



Original article (Orijinal araştırma)

Detection and identification of citrus long-horned beetle, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) a new pest in Antalya Province, Turkey by sequencing of mtCOI region¹

Antalya ilinde yeni bir zararlı Turunçgil uzun antenli böceği, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae)'in tespiti ve mtCOI gen bölgesi sekansına göre tanımlanması

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Summary

In 2016, *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) was recorded for the first time on *Acer negundo* L. in Antalya Province, Turkey. Genetic identification of adult samples was made based on approximate alignment of a 729-bp sequence of the mitochondrial cytochrome oxidase I gene region. The sequence alignment in the BLAST analysis from the NCBI Genbank showed 99% similarity with *A. chinensis*. The sequence data was deposited to NCBI Genbank. A phylogenetic analysis was performed by using the sequence data belonging to *A. chinensis* and some species of same genus obtained from the NCBI. According to the phylogenetic analysis, populations of *A. chinensis* from Antalya and China were placed in the same subgroup and therefore it is postulated that the origin of the Antalya population is China.

Keywords: *Anoplophora chinensis*, DNA barcoding, mtCOI, phylogenetic analysis

Özet

Bu çalışma ile *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae) Antalya ilinde ilk kez 2016 yılında *Acer negundo* L. bitkisi üzerinde tespit edilmiştir. Bununla birlikte, çalışmada *A. chinensis*'e ait ergin bireylerden DNA ekstraksiyonu yapılmıştır. Elde edilen DNA örneklerinin mitokondrial sitokrom oksidaz I gen bölgesinin amplifikasyonu sonucunda yaklaşık 729 bp sekans dizilimi elde edilmiştir. Elde edilen veriler, NCBI Genbank' da BLAST analizine tabi tutulmuş ve *A. chinensis* ile %99 benzerlik gösterdiği tespit edilmiştir. Sekans dizilimi NCBI' a kaydedilmiş, Genbank' ta *A. chinensis* ve aynı cinse ait bazı türlerin sekans verilerinden yararlanılarak filogenetik analiz yapılmıştır. Filogenetik analize göre Antalya *A. chinensis* popülasyonu Çin popülasyonu ile aynı alt grupta yer almış ve Antalya popülasyonunun kökeninin Çin olabileceği ağırlık kazanmıştır.

Anahtar sözcükler: *Anoplophora chinensis*, DNA barkodlama, mtCOI, filogenetik analiz

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Introduction

Being one of the largest families within the Coleoptera, the Cerambycidae includes over 11 subfamilies with more than 35,000 species in 4,000 genera (Lawrence, 1982). The citrus long-horned beetle, *Anoplophora chinensis* (Forster, 1771), a species in the Cerambycidae family, is native to China, Japan and North Korea (Ge et al., 2014). In June 2014, the pest was detected in Şile-Istanbul, which was the first record for Turkey (Hızal et al., 2015).

Adults are black with females (about 40 mm) larger than males (about 25 mm). The antennae have 11 segments, with white or light blue bands. The elytra are narrow in males, wider and rounded in females and there are many irregular white spots on the elytra. The legs are a light blue color (Figure 1).



Figure 1. Adults of *Anoplophora chinensis* (Forster, 1771).

The citrus long-horned beetle is distinguished by tubercles on the elytra from the Asian long-horned beetle, *Anoplophora glabripennis* (Motschulsky, 1853), which has a similar geographic distribution and causes similar damage (Hu et al., 2009). *Anoplophora malasiaca* (Thomson, 1865) is a synonym of *A. chinensis* (Lingafelter & Hoebeke, 2002).

The eggs of *A. chinensis* are on average 5 mm, elongate and creamy white. They turn yellowish brown when ready to hatch (Lieu, 1945). The larvae are legless and full-grown larvae are about 50 mm long. They are creamy white and with a yellowish chitinized pattern on the prothorax. The pupae are 27-38 mm long (Gyeltshen & Hodges, 2005) (Figure 2).

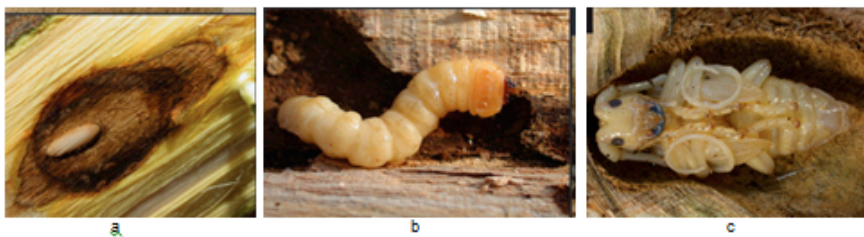


Figure 2. *Anoplophora chinensis* (Forster, 1771): a) Egg, b) larva and c) pupa (Haack et al., 2010).

Anoplophora chinensis commonly feeds on *Acer* spp., *Aesculus hippocastanum* L., *Alnus* spp., *Betula* spp., *Carpinus* spp., *Citrus* spp., *Cornus* spp., *Corylus* spp., *Cotoneaster* spp., *Crataegus* spp., *Fagus* spp., *Lagerstroemia* spp., *Malus* spp., *Platanus* spp., *Populus* spp., *Prunus laurocerasus* L., *Pyrus* spp., *Rosa* spp., *Salix* spp. and *Ulmus* spp. with *Casuarina* spp., *Cryptomeria* spp., *Ficus* spp., *Hibiscus* spp., *Litchi* spp., *Mallotus* spp., *Melia* spp. and *Morus* spp. among the others also recorded as hosts (Anonymous, 2013).

Females feeding and mating on young twigs on the upper parts of host plants move towards the bottom of the tree to search for a suitable place to deposit their eggs. T-shaped cracks are made in the bark during egg deposition with the ovipositor. The females make 3-4-mm long cuts in the bark with their mandibles. Only one egg is deposited into each wound. Eggs are usually deposited at the base of the trunk, root collars or exposed roots (Maspero et al., 2007). It can take one or two years to complete their life cycle under natural conditions and they adults usually live for about 1-3 months between May and August (Anonymous, 2013).

Adults cause damage by feeding on thin branches and leaves. The greatest damage is caused by the feeding of larvae. The freshly-hatched larvae feed on primarily cambium layers, and in time larvae feed on xylem tissues. Since damage to the xylem inhibits the flow of water and nutrients in the host tree, they cause death of branches and ultimately death of the whole tree. Through the attacks of the larvae, trees are weakened and become more susceptible to disease and wind damage (Sjöman et al., 2014).

The objectives of the present study were to identify the Antalya population of *A. chinensis* by molecular methods and to determine its evolutionary relationship with the sibling groups from different countries deposited in the NCBI Genbank database based on mtCOI sequences through phylogenetic analysis. To the best of our knowledge, this was the first study on the identification of *A. chinensis* based on mtCOI region in Turkey. The sequence information of the Antalya population was added to Genbank. Thus, this will contribute to genetic information about the insect and provide a reference source in different studies. In addition, brief information about the detection of the pest in Antalya is been provided.

Material and Methods

Determination of pests and obtaining pest samples

Upon the detection of adult pests on the Akdeniz University Campus on 3 June 2016, trees in the region were examined. Sampling was done randomly at irregular intervals. Male and female of the pest were identified mostly at the lower portions of the trunk and rarely at the top. Adult samples were collected, put into plastic bags, labeled and immediately brought to the laboratory for examination. Some of the samples were kept at -20°C for a short time before molecular analysis.

DNA extraction and PCR amplification

In DNA extraction studies, leg parts belonging to live or just dead insects were used as described by Muraji et al (2011). DNA was extracted in accordance with the CTAB protocol for invertebrates (Folmer et al., 1994) from three individuals. The isopropanol step in the CTAB protocol was modified to allow samples to be kept overnight before being dissolved in 40 µl distilled water.

For the amplification of the mitochondrial cytochrome oxidase I (mtCOI) gene, CI-J-2195 and TL2-N-3014 universal primers reported to amplify this gene in coleopteran insects (Simon et al., 1994) were used. In accordance with to Yükselbaba & Göçmen (2016), the conditions specified were modified (0.5 µl DNA in 24 µl). The PCR cycling protocol for mtCOI was 94°C for 5 mins, followed by 35 cycles (94°C for 50 s, 50°C for 50 s and 72°C for 45 s) and with a final extension at 72°C for 5 mins.

The PCR products obtained were sequenced in both directions by IONTEK (Istanbul, Turkey). Data acquired were inspected and the best sequence registered with NCBI (KX530204).

Phylogenetic analysis

The phylogenetic analyses were done by distance matrix using DNAdist program (Felsenstein, 1993). BLAST analysis for identification of species and seeking homologous *A. chinensis* sequence alignments was done with the NCBI database (<http://www.ncbi.nlm.nih.gov>). The multiple alignment analysis was done online by M-Coffee (<http://www.tcoffee.org>). To reconstruct the phylogenetic tree from evolutionary distance data, a phylogenetic tree was constructed according to the neighbor-joining method and Kimura two-parameter model (Saitou & Nei, 1987). Bootstrap was performed with 1000 repetitions to produce the phylogenetic tree. Species belonging to *Anoplophora* in the genbank used for phylogenetic analysis are detailed in Table 1.

Table 1. Organisms used from Genbank for sequence alignment

Genbank Accession No	Organism	Origin
KX530204	<i>Anoplophora chinensis</i>	Antalya, Turkey
AB500900	<i>Anoplophora chinensis</i>	Oita, Japan
AB533639	<i>Anoplophora chinensis</i>	Japan
AB500897	<i>Anoplophora chinensis</i>	Shizuoka, Japan
AB500901	<i>Anoplophora chinensis</i>	Hiroshima, Japan
AB500896	<i>Anoplophora chinensis</i>	Ibaraki, Japan
AB500902	<i>Anoplophora chinensis</i>	Kagoshima, Japan
KT726932	<i>Anoplophora chinensis</i>	Xiamen, China
AB533640	<i>Anoplophora macularia</i>	Japan
GU003937	<i>Anoplophora horsfieldi</i>	China
DQ768215	<i>Anoplophora glabripennis</i>	unknown
KF737826	<i>Anoplophora lurida</i>	unknown
EU599206	<i>Monochamus galloprovincialis</i>	unknown

Results

During sampling of damaged trees, both females and males were detected, however, no eggs or larvae were found. *Anoplophora chinensis*, native to China, Japan, and North Korea (Ge et al., 2014), was first recording in Antalya in this study. While adults and evidence of their feeding were rarely observed on the upper parts of trees, both females and males were mostly detected around the exit holes (6-10 mm diameter) near the base of the trunk (Figure 3); and it was observed that these adults could fly short distances when disturbed. Similarly, Haack et al. (2010) observed that exit holes were found within 50 cm of the base of the tree and in exposed roots.



Figure 3. Damage to *Acer negundo* L. by larvae and adults of *Anoplophora chinensis* (Forster, 1771).

Molecular studies

The amplified PCR products from the mtCOI gene were approximately 800 bp as expected for the primers used (Figure 4).

From the sequence analysis, an approximate 729 bp nucleotide alignment was obtained and registered to the NCBI Genbank (KX530204). The BLAST analysis showed 98-99% similarity with *A. chinensis* sequences, 96-97% with *Anoplophora macularia* (Thomson, 1865) and 93% with *A. glabripennis*.

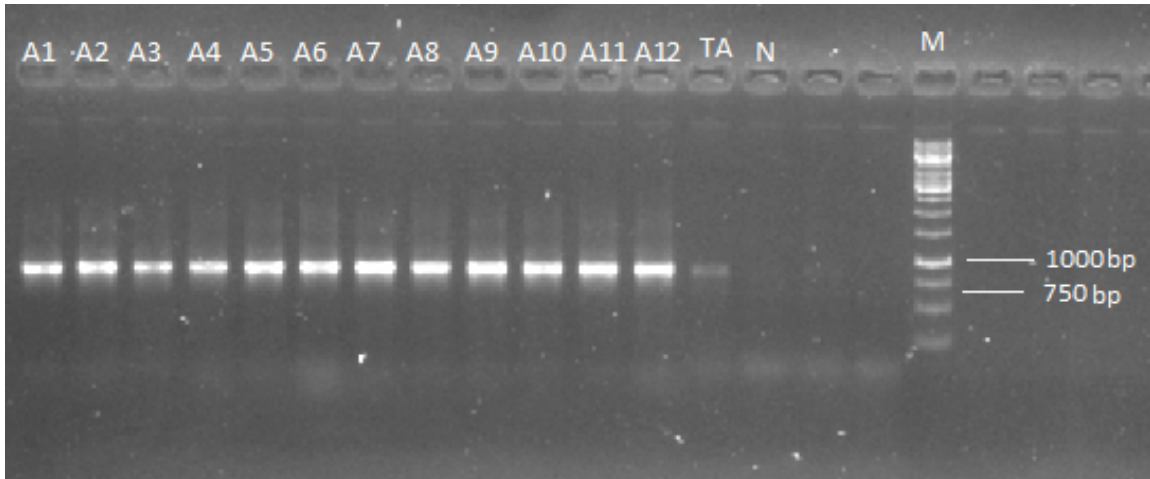


Figure 4. *Anoplophora chinensis* (Forster, 1771) PCR reaction image (1% TAE gel). A1-A12, *A. chinensis*; TA, *Tuta absoluta* (Meyrick, 1917) (for positive control); N, negative control; M, 1 kb marker.

According to the sequence alignments obtained from the Genbank, a phylogenetic tree was constructed (Figure 5). According to the neighbor-joining phylogenetic tree *A. macularia* and *A. chinensis* are in separate groups. Likewise, the Chinese and Japanese populations of *A. chinensis* are in different subgroups. The specimens from Antalya are placed in the same subgroup as those from China. The Japanese populations fall into two separate subgroups.

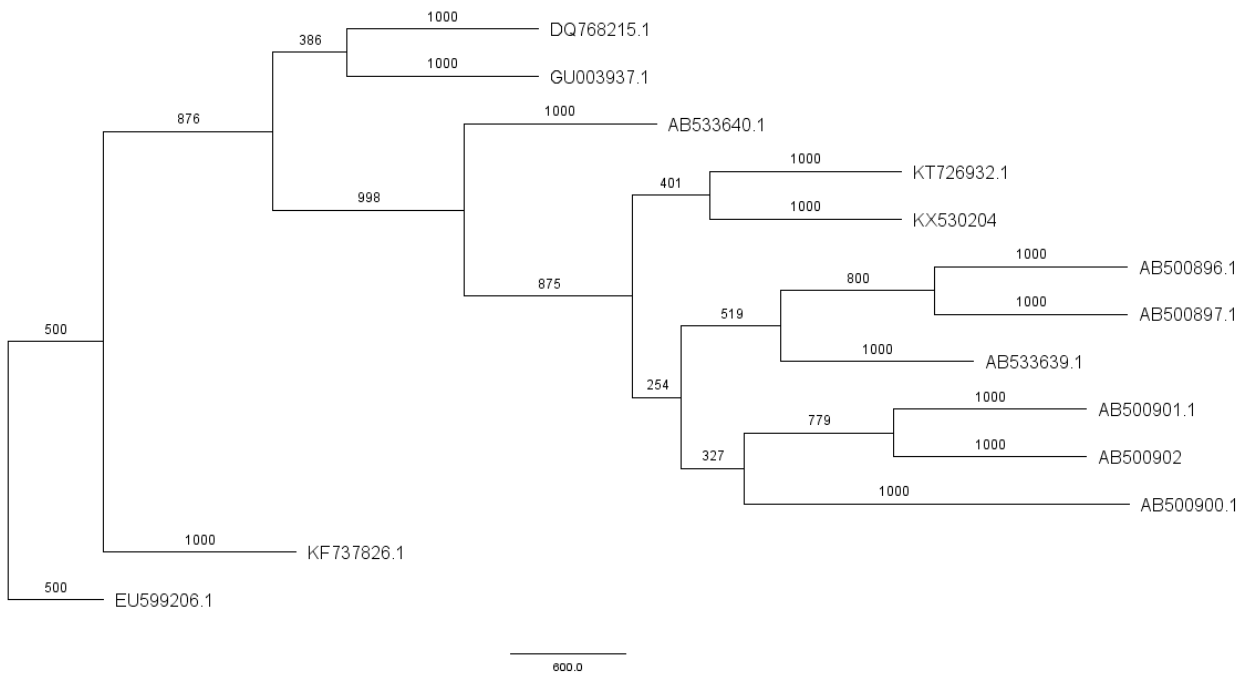


Figure 5. Phylogenetic tree from genbank sequences of *Anoplophora chinensis* (Forster, 1771) populations. The phylogenetic tree was constructed according to the neighbor-joining method and the Kimura two-parameter model with 1000 bootstrap replicates. The Genbank accessions numbers for each sample are as in Table 1.

Discussion

In this study, even though there are a variety of tree species (*Acer* spp., *Albizia julibrissin* Durazz., *Jacaranda mimosifolia* D. Don, *Melia azedarach* L., *Ailanthus* spp. and *Pinus* spp.) on the Akdeniz University Campus where the pest was detected, adult individuals and adult exit holes were only detected in *Acer negundo* L. In observations made in different areas of the province on the same dates, adult exit holes were also mostly found in *Acer* spp. (data not shown). These findings suggest that of the available tree, the first preference of the pest was *Acer* spp.. Similarly, in Europe, in some of the recorded cases, *A. chinensis* seems to attack *Acer* trees first (Herard et al., 2006, Peverieri & Roversi, 2010). Eradication methods for this new pest, *A. chinensis*, for our country are important. It is necessary to determine whether *A. chinensis* has caused any damage, particularly in locations from where plants are imported, and to avoid imports of plant, wooden material and timber from these locations. It is also important to eradicate adults when first detected, and to cut and remove trees intensely damaged by the pest. Potential host plants around trees on which the pest was detected should be examined carefully and any suspected ones should be removed (Hızal et al., 2015). Signs of the infestation include traces of egg laying in the bark (T-shaped cracks), existence of excrements from larvae activity and wood dusts, color change on the bark, deformations, bark grooves, galleries made by larvae, adult exit holes, damage from adult feeding, upward deaths in tree branches and death of whole trees. Adult exit holes occur in the lower 50 cm of the tree trunk, mostly at 15-20 cm (Anonymous, 2013, Maspero, 2015). Strangi et al. (2013) indicated that it would be useful for plant health research to be able to reliably detect *A. chinensis* infestation by molecular analysis of larval excrement and that this method could be used as a detection tool for plant inspection.

In their study on the mitochondrial genome of *A. chinensis*, Li et al. (2015) reconstructed the phylogenetic relations of 12 Lamiinae species based on mtCOI nucleotide sequences using the Bayesian inference method. They determined that the phylogenetic tree was divided into three major clades and that *Anoplophora* [*A. glabripennis*, *A. chinensis* and *Anoplophora lurida* (Pascoe, 1857)] and *Monochamus* [*Monochamus alternatus* Hope, 1842, *Monochamus urussovi* (Fisch., 1806) and *Monochamus sutor* (L., 1758)] were placed in the third lineage. Apart from such molecular studies supporting morphological studies in the Lamiinae, in another study, Muraji et al. (2011) determined that the genetic variation of species belonging to the *Anoplophora* in Japan was identified by mtDNA sequence and the species was split into two main groups. In the same study, they noted that a sample taken in East Honshu was the same as *A. chinensis* from China. Ohbayashi et al. (2009) studied the phylogenetic analysis of *Anoplophora* species and their relatives in the Lamiinae with respect to mtCOI gene region.

This study registered with Genbank the first mtCOI region sequence of *A. chinensis* from Turkey for a population from Antalya. Data recorded in Genbank can be used as a reference for further studies. Phylogenetic analysis was performed to determine that the Antalya population is likely to have originated from China not Japan. More complete knowledge of the dispersal mechanisms and genetic phylogeographical structure of *A. chinensis* is essential for developing effective management strategies.

References

- Anonymous, 2013. EPPO Standard PM 9/16 (1) *Anoplophora chinensis*: procedures for official control. OEPP/EPPO Bulletin, 43:518-526.
- Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3 (5): 294-299.
- Ge, X., S. Zong, S. He, Y. Liu & X. Kong, 2014. Areas of China predicted to have a suitable climate for *Anoplophora chinensis* under a climate-warming scenario. Entomologia Experimentalis et Applicata. 153 (3): 256-265.

- Gyeltshen, J. & A. Hodges, 2005. Citrus Longhorned Beetle, EENY-357. University of Florida: (Web page: <http://edis.ifas.ufl.edu/IN633>) (Date accessed: 11.05.2017).
- Haack, R. A., F. Hérard, J. Sun & J. J. Turgeon, 2010. Managing invasive populations of Asian longhorned beetle and citrus longhorned beetle: A worldwide perspective. *Annual Review of Entomology*, 55: 521-546.
- Herard, F., M. Ciampitti, M. Maspero, H. Krehan, U. Benker & C. Boegel, 2006. *Anoplophora* species in Europe: Infestations and management processes. *OEPP/EPPO Bulletin*, 36: 470-474.
- Hızal, E., Z. Arslangündoğdu, A. Göç & M. Ak, 2015. The new record for Turkish invasive alien insect fauna *Anoplophora chinensis* (Forster, 1771) (Coleoptera: Cerambycidae). *Journal of the Faculty of Forestry Istanbul University*. 65 (1): 7-10.
- Hu, J., S. Angeli, S. Schuetz, Y. Luo & A. E. Hajek, 2009. Ecology and management of exotic and endemic Asian longhorned beetle *Anoplophora glabripennis*. *Agricultural and Forest Entomology*, 11: 359-375.
- Lawrence, J. F., 1982. "Coleoptera, 482-553". In: *Synopsis and Classification of Living Organisms* (Ed. S. Parker). McGraw Hill, New York, 1232 pp.
- Li, W., X. Yang, L. Qian, Y. An & J. Fang, 2015. The complete mitochondrial genome of the citrus long-horned beetle, *Anoplophora chinensis* (Coleoptera: Cerambycidae). *Mitochondrial DNA*, Early Online: 1-3.
- Lieu, K.O.V., 1945. The study of wood borers in China I: Biology and control of the citrus-root-cerambycids, *Melanauster chinensis*, Forster (Coleoptera). *The Florida Entomologist*, 27: 61-101.
- Lingafelter, S. W. & E. R. Hoebeke, 2002. Revision of *Anoplophora* (Coleoptera: Cerambycidae). *Entomological Society of Washington, D.C.* 236 pp.
- Maspero, M., 2015. Managing Invasive Populations of *Anoplophora chinensis* and *A. glabripennis* in Lombardy Region. Dottorato Di Ricerca in Scienze e Tecnologie Agrarie, Ambientali e Alimentari. Alma Mater Studiorum - Università di Bologna, 76 pp.
- Maspero, M., G. Cavalieri, G. D'Angelo, C. Jucker, M. Valentini, M. Colombo, F. Herard, J. Lopez, N. Ramualde, M. Ciampitti, G. Caremi & B. Cavagna, 2007. *Anoplophora chinensis* - Eradication programme in Lombardia (Italy), European and Mediterranean Plant Protection Organization (Web page: http://www.eppo.int/QUARANTINE/special_topics/anoplophora_chinensis/chinensis_IT_2007.htm) (Date accessed: 19.09.2015).
- Muraji, M., S. Wakamura, H. Yasui, N. Arakaki, Y. Sadoyama, S. Ohno, & K. Matsuhira, 2011. Genetic variation of the white-spotted longicorn beetle *Anoplophora* spp. (Coleoptera: Cerambycidae) in Japan detected by mitochondrial DNA sequence. *Applied Entomology and Zoology*, 46 (3): 363-373.
- Ohbayashi, N., J. Ogawa & Z. H. Su, 2009. Phylogenetic analysis of the Lamiinae genus *Anoplophora* and its relatives (Coleoptera: Cerambycidae) based on the Mitochondrial COI Gene. *Special Bulletin of the Japanese Society of Coleopterology*, 7: 309-324.
- Peverieri, G. S. & P. F. Roversi, 2010. Feeding and oviposition of *Anoplophora chinensis* on ornamental and forest trees. *Phytoparasitica*, 38 (5): 421-428.
- Saitou, N. & M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.
- Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu & P. Flook, 1994. Evaluation, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87: 651-701.
- Sjöman, H., J. Ostberg & J. Nilsson, 2014. Review of host trees for the wood-boring pests *Anoplophora glabripennis* and *Anoplophora chinensis*: An urban forest perspective. *Arboriculture & Urban Forestry*, 40 (3): 143-164.
- Strangi, A., G. Sabbatini Peverieri & P. F. Roversi, 2013. Managing outbreaks of the citrus long-horned beetle *Anoplophora chinensis* (Forster) in Europe: molecular diagnosis of plant infestation. *Pest Management Science*. 69 (5): 627-63.
- Yükselbaba, U., H. Göçmen, 2016. Determination of genetic variation of tomato leafminer [*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)] populations from West Mediterranean Region of Turkey based on mitochondrial cytochrome oxidase I (mtCOI). *Mediterranean Agricultural Sciences*, 29 (1): 5-7.