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**CELLULAR AND MOLECULAR BIOLOGY**

# An integrative study of the invasive jumping-snail *Ovachlamys fulgens* (Gastropoda, Helicarionidae) in Rio de Janeiro and its fast spreading in Southeastern and Southern Brazil

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**Abstract:** The Japanese invasive jumping snail *Ovachlamys fulgens* is a pest of ornamental plants and an intermediate host of a nematode that causes eosinophilic meningitis. We expand its distribution to eight municipalities from Rio de Janeiro State, and one locality from the Paraná State, and generated for the first time partial sequences of the cytochrome c oxidase subunit I (COI) gene for Brazilian populations. External morphology, reproductive system, shell, radula, and jaw were also analyzed and described. Twenty-one lots were collected from Rio de Janeiro, Niterói, Magé, Miguel Pereira, Petrópolis, Teresópolis, Nova Friburgo, Bom Jardim and Paraty, in Rio de Janeiro State, and from Foz do Iguaçu, Paraná State. External morphology, shell and reproductive system were typical of *O. fulgens*, with some peculiarities found in the shell and radula. A single haplotype was found, which was 100% similar to sequences of COI available in GenBank for specimens from Japan and Argentina. The species seems to be adapted to many habitats and be rapidly expanding its distribution in Southeastern and Southern Brazil, and other South America countries. We highlight the importance of monitoring *O. fulgens*, considering its potential to compete with native mollusks, attack several plants, and be a transmitter of diseases.

**Key words:** Invasive species, ornamental plant pest, rapid spreading, terrestrial mollusk.

## INTRODUCTION

The genus *Ovachlamys* Habe, 1946 has traditionally been classified in the family Helicarionidae Bourguignat, 1877, although Schileyko (2002) has classified it in Euconulidae H. B. Backer, 1928. In a recent study, Páll-Gergely et al. (2017), based on molecular studies, solved this conflict by classifying the genus *Ovachlamys* in the family Helicarionidae.

*Ovachlamys fulgens* (Gude, 1900) is a small terrestrial gastropod whose shell measures around 6-7 mm in diameter and 4.5 in height, popularly known as jumping snail because it possesses the ability to jump when threatened (Capivera & White 2011). It was originally described from Ryukyu Islands (also known as Loo-Choo), an archipelago currently administered by Japan (Barrientos 2000). This territory is formed by subtropical forests with a great biodiversity of organisms (Yasuhiro et al. 2004).

Currently, *O. fulgens* is widely distributed, occurring in Southeast Asia in countries such as Singapore and Thailand (Capivera & White 2011) and China (Formosa Island) (Hwang 2014). In the

Pacific Ocean is registered to Australia (Smith et al. 2002), French Polynesia (Mo'Orea) (Lovenburg 2009), American Samoa (Tutuila Island), Vanuatu and Hawaii (Cowie 2001). In continental United States occurs in Florida, Miami and Chicago (Robinson & Slapcinsky 2005), while in Central America it is reported to Nicaragua (Pérez et al. 2005) and Costa Rica (Barrientos 1998, 2000, 2003). It is also reported to Trinidad and Tobago, Lesser Antilles (Stange 2004, Capivera & White 2011). In South America, it has been reported to Colombia (Robinson & Slapcinsky 2005), Argentina (Beltramino et al. 2018) and Brazil. In Brazil *O. fulgens* is cited to São Vicente (Porchat Island and Japuí) and Santos (São Vicente Island), São Paulo State (Teixeira et al. 2017), Teresópolis and Guapimirim (Salles et al. 2018), Rio de Janeiro State, in the Serra dos Órgãos Park National (Parnaso), and Blumenau, Botuverá and Bombinhas, Santa Catarina State (Agudo-Padrón 2019).

The species *O. fulgens* was already found naturally infected with *A. cantonensis* in Hawaii (Kim et al. 2014), which causes the zoonosis eosinophilic meningitis, currently considered an emergent disease in Brazil (Morassutti et al. 2014). Also, several species of terrestrial mollusks have been found naturally infected with *A. costaricensis* that causes the human abdominal angiostrongyliasis (Chen 1935) (Graeff-Teixeira et al. 1993, Ohlweiler et al. 2010, Thiengo & Fernandez 2013, Rodriguez et al. 2018). *O. fulgens* is a pest of orchids (Stange 2004) and of a wide variety of plants exported and imported by several countries (Robinson & Slapicinsky 2005).

This study aimed to expand the geographic distribution of *O. fulgens* to eight municipalities from Rio de Janeiro State, and one locality from the Paraná State, and generate for the first time sequences of the cytochrome c oxidase subunit I (COI) gene for Brazilian populations. External morphology, reproductive system, shell, radula and jaw were also analyzed and described.

## MATERIALS AND METHODS

Specimens were collected in the municipalities of Rio de Janeiro, Niterói, Magé, Miguel Pereira, Petrópolis, Teresópolis, Nova Friburgo, Bom Jardim and Paraty, from 2016 to 2019, and deposited in the Collection of Mollusks of the Oswaldo Cruz Institute (CMIOC 10983 to CMIOC 11000, CMIOC 11536, CMIOC 11564). Two specimens from Foz do Iguaçu, Paraná State, were also examined and included in the study (CMIOC 11748). A map was constructed using Quantum Gis Girona 3.0. program, based on coordinates obtained for each sampled site using a GPS (Global Positioning System).

Alive specimens were previously fixed for the morphological analyses, according to Thomé (1975). At least two specimens of each population were dissected under stereomicroscope (except those represented only by shells). External morphology and reproductive system characteristics were compared to the specialized bibliography (Gude 1900, Stange 2004, Capivera & White 2011, Salles et al. 2018). The specimens were analyzed under stereomicroscope Stemi SV6 Zeiss with an attached digital camera to obtain photos of shell and reproductive system. Subsequently, drawings were developed based on these pictures using the CorelDrawX8 and Photoshop CC 2018 programs. Thirty-six shells from different municipalities were measured based on Thomé et al. (2006) methodology, using a stereomicroscope (Leica M205C) with an image capture System (DMC2900) and Leica Application Suite V4.7 Program (Figure 1). Only specimens from Paraty were not measured because they were newly hatched snail and their shells were damaged. Other terrestrial snails and

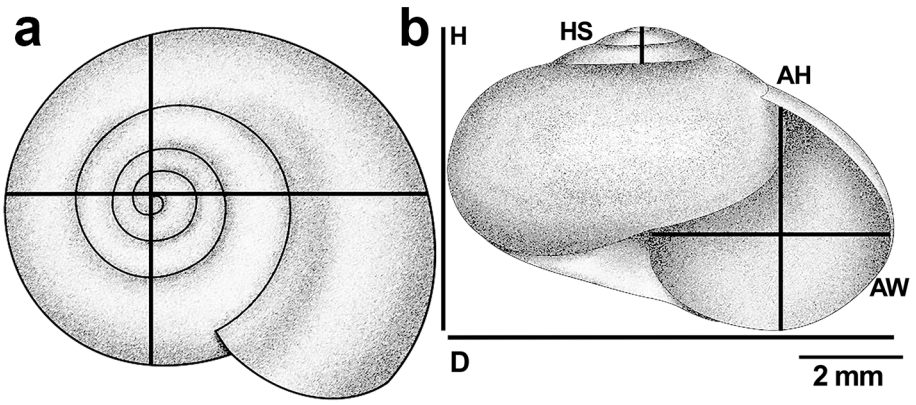


**Figure 1. Shell measurements of *Ovachlamys fulgens*. a. Number of whorls of the shell; b. Shell height (H), spire height (HS), shell width (D), aperture height (AH), aperture width (AW).**

slugs collected in the same environment of *O. fulgens* were also taxonomically identified and deposited at the CMIOC (CMIOC 10887, CMIOC 11376, CMIOC 11378, CMIOC 11381, CMIOC 11284, CMIOC 11320, CMIOC 11323, CMIOC 11593).

Preserved shells of two specimens from Miguel Pereira and Rio de Janeiro (Vargem Grande), as well as the radulae and jaws of three individuals from Magé, Miguel Pereira and Petrópolis were analyzed using a Jeol JSM 6390LV scanning electron microscope, at the Rudolf Barth Electron Microscopy Platform of the Oswaldo Cruz Institute/Fiocruz, Rio de Janeiro, RJ. The shells were cleaned with distilled water and naturally dried. They both were removed from the buccal bulb of specimens fixed in alcohol 70%. After removing radulae and jaws, they were dipped in a solution of sodium hypochlorite at 0.5% for five seconds to completely remove the tissues. Subsequently, both were washed at least three times in distilled water for the total withdrawal of sodium hypochlorite. After having the shells dried and the radulae and jaws cleaned, the material was mounted, using a carbon ribbon on aluminum stubs and coated with an approximately 20-nm layer of gold, before the SEM analysis.

For the molecular analysis, DNA was extracted from specimens from six localities: Petrópolis (Quitandinha and Vila São Luiz), Magé (Pau Grande), Rio de Janeiro (Grumari and Jacarepaguá) and Niterói (Sapê) (Table I). The DNA was obtained from a small portion of the foot tissue and/or buccal bulb. These were removed from the mollusks already fixed in 70% alcohol, washed in ultra-pure water, and preserved by freezing until DNA extraction. The DNA extraction was performed using Qiagen's "Dneasy Blood and Tissue" Kit, following the protocol recommended by the manufacturer for the isolation of the genetic material.

For the molecular identification of the species, the Cytochrome Oxidase I (COI) was amplified by Polymerase Chain Reaction (PCR), using primers described by Folmer et al. (1994). Invitrogen reagents were used in the following final concentrations: Buffer 1x, DNTP 0.2 mM, $MgCl_2$ 2.5 mM, 0.2 µM of each primer, Taq 1 unit per reaction and 1µl of DNA. The remain was completed with ultra-pure water to complete 25 µl per sample. The separate samples in different eppendorfs were placed in a Mastercycler thermocyclator, where the cycling was programmed with the following steps: 94 °C for 5 minutes, after 35 cycles at 94 °C for 1 minute, 52 °C for 1 minute, 72 °C for 1 minute, ending with 1 Cycle of 72 °C for 5 minutes. Subsequently, the electrophoresis of the PCR products was visualized in a 1.5% agarose gel. After that, the PCR product was purified with GE Healthcare's gfx PCR DNA and Gel Band Purification Kit. Sequencing was carried out at the Genomic

**Table I. List of the lots analyzed in the present study and its respective CMIOC Number, GenBank Access Number, collecting date, indication of the kind of sample (live specimen or/and shell), geographic coordinates and altitude of each site. Sequenced specimen was individualized in its respective CMIOC lot.**

| CMIOC | GenBank Accession | Collecting | Municipalities | Sampled | mollusks | Geographic | coordenates | Altitude |
|---|---|---|---|---|---|---|---|---|
| Number | Number | Date | | Shell | Live specimens | S | W | Meters |
| 10983 | - | 23/11/2016 | Nova Friburgo | 1 | - | 22°20'47.4" | 42°19'33.4" | 644,6 |
| 10984 | - | 23/11/2016 | Nova Friburgo | 1 | - | 22°21'05.8" | 42°22'39.9" | 792,3 |
| 10985 | - | 29/05/2018 | Teresópolis | 1 | - | 22°23'12.2" | 43°00'02.4" | 885 |
| 10986 | - | 14/03/2017 | Petrópolis | 4 | 1 | 22°28'44.1" | 43°09'32.9" | 837,6 |
| 10987 | MK279357 | 07/06/2017 | Petrópolis | 18 | 12 | 22°28'44.1" | 43°09'32.9" | 837,6 |
| 10988 | MK279361 | 07/06/2017 | Petrópolis | 11 | 7 | 22°32'33.6" | 43°14'02.2" | 622,7 |
| 10989 | - | 13/07/2017 | Rio de Janeiro | 4 | - | 22°58'32.6" | 43°30'18.6" | 43,5 |
| 10990 | - | 13/07/2017 | Rio de Janeiro | 10 | - | 22°58'31.3" | 43°30'14.1" | 37,3 |
| 10991 | MK279356 | 13/07/2017 | Rio de Janeiro | - | 1 | 23°03'02.8" | 43°32'23.9" | 8 |
| 10992 | - | 02/08/2017 | Magé | 2 | - | 22°35'53.7" | 43°06'29.8" | 9,6 |
| 10993 | MK279358 | 02/08/2017 | Magé | 2 | 4 | 22°35'17.0" | 43°09'59.4" | 21,9 |
| 10994 | MK279360 | 16/05/2017 | Rio de Janeiro | 2 | 1 | 22°57'39.7" | 43°19'47.3" | 23,8 |
| 10995 | - | 04/12/2017 | Nova Friburgo | 1 | - | 22°21'30.1" | 42°21'26.8" | 702,3 |
| 10996 | - | 07/08/2017 | Petrópolis | 8 | 4 | 22°28'44.1" | 43°09'32.9" | 837,6 |
| 10997 | - | 07/08/2017 | Petrópolis | 2 | 14 | 22°31'31.7" | 43°10'38.0" | 916,7 |
| 10998 | - | 15/12/2017 | Niterói | 2 | - | 22°55'59.1" | 43°03'13.1" | 20,8 |
| 10999 | MK279359 | 15/12/2017 | Niterói | 9 | 4 | 22°53'12.3" | 43°03'20.3" | 55,6 |
| 11000 | - | 06/02/2018 | Miguel Pereira | - | 3 | 22°29'01.3" | 43°30'29.5" | 660,7 |
| 11536 | - | 18/01/2018 | Parati | 8 | - | 23°12'57.6" | 44°42'36.4" | 8 |
| 11564 | - | 24/10/2018 | Bom Jardim | 4 | - | 22°09'01.3" | 42°25'26.2" | 601 |
| 11748 | - | 20/03/2019 | Foz do Iguaçu | - | 2 | 25°33'33.2" | 54°33'35.6" | 226 |

Platform - DNA Sequencing RPT01A (Network of Fiocruz Technological Platforms), according to Otto et al. (2008) methodology.

Each site of the sense and anti-sense sequences obtained from the sequenced samples was manually verified in the Seqman Lasergene program (version 7) (DNAStar, INC). The consensus sequences of the sense and anti-sense sequences of all specimens were compared to each other and with two *O. fulgens* sequences available in the GenBank database, one from Japan and another from Argentina (Hyman et al. 2007, Beltramino et al. 2018) using the BLAST tool (Basic Local Alignment Search tool). All sequences obtained in this study were deposited in the GenBank (MK279356 - MK279361). The CLUSTALW tool available in the program Mega v. 7.0 (Kumar et al. 2016) was used for the alignment of the sequences studied. jModeltest 2.1.10 (Darriba et al. 2012) program was used to infer the best-fit model of nucleotide substitution based on the lowest

Akaike and Bayesian Information Criterion value (and therefore delta=0) for phylogenetic reconstruction. A Bayesian phylogenetic tree was built based on sequences of seven taxa classified into Helicarionoidea (Hyman et al. 2007) from GenBank, and sequences of four individuals from Rio de Janeiro State, using BEAST 1.8 (Drummond et al. 2012) and a two-partitions [(1+2),3] scheme of codon position. A prior tree was randomly generated, and a Yule process of speciation was imposed for all tree reconstructions. Three independent runs were performed for $10^7$ generations, with a burn-in of $10^6$ generations. Convergence of parameters and proper mixing were confirmed by calculating the effective sample size (ESS) in TRACER 1.6 (Rambaut et al. 2014), excluding the initial 10% (burn-in) of each run. All considered parameters had ESS > $10^3$. Runs were combined using LogCombiner and a maximum credibility tree based on the 30,000 trees (burn-in= 9,000) was generated with a posterior probability limit of 0.6 using Tree Annotator (both part of the BEAST package). Statistical support for clades was assessed by the posterior probability method.

## RESULTS

A total of 90 shells and 53 live specimens of *O. fulgens* were collected. The species is recorded for the first time in eight municipalities in the state of Rio de Janeiro: Miguel Pereira, Niterói, Nova Friburgo, Petrópolis, Magé, Rio de Janeiro (neighborhoods of Grumari, Jacarepaguá and Vargem Grande), Bom Jardim, Parati. It was also collected in Teresópolis. The species is also for the first time reported to Paraná State, in the municipality of Foz do Iguaçu (Figure 2).

Specimens were collected in urban and anthropized environments. They were collected on the ground and plants of gardens, wastelands, walls of houses, sidewalks, including sites with waste accumulation, and/or under stones, bricks and fallen trunks, on shrubs, inside trunk holes, and in plant nurseries.

*Ovachlamys fulgens* was found occuring simpatrically with several other terrestrial mollusks, including exotic and native species. Some examples of exotic and sinantropic species were *Bradybaena similaris* (Férussac, 1821) (CMIOC 11381), *Leptinaria unilamellata* (d'Orbigny, 1835) (CMIOC 11376), *Beckianum beckianum* (Pfeiffer, 1846) (CMIOC 11378). *Phyllocaulis boraceiensis* Thomé, 1972 (CMIOC 10887), *Megalobulimus ovatus* (Müller, 1774) (CMIOC 11284, CMIOC 11323) and *Drymaeus papyraceus* (Mawe, 1823) (CMIOC 11320) were among the native species.

### Shell

The shell presented up to four whorls and ¼ in the analyzed populations. It is subglobose, dextrogyre, amber colored, translucent, shiny and fragile. The shells presented an average of 6.15 mm ± SD 2.12 mm in diameter, and 4.24 mm ± SD 1.47 mm of total height. The largest specimen was collected in the municipality of Magé, reaching 9.72 mm in diameter and 6.76 mm in height (Table II).

The shell is depressed, with its first whorls forming an obtuse apex. The sutures are simple and the whorls gradually increase in diameter. The average of the spire height was 0.51 mm ± SD 0.20 mm. The last whorl is convex, slightly inflated and has more than double the width of the penultimate whorl. The shell is minutely striated with fine microscopic spiral grooves that start shortly after a small smooth portion of the protoconch (Figure 3-4). Subtle growth lines can be seen, however, do not present a pattern in its spacing or continuity (Figure 4).

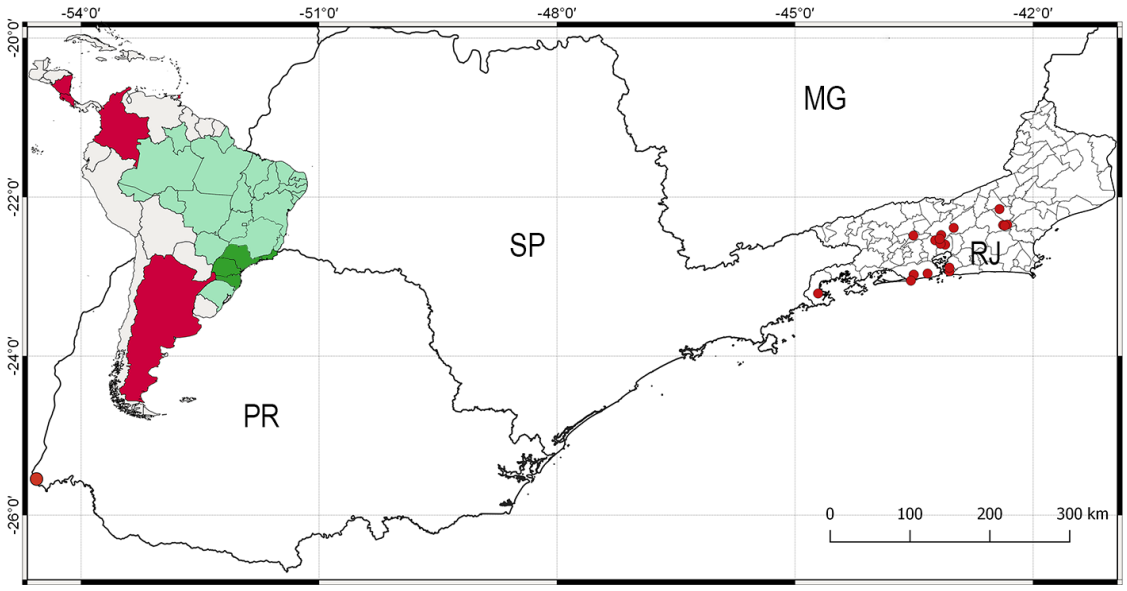


**Figure 2. Distribution of *Ovachlamys fulgens* in Central America (Nicaragua and Costa Rica), Antilles (Trinidad and Tobago) and South America (Colombia, Argentina and Brazil). Brazil is in light green and States where the species has been reported are in dark green (considering previous reports and the present study). Red circles represent the new occurrences of O. *fulgens* in Rio de Janeiro and Paraná State, Brazil.**

## External morphology

The analyzed specimens presented blackish to grayish colored body (Figure 5). The dorsum is darker in comparation to its lateral. There are two darker black stripes located dorsally along the upper tentacles. The lateral region the body nearer the ventral side has lighter coloration.

The foot is narrow and light beige, delimited by two thin and shallow grooves that are flanked by two areas of tissue, one on each side of the foot (Figure 5c). Each one of these areas has a little less than two times the foot width. The foot is long and presents visible muscular striations on the dorsum and lateral areas. There is a structure shaped as a "horn" in its dorsal final portion, which has a longitudinal slit (Fig. 5b). Observations of the live animal allowed us to verify that when the animal is moving the mucus accumulate in this slit forming spheres.

The mantle border is whitish in color. The mantle at the roof of the pulmonary cavity, which is in contact with the inner part of the shell, presents black spots and stripes, which can be seen by the shell transparency (Figure 5).

The mantle has two lateral expansions that partially cover the shell dorsally. In live specimens they are almost imperceptible, but they become easily visible in fixed animals. Such extensions have blackened stains or fine points (Figure 5a). During the displacement of the living animal, they can be seen moving slowly.

## Reproductive system

*Ovachlamys fulgens* is hermaphroditic, with a common atrium for the penis and vagina, which open in a single pore located on the right side of the animal, near the base of the right tentacle (Fig. 5d). The hermaphroditic portion is distally located and is constituted by the gonad and hermaphroditic duct o (=ovulispermioduct, that is the duct that leads the spermatozoids and ovules), besides the *carrefour* and talon.

The atrium is well defined, with a median length and uniform width from its opening to

its distal final portion. The bursa copulatrix is small, has elliptical form and is connected to the end of the vagina, near the atrium, through a short and narrow duct, with approximate the same length to the pouch itself, although a little shorter.

The oviduct has differentiated areas. They present different levels of wrinkles and even different coloration. The degree of development of the oviduct varied among the analyzed specimens, as result of their degree of maturity.

The prostate is disposed along the concave side of the oviduct as a narrow and very subtle longitudinal band of acinus and runs along the autosperm groove that begin in the carrefour. The vas deferens exteriorizes from the prostate and penetrates the epiphallus base. The epiphallus is relatively small and slightly enlarged, tapering near its connection with the vas deferent. The penis has a dilated portion close to its apex, close to the beginning of the epiphallus. In young specimens, these peculiarities are already visible, although the penis is slender. Externally to this dilated region, it is possible to observe the vas deferens by transparency. The retractor muscle is long and connects to the apex of the penis.

The albumen gland is large and resembles a bean shape. The *carrefour* is located approximately in the middle of the albumen gland, where the hermaphroditic duct is connected, coming from the ovotestis (= gonad or hermaphroditic gland), which is posteriorly located at the body. In the concave side of the albumen gland, it is possible to observe the capsule gland and a very small talon immersed in this structure (Figure 6). The capsule gland secretes a capsular membrane, which surrounds every albuminate egg, based on Dayrat & Tillier (2002). The latter can be distinguished from the albumin gland by a slightly lighter coloration and by a more compact appearance. The ovotestis is formed by three distinct lobes connected through fine ducts between them and the hermaphroditic duct, and is surrounded to the lobes of the digestive gland.

## Radula and jaw

The jaw is a membranous structure in the form of a half moon, with rounded ends. It has a smooth appearance, although in higher magnification (SEM) subtle horizontal lines arranged throughout its extension are observed (Figure 7a).

The radula presented the following dentary formula: (61-63) + (9-10) + (1) + (9-10) + (61-63), that include central, lateral and marginal teeth organized in straight lines (Figure7b-d). Central teeth are symmetric and tricuspid. They have a more developed central cuspid (mesocone) and the two smaller ones that are located in around the half of the total length of the tooth (ectocones), in each side of the central cuspid. Lateral and marginal tooth are asymmetrical. Two types of lateral teeth were found, being noticed a transition between them and the marginals. The lateral teeth closest to the central tooth are larger and have three well-developed cusps (Figure 7b). They differ of the central tooth because their ecotones present different sizes and positions. The lateral teeth closest to the marginals are slightly more curved and have less prominent ecotones (Figure 7b). The marginal teeth are curved with the cuspid facing the radula margin, having from two to six cuspids.

## Molecular identification

The COI sequences obtained for *O. fulgens* revealed a fragment of 625 pairs of bases (Table I). These were identical to each other and when analyzed by BLAST they were 100% similar to the Japanese and Argentine sequences available in GenBank database for *O. fulgens*. The GTR +Γ

**Table II. Shell measurements obtained under a stereomicroscope with image capture system, using Leica *Application Suite* program (v 4.8). A. Shell height; D. Shell width; HS. Spire height; AH. Aperture height; AW. width of aperture.**

| Municipalities | Shell measurements | | | | |
|---|---|---|---|---|---|
| | A | D | HS | AH | AW |
| Magé | 6,76 | 9,72 | 0,55 | 4,62 | 5,23 |
| Magé | 5,97 | 8,52 | 0,80 | 3,72 | 4,39 |
| Magé | 5,82 | 8,37 | 0,69 | 3,87 | 4,41 |
| Magé | 5,78 | 8,81 | 0,87 | 4,08 | 4,74 |
| Miguel Pereira | 4,97 | 7,52 | 0,54 | 3,63 | 3,16 |
| Miguel Pereira | 2,75 | 3,82 | 0,14 | 2,17 | 2,08 |
| Niterói | 5,19 | 7,16 | 0,65 | 3,72 | 3,97 |
| Niterói | 6,15 | 8,71 | 0,65 | 4,22 | 4,46 |
| Niterói | 5,50 | 8,18 | 0,80 | 3,91 | 4,49 |
| Niterói | 5,09 | 7,66 | 0,57 | 3,84 | 4,11 |
| Niterói | 5,35 | 7,87 | 0,64 | 3,71 | 4,20 |
| Niterói | 5,92 | 8,55 | 0,63 | 4,14 | 4,60 |
| Niterói | 4,50 | 6,43 | 0,46 | 3,37 | 3,27 |
| Nova Friburgo | 3,21 | 4,66 | 0,63 | 2,11 | 2,25 |
| Nova Friburgo | 5,89 | 8,38 | 0,61 | 3,57 | 3,61 |
| Petrópolis | 2,10 | 2,93 | 0,22 | 1,40 | 1,44 |
| Petrópolis | 2,08 | 2,97 | 0,24 | 1,37 | 1,56 |
| Petrópolis | 1,97 | 2,75 | 0,23 | 1,38 | 1,44 |
| Petrópolis | 2,03 | 2,95 | 0,17 | 1,46 | 1,58 |
| Petrópolis | 1,95 | 2,80 | 0,20 | 1,38 | 1,49 |
| Petrópolis | 1,99 | 3,03 | 0,26 | 1,45 | 1,66 |
| Petrópolis | 1,85 | 2,72 | 0,22 | 1,34 | 1,45 |
| Rio de Janeiro | 4,30 | 6,11 | 0,56 | 2,71 | 3,36 |
| Rio de Janeiro | 3,80 | 5,84 | 0,48 | 3,05 | 3,08 |
| Rio de Janeiro | 3,99 | 5,97 | 0,52 | 2,77 | 3,19 |
| Rio de Janeiro | 3,96 | 5,69 | 0,50 | 2,57 | 2,91 |
| Rio de Janeiro | 3,85 | 5,92 | 0,43 | 2,91 | 3,01 |
| Rio de Janeiro | 6,06 | 8,37 | 0,59 | 4,33 | 4,74 |
| Rio de Janeiro | 4,16 | 6,28 | 0,55 | 2,73 | 3,25 |
| Teresópolis | 2,44 | 3,64 | 0,28 | 1,88 | 1,91 |
| Foz do Iguaçu | 4,50 | 6,57 | 0,53 | 2,88 | 3,57 |
| Foz do Iguaçu | 3,87 | 5,46 | 0,41 | 2,65 | 2,79 |
| **Total average** | **4,18** | **6,07** | **0,49** | **2,90** | **3,17** |
| **Standard deviation** | **1,55** | **2,23** | **0,20** | **1,05** | **1,19** |


**Figure 3.** Shell of *Ovachlamys fulgens*: frontal (**a**), apical (**b**) and ventral (**c**) (CMIOC 10990).

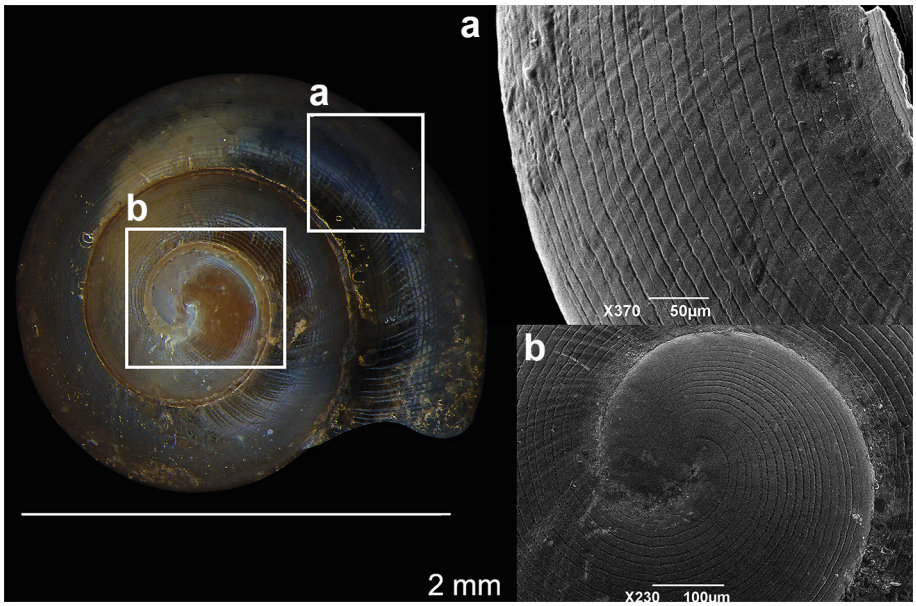


**Figure 4.** Scanning electron-micrographs of the shell of *Ovachlamys fulgens* (CMIOC 11000). **a.** Fine spiral grooves that are present in all shell whorls, except in the beginning of the protoconch (**a-b**), and growth-lines (**b**).

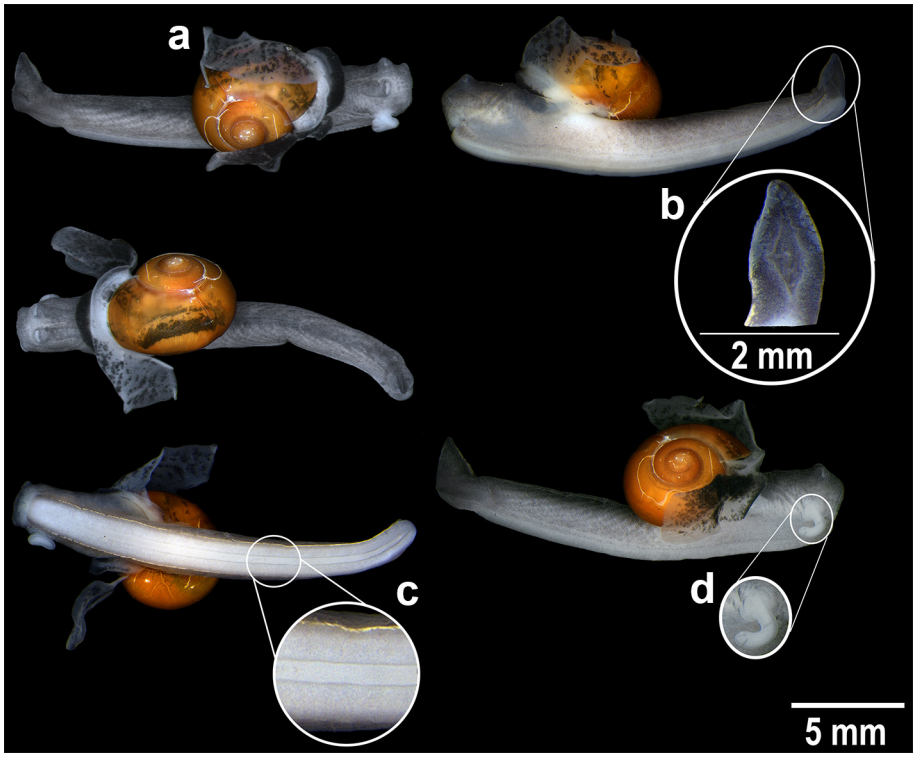


**Figure 5.** Photography of a specimen of *Ovachlamys fulgens* showing its general external morphology after being killed in water and subsequently fixed in alcohol 70%, obtained under a stereomicroscope (CMIOC 10985). **a.** Dorsal view, showing the mantle expansions. **b.** Detail of the caudal horn and its longitudinal groove. **c.** Ventral view, showing the foot. **d.** Lateral view, with part of the phallus extroverted in the anterior portion of the body.

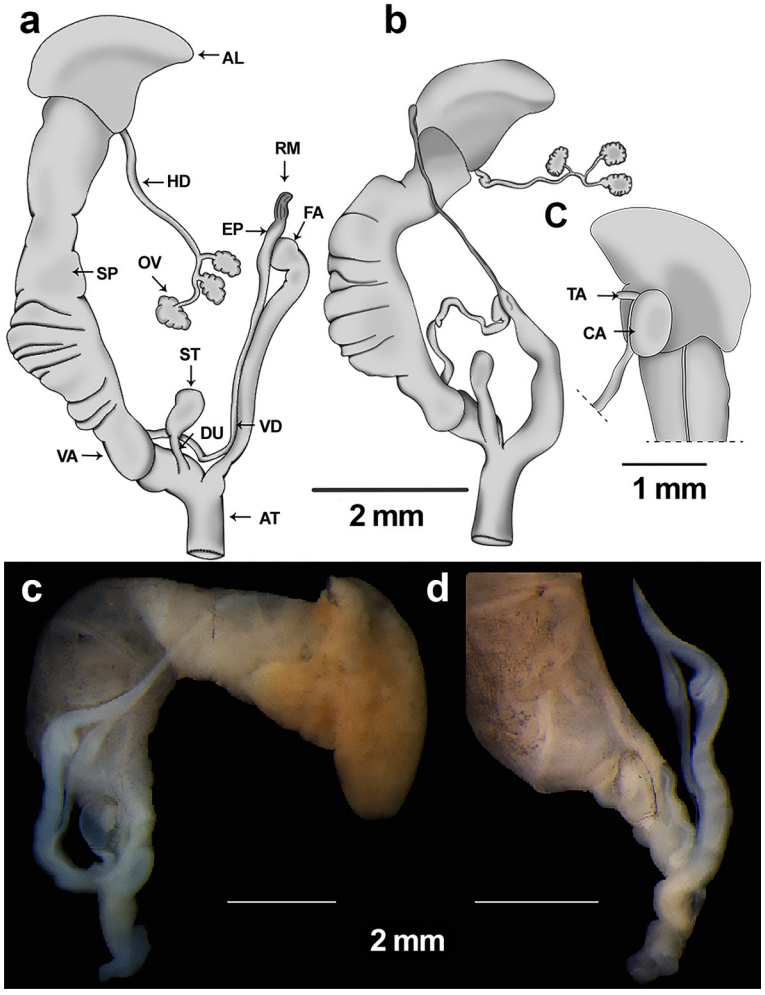


**Figure 6.** Drawing and photography of the complete reproductive system of *Ovachlamys fulgens* under stereomicroscope. **a.** Specimen from Petrópolis (CMIOC 10986). **b.** Specimen from Niterói (CMIOC 10.999). **d-e.** Specimen from Magé (CMIOC 10993). AL. Albumen gland; AT. Genital atrium; CA. Capsule gland; EP. Epiphalus; DU. Bursa copulatrix duct; HD. Hermadrodict duct (or ovulispermioduct); FA. Penis, and its expansion proximally located; SP. Oviduct; OV. Ovotestis (=gonad or hermafrodict gland). RM. Retractor muscle of the penis inserted on the lower part of the epiphallus; ST. Bursa copulatrix; VD. Vas deferens; VA. Short vagina; TA. Talon.

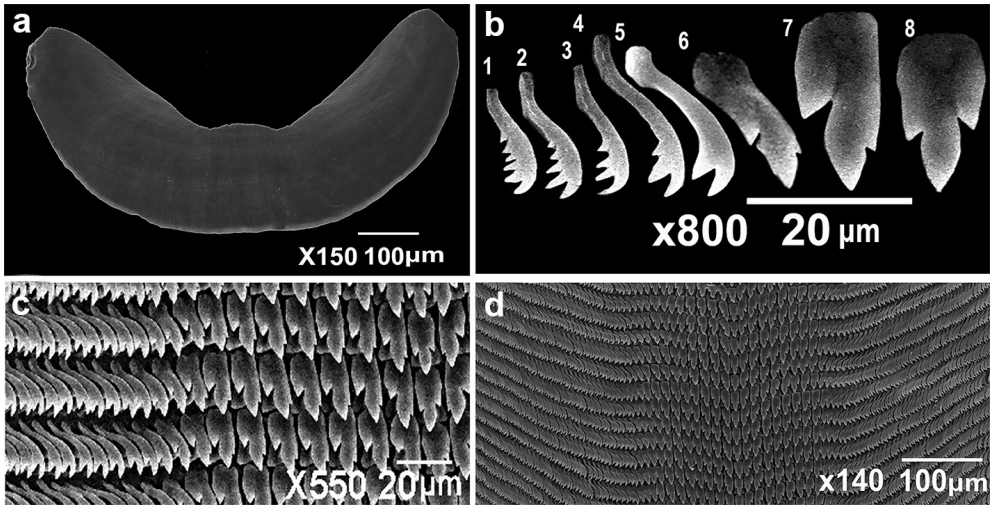


**Figure 7.** Scanning electron-micrographs of the jaw (**a**) and radula (**b, c, d**). **b.** Different types of teeth found in the *Ovachlamys fulgens* radula. **c.** Left side of the radula, showing lateral and marginal teeth (COMIC 11000). **d.** Left and right sides of the radula.

model was the best nucleotide substitution model (lower AIC and BIC value) for the phylogenetic reconstruction of the data. The Bayesian phylogenetic tree (Figure 8) grouped all analyzed sequences, including those from Japan and Argentina, in a well supported monophyletic clade, corroborating the taxonomic identity of the studied individuals and the detection of a single haplotype.

## DISCUSSION

In this study, we expand the distribution of *O. fulgens* to eight municipalities in Rio de Janeiro State, and one locality from the Paraná State, where the species is for the first time reported. In Rio de Janeiro State, it was found in three different regions (Serrana, Centro-Sul Fluminense and Metropolitan), ranging from 8 m to 916, 7 m of altitude. In Paraná, it was collected in a hotel garden, in Vila Olimpia neighborhood. This last report is the closest to Eldorado, in Missiones, Argentina, where the species is also recorded (Beltramino et al. 2018).

Previous studies in Rio de Janeiro State recorded *O. fulgens* in Serra dos Órgãos National Park (Parnaso), in the municipalities of Teresópolis and Guapimirim (Salles et al. 2018). We highlight the fact that *O. fulgens* was not found in previous surveys performed in the Rio de Janeiro State (Lopes et al. 2012, Rodrigues et al. 2016, Alexandre et al. 2017). It is also interesting to notice that there are almost simultaneous reports of *O. fulgens* in São Paulo, Rio de Janeiro and Santa Catarina in Brazil (Teixeira et al. 2017, Salles et al. 2018, Agudo-Padrón 2019) and Argentina (Beltramino et al. 2018).

Alexandre et al. (2017) reported *O. fulgens* for the first time in Brazil based on specimens from São Paulo, although only based on analysis of alive specimens, general aspects of the shell, and

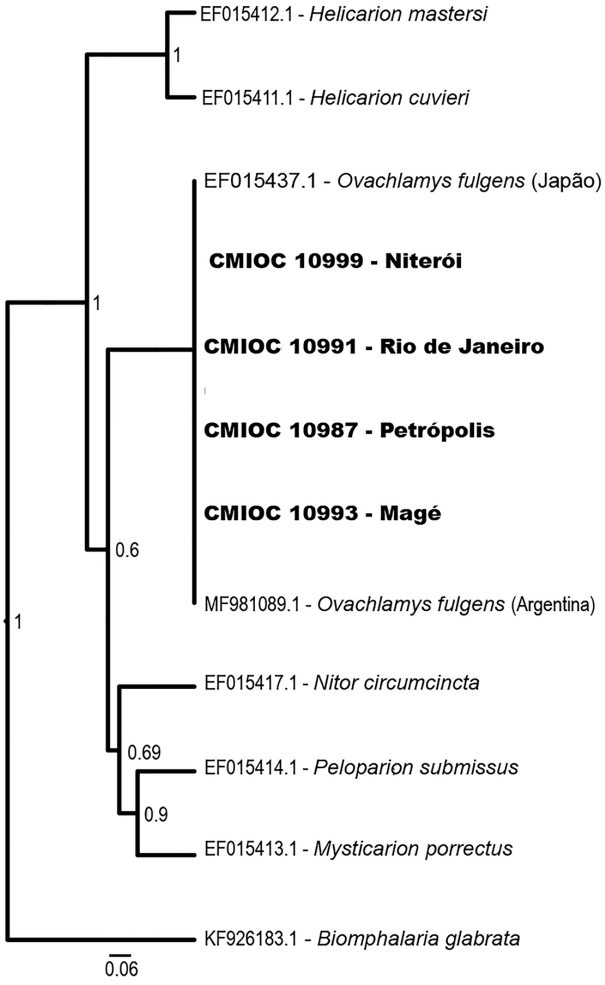


**Figure 8. A phylogenetic species tree reconstructed with 625 base pair of COI sequences of seven taxa classified in Helicarionoidea and sequences of four individuals from Rio de Janeiro. Posterior probabilities above 0.6 are show for key nodes.**

jumping behavior. Despite the authors consider their identification a tentative, it is a valid record, since the few described morphological aspects and the pictures of the alive specimen allow confirming its identification. In other side, Agudo-Padrón (2019) states that the species would have been present in Santa Catarina State since 2013, but it was being misidentified as a species of the family Eucolunidae.

Although Teixeira et al. (2017) has mentioned that *O. fulgens* has been introduced

in Venezuela, they based this affirmation on a list of quarentenary species (República Bolivariana de Venezuela, 2017), what do not mean that *O. fulgens* occurs in this country. Thus, the species is so far considered reported to Brazil, Argentina, and Colombia. According to Robinson & Slapcinsky (2005) *O. fulgens* has been intercepted in shipments of flowers from Colombia in the United States.

According to Stange (2004) and Robinson & Slapcinsky (2005), *O. fulgens* is commonly intercepted in a wide variety of plants imported from several countries. This pest condition of ornamental plants has probably influenced the apparent rapid propagation of this species in South America. Another fact that certainly has influenced its spreading is its small size and the ability to perform almost daily self-fertilization and oviposition, which start approximately 42-45 days after hatching, when the shell has a diameter varying 4.73 to 5.12 mm (Barrientos 1998).

In the present study specimens were collected in urban areas, in highly disturbed environments, as well as in as in plant nurseries, in agreeing with the literature. In Costa Rica, Barrientos (2000) related the presence of the species to humans and to agriculture. Teixeira et al. (2017) found specimens in leaf litter of a very disturbed patch of Atlantic Forest and in a orchid nursery, occuring in the same enviroment of native species in São Paulo. Beltramino et al. (2018) registered the species in an urban environment, in Eldorado, Argentina, located in a mosaic of native forest, plantations, agriculture and pastures. Besides, Salles et al. (2018) collected the species within the Atlantic forest area, within Parnaso Park, mainly on the margins of trails. Our studied showed that this species is adapted to different environments, since it was collected in urban and synanthropic areas (in most cases), near in vegetation areas near the beach, but also in high altitudes, in dirty places, but also in places near preserved forests and in plant nurseries.

The shell of *O. fulgens* is very typic, and the conchological characteristics described in the present study agree with those already known for this species, with few variations. The maximum size found for the shell, according to literature data, is 7 mm in diameter (Barrientos 1998, Capivera & White 2011, Hwang 2014, Teixeira et al. 2017, Salles et al. 2018), much smaller than the specimens from Magé that reached 9.72 mm. The analysis of the shell allowed to observe thin lines little spaced one from each other parallel to the suture, in spiral form, in accordance to other authors (Salles et al. 2018, Beltramino et al. 2018).

Among the external characteristics of the animal's body, a characteristic that draws attention and allows to easily distinguish *O. fulgens* from other native Brazilian species, such as Euconulidae species, with which they can be mistaken, is the presence of a caudal horn, located distally and dorsally in the foot, not found in Brazilian families of terrestrial mollusks. Other families, in addition to Helicarionidae, also present a caudal horn, such as Urocyclidae and Ariophantidae (Herbert & Kilburn 2004), for example, but they do not occur in Brazil (Salgado & dos Santos Coelho 2003). Among the possible suggested functions for this structure for Urocyclidae is the resorption of mucus that is left behind during displacement, the discouragement of predators by this structure become harder the ingestion of the mollusk, and the use as a rich sexual stimulator (Herbert & Kilburn 2004). According to the authors, this structure had already been also associated to absorption of nutrients, pheromones production and to locomotion, allowing the specimen to hung suspended in the air through a thin thread of mucus from a place

to another one (Herbert & Kilburn 2004). When live specimens of *O. fulgens* are in movement it is possible to notice that besides the mucus that is being liberated during the locomotion, the horn become partially curved and accumulate spheres of mucus in its slit.

The radula has a central teeth line, centrally located in the radula, flanked by rows of lateral teeth, followed by rows of marginal teeth, on each side of the central teeth. In the present study, a highest number of teeth per row was found for *O. fulgens*, with the following dental formula (61-63) + (9-10) + (1) + (9-10) + (61-63). Salles et al. (2018) found 127 teeth per row: (55) + (8) + (1) + (8) + (55), while Beltramino et al. (2018) found the following dental formula for a population from Argentina: (42–40) + (8–7) +1+ (7–8) + (40–42). Variations found in the number of cusps of the lateral and marginal teeth were not previously described. Other morphological characteristics related to external features and reproductive system agree with that described by several authors for this species (Schileyko 2002, Salles et al. 2018).

This is the first time that Brazilian populations are molecularly analyzed, what can be useful to track its invasion pathways in future investivagions. A single haplotype was found for the marker analyzed. According to Lounnas et al. (2017), invasive species commonly have loss of genetic variability, especially those that perform self-fertilization. These authors observed a low or almost no genetic variability in most populations of the freshwater snail *Pseudosuccinea columella* (Say, 1817), with the detection of a single "World Invader" genotype. Patiño-Montoya & Giraldo (2017) has observed similar results to the giant African terrestrial snail *Achatina fulica* Bowdich, 1822, that presented low genetic diversity compared to populations in its natural distribution in Africa, due to a founding effect occurring in each introduction event and the bottlenecks occurring after the establishment and acclimatization to the conditions of the new habitat.

The species *O. fulgens* was already found naturally infected with *A. cantonensis* in Hawaii (Kim et al. 2014). In the urban areas, *O. fulgens* was found ocurring simpatrically with other exotic and invasive species already found naturaly infected with helminths of medical interest, such as *B. similaris* and *D. laeve* (Ohlweiler et al. 2010). Teixeira et al. (2017) also highlighted that *O. fulgens* was collected in places where species that have medical importance as *A. fulica* and *Subulina octona* (Bruguière, 1792), were also found. The enviroment where *O. fulgens* was found is suitable for rodents that act as final host of both *A. cantonensis* and *A. costaricensis*, what demonstrate the potential of *O. fulgens* becomes part of the life cycle of these parasites also in Brazil.

Our results demonstrate that *O. fulgens* presents a great potential to rapidly expand its distribution to other Brazilian states, and to South American countries, and that probable it is more widespread than here reported. The specimens demonstrate to be adapted to variety of environments, what with its pest condition, small size, and self-fertilization capacity, potentiate its spreading capacity. We highlight the importance of continuous monitoring of this species, since it can compete with native species of mollusks, attack innumerous species of ornamental plants, and become intermediate host of nematodes of medical interest.

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## Author contributions

CRM developed the project, performing morphological, conchological and molecular analyses of the samples, and drafted the manuscript. JCA obtained the COI sequences and performed the phylogenetic analysis. CRM, SCT, MAF, PSR, SCT, SRG planned and participated in the field expeditions for obtention of samples. HSB obtained scanning electron-micrographs of the radula and jaw. SRG supervised the project's development and the manuscript writing. All authors discussed the results and reviewed the final manuscript.