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9 ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

10 PARASITIC INFECTION IN CAPTIVE ANURANS FROM FREE-LIVING

11 ENVIRONMENTS: DIAGNOSIS AND TREATMENT

12 INFECCIÓN PARASITARIA EN ANUROS EN CAUTIVERIO PROCEDENTE DE

13 AMBIENTES DE VIDA LIBRE: DIAGNÓSTICO Y TRATAMIENTO

14 INFECÇÃO PARASITÁRIA EM ANUROS EM CATIVEIRO PROVENIENTES DE

15 AMBIENTES DE VIDA LIVRE: DIAGNÓSTICO E TRATAMENTO

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26 Running Head: Diagnosis and treatment of captive anurans

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32

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39

40 **ABSTRACT**

41 Free-living amphibians naturally harbor a wide variety of endoparasites, many of which
42 do not cause deleterious effects in healthy individuals. The present study aimed to report
43 the parasites found in coproparasitological examinations of *Odontophrynus carvalhoi*
44 Savage & Cei, 1965 and *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-
45 Pimentel, Toledo & Bruschi, 2020, originating from the wild and kept under human care
46 at the Núcleo Regional de Ofiologia (NUROF-UFC), Ceará state, Brazil, as well as the
47 control measures implemented through an antiparasitic program. Direct examination
48 and flotation methods were employed. Protozoa were detected, such as coccidian
49 oocysts and *Nyctotheroides* sp. at various stages, cestode eggs, nematode eggs, and
50 adult nematodes identified as *Aplectana hylambatis* Baylis, 1927. The animals were
51 treated with Fenbendazole (50 mg/kg), and monitoring was performed through fecal
52 examinations following deworming. A reduction in the number of parasite species was
53 observed, thus indicating that the protocol was effective.

54 **Keywords:** Anura – Biological Monitoring – Infection Control – Parasitic diseases –
55 Specimen Handling – Wilderness Medicine

56 **RESUMEN**

57 Los anfibios de vida libre albergan naturalmente una amplia variedad de endoparásitos,
58 muchos de los cuales no causan efectos deletéreos en individuos sanos. El presente
59 estudio tuvo como objetivo reportar los parásitos encontrados en exámenes
60 coproparasitológicos de *Odontophrynus carvalhoi* Savage & Cei, 1965 y *Pithecopus*
61 *gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo & Bruschi, 2020
62 provenientes de la naturaleza y mantenidos bajo cuidado humano en el Núcleo Regional
63 de Ofiología (NUROF-UFC), estado de Ceará, Brasil, así como las medidas de control
64 implementadas mediante un programa antiparasitario. Se emplearon los métodos de
65 examen directo y de flotación. Se detectaron protozoarios, como ooquistes de coccidios
66 y *Nyctotheroides* sp. en diferentes estadios, huevos de cestodos, huevos de nematodos
67 y nematodos adultos identificados como *Aplectana hylambatis* Baylis, 1927. Los
68 animales fueron tratados con Fenbendazol (50 mg/kg) y el monitoreo se realizó
69 mediante exámenes coproparasitológicos posteriores a la desparasitación. Se observó
70 una reducción en el número de especies de parásitos, lo que indica que el protocolo fue
71 efectivo.

72 **Palabras clave:** Anura – Control de infecciones – Enfermedades parasitarias – Manejo
73 de muestras – Medicina de la Vida Silvestre – Monitoreo biológico

74

75 **RESUMO**

76 Anfíbios de vida livre naturalmente abrigam uma ampla variedade de endoparasitos,
77 muitos dos quais não causam efeitos deletérios em indivíduos saudáveis. O presente
78 estudo teve como objetivo relatar os parasitos encontrados em exames
79 coproparasitológicos de *Odontophrynus carvalhoi* Savage & Cei, 1965 e *Pithecopus*
80 *gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo & Bruschi, 2020, oriundos
81 da natureza e mantidos sob cuidados humanos no Núcleo Regional de Ofiologia

82 (NUROF-UFC), estado do Ceará, Brasil, bem como as medidas de controle
83 implementadas por meio de um programa antiparasitário. Foram empregados os
84 métodos de exame direto e de flutuação. Foram detectados protozoários, como oocistos
85 de coccídios e *Nyctotheroides* sp. em diferentes estágios, ovos de cestoides, ovos de
86 nematoides e nematoides adultos identificados como *Aplectana hylambatis* Baylis,
87 1927. Os animais foram tratados com fenbendazol (50 mg/kg), e o monitoramento foi
88 realizado por meio de exames fecais após a vermifugação. Observou-se redução no
89 número de espécies parasitárias, o que indica que o protocolo foi eficaz.

90 **Palavras-chave:** Anura – Controle de infecção – Doenças parasitárias – Manejo de
91 espécimes – Medicina da vida silvestre – Monitoramento biológico

92

93 INTRODUCTION

94 In wildlife, parasitic infections are common in anurans and are intrinsically related
95 to environmental conditions and biological aspects of the host, such as behavior, diet,
96 and body size (Campião & Dáttilo, 2020). Parasites can interfere with processes such
97 as migration, predation, dispersal, and speciation, playing a significant ecological role in
98 regulating nature (Vitt & Caldwell, 2009; Matias *et al.*, 2018; Oliveira *et al.*, 2022).

99 It is observed that many endoparasite species may not cause deleterious effects,
100 whereas others are associated with the occurrence of gastrointestinal diseases and
101 mortality in amphibians (Ellerd *et al.*, 2022). In captivity, stressors can cause
102 immunosuppression, which tends to increase susceptibility to the development of
103 parasitic diseases (Densmore & Green, 2007). Clinical signs are only noticeable in high-
104 intensity infections, which can cause mechanical obstructions, hemorrhagic ulcers,
105 enteritis, and pneumonia, and may lead to death, depending on the parasite species and
106 the host's overall health (Garner & Jacobson, 2021; Ellerd *et al.*, 2022).

107 *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo &
108 Bruschi, 2020 and *Odontophrynus carvalhoi* Savage & Cei, 1965 are species of anuran
109 amphibians distributed in northeastern Brazil and are currently not threatened with
110 extinction, according to the Ceará Threatened Fauna List (SEMA, 2025) and the IUCN
111 Red List (IUCN, 2023).

112 *Pithecopus gonzagai* is a small-sized species from the family Phyllomedusidae,
113 belonging to the *Pithecopus hypochondrialis* group, distributed north of the São
114 Francisco River (Andrade *et al.*, 2020). It has an arboreal habit, is predominantly
115 nocturnal, and is mainly associated with lentic environments (Oliveira *et al.*, 2018). In
116 turn, *O. carvalhoi* is a medium-sized frog from the family Odontophrynidae, part of the
117 *Odontophrynus cultripes* group, occurring in areas of the Atlantic Forest, Cerrado, and
118 Caatinga. Individuals of this species have terrestrial habits, often burrow to avoid drying
119 out, and occupy both lotic and lentic environments (Costa *et al.*, 2017).

120 In this context, the present work aimed to record the occurrence of endoparasites
121 in free-living anurans from the state of Ceará under handling at the facilities of the Núcleo
122 Regional de Ofiologia, Universidade Federal do Ceará (NUROF-UFC), as well as to
123 evaluate parasitic control through the application of an antiparasitic protocol combined
124 with environmental management.

125

126 **MATERIAL AND METHODS**

127 Seven frogs were collected by manual capture during active searches during
128 scientific expeditions from June to July 2024 and sent to the Núcleo Regional de
129 Ofiologia - NUROF (UFC) in Ceará state, Brazil, for the purpose of studying behavior
130 and adaptation to the *ex situ* environment and for inclusion in environmental education
131 projects.

132 Four individuals of the species *P. gonzagai* from Uruburetama (-3.648399, -
133 39.472543, WGS 84), which is located in the Morphoclimatic Domain of the Brazilian

134 Caatingas, characterized by the predominance of the semi-arid climate typical of the
135 Caatinga (Ab'saber, 1974; IPECE, 2009), and three *O. carvalhoi* from Mulungu (-
136 4.265315, -38.932152, WGS 84), situated in the Baturité massif, classified as an
137 "highland marshes" a fragment of the Atlantic Forest, a humid tropical forest (Santos-
138 Cavalcante *et al.*, 2024), both areas from the state of Ceará, Northeast of Brazil.

139 The individuals were kept in glass terrariums specific to each species, with
140 average dimensions of 0.75 x 0.4 x 0.4 m. These environments were enriched with
141 substrates such as moss, soil, leaves, and ornamental plants like anthurium,
142 chlorophytum, and fern, as well as vegetal hideouts. To monitor the environmental
143 conditions, digital thermohygrometers were used, along with spray bottles and
144 humidifiers with dechlorinated water to maintain proper humidity.

145 To collect fresh feces for direct parasitological examination from *P. gonzagai* a
146 thin layer of water was poured in the bottom of containers to place the individuals
147 (Ministério da saúde, 2012). For *O. carvalhoi*, two methods were used: direct collection
148 of fresh feces from the terrarium and cloacal lavage (Divers & Stahl, 2019), with a 04
149 urethral catheter and about 0.5 mL of saline solution. The collected material was
150 centrifuged for five minutes at 1200 rpm, and the precipitate was used to prepare slides,
151 sometimes with a drop of Lugol's iodine for better contrast during microscope use. The
152 nematodes were processed using methods outlined in Ferreira-Silva *et al.* (2022). The
153 slides were examined under a light microscope.

154 The antiparasitic treatment protocol used was Fenbendazole 50 mg/kg,
155 administered orally every 24 hours for 5 days (Walker & Whitaker, 2000; Divers & Stahl,
156 2019). The drug was diluted and reconstituted on-site, and administered orally using
157 syringes. Follow-up serial parasitological examinations were performed after treatment
158 to assess efficacy at 15, 30, and 60 days. After the last day of treatment, the animals
159 were transferred from their environment to terrariums with new or sterilized substrate
160 and materials that were autoclaved and disinfected.

161 **Ethical aspects**

162 The collections were authorized by the Chico Mendes Institute for Biodiversity
163 Conservation (ICMBio) through the Biodiversity Authorization and Information System
164 (SisBio license N°. 29613). The procedures for capture, handling, collection,
165 transportation, and ex situ management were authorized by the Ethics Committee for
166 Animal Use in Research at the Federal University of Ceará (CEUA-UFC protocol N°.
167 6314010321).

168

169 **RESULTS**

170 The examined individuals showed no noticeable clinical changes, either
171 behavioral or physical. The feces of both species were dark brown, semi-solid, well-
172 formed, and without macroscopic alterations.

173 The *P. gonzagai* samples collected revealed the presence of protozoa, cestode
174 eggs, and nematode adults and eggs (Table 1). Among the protozoa, unsporulated
175 coccidian oocysts (Fig. 1h), the ciliate *Nyctotheroides* sp. was recorded in the
176 trophozoite (Fig. 1a) and cyst stages (Fig. 1b), with a discarded cyst wall (Fig. 1c).
177 Additionally, rounded-to-oval structures with transparent to translucent contents,
178 suggestive of cysts of *Entamoeba* sp. or *Giardia* sp., were visualized. The cestode eggs
179 had a thick, striated shell and were embryonated (Fig. 1f). Embryonated eggs with a thin
180 shell were also recorded (fig. 1d). The adult nematodes were identified as *Aplectana*
181 *hylambatis* Baylis, 1927 (Cosmocercidae) (Fig. 2a–f). This species is characterized by
182 females being slightly larger than males and by the presence of lateral wings in both
183 sexes. Males have a conical tail, numerous caudal papillae, and heavily chitinized
184 spicules (Fig 2e), with the posterior end surrounded by a membranous sheath forming
185 an extension of the spicule (Fig. 2f), as well as a heavily chitinized gubernaculum (Fig.
186 2e). Females have a post-equatorial vulva with a mamillated cuticular protrusion on the
187 anterior lip of the vulva, a strongly muscular vagina oriented anteriorly (Fig. 2c),

188 prolapsed ovaries (both located anterior to the vulva), and a conical tail (Fig. 2b).
189 Although the direct parasitological examination is qualitative, the findings suggest mild
190 infections with no apparent clinical significance.

191 The samples collected from *O. carvalhoi* revealed the presence of trophozoites,
192 and *Nyctotheroides* sp. cysts were again identified, being the cysts rounded,
193 embryonated, with thin, smooth shells, and presenting a polar operculum (Fig. 1a–c).
194 Additionally, moderate numbers of unflagellated protozoa were observed (Fig. 1e). Also,
195 embryonated eggs with thin, smooth shells, suggestive of trichostrongylids or species
196 with similar morphology (Fig. 1i), were also observed, and a rounded larvated nematode
197 egg with a thick shell (Fig. 1g). Specimens of *A. hylambatis* were observed (Fig. 2a–f),
198 as well as in *P. gonzagai*.

199 Given these findings, antiparasitic treatment was initiated, and
200 coproparasitological exams were repeated at the end of the protocol. In analyses
201 performed after the end of deworming in *P. gonzagai*, neither nematodes nor cysts of
202 *Entamoeba* sp. or *Giardia* sp. were observed; however, trophozoites of *Nyctotheroides*
203 sp. were still detected in moderate numbers. The coccidian oocysts were observed after
204 deworming, but in smaller quantities.

205 In subsequent coproparasitological exams of *O. carvalhoi*, a residual presence
206 of embryonated and larvated nematode eggs was observed, with a rounded shape and
207 thick shell, as well as embryonated eggs with a thin, smooth shell, in lower quantities.
208 Also recorded were flagellated protozoa, along with trophozoites and cysts of the ciliated
209 *Nyctotheroides* sp. Adult nematodes of *A. hylambatis* were no longer observed after
210 treatment.

211

212 **DISCUSSION**

213 Before the taxonomic differentiation between *P. gonzagai* and *Phyllomedusa*
214 *nordestina* Caramaschi, 2006, which occurred in 2020 (Andrade *et al.*, 2020), reports of

215 nematodes from the families Cosmocercidae (*Cosmocerca parva* Travassos, 1925,
216 *Cosmocercella phyllomedusae* Baker and Vaucher, 1983, and *Oxyascaris caudacutus*
217 Freitas, 1958) and Rhabdiasidae (*Rhabdias* sp.) were documented in forest remnants
218 from the states of Pernambuco and Ceará (Sena *et al.*, 2018). Another study conducted
219 in the state of Ceará reports nematodes from the families Cosmocercidae (*Aplectana*
220 *membranosa* Miranda, 1924 and *Raillietnema spectans* Gomes, 1964) and Molineidae
221 (*Oswaldocruzia mazzai* Travassos, 1935) in specimens of *P. gonzagai* (Sampaio *et al.*,
222 2022). Therefore, all morphotypes recorded in *P. gonzagai* represent new records for
223 this species.

224 For *O. carvalhoi*, there are few studies investigating endoparasites of this
225 species, with reports of the occurrence of *A. hylambatis*, *Cosmocerca brasiliense*
226 Travassos, 1925, *Gorgoderina parvicava* Travassos, 1922, *Ochoterenella* sp., *O.*
227 *mazzai*, *Oxyascaris* sp., *Parapharyngodon* sp., *Physaloptera* sp., *Raillietnema* sp.,
228 *Rhabdias* sp., and *Strongyloides* sp. (Quirino *et al.*, 2023). Therefore, with the exception
229 of *A. hylambatis*, all morphotypes here recorded in *O. carvalhoi* represent new records
230 for this species.

231 Free-living amphibians commonly to carry a moderate load of protozoans and
232 commensal metazoans in the gastrointestinal tract with no harm to their health
233 (Densmore & Green, 2007). Interactions and competition among parasites within the
234 host reduce morbidity rates and regulate the risk of developing parasitic diseases
235 (Johnson *et al.*, 2013). The most common parasites of amphibians are *Microsporidia*,
236 *Myxosporea*, and *Coccidia*, which have different impacts on amphibians depending on
237 the species, life stage, and degree of infection.

238 However, immunosuppressive conditions, often associated with environmental
239 constraints in ex situ settings, may favor the progression to clinically relevant infections
240 (Densmore & Green 2007). In this context, the ex situ management of wild animals
241 requires the implementation of preventive veterinary measures as a fundamental

242 strategy to ensure animal health and welfare (Ossiboff *et al.*, 2020). In the present study,
243 coproparasitological examinations were performed for prophylactic purposes, followed
244 by anthelmintic treatment, considering the potential for fecal contamination within the
245 enclosures, which may promote new infections or reinfections and eventual clinical
246 manifestations (Bais *et al.*, 2017). Although no clinical alterations were observed, high
247 parasite loads of *A. hylambatis* were recorded in *O. carvalhoi*, as well as a significant
248 presence of coccidian oocysts in some individuals of *P. gonzagai*.

249 Confinement may represent a risk factor for the development of parasitic
250 diseases associated with parasites that have direct life cycles, due to the increased
251 likelihood of continuous exposure to infective stages in the environment (Bartošová-
252 Sojková *et al.*, 2015; González *et al.*, 2021). Therefore, the importance of systematic
253 health monitoring of anurans maintained *ex situ* is emphasized.

254 Studies report that nematodes of the genus *Aplectana* sp. are common and
255 considered non-pathogenic in amphibians. Similarly, species of *Nyctotheroides* live in a
256 commensal relationship in the posterior portion of the amphibians' gastrointestinal tract
257 (Şenler, 2000) and are considered to have low pathogenicity. An increase in their
258 numbers in the feces may indicate dysbiosis (Divers & Stahl, 2019).

259 Treatment with fenbendazole is considered safe and is frequently prescribed,
260 particularly for symptomatic infections (Walker & Whitaker, 2000). Fenbendazole is
261 widely used in veterinary clinics (Carlos *et al.*, 2011). With anti-helminthic and
262 antiprotozoal action, fenbendazole belongs to the benzimidazole group and works by
263 inhibiting the synthesis of adenosine triphosphate (ATP) and the production of cellular
264 microtubules (Lanusse *et al.*, 2009) in parasites (Panarella, 2002). In frogs of the genus
265 *Bufo* sp., the efficacy of fenbendazole was tested and compared with that of other drugs,
266 such as levamisole, with no significant differences in helminth efficacy (Bianchi *et al.*,
267 2014). Fenbendazole, thiabendazole, and ivermectin are important drugs in the
268 treatment of parasitoses in amphibians (Densmore & Green, 2007).

269 In this study, the use of the fenbendazole protocol showed promising treatment
270 for mixed parasitic infections in the studied anuran species. However, due to the lack of
271 elimination of the parasitic load in individuals after the first treatment, maybe booster
272 treatments, fenbendazole dose adjustment, or the use of standardized veterinary
273 preparations could achieve better control of drug absorption and pharmacokinetics.
274 Also, further use of fenbendazole is indicated after monitoring clinical and health signs
275 of the animals, combining information from both parasite load, symptoms, and
276 anamnesis. Since it was not possible to monitor laboratory toxicity in the specimens due
277 to their small body size, and no toxicity signs were observed in this sample, further
278 studies are needed to define an optimal fenbendazole protocol for the species studied.
279 Another issue raised and supported by Li *et al.* (2020) is that infection by *Nyctotheroides*
280 sp. might persist because the insects used as food for the anurans were possibly
281 previously infected, given that these insects inhabit the gastrointestinal tracts not only of
282 vertebrates but also of invertebrates. In other words, it would not be possible to evaluate
283 the efficacy of fenbendazole against the observed ciliates, as reinfection could be
284 occurring through the diet.

285 Overall, this study contributes to the knowledge that coproparasitological
286 examination has been effective in monitoring the health of captive amphibians.
287 Treatment with fenbendazole eliminated the adult nematodes of *A. hylambatis* and
288 reduced the protozoa in the feces of *P. gonzagai* and *O. carvalhoi*, warranting monitoring
289 via coproparasitological exams and subsequent booster doses to assess parasitic load.

290

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305

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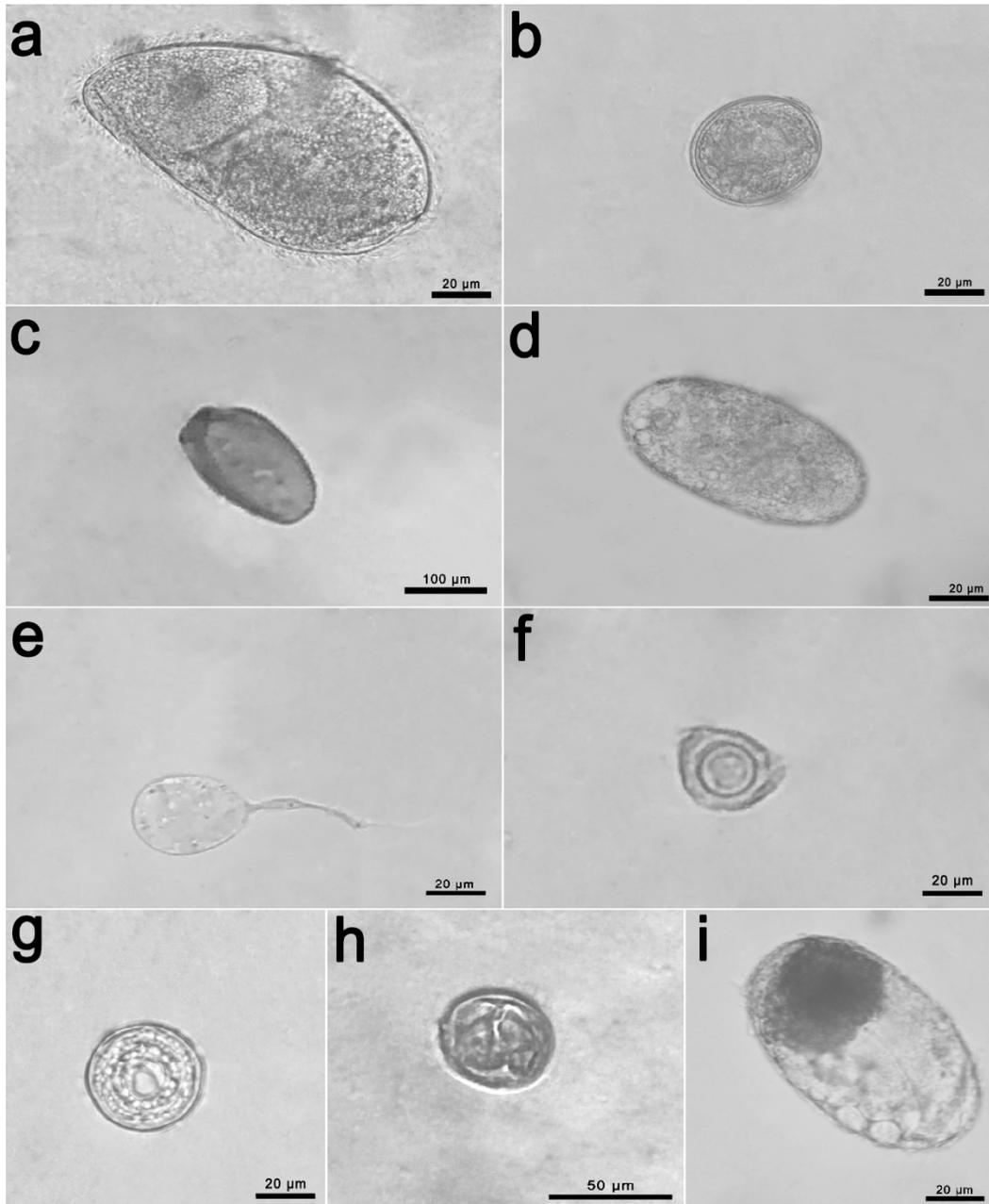
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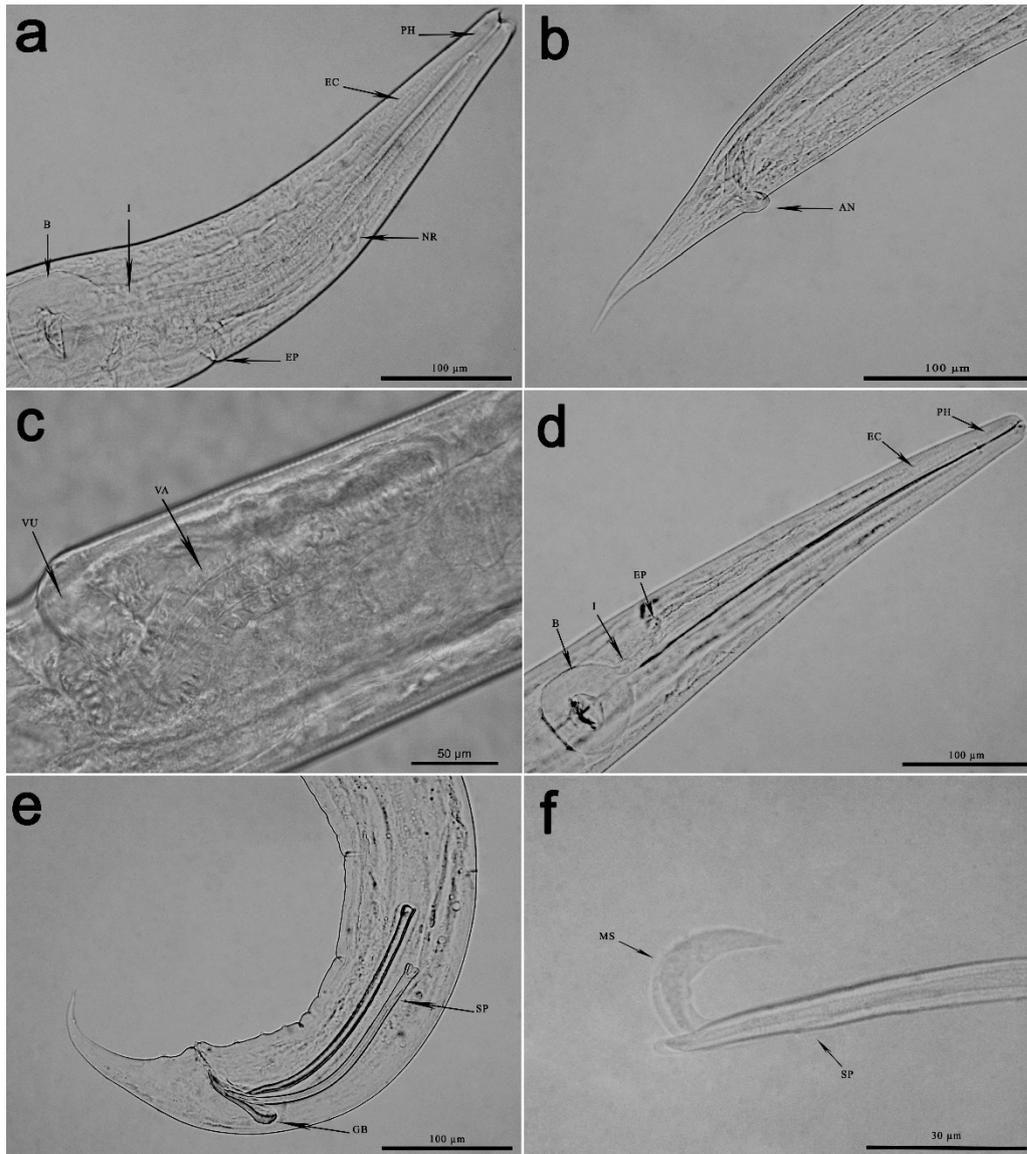
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429 **Figure 1:** Microscopic findings in the direct parasitological stool exam of *Pithecopus*
 430 *gonzagai* and *Odontophrynus carvalhoi*. **a** - Ciliophora, *Nyctotheroides* sp., trophozoite;
 431 **b** - Ciliophora, *Nyctotheroides* sp., cyst with apical operculum; **c** - Ciliophora,
 432 *Nyctotheroides* sp., excysted cyst; **d** - Embryonated egg with thin shell; **e** - Uniflagellated
 433 protozoan; **f** Embryonated egg cestode; **g** - Rounded larvated egg with thick shell; **h** -
 434 Coccidian oocyst; **i** - Embryonated egg with thin, smooth shell, suggestive of
 435 trichostrongylids or species with similar morphology.



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437 **Figure 2.** *Aplectana hylambatis* registrada em *Pithecopus gonzagai* e *Odontophrynus*
 438 *carvalhoi*. **a** - Female anterior region, showing pharynx (PH), esophagus corpus (EC),
 439 isthmus (I), bulbo (B), nerve ring (NR), and excretory pore (EP), lateral view; **b** - Female
 440 tail with the anus (AN) opening in lateral view; **c** - Female with detail of the vulva (VU),
 441 muscular vagina (VA), lateroventral view; **d** - Male anterior region, showing pharynx
 442 (PH), esophagus corpus (EC), isthmus (I), bulbo (B), and excretory pore (EP), lateral
 443 view; **e** - Male tail showing spicules (SP) and gubernaculum (GB), lateral view; **f** -
 444 Spicules (SP) with membranous sheath (MS), dissected male for visualization of the
 445 membranous sheath.

446 **Table 1.** Microscopic findings in the direct parasitological stool exam of *Pithecopus*
 447 *gonzagai* and *Odontophrynus carvalhoi*.

Host	Parasite
<i>Pithecopus gonzagai</i>	<p>Protozoa</p> <p><i>Nyctoteroides</i> sp. (Trophozoites and cysts) Cyst of <i>Entamoeba</i> sp. or <i>Giardia</i> sp. Coccidian oocyst</p> <p>Cestoda</p> <p>Embryonated egg</p> <p>Nematoda</p> <p><i>Aplectana hylambatis</i> Embryonated egg, thin shell</p>
<i>Odontophrynus carvalhoi</i>	<p>Protozoa</p> <p><i>Nyctotheroides</i> sp. (Trophozoites and cysts) Uniflagellate protozoan</p> <p>Nematoda</p> <p><i>Aplectana hylambatis</i> Larvated egg Embryonated egg, thin and smooth shell, suggestive of trichostrongylids</p>

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