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Molecular Identification and Morphology of the Larva of Fig Stem Borer, *Trichoferus fissitarsis* Sama, Fallahzadeh and Rapuzzi, 2005 (Coleoptera, Cerambycidae) from Kurdistan Region, Iraq

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ABSTRACT

The fig stem borer *Trichoferus fissitarsis* Sama, Fallahzadeh, and Rapuzzi, 2005 (Coleoptera: Cerambycidae) considered the widely distributed and harmful fig insect in the Kurdistan Region-Iraq. Larvae are dangerous, and create large-diameter exit holes and tunnels that can pointedly reduce the tree's vitality. Incontrast to the adult beetle, there is little evidence of larval identification. During the survey, 15 larval specimens were collected. mtDNA sequence of the *COI* specimens was obtained and analyzed for the species reported in NCBI Gen Bank from Kurdistan Region- Iraq. Besides, local distribution, taxonomy, host plants, and economic impact of the species were demonstrated. Moreover, the last larval instar and pupa morphologically had been described. Trunk significance and dried wood as a possible pathway for damaging wood borer species were highlighted.

Keywords: Trichoferus fissitarsis, COI, Gen bank, Larva, Morphology.

1 Introduction

Cerambycidae form the most important family of predominantly xylophagous insects on the land and are most species-rich in the tropics with an estimated 5300 genera, and 36300 extant species [1, 2]. Presently eight subfamilies of Cerambycidae have been documented: Cerambycinae, Dorcasominae, Lamiinae, Lepturinae, Necydalinae, Parandrinae, Prioninae and Spondylidinae [3, 4]. Longhorn beetles are habitually related to Buprestidae (flat-headed borers) (>15000 described species), which are principally wood borers and diversely distributed [5]. Genus *Trichoferus* Wollaston, 1854 has 28 species. Only four species of this number have been reported in Iraq, namely: *T. fasciculatus* (Faldermann, 1837), *T. griseus* (Fabricius, 1792), *T. preissi* (Heyden, 1894), and *T. fissitarsis* Sama, Fallahzadeh & Rapuzzi, 2005 [6, 7, 8]. Cerambycid larvae, also known as round-headed borers are particularly wood borers, often tedious deep into inner tissues [9]. Most of its species have an essential role in wild forests and horticultural areas, especially in northern Iraq, where they bore the host plant stems and destroy wood constructions [10]. Habitually, their distribution is more abundant in the Oriental and Neotropical regions and slightly less extent in the Palearctic region [11, 12]. While some authors have previously conducted valuable studies on the Cerambycidae species in Iraq [10, 13, 14, 15], the Iraqi Cerambycidae checklist still needs updating, enriching, and introducing new species. Hence, surveillance, precise description of original species, and GenBank registering are required. Thus, the classification and phylogeny of Cerambycidae remain debated on many points, due to the problems of current morphological and molecular data, which suffer from taxon sampling inequity [16]. Iraqi fauna of longhorn beetles contains 64 species inside 33 genera and five subfamilies [17]. Accordingly, most new Cerambycidae species have been recorded in northern Iraq, recently new records have been added in the southern part (Sulaymaniyah Governorate) [18]. Adding new species of Cerambycidae across the Provinces of Iraq expands its geographical distribution. Therefore, the introduction of inherited registration and morphological characteristics of *T. fissitarsis* is needed to identify the right taxonomic position. This study aims to report this dangerous species based on elucidations of DNA sequences from the mitochondrial partial gene (*COI*), and morphological description which will help fig plantation farmers of Kurdistan region- Iraq.

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2 Materials and Methods

2.1 Collecting Specimens and Identification

A total of 15 Larvae were collected in the stalks and branches of a fig tree, *Ficus carica* L. 1753 in the gardens and orchards of Erbil Governorate, Kurdistan Region-Iraq during late April to late June 2023. Collected stems and trunks were transferred to the rearing cage and placed in field condition to allow adult emergence. Adult and larval specimens were identified using the available keys [19, 20, 21]. Specifically, the species were certified by the Cerambycid expert Dr. Pierpaolo Rapuzzi (Geneva Natural History Museum, Italy).

2.2 Genomic DNA Extraction and Amplification Process

Based on the manufacturer's protocol, genomic DNA was extracted from the larval tissue using the ZYMO-Quick DNA Tissue/Insect Micro-Prep Kit (USA) Catalog number D6015. The target size was set at 700 base pairs. *COI* gene segment was amplified using PCR with a total reaction volume of 50 µl, including 2xTaq DNA Polymerase Master Mix from AMPILIQON A/S, 10 pmol each of the forward and reverse primer primer C1J-1718, DNase-free water, and template DNA. Amplification was performed in a Bio-Rad PTC-200 Gradient Thermal Cycler, with an initial denaturation at 95 °C for 5 minutes, followed by 34 cycles of denaturation at 95 °C for 30 seconds, annealing at 46 °C for 30 seconds, and extension at 72 °C for 60 seconds, with a final extension at 72 °C for 10 minutes [22, 23]. The last product was run in 1.5% agarose gel of electrophoresis (Fig. 1), and upon observing a distinct band, the samples were purified via a NanoDrop 1000 spectrophotometer by Thermo Scientific that measured DNA concentration in 1 µl of the sample and then sequenced by the Macro Gene company from Republic of South Korea. Sequence results were submitted to the NCBI GenBank with the accession number OR268947.

2.3 Morphological studies

For the morphological illustration, the procedures of [24, 25, 26] followed, the larval body was detached and bubbled in 15% KOH aqueous solution till their structures moderated and melted fat bodies. Next, the structures were washed three times with distilled water and determined under a dissecting microscope (OPTIKA, Italy). Later, the mouthparts detached from the head along the posterior margin of the epistome, and the cuticle cuts along a pleural line from the thorax to the end of the body to separate pronotal and pro-sternal plates, leg rudiments, mesothoracic, and abdominal spiracles.

3 Results and Discussions

3.1 Molecular Identification

To authenticate the specimen's identity, an analysis of the mitochondrial *COI* gene was conducted through PCR amplification. The PCR assay revealed a 700 base pair band corresponding to the sample, which was observed through 1.5% agarose gel (Fig. 1).

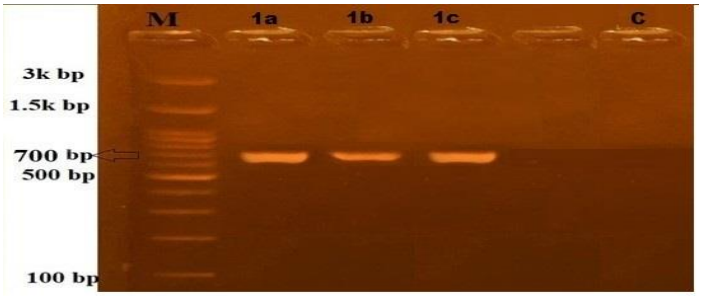

Figure 1: Agarose gel electrophoresis for PCR amplified COI gene, run by 1.5% agarose gel; Lane M represents ladder (100-3000bp). The lane1-5 amplified PCR products for the replicated sample of *Trichoferus fessitarsus*, and C is a negative control.

Sequencing was produced by Micro-gene Company in South Korea. Following this, a BLAST of the sequences was performed in the NCBI database. Analysis of BLAST results of sequenced DNA for the species presented a high similarity of 100% with the reference sequences that occurred in NCBI, and it was confirmed that the result matched with *Trichoferus fissitarsis*, corroborating the species identification based on pairwise genetic separation. This query sequence has been duly registered in NCBI GenBank, accompanied by a certain accession number (Table 1).

3.2 Phylogenetic Implication

The construction of a phylogenetic tree enables the determination of species relatedness through the application of molecular techniques. MEGA11 software was utilized based on the mitochondrial (*COI*) gene, which has frequently been employed due to its conserved nature and relatively rapid rates of evolution. Phylogenetic analyses based on DNA sequences rely on the assumption that closely related species share a more recent common ancestor than distantly related ones. By comparing DNA sequences, researchers can construct phylogenetic trees that depict the branching patterns of species, reflecting their evolutionary relationships [27]. This tree not only elucidates the evolutionary history of the Hesperophanini species but also provides a framework for understanding their taxonomic classifications, ecological interactions, and evolutionary processes. Through the neighbor-joining method, it was ascertained that the studied species is indeed *T. fissitarsis*. Concurrently, the tribe Hesperophanini, along with other related species, coalesced into a singular clade, showcasing a noticeable phylogenetic similarity and important divergence within the same genus's species lineages (Fig. 2).

3.3 Morphological Study

Material Examined: The specimens were collected from different locations of Erbil Province: Mergasor, Khalifan, Kore, Chiwa-Betwata, Shaqlawa, Gird-Jutyar, Ainkawa, Qushtapa, and Sebiran. All the larval specimens were categorized and grouped. The last instar larvae were chosen for morphological study.

Trichoferus fissitarsis: Sama, Fallahzadeh and Rapuzzi, 2005

Hesperophanes preissi: Khiri, 1974 (misidentification)

Table 1: Confirmation identity of *Trichoferus fissitarsis* according to a BLAST analysis correlated with GenBank records of the Mitochondrial *COI* gene fragment.

Insect Identified	Accession Number	Query Cover %	Identic Number %	Accession Number of BLAST Alignment	Insect Alignment	Country
Trichoferus fissitarsis	**OR268947**	100	84.73	FJ559059	*Dorysthenes pici*	Taiwan
		100	84	MN609297	*Oedemera amurensis*	Korea
		100	84	MN609298	*Oedemera amurensis*	Korea
		100	84	MN609299	*Oedemera amurensis*	Korea
		100	84	MN609300	*Oedemera amurensis*	Korea
		100	83.81	KM286000	*Prionus coriarius*	France
		100	83.27	MH157997	*Pimelia maura*	Germany
		100	83.27	MH157998	*Pimelia maura*	Germany
		100	82.91	MH925771	*Batocera lineolata*	China
		100	83.27	KF247262	*Batocera* sp.	China

Larval habitus: larval body relatively large and flat with an off-brown color, body length about 2.5 – 2.7 cm, width of pronotum 0.7-0.9 cm (Pl.1 A, B). Pronotal and prosternal plates are wide and moderately sclerotized (Pl.1 C, D). Head: wider than long with sclerotized epistome, hypostome, and pleurostome that surround antennae and mouthparts. Epistome: convex and rectangular shape, well sclerotized, almost 5 times as wide as long, frontal borders semi-globular, and marginally concave between condyles. The middle part carried two groups of stringy sensillae, each group consisted of two long sensillae and a single campaniform sensillum. A pair of long setae rise from the anterior edge of the epistome with a long seta behind the condyles near the antennal incision (Pl. 2 A). Hypostome: elongated, smooth, and bright, anterior margin brown, narrowed in the middle part, divided widely by vertical suture; it bears a campaniform sensillum and trichoid behind hypostome condyles. Antennae: three-segmented, basal segment cylindrical, and more expansive than long. The middle segment almost as long as wide; smaller than the basal segment, and marginally provides several short setae and a campaniform sensillum. The apical segment is tubular shaped and nearly twice times as long as wide, upper cavity holds two sizable and thin palmate sensillae with a large conical sensillum (Pl. 2 B).

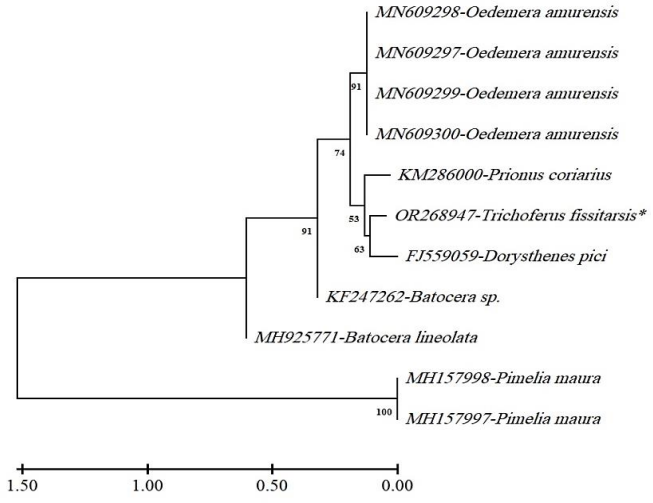

Figure 2: Phylogenetic tree of *T. fissitarsis* with its documented species; the Maximum Likelihood technique constructed on the (MEGA11) program and bootstrap exploration with 100 re-samplings and using DNA sequences of fractional mitochondrial gene COI as contribution information.

Ante clypeus: membranous and strongly transverse, wider than long, its anterior borders marginally arcuate toward the posterior part. Labrum: transverse, lateral branch of palatine sclerite strong and carries numerous long trichosensillae, anterior margin arched and provided with a row of dense and long setae (Pl. 2 C). Mandibles: triangular and strongly sclerotized, with three blunt and arched apical teeth; inner concavity where epicranial apophysis is articulated. Molar area is vast and slightly convex (Pl. 2 D). Prementum: obvious and bears six trichosensillae, mentum marked by basal pigmentation with long setae and sub-mentum slightly pigmented with two basal setae. Ligula: conically shaped and provided with long setae. Labial palp: two segmented with strong palpiger, basal segment larger than terminal one. Long apical trichosensilla with two short thin setae raised from its outer margin; terminal segment cylindrical and longer than wide with sensory cone (Pl. 2 E).

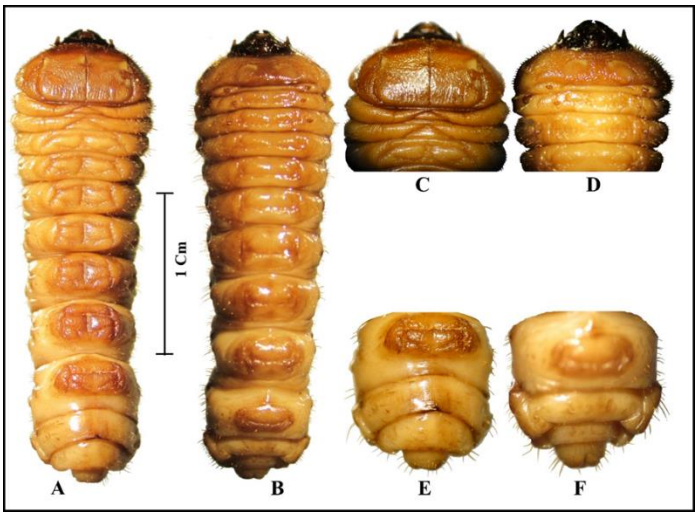

Plate 1: Larval body of *Trichoferus fissitarsis*;(A) Dorsal view, (B) Ventral view, (C) Pronotal plate, (D) Prosternal plate, (E) Dorsal view of the anal segment, (F) Ventral view of the anal segment.

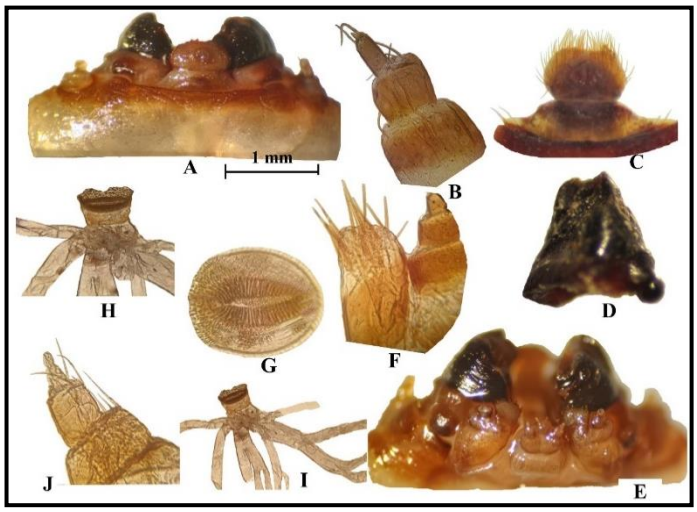

Plate 2: Larva of *Trichoferus fissitarsis*, A, Epistom; B, Antenna; C, Anteclypeus and labrum; D, Mandible; E, Labio-maxillary complex; F, Maxilla; G, Spiracle; H, Trachea of mesothoracic spiracle; I, Trachea of abdominal spiracle; J, leg rudiment.

Maxillae: cardo triangular and sclerotized; stipes arcuated with long lateral setae. Maxillary palps consist of three barrel-shaped segments, every segment wider than the next one with gradual contracting from bottom to upper. Terminal margin of segments surrounded by long sparse setae. Mala: cylindrical and strongly sclerotized, upper surface longitudinally expanded and provided with dense and long trichosensillae (Pl. 2 F).

Thorax (Pl. 1, C, D): Prothorax transversely elongate, with well-developed pronotal plate. Pronotal plate surface designed by short and sparse setae. Pronotal groove appears as a straight line. Episternum distinctly appears near the hind margin. The spiracle system is peripneustic without marginal chambers; mesothoracic spiracle functional, nearly 3 times longer than wide and 1.5 times wider than the abdominal spiracles, with the strongly branched trachea (Pl. 2 G, H). Abdominal spiracles are smaller and present on the dorsolateral sides of segments (1 - 8) only. Tracheal system is relatively narrower and feebly branched (Pl. 2 I). Leg rudiments present, coxal lobes developed on thoracic segments, trochanter not found, femur appears as two setae and tibia with one long and one short seta (Pl. 2 J). Meso and meta-thorax are ring-shaped with medial diverging and shorter than the pro-thorax and abdominal segments. Tergum surface is rough, with several short setae.

Abdomen; relatively large and dorsally has more retractile and often structurally sculptured; protuberances are maintained in tunnels; abdominal segments (1 - 7) with dorsal ampullae. The segments 6^{th} - 10^{th} gradually decreased in size. Each segment provided a moderately developed dorsal fold. Last abdominal segment is round, smallest, and opened by three radiated anal clefts (Pl.1, E, F).

Pupa: Exarate type, with bright yellow color, length about 2-2.3 cm. Head: without setae, c1ypeus, and eyes distinctly developed. The antennal tubercles are poorly distinct, with basal grooves. Flagellum: fitting backward, overcoming the metathorax to the first abdominal segments. Thorax: pronotum rounded, transverse and soft. The posterior margin with short tubercle fit retrograde. Mesothorax: transverse and dorsally arcuated. Elytra theca is long without setae and procurved behind forelegs (Pl. 3, A). Metathorax: slightly transverse and longer than mesothorax. The wing theca is smooth without setae and bent behind meddle legs. Ventral side is smooth and covered by the antenna, legs, and wing theca (Pl. 3, B). Abdomen: consists of nine visible segments. Segments 1– 6 transverse with short setae, and the segments 6^{th} to the end of the body tapered moderately. Last abdominal segment is round and visible on the ventral side; functional spiracles also have been noticed on the plural sclerites with the same numbers as in larva but marginal chambers had not developed entirely.

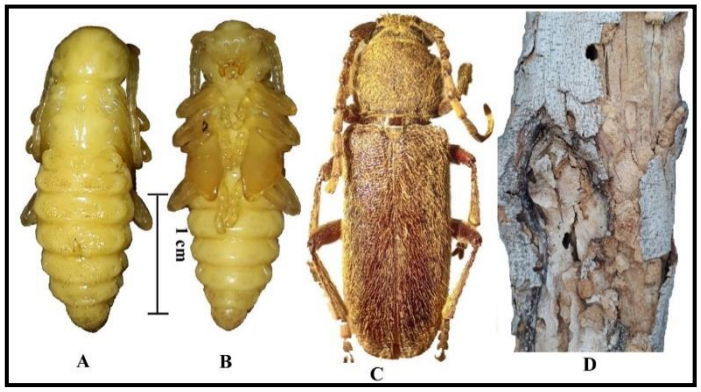

Plate 3: *Trichoferus fissitarsis*, A, Pupa in dorsal view; B, Pupa in ventral view; C, Adult beetle; D, Damage signs with existing holes.

3.4 Distribution

Trichoferus fissitarsis has a limited distribution compared with other species. In the light of many research attempts during the study period, [28, 29, 30], it was concluded that this species was found merely in Iran, Iraq, and Turkey.

3.5 Host Plants

Larva of *Trichoferus fissitarsis* is xylophagous and associated with living timber of the Fig tree, *Ficus carica* L. 1753 [31], but the adults are polyphagous and feed on the plant buds and leaves.

3.6 Damage and Economic Impact

Adult beetles and larvae are injurious (Pl. 3, C). Larvae start to feed after a few days of hatching, making tunnels through the host's trunk and branches, and gradually moving toward older branches or the main stem. Consequently, this movement might cause dying branches, decaying, and the demise of the host tree (Pl. 3, D). The exit holes' existence in the branch and trunk, frass exclusion, and the lesions produced by the mature female might also assist an opening for infection by inferior microorganisms and pests. *Trichoferus fissitarsis* affects the cultivation of fig trees in Iraq. Larvae hurt the stems of fig trees and destroy the branches and main trunk. Larval holes and tunnels weaken the trees and decline too [9, 10]. Thus, infected branches will be cut off when exposed to mechanical influences. Economically, it leads to fig yield reduction in the Kurdistan Region.

4 Discussion

The Hesperophanini Mulsant, 1839 tribe includes 23 species distributed worldwide. However, *T. fissitarsis* had been stated in the prior checklist of Iraqi Cerambycidae. Meanwhile, two other species *Hesperophanes griseus* and *H. preissi* had been reported as a major insect pest of fig tree [10]. In the recent Catalog of Cerambycidae, the genus *Trichoferus* Wollaston, 1854 contains fifteen species in the Palearctic Region, and from this contribution, three species were extant in Iraq only [2, 7,]. Recently, Rapuzzi and Khudhur updated the Cerambycidae checklist, added new species records for the Iraqi fauna, and included an additional Southern distributional range [18]. The number of species and their records in the Iraqi checklist need reliable comprehensive documentation. Hence, the precise description of its species is deficient. To solve this problem, a molecular method depended on the mitochondrial fractional gene *COI* was used which is considered a successful practice for insect identification. Molecular analysis of the current investigation successfully matched 100% of *T. fissitarsis*. After comparing and checking the studied specimen with the preserved specimen in the museum, we have reached the result that the specimen is *T. fissitarsis* which is similar, and morphological dissimilarities had not been noticed. The primary issue is the reporting of this species by its synonym, *Hesperophanes sericeus* (Fab., 1787). Concerning the structural exploration, the study consequences were approved with the results of Sama *et al*., [32]. However, the characteristic depiction was insufficient, and a comprehensive colorful clarification of the taxa was not clarified.

Conclusions

In this study *Trichoferus fissitarsis* was genetically registered in NCBI GenBank for the Iraq-Kurdistan Region. The molecular diagnostic technique confirmed the identification and taxation of evolutionary relatives of the species based on the sequences of mitochondrial *CO1* and agreed with these results by adding modern data of related morphology. A comprehensive description of the most indicative characteristics of the last larval instar and pupa that have significance in taxonomy and identifying this species was illustrated.

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Conflict of Interest

No

Author contribution

All parts of this article were conducted by the author.

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Data Availability

The datasets generated and analyzed during the present study are available from the author upon reasonable request.

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