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

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

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

Introduction

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

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

Lasius neglectus has a climatic preference which makes it the most threatening among the outdoor invasive ant species for the largest part of Europe. It also has found suitable places in other temperate regions (Bertelsmeier et al. 2014). The present range of *L. neglectus* in Eurasia extends from 36°N to 54°N and from 1°E to 74°E, including about 160 known localities. In Europe, the species has been observed in most countries (Espadaler and Bernal 2016), and it occurs mainly in urban and suburban 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 for this supposedly originally Mediterranean species (Schultz and Seifert 2005). Furthermore, the proportion of climatically suitable areas for *L. neglectus* in Europe is predicted to increase with climate change in the future (Bertelsmeier et al. 2014).

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.

Materials and Methods

Present states of 29 *L. neglectus* supercolonies in Bulgaria, Hungary, Poland and Spain were compared with their known earlier sizes. For this purpose we searched for *L. neglectus* individuals and nests meter by meter along the streets (as transects) within the latest known area of the supercolonies. Private zones were usually not included in our work. This monitoring was done in summers of 2013-2015 under weather circumstances favourable for activity of the species. This work practically meant turning up stones, digging the soil, and checking kerbs (see Fig. 1 of Online Resource) for nests. Parallel with this, ant individuals were searched for on the roadways, pavements, hedges, bushes and

tree trunks. By these methods we have already surveyed these supercolonies in their earlier stages (for details see Online Resource) and realised that it is easy to record this ant species these ways (Tartally 2006; Espadaler et al. 2007). According to the registered changes of the state of the colonies, they were arranged into four categories: EXPANDED, STAGNATED, DECLINED and NOT FOUND. In the case of the "not found" category we searched for *L. neglectus* individuals especially intensively on the former known area of the supercolonies but have recorded no specimens. We do not call this category "disappeared" because both disappearance and probably unlucky samplings can be in the background of such negative results (see the story of the supercolony at Orom Str. in the Online Resource), especially in the case of previously huge supercolonies occupying private zones.

112 Results

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

Discussion

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.

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

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

fragmentation, reduced genetic heterogeneity due to isolation and inbreeding, which leads to reduced adaptability to changing external conditions), (5) confrontation with highly competitive native species, and (6) lack of suitable nesting microhabitats (Haines and Haines 1978; Gerlach 2005; Espadaler et al. 2007; Cooling et al. 2012; Cooling and Hoffmann 2015). Each factor (or group of factors) may be true for an individual case. Some factors may impact the others, and this makes the individual situations difficult to interpret. On the other hand, in every locality, the phase of population growth, which precedes the phase of decline, may proceed differently. It depends on local conditions, such as climate, management, urbanization processes, etc. (Espadaler et al. 2007). Therefore it should be emphasized that it is not immediately obvious how to determine even an approximate age of given ant supercolonies based simply on their sizes.

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

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

undergoing. It will be especially important to monitor the localities where *L. neglectus* individuals were not found recently at the area of the former supercolonies. It would help to realise whether such supercolonies can disappear or just drastically collapse (see the story of the supercolony at Orom Str. in the Online Resources). Further studies of the possible factors causing the expansion, stagnation or collapse based on adequate quantitative data and the ecological characteristics of this invasive species, could be used in order to model the populations' dynamics in more countries.

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Table 1 The direction of development of the investigated *L. neglectus* supercolonies in four European countries (for details see Online Resource)

Country	Expanded	Stagnated	Declined	Not found	In total
Bulgaria	0	0	0	8	8
Hungary	0	5	5	1	11
Poland	1	1	2	1	5
Spain	1	0	4	0	5
In total	2	6	11	10	29

Online Resource: Tartally A*, Antonova V, Espadaler X, Csősz S, Czechowski W: Collapse of the invasive garden ant, *Lasius neglectus*, populations in four European countries – *Biological Invasions*

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A detailed history of the investigated supercolonies of the invasive garden ant (*Lasius neglectus* van Loon, Boomsma *et.* Andrasfalvy, 1990; Hymenoptera: Formicidae):

Bulgaria

Eight locations are known in Bulgaria (Espadaler et al. 2007; Cremer et al. 2008; Espadaler and Bernal 2016). We did not have any pieces of information from the previous authors neither about the microhabitats or number and size of the populations within a locality, nor about their invasive status at the time of discovery. In July 2013 and August 2014 we searched thoroughly in about 100 m radius from the known geographical coordinates. Despite we have found nests of other *Lasius* s.str. species [*L. niger* (Linnaeus, 1758), *L. alienus* (Foerster, 1850) and *L. brunneus* (Latreille, 1798)], we have not found any colonies of *L. neglectus* at these localities:

- 1. "Albena" (43°12'0"N, 27°4'12"E), NOT FOUND: Discovered in 1984 (Seifert 2000).
- 2. "*Balchik 1*" (= "*Bhot*" in Espadaler and Bernal 2016, unknown name with geographical coordinates in Balchik; 43°24'0"N, 28°7'48"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
- 3. "Balchik 2" (43°24'36"N, 28°9'36"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
- 4. "*Dobrich*" (= ex "*Tolbuhin*" in Espadaler and Bernal 2016; 43°33'36"N, 27°49'48"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
- 5. "*Kavarna*" (43°25'48"N, 28°19'48"E), NOT FOUND: Discovered in 2004 (Cremer et al. 2008; Seifert in litt. in Espadaler and Bernal 2016).
- 6. "Kranevo" (43°20'24"N, 28°3'0"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
 - 7. "Senokos village" (41°49'12"N, 23°13'48"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).
- 8. "*Varna Municipality*" (43°12'36"N, 27°54'36"E), NOT FOUND: Discovered in 2004 (leg, K.S. Petersen., pers. comm. in Espadaler and Bernal 2016).

Hungary

- In total, 21 *L. neglectus* (super)colonies are known in Hungary (see all of them in Table 1 in Tartally and Báthori 2015). Some data about the earlier area were available from 11 ones:
- 1. "Budapest, Árpád-bridge" (47°31'57"N, 19°3'54"E), STAGNATED: A colony a few square meters in size was recorded here in 1999 (Tartally 2000a). The colony was examined again 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.

2. "Budapest, Budatétény" (47°24'17"N, 19°0'30"E), DECLINED: This is the type locality of L. neglectus in which the supercolony was estimated to cover an area of c.a. 2 km² in 1988 (van Loon et al. 1990). When the borders, published by van Loon et al. (1990), were compared along six transects with the borders found in August 2005, the mean expansion was 89 m year-1 (Espadaler et al. 2007; see also Appendix 1 of Nagy et al. 2009). Both in 1988 (van Loon et al. 1990) and in 2002 (see Nagy et al. 2009 and its Appendixes), L. neglectus was found to be the dominant ant, and within this period it was often the only visible ant species in most of the supercolony. It typically was visible in irregularly high density (AT, pers. observ.). This dominance of L. neglectus was evident for about 20 years, from 1988 (van Loon et al. 1990; Nagy et al. 2009) to c.a. 2005, as AT regularly visited the supercolony from 1990 at least once every two or three years. However, in 2009 it was shocking when no L. neglectus workers or nests were visible in the localities in which e.g. the type individuals had been collected in 1988 (van Loon et al. 1990) and in which this ant had occurred in high densities (Nagy et al. 2009; Fig. 1) for about 20 years. During a c.a. one hour-long search in 2009, only two small nests were found in the area under two distinct stones, c.a. 900 meters from each other. In September 2014, the colony was visited again (Tartally and Báthori 2015) and the situation had not changed substantially. These two small, refuge-like colonies and a third one (see Table 1 of Tartally and Báthori 2015) were found on that occasion during a c.a. one hour-long search. However, it should be noted that one would need to spend several months within the area of the largest known size (in 2005, see Appendix 1 in Nagy et al. 2009) of this supercolony in order to perform a thorough search for this ant. Both in 2009 and in 2014, the typical suburban ant fauna, dominated by the Lasius s.str. Fabricius,

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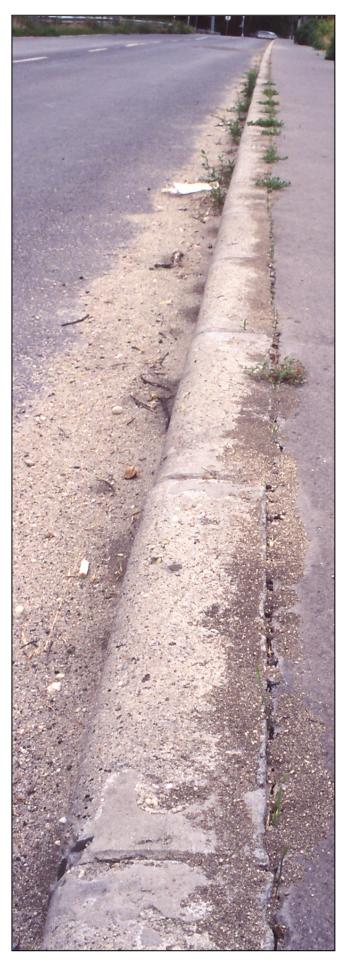
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Fig. 1 The entrances of polydomous *L. neglectus* nests were well visible almost continuously along the kerbs at Budatétény (Növény Str., Budapest, Hungary) in 1998. This place was about the centre of the type supercolony that time (van Loon et al. 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).



375 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.).

- 3. "Budapest, Castle" (47°29'40"N, 19°2'30"E), DECLINED: Workers of L. neglectus were found only on and around one tree in 1988 at this locality (van Loon at al. 1990). The area of this supercolony was estimated as 102,450 m² in 2005, and the local average expansion rate was estimated at 10.6 m per year-1 between 1988 and 2005 (Espadaler et al. 2007). However, the area of this supercolony appeared to be c.a. the same size in September 2014, when it was visited again (Tartally and Báthori 2015). In 2010, the observation was made (Cs. Nagy, pers. comm.) that the local ant species had appeared again and L. neglectus had disappeared in some parts of this supercolony that had been well colonized by L. neglectus earlier. This phenomenon and similar "new wholes" were also found within the supercolony in September 2014.
 - 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).
 - 5. "Budapest, Orom Str." (47°29'24"N, 19°2'29"E), STAGNATED: A colony of a few m² in size was recorded here in 2000 (Tartally 2000a). The colony was thought to have disappeared by 2004 (Tartally et al. 2004), but a similarly small colony was rediscovered (or a new one was discovered) less than one-hundred meters from the original locality in September 2014 (Tartally and Báthori 2015). So, it seems very likely that here are some remnants of an original invasion which have not totally disappeared.
 - 6. "Budapest, Pázmány P. Promenade" (47°28'10"N, 19°3'50"E), STAGNATED: A colony of a few m² 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 m² 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.
 - 9. "Debrecen, Botanical garden" (47°33'28"N, 21°37'17"E), STAGNATED: The colony was discovered in 1997 (Tartally 2000a; Tartally 2000b). In 1998, its size was estimated at c.a. 0.1 km², and it expanded an average 13 m per year¹ along 4 transects until 2002 (Tartally 2006, see its Fig 1 for a map showing the expansion in detail) and the level of expansion was the same until 2005 (Espadaler et al. 2007). However, the colony was revisited more times in 2014 (Tartally and Báthori 2015; AT, unpublished data) and its area had not changed much since 2005. The expansion level declined, e.g. while the average expansion rate was 3.125 m per year¹ between 1998 and 2002, this rate was only 1.667 m per year¹ between 2002 and 2014, in general in the two directions of the transect between the entrance and the observatory (see the transect in Fig 1-2 in Tartally 2006). The pattern of co-occurrence of L. neglectus and the native ant species here was studied several times (Tartally 2000b; Tartally 2006), and the most important competitors were L. niger, L. fuliginosus (Latreille, 1798), 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).

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).

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

In Poland, *L. neglectus* is known only from Warsaw, where it was formally reported in 1999 (Czechowska and Czechowski 1999), but in all likelihood it appeared there no later than the beginning 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 dwelling place. In total, five polydomous systems of the species were found, all situated in a central part of the city within a radius of 3 km (Czechowska and Czechowski 2003). A later search for *L. neglectus* done in 2009–2010 in other areas of the urban and suburban greenery of Warsaw, which run the highest risk of being infested by foreign ant species, such as parks, two botanical gardens (including greenhouses) and the zoo (including pavilions), did not yield any results (H. Babik, pers. comm.).

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:

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

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

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

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

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

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.

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:

1. "Bellaterra" (41°30'8"N, 2°6'15"E), DECLINED about 24 %, see the $\underline{\text{map}}$ 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 Bernal (2016) for details.
- 3. "Sant Cugat" (41°30'0"N, 2°6'0"E), DECLINED about 18%, see the map of Espadaler and Bernal (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 (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.

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