



# 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](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).

## *Pacifastacus leniusculus*

(Dana, 1852)

Common names:

English: (American) signal crayfish

French: écrevisse de Californie, écrevisse signal, écrevisse du Pacifique

German: Signalkrebs

Dutch: signaalkreeft, Californische rivierkreeft

Last update: November 2018



## General information on *Pacifastacus leniusculus*

### Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Malacostraca	Decapoda	Astacidae	<i>Pacifastacus</i>

### Species in the same genus: N = 4 [2-6]

Note: The genus comprises 4 extant species. Recent studies from the native range of genus *Pacifastacus* revealed that multiple genetically highly divergent lineages have been pooled under *P. leniusculus*.

### Infra-species level: N = 3 [3-5]

Three subspecies, originally described as distinct species, have been formally recognized: *Pacifastacus leniusculus leniusculus*, *P.l. klamathensis*, and *P.l. trowbridgii*. Recent studies, however, have demonstrated that the morphology-based assignment to these subspecies does not match assignments to genetically defined *Pacifastacus* clades.



### Native range: [7]

Canada and the west coast of the United States of America.

### Invasive range: [4,7,8]

#### Europe (geographical):

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

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

<http://alien.jrc.ec.europa.eu/SpeciesMapper>

<https://www.gbif.org/species/2226990>

<https://www.cabi.org/isc/datasheet/70581>

<http://www.europe-aliens.org/speciesFactsheet.do?speciesId=53415#>

<http://issg.org/database/species/ecology.asp?si=725&fr=1&sts=sss>

#### Outside Europe (geographical):

Japan.

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

For more information on *Pacifastacus leniusculus* see the references and online information listed at the end of this document.



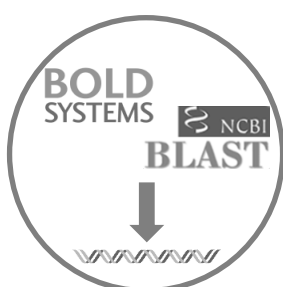
## Species identification based on DNA barcodes

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

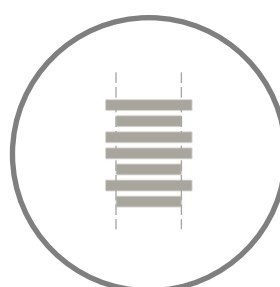
### Material and Methods [1]



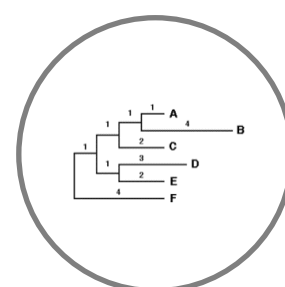
Download all sequence data available for the genus



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, COI is a reliable DNA marker for the identification of *Pacifastacus leniusculus*.**

### Discussion

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

For each DNA marker, all four *Pacifastacus* species are represented in the analyses (Table 2), yet *P. leniusculus* is never recovered as a single cluster. This might be because *P. leniusculus* potentially comprises multiple cryptic groups according to Larson *et al.* [4,6] and Petrusek *et al.* [8].

The *P. leniusculus* COI sequences form one cluster containing all non-native sequences and multiple other clusters with sequences from the native region. This is in accordance with a study comparing COI sequences of European *P. leniusculus* populations and native COI sequences, suggesting that only one of the native lineages spread across Europe [8]. This implies that invasive *P. leniusculus* can be identified using COI. To allow for a better evaluation the performance of COI, additional sequences of the congeners should be added.

For both 16S and GAPDH fewer *P. leniusculus* sequences from the invasive range are available and there is little sequence variation among the different species. Therefore it is currently impossible to assess the ability of these DNA markers to identify *P. leniusculus*.

**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					
16S			X		



<b>GAPDH</b>		X	X	
<b>Table 2:</b> Publicly available sequences downloaded (November 2018) 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 name with '+' is considered extinct [5].				
Species in genus	<b>COI</b>	<b>16S</b>	<b>GAPDH</b>	
<i>Pacifastacus connectens</i>	X	X	X	
<i>Pacifastacus fortis</i>	X	X	X	
<i>Pacifastacus gambelii</i>	X	X	X	
<b><i>Pacifastacus leniusculus</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	
<i>Pacifastacus nigrescens</i> †				
<b>TOTAL species</b>	<b>4/4</b>	<b>4/4</b>	<b>4/4</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/70581>  
[https://www.nobanis.org/globalassets/speciesinfo/p/pacifastacus-leniusculus/pacifastacus\\_leniusculus1.pdf](https://www.nobanis.org/globalassets/speciesinfo/p/pacifastacus-leniusculus/pacifastacus_leniusculus1.pdf)  
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2498>  
<http://issg.org/database/species/ecology.asp?si=725&fr=1&sts=sss>  
<http://www.iucngisd.org/gisd/species.php?sc=725>  
<https://www.fws.gov/fisheries/ans/erss/highrisk/Pacifastacus-leniusculus-ERSS-revision-June2015.pdf>  
<https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=200>  
<https://www.naturaldevon.org.uk/wp-content/uploads/2017/10/Signal-Crayfish-DISI-Species-Factsheet.pdf>  
<http://species.biodiversityireland.ie/profile.php?taxonId=21754&taxonName=signal&keyword=Invasive%20Species%20Of%20Union%20Concern>  
<https://www.nvwa.nl/binaries/nvwa/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-californische-rivierkreeft/factsheet-californische-rivierkreeft-nvwa-20180321.pdf> [NL]

### Picture credits

Page 1: Signal Crayfish (*Pacifastacus leniusculus*). River Arlanza, near Santo Domingo de Silos, Burgos (Spain) By David Perez [CC BY 3.0]  
Page 2 (left): *Pacifastacus leniusculus*, Ecrevisse de Californie Lac du Bourget (France) By Jean Guérin [CC BY-NC-SA 2.0]  
Page 2 (right): Signal crayfish female *Pacifastacus leniusculus* captured from the Iller River, southern Germany By Astacoides [CC BY-SA 3.0]

### References

- [1] N. Smits, S. Gombeer, K. Meganck, A. Vanderheyden, 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 from: <http://bopco.myspecies.info/content/invasive-alien-species-ias-factsheets>.
- [2] K. A. Crandall and S. De Grave, "An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list" *J. Crust. Biol.*, vol. 37, no. 5, pp. 615–653, Sep. 2017.
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