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Achatina fulica Bowdich, 1822 (Mollusca, Gastropoda, Achatinidae) carrier of Helminthes, Protozoa and Bacteria in northeast Venezuela

Achatina fulica *Bowdich, 1822 (Mollusca, Gastropoda, Achatinidae) hospedador de helmintos, protozoarios y bacterias en el noreste de Venezuela*

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Dedicated to *Dr. Rafael Martínez Escarbassiere for his pioneering research on* Achatina fulica *in America.*

RESUMEN

Por cuanto el molusco *Achatina fulica* nativo del África es vector de helmintos, pero su relación con protozoarios y bacterias es poco conocida, decidimos estudiar las excretas de 1.200 ejemplares capturados en los estados Anzoátegui, Monagas, Sucre y Nueva Esparta, del noreste de Venezuela. Su moco pedal y heces mostraron infección por los protozoarios *Chilomastix* spp., *Trichomonas* spp., *Giardia* spp., *Balantidium* spp., *Entamoeba* spp., *Iodamoeba* spp., *Blastocystis* spp. Y por los helmintos de los grupos Ascarioidea, Trichuroidea, Ancylostomatidae y Cestoda. El moco céfalopodal mostró únicamente larvas de Rhabditida. Las bacterias *Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, K. azaenae, Aeromonas hydrophila, Acinetobacter baumannii, Pseudomonas aeruginosa, Campylobacter* spp. infectaron a las tres excretas. Los mecanismos de transmisión y la composición de las excretas, como nichos fisiológicamente apropiados para los organismos encontrados, son discutidos en relación con el riesgo epidemiológico que el molusco representa en salud pública y veterinaria.

Palabras clave: *Achatina fulica*, carrier, helmintos, protozoarios, bacterias, Venezuela.

SUMMARY

The mollusk Achatina fulica*, native to Eastern Equatorial Africa, has been incriminated as a carrier or vector of helminthes. Nevertheless, information in the literature as regards its status as a carrier for bacteria is scarce, and we could find no reference at all for its relation to protozoa. We studied microscopically the excreta from 1200 snails captured in Anzoátegui, Monagas, Sucre and Nueva Esparta states, in northeast Venezuela. The pedal mucus and feces were infected by the protozoa* Chilomastix *spp.,* Trichomonas *spp.,* Giardia *spp.,* Balantidium *spp.,* Entamoeba *spp.,* Iodamoeba *spp.,* Blastocystis *spp., as well as helminthes of Ascarioidea, Trichuroidea, Ancylostomatidae and Cestoda groups. The only helminthes found in the cephalopodal mucus were Rhabditida larvae. The three excreta were also infected by the bacteria:* Citrobacter freundii*,* Escherichia coli*,* Klebsiella pneumoniae*,* K. azaenae*,* Aeromonas hydrophila*,* Acinetobacter baumannii*,* Pseudomonas aeruginosa*,* Campylobacter *spp. Risk of infection and transmission mechanisms as well as the composition of the excreta as appropriate physiological niches for the organisms mentioned, are discussed with regard to the epidemiological importance of this snail for in human and veterinary health.*

Key words: Achatina fulica *carrier, helminthes, protozoa, bacteria, Venezuela.*

INTRDUCCIÓN

Achatina fulica (the Giant African snail, called in Venezuela "caracol africano") is an arboreal and terrestrial mollusk that frequently invades the vegetated shores of tropical and subtropical water bodies. The snails were introduced from Eastern Equatorial Africa by humans and are currently found in Asia (India, China, Japan, Philippines), the Pacific, Madagascar, Indonesia, Australia, the Caribbean Basin, the United States and South America (Colombia, Venezuela, Ecuador, Brazil,

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Argentina) (Martínez Escarbassiere & Martínez, 1997; Correoso, 2006; USDA, 2008; Gutiérrez *et al.*, 2011).

Achatina fulica snails are polyphagous (herbivores, carnivores, necrophages), and along with organic detritus, their preferred food list to date consists of over 500 known plant species, ranging from coffee and bananas in the tropics to potatoes and tobacco in more temperate zones. The snails are voracious eaters and competitors of native snails as well as habitat modifiers. Their importance in agricultural systems gardens and as agents for the reduction of biological diversity is increasing at an alarming rate making them one of the 100 most important pests worldwide (USDA, 2007; Zanol *et al.*, 2010; Cardoso *et al.*, 2012).

Furthermore, this species is an intermediate host for nematode pathogens such as *Angiostrongylus cantonensis*, the causal agent of zoonotic meningoencephalitis and *A. costaricensis*, which produces abdominal ileocolitis. They are thus also considered to represent a potential zoonotic risk and a possible danger to public health (Martínez Escarbassiere & Martínez, 1997; Teles *et al.*, 1997).

Achatina fulica, like all Achatinidae, needs calcium for the formation of its shell and for reproduction. It thus prefers environments rich in calcium carbonate, such as limestone landscapes with a pH of 7.0-8.0 and urban areas with abundant cement or concrete. Snails are active in the early morning, late afternoon, on cloudy, damp, or rainy days and avoid direct sunlight (USDA, 2007).

The muci of the Mollusca are produced by several glands that secrete the mucus through pores that pass between the epidermal cells. Pedal mucus is a gel produced when the foot gland of the snail, which can detect vibrations, is stimulated mechanically or chemically. This gel acts as a lubricant that permits the snails to slide over the surface of terrestrial or aquatic habitats by reducing friction between the foot (sole) and the ground (the slime trail). It also has strong adhesive properties that enable the snails to crawl up vertical surfaces such as walls and roofs without falling off. Furthermore, the mucus acts as a means by which information regarding locomotion direction, osmoregulation and the reparation of skin lesions can be conveyed as well as the type of food travelled over and the snail's sexual state (Skingsley *et al.*, 2000).

It is frequently difficult to differentiate where the head of the snail ends and the foot begins, so they are collectively called head-foot or cephalopodium which is a typical morphological character of all gastropods (Nordsieck, 2011) If the snail is disturbed continuously or even violently it discharges a clear foamy mucus by the synchronised contraction of the cephalopodal gland, located in the upper lateral region of the cephalopodium. This mucus is composed of acidic, neutral and sulphated mucins which behave as chemical barriers and form part of the defense mechanisms of the innate immunological system of the snails (Skingsley *et al.*, 2000; Pinchuck & Hodgson, 2009, 2012).

Achatina fulica was captured for the first time in Venezuela in Caracas, the capital city, and subsequently in the states of Miranda, Lara, Portuguesa, Carabobo, Aragua, Nueva Esparta (Margarita Island), Sucre, Monagas and the Delta Amacuro and most recently in Zulia, Anzoátegui and Yaracuy states. The snails have been found in diverse habitats; from forests to mountains and in both rural and strictly urban areas (Martinez Escarbassiere & Martinez, 1997; Martínez Escarbassiere *et al.*, 2008; Oletta & Carvajal, 2011). In Venezuela *A. fulica* has been found infected with *Schistosoma mansoni*, *Strongyloides* spp., *Trichuris* spp. and *Hymenolepis* spp. (Liboria *et al.*, 2010); the first case of human infection by *A. costaricensis* was reported by Incani *et al.* (2007).

To our knowledge, despite the extensive geographical distribution and the importance of *A. fulica* as a carrier or vector of helminthes, studies regarding its status for bacteria are scarce and infrequent, and as far as we know there are none incriminating it as a carrier for protozoa (Utomo *et al.*, 1991; Cardoso *et al.*, 2012). This, coupled with the explosive dispersion of dense populations of the snails in Venezuela, prompted this investigation. We chose the northeast of Venezuela as our study area due to the lack of investigations undertaken in this region, despite continuous complaints of the snails' presence made to the Eastern Center for Tropical Medicine, Eastern University, (Anzoátegui state) by the local inhabitants.

MATERIAL AND METHODS

Capture, maintenance, identification and parasitological and microbiological analyses of the snails

Snails were captured in the afore mentioned habitats in the evening (USDA, 2007) from urban areas in Anzoátegui, Monagas, Sucre and Nueva Esparta (Margarita Island) states during the months of June to August 2011 inclusive. Adult snails (average length 18 cm, able to copulate and oviposit) were collected by hand from plants, fruits and vegetables in small farms and residential gardens, as well as on walls outside human dwellings and pavements (Fig. 1 A/F). Latex gloves and safety glasses were worn at all times to prevent any risk from infection.

After collection, snails were placed in plastic bags, labeled according to date and collection site and kept in the laboratory in darkness at a relative humidity of 70%, and temperature 24-26ºC. They were fed with lettuce leaves (*Lactuca sativa*) *ad libitum* (Liboria *et al.*, 2010) thoroughly washed with sterile saline solution and vinegar. Species were identified according to Martínez Escarbassiere & Martínez (1997).

For the parasitological study, three hundred snails were collected from each of the four states mentioned (n=1200). They were separated into groups of 150 mollusks each from two municipalities per state and placed in plastic containers 60 x 40 x 20 cm, divided internally into five compartments with stiff plastic sheets and covered with perforated lids so that the snails could breathe easily (Fig. 2A, B). Each container was labeled according to the collection site of the snails placed in it. Snails were washed with sterile saline solution before placing them individually in each of the five compartments and the different types of mucus were obtained sequentially using a sterile pipette, as follows: pedal mucus (3

Fig. 1. *Achatina fulica*. A) Specimens undergoing intercourse; B) eggs inside the snail observed after dissection (400 X); C) eggs extracted by dissection (400 X); D) specimens attached to a plantain plant (*Musa paradisiaca*) in a garden; E) snails and excreted feces attached to a taro leaf (*Xanthosoma sagiittifolium*); F) individuals attached to wall of a house.

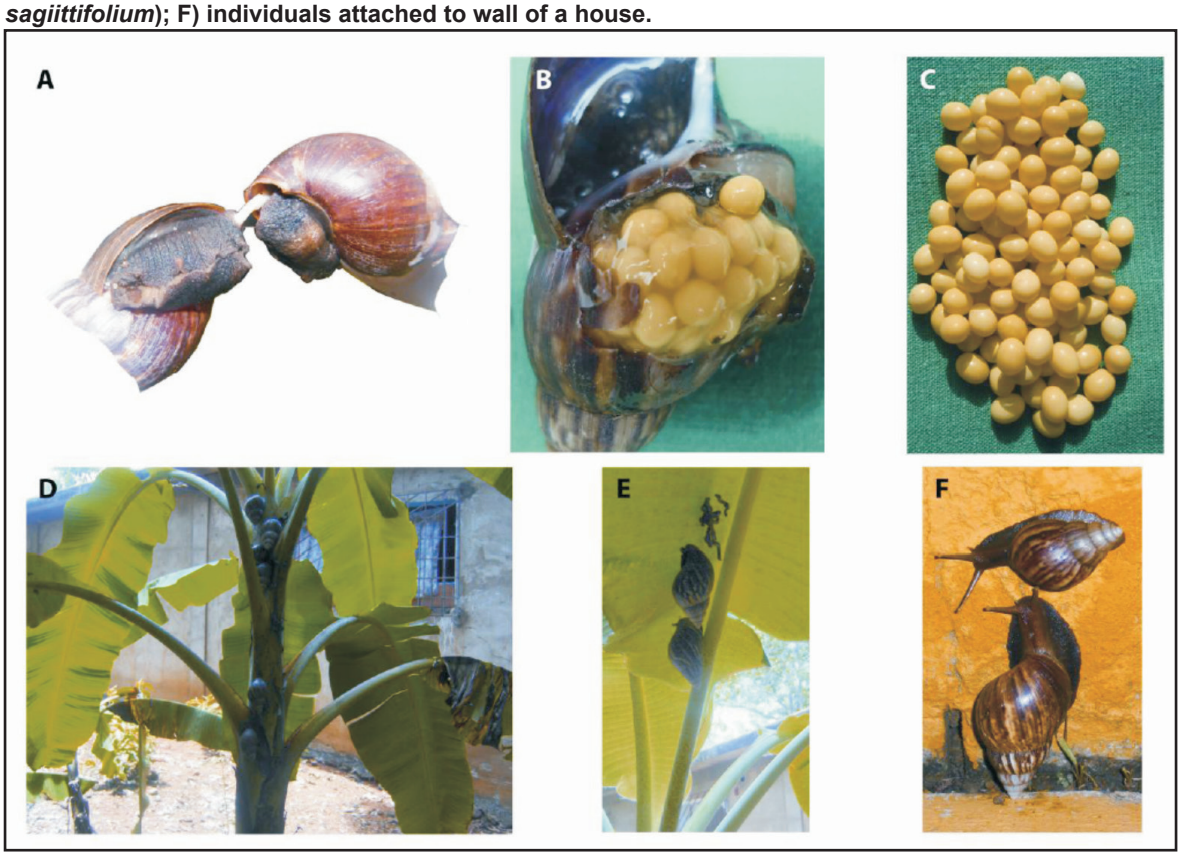
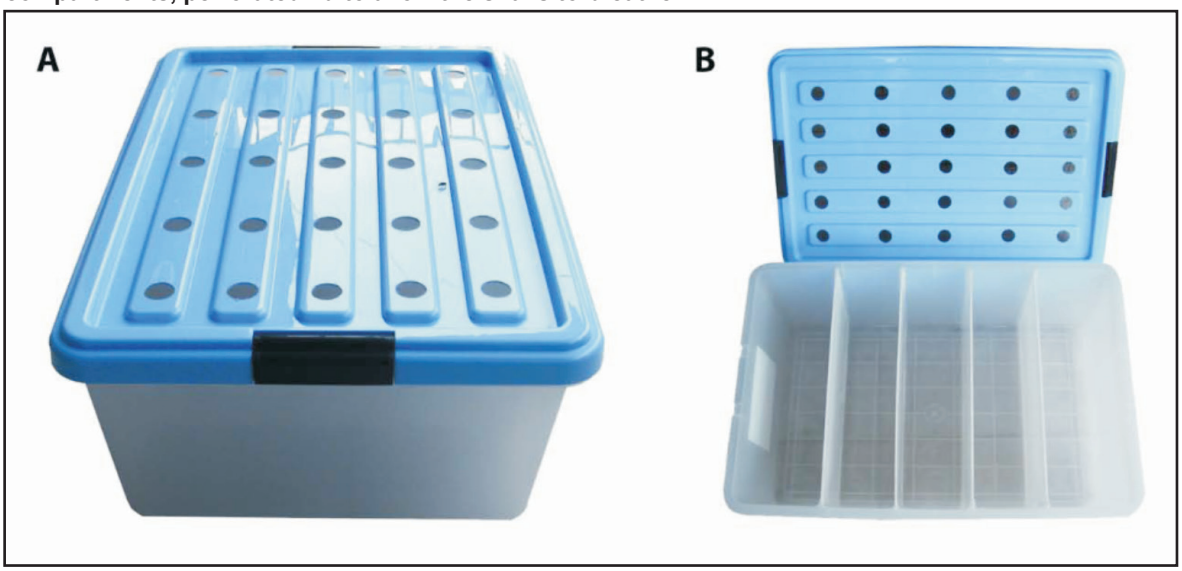


Fig. 2. A) container used for maintaining the snails in the laboratory; B) container divided into five compartments, perforated lid to allow the snails to breathe.



ml average) by lightly rubbing the snails feet with a small, sterile applicator stick; cephalopodal mucus (5 ml average) expressed by manual compression on the cephalopodal gland and finally the feces (50 mg average). In order to avoid fecal contamination, feces released during the collection of the other excreta were immediately recovered and both the compartment and the snail were thoroughly washed before continuing with their extraction. Samples of the three excreta were taken with small sterile applicator sticks and prepared as wet mounts by mixing them with drops of sterile isotonic solution. They were examined immediately for protozoa (trophozoites or cysts) and helminthes (eggs, larvae or adults). The material was also stained using Lugol´s solution in order to confirm the identification of cysts and eggs. The presence of coccidians was determined from samples stained with Kinyoun's solution. Fecal samples were prepared according to the Kato-Katz method for qualitative diagnosis. All preparations were sufficiently thin so as to be spread smoothly and evenly under the cover glass (75 x 37 mm) (Spencer & Monroe, 1961). Preparations were examined immediately with an Olympus CH20 microscope (400X; 1,000X magnification) and micrographs were taken using a digital camera (Nikon Coolpix P550, resolution 16.1 megapixels) attached to the microscope.

The presence and identification of the bacteria observed in the three types of excreta taken from ten snails captured from each of the four states surveyed (n = 40), was done using Gram coloration and by inoculation in gelatin agar Thiosulfate-Citrate-Bile- Sucrose (TCBS), Xylose-lysine-desoxycholate (XLD) agar, Salmonella-Shigella (S-S) agar, MacConkey agar and MacConkey broth (incubated at 35ºC/24hs, under facultative aerobic conditions); blood agar and chocolate agar (incubated at 35ºC/24hs under microaerophilic conditions); Preston agar and chrome agar (incubated at 35ºC/48hs under microaerophilic conditions).

Ethics Considerations

All experiments involving animals were conducted according to the current Bioethical Laws as laid down by the Venezuelan Science and Technology Ministry, and were approved by the Ethics Committee and the Committee for Animal Care of the National Fund for Science and Technology (FONACIT, Caracas, Venezuela).

RESULTS

Results of the parasitological analyses of the mollusks

All three excreta types in every one of the 1,200 *A. fulica* specimens examined were infected (Tables I, II). Both, the pedal mucus and the feces, contained protozoa and helminthes taxa of human and

Fig. 3. A) *Chilomastix* spp., trophozoite; B) *Trichomonas* spp., trophozoite; C) *Giardia* spp., cyst; D) *Balantidium* spp., trophozoite; E) *Entamoeba* spp., cyst; F) *Entamoeba* spp., trophozoite; G) *Iodamoeba* spp., cyst; H: *Blastocystis* spp., vacuolar stage (1000X; bar= 10um).

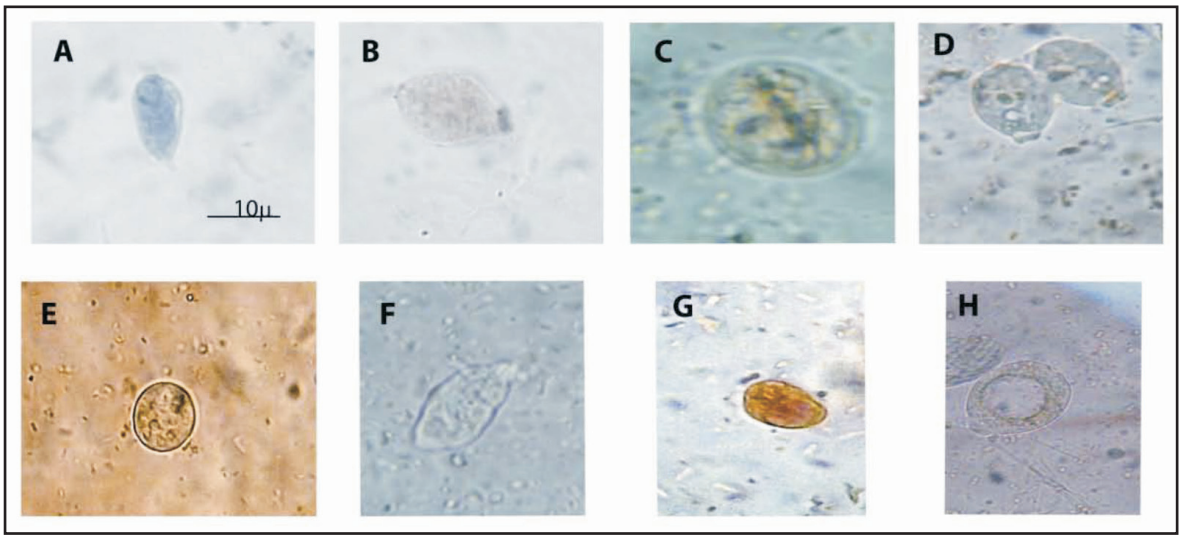


Fig. 4. Eggs of: A) Ascaridida; B) Trichuroidea; C) Ancylostomatidae; D) Toxocaridae; E) *Toxascaris* spp.; F) *Hymenolepidae* spp.; G) Rabdithida, female; H) Rabdithida, male, posterior end; I) Rabdithida male; anterior end.

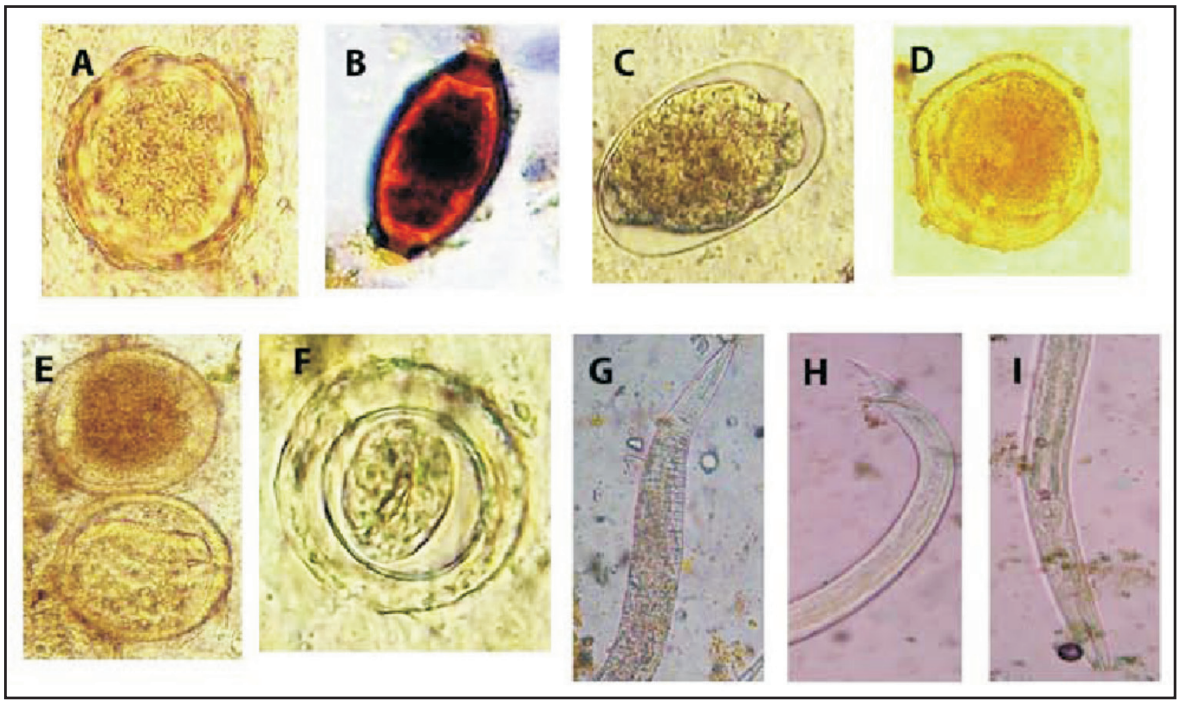


Table I. Proportion of Protozoa and Helminthes observed in the pedal mucus, and feces of *Achatina fulica* captured in Anzoátegui and Monagas states (northeast Venezuela).

Organisms	Excreta	Taxa	Anzoátegui			Monagas		
			Bolívar Municipality Examined snails / infected snails (%)	Sotillo municipality Examined snails / infected snails (%)	Total Examined snails / infected snails (%)	Bolívar Municipality Examined snails / infected snails (%)	Piar municipality Examined snails / infected snails (%)	Total Examined snails / infected snails (%)
Protozoa	Pedal mucus	*Chilomastix* spp.	150/14 (9%)	150/13 (9%)	300/27 (9%)	150/11(7%)	150/9 (6%)	300/20 (7%)
		Trichomonas spp.	150/19 (13%)	150/18 (12%)	300/37 (12%)	150/6 (4%)	150/6 (4%)	300/12 (4%)
		Giardia spp.	150/7 (5%)	150/12 (8%)	300/19 (6%)	150/0 (0%)	150/3 (2%)	300/3 (1%)
		Balantidium spp.	150/2 (1%)	150/1 (1%)	300/3 (1%)	150/2 (1%)	150/1 (1%)	300/3 (1%)
		Entamoeba spp.	150/3 (2%)	150/2 (1%)	300/5 (2%)	150/5 (3%)	150/5 (3%)	300/10 (3%)
		Iodamoeba spp	150/4 (3%)	150/7 (5%)	300/11 (4%)	150/4 (3%)	150/9 (6%)	300/13 (4%)
		Blastocystis spp.	150/20 (13%)	150/23 (15%)	300/43 (14%)	150/9 (6%)	150/8 (5%)	300/17 (6%)
	Feces	*Chilomastix* spp.	150/8 (5%)	150/7 (5%)	300/15 (5%)	150/7 (5%)	150/8 (5%)	150/8 (5%)
		Trichomonas spp.	150/9 (6%)	150/11 (7%)	300/20 (7%)	150/5 (3%)	150/4 (3%)	150/4 (3%)
		Giardia spp.	150/13 (9%)	150/7 (5%)	300/20 (7%)	150/0 (0%)	150/0 (0%)	150/0 (0%)
		Balantidium spp.	150/2 (1%)	150/1 (1%)	300/3 (1%)	150/0 (0%)	150/0 (0%)	150/0 (0%)
		Entamoeba spp.	150/2 (1%)	150/2 (1%)	300/4 (1%)	150/5 (3%)	150/5 (3%)	150/5 (3%)
		Iodamoeba spp	150/1 (1%)	150/6 (4%)	300/7 (2%)	150/5 (3%)	150/6 (4%)	150/6 (4%)
		Blastocystis spp.	150/5 (3%)	150/4 (3%)	300/9 (3%)	150/9 (6%)	150/10 (7%)	300/19 (6%)
Helminths	Pedal mucus	Ascarioidea	150/9 (6%)	150/4 (3%)	300/13 (4%)	150/7 (5%)	150/6 (4%)	300/13 (4%)
		Trichuroidea	150/5 (3%)	150/7 (5%)	300/12 (4%)	150/9 (6%)	150/7 (5%)	300/16 (5%)
		Ancylostomatidae	150/4 (3%)	150/3 (2%)	300/7 (2%)	150/3 (2%)	150/1 (1%)	300/4 (1%)
		Toxocaridae	150/10 (7%)	150/0 (0%)	300/10 (3%)	150/0 (0%)	150/4 (3%)	300/4 (1%)
		Hymenolepidae	150/9 (6%)	150/2 (1%)	300/11 (4%)	150/3 (2%)	150/6 (4%)	300/9 (3%)
		Rhabditidae	150/2 (1%)	150/2 (1%)	300/4 (1%)	150/3 (2%)	150/1 (1%)	300/4 (1%)
	Feces	Ascarioidea	150/0 (0%)	150/0 (0%)	300/0 (0%)	150/9 (6%)	150/7 (5%)	300/16 (5%)
		Trichuroidea	150/5 (3%)	150/3 (2%)	300/8 (3%)	150/7 (5%)	150/5 (3%)	300/12 (4%)
		Ancylostomatidae	150/6 (4%)	150/9 (6%)	300/15 (5%)	150/3 (2%)	150/3 (2%)	300/6 (2%)
		Toxocaridae	150/4 (3%)	150/1 (1%)	300/5 (2%)	150/5 (3%)	150/9 (6%)	300/14 (5%)
		Toxascaris (?)	150/0 (0%)	150/0 (0%)	300/0 (0%)	15/4 (3%)	150/3 (2%)	300/7 (2%)
		Cestoda.	150/3 (2%)	150/2 (1%)	300/5 (2%)	150/3 (2%)	150/2 (1%)	300/5 (2%)

Table II. Proportion of Protozoa and Helminthes observed in the pedal mucus, cephalopodal mucus and feces of *Achatina fulica* captured in Sucre and Nueva Esparta states (northeast Venezuela).

Organisms	Excreta	Taxa	Anzoátegui			Monagas		
			Bolívar Municipality Examined snails / infected snails (%)	Sotillo municipality Examined snails / infected snails (%)	Total Examined snails / infected snails (%)	Bolívar Municipality Examined snails / infected snails (%)	Piar municipality Examined snails / infected snails (%)	Total Examined snails / infected snails (%)
Protozoa	Pedal mucus	*Chilomastix* spp.	150/14 (9%)	150/9 (6%)	300/ 23 (8%)	150/5 (3%)	150/8 (5%)	300/13 (4%)
		Trichomonas spp.	150/8 (5%)	150/6 (4%)	300/14 (5%)	150/8 (5%)	150/7 (5%)	300/15 (5%)
		Giardia spp.	150/17 (11%)	150/11 (7%)	300/28 (9%)	150/1 (1%)	150/0 (0%)	300/1 (0%)
		Balantidium spp.	150/1 (1%)	150/4 (3%)	300/5 (2%)	150/4 (3%)	150/5 (3%)	300/9 (3%)
		Entamoeba spp.	150/5 (3%)	150/3 (2%)	300/8 (3%)	150/6 (4%)	150/7 (5%)	300/13 (4%)
		Iodamoeba spp.	150/3 (2%)	150/5 (3%)	300/8 (3%)	150/9 (6%)	150/6 (4%)	300/15 (5%)
		Blastocystis spp.	150/6 (4%)	150/7 (5%)	300/13 (4%)	150/4 (3%)	150/9 (6%)	300/13 (4%)
	Feces	*Chilomastix* spp.	150/9 (6%)	150/6 (4%)	300/15 (5%)	150/6 (4%)	150/8 (5%)	300/14 (5%)
		Trichomonas spp.	150/8 (5%)	150/10 (7%)	300/18 (6%)	150/11 (7%)	150/13 (9%)	300/24 (8%)
		Giardia spp.	150/4 (3%)	150/0 (0%)	300/4 (1%)	150/0 (0%)	150/0 (0%)	300/0 (0%)
		Balantidium spp.	150/4 (3%)	150/3 (2%)	300/7 (2%)	150/5 (3%)	150/4 (3%)	300/9 (3%)
		Entamoeba spp.	150/3 (2%)	150/7 (5%)	300/10 (3%)	150/7 (5%)	150/7 (5%)	300/14 (5%)
		Iodamoeba spp.	150/4 (3%)	150/7 (5%)	300/11 (4%)	150/2 (1%)	150/13 (9%)	300/15 (5%)
		Blastocystis spp.	150/7 (5%)	150/8 (5%)	300/15 (5%)	150/8 (5%)	150/11 (7%)	300/19 (6%)
Helminths	Pedal mucus	Ascarioidea	150/5 (3%)	150/7 (5%)	300/12 (4%)	150/14 (9%)	150/17 (11%)	300/31 (10%)
		Trichuroidea	150/8 (5%)	150/9 (6%)	300/17 (6%)	150/7 (5%)	150/12 (8%)	300/19 (6%)
		Ancylostomatidae	150/3 (2%)	150/5 (3%)	300/8 (3%)	150/3 (2%)	150/1 (1%)	300/4 (1%)
		Toxocaridae	150/7 (5%)	150/2 (1%)	300/9 (3%)	150/2 (1%)	150/1 (1%)	300/3 (1%)
		Hymenolepidae	150/1 (1%)	150/2 (1%)	300/3 (1%)	150/5 (3%)	150/1 (1%)	300/6 (2%)
		Rhabditidae	150/2 (1%)	150/1 (1%)	300/3(1%)	150/1 (1%)	150/2 (1%)	300/3(1%)
	Feces	Ascarioidea	150/4 (3%)	150/5 (3%)	300/9 (3%)	150/11 (7%)	150/6 (4%)	300/17 (6%)
		Trichuroidea	150/7 (5%)	150/5 (3%)	300/12 (4%)	150/5 (3%)	150/2 (1%)	300/7 (2%)
		Ancylostomatidae	150/1 (1%)	150/2 (1%)	300/3 (1%)	150/1 (1%)	150/1 (1%)	300/2 (1%)
		Toxocaridae	150/10 (7%)	150/2 (1%)	300/12 (4%)	150/3 (2%)	150/2 (1%)	300/5 (2%)
		Hymenolepidae	150/3 (2%)	150/1 (1%)	300/4 (1%)	150/2 (1%)	150/3 (2%)	300/5 (2%)

or animal importance (Figs. 3A/H; 4A/I); coccidia were not observed. The cephalopodal mucus was infected by adult of one imputed Rhabditida. Protozoa and helminthes were common in the pedal mucus and feces of snails collected from all the states surveyed, although the percentages of infection did vary. In addition, these excreta contained larval stages of Nematodes, highlighting the need for further studies for their identification to gender or species level.

Results of the microbiological analyses of the mollusks

The microbiological study (Tables III, IV) showed that all three types of excreta sampled from the 40 individuals examined, contained gram-negative facultative anaerobic bacilli and coccobacilli Enterobacteriaceae and Aeromonadaceae, as well as other bacteria whose taxonomic status is not well defined (Murray *et al.*, 2009). All three types of excreta were negative for levaduriforme yeasts.

DISCUSSION

All the helminthes found in *A. fulica* snails belong to taxa that require soil to complete their development cycles. The Enterobacteriaceae, as well as *A. hidrofila*, *P. aeruginosa*, *A. baumanni*, *E. coli* and *Campylobacter* spp. also develop and proliferate in soil, vegetation, decomposing organic material and water (Murray *et al.*, 2009), all of which are inhabited by *A. fulica*.

On the other hand, it has been estimated that 5.3 billion people are at risk of contracting geohelminthiasis mainly caused by *Ascaris*, *Trichuris*, *Necator/Ancylostoma*, *Toxocara* and *Toxascaris*, with the highest prevalence reported from Africa, Asia and Latin America. These helminthes are endemic in America and the Caribbean, with around 100, 84 and 50 million people infected with the first three helminthes listed above, respectively. Venezuela has been reported as being among the countries with the highest risk of infection (27% for *Trichuris* spp., indicating an urgent need for the implementation of control measures according to the latest recommendations drawn up by the PAHO (Pullan & Brooker, 2012; Chammartin *et al.*, 2013).

Similar health risks have been linked to the presence of the protozoa and bacteria found in the snail's excreta during this investigation, which taken together, are responsible for three of the greatest current global public health problems. This is especially true for populations with a high degree of poverty, an inadequate water supply and poor sanitation and health education (Murray *et al.*,2009; Duc *et al.*, 2011, Pullan & Brooker, 2012); a situation typical of many tropical countries, including Venezuela.

Furthermore, two of the bacteria identified in the snails examined, *E. coli* and *Campylobacter* spp., have been singled out as being among the seven most important bacterial pathogens for humans (Faruque, 2012).

Investigations on the composition, characterization and functions of the microbiota in the gastrointestinal tract of the Mollusca and in particular *A. fulica*, are scarce. According to Charrier *et al.* (2006) in *Helix* spp. the digestive tract is acidic in the crop but neutral or alkaline in the intestine. This highlights the dependence of these snails on the high biochemical potential of their diverse bacterial microbiota for the degradation and fermentation of the principal vegetable components (soluble sugars and polymers) that make up their diet.

Cardoso *et al.* (2012) also found diverse, abundant and metabolically active bacterial communities that included, among others, *Aeromonas*, *Citrobacter*, *Klebsiella*, *Acinetobacter* and *Pseudomonas* in the intestinal tracts of Giant African snails starved for 72 hours in order to minimize the presence of transient bacteria. This leads us to suppose that these bacteria are native to the intestinal tracts of *A. fulica* making it *"per se"* a very effective disperser of these organisms.

The bacteria are probably acquired by *A. fulica* through its diet, the polyphagous nature of which results in the development of a structurally complex and highly diverse microbiotic community. The impact of biotic and abiotic factors on this bacterial community should, however, be investigated. These bacteria are responsible for the digestion of complex polysaccharides, by converting sugars to short-chain fatty acids and synthesizing amino acids and essential vitamins. Cardoso *et al.* (2012) suggest that this microbiota enables the snails to adapt to different diets. It is thus important to determine their species

Table III. Presence (+) or absence (-) of bacteria in the pedal mucus, cephalopodal mucus and feces of *Achatina fulica* captured in Anzoátegui and Monagas states (Northeast Venezuela).

Excreta	Taxa	Anzoátegui	Monagas State
		Number of examined exemplars. N=10	Number of examined exemplars. N=10
Pedal mucus	*Escherichia coli* *	+	+
	Citrobacter freundii *	+	+
	Klebsiella azaenae *	+	+
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	+
	Acinetobacter baumannii ***	+	+
	Pseudomonas aeruginosa ***	+	+
	Campylobacter spp.***	+	+
Cephalopodal mucus	*Escherichia coli* *	-	-
	Citrobacter freundii *	+	+
	Klebsiella azaenae *	-	-
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	+
	Acinetobacter baumannii ***	-	-
	Pseudomonas aeruginosa ***	+	-
	Campylobacter spp.***	-	+
Feces	*Escherichia coli* *	+	+
	Citrobacter freundii *	-	+
	Klebsiella azaenae *	+	+
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	+
	Acinetobacter baumannii ***	+	+
	Pseudomonas aeruginosa ***	-	-
	Campylobacter spp.***	-	-

*=Enterobacteriaceae; **=Aeromonadaceae; ***=Taxonomic status is not well defined (Murray *et al.*, 2009)

composition, in order to provide knowledge that could contribute to the elaboration of strategies for snail control. With this in mind, we would like to mention the fact that *A. hydrophila* (Aeromonadaceae) the causal agent of diseases in humans, fish and mollusks and identified in all three excreta types collected from *A. fulica*, has been implicated in its decline in several geographic regions (Yamada *et al.*, 2011).

The polyphagous diet of *A. fulica*, together with its physiologically high environmental adaptability and capacity to proliferate in an eclectic range of habitats over a wide geographic range (in Venezuela, snails have been reported as having invaded 60% of the national territory; Martinez Escarbassiere *et al.* (2008); Oletta & Carvajal (2011)) are characteristics that make it a very important carrier and disperser of protozoa, helminthes and bacteria. So much so that has been reported to "sow" terrestrial environments and contaminate water bodies with these organisms and has thus become an excellent indicator for their presence (Liboria *et al.*, 2010).

These pathogens may be transmitted from *A. fulica* to humans and animals by the ingestion of raw plant products and water infected by their excreta (Faruque, 2012), eating raw or undercooked snails (Zanol *et al.*, 2010), or possibly from the handling of live snails, especially if the mucous membranes of the eyes, nose or mouth come into contact with the snails' excreta (USDA, 2008).

Table IV. Bacteria observed in the pedal mucus, cephalopodal mucus and feces of *Achatina fulica* captured in Sucre and Nueva Esparta states (Northeast Venezuela).

Excreta	Taxa	Sucre State	Nueva Esparta State
		Snails examined N=10	Snails examined N=10
Pedal mucus	*Escherichia coli* *	+	+
	Citrobacter freundii *	+	+
	Klebsiella azaenae *	+	+
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	+
	Acinetobacter baumannii ***	+	+
	Pseudomonas aeruginosa ***	+	+
	Campylobacter spp.***	+	+
Cephalopodal mucus	*Escherichia coli* *	-	-
	Citrobacter freundii *	+	-
	Klebsiella azaenae *	-	-
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	+
	Acinetobacter baumannii ***	+	+
	Pseudomonas aeruginosa ***	-	-
	Campylobacter spp.***	-	-
Feces	*Escherichia coli* *	+	+
	Citrobacter freundii *	+	+
	Klebsiella azaenae *	+	+
	Klebsiella pneumoniae *	+	+
	Aeromonas hydrophila **	+	-
	Acinetobacter baumannii ***	+	+
	Pseudomonas aeruginosa ***	+	+
	Campylobacter spp.***	+	+

*=Enterobacteriaceae; **=Aeromonadaceae; ***=Taxonomic status is not well defined (Murray *et al.*, 2009)

Despite the warnings given by Martinez Escarbassiere & Martinez (1997) about the risks associated with the presence of *A. fulica* in Venezuela, few studies have determined the impact of this snail species either on the natural environment or human and veterinary health. Thus a low priority has been given to the proper surveillance of the zones invaded by this mollusk by the health authorities and there have been few measures taken to restrict its invasion of the rest of the country. There have also been a lack of control measures as regards food security issues and little attempt to educate the population as regards the health risks involved. Implementation of these aspects would contribute to mitigating the number of cases of infection reported as well as the emergence of new diseases due to the intense and rapid invasion and proliferation of *A. fulica* in Venezuela. A greater knowledge of the snail distribution would enable the instigation of effective measures for the control of the protozoa, helminthes and bacteria that they harbor. We suggest that these considerations underline the epidemiological importance of *A. fulica* for human and animal health.

Two mucopolysaccharide groups, Glycosaminoglycans (GAGs, heparin-, chondroitin-, dermatan sulfate and hyaluronic acid) and glycoproteins, suspended in 80-99% water have been found ubiquitously in the animal kingdom, from the most primitive invertebrates (Hydra, Cnidaria) to vertebrates, demonstrating their conserved status and role in fundamental biological processes. In

the *A. fulica* pedal mucus, these structures are responsible for regulating cell growth, proliferation, differentiation, morphogenesis and migration, and are also involved in the transport of substances, thus providing energy and metabolic resources basic to the snail´s survival (Skingsley *et al.*, 2000; Berniyanti *et al.*, 2007; Yamada *et al.*, 2011).

In addition, it has been demonstrated that this mucus contains a broad spectrum antibiotic glycoprotein effective against *E. coli*, *Streptococcus mutans*, *Staphylococus aureus* and *S. epidermidis* (Berniyanty *et al.*, 2007; Santana *et al.*, 2012) indicating its importance for the mollusk immune system. With regard to this we should add that a highly sulfated product -acharan sulfate, related to heparin/heparin sulfate but structurally unique to *A. fulica*, may also be responsible for protecting *A. fulica* from foreign bodies such as bacteria and virus (Yamada *et al.*, 2011).

Mucins, a family of highly glycosylated proteins found throughout the animal kingdom including mollusks, are basic components of gel secretions. They have diverse functions, ranging from lubrication and the regulation of the calcification process as constituents of the snail´s shell, to the formation of physical barriers for defence including inhibitory chemicals which bind to parasites, commensals and predators (Skingsley *et al.*, 2000, Pinchuck & Hodgson, 2009, 2012). These aspects could explain the role of the snails´ excreta as appropriate niches for the organisms mentioned.

CONFLICT OF INTERESTS: None.

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