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# Challenges for detection of contaminants: the case of the New-Zealand flatworm

Nikol Kmentová, Stewart Rosell, Jane Reniers, Arnaud Jacobs,  
Sytske de Waart, Archie Murchie & Maarten Vanhove



# List of Invasive Alien Species of Union concern

European Commission | English | Search

Energy, Climate change, Environment

## Environment

Home > Topics > Nature and biodiversity > Invasive alien species

### Invasive alien species

Preventing and minimising the effects on invasive alien species on Europe's biodiversity.

#### In the EU

<b>88</b> invasive alien species are strictly regulated	<b>47</b> animal species of Union concern	<b>41</b> plant species of Union concern
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## Flower pot trade as a source of invasive species



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# Flower pot trade as a source of invasive species



**Soft tree fern** (*Dicksonia antarctica*)



*Fletcheria quinquelineata*



# List of Invasive Alien Species of Union concern

## The threat posed by invasive alien flatworms to EU agriculture and the potential for phytosanitary measures to prevent importation

A report for the IUCN

by

Archie K. Murchie

THE CONVERSATION  
L'expertise universitaire, l'engagement journalistique



Obama nungara in a garden in France. Photo by Pierre Gros, CC BY

### Obama nungara: How a flatworm from Argentina crossed the Atlantic and invaded France

SMART NEWS

## Hammerhead Flatworms Have Been Quietly Invading France for Two Decades

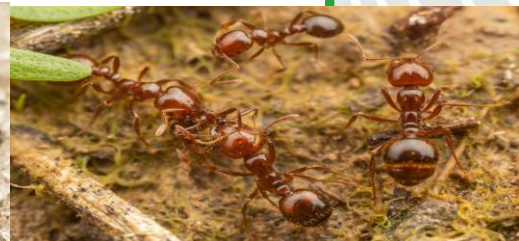
And that could spell trouble for the country's soils



INDEPENDENT

## Giant predatory worms invading France and threatening local wildlife

Creatures that grow up to 40cm have been living virtually unnoticed across the region for decades



NEWS PLANTS & ANIMALS

## Red fire ants, a dreaded pest, have invaded Europe

After discovering one of the worst invasive species in the world in Italy, researchers plan swift eradication

## Flatworm on the EU Invasive alien species list



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**New-Zealand flatworm (*Arthurdendyus triangulatus*)**

# Where are they?



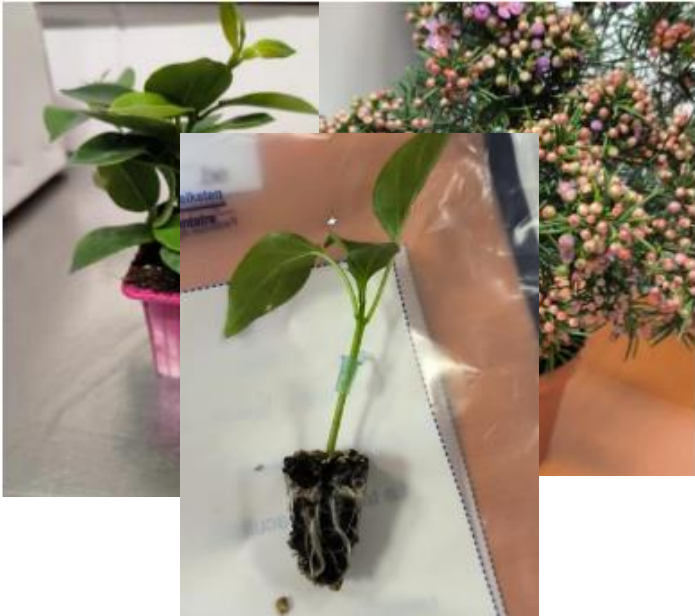
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# How did they get there?

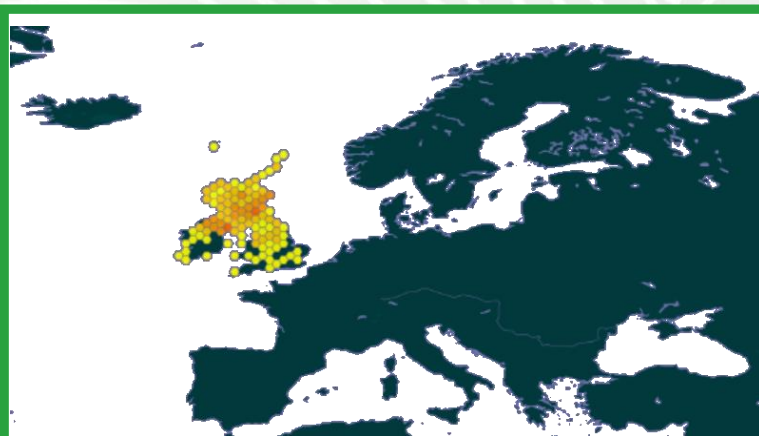


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# Where are they?



1963

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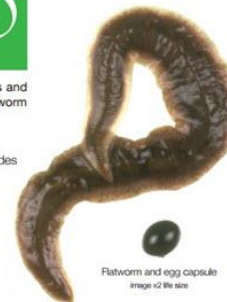
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## The OPAL New Zealand Flatworm Survey



The New Zealand Flatworm was introduced into the UK in the 1960s and feeds on earthworms. We need your help to find out how far this flatworm has spread and what influence it is having on the environment.

- Flat and ribbon-like
- Dark purple-brown on top, pale underneath and along the sides
- Adults usually 0.3-1cm wide and 5-15cm long
- Pointed at both ends and covered in sticky mucus
- Egg capsules look like blackcurrants but smaller
- Found in dark, damp places such as under wood, stones and plastic, or on soil
- Leave slime circles where they've been resting
- Only handle if you are wearing gloves
- Don't try to turn over any heavy stones or logs



Flatworm and egg capsule  
image © life size

### We need your help!



**Activity 1:** If you find a New Zealand Flatworm, please **take a photo** (alongside the ruler on this card) and submit this together with its **location** to [www.opalexplornature.org](http://www.opalexplornature.org)



**Activity 2:** If you have 10 minutes to spare, please turn over for activity 2, even if you have not found a flatworm.

Scan the QR code to go straight to the OPAL website



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# An experimental approach to develop a detection protocol

## Goals:

- 1) propose the authorities a protocol for detection of the New Zealand flatworm at border control
- 2) validate it experimentally



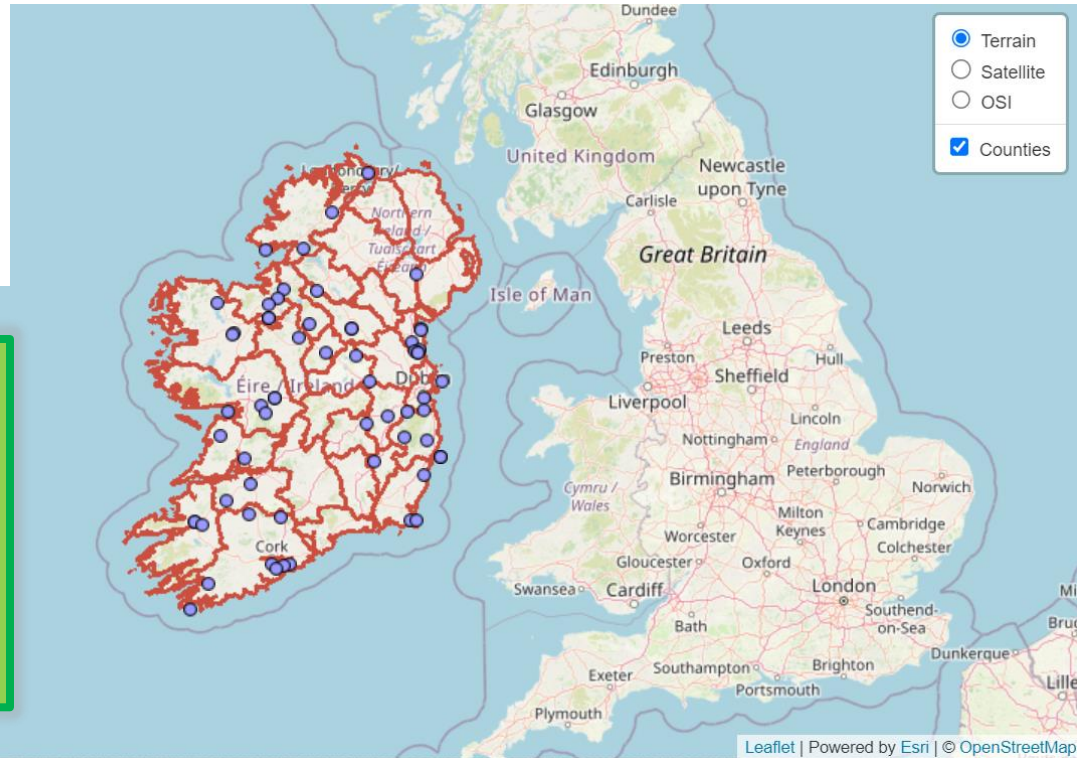
**AFSCA**  
**FAVV**



# Where to get them, where to experiment on them?



**DNA-based  
barcoding to  
facilitate routine  
species-level  
identification by  
non-specialists**



**National  
Biodiversity  
Data Centre**

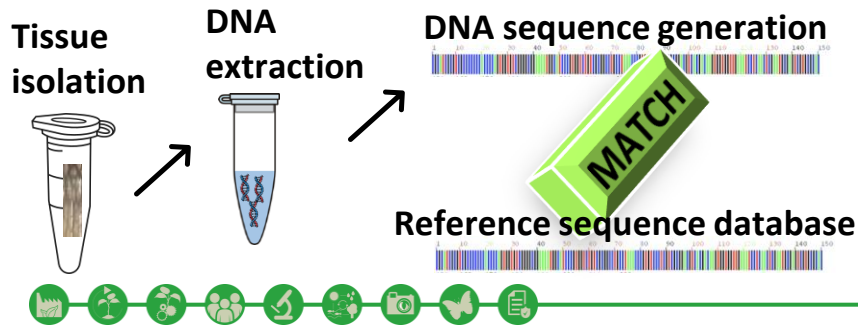
Documenting Ireland's Wildlife



# STEP 1: DNA sequence database



Species	COX1 mtDNA	ITS1 rDNA	ITS2 rDNA	5.8S rDNA	18S rDNA	28S rDNA	EF1 alpha
<i>Arthurdendyus triangulatus</i>	48	104	1	1	6	2	1
<i>Australoplana sanguinea alba</i>	0	1	-	-	3	0	-
<i>Bipalium kewense</i>	19	-	-	-	3	4	-
<i>Diversibipalium mayottensis</i>	4	-	-	-	-	1	-
<i>Diversibipalium multilineatum</i>	13	-	-	-	3	4	2
<i>Caenoplana variegata</i>	7	-	-	-	1	-	-
<i>Caenoplana coerulea</i>	27	-	-	-	15	1	-
<i>Caenoplana decolorata</i>	1	-	-	-	-	-	-
<i>Dolichoplana striata</i>	4	-	-	-	1	2	1
<i>Humbertium covidum</i>	1	-	-	-	-	-	-
<i>Kontikia atrata</i>	5	-	-	-	-	-	-
<i>Parakontikia ventrolineata</i>	4	-	-	-	-	2	1
<i>Obama nungara</i>	50	6	-	6	4	2	3
<i>Platydemus manokwari</i>	49	-	-	-	3	1	1
<i>Amaga expatria</i>	8	-	-	-	1	2	-
<i>Marionfyfea adventor</i>							Data not available
<i>Arthurdendyus albidus</i>							Data not available
<i>Arthurdendyus australis</i>							Data not available
<i>Artioposthia exulans</i>							Data not available
<i>Kontikia andersoni</i>							Data not available
<i>Kontikia bulbosa</i>							Data not available
<i>Parakontikia coxii</i>							Data not available



# STEP 1: DNA sequence database



**DNA-based barcoding to facilitate routine species-level identification by non-experts**

Species	# available
<i>Anisorhynchodemus</i> sp.	15
<i>Arthurdendylus triangulatus</i>	12
<i>Bipalium kewense</i>	2
<i>Caenoplana coerulea</i>	9
<i>Caenoplana micholitzii</i>	2
<i>Caenoplana variegata</i>	8
<i>Diversibipalium multilineatum</i>	3
<i>Dolichoplana</i> sp.	4
<i>Dolichoplana striata</i>	1
Geoplanidae indet.	2
<i>Marionfyfea adventor</i>	3
<i>Microplana</i> cf. <i>scharffi</i>	1
<i>Microplana terrestris</i>	9
<i>Obama nungara</i>	10
<i>Parakontikia ventrolineata</i>	2
<i>Platydemus manokwari</i>	2
<i>Rhynchodemus sylvaticus</i>	8
<b>total</b>	<b>82</b>



## STEP 2: Experimental validation




**Soil** versus  
**water** as a  
source of  
eDNA

**DNA-based  
detection  
following the  
flatworm's  
removal**



# Challenges of species-specific DNA-based diagnostics

## Problem 1: contamination with prey

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### Article

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**ZOOTAXA**  
ISSN 1175-5334 (online edition)

### Genetic variability of *Arthurdendyus triangulatus* (Dendy, 1894), a non-native invasive land planarian

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## Problem 2: false positives: cross-detection of related species

## Problem 3: false negatives: capture DNA sequence variation

## Problem 4: flatworm eggs not detectable



# Challenges of species-specific DNA-based diagnostics

**Problem 1: contamination with prey**

**Problem 2: false positives: cross-detection of related species**

**Problem 3: false negatives: capture DNA sequence variation**

**Problem 4: flatworm eggs not detectable**

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<i>Dolichoplana striata</i>	1
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<i>Marionfyfea adventor</i>	3
<i>Microplana</i> cf. <i>scharffi</i>	1
<i>Microplana terrestris</i>	9
<i>Obama nungara</i>	10
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<b>total</b>	<b>82</b>





# Challenges of species-specific DNA-based diagnostics

**Problem 1: contamination with prey**

**Problem 2: false positives: cross-detection of related species**

**Problem 3: false negatives: capture DNA sequence variation**

**Problem 4: flatworm eggs not detectable**



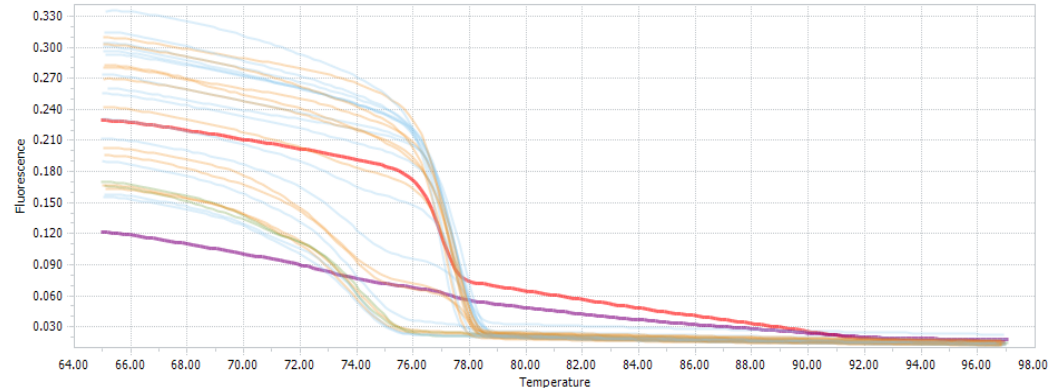
# Border control and diagnostics of the New Zealand flatworm



**Visual inspection combined with DNA-based species validation**



**eDNA capture followed by qPCR species-specific diagnostics**



# Take-home message

**First species-specific phytosanitary protocol for invasive land planarian detection – visual inspection supported by eDNA methodology**

**Egg capsules not-detectable by molecular-based barcoding techniques**

**New lineages of flatworms revealed by molecular characterisation**

**Genomic data for detection of related species through barcoding of Dutch non-native planarians**

# Future directions

**Discussion on species-specific versus general protocols in terms of time and budget efficiency**



# Funding



Invasive Alien Species  
National Scientific Secretariat

CMK  
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ENVIRONMENTAL SCIENCES

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**IAS Secretariat - Belgium**  
@Secretariat\_IAS Volgt jou

Health  
Food Chain Safety  
Environment

**Thank you for your attention!**

**Questions? Feedback?**