## Biological Invasions, 2016, 18: 3127-3131 (accepted version)

# DOI: <u>10.1007/s10530-016-1227-x</u>

Title: Collapse of the invasive garden ant, Lasius neglectus, populations in four European countries

Authors: András Tartally<sup>1,\*</sup>, Vera Antonova<sup>2</sup>, Xavier Espadaler<sup>3</sup>, Sándor Csősz<sup>4</sup>, Wojciech Czechowski<sup>5</sup>

10 1: Department of Evolutionary Zoology and Human Biology, University of Debrecen, Egyetem Tér 1,
H-4032 Debrecen, Hungary
12

13 2: Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Y. Gagarin
14 Str., 1113 Sofia, Bulgaria
15

16 3: Ecology Unit and CREAF, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, E-08193,
17 Spain
18

4: Entomology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA
94118, U.S.A.

5: Laboratory of Social and Myrmecophilous Insects, Museum and Institute of Zoology, Polish
 Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

 25 \*: Corresponding author: Tel: +36 52 316 666 / 62349; Fax: +36 52 512 941; E-mail: tartally.andras@science.unideb.hu
 27

28 Abstract: The invasive garden ant Lasius neglectus (Hymenoptera: Formicidae) has been spreading 29 rapidly in Europe ever since the 1990s. This ant established enormous supercolonies in many 30 European cities and poses a serious threat to the local native faunas. The spread of this species has not 31 slowed down in the last decades, but in the recent years the sizes of the known L. neglectus 32 populations have generally been declining or have stagnated. For 29 supercolonies checked in four 33 countries, in 10 cases L. neglectus individuals have not been found on the former area of their 34 occurrence. On the other hand, only two supercolonies have expanded. In this paper, we summarize 35 these monitoring data collected by the personal independent, diligent monitoring activities of 36 myrmecologists on populations of the invasive garden ant in Bulgaria, Hungary, Poland and Spain. 37 The reasons for this collapse are thought to be: (1) depletion of the local resources, (2) gradation of 38 pathogens and (social)parasites, (3) climatic factors, (4) intra-population mechanisms, (5) 39 confrontation with highly competitive native species, (6) and lack of suitable nesting microhabitats. As 40 similar phenomena were observed in the cases of supercolonies of other invasive ant species, it seems 41 that they decline more generally than has been thought.

42

1 2 3

4 5

6 7

8

9

43 Keywords: polygyny, supercolony, population dynamic, pest species, declining, disappearance

## 45 Introduction

46

47 The invasive garden ant (Lasius neglectus van Loon, Boomsma et Andrásfalvy, 1990; Hymenoptera: 48 Formicidae, subgenus Lasius s.str.) is among the 19 ant species considered the most problematic by the 49 Invasive Species Specialist Group (ISSG) of the International Union for the Conservation of Nature 50 (IUCN) (Bertelsmeier et al. 2014). This polygynous and polydomous species has been described on the 51 basis of specimens from Budapest (Hungary) (van Loon et al. 1990). Its known presence there for 52 nearly two decades as an unnamed outdoor foreign species dates back to the beginning of the 1970s 53 (van Loon et al. 1990). The species probably originated in Asia Minor (Seifert 2000) and quickly revealed 54 its expansive and invasive nature through its fast spread across Europe and part of Middle Asia 55 (Espadaler et al. 2007; Espadaler and Bernal 2016). This ant is known to be transported to new sites in 56 potted plants, soil and organic materials (van Loon et al. 1990; Tartally et al. 2004).

57

58 On the newly established bridgeheads, the species expands its range mainly by budding, since in the case 59 of L. neglectus the nuptial flights have been replaced by intranidal mating (van Loon et al. 1990; Seifert 60 2000; Cremer et al. 2008). Thus, the areas of the supercolonies (i.e., huge polydomous systems) can come 61 to cover areas of several square kilometres by expansion (Espadaler et al. 2007). In invaded areas, L. 62 neglectus can outnumber native ants by a factor of 100 (Tartally 2000, 2006; Nagy et al. 2009; Paris and 63 Espadaler 2012). It constitutes a serious hazard to the local myrmecofaunas, since it is highly competitive 64 towards other ant species and effects negatively or, in the case of a few species, positively the density 65 of other arthropods (Nagy et al. 2009; Boase 2014). It both occupies most available nest microhabitats 66 and monopolizes ornamental plants, mainly trees (Czechowska and Czechowski 2003; Tartally 2006; 67 Paris and Espadaler 2012). It is also reported to infest houses in large numbers and to cause damage in 68 greenhouses, parks and gardens by protecting aphids (van Loon et al. 1990; Seifert 2000; Espadaler and 69 Rey 2001). This ant is also found in a high density within electro-mechanical devices, including 70 electrical plugs, and this can cause fire hazards by creating electrical short circuits (Rey and Espadaler 71 2005).

73 Lasius neglectus has a climatic preference which makes it the most threatening among the outdoor 74 invasive ant species for the largest part of Europe. It also has found suitable places in other temperate 75 regions (Bertelsmeier et al. 2014). The present range of L. neglectus in Eurasia extends from 36°N to 76 54°N and from 1°E to 74°E, including about 160 known localities. In Europe, the species has been 77 observed in most countries (Espadaler and Bernal 2016), and it occurs mainly in urban and suburban 78 habitats. It is known that L. neglectus can survive in areas in which mean temperatures in January are between -4.5 and -6 °C, and one may expect that mean January temperatures below -7 °C are critical 79 80 for this supposedly originally Mediterranean species (Schultz and Seifert 2005). Furthermore, the 81 proportion of climatically suitable areas for L. neglectus in Europe is predicted to increase with 82 climate change in the future (Bertelsmeier et al. 2014).

83

Until recently, most publications (for details see References) on *L. neglectus* reported its continuing invasion of more and more towns and countries in Europe and the increases in the size of the supercolonies. However, in recent years, it has been informally detected that at least some European populations/supercolonies are going through a crisis. The present contribution examines the data concerning this phenomenon with the goal of drawing the attention of myrmecologists to the importance of further, more focused research.

90

91

#### 92 Materials and Methods

93

94 Present states of 29 *L. neglectus* supercolonies in Bulgaria, Hungary, Poland and Spain were compared 95 with their known earlier sizes. For this purpose we searched for *L. neglectus* individuals and nests 96 meter by meter along the streets (as transects) within the latest known area of the supercolonies. 97 Private zones were usually not included in our work. This monitoring was done in summers of 2013-98 2015 under weather circumstances favourable for activity of the species. This work practically meant 99 turning up stones, digging the soil, and checking kerbs (see Fig. 1 of Online Resource) for nests. 90 Parallel with this, ant individuals were searched for on the roadways, pavements, hedges, bushes and 101 tree trunks. By these methods we have already surveyed these supercolonies in their earlier stages (for 102 details see Online Resource) and realised that it is easy to record this ant species these ways (Tartally 103 2006; Espadaler et al. 2007). According to the registered changes of the state of the colonies, they 104 were arranged into four categories: EXPANDED, STAGNATED, DECLINED and NOT FOUND. In 105 the case of the "not found" category we searched for L. neglectus individuals especially intensively on 106 the former known area of the supercolonies but have recorded no specimens. We do not call this 107 category "disappeared" because both disappearance and probably unlucky samplings can be in the 108 background of such negative results (see the story of the supercolony at Orom Str. in the Online 109 Resource), especially in the case of previously huge supercolonies occupying private zones.

- 110
- 111
- 112 **Results**
- 113

114 Only two (6.9%) of the 29 investigated *L. neglectus* supercolonies fitted the category EXPANDED 115 showing invasive features. Most of them (27) belonged to the DECLINED, STAGNATED or NOT 116 FOUND categories (Table 1; for details see Online Resource). Thus, as much as 93.1% of the 117 supercolonies did not show invasive features in recent years. When the frequencies of EXPANDED + 118 STAGNATED vs. DECLINED + NOT FOUND supercolonies were compared by the chi-square test, 119 the two classes were not randomly distributed (8 vs. 21;  $\chi = 5.82$ , p = 0.015). Instead, the collapsing 120 class (DECLINED + NOT FOUND) was more frequent than expected.

- 121
- 122
- 123 **Discussion**
- 124

The results show that *L. neglectus* seems to have decreased or maybe even stopped its invasiveness in most of the investigated supercolonies. Even some huge supercolonies seem to have declined and often no *L. neglectus* have been found on their former area. Such phenomena were observed in four European countries by different researchers, independently from one another.

Despite we did not have the possibility to search in private properties, the declining of all of the "DECLINED" supercolonies was clear in the public areas. Furthermore, every "NOT FOUND" supercolony was previously found in public areas. So, the problem about having no entrances to private properties do not affect our conclusion that *L. neglectus* decreased its invasiveness at most of the examined supercolonies.

135

136 The observed decline of the European populations of L. neglectus tallies with that of other populations 137 of invasive ant species in the World. (1) The collapse of the Argentine ant Linepithema humile (Mayr, 138 1868) in New Zealand was recently reported by Cooling et al. (2012). The biology of this species is 139 quite similar to that of L. neglectus (Seifert 2000; Espadaler et al. 2007) and may provide an excellent 140 basis for comparison. Observations of Argentine ants' populations showed that their mean survival 141 time is about 14 years. After that, the supercolonies scatter and ultimately disappear (Cooling et al. 142 2012). (2) In Australia, seven populations of the yellow crazy ant Anoplolepis gracilipes (Smith, 1857) 143 declined or disappeared completely without human intervention (Cooling and Hoffmann 2015), and a 144 101-hectare supercolony of this species fragmented into 10 small isolated colonies (Gerlach 2005). (3) 145 The big-headed ant *Pheidole megacephala* (Fabricius, 1793) was known as the only ant species on the 146 island of Culebrita. However, 76 years later 16 other ant species co-occurred there, and P. 147 megacephala was restricted to a small patch in the centre of the island (Torres and Snelling 1997). 148 According to another observation, the proportion of this ant increased as of the second year of site 149 rehabilitations for a period of five years, after which it came to comprise 97% of the catch, but by year 150 13 its abundance had dropped to very low levels (Majer and de Kock 1992).

151

According to these data and our recent findings, it is not rare for some supercolonies of invasive ants to decline more generally than was thought (a phenomenon well-known with regards to invasive species of other taxa, see e.g.: Simberloff and Gibbons 2004). The reasons for such population collapses may be diverse: (1) depletion of the local resources, (2) gradation of pathogens and (social)parasites, (3) climatic factors, (4) intra-population mechanisms (intra-colony social 157 fragmentation, reduced genetic heterogeneity due to isolation and inbreeding, which leads to reduced 158 adaptability to changing external conditions), (5) confrontation with highly competitive native species, 159 and (6) lack of suitable nesting microhabitats (Haines and Haines 1978; Gerlach 2005; Espadaler et al. 160 2007; Cooling et al. 2012; Cooling and Hoffmann 2015). Each factor (or group of factors) may be true 161 for an individual case. Some factors may impact the others, and this makes the individual situations 162 difficult to interpret. On the other hand, in every locality, the phase of population growth, which 163 precedes the phase of decline, may proceed differently. It depends on local conditions, such as climate, 164 management, urbanization processes, etc. (Espadaler et al. 2007). Therefore it should be emphasized 165 that it is not immediately obvious how to determine even an approximate age of given ant 166 supercolonies based simply on their sizes.

167

168 Irrespective of the underlying reasons, the reported cases of collapse of the L. neglectus supercolonies 169 in Europe explicitly show that population growth of the introduced L. neglectus supercolonies is not an 170 irreversible process. The appearance of *L. neglectus* within a native ant community is not necessarily 171 followed by persistent invasion. On the contrary, we are faced with a very dynamic system. A 172 supercolony can collapse, but some its isolated refugial fragments might survive, maintaining the 173 capacity of the population to expand again under favourable circumstances. When expanding, such 174 "sister refuge fragments" meet, and they presumably can merge again into one huge supercolony, 175 because L. neglectus workers originated from related colonies do not recognize one another as 176 intruders and the aggression-level between them is reported to be very low (Cremer et al. 2008; 177 Ugelvig et al. 2008). The general applicability of the hypothesis of the revival of the invasive L. 178 neglectus supercolonies from small "refugial" spots is worth thorough testing.

179

Finally, we consider it important to stress that the outcome of this process is not predictable on the basis of our data, and there is no reason to believe that the decline in the *L. neglectus* populations will lead to their extinction in the European cities. On the contrary, we underline the importance of better and continuous monitoring of the invasive populations, because they can be most effectively controlled only if we ensure up-to-date awareness of the changes that these populations are

185	undergoing. It will be especially important to monitor the localities where L. neglectus individuals				
186	were not found recently at the area of the former supercolonies. It would help to realise whether such				
187	supercolonies can disappear or just drastically collapse (see the story of the supercolony at Orom Str				
188	in the Online Resources). Further studies of the possible factors causing the expansion, stagnation or				
189	collapse based on adequate quantitative data and the ecological characteristics of this invasive species,				
190	could be used in order to model the populations' dynamics in more countries.				
191					
192					
193	Acknowledgements				
194					
195	AT was supported by the 'AntLab' Marie Curie Career Integration Grant within the 7th European				
196	Community Framework Programme and by a 'Bolyai János' scholarship of the Hungarian Academy				
197	of Sciences (MTA).				
198					
199					
200	References				
201					
202	Bertelsmeier C, Luque GM, Hoffmann BD, Courchamp F (2014) Worldwide ant invasions under				
203	climate change Biodiversity and Conservation 24:117-128 doi:10.1007/s10531-014-0794-3				
204	Boase C (2014) Lasius neglectus (Hymenoptera: Formicidae) in the UK: status, impact and				
205	management. In: Müller G, Pospischil R, Robinson WH (eds) Proceedings of the 8th				
206	International Conference on Urban Pests. OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a,				
207	Hungary, pp 223-228				
208	Cooling M, Hartley S, Sim DA, Lester PJ (2012) The widespread collapse of an invasive species:				
209	Argentine ants (Linepithema humile) in New Zealand Biology Letters 8:430-433				
210	doi:10.1098/rsbl.2011.1014				

- Cooling M, Hoffmann BD (2015) Here today, gone tomorrow: declines and local extinctions of
  invasive ant populations in the absence of intervention Biological Invasions 17:3351-3357
  doi:10.1007/s10530-015-0963-7
- Cremer S et al. (2008) The evolution of invasiveness in garden ants PloS one 3:e3838
  doi:10.1371/journal.pone.0003838
- Czechowska W, Czechowski W (2003) Further record of *Lasius neglectus* van Loon, Boomsma &
   Andrásfalvy (Hymenoptera: Formicidae) from Warsaw, with a key to the Polish species of the
   subgenus *Lasius* s.str. Fragmenta Faunistica 46:195-202
- Espadaler X, Bernal V (2016) *Lasius neglectus* a polygynous, sometimes invasive, ant.
   http://www.creaf.uab.es/xeg/Lasius/index.htm Accessed 27.02 2016
- Espadaler X, Rey S (2001) Biological constraints and colony founding in the polygynous invasive ant
   *Lasius neglectus* (Hymenoptera, Formicidae) Insectes Sociaux 48:159-164
   doi:10.1007/p100001760
- Espadaler X, Tartally A, Schultz R, Seifert B, Nagy C (2007) Regional trends and preliminary results
   on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera,
   Formicidae) Insectes Sociaux 54:293-301 doi:10.1007/s00040-007-0944-7
- Gerlach J (2005) Social breakdown as a population regulating process in invasive ant species
   Phelsuma 13:80-85
- Haines IH, Haines JB (1978) Colony structure, seasonality and food requirements of the crazy ant,
   *Anoplolepis longipes* (Jerd.), in the Seychelles Ecological Entomology 3:109-118
   doi:10.1111/j.1365-2311.1978.tb00909.x
- Majer J, de Kock AE (1992) Ant recolonisation of sand mines near Richards Bay, South Africa: an
  evaluation of progress with rehabilitation South African Journal of Science 88:31-36
- Nagy C et al. (2009) Effects of the invasive garden ant, *Lasius neglectus* van Loon, Boomsma &
   Andrásfalvy, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in
   the type supercolony Myrmecological News 12:171-181

- Paris C, Espadaler X (2012) Foraging activity of native ants on trees in forest fragments colonized by
  the invasive ant *Lasius neglectus* Psyche: A Journal of Entomology 2012:1-9
  doi:10.1155/2012/261316
- Rey S, Espadaler X (2005) Area-wide management of the invasive garden ant *Lasius neglectus*(Hymenoptera: Formicidae) in Northeast Spain Journal of Agricultural and Urban Entomology
  242 21:99-112
- Schultz R, Seifert B (2005) *Lasius neglectus* (Hymenoptera: Formicidae) a widely distributed tramp
   species in Central Asia Myrmecologische Nachrichten 7:47-50
- Seifert B (2000) Rapid range extension in *Lasius neglectus* (Hymenoptera, Formicidae) an Asian
   invader swamps Europe Mitteilungen Museum Naturkunde Berlin, Deutsche Entomologische
   Zeitschrift 173-179
- Simberloff D, Gibbons L (2004) Now you see them, now you don't! Population crashes of
  established introduced species Biological Invasions 6:161-172
  doi:10.1023/b:binv.0000022133.49752.46
- Tartally A (2000) Notes on the coexistence of the supercolonial *Lasius neglectus* van Loon, Boomsma
   et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species Tiscia (Szeged) 32:43 46
- Tartally A (2006) Long term expansion of a supercolony of the invasive garden ant *Lasius neglectus*(Hymenoptera: Formicidae) Myrmecologische Nachrichten 9:21-25
- Tartally A, Hornung E, Espadaler X (2004) The joint introduction of *Platyarthrus schoblii* (Isopoda:
  Oniscidea) and *Lasius neglectus* (Hymenoptera: Formicidae) into Hungary Myrmecologische
  Nachrichten 6:61-66
- 259 Torres JA, Snelling RR (1997) Biogeography of Puerto Rican ants: a non-equilibrium case?
  260 Biodiversity and Conservation 6:1103-1121 doi:10.1023/a:1018332117719
- Ugelvig LV, Drijfhout FP, Kronauer DJ, Boomsma JJ, Pedersen JS, Cremer S (2008) The introduction
   history of invasive garden ants in Europe: integrating genetic, chemical and behavioural
   approaches BMC Biology 6:11 doi:10.1186/1741-7007-6-11

264	van Loon AJ, Boomsma JJ, Andrásfalvy A (1990) A new polygynous Lasius species (Hymenoptera;
265	Formicidae) from central Europe Insectes Sociaux 37:348-362 doi:10.1007/bf02225997
266	
267	
268	
269	Table 1 The direction of development of the investigated L. neglectus supercolonies in four European
270	countries (for details see Online Resource)

Expanded	Stagnate d	Declined	Not found	In total
0	0	0	8	8
0	5	5	1	11
1	1	2	1	5
1	0	4	0	5
2	6	11	10	29
	0 0 1 1	0         0           0         5           1         1           1         0	0         0         0           0         5         5           1         1         2           1         0         4	0     0     0     8       0     5     5     1       1     1     2     1       1     0     4     0

274	Online Resource: Tartally A*, Antonova V, Espadaler X, Csősz S, Czechowski W: Collapse of the
275	invasive garden ant, Lasius neglectus, populations in four European countries – Biological Invasions
276	
277	*: Corresponding author: Department of Evolutionary Zoology and Human Biology, University of
278	Debrecen, Egyetem Tér 1, H-4032 Debrecen, Hungary. E-mail: tartally.andras@science.unideb.hu
279	Debreeen, Egyetein fei 1, 11 1052 Debreeen, 11ungur y. E mun. urrany.unarus e seienee.unaeo.nu
280	
281	A detailed history of the investigated supercolonies of the invasive garden ant (Lasius neglectus van
282	Loon, Boomsma et. Andrasfalvy, 1990; Hymenoptera: Formicidae):
283	
284	
285	Bulgaria
286	
287	Eight locations are known in Bulgaria (Espadaler et al. 2007; Cremer et al. 2008; Espadaler and Bernal
288	2016). We did not have any pieces of information from the previous authors neither about the
289	microhabitats or number and size of the populations within a locality, nor about their invasive status at
290	the time of discovery. In July 2013 and August 2014 we searched thoroughly in about 100 m radius
291	from the known geographical coordinates. Despite we have found nests of other <i>Lasius</i> s.str. species
292	[L. niger (Linnaeus, 1758), L. alienus (Foerster, 1850) and L. brunneus (Latreille, 1798)], we have not
293	found any colonies of L. neglectus at these localities:
294	
295	1. "Albena" (43°12'0"N, 27°4'12"E), NOT FOUND: Discovered in 1984 (Seifert 2000).
296	
297	2. "Balchik 1" (= "Bhot" in Espadaler and Bernal 2016, unknown name with geographical coordinates
298	in Balchik; 43°24'0"N, 28°7'48"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers.
299	comm. in Espadaler and Bernal 2016).
300	I ,
301	3. "Balchik 2" (43°24'36"N, 28°9'36"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers.
302	comm. in Espadaler and Bernal 2016).
302	commit. In Espadator and Dornar 2010).
303	4. "Dobrich" (= ex "Tolbuhin" in Espadaler and Bernal 2016; 43°33'36"N, 27°49'48"E), NOT
305	FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
	round. Discovered in 2004 (leg, K.S. Felersen, pers. comm. in Espadaler and Demai 2010).
306	
307	5. "Kavarna" (43°25'48"N, 28°19'48"E), NOT FOUND: Discovered in 2004 (Cremer et al. 2008;
308	Seifert in litt. in Espadaler and Bernal 2016).
309	
310	6. "Kranevo" (43°20'24"N, 28°3'0"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers.
311	comm. in Espadaler and Bernal 2016).
312	
313	7. "Senokos village" (41°49'12"N, 23°13'48"E), NOT FOUND: Discovered in 2004 (leg, K.S.
314	Petersen., pers. comm. in Espadaler and Bernal 2016).
315	
316	8. "Varna Municipality" (43°12'36"N, 27°54'36"E), NOT FOUND: Discovered in 2004 (leg, K.S.
317	Petersen., pers. comm. in Espadaler and Bernal 2016).
318	retersent, pers. comm. In Espadater and Demar 2010).
319	TT
320	Hungary
321	
322	In total, 21 L. neglectus (super)colonies are known in Hungary (see all of them in Table 1 in Tartally
323	and Báthori 2015). Some data about the earlier area were available from 11 ones:
324	
325	1. "Budapest, Árpád-bridge" (47°31'57"N, 19°3'54"E), STAGNATED: A colony a few square meters
326	in size was recorded here in 1999 (Tartally 2000a). The colony was examined again in September
327	2014 (Tartally and Báthori 2015) and its area had not changed considerably, or if it had, at most it had
328	shrunk a little.

329 2. "Budapest, Budatétény" (47°24'17"N, 330 19°0'30"E), DECLINED: This is the type 331 locality of L. neglectus in which the 332 supercolony was estimated to cover an area 333 of c.a. 2 km<sup>2</sup> in 1988 (van Loon et al. 1990). 334 When the borders, published by van Loon et 335 al. (1990), were compared along six transects 336 with the borders found in August 2005, the 337 mean expansion was 89 m year-1 (Espadaler 338 et al. 2007; see also Appendix 1 of Nagy et 339 al. 2009). Both in 1988 (van Loon et al. 340 1990) and in 2002 (see Nagy et al. 2009 and 341 its Appendixes), L. neglectus was found to be 342 the dominant ant, and within this period it 343 was often the only visible ant species in most 344 of the supercolony. It typically was visible in 345 irregularly high density (AT, pers. observ.). 346 This dominance of L. neglectus was evident 347 for about 20 years, from 1988 (van Loon et 348 al. 1990; Nagy et al. 2009) to c.a. 2005, as 349 AT regularly visited the supercolony from 350 1990 at least once every two or three years. 351 However, in 2009 it was shocking when no L. 352 *neglectus* workers or nests were visible in the 353 localities in which e.g. the type individuals 354 had been collected in 1988 (van Loon et al. 355 1990) and in which this ant had occurred in 356 high densities (Nagy et al. 2009; Fig. 1) for 357 about 20 years. During a c.a. one hour-long 358 search in 2009, only two small nests were 359 found in the area under two distinct stones, 360 c.a. 900 meters from each other. In 361 September 2014, the colony was visited again 362 (Tartally and Báthori 2015) and the situation 363 had not changed substantially. These two 364 small, refuge-like colonies and a third one (see Table 1 of Tartally and Báthori 2015) 365 366 were found on that occasion during a c.a. one hour-long search. However, it should be 367 noted that one would need to spend several 368 369 months within the area of the largest known 370 size (in 2005, see Appendix 1 in Nagy et al. 371 2009) of this supercolony in order to perform 372 a thorough search for this ant. Both in 2009 373 and in 2014, the typical suburban ant fauna, 374 dominated by the Lasius s.str. Fabricius,

**Fig. 1** The entrances of polydomous *L. neglectus* nests were well visible almost continuously along the kerbs at Budatétény (Növény Str., Budapest, Hungary) in 1998. This place was about the centre of the type supercolony that time (van Loon et al. 1990) and also in 2005 (see "site 6" in Nagy et al. 2009), where AT regularly found similar nest entrance patterns between 1990 and 2005. However, we did not find any *L. neglectus* individuals or nests here neither in 2009 nor in 2014 (scanned from the 18-years-old photo taken by G. Szövényi).



1804, *Tertamorium* cf. *caespitum* (Linnaeus, 1758) and *Serviformica* Forel, 1913 species, was clearly
visible in the area (AT, pers observ.) in which these native ant species had been being outdone by the
invasive *L. neglectus* for at least 15 years (van Loon et al. 1990; Nagy et al. 2009; AT, pers. observ.).

379 3. "Budapest, Castle" (47°29'40"N, 19°2'30"E), DECLINED: Workers of L. neglectus were found 380 only on and around one tree in 1988 at this locality (van Loon at al. 1990). The area of this 381 supercolony was estimated as 102,450 m<sup>2</sup> in 2005, and the local average expansion rate was estimated 382 at 10.6 m per year-1 between 1988 and 2005 (Espadaler et al. 2007). However, the area of this 383 supercolony appeared to be c.a. the same size in September 2014, when it was visited again (Tartally 384 and Báthori 2015). In 2010, the observation was made (Cs. Nagy, pers. comm.) that the local ant 385 species had appeared again and L. neglectus had disappeared in some parts of this supercolony that 386 had been well colonized by L. neglectus earlier. This phenomenon and similar "new wholes" were also 387 found within the supercolony in September 2014.

388

402

4. "*Budapest, Galvani Str.*" (47°27'20"N, 19°2'29"E), NOT FOUND: The colony was discovered in 1994 (Tartally 2000a) along a c.a. 250 m-long part of the street. The colony was still present in a stretch of about the same length in 2001 (Tartally et al. 2004), but no *L. neglectus* workers were found there in September 2014, though a thorough search was performed (Tartally and Báthori 2015). This supercolony appears now to have disappeared, but one should be careful with these kinds of statements (see the story of the following colony).

- 395
  396 5. "*Budapest, Orom Str.*" (47°29'24"N, 19°2'29"E), STAGNATED: A colony of a few m<sup>2</sup> in size was
  397 recorded here in 2000 (Tartally 2000a). The colony was thought to have disappeared by 2004 (Tartally
  398 et al. 2004), but a similarly small colony was rediscovered (or a new one was discovered) less than
  399 one-hundred meters from the original locality in September 2014 (Tartally and Báthori 2015). So, it
  400 seems very likely that here are some remnants of an original invasion which have not totally
  401 disappeared.
- 6. "*Budapest, Pázmány P. Promenade*" (47°28'10'N, 19°3'50'E), STAGNATED: A colony of a few
  m<sup>2</sup> in size was recorded here in 2002 (Tartally et al. 2004). The colony was revisited in September
  2014 (Tartally and Báthori 2015) and its area had not changed considerably, or if it had, at most it had
  shrunk a little.

7. "*Budapest, Pétervárad Str.*" (47°31'8"N, 19°6'30"E), DECLINED: The colony had been observed
in several streets around this street in 1988 (van Loon et al. 1990) and was still present in more streets
in 2003 (Tartally et al. 2004). However, *L. neglectus* workers were found only under a stone in
September 2014, though a thorough search was performed (Tartally and Báthori 2015).

8. "*Budapest, Tigris Str.*" (47°29'32'N, 19°1'53"E), STAGNATED: A colony a few m<sup>2</sup> in size was recorded (A. Andrásfalvy, pers. comm.) here in 1999 (Tartally 2000a). The colony was revisited in September 2014 (Tartally and Báthori 2015) and its area had not changed considerably, or if it had, at most it had shrunk a little.

417

9. "Debrecen, Botanical garden" (47°33'28"N, 21°37'17"E), STAGNATED: The colony was 418 419 discovered in 1997 (Tartally 2000a; Tartally 2000b). In 1998, its size was estimated at c.a. 0.1 km<sup>2</sup>, 420 and it expanded an average 13 m per year<sup>1</sup> along 4 transects until 2002 (Tartally 2006, see its Fig 1 for 421 a map showing the expansion in detail) and the level of expansion was the same until 2005 (Espadaler 422 et al. 2007). However, the colony was revisited more times in 2014 (Tartally and Báthori 2015; AT, 423 unpublished data) and its area had not changed much since 2005. The expansion level declined, e.g. 424 while the average expansion rate was 3.125 m per year<sup>1</sup> between 1998 and 2002, this rate was only 425 1.667 m per year<sup>1</sup> between 2002 and 2014, in general in the two directions of the transect between the 426 entrance and the observatory (see the transect in Fig 1-2 in Tartally 2006). The pattern of co-427 occurrence of L. neglectus and the native ant species here was studied several times (Tartally 2000b; 428 Tartally 2006), and the most important competitors were L. niger, L. fuliginosus (Latreille, 1798), 429 Tetramorium cf. caespitum, Liometopum microcephalum (Panzer, 1798) and Serviformica species

[sometimes as hosts of *Polyergus rufescens* (Latreille, 1798)]. These ant species were still present in
September 2014 in more places within the area of the supercolony, e.g. *L. fuliginosus* and *L. microcephalum* are still present on the oak trees, as was the case in 1998-2002, and the supercolony is
still not present to the southeast of these trees (see Fig 1 in Tartally 2006).

434

10. "Debrecen, Csap Str." (47°31'50'N, 21°36'49'E), DECLINED: The colony was discovered in
2007 along a stretch of the street roughly 70 m long and in the yard of a detached house (AT,
unpublished data). However, in September 2014 *L. neglectus* workers were only found under a stone
in the yard, though a thorough search was performed along the street and in the yard (Tartally and
Báthori 2015).

440

453 454

455

11. "Érd, Felső Str." (47°22'13"N, 18°55'23"E), DECLINED: The colony was discovered in 1998
(Tartally 2000a) in front of a detached house and in its yard. The colony was still widely present in
front of the house (the yard was not checked on that occasion) in 2001 (Tartally et al. 2004). However,
in September 2014 *L. neglectus* workers were only found on a tree in front of the house, though a
thorough search was performed in the yard and in front of the house (Tartally and Báthori 2015).

In the case of the other 10 Hungarian supercolonies (see Tartally and Báthori 2015), no useful data about this topic were recorded in the earlier research, as the borders of these supercolonies have not been established yet. However, they can all be treated as supercolonies because they were more than twenty square meters in size when the first investigations were made and also in September 2014 (see Tartally and Báthori 2015).

## Poland

456 In Poland, L. neglectus is known only from Warsaw, where it was formally reported in 1999 457 (Czechowska and Czechowski 1999), but in all likelihood it appeared there no later than the beginning 458 of the 1990s, as suggested by an observation made by the late Prof. Bohdan Pisarski (pers. comm. to W. Czechowski) concerning "the strange small L. niger" which occurred in masses close to his 459 460 dwelling place. In total, five polydomous systems of the species were found, all situated in a central 461 part of the city within a radius of 3 km (Czechowska and Czechowski 2003). A later search for L. 462 neglectus done in 2009–2010 in other areas of the urban and suburban greenery of Warsaw, which run 463 the highest risk of being infested by foreign ant species, such as parks, two botanical gardens 464 (including greenhouses) and the zoo (including pavilions), did not yield any results (H. Babik, pers. 465 comm.). 466

In summer 2015, the five known *L. neglectus* colonies were examined, 13–16 years after having been
discovered. Consecutively as in Czechowska and Czechowski (2003), the states of the colonies were
as follows:

471 1. "Solec Str." (52°14'09"N, 21°02'10"E), NOT FOUND: The colony was discovered in 1999. Since
472 then, the street has been partly redeveloped, including new houses, paved stretches and reorganized
473 (reduced) greenery. No trace of *L. neglectus* was found, neither in the sites in which it had previously
474 been observed nor in the vicinity.

475

476 2. "*Furmańska Str.*" (52°14'39"N, 21°01'93"E), EXPANDED: The colony most probably was
477 accidentally discovered by B. Pisarski a quarter of a century ago (see above) and then formally
478 reported in 1990. At present, it is still in very good condition, apparently even better than previously,
479 both in respect of its range and individual colony-sizes. The ant workers are noticeably bigger than
480 those from other *L. neglectus* colonies in Warsaw (W. Czechowska, pers. comm.).

3. "*The Marshal Edward Rydz-Śmigły Park*" (52°13'42"N, 21°01'48"E), DECLINED: The colony
was discovered in 2002. Originally (in the early 2000s), it was the biggest known *L. neglectus* colony
in Warsaw; material taken from it represented the Warsaw population of the species in comparative

population studies on *L. neglectus* (Cremer et al. 2008; Ugelvig et al. 2008). Now, the former supercolony occurs in a vestigial form. Some single colonies survived on or near a few trees with incomparably weaker activity on the trunks, and the ants seem visibly smaller than in a prosperous period in their life. Originally, there was also a big *L. neglectus* nest density around the fountain in the park on the area paved with a granite sett. Now, there are only a few nests of the native *L. niger* instead of crowds of *L. neglectus* (W. Czechowska, pers. comm.).

491

492 4. "Emilii Plater Str." (52°13'35"N, 21°00'23"E), DECLINED: The colony was discovered in 2002. 493 At the time, it "stretched for about 300 malong the street where the ants visited canopies of several 494 trees [...]. The main [individual] colony there seemed to be that at the foot of the old maple. This tree, 495 invaded by ants in masses, was situated at the crossing of E. Plater St and Nowogrodzka St at a very 496 small patch of dense ornamental shrubby and herb vegetation, completely encircled by concrete or 497 asphalt surface" (Czechowska and Czechowski 2003). At present, this main single colony seems to 498 have died out completely. Even under weather conditions especially favorable for L. neglectus, not a 499 single ant can be seen. And the whole linear polydomous system is now limited to nests at the base of 500 only a few trees (with no ants visible on tree stems). The rest of the trees of the former L. neglectus 501 system are, most probably, occupied by the native dendrophilic L. brunneus - only external signs of 502 nests typical of *L. brunneus* presence (brown sawdust) were visible.

503

504 5. "Opaczewska Str." (52°12'34'N, 20°58'12'E), STAGNATED: The colony was discovered in 2002.
505 Originally, the following description was given: "the polydomous system of L. neglectus stretched for
506 about 1 km in the green belt along the street" (Czechowska and Czechowski 2003). Now it seems to
507 retain its previous state, more or less.

509 At the same time (in 2015), some new L. neglectus nests were found in Warsaw (G. Trigos Peral, 510 pers. comm.). Two of them (ca. 200 m from each other) were located in the same city quarter as the 511 colony no. 5 (ca 1 km to the south). A few nests were in the Ujazdowski Park, at a distance ca. 600 m 512 (to the southwest) from the supercolony no. 3 (which has now deteriorated). The latter place was 513 searched in 2009 with no results, though close attention was paid to possible occurrence of L. 514 neglectus (H. Babik, pers. comm.). It seems, therefore, that when old supercolonies in Warsaw tend to 515 collapse, new ones can successfully come into being in other place. Alternatively, these small colonies 516 can be remnants of some earlier collapsed supercolonies (see above the stories of the supercolonies at 517 Budatétény and Orom Str.). Besides, a single L. neglectus worker was found in one of the pitfall traps 518 set in the Pole Mokotowskie (G. Trigos Peral, pers. comm.) - a park where the search for this species 519 were not been carried out earlier.

520 521

522

523

## Spain

In Spain area mappings for the changes that have taken place to five supercolonies were done from 2002 to 2009. The change between 2002 and 2009 was expressed as a percentage, in %, as the difference between the estimates in both years divided by the estimate in 2002.

527

The physical structure of the habitat (freely accessible, private properties, public gardens, streets) and exact microhabitats where the ants were detected (walls, trees, nests under stones or in concrete cracks or crevices) allowed and required different procedures in order to arrive at estimates. For two populations (Bellaterra, Sant Cugat), the number of trees with ant trails was the unit used to estimate colony presence. For the populations from Seva, Taradell and Matadepera, the area occupied was estimated using the perimeter of the polygons limiting ant colonies. Four of these supercolonies were observed as being in decline, while one of them showed an expansion of the infested area:

536 1. "*Bellaterra*" (41°30'8'N, 2°6'15''E), DECLINED about 24 %, see the <u>map</u> of Espadaler and Bernal (2016) for details.

- 539 2. "*Matadepera*" (41°36'36''N, 2°18'0''E), DECLINED about 7 %, see the <u>map</u> of Espadaler and 540 Bernal (2016) for details.
- 542 3. "*Sant Cugat*" (41°30'0'N, 2°6'0'E), DECLINED about 18%, see the <u>map</u> of Espadaler and Bernal 543 (2016) for details.
- 545 4. "*Taradell*" (41°52'48"N, 2°18'0"E), DECLINED about 18 %, see the <u>map</u> of Espadaler and Bernal
  546 (2016) for details.
- 548 5. "Seva" (41°48'0"N, 2°15'36"E), EXPANDED about 14%, see the map of Espadaler and Bernal
  (2016) for details.

#### 552 **References**

541

544

551

- 554 Cremer S et al. (2008) The evolution of invasiveness in garden ants PloS one 3:e3838 555 doi:10.1371/journal.pone.0003838
- 556 Czechowska W, Czechowski W (1999) Lasius neglectus van Loon, Boomsma et Andrasfalvy, 1990
   557 (Hymenoptera, Formicidae), nowy dla Polski gatunek mrówki, w Warszawie Przegląd
   558 Zoologiczny 43:189-191
- Czechowska W, Czechowski W (2003) Further record of *Lasius neglectus* van Loon, Boomsma &
   Andrásfalvy (Hymenoptera: Formicidae) from Warsaw, with a key to the Polish species of the
   subgenus *Lasius* s.str. Fragmenta Faunistica 46:195-202
- 562 Espadaler X, Bernal V (2016) Lasius neglectus a polygynous, sometimes invasive, ant.
   563 http://www.creaf.uab.es/xeg/Lasius/index.htm Accessed 27.02 2016
- Espadaler X, Tartally A, Schultz R, Seifert B, Nagy C (2007) Regional trends and preliminary results
   on the local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera,
   Formicidae) Insectes Sociaux 54:293-301 doi:10.1007/s00040-007-0944-7
- Nagy C et al. (2009) Effects of the invasive garden ant, *Lasius neglectus* van Loon, Boomsma &
   Andrásfalvy, 1990 (Hymenoptera: Formicidae), on arthropod assemblages: pattern analyses in
   the type supercolony Myrmecological News 12:171-181
- Seifert B (2000) Rapid range extension in *Lasius neglectus* (Hymenoptera, Formicidae) an Asian
   invader swamps Europe Mitteilungen Museum Naturkunde Berlin, Deutsche Entomologische
   Zeitschrift 173-179
- 573 Tartally A (2000a) A Magyarországról leírt invazív Lasius neglectus van Loon, Boomsma et
   574 Andrásfalvy, 1990 (Hymenoptera: Formicidae) újabb hazai lelőhelyei Folia Entomologica
   575 Hungarica 59:298-300
- Tartally A (2000b) Notes on the coexistence of the supercolonial *Lasius neglectus* van Loon,
   Boomsma et Andrásfalvy 1990 (Hymenoptera: Formicidae) with other ant species Tiscia
   (Szeged) 32:43-46
- Tartally A (2006) Long term expansion of a supercolony of the invasive garden ant *Lasius neglectus* (Hymenoptera: Formicidae) Myrmecologische Nachrichten 9:21-25
- Tartally A, Báthori F (2015) Does *Laboulbenia formicarum* (Ascomycota: Laboulbeniales) fungus
   infect the invasive garden ant, *Lasius neglectus* (Hymenoptera: Formicidae), in Hungary? e Acta Naturalia Pannonica 8:117-123
- Tartally A, Hornung E, Espadaler X (2004) The joint introduction of *Platyarthrus schoblii* (Isopoda:
   Oniscidea) and *Lasius neglectus* (Hymenoptera: Formicidae) into Hungary Myrmecologische
   Nachrichten 6:61-66
- Ugelvig LV, Drijfhout FP, Kronauer DJ, Boomsma JJ, Pedersen JS, Cremer S (2008) The introduction
   history of invasive garden ants in Europe: integrating genetic, chemical and behavioural
   approaches BMC Biology 6:11 doi:10.1186/1741-7007-6-11
- van Loon AJ, Boomsma JJ, Andrásfalvy A (1990) A new polygynous *Lasius* species (Hymenoptera;
   Formicidae) from central Europe Insectes Sociaux 37:348-362 doi:10.1007/bf02225997
  - 16