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Pleistocene events and ongoing population outbreaks affect the genetic structure of the Eurasian spruce bark beetle *Ips typographus* in the Southern Alps and the Apennines

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Abstract

The bark beetle *Ips typographus* (L.) is the most important pest in European Norway spruce forests. Especially after abiotic disturbance events, like storms or snowfall, this beetle can utilise vast amounts of trees with impaired defences, leading to mass outbreaks and further mortality of spruce. In 2018, the 'Vaia' storm damaged huge areas of Norway spruce-dominated forests in the Southern Alps. In the following years, the same regions were additionally affected by heavy snowfalls providing high amounts of suitable material for *I. typographus* brood establishment and development. Since then, these forests have faced unprecedented population outbreaks of *I. typographus* with severe effects for forest ecosystems, from both an ecological and an economic perspective. A comprehensive understanding of this beetle's genetic structure, including effects of the last ice ages as well as consequences of current population outbreaks, can give insights into the population dynamics of an eruptive species. Here, we studied the genetic structure of five epidemic populations from the Southern Alps and one from a potential glacial refugium in the Apennines. Our results revealed a low genetic diversity and a weak population structure among all studied locations. In the six analysed populations, four already described haplotypes, belonging to three slightly divergent haplogroups, were found. These haplotypes were evenly distributed among populations, including the potential glacial refugium in the Apennines. High gene flow among populations is probably maintained by the beetle's high dispersal capacity and high population densities, resulting in a panmictic population structure.

Key words: mitochondrial markers, COI, Scolytinae, haplotypes, *Picea abies*, forest pest, phylogeography, ices ages, Pleistocene, evolution.

Introduction

Over the last decades, extreme weather events caused severe damages in Norway spruce-dominated forests throughout Europe (Wermelinger, 2004; Raffa *et al.*, 2015). Storms, drought and snowfall provided huge amounts of trees with impaired defences, making them more susceptible to biotic stressors and predisposing them to pest infestations (Schlyter *et al.*, 2006; Marini *et al.*, 2017). These trees were later colonised by the spruce bark beetle *Ips typographus* (L.) (Coleoptera Curculionidae Scolytinae) with subsequent population outbreaks, due to the increased availability of breeding material (Bakke, 1983; 1989; Wermelinger, 2004; Mezei *et al.*, 2014). In regions with high population densities even moderately stressed trees were successfully infested, resulting in vast damages in these forests both from an economic and ecological point of view (Bakke, 1983; 1989; Wermelinger, 2004; Mezei *et al.*, 2014; Raffa *et al.*, 2015; Netherer *et al.*, 2021; Schebeck *et al.*, 2023).

The 'Vaia' storm in 2018 and heavy snowfalls and drought in the following years led to huge amounts of damaged and weakened trees in the Dolomites, followed by unprecedented mass outbreaks of *I. typographus* with severe effects on forest ecosystems (Chirici *et al.*, 2019; Pilli *et al.*, 2021; Udali *et al.*, 2021; Vaglio Laurin *et al.*, 2021). *I. typographus* is a widespread bark beetle in Eurasia, usually found on weakened individuals of *Picea abies* (L.) Karsten. It is a polygynous phloem breeder where males are the initial tree colonisers (Postner, 1974; Sauvard, 2004; Wermelinger, 2004; Schebeck *et al.*, 2023). After successfully overcoming tree defences, males bore a mating chamber in the phloem and release aggregation pheromones which attract male and female conspecifics (Schlyter *et al.*, 1987a; 1987b). One male usually mates with two to three females, which individually build their mother tunnels and establish a new offspring generation (Wermelinger, 2004; Schebeck *et al.*, 2023). It is a multivoltine species, depending on latitude and altitude, with up to three generations plus sister broods under favourable environmental conditions (Wermelinger, 2004; Baier *et al.*, 2007; Faccoli, 2009; Jönsson *et al.*, 2011; Schebeck *et al.*, 2023). In addition, its high fecundity, its pheromone communication and its association with bacterial and fungal symbionts are important traits that make this bark beetle an important pest species (Schlyter *et al.*, 1987a; 1987b; Kirisits, 2004; Wermelinger, 2004; Mayer *et al.*, 2015; Tanin *et al.*, 2021; Kandasamy *et al.*, 2023; Schebeck *et al.*, 2023).

A comprehensive understanding of the biology of

I. typographus can help to assess its current population outbreaks and is also the basis to infer management strategies. In addition to key life-history traits, including reproductive behaviour, pheromone communication or fungal associates, data on the species' population genetic structure and recent evolutionary history are crucial to get insights into the species' natural history (Sallé *et al.*, 2007; Avtzis *et al.*, 2012; Bertheau *et al.*, 2013; Mayer *et al.*, 2015).

The population genetic structure and phylogeographic patterns of most animal and plant species were shaped by past climatic events. Cycles of glacial and interglacial periods during the Pleistocene had a strong impact on the distribution of various biota. During the last glacial maximum major parts of the Northern hemisphere were covered by a thick ice shield and therefore uninhabitable for life (Hewitt, 1996; 2000; Taberlet *et al.*, 1998; Mix, 2001; Schmitt, 2007; Clark *et al.*, 2009; Habel *et al.*, 2010; Ehlers *et al.*, 2018). Thus, species retracted to refugial areas where environmental conditions were more favourable. Important refugial areas for many European species were located in the Mediterranean region, for example, the Italian Peninsula, the Iberian Peninsula or the Balkan Peninsula (Hewitt, 1999; Habel *et al.*, 2010). In addition, several extra-Mediterranean refugia have been described, for instance, the Carpathian Mountains (Varga, 2010; Schmitt and Varga, 2012). The retraction of a species to multiple geographically separated refugia led to isolation and limited gene flow among populations. After the last ice ages, climatic conditions got more favourable again, resulting in recolonization of previously uninhabitable regions and the re-establishment of gene flow among separated populations. These Pleistocene events and processes were major evolutionary drivers in many species, shaping their present genetic structure (Taberlet *et al.*, 1998; Hewitt, 2000; Habel *et al.*, 2010).

The genetic population structure of *I. typographus* was strongly influenced by both, climatic fluctuations during the Pleistocene and its strong dependency on *P. abies*. As *I. typographus* mainly utilises Norway spruce as a host, it shared certain refugial areas with this tree species (Stauffer *et al.*, 1999; Sallé *et al.*, 2007; Bertheau *et al.*, 2013; Mayer *et al.*, 2015; Schebeck *et al.*, 2023). Based on pollen records and genetic data, Norway spruce survived the last ice ages in four major glacial refugia: the Apennines, the Dinaric Alps on the Balkan Peninsula, the Carpathian Mountains and in the Russian plain. In addition, several small refugial areas in Eurasia have been described (Schmidt-Vogt, 1977; Lagercrantz and Ryman, 1990; Giannini *et al.*, 1991; Terhürne-Berson, 2005; Tollefsrud *et al.*, 2009).

The glacial refugium in the Apennines was of outstanding importance for many European animals and plants, with implications for the genetic structure of various species (Watson, 1996; Bartolini *et al.*, 2014; Bogdanowicz *et al.*, 2015). Common European bark beetles, like the six-toothed spruce bark beetle *Pityogenes chalcographus* (L.) or the pine shoot beetle *Tomicus piniperda* (L.) shared this refugial area with their conifer hosts (Ritzerow *et al.*, 2004; Horn *et al.*, 2009; Bertheau *et al.*, 2013; Schebeck *et al.*, 2018; 2019). Due to its great economic and ecological importance, the phylogeography of *I. typographus* has been investigated in various studies using a part of the mitochondrial COI gene (Stauffer *et al.*, 1999; Avtzis *et al.*, 2012; Bertheau *et al.*, 2013; Krascsenitsová *et al.*, 2013; Mayer *et al.*, 2015). In general, the genetic structure and diversity of *I. typographus* is low, but patterns indicating a North-South structure have been discovered (Stauffer *et al.*, 1999; Bertheau *et al.*, 2013; Mayer *et al.*, 2015). *I. typographus* can be grouped in three slightly differing COI haplogroups It-A, It-B and It-C, which consist of various mitochondrial haplotypes (Bertheau *et al.*, 2013). Except of two small refugial areas in the Carpathians, the exact number and location of other glacial refugia are elusive yet; other likely glacial refugia were the Apennine Peninsula, the Russian plain or the Dinaric Alps (Bertheau *et al.*, 2013; Krascsenitsová *et al.*, 2013; Schebeck *et al.*, 2023). In particular, the Apennines are an interesting biogeographic region for both, *I. typographus* and Norway spruce, as they represent the southern edge of their current distribution. Moreover, the importance of the Apennines as a glacial refugium for the genetic structure of adjacent *I. typographus* populations has not been clearly resolved yet. It was proposed that *I. typographus*' high dispersal capacity and rather young evolutionary age of recent haplotypes, compared to other scolytine species, might explain its low genetic diversity and shallow population structure (Bertheau *et al.*, 2013; Krascsenitsová *et al.*, 2013; Mayer *et al.*, 2015).

When studying population genetics using mitochondrial markers, cryptic nuclear copies of mitochondrial DNA (NUMTs) can cause misinterpretations of results and an overestimation of genetic diversity (Song *et al.*, 2008; Arthofer *et al.*, 2010; Hazkani-Covo *et al.*, 2010; Bertheau *et al.*, 2011; Jordal and Kambestad, 2014). The insertion of mitochondrial DNA into the genome is a biological process which is of great evolutionary interest as it plays a role in cell evolution and is a key source of genetic variation (Puertas and González-Sánchez, 2020). NUMTs often only differ in 1-3 bp from described haplotypes, which can lead to co-amplifications and misleading conclusions of a species' evolutionary history (Song *et al.*, 2008; Arthofer *et al.*, 2010; Hazkani-Covo *et al.*, 2010; Bertheau *et al.*, 2011; Jordal and Kambestad, 2014). When investigating the phylogeography of *I. typographus*, one must be aware of the presence of recently evolved cryptic NUMTs, which can hinder phylogeographic analyses in this species (Arthofer *et al.*, 2010; Bertheau *et al.*, 2011; Jordal and Kambestad, 2014).

Here, we investigated the genetic structure of *I. typographus* of an Apennine location and five sites in the Southern Alps, a region with current strong population outbreaks. A fragment of the mitochondrial COI gene of 190 individuals was analysed. Data were compared to previous work studying the population genetics of other European *I. typographus* populations, to understand the significance of the Apennines as a potential glacial refugium, its implications for adjacent regions in the Southern Alps and the effects of epidemic population events on the genetic structure. This study contributes to a comprehensive understanding of *I. typographus*' recent evolutionary past and adds important knowledge to the natural history of this widespread spruce bark beetle.

Table 1. Overview of locations, abbreviations of populations, coordinates and sample sizes (n) of *I. typographus* collected from the Southern Alps and the Apennines.

Site	Abbreviation	Coordinates	n
Kals (Austria)	AtKa	46.995611, 12.630144	24
Obertilliach (Austria)	AtOb	46.687870, 12.626397	24
Innichen (Italy)	ItIn	46.742894, 12.336625	22
Ahrntal (Italy)	ItAh	46.888169, 11.872553	24
Agordo (Italy)	ItAg	46.341372, 11.903494	48
Abetone (Italy), Apennines	ItAb	44.144906, 10.664202	48

Materials and methods

Sampling of beetles

To study the population genetics and phylogeography of *I. typographus* on a small geographic scale a total of 190 individuals were collected from five locations in the Southern Alps in 2020 and from one population in the Apennines in 2018 (table 1). The mean distance between the Southern Alps populations was 45 km, the geographic distance from the Apennine population to the closest Southern Alps population (Agordo) was 260 km. To prevent sampling of siblings only one individual per mother gallery was taken. Per location, individuals were collected from one or two wind-felled trees that were placed directly next to each other. After collection, alive beetles were immediately transferred to absolute ethanol and stored at −20 °C.

DNA extraction

Genomic DNA was extracted from the beetles' head and thorax using the Gentra Puregen Kit (QIAGEN), following the manufacturers protocol for DNA purification from tissue. The head and thorax were homogenised using a formed pipette tip. DNA was dissolved in 100 µl hydration solution, and the extracted DNA was stored at 4 °C.

PCR amplification and sequencing

A ~564 bp fragment of the mitochondrial COI gene was PCR-amplified using the primer pair ItCOI-F and UEA10 (Juan *et al.*, 1995; Lunt *et al.*, 1996). The total volume of the reaction mix was 20 µl, containing 12.66 µl ddH$_2$O, 4.0 µl reaction buffer, 0.4 µl dNTPs, 0.4 µl ItCOI-F, 0.4 µl UEA10, 0.14 µl Taq DNA polymerase (5 U/µl) and 2 µl template DNA. An initial denaturation step (94 °C for 3 minutes) was followed by 32 cycles of [denaturation at 94 °C for 30 sec, annealing at 51 °C for 1 minute, elongation at 72 °C for 1 minute] and completed by a final extension step at 72 °C for 7 minutes. To assure the formation of amplicons, the PCR products were run on a 1.5% agarose gel stained with GelRed Nucleid Acid Gel Stain (BIOTIUM, Fremont). PCR product purification and Sanger sequencing were performed by an external provider (Eurofins Genomics, Eberswalde, Germany).

Sequence analysis

First, sequence quality was checked by eye for ambiguous sites using Chromas 2.6.6 (Technelysium Pty Ltd, 1998-2018), particularly controlling for double peaks and potential NUMT co-amplification (Bertheau *et al.*, 2011). Forty-one sequences were discarded due to the presence of double peaks. The remaining 149 sequences were aligned using ClustalW, implemented in MEGA version 11 (Tamura *et al.*, 2021). To investigate the population genetic diversity and structure and exclude NUMTs, the obtained sequences were compared to haplotypes and NUMTs described by Stauffer *et al.* (1999) and Bertheau *et al.* (2011; 2013) (GenBank accession numbers: ITU82589, AF036150-AF036156, JN133852-JN133881, JN133882-JN133897) and assigned manually to described haplotypes, haplogroups and NUMTs. The total genetic diversity, average within-population diversity, mean number of pairwise differences and nucleotide diversity of the populations were calculated in MEGA 11 (Tamura *et al.*, 2021). The haplotype diversity Hd and nucleotide diversity π were calculated in DnaSP (Rozas *et al.*, 2017).

Results

In total, 58 sequences out of 190 had to be excluded from the genetic data set: 41 sequences showed double peaks being significant signs for NUMT co-amplification and 17 sequences aligned with described NUMT sequences by Bertheau *et al.* (2011). Most NUMTs were detected in ItAg (81.25% of all sequences from this population), followed by AtKa (20.83%), ItIn (18.18%), ItAh (16.67%), ItAb (10.42%) and AtOb (4.17%) (figure 1a).

The final COI data set comprised 132 sequences with a fragment length of 546 bp with a total of three polymorphic nucleotides resulting in four different haplotypes (figure 1b). All present haplotypes were described by Stauffer *et al.* (1999) and Bertheau *et al.* (2013). All mutations were transitions and the overall nucleotide frequencies were T (37.3%), C (19.1%), A (31.1%) and G (12.5%). The overall nucleotide diversity π was 0.00097 and the overall haplotype diversity Hd was 0.511 (table 2). The population with the highest haplotype and nucleotide diversity was ItAh, followed by ItIn, AtOb, AtKa, ItAb and ItAg.

Overall, HTI was the most common haplotype with 58.3%, followed by HTII with 39.4%, It1 with 1.5% and It19 with 0.8% (figure 1b). HTI and It19 belong to haplogroup It-A, HTII to haplogroup It-B, and It1 to haplogroup It-C, as described in Stauffer *et al.* (1999) and Bertheau *et al.* (2013). Haplogroup It-A was predominant in the Apennine population ItAb with 72.1% and in the Southern Alp populations AtKa with 73.7%, ItIn with

61.1%, AtOb with 52.2% and ItAh with 50%. Interestingly, the haplogroup It-B was the only present haplogroup in the population ItAg, which also had the highest amount of NUMTs. Haplogroup It-B was the second common in the populations AtKa with 26.3%, ItIn with 38.9%, AtOb with 47.8%, ItAb with 25.6% and ItAh with 45.0%. The haplogroup It-C was only found in small amounts in the north-western population ItAh with 5.0% and in the Apennine location ItAb with 2.3% (table 3, figure 2).

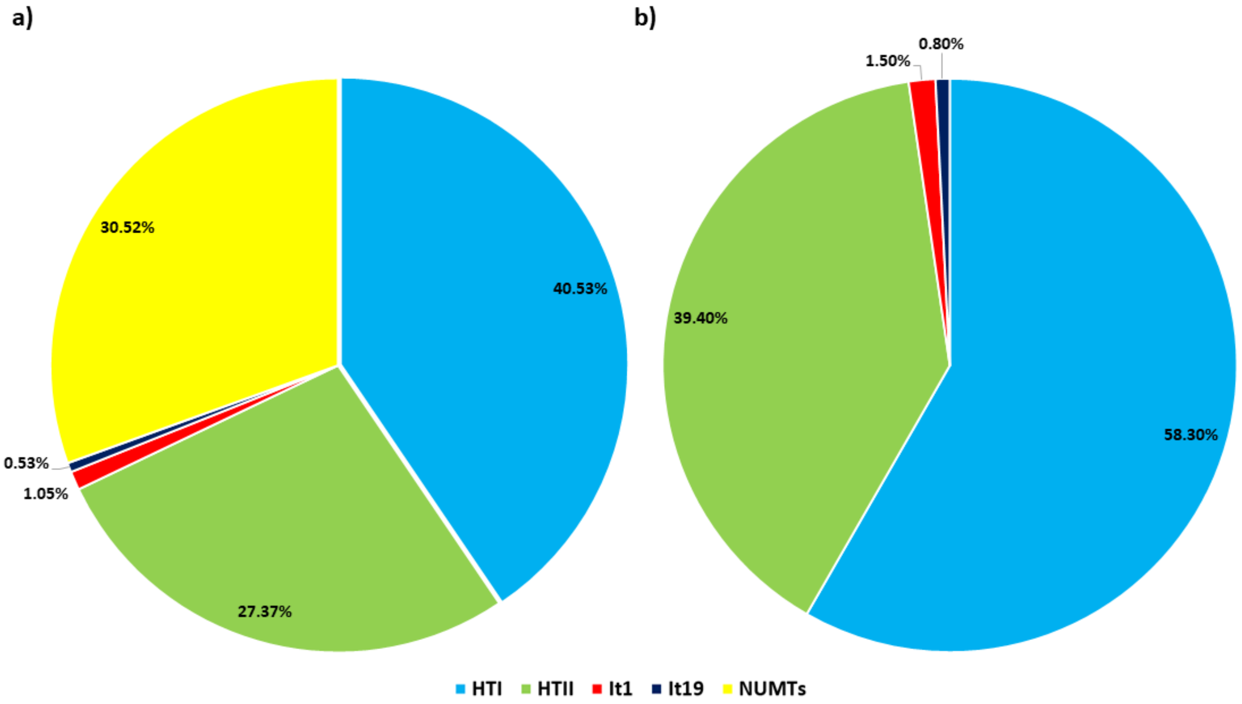

Figure 1. a) Overview of the distribution of haplotypes HTI, It19 (both haplogroup It-A), HTII (haplogroup It-B) and It1 (haplogroup It-C), and nuclear copies of mitochondrial DNA (NUMTs) of 190 individuals. **b)** Overview of the distribution of haplotypes of the analysed 132 sequences, assigned to the haplotypes HTI (haplogroup It-A), HTII (haplogroup It-B), It1 (haplogroup It-C) and It19 (haplogroup It-A). Haplotype designation after Stauffer *et al.* (1999) and Bertheau *et al.* (2013); colour scheme as in Bertheau *et al.* (2013).

Table 2. Sample sizes (n), overview on the distribution of haplotypes and haplogroups and intra-population diversity indices [nucleotide diversity and standard deviation (π ± SD) and haplotype diversity and standard deviation (Hd ± SD)].

Population	n	Haplotypes	Haplogroups	π ± SD	Hd ± SD
AtKa	19	3	2	0.00094 ± 0.00023	0.485 ± 0.104
AtOb	23	2	2	0.00096 ± 0.00006	0.522 ± 0.033
ItIn	18	2	2	0.00096 ± 0.00009	0.523 ± 0.048
ItAh	20	3	3	0.00114 ± 0.00017	0.574 ± 0.055
ItAg	9	1	1	0	0
ItAb	43	3	3	0.00080 ± 0.00014	0.424 ± 0.068
Total	132	4	3	0.00097 ± 0.00005	0.511 ± 0.020

Table 3. Overview of the distribution of haplotypes and haplogroups in the populations. Haplotype designation after Stauffer *et al.* (1999) and Bertheau *et al.* (2013).

Population	HTI (It-A)	HTII (It-B)	It1 (It-C)	It19 (It-A)	Total
AtKa	13	5	0	1	19
AtOb	12	11	0	0	23
ItIn	11	7	0	0	18
ItAh	10	9	1	0	20
ItAg	0	9	0	0	9
ItAb	31	11	1	0	43
Total	77	52	2	1	132

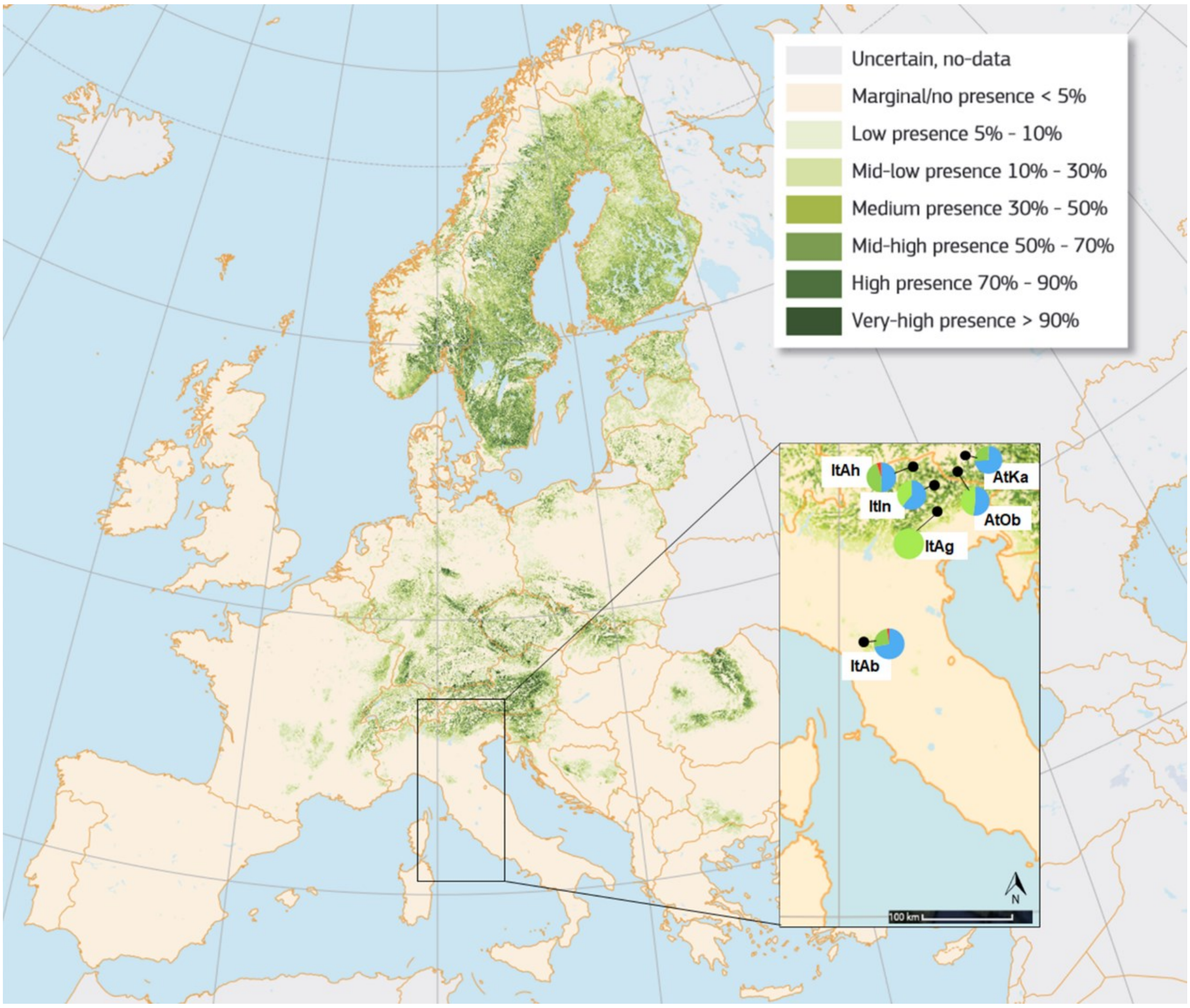

Figure 2. Distribution of haplogroups It-A (blue), It-B (green) and It-C (red) (colour scheme as in Bertheau *et al.*, 2013), in five Southern Alps locations and one Apennine location embedded into a distribution map of *P. abies* (adapted from Caudullo *et al.*, 2016).

Discussion

Here, we studied the population genetics of the Eurasian spruce bark beetle *I. typographus* and describe potential effects of Pleistocene events and current population outbreaks on the genetic structure of this widespread bark beetle. We focused on an interesting part of the species' range, i.e. the Apennines in Italy, a potential glacial refugium, and the Southern Alps, a region with current severe population outbreaks in close geographic vicinity. In general, a weak genetic structure among locations was observed. In total, we found a generally low genetic diversity with a total of four haplotypes, belonging to the three major haplogroups previously described. The weak genetic structure among populations suggests a strong and frequent exchange of genetic material among locations. The question whether the Apennines were a glacial refugium of *I. typographus* remains elusive.

The population genetic structure of *I. typographus* in Europe is relatively well resolved. Using a part of the mitochondrial COI gene, various authors described a shallow population genetic structure and low genetic diversity. Various mitochondrial haplotypes, which are assigned to three major haplogroups, have been described (Stauffer *et al.*, 1999; Bertheau *et al.*, 2013; Krascsenitsová *et al.*, 2013; Mayer *et al.*, 2015). Haplotype analysis, as well as the combination of nuclear and mitochondrial markers suggest a grouping in two major genetic clusters along a North-South gradient in Europe (Bertheau *et al.*, 2013; Mayer *et al.*, 2015). The haplogroup It-C is present in a small portion in both Northern and Southern European populations, whereas It-B is dominant in Southern European populations and It-A is prevalent in Northern European populations (Bertheau *et al.*, 2013). In our study, It-C was only present in a small number in two populations, It-B was the second common haplogroup, whereas It-A was the dominant haplogroup in all populations, except in ItAg, which only consisted of one haplotype, HTII (It-B), but over 80% of this population's sequences were NUMTs.

The geographic variation of the presence of NUMTs is a notable part of this study. Overall, about 30% of sequences were assigned to these nuclear copies of mitochondrial haplotypes and one population harboured an extraordinarily high number of NUMTs. Bertheau *et al.* (unpublished data, C. Bertheau) analysed COI sequences of *I. typographus* from a wide range and the distribution of NUMTs showed a geographic pattern, with populations from Italy and France having the highest prevalence. While the geographic variation of NUMTs is interesting, its functional significance remains unclear, particularly as no clear pattern among populations was found here.

The genetic pattern of *I. typographus* described here was likely shaped by past events, i.e. climate-driven range changes during the Pleistocene (Hewitt, 1999; 2000; Habel *et al.*, 2010; Bertheau *et al.*, 2013; Schebeck *et al.*, 2023). Like many other bark beetles with a strong host plant dependency, *I. typographus* shared certain refugial areas with its preferred host Norway spruce (Bertheau *et al.*, 2013; Schebeck *et al.*, 2023). Main *P. abies* glacial refugia were in the Apennines, the Carpathians, the Dinaric Alps as well as in the Russian plain, with some additional minor refugia (Schmidt-Vogt, 1977; Lagercrantz and Ryman, 1990; Giannini *et al.*, 1991; Stewart and Lister, 2001; Terhürne-Berson, 2005; Tollefsrud *et al.*, 2009). Using molecular markers, it is possible to detect the number and location of certain - even cryptic - refugia. For example, in a small-scale genetic study on the bark beetle *P. chalcographus* private, unique haplotypes (typical for refugia), were found, helping to resolve the species' Pleistocene history (Schebeck *et al.*, 2019). A distinct determination of the Apennines as *I. typographus* refugium is difficult as no private haplotypes have been detected for this population. Haplotypes of the rare haplogroup It-C were also reported from populations close to other putative refugia, i.e. the Carpathians or the Dinaric Alps, making a conclusive statement challenging (Bertheau *et al.*, 2013; Mayer *et al.*, 2015).

Bertheau *et al.* (2013) also analysed the COI gene of 35 individuals of an Apennine *I. typographus* population, reporting a haplotype diversity of 0.48 and three haplotypes belonging to the haplogroups It-A and It-B. In comparison, here 43 individuals from ItAb were analysed, revealing three haplotypes contributing to three haplogroups, and a slightly lower haplotype diversity (0.42). In both studies, the Apennines were not revealed as an *I. typographus* glacial refugium. The use and combination of other molecular markers (as applied in e.g. Mayer *et al.*, 2015; Schebeck *et al.*, 2018; Dzurenko *et al.*, 2021) and incorporating populations from a wide part of the species' range might help to shed more light on this open question in bark beetle phylogeography.

Various life-history traits might explain the small-scale genetic structure of *I. typographus* in the Southern Alps and the adjacent Apennine mountains. One of the most important, species-specific traits is its dispersal capacity. Like various other outbreeding conifer bark beetles, such as *P. chalcographus*, young adults can disperse several hundred meters to colonise new hosts, even extreme distances of up to 43 km have been reported for *I. typographus* (Nilssen, 1984; Forsse and Solbreck, 1985). The high population densities in the current eruptive bark beetle populations in the Southern Alps and the high dispersal capacities facilitate the exchange of genetic material among populations, resulting in panmictic populations with a weak genetic structure (Peterson and Denno, 1998; Sallé *et al.*, 2007). Additionally, when studying the phylogeography of *I. typographus*, the effects of human-mediated dispersal on the genetic structure via beetle-infested timber cannot be ruled out completely. For example, the absence of genetic structure in another bark beetle species, the pine shoot beetle *T. piniperda*, was hypothesised to be influenced by human trade (Kerdelhué *et al.*, 2006).

Moreover, the species' mostly monophagous feeding behaviour and the largely unlimited availability of hosts prevents a host-mediated effect on the genetic structure as it is suggested for other bark beetles, which might occur when they face a lack of breeding material under epidemic conditions (Sequeira *et al.*, 2000; Bertheau *et al.*, 2013). In addition, the relatively young evolutionary age of present haplogroups is likely another reason for a weak genetic structure among European populations of *I. typographus* (Bertheau *et al.*, 2013; Mayer *et al.*, 2015).

Other widespread European conifer bark beetles, like *P. chalcographus* or *T. piniperda*, show a stronger genetic structure and diversity (Horn *et al.*, 2009; Schebeck *et al.*, 2019). *P. chalcographus* main refugial areas are well known: the Russian plain, the Carpathian Mountains, the Italian-Dinaric region and some smaller refugia, e.g. the Apennine mountains (Avtzis *et al.*, 2008; Bertheau *et al.*, 2013; Schebeck *et al.*, 2018; 2019; 2023). In comparison, the knowledge on *I. typographus*' recent evolutionary history is scarce, a slight North-South structure across Europe and two small potential glacial refugia in the Carpathians are known (Bertheau *et al.*, 2013; Krascsenitsová *et al.*, 2013; Mayer *et al.*, 2015; Schebeck *et al.*, 2023). Different life-history traits of *I. typographus* and *P. chalcographus* could have led to different evolutionary histories. As mentioned above, *I. typographus* is a mostly monophagous species, whereas *P. chalcographus* is considered more oligophagous: both species can successfully breed in different Pinaceae species and prefer Norway spruce, but *P. chalcographus* has been observed on secondary species more often than *I. typographus* (Eidmann, 1987; Mayer *et al.*, 2015; Schroeder and Cocoş, 2018; Schebeck *et al.*, 2023). One hypothesis is that the shortage of suitable host plants forces *I. typographus* individuals to disperse across long distances when searching for appropriate hosts, leading to increasing gene flow and decreasing genetic structure (Postner, 1974; Austarå and Midtgaard, 1986; Bertheau *et al.*, 2013). Furthermore, high population densities in outbreaking populations paired with the high dispersal capacity of individuals maintain frequent gene flow among populations, explaining a low genetic structure among populations. Moreover, the time of divergence from the most recent common ancestor of *I. typographus* present haplotypes was dated 19,000 years ago, in comparison the divergence of *P. chalcographus* haplotype lineages happened about 101,000 years ago, which may have influenced the species' present genetic structure (Bertheau *et al.*, 2013; Schebeck *et al.*, 2023).

Conclusion

The analysis of the phylogeography of *I. typographus* in the Apennines and the influence of this potential glacial refugium on adjacent regions in the Southern Alps revealed a low genetic structure and diversity, supporting results of previous studies. The question to what extent the Apennine refugial area influenced the genetic structure of *I. typographus* in the Southern Alps remains elusive, as no unique haplotypes were detected in this population. Our study adds additional data on the phylogeography of this important pest species and the population genetic structure of outbreaking populations, contributing to a comprehensive picture of the species' recent evolutionary history.

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