

## From the estuary to the Amazon basin: *Corbicula fluminea* (O.F. Müller, 1774) (*Bivalvia Venerida Cyrenidae*) in Ecuador

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### ABSTRACT

The Asian clam *Corbicula fluminea* (O.F. Müller, 1774) (*Bivalvia Venerida Cyrenidae*) is a non-indigenous invasive species with a vast record of new occurrences worldwide. The salinity and thermal tolerance of the clam led us to look for evidences on the presence of *C. fluminea* in the Guayas Estuary, Ecuador. The inspection of the upper estuary confirmed our hypothesis. Fresh remains of the clam, along with degraded shells and different shell class sizes ( $14.62 \pm 2.67$  mm, mean shell length) were observed. It was concluded that this estuary corresponds most likely to the introduction of *C. fluminea* in Ecuador. Its occurrence at the upper Amazon basin is attributed to a human mediated introduction linked to the trading use of the clam.

### KEY WORDS

Ballast water; Ecuador; human mediated; invasibility; invasiveness.

Received 02.11.2017; accepted 19.11.2017; printed 30.12.2017

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### INTRODUCTION

The Asian clam *Corbicula fluminea* (O.F. Müller, 1774) (*Bivalvia Venerida Cyrenidae*) is a highly invasive species (McMahon, 1999). Its native distribution range is thought to extend from eastern Asia (reaching the north in eastern Russia and the Philippines in the South, including China, the Korean Peninsula as well as Japan (Karatayev et al., 2007). As a result of its invasive potential, the species occurs now in all the continents except the Antarctic (Gama et al., 2016). The invasive occurrence of *C. fluminea* in South America is not so overwhelmingly present as in Europe or North America, but the information about its distribution range in South America is somewhat contradictory. Some published data situate *C. fluminea* exclusively at the east of the Andean Cordillera (Gama et al., 2016) while others authors locate populations of the clam

between the Cordillera and the Pacific Ocean (Crespo et al., 2015).

The spreading of *C. fluminea* in estuaries and freshwater ecosystems depends on key environmental parameters such as salinity, temperature and oxygen concentration values. The species is able to inhabit estuarine environments but it is rarely observed beyond the oligohaline zone, with a reported maximum of 17 psu (Verbrugge et al., 2012) in which its survival may be compromised (Morton & Tong, 1985). Verbrugge et al. (2012) limit the thermal tolerance of the Asian clam at 37 °C, while Crespo et al. (2015) provide information on thermal survivorship within the range 2–34.8 °C. This clam has low tolerance to reduced dissolved oxygen conditions, especially adult individuals (Mathews & McMahon, 1999). Values of 3% in oxygen saturation during more than a week may cause a significant increase in mortality

(Matthews & McMahon, 1999). Besides, adequate concentrations of calcium and satisfactory pH values are also important for the establishment of *C. fluminea* populations (Cooper, 2007). Decimated population may recover easily or isolated individuals may found new populations because *Corbicula* Megerle von Mühlfeld, 1811 specimens have high reproductive capacity and plasticity undergoing hermaphroditic, cross-fertilization and self-fertilization (McMahon, 1999; Lee et al., 2005).

Ballast water discharge, shellfish transplantation and intentional releases are frequently reported among the most common vectors for aquatic species introductions (Roman & Darling, 2007). The completion of an aquatic invasion depends on a number of factors such as vector dynamics at the origin and destination points, species endurance during transport, the existence of advantageous environmental or biological conditions in favor of an invasive species or even the invasive susceptibility of a habitat. For instance, large ports receiving transoceanic ships facilitate the introduction of invasive species because of the increased risk of ballast waters discharge (Keller et al., 2011). In the same vein, species adapted to dynamic environments may become more easily established in destination habitats than species with lesser physiological plasticity (Davis, 2009; Liu et al., 2012). Species invasiveness may be also enhanced or limited by biological interactions such as, for instance, predator-prey dynamics (Hunt & Yamada, 2003). Invasive species could additionally benefit from their edible features to spread further: unrestricted harvesting of introduced species for human consumption may create the temptation to introduce the species to uninvaded regions as an economic resource (Nuñez et al., 2012).

The aim of this study was to find evidences of the occurrence of *C. fluminea* in the largest estuarine system in Ecuador, the Guayas estuary. This work also intends to clarify the introduction of the clam in the country and advance the most likely pathway followed by the Asian clam to reach the upper Amazon basin.

## MATERIAL AND METHODS

### *Study area*

The Mocolí Island is located in the upper Guayas estuary, where the Babahoyo River flows into the estuary (Fig. 1). The study site is not far from the port of Guayaquil. The port has been historically located in the Guayas estuary, but new port terminals were built in a nearby creek interconnected with the estuary. The Mocolí Island has a surface of 295.4 hectares and it is used as a residential area. The island is mainly a private property and access to the intertidal area is very difficult or even forbidden for government's officials. This situation is recurrent in the upper estuary where real estate businesses own properties around the margins of the estuary. Our first plan was to inspect parts of the Island where access to the shore was easily granted. However, guards stopped us in our way and we were directed to the property managers. After meeting with a number of managers by more than an hour and a half, access was finally granted to part of the intertidal zone nearby the bridge that connect the Island with the mainland (02°06'09.88"S, 079°52'05.60"W; Fig. 1).

### *Sampling, lab procedures and data analysis*

Sampling was carried out, during ebb tide, at the beginning of February 2017. The mid height of the intertidal region was inspected within a surface of approximately 75 m<sup>2</sup> during 30 min by three people. All the retrieved sampled elements were stored in 70% alcohol solution. Next, 150 m of the upper intertidal region were inspected in the opposite direction of the bridge during 20 min by four people, when the tide prevented continuing the survey in the mid intertidal. Many debris brought by currents and tides were present in this zone, including dead clams. Water samples were collected to measure salinity in the lab and water temperature was measured in situ with a digital thermometer (almost at noon).

Shell length was measured in the right valve, with a Vernier caliper, to the nearest 0.01 mm. Salinity was measured optically in the lab with a refractometer. Shells were washed up and opened to look for living specimens. Pictures were taken to highlight key features of the sampled individuals. Shell's class sizes were displayed in a histogram with 1mm bin's width. The mean and standard deviation of shells with a normal distribution were de-

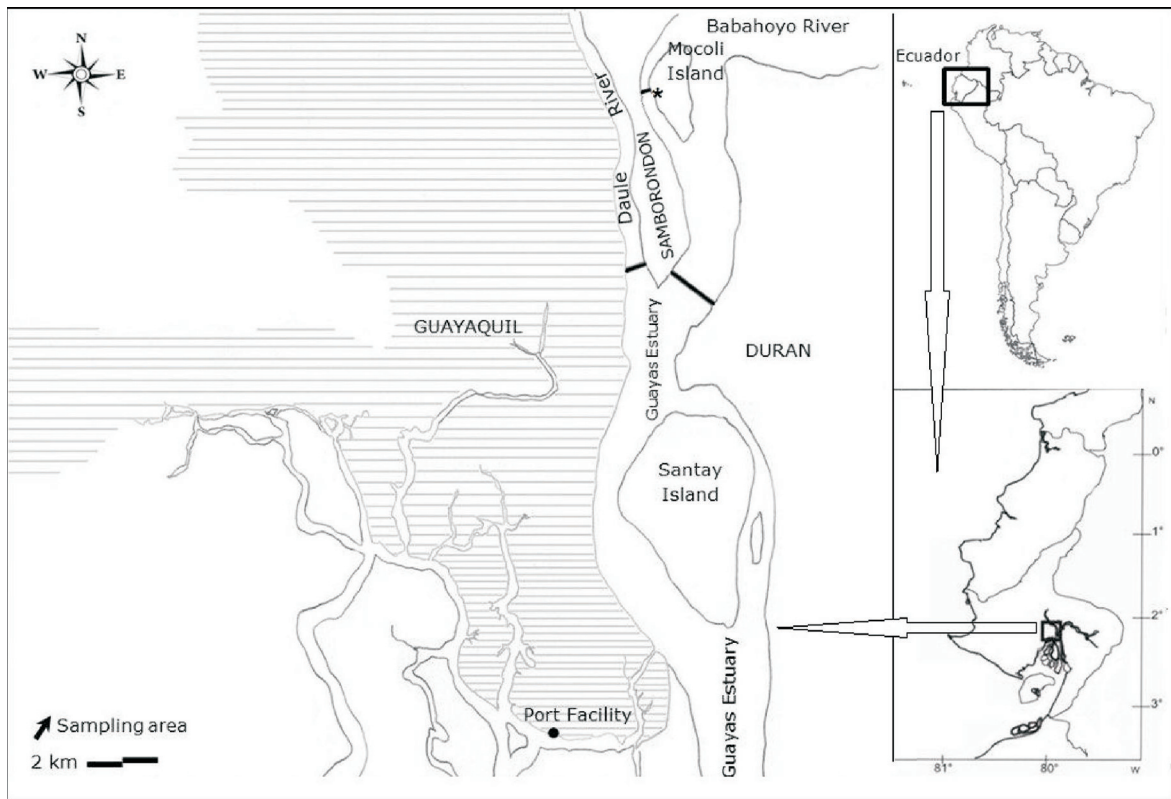
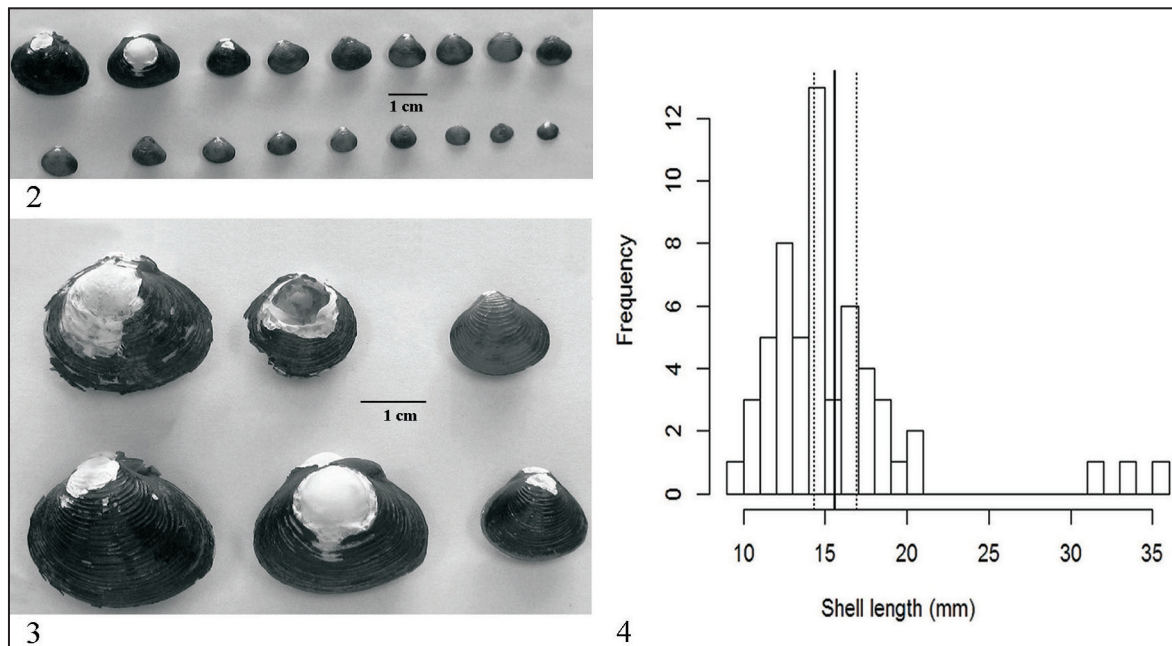


Figure 1. Map showing the study site and the location of the Port of Guayaquil in relation to the Guayas estuary. Gross dark lines over the estuary are bridges. The striped area represents the city of Guayaquil.

Location	Coordinates	References	Survey
Pastaza River	2°21'33"S, 77°05'03"W	Willink et al., 2005	1999
Pastaza River	1°55'09"S, 77°48'52"W	Lee et al., 2005	1999
Pastaza River	2°14'09"S, 77°15'10"W	Lee et al., 2005	1999
Esmeraldas River	0°55'01"N, 79°39'17"W	INP, 2002	2001
Taura River Basin	2°18'00"S, 79°43'60"W	Mora, 2005	2005
Taura River Basin	2°15'26"S, 79°22'12"W	Mora, 2005	2005
Baba River Basin	0°29'37"S, 79°19'21"W	Cárdenas, 2011	2010-2011
Baba River Basin	1°01'02"S, 79°27'49"W	Cárdenas, 2011	2010-2011
Los Rios Province	1°20'43"S, 79°22'33"W	Muzzio, 2011	Before 2011
Los Rios Province	1°47'43"S, 79°18'05"W	Muzzio, 2011	Before 2011
Los Rios Province	1°40'26"S, 79°38'54"W	Muzzio, 2011	Before 2011
Búa River	0°04'14"S, 79°26'32"W	Muzzio, 2011	Before 2011
Isla Mocoli	2°06'10"S, 79°51'41"W	This study	2017

Table 1. Locations, coordinates, references and dates of the survey at study sites where the occurrence of *Corbicula fluminea* was reported in Ecuador.



Figures 2, 3. Photographs showing (Fig. 2) the whole set of shell's sizes collected at the sampling site and (Fig. 3) state of conservation of the shells. Figure 4. Histogram showing shell length frequencies at bins of 1 mm. The dark vertical line is coincident with the mean length of the whole set of shells after excluding the three shells located at the right side of the histogram. Vertical discontinuous lines represent the standard deviation.

picted in the histogram. Normality was assessed with a Shapiro test (Crawley, 2007). The Chebyshev's inequality was used to calculate the proportion of the population at a distance of  $k$  standard deviations from the mean (Beasley et al., 2004). The Chebyshev's inequality is a conservative approach that may be applied to any distribution type (Beasley et al., 2004). Data analysis was carried out using R statistical software. The systematic follows WoRMS (2017).

## RESULTS

The mean salinity in the study site was between 0-0.5 psu and mean temperature  $26.8 \pm 0$  °C. None of the shells collected corresponded with a living clam. However, fourteen shells were observed in living position at the study site, indicating that the clams settled, lived and died in the sampling area at the upper reaches of the Guayas estuary. Shell's morphology match the expected features for *C. fluminea*, though the taxonomy of the genus *Corbicula*

is a matter of controversy and debate (Lee et al., 2005). The whole set of shell's sizes is shown in figure 2. The picture includes 18 out of 57 collected shells. The largest shell had a length of 35.23 mm and the smallest 9.48 mm. The state of conservation of the shells (Fig. 3) indicates that the time of death was not the same for all the clams. Overall, the information provided by figure 2 suggest that not all the collected shells belong to the same reproductive event.

The existence of at least two clam cohorts is well represented in the histogram shown in figure 3 (the whole set of shell were included). There is a big gap between the shells in the left side of the histogram and the shells in the right side. The whole set of shells do not have a normal distribution (Shapiro test,  $W = 0.741$ ,  $p < 0.001$ ) but the population is normally distributed when shells in the right side of the histogram are not considered (Shapiro test,  $W = 0.975$ ,  $p = 0.307$ ). The mean and the standard deviation ( $14.62 \pm 2.67$  mm) for the population of clams with a normal distribution are shown in the histogram (Fig. 4). The smallest shell

in the group located in the right side of the histogram has a length of 31.73 mm, or  $k=6.41$  standard deviations from the shell's length mean in the left side of the histogram.  $K$  was obtained after solving the equation: mean +  $k$  (standard deviations) = 31.73. In accordance to the Chebyshev's inequality, six standard deviations should include 97% of the population (Beasley et al., 2004). If this proportion is thought as likelihood, then the probability of belonging to a population of shells with a mean length of 14.62 mm for a shell with 31.73 mm length would be less than 0.03%. Thus, differentiate reproductive events emerge again, indicating that *C. fluminea* has reproductive populations in the Guayas estuary.

## DISCUSSION

The Asian clam *C. fluminea* was able to colonize the oligohaline portion of the Guayas estuary. The clam has also the capacity to reproduce in the estuary, though their populations demonstrate to be vulnerable in this environment. Concurrently, we did not find living specimens, but the collections of shell in living position prove the ability of the clam to establish populations within the estuary. Collection of shells in living position has been used to characterize and study clam populations in sampling sites (Palacios et al., 2000; Conde et al., 2012). The shell size range indicates that most of the clams were likely within their first year at the time of death, except the three larger individuals (Cataldo & Boltovskoy, 1998; Fig. 4). Brown et al., (2007) found reproductive individuals within the shell size range 6–10 mm (see also McMahon, 1999), strongly suggesting that the clams in this study were able to reproduce before dying. Additionally, the different conservation state of the retrieved shells suggests that not all the clams belonged to the same generation. Thereby, the ability of the clam to reproduce in the Guayas estuary seems to be very likely.

*Corbicula fluminea* was probably transported from North America to Ecuador rather than from eastern South America (Crespo et al., 2015) because of the proximity of ports and the higher number of donor areas in western North America. The largest commercial port in Ecuador is located in the Guayas estuary (Fig. 1) emerging as the

most likely recipient of marine vectors transporting the clam (merchant ships; Roman & Darling, 2007; Keller et al., 2011). The morphotype Form A described for the Ecuadorian *Corbicula* in Lee et al. (2005) is frequent in North America with only one record in Argentina, at the south of the continent. The introduction of *C. fluminea* in the Guayas estuary may be explained by the release of individuals from bilge or ballast water. Additionally, the invasibility of oligohaline zones in estuaries may facilitate the occurrence of the Asian clam. This estuarine region in the marine-riverine confluence provides a suitable environment, since it is not generally limited by food resources and few predators may cope with saline stress (preconditions highlighted by Davis, 2009). Conversely, *C. fluminea* can osmoregulate at salinities below 13 psu, with little mortality for at least 7 days (Morton & Tong, 1985) or even establishing permanent populations in oligohaline estuarine zones



Figure 5. Fisherman showing a partial catchment of *Corbicula fluminea* inside a net used to gather clams. *C. fluminea* is caught by diving before being brought on board within the net. Note that the fisherman stands over a larger catchment already stored at the bottom of his boat.

(Conde et al., 2013). Once established in an estuary, the clam is prone to spread further. Tidal currents allow the clam to dislodge upstream or downstream. To do so, small individuals of *C. fluminea* secrete long mucous threads through their exhalent siphons in order to be transported by currents (Prezant & Chalermwat, 1984). Additional vectors of dispersal have been pointed out such as local pedal locomotion, entanglement with drifting plant (water hyacinth is a drifting plant in the Guayas estuary), carriage by birds, angling, commercial fishing, fish stocking or dredging among others (Karatayev et al., 2007; Roman & Darling, 2007; Minchin, 2014).

Gama et al. (2016) did not include Ecuador as a world's known location where the Asian clam has been reported, though previously, Crespo et al., (2015) referred the occurrence of the Asian clam in the country. The latter authors provide both mean and maximum shell length in relation to latitude and temperature that approach the values described in this study. Moreover, the presence of the clam in Ecuador led us to look for more detailed information, including grey literature. The outcome of this search revealed that the distribution of the clam, rather than occasional or localized (Crespo et al., 2015) is widespread from north to south in watersheds at the west of the Andean Cordillera (Table 1). Cárdenas (2013) even reported the clam as a fishery for human consumption and also refers the selling of *C. fluminea* in local markets. Indeed, the fishery of the clam in Ecuador has been documented by our institution in the Baba River (Fig. 5).

The exploitation of *C. fluminea* as a commercial valuable species in Ecuador may clarify the main reason for its widespread distribution in the country. Moreover, this tradable feature of the clam may elucidate its introduction to the east of the Andean Cordillera (Crespo et al., 2015) at the Pastaza River, Amazon basin (including Ecuador and Peru; Lee et al., 2005). Though Lee et al. (2005) referred only to the genus *Corbicula*, most likely the collected individuals from the Pastaza River pertained to the same species as the one describe in this study. A human mediated introduction from the western to the eastern Andes emerges as a necessary transport vector to overpass this natural barrier with a mean height of 4000 meters. The Asian clam has been previ-

ously described as upstream as in Manaus, lower Amazon (Beasley et al., 2003) in the west of the Amazon basin. We hypothesize that the long way from the Pastaza River to Manaus is highly susceptible of being colonized by *C. fluminea*. The clam may benefit from the downstream current along with its dispersion capabilities, an edible reputation and a high biological plasticity (Lee et al., 2005). If the clam was able to cross the Andean Cordillera, the allegedly high autochthonous aquatic diversity and the anoxic waters in the Amazon basin (Crespo et al., 2015 and reference therein) seem to be minor barriers to the dispersal of the clam towards the west. A follow up of the invasion in the Amazon basin for ecosystem conservation purposes would be advisable.

## ACKNOWLEDGEMENTS

We are grateful to all these colleagues worked at the National Institute for Fisheries (INP) in Guayaquil (Ecuador): Jackeline Cajas and Xavier Icaza for field assistance, to Dr. Elba Mora for her guidance, to Walter Méndez for the drawing of figure 1 and to Dr. Willan Revelo for providing the photographic material (Fig. 4). The first author was granted by the Secretary of Higher Education, Science, Technology and Innovation (SENESCYT), Ecuadorian Government, as a Prometeo Researcher.

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26.10.2017.