# “Enemy release” Ausência de parasitas nas populações do primeiro anfípode invasor da Península Ibérica

**Filipe BANHA**, Pedro M. Anastácio, Michal Rachalewski, Karolina Bacela-Spychalska and Michal Grabowski

Life INVASEP

LUCHA CONTRA ESPECIES INVASORAS EN LAS CUENCAS HIDROGRÁFICAS DEL TAJO Y DEL GUADIANA EN LA PENÍNSULA IBÉRICA

UNIVERSIDADE DE ÉVORA

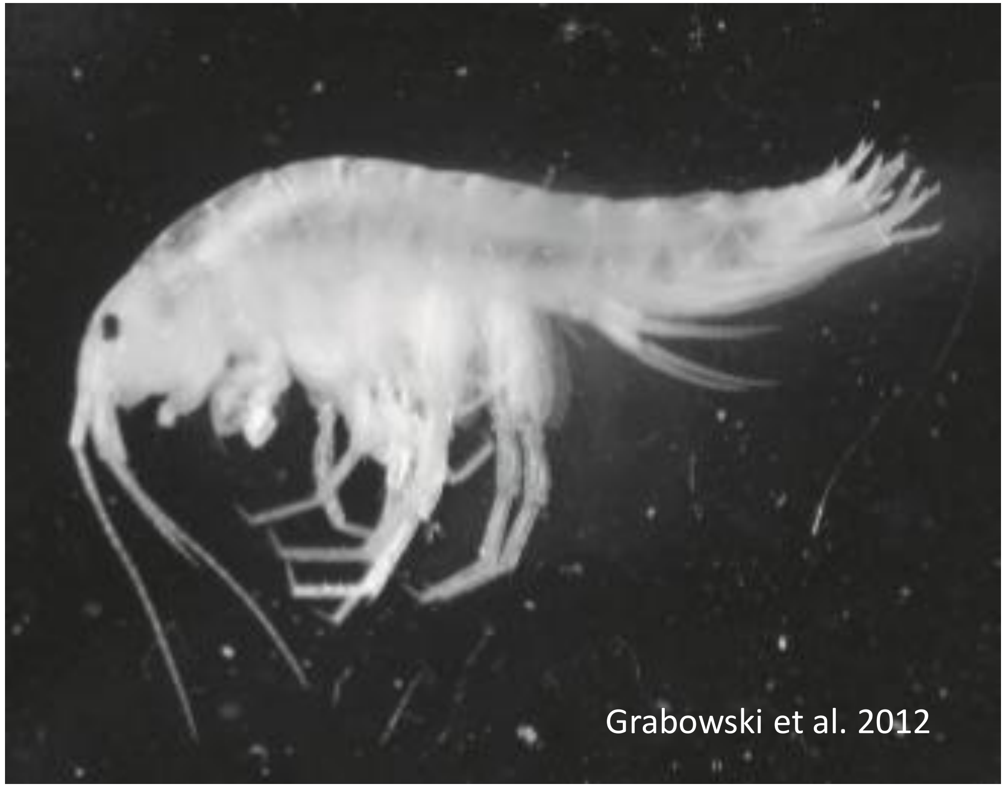
MARE

University of
LODZ

The University of Lodz

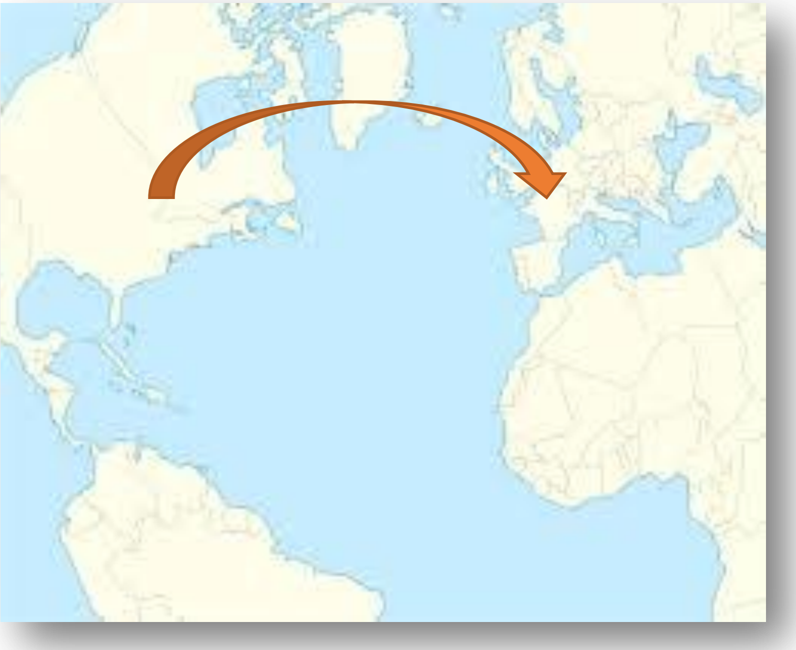
Department of Invertebrate Zoology
& Hydrobiology

# Introdução



Grabowski et al. 2012

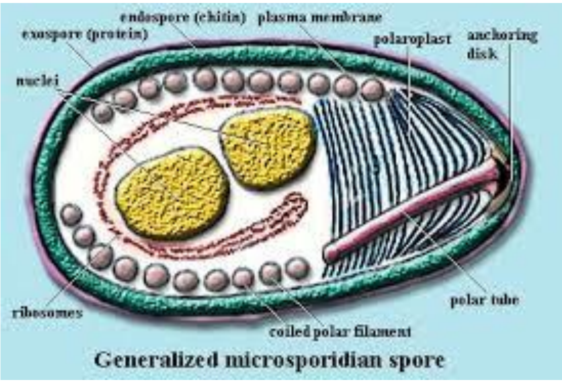
*Crangonyx pseudogracilis* Bousfield, 1958

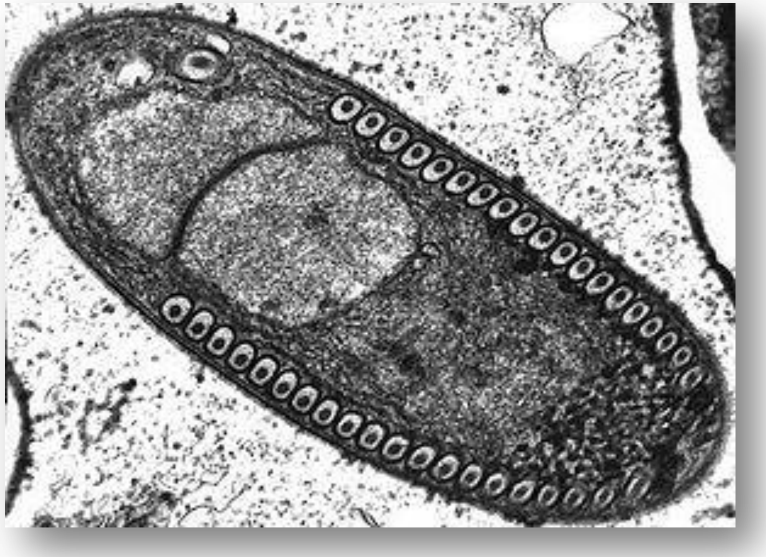


# Introdução

Microsporidia

- Eucariontes (Opisthosporidia)
- Parasitas intracelulares obrigatórios
- Esporos não móveis

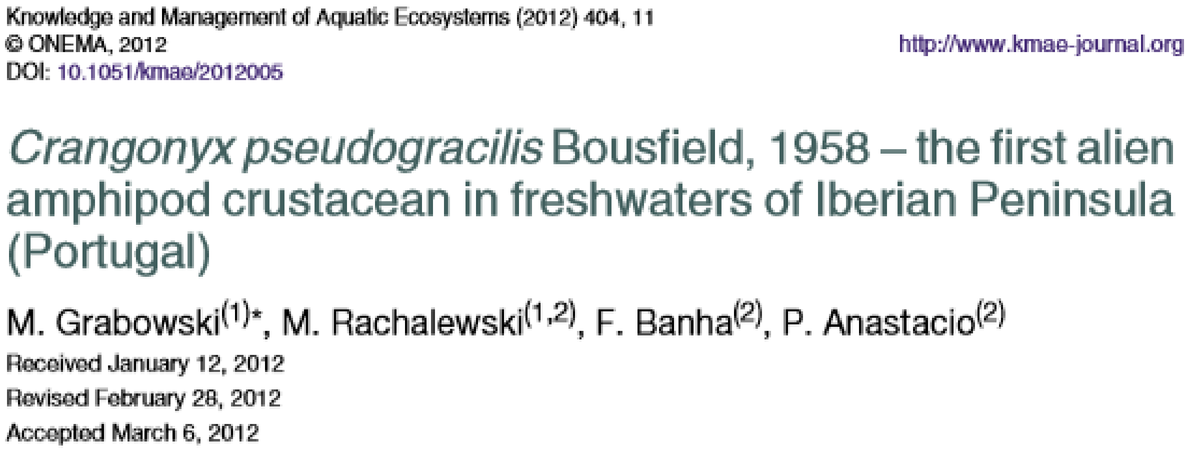

endospore (chitin)
plasma membrane
exospore (protein)
polaroplast
anchoring disk
nuclei
ribosomes
polar tube
coiled polar filament
Generalized microsporidian spore



Esporoblasto

*Fibrillanosema crangonycis*

# Introdução



Knowledge and Management of Aquatic Ecosystems (2012) 404, 11
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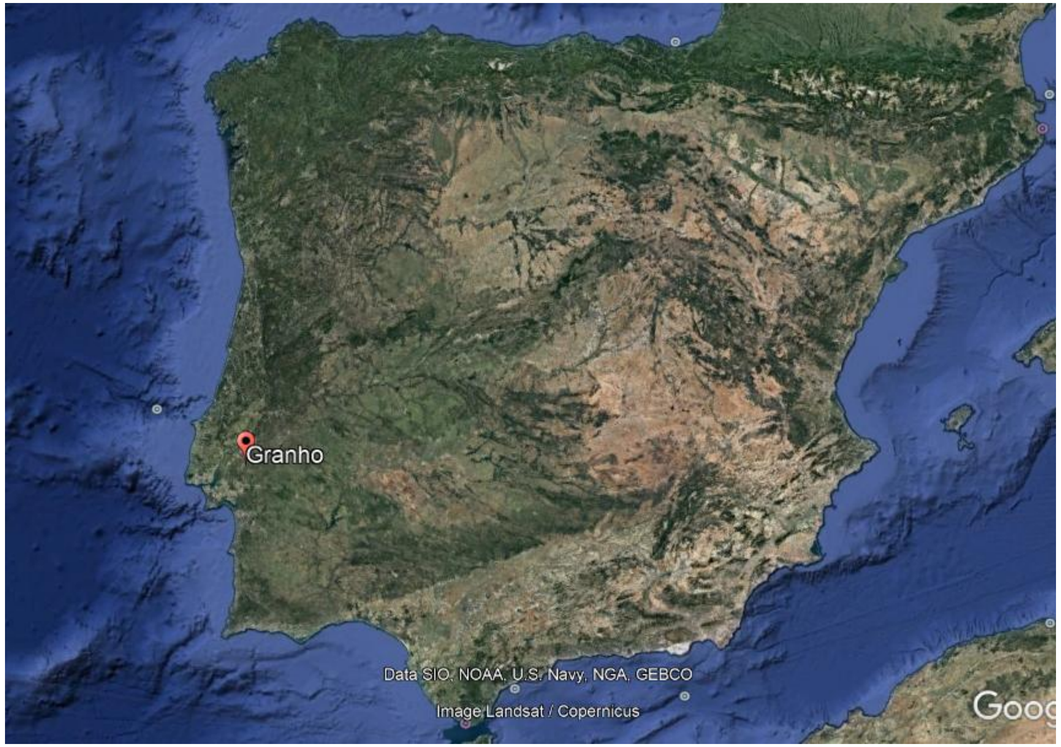
DOI: 10.1051/kmae/2012005
http://www.kmae-journal.org

## *Crangonyx pseudogracilis* Bousfield, 1958 – the first alien amphipod crustacean in freshwaters of Iberian Peninsula (Portugal)

M. Grabowski(1)*, M. Rachalewski(1,2), F. Banha(2), P. Anastacio(2)

Received January 12, 2012
Revised February 28, 2012
Accepted March 6, 2012


Granho
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image Landsat / Copernicus
Goog

# Objetivo

Neste trabalho pretendeu-se estudar:

- A origem de *C. pseudogracilis* no Sudeste da Península Ibérica e a extensão da área invadida.
- A estrutura populacional desta espécie.
- A prevalência de parasitas nesta população

Knowl. Manag. Aquat. Ecosyst. 2018, Vol, No
© F. Banha *et al.*, Published by EDP Sciences 2018
https://doi.org/10.1051/kmae/2018003

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# Enhanced fecundity and parasite release in the first amphipod invader on the Iberian Peninsula

Filipe Banha[1,*], Pedro M. Anastácio[1], Michal Rachalewski[2], Karolina Bacela-Spychalska[2] and Michal Grabowski[2]

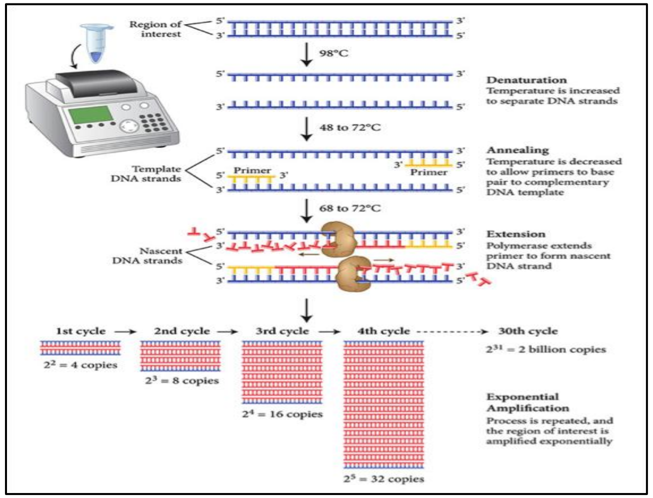
# Métodos

- 48 locais amostrados em 3 bacias hidrográficas (Rio Tejo, Rio Sado; Rio Guadiana)
- Caracterização local: vegetação/substrato/velocidade da corrente/profundidade

# Métodos

**Microscopia**

- Identificação anfípodes capturados
- Medição comprimento
- Determinação do sexo
- Número de ovos e embriões em fêmeas


Region of interest
5'
3'
98°C
Denaturation
Temperature is increased to separate DNA strands
48 to 72°C
Annealing
Temperature is decreased to allow primers to base pair to complementary DNA template
Template DNA strands
Primer
Primer
68 to 72°C
Extension
Polymerase extends primer to form nascent DNA strand
Nascent DNA strands
1st cycle
2nd cycle
3rd cycle
4th cycle
30th cycle
2^2 = 4 copies
2^3 = 8 copies
2^4 = 16 copies
2^5 = 32 copies
2^31 = 2 billion copies
Exponential Amplification
Process is repeated, and the region of interest is amplified exponentially

**PCR**

- Diversidade genética (mtDNA) gene cytochrome oxidase subunit 1 (COI)

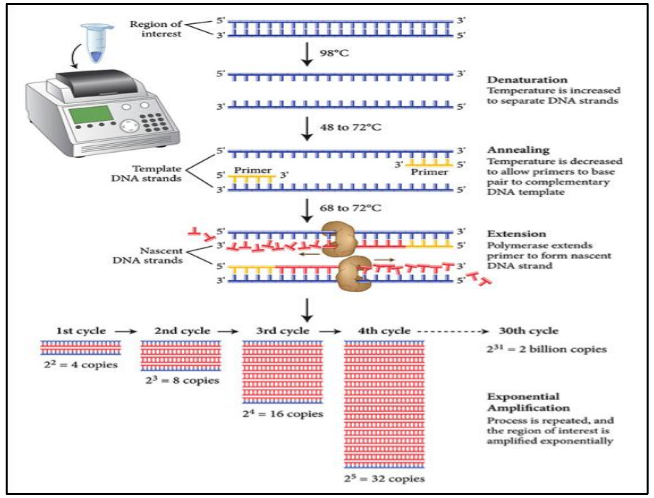
Primers LCO1490-JJ e HCO2198-JJ (Astrin and Stüben 2008; Wattier et al., 2007)

# Métodos

## Deteção de parasitas

**Microscopia**

- Macroparasitas (helmintes)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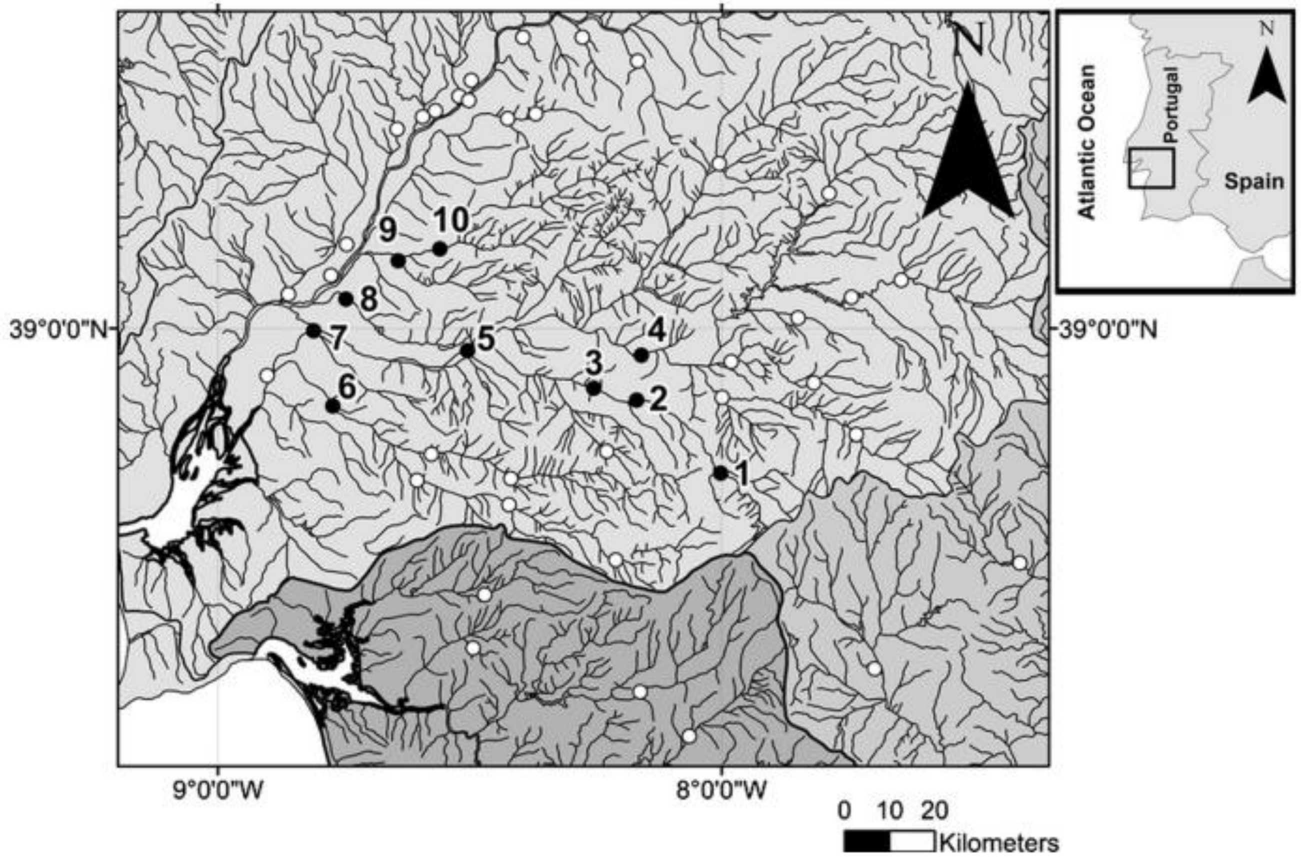
**PCR**

- Microsporidia SSU rDNA

Primers v1f (Baker et al., 1994) e 530r (Vossbrinck et al., 1993)

# Resultados

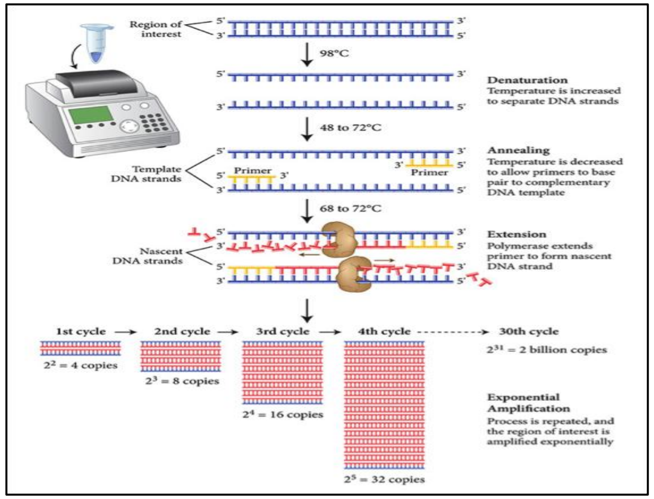

9
10
8
7
5
4
3
2
6
1
39°0'0"N
39°0'0"N
9°0'0"W
8°0'0"W
0 10 20
Kilometers
N
Atlantic Ocean
Portugal
Spain
N

Locais de amostragem: Branco: não detetado; Negro: detetado.

# Resultados

- Densidade média = 23,2 indivíduos $m^2$; Rácio (M/F) = 0,51
- Fecundidade – 30 % pop. - média 33 ovos – 7,14-11,46 mm – $Y=3.055x+26,005$ ($r^2=0.8417$)
- Tamanho – F (7,68 mm) > M (5,23 mm) ($p<0.001$)
- Genética - 1 haplotipo COI idêntico ao encontrado na Europa (AY529053) e no Lago Charles (EUA) (AJ968893)

# Resultados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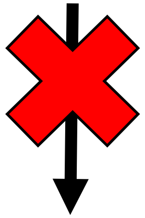
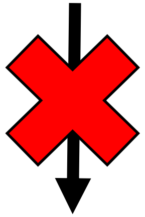
**Microscopia**



macroparasitas

**PCR**



microsporidia amplicões

# Conclusão

- *C. pseudogracilis* apresenta uma área de invasão limitada.
- Baixo risco de dispersão de patogénicos.
- 2 cenários possíveis para a origem desta espécie em Portugal.

# Agradecimentos

National
Science
Centre
Poland

FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

**Plano estratégico do MARE – Centro de Mar e Ambiente (UID/MAR/04292/2013)**

**Department of Invertebrate Zoology & Hydrobiology, University of Lodz.**

**the Polish National Science Centre (Grant 447 No. 2011/03/D/NZ8/03012).**

Obrigado pela vossa atenção!