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MOLECULAR IDENTIFICATION OF CARPENTER BEE *XYLOCOPA PUBESCENS* SPINOLA FROM CENTRAL SULAWESI, INDONESIA

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ABSTRACT

***Xylocopa pubescens* Spinola was identified at subgenus level by its external morphology. This can be easily differentiated by the mesosoma covered dorsally with yellow hairs. Males are smaller than females, and they can be distinguished by a narrow head and yellow pubescence covering their entire body. An individual from Central Sulawesi was successfully sequenced for the barcode region of mitochondrial cox1. Sequence length was 673 bp (0% ambiguities) with no evidence of stop codons. Comparison with the DNA barcode library using the BOLD ID Engine resulted in a 99.61% of similarity with an exemplar of *X. pubescens*. NCBI Blast resulted in 93.46% of similarity with the related species *X. aestuans*.**

Key words: Apidae, carpenter bee, Central Sulawesi, COI, diversity, DNA barcoding, identification, Indonesia, insect, pollination, taxonomic, *Xylocopa pubescens*

Xylocopa are distinguished by their huge size (13-30 mm) and strong jaws, which they use to cut the corolla of tubular flowers in order to extract the nectar. They also have a preference for building their nests on trees and wooden buildings. The genus contains 469 species that are divided into 38 subgenera. It is found all over the world, with tropical and subtropical regions having the highest levels of diversification. The genus has seven species in Europe and six species that are known to exist in North Africa. Due mostly to wood imports, a number of *Xylocopa* species have been introduced into island (Okabe, 2010) and continental (Dahlberg et al., 2013) ecosystems. *Xylocopa sonorina* Smith, 1874, for instance was brought to a number of Pacific islands (Barrows, 1980). More recently, Japan has reported *Xylocopa tranquebarorum* (Swederus, 1787) for the first time (Okabe, 2010). The vulnerability of island ecosystems to invasive species, which pose the biggest danger to island invertebrates (Leclerc et al., 2018), can intensify these effects in insular ecosystems. The lowers costs and possible damage, early detection of invasive species is therefore one of the most crucial steps to assure the success of control or eradication programs. Regardless of an exotic species propensity for invasion, citizen science has shown to be a useful method for early identification in this setting (Thomas et al., 2017; Poland and Rassati, 2019). This study reports the discovery of *Xylocopa pubescens* Spinola from Central Sulawesi, based on evidence validated by DNA barcoding.

MATERIALS AND METHODS

The carpenter bee examined were collected from Central Sulawesi, Indonesia during 2023. Materials examined: 1 examples, Registration Number: ENTO/UNTAD-0001, collected by: Rocky Reviko T. Lembah and Manap Trianto, Adults: ♀ small-sized; body length 4.2 cm. For DNA barcoding, the specimens were collected and preserved in 95% ethanol and kept at -20°C till further use. In order to confirm the identification, DNA from a single leg of a captured individual was extracted and amplified in a single step using Phire Animal Tissue Direct PCR Kit (Thermo Scientific) and standard barcoding primers (LCO1490 and HCO2198). PCR amplification conditions were 2 min predenaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 50°C for 30 sec, and extension at 72°C for 30 sec. A final extension of 5 min at 72°C was performed (Arisuryanti et al., 2020). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. This analysis involved 33 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 617 positions in the

final dataset. Evolutionary analyses were conducted in MEGA X.

RESULTS AND DISCUSSION

***Xylocopa pubescens* (Spinola, 1838)** (Fig. 1)

Xylocopa pubescens was identified at subgenus level by its external morphology. From this last species, *X. pubescens* can be easily differentiated by the mesosoma covered dorsally with yellow hairs. Males are smaller than females, and they can be distinguished by a narrow head and yellow pubescence covering their entire body (Fig. 1). However due to morphological similarities with related species such as *X. modesta* Smith, 1854 from Cape Verde, *X. aestuans* Linnaeus, 1758 from Southeast Asia or *X. appendiculata* Smith, 1852 (introduced in North America), a molecular identification through barcode was accomplished. An individual from Central Sulawesi was successfully sequenced for the barcode region of mitochondrial cox1. Sequence length was 673 bp (0% ambiguities) with no evidence of stop codons. Comparison with the DNA barcode library using the BOLD ID Engine resulted in a 99.61% of similarity with an exemplar of *X. pubescens*. NCBI Blast resulted in 93.46% of similarity with the related species *Xylocopa aestuans*.

The occurrence of *X. pubescens* in the island of Sulawesi can be explained by two alternative scenarios. It may have arrived in a shipment of wood in the port. This hypothesis is supported by its wide distribution around the port area of the capital. Wood- or stem-nesting bees, such as those of the family *Megachilidae* or the genus *Xylocopa*, are known to disperse further by transported nests to isolated islands than by flight (Poulsen and Rasmussen, 2020). Globalization has accelerated this process, thus favouring the introduction of species as carpenter bees that nest in wood or other commercial substrates. In the last decade, several exotic wood nesting bees have been reported in the Sulawesi Islands such as *Xylocopa confusa* Latreille and *X. latipes* Drury (Windarsih and Trianto, 2021). Alternatively, it's possible that *X. pubescens* came by itself. Due to its adaptation to xeric circumstances, *X. pubescens* is presumably increasing its native range. Over the past ten years, it has colonized two separate locations, most likely in response to changes in the global climate. Thus, it is impossible to rule out a natural spread to the Sulawesi Islands. The species discovery in a remote area in central Sulawesi may also be explained by this procedure.

Fig. 1. *Xylocopa pubescens* Spinola

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AUTHOR CONTRIBUTION STATEMENT

Contributed in molecular confirmation (IMB, MM, FD) and Field surveys (MT).

CONFLICT OF INTEREST

No conflict of interest.

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