

Globocephalus urosubulatus (Alessandrini, 1909) (Nematoda: Ancylostomatidae) in Brazil: a morphological revisitation

Globocephalus urosubulatus (Alessandrini, 1909) (Nematoda: Ancylostomatidae)
no Brasil: uma revisitação morfológica

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Abstract

Helminthiasis occurs frequently in wild or domestic pigs in the most varied environments or production systems; however, the literature includes few taxonomic studies for the purpose of expanding this knowledge about the parasitic diversity in these animals. Thus, in order to expand this knowledge regarding parasitic diversity in these animals, the present study reports the occurrence and redescribes *Globocephalus urosubulatus* infecting domestic pigs in the state of Pará, Brazil, using microscopy. Four hundred and ten specimens of *Sus scrofa* intestinal nematodes were collected extensively in the municipality of Moju, northeastern mesoregion of Pará and observed under light and scanning electron microscopy. Based on morphological characteristics, the nematodes were identified as *G. urosubulatus*, and new morphological data were added by light microscopy and scanning electron microscopy for the diagnosis of this parasite.

Keywords: Biodiversity, nematodes, *Sus scrofa*, Pará, Amazon.

Resumo

As helmintíases ocorrem frequentemente em porcos selvagens ou domésticos nos mais variados ambientes ou sistemas de produção, porém a literatura contempla poucos estudos taxonômicos. Assim, com a finalidade de ampliar esses conhecimentos sobre a diversidade parasitária nesses animais, o presente estudo relata a ocorrência e redescreve *Globocephalus urosubulatus* infectando porcos domésticos no estado do Pará, Brasil, utilizando-se microscopia. Foram recolhidos 410 exemplares de nematoides do intestino de *Sus scrofa*, criados extensivamente no município de Moju, mesorregião nordeste do Pará e observados em microscopia de luz e eletrônica de varredura. Com base em características morfológicas, os nematoides foram identificados como *G. urosubulatus*, sendo adicionados novos dados morfológicos por microscopia de luz e microscopia eletrônica de varredura para o diagnóstico desse parasito.

Palavras-chave: Biodiversidade, nematoides, *Sus scrofa*, Pará, Amazônia.

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Introduction

Helminthiasis frequently occurs in wild or domestic pigs in the most varied environments or production systems around the world, but has generally received less attention when compared to parasitological studies in ruminants (Thamsborg et al., 2017). For Hale & Stewart (1979) and Roepstorff et al. (2011), helminth infections in these animals are not always apparent and persist at subclinical levels for extended periods, often leading to death for the animals.

Parasitism in pigs can affect performance in terms of efficient feeding, growth rate, general health and condemnation after slaughter (Weng et al., 2005). There are few studies that detail or inventory the parasitological fauna of wild and domestic species, especially in taxonomic assessments and that trace differences and similarities between different groups of parasites. Therefore, the accumulation of information over time will enable validation of the use of helminths in monitoring the health of ecosystems and pig breeding systems (Bongers & Ferris, 1999; Brandão et al., 2009).

Due to the economic and ecological importance of pigs, especially those destined for slaughter, many researchers in different countries such as France (Humbert & Henry, 1989), India (Yadav & Tandon, 1989; Laha et al., 2014a, b), Tanzania (Esrony et al., 1997), Mexico (Ortega-Pierres et al., 2000), Croatia (Rajković-Janje et al., 2002), Iran (Solaymani-Mohammadi et al., 2003), Burkina Faso (Tamboura et al., 2006), Zimbabwe (Marufu et al., 2008), Kenya (Kagira et al., 2012), Ethiopia (Kumsa & Kifle, 2014), Philippines (Padilla & Ducusin, 2015), Azerbaijan (Ali Gyzy & Ilyas Oglu, 2016), Bulgaria (Panayotova-Pencheva & Dakova, 2018) and Colombia (Chaparro-Gutiérrez et al., 2018), have been studying their parasitic communities in order to get to know its parasitic community, as well as its relationship with public health and with the high rates of morbidity and mortality in different production systems.

Despite the Brazilian literature involving several studies on the parasitic fauna of pigs, these data are scarce for the Amazon region, mainly regarding helminth diversity in pigs raised extensively and not submitted to the hygienic-sanitary inspection process. Among the nematodes present as fauna parasites in pigs in Brazil, the most genera most often recorded genera are *Ascaris*, *Ascarops*, *Globocephalus*, *Hyostrongylus*, *Metastrongylus*, *Oesophagostomum*, *Physocephalus*, *Stephanurus*, *Strongyloides*, *Trichostrongylus* and *Trichuris* (D'Alencar et al., 2006; Pinto et al., 2007; Brito et al., 2012; Anjos et al., 2015; Mattos et al., 2020).

The genus *Globocephalus* is one of the least known and is represented in Brazilian territory by two species *G. urosubulatus* (Alessandrini, 1909) and *G. marsupialis* Freitas & Lent, 1936 (Vicente et al., 1997). Only *G. urosubulatus* has been recorded as parasitizing pigs in different rearing systems in Brazil (Freitas & Costa, 1967; Francis, 1978; Carneiro et al., 1979, 1980; Pinto et al., 2007). Thus, the aim of the present study was to report the occurrence and redescribe *Globocephalus urosubulatus* infecting domestic pigs in the state of Pará, Brazil, using microscopic tools.

Materials and Methods

Survey data

The nematodes were recovered from the small intestine of a single pig slaughtered at a market in the municipality of Moju (01°53'02" S; 48°46'08" W), mesoregion of northeastern Pará, with slaughter done without the sanitary control. The intestine was placed in a basket and washed with water, and macro-residues were separated with the aid of a steel granulometric sieve (9.50mm opening). What passed through the sieve was then fixed in 70% ethanol, stored at room temperature according to (Ahn et al., 2015; Kuzmin et al., 2019; Morais et al., 2020) and transported in a 30L bag to the Laboratório de Parasitologia Animal, Instituto da Saúde e Produção Animal, Universidade Federal Rural da Amazônia. Aliquots of the material were separated into petri dishes, observed with the aid of a stereomicroscope (LEICA-ES2) and the parasites collected.

Light microscopy

For morphological and morphometric analysis twenty-five nematodes (15 females, 10 males) were dehydrated in an ethanol series, clarified with Aman's Lactophenol (20%) and observed using a light microscope, and LEICA DM2500 camera with an imaging capture system. Measurements are shown in micrometers as the mean followed by the range, or as otherwise indicated. Taxonomic classification of nematodes was in accordance with Cameron (1924), Freitas & Lent (1936), Vicente et al. (1997) and Anderson et al. (2009).

Scanning electron microscopy

For scanning electron microscopy (SEM), twenty nematodes (10 females and 10 males) were post-fixed in 1% osmium tetroxide, dehydrated to the critical point for CO₂, metalized with gold-palladium, and analyzed with the VEGA 3 LMU/TESCAN scanning electron microscope at the Laboratório de Microscopia Eletrônica de Varredura, Instituto de Saúde e Produção Animal da Universidade Federal Rural da Amazônia.

Results

A total of 410 [females= 264, males= 146] nematodes were recovered from *Sus scrofa*. All specimens collected showed characteristics compatible with the genus *Globocephalus* (Nematoda: Ancylostomatidae). The morphological and morphometric characteristics of the nematodes recovered from domestic pigs are presented below and in Table 1.

Table 1. Morphological and morphometric comparison of *Globocephalus urosubulatus* collected in *Sus scrofa* from State of Pará, Brazil.

| Character | <i>Globocephalus urosubulatus</i> | | <i>G. urosubulatus</i> | | <i>G. urosubulatus</i> | | <i>Globocephalus marsupialis</i> | |
|---------------------------------------|-----------------------------------|-------------|----------------------------------|------------|----------------------------|------------------------|--|-------------|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Hosts Type locality | <i>Sus scrofa</i> Moju-Pará | | <i>Sus scrofa</i> Rio de Janeiro | | <i>Sus scrofa</i> Bulgaria | | <i>Metachirops opossum</i> Petrópolis-Rio de Janeiro | |
| Length (mm) ^a | 4-5 | 6-8 | 3.16-5.32 | 5.02-6.53 | 3.5-5 | 4.5-8 | 4.4-6.18 | 10.47-11.44 |
| Width | 167-300 | 429-514 | 320-400 | 490-570 | 360-370 | 420-500 | 237-316 | 400-430 |
| Buccal capsule ^{b, L} | 125-150 | 167-227 | 119-127 | 192-220 | 140-200 | 140-200 | 104 | 129-143 |
| Buccal capsule ^{b, W} | 100-140 | 140-160 | 162-186 | 159-178 | 150-170 | 150-170 | 96 | 114-129 |
| Nerve ring | 317-367 | 387-500 | 290-460 | 380-460 | 380-520 | 380-520 | 360 | 429 |
| Excretory pore | 317-417 | 433-547 | 320-500 | 320-530 | - | - | - | - |
| Deirids | 370-533 | 433-547 | - | - | 430-610 | 430-610 | - | - |
| Muscular esophagus ^{a, b, L} | 0.487-0.540 | 0.593-0.687 | 0.550-0.710 | 0.680-0.75 | 0.560-0.690 | 0.560-0.690 | 0.789-0.800 | 1-1.3 |
| Muscular esophagus ^{b, W} | 93-133 | 147-173 | 110-170 | 110-160 | 120-150 | 120-150 | - | - |
| Vulva (mm) | - | 3-5 | - | 1.73-2.91 | - | 2.20-2.40 ^c | - | 4.4-5.7 |
| Spicule | 337-527 | - | 490-550 | - | 420-580 | - | 338-378 | - |
| Gubernaculum | 60-88 | - | 89-110 | - | 70-80 | - | 38-48 | - |
| Tail | - | 130-200 | - | - | 120-180 | 120-180 | - | 304-329 |
| Mucron | Absent | | - | | - | 40 | - | |
| Reference | From this study | | Francis (1978) | | Nanev et al. (2007) | | Freitas & Lent (1936) | |

^aMeasurements in micrometers unless indicated and the parameter number of buds is given in amplitude; ^bAbbreviations: L: length, W: width; ^cDistance between the vulva and the posterior body end.

Ancylostomatidae Looss, 1905

Globocephalus Molin, 1861

Globocephalus urosubulatus (Alessandrini, 1909)

(Based on light microscopy and scanning electron microscopy examination: Figures 1-3)

Medium size nematode. Females larger than males, the morphology of the anterior region being similar in both sexes (Figures 1a, b, 3a). Thick cuticle, with thin transverse streaks along the body. Long, rounded cephalic end, circular mouth opening, surrounded by a delicate cuticular ring (Figure 1c, 3b). Subglobular buccal capsule, without cutting plates or teeth on its margin, a pair of large sub-ventral teeth near the base of the capsule (Figure 1d). A rib with small spines reaching the top of the inner surface of the mouth capsule on its back (Figure 3c). Four external cephalic papillae and four internal papillae and a pair of lateral amphids. Well-developed claviform muscular esophagus, opening to the intestine through a strong valve. Excretory pore and spoon-shaped deirids located near the nerve ring (Figures 1a, b, 3a).

Females with eggs (based on 10 specimens): body 6mm (6-8mm), maximum width at esophageal/intestinal junction 469µm (429-517µm). Length of buccal capsule 200µm (167-227µm) × 148µm (140-160µm). Maximum length/

width ratio of buccal capsule 1:0.7. Muscular esophagus with a size of 645µm (593-687µm) × 157µm (147-173µm). The esophagus represents 10% (8-11%) of body length. Length of entire esophagus and buccal capsule representing 13% (11-14%) of body length. Nerve ring, excretory pore and deirids at 452µm (387-500µm), 482µm (433-547µm) and 495µm (433-547µm), respectively, from anterior extremity. Vulva situated at, 4mm (3-5mm) from anterior end, at about 63% (59-67%) of body length; vulval lips not elevated (Figure 1e, 3d). Muscular vagina directed posteriorly; uterus filled with eggs elliptical or round shape, shell single, thin, smooth and transparent and morulated embryo (Figure 1g). The eggs have a size of 49µm (32-67µm) long by 32µm (23-47µm) wide. Tail conical has a length of 162µm (130-200µm) (Figure 1f), with two small papillae near the end (Figure 3e). Anus with prominent upper lip (Figure 3e).

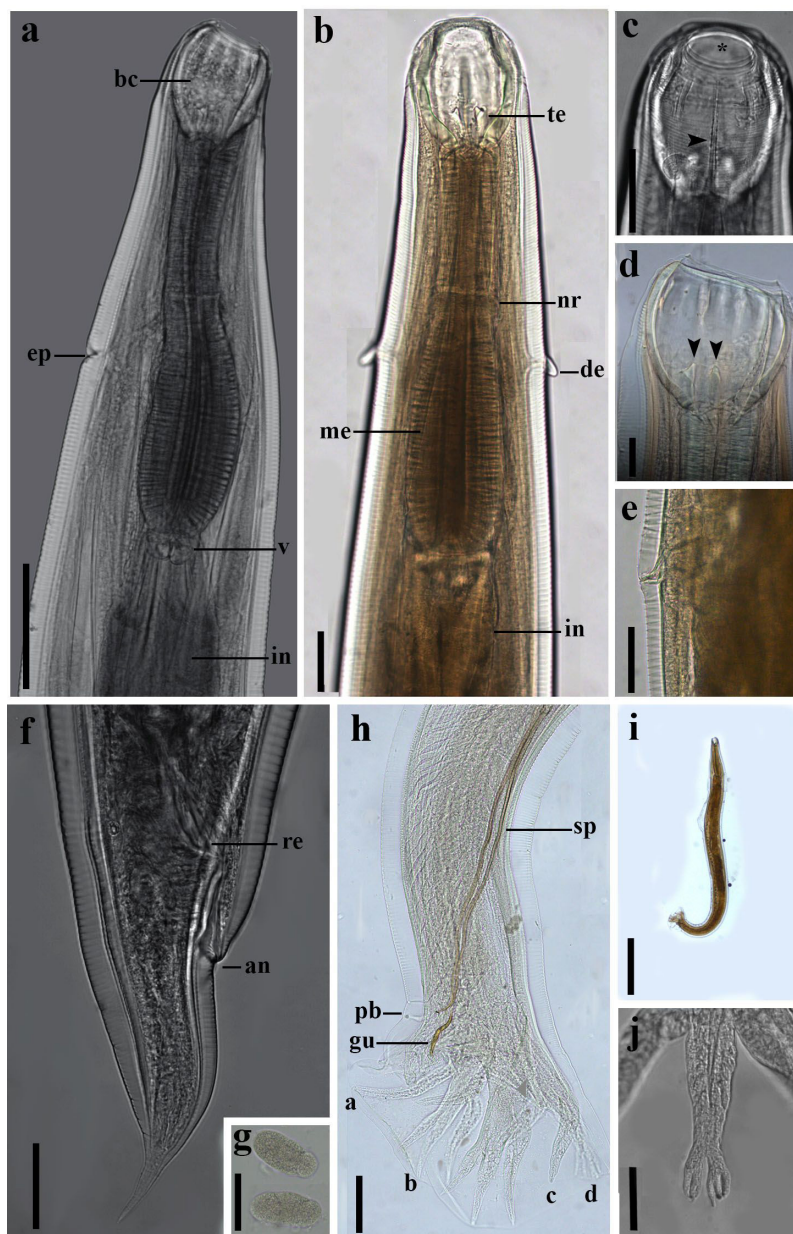


Figure 1. Light microscopy of *Globocephalus urosubulatus*: (a) Lateral view of cephalic region of female showing buccal capsule (bc); excretory pore (ep); posterior region of the esophagus is expanded and opens into the intestine by a strong valve (v) and intestine (in). Bar = 200µm; (b) Ventral view of cephalic region showing buccal capsule; teeth (te); nerve ring (nr); deirids (de); muscular esophagus (me) and intestine (in). Bar = 100µm; (c) Detail of circular oral opening (*) and rib with small spines reaching the inside tip of the buccal capsule on the back (arrow points). Bar = 100µm; (d) buccal capsule armed with two sclerotized tooth-like structures (arrow points). Bar = 50µm; (e) Detail of vulva. Bar = 100µm; (f) Posterior end of female lateral view: rectum (re) and anus (an). Bar = 50 µm; (g) Detail of eggs. Bar = 50µm; (h) Posterior end of male, lateral view spicules (sp); pre-bursal papillae (pb); Gubernaculum (gu) and papillae a = ventral and lateroventral rays, b = anterolateral, mediolateral, posterolateral rays, c = externodorsal rays and d = dorsal trunk. Bar = 100µm; (i) Overview of a male specimen. Bar = 12µm; (j) Detail of the trifurcated final part of the dorsal trunk. Bar = 50µm.

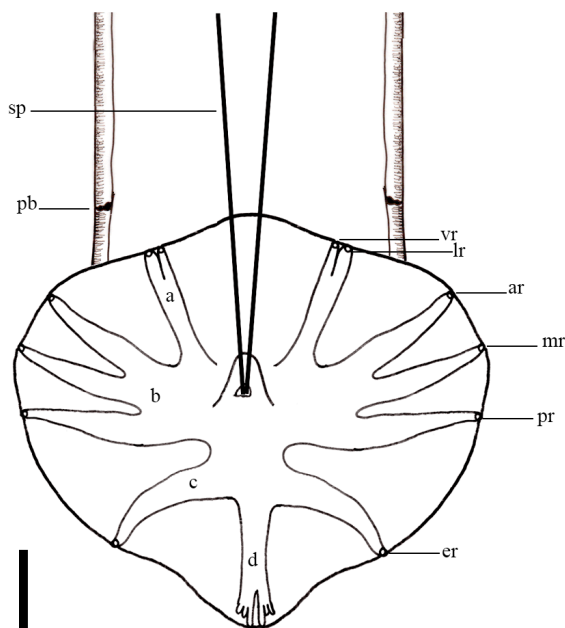


Figure 2. Light microscopy of *Globocephalus urosubulatus* in ventral view of the caudal bursa showing the spicules (sp); pre-bursal papillae (pb) and papillae a = ventroventral (vr) and lateroventral rays (lr), b = anterolateral (ar) mediolateral (mr), posterolateral rays (pr), c = externodorsal rays (er) and d = dorsal trunk. Bar = 100µm.

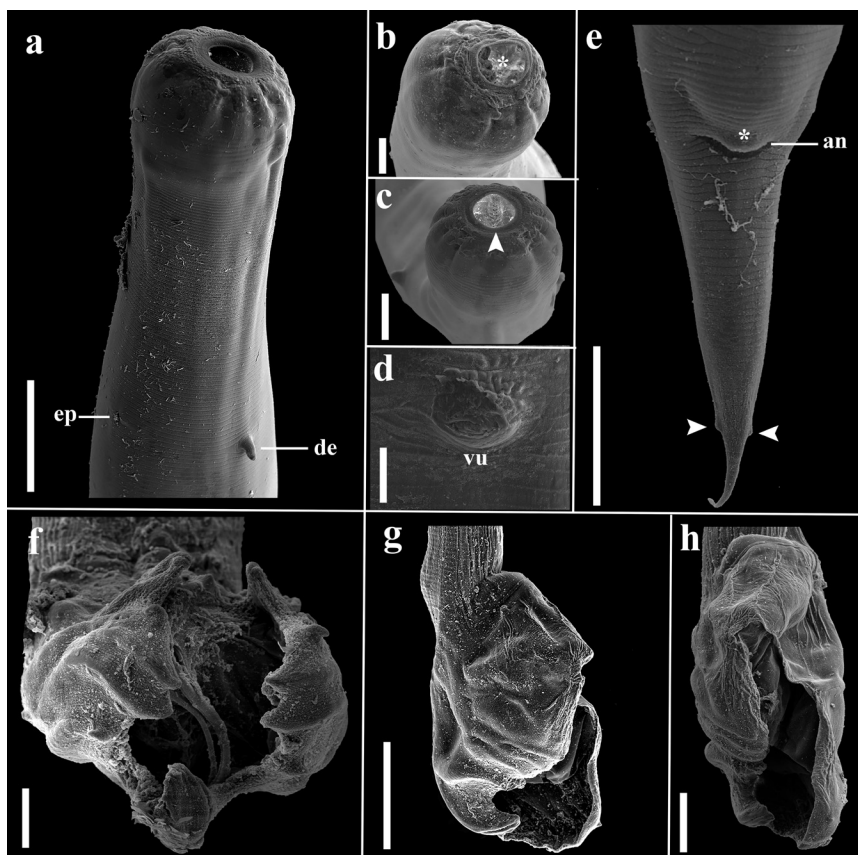


Figure 3. Scanning electron microscopy of *Globocephalus urosubulatus*. (a) Lateral view of cephalic region of female showing circular oral opening, excretory pore (ep) and deirid (de). Bar = 100 µm; (b) Frontal view of the oral opening (*) of males. Bar = 20 µm; (c) View of the rib with small spines reaching the inside tip of the buccal capsule on the back. Bar = 50 µm; (d) Detail of Vulva (vu). Bar = 20µm; (e) Posterior end of female ventral view anus (an), lip elevated (*) and on pair of papillae lateral (arrow points). Bar = 50 µm; (f, g, h) Different views of the male caudal region. Bar = 20 µm, Bar = 100 µm, Bar = 50 µm.

Females without eggs (based on 5 specimens): body 6mm (4-8mm) long, maximum

width at esophageal/intestinal junction 474 μ m (457-514 μ m). Length of buccal capsule 209 μ m (193-227 μ m) \times 151 μ m (140-160 μ m). Maximum length/width ratio of buccal capsule 1:0.7. Muscular esophagus with a size of 512 μ m (487-540 μ m) \times 116 μ m (93-133 μ m). The esophagus represents 10% (8-11%) of body length. Length of entire esophagus and buccal capsule representing 13% (11-15%) of body length. Nerve ring, excretory pore and deirids at 476 μ m (447-500 μ m), 488 μ m (447-547 μ m) and 512 μ m (480-573 μ m), respectively, from anterior extremity. Vulva situated at, 4mm (3-5mm) from anterior end, at about 64% (60-69%) of body length; vulval lips not elevated. Muscular vagina directed posteriorly, uterus without eggs. Tail conical has a length of 150 μ m (143-157 μ m), with two small papillae near the end. Anus with prominent upper lip.

Males (based on 10 specimens): body 5mm (4-5mm), maximum width at esophageal/intestinal junction 260 μ m (167-300 μ m). Length of buccal capsule 132 μ m (125-150 μ m) \times 111 μ m (100-140 μ m). Maximum length/width ratio of buccal capsule 1:0.8. Muscular esophagus with a size of 512 μ m (487-540 μ m) \times 116 μ m (93-133 μ m). The esophagus represents 11% (10-13%) of the total body length. Length of entire esophagus and buccal capsule representing 14% (12-16%) of body length. Nerve ring, excretory pore and deirids at 347 μ m (317-367 μ m), 369 μ m (317-417 μ m) and 456 μ m (370-533 μ m), respectively, from anterior extremity. Spicules long, filiform, sub-equal and sclerotized 437 μ m (337-527 μ m) long, representing 9% (7-11%) of total body length. The parasite had dorsally curved and sometimes S-shaped points. Spoon-shaped sclerotized gubernaculum sclerotized in lateral view, with a length of 76 μ m (60-88 μ m). One pair of pre-bursal papillae present. Copulatory bursa well developed (Figures 3f, g, h), broader than long, supported by five rays emerging from a dorsal trunk: thick dorsal trunk, bifurcated from two thirds of the length, of which each sub-ray is trifurcated at the end (Figures 1h, i, 2). Bifurcated first arched rays (ventroventral and lateroventral), three slender and arched lateral rays with a common rod. The anterolateral, mediolateral and posterolateral rays merge at the base and then divide, projecting in parallel, to the edge of the bursa (Figure 1j). The externodorsal rays emerge from the trunk and project parallel to the edge of the bursa.

Discussion

The nematodes parasitizing the intestines of pigs, collected in the municipality of Moju, state of Pará, have similar characteristics to those of other species of the family Ancylostomatidae (Looss, 1905) and genus *Globocephalus* (Molin, 1861). Based on these morphological features, the nematodes were identified as *G. urosubulatus* (Alessandrini, 1909). Taxonomically the genus *Globocephalus* has undergone different classification proposals: *Cystocephalus* Railliet (1895), *Characostomum* Railliet (1902), *Crassisoma* Alessandrini (1909), *Raillietostromylus* (Lane, 1923) and *Globocephaloides* (Yorke & Maplestone, 1926), but today the denomination *Globocephalus* is accepted under the rules of zoological nomenclature.

Worldwide, there are few species proposed for *Globocephalus* and its well-defined biogeographic distributions: *G. longemucronatus* Molin, 1861 (Germany and Japan), *G. connorfilii* Lane 1922 (India, Samoa, China and Taiwan), *G. samoensis* (Lane, 1922) (Samoa, New Guinea, India, China and Japan), *G. sichuanensis* Wu 1984 (China), *G. amucronatus* (Smit & Noto-soediro, 1926) (Netherlands), *G. versteri* Ortlepp, 1964 (Africa), *G. madagascariensis* Chanaid 1966 (Madagascar), *G. urosubulatus* (Alessandrini, 1909) (Austria, Turkey, New Zealand, France, Germany, Bulgaria, Zaire, Iran, Guyana, Africa, New Guinea, India and America) (Ahn et al., 2015) and *G. marsupialis* Freitas & Lent, 1936 (America), the last two being recorded in Brazil (Vicente et al., 1997). *G. marsupialis* the latter being described as a parasite of the small intestine of *Philander opossum* (Linnaeus, 1758) (Syn. *Metachirops opossum*) (Mammalia: Didelphidae) in the State of Rio de Janeiro.

There are few taxonomic studies for the genus *Globocephalus*. Even the original descriptive articles have limitations in their morphological and morphometric data, which makes it necessary to update these data again (Lane, 1922; Cameron, 1924; Freitas & Lent, 1936), as in studies by Nanev et al. (2007) for *G. urosubulatus* collected from wild boars in Bulgaria and Ahn et al. (2015) to provide morphological features, and measurements of various parts of the adult nematodes by both light and scanning electron microscopies from *G. samoensis* in wild boars from South Korea.

This is the first study to describe ultrastructural aspects of *G. urosubulatus* in Brazil, in the world most studies for this species were based on light microscopy, but in this study the use of SEM made it possible to observe the mouth opening, distribution of cephalic papillae, deirids, vulva and a panorama of the male's tail, in addition to the presence of a pair of papillae present in the female's tail. According to Cesaroni et al. (2017) nematodes are small and usually have small external morphological characters, although the use of SEM has been used in taxonomic

and systematic publications for helminths, its use for large groups such as nematodes this technique is still limited, even if it allows for observation of details that light microscopy hardly detects.

In this study we present new morphological and morphometric data and the first record of *G. urosubulatus* recovered from pigs in the State of Pará. Different authors have reported the presence of this parasite in the same host in Brazil: Amazonas (Freitas & Costa, 1967), Goiânia and Distrito Federal (Carneiro et al., 1979, 1980), Rio de Janeiro (Francis, 1978) and Bahia (Pinto et al., 2007).

Species of *Globocephalus* are taxonomically characterized by the morphology and morphometry of the oral capsule, teeth, position of the vulva and the distribution of rays in the male copulatory bursa, as well as the morphology of the dorsal ray (Freitas & Lent, 1936; Ahn et al., 2015). For Nanev et al. (2007) characteristics such as body size, esophagus, nerve ring, length of specimens and gubernaculum used independently as taxonomic criteria for species identification are not reliable.

Based on these morphological features, the nematodes were identified as *Globocephalus urosubulatus*. In Brazil, only Francis (1978) presented morphometric data for this species recovered from the intestine of pigs in the state of Rio de Janeiro, the morphometry being very similar to this study, with only differences in the location of the vulva in females (3-5mm), while that for the specimens found in Rio de Janeiro, the location was closer to the cephalic region (1.73-2.91mm), which can also be observed in the study by Nanev et al. (2007) for female specimens of *G. urosubulatus* (2.20–2.40mm) in pigs in Bulgaria. However, the use of young females can justify such variation, which in this work we have resolved by presenting the morphometry of ovigerous females and juvenile females separately.

Globocephalus urosubulatus differs from *G. marsupialis*, in addition to the host group, in morphology and morphometry, especially the gubernaculum (*G. urosubulatus* = 60-88µm × *G. marsupialis* = 38-48µm) and spicules (*G. urosubulatus* = 337-527µm × *G. marsupialis* = 338-378µm), in addition to the size of the female esophagus and tail. For Freitas & Lent (1936), although the genus *Globocephalus* presents a small number of species, it presents difficulties regarding the literature described for the genus, especially due to the impossibility of studying the species, since Molin drawings have weak taxonomic arguments, which would hardly support the species distinction. Morphological and morphometric data for *G. urosubulatus* and *G. marsupialis* are presented in Table 1.

Morphologically the *G. urosubulatus* eggs observed in the womb presented an elliptical or round shape, shell single, thin, smooth and transparent and morulated embryo. Even though the description of the eggs of this species is presented, we do not believe that it is a strong character for differentiation or diagnosis of this parasite, since egg morphology is very similar to that of other species within the Ancylostomatidae. So far, no coprological diagnosis of *G. urosubulatus* has been made in Brazil, with most of the positive specimens obtained from necropsies (see Freitas & Costa, 1967; Francis, 1978; Carneiro et al. 1979, 1980; Vicente et al., 1997; Pinto et al., 2007). However, the records of occurrence and prevalence of *G. urosubulatus* by coprological examinations are also few in the literature: Permin et al. (1999) registered in Ghana (2.7% for eggs and 20% for adults after necroscopic examination); Dadas et al. (2016) in Mumbai demonstrated low prevalence (0.74%) of parasitism. Nwoha & Ekwurike (2011) registered in Abia State (Nigeria) 68% of the eggs of *Globocephalus* sp. in fecal samples from pigs of different ages. Stojanov et al. (2018) registered a prevalence of 30% of *Globocephalu* sp. in pigs in Serbia. Pinto et al. (2007) emphasize the importance of nematodes rarely reported in the parasite of pigs literature, especially in Brazil, and they use *G. urosubulatus* as an example, emphasizing that the conditions of the reproduction environment, especially the free-living ones, favor the maintenance of the parasites in the cycle.

Nejsum et al. (2012), states that in addition to the close similarity in the morphology of eggs of different species present and the same host, cases of cross-infection would not be detected by standard coprology methods, and mentions that studies based only on coprological exams, for example, may be the reason that the zoonotic potential of certain nematodes such as *T. suis* has been ignored so far.

Conclusion

This study describes the occurrence of *Globocephalus urosubulatus* adding data in light microscopy and scanning electron microscope, in addition to expanding the knowledge about this species and contributing to a better understanding of the diversity of nematodes in pigs in Brazil.

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