

Short note

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**On the presence of the invasive planarian *Obama nungara*
(Carbayo, Álvarez-Presas, Jones & Riutort, 2016)
(Platyhelminthes: Geoplanidae) in an urban area in Belgium**

**Jan Soors^{1,*}, Tom Van den Neucker², David Halfmaerten¹,
Sabrina Neyrinck¹ & Marc De Baere³**

¹ Research Institute for Nature and Forest (INBO), Herman Teirlinck Havenlaan 88 bus 73,
1000 Brussels, Belgium.

² University of Antwerp, Department of Biology, Ecosystem Management Research Group,
Universiteitsplein 1C, B-2160 Wilrijk, Belgium.

³ Alemstraat 12, 2811 Leest, Belgium.

* Corresponding author: jan.soors@inbo.be

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We report the first records of the invasive terrestrial planarian *Obama nungara* in Belgium. Since spring 2017, the species has frequently been found in Elsene (50°50'2.8" N, 4°21'52.0" E), a municipality of the Brussels Capital Region (Belgium). It was observed for the first time on June 15th, 2017 under stone floor tiles in a private courtyard in an urbanized area. Additional observations were made regularly, with a maximum of seven specimens observed on January 5th, 2018.

Obama nungara is a fairly large terrestrial planarian, up to about 70 mm long and 5 mm wide. Its sheer size distinguishes *O. nungara* from the three terrestrial planarians that are native to Belgium, *Microplana terrestris* (Müller, 1773), *M. scharffi* (Graff, 1896) and *Rhynchodemus sylvaticus* (Leidy, 1851). *Microplana terrestris* is usually smaller than 20 mm when extended, *M. scharffi* can be 40 mm long and the length of *R. sylvaticus* rarely exceeds 35 mm [1,2]. Besides its larger size, differences in body colour distinguish *O. nungara* from the three native species. The colour of the dorsal side of *O. nungara* is marbled light brown to dark brown, sometimes almost black, and is covered with longitudinal black striae. The marbling in dark specimens is only visible under good light conditions (Fig. 1). The ventral side is greyish white [3]. *Microplana terrestris* on the other hand is white bellied and without visible markings or lines on the dorsal side, *M. scharffi* is mainly yellow and *R. sylvaticus* has a brownish colour, with two dark lines on its dorsum and a dark spot halfway along its body [1,2].

Obama nungara has only recently been described [3]. The species had previously been confused with its congener *Obama marmorata* (Schultze & Müller, 1857). However, histological and molecular data revealed that *O. nungara* is a separate species [3]. There are also several external morphological features that distinguish *O. nungara* and *O. marmorata*. *Obama marmorata* is a larger species, up to 100 mm

when extended and has an overall lighter colour, its eyes are arranged in two lateral bands on the dorsum that comprise one fifth of the body width instead of one third, and its mouth and gonopore are generally positioned more posteriorly than in *O. nungara*. Some specimens of *O. nungara* have a narrow pale midline of the ground colour, which is devoid of dark pigment spots [3].

Considering the initial confusion of *O. nungara* and *O. marmorata* [3] and because the identification of terrestrial planarians can be challenging, especially when it is based solely on external morphological features, we also performed genetic analysis to confirm the identity of the planarians collected in Elsene. Two specimens were subjected to genetic analysis. A first voucher specimen INBO_U2017E10013 was collected on August 30th, 2017 and a second voucher specimen INBO_U2017E10014 on October 27th, 2017 and killed by direct immersion in absolute ethanol. Genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol with lysis overnight. We amplified an 899 bp fragment covering 850 bp of the mitochondrial COI gene by polymerase chain reaction (PCR), using the following primers: BarT (ATGACDGCSCATGGTTTAATAATGAT) [4] and COIR (CCWGYARMCCCHCCWAYAGTAAA) [5]. PCRs were performed in triplicate in 26 μ L of reaction mixture containing 6 μ L of extracted DNA, 0.4 μ M of each primer, 1 \times Taq buffer with KCl, 2 mM MgCl₂, 150 μ M of each dNTPs and 0.8 U Taq polymerase (Thermo Fisher Scientific). Each PCR was composed of an initial denaturation at 94°C for 2 min followed by 35 amplification cycles (denaturation at 94°C for 30 s, annealing at 43°C for 40 s and elongation at 72°C for 1 min) and a final elongation step at 72 °C for 5 min. Amplified DNA was purified using the ExoSAP-IT method (Thermo Fisher Scientific) and quantified on a 2% agarose gel. Sequencing reactions with both primers were performed using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) in a 10 μ L volume containing 25 ng of purified DNA, 0.4 μ M of BarT or COIR primer, 0.5 \times Ready Reaction mix and 0.5 \times Sequencing buffer. The cycling profile for both directions started with an initial denaturation at 96°C for 1 min followed by 35 amplification cycles (10 s at 96°C, 5 s at 50°C and 4 min at 60°C). After purification with the BigDye XTerminator Purification kit (Thermo Fisher Scientific) products were analyzed on an ABI 3500 genetic analyzer (Thermo Fisher Scientific). Sequences were checked for quality, aligned and a consensus sequence from both directions generated in Geneious ver. 8.1.9 (Biomatters Ltd.). Sequences were submitted to the European Nucleotide Archive (ENA)



Fig. 1 – A live specimen of *Obama nungara* collected in Elsene (Belgium) on August 30th 2017. Ruler scale in cm.

with accession numbers LS992235 and LS992236 (<http://www.ebi.ac.uk/ena/data/view/LS992235> and <http://www.ebi.ac.uk/ena/data/view/LS992236>).

Our 850 bp COI sequences contain the 822 bp that were previously used for phylogenetic analysis to delimit and distinguish *O. nungara* from other closely related species [3]. Moreover, sequences from both voucher specimens showed a complete match with known sequences from *O. nungara* paratypes [3]. Sequence LS992235 from the first specimen INBO_U2017E10013 was identical to accession number KT714108 from *O. nungara* paratype MZUSP PL 1599 and sequence LS992236 from the second specimen INBO_U2017E10014 to that of accession number KT714105 from *O. nungara* paratype MZUSP PL 1598 [3]. This confirms that the two planarians found in Elsene can be assigned to the species *O. nungara*. To further illustrate this, a Tamura-Nei neighbour-joining tree was constructed placing our sequences together with available COI sequences from other *O. nungara* specimens, a selection of voucher specimens of other *Obama* species and a specimen of *Cratera crioula* as outgroup (Fig. 2) [3,4,6,7].

Obama nungara is native to Santa Catarina and Rio Grande do Sul, the two southernmost states of Brazil and is also found in Argentina [6,8]. Published information about the habitat preferences of *O. nungara* is scarce, which is probably related to the fact that the species has only recently been described. BOLL & LEAL-ZANCHET [9] collected specimens of *O. nungara* in human-disturbed areas for a prey selection experiment, but the authors did not provide further details regarding habitat characteristics. *Obama nungara* feeds on a wide variety of prey items, including snails, earthworms and other planarians, although woodlice (Isopoda) do not appear to be a part of their diet [9]. Despite the fact that *O. nungara* has until recently been confused with *O. marmorata*, non-native populations are known to occur in several European countries, including Great Britain, Spain, France and Italy [3,10]. LAGO-BARCIA

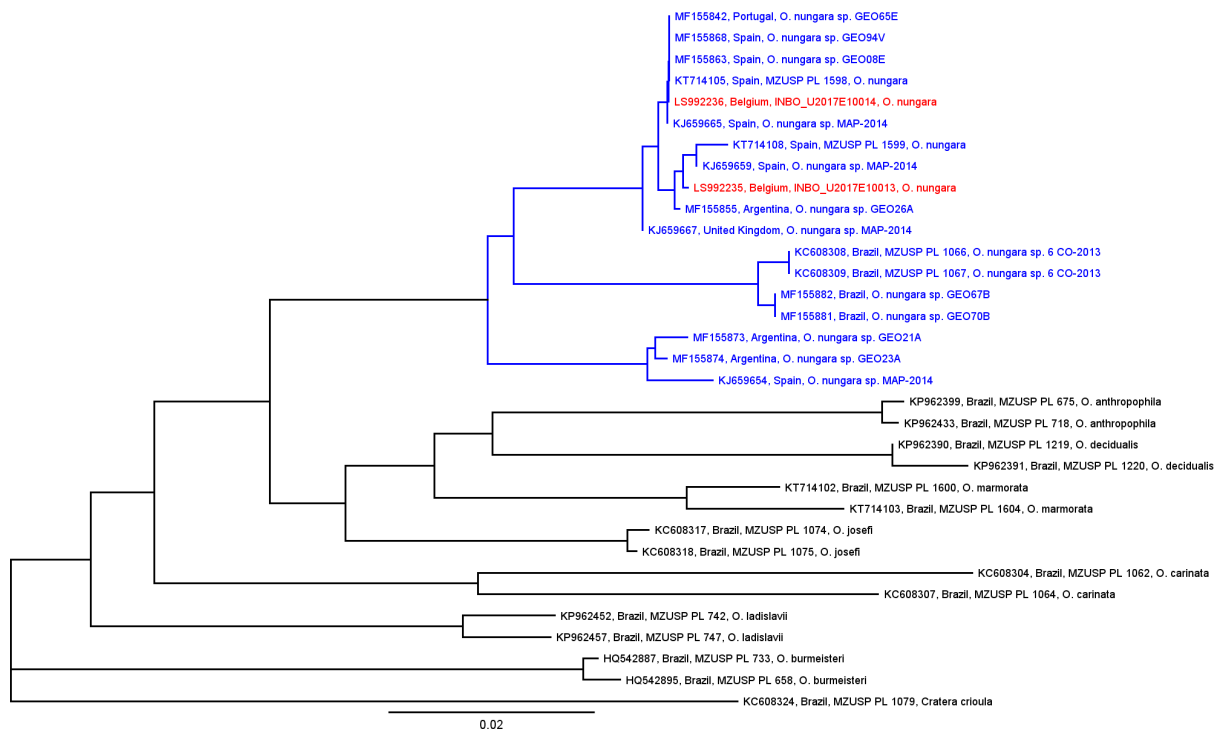


Fig. 2 – A Tamura-Nei neighbour-joining tree showing that the two Belgian specimens of *O. nungara* (red) are placed in the *O. nungara* clade (blue) together with other specimens from Argentina, UK and the Iberian Peninsula. COI sequences are indicated by their ENA accession number, country, specimen code (vouchers only) and species. The scale bar indicates substitutions per site.

et al. [6] recently established that the Iberian populations of *O. nungara* originate from Argentina and since our specimens have identical sequences to specimens found in Spain they might have the same origin (Fig. 2).

The most probable source of introduction into Europe is the ornamental plant trade [7,11]. It is likely that *O. nungara* has hitchhiked to the isolated courtyard in Elsene with garden plants. In an effort to prevent further spread, all specimens of *O. nungara* detected at the locality in Elsene were euthanized. Despite these eradication efforts, additional specimens of *O. nungara* continued to be found and the highest number of individuals was collected seven months after its discovery, during winter. This indicates that the Belgian population is persistent and that the species is capable of surviving in a temperate climate.

Its tolerance for a broad temperature range had already been observed in France, where it is the most widely distributed invasive land planarian [12]. The temperature tolerance of *O. nungara*, its presence in highly disturbed areas and its broad food niche suggest a high tolerance for environmental disturbance and a significant potential to become an invasive species [9]. Therefore, it is likely that *O. nungara* will continue to expand its range throughout Europe, aided by the increasing mobility of people and the global trade in ornamental plants [11]. The invasive potential of planarians is illustrated by the presence of up to six species of alien planarians in plant nurseries in Spain [7].

The invasive potential and the carnivorous feeding habits have increased worldwide attention for terrestrial planarians, because they may have significant adverse effects on indigenous lumbricids and / or molluscs. Several authors suggest that planarians may have a consequential indirect impact on soil fertility, soil drainage and thus on ecosystem functioning [1,7,11]. Because of these concerns, it is important to carefully monitor the Belgian population of *O. nungara* and to continue the eradication efforts to prevent further spread. Implementation of strict phytosanitary measures in the horticultural trade could prevent the further spread of *O. nungara* and the introduction of other potentially invasive planarians in Belgium and elsewhere in Europe.

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