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Research Article

Going Dutch: European distribution of non-native land flatworm species belonging to Geoplaninae and Bipaliinae with focus on the Netherlands

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Abstract

Non-native land flatworms can have a negative impact on local ecosystems, due to their voracious appetites for earthworms or snails. Accurate knowledge on the distribution of non-native populations of land flatworms is necessary to design effective policy to control their spread across Europe. The aim of this study is to address the spatiotemporal distribution of selected species of non-native land flatworms (Geoplaninae and Bipaliinae) in the Netherlands, and provide their current distribution and introduction pathways in a pan-European perspective.

Specimens of *Obama* spp., *Bipalium kewense* and *Diversibipalium multilineatum* were reported across selected Dutch gardens, greenhouses, plant nurseries or garden centers. European distribution of these planarians species was reconstructed using previously published datasets and from records available on GBIF. Morphological species identification was supported by DNA barcoding using a portion of the 28S rDNA marker. Introduction pathways were addressed via haplotype networks based on COI mtDNA.

In total, 27 specimens of non-native land flatworms were collected in the Netherlands. Their different spatiotemporal distribution pattern indicates differences in tolerance to environmental conditions in Northern Europe between *B. kewense* restricted to greenhouses and *D. multilineatum* found in gardens. Generally, an increasing trend in the number of records of *Obama nungara* is observed in the Netherlands and in Europe, with the highest number of records per country reported in France (1.428) followed by the Netherlands (149) and Italy (64). The high numbers of France are, however, artificial and originate from communication towards the public, which has not been as pronounced in other European countries. Genetic analyses suggest multiple introductions of *O. nungara* in Europe. The combination of morphological and molecular species identification revealed the presence of *Obama anthropophila* being the first record of this species outside its native range in Brazil. Our results further support the established status of these species in Europe and highlight the importance of citizen scientists in non-native species research.

Key words: Citizen science, land planarians, *Obama anthropophila*, potted plant trade

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Introduction

Worldwide, more than 960 species of land flatworms or terrestrial planarians (Geoplanidae) have been described in scientific literature (Cardoso et al. 2023). Most species have been found in (sub)tropical areas in Australia, New Zealand, South America, South East Asia and Africa.

Some species of non-native land flatworms with a generalist diet and high tolerance to environmental disturbances have a high potential to have a negative impact in their new environment. Land flatworms are voracious top-level predators of soil organisms. Introduction of non-native land flatworms can change nutrient cycling, endanger native species like snails, and diminish soil fertility by reducing earthworm numbers (Sluys 2016). The New Zealand flatworm *Arthurdendyus triangulatus* (Dendy, 1894) is a non-native land flatworm in Great Britain, Ireland and the Faroe Islands that predates on native earthworms and other soil invertebrates that are important for maintaining soil fertility (Murchie 2009). This flatworm species has been recently added to the EU-list of Invasive Alien Species of Union concern (European Commission 2022a). Another harmful species is the New Guinea flatworm *Platydemus manokwari* Beauchamp, 1962, which is considered to be one of the 100 worst invasive species by the IUCN (Lowe et al. 2000). There is one record of *P. manokwari* in Europe in a greenhouse in Caen, France in 2013 (Justine et al. 2014).

International trade in potted plants has been identified as the main transport mode and source of introduction of land flatworms into non-native areas (Álvarez-Presas et al. 2014). Other means of transport where plants are not involved have been suggested, such as the import of stones (plates of travertine) and plastic bags containing potted soil or fertilizer (Justine et al. 2022b; Justine personal observation). In addition to the international horticulture trade, transport of inert and sufficiently moisted materials should therefore also be considered as a means of transport contributing to the introduction of land flatworms.

In total, 22 species of non-native land flatworms have been reported in Europe so far (Thunnissen et al. 2022). Recently, an increasing number of studies have given an insight into their distribution in Europe, especially in France, Spain and Italy (Vila-Farré et al. 2011; Álvarez-Presas et al. 2014, 2023; Carbayo et al. 2016; Justine et al. 2018; Jones et al. 2020; Mori et al. 2022a, b; Thunnissen et al. 2022; Solà et al. 2023). This caused increased awareness on the possible negative impact of non-native land flatworms in Europe, including among the authorities, e.g. the first EU level conference on Border controls of Invasive Alien Species in March 2024 (Belgian presidency Council of European Union 2024). However, an overview of their temporal distribution patterns in Europe is lacking.

Geographically, small-scale studies are of a pivotal role to understand the spread of non-native species in introduced areas (Jeschke et al. 2014). In case of the New Zealand flatworm the first report of this species outside of New Zealand was on plants in the Edinburgh Botanic Gardens in 1965, later mentioned as a possible source of dispersal across Great Britain (Cannon et al. 1999). However, analyses of genetic variation in *A. triangulatus* suggest multiple introductions into the United Kingdom (Dynes et al. 2001). Since then populations of *A. triangulatus* have been reported in other island territories of Ireland and the Faroe Islands (Murchie 2009). Plants introduced into botanical gardens, often exchanged with other botanical gardens, can figure as primary entries of introduction of non-native species such as land flatworms (Galbraith and Cavallin 2021). There are, however, other pathways, like garden centers, from where land flatworms are distributed to private gardens, and if they are tolerant to external environmental conditions, from there on into natural areas. Insight into the primary entries is crucial for effective monitoring and management strategies of land flatworms.

To monitor distribution of non-native species, expertise on accurate species-level identification is of crucial importance. Given the high morphological resemblance between species in the same genus of land flatworms, a combination of morphological characterisation of the internal anatomy and DNA barcoding is often necessary to reveal species-level distribution patterns and hence introduction pathways (Winsor 1983; Sluys and Riutort 2018). So far, land flatworms in both native and introduced areas of distribution have been only poorly represented in genetic databases.

Accurate knowledge on the distribution of non-native populations of land flatworms is necessary to design effective policy to control their spread across Europe. The aim of the present study is to provide an overview of the current known distribution of species of Geoplaninae (Neotropical land flatworms) and Bipaliinae (hammerheads) as known invaders in Europe, with a detailed focus on their spatio-temporal spread and invasion pathways across the Netherlands. This paper is limited to these two subfamilies, but there are two other subfamilies of Geoplanidae: Microplaninae and Rhynchodeminae. Some species of these other two subfamilies are also introduced in Europe (Thunnissen et al. 2022).

Materials and methods

Collection of specimens

We combine information of specimens collected during a pilot search for land flatworms in greenhouses in the Netherlands (Thunnissen et al. 2020), and in a follow-up study conducted for the Nederlandse Voedsel- en Warenautoriteit (Dutch Food and Consumer Product Safety Authority) (De Waart and Thunnissen 2023). Also Dutch citizen scientists that reported their findings on the presence of *Obama* spp. and *Diversibipalium* spp. on Waarneming.nl were contacted and visited to collect the specimens. A single specimen of *B. kewense* from Belgium was found by the first author on a field trip, and a single specimen of *Obama nungara* Carbayo, Alvarez-Presas, Jones & Riutort, 2016 from Italy was received from a fellow researcher. To establish pathways of introduction, information of the type of environment of each record of non-native land flatworms was recorded.

All collected specimens were photographed and subsequently stored in 97% molecular-grade ethanol. Photos and metadata are available as part of the Suppl. material 1. Specimens were deposited in the collection of Naturalis Biodiversity Center in Leiden, the Netherlands, under institution- and specimen code RMNH.VER.19979 - RMNH.VER.21296.

Terminology of invasion: the case of land flatworms

Invasion biology is a dynamic and rapidly evolving discipline. Technical terminology has proliferated over the decades. Consistency in this terminology is important for clarity of interpretation and communication. The terms 'invasive', 'alien', 'non-native', 'exotic', 'imported' and 'introduced' are often used interchangeably. Confusion can occur when 'invasive' refers to species that have successfully established and spread to new areas, regardless of their impacts, or when it refers to species that cause ecological or socio-economic harm in their new environment regardless of the stage of the invasion process (Richardson et al. 2000; Leung et al. 2002; Blackburn et al. 2011; Lockwood et al. 2013; Soto et al. 2024).

Soto et al. (2024) propose a streamlined framework based on the terms (i) 'non-native', denoting species transported beyond their natural biogeographic range, (ii) 'established non-native', i.e. those non-native species that have established self-sustaining populations in their new locality(ies) in the wild, and (iii) 'invasive non-native' (or: 'invasive') – populations of established non-native species that have recently spread or are spreading rapidly in their invaded range actively or passively with or without human mediation (Soto et al. 2024). In this article, we adopt this standardized terminology.

Habitat designation is important to assess potential introduction pathways and the risk of land flatworms spreading across the non-native area. Given the lack of official differentiation in habitat designation of land flatworms we propose the following terminology on the type of environment in which these are found. The terminology focuses particularly on the available humidity:

- Outside in a natural area: climate, fauna and flora are all natural;
- Outside in a garden (private or public): environment is modified by humans, but the climate is almost natural; the difference with natural area includes watering in summer; control over flora and often also fauna;
- Inside in a plant nursery and garden center: even though the plants can be outside, the climate is modified by misting and watering in summer and measures against cold in winter; high control over flora and fauna;
- Inside in a greenhouse: the internal climate is disconnected from the surrounding natural climate; high control over flora and fauna; in this category belong also private terraria, something of the size of an aquarium, often with reptiles or amphibians.

Data on geographical distribution

In order to provide a comprehensive overview of the current distribution of studied non-native species of land flatworms, data from all previously published records of members of Geoplaninae and Bipaliinae in Europe (Moseley 1878; Scharff 1895; Jaenichen 1896; Van de Woestijne 1907; Arndt 1934; Pfitzner 1958; Marcus and Marcus 1959; Luther 1961; Sneli 1969; Kirkegaard 1971; Winsor 1983; Kosel 2002; Anderson 2003; Álvarez-Presas et al. 2014; Lago-Barcia et al. 2015; Solà et al. 2015; Aldred 2016; Carbayo et al. 2016; Mazza et al. 2016; Sluys et al. 2016; Justine et al. 2018; Lago-Barcia et al. 2019; Soors et al. 2019; Justine et al. 2020; Negrete et al. 2020; Čapka and Čejka 2021; De Waart et al. 2021; Kutschera and Ehnes 2021; De Waart 2022; European Commission Directorate-General for Environment 2022b; Justine et al. 2022a; Mori et al. 2022a, b; Rabitsch and Nehring 2022; Suárez et al. 2022; Álvarez-Presas et al. 2023; Mori et al. 2023; Cilia 2024; Glaw et al. 2024; Jones personal communication 2024; Lazányi et al. 2024; Ritter 2025; Bouguerche et al. (2025)) were combined with datasets of these species from GBIF.org (accessed between July 24th and March 2025). This method has shortcomings, which we explain in the Discussion paragraph.

For the Dutch distribution records of the non-native land flatworms the following datasets were combined and corrected where necessary (see also Fig. 1):

1. Specimens deposited in natural history collections;
2. Published records in scientific journals;
3. Citizen science platforms such as Waarneming.nl, iNaturalist.org and Observation.org;
4. GBIF.org (Global Biodiversity Information Facility);
5. Not yet published data from private databases or private collections.

This gives a more correct dataset, which was possible because the first author of the present study is the national coordinator of the land flatworm-working group in the Netherlands with species validation role.

Distribution maps were produced using the Google Maps API on Google Cloud Platform (GCP). Data collection corresponds to the stage of November 17th 2024.

Molecular barcoding and population genetics

The specimens of Geoplaninae and Bipaliinae from the Netherlands, and single specimens from Italy and Belgium, respectively, that have been used for molecular analyses, are listed in Table 1.

DNA extraction

First, a small piece of each individual was cut using a sterile scalpel at its anterior end (to avoid contamination with food items) and subsequently transferred to a 1.5 mL tube with ethanol 96%. The remaining part of the body was deposited in the triclads collection of Naturalis Biodiversity Center in Leiden, the Netherlands (see Table 1 for institution- and specimen codes). The whole genomic DNA was extracted using the following protocol based on Kmentová et al. (2021): TNES buffer (195 µL; 400 mM NaCl, 20 mM EDTA, 50 mM Tris (pH 8, 0.5% SDS)), and 5 µL of Thermo Scientific™ proteinase *K* (20 mg/mL) (Thermo Fisher Scientific, United States) were added to the sample. The incubation time varied between 3 hours to overnight at 55 °C. In case of the presence of indigestible debris, samples were short spun (until 7000 rpm) following transfer of the supernatant into a new 1.5 mL tube. Subsequently, 2 µL of Invitrogen™ yeast RNA (10 mg/mL) (Thermo Fisher Scientific) was added as a DNA carrier. Next, 65 µL of NaCl (5 M) and 290 µL of ethanol (96% EtOH) were added, and the sample was cooled for 60 min at −20 °C. Following the incubation period in the freezer, the sample was spun down for 15 min at 18,000 g to a small pellet. The supernatant was removed and substituted by 1 mL of chilled ethanol (70% EtOH). Next, the samples were centrifuged for 5 min at 18,000 g. The ethanol rinsing step (removal of supernatant, addition of ethanol and centrifugation) was repeated once. The ethanol was removed, and the DNA was eluted in 100 µL of 0.1× TE buffer (0.02% Thermo Scientific™ Tween 20 washing buffer). The DNA extract was placed at 4 °C for resuspension overnight, and stored at a temperature of −20 °C.

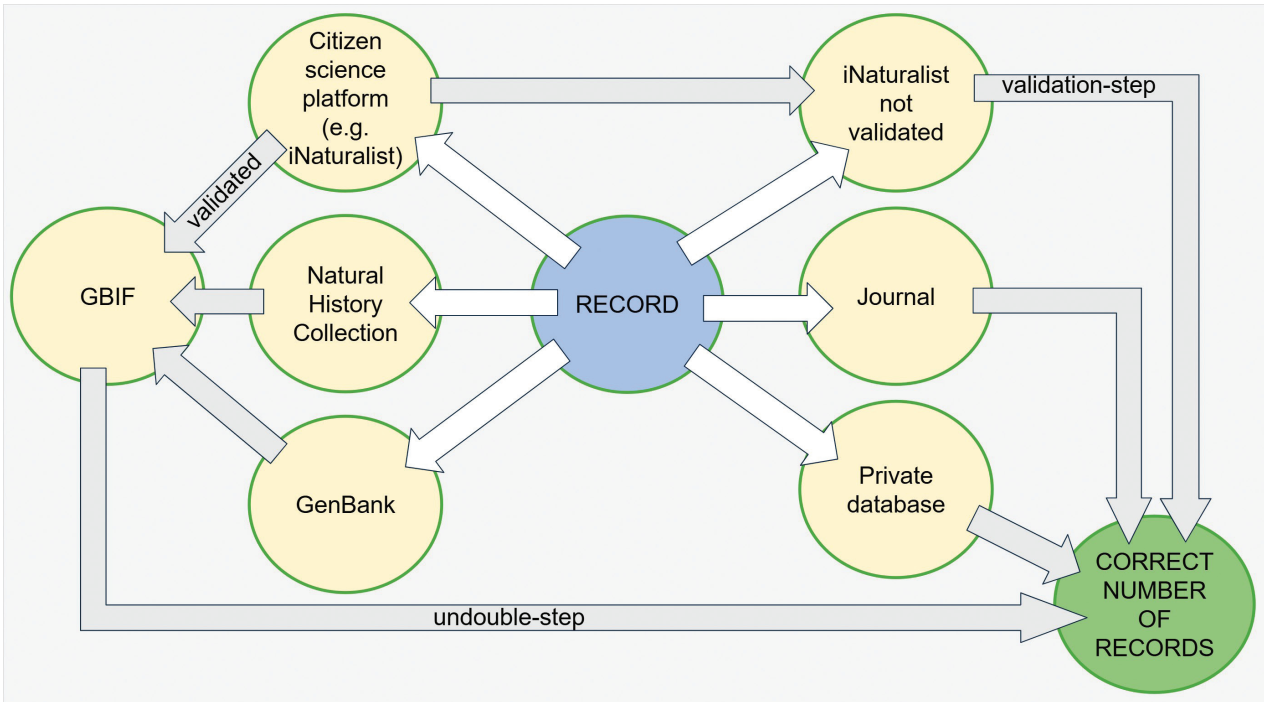

Figure 1. Graphical representation of data sources leading to a correct number of records.

Table 1. Specimens of Geoplaninae and Bipaliinae from the Netherlands, Italy and Belgium used for molecular analysis.

Species	Institution- and specimen code	GenBank accession numbers	Date	Locality	Habitat-type
Obama nungara	RMNH.VER.19979	PV537453	18.v.2021	Sinderen	garden center/ plant nursery
Obama nungara	RMNH.VER.19990	–	19.ix.2021	Hendrik-Ido-Ambacht	garden
Obama nungara	RMNH.VER.19998	–	8.xi.2021	Zeist	garden
Obama nungara	RMNH.VER.20001	PV537451	25.xi.2021	Emmen	greenhouse
Obama nungara	RMNH.VER.21291	PV544840	4.i.2022	Leidschendam	greenhouse
Obama nungara	RMNH.VER.20216	PV537456	28.vi.2022	Ridderkerk	garden
Obama nungara	RMNH.VER.21281	PV537458	7.i.2023	Melderslo	garden center/plant nursery
Obama anthropophila	RMNH.VER.21236	PV537458	7.i.2023	Melderslo	garden center/plant nursery
Obama nungara	RMNH.VER.21178	PV537460	1.iv.2023	Boskoop	garden center/plant nursery
Obama nungara	RMNH.VER.21194	PV537462	4.vi.2023	Wilhelminaoord	garden center/plant nursery
Obama nungara	RMNH.VER.20214	PV537455	24.iv.2022	Casinalbo, Italy	garden
Bipalium kewense	RMNH.VER.19999	PV537450	25.xi.2021	Emmen	greenhouse
Bipalium kewense	RMNH.VER.21185	PV537461	30.iv.2023	Meise, Belgium	greenhouse
Diversibipalium multilineatum	RMNH.VER.20201	PV537452	14.xii.2021	Aagtdorp	garden
Diversibipalium multilineatum	RMNH.VER.20205	PV537454	20.i.2022	Aagtdorp	garden
Diversibipalium multilineatum	RMNH.VER.20217	PV537457	21.vii.2022	Zwijndrecht	garden

Genetic characterization

The existing PCR protocols were followed in order to obtain sequences of a portion of the mitochondrial cytochrome *c* oxidase subunit 1 (COI mtDNA) gene as a commonly used barcoding marker for land flatworms. Part of the mitochondrial COI mtDNA gene was amplified using the primers ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') (Littlewood et al. 1997) combined with Asmit2 (5'-TAAAGAAAGAACATAATGAAAATG-3') (Littlewood et al. 1997), or the combination of primers BarS (forward 5'-GTTATGCCT-GTAATGATTG-3') (Álvarez-Presas et al. 2011) and COIR (reverse 5'-CCW-GTYARMCCHCCWAYAGTAAA-3') (Lázaro et al. 2009; Mateos et al. 2013). The amplification reaction contained 24 μL of PCR mix (12.5 μL of 2× u MangoMix (GC Biotech), 0.5 μL of 5 mM $MgCl_2$, 1 μL of each primer at 10 μM, 9 μL of double distilled (dd) H_2O with 1 μL of isolated DNA (concentration was not measured) in a total reaction volume of 25 μL. The amplification was performed under the following conditions: initial denaturation at 94 °C for 4 min, 40 cycles of 30 sec at 94 °C, 40 sec at 48 °C and 50 sec at 72 °C, and a final extension of 7 min at 72 °C. PCR amplification success was verified by agarose gel electrophoresis. A volume of 10 μL was purified using the GeneJET extraction kit (Fisher Scientific) following the manufacturer's instructions. Purified PCR products were sent out to Macrogen Europe B.V. for Sanger sequencing; amplification primers were used for sequencing reactions. The acquired sequences for each marker were visually inspected and trimmed using the software Geneious Prime v2023.2.1.

Intraspecific genetic variation

Open reading frames of the COI mtDNA sequences were visualised and their protein translation was checked using the flatworm mitochondrial code in Geneious Prime v2023.2.1. Notably, previously published sequences of *D. multilineatum* (GenBank accession numbers FR989851-4) were corrected as the insertion of triple N appeared artificial in the alignment. The intraspecific genetic variation was characterized as max. uncorrected p-distances (%). The sequences available from both native and non-native ranges of each species were included in the respective analysis. Given the scarcity of available sequences for other species, population-level analyses were performed only for *O. nungara* and were based on the most recently published results of validated records (Justine et al. 2022b). Haplotype networks were constructed using a Median-joining algorithm as implemented in PopArt v1.7 (www.popart.otago.ac.nz). Results of two different datasets were compared: a) the complete alignment of all available COI mtDNA sequences of *O. nungara* (n = 93, 1,737 bp) and b) a subset of available COI mtDNA sequences of *O. nungara* (n = 83, 441 bp) minimizing the number of gaps and including variable positions otherwise masked during the construction of haplotypes network as gaps in the dataset (a). The number of haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity were calculated in DnaSP v6 (Rozas et al. 2017). Accession numbers of the previously published COI mtDNA sequences of the land flatworm species used in the comparative analyses are presented in Suppl. material 3. Newly acquired COI mtDNA sequences were submitted to the NCBI GenBank online database under the following institution- and specimen codes RMNH.VER.19979 - RMNH.VER.21296 (see also Table 1).

Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility, and are available at https://doi.org/10.15468/ggfbvv.

Results

Temporal and spatial distribution of non-native land flatworms in the Netherlands

In total, there were 89 records of species of Geoplaninae and 12 records of species of Bipaliinae in the Netherlands with most specimens being found in gardens (see Table 2 and Suppl. material 1). From these records, 24 individuals of Geoplaninae were collected and three individuals of Bipaliinae.

It is noteworthy that out of the 101 records of non-native flatworms of Geoplaninae and Bipaliinae in the Netherlands, none were so far found in natural areas.

Species of Geoplaninae

Morphological determination through the external appearance of eight specimens collected as part of the present study, accompanied by genetic barcoding revealed the presence of two species of Geoplaninae in the Netherlands. Our specimens matched the following characteristics, described in Carbayo et al. (2016). *Obama nungara* is a large (50 to 70 mm), broad and leaf-shaped land flatworm. Eyes are placed in a single row around the leading edge and in multiple rows further back along the side of the animal. The dorsal surface is marbled light to dark brown, sometimes almost black, and provided with longitudinal black striae (see Fig. 2A). A pale midline may be present due to the absence of these stripes (Carbayo et al. 2016). *Obama anthropophila* Amaral, Leal-Zanchet & Carbayo, 2015 is described in Álvarez-Presas et al. (2015) as a large (up to 70 mm), broad and leaf-shaped land flatworm. The back is evenly brown (see Fig. 2B), slightly lighter towards the sides, the belly is light brown. Eyes are placed in a single row around the leading edge and in multiple rows further back along the side of the animal (Álvarez-Presas et al. 2015). Our specimen matched these characteristics, but only after the outcome of the DNA-analysis could we confirm the characteristics as seen on the picture we took.

According to Carbayo et al. (2016) and Soors et al. (2019) the marbling in dark specimens of *O. nungara* is only visible under good light conditions. Correct species determination of our records from some of the photos of the citizen science-records was therefore not always possible. These land flatworms can belong to another species, like *O. anthropophila* or even be a member of another genus like *Amaga pseudobama* (Justine et al. 2024a). They are indicated as '*Obama* sp'. in this paper.

The present study provides the first report of *O. anthropophila* in Europe, and outside its native range in Brazil. This specimen was found in a greenhouse, but there is no information if plants from this greenhouse were imported from Brazil. An overview of the distribution of *O. nungara* and *O. anthropophila* in the Netherlands is presented in Fig. 3. Only a single specimen of *O. anthropophila* has been found in the Netherlands while 334 specimens of *O. nungara* have been found in 79 Dutch localities so far.

The first record of *O. nungara* in the Netherlands is dated back to 2020 in a garden center in Gilze, found by a garden center employee (De Waart et al. 2021). In the following years there has been a large increase in the records of this non-native land flatworm in the Netherlands (see Fig. 4). Most of them were found in gardens; other habitats were greenhouses and garden centers/plant nurseries (see Table 2 and details in Suppl. material 1). Moreover, 93% of the records came from citizen scientists.

Table 2. Number of records of representatives of Geoplaninae and Bipaliinae in the Netherlands with the designation of habitat types. The data presented in this table are based on literature search, and on data presented in this article.

Habitat type	*O. nungara*	*O. anthropophila*	*Obama* sp.	*B. kewense*	*D. multilineatum*	Total
Garden	59[1)]	–	4[1)]	–	5[1)3)]	68
Garden center/ plant nursery	23[1)2)]	1[1)]	–	–	–	24
Greenhouse	2[1)]	–	–	7[1)]	–	9
Total	84	1	4	7	5	101

1) this article; 2) De Waart et al. 2021; 3) De Waart et al. 2022.

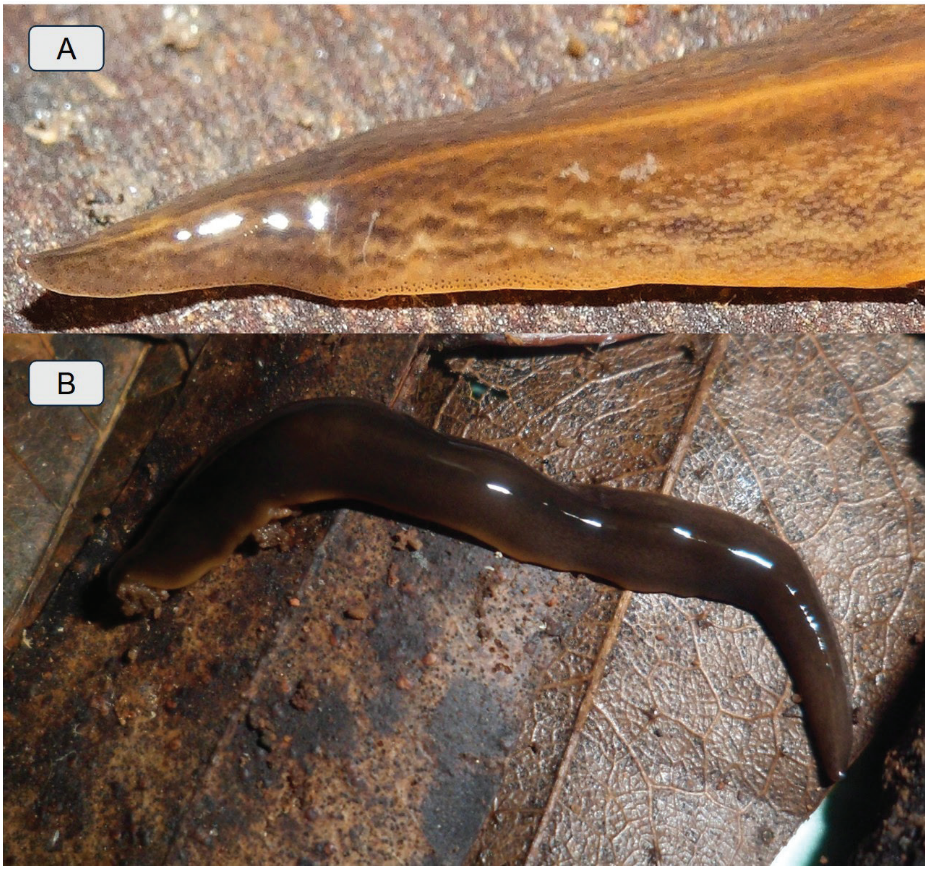

Figure 2. A detail of *Obama nungara*, found in Brazil showing the anterior end. Scale bar not available, but adult specimens can reach a length of 5 to 7 cm (Carbayo et al. 2016). Photo by Piter Kehoma Boll, CCBY-SA 4.0 **B** *Obama anthropophila*, found in Brazil. Photo by Piter Kehoma Boll, CCBY-SA 4.0. Scale bar not available, but adult specimens can reach a length of 7 cm (Álvarez-Presas et al. 2015).

Species of Bipaliinae

Two species of Bipaliinae have been found in the Netherlands to date. Our specimens matched the following characteristics, described in Moseley (1878) and Winsor (1983). *Bipalium kewense* is a species of land flatworms whose body shape is characterized by a typical "hammerhead" (see Fig. 5A). Living specimens may attain a length of up to 450 mm. The dorsal ground color is usually a light ocher, with five black to gray-brown-colored longitudinal stripes. The paired lateral and marginal stripes unite just behind the neck to form an incomplete black transverse neck band, interrupted dorsally by a small median gap, and ventrally by the creeping sole. The ventral surface is a light ocher color, with a distinct off-white creeping sole, delineated by paired, narrow, longitudinal diffuse gray-violet stripes (Moseley 1878; Winsor

Figure 3. Map with records of *Obama nungara* and *Obama anthropophila* in the Netherlands.

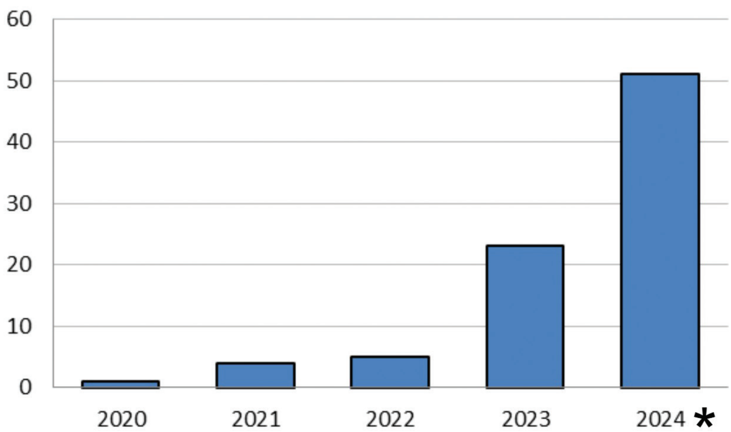

Figure 4. Number of records of *Obama nungara* in the Netherlands (from 2020 to November 17th 2024).* 2024 is not a full year, but only up to November 17th.

1983). *Diversibipalium multilineatum* (Makino & Shirasawa, 1983) is also a member of the hammerheads (see Fig. 5B). Our specimens matched the following characteristics, described in Makino and Shirasawa (1983). Living specimens may attain a length of up to 210 mm. The dorsal ground color is ocher with five black to dark brown dorsal stripes, the middle of which extends over the characteristic triangular

Figure 5. **A** *Bipalium kewense*, found in France. Scale bar not available, but adult specimens can reach a length of 45 cm (Winsor 1983). Photo by Pierre Gros, CC BY-SA 4.0 **B** *Diversibipalium multilineatum*, found in the Netherlands. Scale bar not available, but adult specimens can reach a length of 21 cm (Makino and Shirasawa 1983). Photo by Roy Kleukers.

hammer head. Characteristic of this hammerhead is that the start of the middle dorsal stripe on the head is somewhat thickened. The ventral side is lighter colored with three longitudinal stripes (Makino and Shirasawa 1983). *Bipalium kewense* Moseley, 1878 has been reported in five localities in the Netherlands, and *Diversibipalium multilineatum* has been found in two localities in the Netherlands (see Fig. 6).

The first record of *B. kewense* in the Netherlands is dated back to 1912 in a greenhouse of the Botanical Garden in Amsterdam (Thunnissen et al. 2022). Following the initial record, this species has been found in a tropical greenhouse of two zoos, and in greenhouses of two other botanical gardens (for details see Suppl. material 1). Not all collectors are registered, and of the registered collectors we do not know if they are citizen scientists or professional experts.

The first record of *D. multilineatum* in the Netherlands is dated back to 2021 in a garden in Aagtdorp followed by the second record in 2022 in a garden in Zwijndrecht (De Waart 2022) (see Fig. 6). The species has been found at the same locality in Aagtdorp in three consecutive years (2021, 2022, 2023) and in Zwijndrecht at the same locality again in 2024 (present study). All records were reported by citizen scientists.

Figure 6. Map with records of *Bipalium kewense* and *Diversibipalium multilineatum* in the Netherlands.

Temporal distribution in Europe

In total, 6 species of Geoplaninae and Bipaliinae have been so far reported in 25 European countries, see overview in Table 3.

Species of Bipaliinae

In total, four species of Bipaliinae have been found in Europe to date: *B. kewense*, *B. vagum*, *D. multilineatum* and *V. covidum*. Our literature search shows that these four hammerheads have been found in 23 European countries in total with the most records present in France (216) and Italy (89) (see Table 4, Figs 7, 8 and Suppl. material 3). *Bipalium kewense* appears to be the most successful hammerhead invader in Europe with 345 records from 18 countries with the first record dated back to 1862 in a greenhouse in Copenhagen, Denmark (GBIF.org 2024a). We have left out the record from the greenhouse of the botanical garden in Graz (Austria) from 1899 (Arndt 1934) since the record was subsequently marked as misidentification of *Dolichoplana feildeni* Graff, 1899 by Winsor (1983). The other three hammerhead species beside *B. kewense* have been, to date, only found in a handful of European countries, and only as of 2010 (see Table 4).

Table 3. Overview of all records of non-native bipaliin and geoplanin land flatworms in Europe from 1862 (oldest record of *Bipalium kewense*) till November 2024. See Supplement C for references.

Species	Number of European countries	Year of the first record	Total number of records in Europe
Bipalium kewense	18	1862 (GBIF.org 2024a)	345
Obama nungara	19	2008 (Carbayo et al. 2016)	1845
Diversibipalium multilineatum	10	2010 (Justine et al. 2018)	143
Vermiviatum covidum	2	2013 (Justine et al. 2022a)	6
Bipalium vagum	2	2014 (Mori et al. 2022a)	5
Obama anthropophila	1	2023 (this article)	3

Table 4. Records of members of Bipaliinae in Europe since 1862 (oldest record of *Bipalium kewense*) divided by country.

	Bipalium kewense		*Diversibipalium multilineatum*		*Bipalium vagum*		*Vermiviatum covidum*	
Country	**N. of records**	**Year of the first record**	**N. of records**	**Year of the first record**	**N. of records**	**Year of first record**	**N. of records**	**Year of first record**
France	148	1999 (GBIF.org 2024b)	64	2010 (Justine et al. 2018)	–	–	4	2013
Italy	37	2010 (Mori et al. 2022a)	46	2014 (Mazza et al. 2016)	4	2014 (Mori et al. 2022a)	2	2014
Spain	31	1983 (Álvarez-Presas et al. 2014)	–	–	–	–	–	–
Germany	31	1886 (Arndt 1934)	–	–	–	2024 (GBIF.org 2025)	–	–
Portugal	26	1895 (Marcus and Marcus 1959)	–	–	–	–	–	–
UK	20	1877 (Moseley 1878)	2	2021 (GBIF.org 2024d)	–	–	–	–
Netherlands	15	1912 (this article)	14	2021 (De Waart 2022)	–	–	–	–
Switzerland			10	2016 (Justine et al. 2018)	–	–	–	–
Belgium	11	1906 (Van de Woestijne 1907)	2	2024 (GBIF.org 2024e)	–	–	–	–
Finland	6	1935 (GBIF.org 2024c)	–	–	–	–	–	–
Poland	6	1898 (Arndt 1934)	–	–	–	–	–	–
Ireland	4	1892 (Winsor 1983)	–	–	–	–	–	–
Denmark	3	1862 (GBIF.org 2024a)	–	–	–	–	–	–
Norway	2	1969 (Sneli 1969)	–	–	–	–	–	–
Sweden	2	2009 (GBIF.org 2024f)	–	–	–	–	–	–
Croatia	–	–	2	2022 (Mori et al. 2023)	–	–	–	–
Slovenia	–	–	2	2023 (Mori et al. 2023)	–	–	–	–
Czech Republic	1	1903 (Kosel 2002)	–	–	–	–	–	–
Malta	1	(European Commission: Directorate-General for Environment 2022b)	–	–	–	–	–	–
Monaco	1	2014 (Justine et al. 2018)	–	–	–	–	–	–
Slovakia	1	1997 (Kosel 2002)	–	–	–	–	–	–
Austria	–	–	1	(Ritter 2025)	–	–	–	–
Hungary			1	2023 (Lazányi et al. 2024)	–	–	–	–

The most northern outside record (in gardens or in natural areas) of *B. kewense* to date in continental Europe is in Monéteau, France (see Fig. 8) (GPS coordinates 47.8; 3.6). At roughly the same latitude *B. kewense* has been found in greenhouses in France (Auxerre, GPS coordinates 47.8; 3.6) (Justine et al. 2018) and Slovakia (GPS coordinates 48.1; 17.1) (Kosel 2002). Only four records of *D. multilineatum* are from greenhouses (two in France, one in Italy and one in Hungary). The other 140 records were found outside in gardens or natural areas: even the most northern records in The Netherlands were from gardens, even in the winter; in Austria and Switzerland this species has been found outside (Ritter 2025; personal observation Justine). The European records from *B. vagum* in the north of Italy are all from gardens. The record from Germany is from a greenhouse. The European records of *V. covidum* in the middle and south of Italy and the south of France are all from gardens.

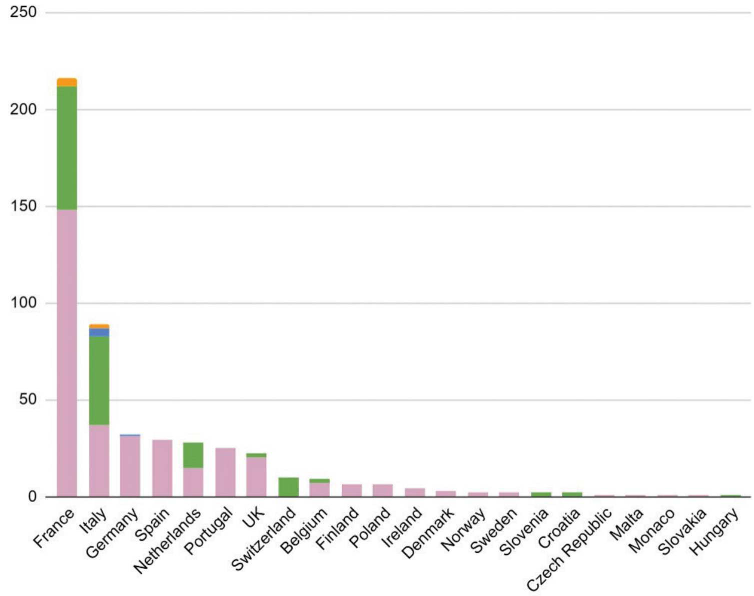

Figure 7. Overview of cumulative records of *Bipalium kewense* (in purple), *Diversibipalium multilineatum* (in green), *Vermiviatum covidum* (in orange) and *Bipalium vagum* (in blue) in Europe between 1862 (the oldest record of *B. kewense* in a greenhouse in Copenhagen) and November 2024.

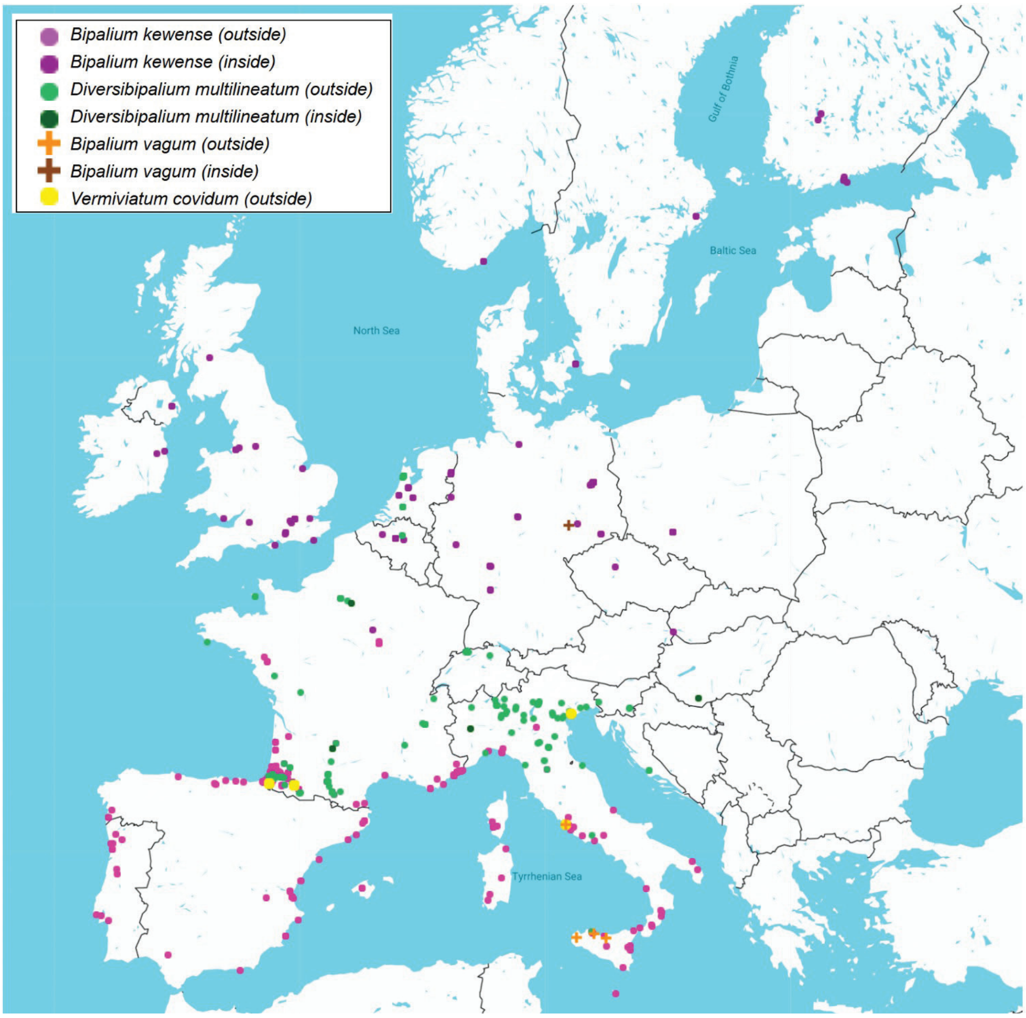

Figure 8. Map with records of species of Bipaliinae in Europe.

Species of Geoplaninae

The present study provides the first record of *O. anthropophila* in Europe. Our literature search shows that *O. nungara* has been found in 19 European countries so far. The first record of this species dates back to 2008 in Guernsey (UK) (Carbayo et al. 2016) with the highest total number of records present in France (1.428 records). See Table 5, Fig. 9 and Suppl. material 3 for details. The map in Fig. 9 shows that Sweden is the northern boundary for *O. nungara* in continental Europe.

Barcoding results and intraspecific genetic variation

In total, 14 COI mtDNA sequences were generated from two specimens of *B. kewense*, three specimens of *D. multilineatum*, eight specimens of *O. nungara* and a single specimen of *O. anthropophila* recovered from 11 locations in the Netherlands, one specimen of *O. nungara* from Italy and one specimen of *B. kewense* from Belgium (see Table 1).

The highest observed intraspecific genetic variation (%) in the COI mtDNA region differed between the land flatworm species analyzed in this study. The alignment of *B. kewense* comprises 25 sequenced individuals (including previously

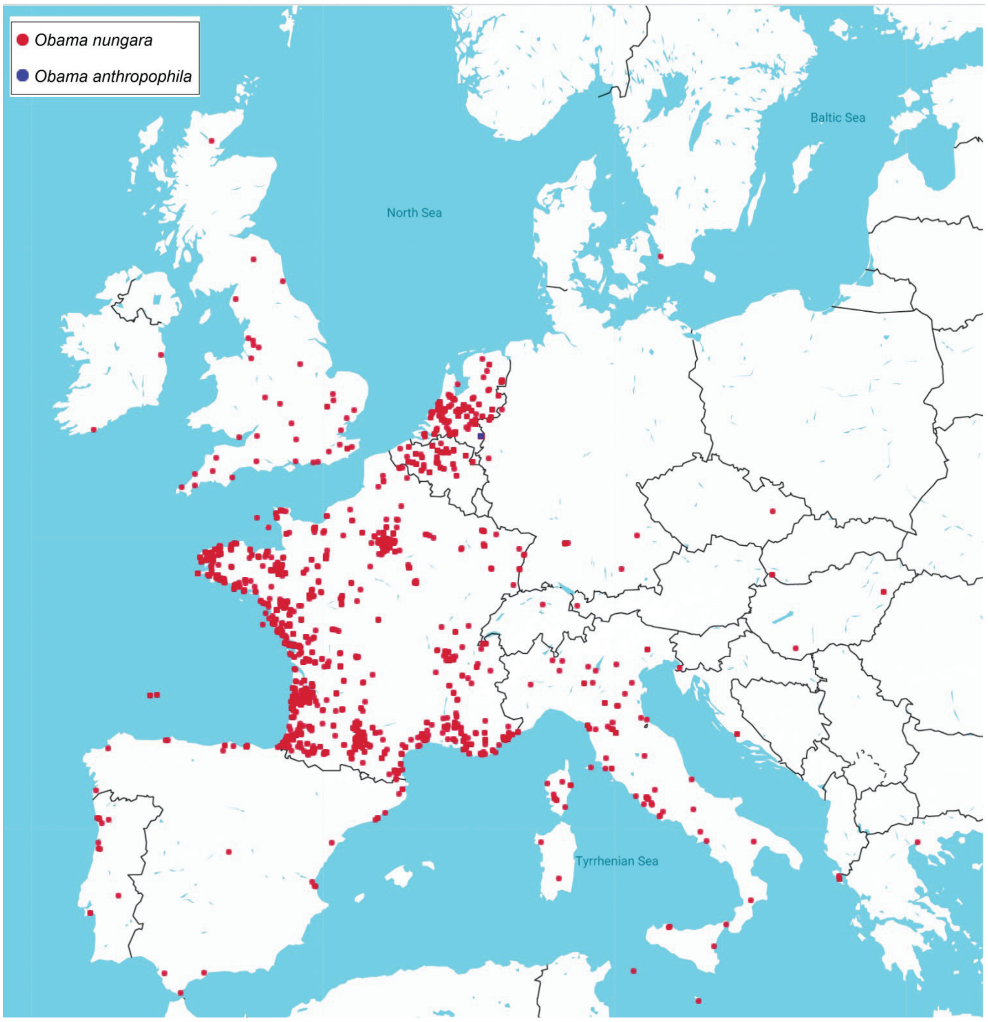

Figure 9. Map with records of species of Geoplaninae in Europe.

Table 5. Records of *Obama nungara* in Europe since 2008, divided by country.

Country	Number of records	Year of first record
France	1.428	2013 (Justine et al. 2020)
Netherlands	149	2020 (De Waart et al. 2021)
Belgium	68	2017 (Soors et al. 2019)
Italy	64	2012 (Carbayo et al. 2016)
UK	40	2008 (Carbayo et al. 2016)
Portugal	39	2014 (Lago-Barcia et al. 2019)
Spain	29	2010 (Solà et al. 2015)
Germany	7	2020 (GBIF.org 2024g)
Greece	4	2022 (GBIF.org 2024h)
Switzerland	4	2014 (Justine et al. 2020)
Croatia	2	2021 (Mori et al. 2023)
Hungary	2	2024 (Lazányi et al. 2024)
Ireland	2	2009 (Justine et al. 2020)
Slovakia	2	2021 (Čapka and Čejka 2021)
Czech Republic	1	2022 (GBIF.org 2024i)
Austria	1	2024 (Glaw et al. 2024)
Malta	1	2023 (Cilia 2024)
Slovenia	1	2023 (Mori et al. 2023)
Sweden	1	2024 (Bouguerche et al. 2025)
Total number of records	1.845	

published and newly generated sequences) and constituted 903 bp with a max. pairwise distance of 0.83% including four variable positions. A single variable position was observed in the alignment (930 bp) of *D. multilineatum* accounting for a max. pairwise distance of 0.14% comprising sequences of 16 individuals. In the case of *O. anthropophila*, the alignment constituted 882 bp and the sequence generated as part of the present study differed in four nucleotide positions with the previously sequenced individuals (n = 12) accounting for 1.20% and the overlapping positions of 333 bp. The total alignment of the COI mtDNA region of *O. nungara* consisted of 1,737 bp with a max. pairwise distance of 6.20%.

New sequences for COI mtDNA were obtained from seven individuals of *O. nungara* from the Netherlands, comprising two different haplotypes and containing a single polymorphic site with a maximum sequence length of 441 bp. Overall, the dataset (a) (n = 93, 1,737 bp) comprised 17 different haplotypes with 21 polymorphic sites. Haplotype and nucleotide diversity of the dataset (a) was estimated at 0.732+-0.041 and 0.01374, respectively. In the case of dataset (b) (n = 83, 441 bp), 19 different haplotypes with 23 polymorphic sites were reported. Haplotype and nucleotide diversity of the dataset (b) was estimated at 0.809+-0.034 and 0.01068, respectively. The haplotype networks of *O. nungara* of both datasets show a core-satellite structure with three central haplotypes. The position of newly generated sequences in respect to the ones reported in the native areas, which are Argentina and Brazil (Carbayo et al. 2016), and other European countries (non-native areas of distribution) suggest an Argentinian origin of both lineages/haplotypes found in the Netherlands. Moreover, analyses of the subset of available COI mtDNA sequences presented in the dataset (b) revealed presence of a rare haplotype in the Netherlands shared with a single specimen of *O. nungara* recovered from France and Italy, respectively.

Discussion

The Netherlands: gateway of horticulture to the rest of Europe

Contamination of containerised potted plants in the international potted plant trade was identified as one of the main introduction pathways of land flatworms; first records of these non-native flatworms often originate from nurseries and botanic gardens (Blackshaw and Stewart 1992; Álvarez-Presas et al. 2014; Sluys et al. 2016). The trade of ornamental plants and soil as an invasion pathway creates an enormous reservoir of living soil microorganisms such as nematodes which remain undetected (Smith et al. 2007; Kenis et al. 2009; Bradley et al. 2011; Santini et al. 2012). The commercial plant trade was also identified as the main source of invasion and subsequent spread of *A. triangulatus* in Scotland and the UK (Boag and Neilson 2014), being the single species of flatworm on the List of Invasive Species of EU concern. The USDA Foreign Agricultural Service puts the Netherlands as the number one exporter of floriculture: cut flowers, bulbs and live plants, with about a 50 percent share of the global market. The trade mostly takes place between European countries with the Netherlands having a particularly high role. Unfortunately, the import origin of these plants to Europe is hardly traceable (USDA Foreign Agriculture Service 2016). In 2021 the Netherlands imported 54,7 million kilograms of live outdoor plants, mostly from other European countries (54,4 million kilograms). In the same year the Netherlands exported 295 million kilograms of live outdoor plants, also mostly to other European countries (291 million kilograms) (CBS Statline 2022).

Overall higher ratio of export versus import (5×) reported in the commercial potted plant trade highlights the importance of the Netherlands as a potential nursery ground and gateway for soil-born non-native taxa including flatworms. So far no research has been done into this mechanism, but given the fact that the Netherlands is the world leader in exporting floriculture, it might play an important role in spreading land flatworms in Europe but also worldwide. Monitoring land flatworms in the Netherlands is thus important to prevent them spreading to other (European) countries. Plant material (potted plants, vegetables and fruit) imported to the Netherlands is randomly checked by the NVWA (The Netherlands Food and Consumer Product Safety Authority) for non-native exotic species such as nematodes (Nederlandse Voedsel- en Warenautoriteit nd). The Dutch government (and other European countries like Belgium) are exploring methods to monitor and detect land flatworms during import controls, in which molecular diagnostics (DNA-analyses) play an important role (Movares 2022).

Negative impact of the particular non-native land flatworms in this study

Land flatworms are predators of soil organisms. The introduction of non-native land flatworms can have a negative impact on native species like snails, and diminish soil fertility by reducing earthworm numbers (Sluys 2016).

The focus in the current study is on species of Bipaliinae and Geoplaninae. Two species of these subfamilies (*B. kewense* and *O. nungara*) are currently under investigation to be added to the list of non-native alien species of Union Concern (the Union list) under the EU IAS Regulation. This list includes a selection of species that cause the most damage to native biodiversity, and for which concerted measures are required across the EU (European Commission 2022a). Both species have no known natural enemies or means of control other than phytosanitary measures. The motivation to add *B. kewense* to this list, is that this species is the most widely dispersed flatworm species in the world. Although no specific deleterious consequences of this species are known, deleterious impacts on soil invertebrates are not as readily observed as above-ground impacts and it remains possible that *B. kewense* predation could have unforeseen effects on soil fauna. Motivation to add *O. nungara* to this list, is that this species has established itself in Europe and is spreading. There is a high risk that *O. nungara* could impact biodiversity in a similar way to the non-native land flatworm *A. triangulatus* and deplete native/endemic snail and earthworm populations (European Commission 2022b).

Bipalium kewense: from indoors to outdoors

The first record of *B. kewense* in Europe is dated to 1862 from a greenhouse in Denmark (GBIF.org 2024a), but the species was described from a specimen found in 1877 in a greenhouse in the United Kingdom (Moseley 1878). After that specimens were found in greenhouses in Belgium (Van de Woestijne 1907), Germany (Arndt 1934), Poland (Arndt 1934), Portugal (Marcus and Marcus 1959), Ireland (Winsor 1983) and Czech Republic (Kosel 2002); the first records in the Netherlands are provided here.

According to a pan-European study from 2022 on the spread of three species of non-native land flatworms in southern European countries, *B. kewense* has been found in private gardens in France, Italy, Portugal, Malta and Spain. In the following northern countries *B. kewense* has been only found in greenhouses so far: Belgium, Czech Republic, Denmark, Finland, Germany, Ireland, the Netherlands, Poland, Slovakia and the United Kingdom (European Commission 2022a; Jones personal communication 2024). In the present study, we update the record lists of *B. kewense* with specimens found in greenhouses in Norway (Sneli 1969) and Sweden (GBIF.org 2024f) (see overview in Fig. 8, Tables 3, 4 and Suppl. material 3). With global warming this distribution pattern might change in the coming decades (Thunnissen et al. 2022).

A modeling study has been done on the future spread of five hammerhead species based on several climate change models (Fourcade et al. 2022). Precipitation and minimum temperature were the most important environmental variables predicting the distribution of these hammerhead species. Overall, all five species had higher suitability in the current climate than their present distribution, among which western Europe, but only small areas were suitable for two or more species. Measures of overlap revealed that *D. multilineatum* and *B. kewense* were the most dissimilar in their environmental demands (Fourcade et al. 2022). This does not, however, correspond with our findings, where *D. multilineatum* and *B. kewense* do appear in the same area, outside in gardens or in natural areas, in the southern part of Europe (see Fig. 8). So far three other species of Bipaliinae besides *B. kewense* have been found in Europe. Their history is much shorter: as of 2010, and their European distribution much more limited than that of *B. kewense* (see Table 4 for an overview). Bipaliin species originate from Southeast Asia and Madagascar, but some species like *Bipalium vagum* Jones & Sterrer, 2005 and *Vermiviatum covidum* (Justine, Gastineau, Gros, Gey, Ruzzier, Charles & Winsor, 2022) have only been found as non-native species in other countries, with the area of origin remaining unknown (Winsor 1983; Jones and Sterrer

2005; Mazza et al. 2016; Justine et al. 2022a). Of these three hammerheads beside *B. kewense*, *D. multilineatum* seems to have the highest potential to establish as it has adapted to the cold weather in Northern Europe more successfully than *B. kewense*: the Netherlands is the most northern boundary for *D. multilineatum*, where it has been found at two locations in gardens several years in a row.

Rapid rise of *O. nungara* and the first report of *O. anthropophila* in Europe

France and the Netherlands are the countries with the most records of specimens of Geoplaninae in Europe. The Neotropical region is the native range of geoplanin species (Ogren and Kawakatsu 1990; Ogren et al. 1992; Negrete et al. 2020) which spans from central Mexico and the Caribbean islands to southern South America (Ricardo and Novillo 2024). The first specimen of *O. nungara* found in Europe was in 2008 in Guernsey (UK) (Carbayo et al. 2016). Since then there has been a rapid increase in the number of findings in some European countries like France (1.428), the Netherlands (149), Italy (64), Belgium (68) and the UK (40). Until 2020 there were no records of *O. nungara* in Germany or any European country east of Germany. Recently, specimens of *O. nungara* were recorded in the Czech Republic, Hungary, Slovenia, Poland and Austria. In the southern part of Sweden (Malmö) specimens of *O. nungara* were recently reported as being imported with potted plants from Germany (Bouguerche et al. 2025). In other Scandinavian countries (Denmark, Norway and Finland) so far *O. nungara* has not yet been found (see Table 5 and Suppl. material 3). The reason behind this discrepancy in distribution is unclear. The influence of the climate, less import of potted plants, or geographical/cultural biases as observed by Justine et al. (2020) were suggested as potential players. Also the influence of the media on the number of records by citizen scientists is enormous (Justine et al. 2024b).

In the Netherlands, most specimens of *O. nungara* have been found in private gardens by citizens (59 of the 84 records). The expert-findings originate mostly from garden centers, plant nurseries and greenhouses. This is a common discrepancy found in the reporting history of non-native land flatworms as suggested in previous studies conducted in the Netherlands (Thunnissen et al. 2020; De Waart and Thunnissen 2023).

The stage of invasion is measured by the level of establishment of the invader in the non-native area. In Europe *O. nungara* is considered as established: since the first record in 2008 in Guernsey (UK), the species has rapidly spread over 18 other European countries with up to a hundred records every year, as shown in Fig. 11. There are more records than presented here, especially in France for the last few years (Justine personal observation). These records can be found in the INPN data (https://inpn.mnhn.fr) but there is some delay in uploading them to GBIF (as highlighted in the methods section).

The preponderance of records in France, adding to the high numbers in 2014 and 2018, is artificial and originates in efforts in communication towards the public which have not been done in countries where flatworms are probably as abundant (i.e. Spain and Italy). Justine et al. (2020) noted that waves of records were received within days of certain media reports featuring their research (either radio, television or newspapers) in 2014 and 2018. These temporal biases occur when more reports are received at certain times of the year, independent of the actual abundance of the species. This creates what Justine et al. (2024b) call a type of virtuous circle: the press releases about a scientific paper create media-attention, which results in more records and specimens from citizen science. Another scientific paper follows, which creates media attention etcetera. In other words: communication with the public is the means of obtaining more data (Justine et al. 2024b).

The facts that support the establishment status of *O. nungara* in the Netherlands are that (1) there has a rapid increase over the last five years in records; (2) at five localities they have been found in consecutive years (see Suppl. material 1: tables S9, S15, S19, S27, S37) and (3) five records were made during the winter months (November till February). On the contrary, this status is not supported by (1) presence only in the urban environment (gardens, garden centers/plant nurseries, greenhouses), not in natural areas and (2) lack of persistence in some of the revisited localities across the timeframe of several years. Therefore, a possibility of ongoing introduction events might explain the current situation of *O. nungara* in the Netherlands and in Europe in general. The presence of established populations in nurseries and subsequent recurrent invasions of the surrounding gardens with the purchase of pot plants is proposed as an alternative or co-occurring scenario.

In the present study, we also provide the first report of *O. anthropophila* in Europe. This species originates in Brazil and has not been previously found outside its native distribution. In Brazil it was mainly found in anthropogenically impacted areas, as its name indicates, and on the border of remnants of Brazilian rainforests (Álvarez-Presas et al. 2015; GBIF.org).

Land flatworms have strict ecological requirements concerning humidity and temperature (Sluys 1999). Suitable habitats for *O. nungara* can be deducted by the climatological parameters of its natural occurrence, and climatological parameters of invaded areas where it can survive outside in gardens and natural areas (Fourcade 2021). The main predictor for the ecological niche of *O. nungara* is the minimum temperature of the coldest month. The optimal climatic conditions correspond to temperatures that remain above 0 in winter, but not too hot either (optimal minimum temperature around 5–12 °C). The second most important variable is the maximum temperature of the warmest month. Summer temperature for *O. nungara* must remain below 28 °C. Precipitation in summer and soil pH play a limited role but can be summarized as follows: summer precipitation not less than 50 mm/month, and pH not too acid (Fourcade personal communication). Daily monitoring in a four-year survey of *O. nungara* in a French garden confirmed this: population growth is favored by mild winters and precipitation, while it is disadvantaged by drought. The research also revealed that populations of *O. nungara* are able to recover from a period of severe drought (Noel et al. 2025). Incorporation of climate change models predicted an increase of suitable areas for *O. nungara* globally in current and future climate, including Africa and a larger part of Central and South America (Fourcade 2021). *Obama nungara* has already been found in Africa at the island of Réunion (Justine et al. 2022b). To date, the south of Sweden is the most northern place in continental Europe with a suitable climate for *O. nungara*. The milder winters on the British Isles compared with continental climate of a similar latitude (Royal Meteorological Society 2024) are also suitable for the presence of this species. Due to climate change, the spread of *O. nungara* and potentially also other land flatworms with similar environmental preferences towards the northern areas has been predicted (Fourcade 2021).

Genetic variation and origin of *Obama nungara* in Europe

So far, only few plant host-alien flatworm species co-introduction pathways have been traced e.g. tree ferns belonging to *Dicksonia antarctica* Labill 1806 imported from Australia and flatworm species of *Fletchamia* (Matthews 2005; Cannon and Baker 2007). Population genetic analyses have been used as an approach to trace the origin of species outside their native range potentially to be linked with concrete routes of introduction. In practical terms, results of genetic tracing of introduction pathways can facilitate the design of strategies for controlling or preventing invasions (Estoup and Guillemaud 2010). Among the non-native land flatworms included in the present study, *O. nungara* is represented by the largest number of genetic barcodes and shows the highest level of intraspecific genetic variation. The existence of different clusters with close genetic similarity to both native countries of origin with available data and specimens retrieved from Europe (see Fig. 10) suggest multiple introduction events (Justine et al. 2022b). In particular, specimens retrieved from the Netherlands cluster together with the individuals from other European countries and Argentina. The introduction pathway of *O. nungara* to Europe is unknown, but given the higher haplotype diversity recovered from Spain (Lago-Barcia et al. 2019; Justine et al. 2020) and their similarity to haplotypes collected in Argentina being part of the native range (see Fig. 10), Spain is suggested as a gateway of *O. nungara* in Europe. However, given the scarcity of genetic information available from the native range, alternative scenarios cannot be excluded. Primary entry of *O. nungara* into Europe might have been via direct trade between Argentina and Spain (Justine personal observation), but given the previously postulated link between international potted plant trade and invasion of non-native land flatworms in Europe (Blackshaw and Stewart 1992; Álvarez-Presas et al. 2014; Sluys et al. 2016), and the above mentioned importance of the Netherlands in the import of horticulture products, the Netherlands is most likely now an important gateway of non-native land flatworms in Europe. Given the absence of genetic barcodes from their native range of distribution, correct habitat designation is a key method to track establishment of non-native flatworm species currently present in Europe.

Importance of citizen science and museum collections

Records from citizen scientists provide an important source to monitor the distribution of non-native species, as illustrated by the fact that in the Netherlands 93% of the records of *O. nungara* originate from citizen scientists. Moreover, participation of the public in research and management boosts awareness, engagement and scientific literacy and can reduce conflict in Invasive Alien Species-management (Price-Jones et al. 2022).

Nature observation-platforms such as iNaturalist.org and Observation.org are a preferred way to monitor species records from citizens (Kullenberg and Kasperowski 2016; Pernat et al. 2024). These platforms provide a valuable source to track distribution of non-native land flatworms. As the information reported in these observation-platforms is validated by experts in the respective field and/or has community consensus, datasets are widely used in scientific publications (Silvertown 2009; López-Guillén et al. 2024). However,

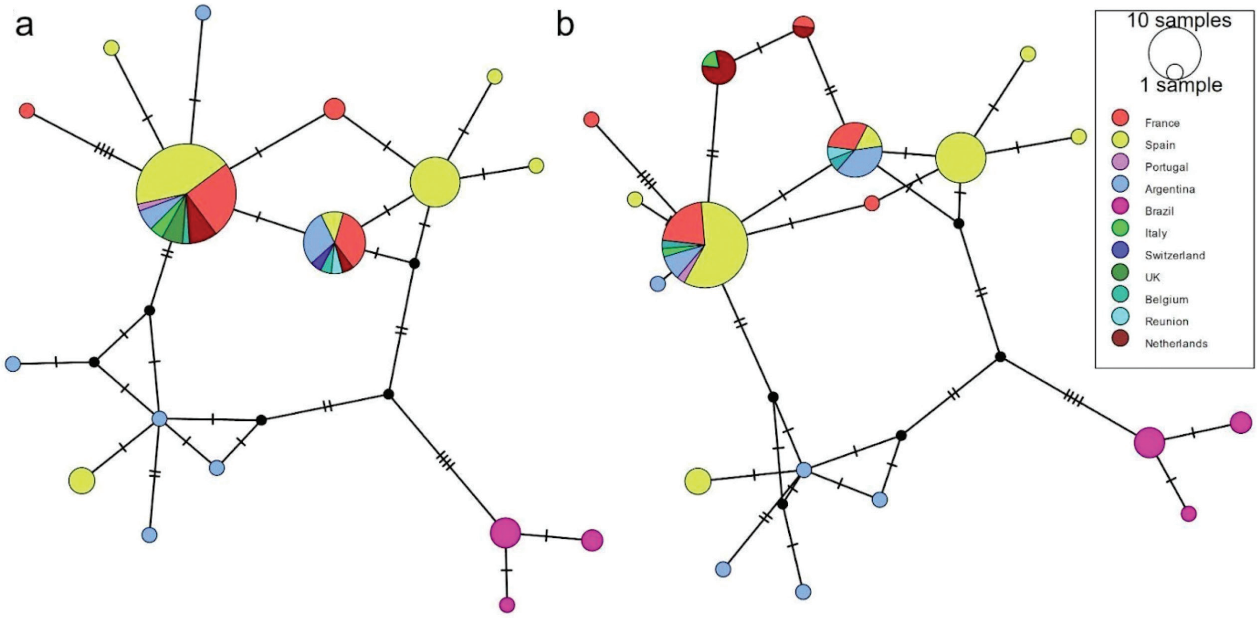

Figure 10. Genetic population structure of *Obama nungara* based on the mitochondrial cytochrome *c* oxidase subunit I (COI) sequences. Median-joining haplotype networks combining previously published specimens across native (Argentina, Brazil) and non-native range designated by the country of origin with the newly acquired sequences as part of this study (the Netherlands) presented as dataset (**a**) and (**b**). Colored circles represent observed haplotypes where the size is proportional to the number of individuals sharing a haplotype. The number of mutations (nucleotide differences) between haplotypes is indicated via black lines with putative haplotypes depicted as black-filled circles.

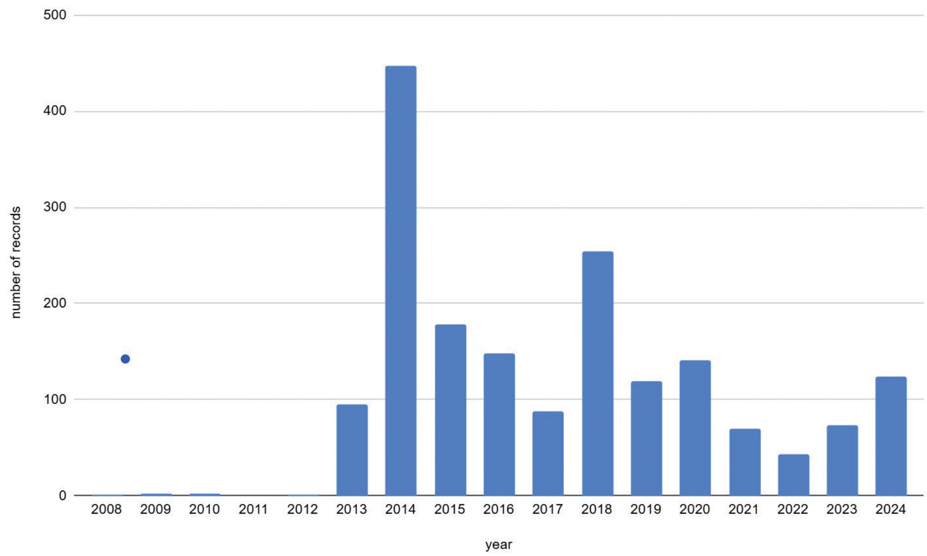

Figure 11. Records of *Obama nungara* in Europe per year.

a backlog of non-validated records on these platforms hampers full understanding of invasion biology and distribution patterns of non-native species (personal observation of the first author; see an example of the backlog in records of *O. nungara* in Spain in Suppl. material 2). A growing collection of validated records on these platforms will therefore enhance our understanding of invasion biology and distribution patterns of non-native species.

Two aspects in the use of citizen science data for land flatworm research have to be taken in consideration. The first one is that land planarians are strongly seasonal in their activity. When conditions are not optimal (temperature, humidity) they can retreat into the soil or into rotten timber. Some species can spend such periods in a prolonged state of torpor or dormancy within a protective sheath of hardened epidermal secretions (Winsor et al. 2004). The second consideration is that identification of land flatworms in this article was based on external appearance, sometimes only from pictures (see Suppl. material 1). Only in a few cases the identification was confirmed by DNA analysis. Identification of land flatworms based on external features alone is not entirely reliable. As taxonomic studies progress, more cases of cryptic land planarian species are being discovered, so there is the possibility of misidentifications (Carbayo personal communication).

Another valuable source of knowledge on distribution patterns in invasion biology research are specimens stored in the natural history collections. Since most natural history collections suffer from a backlog in registration (Johnson et al. 2023), clearing this backlog will also help our understanding of the distribution of non-native species. In the collection of Naturalis Biodiversity Center in Leiden, the Netherlands, only 21% of the specimens has been registered (see https://bioportal.naturalis.nl/nl/dashboard). In the present study, four records of *B. kewense* originate from samples stored in the museum collection of Naturalis Biodiversity Center that were never mentioned in literature. In the case of *B. kewense*, the first record in the Netherlands is dated back to 1912 when a single specimen was found in a greenhouse in the Artis Zoo (Thunnissen et al. 2022). However, before being published by Thunnissen et al. (2022) this record has only been recorded on bioportal.naturalis.nl, a website from Naturalis Biodiversity Center that serves as the national access portal for all information relating to natural history (https://bioportal.naturalis.nl). This gap is now filled with the publication of nine records based on the Dutch specimens of *B. kewense* stored in the collection of Naturalis Biodiversity Center in the Netherlands, ranging from 1912 to 2021 (see Suppl. material 1).

Challenges in data collection

A correct dataset of non-native land flatworms is crucial in order to make any statement about distribution and introduction pathways, as is described in the methodology section (see Fig. 1). Combining data published in scientific journals with the records from GBIF.org, as we did for the European dataset, has the following shortcomings (see Fig. 12):

1. Non-validated (i.e. records with no community consensus on a precise identification) and recent records from iNaturalist.org are missing;
2. Records from private collections and private databases are missing;
3. If a validated record from iNaturalist.org is published in a journal, the specimen is deposited in a natural history collection and genetic data has been deposited in a genetic database, the same record will be three times in GBIF, and four times in total in our dataset.

In Suppl. material 2 is an illustration of this shortcoming for distribution data in Spain of the land flatworm *O. nungara*.

Given the multimodularity of data available on GBIF, the level of data curation generating a correct dataset as we did for the Dutch distribution of non-native land flatworms (see Fig. 1) costs a lot of time and is prone to assumption driven error, and thus not applicable for the (much larger) European dataset.

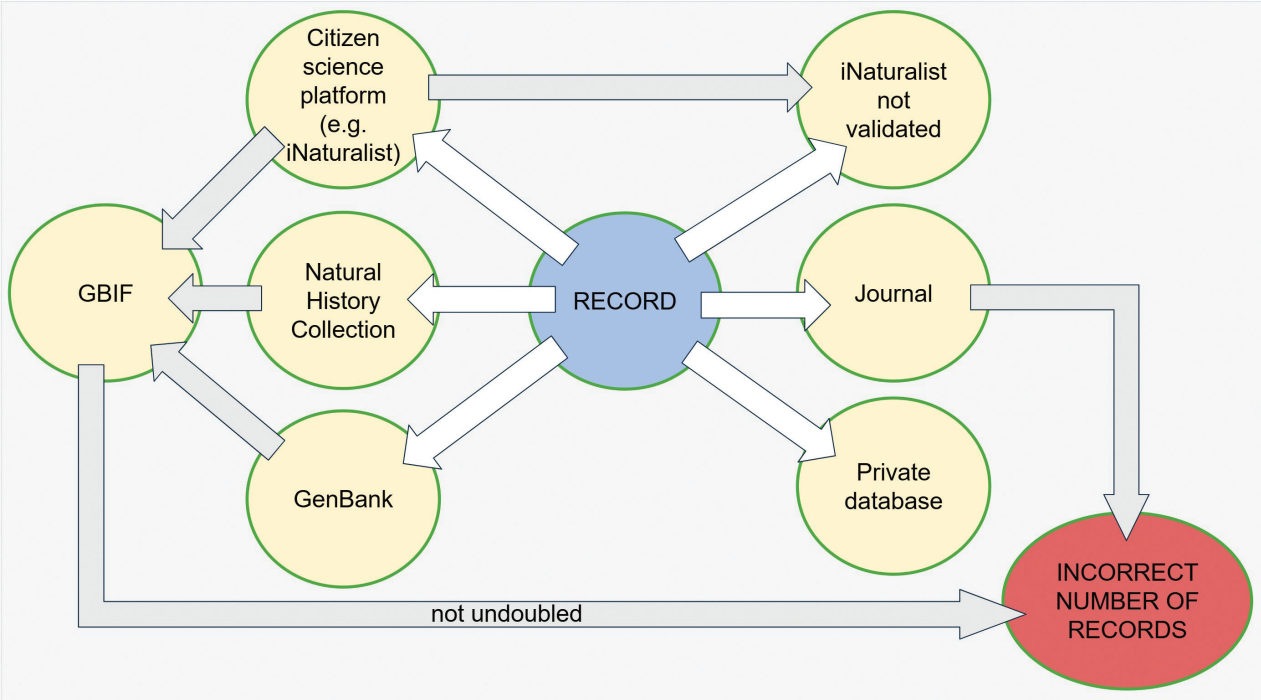

Figure 12. Graphical representation of the shortcomings of using only data published in scientific journals and GBIF.org, leading to an incorrect number of records.

Importance of habitat definitions

To understand the (negative) impact of non-native species it is important to establish their introduction pathway and ability to spread across the non-native region (Thunnissen et al. 2022). However, information on habitat is often missing in published records of non-native flatworms in Europe. Moreover, different habitat definitions have been used interchangeably in previous studies. Álvarez-Presas et al. (2014) made a subdivision in the habitats into nursery, garden, plantation, orchard and semi-natural area. Mori et al. (2022a,b, 2023) used botanical garden, plant nursery/nurseries and plantations, private garden, urban park/urban areas including urban parks, human indoor, port area, semi-natural area, city street, private balcony, salad of the supermarket and tropical greenhouse as habitat definitions. Not only is there no unified habitat designation of non-native land flatworms in Europe but the difference between inside (in a greenhouse or plant nursery) and outside (in a garden or natural area) is often unclear. This information is crucial since environmental conditions such as humidity and temperature play a pivotal role in the survival of land flatworms (Sluys and Riutort 2018) and potential spread in outdoor habitats like gardens and natural areas. For example, if a land flatworm is only found in greenhouses, like *B. kewense* in the Netherlands, it is not likely it will spread to natural areas and have negative effects on native ecosystems. To assess the risk a land planarian poses to the natural habitat where it is introduced, it is important to know the diet of each particular species (Thunnissen et al. 2022). Beside that, knowledge about introduction pathways is also important to assess the risk of land flatworms spreading across the non-native area. Based on metadata of the previously published records of land flatworms in the Netherlands (De Waart et al. 2021; De Waart 2022; Thunnissen et al. 2022) and specimens collected as part of the present study (see Suppl. material 1), a simplified scheme depicting proposed primary entries and secondary habitats is presented in Fig. 13.

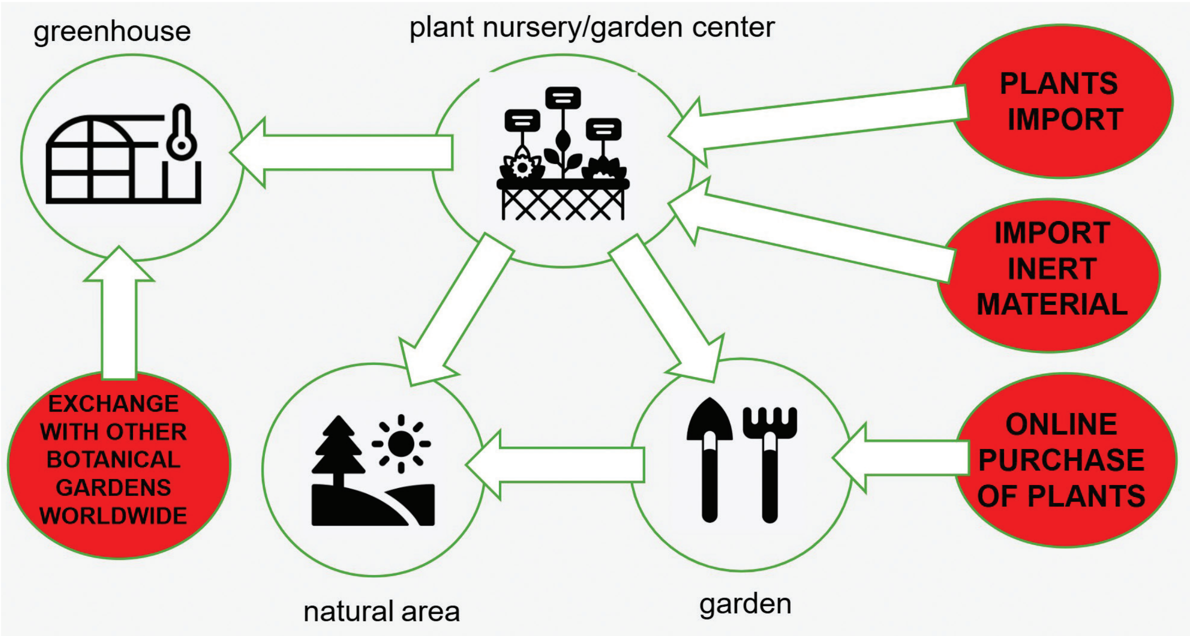

Figure 13. Proposed invasion pathways of land flatworms in the Netherlands. Red = primary entry. Source of icons www.thenounproject.com: Adrian Coquet (natural area icon), Azam Ishaq (garden center icon), Igraphics (greenhouse icon), Amethyst Studio (private garden icon) used under creative common license.

The habitat types used in the present study (see the methodology section and Fig. 13) provide crucial information to understand introduction pathways and the extent to which the targeted species are able to survive and spread across outdoor habitats like gardens and natural areas, and are proposed as a standard to be used in future studies on non-native land flatworms.

Conclusion

Land flatworms show rapid spread in their non-native areas of distribution in recent decades. So far an overview of introductions of non-native land flatworms into the Netherlands and Europe was missing. In the present study we provide the distribution of representatives of two subfamilies (Geoplaninae and Bipaliinae) across space and time in the Netherlands and Europe.

Obama nungara has been on the rise in the Netherlands in the last five years. The south of Sweden is the most northern part of continental Europe where *O. nungara* occurs outside in gardens or natural areas, but global warming is likely to shift suitable habitats up north. In case of *D. multilineatum* the Netherlands is the most northern part of Europe where it has been found outside, in gardens. Our study revealed the presence of *O. anthropophila*. It was found in a garden center in the Netherlands, and is the first record of this species outside its native range in Brazil. The presence of *B. kewense* outside in gardens or natural areas has been limited to the Southern European countries.

To understand the (negative) impact of non-native species it is important to establish their introduction pathways and their ability to spread across the non-native region. In this study we propose a terminology of habitat types that provide crucial information to understand the introduction pathways and the extent to which the targeted species are able to survive and spread across habitats as a standard to be used in future studies on non-native land flatworms.

Non-native land flatworms are found all over Europe. Species of Geoplaninae and Bipaliinae occur in 24 European countries (see overview in Table 3). The international potted plant trade was identified as one of the main introduction pathways of land flatworms. In the present study we highlight the probable role of the Netherlands as a nursery ground for soil-born non-native taxa including land flatworms for the rest of Europe as the number one exporter of floriculture worldwide.

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The observers that found a land flatworm in their garden have been asked permission by email or via Waarneming.nl to publish their name and photo. In case of no response the photos were published under Creative Commons. With other records that are marked as "consent not obtained", sometimes the photo can be found in the URL of the reference.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Sytske de Waart collected most of the data, analyzed the data, made live photographs, prepared figures and/or tables, drafted the manuscript and coordinated the writing and approved the final draft. Nikol Kmentová conceptualised the study, supervised data collection and results visualisation, performed the genetic part of the study, authored or reviewed drafts of the manuscript and approved the final draft. Maarten Vanhove helped with conceptualisation of the manuscript, co-supervised data analyses, reviewed drafts and approved the final draft of the manuscript. Jean-Lou Justine collected part of the data, co-supervised data analyses, reviewed drafts and approved the final draft of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

New records of land flatworms for the Netherlands, Belgium and Italy

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Data type: docx

Explanation note: **Text 1**. Rationale and procedure of the source and status of the new records of *Bipalium kewense*, *Diversibipalium multilineatum*, *Obama nungara* and *Obama anthropophila* found in the Netherlands, Italy and Belgium. **table S1.** *Bipalium kewense* from Amsterdam. **table S2**. *Bipalium kewense* from Amsterdam. **table S3**. *Bipalium kewense* from Utrecht Hortus Botanicus. **table S4**. *Bipalium kewense* from Leiden. **table S5**. *Bipalium kewense* from Emmen. **fig. S1.** *Bipalium kewense* from Emmen, zoo (photo Sytske de Waart). **table S6.** *Bipalium kewense* from Meise, Belgium. **fig. S2.** *Bipalium kewense* from Belgium Botanical Garden Meise (photo Sytske de Waart). **table S7.** *Diversibipalium multilineatum* from Aagtdorp. **fig. S3.** *Diversibipalium multilineatum* from Aagtdorp (photo Roy Kleukers). **table S8.** *Diversibipalium multilineatum* from Zwijndrecht. **fig. S4.** *Diversibipalium multilineatum* from Zwijndrecht (photo Maurits Waaijenberg). **table S9.** *Obama nungara* from Hendrik Ido Ambacht. **fig. S5**. *Obama nungara* from Hendrik Ido Ambacht (photo Sytske de Waart). **table S10.** *Obama nungara* from Zeist. **fig. S6**. *Obama nungara* from Zeist (photo Sytske de Waart). **table S11.** *Obama nungara* from Emmen. **fig. S7**. *Obama nungara* from Emmen (photo Jan Schimmel). **table S12.** *Obama nungara* from Leidschendam. **fig. S8**. *Obama nungara* from Leidschendam (photo Sytske de Waart). **table S13.** *Obama nungara* from Casinalbo, Italy. **fig. S9**. *Obama nungara* from Casinalbo, Italy (photo Antonio Todaro). **table S14.** *Obama nungara* from Zuidplaspolder. **table S15.** *Obama nungara* from Ridderkerk. **fig. S10**. *Obama nungara* from Ridderkerk (photo Sytske de Waart). **table S16.** *Obama nungara* from Witharen. **fig. S11**. *Obama nungara* from Witharen (photo Dewi Polak). **table S17.** *Obama anthropophila* from Melderslo. **fig. S12**. *Obama anthropophila* from Melderslo (photo Sytske de Waart). **table S18.** *Obama nungara* from Melderslo. **fig. S13**. *Obama nungara* from Melderslo (photo Sytske de Waart). **table S19.** *Obama nungara* from Boskoop. **fig. S14**. *Obama nungara* from Boskoop (photo Sytske de Waart). **table S20.** *Obama nungara* from 's Gravenpolder. **fig. S15**. *Obama nungara* from 's Gravenpolder (photo Gert Jan). **table S21.** *Obama nungara* from Ridderkerk. **fig. S16**. *Obama nungara* from Ridderkerk (photo Wilbert de Kok. CC-BY-NC-ND). **table S22.** *Obama nungara* from Wilhelminaoord. **table S23.** *Obama nungara* from Alphen aan den Rijn. **fig. S17**. *Obama nungara* from Alphen aan den Rijn (photo Eulalia Gasso). **table S24.** *Obama nungara* from Amsterdam. **fig. S18**. *Obama nungara* from Amsterdam (photo Koen van Tilburg). **table S25.** *Obama nungara* from Maastricht. **fig. S19**. *Obama nungara* from Maastricht (photos Frank Collas). **table S26.** *Obama nungara* from Veghel. **fig. S20**. *Obama nungara* from Veghel (photo Mark van Heijst). **table S27.** *Obama nungara* from Borculo. **fig. S21**. *Obama nungara* from Borculo (photo Marion). **Table S28.** *Obama nungara* from Halsteren. **fig. S22**. *Obama nungara* from Halsteren (photo Amanda Dubois de Waal). **table S29.** *Obama nungara* from Den Haag. **fig. S23**. *Obama nungara* from Den Haag (consent to use photo obtained). **table S30.** *Obama nungara* from Didam. **fig. S24**. *Obama nungara* from Didam (photo Bart de Klaver). **table S31.** *Obama nungara* from Leusden. **fig. S25**. *Obama nungara* from Leusden (photo Hugo Jansen). **table S32.** *Obama nungara* from Noordwijkerhout. **fig. S26**. *Obama nungara* from Noordwijkerhout (photo Inge Schallenberg). **table S33.** *Obama nungara* from Hardinxveld-Giessendam. **fig. S27**. *Obama nungara* from Hardinxveld- Giessendam (photo Karina). **table S34.** *Obama nungara* from Didam. **fig. S28**. *Obama nungara* from Didam (photo Tsjidsger Terpstra. CC-BY-NC-ND). **table S35.** *Obama nungara* from Rotterdam. **fig. S29**. *Obama nungara* from Rotterdam (photo Sam van der Tuin). **table S36.** *Obama nungara* from Arnhem. **table S37.** *Obama nungara* from Boskoop. **fig. S30.** *Obama nungara* from Boskoop (photo Wilfred Verkerk). **table S38.** *Obama nungara* from Boskoop. **fig. S31**. *Obama nungara* from Boskoop (photo Sytske de Waart). **table S39.** *Obama nungara* from Tilburg. **fig. S32**. *Obama nungara* from Tilburg (photo Lara B. CC-BY-NC-ND). **table S40.** *Obama nungara* from Oegstgeest. **fig. S33**. *Obama nungara* from Oegstgeest (photo Sylvia Holverda. CC-BY-NC-ND). **table S41.** *Obama nungara* from Loosbroek. **fig. S34**. *Obama nungara* from Loosbroek (photo Eva Buné). **table S42.** *Obama nungara* from Goes. **fig. S35**. *Obama nungara* from Goes (photo Barry van den Berge). **table S43.** *Obama nungara* from Amersfoort. **fig. S36**. *Obama nungara* from Amersfoort (photo Karin). **table S44.** *Obama nungara* from Amersfoort. **fig. S37**. *Obama nungara* from Amersfoort (photo Harm Jan Berendsen). **table S45.** *Obama nungara* from Emmen. **fig. S38**. *Obama nungara* from Emmen (photo Jeroen Onrust). **table S46.** *Obama nungara* from Leiden. **fig. S39**. *Obama nungara* from Leiden (photo Sarah Mawhorter). **table S47.** *Obama nungara* from Utrecht. **fig. S40**. *Obama nungara* from Utrecht (photo Maaike de Vos). **table S48.** *Obama nungara* from Amersfoort. **fig. S41**. *Obama nungara* from Amersfoort (photo Gert Jan Leving). **table S49.** *Obama nungara* from Melderslo. **fig. S42**. *Obama nungara* from Melderslo (photo Teun. CC-BY-NC-ND). **table S50.** *Obama nungara* from Noordwijkerhout. **fig. S43**. *Obama nungara* from Noordwijkerhout (photo Henry Star). **table S51.** *Obama nungara* from Bunnik. **fig. S44**. *Obama nungara* from Bunnik (photo Sander Beltman). **table S52.** *Obama nungara* from Weespersluis. **fig. S45**. *Obama nungara* from Weespersluis (photo Steven Wytema). **table S53.** *Obama nungara* from Monster. **fig. S46**. *Obama nungara* from Monster (photo Marja & Dries. CC-BY-NC-ND). **table S54.** *Obama nungara* from Teteringen. **table S55.** *Obama nungara* from Barendrecht. **fig. S47**. *Obama nungara* from Barendrecht (photo Maurits Waaijenberg). **table S56.** *Obama nungara* from Made. **fig. S48**. *Obama nungara* from Made (photo Frank. CC-BY-NC-ND). **table S57.** *Obama nungara* from Barneveld. **fig. S49**. *Obama nungara* from Barneveld (photo Evert. CC-BY-NC-ND). **table S58.** *Obama nungara* from Oosterwolde. **fig. S50**. *Obama nungara* from Oosterwolde (photo Arjan Haaijema). **table S59.** *Obama nungara* from Oegstgeest. **fig. S51**. *Obama nungara* from Oegstgeest (photo Luuk. CC-BY-NC-ND). **table S60.** *Obama* sp. from Nunspeet. **fig. S52**. *Obama* sp. from Nunspeet (photo Matthijs Top).

table S61. *Obama nungara* from Dinteloord. **fig. S53**. *Obama nungara* from Dinteloord (photo Lara. CC-BY-NC-ND). **table S62.** *Obama nungara* from Malden. **fig. S54**. *Obama nungara* from Malden (photo Berend Flamink. CC-BY-NC-ND). **table S63.** *Obama nungara* from Aldwald. **fig. S55**. *Obama nungara* from Aldwald. Specimen was accidentally cut in half (photo Reni van der Meulen). **table S64.** *Obama nungara* from Zaandam. **fig. S56**. *Obama nungara* from Zaandam (photo Erik de Vries). **table S65.** *Obama nungara* from Ede. **fig. S57**. *Obama nungara* from Ede (photo Marjolein Postma). **table S66.** *Obama nungara* from Noorden. **fig. S58**. *Obama nungara* from Noorden (photo Ghislaine Holswilder. CC-BY-NC-ND). **table S67.** *Obama* sp. from Limmen. **fig. S59**. *Obama* sp. from Limmen (photo Carina Huis). **table S68**. *Obama nungara* and *Obama* sp. from Malden. **fig. S60A**. *Obama* sp. from Malden 13 August 2024 (photo Werner). **fig. S60B**. *Obama nungara* from Malden 18 August 2024 (photo Werner). **table S69.** *Obama nungara* from Leek. **fig. S61**. *Obama nungara* from Leek (photo Barbera Star). **table S70.** *Obama nungara* from Oosterland. **fig. S62**. *Obama nungara* from Oosterland (photo Paulien). **table S71.** *Obama nungara* from Amsterdam. **fig. S63**. *Obama nungara* from Amsterdam (photo Nolda Vrielink). **table S72.** *Obama nungara* from Tilburg. **fig. S64**. *Obama nungara* from Tilburg (photo Dave van B. CC-BY-NC-ND). **table S73.** *Obama nungara* from Barneveld. **fig. S65.** *Obama nungara* from Barneveld (photo Vera van der Mijden). **table S74.** *Obama* sp. from Noordwijk. **fig. S66**. *Obama* sp. from Noordwijk (photo Martin van der Plas). **table S75.** *Obama nungara* from Apeldoorn. **table S76.** *Obama nungara* from Brielle. **fig. S67**. *Obama nungara* from Brielle (photo Kevin Varekamp). **table S77.** *Obama nungara* from Hazerswoude. **fig. S68**. *Obama nungara* from Hazerswoude (photo Mike). **table S78.** *Obama nungara* from Lienden. **fig. S69**. *Obama nungara* from Lienden attacking an earthworm (photo Lesley Bezemer). **table S79.** *Obama nungara* from Megchelen. **fig. S70**. *Obama nungara* from Megchelen (photo Eugene Raben). **table S80.** *Obama* sp. from Austerlitz. **fig. S71**. *Obama* sp. from Austerlitz (photo Roland Zoer). **table S81.** *Obama nungara* from Noordwijkerhout. **fig. S72.** *Obama nungara* from Noordwijkerhout (photo Laura Duivenvoorde). **table S82.** *Obama nungara* from Emmen. **fig. S73**. *Obama nungara* from Emmen (photo Stefan Wiebing). **table S83.** *Obama nungara* from Wijchen. **fig. S74**. *Obama nungara* from Wijchen; not visible on photo, but clearly visible on specimen are the characteristic striae of *O. nungara* (photo Rosanna Vos). **table S84.** *Obama nungara* from Chaam. **fig. S75**. *Obama nungara* from Chaam (photo Damon). **table S85.** *Obama nungara* from Geesteren. **fig. S76**. *Obama nungara* from Geesteren (photo Ans Smeets Oude Luttikhuis). **table S86.** *Obama nungara* from Culemborg. **fig. S77A**. *Obama nungara* from Culemborg 9 October 2024 (photo Yfke van Viersen). **fig. S77B**. *Obama nungara* from Culemborg 13 October 2024 (photo Yfke van Viersen). **table S87.** *Obama nungara* from Nijmegen. **fig. S78**. *Obama nungara* from Nijmegen (photo Arjun Aalbers). **table S88.** *Obama nungara* from Driebergen. **fig. S79**. *Obama nungara* from Driebergen (photo Annelies). **table S89.** *Obama nungara* from Vinkeveen. **fig. S80**. *Obama nungara* from Vinkeveen (photo Deanne Licht). **table S90.** *Obama nungara* from Hoogkarspel. **fig. S81**. *Obama nungara* from Hoogkarspel (consent to use photo obtained). **table S91.** *Obama nungara* from Schaik. **fig. S82**. *Obama nungara* from Schaik (photo Mireille Kouwer). **table S92.** *Obama nungara* from Weesp. **fig. S83**. *Obama nungara* from Schaik (consent to use photo obtained).

Link: https://doi.org/10.3897/neobiota.99.145703.suppl1

Supplementary material 2

Obama nungara records in Spain: an analysis

Authors: Sytske A. de Waart, Maarten P. M. Vanhove, Jean-Lou Justine, Nikol Kmentová

Data type: docx

Explanation note: **Text 2.** Illustration of the shortcomings when combining records from GBIF.org and those published in scientific journals. **table S93**. Number of *Obama nungara* records from Spain between 2010 and 2024 from different datasets.

Link: https://doi.org/10.3897/neobiota.99.145703.suppl2

Supplementary material 3

Metadata of all records of Bipaliinae and Geoplaninae reported in Europe to date

Authors: Sytske A. de Waart, Maarten P. M. Vanhove, Jean-Lou Justine, Nikol Kmentová

Data type: xlsx

Explanation note: **table S94**. Metadata of all records of *Bipalium kewense* reported in Europe to date. **table S95.** Metadata of all records of *Bipalium vagum* reported in Europe to date. **table S96.** Metadata of all records of *Diversibipalium multilineatum* reported in Europe to date. **table S97.** Metadata of all records of *Vermiviatum covidum* reported in Europe to date. **table S98.** Metadata of all records of *Obama nungara* reported in Europe to date. **table S99.** Metadata of all records of *Obama anthropophila* reported in Europe to date.

Link: https://doi.org/10.3897/neobiota.99.145703.suppl3

Supplementary material 4

List of previously published COI mtDNA gene fragments being part of the present study

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Data type: docx

Explanation note: **table S100**. List of previously published COI mtDNA gene fragments being part of the present study.

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