



RESEARCH ARTICLE

REVISED Population characterization and parasitological assessment of the giant African snail (*Achatina fulica*) in urban areas of Cartagena, Colombia [version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background: The giant African snail, *Achatina fulica*, is an invasive species recognized for being a serious agricultural pest and an intermediary vector for diverse parasites that cause diseases in humans. The knowledge of the state of African snail populations in urban areas is of great ecological and public health importance. Therefore, our objective was to characterize the status of giant African snail populations present in the city of Cartagena, Colombia, including the assessment of nematode parasites in the specimens.

Methods. Sites were visited following information from citizens affected by the presence of the African snail. The specimens were collected and transported to the laboratory; subsequently, they were weighed, measured, and classified by size. Dissections of lung tissue and soft organs were performed to search for and identify nematode parasites. Size measurement between the sampled sites was statistically compared and density and biomass indicators were established.

Results. In total, 204 snails were collected distributed among four sites within Cartagena city. Of these, 50% were juvenile specimens (10-40 mm). The size of the specimens showed significant differences between the sampling areas. The calculated density was between 0.0019-0.68 ind/m² and the biomass between 3.92-48.75 kg/ha. No presence of nematode parasites was observed in these specimens.

Conclusions. Densities and biomasses of *A. fulica* in Cartagena do not reach levels considered highly harmful. On the other hand, although no parasites were found in these snails, it is relevant to continue with

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studies on the human health risks that represent the presence of this invasive species in urban areas of Cartagena.

Keywords

Achatina fulica, biomass, population density, invasive species, plague, intermediary vector

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REVISED Amendments from Version 1

In this version we have corrected the year of the first report of the giant African snail in Colombia, we have also provided more details on the collection methods, data analysis, and the characteristics of the sites where the snails were found. Finally, in Table 1, it was indicated that "total" refers to the total of individuals collected.

Any further responses from the reviewers can be found at the end of the article

Introduction

One of the greatest threats to global biodiversity, livelihoods, human and animal health, agriculture, and the local economy are invasive alien species¹. The giant African snail, *Achatina fulica*, (Bowdich 1822) is a gastropod native to Africa and considered one of the 100 worst invasive alien species in the world^{2,3}. Currently, this species is widespread in tropical and subtropical areas on all continents due to the growth of international trade and increased transport that facilitate the introduction of this species, accidentally through agricultural or horticultural products or intentionally as a source of food or as a pet^{2,4,5}.

The giant African snail is recognized as an agricultural and garden pest⁵. Its great reproductive capacity allows it to reach high densities and biomass in short times^{3,5}, and its broad spectrum herbivorous diet allows it to consume more than 50 species of native and agricultural plants, all of which makes this species a serious pest with the ability to modify habitats and displace native species⁵. The giant African snail is an intermediate host for various parasites, among which the nematodes *Angiostrongylus cantonensis* and *Angiostrongylus costaricensis* stand out, which cause eosinophilic meningoencephalitis and abdominal angiostrongyliasis in humans, respectively^{6,7}. Recently, the presence of *A. cantonensis* in African snails in Colombian territory was confirmed, putting the population on alert due to the exposure of humans to the parasitic load of this snail, leading to the consideration of eosinophilic meningitis as a possible emerging disease in Colombia⁸. Risk factors for infection in humans and pets by this and other nematodes include eating infected snails raw or undercooked, or food contaminated by snail slime or feces^{9,10}.

In Colombia, the presence of *A. fulica* was registered for the first time in 2009¹¹, since then the mollusk has been distributed in more than 20 departments, registering in all regions of the country¹². Particularly in the Colombian Caribbean Region, the growth of *A. fulica* populations has been documented in the department of Sucre¹²⁻¹⁴. In Cartagena, Bolívar, journalistic media reported the presence of the giant African snail south of the city in 2016. However, since its appearance, there has been no scientifically documented record of the presence and distribution of this species in urban areas of the city, as well as its impact on human health. Therefore, the objective of this study was to generate the baseline of reference information on the African snail populations present in the Cartagena city, including the assessment of nematode parasites in the specimens.

Methods

Study area

Cartagena (10 ° 23'59 " N 75 ° 30'52 " W), capital of the department of Bolívar located within the Colombian Caribbean region, is a city of low ecotone or coastal, presenting altitudes between 1 and 9 meters above sea level, a temperature average of 27.5°C, a relative humidity of 80% and an annual rainfall of 870 mm.

Collection of specimens

The search for, and collection of, the snails was carried out between July and October 2019, a period that included the rainy season in the region and which the proliferation of the giant African snail is favored. The sampling consisted of visiting the zones within the neighborhoods of the city Cartagena previously informed by citizens affected by the presence of the snail in their homes and surroundings. At each place, a 50-minute sampling effort was made by two people in a single visit. During the inspections, searches were carried out in gardens, under undergrowth and rubble, or between crevices where they could take refuge. The specimens were collected manually using gloves and face masks and transported in easy-sealing bags to the laboratory of the UNIMOL research group in the University of Cartagena. As this was a monitoring study, all the snails available during collections were included for analyzes, no exclusion criteria were applied for the collection of the snails.

Morphometric analysis

The collection included live individuals of all sizes, which were measured using a digital caliper (0.01 mm) and weighed in a weighing machine (1 gr). The length of the shell was used as a descriptor of the total size of each individual, so size classes were defined as the following: class 1, to which newly hatched individuals and up to 10 mm were assigned; class 2, juveniles (10–40 mm); class 3, young adults (40–70 mm) and class 4, adults (> 70 mm)¹⁵.

Parasitological analysis

Specimens larger than 50 mm have a greater probability of carrying nematode larvae¹⁶, even so, we decided to evaluate specimens greater than 30 mm to increase the probability of findings. The snails were washed externally and were put to sleep by thermal shock by introducing them in ice water, once they were numb, the shell was broken by using dissection scissors to extract the lung tissue and the rest of the soft organs. The search and collection methods for nematode parasites described by Córdoba *et al.*¹⁷ were used. Briefly, the lung tissue was cut in 1 × 1 cm pieces and placed on microscope slides for observation under a stereoscope. Likewise, the other organs were cut and examined by compression between two glass pieces.

Data analysis

As data were not normally distributed, the snail size measurements between the sites were compared using the Kruskal-Wallis and Bonferroni tests post hoc, using the statistical software SPSS v.19.0 (IBM Corp, Armonk, NY) with a statistic significance defined as $p < 0.05$. Also, the population density of each zone was calculated according to the number of individuals collected per effective area sampled (Individuals/m²) and the biomass

per unit area (kg/hectares) was established. The google earth Pro 7.3.3.7786 polygon was used to determine the sampled area of each zone.

Ethical aspects

Giant African snail has been declared in Colombia as an invasive species, for which legal research permits are not required. This study was adjusted to the management regulations for *A. fulica* issued by the Ministry of Environment, 2011¹⁸. Also, during the execution of the study, efforts were made to reduce animal suffering during sacrifices, for this, the snails were put to sleep in ice water until their death before the dissections.

Results

During the period studied 204 snails were collected, distributed in four zones of four Cartagena neighborhoods, for which the presence of *A. fulica* was indicated by the community (Figure 1). The inspected sites where the snails were collected

corresponded principally to extensive gardens and other spaces inside of private properties and residential condominiums (Manga, Las Gavias, and Serena del Mar). At these sites the snails were found in different substrates, such as plants, tree roots, grasses, under litter, flowerpots, and ornamental plants; In Zaragocilla, snails were found aggregated in rubble inside an educational institute.

Overall, 50% of the total material collected corresponded to class 2 snails, followed by class 3 (42.64%) and class 4 (7.35%). In particular, in the Zaragocilla and Las Gavias sites, class 3 individuals predominated with 44.11% and 52.33%, respectively, in Manga, class 2 specimens were more abundant with 73.33% (Table 1).

Mean size and weight per site is shown in Table 2. The Kruskal-Wallis test showed a significant difference between the sizes of the most abundant African snail population groups

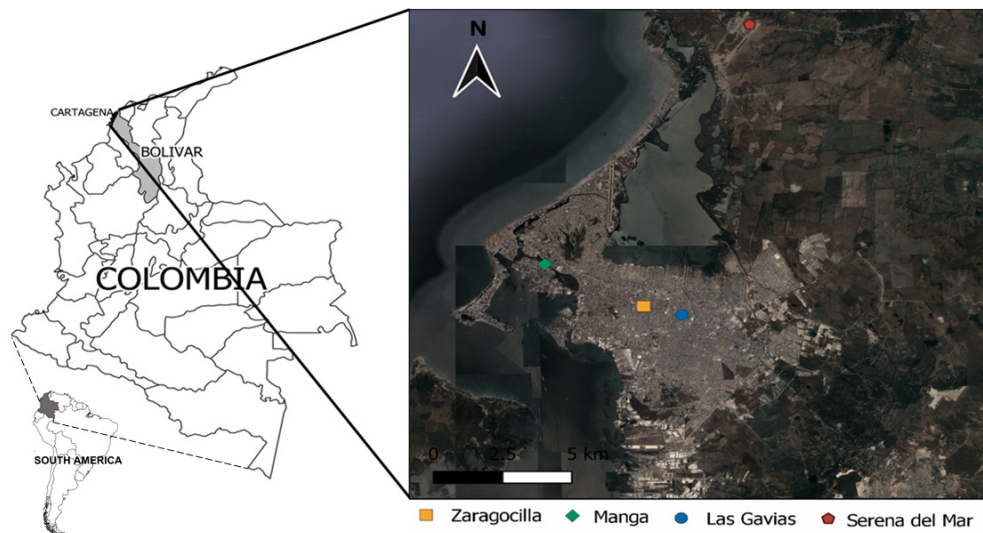


Figure 1. Sites where snails were found in Cartagena, Colombia.

Table 1. Size class distribution of *A. fulica* in Cartagena, Colombia.

Site	Class 1	Class 2	Class 3	Class 4	Total
Zaragocilla	0	10	15	9	34
Manga	0	44	15	1	60
Las Gavias	0	48	56	3	107
Serena del Mar	0	0	1	2	3
Total	0	102	87	15	204

Class 1: newly hatched individuals (up to 10 mm); class 2: juveniles (10 to 40 mm); class 3: young adults (40 to 70 mm); class 4: adults (> 70 mm); total: total of snails collected

Table 2. Analysis morphometric the population groups of *A. fulica* in Cartagena, Colombia.

Parameters	Site	n	Mean	Range	SD
Size (mm)	Zaragocilla	34	57.2	24.3–87.9	17.99
	Manga	60	36.2	20.5–78	11.44
	Las Gavias	107	42.6	28.3–80	8.08
	Serena del Mar	3	69	59.4–77.6	9.15
Weight (g)	Zaragocilla	34	26.4	3–86	20.8
	Manga	60	7.1	1–25	5.56
	Las Gavias	107	9,7	3–38	5.36
	Serena del Mar	3	21	17–25	4

n: number of snails collected, SD: standard deviation.

(Zaragocilla, Manga, and Las Gavias sites) ($p < 0.01$). Likewise, Bonferroni-adjusted pairwise comparisons indicated that differences existed between each site ($p < 0.01$ between each pairing). The population density established in each point, as well as the estimated biomass according to the calculated total weight, is presented in Table 3.

Regarding the parasitological analyzes, all the observed lungs were found healthy (free of cysts), likewise, no free larvae were observed in the slime or the rest of the soft organs, showing that these specimens were not infected with nematode parasites according to the methodology used.

Discussion

Understanding the populations of giant African snail present in different areas of Colombia is of great ecological, agricultural, and public health importance. The current study constitutes the first scientifically documented record of the presence of the species *A. fulica* in urban areas of the city of Cartagena.

It is known that the African snail can reach sizes of up to 300 mm in the length of the shell¹⁹. In contrast, other studies establish that the size naturally reached by *A. fulica* is around 100 mm^{20,21}. Even so, the mean values reported are usually much smaller and vary between 30 and 60 mm^{13,14,22–25}. Comparatively, our results from Cartagena agree with these last values, obtaining average sizes between 36.2–69 mm. Patterns in snail size can reflect the age structure of the population in a given area. It has been proposed that the establishment of *A. fulica* in new areas takes place in three stages: exponential, known as a long phase with vigorous individuals; stable, with a prevalence of a variable shell size among young and adult, and the decline phase, where young individuals are prevalent²². This suggests that the populations obtained from *A. fulica*

collected in Cartagena are in the stable phase, presenting a variety of young and adult individuals.

The population density of *A. fulica* reported in this study was between 0.0019–0.6818 ind/m², these density values are similar to those obtained by De la Ossa *et al.* in studies carried out with snails collected in urban areas of the city of Sincelejo and other municipalities of the department of Sucre, these authors reported densities in a range of 0.0031–0.205 ind/m²^{21,14,24}. Other similar values were reported in a study performed in Ilha Porchat, Brazil, obtaining a density of 0.07 ind/m²²³. Investigations such as those carried out in Havana, Cuba, showed a considerably lower density of snails (0.00015 ind/m²)²⁶. In contrast, densities of 1.1–4.6 ind/m² have been reported in different departments of Colombia²⁷, 0.06–8 ind/m² in Northeast Brazil²⁸, and an average of 8.4 ind/m² in Puyo, Ecuador²². On the other hand, studies performed in Puerto Iguazú y Corrientes, Argentina, recorded a much higher average density, which reaches 107.6 ind/m² and 118.6 ind/m², respectively^{15,29}. It has been mentioned that the areas highly affected by the giant African snail present densities of 10 ind/m² or more^{12,30}; this similarly occurs with regards to biomass, where devastating values of up to 780 kg/ha are estimated for areas which are highly affected^{12,31}. If that is the case, the densities and biomasses obtained in the snail populations of Cartagena are not very harmful.

Nematodes such as *A. cantonensis* and *A. costaricensis* cause eosinophilic meningoencephalitis and abdominal angiostrongyliasis, respectively, in humans. The life cycle of these nematodes involves rodents as the definitive host and mollusks of various species, including *A. fulica*, as intermediate hosts⁷. The snails examined in this work did not show evidence of infection by nematode parasites, one of the reasons could be the environment in which these snails were found. It has been noted

Table 3. Density and biomass calculated in the population groups of *A. fulica* in Cartagena, Colombia.

Site	n	Sampled area (m ²)	Density (ind/m ²)	Total weight (g)	Biomass (kg/ha)
Zaragocilla	34	747	0.0455	898	12.02
Manga	60	88	0.6818	429	48.75
Las Gavias	107	2443	0.0438	1048	4.28
Serena del Mar	3	1605	0.0019	63	3.92

n: number of snails collected.

that the African snail prefers sites such as garbage dumps, landfills, and empty lots, places where there is a greater probability of contact with rats, necessary for the nematodes to complete their life cycle¹⁷. In our case, more than 80% of the analyzed snails were collected in gardens of remarkably well-kept properties given the exclusivity of the sites, and according to interviews with various neighbors, rodents do not frequent these spaces.

In conclusion, although the densities of *A. fulica* estimated do not reach values considered highly harmful, the presence of this invasive species in properties and residential areas of Cartagena is worrying. Most of the snails were found associated with gardens, which suggests that the introduction and dispersal of this species within the city are related to the transport of organic fertilizers or other garden products, which could convert this species into a serious pest. Similarly, although no parasites were found in these snails, it is necessary to continue carrying out studies on the effect of this species on human health, as well as training campaigns on the management and control of the giant African snail in the city.

Data availability

Figshare: Raw data file_African Snail Project.csv, <https://doi.org/10.6084/m9.figshare.13288808>³².

This project contains the following underlying data:

- Raw data file_African Snail Project.csv (original raw data)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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In the article "Population characterization and parasitological assessment of the giant African snail (*Achatina fulica*) in urban areas of Cartagena, Columbia, Eder Cano-Perez and co-authors attempt to identify Giant African snail (GAS) population in the city Cartagena, Columbia and investigate the presence of nematode parasites in the collected specimens. Giant African snails are a highly invasive species and if remained unchecked can affect agriculture, native biodiversity, human health, and the economy. The authors in this paper provide an initial status of this invasive species since its introducing in 2016, whether they are carriers of nematode parasites and if they pose any potential human health risk. This article addresses the need for early assessment and collecting distribution information of invasive species to prepare and make better management plans. Despite the small Spatio-temporal distribution of the study, it makes an important contribution to invasive species distribution and its potential environmental and health risks.

The authors have collected specimens and location information for 204 snails from four zones within the city of Cartagena. They recorded their body weight, shell size, snail density per area, presence-absence of parasites within organs of each specimen. The authors found that 50% of the snails were juveniles (10-40 mm) followed by adults (40-70 mm and >70 mm). They also found a significant differences in sizes across the different zones. Interestingly the specimens collected were healthy and free from any cysts or larvae. The authors have clearly cited the current literature surrounding the invasion of giant African snails, especially across South America. The study design, sample collection and analysis as well as limitations of the collection sites are also well explained and justified. However, the raw data presented seems to be incomplete as a result the use and interpretation of the statistical models and analysis do not ensure full reproducibility of the data.

Here are some queries suggestions:

1. The authors indicate the number of samples collected is 204 but the raw data file lists only 50 individuals. As a result, I was not able to analyze the data myself. Additionally, can they

provide details of how the variables were analyzed in the model?

2. It would perhaps be helpful if the authors provided information (in form of the column data) of the calculated population density and biomass per unit area in the raw data file.
3. Can the authors clarify why they decided not to normalize the data? The weight data can be ln-transformed, and the shell length can normalize using sqrt function.
4. The column "number of snails collected per site" is missing or if it is in the data file, the column title is missing.
5. Can the authors please elaborate on the column shell length? What do the two values indicate and some rows do not have two values.
6. Graphical representation of the statistical analysis will help in understanding the paper more as it will indicate the significant differences between the population density across the different collection sites.
7. The authors mention the Giant African snail affect the citizens of Cartagena; it would be helpful if they can shed light on what those effects are? For example, they are in general a nuisance or their presence or the sight of the snails.
8. Under the section "Morphometric analysis" it can be useful if the authors provide information as standard errors as "+/-" for digital callipers and weighing machine.
9. I would like to see some more discussion about the different phases of the *A.fulica* establishment and its potential impact (if any) in the future in Cartagena.
10. I would like to see some more discussion on the population density of the snails in Cartagena and what it can possibly imply for the future.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Invasive species, Data analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 12 July 2021

<https://doi.org/10.5256/f1000research.55535.r87814>

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Jenny J Chaparro Gutiérrez 

CIBAV Research Group, Veterinary Medicine School, Faculty of Agrarian Sciences, University of Antioquia, Antioquia, Colombia

The study of the African giant snail, *Lissachatina fulica*'s distribution and possible source of zoonotic agents is critical. This study describes the pest's distribution in Cartagena, and its findings are valuable as primary data in the area.

Several parts of the manuscript, however, need to be supplemented or clarified:

1. The snail's genus and species are *Lissachatina fulica*.
2. It is unclear how they determined the sample size or the number of calls they would answer to define sample size.
3. Although the dissection method was chosen to search for nematodes in snails, the limitations of this methodology in comparison to enzymatic digestion methods were not mentioned. It is critical to include this when discussing the effects of parasite absence.
4. The cited literature is incomplete; it does not include all reports of studies on the presence of the snail in Colombia, and studies with relevant findings of nematodes with or without pathogenic potential in animals are omitted e.g:
 - Guerrero *et al.* (2018)¹
 - Penagos-Tabares *et al.* (2019)²
 - Heredia *et al.* (2018)³
 - Lange *et al.* (2018)⁴
5. Interviews with neighbours are mentioned in the discussion, but this is not reflected in the

materials and methods, nor in the information analysis. However, no mention is made of the community's reaction to the presence of the snail. There is mention of a potential impact on public health, but no mention of consumption habits or home gardens with the data of interviews. Please clarify.

6. The conclusion emphasizes the significance of continuing to conduct studies and management and control campaigns; I propose that it be stated whether they exist in Colombia or should be designed, and whether they should be articulated with the ICA or the National Ministry of Health.

References

1. Guerrero R, Rincon-Orozco B, Delgado NU: *Achatina fulica* (Mollusca: Achatinidae) Naturally Infected with *Caenorhabditis briggsae* (Dougherty and Nigon, 1949) (Nematoda: Rhabditidae). *J Parasitol.* **104** (6): 679-684 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Penagos-Tabares F, Lange MK, Vélez J, Hirzmann J, et al.: The invasive giant African snail *Lissachatina fulica* as natural intermediate host of *Aelurostrongylus abstrusus*, *Angiostrongylus vasorum*, *Troglostrongylus brevior*, and *Crenosoma vulpis* in Colombia. *PLoS Negl Trop Dis.* **13** (4): e0007277 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Heredia NS, Ávila AS, Velásquez LE: In vitro culture of L3 larvae of nematodes obtained from the African giant snail *Lissachatina fulica* (Mollusca: Gastropoda) in Santa Fe de Antioquia. *Biomedica.* 2018; **38** (0): 24-29 [PubMed Abstract](#) | [Publisher Full Text](#)
4. Lange MK, Penagos-Tabares F, Vélez J, Gutiérrez J, et al.: Regional report on *Angiostrongylus vasorum* in Colombia: Genetic similarity to European lineage. *Vet Parasitol Reg Stud Reports.* **13**: 21-23 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Veterinary Parasitology

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 April 2021

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Diego E. Gutiérrez Gregoric 

¹ División Zoología Invertebrados, Museo de La Plata, Buenos Aires, Argentina

² CONICET, Buenos Aires, Argentina

Responses to comments have been satisfactory.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 05 March 2021

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The present study focuses on an invasive alien species with potential effects on human health and agriculture. The population and parasitological analysis in urban areas of Cartagena, Colombia is highlighted, in such a way as to evaluate the role of these snails as potential transmitters of parasites. As the authors postulate, these studies must be constant, since in Colombia itself parasites have already been registered in this snail.

With regard to data collection, the estimate of density is not clear, nor is the number of samplings. Did they go to the same site more than once? Is a site the same as a house? These points should be better clarified in the methodology and in the results tables.

Minor Comments

- Keywords: *Achatina fulica* must go in italic.
- Introduction: The authors mention the first citation of *Achatina fulica* in Colombia for 2010, however in 2009 it had already been cited by Borrero et al. (2009, Tentacle N ° 17).
- In Table 1 you must indicate if it is the total number of individuals collected.
- For further parasitological studies on snails, the authors should use other techniques for the observation of larvae, in addition to those used here (see Valente *et al* 2017, Veterinary Parasitology).

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

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Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malacology

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Author Response 16 Mar 2021

Eder Cano Pérez, Universidad de Cartagena, Cartagena de Indias, Colombia

Dear reviewer,

Thank you for the comments and suggestions to our manuscript.

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Author Response 16 Mar 2021

Eder Cano Pérez, Universidad de Cartagena, Cartagena de Indias, Colombia

Dear reviewer,

Thank you for the comments and suggestions to our manuscript.

Competing Interests: No competing interests were disclosed.

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