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.

Alopochen aegyptiaca

(Linnaeus, 1766)

Common names:
English: Egyptian goose, Nile goose
French: ouette d'Egypte
German: Nilgans
Dutch: nijlgans

Last update: September 2019

General information on *Alopochen aegyptiaca*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Chordata	Aves	Anseriformes	Anatidae	*Alopochen*

Species in the same genus: N = 1 [2–4]
Note: The genus *Alopochen* includes one species: *Alopochen aegyptiaca* and belongs to the subfamily Tadorninae.
The species name on the EU Regulation should be corrected from *A. aegyptiacus* to *A. aegyptiaca* to agree with naming conventions.

Infra-species level: N = 0 [2, 5, 6]
Note: To our knowledge, no subspecies have been described.
Hybridization with other geese and duck species has been observed in invasive regions.

Native range: [2, 6, 7]
Sub-Saharan Africa and Nile valley.

Invasive range: [5, 6]
Europe (geographical):
Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Italy, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, United Kingdom.

For more detailed locality information and the most recent distribution updates, please visit:
https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R00644
https://www.gbif.org/species/2498252

Outside Europe (geographical):
Australia, China, Israel, Syria, United Arab Emirates, United States of America.

Morphology, biology, invasion, negative effects and remedies
For more information on *Alopochen aegyptiaca* please see the references and online information listed at the end of this document.

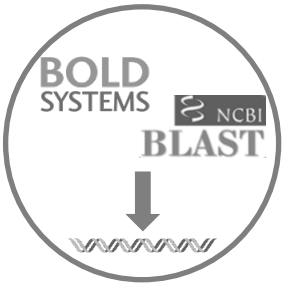
Species identification based on DNA barcodes

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.

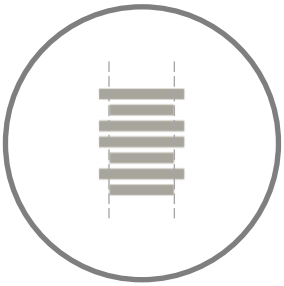
In this context, BopCo evaluated the inclusion of the IAS and their close relatives in both publicly available reference libraries BOLD (www.boldsystems.org/) and GenBank (www.ncbi.nlm.nih.gov/nuccore/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

Material and Methods [1]

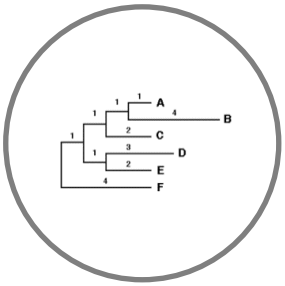


Download all sequence data available for the genus

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, cytb is the most reliable DNA marker for the identification of *Alopochen aegyptiaca*. To allow for a better evaluation of the performance of this marker for species identification, additional sequences should be added to the analyses.

Discussion

Since *Alopochen* is a monotypic genus, DNA markers for which *Alopochen aegyptiaca* sequences were available, were downloaded from GenBank and BOLD for all represented species of the tribe Tadornini. Five DNA markers were evaluated (Table 1).

In the NJ-tree for **cytb,** the *A. aegyptiaca* sequences (from both native and invasive locations) cluster together with high support, however, only a small number of sequences is available. Within the tribe Tadornini, this marker has the highest species coverage (Table 2). To allow for a better evaluation of this marker additional sequences for *A. aegyptiaca* and the missing Tadornini species should be added to the analyses.

The universal barcode marker **COI** clusters the available tribe Tadornini species with high support, but the *A. aegyptiaca* cluster is only represented by two sequences from Djibouti (native region). Additional sequences for *A. aegyptiaca* (from the invasive regions) and missing Tadornini species would allow for a better evaluation of the performance of this marker for species identification. Huang *et al.* [8] conclude that COI is effective for species identification (but without including *A. aegyptiaca*). BOLD contains additional *A. aegyptiaca* sequences under Private status, so they could not be included in the present analyses.

For the **D-loop**, **12S** and the **ND2** gene, only one *A. aegyptiaca* sequence is available for each marker. The sequences of other species cluster with high support, but it is currently impossible to assess the ability of these markers to identify *A. aegyptiaca*. The phylogenetic use of the D-loop is further discussed in Donn-Goussé *et al.* [9] and Warzecha *et al.* [10].

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 species of the subfamily are represented in the final NJ-tree. An 'X' indicates that the issue was encountered, a '1' indicates only one *A. aegyptiaca* sequence was available, n/a: not applicable.

Markers analysed	**1**	**2**	**3**	**4**	**5**
COI	X	X			X
cytb	X				X
D-loop	X	1	n/a		X
12S	X	1	n/a		X
ND2	X	1	n/a		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 names follow [11]. An 'X' indicates that at least two sequence were used in the final alignment, a '1' indicates only one sequences was available for the final alignment. Species were grouped by genus, with the number in brackets indicating the number of species recognized following [11].

Genera (species) in subfamily	**COI**	**cytb**	**D-loop**	**12S**	**ND2**
Alopochen aegyptiaca	**X**	**X**	**1**	**1**	**1**
Chloephaga (5)	X	X	X	X	X
Neochen (1)	X	X		X	
Radjah (1)		X		X	X
Tadorna (6)	X	X	X	X	X
TOTAL species	**8/14**	**11/14**	**5/14**	**6/14**	**8/14**

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://datazone.birdlife.org/species/factsheet/egyptian-goose-alopochen-aegyptiaca
https://www.fws.gov/fisheries/ANS/erss/highrisk/ERSS-Alopochen-aegyptiaca-FINAL-April2018.pdf
http://animalia.bio/egyptian-goose
http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=140
http://www.oiseaux.net/oiseaux/ouette.d.egypte.html [FR]
https://waarnemingen.be/pda/shellphp/exo/be/nl/152.pdf [NL]

Picture credits

Page 1: Egyptian Goose (*Alopochen aegyptiaca*), Hyde Park, London By Charles J Sharp [CC BY-SA 3.0]
Page 2 (left, top): Egyptian Goose duckling in KwaZulu-Natal, South Africa By GalliasMJ [Public Domain]
Page 2 (left, bottom): Egg of Egyptian goose Collection of Jacques Perrin de Brichambaut By Roger Culos [CC BY-SA 4.0]
Page 2 (right): Nilgänse Alopochen-aegyptiaca-oie-2348007 By lapping [CC0]

References

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