American Journal of Entomology
2024, Vol. 8, No. 3, pp. 102-108
https://doi.org/10.11648/j.aje.20240803.14

Research Article

Enzyme-Mediated Duplex Exponential Amplification: A New Platform for Rapid Screening of *Hylurgus ligniperda*

Wang Jiaying[1, 2], Cui Junxia[1, 2], Yan Shuyi[5], Liu Li[4], Chen Xianfeng[3, *]

[1]Technical Center, Ningbo Academy of Inspection and Quarantine, Ningbo, China
[2]Technical Center, Ningbo Customs, Ningbo, China
[3]Animal and Plant Quarantine Office, Ningbo Customs, Ningbo, China
[4]Technical Center, Ningbo Zhongsheng Product Inspection Co., Ltd, Ningbo, China
[5]College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo, China

Abstract

As the world's second largest timber importer, wood demand in China has been growing extremely rapidly, leading to an increase of 163% from 2009 to 2018. The plant quarantine pest *H. ligniperda Fabricius, 1787* is an invasive species frequently intercepted at ports. *H. ligniperda* causes damage mainly to pine and spruce. To improve the efficiency of on-site inspection and the efficacy of early detection, tight quarantine in ports, time-effective identification, and a national surveillance program for high-risk invasive bark beetles are in urgent need. In this study, a simple, fast and accurate classification method for *H. ligniperda* is established based on the enzyme-mediated duplex exponential amplification (EmDEA) technique. Partial region from *inhibitor of apoptosis 2* (*IAP2)* gene was selected as the target and 6 primer/probe combinations were designed. Through selection, the combination of 3-HY-F3, 3-HY-R2 and 3-HY-RNA5 was chosen as the final primer-probe set, as it showed the lowest Ct with highest final fluorescence signal. Method validation and specificity test using 6 other beetle species living on coniferous wood showed that this method is result reliable and specific. Through parameter analysis with positive plasmid, the detection limit was calculated to be 13.6 copies/μL (9×10^{-7} ng DNA/ reaction), much higher than conventional molecular methods such as PCR. The whole process including isothermal amplification, data analysis, and result output can be finished in 30 min, which is highly time-effective. Besides, the operation is simple and little training is needed for non-professionals. The application prospects of this rapid screening system include customs screening in ports, wild survey in non-lab situations and early warning system development. The new analysis platform EmDEA, can also be implemented in rapid detection and identification of other forestry pests.

Keywords

Enzyme-Mediated Duplex Exponential Amplification, Rapid Screening, Quarantine Plant Pest, *H. ligniperda Fabricius, 1787*

*Corresponding author: c@nbyjg.com (Chen Xianfeng)

Received: 23 July 2024; **Accepted:** 12 August 2024; **Published:** 27 August 2024

1. Introduction

The important plant pest red-haired pine bark beetle (*H. ligniperda Fabricius, 1787)*, belongs to Coleoptera, Curculionidae, Hylurgini taxonomically [1]. Originated from southern Europe and the Mediterranean coast of northern Africa, it has been spread to all the continents due to increasing international trade and social communication globally [2]. *H. ligniperda* adults live in soil, invade the roots of newly cut or healthy trees and finally kill them. Gifted with an outstanding natural transmission ability, this wood-boring beetle forms a major threat to pine and spruce [3]. Timber imports have been rising year by year, resulting in an increase of 163% from 2009 to 2018, thus China has become the world's second largest timber importer [4]. Moreover, it achieves long-distance transmission via international transportation of wood, wood packaging and bedding materials [5]. Once across the borderline and established, the spread of *H. ligniperda* would significantly harm the local ecology and lead to considerable economic loss in China [6]. Rapid, and accurate identification would contribute to efficient screening in ports and further monitoring of *H. ligniperda*.

Traditional identification methods for insects based on morphological characteristics have several drawbacks [7]. Most of all, morphological discrimination is largely based on complete adults, whereas samples collected in ports are often in the early growth stages (eggs, larvae, or pupae), or even in an incomplete condition. Besides, species classification requires solid taxonomic background and much training experience. The identification of many pests, especially unknown ones based on morphological traits needs improvement in precision and efficiency.

Molecular analysis platforms are useful tools that can aid in precise identification of plant pests [8]. Barcoding system has become an useful way for domestic/invasive species delimitation, with progresses in accuracy, convenience and time-cost [9]. Gene regions from mitochondrial DNA, such as COI and COII, are the most popular barcodes due to several advantages such as near-neutrality, universality and minimal recombination [10-12]. However, commonly used molecular methods such as PCR, are often time-consuming, relying on thermostable DNA polymerase and expensive thermal cycler, and professional training [13]. Thus, they are not suitable for on-site applications. In this background, isothermal amplification was proposed as an efficient supplement for nucleic acid amplification working at one temperature. It succeeds in *in vitro* amplification independent of conventional three steps of high-temperature denaturation, annealing, and extension. In other words, it does not rely on complex equipment such as PCR cycler [14].

In this study, a fast, simple and precise identification method for *H. ligniperda* is developed based on the EmDEA technique. The whole process including isothermal amplification, data analysis, and result output can be finished in 30 min, which is highly time-effective. Besides, the operation is simple and little training is needed for non-professionals. This method is aimed to assist rapid screening of *H. ligniperda* and fast customs clearance in ports.

2. Materials & Methods

2.1. Sample Preparation & DNA Extraction

Altogether 16 insect samples were used in method development, including 10 *H. ligniperda* and 6 other insect species (Table 1). Some of them were reserved samples in our lab, and others were donated by other institutions. *Ips grandicollis*, *I. calligraphus*, and *I. typographus* all belong to Hylurgini, which are closely related to *H. ligniperda* taxonomically. Nucleic acid extraction from insect samples was conducted using a fast method [15]. The DNA solutions were analyzed via NanoDrop 2000C (ThermoFisher, China) and stored at -20 ℃.

Table 1. *Sample list.*

No.	Order, Family	Species	Host	Origin
1	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
2	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	/	Reserved sample
3	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
4	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus sylvestris*	Reserved sample
5	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	/	Reserved sample
6	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
7	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
8	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample

No.	Order, Family	Species	Host	Origin
9	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
10	*Coleoptera, Curculionidae*	*H. ligniperda Fabricius, 1787*	*Pinus radiata*	Reserved sample
11	*Coleoptera, Scolytidae*	*I. grandicollis Eichhoff*	*Pinus taeda*	Donated by Guangzhou Customs
12	*Coleoptera, Curculionidae*	*X. compactus Eichhoff, 1875*	*Rhododendron simsii*	Reserved sample
13	*Coleoptera, Curculionidae*	*X. germanus Blandford*	/	Reserved sample
14	*Coleoptera, Curculionidae*	*X. crassiusculus Motschulsky*	/	Donated by Guangzhou Customs
15	*Coleoptera, Scolytidae*	*I. calligraphus Germar*	/	Reserved sample
16	*Coleoptera, Scolytidae*	*I. typographus L.*	*Picea asperata*	Donated by Nanjing Customs

2.2. Target Region Selection & Primer Design

Partial region from *inhibitor of apoptosis 2* (*IAP2)* gene (Accession No. MF771792.1) was selected as the target and corresponding primers were designed by GeneVide Biotech Co., Ltd. (China). DNA primers and recombinant plasmid inserted with the target gene region were synthesized by BGI Co., Ltd (Guangdong), and RNA probes were provided by GeneVide Biotech Co., Ltd. (China).

2.3. Establishment of EmDEA Assay

The reaction mixture (in a total volume of 20 μL) consists of 1 μL upstream primer (10 μM), 1 μL downstream primer (10 μM), 1 μL RNA probe (1 μM), 7 μL DNA template, 10 μL activating buffer, and one tube of dried enzyme powder. The activating buffer and dried enzyme powder were provided by GeneVide Biotech Co., Ltd. (China) in a commercial kit (product code: RR032). Amplification was performed using a LightCycler480II (Roche, Switzerland). Totally 30 cycles of amplification were carried out, each at 42 ℃ for 1 min. The reporter was FAM (carboxyfluorescein). Fluorescence signal was collected at the end of each cycle.

Primer-probe combinations were subject to 3 rounds of selection using recombinant plasmid as template to obtain the most efficient primer-probe set. The most effective RNA probe was determined after the first round based on combination series (Table 2). Then came to the selection of most productive downstream and upstream primers in a similar way.

Table 2. *Primer-probe selection combination.*

No.	Combination	RNA probe	Upstream Primer	Downstream Primers
1	RNA1F3R3	3-HY-RNA1	3-HY-F3	3-HY-R3
2	RNA1F3R4	3-HY-RNA1	3-HY-F3	3-HY-R4
3	RNA1F4R3	3-HY-RNA1	3-HY-F4	3-HY-R3
4	RNA1F4R4	3-HY-RNA1	3-HY-F4	3-HY-R4
5	RNA2F3R3	3-HY-RNA2	3-HY-F3	3-HY-R3
6	RNA2F3R4	3-HY-RNA2	3-HY-F3	3-HY-R4
7	RNA2F4R3	3-HY-RNA2	3-HY-F4	3-HY-R3
8	RNA2F4R4	3-HY-RNA2	3-HY-F4	3-HY-R4
9	RNA3F3R3	3-HY-RNA3	3-HY-F3	3-HY-R3
10	RNA3F3R4	3-HY-RNA3	3-HY-F3	3-HY-R4
11	RNA3F4R3	3-HY-RNA3	3-HY-F4	3-HY-R3
12	RNA3F4R4	3-HY-RNA3	3-HY-F4	3-HY-R4
13	RNA4F3R3	3-HY-RNA4	3-HY-F3	3-HY-R3
14	RNA4F3R4	3-HY-RNA4	3-HY-F3	3-HY-R4
15	RNA4F4R3	3-HY-RNA4	3-HY-F4	3-HY-R3
16	RNA4F4R4	3-HY-RNA4	3-HY-F4	3-HY-R4
17	RNA5F3R3	3-HY-RNA5	3-HY-F3	3-HY-R3
18	RNA5F3R4	3-HY-RNA5	3-HY-F3	3-HY-R4
19	RNA5F4R3	3-HY-RNA5	3-HY-F4	3-HY-R3
20	RNA5F4R4	3-HY-RNA5	3-HY-F4	3-HY-R4
21	RNA6F3R3	3-HY-RNA6	3-HY-F3	3-HY-R3
22	RNA6F3R4	3-HY-RNA6	3-HY-F3	3-HY-R4
23	RNA6F4R3	3-HY-RNA6	3-HY-F4	3-HY-R3
24	RNA6F4R4	3-HY-RNA6	3-HY-F4	3-HY-R4

2.4. Method Validation, Specificity and Sensitivity Analysis

Ten *H. ligniperda* samples were used for method validation. The recombinant plasmid was employed in sensitivity analy-

sis, which was diluted into a series of concentrations ranging from 1.36×10^5 copies/μL to 1.36×10^0 copies/μL. Six related beetle species were collected for specificity analysis.

RNase free ddH_2O was adopted as the negative control. Three repetitions were set for each treatment.

3. Result & Analysis

3.1. Primer Selection

IAP2 gene was highly diverse inter-species, while conserved intra-species for *H. ligniperda*. Based on *IAP2* gene sequence, 6 upstream and 6 downstream DNA primers along with 6 RNA probes were generated (Table 3). Each upstream primer was decorated with a T7 promoter. To obtain the most efficient combination of primers and probe, screening analysis was conducted (Table 2). The lowest Ct with highest final fluorescence signal came from the combination of 3-HY-F3, 3-HY-R2 and 3-HY-RNA5, which were selected as the final primer-probe set.

Table 3. *Primer list.*

Primer		Sequence (5'-3')
Upstream Primer	3-HY-F1	AAGCTAATACGACTCACTATAGGGCAGACTCCAGACATACTGGCGCAAGCAG
	3-HY-F2	AAGCTAATACGACTCACTATAGGGCCAGACATACTGGCGCAAGCAGGATTTT
	3-HY-F3	AAGCTAATACGACTCACTATAGGGATACTGGCGCAAGCAGGATTTTACTATG
	3-HY-F4	AAGCTAATACGACTCACTATAGGGGCGCAAGCAGGATTTTACTATGAAGGTA
	3-HY-F5	AAGCTAATACGACTCACTATAGGGTCATTGGTTTACTCTATAGAGCCAAAGT
	3-HY-F6	AAGCTAATACGACTCACTATAGGGGTTTACTCTATAGAGCCAAAGTTTCACT
Downstream primers	3-HY-R1	CTAGTCCTAAAACAGTACTTTTCTTATT
	3-HY-R2	GATCGCCTAGTCCTAAAACAGTACTTTT
	3-HY-R3	GCACCTGATCGCCTAGTCCTAAAACAGT
	3-HY-R4	AACATCGCACCTGATCGCCTAGTCCTAA
	3-HY-R5	AATGGAAACATCGCACCTGATCGCCTAG
	3-HY-R6	CATCACAATGGAAACATCGCACCTGATC
RNA probe	3-HY-RNA1	FAM-UUGAGUAAAUCAAGGAAUUGUCCUGAAG-BHQ1
	3-HY-RNA2	FAM-GCUUGUUUGAGUAAAUCAAGGAAUUGUC-BHQ1
	3-HY-RNA3	FAM-UUCUGUGCUUGUUUGAGUAAAUCAAGGA-BHQ1
	3-HY-RNA4	FAM-GGGCAUUUCUGUGCUUGUUUGAGUAAAU-BHQ1
	3-HY-RNA5	FAM-AAAAUGGGGCAUUUCUGUGCUUGUUUGA-BHQ1
	3-HY-RNA6	FAM-GAGUUGAAAAUGGGGCAUUUCUGUGCUU-BHQ1

Sequences with underlines are T7 promoter.

3.2. Method Parameter Analysis

Ten *H. ligniperda* samples were used in method validation and specificity test along with 6 related beetle species. According to the result (Figure 1), only *H. ligniperda* samples showed positive amplification curves with Ct below 20, which demonstrates that this new assay is highly specific and reliable. Six concentrations of recombinant plasmid solutions ranging from 1.36×10^5 copies/μL to 1.36×10^0 copies/μL were employed in method sensitivity test. All of the concentrations had positive amplification curves with Ct below 25 except the lowest degree (Figure 2). It means the detection limit is 13.6 copies/μL (9×10^{-7} ng DNA/ reaction), much higher than commonly used molecular methods such as PCR.

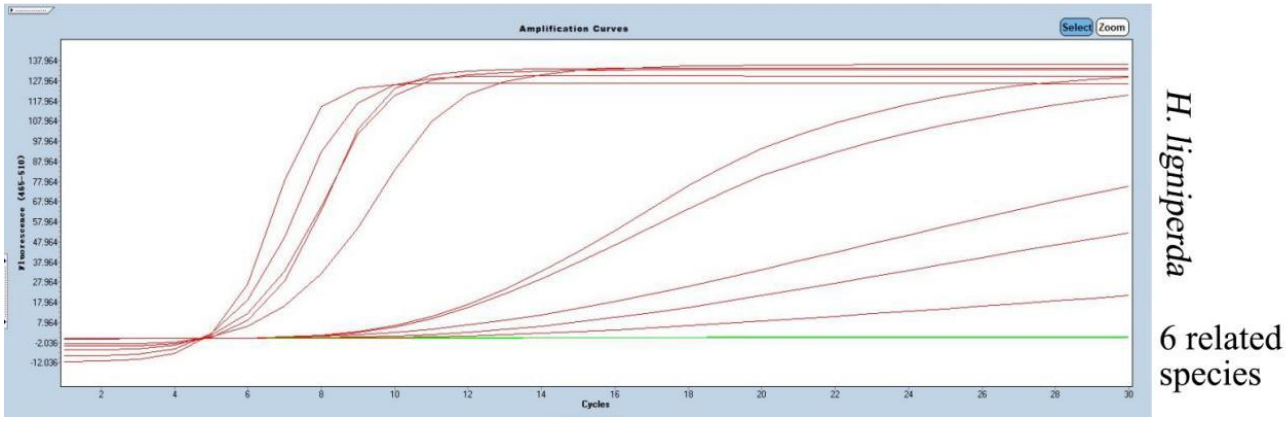

Figure 1. *Method validation and specificity analysis result.*

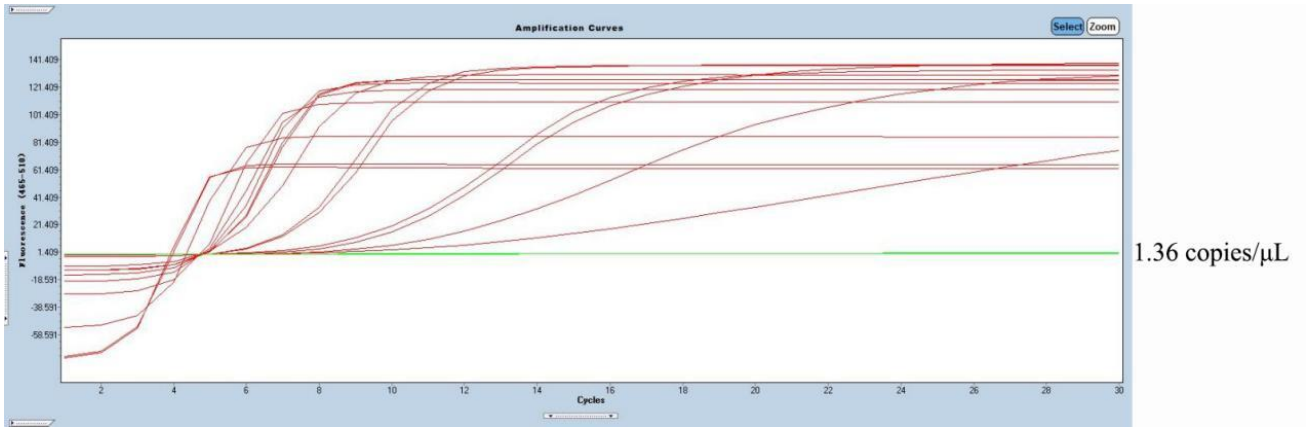

Figure 2. *Sensitivity analysis result.*

4. Discussion

As the building, furniture, packaging and other raw materials, wood demand in our country is growing extremely rapidly, leading to a huge domestic timber consumption [16]. A lack of forest resources, coupled with implementation of the sustainable development strategy makes the domestic timber supply fall behind the need largely [17]. The ever-increasing economic and trade links accelerate the migration of related alien species from one place to another globally. One step further, growing timber imports create opportunities for invasion by alien wood pests.

Being a major threat to pine forests, *H. ligniperda* takes advantage of nutrient deficiencies, mechanical injuries, diseases and pest attack and finishes feeding on weakened pine trees. Through maturational feeding, they result in as high as 10% mortality of young pine plantations [3]. This beetle can also be a vector for serious diseases such as blackstain root caused by *Leptographium* sp. [18]. *H. ligniperda* is an invasive quarantine pest in China. With 10199 interceptions in ports from 2003 to 2016, it has been the second most frequently intercepted plant pest in China [2]. Unfortunately, it has been reported that *H. ligniperda* recently invaded and established in some parts of Shandong Province, China [19]. Alien bark beetles are strong candidates for invasion. As bark beetles are usually wood-boring, they can escape from inspection via hiding under the bark and succeed in long-distance spread with wood cargoes [20]. International ports with large quantities of timber importation are one of the major sites with high invasion risks. To improve the efficacy of early detection and subsequent prevention, tight quarantine in ports, efficient inspection and monitoring, and a national surveillance program for invasive bark beetles are in urgent need.

Researchers have applied the Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology in rapid identification of *H. ligniperda*, which do not rely on specialized entomological expertise [21]. This new method is logically based on protein profiles of specific species, and a reference spectra database of high quality needs to be constructed before application. The quality of protein fingerprint database is largely influenced by specimen site, protein stability, developmental period, and lysis solution. Because of poor stability of proteins in target species, the consistency and repeatability of this method need to be improved.

In this study, a simple, time-effective and accurate classifi-

cation method for *H. ligniperda* is established based on the EmDEA technique. Partial region from *IAP2* gene was selected as the target and 6 primer/probe combinations were designed. Through selection test, the combination of 3-HY-F3, 3-HY-R2 and 3-HY-RNA5 were chosen as the final primer-probe set, as it showed the lowest Ct with highest final fluorescence signal. Method validation and specificity test using beetle samples showed that this method is result reliable and specific. Through parameter analysis with positive plasmid, the detection limit was calculated to be 13.6 copies/μL (9×10^{-7} ng DNA/ reaction), much higher than conventional molecular methods such as PCR. The whole process including isothermal amplification, data analysis, and result output can be finished in 30 min, which is highly time-effective. Besides, the operation is simple and little training is needed for non-professionals.

The application prospects of this new method include rapid screening for customs in ports, wild survey in non-lab situations and early warning system development. To prevent and control exotic invasive species, early detection and in-time warning is fundamental. The recognition of locations where invasion and colonization of exotic species is more probable, followed by an efficient trapping strategy, can significantly increase the chance of early detection. Based on this, timely countermeasures to eradicate or contain alien invasions can work efficiently. The EmDEA technology can further be implemented in rapid detection and identification of other forestry pests.

5. Conclusion

The booming economic communications accelerate the migration of alien pests from one site to another around the world. Increasing timber imports create opportunities for invasion by exotic wood beetles. The harmful red-haired pine bark beetle plays a role in transmission of several fungi such as *L. wagneri* causing blackstain root disease. Efficient on-site inspection and early detection should be confirmed in order to guarantee the safety of domestic ecosystem. In this study, a simple, fast and precise identification method for *H. ligniperda* is established based on the EmDEA technique. Partial region from *inhibitor of apoptosis 2* (*IAP2)* gene was selected as the target and 6 primer/probe combinations were designed. Through selection, the combination of 3-HY-F3, 3-HY-R2 and 3-HY-RNA5 was chosen as the final primer-probe set, as it showed the lowest Ct with highest final fluorescence signal. Method validation and specificity test using beetle samples showed that this method is result reliable and specific. Through parameter analysis with positive plasmid, the detection limit was calculated to be 13.6 copies/μL (9×10^{-7} ng DNA/ reaction), much higher than conventional molecular methods such as PCR. The whole process including isothermal amplification, data analysis, and result output can be finished in 30 min, which is highly time-effective. Besides, the operation is simple and little training is needed for non-professionals. This rapid screening system can be further applied in customs screening in ports, wild survey in non-lab situations and early warning system development. The new analysis platform EmDEA, can also be implemented in rapid detection and identification of other forestry pests, such as invasive ants and exotic insects.

Abbreviations

EmDEA	Enzyme-Mediated Duplex Exponential Amplification
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
IAP2	Inhibitor of Apoptosis 2

Author Contributions

Wang Jiaying: Funding acquisition, Investigation, Writing – original draft

Cui Junxia: Project administration

Yan Shuyi: Experiment conduct

Liu Li: Data curation, Investigation, Resources

Chen Xianfeng: Conceptualization, Supervision

Funding

This study received support from the Ningbo Public Interest Plan (2023S182), the Research Foundation of the General Administration of Customs of the People's Republic of China (2023HK048) and National Key R&D Program of China (2022YFF0608804).

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Clare GK, George EM. Life cycle and massrearing of Hylurgus ligniperda using a novel eggcollection method. New Zealand Plant Protection. 2016, 69: 143-152. https://doi.org/10.30843/nzpp.2016.69.5895

[2] Lin W, Park S, Jiang ZR, et al. Native or invasive? The red-haired pine bark beetle Hylurgus ligniperda (Fabricius) (Curculionidae: Scolytinae) in East Asia. Forests. 2021, 12(7): 950. https://doi.org/10.3390/f12070950

[3] Hoebeke ER. Hylurgus ligniperda: a new exotic pine bark beetle in the United States. Newsl Mich Entomol Soc. 2001, 46: 1-2.

[4] Zhang JR, Yang J, Yi JX. Structure analysis and suggestions of China timber imports. World Journal of Forestry. 2019, 8(4): 129-136. https://doi.org/10.12677/WJF.2019.84018

[5] Wu Z, Gao T, Luo Y, et al. Prediction of the global potential geographical distribution of Hylurgus ligniperda using a maximum entropy model. Forest Ecosystems. 2022, 9: 100042. https://doi.org/10.1016/j.fecs.2022.100042

[6] Zhou YT, Ge XZ, Zou Y, et al. Prediction of the potential geographical distribution of Hylurgus ligniperda at the global scale and in China using the MaxEnt model. Journal of Beijing Forestry University. 2022, 44(11): 90-99. https://doi.org/10.12171/j.1000−1522.20210345

[7] Lucas L, Gómez D, Braccini CL, et al. Morphological and molecular identification of the invasive Xylosandrus crassiusculus (Coleoptera: Curculionidae: Scolytinae) and its South American range extending into Argentina and Uruguay. Annals of the Entomological Society of America. 2017, 110(3): 344-349. https://doi.org/10.1093/aesa/sax032

[8] Albo JE, Marelli JP, Puig AS. Rapid Molecular Identification of Scolytinae (Coleoptera: Curculionidae). International Journal of Molecular Sciences. 2019, 20(23): 5944. https://doi.org/10.3390/ijms20235944

[9] Madden MJL, Young RG, Brown JW, et al. Using DNA barcoding to improve invasive pest identification at US ports-of-entry. PLoS One. 2019, 14(9): e0222291. https://doi.org/10.1371/journal.pone.0222291

[10] Hendrich L, Morinière J, Haszprunar G, et al. A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. Molecular Ecology Resources. 2015, 15(4): 795-818. https://doi.org/10.1111/1755-0998.12354

[11] Roe AD, Sperling FAH. Patterns of evolution of mitochondrial cytochrome c oxidase I and II DNA and implications for DNA barcoding. Molecular Phylogenetics and evolution. 2007, 44 (1): 325-345. https://doi.org/10.1016/j.ympev.2006.12.005

[12] Dong Z, Wang Y, Li C, et al. Mitochondrial DNA as a molecular marker in insect ecology: Current status and future prospects. Annals of the Entomological Society of America. 2021, 114 (4): 470-476. https://doi.org/10.1093/aesa/saab020

[13] Moeini-Zanjani A, Pournajaf A, Ferdosi-Shahandashti E, et al. Comparison of loop-mediated isothermal amplification and conventional PCR tests for diagnosis of common Brucella species. BMC research notes. 2020, 13: 1-5. https://doi.org/10.1186/s13104-020-05377-8

[14] Jiang H, Li Y, Lv XF, et al. Recent advances in cascade isothermal amplification techniques for ultra-sensitive nucleic acid detection. Talanta. 2023, 260: 124645. https://doi.org/10.1016/j.talanta.2023.124645

[15] Wang JY, Cui JX, Liu L, et al. A Fast, Simple and Low-cost DNA Extraction Protocol from Common Ants and Beetles for Multiple Molecular Applications. American Journal of Bioscience and Bioengineering. 2024, 12(3): 48-56.

[16] Barbu MC, Tudor EM. State of the art of the Chinese forestry, wood industry and its markets. Wood Material Science & Engineering. 2022, 17(6): 1030-1039. https://doi.org/10.1080/17480272.2021.1891457

[17] Liu F, Wheiler K, Ganguly I, et al. Sustainable timber trade: A study on discrepancies in Chinese logs and lumber trade statistics. Forests. 2020, 11(2): 205. https://doi.org/10.3390/f11020205

[18] Kim S, Harrington TC, Lee JC, et al. Leptographium tereforme sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle Hylurgus ligniperda in California. Mycologia. 2011, 103(1): 152-163. https://doi.org/10.3852/10-096

[19] Li C, Wang B, Ji Y, et al. Mitochondrial genome provides species-specific targets for the rapid detection of early invasive populations of Hylurgus ligniperda in China. BMC genomics. 25(1): 90. https://doi.org/10.1186/s12864-024-10011-z

[20] Forgione L, Bacher S, Vimercati G. Are species more harmful in their native, neonative or alien range? Insights from a global analysis of bark beetles. Diversity and Distributions. 2022, 28(9): 1832-1849. https://doi.org/10.1111/ddi.13585

[21] Wang J, Tao J, Dong Z, et al. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry: An Innovative Tool for Rapid Identification of Hylurgus ligniperda, an Invasive Pest. Forests. 2024, 15(4): 729. https://doi.org/10.3390/f15040729