Journal homepage
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Anbar Journal of Agricultural Sciences
(University of Anbar – Agriculture College)

FIRST MITOCHONDRIAL GENOME REPORT OF TRICHOFERUS FISSITARSIS SAMA, FALLAHZADEH AND RAPUZZI 2005 (COLEOPTERA: CERAMBYCIDAE) WITH MORPHOLOGICAL IMPLICATIONS

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Article info

Received: 2023-08-18
Accepted: 2023-09-20
Published: 2023-12-31

DOI-Crossref:
10.32649/ajas.2023.142348.1067

Cite as:
Jalil, P. A. (2023). First mitochondrial genome report of Trichoferus fissitarsis Sama, Fallahzadeh and Rapuzzi 2005 (Coleoptera: Cerambycidae) with morphological implications. Anbar Journal of Agricultural Sciences, 21(2): 354-365.

Abstract

The long-horned beetle, Trichoferus fissitarsis Sama, Fallahzadeh, and Rapuzzi 2005, is one of the most dangerous pests on fig trees, Ficus carica, in the Kurdistan Region- Iraq. In the current study, this species had been genetically reported in GenBank for the first time. However, it was recorded in Cerambycidae checklists from Iraq by its synonym Hesperophanes sericeus (Fab., 1787). Hereby, for the misidentification of this species and to solve the problem of identification, the molecular technique based on the mitochondrial partial gene mt COXI was exposed, and via this technique, and based on the COXI nucleotide sequence, the phylogenetic analysis was constructed for illuminating the species group, and their relations with other recorded species which belonged to the tribe Hesperophanini Mulsant, 1839. Also, the taxonomic status of this species has been discussed. Meanwhile, some illustrated evidence on the damage signs, host plants and morphological characteristics were constructed, described and illustrated clearly.

Keywords: Cerambycidae, Mitochondrial gene, Trichoferus fissitarsis, Morphology.

أول تسجيل للحشرة *Trichoferus fissitarsis* Sama, Fallahzadeh and Rapuzzi 2005 عن طريق الجين المتقدرة (Coleoptera: Cerambycidae) مع ملاحظات عن مظهره الخارجية

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الخلاصة

الخنفساء ذو القرون طويلة *Trichoferus fissitarsis* Sama, Fallahzadeh and Rapuzzi 2005 تم تسجيلها وراثيًا في بنك الجينات للعراق إقليم كردستان. مع ان سجل هذا النوع الخطير في البيئة الحشرية العراقية. تم تأكيد التشخيص عن طريق التقنية الجزيئية المعتمدة على جين المايتوكوندريا (COXI)، وتمت مناقشة الحالة التصنيفية لهذا النوع. وفي الوقت نفسه، تم إنشاء بعض الأدلة الموضحة على علامات الأصابة والعوائل النباتية والخصائص المظهر الخارجي ووصفها بوضوح.

كلمات مفتاحية: خنفساء ذو القرون الطويلة، جين الميتوكوندريا، Trichoferus fissitarsis، المظهر الخارجي.

Introduction

The family Cerambycidae, commonly known as longhorn beetles, covers one of the most diverse and economically important families of the order Coleoptera, where about 36,300 species in more than 5,300 genera were described worldwide (6, 35, and 38). Its species are widely distributed worldwide as some species were imported and became traditional outside their natural distribution range (40). The species of this family are also known as wood-borers due to their larval habits of boring the host plant stems. Thus, they cause great economic damage to forests and fruit trees (14). Fig stem borer is the main reason for decreasing fig production; therefore, further research is required to find the practicable technique to manage this destructive pest. The genus *Trichoferus* Wollaston, 1854, contains seven species in central Europe: *T. fasciculatus* (Faldermann, 1837), *T. griseus* (Fabricius, 1792), *T. holosericeus* (Rossi, 1790), *T. magnanii* Sama, 1992, *T. pallidus* (Olivier, 1790), *T. spartii* (G. Müller, 1948) and *T. campestris*. In Iraq only two species had been recorded *T. fasciculatus fasciculatus* (Faldermann, 1837) and *T. griseus* (Fabricius, 1792) (27 and 31). (36) have recently stated that *T. fissitarsis* is found in Iraq without any morphological explanation or clarifying detailed description (36). Also, this species was recorded as a new species from Iran (34). This species was reported as the first record in Turkey

during entomological surveys 2010 and 2011 by (37 and 39). Accordingly, great, known works were established like thesis, preliminary data or checklists by some Iraqi authors such as (1, 2, 4, 11, 17, 19 and 20). Awkwardly, the Iraqi fauna of long-horned beetles is incomplete and has not been corrected so far; therefore, new collecting, detailed description of new taxa, and Gene Bank registration of the Iraqi specimens are needed. Fragmentary data about longhorn beetle records in different country provinces are available in many publications. However, there is insufficient information about detailed morphology and molecular identification of cerambycid species in Iraq. This investigation has dealt with the genetic identification depending on inferences of DNA sequences from cytochrome oxidase (COXI) of *Trichoferus fissitarsis,* and morphological description has been implemented besides collecting data on the taxonomy, local distribution, and host plants.

Materials and Methods

Specimen Collection: The specimens were collected on the stems and trunks of Fig trees, *Ficus carica* in gardens, and orchard trees from different localities of Erbil province, Kurdistan Region-Iraq from the beginning of May to late August 2021-2022.

Specimens Identification: To be certain about the correct identification of this species in the larval and adult stage, we would carry out the cutting process if those bushes and parts were infected with the larvae of this species. We put it in the rearing cage which was covered by very thin clothes from the outer weather circumstances to allow the emergence of the adults (P. 3, A). Then, we sent the beetle to the connected museums to compare it with the identified species, and available keys were depended on (7, 8, 9, 29 and 35). Ultimately, Cerambycidae expert Dr. Pierpaolo Rapuzzi (Cividale del Friuli UD, Italy) confirmed the genus and species name. All specimens have been preserved and labelled in a specific insect box, then placed in the Museum of Plant Protection, College of Agricultural Engineering Sciences, Salahaddin University, Erbil.

DNA extraction, amplification, and sequencing: According to the manufacturer's protocol, the whole genomic DNA was extracted from an insect's body (i.e. thorax muscle and hind femur of the adult beetle) using ZYMO Quick-DNA Tissue/Insect Micro preparing Kit (USA) No. D6015. The target is 700 bp fragments. The sequence of *COXI* was amplified by polymerase chain reaction (PCR), and the mitochondrial partial gene was prepared in a total volume of 50 µl of reaction mixture including 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 pmol of forward primer C1J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3'), and 10 pmol of reverse primer C1-J-1718 (5'-ACTGTAAATATATGATGAGCTCA-3'), DNase free water and template DNA (Table 1), the mixture was run by Bioresearch PTC-200 Gradient thermocycler (15 and 21). According to the following procedures: initial denaturation at 95 °C for 5 min, followed by 34 cycles at 95 °C for 30 s; annealing temperature at 46 °C for 30 s; and extension at 72 °C for 1 hr. and final extension at 72 °C for 10 min. PCR products were tested by electrophoresis on a 1.5% agarose gel. If a single band were clarified (12), then it would be purified using

a spectrophotometer model NanoDrop 1000 manufactured by Thermo Scientific designed for measuring nucleic acid concentrations in sample volumes of one microliter was used and then sequenced by the molecular and genetic company (Macro Gene); Republic of Korea (South): http://www.macrogene.com/en/main/index.php. The sequences generated in this study were all deposited to the National Center for Biotechnology Information (NCBI) with the GenBank accession number OR268947.

Table 1 The volumes and reagents of PCR Amplification.

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	10 Pmol	2
3	Reverse Primer	10 Pmol	2
4	DNase Free Water	-	18
5	Template DNA	50ng/µl	3
	Total		**50**

Results and Discussion

Material Examined: Holotype (♂♀): In the present study, both genders of the adult beetle were studied for dealing with the morphology and taxonomic investigations. The species were collected from Shaqlawa, 36°24’15.585” N / 44°15’32” E, 834 m, Betwata, 36°20’47” N/44°34’22” E, 849m, Khalifan 36°35’01” N /44°23’58” E, 772, Qushtapa, 36°01’19” N /43°57’26” E, 772.

Molecular identification: When the sequence of the examined species was blasted in (NCBI), it was indicated that this species was identical to those of the *Trichoferus fissitarsis* based on pairwise genetic separations. (Table. 2).

Table 2 Blast information about the target species *Trichoferus fissitarsis* according to Genbank registration of partial mitochondrial COXI

Accession Number	Query Cover %	Identic Number %	Gene bank Species Identification	NCBI Registration	Country Identification
OR268947	100%	100%	*Trichoferus fissitarsis*	New record in NCBI	New record for Iraq

Phylogenetic Inferences: The phylogenetic analysis was constructed via (MEGA11) program based on the *COXI* nucleotide sequence illuminating the group of four specimens. The results of the current study are compatible with (13). From the sequence difference and similarity, the phylogeny was formed, and it was revealed that species belonging to relevant genera were close to each other. The neighbour-joining tree indicated that the examined material was *T. fissitarsis*, and the tribe Hesperophanini of the four species was grouped in one cluster with high similarity to their ancestors and considerable differences between the same genus species (Fig. 1).

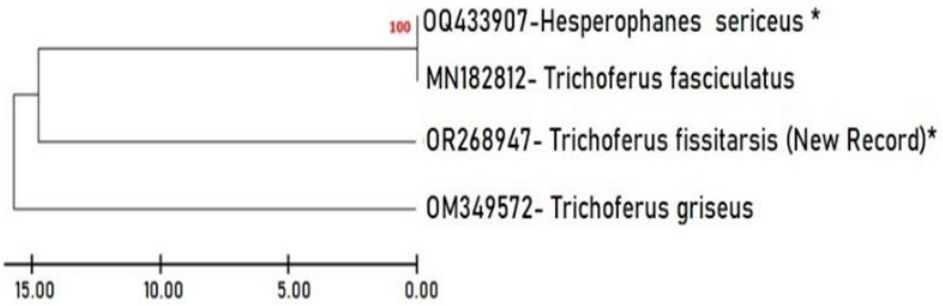

Figure 1 Phylogenetic tree of examined species by using the Maximum Likelihood method based on the (MEGA 11) software and bootstrap analysis with 100 re-samplings, and using partial DNA sequences of partial mitochondrial gene COXI as input data.

Trichoferus fissitarsis (34).
Synonyms: *Trichoferus preissi* Villiers, 1967; (misidentification).
Hesperophanes preissi: (20) (misidentification).

Morphology of holotype: The body of a beetle (P1. A) elongate characterized by being 17-.8 mm in length and 5.5-6.0 mm in width. The females are bigger than males in size, there is an end of the abdomen round in females, while in contrast, males are known for their sub-conical shape and dramatically narrowed. The body color is dark brown, and it appears brighter than its real color due to its being covered by dense yellowish hairs on the body and elytra. Head, noticeably oval-shaped, anteriorly refracted covered with dense yellowish hairs; antennal socket bordered by compound eyes and far from mandibles bases. Compound eyes are triangular and located laterally. Antennae (P2. A) filiform, not reaching the body's middle, comprises 11 flattened segments except 1st and 2nd segment. Further, the second segment, the largest segment, is elongated and trapezoid, and. Each segment provided several golden hairs that condensed several golden hairs at the apical outer margin. Mandibles (P.2 B) stout, strongly sclerotized with three sharp arched apical teeth, and widened basally. Labrum (P.2 C) transverse, medially rounded, long and dense setae covered the dorsal surface and condensed on the outer margin; anti-clypeus membranous and wider than long. Labium (P.2 D) is obvious, and its parts are well developed; labial palps have three segments, the terminal segment is larger and darker than 1st and 2nd segments. Long and sparse setae raised on apical outer margins of 1st and 2nd segments. Ligula membranous are developed as two terminal lobs and provided with long and dense setae.

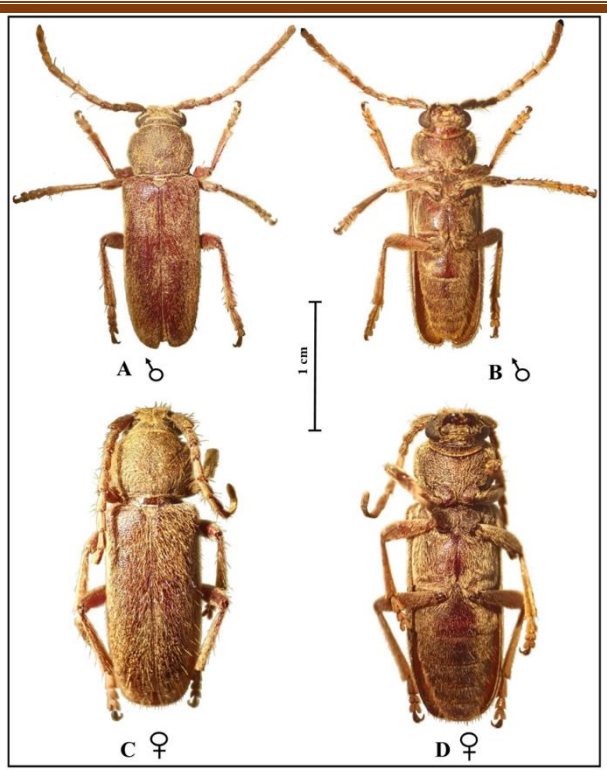

Plate 1 *Trichoferus fissitarsis* (34), A, Dorsal view of Male; B, Ventral view of Male; C, Dorsal view of female; D, Ventral view of female.

Antennae (P2. A) filiform, not reaching the body's middle, comprises 11 flattened segments except 1st and 2nd segment. Further, the second segment, the largest segment, is elongated and trapezoid, and. Each segment provided several golden hairs that condensedseveral golden hairs at the apical outer margin. Mandibles (P.2 B) stout, strongly sclerotized with three sharp arched apical teeth, and widened basally. Labrum (P.2 C) transverse, medially rounded, long and dense setae covered the dorsal surface and condensed on the outer margin; anti-clypeus membranous and wider than long. Labium (P.2 D) is obvious, and its parts are well developed; labial palps have three segments, the terminal segment is larger and darker than 1st and 2nd segments. Long and sparse setae raised on apical outer margins of 1st and 2nd segments. Ligula membranous are developed as two terminal lobs and provided with long and dense setae. Maxillae (P.2 E), cardo triangular and sclerotized; maxillary palp 4 segments, the apical segment is clavate shape, darker, and twice as long as second and third segments. Mala cylindrical is well sclerotized, and the apical part is expanded and covered by long and dense setae, stipes crescent shape and provided with dense, long setae. The pro-notum is slightly round without marginal appendages, whereas the posterior margin is nearly straight. Likewise, Pro-notum surface is designed by five fine manners covered with dense, whitish yellow, and long pubescence. Meanwhile, Scutellum is triangular shield-shaped, providing dense whitish-yellow pubescence. Elytra elongate, wider than pro-notum with parallel sides, apically plumped and narrowed toward ends. Further, the whole surface of the elytra is covered by dense long setae. Pro-sternum moderately prolonged toward the cervix while Meso-sternum narrow, depressed, and restricted between pro- and meta-sternum. Meta-sternum quadrate is shaped larger than pro and meso-sternum and the

posterior margin is characterized by a partial longitudinal design. Legs similar in shape completely covered with dense hairs. Fore and middle coxae are globose, and hind coxae are triangular and transverse with a round, shrill lateral side. Femur long and gradually broad toward the tibia. Tibia relatively large and dramatically widened toward the tarsus and terminated with two long sparse setae. Tarsus, three segments, all segments extremely bilobed medially, provided by long moderate setae, and ended by two acute hawk-shaped claws. The abdomen includes five noticeable segments, the first larger than other segments while the last is smaller. The outer margin is rounded in females. In contrast, it is tubular and tapered in males (P.1 B & D).
Male genitalia, aedeagus cylindrical and apically tapered, pale brown, lateral lobes long, deeply split, apically moderately dilated, bearing long, dense setae (P.2. F & g), internal sac with numerous small irregular sclerites.

Distribution: This species was distributed among the Mediterranean region, south Europe (France, Italy, Ukraine, and Crimea), North Africa (Egypt), and Iberian Peninsula to Bulgaria, Crete, Cyprus, Greece, eastern wards to Iran, Iraq, Jordan, Palestine, south Turkey, and Syria (3, 5, 18, 26, 32 and 33).

Host Plants: Larvae of *Trichoferus fissitarsis* are xylophagous and attached to dried wood or decayed branches of fig, *Ficus carica*, but the adult is polyphagous and feeds on broad-leaf trees (5, 22 and 23).

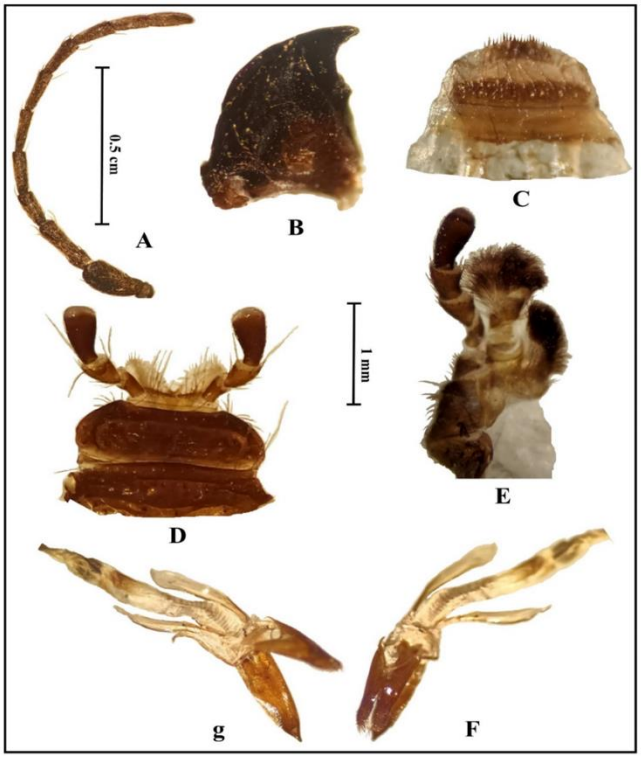

Plate 2 Trichoferus fissitarsis (34), A, Antenna; B, Mandible; C, Labrum with anti-clypeus; D, Labium; E, Maxilla; F, Lateral lobes of male genetalia; g, aedeagus.

Damage and Economic Impact: Both adults and larvae are injurious. That is, larvae start to create tunnels through the trunk and older branches of the tree. Thus, this activity may lead to a weakening crown, decaying, and finally, the host tree's death

(P.3, B). The presence of exit holes in the trunk, frass expulsion, and the lesions caused by the female beetle might also provide an opening for infection by inferior pathogens and pests (P.3, C). While *Trichoferus fissitarsis* is a serious pest in Asia, and its effects have generally been modest in Iraq, their damage could lead to reduced fruit harvest, bad quality of wood, and incompletion of tree lifecycles. The mechanical injury affected by larval holes and tunnels can also decline the trees so that in the case of unfavourable weather conditions and strong winds, branches and stems will be cut off. Given its preference for fig tree, *Ficus carica,* the larval stage (P.3, D) has a destructive impact on fig tree decaying and low production in addition to the horticulture, and it affects the farmers in the Kurdistan region.

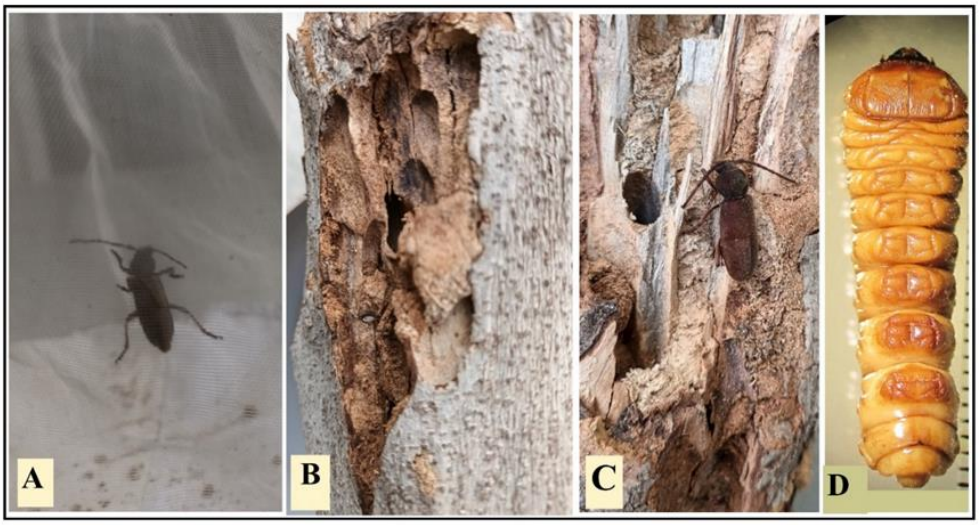

Plate 3 *Trichoferus fissitarsis* (34); A, Rearing Cage; B, Damage signs; C, Exit holes and adult emergence; D, Larval stage.

Discussion: The species *Trichoferus fissitarsis* belonged to the subfamily of Cerambycinae Latreille 1802 and the tribe Hesperophanini Mulsant, 1839. It is distributed worldwide among the Cerambycine family, which includes 23 species. In the Iraqi prior checklists, *Trichoferus fissitarsis* was not recorded. Instead of this species, two other species *Hesperophanes griseus* and *H. preissi* had been recorded on the fig tree (20). Besides, misidentification in our checklist refers to the lack of taxonomic studies and specific research on the Cerambycidae species and the lack of communication between the museums of developed countries and neighbouring countries where mostly the species is registered by their synonymous. Likewise, the convinced reason refers to the development of taxonomy. Recently, (28) recorded this species for the Iraqi fauna.

According to a recent catalogue, the genus *Trichoferus* Wollaston, 1854 includes 15 species in the Palearctic region, and only three species were present in Iraq (10, 29 and 30). Species number and their recorded names on the Iraqi Cerambycidae checklist are un-updated to date. Even a detailed description of its species is poor. For this reason, and the ruins of this problem, we resort to using the molecular technique based on the mitochondrial partial gene COXI, considered the best and most successful technique to solve the identification problem (16). The molecular analysis of the present study successfully identified 100% of our specimen as that of *T.*

fissitarsis. Thus, we investigate that the present and insect museum specimens are similar. After the comparison process, no taxonomic differences were observed except for the color and surface of the elytra, which might be changed belonged to the preservation period. On the one hand, we a misidentification problem because the museum specimen was collected in 1976 and was very old. Moreover, the species was identified by its synonymous *Hesperophanes sericeus* (Fab., 1787). Regarding the morphological investigation, the results of the current study agreed with the results of (25 and 34), although the morphological illustration was not available, and a precise description of *T. fissitarsis* was not reported.

Conclusions: Fig stem borer, *Trichoferus fissitarsis* was identified based on the molecular diagnostic technique and genetically reported as a new record from GenBank, and assessment of the evolutionary relations of examined materials based on mitochondrial *COX1* sequences and confirming these results by integrating contemporary knowledge of relevant morphological features. A detailed description of the diagnostic characteristics of the adult beetle that have taxonomic importance in identifying this species was illustrated.

Conflict of Interest: The author confirms that this article's content has no conflict of interest.

Acknowledgments: We thank Dr. Pierpaolo Rapuzzi (Cividale del Friuli UD, Italy) for identifying the species and providing valuable recommendations. We thank Mr. Abdul-Qadir Salih Khidhir, Manager of Insect Museum, Directorate of Agriculture Researches, Ministry of Agriculture and Water Resources, Erbil, Iraq, for comparing *Trichoferus fissitarsis* with the preserved species and for giving information about the checklist of Iraqi Cerambycidae and guiding the localities of fig orchards within Erbil Province.

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