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Morphological and Molecular Characterization of *Psytalia* sp. (Hymenoptera: Braconidae); a Parasitoid of the Peach Fruit Fly *Bactrocera zonata* (Diptera: Tephritidae), in Sudan

Manal H. Elbashir¹, Sumaia M. Abukashawa² and Mohamed E. E. Mahmoud^{1,*}

¹Agricultural Research Corporation (ARC), IPMRC, National Insect Museum, Wad Medani, Sudan

²Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan

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ABSTRACT

The fruit fly *Bactrocera zonata* (Diptera: Tephritidae) is a serious pest of horticultural crops that causes severe losses to the production and affects adversely economic and hindered exportation. Different species of parasitoids were found very effective in controlling fruit flies in biological control programs. This study was carried out to characterize morphologically and molecularly *Psytalia* sp a parasitoid reared out from *B. zonata* in Sudan. The parasitoid was morphologically identified up to the genus level using specific key. Molecular analysis using quantified and amplified by Polymerase Chain Reaction (PCR) using mitochondrial cytochrome oxidase I (COI) gene. As primer gave 700 bp DNA fragment for test *Psytalia* species. Absence of *Psytalia* species from the entomological records of Insect Collection Unit, Agricultural Research Corporation points to the recent introduction of the wasps and necessity for more surveys of the native and introduced wasp parasitoid species in Sudan.

*Corresponding Author

Email: nazeerazo@yahoo.com

1. Introduction

Fruit flies (Diptera: Tephritidae) are among the most destructive insect pests affecting horticultural production worldwide, causing substantial economic losses through direct fruit damage, reduction in fruit quality, and restrictions on international trade [1-4]. Among these pests, the peach fruit fly, *Bactrocera zonata* (Saunders), has emerged as an important invasive species in Africa and the Middle East. The pest attacks a wide range of economically important fruits and vegetables including guava, mango, citrus, peach, and cucurbits, leading to severe yield reduction and market losses [5]. Since its introduction into Sudan in 2012, *B. zonata* has become a serious threat to horticultural production systems, particularly in irrigated agricultural regions such as Gezira State [6, 7].

The extensive use of chemical insecticides for fruit fly management has resulted in several environmental and economic concerns, including pesticide resistance, destruction of non-target organisms, environmental contamination, and residue accumulation on fruits intended for export markets [8-10]. Consequently, integrated pest management (IPM) approaches emphasizing environmentally friendly and sustainable methods have gained increasing importance. Among these approaches, biological control using parasitoid wasps has proven highly effective in suppressing fruit fly populations under both natural and managed ecosystems [11].

Parasitoid wasps belonging to the family Braconidae are among the most important natural enemies of tephritid fruit flies. The family Braconidae represents one of the largest and most diverse hymenopteran families, comprising more than 20,000 described species worldwide, with many additional undescribed taxa [12-14]. Members of the subfamily Opiinae are particularly important because most species are larval-pupal endoparasitoids of cyclorrhaphous Diptera, especially fruit flies [15]. Species of the genus *Psytalia* Walker have received considerable attention due to their effectiveness in biological control programs targeting invasive fruit flies [7, 16, 17].

The genus *Psytalia* includes numerous parasitoid species distributed across tropical and subtropical regions of Africa, Asia, and the Mediterranean basin [15]. Several species, including *P. concolor*, *P. humilis* and *P. cosyrae*, have been widely studied for their potential in suppressing populations of *Bactrocera* [12] and [18] and *Ceratitis* species [14, 19-21]. These parasitoids are characterized by their high host specificity, adaptability to different environmental conditions, and ability to establish stable populations in fruit-growing areas [11, 22, 23].

In Sudan, information concerning indigenous and introduced parasitoids associated with invasive fruit flies remains limited. The shortage of specialized taxonomists and the lack of comprehensive molecular databases for parasitoids have hindered accurate identification and documentation of parasitoid fauna. Traditional morphological identification remains fundamental in insect taxonomy; however, morphological similarities among closely related species often complicate accurate diagnosis [24, 25]. Therefore, the integration of molecular techniques with classical taxonomy has become essential for accurate species identification and phylogenetic characterization.

Molecular identification based on mitochondrial DNA markers, particularly the cytochrome c oxidase subunit I (COI) gene, has become a widely accepted tool for DNA barcoding and species discrimination in insects [26]. The COI gene possesses sufficient interspecific variation while remaining relatively conserved within species, making it highly suitable for distinguishing closely related parasitoid taxa [27]. Polymerase Chain Reaction (PCR)-based amplification of COI sequences has been successfully applied for the identification and differentiation of several species of *Psytalia* associated with fruit flies [28, 29].

The accurate identification of parasitoid species associated with *B. zonata* in Sudan is important for developing effective biological control programs and improving integrated management strategies against invasive fruit flies. Therefore, the present study aimed to characterize *Psytali* species associated with *B. zonata* in Sudan using both morphological and molecular approaches. The study also sought to provide baseline information on parasitoid diversity and contribute to future biological control research programs in Sudan.

2. Materials and Methods

2.1. Sample Collection

Guava fruits infested by fruit flies were collected from Fadasi and Gazirat Elfil in the Gezira State from June to August 2017 [21]. Infested fruits were kept in plastic containers 20x10x15 cm, containing sterile soil, and closed with cotton cloth and rubber bands to avail ventilation. The soil was moistened to keep suitable environment for the emergence of the puparia. After ten days, the soil was sifted to collect puparia and sort them out depending on their color. The puparia that showed signs of parasitism (black) were placed in 9 cm diameter Petri dishes, with wet soil, and kept in special transparent cages 30x30x30 cm at the Integrated Pest Management Research and Training Center (IPM) laboratory in the headquarters of (ARC) Wad Medani. Twenty samples of *emerged Psytalia* sp. were collected from the guava fruits. The emerged parasitoids were left for 24 hours to feed on honey and then collected by using an aspirator. Parasitoids were preserved in 70% alcohol (ethanol) in Eppendorf tubes. Preserved samples were used later for morphological and molecular identification.

2.2. Morphological Identification

Collected parasitoid specimens were investigated for their presence at the insect taxonomy unit. Parasitoid specimens were viewed individually under a dissecting microscope. Parasitoid specimens were identified depending on the morphological characters of wings, head, thorax, eyes, antennae shape, legs, and hind abdomen following the key of the hymenopteran species [24].

2.3. Molecular Identification

Whole genomic DNA of single insect was extracted from 17 specimens of *Psytalia* sp; that are morphologically similar, using a modified potassium-acetate following protocol described by [18]

2.4. DNA Amplification by Polymerase Chain Reaction (PCR)

Cytochrome Oxidase subunit one (COI) gene regions of the Genomic DNA of the samples of *Psytalia* sp was amplified using analytical thermo-cycler TAS™ (Thermo Assists, Pettier technology). The universal primer LCO1490 (5' GGTCAACAAATCATAAAGATATTGG-3') paired with reverse (HCO 2198) (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) were used for DNA amplification. DNA amplifications (PCR) were performed using ISTARtaq™DNA polymerase (5U/μl). Total reaction volume of 25μl contained: 2μl DNA template, 2μl (10x) PCR buffer (300mM Tris HCl (pH 9.0); 300mM salt containing K⁺ and NH₄⁺ w/20Mm Mg²⁺), 2μl dNTPs, 1μl forward and reverse primers, 0.3μl Taq Polymerase and 16 μl dd. sterile water. The reaction solution was put in the thermo-cycler machine programmed to 95°C for 5 minutes, followed by 35cycles of 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 45 seconds and a final extension at 72°C for 6 minutes. Amplified products were separated on 2% agarose gels (ACROS). Gels were stained using ethidium bromide and the PCR DNA was documented using UV documentation system (Ingenious/Syngeneic Bio Imaging).

3. Results and Discussion

3.1. Morphological Identification of *Psytalia* sp.

The parasitoid specimens collected from the infested guava fruits were identified morphologically as belonging to the genus *Psytalia* Walker (Hymenoptera: Braconidae: Opiinae). Identification was confirmed by [8] at the International Centre of Insect Physiology and Ecology (icipe), Kenya. Examination of the records available at the Insect Collection Unit of the Agricultural Research Corporation (ARC), Sudan, revealed that this parasitoid species had not previously been documented, suggesting either a recent introduction or an underexplored parasitoid fauna associated with fruit flies in Sudan.

The examined female specimens exhibited several characteristic morphological features typical of the genus *Psytalia*. Adult wasps were generally small, measuring approximately 2-5 mm in body length, with a slender body

and brownish-yellow coloration (Fig. 1). The antennae were filiform and composed of fewer than 35 antennal segments, a characteristic feature closely associated with *P. concolor*. The clypeus was relatively short and protruding, while the mandibles displayed the characteristic structure described for members of the subfamily Opiinae.



Figure 1: Morphological features of female of *Psyttalia* sp. showing natural colour.

The forewing venation pattern represented one of the most important diagnostic characters observed in the specimens. The second submarginal cell appeared relatively elongated, and the forewing stigma was broad and well-developed. The hind wing vein RS was absent or highly reduced, consistent with previously described diagnostic characteristics of *Psyttalia* species (Fig. 2 and 4). Furthermore, the vein located between m-cu and 2RS was distinctly thickened, while the m-cu vein originated basally or directly in line with 2RS.

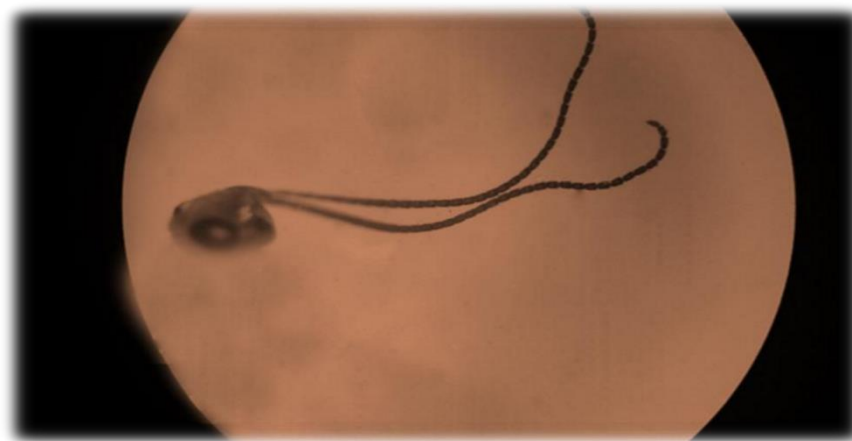


Figure 2: Distinguishing morphological features of *Psyttalia* sp., the antenna and antennal base.

The metasomal tergites provided additional important diagnostic features. The second metasomal tergum (T2) was distinctly shorter than the third metasomal tergum (T3), which clearly differentiated the specimens from other closely related genera such as *Opius* (Fig. 3). In addition, the hypopygium was elongated and strongly attenuated medially, a feature commonly reported in *Psyttalia* species. These combined morphological characters strongly indicated that the collected parasitoid belongs to the genus *Psyttalia* and shares close similarity with *P. concolor*.

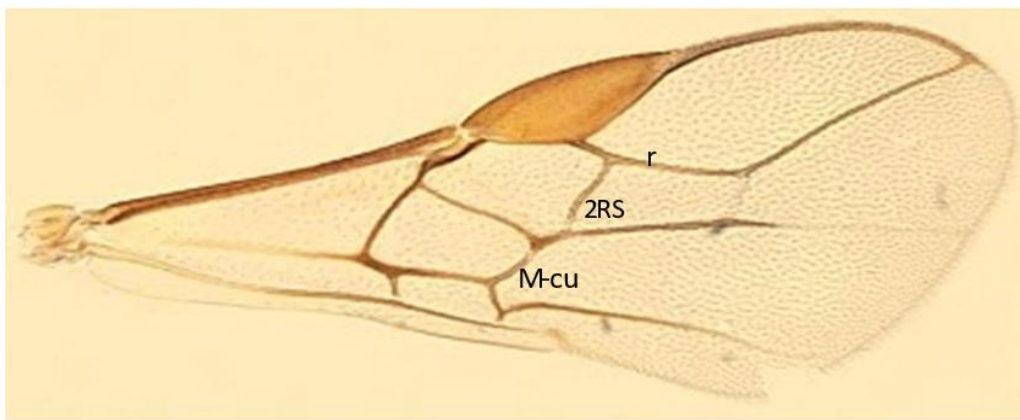


Figure 3: Morphological distinctive wings of *Psytalia concolor* showing forewing.

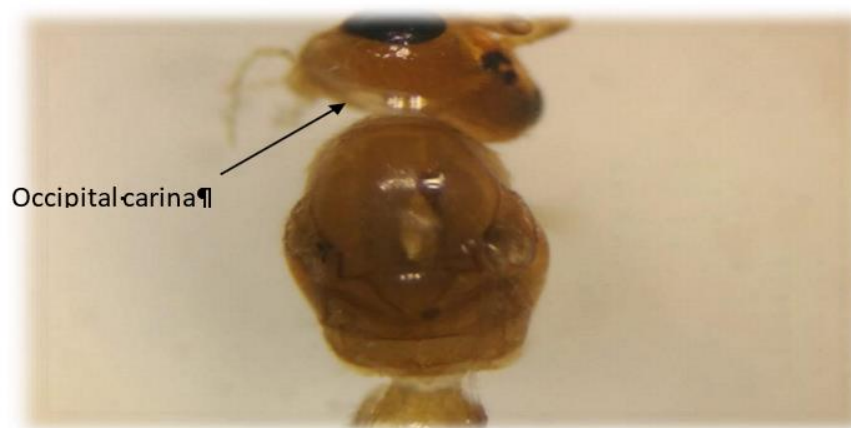


Figure 4: Anterior view of head capsule showing the occipital carina and shortened second metasomal tergum (T2) typical of *Psytalia* sp.

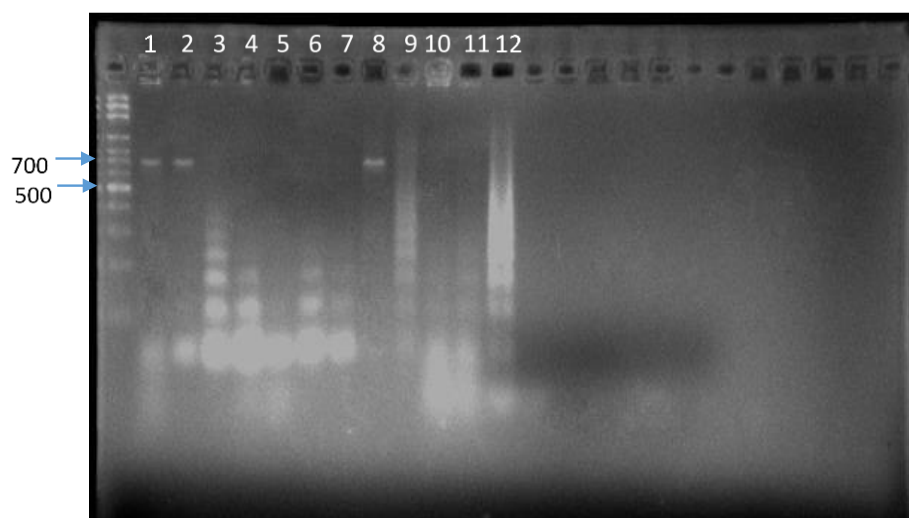
3.2. Molecular Identification of *Psytalia* sp.

A total of twenty PCR amplification trials were conducted using genomic DNA extracted from seventeen parasitoid specimens. Successful amplification of the mitochondrial cytochrome oxidase subunit I (COI) gene was achieved in three samples under the optimized PCR conditions described in the Materials and Methods section.

The universal primer pair LCO1490 and HCO2198 successfully amplified a DNA fragment ranging from approximately 700 to 710 base pairs (bp) (Fig. 5). The amplified products appeared as clear and distinct bands on 2% agarose gel electrophoresis, confirming successful amplification of the target COI region. The obtained fragment size was consistent with previously reported COI amplification products for *Psytalia* species and particularly *P. concolor* [16, 29, 30].

Among the tested samples, only three produced positive amplification products, while the remaining samples failed to generate visible bands. Alteration of the PCR conditions resulted in the absence of specific amplification products, indicating that successful amplification depended strongly on optimized reaction conditions. Nevertheless, the successful amplification obtained in the positive samples confirmed the suitability of the selected universal primers for molecular characterization of *Psytalia* species.

The molecular results supported the morphological identification and demonstrated the usefulness of COI-based PCR amplification as a reliable diagnostic tool for parasitoid identification. The combination of morphological and molecular evidence strengthened the identification of the collected parasitoid as a member of the genus *Psytalia*.



Lane 1, 100 bp DNA ladder standard.

Lanes 2, 8, 11 positive amplification products of COI gene.

Lanes 3, 4, 5, 6, 7, 9, 10, 12 negative amplification products of COI gene.

Figure 5: Agarose gel showing PCR amplification products of COI gene. The produced 700 bp bands were specific for *Psytalia* species.

The present study successfully characterized a parasitoid species associated with *B. zonata* in Sudan using both morphological and molecular approaches. The results demonstrated that the collected parasitoid belongs to the genus *Psytalia* (Hymenoptera: Braconidae: Opiinae), a group recognized worldwide for its important role in the biological control of tephritid fruit flies.

Morphological examination revealed several diagnostic characteristics that clearly distinguished the studied specimens from other members of the subfamily Opiinae. Among the most important characters observed were the shortened second metasomal tergum (T2), elongated second submarginal cell, broad forewing stigma, reduced hind wing venation, and attenuated hypopygium. These characteristics agree closely with the descriptions provided by [15, 28] for *Psytalia* species associated with fruit flies.

The observed morphological similarity between the Sudanese specimens and *P. concolor* suggests a close taxonomic relationship. Similar findings were reported by [20], who documented the effectiveness and widespread distribution of *P. concolor* and related species in biological control programs targeting tephritid fruit flies. The relatively low number of antennal segments and the characteristic wing venation observed in the present study further support this relationship.

4. Conclusion

To improve identification of insect species, further taxonomical work should be considered. This study paved the way for future research in parasitoid wasps in Sudan. The molecular identification is true additive and supportive to morphological identification. Its high time to conduct survey regarding natural enemies especially parasitic hymenoptera for their importance role in biological control programs in Sudan.

Conflict of Interest

The authors declare that they have no conflict of interest.

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