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Chapiniella variabilis (Nematoda) parasitizing *Chelonoidis carbonarius* and *C. denticulatus* (Testudinidae) in the state of Piauí

Chapiniella variabilis (Nematoda) parasitando *Chelonoidis carbonarius* e *C. denticulatus* (Testudinidae) do estado do Piauí

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Abstract

Chapiniella variabilis (Chapin, 1924), a strongylid nematode, was collected parasitizing the large intestine of the tortoises *Chelonoidis carbonarius* (Spix, 1824) (*Cc*) and *C. denticulatus* (Linnaeus, 1766) (*Cd*) in the Zoobotanical Park of the municipality of Teresina, state of Piauí, Brazil. The taxonomic identification was based on morphological and morphometric features, using bright-field and scanning electron microscopy. The present study adds new observations on the morphology, mainly relating to the mouth papillae, external and internal leaf-crown elements, excretory pore, deirids and male and female posterior end. The parasitic indices of prevalence (P), mean intensity (MI), mean abundance (MA) and range of infection (RI) of *C. variabilis* in these two tortoise species were: P = 100%, MI = 833.3, MA = 833.3, RI = 500-1,500 (*Cc*); P = 100%, MI = 472.2, MA = 472.2, RI = 333-500 (*Cd*). This record expands occurrences of *C. variabilis* to a new host, *C. carbonarius*, and to another state in Brazil, in the Neotropical region of South America. Adjustment to host management with the aim of improving hygiene and health conditions is suggested.

Keywords: Nematode, strongylid, *Chapiniella variabilis*, Testudinid, Brazil.

Resumo

Chapiniella variabilis (Chapin, 1924), um nematoide estrongilídeo, foi coletado parasitando o intestino grosso de jabutis, *Chelonoidis carbonarius* (Spix, 1824) e *C. denticulatus* (Linnaeus, 1766) do Parque Zoobotânico, município de Teresina, estado do Piauí, Brasil. A identificação taxonômica foi baseada nos caracteres morfológicos e morfométricos usando microscopias de campo claro e eletrônica de varredura. O presente estudo adiciona novas observações na morfologia, principalmente relacionadas as papilas bucais, elementos externos e internos da coroa-foliar, poro excretor, deirídeos, e extremidade posterior de machos e fêmeas. Os índices parasitários de prevalência (P), intensidade média (IM), abundância média (AM) e amplitude de variação de infecção (AI) de *C. variabilis* em ambos jabutis foram P = 100%, IM = 833,3, AM = 833,3, AI = 500-1.500 (*Cc*); P = 100%, IM = 472,2, AM = 472,2, AI = 333-500 (*Cd*). Este registro aumenta a ocorrência de *C. variabilis* a um novo hospedeiro, *C. carbonarius*, e a um outro estado do Brasil, na região Neotropical da América do Sul. É sugerido um ajuste no manejo dos hospedeiros objetivando melhora das suas condições higiênico sanitárias.

Palavras-chave: Nematoide, estrongilídeo, *Chapiniella variabilis*, Testudinídeos, Brasil.

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Introduction

The tortoise *Chelonoidis carbonarius* (Spix, 1824) occurs in the southernmost part of Central America (southeastern Panama), in part of the northern half of South America (west of the Andes in Chocó, Colombia, and east of the Andes in Colombia, Venezuela, the Guianas and northeastern Brazil, south to Rio de Janeiro, and west to eastern Bolivia, Paraguay and northern Argentina), and in Trinidad and some islands of the Lesser Antilles. The tortoise *Chelonoidis denticulatus* (Linnaeus, 1766) occurs in the northern half of South America east of the Andes, also until to the south of Brazil, and on Trinidad island (FRITZ & HAVAŠ, 2007; RUEDA-ALMONACID et al., 2007).

In a parasitological helminth survey, a strongylid nematode species was recovered from *C. carbonarius* and *C. denticulatus*, which were kept at the Zoobotanical Park, Teresina, state of Piauí, Brazil. The aims of this study were to identify this strongylid taxonomically, based on morphological and morphometric features, using bright-field and scanning electron microscopy; calculate their parasitic indices, i.e. prevalence, mean intensity, mean abundance and range of infection; and point out its importance with regard to jeopardizing the health of these reptiles in captivity.

Materials and Methods

Between June 2014 and June 2015, a total of 12 adult tortoises (six *C. carbonarius* and six *C. denticulatus,* three males and three females from each) at the Zoobotanical Park, municipality of Teresina, state of Piauí, Brazil (05º 04' 10" S; 42º 76' 87" W) were examined. They formed part of a group of 102 specimens of *C. carbonarius* and 40 of *C. denticulatus* that were living under confinement in the same area of captivity, all specimens were from the state of Piauí, coming from seizures carried out by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) or the state environmental police or by donations from private individuals. The tortoises were subjected to stool analysis by means of the Hoffmann and Willis methods (KNOFF & GOMES, 2012), and the twelve individuals with the highest rates of parasitism were chosen for sacrifice. The chelonians were identified based on Rueda-Almonacid et al. (2007), and the combinations of specific epithets are according to the nomenclatural adjustments suggested by Olson & David (2014) for the genus *Chelonoidis* Fitzinger, 1835 and accepted by Costa & Bernils (2015). To sacrifice the animals, they were first sedated using an association of ketamine (15 mg/kg) and xylazine (10 mg/kg), applied intramuscularly. Forty-five minutes later, an overdose of 25 mg/kg of 1% sodium thiopental was applied intravenously.

The chelonians were then necropsied. The nematodes recovered through this process were placed in Petri dishes with 0.65% NaCl solution, fixed in hot AFA (glacial acetic acid, formaldehyde and 70% ethanol in the proportions of 2:3:95), preserved in a solution of 70% ethanol plus 5% glycerin and clarified with Amman's lactophenol, Some specimens were mounted in Canada balsam as described by Knoff & Gomes (2012).

The nematodes were identified taxonomically in accordance with Lichtenfels & Stewart (1981), De Ley & Blaxter (2004) and Lichtenfels (2009). Morphometric analyses were performed using a bright-field Olympus BX41 microscope with a drawing tube connected. Measurements are shown in millimeters (mm) with the averages in parentheses, unless otherwise indicated; images were obtained using a Canon digital camera (Power Shot A640) coupled to a Zeiss Axiophot microscope.

For topographic characterization of the cuticular surface, nematodes were analyzed using a scanning electron microscope (SEM). Samples were fixed in 2.5% glutaraldehyde with 0.1 M sodium cacodylate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated in an ethanol series, dried using the CO_2 critical point drying method, coated in gold, examined and photographed using a Vega-3 scanning electron microscope (Tescan), under 15 kV acceleration voltage.

Prevalence (P), mean intensity (MI), mean abundance (MA), and range of intensity of infection (RI) were calculated as described by Bush et al. (1997). The representative specimens (vouchers) were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, state of Rio de Janeiro, Brazil. This study was authorized by the Ethics Committee for Animal Experimentation of the Federal University of Piauí, Teresina, state of Piauí, Brazil, under number 09/2015, and by the Biodiversity Authorization and Information System (SISBIO), under number 44782-1.

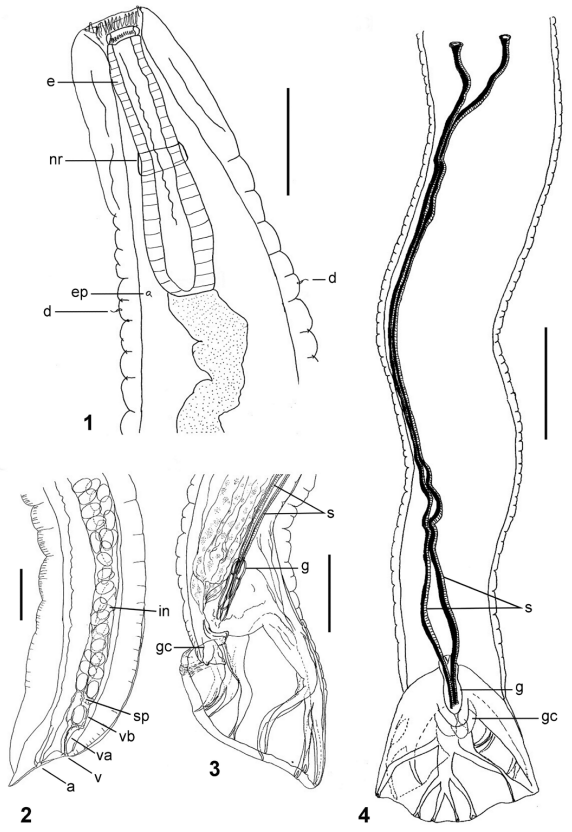
Results

All hosts (prevalence = 100%) were parasitized with adult nematodes. A total of 7,333 nematodes were collected, including 4,500 from six specimens of *C. carbonarius* and 2,833 from six of *C. denticulatus*. All the nematodes were alive and showed high motility.

Rhabditida Chitwood, 1933
Rhabditina Chitwood, 1933
Rhabditomorpha De Ley and Blaxter, 2002
Strongyloidea Baird, 1853
Strongylidae Baird, 1853
Cyathostominae Nicoll, 1927
Sauricolini Popova, 1952, emend. Lichtenfels, 1980
Chapiniella Yamaguti, 1961, emend. Lichtenfels, 1980, redef. Lichtenfels and Stewart, 1981
Chapiniella variabilis (Chapin, 1924) (Figures 1-10)

General description

Short, thin nematodes with inflated cuticle marked by coarse annulation. Four submedian cephalic papillae with elongated digitiform tips. Two lateral papillae (amphids), slightly elevated. Buccal capsule short and shallow, ring-shaped, wide and circular, with 18 long slender external leaf-crown elements and 18 short internal leaf-crown elements, externally inconspicuous, but conspicuous to bright-field microscopy, inserted alternately at base of the buccal capsule; border of oral opening divided into numerous extensions of external leaf-crown. Dorsal gutter absent. Esophagus slender and long, six times as long as wide, enlarged slightly posteriorly. Nerve ring at middle of esophagus, where



Figures 1-4. *Chapiniella variabilis* collected from *Chelonoidis carbonarius*. 1. Male, anterior end showing buccal capsule, esophagus (e), nerve ring (nr), cervical papillary deirids (d) and excretory pore (ep), in lateroventral view. 2. Female, posterior end showing anus (a), vulva (v), vagina (va), vestibule (vb), sphincter (sp), infundibulum (in) and eggs, in lateral view. 3. Male, posterior end showing copulatory bursa, genital cone (gc), gubernaculum (g) and distal portion of spicules (s), in lateral view. 4. Male, posterior end showing copulatory bursa, genital cone (gc), gubernaculum g) and entire spicules (s), in dorsal view. Scale bars: 1 = 100 μm; 2-4 = 200 μm.

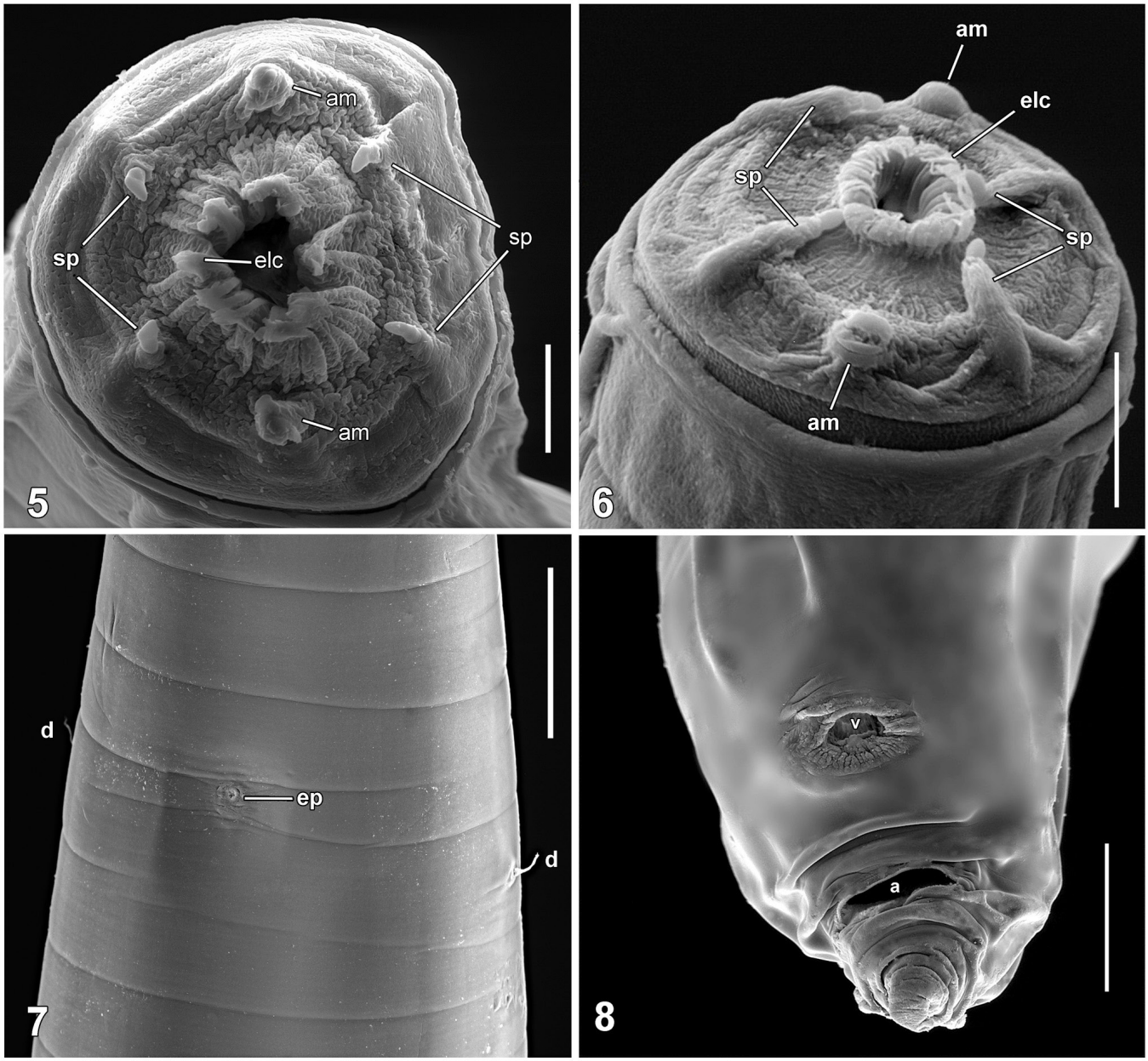
there is a slight narrowing of its width. Ventral excretory pore and lateral cervical papillae (deirids), bristle-shaped (at an annule beneath the excretory pore), near base of esophagus. Spicules alate with slightly curved tips, covering 23.33 to 28.57% (24.5%) of body length. Gubernaculum short, trough-shaped with proximal, thick, spongy, lightly sclerotized portion and a distal, thin, densely sclerotized plate with lateral flanges; proximal portion with medial projection between spicules. Genital cone bearing two digitiform dorsolateral projections, two pear-shaped internal dorsal projections and a ventral triangular projection with membranous skirt. Dorsal lobe of copulatory bursa longer than lateral lobes; dorsal lobe not separated from lateral lobes; lateral lobes not completely separated ventrally. All rays of bursa reach edge of bursa except external lateral and external dorsal rays. Ventral-ventral and lateroventral rays parallel, curving ventrally. Dorsal bursal ray with central bifurcate ramus and paired lateral rami that bifurcate near distal ends. External dorsal rays originating from the stem of dorsal ray. Prebursal papillae absent. Two papillae on the dorsal surface of copulatory bursa near the edge at level of external dorsal rays. Female tail short; vulva near anus; ovijectors parallel, with thin muscles. Ovijectors long, sphincters and infundibula not markedly differentiated, together accounting for 2.7 to 5.5% of body length. Ova large, unembryonated.

Male, measurements of 10 specimens collected from *C. carbonarius*: total length 3.85-7.52 (5.62); esophagus length 0.32-0.46 (0.37); anterior end to nerve ring 0.20-0.29 (0.25) (n = 6); excretory pore 0.33-0.65 (0.43) (n = 8); and cervical papillae (deirids) 0.35-0.54 (0.43) (n = 7). Buccal capsule depth 5-15 (9) μm; and diameter 22-47 (33) μm. Body width at base of cephalic collar 0.047-0.120 (0.067); at base of esophagus 0.140-0.260 (0.190); and at midbody 0.210-0.420 (0.300). Width of cuticular annule at base of esophagus 0.027-0.067 (0.04); and at midbody 0.02-0.08 (0.04). Spicules 1.10-1.81 (1.34) long and 0.010-0.020 (0.013) wide at proximal ends. Gubernaculum length 0.05-0.11 (0.07) (n = 7). Genital cone length 0.02-0.03 (0.02) (n = 8). Dorsal ray length 0.12-0.22 (0.16) (n = 9); and external rays 0.19-0.26 (0.21) (n = 7), originating from distal end.

Female, measurements of 10 specimens collected from *C. carbonarius*: total length 5.62-9.70 (7.70); esophagus length 0.35-0.61 (0.45); anterior end to nerve ring 0.164-0.237 (0.201) (n = 5); excretory pore 0.37-0.58 (0.48) (n = 8); and cervical papillae (deirids) 0.37-0.56 (0.46) (n = 6). Buccal capsule depth 5-10 (10) μm; and diameter 25-50 (40) μm. Body width at base of cephalic collar 0.05-0.08 (0.07); at base of esophagus 0.13-0.37 (0.32); and at midbody 0.28-0.50 (0.40). Width of cuticular annule at base of esophagus 0.025-0.090 (0.050); and at midbody 0.02-0.10 (0.05). Distance from vulva to anus 0.025-0.100 (0.050). Vagina length 0.050-0.055 (0.052) (n = 3); vestibule length 0.05-0.10 (0.09) (n=7); and combined length of sphincter and infundibulum 0.200-0.500 (0.300) (n = 2). Egg length 0.060-0.080 (0.070) (n = 20); and width 0.040-0.045 (0.044) (n = 20). Tail length 0.197- 0.200 (0.190).

Male, measurements of 10 specimens collected from *C. denticulatus*: total length 5.3-6.9 (6.14); esophagus length 0.31-0.47 (0.41); anterior end to nerve ring 0.214-0.231 (0.219); excretory pore 0.420-0.510 (0.450) (n = 3); and cervical papillae (deirids) 0.41-0.52 (0.43) (n = 3). Buccal capsule depth 12.5-20 (14) μm (n = 9); and diameter 35-55 (46) μm (n = 9). Body width at base of cephalic collar 0.06-0.09 (0.07) (n = 7); at base of esophagus 0.20-0.32 (0.25) (n = 8); and at midbody 0.26-0.47 (0.35). Width of cuticular annule at base of esophagus 0.037-0.072 (0.05) (n = 8); and at midbody 0.04-0.09 (0.06). Spicules 1.30-1.61 (1.49) long and 0.015-0.020 (0.018) wide. Gubernaculum length 0.087-0.125 (0.097) (n = 8). Genital cone length 0.02- 0.03 (0.02) (n = 4). Dorsal ray length 0.13-0.22 (0.17) (n = 9); and external rays 0.10-0.20 (0.12) (n = 8), originating from distal end.

Female, measurements of 10 specimens collected from *C. denticulatus*: total length 7.25-9.07 (7.90); esophagus length 0.42-0.51 (0.45); anterior end to nerve ring 0.200-0.269 (0.231); excretory pore 0.46-0.53 (0.48) (n = 6); and cervical papillae (deirids) 0.43-0.55 (0.49) (n = 7). Buccal capsule depth 10-40 (30) μm; and diameter 40-80 (60) μm. Body width at base of cephalic collar 0.07-0.08 (0.08); at base of esophagus 0.20-0.29 (0.23); and at midbody 0.34-0.59 (0.44). Width of cuticular annule at base of esophagus 0.05-0.06 (0.05) (n = 8); and at midbody 0.05-0.08 (0.06) (n = 6). Distance from vulva to anus 0.030-0.060 (0.04)



Figures 5-8. *Chapiniella variabilis* collected from *Chelonoidis carbonarius* (Cc) and *C. denticulatus* (Cd), by means of SEM. 5-6. Male and female (Cc), cephalic end showing four submedian papillae with digitiform tips (sp), two slightly elevated amphids with C-shaped openings (am) and elements of the external leaf-crown (elc), and their overlying cuticular extensions, in en-face view. 7. Female (Cc), part of anterior region showing excretory pore (ep) and deirids (d), in ventral view. 8. Female (Cd), tail showing vulva (v) and anus (a), in ventral view. Scale bars: 5-6 = 10 µm; 7 = 50 µm; 8 = 40 µm.

(n = 8). Vagina length 0.050-0.055 (0.052) (n = 3); vestibule length 0.075-0.100 (0.08) (n = 7); and combined length of sphincter and infundibulum 0.250-0.500 (0.300) (n = 4). Egg length 0.060-0.080 (0.070) (n = 20); and width 0.040-0.045 (0.044) (n = 20). Tail length 0.200- 0.205 (0.200).

Hosts: *C. carbonarius* (*Cc*) and *C. denticulatus* (*Cd*).

Locality: Zoobotanical Park, municipality of Teresina, state of Piauí, Brazil.

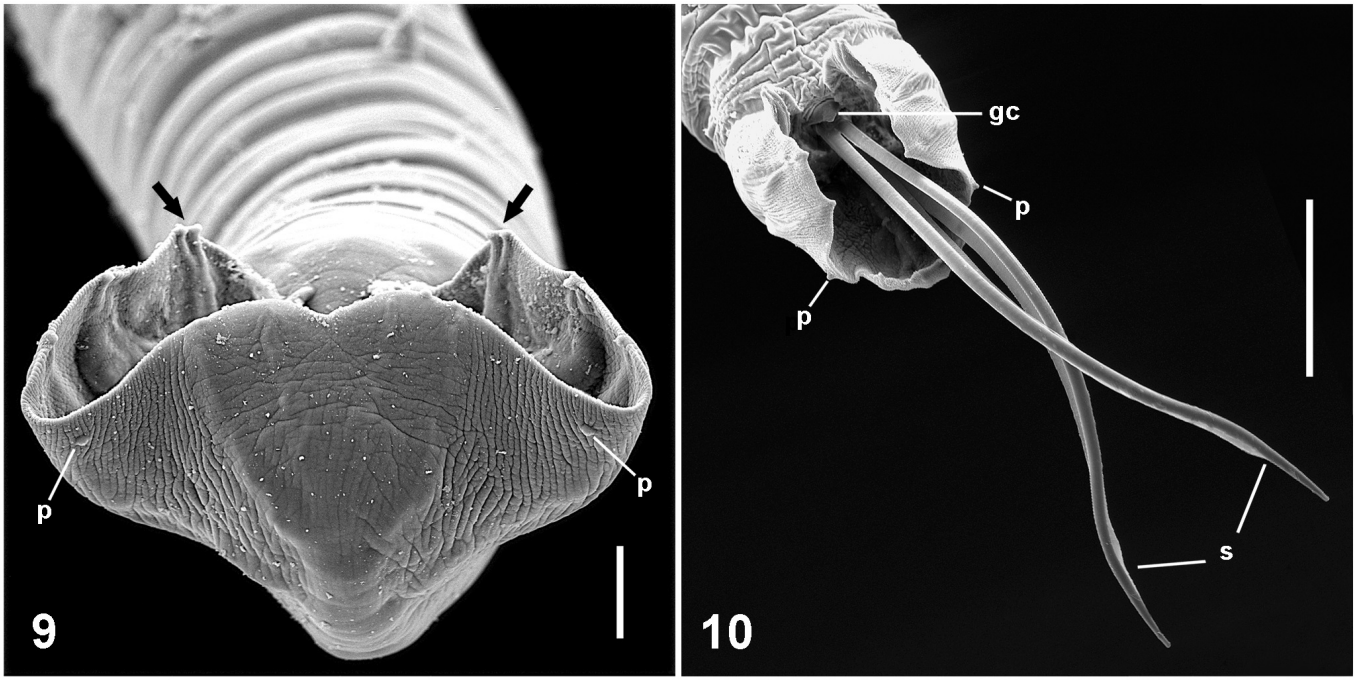
Parasitic indices: P = 100%, MI = 833.3, MA = 833.3, RI = 500-1,500 (*Cc*); P = 100%, MI = 472.2, MA = 472.2, RI = 333-500 (*Cd*).

Infection site: large intestine in both hosts.

Specimens deposited: CHIOC 38142 (6 males and 6 females) (*Cc*) wet material; CHIOC 38143 (7 males and 5 females) (*Cd*) wet material, vouchers.

Remarks

Only two genera of strongylid nematodes, both in Cyathostominae Nicoll, 1927, Sauricolini Popova, 1952, have been reported in reptiles: *Sauricola* Chapin, 1924 and *Chapiniella* Yamaguti, 1961,



Figures 9-10. *Chapiniella variabilis* collected from *Chelonoidis carbonarius*, by means of SEM. 9. Male, tail showing copulatory bursa, with two papillae (p) on its dorsal surface, and ventral-ventral rays bifurcated near distal ends (indicated by arrows), next to lateroventral rays with the same features, in ventral and dorsal views. 10. Male, tail showing copulatory bursa, with two papillae on its dorsal surface (p), genital cone (gc) and extroverted spicules (s), in ventral view. Scale bars: 9 = 20 μm; 10 = 100 μm.

emend. Lichtenfels, 1980 and redefined by Lichtenfels and Stewart, 1981. The latter differs mainly by having a slender esophagus (six times as long as wide), enlarged slightly posteriorly, and a dorsal bursal ray with central bifurcated ramus and paired lateral rami that usually bifurcate near the distal ends (LICHTENFELS & STEWART, 1981; LICHTENFELS, 2009). These features were observed in the nematode specimens collected in the present study, which made it possible to identify them as belonging to this genus.

Among the six known *Chapiniella* species, three were described infecting the tortoise *C. denticulatus* in South America, as follows: *Chapiniella variabilis* in the state of Pará, Brazil, and *C. larensis* Diaz-Ungria and Gallardo, 1968 and *C. diazi* Chabaud and Tcheprakoff, 1977, in Venezuela (CHAPIN, 1924; DIAZ-UNGRIA & GALLARDO, 1968; CHABAUD & TCHEPRAKOFF, 1977; VICENTE et al., 1993). Among the other three species, *C. chitwoodae* Lichtenfels and Stewart, 1981, and *C. gallatii* Lichtenfels and Stewart, 1981, were described in the gopher tortoise, *Gopherus polyphemus* (Daudin, 1802) (Testudines, Cryptodira, Testudinidae) in Georgia, USA, and *C. jellisoni* Lichtenfels and Stewart, 1981, in an unidentified tortoise in Southeast Asia (LICHTENFELS & STEWART, 1981).

The descriptions of the adult nematode specimens that were collected from *C. carbonarius* and *C. denticulatus* in Piauí are concordant with the original description of *C. variabilis* based on adults recovered from *C. denticulatus* in the state of Pará, Brazil (CHAPIN, 1924). Comparing the specimens from Pará with those from Piauí, the ranges of body, spicule and gubernaculum lengths were considered to be intraspecific variations, taking into account that the largest specimens of the present study were similar in size to the Pará specimens. The same morphological pattern was observed, with the same cephalic papilla distribution, buccal capsule with 18 long slender external leaf-crown elements, esophagus, nerve ring, excretory pore, ventral and cervical papillae (deirids); the same male structures, i.e. spicules, gubernaculum genital cone, dorsal and lateral lobes of the copulatory bursa and bursal rays; and the same female structures, i.e. short tail, vulva, anus and ovijectors. In the original description, the ova were not described (CHAPIN, 1924), but have now been observed and described.

The main features that distinguish *C. variabilis* from the other five *Chapiniella* species are the leaf-crowns of 18 elements each, on the cephalic end; the ratio of the length from the nerve ring to the anterior end divided by the length of the esophagus of 50%; the ratio of spicule length to body length of close to 20%; and the diameter of the buccal capsule of 40 μm. These parameters served to reinforce the species identification of the specimens collected in the present study (CHAPIN, 1924; DIAZ-UNGRIA & GALLARDO, 1968; CHABAUD & TCHEPRAKOFF, 1977; LICHTENFELS & STEWART, 1981).

Based on observations from bright-field microscopy (BF) and scanning electron microscopy (SEM), new details of the morphology of these species have been provided. At the anterior end, it was possible to show the morphological features and locations of two submedian papillae with digitiform tips and amphids with C-shaped openings on the subventral surfaces, and two submedian papillae on the dorsal surface. These formed a hexagonal subdivision between the papillae at the cephalic end and the 18 elements of the external leaf-crown, and were

symmetrically distributed with a set of three elements in each interval formed between the cephalic papillae and amphids. Another study using SEM showed a similar pattern at the cephalic end, in a description of *C. chitwoodae* and *C. gallatii,* which are parasites of the gopher tortoise (*G. polyphemus*), in Georgia, USA. These two species differ by having 19 and 17 leaf-crown elements, respectively (LICHTENFELS & STEWART, 1981). *Chapiniella larensis* has 18 leaf-crown elements, a buccal capsule diameter of 80 µm and a spicule length/body length ratio of 46% (DIAZ-UNGRIA & GALLARDO, 1968). *C. diazi* has 30 leaf-crown elements, a buccal capsule diameter of around 20 µm and a spicule length/body length ratio of 33% (CHABAUD & TCHEPRAKOFF, 1977).

In the *C. variabilis* specimens of the present study, in comparison with the original description (CHAPIN, 1924), it was possible to add morphological details through SEM, such as the shallow and rounded excretory pore surface; bristle-shaped deirids at an annule beneath the excretory pore; tail corrugations around the vulva and a smooth surface around the anus in females; posterior end showing the nature of the copulatory bursa curve ventrally, with dorsal lobe not separated from lateral lobes, in males; lateral lobes not completely separated ventrally; and, through both BF and SEM, the dorsal bursal ray with central bifurcated ramus and paired lateral rami bifurcating near the distal ends. In addition, SEM showed the presence of two papillae on the dorsal surface of the copulatory bursa near the edge, at the level of the external dorsal rays, a characteristic that had not been reported in previous descriptions of the genus. In one specimen that had extroverted spicules, it was observed through SEM that these were slightly recurved at the distal end.

Discussion

Chapiniella variabilis had previously been reported only in *C. denticulatus* in the state of Pará, Brazil (CHAPIN, 1924). Therefore, this is the first report of this species parasitizing this tortoise in the state of Piauí. This report expands the occurrence of this parasite in this tortoise in Brazil, and expands it to the tortoise *C. carbonarius* as a new host.

In the present study, the parasitic indices of *C. variabilis* were high in both tortoise species. Considering that the individuals examined were selected from group of 142 tortoises, living in the same area of captivity (covering 225 m^2), and the fact that *Chapiniella* species have a direct life cycle (MADER, 2006), transmission could facilitate spreading of this nematode between these tortoises. According to Primack & Rodrigues (2001), grouping hosts together may cause greater direct pressure towards occurrences of parasites and diseases. Placing host populations in small-sized areas may decrease habitat quality and food availability, which will give rise to low nutritional values, malnourished animals and, consequently, increased susceptibility to infections. Groupings in large numbers can lead to social stress in populations followed by decreased disease resistance. Therefore, adjustments to host management should be made, with application of anthelmintics and reduction of the number of reptiles per m^2, hereby increasing the space available for them, in order to avoid higher parasitic indices. Without such measures, the health of these reptiles at the Zoobotanical Park of Teresina may be jeopardized.

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