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THÈSE présentée par :

Yuan YUAN

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Comparative approach to invasiveness in congeneric bark beetles: the case of the Hylurgini group

THÈSE dirigée par :

Pr ROUX Géraldine

Professeure, Université d'Orléans, France

Dr AUGER-ROZENBERG Marie-Anne

Directrice de recherche, INRAE Centre-Val de Loire, France

RAPPORTEURS :

Dr KERDELHUE Carole

(Présidente de jury) Directrice de recherche, INRAE

Montpellier Occitanie, France

Pr BATTISTI Andrea

Professeur, University of Padova, Italy

JURY :

Dr BERTHEAU Coralie

Maîtresse de conférences,

Université Marie et Louis Pasteur Montbéliard, France

Dr BANKHEAD Stéphanie

Maîtresse de conférences, Université d'Orléans, France

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My contributions in the different chapters presented in my thesis manuscript (Green = Major contribution / Yellow = Secondary contribution / Dark gray = Not contributed).

<u>Thesis Chapter</u>	<u>Associated Publication Title</u>	<u>Design</u>	<u>Fieldwork and Laboratory Work</u>	<u>Data Processing</u>	<u>Bioinformatics Analysis</u>	<u>Interpretations</u>	<u>Writing</u>
<u>Chapter 1</u>							
<u>Chapter 2</u>	Seven mitochondrial genomes of tribe Hylurgini (Coleoptera: Curculionidae: Scolytinae) in Eurasia and their phylogenetic analysis						
<u>Chapter 3</u>	One is a worldwide invader, the other never moved: contrasted phylogeographic patterns of two congeneric <i>Hylurgus</i> conifer bark beetles						

Key terms and concepts

Various terms, such as alien, exotic, introduced, nonindigenous, immigrant, and nonnative, have been used interchangeably in discussions of species invasions. [Pureswaran et al. \(2022\)](#) provided a list of terms and their definitions (Text box 1). To ensure clarity and consistency, we adopt the same terminology in this study, minimizing potential disputes and confusion arising from the diverse and sometimes subjective interpretations found in the invasive species literature ([Valéry et al. 2008](#)).

TEXT BOX 1 Definition of key terms. ([Pureswaran et al. 2022](#))

- **Native species.** A species living within its natural range as a naturally occurring and self-sustaining population (observed in historical times), including the area which it can reach and occupy using its natural dispersal systems. Synonym: indigenous.
- **Nonnative species.** A species or lower taxon that is not native to an area and that has entered by human agency, either intentionally or accidentally, into the area. A species that has entered an area through natural means is generally not considered a nonnative species but a species extending its natural range. Synonyms: nonindigenous, alien, exotic.
- **Entry.** Movement of a species or lower taxon into an area where it is not yet present, or present but not widely distributed. Synonym: arrival.
- **Establishment.** The process of a nonnative species population perpetuating in a habitat in the area it has entered, by successful reproduction. A single generation of reproduction can generally be considered establishment, provided the offspring have a likelihood of continued survival.
- **Introduction.** The entry and establishment of a species into an area where it is nonnative, through movement by human agency, either directly from an area where the species is native, or indirectly (by successive movement from an area where the species is native through one or several areas where it is not).
- **Invasive species.** Nonnative species whose introduction and/or spread cause ecological, environmental, and/or economic damage.
- **Established species.** Nonnative species that have sustained populations over several reproductive cycles and are not necessarily invasive in their novel ecosystem.
- **Spread.** Expansion of the geographical distribution of a species or lower taxon within an area.
- **Incursion.** Isolated population recently detected in an area, not known to be established, but expected to survive in the near future. Note distinction with invasion where the isolated population has established and started spreading.
- **Invasion.** Spread of a nonnative species to areas outside sites of introduction. Invasion may occur in various ecosystems and habitats, i.e., natural, seminatural, artificial, and with various consequences, i.e., positive, negative, and neutral.

[The definition of "invasion" here remains subject to debate. A substantial body of literature suggests that invasion should be primarily characterized by the ecological impact on native ecosystems and biodiversity, rather than by the extent of spatial spread. In this view, a species may be considered invasive even if it does not exhibit significant range expansion, provided it causes measurable ecological disruption at the site of introduction.]

- **Invasiveness.** Propensity of nonnative species to invade a new area. Traits that explain differences among species in invasiveness include ability for long-distance dispersal (by natural means or human agency), to establish with small numbers of propagules, and in diverse environments.
- **Invasibility.** Susceptibility of an area of introduction to invasion by a nonnative species. Environmental characteristics that explain differences in invasibility among habitats include community structure and disturbance factors.
- **Pest.** A species or lower taxon of plant, animal, or pathogenic agent injurious to plants or plant products.

General introduction

The expansion of global trade has significantly contributed to the movement of organisms beyond their native geographic ranges, both intentionally and unintentionally (Seebens *et al.* 2017). Between 1800 and 2000, the rate of species introductions steadily increased globally, a trend that is projected to persist in the coming decades (Seebens *et al.* 2017, 2021). Such introductions often result in biological invasions, which are a key driver of global biodiversity loss (Bellard *et al.* 2016) and a substantial source of economic damage (Holmes *et al.* 2009). Insects represent a large part of these organisms translocated from one continent to another, their number of non-native species established outside the native range outnumbering those from all other animal groups, with recent estimates ranging from 4992 to 9835 (Liebhold *et al.* 2024). Moreover, this recent influx of non-native insects appears to involve predominately accidentally-introduced herbivores and, among them, ca. 75% associated to woody plants (Roques *et al.* 2020). This is probably related to recent changes in trade practices, especially the increased magnitude in the movement of live plants globally (Liebhold *et al.* 2012, Eschen *et al.* 2015, Essl *et al.* 2015).

This international movement of non-native insects associated to woody plants poses a significant threat to forests worldwide (Holmes *et al.* 2009, Aukema *et al.* 2011). Non-native herbivores, including wood-borers, sap-feeders, foliage-feeders, and seed eaters, are regarded as the most destructive (Brockerhoff and Liebhold 2017). Among the most successful invasive species, a large number of scolytid species (Coleoptera: Curculionidae: Scolytinae), i.e. bark and ambrosia beetles (Wood 1982) have established populations outside their native range, partly due to their cryptic nature and close association with bark and wood. This enables their unintentional spread across continents through the trade of plants, timber, raw wood, transport in wood products, and packaging materials (Brockerhoff *et al.* 2006, Rassati *et al.* 2018, Meurisse *et al.* 2019, Pureswaran *et al.* 2022).

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a major group of wood-boring insects known for their ability to tunnel into tree bark, where they lay eggs and develop within the phloem (Kirkendall *et al.* 2015). While they play essential roles in forest ecosystems by decomposing dead wood, their introduction into new regions driven by global trade and climate change over the past two centuries has led to severe outbreaks, causing mass mortality of healthy trees (Seebens *et al.* 2017, 2021). Currently, about 40 bark beetles are known to have established populations in countries outside their native range worldwide.

Understanding the drivers behind the invasion success of non-invasive bark beetles remains a pivotal area of research in ecology and invasion biology (Pureswaran *et al.* 2022). This research has profound implications for predicting high-risk species and regions, mitigating ecological and economic consequences, and enhancing strategies for managing invasive populations (Liebhold and Tobin 2008). This PhD thesis has been initiated after the record of the introduction in China of an invasive species of bark beetle native to Europe, *Hylurgus ligniperda* (Coleoptera: Curculionidae: Scolytinae). It stands out as the most widely distributed

species across invaded regions. A global multispecies survey of invasive Mediterranean pine bark beetles conducted between 2016 and 2017 deployed a network of interception traps (six black cross-vane traps in each country) across six non-European countries (Argentina, Australia, New Zealand, South Africa, the United States, and Uruguay) and six European countries (France, Greece, Hungary, Italy, Portugal, and Spain) for comparison (Faccoli *et al.* 2020): in Mediterranean regions, *Orthotomicus erosus* (12,063 specimens), *H. ligniperda* (3,920), *Hylurgus angustatus* (2,953), *Hylurgus micklitzi* (2,121), and *Hylastes ater* (303) were the most frequently detected species; by contrast, within non-native regions of the Southern Hemisphere, *H. ligniperda* dominated trap catches (14,544 specimens, including 11,312 in New Zealand, 2,600 in Uruguay, 436 in Australia, 195 in Argentina, 1 in South Africa), followed distantly by *H. ater* (303), highlighting the exceptional invasive success of *H. ligniperda* outside its native range. After its introduction to New Zealand in 1974, *H. ligniperda* spread nationwide within just three years of its initial detection (Bain 1977), a rapid expansion largely attributed to its exceptional dispersal capacity (Chase *et al.* 2017, Meurisse and Pawson 2017, Brockerhoff *et al.* 2025). In New Zealand's plantation forests, *H. ligniperda* populations surged dramatically within the first two to three years following harvesting, with more than 687,000 individuals captured in funnel traps (even-aged *Pinus radiata* stands) (Brockerhoff *et al.* 2025). During a 12-month monitoring period (2012-2013) in Uruguay (*Pinus taeda* stands), 7,673 scolytine beetles were captured, with bark beetles comprising 98.9%, among which *H. ligniperda* was the most abundant (57%), followed by *O. erosus* (30%) and *Hylastes luteus* (12%) (Gómez *et al.* 2017). *H. ligniperda* (1,762 individuals) was the most frequently captured scolytid in a 2001 trap comparison study conducted in a managed Christmas tree plantation in New York dominated by *Pinus nigra*, *Pinus strobus*, and *Pinus sylvestris*. Other frequently collected species included *Orthotomicus caelatus* (194), *Tomicus piniperda* (187), *Ips grandicollis* (155), and *Hylastes opacus* (82) (Petrice *et al.* 2004). These data clearly demonstrate that *H. ligniperda* exhibits exceptional invasive success across diverse pine forest ecosystems worldwide, highlighting its formidable dispersal capacity and rapid reproductive capabilities. They also reveal that the species has achieved significant ecological release in novel environments, posing a long-term threat to local forestry production and ecological security.

The principal objective of this thesis is to identify the key biological and genetic traits underlying the global invasion success of bark beetles, with a particular emphasis on *H. ligniperda*. To address this objective, the research is structured into two complementary components: a comparative analysis of biological traits between invasive and non-invasive bark beetles, and a genetic investigation focusing on species within the genus *Hylurgus*. Ultimately, this work aims to elucidate the mechanistic drivers of differential invasiveness among species within the tribe Hylurgini, seeking to answer the central question of why certain species exhibit strong invasive potential while others do not.

1) Comparing the biological and ecological traits of invasive and non-invasive congeners within the tribe Hylurgini (Chapter1 & Chapter3)

H. ligniperda has a global invasion history of over half a century (Murayama and others 1936), *Dendroctonus valens* has invaded China since 1998 (Song 2000), and *T. piniperda* has been established in North America since 1992 (Poland and Haack 2000), causing significant damage to pine forests. However, their closely related congeneric species (*Dendroctonus ponderosae*, *Tomicus destruens*, *H. micklitzi*) have not successfully established populations across continents. To identify invasion-related traits, we conducted a comparative analysis of ecological, physiological, and genetic characteristics among these invasive and non-invasive bark beetles, all of which feed on *Pinus* spp. and belong to the tribe Hylurgini.

2) Mitochondrial genomics and phylogenetics of seven Hylurgini species (Chapter 2)

Mitochondrial genomes are widely used in species identification, molecular evolution, and population genetics due to their ease of amplification, maternal inheritance, low recombination rate, and rapid mutation rate (Cameron 2014). Currently, GenBank contains mitochondrial genomes for 572 Coleoptera species, including 39 from Scolytinae. Within Hylurgini, only *D. valens*, *Dendroctonus rufipennis*, and *H. ligniperda* have complete mitochondrial genomes (Guo et al. 2023, Yu et al. 2023). However, mitochondrial data beyond COI remains scarce, limiting phylogenetic studies. To address this gap, we sequenced, assembled, and annotated the mitochondrial genomes of seven key Hylurgini species (*D. valens*, *H. ligniperda*, *H. micklitzi*, *T. piniperda*, *Tomicus brevipilosus*, *Tomicus minor*, and *Tomicus yunnanensis*) that cause significant damage to conifers. Our goal is to analyze mitochondrial genome structure, base composition, and gene evolution, while clarifying the phylogenetic relationships between Hylurgini and other Scolytinae groups.

3) Comparative mtDNA gene phylogeographic patterns of *Hylurgus* spp. (Chapter 3)

H. ligniperda and *H. micklitzi* are congeneric bark beetles native to the Western Palearctic, where they develop on pine trees, often in sympatry, with similar life histories (Fabre and Carle 1975, El Khoury et al. 2019). *H. ligniperda* has successfully invaded multiple continents, whereas *H. micklitzi* remains restricted to its native range. This provides an excellent model for exploring historical species differentiation and invasion-related traits. By comparing mitochondrial COI genetic diversity and phylogeographic patterns of these two species across the Western Palearctic, we aim to assess whether key ecological or genetic differences contributed to the invasion success of *H. ligniperda*.

Together, these chapters provide an integrated perspective on the ecological, genetic, and evolutionary factors contributing to the global invasion success of *H. ligniperda*. By comparing invasive and non-invasive congeners, assembling complete mitochondrial genomes, and analyzing phylogeographic patterns, this research will lay the groundwork for more targeted surveillance and management strategies of high-risk bark beetle species.

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Chapter 1: Invasion and non-invasion in bark beetles: a trait-based perspective

Chapitre 1: Invasion et non-invasion chez les coléoptères de l'écorce: une perspective basée sur les traits

Résumé en français du contexte, des objectifs de l'étude et des méthodes utilisées

Résumé

Ce chapitre présente un aperçu de l'état mondial des invasions des coléoptères de l'écorce (Scolytinae) et passe en revue les bases de données et la littérature actuelles, en introduisant plus de 40 espèces de coléoptères de l'écorce établies en dehors de leurs aires de répartition natives. Bien que la plupart des coléoptères de l'écorce soient écologiquement inoffensifs ou bénéfiques dans leurs forêts d'origine, certaines espèces causent des dommages écologiques et économiques importants dans de nouveaux environnements. La propagation des coléoptères de l'écorce invasifs est étroitement liée au transport de produits en bois et au changement climatique, avec des exemples notables comme *Dendroctonus valens*, *Tomicus piniperda* et *Hylurgus ligniperda*.

Le chapitre explore également les caractéristiques biologiques, écologiques et évolutives susceptibles de favoriser le succès de l'invasion. Parmi les traits courants associés à l'invasion figurent une grande taille corporelle, la multivoltinisme (plusieurs générations par an), une large gamme d'hôtes, une tolérance au froid et une forte capacité de dispersion. Cependant, aucun trait unique ne permet de prédire de manière fiable l'invasibilité d'une espèce. Le succès de l'invasion semble résulter d'une combinaison de traits spécifiques à l'espèce, de l'historique d'introduction (par exemple, la pression de propagule, les introductions multiples) et des caractéristiques de l'écosystème récepteur (par exemple, la compatibilité climatique, la disponibilité des hôtes et la résistance biotique). Un cadre de comparaison fondé sur une approche multifactorielle est proposé pour mieux comprendre les facteurs moteurs des invasions des coléoptères de l'écorce.

Dans la section 3, l'auteur procède à une comparaison systématique des caractéristiques écologiques et génétiques de trois paires d'espèces sœurs : *Dendroctonus valens* et *D. ponderosae*, *Tomicus piniperda* et *T. destruens*, ainsi que *Hylurgus ligniperda* et *H. micklitzi*. Les résultats montrent que les espèces invasives tendent à avoir une plus grande gamme d'hôtes, une plus grande plasticité écologique et une structure de population plus complexe, des caractéristiques qui contribuent en partie à leur succès en tant qu'espèces envahissantes. Cependant, cette relation n'est pas absolue, soulignant l'importance des facteurs environnementaux et de l'aléa historique.

Mots-clés: coléoptères de l'écorce; espèces invasives; succès de l'invasion; caractéristiques des espèces; Hylurgini

Introduction chapter: Could biological and evolutionary traits predict the invasion success of non-native species? Study cases in invasive and non-invasive congeneric bark beetles

1 Introduction

1.1 Invasive scolytids

Scolytids are broadly classified into two groups based on their feeding habits: bark beetles and ambrosia beetles (Wood 1982). Bark beetles are relatively host-specific, constructing galleries primarily in the phloem or occasionally in the sapwood, from which they obtain nutrients (Kirkendall *et al.* 2015). In contrast, ambrosia beetles have a broader host range, cultivating symbiotic fungi within their wood galleries, which serve as their primary food source throughout both larval and adult stages (Batra 1966). Bark beetles have a more homogenously global distribution, less dependent on strict climates, and largely influenced by host specificity, may colonize larger areas and establish in distant regions if transported, while ambrosia beetles are more diverse and abundant in warm, humid regions, particularly tropical areas, despite being polyphagous, largely influenced by climate and establish only in climates suitable for reproduction (Brockerhoff *et al.* 2006, Rassati *et al.* 2016, Gohli and Jordal 2017). While sib-mating is relatively rare in bark beetles, where most species outbreed as females locate mates at the entrance of new galleries, it is more common in ambrosia beetles, with approximately half of the species inbreeding by mating with their brothers within maternal galleries (Kirkendall *et al.* 2015). Inbreeding species mitigate the risks of inbreeding depression that can arise in outbreeding species when mating partners are too closely related (Peer and Taborsky 2005, Andersen *et al.* 2012), making them less vulnerable to genetic constraints typically associated with small founder populations. At present, 123 Scolytinae species (42 bark beetles, 51 ambrosia beetles, 29 seed- and twig-feeding species, and one root-feeding species) have established outside their native range in the world (Lantschner *et al.* 2020), posing economic and ecological threats to forests and plantations (Hulcr and Dunn 2011), yet they represent only a small fraction of the described Scolytinae fauna (i.e., about 6000 species) (Rabaglia *et al.* 2019, Lantschner *et al.* 2020, Pureswaran *et al.* 2022).

1.2 Known drivers of scolytid invasion success

Understanding the drivers behind the invasion success of alien insects is a critical area of research in ecology and invasion biology (Pureswaran *et al.* 2022), with implications for predicting high-risk species and regions, mitigating ecological and economic impacts, and improving invasive population management (Liebhold and Tobin 2008). The success of non-native species invasions is driven by three key factors: propagule pressure (frequency and number of introductions) (Brockerhoff *et al.* 2014), invasiveness (species traits affecting survival and reproduction) (Kolar and Lodge 2001), and invasibility (biotic and abiotic conditions of the new habitat) (Fridley 2011).

This chapter focuses on propagule pressure and invasiveness, but does not delve deeply into invasibility.

Long-distance transportation of Scolytines primarily occurs through individuals concealed in live woody plants, fresh or processed timber, and wood packaging materials such as dunnage, crates, pallets, and drums, as well as wood products like furniture, toys, and crafts (Brockerhoff *et al.* 2006, Meurisse *et al.* 2019). Additionally, adult beetles may spread via hitchhiking, unintentionally transported on vehicles, shipping containers, trailers, or other traded goods such as heavy machinery and small packaged items, allowing them to travel undetected over long distances while being shielded from adverse environmental conditions (Pureswaran *et al.* 2022). While international travelers are unlikely to be a major vector, occasional transport may occur through outdoor equipment, contaminated personal belongings, or infested wood crafts. Scolytines also disperse actively or passively over long distances (Chase *et al.* 2017, Meurisse and Pawson 2017), but flying individuals are rapidly diluted in the environment, limiting their role to short-range expansion or long-distance invasions by inbreeding and parthenogenetic species like ambrosia beetles (Pureswaran *et al.* 2022). As a result, traded commodities remain the primary driver of long-distance Scolytinae species movement, with round wood imports identified as a major invasion risk. Traded commodities are the primary vectors for long-distance Scolytinae species dispersal, with round wood imports identified as a major invasion risk (United States. Forest Service 1992, 1993, Kliejunas *et al.* 2003, EPPO 2020). In 2014, international trade volumes reached 133.5 million m³ for industrial round wood and 9.3 million m³ for fuelwood (Allen *et al.* 2017). Among the various pathways, inter-continental trade of fresh wood poses one of the greatest risks for the spread of woodboring beetles (Meurisse *et al.* 2019). Evidence from interception records highlights the strong association between bark and ambrosia beetles and traded wood products. In Finland, Siitonen (2000) found that 23 Scolytinae species, out of 43 bark- and wood-boring beetle species, were intercepted in Russian round wood imports. A study by Haack (2006) in the USA identified 40 Scolytinae genera intercepted on imported wooden commodities and packaging materials between 1985 and 2000. Similarly, Brockerhoff *et al.* (2006) reported 103 bark and ambrosia beetle species intercepted on sawn timber, logs, and other wooden imports and packaging materials in New Zealand between 1950 and 2000.

Analyses performed at the continental scale indicate that the likelihood of establishment in an invaded area is primarily a function of import volume for bark beetles, like using interception data as a proxy for propagule pressure (Brockerhoff *et al.* 2014), but that it is also strongly affected by other factors (Marini *et al.* 2011). *Ips* and *Dendroctonus* bark beetles are often intercepted outside their native ranges (Haack 2002, 2006), but with the exception of *Dendroctonus valens*, most have failed to establish successfully. For example, the Eurasian spruce bark beetle, *Ips typographus*, infests many native and alien *Picea* trees, has been intercepted 727 times globally (Turner *et al.* 2021), including 253 occurrences in North America between 1985 and 2005 (Haack 2006), yet it has not established despite the widespread availability of suitable hosts across the continent. And

the mountain pine beetle (*Dendroctonus ponderosae*), a highly aggressive pest of *Pinus* spp. in western North America, has yet to be intercepted in Europe but has been recorded 292 times in South Korea and five times in Japan (Turner *et al.* 2021), highlighting its potential to establish in pine-growing regions beyond its native range.

A global study found that high reproductive rates and longer reproductive lifespans are strongly associated with invasion success in mammals. These life history traits make it easier for such species to establish and spread in new environments (Capellini *et al.* 2015). Research has also shown that cognitive traits such as behavioral flexibility, innovation, and problem-solving abilities play a key role in animal invasions. For example, the North American raccoon (*Procyon lotor*) demonstrates a high level of problem-solving ability and behavioral adaptability, allowing it to thrive in a wide range of environments (Szabo *et al.* 2020). The rusty crayfish (*Orconectes rusticus*) exhibits faster growth and greater competitive ability than native species in invaded areas, enabling it to quickly occupy shelters and evade predators. These evolved traits amplify its ecological impact, leading to declines in the diversity of native plants and invertebrate (Therry *et al.* 2019). Certain biological traits are also overrepresented among non-native plant species that have successfully invaded new areas (Price and Jain 1981, Pyšek and Richardson 2007). It is very likely that functional traits of species (i.e, plants, birds, insects), such as the biology, the numbers of introduction pathways and habitats, life history and genomic traits, size and fitness, and the number of enemies, promote invasiveness (Kolar and Lodge 2001, Van Kleunen *et al.* 2010, Zhao *et al.* 2023). One possible reason is that, aside from a few exceptions, the majority of studies investigating species traits associated with invasiveness have primarily focused on relatively simple attributes such as voltinism and habitat type, as these traits are commonly documented across a wide range of species. Many potentially important functional traits for invasiveness, however, have to be measured on invasive and non-invasive species grown under common environmental conditions (Van Kleunen *et al.* 2010). Actually, it appears that within scolytids congeneric species could be either highly invasive or conversely never move from the native range (Vega and Hofstetter 2014).

2 Overview of scolytids invasions

2.1 Materials and Methods

Study areas

This study was conducted on a global scale, dividing all land areas (except Antarctica) into six biogeographic regions: Nearctic, Neotropic, West-Palearctic, Pest-Palearctic, Indo-Malay, Afrotropic, and Austro-Pacific (Figure 1). These regions are adapted from Lantschner *et al.* (2020)'s classification and Wallace's classification, with slight modifications to align with national political boundaries. Due to the relatively low number of bark beetle species in Oceania,

Australasia and Oceania were combined into a single Austro-Pacific region. We visualized the map in R (version 4.4.3), employing packages such as ggplot2.

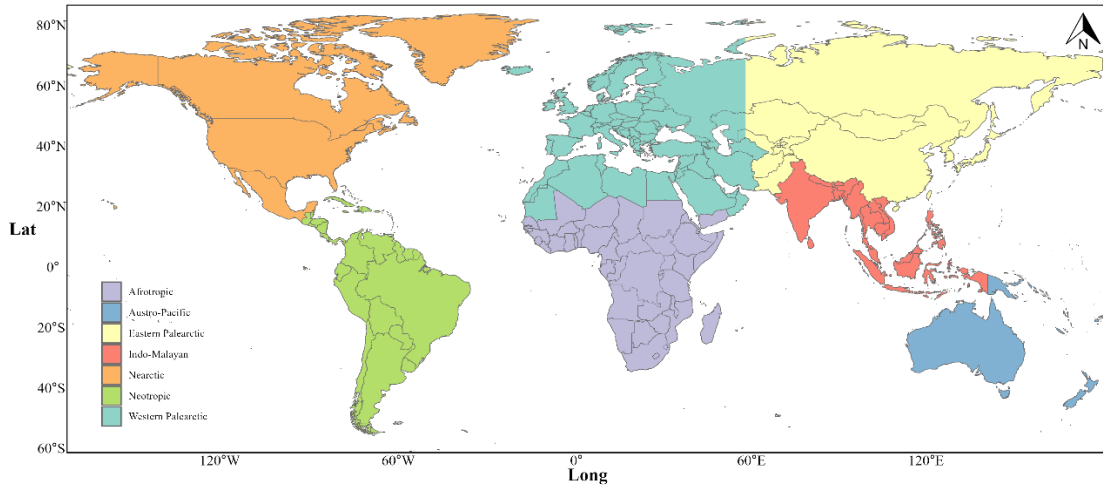


Figure 1 Geographical ranges of the world's biogeographical regions

Non-native Scolytinae species data collection

We collated records of bark beetle species established outside their native ranges from diverse sources, including peer-reviewed scientific literature and publicly accessible online databases documenting first detections prior to 2025 (Table S1). The study focuses exclusively on nonnative species originating from geographically distant biogeographic regions or separate continents. Species that expanded their ranges within the same native biogeographic region, such as *Polygraphus proximus*, were excluded from the analysis. Our search focused primarily on regional monographs and catalogs (Wood 1972, 1986, 2007, Wood and Bright 1992), in addition to online databases (Zenodo, CABI, GBIF) and published compilations of nonnative species (Lantschner *et al.* 2020). Our analysis was restricted to records of species that have established self-sustaining populations in nonnative regions, defined as those consistently detected over multiple years and across different locations within the invaded area. For each nonnative species, we compiled data on both native and invaded ranges, classified into seven biogeographic regions, based on information from the same sources, including literature and databases. It should be noted that the native range of *Scolytogenes jalapae* remains uncertain. In such cases, we adopted the native region deemed most likely based on the criteria established by experts (mainly Wood (1972, 1986, 2007), Wood and Bright (1992b), Haack and Rabaglia (2013), Lantschner *et al.* (2020), Marchioro *et al.* (2022), as well as other updated specific publications. See Table S1 for more detail). Species with a wide distribution and highly uncertain native ranges were excluded from analyses concerning regions of origin and destination. However, they were retained in assessments of the total number of colonized regions (see relevant section for further details).

Data analysis

Each species was further categorized by its feeding habit and mating system, as these biological traits are thought to influence both the likelihood of introduction and the success of establishment. Mating systems were classified as either (1) polygyny or (2) monogamy. Host types were grouped into (1) coniferous trees and (2) broadleaved trees. In terms of impact, species were assigned to one of three categories: (1) primary bark beetles, which have been observed attacking and successfully killing healthy hosts; (2) secondary bark beetles, which typically attack weakened or already dead hosts. And we compiled information on the number of host plant families for each bark beetle as a proxy of their diet breadth.

We calculated the total numbers of species invading two categories of numbers of colonized regions (including the native range): two to three and four to six. We classified species by their number of hosts in two categories: oligophagous (species that use hosts from one or two plant families) and polyphagous (species that use hosts from three or more plant families). In the case of host type, we used two categories: conifer trees and broadleaf trees. For the mating system, we considered two categories: polygyny and monogamy. They were categorized based on the severity of the damage they cause into primary pest and secondary pest. We then used chord diagrams and alluvial plots to visualize the results in R (version 4.4.3), employing packages such as *circlize*, *ggalluvial*, and *ggplot2*.

2.2 Results and discussions

Based on range data presented primarily in previous lectures and data, at least 40 bark beetles are known to be established in countries outside their native range worldwide (Table S1). These 40 bark beetle species belong to 20 genera across 12 tribes, among which the genera *Scolytus*, *Orthotomicus*, and *Hylastes* contain a greater number of invasive species than other genera. Among the 40 invasive bark beetle species, the Palearctic region is the most common origin, followed by the Nearctic and Indo-Malayan regions. In contrast, the main recipient regions are the Neotropic, Nearctic, and Afrotropic zones (Figure 2). Of these 40 species, 27 infest conifers, while 13 target broad-leaved trees. In many countries in the Southern Hemisphere, the extensive planting of exotic tree species, particularly *Pinus* and *Eucalyptus*, has dramatically increased over the past century due to their outstanding growth and adaptability in new environments (Sedjo 1999). These pine forest plantations native to the Northern Hemisphere (for example, *Pinus radiata*, *Pinus elliottii*, *Pinus taeda*, *Pinus patula*, *Pinus ponderosa*, *Pinus contorta*) provide a vast and suitable habitat for conifer-feeding bark beetles, facilitating their potential for wide distribution (Lantschner et al. 2017). Most invasive conifer-infesting bark beetles utilize pine (*Pinus*) as their primary or secondary host for breeding. Most invasive bark beetles that infest conifers utilize *Pinus* species as either primary or secondary hosts for reproduction. The predominance of conifer-infesting species among invasive bark beetles globally can be attributed to three main factors: (1) nearly every conifer species hosts at least one bark beetle species; (2) conifer wood is widely used in

international trade, particularly in solid wood packaging materials; and (3) many countries in the Southern Hemisphere have established extensive plantations of exotic conifers, primarily using species native to the Northern Hemisphere (Haack 2002, Faccoli *et al.* 2020). The introduction of non-native pines has led to unforeseen pest issues, with Mediterranean pine bark beetles posing a significant threat to these plantations, which lack native pine pests and natural enemies for biological control (Colautti *et al.* 2004, Sopow *et al.* 2015, Gómez *et al.* 2017). Regarding the mate behavior of the 40 bark beetle species that have become established in other continents or world regions, 22 are monogamous species where the females initiate attack, whereas 17 are polygamous species where the males initiate attack (Figure 3, Figure S1).

Most conifer-infesting bark beetles that have established populations outside their native range (Table S1) are typically classified as either secondary bark beetles or solitary colonizing bark beetles. Secondary bark beetles primarily infest weakened or recently dead host trees. Examples include various species from the genera *Crypturgus*, *Dryocoetes*, *Hylastes*, *Hylurgops*, *Hylurgus*, *Ips*, *Orthotomicus*, *Phloeosinus*, *Pityogenes*, *Polygraphus*, *Scolytus*, and *Tomicus* (Haack 2002). The genera *Hylastes*, *Hylurgus*, and *Orthotomicus* are among the most abundant and widespread species infesting pines in the Mediterranean region, though they are rarely considered pests (Faccoli *et al.* 2020). Since solid wood packing materials are often made from freshly cut trees, which may already be infested with secondary bark beetles before processing, these beetles have the potential to spread through international trade. Among these genera, many bark beetles have already invaded new continents and established populations with less economic damage, like *Hylastes angustatus*, *Hylastes ater*, *Hylastes opacus*, *Hylurgops palliates*, *Hylurgus ligniperda*, *Ips grandicollis*, *Orthotomicus erosus*, *Phloeosinus armatus*, *Phloeosinus cupressi*, *Phloeosinus dubiosus*, *Pityogenes bidentatus*, *P. proximus*, *Tomicus piniperda* (Table S1). Among them, *H. ligniperda* and *H. ater* were the most widespread species found in several invaded regions (Faccoli *et al.* 2020). Unlike secondary bark beetles, primary bark beetles, such as many North American *Dendroctonus* species, primarily attack and kill healthy, standing trees. They typically launch mass attacks by releasing aggregation pheromones, coordinating their assault on individual trees to overwhelm the host's natural defenses. *D. micans* and *D. ponderosae* are examples of solitary colonizing bark beetles that typically attack the lower trunk of living trees, near the ground line. Among the 40 invasive bark beetle species, only a few, including *D. valens*, *Orthotomicus erosus*, *Pityophthorus juglandis*, and *Scolytus kirschii*, are considered primary pests in certain regions, as they are capable of attacking and killing healthy trees. At present, *D. valens* has successfully established a large population in its introduced range (North China) and has transitioned into a primary pest (Qiu 2013). *O. erosus* is native to the Mediterranean Basin, the Middle East, Central Asia and China, and has become globally invasive, establishing in South Africa, Swaziland, Uruguay and California (Seybold *et al.* 2016, Kirkendall 2018); it primarily infests pines weakened by drought and disturbance yet can colonize and kill healthy pines under arid or outbreak conditions (Sarıkaya and Sen 2017) as documented in Zhejiang Province, China, where it

served as the primary cause of mortality (Jiang *et al.* 1992); it reproduces mainly on *Pinus* spp. and ranks as the second most frequently intercepted bark beetle at US ports with 385 interceptions (Seybold *et al.* 2016). In invaded regions such as eastern North America and parts of Europe, *P. juglandis* is widely considered a primary pest because it acts as the key vector of *Geosmithia morbida*, the pathogen responsible for thousand cankers disease, which causes severe decline and death of walnut trees. In its native range (southwestern North America), however, the beetle typically infests stressed or dying twigs and is regarded as a secondary pest with relatively minor impact (Moricca *et al.* 2019, Marchioro and Faccoli 2022). *S. kirschii*, native to the Western Palearctic region, has invaded Brazil and South Africa, primarily infesting elms (*Ulmus* spp.) and is considered a vector for the pathogens causing Dutch Elm Disease (DED), with reports of it causing the death of healthy English elms (*Ulmus procera*) in South Africa (Six *et al.* 2005, Kirkendall 2018, Özgen 2019). If trees used for wood packing materials were already infested before harvesting and the bark was not completely removed, these beetles could be transported along with the wood. Because solitary colonizers do not rely on large populations to establish

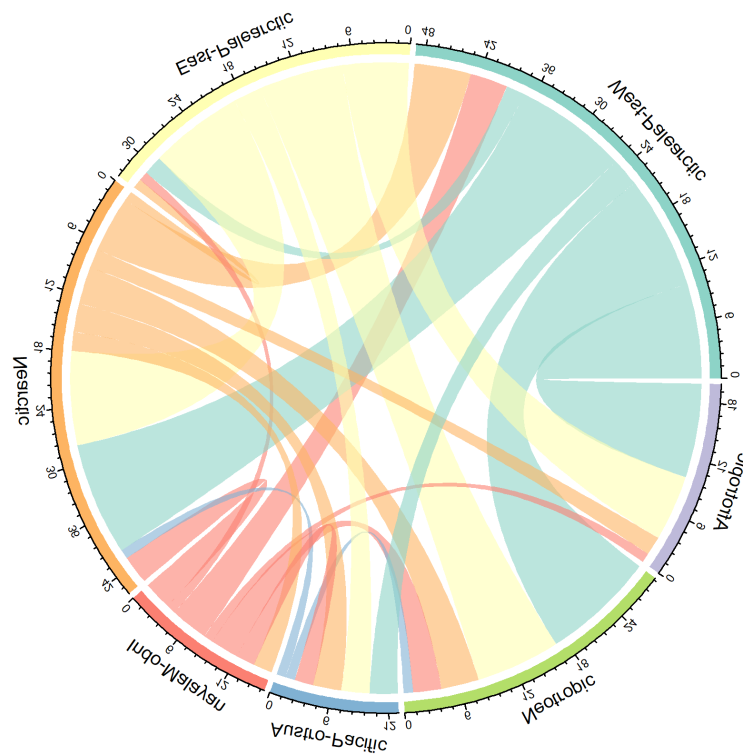


Figure 2 Chord graph showing the source and recipient regions of invasive bark beetles (excluding natural dispersal). This chord diagram illustrates the biogeographic origins and target regions of bark beetle invasions worldwide. Each segment on the outer circle represents a biogeographic region, while the connecting arcs indicate the direction and frequency of species introductions. Arc thickness reflects the number of invasive species involved. Thicker lines represent a higher number of species moving between two regions. Arc color indicates the source region, allowing visual tracking of invasion origins (same in Figure 1).

Themselves, even a small number of individuals introduced into a new environment could lead to successful colonization. Although wood packing materials could theoretically be made from trees infested with primary bark beetles, no such species has successfully established populations outside their native range. This suggests that insufficient numbers of primary bark beetles have been introduced at any given time to trigger a successful mass attack, preventing their establishment in new regions (Haack 2002).

Understanding whether these invasive insect species differ from non-invasive congeneric insect species, and moreover whether these species characteristics are related to invasiveness, remain a challenge. A meta-analysis of 117 studies comparing life-history traits between 125 invasive and 196 non-invasive plant species found that invasive species generally exhibit higher trait values (Van Kleunen *et al.* 2010). For insects, a meta-analysis comparing species characteristics between the 100 worst invasive species and the related non-invasive species showed that invasive insects overall have more pathways of introduction, occur in more habitats,

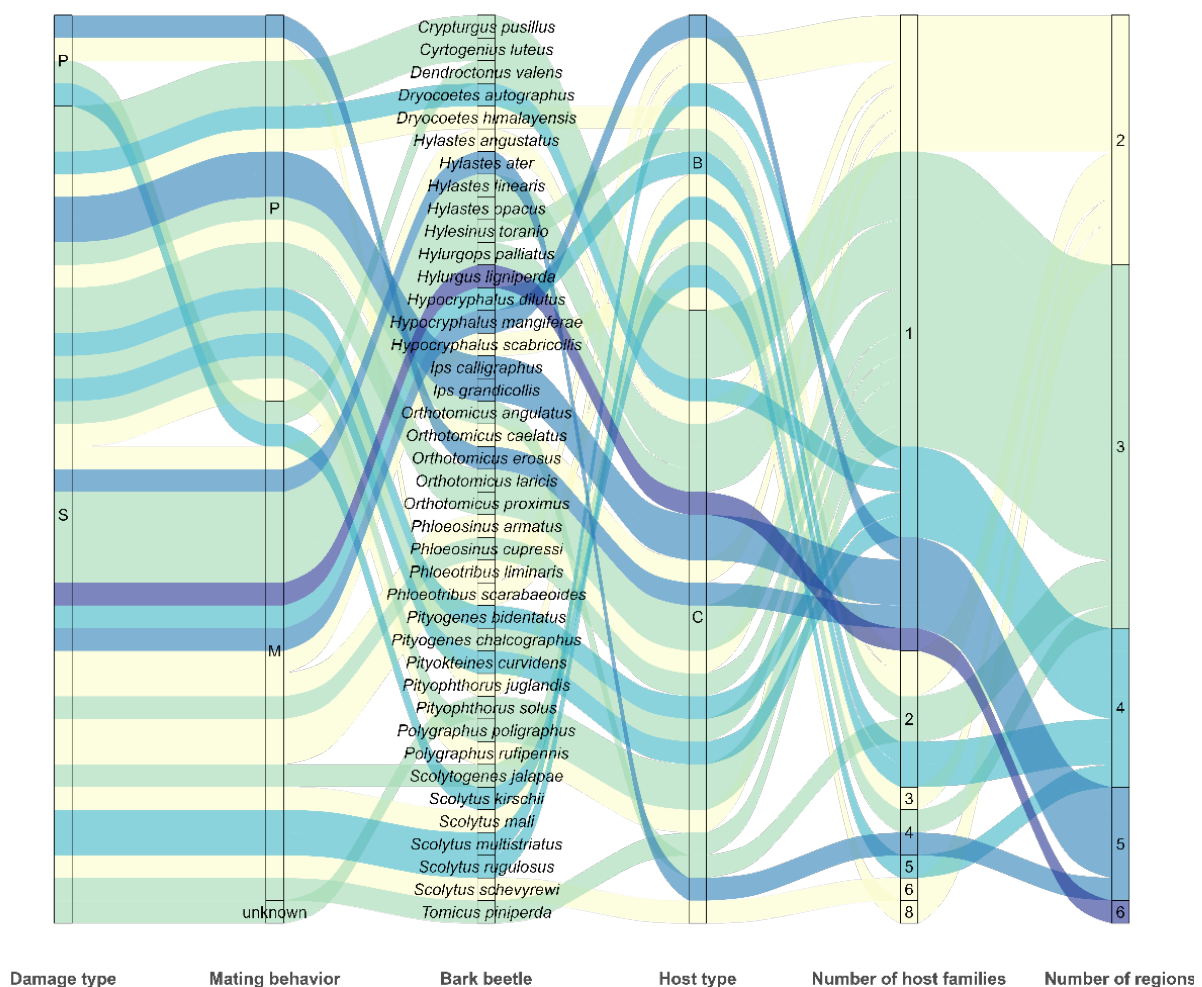


Figure 3 Alluvial diagram showing the number of invasive bark beetles of each trait. Damage: P: primary pest, S: secondary pest; Mating behavior: M: monogamous species, P: polygamous species; Host type: C: coniferous trees; B: broadleaf trees.

have higher fecundities, higher voltinism, more genes, shorter lifespans, and faster development from egg to adult (Zhao *et al.* 2023). Current understanding remains uncertain, largely due to limitations in experimental data and the scope of analyzed invasion traits (Brockerhoff and Liebhold 2017).

3 Focusing on pairs of congeneric bark beetles, including invaders and non-invaders

The tribe Hylurgini (Coleoptera: Curculionidae: Scolytinae), which comprises approximately 130 species in 14 genera (Kirkendall *et al.* 2015), includes some remarkable examples of pairs of congeneric species including invasive and non invasive species. In this chapter, we reviewed two well documented pairs of bark beetles, *Dendroctonus valens* & *D. ponderosae*, and *Tomicus piniperda* & *T. destruens*. As these closely related species pairs infest primarily *Pinus* species, they may share common phylogenetic and ecological characteristics, and thus constitute interesting study cases to identify traits associated with invasiveness. In these examples, each invasive species is paired with a non-invasive congener that co-occurs in its native range and for which relatively abundant biological and ecological data are available, enabling comparative analyses of invasive versus non-invasive life-history traits.

3.1 *Dendroctonus* genus: the invasive species *D. valens* (RTB) vs the non-invasive *D. ponderosae* (MPB)

The red turpentine beetle (RTB), *D. valens* LeConte, is a secondary bark beetle that does not commonly cause tree mortality in its native range in North America, but since its introduction into China in the 1980s, it has caused over 10 million pine trees in northern provinces (Qiu 2013). The mountain pine beetle (MPB) is a primary tree-killing *Dendroctonus* bark beetle in North America and has a long recorded history of major outbreaks (Safranyik *et al.* 2010).

3.1.1 Distribution and host range

***D. valens*, widely distributed with the broad pine hosts in North America, has largely invaded China at present**

In its native areas, RTB exhibits the broadest host range among *Dendroctonus* beetles, a trait likely driven by its expansive geographic distribution, which extends from Nova Scotia (Canada) west to California (USA) through Mexico south to Guatemala and Honduras (Smith 1971, Wood 1982). The native range of *D. valens* extends broadly from 15°N to beyond 60°N latitude, with population concentrated predominantly between 30°N and 50°N (Figure 4) (Zhou *et al.* 2024). In central Mexico, RTB typically occurs at elevations below 3000 meters, whereas in Guatemala it is found at altitudes ranging from 1500 to 3000 meters (Wood 1982). In Arizona, RTB shows a strong preference for high elevations (2,505–2,651m) (Williams *et al.* 2008), contrasting with observations from Mexico, where the species is more commonly encountered at elevations between 2100 and

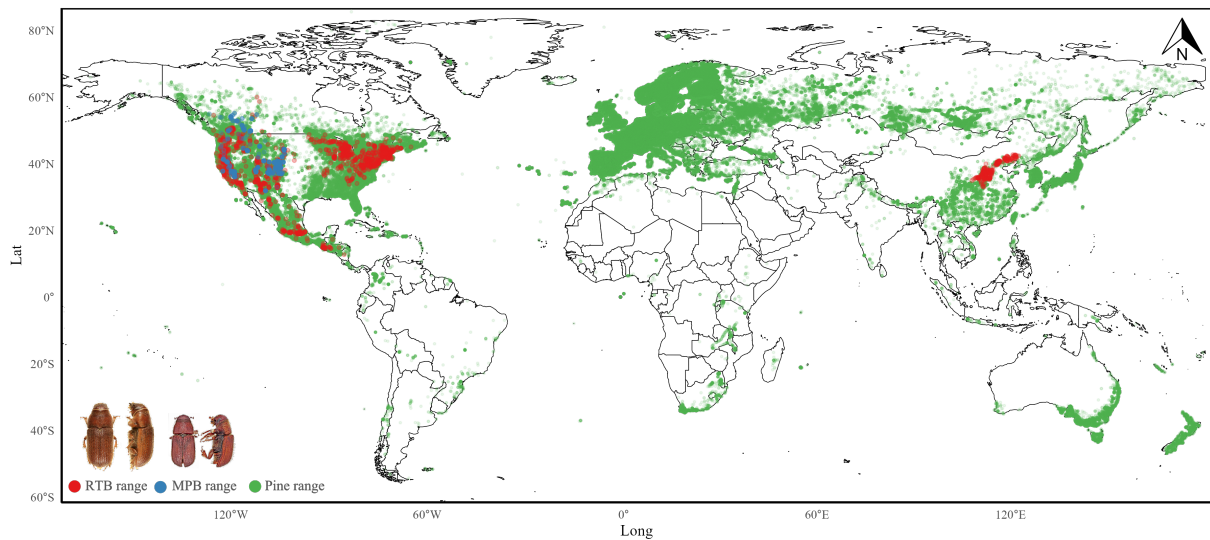


Figure 4 Percent distribution of *D. valens* and *D. ponderosae*. The sites of RTB refer to [Zhou et al. \(2024\)](#). The sites of MPB and pine trees refer to GBIF. The photos of insects are from the website (www.invasive.org).

2500 meters ([Salinas-Moreno et al. 2004](#)). Across North America, *D. valens* attacks all species of *Pinus* within its range and occasionally targets *Picea* (spruce) and *Larix* (larch) species ([Bright Jr. 1976](#), [Furniss and Carolin 1977](#), [Cibrián Tovar et al. 1995](#)) (Table 1). In the western United States, *P. ponderosa*, *P. contorta*, *Pinus jeffreyi*, *Pinus lambertiana*, *Pinus monticola*, and *P. radiata* are preferred hosts, with Monterey pine (*P. radiata*) reported as the tree most frequently killed and ponderosa pine (*P. ponderosa*) is the tree most frequently attacked ([Smith 1971](#)). In Mexico, RTB infests 20 *Pinus* species and shows a marked preference for *Pinus leiophylla* within its optimal elevational range (2100–2500 m) ([Salinas-Moreno et al. 2004](#)).

Following its initial detection in Shanxi province, *D. valens* has established infestations across seven provinces in China (33°N–41°N), primarily colonizing *Pinus tabuliformis* as its primary host and *Pinus sylvestris* var. *mongolica* as its preferred host, with occasional infestations recorded in *Pinus bungeana* and *Pinus armandii* in Shanxi province ([Yan et al. 2005](#), [Zhao et al. 2008](#), [Liu 2020](#), [Liu et al. 2021](#), [Zhang et al. 2022](#), [Zhou et al. 2024](#)) (Figure 4). Field observations in Inner Mongolia revealed severe damage to *P. sylvestris* var. *mongolica* in mixed forests, contrasting with lower susceptibility of *P. tabuliformis* ([Liu 2020](#)) (Table 1). Laboratory experiments further confirmed a host preference hierarchy, with *Pinus sylvestris* var. *mongolica* ranked highest, followed by *Pinus tabuliformis*, and then *Pinus bungeana* (wild conditions); under laboratory conditions, *P. sylvestris* var. *mongolica* and *Pinus massoniana* were preferred over *P. tabuliformis* ([Liu 2020](#)). Despite no wild infestations reported on *P. massoniana*, this species exhibited the highest phloem consumption in laboratory assays ([Liu 2020](#)). Adult beetles successfully reproduced in all four tested host species (*P. sylvestris* var. *mongolica*, *P. massoniana*, *P. tabuliformis*, and *P. bungeana*), highlighting their broad host adaptability ([Liu 2020](#)).

RTB, typically a secondary pest that infrequently kills hosts in its native regions, becomes a primary pest responsible for substantial damage in invasive areas.

D. ponderosae*, mainly existed in western North America, infesting almost all pine trees except *P. jeffreyi

MPB is one of the most economically significant native pests in western North American forest ecosystems. Its expansion into higher elevation ranges, more northern latitudes, and new host species is ongoing (Mock *et al.* 2007, Safranyik *et al.* 2010, Cullingham *et al.* 2011, Raffa *et al.* 2013), with MPB currently distributed from the Baja California Peninsula in Mexico to northern British Columbia and western Alberta in Canada (Wood 1982, Salinas-Moreno *et al.* 2004) (Figure 4).

D. ponderosae colonizes all native pines within its range, except *P. jeffreyi* (Wood 1982), and also targets several exotic pine species (Table 1). Among its hosts, lodgepole pine (*P. contorta*) is the most commonly infested, supporting the largest and most severe outbreaks (Safranyik and Carroll 2006). Although outbreaks also affect *P. ponderosa*, *Pinus flexilis*, and *Pinus albicaulis*, they are generally more localized, partly due to the restricted ranges of these species (Six and Bracewell 2015). Research shows that MPB exhibits similar infestation rates and reproductive success in its natal host, lodgepole pine (*P. contorta*), and in alternate hosts such as ponderosa pine (*P. ponderosa*) under favorable conditions (West *et al.* 2016). Historical records from 1967 document MPB infestations in seven non-traditional hosts at the University of Idaho's Shattuck Arboretum, including non-native pine hosts jack pine (*P. banksiana*), Austrian pine (*P. nigra*), and native host red pine (*Pinus resinosa*), with confirmed tree mortality (Furniss and Schenk 1969). Furthermore, non-native Eurasian and Asian pines in North American arboreta, such as Chir pine (*Pinus roxburghii*) and Japanese red pine (*Pinus densiflora*), have also been attacked by MPB (Furniss and Schenk 1969, Smith 1981). Subsequent research reveals that MPB successfully colonizes freshly cut logs of novel northeastern North American pines, such as *Pinus strobus* (eastern white pine) and *Pinus sylvestris* (Scots pine) (Cook and Martinez 2018). While reproductive efficiency in these hosts remains lower than in historical hosts like lodgepole pine (*P. contorta*) and ponderosa pine (*P. ponderosa*), their weak constitutive chemical defenses make them highly susceptible to invasion (Rosenberger *et al.* 2017b). MPB exhibits cross-generic host expansion, colonizing spruce species (e.g., MPB exhibits cross-generic host expansion, colonizing spruce species (e.g., *Picea abies*, *P. glauca*) with emergence densities comparable to pine hosts, a phenomenon also observed in other *Dendroctonus* species during outbreaks when high beetle abundance and olfactory confusion caused by aggregation pheromones from attacked hosts trigger spillover attacks on nonhost trees (Furniss and Schenk 1969, Cook and Martinez 2018).

3.1.2 Species biological and ecological traits

As congeneric beetles, RTB and MPB share many similar ecological and biological species characteristics. However, when comparing their traits from previous lectures, RTB exhibits a bigger

body size, higher voltinism, earlier flight time, and a lower supercooling point compared to MPB (Table 1).

Body size

For RTB, the body mass of *D. valens* is 31.13 ± 0.99 mg, and there were no significant differences between the body mass of females (32.06 ± 1.06 mg) and males (29.59 ± 0.81 mg) in China (Shi and Sun 2010a). Its body length ranges from 5.3-9.2 mm (mean 7.3 mm), making it the largest beetle in the *Dendroctonus* genus (Zhang et al. 2002). The body length ranges from 7.5-9.6 mm (mean 8.28 mm) in females and 5.9-8.1 mm (mean 6.47 mm) in males (Miao et al. 2001).

For MPB, the body mass ranges from 6.3-19.0 mg and body length ranges from 4.41-6.09 mm in females and 4.1-13.6 mg and 3.78-5.46 mm in males (Pureswaran and Borden 2003), confirming the consistent sexual size dimorphism in this species (Bracewell et al. 2013, Bentz et al. 2014).

Beetle preflight weight influenced flight initiation, distance, and duration; larger beetles were more likely to fly and, once in flight, traveled longer and farther than smaller beetles, utilizing lipids as their energy source, as evidenced by lower lipid content postflight, with flight distance negatively correlated with postflight lipid levels (Evenden et al. 2014). Wu (2024) found that pre-flight body weight significantly influenced flight propensity and flight distance, with heavier beetles more likely to disperse and colonize new region.

Voltinism

RTB, in most native areas, has at least one generation per year, with up to two years for one generation in northern high-elevation regions and as many as three generations per year in southern low-elevation areas (Smith 1971). In most regions, RTB completes one generation annually, but this extends to two years in cold or high-elevation regions, while warm or low-elevation regions may support up to three generations per year (Smith 1971, Zhang et al. 2002). In China, it's univoltinism in the northern regions (North to 36.5 °N) but shifts to bivoltinism in the southern regions (South to 36.5° N) (Zhao et al. 2008).

MPB is predominantly univoltine throughout most of its range in western North America, with semivoltinism (taking 2 years for a single generation) at the highest elevations, and bivoltinism being rare or absent, meanwhile northern populations developing faster than southern populations to conform to relatively shorter growing seasons (Bentz and Powell 2014, Bentz et al. 2014). A univoltine life cycle allows the insect to synchronize the emergence of brood adults, facilitating coordinated mass attacks on host trees and ensuring that offspring enter winter as larvae, the stage best adapted to survive cold temperatures. In contrast, a semivoltine life cycle is maladaptive, as it disrupts seasonal synchronization, leading to more brood entering winter at stages vulnerable to freezing. Additionally, it prolongs the feeding period in a host that is progressively declining in nutrients and moisture, and increases the duration of exposure to natural enemies (Six and Bracewell 2015).

Reproductive strategies and sex dynamics of parent adults in brood systems

In the native range, the sex ratio (male to female) of RTB is 1.38:1 in Mexico (Rose 1966). In the Sierra Nevada of California, the eggs, the larvae, and the pupa last 2 weeks, 8 weeks, and 1 week (total 11 weeks), respectively (Smith 1971). In China, the sex ratios (male to female) of RTB and 1.02:1 (Zhang *et al.* 2002) or 1:1 (Ming *et al.* 2003). Females attract males through volatiles and acoustic signals, while males release pheromones to maintain monogamy, with these mechanisms ensuring reproductive success and reducing competition (Liu *et al.* 2020). Of the 132 trapped RTB females, only five (~3%) were found to have sperm in their spermathecae, which may result from pre-emergence mating with siblings or unrelated males, although mature females are capable of avoiding sibling mating (Chen *et al.* 2012, Chen 2017). RTB maintains a monogamous system through various mechanisms to enhance reproductive success. Females detect whether a tunnel is occupied through physical contact and, if occupied, leave to find a new tunnel, while males repel competitors through acoustic signals, volatiles, and physical pushing, and blocking the tunnel entrance (Liu *et al.* 2020). Maternal galleries range from 30–65 cm long and 1.5–2.0 cm wide, filled with reddish-brown granular frass and wood debris (Miao *et al.* 2001). Pairs of red turpentine beetles introduced into pine bolts yielded ranges from 16–281 eggs with an average of 106, with the egg, larval (five instars), and pupal stages lasting 10, 84, and 10 days (~100 d totally), respectively (Yan *et al.* 2005, Liu *et al.* 2014). Under laboratory conditions, the egg stage lasts 10–15 days (average 11.2 days), the larval stage 60–75 days, the pupal stage 11–13 days (average 12.4 days), and newly emerged adults remain in the pupal chamber for 6–9 days (Miao *et al.* 2001).

For MPB, the polygamous mating system better explains the relationships among individuals within a gallery than the previously reported female monogamous/male polygynous system (Janes *et al.* 2016). The adult sex ratio (male to female) of MPB is 1:2 in North America (Lachowsky and Reid 2014). Pre-emergence mating (which may include neighbors, siblings, or both) by MPB is approximately 1–2% (Safranyik *et al.* 2010) and 3%–12% (Bleiker *et al.* 2013), with the number of individuals resulting from mating among siblings being approximately 5% (Janes *et al.* 2016). Pre-emergence mating is more common in low-density endemic populations, while higher beetle densities provide increased mate choice and opportunities (Janes *et al.* 2016). McManis *et al.* (2019) provided a model of MPB oviposition, showing mean realized fecundity during the 30-day experimental period was 73.6 eggs per female while the estimated potential population fecundity was 91.0 eggs per female. Divergent selection and local adaptation in developmental time have led to longer generation times for southern mountain pine beetles (median = 149 days) compared to their northern counterparts (median = 76 days) in 22°C common garden experiments (Bentz *et al.* 2011, Bracewell *et al.* 2013).

Ecological niches

In North America, RTB attacks are typically concentrated in the lower 6 feet (~183cm) of the tree but sometimes occur above 12 feet (~365cm) (Smith 1971). Beetles emerging from recently cut stumps and dying trees attack trees, exposed roots, or freshly cut stumps (Smith 1971). In

contrast, in China, behavioral assays showed that RTB preferred the bark of large-diameter trees (diameter at breast height, DBH $\geq$ 30 cm) and had a higher attack rate on the bolts of these trees (Liu *et al.* 2011). RTB adult beetles typically invade the trunk about 1 m above the root base, but they are often found near the ground surface (Zhang *et al.* 2002). The highest number of entry holes is found 30–50 cm above the ground (Miao *et al.* 2001).

The average DBH of lodgepole pine (*P. contorta*) killed by MPB (27.0 ± 0.6 cm [SEM]) was significantly greater compared to live lodgepole pine remaining in infested plots (12.8 ± 0.4 cm [SEM]) ($p < 0.001$), showing that MPB exhibits a preference for larger diameter trees within infested stands (Klutsch *et al.* 2009). Trees with larger diameters possess thicker phloem, providing a more favorable environment for insect population growth (Amman *et al.* 1977).

Dispersal capacity and flight time

In North America, RTB's peak flight and attack activity usually occurs in spring (Smith 1971) with a consistent flight season from April to October in Arizona, peaking in May (Williams *et al.* 2008). The flight distance of RTB can exceed 16 km in USA (Smith 1971). In China, RTB flights begin in late spring (late April) and end in early autumn (early October), including two flight peaks in late May and late July in Yulin City and Taiyue Mountain in Shaanxi province (Miao *et al.* 2001). Beetles begin to fly out on the 10th day and continue for 20 days, with peak flight occurring on the 21st day (Liu *et al.* 2014). The flight distance of RTB can reach about 35 km (generally 20 km) in China (Zhang *et al.* 2002). In China, adults have strong flight capabilities, with flight heights exceeding 10 meters and distances reaching over 10 kilometers (Miao *et al.* 2001). Both adults and larvae can survive up to two weeks without feeding, enabling RTB to persist and spread in regions without suitable hosts (Zhao *et al.* 2008).

MPB flight activity was reported from early August to early September (Williams *et al.* 2008). In a 23-hour bioassay, mountain pine beetles exhibited mean flight distances ranging from 2.12 to 5.95 km, with the longest recorded flight by an individual beetle exceeding 24 km (Evenden *et al.* 2014). Despite variations in individual flight capacity, MPB maintain a relatively consistent flight speed, ranging from 1.55 to 1.93 km/h (Evenden *et al.* 2014). Shagelski *et al.* (2021) investigated the relationship between beetle flight phenotypes and gene expression through a combination of flight bioassays and transcriptome analyses. The flight assays revealed that all female samples ($n = 124$) flew an average of 7.4 km and initiated flight 176 times, while the subset used for differential expression analysis ($n = 14$) averaged 10.08 km and 72.8 flight initiations. Notably, beetles with a strong flight phenotype flew an average of 19.9 km compared to only 0.231 km for those with a weak flight phenotype, a difference that was statistically significant ($p < 0.0001$), and strong fliers exhibited a significantly higher proportion of flight during dark periods. The founder effect detected in northern Canada by Mock *et al.* (2007) had largely disappeared within just three generations (approximately three years), as shown by Cullingham *et al.* (2012). Moreover, other studies have demonstrated that rapid population expansion following a founder or bottleneck event can quickly restore high levels of genetic diversity (Roderick 1996, Cai *et al.* 2008, Kaňuch *et al.* 2014).

Consequently, Canadian populations have had sufficient time to either preserve or recover their genetic diversity, thereby erasing the typical genetic signatures of recent expansion (Janes *et al.* 2018).

Pheromone and volatiles

In its native North American range, RTB typically colonizes stumps or stressed trees through solitary or limited attacks in response to host volatiles released by the host trees rather than aggregation pheromones (Owen 2003, Aukema *et al.* 2011). Host-derived monoterpenes such as α -pinene, β -pinene, and 3-carene attract RTB adults, with regional differences in enantiomeric preferences and lure effectiveness (Hobson *et al.* 1993, Erbilgin *et al.* 2007, Kelsey *et al.* 2023). Synergism with ethanol occurs at moderate doses (Kelsey and Westlind 2022, 2023), and low-dose *frontalin* (<3 μ g/day) can enhance the beetles' trap catches, whereas high-dose *frontalin* (>1 mg/day) often reduces RTB attraction (Fettig *et al.* 2004, Zhang and Sun 2006, Kelsey and Westlind 2022). In contrast, in China, where RTB has established as an invasive species, beetle attacks both healthy trees and stumps (Liu *et al.* 2008, 2011, Xu *et al.* 2014). Females may release *frontalin*, which is attractive to both sexes (Liu *et al.* 2013), and both sexes emit oxygenated monoterpenes such as *trans-verbenol* and *myrtenol* during feeding (Zhang and Sun 2006, Liu *et al.* 2013, 2017), with field evidence confirming the emission of these volatiles (Shi and Sun 2010b). Similarly to North American populations, low-dose *frontalin* enhances the RTB response to 3-carene (Liu *et al.* 2013), while high-dose *frontalin* either reduces or has no effect on their attraction (Zhang and Sun 2006). Additionally, *trans-verbenol* and *myrtenol* increase RTB attraction in combination with host volatiles (Zhang and Sun 2006). At higher beetle densities, pheromone-mediated communication shifts: both sexes produce agreement sounds in response to *frontalin*; males generate territorial signals to defend feeding sites; and anti-aggregation pheromones such as *exo-brevicomin* are released to reduce further colonization pressure (Liu *et al.* 2017).

The pheromone system of *MPB* varies by sex and host. Females produce *trans-verbenol*, peaking during attack and decreasing after mating (Pitman *et al.* 1968, Pureswaran *et al.* 2000). Males initially produce *exo-brevicomin*, an anti-aggregation pheromone, which shifts to *frontalin* after mating (Ryker and Libbey 1982). Host-specific differences occur, with no *exo-brevicomin* or *frontalin* detected in *P. banksiana* (Cale *et al.* 2015). *trans-verbenol* attracts beetles and synergizes with myrcene (Pitman *et al.* 1968). Enantiomeric preferences vary regionally (Libbey *et al.* 1985). Both *exo-brevicomin* and *frontalin* show biphasic effects, enhancing attraction at low doses but inhibiting aggregation at higher levels (Rudinsky *et al.* 1974, Borden *et al.* 1987).

Table 1 The main species traits of *D. valens* and *D. ponderosae*

Species traits	<i>Dendroctonus valens</i>	References	<i>Dendroctonus ponderosae</i>	Reference
Body mass	31.13 ± 0.99 mg F: 32.06 ± 1.06 mg M: 29.59 ± 0.81 mg	(Shi and Sun 2010a)	F: 6.3-19.0 mg M: 4.1-13.6 mg	(Pureswaran and Borden 2003)
Body length	5.3-9.2mm (mean 7.3mm)	(Zhang <i>et al.</i> 2002)	F: 4.41-6.09 mm M: 3.78-5.46 mm	(Pureswaran and Borden 2003)
Invaded range	China 1998	(Song 2000)	/	/
Native range	In North America, it extends from Nova Scotia (Canada) west to California (USA) and south to Honduras	(Smith 1971, Wood 1982)	In western North America, it occurs from Peninsula Baja California Peninsula in Mexico to northern British Columbia and western Alberta in Canada	(Wood 1982, Salinas-Moreno <i>et al.</i> 2004)
Host species specificity	Oligophagous	(Six and Bracewell 2015)	Oligophagous	(Six and Bracewell 2015)
Host plants	Native areas: all native pines, mainly <i>P. radiata</i> and <i>P. ponderosa</i> , other <i>P. contorta</i> , <i>P. jeffreyi</i> , <i>P. lambertiana</i> , <i>P. monticola</i> ; China: main <i>P. tabuliformis</i> . preferred <i>P. sylvestris</i> var. <i>mongolica</i> . Occasionally <i>P. bungeana</i> and <i>P. armandii</i>	(Smith 1971, Bright Jr. 1976, Furniss and Carolin 1977, Cibrián Tovar <i>et al.</i> 1995, Yan <i>et al.</i> 2005, Zhao <i>et al.</i> 2008, Liu 2020, Zhang <i>et al.</i> 2022, Zhou <i>et al.</i> 2024)	All native pines except <i>P. jeffreyi</i> , mainly <i>P. contorta</i> , Other: <i>P. ponderosa</i> , <i>P. flexilis</i> , <i>P. albicaulis</i> , <i>P. resinosa</i> ; Exotic trees: <i>P. banksiana</i> , <i>P. nigra</i> , <i>P. strobus</i> , <i>P. sylvestris</i> , <i>P. roxburghii</i> , <i>P. densiflora</i>	(Furniss and Schenk 1969, Smith 1981, Wood 1982, Safranyik and Carroll 2006, Six and Bracewell 2015, Cook and Martinez 2018)
Initiate host location	Female colonizers of trees via solitary attacks, responding to volatiles in native areas; Females release <i>frontalin</i> to attract both sexes for mass attacks on healthy pine trees in China	(Owen 2003, Aukema <i>et al.</i> 2011, Liu <i>et al.</i> 2013)	Female produce trans-verbenol to attract both males and other females to mass attack	(Pitman <i>et al.</i> 1968)

Feeding mode	Phloephagous	(Six and Bracewell 2015)	Phloephagous	(Six and Bracewell 2015)
Biological niches	Root and lower stem	(Smith 1971, Liu <i>et al.</i> 2011)	Root and lower stem	(Klutsch <i>et al.</i> 2009)
Sex ratio	Adult F: M $\approx$ 1:1	(Rose 1966, Zhang <i>et al.</i> 2002, Ming <i>et al.</i> 2003)	Adult F: M = 2:1 egg F: M $\approx$ 0.86:1	(Lachowsky 2016)
Mating behavior	Outbreeding and few Inbreeding (3%)	(Chen <i>et al.</i> 2012)	Outbreeding and little inbreeding (1-12%)	(Safranyik <i>et al.</i> 2010, Bleiker <i>et al.</i> 2013, Janes <i>et al.</i> 2016)
Parent adults in brood systems	Monogamous	(Liu <i>et al.</i> 2020)	Polygamous	(Janes <i>et al.</i> 2016)
Voltinism	Uni- to multivoltine (1~3 generations/year)	(Smith 1971, Zhang <i>et al.</i> 2002, Zhao <i>et al.</i> 2008)	Semi- to Univoltine, rare bivoltinism	(Bentz and Powell 2014, Bentz <i>et al.</i> 2014)
Fecundity	Over 100 eggs per female	(Yan <i>et al.</i> 2005)	The estimated maximum 91 eggs per female in the model	(McManis <i>et al.</i> 2019)
Dispersal capacity	Up to 35km in China Over 16km in USA	(Smith 1971, Zhang <i>et al.</i> 2002)	Up to 24 km in flight bioassays 1.55-1.93 km/h	(Evenden <i>et al.</i> 2014)
The mean supercooling point	Larvae: -18.34 $\pm$ 0.26°C; Adult:-18.59 $\pm$ 0.63°C	(Dong <i>et al.</i> 2021)	Larvae: -33.2°C	(Rosenberger <i>et al.</i> 2017a)
Primary (attack living trees)	Secondary in native areas; Primary in China	(Six and Bracewell 2015)	Secondary and primary; Yes when at a high population density	(Six and Bracewell 2015)
Tree killers	Usually no in native areas; Yes when at high population density in China	(Six and Bracewell 2015)	Yes when at high population density	(Six and Bracewell 2015)
Outbreaks	No in native areas; Yes in China, usually after thinning, drought and fire	(Six and Bracewell 2015)	Yes, usually in a warm and dry climate	(Six and Bracewell 2015)

Table 2 Evolutionary traits of *D. valens* and *D. ponderosae*

Species	Populations	N/N _{MT}	$\pi \pm SD$ (%)	Hd $\pm$ SD	A	He $\pm$ SD (Nei, 1973)	Pi	Molecular tool (reference)
RTB	USA	61/41	3.07 $\pm$ 1.55	0.97 $\pm$ 0.01				
	Mexico (including Guatemala)	18/12	6.20 $\pm$ 3.18 (×)	0.86 $\pm$ 0.08 (×)				MtDNA (Cai et al. 2008)
	China	24/6	0.66 $\pm$ 0.40	0.60 $\pm$ 0.11				
	Total	103/57	4.32 $\pm$ 2.13 (×)	0.97 $\pm$ 0.01 (×)				
	West USA	216/-			6.778	0.556 $\pm$ 0.281		
	Central USA	34/-			3.111	0.374 $\pm$ 0.264		
	Northeast USA	102/-			5.778	0.360 $\pm$ 0.307		Microsatellite (Taerum et al. 2016)
	Mexico	50/-			4.111	0.430 $\pm$ 0.289		
	China	62/-			4.333	0.464 $\pm$ 0.224		
	Total	464/-			3.111-6.778	0.360-0.556		
	Arizona-Colorado	19/-					2.3e ⁻³	
	California-Montana	20/-					2.64e ⁻³	Population
	Minnesota-Wisconsin	20/-					2.47e ⁻³	genome
	China	48/-					3.00e ⁻³	(Liu et al. 2022)
	Total	107/-					3.00e⁻³-2.3e⁻³	
MPB	North NA	173	0.65 $\pm$ 0.34	0.9035 $\pm$ 0.0109				MtDNA (Mock et al. 2007 , Cullingham et al. 2012)
	Central NA	78	0.89 $\pm$ 0.45	0.9624 $\pm$ 0.0106				
	South NA	87	0.87 $\pm$ 0.45	0.9588 $\pm$ 0.0096				
	Total	338	0.65-0.87	0.9035-0.9624				
	Rocky Mountains	1739			4.69-11.31	0.496-0.644		Microsatellite (Samarasekera et al. 2012)
	Northeast of Rocky Mountains	1302			4.77-7.46	0.478-0.515		
	Nechako Plateau	198			4.46-5.00	0.461-0.484		

West of Rocky Mountains	469	3.46-9.23	0.476-0.614
Cariboo-Chilcotin	308	5.31-7.08	0.490-0.620
Coast Mountains	43	5.69	0.621
Cascade Mountains	46	7.15	0.638
Thompson-Okanagan	237	6.77-7.23	0.585-0.642
Kootenays	88	7.15	0.606-0.641
Total	4484	3.46-11.31	0.461-0.644

N. number of samples;

MtDNA: N_{HT} , number of mtDNA haplotype; Hd, haplotype diversity; π , nucleotide diversity;

Microsatellite: A, mean number of alleles; H_e , mean expected heterozygosity;

Population genome: P_i , genome-wide median nucleotide diversity;

(x), the results are incorrect due to the inclusion of the cryptic species *Dendroctonus beckeri*

Overwinter and cold tolerance

RTB overwinters differently depending on region. In colder areas of North America, winter is passed primarily by adults and, to a lesser extent, by larvae, while pupae and eggs rarely survive the winter (Smith 1971). In China, the majority overwinter as mature larvae (52.5%), adults (25%), and third-instar larvae (18.2%) within the bark at the base of the trunk or roots, with smaller proportions overwintering as second-instar larvae or pupae (Miao *et al.* 2001). Zhao *et al.* (2009) measured the supercooling point (SCP) and short-term lethal low temperature (LLT) of RTB under laboratory conditions and predicted that larvae can safely overwinter at atmospheric temperatures as low as -23.5°C . Additionally, they found that the average SCP of overwintering larvae in Shanxi is $-11.98 \pm 2.55^{\circ}\text{C}$, classifying RTB as a freeze-tolerant insect. Dong *et al.* (2021) further investigated cold resistance in Inner Mongolia, measuring the supercooling and freezing points of both overwintering larvae and adults, along with the levels of antifreeze substances. The supercooling points of larvae ($-18.34 \pm 0.26^{\circ}\text{C}$) and adults ($-18.59 \pm 0.63^{\circ}\text{C}$) showed no significant difference, but the freezing points differed significantly ($-10.17 \pm 0.36^{\circ}\text{C}$ for larvae and $-15.90 \pm 0.70^{\circ}\text{C}$ for adults), suggesting that populations at higher latitudes may possess stronger cold tolerance. Overwintering larvae exhibited significantly higher levels of fat, glycerol, trehalose, and sorbitol, whereas overwintering adults accumulated greater amounts of glycogen, glycerol, trehalose, and sorbitol. Among these, glycerol, trehalose, and sorbitol act as key antifreeze substances, with fat being critical for larval overwintering and glycogen for adult overwintering (Dong *et al.* 2021). Hou *et al.* (2021) reported that the abundances of gut microbiota such as *Enterobacter*, *Serratia*, *Erwinia*, and *Klebsiella*, as well as the activities of antioxidant enzymes catalase and peroxidase, decreased significantly during overwintering. Zhao *et al.* (2021) identified 140 candidate genes associated with cold tolerance, including genes encoding trehalose transporter, fructose-1,6-bisphosphatase, and trehalase.

MPB cold tolerance varies across life stages and geographic regions. Régnière and Bentz (2007) developed a model identifying three cold-hardening states: (1) non-cold-hardened summer larvae with a mean supercooling point (SCP) of -9.8°C , (2) intermediate larvae with an SCP of -21.2°C , and (3) cold-hardened, glycerol-rich larvae with an SCP of -32.3°C . The model, validated with field data ($R^2 = 0.97$), revealed greater cold tolerance in high-latitude populations. Climate data (1980-2006) indicated a warming trend ($0.34^{\circ}\text{C}/\text{year}$ increase in extreme winter temperatures), resulting in higher overwinter survival rates ($1.3\%/\text{year}$) and potential range expansion. Rosenberger *et al.* (2017a) observed that MPB larvae develop faster in eastern hosts (e.g., red pine, Scots pine), leading to earlier, less cold-tolerant pupal or adult stages, increasing overwintering mortality. In contrast, larvae in historical hosts (e.g., ponderosa pine) overwinter as cold-hardened late-instar larvae with higher survival rates. Fourth instars are more cold-tolerant than pupae or teneral adults, and female adults are more cold-tolerant than males, suggesting sex-specific adaptations. Significant differences in mean SCP were observed among hosts, with Scots pine larvae having a higher SCP (-28.2°C) than lodgepole pine (-33.2°C). LLT-based models were

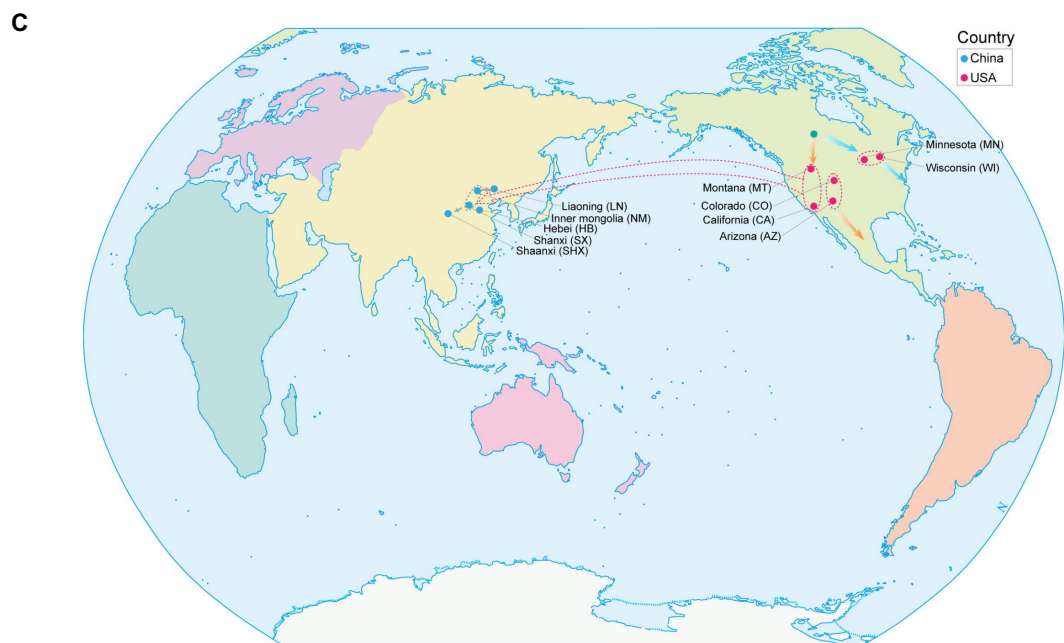
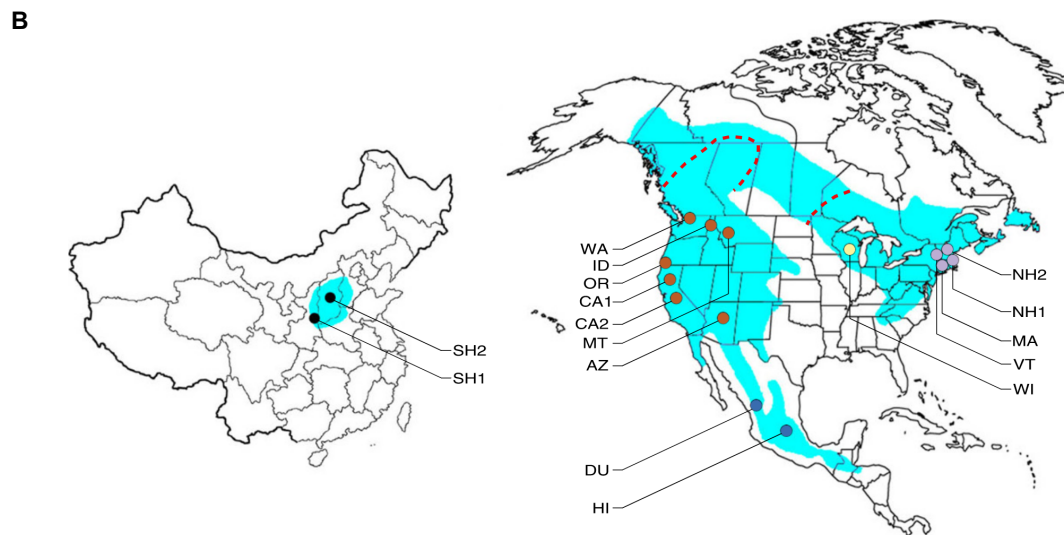
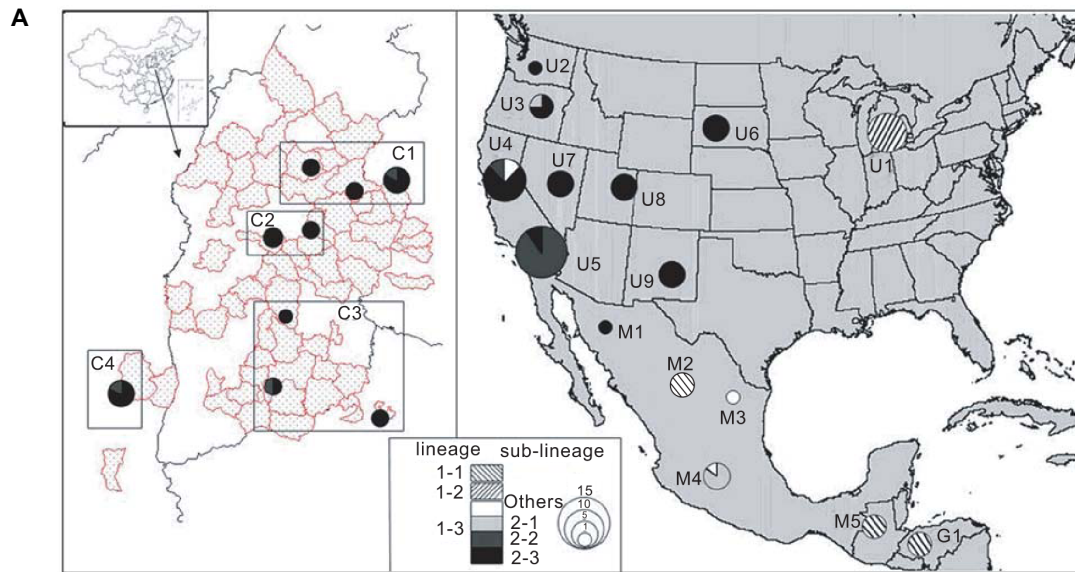
more accurate in predicting mortality than SCP models, as red pine larvae had a mean LLT₅₀ of -33.3°C. About 25% of larvae in hosts like red pine survived freezing, indicating partial freeze tolerance. Despite host-specific effects, LT₉₀ values across all hosts remained above the critical threshold of -40°C. These findings highlight the trade-off: novel hosts enhance development but increase overwintering risks. Continued winter warming could lead to rapid MPB population expansion in eastern hosts, threatening boreal forests. This emphasizes the need to integrate host-specific cold tolerance data into climate suitability models for risk assessments and adaptive forest management.

3.1.3 Phylogeography and evolutionary traits

The genome size of *D. valens* is 322.41 Mb (Liu *et al.* 2022), notably larger than the 252.85 Mb genome of *D. ponderosae* (Keeling *et al.* 2013). Additionally, repetitive sequences make up 45.22% of the *D. valens* genome, which is significantly higher than the 21.12% observed in *D. ponderosae* (Keeling *et al.* 2013, Liu *et al.* 2022). *D. valens* has 12 autosomes plus an XY sex chromosome, while *D. ponderosae* possesses 11 autosomes and an XY sex chromosome (Keeling *et al.* 2013, Liu *et al.* 2022). Their evolutionary traits are summarized in Table 2; however, due to the presence of cryptic species, direct comparisons are challenging based on mtDNA data. RTB shows a higher mean number of alleles and mean expected heterozygosity (He) than MPB based on microsatellite data. Notably, RTB shows a more complex genetic structure than MPB within its native range (Cai *et al.* 2008, Taerum *et al.* 2013, 2016, Janes *et al.* 2018, Liu *et al.* 2022).

The primary pest *D. valens* in the invaded range, having a strong genetic structure in native areas

In native areas, *D. valens* has an obvious genetic structure obtained by different gene tools, like the mtDNA (Cognato *et al.* 2005, Cai *et al.* 2008), microsatellite (Taerum *et al.* 2016), and population genomics (Liu *et al.* 2022) (Figure 5). Due to limited and biased sampling locations, the complete phylogeographic structure in native range remains uncertain. However, mtDNA analysis has divided 22 populations into three lineages (45 haplotypes): Western NA, Michigan, and Mexico and Guatemala (Cognato *et al.* 2005, Cai *et al.* 2008) (Figure 5A, 5D). The latter showed over 10% p-distance from the other two and is considered a cryptic species, *Dendroctonus beckeri* Thatcher (Cai *et al.* 2008, Ramírez-Reyes *et al.* 2023) (Figure 5A, 5D). Microsatellite data revealed three lineages: West US, Central US, and Mexico, and added a fourth in the Northeast US (Taerum *et al.* 2016) (Figure 5B, 5D). Population genomics further split the western cluster into two lineages, an admixed California and Montana group and a pure Arizona and Colorado group, and confirmed the Minnesota and Wisconsin lineage (Liu *et al.* 2022) (Figure 5C, 5D). In conclusion, I hypothesized present studies separated RTB's native populations into four clades: Northeast US clade (appeared in microsatellite research), Northcentral US clade (appeared in three above studies), Mexico lineage (appeared in mtDNA and microsatellite research), West NA clade



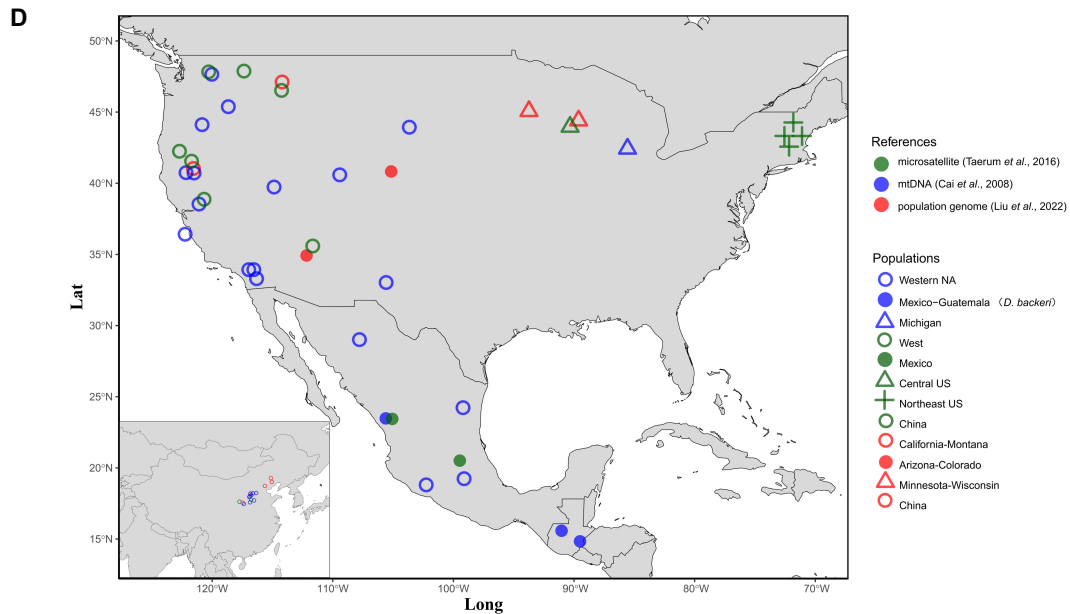


Figure 5 Phylogeography of *D. valens*. **A.** Map of *D. valens* distribution regions and sampling sites and haplotypes distribution in China and in native ranges respectively. The dotted patches show the distribution regions in China. The circles with different sizes and colors indicate the sample size and different lineages or sub-lineages to which they belong (Cai *et al.* 2008). **B.** Distributions of *D. valens* in China and North America, shown with the sampling locations for this study. Red dashed lines demarcate the eastern and western North American ranges (Taerum *et al.* 2013). Circles represent sampling locations, and the colors of the circles represent the population to which the sampling locations belong brown for the West population, yellow for the Central population, purple for the Northeast population, blue for the Mexico population, and black for the China population. Abbreviations are as follows: AZ - Arizona, CA1 - California 1, CA2 - California 2, DU - Durango, HI Hidalgo, ID - Idaho, MA - Massachusetts, MT - Montana, NH1 - New Hampshire 1, NH2 - New Hampshire 2, OR - Oregon, SH1 - Shaanxi, SH2 - Shanxi, VT - Vermont, WA - Washington, WI - Wisconsin (Taerum *et al.* 2016). **C.** Global representation of sampling sites of different geographical populations and RTB movement. The putative invasion and spread routes was also indicated in the map based on data collected from literature (The green dot denotes the area Canada where RTB originates. The orange solid arrows indicate the west route of RTB spread in North America, and the blue solid arrows show the east route of RTB spread in North America. The red dotted arrow represents the putative invasion route from the west coast of North America to Shanxi province of China). Resequencing samples were collected from six states (red dots) in the original country North America (including Arizona [AZ], Colorado [CO], California [CA], Montana [MT], Wisconsin [WI], and Minnesota [MN]) and five provinces (blue dots) in the invaded country China (including Liaoning [LN], Inner Mongolia [NM], Hebei [HB], Shanxi [SX], and Shaanxi [SHX]) (Liu *et al.* 2022). **D.** Geographic distribution of genetic sampling sites for *Dendroctonus valens* across its native and introduced range in A, B and C. Color represents different literature sources or types of genetic data, while shape indicates the population groups defined in each study.

(appeared in three research) which is separated into two sub-clades: California-Montana lineage (appeared in genomic research), Arizona-Colorado lineage (appeared in genomic research) (Figure 5). The presence of multiple deep branches and differences in genetic diversity patterns within lineages may indicate the existence of multiple refugia during periods of lineage divergence (Hewitt 1996). Western America (California and southern Oregon) with the highest diversity and Mexico may provide two or more southern refugia for RTB in the glacial period (Soltis *et al.* 1997, Cai *et al.* 2008). Geographic barriers (eg., the Sierra Madre Occidental and Great Plains) in North America and different recolonization routes from distinct glacial refugia shaped the complex genetic structure (Taerum *et al.* 2016). Previous breeding experiments with RTB showed that while beetles from eastern and western North America can mate, they do not produce brood, suggesting limited admixture between populations, though beetles from Central or Mexico were not tested (Pajares and Lanier 1990).

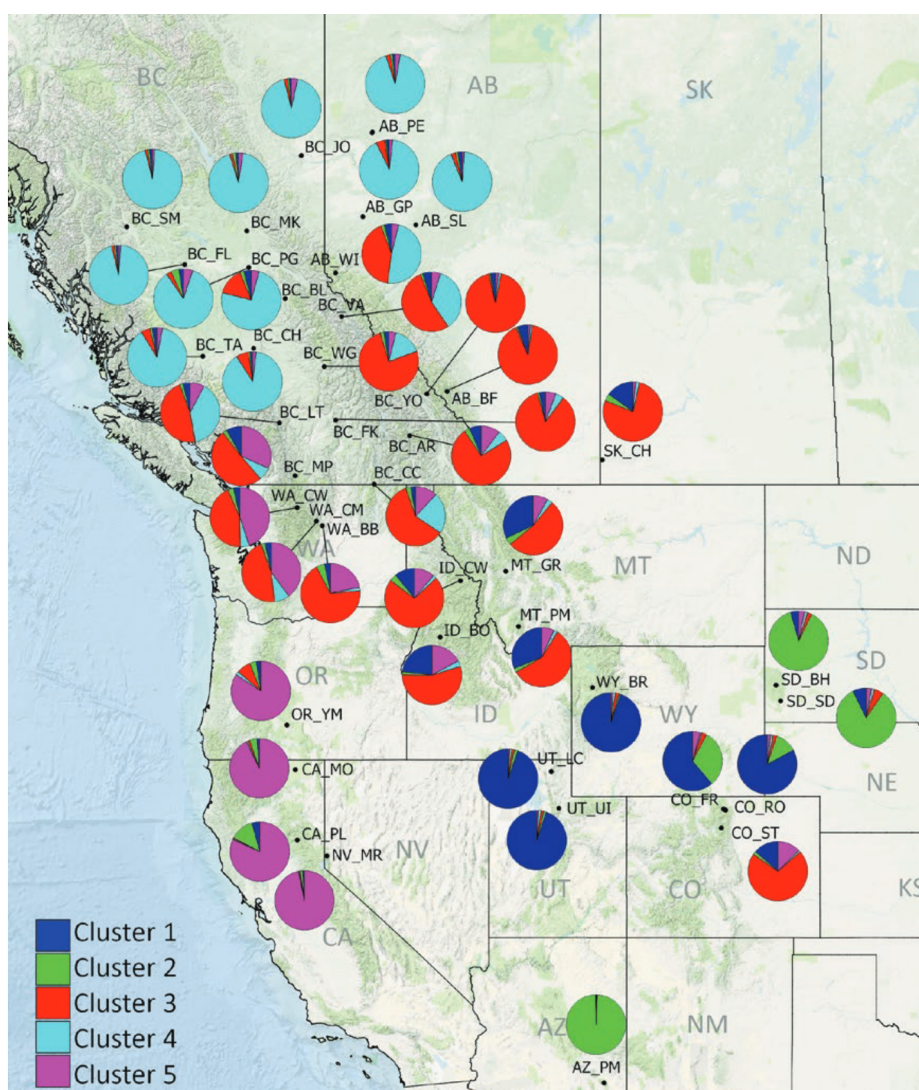


Figure 6 Phylogeography of *D. ponderosae*. Map of MPB sites throughout western North America. Each site is represented by a pie chart depicting the average assignment (Q values) of individuals to one of five STRUCLURE clusters (K = 5) (Janes *et al.* 2018).

In invasive areas (China), *D. valens* has only one lineage with relatively high diversity but doesn't have an obvious genetic structure by different gene technological tools (Cognato *et al.* 2005, Cai *et al.* 2008, 2011, Taerum *et al.* 2016, Cui 2018, Liu *et al.* 2022, Zhang *et al.* 2022). Four studies consistently suggest that the Chinese population of *D. valens* likely originated from the Pacific Northwest with multiple invasions from different source populations (Cognato *et al.* 2005, Cai *et al.* 2008, Taerum *et al.* 2016, Liu *et al.* 2022), while genetic variation was initially reduced due to a bottleneck effect, it was subsequently maintained at moderate levels following rapid post-invasion population expansion, like the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Rispe *et al.* 2020).

The primary pest *D. ponderosae* in western North America, has a simple and weak genetic structure

In Alberta (AB) and British Columbia (BC) in western Canada and South Dakota in the USA, MPB exhibits a clear genetic structure (Figure 6), a well-defined north-south division likely driven by postglacial colonization and recent climate changes, as revealed by AFLP (Mock *et al.* 2007), mtDNA (Mock *et al.* 2007, Cullingham *et al.* 2012), microsatellite loci (Davis *et al.* 2009, Samarasekera *et al.* 2012), SNPs analyses (Janes *et al.* 2014, 2018, Batista *et al.* 2016, Trevoy *et al.* 2019), meanwhile RAD sequencing uncovers distinct population structures for autosomes and sex chromosomes (Dowle *et al.* 2017). The nucleotide diversity of southern populations is higher than that of northern populations in western North America (Cullingham *et al.* 2012) (Table 2). Climate change has driven MPB's range expansion northward and eastward in Canada, introducing it to new landscapes and host plants and facilitating adaptive radiation.

3.2 *Tomicus* genus: the invasive *T. piniperda* vs the non-invasive *T. destruens*

The larvae of *T. piniperda* develop in weakened or freshly dead trees, as the species is unable to overcome the defenses of healthy conifers (Lieutier 2002, Kerdelhué *et al.* 2006). However, it can successfully attack and reproduce in trees stressed by external factors such as insect-induced defoliation or drought, thereby contributing to increased tree mortality (Wainhouse and Inward 2016).

T. destruens also infest colonizes weakened or freshly dead trees, but is considered a primary pest and ranks among the most destructive insects affecting Mediterranean pine forests in Spain (Monleón *et al.* 1996) and Turkey (Sarıkaya and Avci 2010).

3.2.1 Taxonomic status of the two Mediterranean *Tomicus* sibling species

For many years, *T. piniperda* and *T. destruens* were regarded as synonyms, particularly where they coexist in the Mediterranean basin (Winkler 1932, Gallego and Galián 2001, Kerdelhué *et al.* 2002). Recent studies have identified morphological traits that can differentiate these sibling species; however, accurately determining their identity based solely on morphology remains extremely challenging (Kerdelhué *et al.* 2002, Faccoli 2006). Kerdelhué *et al.* (2002) definitively clarified the taxonomic status of the Mediterranean *T. piniperda* s.l., demonstrating that *T.*

piniperda, predominantly found in continental France on *P. sylvestris*, *P. pinaster*, and *P. uncinata*, and *T. destruens* associated with *P. pinea* and *P. halepensis*, primarily in Southern France and Corsica, are two distinct species based on analyses of ITS1 and COI markers. [Faccoli et al. \(2005\)](#) confirmed the distinct species status of *T. piniperda* and *T. destruens* using SSCP and COI markers, noting that they were not found in sympatry within the sampled Italian populations, with *T. piniperda* associated with continental pines (*P. sylvestris*: main host, *P. nigra*) and *T. destruens* restricted to Mediterranean pines (*P. pinea*, *P. pinaster*, *P. halepensis*).

3.2.2 Distribution and host range

Tomicus is a Palearctic genus with species distribution mainly in Europe and Asia ([Ritzerow et al. 2004](#)).

T. piniperda and *T. destruens* have been reported in northwestern Africa, likely due to natural post-glacial colonization from Europe via Spain ([Lieutier et al. 2015](#)). As a thermophilous species adapted to circum-Mediterranean conditions, *T. destruens* is commonly found along the northern and southern coasts of the Mediterranean Sea and in Asia Minor, making its presence in North Africa unsurprising. However, records of *T. piniperda* in these regions, typically a species associated with colder climates and continental hosts, are likely the result of past misidentifications, as *T. destruens* has now been confirmed as a distinct species ([Kerdelhué et al. 2002](#), [Kohlmayr et al. 2002](#)). While both species can occur within the same country (e.g., Italy, France, Spain, and Balkan countries), they generally inhabit different environments, with *T. piniperda* favoring continental pine species and *T. destruens* being associated with Mediterranean pine species. True sympatry, where populations of both species coexist in the same area or even on the same tree, has only been documented in North Portugal ([Vasconcelos et al. 2005](#)), in northern Spain ([Gallego et al. 2004](#)), and southwestern France ([Kerdelhué et al. 2002](#)) in stands of maritime pine (*P. pinaster*) at elevations of 400–600 meters above sea level. This observation is further supported by a recent climatic model proposed by [Horn et al. \(2012\)](#), showing temperature appeared to be most correlated with both species distributions among their investigated climate variables.

***T. piniperda*, majorly infest continental pines and are widely distributed in the Eurasian forests**

T. piniperda L. (Coleoptera, Scolytidae) has the most extensive geographic range within its genus, covering a large part of Europe from Scandinavia to France, Italy, and the northern half of the Iberian Peninsula ([Kerdelhué et al. 2002, 2006](#), [Faccoli et al. 2005](#), [Vasconcelos et al. 2006](#), [Horn et al. 2012](#), [Lieutier et al. 2015](#)), as well as Northeastern Asia, including Japan ([Masuya et al. 1998, 1999](#)), Korea ([Ueki 1911](#), [Park 2016](#)), and Northeastern China ([Ritzerow et al. 2004](#)) (Figure 7a). During the 16-year period from 1985 to 2000, *T. piniperda* was intercepted 151 times on cargo arriving from at least 18 different European countries ([Haack and Poland 2001](#)). *T. piniperda* populations were first identified in North America in Ohio in 1992 ([Haack et al. 1997](#)). It has also been identified as an invasive species in North America, where it has expanded its range

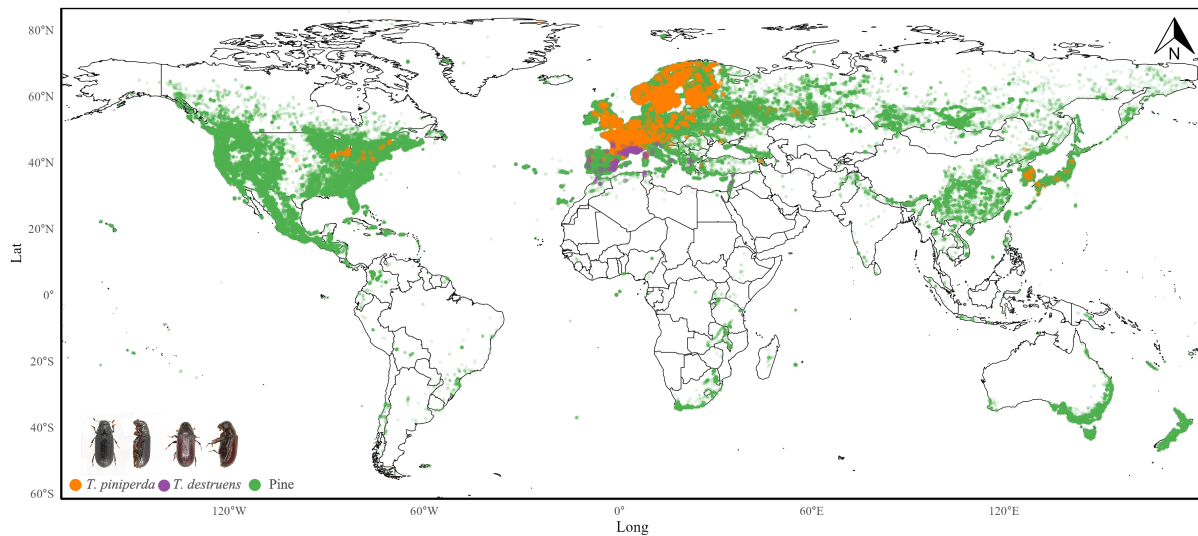


Figure 7 Percent distribution of *T. piniperda* and *T. destruens*. The sites of two insects refer to [Horn et al. \(2012\)](#), [Ritzerow et al. \(2004\)](#), and GBIF. The sites of pine trees refer to GBIF. The photos of insects are from the website (www.invasive.org, www.tomicusdestruens.com/index.html)

in the north-central and northeastern United States (U.S.) and adjacent regions in Canada ([Poland and Haack 2000](#), [Haack and Poland 2001](#), [Poland et al. 2002](#), [Lieutier et al. 2015](#)). The distribution of *T. piniperda* is shown in Figure 3a.

T. piniperda is capable of infesting a range of conifer species: *P. sylvestris*, *P. pinaster*, *P. nigra*, *P. pinaster*, and *P. contorta* in Europe ([Faccoli et al. 2005](#), [Lieutier et al. 2015](#)), *P. thunbergia* and *P. tablifformis* in Northeastern China ([Ritzerow et al. 2004](#)), *P. densiflora* in Japan ([Masuya et al. 1998, 1999](#)), Korea, *P. strobus*, *P. sylvestris*, *P. ponderosa*, *Pinus banksiana*, and *P. resinosa* in North America ([Långström et al. 1994](#), [Sadof et al. 1995](#), [Haack and Poland 2001](#)). However, Scots pine (*P. sylvestris*) serves as its primary host across its geographic range ([Hanson 1937](#), [Lieutier et al. 2015](#)).

***T. destruens*, a strict Mediterranean species restricted to Mediterranean pines**

T. destruens is exclusively found in the coastal regions surrounding the Mediterranean countries and Portugal (Figure 7b) ([Chakali 2005](#), [Sarıkaya and Avci 2010](#)). *T. destruens* primarily targets Mediterranean pine species, including *P. halepensis*, *P. pinaster*, *P. pinea*, *P. brutia*, and *P. canariensis* ([Gallego et al. 2004](#)). However, it has also been observed infesting exotic pines such as *P. radiata* and occasionally *P. nigra* in Turkey ([Sarıkaya and Avci 2010](#)) and Spain ([Gallego et al. 2004](#)). Laboratory experiments in [Faccoli \(2007\)](#) assessed the performance of *T. destruens* on three Mediterranean pines (*Pinus pinea*, *P. pinaster*, *P. halepensis*) and two continental pines (*P. nigra*, *P. sylvestris*), finding that the beetle colonized all species but failed to reproduce on *P. sylvestris*, with *P. halepensis* showing the highest fecundity, *P. pinaster* the lowest, and adults from *P. pinea* exhibiting the longest longevity.

3.2.3 Species biological and ecological traits

Both species are monogamous, with females initially boring into the bark, later joined by males for mating within the nuptial chamber. *T. piniperda* is found throughout its native range and in North America, with sister generations increasing from the north to the south (Långström 1983, Sauvard 1988, Poland and Haack 2000, Ryall and Smith 2000). *T. destruens* had one generation per year in southwestern France (Larroche 1975), southeastern France (Carle 1974), northeastern Spain (Monleón *et al.* 1996), central and northern Italy (Nanni *et al.* 1997), and Turkey (Chakali 2005, Vasconcelos *et al.* 2006, Sarikaya and Avci 2010) with sister generations. Newly emerged adults of all *Tomicus* species require a period of maturation feeding to reach sexual maturity. This feeding typically occurs on the shoots or twigs of healthy trees, usually of the same host species, where the beetles completed their development. *T. piniperda* has more host plants, stronger cold-tolerant, an early flight time and longer seasonal flight periods than *T. destruens* (Table 3).

Sex ratio and Fecundity

For *T. piniperda*, the sex ratio remained approximately 1:1, with a notable increase in females observed during early summer (Långström 1983).

For *T. destruens*, in a *Pinus halepensis* stand in Algeria, Chakali (2005) recorded an average fecundity of 58.6 eggs per female (range: 26–139) and a mean tunnel length of 10 cm (range: 5.3–17.2 cm). These values were influenced by the host species, with *Pinus halepensis* showing higher fecundity and longer tunnels than maritime and stone pines (Faccoli 2007). In Turkey, egg gallery lengths averaged 9.8 cm in *P. brutia* (range: 6–12.4 cm), with the longest measuring in *P. nigra* (Sarikaya and Avci 2010).

Ecological niches

In the first dispersal period, *T. piniperda* predominantly breeds in dead trees and timber but can occasionally infest live, weakened trees (Långström and Hellqvist 1993, Haack and Poland 2001). It has been observed colonizing the lower portions of trees, typically below 5 meters in height in Japan (Masuya *et al.*, 1999), preferentially attacking the lower stems with thick, corky bark in Sweden (Långström 1984, Långström and Hellqvist 1993) and North America (Haack and Poland 2001).

In contrast, the adults of *T. destruens* predominantly infest the middle to upper parts of the stems of mature pine trees predominantly infest the middle to upper parts of the stems of mature pine trees (Carle 1974, Stergulc 2002, Kocabişik 2019).

Two dispersal periods and dispersal capacity

A distinctive characteristic of the genus *Tomicus* is its two dispersal phases: an early reproductive attack targeting weakened or dead trees, followed by a later maturation attack on the shoots or twigs of healthy trees.

Studies of the primary dispersal period, also called the reproductive flight period, have revealed distinct differences between *T. piniperda* and *T. destruens*. During this phase, adults

Table 3 The main species traits of *T. piniperda* and *T. destruens*

Species traits	<i>T. piniperda</i>	References	<i>T. destruens</i>	References
Invaded distribution	North America	(Poland and Haack 2000, Haack and Poland 2001, Poland <i>et al.</i> 2002, Lieutier <i>et al.</i> 2015)	/	/
Native distribution	Covering much of Europe, from Scandinavia to France, Italy, and northern Iberia	(Kerdelhué <i>et al.</i> 2002, 2006, Faccoli <i>et al.</i> 2005, Vasconcelos <i>et al.</i> 2006, Horn <i>et al.</i> 2012, Lieutier <i>et al.</i> 2015)	The coastal regions of Mediterranean countries and Portugal	(Chakali 2005, Sarikaya and Avci 2010)
Host species specificity	Larvae develop in the phloem, adults mature in shoots Europe: <i>P. sylvestris</i> (primary), <i>P. pinaster</i> , <i>P. nigra</i> , <i>P. pinaster</i> , and <i>P. contorta</i> ;	(Lieutier <i>et al.</i> 2015)	Larvae develop in the phloem, adults mature in shoots	(Lieutier <i>et al.</i> 2015)
Host plants	East Asia: <i>P. thunbergia</i> , <i>P. tablifomis</i> , <i>P. densiflora</i> ; North America: <i>P. strobus</i> , <i>P. sylvestris</i> , <i>P. ponderosa</i> , <i>P. banksiana</i> , and <i>P. resinosa</i>	(Långström <i>et al.</i> 1994, Sadof <i>et al.</i> 1995, Masuya <i>et al.</i> 1998, 1999, Haack and Poland 2001, Ritzerow <i>et al.</i> 2004, Faccoli <i>et al.</i> 2005, Lieutier <i>et al.</i> 2015)	<i>P. halepensis</i> , <i>P. pinaster</i> , <i>P. pinea</i> , <i>P. brutia</i> , <i>P. canariensis</i> , <i>P. radiata</i> , occasionally reported on <i>P. nigra</i>	(Gallego <i>et al.</i> 2004, Sarikaya and Avci 2010)

Ecological niches	The lower stems	(Långström 1984, Långström and Hellqvist 1993, Masuya <i>et al.</i> 1999)	The medium-upper stems	(Carle 1974, Stergulc 2002)
Initiate host location	Female	(Lieutier <i>et al.</i> 2015)	Female	(Lieutier <i>et al.</i> 2015)
	Larvae are phloephagous;		Larvae are phloephagous;	
	Adults feed on shoots for maturation	(Lieutier <i>et al.</i> 2015)	Adults feed on shoots for maturation	(Lieutier <i>et al.</i> 2015)
Sex ratio	F:M $\approx$ 1:1	(Långström 1983)	unknown	/
Mating behavior	Monogamous	(Lieutier <i>et al.</i> 2015)	Monogamous	(Lieutier <i>et al.</i> 2015)
Fecundity	Unknown	/	Mean 58.6 eggs per female (range: 26–139)	(Chakali 2005)
				(Carle 1974, Larroche 1975, Monleón <i>et al.</i> 1996, Nanni <i>et al.</i> 1997, Chakali 2005, Vasconcelos <i>et al.</i> 2006, Sarikaya and Avci 2010)
Voltinism	Univoltine	(Långström 1983, Sauvard 1988, Poland and Haack 2000, Ryall and Smith 2000)	Univoltine	
Dispersal capacity	Adults disperse twice per generation (once for maturation feeding and once for oviposition)	(Kerdelhué <i>et al.</i> 2006)	Adults disperse twice per generation (once for maturation feeding and once for oviposition)	(Lieutier <i>et al.</i> 2015)
Flight temperature	$\geq 12^{\circ}\text{C}$	(Saarenmaa 1989)	$\geq 24^{\circ}\text{C}$	(Monleón <i>et al.</i> 1996)

Primary (attack living trees)	Secondary	(Lieutier <i>et al.</i> 2015)	Secondary	(Lieutier <i>et al.</i> 2015)
Tree killers	No	(Lieutier <i>et al.</i> 2015)	No	(Lieutier <i>et al.</i> 2015)
Outbreaks	No	(Lieutier <i>et al.</i> 2015)	No	(Lieutier <i>et al.</i> 2015)

Table 4 Evolutionary traits of *T. piniperda* and *T. destruens*

Species	Country	Populations	N _{MT} /N _{HT}	π	Hd	N _{MI} /AR	A	He	Molecular tool (reference)
<i>T. piniperda</i>	Austria	Kühnsdorf	5/1	0	0				
	Croatia	Korenica	5/2	0.4	0.4				
	Czech Republic	Stara Boleslav	2/1	-	-				
	Estonia	Tahkurana	5/3	2.4	0.7				
	Finland	Tuusala	5/2	2	0.4				
	France	Narbonne	5/4	3	0.9				
		Orléans	5/2	1.2	0.6				
		Mulhouse	5/2	0.4	0.4				
		St André les Alpes	5/3	0.8	0.7				MtDNA + microsatellite (Horn <i>et al.</i> 2009)
		Quillan	5/4	4.2	0.9				
		Mont Louis	5/5	3.4	1				
		Brioude	5/4	1.2	0.9				
		Bordeaux	5/2	0.6	0.6				
		Oléron	4/2	2	0.5				
		Bispingen	5/3	2.8	0.7				
	Italy	Roccia	5/2	0.4	0.4				
		Melone							
		Passo del Bocco	3/2	1.33	0.67				

	Sonico	2/2	-	-				
	Villasantina	1/1	-	-				
Norway	Skril	5/1	0	0				
	Borkam	5/1	0	0				
Poland	Hajno' Wka	5/4	2.8	0.9				
Portugal	Vila Real	5/4	2.8	0.9				
Russia	Lomonosor	5/2	2	0.4				
	Zelenogradsk	2/1	-	-				
	Sebezh	5/3	2	0.7				
Slovenia	Kranj-Mlaka	5/3	2.2	0.7				
Spain	Uriz-Begonte	5/4	2.4	0.9				
	Vitoria	5/5	3.4	1				
	Segovia	4/3	3.16	0.83				
	La Torre de Cadi	5/5	2.6	1				
Sweden	Uppsala	4/1	0	0				
	Overkalix	5/2	0.8	0.4				
Switzerland	Liesberg	3/2	1.33	0.67				
Total		150/36		0.82				
Britian	Scotland	22/8	0.0039 ±	0.8398 ±	23.8/5.084	7.400 ±	0.720 ±	MtDNA +
			0.0024	0.0462				
	Wales	45/5	0.0029 ±	0.4737 ±	53.8/4.921	9.600 ±	0.668 ±	(de Becquevort
			0.0018	0.0824				
						3.203	0.103	<i>et al. 2024)</i>

	South of England	15/6	0.0038 ±	0.7905 ±	14.2/3.971	4.800 ±	0.596 ±
			0.0024	0.0785		1.241	0.095
	North of England	9/4	0.0045 ±	0.7500 ±	12.8/4.349	5.000 ±	0.676 ±
			0.0029	0.1121		1.342	0.053
	Switzerland	7/3	0.0011 ±	0.7143 ±	15.8/4.054	4.800 ±	0.637 ±
			0.0010	0.1267		1.020	0.064
	Belgium	2/1	0	0	8.4/4.083	4.200 ±	0.648 ±
						0.583	0.057
	The Netherlands	15/5	0.0027 ±	0.6952 ±	15.8/5.099	6.600 ±	0.617 ±
			0.0018	0.1092		1.860	0.132
	Total		115/17	0.003 ±	0.712 ±		
				0.004	0.042		

<i>T. destruens</i>	Algeria	Senalba	5/4	2.00	0.90	MtDNA (Horn <i>et al.</i> 2006)
	Croatia	Pula	5/1	0.00	0.00	
	Spain	Uriz-Begonte	5/2	0.40	0.40	
		Roquez	5/5	1.80	1.00	
		Bilbao	5/3	1.00	0.80	
		Madrid	5/3	0.80	0.70	
		Valence	3/3	-	-	
		Baleares	5/3	1.80	0.70	
		Seville	5/3	1.20	0.70	
	France	Lubéron	5/2	0.80	0.70	
		Les Arcs	5/3	0.80	0.70	
		Pietrosella	4/2	0.50	0.50	

MtDNA
(Horn *et al.*
2006)

	Calvi	4/3	1.17	0.83
	Toulon	5/3	1.80	0.80
	Pautilles	4/3	2.67	0.83
	Collioures	5/3	1.60	0.70
	St Chinian	5/2	0.40	0.40
	Oléron	1/1	-	-
	Pierroton	5/2	0.40	0.40
	Forêt de la Coubre	5/1	0.00	0.00
Greece	Polygyros	1/1	-	-
	Domokos	5/1	0.00	0.00
	Agia- Anastasia	5/3	0.80	0.70
Israel	Jérusalem	4/2	2.00	0.67
Italy	Gioiosa — Taranto	5/3	1.20	0.70
	Alberese — Grossetto	5/4	1.20	0.90
	Vallevecchia — Venezia	5/1	0.00	0.00
	Eraclea Mare — Venezia	5/2	4.80	0.60

	Ginosa			
	Marina —	5/3	3.80	0.80
	Taranto			
Lebanon	Aley	4/3	1.67	0.83
	Broumana	5/5	2.80	1.00
Morocco	Tamrabta	5/3, 5/4	1.16	0.76
	Larache- Leghdira	5/3, 5/2	0.80	0.53
	La Mamora	5/4, 5/4	2.29	0.91
Portugal	Ota	5/2	0.60	0.60
	Ota	5/4	1.80	0.90
	Aveiro	5/2	0.40	0.40
	Ponte Lima	5/1	0.00	0.00
	Alcacer do Sal	5/3	1.40	0.70
Tunisia	Sakia	5/4, 5/4	2.18	0.96
	Tabarka	5/2, 4/3	1.17	0.69
	Teknepinar	5/3	2.20	0.70
Total		219/53		0.87

MtDNA: N_{MT}, number of mtDNA sequences; N_{HT}, number of mtDNA haplotype; Hd, haplotype diversity; π, nucleotide diversity;

Microsatellite: N_{MI}, values across loci of sample size; AR, allelic richness; A, mean number of alleles; He, expected heterozygosity;

emerge from their overwintering sites and seek breeding material, such as recently cut pine trees, logs, branches, and stumps, to oviposit in the inner bark. In Central Portugal, at the same sites, *T. piniperda* reproduces in early spring (mainly March, occasionally as early as December), whereas the reproductive flight of *T. destruens* occurs in late autumn (September and October), with no overlap between their flight periods, possibly to avoid hybridization (Vasconcelos *et al.* 2005).

In Canada, mark-release-recapture experiments showed that most *T. piniperda* dispersed within 400 m, with an average dispersal distance of 900 m, and some individuals reaching up to 2,000 m under favorable conditions (Barak *et al.* 2000). Furthermore, the spring flight of *T. piniperda* is strongly influenced by maximum daily temperatures, with a flight threshold around 12 °C according to the swarming model of Saarenmaa (1989), which was validated against swarming data from Europe (Bakke 1968) and northeastern United States (Haack *et al.* 1997).

In contrast, distinct differences have been revealed between *T. piniperda* and *T. destruens*. During this phase, adults emerge from their overwintering sites and seek breeding material, such as recently cut pine trees, logs, branches, and stumps, to oviposit in the inner bark (Monleón *et al.* 1996).

The secondary dispersal phase occurs after nymphosis, when young adults disperse to the crowns of healthy trees to feed on shoots or twigs during maturation feeding (Horn *et al.* 2009).

Overwinter and cold tolerance

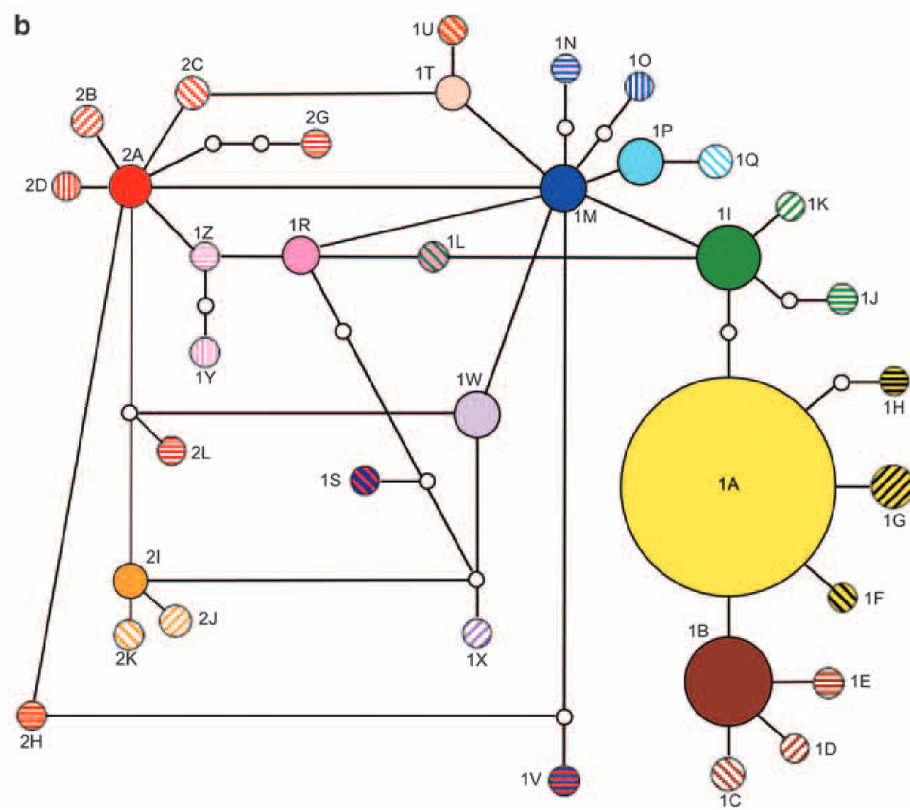
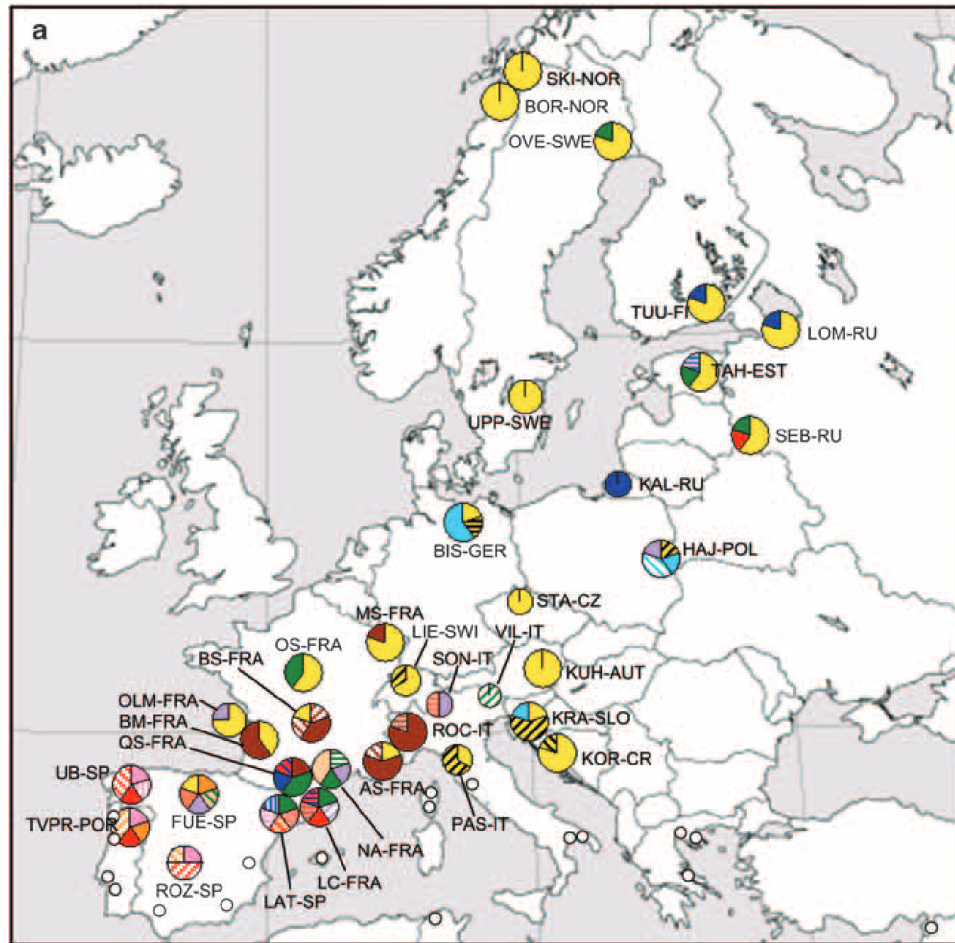
T. piniperda (Ryall & Smith, 2000) is a cold-tolerant species adapted to cooler climates, unable to thrive in warm and dry environments adapted to cooler climates, unable to thrive in warm and dry environments (Gallego *et al.* 2004, Horn *et al.* 2012). It develops optimally within a temperature range of 10–15 °C (Ryall and Smith 2000). In Ontario, Canada, the total thermal requirement for development was estimated at 1250 ± 73 degree-days (Ryall and Smith 2000). In North America, adult beetles overwinter by boring into the bark at the base of live pine tree (Barak *et al.* 2000, Haack *et al.* 2001), whereas in Sweden, they overwinter in the forest duff (Långström 1983).

For *T. destruens*, overwintering strategies are more variable: part of the population, including adults, larvae, and pupae, overwinters under the bark of infested pines, while some adults overwinter within pine shoots, as observed in Central Italy (Peverieri *et al.* 2008).

3.2.4 Phylogeography and evolutionary traits

As indicated in Table 4, *T. piniperda* and *T. destruens* display comparable levels of mtDNA haplotype diversity. *T. piniperda* (150 individuals, 36 haplotypes, 0.82 haplotype diversity) shares similar mtDNA evolutionary traits with *T. destruens* (219 individuals, 53 haplotypes, 0.87 haplotype diversity). *T. destruens* exhibits a more complex genetic structure than *T. piniperda* in its native range (Horn *et al.* 2006, 2009).

A



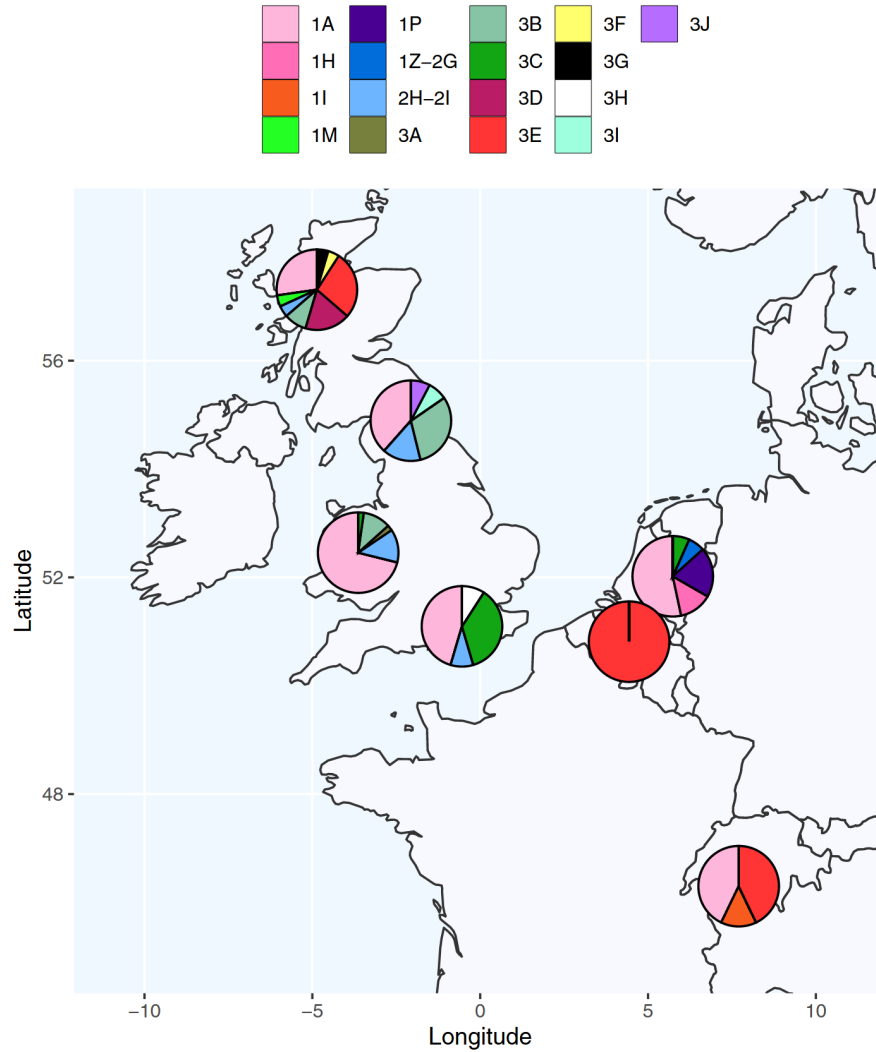
B

Figure 8 Phylogeography of *T. piniperda*. **A.** Geographical distribution of *T. piniperda* populations and their associated haplotypes of the cytochrome oxidase I and II sequences. (a) Geographic distribution of the 34 populations represented with the proportion of different haplotypes for each population. Small open circles show unsuccessful trappings. Codes for the localities are given in Table 1. Color codes refer to the color used in the haplotype network. (b) Haplotype network of the 36 haplotypes. Each line corresponds to a mutational step, and each empty circle to a missing intermediate. Haplotype frequencies are represented by the size of the circle (Horn *et al.* 2009); **B.** Distribution of each haplotype found in *T. piniperda* (present study results only) and its frequency (de Becquevort *et al.* 2024).

Temperate species *T. piniperda*, a weak genetic structure in Europe influenced by the primary host *P. sylvestris*

T. piniperda has a weak genetic structure obtained by the mtDNA and microsatellite (Figure 8). Kerdelhué *et al.* (2002) obtained 21 COI-COII haplotypes from 38 individuals and 16 populations throughout France, finding the effect of the host plant was significant for *T. piniperda* only, while the effect of geographical isolation was not. Ritzerow *et al.* (2004) obtained 25 COI haplotypes from 74 samples in Europe, China, and USA, with eight haplotypes (72% of individuals)

restricted to specific regions (China, France, Spain, etc.). European populations show low diversity in Central and Northern Europe, Chinese populations are highly diverse and isolated, while USA populations resemble European ones, indicating a European origin. *T. piniperda*'s refugial areas likely existed in Southern Europe and Western Russia, with the Pyrenees acting as a migration barrier post-Ice Age, while Chinese and European populations have been separated for at least 0.6 mya. [Faccoli et al. \(2005\)](#) identified 11 COI haplotypes from 24 individuals across four Italian populations, revealing a lack of strong genetic structure in *T. piniperda*, likely due to the continuous distribution of its primary host, *P. sylvestris* and the intensive trade of timber among different countries. The high polymorphism observed in *T. piniperda* was attributed to the existence of multiple distinct refugial areas during the last ice age, with Italy suggested as one such potential refugium. [Kerdelhué et al. \(2006\)](#) genotyped 210 samples from seven populations covering the main regions of France for five microsatellite loci ([Kerdelhué et al. 2003](#)), revealing its populations were very weakly differentiated, with a limited effect of isolation by distance possible due to its dispersal abilities. This pattern suggests that host specialization has limited influence on the evolution of *T. piniperda* within this restricted group of host trees. However, COI-COII markers indicate a weak but significant effect of host species as a barrier to gene flow ([Kerdelhué et al. 2002](#)). The different results between nuclear and mitochondrial markers could be explained by sex-biased dispersal, as proposed for *I. typographus* ([Sallé et al. 2007](#)). [Horn et al. \(2009\)](#), combining data from [Kerdelhué et al. \(2002\)](#) identified 36 COI-COII haplotypes across 34 European populations, with most haplotypes restricted to single sites. The Iberian Peninsula showed highest diversity and acted as a major glacial refugium, while northern populations were less diverse, dominated by a single haplotype, reflecting postglacial recolonization. [de Becquevort et al. \(2024\)](#) found 17 haplotypes and two nuclear DNA groups across UK and mainland Europe, with higher diversity in Scottish populations, suggesting historical fragmentation and proximity to a Pleistocene refuge.

In summary, studies have identified multiple glacial refugia for *T. piniperda*, including the Iberian Peninsula, the Pyrenees, Central Europe, and Scandinavia. These refugia served as sources for postglacial recolonization of Europe ([Faccoli et al. 2005](#), [Horn et al. 2009](#), [de Becquevort et al. 2024](#)). Postglacial expansion from these refugia shaped its current distribution, with northern populations often showing lower genetic diversity due to founder effects during recolonization ([Kerdelhué et al. 2002](#), [Horn et al. 2009](#)). The phylogeography is tightly linked to the distribution of its primary host, *P. sylvestris*, which influenced its survival in glacial refugia and subsequent migration patterns ([Kerdelhué et al. 2002](#), [de Becquevort et al. 2024](#)). High genetic diversity was observed in southern populations (e.g., the Iberian Peninsula), where refugia were concentrated, while northern populations are genetically less diverse ([Kerdelhué et al. 2002](#), [Horn et al. 2009](#)). Populations in the UK exhibited distinct genetic structure due to limited forest cover and fragmented habitats, restricting gene flow ([Horn et al. 2009](#), [de Becquevort et al. 2024](#)). Physical barriers, such as the Pyrenees and the Alps, have played significant roles in limiting gene

flow between populations, leading to regional differentiation (Kerdelhué *et al.* 2002, 2006, de Becquevort *et al.* 2024). Long-distance dispersal facilitated by timber trade may have contributed to the mixing of populations in certain areas, reducing regional genetic differentiation (Kerdelhué *et al.* 2002, Faccoli *et al.* 2005). Past gene flow during interglacial periods allowed some haplotypes to spread across regions, while local environmental conditions (e.g., temperature, forest composition) shaped adaptation and differentiation (Kerdelhué *et al.* 2002, de Becquevort *et al.* 2024). The phylogeographical pattern presented here partially reflects the postglacial history of the beetles' primary host tree, *P. sylvestris* (Horn *et al.* 2009).

The phylogeographic structure of *T. piniperda* results from glacial refugia, postglacial expansion, host tree distribution, geographic barriers, human activity, and local adaptation. These factors collectively shaped the genetic diversity and spatial distribution of its populations across Europe.

***T. destruens*, a circum-Mediterranean species, shows strong genetic structure shaped by geographic barriers around the Mediterranean Sea**

T. destruens has a strong genetic structure obtained by the mtDNA and microsatellite (Figure 9). Kerdelhué *et al.* (2002) obtained nine haplotypes from 34 individuals and 16 populations, finding the effect of the host plant and geographical isolation was not significant for *T. piniperda* only. Faccoli *et al.* (2005) obtained seven COI haplotypes from 16 individuals and four populations in Italy and a strong phylogeography in this species possibly due to high fragmentation of the hosts range. *T. destruens* may have a contact zone between the western and eastern Mediterranean in Italy. Horn *et al.* (2006) identified 53 COI-COII haplotypes from 219 samples across 42 Mediterranean and Atlantic populations, revealing two distinct clades: eastern and western. The eastern clade, primarily associated with *P. halepensis* and *P. brutia*, exhibited strong genetic structure and limited gene flow, possibly recognizing from eastern coast of Italy and the Balkan Peninsula. In contrast, the western clade, predominantly linked to *P. pinaster*, showed a weaker structure, likely influenced by host species, climatic factors, and geographical barriers, possibly from the Iberian Peninsula (Portugal and Spain) refugium. The Adriatic coast of Italy was identified as a contact zone between populations from these two refugia. Vasconcelos *et al.* (2006) identified 26 COI-COII haplotypes from 84 samples and 26 populations from Portugal, France, and Spain, together with previous data in Kerdelhué *et al.* (2002). The spatial genetic structure revealed two glacial refugia: one in Portugal, associated with *P. pinaster*, and another in the eastern range, linked to *P. halepensis* and *P. pinea*. Genetic variation and distribution patterns were strongly influenced by host tree dominance, with *P. pinaster* prevailing in the west and *P. halepensis*/*P. pinea* in the east. Gallego & Galián (2008) compiled and analyzed 116 COI-COII haplotypes from 323 specimens across 61 populations spanning the entire Mediterranean Basin, integrating new

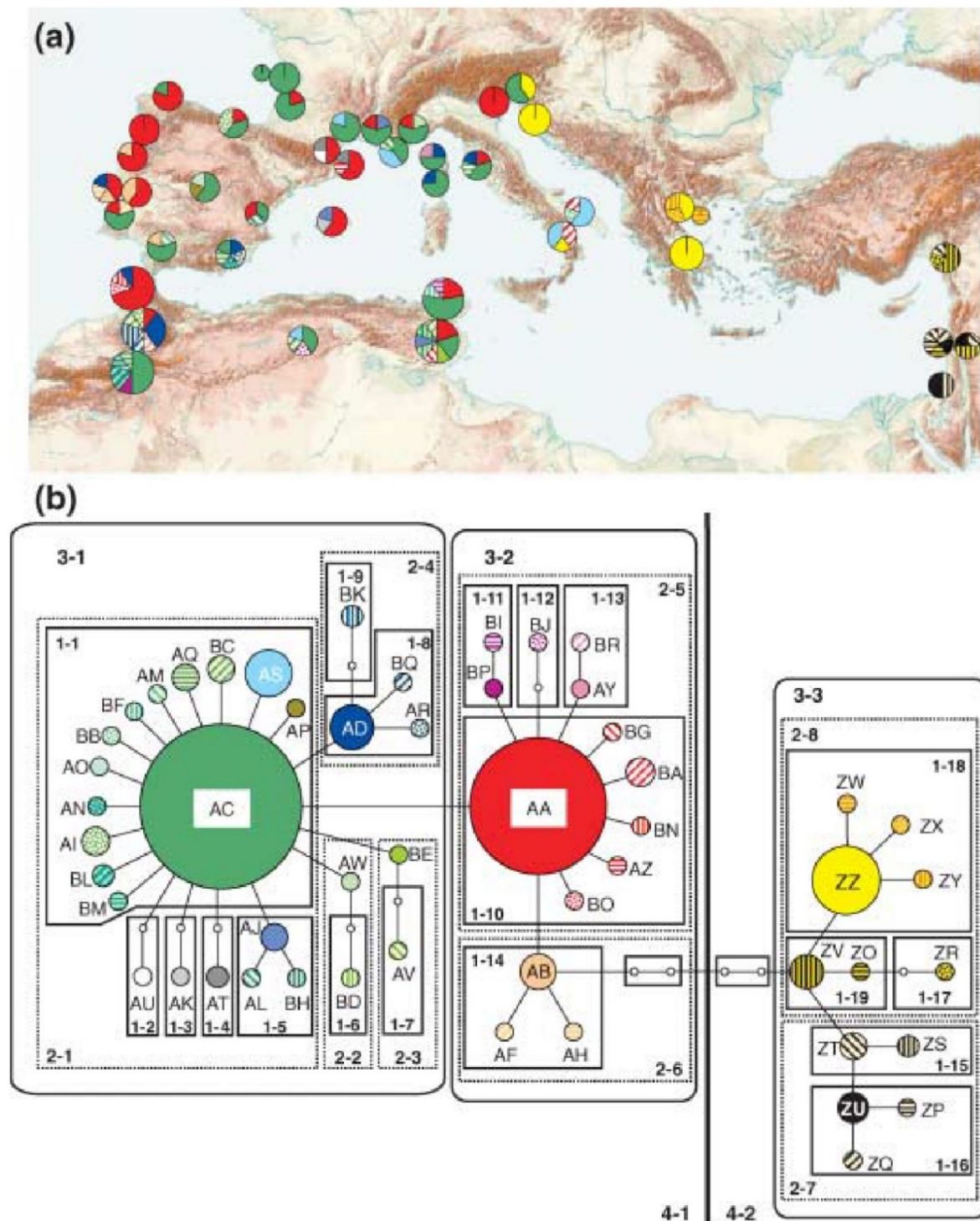


Figure 9 Phylogeography of *T. destruens*: Haplotype distribution and haplotype network of 219 *T. destruens* cytochrome oxidase I and II sequences. **a.** Geographic distribution of the haplotypes among the 42 sampled populations. **b.** Haplotype network of the 53 haplotypes with corresponding colour codes and nested design for the NCPA. Haplotype frequencies are represented by the area of the circle. Each line corresponds to a mutational step and each empty circle to a missing intermediate. Ambiguous haplotype AP is presented here connected to haplotype AC (option APAC, see text for details). Boxes represent the n-step clades. The thick line separates the 4-step clades (clades 4-1 and 4-2) (Horn *et al.* 2006).

collections with data from previous studies to create a comprehensive dataset for phylogeographic research (Schroeder 1988, Byers 1992, Gallego and Galián 2001, Sequeira and Farrell 2001, Kerdelhué *et al.* 2002, Kohlmayr *et al.* 2002, Faccoli *et al.* 2005, Horn *et al.* 2006, Vasconcelos *et al.* 2006). The current molecular diversity of *T. destruens* arises from postglacial expansion from two principal refugia in the West and East Mediterranean (Horn *et al.* 2006), followed by local lineage fixation driven by environmental heterogeneity like altitude (Silvertown *et al.* 2006) for plant communities. Genetic diversity is closely tied to the micro-environmental structure of the landscape, shaped by competitive exclusion between mitochondrial lineages. Older lineages dominate average environmental conditions, while younger lineages have been displaced to extreme habitats through niche differentiation. This evolutionary scenario suggests that the phylogeography and molecular diversity of *T. destruens* are the result of recolonization from these refugia and habitat heterogeneity facilitating lineage fixation.

Studies identify two main glacial refugia: the western Mediterranean (Portugal and Iberian Peninsula, linked to *P. pinaster*) and the eastern Mediterranean (Italy and Balkans, associated with *P. halepensis* and *P. brutia*). Postglacial expansions from these refugia shaped the current population distribution, with the Adriatic coast acting as a contact zone between the two lineages (Horn *et al.* 2006, Vasconcelos *et al.* 2006, Sánchez-García *et al.* 2015). High genetic diversity is observed, with significant spatial structure influenced by local environmental conditions and geographic barriers (Horn *et al.* 2006, Gallego and Galián 2008, Sánchez-García *et al.* 2015). Western populations exhibit weak phylogeographic structure due to long-distance dispersal and timber trade, while eastern populations show stronger structure and low gene flow (Horn *et al.* 2006, Sánchez-García *et al.* 2015). Environmental heterogeneity (e.g., altitude, climate) drives local lineage fixation and genetic diversity. The distribution of host species (*P. pinaster*, *P. halepensis*, *P. brutia*) is a key factor shaping population dynamics, with western populations linked to *P. pinaster* and eastern populations associated with *P. halepensis* and *P. brutia* (Vasconcelos *et al.* 2006, Sánchez-García *et al.* 2015, de Becquevort *et al.* 2024). Competitive exclusion between mitochondrial lineages contributes to the genetic structure, with older lineages occupying average habitats and newer lineages confined to extreme environments (Gallego and Galián 2008, Sánchez-García *et al.* 2015). Research combines mitochondrial DNA (COI-COII) analysis with phylogeographic methods like Nested Clade Phylogeographic Analysis (NCPA) and regression models (Vasconcelos *et al.* 2006, Sánchez-García *et al.* 2015). Integration of genetic data with environmental and ecological models has provided a comprehensive understanding of the species' historical and contemporary distribution (Gallego and Galián 2008, Sánchez-García *et al.* 2015).

The phylogeographic patterns of *T. destruens* result from the interplay of historical glacial refugia, host tree distributions, environmental heterogeneity, competitive exclusion, human activities, and geographic barriers. Together, these factors shape its genetic diversity and spatial distribution.

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Chapter 2: Seven mitochondrial genomes of tribe Hylurgini in Eurasia and their phylogenetic analysis

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Chapter 2: Sept génomes mitochondriaux de la tribu Hylurgini en Eurasie et leur analyse phylogénétique

Résumé en français du contexte, des objectifs de l'étude et des méthodes utilisées

Résumé

La tribu des Hylurgini (Coleoptera : Curculionidae : Scolytinae) comprend des scolytes d'importance économique, notamment des espèces envahissantes appartenant aux genres *Dendroctonus* et *Hylurgus*. Ces espèces envahissantes coexistent avec les espèces indigènes du genre *Tomicus* au sein des Hylurgini et attaquent ensemble les arbres hôtes en Chine. Toutefois, les données disponibles sur les génomes mitochondriaux des Hylurgini restent insuffisantes pour mener des études phylogénétiques approfondies, clarifier les relations évolutives entre ces espèces et mieux comprendre la divergence des niches ainsi que les risques communs qu'elles représentent. Dans cette étude, nous avons séquencé et analysé les génomes mitochondriaux de sept espèces d'Hylurgini: *Dendroctonus valens*, *Hylurgus ligniperda*, *Hylurgus micklitzi*, *Tomicus piniperda*, *Tomicus brevipilosus*, *Tomicus minor* et *Tomicus yunnanensis*. Les génomes mitochondriaux obtenus varient entre 15 339 pb et 17 545 pb, avec une teneur en AT comprise entre 73,24 % et 78,81 %. La structure de ces génomes est cohérente avec celle des insectes ancestraux. En nous basant sur les 13 gènes codant pour des protéines de 29 espèces de scolytes déjà publiées, nous avons construit des arbres phylogénétiques selon les méthodes de maximum de vraisemblance et d'inférence bayésienne. Les topologies des deux arbres sont quasiment identiques. Ces résultats clarifient la classification taxonomique des Hylurgini ainsi que les relations évolutives avec leurs groupes frères au sein des Scolytinae. Cette étude offre des perspectives utiles pour mieux comprendre les liens évolutifs entre scolytes envahissants et indigènes, ainsi que pour l'identification moléculaire et la détection de nouvelles espèces envahissantes.

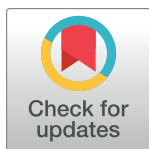
RESEARCH ARTICLE

Seven mitochondrial genomes of tribe Hylurgini (Coleoptera: Curculionidae: Scolytinae) in Eurasia and their phylogenetic analysis

Na An^{1,2}, Yuan Yuan^{2,3}, Sixun Ge^{1,2}, Xudong Zhang^{1,2}, Lili Ren^{1,2*}, Alain Roques^{2,3}, Youqing Luo^{1,2*}

1 Beijing Key Laboratory for Forest Pest Control, College of Forestry, Beijing Forestry University, Beijing, China, **2** IFOPE, Sino-French Joint Laboratory for Invasive Forest Pests in Eurasia, INRAE URZF and Beijing Forestry University, Beijing, China, **3** INRAE URZF, Orléans, France

* lily_ren@bjfu.edu.cn (LR); youqingluo@126.com (YL)



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Abstract

The Hylurgini tribe (Coleoptera: Curculionidae: Scolytinae) comprises commercially significant bark beetles, including invasive species within the genera *Dendroctonus* and *Hylurgus*. These invasive species coexist with native *Tomicus* species of Hylurgini and cooperatively infest host trees in China. However, we lack sufficient mitochondrial genome data of Hylurgini to conduct phylogenetic studies, clarify the phylogenetic relationships of the above species, and improve the understanding of niche divergence and common hazards. Here, we sequenced and analyzed the mitochondrial genomes of seven Hylurgini species, including *Dendroctonus valens*, *Hylurgus ligniperda*, *Hylurgus micklitzi*, *Tomicus piniperda*, *Tomicus brevipilosus*, *Tomicus minor* and *Tomicus yunnanensis*. All sequenced mitochondrial genomes ranged from 15,339 bp to 17,545 bp in length, and their AT contents ranged from 73.24% to 78.81%. The structure of the seven mitochondrial genomes was consistent with that of ancestral insects. Based on 13 protein-coding genes from the reported mitochondrial genomes of 29 species of bark beetles, we constructed phylogenetic trees using maximum likelihood and Bayesian inference methods. The topology of the two phylogenetic trees was almost consistent. The findings elucidated the taxonomy classification of Hylurgini and the evolutionary connections of its sister taxa within the Scolytinae. This study offers insights for examining the evolutionary connections between invasive and native bark beetles, as well as the molecular identification and detection of newly invading species.

1. Background

Bark and ambrosia beetles comprise the subfamilies Scolytinae and Platypodinae in the family Curculionidae [1–7]. Hylurgini (Coleoptera: Curculionidae: Scolytinae), is a large group of Scolytinae which contains 14 genera: *Chaetoptelius* Fuchs, *Dendroctonus* Erichson, *Dendrotrupes* Broun, *Hylurdrectonus* Schedl, *Hylurgonotus* Schedl, *Hylurgopinus* Swaine, *Hylurgus*

Competing interests: The authors have declared that no competing interests exist.

Latreille, *Pachycotes* Sharp, *Pseudohylesinus* Swaine, *Pseudoxylechinus* Wood & Huang, *Sinophloeus* Brèthes, *Tomicus* Latreille, *Xylechinosomus* Schedl, and *Xylechinus* Chapuis [4, 5, 8–12]. There are three genera in the tribe Hylurgini occurring in China, i.e., *Tomicus*, *Dendroctonus* and *Hylurgus*.

Many investigations have identified two or more species within the tribe Hylurgini that cooperatively damage their hosts in the same location. In some areas, the simultaneous species of Hylurgini are all native and belong to the same genus. For example, in 1997, a survey was conducted in Kunming City and Shuangbai County, Chuxiong City, Yunnan Province. *Tomicus minor* (Hartig) and *T. yunnanensis* concurrently inflicted damage on *Pinus yunnanensis*, leading to tree vulnerability and expedited mortality [13–15]. In 2011, *Tomicus brevipilosus*, *T. minor* and *T. yunnanensis* were reported to co-occur in Qujing County, Jianshui City and Chuxiong City of Yunnan Province [16, 17]. In Arizona, USA, *Dendroctonus frontalis* Zimm. and *Dendroctonus brevicomis* LeConte were found on the same tree [18, 19]. In some areas, the invasive species of Hylurgini became co-damaging with indigenous species. *T. piniperda* and *H. ligniperda* were found to damage the host together in the Muping District of Yantai City, Shandong Province. This phenomenon was also observed in *Dendroctonus valens* and *T. piniperda* at Heilihe National Nature Reserve, Inner Mongolia.

There are many important forest pests in the tribe Hylurgini. Some species could cause massive damage during outbreaks. For example, *Dendroctonus ponderosae* Hopkin, a native species of western North America, caused massive forestry damage to more than 1.2 million hectares in 2004 [20]. *D. valens* and *H. ligniperda*, have been introduced from their indigenous habitats and have become significant invasive species, both caused significant destruction to forests following their introduction to China [7, 21–23]. Since its initial identification in 2020, *H. ligniperda* has inflicted significant damage on *Pinus thunbergii* inside the coastal shelter belt of Moping District, Yantai City, Shandong Province. [24]. The invasive *D. valens* was introduced into China in 1998, mainly damaging healthy *Pinus tabulaeformis* with a diameter at breast height greater than 10 cm or more than 30 years old [25, 26]. *Tomicus* spp., found in many regions of China, damage pine trees by feeding on the shoot and trunk [27]. Since the 1980s, in Yunnan Province alone, the damaged area has reached more than 10×10^4 hm² all year round [28].

In the study by Johnson *et al.* [29], 251 protein-coding genes from the subfamily Scolytinae were analyzed to establish the phylogenetic relationships of the subfamily Scolytinae, which consists of 22 tribes, including two species of *Dendroctonus* in the tribe Hylurgini. However, in morphological classification, the tribes of Xyleborini, Corthylini and Cryphalini are monophyletic [29–32]. According to the findings of Johnson *et al.*, Xyleborini is associated with Dryocoetini, Corthylini is polyphyletic, and Cryphalini is significantly polyphyletic [29]. The phylogenetic relationships of this subfamily are still confusing due to the conflicting phylogenetic frameworks mentioned above, and data on Hylurgini are lacking, so further clarification is needed.

At present, mitochondrial genomes have been used in a wide range of studies such as species identification, molecular evolution and molecular markers for comparative genomics, and are of great significance in population genetics and evolution [26, 33–39]. Insect mitochondrial genome is more easily amplified than the nuclear genome, lacking non-coding regions (i.e., introns), maternal inheritance, low recombination rate, small size, rapid mutation rate, and so on [40–42]. Currently, the mitochondrial genomes of Coleoptera have been released in GenBank with 572 species, and 39 species of Scolytinae have complete mitochondrial genome data [43, 44]. Three species of Hylurgini have complete mitochondrial genomes, including *D. valens*, *D. rufipennis* and *H. ligniperda* [45]. The studies on Hylurgini were mainly focused on morphology and molecular identification based on the cytochrome c oxidase subunit I (COI)

gene [46]. However, morphological characteristics are ineffective in solving intergeneric and interspecific phylogenetic relationships [16, 47–50]. The mitochondrial gene data of Hylurgini in GeneBank was seriously lacking except for the cytochrome oxidase I (*COI*) gene, which hindered the development of phylogenetic studies on it.

Here, we sequenced, assembled, and annotated the mitochondrial genomes of seven Hylurgini species, including *D. valens*, *H. ligniperda*, *H. micklitzi*, *T. piniperda*, *T. brevipilosus*, *T. minor*, *T. yunnanensis* and *H. micklitzi*. They have caused severe damage in China except for *H. micklitzi*. *H. micklitzi* currently does not distribute in China, but has extensive distribution in Europe. The mitochondrial genomes of seven Hylurgini species were compared and analyzed to understand their structure and base composition and to clarify the phylogenetic relationship between the tribe Hylurgini and other sister groups in Scolytinae. Phylogenetic studies of the tribe Hylurgini can provide us with a possible evolutionary framework to clarify the phylogenetic relationships of these species. Understanding phylogenetic relationships can also further advance knowledge of their niche changes and common hazards. Moreover, the members of the tribe Hylurgini are generally small in size, with close morphology of sympatric species. Its identification relies on taxonomists and strict identification conditions. Sequencing the mitochondrial genome could also advance the development of molecular markers for the tribe Hylurgini. Our results have important implications for studying mitochondrial genomes and clarifying the phylogenetic relationship.

2. Materials and methods

2.1 Sampling and genomic DNA extraction

Samples of seven species of bark beetle adults were collected from China and France. From October 16th to 23th, 2021. More than 600 individuals of *T. yunnanensis*, *T. minor*, *T. brevipilosus* were collected from three sites in Yunnan Province. *D. valens* was collected in Heilihe National Nature Reserve, Inner Mongolia. *H. ligniperda* and *T. piniperda* were collected from Muping, Shandong Province. *H. micklitzi* has been collected by the Orléans Forest Zoology Research unit (French Institute for Agri-Food and Environmental Research- INRAE), at Le Thoronet, Southern France by attractive traps on July 15th 2021 (Table 1). The above specimens were stored in the laboratory (College of Forestry, Beijing Forestry University) refrigerator in absolute ethyl alcohol at −20°C. A Zeiss AxioZoom V16 type microscope was used to observe and take photos of the samples (Figs 1 and 2). We identified the specimens with reference to these previous studies [9, 16, 49, 51]. According to the instructions, the AxyPrep Multi-source Genomic DNA Miniprep Kit (Axygen, USA) was used to extract the genomic DNA

Table 1. Collection information of Hylurgini species in this study.

Name	Location	Country	Longitude	Latitude	Accession number
<i>Dendroctonus valens</i> LeConte	Heilihe National Nature Reserve, Inner Mongolia	China	118.466830	41.407369	OP651189
<i>Hylurgus ligniperda</i> (Fabricius)	coastal shelterbelt in Moping District, Yantai City, Shandong Province	China	121.851217	37.457241	OP651193
<i>Hylurgus micklitzi</i> Wachtl	Le Thoronet	France	6.303889	43.451944	OP651194
<i>Tomicus brevipilosus</i> (Eggers)	Ninger Hani and Yi Autonomous County, Pu'er City, Yunnan Province	China	101.238255	22.964431	OP651191
<i>Tomicus minor</i> (Hartig)	Pupeng Town, Xiangyun County, Dali Bai Autonomous Prefecture, Yunnan Province	China	100.915525	25.315671	OP644291
<i>Tomicus piniperda</i> (Linnaeus)	coastal shelterbelt in Moping District, Yantai City, Shandong Province	China	121.851217	37.457241	OP651192
<i>Tomicus yunnanensis</i> Kirkendall & Faccoli	Anaconda Pit, Panlong District, Kunming City, Yunnan Province	China	102.882109	25.202455	OP651190

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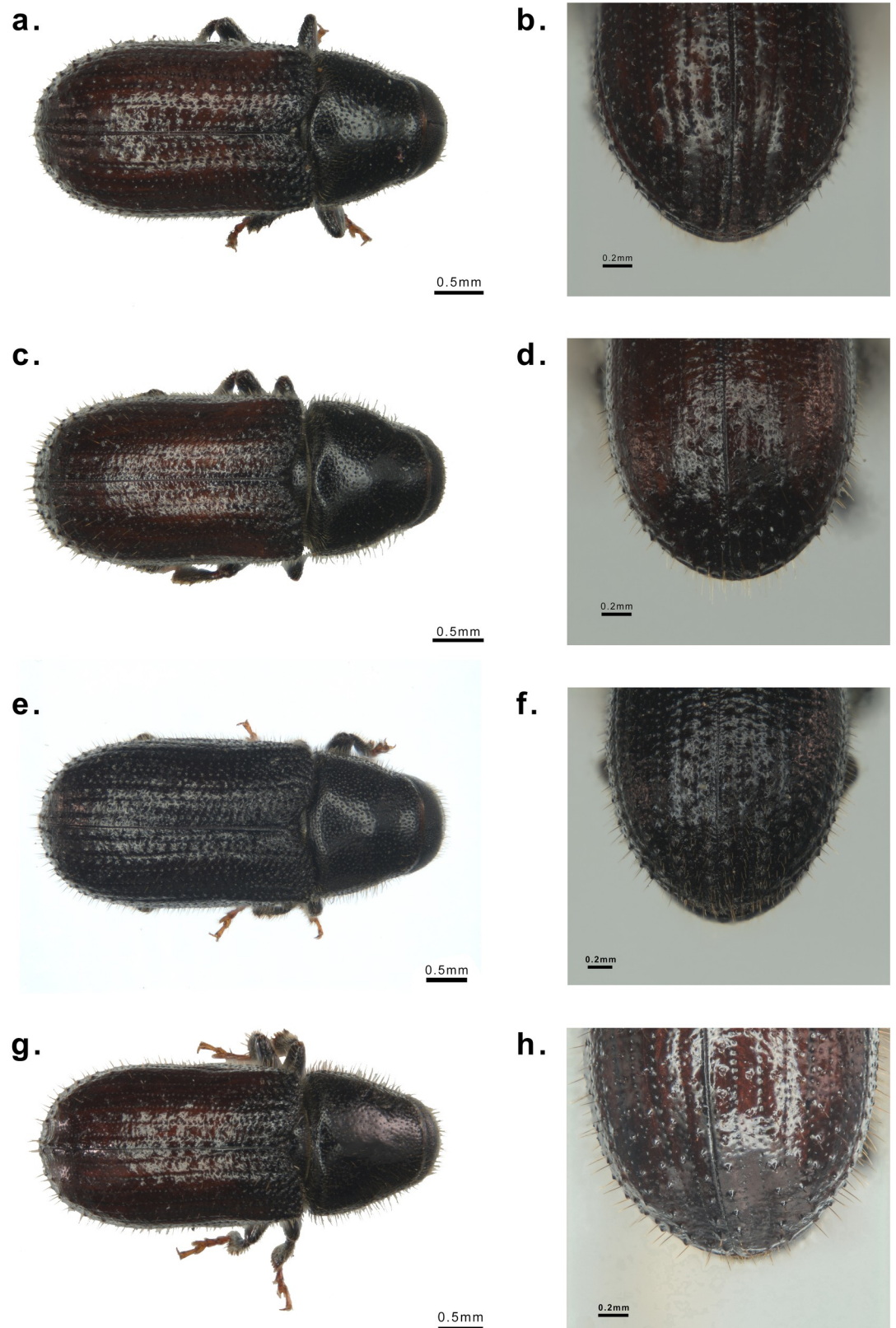


Fig 1. Morphological characteristics of Hylurgini. a. Adult dorsal view of *Tomicus brevipilosus*, b. Eytral declivity of *Tomicus brevipilosus*, c. Adult dorsal view of *Tomicus minor*, d. Eytral declivity of *Tomicus minor*, e. Adult dorsal view of *Tomicus piniperda*, f. Eytral declivity of *Tomicus piniperda*, g. Adult dorsal view of *Tomicus yunnanensis*, h. Eytral declivity of *Tomicus yunnanensis*.

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from the entire body of specimens from the same locality of each Hylurgini species. The total DNA quality was examined with 1% agarose gel, and the concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.2 Mitochondrial genome sequencing and assembly

Samples with qualified mass and concentration greater than 2 ng/μl were sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd for high-throughput sequencing. The library was constructed and sequenced using the Illumina HiSeq X platform based on Sequencing by Synthesis (SBS) technique. Filter the raw data using *Hylastes attenuatus* (KX035212.1) as the reference sequence. Clean reads were assembled with the software GetOrganelle (<https://github.com/Kinggerm/GetOrganelle>) [52] under the K-mer = 21, 45, 65, 85, 105. To ensure the accuracy of the results, NOVOPlasty4.2 (<https://github.com/ndierckx/NOVOPlasty>) [53] was also used with *Hylastes attenuatus* (KX035212.1) as a seed sequence for mitochondrial genome assembly under K-mer = 33. Then the results of the two software were compared, and the full length of mitochondrial genome sequences of the seven species were obtained.

2.3. Mitochondrial genome annotation and analysis

MITOS (<http://mitos2.bioinf.uni-leipzig.de/index.py>) [54] was used to annotate the mitochondrial genome results of seven species. The type of reference gene set was Metazoa, and the Genetic Code was Invertebrate. The BLAST function of NCBI was used to confirm the accuracy of the annotation results. Based on the available mitochondrial genome data of the family Scolytinae, the start codons and stop codons with problems in 13 PCGs were manually adjusted. ARWEN (www.acgt.se/online.html) [55] and tRNAscan—SE 2.0 (<http://trna.ucsc.edu/tRNAscan-SE/>) were used to predict the secondary structure of tRNA and annotate the undiscovered tRNA [56]. Using the Tandem Repeats the Finder online server (<http://tandem.bu.edu/trf/trf.html>) to find concatenation repeats sequences [57]. The two rRNA genes were compared with *Dendroctonus rufipennis*, *Hylastes attenuatus* Erichson and *Polygraphus poligraphus* (Linnaeus) of the subfamily Scolytinae, and manually adjusted by them. MEGA11 [58] was used to analyse the nucleotide codon usage frequency (RSCU) and nucleic acid composition. According to the formula, the AT-skew = [(A - T)/(A + T)] and GC-skew = [(G - C)/(G + C)] calculating nucleotide differences (AT deflection and GC deflection). The synonymous substitution rate (dS) and non-synonymous substitution rate (dN) were calculated by PALM 4.9j [59]. The heatmap was plotted using TBtools (Toolbox for biologists) [60]. Finally, CGView Server (http://stothard.afns.ualberta.ca/cgview_server) [61] was used to draw the mitochondrial genome maps.

2.4 Sequence alignment and phylogenetic analysis

Complete published mitochondrial genome sequences of 29 species from 10 tribes of Scolytinae were downloaded from the GenBank (S1 Table), and 13 protein-coding genes (PCGs) were extracted using the Phylosuite1.2.2 [62] software. MAFFT [63] was used to conduct multiple sequence alignment. Then, the sequences were concatenated to obtain a nucleotide sequence data set of protein-coding genes. The sequence of *Acridia cinerea* (NC_014887.1) was selected as an outgroup. We used IQ-TREE (maximum likelihood, ML) [64] and MrBayes

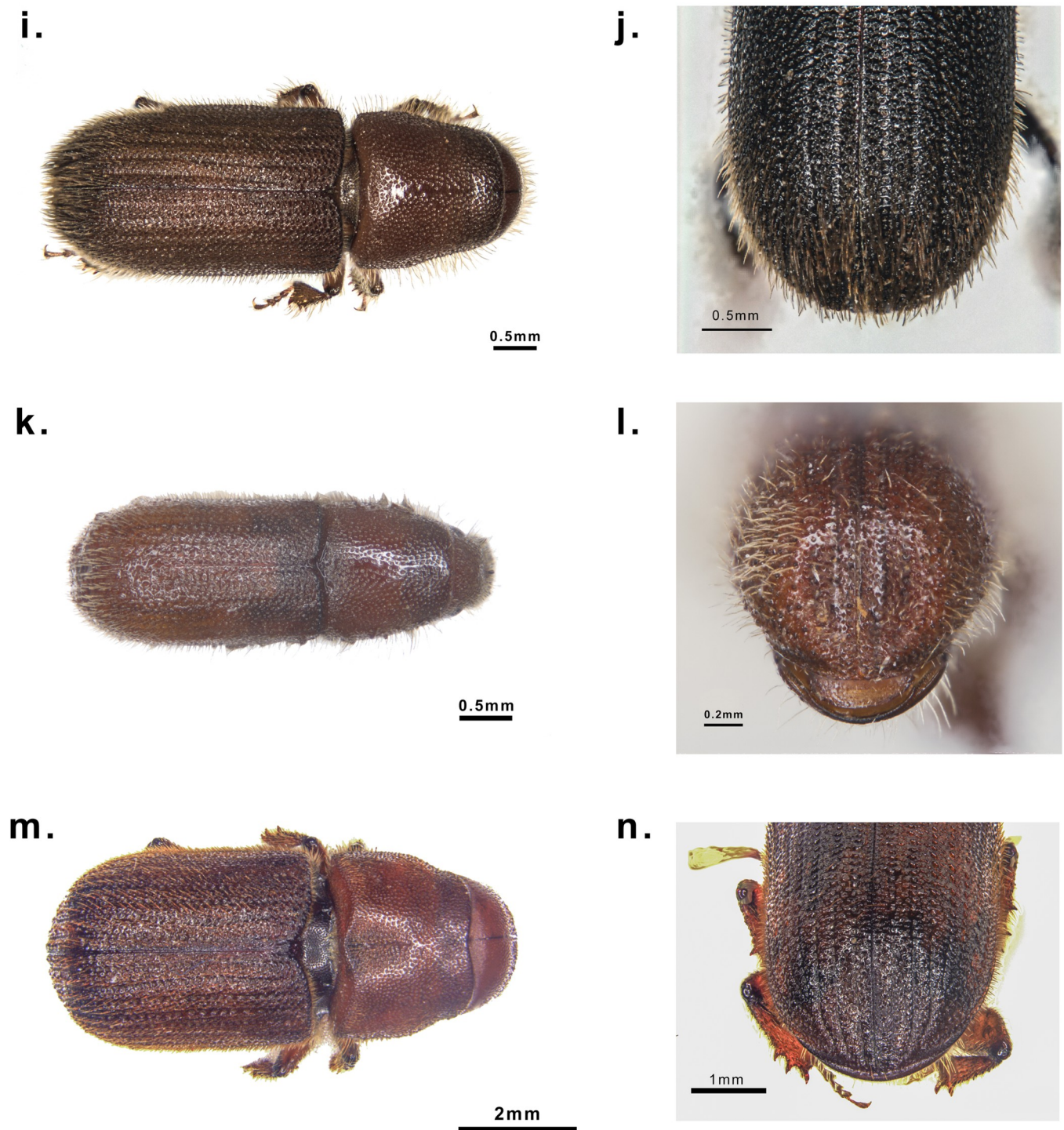


Fig 2. Morphological characteristics of Hylurgini. i. Adult dorsal view of *Hylurgus ligniperda*, j. Eytral declivity of *Hylurgus ligniperda*, k. Adult dorsal view of *Hylurgus micklitzi*, l. Eytral declivity of *Hylurgus micklitzi*, m. Adult dorsal view of *Dendroctonus valens*, n. Eytral declivity of *Dendroctonus valens*.

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3.2.6 (Bayesian inference, BI) [65] of Phylosuite1.2.2 software to construct phylogenetic trees. In IQ-TREE, we selected the “Auto” model. The confidence level of the branch node was estimated using the 1,000 ultrafast bootstrap replicates. With the help of ModelFinder [66], the optimal nucleotide substitution model was selected as GTR + I + G + F, and MrBayes performed Bayesian inference under the GTR + I + G + F model, operating for 2,000,000 generations with two simultaneous runs, sampling once every 1,000 generations, and the burn-in fraction was set to 0.25. The value of the average standard deviation of split frequencies was less than 0.01, and there was little difference between the results of the two runs, and the parameters converged [67]. The tree files were visualized by iTOL (Interactive Tree Of Life) (<http://itol.embl.de>) [68]. Finally, MEGA11 software was used to calculate the genetic distance of Hylurgini.

3. Results

3.1 Mitochondrial genome structure and base composition of seven Hylurgini species

The mitochondrial genome sequences of seven Hylurgini species ranged from 15,339 bp to 17,545 bp. The shortest was *T. piniperda* and the longest was *T. minor*. *T. minor*, *T. yunnanensis*, and *D. valens* were composed of a control region and 37 genes (13 protein-coding genes, 22 tRNA genes and two rRNA genes). The other four species, *T. brevipilosus*, *T. piniperda*, *H. ligniperda* and *H. micklitzi*, were missing *tRNA^{Ile}* genes, all of which were 36 genes (Figs 3 and 4). Among the seven newly sequenced mitochondrial genomes, nine protein-coding genes (*ND2*, *COI*, *COII*, *ATP8*, *ATP6*, *COIII*, *ND3*, *ND6*, *Cytb*), 14 tRNA genes (*tRNA^{Ile}*, *tRNA^{Met}*, *tRNA^{Trp}*, *tRNA^{Leu(UUR)}*, *tRNA^{Lys}*, *tRNA^{Asp}*, *tRNA^{Gly}*, *tRNA^{Ala}*, *tRNA^{Arg}*, *tRNA^{Asn}*, *tRNA^{Ser(AGN)}*, *tRNA^{Glu}*, *tRNA^{Thr}*, *tRNA^{Ser(UCN)}*) were distributed on the J strand (majority strand, encodes more genes in the DNA double strand), while the remaining 14 genes were on the N strand (minority strand, encodes a relatively small number of genes in the DNA double strand) [69, 70].

There were 13 non-coding regions in *D. valens*, 13 in *H. ligniperda* and *H. micklitzi*, 16 in *T. minor*, *T. brevipilosus* and *T. piniperda*, and 17 in *T. yunnanensis* at most. Except for the control region, the most extended non-coding region was in *tRNA^{Ile}-tRNA^{Gln}* of *T. yunnanensis*, with a length of 281 bp. The overlap of the seven Hylurgini species varied from 8 to 14 places. Two overlapping regions, *tRNA^{Lys}-tRNA^{Asp}* and *ATP8-ATP6*, were found in all seven bark beetle species.

The content of A+T in the whole mitochondrial genome of the seven Hylurgini species ranged from 73.24% to 78.81%, with the lowest content of *D. valens* and the highest content of *H. micklitzi*, showing an obvious AT bias. The AT-skew of the seven species was 0.024 to 0.080, and the GC-skew was negative, ranging from -0.239 to -0.158.

3.2 Mitochondrial protein-coding genes and relative synonymous codon usage

In seven mitochondrial genomes, the total length of 13 protein-coding genes ranged from 11,082 bp to 11,138 bp, accounting for 61.84% to 72.61% of the total sequence, in which *ND5* of *T. brevipilosus* was the longest, 1,723 bp. *ATP8* of *H. micklitzi*, *D. valens*, and *H. ligniperda* was the shortest, 156 bp. Most PCGs were started by the insect standard start codon ATN. However, the start codon of *T. minor*'s *COI* gene was TCG; the start codon of the protein-coding gene *ND1* of *T. yunnanensis*, *T. brevipilosus*, *H. micklitzi* and *T. piniperda* was TTG respectively. As for stop codon, five PCGs (*ND2*, *COI*, *ATP6*, *COIII*, *ND6*) ended with TAA in seven

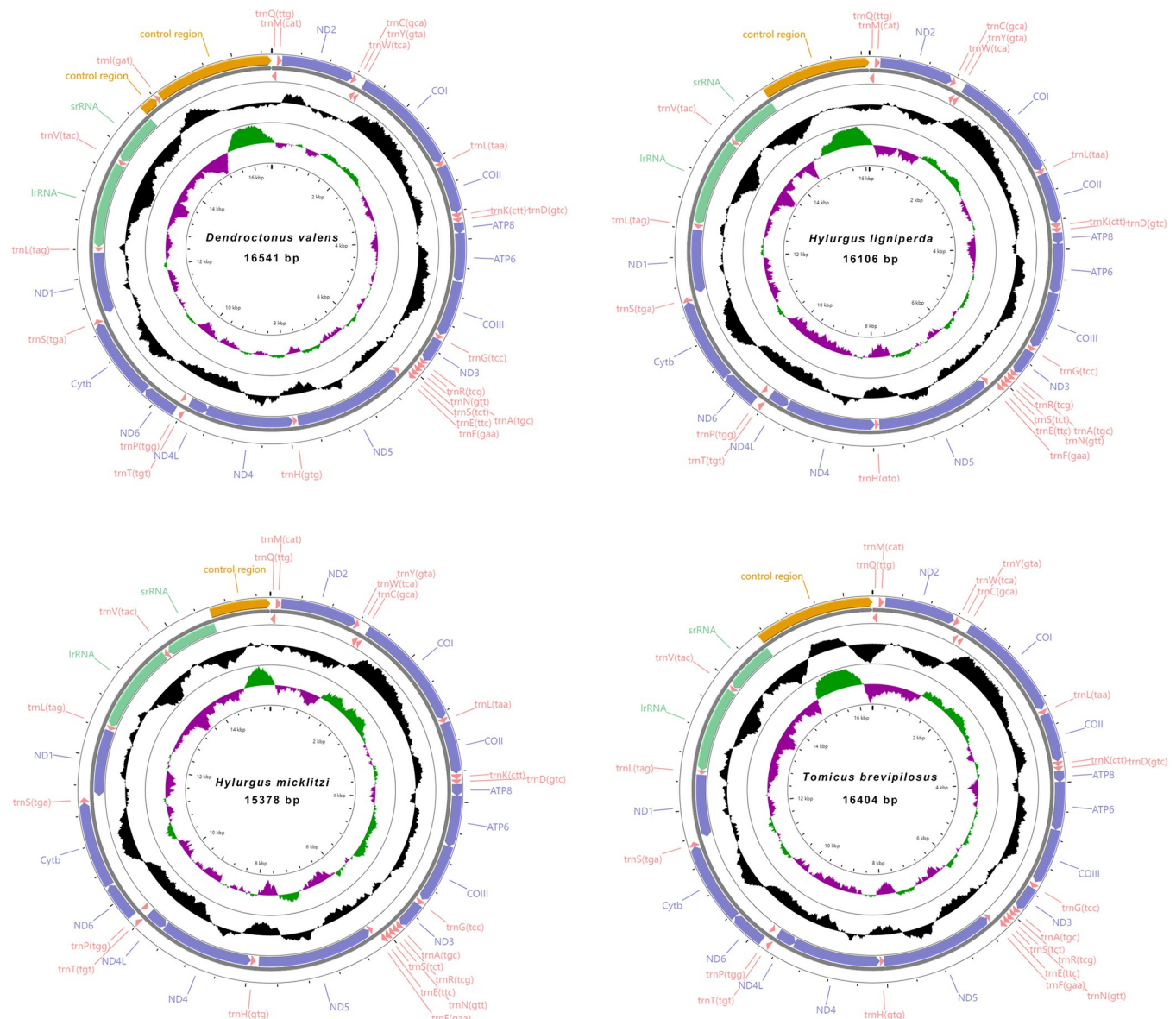


Fig 3. Mitochondrial genome circular maps of *Dendroctonus valens*, *Hylurgus ligniperda*, *Hylurgus micklitzi* and *Tomicus brevipilosus*.

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mitochondrial genomes sequenced in this study. *Cytb* of *T. brevipilosus* and *H. micklitzi*, *ND4L* of *H. ligniperda*, *ND1* of *T. yunnanensis* and *D. valens*, *ND3* of *H. micklitzi*, *D. valens* and *T. minor* all had TAG as the stop codon. *ND5* of *H. ligniperda* was terminated by TA residue. In contrast, the remaining PCGs including *COII*, *ND5*, *ND4* and *ND1* appeared to terminate with a T residue in seven species. For example, *ND5*, *ND4* and *ND1* of *H. micklitzi*, *ND5*, *ND4* of *D. valens*.

The AT content of PCGs was 71.6% to 77.9%. Contents of the third codon position AT *H. micklitzi*, *H. ligniperda*, *T. minor*, *T. yunnanensis* and *T. piniperda* (77.4% to 81.8%) were higher than those of the first (70% to 77.5%) and the second (69.5% to 78.3%) codon positions. In contrast, *D. valens* and *T. brevipilosus* had higher AT content at the first codon position (73% and 79.2%) than at the second (71.3% and 72.3%) and third (70.6% and 77.2%) codon

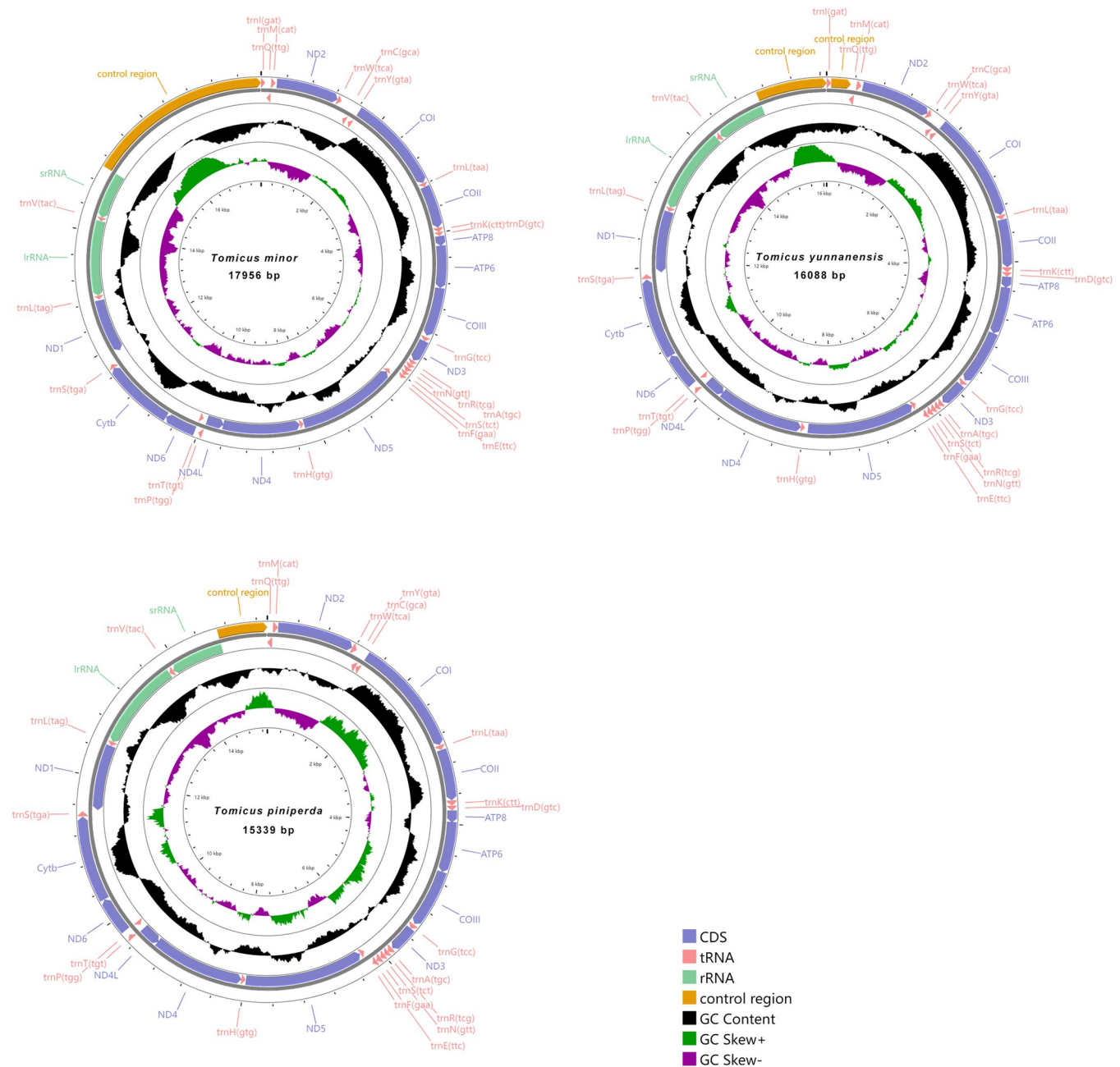


Fig 4. Mitochondrial genome circular maps of *Tomicus minor*, *Tomicus yunnanensis* and *Tomicus piniperda*.

<https://doi.org/10.1371/journal.pone.0313448.g004>

positions. The AT-skew range of PCGs for the seven species was -0.152 to -0.129 , and the range of GC-skew was -0.067 to -0.008 .

In addition, the relative synonymous codon usage (RSCU) analysis results showed that in the mitochondrial genome PCGs of seven Hylurgini species, there were 22 codon families containing 62 codons (excluding two stop codons). The codon families encoding amino acids Ala, Arg, Gly, Pro, Ser2, Thr, and Val are frequently used in all seven mitochondrial genomes (Fig 5). Among the seven mitochondrial genomes, UUU (Phe), UUA (Leu), CCU (Pro), GUU (Val), GCU (Ala), CGA (Arg), ACU (Thr), GGA (Gly) were the most frequently used. The five

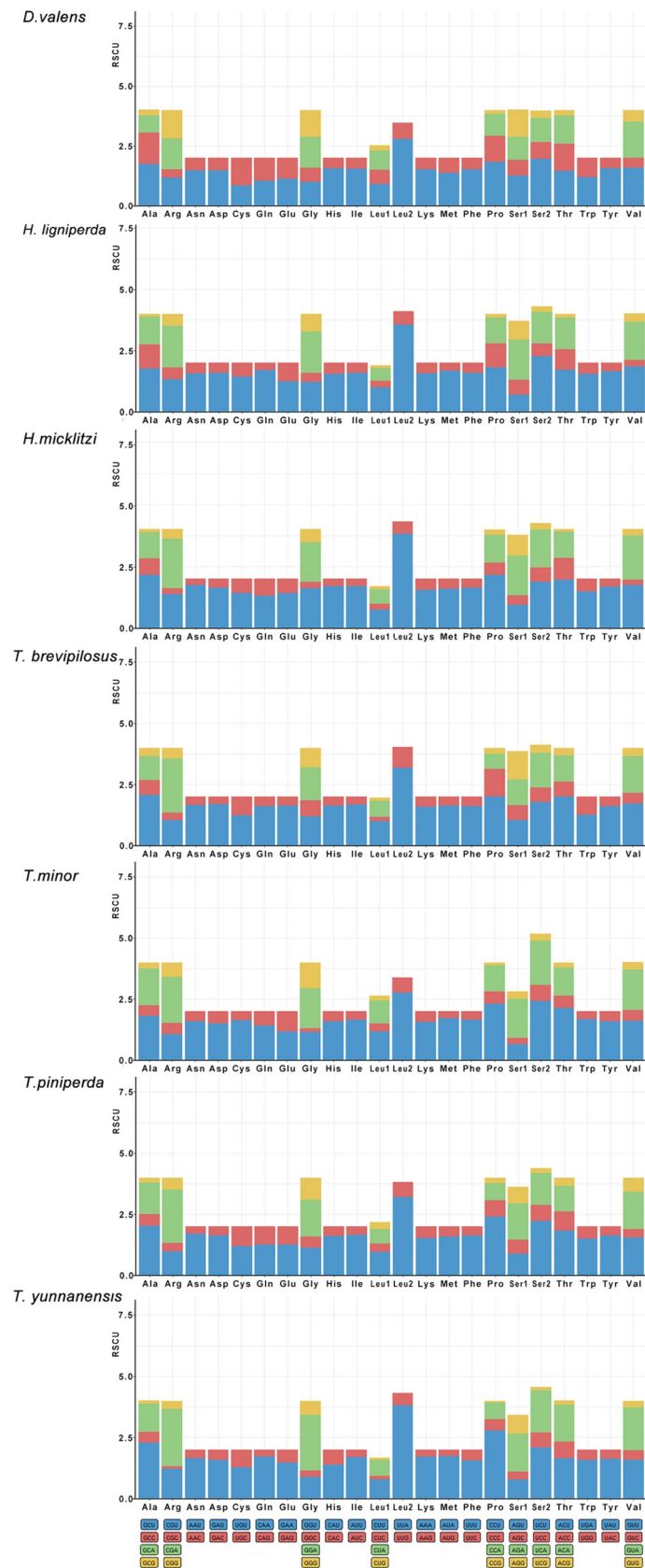


Fig 5. The relative synonymous codon usage (RSCU) in the mitochondrial genomes of seven Hylurgini species.

<https://doi.org/10.1371/journal.pone.0313448.g005>

most frequent codons make up 10.94% to 15.16% of all PCG codons. The least frequently used codon was the CCG (Pro) codon of *T. yunnanensis*, with an RSCU of 0.07.

Compared with other synonymous codons, A or T ranked third in the double and quadruple degenerate synonymous codons were overused. In this study, there was a preference for codons, except CCU (Pro) (1.14), AGG (Ser1) (1.15), and GGG (Gly) (1.06), all of which ended in A or T (U). This suggests that, in addition to the effect of selection on the bias of codon usage, the mutation pressure at the third codon position from GC to AT is higher than in the opposite direction (AT to GC), as well as at the first and second codon positions [71]. In addition, the five most frequently used codons in each of the seven mitochondrial genomes were not identical. For example, the five codons with the highest frequency (maximum RSCU value) of *D. valens* and *H. ligniperda* were UUA(Leu), CCU(Pro), UCU(Ser2), GUU(Val), and GCU(Ala), that of *H. micklitzi* and *T. brevipilosus* were UUA (Leu), CCU (Pro), CGA (Arg), ACU (Thr) and GCU (Ala), and that of *T. minor* were UUA (Leu), CCU (Pro), UCU (Ser2), CGA (Arg) and ACU(Thr). It could be seen that different species of the same family may have different preferences for using synonymous codons. However, the specific situation needs further analysis.

3.3 Synonymous substitution rate and the non-synonymous substitution rate of mitochondrial protein-coding genes

To determine the molecular sequence evolution mechanism of Hylurgini, we also analysed the Synonymous substitution rate (dS) and the Non-synonymous substitution rate (dN) of each PCGs (Fig 6). The results showed that all PCGs of seven mitochondrial genomes evolved under purification selection ($dN/dS < 1$). On the one hand, the dN/dS values of all PCGs were very low, ranging from 0.0021 to 0.4001, indicating strong purification selection. The *ATP8* ($dN/dS = 0.3703$) of *H. micklitzi* vs. *D. valens* and the *ATP8* ($dN/dS = 0.4001$) of *H. ligniperda* vs. *D. valens* were closer to 1, indicating that the purification selection of this gene was looser. In the Hylurgini, the purified selected genes were sorted by decreasing selection intensity (increasing dN/dS value) as follows: *COI* > *COII* > *Cytb* > *COIII* > *ATP6* > *ND5* > *ND1* > *ND3* > *ND4* > *ND4L* > *ND2* > *ND6* > *ATP8*. None of the PCGs had $dN/dS > 1$, indicating that Hylurgini PCGs were mainly influenced by purification selection in the evolutionary process, while *ATP8* encountered relatively small purification selection pressure. HeatMap shows that *ND2*, *ND4L* and *ND6* were under loose purification selection except for *ATP8* (Fig 7). While CO genes (*COI*, *COII* and *COIII*) were under relatively strong purification selection pressure. In conclusion, purification selection would eliminate harmful mutations in the population, and all PCGs of the Hylurgini can be used for our phylogenetic analysis.

3.4 Transfer and ribosomal RNA genes of mitogenome

A total of 22 tRNA genes were annotated by MITOS2. The length of tRNA genes ranged from 60 bp (*tRNA^{Cys}* of *D. valens*) to 71 bp (*tRNA^{Lys}* of *D. valens*, *H. micklitzi*, *T. minor*, *T. brevipilosus*, *T. yunnanensis* and *T. piniperda*). The average length of t-RNA is 64 bp. The secondary structure of 21 tRNA genes was typical of the cloverleaf, and only the *tRNA^{SER(AGN)}* was atypical in all seven Hylurgini, lacking a dihydrouracil (DHU) arm (Fig 8).

In addition, the secondary structure prediction results showed that unmatched base pairs occurred on the stem of some tRNA genes. The mismatched pairs of G-U occurred on 11 tRNA genes, a total of 84 pairs. *H. ligniperda* had the most mismatches and *T. brevipilosus* had the least. The G-U mismatched pairs of *tRNA^{Ala}*, *tRNA^{Phe}*, *tRNA^{His}*, *tRNA^{Leu(CUN)}*, and *tRNA^{Val}* occurred on acceptor stems. The mismatch pairs of *tRNA^{Gln}*, *tRNA^{Tyr}*, *tRNA^{Phe}* and

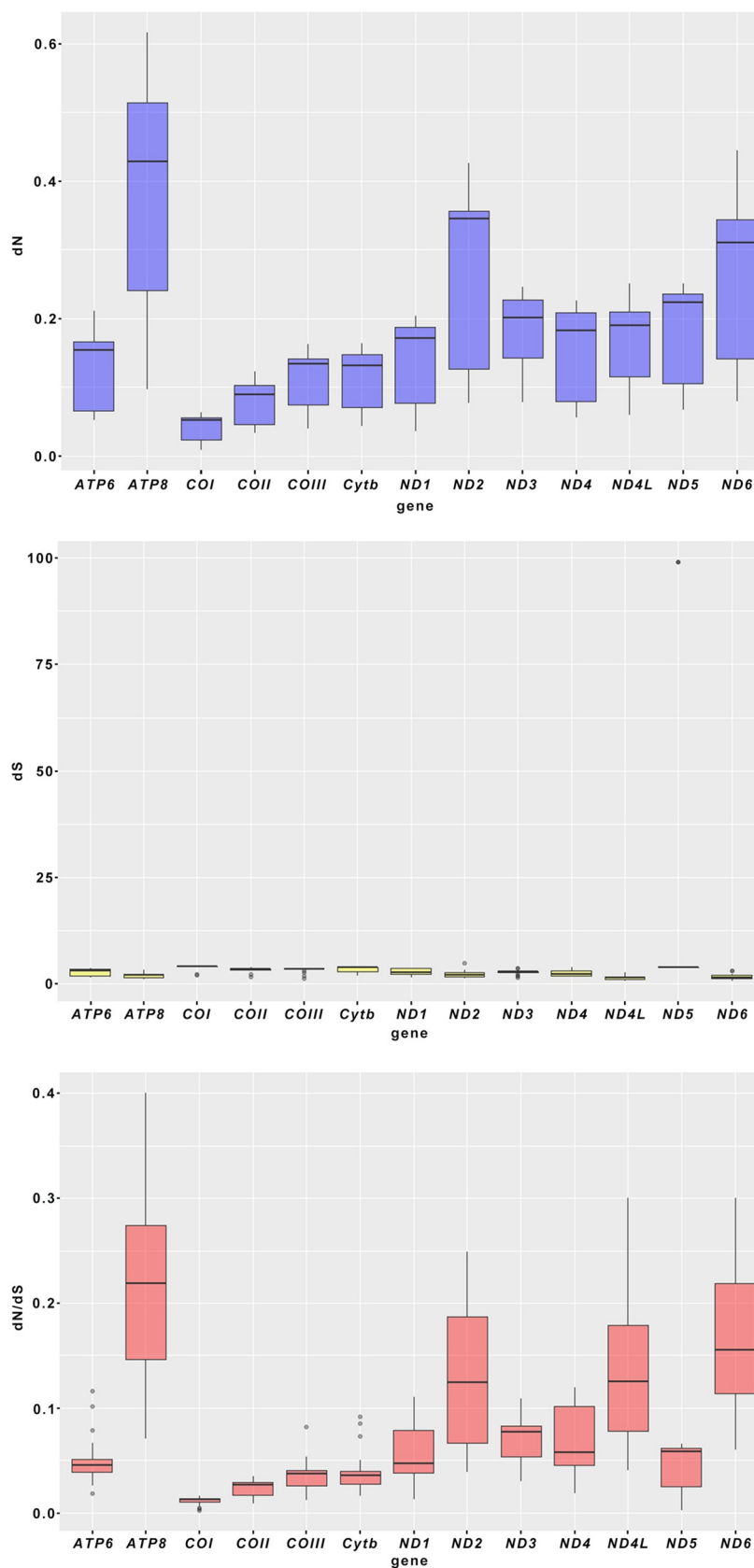


Fig 6. The dN, dS, and dN/dS values of 13 PCGs in the mitochondrial genomes of Hylurgini species.

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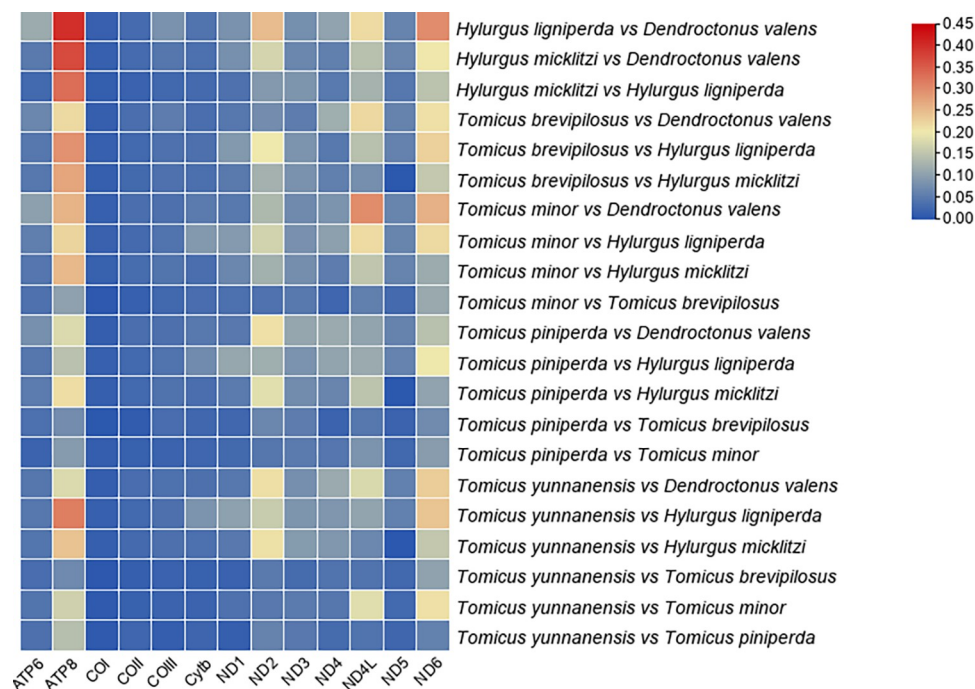


Fig 7. The HeatMap of 13 PCGs in the mitochondrial genomes of Hylurgini species.

<https://doi.org/10.1371/journal.pone.0313448.g007>

$tRNA^{Pro}$ occurred on DHU arm, $tRNA^{Ile}$ on the anticodon arm, and $tRNA^{Trp}$, $tRNA^{Ser(AGN)}$ occurred on TΨC stem (Fig 8).

The two rRNA, *srRNA* and *lrRNA* genes, were on the N-strand. The length of the *lrRNA* ranged from 1,283 bp to 1,301 bp. The shortest was *H. micklitzi* and the longest was *T. yunnanensis*. The length of *srRNA* ranged from 765 bp to 782 bp. The shortest was *H. micklitzi* and the longest was *T. minor*. The AT content of *lrRNA* ranged from 80.6% to 82.5%, which was higher than that of *srRNA* (79.3% to 80.6%). All of them had high A + T preference.

3.5 Phylogenetic analysis

We performed a phylogenetic analysis of 13 PCGs from 37 mitochondrial genomes (from 11 tribes of the subfamily Scolytinae). The same topologies were presented by constructing phylogenetic trees from two methods, likelihood (ML) and Bayesian inference (BI) (Figs 9 and 10). In the Bayesian inference, the tribe of Scolytini, Hylastini, Hylurgini, Xyloterini, Ipini, Xyleborini and Corthylini showed high bootstrap values. In the likelihood analysis, the tribe of Scolytini, Hylastini, Xyloterini, Ipini, Corthylini and Hylurgini showed high bootstrap values. These results allowed better support for the monophyly of the tribe and genera of our studied species. The results of the two methods showed consistent phylogenetic relationships. Scolytini was the first to separate from the other groups and clustered as a monophyletic branch. *Phloeosinus perlatus* of the tribe Phloeosinini and *Polygraphus poligraphus* of the tribe Polygraphini clustered as a monophyletic branch. The Hylastini and Hylurgini are monophyletic. Cryphalini and Xyloterini clustered into a monophyletic clade. Ipini with Dryocoetini, and Xyleborini formed a monophyletic lineage with Ipini sister to Dryocoetini and Xyleborini. *Gnathotrichus materiarius* and *Pityophthorus pubescens* of the tribe Corthylini formed a monophyletic branch. These results were consistent with morphological and single gene/multiple genes of the mitochondrial genomic PCGs tree [72].

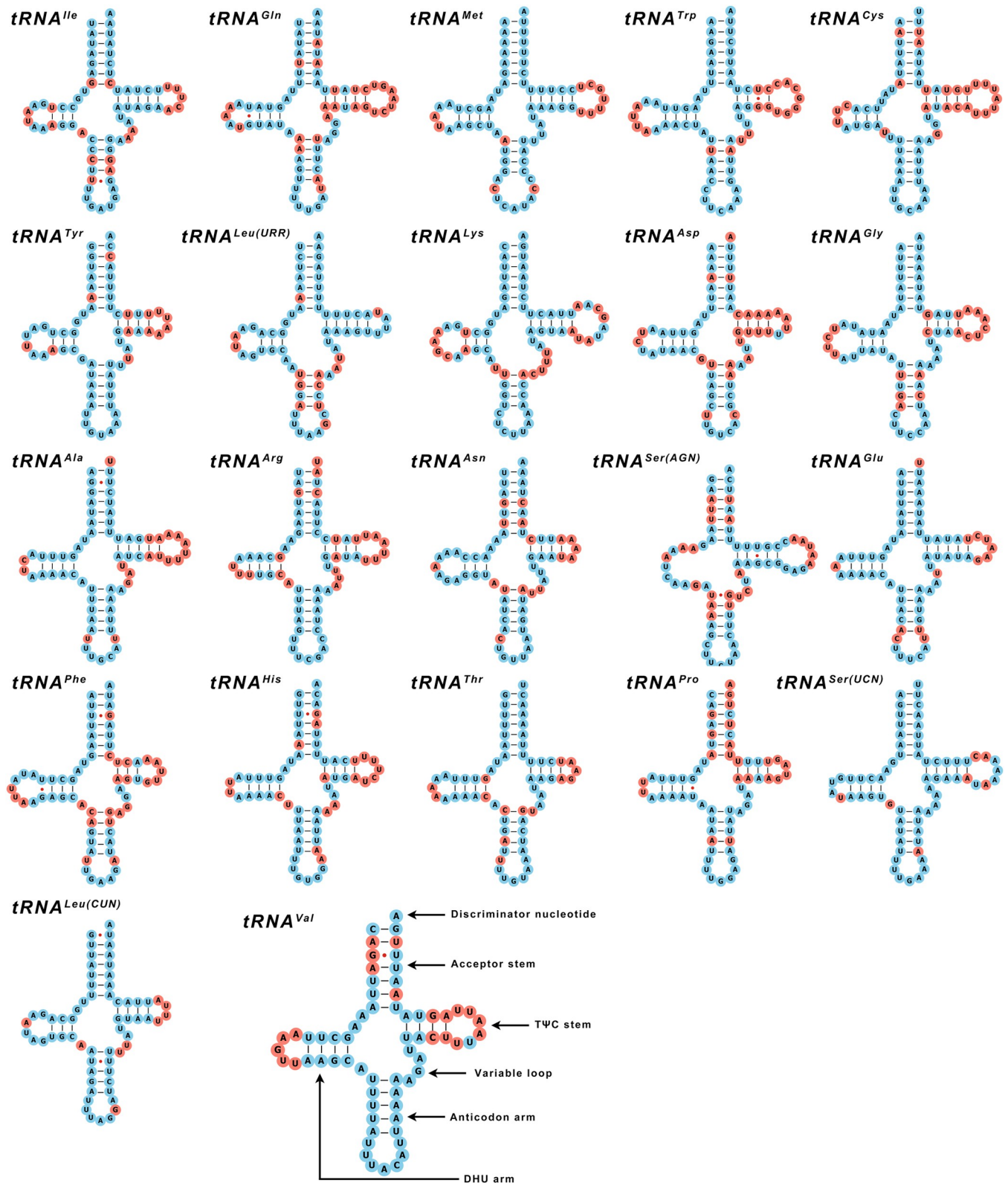


Fig 8. Secondary structure of 22 tRNA genes in the mitochondrial genome from seven species of Hylurgini. Conserved sites and mutation sites were indicated in blue and red, respectively, and mismatched base pairs were marked with red solid dots.

<https://doi.org/10.1371/journal.pone.0313448.g008>

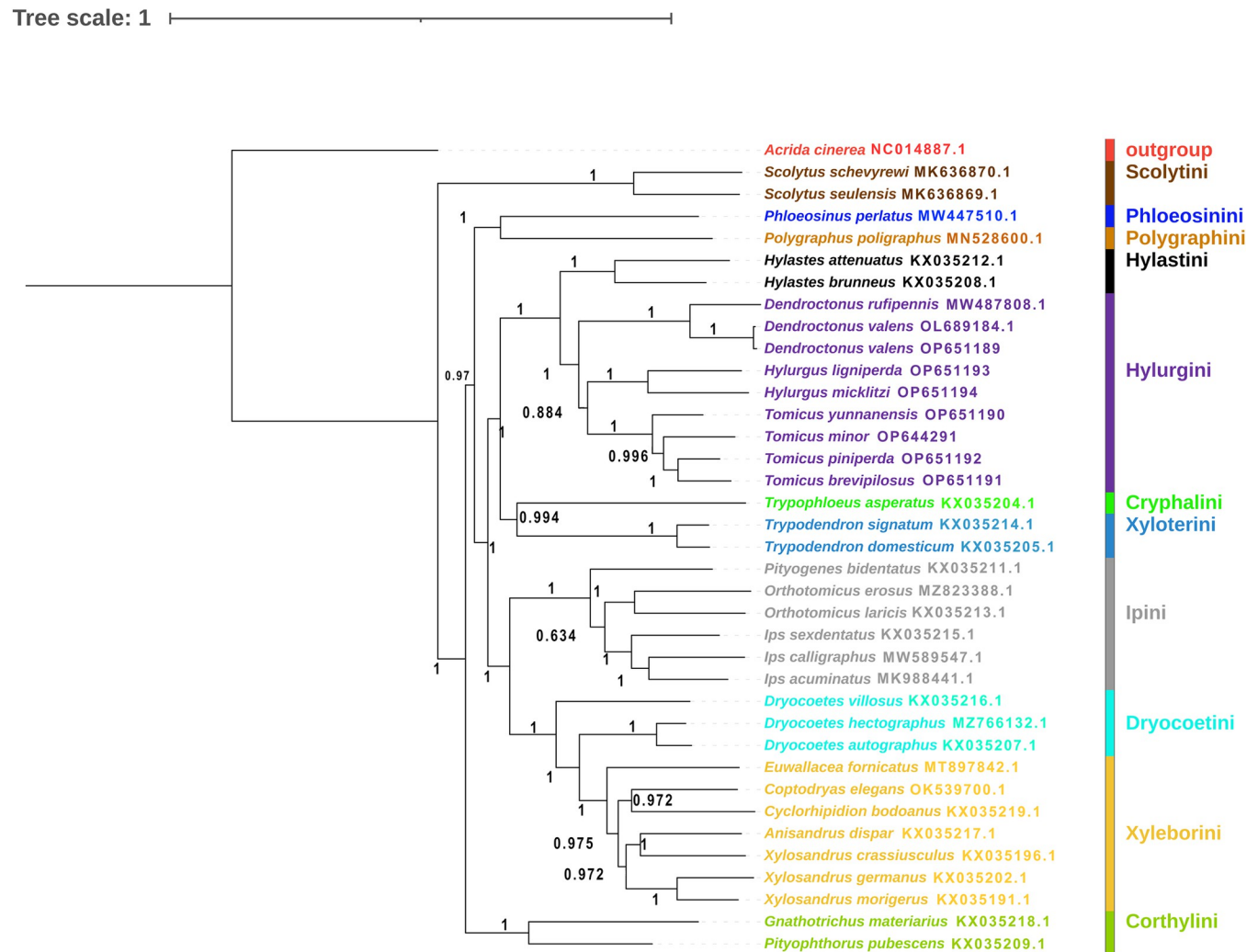


Fig 9. A phylogenetic tree constructed based on 13 PCGs of the mitochondrial genomes. The numbers at nodes indicate Bayesian posterior probabilities.

<https://doi.org/10.1371/journal.pone.0313448.g009>

4. Discussion

4.1 Mitochondrial genomes comparison between Hylurgini and the other tribes of Scolytinae

The seven mitochondrial genomes newly sequenced were shorter than those of their sister groups published to NCBI (S2 Table). The mitochondrial genome structure of the seven bark beetles, as determined through our assembly and annotation, was congruent with previously known species of the tribes Phloeosinini and Ipini [72]. The mitochondrial genome structure was identical to that of ancestry insects [33]. All 22 tRNA genes of *T. minor*, *T. yunnanensis* and *D. valens*, were successfully annotated by MITOS2. However, *tRNA^{Ile}* of *T. brevipilosus*, *T. piniperda*, *H. ligniperda* and *H. micklitzi* were not annotated. The proximity of *tRNA^{Ile}* to the regulatory region and its abundance of A and T bases restricts Illumina sequencing runs, resulting in the detection of only 36 genes without comprehensive sequencing. The content of A + T of Hylurgini ranged from 73.24% to 78.81%, the content of *D. valens* was the lowest, and *H. micklitzi* was the highest, showing an obvious AT bias. In general, the species of Hylurgini

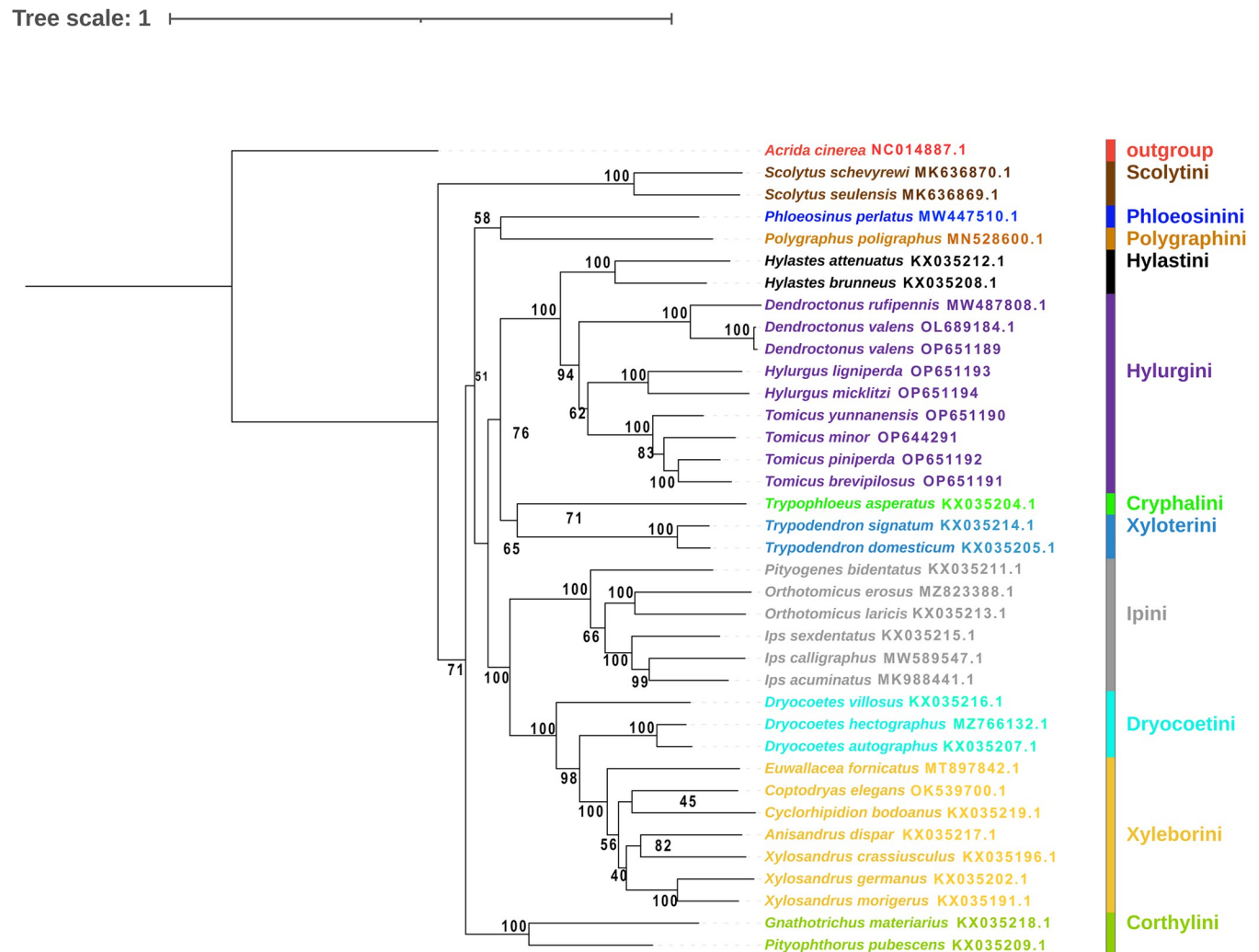


Fig 10. A phylogenetic tree constructed based on 13 PCGs of the mitochondrial genomes. The numbers at nodes indicate ML bootstrap support values.

<https://doi.org/10.1371/journal.pone.0313448.g010>

had a low content of A + T compared with other species of Scolytinae (S2 Table). The AT-skew of the whole mitochondrial genome of the seven species was positive, indicating that there were more bases A than C. The GC-skew was negative, indicating that the content of G in the mitochondrial genome of Hylurgini was less than C, which was consistent with the results of other published Scolytinae [72].

In the 13 PCGs of seven mitochondrial genomes, TCG and TTG were also used as the start codons in addition to the standard ATN start codon. The *COI* gene of *T. minor* was taken TCG as the start codon, while the mitochondrial genomes of other bark beetles have not been reported, but the *COI* gene of other insects, such as Diptera, has the same occurrence [73, 74]. TTG was the start codon of the *ND1* of *T. yunnanensis*, *T. brevipilosus*, *H. micklitzi* and *T. piniperda*, which also occurs on the *ND1* gene of the genus *Ips* [72]. This study also discovered stop codons, including TAG, T residues, and TA residues, in addition to TAA. TAG was the stop codon of *Cytb*, *ND4L*, *ND1* and *ND3*, which also occurred in the PCGs of the published species of the Scolytinae [72, 75]; *ND5* of *H. ligniperda* was terminated by TA residue. *COII*, *ND5*, *ND4* and *ND1* all are terminated with a T residue. This is also consistent with the mitochondrial genome of other published bark beetles [72].

The total AT content of the 13 PCGs ranged from 71.6% to 77.9%, higher than the average AT content of the 29 published mitochondrial genome PCGs we referred to: 70.37% (S2 Table). We also compared our nucleotide composition of the codon position of the concatenated PCGs with other published related species. The results of *H. micklitzi*, *H. ligniperda*, *T. minor*, *T. yunnanensis*, and *T. piniperda* were consistent with the published related species such as *Hylastes* and *Ips*. The content of the third codon positions was higher than that of the first and the second codon positions (S2 Table). The AT-skew and GC-skew values of PCGs of seven mitochondrial genomes were negative, indicating that the number of base T was greater than A. The number of base C was greater than G in PCGs, which was consistent with the published results of the tribe Ipinini and Hylastini [72].

RSCU is the ratio between the frequency at which a codon is observed and the expected frequency at which the codon of any particular amino acid is equally used [76]. Thus, RSCU directly exhibits a using deviation between synonymous codon and uniform. RSCU = 1 indicates that the codon is randomly used (unbiased), RSCU > 1 suggests that the codon is used more frequently (biased), and RSCU < 1 indicates that the codon is used less frequently [77]. In the seven mitochondrial genomes, the number of synonymous codons with high using frequency was 26 (*T. yunnanensis* and *T. piniperda*), 28 (*H. micklitzi*, *T. brevipilosus* and *H. ligniperda*), and 30 (*T. minor*), 32 (*D. valens*), indicating a preference for these codons in their respective species. This indicates that species preferentially select optimal synonymous codons, as some chosen codons are more frequently found at conserved places [78].

Only 21 tRNA genes were detected in four of the seven sequenced mitochondrial genomes, and the missing gene was *tRNA^{Ile}*. This phenomenon has also occurred in other species of Scolytinae [79]. The loss of *tRNA^{Ile}* is due to the sequencing method. The second-generation sequencing sometimes fails to detect the AT-rich area, resulting in the loss of the gene near the AT-rich area. In the insect mitochondrial genome, *tRNA^{SER(AGN)}* usually misses the DHU arm rather than the classic cloverleaf structure. This phenomenon has also been observed in the mitochondrial genomes of other species of Scolytinae, e.g., the genus of *Ips* [72]. According to the results, the base mismatch in the secondary structure of tRNA mainly occurred in the DHU arms, and little in the Anticodon arms. Consequently, we can infer that various regions of tRNA exhibit distinct conservative traits, with the Anticodon arm being the most conserved. In addition, most mutation sites were located on the ring, while fewer were on the arm, indicating that the stem was more conservative.

4.2 Phylogeny of Hylurgini

The phylogenetic relationships among some taxa of Scolytinae were still unclear. For example, the tribe Dryocoetini was shown to be paraphyletic, with *D. villosus* being sister to the other *Dryocoetes* species and the tribe Xyleborini. Among Hylurgini, our results showed that all three genera exhibit good monophyly. In the genus *Tomicus*, *T. brevipilosus*, *T. piniperda* and *T. minor* clustered into a monophyletic branch, which sister to *T. yunnanensis*. The *T. brevipilosus* and *T. piniperda* clustered into a monophyletic branch, separated from *T. minor*.

Hylurgini was sister to the tribe Hylastini in our phylogenetic analysis, aligning closely with the classification based on morphological characteristics [9]. The position of *Dryocoetes villosus* was undecided. Jordal [80] and Pistone [81] also indicated that the genus *Dryocoetes* was not monophyletic by molecular phylogeny and constitutes at least two sister groups, which requires a revision of it.

The genetic distance data of 37 species of beetles calculated by Mega 11 are shown in S3 Table. *T. piniperda* is comparatively more distantly related to *H. ligniperda* in the *Hylurgus* genus. *H. ligniperda* is more closely related to *T. yunnanensis*, a member of the *Tomicus* genus.

T. minor is the most distantly related to *T. yunnanensis* and is distantly related to three other beetle species in the same genus. The results were not consistent with the phylogenetic results. In the phylogenetic results, *T. yunnanensis* was the most distant from the other three species of the same genus. *T. brevipilosus* is closer to *T. piniperda* than *T. yunnanensis* and *T. minor*. *D. valens* is most closely related to *T. piniperda* in the genus *Tomicus* and *Hylurgus*, and most distantly related to *H. ligniperda*. Based on the analysis of the aforementioned results, it is possible to tentatively conclude that the Hylurgini beetles located in the same region are not closer to the genetic distance. Although their ecological niches are much closer, they have no obvious connection. However, since we only discussed a limited number of data of the Hylurgini, there is no evidence of other tribes, so further in-depth research is needed.

5. Conclusion

We analysed the structure and base composition, relative synonymous codon usage of protein-coding genes, secondary structure of tRNAs and synonymous substitution rate of the mitochondrial genomes of seven bark beetles. The conserved structure of the subfamily Scolytinae's mitochondrial genome may help us elucidate the phylogenetic relationships at a relatively high taxonomic level. We constructed a phylogenetic tree based on its mitochondrial genome data. The phylogenetic analysis was conducted to understand the evolutionary relationships within the Hylurgini tribe and Scolytinae. The taxonomic position of Hylurgini is further clarified, as well as its relationship to other sister groups. The mitochondrial genome of Hylurgini will facilitate our search for more reliable regions to develop molecular markers, and not just the commonly used *COI* genes. The complex co-existence phenomenon of the Hylurgini is present not only in the same genus but also between different genera (native and non-native). This phenomenon complicates the identification of bark beetles further. The novel molecular markers will facilitate the challenging species identification of the Hylurgini.

Supporting information

S1 Table. The species information from GeneBank used in the article.
(DOCX)

S2 Table. Base composition of mitochondrial genomes of 37 species.
(XLSX)

S3 Table. Genetic distance of 37 species of beetles.
(XLS)

S4 Table. Organization of the mitochondrial genome of *Dendroctonus valens*.
(DOCX)

S5 Table. Organization of the mitochondrial genome of *Hylurgus ligniperda*.
(DOCX)

S6 Table. Organization of the mitochondrial genome of *Hylurgus micklitzi*.
(DOCX)

S7 Table. Organization of the mitochondrial genome of *Tomicus brevipilosus*.
(DOCX)

S8 Table. Organization of the mitochondrial genome of *Tomicus piniperda*.
(DOCX)

S9 Table. Organization of the mitochondrial genome of *Tomicus minor*.
(DOCX)

S10 Table. Organization of the mitochondrial genome of *Tomicus yunnanensis*.
(DOCX)

S11 Table. The data of dN, dS and dN/dS boxplot.
(XLSX)

S12 Table. The data of heatmap.
(XLSX)

S13 Table. World distribution of seven Hylurgini species.
(DOCX)

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Author Contributions

Data curation: Na An.

Formal analysis: Na An.

Writing – original draft: Na An.

Writing – review & editing: Yuan Yuan, Sixun Ge, Xudong Zhang, Lili Ren, Alain Roques, Youqing Luo.

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Chapter 3: Comparative phylogeographic patterns of two congeneric *Hylurgus* species

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Chapter 3: Modèles phylogéographiques comparatifs de deux espèces congénériques de *Hylurgus*

Résumé en français du contexte, des objectifs de l'étude et des méthodes utilisées

Résumé

Dans la région paléarctique occidentale, les scolytes congénériques *Hylurgus ligniperda* et *Hylurgus micklitzi* se développent sur les pins. Bien que *H. ligniperda* possède une aire de répartition plus vaste en Europe, les deux espèces coexistent souvent en sympatrie dans le bassin méditerranéen. Elles partagent des histoires de vie relativement similaires, ce qui en fait un excellent modèle pour des études comparatives sur la différenciation des espèces et les traits écologiques. De plus, avec l'intensification des activités humaines et du commerce international, *H. ligniperda* a envahi tous les continents, tandis que *H. micklitzi* reste limité au bassin méditerranéen. Notre étude a utilisé des marqueurs COI pour évaluer la diversité et la structure génétique ainsi que l'histoire démographique des deux espèces, révélant des différences marquées. *H. micklitzi* présente une faible diversité génétique et des structures de population peu profondes avec une expansion restreinte. En revanche, *H. ligniperda* montre une histoire évolutive postglaciaire plus longue et plus complexe, avec quatre clades distincts issus de refuges glaciaires séparés, accompagnée d'une variabilité génétique importante à travers l'Europe. Les analyses comparatives entre les deux espèces suggèrent que certains traits, comme une taille corporelle plus grande, un nombre de générations plus élevé (voltinisme), une niche écologique plus large, et des introductions fréquentes, pourraient avoir facilité l'invasion rapide et la dispersion mondiale de *H. ligniperda* au cours du siècle dernier.

Mots-clés: COI, phylogéographie comparative, structure génétique, *Hylurgus ligniperda*, *Hylurgus micklitzi*, invasivité, traits d'espèce.

Research Article

One is a worldwide invader, the other never established in other continents: contrasted phylogeographic patterns of two congeneric *Hylurgus* conifer bark beetles

Yuan Yuan^{1,2,3}, Alain Roques^{1,3}, Emmanuelle Magnoux^{1,3}, Lili Ren^{3,4}, Eckehard G. Brockerhoff⁵, Juan Shi^{3,4}, Youqing Luo^{3,4}, Na An^{3,4}, Marie-Anne Auger-Rozenberg^{1,3}, Géraldine Roux^{1,2,3}

1 INRAE, Zoologie Forestière, Orléans, 45075, France

2 Laboratoire Physiologie, Ecologie et Environnement P2E, Université d'Orléans, 1 rue de Chartres, B.P. 6759, 45067 Orléans cedex 2, France

3 Sino-France Joint Laboratory for Invasive Forest Pests in Eurasia, Beijing Forestry University, Beijing, 100083, China

4 Beijing Key Laboratory for Forest Pest Control, Beijing Forestry University, Beijing, 100083, China

5 Swiss Federal Institute for Forest, Snow and Landscape Research WSL, CH-8903 Birmensdorf, Switzerland

Corresponding authors: Marie-Anne Auger-Rozenberg (marie-anne.auger-rozenberg@inrae.fr); Géraldine Roux (geraldine.roux@univ-orleans.fr)



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Abstract

In the Western Palaearctic, the congeneric bark beetles *Hylurgus ligniperda* and *Hylurgus micklitzi* are developing on pine trees. Although *H. ligniperda* has a larger native range in Europe, both species often occur in sympatry in the Mediterranean Basin. They share rather similar life histories, thus providing an excellent model for comparative studies on species differentiation and ecological traits. Moreover, along with increasing human activities and international trade, *H. ligniperda* has invaded all continents, whereas *H. micklitzi* remains confined to the Mediterranean Basin. Our research utilised COI markers to assess the genetic diversity and structure, alongside the demographic history, of both species and revealed marked differences. *H. micklitzi* exhibited low genetic diversity and shallow population structures with restricted expansion. In contrast, *H. ligniperda* displayed a longer and more complex post-glacial evolutionary route with four distinct clades originating from separate glacial refugia, coupled with considerable genetic variability across Europe. Comparative analyses about the two species suggest that species traits, such as larger body size, increased voltinism, broader ecological niches and frequent introduction events may have helped the fast global invasion and spread of *H. ligniperda* over the past century.

Key words: COI, comparative phylogeography, genetic structure, *Hylurgus ligniperda*, *Hylurgus micklitzi*, invasiveness, species traits

Introduction

Globalisation has dismantled biogeographical barriers, accelerating the introduction of non-native insect species at an unprecedented rate over the past century without any sign of saturation for the future (Ricciardi 2007; Seebens et al. 2018). Plant trade has emerged as the predominant pathway for insect introductions (Liebhold et al. 2012; Fenn-Moltu et al. 2023), facilitating the establishment of numerous non-native species populations (Blackburn et al. 2011). When some of these species have become invasive, they pose significant threats to ecological

* These authors contributed equally to this work.

systems and native biodiversity (CBD 2000). Additionally, they are responsible for substantial agricultural and forestry losses, incurring considerable economic costs related to their management and control (Ricciardi 2007; Bonnamour et al. 2021).

While some species successfully establish invasive populations across new regions, others, despite their phylogenetic and similar biological and ecological traits, remain confined to their native habitats. Comparing traits of related species with such differences would, thus, provide an opportunity to explore the mechanisms underlying the invasion processes. For instance, genomic comparisons of two congeneric species of *Spodoptera* (Lepidoptera, Noctuoidea) revealed potential factors contributing to the fall armyworm's successful invasion (Cui et al. 2020). Furthermore, Zhao et al. (2023) conducted a meta-analysis comparing traits of 100 invasive insects (including 25 Hemipterans, 23 Coleopterans, 18 Lepidopterans, 15 Hymenopterans and 12 Dipterans) with those of their non-invasive relatives, finding many differences in the number of introduction pathways, the number of habitats, life history traits, natural enemies and genomic traits. Compared to its congener *Dendroctonus ponderosae*, the invasive bark beetle *Dendroctonus valens* has a larger body size (Zhang et al. 2002; Pureswaran and Borden 2003; Shi and Sun 2010), more annual generations (Smith 1971; Bentz and Powell 2014), more and broader habitats (Smith 1971; Wood 1982; Salinas-Moreno et al. 2004), a larger genome size (Keeling et al. 2013; Liu et al. 2022) and a more complex phylogeographic pattern (Cai et al. 2008; Cullingham et al. 2012; Samarasekera et al. 2012; Taerum et al. 2016; Janes et al. 2018; Liu et al. 2022) in native areas.

Comparative phylogeography, which benefits from the improved integration of numerous complementary disciplines, including genetics, ecology, palaeontology, palynology and climatology, can identify geographical distributions and evolution of genetic lineages in sympatric species (Taberlet et al. 1998). On a large scale, the general trends across one certain region could be analysed and elucidated by comparing over the same area intraspecific phylogeographic patterns of several taxa (e.g. birds, reptiles, crustaceans etc.), including glacial refugia, post-glacial colonisation routes, presence of geographic barriers, main suture-zones and others (Avice et al. 1987; Bermingham et al. 1992; Taberlet et al. 1998). On a smaller scale, comparative phylogeography of species in sympatry could infer general or special patterns of evolutionary history associated with specific regions and their biotic or abiotic characteristics (Bermingham and Moritz 1998; Avice 2009; Mayer et al. 2015). Due to its rapid mutation rate and short coalescence time, mitochondrial DNA (mtDNA) has obvious advantages in determining a species' genetic diversity and phylogeographic structure. MtDNA sequences are the most popular genetic markers for phylogeographic research because they allow the application of phylogenetic tools and the genealogical approach to population-level enquiries (Brito and Edwards 2009). This approach has also been used to compare genetic diversity and population structure in invading populations versus native ones to identify potential source areas of invasion (Javal 2017; Bras et al. 2019; Lesieur et al. 2019; Urvois et al. 2023).

The red-haired bark beetle *Hylurgus ligniperda* J. C. Fabricius (Coleoptera, Curculionidae, Scolytinae) and its sister species *Hylurgus micklitzi* Wachtl constitute an interesting study case. Native to the Western Palaearctic, these phloeophagous pests are often found in sympatry on pine trees, mainly in the Mediterranean parts of their distributions. *H. ligniperda*, a temperate species, has expanded its range to nearly all continents (CABI 2021; Brockerhoff et al. 2025b), contrasting

sharply with *H. micklitzi*, which remains confined to the Mediterranean Basin without any records of introduction so far (El Khoury et al. 2019). Brockerhoff et al. (2014) used 13,828 border interception records from the U.S. and New Zealand (1949–2008) for 444 beetle species as proxies for arrival rates to model the arrival-establishment relationship, *H. ligniperda* and *H. micklitzi* at 244.5 and 0.4, respectively. All the scolytids interception data at U.S. ports of entry during 1985–2000 showed 217 interceptions for *H. ligniperda* and a single interception for *H. micklitzi* from five European countries (Haack 2002). Between 2003 and 2016, *H. ligniperda* was the second most frequently intercepted scolytids in ports of China, with 10,199 interceptions (Wang et al. 2018).

Different species' characteristics might explain their different invasiveness to some extent. In the native range, both *Hylurgus* species have been observed to be capable of development on five species of pine trees: *Pinus pinaster* Aiton, *P. pinea* L., *P. halepensis* Mill., *P. brutia* Ten. and *P. nigra* J.F. Arnold (Fabre and Carle 1975; Mendel et al. 1985; El Khoury et al. 2019; Gencal and Sarikaya 2023). Since two bark beetles are exclusively dependent on pines for feeding and reproduction, it could be hypothesised that their respective phylogeographic distribution, glacial refugial areas and further population expansions are consistent with their main host pines. Besides, the two *Hylurgus* species share similar feeding habits, ecological niches and reproduction systems, but differ in adult body length (*H. ligniperda*: 4–5.7 mm, *H. micklitzi*: 3.5–3.8 mm) (Grüne 1979), voltinism (*H. ligniperda*: 1–4 generations/year, *H. micklitzi*: 1 generation/year) (Fabre and Carle 1975; Mendel et al. 1985; Parra Emilfork 1996), native distribution (*H. ligniperda*: Europe, North Africa, West Asia, *H. micklitzi*: Mediterranean Basin) (El Khoury et al. 2019; CABI 2021), host range in native habitats (*H. ligniperda*: six main pine species, *H. micklitzi*: two main and three minor pine species) (Fabre and Carle 1975; Mendel et al. 1985; El Khoury et al. 2019; Gencal and Sarikaya 2023).

In this study, we hypothesise that differences in genetic diversity in the native range, combined with species-specific biological and ecological traits, have contributed to the distinct invasive capacities of these two congeneric species. Our study aimed to compare the genetic diversity and phylogeographic patterns of the two *Hylurgus* species in their native Western Palaearctic and to discuss whether contrasting species' characteristics between *H. ligniperda* and *H. micklitzi* would have influenced their respective success or failure in invasion.

Materials and methods

Insect sampling

From 2013 to 2024, adults of *H. ligniperda* and *H. micklitzi* were sampled from traps deployed in ports-of-entry and pine forests baited with either bark beetle pheromones or cerambycid pheromone blends supplemented with ethanol and α -pinene as described in Roques et al. (2023). Specimens of *H. ligniperda* were obtained from 120 sites located in 10 native European regions (mainland France, Corsica, mainland Italy, Sardinia, Hungary, Czechia, Spain, Portugal, Switzerland and Greece) and nine invaded countries distributed over five continents (Chile, Argentina, China, USA, New Zealand, South Korea, South Africa, Uruguay and Australia). *H. micklitzi* was obtained from 53 sites in seven native regions distributed around the Mediterranean Basin (mainland France, Corsica, mainland Italy, Sardinia, Spain

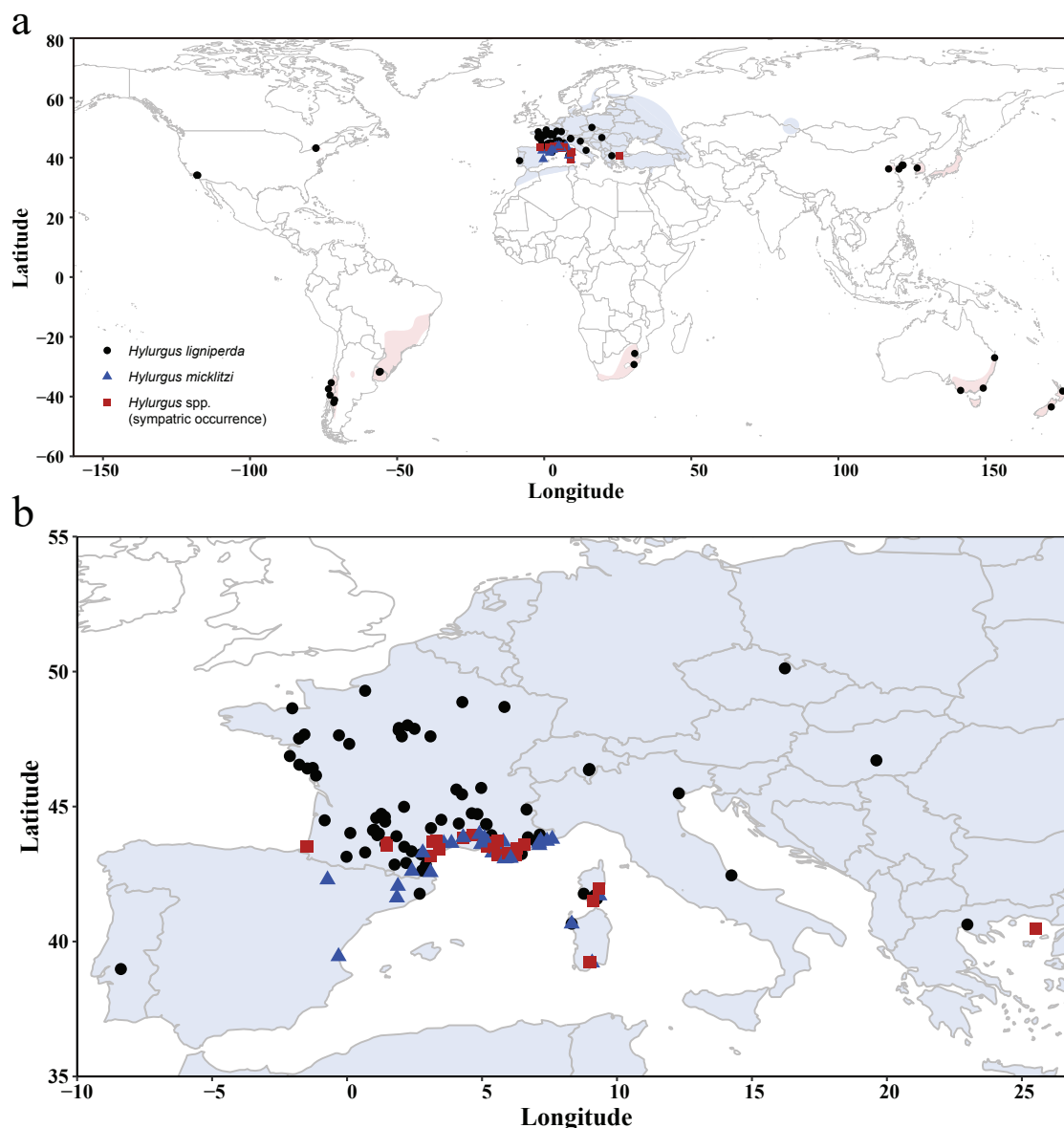


Figure 1. **a.** The sampling sites of *H. ligniperda* and *H. micklitzi* in the world; **b.** The sampling sites of *H. ligniperda* and *H. micklitzi* in Europe. Blue areas represent native ranges and red areas represent invasive ranges according to CABI and previous records for *H. ligniperda* (Parra Emilfork 1996; Kvamme and Lindelöw 2014; El Khoury et al. 2019; Lin et al. 2021; Lantschner et al. 2024; Brockerhoff et al. 2025b).

and Greece). The sampling locations are detailed in Fig. 1 and Suppl. material 1. Individuals were morphologically identified using the key available in Grüne (1979). All samples were stored in 96% ethanol and at -20 °C until DNA extraction.

DNA protocols, amplification and sequencing

For both species, DNA was extracted for 1–10 individuals from each sampling site using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The PCR reaction was amplified via the universal COI primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') for all individuals of two species (Folmer et al. 1994). The PCR was performed as follows: denaturation for 5 min at 94 °C followed by 35 cycles of amplification of 45 s at 94 °C, 50 s at 47 °C, 90 sec at 72 °C and final elongation for 5 min at 72 °C.

In order to check if the individuals were properly assigned to each species, we also amplified and sequenced a section of the conserved D1D3 domain of 28S rDNA from a representative number of specimens (*H. ligniperda*: N = 4; *H. micklitzi*: N = 1), using conserved forward primer D1F (5'-ACCCGCTGAATT'TAAG-CATAT-3') and reverse primer D3R (5'-TAGTTCACCATCTTTTCGGGTC-3') (Harry et al. 1996, Lopez-Vaamonde et al. 2001). The PCR amplification consisted of the denaturation for 5 sec at 95 °C, followed by 35 cycles of 30 sec at 95 °C, 30 sec at 53 °C and 1 min 30 sec at 72 °C.

The purification of amplicons was performed with NucleoSpin gel and a NucleoFast 96 PCR clean-up kit (Macherey-Nagel, Düren, Germany). All sequences were obtained in the forward and reverse directions on an ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific) using the ABI Prism BigDye 3.1 Terminator sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the primers used for PCR amplification. All sequence electropherograms were checked and we created contigs and trimmed all sequences to the same length using Codon Code Aligner 6.0.2 (Codon-Code Corporation, Dedham, MA, USA). DNA sequences were aligned using ClustalW in MEGA11 (Tamura et al. 2021). The alignment was completed with seven COI sequences of *H. ligniperda* available from GenBank, from two sources, including sequences JQ015128–JQ015131 (Shukle et al. 2019) and MZ562956 and MZ562958 (Lin et al. 2021), one sequence of *H. micklitzi* is KM285773 (Rougerie et al. 2015).

Molecular data analyses

All sequences were translated into amino acids to check that no stop codons occurred; to quantify the divergence between haplotypes, the pairwise nucleotide *p*-distances were calculated by MEGA11 (Tamura et al. 2021). A Maximum-Likelihood phylogenetic tree using the Tamura 3-parameter (T92) + G Model was performed with 1,000 bootstraps by MEGA11 (Tamura et al. 2021). *H. micklitzi* was selected as the outgroup for the population genetic analyses of *H. ligniperda* due to its close phylogenetic relationship, while being a distinct, non-invasive congeneric species. Indices of genetic diversity in the studied species (gene diversity and nucleotide diversity per site) were estimated using DNAsp6 (Rozas et al. 2017). To identify groups of the population that were geographically homogeneous and maximally differentiated by a spatial analysis of variance, we used a spatial analysis of variance (SAMOVA 2.0) (Dupanloup et al. 2002). The programme was run for 10,000 permutations from 100 random initial conditions for 2 to 15 differentiated groups (K = 2 to K = 15). A TCS haplotype network, based on statistical parsimony, was built and lineage and haplotype maps were performed using R version 4.5.1 (R Core Team 2024). To infer the demographic histories and detect signs of recent population expansions, we used the frequency-based indicators Tajima's D and Fu's FS statistics available in DNAsp6 (Rozas et al. 2017). Significantly negative Tajima's D and Fu's FS values were taken as evidence of a population expansion.

Results

Interspecific study

The alignment of the 274 and 174 partial COI sequences of *H. ligniperda* and *H. micklitzi*, respectively, revealed 139 polymorphic sites (no deletions nor insertions),

of which 75 appear as diagnostic mutations separating the two species. The alignment of 898 bp sequences belonging to the 28S rDNA gene separated *H. ligniperda* and *H. micklitzi* by 32 polymorphic sites, all of which appeared as diagnostic mutations. In order to confirm the specific status of all specimens identified as *H. ligniperda*, four individuals of *H. ligniperda*, representative of the four main mtDNA haplogroups and one individual of *H. micklitzi*, were sequenced for the 28S rDNA gene. Sequences corresponding to two individuals from Gruissan (France) belonging to HLD07 mtDNA lineage, one from Yantai (China) belonging to HLA01 and one from Lupiac (France) belonging to HLA02 displayed the same 28S genotype, except for one ambiguous site showing a double peak (one from Gruissan belonging to HLD07, one from Yantai belonging to HLA01) at the diagnostic nucleotide. The interspecific genetic distances ranged from 15.9% to 17.5% for the COI gene and ranged from 3.6% to 3.7% for the nuclear gene. All identified haplotypes were deposited in GenBank (accession references PV701517–PV701783 of *H. ligniperda* and PV691892–PV692064 of *H. micklitzi* for the COI gene and PV888900–PV888903 of *H. ligniperda* and PV888956 of *H. micklitzi* for the 28S gene).

Hylurgus ligniperda genetic study

We obtained 46 mtDNA haplotypes from 120 populations and 274 individuals, with 66 variable sites out of the 578 bp sequenced. Overall haplotype and nucleotide diversities were 0.767 and 0.01276, respectively, 0.785 and 0.01226 in the native area and 0.681 and 0.0143 in the invaded area. We found two main haplotypes HLA01 (100 individuals from 45 populations) and HLD07 (86 individuals from 52 populations) and 25 local haplotypes (Suppl. material 1). In the native area, 41 haplotypes were found, including the two dominant haplotypes HLA01 (67 individuals from 33 populations) and HLD07 (69 individuals from 44 populations) and 21 local haplotypes (Suppl. material 1; Fig. 2). In the invaded area, 10 haplotypes were identified, including the two main and widely introduced haplotypes HLA01 (23 individuals from 11 populations) and HLD07 (17 individuals from 8 populations), four limited distributed haplotypes (HLC07, HLC10, HLD03, HLD08) and the other four unknown locations haplotypes (HLE01, HLE02, HLE03, HLE04) (Suppl. material 1; Figs 2, 3).

Overall, the Maximum Likelihood tree and the haplotype TCS network revealed five major groups of haplotypes: HLA, HLB, HLC, HLD and HLE (Figs 2a, 3). Clade HLA grouped haplotype HLA01 and several other less frequent haplotypes (HLA02–14), mostly from south-eastern France. Clade HLB and clade HLC included six (HLB01–HLB06) and 13 (HLC01–HLC13) less frequent haplotypes, respectively, widely distributed in Europe. Clade HLD gathered haplotype HLD07 and some other low-frequency haplotypes (HLD01–06, HLD08–09), mainly from south-western France. Clade HLE grouped four unique haplotypes (HLE01–04) corresponding to the GenBank sequences. The HLE haplotypes used in this study were derived from GenBank records, based on unidentified museum specimens and likely do not originate from USA populations.

The genetic distances ranged from 0.17% to 1.9% within clades and from 0.69% to 2.6% between clades, except for HLE, for which the inter-clade genetic distance reached 4.84% (Table 2).

We conducted demographic inferences within the whole dataset and clades, except HLE. For the whole data, Fu's F_s (−9.515, $P < 0.05$) was significantly negative

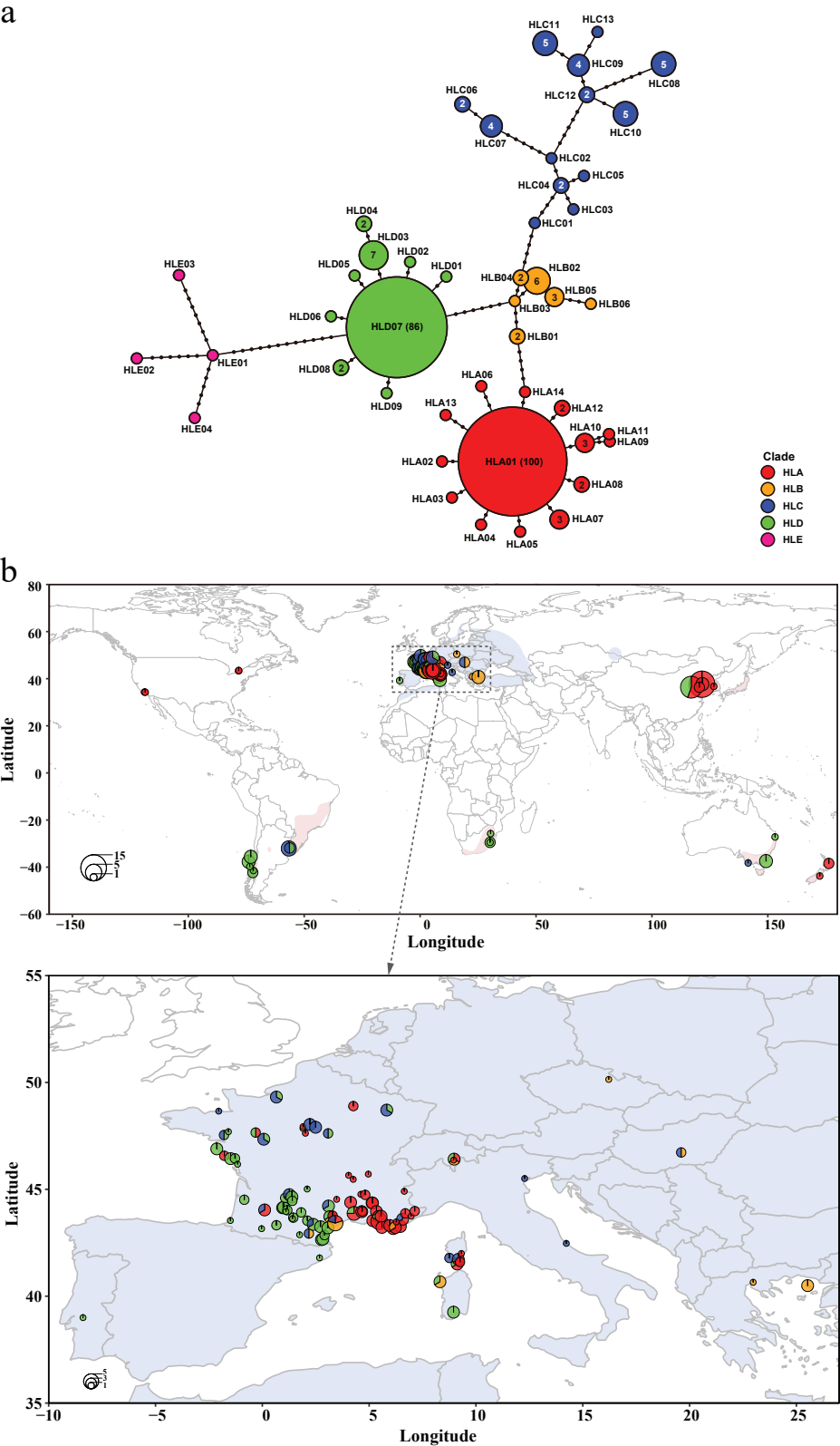


Figure 2. Geographical distribution of *H. ligniperda* populations and their associated COI haplotypes. **a.** TCS haplotype network found by the present study. The area of the circle represents haplotype frequencies (haplotypes without frequency annotations represent singleton sequences). Each black dot corresponds to a mutational step and each empty circle to a missing intermediate; **b.** The geographical distribution of the corresponding clades. The colours are the same as for the haplotype network above. Blue areas represent native ranges and red areas represent invasive ranges according to CABI and previous records (Parra Emilfork 1996; Kvamme and Lindelöw 2014; Lin et al. 2021; Brockerhoff et al. 2025b). HLE is not shown due to its unknown origin.

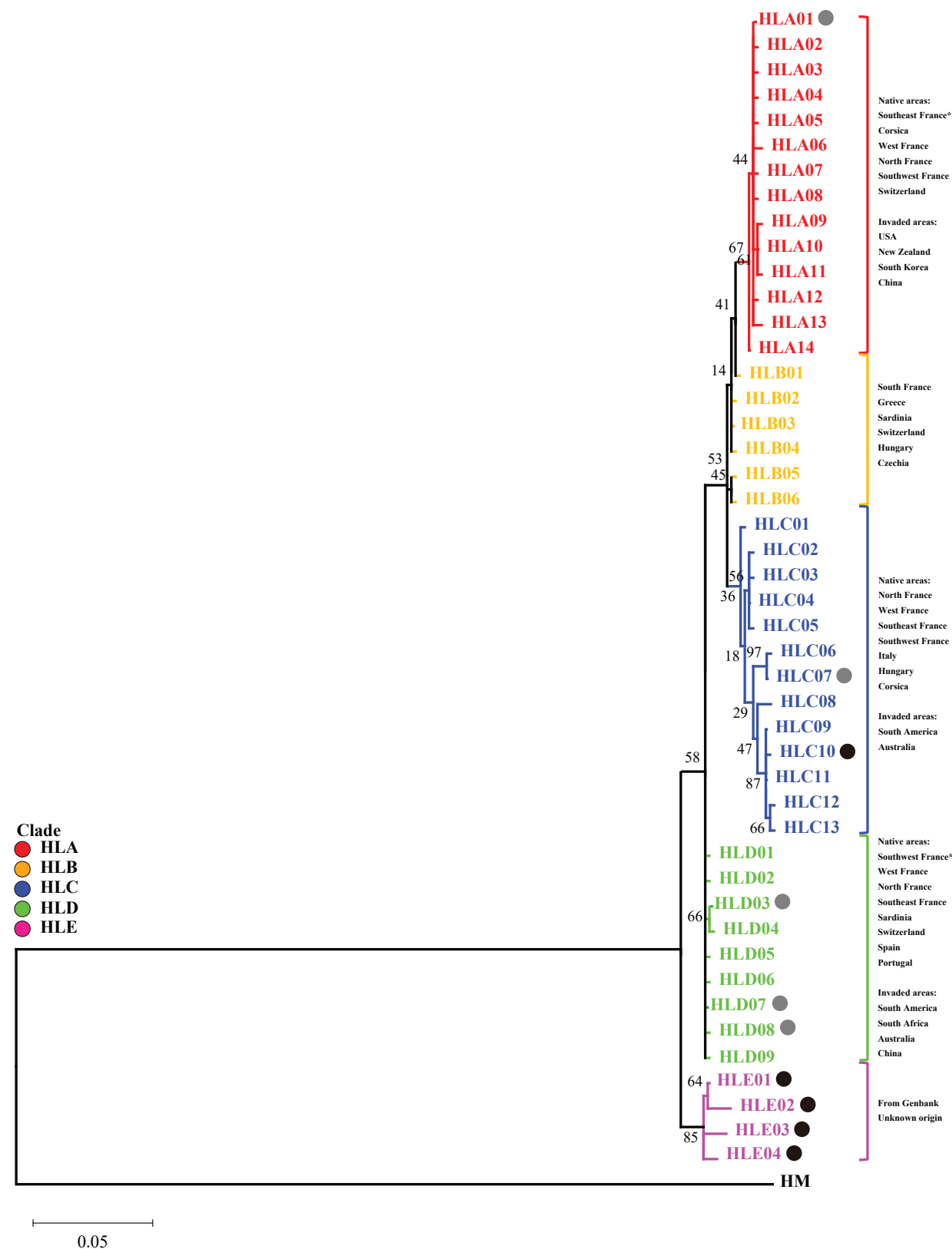


Figure 3. Maximum Likelihood tree (T92 + G Model), based on *H. ligniperda*'s COI sequences built with MEGA 11. *H. micklitzi* (HM) was used as an outgroup. The number beside the branch represents the the statistical support for that branch split. The colour of the squares next to the haplotypes shows whether they were identified in the native area only (no squares), in the invaded area only (black) or in both areas (grey). Asterisk indicates areas of widespread distribution for this lineage.

and for HLA and HLD, both Tajima's D values (HLA: -2.33698, $P < 0.01$; HLD: -1.88720, $P < 0.05$) and Fu's F_s (HLA: -18.446, $P < 0.01$; HLD: -9.058, $P < 0.05$) were significantly negative, indicating population expansion for these groups. By contrast, for HLB and HLC groups, results were not significant, indicating rather a long-term population demographic stability (Table 3).

Hierarchical analyses of molecular variance were performed using all individuals sampled from native areas (Europe). The five groups obtained with the SAMOVA analysis are shown in Fig. 4: (i) a 'south-eastern France' group comprising the 43 populations mainly in the east of Massif Central, Czechia, Switzerland and Corsica; (ii) a 'northern France' group clustering the six populations from northern France; (iii) a 'Greece' group including two populations from Thessaloniki, Greece; (iv) a 'central Europe' group comprising the four populations from north-western France, Hungary and mainland Italy; (v) a 'south-western France' group clustering the 39 populations from west of the Massif Central to the Pyrenees, the North Atlantic coast of western France, Spain, Portugal and Sardinia. The result showed that most of the molecular variance could be attributed to five geographical groups and within populations (67.19% and 32.38% respectively, $P < 0.001$).

Hylurgus micklitzi genetic study

MtDNA sequencing of 174 individuals from 53 populations resulted in an alignment of a 578 bp COI fragment, including 15 polymorphic sites and 16 haplotypes (Fig. 5a, Suppl. material 1). Haplotype and nucleotide diversities were 0.465 and 0.00096. One main haplotype, HM02 ($N = 124$), shared by three-quarters of the individuals, was distributed in most sampling locations in the western part of the Mediterranean Basin from Spain, southern mainland France and Corsica. A second, less frequent haplotype HM01 ($N = 29$) showed an extensive geographical distribution, occurring in the western (Spain, southern mainland France, Corsica and Sardinia) and eastern (Greece) part of the Mediterranean Basin.

The remaining 14 haplotypes (one to two individuals per haplotype) were observed in southern France (Fig. 5b). All other haplotypes were separated from the main haplotype HM02 by two mutation steps at most (Fig. 5a). The haplotype network was star-shaped and did not show any phylogenetic structure. We could identify one haplogroup of linked haplotypes (HM01-HM03-HM12-HM14-HM16) that mainly occurred in the western (Spain, southern mainland France, Corsica and Sardinia) and eastern (Greece) part of the Mediterranean Basin.

Given the absence of phylogenetic structure in *H. micklitzi*, the tests were only performed at the species level. Both Tajima's D (-2.03939, $P < 0.05$) and Fu's F_s (-17.176, $P < 0.05$) were negative and significant and unambiguously suggested a recent demographic expansion in this species.

Hylurgus species genetic study in sympatric sites

From 22 sites where the two species were trapped in sympatry (Fig. 1), we identified 14 COI haplotypes from 66 individuals of *H. ligniperda* and eight haplotypes from 50 individuals of *H. micklitzi* (Suppl. material 1). Overall, haplotype and nucleotide diversities were 0.675 and 0.00914 for *H. ligniperda* and 0.491 and 0.00112 for *H. micklitzi*. When comparing haplotype richness between the two

Table 1. The main species traits of *H. ligniperda* and *H. micklitzi*.

Species traits	<i>H. ligniperda</i>	References	<i>H. micklitzi</i>	References
Body size	4–5.7mm	(Grüne 1979)	3.5–3.8mm	(Grüne 1979)
Invaded distribution	China 2019, Argentina 2007, South Korea 1999, The United States 1995, Brazil 1991, Chile 1985, Sri Lanka (unknown date), New Zealand 1974, South Africa 1973, Uruguay 1956, Australia 1942, Japan 1935	(Murayama 1936; Bain 1977; Geertsema 1982; Ciesla and Ramírez Grez 1988; Wood and Bright 1992; Hoebeke 2001; Park et al. 2017; Kirkendall 2018; CABI 2021; Lin et al. 2021; Ren et al. 2021; Lantschner et al. 2024; Brockerhoff et al. 2025b)	/	/
Native distribution	Europe, northern Africa and West Asia	(Fabre and Carle 1975; CABI 2021; Brockerhoff et al. 2025b)	Around the Mediterranean Sea	(El Khoury et al. 2019)
Host plants	<i>P. pinaster</i> , <i>P. halepensis</i> , <i>P. sylvestris</i> , <i>P. pinea</i> , <i>P. brutia</i> , <i>P. nigra</i> in native areas; South Africa: <i>P. patula</i> , <i>P. elliottii</i> ; Oceania: <i>P. radiata</i> ; North America: <i>P. halepensis</i> , <i>P. pinea</i> ; South America: <i>P. contorta</i> , <i>P. elliottii</i> , <i>P. ponderosa</i> , <i>P. radiata</i> , <i>P. sylvestris</i> , <i>P. taeda</i> ; East Asia: <i>P. thunbergii</i> , <i>P. densiflora</i>	(Fabre and Carle 1975; Bain 1977; Zhou et al. 2001; Kim et al. 2011; Clare and George 2016; Lin et al. 2021; Ren et al. 2021; Gencal and Sarikaya 2023; Lantschner et al. 2024)	Mainly <i>P. halepensis</i> and <i>P. brutia</i> ; Other: <i>P. pinaster</i> , <i>P. pinea</i> , <i>P. nigra</i>	(Mendel et al. 1985; Sarikaya and Yildirim 2011; Faccoli et al. 2020)
Initiate host location	Female	(Fabre and Carle 1975; Ren et al. 2021)	Female	(Mendel et al. 1985)
Feeding mode	Phloeophagous	(Vega and Hofstetter 2014)	Phloeophagous	(Vega and Hofstetter 2014)
Biological niches	Root and lower stem	(Fabre and Carle 1975)	Root and lower stem	(Mendel et al. 1985)
Sex ratio	F: M = 1: 1	(Lanfranco et al. 2001)	Unknown	/
Mating behaviour	Outbreeding and Inbreeding (to some extent)	(Fabre and Carle 1975)	Unknown	/
Parent adults in brood systems	Monogamous	(Ren et al. 2021)	Monogamous	(Mendel et al. 1985)
Voltinism	Uni- to multivoltine (1 – 4 generations/year)	(Fabre and Carle 1975; Tribe 1991; Parra Emilfork 1996; Ren et al. 2021)	Univoltine	(Mendel et al. 1985)
Fecundity	59 – 506 eggs per female in different host and temperature	(Fabre and Carle 1975; Ren et al. 2021)	Unknown	/
Dispersal capacity	Long distance (in the field): individuals were found ≥ 25 km from the nearest host patch; Short distance in the model prediction: 50% of the beetles were caught at 20 m, 305 m and 1150 m with GAMM and 123 m with GAM	(Chase et al. 2017; Meurisse and Pawson 2017; Brockerhoff et al. 2025a)	Unknown	/
Emergence temperature	9–20 °C (optimal 15 °C)	(Tribe 1991)	Unknown	/
Cold tolerance	The lowest SCP: -6.45 ± 0.18 °C (Shandong, China)	(Cheng et al. 2024)	Unknown	/
Development temperature	Eggs to larvae: 9–33 °C (optimal 27.5 °C); Larvae to pupae: 15–33 °C (optimal 28 °C); Pupae to adults: 15–33 °C (optimal 30 °C)	(Pugh et al. 2023)	Unknown	/
Aggregation pheromones	Unknown	/	Unknown	/
Primary (attack living trees)	Secondary	(Fabre and Carle 1975; Ren et al. 2021)	Secondary	(Mendel et al. 1985)
Tree killers	No	(Fabre and Carle 1975; Ren et al. 2021)	No	(Mendel et al. 1985)
Outbreaks	No	(Fabre and Carle 1975; Ren et al. 2021)	No	(Mendel et al. 1985)

Table 2. Minimum – maximum *p*-distances within and between species or clades of *H. ligniperda* and *H. micklitzi* (derived from the mitochondrial haplotypes).

Distance	HL	HLA	HLB	HLC	HLD	HLE	HM
HL	0.2%–4.9%						
HLA		0.2%–0.7%					
HLB		0.7%–1.7%	0.2%–0.9%				
HLC		1.0%–2.6%	0.7%–2.2%	0.2%–1.7%			
HLD		1.6%–2.6%	1.0%–1.7%	1.4%–2.4%	0.2%–0.5%		
HLE		3.8%–4.8%	3.1%–4.0%	3.5%–4.8%	2.1%–3.2%	0.9%–1.9%	
HM	15.9%–17.5%						0.2%–0.7%

Table 3. Tajima's D and Fu's FS values estimated for *H. ligniperda* (whole data set, HLA, HLB, HLC, HLD).

	n	Fu's FS	Tajima's D
whole dataset	274	-9.515*	-0.9425
HLA	119	-18.446**	-2.33698**
HLB	15	-0.274	0.50909
HLC	34	-1.166	0.30782
HLD	102	-9.058*	-1.88720*

n, number of individuals. Values in brackets correspond to the *P*-value. Asterisks indicate significant values (*: *P* < 0.05, **: *P* < 0.01).

species across the seven sympatric sites with equivalent sampling, *H. ligniperda* exhibited a greater number of haplotypes in three locations (La Londe-les-Maures, Toulouse and Bédarieux), whereas *H. micklitzi* showed higher richness only in Le Puy-Ste-Réparate.

Discussion

The observed patterns of population structure and genetic diversity of the two studied species gave additional support that species-specific traits may play a pivotal role in shaping genetic variation and distribution, both directly and indirectly, while also facilitating the survival, establishment and rapid dispersal of non-native insect species (Mayer et al. 2015; Zhao et al. 2023). We investigated the genetic structure and the demographic history of two congeneric species that could be found in sympatry at small geographic scales. Our results revealed: 1) contrasting phylogeographic patterns between *H. ligniperda* and *H. micklitzi* in their native Western Palaearctic ranges; 2) more genetic variability and structure in the invasive species *H. ligniperda* than in non-invasive species *H. micklitzi*; and 3) a probable relation between diverse species traits, such as body length, voltinism and ecological niche and the invasiveness of our focal species (*H. ligniperda*), that its species-specific characteristics having potentially facilitated its invasion in new regions.

Two congeneric species in sympatry in the Western Palaearctic

Both molecular markers, the 28S rDNA gene and COI mtDNA gene, allow an accurate delimitation of *H. ligniperda* and *H. micklitzi*. COI genetic distances be-

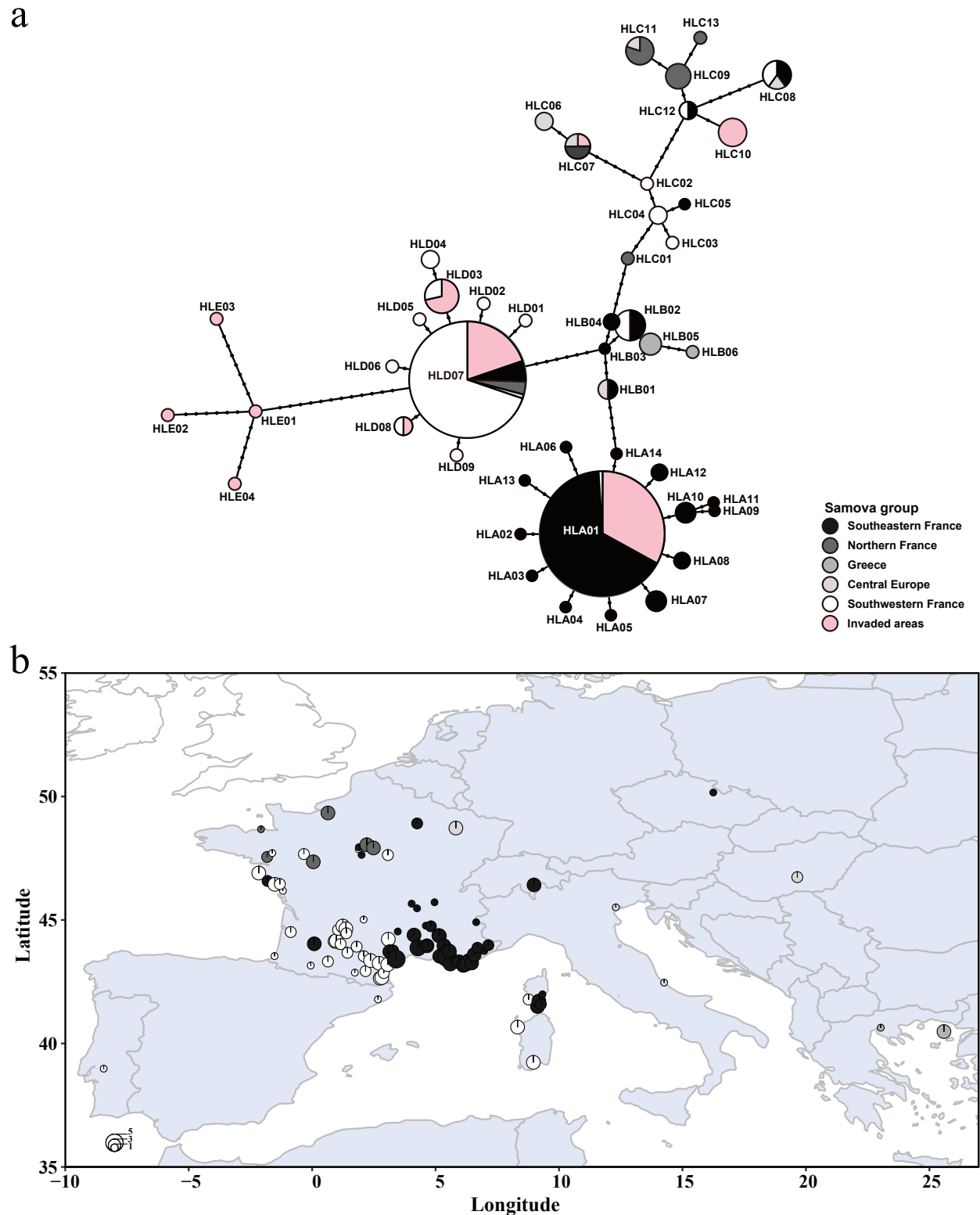
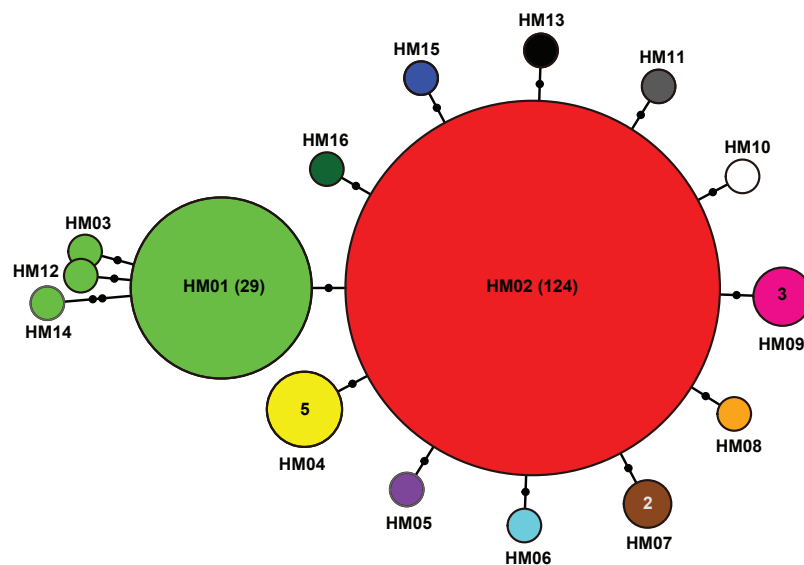


Figure 4. The Spatial Analysis of Molecular Variance (SAMOVA) results. **a.** Haplotype network of the 46 haplotypes of *H. ligniperda* shows the proportion of individuals belonging to each of the five groups and invaded areas; **b.** Geographical distribution of the five identified groups (see text for details) in native areas. The colours are the same as for the haplotype network above.

tween the two species ranged from 15.9% to 17.5%, which were higher than observed between other congeneric species of scolytids, based on the p -distance model. Within *Ips* bark beetles, Chang et al. (2012) found an interspecific genetic distance

a



b

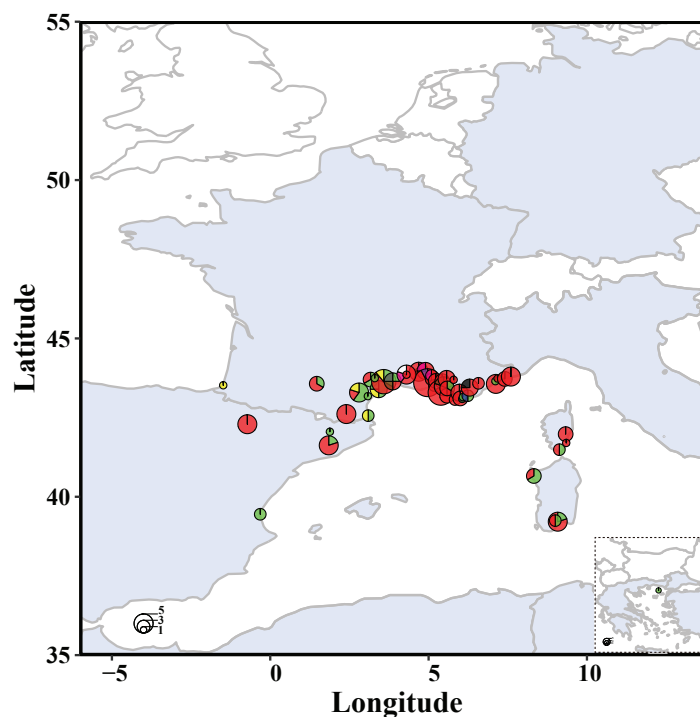


Figure 5. Geographical distribution of *H. micklitzi* populations and their associated COI haplotypes. **a.** TCS haplotype network found by the present study. The area of the circle represents haplotype frequencies (haplotypes without frequency annotations represent singleton sequences). Each black dot corresponds to a mutational step and each empty circle to a missing intermediate; **b.** The geographical distribution of the corresponding clades. The colours are the same as for the haplotype network above. Blue areas represent native ranges according to CABI and previous records (El Khoury et al. 2019; Lantschner et al. 2024).

between 5.6% and 43.1%, based on the Kimura 2-parameter (K2P) model for the COI gene. Du et al. (2021) found mitochondrial genome distances were 22.0% (*Hylastes* species), 9.0% (*Trypodendron* species), 16.9% ~ 23.6% (*Xylosandrus* species), 9.0% ~ 26.3% (*Dryocoetes* species), 24.5% (*Orthotomicus* species) and 3.7% ~ 22.2% (*Ips* species), based on the K2P model. Likewise, COI and COII genetic dis-

tances separating the *Tomicus* bark beetle species ranged from 10.7% to 12.4% between *Tomicus piniperda*, *Tomicus destruens* and *Tomicus minor* and reached 22.9% between *Tomicus* spp. and *Dendroctonus* spp., based on the K2P model (Kerdelhué et al. 2002). Analysing 33 genera and 258 species of ambrosia beetles (Xyleborini tribe) of southeast Asia, Cognato et al. (2020) observed an average interspecific difference for congeners ranging from 9.3–16.3% for COI, based on *p*-distance model. The two species of *Hylurgus* under study may have been separated a long time ago. Due to a faster evolutionary rate (Saccone et al. 1999), mtDNA showed greater intraspecific variability than the nuclear gene and revealed several clades within *H. ligniperda*. This was also observed in the cave beetle *Speonomus normandi hydrophilus* (Coleoptera, Leiodidae) (Kocot-Zalewska et al. 2021). Surprisingly, intraspecific distances within *H. ligniperda* reached 4.84% (when comparing clade HLE to other clades), but these results should be taken with caution because our study included some sequences obtained from GenBank which could result from species misidentifications.

Contrasting phylogeographic patterns between the two sympatric *Hylurgus* species in the native areas

Consistent with our hypothesis, patterns of genetic structure contrasted between the two species of bark beetles. *H. micklitzi* had much less genetic diversity than *H. ligniperda* locally (i.e. within sample sites), regionally (i.e. sympatric areas) and over the whole sampling range of the species (Suppl. materials 1, 2).

H. ligniperda, a diverse and strongly structured bark beetle in its native range

The genetic structure of continental European populations of *H. ligniperda* consisted of four groups, two mainly in southern France and two spread over the sampling sites in Europe. MtDNA haplotype richness was greater in populations sampled around the Mediterranean Basin than those sampled in the northern latitude, consistent with the “southern richness and northern purity” pattern, in which many European species survived in southern refugia and experienced successive bottlenecks during post-glacial northward expansion (Hewitt 1999). In the western side of the Mediterranean Basin, the geographical distribution of *H. ligniperda* partially matched that of its main hosts there, namely *Pinus halepensis* (Gómez et al. 2001; Vendramin et al. 2021; Kurt et al. 2023) and *Pinus pinaster* (Theraroz et al. 2024). The beetle’s genetic diversity distribution may be influenced by historical and environmental constraints, rather than direct host association and local adaptation, similar to the Mediterranean scolytid *T. destruens* (Horn et al. 2006). The split into two groups (‘South-eastern France’ and ‘South-western France’) forming a contact zone in the middle of the Massif Central, where no obvious physical barrier to dispersal occurs, was consistent with the general patterns of genetic structure and diversity observed in European insect taxa such as *Monochamus galloprovincialis* (Haran et al. 2018) and *Thaumatopoea pityocampa* (Rousselet et al. 2010), which followed the classical routes of European post-glacial history (Taberlet et al. 1998; Schmitt 2007; Habel et al. 2010). This suture zone might result from two distinct refugia reaching secondary contact, i.e. when populations with different genomes spread from their refugia encounter (Hewitt 1999). Alternatively, this secondary contact zone could be of a relatively recent origin, as artificial plantations have established a connection

between the native natural forests of *P. pinaster* in the west and *P. sylvestris* L. in the Massif Central (Rousselet et al. 2010). Greece showed two endemic haplotypes that could originate from another classical refuge in the Balkan Peninsula in the eastern Mediterranean (Taberlet et al. 1998), but our limited sampling could not allow further conclusions. Interestingly, Corsica and Sardinia included the two main haplotypes plus four other haplotypes that were shared by the nearby coast of France and Italy, suggesting gene flow between the mainland and these islands.

Based on our sampling in native regions, we observed a strong diversity and genetic structure of invasive populations of *H. ligniperda* in non-native countries. Amongst the 41 haplotypes identified in the native range, at least one of the two most dominant haplotypes (HLA01 and HLD07) was also present in many invaded countries. In addition, four distant lineages were observed over the invaded regions, even within the same small geographic area. These results suggested that *H. ligniperda* most likely repeatedly invaded different locations from various sources before dispersing locally, as it has been shown in other invasive species, such as the woodwasp *Sirex noctilio* (Boissin et al. 2012), the longhorned beetle *Anoplophora glabripennis* (Javal 2017) and the ambrosia beetle *Xylosandrus crassiusculus* (Urvois et al. 2023) in new regions.

Except for HLC10, all haplotypes observed in the invaded area could be found in native regions. Haplotype HLA01 was observed in New York, California, New Zealand, China and South Africa. HLD07 was detected in South America and China. About four other haplotypes, HLC07 was observed in Australia, HLC10 in Uruguay and HLD03 in Uruguay and South Africa. Various haplotypes co-occurred in Australia, Uruguay, Chile and China, suggesting multiple invasions of this species have occurred, like in the box tree moth *Cydalima perspectalis* (Bras et al. 2019). However, even if we observed a population genetic structure of *H. ligniperda* in its native range, mtDNA does not allow determining accurately the native origin of the invaded haplotypes and secondary spreads, nor haplotypes involved in potential bridgehead scenario. Using methods to infer invasion pathways (Guillemaud et al. 2010), together with higher resolution molecular markers, such as RAD sequencing, would permit the accurate determination of the genetic structure of this species in its native range, that would help in the understanding of the dynamics of the introductions.

H. micklitzi, a specialist bark beetle with low genetic diversity and a weak genetic structure

H. micklitzi exhibited drastically different genetic features when compared to *H. ligniperda*: a shallow genetic distribution pattern, as could be seen in other insect species, such as the bark beetle *Ips sexdentatus* (Avtzis et al. 2019) and two geometrid moths *Ourapteryx szechuana* and *Thyrinteina arnobia* (Lepidoptera, Geometridae) (Cheng et al. 2021; Nanini et al. 2024). Only 15 haplotypes were identified over the Mediterranean Basin with low genetic diversity. *H. micklitzi* was characterised by a widespread major haplotype (HM02) all over the sampling sites and many haplotypes only occurred in a single location. The star-shaped network with one over-represented haplotype and an excess of rare ones, as well as the demographic indices, suggested a recent expansion history following a drastic bottleneck (Hickerson et al. 2010) and local occurrence of new, recent haplotypes that probably emerged through mutation during the rapid expansion process (Petit et al. 2005). Genetic diversity was mostly found in Montpellier in southern France, as it was ob-

served in the nearby City of Bessan for *H. ligniperda*. The genetic structure patterns of *H. micklitzi* closely matched its main host pine *P. halepensis* in the western Mediterranean (Kurt et al. 2023). The populations of *H. micklitzi* were mostly monomorphic located on the line from the Pyrenees to Provence and Cote d'Azur. For the eastern Mediterranean, only one haplotype (HM01) was detected in Greece, where *Pinus brutia* and *Pinus nigra* were present. It is worth noting that Corsica and Sardinia contained two main haplotypes (HM01, HM02), but lacked endemic haplotypes, suggesting this species possibly did not have a long history there. Even after experiencing severe population declines, this species' ability to utilise a variety of hosts under survival pressure helped to sustain local populations. Given the apparent lack of genetic diversity and structure in the sampled populations, one could hypothesise that *H. micklitzi* may have recolonised from a single glacial refuge in the post-glacial period and that populations had experienced severe bottlenecks during interglacial periods. To date, no published data are available. Distribution data majorly based on French populations, additional Mediterranean population sampling, such as Spain, Greece, Italy, Turkey, North African countries, are needed to better understand the phylogeographic patterns of this species.

Are there traits favouring invasion potential in *H. ligniperda* and not in *H. micklitzi*?

By comparing species characteristics reported in literature between the invasive *H. ligniperda* and the non-invasive *H. micklitzi*, our findings support the hypothesis that *H. ligniperda* has a larger body size, higher voltinism, larger ecological niches and displays more events of introduction.

The body length of *H. ligniperda* and *H. micklitzi* ranges from 4 to 5.7 mm and 3.5 to 3.8 mm, respectively (Grüne 1979). Adult females of *H. ligniperda* are always the first to locate and attack pine hosts (Ren et al. 2021). A large body size in females is frequently linked to a high potential fecundity because of a larger abdomen that could hold more eggs. Coupled with the capability of inbreeding to some extent, it may help such a non-native species to quickly establish populations in invaded sites (Preziosi et al. 1996). Moreover, large beetles are able to infest large mature trees, while small beetles infest predominantly younger plants. In *Phloeosinus* bark beetles, the small European species *P. aubei* (= *P. bicolor*) and *P. thujae* infest young plants, while the larger Japanese congener *P. rudis* can kill mature trees (Moraal 2010).

Voltinism also differs between the two species. *H. ligniperda* shows one generation per year under cold climates, but their number could be up to four under warmer climates, while *H. micklitzi* only presents one generation per year in all parts of its range. *H. ligniperda* overwinters as an adult in Shandong, China, with its supercooling point (SCP) reaching the lowest (-6.45 ± 0.18 °C) in the mid-to-pose overwintering period; it does not survive beyond one day at -15 °C (1-day $LLT_{50} = -7.1$ °C) and its cold tolerance and overwintering survival are directly influenced by glycogen, lipids, proteins and cryoprotectants (trehalose and sorbitol) (Cheng et al. 2024). To date, no published data are available regarding the overwintering behaviour or thermal tolerance parameters (e.g. critical thermal maxima and minima or SCP) of *H. micklitzi*. The number of generations per year in an insect's life cycle is positively associated with the rate of spread (Fahrner and Aukema 2018) and a high fecundity required for fast population growth allows the initially small populations to establish

and expand rapidly (Jelbert et al. 2019). The same pattern is observed in *Dendroctonus* bark beetles where the invasive *D. valens* can produce up to three generations per year (Yan et al. 2005), while its congener *D. ponderosae*, a dominant pest in western North America, but without any invasion record elsewhere, is mostly univoltine, with semi-voltinism at high elevations and rare bivoltinism (Bentz et al. 2019).

H. ligniperda is also observed in more extensive ecological niches and infests more pine hosts than *H. micklitzi*, the latter mainly present close to the coast around the Mediterranean. *H. ligniperda* has a broader host range, infesting over 18 *Pinus* species. This increases its chances of being transported via wood trade, leading to more frequent interceptions and multiple introductions compared to *H. micklitzi* (Table 1). While no evidence is currently available, *H. micklitzi* might potentially expand its host range if introduced into novel environments, although this remains speculative and would require experimental validation. Similarly, the invasive bark beetle, *D. valens*, infests all pine species in the native areas (extending from Nova Scotia, Canada, west to California, USA and south through Mexico to Guatemala and Honduras) (Bright 1976; Furniss and Carolin 1977; Wood 1982; Cibrián Tovar et al. 1995) and four pine species in the invaded areas (China) (Yan et al. 2005; Liu 2020; Liu et al. 2021) and the non-invasive bark beetle *D. ponderosae*, infesting all native pine trees, except *Pinus jeffreyi* in the native areas (extending from Baja California Norte, Mexico, to northern British Columbia and western Alberta, Canada) (Wood 1982). Another example is given by the invasive bark beetle *T. piniperda*, infesting eight pine species in the native areas (the Palaearctic area from western Europe (Portugal) to east Asia (Japan)) (Masuya et al. 1998, 1999; Ritzlerow et al. 2004; Faccoli et al. 2005; Lieutier et al. 2015) and five pine species in the invaded area (North America) (Långström et al. 1994; Sadof et al. 1995; Haack and Poland 2001; Haack et al. 2001) and non-invasive bark beetle *T. destruens*, infesting seven pine species in the Mediterranean Basin known at present (Gallego et al. 2004; Sarikaya and Avci 2010).

Conclusion

Contrasting genetic diversity and phylogeographic structures of two *Hylurgus* species in sympatric sites across the Mediterranean Basin revealed their different expanding directions and recolonisation routes in contemporary and post-glacial times. *H. ligniperda* showed higher genetic diversity and structure than *H. micklitzi*, locally (i.e. within sample sites), regionally (i.e. sympatric areas) and over the whole sampling range of the species. *H. ligniperda* has invaded new regions all over the world, while its congener has remained confined in its natural range. Together with the species' traits, i.e. a larger body size, a higher voltinism and a larger ecological niche, these characteristics may have enhanced its invasiveness, leading to recurrent invasion and spread of *H. ligniperda* all over the world.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Data curation: EM. Funding acquisition: MAAR. Project administration: MAAR, GR. Resources: LR, AR, EGB. Software: EM. Supervision: GR. Writing – original draft: YY. Writing – review and editing: NA, EGB, LR, JS, YL, GR, AR, MAAR.

Author ORCIDs

Yuan Yuan  <https://orcid.org/0009-0004-1394-9423>

Alain Roques  <https://orcid.org/0000-0002-3734-3918>

Emmanuelle Magnoux  <https://orcid.org/0000-0003-0990-5511>

Lili Ren  <https://orcid.org/0000-0003-0333-0681>

Eckehard G. Brockhoff  <https://orcid.org/0000-0002-5962-3208>

Juan Shi  <https://orcid.org/0000-0001-5647-625X>

Marie-Anne Auger-Rozenberg  <https://orcid.org/0000-0002-2455-2438>

Géraldine Roux  <https://orcid.org/0000-0002-1116-2799>

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Summary of the localities sampled and specimens used in the COI sequencing analyses of *H. ligniperda* and *H. micklitzi*

Authors: Yuan Yuan, Alain Roques, Emmanuelle Magnoux, Lili Ren, Eckehard G. Brockerhoff, Juan Shi, Youqing Luo, Na An, Marie-Anne Auger-Rozenberg, Géraldine Roux

Data type: xlsx

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Link: <https://doi.org/10.3897/neobiota.101.150337.suppl1>

Supplementary material 2

Summary of the localities sampled and specimens used in the COI sequencing analyses of *H. ligniperda* and *H. micklitzi* in sympatry

Authors: Yuan Yuan, Alain Roques, Emmanuelle Magnoux, Lili Ren, Eckehard G. Brockerhoff, Juan Shi, Youqing Luo, Na An, Marie-Anne Auger-Rozenberg, Géraldine Roux

Data type: xlsx

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General discussion

Discussion Générale

Résumé en français du contexte, des objectifs de l'étude et des méthodes utilisées

Résumé

Cette thèse de doctorat explore les facteurs biologiques, écologiques et évolutifs qui influencent le succès des invasions des coléoptères de l'écorce (Scolytinae), en se concentrant sur trois paires d'espèces congénériques: *Hylurgus ligniperda* vs. *H. micklitzi*, *Dendroctonus valens* vs. *D. ponderosae*, et *Tomicus piniperda* vs. *T. destruens*. L'étude combine la phylogéographie comparative, l'analyse des traits et les modèles mondiaux d'invasion pour identifier les facteurs clés des invasions.

H. ligniperda, une espèce eurasiatique invasive, montre une large gamme d'hôtes, un multivoltinisme et une grande diversité génétique, facilitant son adaptation dans des régions non natives. En revanche, *H. micklitzi* est limité à la Méditerranée en raison de sa faible variabilité génétique. *D. valens*, invasif en Chine, présente une plus grande plasticité génomique que *D. ponderosae*, tandis que *T. piniperda* surpasse *T. destruens* grâce à sa capacité de dispersion et son utilisation plus large des hôtes.

Une synthèse de 58 espèces invasives de coléoptères de l'écorce montre que la majorité provient de la zone Paléarctique et envahit des régions avec une forte connectivité commerciale. Les espèces oligophages, se nourrissant de conifères, dominent ces invasions, mais aucune espèce n'est présente dans toutes les régions biogéographiques. Seules quatre espèces agissent comme ravageurs primaires ?, tuant des arbres en bonne santé.

Les limitations méthodologiques incluent la dépendance à l'ADN mitochondrial et des échantillons inégaux. Les recherches futures devraient intégrer des outils génomiques et des approches interdisciplinaires pour affiner l'évaluation des risques et les stratégies de gestion. Les principales questions non résolues incluent le rôle de la dispersion humaine et les dynamiques éco-évolutives.

Cette étude améliore la compréhension des invasions des coléoptères de l'écorce en reliant traits des espèces, biogéographie historique et facteurs anthropiques, soulignant la nécessité de renforcer la biosécurité dans les écosystèmes forestiers vulnérables.

Mots-clés: Invasions biologiques, *Hylurgus*, *Dendroctonus*, *Tomicus*, phylogéographie, analyse des traits, ravageurs forestiers, commerce mondial.

1 Discussion and critical analysis of my thesis

My PhD thesis is part of a joint research initiative between China and France, carried out under the auspices of the International Joint Laboratory for Eurasian Forest Invasive Species (IFOPE). In October–November 2020, the Quarantine Laboratory of the Forest Protection Department at Beijing Forestry University captured a bark beetle that was severely infesting the roots of *Pinus thunbergii* (Japanese black pine) in a coastal shelterbelt in Muping District, Yantai City, Shandong Province, China (37.457241°N, 121.851217°E) (Ren *et al.* 2021). The species was later identified as *Hylurgus ligniperda*, a bark beetle native to the Western Palaearctic that has invaded every continent except the polar regions, making it the most widely distributed invasive bark beetle (Faccoli *et al.* 2020). Preliminary observations indicated that adults can directly enter the soil and invade tree roots, even infest fine roots as small as 0.5 cm in diameter located more than 7 meters away from the base of the tree. The beetle is capable of attacking sub-healthy trees in advance and may ultimately cause tree mortality. Due to its concealed mode of infestation and the absence of obvious above-ground indicators such as frass or other easily recognizable symptoms, monitoring and control of this pest pose significant challenges. After the initial report, the red-haired bark beetle was designated as an important quarantine pest in China. Between 2020 and 2024, the species was subsequently detected in several nearby cities, including Weihai, Qingdao, and Tai'an, all within the same province. These findings have raised concerns about the beetle's origin and potential for widespread dispersal, prompting the initiation of this research project. *H. ligniperda* is a recently established invasive species in China, originally native to the Mediterranean region, with an invasion history spanning over a century. Around the Mediterranean Sea, *Hylurgus micklitzi*, a congeneric species, is co-distributed with *H. ligniperda*. However, while *H. ligniperda* has invaded all continents, *H. micklitzi* remains confined to the Mediterranean basin. They share similar life histories, making them an excellent model for comparative studies on species differentiation and key traits associated with invasive ability.

Although my main species pair belongs to the genus *Hylurgus*, the genus is relatively small and offers limited ecological and biological diversity (Chapter 3, Table 1). In the tribe Hylurgini (Coleoptera: Curculionidae: Scolytinae), species such as *H. ligniperda* (which has been invasive for over 100 years) and *H. micklitzi*, *Dendroctonus valens* (which invaded China more than 20 years ago) and *Dendroctonus ponderosae*, *Tomicus piniperda* (which invaded North America over 20 years ago) and *Tomicus destruens* are closely related both phylogenetically and ecologically, and occur sympatrically in their native ranges. These species share a common origin, yet one is invasive while the other is not, and there is more fundamental study about the other two pairs, making them an excellent comparative model for studying invasion-related traits.

In addition, all above three congeneric species belongs to one group, bark beetle, are insects that tunnel beneath tree bark to feed on the phloem, significantly impacting tree health,

forest ecosystems, and global trade by infesting weakened trees and transmitting plant pathogens that can trigger widespread tree mortality (Vega and Hofstetter 2014). A systematic investigation of all invasive bark beetles provide insights into general species characteristics and damage assessments, supporting the development of effective quarantine measures and informed policy decisions.

My doctoral research focuses on two main objectives: (1) to identify key biological and ecological traits that contribute to the invasion success of bark beetles, and (2) to compare the biological, ecological, and evolutionary traits of congeneric species pairs of Hylurgini (invasive versus non-invasive) to uncover the drivers of successful establishment in invasive scolytids.

During my doctoral research, *H. ligniperda* continued its expansion into new regions, with recent records in China. These new occurrences provide a wealth of additional samples, allowing us to determine whether they result from intrinsic expansion or new introduction events. Furthermore, the growing body of research on invasive bark beetles, particularly the focal species of my study, has also provided substantial data to support my thesis.

1.1 Global invasive bark beetles

Summary of results

Globalization drives the invasion of scolytids, and trade differences between regions worldwide influence the variation in their global invasion dynamics (Seebens *et al.* 2018, Lantschner *et al.* 2020). Before thoroughly studying our three main pairs of bark beetles, a comprehensive understanding of the species characteristics within the bark beetle group is essential for the subsequent analysis. I hypothesize that 40 invasive bark beetles share certain key traits, including broader ecological niches and distinct feeding preferences. Building upon the framework established by Lantschner *et al.* (2020), I expanded the global invasion dataset of bark beetles using information from peer-reviewed publications, books, and databases. I further classified their impact levels and synthesized species-level traits such as damage type, host type, number of host families, number of biogeographic regions, and mating systems that are potentially linked to invasiveness. The results showed that:

- 1) The Palearctic region is the most common origin of invasive bark beetles, followed by the Nearctic and Indo-Malayan regions, while the main recipient zones are the Neotropical, Nearctic, and Afrotropical regions.

Although the global establishment patterns of bark beetles are relatively consistent across regions, their origins differ markedly. Temperate regions such as the Palearctic and Nearctic are major sources of bark beetle introductions, reflecting the tree species composition and ecological characteristics of these zones (Hulcr *et al.* 2015, Kirkendall *et al.* 2015). Globally, plant taxa exhibit strong biogeographic structuring, with the greatest phylogenetic differences occurring between the northern and southern hemispheres (Box 2002). Since bark beetles are typically specialized on phylogenetically related hosts (Futuyma and Agrawal 2009), invasions

across hemispheres, especially for non-tropical species, are less likely to succeed. This limitation is further reinforced by opposing seasonality between hemispheres, which poses a barrier to establishment, particularly for species with obligate diapause requirements (Schebeck *et al.* 2017).

While host-tree phylogenetic similarity could help explain invasion routes, current data are insufficient for quantifying its effect, and it is often confounded with trade patterns. For instance, the Palearctic and Nearctic not only share similar host-tree ranges but also maintain the strongest global trade connections. With increasing global trade and the widespread use of nonnative trees in forestry and urban landscapes (Wingfield *et al.* 2015), the invasion risk posed by bark beetles is likely to rise. Therefore, it is crucial to enhance phytosanitary measures and border surveillance in previously isolated regions to mitigate future invasion risks (Branco *et al.* 2015, Hurley *et al.* 2016, Poland and Rassati 2019).

2) About two-thirds of invasive bark beetles feed on conifers and are oligophagous, typically invading one to two new regions. No bark beetle species has established populations across all biogeographic regions. *H. ligniperda* (oligophagous, conifer-feeding, monogamous) has been established in six regions, with the exception of the Indo-Malayan region. In the same way, three other bark beetle species are widely distributed, i.e. *Hypocryphalus mangiferae* (oligophagous, broadleaf-feeding, monogamous), and *Ips grandicollis* (oligophagous, conifer-feeding, polygamous), *Hylastes ater* (oligophagous, conifer-feeding, monogamous). In terms of invasive bark beetles' mating systems, monogamous and polygamous species are roughly equally represented.

The widely distributed invasive bark beetles are oligophagous species and outbreeders. In contrast, ten ambrosia beetles or seed and twig feeders, typically host generalists (utilizing more than five plant families) and inbreeders, have established populations across all biogeographic regions (Lantschner *et al.* 2020). Compared to these groups, invasive bark beetles show notable differences in host range and reproductive strategies, highlighting distinct invasion patterns relative to ambrosia beetles and seed/twig feeders.

The success of non-native species invasions is influenced by propagule pressure (the frequency and number of introductions) (Brockerhoff *et al.* 2014). Using cumulative interregional import value as a proxy for propagule pressure, Lantschner *et al.* (2020) assessed invasion risk by incorporating forest cover and climatic similarity, and found that trade intensity is the primary driver of non-native bark beetle spread, while climatic similarity and forest area moderately influence establishment success. *H. ligniperda* has shown 30 interceptions at New Zealand's borders between 1950 and 2000 (Brockerhoff *et al.* 2014), 217 interceptions at U.S. ports from 1985 to 2000 (Haack 2002), and 10,199 interceptions in China from 2003 to 2016 (Wang *et al.* 2018), making it one of the most frequently intercepted scolytids globally. *H. mangiferae* was intercepted four times at U.S. ports between 1984 and 2008 in shipments originating from India and Brazil (Haack and Rabaglia 2013). Between 1950 and 2000, a total of 49 interceptions of *I.*

grandicollis were recorded at New Zealand's borders, including 20 originating from Australian consignments (Brockerhoff *et al.* 2006). Between 1985 and 2000, *H. ater* was intercepted 67 times at U.S. ports (Haack and Rabaglia 2013), three times in Canadian wooden consignments in 1997 (Canada Food Inspection Agency 1997).

3) Most invasive bark beetles are secondary pests that infest stressed or dying trees and generally cause relatively minor damage. Only four species: *Dendroctonus valens* (oligophagous, conifer-feeding, monogamous), *Orthotomicus erosus* (oligophagous, conifer-feeding, polygamous), *Pityophthorus juglandis* (oligophagous, broadleaf-feeding, polygamous), and *Scolytus kirschii* (polyphagous, broadleaf-feeding, monogamous) are considered primary pests in certain regions and are capable of attacking and killing healthy trees. *Hypocryphalus scabricollis*, native to the Indo-Malayan region, is the most highly polyphagous invasive bark beetle, feeding on eight plant families and having invaded Tunisia and Algeria (Gaaliche *et al.* 2018, Mellal *et al.* 2023).

Although these four species have not established populations in many regions, their demonstrated ability to cause significant damage highlights the need for stricter quarantine measures and management strategies to limit their further spread.

In summary, Chapter 1 underscores that global trade patterns and species-specific biological traits collectively shape the invasion pathways and impacts of bark beetles. While most invasive species remain limited in distribution and damage potential, the emergence of primary pests highlights the ongoing risk posed by these insects. Enhanced biosecurity measures and continued monitoring are imperative to mitigate future invasions, particularly in regions with high trade connectivity and suitable host availability.

Methodological limitations

Our global assessment of bark beetle invasions has several methodological limitations. First, it depends primarily on published presence-only records and online databases, which are inherently subject to publication bias, uneven spatial and temporal sampling, and potential underreporting of non-native establishments (Haack 2002, Lantschner *et al.* 2020). The division of global landmasses into broad biogeographic regions, though necessary for a global analysis, may obscure finer-scale ecological barriers and pathways (Lantschner *et al.* 2020). Furthermore, species with highly uncertain native ranges were excluded from origin–destination analyses, potentially leading to the loss of informative invasion events. Trait data, including mating systems and host range, were compiled from diverse sources (regional monographs and catalogs, online databases and published compilations of nonnative species), without standardized measurements, and phylogenetic relatedness among species was not accounted for, raising concerns about pseudo-replication and bias in trait–invasiveness associations (Brockerhoff and Liebhold 2017). Importantly, species identifications were based on literature and not verified through molecular methods, and our study did not incorporate population

abundance, dispersal capacity, or quantitative impact measures, all of which are key for understanding invasion success (Van Kleunen *et al.* 2010, Zhao *et al.* 2023). Consequently, while this synthesis provides a valuable baseline, it represents a static view of invasion patterns that may not fully capture ongoing processes or future changes.

Future research would benefit from integrating standardized field surveys, genetic analyses to verify species and reconstruct pathways, explicit consideration of phylogenetic relationships, and inclusion of quantitative metrics such as propagule pressure, population growth rates, and ecological impacts. Such approaches would provide a more dynamic and mechanistic understanding of bark beetle invasion biology (Brockerhoff and Liebhold 2017, Marchioro and Faccoli 2022).

1.2 Ecological traits of three invasive and non-invasive species pairs in the tribe Hylurgini

Summary of results

In Chapters 1 and 3, we systematically compare key traits across these bark beetle species.

Comparative analyses of invasive bark beetles and their non-invasive congeners reveal consistent trait advantages that likely underpin invasion success. *D. valens*, for example, has a bigger body, developed a flexible voltinism pattern ranging from univoltine to bivoltine, and has long-range dispersal capabilities reaching up to 35 km. It also shows refined semiochemical responses and maintains high genetic diversity, which enhances its adaptability across China's diverse pine forests. In contrast, *D. ponderosae* remains largely univoltine, exhibits narrower host and climatic tolerance, and possesses a limited dispersal capacity of 2 to 6 km on average and about 24km in max.

Similarly, *T. piniperda* demonstrates superior ecological plasticity compared to *T. destruens*. It has expanded its host range upon introduction to North America, retained cold-tolerant traits, and exhibits earlier and longer seasonal flight periods that facilitate broader colonization. Its dispersal distance also exceeds those of *T. destruens*, which is constrained by temperature-dependent flight behavior, narrow host use, and limited ecological flexibility.

Finally, *H. ligniperda* outcompetes its congener *H. micklitzi* through a broad host range, multivoltine reproduction, and exceptional adaptability to diverse climatic zones, including regions in the Southern Hemisphere. Its higher genetic diversity and larger body size may further contribute to its ecological competitiveness and resilience. In contrast, *H. micklitzi* exhibits a restricted host spectrum, a univoltine life cycle, and low genetic diversity, which collectively reduce its invasive potential.

Together, these comparisons highlight key traits associated with successful invasions in bark beetles: life-history plasticity, broad host range, and enhanced dispersal.

D. valens* vs. *D. ponderosae

After its invasion into China, *D. valens* has shown several life-history and behavioral shifts compared to its native range. These changes include a shift from a native 1–3-generation/year cycle to a synchronized univoltine–bivoltine pattern, with univoltine populations observed north of approximately 36.5°N and bivoltine populations to the south (Smith 1971, Zhang et al. 2002, Shi and Sun 2010). Furthermore, the species adopted root overwintering and exhibited enhanced cold tolerance (Miao et al. 2001, Dong et al. 2021, Zhao et al. 2021). Semiochemical responses to local pine monoterpenes were also refined (Owen 2003, Aukema et al. 2011), and dispersal distances were found to increase to approximately 35 km (Smith 1971, Zhang et al. 2002). These changes reflect adaptation to China's distinct seasonality and vast *Pinus tabulaeformis* plantations, coupled with multiple introductions from the Pacific Northwest that have preserved high genetic diversity (Cai et al. 2008, Taerum et al. 2016, Liu et al. 2022).

In terms of morphology, *D. valens* is the largest species in its genus, with females measuring 7.5–9.6 mm and males 5.9–8.1 mm in length (Zhang et al. 2002, Shi and Sun 2010). This contrasts with *D. ponderosae*, which is smaller, with females measuring 4.41–6.09 mm and males 3.78–5.46 mm (Pureswaran and Borden 2003). Although body size might theoretically influence factors like dispersal and cold tolerance, it is essential to note that size alone does not account for invasion success. The ecological implications of these size differences must be considered in the broader context of each species' life-history strategies and their interactions with the environment.

Additionally, *D. ponderosae* generally remains univoltine or semivoltine at high elevations (Bentz and Powell 2014, Bentz et al. 2014) and exhibits a narrower host range (Chapter 1, Table 1), which limits its ecological flexibility. The species also relies on less flexible pheromone synergism (Pitman et al. 1968, Pureswaran et al. 2000), disperses over shorter distances (2–6 km on average, with a maximum of ~24 km) (Evenden et al. 2014), and has a historically structured gene pool, which may slow adaptation (Janes et al. 2018). These traits suggest that *D. valens* may possess greater invasion potential in China compared to *D. ponderosae*, although caution is required when interpreting these results. Invasive success is not solely determined by morphological or life-history traits but involves a complex interaction of ecological, genetic, and environmental factors.

In conclusion, while the comparative data between *D. valens* and *D. ponderosae* provide valuable insights, they should be interpreted with care. The successful invasion of *D. valens* in China is likely a result of multiple factors, including its genetic diversity, life-history plasticity, and adaptation to local environmental conditions, rather than any single trait. Furthermore, the complexity of biological invasions requires further investigation into how various species traits interact with changing ecological conditions.

T. piniperda* vs *T. destruens

Upon invading North America, *T. piniperda* exhibited significant shifts in its biological traits compared to its native European populations. In Europe, *T. piniperda* primarily infests *P. sylvestris* and *P. pinaster*, with a balanced 1:1 sex ratio and relatively limited dispersal capacity, averaging around 900 meters but reaching up to 2 km. Upon introduction to North America, *T. piniperda* quickly adapted, expanding its host range to include *Pinus strobus* and *Pinus ponderosa*. This broadening of host flexibility played a critical role in the species' success in its new environment, allowing it to take advantage of a variety of susceptible trees that were not available in its native range.

T. piniperda retained its cold-adapted development traits, thriving in temperatures between 10–15°C and overwintering in the bark of live pines (Ryall and Smith 2000). This ability to withstand cold climates likely gave it a crucial edge in North America, where winters can be harsher than in its native range. The species maintained a similar reproductive and maturation attack strategy, which has allowed for its rapid spread across the northeastern and north-central United States and Canada.

The difference in dispersal capacity between *T. piniperda* and its relative *T. destruens* is particularly noteworthy. While *T. destruens* is more restricted in its flight periods, only flying when temperatures exceed 24°C in late autumn, and shows limited dispersal capabilities (Peverieri et al. 2008), *T. piniperda* flies earlier and longer in the season, with stronger dispersal potential (Kerdelhué et al. 2006, Vasconcelos et al. 2006). This greater mobility has likely been a key factor in *T. piniperda*'s success as an invader, allowing it to rapidly colonize new areas and find suitable hosts.

T. piniperda's ability to adapt to new climates, its broader host range, and its longer, more flexible flight periods give it an edge over species like *T. destruens*. The genetic advantages resulting from multiple introduction events likely further bolster its ability to invade new regions (Haack et al. 2001). This combination of traits, such as plasticity in life-history strategies, broad ecological tolerance, and the ability to outcompete other species, helps explain why *T. piniperda* has been so successful in North America. Its ability to exploit ecological opportunities, such as abundant susceptible hosts and reduced biotic resistance, has facilitated its rapid expansion, making it a prime example of a highly effective invasive species.

H. ligniperda* vs *H. micklitzi

From my perspective, *H. ligniperda* and *H. micklitzi* exhibit significant differences in their ecological traits that profoundly influence their invasion potential. *H. ligniperda* demonstrates remarkable ecological adaptability, broad host range, and multivoltine reproduction, allowing it to rapidly spread in newly invaded regions and adapt to various environmental conditions. These traits enable it to effectively compete and establish itself within foreign ecosystems. For example, its ability to infest a variety of pine species (such as *P. pinaster* and *P. halepensis*) provides

ample resources for rapid reproduction and population growth. This is particularly evident when it invades regions such as North America, New Zealand, and China (Brockerhoff et al. 2014, Wang et al. 2018).

I believe that the high genetic diversity of *H. ligniperda* is a crucial factor in its invasion success. Compared to *H. micklitzi*, it harbors greater genetic variation and stronger population structure, which enhances its adaptability and resilience in novel environments. This genetic flexibility allows it to overcome ecological challenges and expand beyond its native range. It is clear to me that genetic diversity plays a key role in the rapid adaptation and expansion of invasive species.

In contrast, *H. micklitzi* has much lower invasion potential. Its host range is narrow, limited to a few pine species in the Mediterranean region, which restricts its resource access and population growth. Furthermore, it is univoltine, producing only one generation per year, which significantly limits its reproductive capacity and ability to spread. Additionally, its lower genetic diversity and smaller body size make it much less competitive than *H. ligniperda* (Gallego et al. 2004, Sarikaya and Avci 2010).

These comparisons underscore the importance of ecological adaptability, reproductive strategies, and genetic diversity in determining the invasion success of bark beetles. *H. ligniperda*'s ability to exploit a wide range of habitats, reproduce multiple times per year, and benefit from high genetic diversity collectively explain why it is a more successful invader than *H. micklitzi*.

Methodological limitations

Through the systematic comparison of three congeneric bark beetle species pairs (*D valens* vs. *D. ponderosae*, *T. piniperda* vs. *T. destruens*, and *H. ligniperda* vs. *H. micklitzi*), we gained important insights into how traits such as host range, voltinism, dispersal ability, and genetic diversity shape invasion success. However, as I worked through these comparisons, it became increasingly clear that there are notable limitations to our current understanding.

First, much of the evidence is drawn from observations in specific regions, such as North American pine plantations, Mediterranean pine forests, or large-scale *P. tabuliformis* plantations in northern China. Findings under these relatively simplified conditions may not easily generalize to more diverse or mixed forest systems elsewhere. A species that spreads aggressively in a monoculture may behave very differently in a mixed-species native forest.

Second, many studies focus on individual traits, such as flight capacity or cold tolerance, while often overlooking the fact that traits interact in complex ways. Traits like body size, developmental timing, and thermal tolerance frequently co-evolve and jointly shape ecological performance (Bentz et al. 2014, Janes et al. 2014). Especially under rapidly changing climates, predicting invasion success based on a single trait risk oversimplifying the broader adaptive potential of a species.

Another important limitation is that human influences are often underrepresented. Trade routes, forest management practices, and habitat fragmentation caused by urban expansion all profoundly affect bark beetle movement and establishment (Brocknerhoff et al. 2006, Wingfield et al. 2015). Nevertheless, most ecological models still treat these socio-economic factors as static background conditions, rather than dynamic drivers of invasion processes.

Therefore, a better understanding of bark beetle invasions will require the integration of biology, landscape ecology, climate change science, and socio-economic analysis. Invasions are complex and multidimensional, and capturing this complexity calls for interdisciplinary collaboration. It is no longer sufficient for entomologists and forest ecologists to work independently. We must bring together expertise from fields such as trade policy, urban planning, and social sciences. Only by breaking through disciplinary boundaries and framing invasions within dynamic, realistic systems can we hope to truly understand, and perhaps anticipate, the biological invasions that are reshaping forest ecosystems.

1.3 Comparative phylogeography in the native range of three invasive and non-invasive species pairs in the tribe Hylurgini

In my doctoral research, I focused on experimental studies of *Hylurgus* species, specifically *H. ligniperda* and *H. micklitzi*. Through field surveys and genetic analysis, I examined their key biological, evolutionary and ecological traits to better understand their spread and adaptation. I explored aspects such as life history, host preferences, reproductive strategies, and genetic variation, with the goal of uncovering factors that might explain their invasive success in non-native regions. Additionally, I compared the phylogeographic patterns of different populations to see how these species have evolved and expanded both in their native areas and in newly invaded locations.

For the *Dendroctonus* and *Tomicus* species, my approach was based on synthesizing existing research. I collected and reviewed a wide range of studies on these species, focusing on their ecological characteristics, population dynamics, and evolutionary histories. By compiling data from multiple sources, I identified key patterns and trends in their invasive behavior, helping to build a comparative understanding of how they relate to *Hylurgus* species in terms of their spread and ecological adaptability.

This combined approach allowed me to gain insights into the invasion processes of different bark beetle species and provided a solid basis for future research and management efforts.

Summary of results

In comparing invasive bark beetles with their non-invasive congeners, several consistent traits emerge that appear to facilitate invasion success. The invader *D. valens* exhibits greater genomic plasticity, deeper historical subdivision, and higher genetic diversity than the non-invader *D. ponderosae*, suggesting that its complex evolutionary history and genomic

architecture may have pre-adapted it for rapid expansion in novel environments. Similarly, the invader *T. piniperda* combines weak population structure with broad ecological tolerance and a history of genetic admixture, traits that contrast with the more specialized and geographically structured *T. destruens*, whose narrower host associations may constrain its invasive potential. Among *Hylurgus* species, *H. ligniperda* stands out for its high haplotype richness, ecological generalism, and multiple independent introductions across continents, whereas *H. micklitzi* shows limited diversity and a restricted postglacial expansion pattern. Collectively, these comparisons reinforce the notion that high genetic diversity and ecological flexibility (which confer high degree of plasticity and adaptability), and frequent admixture are recurrent features of successful insect invaders, particularly within Scolytinae.

D. valens* vs. *D. ponderosae

The comparison between *D. valens* (red turpentine beetle, RTB) and *D. ponderosae* (mountain pine beetle, MPB) highlights substantial contrasts in species diversity, genomic architecture, and evolutionary dynamics. *D. valens* possesses a larger genome (322.41 Mb) with a higher proportion of repetitive sequences (45.22%), while *D. ponderosae* maintains a smaller genome (252.85 Mb) with only 21.12% repeats (Dowle *et al.* 2017, Liu *et al.* 2022). In my view, the abundance of repetitive elements in *D. valens* likely endows it with greater genomic plasticity, potentially enhancing its capacity to adapt to novel environments, which may represent an adaptive advantage less developed in *D. ponderosae*.

Through analyzing the genetic structures reported in previous studies, I observed that *D. valens* displays a highly structured and geographically complex native population. Multiple distinct lineages have been identified based on mitochondrial DNA, microsatellites, and genome-wide SNP data, reflecting the historical influence of glacial refugia and geographic barriers such as the Sierra Madre Occidental (Cognato *et al.* 2005, Cai *et al.* 2008, Taerum *et al.* 2016, Liu *et al.* 2022). In contrast, *D. ponderosae* exhibits a much simpler pattern: a weak north–south division primarily shaped by postglacial range shifts and recent climatic changes (Mock *et al.* 2007, Cullingham *et al.* 2012, Janes *et al.* 2018). This contrast reinforces my impression that historical environmental heterogeneity played a critical role in shaping the present-day diversity of *D. valens*, whereas *D. ponderosae* evolved under relatively uniform selective pressures.

From a species diversity perspective, I find it striking that *D. valens* maintains high genetic diversity in western North America and Mexico, suggesting the existence of multiple glacial refugia during Pleistocene climatic oscillations (Soltis *et al.* 1997, Cai *et al.* 2008). By contrast, *D. ponderosae* exhibits higher nucleotide diversity in its southern populations, which have served as reservoirs for its northward expansion into new ecological niches (Cullingham *et al.* 2012). This observation aligns with the idea that climatic factors, rather than deep historical divergence, have been the dominant forces shaping *D. ponderosae*'s evolutionary path.

In their respective expansion events, *D. valens* populations invading China arose from multiple introductions originating from the Pacific Northwest, experienced an initial genetic bottleneck, and subsequently maintained moderate diversity through rapid population expansion (Cognato *et al.* 2005, Cai *et al.* 2008, Taerum *et al.* 2016, Liu *et al.* 2022). Reflecting on this, I consider *D. valens* an excellent example of how pre-existing genetic complexity can facilitate invasion success. In contrast, *D. ponderosae*'s expansion across Canada seems to be driven more by ecological opportunity than by genetic novelty (Cullingham *et al.* 2012, Janes *et al.* 2018).

In summary, based on my analysis, *D. valens* demonstrates higher genetic diversity, deeper historical subdivision, and greater genomic plasticity than *D. ponderosae*. These traits likely contribute to its invasive potential and adaptive capacity. Meanwhile, *D. ponderosae* exemplifies how species with simpler genetic structures can nevertheless achieve rapid range expansion when facilitated by environmental changes. These contrasting models offer valuable insights into how genomic traits and historical processes interact to shape invasion outcomes in bark beetles.

T. piniperda* vs *T. destruens

Our comparative analysis of *T. piniperda* and *T. destruens* highlights how different ecological contexts and historical processes have shaped their evolutionary trajectories. Although both species present comparable levels of mtDNA haplotype diversity, as summarized in Table 4 (Chapter 1), the spatial structures and underlying dynamics differ in ways that I find quite revealing.

For *T. piniperda*, a temperate species, phylogeographic studies consistently show a weak genetic structure across Europe. This pattern seems closely linked to the distribution of its primary host, *Pinus sylvestris*, and the historical movements of both trees and timber. Early mitochondrial and nuclear studies (Kerdelhué *et al.* 2002, 2003, Ritzerow *et al.* 2004, Horn *et al.* 2009) already suggested limited differentiation across broad geographic scales, though some structure is detectable in southern Europe. In my opinion, the main drivers here are extensive gene flow and human-mediated dispersal, which have blurred the genetic signatures that might otherwise have been more pronounced. Mitochondrial data further hint at subtle host-associated effects, perhaps linked to sex-biased dispersal, a phenomenon observed in other bark beetles (Sallé *et al.* 2007).

Evidence for multiple glacial refugia, especially in regions like the Iberian Peninsula, the Pyrenees, the southern Alps, and the Balkans, adds another layer to the story (Horn *et al.* 2009, De Becquevort *et al.* 2023). Postglacial expansion from these refugia seems to explain the latitudinal gradient in genetic diversity we see today, with southern populations retaining more diversity and northern ones showing signs of founder effects. A particularly interesting case

comes from the United Kingdom, where localized structuring likely reflects historical habitat fragmentation (De Becquevort *et al.* 2023).

I believe these findings illustrate that even a species often described as a generalist can be shaped by fine-scale ecological and historical processes. In my view, the modest host-associated differentiation in *T. piniperda* deserves more attention, as it suggests that local adaptation may be more important than previously assumed.

Moreover, I argue that *T. piniperda*'s ecological flexibility, weak population structure, and historical admixture have combined to enhance its potential for invasion. Genetic mixing among different glacial lineages might have broadened the species' adaptive range, pre-adapting it to survive and thrive in new environments. This helps explain its rapid establishment and spread after its introduction to North America. From my perspective, *T. piniperda* is a clear example of how evolutionary history can set the stage for biological invasions, even before human activities accelerate the process.

In contrast, *T. destruens*, which is native to the Mediterranean Basin, presents a much stronger genetic structure. This difference, in my opinion, reflects the fragmented landscapes and environmental variability typical of Mediterranean ecosystems. Mitochondrial and nuclear markers (Faccoli *et al.* 2005, Horn *et al.* 2006, Gallego and Galián 2008) reveal two well-differentiated lineages: one in the western Mediterranean and the other in the eastern parts. Their current contact zone along the Adriatic coast points to historical separations and later secondary contact events.

Interestingly, the genetic structure of *T. destruens* seems tightly linked to its host trees. Western populations mostly associate with *Pinus pinaster*, while eastern ones are connected with *Pinus halepensis* and *Pinus brutia* (Vasconcelos *et al.* 2006, Sánchez-García *et al.* 2015). This contrasts with *T. piniperda*, where the influence of host association appears much weaker. *T. destruens* also shows strong spatial structuring shaped by local environmental factors like altitude and climate, with signs of competitive exclusion between mitochondrial lineages (Gallego and Galián 2008). Older lineages tend to dominate average environments, while younger ones are more often found in marginal habitats, suggesting an ecological sorting process over time.

Based on these patterns, I consider *T. destruens* less likely to become invasive than *T. piniperda*, at least under current conditions. Its strong specialization on particular hosts and environments would naturally limit its ability to colonize new regions. However, I also see signs of underestimated ecological plasticity. Its presence in a range of stressful environments suggests that, under the right circumstances, particularly with global climate change or introduction into Mediterranean-type ecosystems elsewhere, *T. destruens* might exceed our expectations. I would argue that a careful reassessment of its invasion risk is warranted, especially given the increasing movement of pine material worldwide.

Taken together, the phylogeographic patterns of *T. piniperda* and *T. destruens* underscore how species' evolutionary histories, ecological interactions, and human activities interact to shape contemporary distributions. In my view, *T. piniperda* reflects a case where wide ecological amplitude and human facilitation have largely erased genetic boundaries, while *T. destruens* shows the enduring impact of historical fragmentation and niche specialization.

H. ligniperda* vs *H. micklitzi

At the beginning of my doctoral research, I hypothesized that the two *Hylurgus* species possess key ecological and biological traits that shape their distinct genetic structures and demographic histories in their native ranges. Based on preliminary observations and known host associations, I predicted that *H. ligniperda* would display higher genetic diversity and a more complex population structure than *H. micklitzi*. To address this question and fill a notable gap in phylogeographic research on these bark beetles, I used an extensive sampling plan, supported through international collaborations, and screened different mitochondrial markers.

The results presented in Chapter 3 confirm my initial expectations. *H. ligniperda* exhibits higher genetic diversity and pronounced population subdivision within its native range, whereas *H. micklitzi* shows lower genetic diversity and limited genetic structure. In *H. ligniperda*, four distinct genetic groups were identified in Europe, two centered in southern France and two distributed more broadly. The higher haplotype richness observed in Mediterranean populations (mainly in mainland France) seems consistent with the "southern richness and northern purity" pattern recognized in many European taxa following the last glaciation (Hewitt 1999, Vendramin *et al.* 2021). A contact zone in the Massif Central reflects secondary admixture between southwestern and southeastern refugial lineages (Taberlet *et al.* 1998, Schmitt 2007, Rousselet *et al.* 2010), while island populations such as Corsica and Sardinia contain both dominant mainland haplotypes and private variants, suggesting historical gene flow facilitated by land bridges or anthropogenic movement (Horn *et al.* 2006, Vendramin *et al.* 2021). Furthermore, the geographic distribution of *H. ligniperda*'s genetic groups appears to correlate with the distribution of major host species such as *P. halepensis* and *P. pinaster* (Gómez *et al.* 2001, Vendramin *et al.* 2021), suggesting that host availability may have influenced population structure.

By contrast, *H. micklitzi* exhibits low genetic diversity across its range. A single dominant haplotype (HM02) was detected across most sites, with additional haplotypes confined to single locations. This pattern is consistent with a recent population expansion following a bottleneck, supported by the star-like haplotype network and demographic indices (Hickerson *et al.* 2010). In my interpretation, *H. micklitzi* likely persisted in a single or very few glacial refugia and recolonized the Mediterranean region rapidly. Its low genetic diversity aligns with its narrow host range and apparent lack of strong local adaptation (Petit *et al.* 2005). In the two islands, Corsica and Sardinia, *H. micklitzi* populations contained multiple haplotypes but no endemic lineages,

suggesting relatively recent colonization. This finding, together with its overall low differentiation, supports the hypothesis of post-glacial expansion from a restricted source population.

Regarding invasiveness, my analyses indicate that *H. ligniperda*'s higher genetic diversity, greater ecological flexibility, and broader host range may contribute to its invasion success. In non-native regions, I detected multiple independent introductions, as reflected by the presence of two dominant haplotypes (HLA01 and HLD07) across New York, California, New Zealand, China, and South Africa. Similar invasion patterns have been observed in other wood-boring pests, such as *Sirex noctilio* and *Anoplophora glabripennis* (Boissin *et al.* 2012, Javal 2017), and suggest that genetic admixture from multiple source populations can enhance colonization potential and adaptive capacity (Urvois *et al.* 2023).

In contrast, *H. micklitzi* has not been recorded as an invasive species outside its native range. Its low genetic diversity, narrow host specialization, and apparent lack of strong demographic expansion signals may limit its capacity to establish and spread in new environments. Based on these findings, I infer that *H. ligniperda* possesses several traits commonly associated with successful biological invasions, including high propagule pressure, ecological generalism, and the ability to maintain genetic variation during colonization (Guillemaud *et al.* 2010, Blackburn *et al.* 2011), while *H. micklitzi* does not.

Additionally, I identified an unexpectedly divergent haplogroup (HLE) in *H. ligniperda*, retrieved from GenBank sequences. The inter-clade divergence (4.84%) is markedly higher than the genetic distances among other groups (0.69%–2.60%). Due to incomplete metadata and potential misidentifications, I consider the interpretation of HLE as provisional and recommend further genomic work to clarify its significance.

Methodological limitations

In my doctoral research, I focused on experimental studies of *Hylurgus* species, specifically *H. ligniperda* and *H. micklitzi*. Through field surveys and genetic sampling, I examined their key biological and ecological traits to better understand their spread and adaptation. I explored aspects such as life history, host preferences, reproductive strategies, and genetic variation, with the goal of uncovering factors that might explain their invasive success in non-native regions. Additionally, I compared the phylogeographic patterns of different populations to investigate how these species have evolved and expanded both in their native areas and in newly invaded locations.

For the *Dendroctonus* and *Tomicus* species, my approach was based on synthesizing existing research rather than conducting new experiments. I collected and reviewed a wide range of studies on these species, focusing on their ecological characteristics, population dynamics, and genetic profiles. By compiling data from multiple sources, I identified key patterns and trends in their invasive behavior, helping to build a comparative understanding of how they relate to *Hylurgus* species in terms of their spread and ecological adaptability.

This combined approach allowed me to gain insights into the invasion processes of different bark beetle species and provided a solid basis for future research and management efforts.

However, several limitations should be acknowledged:

First, the genetic data for *Hylurgus* species were mainly derived from mitochondrial markers. While mitochondrial DNA provides useful information for detecting population structure and historical demographic events, it reflects only maternal lineages and may not capture the full extent of genetic variation shaped by nuclear genes. Future studies incorporating genome-wide markers, such as SNPs or whole-genome sequencing, would provide a more comprehensive picture of the species' evolutionary history and adaptive potential.

Second, the field sampling design, although extensive, was constrained by geographic accessibility and resource availability. Some potentially important regions within the native ranges of *H. ligniperda* and *H. micklitzi* were under-sampled or not sampled at all, which could have limited the detection of fine-scale population structure or rare lineages. Expanding the spatial coverage of sampling in future studies would help address this gap.

Third, while the phylogeographic interpretations for *Tomicus* and *Dendroctonus* species were based on thorough literature synthesis, the reliance on previously published datasets introduces potential inconsistencies in marker types, sampling strategies, and analytical methods among studies. This heterogeneity could have influenced comparative conclusions. Coordinated, standardized multi-species genetic studies would improve the reliability of cross-species comparisons.

Fourth, although historical and ecological contexts were considered when interpreting genetic patterns, some factors such as the precise roles of host shifts, climate variability, and human mediated dispersal remain difficult to disentangle based solely on genetic evidence. Integrating ecological niche modeling, dendrochronological studies, and experimental approaches could complement genetic inferences and provide a fuller understanding of the invasion dynamics.

Finally, some unexpected findings, such as divergent haplogroups detected in public databases, highlight the need for caution when interpreting data with incomplete metadata. Misidentifications, cryptic species, or technical errors could confound phylogeographic interpretations. Future research should prioritize high-quality genomic datasets accompanied by verified voucher specimens.

In summary, while the research provides valuable insights into the invasion biology and evolutionary dynamics of bark beetles, addressing these limitations through broader sampling, genomic approaches, standardized data collection, and interdisciplinary methods will be critical for advancing our understanding of these important forest pests.

2 Perspectives and unresolved problems

While this study sheds light on key differences in invasive potential between *H. ligniperda* and *H. micklitzi*, it also leaves several critical questions unresolved and opens new avenues for inquiry. The following section outlines some of these outstanding challenges and suggests promising directions for future research.

2.1 Global invasion history of the widest invasive bark beetle, *H. ligniperda*

2.1.1 Its management in invaded regions

In the management of *H. ligniperda*, frequent use of clear-cutting and burning, methyl bromide fumigation, or pyrethroid insecticides can severely disrupt ecosystems, reduce biodiversity (Huanquilef *et al.* 2021), and accelerate the development of insecticide resistance (D’Incao *et al.* 2012). As such, an urgent shift toward ecologically based control strategies centered on natural enemies and botanically derived compounds is needed. However, available biological control agents remain limited. In the 1970s, New Zealand released the predatory beetle *Thanasimus formicarius* and the parasitic wasp *Rhoptrocentrus piceus* against *H. ligniperda* and *H. ater*, but with limited success (Zondag 1979, Reay *et al.* 2012). In China, only laboratory studies have demonstrated the pathogenic potential of the entomopathogenic nematode *Steinernema feltiae* against *H. ligniperda* (Hu *et al.* 2004). Similarly, research on plant-derived compounds remains sparse, with Chilean scientists identifying ethanol extracts of *Cestrum parqui* as effective antifeedants under laboratory conditions (Huanquilef *et al.* 2021). In New Zealand’s largest plantation forest, *H. ligniperda* populations increased sharply within 2–3 years following harvesting due to an abundance of breeding material, with dispersal largely confined within a 400-meters radius. Sequential harvesting of adjacent stands generates repeated resource pulses that facilitate population build-up, highlighting the need for forest management strategies that maintain a 1–2 km spacing between harvest sites to mitigate outbreak and spread risk (Brockhoff *et al.* 2025a). Moving forward, systematic investigations into native natural enemies, field deployment of entomopathogenic nematodes, and the development of repellents and plant-based semiochemicals will be critical to advancing sustainable and ecologically sound control of *H. ligniperda*.

2.1.2 Invasion routes and trace of *H. ligniperda* with more resolute genetic markers

We are currently collaborating with Eckehard G. Brockhoff on a comprehensive study of *H. ligniperda*’s global spread (Brockhoff *et al.* 2025b). In his forthcoming publication, Brockhoff and his colleagues aim to clarify the true native and non-native ranges of this species by critically reviewing and reconciling historical literature records. Based on this revised understanding, they will use mitochondrial DNA markers to trace the origins of invasive populations. Meanwhile, our team has generated Restriction site Associated DNA tag

sequencing (RADseq) libraries in order to analyse the resulting Single Nucleotide Polymorphism (SNP) data.

2.1.2.1 Employing RADseq to reconstruct the global invasion pathways of *H. ligniperda*

RADseq enables high-throughput discovery of thousands of genome-wide SNPs by targeting loci adjacent to restriction enzyme cut sites, thus providing the resolution necessary to distinguish closely related source and invasive populations of non-model species (Willing et al. 2011, Andrews et al. 2016, 2018). Moreover, by utilizing codominant nucleotide substitutions, RADseq allows accurate differentiation between heterozygous and homozygous genotypes, ensuring data standardization and reproducibility across laboratories (Peterson et al. 2012, Excoffier et al. 2013). Moreover, RADseq is particularly well-suited for organisms lacking a high-quality reference genome, since *de novo* assembly of RAD tags can generate robust SNP datasets without prior genomic resources (Etter et al. 2011, Gauthier et al. 2020). Compared to whole-genome resequencing (WGS), which depends on a high-quality reference genome, RADseq is particularly well suited for non-model organisms as it enables reliable SNP discovery through *de novo* assembly of RAD tags and reduces per-sample sequencing costs by approximately 5–10-fold, significantly improving the cost-effectiveness of medium- to large-scale studies while retaining sufficient marker density for rigorous population genomic inference (Miller et al. 2007, Peterson et al. 2012, Inbar et al. 2020, Qu et al. 2020). Compared to microsatellites (SSRs), which typically include only dozens of loci and are prone to electrophoretic errors, and mitochondrial DNA (mtDNA) markers, which trace only maternal lineages, RADseq captures genome-wide genetic variation and is better suited for resolving recent or multiple-source invasion histories. Moreover, in contrast to AFLP, which produces dominant presence/absence markers with relatively lower reproducibility, RADseq offers more stable data quality and benefits from well-established downstream bioinformatics pipelines (Davey et al. 2011, Peterson et al. 2012, Andrews et al. 2016).

In the context of invasive insects, RADseq and related reduced-representation approaches have been successfully applied to reconstruct introduction histories and detect fine-scale genetic structuring. For example, genotype-by-sequencing (GBS; a RADseq derivative) resolved the complex European invasion history of the Asian longhorned beetle *Anoplophora glabripennis*, distinguishing at least three independent introduction routes into Italy and Germany (Filipović 2023). RADseq-derived SNPs have also traced the demographic expansion of the cotton mealybug *Phenacoccus solenopsis*, revealing a single primary source population in India and subsequent rapid differentiation in China (Poveda-Martínez et al. 2022). Similarly, reduced-representation sequencing of the invasive lady beetle *Harmonia axyridis* demonstrated subtle genetic divergence among North American source populations and multiple bridgehead introductions into Europe (Li et al. 2020).

Beyond beetles and Hemiptera, RADseq has elucidated invasion pathways in Lepidoptera: population genomic analyses of the fall webworm *Hyphantria cunea* in China uncovered geographic barriers that limited gene flow between southern and northern clades immediately following introduction (Poveda-Martínez et al. 2022). In bark beetles, RAD markers provided new insights into the evolutionary history of the genus *Dendroctonus*, enabling reconstruction of divergence events between native North American lineages and invasive Chinese populations of the red turpentine beetle (*D. valens*) (Godefroid et al. 2019).

Finally, integration of RADseq-derived SNPs within coalescent-based and Approximate Bayesian Computation (ABC) frameworks enables formal testing of competing demographic models and precise inference of invasion source populations and gene-flow histories (Li et al. 2020, Resh et al. 2021). Together, these examples underscore RADseq's capacity to unravel the complex invasion dynamics of *H. ligniperda* and other non-model invasive insects.

2.1.2.2 Current research progress

At present, we use RADseq to investigate the global invasion history of *H. ligniperda*, identifying its origins, genetic structure, and potential bridgehead events. By analyzing multiple invasive populations, we aim to determine whether introductions occurred from single or multiple sources and whether secondary invasions facilitated its spread.

We analysed 124 individuals of *H. ligniperda* obtained from 88 sites (Figure S1a, Table S1) located in nine native European regions (mainland France, Corsica, mainland Italy, Sardinia, Hungary, Czechia Portugal, Switzerland, and Greece) and eight invaded countries distributed over five continents (Chile, Argentina, China, USA, New Zealand, South Africa, Uruguay, and Australia). And we also added seven individuals of *H. micklitzi* as outgroup obtained from six sites (Figure S1b, Table S2) located in Europe.

In summary, by integrating mitochondrial and nuclear genomic approaches, we aim to resolve fine-scale population structure, identify secondary introduction pathways, and detect genetic signatures of adaptation to novel environments. Collectively, these efforts will deepen our understanding of *H. ligniperda*'s invasion history and guide the development of more effective biosecurity and management strategies.

2.2 Global invasive scolytids data collection and analysis

One of the most pressing future tasks is to resolve inconsistencies and fragmentation in existing literature and databases related to bark beetle invasions. Currently, species origin status, introduction pathways, and trait data are often derived from heterogeneous sources that vary in accuracy, taxonomic reliability, and geographic scope (Early et al. 2016, Pyšek et al. 2020). These inconsistencies hinder comparative analysis and risk perpetuating errors across studies (Seebens et al. 2018). Therefore, a coordinated effort is needed to build a standardized and curated global database that consolidates verified occurrence records, trait measurements,

and introduction history based on clear criteria and, where possible, supported by molecular evidence (Roy et al. 2014, Pagad et al. 2018).

Such a platform would enable meta-analyses that go beyond narrative syntheses, allowing quantitative testing of hypotheses on invasion routes, trait correlates of spread, and biogeographic trends (Gurevitch et al. 2018, Lantschner et al. 2020). Meta-analytical methods can account for variation among studies and help identify robust patterns from noisy datasets. Importantly, better database infrastructure would also facilitate dynamic updates, helping researchers and policymakers track invasion processes in near-real time (Crall et al. 2012). In this way, addressing data fragmentation is not only a technical challenge but a foundational step toward more accurate, transparent, and reproducible invasion biology.

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Appendix

List of publications

Peer-reviewed Publications

- Yuan Y.**, Roques A., Magnoux E., Ren L., Brockerhoff E.G., Shi J., Luo Y., An N., Auger-Rozenberg M.-A., Roux G. (2025). One is a worldwide invader, the other never established in other continents: contrasted phylogeographic patterns of two congeneric *Hylurgus* conifer bark beetles. *NeoBiota*, 101, 91-117. <https://doi.org/10.3897/neobiota.101.150337>
- An N., **Yuan Y.**, Zhang X., Ge S., Ren L., Roques A., Luo Y. (2024). Seven mitochondrial genomes of Tribe Hylurgini (Coleoptera: Curculionidae: Scolytinae) in Eurasia and their phylogenetic analysis. *PLOS ONE*, 19(11): e0313448. <https://doi.org/10.1371/journal.pone.0313448>
- Roques A., Ren L., Rassati D., Shi J., ..., **Yuan Y.**, ..., Millar J.G. (2023). Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species. *NeoBiota*, 84, 169–209. <https://doi.org/10.3897/neobiota.84.91096>
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- Yuan Y.**, Ren L., Yu R., Li J., Shi F., Zeng J., Luo Y. (2022). Investigation of cerambycid beetle species in pine forests invaded by pine wood nematode in Fushun, Liaoning province. *Chinese Journal of Applied Entomology*, (02), 446–456. (Chinese)

Preprints

- Brockerhoff E.G., Schläfli L., Cornejo C., Kappeler J., [...], **Yuan Y.**, Prospero S. (2025). Worldwide spread of *Hylurgus ligniperda* (Coleoptera: Scolytinae), and the potential role of bridgehead invasions. *BioRxiv*. <https://doi.org/10.1101/2025.05.17.654641> (Preprint)

Conference Presentations

Oral Presentations

- Yuan Y.**, Roques A., Magnoux E., Auger-Rozenberg M.-A., Roux G. (2024, September). Comparative phylogeographic histories of two congeneric *Hylurgus* conifer bark beetles. Pest in Changing World, Beijing, China. (*International Conference*)
- Yuan Y.**, Roques A., Magnoux E., Auger-Rozenberg M.-A., Roux G. (2023, September). Comparative phylogeographic histories of two conifer-damaging *Hylurgus* bark beetles in Eurasia. Global challenges and innovative management of bark and wood borers in planted forests, Bordeaux, France. (*International Conference*)

Poster Presentations

Yuan Y., Roques A., Magnoux E., Auger-Rozenberg M.-A., Roux G. (2023, October). Comparative phylogeographic histories of two congeneric *Hylurgus* conifer bark beetles in Eurasia. 35th Biotechnocentre symposium: Meetings in the fields of Life Sciences, Health, and Well-being in the Centre-Val de Loire region, France.

Supplementary material of chapter 1

Supplementary Table 1 The list of invasive bark beetles around the world

Bark beetle	Native region	Established nonnative region	Host type	Number of host families	Damage type	Mating behaviour	Sources
<i>Crypturgus pusillus</i>	PA	NEA	C	1	S	P	(Wood and Bright 1992, Kirkendall et al. 2015)
<i>Cyrtogenius luteus</i>	IM	NEO, WPA	C	1	S	P	(Wood and Bright 1992, Beaver and Lan-Yu 2010, Faccoli et al. 2012, Lantschner et al. 2024)
<i>Dendroctonus valens</i>	NEA	PA	C	1	P	M	(Wood and Bright 1992, Six and Bracewell 2015, Liu 2020)
<i>Dryocoetes autographus</i>	PA, NEA	NEO	C	1	S	P	(Wood and Bright 1992, Johansson et al. 1994, Wood 2007, Smith and Hulcr 2015)
<i>Dryocoetes himalayensis</i>	IM	WPA	B	2	S	P	(Wood and Bright 1992, Kirkendall et al. 2015, Kašák et al. 2023)

<i>Hylastes angustatus</i>	WPA	AFR	C	1	S	M	(Wood and Bright 1992, Lopez et al. 2007)
<i>Hylastes ater</i>	PA	NEO, AP, AFR	C	1	S	M	(Wood and Bright 1992, Kirkendall 2018, Faccoli et al. 2020, Lantschner et al. 2024)
<i>Hylastes linearis</i>	WPA	AFR, NEO	C	1	S	M	(Wingfield and Knox-Davies 1980, Wood and Bright 1992, Kirkendall 2018)
<i>Hylastes opacus</i>	PA	NEA	C	1	S	M	(Wood and Bright 1992)
<i>Hylesinus toranio</i>	WPA	EPA, NEO	B	2	S	M	(Wood and Bright 1992, Holgado 2007, Курко and Бачинська n.d.)
<i>Hylurgops palliatus</i>	PA	NEA	C	1	S	M	(Wood and Bright 1992, Mercado-Velez and Negron 2014)
<i>Hylurgus ligniperda</i>	WPA	NEA, NEO, AFR, AP, EPA	C	1	S	M	(Wood and Bright 1992, CABI 2021a, Lin et al. 2021, Ren et al. 2021, Lantschner et al. 2024)

<i>Hypocryphalus dilutus</i>	IM	PA, NEA	B	2	S	M	(Vega and Hofstetter 2014, Barnouin et al. 2020, Yang 2020, Gugliuzzo et al. 2023, Justesen et al. 2023)
<i>Hypocryphalus mangiferae</i>	IM	NEA, NEO, AFR, AP	B	1	S	M	(Al Adawi et al. 2013, Johnson et al. 2017)
<i>Hypocryphalus scabricollis</i>	IM	WPA	B	8	S	M	(Vega and Hofstetter 2014, Kirkendall 2018)
<i>Ips calligraphus</i>	NEA	IM, WPA, NEO	C	1	S	P	(Wood and Bright 1992, Griffin et al. 2020, Li et al. 2021, Mas and Johnson 2023, Bobadilla Peñaló and Pérez 2025)
<i>Ips grandicollis</i>	NEA	AP, IM, WPA, NEO	C	1	S	P	(Wood and Bright 1992, Griffin et al. 2020, Li et al. 2021, Mas and Johnson 2023)

							(Wood and Bright 1992,
<i>Orthotomicus angulatus</i>	EPA, IM	AP	C	2	S	P	Brockerhoff et al. 2006, Kirkendall et al. 2015)
<i>Orthotomicus caelatus</i>	NEA	AFR	C	1	S	P	(Wood and Bright 1992, Kirkendall et al. 2015)
<i>Orthotomicus erosus</i>	PA	NEA, NEO, AFR	C	1	P	P	(Brockerhoff et al. 2006, Kirkendall et al. 2015, Seybold et al. 2016, Kirkendall 2018)
<i>Orthotomicus laricis</i>	PA	NEO	C	1	S	P	(Wood and Bright 1992, Kirkendall et al. 2015, Kirkendall 2018)
<i>Orthotomicus proximus</i>	PA	AFR	C	1	S	P	(Wood and Bright 1992, Kirkendall et al. 2015)
<i>Phloeosinus armatus</i>	WPA	NEA	C	1	S	M	(Lieutier et al. 2016)
<i>Phloeosinus cupressi</i>	NEA	NEO, AP	C	1	S	M	(Wood and Bright 1992, Kirkendall et al. 2015)

<i>Phloeotribus liminaris</i>	NEA	WPA	B	1	S	M	(Wood and Bright 1992, Pennacchio et al. 2004, Ethington et al. 2021)
<i>Phloeotribus scarabaeoides</i>	WPA	NEA	B	3	S	M	(R. Gonzalez and M. Campos 1990, Wood and Bright 1992, Pennacchio et al. 2004, Mifsud and Knížek 2009, Anon 2024)
<i>Pityogenes bidentatus</i>	PA	NEA, AFR	C	1	S	P	(Hoebeke 1989, Wood and Bright 1992, Byers 2012, Kirkendall et al. 2015)
<i>Pityogenes chalcographus</i>	PA	NEO	C	1	S	P	(Wood and Bright 1992, Brockhoff et al. 2006)
<i>Pityokteines curvidens</i>	WPA	EPA, AFR, NEO	C	1	S	P	(Noboch 1974, Wood and Bright 1992, Kirkendall et al. 2015, CABI 2021b)
<i>Pityophthorus juglandis</i>	NEA	WPA	B	1	P	P	(Parker 2020, Marchioro and Faccoli 2022)

<i>Pityophthorus solus</i>	NEA, NEP	WPA	C	1	S	unknown	(Bright 1981, Goldarazena et al. 2014)
<i>Polygraphus poligraphus</i>	PA	AFR	C	1	S	P	(Wood and Bright 1992, Viklund et al. 2025)
<i>Polygraphus rufipennis</i>	NEA	AFR	C	1	S	P	(Bowers et al. 1991, Wood and Bright 1992, Park 2002)
<i>Scolytogenes jalapae</i>	IM, EPA, AP(?)	NEO, NEA	B	4	S	M	(Wood 2007, Ferro and Gimmel 2011, de Souza Covre et al. 2015)
<i>Scolytus kirschii</i>	PA	AFR, NEO	B	5	P	M	(Kraatz 1876, Wood and Bright 1992, Six et al. 2005, Wood 2007, Özgen 2019)
<i>Scolytus mali</i>	WPA	NEA	B	2	S	M	(Rudinsky et al. 1978, Wood and Bright 1992, K. B. Isayeva 2021)
<i>Scolytus multistriatus</i>	WPA	NEA, NEO, AP	B	1	S	M	(Wood and Bright 1992, Smith and Cognato 2014)
<i>Scolytus rugulosus</i>	PA	NEA, NEO	B	2	S	M	(Wood and Bright 1992, Smith and Cognato 2014)

							(Wood and Bright 1992, Smith and
<i>Scolytus schevyrewi</i>	EPA	NEA	C	6	S	M	Cognato 2014)
<i>Tomicus piniperda</i>	PA	NEA	C	1	S	M	(Lieutier et al. 2015)

Abbreviation: Biogeography: AFR: Afrotropic, AP: Austro-Pacific, IM: Indo-Malaya, NEA: Nearctic, NEO: Neotropic, WPA: West-Palearctic, EPA: East-Palearctic; Host type: C: coniferous trees; B: broadleaf trees; Damage type: P: primary pest, S: secondary pest; Mating behavior: M: monogamous species, P: polygamous species.

Supplementary material of chapter 3

Supplementary Table 1 Summary of the localities sampled and specimens used in the COI sequencing analyses of *H. ligniperda* and *H. micklitzi*

Species	Range	Sender (Genbank/accession number/uffelD)	Country	State/Province/ Region	Locality (*)	Forest or port	Year	Lat.	Long.	No. of individuals collected	No. in COI analysis (haplotype)	Hd	Nd	Mitochondrial cluster
<i>Hylurgus ligniperda</i>	North America	Genbank (JQ015128, JQ015129, JQ015130, JQ015131)	Museum collection (unknown origin)	-	-	-	-	-	-	-	4 (HLE01, HLE02, HLE03, HLE04)	1	0.01326	HLE
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701596)	Portugal	Alentejo	Coruche	no record	2016	38.98	-8.38	3	1 (HLD03)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701583, PV701584, PV701585)	Italy	Sardinia	Alghero	no record	2023	40.66	8.32	30	3 (HLB02, HLD07)	0.667	0.00807	HLB, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701602)	France	Alpes-Maritimes	Le Bar-sur-Loup	<i>Pinus halepensis</i>	2021	43.73	6.97	1	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701598, PV701599)	France	Alpes-Maritimes	Malaussène	<i>Pinus halepensis</i>	2021	43.96	7.14	2	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701600, PV701601)	France	Alpes-Maritimes	Seranon	<i>Pinus halepensis</i>	2021	43.86	6.70	2	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701603)	France	Ardèche	Coux	no record	2021	44.75	4.62	13	1 (HLA10)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701604)	France	Ardèche	Saint-priest	no record	2021	45.69	4.97	1	1 (HLA10)	-	-	HLA

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701605)	France	Ariège	Lordat	no record	2021	42.85	1.76	1	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701606, PV701607, PV701608)	France	Aude	Conques sur Orbiel	no record	2021	43.34	2.39	14	3 (HLC12, HLD07)	0.667	0.01384	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701612, PV701613, PV701614)	France	Aude	Ferrals-les Corbières	<i>Pinus halepensis</i>	2021	43.23	2.74	20	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701609, PV701610, PV701611)	France	Aude	Gruissan*	<i>Pinus halepensis</i>	2021	43.18	3.08	5	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701615, PV701616)	France	Aude	Quillan	<i>Pinus halepensis</i>	2021	42.91	2.19	2	2 (HLB02, HLC04)	1	0.01211	HLB, HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701617, PV701618, PV701619)	France	Aveyron	Millau	no record	2021	44.20	3.11	5	3 (HLC04, HLD07)	0.667	0.01153	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701623, PV701624, PV701625, PV701626, PV701627, PV701628)	France	Bouches-du-Rhône	Gardanne*	<i>Pinus halepensis</i>	2021	43.49	5.48	27	6 (HLA01, HLA05, HLA11, HLD07)	0.8	0.00807	HLA, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701620, PV701621, PV701622)	France	Bouches-du-Rhône	La Ciotat*	<i>Pinus pinea</i>	2021	43.20	5.61	17	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701635, PV701636, PV701637)	France	Bouches-du-Rhône	Le Puy-Ste-Réparate*	<i>Pinus halepensis</i>	2021	43.71	5.45	9	3 (HLA01)	0	0	HLA

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701632, PV701633, PV701634)	France	Bouches-du-Rhône	Marignane*	<i>Pinus halepensis</i>	2021	43.51	5.18	5	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701629, PV701630, PV701631)	France	Bouches-du-Rhône	Tarascon*	<i>Pinus halepensis</i>	2021	43.94	4.67	14	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701638)	France	Charente-Maritime	La Rochelle	port	2018	46.15	-1.15	1	1 (HLD09)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701647, PV701648)	France	Corse-du-Sud	Coti-Chiavari	no record	2023	41.77	8.77	2	2 (HLC08)	0	0	HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701639, PV701640, PV701641)	France	Corse-du-Sud	Figari*	<i>Pinus nigra laricio</i>	2021	41.49	9.13	30	3 (HLA01, HLD07)	0.667	0.01269	HLA, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701642, PV701643)	France	Corse-du-Sud	Porto-Vecchio	<i>Pinus nigra laricio</i>	2021	41.58	9.26	26	2 (HLA01, HLA12)	1	0.00173	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701644, PV701645, PV701646)	France	Corse-du-Sud	San-Gavinu-di-Carbini	<i>Pinus nigra laricio</i>	2021	41.71	9.18	16	3 (HLA01, HLC08)	0.667	0.01384	HLA, HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701649)	France	Haute-Corse	Serra-di-Fiumorbo*	no record	2023	41.98	9.33	1	1 (HLA08)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701650, PV701651, PV701652)	France	Drôme	Châteauneuf-de-Bordette	no record	2021	44.34	5.17	4	3 (HLA01, HLA12)	0.667	0.00115	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701653, PV701654)	France	Drôme	Cliousclat	no record	2021	44.72	4.83	2	2 (HLA01)	0	0	HLA

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701655, PV701656, PV701657)	France	Drôme	Nyons	no record	2021	44.35	5.15	20	3 (HLA01, HLA06, HLA10)	1	0.00346	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701658, PV701659, PV701660)	France	Eure	Montfort-sur-Risle	no record	2021	49.29	0.67	7	3 (HLC07, HLC11, HLD07)	1	0.01615	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701665, PV701666)	France	Gard	Aramon*	<i>Pinus halepensis</i>	2021	43.95	4.69	9	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701661, PV701662, PV701663, PV701664)	France	Gard	Milhaud*	<i>Pinus halepensis</i>	2021	43.85	4.30	5	4 (HLA01, HLD07)	0.5	0.00952	HLA, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701672, PV701673, PV701674)	France	Gers	Lupiac	no record	2021	44.02	0.12	5	3 (HLA01, HLC12)	0.667	0.01499	HLA, HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701675, PV701676)	France	Gironde	Bordeaux	<i>Pinus halepensis</i>	2023	44.49	-0.83	16	2 (HLD01, HLD07)	1	0.00173	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701669, PV701670)	France	Gironde	Toulouse*	no record	2021	43.65	1.46	14	2 (HLD05, HLD07)	1	0.00173	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701671)	France	Haute Garonne	Lagrâce-Dieu	no record	2021	43.61	1.42	1	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701667, PV701668)	France	Haute Garonne	Saint-Gaudens	no record	2021	43.30	0.67	2	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701597)	France	Haute-Alpes	Guillestre	<i>Pinus sylvestris</i>	2021	44.89	6.65	1	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	North America	WSL collaborators (PV701519)	USA	California	San Dimas	no record	2020	34.08	-117.78	3	1 (HLA01)	-	-	HLA

<i>Hylurgus ligniperda</i>	North America	WSL collaborators (PV701520)	USA	California	San Marino	no record	2020	34.13	-118.12	3	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701579)	Greece	Central Macedonia	Thessaloniki	no record	2020	40.63	22.98	5	1 (HLB06)	-	-	HLB
<i>Hylurgus ligniperda</i>	Europe	Dimitrios N. Avtzis (PV701576, PV701577, PV701578)	Greece	Central Macedonia	Thessaloniki*	no record	2023	40.48	25.53	32	3 (HLB05)	0	0	HLB
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701531, PV701532, PV701533)	Chile	Central Region	Sawmill Colorado	no record	2020	-37.43	-73.35	3	3 (HLD07, HLD08)	0.667	0.00115	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701689)	France	Loire	Champdieu	no record	2021	45.63	4.05	1	1 (HLA09)	-	-	HLA
<i>Hylurgus ligniperda</i>	Asia	Genbank (MZ562958)	South Korea	Chungcheongnam-do	Buyeo-gun	no record	2008	36.52	126.79	-	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701724, PV701725)	France	Marne	Nuisement-sur-Coole	no record	2021	48.87	4.27	6	2 (HLA04, HLA07)	1	0.00346	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701730)	France	Hautes-Pyrénées	Ade	no record	2022	43.14	-0.02	1	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701677, PV701678, PV701679)	France	Hérault	Bédarieux*	<i>Pinus halepensis</i>	2021	43.70	3.17	9	3 (HLA01, HLB02, HLD07)	1	0.01384	HLA, HLB, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701682, PV701683, PV701684, PV701685, PV701686)	France	Hérault	Bessan*	<i>Pinus halepensis</i>	2021	43.40	3.42	30	5 (HLA14, HLB02, HLB04, HLC05)	0.9	0.00865	HLA, HLB, HLC

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701680, PV701681)	France	Hérault	Olmet-et-Villecun*	<i>Pinus halepensis</i>	2021	43.75	3.30	2	2 (HLA01, HLA13)	1	0.00346	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701687)	France	Ille-et-Vilaine	Saint-Malo	no record	2017	48.64	-2.03	1	1 (HLC01)	-	-	HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701698, PV701699, PV701700)	France	Loiret	Ingrannes	<i>Pinus sylvestris</i>	2021	48.01	2.24	12	3 (HLC09, HLC11)	0.667	0.00115	HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701701, PV701702)	France	Lot	Labastide- Marnhac	no record	2021	44.44	1.41	2	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701695, PV701696, PV701697)	France	Loiret	Lorris	<i>Pinus sylvestris</i>	2021	47.88	2.50	37	3 (HLC07, HLC11, HLC13)	1	0.00923	HLC
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701693)	France	Loiret	Orléans Nord	<i>Pinus sylvestris</i> / <i>P. nigra</i>	2017	47.91	1.92	1	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701694)	France	Loiret	Orléans Inrae	<i>Pinus sylvestris</i> / <i>P. nigra</i>	2017	47.83	1.91	1	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701688)	France	Loir-et-Cher	Lamotte-Beuvron	<i>Pinus sylvestris</i> / <i>P. nigra</i>	2021	47.60	2.03	1	1 (HLA07)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701709)	France	Lot	Figeac	<i>Pinus sylvestris</i>	2021	44.99	2.11	1	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701710, PV701711)	France	Lot	Montcabrier	no record	2021	44.58	1.08	2	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701703, PV701704, PV701705)	France	Lot	Saint-Denis- Catus	<i>Pinus sylvestris</i>	2021	44.62	1.40	4	3 (HLD04, HLD07)	0.667	0.00231	HLD

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701706, PV701707, PV701708)	France	Lot	Salviac	<i>Pinus sylvestris</i>	2021	44.73	1.27	13	3 (HLC02, HLD04, HLD07)	1	0.01269	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701713, PV701714, PV701715)	France	Lozère	Barre-des-Cévennes	no record	2022	44.37	4.14	12	3 (HLA01, HLA07)	0.667	0.00115	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701716)	France	Lozère	Mende	no record	2022	44.51	3.49	1	1 (HLA08)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701712)	France	Lozère	Saint Etiennev	no record	2021	45.45	4.26	2	1 (HLA03)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701595)	Spain	Girona	Maçanet de la Selva	no record	2021	41.77	2.69	3	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701722, PV701723)	France	Maine-et-Loire	Montreuil-sur-Loir	no record	2021	47.60	3.09	2	2 (HLC03, HLD07)	1	0.01557	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701726, PV701727, PV701728)	France	Meurthe-Moselle	Ecrouves	no record	2021	48.69	5.83	3	3 (HLC07, HLC11, HLD07)	1	0.01615	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701729)	France	Pyrénées-Atlantiques	Bayonne*	<i>Pinus pinaster</i>	2017	43.52	-1.49	2	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701736, PV701737, PV701738)	France	Pyrénées-Orientales	Canohès	no record	2021	42.65	2.84	30	3 (HLD06, HLD07)	0.667	0.00115	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701731, PV701732, PV701733)	France	Pyrénées-Orientales	Llupia	no record	2021	42.63	2.78	13	3 (HLD07)	0	0	HLD

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701734, PV701735)	France	Pyrénées-Orientales	Salses-le-Château	no record	2021	42.84	2.92	5	2 (HLD07, HLD08)	1	0.00173	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701741, PV701742)	France	Tarn	Saurs	no record	2021	43.90	1.83	18	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701739, PV701740)	France	Tarn	Saint Avit	no record	2021	43.51	2.12	2	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701746)	France	Tarn-et-Garonne	Montaïn	no record	2022	43.94	1.12	2	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701743, PV701744, PV701745)	France	Tarn-et-Garonne	Saint-Paul-d'Espis	no record	2021	44.14	1.01	22	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701747, PV701748)	France	Tarn-et-Garonne	Saint-Porquier	no record	2021	44.00	1.18	2	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701749, PV701750, PV701751)	France	Tarn-et-Garonne	Saint-Vincent-Lespinnasse	no record	2021	44.12	0.96	8	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701753, PV701754, PV701755)	France	Var	Hyères	<i>Pinus pinea</i>	2018	43.14	6.13	4	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701756, PV701757, PV701758)	France	Var	La Londe-les-Maures*	<i>Pinus pinea</i>	2021	43.19	6.24	25	3 (HLA01, HLB04)	0.667	0.00692	HLA, HLB
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701765, PV701766, PV701767)	France	Var	La Môle	<i>Pinus pinea</i>	2021	43.24	6.47	12	3 (HLA01)	0	0	HLA

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701759, PV701760, PV701761)	France	Var	La Valette*	<i>Pinus pinea</i>	2021	43.29	5.97	10	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701752)	France	Var	Le Thoronet*	<i>Pinus halepensis</i>	2018	43.45	6.28	1	1 (HLA02)	-	-	HLA
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701582)	Czech Republic	Hradec Králové	Trebechovice pad Orebem	no record	2021	50.12	16.22	3	1 (HLB01)	-	-	HLB
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701762, PV701763, PV701764)	France	Var	Roquebrune sur Argens*	no record	2021	43.58	6.58	17	3 (HLA01, HLC08)	0.667	0.01384	HLA, HLC
<i>Hylurgus ligniperda</i>	Africa	WSL collaborators (PV701537, PV701538, PV701539)	South Africa	KwaZulu-Natal	Winterhaven	no record	2021	-29.27	30.54	3	3 (HLD03)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701771, PV701772, PV701773)	France	Vaucluse	Gargas	no record	2021	43.94	5.35	13	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701768, PV701769, PV701770)	France	Vaucluse	La Bastidonne*	no record	2021	43.72	5.57	27	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701717, PV701718, PV701719)	France	Maine-et-Loire	Brain-sur-Allonnes	no record	2020	47.32	0.08	5	3 (HLC09, HLD07)	0.667	0.01269	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701781, PV701782, PV701783)	France	Vendée	La Barre-de-Monts	no record	2021	46.87	-2.12	11	3 (HLD07)	0	0	HLD

<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701690, PV701691)	France	Loire-Atlantique	Le Gâvre	no record	2021	47.52	-1.78	4	2 (HLC09, HLD07)	1	0.01903	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701720, PV701721)	France	Maine-et-Loire	Lézigné	no record	2021	47.64	-0.30	2	2 (HLA01, HLD07)	1	0.01903	HLA, HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701775, PV701777, PV701778)	France	Vendée	Longeville-sur- Mer	no record	2020	46.41	-1.47	8	3 (HLD02, HLD07)	0.667	0.00115	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701692)	France	Loire-Atlantique	Lusanger	no record	2021	47.67	-1.58	4	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701774, PV701776)	France	Vendée	Olonne-sur-Mer	no record	2020	46.55	-1.77	3	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Europe	INRAE collaborators (PV701779, PV701780)	France	Vendée	La Faute-sur-Mer Pointe d'Arçay	no record	2021	46.43	-1.27	4	2 (HLD03, HLD07)	1	0.00173	HLD
<i>Hylurgus ligniperda</i>	Africa	WSL collaborators (PV701540)	South Africa	Mpumalanga	Kaapschehoop, Mbombela	no record	2020	-25.57	30.76	3	1 (HLD03)	-	-	HLD
<i>Hylurgus ligniperda</i>	Oceania	WSL collaborators (PV701541, PV701542, PV701543)	Australia	New South Wales	Bondi Forest	no record	2021	-37.17	149.25	3	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	North America	WSL collaborators (PV701518)	USA	New York	Monroe County, Rochester, Genesee Valley Park	no record	2021	43.12	-77.63	3	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	North America	WSL collaborators (PV701517)	USA	New York	Monroe County, Rochester, West Durand Eastman Park	no record	2021	43.23	-77.57	3	1 (HLA01)	-	-	HLA

<i>Hylurgus ligniperda</i>	Oceania	WSL collaborators (PV701546, PV701547)	New Zealand	North Island	Bay of Plenty, Rotorua	no record	2020	-38.16	176.27	4	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701527, PV701528, PV701529)	Chile	North Region	Sawmill Viñales	no record	2020	-35.37	-72.4	3	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701587)	Italy	Pescara	Pineta d'Annunzio	no record	2019	42.45	14.24	3	1 (HLC08)	-	-	HLC
<i>Hylurgus ligniperda</i>	Oceania	WSL collaborators (PV701544)	Australia	Queensland	Bribie Island	no record	2021	-26.99	153.13	3	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701534)	Argentina	Rio Negro	Bariloche	no record	2020	-41.12	-71.25	3	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701535, PV701536)	Argentina	Rio Negro	El Bolsón	no record	2018	-42	-71.52	3	2 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701588, PV701589, PV701590)	Italy	Sardinia	Macchiareddu*	no record	2023	39.23	8.96	4	3 (HLD07)	0	0	HLD
<i>Hylurgus ligniperda</i>	Asia	Genbank (MZ562956)	China	Shandong	Yantai	no record	44105	37.46	121.99	-	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	Asia	BJFU collaborators (PV701560, PV701561, PV701562)	China	Shandong	Qingdao	<i>Pinus thunbergii</i>	2023	36.17	120.58	11	3 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Asia	BJFU collaborators (PV701549, PV701550, PV701551, PV701552, PV701553, PV701554,	China	Shandong	Tai'an	<i>Pinus thunbergii</i>	2022, 2023	36.21	117.09	35	9 (HLA01, HLD07)	0.556	0.01057	HLA, HLD

		PV701555, PV701556, PV701557)												
<i>Hylurgus ligniperda</i>	Asia	BJFU collaborators (PV701558, PV701559)	China	Shandong	Weihai	<i>Pinus thunbergii</i>	2023	37.47	121.87	20	2 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Asia	BJFU collaborators (PV701563, PV701564, PV701565, PV701566, PV701567, PV701568, PV701569, PV701570, PV701571, PV701572, PV701573, PV701574, PV701575)	China	Shandong	Yantai	<i>Pinus thunbergii</i>	2021, 2022	37.45	121.70	206	13 (HLA01)	0	0	HLA
<i>Hylurgus ligniperda</i>	Oceania	WSL collaborators (PV701548)	New Zealand	South Island	Canterbury, West Melton	no record	2020	-43.47	172.38	4	1 (HLA01)	-	-	HLA
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701530)	Chile	South Region	Sawmill Valdivia	no record	2020	-39.52	-72.84	3	1 (HLD07)	-	-	HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701580, PV701581)	Hungary	Bacs-Kiskun	Bugacpusztahaza	no record	2021	46.71	19.61	2	2 (HLB01,HLC06)	1	0.01903	HLB, HLC
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701523, PV701524, PV701525, PV701526)	Uruguay	Tacuarembó	Agroempresa Forestal	no record	2020	-31.83	-56.06	4	4 (HLC10)	0	0	HLC
<i>Hylurgus ligniperda</i>	South America	WSL collaborators (PV701521, PV701522)	Uruguay	Tacuarembó	Lumin	no record	2020	-31.62	-55.72	4	2 (HLC10, HLD03)	1	0.02076	HLC, HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701591)	Switzerland	Ticino	Irana	no record	2022	46.35	8.96	3	1 (HLA01)	-	-	HLA

<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701592, PV701593, PV701594)	Switzerland	Ticino	Leggiuna	no record	2022	46.39	8.98	3	3 (HLA01, HLB03, HLD07)	1	0.01269	HLA, HLB, HLD
<i>Hylurgus ligniperda</i>	Europe	WSL collaborators (PV701586)	Italy	Veneto	Mestre	no record	2021	45.49	12.29	3	1 (HLC06)	-	-	HLC
<i>Hylurgus ligniperda</i>	Oceania	WSL collaborators (PV701545)	Australia	Victoria	Hotspur	no record	2021	-37.94	141.56	3	1 (HLC07)	-	-	HLC
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691910, PV691911, PV691912, PV691913, PV691914)	Italy	Sardinia	Cagliari	no record	2023	39.21	9.08	136	5 (HM01, HM02)	0.4	0.00069	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691915, PV691916)	Italy	Sardinia	Macchiareddu*	no record	2023	39.25	9.00	153	2 (HM01, HM02)	1	0.00173	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691917, PV691918, PV691919)	Italy	Sardinia	Zona Industriale San Marco	no record	2023	40.66	8.32	106	3 (HM01, HM02)	0.667	0.00115	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691898, PV691899, PV691900, PV691901, PV691902)	Spain	Aragon	Huesca	Pinus halepensis	2013	42.29	-0.72	408	5 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691892, PV691893, PV691894, PV691895, PV691896)	Spain	Catalonia	Barcelone	Pinus halepensis	2013	41.62	1.85	23	5 (HM01, HM02)	0.4	0.00069	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691897)	Spain	Catalonia	Berga	Pinus nigra/P. pinea	2013	42.05	1.88	4	1 (HM03)	-	-	-

<i>Hylurgus micklitzi</i>	Europe	Dimitrios N. Avtzis (PV691920, PV691921, PV691922) INRAE collaborators	Greece	Central Macedonia	Thessaloniki*	no record	2023	40.48	25.53	40	3 (HM01)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	(PV691905, PV691906, PV691907, PV691908, PV691909) INRAE collaborators	Italy	Liguria	Vintimille	<i>Pinus halepensis</i>	2017	43.80	7.60	39	5 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	(PV691929, PV691930, PV691931, PV691932, PV691933) INRAE collaborators	France	Alpes-Maritimes	Antibes	<i>Pinus halepensis</i>	2018	43.56	7.12	17	5 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	(PV691934, PV691935, PV691936) INRAE collaborators	France	Alpes-Maritimes	Cap-d'ail	<i>Pinus halepensis</i>	2018	43.72	7.41	7	3 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	(PV691937) INRAE collaborators	France	Alpes-Maritimes	Nice	<i>Pinus halepensis</i>	2018	43.73	7.18	2	1 (HM02)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	(PV691938) INRAE collaborators	France	Alpes-Maritimes	Villeneuve-Loubet	<i>Pinus halepensis</i>	2018	43.65	7.11	2	1 (HM01)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	(PV691939) INRAE collaborators	France	Aude	Gruissan*	<i>Pinus halepensis</i>	2021	43.18	3.08	25	1 (HM01)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	(PV691940, PV691941, PV691942, PV691943, PV691944) INRAE collaborators	France	Aude	Pouzols-Minervois	<i>Pinus halepensis</i>	2021	43.29	2.80	24	5 (HM01, HM02, HM04)	0.7	0.00173	-

<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691968, PV691969, PV691970)	France	Bouches-du-Rhône	Aix-en-Provence	<i>Pinus halepensis</i>	2021	43.50	5.42	29	3 (HM02, HM06)	0.667	0.00115	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691979, PV691980, PV691981)	France	Bouches-du-Rhône	Belcodène	<i>Pinus halepensis</i>	2021	43.42	5.59	19	3 (HM01, HM02)	0.667	0.00115	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691945, PV691946, PV691947, PV691948, PV691949, PV691950, PV691951, PV691952, PV691953, PV691954, PV691955, PV691956, PV691957)	France	Bouches-du-Rhône	Fos-sur-Mer	<i>Pinus halepensis</i>	2016, 2017, 2018	43.60	4.97	85	13 (HM02, HM05)	0.154	0.00027	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691971, PV691972, PV691973, PV691974, PV691975)	France	Bouches-du-Rhône	Gardanne*	<i>Pinus halepensis</i>	2021	43.49	5.48	20	5 (HM02, HM07)	0.4	0.00069	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691985, PV691986, PV691987)	France	Bouches-du-Rhône	La Ciotat*	<i>Pinus pinea</i>	2021	43.19	5.58	25	3 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691988, PV691989)	France	Bouches-du-Rhône	Lamanon	<i>Pinus halepensis</i>	2021	43.71	5.08	25	2 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691982, PV691983, PV691984)	France	Bouches-du-Rhône	Lambesc	<i>Pinus halepensis</i>	2021	43.66	5.23	25	3 (HM02)	0	0	-

<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691990, PV691991, PV691992)	France	Bouches-du-Rhône	Le Puy-Ste-Réparate*	<i>Pinus halepensis</i>	2021	43.65	5.43	20	3 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691976, PV691977)	France	Bouches-du-Rhône	Marignane*	<i>Pinus halepensis</i>	2021	43.51	5.18	21	2 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691958, PV691959, PV691960, PV691961, PV691962, PV691963, PV691964, PV691965, PV691966, PV691967)	France	Bouches-du-Rhône	Marseille	<i>Pinus halepensis</i>	2016, 2017, 2018	43.29	5.38	188	10 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	Genbank (KM285773)	France	Bouches-du-Rhône	Saint-Paul-lez-Durance	no record	2010	43.69	5.79	-	1 (HM02)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691978)	France	Bouches-du-Rhône	Tarascon*	<i>Pinus halepensis</i>	2021	43.94	4.67	20	1 (HM08)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691903, PV691904)	Spain	Valencian Community	Valencia	port	2023	39.45	-0.32	2	2 (HM01)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691924, PV691925)	France	Corse-du-Sud	Figari*	<i>Pinus pinaster</i>	2021	41.49	9.13	5	2 (HM01, HM02)	1	0.00173	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691923)	France	Corse	Sainte-Lucie-de-Porto-Vecchio	<i>Pinus nigra laricio</i>	2016	41.70	9.35	1	1 (HM02)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691926, PV691927, PV691928)	France	Corse	Serra di Fiumorbu*	no record	2023	41.98	9.33	41	3 (HM02)	0	0	-

<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692037, PV692038, PV692039)	France	Creuse	Le Pradet	no record	2021	43.11	6.00	58	3 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691994, PV691995, PV691996, PV691997, PV691998)	France	Gard	Aramon*	<i>Pinus halepensis</i>	2021	43.95	4.69	25	5 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691993)	France	Gard	Milhaud*	<i>Pinus halepensis</i>	2021	43.85	4.30	31	1 (HM02)	-	-	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV691999, PV692000, PV692001, PV692002, PV692003)	France	Gard	Nîmes	<i>Pinus halepensis</i>	2021	43.86	4.30	31	5 (HM02, HM09, HM10)	0.7	0.00138	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692004, PV692005, PV692006)	France	Gironde	Toulouse*	no record	2021	43.57	1.47	18	3 (HM01, HM02)	0.667	0.00115	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692025, PV692026, PV692027)	France	Hérault	Bédarieux*	no record	2021	43.70	3.17	4	3 (HM01, HM02)	0.667	0.00115	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692020, PV692021, PV692022, PV692023)	France	Hérault	Bessan*	no record	2021	43.40	3.42	31	4 (HM01, HM04, HM12)	0.833	0.0026	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692007, PV692008, PV692009, PV692010, PV692011, PV692012,	France	Hérault	Gignac	<i>Pinus halepensis</i>	2014, 2016, 2017, 2018	43.63	3.58	143	9 (HM01, HM02, HM04)	0.639	0.00125	-

		PV692013, PV692014, PV692015)												
		INRAE collaborators									4 (HM01,			
<i>Hylurgus micklitzii</i>	Europe	(PV692016, PV692017, PV692018, PV692019)	France	Hérault	Montpellier	no record	2021	43.64	3.87	5	HM02, HM07, HM11)	1	0.0026	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692024)	France	Hérault	Olmet-et-Villecun*	no record	2021	43.75	3.30	1	1 (HM01)	-	-	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692028)	France	Pyrénées- Atlantiques	Bayonne*	<i>Pinus pinaster</i>	2017	43.52	-1.49	2	1 (HM04)	-	-	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692029, PV692030, PV692031, PV692032, PV692033)	France	Pyrénées-Orientales	Prades	no record	2013	42.61	2.40	662	5 (HM02)	0	0	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692034, PV692035)	France	Pyrénées-Orientales	Vendres	port	2016, 2017	42.56	3.09	4	2 (HM01, HM04)	1	0.00346	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692054)	France	Var	Carqueiranne	<i>Pinus halepensis</i>	2021	43.09	6.06	25	1 (HM16)	-	-	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692036)	France	Var	Crau	<i>Pinus halepensis</i>	2018	43.15	6.08	2	1 (HM01)	-	-	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692052, PV692053)	France	Var	La Londe-les- Maures*	<i>Pinus pinea</i>	2021	43.19	6.24	25	2 (HM14, HM15)	1	0.00692	-
<i>Hylurgus micklitzii</i>	Europe	INRAE collaborators (PV692048, PV692049, PV692050, PV692051)	France	Var	La Valette*	<i>Pinus pinea</i>	2021	43.29	5.97	25	4 (HM02)	0	0	-

<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692040, PV692041, PV692042, PV692043)	France	Var	Le Thoronet*	<i>Pinus halepensis</i>	2018	43.45	6.30	184	4 (HM02, HM13)	0.5	0.00087	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692046, PV692047)	France	Var	Roquebrune sur Argens*	<i>Pinus halepensis</i>	2021	43.58	6.58	25	2 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692044, PV692045)	France	Var	Six-Fours-les-Plages	<i>Pinus halepensis</i>	2021	43.07	5.84	25	2 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692057, PV692058, PV692059, PV692060)	France	Vauclus	La Bastidonne*	<i>Pinus halepensis</i>	2021	43.72	5.57	30	4 (HM02)	0	0	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692055, PV692056)	France	Vauclus	Taillades	<i>Pinus halepensis</i>	2021	43.83	5.09	5	2 (HM02, HM10)	1	0.00173	-
<i>Hylurgus micklitzi</i>	Europe	INRAE collaborators (PV692061, PV692062, PV692063, PV692064)	France	Vauclus	Vedène	<i>Pinus halepensis</i>	2021	43.97	4.90	5	4 (HM02, HM10)	0.5	0.00087	-

Abbreviations: Hd, haplotype diversity; Nd, nucleotide diversity.

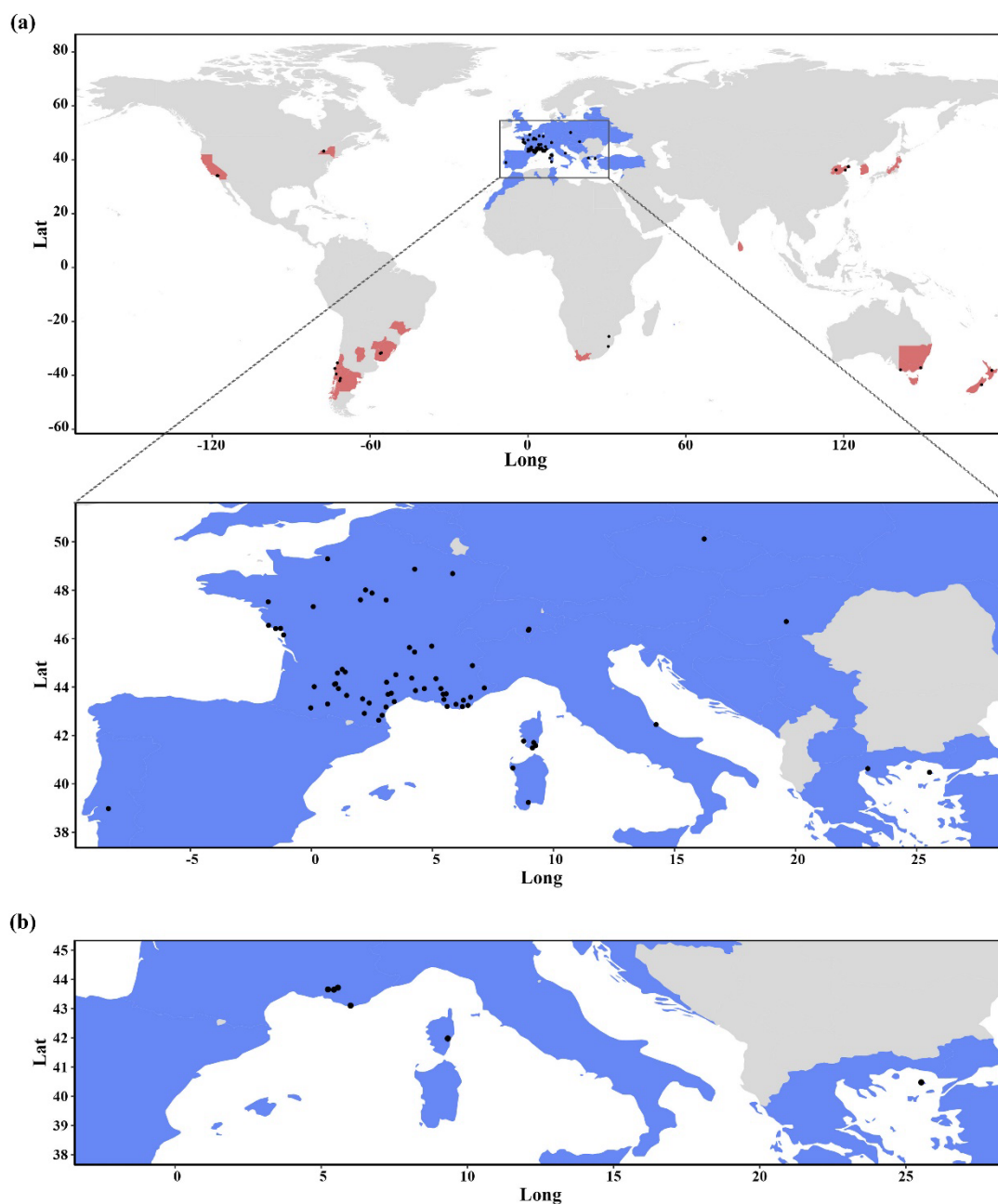
Supplementary Table 2 Summary of the localities sampled and specimens used in the COI sequencing analyses of *H. ligniperda* and *H. micklitzi* in sympatry

Range	Country	State/Province/ Region	Locality(*)	Forest or port	Year	Lat.	Long.	<i>Hylurgus ligniperda</i>				<i>Hylurgus micklitzi</i>			
								No. in COI analysis	No. of	Hd	Nd	No. in COI analysis	No. of	Hd	Nd
								(haplotype)	haplotype			(haplotype)	haplotype		
Europe	Italy	Sardinia	Macchiareddu*	no record	2023	39.23	8.96	3 (HLD07)	1	0	0	2 (HM01, HM02)	2	1	0.00173
Europe	Greece	Central Macedonia	Thessaloniki*	no record	2023	40.48	25.53	3 (HLB05)	1	0	0	3 (HM01)	1	0	0
Europe	France	Corse-du-Sud	Figari*	<i>Pinus nigra</i> <i>laricio</i>	2021	41.49	9.13	3 (HLA01, HLD07)	2	0.667	0.01269	2 (HM01, HM02)	2	1	0.00173
Europe	France	Corse	Serra di Fiumorbu*	no record	2023	41.98	9.33	1 (HLA08)	1	-	-	3 (HM02)	1	0	0
Europe	France	Aude	Gruissan*	<i>Pinus</i> <i>halepensis</i>	2021	43.18	3.08	3 (HLD07)	1	0	0	1 (HM01)	1	-	-
Europe	France	Var	La Londe-les- Maures*	<i>Pinus pinea</i>	2021	43.19	6.24	3 (HLA01, HLB04)	2	0.667	0.00692	3 (HM02)	1	0	0
Europe	France	Bouches-du-Rhône	La Ciotat*	<i>Pinus pinea</i>	2021	43.20	5.61	3 (HLA01)	1	0	0	2 (HM14, HM15)	2	1	0.00692
Europe	France	Var	La Valette*	<i>Pinus pinea</i>	2021	43.29	5.97	3 (HLA01)	1	0	0	4 (HM02)	1	0	0
Europe	France	Hérault	Bessan*	<i>Pinus</i> <i>halepensis</i>	2021	43.29	3.42	5 (HLA14, HLB02, HLB04, HLC05)	4	0.9	0.00865	4 (HM01, HM04, HM12)	3	0.833	0.0026
Europe	France	Var	Le Thoronet*	<i>Pinus</i> <i>halepensis</i>	2018	43.45	6.28	1 (HLA02)	1	-	-	4 (HM02, HM13)	2	0.5	0.00087
Europe	France	Bouches-du-Rhône	Gardanne*	<i>Pinus</i> <i>halepensis</i>	2021	43.49	5.48	6 (HLA01, HLA05, HLA11, HLD07)	4	0.8	0.00807	5 (HM02, HM07)	2	0.4	0.00069
Europe	France	Bouches-du-Rhône	Marignane*	<i>Pinus</i> <i>halepensis</i>	2021	43.51	5.18	3 (HLA01)	1	0	0	2 (HM02)	1	0	0
Europe	France	Pyrénées-Atlantiques	Bayonne*	<i>Pinus</i> <i>pinaster</i>	2017	43.52	-1.49	1 (HLD07)	1	-	-	1 (HM04)	1	-	-

Europe	France	Var	Roquebrune sur Argens*	no record	2021	43.58	6.58	3 (HLA01, HLC08)	2	0.667	0.01384	3 (HM01, HM02)	2	0.667	0.00115
Europe	France	Gironde	Toulouse*	no record	2021	43.65	1.46	2 (HLD05, HLD07)	2	1	0.00173	2 (HM02)	1	0	0
Europe	France	Hérault	Bédarieux*	<i>Pinus halepensis</i>	2021	43.70	3.17	3 (HLA01, HLB02, HLD07)	3	1	0.01384	3 (HM02)	1	0	0
Europe	France	Bouches-du-Rhône	Le Puy-Ste-Réparate*	<i>Pinus halepensis</i>	2021	43.71	5.45	3 (HLA01)	1	0	0	3 (HM01, HM02)	2	0.667	0.00115
Europe	France	Vaucluse	La Bastidonne*	no record	2021	43.72	5.57	3 (HLA01)	1	0	0	4 (HM02)	1	0	0
Europe	France	Hérault	Olmet et Villecun*	<i>Pinus halepensis</i>	2021	43.75	3.30	2 (HLA01, HLA13)	2	1	0.00346	1 (HM01)	1	-	-
Europe	France	Gard	Milhaud*	<i>Pinus halepensis</i>	2021	43.85	4.30	4 (HLA01, HLD07)	2	0.5	0.00952	1 (HM02)	1	-	-
Europe	France	Bouches-du-Rhône	Tarascon*	<i>Pinus halepensis</i>	2021	43.94	4.67	3 (HLA01)	1	0	0	1 (HM08)	1	-	-
Europe	France	Gard	Aramon*	<i>Pinus halepensis</i>	2021	43.95	4.69	2 (HLA01)	1	0	0	5 (HM02)	1	0	0
Total								63	14	0.675	0.00914	50	8	0.491	0.00112

Abbreviations: Hd, haplotype diversity; Nd, nucleotide diversity.

Supplementary material of general discussion



Supplementary Figure 1 **a** The sampling sites of *H. ligniperda*. **b** The sampling sites of *H. micklitzi*. Blue areas represent native ranges and red areas represent invasive ranges according to CABI and previous records (Parra Emilfork 1996, Kvamme and Lindelöw 2014, El Khoury et al. 2019, Lin et al. 2021, Lantschner et al. 2024).

Supplementary Table 1 Summary of the localities sampled and specimens used in the RADseq analyses of *H. ligniperda*.

Range	Sender (Genbank/acce ssion number/uffeld)	Country	State/Province/ Region	Locality	Year	Lat	Long	n
Europe	WSL collaborators	Hungary	Southern Great Plain	6114 Bugacpusztaháza	2021	46.7	19.6	2
Europe	INRAE collaborators	France	Hautes-Pyrénées	Ade	2022	43.1	0.0	1
South America	WSL collaborators	Uruguay	Tacuarembó Department	Agroempresa Forestal (A.F.)	2020	-31.8	-56.1	3
Europe	INRAE collaborators	France	Lozère	Banne des Cevemes	2022	44.4	4.1	1
Oceania	WSL collaborators	Argentina	Rio Negro	Bariloche	2020	-41.1	-71.3	1
Oceania	WSL collaborators	New Zealand	North Island	Bay of Plenty, Rotorua	2020	-38.2	176.3	1
Europe	INRAE collaborators	France	Hérault	Bédarieux	2021	43.7	3.2	2
Europe	INRAE collaborators	France	Hérault	Bessan	2021	43.4	3.4	5
Oceania	WSL collaborators	Australia	New South Wales	Bondi Forest S.F., Bombala	2021	-37.2	149.3	2
Europe	INRAE collaborators	France	Vendée	Brain sur Allonnes	2020	47.3	0.1	1
Europe	INRAE collaborators	France	Loir-et-Cher	Camotte Beuvron	2021	47.6	2.0	1
Oceania	WSL collaborators	New Zealand	South Island	Canterbury, West Melton	2020	-43.5	172.4	1
Europe	INRAE collaborators	France	Loire	Champdieu	2021	45.6	4.0	1
Europe	INRAE collaborators	France	Aude	Conques sur Orbriel	2021	43.3	2.4	2
Europe	WSL collaborators	Portugal	Alentejo	Coruche	2016	39.0	-8.4	1

Europe	INRAE collaborators	France	Corse	Coti-Chiavari	2023	41.8	8.8	2
Europe	INRAE collaborators	France	Meurthe-et- Moselle	Ecrouves	2021	48.7	5.8	1
Oceania	WSL collaborators	Argentina	Rio Negro	El Bolsón	2018	-42.0	-71.5	2
Europe	INRAE collaborators	France	Corse	Figari	2021	41.5	9.1	1
Europe	INRAE collaborators	France	Bouches-du- Rhône	Gardanne	2021	43.5	5.5	2
Europe	INRAE collaborators	France	Vaucluse	Gargas	2021	43.9	5.4	1
Europe	INRAE collaborators	France	Aude	Gruissan	2021	43.2	3.1	1
Europe	INRAE collaborators	France	Hautes-Alpes	Guillestre	2021	44.9	6.7	1
Oceania	WSL collaborators	Australia	Victoria	Hotspur	2021	-37.9	141.6	1
Europe	INRAE collaborators	France	Loiret	Ingrannes	2021	48.0	2.2	2
Europe	WSL collaborators	Switzerland	Ticino	Irana	2022	46.3	9.0	1
Europe	INRAE collaborators	France	Vaucluse	La Bastidonne	2021	43.7	5.6	1
Europe	INRAE collaborators	France	Bouches-du- Rhône	La Ciotat	2021	43.2	5.6	1
Europe	INRAE collaborators	France	Var	La Londe des Maures	2021	43.2	6.2	1
Europe	INRAE collaborators	France	Var	La Môle	2021	43.2	6.5	1
Europe	INRAE collaborators	France	Charente-Maritime	La Rochelle	2018	46.2	-1.1	1
Europe	INRAE collaborators	France	Var	La Valette	2021	43.3	6.0	1
Europe	INRAE collaborators	France	Vendée	Le Gavre	2021	47.5	-1.8	1

Europe	INRAE collaborators	France	Bouches-du-Rhône	Le Puy Ste Réparate	2021	43.7	5.4	1
Europe	INRAE collaborators	France	Var	Le Thoronet	2018	43.5	6.3	1
Europe	WSL collaborators	Switzerland	Ticino	Leggiuna	2022	46.4	9.0	1
Europe	INRAE collaborators	France	Pyrénées-Orientales	Llupia	2021	42.6	2.8	1
Europe	INRAE collaborators	France	Vendée	Longeville sur Mer	2020	46.4	-1.5	2
Europe	INRAE collaborators	France	Loiret	Lorris	2021	47.9	2.5	2
South America	WSL collaborators	Uruguay	Tacuarembó Department	Lumin	2020	-31.6	-55.7	2
Europe	INRAE collaborators	France	Gers	Lupiac	2021	44.0	0.1	2
Europe	INRAE collaborators	Italy	Sardegna	Macchiareddu	2023	39.2	9.0	1
Europe	INRAE collaborators	France	Alpes-Maritimes	Malaussène	2021	44.0	7.1	1
Africa	WSL collaborators	South Africa	Mpumalanga Region	Mbombela Local Municipality/Kaapshehoop	2020	-25.6	30.8	1
Europe	INRAE collaborators	France	Lozère	Mende	2022	44.5	3.5	1
Europe	INRAE collaborators	France	Gard	Milhaud	2021	43.9	4.3	1
Europe	INRAE collaborators	France	Aveyron	Millau	2021	44.2	3.1	1
North America	WSL collaborators	USA	New York	Monroe County, Rochester, Genesee Valley Park	2021	43.1	-77.6	1
North America	WSL collaborators	USA	New York State	Monroe County, Rochester, West Durand Eastman Park	2021	43.2	-77.6	1
Europe	INRAE collaborators	France	Tarn-et-Garonne	Montain	2022	43.9	1.1	1
Europe	INRAE collaborators	France	Tarn-et-Garonne	St Vincent l'Espinasse	2021	44.12	0.96	1

Europe	INRAE collaborators	France	Lot	Montcabrier	2021	44.6	1.1	1
Europe	INRAE collaborators	France	Eure	Montfort sur Risle	2021	49.3	0.7	2
Europe	INRAE collaborators	France	Maine-et-Loire	Montreuil sur Loir	2021	47.6	3.1	1
Europe	INRAE collaborators	France	Marne	Nutsement sous Coole	2021	48.9	4.3	1
Europe	INRAE collaborators	France	Drôme	Nyons	2021	44.3	5.1	1
Europe	INRAE collaborators	France	Hérault	Olmet et Villecun	2021	43.7	3.3	1
Europe	INRAE collaborators	France	Vendée	Olonne sur Mer	2020	46.5	-1.8	1
Europe	WSL collaborators	Italy	Pescara	Pineta d'Annunzio	2019	42.5	14.2	1
Europe	INRAE collaborators	France	Vendée	Pointe d'Arçay	2021	46.4	-1.3	1
Europe	INRAE collaborators	France	Corse	Porto Vecchio	2021	41.6	9.3	2
Asia	BJFU collaborators	China	Shandong Province	Qingdao	2023	36.2	120.6	3
Europe	INRAE collaborators	France	Aude	Quillan	2021	42.9	2.2	2
Europe	INRAE collaborators	France	Var	Roquebrune sur Argens	2021	43.6	6.6	2
Europe	INRAE collaborators	France	Haute-Garonne	Saint Gaudens	2021	43.3	0.7	1
Europe	INRAE collaborators	France	Lot	Saint-Denis Catus	2021	44.6	1.4	1
Europe	INRAE collaborators	France	Pyrénées- Orientales	Salses Le Chateau	2021	42.8	2.9	1
Europe	INRAE collaborators	France	Lot	Salviac	2021	44.7	1.3	1
North America	WSL collaborators	USA	California	San Dimas	2020	34.1	-117.8	1

Europe	INRAE collaborators	France	Corse	San Gavinu di Carbini	2021	41.7	9.2	2
Europe	INRAE collaborators	Italy	Alghero	San Marco sawmill	2023	40.7	8.3	1
North America	WSL collaborators	USA	California	San Marino	2020	34.1	-118.1	1
South America	WSL collaborators	Chile	Central Region	Sawmill Colorado	2020	-37.4	-73.4	1
South America	WSL collaborators	Chile	South Region	Sawmill Valdivia	2020	-39.5	-72.8	1
South America	WSL collaborators	Chile	North Region	Sawmill Viñales	2020	-35.4	-72.4	1
Europe	INRAE collaborators	France	Lozère	St Etiennev	2021	45.4	4.3	1
Europe	INRAE collaborators	France	Tarn-et-Garonne	St Paul d'Espts	2021	44.1	1.0	1
Europe	INRAE collaborators	France	Tarn	Stavit	2021	43.5	2.1	1
Europe	INRAE collaborators	France	Ardèche	Stpriet	2021	45.7	5.0	1
Asia	BJFU collaborators	China	Shandong Province	Tai'an	2023	36.2	117.1	3
Europe	INRAE collaborators	France	Bouches-du-Rhône	Tarascon	2021	43.9	4.7	1
Europe	Dimitrios N. Avtzis	Greece	Central Macedonia	Thessaloniki	2023	40.5	25.5	3
Europe	WSL collaborators	Greece	Central Macedonia	Thessaloniki	2020	40.6	23.0	1
Europe	INRAE collaborators	France	Gironde	Toulouse	2021	43.7	1.5	1
Europe	WSL collaborators	Czech Republic	Hradec Králové Region	Trebechovice pad Orebem	2021	50.1	16.2	1
Asia	BJFU collaborators	China	Shandong Province	Weihai	2023	37.5	121.9	2
Africa	WSL collaborators	South Africa	KwaZulu-Natal Region	Winterhaven	2021	-29.3	30.5	3

Asia	BJFU collaborators	China	Shandong Province	Yantai	2021	37.5	121.7	5
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n: Number of samples in RADseq analysis

Supplementary Table 2. Summary of the localities sampled and specimens used in the RADseq analyses of *H. micklitzi*.

Range	Sender (Genbank/accession number/uffeld)	Country	State/Province/Region	Locality	Year	Latitude	Longitude	n
Europe	INRAE collaborators	France	Vaucluse	La Bastidonne	2021	43.72	5.57	1
Europe	INRAE collaborators	France	Bouches-du-Rhône	Lambesc	2021	43.66	5.23	1
Europe	INRAE collaborators	France	Creuse	Le Pradet	2021	43.11	6.00	1
Europe	INRAE collaborators	France	Bouches-du-Rhône	Le Puy Ste Réparate	2021	43.65	5.43	1
Europe	INRAE collaborators	France	Corse	Serra di Fiumorbu	2023	41.98	9.33	1
Europe	Dimitrios N. Avtzis	Greece	Central Macedonia	Thessaloniki	2023	40.48	25.53	2

n: Number of samples in RADseq analysis

**Approche comparative du caractère invasif chez des scolytes congénériques:
le cas des Hylurgini**

Résumé

Les scolytes (Coleoptera : Curculionidae : Scolytinae) comprennent plusieurs espèces envahissantes qui causent d'importants dommages écologiques et économiques. Cette thèse explore les traits associés au succès de l'invasion chez les scolytes congénériques du groupe des Hylurgini, en comparant trois paires d'espèces, chacune composée d'une espèce envahissante et d'une espèce non envahissante. Le chapitre 1 passe en revue les traits d'histoire de vie et montre que les espèces envahissantes présentent souvent une gamme d'hôtes plus large, une plus grande plasticité écologique et un historique d'introductions multiples. Toutefois, le succès de l'invasion dépend d'une interaction complexe entre les traits intrinsèques des espèces et les facteurs environnementaux et anthropiques externes. Le chapitre 2 présente les génomes mitochondriaux complets de sept espèces d'Hylurgini, et reconstruit leur phylogénie à partir de 13 gènes codant pour des protéines. Ces données clarifient les relations évolutives au sein du groupe et facilitent l'identification moléculaire et les études phylogéographiques. Le chapitre 3 compare deux espèces du genre *Hylurgus*, i.e. *H. ligniperda* invasive et *H. micklitzi* non invasive, en sympatrie dans différentes régions en Europe. L'étude révèle une importante diversité génétique au sein de l'espèce invasive *H. ligniperda*, associée à une structuration phylogéographique de ses populations, probablement issues de plusieurs refuges postglaciaires, tandis que *H. micklitzi* montre une diversité et une structuration plus faible. Cette thèse souligne l'importance d'approches comparatives et intégratives pour mieux comprendre les invasions et renforcer les stratégies de biosécurité forestière.

Mots-clés : scolytes, Hylurgini, invasivité, phylogéographie, génome mitochondrial, traits d'histoire de vie

**Comparative approach to invasiveness in congeneric bark beetles:
the case of the Hylurgini group**

Abstract

Bark beetles (Coleoptera: Curculionidae: Scolytinae) include several invasive species that cause significant ecological and economic damage. This thesis investigates the traits associated with invasion success in congeneric bark beetles of the Hylurgini tribe by comparing three species pairs, each consisting of one invasive and one non-invasive species. Chapter 1 reviews life-history traits and shows that invasive species often exhibit a broader host range, greater ecological plasticity, and a history of multiple introductions. However, invasion success depends on a complex interaction between intrinsic species traits and external environmental and anthropogenic factors. Chapter 2 presents the complete mitochondrial genomes of seven Hylurgini species and reconstructs their phylogeny based on 13 protein-coding genes. These data clarify evolutionary relationships within the group and facilitate molecular identification and phylogeographic studies. Chapter 3 compares two species of the genus *Hylurgus*, namely invasive *H. ligniperda* and non-invasive *H. micklitzi*, which coexist in various regions of Europe. The study reveals substantial genetic diversity in *H. ligniperda*, along with phylogeographic population structuring likely shaped by multiple postglacial refugia, whereas *H. micklitzi* shows lower diversity and weaker structure. This thesis highlights the importance of comparative and integrative approaches to better understand biological invasions and to strengthen forest biosecurity strategies.

Keywords: bark beetles, Hylurgini, invasiveness, phylogeography, mitochondrial genome, life history traits