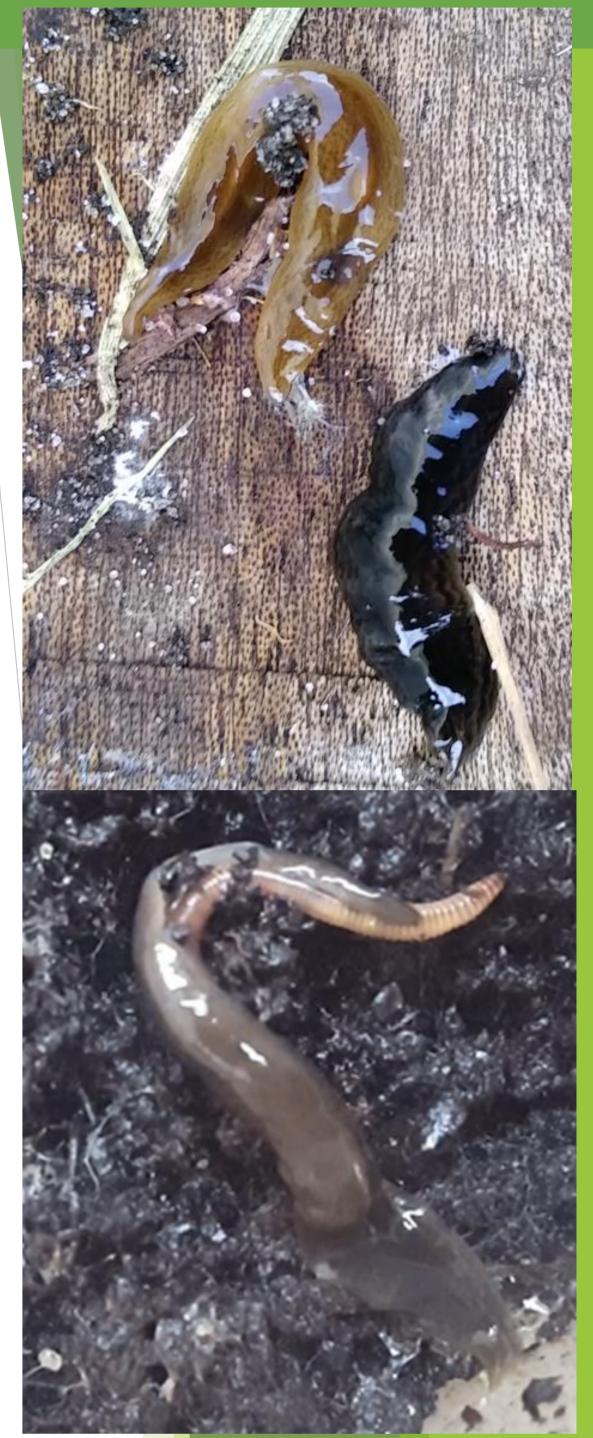


Metabarcoding of earthworm species in gut content of the recently introduced terrestrial flatworm Obama nungara as a tool to assess the impact of this invasive predator

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Introduction

Invasion by terrestrial flatworms (Plathyhelminthes: 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 predates 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.



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Aims

- 1. 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)
- 2. 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



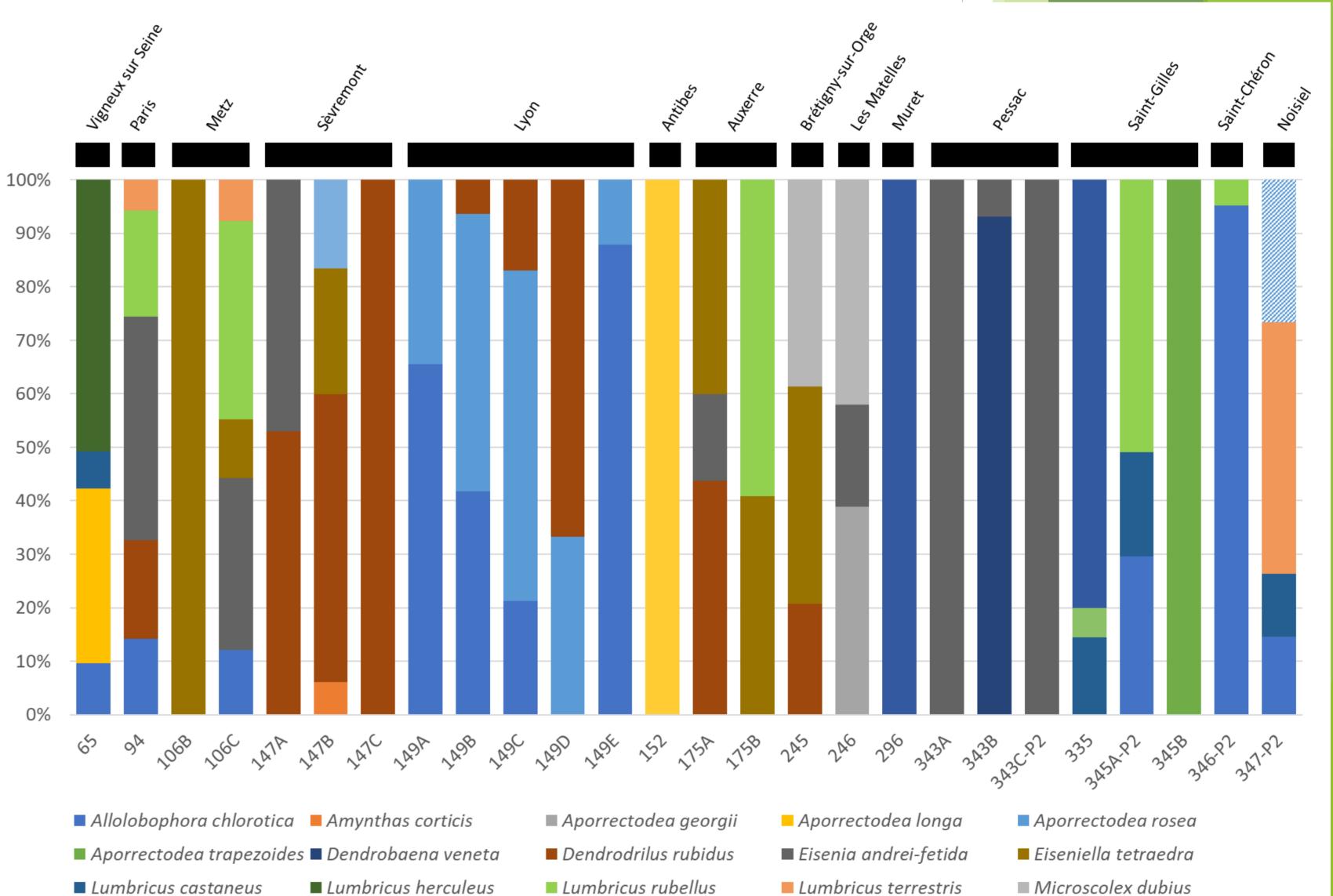
- 35 samples collected by citizen science participants
- 26 samples amplifying the earthworm-specific fragment (Fig. 2) \rightarrow 74% that fed on earthworms before their capture

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

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

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

- Allolobophora chlorotica, epi-endo-anecic
- Dendrodrilus rubidus, epigeic
- Eisenia fetida/andrei, epigeic
- Eiseniella tetraedra, epigeic
- Aporrectodea rosea, endogeic



Lumbricus rubellus, epigeic (Fig.3)

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-anecic ones. These results expand the range of food possibilities known for this species and confirms its status of threat for native earthworms.





Satchellius mammalis Microscolex phosphoreus Octolasion cyaneum Octolasion lacteum 🖉 Lumbricidae 1

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

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