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Turgida turgida (Nematoda: Physalopteridae) parasitizing Water Opposssum (*Chironectes minimus* Zimmermann, 1780) in the Western Cordillera of Colombia and updated checklist of its associated parasites

Turgida turgida (Nematoda: Physalopteridae) parasitando zarigüeya acuática (*Chironectes minimus* Zimmermann, 1780) en la Cordillera Occidental de Colombia y lista actualizada de sus parásitos asociados

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Chironectes minimus is the only marsupial species of a semi-aquatic nature and is widely distributed in tropical and subtropical habitats from Central to South America. Although it has a wide distribution, little is known about aspects of its biology, as well as its parasites because it is considered a difficult species to sample. In this study, we report for the first time the presence of the nematode *Turgida turgida* in *C. minimus* in an area of the Western Cordillera in Colombia and compile a list of its other associated parasites. While preparing a specimen of *C. minimus* to enter the Mammal Collection of the Corporación Universitaria Santa Rosa de Cabal (CUS-M), six individuals of *T. turgida* were found when their stomach contents were checked. For scanning electron microscope analysis (SEM), all the samples were cleaned in saline solution and mounted on supports using carbon adhesive tape, coated with gold by sputtering in a cathodic sputtering machine. The nematodes found in the stomach of *C. minimus* were identified as *Turgida turgida* and correspond to five males and one female with a mean length of 2.45 cm and 4.59 cm, respectively. SEM revealed structures that characterize this species, such as ventrocaudal ornamentation patterns, and the presence of the 22nd broad, truncated caudal papilla on the male tail. We emphasize the importance of biological collections and recommend closer examination of specimens during the collection process, given the possibility of gathering relevant information on specimen-associated parasites, which could provide valuable insights into host-parasite relationships, ecological interactions and potential disease transmission pathways, ultimately allowing us to understand their implications for both public health and animal health.

Key words: Cloud forests; biological collections; neotropical marsupial; parasitic helminths; scanning electron microscope.

Chironectes minimus es la única especie de marsupial de carácter semiacuático y se encuentra ampliamente distribuida en hábitats tropicales y subtropicales desde Centroamérica hasta Sudamérica. A pesar de su amplia distribución, poco se conoce sobre aspectos de su biología, así como de sus parásitos debido a que es considerada una especie difícil de muestrear. En este estudio reportamos por primera vez la presencia del nematodo *Turgida turgida* en *C. minimus* en un área de la Cordillera Occidental en Colombia y compilamos una lista de sus otros parásitos asociados. Mientras se preparaba un espécimen de *C. minimus* para ingresar a la Colección de Mamíferos de la Corporación Universitaria Santa Rosa de Cabal (CUS-M), se encontraron seis individuos de *T. turgida* al revisar su contenido estomacal. Para el análisis de microscopía electrónica de barrido (MEB), todas las muestras fueron limpiadas en solución salina y montadas sobre soportes utilizando cinta adhesiva de carbono, recubiertas con oro por pulverización catódica. Los nematodos encontrados en el estómago de *C. minimus* fueron identificados como *Turgida turgida* y corresponden a cinco machos y una hembra con una longitud media de 2,45 cm y 4,59 cm, respectivamente. La MEB reveló estructuras que caracterizan a esta especie, como los patrones de ornamentación ventrocaudal y la presencia de la 22 papila caudal ancha y truncada en la cola del macho. Destacamos la importancia de las colecciones biológicas y recomendamos un examen más minucioso de los especímenes durante el proceso de recogida, dada la posibilidad de recopilar información relevante sobre los parásitos asociados a los especímenes, lo que podría aportar información valiosa sobre las relaciones huésped-parásito, las interacciones ecológicas y las posibles vías de transmisión de enfermedades, permitiéndonos en última instancia comprender sus implicaciones tanto para la salud pública como para la sanidad animal.

Palabras clave: Bosque de niebla; colecciones biológicas; helmintos parásitos; marsupial neotropical; microscopía electrónica de barrido.

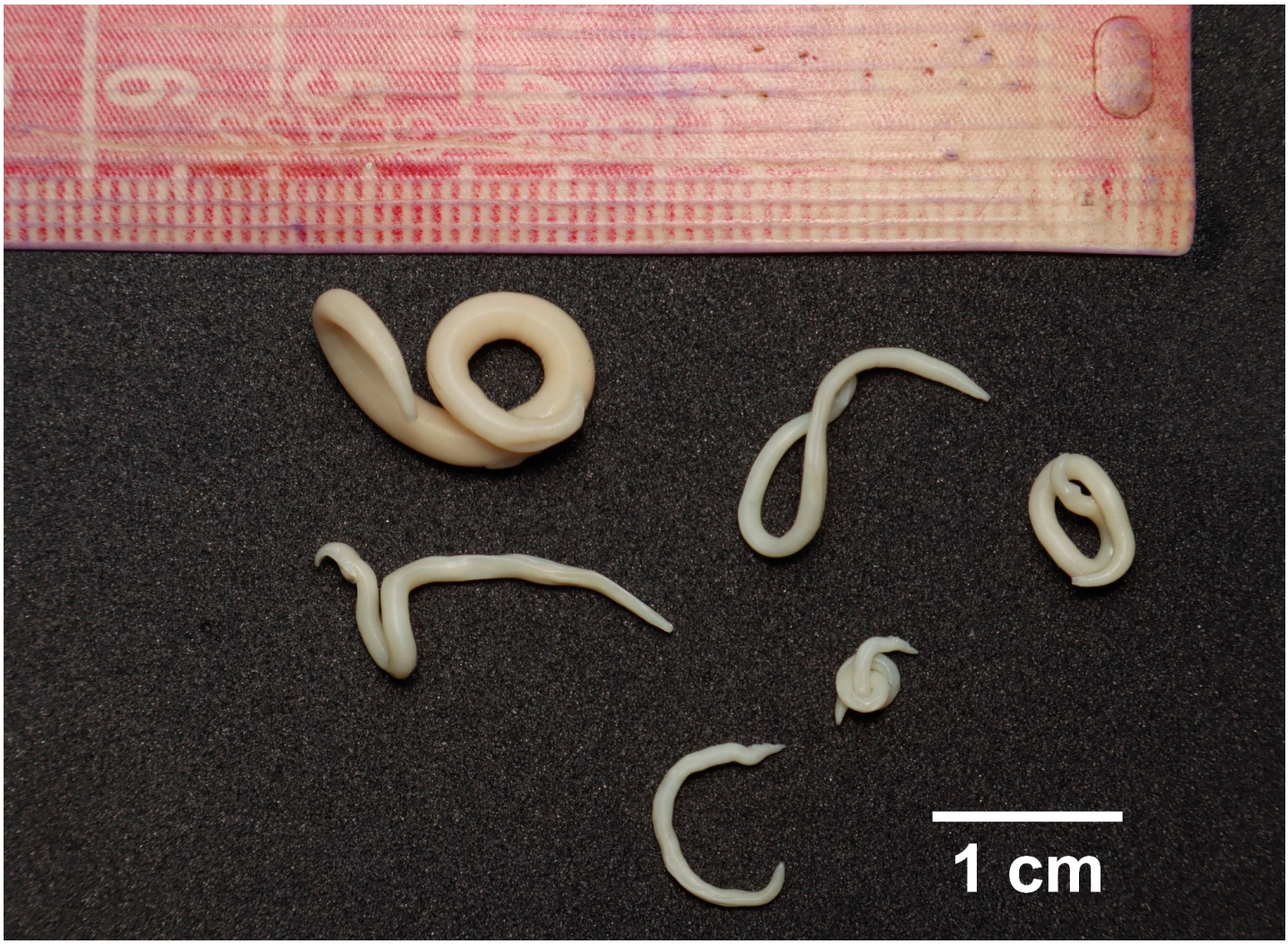

Figure 1. Adult individuals of *Turgida turgida* found in the stomach wall of *Chironectes minimus* (CUS-M 0514) in Pueblo Rico, Risaralda, Colombia.

The Water Opossum, *Chironectes minimus* Zimmermann, 1780 (Mammalia: Didelphidae) is the only marsupial species of a semi-aquatic nature and is widely distributed in tropical and subtropical habitats from Central America to South America (Ardente *et al.* 2013). It is mainly associated with riverbeds with stony substrates, clear water, and preserved riparian vegetation (Prieto-Torres and Pinilla-Buitrago, 2017). Although it has a wide distribution, little is known about aspects of its biology, as well as its parasites (Fernandez *et al.* 2015) because it is considered a difficult species to sample given that it is not attracted by conventional baits and because it mainly inhabits water sources, which makes it difficult to set traps (Bressiani and Graipel 2008). Parasites are essential in ecosystems because they participate in the regulation and structuring of the host population, intervening in processes such as competition, migration, reproduction and speciation (Bennati *et al.* 2023), and both biotic and abiotic factors influence their interaction with hosts (Cirino *et al.* 2020).

The literature documents that parasitic helminths in wildlife are rare and there are few reviews of them (Beveridge and Spratt 2015). This may be due to the fact that many species are cryptic and require molecular techniques for identification, and to the scarce availability of active taxonomists who can determine them by morphological characteristics (Kim and Byrne 2006). This results in the fact that many helminth species deposited in biological collections have not yet been described and numerous marsupial species have not been examined for parasites (Beveridge and Gasser 2014), making it difficult to understand the biology and interaction between the two groups. The family Physalopteridae (Nematoda: Spiruroidea) comprises 22 genera and around 314 valid species (Hodda 2022), among which *Turgida turgida* Rudolphi, 1819 stands out as it is widely recorded in marsupials such as *Didelphis aurita* (Gomes *et al.* 2003), *D. virginiana* (Gray and Anderson 1982), among others. In this study, we report for the first time the presence of *T. turgida* in *C. minimus*, confirmed by scanning electron microscopy (SEM) in an area of the Western Cordillera in Colombia and compile a list of its other associated parasites.

While preparing a specimen of *C. minimus* to enter the Mammal Collection of the Corporación Universitaria Santa Rosa de Cabal (CUS-M), six individuals of *T. turgida*

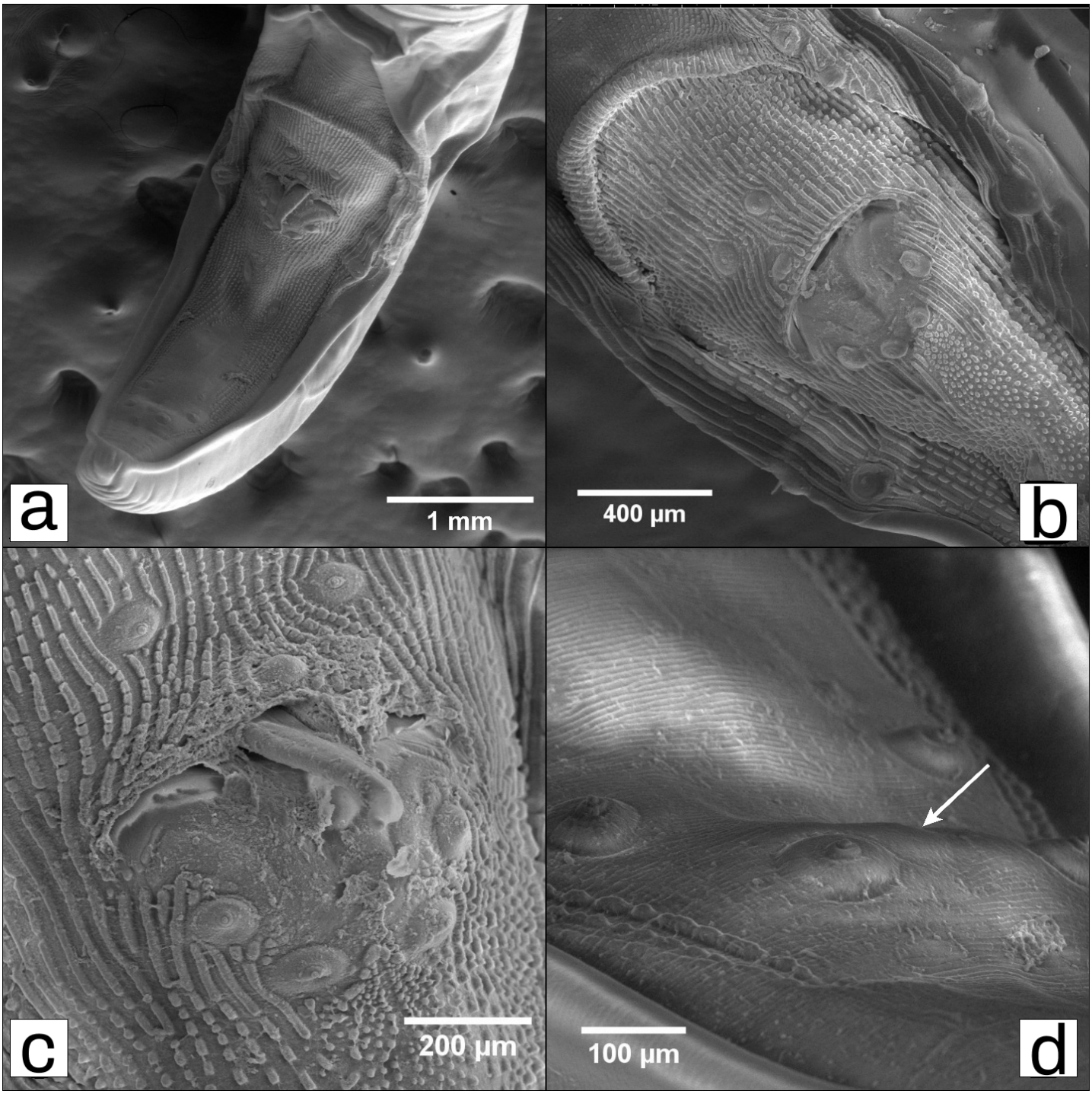

Figure 2. SEM micrographs of male specimens of *Turgida turgida*. (a) General view of the posterior end of the male, ventral side. The distribution of papillae can be seen, (b, c) Cloacal region, (D) Postcloacal papillae, including the broad, truncated papilla (white arrow).

were found when their stomach contents were checked (Figure 1). The specimen, a male *C. minimus* from the municipality of Pueblo Rico, Risaralda, Colombia (5° 16′ 21.0″ N, 76° 1′ 36.0″ W; 1169 m), was collected under the Framework Permit for the collection of wildlife specimens for scientific research purposes, Res 2004/2016 granted by the Corporación Autónoma Regional de Risaralda (CARDER), then was catalogued with the code CUS-M 0514; and samples of its parasitic helminths were preserved and fixed in a solution of 70 % alcohol and 5 % formaldehyde. For scanning electron microscopy analysis, the samples were cleaned in saline solution and mounted on supports using carbon adhesive tape, coated with gold by sputtering in a cathodic sputtering machine (Quorum model SC7620) and examined with a scanning electron microscope (Fei Quanta 250) at 12.5kV at the Laboratorio de Microscopía Electrónica of the Universidad de Caldas. Subsequently, the works of Matey *et al.* (2001), Chaubad (2009) and Humberg *et al.* (2011) were used for taxonomic identification.

A literature review was conducted to compile the species of parasites reported in *C. minimus*, and the information is drawn from nine scientific articles retrieved from the Dialnet, Elsevier, Google Scholar, PubMed, Redalyc, and SciELO databases using the following keywords: arthropods, *Chironectes minimus*, Didelphidae, Didelphimorphia, helminths, marsupials, nematodes, parasites, hosts, Physalopteridae, *Turgida turgida*. The records were obtained from papers that addressed aspects of the natural history of the species (Marshall 1978; Smith 2009) and checklists or reports of specific parasite groups (Miyazaki *et al*. 1974; Pinto *et al*. 2011; Lindardi 2012; Chero *et al*. 2017; Noronha *et al*. 2002; Varella *et al*. 2022; Jiménez *et al*. 2024).

The nematodes found in the stomach of *C. minimus* were identified as *Turgida turgida* and correspond to males (n = 5) and one female (n = 1), with a mean length of 2.45 cm (2.90; 3.18; 2.42; 1.85; 1.88 cm) and 4.59 cm, respectively (Figure 1). No other parasites were found in the specimen. SEM revealed structures that differentiate *T. turgida* from other Physalopteridae species, such as ventrocaudal ornamentation patterns (Figure 2a, Figure 2b, Figure 2c), and the presence of the 22nd broad, truncated caudal papilla on the male tail (Figure 2d). The specimen examined had no apparent lesions on the stomach.

According to the literature review, *C. minimus* has been reported to have 18 parasites in different areas of the body (Table 1). Most of them are helminths, with 14 species (77.78 %) predominantly belonging to the genera *Aspidodera* and *Cruzia*, and the remaining four are arthropods. This distribution highlights a marked predominance of helminth parasites in *C. minimus*, suggesting that gastrointestinal and other internal parasitic infections constitute the most commonly documented host-parasite associations in this species. In contrast, arthropod parasites account for 22.22 % of the total diversity of parasites documented in the literature. Records indicate that helminths are found in internal anatomical regions, particularly associated with the gastrointestinal tract, while arthropods are mainly associated with external regions of the body.

This data corresponds to information collected over more than 20 years, most of which comes from occasional records. They appear in brief mentions in studies on specific parasites and host lists and are often part of supplementary information when the main subject of the study is on other species of Didelphimorphia. Although not all papers specify the location where the parasite and host were recorded, there is information on the parasites *Rhopalias caballeroi*, *Spirura guianensis* and *Travassostrongylus callis* in Bolivia, *Adoratopsylla antiquorum*, *Aspidodera raillieti*, *Cruzia tentaculata*, *Dipetalonema* sp., *Litomosoides* sp., *Trichuris minuta* and *Turgida turgida* in Brazil; and *Paragonimus amazonicus* in Peru.

Turgida turgida is a parasite reported in several species of marsupials such as *Didelphis albiventris*, *D. aurita*, *D. marsupialis, D. virginiana and Philander opossum*, being

Table 1. List of parasites found in *Chironectes minimus*.

Species/Taxon	Infection site	References
Arthropoda		
Ctenophthalmidae		
Adoratopsylla antiquorum	Tegument	(Linardi 2012)
Adoratopsylla sp.	Tegument	(Marshall 1978; Smith 2009)
Doratopsylla sp.	Tegument	(Marshall 1978; Smith 2009)
Trombiculidae		
Doloisia sp.	Tegument	(Marshall 1978; Smith 2009)
Cestoda		
Diphyllobothriidae		
Ligula sp.	Small intestine	(Marshall 1978; Smith 2009)
Sparganum sp.	Muscle and subcutaneous tissue	(Marshall 1978; Smith 2009)
Nematoda		
Aspidoderidae		
Aspidodera raillieti	Large intestine	(Chero *et al.* 2017; Noronha *et al.* 2002, Pinto *et al.* 2011; Varella *et al.* 2022)
Heligmosomidae		
Travassostrongylus callis	Small intestine	(Jiménez *et al.* 2024)
Kathlaniidae		
Cruzia tentaculata	Excrements	(Noronha *et al.* 2002; Pinto *et al.* 2011)
Onchocercidae		
Dipetalonema sp.	Peritoneum	(Noronha *et al.* 2002; Pinto *et al.* 2011)
Litomosoides sp.	Lungs	(Noronha *et al.* 2002; Pinto *et al.* 2011)
Physalopteridae		
Turgida turgida	Stomach	(Noronha *et al.* 2002; Pinto *et al.* 2011; this work)
Spiruridae		
Spirura guianensis	Stomach	(Jiménez *et al.* 2024)
Trichuridae		
Trichuris minuta	Cecum	(Noronha *et al.* 2002; Pinto *et al.* 2011)
Trematoda		
Opisthorchiidae		
Amphimerus sp.	Bile ducts	(Marshall 1978; Smith 2009)
Paragonimidae		
Paragonimus amazonicus	Lungs	(Chero *et al.* 2017; Miyazaki *et al.* 1974)
Rhopaliidae		
Rhopalias sp.	Small intestine	(Marshall 1978; Smith 2009)
Rhopalias caballeroi	Small intestine	(Jiménez *et al.* 2024)

present from North America to South America and normally associated with the greater curvature of the stomach (Gray and Anderson 1982). In the family Physalopteridae the species appear to be selective to the site of infection, differing from the genus *Physaloptera*, which can infect the lesser covering of the stomach, the pylorus, or the fundic region (Humberg *et al.* 2011). These parasitic helminths can cause gastric perforations and sepsis, thus Nichelason *et al.* (2008) highlight *T. turgida* as a species with the ability to significantly influence morbidity and mortality in didelphids. Its life cycle is indirect, beginning when an insect ingests the parasite's eggs, whose larvae subsequently become encysted in the external wall of the intestine (Anderson 2000), the definitive host consumes the insect and then the nematode matures sexually and begins to produce eggs that will be released into the environment through the feces, thus completing the cycle (Gray and Anderson 1982). As intermediate hosts, the orders Blattodea, Coleoptera, and Orthoptera have been reported (Zago-Filho 1958; Anderson 2000). On the other hand, paratenic hosts such as amphibians and reptiles have also been reported (Widmer 1970; Humberg *et al.* 2011). *Chironectes minimus* has mainly a carnivorous diet, frequently consuming different aquatic organisms such as fish, crustaceans, frogs and insects (Hume 2005), therefore, it is possible that in the case of *T. turgida* the form of infection towards *C. minimus* is through its diet, given the wide diversity in its diet which could be a factor for this species to be more vulnerable to prey that are intermediate hosts, thus enhancing the spectrum of infections (Álvarez-Coto 2018), in the same way that happens in other didelphids (Gomes *et al.* 2003). Previously, Noronha *et al.* (2002) and Pinto *et al.* (2011) report *T. turgida* as a stomach parasite of *C. minimus* in Brazil in State of Rio de Janeiro, municipality of Santo Antônio, State of Pará. In addition, there are records of arthropods, cestodes, trematodes, and other nematodes parasitizing different areas of the body (Table 1). According to the literature, *T. turgida* has high prevalence rates in other Didelphimorphia (Table 2) in countries such as Bolivia (Jiménez *et al.* 2024), Brazil (Silva and Costa 1999; Boullosa *et al.* 2017; Costa-Neto *et al.* 2019; Freitas *et al.* 2022), Mexico (Cañeda-Guzmán 1997; Monet-Mendoza *et al.* 2005), and the United States (Gray and Anderson 1982). This high prevalence may be related to the ecological and trophic characteristics of these marsupials, which could increase their exposure to infectious stages present in the environment or in intermediate hosts. It may also suggest that this nematode is well adapted to these hosts and that its transmission is relatively efficient, playing a significant role within the parasitic community associated with them (Freitas *et al.* 2022). From a population perspective, infections with high prevalence could influence the physiological condition of individuals, especially when parasite loads are high, which could potentially affect their survival or reproductive success (Boullosa *et al.* 2017). The higher number of *T. turgida* records in countries such as Bolivia, Brazil, Mexico and the United States does not necessarily reflect its actual distribution on the continent, but rather differences in sampling effort and the intensity of parasitological studies conducted in these areas. In this regard, the absence of records in other countries of the Americas could be associated with a smaller number of studies focused on the parasitic fauna of Didelphimorphia, rather than a true absence of the nematode.

Table 2. Prevalence of *Turgida turgida* in other Didelphimorphia.

Species	Prevalecence	Country	Reference
Didelphis albiventris	33%	Bolivia	(Jiménez *et al.* 2024)
	73%	Brazil	(Silva and Costa 1999)
Didelphis aurita	70%	Brazil	(Boullosa *et al.* 2017)
	75%	Brazil	(Costa-Neto *et al.* 2019)
Didelphis marsupialis	75%	Bolivia	(Jiménez *et al.* 2024)
	59%	Brazil	(Freitas *et al.* 2022)
	50%	Mexico	(Cañeda-Guzmán 1997)
Didelphis virginiana	50%	Mexico	(Cañeda-Guzmán 1997)
	60%	Mexico	(Monet-Mendoza *et al.* 2005)
	72%	United States	(Gray and Anderson 1982)
Philander opossum	9.5%	Mexico	(Cañeda-Guzmán 1997)

Spratt and Beveridge (2016) estimate that about 26 % of the global marsupial fauna has not yet been examined for parasites. The lack of representativeness of this group in some biological collections may be because they are rare or threatened, limiting the opportunity to collect parasites from them (Beveridge and Spratt 2015). Although the diversity of helminths and other parasites is well documented in Australian marsupials (Beveridge and Gasser 2014; Beveridge and Spratt 2015; Spratt and Beveridge 2016), in contrast, for the Neotropics, having fewer species, efforts to know the parasites of marsupials have been focused mainly on the genus *Didelphis* (Jiménez *et al.* 2011; Acosta-Virgen *et al.* 2015; Freitas *et al.* 2022) because these species inhabit peri-urban areas, in addition to being compatible hosts and reservoirs of diseases that affect humans (Bezerra-Santos *et al.* 2021). Previous studies suggest that the family Didelphidae are frequent hosts of helminths (Benatti *et al.* 2023).

Chironectes minimus has been considered a rare or infrequent species, however, the capture method proposed by Bressiani and Graipel (2008) demonstrates that it may be more common than previously thought; representing an opportunity to better understand the parasites associated with this species and other aspects of its biology. Considering that the last recorded occurrence of the helminth *T. turgida* in *C. minimus* was

more than two decades ago, this record contributes to the knowledge of parasite-host relationships between these species, in addition to being the first record in Colombia. Furthermore, we emphasize the importance of biological collections and recommend further examination of specimens during the collection process, given the possibility of gathering relevant information regarding the parasites associated with the specimens, allowing us to understand their possible implications for public and animal health (Benatti *et al.* 2023).

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