



A survey and risk screening of non-native ant species colonising greenhouses in Hungary

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Received: 20 July 2023 / Accepted: 4 December 2023
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Abstract Invasive species represent a severe threat for ecosystem health worldwide. With increasing global trade and ongoing climate change, monitoring non-native species and their hotspots of potential spread is becoming increasingly important. Invasive ants are one of the most problematic groups of organisms costing billions of dollars a year globally to control. Therefore, emerging ant invasions require more focused engagement to assess their extent, and effective measures to prevent the spread of non-native invasive species can be time consuming and expensive to implement. In addition to places with high commercial traffic, greenhouses are potential hotspots for non-native species as they can be entry points for invasions. However, the role of greenhouses in

ant invasions is still understudied. In this study, an extensive survey of greenhouses in zoos and botanical gardens of Hungary was conducted to search for non-native ant species. The five species found in the surveys and an additional two known from Hungary were then screened for their risk of invasiveness in the country under both current and predicted climate conditions. Three species were found to pose a considerable conservation and economic risk for Hungary, and one is already present in the wild. Increased monitoring of greenhouses and other heated premises for the presence of non-native species and targeted chemical eradication are needed to prevent their spread. The risk screening method employed in this study can be applied to a wide range of terrestrial animal taxa, thereby providing a basis for developing more effective prevention and control strategies against invasive species.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10530-023-03227-9>.

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Keywords Biological invasions · Introduced species · Terrestrial species invasiveness screening kit (TAS-ISK) · Tramp species

Introduction

The accelerated and increased movement of people and goods between distant locations as a result of globalisation has contributed to the rapid spread of non-native species (Meyerson and Mooney 2007; Westphal et al. 2008; Hulme 2009, 2021; Perrings et al. 2010). By overcoming natural barriers, organisms can move thousands of kilometers from their natural range (Carey 2002; Senok et al. 2012; Guo et al. 2013; Turbelin et al. 2017). However, for most of these ‘stowaways’, their new environment is usually unsuitable for permanent establishment because of different climatic and environmental factors from their native range (Vilà et al. 2008; Tinsley et al. 2015; Tartally et al. 2016). On the other hand, those non-native species that do find suitable conditions in the new environment may eventually be able to establish viable populations. In the absence of effective natural enemies, these species may then become invasive and cause substantial damage to both natural and urbanised environments (Pimentel et al. 2005; Reaser et al. 2007; Keller et al. 2011).

The high diversity of non-native species is particularly marked in botanical and zoological gardens and collections, which often provide specific habitat conditions such as non-natural temperature and humidity ranges (Wang et al. 2015; Roy et al. 2020). These artificial enclosures have been shown in several cases to host inadvertently introduced non-native organisms that might become invasive (Hänggi and Straub 2016; Oszust et al. 2020). These include arthropods (Wang et al. 2015; Blatrix et al. 2018; Wong et al. 2023), molluscs (Yeung et al. 2019) and vertebrates (Heinicke et al. 2011). Zoological and botanical gardens with greenhouses usually expand their stock with species from exotic countries. Such expansions typically import copious amounts of soil and wood with the newly acquired plants and can therefore function as potential hiding places for several small-bodied organisms including ants, which may subsequently establish permanent colonies in buildings (Blatrix et al. 2018). Ants can disturb or harm the animal and plant species living in zoos and botanical gardens

and can also contaminate their food thereby causing financial damage (Kenis and Branco 2010). Even worse, artificial enclosures may provide long-term refuges for potentially invasive ant species until the environment becomes suitable for their establishment in the wild (Blatrix et al. 2018).

The most difficult invasive species to control are those that are hard to detect because of their size or life-history traits. These species can move into new areas while remaining undetected (Stohlgren and Schnase 2006; Mehta et al. 2007). Among these small organisms, the Global Invasive Species Database lists 19 invasive ant species (GISD 2023). Because of their high adaptability and dominance in ecosystems due to their social lifestyle, ants stand as a priority in invasive species monitoring protocols (Ujiyama and Tsuji 2018; Vanderwoude et al. 2021; Wong et al. 2023). Despite representing only $\approx 0.68\%$ of the currently known ≈ 1.49 million animal species (Bánki et al. 2023), five of the 100 most problematic invasive species are ants (Global Invasive Species Database 2023). These can cause severe ecological damage by suppressing native species and are also harmful as secondary agricultural pests in urbanised habitats, costing millions of dollars to prevent and manage worldwide (O’Dowd et al. 2003; Gutrich et al. 2007).

In Europe, nine invasive ant species are present in the wild (Janicki et al. 2016; Guénard et al. 2017). However, far more ant species with limited invasion potential can be found in various artificial habitats, such as heated buildings in urbanised areas (Boer and Vierbergen 2008; Blatrix et al. 2018). In Hungary, there are two invasive ant species with outdoor occurrence: the invasive garden ant *Lasius neglectus*, which has been recorded several times over the last three decades (Nagy et al. 2009; Tartally and Báthori 2015), and the recently found *Tapinoma magnum*, which is only known from the urban habitats of Paks (Hunor Dávid Sebestyén, unpublished data). Additionally, five ant species of non-native origin (i.e. *Hypoponera punctatissima*, pharaoh ant *Monomorium pharaonis*, ghost ant *Tapinoma melanocephalum*, Guinea ant *Tetramorium bicarinatum* and *Tetramorium insolens*) have been reported from different heated premises (Csősz et al. 2021). Yet, the non-native ant species detected so far in Hungary are based on relatively old data (see Csősz et al. 2021), and no extensive survey of local greenhouses has ever been conducted.

This study focused on non-native ants in major greenhouses across Hungary to assess what species are present and what threats they are likely pose to the environment—noting that ants’ invasion potential differs between species and can change over time. Thus, a species with minimal invasion potential can be highly invasive after a relatively short time due to post-introduction evolutionary changes (Suarez and Tsutsui 2008). To estimate more accurately this risk of invasiveness, a risk screen of Hungarian non-native ant species was also conducted using a recently released decision-support tool.

Materials and methods

Survey of greenhouses

The greenhouses of eleven institutions across Hungary were surveyed for the presence of non-native ant species. Surveys were carried out between 11 October 2022 and 24 May 2023. The primary method used was hand collecting during which potential habitats in greenhouses were scanned, such as the undersides of decoys and tree trunks, stems and leaves of living plants, though monitoring of bare ground surfaces was also used (Fig. 1). The collected specimens were stored in 99% absolute ethanol for later preparation and identification (pinned specimens are stored



Fig. 1 The greenhouses surveyed across Hungary for the presence of non-native ant species provide diverse climatic conditions and microhabitats for non-native organisms. **a, b** Some greenhouses are inspired by tropical habitats, typically with high humidity, providing a diverse habitat with planted plants

that contributes to a levelling vegetation. **c, d** Other greenhouses are more disturbed, have more open ground surfaces and sandy or forest soils as opposed to the peaty loamy soils typical of tropical greenhouses. Figure created using the free PhotoScape v3.7 software

in the personal collection of FB at Eötvös Loránd University, Hungary). In the laboratory, the specimens were taxonomically identified by morphological examination using a range of identification keys (Seifert 2002, 2013, 2018; Bolton 2007; Lapolla et al. 2011; Wagner et al. 2017). The following eleven institutions were surveyed: Botanical Garden Szeged (Szeged), Budapest Zoo and Botanical Garden (Budapest), Debrecen University Botanical Garden (Debrecen), Debrecen Zoo (Debrecen), ELTE Botanical Garden (Budapest), Pécs Zoo (Pécs), Sóstó Zoo (Nyíregyháza), Szeged Zoo (Szeged), Tuzson János Botanical Garden (Nyíregyháza), University of Pécs Botanical Garden (Pécs), Vácrátót Botanical Garden (Vácrátót). The institutions were classified into two types: botanical gardens (BG) and zoos (Z). Notably, the Budapest Zoo and Botanical Garden was classified as a zoo based on its main activities. The institutions surveyed are fairly representative of those across the country as a whole. Two large institutions were not surveyed, namely the Xantus János Zoo in Győr, whose management could not be approached about the surveys, and the Miskolc Zoo, which did not have a heated greenhouse all year round. Other institutions in Hungary are rather small, and most of them do not have year-round heated greenhouses, so they were not included in the survey.

Differences in number of ant species found in botanical gardens and zoos were tested by permutational (univariate) analysis of variance (Anderson et al. 2001; Anderson and Robinson 2001). This was based on a one-way design including factor ‘Institution’ with two levels (BG and Z). Analysis was implemented in PERMANOVA+ for PRIMER v7 (Clarke and Gorley 2015) after normalisation of the data, using a Euclidean distance, 9999 Monte-Carlo permutations of the raw values (because of the low sample sizes), and with the level of significance at $\alpha=0.05$. The advantage of using a permutational approach relative to a parametric one is that the stringent assumptions of normality, homoscedasticity and ‘representative’ sample sizes are overcome (Anderson et al. 2001).

Risk screening

The recently developed Terrestrial Animal Species Invasiveness Screening Kit (TAS-ISK) was employed for the screening component of this study (Vilizzi

et al. 2022a: www.cefas.co.uk/nns/tools), with Hungary as the risk assessment area. A pre-release v2.4 of the toolkit with enhancements to the questionnaire, which is available as part of the report provided in the Supplementary Material, was used to conduct the screenings. The TAS-ISK is an electronic decision-support tool consisting of 55 questions that attempt to predict the risk of a species’ introduction, establishment, spread and impact based on its biogeographical/historical and biological/ecological traits under both current (first 49 questions comprising the Basic Risk Assessment: BRA) and predicted climate conditions (last six questions comprising the BRA + Climate Change Assessment: BRA + CCA). A detailed description of the methodology for evaluating the results obtained and of the toolkit operation is given in Vilizzi et al. (2022a, b). Briefly, to achieve a valid screening, the assessor must provide for each question a response, a confidence level and a justification, with two resulting score outcomes (BRA and BRA + CCA). Scores < 1 suggest a ‘low risk’ of the species being or becoming invasive in the risk assessment area, whereas scores ≥ 1 indicate a ‘medium risk’ or a ‘high risk’. Distinction between medium and high risk is defined using a calibrated threshold (Vilizzi et al. 2022b). However, given the recent release of the TAS-ISK and the current lack of any calibration, evaluation of the BRA and BRA + CCA risk scores in this study was based on a qualitative evaluation (cf. Castellanos-Galindo et al. 2018; Suresh et al. 2019).

Species selection

In total, seven ant species non-native to Hungary were included in the screening (Table 1). These comprised the five species found during the survey (see *Results*) and another two, namely *M. pharaonis* and *T. magnum*, which were found in 2022 in Paks (Hunor Dávid Sebestyén, pers. obs.). As a result, all currently occurring non-native ant species in Hungary, both indoors and in the wild, were screened for their risk of invasiveness. Notably, two previously detected non-native *Tetramorium* species (i.e. *T. bicarinatum* and *T. insolens*) that used to occur in the Budapest Zoo and Botanical Garden (Mocsáry 1897) were no longer present at the time of this study, probably because a heating system was not in operation for one winter due to a rebuilding project. Additionally, the presence

Table 1 Scoring output for the non-native ant species evaluated with the Terrestrial Species Invasiveness Screening Kit for Hungary (see Supplementary Table 1)

Section/category	In (RS)					Out (AD)	
	<i>Cardiocondyla obscurior</i>	<i>Hypoconera ergatandria</i>	<i>Lasius neglectus</i>	<i>Nylanderia vividula</i>	<i>Technomyrmex vitiensis</i>	<i>Monomorium pharaonis</i>	<i>Tapinoma magnum</i>
<i>Biogeography/Historical</i>	4.0	13.0	21.0	7.5	7.0	18.0	17.0
Domestication/cultivation	2.0	2.0	2.0	2.0	2.0	2.0	0.0
Climate, distribution and introduction risk	0.0	1.0	1.0	1.0	1.0	2.0	1.0
Invasive elsewhere	2.0	10.0	18.0	4.5	4.0	14.0	16.0
<i>Biology/Ecology</i>	21.0	18.0	24.0	25.0	19.0	26.0	25.0
Undesirable (or persistence) traits	4.0	7.0	7.0	7.0	5.0	8.0	8.0
Resource exploitation	5.0	0.0	7.0	7.0	7.0	7.0	7.0
Reproduction	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dispersal mechanisms	2.0	1.0	2.0	1.0	1.0	1.0	2.0
Tolerance attributes	7.0	7.0	5.0	7.0	3.0	7.0	5.0
BRA Score	25.0	31.0	45.0	32.5	26.0	44.0	42.0
<i>Climate change</i>	2.0	10.0	12.0	12.0	4.0	12.0	12.0
BRA + CCA Score	27.0	41.0	57.0	44.5	30.0	56.0	54.0
<i>Confidence</i>							
BRA	0.66	0.67	0.71	0.63	0.60	0.68	0.62
CCA	0.54	0.50	0.50	0.42	0.33	0.46	0.50
BRA + CCA	0.65	0.65	0.69	0.61	0.57	0.66	0.60

BRA basic risk assessment, CCA climate change assessment. In = Found in greenhouses based on a recent survey (RS) of institutions in this study; Out = Found out of greenhouses based on additional data (AD) not coming from the survey

of *H. punctatissima* (Gallé et al. 1998) was probably a result of misidentification as the *Hypoconera* sp. specimens found during the surveys in this study actually belong to the *H. ergatandria*. Further, *T. melanocephalum* was found in a residential building in Budapest (Csósz et al. 2011), but biocontrol targeted on this population started right after its detection so that the presence of this species is questionable (i.e. probably eradicated). The above four species, which were previously detected but are now considered to be eradicated or with uncertain occurrence data, were therefore not included in the screening.

Results

Five non-native ant species were found during the surveys of which four new to the fauna of Hungary (*Cardiocondyla obscurior*, *H. ergatandria*, Nylander's crazy ant *Nylanderia vividula*, Fijian

white-footed ant *Technomyrmex vitiensis*) and one known from previous studies (*L. neglectus*). In addition to these non-native species, 16 native and mostly common ant species were found in the greenhouses (Table 2). *Camponotus fallax*, *Cryptopone ochracea*, *Formica cunicularia*, silky ant *Formica fusca*, central European bicolored ant *Lasius emarginatus*, *L. neglectus*, *Lasius plathythorax*, grass ant *Myrmecina graminicola*, *Plagiolepis pygmaea*, erratic ant *Tapinoma erraticum*, *T. vitiensis* and *Tetramorium sterckeii* were only found in botanical gardens, whereas *C. obscurior* and *N. vividula* were only found in zoos (Table 2). The other species (carpenter ant *Camponotus vagus*, *Dolichoderus quadripunctatus*, *Hypoconera ergatandria*, black garden *Lasius niger*, *Solenopsis fugax*, immigrant pavement ant *Tetramorium immigrans*) were found in both types of institutions. The mean number of species found in the institutions was 5.81, whereas the largest number of species found in an institution was ten (Debrecen University

Table 2 List of ant species (non-native in bold) found in greenhouses (+) across Hungary with corresponding type of facility

Species name	Botanical gardens					Zoos					
	Botanical Garden Szeged	Debrecen University Botanical Garden	ELTE Botanical Garden	Tuzson János Botanical Garden	University of Pécs Botanical Garden	Vácrátót Botanical Garden	Budapest Zoo and Botanical Garden	Debrecen Zoo	Pécs Zoo	Sóstó Zoo	Szeged Zoo
<i>Camponotus fallax</i>			+	+		+					
<i>Camponotus vagus</i>						+	+				
<i>Cardiocondyla obscurior</i>							+				
<i>Cryptopone ochracea</i>			+								
<i>Dolichoderus quadripunctatus</i>					+			+			
<i>Formica cunicularia</i>		+			+						
<i>Formica fusca</i>			+								
<i>Hypoponera ergatandria</i>	+	+	+	+		+	+	+	+	+	+
<i>Lasius brunneus</i>	+								+		+
<i>Lasius emarginatus</i>			+			+	+				
<i>Lasius neglectus</i>		+									
<i>Lasius niger</i>	+	+	+	+	+	+	+		+		
<i>Lasius plathythorax</i>		+				+					
<i>Myrmecina graminicola</i>		+		+		+					
<i>Nylanderia vividula</i>										+	
<i>Plagiolepis pygmaea</i>					+						

Table 2 (continued)

Species name	Botanical gardens					Zoos						
	Botanical Garden Szeged	Debrecen University Botanical Garden	ELTE Botanical Garden	Tuzson János Botanical Garden	University of Pécs Botanical Garden	Vácrátót Botanical Garden	Budapest Zoo and Botanical Garden	Debrecen Zoo	Pécs Zoo	Sóstó Zoo	Szeged Zoo	Total number of species
<i>Solenopsis fugax</i>	+		+		+	+	+					8
<i>Tapinoma erraticum</i>					+							1
<i>Technomyrmex vitiensis</i>		+										1
<i>Tetramorium immigrans</i>		+	+	+		+						4
<i>Tetramorium sterckei</i>	+				+	+						3
Total number of species	4	10	8	5	8	10	8	2	3	3	3	3

Botanical Garden and Vácrátót Botanical Garden) and the lowest was two (Debrecen Zoo). The mean number of species found in botanical gardens was higher than in zoos (7.5 ± 3.1 SE vs. 3.8 ± 1.7 SE; $F_{1,9}^{\#} = 6.18$, $P^{MC} = 0.034$; # = permutational value, MC = Monte-Carlo value).

Following risk screening, the species with the highest BRA scores were *L. neglectus*, *M. pharaonis* and *T. magnum* (Table 1). These three species were classified as ‘invasive elsewhere’ and scored highest in the Biology/Ecology section of the TAS-ISK questionnaire. These three species also achieved the highest possible increase (+12) for the CCA, which increased the BRA score for all species. *Cardiocondyla obscurior* had the lowest BRA score. The highest confidence value for the BRA was for *L. neglectus*, and for the CCA was for *C. obscurior*. The TAS-ISK combined report for the seven screened species is provided as Supplementary Material.

Discussion

This study is one of the first to investigate extensively the greenhouses of a country as potential habitat for non-native ants and the potential threat to the environment posed by the species found therein. During the present surveys, 16.6% of the ant fauna in Hungary (Csősz et al. 2021) was found in greenhouses, including five non-native ant species, four of which are new to the Hungarian fauna. Using the TAS-ISK decision-support tool, it can be concluded that at least three of the non-native ant species found in Hungary, namely *L. neglectus*, *M. pharaonis* and *T. magnum*, are likely to pose a high risk of invasion to native ecosystems or agricultural land under both current and predicted future climate conditions. The present results also highlight the fact that by systematically surveying greenhouses, non-native ant (and, most probably, other) species can be easily detected and that greenhouses may be habitats for potentially invasive ant species.

Institutions often thematically design their greenhouses by arranging them according to a specific biogeographical region and by shaping the microclimate inside. This is the case of e.g. Mediterranean house, tropical house and ‘cactus’ house microclimates, where ants and other species adapted to different environmental conditions can find ideal living

conditions for long-term settlement. Because of the strict regulations on animal and plant trade (Hulme 2009), these institutions often expand their collections by bartering between themselves, thereby causing, without proper care, the cross-spreading of non-native (invasive) species. In this regard, two different ways of introduction of non-native ant species can be assumed. The first is that institutions accidentally infect each other with non-native species *via* trade; the second is that new non-native ant species are occasionally introduced through the rare but still occurring collection extension from native regions. This seems to be supported by the fact that there were ‘common’ non-native species found in almost every surveyed institution (e.g. *H. ergatandria*) and also species found in only one institution (e.g. *T. vitiensis*). Among the institutions surveyed in this study, botanical gardens typically hosted more species than zoos. During the surveys, botanical gardens seemed to have more potentially suitable microhabitats for ants. Because of this and their more frequent collection expansion, these institutions were found to be more species rich. This pattern was present irrespective of the fact that pesticides are used more frequently in greenhouses due to the absence of animals sensitive to various chemicals. However, most of the non-native ant species found in the survey (i.e. *C. obscurior*, *H. ergatandria*, *N. vividula*) were from a single zoo (namely, Budapest Zoo and Botanical Garden). As pesticides are not used frequently in this institution, the survival of these non-native species (in the absence of other effective control strategies) seems assured at least in the medium term.

Based on the present screening outcomes, the most threatening species found in the risk assessment area were *L. neglectus*, *M. pharaonis* and *T. magnum*. *Lasius neglectus* is so far only known from urbanised habitats of Hungary, namely in Budapest (Tartally and Báthori 2015), Mátrafüred (AT unpublished data) and from a botanical garden in Debrecen (Tartally and Báthori 2015). A study of the latter site suggests that this species can exert serious impact on native communities (Nagy et al. 2009), as reported in other areas of Europe (Paris and Espadaler 2012). Although declines in *L. neglectus* have been observed in some non-native areas (Tartally et al. 2016), the increased BRA score from the CCA suggests that future expansion of the species in the risk assessment area is worth monitoring. *Monomorium pharaonis* is found

only in heated buildings in Hungary, although non-native outdoor populations are known in the southern Mediterranean, where climate change and heat island effect in urbanised habitats (Deilami et al. 2018) may cause further expansion and outdoor occurrence. The outdoor presence of *T. magnum* in the risk assessment area is of great concern given that it is already found in the wild in many European countries and could have severe impacts. *Tapinoma magnum* is effective in protecting agricultural pests from a variety of predators and parasitoids (Mansour et al. 2012), but it has also been shown to cause direct damage to horseradish, where the ant has caused extensive damage by licking phloem sap (see Seifert et al. 2017). This species is also capable of disturbing human outdoor activity with its large polygynous colonies (Freitag and Cherix 2019). Risk screening for *C. obscurior*, *H. ergatandria*, *N. vividula* and *T. vitiensis* resulted in lower BRA scores compared to the above three species. Despite ongoing climatic change, these species with predominantly tropical distributions are therefore likely to pose a low risk of invasiveness in Hungary.

Overall, this study has provided support to the claim that greenhouses can function as hotspots for potentially invasive non-native ant species. Despite increasingly stringent regulations, many species in the international private and corporate trade still pose a potential threat to native communities and ecosystems (Borsky et al. 2020; Beaury et al. 2021). Although the primary entry points for non-native, potentially invasive species may be mainly places with high international traffic, such as airports and ports (Chapman et al. 2017; Yu et al. 2020), institutions such as zoos and botanical gardens, which play a significant role in environmental education (Miller et al. 2004; Williams et al. 2015), should pay particular attention to the control of potentially dangerous non-native species in their collections. Nevertheless, it is observed that for many taxa, these institutions may serve as potential habitats (Wang et al. 2015) and botanical gardens still host more non-native invasive than endangered plant species requiring protection (Hulme 2015). In addition to consciously kept but problematic invasive plants, these institutions mainly provide habitat for various undesirable or institution-neutral non-native invasive arthropods. In this regard, a wide taxonomic range of invasive arthropods is known

from greenhouses, such as Araneae (Hänggi and Straub 2016), Heteroptera (Macavei et al. 2015) and Hymenoptera (Wong et al. 2023), although other taxa like Fungi (Wojewoda and Karasiński 2010) and Rotifera (Kolicka et al. 2015) are also involved. For this reason, monitoring these institutions and assessing the threats of the non-native species present therein (i.e. by using the newly available TASSISK decision-support tool) would be highly justified. Such screening studies would allow to develop institution- or region-specific protocols for control strategies, as has already been done in some cases (Reichard 2011; Heywood and Sharrock 2013; Driller and Martínez-Muñoz 2022).

Acknowledgements We are grateful to the management and staff of the institutions for allowing us to carry out the surveys: Botanical Garden Szeged (Anikó Németh), Budapest Zoo and Botanical Garden (András Benyó), Debrecen University - Botanical Garden (Dr. László Papp), Debrecen Zoo (Dr. Gergely Sándor Nagy and Antal Nagy), ELTE Botanical Garden (Papp László), Pécs Zoo (Tamás Varga), Sóstó Zoo (Zsuzsa Petró), Szeged Zoo (Anita Ambrus), Tuzson János Botanical Garden (Dr. Judit Krisztina Csabai), University of Pécs Botanical Garden (Dr. Tamás Wirth), Vácraót Botanical Garden (Dr. Vincze Zsigmond and Dr. Krisztián Halász). This study was supported by grant National Talent Programme-Nemzet Fialat Tehetségeiért Ösztöndíj (NTP-NFTÖ-22-B-0063) (FB). This project has received funding from the HUN-REN Hungarian Research Network. This research was co-financed by the National Research, Development, and innovation Fund (Hungary) under Grant No. K 147781 (On behalf of SC).

Author contributions Conceptualisation: FB, SC; Methodology: FB, SC, GH, LV, TJ, CK, MP; Formal analysis and investigation: FB, LV; Writing—original draft preparation: FB; Writing—review and editing: FB, SC, LV, GH. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by Eötvös Loránd University. The study was carried out with the support of grant National Talent Programme-Nemzet Fialat Tehetségeiért Ösztöndíj (NTP-NFTÖ-22-B-0063) (FB). This project has received funding from the HUN-REN Hungarian Research Network. This research was co-financed by the National Research, Development, and innovation Fund (Hungary) under Grant No. K 147781135795 (On behalf of SC).

Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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