Research Article

Interception of non-native *Stromatium longicorne* (Newman, 1842) (Coleoptera: Cerambycidae) in South Korea

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Abstract

Wood-boring beetles, including longhorned beetles, are significant exotic insects that disperse outside their native habitats due to global trade. Detection and accurate identification of these beetles pose challenges due to cryptic behavior of larvae and their similar external morphology. In this study, we focus on the identification of two unidentified longhorned beetle larvae found in a wooden Gymboree toy and a wooden bed frame. Both larvae died during rearing, with confirmed lifespans of at least 6 and 7 years, respectively. Molecular identification using mitochondrial COI gene successfully identified larvae as *Stromatium longicorne* (Newman, 1842) (Coleoptera: Cerambycidae), a species inhabiting various species of dry woods and native to Southern Asia. The detection of *S. longicorne* in South Korea confirmed the case of non-native longhorned beetles introduced through processed wood products. This discovery adds a new non-native longhorned beetle species to South Korea and raises concerns about potential establishment of subtropic insects through processed wood products and under warmer conditions due to global warming.

Key words: molecular identification, larva, longhorned beetle, COI

Introduction

Wood-boring beetles have emerged as prominent exotic insects that disperse outside their native range due to increased global trade (Brockerhoff et al. 2006). These beetles encompass a diverse array of phylogenetically independent lineages, including longhorned beetles (e.g., Cerambycidae, Disteniidae, Vesperidae), weevils (Curculionidae), bark beetles, ambrosia beetles (Curculionidae: Scolytinae), jewel beetles (Buprestidae), and other minor beetle families (Brockerhoff et al. 2006; Wu et al. 2017). They are frequently encountered during quarantine but pose a challenge for detection due to their larval dispersal and cryptic behavior. Detection methods for wood-boring beetle larvae or pupae are currently being researched, utilizing techniques such as vibration (Zorović and Čokl 2015) and acoustic signals (Bilski et al. 2017). Moreover, accurate identification of these beetles is hindered by their similar external morphology, leading to the application of molecular identification as a post-detection method (Harris et al. 2010; Wu et al. 2017; Lee and Lee 2024).

Within the wood-boring beetles, the longhorned beetles (Coleoptera: Cerambycidae) represent one of the most diverse families (Švácha and Lawrence 2014) and include several notorious invasive species, such as the Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky, 1853)) and Citrus longhorned beetle (*Anoplophora chinensis* (Forster, 1771)). These longhorned beetles are commonly transported through various means, including plants for planting, wood packaging materials, finished wood products, timber, and other plant materials (Eyre and Haack 2017). Consequently, numerous non-native longhorned beetles have established populations outside their native range in different parts of the world (Eyre and Haack 2017; Lee et al. 2020, 2021, 2023a, b; Seidel et al. 2021; Broadley et al. 2023). Despite only a small fraction of individuals being detected during quarantine (Wu et al. 2017), it is believed that many more species and individuals have passed through undetected. South Korea, being the seventh-largest exporting nation globally (World Trade Organization 2022), is not exempt from this issue and has experienced the establishment of non-native cerambycid populations, including invasive populations of *A. glabripennis* and *Anoplophora horsfieldii* (Hope, 1843) (Lee et al. 2020, 2023a).

In line with the common occurrence where the majority of introduced species and individuals manage to evade detection during quarantine procedures, South Korea has recently encountered two cases in which non-native longhorned beetle larvae were not initially detected in quarantine but were discovered afterwards. These incidents were discovered by a citizen's report to a broadcasting program regarding a sound emanating from a wooden product. Subsequent X-ray examinations confirmed the presence of larvae within the products (Supplementary material Figure S1). The products, along with the larvae, were then forwarded for further investigation. However, despite the efforts made to rear the larvae, their survival was unsuccessful. As a result, molecular identification techniques were employed to determine the species of the larvae (Lee and Lee 2024). These two cases represent the first confirmed cases of longhorned beetle larvae detected in finished wood products in South Korea. This study will provide valuable insight that some longhorned beetle larvae can persist in dry wood for extended periods, allowing them to develop fully within the storage sites of wood products or timbers and potentially causing damage or dispersing after the quarantine period.

Materials and methods

A total of two unidentified wood-boring beetle larvae samples, labeled as TNE20-126 and TNE22, were collected and utilized in this study. TNE20-16

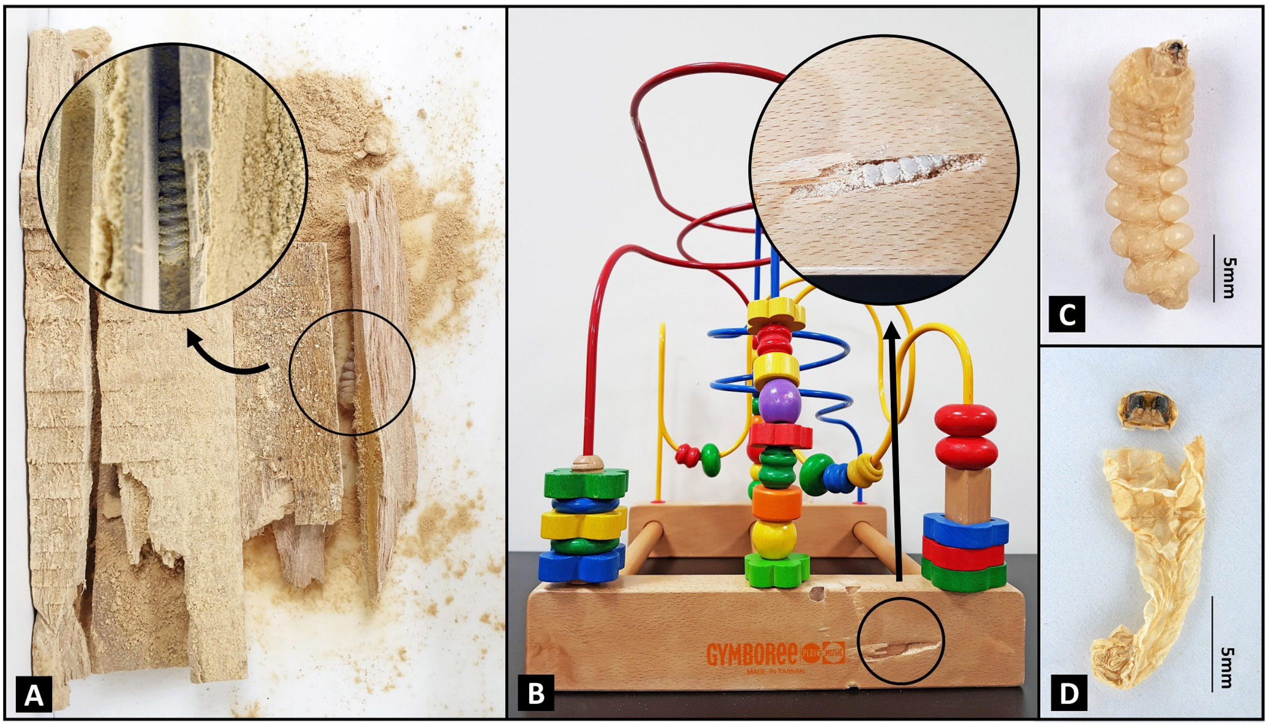

Figure 1. A. Fragment of a wooden bed frame harboring a *Stromatium longicorne* larva (TNE22). Inner circle: Close-up photograph of the larva. B. Gymboree toy harboring *S. longicorne* larvae (TNE20-126). Inner circle: Close-up of the larva. C. Close-up photograph of larva TNE20-126. D. Exuviate of larva TNE22. Scale bars = 5 mm.

was discovered in a wooden Gymboree toy imported from Taiwan, which was purchased in 2014 and reported in 2017 due to scratching sounds within the toy (Figure 1B, C). The larva was found in August 2017 and reared at room temperature within the same wooden structure until its demise in January 2020. TNE22 detected from a bed imported from Mainland China, sourced from an undisclosed location, and was reported in November 2019 (Figure 1A, D). Although two larvae were present in the item, only one (TNE22) was selected for further analysis.

DNA extraction of two unidentified larvae was performed using the DNeasy Blood and Tissue kit (QIAGEN, Inc. Germantown, MD, U.S.A.), following the manufacturer's protocols. We obtained tissues for DNA extraction from larval exuviates to keep the larvae alive and emerge. For PCR amplification, we used two widely-used COI (cytochrome oxidase subunit I) primer sets: LCO (GGTCAACAAATCATAAAGATATTGG), HCO (TAAACTTCAGGGTGACCAAAAAATCA). We carried out PCR amplification with the following thermal conditions: initial denaturation at 95 °C for 5 minutes, followed by 42 cycles of denaturation at 95 °C for 30 seconds, hybridization at 43 °C for 40 seconds, and extension at 72 °C for 40 seconds, and a final elongation at 72 °C for 10 minutes. We sent successfully amplified PCR products to Macrogen (Daejeon, Korea) for purification and sequencing. Both strands were assembled and examined with SEQMAN PRO v.7.1.0 (DNASTAR, Inc., Madison, WI, U.S.A.) and were examined and manually adjusted with MEGA X (Kumar et al. 2016), using the amino acid translation option.

As the two larvae were died before they becoming imagoes, molecular identification was adopted for their species level identification. We adopted both tree-based molecular identification and BLAST that has been widely used (Shin et al. 2015; Wu et al. 2017). For tree-based identification, public COI sequences of cerambycinae were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and BOLD systems (www.boldsystems.org). To facilitate genetic analyses, we deleted perfectly redundant sequences using Jalview 2.1.1 (Waterhouse et al. 2009) and automatically removed short sequences (< 500 bp) using Seqkit v.0.12.0 (Shen et al. 2016). The final matrix included 804 species in total (Table S1). The phylogenetic analysis was conducted using the maximum likelihood method and W-IQ-TREE software (Trifinopoulos et al. 2016). The optimal substitution model (TPM2+F+I+G4) was determined using Modelfinder (Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion. The nodal support value was determined through ultrafast bootstrap with 1,000 replicates.

Results

The two unidentified larvae were both identified as *Stromatium longicorne* (Newman, 1842) through Blast search (Figure S2). TNE20-126 showed 99.24% identification (PI) and 100% query cover (QC) with the Genbank accession number MN315185, followed by PI 98.78% PI and 100 QC with FJ558998. TNE22 also showed its closest affinity with the same two sequences, 99.85% PI and 100% QC with MN315185 and PI 99.39% PI and 100% QC with FJ558998.

The resulting phylogenetic tree included 804 terminals in total (Table S1, Figure S3). TNE20-126, TNE 22, MN315185 and FJ558998 recovered a mono-clade with high supporting value (ultrafast bootstrap support, UF hereafter: 97), again confirming its identity as *S. longicorne* (Figure 2A, Figure S3). KY357579 recovered sister of this clade with low divergence rate implying it is also *S. longicorne*. The *S. longicorne* clade showed its closest affinity with *S. barbatum* with high supporting value (UF: 100). The overall phylogeny showed numerous severely low-supported nodes widely across shallow to deeper relationships which ultimately was recovered polyphyletic lineages (Figure 2B, Figure S3), possibly because of the high saturation of COI sequences (Cruaud et al. 2022).

Discussion

The genus *Stratomatium* Audinet-Serville, 1834 belongs to the order Coleoptera, family Cerambycidae, and includes five recorded species worldwide (Jin et al. 2018; Tavakilian and Chevillotte 2018). During the larval stage, these beetles primarily inhabit dry trees, regardless of the host species (Cocquempot 2006). Their lifespan can range from 1 to 7 years, depending on environmental factors (Cocquempot et al. 2015; Shi et al. 1982).

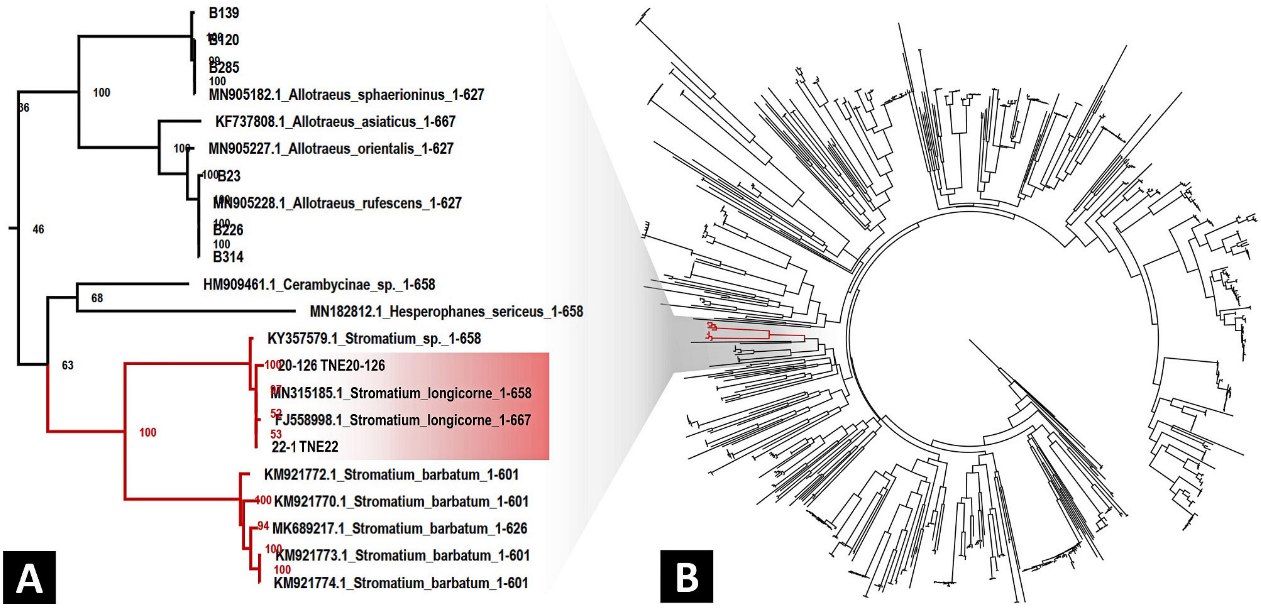

Figure 2. A. Zoom-in figure of the clade that includes TNE22 and TNE20-126. The genus *Stromatium* is indicated by a red line, and *Stromatium longicorne* is highlighted with a red square. B. Complete phylogenetic tree comprising 801 Cerambycinae and 2 outgroup Lamiinae species. The genus *Stromatium* is represented by a red line, and the clade zoomed in on in Figure 2A is highlighted in gray.

Stromatium longicorne has a wide distribution spanning the Southeast Asia to Indian Himalayan region. This species has been reported in various countries outside its native range, including Australia (Duffy 1953, 1968), the United States (furniture originating from Pakistan from 1984 to 2008; Eyre and Haack 2017), France (wooden furniture of unknown origin in 1996; Cocquempot 2006), Austria (timber from China in 2008; Jin et al. 2018), and Belgium (2013 in a wooden toy horse from Germany; Cocquempot et al. 2015). Similar to other *Stromatium* species, *S. longicorne* can utilize a variety of dried hardwoods for survival (Jin et al. 2018; Cocquempot 2006).

TNE20-126 remained in the larval stage inside the toys for at least seven years, while TNE22 remained in the larval stage for over six years. Despite the typical life history of *S. longicorne* being believed to span 1 to 5 years (Shi et al. 1982), observations indicate that larvae can live and develop in processed wood for extended periods, as documented in several studies (Craighead 1923; Duffy 1953; Linsley 1959; Bense 1995; Cocquempot et al. 2015). These findings confirm the ability of *S. longicorne* larvae to survive and molt in processed wood for over seven years, exceeding their typical 1–5 years of life cycle.

The discovery of *S. longicorne* in South Korea adds a new non-native longhorned beetle species to the growing list of non-native species (e.g., Lee et al. 2020, 2023a) in the country and highlights the introduction of insects through processed wood products, such as toys and furniture. The larvae's extended lifespan demonstrates their ability to survive longer than expected under unfavorable environmental conditions and potentially undergo pupation and emergence after additional years. Currently, there is no evidence of established populations of *S. longicorne* outside its native distribution. However, due to the species' broad diet of dried hardwood, irrespective of tree species, similar to the widely distributed house borer *Hylotrupes bajulus* (Linnaeus, 1758), the possibility of its establishment beyond its native range cannot be disregarded (Cannon and Robinson 1982). It is also worth noting that considering the recent discovery of numerous subtropical species in Korea and the presence of an established subtropical species, *A. horsfieldi* (Lee et al. 2023a), it is plausible for the oriental species, *S. longicorne*, to establish itself in Korea under warmer conditions resulting from global warming.

Author's contribution

Research conceptualization – DC, SL, YL; sample and data collection – DC, HL, SN, SS, YL; data analysis and interpretation – SL, YL; writing original draft – SL; funding acquisition – SL, DC, HL; review and editing – all authors.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Genbank Accession number of sequences included in phylogenetic analysis.
Figure S1. Screenshots of samples in broadcasting. A–B. TNE22 C. TNE20-126.
Figure S2. Nucleotide Blast result. A. TNE20-126 B. TNE22.
Figure S3. Phylogenetic tree of Cerambycinae, *Stromatium* clade highlighted in red.

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