



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.

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

Faxonius limosus

(Rafinesque, 1817)

Common names:

English: spiny-cheek crayfish

French: écrevisse américaine

German: Kamberkrebs

Dutch: gevlekte rivierkreeft

Last update: December 2021



General information on *Faxonius limosus*

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 spiny-cheek 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, 5]

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

Invasive range: [4–6]

Europe (geographical):

Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxembourg, Montenegro, Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine, United Kingdom.

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

<https://easin.jrc.ec.europa.eu/spexplorer/species/factsheet/R10443>

<https://www.gbif.org/species/2227000> (*Orconectes limosus*)

<https://www.gbif.org/species/8909595> (*Faxonius limosus*)

<https://www.nobanis.org/globalassets/speciesinfo/o/orconectes-limosus/orconectes-limosus.pdf>

<https://fws.gov/media/ecological-risk-screening-summary-spiny-cheek-crayfish-orconectes-limosus-high-risk>

Outside Europe (geographical):

Morocco.

Morphology, biology, invasion, negative effects and remedies

For more information on *Faxonius limosus* 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/) and GenBank (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]



Conclusion

Based on the present evaluation of the available sequence data, a combination of DNA markers COI and 16S is most reliable for the identification of *Faxonius limosus* (previously placed in genus *Orconectes*). To better evaluate the performance of these markers for species identification, additional sequences for the congeners should be added to the analyses.

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. Five DNA markers were evaluated (Table 1).

The *Faxonius limosus* COI sequences form a cluster in the NJ-tree. Because not all congeneric species are represented (Table 2), however, adding sequences of such missing species might alter the clustering. In addition, most congeneric species represented by two or more sequences do not form clusters in the NJ-tree, which raises doubts about the taxonomic resolution of COI for this genus. To allow for a better evaluation of the performance of COI for species identification, 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 [7] scrutinized the quality of *Orconectes* and *Faxonius* sequences published by Taylor and Knouft [8] 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. limosus* cluster if they are excluded from the analysis.

In the NJ-tree for 16S, the *F. limosus* sequences cluster together, except for one (GenBank accession number GQ168834). This latter sequence was derived from a food item, so morphological identification might have been difficult, therefore this sequence is a possible misidentification. If so, 16S might be useful in differentiating *F. limosus* from the other *Faxonius* species. To allow for a better evaluation of the performance of 16S for species identification, the missing species, as well as extra *F. limosus* sequences should be added to the analysis.



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

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, an '(X)' indicates the non-clustering was due solely to (a) potential misidentified or mislabelled sequence(s), a '1' indicates only one *F. limosus* sequence was available.

| Markers analysed | 1 | 2 | 3 | 4 | 5 |
|------------------|---|---|-----|---|---|
| COI | | | | | X |
| 16S | X | | (X) | X | X |
| 12S | X | X | | | X |
| 28S | X | X | 1 | | X |
| H3 | X | X | 1 | | 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 [7]. Species names with * are not mentioned in [2] but are used on GenBank.

| Species in genus | COI | 16S | 12S | 28S | 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 cristavarius</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 ethieri</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 | | | |
| <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 |



| Species in genus | COI | 16S | 12S | 28S | H3 |
|----------------------------------|-----------------------|--------------|--------------|-------------|-------------|
| <i>Faxonius longitidigitus</i> | X | X | | | |
| <i>Faxonius luteus</i> | X | X | X | X | X |
| <i>Faxonius marcus</i> | 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 | |
| <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 | | |
| <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 virginiensis</i> | X | | | | |
| <i>Faxonius virilis</i> | X | X | X | X | X |
| <i>Faxonius wagneri*</i> | X | | | | |
| <i>Faxonius williamsi</i> | X | X | | | |
| <i>Faxonius wright</i> | X | X | | | |
| <i>Faxonius yanahlindus</i> | X | | | | |
| TOTAL species | 73/89 (75/91)* | 65/89 | 10/89 | 9/89 | 7/89 |

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/72033>

<https://www.nobanis.org/globalassets/speciesinfo/o/orconectes-limosus/orconectes-limosus.pdf>

<http://www.nonnativespecies.org/factsheet/factsheet.cfm?speciesId=2441>

<https://fws.gov/media/ecological-risk-screening-summary-spiny-cheek-crayfish-orconectes-limosus-high-risk>

Picture credits

Page 1: *Orconectes limosus*, Kamberkrebs By A.Berger [CC BY-SA 3.0]

Page 2 (left): Kamberkrebs spiny-cheek crayfish *Orconectes limosus* male By Astacoides [CC BY-SA 3.0]

Page 2 (right): Ecrevisse américaine (*Orconectes limosus*) By Christophe Quintin [CC BY-NC 2.0]

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: <https://bopco.be/output/iasfactsheets>.
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