



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.

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

Threskiornis aethiopicus

(Latham, 1769)

Common names:

English: (African) sacred ibis, black-necked ibis

French: ibis sacré

German: Heiliger Ibis

Dutch: heilige ibis

Last update: September 2019



General information on *Threskiornis aethiopicus*

Classification

| Kingdom | Phylum | Class | Order | Family | Genus |
|----------|----------|-------|---------------|-------------------|---------------------|
| Animalia | Chordata | Aves | Ciconiiformes | Threskiornithidae | <i>Threskiornis</i> |

Species in the same genus: N = 5 [2]

Note: The often listed subspecies *T.a. bernieri* and *T.a. abbotti* are here recognized as a full species and its subspecies.

Infra-species level: N = 0

Note: To our knowledge, no subspecies have been described.



Native range: [3, 4]

Sub-Saharan Africa, Iraq and Kuwait.

Invasive range: [5, 6]

Europe (geographical):

Austria, Belgium, Czech Republic, France, Germany, Greece, Italy, Latvia, Portugal, Spain (incl. Canary Islands), United Kingdom.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R15136>

www.gbif.org/species/2480764

<https://avibase.bsc-eoc.org/species.jsp?lang=NL&avibaseid=550DE745B77C8079&sec=map>

Outside Europe (geographical):

Australia, India, Taiwan, United Arab Emirates, United States of America.

Morphology, biology, invasion, negative effects and remedies

For more information on *Threskiornis aethiopicus* 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]



Conclusion

Based on the present evaluation of the available sequence data, no marker can reliably identify *Threskiornis aethiopicus*. COI seems most promising to further investigate once new sequence data becomes available.

Discussion

DNA markers, for which *Threskiornis* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Threskiornis*. Four DNA markers were evaluated (Table 1). The species coverage was low for each DNA marker. Therefore, these DNA markers are currently not useful for the identification of *T. aethiopicus*

The NJ-tree based on COI shows three highly supported clusters of *T. aethiopicus* sequences. One cluster includes a sequence of *T. aethiopicus* from Australia, together with a *T. molucca* sequence, which is only known from Australia; the former might be mislabelled/misidentified. A second supported cluster exclusively includes Indian *T. aethiopicus* sequences; this could possibly be mislabelled *T. melanocephalus* samples from India. The potential mislabelling might be linked to the fact that some authors still treat *T. molucca* and *T. melanocephalus* as subspecies of *T. aethiopicus* and that these three species have overlapping distributions. The third cluster groups sequences from *T. aethiopicus* collected in both its native and invasive range (i.e., Djibouti, South Africa and Taiwan). In order to better evaluate the performance of COI to identify *T. aethiopicus* s.s., additional voucher specimen sequences, should be added. It is currently impossible to confirm the ability of this marker to identify *T. aethiopicus*.

For marker **cytb**, the genetic variation is low raising doubts about the taxonomic resolution of the marker for the genus *Threskiornis*. Hence it is not advisable to apply this marker for species identification.

For the markers **12S** and **16S**, few sequence data is available. Hence, it is premature to assess the ability of these DNA markers to identify *T. aethiopicus* from other *Threskiornis* species.



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, n/a: not applicable.

| Markers analysed | 1 | 2 | 3 | 4 | 5 |
|------------------|---|---|-----|---|---|
| COI | | | X | X | X |
| cytb | X | X | X | | X |
| 12S | X | X | n/a | | X |
| 16S | X | X | | | X |

Table 2: Publicly available sequences downloaded (September 2019) from BOLD and GenBank which were withheld as reliable and informative in the final alignment that was used for building the NJ-trees. The species names follow [2]. An 'X' indicates that at least one sequence was used in the final alignment, a '1' indicates only one sequence was available.

| Species in genus | COI | cytb | 12S | 16S |
|--|------------|------------|------------|------------|
| <i>Threskiornis aethiopicus</i> | X | X | X | X |
| <i>Threskiornis bernieri</i> | | | | |
| <i>Threskiornis melanocephalus</i> | | | | |
| <i>Threskiornis molucca</i> | 1 | 1 | | 1 |
| <i>Threskiornis spinicollis</i> | | | | |
| TOTAL species | 2/5 | 2/5 | 1/5 | 2/5 |

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

<http://www.oiseaux.net/oiseaux/ibis.sacre.html> [FR]
<https://avibase.ca/550DE745>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=3537>
http://aviansag.org/Fact_Sheets/Cicag/Sacred_Ibis.pdf
<https://species.biodiversityireland.ie/profile.php?taxonId=12088&taxonName=ibis>
<https://avibirds.com/sacred-ibis/>

Picture credits

Page 1: Szent íbisz (*Threskiornis aethiopicus*) By Veszprémi Állatkert [CC BY-SA 3.0]
Page 2 (left): A Sacred Ibis (*Threskiornis aethiopicus*) at the Honolulu Zoo By cliff1066 [CC BY 2.0]
Page 2 (middle): Over the creek By Michael Jefferies [CC BY-NC 2.0]
Page 2 (right): Eggs of African sacred ibis Two specimens of the same spawn; collection of Jacques Perrin de Brichambaut By Roger Culos [CC BY-SA 4.0]

References

- [1] N. Smits, S. Gombeer, K. Meganck, 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: <https://bopco.be/output/iasfactsheets>.
- [2] 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>.
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