Bulletin of Entomological Research

cambridge.org/ber

Research Paper

Cite this article: Schuler H, Elsler D, Fischnaller S (2021). Population genetics of the brown marmorated stink bug *Halyomorpha halys* in the early phase of invasion in South Tyrol (Northern Italy). *Bulletin of Entomological Research* **111**, 394–401. https://doi.org/10.1017/S0007485320000553

Received: 9 April 2020
Revised: 11 August 2020
Accepted: 14 August 2020
First published online: 27 October 2020

Keywords:
Agricultural pest; *Halyomorpha halys*; invasion dynamics; invasive species; marmorated stink bug

Author for correspondence:
Hannes Schuler,
Email: hannes.schuler@unibz.it

Population genetics of the brown marmorated stink bug *Halyomorpha halys* in the early phase of invasion in South Tyrol (Northern Italy)

Hannes Schuler[1], David Elsler[1] and Stefanie Fischnaller[2]

[1]Faculty of Science and Technology, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy and [2]Research Centre Laimburg, Pfatten, Italy

Abstract

The brown marmorated stink bug *Halyomorpha halys* is one of the most harmful invasive species in the world. Native to East Asia, this insect was introduced into North America in the 1990s and into Europe in the 2000s where it subsequently established and spread across the continent. Previous population genetic studies determined the invasion pathways at continental and national levels. However, information on the dynamics on a small-scale is currently scarce. Here we study the genetic diversity and population dynamics of *H. halys* in South Tyrol, a region in Northern Italy, since its arrival to its widespread establishment over a period of four years. By haplotyping 162 individuals from ten populations (including six previously published individuals) we found a high haplotype diversity in most populations with an increasing diversity across the different years. Most haplotypes were previously found in other regions of Northern Italy, providing evidence for migration from neighboring regions. However, the presence of four previously undescribed haplotypes as well as a haplotype previously found exclusively in Greece highlights additional long-distance dispersal across the continent. Phylogenetic analysis of the haplotypes found in South Tyrol showed that the majority of haplotypes clustered with haplotypes predominantly found in Japan. This suggests a potential recent introduction of *H. halys* individuals from Japan into Europe, and thus an additional invasion pathway that was previously unidentified.

Introduction

The brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) is an invasive insect pest introduced from Asia into several countries in the world (Cianferoni *et al.*, 2018). Outside its native range it was first described in Pennsylvania, USA in 1996. It subsequently spread across the continent, invading most states in the USA before it was intercepted in Canada in 2010 (Gariepy *et al.*, 2014; Xu *et al.*, 2014). In Europe, *H. halys* was introduced initially into Switzerland and Liechtenstein in 2004 (Arnold, 2009; Haye *et al.*, 2014) and was subsequently recorded in neighboring countries including Germany, France, Italy and Greece in the following years (Heckmann, 2012; Callot and Brua, 2013; Maistrello *et al.*, 2014; Milonas and Partsinevelos, 2014). *H. halys* has invaded Russia, Abkhazia, Georgia (Gapon, 2016) and Chile (Faundez and Rider, 2017), and was intercepted multiple times in New Zealand and Australia but no populations have established in these latter countries (Gariepy *et al.*, 2014; EPPO 2019).

Detailed knowledge of invasion routes is of considerable interest to prevent and control introductions and establishments of novel invasive species (Estoup and Guillemaud, 2010). In particular, the use of molecular and population genetic tools has shown to provide substantial information about the reconstruction of invasion routes. Several studies have described the invasion history and genetic diversity of *H. halys* using molecular tools with a particular focus on various mitochondrial regions (Cesari *et al.*, 2014, 2018; Gariepy *et al.*, 2014; Xu *et al.*, 2014; Zhu *et al.*, 2016; Valentin *et al.*, 2017; Lee *et al.*, 2018; Kapantaidaki *et al.*, 2019).

The comprehensive characterization of the genetic diversity of *H. halys* showed a high diversity within Asian populations and a significant genetic structure between populations from China, Korea and Japan (Xu *et al.*, 2014). In contrast, invasive populations in the USA showed much lower genetic diversity than in the native range with only two haplotypes present before 2008 (Xu *et al.*, 2014; Valentin *et al.*, 2017) and three additional haplotypes described in 2016 (Valentin *et al.*, 2017). In particular, the presence of a single haplotype (H1) in the east coast suggests that the invasion resulted from a single introduction event of a few founder individuals (Xu *et al.*, 2014). Since in Asia H1 is exclusively present in China, it is assumed that the first individuals on the east coast were introduced from China (Valentin *et al.*, 2017). In contrast, higher levels of haplotype diversity in the west coast indicate that multiple introduction events have occurred (Xu *et al.*, 2014; Valentin *et al.*, 2017). Europe, in contrast shows a different picture: while populations in Switzerland belong mainly to haplotype H3 and the less common haplotype H8, individuals in Northern Italy across different populations in the Emilia Romagna region are mainly H1 (Cesari *et al.*, 2014; Gariepy *et al.*, 2014, 2015). This suggests that while the first introduction of *H. halys* into Switzerland resulted from a direct interception from China, a second introduction occurred in Northern Italy, probably from an eastern US population (Valentin *et al.*, 2017). Moreover, a high haplotype diversity was found in Greece. Since several haplotypes – including the most common haplotype H33 – were outside of their native range only described in Greece, it is assumed that populations in Southeastern Europe resulted from an independent introduction event from China (Cesari *et al.*, 2014; Gariepy *et al.*, 2014, 2015; Valentin *et al.*, 2017).

A recent characterization of populations across Italy compared the haplotype frequency across different years and provided new insight into invasion dynamics on a smaller scale (Cesari *et al.*, 2018). In 2013 just two haplotypes were identified in Italy, H1 in the Emilia Romagna region and H3 in the Lombardy region (Cesari *et al.*, 2014, 2018). While the population in Lombardy likely resulted from an introduction from Switzerland, *H. halys* in Emilia Romagna was probably introduced from Eastern USA (Valentin *et al.*, 2017; Cesari *et al.*, 2018). The number of haplotypes increased in just a few years with a total of 22 haplotypes recorded in 2016, likely as a result of multiple introduction events and range expansions from other European populations (Cesari *et al.*, 2018).

Although the invasion history of *H. halys* on a global (Xu *et al.*, 2014; Valentin *et al.*, 2017; Lee *et al.*, 2018; Kapantaidaki *et al.*, 2019), continental (Cesari *et al.*, 2014; Gariepy *et al.*, 2015) and national (Cesari *et al.*, 2018) scale has been well-investigated, studies describing the invasion dynamics on a smaller scale are currently scarce. Here we focus on the genetic diversity of *H. halys* in South Tyrol (Northern Italy). This region is of special interest as it is one of the largest contiguous areas of apple production in Europe and therefore threatened by this emerging agriculture pest. The first exemplars of *H. halys* were found in South Tyrol in 2016 on a cargo from Bergamo (Lombardy, Italy) (Unterthurner, 2016). Since 2016 *H. halys* was frequently found in South Tyrol suggesting a successful establishment in this region (Unterthurner *et al.*, 2017). Moreover, from 2016 the frequency of *H. halys* detected in a monitoring program increased across the region, confirming the successful establishment and rapid spatial spread in this region (Fischnaller *et al.*, unpublished). Whether the current population in South Tyrol resulted from a single introduction in 2016 and a subsequent spread or from multiple introduction events across different years is currently not known. We therefore aim to study the genetic diversity across different populations sampled across 4 years and compare our data to previously published global, continental and national data to understand the population dynamics of *H. halys* in this area.

Materials and methods

Insect collection, DNA extraction and sequencing

A total of 156 individuals of *H. halys* collected from 2016 to 2019 across different localities in South Tyrol were analyzed. Localities within an area of 3 km were clustered into populations resulting in a total of nine different geographic populations (table 1). The number of individuals per population ranged on average from 14 to 16 individuals with the exceptions of Klausen (population 9, $n = 18$), Montan (population 9, $n = 5$) and Laimburg (population 7, $n = 43$). In total 21 individuals were sampled in 2016, 17 in

Table 1. Sampling sites, geographical coordinates, year captured and abundance of different haplotypes

	Locality	Latitude, longitude	Year	*n*	H1	H3	H8	H23	H33	H41	H78	H80	H152	H153	H155	H164	H165	H166	H167
1	Plaus	46.654831, 11.042168	2016, 2018, 2019	14	6	1	2						1	3				1	
2	Meran	46.645792, 11.182573	2016, 2019	18	6	2			1		3	2		3			1		
3	Tscherms	46.624479, 11.145627	2017, 2018	14	8	3					1			2					
4	Völlan	46.594156, 11.147902	2019	14	9	3	1							1					
5	Terlan	46.502633, 11.277485	2016, 2018	16	5	4	1	2			2			1		1			
6	Bozen	46.473036, 11.300277	2018, 2019	14	5	2				1		1	1	3			1		
7	Laimburg	46.384228, 11.288544	2016, 2017, 2018, 2019	43	30	7		2		1	1			1		1			
8	Montan	46.318444, 11.293250	2019	5	4													1	
9	Klausen	46.641716, 11.565951	2019	18	8	1		2	1	1				3	1				1
	*Brixen	Cesari *et al.* (2018)	2016	6	5	1													
				162	**86**	**24**	**4**	**6**	**2**	**3**	**7**	**3**	**2**	**17**	**1**	**2**	**2**	**2**	**1**

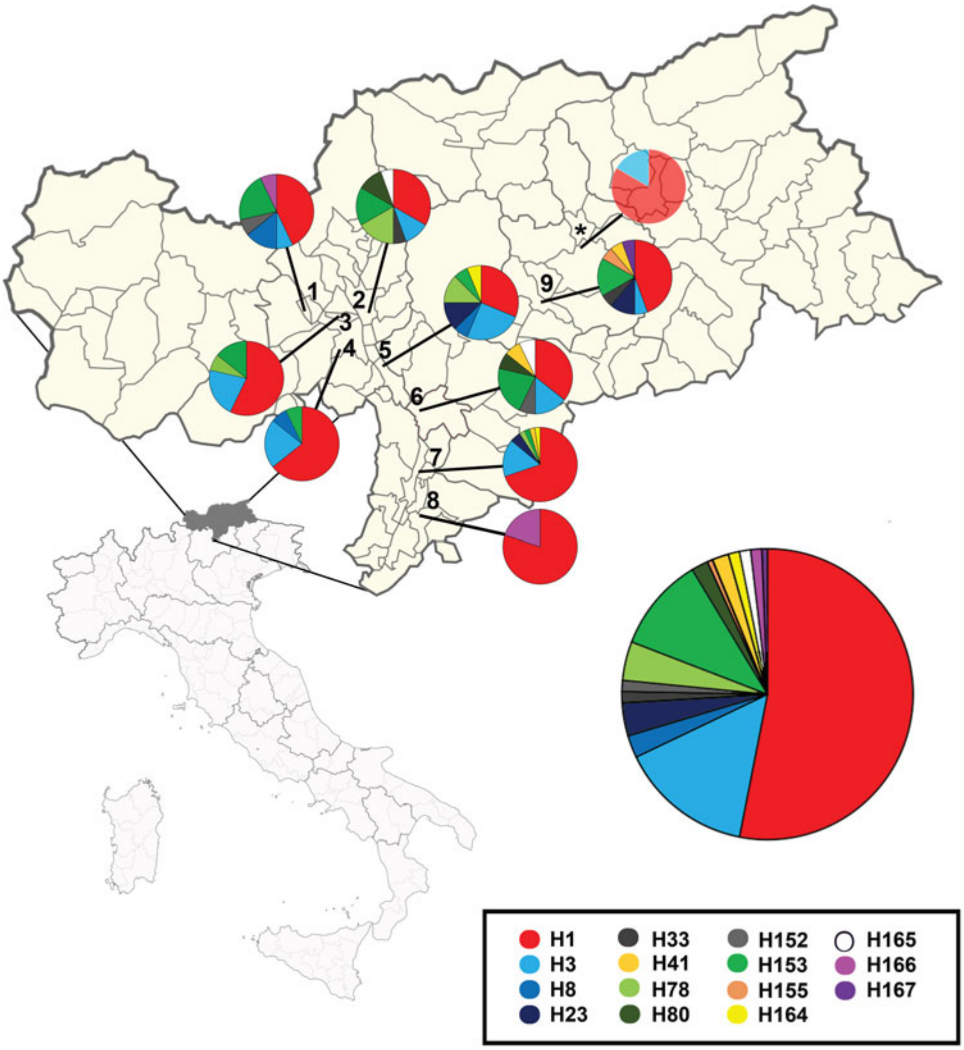

Figure 1. Geographical distributions of *H. halys* haplotypes across South Tyrol. Population localities are listed in table 1, the pie chart indicated by an asterisk represents a population studied by Cesari *et al.* (2018). The larger pie chart represents the total frequency of all haplotypes.

2017, 33 in 2018 and 85 in 2019. Additionally, six individuals that were analyzed previously in South Tyrol by Cesari *et al.* (2018) were included in our dataset (population *Brixen).

After taxonomic determination all individuals were stored in absolute ethanol at −20°C. DNA was extracted from a single hindleg using the GenElute Mammalian Genomic DNA miniprep kit (Sigma-Aldrich, Steinheim, Germany). DNA was eluted in 100 µl elution solution (10 mM Tris, 1 mM EDTA) and stored at 4°C. A 615 bp fragment of the mitochondrial COI was polymerase chain reaction (PCR) amplified using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). This region has been characterized already in other studies (Gariepy *et al.*, 2014, 2015; Xu *et al.*, 2014; Zhu *et al.*, 2016; Cesari *et al.*, 2018; Lee *et al.*, 2018) allowing a direct comparison with previously published data.

Each PCR reaction was performed in a total volume of 25 µl containing 7.5 µl H_2O, 12.5 µl UCP HiFidelity PCR Master Mix (Qiagen, Hilden, Germany), 0.25 µM of each primer and 2.5 µl template DNA. All reactions were performed in an Eppendorf Mastercycler Gradient (Eppendorf; Hamburg, Germany) with the following conditions: 35 cycles with 30 s at 98°C, 10 s at 98°C, 10 s at 51°C, 15 s at 72°C followed by a final extension at 72°C for 2 min. All PCR products were run on a 1% agarose gel stained with SYBRsafe (Thermo Fisher Scientific) and Sanger-sequenced by Eurofins MWG Operon (Ebersberg, Germany).

Population genetic analysis

Sequences were edited manually and aligned with CodonCode Aligner v8.0 (CodonCode Dedham, MA, USA). Our sequences were compared to sequences available in Genbank using the BLAST algorithm in NCBI. To exclude PCR artifacts, sequences with ambiguous polymorphisms and individuals with unique haplotypes (see 'Results' section) were confirmed by sequencing the product of two independent PCR runs. New haplotypes were deposited in the Genbank under the accession numbers MT199091–MT199094. The number of haplotypes (H), haplotype diversity (h) and nucleotide diversity (p) were determined using the program DNAsp v6 (Librado and Rozas, 2009). Neighbor-joining tree analysis was performed with Mega X (Kumar *et al.*, 2018) using the Tamura 3-parameter model (T92+G).

Results

We found a total of 15 different haplotypes across all populations of *H. halys* in South Tyrol. Eleven haplotypes have been described already in several other native and invasive populations, whereas four haplotypes (H164–167) were detected here for the first time. The most prevalent haplotype H1 was found in 86 out of 162 individuals (53.37%) in each population in South Tyrol (fig. 1; table 1). Haplotype H3 was found in 24 individuals (14.72%) across all populations, and was absent only in Montan (population 8). Haplotype H153 was found in 17 individuals (10.43%) across all populations except for Montan and Brixen (Cesari *et al.*, 2018). The other 12 haplotypes were found in low frequencies in one (0.61%; H155 and H167) to seven (4.29%; H78) individuals.

Overall, we found a high genetic diversity of *H. halys* in South Tyrol with a haplotype diversity of 0.684 ± 0.00128 and a nucleotide diversity of 0.00455 ± 0.00037(table 2). The number of haplotypes per population ranges from two in Montan (population 8) to eight in Klausen (population 9). Apart from the low haplotype and nucleotide diversity in Brixen in 2016 (Cesari *et al.*, 2018; table 2), the lowest haplotype diversity in our study was found in Montan (population 8) with a haplotype diversity of $h = 0.4 \pm 0.237$. The lowest nucleotide diversity was found in Laimburg (population 7) with $p = 0.0024 \pm 0.00065$ (table 2). In contrast, the highest haplotype diversity was found in Meran (population 2) and Terlan (population 5) with $h = 0.85 \pm 0.057$, while the highest nucleotide diversity was found in Meran with $p = 0.00694 \pm 0.0008$ (table 2).

The comparison between individuals collected in different years shows an increasing number of haplotypes across the different years: while in 2016 six haplotypes and in 2017 four haplotypes were found, the number of haplotypes increased to nine in 2018 and 14 in 2019 (fig. 2). This is reflected by a lower haplotype diversity (0.453 ± 0.116 and 0.419 ± 0.141) and nucleotide diversity (0.00321 ± 0.00101 and 0.00341 ± 0.00137) in 2016 and 2017, respectively, compared to 2018 ($h = 0.716 \pm 0.067$ and $p = 0.00368 \pm 0.00073$) and 2019 ($h = 0.768 \pm 0.039$ and $p = 0.00538 \pm 0.00044$; table 2).

The most widespread haplotype across all years and regions in South Tyrol is H1. This haplotype is the most common haplotype in the native range in Asia but also in most countries of the world. The haplotype is present in China and Japan but not in Korea, it is the most widespread in the USA, Canada and most European countries (Gariepy *et al.*, 2015; Cesari *et al.*, 2018) (table 3). In 2016 five other haplotypes were already present in South Tyrol. Haplotype H3 has been described in its native range in China (Gariepy *et al.*, 2015) and is present in Hungary, Greece and Italy, and is described as the most prevalent haplotype in Switzerland and France (Gariepy *et al.*, 2015; Cesari *et al.*, 2018; Lee *et al.*, 2018). In Italy this haplotype is frequent in Lombardy and has been recorded also in Piedmont, Emilia Romagna, Lazio and Calabria (Cesari *et al.*, 2018). Haplotype H23 was described in Switzerland, France (Gariepy *et al.*, 2014) and in Italy where it was exclusively found in Veneto (Morrison *et al.*, 2017) while H78 was described in China (Zhu *et al.*, 2016) and is especially present in Liguria and southern Piedmont. In contrast, H153 has been solely found in Italy where it was exclusively recorded in Veneto (Cesari *et al.*, 2018; table 2). One individual in 2016 belonged to a haplotype (H164) which has not been described yet.

Haplotypes H1, H3, H151 and H153 were still present in 2017 and all six haplotypes described in 2016 were found again in 2018. Three additional haplotypes were found in 2018: haplotype H8 has been described in low frequency in France and Switzerland as well as in Italy in low frequencies in Lombardy, Piedmont, Emilia-Romagna and Veneto (Cesari *et al.*, 2018; table 3). Haplotype H152 was exclusively found in Piedmont in low frequencies, whereas H41, a haplotype that originates in Japan, has been described outside its native range exclusively in Veneto (Cesari *et al.*, 2018; table 3).

Table 2. Genetic diversity of *H. halys* in South Tyrol

	n	*N*	*h* ± SD	*p* ± SD
All	162	15	0.684 ± 0.00128	0.00455 ± 0.00037
1. Plaus	14	6	0.791 ± 0.089	0.00598 ± 0.00094
2. Meran	18	7	0.85 ± 0.057	0.00694 ± 0.0008
3. Tscherms	14	4	0.648 ± 0.116	0.00419 ± 0.00421
4. Völlan	14	4	0.571 ± 0.132	0.00257 ± 0.00119
5. Terlan	16	7	0.85 ± 0.06	0.00516 ± 0.00093
6. Bozen	14	7	0.846 ± 0.074	0.00658 ± 0.00092
7. Laimburg	43	7	0.494 ± 0.085	0.0024 ± 0.00065
8. Montan	5	2	0.4 ± 0.237	0.00317 ± 0.00188
9. Klausen	18	8	0.791 ± 0.087	0.00574 ± 0.00067
*Brixen	6	2	0.333 ± 0.215	0.00051 ± 0.00033
2016	27	6	0.453 ± 0.116	0.00321 ± 0.00101
2017	17	4	0.419 ± 0.141	0.00341 ± 0.00137
2018	33	9	0.716 ± 0.067	0.00368 ± 0.00073
2019	85	14	0.768 ± 0.039	0.00538 ± 0.00044

n, number of individuals analyzed; *N*, number of haplotypes; *h*, haplotype diversity; *p*, nucleotide diversity; data of *Brixen were taken from Cesari *et al.* (2018).

In 2019, besides H164 all haplotypes have been found again, whereas six haplotypes appeared for the first time. Haplotype H33 was described already in China and outside its native range exclusively in Greece (Gariepy *et al.*, 2015). In 2019 this haplotype was found in Meran (population 2) and Klausen (population 9). Haplotype H180 occurs in low frequencies in Italy, in Lombardy, Piedmont, Veneto and has been found in Meran (population 2) and Bozen (population 6), whereas H155 outside its native range occurs only in low frequencies in Veneto, Trentino (Cesari *et al.*, 2018) and has been found in Klausen (population 9). Finally, three haplotypes (H165, H166 and H167) that have not been previously characterized in any other region in the world are scattered across South Tyrol.

Phylogeny and network analysis of the different COI haplotypes described in previous studies showed that haplotypes described in China and Korea formed a different clade than those found in Japan (Zhu *et al.*, 2016; Valentin *et al.*, 2017; Lee *et al.*, 2018). The incorporation of the 15 haplotypes in South Tyrol revealed that six haplotypes (H1, H3, H8, H78, H80 and H152) clustered with haplotypes described in China,

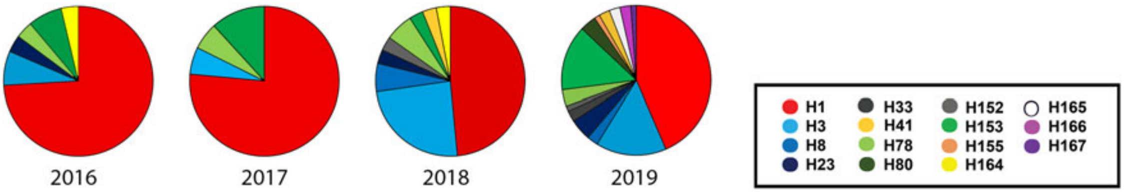

Figure 2. Frequency of *H. halys* haplotypes across different years.

Table 3. Overview of the haplotypes found in South Tyrol compared with descriptions in their native range (Xu *et al*., 2014, Valentin *et al*., 2017), invasive range in Europe and North America (Gariepy *et al*., 2014, 2015; Morrison *et al*., 2017, Lee *et al*., 2018, Kapantaidaki *et al*., 2019) and different regions in Italy (Cesari *et al*., 2018)

	Native	Invasive	Invasive Italy
H1	**China**, Japan	**USA**, **Canada**, Switzerland, France, **Hungary**, **Romania**, Greece, Italy	Piedmont, **Lombardy**, **Emilia-Romagna**, **Friuli V.G.**, **Tuscany**, **Trentino-S.T.**, Veneto
H3	China	USA, **Switzerland**, **France**, Hungary, Greece, Italy	Emilia-Romagna, **Lombardy**, Piedmont, Veneto, Lazio, Calabria, Trentino-S.T.
H8	–	France, Switzerland, Italy	Lombardy, Piedmont, Emilia-Romagna, Veneto
H23	Japan	France, Switzerland, Italy	Veneto
H33	China	**Greece**	–
H41	Japan	Italy	Veneto
H78	China	Italy	Lombardy, Piedmont, **Liguria**, Tuscany
H80	China	Italy	Lombardy, Piedmont, Veneto
H152	–	Italy	Piedmont
H153	–	Italy	Veneto
H155	–	Italy	Veneto, Trentino-S.T.
H164	–	–	–
H165	–	–	–
H166	–	–	–
H167	–	–	–

Countries highlighted in bold represent the country/region where the corresponding haplotype is predominant.

one haplotype (H33) clustered with a branch containing individuals from China and Japan, and eight haplotypes (H23, H41, H153, H155, H164, H165, H166 and H167) clustered with haplotypes described primarily in Japan (fig. 3).

Discussion

The invasion history of the brown marmorated stink bug *H. halys* in North America and Europe has been investigated in several recent studies (Gariepy *et al*., 2014, 2015; Valentin *et al*., 2017; Cesari *et al*., 2018; Lee *et al*., 2018). By comparing mitochondrial haplotypes of native populations from Asia with populations outside its native range, the invasion history was studied on a global (Gariepy *et al*., 2014; Valentin *et al*., 2017; Lee *et al*., 2018), continental (Gariepy *et al*., 2015; Cesari *et al*., 2018) and national (Cesari *et al*., 2014, 2018; Xu *et al*., 2014) scale. In the present study, we document for the first time the invasion history of *H. halys* on a regional scale in a time period of 4 years. We describe a remarkably high haplotype diversity across the whole range in South Tyrol with a high number of haplotypes that were increasing across the different years. The comparison of the haplotypes found in South Tyrol with previously published studies highlight a complex invasion history in this region, challenging the previously believed introduction hypothesis of *H. halys* in Europe.

The most striking result of our study is the high number of haplotypes present in South Tyrol. General invasive populations express a lower genetic diversity than populations in their native range after experiencing a strong population bottleneck (e.g. Dlugosch and Parker, 2008). The founder effect was particularly strong in *H. halys* populations in North America where initially just one to two haplotypes were described in the USA (Gariepy *et al*., 2014; Xu *et al*., 2014) and just three haplotypes in Canada (Gariepy *et al*., 2014; Valentin *et al*., 2017). Although the number of haplotypes in North America increased likely because of additional introductions (Morrison *et al*., 2017; Valentin *et al*., 2017; Lee *et al*., 2018; Kapantaidaki *et al*., 2019), genetic diversity of *H. halys* in North America is still limited with a maximum of five haplotypes described (Valentin *et al*., 2017). Similarly, the number of haplotypes in various European countries is low, especially in Romania (one haplotype), Hungary (two haplotypes), France (three haplotypes) and Switzerland (four haplotypes) (Gariepy *et al*., 2015; Morrison *et al*., 2017; Valentin *et al*., 2017; Cesari *et al*., 2018; Lee *et al*., 2018). In contrast, populations in Greece show considerably

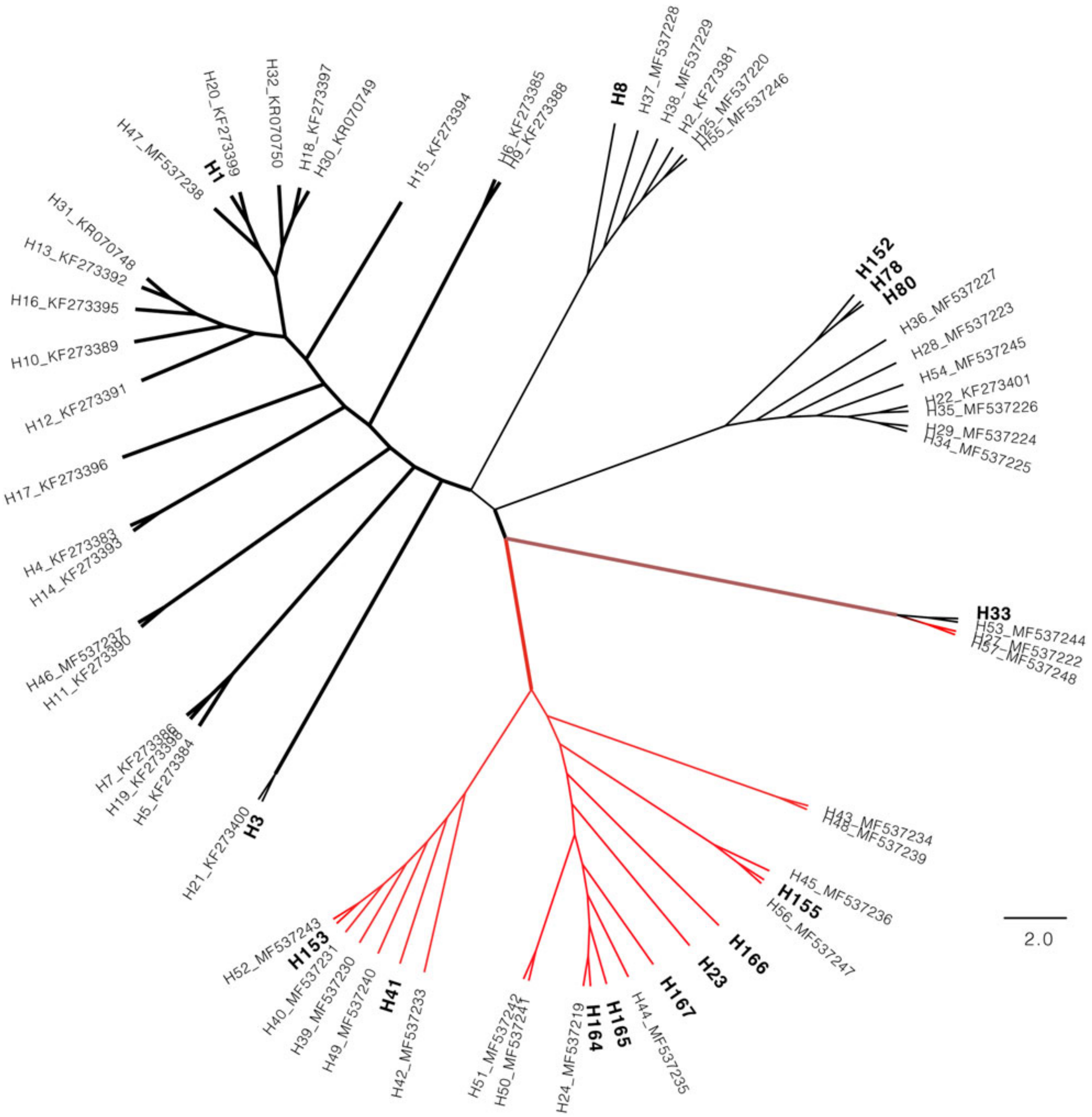

Figure 3. Neighbor-joining tree of *H. halys* haplotypes described in Xu *et al*. (2014) and Valentin *et al*. (2017) with the corresponding Genbank accession number, haplotypes present in South Tyrol are highlighted in bold. Black branch colors represent haplotypes predominantly found in China whereas red branches include haplotypes mainly found in Japan based on Xu *et al*. (2014) and Valentin *et al*. (2017).

high genetic diversity with up to ten haplotypes described (Gariepy *et al*., 2015; Valentin *et al*., 2017; Lee *et al*., 2018; Kapantaidaki *et al*., 2019). Moreover, the use of multiple molecular markers revealed up to 22 haplotypes in Italy (Cesari *et al*., 2018).

On a regional scale up to ten different haplotypes were described in Piedmont and Lombardy based on a combined analysis of the mitochondrial COI and COII regions (Cesari *et al*., 2018). Considering only the COI locus used in our study a maximum of eight haplotypes where described in Piedmont, seven in Lombardy and six in Veneto (Cesari *et al*., 2018). Thus, with 15 haplotypes described in South Tyrol, this region represents the highest number and diversity of haplotypes outside the native range of *H. halys*.

H. halys was recorded in Italy for the first time in 2012, in the province of Reggio Emilia (Maistrello *et al*., 2014). The first individuals in South Tyrol were found four years later in March 2016, with some individuals detected in open fields and remarkably high numbers found in packing materials in shipments from Bergamo, Northern Italy (Unterthurner *et al*., 2017). By haplotyping 27 individuals (including six individuals studied by Cesari *et al*. (2018)) from 2016 we found six haplotypes present in South Tyrol: H1, H3, H8, H78, H153 and H164. By that time just two haplotypes, H1 and H3, were described in Bergamo, whereas H78 and H8 have been described in Brescia and in Como, respectively, approximately 50 km next to Bergamo.

However, neither H153 nor H164 have been found in this geographic area (Cesari *et al*., 2018). Although these findings are generally in concordance with a primary introduction of *H. halys* from Lombardy, the presence of haplotype H153 that has been described exclusively in Veneto (Morrison *et al*., 2017; Cesari *et al*., 2018) and H164, a haplotype that has not been described outside of South Tyrol indicates that additional introductions, likely secondary invasions from other areas have occurred.

Generally, we could not find a significant impact of geography on the haplotype composition, with H1 present in the majority of individuals from all populations and H3 and H153 that were present in most populations in minor frequencies. The other haplotypes were scattered across the region with no spatial structure. The haplotype diversity was generally higher in cities (Meran, Bozen and Klausen) than in villages with a particularly low nucleotide diversity in remote areas in Völlan, Laimburg and Montan. This emphasizes the important role of active and passive human-mediated transportation for the dispersal of *H. halys* but might also highlight a general preference for urban areas where a higher diversity of host plants and the presence of suitable overwintering shelter locations might benefit the insect (Hoebeke and Carter, 2003; Leskey *et al*., 2012; Wallner *et al*., 2014; Leskey and Nielsen, 2018).

Essentially all haplotypes described in different years were still present in the following years suggesting that introduced *H. halys* individuals were able to establish into the new territory. However, the number of haplotypes and the general haplotype diversity increased across the different years. While the number of haplotypes in 2016 and 2017 with six and four haplotypes was relatively low, it increased to nine and 14 haplotypes in the following two years. Although the higher sample size in 2019 might have affected this outcome, both haplotype and nucleotide difference increased across the years (table 2). This is in line with Cesari *et al*. (2018) who described an increasing haplotype diversity in Italy within a few years. We interpret this result as a consequence of repeated introduction of *H. halys* from different populations across Italy and other countries. Especially the detection of H33, a haplotype that outside its native range was found exclusively in Greece indicates that secondary invasions likely due to long-range human-mediated transportation might have occurred.

It is unclear, why the populations in a restricted area in South Tyrol show a higher haplotype diversity than other areas previously studied. Most of the studies describing the haplotype diversity of *H. halys* in invasive areas in Europe and North America describe populations sampled between 2011 and 2016 (Gariepy *et al*., 2014; Morrison *et al*., 2017; Valentin *et al*., 2017; Cesari *et al*., 2018; Lee *et al*., 2018; Kapantaidaki *et al*., 2019) or earlier (Xu *et al*., 2014). It might be possible that additional introductions from the native range into Europe occurred recently and increased the haplotype diversity in other areas as well. Valentin *et al*. (2017) analyzed the invasion history of *H. halys* and concluded that most invasive populations in North America and Europe established from a direct introduction of *H. halys* populations from China with separate introductions into Eastern and Western USA and Canada, as well as into Switzerland and Greece. In contrast, the population in Northern Italy was established by a secondary introduction from Eastern USA into Emilia Romagna and the spread of the founder population in Switzerland to the neighboring countries. The authors found little evidence that populations outside China such as Japan and Korea established outside their native range (Valentin *et al*., 2017). Since half of the haplotypes found in South Tyrol have not been described in their native range, we performed a phylogenetic analysis to reconstruct their potential origin. Surprisingly, half of the haplotypes described in South Tyrol clustered with haplotypes that were previously described in Japan (Xu *et al*., 2014). This includes all four newly described haplotypes as well as four haplotypes that have been described previously in other regions in Italy (Cesari *et al*., 2018). As limited commercial relations between South Tyrol and Japan exist, we consider a direct introduction of *H. halys* into South Tyrol unlikely. Nevertheless, our results suggest that an introduction of *H. halys* individuals from Japan might have occurred in Italy or neighboring countries recently, a hypothesis that needs to be tested in future studies.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485320000553.

Acknowledgements. We thank Evi Deltedesco, Hannes Elsler, Markus Ladurner, Manuel Messner, Toni Riegler, Anna Rottensteiner, Arnold Schuler, Tanja Schuler, Raffael Tiziani and Michael Unterthurner for providing insect specimens, Manfred Wolf, Thomas M. Wolfe, Christian Stauffer and two anonymous reviewers for helpful comments and suggestions and Michele Cesari for clarification of previously published data. This study was funded by the Austrian Science Fund (project number P31441) and internal funds of the Free University of Bozen-Bolzano to HS and internal funds of the Research Centre Laimburg and the South Tyrolean Apple Consortium (SAK) to SF. The authors thank the Department of Innovation, Research and University of the Autonomous Province of Bozen/Bolzano for covering the Open Access publication costs.

Author contributions.

This work is part of the bachelor thesis of DE, supervised by HS and SF. HS and SF designed the study, HS, DE and SF collected and taxonomically identified the samples, DE and HS conducted lab work and analyzed the data. HS wrote the first draft of the manuscript and all authors contributed to the final version.

Conflict of interest. The authors declare no competing interests.

Data availability. Sequence data will be deposited in public databases after manuscript acceptance.

References

Arnold K (2009) *Halyomorpha halys* (Stål, 1855), eine für die europäischen Fauna neu nachgewiesene Wanzenart (Insecta: Heteroptera, Pentatomidae, Pentatominae, Cappaeini). *Mitteilungen des Thüringer Entomologenverbandes c.V* **16**, 19.

Callot H and Brua C (2013) *Halyomorpha halys* (Stål, 1855), la Punaise diabolique, nouvelle espèce pour la faune de France (Heteroptera Pentatomidae). *L' Entomologiste* **69**, 69–71.

Cesari M, Maistrello L, Ganzerli F, Dioli P, Rebecchi L and Guidetti R (2014) A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug *Halyomorpha halys* populations in Italy. *Journal of Pest Science* **88**, 1–7.

Cesari M, Maistrello L, Piemontese L, Bonini R, Dioli P, Lee W, Park CG, Partsinevelos GK, Rebecchi L and Guidetti R (2018) Genetic diversity of the brown marmorated stink bug *Halyomorpha halys* in the invaded territories of Europe and its patterns of diffusion in Italy. *Biological Invasions* **20**, 1073–1092.

Cianferoni F, Graziani F, Dioli P and Ceccolini F (2018) Review of the occurrence of *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae) in Italy, with an update of its European and World distribution. *Biologia* **73**, 599–607.

Dlugosch KM and Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**, 431–449.

EPPO Global Database (2019) Available at https://gd.eppo.int.

Estoup A and Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* **19**, 4113–4130.

Faundez EI and Rider DA (2017) The brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in Chile. *Arquivos Entomoloxicos* **17**, 305–307.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Gapon DA (2016) First records of the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. *Entomological Review* **96**, 1086–1088.

Gariepy TD, Haye T, Fraser H and Zhang J (2014) Occurrence, genetic diversity, and potential pathways of entry of *Halyomorpha halys* in newly invaded areas of Canada and Switzerland. *Journal of Pest Science* **87**, 17–28.

Gariepy TD, Bruin A, Haye T, Milonas P and Vétek G (2015) Occurrence and genetic diversity of new populations of *Halyomorpha halys* in Europe. *Journal of Pest Science* **88**, 451–460.

Haye T, Abdallah S, Gariepy T and Wyniger D (2014) Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, *Halyomorpha halys*, in Europe. *Journal of Pest Science* **87**, 407–418.

Heckmann R (2012) Erster Nachweis von *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) für Deutschland. *Heteropteron* **36**, 17–18.

Hoebeke ER and Carter ME (2003) *Halyomorpha halys* (Ståhl) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. *Proceedings of the Entomological Society of Washington* **105**, 225–237.

Kapantaidaki DE, Evangelou VI, Iii WRM, Leskey TC, Brodeur J and Milonas P (2019) *Halyomorpha halys* (Hemiptera: Pentatomidae) Genetic Diversity in North America and Europe. *Insects* **10**, 174.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

Lee W, Guidetti R, Cesari M, Gariepy TD, Park Y-L and Park C-G (2018) Genetic diversity of *Halyomorpha halys* (Hemiptera, Pentatomidae) in Korea and comparison with COI sequence datasets from East Asia, Europe, and North America. *Florida Entomologist* **101**, 49–54.

Leskey TC and Nielsen AL (2018) Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. *Annual Review of Entomology* **63**, 599–618.

Leskey TC, Hamilton GC, Nielsen AL, Polk DF, Rodriguez-Saona C, Christopher Bergh J, Ames Herbert D, Kuhar TP, Pfeiffer D, Dively GP, Hooks CRR, Raupp MJ, Shrewsbury PM, Krawczyk G, Shearer PW, Whalen J, Koplinka-Loehr C, Myers E, Inkley D, Hoelmer KA, Lee DH and Wright SE (2012) Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. *Outlooks on Pest Management* **23**, 218–226.

Librado P and Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Maistrello L, Dioli P, Vaccari G and Caruso S (2014) First records in Italy of the Asian stinkbug *Halyomorpha halys*, a new threat for fruit crops. *Atti Giornate Fitopatologiche* **1**, 283–288.

Milonas PG and Partsinevelos GK (2014) First report of brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in Greece. *EPPO Bulletin* **44**, 183–186.

Morrison WR, Milonas P, Kapantaidaki DE, Cesari M, Di Bella E, Guidetti R, Haye T, Maistrello L, Moraglio ST, Piemontese L, Pozzebon A, Ruocco G, Short BD, Tavella L, Vétek G and Leskey TC (2017) Attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae) haplotypes in North America and Europe to baited traps. *Scientific Reports* **7**, 16941.

Unterthurner M (2016) Die Marmorierte Baumwanze schmuggelt sich ein. *Obstbau*Weinbau* **4**, 21–23.

Unterthurner M, Ladurner M, Zelger A, Fischnaller S, Walcher M and Haye T (2017) Marmorierte Baumwanze im Visier. *Obstbau*Weinbau* **2**, 5–11.

Valentin RE, Nielsen AL, Wiman NG, Lee DH and Fonseca DM (2017) Global invasion network of the brown marmorated stink bug, *Halyomorpha halys*. *Scientific Reports* **7**, 9866.

Wallner AM, Hamilton GC, Nielsen AL, Hahn N, Green EJ and Rodriguez-Saona CR (2014) Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), after arrival in the United States. *PLoS One* **9**(5), e95691.

Xu J, Fonseca DM, Hamilton GC, Hoelmer KA and Nielsen AL (2014) Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*. *Biological Invasions* **16**, 153–166.

Zhu GP, Ye Z, Du J, Zhang DL, Zhen YH, Zheng CG, Zhao L, Li M and Bu WJ (2016) Range wide molecular data and niche modeling revealed the Pleistocene history of a global invader (*Halyomorpha halys*). *Scientific Reports* **6**, 1–10.