

Rapid Communication**Aesthetic aliens: invasion of the beauty rat snake, *Elaphe taeniura* Cope, 1861 in Belgium, Europe**

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Abstract

We report on an established population of the beauty rat snake, *Elaphe taeniura* Cope, 1861, a large, oviparous colubrid native to Southeastern Asia, in Belgium. The snakes have invaded a railroad system next to a city in the northeast of the country. Our report is based on validated citizen science observations, supplemented with directed surveys. The species has been recorded in the wild since 2006, most probably following an introduction linked to the pet trade. Genetic identification, based on the COI gene, confirms that the sampled individuals belong to *E. taeniura*. In addition, the snakes recorded in Belgium phenotypically match *E. t. taeniura*, a Chinese subspecies. Exact date of introduction, invasion extent and population size are currently unknown, but the number of observations has increased in recent years. Sightings exist from an area of 208 km², yet the core distribution is estimated to be no more than 2 km². Based on what is currently known on its ecology and distribution, we estimate that the species represents medium environmental risk. However, the species' distribution and invasive potential in Belgium remain largely unknown. As management of more widely established snake populations is notoriously difficult, we advocate a rapid response as the most appropriate risk management strategy.

Key words: reptile, established species, invasive species, pet trade, impact assessment, risk assessment, risk management, ISEIA protocol

Introduction

The introduction rate of alien reptiles and amphibians has increased globally (Seebens et al. 2017). In Europe, several invasive herpetofauna species have far-reaching impacts. Marked examples are terrapin species originating from North America, currently widespread and reproducing in southern parts of the continent (Cadi and Joly 2003; Standfuss et al. 2016), and invasive populations of American bullfrog (*Lithobates catesbeianus*), African clawed frog (*Xenopus laevis*) and water frogs from eastern Europe, the Near East and North Africa (*Pelophylax* spp.) that devastate populations of native amphibians across large areas (Holsbeek et al. 2008; Measey et al. 2012; Kopecký et al. 2016). An example of an invasive snake in the European

Union, albeit with no current populations in continental Europe, is the California kingsnake (*Lampropeltis getula californiae*), a popular pet snake, recorded since 2007 on Gran Canaria, Spain, following intentional or accidental release in two separate locations (Monzón-Argüello et al. 2015). Autopsies showed that endemic lizard species are the primary food source. Despite population control efforts, the expansion on Gran Canaria has increased markedly and coincided with declines in endemic prey species (Cabrera-Pérez et al. 2012). As a consequence, the species is considered to represent high environmental risk for continental Europe (Verzelen et al. 2018).

The main pathways for the introduction of snakes are deliberate releases or involuntary escapes of pet snakes, as cargo contaminants or as hitchhikers (Ruiz and Carlton 2004; Bomford et al. 2009). For example, in Europe, the trade in olive trees is of significance, allegedly enabling the introduction and establishment of several snake species from the Iberian Peninsula to the Balearic Islands (Silva-Rocha et al. 2015). Likewise, the western whip snake (*Hierophis viridiflavus*) was introduced from southern Italy to southwestern Germany as a waste contaminant, with ongoing eradication efforts (Laufer 2019). Additionally, several non-European snake species succeeded to establish reproducing populations on the continent. The Algerian false smooth snake (*Macroprotodon cucullatus*), putatively introduced to the Balearic Islands during Roman times, is believed to have contributed to the extinction of Lilford's wall lizard (*Podarcis lilfordi*) on Mallorca and Menorca (Silva-Rocha et al. 2015). More recently, the Algerian whip snake (*Hemorrhois algirus*) was introduced to Malta, supposedly as a transport contaminant during World War I (Speybroeck et al. 2016). In 1995, the Amur rat snake (*Elaphe schrenckii*), classified as a species with moderate environmental risk, successfully established in the northern part of the Netherlands, after deliberate release of pet snakes in a private garden (Van de Koppel et al. 2012). Besides the aforementioned three established colubrid species, there are recorded populations of a fourth non-European snake species in Europe since 2010 (Zamora 2017) – the Brahminy blind snake (*Indotyphlops braminus*), introduced as a contaminant in potted ornamental plants. A parthenogenetic typhlopoid, *I. braminus* establishes easily and, consequently, has been found in several southern European countries (Paolino et al. 2019).

In recent years, the pet trade has become a key pathway for the introduction of herpetofauna, with the potential for many more (un)intentional introductions (Hulme et al. 2008; Filz et al. 2018), as first record rates are correlated with trade values of the imported commodities (Seebens et al. 2017). As such, in Belgium, 24 non-European snake species have been recorded in the wild (Supplementary material Table S2, adapted from van Doorn et al. 2021), belonging to seven families. Escaped or released pets account for 82% of the species, while three species were introduced as stowaways and one as a wood contaminant.

Here, we report on the invasion and establishment of a fifth alien snake species in Europe, the beauty rat snake (*Elaphe taeniura* Cope, 1861) in Belgium. We present data on its invasion history, taxonomy, distribution, life-history and ecology. Furthermore, we present a preliminary impact assessment and briefly discuss options for risk management.

Materials and methods

Observational data

For the period 2006–2019, observational records of *Elaphe taeniura* in Belgium from the online citizen science platform “waarnemingen.be” are available through GBIF (Vanreusel et al. 2020). Each single record was validated before inclusion in the dataset. The majority of the records were performed by a handful of experienced observers and accompanied with clear pictures, location data and sighting details. When necessary, additional information was requested. In addition the authors recorded field data during 31 dedicated field surveys in 2018–2019. No native snake species occur in the surveyed locations. Each survey consisted of a visit to locations where beauty rat snakes had been documented before. At each site, beauty rat snakes were visually located above ground and under artificial cover. During these survey rounds observed snakes were caught by hand, and a subset of the captured snakes were sexed (N = 15), measured (N = 10) and weighed (N = 10). Counts of dorsal, ventral, subcaudal and subocular scales were carried out for five captured individuals and intact shed skins. Comparative scale count data were obtained from Schulz (2010). All captured snakes were removed from the wild and rehomed. Analyses and visualizations of the data were done with R (R Core Team 2020) in RStudio (RStudio Team 2020).

Molecular analyses

From a subset of five removed specimens, tail tips were taken for genetic identification. Genomic DNA was extracted from tissue material with the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer’s protocol, with 1 hour lysis and final elution in 140 μ L AE buffer. Next, a 658 bp barcoding fragment of the mitochondrial cytochrome oxidase subunit I gene (COI) was amplified via polymerase chain reaction (PCR), using the degenerated Folmer primers dgLCO-1490 (GGT CAA CAA ATC ATA AAG AYA TYG G) and dgHCO-2198 (TAA ACT TCA GGG TGA CCA AAR AAY CA) (Meyer et al. 2003). PCRs were performed in 26 μ L of reaction mixture containing 6 μ L of extracted DNA (10 ng/ μ L), 0.4 μ M of each primer, 1x Taq buffer with KCl, 2 mM MgCl₂, 200 μ M of each dNTPs and 0.8 U Taq polymerase (Thermo Fisher Scientific). Each PCR reaction included an initial denaturation at 94 °C for 2 min, followed by 35 amplification cycles (denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s and elongation

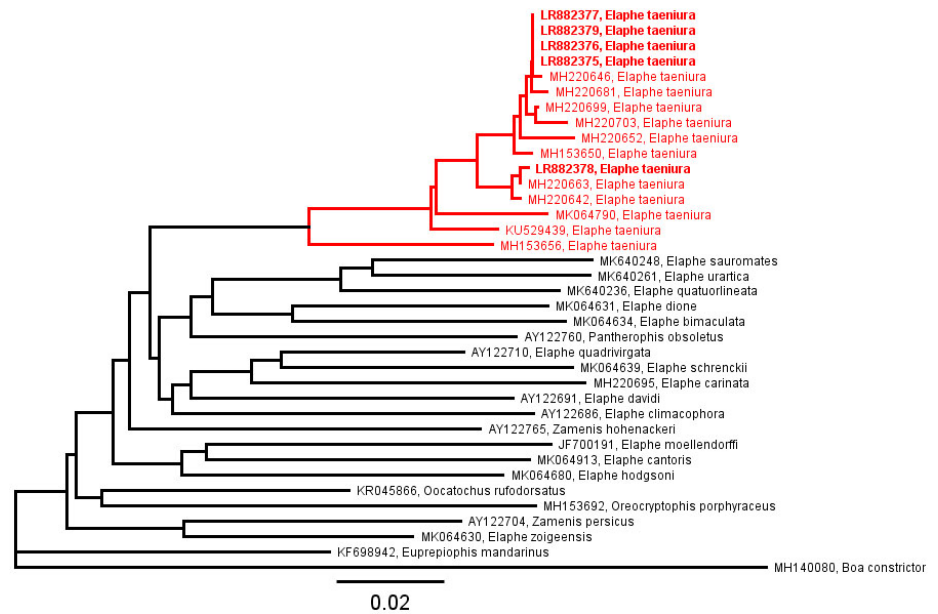


Figure 1. Tamura-Nei neighbour-joining tree showing the Belgian *E. taeniura* specimens (in bold) together with COI sequences from *E. taeniura* (in red), a selection of other closely related rat snake species and *Boa constrictor* as the outgroup. The scale bar indicates substitutions per site. Sequences are indicated by their INSDC accession number and scientific name.

at 72 °C for 1 min) and a final elongation step at 72 °C for 5 min. Amplified DNA was then purified using the ExoSAP-IT method (Thermo Fisher Scientific), followed by a quality check on a 1% agarose gel. These high quality samples were diluted 2.5 times before incorporating them in a sequence reaction for each of both primers. This was done using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) in a 10 µL volume containing 2 µL of purified DNA, 0.4 µM of dgLCO-1490 or dgHCO-2198 primer, 0.5x Ready Reaction mix and 0.5x Sequencing buffer. The cycling profile for both directions started with an initial denaturation at 96 °C for 1 min followed by 35 amplification cycles (10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C). After purification with the BigDye XTerminator Purification kit (Thermo Fisher Scientific), products were analyzed on an ABI 3500 genetic analyzer (Thermo Fisher Scientific). Sequences were checked for quality and aligned, and a consensus sequence from both directions was generated in Geneious v.10.2.6 (Biomatters Ltd.). A neighbour-joining tree was constructed with the Geneious tree builder function using the Tamura-Nei genetic distance model and a 93% similarity cost matrix. Sequences for *E. taeniura* and other species were obtained through Genbank. Pairwise identification of the Belgian samples was carried out with the BOLD Identification System (Ratnasingham and Hebert 2007) and *E. taeniura* sequences from Genbank (Genbank accession numbers incorporated in Figure 1).

Results and discussion

Identification and origin in Belgium

Molecular analyses of the five specimens yielded sequences of the full 658 bp COI fragment. These were identified as *Elaphe taeniura* with 99.85%

Table 1. Lepidosis (scale counts) of beauty rat snakes (*Elaphe taeniura*) found in Belgium and native individuals belonging to different subspecies (Schulz 2010).

	Subspecies	Lepidosis			
		Ventrals	Subcaudals	Suboculars	Dorsals (neck)
	<i>E. taeniura</i> Belgium	228–231	84–93	0–2	21–23
Northern group	<i>E. t. friesii</i>	243–262	96–123	2–4	25
	<i>E. t. mocquardi</i>	251–264	90–125	2	25
	<i>E. t. schmackeri</i>	246–260	104–125	2	25
	<i>E. t. taeniura</i>	225–255	84–112	2	23–25
	<i>E. t. yunnanensis</i>	236–260	89–120	0–4	25
Southern group	<i>E. t. grabowskyi</i>	275–285	92–114	1–2	27
	<i>E. t. ridleyi</i>	287–305	105–122	0–2	29
	<i>E. t. helfenbergeri</i>	282–292	82–97	0–1	27
	<i>E. t. callicyanous</i>	272–279	116–125	0–4	25–29

pairwise identity. Moreover, a neighbour-joining analysis clustered the Belgian *E. taeniura* sequences along with curated *E. taeniura* sequences (Figure 1).

Elaphe taeniura Cope, 1861 (syn. *Orthriophis taeniurus* Wallach et al. 2014) is widely distributed in (sub)tropical and temperate Southern and Eastern Asia (see Schulz 1996 for range maps of the subspecies). Currently, nine subspecies have been described that vary in morphological and ecological characteristics (Schulz 2013; Schulz et al. 2015). Beauty rat snakes are non-venomous, oviparous colubrids reaching lengths of up to 250 cm. *Elaphe taeniura* is a generalist feeder with a fast metabolism, preying on a wide range of small mammals, birds and lizards (Oxtoby 1988). The species is flexible in its habitat selection and can be found in primary forests as well as agricultural areas and within city limits, often occurring in close proximity to humans. Populations of the northern subspecies group may hibernate for several months, depending on climatic conditions (Gumprecht 2004). Identification of the subspecies occurring in Belgium can inform risk assessment. Yet, as detailed genetic reference material is lacking, molecular distinction of the subspecies is currently not possible. However, scale counts of all examined individuals reveal relatively low numbers when compared to those reported from most subspecies, overlapping only with those of *E. t. taeniura*. (Table 1). Other phenotypic traits of the Belgian specimens (Figure 2B) also match this subspecies (following Schulz 2010). The body is laterally compressed, ground coloration is light brown, with a black interconnected H-shaped dorsal pattern starting 3–4 head lengths behind the cranium, fading into a light stripe caudally. The sides sport black blotches and markings, gradually changing into two solid black lateral stripes caudally, with some specimens showing white transverse bands on the posterior end of the body. The venter is pale yellow, occasionally exhibiting darker speckling. The head is elongated, with a pronounced postocular black streak. The tongue is black and blue. No individuals longer than 142 cm have been found, which puts the retrieved snakes well within the range of *E. t. taeniura*, reported to grow to total lengths of 130–160 cm (Gumprecht 2004).

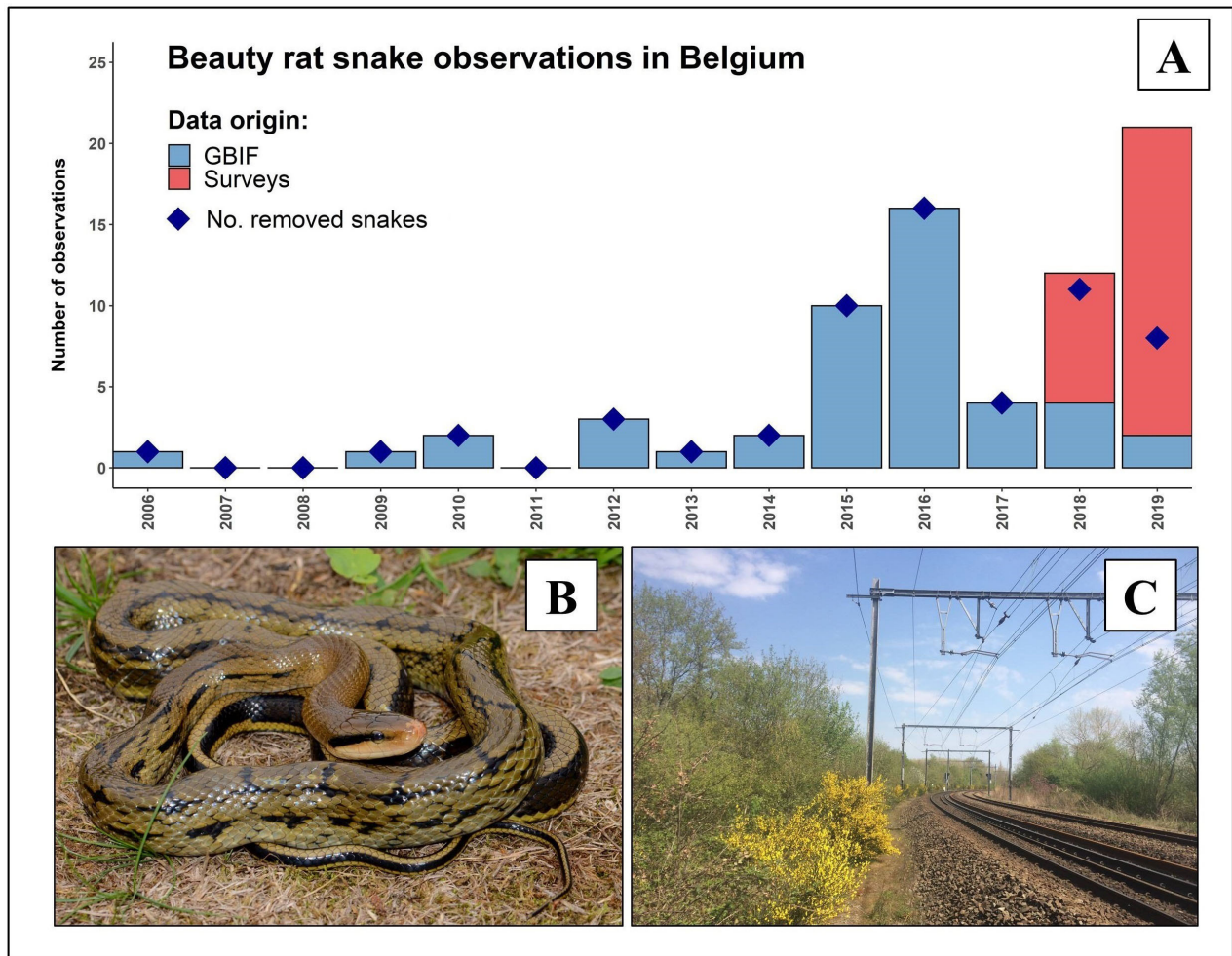


Figure 2. *Elaphe taeniura* in Belgium. (A) Number of observations (color depicts data origin) and removed snakes (points) per year. (B) An adult female collected by the first author (September 4th 2018) and (C) railroad habitat of *E. taeniura* in Belgium, a typical habitat for many ectotherms in central and northern Europe, in this particular location shared with native slow worm (*Anguis fragilis*) and non-native common wall lizard (*Podarcis muralis*). Photographs by LvD.

Beauty rat snakes are popular pet animals due to their appealing appearance (Gumprecht 2004) and ease of husbandry (Schulz 1996). As such, released or escaped individuals are the most plausible origin of this population in Belgium. Presence of a reptile shop that imported, distributed and sold beauty rat snakes located in the area where most of the observations are recorded (Figure 3) adds likeliness to this hypothesis. Consulted prices of online retailers offering *E. taeniura* for sale (N = 27) range from € 39 to € 504 per animal, depending on country, subspecies, size, scarcity and aberrant coloration. The subspecies *E. t. friesii* (N = 11), *E. t. callicyanous* (N = 8) and *E. t. taeniura* (N = 5) were more often found to be for sale than *E. t. grabowskyi* (N = 1), *E. t. ridleyi* (N = 1) and *E. t. mocquardi* (N = 1). While both scale counts and phenotypic characteristics indicate that the specimens in Belgium belong to the taxon *E. t. taeniura*, care should be taken with introductions potentially originating from the pet trade, as inter- and intraspecific hybrids are common in captivity, even when morphologically seemingly belonging to a single (sub)species (e.g. *Pituophis* sp. in the Netherlands, Struijk 2018).

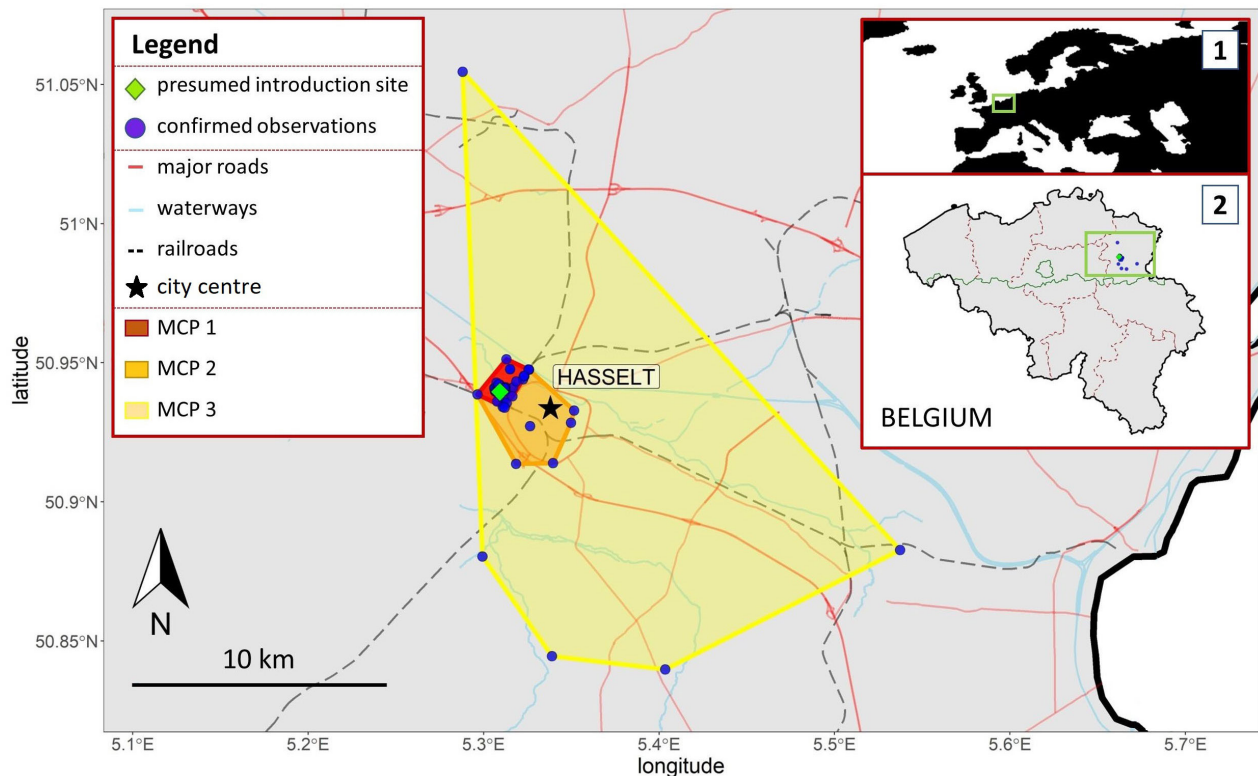


Figure 3. Distribution map of *Elaphe taeniura* in Belgium (inset 1, 2), Europe. Minimum convex polygons (MCP) added for the core distribution area (MCP1), the observations around the city (MCP2) and all observations (MCP3).

Distribution in Belgium

The first confirmed observation of *E. taeniura* in the wild in Belgium was on 26 October 2006. In subsequent years, the number of observations increased (Figure 2A), with a cumulative total of 73 observations by the end of 2019. These observations consisted of 64 live snakes (50 were removed from the wild), three dead snakes, six eggs (in a single clutch) and three shed skins. As snakes of all life stages have been observed in the wild, it is likely that the population is established, i.e. reproducing and self-sustaining without further introductions.

Most observations cluster around the railroads in the western part of the city of Hasselt, Limburg Province (Figure 3, Table S1). However, five observations were recorded up to 15 kilometers outside of Hasselt. Further research is needed to assess the natural dispersal capacity of this species in Belgium and the possible effect of hitchhiking in trains or cars to explain occurrences further away from the introduction site. When taking all observations into account, the area of occupancy (minimum convex polygon) is 208 km². However, when the few observations outside the main cluster (N = 5) are removed, the area of occupancy is reduced to 10 km². If in addition the observations in the city center (N = 5) are excluded, only 2 km² remains as the core distribution area. The majority (86%) of the observations (N = 63) are thus from a relatively small area (Figure 3). This is, however, most likely an underestimation of the actual distribution of the species in Belgium, as the data presented here is largely opportunistic and

surveys were only conducted in areas where observations had been recorded. Furthermore, the train tracks are off limits to the public, and observations by railway personnel have not been systematically recorded.

Ecology and life history in Belgium

Within the occupied area in Belgium, most observations are recorded along railroad embankments (Figure 2C) and in adjacent areas. Due to a warmer microclimate, railroads are also a preferred habitat of native snakes (*Vipera berus*, *Natrix helvetica* and *Coronella austriaca*), where these species often occur in higher densities than in other habitats, especially in southern Belgium (Graitson et al. 2020). The railroad where most of the observations were made is exposed to direct sunlight, provides shelter (e.g. cable trays) and houses ample prey species (such as rodents). Isolated observations stem from (ware)houses, gardens, and as roadkills. During targeted searches the authors found only one snake active on the surface, with all other specimens located under artificial cover. A (semi-)fossorial ecology is also reported in the wild (Gumprecht 2004; Moseley 2008). In their native range, *E. t. taeniura* inhabits (sub)tropical, temperate and continental climate zones from sea level up to 1,500 m (Schulz 1987) and hibernates for up to five months (Gumprecht 2004). Hibernation in Belgium is suspected to occur in locations where railroad cables allow deeper underground penetration. The latest date in the year we recorded a beauty rat snake during directed surveys was October 8th (2019); the earliest was March 12th (2020), indicating a comparable hibernation period to that of native Belgian snake species. The average summer air temperature in Shenyang, northeast China, close to the natural northern range limit of *E. t. taeniura* is higher (June–August: 23.8 °C, Climate-data 2020) than the average summer air temperature in Hasselt, Belgium (June–August: 17.6 °C, KMI 2020). Consequently, the major bottleneck for beauty rat snakes in Belgium is thought to be the temperature necessary for egg development. At a temperature of 22–28 °C the eggs hatch after 70–75 days (Schulz 1987). As such, in Belgium, *E. taeniura* is limited to specific microclimates for egg-laying. Six eggs were discovered buried under wood on top of the railway embankment on July 1st 2016 (Engelen 2016). However, other anthropogenic structures are capable of providing suitable temperatures. Examples of European snake species relying on anthropogenic heat sources for egg deposition are grass snakes (*Natrix* spp., including native *Natrix helvetica*) which use manure and compost heaps and can therefore occur at higher latitudes than other egg-laying species, escaping natural thermal limits (Löwenborg et al. 2010). Likewise, Aesculapian snake (*Zamenis longissimus*), a European rat snake species, uses manure and compost heaps as egg deposition sites at the northern edge of its range (Kovar et al. 2016). It is thus likely that beauty rat snakes will readily use anthropogenic structures.

Furthermore, climate change can increase reproductive success and distribution of *E. taeniura* in Belgium.

Life stage information was obtained for 38 snakes, comprising 24 adults, seven subadults and seven neonates. Sex was recorded for 15 snakes, of which ten males and five females. The average weight (\pm SD) of ten measured adults was 380 ± 144 g, with a maximum of 551 g. The average length (\pm SD) was 118 ± 18 cm, with a maximum of 142 cm. Captured snakes were all healthy and behaved naturally. Diet analyses of Belgian *E. taeniura* have not yet been performed. Presumably, the population largely subsists on rodents, plentiful in their subterranean habitat. Corroborating this assumption are the observations of two subadult brown rats, *Rattus norvegicus*, regurgitated post-capture by two adult snakes: one in September 2018 and one in July 2020.

Impact assessment

Due to an alleged mismatch between the native range and European climate (Bugter et al. 2014), and uncertainty regarding its native climate (Kopecký et al. 2016), previous risk assessments for beauty rat snakes in Europe have not considered the species to be of concern. Overall, species distribution models tend to be poor predictors of invasive potential (Liu et al. 2020). The here presented case of establishment in Belgium exemplifies the ecological flexibility of the species to adapt to man-made habitats and underlines the importance of including microclimatic variation in risk assessments. Climate envelope models, often used for ectotherms, comparing climate of the native range to the climate of the assessment area rely on standardized minima, maxima and average temperatures (Bomford et al. 2009), overlooking key local climatic variations, in this case railways with a warmer microclimate, dispersal opportunities (Ascensão and Capinha 2017) and urban heat island effects (Oke 1973). Likewise, alien populations of the common wall lizard (*Podarcis muralis*), have spread and established themselves outside of their native range by virtue of the railways and assisted train dispersal in Belgium (Jooris and Lehouck 2007).

The closely related *E. schrenckii* is an established alien species in the Netherlands with an Invasive Species Environmental Impact Assessment (ISEIA) rating of B1 (Branquart 2009), i.e. moderate environmental risk occurring in isolated populations (Van de Koppel et al. 2012). In China, the populations of *E. taeniura* from Hong Kong and Beijing are presumed to be non-native (Francis and Ferguson 2018; Midtgaard 2019), highlighting the risk for the establishment of non-native populations in Asia. Records of *E. taeniura* in Russia and Korea are probably human-mediated (Schulz 1996), as well as an observation of *E. taeniura* in Chinese cargo in New Zealand (Gill et al. 2010). Consequently, *E. taeniura* is regarded as a threat, potentially spreading via military pathways in Asia (Pitt et al. 2010). In addition, *E. taeniura* is reported as an invasive species in Japan, originating

from captive animals. Here, the Taiwanese subspecies (*E. t. friesi*) has been recorded in the south-central part of Okinawa island since 2003 and is considered of conservation concern through potential predation on endemic mammals and birds (Ota et al. 2004). Therefore, *E. t. friesi* has been banned for import, transport and keeping (Mito and Uesugi 2004) and wild caught specimens are bought and removed by the environmental department of Okinawa as a mitigation measure (Okinawa Prefecture 2019).

Using the ISEIA-protocol (an environmental impact assessment following Branquart 2009), for a worst-case scenario where *E. taeniura* occupies all potential habitats in Belgium, we tentatively classify *E. taeniura* as a B1 species (Table S3), i.e. a watchlist species with moderate environmental risk currently occurring in isolated populations. However, to perform a full risk assessment to evaluate its invasive potential under climate change, more systematic surveys are needed to determine the distribution extent and size of this population, and to gather more data on its ecology. Social impact is presently limited, as the habitat is largely inaccessible to the public. However, observations of beauty rat snakes in new housing estates and properties close to the railways have caused some social unrest. Health hazards for railroad workers due to fear responses are a concern, as beauty rat snakes are frequently encountered during construction works. Economic impact has not been reported, but potential disruption of train traffic is possible due to wire shorting. Zoonotic disease transmission is unlikely (Bugter et al. 2014).

Future outlook

Regardless of environmental and other risks associated with the introduction of *E. taeniura*, risk management should complement risk assessment (Booy et al. 2017, 2020). On the prevention side, from 2019, a Positive List of reptiles is in force (Flemish Government 2019) which forbids the sale and possession of, among other species, beauty rat snakes in the Flemish region, effectively limiting the potential for future introductions. Although some positive results have been achieved with the eradication of alien snakes from restricted localities, attempts to eradicate snakes from larger areas have not been successful (Rodda et al. 2002). Hand capture has been documented as an effective method to capture significant numbers of snakes (Cabrera-Pérez et al. 2012), although other methods may be needed to ensure complete removal, including artificial refugia or specific traps (Rodda et al. 1999). The specific site conditions in this case, an active railway inaccessible to the public, pose additional management challenges. Current trapping methods are also untested for use on this species, which would warrant an adaptive management approach. Clearly, there is a strong link between eradication feasibility and invasion extent (Booy et al. 2020). Therefore, for eradication to work, the time to act is now. For eradication efforts to be effective, knowledge gaps on distribution need to

be closed through dedicated surveys. Other factors important for a successful control campaign include clear lines of responsibility, dedicated budgets and perseverance, political and stakeholder support, clear risk communication, as well as thorough scientific follow-up (e.g. Adriaens et al. 2015).

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Authors' Contribution

LvD, JS and TA conceptualized the research and wrote the original draft. LvD and PE performed the data collection. RB, DH and SN performed the genetic analysis. All authors contributed to the writing and revisions of the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Consolidated observations of *Elaphe taeniura* in Belgium from 2006–2019.

Table S2. Recorded non-European snake species in Belgium.

Table S3. ISEIA scoring of the beauty rat snake, *Elaphe taeniura* under a worst case scenario in Belgium.

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