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Chrysosoma nellyae (Diptera: Dolichopodidae), a New Fly Species Discovered from Bohol Island, Philippines

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ABSTRACT

A new fly species, *Chrysosoma nellyae*, has been recently discovered in Bohol, Philippines. Aside from the metallic blue head with black antennae which are reminiscent of *Chrysosoma* species, its thorax is metallic blue with a greenish and brown prescutellar patch, scutellum brown, blue green border with hair like bristle. In addition, based on the similarity of the male genitalia, cercus morphology, and legs, the new species appears to be related to *C. crinicorne.* To increase the accuracy of species identification, DNA barcoding of the mitochondrial cytochrome oxidase I gene was employed and further inferred for its phylogenetic relationship with other *Chrysosoma* species.

Key words: Diptera, *Chrysosoma*, phylogenetic analysis, DNA barcoding, Bohol Island.

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INTRODUCTION

Dolichopodidae, or the long-legged flies are known to be the largest dipteran family with about 7,500 species being described globally (Bickel, 2009). *Chrysosoma* (Dolichopodidae) is a large and complex genus of the Old-World tropics where major species groups are defined (Bickel & Lian-Meng, 1996). There are 84 dolichopodid species recorded from the Philippines, of which only 36 species have their type locality in the country (Ramos, Meier, Nuneza, & Grootaert, 2018). According to Delfinado and Hardy (1969), at least ten species of *Chrysosoma* were reported and described by Frey (1925) in the country, and three of these were found in Leyte, Samar, and Dumaguete. Ramos et al. (2018) listed 12 species of *Chrysosoma* in the country. Considering the underestimation of the true diversity in the Philippines based on a global inventory of Dolichopodidae (Yang, Zhu, Wang, & Zhang, 2006), we explored the species diversity in Bohol Island, a mega-biodiversity hotspot in the Philippines, which has never been dealt with comprehensively. Using morphological and DNA barcoding approaches, we report a new fly species collected in the forests of Rajah Sikatuna Protected Landscape (RSPL) of Bohol Island.

MATERIALS AND METHODS

Taxonomy

With permission to sample and later an issuance of a gratuitous permit from Department of Environment and Natural Resources-Region VII (Wildlife GP 2019-11, series of 2019), collections were made at the Magsaysay Park, Bilar, Bohol with malaise traps deployed in areas with minimum disturbance: Trap 1 (9.70431°N, 13 24.1239 E) and Trap 2 (9.70359°N, 124.1252°E) from June to August 2016. The traps were allowed to remain open during the entire sampling period and the bottles containing 70% ethanol alcohol were changed every week. Collected specimens were sorted into various families and placed in separate vials with screw caps and labels/codes for molecular work. Sorting of specimens was based on the guidebook by Oosterbroek (1998) and verified by taxon-specific experts from the National University of Singapore (NUS).

Images of *C. nellyae* were acquired using a Dun Inc. Passport II microphotography system, fitted with a Canon 65mm 5X MPE lens. The images were compiled using Zerene Stacker and digitally processed using Photoshop CS5.

To confirm new species identification, taxonomic experts at NUS compared the undetermined specimens with specimens from a digital reference collection, a physical reference collection of specimens used for taxa identification that needs to be identified routinely by taxonomists from different backgrounds and updated identification tools for dipterans (Ang et al., 2012).

Molecular data

DNA extraction and sequencing

DNA extraction from the entire leg of a sample specimen was done using QuickExtract™ DNA Extraction solution (Lucigen) and based on the methods of Wong et al (2014). Primer pairs (IDTdna) used to target the mitochondrial COI gene for PCR amplification were as follows: Forward: GGWACWGGWTGAACWGTWTAYCCYCC and Reverse: TAWACYTCWGGRTGNCCRAARAAYCA. PCR conditions were set at the following: initial denaturation at 95°C (3 mins); 1 cycle of 94°C (1 min), annealing 47°C (1 min), and 72°C (1 min), followed by 40 cycles with final extension set at 72°C (5 mins). The PCR products were purified using Sure Clean (Bioline), quantified in equimolar ratios using Nanodrop (Quiagen), and pooled prior to library preparation and Next-Generation Sequencing with Illumina MiSeq and HiSeq 2500 sequencing platforms. Sequencing libraries were prepared by AITbiotechusing the TruSeq Nano DNA Library Preparation Kit (Illumina), according to the manufacturer's protocol. Illumina MiSeq runs were provided by AITbiotech with the use of MiSeq Reagent Kit v3 (2 X 300 bp read lengths) while HiSeq runs were provided by SCELSE with HiSeq 2500 System and Rapid SBS Kit v2 (2 X 250 bp read lengths). The respective partial sequence of the COI mitochondrial DNA has been submitted to NCBI GenBank for data referencing with Accession No. ON023659.

Phylogenetic analysis

The DNA analysis pipeline was based on the method of Meier, Wong, Srivathsan, & Foo (2016). Paired-end reads were merged using PEAR 0.9.6 (Zhang, Kobert, Flouri, & Stamatakis, 2014). Reads from each PCR product were assigned to their corresponding specimen using a uniquely labeled primer pair, and the dominant read was identified as the specimen barcode. A Python script was implemented (Srivathsan & Meier, 2012) to demultiplex the data, count the number of reads per sample, identify and group identical reads of these amplicons into sets, identify the dominant set of reads and combine it with otherwise identical length-variants, and counting the number of reads in the largest identity set, and comparing it with the count of the set with the second-highest number of reads. For purposes of quality control, barcoding of a particular sample was successful if: (i) the total read count was > 50x, (ii) the total barcode count was > 10x, and (iii) and the most dominant read was at least five times that of the second most dominant read (Meier et al., 2016).

The sequences were then used for NCBI-BLAST (Basic Local Alignment Search Tool) to search for sequences that match (>97% identity) the taxa. The top twenty specimens with the highest percentage match (86-88%) were retrieved for phylogenetic analyses. All retrieved sequences were aligned using MAFFT version 7 (https://mafft.cbrc.jp/alignment/software/). Phylogenetic inferencing of the aligned sequences was done using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Evolutionary relationships of each taxon were inferred using the Neighbor-Joining method (Saitou and Nei 1987) with 1000 bootstrap support. Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei, & Kumar, 2004).

RESULTS AND DISCUSSION

Chrysosoma nellyae sp. nov.

Type material. Philippines, island of Bohol, province of Bilar, Magsaysay Park, 295 meters asl, 9.70431°N, 13 24.1239 E, 08.30.2006, 1♂ (Fig. 1), leg. RP Jose, Entomology Laboratory, National University of Singapore (after the photo was taken, the specimen was stored dry, withered, and stored in a collection).

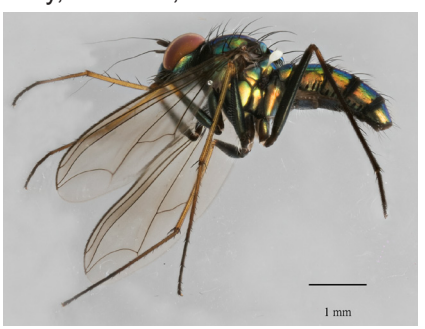

Fig. 1. Images of a new fly species, *Chrysosoma nellyae,* discovered from Bohol Island**.** Holotype, male (P20F99R24) showing the (A) dorsal and (B) lateral habitus of the specimen. Scale bar was set at 1 mm.

Dimensions. Body Length: 4.0-5.0 mm, Height: 5.0-6.0 mm, Wings: 10.0-11.0 mm.

Description. Male. Atenna: Arista with basal segment black, base of apical aristal segment pale becoming black towards tip. Palpus light brown elongate with bristling. Head: brightly metallic blue with yellowish metallic near antenna; Thorax: is metallic blue. with a greenish and brown prescutellar patch. Scutellum brown, blue green border with hair like bristle. Wings: Its spotless hyaline wing, length of 4.5 mm, has dark brown cross veins; Abdomen: With first tergite to last tergite is metallic blue in color with blue green and yellow brown marginal patch at the center. Legs: Black femur with shades of metallic blue; light brown tibia; dark brown tarsus; and long slender legs, relative length of foreleg (femur: 3.3 mm; tibia: 3.5 mm; tarsus: 3.1 mm); mid leg (femur: 4.0 mm; tibia: 5.5 mm; tarsus: 3.5 mm) hind leg (femur: 4.5 mm; tibia: 6.0 mm; tarsus: 2.5 mm). An image of the index species is shown in Fig. 1.

Diagnosis. Ratio of length to height of 1st flagellomere to length of arista, 0.8: 0.8: 13.4. Ratio of epistome to clypeus height, 28: 22. Four or five dorso-central setae. Fore coxa yellow; middle and hind coxae dark. Middle tibia and tarsus without erect pubescence and long setae. Length ratio of fore tibia to tarsus (segments from first to third), 125: 89: 19: 15. Length ratio of middle tibia to tarsus (segments from first to fourth), 115:39:26:12: 10. Length ratio of hind tibia to tarsus (segments from first to fifth), 225: 104: 39: 26: 15: 11. Wing without distinct spots. Ratio of parts of costa between R2+3 and R4+5 to those between R4+5 and M1, 21: 6. Ratio of crossvein m-cu to apical part of CuA 1, 53: 30. Ratio of apical part of M1 +2 (fork-handle) to M2, 61: 37. Cross vein m-cu sinuate; M1 gently curved; M2 poorly developed. Anal lobe well developed; anal angle acute. Length: body 5.1 mm, wing 5.3 mm. Morphologically,

the unidentified species is related to *Chrysosoma crinocorne*. Nonetheless, this new species differs morphologically with thorax is metallic blue with a greenish and brown prescutellar patch. Scutellum brown, blue green border with hair like bristle.

Molecular characterization

To further confirm the identification of the unknown Diptera, DNA barcoding was conducted using mitochondrial CO1 as the gene marker. A partial sequence of 342bp from the CO1 region was amplified and compared to other dipteran DNA sequences. Two DNA-sequence based approaches were undertaken for species discrimination and identification namely, the "best match" approach where the DNA sequence is directly compared to all species barcodes and the tree-based identification (Meier, Shiyang, Vaidya, & Ng, 2006; Blaxter et al, 2005; De Salle, Egan, & Siddall, 2005; Barrett & Herbert 2005).

For the "best match" approach, the DNA sequence of the unknown species was queried for exact match or highly similar sequences in the NCBI GenBank database using the BLAST search engine. Based on the results, there were no exact matches. The closest and most similar known sequence is *Chrysosoma crinocorne* sharing only 87% similarity to the unidentified species. The 13% variation in its DNA sequence is significant enough to indicate that *Chrysosoma nellyae* is new compared to the collection of DNA barcodes in the database.

To further discriminate and identify *Chrysosoma nellyae* using its DNA barcode, tree-based identification approach using the Neighbor-Joining tree was applied. An unidentified species or query sequence is considered successfully identified when it clusters to conspecific sequences. Clustering sequences by similarity utilizes pair wise distances. Thus, we retrieved and analyzed 20 other mitochondrial COI sequences of different fly species sharing the highest similarity with our unidentified species.

Fig. 2 displays a phylogenetic tree with 20 of its closest species that share 86-87% similarities to illustrate the evolutionary relationships within the taxa. Our result of the Neighbor-Joining method with 1000 bootstrap support further confirms that the unidentified species is closely related to *Chrysosoma crinicorne* with bootstrap support of 46% and shares high relatedness to *Chrysosoma vittatum* clustering as one group. Both *C. crinicorne* and *C. vittatum* have been documented to be present in the Philippines together with the other 10 recorded *Chrysosoma* species (*C. annuliferum*, *C. chrysoleucum*, *C. excitatum*, *C. fistulatum*, *C. fusiforme*, *C. pelagica*, *C. philippinense*, *C. proliciens*, *C. schistellum*, and *C. terminatum*) (Yang et al, 2006). Unfortunately, only *C. crinicorne* and *C. vittalum* have partial COI DNA sequences deposited in NCBI for molecular identification both emerging to share a high similarity as well as relatedness to *Chrysosoma nellyae*. Although it is expected that *Chrysosoma bearni* should have clustered together with the rest of the *Chrysosoma* species, *C. bearni* was never recorded to be found in the Philippines; thus, it may have variable sequences or morphological phenotypes that are not reminiscent of Philippine *Chrysosoma* species. Numbers next to branches of the Neighbor-Joining tree indicate the percentage of replicate trees where associated taxa clustered together in the bootstrap test.

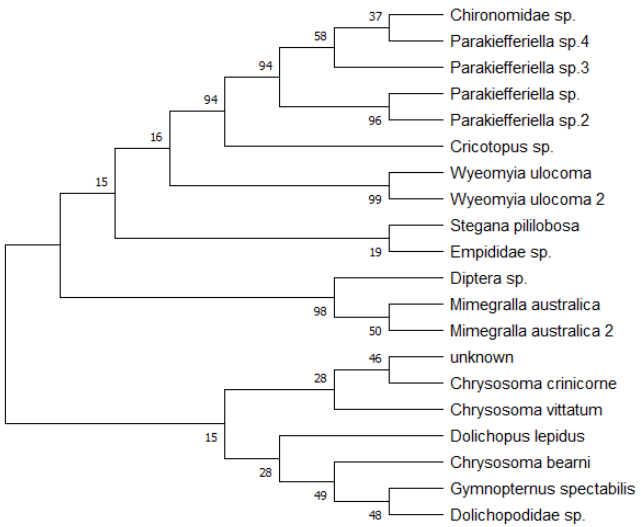

Fig. 2. Phylogenetic analysis using MEGA X. The top twenty species sharing the highest similarity to the DNA sequence of *Chrysosoma nellyae* sp. nov. were inferred for phylogenetic analysis using the Neighbor-Joining method with 1000 bootstrap support. *Chrysosoma nellyae* sp. nov. is more closely related to *Chrysosoma crinicorne* with a bootstrap support of 46% and shares high relatedness to *Chrysosoma vittatum* clustering as one group. Numbers next to branches indicate percentage of replicate trees where associated taxa clustered together in the bootstrap test.

Thus, by combining the morphological descriptors with molecular characterization, the unidentified fly species is undoubtedly classified under the genus *Chrysosoma* and is a new species ubiquitously abundant in Bohol Island, a mega-biodiversity hotspot in the Philippines. The insect will be the 13th species of *Chrysosoma* discovered in the Philippines.

Etymology. The species name is in memory of the mother of RPJ.

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