

Vespa velutina nigrithorax Lepeltier, 1836 from Hamburg (Northern Germany) shares the same COI haplotype with other European populations

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Abstract

The yellow-legged, or Asian hornet, *Vespa velutina*, has invaded large parts of South-Western Europe and now starts spreading further north. Recently, it has been reported from Hamburg, Northern Germany. Yet, the origin of this new invasion is not clear as the founders may have come either from Southern Europe or from Asia. We here use COI sequences to test these alternatives and find that the specimens from Hamburg share the same haplotype with all Southern European samples. This suggests that the species has been transported long distance from other Europe to Northern Germany and shows further its high invasion potential.

Keywords

Asian hornet, France, honey bees, Italy, yellow legged hornet

Introduction

The Asian yellow legged hornet (*Vespa velutina* Lepeltier, 1836) was introduced to Southern Europe via pottery from China before 2004 (Haxaire et al. 2006) and has since then spread across large parts of Europe (Villemant et al. 2006; Rome and Villemant 2020). Previous genetic studies suggested that few or a single mated female gave rise to the invasion (Arca et al. 2015), suggesting an extreme founder effect reflected in the presence of

a single mitochondrial haplotype in the invaded regions. Most recently, the species was found in Hamburg, Northern Germany. While only a single individual was described in the original first record (Husemann et al. 2020), subsequently several more specimens from a second location in Hamburg close to the original record and a nest were found. Later in 2020 more individuals at another location more distant of the original find were recovered. However, the origin of these specimens remained unclear. The founding specimens may either have been transported from Southern Europe or, possibly, a second time from the Asian native range of *V. velutina*. The latter may be a valid alternative as Hamburg is one of the major harbors in Germany and as such is a hub for many alien species (e.g. Kraepelin 1900; Weidner 1981; Nehring 2006). Therefore, we sequenced the COI gene of nine specimens recently collected in Hamburg and compared them to all publicly available sequence data of the species to test the two alternative hypotheses of 1) an invasion from Southern Europe, or 2) a second invasion from Asia.

Materials and methods

We sequenced the COI gene for nine specimens from Hamburg from five locations and two specimens from Paris in our analyses: two specimens from 53.543056°N, 10.073639°E, collected in fall 2019 in a wasp trap; two specimens from 53.570915°N, 9.850126°E (17.08.2020); three specimens from 53.569317°N, 9.850461°E (18.08.2020); one specimen from 53.575623°N, 9.856934°E (14.08.2020); one specimen from 53.539700°N, 10.058650°E (27.07.2020); and two specimens from Paris, Jardin des Plantes (48.844708°N, 2.362767°E; 23.07.2020, leg. Q. Rome). We extracted DNA from one leg of each specimen using a Chelex protocol (Walsh et al. 1991) and amplified the DNA barcoding fragment of the cytochrome C oxidase subunit I (COI) using the primers HCO / LCO (Folmer et al. 1994) using a standard DNA barcoding protocol. Sequencing was performed by MacroGen, Inc. (Amsterdam, NL).

We further downloaded all available sequences for *Vespa velutina* from BOLD systems (90 sequences, plus 11 newly generated sequences) with sequences of *Vespa crabro* from Canada and of *Vespa mandarinia* from Nepal as outgroups. All sequences were aligned using MUSCLE (Edgar 2004) as implemented in Geneious v. 6.1.8. (Kearse et al. 2012). The alignment was trimmed to 544 bp. For the haplotype network analysis, the two outgroup sequences and two BOLD sequences of *V. velutina* which showed to be strongly divergent were removed (N = 97 sequences remained).

We estimated the most suitable substitution model using MrModeltest v.2.3 (Nylander 2004). The most suitable model was determined as GTR+G based on the AIC. In order to adjust for rate heterogeneity we used the reversible jump model in MrBayes v. 3.2.6 (Ronquist et al. 2012). We ran the simulation for 10 million generations sampling every 1,000 generations for a total of 10,000 final samples. Convergence was confirmed with average split frequencies being below 0.01. Trees were visualized with FigTree v. 1.4.2 (Rambaut 2008). We further constructed a TCS network using PopArt (Leigh and Bryant 2015) using the default parameters and different color coding for each country.

Results and discussion

Nine specimens from Hamburg and two from Paris were successfully sequenced (NCBI Accession numbers [MT941771](#)–[MT941781](#)), and we confirmed the species identity as *Vespa velutina* (in line with the morphological identification) using the BLAST algorithms of the NCBI and BOLD databases. All 11 new sequences were identical. The best hits were for *V. velutina* without subspecies designation in both databases. The best hits with subspecies designation were for *V. v. nigrithorax*. The Bayesian phylogenetic tree was poorly resolved due to the low divergence of sequences (data not shown). The haplotype network clearly separates haplotypes from Indonesia and Malaysia (Fig. 1); these represent the subspecies *V. v. velutina* Lepeletier, 1836, *V. v. ardens* Buysson, 1905, and *V. v. celebensis* Perkins, 1910 from Indonesia and *V. v. divergens* Perkins, 1910 from Malaysia. The specimen from Taiwan represents the subspecies *V. v. flavitarsus* Sonan, 1939, which is less clearly separated from the majority of haplotypes representing the European invasive *V. v. nigrithorax* Buysson, 1905. The Indian samples, formerly described as *V. v. auraria* Smith, 1852 and *V. v. variana* Vecht, 1957 from Thailand and Vietnam are not clearly separated from haplotypes of *V. v. nigrithorax*. *Vespa v. nigrithorax* shows some diversity with several haplotypes in Asian samples, yet, all European invasive locations share a single haplotype in accordance with the hypothesis of Arca et al. (2015) and Granato et al. (2019) that a single

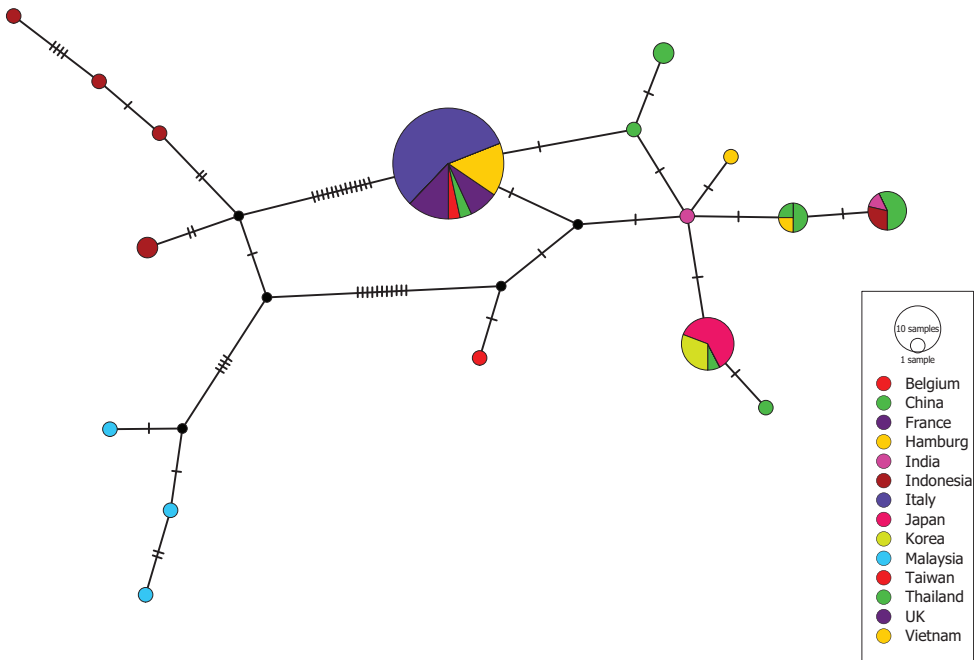


Figure 1. Haplotype network showing the relationships of the specimens from Hamburg compared to specimen entries from the BOLD database (Network created in PopArt).

or few females founded all invasive populations in Europe. Also, the specimens from Hamburg share this haplotype clearly pointing to a southern European origin of these specimens and hence making a second invasion from Asia unlikely.

The presence of *V. v. nigrithorax* in Hamburg remains surprising given the large distance to any other European populations. Considering the interest in the species and the reporting duties by the EU, it remains unlikely that the species has reached Northern Germany on natural routes without being seen. The large gap in occurrence in central Germany hence suggests that the species likely was anthropogenically transported over a long distance from Southern Europe to Northern Germany. This once more demonstrates the high invasion potential of the species.

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