SHORT COMMUNICATION

Urban structures are poor stepping stones for crevice-nesting ants in fragmented landscapes

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Abstract

Despite the high fragmentation of its natural habitats—grasslands with limestone rocks—a previous study revealed that the ant species *Temnothorax nigriceps* show no evidence of genetic isolation between populations separated by kilometers of presumably unsuitable landscape. In the present study, we hypothesized that urban anthropogenic structures might allow the species to nest and maintain efficient gene flow in spite of the ongoing fragmentation. To test this, we surveyed the presence of *T. nigriceps* in many putatively suitable anthropized habitats—such as stone walls, ornamental devices, or urban park structures—in an urban area near a continuous source of propagules from natural habitats. We found very few occurrences of *T. nigriceps* in this urban area, suggesting either a low density of nests in such habitats or difficulty in detecting them in anthropized environment. We discuss the importance of surrounding vegetation and water sources for nesting, especially in anthropized environment where temperatures on mineral surfaces can exceed those in the species' natural habitats. We conclude that *T. nigriceps* tend to nest in the most natural-like structures, implying that urban habitats provide poor substitute for the species' native environment.

Keywords Ants · Human-mediated dispersal · Nest material · Nest-site selection · Temnothorax species · Urbanization

Introduction

The ant species *Temnothorax nigriceps* (Mayr 1855) typically inhabits sunny, xerothermic environments with sparse vegetation and bare rocks. Colonies consisting of a few dozen individuals nest in narrow rock crevices (Seifert 2018). Despite the extensive fragmentation of its natural habitat due to anthropogenic activities, such as agriculture or urbanization (Poschlod & WallisDeVries, 2002; Poschlod et al. 2008), a previous study surprisingly found only limited genetic differentiation among *T. nigriceps* populations (Cordonnier et al. 2022). This pattern could be attributed to either high dispersal capabilities mitigating the negative effects of fragmentation or the species' adaptability to utilize alternative, man-made nesting sites in urban areas. Another

potential explanation is the presence of large effective population sizes. However, the detection of local non-random mating suggests that this hypothesis is less likely (Cordonnier et al. 2022).

Habitat fragmentation is expected to impact *Temnothorax* species even at small spatial scales (e.g., Cordonnier et al. 2023, but see Khimoun et al. 2020). Mating in these small ants is presumably a local event, with nuptial flights occurring over short distances. For example, sexuals of *Temnothorax nylanderi*, a forest-dwelling species, fly within forests or at their edges, avoiding open areas (Noordijk et al. 2008). Though *Temnothorax* sexuals have been recorded more than 100 m above ground and might be occasionally drifted over large areas by wind (Helms et al. 2016), it is unlikely that the number of *T. nigriceps* reproductives traversing large distances is sufficient to maintain gene flow between isolated populations.

The present study explored the hypothesis that *T. nigriceps* utilizes secondary habitats, particularly urban areas, to persist and maintain gene flow despite ongoing anthropogenic fragmentation. Most ant species require minimal resources to establish colonies, suggesting adaptability to various environments. Previous works have shown that *T.*

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nigriceps readily nests in crevices of man-made stone walls in orchards or vineyards at the edge of villages (Seifert, 2018; Suefuji and Heinze 2014), but the use of such sites in highly modified anthropized environments remains underexplored. For example, *T. nigriceps* might be able to use urban microhabitats, such as rocky elements of urban settlements. The utilization of these artificial structures can provide alternative shelter for wildlife in fragmented environments (Lowry et al. 2013), with some previous evidence also in ants (Cordonnier et al. 2019).

In this study, we investigated the capacity of *T. nigriceps* to utilize man-made structures in urban areas, such as stone walls, masonry, or ornamental structures as alternative nesting sites. This adaptability might facilitate the species' survive in small suitable patches of habitat and provide an explanation for the absence of genetic isolation observed by Cordonnier et al. (2022). If *T. nigriceps* is able to use anthropized habitats, we expect to detect its presence in urban settlements near its natural habitats, with the latter constituting a continuous source of propagules. To test this hypothesis, various stone structures were examined for the presence of *T. nigriceps* to establish the typology of its anthropogenic habitats.

Materials and methods

Study sites and sampling

Fieldwork was conducted in Kallmünz, Germany, a city surrounded by natural *T. nigriceps* habitats where the species is densely present (48 colonies spread all around the city previously collected; green areas in Fig. 1; Cordonnier et al. 2022). The choice of Kallmünz as a study site makes, therefore, particular sense because of the high densities of nests in the direct surroundings (at a few meters from the
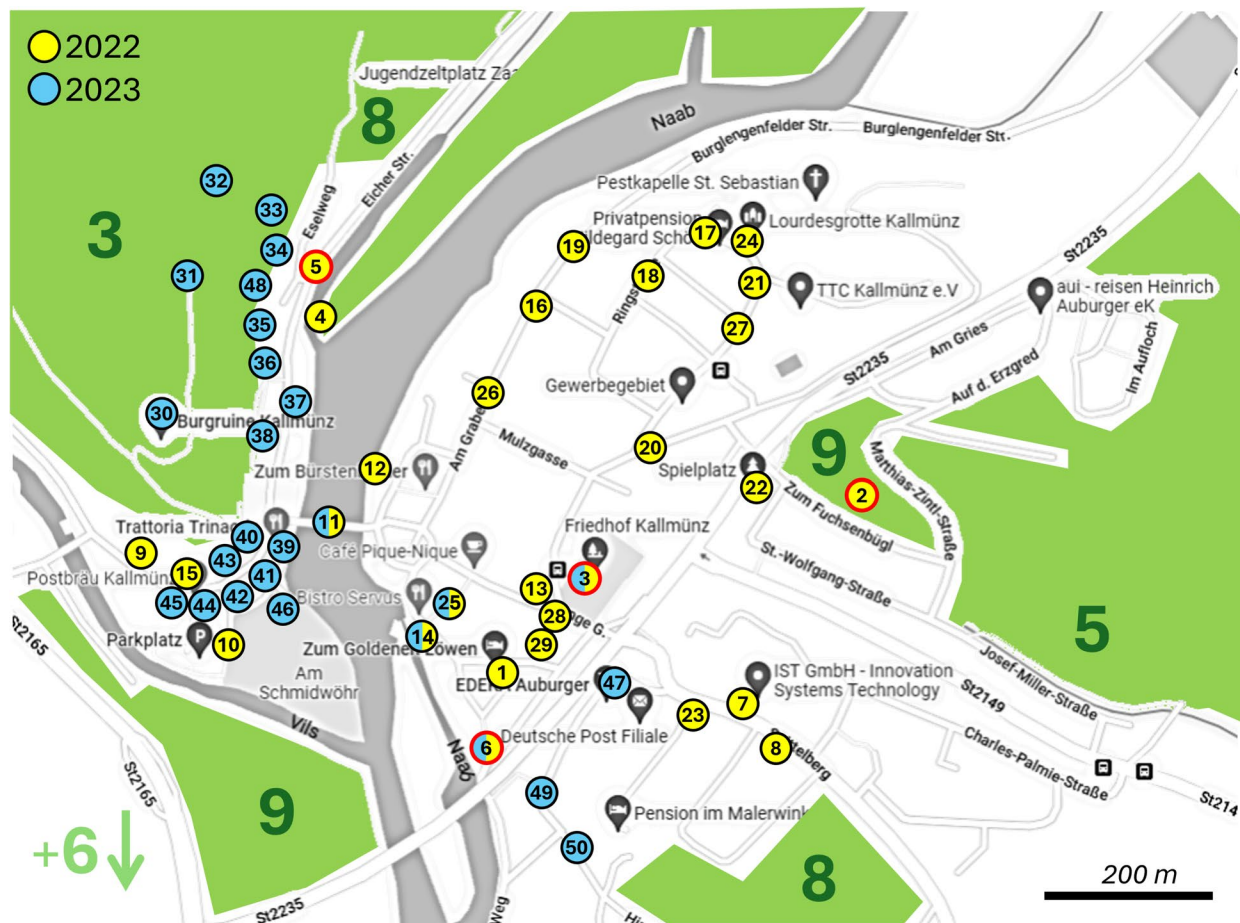

Fig. 1 Map of sampling sites in Kallmünz (Germany). Each sampling site corresponds to one suitable mineral element (except sites 1, 2, 3, and 30 with resp. 10, 10, 8, and 15 elements investigated). Yellow: sites investigated in 2022. Blue: sites investigated in 2023. Green areas are natural habitats where *T. nigriceps* is known to occur (based on Cordonnier et al. 2022) with the number of colonies previously collected (note that this corresponds to approx. 10 colonies sampled per day per person, AB pers. comm.). The sites where *T. nigriceps* has been collected (see "Results" section) are circled in red

city center), in all directions around the city. No other site presenting a similar potential exposure (i.e., likely to receive such a propagule pressure of *T. nigriceps* sexuals) is known.

Within the city, we surveyed all man-made structures that appeared suitable for the species using the same protocol successfully applied in Cordonnier et al. (2022). Urban mineral elements, such as rocks, bridge fences, and walls (Figure S1 for exhaustive list) which correspond to potential nest sites, were carefully inspected by two people for the presence of nests or ant activity. Surveys were conducted in early June 2022 ($n = 29$ sites) and July 2023 ($n = 25$ sites; Fig. 1) at temperatures ranging from 21 to 31 °C. Four sites were revisited in both years. Each site was mapped and photographed (Fig. 1, Fig S1). For all potential mineral elements, the presence of grass and the overall texture of the mineral surface were recorded. To confirm the occurrence of the species during the sampling period, one site (number 2, Fig. 1) within the natural habitat was included and sampled.

In this natural site and in three urban sites (1, 3 and 30), more than one mineral element was visited (10 elements in the natural site and 10 elements in site 1, eight elements in site 3, and 15 elements in site 30, Fig. S2 and S3). In addition, an alternative protocol using baits (tuna and honey) was used on the same elements from the sites 1 to 3. Each bait was left in place for three hours and visited every 10 min the first hour and then every 20 min the last 2 h. All *Temnothorax* ants present on the bait or within a 10 cm radius were collected for further analysis.

Of the total samples collected for this study, only 10 contained one or more *Temnothorax* ants (seven samples in 2022, three samples in 2023). None of these records resulted from baiting; all were obtained through direct searches on mineral surfaces, using the same method as in Cordonnier et al. (2022). This suggests that baiting is not an effective sampling method for detecting slow-moving species of the *Temnothorax* genus.

Morphological and genetic identification

Morphological characteristics of all *Temnothorax* individuals (minimum five when more individuals were collected per collection site) were examined using a Leica binocular microscope (magnification: 30x). Particular attention was given to femora coloration (central portions darkened in *T. nigriceps*), overall body color (brown to almost black), and head-mesosoma contrast (head distinctly darker than mesosoma) (Salata & Borowiec 2013).

To confirm the identity of the species, genomic DNA was extracted from a single worker per sample ($n = 10$) using a CTAB method (modified from Sambrook & Russell, 2001). All samples were Sanger sequenced for a stretch covering the mitochondrial genes CO I and II including tLeu (primers C1-J-2183 Jerry: 5'-CAACATTTATTTTGATTTTTGG-3'

/ Cw.3031: 5'-TTTGCMCTWATCTGCCMTATT-3'). The 25 µl PCR reaction volume consisted of 12.5 µl buffer with Taq DNA polymerase, 9.5 µl ddH2O, 1 µl reverse primer, 1 µl forward primer (final concentration of 0.5 µM) and 1 µl DNA (2–10 ng). PCR consisted of initial denaturation at 94 °C (4 min), 37 cycles at 94 °C (denaturation, 45 s), 50 °C (annealing, 45 s) and 72 °C (elongation, 60 s), and a final step at 72 °C (5 min). PCR products were cleaned up using the NucleoSpin Gel and PCR Macherey–Nagel's Clean-up Kit and sequencing was conducted by LCG Genomics GmbH (Berlin, Germany).

Phylogenetic relationships among the mitochondrial haplotypes were based on sequence fragments ranging from 799 to 1409 bp in length (Genebank accession numbers: [PQ672614-PQ672622]). The sequences generated in this study were compared with sequences obtained from GenBank, including 9 *Temnothorax* species present in Germany and 2 *Leptothorax* species, covering all morphologically identified species except *T. albipennis*, for which no sequence was available. *T. albipennis*, along with *T. nigriceps* (Mayr), *T. tuberum* (F.), and *T. unifasciatus* (Latr.) forms a group of closely related species with tendencies for hybridization, often co-occurring in the same habitat (Douwes & Stille 1991, Salata & Borowiec 2013). Sequences were aligned using default options in MUSCLE v3.8.31 (Edgar 2004) as implemented in SeaView v4.2.9 (Gouy et al. 2010). The relationships were evaluated based on a tree constructed using the PhyML algorithm with the GTR distance without invariable sites, optimized nucleotide equilibrium frequencies, and tree-searching operations involving best of NNI and SPR.

Results

Among the 10 samples analyzed, five were identified as *T. nigriceps* based on morphological traits, which were subsequently confirmed by their mitochondrial DNA sequences (Fig. 2). This included one individual collected from the species' natural habitat (sampling site 2 in Fig. 1). *T. nigriceps* was thus sampled in only four human-made or human-modified environments (two samples in site 3, one sample from site 5 and one sample from site 6, Figs. 1 and 3). The five other samples belonged to *T. interruptus* (site 4), *Leptothorax gredleri* (site 8), *L. acervorum* (site 30), and unidentified *Temnothorax* species (sites 7 and 30—morphologically similar to *T. albipennis*). As a result, over the 3 days of sampling, the targeted species was recorded only four times, indicating either a low density of nests in urban habitats and/or a relatively hard detection in anthropized environments.

Among the suitable mineral elements investigated, those where *T. nigriceps* was collected probably were most similar to the species' natural habitat (see example in site 2 picture,

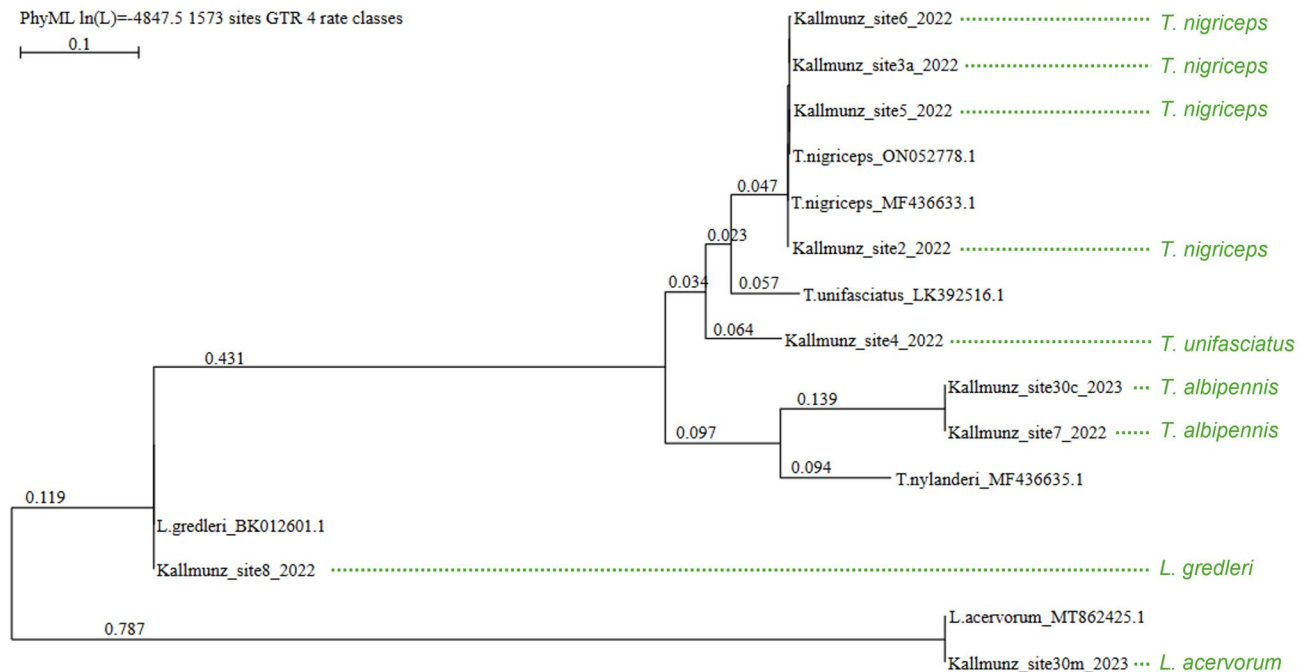Sequencing   Morphology

PhyML ln(L)=-4847.5 1573 sites GTR 4 rate classes

Fig. 2 Tree obtained from the COI–COII sequences built using the PhyML algorithm with the GTR distance without invariable sites, optimized nucleotide equilibrium frequencies, and tree-searching operations involving best of NNI and SPR. For all sequences gener-ated in this study, the associated morphological identification is indicated in green. All other sequences are named with the species ID and their GenBank accession number

Fig. 3). These collection sites, except for site 6 (Fig. 3), typically were older, unprocessed stones with rough, porous surfaces and associated vegetation. Two samples were collected within 50 m of each other at site 3 (graveyard of the city), in the same type of nesting material (Fig. 3).

Discussion

The rare detection of *T. nigriceps* suggests that it is absent from the urban area. The species may be highly selective in its habitat requirements and, therefore, may avoid nesting in highly transformed mineral surfaces. This result could be extended to other species within the genus as none of the records of *Temnothorax* or *Leptothorax* sp. was from material processed by humans. In addition, the presence of competing ant species in urban areas, often characterized by aggressive behavior and larger colony sizes, may hinder the establishment of *Temnothorax* colonies through competition by interference or by direct interaction (Gibb and Hochuli 2003; Holway and Suarez 2006; Stringer et al. 2009).

Temnothorax species are generally considered predators or scavengers (Seifert 2018; Giannetti et al. 2022), but some of them can also feed on dropped-off honey dew, nectar of floor plants or crushed nuts (Fokuhl et al. 2012).

In addition, species, such as *T. nylanderi*, exhibit a limited foraging range around their nest (Heinze et al., 1996). *T. nigriceps*, therefore, likely depends on microhabitats offering sufficient prey or alternative food sources. While the presence of suitable nesting sites is essential, the availability of adequate food resources is another critical factor for colony establishment and persistence in urban areas.

The low number of *T. nigriceps* samples collected in urban area during the study period may also be partly attributed to a low detection of the nests rather than an absence of the species. The relatively small colony size and cryptic behavior typical of *Temnothorax* and *Leptothorax* species likely contribute to their low detectability in complex urban environments. In addition, high ambient temperatures (> 30 °C) may have reduced ant activity on overheated mineral substrates. Urban areas tend to favor ant species adapted to warmer, drier environments (Menke et al. 2011), which could influence the distribution of *T. nigriceps* within the city. The possibility of seasonal nest displacement in microhabitats with cooler conditions cannot be excluded. Earlier sampling, such as in early May, might have yielded higher detection rates. However, the detection of the species in its natural habitat under similar high-temperature conditions suggest that the low presence

Fig. 3 Pictures of the collection sites where *Temnothorax* or *Leptothorax* individuals have been collected. Site 2 (top left) is a rock located in natural habitat of *T. nigriceps*. All the other mineral elements are located in the urban area of Kallmünz, Germany (see Fig. 1 for the map). The species identified based on mtDNA (or morphology for *T. albipennis*) are indicated below each picture

of *T. nigriceps* in urban areas is primarily linked to its limited occupation of anthropogenic structures.

Consistent with the previous factors (i.e., urban areas providing drier and warmer shelter, and necessity to find food resources around the suitable nest material), cemeteries could represent refuges for *T. nigriceps*, offering a combination of nesting substrates, vegetation, and water resources. These conditions, combined with reduced human disturbance, may create favorable habitats for both the ants and their nutritional resources. The role of cemeteries in maintaining biodiversity within cities is known for a large range of taxa (Löki et al. 2019), with previous examples in ants (e.g., Klesniaková et al. 2016).

Based on our findings, urban habitats appear to be poor substitutes for the natural habitats of *Temnothorax nigriceps*. A wide utilization of human-modified mineral elements as alternative shelters to prevent isolation and maintain the gene flow between fragmented populations of *T. nigriceps* is, therefore, unlikely. Punctual human-mediated dispersal by translocation of nesting material from natural habitats, or the use of other, right now unidentified types of habitats would be forthcoming more efficient to prevent the genetic isolation of the population.

Declarations

Conflicts of interests The authors have no competing interests to declare that are relevant to the content of this article.

References

Cordonnier M, Gibert C, Bellec A, Kaufmann B, Escarguel G (2019) Multi-scale impacts of urbanization on species distribution within the genus Tetramorium. Landscape Ecol 34:1937–1948

Cordonnier M, Felten D, Trindl A, Heinze J, Bernadou A (2022) Absence of genetic isolation across highly fragmented landscape in the ant *Temnothorax nigriceps*. BMC Ecology and Evolution 22(1):1–11

Cordonnier M, Lindner T, Heinze J (2023) Fragmentation shapes nest density and social structure but not genetic diversity of *Temnothorax crassispinus* (Formicidae). Popul Ecol 65(3):192–202

Douwes P, Stille B (1991) Hybridization and variation in the Leptothorax tuberum group (Hymenoptera: Formicidae). J Zool Syst Evol Res 29(3):165–175

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32(5):1792–1797

Fokuhl G, Heinze J, Poschlod P (2012) Myrmecochory by small ants–Beneficial effects through elaiosome nutrition and seed dispersal. Acta Oecologica 38:71–76

Giannetti D, Schifani E, Gugliuzzo A, Zappalà L, Biondi A, Grasso DA (2022) Native European ants can discourage host colonization and reduce reproductive success of the invasive ambrosia beetle Xylosandrus compactus. Biol Control 174:105032

Gibb H, Hochuli DF (2003) Colonisation by a dominant ant facilitated by anthropogenic disturbance: effects on ant assemblage composition, biomass and resource use. Oikos 103(3):469–478

Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27(2):221–224

Heinze J, Foitzik S, Hippert A, Hölldobler B (1996) Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. Ethology 102(3):510–522

Helms JA, Godfrey AP, Ames T, Bridge ES (2016) Predator foraging altitudes reveal the structure of aerial insect communities. Sci Rep 6(1):28670

Holway DA, Suarez AV (2006) Homogenization of ant communities in Mediterranean California: the effects of urbanization and invasion. Biol Cons 127(3):319–326

Khimoun A, Doums C, Molet M, Kaufmann B, Peronnet R, Eyer PA, Mona S (2020) Urbanization without isolation: the absence of genetic structure among cities and forests in the tiny acorn ant Temnothorax nylanderi. Biol Lett 16(1):20190741

Klesniaková M, Holecová M, Pavlíková A (2016) Interesting ant species in the urban greenery of Bratislava. Folia faunistica Slovaca 21(3):235–238

Löki V, Deák B, Lukács AB, Molnár A (2019) Biodiversity potential of burial places–a review on the flora and fauna of cemeteries and churchyards. Global Ecology and Conservation 18:e00614

Lowry H, Lill A, Wong BB (2013) Behavioural responses of wildlife to urban environments. Biol Rev 88(3):537–549

Menke SB, Guénard B, Sexton JO, Weiser MD, Dunn RR, Silverman J (2011) Urban areas may serve as habitat and corridors for dry-adapted, heat tolerant species; an example from ants. Urban Ecosystems 14:135–163

Noordijk J, Morssinkhof R, Boer P, Schaffers AP, Heijerman T, Sýkora KV (2008) How ants find each other; temporal and spatial patterns in nuptial flights. Insectes Soc 55:266–273

Poschlod P, WallisDeVries MF (2002) The historical and socioeconomic perspective of calcareous grasslands—lessons from the distant and recent past. Biol Cons 104(3):361–376

Poschlod P, Karlík P, Baumann A, Wiedmann B (2008) The history of dry calcareous grasslands near Kallmünz (Bavaria) reconstructed by the application of palaeoecological, historical and recent-ecological methods. Human nature: studies in historical ecology and environmental history. Institute of Botany of the Czech Academy of Sciences, Brno, CZ, 130–143.

Salata S, Borowiec L (2013) *Temnothorax albipennis*(CURTIS, 1854) in Poland and identification of the T. *tuberum* species complex (Hymenoptera: Formicidae). Genus 24(3–4):403–413

Sambrook J, Russell DW (2001) Detection of DNA in agarose gels. Molecular Cloning, A Laboratory Manual,(3rd Ed.) Cold Spring Harbor Laboratory Press, New York, 5–14.

Seifert B (2018) The ants of Central and North Europe. Lutra Verlags- und Vertriebsgesellschaft, Tauer, Germany, p 408

Stringer LD, Stephens AE, Suckling DM, Charles JG (2009) Ant dominance in urban areas. Urban Ecosystems 12:503–514

Suefuji M, Heinze J (2014) The genetic population structure of two socially parasitic ants:"the active slave-maker" *Myrmoxenus ravouxi* and the "degenerate slave-maker" *M. kraussei*. Conserv Genet 15:201–211