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MOLECULAR GENETIC DIAGNOSIS OF *TRIBOLIUM CASTANEUM* AND *TRIBOLIUM CONFUSUM* (COLEOPTERA : TENEBRIONIDAE) BY SEQUENCING OF COI GENE ANALYSIS

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ABSTRACT : Molecular genetic comparison between two species of flour beetles of the genus *Tribolium* was studied in Diyala Governorate, Iraq using the COI gene sequence sequencing. The study was conducted on 20 specimens of insects, 10 specimens for the *Tribolium castaneum* and 10 specimens for the *Tribolium confusum*, which were collected from the grain store of Diyala Governorate - Iraq. DNA was extracted from adult insects of both species using genomic DNA mini kit, the COI gene was amplified using polymerase chain reaction, and the molecular weight of the gene amplification product in both species of insects was 1000 base pairs. The results of the statistical analysis using Bioedit program showed that there is a difference in the sequence of nitrogenous bases of the COI gene between the two species on the one hand and the reference sequence of the gene on the other hand. The percentage of congruence of the gene sequence in the studied samples with the sample of the gene bank was 90%. The results of the phylogenetic tree based on the sequence of the COI gene showed the separation of the samples of *Tribolium castaneum* from the samples of *Tribolium confusum*, as well as a slight heterogeneity between samples of one species, samples of both species are very close to the sample of the gene bank.

Key words : *Tribolium castaneum*, *Tribolium confusum*, COI, Diyala, Iraq.

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INTRODUCTION

The red flour beetle, *T. castaneum* (Herbst) and the confused flour beetle, *T. confusum* (Jacquelin du Val) (Coleoptera : Tenebrionidae). It is one of the stored crop pests, which are similar in morphological form. The last insect was named by this name because of the confusion in knowing its identity because it resembles the last insect to a very large degree from the morphological point of view (Walter, 1990; Zainab *et al*, 2021). They are two of the most widespread pests found in flour mills as well as in places where grain and dried foods are processed and stored and were recently classified as one of the most important insect pests that live in grain processing factories and storage facilities (Campbell *et al*, 2004). There are several methods we use to protect stored goods from being infected with these pests, including the use of fumigants and chemical pesticides. Previous research showed that *T. castaneum* is more affected than *T. confusum* for two types of aerosols, namely of two types to pyrethrin aerosol, hydroprene and pyriproxifen (Arthur, 2001, 2008; Arthur and Hoernemann, 2004; Arthur *et al*, 2009; Sutton *et al*, 2011). The previous research also showed that the immature instars i.e. the larvae and pupae of both *T. castaneum* and *T. confusum* were more affected than the mature stages i.e. the adults (Arthur, 2008; Arthur and Fontenot, 2012). Inanition to, the effectiveness of insecticides is affected by the type of insect and its stages of development. Therefore, the differential sensitivity of the insecticide between insect species and the stages of development of the insect must be taken into account when using chemical pesticides to eliminate this pest in grain stores (Arthur, 2001). When the pest manager is fully aware of the pest to be controlled, this leads to the legalization of the use of chemical pesticides, as well as knowledge of the extent of pest resistance to pesticides and improving the level of pest management. It is not possible to distinguish between *T. castaneum* and *T. confusum* in the incomplete instar stage, i.e. egg, larva, pupa and even in the adult stage without special training on how to use the taxonomic keys based on morphology. Therefore, it is better to find another better methods to distingue depends on DNA were used

to diagnose insect species, and these parameters are random polymerase chain reaction, polymorphism of amplification of fragment length, polymorphism of length of the restriction fragment and simple sequence repetition. The use of these parameters adds significant value in diagnosing species as well as alternative methods for diagnosing the phenotype of insectivores. The method of amplifying a gene from the DNA genes, whether they are nuclear DNA genes or mitochondrial DNA genes using the polymerase chain reaction, and comparing the alignment of nitrogenous bases from the sequence of the amplified gene to distinguish between insect species One of these methods is the use of COI gene sequencing analysis to compare between *T. castaneum* and *T. confusum*. Nucleotide sequencing of many mitochondrial DNA genes has been used to study the genetic relationships between species or populations of genetically heterogeneous insect pests of the same genus, because they may show high variances among themselves, especially in the replacement of some nucleotides (Havill *et al*, 2007). The current research aims to amplify and make a nucleotide sequence for a partial of the COI gene in *T. castaneum* and *T.confusum* and compare the sequence of the gene between *T. castaneum* and *T.confusum* to reveal the phylogenetic relationship between the two species.

Specimens collection

This research was carried out in the Molecular Genetics Laboratory of the Biology Department at the Faculty of Education for Pure Sciences, University of Diyala. The study included 20 specimens, 10 specimens for *T.castaneun* and 10 specimens for *T.confusum*. Specimens of both species were collected from grain stores in Diyala Governorate, Iraq. The specimens were placed in 70% ethanol alcohol and transported to the laboratory for the purpose of DNA extraction.

DNA extraction

DNA was extracted using Genomic DNA Mini kits manufactured by the Korea company Bioneer and stored at 4°C. Then the quality and quantity of the extracted DNA was estimated by spectrophotometry. The spectrophotometric method is used for the accurate quantification of DNA. The DNA concentration are determined by measuring the optical density (OD) in spectrophotometer at the wavelength 260 nm knowing that the DNA has a maximum UV absorption spectrum at 260 nm.

COI gene amplification

The primer used amplification for COI gene was COIF (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') and COIR (5'-ATC TCC CAC ATT ATT AGA CAA G-3') (Simon *et al*, 1994). The total volume of the PCR mixture is 25 µL and consists of 5 µL Master mix, 1.5 µL forward primer, 1.5 µL revers primer, 5 µL DNA and 12 deionized water. The thermopolymer programmed includes: initial denaturation 94°C for 5 min, number of cycles 35 of denaturation 94°C for 50 s, annealing 55°C for 30 s and extension 72°C for 1 min and final extension 72°C for 10 min. The PCR products were electrophoresed on 1% agarose gel, stained with ethidium bromide 2ìl and visualized under a UV transilluminator in a LG2020 Gel Documentation System. Ten PCR product specimens of each species was sent to Macrogene company in south Korea for direct sequencing.

Sequencing of COI gene

The nucleotide sequence alignment of the COI gene was done in specimens of both *T. castaneum* and *T. confusum* using the seventh version of the bioedit program., and Similar sequences were combined into a single haplotype. Phylogenetic tree analyzes based on the neighborhood association method and the maximum likelihood method were performed separately for the COI gene region using MEGA 5.05 (Tamura *et al*, 2011).

RESULTS

COI amplification of *T. castaneum* and *T. confusum* is given Figs. 1 and 2.

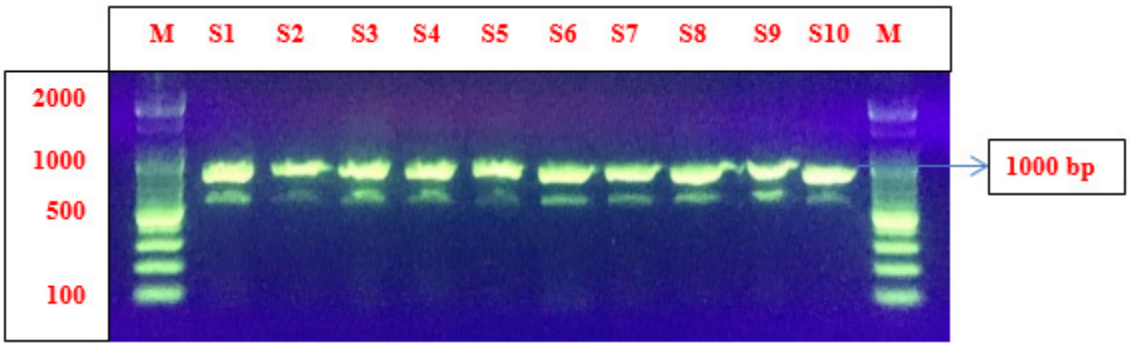
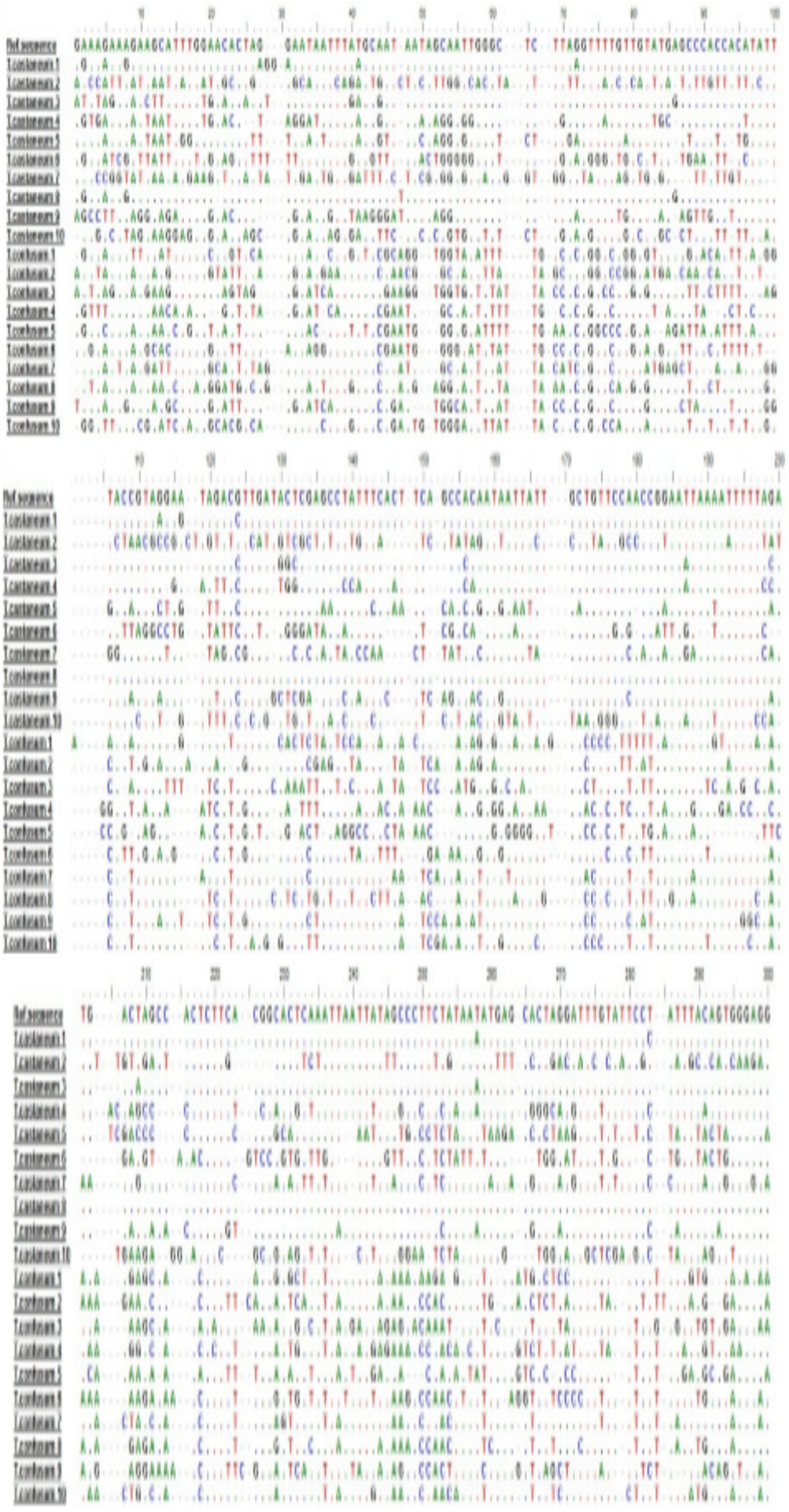


Fig. 1 : COI amplification product of *T. castaneum* and *T. confusum* carried over on agarose gel at a concentration of 1% for 1.5 hours after staining with silver stain and photographed under UV light. S1 to S5 in the pictures refer to *T. castaneum*, S6 to S10 in the pictures refer to *T. confusum*.



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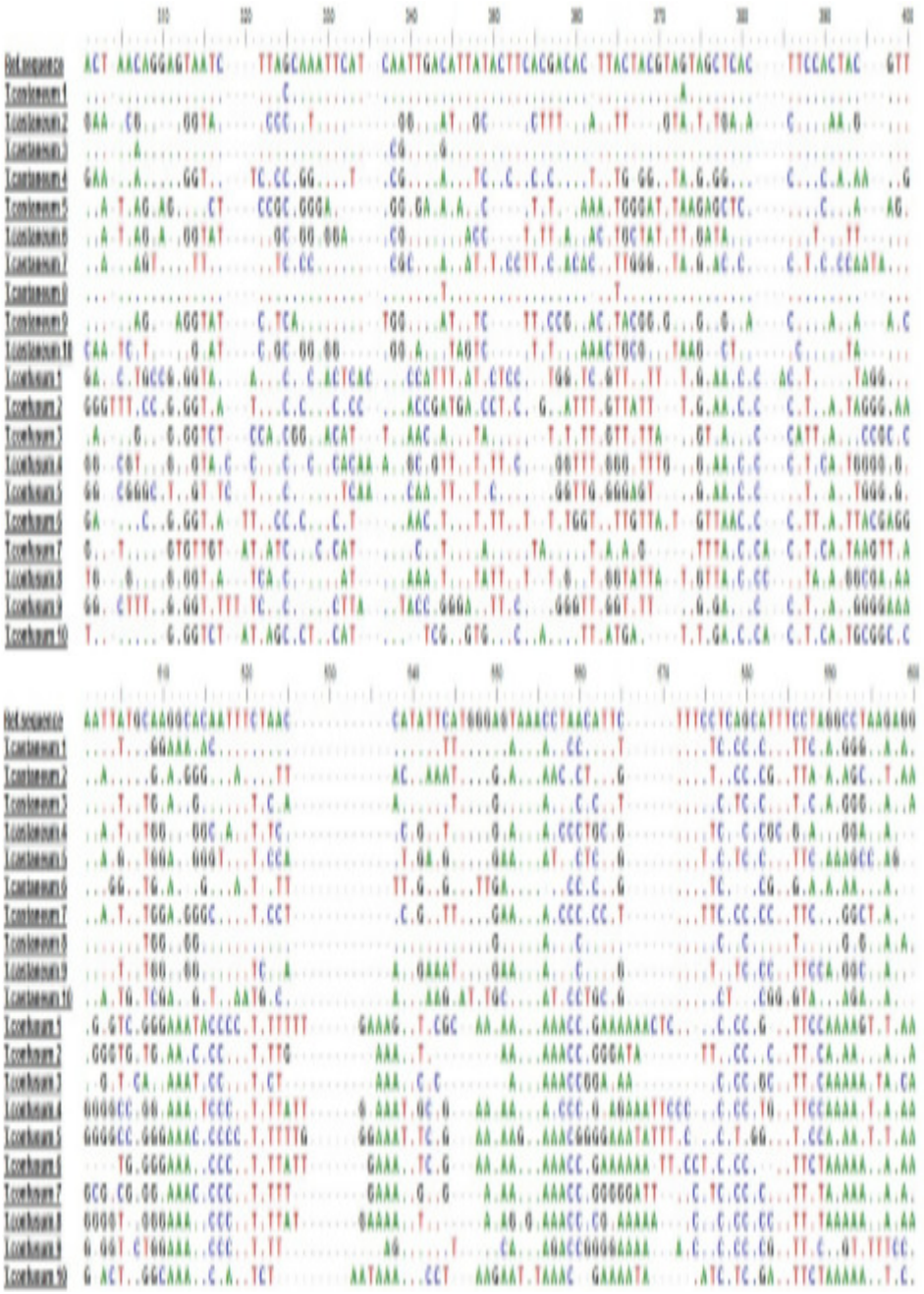

DISCUSSION

The result of amplification of the COI gene in specimens of *T. castaneum* and *T. confusum* reached 1000 bp (Fig. 1). There is no difference in the molecular weight of the gene between specimens of both species . The results of this research agree with the results of the researcher (Ming *et al*, 2014) included genetic relationships between *Tribolium castaneum* and *T. confusum* based on mitochondrial DNA sequences. The results of the COI gene sequencing analysis showed that the percentage of congruence of the gene sequence in specimens of *T. castaneum* and *T. confusum* was 90% and 80% with the reference sequence, respectively. While the *T. castaneum* specimens were very different from the *T. confusum* specimens in the sequence matching of the COI gene in terms of replacing many of the nitrogen bases along the gene (Fig. 2). The size and morphology of both *T. castaneum* and *T. confusum* are very similar (Bousquet, 1990). It is not possible to distinguish between the two species phenotypically, but from a molecular point of view, especially when studying the sequence of one of the mitochondrial DNA genes, such as the COI gene used in the current research, we were able to distinguish between the two species with very high accuracy. The

Fig. 2 continued...

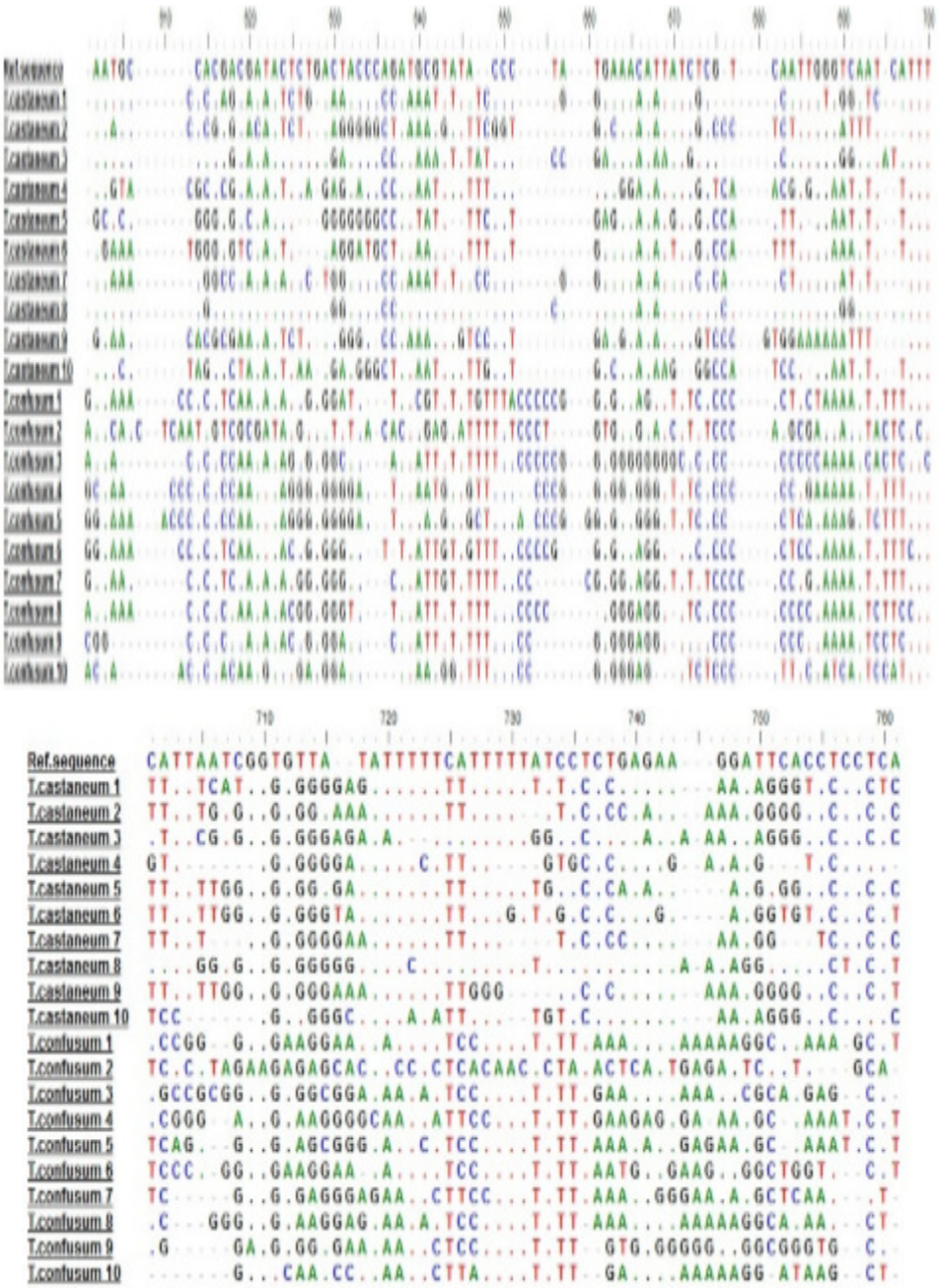

Fig. 2 : Comparison of the nitrogenous base alignment of a part of the COI gene both of *T. castaneum* and *T. confusum*.

results of the phylogenetic tree analysis based on the sequence of the COI gene showed that the 10 specimens of *T. castaneum* descended from one branch, as well as 10 specimens of *T. confusum* also descended from one branch. There is also a heterogeneity between samples of the same species, as shown in Fig. 3.

CONCLUSION

The *T. castaneum* differs from *T. confusum* genetically, but they are very similar from the phenotypic point of view. The COI genetic region is very important in measuring genetic heterogeneity and phylogenetic relationships among morphologically similar species. Moreover, the genetic ratios obtained do not completely match with the phylogenetic relationship inferred from the morphological data for both *T. castaneum* and *T. confusum*.

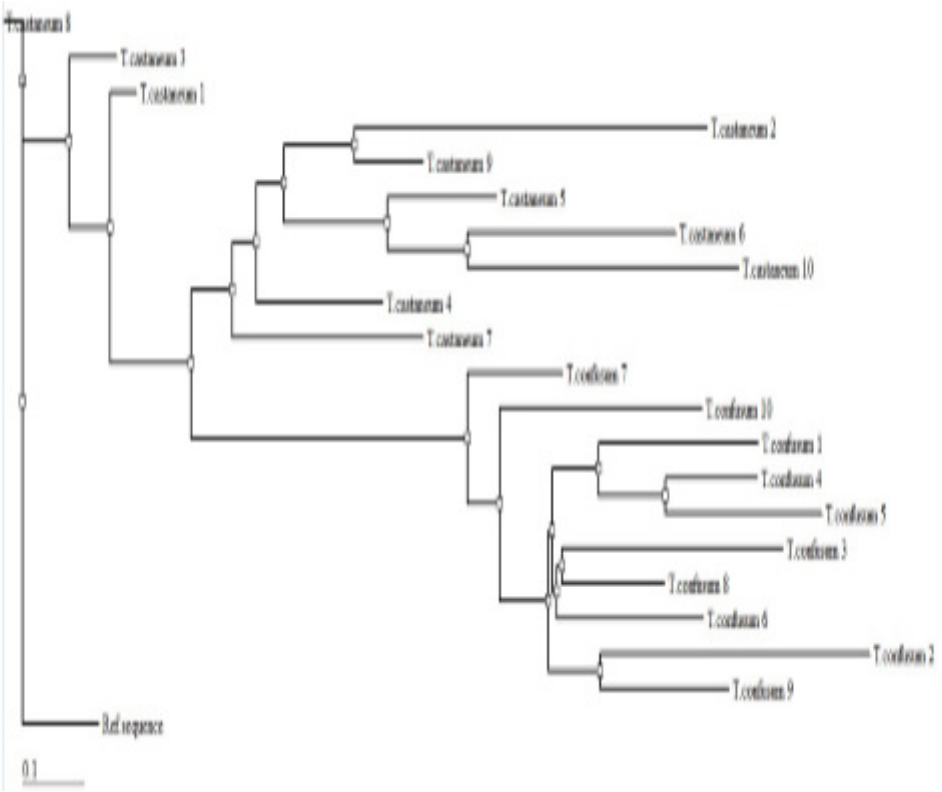

Fig. 3 : Phylogenetic tree of *T. castaneum* and *T. confusum* depending on the sequence of COI gene.

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