

# Comparative morphology of the species of *Libyostrongylus* and *Codiostomum*, parasites from ostriches, *Struthio camelus*, with a identification key to the species

Morfologia Comparativa das espécies de *Libyostrongylus* e *Codiostomum*, parasitas de avestruzes, *Struthio camelus*, com uma chave para identificação das espécies

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## Abstract

One of the most common problems in breeding of ostriches in captivity is the control of parasitic diseases. This work presents keys for the identification of adult nematodes and infective larvae by morphologic and morphometric characteristics. These keys will allow the scientific community to identify the species that infect the ostriches either based on the characteristics of the posterior end of the infective larvae found through a simple fecal exam or by observing the morphology and morphometry of adult worms recovered during necropsies. These keys will facilitate ecological and systematic studies, as well as increase the understanding of the epidemiology of these parasitosis in ostriches.

**Keywords:** Ratites, Nematoda, Strongylida, infective larvae.

## Resumo

Um dos problemas mais comuns na criação de avestruzes em cativeiro é o controle das doenças parasitárias. Este trabalho apresenta chaves para a identificação de Nematoda adultos e larvas infectantes através de caracteres morfológicos e morfométricos. Essas chaves de identificação permitirão à comunidade científica o diagnóstico das espécies que infectam as avestruzes com base nas características da extremidade posterior das larvas infectantes encontradas por meio de simples exames fecais ou pela observação da morfologia e morfometria dos espécimes adultos recuperados durante necropsia. Dessa forma, as chaves de identificação facilitarão os estudos ecológicos e sistemáticos, bem como a melhor compreensão da epidemiologia dessas infecções em avestruzes.

**Palavras-chave:** Ratitas, Nematoda, Strongylida, larva infectante.

The ostriches, *Struthio camelus* Linnaeus, 1758 are birds belonging to the group of the ratites. These birds originated from Africa, and currently, their commercial breeding has gained economic importance worldwide due to the ability of these birds to adapt to different environments and their lucrative agricultural potential (TULLY; SHANE, 1996). One of the most common problems in breeding ostriches in captivity is the control of parasitic diseases, particularly those caused by parasites that have a direct lifecycle (HUCHZERMEYER, 2005). A diverse helminthic fauna has been reported in ostriches (Table 1), of these, the nematodes are important organisms that limit the development of the ostrich flock.

In 1880, small nematodes were collected from an ostrich proventriculum in South Africa by Arthur Douglass and sent to Dr. Cobbold, who identified the worms and named the new species *Strongylus douglassii* in honor of their collector (COBBOLD, 1882). Later, Lane (1923) established the genus *Libyostrongylus* for some African Trichostrongylidae, where *L. douglassii* is the type species from ostriches and *L. hebreanicutus* Lane, 1923, as the type species for gorillas. Gilbert (1937) described a new species *L. magnus*, being found to-date only in ostriches from the African continent, without recent reports in the world. In this species, unlike others of this genus, the females are smaller than males. *Libyostrongylus douglassii* and *L. hebreanicutus* differ in the structure of the terminal bifurcations of the dorsal ray and in the pattern of lateral rays (rays 3 to 5) of the copulatory bursa. Ortlepp (1939) established the genus *Paralibyostrongylus*; being *P. vondwei* Ortlepp, 1939, the type species and *P. hebreanicutus*, and *P. nigeriae* Baylis, 1928

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**Table 1.** Main parasites reported parasitizing ratites (ostrich, *Struthio camelus*; Rhea, *Rhea americana*; Emu, *Dromaius novaehollandiae*; Cassowary, *Casuaris casuarius*; Kiwi, *Apterys* spp.).

Parasites	Site of Infection	Host	Author (s)
<b>Protozoa</b>			
<i>Balantidium</i> sp.	Caecum/Intestine	Ostrich, Rhea	1, 2
<i>Cryptosporidium</i>	Intestine	All	1, 3, 4
<i>Eimeria</i> spp.	Intestine	All	1, 3
<i>Entamoeba</i> sp.	Caecum/Intestine	Ostrich, Rhea	1
<i>Giardia</i>	Caecum/Intestine	Ostrich, Rhea, Emu	1, 3
<i>Histomonas meleagridis</i>	Liver	Ostrich, Rhea	1, 3, 5
<i>Isospora</i> spp.	Intestine	Ostrich	1, 3
<i>Leucocytozoon struthionis</i>	Blood	Ostrich	3
<i>Plasmodium struthionis</i>	Blood	Ostrich	3
<i>Pleuromonas</i>	Large Intestine	Ostrich, Rhea	1
<i>Tetratrichomonas</i>	Caecum/Intestine	Ostrich, Rhea	1
<i>Toxoplasma gondii</i>	CNS	All	3
<i>Trichomonas</i>	Esophagus	Ostrich, Emu	3
<b>Trematoda</b>			
<i>Fasciola</i> sp.	Liver	Rhea, Emu	6, 7
<i>Philophthalmus gralli</i>	Eyes	Ostrich	3
<i>Philophthalmus aweerensis</i>	Eyes	Rhea	8
<b>Cestoda</b>			
<i>Chapmania tauricolis</i>	Small Intestine	Rhea	9
<i>Davainea</i> spp.	Small Intestine	Rhea, Emu, Cassowary	3
<i>Houttuynia struthionis</i>	Small Intestine	Ostrich, Rhea	1, 3, 10
<i>Monoecocestus</i> sp.	Small Intestine	Rhea	11
<i>Raillietina</i> spp.	Intestine	Emu	12
<b>Nematoda</b>			
<i>Ascaridia orthocerca</i>	Intestine	Rhea	3
<i>Baylisascaris</i> sp.	CNS (Larvae)	Ostrich, Rhea	3
<i>Capillaria</i> spp.	Intestine	Ostrich, Rhea	1, 13
<i>Chandlerella quiscalis</i>	CNS (larvae)	Emu	3
<i>Codistomum struthionis</i>	Caecum	Ostrich	3, 14
<i>Cyathostoma variegatum</i>	Lungs/Trachea	Emu	15
<i>Cyrnea colini</i>	Proventriculum/Gizzard	Ostrich	3
<i>Deletrocephalus dimidiatus</i>	Large Intestine	Rhea	3, 11, 16, 17, 18
<i>Deletrocephalus cesarpintoii</i>	Large Intestine	Rhea	3, 9, 17, 19
<i>Dicheilonema rhae</i>	Connective Tissue	Ostrich, Rhea	3, 20
<i>Dromastrongylus bicuspis</i>	Small Intestine	Emu	3
<i>Habronema incerta</i>	Proventriculum/Gizzard	Rhea	3
<i>Libyostrongylus</i> spp.	Proventriculum/Gizzard	Ostrich	1, 3, 21, 22, 24
<i>Odontospirura cetiopenis</i>	Proventriculum/Gizzard	Rhea	25
<i>Paradeletrocephalus minor</i>	Large Intestine	Rhea	17, 26
<i>Paranchocerca struthiononus</i>	Lungs	Ostrich	3
<i>Sicarius uncinipenis</i>	Gizzard	Rhea	9, 27
<i>Sicarius waltoni</i>	Gizzard	Rhea	27
<i>Struthiofilaria megalcephala</i>	Thoracic cavity	Ostrich	3
<i>Syngamus trachea</i>	Trachea	Ostrich, Rhea, Emu	3
<i>Torquatoides crotophaga</i>	Gizzard	Rhea	9
<i>Vaznema zschokkei</i>	Proventriculum	Rhea	27

Authors: <sup>1</sup>Ponce Gordo et al. (2002); <sup>2</sup>Ederli and Oliveira (2008); <sup>3</sup>Foreyt (2005); <sup>4</sup>Oliveira et al. (2008); <sup>5</sup>Dhillon (1983); <sup>6</sup>Soares et al. (2007); <sup>7</sup>Vaughan et al. (1997); <sup>8</sup>Schuster (2011); <sup>9</sup>Zettermann et al. (2005); <sup>10</sup>Pintori et al. (2000); <sup>11</sup>Giossa et al. (2004); <sup>12</sup>O'Callaghan et al. (2000); <sup>13</sup>Railliet and Henry (1911); <sup>14</sup>Ederli et al. (2008c); <sup>15</sup>Rickard et al. (1997); <sup>16</sup>Monteiro et al. (2002); <sup>17</sup>Freitas and Lent (1947a); <sup>18</sup>Travassos (1933); <sup>19</sup>Vaz (1936); <sup>20</sup>Vakarenko and Sharpilo (2000); <sup>21</sup>Ederli et al. (2008); <sup>22</sup>Ederli et al. (2008a); <sup>23</sup>Bonadiman et al. (2006); <sup>24</sup>Hoberg et al. (1995); <sup>25</sup>Wehr (1934); <sup>26</sup>Acomolli et al. (2006); <sup>27</sup>Freitas and Lent (1947b).

included in this new genus (HOBERG et al., 1995). Hoberg et al. (1995) described a third species, named *L. dentatus* Hoberg, Lloyd and Omar, 1995, due to the presence of prominent esophageal teeth. Thus, the genus *Libyostrongylus* is actually comprised of 3 species: *L. douglassii*, *L. magnus* and *L. dentatus*. Of these, the most common species is *L. douglassii*, which has been reported in South African flocks (FOCKEMA et al., 1985; MALAN et al., 1988; REINECKE, 1983); Australia (BARTON; SEWARD, 1993; BUTTON et al., 1993; MORE, 1996); the United States (HOBERG et al., 1995); Italy (PINTORI et al., 2000); Scotland (PENNYCOTT; PATTERSON, 2001); Spain, Belgium, Portugal and the Netherlands (PONCE GORDO et al., 2002); Sweden (JANSSON et al., 2002); New Zealand (MACKERETH, 2004; MCKENNA, 2005); Zimbabwe (MUKARATIRWA et al., 2004) and Brazil (BONADIMAN et al., 2006; EDERLI et al., 2008a, b). There have been few reports of 2 other species of the genus *Libyostrongylus*. *Libyostrongylus magnus* has been reported only in South Africa (GILBERT, 1937) and *L. dentatus* only in the United States (HOBERG et al., 1995) and Brazil (BONADIMAN et al., 2006; EDERLI et al., 2008a, b). Because most reports are based only on fecal exams, the low reported rate of infections by these 2 species is probably due to errors in diagnosis because of the similarities in the infective larvae.

Data about the life cycle of the genus *Libyostrongylus* refer only to *L. douglassii*, which has a direct life cycle typical of Trichostrongylidae, with a pre-patent period of approximately 36 days (THEILER; ROBERTSON, 1915) (Figure 1). The eggs are shed in the feces of the infected hosts and develop in the environment into larvae of first (L<sub>1</sub>), second (L<sub>2</sub>) and third (L<sub>3</sub>)

stages (infective larvae). This development occurs at a minimum temperature of 7 to 10 °C and maximum of 37 °C. Infective larvae display negative geotropism, tending to climb to the tip of vegetation through the moisture layer (MCKENNA, 2005). Under optimal temperature conditions (36 °C), the development to infective larvae occurs in approximately 60 hours (BARTON; SEWARD, 1993). Ostriches become infected by the ingestion of the infective larvae, which develops into the fourth larval stage (L<sub>4</sub>) within the proventricular glands in a period of 4 to 5 days. After approximately 20 days, these larvae develop into juvenile larvae. After the maturation process, copulation occurs and the females release eggs in the proventriculum approximately 33 days after the infection. It takes about 4 days for eggs appear in the feces of the host (THEILER; ROBERTSON, 1915).

Another species often cited as a parasite from ostriches, although with few reports, is *Codiostomum struthionis*. It was originally described as *Sclerostoma struthionis* by Horst in 1885 (POPOVA, 1955). In 1911, Railliet and Henry reclassified this species, and established the genus *Codiostomum*, being *C. struthionis* the type species and the only species in the genus (RAILLIET; HENRY, 1911). Since that time, there have been an extremely small number of studies regarding this nematode. However, this species is cited in several scientific publications describing the similarity of its eggs with that of the genus *Libyostrongylus* (BARTON; SEWARD, 1993; BLACK, 2001; PENNYCOTT; PATTERSON, 2001; SOTIRAKI et al., 2001; PONCE GORDO et al., 2002; HUCHZERMAYER, 2002, 2005; MCKENNA, 2005; TIŠLJAR et al., 2007).

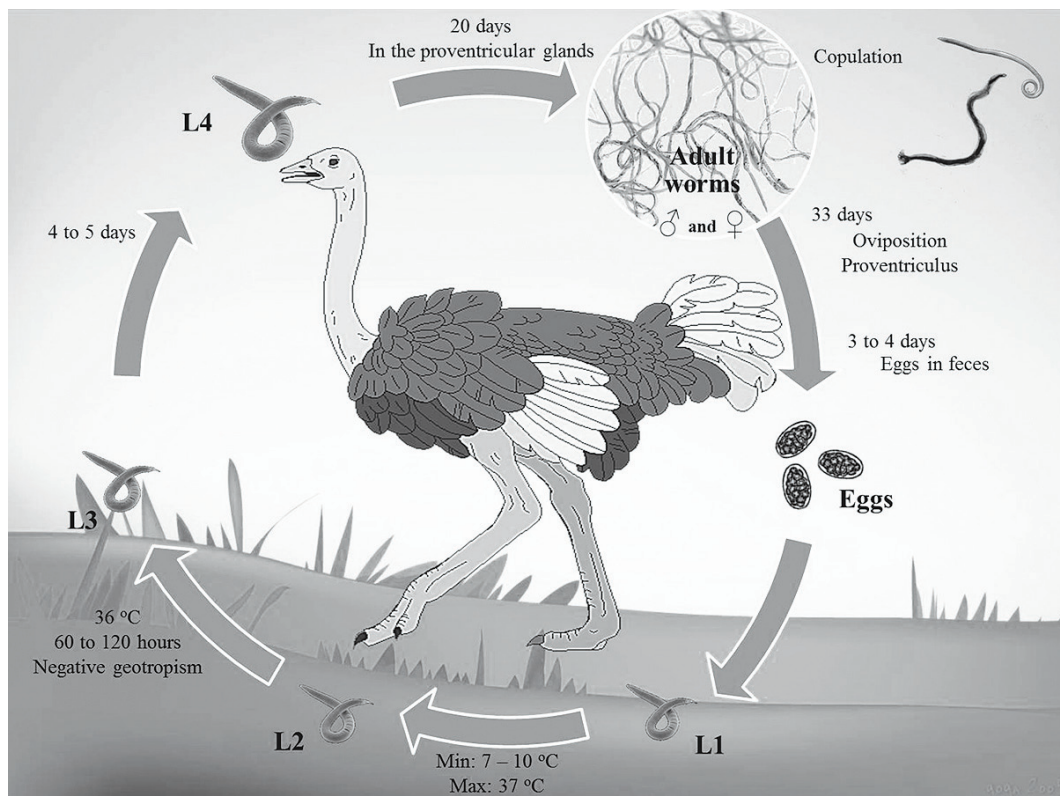


Figure 1. Life cycle of *Libyostrongylus* spp., according to Theiler and Robertson (1915).



The life cycle of *C. struthionis* has not been determined. Oliveira et al. (2009) believe that these nematodes have a direct life cycle and the larvae migrate into the host tissue, appearing in the nodules found along the cecum of infected birds, however, this fact has not been confirmed.

According to Ponce Gordo et al. (2002), the occurrence of *C. struthionis* is often overlooked in Europe due to a possible confusion in the diagnosis by the observation of eggs in the feces because the eggs are similar to those of the genus *Libyostrongylus*, this misdiagnosis is probably happening on other continents.

There are few reports of the occurrence of *C. struthionis* infecting ostrich flocks in South Africa (POPOVA, 1955); Europe (PONCE GORDO et al., 2002); Brazil (EDERLI et al., 2008c) and possibly in Greece, where Sotiraki et al. (2001) found eggs typical of the family Strongylidae in feces of ostriches but did not analyze fecal cultures to confirm the diagnosis. This low incidence of infection is probably due to misdiagnosis because of the similarity of *C. struthionis* eggs with those from the genus *Libyostrongylus*. Additionally, there is similarity between the infective larvae of *C. struthionis* and *L. dentatus* (EDERLI et al., 2008a, c).

Until recently, the specific diagnosis of these nematodes was possible only through the identification of adult worms collected during necropsies. Studies carried out by Ederli et al. (2008a) and Ederli et al. (2008c) allowed the distinction of *C. struthionis*, *L. douglassii* and *L. dentatus* through the observation of infective larvae recovered from fecal cultures, thus facilitating diagnosis.

*Libyostrongylus* spp. is considered the most pathogenic nematode, responsible for 50% of the mortality of juvenile birds and occasionally killing adults (REINECKE, 1983). Until recently, *C. struthionis* was considered a nonpathogenic parasite (HUCHZERMAYER, 2005); however, a recent study by Oliveira et al. (2009) verified that *C. struthionis* is responsible for macroscopic lesions in the ostriches' cecum, characterized as mucosal thickening nodules distributed throughout the cecum, and the presence of hemorrhages caused by small ulcers circumscribed to the mucosal edema. Due to these observations, these authors hypothesize that a high parasite load by *C. struthionis* could result in loss of fitness.

These parasites appear to be specific to ostriches; however, there was a single report of the occurrence of *Libyostrongylus* in emu, *Dromaius novaehollandiae* (Lathan, 1790) in Sweden (JANSSON; CHRISTENSSON, 2000; PONCE GORDO et al., 2002), which may have been due an error in diagnosis. The cross-transmission potential of these nematodes to other domestic birds (in particular, other ratites) has not yet been determined. Studies carried out by Zettermann et al. (2005) to detect helminthes in rheas, *Rhea americana* Linnaeus, 1758, showed that none of the 8 free-living adult rheas or 12 captive juveniles rheas were infected by *Libyostrongylus* spp. or *C. struthionis*. They found 7 other species of parasites in the examined rheas: *Sicarius uncinipenis* (Molin 1860), *Torquatoides crotophaga* Williams, 1929, *Deletrocephalus dimidiatus* Diesing, 1851, *D. cesarpintoi* Vaz, 1936, *Paradeletrocephalus minor* Freitas and Lent, 1947, *Capillaria venteli* Freitas and Almeida, 1935 and *Dicheilonema rhaeae* (Owen, 1843). Gallo et al. (2010) conducted a study to evaluate the effects of an experimental infection of chickens by *Libyostrongylus* spp., where they infected normal and immunosuppressed one-day-old chicks with infective larvae of *L. douglassii* and *L. dentatus*; nonetheless, the infection

was not established. In some birds they observed the elimination of unshathed larvae in birds' feces from 1 to 5 days following the infection. Moreover, the presence of eggs of Trichostrongylidae was not observed until the end of the experiment (52 days). Because the unshathed larvae cannot survive in the environment, chickens are not at risk for the transmission or for acting as a paratenic host. However, a possibility of transmission of these parasites to other birds, mainly other ratites, cannot be discounted.

The present article presents keys for the identification of the main genus (infective larvae and adults) in ostriches to facilitate diagnosis by fecal exams and the observation of adults recovered in necropsies.

## Adult Nematodes

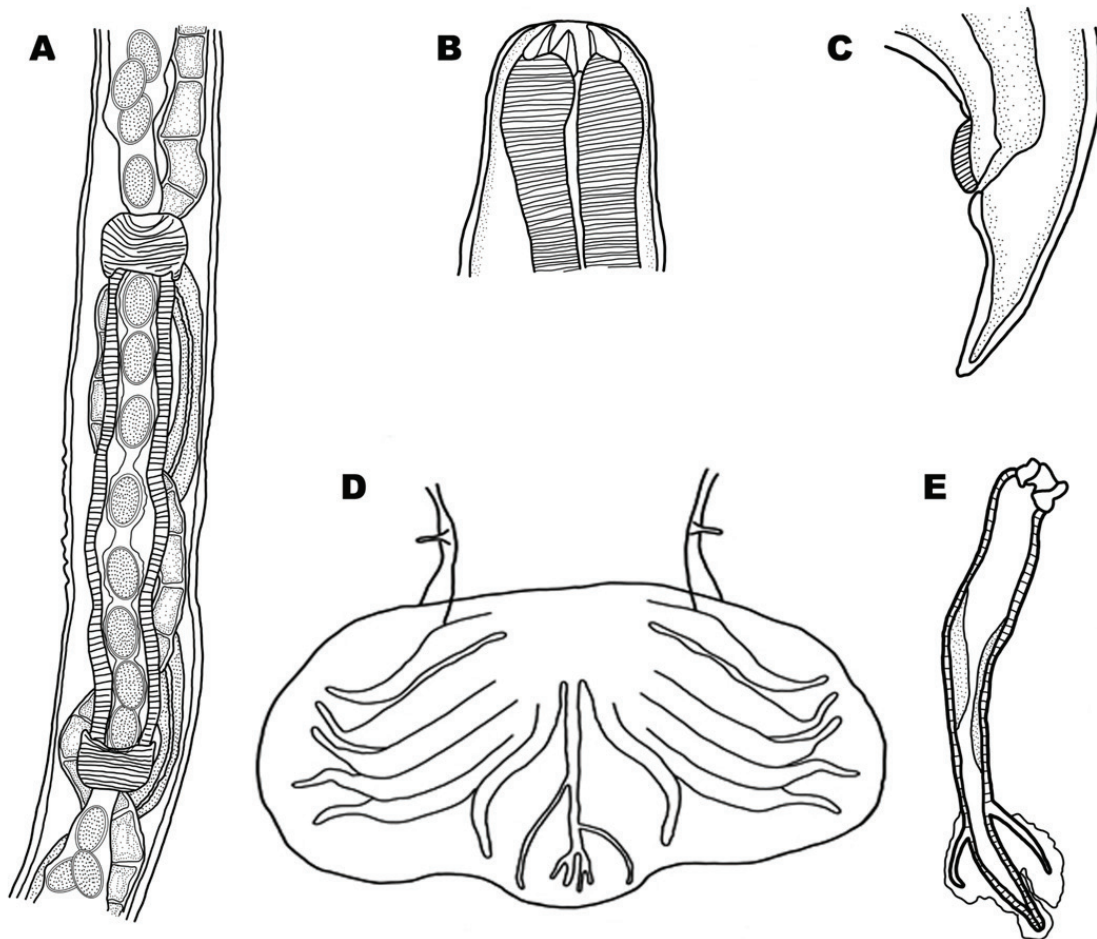
The parasites of the genus *Libyostrongylus* are small nematodes, of a reddish color *in vivo* and found in the proventriculus and gizzard of ostriches. The genus *Libyostrongylus* is cited as being found under the koilin layer (HOBERG et al., 1995; HUCHZERMAYER, 2005; EDERLI et al., 2008b); however, a recent study by Ederli and Oliveira (2009) observed differential localization of *L. douglassii* and *L. dentatus* within the proventriculus. *Libyostrongylus douglassii* is found on the mucosa just under the koilin layer, as described in the literature, while *L. dentatus* is found inserted into the koilin layer. The distinct localization of these 2 species in the proventriculus may be the reason there are so few reports of the presence of *L. dentatus*. Until the publication of these observations, *L. dentatus* was sought along with *L. douglassii* under the koilin layer, possibly resulting in false negative diagnoses for *L. dentatus* because the koilin layer is removed and discarded during the collection of the parasites. The description of the differential localization of these 2 species of nematodes in the proventriculus may result in an increase in the number of reports of *L. dentatus*.

The morphological description of *L. magnus* is published in Greek (GILBERT, 1937), which hinders its access. There are no further studies about the morphology of this species. Hoberg et al. (1995) was able to re-examine some specimens deposited in the International Institute of Parasitology (LSHTM 1317) which was previously identified as *L. douglassii* in 1933 in Ethiopia. Hoberg et al. (1995) identified those nematodes as *L. magnus*. According to Hoberg et al. (1995), the measurements of some male and female specimens of *L. douglassii* described by Durette-Desset and Denke (1978) from an ostrich in Somalia should classify them as *L. magnus* (Table 2). Unlike other species of the genus, the females of *L. magnus* are smaller than males and have a larger ovejector when compared with *L. douglassii* and *L. dentatus* (Table 2). *Libyostrongylus douglassii* and *L. dentatus* can be distinguished by the observation of some morphological characteristics. *Libyostrongylus dentatus* can be distinguished from congeners by the presence of 3 prominent esophageal teeth (Figure 2B). Both males and females of *L. dentatus* are longer than *L. douglassii* (HOBERG et al., 1995; EDERLI et al., 2008b) (Table 2). These 2 species also have differences in the morphology on the cephalic end: *L. dentatus* has a flat extremity with 3 pairs of rounded cephalic papillae, while *L. douglassii* has a rounded end with 3 pairs of elongated papillae (EDERLI et al., 2008b).

**Table 2.** Comparison of adult males and females from the species of genus *Libystrongylus* Cobbold, 1882. Measurements in micrometers.

Characters	<i>L. douglassii</i> <sup>1</sup>	<i>L. magnus</i> <sup>2</sup>	<i>L. dentatus</i> <sup>1</sup>
Total length (Females)	3,340-5,120	7,560-11,430	7,192-10,450
Total length (Males)	2,240-4,928	13,860-14,580	4,752-5,156
Ovejector length	188-335	1,725	407-503
Spicules length	144-171	220-240	103-130
Prominent esophageal teeth	Absent	Absent	Present
Cuticular inflation at anus level	Absent	Absent	Present
Dorsal ray symmetry	Symmetric	Symmetric	Asymmetric

<sup>1</sup>Ederli et al. (2008b). <sup>2</sup>Skrjabin (1961).

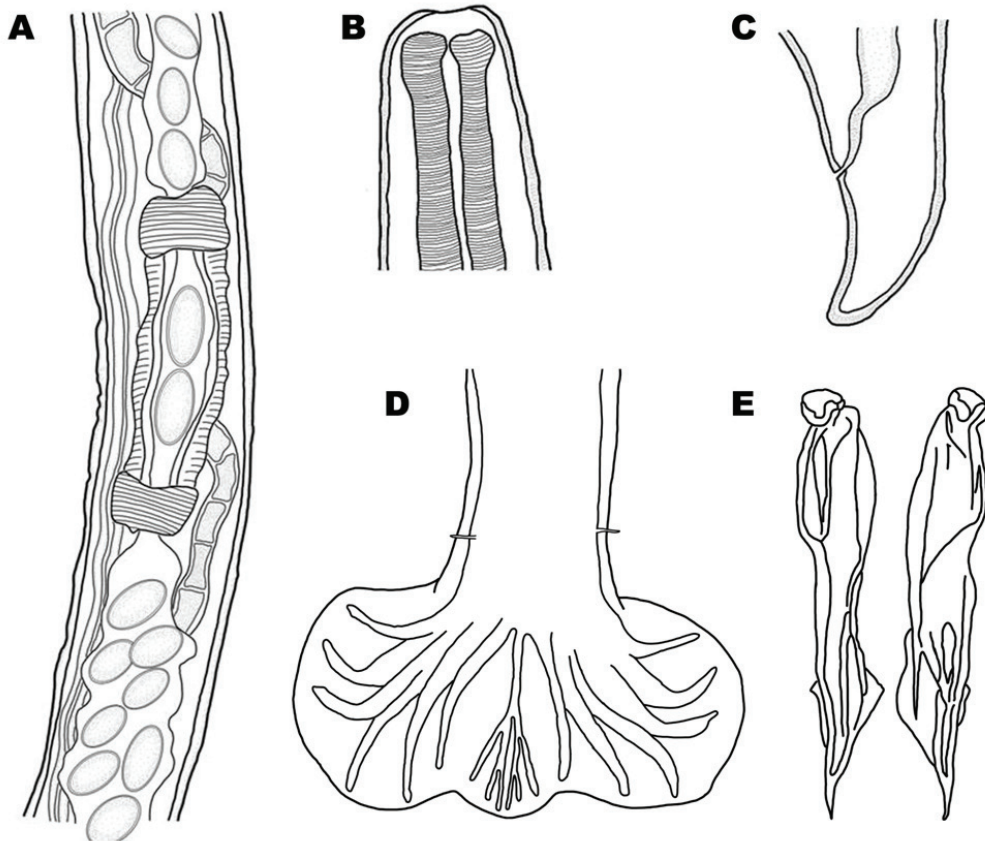


**Figure 2.** *Libystrongylus dentatus*. (A) Ovejector; (B) Anterior end; (C) Female posterior end; (D) Male copulatory bursa; and (E) Spicules.

Female *L. dentatus* can be distinguished from *L. douglassii* by its longer ovejector (Figures 2A and 3A) and by the presence of a cuticular inflation at the anal level (Figures 2C and 3C). *Libystrongylus magnus* was described as having long ovejector, but it can be distinguished from *L. dentatus* by the larger number of eggs inside (Figure 4A) (SKRJABIN, 1961). Male *L. dentatus* can be distinguished by an asymmetric dorsal ray (Figure 2D), while other species have a symmetric dorsal ray (Figure 3D and 4D). Moreover, *L. dentatus* have spicules with the main shaft ending in a rounded point (Figure 2E), while the spicules of *L. douglassii*

have a main shaft ending in a point (Figure 3E) (HOBERG et al., 1995; EDERLI et al., 2008b).

*Codiostomum struthionis* are large and robust nematodes, with white coloration *in vivo*, observed easily in the cecum of ostriches. These nematodes can be easily distinguished from the genus *Libystrongylus* because of their greater length. Recently, this species had its morphology and ultrastructure redescribed by Ederli et al. (2008c). *Codiostomum struthionis* has a well-developed buccal capsule with 2 rows of corona radiata. Males have a strongly curved ventral copulatory bursa, with a large projection of the



**Figure 3.** *Libyostrongylus douglassii*. (A) Ovejector; (B) Anterior end; (C) Female posterior end; (D) Male copulatory bursa; and (E) Spicules.

dorsal lobe and long and slender spicules. Females have prodelphic uteri and a pointed tail tip (EDERLI et al., 2008c).

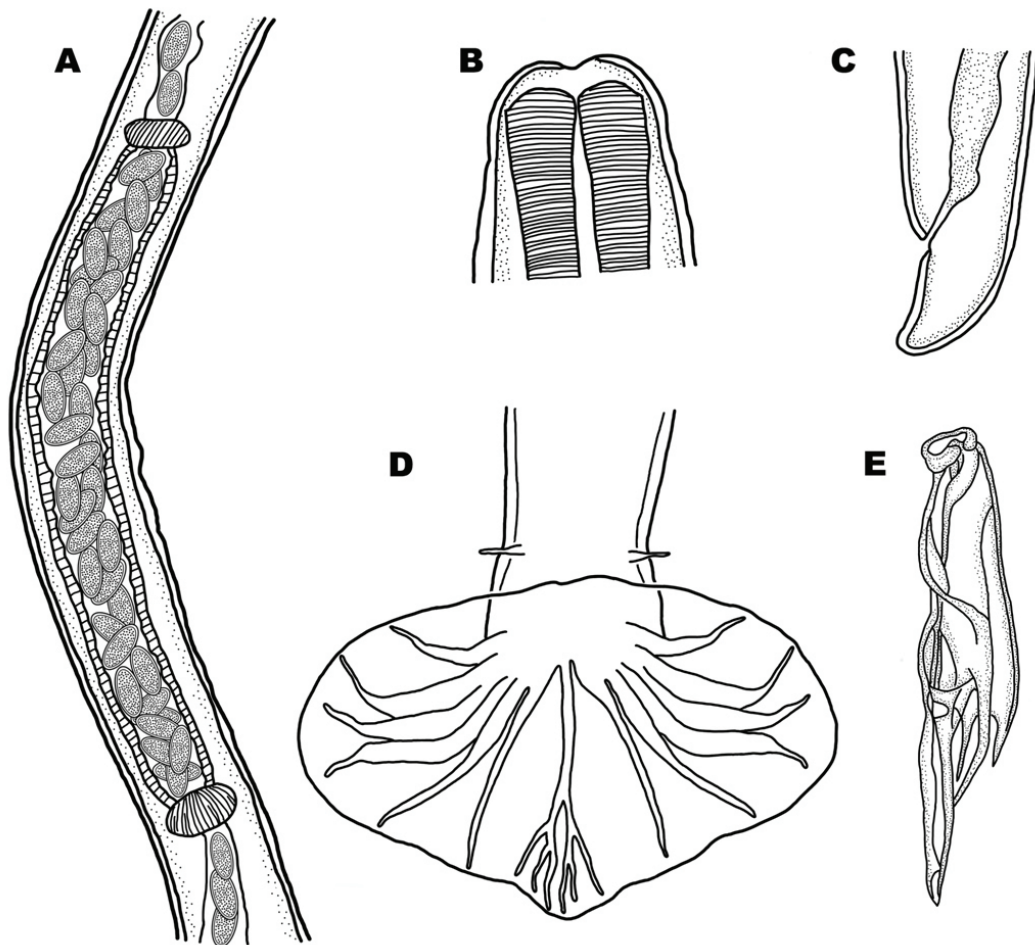
To facilitate the differentiation of adult nematodes from the genus *Libyostrongylus*, an identification key is described below:

- |    |  |  |
|----|--|--|
| 1a | Specimens larger than other species from the genus (Table 2), being females smaller than males. Ovejector long with a considerable number of eggs inside, overlapping (Figure 4A)  | <i>Libyostrongylus magnus</i> Gilbert, 1937                  |
| 1b | Females larger than males (Table 2). Eggs arranged linearly within the ovejector (Figures 2A and 3A)   | 2  |
| 2a | Absence of prominent esophageal teeth, or poorly developed (Figure 3B); Females with a short ovejector with a small number of eggs inside (normally 2 eggs) (Figure 3A), absence of a cuticular inflation at anus level (Figure 3C). Males with a symmetric dorsal ray (Figure 3D) and the spicules main shaft ending in a sharp tip (Figure 3E) | <i>Libyostrongylus douglassii</i> (Cobbold, 1882)            |
| 2b | Presence of 3 prominent esophageal teeth (Figure 2B); Females with a long ovejector, with a median number of eggs inside (Figure 2A), and presence of a cuticular inflation at anus level (Figure 2C). Males with an asymmetrical dorsal ray (Figure 2D) and the spicules main shaft ending in a rounded tip, with an hyaline cap (Figure 2E)    | <i>Libyostrongylus dentatus</i> Hoberg, Lloyd and Omar, 1995 |

## Infective Larvae

The infective larvae of the genus *Libyostrongylus* are characterized by the presence of a knob in the larvae tip tail (BARTON; SEWARD, 1993). Bonadiman et al. (2006) reported the presence of *L. douglassii* and *L. dentatus* in the northern region of the state of Rio de Janeiro, Brazil, by observing adult worms collected from the proventriculum during necropsies in 3 adult ostriches. They also observed differences in the length and morphology of the infective larvae sheath tail recovered from fecal cultures of samples from the same region. Some larvae had short sheath tail with an acute termination and others had a long and filamentous sheath tail. The authors described these differences as a possible way to distinguish these 2 species, as both were found parasitizing ostrich flocks in the analyzed region (BONADIMAN et al., 2006). Ederli et al. (2008a) confirmed this observation, showing the infective larvae of *L. douglassii* which has a short sheath tail (average of  $29.52 \mu\text{m} \pm 4.11$ ; ranging from 20.62 to 36.31  $\mu\text{m}$ ), while the infective larvae of *L. dentatus* has a long sheath tail (average of  $61.20 \mu\text{m} \pm 2.17$ ; ranging from 40.01 to 84.01  $\mu\text{m}$ ) (Figures 5A, B). Following this study, it was possible to differentiate these 2 species by the morphology and morphometry of the infective larvae sheath tail recovered from fecal cultures. Because it was no longer necessary to perform a necropsy to collect adult worms, specific diagnosis was facilitated. However, the infective larvae of *C. struthionis* also have a long and filamentous sheath tail (average of  $110.74 \mu\text{m} \pm 13.46$ , ranging from 85.87 to 143.18  $\mu\text{m}$ ), but





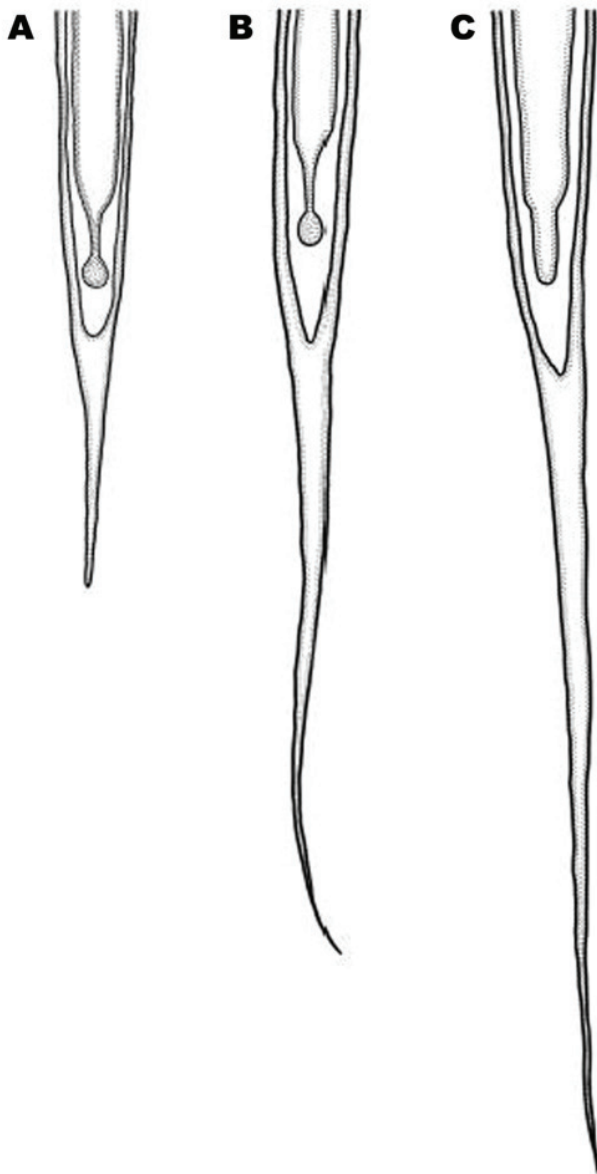
**Figure 4.** *Libyostrongylus magnus* (A) Ovejector; (B) Anterior end; (C) Female posterior end; (D) Male copulatory bursa; and (E) Spicules. Re-drawn from Skrjabin (1961).

the tail of the larvae inside the cuticle has an acute termination (Figure 5C) (EDERLI et al., 2008c), which is different from the genus *Libyostrongylus*, which has a knob on the larvae tip tail (EDERLI et al., 2008a, b) (Figures 5A, B). The total length of the infective larvae is not a good parameter to distinguish the species due to the similarity between nematodes. *Libyostrongylus douglassii* has a total length of  $874.33 \mu\text{m} \pm 33.80$ , ranging from  $784.47$  to  $957.90 \mu\text{m}$ ; *L. dentatus*  $784.47 \mu\text{m} \pm 43.63$ , ranging from  $735.84$  to  $947.01 \mu\text{m}$  and *C. struthionis*  $598.25 \mu\text{m} \pm 25.15$ , ranging from  $511.92$  to  $642.41 \mu\text{m}$  (EDERLI et al., 2008a, c).

The infective larvae of *L. douglassii* have a less-rounded cephalic end and became thinner more abruptly than *L. dentatus* infective larvae (BONADIMAN et al., 2006), while the infective larvae of *C. struthionis* have a flat, rounded cephalic end (EDERLI et al., 2008c). These parameters are not reliable in the specific diagnosis of these infective larvae due the similarity between the cephalic ends.

*Libyostrongylus douglassii* is easily distinguished from *L. dentatus* and *C. struthionis* by the infective larvae sheath tail: it is short with an acute termination, differing from the other 2 species, which have a long and filamentous sheath tail (Figure 5). The differential

diagnosis between these 2 species is made by the observation of the shape of the larvae tail: *L. dentatus* has a knob in the tip of the tail (Figure 5B), while *C. struthionis* has an acute termination in the tip of the tail (Figure 5C) (EDERLI et al., 2008a, c). The comparison of the mean lengths of the infective larvae sheaths tail of *L. douglassii* and *L. dentatus* was considered extremely significant (EDERLI et al., 2008a); however, this parameter must be associated with the tail morphology of the larvae, because *L. dentatus* can be confused with *C. struthionis*, as mentioned previously. Due to these similarities, the identity of infective larvae can be unclear in fecal examinations, resulting in an error in the diagnosis. This misdiagnosis may be occurring with *C. struthionis* because there are few reports of this nematode in ostrich flocks (POPOVA, 1955; SOTIRAKI, et al., 2001; PONCE GORDO et al., 2002; EDERLI et al., 2008a). A recent reported the occurrence of *C. struthionis*, correlating its presence with dry and rainy seasons. However, the authors demonstrated images of infective larvae of *L. dentatus*, with a knob on the larvae tip tail and a long and filamentous sheath tail (FAGUNDES et al., 2012).



**Figure 5.** Posterior end of infective larvae. (A) *Libyostrongylus douglassii*; (B) *Libyostrongylus dentatus*; and (C) *Codiostomum struthionis*.

Below is a key to the identification of the infective larvae of nematodes of the genera *Libyostrongylus* and *Codiostomum*.

- |    |   |  |
|----|---|--|
| 1a | Presence of a knob at the infective larvae tip tail (Figures 5A, B) | 2  |
| 1b | Absence of a knob at the infective larvae tip tail (Figure 5C)      | <i>Codiostomum struthionis</i> (Horst, 1885)                 |
| 2a | Short sheath tail (Figure 5A)                                       | <i>Libyostrongylus douglassii</i> (Cobbold, 1882)            |
| 2b | Long sheath tail (Figures 5B, C)                                    | <i>Libyostrongylus dentatus</i> Hoberg, Lloyd and Omar, 1995 |

## Conclusion

It is possible to distinguish between species of ostrich parasites by the morphological and morphometric characteristics of both adult nematodes and infective larvae, thus facilitating the diagnosis, ecology, systematic and epidemiology study of these parasites.

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