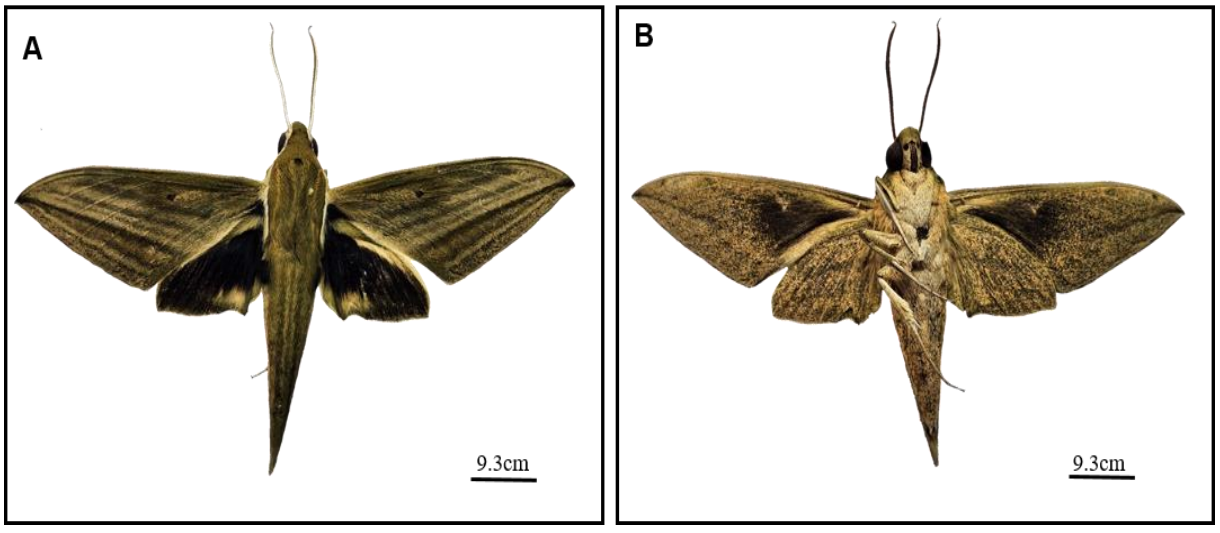

Figure 5. ***Theretra acuta*** **(Vaglia & Liyous, 2010) A) Dorsal view B) Ventral view**

Theretra acuta (Vaglia & Liyous, 2010) Fig. 5

Measurements (cm): Head: antenna: 1.6cm-, proboscis: 5.5cm, eyes: 0.3cm-, Thorax: prothorax: 0.6cm, mesothorax: 0.4cm, metathorax: 0.3cm, Abdomen: eight segment: 2.1cm, setae: 0.5cm-, Legs: foreleg: 1.8cm-, middle leg: 2.5cm, hind leg: 2.7cm-. Forewing: base to apex: 4.4cm, apex to tornus: 2.4cm, tornus to base: 2.5cm, base: 0.5cm. Hindwing: base to apex: 2.8cm, apex to tornus: 1.9cm, tornus to base: 1.1cm, base: 0.3cm.

Morphological Description

The head of *T. acuta* is dominantly covered heavy green with white line at the upper part of the eyes continuous to the base of the forewing with black eyes. Dorsal part of the thorax is covered with green, with golden yellow patches and black small circle at the center while ventral part is lighter color than dorsal part. Abdomen is dominantly covered with green, same coloration with the thorax. Distinct lines observed down to anal area while ventral area is lighter color with many black spots. Dorsal part of the forewing is dominantly colored greenish. Costal margin is coated with black. Outer margin is straight with dark fringe. Inner margin slightly curves near the tornus coated with yellow hue. Several black transverse lines running from mid of inner margin to the apical region. Terminal area is covered with black spots. Black orbicular spot is present. While ventral part has discal area coated with black color, while median to terminal area is covered with heavy black spots. Black discontinuous line running from the apex. Its hindwing dorsal part is dominantly covered with black. Costal margin coated with yellow. Undulate outer margin with yellow-black fringe. Tornal area with yellow shade while ventral part has coloration the same with forewing ventral part, but mostly covered with heavy small dark spots.

Diagnosis: This species were not recorded on the work of Hogenes and Treadaway (1998) who studied Philippine Family Sphingidae. This species may be confused with *T. rhesus*; however, they differ in color with *T. rhesus* lighter brown than *T. acuta* (greenish).

Materials examined: PHILIPPINES • (1/1) 1♂ Adult. Mount Melibengoy, T'boli, South Cotabato, Philippines, 1116.3 masl - 1602.5 masl, 6°5'48.2239" N 124°52'55.14301" E, 16-19 February 2023, 6 PM - 4 AM.

CONCLUSION

The present study reports a new record of *Cechenena transpacifica* (Clark 1923) and *Theretra acuta* (Vaglia & Liyous, 2010) with morphological description supported by DNA barcoding in Mindanao, Philippines, collected in two mountain ecosystems namely Mt. Balatukan and Mt. Melibengoy. This provides additional data on the distribution of Family Sphingidae throughout the Philippines. Especially here in Mindanao.

ACKNOWLEDGEMENT

This study was funded by the Department of Science and Technology-Science Education Institute (DOST-SEI). The authors also thank Cindy Love Cagas, Rona Mae Viernes, Kent Sean Alan Dargantes for the help during sampling. To Sir Dave Mohagan, of Central Mindanao University (CMU) Museum who facilitates the morphological identification of the specimens.

References

1. Ashfaq M, Akhtar S, Khan AM, Adamowicz, SJ, Hebert P (2013) DNA barcode analysis of butterfly species from Pakistan points towards regional endemism. Molecular Ecology Resources, 13: 832-843. doi:10.1111/1755-0998.12131
2. Brechlin R Kitching I (1996) New species of the genera Rhodoprasina Rothchild & Jordan and Acosmeryx Boisduval from Thailand and Vietnam, with a redescription of R. corolla Cadiou & Kitching Nachr. Ent. Ver. Apollo NF 17 (1) : 51-66
3. Cho S, Epstein SW, Mitter K, Hamilton CA, Plotkin D, Mitter C, Kawahara AY (2016) Preserving and vouchering butterflies and moths for large-scale museum-based molecular research. PeerJ. Doi 10.7717/peerj.2160
4. De Camargo AJA, de Camargo NF, Corrêa DCV, de Camargo WRF, Vieira EM, Marini-Filho O, Amorim FW (2016) Diversity patterns and chronobiology of hawkmoths (Lepidoptera, Sphingidae) in the Brazilian Amazon rainforest. Journal of Insect Conservation, 20(4): 629–641. doi:10.1007/s10841-016-

5. Fontanilla IKC, Torres AF, Cañasa ADG, Yap S, Ong PS (2014) State of animal DNA barcoding in the Philippines: A review of COI sequencing of Philippine native fauna. Philippine Science Letters, 7(1):104-137. Retrieved from https://www.researchgate.net/publication/273064221
6. Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics: TIG 23 (4): 167-72. https://doi.org/10.1016/j.tig.2007.02.001
7. Hogenes W, Treadaway CG (1998) The Sphingidae (Lepidoptera) of the Philippines. Entomologischer Verein Apollo e.v. Frankfurt am main, 17:17-132. Retrieved from www.zoobodat.at
8. Jalali SK, Ojha R, Venkatesan T (2015) DNA barcoding for identification of Agriculturally Important Insects. New Horizons in Insect Science: Towards Sustainable Pest Management, 13-22. doi:10.1007/978-81-322-2089-3_2
9. Jinbo U, Kato T, Ito M (2011) Current progress in DNA Barcoding and future implications for entomology. Entomological Science, 14: 107-124. doi:10.1111/j.1479-8298.2011. 00449.x
10. Karthika P, Krishnaveni N, Vadivalagan C, Murugan K, Nicoletti M, Benelli G (2016) DNA barcoding and evolutionary lineage of 15 insect pests of horticultural crops in South India. Karbala International Journal of Modern Science, 2(3): 156–168. doi: 10.1016/j.kijoms.2016.03.0
11. Kelnarova I, Jendek E, Grebennikov VV, Bocak L (2018) First molecular phylogeny of Agrilus (Coleoptera: Buprestidae), the largest genus on Earth, with DNA barcode database for forestry pest diagnostics. Bulletin of Entomological Research 109 (2): 200-211. https://doi.org/10.1017/S0007485318000330
12. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
13. Lara-Pérez LA, Campos-Domínguez J, Díaz-Fleischer Fet al (2017) Species Richness and Abundance of Satur-niidae (Lepidoptera) in a Tropical Semi-deciduous Forest of Veracruz, Mexico and the Influence of Climatic Variables. Revista Mexicana de Biodiversidad 88 (1): 173 –182. doi: 10.1016/j.rmb.2016.10.020
14. Marullo R, Mercati F, Vono G 2020 DNA Barcoding: A Reliable Method for the Identification of Thrips Species (Thysanoptera, Thripidae) Collected on Sticky Traps in Onion Fields. Insects. 11(8):489. https://doi.org/10.3390/insects11080489
15. Mape N, Alisto L, Kitching I (2022) Hawkmoths of Baguio City, Philippines: a Preliminary Checklist Derived from Photo-based Observation Records Available on iNaturalist and Philippine Lepidoptera Internet Platforms. Philippine Journal of Science. 151: 1447-1453. 10.56899/151.04.12.
16. Messenger C (1997) The Sphinx Moths (Lepidoptera:Sphingidae) of Nebraska. Transactions of the Nebraska Academy of Sciences and Affiliated Sciences, 24:89-141. Retrieved from http://digitalcommons.un/.edu/tnas/72
17. Pittaway AR, Kitching IJ (2020) Sphingidae of the Eastern Palaearctic (including Siberia, the Russian Far East, Mongolia, China, Taiwan, the Korean Peninsula and Japan). http://tpittaway.tripod.com/china/china.htm.
18. Primo LM, Duarte JA, Machado IC (2013) Hawkmoth fauna (Sphingidae, Lepidoptera) in a semi-deciduous rain forest remnant: composition temporal fluctuations, and new records for northeastern Brazil. Annals of the Brazilian Academy of Sciences, 85(3):1177-1188. Retrieved from www.scielo.br/aabc
19. Sebua CMD, Nuñeza OM (2020) Species Diversity of Lepidoptera in Western Mindanao State University – Experimental Forest Area, Zamboanga City, Philippines, Entomol Appl Sci Lett, 7 (1): 33-43.
20. Stork NE (2017) How Many Species of Insects and Other Terrestrial Arthropods Are There on Earth? Annual Review of Entomology, 63(1):31–45. doi:10.1146/annurev-ento-020117-043348
21. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.
22. van Nieukerken EJ, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, Wahlberg N (2011) Order Lepidoptera Linnaeus, 1758. In: Zhang, Q. Animal biodiversity an outline of higher-level classification and survey of taxonomic richness. Zootaxa. 3148(1):212-21.
23. Zwick A, Treadaway CG (2001) Notes on the hawkmoths of the Philippines (Lepidoptera:Sphingidae). Nachr. entomol. Ver. Apollo, N. F. 22(3):177-18