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The invasive land flatworm *Obama nungara* in La Réunion, a French island in the Indian Ocean, the first report of the species for Africa

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Abstract

The land flatworm *Obama nungara*, a species originating from South America and already invasive in many European countries, is recorded from La Réunion, a French island in the Indian Ocean. This is the first record of *O. nungara* from this locality and also the first record of the species for Africa. Three specimens were collected in 2021 and 2022, in the communes of Saint Paul, Saint Joseph and Le Tampon, respectively; the three localities are widely separated, with two in the Western part and one in the South-eastern part of the island. This suggests that the species is already present in several locations in La Réunion, and it is likely that the species is already present since 2020. The specimen from Saint Paul had the same *cox1* haplotype as specimens previously recorded from several countries of Europe; it is hypothesized that the species was imported from Europe, probably from France. We mapped climatic suitability of the species in La Réunion and found that *O. nungara* could potentially invade a large part of the island. One record was apparently associated with the transport of plates of travertine, a construction material which has numerous cavities thus suitable for the transport and survival of adult or cocoons of land flatworms.

Key words: Invasive Alien Species, flatworm, citizen science, new records, La Réunion

Résumé

Le Plathelminthe terrestre *Obama nungara*, une espèce originaire d'Amérique du Sud et déjà envahissante dans de nombreux pays européens, est signalé à La Réunion, une île française de l'Océan Indien. Ceci est le premier signalement d'*O. nungara* dans cette localité et aussi le premier signalement de l'espèce pour l'Afrique. Trois spécimens ont été collectés en 2021 et 2022, respectivement dans les communes de Saint Paul, Saint Joseph et Le Tampon; les trois localités sont largement séparées, avec deux dans la partie occidentale et une dans la partie sud-est de l'île. Ceci suggère que l'espèce est déjà présente dans plusieurs localités de La Réunion, et il est probable que l'espèce soit déjà présente depuis 2020. Le spécimen de Saint Paul avait le même haplotype du *cox1* que les spécimens précédemment récoltés dans plusieurs pays d'Europe; nous faisons l'hypothèse que l'espèce a été importée d'Europe, probablement de France. Nous avons cartographié l'adéquation climatique de l'espèce à La Réunion et constaté qu'*O. nungara* pourrait potentiellement envahir une grande partie de l'île. Un signalement était apparemment associé au transport de plaques de travertin, un matériau de construction qui possède de nombreuses cavités, donc adapté au transport et à la survie des adultes ou des cocons de vers plats terrestres.

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Introduction

Invasive species are permeating every part of the planet, even the most remote sites. Land flatworms are representative of this trend (Sluys, 2016). The New Guinea flatworm, *Platydemus manokwari* de Beauchamp, 1963, has been included in the list of the "100 world's worst invasive alien species" (Lowe *et al*., 2000); it is present in many territories in the Pacific (Justine *et al*., 2014), has recently invaded continental USA (Justine *et al*., 2015), and is associated with the decline of native snails (Gerlach *et al*., 2021). Other examples include the hammerhead flatworms, especially *Bipalium kewense* Moseley, 1878, now in dozens of countries, or *Bipalium vagum* Jones & Sterrer, 2005, now widespread in many tropical countries (Justine *et al*., 2018; Winsor, 1983).

Obama nungara Carbayo *et al*., 2016 was recently described from a series of specimens, both from their land of origin, South America, and from Europe (Carbayo *et al*., 2016). It has now invaded several countries in Europe (Justine *et al*., 2020b). Molecular studies have shown that the species has three *cox1* haplotypes, designated as "Argentina 1", "Argentina 2" and "Brazil" according to their region of origin. The Brazil haplotype has never been found outside of Brazil; the Argentina 2 haplotype has been recorded only in Argentina and a few localities in Spain; in contrast, the Argentina 1 haplotype has been found in several countries in Europe, including France, Spain, Portugal, Italy, Switzerland and UK (Justine *et al*., 2020b).

Obama nungara is a predator of earthworms and molluscs and thus is a potential threat for the biodiversity of soils. It is able to proliferate at high rates in gardens in temperate climates, with thousands of specimens in a single garden (Justine *et al*., 2020b).

In this paper, we report the first finding of *O. nungara* in La Réunion, a French island in the Indian Ocean. We show that the *cox1* haplotype of this specimen is compatible with an importation from Europe, probably metropolitan France. In addition, we mapped climatic suitability of the species in La Réunion, projected from a previously published species distribution model (Fourcade, 2021), showing that this species could invade a large part of the island.

Material and methods

Collection of specimens

Citizen science is highly effective for collecting information of land flatworms (Justine *et al*., 2014, 2015, 2018, 2020a, b, 2022a; Mori *et al*., 2022). One of us (ADM) initiated a citizen science project in La Réunion about land flatworms, supported by the local association ARBRE (Agence de Recherche pour la Biodiversité à La Réunion *https://arb-reunion.fr/*). Among the many records obtained from participating individuals, a few flatworms were morphologically similar to *Obama nungara*. Photographs of the living flatworms were taken, and a few specimens of flatworms were collected and fixed in ethanol. The specimen from Petite France (Saint Paul) was registered in the collections of the Muséum national d'Histoire naturelle under number MNHN JL449, and then a part of the body was taken for molecular study and sent to Poland (RG). The specimen from Saint Joseph was registered as MNHN JL452 and no molecular study was undertaken.

Molecular methods: sequencing, alien DNA, and haplotype network

The complete mitogenome of specimen JL449 was sequenced in the context of a project in progress. Sequencing was performed following the exact same protocol as described in detail in a recent paper (Justine *et al*., 2022a), with a k-mer of 125. The sequence corresponding to the *cox1* gene was identified using ORF finder on the NCBI portal (https://www.ncbi.nlm.nih.gov/orffinder) and by comparison with a published complete mitogenome (Solà *et al*., 2015). The *cox1* sequence was deposited in GenBank as OM451228.

The pool of contigs obtained after assembly was also datamined to look for the presence of alien DNA following Justine *et al*. (2022a). The phylogenetic tree (not shown) obtained on this contaminant DNA was based on the partial 5.8S and ITS1, which were aligned with corresponding sequences of slugs (Quinteiro *et al*., 2005; Rowson *et al*., 2014), trimmed and computed for a Maximum Likelihood (ML) phylogeny with the General Time Reverse model and 100 bootstrap replicates, all steps performed by MEGAX (Kumar *et al*., 2018).

We constructed an haplotype network using a matrix made public (Justine *et al*., 2020b) and simply added one taxon (the *cox1* sequence of specimen JL449) and one trait label ("La Réunion"). In the nexus file, traits were the

geographical origin of the species, with number of traits set to 10, and trait labels set as: Argentina, Brazil, UK (including Guernsey), Portugal, Spain, Italy, Switzerland, Belgium, Metropolitan France, and La Réunion. The matrix had 92 taxa and was 255 bases in length. We used PopART (Leigh & Bryant, 2015) to create the haplotype network; the method of Templeton, Crandall and Sing ("TCS") was used to infer relationships among samples (Clement *et al*., 2002).

Species distribution modelling

We produced a map of climatic suitability of *O. nungara* in La Réunion using an already published species distribution model (Fourcade, 2021). Briefly, a MaxEnt model (Phillips *et al*., 2017) was fitted via the ENMeval R package (Kass *et al*., 2021) using 144 georeferenced records of *O. nungara* both from the species' native range and from Europe, as well as global bioclimatic predictors at 2.5 arc-min resolution. Here, we obtained the same bioclimatic variables at a finer resolution (30 arc-sec) and used them to project model predictions in La Réunion. Full details of the modelling parameters are available in Fourcade *et al*. (2021), where the employed model is referred to as 'fully tuned'.

Results

As part of our citizen science initiative, we obtained three records of flatworms resembling *Obama nungara* from La Réunion.

- Régis Bretzner found several specimens of flatworms in his house located in Petite France (commune of Saint Paul) in the western part of the island. The specimens, ranging from a few millimetres to 4 cm, were seen mainly in his bathroom. The first specimen was found on 10 June 2021 **(Figure 1)**, and the participant recalled seeing flatworms until the end of July, but not since (telephone interview in January 2022). The bathroom had been recently redone using plates of travertine. The travertine had many holes and cavities; it was bought locally, its origin was unknown, but it is likely that the stones were imported from France.
- Renaud Hoarau collected a specimen on 22 April 2021 in the garden of his house in la Plaine des Grègues (commune of Saint Joseph), in the eastern part of the island. He moved into this house in 2020 and remembers seeing flatworms since he first arrived, and continually, up to January 2022 when we interviewed him by telephone. The April 2021 specimen was photographed by Nicolas Huet **(Figure 2)**. In addition, Renaud Hoarau provided a photograph (not shown) of another specimen, not collected, seen 23 January 2022 close to his home.
- Magali Poirier photographed a specimen on 19 September 2021 in the garden of the Gîte de la Bergerie (Commune of Le Tampon).

Coordinates for records were thus: Petite France, 55.34063 E, 21.01470 S; Plaine des Grègues, 55.59410 E, 21.32402 S; Gîte de la Bergerie, 55.58497 E, 21.25391 S.

The living specimens on the photographs sent by both collectors showed the general characteristics of *Obama nungara*, with a brown dorsal colour with darker striae (**Figures 1–2**). The preserved specimens showed a green-brown dorsal colour and a beige ventral colour (not shown).

The complete *cox1* gene was 1737 bp long, which is identical to the reference from Solà *et al*. (2015) and had the same start and stop codons (GTG and TAA respectively). The haplotype network (**Figure 3**) showed that the specimen from La Réunion was identical to one of the haplotypes included within the Argentina 1 network. This haplotype includes samples from France, Spain, Argentina, Switzerland, and Belgium.

In our research of alien DNA (from prey in the gut of the flatworm), only few contigs with low coverage returned positive blastn results. Megablast queries showed that all these contigs belong to the cluster of nuclear ribosomal RNA genes of a slug of the genus *Arion*. The ML tree associated the alien DNA in a highly supported clade with *Arion intermedius* (Normand, 1852) isolate 56D (GenBank accession number AY316286.1), a specimen from Aran Valley, Lleida, Spain.

Modelling (**Figure 4**) showed that ca. 57% of the island of La Réunion was suitable for *O. nungara* (based on the threshold that maximises model sensitivity and specificity); in addition, about 38% of the island had a very high

climatic suitability (> 0.8). Based on the analysis of the model's response curves (Fourcade, 2021), it appears that the limiting factor was mainly the mean temperature of the warmest quarter (BIO 10 bioclimatic variable), coastal areas being too warm while high-altitude areas are too cold. It is noteworthy that all three localities where *O. nungara* specimens were found are inside zones of high suitability (Plaine des Grègues: 0.92; Petite France: 0.89; Gîte de la Bergerie: 0.73).

FIGURE 1. Photo of a live specimen of *Obama nungara*, taken in Petite France in the commune of Saint Paul, La Réunion. Photo by Régis Bretzner, taken 10 June 2021. The specimen is brown on the back, with darker brown striae, a reddish tip, and with a cream stripe running a short distance from the head towards the tail, but which soon disappears into the general colour of the back.

FIGURE 2. Photo of a live specimen of *Obama nungara*, taken in la Plaine des Grègues in the commune of Saint Joseph, La Réunion. Specimen collected 22 April 2021 by Renaud Hoarau, photograph taken by Nicolas Huet. The photo clearly shows the dark brown striae on the brownish coloured back, and the whitish under surface.

Discussion

Obama nungara, originating from South America (Brazil and Argentina) (Carbayo *et al*., 2016) has now invaded many countries in Europe. In 2020, a study found that Guernsey Island, UK, Spain, Portugal, France, Belgium, Italy, Ireland, Switzerland, and Madeira Island were already invaded (Justine *et al*., 2020b). More recently, the species was reported from Azores Islands (Lago-Barcia *et al*., 2020), The Netherlands (de Waart *et al*., 2021), and finally Austria and Germany (Justine *et al*., unpublished) and Slovakia (Čapka & Čejka, 2021), therefore falsifying a previous statement (Justine *et al*., 2020b) that "no record was known from Germany or any country East of Germany". There are also reports of specimens resembling *O. nungara* from USA, Mexico, Costa Rica, Bolivia, and Chile in iNaturalist (*www.iNaturalist.org*), but none of these citizen science records has been published nor confirmed by molecular methods.

The present record in the island of La Réunion, a French island located east of Madagascar in the Indian Ocean,

is the first for this territory. As such, it is also the first report of *Obama nungara* for Africa. Another alien flatworm, *Bipalium vagum*, has been recorded in La Réunion (Justine *et al*., 2018). Our current citizen science initiative has shown that other flatworm species are present, and these records will be published in papers to come.

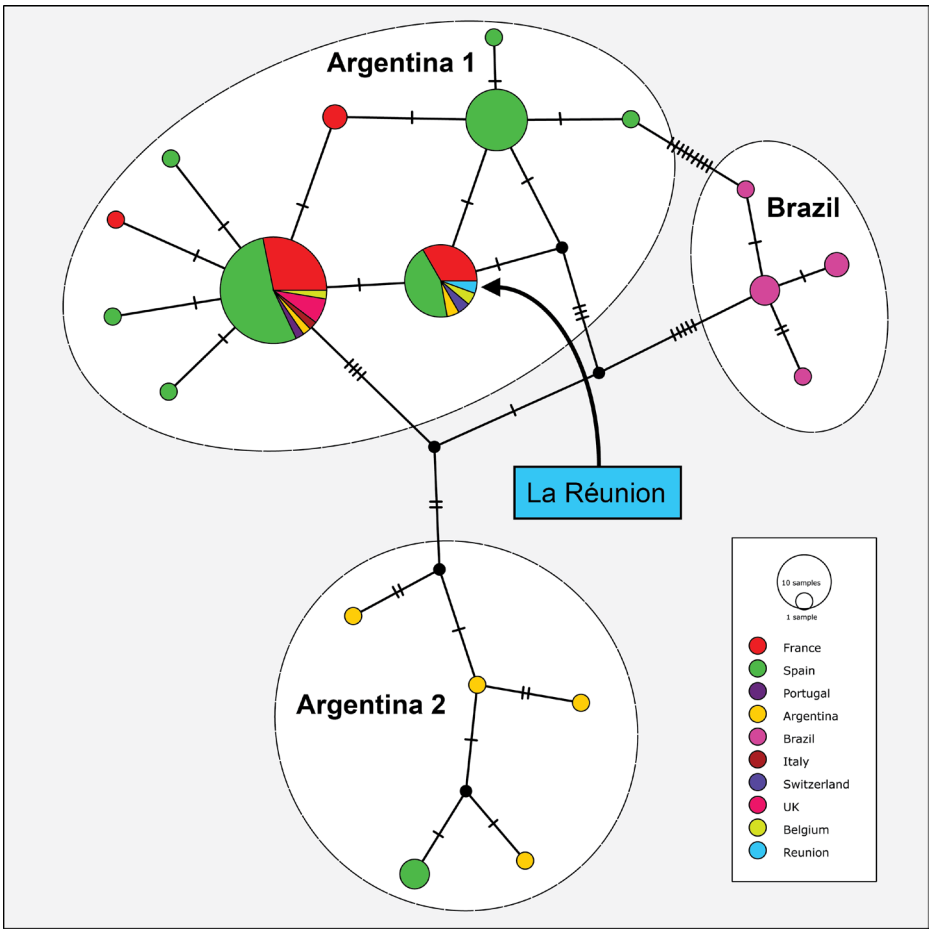

FIGURE 3. Haplotype network. The network was obtained by using a matrix made public (Justine *et al*., 2020b) and adding a single sequence, from the specimen MNHN JL449 from Petite France, Saint Paul, La Réunion.

There are two studies on the potential distribution of *Obama nungara* worldwide. One used built-in parameters in the software (Negrete *et al*., 2020) and one used more elaborate methods (Fourcade, 2021). In this second study, it was shown that *O. nungara* had the potential to invade a large number of territories in the world. This includes a significant part of Africa, in particular south and east Africa, as well as Madagascar and its neighbouring islands. We were able, here, to specify at a finer scale the localities of La Réunion that appear climatically suitable for *O. nungara*. Model projections reveal that most of the areas of intermediate altitude have the potential to be invaded, because they harbour mild temperatures during the hot season that are needed for *O. nungara* survival. A more precise assessment of invasion risk would require a finer characterisation of *O. nungara*'s ecological niche, e.g. with regard to microclimate, soil properties, as well as biological interactions with its preys and predators.

The present report is based on only three records, but we have no doubt that these are only the visible part of the iceberg. In the two islands of Martinique and Guadeloupe (Antilles) a similar situation was found for the New Guinea flatworm, *Platydemus manokwari*. Only two years after the first report (Justine & Winsor, 2020), it was found that the flatworm had already invaded most of Guadeloupe (Justine *et al*., 2021). Similarly, the invasive flatworm *Amaga expatria* was found in many locations in the two islands (Justine *et al*., 2020a). This is again an illustration that islands are fragile ecosystems which are more sensitive to invasion by alien species. We fear that the same will happen in La Réunion with *O. nungara* (and possibly other flatworm species).

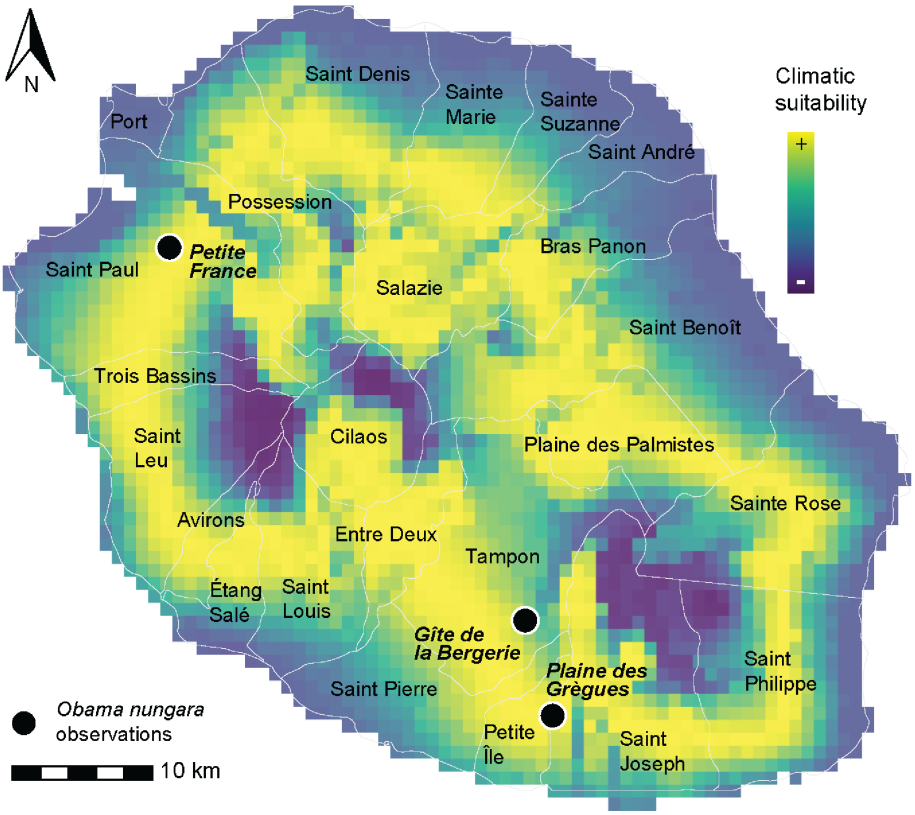

FIGURE 4. Climatic suitability of *Obama nungara* in La Réunion. Names of communes and their limits (white lines) are indicated. The three localities where the specimens were found (Petite France, Plaine des Grègues and Gîte de la Bergerie) are indicated.

Since the feeding behaviour of *O. nungara* has not been yet directly observed in la Réunion, the results we obtained on the alien DNA associated to the specimen have to be taken with care, keeping in mind that they concern a single specimen. Detecting a European species, the Hedgehog Slug *Arion intermedius* as prey, was unexpected. However, this species is a known introduction in many countries and territories, including La Réunion (Auffenberg *et al*., 2021).

In the case of the invasion of La Réunion by *O. nungara*, it is noteworthy that the specimens most probably do not come from their land of origin (Brazil or Argentina) but from another continent (Europe) and, because of the high level of commerce between La Réunion and France, probably from the latter.

Land flatworms are usually considered to be spread internationally by the transport of plants (Murchie & Justine, 2021; Sluys, 2016). Indeed, adults flatworms can survive in the earth of potted plants, and cocoons can also go undetected in the same situation. Our current study suggests that the transport of travertine stone was implicated in the possible spread of *O. nungara*. We do not know whether the flatworms were directly imported from Europe to La Réunion in a stack of travertine stone, or if following delivery to La Réunion, the stack was colonized by a local proliferation of the flatworm in an area of the island where the stone was kept before being sold to the public; the finding of many specimens by the observer in Saint Joseph favours the second hypothesis. In both cases, this hypothesis of transport of the flatworms together with the stone deserves consideration. Indeed, decorative stone such as travertine is often transported as stacks with the plates separated from each other by very thin spaces. Considering that travertine offers many spaces and cavities, which can harbour either adults or cocoons, this stone offers ideal conditions for the transport of flatworms such as *Obama nungara*. Transport of inert materials such as flat slices or tiles of stone should be considered a possible way of transport for invasive flatworms, in addition to the transport of plants.

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