

First Czech record of the Asian hornet (*Vespa velutina*) and a climatic prediction of its spread in the Czech Republic

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Abstract

The Asian or yellow-legged hornet *Vespa velutina nigrithorax* has been accidentally introduced into several European countries. This invasive species has an impact on entomofauna, mainly apiculture, and plant biodiversity through reduction of pollinators. We present the first record of the species in the Czech Republic, notes associated with this record, its COI barcode, and modelled potential distribution. A specimen of *Vespa velutina nigrithorax* was recorded in October 2023 in Pilsen city (West Bohemia, Czech Republic). The nest was traced by observing released individuals, detected on Black locust (*Robinia pseudoacacia*), and removed. The nest comprised 361 workers, 459 males, five queens, and larval and pupal stages. The COI barcode confirmed the European origin of this nest. The species either followed natural routes from Bavaria (Germany) or was introduced by transportation. A species distribution model (Maxent) trained on European distribution showed that the western Czech Republic is more suitable for the species than its eastern parts, as the species likely prefers lower continentality. Recently, this record represented an isolated occurrence in the Czech Republic; however further expansion of this invasive species is expected.

Key message

Vespa velutina is an invasive pest spreading in Europe and which could have a negative impact on beekeeping

We presented the complex study relating to the first record of *Vespa velutina* in the Czech Republic.

Czech sample of *Vespa velutina* belongs to the same haplotype as other European countries, and it has suitable conditions for living in the Czech Republic whereas it formed a large nest with 6750 cells here.

Introduction

The Asian hornet *Vespa velutina* Lepeletier, 1836 (Hymenoptera: Vespidae) is an invasive species in Europe with immense impact on ecosystems. It is naturally distributed in south-east Asia: Afghanistan, Bangladesh, Bhutan, China, Hong Kong, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Taiwan, Thailand, and Vietnam (Lioy et al. 2022). The subspecies *Vespa velutina nigrithorax* Buysson, 1905 was introduced by accident into France near Agen in 2004 (Haxaire et al. 2006; Villemant et al. 2006). Since then, it has spread rapidly across south-western Europe. It has established colonies in Spain (Castro and Pagola-Cardé 2010), Portugal (Grosso-Silva and Maia 2012), Belgium (Bruneau 2011), Italy (Demichelis et al. 2014), Germany (Witt 2015), Great Britain (Budge et al. 2017), the Balearic Islands (Leza et al. 2018), the Channel Islands (States of Guernsey Government 2016), the Netherlands (Smit et al. 2017), Switzerland (Ebener 2017), Luxemburg (Ries et al. 2021), Ireland (Dillane et al. 2022), and Hungary (Márta and Vas 2023). In France, the recorded invasion speed was around 78 km/year (Robinet et al. 2017). The invasion risk could be magnified through anthropogenic transportation (Robinet et al. 2018), which plays an important role in invasive species spread across the world (Pyšek et al. 2010).

Vespa velutina forms a large nest with one queen and thousands of individuals during the season. From autumn, the nest produces young queens, which establish new colonies after hibernation. It is a generalist predator of insects. The species has a considerable impact on apiculture, as the domestic honeybee (*Apis mellifera*) is its important prey in Europe, representing a readily available protein resource (Husemann et al. 2020b). *Vespa velutina* also affects the genus *Vespula* (Vespidae) due to competition (Verdasca et al. 2021), and indirectly plant biodiversity by reducing the pollinator populations (Monceau and Thiery 2016).

In this study, we present the first record of *V. velutina nigrithorax* in the Czech Republic (Central Europe), along with remarks about discovery of its nest, genetic confirmation of its origin, and climatic models forecasting the species' invasion across the country.

Methods

Tracing

On 5 October 2023, a member of the public photographed an individual of a hornet in Pilsen city (West Bohemia), urban district Škvřany. He sent the photo to the Nature Conservation Agency of the Czech Republic [NCA CR], which monitors invasive species in the country. The photographed individual was determined as *Vespa velutina nigrithorax*. After the identification, we visited the site and confirmed the first record. We observed a few foraging and feeding individuals (49°44'49.7"N, 13°21'3.4"E).

On 9 October 2023, we tracked *V. velutina nigrithorax* from the foraging and feeding site to the nest (Fig. 1) following the method of Leza et al. (2018). First, we caught 10 individuals with an entomological net and placed them into a plastic box, where they were fed with honey. After, we gradually let these individuals out, and traced the nest according to the direction of their flight (Fig. 2). At the beginning, all individuals flew in an approximately similar direction. When we let out the last two individuals, they flew in a different direction. We predicted that the nest had to be placed at a site between these two directions using a triangulation method (Leza et al. 2018). Tracking the nest took one hour. On the next day, the nest was removed by firefighters. The individuals in the nest were euthanised by Effect (UNICHEM D.O.O), an insecticide. The nest was preserved and subsequently analysed in the laboratory. It was scanned by X-ray machine and CT scanner (Fig. 3) to analyse its inner structure.

Habitat

We conducted a standard phytosociological relevé in the close vicinity of the nest comprising a polygon of 400 m², and in the wider environ of 900 m² (Fig. 1), listing all vascular plant species (Braun-Blanquet 1964) and biotope types (Chytrý et al. 2010). We defined the percentual cover of herb (E1), shrub (E2), and tree (E3) layer. The nomenclature of vascular plants follows Kaplan et al. (2019).

DNA analysis

To confirm that the Czech *V. velutina nigrithorax* originated from the colonies established in Europe, we used DNA barcoding (sequencing of cytochrome c oxidase subunit I gene, COI). We extracted DNA from two legs of one individual with the Genomic DNA Mini Kit–Tissue (Geneaid) following the manufacturer's protocols. We amplified the first part of COI using primer pair hybLCO (5'-TAATACGACTCACTATAGGGGGTCAACAAATCATAAAGATATTGG-3') and hybHCO (5'-ATTAACCCTCACTAAAGGGTAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The PCR mixture contained 4 µl of PCR H₂O, 0.625 µl of each primer, 6.25 µl of Bioline 2x MyTaq HS Red Mix (Meridian Bioscience Inc.), and 2 µl of extracted DNA. The PCR protocol consisted of 95°C for 5 min; followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s; with a final extension of 72°C for 10 min. The PCR product was cleaned with enzymes FastAP and ExoI (ThermoFisher) and sequenced from the 5' end in Macrogen Inc. on ABI3730XL DNA analysers. The sequence was checked visually in Geneious v. 8.0.5 (Kearse et al. 2012). The GenBank accession number is PP238379. We downloaded COI sequences for *Vespa velutina* from the BOLD database (Ratnasingham and Hebert 2007) (on 16 November 2023) which were sequenced from the 5' end, and aligned them together with the newly produced sequence. We constructed a haplotype network using the TCS algorithm in POPART (Leigh and Bryant 2015).

Predicting species distribution

To predict potentially suitable areas for the species in the Czech Republic (areas prone to invasion), we performed species distribution modelling based on the Maxent algorithm. Occurrence data of *Vespa velutina* were extracted from the GBIF.org (GBIF.org) and iNaturalist.org (iNaturalist.org) databases. These points were filtered and thinned through the Wallace application (Kass et al. 2023) and 2,339 points were left for modelling input. The model training area included all its European occurrences (without the new Czech record) available as of April 2022 with a 78 km buffer around them (invasion speed per year, Robinet et al. 2017).

We utilized a total of 19 raster variables: 15 bioclimatic variables for the climate normal 1991–2020, continentality, elevation, slope gradient, and land cover categories. Bioclimatic variables were sourced using ClimateEU software (Marchi et al. 2020), which generates raster variables at a desired resolution and adjusts them according to altitude. Detailed land cover and land use data were derived from the Land Cover Map of Europe 2017 (LCME 2017) dataset (Malinowski et al. 2020) which has a resolution of 10 x 10 m per pixel. To detect the preferred land cover, we used 2017 data containing 13 land cover classes. Newer data were not available at the desired resolution, and as the land cover can change relatively quickly, some uncertainty needs to be considered (Brus et al. 2018). All 19 raster variables were exported into ArcGIS Pro software (Esri 2023) at a resolution of 250×250 metres per pixel. Correlating variables were eliminated by a post-correlation matrix evaluation in ArcGIS Pro. Subsequent variable selection was performed by applying Principal Component Analysis (PCA) using RStudio and R packages corr (Kuhn et al. 2022), ggcorrplot (Kassambara 2023), and FactoMineR (Lê et al. 2008), to transform the original variables into principal components with the least loss of information. Two sets of data entered the PCA – climatic variables in locations of species occurrence (already colonised areas) and in a random sample of 100,000 points from the environmental background. PCA was used to find the best explanatory

variables describing the variability between the environment and *V. velutina* habitat locations. The variables that contributed most to the first two components were selected.

Using the occurrence data and raster variables, several models based on the Maxent algorithm were developed through the Wallace plugin using R packages ENMeval (Kass et al. 2021), dismo (Hijmans et al. 2023), and maxent (Phillips 2021). As a machine learning algorithm, Maxent internally decides variable selection and model fit (James et al. 2013); however, different settings affect model complexity and the resulting predictions: feature classes (modelling functions) and regularization multipliers (RM; penalizations against model complexity). Twelve different models were developed, differing in the feature classes (hinge function and linear-quadratic-product combination) and regularization multipliers (0.5, 1.5, 2.5). Models' applicability was statistically evaluated using Area Under the Curve (AUC), Omission Rate (OR), Akaike Information Criterion (AIC), and Continuous Boyce Index (CBI). The model selection was performed in two steps: first, models with the most accurate feature classes in each RM category were selected (RM determines the tightness of the training data – the smaller RM, the greater the tightness). Second, we considered the apparent adaptability of *V. velutina*, and the model with the RM category which favoured the least strict feature classes model was chosen.

Based on this model, a habitat suitability measure was generated for the model training area. The trained model was subsequently projected to the Czech Republic for the same climate normal (1991–2020).

Results

During a thorough field investigation in the vicinity of the nest site, we compiled the following records: (1) a local beekeeper photographed *Vespa velutina nigrithorax* already on 30 July 2023, but this record was not reported; 30 July 2023, 1 worker, near a beehive, 100 metres from the nest. (2) Record sent to the NCA CR; 5 October 2023, 9 workers, feeding on Common Snowberry (*Symphoricarpos albus*), 550 metres from the nest; (3) 6 October 2023; 10 workers, feeding and foraging on *S. albus*, 550 metres from the nest; (4) 8 October 2023, 11 workers, feeding and foraging on Ivy (*Hedera helix*) 450 metres from the nest; and the nest. The nest was built on a Black Lotus (*Robinia pseudoacacia*), 15 metres high in the tree crown, GPS: 49°44'35.6"N, 13°20'47.2"E, height: 75 cm; diameter: 50 cm, and 8 galleries with 6750 cells.

The nest harboured 361 adult workers, 459 adult males, and 5 adult queens. The majority of the colony was comprised of larval and pupal stages. Most of the pupae of young queens remained unhatched.

Habitat

In the close vicinity of the nest, the biotope was X12B, which are other stands of early successional woody species (Chytrý et al. 2010), with the presence of *Betula pendula*, *Fraxinus excelsior*, *Populus nigra*, and a rich nectareous tree, *Acer pseudoplatanus*.

Phytosociological relevés in the wider environs of the nest showed that the site represented biotope X9B, forest plantations of allochthonous deciduous trees; slope 40°, WNW aspect: E3 layer: *Robinia*

pseudoacacia 4, *Acer pseudoplatanus* +, *Fraxinus excelsior* +, *Prunus avium* r; E2 layer: *Acer pseudoplatanus* 1, *Crataegus monogyna* +, *Robinia pseudoacacia* +, *Sambucus nigra* +, *Symphoricarpos albus* +; E1 layer: *Alliaria petiolata* +, *Chelidonium majus* +, *Geum urbanum* +, *Poa nemoralis* +, *Rubus caesius* +, *Rubus fruticosus* agg. +, *Urtica dioica* +, *Vinca minor* +, *Dryopteris filix-mas* r, *Galeobdolon argentatum* r.

DNA analyses

The haplotype network (Fig. 4) revealed that the Czech sample belongs to a haplotype containing all sequences from other European countries, and a few from China.

Predicting species' distributions

The final variables incorporated into the model were BIO2 (Mean Diurnal Range), BIO3 (Isothermality), BIO4 (Temperature Seasonality), BIO10 (Mean Temperature of Warmest Quarter), BIO11 (Mean Temperature of Coldest Quarter), BIO15 (Precipitation Seasonality), BIO18 (Precipitation of Warmest Quarter), BIO19 (Precipitation of Coldest Quarter), and the categorical land cover variable.

In the first step of the model selection, models H0.5, H1.5 and LQH2.5 were selected (Table 1). In the second step, we selected the model with the least strict feature class, LQH2.5. This model combines less clamping on the input data, which gives room to consider the adaptation, with reasonably good computational performance.

The final model projected to the Czech Republic (Fig. 5) showed a distinctively increased habitat suitability in the western part of the country, decreasing towards the east. Upon visual comparison of the individual input climatic variables, it was determined that this phenomenon is influenced, among other factors, by the increasing precipitation seasonality towards the east of the Czech Republic. In the west, annual precipitation totals are more consistent, while in the east, there are pronounced differences between summer and winter totals.

Discussion

All recorded specimens of *Vespa velutina* in the Czech Republic belong to the subspecies *V. velutina nigrithorax*, similar to other European countries. We recorded individuals feeding and foraging on *H. helix* and *S. albus*. Pollen and nectar of *H. helix* are major autumn and winter resources for many insects (Metfalfe 2005) and individuals of *V. velutina* are frequently observed feeding on it in other countries (Monceau et al. 2013; Ueno 2015). The Czech nest predominantly contained larvae and pupae; thus the nest was likely eliminated before the production of young queens. We detected only five adult queens in the nest, but many other queens were close to hatching (Fig. 3C). We also determined 459 males that had reached the adult stage before the new queens (Darrouzet et al. 2015).

The Czech nest was built on an invasive tree, *R. pseudoacacia*, surrounded by forest plantations of allochthonous deciduous trees. *Robinia pseudoacacia* represents a rich source of nectar for insects.

Nests of *V. velutina* are commonly built on *R. pseudoacacia*, recorded, e.g., in northern Spain (Leza et al. 2018) and in Hungary. The secondary nests in European countries were built predominantly on trees (90%), with a minority in buildings (10%), and underground (< 1%) (Franklin et al. 2017). Moreover, Monceau et al. (2012) corroborated that *V. velutina* built their nests in the vicinity of water resources, and more individuals were caught in traps located closed to water. The Czech nest was located 80 metres from the Vejprnický stream.

The whole population of *V. velutina* in Europe likely descended from a single mated queen introduced to France from eastern China, as a strong bottleneck was revealed by mitochondrial and microsatellite markers (Arca et al. 2015). Other European populations were found genetically similar to the French (Granato et al. 2019; Husemann et al. 2020a; Dillane et al. 2022; Herrera et al. 2023), all pointing to the single origin of the invasion and subsequent spread across Europe. We confirmed that the sequenced Czech specimen also belongs to this group.

The exact route of *V. velutina* arrival to the Czech Republic is disputable and probably occurred through transportation. On the other hand, the species could have also utilised natural routes from Bavaria in Germany where *V. velutina* was observed (Höcherl and Berg 2020). The Czech Republic may have been colonised through the Všeruby pass, passing through the Bohemian Forest chain from foothills in Germany (Pařil et al. 2008).

The occurrence in West Bohemia was expected, based on species distribution modelling (Fig. 5). The western part of the Czech Republic is more climatically stable, which appears to be favoured by *V. velutina*. Simultaneously, there is a pronounced temperature seasonality in the Czech Republic (correlating with continentality) that increases from west to east. The hornets likely prefer lower continentality, i.e., smaller differences between maximum and minimum temperatures throughout the year (greater thermal stability). Without human mediated dispersal, the occurrence was expected mainly in the west part of the Czech Republic. The potential future distribution of *V. velutina* in the Czech Republic can be classified as moderate under current conditions and future climate change scenarios (Kim et al. 2021; Abou-Shaara and Al-Khalaf 2022); however, with human-mediated dispersal, a higher geographic distribution of the nests can be expected, similarly to other European countries (Robinet et al. 2018).

The crucial factor for the prevention of the spread of *V. velutina* is its early detection. In this case, civic collaboration, i.e., citizen science, may offer key information about recent records of invasive species and thus participate in data collection (Encarnação et al. 2021). Invasive species in the Czech Republic are monitored by the Nature Conservation Agency of the Czech Republic, which collaborated on preparation of an eradication plan for *V. velutina* (Skuhrovec and Pergl 2023), and administers the e-mail address invasivnidruhy@aopk.cz designed for reporting invasive species. The eradication plan outlines the procedures for monitoring and mapping of this species. Moreover, scientists and amateurs alike may use the application Nahlaš Sršeň [Report the Hornet] (Zaoral 2023).

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jan Walter, Jan Bureš, Jan Brus, Ondřej Biemann and Alena Sucháčková Bartoňová. The first draft of the manuscript was written by Jan Walter, Tomáš Görner, Jan Brus, Jan Bureš and Alena Sucháčková Bartoňová. All authors read and approved the final manuscript.

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Data availability statement

The collected specimens and the nest are deposited in the Museum of West Bohemia, Plzeň, Czech Republic. The filtered dataset of distribution records is available from the authors upon a reasonable request. The COI sequence was deposited in GenBank (accession number PP238379).

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Table 1

Table 1 is available in the Supplementary Files section.

Figures



Figure 1

Map of the study site in Pilsen city (Czech Republic, West Bohemia) with marked site of the first record of *Vespa velutina nigrithorax* (1) and the location of their nest (2). In the environs of the nest, we documented the biotopes X12B – other stands of early successional woody species and X9B – forest plantations of allochthonous deciduous trees (Chytrý et al. 2010).

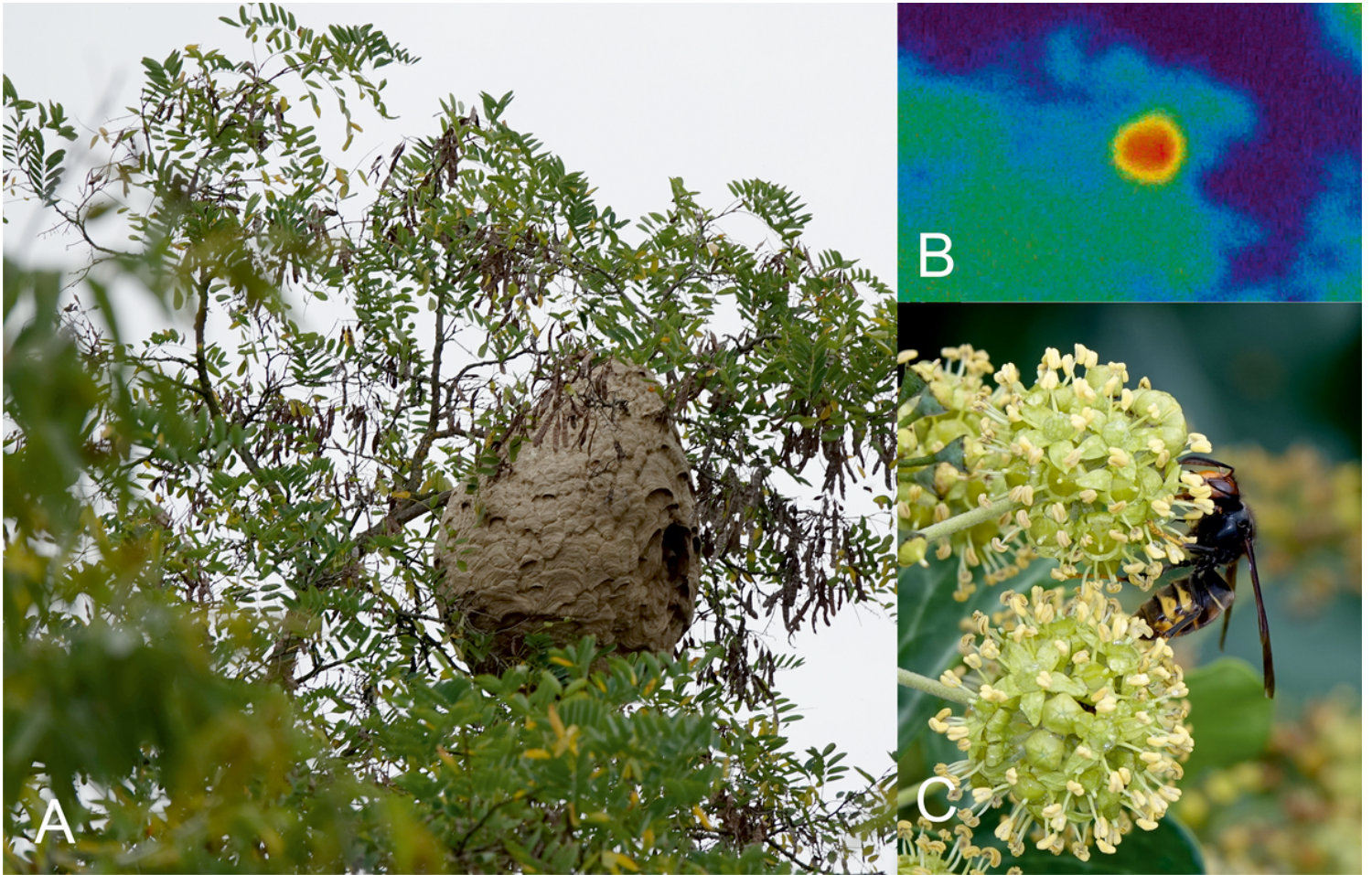


Figure 2

Nest (A) of *Vespa velutina nigrithorax* in Pilsen city (Czech Republic, West Bohemia), thermal image of the nest (B), and a worker (C) feeding on Ivy (*Hedera helix*).

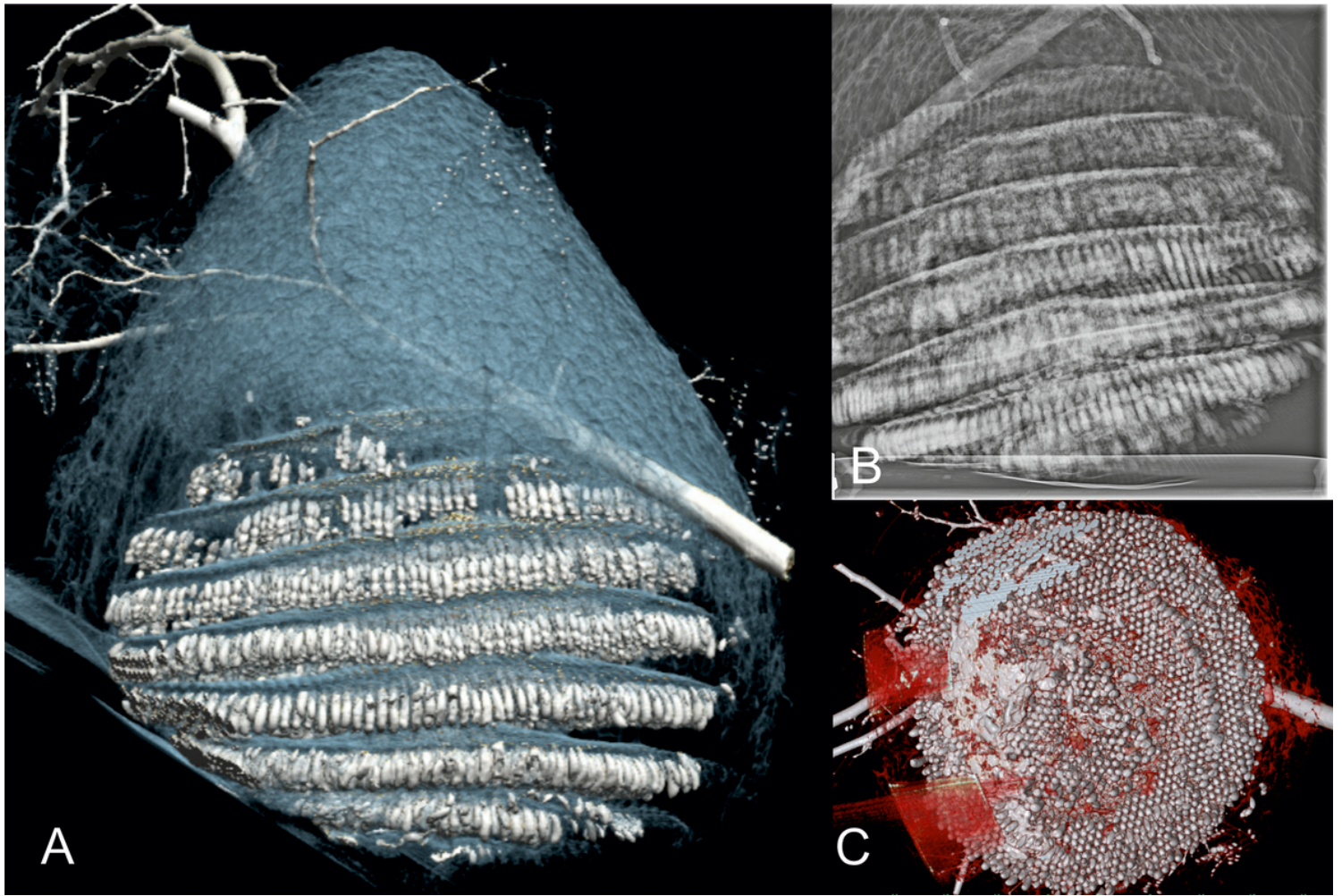


Figure 3

Images of the nest of *Vepsa velutina nigrithorax* from Pilsen city (Czech Republic, West Bohemia) using CT scanner (A) and X-ray machine (B), and a CT image of a horizontal cut of one gallery (C) from the nest.

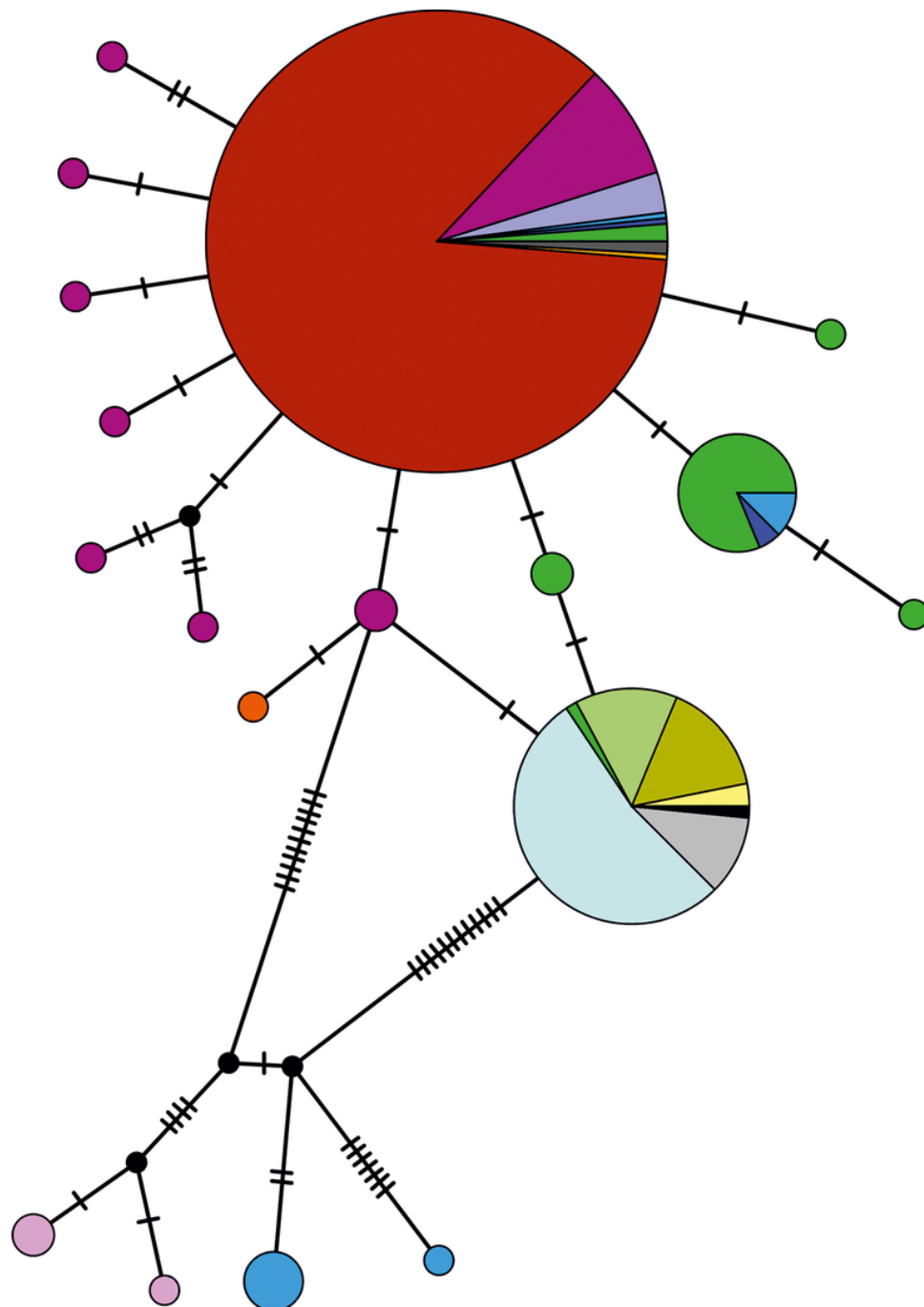


Figure 4

Haplotype network of the COI gene for *Vespa velutina nigrithorax*. Mutations are depicted as black dots and hatch marks. All European samples, together with the Czech specimen, form a single haplotype, pointing to European origin of the newly discovered Czech records.



Figure 5

Map of potential distribution of *Vespa velutina nigrithorax*, based on Maxent modelling.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)