

First report and molecular identification of *Chilo partellus* (Swinhoe, 1885) in South-eastern Türkiye: invasion continues (Lepidoptera: Crambidae)

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Abstract

Chilo partellus (Swinhoe, 1885) (Lepidoptera: Crambidae), is an invasive maize pest that can cause high yield losses. The pest has recently been introduced and established in eastern Mediterranean countries such as Türkiye. However, research regarding its expansion towards other regions in Türkiye and other countries is required. This study is the first to report the presence and abundance of the pest in South-eastern Türkiye provinces, Diyarbakır, Mardin, Şanlıurfa, and Şırnak as well as the infestation rate and its contribution to the stemborer-dependent infestation rates. There were significant differences between the abundances of the pest in different counties, while the infestation rate, the contribution to the stemborer-dependent infestation rate, and the contribution to the total stemborer population were not statistically different between counties. The pest was only recorded in three southern counties of Şanlıurfa, during routine September surveys in 2022 and was absent in Diyarbakır, Mardin, and Şırnak. Specimens were morphologically diagnosed, and for molecular identification and phylogenetic analysis, nucleotide sequencing of the mitochondrial cytochrome oxidase subunit I (COI-I) gene was performed. All specimens were confirmed as *C. partellus*. Further, phylogenetic analysis revealed intraspecific genetic variation and the similarity of the specimens to reference sequences from Africa.

Keywords: Lepidoptera, Crambidae, invasive pests, maize, phylogenetic analysis, spatial distribution, Türkiye.

Primer informe e identificación molecular de *Chilo partellus* (Swinhoe, 1885) en el sureste de Türkiye: continúa la invasión (Lepidoptera: Crambidae)

Resumen

Chilo partellus (Swinhoe, 1885) (Lepidoptera: Crambidae), es una plaga invasora del maíz que puede causar grandes pérdidas de rendimiento. La plaga se ha introducido y establecido recientemente en países del Mediterráneo oriental como Turquía. Sin embargo, es necesario investigar su expansión hacia otras regiones de Turquía y otros países. Este estudio es el primero que informa de la presencia y abundancia de la plaga en las provincias del sureste de Turquía, Diyarbakır, Mardin, Ranlıurfa y Rırnak, así como de la tasa de infestación y su contribución a las tasas de infestación dependientes del barrenador del tallo. Había diferencias significativas entre las abundancias de la plaga en los distintos condados, mientras que la tasa de infestación, la contribución a la tasa de infestación dependiente de la broca y la contribución a la población total de brocas no eran estadísticamente diferentes entre condados. La plaga sólo se registró en tres condados del sur de Ranlıurfa, durante las encuestas rutinarias de septiembre de 2022, y estuvo ausente en Diyarbakır, Mardin y Rırnak. Los especímenes se diagnosticaron morfológicamente y, para la identificación molecular y el análisis filogenético, se realizó la secuenciación de nucleótidos del gen mitocondrial de la subunidad I de la citocromo oxidasa (COI-I). Todos los especímenes se

confirmaron como *C. partellus*. Ademas, el analisis filogenetico revelo la variacion genetica intraespecifica y la similitud de los especimenes con las secuencias de referencia de frica.

Palabras clave: Lepidoptera, Crambidae, plagas invasoras, maz, analisis filogenetico, distribucion espacial, Turkiye.

Introduction

Maize constitutes one of the most significant sources of human and animal nutrition in the world. In the last quarter century, maize has become the second most widely cultivated crop in South-eastern Turkiye (TUIK, 2022). Preferred by both small- and large-scale farmers, maize is cultivated either as a first crop, sown in early spring and harvested at midsummer or as a second crop, sown in early summer and harvested at the end of the autumn (TonĀa, 2021). Therefore, maize plants are available throughout the whole growing season in the region and, consequently, are threatened by insect pests, the most significant group of which is represented by lepidopteran stemborer species, mainly *Sesamia nonagrioides* (Lefebvre, 1827), *Sesamia cretica* Lederer, 1857 (Lepidoptera: Noctuidae), *Ostrinia nubilalis* (Hubner, 1796) and a recently established competitive species, *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae) (Bayram & TonĀa, 2016).

Early-growing stage host feeding by *C. partellus* larvae causes characteristic holes and mechanical damage to the leaves of the host plants. Following that, the damage may result in the death of the growing point of host seedlings, which is called “dead heart”. Older larvae feed on and tunnel into the maize stems and cobs, causing extensive yield losses. The pest attacks both cereal crops and uncultivated grasses. *Chilo partellus* originated in Asia and invaded Africa from India before 1930 (Kfir et al. 2002). A climate change scenario with advanced insect life cycle modelling software predicted the expansion of the pest towards regions including eastern Mediterranean countries including Turkiye and Israel (Khadioli et al. 2014). The first larval specimens of *C. partellus* were detected in maize and sorghum fields in the Western Galilee region of Israel in 2010 with a rapid spread to all northern part of the country by the end of 2011 (Ben-Yakir et al. 2013). The spread of the pest took no longer than 4 years to reach a zoogeographically significant country, Turkiye (Bayram & TonĀa, 2016). Surveys performed in the Mediterranean region of Turkiye yielded the first detection of the pest in the maize fields in the provinces of the Eastern Mediterranean region of Turkiye, Adana, Hatay, and Osmaniye in September and October 2014, and the pest was predicted to spread to other surrounding provinces and regions with low and high altitudes (Bayram & TonĀa, 2016).

Previous surveys conducted between 2018-2020 investigating the stemborer in the region confirmed the region-wide presence of *Sesamia nonagrioides*, *S. cretica*, and *Ostrinia nubilalis* (TonĀa, 2021). The surveys also aimed to confirm the presence or absence of invasive maize stemborers such as *C. partellus*, and the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), both of which were not recorded during the course of the study (TonĀa, 2021). Continued surveys in the following years confirmed their absence in 2021, while only *C. partellus* was detected in Southeastern Turkiye in September 2022.

Chilo partellus is of great agricultural importance due to its high yield-reducing feeding damage in maize and sorghum (Kfir et al. 2002). The yield losses continue to grow because the pest has a great capability of spreading as a consequence of its relatively shorter life cycle, high reproduction rate, and ability to adapt to lower and higher altitudes (Khadioli et al. 2014). Despite its agricultural relevance and invasiveness, the number of studies investigating genetic variation within *C. partellus* populations is scarce. For example, employing the mitochondrial cytochrome oxidase subunit I (COI-I) gene, previous studies have revealed that there were no detectable intraspecific genetic variations between the populations of *C. partellus* in Swaziland and South African countries and between the populations from Eastern Countries such as Pakistan and India (Assefa & Dlamini, 2016; Donga & Meadow, 2018; Lee et al. 2019). However, there were indeed genetic variations between *C. partellus* populations from Africa and Asia, which suggests intercontinental genetic variation between the populations of *C. partellus* (Lee et al. 2019). Normally, genetic variations may be expected between the populations of

Africa, and Asia, in a transcontinental manner, as these continents individually differentiate in terms of climatic and agricultural landscapes. As expected, genetic variations may not occur within populations from Asia, where the pest originated, and within Africa, where the pest is well-established in a long-term manner. Both situations have yielded continuous interpopulation gene flow on both continents (Assefa & Dlamini, 2016; Donga & Meadow, 2018).

To the best of our knowledge, this work is the first to alert about the continuing geographic spread of *C. partellus* towards the northern part of the Fertile Crescent reporting the presence and abundance of the pest in South-eastern Türkiye. Furthermore, we accurately identified the specimens collected from maize fields during surveys, using the COI-I gene, and constructed a phylogenetic tree to compare collected samples with the published reference sequences from GenBank, and, thus, the genetic diversities of the newly-established populations in the region in comparison with the sequences of world populations are documented.

Materials and Methods

SURVEY PROGRAM

During routine September surveys in South-eastern Türkiye in 2022, aiming to define the abundance and infestation levels of maize stemborers, *Sesamia* spp. and *Ostrinia nubilalis*, larval and pupal specimens having different morphological characteristics than *Sesamia* spp. and *O. nubilalis* were detected. Based on the experience of the first author in reporting the first occurrence of *C. partellus* in Türkiye, the larval specimens that belonged to *C. partellus* were easily noticed (Bayram & Tonga, 2016). The fully-grown larvae of the pest have a black/dark brown head, a yellow to pink body colour with black spots, four brown longitudinal stripes along the back, and a shiny brown or black prothorax (CABI, 2022). The pupal specimens were also considered *C. partellus* by count since there were no larval specimens of conflicting species such as *O. nubilalis* in the surveyed localities during sampling dates, which was, afterward, confirmed upon hatching during incubation in laboratory chambers with constant climatic conditions (25 ± 1 °C, 60 ± 5 RH, 16:8 h illumination) conditions. Each maize field was entered from the side, and a 30 x 30 m² area was considered a sampling unit. In each sampling unit, maize plants were rapidly checked for any stemborer damage on leaves, stems, and cobs. The infested maize plants were uprooted and dissected carefully. The number of specimens was recorded for each pest. The larval and pupal specimens were collected and grouped separately in plastic containers with purpose-relevant labels. All materials were transferred to the laboratory chambers. The larval specimens were provided with fresh cobs and stalks, and the containers were renewed daily. All samplings were performed on second-crop maize vegetation, and plants were at the reproductive stages between the silking and the end of fruit development stages, referring to BBCH scale 63-79 codes (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) (Weber & Bleiholder, 1990; Lancashire et al. 1991).

STATISTICAL ANALYSIS

The number of larvae and pupae of the pest was subjected to generalized linear models since log-transformation is not suggested for ecological count data (O'Hara & Kotze, 2010). Previous studies also demonstrated that generalized linear models, especially the models including negative binomial distribution constitute the best fit for the ecological count data (Sokame et al. 2019; Tonga, 2021). The number of insects between counties was compared using negative binomial generalized linear models with `glm.nb` function from the `mass` package (Venables & Ripley, 2002). The percent data regarding infestation rate and contribution rates were analysed using binomial distributed generalized linear models. County was the factor and the mean number of each parameter per county was separated using Tukey multiple comparison post hoc tests via the `lsmeans` package (Lenth, 2016). All statistical

procedure was implemented in R statistical environment. The figure regarding the ecological data and the map were generated using GraphPad Prism 8 and QGIS 3.28.0, respectively.

DNA EXTRACTION

The total genomic DNA of three adult *C. partellus* specimens collected in Türkiye was extracted by grinding the entire body of the specimens using the PureLink™ Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. Following the final wash, 100 L of elution buffer was used to elute genomic DNA. The concentration and caliber of the isolated DNA were assessed via a Qubit 4 fluorometer. All DNA extracts were stored at -20 °C until the PCR procedure.

PCR AMPLIFICATION

To specifically amplify a 708-bp fragment of the COI-I gene, universal primer pairs (Arthropod_F (LCO1490): 5' GGT CAA CAA ATC ATA AAG ATA TTG G3' and Arthropod_R (HCO2198): 5' TAA ACT TCA GGG TGA CCA AAA AAT CA3') were used (Folmer et al. 1994). The PCR cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 45 s, elongation at 72 °C for 45 s, and a final elongation step at 72 °C for 7 min.

All polymerase chain reactions (PCR) were conducted in a volume of 25 µL consisting of 2,5 µL of 10xPCR buffer, 1,5 µL of MgCl₂, 0,5 µL of dNTP, 0,5 µL of each primer, 0,2 µL of Taq DNA polymerase (Thermo), and 1 µL of extracted DNA and nuclease-free water. The resultant PCR products were purified with the HighPrep PCR clean-up system (MagBio Genomics Inc.) and sequenced by MedSanTek Inc. (Istanbul, Türkiye). All specimens were sequenced on both strands to reduce PCR artifacts and ambiguities. Using the Geneious v6 software (accessible at www.geneious.com), complementary strand sequences were edited and reconciled. All sequences were aligned using MAFFT v7 (Katoh et al. 2019) with the default option settings. There were no gaps in the corresponding alignment. The coding frame was then examined for potential errors or stop codons using Mesquite v3.70 (Maddison and Maddison 2018), and the alignments of the sequences from Suruç, Ceylanpınar, and Akçakale counties of Şanlıurfa, Türkiye were submitted to NCBI with accession numbers OQ154267, OQ154268, and OQ154269, respectively.

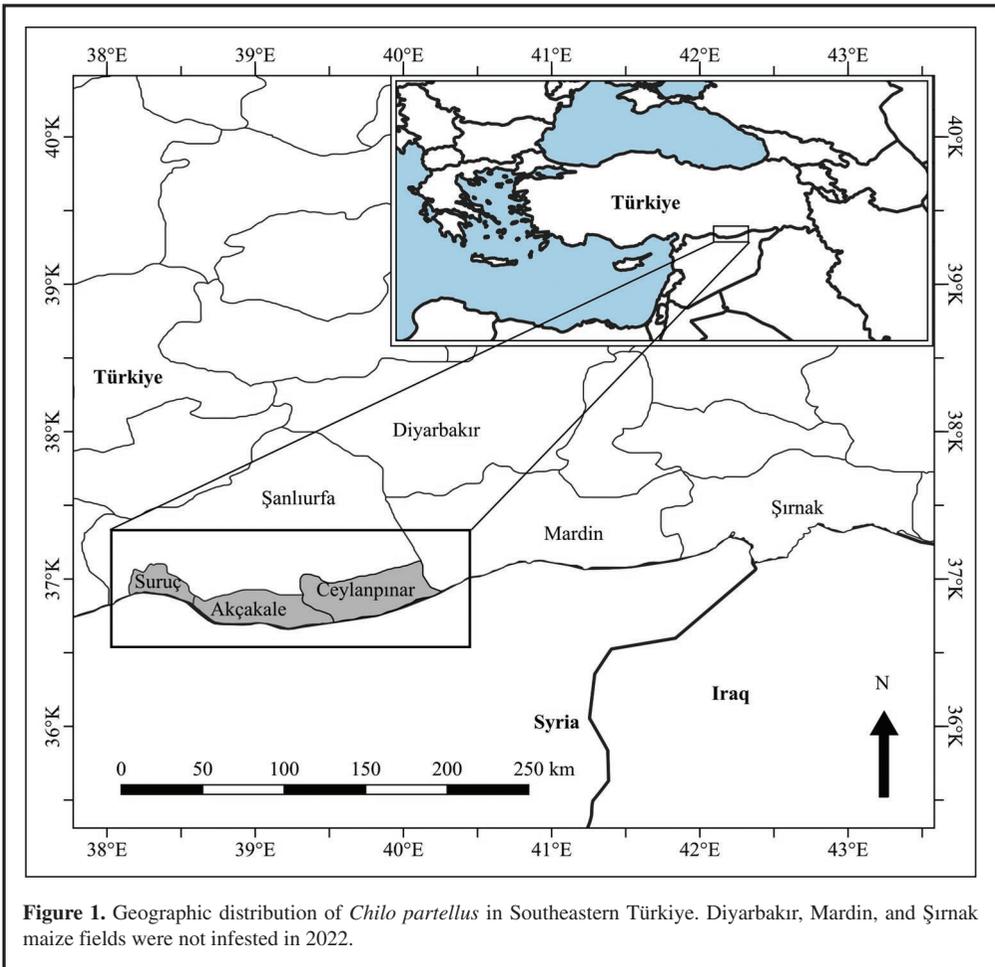
PHYLOGENETIC ANALYSIS

The COI gene-based phylogenetic tree was constructed using the sequences obtained here and those from the public GenBank database. Using Clustal X v2.0 (Larkin et al. 2007) and BioEdit v7.0.5 (Hall, 1999), all sequences were aligned and subsequently trimmed to obtain sequences of equal length. Utilizing MEGA 11 (Molecular Evolutionary Genetics Analysis), a maximum likelihood (ML) phylogenetic tree was constructed (Tamura et al. 2021). MEGA 11 determined that, based on Bayesian information criterion (BIC) scores, the best-fitting substitution model for COI was TN93+G. Intraspecific and interspecific genetic distance analyses were conducted by selecting different groups and then computing the mean distance within or between groups using MEGA 11 (Tamura et al. 2021).

Results

CHILO PARTELLUS EXPANSION TOWARD SOUTH-EASTERN TÜRKİYE

In total, 24 locations from South-eastern Türkiye maize fields were sampled to check the presence of *C. partellus* among which only 5 fields were infested. In total, three counties in Şanlıurfa, Türkiye were infested with the pest. The maize fields checked in Diyarbakır, Mardin, and Şırnak provinces were uninfested (Figure 1).



The total number of larvae + pupae counted was 1144 collected from 170 plants: with a total number of 451 (57 plants), 521 (75 plants), and 172 (38 plants) from Suruç, Akçakale, and Ceylanpınar, respectively. The pest was present in two of three locations in both Suruç and Akçakale and absent in two of three locations in Ceylanpınar. The mean numbers of the pest per plant were different between counties and the pest was equally more abundantly present in Suruç and Akçakale than in Ceylanpınar ($X^2_{(2)}=14.08$, $P<0.001$). Neither the contribution of the pest to the total number of stemborers per field ($X^2_{(2)}=0.54$, $P=0.763$) nor the contribution of *C. partellus* to total stemborer-dependent infestation rate of the pest per field differed between counties ($X^2_{(2)}=0.37$, $P=0.83$). Similarly, the infestation rate of the pest was not statistically different between counties ($X^2_{(2)}=0.39$, $P=0.82$) (Figure 2).

MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS

DNA was successfully extracted, amplified, and sequenced for three samples collected in South-

eastern Türkiye. Based on initial BOLD and GenBank investigations, all four sequences were identified as *C. partellus* (>99.0%) using the COI-I barcode gene. Sequences with 708 bp length were generated and submitted to NCBI under the accession numbers OQ154267 (Suruç, Türkiye), OQ154268 (Ceylanpınar, Türkiye), OQ154269 (Akçakale, Türkiye). In addition to sequences of this study (n=3), various *C. partellus* sequences (n=24) along with an outer reference sequence of another *Chilo* species, *C. diffusilineus* (Joannis, 1922) were downloaded from GenBank along with their accession numbers and trimmed to 539 bp which were, afterward, subjected to phylogenetic analysis. In total, 28 sequences were involved in the maximum likelihood tree. Identical topologies were discovered in the phylogenetic tree starting to discriminate two clades for two species i.e., the first branch that included all sequences of the species of interest, *C. partellus*, and the other branch consisted of the sequence of the outer reference species, *C. diffusilineus*. The *C. partellus* clade had two main groups, the first of which included our samples (n=3) and all African samples along with two Indian samples (bootstrapping support value=98%), and the second contained the specimens only from Asian countries, Pakistan and India (bootstrapping support value=80%) (Figure 3).

Discussion

A large-scale survey program to sample maize stemborer species in Southeastern Türkiye was carried out in recent years, 2018-2020 (TonĖa, 2021) which continued with surveys in 2021 and 2022. The stemborer species *Sesamia* spp. and *O. nubilalis* were recorded while *C. partellus* was never recorded until samplings in September 2022. During this study, in total, 5 locations from 3 counties from Şanlıurfa, were found infested. The attempts to check the status of the pest in the region confirmed its absence in other South-eastern provinces of Türkiye such as Diyarbakır, Mardin, and Şırnak.

A previous study reported the introduction of *C. partellus* into the eastern Mediterranean region of Türkiye assuming the invasion route of the pest was the Southern border as the pest was earlier reported in Israel, another southern country (Ben-Yakir et al. 2013; Bayram & TonĖa, 2016). The pest has arrived in Türkiye by infesting maize, sorghum, and uncultivated gramineous host plants along the Mediterranean line because of ideal climatic conditions, for example, warm and dry summers followed by humid and mild winters that constitute an ideal habitat for maize stemborers. Besides, the expansion of the pest towards South-eastern Türkiye suggests a definite ongoing expansion of the pest toward the large-scale maize cultivated lands. Further studies are scheduled to report its status in South-eastern Türkiye as the pest possess a threat to maize cultivation in infested and uninfested provinces. The pest has expanded its geographic distribution to the east since its introduction into Türkiye from the Mediterranean region. Therefore, the monitoring and survey studies should be performed in western regions of Türkiye such as the Aegean region, and Southeast European countries such as Greece.

In a very short time (last few months), the pest was able to establish its population in detected areas where other pests, such as *Sesamia* spp. and *O. nubilalis* already infest. The contribution of *C. partellus* and *Sesamia* spp. to stemborer-dependent plant infestation were not different and the remaining specimens were identified as *Sesamia* spp. while there were no *O. nubilalis* specimens in sampled fields even though previous efforts yielded the consistent presence of *O. nubilalis* in the southern counties in the region (TonĖa, 2021). Possibly, *C. partellus* dominated *O. nubilalis* which, for sure, requires further surveys targeting more fields. However, the invasive ability of the pest may result in interspecific competition with other stemborers because the contribution of *C. partellus* to the stemborer population and stemborer-dependent infestation rate reached higher than 50% levels in the sampled counties. Although this is the first year of invasion toward the region, the pest may likely dominate other stemborers as it has a faster life cycle, high level of population growth, and earlier niche

establishment when compared with other stemborers from the families Noctuidae and Crambidae of Lepidoptera (Moeng et al. 2018).

The altitudes of fields in which the pest was recorded were between 340-490 m as the lowest and highest respectively. The invasion of the pest in such altitudes is not surprising as it can survive in the zones with 1600 m elevation (Kfir et al. 2002). In this study, we were unable to record any natural enemies of the pest especially, the egg and larval parasitoids. However, previous studies reported the presence of egg parasitoid, *Trichogramma evanescens* with efficiency on the eggs of *Sesamia* spp. in Southeastern Türkiye (Tonğa & Bayram, 2021). The parasitoid is already known to parasitize *C. partellus* eggs in the Mediterranean region (Achiri et al. 2019) and the situation of larval parasitoids in Türkiye is unknown. Even though several braconids and ichneumonids were reported parasitizing larvae and pupae of the lepidopteran maize pests in Southeastern Türkiye, there is still a need for studies investigating their efficiency on *C. partellus* (Gözüaçık et al. 2009).

Accurate species identification of pests is one of the key components for effective pest control. The employment of the COI-I barcode gene has yielded successful identification and population genetics of stemborers (Donga & Meadow, 2018). We, based on a phylogenetic analysis of adult specimens, identified all samples as *C. partellus* using the COI-I barcode gene. The establishment of the pest species in Harran plain that include infested counties is very recent, and there was within-species genetic variation although the specimens were collected from maize fields in neighbouring counties. Similar clustering observed among reference African samples suggest the possibility of invasion of the South-eastern region by several lines altogether. Further efforts should be devoted to investigating whether further within-species variation occurs in the populations in the region in upcoming years as the pest may exhibit distinct biological and molecular responses to the recently invaded lands. Interestingly, among the African reference sequences (clade-1) are two Indian samples while no African samples clustered among Asian samples. The origin of the pest is Asia and the presence of African samples in Asia may suggest that the pest might have reversely invaded Asia from Africa after settlement of nearly a century (Kfir et al. 2002). The confirmation of this assumption requires specific attention.

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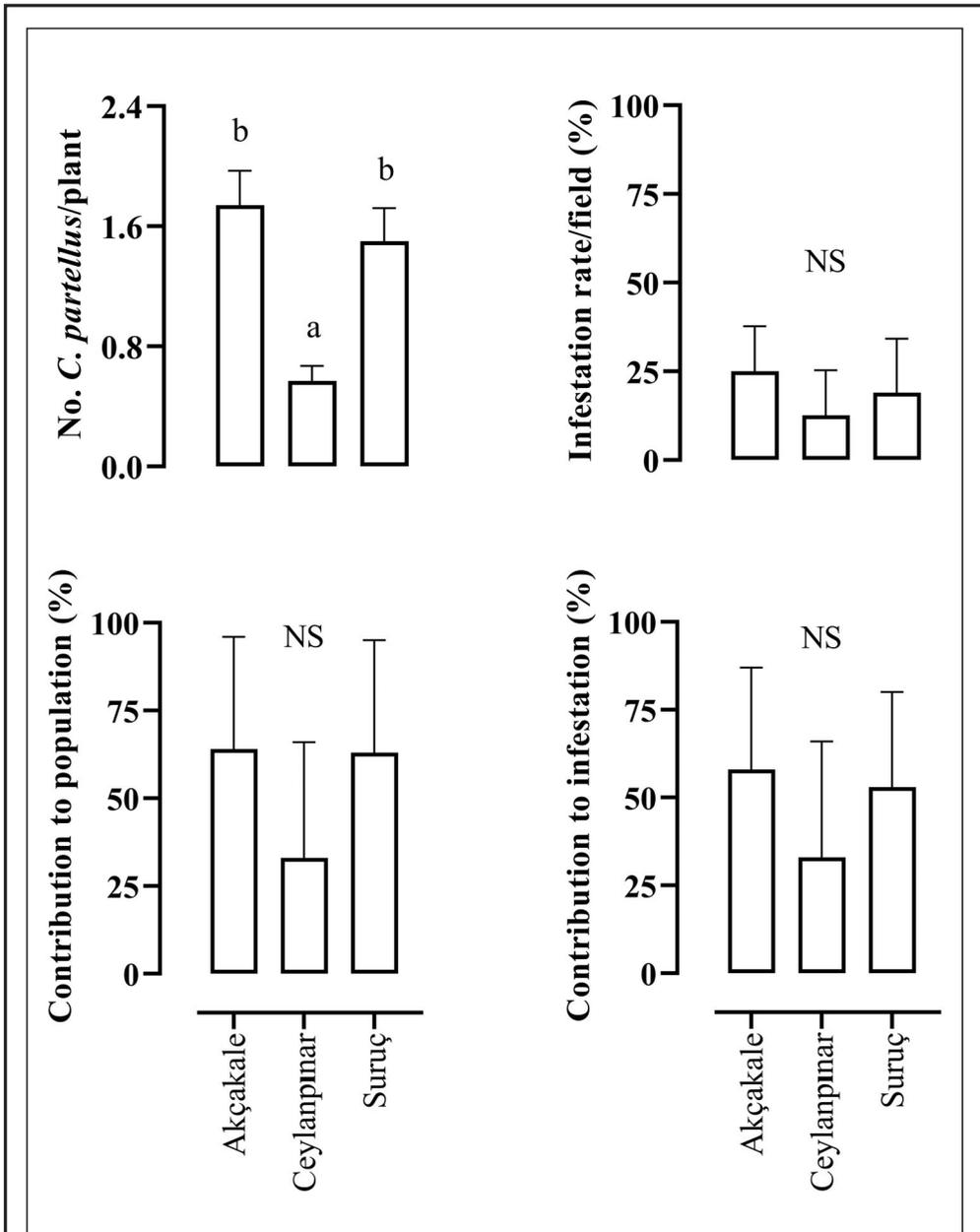


Figure 2. Mean number per plant, infestation rate per field, and contribution to field-scale stemborer population and contribution to field-scale stemborer-dependent infestation for *Chilo partellus*. Different letters on the top of the bars depict significant differences while NS represents insignificant results. Mean densities of the pest in counties were compared using Tukeys post hoc test

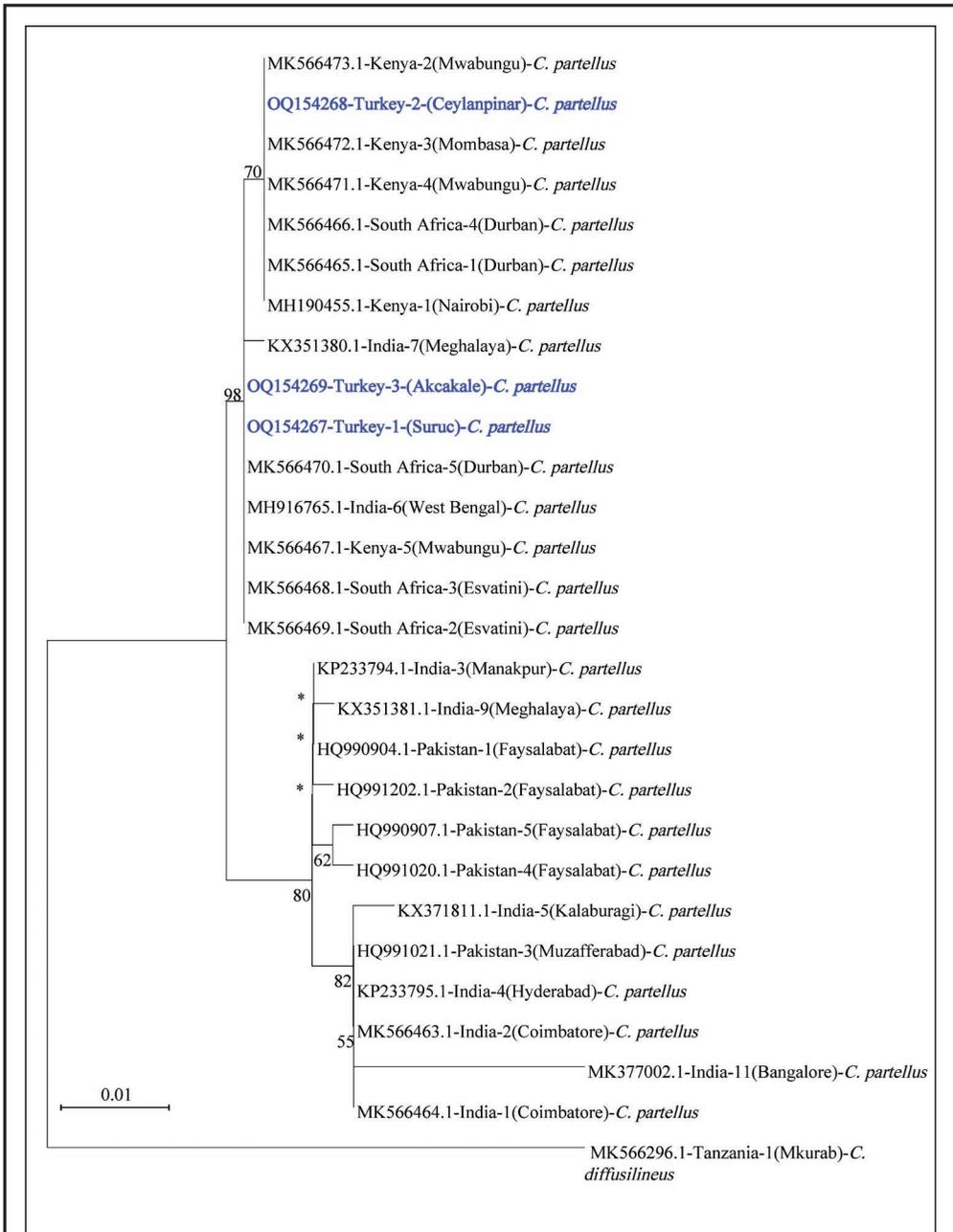


Figure 3. Maximum likelihood phylogenetic analysis of partial mitochondrial COI sequences of *Chilo partellus* specimens collected from maize fields in Türkiye along with sequences from other countries. Bootstrap values lower than 50 are not presented on branches (*). The reference species is *Chilo diffusilineus*.