

Research Article

Distribution and invasion risk of non-native amphibians and aquatic reptiles in the Pannonian Biogeographical Region of Central Europe

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Abstract

Global declines in amphibians and reptiles have been well documented for decades, with invasive species being one of the major threats. In the Pannonian Biogeographical Region (PBR) of Central Europe, which includes the whole territory of Hungary and peripheral areas of Austria, Croatia, Czech Republic, Romania, Slovakia, Slovenia, Serbia, and Ukraine, there is currently no systematic overview of the occurrence and potential risk of invasiveness of non-native amphibians and aquatic reptiles. Here, we (i) collated observations on non-native species occurrence in the PBR, (ii) ran a monitoring program focused on the urban areas around Budapest, the capital of Hungary, and (iii) screened 16 amphibian and 28 aquatic reptile non-native species found across the PBR and in the local pet trade market for risk of invasiveness under both current and predicted future climate conditions. The risk screening was carried out using the Aquatic Species Invasiveness Screening Kit v2.4 (AS-ISK). We collected 5,134 observations of five amphibian and 14 aquatic reptile non-native species in the PBR, with the highest diversity and densities observed in urban areas. Screening revealed that six amphibians and 23 aquatic reptiles can be regarded as posing a high to very high risk of invasiveness, especially after accounting for the effects of climate change. Combining occurrence data and screening results, the highest-risk taxa include the three subspecies of pond slider *Trachemys scripta*, the common snapping turtle *Chelydra serpentina*, the Eastern river cooter *Pseudemys concinna*, the Chinese soft-shelled turtle *Pelodiscus sinensis*, the false map turtle *Graptemys pseudogeographica*, the African clawed frog *Xenopus laevis*, and the western dwarf clawed frog *Hymenochirus curtipes*. *Trachemys scripta elegans* and *T. s. scripta* can already be considered invasive. We conclude that pet keeping presents a serious conservation threat for the PBR, where urban areas act as incubators for potential invasion. We emphasize the importance of public campaigns aimed at raising awareness of the risk posed by invasive species and of continuous targeted monitoring of urban areas to prevent future invasions.

Key words: Aquatic Species Invasiveness Screening Kit (AS-ISK), biological invasion, citizen science, conservation, herpetofauna

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Introduction

Starting in the 1990s, conservation biologists and herpetologists began to raise concerns about a possible ‘global amphibian decline’ (Barinaga 1990; Wake 1991; Blaustein et al. 1994). This has since been confirmed by subsequent studies (Houlahan et al. 2000; Stuart et al. 2004) that have also included reptiles (Gibbons et al. 2000). The factors behind species declines are numerous, complex, and typically act synergistically (Green et al. 2020; Biber et al. 2023). Besides climate change and human-induced habitat disruption, biological invasions are important contributors, particularly affecting the more vulnerable native species (e.g., Gibbons et al. 2000; Kiesecker et al. 2001; Collins and Storfer 2003; Kraus 2009, 2015; Falaschi et al. 2019). Further, human-induced disturbance and climate change directly contribute to the spread of invasive species (Vandekerkhove and Cardoso 2010; Falaschi et al. 2020; Gibbons et al. 2000; Pyšek et al. 2020, 2021; Maximo et al. 2021; Reshetnikov et al. 2023). Biological invasions pose serious problems from the population to the ecosystem level (Pyšek et al. 2020, 2021).

Global declines in native herpetofauna and overall issues related to the impacts of invasive species have also affected several regions of the European Union (Falaschi et al. 2019, 2020; Haubrock et al. 2023). The problem of invasive species has already been highlighted in several areas, such as the Mediterranean region (e.g., Poch et al. 2020; Esposito et al. 2022; Soto et al. 2025), the British Isles (Langton et al. 2011; Cathrine 2024), the southern region of the Carpathians and the western part of the Ponto-Caspian region (Sos 2007, 2009; Stănescu et al. 2020; Iftime and Iftime 2021, 2022), and Central Europe (Kraus 2009), including the Pannonian Biogeographical Region (PBR), which harbors a rich herpetofauna compared to other European regions (Sillero et al. 2014). The PBR includes the whole area of Hungary and peripheral areas of Czech Republic, Romania, Slovakia, Serbia, and Ukraine (Mozsár et al. 2021). In Hungary, where all native amphibians and reptiles are protected by law (Puky et al. 2005), knowledge of invasive aquatic species has recently increased based on targeted research programs for crayfishes and fishes (Szalóky et al. 2015; Takács et al. 2017, 2025; Mozsár et al. 2021), but not for amphibians and reptiles (Haraszthy 2022). In the other countries of the PBR, most studies on non-native species have focused on the conservation of targeted native aquatic species (e.g., Koščo et al. 2010; Lipták et al. 2023; Nekrasova and Marushchak 2023). The number of studies on non-native species in these countries is, however, limited overall (e.g., Kopecký et al. 2013, 2016, 2019; Vilizzi et al. 2021).

The lack of comprehensive information on non-native amphibians and aquatic reptiles in the PBR is a crucial problem for conservation. This is because several of these non-native species are very popular as pets, which makes their introduction into natural ecosystems likely. For instance, the red-eared and yellow-bellied sliders *Trachemys scripta elegans* and *T. s. scripta* have been routinely recorded from several areas (e.g., Puky et al. 2004, 2005; Bódis et al. 2012; Urošević et al. 2016; Iftime and Iftime 2021, 2022), where their successful reproduction has been documented (Iftime and Iftime 2021; Weiperth 2022). These turtles are well known for their invasiveness, which is causing conservation issues worldwide (Invasive Species Specialist Group of the IUCN Species Survival Commission 2011; Kopecký et al. 2013, 2019; Vilizzi et al. 2021; Reshetnikov et al. 2023; Nerozzi et al. 2024; Rato et al. 2025). Targeted monitoring and research programs on the impacts of these two subspecies on the native European pond turtle *Emys orbicularis* have

been launched in Hungary by non-governmental (e.g., WWF Hungary, BirdLife Hungary) and governmental nature conservation bodies (Farkas 2008; Farkas et al. 2013). Furthermore, human-related activities, including urbanization and pollution, can create favorable conditions for the establishment and spread of non-native species, making urban areas suitable hotspots for biological invasions (e.g., Kopecký et al. 2013, 2019; Balzani et al. 2016; Stănescu et al. 2020; Vilizzi et al. 2022; Fănararu et al. 2024; Kuhn et al. 2025). Therefore, not only (quasi)natural habitats but also urban environments should be monitored and given special attention to conserve biodiversity. We note that we use the term “(quasi)natural” instead of the commonly used “natural” to acknowledge that fact that there are no truly natural (i.e. human influence free) habitats left in the PBR. There are various habitats characterized by low technological transformation, such as meadows, hedgerows, regulated streams, channels, old ponds, and oxbows that can act as bridges between (quasi)natural and artificial landscapes (e.g., Haraszthy 2014; Špulerová et al. 2022). These habitats have been modified in the long-term by different human activities but nevertheless retain much of their natural biodiversity and processes (e.g., Puky et al. 2004, 2005; Haraszthy 2014; Hamer et al. 2023).

To date, a comprehensive review of the occurrence and distribution of non-native amphibians and aquatic reptiles in the PBR is missing, including an evaluation of their risk of invasiveness. Risk screening, the first step in the risk analysis process (Vilizzi et al. 2022), provides valuable information about which non-native species are likely to become invasive, thus posing a threat to native ecosystems. Knowledge of the scientific literature on species is essential for accurate analysis and evaluation of results. Risk screening, whenever feasible, should be supplemented with field-based data, as the distribution and risk of invasiveness of non-native species are both necessary to inform decision-makers, lawmakers, and environmental managers about actions for eradication, control, or prevention, as well as for long-term management plans. In many cases, the results of risk screening can also be used in the legislative process (e.g., Tricarico et al. 2010; Rato et al. 2025).

In this study, we aimed to provide a comprehensive evaluation of the occurrence, distribution, and risk of invasiveness of non-native amphibians and aquatic reptiles in the PBR. Our objectives were threefold: to (i) collect and collate data on the occurrence and distribution of non-native amphibians and aquatic reptiles in the PBR to gain an understanding of their current status; (ii) run targeted monitoring in a highly populated urban area to evaluate the role of urban areas in non-native amphibian and aquatic reptile establishment; and (iii) conduct a risk screening of the non-native amphibians and aquatic reptiles that are part of the local pet trade market under both current and future climate change scenarios. Based on the outcomes of this study, the species at higher risk of invasiveness are identified as requiring prompt attention by conservationists and decision-makers across all countries of the PBR.

Methods

Study area

The PBR is located in the Carpathian Basin of Central Europe (European Environmental Agency 2009; Fig. 1A, B), with a direct connection via water courses to Austria, Croatia, and Slovenia. Two-thirds of the PBR are located in lowlands (200 m max altitude), with the rest in mid-altitude regions (200–800 m) and, in

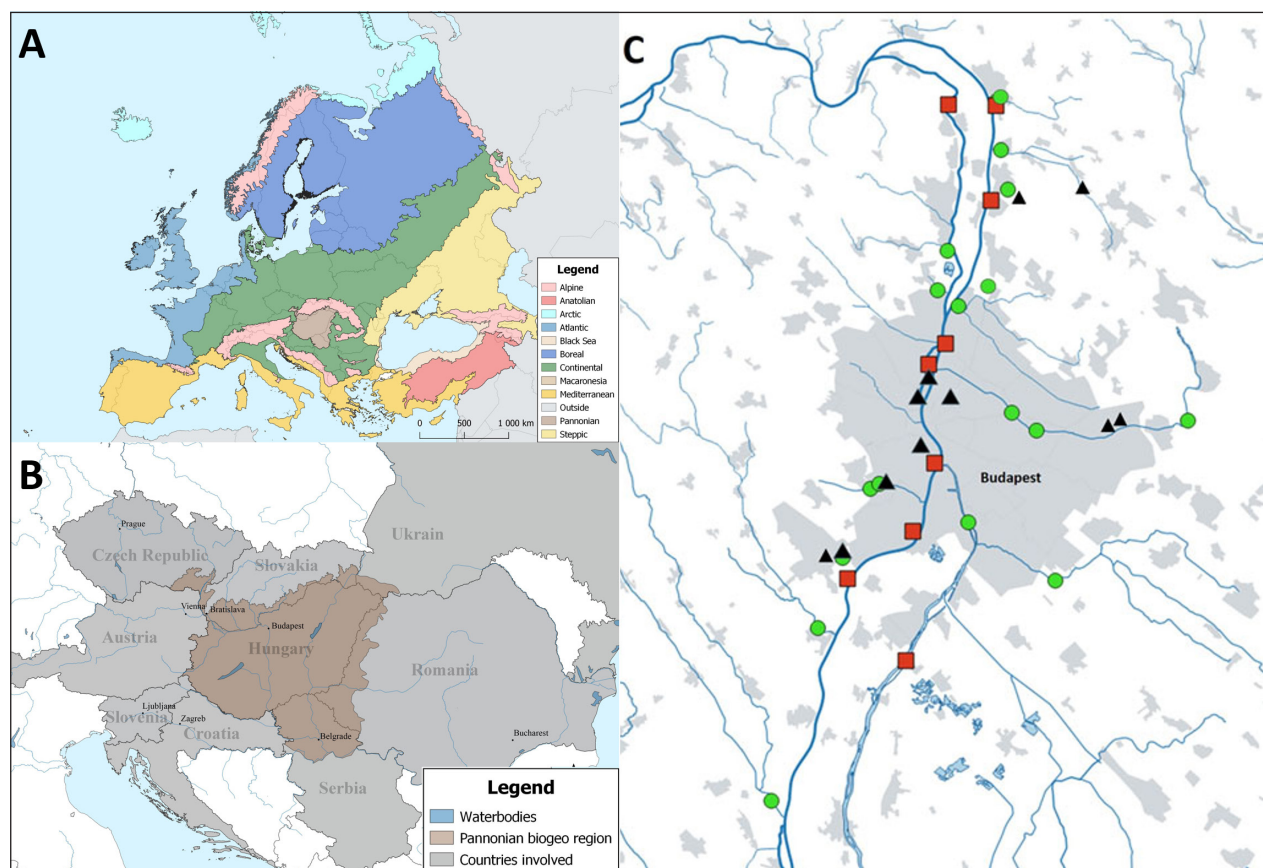


Figure 1. A. Biogeographical Regions of Europe (from <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>); B. Countries of the Pannonian Biogeographical Region; C. Location of the sampling sites in the urbanized region of Budapest in Hungary. Green circles = sampling sites in tributaries of the River Danube; red squares = sampling sites in side arms of the River Danube; black triangles = sampling sites in artificial and thermal ponds. For a list of the water bodies (Suppl. material 3).

smaller proportions, submontane regions (> 500 m). Before the highest intensity of river regulation, which began in the mid-19th century (starting around 1846) and continued into the 20th century, almost the entire lowland area of the PBR formed the floodplain of the large alluvial rivers draining into the Carpathian Basin. The PBR also hosts a large number of different artificial habitats, including thermal and industrial warm-water outflows and ponds, rain- and storm-water creeks, channels, and ponds. While these habitats are important for the local biodiversity, they are dominated by non-native species (e.g., Haraszthy 2014, 2022; Takács et al. 2017; Weiperth et al. 2020; Blaha et al. 2022).

There are 18 species of native amphibians (12 frogs, one salamander, and five newts) and 16 species of native reptiles (eight lizards, seven snakes, and one turtle), of which three are aquatic (two snakes and one turtle), in the PBR (Suppl. materials 1, 2) (Sillero et al. 2014). This species-rich herpetofauna has suffered declines since the second half of the 20th century (Puky et al. 2005; Tomović et al. 2014; Vörös et al. 2015; Hamer et al. 2021).

Species occurrence and distribution

Data on the occurrence and distribution of non-native amphibians and aquatic reptiles in the PBR were retrieved from five sources: (i) non-governmental organizations' (NGOs) databases, (ii) expert opinion, (iii) citizen science, (iv) literature

data, and (v) field observations. These were complemented by an additional source represented by (vi) a long-term targeted monitoring program in the area of Budapest, the capital of Hungary (described separately below).

For NGO databases, data (with exact localities and time) were retrieved from (i) the Amphibian and Reptile Conservation Section of BirdLife Hungary (1996–2024), resulting in 1,694 non-native species observations; (ii) the Dr. Puky Miklós Toad Action Group Association (1992–2024), resulting in 665 non-native species observations; and (iii) region-specific databases (Herpetology.cz: <https://herpetology.cz>; Open Herp Maps: <https://openherpmaps.ro/>; Österreichische Gesellschaft für Herpetology Societas: <https://www.herpetozoo.at/>; Europaea Herpetologica: <https://www.seh-herpetology.org/>), resulting in 61 non-native species observation from the earliest data all up to the end of 2024.

For expert opinion, based on field observations and notifications from civilians, anglers, and fishermen, interviews were conducted in 2021–2024 involving 16 nature conservation experts from Hungarian national park directorates and eight experts from public companies working in the management and maintenance of wetlands and artificial aquatic habitats in Hungary. The interviews resulted in 633 non-native species observations with accurate location and time. To gather data from all areas of the PBR, data on non-native amphibians and aquatic reptiles were also collected from community groups and social media forums in 2020–2024, with accurate location, time, and photos that helped identify the species. This resulted in 314 observations.

For citizen science, three questionnaires were circulated during 2019–2021 primarily to evaluate the knowledge of the respondents about the local pet trade market of non-native amphibians and aquatic reptiles but also about their field observations of non-native species. For the purposes of this study, only occurrence data of non-native amphibians and aquatic reptiles were extracted from the questionnaires. This yielded 25 observations of well-identified species with exact locality and time.

For literature data, searches were conducted for 20 years (2004–2024) in Google Scholar (<https://scholar.google.com/>) and Web of Science (<https://www.webofscience.com/>) using the keywords “Pannonian Ecoregion,” “Pannonian Biogeographical Region,” the names of the countries within the PBR (see Section Study area), “alien,” “invasive,” “non-native,” “amphibian,” “reptile,” and the names of the relevant taxa. Both the title and abstract of the papers retrieved in the search were checked, and, whenever necessary, the whole text was carefully read, including an additional search for further potentially relevant published sources in the cited literature. This resulted in 14 relevant scientific publications for a total of 359 observations of non-native species with exact locations and time (Puky et al. 2004, 2005; Kovács et al. 2004; Kleewein and Wöss 2009; Bódis et al. 2012; Dimancea 2013; Dordević and Andelković 2015; Weiperth et al. 2015; Jablonsky et al. 2016; Urošević et al. 2016; Teran et al. 2017; Koren et al. 2018; Iftime and Iftime 2021; Nekrasova et al. 2022). Since more and more occurrence data are published on different media platforms (e.g., local and national media and websites), national press and website data searches were conducted in Google (Angulo et al. 2021). This resulted in 71 relevant media publications, but these could not be used because the exact locations of the non-native species observations could not be determined. During the Boolean search, which was used for both scientific publications and

other media sources, we attempted to examine all word combination systems in both English and the national languages of the PBR countries.

For field observations, we added our unpublished personal data based on field-work conducted in for a decade (2014–2024) for various purposes. From this source, 245 non-native species observations were added to the database with exact times and localities.

Long-term monitoring of a highly populated urban area

A 10-year (March 2015–November 2024) field monitoring program was conducted to determine the occurrence of non-native amphibians and aquatic reptiles in the vicinity of Budapest (Fig. 1C). Although data from this monitoring are included in the overall species distribution assessment, they are also discussed separately to evaluate the importance of urban areas in the establishment and potential spread of non-native species. The sampled area covers approximately 8,200 km² and has more than 3.3 million inhabitants, with a population density of 400 people/km² (Kondor et al. 2022). In the last three decades, land use conversion and the rate of urban spread have increased, and the proportion of artificial areas is currently at \approx 20% (Kovács et al. 2019). For the monitoring, we followed the Hungarian National Biodiversity Monitoring Protocol (Korsós 1997). Field surveys were conducted three times per year (March–April, June–July, October–November) using various sampling methods consisting of visual observations, baited traps, nets, and electrofishing. Altogether, 33 water bodies were sampled across 37 sampling sites. The water bodies consisted of 13 tributaries of the River Danube (17 sampling sites), nine side arms of the River Danube (nine sampling sites), and 11 ponds (11 sampling sites) (Fig. 1C, Suppl. material 3). The monitoring resulted in 1,138 non-native species observations with exact time and locality (Suppl. material 3).

During the summary and analysis of the data, we made every effort to avoid data duplication or multiplication. In all cases, we transported all collected individuals to receiving organizations during our monitoring program and other field samplings. In addition, we screened the databases for duplicate or multiple records throughout the entire process.

Risk screening

In total, 44 non-native pet-traded species comprising 16 amphibians and 28 aquatic reptiles were identified for risk screening in the PBR (Table 1). The final list of species for screening was based on our occurrence data, pet shop lists showing the species available for purchase at the time of the study, and overall knowledge about the species available from the pet trade or breeders (Suppl. material 4). In the case of the pond sliders, the three subspecies *T. s. elegans*, *T. s. scripta*, and *T. s. troosti* were screened separately due to their conservation relevance.

Risk screening was carried out with the Aquatic Species Invasiveness Screening Kit v2.4 (AS-ISK: download at www.cefas.co.uk/nns/tools/). This multilingual, taxon-generic decision-support tool complies with the “minimum standards” for screening non-native species under EC Regulation No. 1143/2014, the European Alien Species Information Network (EASIN), the Global Biodiversity Information

Table 1. Non-native amphibians and aquatic reptiles evaluated with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for their risk of invasiveness in the Pannonian Biogeographical Region of Central Europe. For each amphibian and aquatic reptile, the *a priori* categorization into invasive and non-invasive is based on the four step protocol by Vilizii et al. (2022): (1) Global Biodiversity Information Facility (GBIF: www.gbif.org); (2) Global Invasive Species Database (GISD: www.iucngisd.org); (3) European Alien Species Information Network (EASIN: <https://easin.jrc.ec.europa.eu/easin>); (4) Google Scholar literature search. N = no impact/threat; Y = impact/threat; “–” = absent; n.a. = not applicable.

		A priori categorization				
Species name	Common name	GBIF	GISD	EASIN	Google Scholar	Outcome
Amphibians						
<i>Ambystoma mexicanum</i>	axolotl	N	–	N	N	Non-invasive
<i>Ceratophrys ornata</i>	Argentinean horned frog	N	–	–	N	Non-invasive
<i>Cynops orientalis</i>	oriental fire-bellied newt	–	–	–	N	Non-invasive
<i>Cynops pyrrhogaster</i>	Japanese fire-bellied newt	N	–	N	N	Non-invasive
<i>Dendrobates auratus</i>	dart poison frog	N	–	–	N	Non-invasive
<i>Dendrobates tinctorius</i>	dyeing dart frog	–	–	–	N	Non-invasive
<i>Dyscophus guineti</i>	Sambava tomato frog	–	–	–	N	Non-invasive
<i>Hymenochirus curtipes</i>	western dwarf clawed frog	–	–	–	N	Non-invasive
<i>Lithobates catesbeianus</i>	American bull frog	Y	Y	Y	n.a.	Invasive
<i>Pelobates balcanicus</i>	Balkan spadefoot	–	–	–	N	Non-invasive
<i>Pleurodeles waltl</i>	Iberian ribbed newt	N	–	–	N	Non-invasive
<i>Rhinella marina</i>	cane toad	Y	Y	Y	n.a.	Invasive
<i>Trachycephalus resinifictrix</i>	mission golden-eyed tree frog	–	–	–	N	Non-invasive
<i>Tylototriton shanjing</i>	Hoanglien Mountain crocodile newt	–	–	–	N	Non-invasive
<i>Tylototriton verrucosus</i>	Himalayan newt	–	–	–	N	Non-invasive
<i>Xenopus laevis</i>	African clawed frog	Y	Y	Y	n.a.	Invasive
Aquatic reptiles						
<i>Caiman crocodilus</i>	common caiman	Y	N	N	n.a.	Invasive
<i>Chelodina longicollis</i>	common snake-necked turtle	–	–	–	N	Non-invasive
<i>Chelus fimbriata</i>	Amazon mata mata	N	–	N	N	Non-invasive
<i>Chelydra serpentina</i>	common snapping turtle	Y	–	N	n.a.	Invasive
<i>Chrysemys picta</i>	eastern painted turtle	N	–	N	N	Non-invasive
<i>Clemmys guttata</i>	spotted turtle	–	–	–	N	Non-invasive
<i>Cuora flavomarginata</i>	snake-eating turtle	–	–	–	N	Non-invasive
<i>Emydura subglobosa</i>	red-bellied short-necked turtle	–	–	–	N	Non-invasive
<i>Graptemys pseudogeographica</i>	false map turtle	Y	–	N	n.a.	Invasive
<i>Kinosternon subrubrum</i>	common mud turtle	–	–	–	N	Non-invasive
<i>Macrochelys temminckii</i>	alligator snapping turtle	Y	–	N	n.a.	Invasive
<i>Malaclemys terrapin</i>	diamond-backed terrapin	–	–	–	N	Non-invasive
<i>Mauremys caspica</i>	Caspian terrapin	N	–	N	N	Non-invasive
<i>Mauremys reevesii</i>	Chinese tree-keeled pond turtle	N	–	N	N	Non-invasive
<i>Mauremys rivulata</i>	Balkan terrapin	–	–	N	N	Non-invasive
<i>Mauremys sinensis</i>	Chinese stripe-necked turtle	N	–	N	N	Non-invasive
<i>Nerodia fasciata</i>	southern water snake	–	–	–	N	Non-invasive
<i>Nerodia sipedon</i>	common water snake	–	–	–	N	Non-invasive
<i>Paleosuchus palpebrosus</i>	Cuvier's smooth-fronted caiman	N	–	–	N	Non-invasive
<i>Paleosuchus trigonatus</i>	smooth-fronted caiman	–	–	–	N	Non-invasive
<i>Pelodiscus sinensis</i>	Chinese soft-shelled turtle	Y	–	N	n.a.	Invasive
<i>Pseudemys concinna</i>	eastern river cooter	N	–	N	N	Non-invasive
<i>Pseudemys nelsoni</i>	Florida red-bellied cooter	Y	–	N	n.a.	Invasive
<i>Pseudemys peninsularis</i>	peninsula cooter	Y	–	–	n.a.	Invasive
<i>Sternotherus odoratus</i>	common musk turtle	–	–	N	N	Non-invasive
<i>Thamnophis sirtalis</i>	common garter snake	–	–	–	N	Non-invasive
<i>Trachemys decussata</i>	Cuban slider	N	–	N	N	Non-invasive
<i>Trachemys scripta elegans</i>	red-eared slider	Y	Y	Y	n.a.	Invasive
<i>Trachemys scripta scripta</i>	yellow-bellied slider	Y	–	N	n.a.	Invasive
<i>Trachemys scripta troosti</i>	Cumberland slider	Y	–	N	n.a.	Invasive

Facility (GBIF), the Global Invasive Species Database (GISD) of the Convention on Biological Diversity, and the International Union for Conservation of Nature (IUCN) on the prevention and management of the introduction and spread of invasive alien species (Masters and Norgrove 2010; Copp et al. 2016; Boon et al. 2020; Vilizzi et al. 2021). The AS-ISK consists of 55 questions, of which 49 comprise the Basic Risk Assessment (BRA) and six the Climate Change Assessment (CCA). Of the 46 species in total, 44 were screened by BP-S and two jointly by AW and BP-S. Screening followed the protocol by Vilizzi et al. (2022), with the assessors providing for each question a response, a confidence level, and a justification (Vilizzi and Piria 2022). This resulted in two outcome scores: BRA and BRA+CCA. Scores < 1 rank the species as carrying a “low risk” of invasiveness in the risk assessment area; scores ≥ 1 rank the species as “medium risk” or “high risk.” To distinguish between medium- and high-risk species, two separate calibrated thresholds for amphibians and aquatic reptiles were computed by Receiver Operating Characteristic (ROC) curve analysis (Vilizzi and Piria 2022; Vilizzi et al. 2022). An ad hoc threshold (as per Britton et al. 2011) was also used to distinguish “very high risk” species within those classified as high risk for the two groups of organisms.

The *a priori* categorization of species required for ROC curve analysis was implemented following the protocol by Vilizzi et al. (2022). Fitting of the ROC curve was performed with pROC (Robin et al. 2011) for R x64 v4.3.2 (R Development Core Team 2023). Permutational ANOVA with normalization of the data was used to test for differences in the confidence factor (Vilizzi et al. 2022) between the BRA and BRA+CCA using a Bray–Curtis dissimilarity measure, 9,999 unrestricted permutations of the raw data, with statistical effects evaluated at $\alpha = 0.05$. Following identification of the threshold score, evaluation of the risk classifications to identify false-positive and false-negative rankings was not applied to medium-risk species because their further evaluation in a comprehensive risk assessment depends on policy and management priorities and on the availability of financial resources.

Results

Species occurrence and distribution

In all data combined (general occurrence data and the urban monitoring data), we collected 5,134 non-native species observations with exact dates and locations during the period between 1990 and 2024. In total, five non-native amphibian and 16 non-native aquatic reptile taxa (we treat the pond slider subspecies, the red-eared slider *Trachemys scripta elegans*; the yellow-bellied slider *T. s. scripta*; and the Cumberland slider *T. s. troosti*, separately due to their numbers and importance) were recorded in the PBR (Fig. 2, Table 2, Suppl. materials 3, 4, 5). All 5,134 observations with exact locations are displayed in Fig. 2, and a combined heat map is provided in Fig. 3. For more details on the species, particular observations, and habitat types see Table 2. For a full list of 5,134 observations see Suppl. materials 4, 5.

Amphibians

The most abundant species was the African clawed frog *Xenopus laevis*, with 59 observed individuals from 11 water bodies in the region of Budapest. The second most abundant species was the western dwarf clawed frog *Hymenochirus curtipes*, with 33

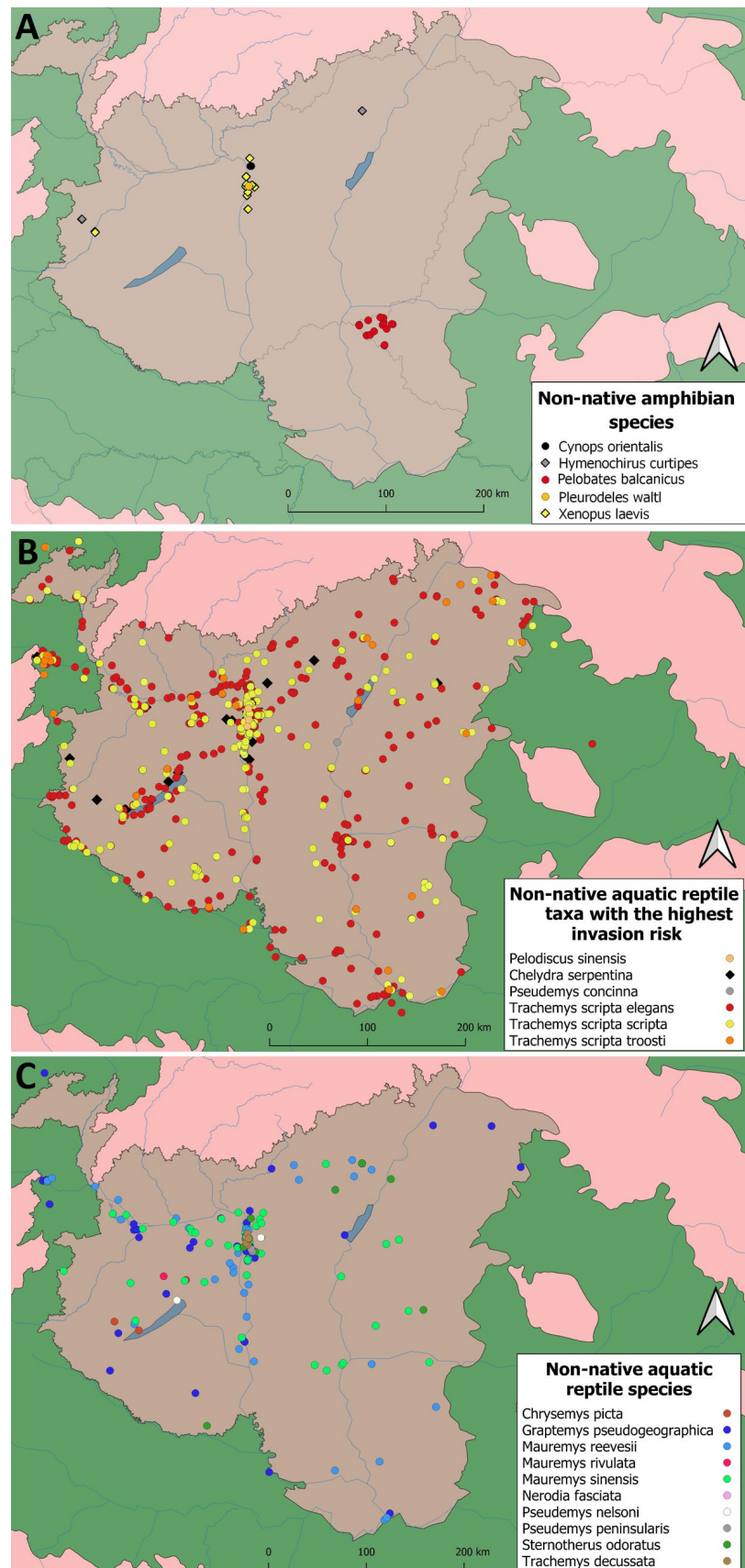


Figure 2. Distribution of non-native amphibians and aquatic reptiles in the Pannonian Biogeographical Region (1990–2024). **A.** Amphibians; **B.** the aquatic reptiles falling into the “very high risk” category in our risk assessment; **C.** the rest of aquatic reptiles. For a list of the water bodies (Suppl. material 4).

Table 2. Observations on the occurrence of non-native amphibians and aquatic reptiles in the Pannonian Biogeographical Region (Fig. 2). I = non-governmental organizations databases; II = expert opinion (GO database); III = citizen science; IV = literature data; V = field observations; VI = monitoring program. Habitat types: 1 = artificial standing water (thermal ponds, artificial ponds, urban lakes, mine lakes, water reservoirs); 2 = artificial running waters (rainwater ditches, channels); 3 = natural standing waters (ponds, lakes, oxbows); 4 = side arms and bays of (quasi)natural running waters; 5 = main arms of (quasi)natural running waters (streams, rivers); 6 = main arm of well-regulated running waters (urbanized sections of streams, rivers); 7 = wetlands.

Species name	I	II	III	IV	V	VI	First record	Localities / (quasi) natural localities	Stage	Habitat type	Literature data of first observation
Amphibians											
<i>Cynops orientalis</i>	N	N	N	N	N	Y	2017 – Göd (Hungary)	3 / 1	adults	1, 2	Szajbert and Weiperth 2022b
<i>Hymenochirus curtipes</i>	N	N	N	N	Y	Y	2015 – Budapest (Hungary)	6 / 1	juveniles, adults	1, 2, 6	Weiperth et al. 2015
<i>Pelobates balcanicus</i>	Y	N	N	Y	Y	N	2015 – Comloșu Mare, Sănnicolau Mare, Saravale, Vălceni (Romania)	15 / 5	tadpoles, adults	1, 2, 3, 7	Teran et al. 2017
<i>Pleurodeles waltl</i>	N	N	N	N	N	Y	2022 – Budapest (Hungary)	1 / 0	larvae, adults	1	Weiperth and Szajbert 2022
<i>Xenopus laevis</i>	N	N	N	N	Y	Y	2016 – Budapest (Hungary)	11 / 6	eggs, adults	1, 4	Szajbert and Weiperth 2022c
Aquatic reptiles											
<i>Chelydra serpentina</i>	Y	Y	Y	Y	Y	Y	2007 – Debrecen, Budapest (Hungary)	30 / 23	juveniles, adults	1, 2, 3, 4, 5, 7	Babocsay 2022a
<i>Chrysemys picta</i>	N	N	N	N	Y	Y	2017 – Budapest (Hungary)	6 / 4	adults	1, 3, 4, 7	-
<i>Graptemys pseudogeographica</i>	Y	Y	Y	Y	Y	Y	2009 – Vienna (Austria)	54 / 13	juveniles, adults	1, 2, 3, 4, 5, 6, 7	Kleewein and Wöss 2009
<i>Mauremys reevesii</i>	N	N	N	N	Y	Y	2017 – Tata and Budapest (Hungary)	45 / 9	juveniles, adults	1, 2, 3, 4, 6	-
<i>Mauremys rivulata</i>	Y	N	N	N	N	N	2012 – Várpalota (Hungary)	2 / 0	adults	1	-
<i>Mauremys sinensis</i>	Y	Y	N	N	Y	Y	2014 – Budapest (Hungary)	59 / 2	juveniles, adults	1	Haraszthy 2022
<i>Nerodia fasciata</i>	N	Y	N	N	N	N	2016 – Budapest (Hungary)	1 / 0	adults	1	-
<i>Pelodiscus sinensis</i>	Y	Y	Y	Y	Y	Y	2007 – River Danube (Hungary)	22 / 19	adults	1, 3, 4, 5, 6	Urošević et al. 2018
<i>Pseudemys concinna</i>	Y	N	Y	Y	Y	Y	2015 – Budapest (Hungary)	7 / 3	adults	1, 4	Kleewein and Wöss 2009
<i>Pseudemys nelsoni</i>	Y	Y	N	N	N	N	2016 – Budapest (Hungary)	2 / 2	adults	1, 6	-
<i>Pseudemys peninsularis</i>	Y	N	N	N	N	Y	2014 – Budapest (Hungary)	5 / 2	adults	1, 4	-
<i>Sternotherus odoratus</i>	N	N	Y	N	Y	Y	2016 – Budapest (Hungary)	12 / 4	juveniles, adults	1, 2, 6	-
<i>Trachemys decussata</i>	Y	N	N	N	N	Y	2017 – Budapest (Hungary)	4 / 2	adults	1, 4	Haraszthy 2022
<i>Trachemys scripta elegans</i>	Y	Y	Y	Y	Y	Y	No exact data	893 / 351	juveniles, adults	1, 2, 3, 4, 5, 6, 7	Kovács et al. 2004; Puky et al. 2004
<i>Trachemys scripta scripta</i>	Y	Y	Y	Y	Y	Y	2009 – Vienna (Austria)	480 / 126	juveniles, adults	1, 2, 3, 4, 5, 6, 7	Kleewein and Wöss 2009
<i>Trachemys scripta troosti</i>	Y	Y	Y	Y	Y	Y	2009 – Vienna (Austria)	37 / 22	adults	1, 2, 3, 4, 5, 6, 7	Kleewein and Wöss 2009

observed individuals from seven locations in Hungary. The third most abundant species was the Chinese fire-bellied newt *Cynops orientalis*, with 16 observed individuals from three locations. The least abundant species was the Iberian ribbed newt *Pleurodeles waltl*, with 10 observed individuals from one location. We found three non-native species in (quasi)natural habitats: *C. orientalis* (Ilka stream), *H. curtipes* (Rákos stream), and *X. laevis* (Barát stream, Gombás stream, Hosszúrét stream, Rákos stream, Ráckevei-Soroksári-Danube, and Lágymányosi Bay) (Figs 2A, 4, Suppl. material 5). In addition to the above pet-trade species, 77 living (adult and tadpole) and road-killed individuals of the Balkan spadefoot toad, *Pelobates balcanicus*, were observed at 15 sites in the southern area of the PBR (Fig. 2A, Suppl. material 5). After the first observation of this species in the Romanian part of the PBR in 2015—identified as *Pel. syriacus* in earlier publications (Székely et al. 2013; Teran et al. 2017)—noting that it is native in the southeastern part of Romania outside

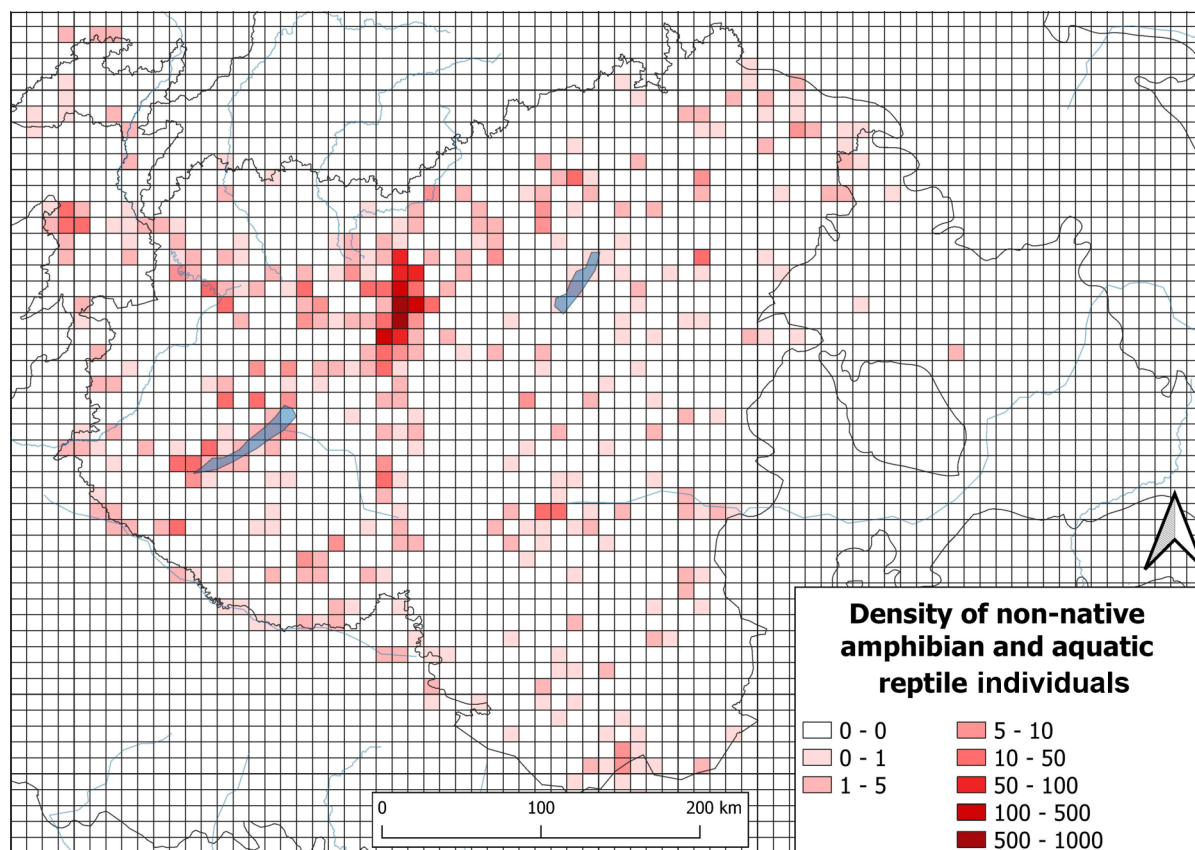


Figure 3. The density of the occurrence data of non-native amphibians and aquatic reptiles in a 10 × 10 km network in the Pannonian Biogeographical Region.

the PBR, the first individuals in the Hungarian region of the PBR in the floodplain area of the River Maros/Mureş were detected on 20 May 2023 (Suppl. material 4).

Aquatic reptiles

All recorded species were turtles, apart from a single observation of the southern water snake *Nerodia fasciata* from the thermal pond on Margaret Island (Japanese Garden) in Budapest. The most abundant were the three North American pond sliders, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti*, with 4,426 observed individuals, representing 86.21% of the total number of observed non-native amphibian and aquatic reptile species in the PBR. Occurrences of individuals belonging to different age classes were reported from 687 artificial and (quasi)natural water bodies across the PBR (Fig. 2B, Table 2, Suppl. materials 3, 4). The most abundant subspecies was *T. s. elegans*, which was detected in all PBR countries, with a total of 3,286 individuals observed. Of these individuals, 2,599 were detected in different artificial habitats. In the last two decades, 687 individuals were observed in (quasi)natural water bodies: main and side arms of rivers, streams, different lakes, and protected wetlands. We detected the breeding of *T. s. elegans* in four bays, 16 side arms of the River Danube in the Hungarian region, six wetland areas of Lake Balaton, and an increasing number of private and open garden ponds (Table 2, Suppl. materials 4, 5). A total of 2,856 observations came from the last decade, suggesting that this subspecies has started to invade the whole PBR (Fig. 2B, Table 2). In total, 1,095 individuals of *T. s. scripta* were observed in artificial and (quasi)natural habitats of

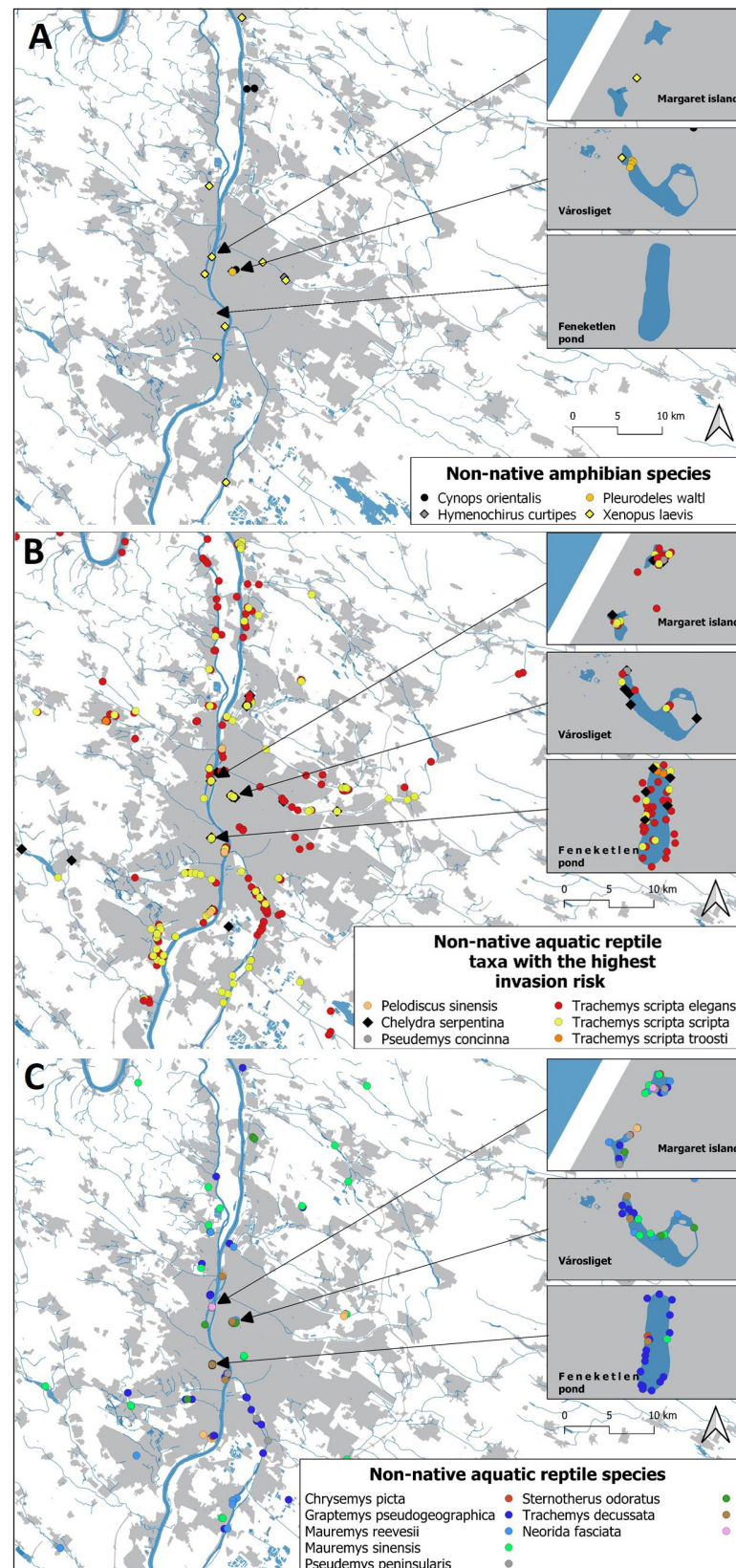


Figure 4. Distribution of non-native amphibians and aquatic reptiles in the central and agglomeration region of Budapest, capital of Hungary (1990–2024), with insets of heavily populated ponds. **A.** Amphibians; **B.** the aquatic reptiles falling into the “very high risk” category in our risk assessment; **C.** the rest of aquatic reptiles. The three insets in all graphs represent three artificial standing water bodies: thermal pond on Margaret Island (Japanese Garden), thermal ponds in Városliget, and Fenekestlen Pond, from top to bottom. For a list of the water bodies (Suppl. material 3).

all PBR countries. The 45 Cumberland slider (*T. s. troosti*) observations all come from the last 14 years, from both (quasi)natural habitats and artificial water reservoirs, such as public ponds (Fig. 2B, Table 2, Suppl. material 5).

The second most abundant species was the false map turtle, *Graptemys pseudogeographica*, with 169 observed individuals from different habitats of 54 artificial and (quasi)natural water bodies and wetlands in the PBR (Fig. 2C, Table 2, Suppl. material 5). Among the other abundant species were two Asian aquatic turtles, namely, the Chinese tree-keeled pond turtle *Mauremys reevesii*, with 83 observed individuals from 45 artificial and (quasi)natural habitats, and the Chinese stripe-necked turtle *M. sinensis*, with 76 observed individuals from two artificial thermal ponds and 59 artificial standing waters (Fig. 2C, Table 2, Suppl. materials 4, 5). Most individuals of both *Mauremys* species were observed in the central and agglomeration regions of Budapest and in 11 artificial water reservoirs in Hungary. The fifth most abundant species was the common snapping turtle *Chelydra serpentina*, with 65 individuals observed in artificial and (quasi)natural standing and running waters, mainly in the Budapest area and in the wetland zone of Lake Balaton. Forty-four individuals were collected in the Budapest area during the last 10 years (Fig. 2C, Table 2, Suppl. materials 2, 3). The remaining species were found in small numbers and occurred predominantly in separate water bodies (Fig. 2C, Table 2, Suppl. materials 4, 5). Twelve non-native species were observed in (quasi)natural habitats: *Ch. serpentina*, *Chrysemys picta*, *G. pseudogeographica*, *M. reevesii*, *Pelodiscus sinensis*, *Pseudemys concinna*, *Pseudemys peninsularis*, *Trachemys decussata*, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti*.

Long-term monitoring of a highly populated urban area

Four species of amphibians and 10 species of aquatic reptiles (all turtles) were recorded for a total of 1,138 individuals across 33 water bodies (Figs 1C, 3, Suppl. material 3). The monitoring program resulted in the first records for the PBR of four non-native amphibians (oriental fire-bellied newt *C. orientalis*, western dwarf clawed frog *H. curtipipes*, Iberian ribbed newt *P. waltil*, and African clawed frog *X. laevis*) and four non-native aquatic reptiles (i.e., eastern painted turtle *Ch. picta*, Chinese stripe-necked turtle *M. sinensis*, peninsula cooter *Ps. peninsularis*, and common musk turtle *Sternotherus odoratus*). We also documented the successful nesting and hatching of *T. s. elegans* and *T. s. scripta* in several artificial and (quasi) natural habitats and collected the first juvenile individuals of *Ch. serpentina*.

Amphibians

We observed 108 individuals in total (Fig. 4A, Suppl. material 3). The most abundant was *X. laevis*, with 56 individuals from nine different aquatic habitats in central Budapest. All individuals were adults, but in one urban public thermal pond near Lukács Bath, we found eggs that successfully hatched under laboratory conditions in May 2020. For *C. orientalis*, 16 individuals were observed, of which 10 were in the Ilka stream at Alsógöd (August 2017) and six between 2019 and 2024 from two public artificial thermal ponds (one near Lukács Bath and another at Szent István Spring in Városliget). Of the 26 individuals of *H. curtipipes*, nine were found in a large thermal pond in Városliget in February 2015, another 11 individuals in thermal ponds near Lukács Bath, three in a thermal pond on Margaret Island (Japanese Garden), and three in the thermal and industrial warm-water-polluted section of the

Rákos stream (Fig. 4A). Finally, 10 individuals, including three living metamorphs and three adult females (plus four dead individuals) of *P. waltl* were collected from the thermal pond of Városliget in April 2022 and June 2024 (Fig. 4A).

Aquatic reptiles

We observed 1,030 individuals in total (Fig. 4B, C, Suppl. material 3). The most common taxa were *T. s. elegans* and *T. s. scripta*, with 486 and 332 individuals, respectively, including many juveniles (82 and 106, respectively), which were found in all studied water bodies (Fig. 4B, Suppl. material 3). During fieldwork, we observed hibernation and successful breeding (as indicated by the presence of nests, eggs, and juveniles) for these two subspecies in five artificial ponds in public areas (Érd rainwater reservoir, Feneketlen Pond, thermal pond on Margaret Island, National Botanical Garden, and pond system in Városliget), as well as in two side arms and two bays of the River Danube (Népsziget side arm, Ráckevei-Soroksári-Danube, Hárosi Bay, and Lágymányosi Bay) (Fig. 4B). Further, we observed 80 individuals of *G. pseudogeographica* and 35 of *Ch. serpentina* (Fig. 4C, Suppl. material 3). In addition to the 51 adults of the former species, the origin of the 29 juveniles is uncertain because only 19 juvenile individuals were collected from (quasi)natural habitats (Lágymányosi Bay and Ráckevei-Soroksári-Danube) and 10 from the urban area of Budapest (Feneketlen Pond, thermal pond on Margaret Island [Japanese Garden], and thermal pond system in Városliget). These juveniles may have originated from natural reproduction or illegal stocking or may have escaped from garden ponds. We detected the co-occurrence of juvenile and adult *Ch. serpentina* in the thermal pond system in Városliget and in Feneketlen Pond in the central area of Budapest, as well as in the Oceán Channel between Dunakeszi and Budapest. Observations for the other species were sporadic (Fig. 4C).

Risk screening

The ROC curves resulted in an AUC of 0.9847 (0.8363–1.0000, 95% CI; CI = confidence interval) for the amphibians and 0.8525 (0.6983–1.0000) for the aquatic reptiles. These AUCs indicated that AS-ISK was able to discriminate with excellent power between invasive and non-invasive amphibians and aquatic reptiles for the risk assessment area. Youden's *J* provided thresholds of 32.5 for amphibians and 27 for reptiles. Accordingly, amphibians and aquatic reptiles with scores within the calibrated threshold intervals [1, 32.5] and [1, 27], respectively, were ranked as medium risk; amphibians and aquatic reptiles with scores within the intervals [32.5, 68] and [27, 68] were ranked as high risk based on the BRA, and those with scores within the intervals [32.5, 80] and [27, 80] as high risk based on the BRA+CCA.

The confidence factors (CF) used the following values. The mean CF_{Total} was 0.616 ± 0.008 SE, the mean CF_{BRA} was 0.625 ± 0.008 SE, and the mean CF_{CCA} was 0.543 ± 0.013 SE. The mean CF_{BRA} was higher than the mean CF_{CCA} ($F_{1,90}^{\#} = 29.52$, $P^{\#} < 0.001$; # = permutational value).

Amphibians

Based on the BRA outcome scores (Table 3, Fig. 5A), four (25.0%) species were ranked as high risk and 12 (75.0%) as medium risk; all three species categorized *a priori* as

Table 3. Risk ranks for the non-native amphibians and aquatic reptiles screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Pannonian Biogeographical Region. For each species, the following information is provided: *a priori* categorization of invasiveness (N = non-invasive; Y = invasive; Table 1); Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (BRA+CCA) scores with corresponding risk ranks (M = medium; H = high; VH = very high) and classification (Class: FP = false positive; TP = true positive; “–” = not applicable as medium risk: see text for details); difference (CCA) between BRA+CCA and BRA scores; confidence factor (CF) for all 55 questions of the AS-ISK (CF_{Total}), for the 49 BRA questions (CF_{BRA}), and for the six CCA questions (CF_{CCA}). Risk ranks are based on the thresholds (Thr) of 32.5 for amphibians and 27 for aquatic reptiles. Risk ranks for the BRA are computed as: M, with score within the interval [1, Thr[; H [Thr, 68]; risk ranks for the BRA+CCA as: M [1, Thr[; H [Thr, 80] (note the reverse bracket notation indicating an open interval). The VH rank is based on an ad hoc threshold of 45 for amphibians and 50 for aquatic reptiles.

BRA					BRA+CCA			CF			
Species name	<i>A priori</i>	Score	Outcome	Class	Score	Outcome	Class	difference (CCA)	CF _{Total}	CF _{BRA}	CF _{CCA}
Amphibians											
<i>Ambystoma mexicanum</i>	N	24.5	M	–	30.5	M	–	6.0	0.65	0.67	0.50
<i>Ceratophrys ornata</i>	N	12.0	M	–	24.0	M	–	12.0	0.61	0.62	0.50
<i>Cynops orientalis</i>	N	32.0	M	–	44.0	H	FP	12.0	0.57	0.58	0.50
<i>Cynops pyrrhogaster</i>	N	12.0	M	–	24.0	M	–	12.0	0.59	0.59	0.58
<i>Dendrobates auratus</i>	N	12.0	M	–	12.0	M	–	0.0	0.60	0.61	0.50
<i>Dendrobates tinctorius</i>	N	14.0	M	–	14.0	M	–	0.0	0.58	0.59	0.50
<i>Dyscophus guineti</i>	N	10.0	M	–	12.0	M	–	2.0	0.62	0.64	0.50
<i>Hymenochirus curtipes</i>	N	41.0	H	FP	53.0	VH	FP	12.0	0.72	0.72	0.75
<i>Lithobates catesbeianus</i>	Y	33.0	H	TP	45.0	VH	TP	12.0	0.74	0.73	0.75
<i>Pelobates balcanicus</i>	N	16.0	M	–	28.0	M	–	12.0	0.55	0.56	0.54
<i>Pleurodeles waltl</i>	N	32.0	M	–	44.0	H	FP	12.0	0.59	0.59	0.54
<i>Rhinella marina</i>	Y	34.0	H	TP	46.0	VH	TP	12.0	0.72	0.71	0.75
<i>Trachycephalus resinifictrix</i>	N	10.0	M	–	10.0	M	–	0.0	0.64	0.65	0.50
<i>Tylototriton shanjing</i>	N	10.0	M	–	10.0	M	–	0.0	0.61	0.62	0.50
<i>Tylototriton verrucosus</i>	N	10.0	M	–	10.0	M	–	0.0	0.60	0.61	0.50
<i>Xenopus laevis</i>	Y	45.0	VH	TP	55.0	VH	TP	10.0	0.72	0.71	0.75
Aquatic reptiles											
<i>Caiman crocodilus</i>	Y	17.0	M	–	23.0	M	–	6.0	0.60	0.62	0.50
<i>Chelodina longicollis</i>	N	25.0	M	–	37.0	H	FP	12.0	0.59	0.61	0.42
<i>Chelus fimbriata</i>	N	16.0	M	–	28.0	H	FP	12.0	0.60	0.61	0.50
<i>Chelydra serpentina</i>	Y	49.0	H	TP	61.0	VH	TP	12.0	0.67	0.67	0.63
<i>Chrysemys picta</i>	N	26.0	M	–	38.0	H	FP	12.0	0.50	0.51	0.50
<i>Clemmys guttata</i>	N	14.0	M	–	16.0	M	–	2.0	0.62	0.63	0.50
<i>Cuora flavomarginata</i>	N	14.0	M	–	18.0	M	–	4.0	0.65	0.67	0.50
<i>Emydura subglobosa</i>	N	12.0	M	–	16.0	M	–	4.0	0.62	0.63	0.50
<i>Graptemys pseudogeographica</i>	Y	35.0	H	TP	47.0	H	TP	12.0	0.63	0.65	0.54
<i>Kinosternon subrubrum</i>	N	17.0	M	–	29.0	H	FP	12.0	0.62	0.64	0.50
<i>Macrochelys temminckii</i>	Y	23.0	M	–	31.0	H	TP	8.0	0.62	0.64	0.42
<i>Malaclemys terrapin</i>	N	12.0	M	–	24.0	M	–	12.0	0.65	0.66	0.54
<i>Mauremys caspica</i>	N	31.0	H	FP	43.0	H	FP	12.0	0.68	0.68	0.67
<i>Mauremys reevesii</i>	N	24.0	M	–	34.0	H	FP	10.0	0.60	0.61	0.54
<i>Mauremys rivulata</i>	N	24.0	M	–	36.0	H	FP	12.0	0.59	0.61	0.46
<i>Mauremys sinensis</i>	N	25.0	M	–	37.0	H	FP	12.0	0.54	0.55	0.50
<i>Nerodia fasciata</i>	N	20.0	M	–	32.0	H	FP	12.0	0.56	0.57	0.50
<i>Nerodia sipedon</i>	N	31.0	H	FP	43.0	H	FP	12.0	0.54	0.54	0.50
<i>Paleosuchus palpebrosus</i>	N	14.0	M	–	16.0	M	–	2.0	0.58	0.59	0.50
<i>Paleosuchus trigonatus</i>	N	16.0	M	–	18.0	M	–	2.0	0.60	0.61	0.50
<i>Pelodiscus sinensis</i>	Y	37.0	H	TP	50.0	H	TP	12.0	0.67	0.67	0.67
<i>Pseudemys concinna</i>	N	40.0	H	FP	52.0	VH	FP	12.0	0.59	0.60	0.50
<i>Pseudemys nelsoni</i>	Y	28.0	H	TP	40.0	H	TP	12.0	0.60	0.61	0.50
<i>Pseudemys peninsularis</i>	Y	31.0	H	TP	43.0	H	TP	12.0	0.60	0.61	0.50
<i>Sternotherus odoratus</i>	N	28.0	H	FP	40.0	H	FP	12.0	0.63	0.64	0.58
<i>Thamnophis sirtalis</i>	N	21.0	M	–	33.0	H	FP	12.0	0.57	0.58	0.50
<i>Trachemys decussata</i>	N	23.0	M	–	35.0	H	FP	12.0	0.48	0.48	0.50

Species name	<i>A priori</i>	BRA			BRA+CCA			CF			
		Score	Outcome	Class	Score	Outcome	Class	difference (CCA)	CF _{Total}	CF _{BRA}	CF _{CCA}
<i>Trachemys scripta elegans</i>	Y	51.0	VH	TP	63.0	VH	TP	12.0	0.73	0.74	0.67
<i>Trachemys scripta scripta</i>	Y	51.0	VH	TP	63.0	VH	TP	12.0	0.73	0.74	0.67
<i>Trachemys scripta troosti</i>	Y	51.0	VH	TP	63.0	VH	TP	12.0	0.60	0.61	0.50

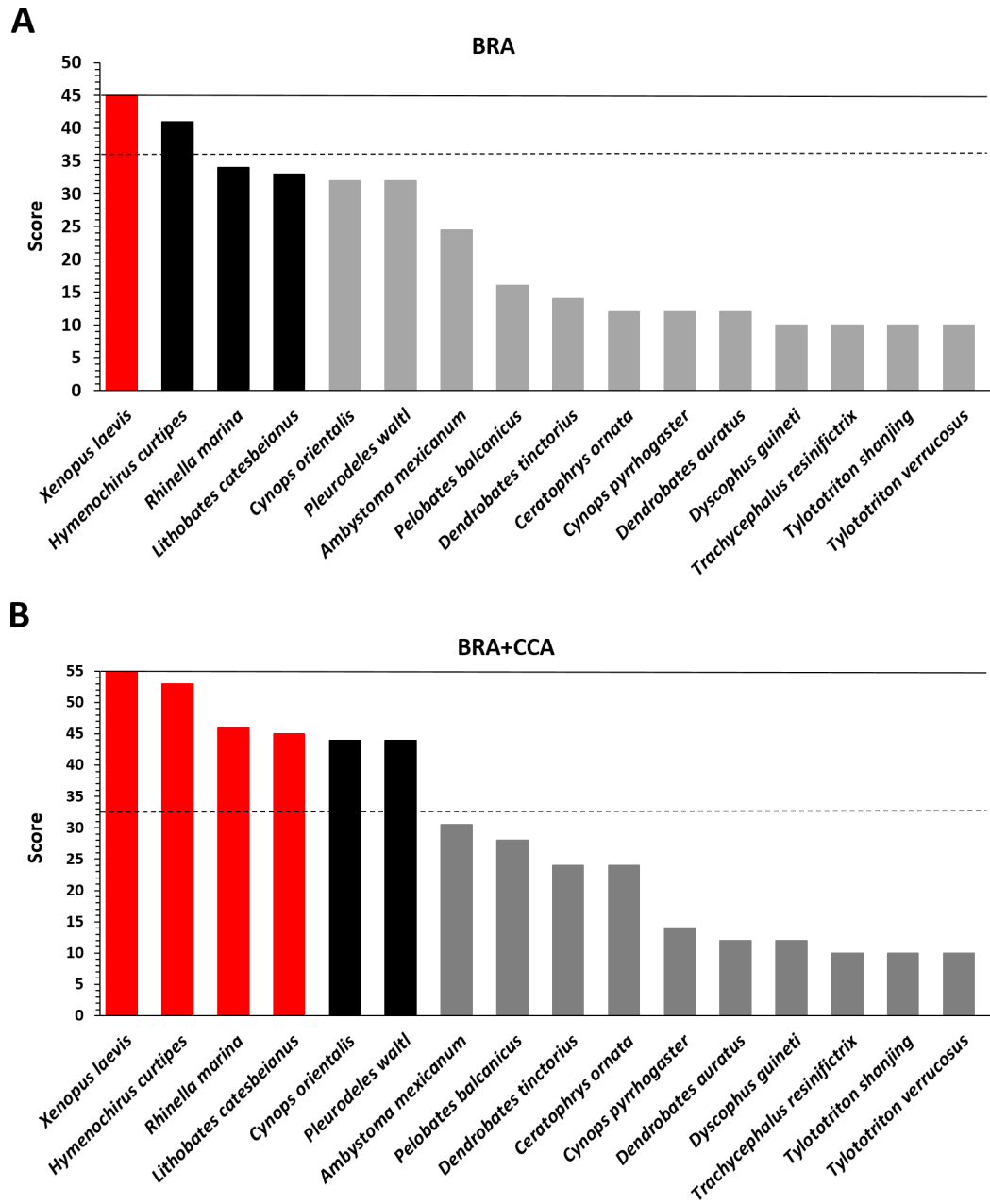


Figure 5. A. The Basic Risk Assessment (BRA) of the amphibian species screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Pannonian Biogeographical Region; **B.** The BRA+CCA (Climate Change Assessment) of the amphibian species. Numerical data is available in Table 3. The level of invasion risk: red: very high; black: high; grey: medium. The horizontal solid lines represent the ad hoc threshold for very high risk (45), and the horizontal dashed lines the threshold for high risk (25.5).

invasive were true positives (American bullfrog *Lithobates catesbeianus*, cane toad *Rhinella marina*, and *X. laevis*); of the 13 species categorized *a priori* as non-invasive, one was a false positive (*H. curtipis*); all 12 medium-risk species were *a priori* non-invasive. Based on the BRA+CCA outcome scores (Table 3, Fig. 5B), six (37.5%) species were ranked as high risk and 10 (62.5%) as medium risk; all species categorized

a priori as invasive were true positives. Of the species categorized *a priori* as non-invasive, three were false positives (*C. orientalis*, *H. curtipes*, and *P. waltl*); all 10 medium-risk species were *a priori* non-invasive.

Overall, the number and proportion of high-risk species increased from four under current climate conditions (BRA) to six under predicted climate conditions (BRA+CCA). The CCA resulted in an increase in the BRA+CCA relative to the BRA score for 11 species and in no change for five (Table 3). The very high-risk taxa (based on an ad hoc threshold of 45) were *X. laevis* for both the BRA and BRA+CCA and *H. curtipes*, *R. marina*, and *L. catesbeianus* for the BRA+CCA only (Table 3, Fig. 5B).

Aquatic reptiles

Based on the BRA outcome scores (Table 3, Fig. 6A), 12 (40.0%) taxa (the three subspecies *T. s. elegans*, *T. s. scripta*, and *T. s. troostii* were screened separately) were ranked as high risk and 18 (60.0%) as medium risk; of the 10 taxa categorized

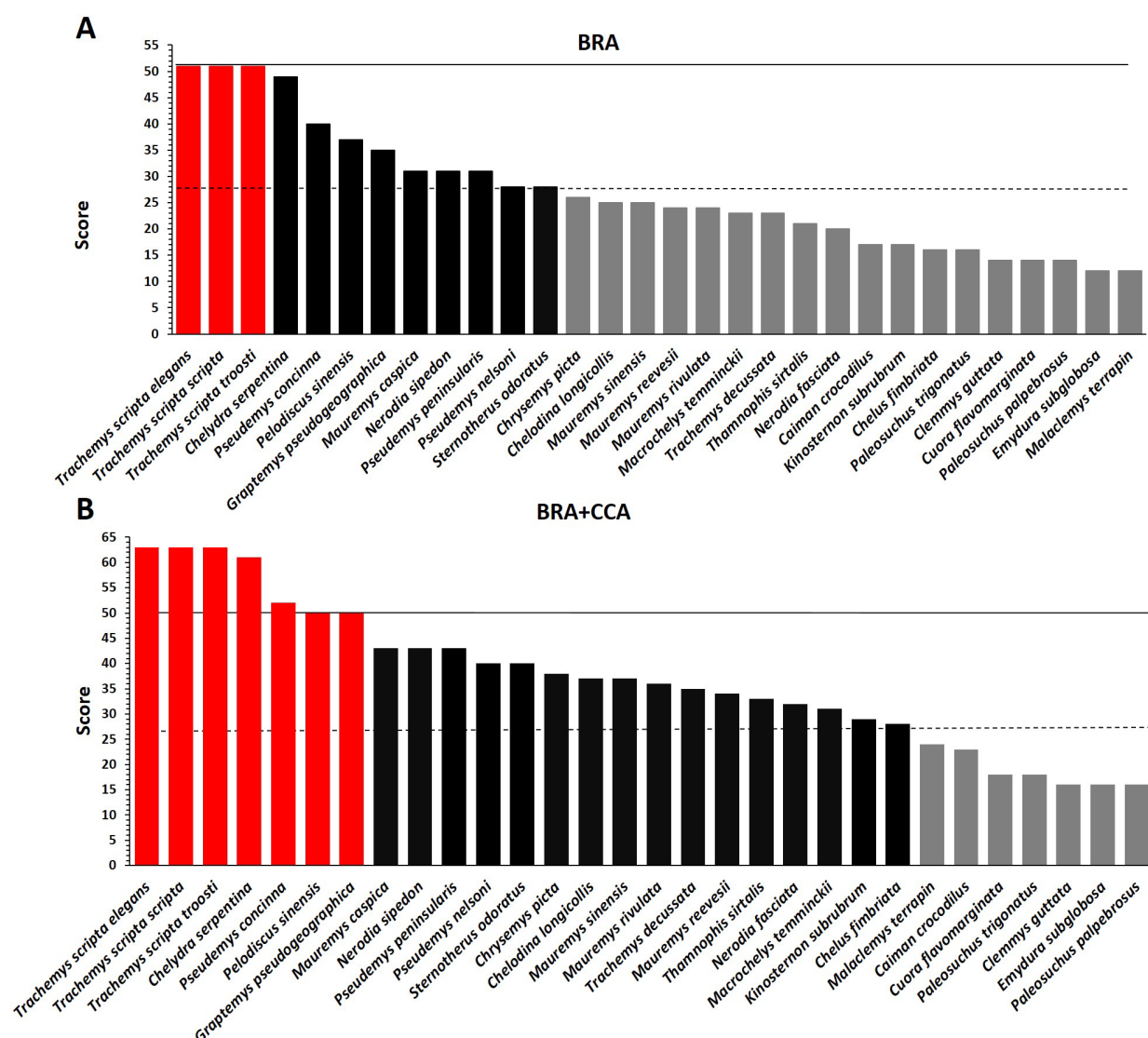


Figure 6. **A.** The Basic Risk Assessment (BRA) of the aquatic reptiles screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Pannonian Biogeographical Region; **B.** The BRA+CCA (Climate Change Assessment) of the aquatic reptiles. Numerical data is available in Table 3. The level of invasion risk: red: very high; black: high; grey: medium.

a priori as invasive, eight were true positives (*Ch. serpentina*, *G. pseudogeographica*, Chinese soft-shelled turtle *Pe. sinensis*, Florida red-bellied cooter *Ps. nelsoni*, peninsula cooter *Ps. peninsularis*, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti*); of the 20 taxa categorized *a priori* as non-invasive, four were false positives (Caspian terrapin *Mauremys caspica*, common water snake *Nerodia sipedon*, Eastern river cooter *Ps. concinna*, and common musk turtle *S. odoratus*); of the 18 medium-risk species, 16 were *a priori* non-invasive and two invasive.

Based on the BRA+CCA outcome scores (Table 3, Fig. 6B), 23 (76.7%) taxa were ranked as high risk and seven (23.2%) as medium risk; of the 10 taxa categorized *a priori* as invasive, nine were true positives (same species as for the BRA plus alligator snapping turtle *Macrochelys temminckii*); of the 20 taxa categorized *a priori* as non-invasive, 14 were false positives (same species as for the BRA plus common snake-necked turtle *Chelodina longicollis*, Amazon mata mata *Chelus fimbriata*, eastern painted turtle *Chrysemys picta*, common mud turtle *Kinosternon subrubrum*, *M. reevesii*, Balkan terrapin *Mauremys rivulata*, *M. sinensis*, southern water snake *N. fasciata*, common garter snake *Thamnophis sirtalis*, and Cuban slider *Trachemys decussata*); of the seven medium-risk species, six were *a priori* non-invasive and one invasive.

Overall, the number and proportion of high-risk taxa increased from 12 (40.0%) under current climate conditions (BRA) to 23 (76.7%) under predicted climate conditions (BRA+CCA). The CCA resulted in an increase in the BRA+CCA relative to the BRA for all 28 species (Table 3). The very high-risk taxa (based on an ad hoc threshold of 50) were *T. s. elegans*, *T. s. scripta*, and *T. s. troosti* for both the BRA and BRA+CCA and *Ch. serpentina*, *Ps. concinna*, *Pe. sinensis*, and *G. pseudogeographica* for the BRA+CCA only (Table 3, Fig. 6B).

Discussion

Biological invasions of non-native species represent the third largest problem contributing to amphibian and reptile declines, following destruction, modification, and fragmentation of habitats and (un)intentional killing (Gibbons et al. 2000). In this study, we have provided the first systematic evaluation of the non-native amphibian and aquatic reptile species for the PBR (large variety of aquatic habitats and a rich natural herpetofauna by European standards), complemented with a risk screening for potentially invasive species either found in the PBR or available from the pet trade market. Our extensive survey revealed a high number of non-native species in the PBR (five amphibians and 14 aquatic reptiles), out of which a worrying number were already observed in (quasi)natural habitats (three anurans and 11 aquatic turtles). Further, the number of non-native taxa (considering species/subspecies already observed in the PBR or available in the pet trade) posing a high risk of establishment and spreading was also high (four anurans and 12 aquatic reptiles), especially when future climate conditions were considered (six anurans and 23 aquatic reptiles).

Non-native amphibian species were observed only in a low number of (quasi) natural habitats. Further, no sign of natural reproduction was observed for human-introduced non-native amphibians. Since non-native amphibians are typically identified upon capture and are almost always immediately removed from their site of capture, overwintering cannot be proven. The observed adult, juvenile, and tadpole individuals of *Pel. balcanicus*, which dispersed into the PBR naturally, support its successful establishment in artificial (e.g., agricultural channels) and (quasi)

natural (e.g., floodplain of River Maros) habitats. Based on the occurrence data of *Pel. balcanicus*, we can expect the appearance of further amphibian and reptile species from the Balkan Region, whose range is increasingly spreading northwards as the climate warms (Dufresnes et al. 2019; Haraszthy 2022; Vilizzi et al. 2022).

The picture is somewhat darker for aquatic reptiles. Unequivocal evidence for overwintering and natural reproduction is known for *T. s. elegans* and *T. s. scripta* (Nikolić and Andjelković 2015; Koren et al. 2018; Weiperth 2022; citizen, nature conservation expert, and our own observations). Both *G. pseudogeographica* and *Ch. serpentina* can overwinter in the PBR. We observed a high number of juvenile *G. pseudogeographica* from both artificial and (quasi)natural habitats; however, we have no information on whether they originated from natural reproduction or intentional release. In *Ch. serpentina*, we are aware that collected adult female individuals laid eggs; however, we do not know whether those eggs were fertile and whether fertilization occurred in nature or before release. However, *Ch. serpentina* successfully overwintered and bred in outdoor enclosures exposed to the local climate in the PBR (Babocsay 2022a; citizen and nature conservation expert communication). We are not aware of natural reproduction in (quasi)natural habitats in any other non-native aquatic reptile species. However, *M. reevesii*, *M. sinensis*, *Pe. sinensis*, *Ps. concinna*, and *S. odoratus* were also successfully overwintered and bred in outdoor enclosures exposed to the local climatic conditions (Haraszthy 2022; Szajbert and Weiperth 2022a; citizen and nature conservation expert communication).

The situation is even more worrying in artificial habitats. Several urban aquatic and wetland habitats contain thermal water, creating favorable conditions for warm-adapted non-native species, and urban environments are in general known to be warmer than (quasi)natural habitats (cf. heat island effects, e.g., Bates et al. 2013; Deilami et al. 2018; Kuhn et al. 2025), aiding the overwintering and reproduction of non-native species for which winters in the PBR are currently too harsh. On top of this, unintentional and intentional release has a higher probability in urban environments, where most exotic pet trade and pet keeping occur (e.g., Weiperth et al. 2015, 2020; Takács et al. 2017, 2025; Iftime and Iftime 2021; Blaha et al. 2022; Haraszthy 2022). Probably as a result of the above factors, the number of occurrences, in terms of species, localities, and individuals, was high in urban areas. We note that we classified not only urban but also continuously maintained agricultural water bodies as artificial. Two non-native amphibian species (*X. laevis* and *H. curtipis*) occurred in 10 artificial habitats (the number of amphibian observations was magnitudes lower than aquatic reptile observations in general), and seven non-native aquatic turtle taxa occurred in more than 10 (*G. pseudogeographica*, *M. reevesii*, *M. sinensis*, *S. odoratus*, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti*). For amphibians, overwintering and successful breeding can be proven only for *Pel. balcanicus*, which appeared in the PBR via natural dispersal. *Xenopus laevis* egg clutches were collected from two localities, Lukács Bath (Budapest) and the thermal pond system in Városliget (Budapest), which later successfully hatched in the laboratory. We caught a female *H. curtipis* developing eggs in Szent István Spring, Városliget (Budapest), which later laid eggs in the laboratory, where the eggs hatched. We caught > 10 cm larvae of *P. waltl* in the largest thermal pond in Városliget (Budapest); however, we cannot be certain that the larvae hatched *in situ*.

Regarding aquatic reptiles, proven overwintering in artificial habitats is known for *Chr. picta*, *G. pseudogeographica*, *Pe. sinensis*, *Ps. concinna*, *Ps. nelsoni*, *T. decussata*, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti*. Further, *T. s. elegans* and *T. s. scripta* are also routinely

reproducing in artificial and (quasi)natural habitats. We found *G. pseudogeographica* juveniles with umbilical scars indicating recent hatching from three localities in Budapest: the thermal pond on Margaret Island (Japanese Garden), Feneketlen Pond, and the largest thermal pond in Városliget. Juvenile *S. odoratus* with umbilical scars were collected in two small thermal ponds near Lukács Bath (Budapest) and Békás Pond (Miskolctapolca). Based on the above detailed distribution data and potential for reproduction, we suggest that artificial, especially urban, habitats can indeed act as incubators for potential invasions in the studied taxa, the prime examples being the *T. scripta* subspecies. Similar conclusions were already reached in the case of several shrimp (Blaha et al. 2022), crayfish (Weiperth et al. 2019, 2020; Lipták et al. 2023), ant (Báthori et al. 2024), fish (Takács et al. 2017, 2025), and two gecko (Babocsay 2022b; Szajbert and Weiperth 2022d) species in the PBR countries. Numerous examples can also be found in other regions (e.g., Barroso de Magalhães and Jacobi 2013; Balzani et al. 2016; Lockwood et al. 2018; Stringham and Lockwood 2018; Nerozzi et al. 2024).

Overall, we can conclude that non-native amphibian and aquatic reptile species occur at the highest diversity and density in artificial habitats, such as urban ponds, lakes, and heat-polluted running and standing waters. This is particularly true for the four capitals (from west to east: Vienna, Bratislava, Budapest, and Belgrade) in the PBR and some popular lake and riverside areas (e.g., Lake Balaton, Ráckevei-Soroksári-Danube, Kisköre water reservoir, several side arms of running waters, and different angling ponds and lakes). Further, based on the dataset of the 10-year monitoring program focusing only on the Budapest area, we find that the resulting 1,138 observations represent 22.16% of the observation data detailed in our study. This is a high percentage, especially considering the regional proportions, because the area of our survey represents only 2.66% of the PBR. Considering amphibians only, we find that 58.38% of the observations come from the monitored urban area. A positive bias in observation probability toward the Budapest area is realistic, since focused monitoring efforts are expected to retrieve more observations than the collection of random observations. However, note that our data from expert interviews are also often based on regular monitoring. In the case of the most abundant taxa (*T. s. elegans*, *T. s. scripta*), their proportion of the total observations was 71.88% in the monitoring program and 86.2% in urban and artificial habitats. Even if the differences between the Budapest area and the whole PBR are numerically smaller than reported here, the higher non-native species diversity and higher local densities in the urbanized environment are evident (Fig. 3, Suppl. material 5) (Haraszthy 2022; Weiperth 2022).

Based on our AS-ISK screenings of amphibians, *X. laevis* poses a very high risk of invasion at present. When factoring in climate change, *H. curtipes*, *L. catesbeianus*, and *R. marina* are expected to pose a very high risk in the near future. *Xenopus laevis* and *H. curtipes* are both present in the PBR despite the fact that both are subtropical/tropical species. *Lithobates catesbeianus* and *R. marina* have no occurrence data from the PBR to date. However, the climate of the PBR is less than optimal for *L. catesbeianus*, similarly to *R. marina*, which are tropical/subtropical species. The risk of invasion by both amphibian species may be high because climate change has caused gradual warming of the whole area of the PBR (e.g., Pieczka et al. 2011; Bartholy et al. 2012; Kopecký et al. 2016; Simon et al. 2023), and the impact of thermal water habitats as invasion hotspots must also be taken into account (e.g., Blaha et al. 2022; Haraszthy 2022; Takács et al. 2025). In addition to very high-risk species, *C. orientalis* should also be mentioned, because it can be a vector of the emerging pathogen *Batrachochytrium salamandrivorans* (Yuan et al. 2018;

Fisher and Garner 2020). Regarding aquatic reptiles, *T. s. elegans*, *T. s. scripta*, and *T. s. troosti* pose a very high risk of invasion at present, all being present in the PBR. *Ch. serpentina*, *Pe. sinensis*, and *Ps. concinna* are expected to pose a very high risk in the near future considering the effects of climate change and are also already present in the PBR. The AS-ISK results and our field data are largely concordant. Taking our results together, the most threatening amphibians are *X. laevis* and *H. curtipes*, while the most threatening aquatic reptiles are *T. s. elegans*, *T. s. scripta*, *T. s. troosti*, *Ch. serpentina*, *G. pseudogeographica*, *Ps. concinna*, and *Pe. sinensis*. *Trachemys s. elegans* and *T. s. scripta* can already be considered invasive in the (quasi)natural habitats of the PBR, where successful adaptation might speed up their future dispersal. Since nesting, successful hatching of eggs, and natural dispersal of young individuals have been observed in numerous habitats based on all five data sources, we can conclude that these subspecies have successfully established themselves in most of the territory of the PBR (Table 2, Suppl. material 3). At the same time, due to illegal releases, stocking, and escapes, new populations can be expected to develop continuously (Reshetnikov et al. 2023). The other aquatic reptile species have not yet established breeding populations in nature. Many of the observed non-native amphibian and aquatic reptile species are present in thermally favorable urban habitats, which can serve as incubators for future invasions. These species hold great invasive potential, especially in light of ongoing climate change. The above-listed non-native species should be priority targets for conservation planning in the PBR, but the numerous other species posing high risk (especially considering climate change) and already detected in urban or other artificial habitats, albeit in small numbers, should also be monitored so that they do not spread unnoticed. We discuss the non-native species with the highest potential impact in detail in the Suppl. material 6.

Conclusion

Successful conservation management of invasive species needs at least two types of data: the distribution of non-native species in the risk assessment area and a scientifically sound invasion risk assessment of the non-native species that are likely to get out of captivity or have already successfully done so. Our results reinforce that AS-ISK is a precise tool, and thus its predictions about future risk, factoring climate change into the estimation, should be taken seriously. Nevertheless, a sound understanding of both target species and climate change underpins the value of risk assessments. Without pertinent data input, it is not possible to determine the exact risks. We recommend implementing our framework (combining extensively collected field data and risk screening analysis) as a necessary first step in assessing the depth of the problem caused by non-native species in other European biogeographical regions as well. Similar risk screening toolkits to what we used here (AS-ISK) are now available for terrestrial animal taxa (TAS-ISK; Vilizzi et al. 2022) and terrestrial plant species (TPS-ISK; Vilizzi et al. 2024), so the framework is not restricted anymore. The large number of non-native amphibians and aquatic reptiles observed in the PBR underlines the permanent risk of new invasions. We suggest that artificial, and particularly urban, environments are indeed potential incubators of non-native species invasions, and thus these environments need continuous high-intensity monitoring and immediate removal of non-native species. Additionally, we emphasize that the PBR is Europe's richest region in artificial and natural thermal springs and therefore has the highest number of potential invasion hotspots

on the continent. Not only our results, but numerous previous studies (e.g., Puky et al. 2005; Bódis et al. 2012; Takács et al. 2017, 2025; Weiperth et al. 2019, 2020; Mozsár et al. 2021; Blaha et al. 2022; Haraszthy 2022) have confirmed that these habitats at different scales function as invasion incubators for many tropical and subtropical taxa. Seeing how (un)intentional pet-trade-related releases can result in the presence of a diverse non-native fauna in the PBR, we argue that public awareness campaigns about the problem are of crucial importance in saving not only the local native herpetofauna but the whole (quasi)natural ecosystem as well.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers). Amphibian and aquatic reptile samplings have been authorized by the Minister of Agriculture and Government Office of Pest County, Permit no.: HHgF/561-1/2016, HHgF/561-1-2018, 1-HAGF/25/2022, HGF/17/2024, and PE/KTFO/1401-11/2022.

Use of AI

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Author contributions

BP-Sz, AW, and GH planned the study. BP-Sz and AW collected the samples and built the database. AW ran the AS-ISK analyses. ZsMB built and managed the GIS database. All authors contributed to the writing of the manuscript.

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Data availability

All data will be uploaded to online repositories upon acceptance.

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Supplementary material 1

Native amphibians and aquatic reptiles of the Pannonian Biogeographical Region (PBR) of Central Europe

Authors: Bettina Pintér-Szajbert, András Weiperth, Zsombor Márk Bányai, Gábor Herczeg

Data type: docx

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Link: <https://doi.org/10.3897/neobiota.107.175797.suppl1>

Supplementary material 2

Native amphibians and aquatic reptiles (common names in Table S1) of the PBR by country

Authors: Bettina Pintér-Szajbert, András Weiperth, Zsombor Márk Bányai, Gábor Herczeg

Data type: docx

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Supplementary material 3

Occurrence data of non-native amphibians and aquatic reptiles

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Data type: docx

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Supplementary material 4

The dataset of the non-native amphibians and aquatic reptiles in the Pannonian Biogeographical Region (PBR) of Central Europe

Authors: Bettina Pintér-Szajbert, András Weiperth, Zsombor Márk Bányai, Gábor Herczeg

Data type: xlsx

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Supplementary material 5

Distribution map of each non-native amphibian and aquatic reptile in the Pannonian Biogeographical Region

Authors: Bettina Pintér-Szajbert, András Weiperth, Zsombor Márk Bányai, Gábor Herczeg

Data type: pdf

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Supplementary material 6

Short description of non-native amphibian and aquatic reptile species at the highest invasion risk in the Pannonian Biogeographical Region

Authors: Bettina Pintér-Szajbert, András Weiperth, Zsombor Márk Bányai, Gábor Herczeg

Data type: docx

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