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Phylogeographic past and invasive presence of *Arion* pest slugs in Europe

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Abstract

Arion vulgaris (syn. _A. lusitanicus_) is the most destructive pest slug in Europe. The species has been regarded a classic case of an ongoing biological invasion with negative economic and ecological impact in many European countries, but this status has recently been contested. In this study, we assessed mitochondrial and autosomal genetic diversity in populations of _A. vulgaris_ across the entire distribution range in order to characterize its evolutionary history. Mitochondrial diversity in _A. vulgaris_ was strongly reduced compared with the closely related and largely codistributed non-invasive species _A. rufus_ and _A. ater_, indicating a very rapid spread of _A. vulgaris_ through Europe. Autosomal diversity assessed in 632 individuals from 32 populations decreased towards eastern and northern Europe which is consistent with the reported expansion of the species towards these regions in the last decades. Demographic simulations supported very recent population founding events in most of the European range. The short periods between the first detection of _A. vulgaris_ in different countries and only a very weak association of genetic structuring among populations with geographical distances suggest a human contribution in the ongoing expansion of the slug. We propose that this contribution may ultimately prevent the exact localization of the debated region of origin of _A. vulgaris_. However, the reclassification of _A. vulgaris_ as noninvasive would be premature. Without counter measures, the Eastern and Northern European countries can expect to see this biological invasion continued in the future.

Keywords: *Arion ater*, *Arion lusitanicus*, *Arion rufus*, *Arion vulgaris*, biological invasion, invasive species, microsatellites, molluscs

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Introduction

Non-native (alien) and invasive species belong to the biggest threats to biodiversity (Mack *et al.* 2000; Sala *et al.* 2000; Lowry *et al.* 2013), and they often cause also large economic losses due to direct damage, costs for damage mitigation or quarantine measures (Pimentel *et al.* 2005; Vila *et al.* 2010). The Convention on Biological Diversity aims to prevent, control and eradicate non-native and invasive species that threaten native ecosystems (UNEP 1992). However, it can be difficult to establish whether a species has been introduced by humans followed by an invasion or whether the species simply experiences a natural range expansion and demographic growth which are a part of every species' evolutionary history. Typically, a natural range expansion is a slow process compared with the rapid expansion of an invader (Hewitt 1999; van der Knaap *et al.* 2005; Ricciardi 2007; Magri 2008). Observational records can hold valuable information about the history of a biological invasion, but data are often sparse or lacking (Estoup & Guillemaud 2010; Fischer *et al.* 2015). Furthermore, invasiveness of the species might be a more important criterion for management decisions than its (non-)native status (Pauli *et al.* 2015).

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Molecular genetic tools have aided significantly in the investigation of biological invasions and their impacts. Genetic data may inform conservation actions directly through the identification of the point of entry of introduced species and their source populations (e.g. Facon *et al.* 2003; Stadler *et al.* 2005; Ascunce *et al.* 2011; Adrion *et al.* 2014; Cristescu 2015). Founder events and bottlenecks during the rapid expansion process often leave a trace of decreased genetic diversity in comparison with a source population (Dlugosch & Parker 2008; White *et al.* 2013). Moreover, genetic reconstruction of the invasion history can provide important clues on a potential human contribution to the initiation and progress of the invasion (Cristescu 2015). Molecular tools may also allow the detection or unambiguous identification of invasive species in a new area at an early stage (Lodge *et al.* 2006).

Here, we focus on the slug *Arion vulgaris* Moquin-Tandon 1855 (syn. *A. lusitanicus* auct. non-Mabille), which is a serious pest for agriculture and horticulture in most European countries with potential impact on native flora and fauna (DAISIE 2009; Blattmann *et al.* 2013). It is classified as one of the hundred most invasive terrestrial invertebrate species in Europe and has been regarded a typical case of an invasive range expansion (DAISIE 2009). *Arion vulgaris* is often referred to as the 'Spanish slug' because it has been assumed native to the Iberian Peninsula (Simroth 1891; Altena Van Regteren 1971; Chevallier 1972) and to have spread from there to the rest of Europe (Rabitsch 2006). However, many *Arion* species cannot be determined reliably based on external morphology, and the study by Quinteiro *et al.* (2005) showed that the taxon originally described as *A. lusitanicus* from the Iberian Peninsula is indeed different from the invasive slugs in other parts of Europe. In the following, we thus refer to the Iberian endemic as *Arion lusitanicus* and use *A. vulgaris* for the pest species. Initial records of *A. vulgaris* in western and Central Europe date back to the 1950s, and the slug has been subsequently reported in the majority of European countries (Fig. 1), suggesting an invasive history over the last decades (Rabitsch 2006; Engelke *et al.* 2011; Păpureanu *et al.* 2014). A recent study contested the southwestern European origin of the pest slug and its classification as an invasive species in many countries (Pfenninger *et al.* 2014), but strongly underestimated its current distribution range. It thus remains unclear whether the species has its origin in southwestern Europe, and whether the recent detection in new countries is indeed due to an ongoing invasion.

In this study, we investigate the population history and spread of *A. vulgaris* slugs through Europe based on a range-wide sampling scheme combined with information from historical records of the first detection in a country. In particular, our sampling covers the (south-) western parts of Europe that were hypothesized to be the source of the *Arion* invasion (Simroth 1891; Altena Van Regteren 1971; Chevallier 1972). In addition to populations from Central Europe where the invasion of the Spanish slug was perceived first, we also cover the regions in eastern Europe where the pest slug has been reported only in the last decade and which would thus represent the current front of the *Arion* invasion (DAISIE 2009; Fig. 1). The identity and history of these slug populations has not been analysed with genetic markers yet, but a potential spread of *A. vulgaris* from eastern Europe to other parts has also been put forward as a possibility (see Pfenninger *et al.* 2014). The specific goals of this study were thus to (i) examine the evidence for the invasive status of *A. vulgaris*, (ii) determine the larger area of its origin (in Western, Central or eastern Europe), and (iii) infer the likely mechanism of its dispersal (natural range expansion after glaciation vs. recent human-mediated introduction).

Our assessment of the history of *A. vulgaris* included as the first step a comparison of mitochondrial diversity patterns with closely related and noninvasive slug taxa. In systems with clear geographic structure in the species and limited recent exchange, phylogeographic analyses based on a few individuals and single genetic markers might be able to resolve the origin of invasive populations (Facon *et al.* 2003; Quaglia *et al.* 2008). In addition, we used multilocus estimates of nuclear DNA diversity in populations of *A. vulgaris* sampled across the range for a finer resolution of the potential invasion process, with a particular focus on the areas with reported recent occurrence. Patterns of genetic diversity might be better captured in such larger population samples (Grapputo *et al.* 2005; Prugnolle *et al.* 2005; Liu *et al.* 2011, 2012; Martinkova *et al.* 2013). Given the very low natural dispersal ability of slugs, a rapid expansion into extensive parts of Europe during the last decades would require a human contribution to the spread, for example through inadvertent transport with agricultural goods (Hulme 2009; Capinha *et al.* 2015). This would probably disturb geographical patterns, such as isolation by distance (IBD), between populations that are expected to establish between stationary populations with limited short-range gene flow over time (Slatkin 1993; Spurgin *et al.* 2014). Knowledge of the genetic structure and invasion history of this species can contribute to elucidation of the mechanisms behind its success (Guiller *et al.* 2012).

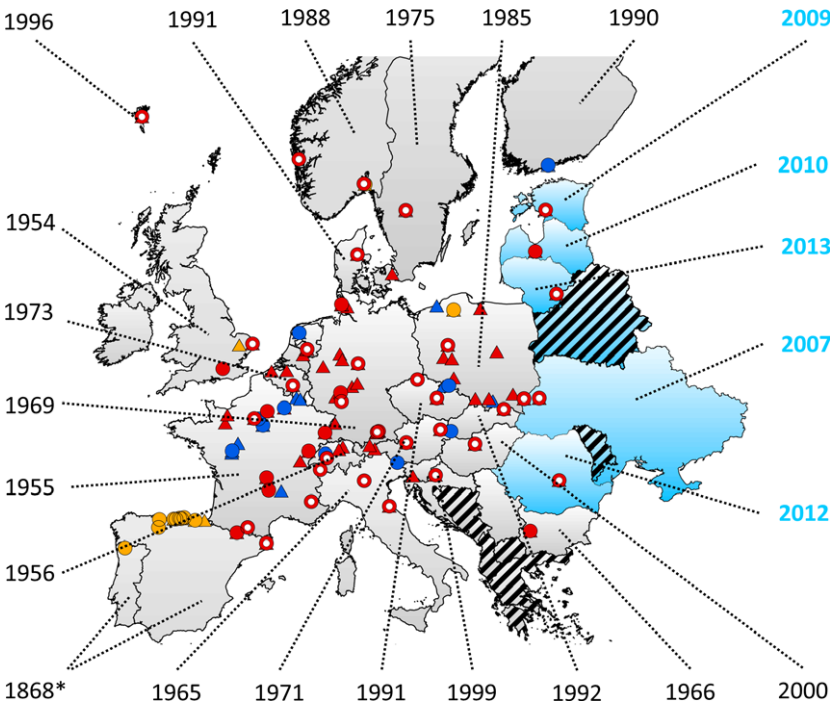

Fig. 1 Map of Europe with the distribution of the invasive slug *Arion vulgaris* (red) and its congeners *Arion rufus* (dark blue) and *Arion ater* (orange) according to phylogenetic analyses of mitochondrial DNA sequences of either ND1 (circles) and COI (triangles) (see Figs S1 and S2). Population samples of *A. vulgaris* are shown as white dots. The year of the first record of *A. vulgaris* in the respective country is given based on data from DAISIE (2009) and Păpureanu *et al.* (2014) and references therein (*note: the date for Portugal and Spain likely reports the endemic Lusitanian slug *Arion lusitanicus* and not *A. vulgaris;* see text and Quinteiro *et al.* 2005). The first records from the current invasion front in eastern Europe from the last decade are in light blue. Striped regions indicate countries from which *A. vulgaris* has not yet been reported.

Materials and methods

Study species

The slug *Arion vulgaris* and its close relatives, *Arion ater* and *Arion rufus,* are 12- to 15-cm-long hermaphrodites laying up to 400 eggs 1–2 times per year (Kozlowski 2012). They have an annual life cycle (Davies 1987). Slugs are dominant seedling predators (Wilby & Brown 2001) capable of altering plant community composition. Moreover, it has been reported that when *A. vulgaris* becomes established in a region, the populations of closely related *A. rufus* or *A. ater* begin to decline, possibly due to interspecific competition (Davies 1987; von Proschwitz 1997).

Sample collection and DNA extraction

Our sampling covered the whole distribution of *A. vulgaris* (Rabitsch 2006) with an emphasis on the recently invaded areas in eastern Europe and the presumed area of origin (Fig. 1). In total, we analysed 692 slugs including invasive *A. vulgaris,* and noninvasive *A. rufus* and *A. ater* that are very similar in external morphology but differ in internal morphology, life history, ecological and genetic traits (Ryser *et al.* 2011; Blattmann *et al.* 2013; Allgaier 2015; Hatteland *et al.* 2015). For mtDNA analysis, we selected up to five *Arion* sp. individuals per location, thereby extending and refining the geographical coverage of previous studies considerably (Fig. 1). For assessment of autosomal diversity in *A. vulgaris* populations, we analysed between 15 and 25 slugs each from 32 locations across Europe (Fig. 1, Table 1). We contacted 56 museums and academic experts on terrestrial molluscs in Spain and Portugal in order to be able to include specimens from the Iberian region in our study. We obtained several hundred slug specimens from the central and southern parts of Iberia (not shown in Fig. 1); however, there were no *A. vulgaris*, *A. rufus* or *A. ater* among them. Only one location in northeastern Spain harboured *A. vulgaris* (Fig. 1). All samples were preserved in pure ethanol, and DNA was extracted from a small piece of foot tissue using a high-salt extraction protocol (Aljanabi & Martinez 1997).

Assessment of mitochondrial DNA diversity

To assess mtDNA diversity across the distribution range, we sequenced the NADH dehydrogenase 1 (ND1) and cytochrome C oxidase 1 (COI) loci of *A. vulgaris/rufus/ater* (Table S1). Specific PCR primers MOL-NAD1F: 5′-CGRAARGGMCCTAACAARGTTGG-3′ and MOL-NAD1R: 5′-GGRGCACGATTWGTCTCNGCTA-3′ (Quinteiro *et al.* 2005) were used to amplify approximately 400 bp of ND1. The polymerase chain reaction (PCR) mix contained ca. 100 ng of DNA, 1 U of GoTaq polymerase (Promega), 1 μM of each primer, 10× buffer, 200 μM of dNTPs and 3 mM of $MgCl_2$ in a total volume of 25 μL. The PCR thermal profile consisted of initial denaturation at 94 °C for 2 min, followed by 40 cycles

Table 1 Locations and size of *Arion vulgaris* population samples (*N*) that were used for microsatellite genotyping (see also Fig. 1), with estimates of allelic richness (A_R), observed (H_O) and expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}). Statistically significant ($P < 0.05$) values of F_{IS} are indicated in bold italics

Country	Location	Latitude	Longitude	*N*	A_R	H_O	H_E	F_{IS}
Austria	Obertraun	47.555	13.693	19	5.10	0.61	0.69	0.036
Austria	Vienna	48.232	16.348	20	3.12	0.49	0.49	−0.027
Belgium	Namur	50.470	4.878	20	3.78	0.53	0.58	***0.114***
Croatia	Tuskanac	45.814	15.969	20	4.59	0.62	0.67	0.002
Czech Republic	Novy Bor	50.762	14.557	19	3.10	0.49	0.49	0.054
Czech Republic	Hluboka	49.845	16.072	18	3.97	0.52	0.57	0.021
Denmark	Kousted	56.515	9.894	19	3.72	0.53	0.56	−0.014
Estonia	Pärnu	58.382	24.521	18	3.15	0.51	0.54	0.049
Faroe Islands	Velbastaður	61.982	−6.852	16	2.98	0.38	0.45	***0.148***
France	Villiers-Saint-Frédéric	48.811	1.882	20	5.42	0.68	0.68	−0.035
France	Boussenac	42.905	1.370	16	5.07	0.53	0.64	***0.090***
France	Selonnet	44.372	6.312	21	3.44	0.49	0.53	0.090
Germany	Planegg	48.107	11.427	20	4.45	0.59	0.63	0.058
Germany	Heppenheim-Hambach	49.663	8.655	15	3.44	0.49	0.54	0.029
Germany	Göttingen	51.549	9.949	19	4.26	0.58	0.62	0.020
Hungary	Budapest	47.480	19.038	22	3.60	0.49	0.56	***0.112***
Italy	Gatteo	44.113	12.379	20	3.83	0.63	0.63	0.000
Italy	Bedizzole	45.509	10.421	20	4.85	0.61	0.67	***0.089***
Lithuania	Vilnius	54.787	25.365	21	2.68	0.40	0.44	0.091
Netherlands	Apeldoorn	52.226	5.992	21	3.07	0.42	0.49	***0.145***
Norway	Egge	59.442	10.401	20	3.11	0.48	0.54	***0.129***
Norway	Bergen	60.387	5.350	25	4.53	0.58	0.64	0.065
Poland	Bolestraszyce	49.813	22.844	20	3.64	0.52	0.57	0.059
Poland	Poznan	52.415	16.928	23	4.31	0.67	0.68	−0.030
Romania	Timişu de Sus	45.524	25.572	21	2.97	0.48	0.49	0.000
Slovakia	Bardejov	49.290	21.277	20	3.62	0.53	0.60	0.066
Spain	Girona	42.013	2.825	18	4.19	0.43	0.54	***0.158***
Sweden	Västergötland	58.367	13.650	21	2.94	0.43	0.48	0.033
Switzerland	Blumenstein	46.742	7.522	25	5.54	0.66	0.73	***0.061***
Switzerland	Salvan	46.111	7.007	25	4.59	0.63	0.66	0.014
UK	Lowestoft	52.485	1.744	15	4.72	0.52	0.62	0.033
Ukraine	Lemberg	49.838	24.030	15	3.54	0.40	0.51	***0.230***

of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min. A last extension step at 72 °C for 10 min was added. The PCR products were checked for correct size on a 1.5% agarose gel by comparison with a 100-bp ladder (Invitrogen). The amplified fragments were purified with a Wizard SV Gel and PCR Clean-Up system (Promega) and dissolved in 40 μL of sterile water. Sequencing was performed in both directions in 10 μL reaction volumes, which contained 2.5 μL of Big Dye Terminator Kit version 3.1 (Applied Biosystems) and 1 μM primer with the following conditions: denaturation at 96 °C for 1 min, 35 cycles of denaturation at 96 °C for 15 s, annealing at 50 °C for 15 s and extension at 60 °C for 3 min.

For COI amplification, we used the primers LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HC02 198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer *et al.* 1994). PCR was identical to the ND1 protocol, but the cycling conditions consisted of initial denaturation at 95 °C for 1.5 min, 5 cycles of 94 °C for 30 s, 45 °C for 1 min and 72 °C for 1.5 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 1 min. The last extension was at 72 °C for 7 min. Sequencing was carried out as for ND1 and reactions from both loci were run on an ABI Prism 3130 system (Applied Biosystems).

Phylogenetic and phylogeographic analyses of mtDNA in Arion species

In order to assess phylogenetic diversity within *Arion* sp. and examine taxonomical consistency with previous studies, we combined our ND1 sequences with 72 sequences of any *Arion* sp. available in GenBank and one *Deroceras* sp. sequence as an outgroup (Tables S2, S4). DNA sequences were aligned in

BIOEDIT 7.1.3 (Hall 1999), and the identity and frequency of haplotypes were determined with DNASP 5 (Librado & Rozas 2009). We reduced the sequence data to the haplotype level for tree reconstruction with Bayesian inference (BI) implemented in MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). Based on the Bayesian information criterion (BIC) implemented in JMODELTEST 0.1.1 (Posada 2008), we selected the TIM3 model with invariable sites and rate variation among sites for the analyses. The following maximum-likelihood parameters were determined in JMODELTEST: base frequencies A = 0.338; C = 0.122; G = 0.139; T = 0.402 and the shape parameter of the gamma distribution (α): 0.801. The analyses were run three times for 10 million generations with every 10th generation sampled using three heated and one cold chain. JMODELTEST and MRBAYES analyses were performed with the CIPRES Science Gateway (Miller *et al.* 2010). The first 25% of the tree samples were discarded as burn-in, and convergence was determined by examining the log-likelihood values and the split frequencies in TRACER 1.6 (Rambaut *et al.* 2014). The trees were visualized in FIGTREE 1.4.2 (Rambaut 2014).

For COI analyses, we added all 462 *Arion* sp. sequences available in GenBank (Tables S3, S5) to our own sequences and processed them as for ND1. Initial phylogenetic analyses showed a very high consistency of the deeper relationships in this large tree with analogous results of Pfenninger *et al.* (2014). We identified the taxa *A. vulgaris*, *A. rufus* and *A. ater* as monophyletic clades in this phylogeny (results not shown). As expected for linked mitochondrial genes, we were able to confirm the identical clustering of our individuals in these clades for ND1 and COI. We therefore focused our further phylogenetic analyses of COI on the monophyletic clades *A. vulgaris*, *A. rufus* and *A. ater* as the taxa particularly relevant for our study of the invasive slug (Table S5). Based on the BIC values from JMODELTEST, we used the HKY model with invariable sites and rate variation among sites with base frequencies A = 0.293, C = 0.164, G = 0.159, T = 0.384 and α = 0.773. Tree reconstruction and visualization were performed as described above for ND1.

Genetic diversity within *A. vulgaris*, *A. rufus* and *A. ater* was assessed by estimating the number of haplotypes, nucleotide and haplotype diversity at the ND1 and COI loci with DNASP. To investigate the geographical distribution and relationships between haplotypes in each species, we reconstructed statistical parsimony haplotype networks in POPART 1 (Leigh & Bryant 2015) from the sequences of the three taxa (Figs S1, S2; Tables S4, S5). For these analyses, we used only those sequences from GenBank where the geographic origin of the sample was clearly specified.

Assessment of nuclear DNA (nucDNA) variation in A. vulgaris populations

NucDNA variation in *A. vulgaris* was analysed in 32 population samples spanning the entire distribution range (Fig. 1, Table 1). Fifteen to 25 individuals per population were genotyped for 15 microsatellite markers (Zemanova *et al.* 2015) in three multiplex PCR mixes: ALU_06_4, ALU_12_2, ALU_34_2, ALU_37_2, ALU_60_2 (Mix 1); ALU_76_2, ALU_79_2, ALU_86_2, ALU_88_2, ALU_92_2 (Mix 2); and ALU_02_3, ALU_11_2, ALU_13_2, ALU_30_2, ALU_96_2 (Mix 3). PCR volumes of 10 μL included 5 μL of Qiagen multiplex kit, 2 μL of H_2O, 1 μL of the primer mix and 2 μL of DNA. The PCR temperature profile was as follows: 15 min of initial denaturation at 96 °C, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min 30 s, extension at 72 °C for 1 min 30 s and the final extension step of 30 min at 60 °C. The PCR product was diluted with 20 μL of distilled H_2O and 1.2 μL of the diluted product was mixed with 12 μL of the internal size standard (GeneScan 500 LIZ; Applied Biosystems) to determine the size of alleles. The amplified fragments were separated on an ABI Prism 3130 Genetic Analyzer and fragment lengths were scored with GENEMAPPER 3.7 (Applied Biosystems). Approximately 10% of the samples were reamplified and genotyped independently to ensure genotyping consistency (Kindler *et al.* 2012). The presence of null alleles and genotyping errors was evaluated with MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004). Linkage disequilibrium between all pairs of loci was assessed in FSTAT 2.9.3.2 (Goudet 1995) with a significance threshold (alpha) of 0.05 and sequential Bonferroni correction for multiple testing (Holm 1979).

Spatial genetic structure among A. vulgaris populations

We used principal coordinate analysis (PCoA) based on pairwise Euclidian distances between microsatellite genotypes performed in GENALEX 6 (Peakall & Smouse 2006) to explore the genetic structure in *A. vulgaris*. Genetic differentiation estimated as F_{ST} between pairs of populations was calculated in ARLEQUIN 3.5 (Excoffier & Lischer 2010) with 1000 permutations and alpha of 0.05 corrected by the sequential Bonferroni method for multiple testing. We tested for isolation by distance (IBD) between populations by applying the Mantel test in PASSAGE 2 (Rosenberg & Anderson 2011) with 999 permutations to matrices of pairwise F_{ST} and geographical distance.

To test for deeper genetic subdivisions within the species, we used the Bayesian clustering methods

implemented in the softwares STRUCTURE 2.3.4 (Pritchard *et al.* 2000) and TESS 2.3 (Chen *et al.* 2007). The analyses in STRUCTURE were run with the admixture model with correlated allele frequencies and without sample location priors and with a burn-in period of 100 000 iterations followed by 500 000 MCMC replicates. The number of clusters (K) was set to range from 1 to 10, with 10 iterations each. For selecting the optimal K value, we used the Evanno approach (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt 2012). Results of the ten STRUCTURE runs were merged in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and visualized in DISTRUCT 1.1 (Rosenberg 2004). The output was displayed on a map produced in ARCMAP 10.2.2 (ESRI, Redlands, CA, USA). In TESS, we ran the CAR model with admixture and 500 000 MCMC replicates after 100 000 burn-in iterations. The number of clusters was set to range from 2 to 20, with 10 iterations each. The optimal K value was selected using the deviance information criterion (DIC) plotted against the maximum number of clusters, which should stabilize close to the optimal value (Durand *et al.* 2009).

NucDNA diversity of A. vulgaris across Europe and expansion scenarios

As gradients of decreasing diversity caused by serial founder events and bottlenecks can indicate the direction of spatial expansions (e.g. Heckel *et al.* 2005; Prugnolle *et al.* 2005; White *et al.* 2013), we assessed patterns of genetic diversity in *A. vulgaris* populations. NucDNA diversity was quantified as allelic richness (A_R) standardized to the lowest sample size of 15 in FSTAT, and observed (H_O) and expected heterozygosity (H_E) in GENALEX. The inbreeding coefficient (F_{IS}) was calculated in ARLEQUIN and the P-values estimated with 10 000 permutations. We investigated geographical patterns of mean A_R, H_O and H_E across the range of *A. vulgaris* by spatial interpolation with inverse distance weighting prediction maps using a smoothing factor of 0.2 in ARCMAP. We further fitted linear models with A_R, H_O and H_E as dependent variables and latitude and longitude as explanatory variables in R 3.1.1 (R Core Team 2014).

We also examined the likelihood of three alternative suggestions for the geographic origin of the invasive slug in Western (Chevallier 1972; Rabitsch 2006), Central or eastern (Pfenninger *et al.* 2014) Europe based on spatial patterns of genetic diversity in populations. We hypothesized that genetic diversity would decrease with increasing distance from the area of origin (Dlugosch & Parker 2008; White *et al.* 2013). We allocated sampling locations into three classes of 500 km with increasing distance from the hypothesized area of origin with the multiple ring buffer tool in ARCMAP (Fig. 2). We tested for differences in A_R, H_O and H_E between the classes with a one-way analysis of variance (ANOVA) in R. Tukey's HSD post hoc tests were applied to determine which classes were different from each other at an alpha of 0.05.

Past demography

We used several methods to investigate changes in the effective population size of *A. vulgaris*. First, we calculated Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) for the ND1 and COI markers in ARLEQUIN. Then, we conducted mtDNA mismatch analysis in the same software under a model of sudden demographic expansion. We calculated the sum of squared deviation (SSD) and Harpending's raggedness index (HRI; Harpending 1994) with 10 000 bootstrap replicates.

The detected large-scale patterns in genetic diversity are consistent with a recent invasion (see Results) and correspond to the years of reported occurrence (oldest in the west, most recent in the east), but in principle this pattern could also be a result of an earlier postglacial expansion from a refugium on the Iberian Peninsula. We therefore tested the relative likelihood of two rather simple demographic scenarios (Fig. S3, Table S6) in the approximate Bayesian computation (ABC) framework (Beaumont *et al.* 2002) implemented in DIYABC 2.1.0 (Cornuet *et al.* 2014) to determine whether ancient or very recent colonization bottlenecks better fit the nucDNA data. In scenario 1, a population went through a relatively old postglacial bottleneck (15 000–20 000 years ago; Clark *et al.* 2009), and in scenario 2, the population experienced a very recent bottleneck (in the last 100 generations = years) as expected for the current invasion. We tested the relative likelihood of these two scenarios in 13 populations spanning the entire European distribution range of the species: Bardejov (Slovakia), Boussenac (France), Egge (Norway), Gatteo (Italy), Girona (Spain), Heppenheim-Hambach (Germany), Novy Bor (Czech Republic), Poznan (Poland), Selonnet (France), Timişu de Sus (Romania), Västergötland (Sweden), Velbastaður (Faroe Islands) and Vilnius (Lithuania).

The base of the parameter estimation consisted of 1 000 000 simulated data sets for each scenario with uniform priors (Table S6). As no specific information on microsatellite mutation rates in gastropods is available, the minimum and maximum values were set to 10^{-5} and 10^{-3}, respectively, and the other parameters were set to default. We used mean number of alleles, mean genetic diversity across loci, mean allele size variance and mean Garza–Williamson's M (Garza & Williamson 2001) as summary statistics, as they are informative about demographic events and divergence time

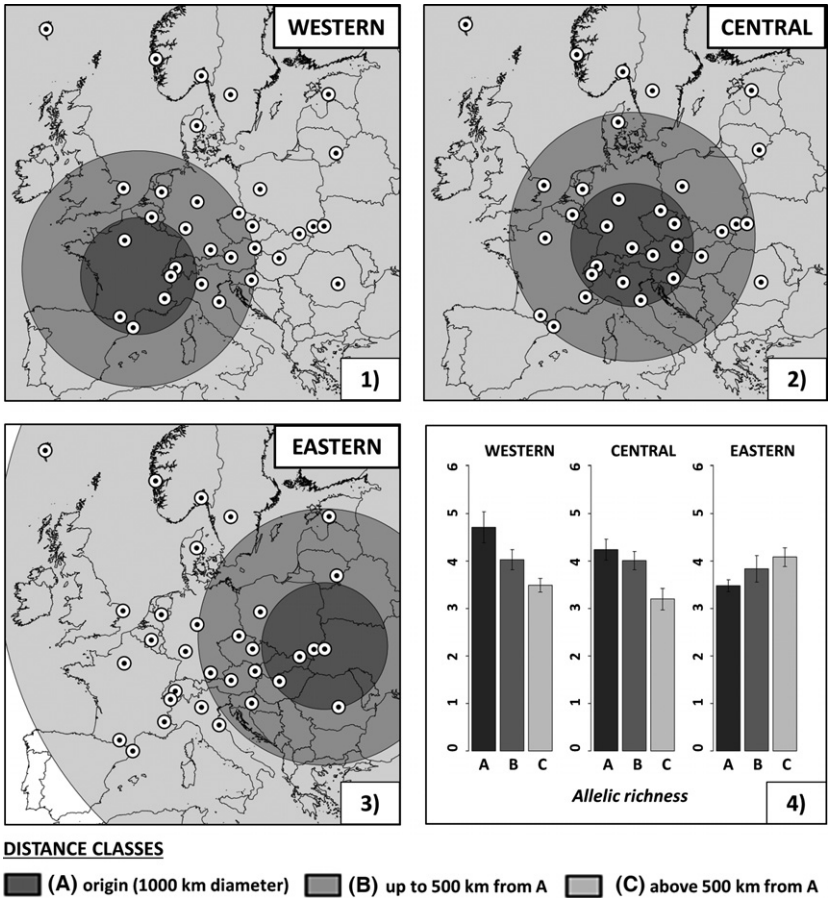

Fig. 2 Schematic representation of three different scenarios of *Arion vulgaris* origin and spread (1-3) that were tested for relationship with allelic richness (4). The sampling locations (white circles with a black dot) were categorized into three different classes according to their distance from the tentative area of origin: Western (1), Central (2) and Eastern (3). Mean allelic richness in nucDNA (4) plotted for each class (A = area of origin (500 km radius), B = up to 500 km distance from A, C = above 500 km distance from A) in each scenario. Error bars represent standard error. The average allelic richness decreased with distance from the class A for Western and Central scenarios, and increased for the Eastern scenario.

(Cornuet *et al*. 2008, 2010). A wide uniform distribution of 10–10 000 was chosen as the prior for effective population sizes (Table S6). For each model, we made sure that the range of the simulated data contained the estimates of the observed summary statistics.

We assessed the discrepancy between the models and observed data by principal component analysis (PCA) implemented in DIYABC with 10 000 simulated data sets. The posterior probability of each scenario was estimated by (i) taking the 500 simulated data sets closest to the observed data set and calculating the proportion that belonged to each scenario (direct approach) and (ii) using logistic regression on the 1% of the simulated data sets closest to the observed data set (logistic approach; Cornuet *et al.* 2010, 2008). Type I and type II errors were estimated for the chosen scenario following Cornuet *et al.* (2010).

Results

Phylogenetic divergence in mtDNA

Both mtDNA sequence markers resolved very high levels of genetic diversity within the *Arion* genus. For ND1, we obtained 130 new sequences resulting in a total data set of 203 sequences together with published data (Tables S1, S4). This data set contained 276 variable sites in 76 haplotypes that we used for phylogenetic reconstructions (Fig. S1; Table S4). For COI, we sequenced 120 *Arion* sp. slugs and complemented these with 222 published sequences from the closer relatives in the genus (Tables S1, S5). We observed 125 variable sites that defined 93 haplotypes in the larger *Arion vulgaris/rufus/ater* clade (Fig. S2; Table S5).

Our phylogenetic analyses revealed in both markers identical, deeply divergent and strongly supported clades despite the differences in taxon coverage (Figs S1, S2). The clustering of some named sequences from the databases was at odds with current taxonomy (see also Quinteiro *et al.* 2005; Pfenninger *et al.* 2014). In general, however, both markers resolved the same monophyletic clades that consisted of multiple individuals that were confirmed as *A. vulgaris*, *A. rufus* or *A. ater*.

Diversity patterns within the A. vulgaris, A. rufus and A. ater mtDNA clades

Our comparative assessment revealed generally much lower levels of mtDNA diversity in *A. vulgaris* than in the closely related and codistributed *A. rufus* and

A. ater. Among 110 *A. vulgaris* individuals from 44 locations covering most of Europe, we found only 11 ND1 and 23 COI haplotypes (Table 2). *Arion rufus* and *A. ater* from across Europe showed very similar numbers of ND1 haplotypes to *A. vulgaris* even though much fewer localities and individuals were covered in our data set (*A. rufus*: 20 individuals, 12 locations; *A. ater*: 18 individuals, 13 locations). Accordingly, haplotype diversity in *A. vulgaris* (ND1: 0.48 and COI: 0.79) was much lower than in *A. rufus* (0.93 and 0.94) and *A. ater* (0.9 and 0.92). Nucleotide diversity was considerably lower in *A. vulgaris* (0.002 and 0.007) and *A. rufus* (0.014 and 0.007) than in *A. ater* (0.043 and 0.045).

In spite of the often sympatric occurrence of the three taxa (Fig. 1), haplotype networks revealed strong differences between species in the distribution and frequencies of haplotypes (Fig. 3). The most frequent ND1 haplotype in *A. vulgaris* (70%) was found in 21 of the 24 countries and 33 of the 44 locations surveyed (Fig. 3). France and Spain harboured several of the few rare haplotypes. Diversity in COI was generally higher, but the most frequent haplotype (41%) was found in 15 of the 28 countries and 35 of the 72 locations surveyed. Rare haplotypes were also mostly detected in France and Spain (Fig. 3). In comparison, the networks of the *A. rufus* and *A. ater* clades were much less biased towards a single, frequent haplotype dominating in many countries (Fig. 3; Table 2).

Genetic diversity in nuclear markers

Microsatellite genotypes were obtained from 632 *A. vulgaris* individuals including recently invaded areas and regions of presumed long-term presence. Polymorphism ranged overall between 7 and 37 alleles per locus with an average of 17. No significant linkage disequilibrium between markers was detected. The loci ALU_06_4 and ALU_30_2 were identified as potentially affected by the presence of null alleles in one quarter of the sampled populations. All further analyses were therefore additionally performed with a data set excluding these two loci. However, results were qualitatively the same and there were only slight quantitative differences compared with the original data set (results not shown).

Differentiation among European populations of A. vulgaris in nucDNA

The PCoA provided no clear separation among invasive slugs from different sampling locations (Fig. S4). Genotypes from the same location clustered often together but different populations overlapped largely and the first two axes of the PCoA explained only 5.74% and 4.7% of the variance. Genetic differentiation among the 32 populations was high overall with $F_{ST} = 0.221$. All pairwise comparisons between populations were significantly different from zero at $P < 0.01$, and populations from the recently invaded range in Estonia, Latvia, Romania and Ukraine showed somewhat higher pairwise F_{ST} values compared with populations from other regions (Table S7). The Mantel test detected a weak but significant association between pairwise genetic and geographical distances ($R^2 = 0.0826$, $P = 0.01$; Fig. S5) across the whole range of *A. vulgaris*, consistent with a pattern of IBD. However, there was no evidence of IBD among only the most recently invaded regions ($R^2 = 0.003$, $P = 0.87$; Fig. S5).

STRUCTURE results showed very high consistency between different runs across all K numbers but without spatial coherence of clusters. Two genetic clusters (Fig. S6) were indicated by the Evanno decision criterion, but the likelihood was not much different for $K = 4$ (Fig. S7; Table S8). For $K = 2$, most populations appeared largely nonadmixed with one cluster dominating Central and northern Europe and the other one western and eastern Europe (Fig. S6). For larger K values, signs of admixture within some populations remained stable, but there was no clear assignment to the clusters for most populations. Complementary analyses with TESS were not conclusive for the most likely K. We observed no plateau in the DIC plotted against

Table 2 Overview of sample sizes and genetic variation in the mitochondrial markers ND1 and COI for the invasive pest slug *Arion vulgaris* (AV) and its congeners *A. rufus* (AR) and *A. ater* (AA)

Marker	Species	Individuals	Locations	Countries	Variable sites	Haplotypes	Haplotype diversity ± SD	Nucleotide diversity ± SD
ND1	AV	111	44	24	11	11	0.48 ± 0.05	0.002 ± 0.0001
	AR	20	12	8	27	13	0.93 ± 0.04	0.014 ± 0.002
	AA	18	13	5	72	12	0.90 ± 0.07	0.043 ± 0.007
COI	AV	225	72	28	22	23	0.79 ± 0.02	0.007 ± 0.001
	AR	41	16	7	21	20	0.94 ± 0.02	0.007 ± 0.001
	AA	21	13	5	69	15	0.92 ± 0.05	0.045 ± 0.004

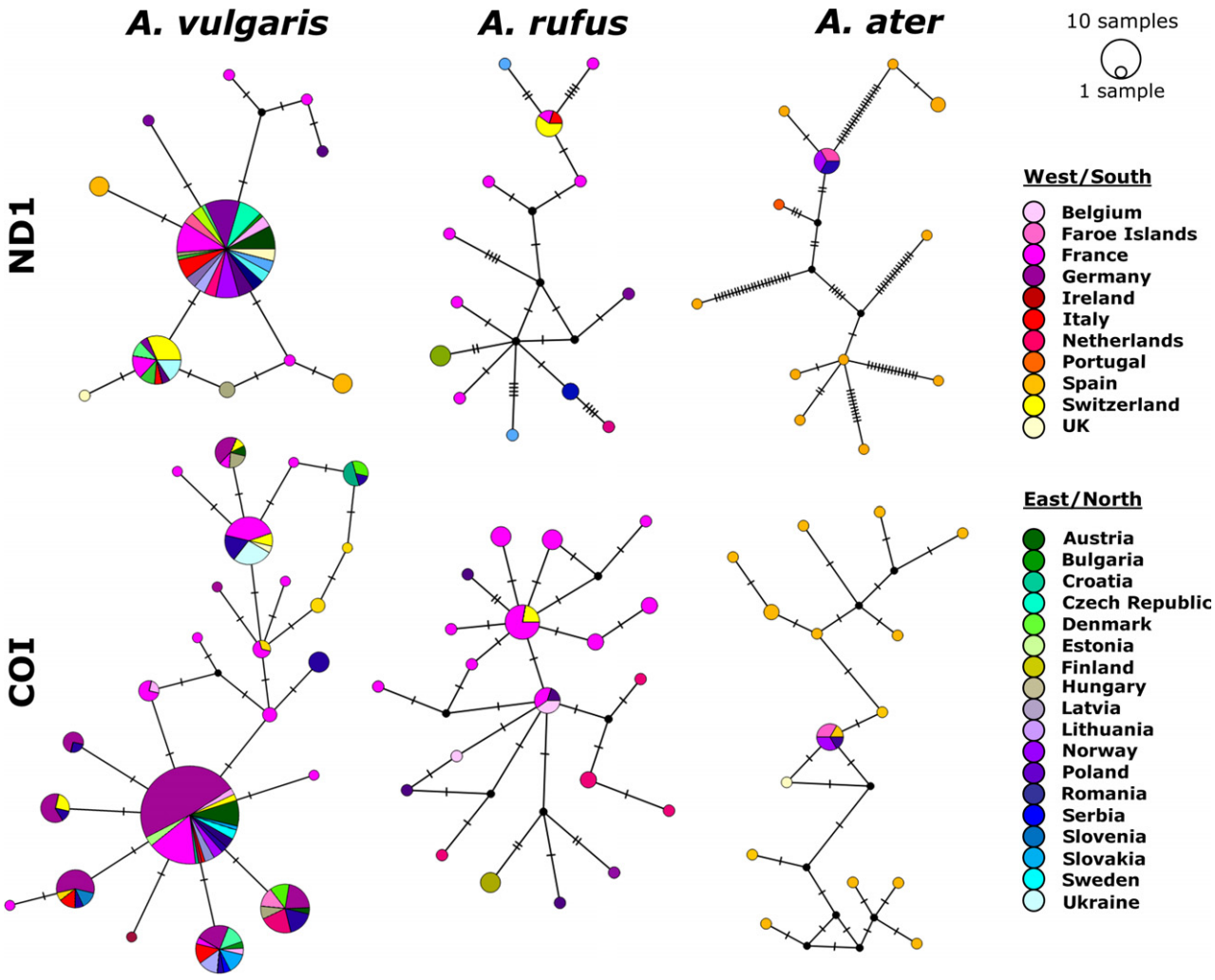

Fig. 3 Statistical parsimony haplotype networks of ND1 (top) and COI (bottom) sequences of *Arion vulgaris* (left), *Arion rufus* (centre) and *Arion ater* (right) sampled across Europe. The size of the circles is proportional to the number of individuals with a particular haplotype. Countries of origin are indicated by colours – western and southern European countries by warm colours, eastern and northern European countries by cool colours. Mutations are shown as hatch marks. Black dots represent inferred haplotypes that are either extinct or not sampled. For invasive *A. vulgaris*, 21 countries (of 24 surveyed) shared the same haplotype in the ND1 marker. Number of individuals, locations and countries of origin are listed in Tables S1, S4, S5.

the maximum number of clusters, which should indicate the optimal value of K (Fig. S8).

Spatial patterns of nucDNA diversity and expansion scenarios

Genetic variability at the population level averaged three to seven alleles per locus with a range from one to 13 alleles for individual loci and populations. Mean A_R ranged between 2.68 and 5.54 per population, mean H_O from 0.384 to 0.684, and H_E between 0.437 and 0.728 (Table 1). F_{IS} estimates, ranging from −0.035 to 0.227, were significantly positive ($P < 0.05$) in 10 of the 32 sampled populations (Table 1).

Genetic diversity was higher in some locations in the western and southern parts of the distribution range and generally lower in the north and the recently invaded areas in eastern Europe (Figs 4 and S9, Table 1). As a consequence, A_R decreased significantly with longitude ($R^2 = 0.1538$, $P = 0.03$; Fig. 5) and latitude ($R^2 = 0.1783$, $P = 0.02$). H_E also decreased significantly with latitude ($R^2 = 0.141$, $P = 0.03$), but the decrease was not significant for H_O ($R^2 = 0.1044$, $P = 0.07$). Association with longitude was not significant for either type of heterozygosity ($P > 0.24$). However, the population on the Faroe Islands – which is an outlier in the longitudinal association (Fig. 5) and clearly a human introduction – had a very strong effect

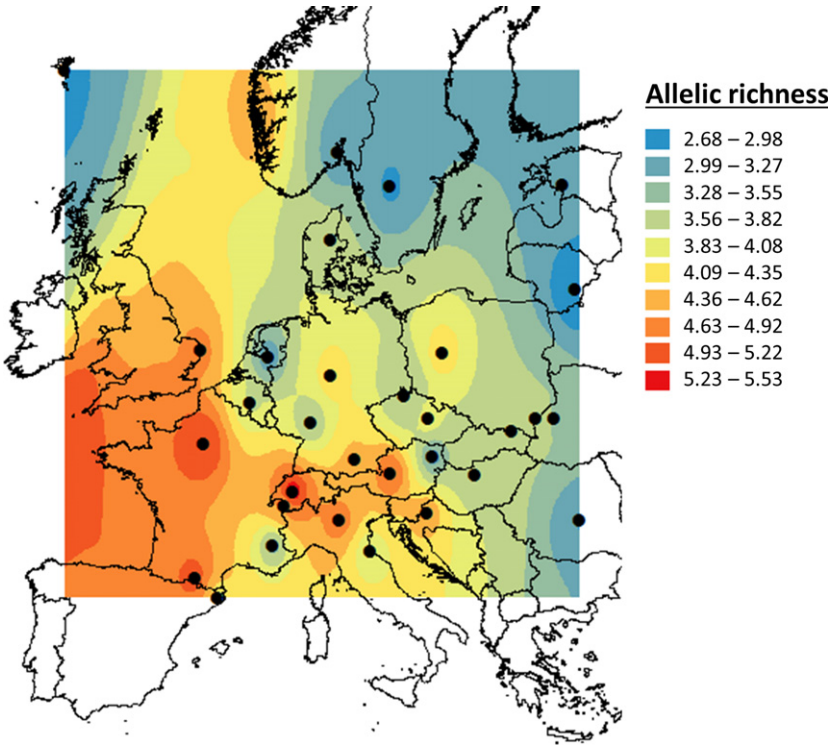

Fig. 4 Inverse distance weighting prediction maps based on mean allelic richness in *Arion vulgaris* populations. Black dots indicate the sampling locations (Table 1). Lowest values are in blue and highest in red. Allelic richness in nucDNA is highest mostly in Western and Central European populations and lowest mostly in Eastern and northern Europe. This corresponds with the recent invasion front in the east (Fig. 1).

on these correlations. Without this population in the North Atlantic, the decrease in genetic diversity was much stronger in the west–east direction (A_R: $R^2 = 0.2997$, $P = 0.001$; H_E: $R^2 = 0.1632$, $P = 0.02$; H_O: $R^2 = 0.0789$, $P = 0.13$) than from south to north (A_R: $R^2 = 0.1409$, $P = 0.03$; H_E: $R^2 = 0.0828$, $P = 0.12$; H_O: $R^2 = 0.0499$, $P = 0.23$).

Our comparison of three scenarios of the invasive slug origin detected a decrease in A_R with the distance from the assumed area of origin for the Western (ANOVA, $F_{2,28} = 7.51$, $P < 0.01$; Fig. 2; Table S9) as well as the Central origin scenario ($F_{2,28} = 4.79$, $P = 0.02$), and a nonsignificant increase with distance for the Eastern origin scenario ($F_{2,28} = 1.14$, $P = 0.34$). Post hoc Tukey's HSD tests revealed that in the Western and Central scenarios, class A with the supposed area of origin (Fig. 2; Western = 4.709 ± SE 0.327; Central = 4.237 ± 0.222) and the farthest class C (Western = 3.483 ± 0.145; Central = 3.194 ± 0.229) were significantly different. Heterozygosity estimates displayed the same patterns as A_R (Fig. S10; Table S9), but the difference between classes A and C was significant only for H_E in the Central scenario ($F_{2,28} = 5.147$, $P = 0.01$).

Demographic history inference

Tajima's D was negative for both mitochondrial markers analysed in *A. vulgaris* (ND1: −1.201; COI: −0.739), but not statistically significant. We observed an analogous pattern in Fu's Fs (ND1: −3.197; COI: −2.273). The mtDNA mismatch analyses indicated that a sudden population expansion in *A. vulgaris* could not be rejected for the ND1 marker (SSD = 0.031, $P = 0.615$; HRI = 0.296, $P = 0.669$), but yielded the opposite result for the COI marker (SSD = 0.096, $P = 0.003$; HRI = 0.256, $P = 0.014$) for which the distribution was bimodal (Fig. S11).

Concerning nucDNA, the evaluation of alternative demographic scenarios of *A. vulgaris* populations with ABC supported a very recent bottleneck in most regions of the distribution area. The model with a bottleneck event and population increase within the last 100 years, which is consistent with a recent introduction or invasion, was favoured in 11 of 13 regions (Table 3). In these analyses, direct and logistic regression approaches yielded in all populations very similar posterior probabilities exceeding 0.7 for the more likely demographic scenario and type I errors <0.2 and type II errors <0.1 (Table 3). Only for two *A. vulgaris* populations – Boussenac (France) and Girona (Spain) located in southwestern Europe close to the Pyrenees – the demographic scenario with a recent bottleneck was less likely than long-term population stability after the last glaciation (Table 3).

Discussion

The patterns of genetic diversity across the distribution range of *A. vulgaris* clearly showed the impact of

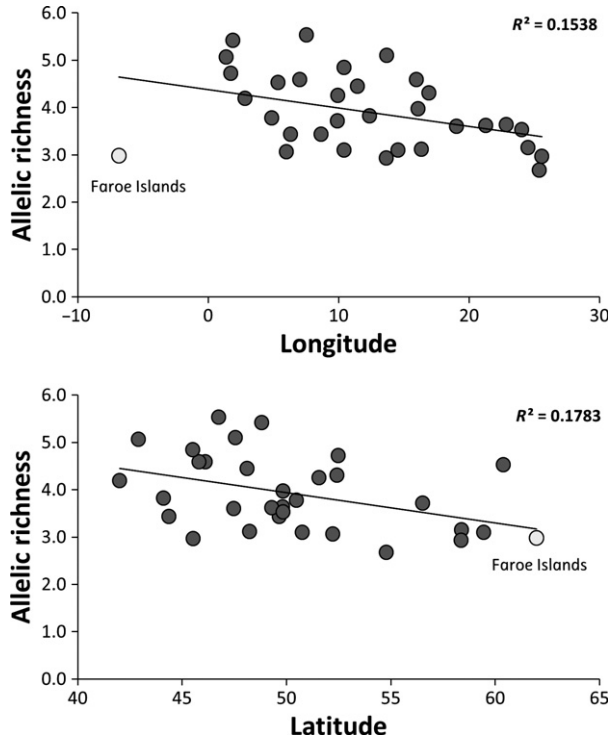

Fig. 5 Plots of allelic richness in *Arion vulgaris* populations vs. longitude (top) and latitude (bottom). European populations of *A. vulgaris* exhibited significant declines in allelic richness from west to east and south to north (see text). Note the outlier position of the population in the Faroe Islands (white dot) in the North Atlantic.

demographic and spatial expansion processes. Slugs in the recently invaded areas of eastern Europe are indeed *A. vulgaris* and they share common mtDNA types with many other European populations. The lack of further phylogeographic patterns together with the gradients of genetic diversity in nucDNA across Europe suggest a recent invasion of the species from south to north and in particular from west to east mediated by human activities.

Genetic signatures of the invasive history and presence of A. vulgaris

We observed large differences in mtDNA haplotype diversity among *A. vulgaris*, *A. rufus* and *A. ater* (Table 2), suggesting that the three species went through different demographic histories. Both mitochondrial markers displayed very little differentiation across the distribution range of *A. vulgaris* (Fig. 3) – a typical feature of biological invasions (Tooman *et al.* 2011; Torres-Leguizamon *et al.* 2011) that has also been found in other invasive molluscs (e.g. Tran *et al.* 2008; Schmidlin *et al.* 2012; Tarnowska *et al.* 2013). Except for the rare haplotypes from France and Spain (Fig. 3),

Table 3 Posterior probabilities of two demographic scenarios on the basis of ABC analyses of 13 populations covering the European range of *A. vulgaris*. The best supported scenario (direct/logistic approach) for a particular location is shown in bold italics. See Fig. S3 and Table S6 for more details on the scenarios

Location	Country	Scenario 1: postglacial bottleneck	Scenario 2: recent bottleneck
Bardejov	Slovakia	0.26/0.19	***0.74/0.81***
Boussenac	France	***0.98/0.94***	0.02/0.06
Egge	Norway	0.02/0.01	***0.98/0.99***
Gatteo	Italy	0.04/0.02	***0.96/0.98***
Girona	Spain	***0.96/0.95***	0.04/0.05
Heppenheim-Hambach	Germany	0.24/0.23	***0.76/0.77***
Novy Bor	Czech Republic	0.28/0.27	***0.72/0.73***
Poznan	Poland	0.25/0.07	***0.75/0.93***
Selonnet	France	0.12/0.12	***0.88/0.88***
Timişu de Sus	Romania	0.22/0.14	***0.78/0.86***
Västergötland	Sweden	0.01/0.02	***0.99/0.98***
Velbastaður	Faroe Islands	0.32/0.29	***0.68/0.71***
Vilnius	Lithuania	0.04/0.05	***0.96/0.95***

there was no clear geographic structure in mtDNA of *A. vulgaris*, which is consistent with a very rapid spread of this species. In stark contrast to the sharing of one haplotype across many European countries in *A. vulgaris*, noninvasive molluscs usually show different haplotypes linked to geographical regions as the result of natural postglacial colonization (Pfenninger & Posada 2002; Pinceel *et al.* 2005b). For example, mtDNA diversity of *A. subfuscus* is relatively high across its native European distribution range (Pinceel *et al.* 2005a) – similar to what we observed for *A. rufus* and *A. ater* – but its genetic diversity is strongly reduced in introduced populations in the USA (Pinceel *et al.* 2005c). Negative Tajima's D and Fu's F hint at the demographic expansion in *A. vulgaris*, but low sequence variability probably limits the signal. The unimodal mismatch distribution in ND1 is fully consistent with a very recent process and only the much extended sampling coverage of COI appears to have picked up some more ancestral structuring in certain parts of the species' range. Particularly, the central and southern parts of France appear most interesting for future sampling in search for further ancestral genetic variation.

Considering nucDNA patterns, the records of recent establishment of the species in many countries are consistent with low genetic diversity in populations probably due to founder events. The population on the Faroe Islands far from the continent is a clear example of a human introduction (*A. vulgaris* detected there in 1996) and very low genetic diversity (Figs 1 and 5). Low

nucDNA diversity in the *A. vulgaris* population in Denmark is consistent with the suggestion of Engelke *et al.* (2011) that some populations in Denmark and Germany have recently gone through a genetic bottleneck. The reduction in the number of individuals during a bottleneck has often a stronger impact on the number of alleles than on heterozygosity levels, because rare alleles can be lost quickly at small population sizes while heterozygosity may still remain at relatively high levels for some time (Nei *et al.* 1975; Luikart & Cornuet 1998). Low diversity in the populations discovered in eastern Europe only in the last decade is also consistent with a recent introduction. The species reproduces preferably by outcrossing (Engelke *et al.* 2011), but the possibility of self-fertilization or mating with close relatives may be beneficial for the initial establishment of an invasive species at low numbers of individuals and might contribute to slight deficits of heterozygosity in some populations (Wright 1921; Table 1). Comparable studies with population-based sampling and highly variable nuclear markers are very rare for terrestrial molluscs, but the expected patterns of decreased genetic variability in recently founded populations and relatively low differentiation between populations have been found in invasive aquatic species (Guiller *et al.* 2012; Miller *et al.* 2013; Riquet *et al.* 2013; Giantsis *et al.* 2014), as well as in other terrestrial invertebrates with low dispersal rates (e.g. Choi *et al.* 2013; Keever *et al.* 2013). The invasive success of *A. vulgaris* despite impoverished genetic diversity conforms to the so-called genetic paradox of invasive species (Frankham 2005). High genetic diversity is not always necessary for the success of invasions (Dlugosch & Parker 2008), and for example, phenotypic plasticity or rapid evolutionary responses might be more important (Lee 2002; Le Roux *et al.* 2007; Knop & Reusser 2012).

Genetic structuring in nucDNA of *A. vulgaris* was significant at the individual and population levels across Europe (Table S7), but similarly to the mtDNA patterns, there was no evidence of major genetic and geographical subdivisions that might be expected for a scenario of mostly natural (re)colonization after the end of the last glaciation around 18 000 years ago. Results from genetic clustering remained largely inconclusive except possibly for an indication for a geographically disjunct cluster mostly in the west (Spain, France, Belgium, UK) and the east (Poland, Ukraine, Lithuania, Estonia) of the distribution range that is separated by the other cluster (Fig. S6). Taken at face value, this could possibly indicate that the recently invaded populations in eastern Europe were founded by individuals from the western part of Europe. However, given the signs of mixing of clusters in many populations, the inconclusiveness of other clustering approaches and the mtDNA patterns of shared haplotypes, this potential west–east connection should be considered with much caution.

Our results have profound ramifications for the recent claim that *A. vulgaris* – one of the often cited and popularized examples of a pest invasion (Rabitsch 2006; Păpureanu *et al.* 2014) – would be native to Central Europe and not invasive (Pfenninger *et al.* 2014). This claim was based on incomplete genetic sampling of the distribution range and results from environmental niche modelling that suggested very similar potential distribution ranges at present and during the last glacial maximum. The detection of the species in new regions of Europe over the last century was supposed to be – at least in part – an artefact of increased awareness towards pest slugs. However, even though some of the older historical records might be incorrect, our data show clearly that slugs in the extensive, recently invaded areas of eastern Europe are indeed *A. vulgaris*. The current distribution range of the species is much larger than the potential range inferred by Pfenninger *et al.* (2014), and the accuracy of the past range reconstructed in that study is unknown. Importantly, the conclusion that *A. vulgaris* is not invasive is in conflict with our much larger data set and needs to be revised. This finding is important for the management of these pest slugs, as effective control measures can only be implemented when invasive species are early and correctly identified (Mack *et al.* 2000; Lodge *et al.* 2006).

Geographical origin of the invasive slugs

Our comparison of alternative scenarios clearly dismisses the suggestion of a potential Eastern origin of the *A. vulgaris* invasion (see Pfenninger *et al.* 2014). We rather found best support overall for the Western origin scenario. The detection of a latitudinal decrease in genetic diversity additional to a longitudinal effect (Fig. 5) could be interpreted with the central–peripheral hypothesis, CPH (Carson 1959; Brown 1984), that states that within a (natural) species distribution range, peripheral populations should show reduced genetic diversity due to bottleneck effects in comparison with central populations (Rowe & Beebee 2003). Studies testing for the CPH typically define the centre as the geographic core of the species range (e.g. Garner *et al.* 2004; Dixon *et al.* 2013), similar to our Central origin scenario (Fig. 2). However, genetic diversity in *A. vulgaris* was highest in the western parts. The predictions of the CPH may not hold for a particular system for various reasons (Micheletti & Storfer 2015), but the longitudinal decrease in nucDNA diversity in populations and the distribution of rare mtDNA haplotypes indicate an eastward expansion consistent with the historical records of the first detection of *A. vulgaris* throughout Europe.

Our high sampling effort shows that Portugal or most of Spain are unlikely to represent the region from where the 'Spanish' slugs originated that have recently invaded most of Europe. Unlike earlier surveys (Quinteiro *et al.* 2005; Pfenninger *et al.* 2014), we were able to detect the invasive slug at least at a single Spanish locality (Girona) in the Pyrenees. A higher probability of population stability in recent decades in our ABC results for this population and Boussenac from southern France (Table 3) could indicate that the origin of the invasion and/or the glacial refugium of the species might have been in this larger area. The particular sampling localities are, however, unlikely to represent the exact invasion origin or glacial refugium of the species and more detailed analyses with extensive sampling of this region could help to clarify this. Moreover, we did not find any *A. vulgaris* in any other part of the Iberian Peninsula (Fig. 1). This means that the species perhaps never existed in most parts of Iberia, has disappeared in recent decades or is at least extremely rare. The first description of an Iberian specimen in 1868 may thus rather represent confusion with the native Lusitanian slug *A. lusitanicus* and not true *A. vulgaris* (see Quinteiro *et al.* 2005). Indeed, several of the modern specimens that we obtained from western and northern Iberia represented the apparently endemic native taxon *A. lusitanicus*.

Human impact on the spread of A. vulgaris

For terrestrial species dispersing without any aid from humans, geography is typically the main factor influencing gene flow between populations which should result in a pattern of isolation by distance (Slatkin 1993). In our case, the correlation between genetic and geographic distance across the whole distribution range was very weak and absent among the most recently invaded populations (Fig. S5). This suggests the relevance of passive transport of *A. vulgaris* to new locations over long distances and that its current distribution has likely been influenced not only by natural dispersal. The presence of *A. vulgaris* in the Faroe Islands and Iceland (Ingimarsdóttir & Ólafsson 2005) is clear evidence of the role of humans in the long-distance spread of this slug. It is not known how the species reached these Atlantic islands, but accidental transport with agricultural or horticultural products appears most likely (Cowie & Robinson 2003).

The speed of range expansion observed in the ongoing invasion of eastern Europe is also implausibly high for the very low natural dispersal abilities of slugs. For example, if we consider the most recently invaded areas in eastern Europe (Lithuania, Estonia, Romania), the slugs would have had to expand their range from the border with the nearest country at a rate of at least 5–30 km per year on average based on the first occurrence records. In comparison with other ongoing invasions, the front of the highly mobile invasive cane toad in Australia has moved on average by 10–55 km per year (Phillips *et al.* 2006, 2007), or the invasive bank vole in Ireland is expanding its range through natural dispersal only by about 2.5 km per year after initial human introduction (White *et al.* 2012, 2013). The combination of natural dispersal and long-distance introduction by humans is a common feature of many biological invasions (Gilbert *et al.* 2004; Aubry *et al.* 2006; Ciosi *et al.* 2011), which has crucial implications for control measures. These can be improved by preventing the establishment of new populations rather than by focusing the eradication efforts on already established fronts (Moody & Mack 1988; Suarez *et al.* 2001). The very recent records of the species from eastern Europe suggest that the current distribution of *A. vulgaris* is not stable and still likely to expand, potentially aided by changes in land use, which often favour synanthropic and pest species (Delattre *et al.* 1992; Kiritani 2007; Kappes & Schilthuizen 2014).

Conclusions

The patterns of nuclear genetic diversity in populations and mtDNA suggest that the history of *A. vulgaris* is significantly impacted by recent demographic and spatial expansion processes. Even though some of the older historical records of first observation might be inaccurate, the overall pattern of a spatial expansion of *A. vulgaris* into the north and east of Europe is certainly genuine, and thus, the reclassification of these voracious pests as noninvasive would be premature. Given its severe impact, stricter control measures on commodities within Europe appear necessary and the further expansion needs to be monitored to enhance our ability to mitigate damage to the ecosystem.

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The idea for this project was conceived by E.K. and G.H. M.A.Z. and E.K. obtained the samples. M.A.Z. conducted the molecular work and data analyses under supervision of G.H. and E.K. All authors contributed to the writing of the manuscript and approved the final version.

Data accessibility

DNA sequences are available in GenBank under Accession nos KX834566–KX834695 (ND1) and KX834696–KX834815 (COI). Genotype data are available in Dryad under doi:10.5061/dryad.5331t. Sampling location information for all samples is provided in the Supplementary Material.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. Bayesian reconstruction of phylogenetic relationships based on 400 bp ND1 sequence haplotypes of *Arion* species with *Deroceras* sp. as an outgroup.

Fig. S2. Bayesian reconstruction of phylogenetic relationships based on 560 bp COI sequence haplotypes of *Arion* species.

Fig. S3. Diagram illustrating the two demographic scenarios tested within the ABC framework.

Fig. S4. Principal coordinates analysis (PCoA) of genetic distances among the 632 individuals of *A. vulgaris* from 32 European locations described in Table 1.

Fig. S5. Mantel test of association of the geographical and codominant genetic distance (pairwise FST) between individuals of *A. vulgaris* in the whole range (top; correlation $R^2 = 0.0826$, $P = 0.01$) and in the most recently invaded regions (Fig. 1).

Fig. S6. Population genetic structure of *A. vulgaris* in Europe.

Fig. S7. Delta *K* values plotted for each number of clusters (*K*), derived from the summarized 100 STRUCTURE iterations for $K = 1$–10 and evaluated by the Evanno method in STRUCTURE HARVESTER.

Fig. S8. Average DIC values from TESS plotted against the maximum number of clusters (*K* max).

Fig. S9. Inverse distance weighting prediction maps based on observed and expected heterozygosity.

Fig. S10. Mean observed heterozygosity (top) and expected heterozygosity (bottom) plotted for each class (A = area of origin (500 km radius), B = up to 500 km distance from A, C = above 500 km distance from A) in Western, Central and Eastern scenario (see Fig. 3 for details).

Fig. S11. Mismatch distributions for *A. vulgaris* using the ND1 (left; unimodal) and COI (right; multimodal) marker.

Table S1. Sequenced individuals of *Arion* spp. with the country of origin, latitude and longitude of the sampling location.

Table S2. List of accession numbers and species (as assigned by the authors of the respective data) of 73 ND1 sequences downloaded from GENBANK.

Table S3. List of accession numbers and species (as assigned by the authors of the respective data) of 462 COI sequences downloaded from GENBANK.

Table S4. List of 203 ND1 sequences used to reconstruct phylogenetic relationships and haplotype network.

Table S5. List of the selected 342 COI sequences used to reconstruct phylogenetic relationships and haplotype network.

Table S6. Priors of the ABC models (illustrated in Fig. S3) used for the ABC analyses.

Table S7. Genetic differentiation between pairs of populations measured as pairwise FST.

Table S8. Summary of the 100 STRUCTURE iterations for K = 1–10 evaluated by the Evanno method in STRUCTURE HARVESTER.

Table S9. Mean and standard error (SE) of allelic richness, observed heterozygosity and expected heterozygosity values in each class (A, B, C; see Fig. 2 for details) in the Western, Central and Eastern scenario.