Invasive Alien Species in Belgium (IAS)

Examining the utility of GenBank and BOLD for species identifications

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INTRODUCTION

The Barcoding facility for Organisms and tissues of Policy COncern (BopCo project - the Belgian federal in-kind contribution to LifeWatch) aims at developing a virtual laboratory, expertise forum and databank network facilitating the identification of biological samples of policy concern for Belgium and Europe. As such, the purpose of the present contribution is to investigate and evaluate the available DNA sequence databases for 37 Invasive Alien Species (IAS) listed in European Regulation 1143/2014. Indeed, in order to protect native biodiversity and ecosystems, and mitigate the potential impact on human health and socio-economical activities, a rapid identification of suspicious biological material is required to assist authorities in their decision process. DNA-based methods are promising, especially in cases where morphological identifications are problematic (e.g. cryptic species, trace material, early life-stages). However, the reliability of species identifications based on DNA-methods are highly dependent on the availability and representativeness of reference sequence databases such as BOLD and GenBank. Therefore, we explored the usefulness of BOLD and GenBank to identify these specific IAS.

MATERIAL, METHODS AND RESULTS

DNA-database compilation and evaluation method

Available DNA sequences of three bird, nine mammal, six crustacean, one insect, two fish, one reptile, one amphibian and 14 plant species, as well as of their congeners, were retrieved from the online repositories. After preliminary filtering and alignment steps, Neighbor-Joining trees were reconstructed (500 BS, Jukes-Cantor distance model) for each marker with sufficient material. To evaluate their capacity at providing a reliable species ID, we classified the different potential issues encountered into eight categories. Each marker of each species was then evaluated based on these criteria:

(1) Taxonomic issues of the target species;
(2) Less than five DNA sequences available for the target species;
(3) Poor geographical coverage (native or invasive range missing or scarce);
(4) Non-recovery or unsupported cluster (< 85 bootstrap value) of the target;
(5) Low overall DNA sequence genetic variation (target and congeners);
(6) Potential species misidentification of a voucher specimen;
(7) Incomplete representation of congener species in the repositories;
(8) Less than three DNA sequences available for each congeneric species.

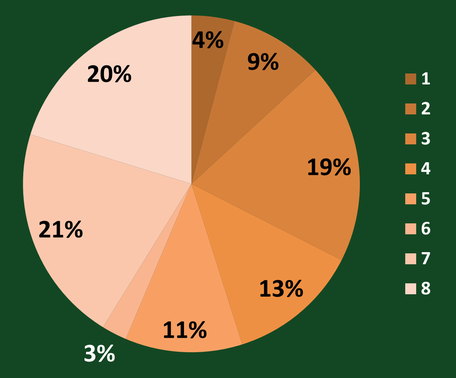


Fig. 1: Average proportion of the occurrence of each category (all DNA sequences, all species included)

Outcomes

35 different markers were evaluated, including COI, Cytb, D-Loop, 12S, 16S, rbcL, ITS, matK and psbA-trnH intergenic spacer. Per species, one to ten different markers (mean of four) were investigated. On average, the most common issues identified were (3), (7) & (8) (Fig. 1). The pattern recorded for the four most common markers are displayed in Fig. 2.

SOME EXAMPLES:

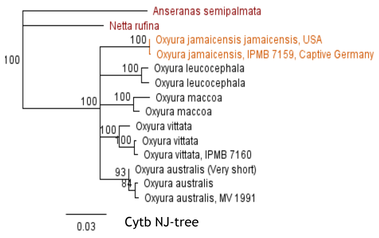
(Cat. 1, 3, 4, 5, 7, 8) Insufficient taxonomic resolution:

Pueraria montana* var. *lobata

Lack of resolving power to distinguish closely related taxa that may hybridize.

(Cat. 2, 3, 6, 8) Limited availability of DNA sequences and geographical coverage of both target and congeners:

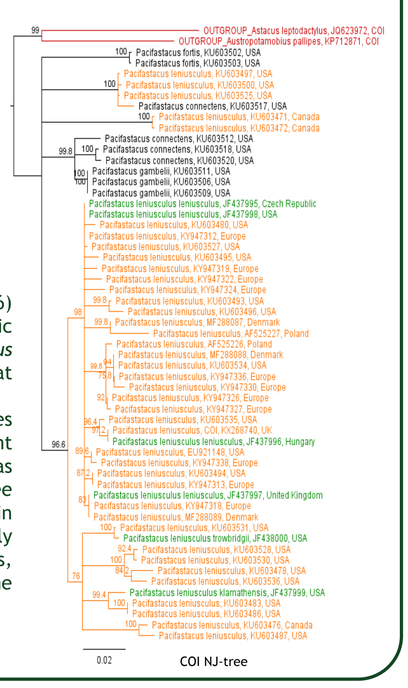
Oxyura jamaicensis



Lack of data from the target species and the congeners, limited geographical coverage. Also, *O. jamaicensis* and *O. ferruginea* were previously split into two distinct species but are now recognized as one, following Avibase (modified on the tree).

(Cat. 1, 4, 6, 8) Non-recovery of the target species as supported cluster:

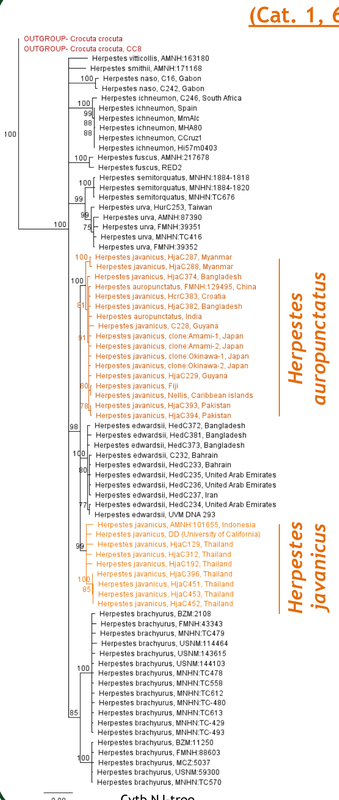
Pacifastacus leniusculus



Larsen *et al*. (2012, 2016) found substantial cryptic diversity within *P. leniusculus* and demonstrated that morphology-based assignment to subspecies does not match assignment to *Pacificastacus* clusters as defined by COI. The three subspecies (indicated in green) were originally described as distinct species, yet they all end up in one cluster in the tree.

(Cat. 1, 6, 8) Taxonomic issue and potential misidentification of vouchers:

Herpestes javanicus



Herpestes auropunctatus

Herpestes javanicus

H. auropunctatus and *H. javanicus* were previously considered as one species but are now split into two distinct species (5% genetic divergence - Veron *et al*. 2007). Moreover, the many introduced populations of *H. javanicus sensu lato* around the world are all believed to be *H. auropunctatus* (Veron *et al*. 2007).

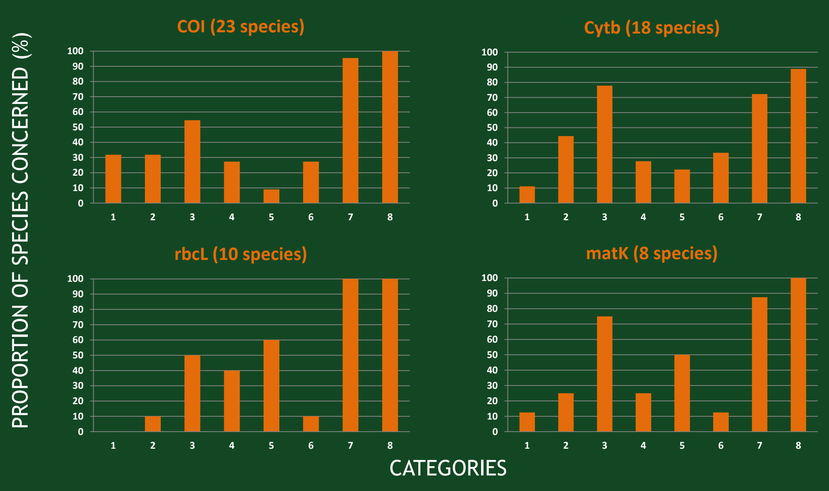


Fig. 2: Proportion of species affected by each potential issue, displayed for the four most represented markers

DISCUSSION

DNA-based identifications

Despite the fact that the ideal situation was never met, 15 of the 37 IAS can be considered reliably identifiable using DNA sequences. For 12 other species, DNA markers with a high potential were identified, yet they are discarded for the time being due to a lack of available material (target and congeners). The remaining ten IAS are presently not considered as identifiable. In conclusion, shortcomings for more than half of the 37 IAS were highlighted in the present work.

- Larson, E. R., Abbott, C. L., Usio, N., *et al*. (2012). The signal crayfish is not a single species: cryptic diversity and invasions in the Pacific Northwest range of *Pacifastacus leniusculus*. *Freshwater Biology*, *57*(9):1823-1838.
- Larson, E. R., Castelin, M., Williams, B. W., *et al*. (2016). Phylogenetic species delimitation for crayfishes of the genus *Pacifastacus*. *PeerJ*, *4*:e1915.
- Veron, G., Patou, M.-L., Pothet, G., *et al*. (2007). Systematic status and biogeography of the Javan and small Indian mongooses (Herpestidae, Carnivora). *Zoologica Scripta*, 36:1-10.