

## A new threat to the European marine environment: The exotic polyclad flatworm *Postenterogonia orbicularis* (Schmarda, 1859)

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### ABSTRACT

Harbours and marinas are optimal places for the detection of the arrival of Non-Indigenous Species (NIS) in the early stages of introduction. Marine traffic serves as a route for species movement among different regions, while the species settlement in the ports is facilitated due to habitat degradation. Among NIS, polyclads flatworms are active hunters of bivalves, posing a threat to recipient environments and aquaculture. They can either use their pharynx and mucus to penetrate the mussels and oysters, or enter molluscs while they filter, making the management of the species difficult. In this regard, this work aims to report the first morphological and molecular identification of a new NIS polyclad native to the Pacific Ocean in Europe, specifically in the Ría de Avilés and the Gijón Port (North of Spain, Cantabrian Sea, Bay of Biscay). The occurrence of mature ovigerous specimens and the collection of specimens of various sizes from different times of the year, evidence a reproductively-active population in the area. The external morphological characters, as the ruffled pharynx and the cephalic eyes, and the internal ones, as the male copulatory apparatus, fit with the description of *Postenterogonia orbicularis*. The phylogenetic analysis based on the mitochondrial *COI* marker, placed the studied specimens from Avilés and Gijón (Spain) and the recently described species *Idiostylochus tortuosus* from Arcachon Bay (France) within the *P. orbicularis* clade (Ilyplanidae family). Therefore, our results suggest that *I. tortuosus* may be a synonym of the New Zealander *P. orbicularis*. Finally, we discuss the possible ecological impacts of its introduction and provide some management recommendations for controlling its influx in the recipient environments.

### 1. Introduction

Nowadays, the main pathway of introduction and spreading of marine Non-Indigenous Species (NIS) is global shipping (Letschert et al., 2021; Shucksmith and Shelmerdine, 2015), mainly via ballast waters (Ashton et al., 2006; Coutts and Taylor, 2004; Ruiz et al., 2000; Shucksmith and Shelmerdine, 2015) and hull fouling (Coutts and Taylor, 2004; Sylvester and MacIsaac, 2010). Consequently, maritime ports are considered the main entrance of aquatic NIS, acting as recipient environments that may facilitate NIS establishment thanks to their

degraded habitats and the different anthropogenic structures and substrates (Hewitt and Minchin, 2009; Seebens et al., 2013). Thus, ports and marinas are the optimal places for the early detection of NIS (Fernández-Rodríguez et al., 2022). Another introduction pathway of NIS is via aquaculture, either directly or indirectly and in this case, ports may also play a role in the secondary spread of these species if they reach port areas (Costello et al., 2022). A good representative of those, is the Japanese oyster (*Magallana gigas* (Thunberg, 1793)), which accounts for 38.7 % of the total exotic species introductions (FAO, 1998; Naylor and Burke, 2005). Also, species such as the arthropods *Monocorophium ueoni*

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(Stephenson, 1932) and *Paranthura japonica* (Richardson, 1909) or the polychaete *Marphysa victori* Lavesque et al., 2017 (Gouilleux and Massé, 2019; Lavesque et al., 2013, 2020) are other examples of introductions related to aquaculture.

Polyclada is an order of free-living marine flatworms (Platyhelminthes) that includes 800–1000 worldwide-distributed species (Gutiérrez et al., 2023; Martín-Durán and Egger, 2012; Rawlinson and Litvaitis, 2008). They are characterised by the presence of a folded pharynx and a ramified intestine with several complex diverticula that account for its etymology (from the Ancient Greek: πολύς (polús), meaning ‘numerous’, and κλάδος (kládos), meaning ‘branch’). The lack of external features and the struggle to capture and preserve the samples, make their identification challenging (Bahia et al., 2017). Moreover, some families such as Enantiidae Graff, 1889 and Boniniidae Bock, 1923 have homoplasy of some morphological and anatomical characters as the pharynx organisation or the position of eyes and tentacles, but they belong to different suborders, Acotylea and Cotylea respectively (Prudhoe, 1985). It was not until the use of phylogenetic analyses, based on nuclear and mitochondrial genetic markers, that this ambiguity was clarified (Laumer and Giribet, 2014). Thus, some genera (e. g., *Theama* Marcus, 1949 and *Cestoplana* Lang, 1884) that were originally included in the Acotylea suborder, were later transferred to the Cotylea one, thanks to the phylogenetic analyses based on the *large subunit of the ribosome* (28 S) nuclear marker and the mitochondrial *Cytochrome c Oxidase Subunit I* (COI) molecular markers (Laumer et al., 2015; Laumer and Giribet, 2014; Rawlinson et al., 2011; Rawlinson and Stella, 2012; Yuki and Hiroshi, 2020). The polyclads encompass different species that feed on invertebrates (e.g., Arthropoda, Cnidaria, Mollusca) (Barton et al., 2020; Gallani et al., 1980; Lee, 2006; Teng et al., 2022) and within molluscs, the commercial bivalves are some of the most threatened by these flatworms, causing massive mortality and serious damage in aquaculture facilities (Vieira et al., 2020). Among cultured bivalves, mussels, oysters and clams are the most common groups actively hunted by polyclads (Lee, 2006; Teng et al., 2022), leading to important economic losses (Vieira et al., 2020). The planktonic larvae or juveniles of polyclads travel in the water column (Martín-Durán and Egger, 2012). They can enter bivalves when they filter and develop inside molluscs, such as *Postenterogonia orbicularis* (Schmarda, 1859) does, making the management of the pest a real challenge for the aquaculture facilities (Castinel et al., 2019; Georgiades et al., 2016). *Postenterogonia* Faubel, 1893 is a monospecific genus within the family Ilyplanidae Faubel, 1983 native to New Zealand and Australian waters and it is also recorded in Japan (Oya, 2023). The only known species, *P. orbicularis*, is described as a hazard for the bivalves of the aquaculture farms in New Zealand (Georgiades et al., 2016). This hazard might be taken into account, since Asturias is a community with an important bivalve production, with a total of 292.531 kg of molluscs captured in 2023, leading to 2.179.702 € of profits in Asturias ([www.sadei.es](http://www.sadei.es)), and the main aquaculture facility is placed in the estuary of Ría del Eo (focused on *M. gigas*) (29 T 59277 21300).

The main goal of this work is to report the first molecular and morphological identification of the non-native flatworm *P. orbicularis* (Ilyplanidae) in European waters, found in the Ría de Avilés and the Gijón Port (Cantabrian Sea, northern Spain). We provide brief notes on its biology, ecology and distribution at its new locality in order to better assess the invasive potential of this alien species. Finally, we discuss the possible ecological impacts of its introduction, as well as some management recommendations for preventing and controlling its influx in the recipient environments.

## 2. Material and Methods

### 2.1. Sampling area and specimens' collection

The Asturian coastline (Bay of Biscay) has an approximate extension of 401 km within the Cantabrian Sea coast from Castropol to Bustio

(MITECO, 2024). The region is geographically divided by Peñas Cape (30 T 70162 37009) which is the northernmost cape in the Principality of Asturias, Spain. The substrate consists of a gently sloping rocky platform, where the upper intertidal is dominated by invertebrates (barnacles and limpets) and the lower intertidal by macroalgae (Fernández, 2011). The W-E coastal current influences the movement of nutrient inputs, with Peñas Cape being the source of upward movement and input in the western part of the cape (Flor, 1977).

This extensive coastline is dominated by 22 fishing villages, each of which has its own port and a strong fishing tradition. In addition, the region has a fleet of more than 250 fishing vessels (SADEI, 2024) and two important commercial ports, Gijón and Avilés. Two fishing ports (Cudillero and Lastres) and two commercial ports (Avilés and Gijón) were sampled for this study.

The port of Cudillero is in the western area of Asturias (29 T 30506 27333). The harbour has 4 floating pontoons and a total of 83 fixed moorings for pleasure craft, and can receive vessels with a maximum length of 8 m and a minimum draught of 3 m. It also has a great fishing tradition, which in the last few decades has decreased to 48 fishing ships (SADEI, 2024) (see [supplementary material 1](#)).

The port of Lastres (30 T 16544 20355), whose main activity is fishing, has a sport dock equipped with 6 pontoons with fixed moorings where boats with a draught of 3.5 m and a maximum length of 14 m can moor. Although it has a strong fishing tradition, like Cudillero, its fleet has been reduced to around 20 fishing ships (SADEI, 2024) (see [supplementary material 1](#)).

The port of El Musel - Gijón is the main bulk port in Spain and the first in the movement of goods by rail, making it one of the most important ports in the Atlantic Arc. This port is divided into 3 different areas: El Musel Port (30 T 81959 25990), Gijón Marina (30 T 83288 26811) and Marina Yates (30 T 82933 25557). The three areas, which are geographically separated, differ in the type of vessels they house and in the economic activity that takes place in them. While El Musel is mainly a commercial port, focused both on the merchant marine sector and the fishing sector, the ports of Marina Yates, which have 156 moorings, and the Gijón Marina with 630 moorings, are focused on the provision of services related to small vessels, such as berthing or maintenance (Puertos del Estado, 2024) (see [supplementary material 1](#)).

The port of Avilés (30 T 64028 27538) is one of the main seaports in the Atlantic Arc. Its location benefits the establishment of domestic traffic between the northern half of the Iberian Peninsula and the Mediterranean Sea, as well as with the Canary Islands. It occupies 980,000 m<sup>2</sup> and has more than 4700 linear m of quays, including areas divided by type of traffic on both sides of the river. The infrastructures of the port make it possible to reach the figure of some 6 million tonnes per year, divided into solid bulk (62 %), liquid bulk (11 %) and general cargo (27 %). These figures are very important considering that it is an estuary port that receives sediments from different sources, having to dredge it annually to maintain the marine draught. The port of Avilés loads/unloads goods, to and from more than 48 countries all over the world (e.g. Australia, Brazil, Canada, USA, France, Italy or Norway). It is also the main fishing port in the whole region in terms of catches unloaded. The port also has a small marina with recreational boats located on the left bank of the river, with about 200 berths distributed over 16 piers for small sport boats (Puerto de Avilés, 2024) (see [supplementary material 1](#)).

Samples were collected from 52 small boats moored in the following Asturian ports: Avilés (3), Gijón (11), Lastres (15), Luanco (5), El Puntal (5), Candás (3), San Esteban de Pravia (2), Llanes (1) Oviñana (1) and Cudillero (6). Among the vessels sampled are fishing boats, pleasure boats and sailing boats, all of which have fibre or steel hulls and sail at speeds of between five and 20 knots (see [supplementary material 1](#)).

Rocky beaches with the same characteristics (e.g. slope and materials) were chosen because of their distance from each of the ports and marinas studied, taking one point next to the harbour and another one far away from them (see [supplementary material 1](#)).

Ports were sampled from 2021 to 2024 every four months and beaches every three months since 2023. Samples from boats were taken during dry-docking, from 2022 to 2023 (see [supplementary material 1](#)).

Polyclads were collected from inside and among mussels (*M. galloprovincialis*) attached to the docks in the harbour of Avilés, located in the Ría de Avilés and Gijón port, Asturias, North of Spain ([Fig. 1](#)), from February 2023 to July 2024 ([Fig. 1](#)). The water temperature during sampling was 13.1 °C and 15.3 °C, respectively. Salinity remained around 33 practical salinity units (PSU) throughout the year. Flatworms were anaesthetised with menthol and stored in 70 % ethanol for histological processing and 100 % ethanol for genetic analyses. The rest of the specimens were deposited in the Zoology Collection of the University of Oviedo ([Table 1](#)).

## 2.2. DNA extraction and amplification

Total DNA was extracted from three specimens using the Illustra Tissue & Cells GenomicPrep Mini Spin Kit (GE Healthcare, Buckinghamshire, United Kingdom) according to the manufacturer's instructions. Briefly, a total of 50 mg of tissue were homogenized in 200 µL of phosphate-buffered-saline (PBS, pH 7.4) in a Precellys 24 Tissue Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) for 2x30s at 5000 rpm, using 1.4 mm ceramic beads (Precellys lysing kit CK14 Bertin Technologies, Montigny-le-Bretonneux, France). Lysis buffer and proteinase K were added to 50 µL of the supernatant and incubated overnight at 56 °C in a thermomixer (Ohaus, Nanikon, Switzerland). DNA was obtained in 0.1 ML of elution buffer and quality and quantity of the extracted DNA were analysed spectrophotometrically (NanoDrop ND-100, ThermoFisher Scientific, Waltham, MA, USA). DNA samples were stored at -20°C until use.

A region of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene (720 bp) was amplified by conventional PCR using the primers Acotylea COI-F (5'-ACTTTATTCTACTAATCATAAGGATATAGG-3') and

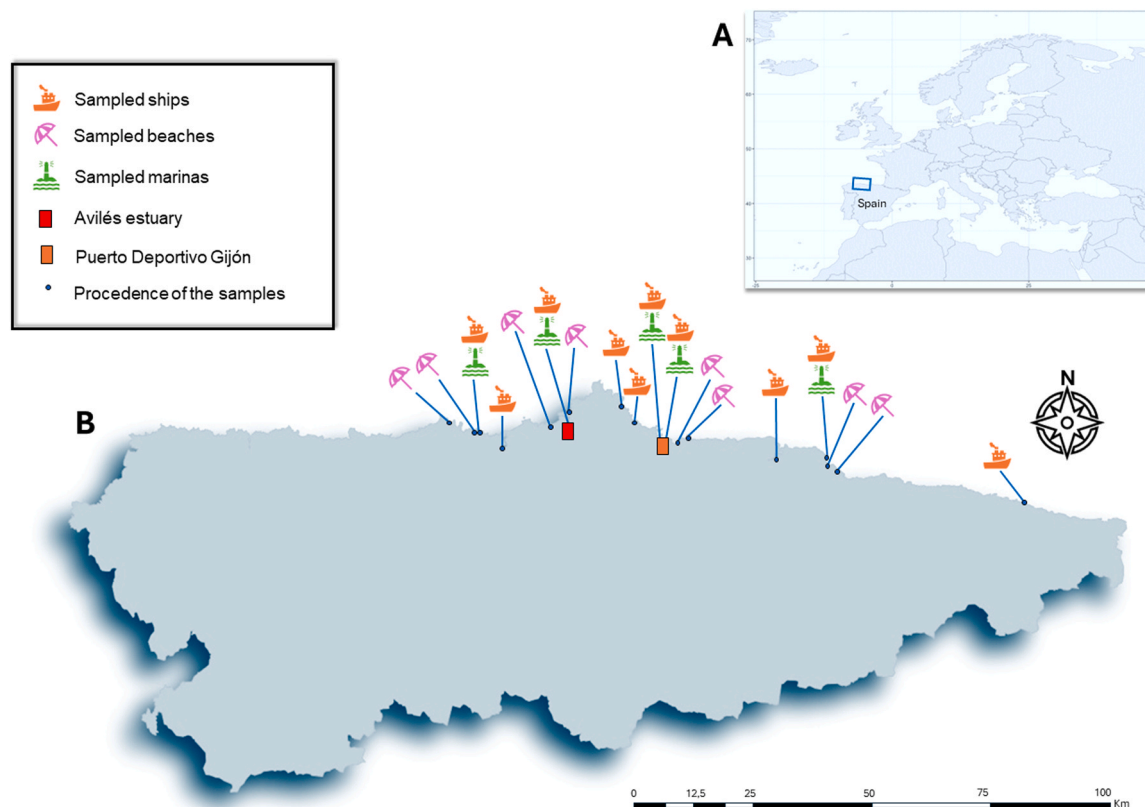
Acotylea COI-R (5'-CTTCTCTATAAAAATGTTACTATTGAGA-3') ([Oya and Kajihara, 2017](#)) PCR amplifications were performed in a 2720 Thermal Cycler (Applied Biosystems, ThermoFisher Scientific, USA) with 12.5 µL of Long Amp Hot Start Taq Master Mix (BioLabs INC, New England), 200 µM of each primer, 5 µL of template DNA and molecular grade water to final volume of 25 µL. Run conditions were as follows: denaturation at 95°C for 1 min, 40 cycles at 95°C for 1 min, 50°C for 1 min and 72°C for 1 min and a final extension of 72°C for 7 min.

Negative controls were included in each assay run. All the PCR products of the expected size were purified using NZYGelpure (NZYTech Lda, Lisboa, Portugal) and sent to an external laboratory (Eurofins Genomics, Köln, Germany) for Sanger sequencing with the same forward and reverse primers used in PCR amplifications (Acotylea COI-F/Acotylea COI-R).

## 2.3. Phylogenetic analyses

The newly generated *COI* sequences were manually reviewed and edited in Geneious Prime V. 2023.2.1 ([Kearse et al., 2012](#)). Ambiguities (i. e. double peaks) were considered "valid" whenever (1) the lower peaks reached at least a third of the total height of the highest peaks and (2) were present in both the forward and reverse sequences.

The edited sequences were used to generate the *COI* dataset that comprised sequences belonging to Ilyplanidae family generated in previous studies (see [supplementary material 2](#)). *Pericelis flavomarginata* [Tsuyuki et al., \(2020\)](#) from the Pericelidae Laidlaw, 1902 and *Cestoplane nopperabo* [Oya and Kajihara, 2018](#) were used as outgroup, since it has been used successfully in previous phylogenetic studies ([Gutiérrez et al., 2023](#)). This *COI* dataset was aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) alignment algorithm as implemented in Jalview 2.11.1.3 ([Edgar, 2004](#); [Waterhouse et al., 2009](#)). The best-fitting substitution model was estimated by the Akaike Information Criterion (AIC) in jModelTest 2.1.10 ([Akaike, 1974](#); [Darriba](#)



**Fig. 1.** Sampling area. A. Location of Asturias within Europe. B. Different ships, beaches and ports sampled in Asturias (central Cantabrian Sea).

**Table 1**  
Identification number of each specimen, date and remarks.

Voucher ID	Species ID	Collection data	Remarks	GenBank accession number
BOS-PLA-00001	<i>Postenterogonia orbicularis</i>	Avilés Port 01/02/24	Specimen used for histological analysis	
BOS-PLA-00002	<i>Postenterogonia orbicularis</i>	Avilés Port 22/11/23	Specimen used for phylogenetic analysis	PP974229
BOS-PLA-00003	<i>Postenterogonia orbicularis</i>	Avilés Port 22/11/23	Specimen used for phylogenetic analysis	PP974230
BOS-PLA-00004	<i>Postenterogonia orbicularis</i>	Avilés Port 22/11/23	Specimen used for phylogenetic analysis	PP974231
BOS-PLA-00005 to BOS-PLA-00045 BOS-PLA-00046	<i>Postenterogonia orbicularis</i>	Avilés Port 22/11/23		
BOS-PLA-00047	<i>Postenterogonia orbicularis</i>	Avilés Port 28/02/23		
BOS-PLA-00047	<i>Postenterogonia orbicularis</i>	Gijón Port 16/02/23	Specimen used for phylogenetic analysis	PP974232
BOS-PLA-00048 to BOS-PLA-00050	<i>Postenterogonia orbicularis</i>	Gijón Port 16/02/23		
BOS-PLA-00051 to BOS-PLA-00053	<i>Postenterogonia orbicularis</i>	San Juan de Nieva 04/07/2024	Specimens used for histological analysis	
BOS-PLA-00054 to BOS-PLA-00072	<i>Postenterogonia orbicularis</i>	San Juan de Nieva 04/07/2024		

et al., 2012), being the General Time Reversible model with gamma distribution and invariable sites (GTR+G+I) the model with the best AIC score (Tavaré, 1986; Rodriguez et al., 1990; Yang, 1994).

The phylogenetic relationships among these taxa were inferred by two different methods: Maximum Likelihood (ML) as implemented in IQtree 1.6.12 webserver (Nguyen et al., 2015; Trifinopoulos et al., 2016) and Bayesian Inference (BI) as implemented in Mr. Bayes 3.2 (Ronquist et al., 2012). The ML analysis was performed by generating an initial Neighbour-Joining (NJ) tree, whose branches were rearranged by the Nearest-Neighbour Interchange (NNI) method, while branch support was estimated by 10,000 ultrabootstrap replications (BT) (Robinson, 1971; Moore et al., 1973; Chernomor et al., 2016; Hoang et al., 2017; Minh et al., 2013). The BI analysis was performed using 2 parallel analyses consisting of 4 Markov Chain Monte Carlo (MCMC), 1 cold and 3 hot chains, that searched for 2,000,000 generations, having a burning fraction of 0.25 (Hastings, 1970). Statistical branch support of the BI analysis was estimated by posterior probability (PP) (Metropolis et al., 1953; Hastings, 1970). Consensus tree branches were collapsed when their PP or BT statistical support of the obtained topologies was below 50 %.

#### 2.4. Histological analysis

Four specimens were employed for histological observation. Specimens were fixed in paraformaldehyde for 24–48 h, washed thereafter in 70 % ethanol for two days prior to dehydration, dehydrated following standard methods, transferred to Histo-Clear and embedded in paraffin wax. Serial, 10 µm thin sagittal sections were cut with a Leica Biosystems HistoCore Biocut microtome and double stained with haematoxylin and eosin and mounted in Eukitt Mounting Medium.

### 3. Results

#### 3.1. Taxonomy

Phylum Platyhelminthes Claus, 1887  
Order Polycladida Lang, 1881  
Suborder Acotylea Lang, 1884  
Family Ilyplanidae Faubel, 1983  
Genus *Postenterogonia* Faubel, 1983  
*Postenterogonia orbicularis* (Schmarda, 1859)

##### 3.1.1. Material examined:

**Avilés** – Ría de Avilés, Avilés harbour: 1 specimen, coll. date 28/02/

2023; 46 specimens, coll. date 22/11/2023; 1 specimen, coll. date 01/02/2024, among and inside *M. galloprovincialis* clusters from the docks of the harbour (30 T 64028 27538); San Juan de Nieva: 22 specimens, coll. date 04/07/2024, inside dead *M. gigas* and behind rocks in a natural beach next to the entrance of the port (30 T 63453 30875). **Gijón** – Puerto Deportivo Gijón, Gijón Port: 4 specimens, coll. date 16/02/23, among and inside *M. galloprovincialis* clusters from the docks of the



**Fig. 2.** Overview of the docks of the Avilés harbour, with a sample of *Postenterogonia orbicularis* inside the shell of *Mytilus galloprovincialis*.

harbour (30 T 83288 26811) (Fig. 2).

3.1.2. Diagnosis

Medium sized flatworm (collected specimens range from 20 to 60 mm in length), oval shape, narrowing towards the posterior margin. Brownish body with dark brown dots on the dorsal surface but not in the ventral one observed alive, ethanol preserved specimens turned cream

in colour. Oviducts and sperm ducts visible in whitish colour, ruffled pharynx located at centre of body (Fig. 3A, B). Cerebral and marginal eyes present (Fig. 4). Seminal vesicles lacking in males, large penis papilla, prostatic vesicle present (Fig. 3C, D). Female copulatory apparatus formed by genito-intestinal duct and vagina, running posteriorly to enter the intestine (Fig. 3C).

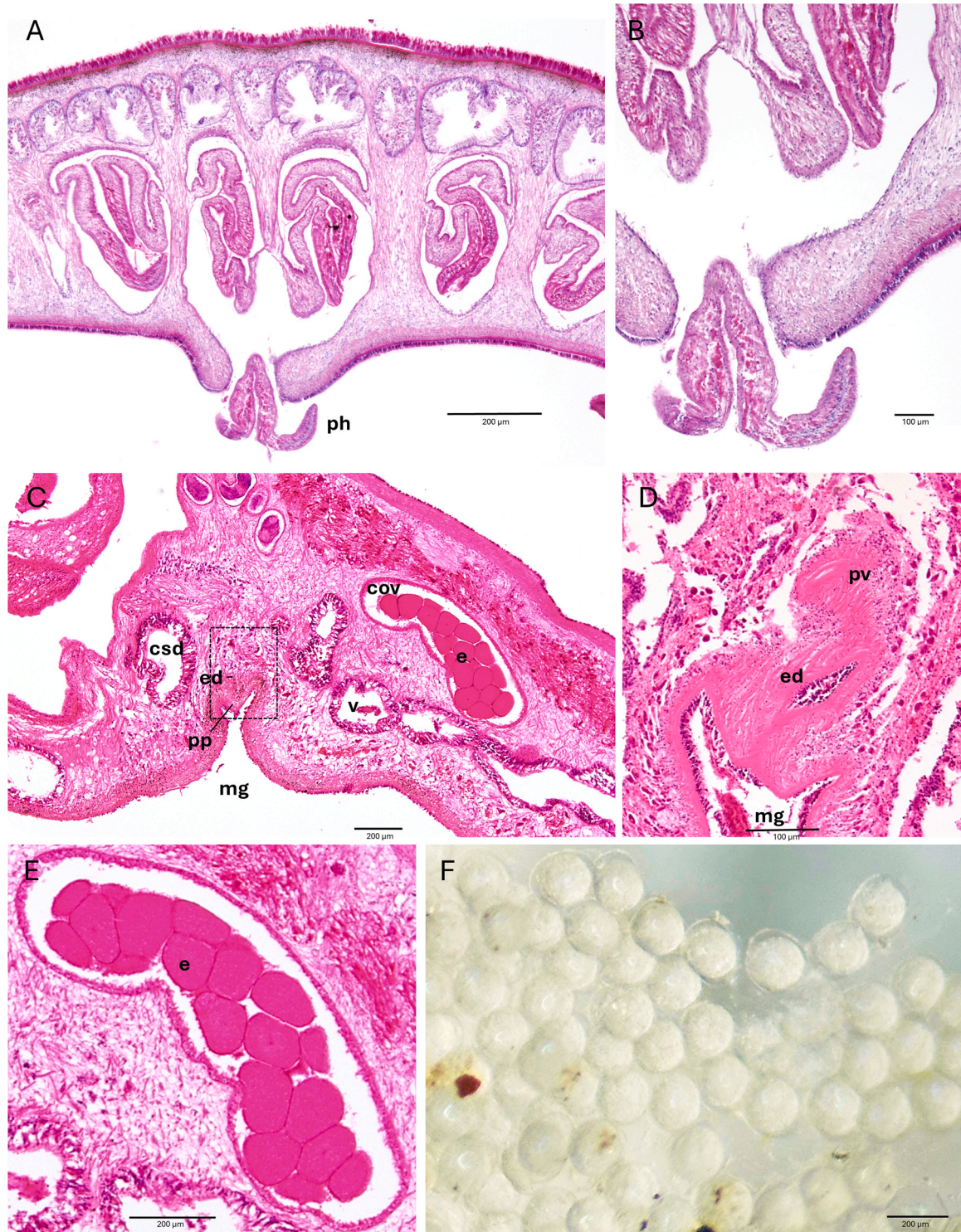


Fig. 3. Photographs of the histological sections of *Postenteregonia orbicularis*. A. sagittal section of the pharyngeal region. B. Detail of the ruffled pharynx. C. sagittal section of male copulatory apparatus. D. Detail of the prostatic vesicle and the ejaculatory duct. E. detail of uterine eggs. F. Released eggs. Abbreviations: cov, common oviduct; csd, common sperm duct; e, eggs; ed, ejaculatory duct; mg, male gonopore; ov, oviduct; ph, pharynx; pp, penis papilla; pv, prostatic vesicle; sd, sperm duct; va, vagina.

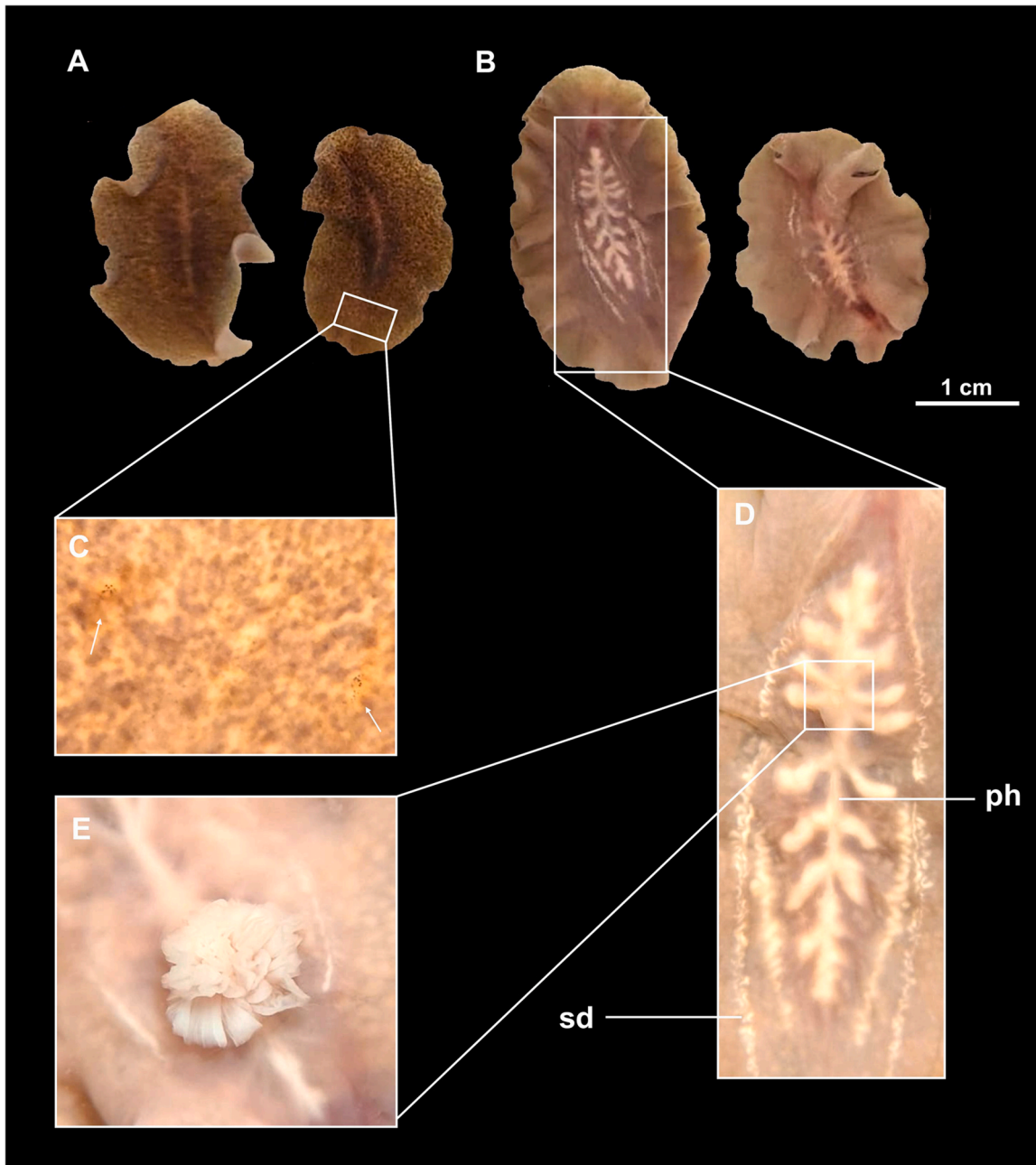


Fig. 4. Photographs of live specimens of *Postenterogonia orbicularis*. A. live specimens, ventral dorsal. B. live specimens, ventral view. C. dorsal view with arrows showing the tentacular eyes. D. enlarged view of the pharynx, E, detailed view of the ruffled pharynx evaginated. Abbreviations: ph, pharynx, sd, sperm duct.

### 3.2. Phylogenetic analyses

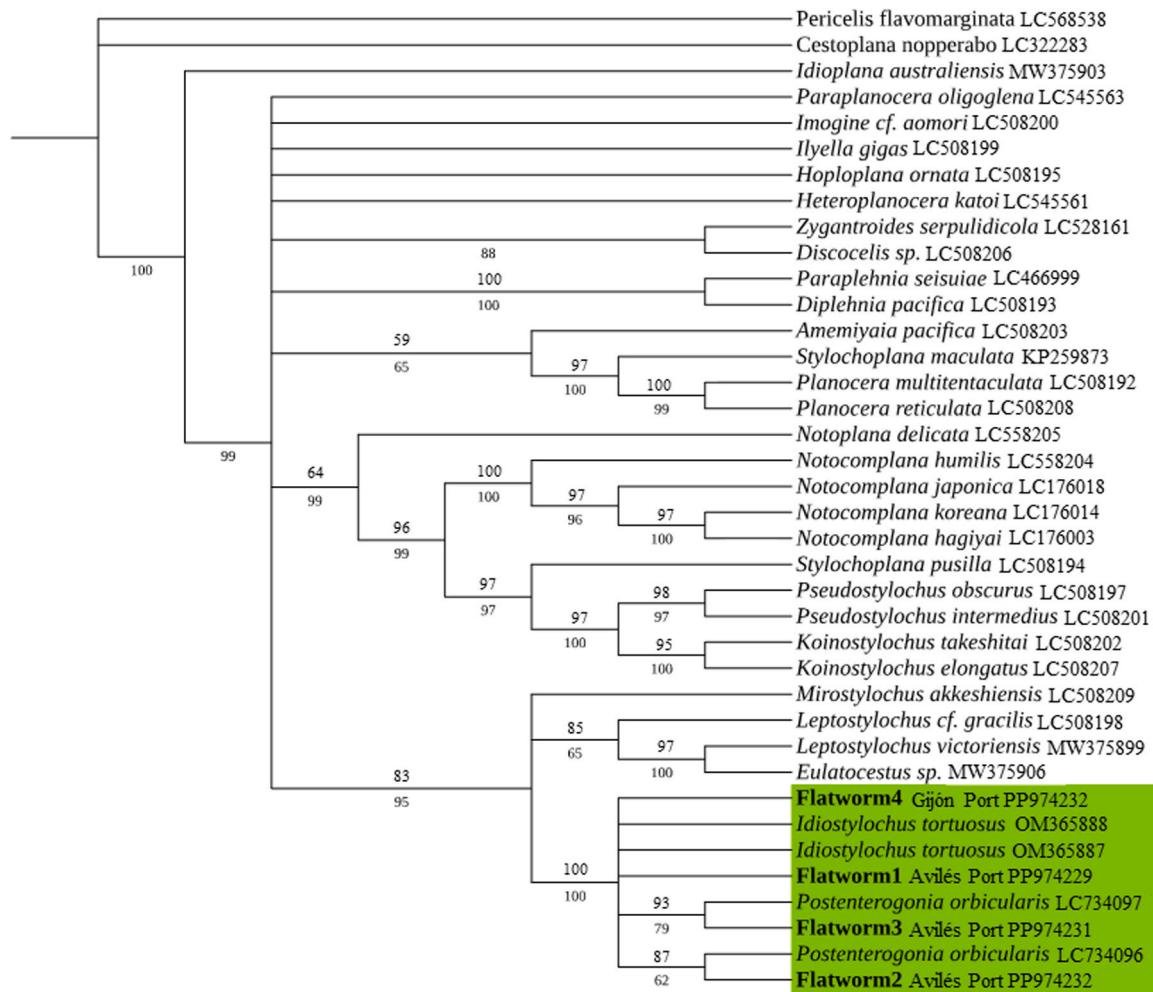
A total of 37 different sequences belonging to 33 taxa formed the *COI* dataset, which had a length of 693 pb, 277 parsimonious-informative sites (see Table 2 for more detail). The ML and BI analysis generated consensus topologies in which our samples were found in a well-supported clade (Fig. 5), comprising sequences from *Mirostylochus*,

*Leptostylochus*, *Eulatocestus*, *Idiostylochus* and *Postenterogonia* (PP: 93 %; BT: 95 %). This clade is formed by two subclades: the low-supported *Leptostylochus-Eulatocestus* subclade (PP: 88 %; BT: 66 %) and the highly supported *Idiostylochus-Postenterogonia* subclade (PP: 100 %; BT: 100 %). Within the latter subclade, the sequences from *I. tortuosus*, *P. orbicularis* and our newly generated sequences (Flatworm 1–4) did not form separate species-specific inner subclades. Furthermore, our

Table 2

Main features of the *COI* dataset alignment. Sites were considered to be variable when at least two different nucleotides were present in the same position, while a site was considered to be Parsimonious-Informative when each of the different nucleotides found at this position has a minimum frequency of two. Outgroups have been excluded.

Number of sequences	Number of taxa	Range of length of sequences (pb)	Alignment length (pb)	C+G (%)	Number of Variable sites	Number of Parsimonious-Informative
37	33	604–688	693	37.3	298	277



**Fig. 5.** Consensus topology obtained from the ML and BI analyses based on *COI*. Numbers under the branches correspond to the Posterior Probability (PP) statistical support from the Bayesian Inference (BI) analysis, while numbers over the branches correspond to the ultrafastBootstrap (BT) statistical support from the Maximum Likelihood (ML) analysis. The subclade in which the samples of this study (Flatworm 1–4) were found has been highlighted in green.

samples did not generate an exclusive clade, being in any case closely related to *P. orbicularis* sequences by weak PP and BS support.

### 3.3. Habitat, distribution and biology

*Postenterogonia orbicularis* specimens ( $N = 72$ ) were found in the harbours of Avilés and Gijón, two of the most important ports in the Atlantic Arc, receiving traffic from 48 different countries and being Avilés dredged once a year. The polyclads were mainly found on mussel clusters in shallow water (Figs. 2, 6). In Avilés, the clusters were mainly constituted by the Mediterranean mussel, *M. galloprovincialis* (Fig. 2), together with the invasive *Xenostrobus securis* (Lamarck, 1819) in smaller proportion. Mussel clusters were attached to the docks and pontoons where the ships and recreational boats moored (Fig. 2). The 22 specimens discovered on San Juan de Nieva beach, located at the entrance of Avilés harbour (within the Ría de Avilés) were collected underneath rocks and within oyster beds of *M. gigas* (Fig. 6). This natural beach is situated in the mouth of the port, at a considerable distance from the primary area where general port activities occur (Fig. 6).

Although *M. galloprovincialis* and *M. gigas* clusters were found in all sampled locations, including the 52 boats sampled, no *P. orbicularis* specimens were detected in the rest of the different sampling points. Neither from the other two small fishing ports sampled (Lastres and Cudillero) nor among the different rocky shores sampled.

Specimens collected in July 2024 (40–60 mm long) were ovigerous,

most carried uterine eggs ranging from 160 to 170  $\mu\text{m}$  in diameter ( $N=40$ ) (Fig. 3C, E) and a small portion of them were mature enough to release their eggs during the magnesium chloride relaxation process. The released eggs had a diameter of between 190 and 210  $\mu\text{m}$  ( $N=40$ ) (Fig. 3F). This, together with the collection of specimens of various sizes from different times of the year, indicate the occurrence of a *P. orbicularis* reproductively-active population in the Ría de Avilés.

## 4. Discussion

Acotylean polyclads suborder enter molluscs and use their mucus and their pharynx to attach themselves to their prey (Hyman, 1951; Gammoudi et al., 2017), allowing the species to travel and disperse with mussels and oysters, as it is suggested for different species of the Polycladida order (Bahia, 2016). Nevertheless, introduction of NIS by ballast waters cannot be ruled out (Tosetto et al., 2023).

The order encompasses different species catalogued as pests in the aquaculture facilities, due to their voracious behaviour and the densities they reach. *Postenterogonia orbicularis*, in New Zealand, is described as a disease of concern for different species of mussels (*Perna canaliculus* (Gmelin, 1791), *M. galloprovincialis*) and oysters (*M. gigas*, *Ostrea chilensis* Küster, 1844) (Castinel et al., 2019; Georgiades et al., 2016). Several marine aquaculture farms, mainly focussed on mussels and fish, are sited in Spain (5012 farms in 2020) (Borja et al., 2018), turning the arrival of this species into a potential hazard to molluscs' farms. The



**Fig. 6.** A. Overview of the San Juan de Nieva beach. B. *Postenterogonia orbicularis* specimen inside a dead *M. gigas* (red circle). C. Death mussels on the bottom of the harbour of Avilés.

larvae of *P. orbicularis* can enter the mussels or the oysters when they are filtering (Georgiades et al., 2016), which might make the species management difficult (Handley and Nelson, 2002). The specimens were only found in the Ría de Avilés and Gijón harbour. No other specimens were detected in other harbours, beaches (except for San Juan de la Nieva) or ships sampled. The harbours do not receive traffic from the places in which this species has been reported, so it might be distributed in other areas apart from the known ones. Thus, it would be advisable for the harbours of Belgium, France, Portugal and The Netherlands to carry out studies to detect the early introduction of this species, because they are the main ports that receive ships from Avilés and Gijón.

The first detection of *P. orbicularis* was in February 2023, with only one specimen found in *M. galloprovincialis* clusters from Avilés harbour and four specimens in Puerto Deportivo Gijón, whereas 45 specimens were collected among empty mussel clusters in a single dock in November 2023. More recently, in July 2024, 22 specimens were collected from a natural beach in the entrance of the Estuary of Avilés inside dead *M. gigas* and underneath rocks.

Remarkably, in a sampling carried out in March 2024 almost the entire mussel populations, including the invader *X. securis*, were found empty in Avilés harbour and no individuals of *P. orbicularis* were detected. However, due to the industrial environment of the ria, a chemical or pollutant spill cannot be discarded as the reason for the massive death of mussels (Fig. 6). The success of *P. orbicularis*, in establishing a reproductively-active population in the Ría de Avilés indicates a high invasive potential of this species in recipient ecosystems, since the species can adapt to environmental conditions and reproduce sexually.

The phylogenetic analyses placed the specimens of Avilés and Gijón within the *Idiostylocus-Postenterogonia* subclade, where neither *P. orbicularis* nor *I. tortuosus* formed species-specific subclades, suggesting that these monospecific taxa may be synonymous and that Spanish specimens belong to one of them. The external anatomic features and the pictures of specimens from Avilés, Gijón and Arcachon (Southwest France), as the ruffled pharynx, the marbled brown dots and

the whitish shape made by the sperm ducts and the pharynx, fit with the morphological description made by Schmarda (1859) and Oya (2023) of *P. orbicularis*. Gutiérrez et al. (2023) described *I. tortuosus* as having a tubular Lang's vesicle, whereas *P. orbicularis* bears a genito-intestinal duct instead of the Lang's vesicle. However, apart from this peculiarity, no morphological differences were found among *I. tortuosus* and *P. orbicularis*. Likewise, COI analysis cannot discriminate between both species. Therefore, we recommend being cautious and take fully into account the possibility that *I. tortuosus* can be a synonym of *P. orbicularis*. A careful revision of the type material of both species (or at least of *I. tortuosus*) is peremptory for clarifying the discrepancy outlined above and for confirming the presumed synonymy between *I. tortuosus* and *P. orbicularis*. Our results represent another example of the usefulness of genetic tools for shedding light on how a presumably native species could actually be an exotic species that, in this case, represents a potential danger for rocky shore bivalves (Castinel et al., 2019; Georgiades et al., 2016).

This is the first molecular and morphological identification of *P. orbicularis* in European waters and in a brackish water ecosystem. The damage that this species can cause has not been evaluated yet but seems to be detected in an early stage of introduction, which confirms that ports are the ideal places for the detection of novel introductions of exotic and invasive species (Fernández-Rodríguez et al., 2022). A similar flatworm species from the same order, *Stylochus mediterraneus* Galleni, 1976, is known to cause important damage in the bivalve aquaculture (Gammoudi et al., 2017). Control of possible adverse effects of *P. orbicularis* as an invasive species would be facilitated by the semi-enclosed nature of Avilés and Gijón Harbours. Thus, we can suggest some management recommendations on the prevention and control of this potential invasive species. It is essential to develop a strategy to involve local shellfish gatherers, fishermen and practitioners to be aware of the danger of exotic flatworms as bivalve predators. Educating local population and port personnel about the possible ecological and economic impacts of invasive species could have immediate benefits (Arias et al., 2013).

Furthermore, a detailed monitoring of the dynamics of *P. orbicularis* should be undertaken in order to establish and understand the evolution of this species in this region and the possible impacts it may cause on the recipient environments and the local aquaculture activities.

#### CRediT authorship contribution statement

**Esteban Pascual-Parra:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ricardo López-Alonso:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana Del Cerro:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Claudia González-Toral:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Jesús García-Maza:** Writing – review & editing, Resources, Methodology. **Iris Carrera-Rodríguez:** Writing – review & editing, Resources, Methodology. **Andres Arias:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Eduardo Cires:** Writing – review & editing, Resources, Methodology, Investigation. **Marta Muñoz:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Deva Menéndez-Teleña:** Writing – review & editing, Resources, Methodology, Investigation. **Verónica Soto-López:** Writing – review & editing, Resources, Methodology, Investigation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.rsma.2024.103758](https://doi.org/10.1016/j.rsma.2024.103758).

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