



# Identification of Invasive Alien Species using DNA barcodes

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## 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](mailto:bopco@naturalsciences.be).

More info on the EU Regulation on [http://ec.europa.eu/environment/nature/invasivealien/index\\_en.htm](http://ec.europa.eu/environment/nature/invasivealien/index_en.htm)

## *Corvus splendens*

Vieillot, 1817

Common names:

English: (Indian) house crow, Colombo crow

French: corbeau d'Inde, corbeau familier, corneille de l'Inde

German: Glanzkrähe

Dutch: huiskraai

Last update: September 2019



## General information on *Corvus splendens*

### Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Aves	Passeriformes	Corvidae	<i>Corvus</i>

### Species in the same genus: N = 40-48 [2-4]

Note: Some discussion remains on the recognition of certain names at either species or subspecies level, hence the range in species number.

### Infra-species level: N = 5 [2, 5, 6]

Note: Five subspecies names are encountered in literature: *C.s. insolens*, *C.s. maledivicus*, *C.s. protegatus*, *C.s. splendens*, *C.s. zugmayeri*. No distinction is made in the European Regulation.

Hybridisation with *C. corone* and *C. albus* is encountered.



### Native range: [5, 7, 8]

Indian subcontinent and neighbouring countries.

### Invasive range: [7-9]

#### Europe (geographical):

Belgium, Cyprus, Denmark, France, Greece, Hungary, Ireland, Latvia, Netherlands, Poland, Spain.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R04121>

<https://www.gbif.org/species/2482499>

<https://www.cabi.org/isc/datasheet/15463>

#### Outside Europe (geographical):

Along the East African coast; Egypt to South Africa, Australia, Caribbean, Madagascar, Malaysia, Middle-East.

### Morphology, biology, invasion, negative effects and remedies

For more information on *Corvus splendens* please see the references and online information listed at the end of this document.



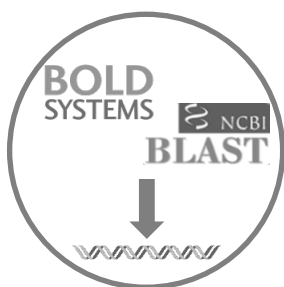
## Species identification based on DNA barcodes

### 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/](http://www.boldsystems.org/)) and GenBank ([www.ncbi.nlm.nih.gov/nuccore/](http://www.ncbi.nlm.nih.gov/nuccore/)) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

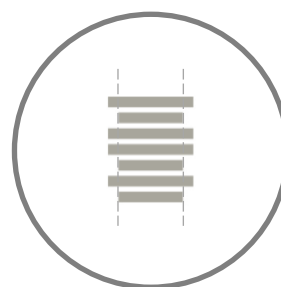
### Material and Methods [1]



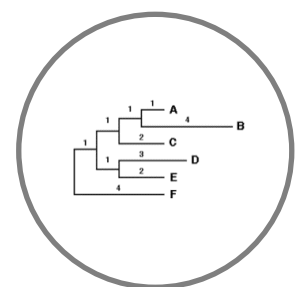
Download all sequence data available for the genus



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, ND2 is the most reliable DNA marker for the identification of *Corvus splendens*.

### Discussion

DNA markers for which *Corvus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Corvus*. Five DNA markers were evaluated (Table 1).

For the **ND2** marker the clustering of *C. splendens* in the NJ-tree is highly supported. This marker is available for nearly all *Corvus* species (Table 2), most with multiple sequences and an overall high genetic variation.

For the universal marker **COI** and for **cytb** the available sequences of *C. splendens* all cluster together with high support. However, for some species in the genus the sequences are not clustering. With many congeners still unrepresented, it is not guaranteed a closely related, unrepresented species would cluster separate from the *C. splendens* cluster. Additional sequences are needed to better evaluate the performance of these markers for species identifications.

Sequences of different lengths from the **Control Region (CR)** or **D-loop** were lumped into a final alignment of 700 bp, similar to Haring *et al.* [3]. The species representation in the alignment of the sequences selected for final evaluation is reported in Table 2. The clustering of *C. splendens* has a low support and not all congeners are represented in the NJ-tree. The data processing (aligning) for this marker is not evident; hence it is not advisable to apply these markers for species identification.

The **ND3** marker has a high species representation, but with few sequences per species. The two sequences of *C. splendens* (both from the invasive region) cluster together with high support. In the currently limited state of the online reference libraries it is impossible to fully assess the ability of this marker to identify *C. splendens*.

For **GAPDH**, **16S** and **trnG** gene only few sequences are available and/or the marker shows little genetic variation among the different species. Therefore, it is currently impossible to assess the performance of these markers to identify *C. splendens*.



**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 'X' indicates that the issue was encountered.

Markers analysed	1	2	3	4	5
COI					X
cytb					X
CR & D-loop					X
ND2					X
ND3	X	X		X	X

**Table 2:** Publicly available sequences downloaded (September 2019) 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 names follow [10]. An 'X' indicates that at least one sequence was used in the final alignment. Species names with '\*' are not mentioned in [10] but used on GenBank.

Species in genus	COI	cytb	CR & D-loop	ND2	ND3
<i>Corvus albicollis</i>	X	X	X	X	X
<i>Corvus albus</i>	X	X	X	X	X
<i>Corvus bennetti</i>			X	X	X
<i>Corvus brachyrhynchos</i>	X	X	X	X	X
<i>Corvus capensis</i>			X	X	
<i>Corvus caurinus</i>	X	X	X	X	X
<i>Corvus compilator</i>					
<i>Corvus corax</i>	X	X	X	X	
<i>Corvus cornix</i> *	X	X	X	X	X
<i>Corvus corone</i>	X	X	X	X	X
<i>Corvus coronoides</i>	X	X	X	X	X
<i>Corvus crassirostris</i>			X	X	X
<i>Corvus cryptoleucus</i>	X	X	X	X	X
<i>Corvus culminates</i> *		X	X	X	
<i>Corvus dauuricus</i>	X		X	X	X
<i>Corvus edithae</i>				X	X
<i>Corvus enca</i>	X		X	X	X
<i>Corvus florensis</i>			X	X	X
<i>Corvus frugilegus</i>	X	X	X	X	X
<i>Corvus fuscicapillus</i>			X	X	X
<i>Corvus hawaiiensis</i>	X	X	X	X	X
<i>Corvus imparatus</i>				X	
<i>Corvus insularis</i>				X	X
<i>Corvus jamaicensis</i>				X	X
<i>Corvus kubaryi</i>	X	X		X	X
<i>Corvus leucognaphalus</i>				X	X
<i>Corvus macrorhynchos</i>	X	X	X	X	X
<i>Corvus meeki</i>				X	X
<i>Corvus mellori</i>			X	X	X
<i>Corvus minutus</i>				X	X
<i>Corvus monedula</i>	X	X	X	X	X
<i>Corvus moneduloides</i>			X	X	X
<i>Corvus moriorum</i> *	X	X			
<i>Corvus nasicus</i>				X	X
<i>Corvus orru</i>		X	X	X	X
<i>Corvus ossifragus</i>	X	X	X	X	X
<i>Corvus palmarum</i>			X	X	X
<i>Corvus pectoralis</i>			X	X	X
<i>Corvus philippinus</i>					
<i>Corvus rhipidurus</i>			X	X	
<i>Corvus ruficollis</i>	X	X	X	X	X
<i>Corvus sinaloae</i>				X	X
<b><i>Corvus splendens</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Corvus tasmanicus</i>			X	X	X
<i>Corvus torquatus</i>					
<i>Corvus tristis</i>			X	X	X
<i>Corvus typicus</i>			X	X	X
<i>Corvus unicolor</i>				X	X



Species in genus	COI	cytb	CR & D-loop	ND2	ND3
<i>Corvus validus</i>			X	X	X
<i>Corvus violaceus</i>				X	X
<i>Corvus woodfordi</i>			X	X	X
<b>TOTAL species</b>	<b>20/40-49</b>	<b>20/40-49</b>	<b>35/40-49</b>	<b>47/40-49</b>	<b>42/40-49</b>

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 DNA sequences for species identification, as well as information on how to send samples for analyses please contact BopCo directly.



## References and online information

### Online information

<https://www.cabi.org/isc/datasheet/15463>  
<http://datazone.birdlife.org/species/factsheet/House-Crow>  
<https://avibirds.com/house-crow/>  
<http://www.oiseaux.net/birds/house.crow.html>  
<https://waarnemingen.be/pda/shellphp/exo/be/nl/261.pdf> [NL]

### Picture credits

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Page 2 (left, bottom): House Crow *Corvus splendens* eggs in Kolkata, West Bengal, India By J.M. Garg [CC BY-SA 3.0]  
Page 2 (right): House Crow (*Corvus splendens*) in flight over Dhrangadrha By Shanthanu Bhardwaj [CC BY-SA 2.0]

### References

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- [10] Christidis *et al.*, "The Howard and Moore Complete Checklist of the Birds of the World, version 4.1," 2018. [Online]. Available: <https://www.howardandmoore.org>.

### To cite this factsheet, please use

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

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