



Infection Detection: Molecular detection of *Angiostrongylus cantonensis* in Uruguayan apple snails

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1905 TIMELINE

1940s 1945 1950s 1960s

2003 2007

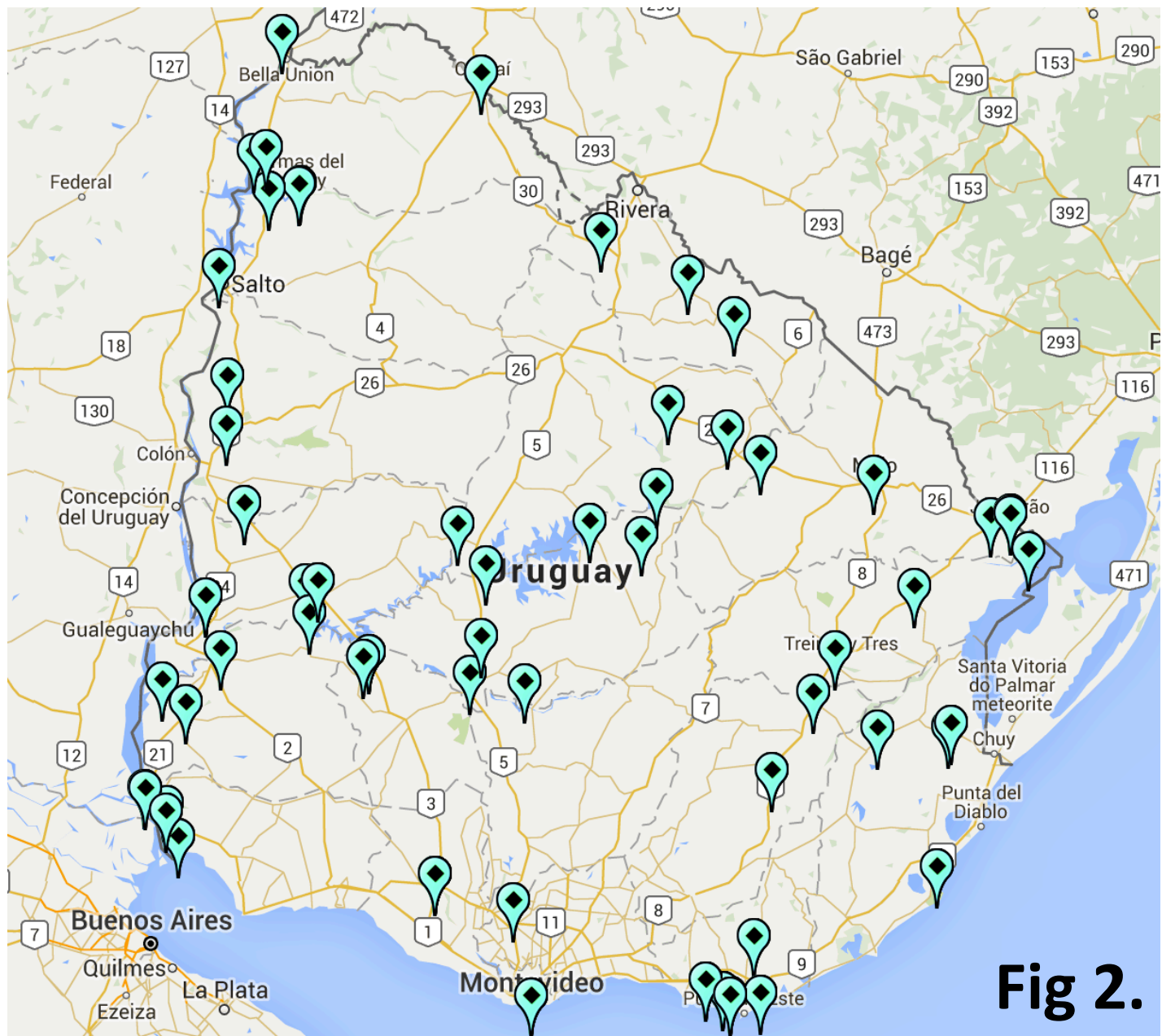
1905: Fourteen species of the genus *Angiostrongylus* described (Kamensky 1905 in Morera & Céspedes 1971)| **1940s:** *Angiostrongylus* spp. introduced and spread in shipments of war material from Southeast Asia to the Western Pacific islands, Micronesia, Melanesia, Australia, and Polynesia (Kliks & Palumbo 1992)| **1945:** First infection of *A. cantonensis* in humans (Beaver & Rosen 1964) | **1950s:** Numerous cases of *Angiostrongylus* identified in Sumatra, the Philippines, Taiwan, Saipan, New Caledonia, and as far east as Rarotonga and Tahiti (Kliks & Palumbo 1992) | **1960s:** Cases of *Angiostrongylus* detected in Vietnam, Thailand, Cambodia, Java, Sarawak, the New Hebrides, Guam, and Hawaii (Kliks & Palumbo 1992) | **2003:** Molecular differentiation of *A. costaricensis*, *A. cantonensis*, and *A. vasorum* (Caldeira et al. 2003) | **2007:** First record of molluscs naturally infected with *A. cantonensis* in Brazil (Caldeira et al. 2007)

ABSTRACT

Freshwater mollusks occupy a central, intermediate position in food webs through direct connections, either as consumers or as prey. Certain mollusks, such as snails, indirectly facilitate trophic interactions by serving as intermediate hosts for parasites, including the nematode *Angiostrongylus cantonensis*. This roundworm causes encephalitic meningitis in humans, or brain swelling, when people consume raw or uncooked, infected gastropods. While humans become accidental hosts after ingestion of infected larvae, mollusks serve as vital intermediate hosts. We sought to quantify *A. cantonensis* infection prevalence within apple snails (*Pomacea* spp.) collected from Uruguay. Uruguay represents the southern limit for *Pomacea* spp., known intermediate hosts for the parasite. We screened for the presence of *A. cantonensis* in apple snails by extracting total genomic DNA from foot tissue and conducting a species-specific PCR targeting the ITS-1 gene (internal transcribed spacer-1). We identified infected individuals through visual comparison against three positive controls using gel electrophoresis. To date, we have found no positives samples but have only screened a small subset of potential hosts. We plan to amplify the ITS-1 gene for *A. cantonensis* in 700+ apple snail tissue samples collected from site locations across Uruguay during previous field excursions (2011-2015). We also want to expand our screen to include 250 samples from Brazil. Other researchers have detected *A. cantonensis* in apple snails from northern Brazil, but no study has yet examined southern Brazil. Our study will provide the first molecular screen for *A. cantonensis* within freshwater apple snails in Uruguay and southern Brazil.

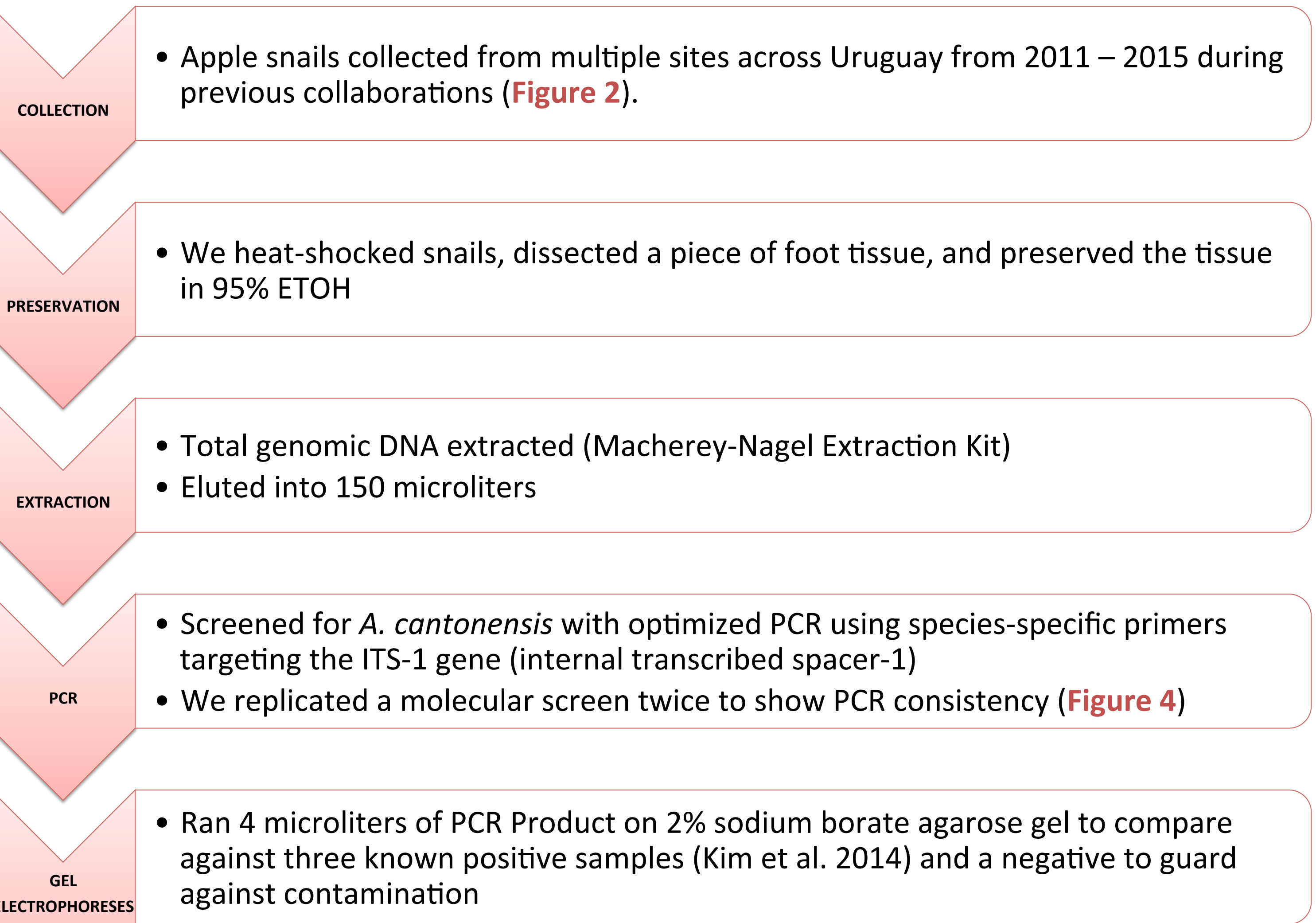
INTRODUCTION

- Snails indirectly facilitate trophic interactions by acting as intermediate hosts for parasites, such as the roundworm *A. cantonensis* (Kim et al. 2014).
- *Angiostrongylus cantonensis* (Figure 1) represents the most common cause for encephalitic meningitis (brain swelling) in humans when people accidentally consume infected hosts (Monte et al. 2012).
- While *A. cantonensis* originates from southeastern China and has spread throughout many tropical and sub-tropical regions of the world during the 20th century (see Timeline), this parasitic nematode spread to the Americas more recently (Martin-Alonso et al. 2015).
- Uruguay (Figure 2) represents the southern limit for *Pomacea* spp., known intermediate hosts for the parasite (Hayes et al. 2008).



Geographic map of all samples collected from Uruguay

METHODS



OBJECTIVES

1. Quantify *A. cantonensis* infection prevalence within Uruguayan apple snails (*Pomacea* spp.)
2. Identify patterns in *Angiostrongylus* literature



Angiostrongylus cantonensis represents the causative agent of human angiostrongyliasis, a potentially fatal disease. Rats serve as definitive hosts for *A. cantonensis* and snails serve as intermediate hosts after eating infected rat feces (Picture A; Figure 1) (Kim et al. 2014). Humans become accidental hosts after consuming raw or undercooked infected mollusks. The parasitic nematode enters the central nervous system and burrows through tissue, indirectly causing hemorrhaging and brain swelling (Picture B) (Thiengo et al. 2013). Freshwater apple snails (*Pomacea* spp.) represent a known intermediate host for *A. cantonensis* (Picture C) (Hayes et al. 2008). Pictures A & B from “Monsters Inside Me”.

RESULTS: LITERATURE REVIEW AND PARASITE SCREEN

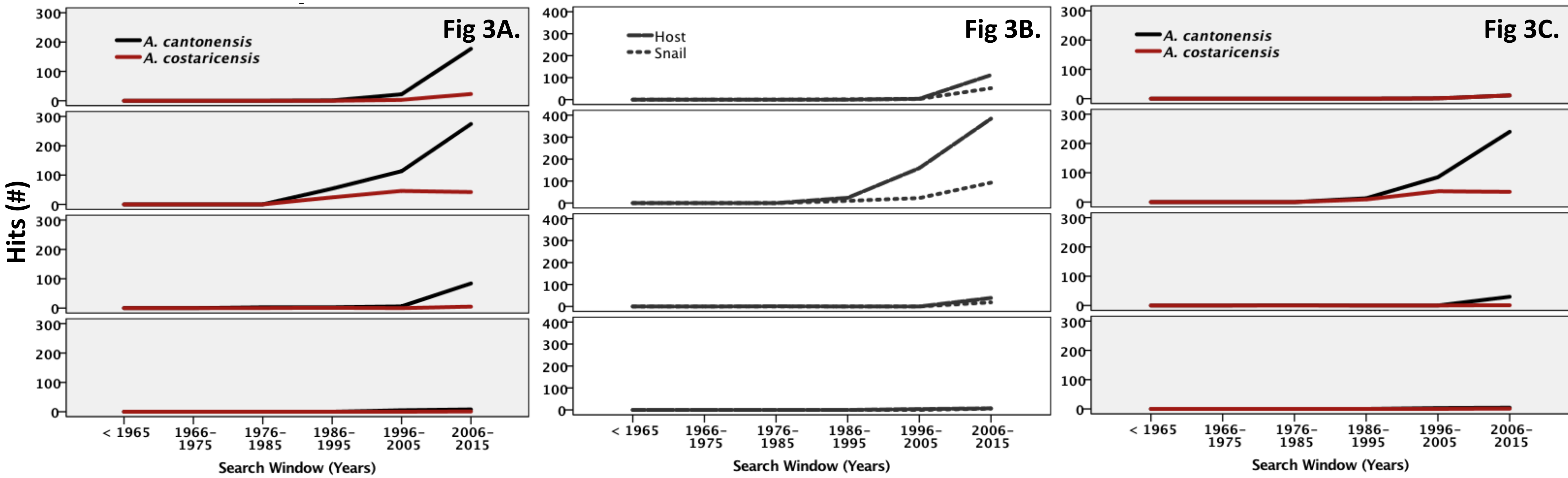
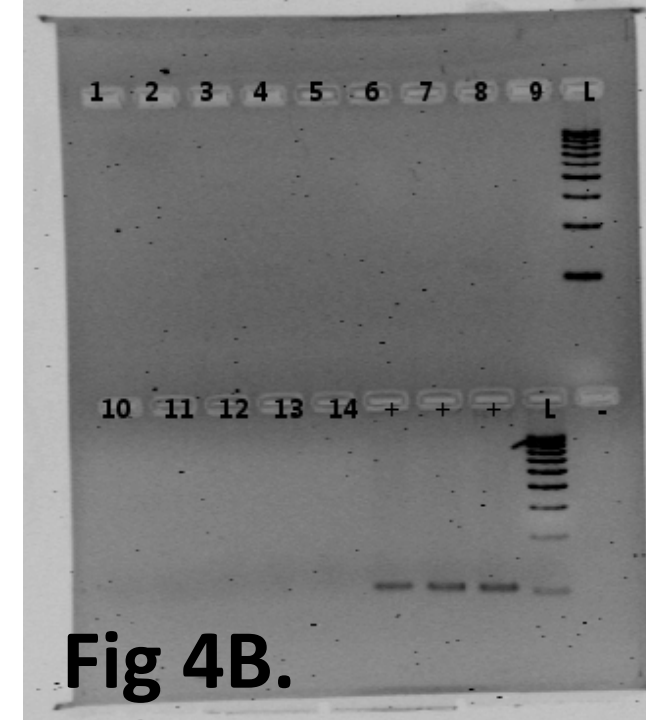
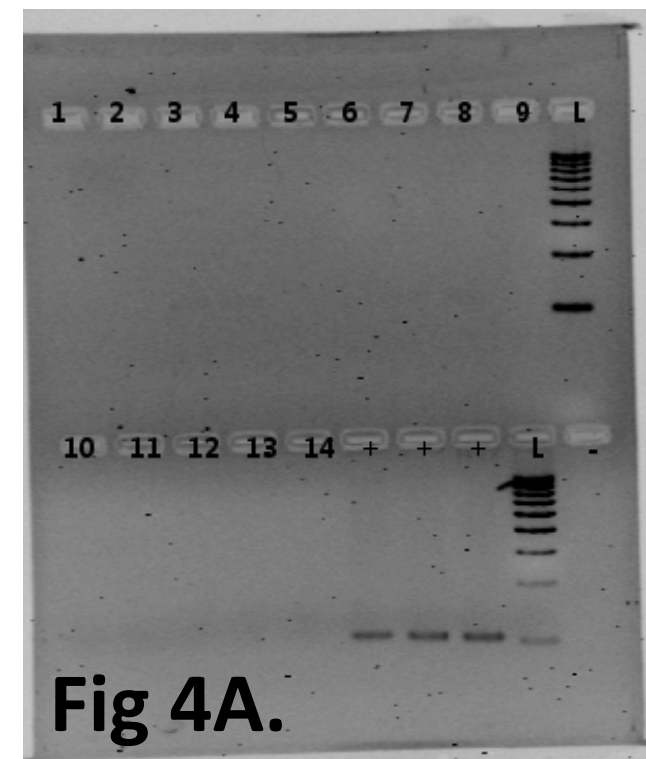


Fig 4. PCR Replications



Using four databases from the Southwestern University Library (“ASC” Academic Search Complete, “BA” Biological Abstracts, “PM” PubMed Central, and “BIO1” BioOne), we searched within abstracts of peer reviewed articles. While *Angiostrongylus* literature increases over time, scholars tend to pay more attention to *A. cantonensis* than *A. costaricensis* by five fold (Figure 3A). Comparing publication specificity revealed that *Angiostrongylus* scholars appear to publish literature on host specificity (broad studies) 3.5x more than snail specificity (narrower studies) (Figure 3B). In addition,, *A. cantonensis* literature tends to show 4x more host specificity than *A. costaricensis* (Figure 3C). Our results have not yet confirmed the presence of *A. cantonensis*, although we carefully monitor our methodological consistency as evident by repeatable gels of an *Angiostrongylus* screen(Figure 4) .

DISCUSSION

To date, none of the 112 Uruguayan apple snails that we have screened indicate a positive infection by *A. cantonensis*, despite the overall increase in attention that this parasite has received in peer-reviewed literature over the last decade. The majority of reports of *A. cantonensis* infection in apple snails include populations in northern and northeastern Brazil (Monte et al. 2012; Thiengo et al. 2010), China (Lv et al. 2009) and Hawaii (Kim et al. 2014). Our results so far may suggest undetectable to minimal infection along the southern border of Uruguay, but we hesitate to extrapolate too much. We have an additional 588 Uruguayan samples to screen and hope to add another 250 samples from southern Brazil. A recent screen of 250 apple snails from the United States also failed to detect any infection (Savrick, unpublished), although those non-native populations may have experienced a severe bottleneck. A comprehensive screen for *A. cantonensis* requires a substantial sample size. Kim et al. (2014) found several positives (70) among snails from 40 sites across the six largest Hawaiian Islands, but that study also included 1,271 samples. We might expect to see positives in future samples given the adjacent geography of Uruguay and Brazil and the number of studies that increasingly focus on the prevalence and distribution of *A. cantonensis*, especially in comparison to the less widely distributed *A. costaricensis* (Yong et al. 2015). Identification of a positive sample would extend the geographical range of this parasite. Furthermore, as global climate change increases suitable habitats for intermediate hosts and more regions with appropriate conditions for parasite transmissions occur (Kim et al. 2014), *A. cantonensis* may represent a more global concern than previously believed.

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