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Original article

Molecular and morphological identification of the invading *Xylosandrus compactus* (Coleoptera: Curculionidae: Scolytinae) and novel host plant records for Türkiye

İstilacı *Xylosandrus compactus*'un (Coleoptera: Curculionidae: Scolytinae) moleküler ve morfolojik tanısı ve Türkiye için yeni konukçu bitki kayıtları

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ABSTRACT

The polyphagous invasive pest *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) has recently been reported in Türkiye. This species causes economic losses for woody ornamental and agricultural plants and forest trees in the lands where it invaded and settled. It should be considered that this pest has a pretty high rate of spread, and its control relies on accurate identification. This species could be distinguished from other ambrosia beetles using both morphological and molecular characteristics provided by the present study. The mtDNA gene region from some specimens collected in Türkiye was used for molecular identification and compared to "barcode" sequences already present in NCBI databases. COI sequences from *X. compactus* individuals obtained from eight distinct host plant species were used in phylogenetic analysis. More research into the genetic diversity of this ambrosia beetle's native and introduced areas is required to understand the origin of this biological invasion in Türkiye. Since its definition in Türkiye, *X. compactus* has been detected in 32 host plant species, and this study has increased that number to 40 by identifying eight new host plant species. Except *Hydrangea macrophylla* (Thunb.) Ser. and *Fraxinus ornus* L., six new species have been added to the world host plant species list for *X. compactus*.

INTRODUCTION

The black twig borer, *Xylosandrus compactus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) is native to Southeast Asia (Chong et al. 2009, Wood 1982), and has been introduced to other continental countries, probably through

the trade of plants and wood. Due to their inhabiting wood tissue, these insects are readily transported to new habitats via timber, wooden pallets, or packaging materials, where their presence can be undetected (Haack 2001, 2006, Lantschner et al. 2020, Mandelshtam et al. 2018). Therefore, cargo shipments represent the principal vector responsible for the extensive spreading of these insects (Brockerhoff et al. 2006). *X. compactus* was found for the first time in Europe in 2011 in Italy (Garonna et al. 2012, Pennacchio et al. 2012), then it spread to other European countries. It is a polyphagous invasive pest of woody plants which has recently been reported from Türkiye (Hızal et al. 2023).

It is determined that the adult females of *X. compactus* are known to attack potential host plants. The beetle characteristically penetrates twigs and small branches, resulting in extensive dieback (Anonymous, 2017). Additionally, this beetle maintains the transportation, inoculation, and cultivation of certain fungal species, including sources of nutrients and various plant pathogenic fungal taxa, into their galleries. The combined impact of colonised insects and pathogenic fungi may represent a further stress factor in attacked plants, which gives rise to concerns from a phytosanitary perspective (Benvenuti et al. 2021, Vannini et al. 2017). It is established that the feeding activities of *X. compactus* have a harmful effect on economically significant plant species, including coffee and cocoa, in the southern hemisphere (Egonyu et al. 2009, Kagezi et al. 2014). The annual economic impact of *X. compactus* on Uganda's coffee production was estimated to be $40 million US in a study conducted by Kagezi et al. (2015). *Laurus nobilis* L. (Francardi et al. 2012) and *Ceratonia siliqua* L. (Gugliuzzo et al. 2019), two species native to the Mediterranean with commercial value, have been identified as preferred host plants. In Türkiye, the economically important species that serve as hosts for *X. compactus* include the laurel (*L. nobilis*), silver linden (*Tilia tomentosa* Moench), chestnut (*Castanea sativa* Mill.), and apple (*Malus domestica* (Suchow) Borkh.) (Acer et al. 2023).

Concerning their small size and remarkably similar appearance, identifying bark and ambrosia beetles using morphological characteristics is difficult and time-consuming, requiring professional abilities (Wood 1986). Furthermore, immature insect stages and limited intercepted samples restrict morphological identification, making identification more challenging. Accurate taxonomic and biogeographic information is crucial for effective phytosanitary monitoring and control of invasive species (Kirkendall and Faccoli 2010, Knižek 2007). In addition, to manage present and future invasions, it is critical to understand the source populations and methods by which invasive species spread to new locations (Hulme 2009, Storer et al. 2017). Molecular methods allow us to reconstitute these circumstances (Le Roux and Wieczorek 2009, Lees et al. 2011).

Environmental variables, development stage of the insect, and the host plant can affect all biochemical markers. Molecular markers, then, seem to be comparatively more dependable techniques (Folmer et al. 1994) and are effective instruments for assessing relationships at the population level. Because of the high evolutionary rate, mitochondrial DNA regions are methodically used as a DNA barcoding system for the identification of animals (Hebert et al. 2004). Due to it being inherited from the mother and having greater variability at the silent sites than nuclear DNA, it is more valuable in phylogenetic and evolutionary analyses (Kim et al. 2000, Kiran et al. 2019). Moreover, mitochondrial genes have several characteristics, including the greater number of copies compared to those in the nucleus (Holland and Parsons 1999), the absence of introns and the presence of many primers (Hebert et al. 2004), maternal inheritance and ease of use (Harrison 1989), and the relatively rapid divergence rates (this feature allows it to be widely used as a genetic marker in insects, both at the species and higher levels) (Simon et al. 1994).

By using the COI gene as a molecular tool, Kiran et al. (2019) summarised the phylogenetic relationships of the collected population of *X. compactus*. In this study, samples of *X. compactus* collected from different species of woody host plants species in İstanbul, Türkiye were subjected to mitochondrial cytochrome oxidase I (COI)-based identification for the first time in Türkiye. Moreover, this study provided details on the morphological characteristics and novel host plants of *X. compactus* found in Türkiye.

MATERIALS AND METHODS

Collection of plant and insect material

In the 2023-2024 period, surveys of parks and gardens in Istanbul revealed the presence of wilted and dried twigs with insect entrance holes in multiple species of plants. The locations where the specimens were collected are illustrated in the Figure 1, and the collection dates are provided in Table 1. The plants were subsequently harvested, placed in plastic containers, and transported to the laboratory for further analysis. Only adult females of *X. compactus* can fly and attack potential host plants. They usually infest twigs and small branches, which are 1-3-year-old twigs up to a maximum diameter of 4-6 cm (Anonymous 2017). Females lay eggs in the breeding chamber, larvae develop, and then, pupae and adults appear in this chamber. Mating is between siblings (Entwistle 1964). Morphometric analyses were

performed on 4 male and 25 female adult individuals from a single breeding chamber of each of the fourteen host plant species. After this, 16 adult female individuals were selected from the same breeding chambers of eight host plant species that had been utilised for morphological identification and underwent DNA analyses. In total, 45 individuals were analysed in this study.

Morphological identification

During field surveys, wilted and dried twigs with insect entrance holes were collected and transported to the laboratory in plastic containers. Twigs were longitudinally dissected with the help of a scalpel to expose the tunnels of insect larvae, pupae, and adults. Diagnostic structures

Table 1. Locations and dates of collection for *Xylosansdrus compactus* and its host plants

Family	Species	Locations	Collection date
Anacardiaceae	*Pistacia terebinthus* L.	Burgaz Ada-Adalar (1)	08.10.2023
Buxaceae	*Buxus sempervirens* L.	Yıldız Grove-Beşiktaş (3)	10.10.2023
Calycanthaceae	*Chimonanthus praecox* (L.) Link	İÜC Faculty of Forestry Campus-Sarıyer (7)	18.09.2023
Fabaceae	*Cergis siliquastrum* L.	Emirgan Grove-Sarıyer (4)	10.10.2023
Fagacea	*Quercus frainetto* Ten.	İstanbul Regional Directorate of Forestry-Maslak Fatih Forest Campus - Sarıyer (6)	20.09.2023
	Castanea sativa Mill.	İstanbul Regional Directorate of Forestry-Maslak Fatih Forest Campus-Sarıyer (6)	20.09.2023
	Fagus orientalis Lipsky	Emirgan Grove-Sarıyer (4)	10.10.2023
Garryaceae	*Aucuba japonica* Thunb.	İÜC Faculty of Forestry Campus-Sarıyer (7)	18.09.2023
Hydrangeaceae	*Hydrangea macrophylla* (Thunb.) Ser.	Atatürk Urban Forest-Sarıyer (5)	20.09.2023
Lauraceae	*Laurus nobilis* L.	Validebağ Grove-Üsküdar (2)	12.10.2023
Magnoliaceae	*Magnolia grandiflora* L.	Yıldız Grove-Beşiktaş (3)	10.10.2023
Oleaceae	*Fraxinus ornus* L.	İÜC Faculty of Forestry Campus-Sarıyer (7)	18.09.2023
Rosaceae	*Spiraea japonica* (L.) Desv.	Atatürk Urban Forest-Sarıyer (5)	20.09.2023
	Malus domestica (Suchow) Borkh.	İÜC Faculty of Forestry Campus-Sarıyer (7)	18.09.2024

Figure 1. The sites where the specimens were collected (Anonymous, 2024)

were examined and documented from 25 adult females and 4 adult males using Leica S8APO stereomicroscope. Specimens, preserved in 96% ethanol, were air-dried, mounted on stubs, and coated with a gold-palladium (Au/Pd) and then imaged using a FEI Quanta FEG 250 scanning electron microscope (SEM) set at 20 kV.

Molecular identification

Molecular identification was made on beetle specimens obtained from 8 different woody plants: *B. sempervirens, C. sativa, C. siliquastrum, F. orientalis, L. nobilis, M. grandiflora, M. domestica*, and *P. terebinthus*. The DNA isolation was performed on two samples collected from each host plant. The DNA of each *X. compactus* specimen was extracted using three legs from each specimen. The legs of the specimens were ground with liquid nitrogen using a mortar and pestle, and the resulting powder was transferred to a microcentrifuge tube. Genomic DNA was extracted employing a commercial kit (PureLink Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The nucleic acid solution was subsequently adjusted to a final volume of 50 µl and stored at -20 °C.

Amplification of the COI gene region in the mitochondrial DNA of *X. compactus* was performed using primers C1J1718/C1N2191. A total of 25 µl was used for each PCR amplification, which included 0.2 µM of each primer and 12.5 µl of commercially available, ready-to-use Master Mix (Dream Taq Hot Start Green PCR Master Mix, Thermo Scientific, USA). The thermal cycling conditions were as follows: in the initial denaturation step at 94 °C for 3 minutes, followed by 35 cycles each consisting of 94 °C for 30 seconds, an annealing step at 50 °C for 30 seconds, an extension step at 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes. The reactions were performed in a C1000 Touch™ Thermal Cycler (Bio-Rad, USA). Products of the PCR process were electrophoresed on a 1.5% agarose gel and seen using a gel documentation system. Amplicons from each sample were purified employing the GeneJet Gel Extraction Kit (Thermo Scientific, USA) following the instructions provided by the manufacturer and sequenced in both directions with the use of amplification primers (BMLabosis, Ankara, Türkiye). The raw sequence data from both reads were controlled to identify ambiguous peaks. The De Novo Assemble plugin in Geneious 2020.0.3 software was used to process and align the forward and reverse sequences into a final consensus sequence (https://www.geneious.com).

The final sequences of the specimens were checked against reference sequences stored in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For the genotyping, the final sequences of each specimen were aligned with all other related sequences using the ClustalW algorithm in MEGA X (Kumar et al. 2018).

Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method with bootstrap values (1000 replicates) in MEGA X. The optimal DNA substitution model for ML was identified using the Akaike Information Criterion (AIC) algorithm, which selected the GTR+G+I model using jModeltest v.0.1.1 (Posada 2008). Haplotype analyses, nucleotide composition and other tests were calculated using DnaSP v.5.1 (Librado and Rozas 2009). The PopART software was employed to construct haplotype networks to determine the correlations between the *X. compactus* and *X. crassiusculus* haplotypes with the default parameters (Leigh and Bryant 2015).

RESULTS AND DISCUSSION

A total of 25 female individuals were measured at 1.4–1.7 mm in length, exhibiting a body form that was longer than its width. The body was brown to dark brown, with the antennae and legs showing a yellowish. The antennae were composed of five funicular segments, and the antennal clubs were obliquely truncated, with the first segment covering the entire posterior face. The pronotum is of equal length and width. The basal pronotum exhibited a dense patch of short, erect setae. The elytra were observed to be 1.3 times longer than their wide and 1.3 times longer than the pronotum. The elytral disc displayed a gradual curve into a convex shape and exhibited six punctate striae with hair-like setae (Figure 2). A total of eight adult male individuals were measured. The adult males were observed to be smaller than the females (0.8–1.0 mm), with a length-to-width rate of approximately 2:1. It was observed that the species was rarely found in twigs. The pronotum was observed to lack a ridge and to have no wings. The spicules were observed to be barely appreciable, almost obsolete. The elytra were observed to have irregular spotting.

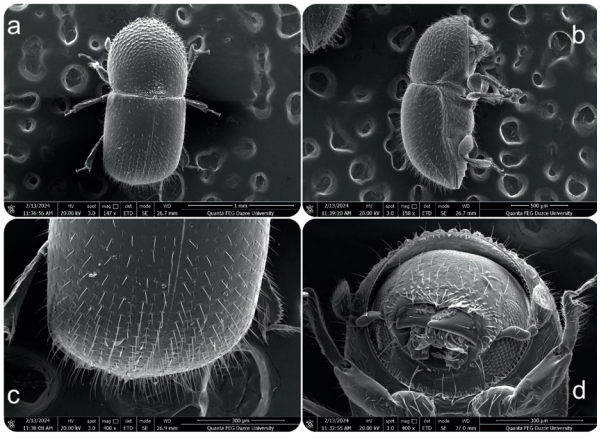

Figure 2. Scanning electron microscope (SEM) photos of female *Xylosandrus compactus* a) overall appearance, b) lateral appearance, c) elytral declivity, d) frontal appearance and antenna

The morphological taxonomic keys created by Dole and Cognato (2010), Gomez et al. (2018), and Smith et al. (2019) were used to identify beetle specimens collected from various woody host plants. Given the absence of morphological variations among the specimens collected from diverse locations in Istanbul, they were all identified as *X. compactus*. This species can be readily distinguished from the *X. germanus* (Blandford), a species of the *Xylosandrus* genus (Ak et al. 2011), which was previously detected in Türkiye, by its 1.36 times smaller size.

The partial COI gene region was successfully amplified from eight *X. compactus* specimens, and sequence analysis revealed that all isolates were completely identical and represented a unique haplotype (TR-Xcomp). The identified haplotype sequence was deposited in GenBank under the accession number OR994505. A comparison of the TR-Xcomp haplotype with existing data revealed a 100% similarity to previously identified haplotypes from Italy (accession numbers OR669531 and MW532748), Spain (MW961427) and France (OK489329). Analysis of the COI data set, constructed with the obtained sequence (n=1) and related *X. compactus* sequences (n=12), were represented totally 5 haplotypes. No insertions, deletions or stop codons were determined. The AT and GC content of the COI dataset was 63.3% and 36.7%, respectively. Haplotype diversity, Tajima's D, and Fu's F values were determined as 0,79, 0,58 and 6,63, respectively. It was also not significant statistically ($p > 0.05$) for Tajima's D or Fu's F in any of the *X. compactus* specimens. Haplotype networks were separated for each group (Figure 3). Phylogenetic tree was constructed by using ML analyses with nucleotide substitution model GTR+G+I and are shown in (Figure 4). The amplified COI gene sequences clearly separated all the haplotypes. Our haplotype coded with TR-Xcomp was grouped with haplotype 1. Five haplotypes are separated from each other by high bootstrap value (96%-100%). *X. crassiusculus* haplotypes were clearly differed from *X. compactus* haplotypes.

Kiran et al. (2019) identified all specimens as a single species, *X. compactus*, based on COI sequence analysis in their study. Similarly, the present study determined that all the samples collected from eight disparate host plants belonged to a single haplotype (GenBank accession number: OR994505, in the study, it was stated as TR-Xcomp in haplotype 1). The DNA sequence of *X. compactus* adults showed 100% identity with other available sequences of the haplotype 1 group. Our results represented that the COI region has potential as an identification tool for the diagnosis of this insect.

As a result of the field and laboratory research, the list of *X. compactus* host plant species has increased by eight, totalling 40 plants (Table 2).

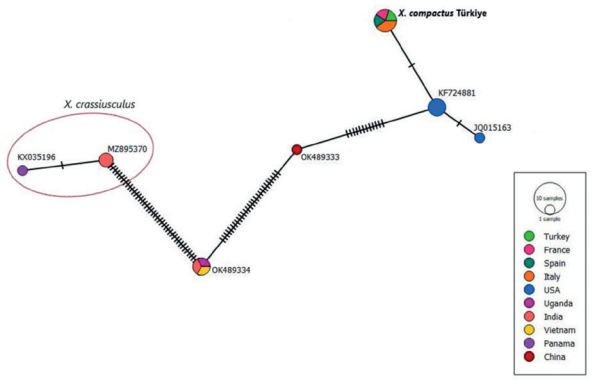

Figure 3. TCS haplotype network for the *Xylosandrus compactus* and *Xylosandrus crassiusculus* COI gene sequences. Between haplotypes, each vertical line represents a single mutation. The circles' sizes correspond to the frequency of haplotypes. The sampled nations are colour-coded

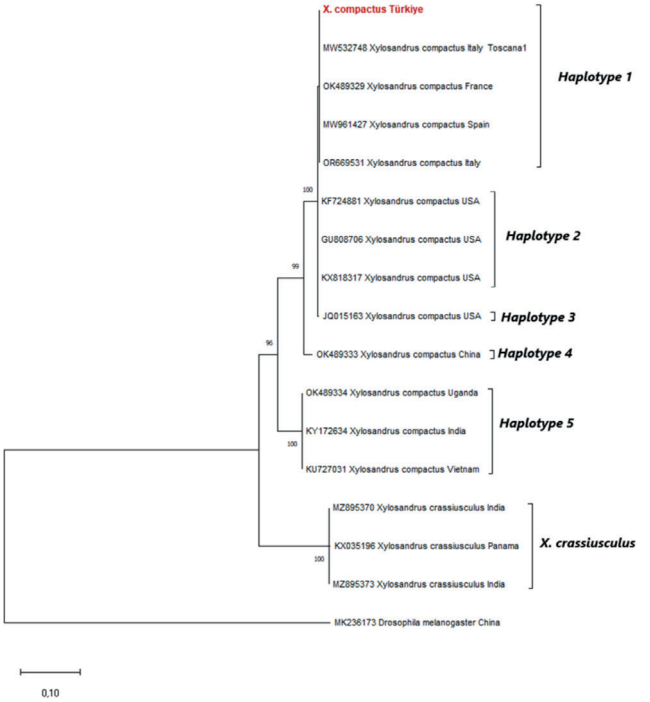

Figure 4. Phylogenetic analysis of *Xylosandrus compactus* specimens. Tree based on the 16 COI sequences belonging to haplotypes available in GenBank and newly characterized one haplotype in this study (in red). The *Drosophila melanogaster* (MK236173) haplotype was used as an outgroup

It is established that *X. compactus* is responsible for inflicting damage upon approximately 340 plant species belonging to 77 families across the world (Hızal et al. 2023). In a recent report by Acer et al. (2023), it was stated that 32 species of ornamental, agricultural, and forest plants have been affected in Türkiye. During the field and laboratory investigations in this study, eight new plants were added to

Table 2. New host plants of *Xylosandrus compactus* for Türkiye

Family	Species	Collection Dates	Life stages		
			Larvae	Pupae	Adult
Anacardiaceae	*Pistacia terebinthus*	08.10.2023	-	+	+
Calycanthaceae	*Chimonanthus praecox*	18.09.2023	-	+	+
Fagaceae	*Quercus frainetto*	20.09.2023	-	-	+
	Fagus orientalis	10.10.2023	-	+	+
Garryaceae	*Aucuba japonica*	18.09.2023	-	-	+
Hydrangeaceae	*Hydrangea macrophylla*	20.09.2023	+	+	+
Oleaceae	*Fraxinus ornus*	18.09.2023	-	-	+
Rosaceae	*Spiraea japonica*	20.09.2023	+	+	+

the *X. compactus* host list, bringing the total number of host plants to 40. The world host plant species list for *X. compactus* does not include any of the hosts recorded in the current study, except *H. macrophylla* and *F. ornus*. It is projected that the distribution areas of this pest will expand in conjunction with global climate change, indicating a need for future surveys in additional areas and on additional hosts.

Author's Contributions

The authors declare that their contributions are as follows: Sabiha ACER (30%), Fahriye ERCAN (20%), Erdem HIZAL (30%), Sevcan ÖZTEMİZ (10%), and Nuri Ercan ÖZTEMİZ (10%).

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Polifag istilacı zararlı *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) yakın zamanda Türkiye'de bildirilmiştir. Bu tür, istila ettiği ve yerleştiği topraklarda odunsu süs ve tarım bitkileri ile orman ağaçları için ekonomik kayıplara neden olur. Bu zararlının oldukça yüksek bir yayılma oranına sahip olduğu ve kontrolünün doğru tanımlamaya dayandığı göz önünde tutulmalıdır. Bu tür, mevcut çalışmada sağlanan morfolojik ve moleküler özellikler kullanılarak diğer ambrosya böceklerinden ayırt edilebilir. Türkiye'de toplanan bazı örneklerden alınan mtDNA gen bölgesi, moleküler tanımlama için kullanılmış ve NCBI veri tabanlarında hali hazırda mevcut olan "barkod" dizileriyle karşılaştırılmıştır. Sekiz farklı konukçu bitki türünden elde edilen *X. compactus* örneklerinden alınan COI dizileri filogenetik analizde kullanılmıştır. Türkiye'deki bu biyolojik istilanın kökenini anlamak için bu ambrosia böceğinin doğal bulunduğu ve sonradan girdiği alanların genetik çeşitliliği hakkında daha fazla araştırma yapılması gerekmektedir. Türkiye'de tespit edilmesinden bu yana *X. compactus* 32 konukçu bitki türünde belirlenmiş olup, bu çalışmada sekiz yeni konukçu bitki türünün tanımlanmasıyla bu sayı 40'a çıkmıştır. *X. compactus* için Dünya konukçu tür listesine *Hydrangea macrophylla* (Thunb.) Ser. ve *Fraxinus ornus* L. hariç altı yeni tür eklenmiştir.

Anahtar kelimeler: ambrosiya böcekleri, moleküler tanılama, COI sekansı, konukçu bitki, istilacı türler

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