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Metabarcoding of earthworm species in gut content of the recently introduced terrestrial flatworm *Obama nungara* as a tool to assess the impact of this invasive predator.

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Introduction

Invasion by terrestrial flatworms (Platyhelminthes: Geoplanidae) is of great concern, as these species may cause severe reductions in native prey populations and major changes in soil fauna community structure [1]. *Obama nungara* is an exotic terrestrial flatworm, which recently invaded the French territory and is only known to date in anthropized environments such as private gardens [2]. *O. nungara* predaes on earthworms (Fig. 1) and thus represents a potential threat for earthworm communities. The metabarcoding of digestive contents represents a powerful approach to study the diet of such newly introduced predatory species, and therefore to forecast their potential impact on native species.

Aims

- To develop a metabarcoding approach to study *O. nungara* gut contents:
 - extracting the digestive contents of flatworms obtained through citizen science contributions
 - amplifying a 70bp earthworm-specific fragment of the 16S RNA gene [3]
 - sequencing amplicons with a high-throughput approach (Illumina MiSeq, paired-end 2 × 150 bp)
- To produce the first ecological results concerning *O. nungara* predation:
 - identifying the species of earthworms consumed
 - investigating whether a predation biased towards some ecological categories exists

Results

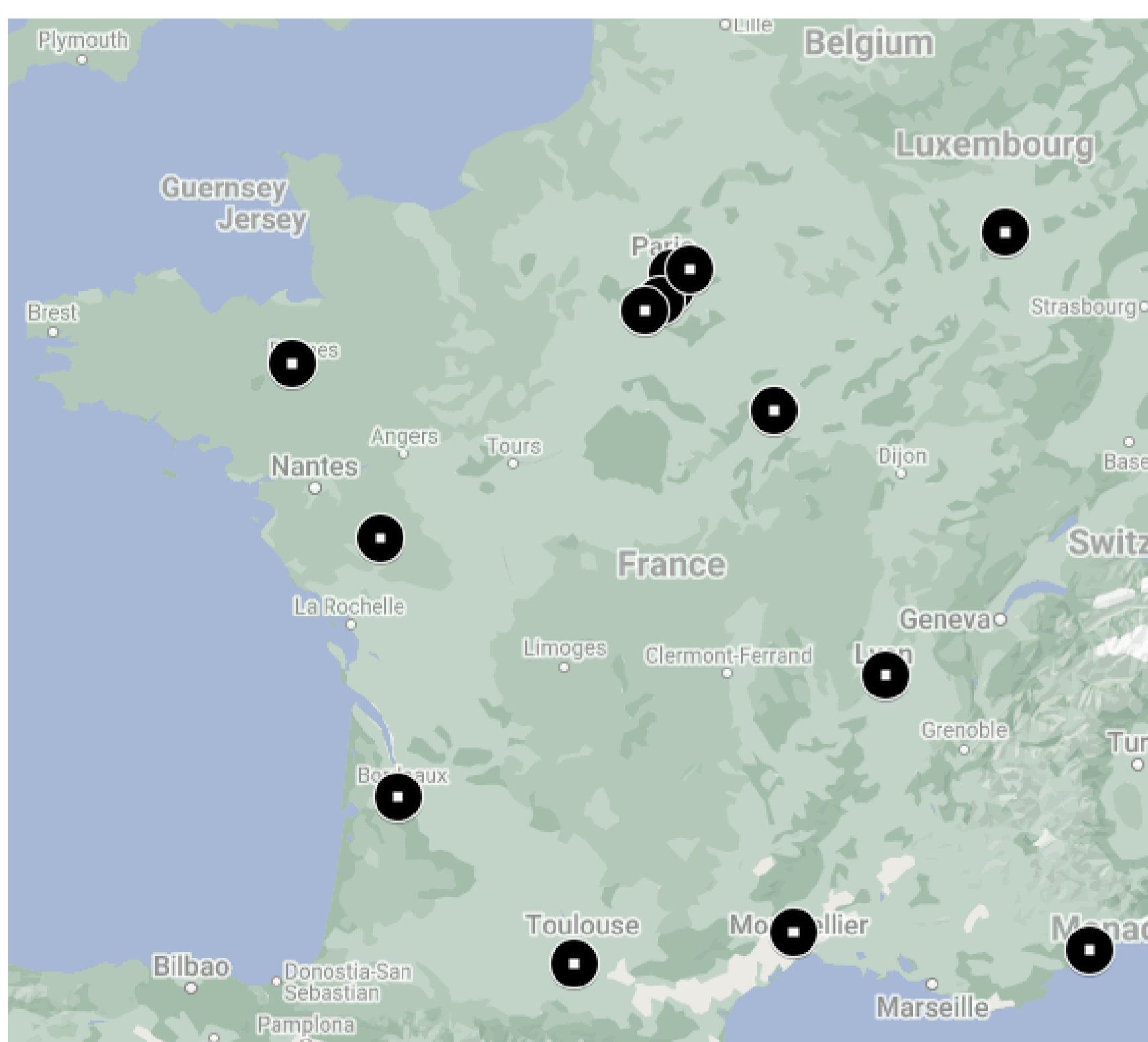


Fig.2. Origin of *O. nungara* samples from 14 private gardens in France

- 35 samples collected by citizen science participants
- 26 samples amplifying the earthworm-specific fragment (Fig. 2) → 74% that fed on earthworms before their capture



Fig.1. *O. nungara* specimens of different colors and attacking an earthworm

The 6 species most frequently consumed by *O. nungara* with their ecological category:

- Allolobophora chlorotica*, epi-endo-aneic
- Dendrodrius rubidus*, epigeic
- Eisenia fetida/andrei*, epigeic
- Eiseniella tetraedra*, epigeic
- Aporrectodea rosea*, endogeic
- Lumbricus rubellus*, epigeic

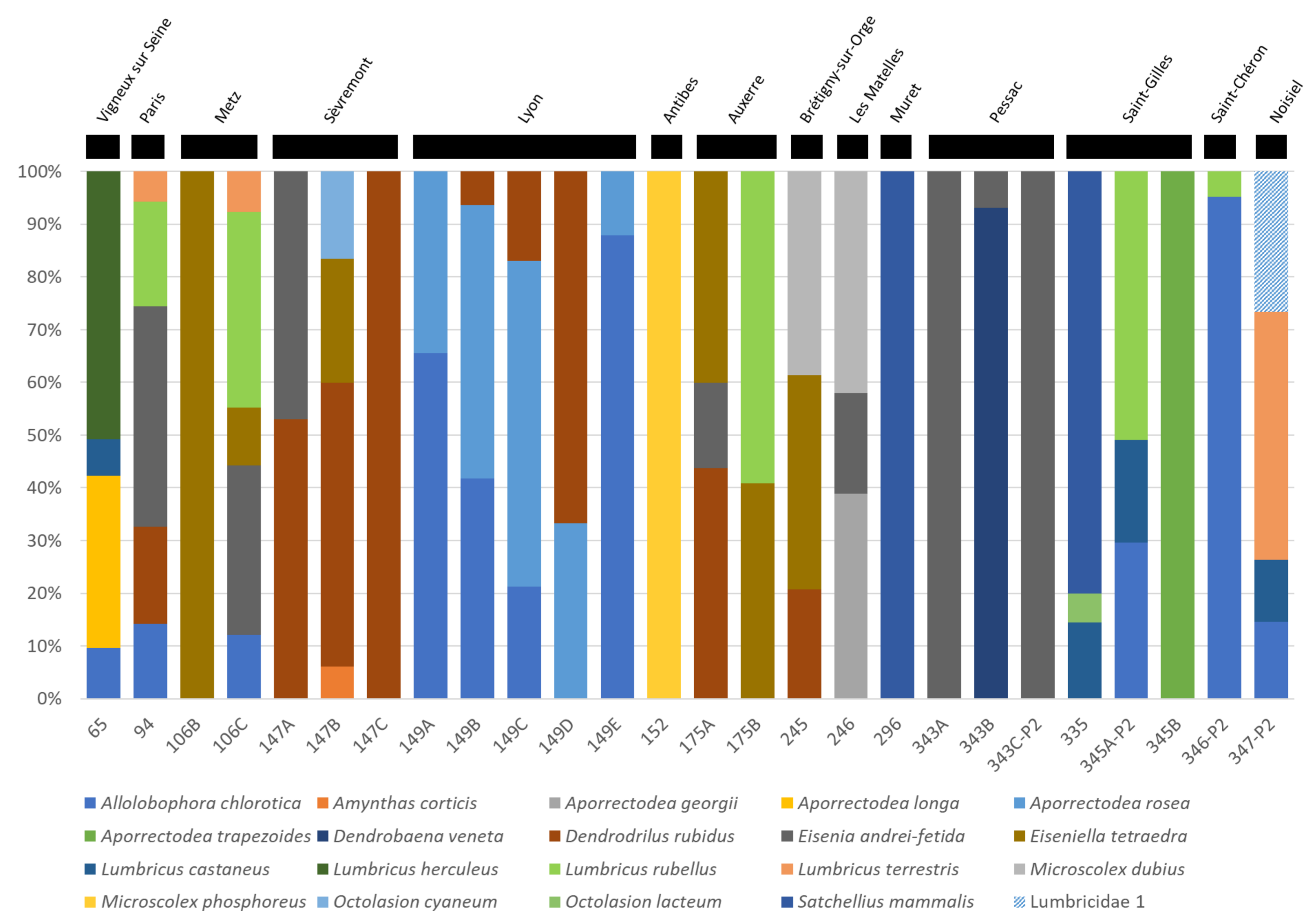


Fig.3. Percentages of sequencing reads for each earthworm species and *O. nungara* gut contents (26 samples, 14 sites)

Conclusion

We validated a metabarcoding approach to identify earthworm prey in the digestive contents of the terrestrial flatworm *O. nungara*. The first results concerning the diet of *O. nungara* in its introduced habitats in France suggest that it can predate on a large panel of earthworm species, including endogeic and epi-endo-aneic ones. These results expand the range of food possibilities known for this species and confirms its status of threat for native earthworms.

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