



## BIOLOGICAL SCIENCES

# Morphological and molecular identification of the invasive freshwater snail *Physa acuta* (Gastropoda: Physidae) into Llanquihue Lake, Chilean Patagonia

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**Abstract:** The sewage snail *Physa acuta*, native to North America, is an effective invasive species around the world. In Chile, it was first reported in 2014 in the north central area of the country. So far, the species has not been recorded in southern Chile. Sampling performed in 2015 in three localities from Llanquihue Lake, Chilean Patagonia, only provided native freshwater snails. However, new collections performed in February 2018 in the same three sites were successful for physid specimens suggesting a biological invasion entailing a large southward range expansion of these snails. Here we performed morphological, microstructural and phylogenetic analyses to investigate whether the new samples belong to *Physa acuta*. The shell morphology, male copulatory complex and radula microstructure of the new material agree with those of the sewage snail. The molecular phylogenetic analyses using the cytochrome c oxidase subunit 1 (COI) gene confirmed morphological identification. We suggest to take prompt measures to prevent the expansion of *Physa acuta* in Llanquihue Lake or nearby aquatic ecosystems.

**Key words:** Chile, invasive species, molecular identification, *Physa*, South America, taxonomy.

## INTRODUCTION

The planet is currently undergoing two processes of global environmental variation, climate change and the globalization of trade, which have impacted the biosphere generating favorable opportunities for an increase in bioinvasions (Lockwood et al. 2007). The introduction of invasive species can generate a series of effects in native ecosystems such as economic loss, damage to human health, landscape alterations and threats to biodiversity (Gordon 1998, Perrings 2001, Bax et al. 2003, Juliano & Lounibos 2005, Lovell et al. 2006). Among a variety of taxa, mollusks are an important source of invasive species and it

is not strange that have appeared published in “the list of 100 of the world’s worst invasive alien species” (Lowe et al. 2000) six species of this phylum, the bivalves *Dreissena polymorpha* (Pallas, 1771), *Mytilus galloprovincialis* (Lamarck 1819) and *Potamocorbula amurensis* (Schrenck, 1861), and the gastropods *Achatina fulica* (Bowdich, 1822), *Euglandina rosea* (Férussac, 1821) and *Pomacea canaliculata* (Lamarck, 1828). Among the invasive mollusks, some fall into the category of “ecosystem engineers” (Jones et al. 1994), as is the case of the aggressive invasion of the golden mussel *Limnoperna fortunei* (Dunker, 1857) in South America (Darrigran & Damborenea 2011).

Physidae are ubiquitous pulmonate gastropods that inhabit a variety of aquatic ecosystems around the world (Appleton 2003, Dillon et al. 2002, Taylor 2003). One of them is the freshwater snail *Physa acuta* Draparnaud, 1805, native to North America (Dillon et al. 2002, 2005, Lydeard et al. 2016), which has profusely invaded all continents, except Antarctic (e.g. Madsen & Frandsen 1989, Ali 1993, Brackenbury & Appleton 1993, Dillon et al. 2002, Zukowski & Walker 2009, Núñez 2010, Vinarski 2017). The species has been commonly called the sewage snail (Aditya & Raut 2002, Appleton 2003, de Kock & Wolmarans 2007, Guo & He 2014) because it is frequently found in polluted habitats which are plentiful in human waste (Brown 1994). *Physa acuta* has also been considered of medical importance since it can potentially acts as an intermediate host of human trematodes (Kanev 1994, Dreyfuss et al. 2002). In South America, *Physa acuta* was identified for the first time by Paraense & Pointier (2003) who reported the synonymy of *Physa cubensis* Pfeiffer, 1839 with *Physa acuta* but it is apparent that the species was present from before in the subcontinent (Miquel 1985, Paraense 1987, Collado 2017, Darrigran et al. 2020).

Despite there is not agreement among the authors, in Chile there has been reported five native physid species: *Physa peruviana* Gray, 1828, *Physa chilensis* Claessin, 1886, *Physa rivalis* Sowerby, 1874, *Physa porteri* Germain, 1913 and *Physa nodulosa* Biese, 1948 (Stuardo 1961, Valdovinos 1999, 2006, Sielfeld 2001). In 2014, the alien *Physa acuta* was added to this list, being recorded in the north central area of the country. The first identification was made from the Elqui River, Región de Coquimbo, and Mataquito River, Región del Maule, both rivers separated by about 560 km (Bousset et al. 2014). More recently, several other populations were reported and collected from different basins

in the country, partially filling the gap in the invasive range of the species (Collado 2017):

- Región de Coquimbo: Choapa River, Illapel River and Consuelo Stream in the Choapa River basin.
- Región de Valparaíso: Santa Juana near Concón in the Aconcagua River basin.
- Región Metropolitana: Parque O'Higgins Spring in the Maipo River basin.
- Región de Valparaíso: El Salto in the Marga Marga River basin.

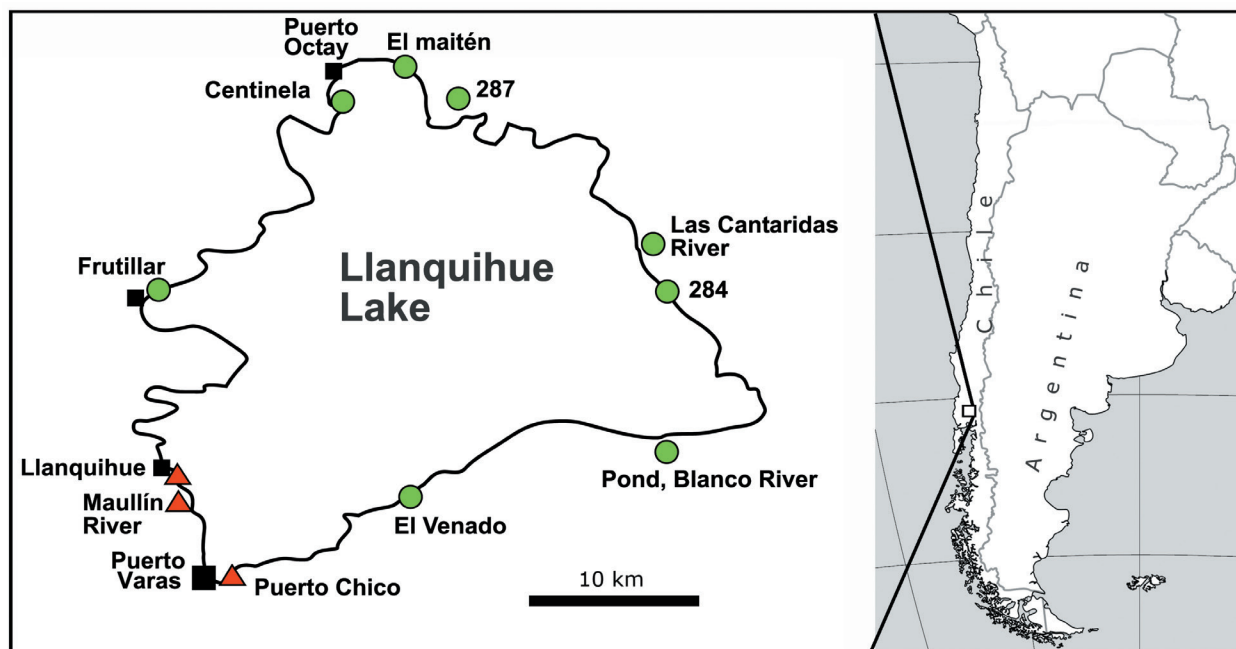
New collections performed in Llanquihue Lake, Región de Los Lagos, Chilean Patagonia, provided physid snails from Puerto Chico, a beach east from Puerto Varas City, Llanquihue City and Maullín River, the effluent of the lake. As these three places were sampled in 2015 by the first author together with assistants, being positive only for native snails (unpublished data), we hypothesized a biological invasion of this species into the lake, including a large dispersion and increase in the range of expansion in the country. This is supported by the fact that physid snails have not been reported for the area in any of the checklist of Chilean mollusk species or other works related to native snails of the lake (Marshall 1933, Biese 1944, Stuardo 1961, Valdovinos 1999, 2006, Sielfeld 2001).

Taxonomic identification of physid species has been historically difficult since species are morphologically similar due to overlapping shell characters due to genetic and environmental influences (Dewitt et al. 2000, Gustafson et al. 2014). The aim of the present study is to assess whether the recently sampled populations of physid snails from Llanquihue Lake correspond to the invasive *Physa acuta* or a closely related species. For this, we performed morphological, microstructural and phylogenetic analyses.

## MATERIALS AND METHODS

Llanquihue Lake is the second largest of the lakes of Chile after Lake General Carrera. It is located between 40°58' and 41° 20' S and between 72°31' and 73°00' W in the Llanquihue Province, comprising an area of about 585 km<sup>2</sup>, with an altitude of 52 m and a depth of 350 m (Solano Asta-Buruaga & Cienfuegos 1899, Chile365 2018). It drains on its Southwest side to the Pacific Ocean nine kilometers to the NW of Puerto Varas giving origin to the Maullín River. Sampling was performed by hand using a sieve from 11 sites along the shoreline of the Llanquihue Lake in February 2018, including streams, irrigation canals and small lagoons, as well as the Maullín River (Fig. 1). The snails were examined and photographed using a Motic SMZ-168 Stereomicroscope adapted with a Moticom 2000 digital camera. The radula was cleaned in a diluted sodium hypochlorite solution and observed using a Hitachi SU3500 scanning electron microscope (SEM). Initial

snail identification was based on the original description and additional literature (Paraense 1987, Paraense & Pointier 2003, Núñez 2011, Collado 2017). Voucher specimens were deposited in the Museo de Ciencias Naturales y Arqueología Profesor Pedro Ramírez Fuentes (MCNPPRF-CC 148-01 to MCNPPRF-CC 148-05), Chillán, Chile. Partial sequences of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene were obtained following Collado (2017), edited in BioEdit (Hall 1999) and aligned in Clustal X (Thompson et al. 1997). Haplotype diversity was estimated using DnaSP 4.0 (Rozas et al. 2003). Maximum parsimony (MP) and Bayesian inference (BI) analyses were implemented in PAUP\* (Swofford 2003) and MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003), respectively, following Collado (2017). For the BI analysis we set the best model of sequence evolution obtained in jModelTest 0.1.1 (Posada 2008) based on the Bayesian information criterion (TPM2uf+I+G). The support nodes were evaluated using 100 bootstrap (bt) pseudoreplicates (Felsenstein



**Figure 1.** Sampling localities in Llanquihue Lake. *Physa acuta* was found in three places (red triangles). Negative sampling sites for the species are shown in green circles. Principal cities are depicted by black squares.

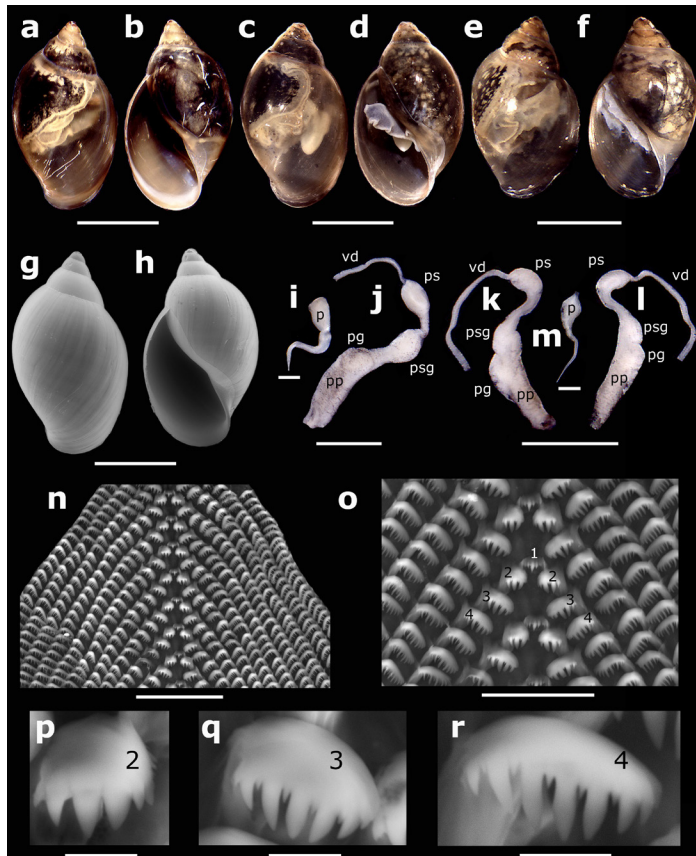
1985) in the MP analysis while in BI analysis were obtained as posterior probability values (p.p). We generated a matrix of 75 COI sequences including those obtained in the present study plus others of *Physa acuta* and related species retrieved from GenBank (Wethington & Lydeard 2007, Pip & Frank 2008, Wethington et al. 2009, Albrecht et al. 2009, 2014, Gates et al. 2013, R. Aguilar, M.B. Ogburn and A.H. Hines, unpublished data, D.S. Park and H.W. Oh, unpublished data). A sequence of the species *Lymnaea stagnalis* (Linnaeus, 1758) was used as outgroup (Remigio 2002).

**RESULTS**

Sampling performed in Llanquihue Lake allowed to collect eight physid specimens in Llanquihue City (41°15'15.00 S; 73°00'06.50" W), forty three in

Puerto Chico (41°19'37.06" S; 72°57'26.64" W) and one in Maullín River (41°15'41.07" S; 73°00'09.14" W), which were identified as *Physa acuta* (Fig. 2 a-r). Shell measurements are shown in Table I. Eight additional sampling sites only provided native specimens of the genus *Potamolithus* Pilsbry, 1896 and/or *Chilina* Gray, 1828.

The shell shape of specimens examined in the present study is elongate-ovate, thin, sinisterly coiled, light brown and translucent (Fig. 2a-h). The mantle is black with white spots, foot gray. The distal portion of the male copulatory complex includes prepuce, penis sheath gland, penis sheath and vas deferens (Fig. 2i-m). The prepuce is a large structure which includes a lenticular preputial gland near its posterior end. The penis sheath encloses an elongated conical penis. The radula is V-shaped (Fig. 2 n-o). It consists of a central tooth composed by



**Figure 2.** a-f. Specimen shells of *Physa acuta* from Llanquihue Lake under stereomicroscope. a, b. Specimen from Maullín River (MCNPPRF-CC 148-01). c, d. Specimen from Puerto Chico (Puerto Varas) (MCNPPRF-CC 148-02). e, f. Specimen from Llanquihue City (MCNPPRF-CC 148-03). g, h. The same specimen of a and b imaged using SEM. i-m. Male copulatory complex of a specimen from Puerto Chico (i, j) (MCNPPRF-CC 148-02) and Llanquihue City (k, l, m) (MCNPPRF-CC 148-03), j,l, left side, k, right side (p: penis, pg: preputial gland, pp: prepuce, ps: penis sheath, psg: penis sheath gland, vd: vas deferens). n. Anterior section of the radular ribbon of a specimen from Llanquihue City (MCNPPRF-CC 148-04). o. Zoom of the central area of n showing the radular teeth augmented (1: central tooth, 2: first pair of lateral teeth, 3: second pair of lateral teeth, 4: marginal teeth). p-r. Radular teeth of a specimen from Puerto Chico (the numbers of the teeth correspond to those denoted in o). Scale bar: a-h= 3 mm, i= 300 μm, j-l= 1 mm, m= 300 μm, n= 50 μm, o= 30 μm, p-r= 5 μm.

three major cusps and other minor ones (Fig. 2o), followed by two pairs of lateral teeth and a series of marginal teeth (Fig. 2p-r).

We yielded 11 COI sequences of *Physa acuta* from Puerto Chico (6) and Llanquihue City (5), which were submitted to GenBank (accession numbers: MK024393-MK024403). The sequence analysis allowed to identify five haplotypes, two from Puerto Chico, two from Llanquihue City and one shared by seven specimens of both localities. Both, the MP (tree not shown) and BI (Fig. 3) analyses recovered the snails from Llanquihue Lake in the *Physa acuta* clade with high node supports (1.00 p.p, 100% bt). Within this clade, the five haplotypes clustered in three different subclades.

## DISCUSSION

The shell shape, male copulatory complex and radular microstructure of specimens from Llanquihue Lake were congruent with the characteristics previously reported in *Physa acuta* (e.g. Draparnaud 1805, Paraense 1987, 2003, Appleton 2003, Paraense & Pointier 2003, Núñez 2011, Collado 2017). Among all the morphological features, the position and characteristic of the preputial gland, lenticular in shape, has been considered as relevant to recognize this species (Paraense 1987, 2003, Paraense & Pointier 2003), although this need to be verified with caution since it is present in other members of the

genus (Wethington & Lydeard 2007). The BLASTn search and phylogenetic analysis based on COI sequences also allowed to recognize the specimens from Llanquihue Lake as belonging to *Physa acuta*. The shared haplotype showed 100% identity to other two sequences of *Physa acuta*, one from Anne Arundel County, Maryland, USA (MH087660) (R. Aguilar, M.B. Ogburn and A.H. Hines, unpublished data) and another from South Korea (KF966541) (D.S. Park and H.W. Oh, unpublished data). Another haplotype recovered in Llanquihue City showed 100% identity to the sequence EU038367 obtained from Santiago de Cuba (Wethington & Lydeard 2007) and several snails from northern and central Chile (Collado 2017). A sequence of Puerto Chico differs in one substitution of sequences from El Salto (KX108847) in central Chile (Collado 2017) and Lake Titicaca (FJ373016) (Albrecht et al. 2009).

The presence of *Physa acuta* in Chile has put in doubt the identity of some native species of the genus. For instance, the native *Physa chilensis* was described in 1886 with samples from Chile as type locality and later collected from El Salto River in the Región de Valparaíso (Sielfeld 2001) but samples from this locality were unambiguously identified as *Physa acuta* (Collado 2017). The same occur in the case of *Physa nodulosa*, originally described in 1948 from the Choapa River, Salamanca and other places in the Región de Coquimbo, where the presence of *Physa acuta* was also verified (Collado 2017).

**Table I. Shell dimensions in mm (minimum, maximum and mean values) of largest specimens of *Physa acuta* sampled in Llanquihue Lake.**

Location	n	Shell length	Shell width
Llanquihue City	8	4.6/5.9/5.1	2.7/3.5/3.1
Puerto Chico	8	3.7/6.7/4.7	2.1/3.9/2.8
Maullín River	1	6.6	3.6



**Figure 3.** Bayesian phylogenetic tree obtained using COI sequences. Snails sequenced in the present study are depicted in bold. Numbers above the nodes indicate posterior probability values obtained in the BI analysis (only values equal to or greater than 0.95 are given) followed by the bootstrap values obtained in the MP analysis (only values above 50% are given).

To become a pest, an invasive species must possess life history traits that confer ability to survive, reproduce and growth in the new ecosystem and then spread to additional places. Among the traits that have allowed *Physa acuta* to overcome the growth of ecologically analogous native species are a higher salinity and temperature tolerances, faster growth rate, higher fecundity, shorter incubation period and continuous reproduction during the year (Brackenbury & Appleton 1991, Zukowski & Walker 2009, Ng et al. 2015). The success of *Physa acuta* as an invader has also been shown by its great resilience capacity to repeated flooding, overcoming the population growth and expansion capability of native species (Brackenbury & Appleton 1991). In some invaded habitats like the Umsindusi River, South Africa, *Physa acuta* reaches densities of up to 4561 individuals/m<sup>2</sup> (Brackenbury & Appleton 1991) while the net reproductive rate has been recorded to be 116.1 in India (Saha et al. 2017). Several authors have shown that this species has replaced populations of endemic species of different genera (de Kock & Wolmarans 2007, Dobson 2004, Gashaw et al. 2008). At present, *Physa acuta* in Llanquihue Lake coexist with two ecologically analogous native freshwater snails, *Potamolithus australis* Biese, 1944 and *Chilina llanquihuensis* Marshall, 1933, with which competitive interactions can be expected as a consequence of niche overlap.

With the data reported in this study, the range of the invasion in Chile is extended several kilometers to the south than previously known (Bousset et al. 2014, Collado 2017), being the southernmost record for the country. Given the high invasiveness of *Physa acuta*, and the absence of previous records in southern Chile, it is possible that climate change may have favored the invasion of the species and subsequent propagation in the lake. Alternatively, it is also

possible that the contamination by sewage waters, which produced several problems to inhabitants of Puerto Varas in 2017 (e.g. <https://www.elciudadano.cl/chile/contaminacion-lago-llanquihue>, <http://www.terram.cl/2017/12/salud-confirma-puntos-de-alta-contaminacion-en-lago-llanquihue/>) could have also favored the spreading of this alien species. In this context, it is worth highlighting an increasingly important variant of dispersal, such as the Jump dispersal model of non-native species, which is associated with humans (MacIssac et al. 2001). This dispersal mode is determined by the probability of movement of propagules transported through human activities from a source of origin to a recipient ecosystems (for example, a tourist center). This type of dispersal is much less dependent on time or on the distance between source and recipient site. Once the non-native species reaches this place, it can spread according to its natural capacity or be transported again by humans through Jump dispersal to a new ecosystem. The positive about establishing the model by which non-native species propagate, lies in, for example, focusing attention on the probabilities of invasion from that receptor site now known to another site, in order to prevent it.

Physids can be spread via different active and/or passive dispersal vectors (Dillon & Wethington 1995, de Kock & Wolmarans 2007, van Leeuwen et al. 2013). The date and mechanism of introduction of *Physa acuta* in Chile is unknown (Collado 2017). The introduction route to Llanquihue Lake is also unknown, but after the summer of 2015, considering that the three places in which the species was found in this study were exhaustively surveyed that year. It is reasonable that there may have been more than one introduction event in the lake, judging from the different haplotypes found, including the arrival of animals from South Korea or

Maryland. However, considering that four of the five haplotypes were already identified in northern and central Chile, we think the most parsimonious interpretation would be that a single introduction of several genetically variable snails occurred from one of these northernmost regions of the country, or both. However, regardless of the causes that entry mechanism of the species, as the invasion is recent, it is advisable to take prompt measures to prevent the expansion of this invasive mollusk in Llanquihue Lake or nearby aquatic ecosystems.

### Acknowledgments

To the staff of the Museo de Ciencias Naturales y Arqueología Profesor Pedro Ramírez Fuentes, Chillán, Chile and Cristian Suarez for technical support. GAC, MAV and CT-D are grateful to Grupo de Investigación Biodiversidad y Cambio Global (GI 170509/EF), Universidad del Bío-Bío. We also thank the CONICYT-FONDEQUIP Program (No. EQM-140088) for acquisition of the Hitachi Scanning Electron Microscope. This work was partially supported by the CONICYT-FONDECYT under Grant 11130697 (GAC).

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#### How to cite

COLLADO GA, VIDAL MA, TORRES-DÍAZ C, CABRERA FJ, ARAYA JF & DARRIGRAN G. 2020. Morphological and molecular identification of the invasive freshwater snail *Physa acuta* (Gastropoda: Physidae) into Llanquihue Lake, Chilean Patagonia. An Acad Bras Cienc 92: e20181101. DOI 10.1590/0001-3765202020181101.

*Manuscript received on October 18, 2018;  
accepted for publication on January 31, 2019*

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Collado GA sampled the snails, performed the phylogenetic analysis and wrote the manuscript, with inputs from Vidal MA, Torres-Díaz C, Araya JF and Darrigran G. Collado GA and Cabrera FJ made the figures. All authors reviewed the final version of the manuscript.

