



Slimy invasion: Climatic niche and current and future biogeography of *Arion* slug invaders

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Abstract

Aim: The current volume of global trade has led to an unprecedented rate of biological invasions, causing severe problems to native ecosystems. The knowledge of species introduction routes and areas suitable for establishment is therefore an important step in preventing future invasions. The situation can be further exacerbated by climate change, which might alter the amount of environmentally suitable areas for establishment of invasive species. Here, we focus on three *Arion* slug species recently introduced to North America and Australia with potentially significant impact—*A. ater*, *A. rufus* and *A. vulgaris*.

Location: Worldwide.

Results: We combined interception records, molecular analyses and species distribution modelling to assess their introduction history and to predict which regions are at highest risk of future invasions. We found extensive sharing of mitochondrial haplotypes among continents in all three species. In concordance with the genetic analyses, interception records suggest that slugs were introduced to the USA and Australia primarily from France, the Netherlands and the UK, but also from other locations in North America. The models predicted climatically suitable regions for the three *Arion* species in several areas across the globe for which management actions can be targeted.

Main conclusions: While the amount of regions with climatic conditions that would be suitable for slug establishment is predicted to slightly decrease under future scenarios, new suitable areas will also emerge. We therefore recommend that prevention efforts to limit new introductions should continue in order to protect vulnerable native ecosystems.

KEYWORDS

Arion ater, *Arion rufus*, *Arion vulgaris*, climate change, invasive species, species distribution modelling

1 | INTRODUCTION

The number of species introductions outside of their native range has grown dramatically over the last 200 years as a consequence of human colonization and trade, and some of these introduced species

become invasive and cause significant impact (Essl, Winter & Pysek, 2012; Mack et al., 2000). In fact, invasive species are currently considered one of the most important drivers of global change and biodiversity loss (Bellard, Cassey & Blackburn, 2016; Bellard, Leclerc & Courchamp, 2015; Clavero & Garcia-Berthou, 2005).

Successful invasions are the result of species overcoming several barriers (Blackburn et al., 2011). The propagule pressure, that is number of individuals introduced and number of introduction events, plays a major role in the likelihood of species establishment (Cardador, Carrete, Gallardo & Tella, 2016). For the later stages, that is becoming invasive and exhibiting negative impact, inherent and environmental factors are deemed more relevant (Richardson & Pysek, 2012). If we want to prevent new invasions and limit further spread of ongoing ones, we need to understand both invasion routes and suitability of local environments through time and space (Broennimann, Mraz, Petitpierre, Guisan & Mueller-Schaerer, 2014).

Interception records at national ports can be used to infer the extent of propagule pressure and the origin of invasive species (Chapple, Whitaker, Chapple, Miller & Thompson, 2013), particularly if they are integrated with molecular analyses (Cristescu, 2015). While multiple introductions may fuel invasive potential, introduced populations often undergo genetic bottlenecks and founder events and are thus genetically less diverse than populations in their native range (Cristescu, 2015). This may decrease a population's ability to establish and persist in the area of introduction (Dlugosch & Parker, 2008), especially if the local environmental conditions differ from the species' native range.

Species distribution models (SDMs; Guisan & Thuiller, 2005; Guisan, Thuiller & Zimmermann, 2017) are a useful tool for predicting areas with suitable environmental conditions by relating occurrence records to climatic variables (Peterson, 2003). SDMs have been successfully used to predict the extent of biological invasions in both plants and animals (e.g., Broennimann & Guisan, 2008; Guisan et al., 2013). The predictions can be made not only for current, but also for future timeframes, which is especially important to anticipate the impact of climate change (IPCC, 2013). It has indeed been predicted that the rate and impacts of biological invasions might be exacerbated by climate change, providing new opportunities for introduction and spread of species into areas that were previously not suitable (Walther et al., 2009).

Here, we apply the above approaches to investigate the recent invasions and potential global spread of three terrestrial slug species of the *Arion* genus (*A. ater*, *A. rufus*, *A. vulgaris*). *Arion vulgaris* (syn. *A. lusitanicus*) belongs to the 100 most invasive species in Europe (DAISIE, 2009), and the large *Arion* species are on the list of potential major pests in the USA (Cowie, Dillon, Robinson & Smith, 2009). The interceptions of *Arion* sp. slugs on shipments from Europe are common at US ports (Barr et al., 2009), and the propagule pressure of introduced slugs can be significant (Robinson, 1999).

Although several *Arion* species have successfully established and invaded parts of most continents during the last two decades (Cadiz & Gallardo, 2007; Gutierrez Gregoric et al., 2013), they have often not been identified to the species level. Moreover, the worldwide potential of *Arion ater*, *A. rufus* and *A. vulgaris* to establish under current and future climate has never been assessed. Previous studies using SDMs predicting slug distribution were conducted only on a local scale and with a single modelling technique (Patrao et al., 2015; Pfenninger, Weigand, Balint & Klussmann-Kolb, 2014).

To address this gap in knowledge, we present here the first global-scale study using mtDNA sequences of the three *Arion* species, combined with data from interception records and with model predictions of their potential current and future distributions. Specifically, in this study, we aimed to: (a) determine the introduction history of *Arion* sp. specimens in North America and Australia, and from this (b) evaluate which areas worldwide are currently and in the future most prone to *Arion* sp. slugs' invasion. This integrative approach can provide new insights into the *Arion* sp. invasions worldwide and enhance our ability to predict and mitigate their impact.

2 | METHODS

2.1 | Study species

Slugs are dominant seedling predators (Nystrand & Granstrom, 1997; Wilby & Brown, 2001), thus capable of altering plant community composition. They can also affect multitrophic interactions among other plant consumers and their predators (Desurmont, Zemanova & Turlings, 2016), and some invasive slugs have been shown to compete with native species (Thompson & Iyengar, 2015; Zemanova, Knop & Heckel, 2017) and predate on seeds rather than disperse them (Blattmann, Boch, Tuerke & Knop, 2013).

Arion ater, *A. rufus* and *A. vulgaris* slugs are 12–15 cm long, hermaphrodites and lay up to 400 eggs 1–2 times per year (Kozłowski, 2012). They primarily outcross but are capable of self-fertilization as well (Engelke, Koempf, Jordaens, Tomiuk & Parker, 2011). *Arion ater* is native to Northern and North-Western Europe and is found mainly in humid biotopes, especially forests, while *A. rufus* occurs in mostly natural habitats of the Central and Western Europe (Kremer, 1990). Outside of Europe, the large black *Arion* slug (unclear whether *A. ater* or *A. rufus*) has been introduced to Alaska probably within the last 20 years and is considered invasive there, with its current occurrence restricted mainly to south-eastern and south-central coastal areas (Meyers, 2006). In Canada, the first report of *A. ater* or *A. rufus* dates back to the 1940's in British Columbia (Forsyth, 2004), more recent observations are from Quebec (Allard, 2012). The occurrence of *A. ater* individuals has also been described from Australia after the year 2000, with one resident reporting to have collected over twenty thousand specimens (records of Museum Victoria). The historical distribution of *A. vulgaris* is not known, because it has spread and become invasive in many European countries in the last 60 years (Rabitsch, 2006; Zemanova, Knop & Heckel, 2016). There is a single unconfirmed report of *A. vulgaris* presence in the USA from Cornell University from 1998 (www.cabi.org/isc/datasheet/6963).

Because all three species can have similar colour morphs, it is very difficult to distinguish them based on their external morphology. The situation is further complicated by the fact that *A. rufus* and *A. ater* used to be regarded as sister-subspecies by some authors (e.g., Gottfried, Dorfman & Wall, 1967) and historical occurrence records may not be reliable. Identification with molecular markers is therefore essential (Zemanova et al., 2016).

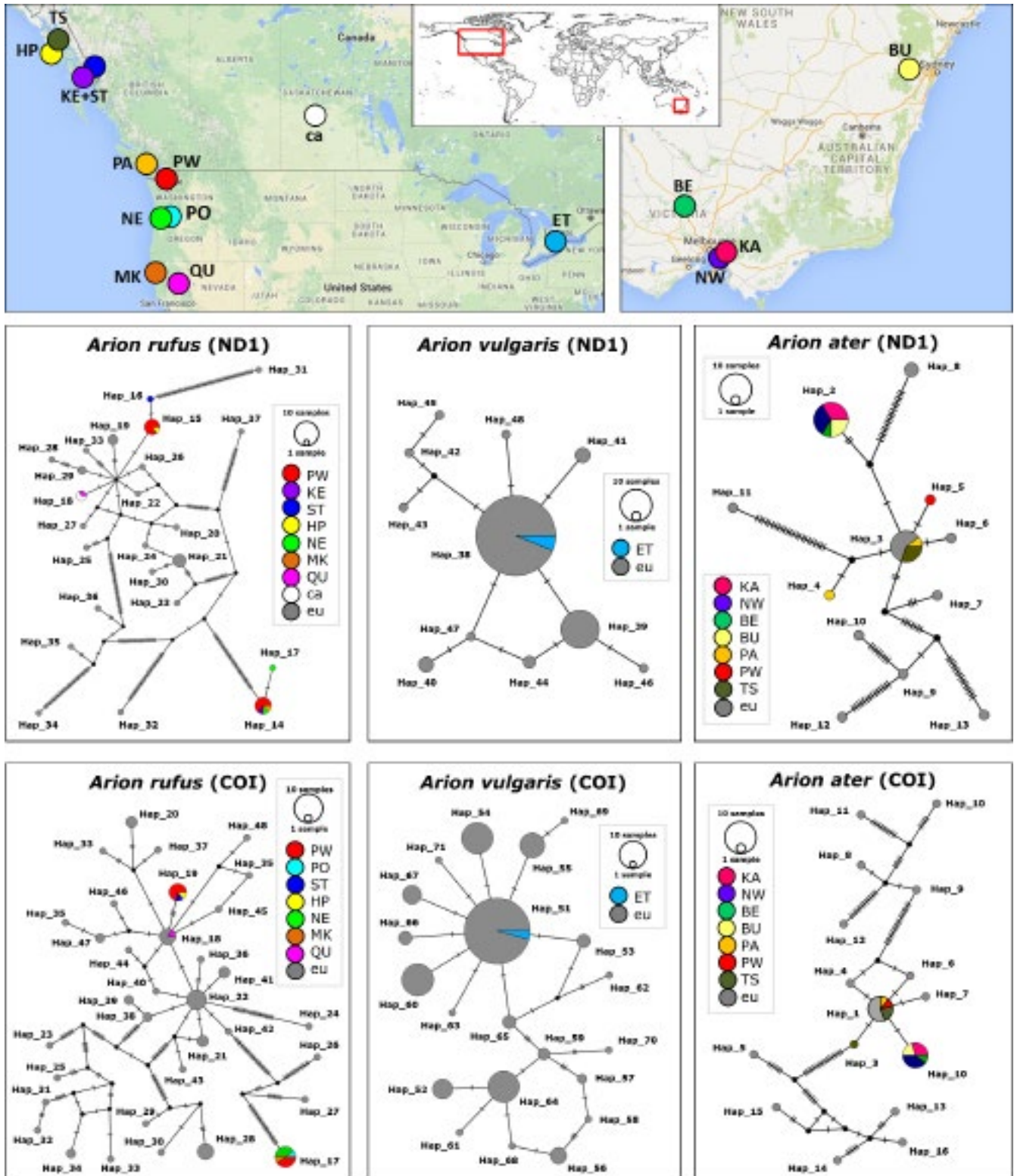


FIGURE 1 Sampling locations and haplotype networks of *Arion rufus*, *Arion vulgaris* and *Arion ater*. Haplotype numbers correspond to Figures S2 and S3 and Tables S2 and S3. List of individuals in each haplotype is in Tables S2 and S3. The size of the circles is proportional to the number of individuals with a particular haplotype. Mutations are shown as hatch marks. Small black dots represent inferred haplotypes that were not sampled. Note that the exact area of origin of the individuals from Canada is not known [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Sampling locations of the three species (identified with ND1 and/or COI markers), with year of sampling and number of individuals sequenced for each marker. Note that the two individuals with Code “ca” were found at a US port on a *Thuja* sp. plant shipment from Canada (exact location of origin is unknown)

Species	Country	Location	Code	Latitude	Longitude	Year	ND1	COI
<i>Arion ater</i>	Australia	Kallista	KA	-37.8836	145.3671	2004	4	3
	Australia	Narre Warren	NW	-38.0025	145.3298	2014	4	4
	Australia	Bendigo	BE	-36.7610	144.2822	2014	1	1
	Australia	Bullaburra	BU	-33.7258	150.4165	2014	3	2
	Canada	Port Alberti	PA	49.2394	-124.7864	2013	2	1
	USA	Point Whitehorn Marine Reserve	PW	48.8864	-122.7796	2013	1	1
	USA	Tenakee Springs	TS	57.7804	-135.2191	2014	3	3
<i>Arion rufus</i>	USA	Point Whitehorn Marine Reserve	PW	48.8864	-122.7796	2013	11	9
	USA	Ketchikan	KE	55.3409	-131.6455	2007	1	0
	USA	Saxman Totem Park	ST	55.3420	-131.6461	2007	1	1
	USA	Halibut Point Road	HP	57.0871	-135.3831	2007	1	1
	USA	Portland	PO	45.5110	-122.6786	2014	0	1
	USA	Nehalem	NE	45.5719	-123.8941	2014	2	4
	USA	McKinleyville	MK	40.9379	-124.1011	2014	1	1
	USA	Quincy	QU	39.9364	-120.9477	2014	1	1
<i>Arion vulgaris</i>	Canada	Etobicoke	ET	43.6325	-79.5076	2009	5	4

2.2 | Interception records and trends in trade volumes

The data on slug interceptions at US ports in the period from 1985 to 2014 were provided by the Animal and Plant Health Inspection Service of the US Department of Agriculture (APHIS USDA). The Department of Agriculture and Water Resources Australia (DAWRA) provided interception records for the period from 1990 to 2014, and the Canadian Food Inspection Agency (CFIA) for years 2001 to 2013. We considered all *Arion* sp. interceptions without distinguishing the species, because often only information at the genus level was available. To test whether there is an association between the type of merchandise that slugs were intercepted on and the country in which the merchandise originated, we performed a chi-squared test of independence (Pearson, 1900) and calculated Cramér's *V* (Cramér, 1946) to approximate the strength of association. Only the US data contained enough observations to conduct the analysis, which was performed in R 3.1.1 (R Core Team, 2014), using the R packages *vcd* (Meyer, Zeileis & Hornik, 2017) and *questionr* (Barnier, Briatte & Larmarange, 2017).

In order to visualize the trends in exports from Europe to the USA, Australia and Canada, we plotted the trade volumes of agricultural commodities (Harmonized System code 06—"live trees and other plants" and code 07—"edible vegetables and certain roots and tubers") for the years 2003–2016. The data were compiled from the European Commission's Market Access Database (http://madb.europa.eu/madb/statistical_form.htm).

2.3 | Sample collection and mitochondrial DNA sequencing

We obtained recently collected samples of 46 *Arion* sp. slugs morphologically resembling *A. ater*, *A. rufus* or *A. vulgaris* from nine locations in the USA, two locations in Canada and four locations in Australia (Figure 1; Tables 1 and S1). Importantly, except for one case of interception on trading commodities (Table 1), all samples were found in the field (i.e., in natural or anthropogenic habitats). The specimens were preserved in pure ethanol. DNA was extracted from a small piece of foot tissue using a high-salt extraction protocol (Aljanabi & Martinez, 1997).

We sequenced the mitochondrial NADH dehydrogenase 1 (ND1) of 43 and cytochrome C oxidase 1 (COI) loci of 37 individuals, respectively (Tables 1 and S1). Specific PCR primers developed by Quinteiro, Rodriguez-Castro, Castillejo, Iglesias-Pineiro and Rey-Mendez (2005) were used to amplify 400 bp of the ND1 gene. To amplify a fragment of the COI gene, we used the primers LCO1490 and HCO2198 (Folmer, Black, Hoeh, Lutz & Vrijenhoek, 1994). The molecular work was done following the protocol described in Zemanova et al. (2016).

2.4 | Phylogenetic analyses, species identification and genetic diversity

Raw sequences were aligned in BIOEDIT 7.1.3 (Hall, 1999) and trimmed to a length of 400 bp (ND1) or 560 bp (COI). To build a

phylogenetic tree and identify *A. ater*, *A. rufus* or *A. vulgaris* individuals, we combined our own sequences and data from our previous sampling of European locations (Zemanova et al., 2016) with ND1 or COI sequences of *Arion* sp. available in the GenBank database (www.ncbi.nlm.nih.gov/genbank). One *A. flagellus* sequence (ND1: AY316247; COI: AY987880) was used as outgroup. After reconstructing the phylogenetic relationships among the available samples (as in Zemanova et al., 2016), we retained those representing the three *Arion* species (ND1: Table S2; COI: Table S3). The final alignment consisted of 198 and 340 sequences for ND1 and COI, respectively.

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 ND1 and the HKY model with invariable sites and rate variation among sites for the COI analyses. The following maximum likelihood parameters were determined for ND1 and COI, respectively: base frequencies A = 0.313 and 0.333; C = 0.158 and 0.144; G = 0.141 and 0.102; T = 0.388 and 0.422, and the shape parameter of the gamma distribution (α): 0.439 and 3.312. 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 using the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 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, Suchard, Xie & Drummond, 2014). The trees were visualized in FIGTREE 1.4.2 (Rambaut, 2014).

We estimated several indices of genetic diversity: number of haplotypes (H), haplotype diversity (H_d) and nucleotide diversity (π) in DNASP 5 (Librado & Rozas, 2009). Genealogical relationships among haplotypes within *A. ater*, *A. rufus* and *A. vulgaris* were reconstructed

by generating a statistical parsimony haplotype network in POPART 1.7 (Leigh & Bryant, 2015).

2.5 | Occurrence data for species distribution modelling

We combined our genetically confirmed occurrence data with information from the Global Biodiversity Information Facility (GBIF; www.gbif.org). In order to minimize the sampling bias reported for the GBIF database (Beck, Böller, Erhardt & Schwanghart, 2014) and to prevent over-fitting and false inflation of model performance because of spatially autocorrelated records (Boria, Olson, Goodman & Anderson, 2014; Veloz, 2009), we removed occurrence records within 150 km of each other. This resulted in 117 retained records of *Arion* sp. (Figure 2).

2.6 | Environmental variables

For the subsequent analyses, we used bioclimatic data available in the WorldClim database (Hijmans, Cameron, Parra, Jones & Jarvis, 2005; www.worldclim.org/bioclim) at 5' resolution (~10 km). The 19 variables were tested for multicollinearity by running a correlation analysis between each pair of variables in R (R Core Team, 2014). From highly correlated variables (Pearson's correlation coefficient >0.8; Mateo et al., 2015) we selected the one that had potentially higher biological relevance for the species' distribution. The final set of variables used to run the models consisted of annual mean temperature (BIO1), temperature seasonality (standard deviation of mean monthly temperatures*100; BIO4), minimum temperature of the coldest month (BIO6), mean temperature of the wettest (BIO8) and driest quarter (BIO9) and annual precipitation (BIO12). These variables are closely correlated with physiological limitations of *Arion* sp. slugs (Crawford-Sidebotham, 1972; Slotsbo, Damgaard, Hansen & Holmstrup, 2013).

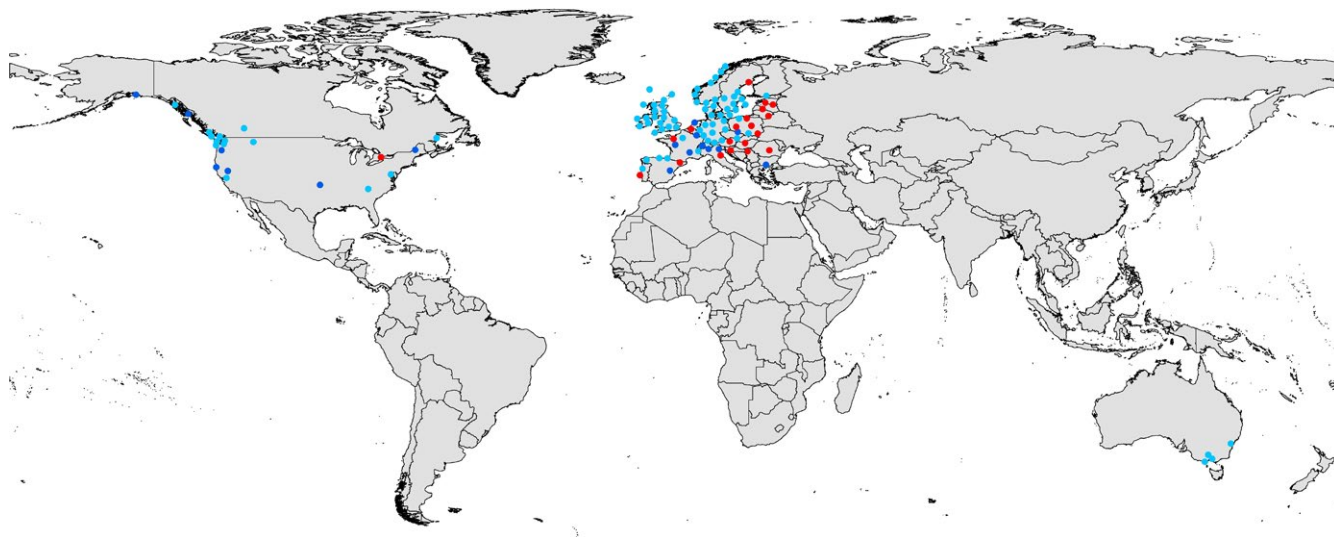


FIGURE 2 Occurrence records of *Arion ater*, *Arion rufus* and *Arion vulgaris* used for the species distribution modelling [Colour figure can be viewed at wileyonlinelibrary.com]

2.7 | Niche overlap analysis

As *Arion* slugs may not have been unambiguously identified to the species level in the available GBIF database and identification in interception records frequently relies on potentially inaccurate morphology, we tested whether the occurrence records of the three species could be pooled together for the SDMs calibration. We assessed the realized niche of each species in their European range by quantifying the first two components of a principal component analysis (PCA) based on the environmental variables described above (Broennimann et al., 2012). Niche overlap was quantified with Schoener's *D* metric (Broennimann et al., 2012; Schoener, 1970), which can range from 0 (no overlap) to 1 (total overlap). We computed the proportion of niche overlap between each species pair in R. All pairs showed at least moderate niche overlap (*A. ater* × *A. rufus*: $D = 0.435$, *A. ater* × *A. vulgaris*: $D = 0.317$, *A. rufus* × *A. vulgaris*: $D = 0.519$; Figure S1). We also ran the SDMs described below for each species separately, but the differences among the outputs were minimal (results not shown). We therefore conducted the following analyses with the pooled data. All niche analyses were run with functions available in the *ecospat* R package (Di Cola et al., 2017).

2.8 | Species distribution models

A worldwide SDM was produced for the three *Arion* species pooled using ensemble modelling (Araujo & New, 2007) implemented in the *biomod2* R package (Thuiller, Lafourcade, Engler & Araujo, 2009). The ensemble modelling consisted of four different techniques: generalized linear model (GLM; McCullagh & Nelder, 1989), gradient boosting machine (GBM; Friedman, 2001), random forest (RF; Breiman, 2001) and maximum entropy implemented in MAXENT (Phillips, Anderson & Schapire, 2006). The extent of geographic background was defined by biomes where the species are presently based on current occurrence records. We randomly sampled 1000 pseudo-absences within the geographic background (Barbet-Massin, Jiguet, Albert & Thuiller, 2012). The sum of weight of occurrences equalled the sum of weights of pseudo-absences. For each technique, 10 iterations of the model were performed. The predictive performance was evaluated using a repeated split-sample approach, with 70% occurrence records used for training the model and 30% for evaluation. Models were evaluated using the area under the receiver operating characteristic curve (AUC; Fielding & Bell, 1997) and the true skill statistic (TSS; Allouche, Tsoar & Kadmon, 2006). A unique ensemble prediction was created using the weighted average of individual model predictions. Predictions were calculated for both current and future climate.

Future projections for 2050 and 2070 were performed with models generated using the IPCC RCP 8.5 trajectory. This relatively extreme scenario predicts continuous increase in greenhouse gases throughout 2100 (Meinshausen et al., 2011), and we chose it because the recent increase in emission rates exceeded the estimations of all previous scenarios (Beaumont, Hughes & Pitman, 2008). We employed the HadGEM2-CC general circulation model (Martin

et al., 2011) that has been previously used in similar studies (e.g., Chang, Hansen & Piekielek, 2014; Shrestha & Bawa, 2014) to project the environmental conditions employed for future SDM scenarios. In order to compare the extent of current and future potential distributions, binary maps of suitable/unsuitable habitats were derived using the threshold maximizing TSS. The fraction of area lost and gained compared to the current distribution in 2050 and 2070 were quantified in R.

3 | RESULTS

3.1 | Trade volumes and interception records

While the volume of agricultural products exported from Europe to Australia and Canada remained relatively constant over the years 2003–2016, we observed an increasing trend in import to the USA, with a growth of over 47,000 tons of agricultural products between 2003 and 2016 (Figure S2).

Between 2001 and 2013 there were only six interceptions of *Arion* sp. slugs recorded in Canada (details not shown). The slugs were intercepted on plant material and all originated in the USA, specifically in Oregon, Michigan and California. At the Australian ports, 50 interceptions of *Arion* species were recorded between the years 1990 and 2014. Thirty of these interceptions were made in goods coming from France, and majority of the slugs were discovered on mushrooms, followed by cut flowers (Figure 3). The interception records at the US ports from the years 1985 to 2014 contained 520 slugs, mostly found on mushrooms, timber and vegetables. The merchandise originated mainly in Canada (170 interceptions), the Netherlands (79) and France (68; Figure 3). There was a statistically significant association between the type of merchandise and the country of origin ($\chi^2 = 851.07$; $df = 175$; $p < 0.001$), with a large effect size (Cramér's $V = 0.572$; Cohen, 1988). For instance, slugs found in mushrooms were for the most part imported from Bulgaria and France, while infested timber originated primarily in Canada.

3.2 | Species identification and haplotype diversity and distribution

The sampled *Arion* sp. slugs formed parts of distinct *Arion ater*, *A. rufus* or *A. vulgaris* clades in the phylogenetic trees based on ND1 (Figure S3) and COI sequences (Figure S4). Across the total sample of 198 sequences we found 48 haplotypes in the ND1 fragment. Within the *A. ater* clade containing 35 sequences we obtained 12 haplotypes; among the 46 *A. rufus* sequences there were 24 haplotypes, and within the *A. vulgaris* clade of 116 sequences, only 11 haplotypes were found (Table S2). The remaining haplotype contained the outgroup sequence of *A. flagellus* (Figure S3, Table S2). The 340 COI sequences contained 72 haplotypes. In this marker we observed 16 haplotypes in *A. ater* (33 sequences), 34 in *A. rufus* (77 sequences), 21 haplotypes in *A. vulgaris* (229 sequences) and one haplotype representing the *A. flagellus* sequence (Figure S4, Table S3). Haplotype and nucleotide diversity was relatively low in

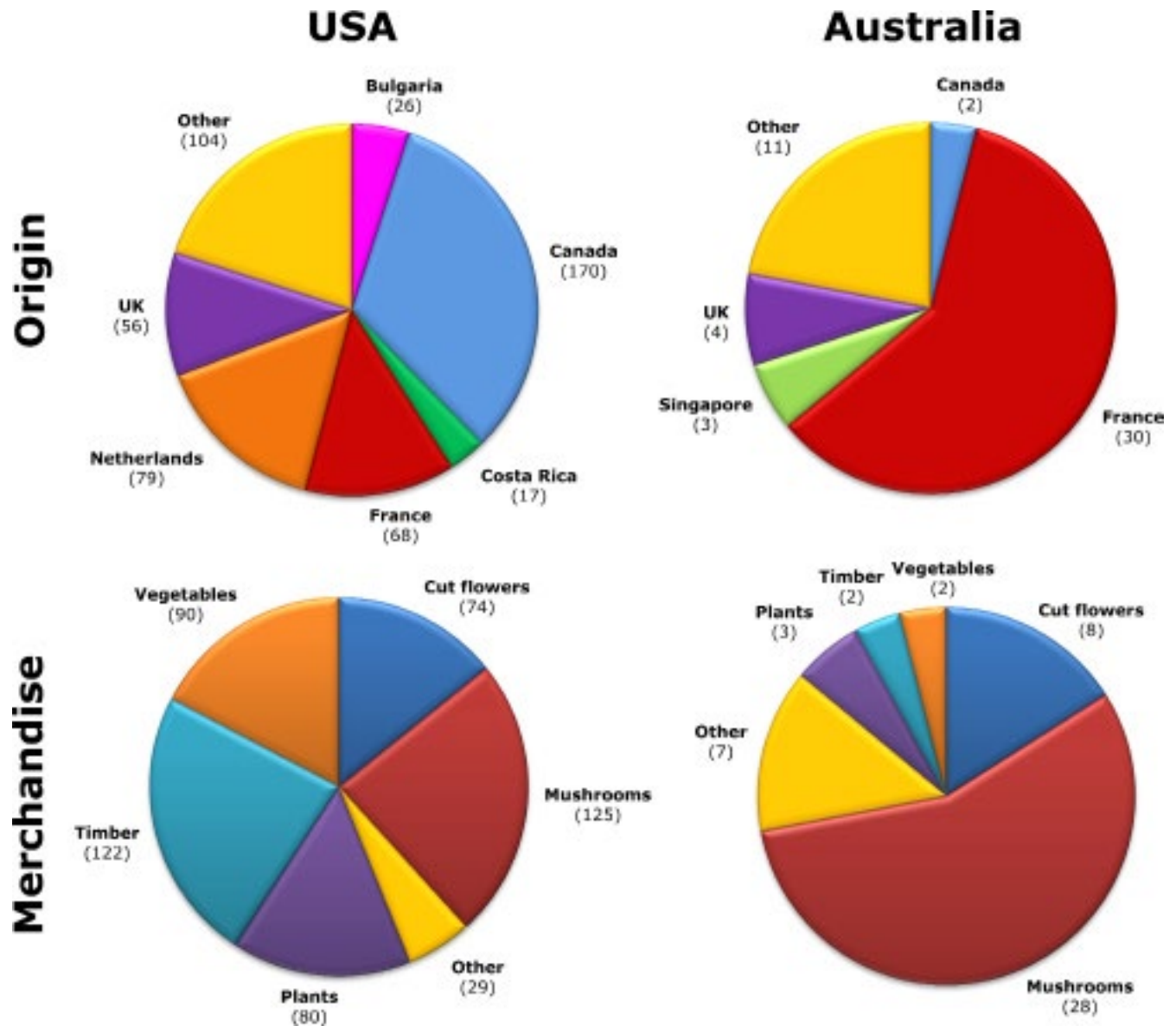


FIGURE 3 Records of *Arion* sp. slugs intercepted at US ports and Australian ports. Origin of the shipment is on top, merchandise in which the slugs were found on the bottom. Numbers in brackets indicate the amount of interceptions in each category. In the USA, the majority of slugs were found in mushrooms and timber, in Australia in mushrooms and cut flowers. The merchandise shipped to the USA originated mostly from Canada, the Netherlands and France, in Australia primarily from France [Colour figure can be viewed at wileyonlinelibrary.com]

the USA, Canada and Australia in both markers, except for the high nucleotide diversity within the *A. rufus* specimens from the USA (Table 2).

All Australian specimens, the two individuals from one Canadian location and four specimens from two sites in the USA belonged to the *A. ater* species (Tables 1 and S1). The sequences clustered in the ND1 marker into four distinct haplotypes—Hap_2-5 (Figures 1 and S3; Table S2). In the COI marker, we observed three haplotypes (Hap_1, 3, 10; Figure 1). The Australian haplotype did not occur among any other available sequences (Figure 1; Tables S2 and S3). One of the *A. ater* individuals found in Canada shared the haplotype (Hap_3 in ND1; Hap_1 in COI; Figure 1) with specimens from Tenakee Springs, USA, and in COI also with an individual from the

Point Whitehorn Marine Reserve, USA, as well as with individuals from Faroe Islands, Norway, Poland and Spain (Tables S2 and S3). The European *A. ater* sequences clustering closest to the individuals in the invaded regions were in ND1 from Spain (Hap_6, 8, 11) and Portugal (Hap_7), and in COI from Spain (Hap_4), Faroe Islands (Hap_6) and the United Kingdom (Hap_7).

All non-European *A. rufus* in our study were found in the USA (Tables 1 and S1). They were differentiated in the ND1 marker into five haplotypes (Hap_14-18) with extensive haplotype sharing among locations (Figures 1 and S3; Table S2). The analysis with the COI marker revealed three haplotypes (Hap_17-19; Figure 1). None of the five *A. rufus* ND1 haplotypes found in the USA was shared with the available European samples (Figure 1, Table S2), but in

TABLE 2 Genetic diversity (H —number of haplotypes, H_d —haplotype diversity, π —nucleotide diversity) within *Arion* species samples from the different geographical regions

Marker	Species	Region	Number of sequences	H	H_d	π
ND1	<i>Arion ater</i>	Australia	12	1	0	0
		Canada	2	2	1	0.005
		USA	4	2	0.5	0.001
		Europe	17	11	0.882	0.044
		TOGETHER	35	14	0.812	0.027
	<i>Arion rufus</i>	USA	20	5	0.726	0.076
		Europe	26	19	0.957	0.061
		TOGETHER	46	24	0.936	0.079
	<i>Arion vulgaris</i>	Canada	5	1	0	0
		Europe	111	11	0.479	0.002
TOGETHER		116	11	0.463	0.002	
COI	<i>Arion ater</i>	Australia	10	1	0	0
		Canada	1	1	0	0
		USA	4	2	0.5	0.001
		Europe	18	14	0.935	0.469
		TOGETHER	33	17	0.862	0.032
	<i>Arion rufus</i>	USA	18	3	0.569	0.055
		Europe	59	32	0.957	0.041
		TOGETHER	77	34	0.95	0.052
	<i>Arion vulgaris</i>	Canada	4	1	0	0
		Europe	225	23	0.794	0.007
		TOGETHER	229	23	0.787	0.007

COI the individual from Quincy clustered together with slugs from Belgium, France and Poland (Table S3). The closest ND1 haplotypes to *A. rufus* slugs in the USA were from individuals found in France (Hap_22, 26), Finland (Hap_19), Poland (Hap_29), Slovakia (Hap_33), Spain (Hap_31) and Germany (Hap_27). The closest COI haplotypes originated in slugs found in Spain (Hap_24, 26, 27), France (Hap_22) and Belgium (Hap_45).

The five slugs from Etobicoke in Canada were identified as *A. vulgaris*, which—to our knowledge—is the first genetically confirmed occurrence outside of Europe. They shared the same haplotype (Hap_38 in ND1 and Hap_51 in COI; Figure 1; Table S2), which was the most common one across all *A. vulgaris* sequences used in this study, accounting in ND1 for 71.5% and in COI for 43%.

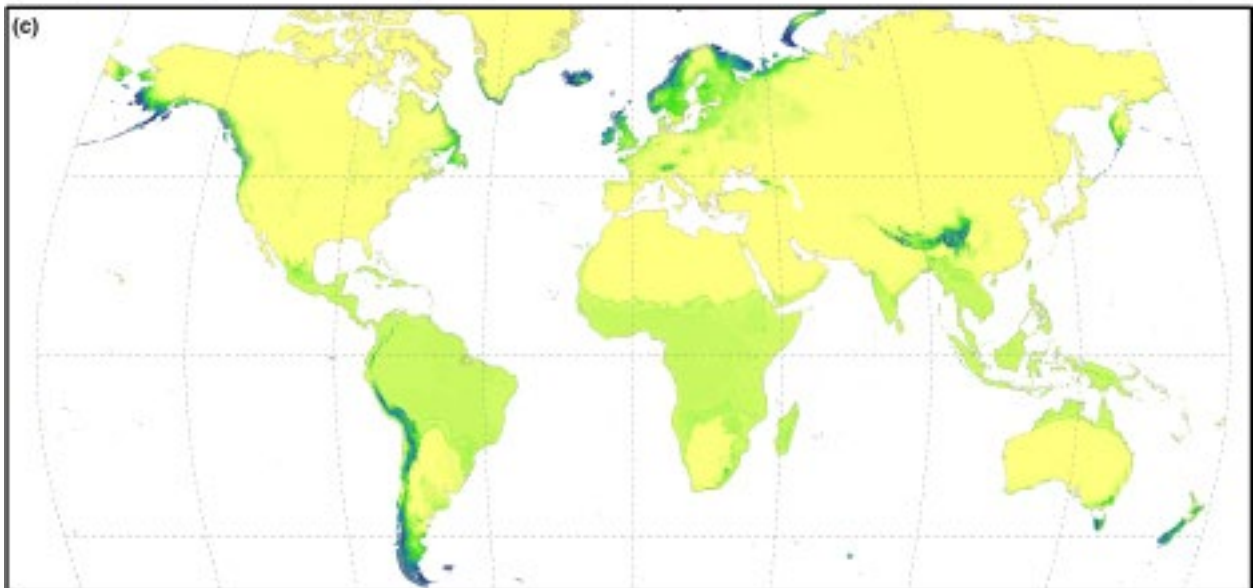
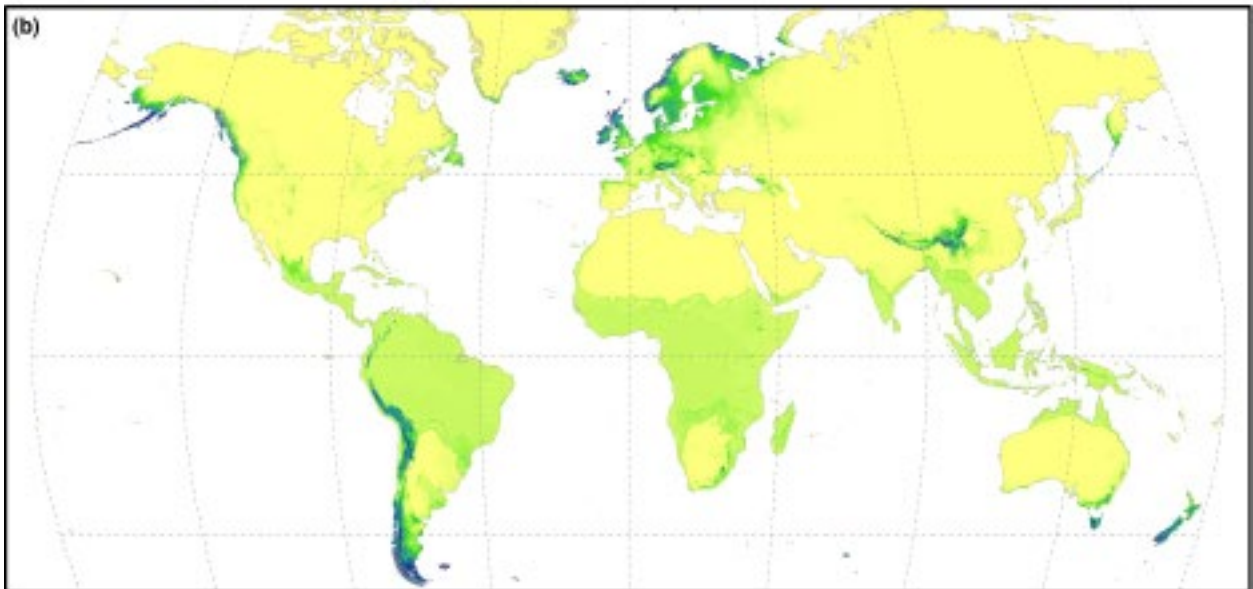
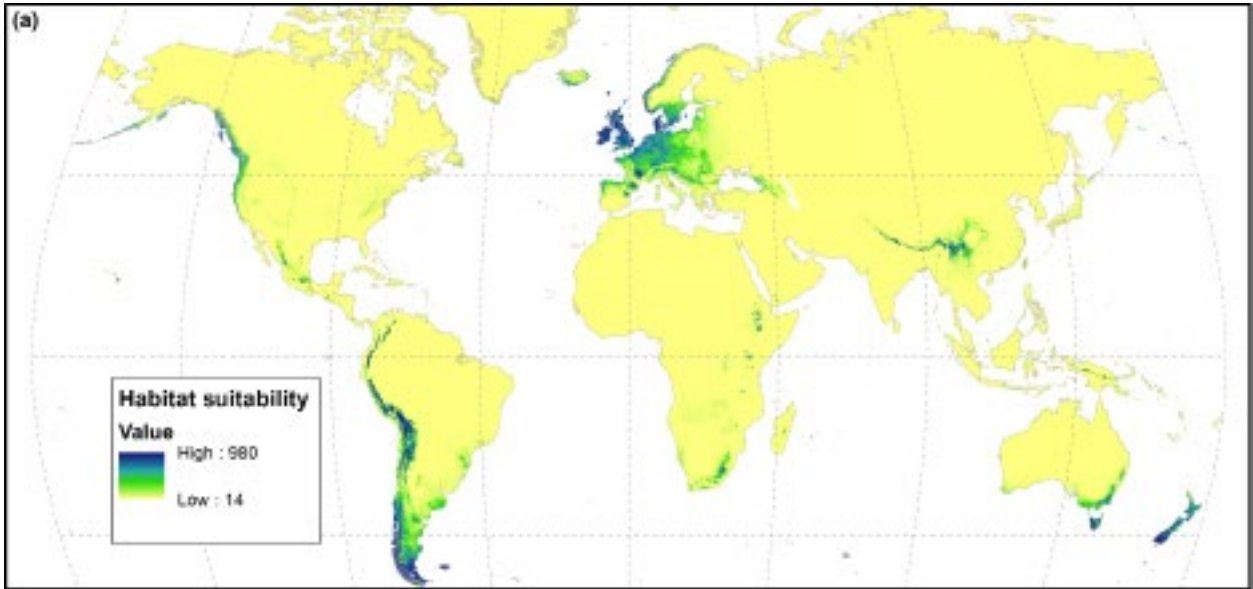
3.3 | Climatically suitable areas under current and future climates

The predictions of ensemble of the four different modelling techniques showed the potential current and future

distributions of *A. ater*, *A. rufus* and *A. vulgaris* (Figure 4). The predictive ability of each model was consistently high, with TSS ≥ 0.8 and AUC ≥ 0.9 . The environmental variables that had consistently the highest influence on species occurrence were BIO1, BIO4 and BIO6 (Table S4). Temperature rather than precipitation therefore seems to be the main influence in the *Arion* sp. slugs' distributions.

The results showed that several parts of the world provide suitable climate for the establishment of *Arion* sp. slugs, in particular the North and South American west coast, southern Argentina and Chile, parts of China, New Zealand, Tasmania and south-eastern Australia (Figure 4). Projections of future conditions under the HadGEM2-CC model revealed that while the amount of the most suitable areas for *Arion* sp. slugs will decrease and the range in Europe will shift towards the north, new climatically suitable areas will also emerge (Figure 4). Specifically, the fraction of area lost/stable/gained (compared to the current distribution) is predicted to be 0.3777/0.6223/0.2736 for 2050 and 0.5493/0.4507/0.4083 for 2070.

FIGURE 4 Global projection of the potential distribution of *Arion* sp. slugs based on the climatic conditions, predicted with the ensemble modelling technique for current conditions and projected to the 2050 and 2070 climate change scenario. Climatic suitability ranges from low to high [Colour figure can be viewed at wileyonlinelibrary.com]



4 | DISCUSSION

In this study, we aimed to assess the genetic identity and introduction history of three invasive *Arion* species in North America and Australia, and the potential for their global spread under current and future conditions by a combination of three distinct methods: interception records, genetic analyses and species distribution modelling. The interception records showed that slugs are introduced not only directly from Europe, but also from successful invasive populations in other locations within the introduced range (Lombaert et al., 2010). The analyses of *A. ater*, *A. rufus* and *A. vulgaris* specimens in their introduced range revealed different genetic signatures, which might be crucial for understanding the introduction process and potentially for prevention of their future establishment. The SDMs predicted several climatically suitable regions for the three *Arion* species in several areas across the globe. The overall extent of the suitable areas is predicted to decrease, but over the coming decades there are likely to be new climatically suitable areas emerging as well due to ongoing climate change.

4.1 | Species assignment and genetic diversity among the introduced *Arion* species

Our analyses revealed a different introduction history among the three *Arion* species. The detection of genetically very divergent sequences in *A. rufus* in the USA indicates multiple or secondary introductions, which are often reported in successful invasions (Cristescu, 2015). The nucleotide diversity was higher than in the European samples, but due to limitations to the current data set more rigorous sampling is required to confirm the observed genetic patterns.

In contrast, the sequenced *A. ater* must have been established from a single introduction, or from repeated introductions of the same source population. The observed low level of haplotype diversity in *A. ater* and *A. vulgaris* seems to be consistent with other studies that have used mtDNA markers to assess genetic variation among introduced molluscs. For example, *A. subfuscus* in the northeastern USA exhibits low genetic diversity, and yet it is one of the most abundant introduced slug taxa in North America (Mc Donnell et al., 2011; Pinceel, Jordaens, Van Houtte, Bernon & Backeljau, 2005). Interestingly, this is in contrast to the predictions of decreased probability of establishment and thriving in genetically depauperate invasive populations (Dlugosch & Parker, 2008; Simberloff, 2009), and further investigations on the population level are warranted.

4.2 | Routes of introduction

France was identified as the source of origin of the majority of merchandise that the *Arion* sp. slugs were intercepted on in Australia and a large source of *Arions* to the USA (Figure 3). Consistently, the three European ND1 haplotypes that clustered closest to the American *A. rufus* haplotypes were derived from French specimens (Hap_22, 23 and 26 in Figure 1 and Table S2). The *A. ater* in North America

clustered with individuals from Western and Northern Europe. Unfortunately, currently, there are not enough *A. ater* sequences available that could be used for comparison with both American and Australian specimens.

The exact origin of *A. vulgaris* would be even more difficult to determine, due to its invasive status and very rapid spread within the last 60 years, which has led to haplotype sharing among many European countries (Zemanova et al., 2016). In our sampling, *A. vulgaris* was found only in one location (Table 1). Therefore, even though *A. vulgaris* is highly invasive in Europe, either border controls were able to prevent *A. vulgaris* introduction, or the other two *Arion* species are more successful in colonizing exotic ranges. Further studies on the differences in the life history traits among *Arion* slugs are therefore necessary.

In combination with the genetic results, the interception records suggest further translocations within North America, that is from Canada to the USA (Figure 3) and *vice versa*. Secondary introductions seem to be common among biological invasions (Chapple et al., 2013). As the interceptions reported here are of living slugs (Barr et al., 2009), even a single specimen is potentially capable of establishing a new population by self-fertilization. Such established populations in the new area might then serve as a source for another invasion wave (Lombaert et al., 2010), spreading, for example in potted plants, being protected from detection and desiccation. Therefore, the priority in preventative and management measures should be directed also towards transport hubs, such as tree nurseries, from which the species might get introduced to other locations (Bergey et al., 2014; Floerl, Inglis, Dey & Smith, 2009).

Slug introductions might be particularly problematic in natural reserves. A recent study on slug abundance in the Terra Nova National Park in Canada found that almost 90% of collected species were non-native taxa (Moss & Hermanutz, 2010). In our own coverage, we also found exotic slugs in a national park—the Point Whitehorn Marine Reserve (PW) harboured both *A. rufus* and *A. ater*. Without control measures, these slug species are likely to spread further given their large reproductive output, nonselective diet and capacity to self-fertilize (Baker, 1955; Briner & Frank, 1998), and their populations might even be complemented with newcomers as the increasing trade volumes (Figure S2) could lead to a higher probability of establishment of new slugs.

4.3 | Predicting the climatically suitable areas and the fate of introduced *Arion* slugs

Using the ensemble SDM approach, we identified several areas that currently have high climatic suitability for *Arion* sp. and might therefore be most prone to slug invasion (Figure 4). These predictions include the known invaded range of *Arion* sp. slugs, but also revealed potential areas that are not yet invaded (Figures 2 and 4).

Even though the origin of the highly invasive *A. vulgaris* has been estimated to lie in South-Western Europe (Zemanova et al., 2016), the models predicted high climatic suitability for *Arion* sp. slugs within this region only in certain parts (Figure 4). While it has been

shown that invasive species may have limited native range (Garzon-Machado, del-Arco-Aguilar & Perez-Paz, 2012), our results could be an artefact of the limited occurrence data set. More thorough sampling in France and Spain might therefore be necessary not only to allow more precise genetic analyses (see above), but also to determine the exact distribution range.

Several studies suggested that, on average, global change will increase the risk of biological invasions and favour invasive species (e.g., Cunningham, Madden, Barnard & Amar, 2016; Knop & Reusser, 2012; Walther et al., 2009). Even though the overall amount of areas with high suitability for *Arion* slugs will decrease, new areas with favourable conditions will also appear (e.g., Iceland and northern Scandinavia, equatorial parts). Similar patterns of emergence of new climatically suitable areas under the climate change have been also observed for another mollusc, the invasive snail *Pomacea canaliculata* (Lei, Chen & Li, 2017).

However, one has to note that the use of SDMs assumes conservation of the ecological niche, and this might not be true during invasions (Broennimann et al., 2007; Guisan, Petitpierre, Broennimann, Daehler & Kueffer, 2014). Additionally, as the invasion of *Arion* sp. slugs outside of Europe is relatively recent, modelling with the current data set might underestimate their potential range (Václavík & Meentemeyer, 2012). The first insight into the potential occurrence of the invasive species provided by SDMs should therefore be complemented by information on other local factors that might influence establishment of the introduced species, such as competitive abilities and lack of predators (Shea & Chesson, 2002). Moreover, further anthropogenic factors, for example land use change and trade, are likely to significantly influence the geographic distribution in biological invasions, but this is difficult to predict based on the environmental variables alone (Chapman et al., 2016; Munoz & Real, 2006). Better understanding of these additional factors and their inclusion would strengthen the predictions in future modelling.

4.4 | Future perspectives

Our study is very relevant to invasion biology and species distribution as it uses information obtained from three different data sources, that is interception records, DNA sequences and spatial distribution models. This joint consideration allows a more assertive interpretation of complex biological phenomena such as those involving the successful establishment of introduced populations. However, we are aware of the limitations of the current data set and propose that for a further understanding of the genetic structure of introduced species, one should test for shifts in allele/haplotype frequencies among locations. Propagule pressure could be tested for instance by modelling different population genetic scenarios of migration among native and introduced locations, compare their likelihoods and calculate number of migrants from the most likely model (Gray et al., 2014; Lombaert et al., 2014; Zemanova et al., 2016). Such assessment, however, requires larger

sample numbers and preferably also more variable markers (e.g., microsatellites or SNPs).

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DATA ACCESSIBILITY

DNA sequences are available in GenBank database under the accession numbers MG837141–MG837220.

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BIOSKETCH

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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