A new record of *Crematogaster scutellaris* (Olivier, 1791) from Landau (Rhineland-Palatinate) with a summary of past findings of the species in Germany

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Kurzfassung
Wir vermelden hier einen neuen Fund der Kippleibameise *Crematogaster scutellaris* für Deutschland aus Landau in Rheinland-Pfalz und betrachten die bisherigen Funde der Art, basierend auf Literaturdaten und bürgerwissenschaftlichen Meldeplattformen. Die Artbestimmung wurde durch DNA-Barcoding bestätigt und die Population wurde anhand der genetischen Daten phylogeographisch eingeordnet. Die Art wurde bisher in sieben westlichen Bundesländern gefunden. Die neue Population konnte 2024 und 2025 am gleich Ort beobachtet werden, somit scheint die Etablierung der Art im Zuge des Klimawandels in Deutschland möglich.

Abstract
Here we report a a new biogeographic record of the Mediterranean acrobat ant *Crematogaster scutellaris* from Germany, Landau, Rhineland-Palatinate, and review recent records based on the literature and citizen science platforms. The species identity was confirmed with DNA barcodes and the population of *C. scutellaris* from Landau was set into phylogeographic context. The species so far has been found in seven federal states of Germany, but only in the western and southern parts of the country. A future permanent establishment of the species in cause of climate change seems possible.

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Introduction

Non-native alien species have been identified as a major threat to biodiversity (Vilà et al. 2010, Schirmel et al. 2016). Increasing global trade and travel, often interacting with climate change, have let to high numbers of newly colonizing species, even in temperate regions (Bertelsmeier 2021, Seebens et al. 2021). Species are often travelling in trading goods, especially in plant material. One of the easily transported groups that have been shown to be both good colonizers and potentially highly detrimental for ecosystems and economies are ants (Williams 1994, Holway et al. 2002, Klotz et al. 2008, Bertelsmeier et al. 2018, Seifert 2020b, Wong et al. 2023).

Some of the best known invasive ants are the red fire ant *Solenopsis invicta* Buren, 1972 (Ascunce et al. 2011, Wetterer 2013a), the Argentine ant *Linepithema humile* (Mayr, 1868) (Wetterer et al. 2009), the pharaoh ant *Monomorium pharaonis* (Linnaeus, 1758) (Wetterer 2010), the little fire ant *Wasmannia auropunctata* (Roger, 1863) (Wetterer 2013b) and the yellow crazy ant *Anoplolepis gracilipes* (Smith, 1857) (Wetterer 2005). Worldwide 19 ant species are currently listed in the IUCN list of invasive species (http://www.iucngisd.org/gisd/). Beyond these very prominent species, many others have been introduced and thrive around the world. Besides negative environmental impacts on other species, some ant species pose severe health risks to humans and other vertebrates (e.g. fire ants, Tschinkel 2006, Klotz et al. 2008). The global economic costs of ant invasions have been estimated to be as high as US$ 52 billion, mainly attributed by the two species *S. invicta* and *W. auropunctata* (Angulo et al. 2022).

In Germany, 50 species of non-native ants were found so far (Seifert 2020b; Rabitsch & Nehring 2023). Most species, however, were only reported from single findings (for example, 26 spp. were intercepted at Hamburg habour, Kraepelin 1901) or were found (16 spp.) in greenhouses. However, most of these introduced species did so far not establish reproductive colonies in the

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wild. Only the following five non-native ant species are considered to be established in Germany, i.e. *Hypoponera punctatissima* (Roger, 1859), *Pheidole pallidula* (Nylander, 1849), *Monomorium gallicum* Seifert, 2025, *Lasius neglectus* Van Loon et al., 1990 and *Tapinoma magnum* Mayr, 1861, the latter two being highly problematic in a number of locations in Germany due to the formation of supercolonies (Felke 2017, 2023, Seifert et al. 2017, Seifert 2018, 2020a,b, 2024). The status of establishment for three other species is not known. This also applies to the acrobat ant *Crematogaster scutellaris* (Olivier, 1791), which occasionally is introduced to Germany (Leininger 1931 in Stitz 1939, Sellenschlo 1993, 2002, 2008, Heller 2004, Lieving in Klotz et al. 2008, Felke & Kleinlogel 2012, Pospischil & Pospischil 2017, Seifert 2018). Originally, the species is known from the whole western Mediterranean including the main islands, from Northern Africa, Spain, Portugal, Southern France, Italy as far north as South Tyrol, and Ticino to the northern part of the Balkans (Slovenia, Croatia, Bosnia and Herzegovina, but possibly not Montenegro) and Hungary. In the northern Balkan region, *C. scutellaris* partly overlaps in distribution and hybridizes with its sister species *Crematogaster schmidti* (Mayr, 1853), which occurs from the Balkans and the Eastern Mediterranean to the Caucasus (Bernard 1967, Baroni Urbani 1971, Poldi et al. 1995, Hellrigl 1996, Poldi et al. 1995, Bračko 2006, 2007, Karaman 2010, Csösz et al. 2011, Borowiec 2014, Seifert 2018). Occasionally, *C. scutellaris* is transported out of its natural range in pieces of cork, wood, fruit or other plant material. Therefore, it has also been found in England (Collingwood 1979), the Netherlands (Boer & Vierwegen 2008), Belgium, Austria (Hölzel 1966, Heller 2004), the Czech Republic (a colony near Brünn; inaturalist.org, 29.8.2025), and even with two single specimens (worker and queen) at two different localities in Finland (inaturalist.org, 29.8.2025).

Although *C. scutellaris* was first introduced to Germany prior to 1937, the species appears not to have established stable colonies (Rabitsch & Nehring 2023). However, in general, besides some reports of populations from different parts of the country, no overview of its occurrence has been provided. Here, we summarize the findings from previous publications and public citizen science portals (observation.org; inaturalist.org) to provide an overview where the species has been found in Germany so far. We also report a new first sighting from Landau, Rhineland-Palatinate, and provide a first DNA barcode of this population in order to suggest a possible biogeographic origin.

Materials and Methods

Description of the finding location

A population of *Crematogaster scutellaris* was observed on a wall and a tree (*Gleditsia triacanthos*) in Landau, Rhineland-Palatinate (49.2010, 8.1115) on July 3, 2024 by Martin Husemann (Fig. 1). The specimens were imaged and first determined with the App ObsIdentify. The identification was confirmed by Gabriel Ziegler in obsidentify.org and Manfred Verhaagh. Later, several specimens were collected by Jens Schirmel at the same location on July 4, 2024, which were subsequently used for DNA barcoding. Several dozens of individuals were observed during several follow-up checks (last check 2024: October 2, 2025: April 29). The new location as well as all previously reported locations from the literature and from the monitoring platforms inaturalist.org and observation.org were georeferenced when exact localities were known and plotted on a map using QGIS (http://www.qgis.org).

Molecular data

DNA was extracted from two specimens using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with an extended overnight incubation time at 56 °C (Thermomixer, with gentle shaking) for cell lysis. For elution 100 µL AE buffer were used. The extracted DNA was stored at -20 °C until later use. Primers used for amplification and sequencing were LCO1490-JJ and HCO2198-JJ (Astrin & Stüben 2008). The amplified mitochondrial region is a part of cytochrome c oxidase subunit I gene (*COI*) and corresponds to the standard barcode region of the animal kingdom (Hebert et al. 2003). A 535 bp long high quality sequence of this fragment could finally be generated.

PCR reactions were carried out in a total volume of 25 µl containing 1.0 µl DNA, 2.0 µl of primers (5 pmol each) and 12.5 µl of 2×Multiplex PCR Plus Master mix (QIAGEN). The PCR protocol consisted of an initial DNA polymerase (HotStar Taq) activation step at 95 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 90 s at 49 °C and 90 s at 72 °C; the last cycle was followed by a final 10 min extension step at 72 °C. 3 µl of the PCR product was visualized on a 1% agarose

Figur 1. Individuals of *Crematogaster scutellaris* observed in Landau on July 3, 2024. – Photo: Jens Schirmel.

gel. The PCR product was purified by using 3 M sodium acetat and ethanol precipitation. Sanger sequencing was performed using the BigDye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific Inc.) and a total amount of 20 ng of purified PCR product. To obtain unequivocal sequences, both sense and antisense strands were sequenced. Sequences were de novo assembled with Geneious v. 11.0.5 (https://www.geneious.com) using a default high sensitivity threshold. The newly generated sequences were submitted to Genbank (Accession numbers: PV574000, PV574001).

All public sequences of *Crematogaster scutellaris* were downloaded from BOLD. We deleted three sequences as these were highly different from all others and likely represented incorrectly identified specimens. Sequences were aligned using the muscle algorithm (Edgar 2004) implemented in Geneious v. 6.1.8. The alignment was trimmed to a final length of 612 bp and used as input for MrBayes v. 3.2.6 (Ronquist et al. 2012). We used the reversible jump model to provide high flexibility in the analyses and ran the analyses for 1 000 000 generations, sampling every 100 generations to generate a sample of 10 000 trees. Convergence was confirmed by average split frequencies below 0.05. The resulting consensus tree was summarized using Figtree v. 1.4.2 (Rambaut 2009).

Results and Discussion

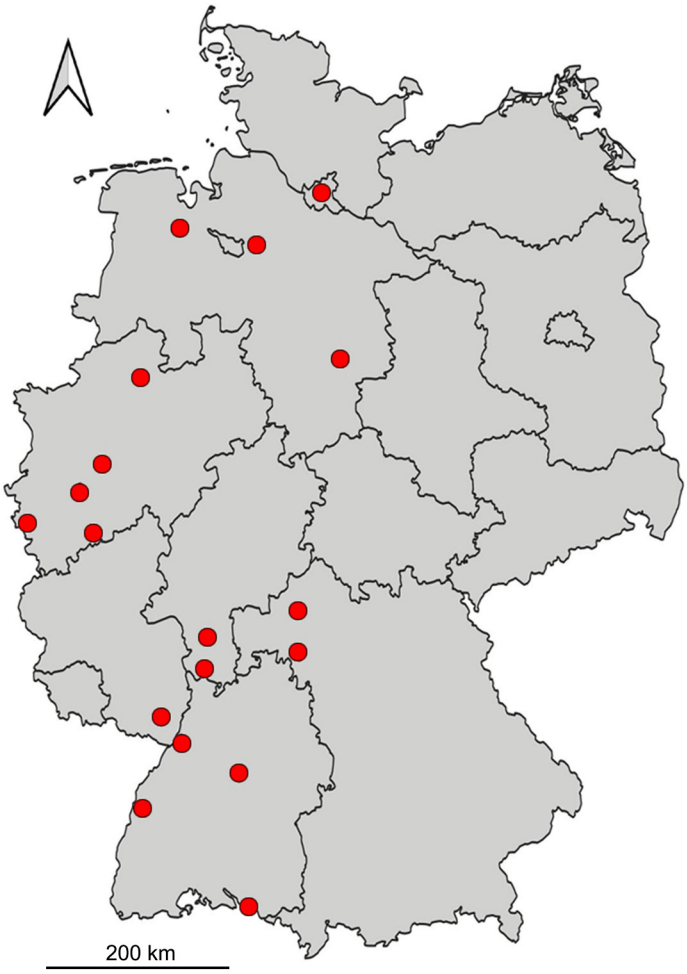
The ant species *Crematogaster scutellaris* is non-native to Germany and is infrequently encountered in different parts of the country. We have discovered a new population in Landau, constituting the first record for Rhineland-Palatinate. At this location, several dozens of individuals regularly occurred outside of a school campus on a wall, house wall and a non-native tree (*Gleditsia triacanthos* L.) with a height of about 18 m and a trunk diameter of about 50 cm (Abteilung Vermessung und Geoinformation, Stadt Landau in der Pfalz; https://maps.landau.de/map22.htm?wor=gu&layers=baeume) (Fig. 1). There were no newly established plants at the site and no transport source could be seen. The population was observed in two consecutive years, suggesting that this may represent a permanently established colony or population.

Table 1. Collection localities of *Crematogaster scutellaris* in Germany. For some locations only the city, but no exact coordinates (latitude and longitude given in decimal degrees, WGS84) or more precise location data were provided (see Fig. 2).

Federal state	Place	Lat	Long	Date of reporting (or discover)	Source
Baden-Württemberg	Immenstaad	47.6745	9.3819	09.05.2024	observation.org
Baden-Württemberg	Stuttgart	48.7640	9.1900	15.07.2023	observation.org
Baden-Württemberg	Laudenbach	49.6090	8.6470	2001	Heller 2004
Baden-Württemberg	Karlsruhe			1931	H. Leininger in Stitz 1939
Baden-Württemberg	Schutterwald	48.4507	7.8865	29.04.2025	Submitted vouchers, det. M. Verhaagh
Bavaria	Bad Kissingen	50.1179	9.8966	08.06.2024	observation.org
Bavaria	Würzburg	49.7766	9.9400	15.09.2023	inaturalist.org
Hamburg	Hamburg			12.2008	Sellenschlo 2008
Hesse	Frankfurt/M.			not given	Heller 2004
Hesse	Darmstadt			06.2012	Felke & Kleinlogel 2012
Lower-Saxony	Lengede	52.2051	10.3270	13.06.2020	Naturgucker.de
Lower-Saxony	Verden	53.1109	9.1414	25.06.2022	inaturalist.org
Lower-Saxony	Ammerland	53.2210	8.1072	26.09.2011	inaturalist.org
North Rhine-Westphalia	Münster-Coerde	51.9895	7.6547	20.03.2024	observation.org
North Rhine-Westphalia	Aachen	50.7537	6.1807	26.03.2024	inaturalist.org
North Rhine-Westphalia	Mettmann	51.0349	6.8887	01.09.2022	inaturalist.org
North Rhine-Westphalia	Wuppertal			2004 (2000 introduced?)	P. Lieving 2006 in Klotz et al. 2008; Körber 2009. Pospischil & Lieving 2012
North Rhine-Westphalia	Bonn			not given	Stitz 1939
North Rhine-Westphalia	Meckenheim			2010 und 2011	Pospischil & Lieving 2012; Pospischil & Pospischil 2017
„Northern Germany“				1991	Sellenschlo 1993, 2002
Rhineland-Palatinate	Landau	49.2010	8.1115	03.07.2024	this study

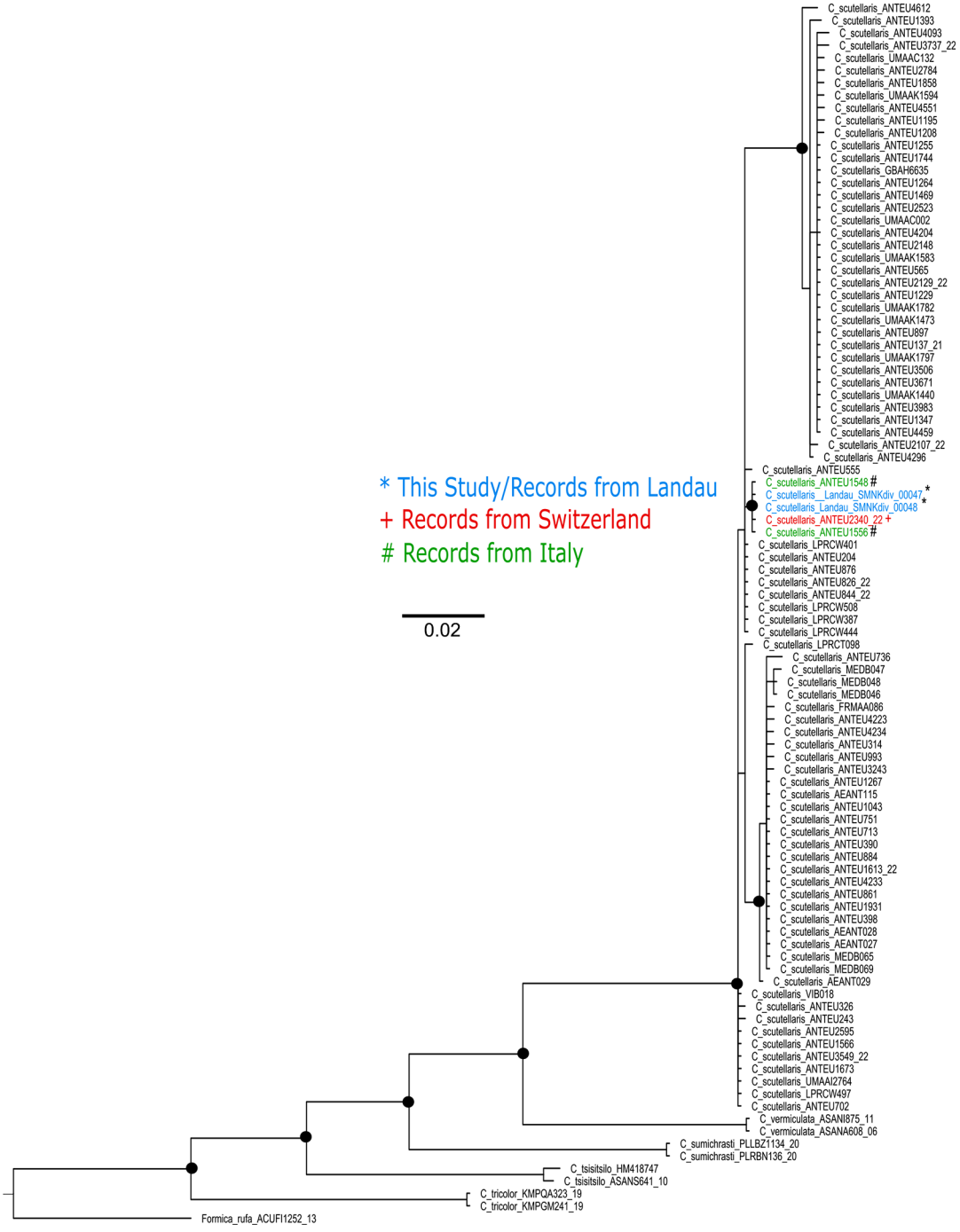
We also checked the literature and recent reports from the citizen science platforms observation.org and inaturalist.org. So far, the species was reported from seven federal states: Baden-Württemberg, Hamburg, Hesse, Lower Saxony, North Rhine-Westphalia, Rhineland-Palatinate and in Bavaria (Table 1, Fig. 2). Interestingly, all findings occurred in the western half of Germany. So far, the species has not formed any permanent occurrences in Germany, but colonies can persist for several years (Heller 2004, Klotz et al. 2008, Felke & Kleinlogel 2012). This western distribution pattern is interesting, as there is a clear border of findings to the east. Whether this is a reporting bias or a real distributional boundary needs to be further investigated. As *C. scutellaris* is a thermophilic species from the Mediterranean, it could benefit from climate warming and become established in the wild at least in the (south-)western parts of Germany in the future. A first tendency might be seen in the fact that 18 out of 21 reported imported colonies occurred since the year 2000. As the species preferably builds its nests (at least in part constructed of a loose carton) in bark and wood e.g. of cork oak *Quercus suber* L. or pine species *Pinus* spp. (Krause 1913, Eidmann 1926, Bernard 1967, Sellenschlo 2008, Seifert 2018), it could then become a serious pest in wooden house constructions (Stitz 1939, Sellenschlo 2002, Felke & Kleinlogel 2012, Pospischil & Lieving 2012). More information about its biology can be found e.g. in Eidmann (1926), Soulié (1955, 1962), Pospischil & Lieving 2012, Frizzi et al. (2020), Gianetti et al. (2021), and summarized in Seifert (2018).

To verify morphological identification, we also generated DNA barcodes (COI) for two specimens from the new population and constructed



Figur 2. Occurrences of *Crematogaster scutellaris* in Germany based on literature data and citizen science platforms. – Grafik: Martin Husemann.

a phylogeny using published data from BOLD. In addition, the mitochondrial phylogeny estimates the relationship to *C. scutellaris* individuals from other locations providing first evidence regarding the biogeographic origin of the Landau colony. BLAST searches resulted in more than 99% matching identity with *C. scutellaris*. Hence, species identification can be genetically confirmed. In our phylogenetic reconstruction, we found no clear geographic structure in the published sequences and our new data. However, the two sequences we generated grouped together in a well-supported clade of five sequences including specimens from Italy and Switzerland (Fig. 3). Therefore, it seems likely, that the newly found colony was brought from one of these areas. *Crematogaster scutellaris* might be introduced with wood, cork, fruits (e.g. peach pit, apple core) and other plant materials or even in coverings of camping vans (Stitz 1939, Sellenschlo 1993, 2002, 2008, Heller 2004, Pospischil & Lieving 2012, Seifert 2018). As the zoo in Landau is close to the collecting location (~ 200 m), the species may have been transported with plant material to the zoo; however, transportation in plant material independent of the zoo is equally likely. From time to time, the weekly market is also held in the immediate vicinity of the site, where, among other things, flowers are sold. For the time being, however, we can only speculate about the origin of the newly discovered population in Landau.



Figur 3. Phylogenetic tree based on Bayesian Inference showing the genetic position of the newly found population from Landau; Black circles indicate posterior probabilities (BPP) above 0.95. – Grafik: Dustin Kulanek.

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