Monitoring the influx of new species through citizen science: The first introduced ant in Denmark

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Climate change and invasive species threaten biodiversity, yet rigorous monitoring of their impact can be costly. Citizen science is increasingly used as a tool for monitoring exotic species, because citizens are geographically and temporally dispersed, whereas scientists tend to cluster in museums and at universities. Here we report on the establishment of the first exotic ant taxon (*Tetramorium immigrans*) in Denmark, which was discovered by children participating in The Ant Hunt. The Ant Hunt is a citizen science project for children that we ran in 2017 and 2018, with a pilot study in 2015. *Tetramorium immigrans* was discovered in the Botanical Garden of the Natural History Museum of Denmark in 2015 and confirmed as established in 2018. This finding extends the northern range boundary of *T. immigrans* by almost 460 km. Using climatic niche modelling and species distribution modelling, we compared the climatic niche of *T. immigrans* in Europe with that of *T. caespitum* based on confirmed observations from 2006-2019. *Tetramorium immigrans* and *T. caespitum* had a 23 % niche overlap, with *T. immigrans* showing stronger occurrence in warmer and drier areas compared to *T. caespitum.* Species distribution modelling identified several, currently uninhabited, areas as climatically suitable for *T. immigrans*, making it likely that this species will continue to spread throughout Europe. Furthermore, *T. immigrans* was sampled almost three times as often in areas with artificial surfaces compared to *T. caespitum*, suggesting that *T. immigrans* may not be native to all of Europe and is being accidentally introduced by humans. Overall, citizen scientists collected data on ants closer to cities and harbours than scientists, and had a stronger bias towards areas of human disturbance. This increased sampling effort in areas of likely introduction of exotic species naturally increases the likelihood of discovering species sooner, making citizen science an excellent tool for exotic species monitoring, as long as trained scientists

are involved in the identification process.

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Abstract

Climate change and invasive species threaten biodiversity, yet rigorous monitoring of their impact can be costly. Citizen science is increasingly used as a tool for monitoring exotic species, because citizens are geographically and temporally dispersed, whereas scientists tend to cluster in museums and at universities. Here we report on the establishment of the first exotic ant taxon (*Tetramorium immigrans*) in Denmark, which was discovered by children participating in The Ant Hunt. The Ant Hunt is a citizen science project for children that we ran in 2017 and 2018, with a pilot study in 2015. *Tetramorium immigrans* was discovered in the Botanical Garden of the Natural History Museum of Denmark in 2015 and confirmed as established in 2018. This finding extends the northern range boundary of *T. immigrans* by almost 460 km. Using climatic niche modelling and species distribution modelling, we compared the climatic niche of *T. immigrans* in Europe with that of *T. caespitum* based on confirmed observations from 2006-2019. *Tetramorium immigrans* and *T. caespitum* had a 23 % niche overlap, with *T. immigrans* showing stronger occurrence in warmer and drier areas compared to *T. caespitum.* Species distribution modelling identified several, currently uninhabited, areas as climatically suitable for *T. immigrans*, making it likely that this species will continue to spread throughout Europe. Furthermore, *T. immigrans* was sampled almost three times as often in areas with artificial surfaces compared to *T. caespitum*, suggesting that *T. immigrans* may not be native to all of Europe and is being accidentally introduced by humans. Overall, citizen scientists collected data on ants closer to cities and harbours than scientists, and had a stronger bias towards areas of human disturbance. This increased sampling effort in areas of likely introduction of exotic species naturally increases the likelihood of discovering species sooner, making citizen science an excellent tool for exotic species monitoring, as long as trained scientists are involved in the identification process.

Introduction

The introduction and establishment of new species outside of their native range, which then go on to become invasive, threaten biodiversity (IPBES, 2019). Monitoring introduced and invasive species (species that have been introduced to an area outside of their range by humans and as invasives have a detrimental impact on nature, economy or human health) has been challenging in general, but has become increasingly problematic and important with accelerating trade, commerce (Meurisse *et al.*, 2019) and climate change (Bellard *et al.*, 2013). Recently, citizen science is being called on as a potential tool for successfully monitoring biodiversity on a large scale (Tulloch *et al.*, 2013; Theobald *et al.*, 2015; Sauer *et al.*, 2017). Citizen science can help to document introduced species in general, but particularly in some of the habitats typically missed by traditional surveys.

Cities and harbours are both hotspots of introduction. In cities, conditions are typically warmer due to the urban heat island effect (Oke, 1973) and are ideal starting points for the spread of new species (von der Lippe & Kowarik, 2007). Likewise, harbours are a major introduction pathway (O'Connor & Weston, 2010), especially of ants (Suarez *et al.*, 2010). Yet studies of ant communities in urban ecosystems are rare (but see Lessard & Buddle, 2005; Toennisson *et al.*, 2011) In Denmark, 80 % of foreign trade goes through Danish industrial harbours (Danske Havne, 2017). Among other products, these ships carry agricultural products, fodder and fertilisers through which new species can be introduced. While general surveys in cities and harbours can be difficult, these are some of the habitats most available to citizen scientists.

Social insects, including ants, are among the most damaging invaders (Holway *et al.*, 2002). Over 150 ant species have been introduced outside their native range and five are listed on the world's 100 most invasive species list by the Invasive Species Specialist Group (ISSG) (Lowe *et al.*, 2000; Sanders & Suarez, 2011). Invasions by non-native ants has been shown to be destructive to ecosystems and financially costly to humans (Holway *et al.*, 2002; Del Toro *et al.*, 2012). Although Denmark currently has no established and widespread invasive ants, thirty non-native species have so far been recorded (Schär *et al.*, 2017), most of them in hothouses in cities.

In 2017 and 2018, with a pilot study in 2015, children, in schools or with their families, collected ants for a project called the Ant Hunt (In Danish, "Myrejagten") at 792 sites Denmark. Although not the main aim of the project, we predicted that due to the expected amount of sampling in urban areas, new species for Denmark were likely to be discovered.

Here we report on the discovery of a newly established ant taxon for Denmark through the citizen science project "the Ant Hunt", *Tetramorium immigrans* (Santschi, 1927). *Tetramorium immigrans* has recently been raised to the level of a cryptic sister species of the morphologically similar *T. caespitum* (Wagner *et al.*, 2017). An even more recent study has demonstrated that *T. immigrans* and *T. caespitum* hybridize (Cordonnier *et al.*, 2019), making identification difficult.

Tetramorium caespitum and *T. immigrans* are both palearctic species (Wagner *et al.*, 2017). However, while *T. caespitum* is common across all of Europe (Seifert, 2007), *T.*

immigrans is primarily found in the Mediterranean, Western Europe, Central Europe, the Balkans, Eastern Europe, Anatolia and Caucasus (Wagner *et al.*, 2017), is more thermophilic and has a more southern and urban distribution than *T. caespitum* (Seifert, 2018; Wagner *et al.*, 2017). Using ecological niche modelling, we compare the climatic niche and habitat use in Europe for these two taxa, in order to determine potential differences in their ecological preferences. Finally, we compare the distance to cities and harbours along with sampled habitat types for data collected through the Ant Hunt with data collected by scientists from 1990-2015 to determine the extent to which data collection by citizens is or is not poised to help document introductions and shifting distributions.

Materials & Methods

Biological data

During the Ant Hunt, families and schools across Denmark collected ants by conducting baiting experiments at a site of their choosing. Participants ranged across all ages, but the average participants were children aged 5-11 years accompanied by a grown-up aged 31-50. They set out six different resources (saltwater, sugar water, oil, dissolved protein powder, a cookie and water) on bait cards and waited for two hours before collecting all the ants that were foraging on the cards. Ants were then frozen and counted before they were sent to the Natural History Museum of Denmark for identification. All experiments were registered in an online database with date and GPS-coordinates (see supplemental material S1 for detailed protocol). In total, families and schools completed 792 experiments, of which 566 contained ants.

The ants were identified using a variety of taxonomic keys (Collingwood, 1979; Seifert, 2007; Douwes *et al.*, 2012; Lebas *et al.*, 2016; Wagner *et al.*, 2017). In total, participants had collected 16,985 specimens from 29 species (Table 1). Of these, specimens from two experiments could not be identified to species level due to missing body parts and specimens from two experiments were flagged as potentially new taxa for Denmark. These were *T. immigrans* and *Technomyrmex albipes*. The establishment of *Technomyrmex albipes* could not be confirmed. However, after the original discovery of *T. immigrans* during the Ant Hunt, trained scientists resurveyed the location several times throughout 2015-2019. The presences of *T. immigrans* was confirmed during every survey and the species was seen to expand to an area of approx. 40 metres, with more than 20 nest entrances along the pavement.

Tetramorium immigrans (Fig. 1a and Fig. 1b) tends to have a larger overall body size, denser striation/sculpturation of the head, thorax and petiolar nodes, as well as a more pronounced microscopic scale pattern on the first gastral tergite than *T. caespitum* (Wagner *et al.*, 2017). Because of the difficulty in distinguishing *T. immigrans* from other species in the *T. caespitum* complex, we visually inspected all specimens found of *T. caespitum* in the Ant Hunt and randomly selected 10 samples from a broad range of localities (Fig. 1c). These were then compared to the sixty-seven specimens of *Tetramorium* workers from the Botanical garden of

Copenhagen (Fig. 1d), which were examined visually for morphological characters distinguishing typical forms of *T. immigrans* and *T. caespitum*. Voucher specimens were stored at the Natural History Museum of Denmark (NHMD 0000188537).

One specimen was chosen to be inspected by X-ray micro computed tomography (MicroCT). The specimen was placed on a designed holder and placed inside a Zeiss Xradia 410 versa system. The system was operated with a high voltage of 40kV and a power of 10W. The data acquisition consisted of 3201 images while rotating 360 degrees, each image had a pixel size of 4.14µm. All data was reconstructed into a 4mm by 4mm by 4mm 3D volume with a voxel size of 4.14µm. The reconstructed image is shown in figure 1b and raw data is available through Figshare (Gundlach, 2019).

DNA analysis

For the DNA analysis of *T. immigrans* we selected two specimens from the confirmed find in the Botanical Gardens of Copenhagen, one from 2015 and one from 2018 and further selected 14 specimens of presumed *T. caespitum*, of which 10 had known coordinates and the remaining four were known to be from somewhere in Denmark.

Up until DNA extraction, all samples from the Ant Hunt were kept in 96 % ethanol in a freezer, while samples from the Natural History Museum of Denmark had been kept as pinned specimens. We extracted DNA by cutting off a small piece of the middle leg of each specimen, to which we added 100 µl of 10 % Chelex in Tris solution. This was mixed and centrifuged for 10 minutes, after which the solution was heated to 99° C for 15 minutes and centrifuged again. The supernatant was used as a template for PCR reactions. We used primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) to amplify mitochondrial COI gene and primer D2B and D3A-r (Saux *et al.*, 2004) to amplify nuclear 28S rDNA gene. PCR reactions were carried out using REDTaq ReadyMix PCR Reaction Mix with 10 µg/mL Bovine serum albumin. The PCR reaction conditions consisted of an initial denaturing step of 94°C for 5 minutes, followed by 35 cycles of 94°C for 40 seconds, 48°C (LCO1490/HCO2198) or 56°C (D2B/D3A-r) for 40 seconds, and 72°C for 60 seconds, and finally an extension step at 72°C for 5 minutes. PCR products were purified using Invitek PCR clean-up MSB spin PCRapace kit and Sanger sequenced in both directions using the Mix2Seq from Eurofins.

Molecular identification was carried out by comparing samples to reference sequences from Wagner et al. 2017 (COI) and Schär et al. 2018 (28S). Raw sequences were edited and aligned using the software geneious v. 2019.0.4. A maximum likelihood tree with the best-fit model automatically selected by modelfinder and 1,000 rapid bootstrap replications was created for the alignment of COI sequences, using the program "IQ-TREE" v. 1.6.1 (Nguyen *et al.*, 2015)).

Tetramorium immigrans has previously been found to be distinguished from similar species (including *T. caespitum*) by having a one base insertion (C) at site 438 of the 28S rDNA fragment amplified by the primers D2B and D3A-r (Saux *et al.*, 2004; Schär *et al.*, 2018). We aligned the sequence of the specimen from Copenhagen to the reference sequences mentioned above to see if the characteristic insertion of *T. immigrans* is present.

Climatic niches of *T. immigrans* and *T. caespitum*

We compared the environmental niche and geographical distribution of *T. immigrans* and *T. caespitum* in Europe using occurrence data from AntMaps (Guénard *et al.*, 2017) and the Ant Hunt along with 10 climatic variables from Worldclim 1.4 at 2.5 arc-minutes (~5 km) resolution (Hijmans *et al.*, 2005).

Due to the recent distinction between *T. caespitum* and *T. immigrans* (Schlick-Steiner *et al.*, 2006; Wagner *et al.*, 2017), we used data points from 2006 onwards from trusted AntMaps sources (Borowiec & Salata, 2018b; Espadaler *et al.*, 2018, Schär *et al.*, 2018; Wagner *et al.*, 2018; Wagner *et al.*, 2017). This resulted in 739 samples of *T. caespitum* and 187 samples of *T. immigrans.* Since *T. Immigrans* was detected in only one location in Denmark, this one location does not contribute much to the overall niche analysis for the species. We selected the climatic variables mean annual temperature, annual temperature seasonality, mean temperature of warmest quarter, mean temperature of coldest quarter, mean annual precipitation, annual precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter and precipitation of coldest quarter because these have been suggested to be the most relevant to *T. immigrans* (Schlick-Steiner *et al.*, 2006; Steiner *et al.*, 2008).

Using the ecospat package (Broennimann *et al.*, 2018) in R (R Core Team, 2018), we compared the environmental niches of *T. caespitum* and *T. immigrans* in a gridded environmental space, where each cell corresponds to a unique set of the 10 climatic variables. We first calculated the density of occurrences and the climatic variables along two climatic axes of a multivariate analysis and then measured the niche overlap along the gradients of this multivariate analysis (as in Broennimann *et al.*, 2012). Niche overlap was calculated using Schoener's overlap metric "D" (Schoener, 1968, 1970), which in this case compares the frequency of observations for each species within the chosen climatic categories (Schoener, 1968). However, we acknowledge that there are other ways to measure niche overlap (see for example Seifert, 2017).

To test for niche equivalency and niche similarity, we compared the observed D metric with 1000 simulated values of D. The niche equivalency test determines whether the overlap between the niches of the two species is higher than two random niches drawn from the same data pool. The niche similarity test determines whether the overlap between the two niches is higher than when one species' niche is randomly drawn in the study area (Broennimann *et al.*, 2012). If the observed value of D falls within the density of 95% of the simulated values, there is no detectable difference in the climatic niche of *T. immigrans* and *T. caespitum*. Finally, we projected the environmental niche of *T. immigrans* and *T. caespitum* onto Europe and visually compared the two species.

Habitat differences

We used the CORINE 100x100 m land cover raster dataset (Copernicus, 2012) and extracted land cover values for all data points for both species using the spatial analysis tool 'extract values to points' in ArcGIS (ESRI, 2010). The CORINE land cover dataset consists of 48 land cover types. For this analysis we excluded all data points that were labelled with no data or one of the water based land cover types ("water bodies", "water courses", "sea and ocean"). The remaining 39 land cover types were reduced to six major classes ("Artificial surface", "Agriculture", "Forest", "Scrub and/or herbaceous vegetation associations", "Open spaces with little or no vegetation" and "Wetlands") following the CORINE land cover nomenclature (Copernicus, 2015).

To test whether sampling of *T. immigrans* and *T. caespitum* were spatially biased, we compared the fraction of samples within each of the six land cover classes with the fraction of these land cover types in Europe using chi-square tests. We then did the same comparing the two species to each other to determine if *T. immigrans* and *T. caespitum* were being sampled in different habitats.

Monitoring for new species

In order to determine the suitability of citizen science for early detection and monitoring of introduced species, we compared the citizen scientist collected dataset of the Ant Hunt with a dataset collated from the Natural History Museum of Denmark, a personal collection by Sämi Schär and the Ph.D. course EuroAnts, which was collected in Denmark from 1990-2015, with 2015 being the most recent year with available data. We compared the datasets based on two measures, 1) distance to likely introduction sites and 2) sampling effort in different land cover types. We calculated the average distance from data points in each dataset to Denmark's seven major cities (cities of 50.000+ inhabitants) and 31 major harbours (harbours with a yearly goods turnover of one million ton) in ArcGIS (ESRI, 2010) and compared the two datasets using a Mann Whitney U-test.

For the land cover analysis, we used the above-mentioned major categories, but also included the category Water as an approximation of samples collected close to the shore of water bodies (Copernicus, 2015). We summarised the number of samples collected in these seven major land cover classes and compared the observed values with expected values based on the availability of each land cover class using Chi-square tests. Based on availability of each land cover class we calculated the ratio of observed samples to expected samples to determine how much more or less a specific land cover type was sampled than what would be expected by chance.

Results

Tetramorium immigrans

Tetramorium immigrans was first discovered in the Botanical Garden of Copenhagen (55.69, 12.57) during a pilot run of the citizen science project in 2015 and was confirmed to be established and spreading in 2018 and 2019.

Based on a maximum likelihood tree of the mitochondrial COI gene (Fig. 2a), the existence of a characteristic one base insertion (C) in the 28S rDNA fragment (Fig. 2b), and visual examination of diagnostic characters we regard the find of *T. immigrans* in Denmark to be verified. This extends the northern limit of *T. immigrans* in Europe by three degrees latitude from Gmina Janowiec Wielkopolski, Poland (52.73, 17.50, Borowiec & Salata, 2018a) to Copenhagen, Denmark (55.69, 12.57), almost 460 km.

Climatic niches of *T. immigrans* and *T. caespitum*

Environmental niche overlap between *T. immigrans* and *T. caespitum*, measured as D, which compares the frequency of observations for each species within the chosen climatic categories, was 23 % with only a slight difference of the niche centroid in environmental space (Fig. 3a). Based on the contribution of the ten climatic variables along the two axes, *T. caespitum* is present in colder and wetter areas, compared to *T. immigrans,* which prefers warmer and drier conditions with stable temperatures (Fig. 3b). *Tetramorium immigrans* and *T. caespitum* differed in mean climatic values for eight of the ten variables (Table S1 and Fig. S1), with no difference in precipitation seasonality and precipitation of coldest quarter. Despite these slight differences, the two species' niches were more similar to each other than two randomly drawn niches within the same data pool (niche equivalency test, $p = 1$) and more similar than when a random niche of either species was drawn within the available climatic space (niche similarity test, $p = 0.3$).

Projection of the climatic niche into geographical space shows that both species appear able to tolerate the climatic conditions of most of Southern and Central Europe, along with the southern part of Northern Europe (Fig. 3cd), although *T. immigrans* may have a slightly more southern distribution than *T. caespitum*.

Habitat differences

The current available data does not allow for an exact determination of the land use of *T. caespitum* and *T. immigrans*, but we can determine in which land cover types the two species are currently observed. *Tetramorium caespitum* was mostly observed in forest and agricultural land use types (34.1 % and 34.96 % of observations, respectively, Table 2). *Tetramorium immigrans* was mostly found in land types with artificial surfaces (48.89 %, Table 2). Both species were rarely found in wetland areas (*T. immigrans*: 1.11 % and *T. caespitum* 0.14 % of observations).

Observations of both species differed significantly from what would be expected if land use reflected availability of the six land cover types in Europe (Chi square tests, $\chi^2 = 322.71$, df = 5, $p < 0.001$ for *T. immigrans* and $\chi^2 = 243.95$, df = 5, $p < 0.001$ for *T. caespitum*).

The two species also showed significant difference in the number of observations in each land cover type compared to each other (Chi-square test, $\chi^2 = 82.66$, df = 5, $p < 0.001$). *Tetramorium immigrans* was found almost three times more often in land use types with artificial surfaces than *T. caespitum* (ratio: 0.35; Table 2). On the other hand, *T. caespitum* was sampled over twice as often in forests than *T. immigrans* (ratio 2.56; Table 2).

Monitoring for new species

Overall, data collected by citizen scientists during the Ant Hunt was significantly closer to cities than data collected by scientists from 1990-2019 (mean 31.27 km ± 29.72 SD and 34.52 km ± 23.21 SD, respectively, W = 127623, $p < 0.001$). For the Ant Hunt, 101 of 667 observations (15 %) were within Denmark's major cities compared to only 4 out of 448 (0.9 %) scientist-collected samples. Data from the Ant Hunt was also significantly closer to major harbours than data from scientists (20.66 km ± 14.90 SD and 28.25 km ± 12.62 SD, respectively, W = 98154, $p < 0.001$). Only six of the major harbours in Denmark were outside of cities with more than 5000 residents (Fig. 4), suggesting a high correlation between industrial harbours and residential areas.

Both scientists and citizen scientists were significantly biased regarding which land cover classes they sampled within (Chi-square test, $\chi^2 = 653.75$, df = 6, $p < 0.001$ and $\chi^2 = 3094.4$, df = 6, $p < 0.001$, respectively). However, although both datasets were biased towards areas with artificial surfaces, the effect was far more pronounced among citizen scientists, who sampled artificially surfaced areas eight times more than expected. Scientists only sampled artificial surface areas three times more than expected by random sampling. Both citizens and scientists avoided agricultural areas, but scientists sampled forests, scrub, coastal areas and wetlands 2-3 times more than expected by random sampling (Table 3).

Discussion

Genetic comparison of the Danish *Tetramorium immigrans* samples with samples from Wagner et al. 2017 (COI) and Schär et al. 2018 (28S) confirmed that *T. immigrans* is established in Denmark. However, genetic analysis of 14 additional *Tetramorium* samples from Denmark collected during the Ant Hunt in 2017 and 2018 conclude that, so far, the distribution of *T. immigrans* in Denmark is limited to the Botanical Garden of Copenhagen.

The climatic niche of the two species only overlapped by 23 % and our study confirms previous assessments that *T. immigrans* prefers warmer and drier climates than *T. caespitum* (Seifert, 2018). Mean temperature of warmest quarter (°C) was 20.27 ± 2.06 SD for *T. immigrans* and 16.77 ± 2.43 SD for *T. caespitum*. This is in accordance with previously recorded standard air temperatures (°C) for May-August for both species (19.9 ± 2.5 and 16.1 ± 2.0 SD, respectively; Wagner *et al.*, 2017). Coupled with the large amount of observation of *T. immigrans* within cities and the large gap in the known distribution from Poland to Denmark, we deem it likely that this species will also establish in other European cities, if it is not there already.

It has already been hypothesized that *T. immigrans* may not be native in most of Europe. Specifically, the species is thought to be introduced in France, Germany and Poland (Gippet *et al.*, 2017; Borowiec & Salata, 2018a; Seifert, 2018; Cordonnier *et al.*, 2019). If true, its' current observed distributional focus in Southern Europe, along with the highest COI variability being in Anatolia and the Caucausus region (Wagner *et al.*, 2017) could be an indication of its' origin. This would also explain the discrepancies between its' current distribution and modelled distribution based on its climatic niche, which suggests the species should be present in eastern Germany (Fig. 3d). However, a second explanation for the suitability of eastern Germany may be that it is an artefact of including records from cities, which are typically warmer than non-urban areas.

Based on the current locations where *T. immigrans and T. caespitum* have been recorded, it appears that *T. immigrans* is limited to areas of human disturbance. However, a more systematic search for *T. immigrans* in areas where it is widespread is needed, since our current study does not account for sampling bias.

While not a main goal of the Ant Hunt, the finding of this species shows how the engagement of untrained volunteers, even children, can be a great asset to the monitoring of biodiversity, especially when it comes to detecting newly introduced species. This is evident from the sample bias of citizen scientists towards cities, harbours and areas of high human disturbance. On the other hand, scientists are more prone to sampling in natural areas. We argue the case that engaging the aid of amateur participants; even as young as 6-10 year-old children, can be a valuable tool for biodiversity monitoring. Citizen scientists are best able to search for species where scientists are most likely to miss them and where introductions are most likely.

Conclusions

We hypothesized that, although not necessarily the main goal of citizen science projects, these projects have a high likelihood of turning up new species, due to the large amount of sampling being carried out in areas of likely introduction, such as harbours and cities.

During the Ant Hunt, a citizen science project, where children set out baiting experiments to help understand the community composition and resource requirements of ants under different environmental conditions, two new species were discovered. One species, *Tetramorium immigrans*, was determined to be established in the Botanical Garden of the Natural History Museum of Denmark in Copenhagen.

Our findings push the distribution of *T. immigrans* north by almost 460 km. Our subsequent analysis of the climatic niche and potential geographical distribution of *T. immigrans* adds some support to the current speculation that this species may not be native to all of Europe and is spreading through introduction to cities, with many currently uninhabited locations identified as climatically suitable. A systematic survey of the land use preferences of *T. immigrans* along with a genetic mapping is needed to fully map and understand the dispersal of *T. immigrans* across Europe.

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References

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M. & Courchamp, F. (2013) 'Will climate change promote future invasions?', Global Change Biology, 19: 3740–3748. doi: 10.1111/gcb.12344.

Borowiec, L. & Salata, S. (2018a) 'Tetramorium immigrans Santschi, 1927 (Hymenoptera: Formicidae) nowy gatunek potencjalnie inwazyjnej mrówki w Polsce', Acta entomologica silesiana 26: 1–5. doi: 10.5281/zenodo.1169156.

Borowiec L. & Salata, S. (2018b) 'Notes on ants (Hymenoptera: Formicidae) of the Euboea Island, Central Greece', Annals of the Upper Silesian Museum in Bytom Entomology 27(5): 1-15.

Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M.-J., Randin, C., Zimmermann, N. E., Graham, C. H. & Guisan, A. (2012) 'Measuring ecological niche overlap from occurrence and spatial environmental data', Global Ecology and Biogeography, 21(4): 481–497. doi: 10.1111/j.1466-8238.2011.00698.x.

Broennimann, O., Cola, V. Di & Guisan, A. (2018) 'ecospat: Spatial Ecology Miscellaneous Methods. R package version 3.0.' Available at: https://cran.r-project.org/package=ecospat.

Collingwood, C. A. (1979) The Formicidae (Hymenoptera) of Fennoscandia and Denmark, Fauna Entomolgica Scandinavica.

Copernicus (2012) 'Corine Land Cover 100m'. European Union, Copernicus Land Monitoring Service.

Copernicus (2015) CORINE Land Cover nomenclature conversion to Land Cover Classification system. Available at: https://land.copernicus.eu/eagle/files/eagle-related-projects/pt_clc-conversion-to-fao-lccs3_dec2010.

Cordonnier, M., Gayet, T., Escarguel, G. & Kaufmann, B. (2019) 'From hybridization to introgression between two closely related sympatric ant species', Journal of Zoological Systematics and Evolutionary Research: 1–11. doi: 10.1111/jzs.12297.

Danske Havne (2017) Danish Harbours. Available at: http://www.danskehavne.dk/ (Accessed: 19 January 2017).

Douwes, P., Abenius, J., Cederberg, B., Wahlstedt, U., Hall, K., Starkenberg, M., Reisborg, C. & Östman, T. (2012) Nationalnyckeln till Sveriges flora och fauna. Steklar: Myror - getingar. Hymenoptera: Formicidae-Vespidae. Uppsala: ArtDatabanken, SLU.

Espadaler X., Pradera, C. & Santana, J.A. (2018) 'The first outdoor-nesting population of Wasmannia auropunctata in continental Europe (Hymenoptera, Formicidae)', Iberomyrmex 10: 1-8.

ESRI (2010) 'ArcGIS'. Environmental Systems Research Institute.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) 'DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates', Molecular marine biology and biotechnology, 3(5): 294–299.

Gippet, J. M. W., Mondy, N., Diallo-Dudek, J., Bellec, A., Dumet, A., Mistler, L. & Kaufmann, B. (2017) 'I'm not like everybody else: urbanization factors shaping spatial distribution of native and invasive ants are species-specific', Urban Ecosystems, 20, pp. 157–169. doi: 10.1007/s11252-016-0576-7.

Guénard, B., Weiser, M. D., Gómez, K., Narula, N. & Economo, E. P. (2017) 'The Global Ant Biodiversity Informatics (GABI) database: Synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae)', Myrmecological News, 24: 83–89.

Gundlach, C. (2019) Tetramorium immigrans visualisation files. figshare. Media.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. (2005) 'Very high resolution interpolated climate surfaces for global land areas', International Journal of Climatology, 25(15): 1965–1978. doi: 10.1002/joc.1276.

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D. & Case, T. J. (2002) 'The causes and consequences of ant invasions', Annual Review of Ecology and Systematics, 33: 181–233. doi: 10.1146/annurev.ecolsys.33.010802.150444.

IPBES (2019) Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science- Policy Platform on Biodiversity and Ecosystem Services. Edited by E. S. Brondizio et al. Bonn: IPBES Secretariat.

Lebas, C., Galkowski, C., Blatrix, R. & Wegnez, P. (2016) Fourmis d'Europe occidentale. Paris: delachaux et niestlé.

von der Lippe, M. & Kowarik, I. (2007) 'Long-distance dispersal of plants by vehicles as a driver of plant invasions.', Conservation biology : the journal of the Society for Conservation Biology, 21(4): 986–96. doi: 10.1111/j.1523-1739.2007.00722.x.

Lessard, J.-P. & Buddle, C. M. (2005) 'The effects of urbanization on ant assemblages (Hymenoptera: Formicidae) associated with the Molson Nature Reserve, Quebec', The Canadian Entomologist, 137(02): 215–225. doi: 10.4039/n04-055.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. (2000) '100 of the world's worst invasive alien species. A selection from the Global Invasive Species Database', The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), p. 12. doi: 10.1614/WT-04-126.1.

Meurisse, N., Rassati, D., Hurley, B. P., Brockerhoff, E. G. & Haack, R. A. (2019) 'Common pathways by which non-native forest insects move internationally and domestically', Journal of Pest Science, 92(1): 13–27. doi: 10.1007/s10340-018-0990-0.

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. (2015) 'IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies', Molecular Biology and Evolution, 32(1): 268–274. doi: 10.1093/molbev/msu300.

O'Connor, S. & Weston, G. P. (2010) 'Box 13.1', in Lach, L., Parr, C. L. & Abbott, K. L. (eds.) Ant Ecology. New York: Oxford University Press, p. 242–243.

Oke, T. R. (1973) 'City size and the urban heat island', Atmospheric Environment Pergamon Pres, 7: 769–779. doi: 10.1016/0004-6981(73)90140-6.

R Core Team (2018) 'R: A language and environment for statistical computing.' Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.r-project.org/.

Sanders, N. J. & Suarez, A. V. (2011) 'Elton's Insights into the Ecology of Ant Invasions: Lessons Learned and Lessons Still to be Learned', in Richardson, D. M. (ed.) Fifty years of invasion ecology - the legacy of Charles Elton. Wiley Blackwell, p. 239–251.

Sauer, J. R. Pardieck, K. L., Ziolkowski, D. J., Smith, A. C., Hudson, M.-A. R., Rodriguez, V., Berlanga, H., Niven, D. K. & Link, W. A. (2017) 'The first 50 years of the North American Breeding Bird Survey', The Condor, 119(3): 576–593. doi: 10.1650/CONDOR-17-83.1.

Saux, C., Fisher, B. L. & Spicer, G. S. (2004) 'Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae:

Amblyoponinae)', Molecular Phylogenetics and Evolution, 33(2): 457–468. doi: 10.1016/j.ympev.2004.06.017.

Schär, S., Talavera, G., Espadaler, X., Rana, J. D., Andersen A. A., Cover, S. P. & Vila, R. (2018) 'Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae', Journal of Biogeography, 45(8): 1917–1928. doi: 10.1111/jbi.13380.

Schär, S., Illum, A. A. & Larsen, R. S. (2017) 'Exotic ants in Denmark (Hymenoptera: Formicidae)', Entomologiske Meddelelser, 85(1–2): 101–109.

Schlick-Steiner, B. C., Steiner, F. M., Moder, K., Seifert, B., Sanetra, M., Dyreson, E., Stauffer, C. & Christian, E. (2006) 'A multidisciplinary approach reveals cryptic diversity in Western Palearctic Tetramorium ants (Hymenoptera: Formicidae)', Molecular Phylogenetics and Evolution, 40(1): 259–273. doi: 10.1016/j.ympev.2006.03.005.

Schoener, T. W. (1968) 'The Anolis Lizards of Bimini: Resource Partitioning in a Complex Fauna', Ecology, 49(4): 704–726.

Schoener, T. W. (1970) 'Nonsynchronous Spatial Overlap of Lizards in Patchy Habitats', Ecology, 51(3): 408–418.

Seifert, B. (2007) Die Ameisen Mittel- und Nordeuropas. Lutra Verlags- und Vertriebsgesellschaft, Tauer, 368 pp.

Seifert, B. (2017) 'The ecology of Central European non-arboreal ants – 37 years of a broad-spectrum analysis under permanent taxonomic control'. Soil Organisms, 89: 1-67.

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

Steiner, F. M., Schlick-Steiner, B. C., VanDerWal, J., Reuther, K. D., Christian, E., Stauffer, C., Suarez, A. V., Williams, S. E. & Crozier, R. H. (2008) 'Combined modelling of distribution and niche in invasion biology: a case study of two invasive Tetramorium ant species', Diversity and Distributions, 14(3): 538–545. doi: 10.1111/j.1472-4642.2008.00472.x.

Suarez, A., McGlynn, T. & Tsutsui, N. (2010) 'Biogeographic and Taxonomic Patterns of Introduced Ants', in Lach, L., Parr, C. L., and Abbott, K. L. (eds) Ant Ecology. New York: Oxford University Press, p. 424.

Theobald, E. J., Ettinger, A. K., Burgess, H. K., DeBey, L. B., Schmidt, N. R., Froehlich, H. E., Wagner, C., HilleRisLambers, J., Tewksbury, J., Harsch, M. A. & Parrish, J. K. (2015) 'Global change and local solutions: Tapping the unrealized potential of citizen science for biodiversity research', Biological Conservation, 181: 236–244. doi: 10.1016/j.biocon.2014.10.021.

Toennisson, T. A., Sanders, N. J. & Klingeman, W. E. (2011) 'Influences on the Structure of Suburban Ant (Hymenoptera: Formicidae) Communities and the Abundance of Tapinoma sessile', Community and Ecosystem Ecology, 40(6), pp. 1397–1404.

Del Toro, I., Ribbons, R. R. & Pelini, S. L. (2012) 'The little things that run the world revisited: A review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae)', Myrmecological News, 17: 133–146.

Tulloch, A. I. T., Possingham, H. P., Joseph, L. N., Szabo, J. & Martin, T. G. (2013) 'Realising the full potential of citizen science monitoring programs', Biological Conservation. Elsevier Ltd, 165: 128–138. doi: 10.1016/j.biocon.2013.05.025.

Wagner, H. C., Arthofer, W., Seifert, B., Muster, C., Steiner, F.M. & Schlick-Steiner, B.C. (2017) 'Light at the end of the tunnel: Integrative taxonomy delimits cryptic species in the Tetramorium caespitum complex (Hymenoptera: Formicidae)', Myrmecological News, 25: 95–129.

Wagner, H. C., Karaman, C., Aksoy, V. & Kiran, K. (2018) 'A mixed colony of Tetramorium immigrans Santschi, 1927 and the putative social parasite Tetramorium aspina sp.n. (Hymenoptera: Formicidae)', Myrmecological News 28: 25-33.

Table 1 (on next page)

List of the number of experiments each species was found in during the pilot study of the Ant Hunt in 2015 and the full study, which ran throughout April-September in 2017 and 2018.

[1]New taxa for Denmark. [2]Individuals that were not determined to species level. An experiment consisted of a baiting trial, where salt, sugar, olive oil, protein powder, water and cookies were left out for two hours after which all ants that had recruited to the baits were collected.

Species	Experiments
Formica cinerea	11
Formica exsecta	1
Formica fusca	89
Formica lugubris	1
Formica picea	1
Formica polyctena	43
Formica pratensis	1
Formica pressilabris	1
Formica rufa	7
Formica rufibarbis	6
Formica sanguinea	2
Formica sp.[2]	1
Formica truncorum	2
Hypoponera punctatissima	1
Lasius flavus	23
Lasius fuliginosus	12
Lasius niger	354
Lasius platythorax	47
Lasius psammophilus	2
Lasius umbratus	1
Myrmecinae sp.[2]	1
Myrmica lobicornis	2
Myrmica rubra	37
Myrmica ruginodis	35
Myrmica rugulosa	6
Myrmica sabuleti	4
Myrmica scabrinodis	4
Myrmica schencki	3
Technomyrmex albipes[1]	1
Tetramorium caespitum	26
Tetramorium immigrans[1]	1

Table 2 (on next page)

Overview of the distribution of observation points for *T. caespitum* and *T. immigrans* across six broad land cover categories.

Land cover categories were derived from CORINE 2012 and their availability was summarized across Europe. The ratio of occurrences in each land cover type by the two species is calculated as *T. caespitum*:*T. immigrans*. Occurrence data was received from antmaps.org and combined with the samples from the Ant Hunt.

1

Land cover class	Europe	*T. caespitum*		*T. immigrans*		Species ratio
	%	counts	%	counts	%	
Artificial surfaces	4.14	121	17.34	88	48.89	0.35
Agriculture	43.03	244	34.96	47	26.11	1.34
Forest	30.06	238	34.1	24	13.33	2.56
Scrub/herbaceous vegetation	14.12	84	12.03	16	8.89	1.35
Open with little-no vegetation	6.06	10	1.43	3	1.67	0.88
Wetlands	2.61	1	0.14	2	1.11	0.13
Total	100	698	100	180	100	

Table 3 (on next page)

Sampling effort across land cover types.

Observed (Obs.) number of samples collected within each land cover type by scientists and citizen scientists (CS) and the expected (Exp.) value if samples had been collected in accordance to availability. The ratio column refers to the ratio of observed:expected number of samples, a value of 1 would mean that a land cover type is sampled equally to its availability.

Land cover class	Denmark	Scientists (n = 448)			CS (n = 667)		
	Proportion	Obs.	Exp.	Ratio	Obs.	Exp.	Ratio
Agriculture	0.76	112	338	0.33	145	504	0.29
Artificial surface	0.08	107	34	3.14	429	51	8.46
Forest	0.09	139	39	3.53	54	59	0.92
Open with little-no veg.	0.00	0	0	0.00	1	1	1.50
Scrub/herbaceous veg.	0.04	53	17	3.11	30	25	1.18
Waterside	0.02	21	10	2.13	5	15	0.34
Wetlands	0.02	16	9	1.79	3	13	0.22

Figure 1

Tetramorium immigrans in Denmark.

a) Photo of *T. immigrans* specimen from the Botanical Garden of Copenhagen. Taken by Rasmus S. Larsen and edited to remove background. Scale: 1 mm. **b)** CT-scan of *T. immigrans* by Carsten Gundlach, 3D Imaging Center, DTU. Scale 0.7 mm. A video of the scan is available as Video S1. **c)** Map of Denmark showing analysed samples of *T. caespitum* (filled orange crosses, 10 localities), observed localities of *T. caespitum* (open orange crosses, 83 localities), the location of *T. immigrans* (blue star) and localities of Ant Hunt experiments where neither *T. caespitum* nor *T. immigrans* was found (open red circles, 735 experiments). **d)** Zoom in of the Botanical Garden at the Natural History Museum of Denmark in Copenhagen from Google Maps. Red circles indicate locations of *T. immigrans*.

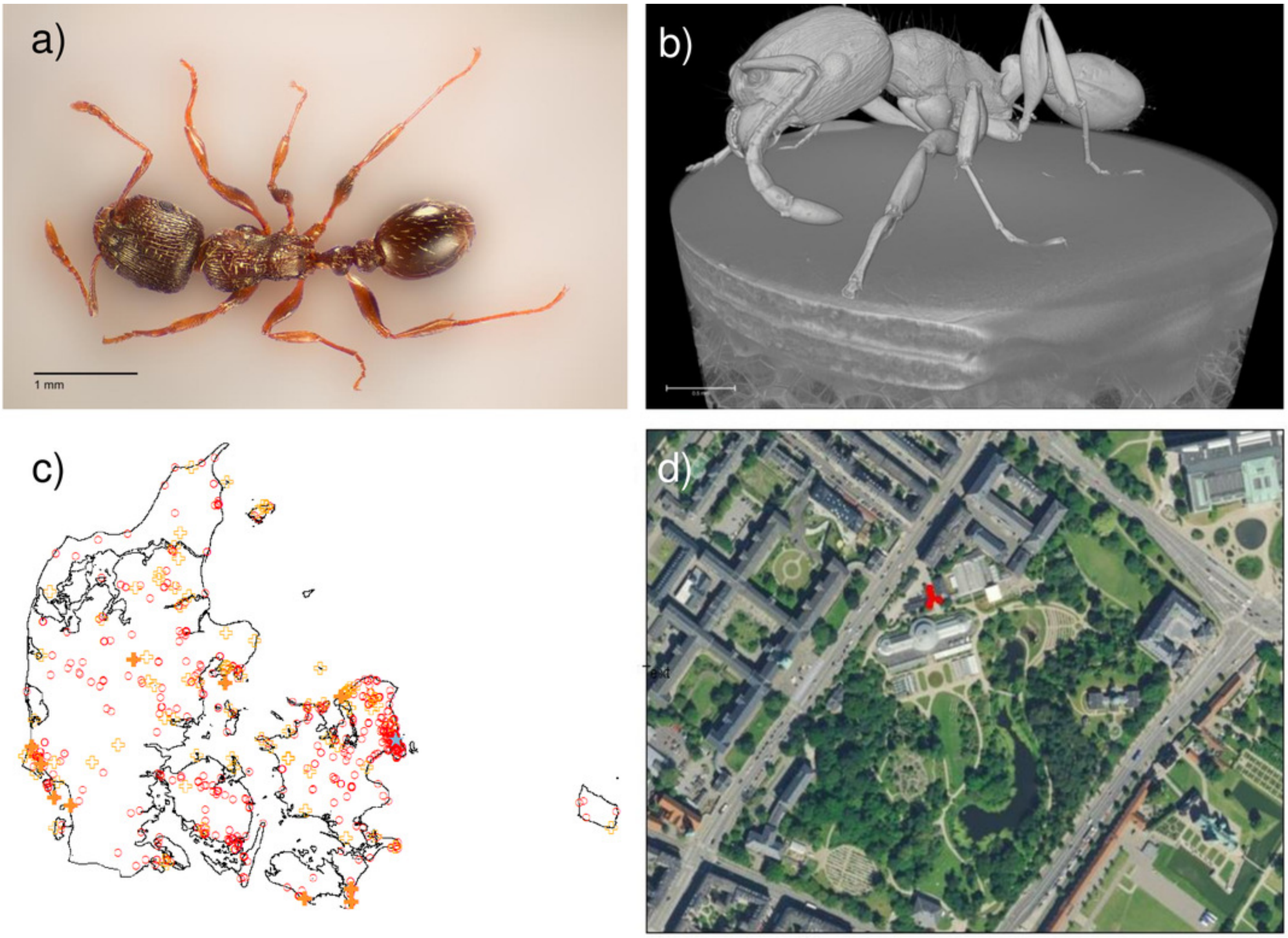
a)
1 mm
b)
c)
d)

Figure 2

Molecular identification of *T. immigrans* from the Botanical Garden of Copenhagen.

a) A maximum likelihood tree for the mitochondrial COI gene. The reference alignment is the full alignment from Wagner et al. 2017 (757 sequences available from GenBank). The red arrow shows the position of the sequence from Copenhagen within mtDNA lineage E, predominantly consisting of *T. immigrans.* Other clades within the tree have been collapsed for simplicity. b) 28S rDNA alignment (*T. immigrans*) showing a characteristic insertion of (C) at position 438 of the fragment amplified by the primers D2B and D3A-r, apparently not shared by *T. caespitum* and other members of the *T. caespitum* complex (Schär et al. 2018). The lowest sequence and chromatogram are from the sample from Copenhagen, showing the typical insertion (C) of *T. immigrans* at site 438.

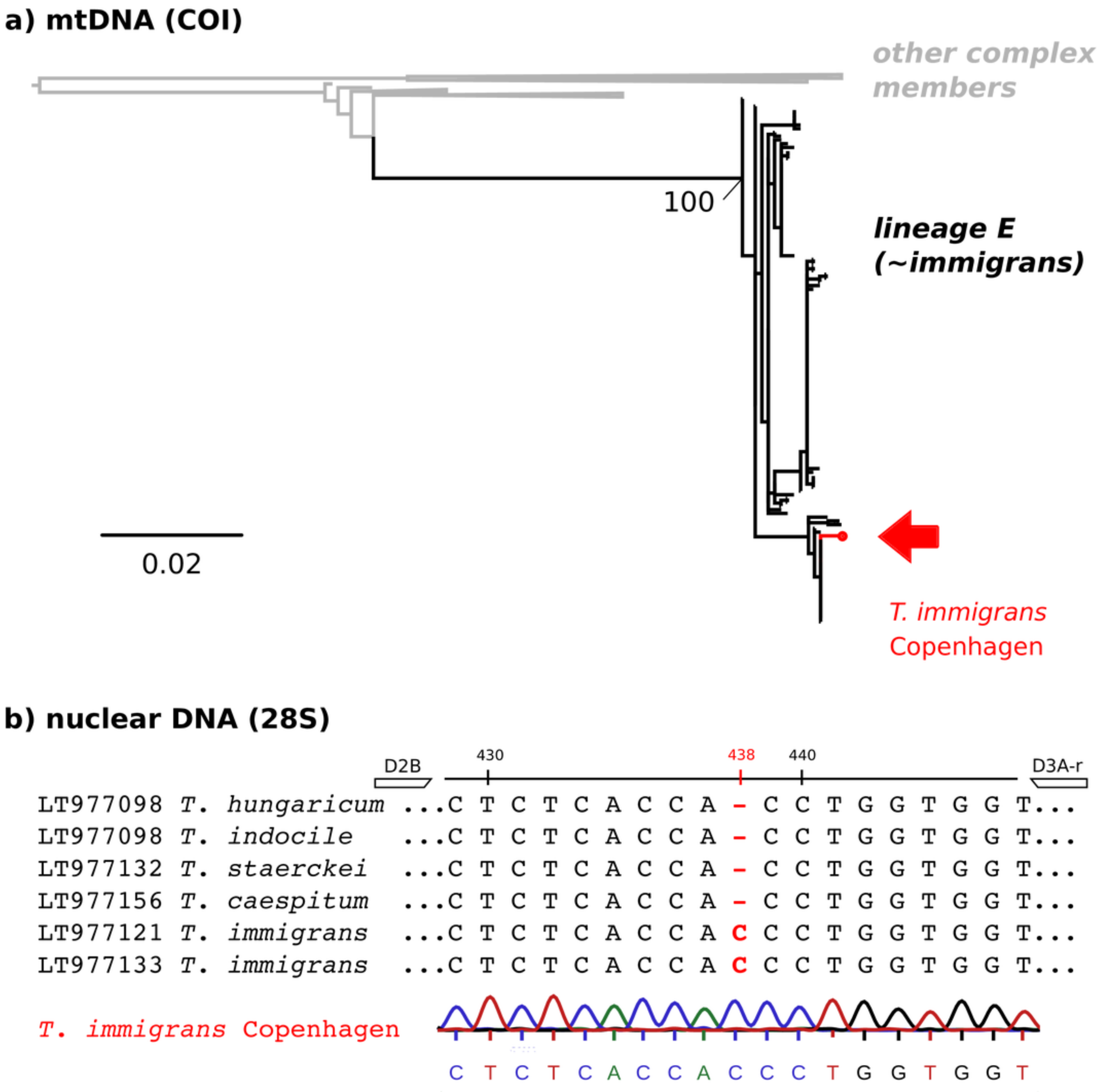
a) mtDNA (COI)
other complex members
100
lineage E (~immigrans)
0.02
T. immigrans Copenhagen
b) nuclear DNA (28S)
D2B
430
438
440
D3A-r
LT977098 T. hungaricum ...C T C T C A C C A – C C T G G T G G T...
LT977098 T. indocile ...C T C T C A C C A – C C T G G T G G T...
LT977132 T. staerckei ...C T C T C A C C A – C C T G G T G G T...
LT977156 T. caespitum ...C T C T C A C C A – C C T G G T G G T...
LT977121 T. immigrans ...C T C T C A C C A C C C T G G T G G T...
LT977133 T. immigrans ...C T C T C A C C A C C C T G G T G G T...
T. immigrans Copenhagen
C T C T C A C C A C C C T G G T G G T

Figure 3

Climatic niche of *Tetramorium caespitum* and *Tetramorium immigrans*.

a) Climatic niche of *T. caespitum* (orange) and *T. immigrans* (blue) along the two first axes of the PCA analysis. Yellow indicates niche space occupied by both species. Black and dashed contour lines indicate 100 % and 75 % available European (background) climate. The arrow marks the difference between the niche centres of the two species. **b)** Correlation circle indicating the contribution of the ten climatic predictors to the PCA axes of Fig. 3a. The ten climatic variables have been grouped into categories indicating temperature, precipitation and seasonality changes along the two axes. **c)** Geographical model of climatic suitability of Europe for *T. caespitum*. Red dots are data points used in the analysis. Darker shading indicates higher suitability. **d)** Geographical model of climatic suitability of Europe for *T. immigrans*. Red dots are data points used in the analysis. Darker shading indicates higher suitability.

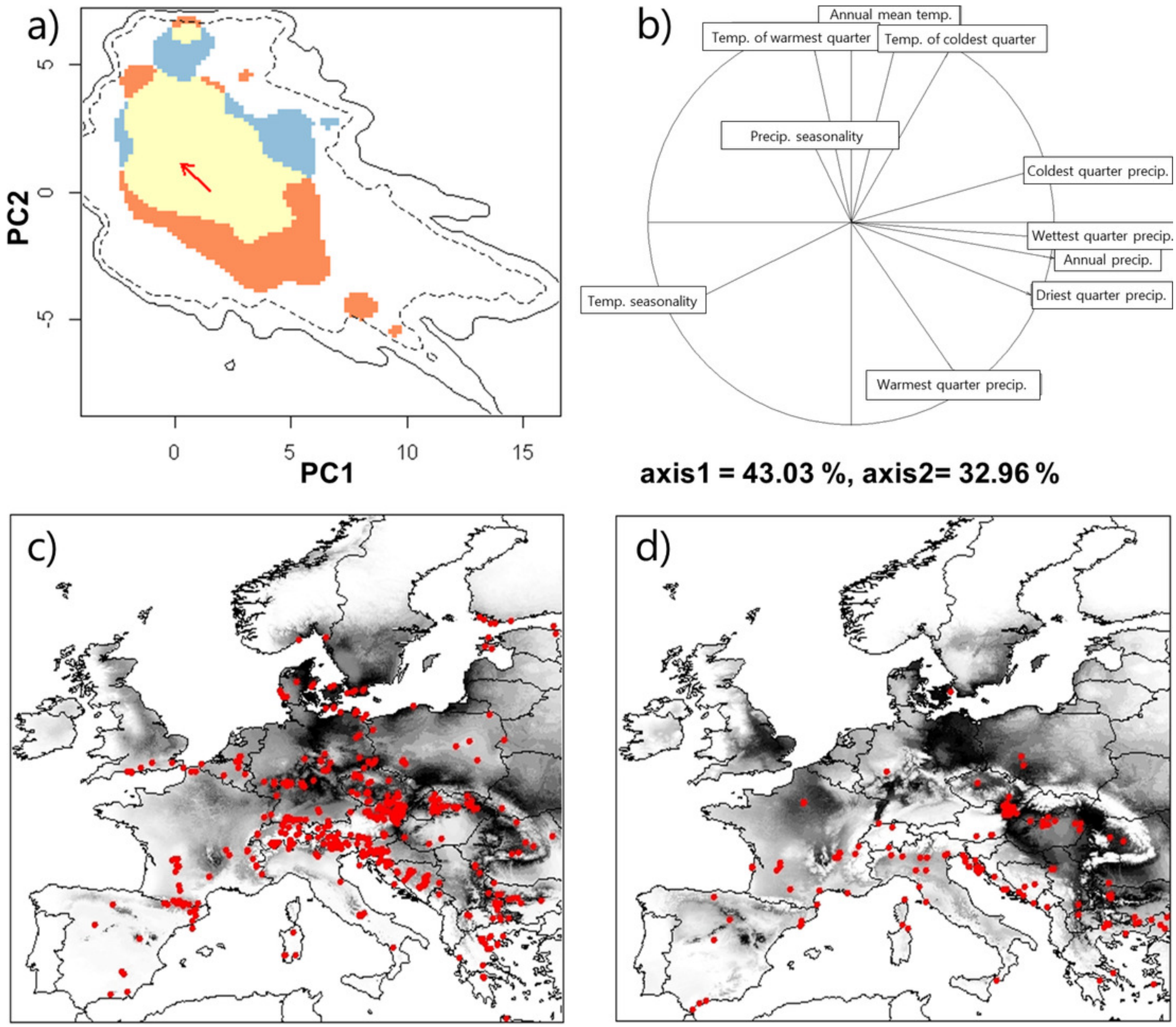
a)
PC2
PC1
0
5
10
15
-5
b)
Annual mean temp.
Temp. of warmest quarter
Temp. of coldest quarter
Precip. seasonality
Coldest quarter precip.
Wettest quarter precip.
Annual precip.
Driest quarter precip.
Temp. seasonality
Warmest quarter precip.
axis1 = 43.03 %, axis2= 32.96 %
c)
d)

Figure 4

Distribution of observations by scientists and citizen scientists in Denmark.

Samples of ants in Denmark collected by scientists are in purple and samples collected by citizen scientists through the Ant Hunt are in yellow. The location of the major harbours are marked by blue triangles and cities with over 5000 residents are marked as red polygons. Six of the major cities used in this study are labelled by name. The seventh, Frederiksberg, is not, because it is a city within København (Copenhagen).