



# Identification of Invasive Alien Species using DNA barcodes

Royal Belgian Institute of Natural Sciences  
Rue Vautier 29,  
1000 Brussels, Belgium  
+32 (0)2 627 41 23

Royal Museum for Central Africa  
Leuvensesteenweg 13,  
3080 Tervuren, Belgium  
+32 (0)2 769 58 54



## 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 Smitz *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).

## *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: November 2018

## 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 = 88 [2]

Note: In 2017, Crandall and De Grave updated the classification of the freshwater crayfishes. The representatives of *Orconectes* form at least two distinct groups, i.e. the “cave-dwelling *Orconectes*” and the remaining “surface-dwelling *Orconectes*” species, and so 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: [3]

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: [3–6]

#### Europe (geographical):

Netherlands, 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/2227064> (*Orconectes virilis*)

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

<http://www.europe-aliens.org/speciesFactsheet.do?speciesId=53409#>

<https://www.nobanis.org/species-info/?taxald=15234>

#### Outside Europe (geographical):

Canada (New Brunswick, Quebec\*, Saskatchewan\*), 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.



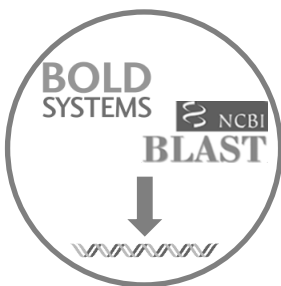
## Species identification based on DNA barcodes

### 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. 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.

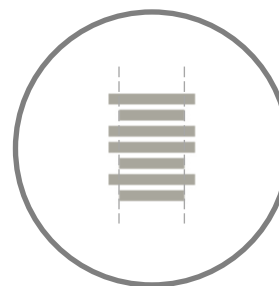
### Material and Methods [1]



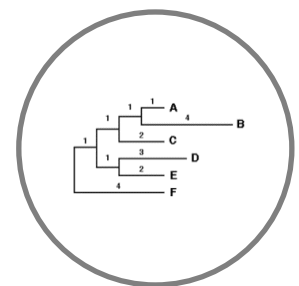
Download all sequence data available for the genus



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 the most promising DNA marker for the identification of the *Faxonius virilis* species complex, yet without separating *F. virilis* s.s. from *F. nais* and *F. deanae* (previously all placed in genus *Orconectes*). To better evaluate the performance of COI, additional sequences for the congeners should be added.**

### Discussion

For the analyses 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* [4, 5]. Only *F. virilis* sensu stricto is 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. This result is similar to the COI tree from Filipová *et al.* [7]. COI thus seems valuable for distinguishing the *F. virilis* species complex from other *Faxonius* species, yet without separating the individual lineages within the complex. To allow for a better 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 only one unique sequence, should be added to the analysis. With regard to the database of COI sequences, Buhay [8] scrutinized the *Orconectes* and *Faxonius* sequences published by Taylor and Knouft [9] 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 and support of the *F. virilis* species complex cluster in the current analyses.

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
COI			X		X
16S			X		X
12S	X	X	X		X
28S	X	X	X		X
ITS1	X	X	X		X
H3	X		X		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]. An 'X' indicates that at least one sequence was used in the final alignment. 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					
<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 durelli</i>	X	X				
<i>Faxonius erichsonianus</i>	X	X				
<i>Faxonius etnieri</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 illinoensis</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				
<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 longitidigitus</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				
<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 virginensis</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>72/88</b>	<b>63/88</b>	<b>10/88</b>	<b>9/88</b>	<b>6/88</b>	<b>7/88</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://www.fws.gov/fisheries/ans/erss/highrisk/Orconectes-virilis-ERSS-revision-June2015.pdf>  
<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2442>  
<http://www.cabi.org/isc/datasheet/72034>

### Picture credits

Page 1: Northern crayfish (*Orconectes virillis*) By Alan Schmierer [CC0 1.0]  
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]

### References

- [1] N. Smitz, 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.
- [3] CABI, "*Orconectes virilis* [original text by Adam Ellis]." *Invasive Species Compendium*. Wallingford, UK: CAB International., 2019. [Online]. Available: <https://www.cabi.org/isc/datasheet/72034>.
- [4] L. M. Mathews, L. Adams, E. Anderson, M. Basile, E. Gottardi, and M. A. Buckholt, "Genetic and morphological evidence for substantial hidden biodiversity in a freshwater crayfish species complex" *Mol. Phylogenetics Evol.*, vol. 48, no. 1, pp. 126–135, Jul. 2008.
- [5] L. Filipová, D. M. Holdich, J. Lesobre, F. Grandjean, and A. Petrusek, "Cryptic diversity within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex: new lineages recorded in both native and introduced ranges" *Biol. Invasions*, vol. 12, no. 5, pp. 983–989, May 2010.
- [6] GISD Global Invasive Species Database, "Species profile: *Orconectes virilis*" 2010. [Online]. Available: <http://www.iucngisd.org/gisd/species.php?sc=218#>.
- [7] L. Filipová, F. Grandjean, C. Chucholl, D. M. Soes, and A. Petrusek, "Identification of exotic North American crayfish in Europe by DNA barcoding" *Knowl. Manag. Aquat. Ecosyst.*, no. 401, p. 11, May 2011.
- [8] J. E. Buhay, "'COI-like' Sequences Are Becoming Problematic in Molecular Systematic and DNA Barcoding Studies" *J. Crust. Biol.*, vol. 29, no. 1, pp. 96–110, Jan. 2009.
- [9] C. A. Taylor and J. H. Knouft, "Historical influences on genital morphology among sympatric species: gonopod evolution and reproductive isolation in the crayfish genus *Orconectes* (Cambaridae)" *Biol. J. Linn. Soc.*, vol. 89, no. 1, pp. 1–12, Sep. 2006.

### To cite this factsheet, please use

Barcoding Facility for Organisms and Tissues of Policy Concern, 2019. Factsheet on *Faxonius virilis*; November 2018. In: Identification of Invasive Alien Species using DNA barcodes. BopCo, Belgium. Available from: [www.bopco.myspecies.info/content/invasive-alien-species-ias-factsheets](http://www.bopco.myspecies.info/content/invasive-alien-species-ias-factsheets), accessed on DD-MM-YYYY.

DISCLAIMER: The information represented in this factsheet has been compiled from many different sources. Every reasonable effort has been made to ensure that the material presented is accurate and reflects the current (see date last update) scientific knowledge. However, recent changes in e.g. taxonomy and distribution, or the publication of additional reference sequences may not be implemented. The views which are expressed in the "Conclusion" are those of the author(s) and have not been peer-reviewed. BopCo does not guarantee the accuracy of the data included in this factsheet. The content of the factsheet is for information only and is not intended as legal advice. BopCo may not be held responsible for the use which may be made of the information contained therein. If you should notice any issues considering the content of this factsheet, or if you would like to contribute any additional information to it, please contact us through [bopco@naturalsciences.be](mailto:bopco@naturalsciences.be).

