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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

A new geographic distribution and morphologic revision of *Mastophorus muris* (Nematoda: Spirurida), a parasite of the stomach of *Rattus norvegicus* in Rio de Janeiro, Brazil

Una nueva distribución geográfica y revisión morfológica de *Mastophorus muris* (Nematoda: Spirurida), un parásito del estómago de *Rattus norvegicus* en Río de Janeiro, Brasil

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Running Head: A new geographic distribution and morphologic revision of *Mastophorus muris* parasite of the *Rattus norvegicus.*

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ABSTRACT

This study provides a comprehensive morphological redescription of *Mastophorus muris* Gmelin, 1790, a spirurid nematode, based on specimens collected from *Rattus norvegicus* Berkenhout, 1769 in Nova Iguaçu municipality, Rio de Janeiro, Brazil. Employing optical and scanning electron microscopy, we detailed morphological characteristics, including previously unreported features such as a pair of ad-cloacal papillae and details of pseudolabia teeth. Additionally, we report a new geographical distribution for *M. muris* in Brazil. The low prevalence of *M. muris* observed in this study highlights the potential impact of anthropic changes on parasite distribution. Further investigations, including molecular analyses, are necessary to elucidate the taxonomic complexity and host-parasite relationships within the genus *Mastophorus*.

Keywords: Morphology – Nematoda – Rodents – Scanning electron microscopy – Spirurida – Taxonomy

RESUMEN

Este estudio proporciona una redescripción morfológica integral de *Mastophorus muris* Gmelin, 1790*,* un nematodo del orden Spirurida, basada en especímenes recolectados de *Rattus norvegicus* Berkenhout, 1769 en el municipio de Nova Iguaçu, Río de Janeiro, Brasil. Empleando microscopía óptica y electrónica de barrido, detallamos características morfológicas, incluidas características no reportadas previamente, como un par de papilas adcloacales y detalles de dientes del pseudolabio. Además, informamos de una nueva distribución geográfica para *M. muris* en Brasil. La baja prevalencia de *M. muris* observada en este estudio resalta el impacto potencial de los cambios antropogénicos en la distribución del parásito. Se necesitan investigaciones adicionales, incluidos análisis moleculares, para dilucidar la complejidad taxonómica y las relaciones huésped-parásito dentro del género *Mastophorus*.

Palabras clave: Microscopía electrónica de barrido – Morfología – Nemátodos – Roedores – Spirurida – Taxonomía

INTRODUCTION

The Spirocercidae Chitwood & Wehr, 1932 family comprises three subfamilies with a global distribution: Spirocercinae Chitwood & Wehr, 1932, encompassing eight genera, parasitizes mammals and birds; Ascaropsinae Alicata & McIntosh, 1933 including ten genera infecting mammals and lastly, Mastophorinae Quentin, 1970, containing the single genus *Mastophorus* Diesing, 1853 found in murid and microtid rodents, as well as various accidental hosts (Anderson, 2009; Bain *et al*., 2014). Actually, there is only one species belonging to this genus: *Mastophorus muris* Gmelin, 1790. This species was initially described as *Ascaris muris*, a parasite of the rat's stomach, by Gmelin (1790), lacking detailed description or illustrations.

This nematode parasitizes the stomach of the definitive hosts and presents an indirect life cycle with insects of the order Orthoptera, Diptera, Coleoptera and Siphonaptera acting as intermediate hosts (Grzybek *et al*., 2014). Insects get infected ingesting larvated eggs eliminated in the feces of the rats (Grzybek *et al*., 2014). The larva of first stage (L1) releases and invades the insect tissues developing into larva of third stage (L3), the infective stage for the definitive host (Lafferty *et al*., 2010). The definite host be infected by the ingestion of the intermediate host with infective larvae. Eggs eliminated in the feces of rodents are larval, ellipsoid-shaped, smooth, and thick-membraned (Rojas & Digiani, 2003).

Inconsistencies in the literature regarding *M. muris* descriptions and classification motivated this study. We employed optical and scanning electron microscopy to provide a comprehensive morphological redescription of the parasite based on specimens collected from *Rattus norvegicus* Berkenhout, 1769. Thus, the present study enhances morphological details of *M. muris* reporting a new geographical distribution obtained from the stomach of *R. norvegicus* from Nova Iguaçu municipality, Rio de Janeiro state, Brazil.

MATERIALS AND METHODS

Helminths were collected from *R. norvegicus* during a 1999 study on intestinal protozoa biodiversity in Nova Iguaçu, Rio de Janeiro, Brazil (22º45’35’’S,43º27’6’’W), resulting in the identification of *Eimeria nieschulzi* Dieben, 1924 and *E. separata* Becker & Hall, 1931 (Bomfim & Lopes, 1999).

Rodents were captured using Tomahawk traps (40.64 cm X 12.70 cm X 12.70 cm) and baits made with banana, sardines, peanut candy and oats. The traps were positioned along a 50-point trail in two distinct habitats located in the Fluminense microregion of Grande Rio (FIBGE, 1985). Captured *R. norvegicus* specimens were transported in plastic containers with access to food and water *ad libitum* to the Laboratory of the Experimental Station for Parasitological Research at the Institute of Biology, Federal Rural University of Rio de Janeiro. The rodents were euthanized in a CO_2 chamber and, following death, they were classified by sex and age according to Calhoun (1962). After that, necropsies were performed.

Nematodes recovered from the stomach were washed in saline, sodium chloride solution (NaCl 0.9%), fixed and conserved in AFA (2% acetic acid, 3% formaldehyde and 95% ethanol) and, posteriorly, clarified in lactophenol (40% lactophenol, 20% lactic acid and 20% phenol in 100 mL q.s.p.) for analysis in this study. Images were captured using Olympus™ BX51 binocular light microscope and the Olympus cellSens Standard software. All the morphological characters were measured in millimeters otherwise stated. Measurements were based on twenty specimens, seven males and thirteen females.

For scanning electron microscopy imaging, four specimens (two males and two females) were selected and fixed using 2.5% glutaraldehyde for one hour. Subsequently, the nematodes were washed and immersed in Na-cacodylate buffer solution and also washed. The specimens were then post-fixed in 1% osmium tetroxide and 0.1M Na-cacodylate for 3 hours at room temperature (Mafra & Lanfredi, 1998). The material was dehydrated using increasing ethanol series and dried with CO_2 using the critical point method. Finally, the mounting was performed using aluminum stubs and sputter coated with a 20 nm thick gold layer on silver cellophane. Thus, the specimens were examined using a Jeol JSM-6390 LV microscope with an accelerating voltage of 15kV at the Oswaldo Cruz Institute (Rudolf Barth Electron Microscopy Platform) in Rio de Janeiro, Brazil. Vouchers specimens were deposited in the Helminthology Collection of the Institute Oswaldo Cruz (CHIOC).

Ethic aspects: This study was conducted according to the Brazilians laws and approved by an ethics committee from Federal Rural University of Rio de Janeiro in 1999. The authors indicate that the guidelines of Brazilian Council for the Control of Animal Experimentation (CONCEA) and the Ethical Committee on the Use of Animals (CEUA) was employed.

RESULTS

TAXONOMIC SUMMARY

Species: Mastophorus muris Gmelin, 1971

Host: *Rattus norvegicus* (Berkenhout, 1769)

Locality: Nova Iguaçu, Rio de Janeiro state (22º45’35’’S,43º27’6’’W)

Site of infection: Stomach

Prevalence: 0.5% (1 rodent infected/ 173 rodents collected)

Intensity: 20 (13 females and 7 males)

Deposited: CHIOC number 39679

General: The nematodes are large, females being longer and thicker than males. Cephalic and body cuticle with transverse striation, males showing ornamentation in the posterior ventral region (Figure 2A). Anterior region composed of two lateral pseudolobias, each formed by three lobes (two submedian lobes slender and slightly rounded and one lateral larger and quadrangular in shape) (Figure 1A). A pair of cephalic papillae near to the external base of each submedian lobe and a porous structure present in submedian lobe (Figure 1B). Amphids at the base of the lateral lobe (Figure 1B). The internal margin of each lobe of the pseudolabia is armed with teeth variable in number (5 to 9), constantly presenting a larger and developed tooth in the center (Figure 1C). Teeth with pointed ends that can be either cleft in two or three cusps in different sizes in the distal region located in the internal border of the pseudolo no bia (Figure 1D, 4D). A rectangular, thick-walled mouth capsule, presence of two derids anterior to excretory pore and nerve ring near excretory pore (Figure 4A, B, E, F). The stoma elongated and cylindrical with thick walls (Figure 4C).

Males. Total body length 27 - 41 (32.7) (n= 7) and wide at mid-body 0.58 – 2.86 (1.08) (n=7). Nerve ring 0.35 – 0.54 (0.42) (n=7), excretory pore 0.41 – 0.57 (0.47) (n=7) and derids 0.29 – 0.47 (0.38) (n= 4) from the anterior end. Stoma 0.15 long and 0.07 wide (n=1). Oesophagus 6.18 length (n=1). Spicules are unequal, sclerotized, filiform with different sizes; right 0.834 long and 0.019 wide (n=1) elongated and blunt-ended and left 0.651 long and 0.018 wide (n=1) slightly smaller and tapered-ended. The length of the shorter spicule is 78.3 percent that of the longer one. Elongated tail with four pairs of pedunculated precloacal papillae and a single papilla at the anterior border of the cloaca (Figure 2A). One pair ad-cloacal papillae and two pairs pedunculated post-cloacal papillae (Figure 2B). Distal end of tail not ornamented with four pairs of sessile papillae and a pair of phasmid (Figure 3A). Gubernaculum present. Tail 0.31-0.51 (0.41) long (n=5).

Females. Total body length 27 - 96 (63) (n=11) and wide at mid-body 0.42 – 3.28 (1.75) (n=11). Stoma 0.17 long and 0.093 wide (n=2). Oesophagus 6.2 length (n=1). Nerve ring 0.29 – 0.93 (0.49) (n=11), excretory pore 0.4 – 0.66 (0.52) (n=11) and derids 0.54 –

0.59 (0.58) (n=4) at anterior end. Vulva at 11 - 31 (24) (n=5) from the anterior end (Figure 3B). Tail 0.28 – 0.74 (0.43) (n=11). Presence of a pair of phasmid in the tip of tail (Figure 3A). Eggs are elliptic with thick shell 41 - 53 µm (44.3 µm) long and 26 - 32 µm (29 µm) wide (Figure 3C).

DISCUSSION

The species belonging to the genus *Mastophorus* Diesing, 1853 are found mainly infecting rats belonging to the families Muridae and Microtidae (Anderson, 2000). This nematode has been reported parasitizing the rats *R. norvegicus* in Israel, Portugal and United States of America (USA) (Firlotte, 1948; Wertheim,1962; Quintal, 2022), *R. alexandrinus* Geoffroy, 1803 in Israel (Wertheim, 1962), *R. assimilis* Gould, 1858 in Australia (Smales, 1997), *R. rattus* Linnaeus, 1758 in New Zealand and Portugal (Charleston & Innes, 1979; Quintal, 2022), *Mus musculus* Linnaeus, 1758 in Lithuania, Serbia and Germany (Mažeika *et al*., 2003; Vukićević-Radić *et al*., 2007; Jost *et al*., 2024), *R. norvegicus* (Syn. *M. decumanus*) in India (Maplestone & Bhaduri, 1942), *Meriones persicus* Blanford, 1875 in Iran (Harandi *et al*., 2016), *Myodes glareolus* Schreber, 1780 in Poland and Germany (Gryzbek, 2014; Jost *et al*., 2024), *Arvicola amphibius* Linnaeus, 1758 in southern Sweden (Neupane, 2018), *Microtus miurus* Osgood, 1901 in Alaska (Haukisalmi *et al*., 1995), *Graomys griseoflavus* Waterhouse, 1837 in Argentina (Rojas & Digiani, 2003), *Geomys breviceps* Baird, 1855, *Peromyscus leucopus* Rafinesque, 1818, *Sigmodon hispidus* Say & Ord, 1825 and *Oryzomys palustris* Harlan, 1837 in USA (Erickson, 1944; Childs & Cosgrove, 1966) and *Apodemus flavicollis* Melchior, 1834 in Germany (Jost *et al*., 2024). In Brazil, *M. muris* was reported infecting *R. norvegicus* and *R. rattus* (Araújo, 1967, Vicente *et al*., 1997). Interestingly, despite this parasite being found majority in rodent species, this nematode was also reported in marsupials

Trichosurus vulpecula Kerr, 1792 and *Hypsiprymnodon moschatus* Ramsay, 1876 in Australia, *Dactylopsila trivirgata* Gray, 1858 in USA, in mustelid *Meles meles* Linnaeus, 1758 in Spain and in carnivores *Lynx pardinus* Temmink, 1827 in Spain, *Vulpes vulpes* Linnaeus, 1758 in Spain and China, *Vulpes ferrilata* Hodgson, 1842 in China and *Canis latrans* Say, 1823 in USA (Johnston & Mawson, 1938; Smales, 1995; Torres *et al*., 1998; Barbosa *et al*., 2005; Chen, 2022) and *Felis silvestris* Schreber, 1777 in Germany (Jost *et al*., 2024). The largest number of rodent species infected with *M. muris* likely acquire it through their diet. Ingestion of intermediate hosts, containing the parasite larvae, makes them susceptible. Additionally, carnivores can become infected by accidentally consuming intermediate or paratenic hosts.

The life cycle of *M. muris* is indirect with a great diversity of insects as intermediate hosts (Grybek *et al*., 2014), likewise, a large number of mammal species can be definitive hosts. The worldwide distribution of the helminth and different host taxa suggests low host specificity both definitive and intermediate. Goldberg & Bursey (2002) suggest that geckos *Hemidactylus turcicus* Linnaeus, 1758 and *H. mabouia* Moreau de Jonnes, 1818 could be paratenic host, since encysted larvae not yet studied have been found in the skeletal muscles of these animals in the same place where there was a high prevalence of the nematode in rodents (Lafferty *et al*., 2010), in fact, further research to elucidate the specific roles of various hosts within it is necessary.

In Brazil, *M. muris* infecting murids was reported as *Protospirura columbiana* Cram, 1926 by Araújo (1967) and Chiefi *et al.* (1980) in the city of São Paulo and as *P. muris* by Brito *et al.* (1969) in the city of Rio de Janeiro. Indeed, the taxonomic classification of the genus *Mastophorus* was historically confused with that of the genus *Protospirura* Seurat, 1914. The genus *Mastophorus* was described by Diesing in 1853, however, York & Maplestone (1926) considered that there was not enough information to create a new genus and relocated eight species of *Mastophorus* in the genus *Protospirura.* After, Chitwood (1938) considered *Mastophorus* a valid genus that differed from *Protospirura* by the arrangement of the pseudolabium teeth and, furthermore, subdivided the species *M. muris* into two varieties: *M. muris* var. *muris* and *M. muris* var. *ascaroides*. Read & Millemann (1953) disagreed with Chitwood's (1938) classification arguing that the chosen characteristics were only valid at the subgenus level. Consequently, they re-established *M. muris* as a distinct species, classifying it and removing it again from the genus *Protospirura*. Examining the morphology of *M. muris* and *P. muricola* larvae, Quentin (1970) confirmed the distinctness of the two genera in accordance to Chitwood (1938). Key differentiating features include the number of teeth in the pseudolabia, the shape of the stoma, the morphology and arrangement of pre- and post-cloacal papillae in males, the length of the male caudal wing, and the position of the vulva in females (Chitwood, 1938; Quentin, 1970). Actually, the synonyms for *M. muris* found in the literature are: *Protospira labiodentata* Linstow, 1899, *P. gracilis* Cram, 1924, *P. columbiana* Cram, 1926, *P. ascaroidea* Hall, 1916, *P. glareoli* Soltys, 1949, *P. marsupialis* Baylis, 1934 and *P. bestiarum* Kreis, 1953.

The species of the present study is *M. muris* by the following characteristics: five to nine teeth in each pseudolabia, long stoma, long tail and pedunculated papillae in males and a pre-equatorial vulva in females in accordance to Chitwood (1938) and Quentin (1970). Inconsistent descriptions of the anterior region, particularly regarding the highly variable tooth shapes, and inaccurate posterior region descriptions on the arrangement of pedunculated and sessile papillae, hindered the accurate classification of *Mastophorus* and *Protospirura* species (Jost *et al*., 2024). Recently, Jost *et al*. (2024) investigated the morphological and molecular variation within *M. muris*, emphasizing the importance of dentition analysis for parasite taxonomy. The study proposed tooth pattern formulas to differentiate *M. muris* specimens based on their hosts. *Mus* hosts were identified by the formula 1–(2 + n)–1–(2 + n)–1, where "n" represents the variable number of smaller denticles. Specimens from non-*Mus* hosts (as *Myodes*, *Rattus*, and *Felis*) shared the formula 1–(2 + n)–1, with a large central tooth and a variable number of smaller denticles. Finally, *Graomys* specimens exhibited a unique formula of 1–3–1–3–1, characterized by three smaller denticles between larger ones in the margin of each lobe as described by Rojas & Digiani (2003). Differently, Wertheim (1962) considered that the denticles would be serrated projections of the thin and flexible membrane covering the pseudolabium noting a high variability in both the number and shape of teeth within the lobes with no dental pattern. In most cases, was observed a centrally placed large tooth and a variable number of teeth, often bifid, juxtaposed to the large central tooth as in specimens of *R. assimilis* from Israel. Our findings are in agreement with Wertheim (1962) that the tooth arrangement on the margin of each lobe is asymmetrical. Notably, a central tooth is consistently present in each lobe, smaller teeth with pointed ends that can be either bifid (divided into two) or trifurcated (divided into three) and pointed teeth of varying sizes can be observed.

Wertheim (1962), Roja & Digiani (2003) and Jost *et al*. (2024) described four pairs of pre-anal and two post-anal pedunculated papillae along with a variable number of sessile papillae (1 to 7 pairs) at the end of the tail and a single papilla just anterior to the edge of the cloaca. Our observations confirm the presence of previously reported papillae, while we additionally identified a pair of ad-cloacal papillae not documented before. *M. muris* exhibits significant morphological and morphometric variation among isolates from different host species. Furthermore, genetic differences have also been identified between isolates, as evidenced by Jost *et al*. (2024). Despite our efforts, genetic material extraction from the samples proved unsuccessful. This limits our ability to contribute valuable insights into the genus' complexity. This study underscores the need for further investigation to clarify host-genus relationships and explore the possibility of reclassifying the parasite into distinct species or even a new genus identity.

The pathogenicity of *M. muris* in definitive hosts have been evaluated as mild pathology mainly related to low parasite burdens. This effect is likely due to the nematodes' consumption of gastric contents, leading to a decline in body condition (Lafferty *et al*., 2010). However, high parasite loads could result in regurgitation, signs of gastritis, obstruction of the gastrointestinal tract, and severe weight loss (Grzybek *et al.*, 2014).

The nematode *M. muris* is worldwide distributed and found in Eurasia, America, and Oceania (Rausch, 1951; Neupane *et al.*, 2018). Study of the prevalence of *M. muris* in *R. rattus* in an island with extensive vegetation cover and coconut trees Palmyra Atoll in the central Pacific Line Islands from North America showed a high prevalence with 59% (97/165) of hosts infected (Lafferty *et al*., 2010). In contrast, a low prevalence of 3.7% (21/567) was observed in *Mus musculus* collected in Germany (Jost *et al*., 2024). In Brazil, the prevalence of this stomach spirurid in studies of the helminth fauna of synanthropic rodents is very low with 2% (4/205) (Chieffi *et al*., 1980) or non-observed in some areas (Simões *et al*., 2016; Carvalho-Pereira *et al*., 2018). In fact, a low prevalence of 0.5% (1/173) was also observed in the present study. Probably, due to the low prevalence of this nematode in Brazil, it is difficult to find infected definitive hosts, moreover, anthropic changes could modify the natural habitats of the intermediate hosts leading to disappearance of parasites. Reinforcing the influence of habitat, Roberts *et al*. (1992)

demonstrated a strong correlation between helminth infections and specific environments. Their study on *Rattus exulans* Peale, 1848 in forests, pastures, and farms found the highest infection rates of the trematode *Brachylaima* sp. Dujardin, 1843 and the nematode *Calodium hepaticum* Bancroft, 1893 (syn.*Capillaria hepatica*), and *M. muris* in forested areas. This suggests that the greater abundance of arthropods, particularly those that create favorable microhabitats for intermediate host survival and reproduction, contributes to higher infection levels in these environments. The prevalence of *M. muris* infection in rodents can be influenced by several factors, such as age, sex, and reproductive status (e.g., adult rodents, mature or lactating females), can play a role (Lafferty *et al*., 2010; Grzybek *et al*., 2014). Additionally, extrinsic factors like season, local temperature, feeding habits, and food availability in the host's environment can also influence infection rates (Lafferty *et al*., 2010; Burlet *et al*. 2011).

This study provided a detailed morphological analysis adding new taxonomic features as a pair of adcloacal papillae and details of pseudolabia teeth for *M. muris* from *R. norvegicus* in Nova Iguaçu municipality, Rio de Janeiro state. Additionally, contributing with a new geographical distribution to *M. muris.* This refined characterization may contribute to future identifications and improve our understanding of the species. To gain a deeper understanding of diversity within the genus *Mastophorus*, integrating molecular characterization analysis would be highly suggested.

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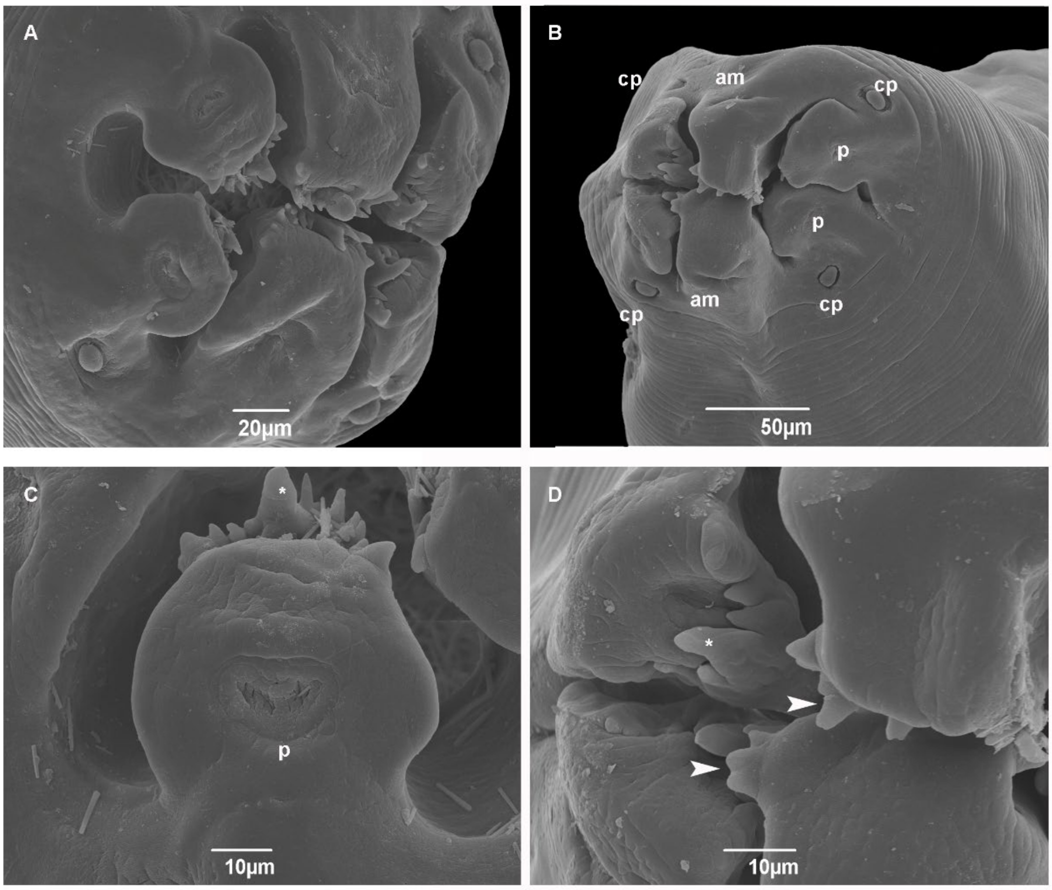

Figure 1. Scanning electron micrographs of *Mastophorus muris* **(**A) Front view from mouth opening. two triangular lateral lobes and square shape medium lobe. (B) Amphids at the base of the lateral lobe (am), a pair of cephalic pap papillae (cp) at the base of each pseudolabia and a porous structure (p) at each median lobe. (C) Lateral lobe; denticulated margin with a central developed teeth (*); porous structure (p). (D) Teeth of different shapes and sizes.

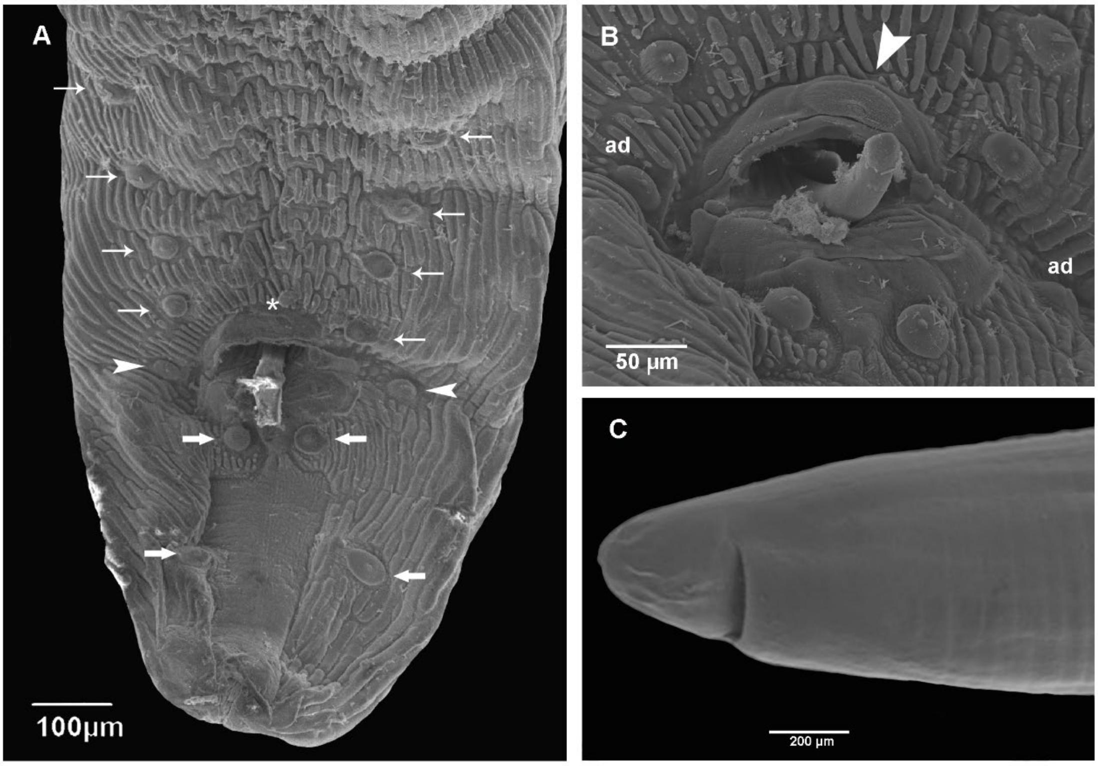

Figure 2. Scanning electron micrographs of *Mastophorus muris* showing ventral view from the posterior end. Male. (A) Four pairs of pedunculated precloacal papillae (arrows) and a single papilla anterior the cloaca (*). One pair ad-cloacal papillae (arrowhead) and two pairs pedunculated post-cloacal papillae (arrows). (B) A single papilla anterior the cloaca (arrowhead) and a pair ad-cloacal papillae (ad). (C) Posterior end female showing the anus.

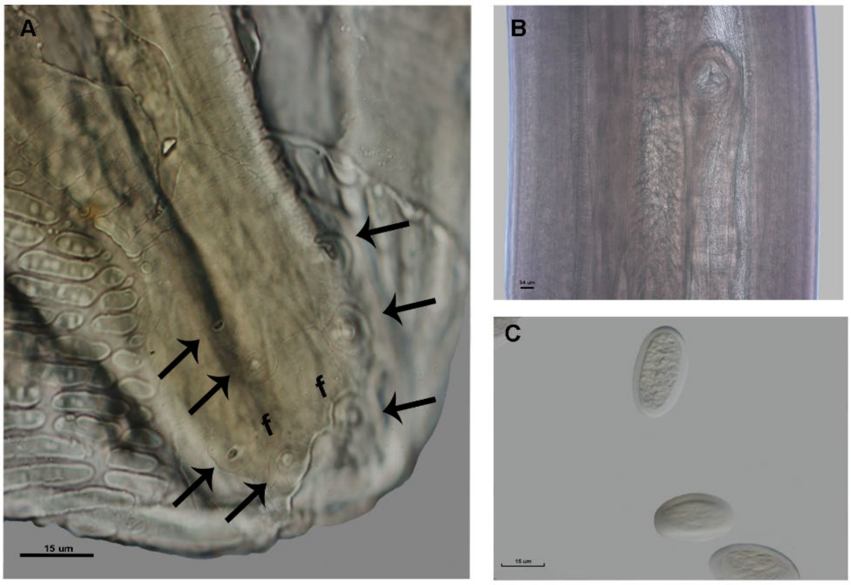

Figure 3. Light microscopy of *Mastophorus muris* (A) Ventral view from the distal end. Tail not ornamented with four pairs of sessile papillae (arrows) and a pair of phasmid (f). (B) Vulva. (C) Eggs.

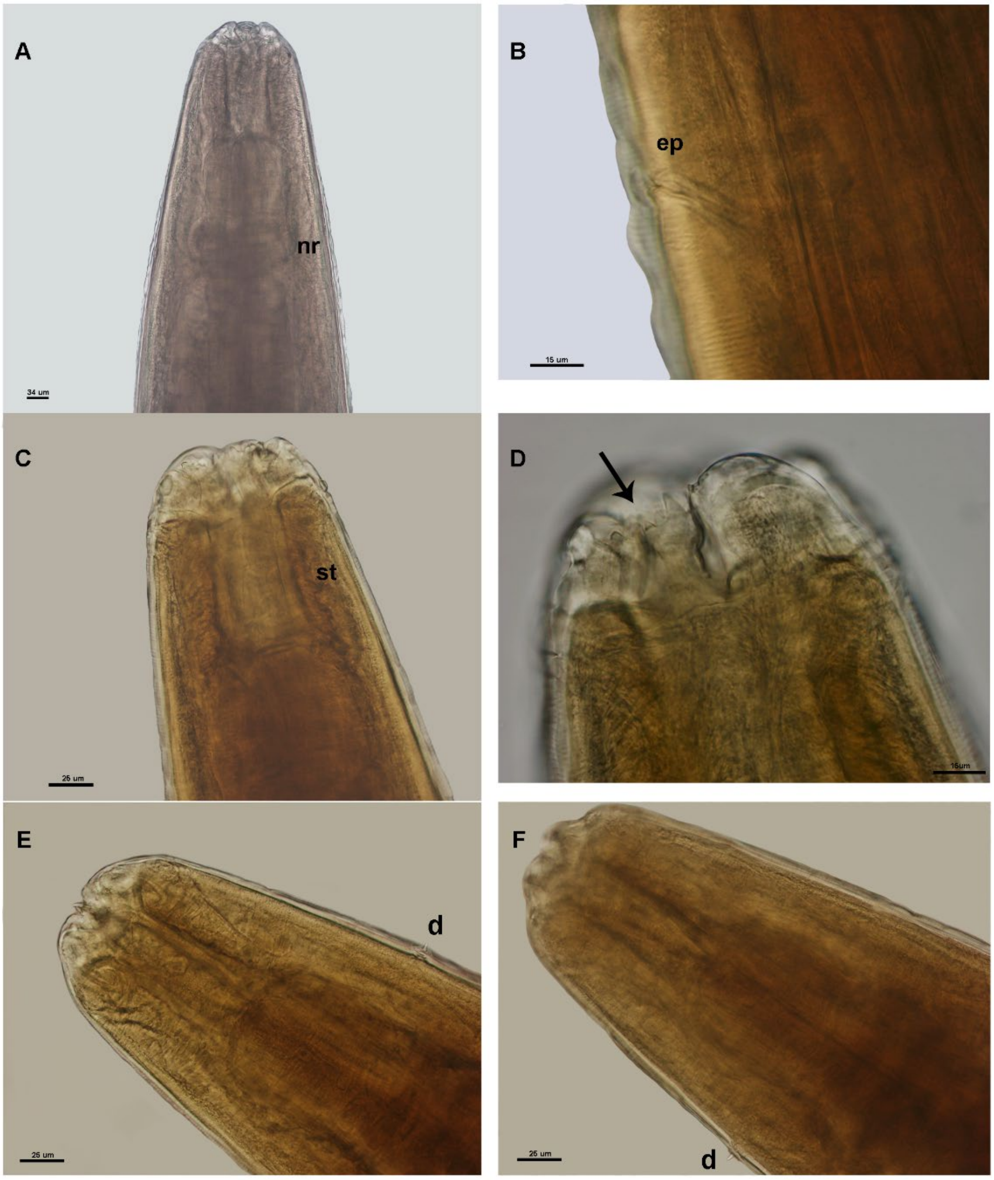

Figure 4. Light microscopy of *Mastophorus muris.* Anterior end of female. (A) Dorsal view. Nerve ring (nr). (B) Excretory pore (ep). (C) Dorsal view. Stoma (st). (D) Teeth of different sizes(arrow). (E, F) Anterior end showing left and right derids (d).