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DNA Barcoding of Scoliid Wasps (Hymenoptera: Scoliidae) From Selected Sites in Luzon Island, Philippines

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Abstract: **Despite the importance of Scoliidae (Hymenoptera) in pest biological control and plant pollination, their taxonomy and systematics are far from clear. Some species are difficult to differentiate because of extreme morphological characteristics similarity. DNA barcoding is a useful tool to delineate species that are difficult to identify using morphological structures. In this study, the six and three samples of Scoliid wasps were collected in Central Bicol State University of Agriculture (CBSUA), Camarines Sur, Bicol, and Nueva Ecija, respectively, were discerned using DNA barcoding of the mitochondrial COI gene. Collected scoliid wasps from two sites have bodies that were fairly large and shouted; length ranged from 16.5 – 19.73mm. Antennae were light red; wings black with purplish reflections; and abdomen with faint blue reflections. Results showed that the three samples (1CBSUA, 2CBSUA, and 4CBSUA) from CBSUA and one sample (2NE) from Nueva Ecija were similar to Scolia kuroiwae. However, only two (1CBSUA, 97.2%; 2NE, 97.8%) from these samples passed the 97.0% cutoff of similarity. On the other hand, the remaining samples (3CBSUA, 5CBSUA, and 6CBSUA; 1NE and 3NE) were similar to Scolia verticalis but failed to hit the 97.0% cutoff of similarity. Samples with hits lower than the 97.0% similarity threshold suggest the possibility of species diversity among the collected Scoliid wasps in the two sites.**

Keywords: **DNA barcoding, scarab beetle, Scolia species, Scoliidae.**

I. INTRODUCTION

White grubs, the root-feeding larvae of various native and introduced scarab beetles (Coleoptera: Scarabaeidae) are one the most destructive and widespread insect pests of crops in the Philippines. Collectively they account for hundreds of millions of pesos in damage and control costs every year. White grubs traditionally have been controlled with soil insecticides. However, pesticide usage in crop areas is increasingly restricted due to perceived hazards and environmental concerns such as groundwater contamination. Scoliid wasps (Hymenoptera: Scoliidae) are pollinators of various plants and larval solitary ectoparasitoids of scarabaeoid beetles (Liu et al., 2021) that include *Anomala sp.* and *Phyllophaga sp.* (Kim, 2009), *Popillia japonica* (Japanese beetle) (Grisell, 2007), and *Oryctes rhinoceros* (coconut rhinoceros beetle) (Paudel et al., 2021). A study by Jepson (1956) in Tanzania reported that large Scoliid wasps, *Campsomeris mansueta*, parasitized the third instar larva of *Cochliois melolonthoides* (sugarcane white grub), and may have caused the 20 – 50% mortality of that stage. Smaller species of *Campsomeris sp., C. felina, C. lachesis,* and *C. caelebs* parasitized second instar larva and may have accounted for the 10 – 25% mortality of this stage.

Meanwhile, another study also showed that Scoliid wasps and *Voria sp.* (Diptera: Tachinidae) parasitoids were able to control the larval population of the taro scarab beetle (*Papuana uninodis*) using a continuous-time simulation model (Faithpraise et al., 2014). Females search for the location of the scarabaeoid larva by vibratory signals (i.e., antennal tapping), then they sting, paralyze and oviposit a single egg on the surface of the host (Liu et al., 2021). The hatched larva develops externally and feeds on its host larva for approximately one to two weeks then spins underground to pupate (Gressell, 2007). Under optimal conditions, the incubation period of eggs is two to three days, the larval period is six to nine days, and the cocoon and the pupal stage is 30 – 40 days in summer. During winter, most species hibernate within the cocoon (CABI, 2019). Scoliid adults of both sexes commonly visit flowers of various plants and some are found to be important pollinators. *Scolia nobilitata* is found to be a common flower visitor of buckwheat (*Fagopyrum esculentum*) in Florida, USA (Campbell et al., 2016). Male *Campsomeris bistrimacula* may be the only pollinator of the terrestrial orchid *Geoblasta penicillata* (Chloraeinae) via pseudocopulation from subtropical South America (Ciotek et al., 2006). The study of Inoue and Endo (2006) in Japan suggested that scoliid wasps may play important role in the pollination of coastal plants in the grassland zones adjacent to the disturbed sand dunes.

Despite the pest control and ecological importance of scoliid wasps, there is limited information, a lack of consolidated pictures of current morphometric data, and no genetic studies of scoliid wasps over the past year from the Philippines. Even though in the past, the country imported *Radumeris tasmaniensis* and *R. radula* for an attempted biological control of native cane grubs (Elliot, 2011). The aim of this study is to identify the species of Scoliid wasps collected from selected sites on the Island of Luzon using mitochondrial DNA barcoding. The use of DNA barcoding in this study is not just to contribute to the DNA database of Scoliid wasps, but rather it is the appropriate tool because some species of Scoliidae are extremely morphologically similar and difficult to identify, especially in males (Liu et al., 2021). Also, recent studies using molecular technology are changing the way we look at species concepts. The tiny parasitoid wasp, *Ormyrus labotus* (Hymenoptera: Ormyridae) was known to science since 1843 and has long been considered generalist with more than 65 host species (Entomological Society of America, 2022). But the study of Sheikh et al. (2022) suggested that *O. labotus* was composed of at least 16 species that are identical in appearance but genetically distinct. On the other hand, a study on white-belted Megaselia (Diptera: Phoridae) found that *M. sulphurizona* includes at least 16 species of the genus (Brown et al., 2022). Therefore, DNA barcoding of Scoliid Wasps (Hymenoptera: Scoliidae) seeks to help in identification of the Taxonomic classifications of the given species which is not just based on its phenotypic characteristics but is also based on its genetic composition on a molecular level.

Species that underwent DNA barcoding were assigned with machine-generated barcode index numbers (BINs) after the aforementioned barcoding process (Kjærandsen, 2022) on a DNA Barcoding Laboratory (DBL) in the Philippines located at UP Diliman. So far, approximately only few species of fauna have undergone DNA barcoding such as the Invasive Leafminers (Diptera: Agromyzidae) in the Philippines (Scheffer, et al., 2006). Since DNA barcoding is a more accurate tool for the identification of species, the current research study shall put emphasis on the further utilization of the DNA barcoding method in Scoliid Wasps. Furthermore, the objectives of the study was focused on- 1) the characterization of the Scoliid wasp collected from selected site in Luzon, Philippines and the 2) determination and comparison of the identity of Scolia spp. using mitochondrial DNA (COI).

II. MATERIALS AND METHODS

A. Collection Sites

Scoliid wasps specimens were collected in six different sites from Camarines Sur, and three sites from Nueva Ecija. Collections sites were usually composed of a large mass of decomposing organic matters from various plant materials and animal dungs, a favorable habitat for scarab grubs.

B. Collection, Storage, and Sending of Specimens

Scoliid adult wasps were collected from August 2019 to December 2019. The specimens were collected early in the morning using a sweeping net while they were cruising on the ground or visiting the flowers of Alagaw (Premna spp.). Each collected scoliid wasp are placed in individual containers filled with 95% ethanol. Each container was tagged properly and brought to the laboratory for morphological diagnosis. From the sample collected, every 5 individuals in each site were taken for DNA analysis. These samples were stored in the refrigerator at four-degree Celsius and later sent to the Philippine Genomic Center at the University of the Philippines Diliman, Quezon City for DNA extraction, PCR, and DNA sequencing.

C. Data to be Gathered:

a. Site description and Scoliid wasp activity

c. Gel electrophoresis pattern of amplified DNA.

d. BLAST result.

III. RESULTS AND DISCUSSION

Observation and Collection of Scoliid Wasp Specimens

As shown in Table 1 the collection sites and number of individuals of both male and female adult Scoliid wasp specimens were collected from the decomposing organic materials. Among the identified organic materials, the highest population were detected from compost heaps in all sites while few numbers of individuals were collected from Alagao flowers.

Table 1: Number of adult individuals and habitat/substrates of *Scolia spp.* population observed and collected from August 2019 to April 2020.

COLLECTION SITE	MALE: FEMALE RATIO	NUMBER OF SPECIMENS	HABITAT
Camarines Sur	14:22	36	-
San Felipe, Naga City	8:12	20	Composted heap/flowers
CBSUA Pili Cam. Sur	4:12	16	Composted leaf litters
San Anres, Candelaria	6:6	12	Composted Leaf Litters
CLSU, Munos, Nueva Ecija	10:16	26	Composted leaf Litters

Scoliid wasps are very important natural agents in the control of green June beetle grubs in the soil. This wasp is noticeable early in the morning and sometimes were seen resting and curled around a plant stalk. Adult wasps were collected during flight near the composed heap using sweeping. Some were gathered from alagaw (Premna sp.) flowers during nectar feeding while some adults were observed busily in gathering nectar from flowers. This behavior of adult starts to feed immediately after mating while the males increase their activity in search of other females (Lopez Pacheco 1984).

Figure 1: A and B are unidentified species of Scarab larvae parasitized by *Scolia sp.* while C is an adult Scoliid Wasp

The wasp was observed flying just a few inches above the ground across the composed heap and soil in search of their host which is the grubs. Once the grub was detected, it started to digs through the compost and soil burrowing its own tunnel or following one made by the grub. According to Barbara and Barratt (2003) that once a grub is located, the female will sting it on the throat and paralyze it. Then the female wasp will lay an egg transversely on the third segment of the grub. The paralyzed grub provides a fresh food supply for the wasp larva after it hatches from the egg. Parasitoids allow the paralyzed host to continue its development and often do not kill and consume the host until the host has reached its maximum size. Adults are most active at in the morning.

Majority of the collecting habitats of wasps found are areas with decomposing leaf litters and other organic materials while some numbers of individuals were collected while visiting on the flower. It was observed during this study, the organic wastes found in the areas were the major determinants of the beetle population and was considered as the most favorable breeding sites for the scarab beetle as scoliid larval hosts (Figure 2). The accessibility of organic materials as food and the richness of nutrients strongly support the existence of scarab for both adult and immature. A pile of rotting wastes, animal manures, compost, rotten palm, and sawdust are the suitable breeding ground for scarab beetle (Bedfort, 1980 & 2013, Zelany, 1969, Manjeri, 2014, Wan et al. 2009 & Moore 2013), Also, adult female scarab beetle lays its eggs in decomposing piles, decayed leaves, and wood debris or in a concealed place rich with organic materials (Sanders et al. 2015). In fact, Norman et al. (2001) found that majority of adult beetle was detected in the decomposed trunk and crown heaping. This may be due to the attraction of the beetles to the volatile compounds produced by the decaying biomass as well as the aggregation pheromone, ethyl – 4 – methyloctanoate, produced by males which was used to trap beetles of both sexes (Jackson et al., 2003).

Diagnosis of Collected Adult Scoliid Wasps

The collected scoliid wasps had a fairly large, stout body with lengths ranging from 16.5 to 19.73mm. The entire antenna is light red; the rest of the integuments black, the abdomen with faint blue reflections. The wings are black with purplish reflections. Abdomen with advanced stinging apparatus. Most of these findings are similar to Scolia (Discolia) vollemhoveni (Saussure) that were reported to occur in Sri Lanka, India, East Indies, Philippines, Indonesia, Malaysia, and Myanmar (Burma) (Gupta & Jonathan, 2003; Krombein, 1978).

Identification of Scoliid Wasps using mitochondrial COI gene

BLAST (www.ncbi.nlm.nihgove/genebank/) results (Table 2) showed that all the collected samples from the nine sites were related to the genus Scolia but they are not per se S. (D.) vollemhoveni. Instead, three samples from Camarines Sur (1 CBSU, AB969812; 2 CBSUA, AB969812; 4 CBSUA, AB969812), and one sample from Nueva Ecija (2 NE, AB969812) were related to S. kuroiwae based on percent Identity. 3 CBSUA (GU596820), 5 CBSUA (GU596820), 6 CBSUA (GU596820), 1NE (GU596820), and 3NE (GU596820) were related to *Scolia verticalis*. The degree of similarity up to which sequences are grouped is known as the cutoff or threshold and needs to be set to a level appropriate for the species level (Chesters & Zhu, 2014).

Table 2: BLAST Results yielding >88 – 97.8% nucleotide identity with *Scolia spp.* collected in selected sites in the Philippines using cytochrome c oxidase I nucleotide sequences.

Sample	Accession	Description	% Identity	E Value
1 CBSUA	AB969812	*Scolia kuroiwae*	97.2	0
2 CBSUA	AB969812	*Scolia kuroiwae*	94.9	0
3 CBSUA	GU596820	*Scolia verticalis*	87.7	0
4 CBSUA	AB969812	*Scolia kuroiwae*	95.5	0
5 CBSUA	GU596820	*Scolia verticalis*	88.0	2.06e-95
6 CBSUA	GU596820	*Scolia verticalis*	90.1	0
1NE	GU596820	*Scolia verticalis*	91.8	0
2NE	AB969812	*Scolia kuroiwae*	97.8	0
3NE	GU596820	*Scolia verticalis*	88.8	0

Sequence similarity employed in many barcoding and metabarcoding studies of insects depends ideally on the taxa being analyzed, but in general, it varies from 95 – 99% (Zenker et al., 2020). As for the species level, the common cutoff used by previous studies such as Marullo et al. (2020), Zenker et al. (2020), Alberdi et al. (2017), Karthika et al. (2016), and Ratnasingham and Hebert (2013), Meier et al. (2006) were ≥97%. From the comparisons between the sample sequences and Gene bank, only 1 CBSUA and 2NE samples passed the percent similarity cutoff. The percent identity or similarity of 1 CBSUA and 2NE samples to S. kuroiwae was 97.2% and 97.8%, respectively. The results of the study showed that although the specimens collected in each site were similar in appearance, they however are not under the same species suggesting a diversity among the Scolia species of Scoliids in Luzon, Philippines.

IV. CONCLUSION

Scoliid wasps are important natural agents in the control of scarabaeoid larva in the soil. Results suggest the presence of scoliid wasp species *Scolia kuroiwae* in the Philippines, particularly in Nueva Ecija and Camarines Sur. Aside from that, the results also suggest a species diversity of the genus Scolia in the country which could be possibly discerned using mitochondrial DNA barcoding.

V. RECOMMENDATIONS

Based on the results of this study, the following recommendations are generated:

1) A comprehensive study of the population genetics using other mitochondrial and nuclear genomes compared with other locations in the Philippines must be done for further confirmation of possible new species;

2) A comprehensive morphometric analysis and comparison of the specimen should be conducted; and

3) Identification of the host species and by specific parasitic scoliid wasp.

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