



Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences
Rue Vautier 29
1000 Brussels, Belgium
+32 (0)2 627 41 23



Royal Museum for Central Africa
Leuvensesteenweg 13,
3080 Tervuren, Belgium
+32 (0)2 769 58 54



General introduction to this factsheet

The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo) provides an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. BopCo is funded by the Belgian Science Policy Office (Belspo), and it represented part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch (November 2015 – February 2022).

Non-native species which are being introduced into Europe, whether by accident or deliberately, can be of policy concern since some of them can reproduce and disperse rapidly in a new territory, establish viable populations and even outcompete native species. As a consequence of their presence, natural and managed ecosystems can be disrupted, crops and livestock affected, and vector-borne diseases or parasites might be introduced, impacting human health and socio-economic activities. Non-native species causing such adverse effects are called Invasive Alien Species (IAS). In order to protect native biodiversity and ecosystems, and to mitigate the potential impact on human health and socio-economic activities, the issue of IAS is tackled in Europe by EU Regulation 1143/2014 of the European Parliament and Council. The IAS Regulation provides for a set of measures to be taken across all member states. The list of *Invasive Alien Species of Union Concern* is regularly updated. However, to implement the proposed actions, methods for accurate species identification are required when suspicious biological material is encountered.

Because morphology-based species identifications are not always possible (e.g. cryptic species, trace material, early life-stages), the purpose of the present work is to investigate and evaluate the usefulness of DNA sequence data to identify each of the IAS included in the EU Regulation. The results are presented as factsheets (one per IAS) compiled using publicly available DNA sequence data and information aggregated from various sources. Each factsheet consists of two major parts: (i) a short introduction to the specific IAS, with information on its taxonomy and current occurrence/distribution in Europe, (ii) an investigation with respect to the usefulness of publicly available DNA sequences to identify this IAS using DNA barcoding to the taxonomic level stated in the EU list. For further information about the reasoning behind the applied approach and details on the materials and methods utilised, please see below and Smitz *et al.* [1].

More info about BopCo on <https://bopco.be> or contact us via bopco@naturalsciences.be.

More info on the EU Regulation on http://ec.europa.eu/environment/nature/invasivealien/index_en.htm.

Vespa velutina nigrithorax

de Buysson, 1905

Common names:

English: Asian black hornet, Asian hornet, yellow-legged hornet

French: frelon asiatique, frelon à pattes jaunes

German: Asiatische Hornisse

Dutch: (donkere) Aziatische hoornaar

Last update: October 2018



General information on *Vespa velutina nigrithorax*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Hymenoptera	Vespidae	<i>Vespa</i>

Species in the same genus: N = 22 [2]

Infra-species level: N = 13 [2-5]

Note: A total of 13 different colour morphs were recognized, including *V. v. nigrithorax*. These forms were long considered as subspecies but are now treated as synonyms of the nominal form.



Native range: [2,3,6-9]

Vespa velutina is widespread in Asia: Afghanistan, Bhutan, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Taiwan, Thailand and Vietnam.

The native distribution of *Vespa velutina nigrithorax* includes east Nepal, west Bhutan, northeast Vietnam and southeast China.

Invasive range: [2,10-14]

Europe (geographical):

Belgium, France, Germany, Italy, Netherlands, Portugal, Spain, Switzerland, United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:

<https://www.gbif.org/species/1311477>

<https://www.gbif.org/species/6247411>

<https://www.cabi.org/isc/datasheet/109164>

<https://gd.eppo.int/taxon/VESPVE/distribution>

<http://www.coloss.org/velutina/>

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R15970>

Outside Europe (geographical):

Japan and South Korea.

Morphology, biology, invasion, negative effects and remedies

For more information on *Vespa velutina nigrithorax* please see the references and online information listed at the end of this document.



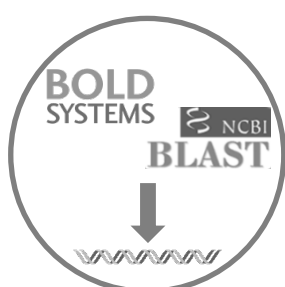
Species identification based on DNA barcodes

Introduction

DNA barcoding is a species identification method that uses a short genetic sequence (DNA barcode) to compare an unknown sample to a database of reference sequences with known species affiliations. The underlying rationale is that the divergence of nucleotide sequences among different species is larger than the nucleotide divergence between sequences within a species. DNA barcoding can facilitate the identification of IAS samples, especially when morphological characteristics are absent or useless. However, to assure correct species identifications, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation to assess the intraspecific genetic divergence, (ii) the closely related species to evaluate the interspecific genetic divergence, and (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS to detect potential population structure or local hybrids.

In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

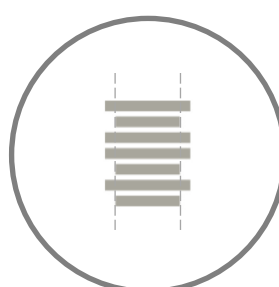
Material and Methods [1]



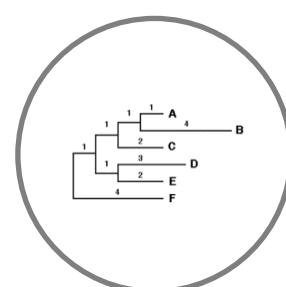
Download all sequence data available for the genus



Filtering the data and selecting 'promising' markers



Aligning and trimming of the sequences



Building Neighbour-Joining tree with Bootstrap support

Conclusion

Based on the present evaluation of the available sequence data, both COI and cytb look promising for the identification of *Vespa velutina*, but cannot distinguish among the different colour morphs. Additional sequences for the congeners would allow for a better evaluation of the performance of these markers.

Discussion

DNA markers for which *Vespa* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Vespa*. Six DNA markers were evaluated (Table 1). Because *Vespa velutina nigrithorax* is mentioned in the EU Regulation, a subspecies level identification is required. However, as reported on CABI [2] and by Carpenter and Kojima [5] the colour forms are now treated as synonyms of the nominal form, so identifications up to the species level are also discussed below. Besides, Arca *et al.* [15] report that most of the *Vespa* species which have been introduced beyond their native ranges failed to become established, except for *Vespa velutina*. Identifications to the species level might therefore be sufficient to assess the potential danger for invasion.

The database of available COI sequences was the most elaborate considering the number of species and colour morphs represented (Table 2), as well as considering the number of sequences per species, followed by cytb. In the NJ-tree for COI, the *V. velutina* sequences from both native and invasive locations cluster together with maximum support, except for two sequences from Vietnam (GenBank accession numbers JQ780459 and KF933081). The latter sequences might involve misidentifications. If so, both DNA markers appear to be useful in differentiating *V. velutina* from the other *Vespa* species (Table 1), yet not all congeneric species are represented. At the infra-species level, there does not seem to be enough sequence variation among the different *V. velutina* colour morphs for either COI or cytb to distinguish them from one another. To allow for a better evaluation of the performance of these markers, the missing species (Table 2) as well as additional sequences for the colour morphs, should be added.

For the other evaluated DNA markers (16S, 12S, COII, and ATP6-8) less sequence data is available for *Vespa velutina* and the congeneric species. Therefore, it is currently impossible to assess the ability of these markers to identify *Vespa velutina nigrithorax*.



Table 1: Overview of the encountered issues concerning the DNA-based identification of the IAS [1]: (1) Insufficient publicly available DNA sequences of the IAS to capture the intra-species divergence; (2) Poor geographical coverage of the IAS sequences (native or invasive range missing); (3) The IAS sequences do not form supported clusters; (4) Potential misidentification of a specimen which influences the clustering of the IAS sequences; and (5) Not all congeneric species are represented in the final NJ-tree. An 'N' indicates the issue is encountered for the colour morph *Vespa velutina nigrithorax*, a 'V' indicates the issue is present when considering *Vespa velutina* at the species level, while a '1' indicates only one *V. v. nigrithorax* sequence was available for analysis.

Markers analysed	1	2	3	4	5
COI			N	V	N V
cytb		N V	N		N V
16S	V	N V	N	V	N V
12S	N V	N V	1	V	N V
COII	N V	N V	1		N V
ATP6-8	N V	N V	1		N V

Table 2: Publicly available sequences downloaded (August 2018) from BOLD and GenBank (including sequences extracted from mitochondrial genomes) which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species and colour morph names follow [2,3,16]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	COI	cytb	16S	12S	COII	ATP6-8
<i>Vespa affinis</i>	X	X	X	X	X	X
<i>Vespa analis</i>	X		X		X	
<i>Vespa basalis</i>	X	X	X	X	X	X
<i>Vespa bellicosa</i>						
<i>Vespa bicolor</i>	X	X	X	X	X	X
<i>Vespa binghami</i>						
<i>Vespa crabro</i>	X	X	X	X	X	X
<i>Vespa ducalis</i>	X	X	X	X	X	X
<i>Vespa dybowskii</i>	X	X		X	X	X
<i>Vespa fervida</i>						
<i>Vespa fumida</i>						
<i>Vespa luctuosa</i>	X					
<i>Vespa mandarinia</i>	X	X	X	X	X	X
<i>Vespa mocsaryana</i>						
<i>Vespa multimaculata</i>						
<i>Vespa orientalis</i>	X	X	X	X	X	X
<i>Vespa philippinensis</i>						
<i>Vespa simillima</i>	X	X		X	X	
<i>Vespa soror</i>	X		X	X		
<i>Vespa tropica</i>	X		X			
<i>Vespa velutina</i>	X	X	X	X	X	X
<i>V. v. ardens</i>	X	X				
<i>V. v. auraria</i>	X	X	X			
<i>V. v. celebensis</i>	X	X				
<i>V. v. divergens</i>	X	X	X			
<i>V. v. flavitarsus</i>	X	X				
<i>V. v. floresiana</i>						
<i>V. v. karnyi</i>						
<i>V. v. nigrithorax</i>	X	X	X	X	X	X
<i>V. v. pruthii</i>						
<i>V. v. sumbana</i>						
<i>V. v. timorensis</i>						
<i>V. v. variana</i>	X	X				
<i>V. v. velutina</i>	X	X	X			
<i>Vespa vivax</i>	X	X	X	X	X	X
TOTAL species	15/22	11/22	12/22	12/22	12/22	10/22
TOTAL colour morphs	8/13	8/13	4/13	1/13	1/13	1/13

For a more elaborate discussion of the available databases, the sequence selection process, the outcome of the NJ-tree analyses, the usefulness of the investigated markers for species identification, as well as for more information on how to send samples for analyses please contact BopCo directly.



References and online information

Online information

<https://www.cabi.org/isc/datasheet/109164>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3826>
<https://secure.fera.defra.gov.uk/nonnativespecies/downloadDocument.cfm?id=646>
<http://www.nationalbeeunit.com/downloadDocument.cfm?id=1111>
http://hymettus.org.uk/downloads/Info_sheets_2010/12_Vespa_velutina_1col_infosheet.pdf
<https://waarnemingen.be/exo/be/nl/8807.pdf> [NL]
<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-aziatische-hoornaar> [NL]
<http://beeaware.org.au/archive-pest/asian-hornet/#ad-image-0>
http://frelonasiatique.mnhn.fr/wp-content/uploads/sites/10/2017/08/BHP-BHP030_Rome_Identification-Vespa-velutina_A4-size.pdf
<http://www.iucngisd.org/gisd/species.php?sc=1803>
<http://issg.org/database/species/ecology.asp?si=1803&fr=1&sts=&lang=EN>

Picture credits

Page 1: *Vespa velutina nigrithorax* en maraude By Alain C. [CC BY-NC-SA 2.0]
Page 2 (top left): *Vespa velutina nigrithorax* - dorsal side By Didier Descouens MHNT [CC BY-SA 4.0]
Page 2 (top right): *Vespa velutina nigrithorax* - ventral side By Didier Descouens MHNT [CC BY-SA 4.0]
Page 2 (bottom left): *Vespa velutina* from France near Limoges under the bark of dead tree for shelter in winter By Siga [CC BY-SA 3.0]
Page 2 (bottom right): *Vespa velutina* nest on the top of a tree, Saint-Laurent-Le-Minier (Gard, France) By Fredciel [CC BY-SA 3.0]

References

- [1] N. Smits, S. Gombeer, K. Meganck, A. Vanderheyden, Y. R. Van Bourgonie, T. Backeljau, and M. De Meyer, "Identifying IAS based on DNA barcoding using currently available sequence data: details on applied material and methods." 2019. [Online]. Available from: <https://bopco.be/output/iasfactsheets>.
- [2] CABI, "*Vespa velutina* [original text by CRC for National Plant Biosecurity, Canberra, Australia]." *Invasive Species Compendium*. Wallingford, UK: CAB International., 2018. [Online]. Available: <https://www.cabi.org/isc>. [Accessed: 09-Mar-2018].
- [3] A. Perrard, M. Arca, Q. Rome, F. Muller, J. Tan, S. Bista, H. Nugroho, R. Baudoin, M. Baylac, J.F. Silvain and J.M. Carpenter, "Geographic variation of melanisation patterns in a hornet species: genetic differences, climatic pressures or aposematic constraints?" *PLoS One*, vol. 9, no. 4, 2014.
- [4] J. van der Vecht, *The Vespinae of the Indo-Malayan and Papuan areas (Hymenoptera, Vespidae)*. Brill, 1957.
- [5] J. Carpenter and J. Kojima, "Checklist of the species in the subfamily Vespinae (Insecta: Hymenoptera: Vespidae)" *Nat. Hist. Bull. Ibaraki Univ.*, vol. 1, pp. 51–92, 1997.
- [6] M. Choi, S. J. Martin, and J. Lee, "Distribution, spread and impact of the invasive hornet *Vespa velutina* in South Korea" *J. Asia. Pac. Entomol.*, vol. 15, no. 3, pp. 473–477, 2012.
- [7] M. Archer, *Vespine Wasps of the World: Behaviour, Ecology & Taxonomy of the Vespinae. Monograph Series*. Manchester: UK: Siri Scientific Press, 2012.
- [8] M. Archer, "Taxonomy, distribution and nesting biology of the *Vespa bicolor* group (Hym., Vespinae)." *Entomol. Mon. Mag.*, vol. 130, no. 1560–63, pp. 149–158, 1994.
- [9] C. Jung, M. Kang, D. Kim, and H. Lee, "Vespid Wasps (Hymenoptera) Occurring Around Apiaries in Andong, Korea - I. Taxonomy and life history" *Korean J. Apic.*, vol. 22, pp. 53–62, 2007.
- [10] LIFE STOPVESPA, "*Vespa velutina*." 2018. [Online]. Available: <https://www.vespavelutina.eu/en-us/>. [Accessed: 09-Mar-2018].
- [11] EPPO, "EPPO Global Database - *Vespa velutina* (VESPE) Distribution." 2018. [Online]. Available: <https://gd.eppo.int/taxon/VESPVE/distribution>. [Accessed: 09-Mar-2018].
- [12] Nederlandse Voedsel- en Warenautoriteit, "Factsheet Aziatische hoornaar (*Vespa velutina*)." 2018. [Online]. Available: <https://www.nvwa.nl/documenten/risicobeoorhttps://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-aziatische-hoornaar>. [Accessed: 09-Mar-2018].
- [13] GB Non-Native Species Secretariat, "*Vespa velutina*, Asian Hornet [text by Marc Botham, David Hubble, Helen Roy]." 2016. [Online]. Available: <http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3826>. [Accessed: 09-Mar-2018].
- [14] L. Turchi and B. Derijard, "Options for the biological and physical control of *Vespa velutina nigrithorax* (Hym.: Vespidae) in Europe: A review" *J. Appl. Entomol.*, vol. 142, no. 6, pp. 553–562, 2018.
- [15] M. Arca, F. Mougél, T. Guillemaud, S. Dupas, Q. Rome, A. Perrard, F. Muller, A. Fossoud, C. Capdevielle-Dulac, M. Torres-Leguizamon and X.X. Chen, "Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe." *Biol. Invasions*, vol. 17, no. 8, pp. 2357–2371, Aug. 2015.
- [16] A. Perrard, K. Pickett, C. Villemant, J. Kojima, and Carpenter J., "Phylogeny of hornets: a total evidence approach (Hymenoptera, Vespidae, Vespinae, *Vespa*)" *J. Hymenopt. Res.*, vol. 32, pp. 1–15, 2013.

To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Vespa velutina nigrithorax*; October 2018. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: <https://bopco.be/output/iasfactsheets>, accessed on DD-MM-YYYY.



DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the “Conclusion” are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through bopco@naturalsciences.be.

