



# 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](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).

## *Faxonius virilis*

(Hagen, 1870)

Common names:

English: virile crayfish, northern crayfish, eastern Crayfish

French: écrevisse à pinces bleues

German: Viril-Flusskrebs

Dutch: geknobbelde Amerikaanse rivierkreeft

Last update: December 2021



## General information on *Faxonius virilis*

### Classification

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

### Species in the same genus: N = 89 [2,3]

Note: In 2017, Crandall and De Grave updated the classification of the freshwater crayfishes. The representatives of genus *Orconectes* form at least two distinct groups, i.e. the “cave-dwelling *Orconectes*” and the remaining “surface-dwelling *Orconectes*” species. Therefore the authors decided to split the genus. Since the type species of *Orconectes*, *O. inermis*, belongs to the cave-dwelling group, the genus is restricted to cave-dwelling species. The surface-dwelling species, including the virile crayfish, are placed in the genus *Faxonius* Ortmann, 1905.

### Infra-species level: N = 0

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



### Native range: [4]

Canada (Alberta, Manitoba, Ontario) and north and central United States of America (Arkansas, Colorado, Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Ohio, Oklahoma, South Dakota, Texas, Wisconsin, Wyoming).

### Invasive range: [4–7]

#### Europe (geographical):

France, Netherlands, 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/R10445>

<https://www.gbif.org/species/2227064> (*Orconectes virilis*)

<https://www.gbif.org/species/8971201> (*Faxonius virilis*)

<https://fws.gov/media/ecological-risk-screening-summary-virile-crayfish-orconectes-virilis-high-risk>

### Outside Europe (geographical):

Canada (New Brunswick, Quebec\*, Saskatchewan\*), China, Mexico and United States of America (Alabama, Arizona, California, Connecticut, Delaware, District of Columbia, Idaho, Maine, Maryland, Massachusetts, Mississippi, Nevada, New Hampshire, New Jersey, New Mexico, New York\*, North Carolina, Pennsylvania, Rhode Island, South Carolina, Tennessee\*, Utah\*, Vermont, Virginia, Washington, West Virginia).

\* *Faxonius virilis* is present, but it is unclear whether this species is native or introduced in these states.

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

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



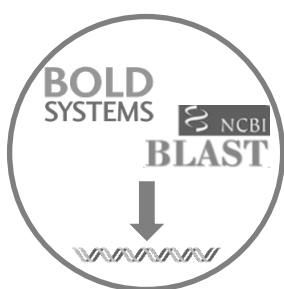
## Species identification based on DNA barcodes

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

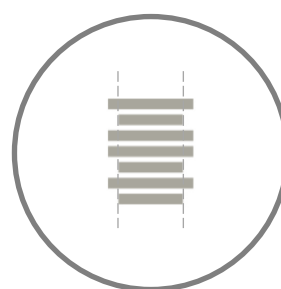
### Material and Methods [1]



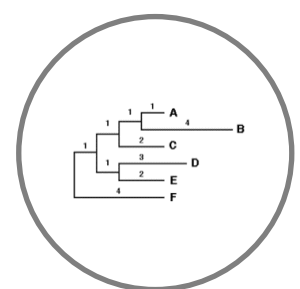
Download all sequence data available for the genus



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, COI is the most promising DNA marker for the identification of the *Faxonius virilis* species complex, separating different *F. virilis* s.s. lineages from *F. nais*, *F. deanae* and from the closely related *F. punctimanus* (previously all placed in genus *Orconectes*). To better evaluate the performance of COI, and to determine if the European *F. virilis* specimens all belong to a single lineage, additional sequences from the European invasive range of *F. virilis* and for the congeners should be added.

### Discussion

We follow Crandall and De Grave [2] and use the genus name *Faxonius* to refer to the surface-dwelling species instead of *Orconectes*, the genus name used in the original EU Regulation on IAS. DNA markers for which sequences were available for species of the genus *Faxonius*, including those still listed as *Orconectes*, were downloaded from GenBank and BOLD. Six DNA markers were evaluated (Table 1).

*Faxonius virilis* represents a species complex. Molecular data revealed the existence of several cryptic lineages within the species complex, including *F. deanae*, *F. nais* and at least four different additional lineages of which one was recently described as *F. quinebaugensis* [5, 6]. Only *F. virilis* sensu stricto is yet reported as invasive.

COI looks promising for the identification of the *Faxonius virilis* species complex, since the *F. virilis* s.s. sequences cluster with high support with all available *F. deanae* and *F. nais* sequences, which is supported by the study of Filipová *et al.* [8]. In our NJ-tree, however, newly generated sequences for *F. punctimanus* [9], a closely related species, also cluster within the species complex, while older *F. punctimanus* sequences cluster with *F. ozarkae*. Yet, COI seems valuable for distinguishing the *F. virilis* species complex (incl. the new *F. punctimanus* sequences) from other *Faxonius* species, and for separating the individual lineages [5,6] within the complex. One lineage comprises the currently available *F. virilis* sequences from Europe, but additional sequences are needed to determine if *F. virilis* in Europe belongs to one lineage only. To allow for a better overall evaluation of the performance of COI for the identification of the *F. virilis* species complex, the missing species (Table 2) as well as additional sequences for the species now represented by few sequence(s) only, should be added. With regard to the database of COI sequences, Buhay [10] scrutinized the



quality of *Faxonius* sequences published by Taylor and Knouft [11] and indicated that multiple sequences were flagged in GenBank as being “COI-like” due to the presence of stop codons and indels. Yet, these sequences do not influence the recovery of the *F. virilis* species complex cluster if they are excluded from the analysis.

For **16S** there are only *F. virilis* s.s. sequences available from North America and these do not form a cluster, neither do the different species from the *Faxonius virilis* species complex.

For **12S**, **28S**, **ITS1**, and **H3** fewer sequences are available for the *F. virilis* species complex and the congeneric species. Therefore, it is currently impossible to assess the ability of these markers to identify *F. virilis* s.s. or the species complex.

**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
<b>COI</b>			X		X
<b>16S</b>			X		X
<b>12S</b>	X	X	X		X
<b>28S</b>	X	X	X		X
<b>ITS1</b>	X	X	X		X
<b>H3</b>	X		X		X

**Table 2:** Publicly available sequences downloaded (December 2021) 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 [2,3]. An ‘X’ indicates that at least one sequence was used in the final alignment. An ‘(X)’ indicates the species is only represented in the dataset containing the “COI-like” sequences identified by Buhay [10]. Species names with \* are not mentioned in [2] but are used on GenBank.

Species in genus	COI	16S	12S	28S	ITS1	H3
<i>Faxonius acares</i>	(X)	X				
<i>Faxonius alabamensis</i>	X	X				
<i>Faxonius alluvius</i>						
<i>Faxonius barrenensis</i>	X	X				
<i>Faxonius bellator</i>		X				
<i>Faxonius bisectus</i>	X					
<i>Faxonius burri</i>	X					
<i>Faxonius carolinensis</i>	X					
<i>Faxonius castaneus</i>						
<i>Faxonius causeyi</i>		X				
<i>Faxonius chickasawae</i>	X	X				
<i>Faxonius compressus</i>	(X)	X				
<i>Faxonius cooperi</i>	X	X				
<i>Faxonius crislavarius</i>	X	X				
<i>Faxonius cyanodigitus</i>						
<i>Faxonius deanae</i>	X	X	X	X		X
<i>Faxonius difficilis</i>	X					
<i>Faxonius durrelli</i>	X	X				
<i>Faxonius erichsonianus</i>	X	X				
<i>Faxonius etneri</i>	X	X				
<i>Faxonius eupunctus</i>	X	X				
<i>Faxonius forceps</i>	X	X				
<i>Faxonius harrisonii</i>	X	X				
<i>Faxonius hartfieldi</i>	X	X				
<i>Faxonius hathawayi</i>						
<i>Faxonius hobbsi</i>	X					
<i>Faxonius holti</i>	X	X				
<i>Faxonius hylas</i>	X	X				
<i>Faxonius illinoiensis</i>	X	X				
<i>Faxonius immunis</i>	X	X				
<i>Faxonius indianensis</i>	X	X				
<i>Faxonius jeffersoni</i>	X					
<i>Faxonius jonesi</i>	X	X				
<i>Faxonius juvenilis</i>	X	X				



Species in genus	COI	16S	12S	28S	ITS1	H3
<i>Faxonius kentuckiensis</i>	X	X				
<i>Faxonius lancifer</i>		X				
<i>Faxonius leptogonopodus</i>	X	X				
<i>Faxonius limosus</i>	X	X	X	X		X
<i>Faxonius longidigitus</i>	X	X			X	
<i>Faxonius luteus</i>	X	X	X	X	X	X
<i>Faxonius marcus</i>	X		X	X	X	X
<i>Faxonius maletae</i>	X	X				
<i>Faxonius marchandi</i>	X	X				
<i>Faxonius margorectus</i>						
<i>Faxonius medius</i>	X	X				
<i>Faxonius meeki</i>	X	X				
<i>Faxonius menae</i>	(X)	X				
<i>Faxonius mirus</i>	X	X				
<i>Faxonius mississippiensis</i>	X	X				
<i>Faxonius nais</i>	X	X				
<i>Faxonius nana</i>		X				
<i>Faxonius neglectus</i>	X	X	X	X	X	
<i>Faxonius obscurus</i>	X	X				
<i>Faxonius occidentalis</i>						
<i>Faxonius ozarkae</i>	X	X				
<i>Faxonius pagei</i>	X	X				
<i>Faxonius palmeri</i>	X	X				
<i>Faxonius pardalotus</i>	X					
<i>Faxonius perfectus</i>	X	X				
<i>Faxonius peruncus</i>	X	X				
<i>Faxonius placidus</i>	X	X				
<i>Faxonius propinquus</i>	X	X				
<i>Faxonius punctimanus</i>	X	X	X		X	
<i>Faxonius putnami</i>	X	X				
<i>Faxonius quadruncus</i>	X	X				
<i>Faxonius quinebaugensis</i>						
<i>Faxonius rafinesquei</i>	X	X				
<i>Faxonius raymondi</i>						
<i>Faxonius rhoadesi</i>	X	X				
<i>Faxonius roberti*</i>	X					
<i>Faxonius ronaldi</i>	X	X	X	X		X
<i>Faxonius rusticus</i>	X	X	X	X		X
<i>Faxonius sanbornii</i>	X	X	X	X		
<i>Faxonius saxatilis</i>	X	X				
<i>Faxonius shoupi</i>	X	X				
<i>Faxonius sloanii</i>	X					
<i>Faxonius spinosus</i>	X	X				
<i>Faxonius stannardi</i>	X					
<i>Faxonius stygocaneyi</i>	X	X				
<i>Faxonius taylori</i>						
<i>Faxonius texanus</i>						
<i>Faxonius theaphionensis</i>						
<i>Faxonius transfuga</i>						
<i>Faxonius tricuspis</i>	X	X				
<i>Faxonius validus</i>	X	X				
<i>Faxonius virginianensis</i>	X					
<b><i>Faxonius virilis</i></b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Faxonius wagneri*</i>	X					
<i>Faxonius williamsi</i>	X	X				
<i>Faxonius wright</i>	X	X				
<i>Faxonius yanahlindus</i>	X					
<b>TOTAL species</b>	<b>73/89 (75/91)*</b>	<b>65/89</b>	<b>10/89</b>	<b>9/89</b>	<b>6/89</b>	<b>7/89</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

<http://www.iucngisd.org/gisd/species.php?sc=218>  
<https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=215>  
<https://fws.gov/media/ecological-risk-screening-summary-virile-crayfish-orconectes-virilis-high-risk>  
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2442>  
<http://www.cabi.org/isc/datasheet/72034>

### Picture credits

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Page 2 (left): Northern Crayfish, Rideau River By D. Gordon E. Robertson [CC BY-SA 3.0]  
Page 2 (right): Northern Crayfish By USFWS Midwest Region [CC BY 2.0]

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