



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) aims at developing an expertise forum to facilitate the identification of biological samples of policy concern in Belgium and Europe. The project represents part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch.

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. In order to implement the proposed actions, however, 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 compiling 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 to the taxonomic level stated in the EU list using DNA barcoding. For further information about the reasoning behind the applied approach and details on the materials and methods utilised, please see below and Smits *et al.* [1].

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

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

Eriocheir sinensis

H. Milne Edwards, 1853

Common names:

English: Chinese mitten crab, Shanghai crab, Chinese river crab

French: crabe chinois, crabe poilu de Shanghai

German: Chinesische Wollhandkrabbe

Dutch: Chinese wolhandkrab

Last update: January 2019



General information on *Eriocheir sinensis*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Malacostraca	Decapoda	Varunidae	<i>Eriocheir</i>

Species in the same genus: N = 4 [2-5]

Note: In the past decades there has been much debate about mitten crab taxonomy. We follow the classification as presented by Ng *et al.* (2008), i.e. the genus *Eriocheir* with 4 species: *E. hepuensis*, *E. japonica*, *E. ogasawaraensis* and *E. sinensis*.

Infra-species level: N = 0

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



Native range: [6, 7]

China (Anhui, Chongqing, Hebei, Hong Kong, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Macau, Shandong, Shanghai, Sichuan, Zhejiang), Japan, North and South Korea, Russia and Taiwan.

Invasive range: [6, 8]

Europe (geographical):

Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, , Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Ukraine, United Kingdom.

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

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

<https://www.gbif.org/species/2225776>

Outside Europe (geographical):

Canada, China (Fujian, Guangdong, Guangxi, Hainan, Henan, Yunnan) and the United States of America.

Morphology, biology, invasion, negative effects and remedies

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



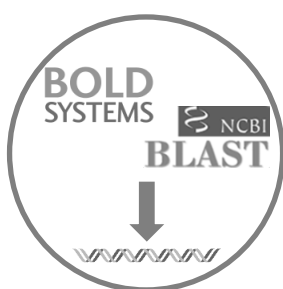
Species identification based on DNA barcodes

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. To assure correct species identifications, however, reference libraries need to include a sufficiently large number of sequences of (i) the IAS under investigation, in order to assess the intraspecific genetic divergence; (ii) the closely related species, in order to evaluate the interspecific genetic divergence; (iii) the different geographical areas covering the distribution range (native and invasive) of the IAS in order to detect potential population structure or local hybrids.

Against this background, 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/nucleotide/) to estimate the reliability with which a species identification can be obtained using DNA barcoding.

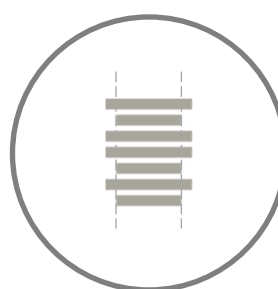
Material and Methods [1]



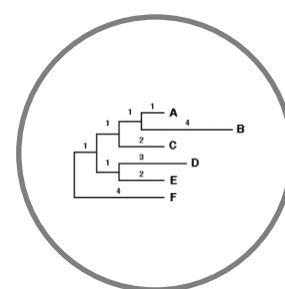
Download all sequence data available for the genus



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 *Eriocheir sinensis*. To allow for a better evaluation of the performance of this marker, additional sequences for *E. sinensis* from the invasive regions should be added to the analyses.

Discussion

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

In the NJ-tree based on **cytb** sequences, all *E. sinensis* sequences end up in one well supported cluster, which also comprises one *E. hepuensis* and several *E. japonica* sequences. The single *E. hepuensis* sequence (GenBank accession number EU137113) was collected in a region of China where *E. sinensis* was introduced [6]. Other sequences from this study cluster as expected, raising the question whether the *E. hepuensis* sequence clustering with *E. sinensis* might involve a misidentification. The *E. japonica* sequences all originate from a study on the genetic distinctiveness of the two previously recognised subspecies, *E. japonica sinensis* and *E. j. hepuensis* [9]. On GenBank these sequences are presently all labelled as *E. japonica* (GenBank accession numbers between DQ114142 and DQ114218), while they should have been labelled *E. sinensis* and *E. hepuensis* after raising the subspecies to the species level. Since the study was published in a Chinese language [9], we are unable to infer which accession numbers belonged to the individual subspecies. If all *E. japonica* sequences ending up in the *E. sinensis* cluster belong to *E. j. sinensis*, *cytb* seems to be able to distinguish *E. sinensis* from the other *Eriocheir* species. The available sequences of *E. sinensis*, however, all come from the native Asian range and additional sequences from the (European) invasive region would allow to better evaluate the performance of this markers for species identification.

The results from the **ND2** analyses look promising, yet all *E. sinensis* sequences with known origin come from the native range, while there is only one sequence for *E. hepuensis* and none for *E. ogasawarensis*. Adding more sequences of the latter two species might influence the clustering in the NJ-trees. In addition, three *E. sinensis* sequences cluster with *E. japonica*, but they were all collected in a hybrid zone of the two species and might involve *E. japonica* - *E. sinensis* hybrids [10].

For the other DNA markers (**COI**, **ITS**, **16S** and **5S**), sequences are available for *Eriocheir sinensis* and (two of) its congeners, but *E. sinensis* never forms a supported cluster. These results are in line with those of Tang *et al.* [11] who found no separation of *E. sinensis*, *E. hepuensis* and *E. japonica* using either COI or the full ITS. The **COI** NJ-tree contains one well supported cluster



including most of the *E. sinensis* sequences, yet a small number of *E. sinensis* sequences are scattered along the NJ-tree. For **ITS** and **16S** there is little sequence variation among the different species, while in the **5S** NJ-tree conspecific sequences did not cluster. Hence, it is not advisable to apply these markers for species identification.

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	X	X	
16S			X		X
ITS1			X		X
ITS2			X		X
Full ITS			X		X
ND2		X	X	X	X
5S		X	X		X

Table 2: Publicly available sequences downloaded (January 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 [2]. An 'X' indicates that at least one sequence was used in the final alignment.

Species in genus	COI	Cytb	16S rDNA	ITS1	ITS2	Full ITS	ND2	5S rDNA
<i>Eriocheir hepuensis</i>	X	X	X	X	X	X	X	X
<i>Eriocheir japonica</i>	X	X	X	X	X	X	X	X
<i>Eriocheir ogasawaraensis</i>	X	X						
<i>Eriocheir sinensis</i>	X	X	X	X	X	X	X	X
TOTAL species	4/4	4/4	3/4	3/4	3/4	3/4	3/4	3/4

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.cabi.org/isc/datasheet/84120>
https://www.nobanis.org/globalassets/speciesinfo/e/eriocheir-sinensis/eriocheir_sinensis.pdf
<https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=182>
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=1379>
<http://www.fao.org/fishery/species/3466/en>
<http://www.iucngisd.org/gisd/species.php?sc=38>
<https://www.naturaldevon.org.uk/wp-content/uploads/2017/10/Chinese-Mitten-Crab-DISI-Species-Factsheet.pdf>
<http://species.biodiversityireland.ie/profile.php?taxonId=22443>
<https://www.invasivespeciesinfo.gov/aquatics/mittencrab.shtml>
<https://www.fws.gov/columbiariver/ANS/factsheets/CMcrab.pdf>
<http://publications.gov.sk.ca/documents/66/89504-English.pdf>
<http://www.gt-ibma.eu/espece/eriocheir-sinensis/>
http://invasivespeciesireland.com/wp-content/uploads/2010/10/Eriocheir_sinensis_ISAP.pdf

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