

Presence of *Paragonimus* species within secondary crustacean hosts in Bogotá, Colombia

Presencia de especies de Paragonimus en huéspedes crustaceos secundarios en Bogotá, Colombia

Presença de espécies de Paragonimus em crustáceos hospedeiros secundários em Bogotá, Colômbia

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Abstract

Background: *Paragonimus* spp. are trematode parasites that infect human populations worldwide. It is believed that infection rates within Asia reach five to ten percent of the total population. Three of the largest areas of possible infection are Asia, Central and South America as well as Africa, where the total population at risk is estimated to be 293 million people. Humans are infected via ingestion of raw or undercooked decapod crustaceans.

Objective: To identify the presence of *Paragonimus* spp. in crabs from Bogotá, Colombia. **Methods:** The native crab *Neostrengeria macropa* and the aquatic invasive crayfish *Procambarus clarkii* in Bogotá, Colombia, were collected from local markets, pet stores and waterways and dissected to assess the presence of *Paragonimus* spp.

Results: The native crab species, *N. macropa* (n=29) had an infection prevalence of 17.2%, while the invasive crayfish species, *P. clarkii* (n=22), had a prevalence of 36.4% combined from both field captured animals and purchased samples.

Conclusion: Although the estimated prevalence is lower compared to previous studies in other cities of

Colombia, *Paragonimus* represent a risk to human health. Several environmental factors may contribute to the difference in prevalence including collecting season, rainfall, temperature, altitude and the El Niño Southern Oscillation.

Keywords: *crabs, invasive species, Neostrengeria macropa, Procambarus clarkii, trematode.*

Resumen

Antecedentes: Los *Paragonimus* spp. constituyen un grupo de parásitos tremátodos que infectan a humanos en todo el mundo. Se considera que entre 5 y 10% de la población humana de Asia está infectada. Las áreas con mayor posibilidad de infección son Asia, Centro y Sur América, así como África. Se estima que 293 millones de personas están en riesgo de infección. Los humanos se pueden infectar al consumir crustáceos decápodos crudos.

Objetivo: Identificar la presencia de *Paragonimus* spp. en crustáceos en Bogotá, Colombia.

Métodos: Una muestra de cangrejos nativos *Neostrengeria macropa* y de decápodos invasores *Procambarus clarkii* fue colectada tanto en mercados locales de Bogotá, como en tiendas de mascotas, ríos, y quebradas. Posteriormente fueron diseccionados para detectar la presencia de *Paragonimus* spp.

Resultados: La prevalencia de la infección en *N. macropa* (n=29) fue de 17,2%, y en la especie invasora, *P. clarkii* (n=22), fue de 36,4% (porcentaje combinado de los animales colectados en el campo y los comprados en tiendas).

Conclusión: Aunque la prevalencia en este estudio fue más baja que la de otras investigaciones relacionadas, se considera que existe riesgo para la salud humana. Es probable que algunos factores medio ambientales hayan contribuido a la diferencia, incluyendo: temporada de colecta, nivel de lluvias, temperatura, altura, y el fenómeno El Niño.

Palabras claves: *cangrejos, especie invasora, Neostrengeria macropa, Procambarus clarkii, tremátodo.*

Resumo

Antecedentes: *Paragonimus* spp. são trematódeos parasitas que infectam populações humanas ao redor do mundo. Acredita-se que as taxas de infecção na Ásia atingem de 5 a 10% da população. As três maiores áreas de infecção se localizam na Ásia, Américas do Sul e Central e África, onde a população total em risco é estimada em 293 milhões de pessoas. Os humanos são infectados pela ingestão de crustáceos decápodes crus ou mal cozidos.

Objetivo: Identificar a presença de *Paragonimus* spp. em Bogotá, Colômbia.

Métodos: Indivíduos de caranguejo nativo *Neostrengeria macropa* e lagostim aquático invasivo *Procambarus clarkii* foram coletados em mercados locais, lojas de animais de estimação e cursos de água em Bogotá (Colômbia) e posteriormente dissecados para verificação da presença de *Paragonimus* spp.

Resultados: A espécie de caranguejo nativa *N. macropa* (n=29) apresentou prevalência de infecção de 17,2%, enquanto a espécie de lagostim invasivo, *P. clarkii* (n=22), apresentou prevalência de 36,4%, quando combinados os animais capturados em campo e os animais comprados.

Conclusão: Embora a prevalência estimada neste estudo foi menor do que a de pesquisas anteriores realizadas em outras cidades da Colômbia, existe um risco para a saúde humana. Há inúmeros fatores ambientais que podem contribuir para a diferença de prevalência, dentre eles: a estação em que a coleta foi realizada, pouca precipitação, temperatura, altitude e a Oscilação Sul-El Niño.

Palabras-chave: *caranguejos, espécies invasivas, Neostrengeria macropa, Procambarus clarkii, trematódeos.*

Introduction

Paragonimiasis is a lung infection caused by trematodes of the genus *Paragonimus* (Braun, 1899). Paragonimiasis is endemic in Asia, Africa, and Central and South America (Davidson, 2005). Infection occurs through consumption of undercooked, raw, or pickled crustaceans (Toledo *et al.*, 2012), which are often aligned with cultural practices or socioeconomic status (Narain *et al.*, 2015). *Paragonimus* spp. penetrates the

gastrointestinal tract and diaphragm, and then matures in the lungs of definitive hosts (Toledo *et al.*, 2012). Clinical symptoms in humans include fever, coughing blood, painful breathing, shortness of breath, and recurrent bacterial pneumonia; chronically infected individuals develop pleural effusion and lung abscesses (Davidson, 2005).

There are numerous mammalian definitive hosts (Liu *et al.*, 2008). Previous studies documented dogs, wild boar, pigs (Kirino *et al.*, 2009), opossum (Little, 1968), foxes, wolves, tigers, lions leopards and civets (Aka *et al.*, 2008) as potential definitive hosts. Apart from raw-infected pork consumption (Procoop, 2009), mammals cannot directly infect other mammals, but they can shed eggs into water sources and continuously re-infect those water sources (Liu *et al.*, 2008). In Colombia, *Paragonimus caliensis* adult trematodes were first described in two species of opossum and in three species of felines, ocelot, margay cat, and jaguar as well as metacercariae being found in the hepatopancreas of crabs (Little, 1968). These definitive hosts allow for maturation of metacercariae and continuation of the life cycle by passing mature oocysts in feces (Liu *et al.*, 2008). Additional identification of mammalian hosts within Colombia would help to identify sources of reinfection in freshwater systems.

Transmission is restricted to areas where the primary intermediate host, molluscs, and secondary intermediate crustacean hosts coexist in freshwater. Eggs are shed into water reservoirs where they hatch and penetrate molluscs such as *Aropygrus* spp. where they mature until they are ready to shed and penetrate crustacean hosts (Vélez *et al.*, 2003). *Neostrengeria macropa* (H. Milne Edwards, 1853) is a brachyuran crab native to the Bogotá mountain range that inhabits freshwater areas between 2,200 and 2,900 m above sea level (Campos, 2005a). *N. macropa* is currently listed as least concern by the IUCN (Cumberlidge, 2008a); however, Campos and Lasso (2015) posit that this species could be vulnerable to human impacts and should be listed as “endangered”. Another potential vector is the Louisiana red-clawed crayfish, *Procambarus clarkii* (Girard, 1852). This invasive species has expanded its range from Mexico and Southeastern United States to other parts of North America as well as South America, Europe, Africa and Asia. *P. clarkii* is documented to carry *Paragonimus* spp. in the United States (Procoop, 2009). Invasive crayfish were introduced from aquaculture operations in Colombia (Álvarez-León and Gutiérrez-Bonilla, 2007). These feed on invertebrates, detritus, macrophytes and algae, which drastically alters native food webs through competition between native and invasive crustacean species (Gherardi, 2006). However, there has been no research identifying the ecological impacts or parasites introduced by crayfish in Colombia. Research of direct effects are limited to single species, concentrated on known definitive hosts and narrow geographic location (Lagrué, 2017).

Uruburu *et al.* (2008) completed a study to identify the prevalence of *Paragonimus* spp. within nine municipalities of Antioquia, Colombia. This yielded a prevalence of 80.8%. Casas *et al.* (2008) identified the prevalence rate for *Paragonimus* spp. to be 55.5% in Medellín. Lastly, work on the lands inhabited by Emberá ethnic groups within Antioquia resulted in a prevalence of 50% in crabs and 1.6% in snails (Vélez *et al.*, 1995; Vélez *et al.* 2003). While human infection rates in Colombia are presently understudied, information about the presence of disease is crucial to estimating the potential risk to vulnerable populations. An example of a local risk factor is “berraquillo”, a smoothie-like blend of raw egg, fruit, and live crab consumed as an aphrodisiac in Bogotá, Colombia (all authors, pers. obs.). Based on prevailing risky food practices and poverty, as well as previous studies from Colombia, this study aimed to identify the prevalence of this trematode in crustaceans found in Bogotá (Colombia).

Materials and Methods

Ethical considerations

Research on invertebrates is not presently required to have an Institutional Animal Care and Use Committee (IACUC) protocol. Documents for Atlanta Metropolitan State College (AMSC) IACUC for animal use off campus were submitted prior to travel. Permission to conduct research on invertebrates was granted through Universidad Nacional de Colombia, Instituto de Ciencias Naturales. Protocols for relaxation of live animals and dissection were found in previous studies of this parasite and host species (Uruburu *et al.*, 2008).

In June 2015, *Neostrengeria macropa* (n=29, adult, male=15, female=14) were purchased from

Paloquemao, a large food market in Bogotá, Colombia. Based on conversations with the store owner, these animals were collected from streams around Gachancipá, north of the city. Juvenile *P. clarkii* were purchased from a pet store and adults were collected in the wild, also in June, from Club El Rincón de Bogotá golf course (n=22, juvenile=8, adult=14, male=13, female=9). The adults were collected with permission of the land owners using a dip net in a manmade lake on the golf course that frequently floods receiving water from the Bogotá river, where this species was likely introduced from.

Crayfish and crabs were placed in an ice bath or water chilled to 2 °C for 10 minutes, after which specimens were measured, and cardiac tissue was removed to ensure cessation of life prior to dissection (Uruburu *et al.*, 2008). Tissues from the heart, hepatopancreas, gill, and large deposits of muscle from claws for both crustacean species, along with abdominal muscle for *P. clarkii*, were placed on slides for examination with stereomicroscopy. From the literature, these tissues were documented to contain metacercariae (Little, 1968; Vélez *et al.*, 2000; Bogitsh *et al.*, 2013). Tissues with metacercariae were placed in 95% alcohol and transferred to Laboratorio de Parasitología Veterinaria at Universidad de Antioquia for staining. Organs were fixed in 10% neutral buffered formalin. These samples were then routinely processed and stained with hematoxylin and eosin for histopathological tissue analysis. Due to permit restrictions on genetic analysis for these samples, *Paragonimus* spp. metacercariae were identified through morphological analysis.

Statistical analysis

We determined whether the prevalence of *Paragonimus* spp. was different from previous findings and a null hypothesis of zero prevalence through Wilcoxon Rank Sum. This non-parametric test was conducted using the statistical package R, with the use of R Commander. Wilcoxon Rank Sum was performed on *N. macropa* to compare our results to the two previous prevalence estimates for Colombia ($\mu=0.555$ and $\mu=0.808$).

A Maximum Entropy (MaxEnt) Species Distribution Model (version 3.3.3k) was applied using Phillips *et al.* (2006) and Hudson *et al.* (2016) as guides. This model uses historical collection data as well as maps of environmental data to predict the probability of geography with similar habitat. The product of this map would help identifying additional collection areas. The environmental layers were downloaded from WorldClim (WorldClim, 2015) for altitude and Bioclim datasets for current data patterns (version 1.3). The data were clipped to the area of interest using the SDMToolbox (version 1.1) within ArcGIS (Version 10.1) and convert raster data downloaded into ASC files to use in the model. The map created contains collection data provided by Prof. Martha H. Rocha de Campos (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá) for *N. macropa*. Due to the scarce collection data for *P. clarkii* in Colombia there was not a sufficient number of observations to create a model. It must be noted that due to limitations to do genetic verification in the country, this portion is effectively a map of potential *Paragonimus* spp. infection for the vector *N. macropa* in the area.

Results

Paragonimus spp. was found in both *N. macropa* and *P. clarkii* (Table 1). Of the market-purchased *N. macropa*, five of 29 (17.2%) had metacercariae present in tissues. While none of the market-purchased *P. clarkii* yielded metacercariae, eight of the 14 wild-caught specimens (57.1%) yielded metacercariae.

Table 1. Prevalence of Parasites Collected from Crustacean Hosts: “Market” *Neostrengeria macropa* were purchased from Paloquemao market in Bogotá, Colombia. *Procambarus clarkii* were purchased from a local pet store designated as market, or were wild-caught from Club El Rincón de Bogotá.

	Number of specimens	<i>Paragonimus</i> spp.
<i>N. macropa</i>		
Market	29	5 (17.2%)
<i>P. clarkii</i>		
Market	8	0
Wild-caught	14	8 (57.1%)

The prevalence of *Paragonimus* spp. of *N. macropa* within Bogotá (17.2%) was significantly different from Casas *et al.* (2008) at 80.8% ($p < 0.001$, Wilcoxon Rank Sum) and Uruburu *et al.* (2008) at 55.5% ($p < 0.001$, Wilcoxon Rank Sum).

Using MaxEnt, we predicted that potential range for *N. macropa* is limited to the Bogotá Plain (Sabana de Bogotá) of the Eastern Cordillera mountain range due to lack of connectivity of suitable habitat (Figure 1). Lack of temperate water connecting mountain ranges in Colombia limits range expansion to other areas of suitable habitat. As water temperature exceeds 26 °C, there is a significant decrease in activity of *N. macropa* (Hudson *et al.* 2016), and therefore it is probably functioning as a vector.

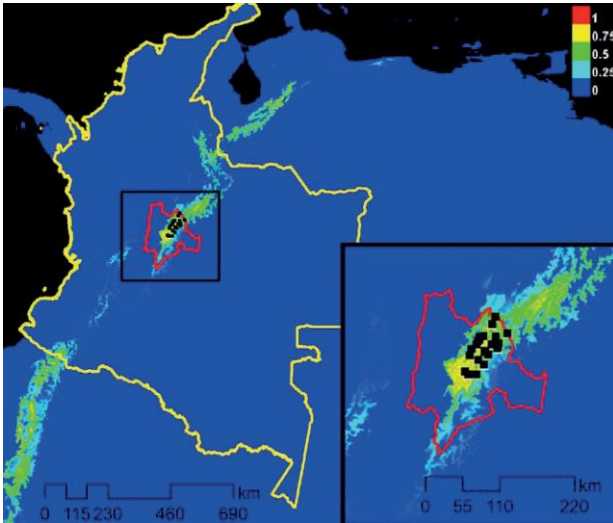


Figure 1. Maximum Entropy Model for the current conditions (Bioclim), and location data of *Neostrengeria macropa* noted by black dots, in Colombia (yellow line) and Cundinamarca province (red line). The map color represents a probability scale, increasing in probability from blue to red, as to the likelihood of suitable habitat for *N. macropa*.

Discussion

Both *N. macropa* and *P. clarkii* make effective vectors for this genus in the Bogotá Plain. *Paragonimus* spp. (Figure 2) prevalence in *N. macropa* endemic crabs was 17.2% (Table 1). Though invasive crayfish, *P. clarkii*, purchased from the pet store were not infected, the animals collected at the golf course had a much higher infection rate (57.1%) than the native crabs of the Bogotá Plain. *Paragonimus* spp. prevalence in crabs from previous studies in Antioquia (Casas *et al.*, 2008; Uruburu *et al.*, 2008) were significantly higher than the prevalence found in this study ($p < 0.001$, Wilcoxon Rank Sum). Despite this lower incidence rate, results are of public health concern because the specimens from this study were purchased at a local market and are meant for consumption.

Due to the range of *N. macropa* in the Eastern Cordillera mountain range around Bogotá, the range for *Paragonimus* spp. infection in that species is also narrow. Prediction of the range of *N. macropa* (Figure 2) shows limited range restriction of this species to the Bogotá Plain (Sabana de Bogotá) due to lack of connectivity of suitable habitat. However, this is not restrictive for these trematodes, as there are other freshwater crustaceans that can serve as intermediate hosts throughout the country and that overlap with the range of *N. macropa* (Campos, 2005a).

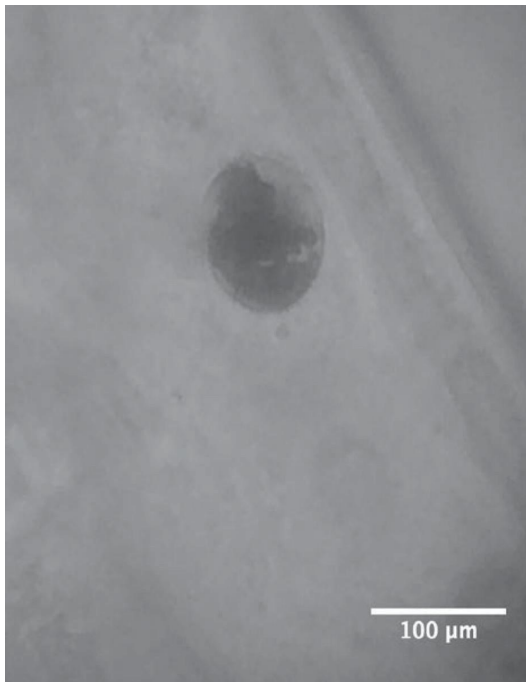


Figure 2. *Paragonimus* spp identified from *N. macropa* using stereomicroscopy. No stain.

For *Paragonimus* spp. to be found in Bogotá, as well as widespread thorough Antioquia, then conduction of this parasite is necessary and could occur through hosts at all stages. Overlap of crustacean species with *N. macropa* and *H. bouvieri* are likely (Campos, 2005a). While the distribution of *N. macropa* is limited (Figure 2), the range of *H. bouvieri* is large and encompasses much of Colombia as well as Venezuela (Cumberlidge, 2008b). *H. bouvieri* has been documented to carry *Paragonimus* spp. in multiple studies in Colombia (Arias *et al.*, 2011; Casas *et al.*, 2008; Vélez *et al.*, 2008). The overlap of habitat ranges could facilitate the spread of this disease.

The role of invasion in providing an additional parasite vectors is prevalent from these results. *P. clarkii* is clearly an effective vector for *Paragonimus* spp. and therefore may impact other native species. Invasive species can drastically alter the natural food web and the effects of disturbances are greater within freshwater ecosystems when compared to terrestrial ecosystems (Lagure, 2017; Sala *et al.* 2000). Crayfish are long-lived crustaceans that can survive in high population densities. Introduction of crayfish into other habitats both accidentally and intentionally as a result of cultivation as a food source is a problem due to escapees from aquaculture operations around the world as well as in Colombia (Álvarez-Léon and Gutiérrez-Bonilla, 2007). *P. clarkii* is invasive in Colombia (Campos and Lasso, 2015), but its distribution in Colombia is unknown. This species is extremely tolerant and can withstand winter temperatures (average high of -9 °C) north of its native range in North America (Gherardi, 2006). This could favour its range expansion into the higher altitudes of the Andes in Colombia.

Abiotic influences between different regions may affect parasitism rates for this species as prevalence from Antioquia and Bogotá are significantly different from each other ($p < 0.001$, Wilcoxon Rank Sum). Altitudinal differences between Bogotá (2600 m) and Medellín (1500 m) may also contribute to differences in parasite prevalence. Other characteristics of lower altitude include warmer temperatures, flatter land, increased vector breeding (Achidi *et al.*, 2008) as well as increased precipitation (Meléndez *et al.*, 2014). Future work should focus on temperature and seasonal aspects of this parasite's life cycle. Temperature differences can also influence parasitic trematodes that have both free-living and host-associated stages, but vary widely within the group. However, temperature does not seem to affect cercaria development within or emergence from the mollusc host due to the limits of relative size and host resources (Poulin, 2006). Some Colombian cultural practices, such as consuming “berraquillo”, carries an increased human risk of ingesting metacercariae. Food preparation in Bogotá is typically warm or cooked food, such as soup;

however, a lack of food knowledge may be reflected in continued practice of “berraquillo” consumption. Two studies conducted interviews of local youth in Antioquia in conjunction with a local university to ascertain food safety knowledge as it relates to crustaceans. The youth were not aware that there was any risk associated with eating raw crabs (Arias *et al.*, 2011; Casas *et al.*, 2008). Areas with lower socioeconomic status as seen in Chocó, Colombia are at higher risk of parasitism as a case study of a seven-year-old girl in this region documents its presence in the region (Múnera *et al.*, 2011). Casas *et al.* (2008) also voiced concerns about risks within indigenous communities such as the Emberá as well as rural areas. Additional food safety education and potential food regulations need to be implemented to prevent unnecessary adverse human health outcomes.

The authors, and previous reviews, recognize the lack of genetic identification as a limitation of this study. However, previous studies have relied solely on morphology for identification. Future directions for this research will be to expand *N. macropa* and *P. clarkii* collections to more wild-caught crustaceans as well as snail species and a broader study of genetic information for species identification. Additionally, determining altitudinal and seasonal differences in prevalence would benefit a more comprehensive view of risk. Most importantly, genetic identification is key for species identification. The first report of the presence of *Paragonimus* sp. in Bogotá, Colombia as well as the host *N. macropa* is important to document due to risky local food practices and lack of knowledge on food safety.

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Conflicts of Interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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Appendix 1: Location data for *Neostrengeria macropa*

Genus	Species	°N	°W
Neostrengeria	macropa	4.828333	-73.892500
Neostrengeria	macropa	5.201944	-73.738056
Neostrengeria	macropa	4.961667	-73.836111
Neostrengeria	macropa	5.209167	-73.901389
Neostrengeria	macropa	5.093889	-73.994167
Neostrengeria	macropa	5.093889	-73.994167
Neostrengeria	macropa	4.698889	-73.991389
Neostrengeria	macropa	5.366667	-73.783333
Neostrengeria	macropa	5.046667	-73.714722
Neostrengeria	macropa	5.112222	-73.701111
Neostrengeria	macropa	5.115000	-73.744167
Neostrengeria	macropa	5.096667	-73.790000
Neostrengeria	macropa	5.011944	-73.878333
Neostrengeria	macropa	5.007778	-73.880556
Neostrengeria	macropa	5.161389	-73.953611
Neostrengeria	macropa	4.890556	-73.969444
Neostrengeria	macropa	5.241389	-73.579444
Neostrengeria	macropa	5.066944	-73.708889
Neostrengeria	macropa	4.961667	-73.836111
Neostrengeria	macropa	4.954722	-74.082500
Neostrengeria	macropa	5.197778	-73.949722
Neostrengeria	macropa	4.700000	-74.100556
Neostrengeria	macropa	5.463056	-73.743889
Neostrengeria	macropa	5.143333	-73.973056
Neostrengeria	macropa	4.735278	-74.143889
Neostrengeria	macropa	5.066944	-73.708889
Neostrengeria	macropa	5.040149	-74.061404
Neostrengeria	macropa	5.040149	-74.061404