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First record of a Physalopteridae parasitizing a specimen of *Bassariscus astutus from* Hidalgo, México

Primer registro de un Physalopteridae parasitando a un ejemplar de *Bassariscus astutus* de Hidalgo, México

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The study of helminths in the Procyonidae of México is scarce, with 4 species of acanthocephalans, 8 species of nematodes, and 6 species of flatworms being recorded. For *Bassariscus astutus*, the cestodes *Mesocestoides bassarisci* and *Taenia pencei* are known, as well as unidentified nematode larvae. The objective of this work is to add a new record of a nematode to the helminth fauna that parasitizes *B. astutus*. Four dead *B. astutus* were obtained from 3 locations in the state of Hidalgo. The viscera of the hosts were examined, and a nematode was found in the stomach of one host. The helminth was fixed and kept in 70 % alcohol for identification. A third-stage larva of the Physalopteridae family was identified by having 2 lateral pseudolabiums in the cephalic region, formed by 3 lips. The middle lip has 4 teeth, 3 internal and 1 external. Each lateral lip has a rough area. Finally, the cephalic region is surrounded by a cuticular fold called a hood. This is the first record of a Physalopteridae parasitizing *B. astutus* in México. The Physalopteridae family includes the genera *Physaloptera* and *Turgida*, both of which have 4 teeth on the middle lip. Our specimen, as it exhibits the above features in addition to the presence of a hood, could be considered a member of the genus *Turgida*. However, due to the absence of the number of uterine branches that differentiate adults of both genera, we cannot confirm that it is a *Turgida*.

Key words: Helminths; larvae; nematode; ringtail.

El estudio de helmintos en los Procyonidae de México es escaso, registrándose 4 especies de acantocéfalos, 8 especies de nematodos y 6 especies de platelmintos. Para *Bassariscus astutus* se conocen los céstodos *Mesocestoides bassarisci* y *Taenia pencei*, así como larvas de nematodos sin identificar. El objetivo del presente trabajo es agregar un nuevo registro de un nematodo a la helmintofauna que parasita *B. astutus*. Se obtuvieron 4 *B. astutus* muertos en 3 localidades del estado de Hidalgo. Las vísceras de los hospederos fueron revisadas, encontrándose un nematodo en el estómago de un hospedero. El helminto fue fijado y mantenido en alcohol al 70 % para su identificación. Una larva, en tercer estadio de desarrollo, de la familia Physalopteridae fue identificada por contar con 2 pseudolabios laterales en la región cefálica, formados por 3 labios. El labio medio tiene 4 dientes, 3 internolabiales y 1 externolabial. Cada labio lateral presenta un área rugosa. Por último, la región cefálica se encuentra rodeada por un pliegue cuticular llamado capuchón. Este es el primer registro de un Physalopteridae parasitando *B. astutus* en México. En la familia Physalopteridae se encuentran los géneros *Physaloptera* y *Turgida*, ambos exhiben 4 dientes en el labio medio, nuestro ejemplar al exhibir los rasgos anteriores además de la presencia de un capuchón, se podría considerar miembro del género *Turgida*. Sin embargo, debido a la ausencia del número de ramas uterinas que diferencian a los adultos de ambos géneros, no podemos confirmar que se trate de *Turgida*.

Palabras clave: Cacomixtle; helmintos; larva; nematodo.

Bassariscus astutus (Lichtenstein, 1830) is a species of procyonid widely distributed in the United States of America and México, ranging from southern Oregon and California to the state of Texas, crossing the southwestern states (Ceballos and Nava 2014). In México, its range covers all the northern and central states of the Mexican Republic up to Oaxaca. Three islands in the Gulf of California are included in its distribution: Tiburón, Espíritu Santo, and San José. In México it has been registered in several states: Baja California, Baja California Sur, Chiapas, México City, Colima, Durango, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Tamaulipas and Veracruz (Ceballos and Nava 2014; León-Paniagua *et al.* 1990). The species has been found at elevations up to 2,900 m (Reid *et al.* 2016) and inhabits mountainous areas and rugged slopes (Ceballos and Nava 2014).

The study of helminths that parasitize *B. astutus* is scarce; in a period of 76 years only 7 studies have been conducted (Table 1) within 24 articles focused on parasites, with ectoparasites standing out (Duran-Irigoyen and

Martínez-Calderas 2023). Regarding endoparasites only 7 species of helminths have been found: 1 acanthocephalan, 3 cestodes and 3 nematodes, along with some unidentified larvae (García-Prieto *et al.* 2012; Duran-Irigoyen and Martínez-Calderas 2023; González-Roa *et al.* 2023; Table 1); therefore, a greater effort is necessary to know the helminths harbored by these mammals. The presence of helminths in a host allows us to understand the biology of the host through the life cycle of the parasite, for example, the Physalopteridae family has a complex life cycle (parasite uses 2 or more hosts in life cycle) that involves intermediate host such as beetles, cockroaches, crickets or grasshoppers; the existence of this family of nematodes indicates the type of food ingested by the host. Nematodes with a simple life cycle (parasite only infects a single host in its life cycle) can be transmitted due to caecotrophy (also known as cecotrophy), a behavior of animals that eat fecal material for nutritional value. This behavior has been observed in rodents from several families: Aplodontidae, Arvicolidae, Bathyergidae, Castoridae, Caviidae, Cricetidae, Geomyidae and Heteromyidae (Kenagy and Hoyt 1980; Anderson 2000; Iturbe-Morgado *et al.* 2017; Bolek *et al.* 2024).

For the reasons mentioned above, the study of helminths in the wild is important because they represent an important component of biological diversity, in addition to helping to understand the history of parasite-host associations, as a means of providing information about the hosts they parasitize and the regions where they live, which can be used in conservation initiatives (Pérez-Ponce de León and García-Prieto 2001a, b). Finally, the objective of the present work is to add a new record of a nematode to the helminth fauna already known as *B. astutus* parasites.

Four specimens of *B. astutus* were found dead, 2 in Cerro de la Rufina, municipality of Mineral del Monte (20º 7′ 50.29″ N, 98º 40′ 8.21″ W; 1 on May, 1 on June 2015); 1 more run over in Santa María la Palma, municipality of Alfajayucan (20º 22′ 21.11″ N, 99º 23′ 4.43″ W; April 2023) and the last one drowned in a cistern in San Antonio Oxtoyucan, municipality of Zempoala (19º 55′ 46.14″ N, 98º 40′ 5.69″ W; January 2023; Figure 1), all in the Mexican state of Hidalgo. The skins and skulls of the specimens collected in Mineral del Monte and Zempoala were deposited in the Museo de Mamíferos del Centro de Investigaciones Biológicas of the Universidad Autónoma del Estado de Hidalgo (UAEH), with the numbers UAEH-CIB-M 2055, 2152 and 2384. The Alfajayucan specimen was dissected at the collection site, obtaining the viscera that were placed in 70 % alcohol to transport them to the laboratory, the ringtail was buried in the collection area due to its advanced state of putrefaction without assigning it a collection number. The digestive tract of the specimens from the other 2 localities was removed and examined in the year of collection. A dead nematode was recovered from the stomach of 1 of the ringtails from the locality of Mineral del Monte, the nematode was fixed in glacial acetic acid and stored in a vial with 70 % alcohol. For identification in 2024, the material was mounted in semi-permanent preparations with lactophenol to clarify and observe important morphological characters. Taxonomic identification was corroborated by comparing the traits with specialized literature, *e.g.*, Anderson (2000) and Chabaud (1975). The measurements are given in millimeters (mm) and micrometers (µm), and the specimen was deposited in the Colección Nacional de Helmintos (CNHE 8485) of the Universidad Nacional Autónoma de México.

The nematode was identified as a third-stage female larva belonging to the Physalopteridae family (prevalence of 25 %). The measurements were: 26.8 mm in length, 680 µm wide at mid-body, esophagus 5.24 mm in length, and anus located 450 µm from the posterior end of the body. The organism is characterized by the presence of 2 lateral pseudolabia in the cephalic region, each formed by 3 lips fused. On the middle lip, 4 teeth can be seen, 3 on the edge of each lip (interlabial teeth [il]) and one behind them (external labial tooth [el]). The single el is large and

Table 1. Records of parasitic helminths of *Bassariscus astutus* and its geographical location. The classification used is the one proposed by Brusca *et al.* 2023.

Phylum	Family	Genus and species	Locality	Reference
Rotifera	Oligacanthorhynchidae	*Macracanthorhynchus ingens*	West Texas	Pence and Willis 1978
Platyhelminthes	Mesocestoididae	*Mesocestoides* sp.	West Texas	Pence and Willis 1978
		M. bassarici	Zoo, México	MacCallum 1921
	Taeniidae	*Taenia pencei*	México City	García-Prieto *et al*. 2012
			West Texas	Rausch 2003
Nematoda	Ancylostomatidae	*Placoconus lotoris*	West Texas	Price 1928; Pence and Willis 1978
	Pneumospiruridae	*Pneumospirura bassarisci*	West Texas	Pence and Stone 1977; Pence and Willis 1978
	Physalopteridae	*Physaloptera* sp.	West Texas	Pence and Willis 1978
		Unknown	Cerro de la Rufina, Hidalgo	This work
	Unknown	Unknown larvae	Puebla, México	González-Roa *et al*. 2023

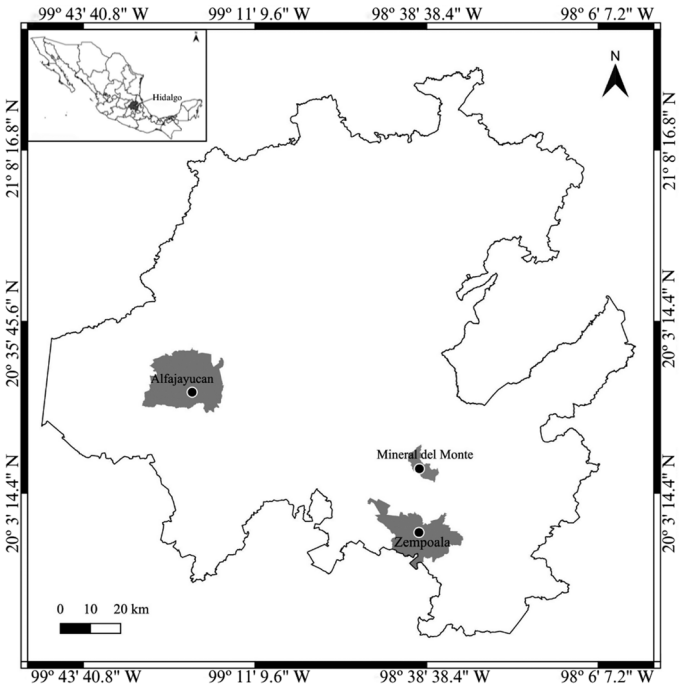

Figure 1. Map of the localities where specimens of *Bassariscus astutus* were found in the state of Hidalgo, México.

pyramid-shaped, and the il are unequal in size (Figure 2a). On the lateral lips, there are areas identified as rugose areas (ra), in an external circle 4 prominent papillae are observed, and at the base of the middle lips are the amphids; finally, the cephalic region is surrounded by a striated cuticular fold that resembles a hood [h] (Figure 2a and 2b). Based on the characteristics described for the organism, we consider that this larva belongs to the genus *Turgida*; however, we cannot confirm this because, in the larval stage in which the nematode is found, the uterine branches have not developed, a feature that differentiates the adults of this genus from those of *Physaloptera* (Anderson 2000).

Additionally, we found in the intestine of the host, pirul (*Schinus molle*) seeds, traces of spiders, caterpillars, insect wings, grasshoppers of the Acrididae family, and flowers. Some of the insects mentioned can serve as intermediate or paratenic hosts for the nematode found. The stomach content found in the *B. astutus* reviewed had already been previously recorded in forest areas and semiarid regions (Nava *et al.* 1999; Castillo-Picazo and García-Collazo 2019), environments where the hosts' bodies were recovered.

In the present work, a larva from the Physalopteridae family was identified in a specimen of *B. astutus* from Cerro de la Rufina, municipality of Mineral del Monte in Hidalgo. The family Physalopteridae includes the genera *Physaloptera* and *Turgida*, both are characterized by having 4 teeth, 3 at the edge of each pseudolip and 1 behind them. The difference between the genera is a cuticular thickening (hood) present in *Turgida* (Chabaud 1975).

The *Physaloptera* genus comprises a total of 144 species that are found parasitizing amphibians, birds, reptiles, and mammals that include in their diet some insects that can be used by the nematode as intermediate hosts (Anderson 2000; Kalyanasundaram *et al.* 2018) since some mammals that are not definitive hosts become occasionally infected by consuming infected insects. On the other hand, in the genus *Turgida*, 2 species have been recorded: *T. turgida*

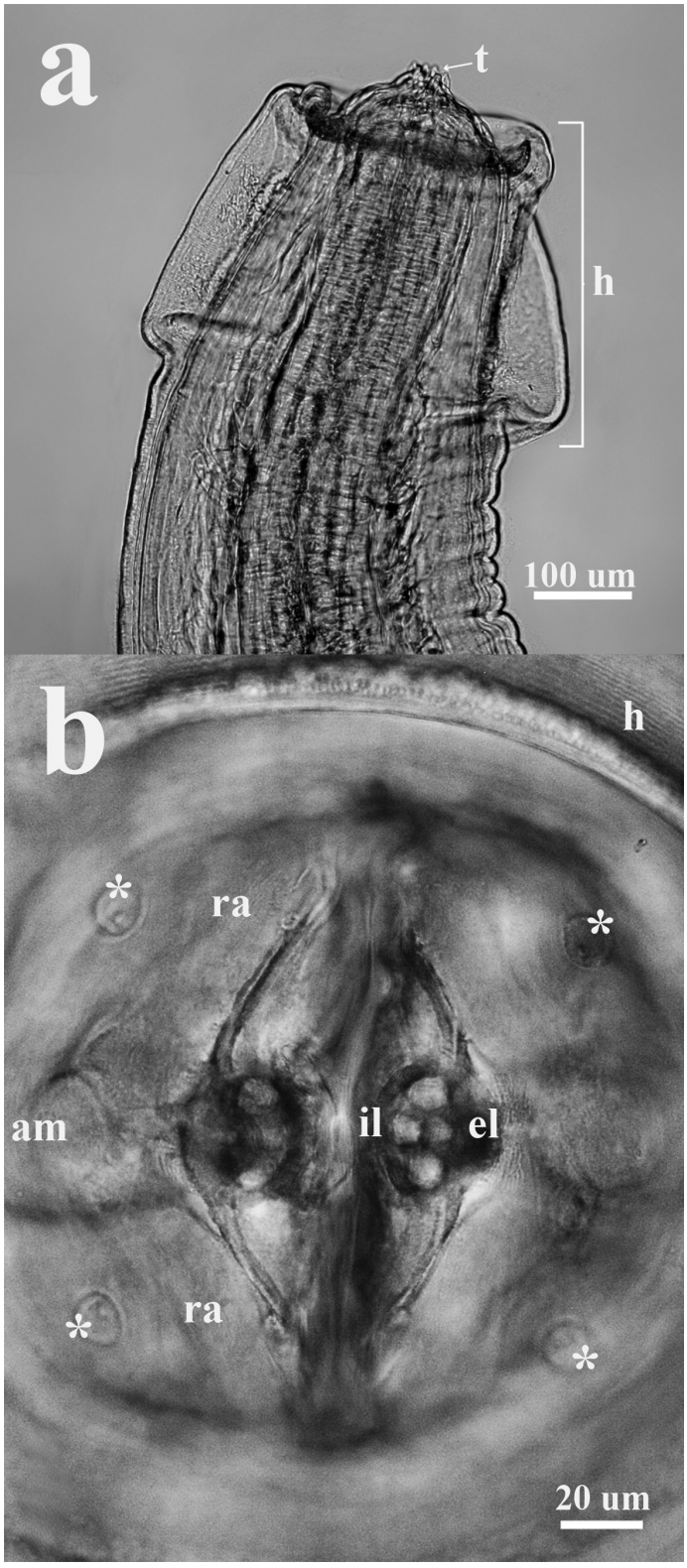

Figure 2. a) Head region of a third instar larva of the Physalopteridae family showing the striated cuticular thickening (h) and the four teeth of the middle pseudolip (t). b) Frontal view of the cephalic region showing the arrangement of the internal labial teeth (il), the external labial tooth (el), rugose area (ra), amphids (am), and papillae (*).

mainly parasitizes representatives of the genus *Didelphis* and *T. torresi* which parasitizes *Cuniculus paca* in neotropical areas (Chabaud 1975; Anderson 2000; Goncalves *et al.* 2006; García-Prieto *et al.* 2012; Hodda 2022).

The larva found has the cephalic characteristics of an adult that allows us to recognize it as Physalopteridae (Anderson 2000), and due to the presence of a hood, we would consider it a larva of the genera *Turgida*, which we cannot confirm because the uterine branches are not developed. In the adult state, the genera can be differentiated by the number of uterine branches present in females, which is 2 to 4 in *Physaloptera* and more than 4 in *Turgida*, (Chabaud 1975), a feature that we could not observe in the organism. This would be the second time that a larva of the Physalopteridae family has been recorded in a specimen of *B. astutus*, the first one was in West Texas as *Physaloptera* sp. (Pence and Willis 1978) where the record was the sole mention of its presence without morphological description, as in the present study.

We consider that the presence of the larva in *B. astutus* is an accidental infection, based on the work of Gray and Gray and Anderson (1982), who obtained adults of *T. turgida* from opossums, and from the collected nematodes they obtained eggs with which they infected previously cultured crickets and fed these insects to different types of hosts such as mammals (*Cavia porcellus*, *Felis catus*, *Marmota monax*, *Procyon lotor*, among others), amphibians (*Rana pipiens*) and reptiles (*Thamnophis sirtalis*), finding numerous undeveloped third-stages larvae in the stomach of all these host. Some of these animals could serve as a host where the development of the nematode does not occur (*i.e.*, it could be a paratenic host), and their role in the transmission of the parasite is unknown (Gray and Anderson 1982; Anderson 2000). *Bassariscus astututs* are likely serving as paratenic hosts, just like the animals mentioned above.

Finally, the study of nematodes that parasitize *B. astutus* is scarce, only larvae of the Physalopteridae family have been recorded in Texas (Pence and Willis 1978), and unidentified larvae from a copro parasitological study in *B. astutus* from Puebla (González-Roa *et al.* 2023). With copro parasitological analysis, the parasites of different hosts can be known; however, it is difficult to characterize helminths morphologically if they are obtained in this way, as happens in the work of González-Roa *et al.* (2023) where they mention them as larvae, while we are reaching a taxonomic level in the organisms obtained. Studies such as the one presented here allow us to learn about the nematofauna of wildlife.

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