SEEFOR SOUTH-EAST EUROPEAN FORESTRY

OPEN ACCESS
ISSN 1847-6481
eISSN 1849-0891

ORIGINAL SCIENTIFIC PAPER

DOI: https://doi.org/10.15177/seefor.25-10

First Record of *Cnestus mutilatus* (Coleoptera, Curculionidae) in Croatia

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Citation: Jukić A, Cvetković F, Krcivoj T, Medak J, Zorić N, Ćelepirović N, Matošević D, 2025. First Record of *Cnestus mutilatus* (Coleoptera, Curculionidae) in Croatia. *South-east Eur for* 16(1): 9-14. https://doi.org/10.15177/seefor.25-10.
Received: 1 Apr 2025; **Revised:** 26 May 2025; **Accepted:** 27 May 2025; **Published online:** 10 June 2025

ABSTRACT

Non-native ambrosia beetles (Coleoptera; Curculionidae; Scolytinae) have been recognized as pests in introduced areas that can significantly influence tree and forest health. *Cnestus mutilatus* (Coleoptera, Curculionidae) is native to Asia and is polyphagous, attacking wide range of deciduous forest trees. Our research has found *C. mutilatus* in traps and on branches in a forest stand in Pleternica in Croatia on *Fagus sylvatica, Quercus rubra* and *Carpinus betulus* in 2024 and 2025. Exit holes, adult galleries and the presence of adult beetles were recorded on branches with diameters ranging from 10 to 25 mm. Adults were also caught in traps baited with a pheromone lure for *Agrilus* spp. This is the first record of *C. mutilatus* in Croatia. Raised population levels of *C. mutilatus* could negatively influence young plants used in forest regeneration. Monitoring will be continued in the affected area, with enhanced data collection through the deployment of pheromone traps in the canopy.

Keywords: ambrosia beetle; alien species; polyphagous; *Fagus sylvatica; Quercus rubra; Carpinus betulus*

INTRODUCTION

Ambrosia beetles (Coleoptera; Curculionidae; Scolytinae) are unique among bark beetles because they do not feed on wood. They live in nutritional symbiosis with ambrosia fungi, boring into trees, making tunnels where they cultivate fungi, their sole source of nutrition. Most ambrosia beetles are not primary tree-killers, but instead they are targeting stressed or dead plants (Rassati et al. 2016).

However, a concerning trend has emerged with a subset of ambrosia beetles, particularly those that have been introduced to regions outside their native range. These non-native species have transitioned from primarily colonizing deadwood to aggressively attacking and killing healthy trees across a wide variety of forest ecosystems globally (Brockerhoff et al. 2006). This shift in behavior has transformed these beetles from benign decomposers to significant pests capable of causing substantial ecological and economic damage (Aukema et al. 2011). The consequences of these invasions are far-reaching, encompassing tree mortality on a large scale, a decline in native biodiversity, and significant financial burdens for various sectors (Brockerhoff et al. 2010).

Ambrosia beetles such as *Xylosandrus germanus* (Blandford), *X. compactus* (Eichhoff), and *X. crassiusculus* (Motschulsky) have been observed attacking healthy trees in regions where they have been introduced as alien species (Six et al. 2009). While some of their symbiotic fungi are harmless, others can be pathogenic causing rapid plant death (Oliver et al 2012, Viloria et al. 2021). *Cnestus mutilatus* (Blandford) (Coleoptera, Curculionidae), also known as camphor shoot borer, is an ambrosia beetle native to Asia and it is recognized as a significant pest in its native range affecting several tree hosts (Colombari et al. 2022). *C. mutilatus* is undergoing a rapid range expansion throughout North America (Gandhi et al. 2009, Oliver et al. 2012, Coyle et al. 2015).

The aim of this paper is to report the first record of *C. mutilatus* in Croatia. While previous records of *C. mutilatus* in Europe have been limited to urban or peri-urban environments, our research documents its occurrence in a natural forest stand. This paper presents a detailed account of the beetle's identification and the environmental conditions of the discovery site, and discusses the potential pathway of its introduction.

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MATERIALS AND METHODS

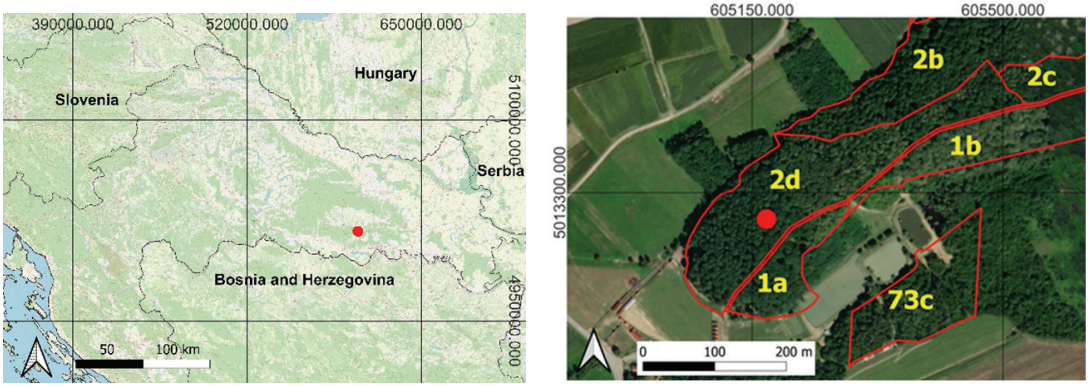
Site Description

Research site was located in Pleternica (Požega-Slavonia County), Sjeverni Dilj Pleternički Management Unit (Figure 1), in Croatia. Monitoring trap was positioned in the southern portion of the management unit (HTRS 96: N 605204.2, E 5013277.7). This is an old mixed stand dominated by red oak (*Quercus rubra* L.) and sessile oak (*Quercus petraea* Matt.), with small-leaved lime (*Tillia cordata* Mill.), common hornbeam (*Carpinus betulus* L.), Turkey oak (*Q. cerris* L.), beech (*Fagus sylvatica* L.), wild cherry (*Prunus avium* L.) and service tree (*Sorbus torminalis* L.). Prior to the storm in the summer of 2023 the stand was generally in good health, featuring tall trees with a fully closed canopy.

Trapping and Collection Method

As part of the yearly quarantine pest surveillance program in forests in Croatia, a trap was deployed for the regular monitoring of the goldspotted oak borer (*Agrilus auroguttatus* Schaeffer). One green WITATRAP multi-funnel trap (Witasek, Austria) was equipped with a collecting cup with dry catch, and an Agriwit pheromone lure specific to *Agrilus* spp. was used for insect monitoring (Figure 2a). The trap was installed at a height of approximately 10 meters in the canopy on a hornbeam tree, set up on 23 April and emptied on 21 August 2024.

Additional sampling was done in March 2025 on the same site. Branches with exit holes were collected manually from trees uprooted during the storm and were still lying on the forest ground. They were inspected in the entomological laboratory of the Croatian Forest Research Institute (Jastrebarsko, Croatia). The branches were cut in line with the exit hole to obtain a cross-section, allowing for an examination of the gallery layout and the determination of the gallery direction (Figure 4b).



(a) **(b)**

Figure 1. (a) The location of the plot marked with a red dot on the map of Croatia; **(b)** The position of the monitoring trap marked with a red dot in a plot with indicated sub-compartment borders.

(a) **(b)**

Figure 2. Surveillance of *Agrillus auroguttatus* near Pleternica, Croatia. **(a)** Green multifunnel trap with pheromone for *Agrilus* spp.; **(b)** Wind-broken trees in a forest stand due to stormy weather.

Morphological Identification

The collected specimens were identified based on both morphological and molecular characteristics. Morphological identification was conducted using the identification keys provided by Gomez et al. (2018) and Smith et al. (2020), while the pictures were taken using DeltaPix DPX IM200-4K. The collected specimens are preserved in ethanol in the collection of Department for Forest Protection and Game Management, Croatian Forest Research Institute, Jastrebarsko, Croatia.

Molecular Identification

Molecular identification was performed on two specimens using DNA analysis. The total genomic DNA from the whole body of 2 sampled insects morphologically detected as *C. mutilatus* was extracted using the Monarch Genomic DNA Purification Kit (New England Biolabs Inc.). Mitochondrial cytochrome oxidase I (mtCOI)-based primers LCO-1490F (5-GGTCAACAAATCATAAA GATATTGG-3) and HCO-2198R (5-TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994) were used for PCR. The PCR amplification was performed in the PCRmax™ Alpha Cycler 1 Thermal Cycler (Fisher Scientific). The cycling conditions used for touch-up PCR were as follows: initial denaturation at 94°C for 5 min, initial 4 cycles at 94°C for 30 s, at 45°C for 60 s, and at 72°C for 1 min, followed by 35 cycles at 94°C for 30 s, at 51°C for 30 s, and at 72°C for 1 min, and the final cycle at 72°C for 10 min. Amplified DNA was purified from the 1.8% agarose gel using the Monarch DNA Gel Extraction Kit (New England Biolabs Inc.). The purified PCR products were sequenced (Macrogen Europa, Amsterdam, Netherlands) in both directions with PCR primers (LCO1490/HCO2198). The sequence was edited with Chromas (Version 2.4.1) computer software, Technelysium Pty Ltd., and used to search for the reference sequence in GenBank (NCBI). The following references were used: JQ015151.2 (Holland et al. 2013), 99.85% identity, and PV257754.1 (country: Slovenia: Radmozanci; collection date: 2024; collector: Tine Hauptman) 99.84% identity.

RESULTS

In August 2024, during the quarantine pest surveillance program, the bark beetle *C. mutilatus* was discovered for the first time in Croatia. The record was discovered in a severely disturbed site, with lots of damaged and broken trees caused by a powerful storm in July 2023 (Figure 2b). Before the storm, the forest was healthy without any biotic or abiotic disturbance. During the examination of the collected samples from monitoring traps on the described locality, eight unknown beetle specimens were detected, distinctly different from other native species.

Morphological and molecular identification confirmed that the species found is *C. mutilatus.*

The sequence obtained showed a similarity of 99.85% and 99.32% with specimens of *C. mutilatus* collected in SE Asia (JQ015151.2) and Europe (Veneto Region, Italy, OL441045.1), respectively.

The sequence is deposited in GenBank under the accession number PQ857761.1.

C. mutilatus is 2.6 to 3.9 mm long (Mandelshtam et al. 2019). It is characterized by elytra that are shorter in comparison to its pronotum (Figure 3). All characteristics of the found specimens in Croatia corresponded to those mentioned in the references. The body is black, punctuated and covered with long golden hairs. Legs, mouthparts (excluding the mandibles) and antennae are reddish or yellowish brown (Schiefer and Bright 2004). The antennal funiculus consists of five segments in females and four in males (Mandelshtam et al. 2018).

C. multilatus spreads by flight from June to August. During this period, it searches for suitable dying hosts that naturally release ethanol-based volatiles (Schiefer and Bright 2004). A study conducted in Japan revealed a univoltine life cycle (Kajimura and Hijii 1992). It primarily attacks branches and thinner trunks, preferring those 1-5 cm thick, where the female can build galleries and lay eggs (Oliver et al 2012). It creates an entrance hole with a diameter of approximately 2 mm and, after drilling, constructs galleries which extend from the entrance chamber along the knot axis, reaching the length of 4 cm (Mandelshtam et al. 2019). The female overwinters with its offspring by blocking the entrance hole on the host with her body. *C. mutilatus* is known as a pest that attacks recently dead host trees. (Kajimura and Hijii 1992, Spears et al. 2022).

During field observation in March 2025, branches exhibiting symptoms of *Cnestus mutilatus* infestation were documented. These symptoms included exit holes, adult

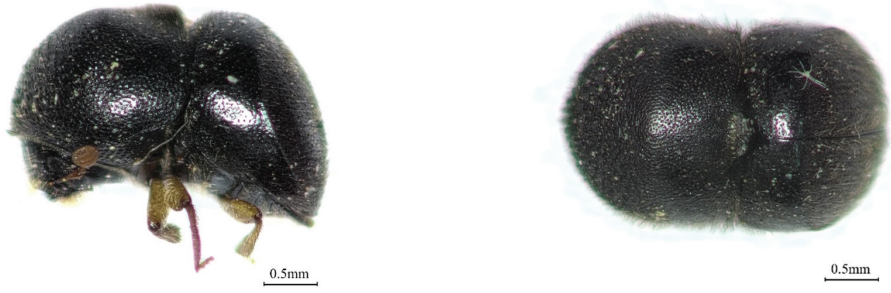


Figure 3. Lateral **(a)** and dorsal **(b)** views of *Cnestus mutilatus* collected in a pheromone trap in Pleternica (Croatia).

galleries and the presence of adult beetles (Figure 4). The infestation was observed on dead wood of *Fagus sylvatica*, *Quercus rubra* and *Carpinus betulus*. Symptoms were observed on branches with diameters ranging from 10 to 25 mm. Figure 4 provides a detailed view of the external (left) and cross-sectional (right) symptoms of infestation in a branch (~20 mm in diameter). The external view shows characteristic circular entrance hole (~2 mm in diameter), where the partially visible elytra of an adult beetle can be observed. The cross-sectional view reveals a radial and irregular gallery system, along with an adult *C. mutilatus* inside the wood. The dark-stained areas surrounding the galleries indicate fungal colonization, thus indicating its symbiotic relationship with ambrosial fungi.

DISCUSSION

The present study documents the first occurrence of *C. mutilatus* in Croatia. This record marks an expansion of the known European distribution of *C. mutilatus*, which until now had been limited to urban or peri-urban settings (Colombari et al. 2022, Ruzier et al 2023; Hauptman, T. personal communication).

In Northeastern part of Slovenia, two adults were caught in traps baited with ethanol in 2024 (Tine Hauptman, personal communication). Croatian and Slovenian site are 172 km apart in a straight line, but the territory between the localities has not been inspected.

The introduction pathway of *C. mutilatus* into Croatia remains unknown, but it can possibly be linked to international trade, untreated timber or ornamental plants from nurseries. These pathways are common vectors for the accidental spread of alien invasive beetles, as the beetles or their larvae can remain hidden within wood materials or plants during transport (Crowl et al. 2008, Hulme et al. 2009, Eschen et al. 2015).

The trees in the affected forest stand in Croatia are under stress due to an extreme weather event that occurred in Croatia in July 2023. A severe storm caused significant damage to the forest, leading to broken branches, uprooted trees and mechanical injures to standing trees. This physical damage weakened the trees, making them more vulnerable to secondary stress factors such as secondary pests and fungal infections. Stressed trees naturally release ethanol, which serves as a cue for ambrosia beetles such as *C. mutilatus* to locate and target suitable hosts for infestation (Oliver et al. 2012). Their small size and the ability to colonize new regions through the transportation of firewood and lumber could contribute to their rapid dispersal (Leavengood 2013). Additionally, females of *C. mutilatus* are pathenogenetic, which is favourable to the rapid establishment and dispersal of the introduced populations (EPPO 2020).

C. mutilatus colonizes branches and the upper part of tree trunks of a small diameter (Schiefer and Bright 2004; Stone et al. 2007), which we also observed in our research. If *C. mutilatus* outbreaks, it will deteriorate the health of young trees used for forest regeneration in Croatia.

According to EFSA's Appendix E, list 3, *C. mutilatus* is categorized as 'introduced' or 'alien' beetle. Despite its classification as a non-native species, there is currently no evidence of significant impact on plant health (EFSA 2024). In the EU, all non-European Scolytinae species, including *C. mutilatus*, are classified as A1 quarantine pests (Commision Implementing Regulation (EU) 2019/2072). *C. mutilatus* is also listed as an ambrosia beetle with potential risks of successful entry, establishment, spread and impact on trees in the EPPO region (EPPO 2020).

It will be important to monitor the further spread of this alien ambrosia beetle species and to evaluate the future damage to forests, especially to sites under rejuvenation. The monitoring should be optimized with multi-funnel traps baited with ethanol lure installed at a height of approximately 10 meters in the canopy, which have shown promise in improving detection rates and monitorng efficacy for such invasive beetles. This method was demonstrated to be effective for detecting *C. mutilatus* and species from the genera *Neoclytus* and *Monochamus* (Cerambycidae) (Miller et al. 2020).

CONCLUSION

The detection of *C. mutilatus* in Croatia raises important questions about its potential ecological and economic impacts on forest ecosystems. While its behavior as a

(a)

(b)

Figure 4. Entrance hole with *Cnestus mutilatus* beetle hidden inside **(a)** and cross-section of a gallery in the branch of European beech (*Fagus sylvatica)* branch **(b)**.

secondary pest has been well documented, further research is needed to clarify whether it can colonize healthy trees and pose a direct threat to forest health. In Croatia, monitoring will be continued in the surrounding area, with enhanced data collection through the deployment of pheromone traps in the canopy. These traps will facilitate the detection of *C. mutilatus* and provide critical insights into its population dynamics, seasonal activity and spatial distribution. Canopy-level placement is expected to target the species' prefferred habitat and optimize trap efficacy. Enhanced surveillance will aid the understanding of the full scope of this species' impact and provide insights for effective forest management and pest control strategies.

Author Contributions

AJ, FC and TK carried out the investigation; TK performed morphology analysis; NĆ performed molecular analysis; DM and JM secured the research funding; AJ, DM, FC, TK, NZ and NĆ helped to draft manuscript; AJ and DM wrote the manuscript.

Funding

This work was funded by the Croatian Ministry of Agriculture and by MIKROBIO project funded by Next Generation EU programme.

Conflict of Interest

The authors declare no conflict of interest.

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