

Biological Invasions

RECONSTRUCTING ROUTES OF INVASION OF OBAMA NUNGARA (PLATYHELMINTHES: TRICLADIDA) IN THE IBERIAN PENINSULA.

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Corresponding Author:	Carolina Noreña Museo Nacional Ciencias Naturales (CSIC) Madrid, Madrid SPAIN	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Museo Nacional Ciencias Naturales (CSIC)	
Corresponding Author's Secondary Institution:		
First Author:	Domingo Lago-Barcia	
First Author Secondary Information:		
Order of Authors:	Domingo Lago-Barcia	
	Fernando-Angel Fernandez-Alvarez	
	Francisco Brusa	
	Iñaki Rojo	
	Cristina Damborenea	
	Lisandro Negrete	
	Cristina Grande	
	Carolina Noreña	
Order of Authors Secondary Information:		
Funding Information:	Spanish Ministry of Economy, Industry and Competitiveness (CGL2011-29916)	Doctor Carolina Noreña
	FCNyM-UNLP (N11/728)	Dr. Lisandro Negrete
	CONICET (PIP 112-201201-00635 CO)	Dr. Cristina Damborenea
	ANPCyT (PICT 2014-0768)	Dr. Cristina Damborenea
Abstract:	<p>Abstract</p> <p>Among other factors, globalization has promoted the spread of alien organisms, posing a great risk to Earth's biodiversity. Land planarians of the family Geoplanidae especially benefit from human-mediated transport. Many species become established in new areas, where they represent threats to the native soil fauna. Obama nungara is a species described from Brazil, but with many well-established populations in Europe. In this study, specimens from Argentina, Brazil, Portugal and Spain were morphologically and molecularly studied to establish the potential origin of the invasive events within the Iberian Peninsula. Analyses of the mitochondrial lineages (haplotype networks) of these populations revealed previously unknown relationships and biogeographical patterns that suggest an Argentine origin for the Iberian populations.</p>	

	<p>Furthermore, comparative analysis of Argentine, Iberian and Brazilian populations revealed three well-defined and distinct Obama nungara clades. Our findings suggest two independent introductions of different populations from Argentina that gave rise to the different Iberian populations. This population diversity suggests hidden biodiversity of alien land planarians in invaded areas and their invasive and adaptive potential.</p>
<p>Response to Reviewers:</p>	<p>COMMENTS TO THE AUTHOR:</p> <p>Dear Dr. Noreña,</p> <p>Please deal with these final issues and I will accept the manuscript right away.</p> <p>Daniel Simberloff, Editor-in-Chief</p> <p>Reviewer #1: The manuscript was revised considering reviewer' suggestions. I have only a few comments inserted in the enclosed file.</p> <p>WE HAVE INTRODUCED THE NEW CHANGES REQUESTED.</p> <p>Reviewer #2: The authors have correctly answered to my questions and have made all necessary changes to the manuscript.</p> <p>Introduction: On proofs, please correct the date for the taxon <i>Platydemus manokwari</i> - it's "de Beauchamp, 1963", not 1962. The explanation for this is available in Wikipedia: https://en.wikipedia.org/wiki/Platydemus_manokwari</p> <p>WE HAVE CHANGED THE DATE OF BEAUCHAMP 1962 TO BEAUCHAMP 1963 AS SUGGESTED BY THE REFEREE AND FOLLOWING THE IZCN, BUT WE BELIEVE THAT PROBLEMS WILL ARISE TO LOCATE THE JOURNAL WHERE PLATYDEMUS IS PUBLISHED, SINCE THE NUMBER AND VOLUME OF THE SAME CORRESPOND TO 1962.</p> <p>Associate Editor: The authors have addressed adequately the comments and suggested edits from the previous round of review. I recommend acceptance after the very minor comments from Reviewers 1 and 2 are addressed. The authors should ensure that submitted figures are high resolution for view online and in print.</p>

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Domingo Lago-Barcia^{1,6}, Fernando Ángel Fernández-Álvarez², Francisco Brusa³, Iñaki Rojo¹, Cristina Damborenea³, Lisandro Negrete³, Cristina Grande⁴ and Carolina Noreña^{1,7}

RECONSTRUCTING ROUTES OF INVASION OF *OBAMA NUNGARA* (PLATYHELMINTHES: TRICLADIDA) IN THE IBERIAN PENINSULA

Running title: Routes of invasion of *Obama nungara*

¹Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain.

² Institut de Ciències del Mar (CSIC), Passeig Marítim 37-49, E-08003 Barcelona, Spain

³ División Zoología Invertebrados, Museo de La Plata (FCNyM-UNLP), Paseo del Bosque s/n, La Plata, Buenos Aires, Argentina & CONICET Consejo Nacional de Investigaciones Científicas y Técnicas.

⁴ Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049, Madrid, Spain.

⁶ Present address: Laboratório de Ecologia e Evolução, Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, Av. Arlindo Bettio, 1000, São Paulo, SP 03828-000, Brazil.

⁷ Corresponding author: norena@mncn.csic.es

Abstract

Among other factors, globalization has promoted the spread of alien organisms, posing a great risk to Earth's biodiversity. Land planarians of the family Geoplanidae especially benefit from human-mediated transport. Many species become established in new areas, where they represent threats to the native soil fauna. *Obama nungara* is a species described from Brazil, but with many well-established populations in Europe. In this study, specimens from Argentina, Brazil, Portugal and Spain were morphologically and molecularly studied to establish the potential origin of the invasive events within the Iberian Peninsula. Analyses of the mitochondrial lineages (haplotype networks) of these populations revealed previously unknown relationships and biogeographical patterns that suggest an Argentine origin for the Iberian populations. Furthermore, comparative analysis of Argentine, Iberian and Brazilian populations revealed three well-defined and distinct *Obama nungara* clades. Our findings suggest two independent introductions of different populations from Argentina that gave rise to the different Iberian populations. This population diversity suggests hidden biodiversity of alien land planarians in invaded areas and their invasive and adaptive potential.

Keywords

Biodiversity; biological invasions; distribution; haplotype networks; Neotropical land planarian

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Introduction

An increase in international trade in the last several decades and a lack of adequate control for alien species has led to the accidental transport of fauna and flora (Kaluza et al. 2010). The spread of invasive species has caused a reduction or extinction of native species, has damaged local ecosystems and has had a negative impact on global biodiversity (Wilcove et al. 1998). The introduction of land planarians (Platyhelminthes, Geoplanidae), recognized as top predators of the invertebrate soil fauna, pose a risk to the native invertebrates in those areas in which they have been accidentally introduced (Murchie and Gordon 2013). For instance, *Platydemus manokwari* Beauchamp, 1963, categorized as one of the 100 worst invasive species (Invasive Species Specialist Group ISSG 2015), has caused a rapid decline in terrestrial invertebrates (land snails, earthworms, isopods) in areas where it has been introduced. The “New Zealand” flatworm *Arthurdendyus triangulatus* (Dendy, 1895) was introduced in the UK, Ireland and Faroe Islands, causing a serious risk to native earthworms (Murchie and Gordon 2013).

The introduction of land planarians into Europe has become increasingly frequent and a matter of concern since the first species of geoplanid, *Paraba multicolor* (Graff, 1899), was found in Hamburg, Germany (Kraepelin 1901). Today, more than 30 species of land planarians have been reported in different countries outside their native range and 21 of them have been found in Europe (Justine et al., 2018). Of these, six species have been reported for Spain, including representatives of the subfamilies Bipaliinae, Rhynchodeminae and Geoplaninae (Vila-Farré et al. 2011; Mateos et al. 2013; Lago-Barcia et al. 2015; Carbayo et al. 2016).

Recently, Lago-Barcia et al. (2015) recorded *Obama marmorata* (Schultze and Müller 1856) in anthropized areas in Argentina and the Iberian Peninsula (IP), mainly in greenhouses and plant nurseries. Previously, *O. marmorata* had only been recorded in southern Brazil (Froehlich 1959). Subsequently, Carbayo et al. (2016) amended the identity of those specimens found in Argentina and Spain by Lago-Barcia et al. (2015) as a new species, *Obama nungara*, with records in Brazil, Argentina, Spain and the United Kingdom, and noted the co-occurrence of *O. nungara* and *O. marmorata* in southern Brazil.

Accurate knowledge of the distribution and origin of introduced populations is imperative to designing effective policy to control introduced species. The presence of *O. nungara* in Europe is significant because it is a member of the subfamily Geoplaninae, which is restricted to the Neotropics with few records outside this region. This species exhibits a ample niche, with earthworms as its main food resource, but also feeding on gastropods and other land planarians. It shows a high tolerance for environmental disturbance (Boll and Leal-Zanchet, 2016). Thus, this species has great potential to become invasive. In this work, we studied new specimens of *O. nungara* from Argentina, Brazil, Portugal and Spain, integrating morphological and molecular data, to elucidate the putative origin of the Iberian populations.

Methods

Specimens were collected by hand from gardens, parks, backyards, greenhouses and human-disturbed native forests in Argentina, Brazil, Portugal and Spain (Table 1). They were collected during the day and night. Live specimens were photographed, then euthanized in boiling water and fixed in 10% formaldehyde or in Bouin’s solution for histological analysis. Before fixation, a small fragment of each specimen was preserved in absolute ethanol for molecular analysis.

Morphological study

Histological sections were cut from body fragments of selected flatworms from Argentina, Brazil and Spain for species identification (Table 1). The tissues were first gradually dehydrated in increasingly concentrated solutions of ethanol, immersed in Butanol, and subsequently embedded in Paraplast® Tissue Embedding Medium. They were then serially sectioned at 6-8 µm thick with a microtome. Sections were stained using Azan (specimens from Spain) and Masson (specimens from Argentina and Brazil) trichrome methods. Sections were observed by optical microscope and the copulatory apparatus reconstructed for identification purposes. Histological features of the reproductive system and other anatomical features were compared between different populations and with the specimens studied by Lago-Barcia et al. (2015) and Carbayo et al. (2016).

The specimens analysed in this work were deposited in the collections of the Museo Nacional de Ciencias Naturales, Madrid (Spain) and Museo de La Plata (Argentina).

Mitochondrial DNA study and haplotype network analysis

Genomic DNA was extracted following a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). A fragment of the cytochrome oxidase I (COI) gene was amplified using the newly designed primer Obama-COIF (forward 5’-GTGATGCCAGTTATGATTGGTGGT-3’) and COIpr-b2 (reverse 5’-

ATGAGCAACAACATAATAAGTATCATG-3') (Bessho et al. 1992). The PCR was performed in a total volume of 25 µl containing 0.125 µl of *Taq* polymerase (5U/µl, 5 PRIME), 2.5 µl of reaction buffer (5 PRIME), 0.5 µl of each primer (10 µM), 2 µl of dNTPs (10 µM) and 2 µl of DNA. The thermocycling program began with an initial denaturation step at 95 °C (10 min), followed by 46 cycles of denaturation at 96 °C (1 min), annealing at 61 °C (1 min) and extension at 72 °C (1 min), ending with a final extension step at 72 °C (7 min). Amplified products were purified with ExoSAP-IT (Affymetrix, OH, USA) and both strands were sequenced on an ABI Prism 3730 (www.secugen.es). DNA sequences were cleaned at the primer ends using Sequencher® 5.2 (Gene Codes Corporation, Ann Arbor, MI, USA; <http://www.genecodes.com>), aligned with MAFFT (Katoh and Standley 2013) and manually checked with BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>).

A total of 121 sequences (each corresponding to a 710 bp fragment) were used for molecular analyses and included sequences from specimens collected for this study and those obtained from GenBank of related *Obama* species (Table 1). A *Pasipha* sp. (Specimen GEO15) sequence was used as an outgroup.

Haplotype networks were constructed using the software PopART (<http://popart.otago.ac.nz>). Specimens from Argentina, Brazil, Portugal and Spain (Table 1) were included in this analysis. TCS methods (Clement et al. 2000) were used to infer relationships among samples.

A COI gene tree was reconstructed by Maximum Likelihood (ML), and implemented in W-IQ-TREE (Trifinopoulos et al. 2016). W-IQ-TREE includes an evolutionary model selection process similar to jModelTest (Darriba et al. 2012), and an extended process using the FreeRate heterogeneity model. The latter model estimates rates freely and moderates the discrete Gamma model. W-IQ-TREE also uses the Bayesian information criterion to select the best-fit model. For phylogenomic data, W-IQ-TREE determines the best-fit partitioning scheme using a fast implementation of Partition Finder (Lanfear et al. 2012).

The RAxML generated tree was used as the input in a Bayesian Poisson Tree Processes (bPTP) analysis, implemented on the bPTP server (<http://species.h-its.org/bptp/>; Zhang et al. 2013). PTP is a single-locus species delimitation method that only uses nucleotide substitution information and implements a model that assumes that gene tree branch lengths are generated by two independent Poisson process classes (within- and among-species substitution events). The bPTP analysis was run using 100,000 MCMC generations, with a thinning of 100 and a burn-in of 10%.

This approach does not require ultrametrization of trees or other species delineation models, such as the Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa and Barraclough 2013). With the PTP model, speciation or branching events are modelled in terms of the number of substitutions (represented by branch lengths), and therefore, only requires a phylogenetic input tree.

Results

Morphological identification

External morphology. Live specimens of *Obama nungara* from Argentina, Brazil and Spain are lanceolate, with the anterior region of the body gradually narrowing towards the tip, which is blunt, and the posterior region ending abruptly. The colour pattern of the dorsal surface varies from light to dark brown with a thin median stripe of yellowish to light brown pigment. The dorsum is also splattered with spots irregularly disposed and anastomosed, lending a marbled appearance (Figs. 1–3). The ventral surface is light gray to whitish. The eyes are monolobated, contour the anterior tip and extend uniserially 3–4 mm from the tip, following marginally for 3–6 mm in two-three irregular rows. They become dorsal pluriserial and trilobated, occupying 60–75% of body width in their maximum extension (between 15% and 30% of body length). Posteriorly, they are more isolated reaching the posterior end. After fixation, the length of specimens is 32–70 mm (mean: 52 mm), and maximum width is 4–8 mm (mean: 6 mm). Mouth and gonopore distance from the anterior tip is 55–66% (mean: 61%) and 69–86% (mean: 77%) relative to body length. However, the external aspect of the specimens of the studied populations did not differ substantially. Specimens from the Iberian Peninsula are slightly smaller than those from Argentina and Brazil, reaching 50mm in length and 6mm in maximum width.

Internal morphology (Figs. 1–3). The cutaneous musculature exhibits the three typical layers of Geoplaninae (circular, diagonal, and longitudinal muscle). The specimens from the Iberian Peninsula show a thicker cutaneous musculature than those from Argentina and Brazil, with the ventral musculature slightly thicker than the dorsal. Parenchymatic musculature is composed of a dorsal layer, with decussate fibers, and supra- and sub-intestinal transverse muscle layers. The pharynx is cylindrical. Posterior to the pharynx, the sperm ducts are dilated and full of spermatozoa, constituting spermiducal vesicles. In the proximity of the penis bulb, the sperm ducts run to the sagittal plane and open into the paired tubular portion of the ventro-dorsally oriented prostatic vesicle. The prostatic vesicle, which is extrabulbar, follows as an unpaired C-shaped part. After a very short section inside the penis bulb, the prostatic vesicle communicates with the ejaculatory duct, which runs proximally straight and distally sinuous. The individuals from the Iberian Peninsula show a more symmetrical penis papilla than those from Argentina and Brazil and the ejaculatory duct is almost straight throughout its extension. The penis papilla, in

1 some specimens from all populations studied herein, is bent to the left, with the dorsal insertion posteriorly
2 displaced, but never reaching the level of the gonopore. On the Iberian specimens, the penis papilla may reach the
3 opening of the gonopore, contrary to the South American individuals where it never reaches the gonopore. The
4 stroma of the penis papilla is pierced by conspicuous erythrophil fine granules of two types, pinkish and
5 erythrophil granules. The latter are more intensely stained and are organized in discrete bundles that traverse the
6 lining epithelium of the papilla. The male atrium presents dorsal folds, one of which separates it from the female
7 atrium. Abundant cyanophil fine granular secretion surrounds the ventro- and dorso-anterior wall of the male
8 atrium, extending to the penis insertions.

9 The ovovitelline ducts arise from the ovaries, and posteriorly to the gonopore, bend dorso-medially and
10 join each other to form a common glandular ovovitelline duct. This duct runs backwards and opens into a short
11 female canal, which is antero-ventrally flexed. The female atrium exhibits a narrow lumen owing to its highly
12 folded walls. Folds in the distal region of the male and female atrium depend on the state of maturity of the studied
13 individuals.

14 Haplotype network analysis and mitochondrial DNA phylogeny

15
16 The haplotype network analysis recognized 21 haplotypes of *Obama nungara*. Three main networks were obtained
17 presenting 18-21 bases that varied between them (Fig. 4). Network 1 (coincident with clade 1) included five
18 haplotypes of specimens from Argentina (marked in blue) and one haplotype of specimens from Spain (marked in
19 green), with the latter haplotype (haplotype 6) being the most frequent. Haplotypes in this network present a
20 minimum of four variable characters between them.

21 Network 2 (coincident with clade 2 and marked in orange) was formed by eleven specimens from Brazil,
22 nine from the present study and two obtained from the Gen Bank database. This network showed five haplotypes
23 presenting one or two variable bases. Haplotype 9 is the most frequent.

24 Finally, network 3 (coincident with clade 3) included specimens from Spain, Portugal (both marked in green), and
25 from Argentina (marked in blue). This group showed eleven haplotypes. Haplotype 17 included only specimens
26 from Argentina and haplotype 12 was shared by specimens from Argentina and Spain (Fig. 4). Haplotypes in this
27 network varied by one or two bases. The haplotypes 16 and 21 were the most frequent, both formed by specimens
28 from different regions of the Iberian Peninsula.

29 ML analyses including all sequences from Table 1 yielded a tree (Fig. 5) with the best-fit model
30 TPM2+I+G4, chosen according to the Bayesian Inference Criterion (BIC). The *O. nungara* sequences were
31 grouped in a well-supported clade (99%). This clade was sister to a clade that included *O. josefi*, *O. decidualis*, *O.*
32 *anthropophila*, and a clade formed by *O. marmorata* and 4 specimens of *Obama* sp. (KP962357, KP962359,
33 KP962430). *O. ladislavii* was the sister group to these two main clades. Although *O. burmeisteri* and *O. carinata*,
34 showed well-supported monophyletic clades with bootstrap values of 100% and 81%, respectively, their
35 relationships with the other clades were not well-supported.

36 The 66 sequences that formed the *Obama nungara* clade, clustered into three well-differentiated lineages
37 (bootstrap values of 93%, 100% and 97%, respectively). Two lineages included sequences from the Iberian
38 Peninsula and Argentina and the third included Brazilian sequences. *Obama nungara* clade 1 (Fig. 5) included
39 specimens from Argentina and from two different localities in Spain; *O. nungara* clade 2 consisted exclusively of
40 Brazilian specimens; and *O. nungara* clade 3 comprised specimens from different localities in Argentina and the
41 Iberian Peninsula.

42
43 *Species delimitation.* Mitochondrial species delimitation results (bPTP) are summarized in Figure 4. Within the
44 main clade, bPTP values also indicated three groups. Representatives of Argentina, together with specimens from
45 Spain, showed a bPTP value of 0.929, thus representing one group. All of the Brazilian specimens of *O. nungara*,
46 constituted a second group with a bPTP value of 0.653. Specimens from various localities in the Iberian Peninsula
47 (Portugal and Spain) and Argentina formed the last group, with a bPTP value of 0.650.

50 Discussion

51 Morphological identification

52
53 The external features and the internal morphology, mainly in regards to the copulatory apparatus, of the specimens
54 from the Iberian Peninsula and those from Argentina and Brazil, agree with the original description of *Obama*
55 *nungara* (Carbayo et al. 2016). The colour pattern of the Iberian specimens varies between beige with dark
56 striations to a dark brown background splattered with almost black striations lending a marbled appearance to the
57 dorsum of *O. nungara*. The ventral region is whitish milky. This heterogeneity in the dorsal colour pattern has also
58 been observed in Brazilian and Argentina populations.

1 However, the populations showed some differences in size (with the Spanish specimens smaller than the South
2 American ones), and in the thickness of the cutaneous musculature (which was more developed in some specimens
3 from Sopelana, Vizcaya, Spain). This feature could be due to diverse maturity stages. Also, the individuals from
4 the Iberian Peninsula showed a more symmetrical penial papilla than those from Argentina and Brazil, which reach
5 the opening of the gonopore, contrary to the South American individuals in which the penial papilla never reached
6 the gonopore. Moreover, the ejaculatory duct in specimens from the Iberian Peninsula was straight. These small
7 differences could represent a slight morphological divergence between the studied specimens of the Iberian
8 Peninsula and those of South America.

9 Haplotype network and mitochondrial DNA analysis

10 The haplotype network analyses indicate the relationships among mitochondrial lineages of different *O. nungara*
11 populations and reveal an Argentine origin of the Iberian Peninsula populations, at least in two different
12 colonization events. The Brazilian clade presents the greatest differences with the other two clades, as it does not
13 share haplotypes with any other clade outside of Brazil. *Obama nungara* seems to be widely distributed within
14 Argentina, and thus a comprehensive sampling from this country is needed to better clarify relationships among
15 populations, since the founding populations are currently unknown.

16 Despite the morphological similarity between individuals from different clades, the molecular differences are
17 remarkable. The question arises whether the three identified clades are comprised of different populations of the
18 same species or three different cryptic species. A similar situation occurs with *Platydemus manokwari*, with two
19 different COI haplotypes, differentiated by 15 variable bases, and few morphological differences (Justine et al.
20 2015). Contrary to Justine et al. (2015), who found only two haplotypes for specimens of *P. manokwari* from
21 different countries and only one population with genetic diversity, we found high levels of genetic diversity in *O.*
22 *nungara* with twenty-one haplotypes.

23 Several species of land planarians have been recognized as introduced and are now found in different
24 parts of the world (Alvarez-Presas et al 2015, Justine et al 2015, 2018). Many of these are from the Southern
25 Hemisphere or from tropical Asia, and some show invasive traits. Successful colonization by land planarians is
26 due to their life history traits such as hermaphroditism, sperm storage, and large capsules containing multiple
27 offspring, which facilitates colonization, allowing only a few individuals to establish new populations (Ducey et
28 al. 2005). *Obama nungara* has been accidentally introduced in the Iberian Peninsula and could have expanded to
29 Europe due to the increase in the commercial exchange amongst European countries and with South America, e.g.
30 Brazil and Argentina (the source of potential new introductions). There is little information on the biological life
31 history of *O. nungara*, but we know the potential adaptive capacity of this species based on the plasticity of its
32 food habits, as a generalist predator of soil invertebrates, such as earthworms, gastropods and other land planarians
33 (Boll and Leal-Zanchet 2016). Invasion success is linked to the diet breadth of the introduced species, and thus *O.*
34 *nungara* could be a successful invader, becoming a threat to the diversity of native invertebrates.

35 Conclusions

36 The similarities between specimens from Argentina and the Iberian Peninsula strongly indicate that the origin of
37 the Iberian populations is Argentina. We believe that identifying routes of introduction of invasive species is key to
38 controlling alien species dispersal. Through the use of haplotype networks, such introductions can be identified as
39 demonstrated in the present study. With the routes identified, special measures can be applied in commerce and
40 transport policies to avoid unnecessary introductions of fauna and flora into foreign countries.

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1 Legends

2 Figure 1+2: 1. *Obama nungara* from the Iberian Peninsula (GEO04) and 2. from Argentina (GEO21). A: Dorsal
3 view of a live specimen. B–G: Selected sagittal sections of the copulatory apparatus. H: Schematic reconstruction
4 of the copulatory apparatus, in sagittal view. In all figures, anterior end of the body is on the left. Abbreviations:
5 cod, common ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female canal; g, gonopore; ma, male
6 atrium; od, ovovitelline duct; pp, penis papilla; pv, prostatic vesicle; sd, sperm duct; sg, shell glands. Cyanophil
7 fine granular secretion is indicated by arrowheads, and erythrophil fine granules are indicated by asterisks. Scale
8 bars: 500 μ m.
9

10 Figure 3+4: 3. *Obama nungara* from Brazil (GEO66). A: Dorsal view of a fixed specimen. B–G: Selected sagittal
11 sections of the copulatory apparatus. H: Schematic reconstruction of the copulatory apparatus, in sagittal view. In
12 all figures, anterior end of the body is on the left. Abbreviations: cod, common ovovitelline duct; ej, ejaculatory
13 duct; fa, female atrium; fc, female canal; g, gonopore; ma, male atrium; od, ovovitelline duct; pp, penis papilla; pv,
14 prostatic vesicle; sd, sperm duct; sg, shell glands. Cyanophil fine granular secretion is indicated by arrowheads,
15 and erythrophil fine granules are indicated by asterisks. Scale bars: 500 μ m.

16 4. TCS network inferred from *Obama nungara* mitochondrial sequences from Argentina, Brazil, Portugal and
17 Spain. Sequence nodes from Argentina are in blue, Brazil in orange and Iberian Peninsula in green.
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19 Figure 5. Best tree of the molecular phylogeny from the ML analyses according to Bayesian Inference Criterion
20 (bootstrap values are indicated on the nodes). Species clusters corresponding to bPTP analyses in column 1,
21 morphological delineations in column 2.
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Fig. 1

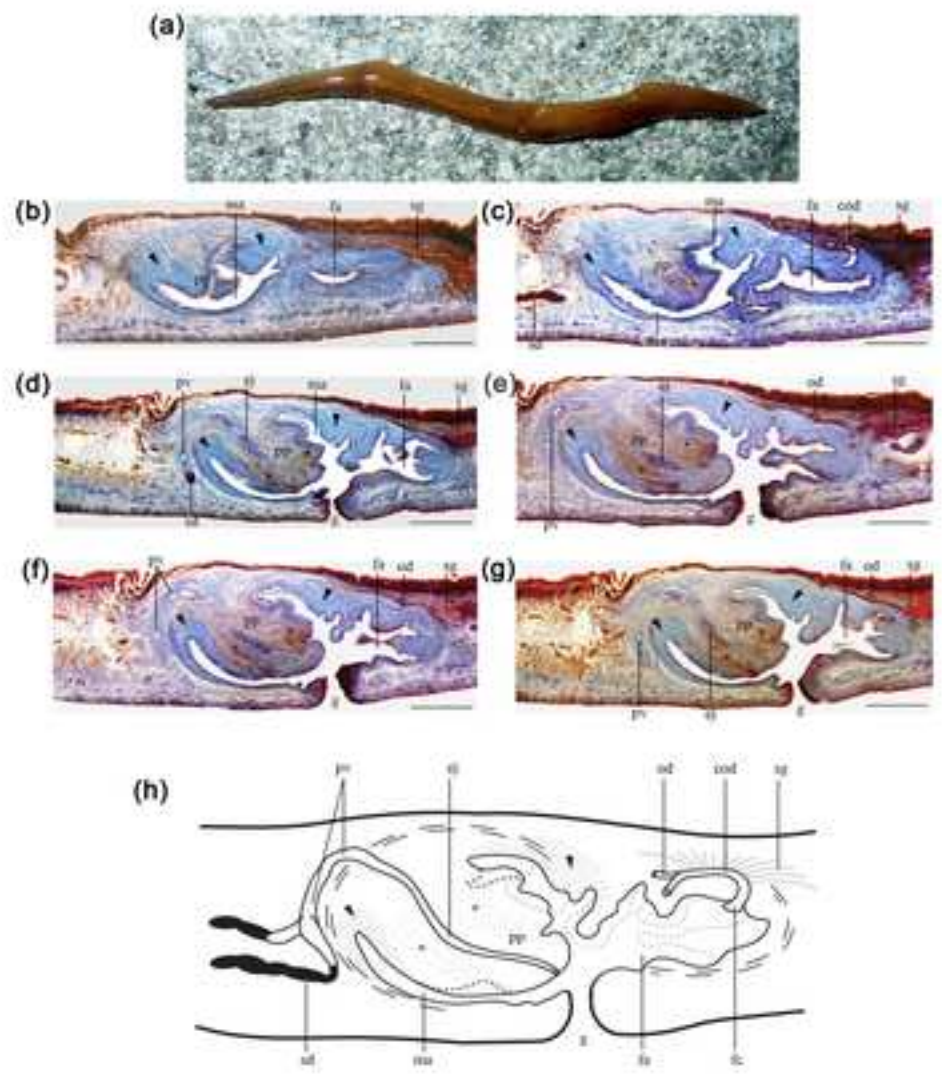


Fig. 2

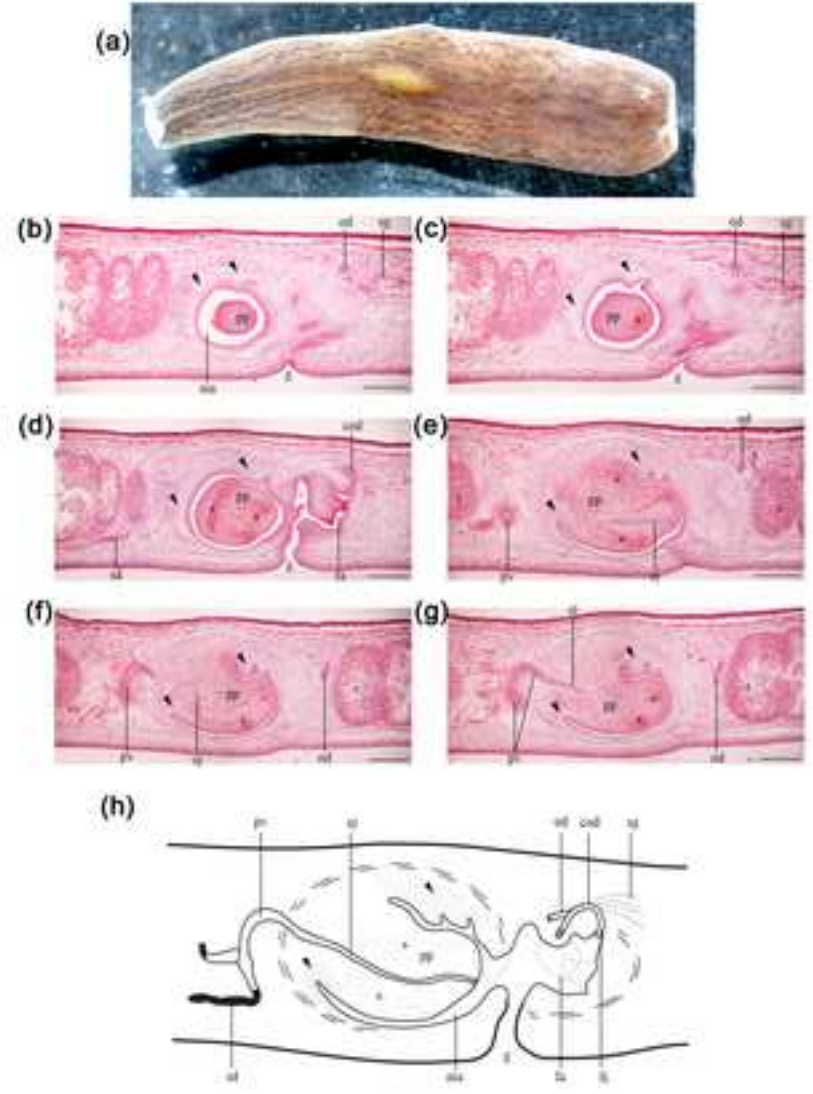


Fig. 3

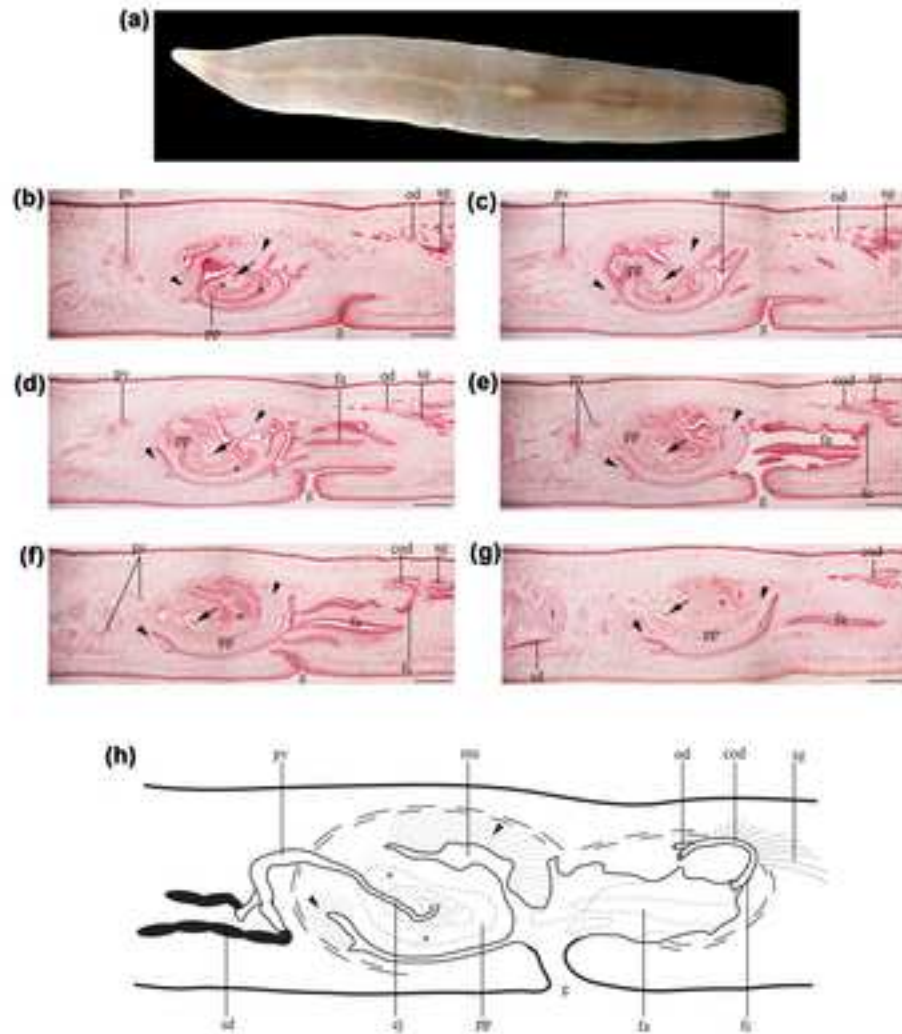


Fig. 4

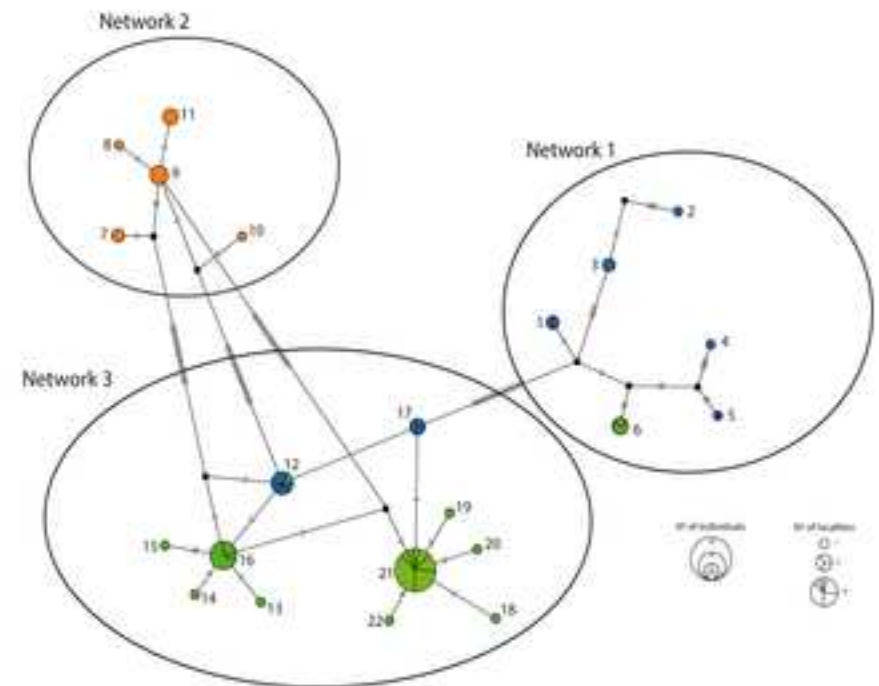


Fig 5

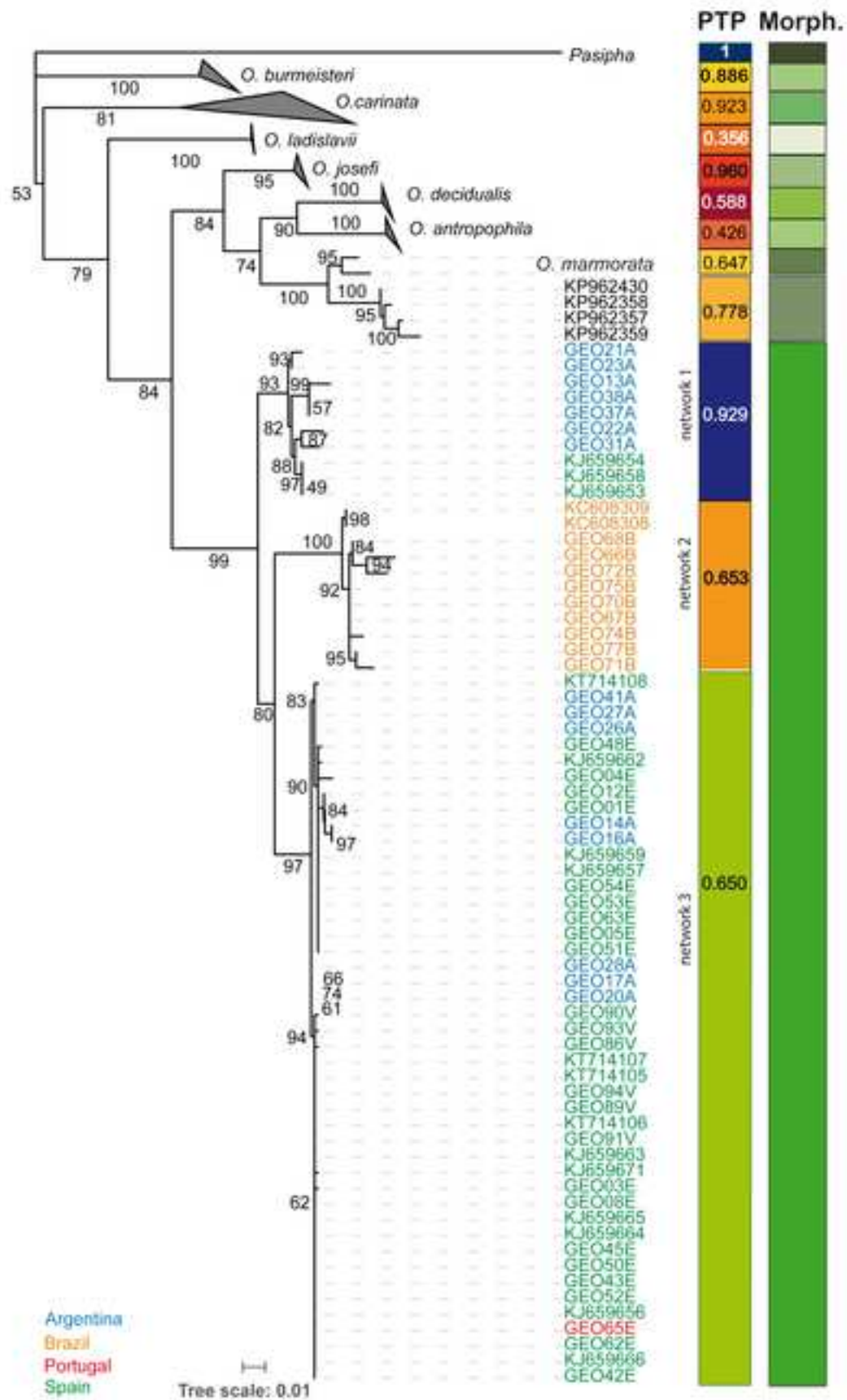


Table 1: Species analysed, with their locality and GenBank accession numbers. (GEOXX code for the processed specimen; letter A for specimens from Argentina; B specimens from Brazil; E for specimens from Asturias, Spain; V specimens from Vizcaya, Spain).

***, sequences from present study.**

Species	Locality	GenBank – accession number
Family Geoplanidae		
<i>Pasipha</i> sp. GEO15A	Vivero Kato, Buenos Aires (Argentina). (Outgroup)	MG639898*
<i>Obama nungara</i> Carbayo, Alvarez-Presas, Jones & Riutort, 2016	Torroella de Fluvià, Girona Spain.	KJ659666
	Torroella de Fluvià, Girona Spain.	KJ659665
	Bordils, Girona, Spain	KJ659664
	Gavà, Barcelona, Spain	KJ659656
<i>Obama nungara</i> GEO50E	Reborio, Muros de Nalón, Asturias (Spain).	MF155841*
<i>Obama nungara</i> GEO65E	Vila do Conde, Oporto (Portugal).	MF155842*
<i>Obama nungara</i> GEO42E	Reborio, Muros de Nalón, Asturias (Spain).	MF155843*
<i>Obama nungara</i> GEO43E	Reborio, Muros de Nalón, Asturias (Spain).	MF155844*
<i>Obama nungara</i> GEO52E	Reborio, Muros de Nalón, Asturias (Spain).	MF155845*
<i>Obama nungara</i> GEO62E	Reborio, Muros de Nalón, Asturias (Spain).	MF155846*
<i>Obama nungara</i> GEO45E	Reborio, Muros de Nalón, Asturias (Spain).	MF155847*
<i>Obama nungara</i> GEO05E	Reborio, Muros de Nalón, Asturias (Spain).	KM053212
<i>Obama nungara</i> GEO54E	Reborio, Muros de Nalón, Asturias (Spain).	MF155849*
<i>Obama nungara</i> GEO63E	Reborio, Muros de Nalón, Asturias (Spain).	MF155850*
<i>Obama nungara</i> GEO53E	Reborio, Muros de Nalón, Asturias (Spain).	MF155851*
<i>Obama nungara</i> GEO51E	Reborio, Muros de Nalón, Asturias (Spain).	MF155852*
<i>Obama nungara</i>	Treto, Cantabria (Spain).	KJ659662
<i>Obama nungara</i> GEO17A	Vivero Ferrari, Buenos Aires (Argentina).	KM053222
<i>Obama nungara</i> GEO20A	Vivero Zoológico, Buenos Aires (Argentina).	MF155854*
<i>Obama nungara</i> GEO26A	Vivero Los Toldos. Buenos Aires (Argentina).	KM053225
<i>Obama nungara</i> GEO27A	Vivero Los Toldos. Buenos Aires (Argentina).	KM053226
<i>Obama nungara</i> GEO28A	Vivero Nueve de Julio. Buenos Aires (Argentina).	KM053227
<i>Obama nungara</i> GEO03E	Reborio, Muros de Nalón, Asturias (Spain).	KM053210
<i>Obama nungara</i> GEO01E	Reborio, Muros de Nalón, Asturias (Spain).	KM053208
<i>Obama nungara</i> GEO14A	Vivero Kato, Buenos Aires (Argentina).	KM053220
<i>Obama nungara</i> GEO16A	Vivero Ferrari, Buenos Aires (Argentina).	KM053221
<i>Obama nungara</i> GEO48E	Reborio, Muros de Nalón, Asturias (Spain).	MF155862*
<i>Obama nungara</i> GEO08E	Viveros La Lloba, Piedras Blancas. Asturias (Spain).	KM053215
<i>Obama nungara</i> GEO12E	Viveros La Lloba, Piedras Blancas. Asturias (Spain).	KM053219
<i>Obama nungara</i> GEO04E	Reborio, Muros de Nalón, Asturias (Spain).	KM053211
<i>Obama nungara</i>	Bordils, Girona, Spain	KJ659663
<i>Obama nungara</i> GEO89V	Sopelana, Vizcaya (Spain).	MF155866*
<i>Obama nungara</i> GEO91V	Sopelana, Vizcaya (Spain).	MF155867*
<i>Obama nungara</i> GEO94V	Sopelana, Vizcaya (Spain).	MF155868*
<i>Obama nungara</i> GEO41A	Córdoba (Argentina).	MF155869*
<i>Obama nungara</i> GEO93V	Sopelana, Vizcaya (Spain).	MF155870*
<i>Obama nungara</i> GEO86V	Sopelana, Vizcaya (Spain).	MF155871*
<i>Obama nungara</i>	Torroella de Fluvià, Girona (Spain).	KT714108
<i>Obama nungara</i>	Torroella de Fluvià, Girona (Spain).	KT714107
<i>Obama nungara</i>	Torroella de Fluvià, Girona (Spain).	KT714106
<i>Obama nungara</i>	Torroella de Fluvià, Girona (Spain).	KT714105
<i>Obama nungara</i> GEO90V	Sopelana, Vizcaya (Spain).	MF155872*
<i>Obama nungara</i> GEO21A	Vivero Zoológico, Buenos Aires (Argentina).	MF155873*
<i>Obama nungara</i> GEO23A	Valeria del Mar, Buenos Aires (Argentina).	MF155874*
<i>Obama nungara</i>	Bordils, Girona (Spain).	KJ659654
<i>Obama nungara</i> GEO38A	Parque Pereyra Iraola, Buenos Aires (Argentina).	MF155875*
<i>Obama nungara</i>	Villasar de Mar, Barcelona (Spain).	KJ659658
<i>Obama nungara</i>	Bordils, Girona (Spain).	KJ659653

<i>Obama nungara</i> GEO22A	Vivero Zoológico, Buenos Aires (Argentina).	MF155876*
<i>Obama nungara</i> GEO37A	Parque Pereyra Iraola, Buenos Aires (Argentina).	MF155877*
<i>Obama nungara</i> GEO31A	Isla Martín García, Buenos Aires, (Argentina).	MF155878*
<i>Obama nungara</i> GEO13A	Vivero Kato, Buenos Aires (Argentina).	MF155879*
<i>Obama nungara</i> GEO66B	Santiago, Rio Grande do Sul (Brazil).	MF155880*
<i>Obama nungara</i> GEO70B	Santiago, Rio Grande do Sul (Brazil).	MF155881*
<i>Obama nungara</i> GEO67B	Santiago, Rio Grande do Sul (Brazil).	MF155882*
<i>Obama nungara</i> GEO75B	Santiago, Rio Grande do Sul (Brazil).	MF155883*
<i>Obama nungara</i> GEO77B	Santiago, Rio Grande do Sul (Brazil).	MF155884*
<i>Obama nungara</i>	Parque Estadual da Serra do Tabuleiro, SC. (Brazil).	KC608309
<i>Obama nungara</i>	Parque Estadual da Serra do Tabuleiro, SC (Brazil).	KC608308
<i>Obama nungara</i> GEO74B	Santiago, Rio Grande do Sul (Brazil).	MF155885*
<i>Obama nungara</i> GEO72B	Santiago, Rio Grande do Sul (Brazil).	MF155886*
<i>Obama nungara</i> GEO68B	Santiago, Rio Grande do Sul (Brazil).	MF155887*
<i>Obama nungara</i> GEO71B	Santiago, Rio Grande do Sul (Brazil).	MF155888*
<i>Obama anthropophila</i> Amaral, Leal-Zanchet & Carbayo, 2015	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962470
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962468
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962466
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962465
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962469
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962467
	Floresta Nacional Sao Francisco de Paula, RS (Brazil)	KP962397
	P.N. Serra do Itajai, SC. (Brazil)	KP962432
	P.N. Serra do Itajai, SC. (Brazil)	KP962431
	P.N. Serra do Itajai, SC. (Brazil)	KP962429
<i>Obama decidualis</i> Amaral & Leal-Zanchet, 2015	Santa Maria, RS (Brazil)	KP962391
	Portao, RS (Brazil)	KP962386
	Santa Maria, RS (Brazil)	KP962389
	Santa Maria, RS (Brazil)	KP962384
	Santa Maria, RS (Brazil)	KP962388
	Santa Maria, RS (Brazil)	KP962385
	Santa Maria, RS (Brazil)	KP962390
	Santa Maria, RS (Brazil)	KP962387
<i>Obama josefi</i> (Carbayo & Leal-Zanchet, 2001)	Floresta Nacional Sao Francisco de Paula, RS. (Brazil)	KP962367
	Floresta Nacional Sao Francisco de Paula, RS. (Brazil)	KC608318
	Floresta Nacional Sao Francisco de Paula, RS(Brazil)	KC608317
<i>Obama sp .</i>	P.N. Serra do Itajai, SC (Brazil)	KP962430
	P.N. Serra do Itajai, SC (Brazil)	KP962358
	P.N. Serra do Itajai, SC (Brazil)	KP962357
	P.N. Serra do Itajai, SC (Brazil)	KP962359
<i>Obama marmorata</i> (Schultze & Müller, 1857)	Parque Estadual da Serra do Tabuleiro, SC, (Brazil).	KT714104
	Parque Nacional da Serra de Itajaí, SC, (Brazil).	KT714103
<i>Obama ladislavii</i> (Graff, 1899)	P.E. Serra do Tabuleiro, SC (Brazil)	KP962492
	P.E. Serra do Tabuleiro, SC (Brazil)	KP962490
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962451
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962450
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962448
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962446
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962444
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962489
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962480
	P.E. Serra do Tabuleiro, SC. (Brazil)	KP962455
<i>Obama burmeisteri</i> (Schultze & Müller, 1857)	P.E. Cantareira, SP. (Brazil)	KP962378
	Sao Paulo, SP. (Brazil)	KP962354
	P.E. Cantareira, SP. (Brazil)	KP962371
	Sao Paulo, SP. (Brazil)	KP962355
	P.E. Cantareira, SP. (Brazil)	KP962373
	Sao Paulo, SP. (Brazil)	KP962362

	P.E. Cantareira, SP. (Brazil)	KP962377
<i>Obama carinata</i> (Riester, 1938)	P.E. Intervalles, SP. (Brazil)	KP962376
	P.E. Intervalles, SP. (Brazil)	KP962375
	P.E. Intervalles, SP. (Brazil)	KP962372
	P.E. Cantareira, SP. (Brazil)	KP962363
	P.E. Cantareira, SP. (Brazil)	KP962364
	P.E. Cantareira, SP. (Brazil)	KP962365
	P.E. Intervalles, SP. (Brazil)	KP962370
	P.E. Cantareira, SP. (Brazil)	KP962361
	P.E. Intervalles, SP. (Brazil)	KP962369
	P.E. Intervalles, SP. (Brazil)	KP962368