

Rapid Communication**First record of the invasive slug *Arion vulgaris* Moquin-Tandon, 1885 (Gastropoda, Stylommatophora, Arionidae) in Quebec (Canada)**

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OPEN ACCESS**Abstract**

Early detection of exotic species and rapid dissemination of information are both essential for risk assessments and preventive measures to be implemented early. In this context, we report the first mention of *Arion vulgaris* in Quebec (Canada). This species is considered as the most important slug pest in Europe and is of particular concern in North America where its presence has rarely been documented. Identifications were based both on a 16S rDNA gene segment and on genital morphology. Our results confirmed the northernmost successful introduction of *A. vulgaris* in North America. This new record emphasizes the need to acquire more data on the invasion of this species in North America.

Key words: DNA barcoding, exotic invasive species, introduced species, Mollusca, genital morphology, North America, 16S rDNA

Introduction

Early detection and identification of exotic species is essential to reduce their impacts and make effective control interventions (Lodge et al. 2006; Vander Zanden et al. 2010; Mehta et al. 2007). This becomes especially relevant when occurrences of invasions by that species have already been documented elsewhere as it allows for risk assessments and preventive measures to be implemented early (Kumschick and Richardson 2013). Multiple events of invasion by the same species can also help to better understand what attributes make an exotic species more or less successful and which environmental conditions influence the invasiveness of a species (Pauchard et al. 2004; van Boheemen et al. 2017).

In North America, the genus *Arion* Férussac, 1819, introduced from Europe, is the most speciose among terrestrial exotic slugs (Barr et al. 2009; Nekola 2014). Many are successful invasive species that are widespread and present in multiple habitats (Chichester and Getz 1969; L'Heureux and Angers 2018; Zemanova et al. 2018). Identification of those taxa to species level is challenging and often requires genetic analyses which makes their detection less accessible than for other animal groups (Pinceel et al. 2004,

2005; Quinteiro et al. 2005; Barr et al. 2009; Rowson et al. 2014a; L'Heureux and Angers 2018).

In Europe, *Arion vulgaris* Moquin-Tandon, 1885, is regarded as the most important slug pest and is listed as one of the 100 most invasive species (DAISIE 2009). Its native distribution has been controversial (Pfenninger et al. 2014; Zemanova et al. 2016), but recent phylogeographic analyses suggested a likely origin in a region ranging from France to Western Germany (Zajac et al. 2020). The distribution of *A. vulgaris* has greatly expanded over the last decades, most probably via human introductions, and it is now widespread throughout most of Europe (Zemanova et al. 2016). *Arion vulgaris* feeds on various plants and is reported to cause important damages in cultivated (including human food, ornamental plants and medicinal herbs) and wild species (Frank 1998; Kozłowski 2005, 2012; Kozłowski and Kozłowski 2011; Blattmann et al. 2013; Kozłowski and Jaskulska 2014). It may also be a potential vector of plant pathogens (genus *Phytophthora* de Bary, 1876), which remain viable after passage through the slug digestive system (Telfer et al. 2015). Antzée-Hyllseth et al. (2020) found a high prevalence of nematodes and trematodes in wild *A. vulgaris* in Norway. This slug species can be the intermediate host of different cardiopulmonary metastrongyloid nematodes infecting cats, dogs and foxes as definitive hosts (Ferdushy et al. 2010; Lange et al. 2018; Penagos-Tabares et al. 2020). It might also be a potential vector of pathogenic bacteria to humans (e.g. Enterobacteriaceae) and farm ruminants through contamination of their food (Stalder et al. 2014; Gismervik et al. 2015a). However, despite the severe decrease in microbial quality of the silage when *A. vulgaris* is accidentally harvested with grass, no pathogenic bacteria were detected in post-fermented silage (Gismervik et al. 2015b).

Arion vulgaris is a species of particular concern in North America (Barr et al. 2009). It is included on the list of *alien non-marine snails and slugs of priority quarantine importance in the United States* (Cowie et al. 2009). So far, this invasive species, present throughout most of Europe, has only been confirmed twice in North America: one record in southern Ontario in Canada (Zemanova et al. 2018) and one record in Mexico (Araiza-Gómez et al. 2021). Considering the potential for invasion by this slug and the numerous impacts reported in Europe, this paper documents the first record of *A. vulgaris* in Quebec and the northernmost successful introduction in North America.

Materials and methods

Specimen sampling

Large specimens of *Arion* sp. were noticed in an urban park in City of Québec, Quebec, Canada (GPS: 46.809375°N, -71.313658°W; Figure 1). Colorations were slightly atypical of other large *Arion* sp. usually observed



Figure 1. Map of Quebec (colored in darker grey) showing sampling location of *Arion vulgaris*. Red tag indicates precise location (City of Québec, Quebec, Canada). Modified from *Forêt ouverte* (Gouvernement du Québec 2019).

in Quebec (L'Heureux *personal observations*), hence four specimens were collected by hand to determine their identity (on August 25, 2021). Specimens were kept on ice in the field, until they were euthanized in a $-20\text{ }^{\circ}\text{C}$ freezer, then placed in 95% ethanol for preservation. No survey was conducted on the collection site or around.

Sequencing

A 4×4 mm piece of foot tissue was dissected from two individuals for total DNA extractions. Tissue pieces were digested overnight at $37\text{ }^{\circ}\text{C}$ in $400\text{ }\mu\text{l}$ of TNES (50 mM Tris pH of 7.5, 400 mM NaCl, 20 mM EDTA and 0.5% SDS) and $5\text{ }\mu\text{l}$ of 20 mg/mL proteinase K. DNA was then extracted using a phenol-chloroform DNA extraction protocol, followed by ethanol precipitation (Sambrook et al. 1989). DNA was resuspended in $200\text{ }\mu\text{l}$ of PCR-grade water.

A 445 bp segment of the 16S rDNA mitochondrial gene was amplified using the following primers: 5'-CGCCTGTTTAWCAAAAACAT-3' and 5'-GGTYTGAACCTCAGATCAGATCAYGT-3', previously tested in L'Heureux and Angers (2018). Those primers were used because they are universal and the segment has been widely used in previous studies on Arionidae identification and phylogeny (e.g. Pinceel et al. 2004, 2005; Barr et al. 2009; Mc Donnell et al. 2011; Breugelmans et al. 2013; Rowson et al. 2014a; L'Heureux and Angers 2018), ensuring sequences of most species are available on the Genbank database (Sayers et al. 2022) for comparison. Polymerase

chain reaction (PCR) amplifications were carried out in a volume of 50 μ l containing 2.5 nmol/L of each dNTP, 0.3 μ mol/L of each primer, 0.8 units of Taq DNA polymerase (Bio Basic Inc., Markham, Ontario, Canada), 5 μ L of 10x Taq polymerase buffer, and approximately 100 ng of DNA. The segment was amplified using the following conditions: one cycle of denaturation at 92 °C for 30 s, then 45 cycles of denaturation at 92 °C for 30 s, annealing at 50 °C for 15 s, and extension at 68 °C for 30 s, with a final extension at 68 °C for 2 min. Each PCR volume was divided in half for the sequencing to be performed in both directions. PCR purifications and Sanger sequencing were performed by Genome Quebec (Montreal, Canada). Forward and reverse sequences were manually aligned and assembled in MEGA 7 (Kumar et al. 2016).

Sequence matching

Sequences were blasted on Genbank (April 12, 2022) to identify the species. The sequence was searched for highest matches using the BLASTN tool. Filters were applied to only retain matches with minimum query coverage of 95%. Sequences not identified to species or identified as hybrids were not considered. Identification was based on highest percent identity and lowest E-value. The best matching sequence of the second species with the highest match is presented for comparison.

Phylogenetic analyses

To verify that the taxonomy of the species identified by BLASTN is properly resolved with the gene 16S rDNA and to verify that sequences associated to the species are properly identified, a phylogenetic analysis was performed using all the 16S rDNA sequences of most species of the subgenus (*Arion*) available on Genbank (imported on February 22, 2022). Key words used were *Arion vulgaris* 16S, *Arion ater* 16S, *Arion rufus* 16S, *Arion lusitanicus* 16S, *Arion flagellus* 16S and *Arion empiricorum* 16S. In addition, three sequences identified as *Arion* sp. on Genbank (KF894080.1; KF894092.1; KF894132.1) were included as they were used in Rowson et al. (2014a) phylogeny. Sequences identified as hybrids were not included. Sequences were aligned by MUSCLE (Edgar 2004) in MEGA 7 (Kumar et al. 2016), and then manually trimmed to fit most of the shortest sequences (395 bp, including indels after alignment). Shorter sequences and sequences containing polymorphic sites (identified as R or W or N) were discarded. Shorter nucleotidic segments allow for the inclusion of more sequences than in the previous analysis. A single sequence was retained when many Genbank sequences were identical. A phylogeny was reconstructed using a neighbour-joining tree (Saitou and Nei 1987) based on the Kimura-2-parameter distance (Kimura 1980) in MEGA7 (Kumar et al. 2016). Support for monophyly was calculated with 1000 bootstrap replications (Felsenstein

Table 1. Top five Genbank sequences matching specimens found in Quebec, sorted by highest percentage identity. Only matches with over 95% coverage are presented. The best match with *A. ater* shows the percent identity gap between both species. Consulted on April 12, 2022.

Species identity	Percent identity	Query coverage	E-value	Accession number	Published in
<i>Arion vulgaris</i>	98.19%	99%	0.0	NC_046044	Doğan et al. 2020
	97.99%	100%	0.0	MN958056	Reise et al. 2020
	97.99%	100%	0.0	MN958055	Reise et al. 2020
	97.54%	99%	0.0	KP835188	–
<i>Arion ater ater</i>	89.80%	100%	3e-156	MN958058	Reise et al. 2020

1985). Gaps were treated as pairwise deletions. Groups were identified based on previous phylogenetic work by Rowson et al. 2014a. The mean distances within and between groups were calculated using MEGA 7 (Kumar et al. 2016).

Dissections

Two specimens were dissected to observe the morphology of their genital apparatus to determine whether morphology is consistent with mitochondrial identification. Procedures for dissections followed Reise et al. (2020) recommendations. Genital apparatus was compared to images and descriptions by Rowson et al (2014b) and Reise et al. (2020). Criteria used for identification were the presence/absence of an oviduct with a dilated part, the presence/absence and position of a retractor muscle on the dilated oviduct, the position (in the dilated part of oviduct or in the atrium) and the shape of the ligula, the position of the openings of epiphallus and bursa copulatrix duct in the atrium and the size of the tubercles on the inner wall of epiphallus (Rowson et al. 2014b; Reise et al. 2020).

Results

Identity based on 16S rDNA segment

A 445 bp segment was obtained by sequencing (Genbank accession number: OP612888). Nucleotide sequences of both specimens were identical. Blasting the sequences on Genbank revealed that the highest matches are belong to sequences identified as *A. vulgaris*, with percent identity up to 98.19% with the 16S rDNA segment of the complete mitochondrial genome published by Doğan et al. (2020) (Table 1). Sequences of other large *Arion* spp. displayed matches lower than 90% identity, the highest one being attributed to *Arion ater* (Table 1).

The neighbour-joining tree contained 71 unique sequences, including the one obtained in this study (Figure 2, Supplementary material Figure S1). *Arion vulgaris* is a phylogenetically well resolved cluster using the 16S rDNA gene. The Quebec sequence clustered with 14 other sequences and this group is supported with a bootstrap value of 100% (Figures 2 and S1). All sequences but one (HQ659926.1) within this group were identified as *A. vulgaris* or *A. lusitanicus* (previously erroneously used as a synonym, see

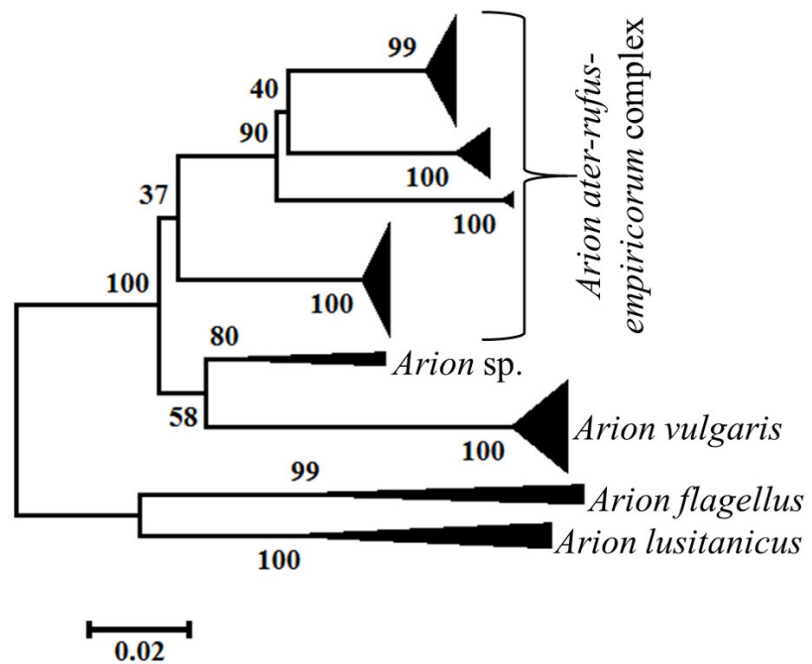


Figure 2. Neighbour-joining tree based on large Arionidae 16S rDNA segments. Percentage bootstrap support values are indicated on branches. Each triangle represents a cluster used to calculate the mean distances within and between groups. See Figure S1 for a detailed version of the tree.

Quinteiro et al. 2005) on Genbank. The identity of individuals within this cluster is also confirmed by the presence of sequences attributed to *A. vulgaris* by Rowson et al. (2014a). Mean genetic distance within this group is 0.010, while distances between this group and other groups range from 0.106 to 0.217. In other clusters, only one sequence was identified as *A. vulgaris* on Genbank and was reattributed to *A. ater* by Rowson et al. (2014a).

Identity based on morphology

External morphology of both specimens prior to dissection is shown in Figure 3A, B. Examination of genital morphology of both specimens were consistent with *A. vulgaris*. The oviduct is strongly dilated, and a retractor muscle is situated on its distal part (Figure 3C, D). The ligula is elongated and situated in the dilated oviduct (Figure 3E, F). The epiphallus and bursa copulatrix duct insert in a lateral bulge of the atrium, beside the insertion of the oviduct (Figure 3C, D). The insertion of the oviduct, epiphallus and bursa copulatrix duct in the atrium is arranged in a straight line (Figure 3C, D). Rows of relatively large tubercles are present on the inner wall of epiphallus (small tubercles are also present for relative size comparison) (Figure 3G, H). Preserved copulating individuals are shown in Figure 4. The individual shown in Figure 3B and one from Figure 4 were used for sequencing.

Discussion

Northernmost record in North America

This article marks the first record of *A. vulgaris* in Quebec, corresponding to the northernmost introduction in North America. Compared to the previous

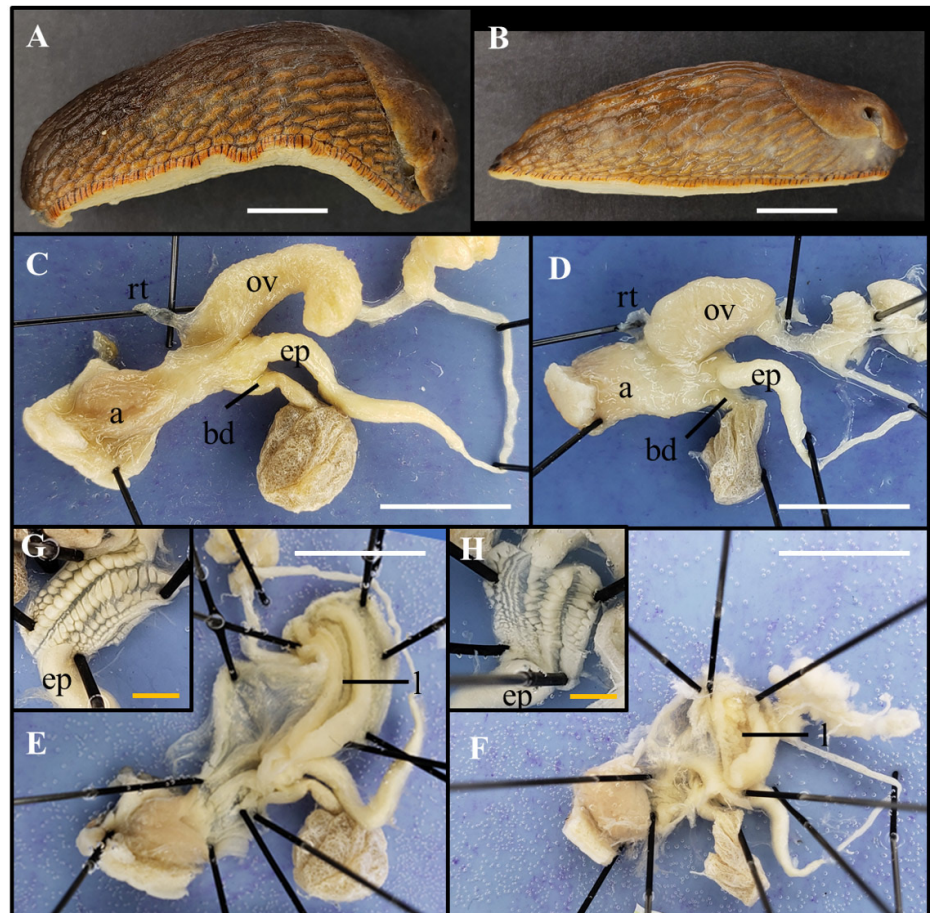


Figure 3. External and internal morphology of two preserved *Arion vulgaris* specimens from Quebec. (A, B) Right lateral view of whole specimens. (C, D) Undissected distal genital apparatus. (E, F) Dissected oviduct showing the ligula. (G, H) Dissected epiphallus showing rows of tubercles on inner wall. Pictures A, C, E, G belong to the same individual, and B, D, F, H belong to a second individual. White scale bars = 1 cm and yellow scale bars = 2 mm. Abbreviations: a – atrium, bd – *bursa copulatrix* duct, ep – epiphallus, l – ligula, ov – oviduct, rt – retractor muscle. Pictures taken with: Samsung Galaxy S9 Model SM-G960W.

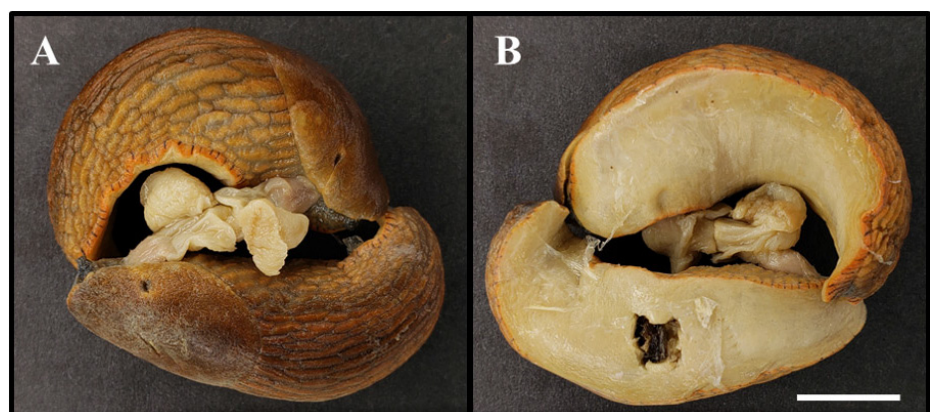


Figure 4. Preserved Individuals in copulation: (A) dorsal view and (B) ventral view. Note that a piece of foot tissue has been removed from one specimen for DNA extractions. Pictures taken with: Samsung Galaxy S9 Model SM-G960W.

record in Ontario (Zemanova et al. 2018), the current observation has a latitude of approximately 3.1769 degrees (approximately 353 km) further north. The species seems established on that site as tens of specimens were

observed, including copulating individuals (Figure 4). At this time, the extent of the species distribution on this site, the presence of the species in other sites in Quebec, the origin(s) of those slugs, the means of introduction and the time since introduction are unknown.

Species identification

The identity of the individuals has been confirmed at the species level based on mitochondrial sequences. The mitochondrial gene 16S rDNA was sequenced because it has been widely used for identification and taxonomy of *Arion* spp., which ensure that most species of the genus would have available sequences on the database (e.g. Pinceel et al. 2004, 2005; Barr et al. 2009; Mc Donnell et al. 2011; Breugelmans et al. 2013; Rowson et al. 2014a; L'Heureux and Angers 2018). However, most recent studies on *A. vulgaris* used other mitochondrial genes such as COI and ND1 (Pfenninger et al. 2014; Zemanova et al. 2016; Zając et al. 2020). To infer the exact European origin of the specimens and to assess if the same haplotype as the ones in Ontario (Zemanova et al. 2018) or Mexico (Araiza-Gómez et al. 2021) are present in Quebec, the sequencing of at least the gene COI would be required. Morphological assessments also support that observed specimens belong to *A. vulgaris*.

Hybridization and subsequent introgression can occur between *Arion vulgaris*, *A. rufus* and *A. ater* in natural conditions (Zemanova et al. 2017; Hutchinson et al. 2021). Exotic *A. rufus* and *A. ater* are already established in North America (Barr et al. 2009; Grimm et al. 2009; Zemanova et al. 2018). Such hybrids could not be detected using mitochondrial genes alone due to maternal inheritance; nuclear genetic data analyses will be required to determine with certainty the status of specimens introduced in Quebec. The morphology of *A. vulgaris* and associated hybrids has been well described and visually represented in the literature (Gural-Sverlova and Gural 2011; Dreijers et al. 2013; Hatteland et al. 2015; Reise et al. 2020; Rowson et al. 2014b). Despite all the available documentation, dissections in this group remain challenging and require a precise expertise. For example, using morphology only, most *A. vulgaris* and *A. rufus* hybrids (confirmed with nuclear DNA data) were assigned to one or the other species instead of hybrids by Zemanova et al. (2017). Pictures of genital apparatus of dissected specimens were included so that our interpretation can be revised in the future if needed.

Species habitat

In its invaded range in Europe, *A. vulgaris* has been recorded from a large variety of anthropogenic and semi-natural habitats such as parks, gardens, agricultural lands and their margins, cemeteries, drainage ditches, roadsides, brush, wastelands, meadows and woodlands (Rowson et al. 2014b; Kozłowski

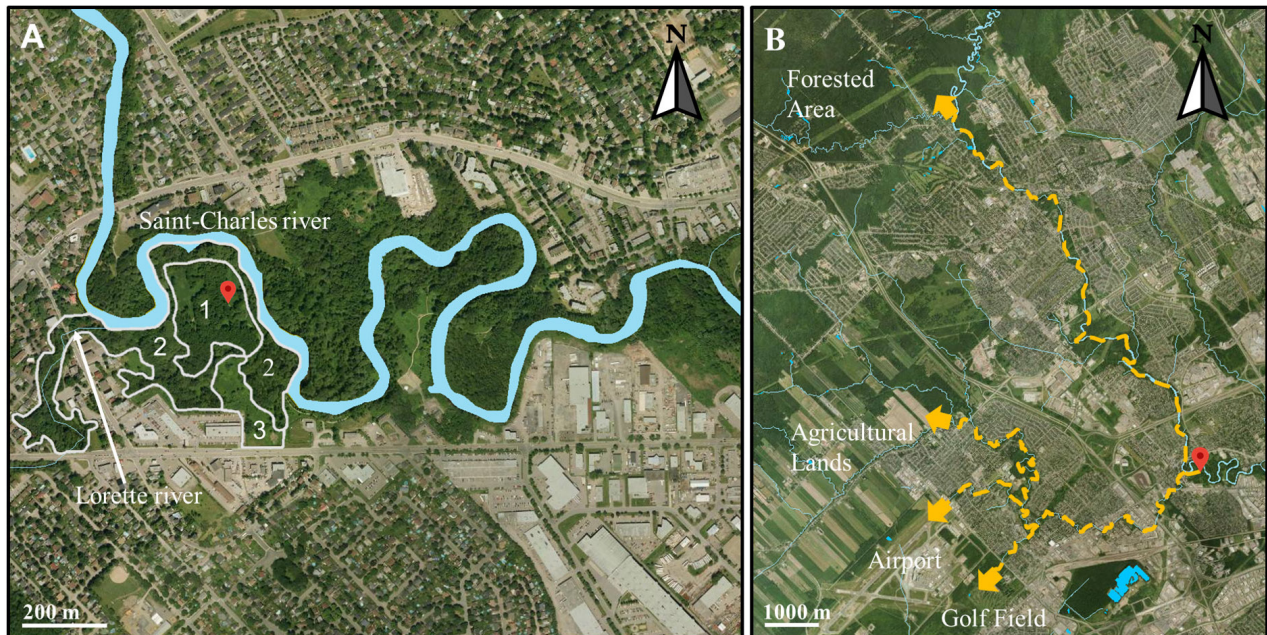


Figure 5. Aerial view of the sampling location of *Arion vulgaris* in Quebec. Red tag indicates precise sampling location. Three ecoforestry habitats of the site are delimited by white lines and numerated. Hydrographic elements (eg. rivers and streams) are in blue. Yellow dotted lines and arrows indicate hypothetical ways to reach new sites following rivers and streams. Modified from *Forêt ouverte* (Gouvernement du Québec 2019).

and Kozłowski 2011; Dörler et al. 2018). The margin of a broadleaved forest is also reported by Kozłowski and Kozłowski (2011). Quebec specimens were collected in an urban forested park, a habitat very similar to some reported for this synanthropic species in Europe. The forested area is mainly composed of deciduous trees and is disturbed by the presence of pedestrian and cycling paths crossing it, and other park infrastructures. According to Quebec ecoforestry data (accessed via Gouvernement du Québec 2019; Figure 5), the forested site is roughly subdivided in three environments. Specimens were collected in a small 3.9 ha forest patch composed of even-aged 50-years-old trees (canopy cover of about 55%) (Figure 5, habitat 1) surrounded by an 8.9 ha old deciduous forest composed of uneven-aged trees (canopy cover of about 75%), in which many specimens were also observed (Figure 5, habitat 2). The third patch is described as a 1.6 ha wasteland that has not been visited by the authors but could constitute a suitable habitat based on European observations (Figure 5, habitat 3). In Europe, the species is very often found in proximity to bodies of water (Kozłowski and Kozłowski 2011), which is a feature of the Quebec site. The drainage of the whole forested area is described as bad to imperfect (Gouvernement du Québec 2019), humidity being an important factor for *A. vulgaris* juvenile and egg survivals (Slotsbo et al. 2011).

Dispersal and potential impacts

Arion vulgaris may rapidly disperse following introduction. For example, the first record of this species in Norway dates back to 1988 and, by 2011,

its presence was recorded in 192 municipalities (Hatteland et al. 2013). Because of the risk of invasion in Quebec, potential ways of dispersal and impacts are discussed in the current context, based on invasion information documented in Europe for this species. This section aims to guide future research on this species in Quebec and should be regarded as a pre-risk assessment until further data on the ecology of *Arion vulgaris* are acquired in the region.

In terrestrial gastropods, large scale dispersal can only be accomplished passively and human activities play an important role in transporting species (Godan 1983; Robinson 1999). In North America, passive dispersal of *Arion* spp. plays an important role in their distribution expansion following their introduction (Mc Donnell et al. 2011; L'Heureux and Angers 2018). As the Quebec site is an urban park, there is human traffic from various regions. The opportunities for accidental or intentional transport (the large size of the specimens makes them a slug which may arouse interest) are great. Moreover, the site is surrounded by residential lands. The transport of slugs or eggs along with diverse objects or materials such as plant materials (e.g. agricultural crops, fruits and vegetables, ornamental plants, seedlings, plant parts for propagation or plant waste and debris), soil (e.g. for gardening or from excavation works) and municipal waste are all routes of passive dispersal identified or suggested for *A. vulgaris* (Kozłowski 2007; DAISIE 2009; Kozłowski and Kozłowski 2011).

Active dispersal can be important locally for the invasion of natural areas where passive dispersal by humans is less likely to take place. For example, in North America, Chichester and Getz (1969) suggested that active dispersal explains the penetration of certain slug species into natural habitats. Kozłowski and Kozłowski (2011) reported that ditches and watercourses are important ways of active dispersal for *A. vulgaris* in Poland. This behavior could have important implications for the spread of *A. vulgaris* in Quebec as the site is bounded at the north by the Saint-Charles River, a tributary of the St. Lawrence River, and the connected Lorette River (Figure 5). The area is surrounded by residential and commercial lands on the other sides (Figure 5), which comprise many barriers to dispersal such as large asphalted areas (e.g. large highways and parkings). Thus, following riversides is the main potential way for long-distance active dispersal on this site. Riversides are continuous ways of dispersal and many forested areas are present along the rivers (Figure 5). Those areas are similar to the forest patch from which specimens were collected in terms of habitat and *A. vulgaris* is likely to establish there (Quebec ecoforestry data accessed via Gouvernement du Québec 2019). Saint-Charles River is connected to continuous forested habitats northwards while the upstream Lorette River, and its tributaries, are connected eastwards to agricultural lands (Figure 5).

By dispersing along riversides, *A. vulgaris* would have to travel approximately 12 km to reach those habitats (Figure 5). Kozłowska and

Kozłowski (2004) reported that the species is able to move up to 7 m in a 2 h period. Under laboratory conditions, Grimm and Schaumberger (2002) recorded a mean distance of 10.8 m in a 24 h period. However, when following PITT-tagged individuals in Sweden, shorter traveled distances were measured per day in average. Watz et al. (2021) reported a daily mean travelled distance of 3.8 m in a fragmented habitat made of alternating short and long grass patches, and Nyqvist et al. (2020) reported a daily mean distance of 1.38 m in forest and 0.81 m in garden habitats. Authors of both studies mentioned that distances might have been underevaluated as a part of the slugs were lost during monitoring and thus might have travelled beyond the studied area.

By reaching and establishing in forests and agricultural lands, *A. vulgaris* could have major impacts. Like other terrestrial slugs, the species have food preferences (Briner and Frank 1998; Kozłowski and Kałuski 2004). As a consequence, Kozłowski and Kozłowski (2011) observed changes in plant composition in natural habitats following *A. vulgaris* introduction. Plant composition changes due to *A. vulgaris* grazing have also been demonstrated experimentally (Buschmann et al. 2005; Lanta 2007). Severe damages caused by *A. vulgaris* feeding were reported in Europe across a wide variety of plant species, parts and stages (Frank 1998; Kozłowski 2005; Kozłowski and Kozłowski 2011; Kozłowski 2012; Blattmann et al. 2013; Kozłowski and Jaskulska 2014). For example, 26 out of 30 vegetable species observed by Kozłowski (2005) presented at least some degree of damage, and among them, plant surface damage of certain species reached almost 70%. *Arion vulgaris* densities reported are variable depending on habitat and locality, but the species can reach very high densities (Kozłowski 2005; Kozłowski and Kozłowski 2011). High densities of up to 65 slugs per square meter in certain gardens in Poland (Kozłowski and Kozłowski 2011) and exceeding 50 slugs per square meter in flower strips and meadows in Switzerland (Briner and Frank 1998) were recorded. This hermaphrodite species produces an average of 405 eggs per slug under field conditions in Poland, after which adults die (Kozłowski 2000; Slotsbo et al. 2013). The large size of the species (Rowson et al. 2014b), its capacity to reach high densities and its preference for anthropogenic habitats like agricultural fields make it a likely agricultural pest in North America as is the case in Europe. Furthermore, it was demonstrated experimentally that the mortality of *A. vulgaris* under laboratory conditions is much lower than that of *A. fasciatus* (Nilsson, 1823) when infected with *Phasmarhabditis hermaphrodita* (Schneider, 1859), a nematode commonly used in slug biological control in Europe (Antzée-Hyllseth et al. 2020). However, this is not the only control method and other options have been studied (e.g. Barua et al. 2021). Numerous European slug species, including several *Arion* spp., are already major agricultural pest in North America (Godan 1983; Hammond and Byers 2002; Grimm et al. 2009).

Conclusion

Considering that *A. vulgaris* is a successful invader in Europe and that several *Arion* species has been successfully introduced in North America, the species should be monitored in North America while its introductions are still recent. In Quebec, the priority should be to inventory the site to precisely map its distribution, especially along local rivers, then to evaluate control actions. Presence of the species on other sites is a possibility that should not be excluded. The introduction of this species offers an opportunity to compare the invasive success in Europe and North America. This may help to better understand the determinants of the invasive success of exotic species.

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Authors' contribution

This project was conceived by É.L.'H. É.L.'H. performed DNA extractions and amplifications, dissections, data analyses and interpretation, made figures and wrote the manuscript under the supervision of B.A. J.L. collected specimens. All authors reviewed and approved the final version of the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Detailed neighbour-joining tree based on large Arionidae 16S rDNA segments.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_LHeureux_et_al_SupplementaryMaterial.pdf