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# THE LUNGWORM, *HALOCERCUS BRASILIENSIS* (NEMATODA: PSEUDALIIDAE), FROM GUIANA DOLPHINS *SOTALIA GUIANENSIS* FROM BRAZIL WITH PATHOLOGICAL FINDINGS

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**KEY WORDS**

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*Halocercus brasiliensis*
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South America
*Sotalia guianensis*

**ABSTRACT**

The Guiana dolphin, *Sotalia guianensis,* is a small cetacean species found on the Atlantic coast of Central and South America from Honduras to Santa Catarina State, Brazil. The nematode *Halocercus brasiliensis* infects this cetacean, resulting in lung pathologies and death. The present study aimed to conduct a morphological and morphometric study of specimens of *H. brasiliensis* collected from *S. guianensis* from the coast of the state of Espírito Santo, Brazil. For this study, 7 dolphins were collected and examined for the presence of lung parasites. Nematodes were collected and processed for light microscopy, and lung fragments were processed for histological analysis. The nematodes were identified as *H. brasiliensis* according to their morphology and morphometry. The histopathological analysis revealed mineralization of the bronchiolar cartilage and inflammatory process. The parasitic infections by *H. brasiliensis* in *S. guianensis* can contribute to the debilitating status of these cetaceans, resulting in their stranding and accidental capture in fishing nets.

*Sotalia guianensis* (Guiana dolphin) is a small cetacean species that is found on the Atlantic coast of Central and South America from Honduras to Santa Catarina State, Brazil (Simões-Lopes, 1988; da Silva and Best, 1996). Brazil is home to the world's largest Guiana dolphin population, located in Sepetiba Bay, on the coast of the state of Rio de Janeiro (IBAMA, 2016). This species is at risk of extinction and is classified as near threatened according to the International Union for Conservation of Nature (Secchi et al., 2018). The species inhabits estuarine environments, mangroves, bays, and coastal areas (Borobia et al., 1991). The Guiana dolphin feeds mainly on teleost fish, mainly those from Clupeidae and Sciaenidae, although the diet also includes cephalopods and crustaceans (Flores and da Silva, 2009).

Parasitic infections in cetaceans can influence their behavior, population size, feeding, and community structure (Raga et al., 2002). Lungworms in cetaceans are responsible for highly debilitating conditions, affecting respiratory capacity and diving (Measures, 2001). In addition, chemical and noise pollution; vessel traffic, gill nets, and seines are listed as threats for these dolphins (Flores and da Silva, 2009).

The genus *Halocercus* was established by Baylis and Daubney in 1925 for nematodes collected from the bronchi of *Delphinus delphis*, named *Halocercus delphini* (Baylis and Daubney, 1925). The genus *Halocercus* comprises 12 species: *Halocercus delphini* Baylis and Daubney, 1925; *Halocercus lagenorhynchi* Baylis and Daubney, 1925; *Halocercus pingi* Wu, 1929; *Halocercus brasiliensis* Lins de Almeida, 1933; *Halocercus taurica* Delyamure, 1942; *Halocercus invaginatus* Quekett, 1841; *Halocercus kirbyi* Dougherty, 1944; *Halocercus dalli* Yamaguti, 1951; *Halocercus sunameri* Yamaguti, 1951; *Halocercus kleinenbergi* Delyamure, 1951; *Halocercus monoceris* Webster, Neufeld and MacNeill, 1973; and *Halocercus hyperoodoni* Gubanov, 1952, being frequently found in the bronchi and bronchioles of cetaceans (Measures, 2001). The species *H. brasiliensis* is reported only in *S. guianensis*. However, some studies identify nematode infections on the coast of South America only at the genus level. Specimens of the genus *Halocercus* were found in cetacean species on the Brazilian coast (Motta, 2006; Carvalho et al., 2010). Some studies identify these nematodes at the species level in *S. guianensis* (Santos et al., 1996; Melo et al., 2006; Carvalho et al., 2010; Marigo et al., 2010; Guimarães et al., 2015; Domiciano et al., 2016). However, these studies report only on the occurrence of this nematode parasitizing dolphins, without describing its morphology and morphometry.

There are few studies on the morphology and pathogenicity of lungworms from Brazilian cetaceans (Lins de Almeida, 1933; Guimarães et al., 2015). Thus, the present study aimed to describe the morphology, morphometry, and pathogenicity of *H. brasiliensis* from *S. guianensis* recovered on the Atlantic coast in the state of Espírito Santo, Brazil to contribute to the correct identification in future studies, highlighting its importance in cetacean health.

## MATERIALS AND METHODS

Seven Guiana dolphins (4 males and 3 females) were recovered dead along the coast of the state of Espírito Santo, Brazil (ICMBio license no. 64724-1) by a nongovernmental organization, the Instituto Organização Consciência Ambiental (ORCA), which develops activities for the conservation of marine species and ecosystems. Cetaceans were sent to ORCA where they were necropsied. The lungs were carefully examined for the presence of parasites. The nematodes that were found were washed in saline solution (0.09% NaCl), fixed in hot (70 C) alcohol–formalin–acetic acid (green liquor ethanol, 93%; formaldehyde, 5%; glacial acetic acid, 2%) overnight, transferred to a solution containing 70% ethanol and 5% glycerin, cleared with Amman's lactophenol (1 part distilled water, 2 parts glycerin, 1 part lactic acid, and 1 part phenolic acid), and mounted onto slides. The nematodes were then observed under a light microscope.

Measurements were performed to the nearest micrometer (mean ± SD; [range]) and were conducted on mature adult specimens (4 males and 7 females) and 20 larvae in utero from multiple animals. Measurements were conducted with an Axioplan Zeiss light microscope (Carl Zeiss, Oberkochen, Germany) equipped with a Canon Power-Shot A640 digital camera (Canon, Beijing, China), and the Zeiss AxionVision Sample Images Software (Carl Zeiss) was used for image analysis. Drawings were performed with the aid of an Axioplan Zeiss light microscope (Carl Zeiss) equipped with a camera lucida, and were digitized using the Adobe Photoshop Elements 8.0 software with the aid of an Intuos4 Wacon® pen tablet (Wacon Co. Ltd., Saitama, Japan). Representative specimens were deposited in the Helminthological Collection of Oswaldo Cruz Institute (CHIOC), Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

For histological examination, fragments of lungs were fixed in 10% formalin and then placed in paraffin. The fixed lung fragments were then sliced into 5- to 6-μm sections, stained with hematoxylin and eosin, and examined using a light microscope.

## RESULTS

### *Halocercus brasiliensis* Lins de Almeida, 1933

(Figs. 1, 2)

*Host: Sotalia guianensis* van Bénéden, 1864 (Cetartiodactyla, Delphinidae).

*Location:* Coast of the Espírito Santo State, Brazil.

*Dates of collection:* Between September and November 2017.

*Site in host:* Lung.

*Prevalence:* 100% (7 of 7 specimens examined).

*Mean intensity:* 19.3 (1–67 worms per host).

*Voucher:* CHIOC 38764.

*General morphology:* Long nematodes, relatively thick bodies, attenuated extremities. No clear difference in male and female specimen length. Nematodes yellow-whitish in vivo; cuticles contained transverse striations, more evident in anterior and posterior regions of the body.

*Males (on the basis of 4 complete specimens and 3 fragments):* Total body length 29,082 ± 16,842 (30,932–49,221) long by 448 ± 76.05 (382–470) wide (Table I). Simple esophagus (Fig. 1A) measured 257 ± 17.7 (232–277) long by 36 ± 14.6 (29–45) wide. Nerve ring (Figs. 1A, 2A) and excretory pore (Figs. 1A, 2A) at 103 ± 19.9 (72–131) and 152 ± 33.1 (128–175) (n = 2) from anterior end, respectively. Tail ventrally curved, membranous copulatory bursa, reduced (Figs. 1C, 2E), without divisions into lobules, measuring 74 ± 8.4 (68–80) long by 122 ± 3.6 (120–125) wide (n = 2), short and stout bursal rays ending in papillae, more evident than rays. Subequal spicules (Fig. 2H) displayed an inflation near the proximal end and a bifid distal end (Figs. 1E, 2H). Right spicule 694 ± 60.2 (605–782) long by 20 ± 2.6 (16–23) wide, measured at the middle of the distance between spicule head and spicular inflation, and 9 ± 0.8 (8.3–11) wide, measured near distal end, anteriorly to its bifurcation. Spicular inflation at 205 ± 25.2 (167–232) from proximal end (Fig. 2D), 42 ± 7.4 (31–50) long by 25 ± 2.9 (21–29) wide. Spicule head (Fig. 2D) well developed, 62 ± 11.1 (50–83) long by 32 ± 6.7 (23–41) wide. Left spicule 682 ± 42.2 (619–741) long by 20 ± 3.8 (14–26) wide, measured at region between spicule head and spicular inflation, and approximately 9 ± 1.4 (6–10) wide, measured near distal end, anterior to bifurcation. Spicular inflation 198 ± 29.9 (158–245) from proximal end, measuring 44 ± 10.5 (33–63) long by 25 ± 3.4 (20–29) wide. Spicule head measured approximately 61 ± 9.1 (43–70) long by 34 ± 6.8 (26–41) wide. Body width measured at spicule level, 376 ± 59.1 (259–451). Gubernaculum (Figs. 1D, 2G) well chitinized, poorly developed, discrete, approximately 105 ± 10.4 (92–124) long by 12 ± 2.1 (8–15) wide. Posterior extremity of body obtuse, with a subterminal large cloaca (Fig. 2H).

*Females (on the basis of 7 complete specimens):* Total body length approximately 28,312 ± 9,522.5 (27,399–55,005) long by 532 ± 78.7 (385–590) wide (Table II). Esophagus simple, 280 ± 35.2 (219–327) long by 40 ± 3.8 (33–45) wide. Nerve ring at 119 ± 25.5 (82–172) from anterior end. Excretory pore not observed on female specimens. Tail with obtuse termination, anus close to vulva (Fig. 2C). Anus (Fig. 2F) nearly terminally located at 20 ± 5.3 (12–28) from posterior end with smooth musculature and well-developed rectal glands. Vulva located immediately anterior to anus, at 35 ± 5.5 (24–43) from posterior end, with delicate, prominent labia. Vagina vera (Fig. 2F) short, rectilinear, parallel to intestine 147 ± 59.8 (85–277) long by 47 ± 10.3 (32–67) wide. Broad, oval, prodelphic ovejector (Fig. 2C) 415 ± 89.5 (321–645) long by 227 ± 57.6 (154–376) wide. Together, vagina and ovejector measured 566 ± 134.9 (414–919) long. Didelphic uterus (Fig. 2C) parallel, occupying almost all the body diameter, more sinuous anteriorly near oviduct. Eggs (Figs. 1F, 2I), containing a developed larva, 88.28 ± 11.27 (62.64–99.76) long by 56.28 ± 5.60 (45.41–66.12) wide, in utero (n = 10). Larvae (Fig. 1F, 2I) 191 ± 23.7 (152–221) long.

*Histopathology:* All analyzed dolphins were parasitized by *H. brasiliensis*, and 60% (4/7) presented pulmonary lesions such as emphysema, atelectasis, mineralization, and inflammatory process. Emphysema and atelectasis were observed associated in all

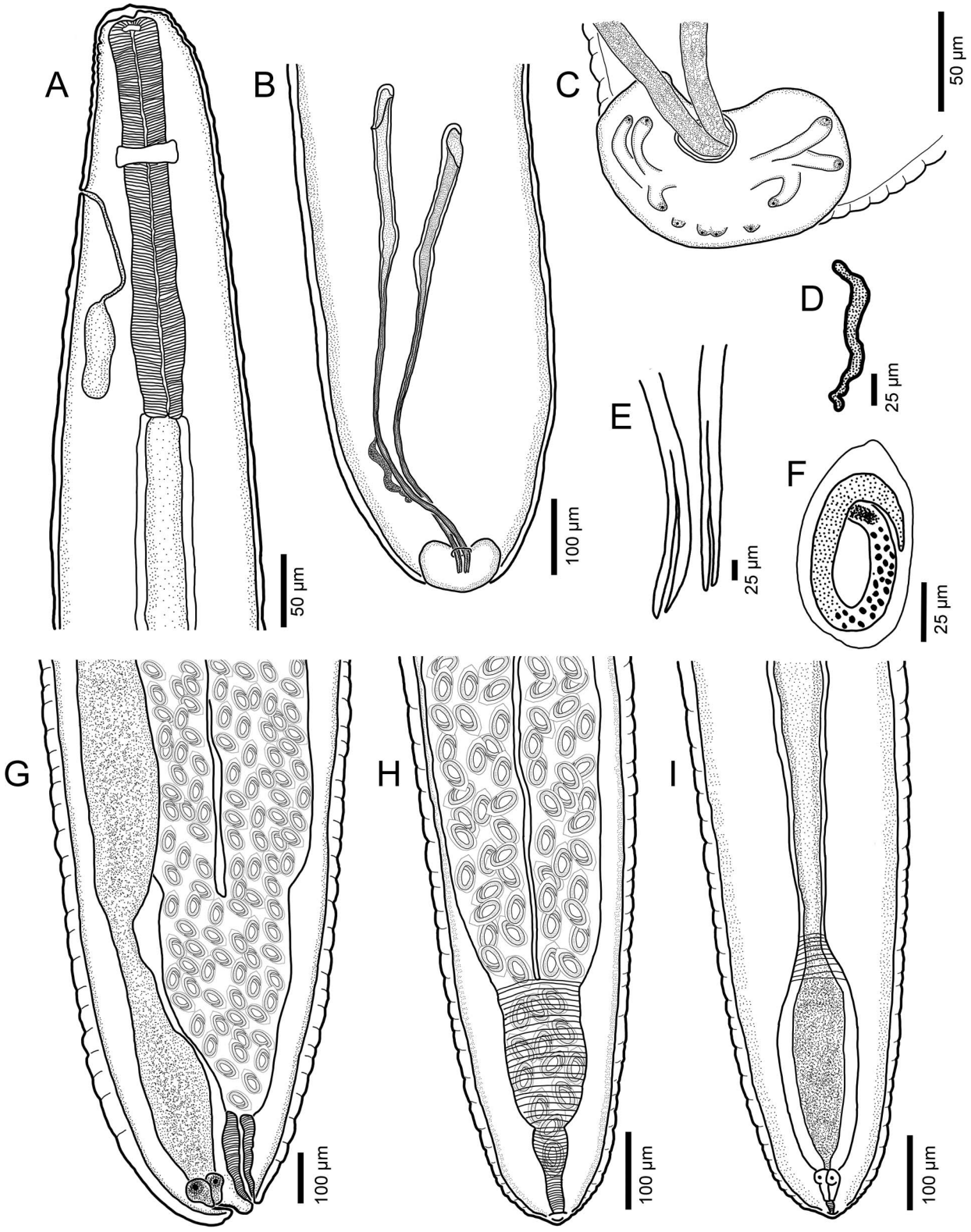


**Figure 1.** Drawings of specimens of *Halocercus brasiliensis* collected from the lungs of *Sotalia guianensis*. (**A**) Anterior region of the body. (**B**) Posterior region of a male. (**C**) Copulatory bursa. (**D**) Gubernaculum, lateral view. (**E**) Distal end of spicules. (**F**) Larvaed egg. (**G**) Posterior region of a female. (**H**) Posterior region of a female, showing the terminal part of the reproductive system. (**I**) Posterior region of a female, showing the terminal part of the digestive system.

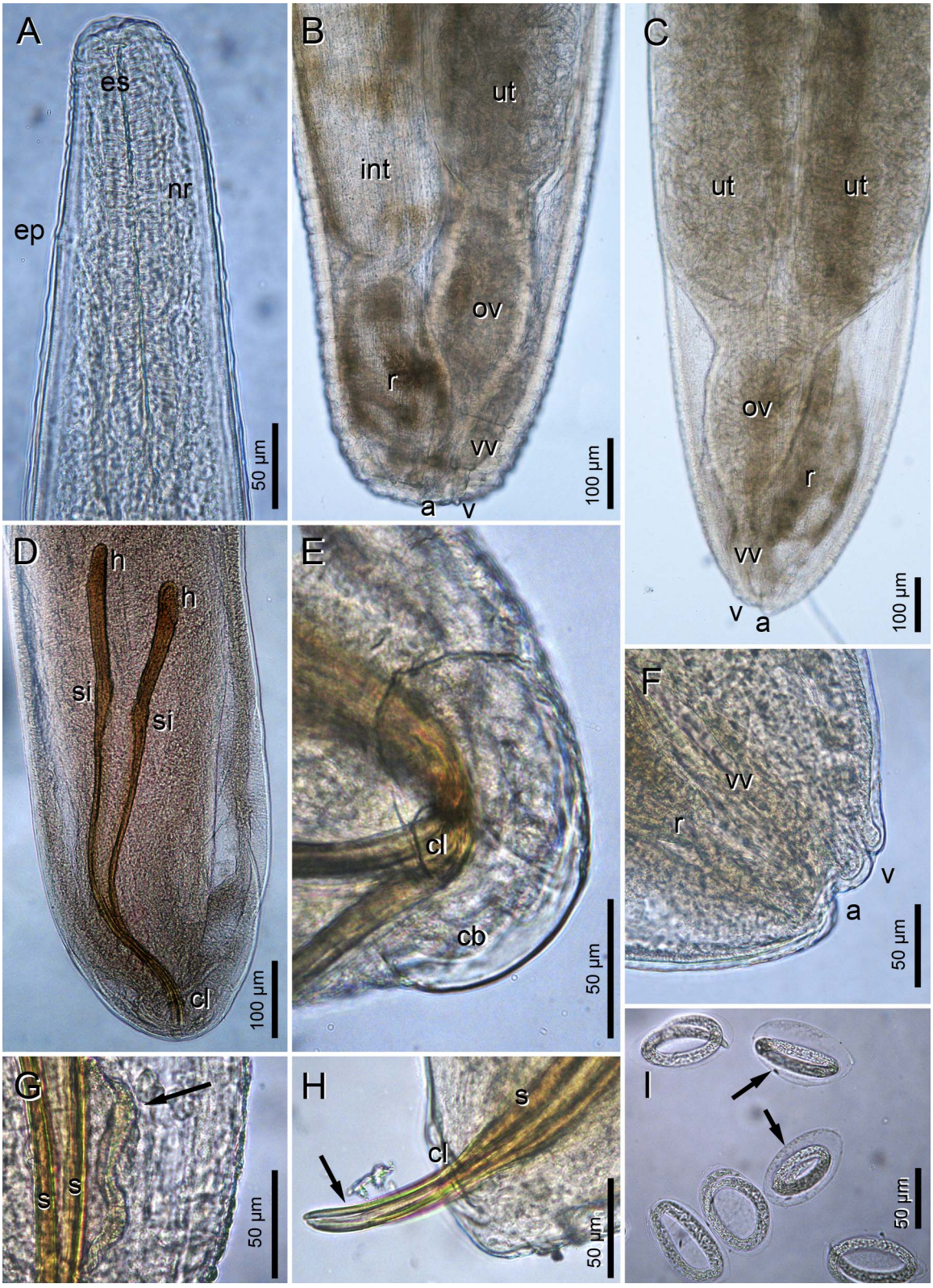


**Figure 2.** Light microscopy of specimens of *Halocercus brasiliensis*, collected from the lungs of *Sotalia guianensis*. (**A**) Anterior region of the body. (**B**) Posterior region of a female, lateral view. (**C**) Posterior region of a female, ventral view. (**D**) Posterior region of a male, ventral view. (**E**) Posterior end of a male. (**F**) Posterior end of a female. (**G**) Gubernaculum (arrow). (**H**) Distal end of spicules, showing bifid termination (arrow). (**I**) Eggs containing larvae inside (arrows). Abbreviations: a, anus; cl, cloaca; cb, copulatory bursa; ep, excretory pore; es, esophagus; h, spicule head; int, intestine; nr, nerve ring; ov, ovejector; r, rectum; s, spicule; si, spicular inflation; ut, uterus; vv, vagina vera; v, vulva. Color version available online.

**Table I.** Mean measurements (minimum and maximum values), in micrometers, of the males of *Halocercus brasiliensis*, parasite of *Sotalia guianensis*, from the coast of state of Espírito Santo, Brazil.

| Character | Present study (n = 7) | Lins de Almeida (1933) |
|---|---|---|
| Body total length | 29,082 (30,932–49,220) | 30,000–35,000 |
| Body width | 448 (382–597) | 390–420 |
| Esophagus length | 257 (232–277) | 220–240 |
| Esophagus width | 36 (29–45) | 28–37 |
| Nerve ring* | 103 (72–131) | 120–150 |
| Excretory pore* | 152 (128–175) | At the level of the esophageal distal end |
| Gubernaculum length | 105 (91–124) | 100–120 |
| Gubernaculum width | 12 (8–15) | 8–10 |
| Spicule length† | 688 (605–833) | 600–700 |

* Measured from the anterior end.
† Based on the mean of right and left spicules.

**Table II.** Mean measurements (minimum and maximum values), in micrometers, of the females of *Halocercus brasiliensis*, a parasite of *Sotalia guianensis*, from the coast of the state of Espírito Santo, Brazil.

| Character | Present study (n = 7) | Lins de Almeida (1933) |
|---|---|---|
| Body total length | 28,312 (27,399–55,005) | 45,000–52,000 |
| Body width | 532 (385–590) | 470–600 |
| Esophagus length | 280 (219–327) | 240–280 |
| Esophagus width | 40 (33–45) | 37–50 |
| Nerve ring* | 119 (82–172) | 120–150 |
| Excretory pore* | Not observed | At the level of the esophageal distal end |
| Vagina length | 147 (85–277) | 210–280 |
| Ovejector length | 415 (321–645) | 230–320 |
| Set: vagina + ovejector | 566 (414–919) | 440–600 |
| Vulva† | 35 (24–43) | 70–96 |
| Anus† | 20 (12–28) | 30–40 |
| Larvae | 191 (152–221) | 210–280 |

* Measured from the anterior end.
† Measured from the posterior end.

cases. Pulmonary edema and thickening of the septum and alveolar muscles (hypertrophy due to overload) were also observed, as well as a decrease in alveolus size due to the thickening of the alveolar wall that was observed in almost all the sites examined, characterizing a massive organ. The mineralization of bronchiolar cartilage was another observed process (Fig. 3A). A focal inflammatory process with mononuclear predominance was also observed (Fig. 3B).

## DISCUSSION

All of the dolphin specimens analyzed were parasitized by *H. brasiliensis*, indicating that this parasite commonly infects these animals. *Halocercus brasiliensis* is a parasite of the superfamily Metastrongyloidea, characterized by grouping members of the species that contain atrophied and a reduced copulatory bursa (Figs. 1C, 2E). Studies on this species of nematode are scarce in the scientific literature. This species was described by Lins de Almeida (1933), and since then, there have been no additional studies on its morphology and morphometry, with studies only reporting its occurrence in marine cetaceans (Santos et al., 1996; Melo et al., 2006; Carvalho et al., 2010; Marigo et al., 2010; Guimarães et al., 2015; Domiciano et al., 2016).

When comparing the measurements of the males and females of *H. brasiliensis* from the present study with those reported by Lins de Almeida (1933), these data were similar, with only a slight difference in measurements of the distance from the vulva and the anus to the posterior extremity (Table II), which were smaller in our study, most likely due to a slight contraction of the posterior region of the parasite's body.

The study from Lins de Almeida (1933) affirms the presence of a visible excretory pore, located at the end of the esophagus. However, this structure was only observed in 2 male specimens, distinctly located posteriorly to the nerve ring (Figs. 1A, 2A). Lins de Almeida (1933) only illustrated the posterior region of a male

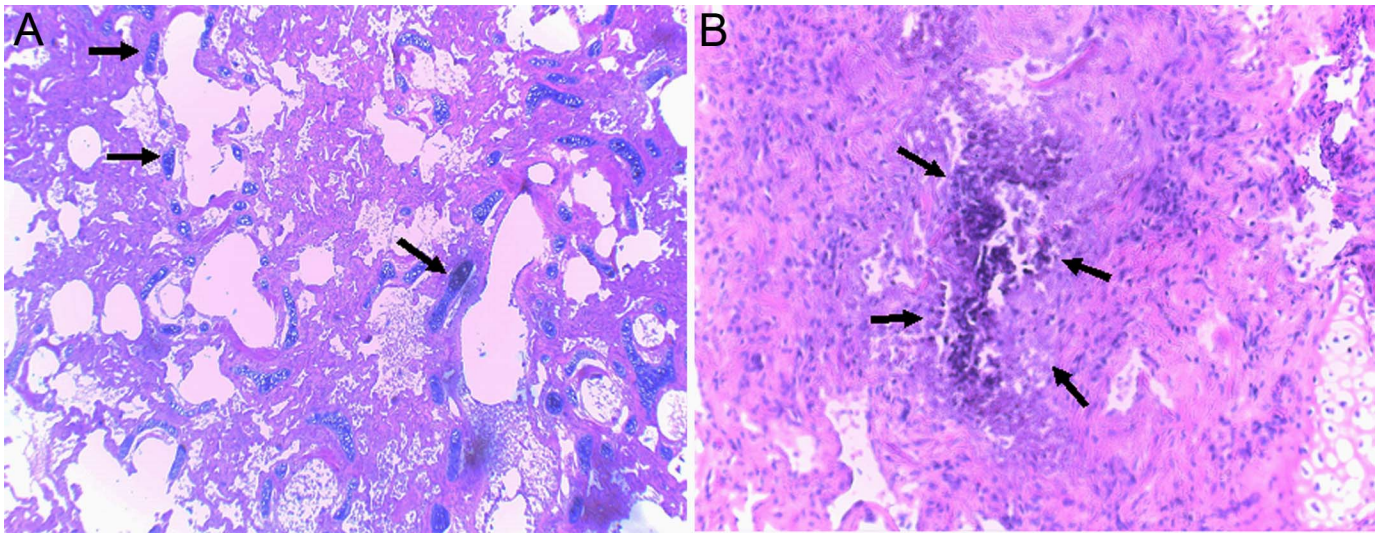


**Figure 3.** Light microscopy of histological findings on lungs of *Sotalia guianensis* infected by *Halocercus brasiliensis*. (**A**) Mineralization of bronchiolar cartilage (arrows). (**B**) Focal inflammatory process with mononuclear predominance (arrows). Magnification ×100. Color version available online.

specimen, without illustrating the excretory pore and without informing the distance of the excretory pore from anterior end. Instead, only its location as a visible circular excretory pore at the end of the esophagus was described. We and Lins de Almeida (1933) did not observe the cervical papillae.

Dougherty (1944) stated that the disposition of the female reproductive system, didelphic and prodelphic, i.e., the distance that the tubes of the female reproductive system run along the female body, extending to near the anterior region or narrowing, reaching the median region of the body, is a taxonomically important characteristic in the genus *Halocercus*. In the specimens of the present study, the characteristics of the female reproductive system agreed with those described by Dougherty (1944), as well as the tubular spicules with a bifid distal end (Figs. 1E, 2H) and a visible inflation near the proximal end of the spicules (Figs. 1B, 2D), which reinforces the diagnosis of *H. brasiliensis* infecting the respiratory system of the dolphins of the coast of the state of Espírito Santo.

Lins de Almeida (1933) mentioned in his work that the species *H. brasiliensis* may be viviparous because of larvae inside the uterus of females. However, when dissecting a female and exposing the uterine contents, we observed the presence of larvaed eggs containing a thin membranous shell (Figs. 1F, 2I), opposing the hypothesis of Lins de Almeida (1933). The lengths of the larvae of our study were similar to those described by Lins de Almeida (1933), as shown in Table I.

In the original description of *H. brasiliensis* by Lins de Almeida (1933), the author only illustrated the posterior region of the male. Although the morphology and morphometry of the females were described in the text, they were not illustrated in that study. Therefore, to complement the study of Lins de Almeida (1933), we expanded the knowledge of this species of parasite with additional data on its description as well as illustrations of optical microscopy, drawings of males and females, and illustrations on the anterior region of the nematode (Figs. 1A, 2A) to contribute to the identification of this species in future studies.

### Histopathology

In our study, lesions similar to those observed by Marigo et al. (2010), who found moderate lesions in 80% (4/5) of the examined lungs, were observed in approximately 60% of the examined lungs. These authors related the presence of infections by *H. brasiliensis* in *S. guianensis* to parasitic pneumonia in 40% of the specimens analyzed, which may have resulted in death. The genus *Halocercus* contains the most common nematodes found in odontocete and is considered pathogenic (Measures, 2001; Rodrigues et al., 2018).

In studies with the dolphin *Tursiops truncatus*, Fauquier et al. (2009) detected the occurrence of pulmonary atelectasis caused by *H. lagenorhynchi* infections. This same pathology was observed in our study on *S. guianensis* infected with *H. brasiliensis*, where the pathology associated with pulmonary emphysema was also observed in some specimens. These pathologies may have led to pulmonary overload, with the appearance of thickening of the septum and muscles beyond the alveolar wall and, consequently, the reduction of the alveoli, characterizing the organ as massive. The mineralization of bronchiolar cartilage was also reported by Guimarães et al. (2015), in *H. brasiliensis* infections in *S. guianensis*. The nematode family Pseudaliidae can cause severe lung infections in cetaceans. *Halocercus* is known to cause injury, with cases of pneumonia and bronchitis observed (Geraci and St. Aubin, 1987).

Although we cannot affirm that the cause of death of the dolphins from our study was due to parasitism, it may contribute to the decline of these animals, as Ruoppolo (2003), when studying the cause of death of the species *Sotalia fluviatilis*, reported that parasitic pneumonia by *H. brasiliensis* was the main cause of dolphin death; lung lesions may weaken the animal and may lead to the death of cetaceans (Rodrigues et al., 2018). Symptoms and lesions caused by pulmonary nematodes may depend on the severity of the infection (Arbelo et al., 2013). According to Measures (2001), contaminants, infectious diseases, and stress make odontocetes more prone to acquiring severe lung diseases from Pseudaliid infection. The present study contributes to the differentiation of *H. brasiliensis* from other species of the genus.

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All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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