



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.

Procambarus virginalis

Lyko, 2017

Common names:

English: marbled crayfish

French: écrevisse marbrée

German: Marmorkrebs

Dutch: marmerkreeft

Last update: January 2019



General information on *Procambarus virginalis*

Classification

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Malacostraca	Decapoda	Cambaridae	<i>Procambarus</i>

Species in the same genus: N = 168 [2-5]

Note: The marbled crayfish (*Procambarus virginalis*) is a triploid descendant of the sexually reproducing slough crayfish (*Procambarus fallax*) [3]. Cross-breeding experiments and parentage analysis showed that marbled crayfish and slough crayfish are reproductively separated [4]. In addition, analysis of complete mitochondrial genomes of marbled crayfish from laboratory lineages and wild populations demonstrates genetic identity and indicates a single origin [4]. This parthenogenetic lineage of *P. fallax* was formally described by Lyko in 2017 [5] as an independent species, *Procambarus virginalis*.

Infra-species level: N = 0

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



Native range: [3, 4]

To this day, the marbled crayfish is only known from aquaria and from its introduced range; there are no known indigenous populations. However, the marbled crayfish is the parthenogenetic form of *P. fallax* whose range comprises southern Georgia and Florida (United States of America).

Invasive range: [3, 6, 7]

Europe (geographical):

Austria, , Croatia, Czech Republic Germany, Hungary, Italy, Netherlands, Romania, Slovakia, Sweden, Ukraine, 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/9442269> (*Procambarus virginalis*)

<https://www.gbif.org/species/8879526> (*Procambarus fallax* f. *virginalis*)

<https://www.gbif.org/species/2227309> (*Procambarus fallax*)

Outside Europe (geographical):

Japan, Madagascar.

Morphology, biology, invasion, negative effects and remedies

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



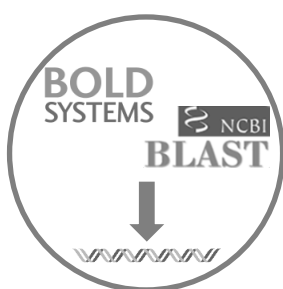
Species identification based on DNA barcodes

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.

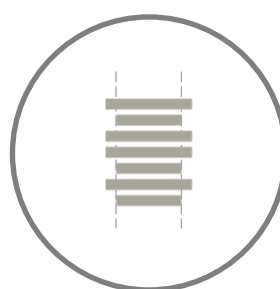
Material and Methods [1]



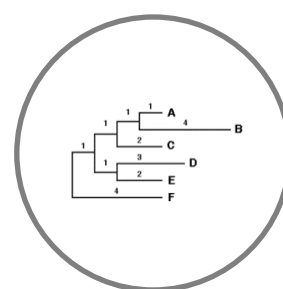
Download all sequence data available for the genus



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, COI is the most reliable DNA marker for the identification of *Procambarus virginalis*. To allow for a better evaluation of the performance of the marker, additional sequences for the poorly or unrepresented congeners should be added.

Discussion

DNA markers for which *Procambarus* sequences were available, were downloaded from GenBank and BOLD for all represented species of the genus *Procambarus*. Five DNA markers were evaluated (Table 1). The sequences did not display intraspecific variation for any of the DNA markers, neither at the level of the full mitochondrial genome [4], which results from its parthenogenetic reproduction strategy.

In the NJ-tree for **COI** *P. fallax* and *P. virginalis* sequences cluster together with maximum support. The differences between the species appeared minimal, yet at least four single nucleotide polymorphisms could be detected for COI [4]. This feature, could be used to diagnose *P. virginalis*. Since only 66 of the 83 species in the NJ-tree are represented by more than one unique sequence and 84 of all *Procambarus* congeners are not represented at all (Table 2), additional COI sequences for the missing congeners, as well as for the species now represented by one unique sequence only, would allow to better evaluate the performance of COI for species identification.

More species are represented in the **16S** NJ-tree, while the number of species in the **12S** NJ-tree is comparable to the COI tree. For both DNA markers *P. fallax* and *P. virginalis* form a cluster, yet with less support than in the COI analysis and without separating both species. The failure to separate *P. virginalis* from *P. fallax* in the NJ-trees can be explained by their recent divergence as the former is a descendant from the latter [3].

The **H3** marker does not cluster *P. virginalis* sequences (with *P. fallax*), while for **28S** there is only one *P. fallax* and no *P. virginalis* sequence available. Therefore it is currently impossible to assess the ability of these markers to identify *P. virginalis*.

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, a '1' indicates only one unique *P. virginalis* sequence was available.

Markers analysed	1	2	3	4	5
COI		n/a			X
16S		n/a	X		X
12S		n/a	X		X
H3	X	n/a	X		X
28S	1	n/a	1		X

Table 2: 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, 5]. An 'X' indicates that at least two sequences were used in the final alignment, a '1' indicates only one unique sequence was available for the final alignment. The list of species is limited to those members of *Procambarus* for which at least one sequence was used in any of the NJ-trees.

Species in genus	COI	16S	12S	H3	28S
<i>Procambarus acherontis</i>	1	1	1	1	1
<i>Procambarus achilli</i>	1	1			
<i>Procambarus acutissimus</i>	1	1	1	1	1
<i>Procambarus acutus</i>	X	X	X	X	X
<i>Procambarus advena</i>	1	1	1	1	1
<i>Procambarus alleni</i>	X	X	X	X	1
<i>Procambarus ancylus</i>	1	1	1	1	1
<i>Procambarus apalachicola</i>	1	1	1	1	1
<i>Procambarus attiguus</i>	1	1	1	1	1
<i>Procambarus barbatus</i>	1		1	1	1
<i>Procambarus bivittatus</i>	1	1	1	1	1
<i>Procambarus blandingii</i>	1	1	1	1	1
<i>Procambarus bouvieri</i>		1	1	1	
<i>Procambarus braswelli</i>		1			
<i>Procambarus caballeroi</i>		1			
<i>Procambarus capillatus</i>	1	1	1	1	1
<i>Procambarus caritus</i>	1	1	1	1	1
<i>Procambarus ceruleus</i>	1	1			
<i>Procambarus chacalli</i>		1			
<i>Procambarus chacei</i>		1	1	1	
<i>Procambarus clarkii</i>	X	X	X	X	X
<i>Procambarus clemmeri</i>		1			
<i>Procambarus contrerasi</i>		1			
<i>Procambarus cubensis</i>		1			
<i>Procambarus cuetzalananae</i>		1			
<i>Procambarus curdi</i>		1	1		
<i>Procambarus delicatus</i>		1	1		
<i>Procambarus digueti</i>		1			
<i>Procambarus dupratzi</i>		1			
<i>Procambarus econfinae</i>	1	1	1	1	1
<i>Procambarus enoplosternum</i>	X	1	1	1	1
<i>Procambarus epicyrtus</i>	1	1		1	1
<i>Procambarus erichsoni</i>	1	1			
<i>Procambarus erythrops</i>	1	1	1	1	1
<i>Procambarus escambiensis</i>	1	1	1	1	1
<i>Procambarus evermanni</i>	1	1	1	1	1
<i>Procambarus fallax</i>	X	X	X	X	1
<i>Procambarus franzi</i>	1	1	1	1	1
<i>Procambarus geminus</i>	1	1	1	1	1
<i>Procambarus geodytes</i>	1	1		1	1
<i>Procambarus gibbus</i>		1			
<i>Procambarus gonopodcristatus</i>		1			
<i>Procambarus hagenianus</i>		1			
<i>Procambarus hayi</i>		1			
<i>Procambarus hidalgoensis</i>		1			
<i>Procambarus hirsutus</i>		1			
<i>Procambarus hoffmanni</i>	1	1			
<i>Procambarus horsti</i>	1	1	1	1	1
<i>Procambarus hortonhobbsi</i>		1			



<i>Procambarus howellae</i>	1	1	1	1	1
<i>Procambarus hubbelli</i>	1	1	1	1	1
<i>Procambarus incilis</i>	1	1	1		1
<i>Procambarus kensleyi</i>	1	1			
<i>Procambarus kilbyi</i>		1			
<i>Procambarus latipleurum</i>	1	1	1	1	1
<i>Procambarus leitheuseri</i>	1	1	1	1	1
<i>Procambarus leonensis</i>	1	1	1	1	1
<i>Procambarus lepidodactylus</i>	1	1	1	1	X
<i>Procambarus liberorum</i>	X	X	X	X	X
<i>Procambarus litosternum</i>	1	1	1	1	1
<i>Procambarus llamasii</i>	X	X	X	X	X
<i>Procambarus lucifugus</i>	X	X	X	X	X
<i>Procambarus lunzi</i>	1	1	1	1	1
<i>Procambarus luxus</i>	1	1	1	1	1
<i>Procambarus mancus</i>	1	1	1	1	1
<i>Procambarus milleri</i>	1	1	1	1	1
<i>Procambarus morrisoni</i>	1	1	1	1	1
<i>Procambarus natchitochae</i>	1	1			
<i>Procambarus nigrocinctus</i>	1	1	1		
<i>Procambarus nueces</i>	1	1	1	1	
<i>Procambarus okaloosae</i>	1	1	1	1	1
<i>Procambarus orcinus</i>	1	1	1	1	1
<i>Procambarus ouachitae</i>		X			
<i>Procambarus paeninsulanus</i>	X	X	1	1	1
<i>Procambarus pallidus</i>	1	1	1	1	1
<i>Procambarus paradoxus</i>		1			
<i>Procambarus parasimulans</i>		1			
<i>Procambarus pearsei</i>		1			
<i>Procambarus pecki</i>		1			
<i>Procambarus pictus</i>	1	1	1	1	1
<i>Procambarus pubescens</i>	1	1	1	1	1
<i>Procambarus pubischelae</i>	X	X	X	X	X
<i>Procambarus pycnogonopodus</i>	1	1	1	1	1
<i>Procambarus pygmaeus</i>	1	1	1	1	1
<i>Procambarus raneyi</i>		1			
<i>Procambarus rathbunae</i>	1	1	1	1	1
<i>Procambarus regalis</i>		1			
<i>Procambarus regiomontanus</i>	1	1	1		1
<i>Procambarus reimeri</i>	1	X	X		
<i>Procambarus riojae</i>	1	1			
<i>Procambarus roberti</i>		1			
<i>Procambarus rogersi</i>	X	X	X	X	X
<i>Procambarus seminolae</i>	1	1	1	1	1
<i>Procambarus shermani</i>	1		1	1	1
<i>Procambarus simulans</i>	X	1	1		1
<i>Procambarus spiculifer</i>	X	X	1	1	1
<i>Procambarus steigmani</i>	1	1	1		1
<i>Procambarus strenthi</i>		1			
<i>Procambarus suttkusi</i>	1	1	1	1	1
<i>Procambarus talpoides</i>	1	1	1	1	1
<i>Procambarus tenuis</i>	X	X	X	X	X
<i>Procambarus teziutlanensis</i>	1	1			
<i>Procambarus tlapacoyanensis</i>	1	1			
<i>Procambarus tolteca</i>	1	1	1	1	1
<i>Procambarus troglodytes</i>	1	1	1	1	1
<i>Procambarus truculentus</i>	1	1	1	1	1
<i>Procambarus tulane</i>		1			
<i>Procambarus vazquezae</i>	X	1			
<i>Procambarus versutus</i>	1	1	1	1	1
<i>Procambarus viaeviridis</i>	1				
<i>Procambarus vioscai</i>		1			
<i>Procambarus virginalis</i>	X	X	X	X	
<i>Procambarus xochitlanae</i>	1	1			



<i>Procambarus youngi</i>	1	1	1	1	1
<i>Procambarus zihuatlensis</i>		1			
<i>Procambarus zonangulus</i>	X	1	1		1
TOTAL species (1 sequence only)	83(66)/168	113(98)/168	73(61)/168	66(55)/168	67(58)/168

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/110477>

<https://www.fws.gov/Fisheries/ANS/erss/uncertainrisk/ERSS-Procamburus-virginalis-final-February2018.pdf>

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<https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/factsheet-marmerkreeft> [NL]

<https://species.biodiversityireland.ie/profile.php?taxonId=187085&taxonGroupName=crustacean&keyword=Invasive%20Species%20of%20Union%20Concern>

Picture credits

Page 1: Marmorkrebs *Procamburus fallax* forma *virginalis* By Chucholl C. [CC BY-SA 3.0]

Page 2 (left): *Procamburus* cf. *fallax*1 By Alexander Mrkvicka [CC BY-SA 3.0]

Page 2 (right): Adult Marmorkrebs (marbled crayfish) By Zfaulkes from Wikimedia Commons [CC0]

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- [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>.
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