Description of the egg and larva of *Raillietiella mottae* (Pentastomida: Raillietiellidae)

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Summary

In the current study, the structural characteristics of the egg and larva of the pentastomid *Raillietiella mottae* (Almeida, Freire, & Lopes 2008), are described and compared with those of other pentastomids. The eggs and larvae were obtained from lizards *Phyllopezus periosus* (Rodrigues 1986) and *Phyllopezus pollicaris* (Spix 1825) which were collected in the environmental protection area of Cachoeira de Missão Velha, Ceará state, northeastern Brazil (7° 13' S; 39° 08' W). Following collection, the specimens were transported to the Laboratório de Zoologia (LZ-URCA) and deposited in terrariums to obtain the feces, which were collected and analyzed for the presence of pentastomid eggs. The eggs found were typical of the genus *Raillietiella*, differing from those of other genera due to the lack of an outer flexible membrane. The larva had two pairs of limbs, each with a pair of terminal hooks. The limbs were unsegmented, ventrally curved, and supported by conical muscle structures with visible segmentation. The tail was bifurcate, and each section had a terminal bristle. Information on egg and larval morphology can be useful in the identification of genera and species. The current study provides a description of the eggs and embryos of a Pentastomida species from the Neotropical Region.

Keywords: parasitism; life cycle; neotropical region

Introduction

Pentastomids are obligatory parasites of the vertebrate respiratory system (Self, 2009). The Pentastomida class comprises two large clusters: Cephalobaenida and Porocephalida. The Cephalobaenida comprises two families: Reighardiidae with a single genus that parasitises species of birds, and Cephalobaenidae, which has three genera: *Cephalobaena, Raillietiella, and Mahafaliella* (Rego, 1983). Adult pentastomids feed largely on blood and can cause severe infections, sometimes resulting in host death (Paré, 2008). The genus *Raillietiella* (Cephalobaenida: Raillietiellidae) comprises approximately 45 species (Christoffersen & De Assis, 2015) and is the most diverse taxon in the Pentastomida. All are small organisms, often <25 mm, that mature principally in the lungs of reptiles (Riley, 1986). The life cycle is unknown for most species, however, it is known that raillietiellids have an indirect life cycle, using insects or other vertebrates to reach their definitive host (Ali & Riley, 1983; Riley, 1986).

Of the species of *Raillietiella*, only *Raillietiella furcocerca* (Diesing 1863), *Raillietiella gehyrae* (Bovien 1927), *Raillietiella frenata* (Ali, Riley, & Self 1981), and *Raillietiella gigliolii* (Winch & Riley 1985), have had their eggs and embryos described. Esslinger (1968) described the initial life stages of *R. furcocerca* collected from the snake *Clelia clelia* (Daudin 1803) in Colômbia, Pence and Canaris (1973) described the morphology of the eggs and larval stages of *R. gehyrae* found in the lungs of the lizard *Mabuya homalocephala*.

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In Brazil, some studies have been carried out on pentastomid infection rates in reptiles (Vrcibradic et al., 2002; Dias et al., 2005; Almeida et al., 2008a, 2008b; Anjos et al., 2008). However, none of them analyzed the egg and larva morphology of the species studied. Recent publications on Raillietiella mottae (Almeida, Freire, & Lopes 2008), have included studies of its taxonomy and occurrence (Almeida et al., 2008a; Silva et al., 2019), and evaluations of its infection rates, prevalence, and mean infection intensity in lizards in northeastern Brazil (Almeida et al., 2008b, 2009; Anjos et al., 2008; Sousa et al., 2010; Brito et al., 2014; Lima et al., 2018).

Knowledge of the morphology of eggs and the initial larvae may aid in identifying infected hosts, since eggs can be encountered in feces (Ali & Riley, 1983; Parê, 2008). In addition, such information might be useful for taxonomic purposes. Although Kelehear et al. (2011) argue that taxonomic studies should include morphological measurements and molecular data, the idea of identifying genera and species from egg morphology has been around since the very earliest pentastomid studies (e.g. Stiles, 1891).

In the current study, we present the description of the egg and larva I of a raillietiellid species (Raillietiella mottae) in the Neotropical region, with the intention of helping parasitologists, veterinarians, and physicians working with wild animals to identify pentastomiasis, as well as providing additional characters that can be used by taxonomists for species identification.

Materials and Methods

Collection of hosts

The initial phase of the study involved the manual collection of 33 lizards (27 Phyllopezus periosus and 6 Phyllopezus pollicaris) in the environmental protection area Cachoeira de Missão Velha, in Ceará state, northeastern Brazil (7° 13’ S, 39° 08’ W). The specimens were transported to the Laboratório de Zoologia da Universidade Regional do Cariri (LZ-URCA) and then allocated to individual terrariums.

Terrarium preparation and feces collection

The terrariums were mounted in the Laboratório de Zoologia da Universidade Regional do Cariri (LZ-URCA), in a 6 square metre space. Each terrarium consisted of a rectangular glass box 50 cm long by 50 cm high and 30 cm wide, covered with a mosquito screen cover supported by lateral and frontal pieces of wood. Inside, the walls near the top edge of each terrarium (approximately 8 cm) were covered with a layer of solid Vaseline to prevent the lizards from escaping. On the outside, a layer of Vaseline (approximately 5 cm) was applied to prevent the passage of insects. The substrate and one of the side walls of each terrarium was covered with an EVA (ethylene vinyl acetate) plate to retain heat. Halogen lamps (70 W) were installed externally and 10 cm from the EVA-covered surface of the four terrariums that surrounded the lamp, so that each lamp simultaneously heated four terrariums. The lamps were connected at 06:00 hrs and disconnected at 16:00 hrs. The room temperature and humidity were checked daily 50 cm from the heat source and in the centre of the laboratory.

Water and food were supplied to the lizards ad libitum, with cockroaches Nauphoeta cinerea (Olivier 1789) provided by the Laboratório de Biotecnologia da Universidade Regional do Cariri (LB-URCA) used as the food source. Cockroaches were provided at a rate equal to the recorded predation rates of individual lizards, with the terrariums being checked every day and the number of cockroaches counted so that there was always food available, but this did not exceed three preys per lizard. This method was adopted to reduce the possibility of the cockroaches feeding on the lizard feces, since the feces was the source of the pentastomid eggs for study.

Feces were collected daily with the aid of metal pincers (always cleaned with 70 % alcohol between uses in each terrarium). The collected material was then stored in individual-specific test tubes and kept at room temperature (25°C). The terrariums were cleaned every 15 days with 70 % alcohol. During this process, the lizards were isolated individually in plastic bags.

Parasitological feces examination and analysis

The lizards were checked for endoparasite infection by the examination of fecal flotation. The feces were weighed, then macerated in chalices with 2 ml of Sheather’s solution (Sheather, 1923). Additional solution was added up to the volume of 10 ml. Each sample was then homogenised for at least 5 min, sieved, placed in test tubes, and centrifuged for 2 min at 2,000 rpm. The test tubes were then placed upright in standard support racks and a small amount of Sheather’s solution was added. The samples were mounted on slides and examined under an optical microscope. A series of five samples was taken from each tube, and the eggs found were counted and measured (length and width). Larvae only ruptured egg membranes with the addition of a small volume (0.1 ml) of 2 % chlorine. Stage I larvae were measured immediately after rupture to prevent the material from being damaged by the action of the chlorine.

In order to perform the egg counts on the ovaries of the females, a grid was plotted on the screen of the image analyzer; each grid was composed of 24 squares (500 µm × 500 µm) and the counting was performed throughout the ovary. The eggs were counted and separated as for maturation into two categories: embryonated eggs, when embryo members, hooks, fulcrum, and buccal cadre were observed; and eggs not embryonated, when these structures were not visualised. Fifteen embryonated eggs were measured for length and width (C/L) and their volume calculated.

At the end of the experiment, the lizards whose feces did not con-
tain pentastomids eggs were euthanised with a lethal injection of lidocaine (2 %) and immediately dissected. Their respiratory system and cavities were analyzed for the presence of pentastomids with a stereomicroscopic loupe. Specimens of pentastomids were preserved in 70 % alcohol and subsequently slide-mounted in Hoyer’s medium and studied using a microscope equipped with an image analyzer (Zen 2 Blue Edition Zeiss). Pentastomids were measured, sexed, and assessed for reproductive maturity. Later, the specimens were deposited in the Parasitological Collection of the Universidade Regional do Cariri (URCA-P: 1187 – 1240). The measurements taken included: length, width, the dimensions of the anterior and posterior hooks AB and BC, and the total area. To obtain the measurements of the hooks AB and BC, we adopted the protocol used by Ali et al. (1981).

Statistics analysis
Data were tested for normality using the Shapiro-Wilk test. We used a linear regression analysis to test the influence of female size on ovary length and whether ovary length influenced the total number of eggs. The egg volume was calculated using the formula for an ellipsoid (V = π × C × L²/6). The volume of eggs with embryos, measured in the ovaries (15 eggs per female), was correlated with the ovary length (Spearman Correlation, following a Z test: Zar, 1999). All analyses were undertaken using the R statistical package (R Core Team, 2011).

Ethical Approval and/or Informed Consent

The specimen study was approved by the Comitê de Uso e Experimentação Animal, CEUA/URCA, under permit number 00011/2016.2.

Results

Egg and embryo morphology

We measured 270 eggs and 88 embryos collected via the fecal flotation test. The eggs (Figs. 1, and 2A, B) were oval shaped, similar to other raillietiellids, with a mean length of 97.68 ± 7.32 μm and mean width of 75.05 ± 5.31 μm (Table 1). The egg capsule consisted of two membranes. The outer membrane had a thickness of 1.92 ± 0.51 μm, low elasticity, and was easily ruptured by cover slip pressure. In contrast, the inner membrane was highly elastic and very resistant to pressure, and was 1.39 ± 0.38 μm thick. On contact with Sheather’s solution, a thick layer usually formed around each egg, which varied in width (4.68 ± 2.93 μm) and was easily ruptured. All the eggs eliminated in the feces contained a fully formed embryo.

Table 1. Length and width data available for Raillietiella spp. eggs (all measurements given in micrometers, μm).

| Species | Length (μ) | Width (μ) | Host | Author (year) |
|---------|-----------|-----------|------|---------------|
| Raillietiella geckonis | 89 | 70 | Gecko verticillatus | Sambon (1922) |
| Raillietiella kochi | 100 | 80 | Varanus ocellatus | Heymons (1926) |
| Raillietiella affinis | 80 | 60 | Gecko verticillatus | Heymons (1935) |
| Raillietiella chamaeleonis | 112 | 71 | Chamaeleo oustaleti / C. verrucosus | Gretillet & Brygoo (1959) |
| Raillietiella congolensis | 75 | 50 | Thelotornis c.atesis | Fain (1961) |
| Raillietiella schoutedeni | 80 | 60 | Monopeltis schoutedeni | Fain (1961) |
| Raillietiella bohlongeri | 85 | 70 | Serpentes do Congo | Fain (1964) |
| Raillietiella furcocerca | 115 | 88 | Clelia clelia | Esslinger (1968) |
| Raillietiella gebyre | 103 | 60 | Mabuya homalocephala | Pence & Canaris (1973) |
| Raillietiella gigliolii | 127 | 92 | Amphisbaena alba | Winch & Riley (1985) |
| Raillietiella trachaea | 167 | 109.5 | Gyps bengalensis | Riley et al. (2003) |
| Raillietiella moreni | 84.2 | 53 | Gallotia atlantica | Abreu-costa et al. (2005) |
| Raillietiella mottae | 97.68 | 75.05 | Ptylopezus periosus/ P. pollicaris | Current study |
The larvae (Fig. 2C) had a length of 149.14 ± 8.84 μm and a width of 78.97 ± 5.12 μm (dorsal organ level), and two pairs of limbs, each with a pair of terminal hooks. The anterior limbs were 17.47 ± 1.81 μm in length, with hooks of unequal sizes (10.17 ± 1.3 μm and 7.64 ± 0.93 μm). The posterior limbs were 16.78 ± 2.52 μm in length, with hooks of 10.73 ± 1.76 μm and 7.88 ± 1.08 μm. The hooks were slightly curved and strongly chitinised, and the limbs were supported by conical muscular structures. The terminal bifurcated tail processes measured 52.80 ± 7.24 μm, and their terminal spines 14.12 ± 2.34 μm. Dorsolateral spines (4.72 ± 1.03 μm) were present close to the anterior pair of limbs. The buccal cadre was 19.19 ± 2.60 μm in length and 12.55 ± 2.18 μm in width, with no distinction between regions. The penetration apparatus (Fig. 2D), located dorso-anteriorly, consisted of a central pair of spines 16.27 ± 1.90 μm in length, with two pairs of cuticular spines (5.74 ± 1.34 μm). The dorsal organ, located anteriorly, measured 7.2 ± 1.11 μm in diameter, and was surrounded by a variable number of giant cells. The larvae had visible segmentation, with non-segmented ventrally curved limbs.

Production of eggs
Between January and June 2017, 23 fecal analyses were performed and 5 geckos (4 P. periosus and 1 P. pollicaris) were identified as hosting pentastomids. A total of 687 eggs were counted in 35.75 grams of feces. The egg volume did not vary between host species (Z = 0.216, p-value > 0.070). Female ovary size and the number of eggs showed normal distributions (W = 0.91799, p-value = 0.1039; W = 0.93853, p-value = 0.248; W = 0.96318, p-value = 0.609, respectively). The regression analysis showed a statistically significant relationship between the ovary length and female length of R. mottae (Fig. 3A) (F = 75.05, R² = 0.815, p-value < 0.0001), and between the ovary size and total number

Fig. 2. Egg and larva of Raillietiella mottae. (A) Egg with larva (dorsal view). (B) Egg with larva (ventral view). (C) Larva (ventral view). (D) Anterior extremity of larva. Abbreviations: AS, Accessory spines; BC, Bucal Cadre; DS, accessory distal spines; GC, giant cell complex; FS, terminal furcal spine; PS, central penetrating spine; FU, furca; HO, hooks; AM, anterior member; EM, external membrane; IM, internal shell membrane; PM, posterior member, and DO, dorsal organ.
of eggs (Fig. 3B) ($F = 13.54$, $R^2 = 0.443$, p-value = 0.0018). Larger females had larger ovaries and more eggs, however, there was no relationship between ovary size and egg volume ($r_s = 0.124$, p-value = 0.612).

**Discussion**

**Morphology of the egg and embryo**

Although the genus *Raillietiella* contains approximately 45 species (Christoffersen & De Assis, 2015), egg production information and larval descriptions are scarce. It is known that cephalobaenid eggs are distinguished by the presence of two membranes, whereas porocephalids have three—two overlapping membranes and one outer membrane—which forms a kind of pouch around the egg (Heymons, 1926; Keegan, 1943; Esslinger, 1962, 1968). The egg length and width are known for some species. However, they are not standardised, with some giving averages while others only raw data, thus making it difficult to compare species.

Esslinger (1968) found that certain aspects, such as characteristics of the penetration apparatus and tail structure, can be used as taxonomic criteria to distinguish *Raillietiella* species. In addition, species of the Cephalobaenida and Porocephalida orders can be separated using the morphology of embryonated eggs and infecting larvae. Cephalobaenids have a narrow and elongated buccal cadre and a completely closed buccal ring, whereas porocephalids have a trapezoid-shaped cadre and U-shaped buccal ring (Heymons, 1926; Fain, 1961, 1964; Nicoli & Nicoli, 1966; Esslinger, 1968). The cadre and buccal ring of *R. mottae* has a shape and length similar to that of other cephalobaenids, but differs from *R. furcocerca* and *R. gehyrae* in that the posterior and anterior parts of the cadre are undivided.

The general morphology of *R. mottae* larvae appears to closely resemble that of other raiieliellids, with previous descriptions of other larvae such as *R. furcocerca* (Esslinger, 1968), *R. gehyrae* (Ali & Riley, 1983), and *R. gigliolii* (Winch & Riley, 1985) generally applying to the larvae described here. The most obvious common similarities these larvae have are: (I) body segmentation, (II) penetrating apparatus located dorso-anteriorly, (III) a bifurcated caudal structure, and (IV) two pairs of anterior and posterior non-segmented limbs. These appendages are located in ventrolateral positions, with laterally curved hooks, and are, as in all raiieliellids, of unequal size. *Raillietiella furcocerca* and *R. gehyrae* have claws fused directly into the limb muscle, whereas in *R. mottae*, the claws are attached to the muscle through a broad base structure. The central penetration apparatus seen here is similar to that so far described for all raiieliellids, with only small differences in length being seen: *R. furcocerca*, 23 μm (Esslinger, 1968), *R. gehyrae*, 22 μm (Ali & Riley, 1983), *R. gigliolii*, 25 μm (Winch & Riley, 1985), and *R. mottae* (16.27 ± 1.90 μm). In all cases, the apparatus is wider at the base and at the tip, with a marked central narrowing. The cuticular spines near the penetration apparatus occur as a pair on each side and a more distal pair located laterally. This characteristic is common to other raiieliellids, although some authors consider these to be a third pair of spines (Fain, 1961; Nicoli, 1963) or papillae (Heymons, 1926). Due to their lateral rather than central location, these paired structures can be considered, in *R. furcocerca*, *R. gehyrae*, and *R. mottae*, as separate to the central series.

The dorsal organ has a uniform appearance, similar to that described for *R. furcocerca* by Esslinger (1968). However, the giant cells differ in quantity between the species; whereas *R. furcocerca* has two pairs and *Porocephalus crotali* (Humboldt 1808), has only one pair of this type of cell (Esslinger, 1962), *R. mottae* has a variable number (11 – 18) of these cells. According to Noc (1923), the dorsal organ secretes a gelatinous substance that protects the larva. In addition to mucus secretion, this structure might also be associated with embryo nutrition (Riley, 1983).

The length of the stage I larval tail can be used to characterise...
genera and possibly species (Esslinger, 1968). In the present study, the R. mottae furca measured 7.24 μm, differing from the 29 μm reported for R. furcocerca by Esslinger (1968). Although it is possible to identify cephalobaenids, raillietiellids, and porocephalids based on the morphology of the eggs and larvae, these aspects have been described only for a few species.

**Egg production**

In Raillietiella spp., eggs are released for several years via host feces. Subsequently, these are ingested by coprophagous insects (intermediate host), which can then be predated by the definitive (vertebrate) host (Lavoipierre & Rajamanickam, 1973; Ali & Riley, 1983; Which & Riley, 1985; Bosch, 1986). The pattern of egg production and release differs between cephalobaenids; Raillietiella orientalis (Hett, 1915), might have as many as 200,000 eggs in the ovary, making it the raillietiellid with the highest known number of eggs per female (Riley, 1983). In contrast, R. mottae females have between 371 and 1,716 eggs in the ovary, a low level of fecundity compared to related species. Such differences might be associated with as-yet unknown factors relating to the life cycle and intermediate hosts used by R. mottae.

Endoparasites can maximise either egg number or size according to their infection strategy and the nature of the definitive host (Daniels et al., 2013). For example, the copepod Diaptomus clavipes (Schacht, 1897) invests in larger eggs and smaller litters. This might be an adaptive response, since larger eggs can generate larger individuals that are more likely to survive to reproduce (Cooney & Gehrs, 1980). Given the size of the body, larger females of R. mottae tend to have larger ovaries and these produce more eggs, however, the eggs do not differ in their volume, showing that R. mottae invests in the quantity of eggs; not in their volume.

Raillietiella mottae has continuous egg production, a characteristic that might well be present in all species in the genus (Ali & Riley, 1983; Riley, 1983). The eggs do not differ in volume among the analyzed hosts, although the use of different host species does result in the production of different numbers and sizes of eggs by some ectoparasites (Daniels et al., 2013) However, this does not seem to be applicable to pentastomids infecting geckos. When R. gehyrae and R. frenatus females reach maturity and begin to release eggs, they will have between 4000 and 5500 eggs in the ovary at any one time, with approximately 25 and 36 % of these being fully embryonated. In our study, no females with less than 20 % of their eggs non-embryonated released their eggs, making it impossible to detect infection using fecal examination.

Egg release in R. mottae, R. gehyrae, and R. frenatus differs from R. sternae. In the latter species, eggs are retained in the uterus until all are capable of infection (Banaja et al., 1976). Ali & Riley (1983) reported in that in R. gehyrae, the uterus can contain 4150 – 6980 eggs in various stages of maturity, and that these are constantly mixed by waves of peristalsis. Since only infectious eggs are released, it is assumed that the vagina has some sort of sieving device that selectively allows the passage of eggs of an appropriate size while preventing the passage of smaller, undeveloped, eggs (Ali & Riley, 1983). It was not possible to locate this structure, and further work is required to identify and describe this selective filter.

**Larval development**

Larval development in raillietiellids occurs progressively and is comparable to the development in hemimetabolic insects, in which the successive stages of larvae differ so that divergence occurs gradually (Bosch, 1986). Esslinger (1962) showed that the larval stage inside the egg of P. crotali ecldes in the alimentary tract of the intermediate host, and that the larva migrates inside the cavity of the body and invades the superficial tissues of the viscera. The larva then changes to become the first of a series of six stages, the last of which can infect the definitive host. Ali & Riley (1983) reported that in Blaberus discoidalis (Serville, 1839), the eggs of R. frenatus and R. gehyrae hatch within a few hours of ingestion, and the primary larvae then migrate to the hemocoel, where they appear to crawl freely in the fat of the body. A few days after infection, third stage larvae can be found, which are capable of infecting geckos. Ingested infectious larvae in the definitive host penetrate the intestinal wall, crawl over the viscera, and soon after, are found in the lung. Winch & Riley (1985) observed strong similarities in the development of R. gigliolii in C. biloba with that of R. gehyrae and R. frenatus. Considering the similarities in larval development among closely-related species, it seems reasonable to assume that, as with R. gehyrae and R. frenatus, the third larval stage of R. mottae is the one capable of infecting the final host. Unfortunately, it was not possible to monitor the development of all larval stages in this study. However, we assume that the development of R. mottae in the definitive host will be similar to that of R. gehyrae and R. frenatus in other gecko species, where five and six moults occur for males and females, respectively.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Ali, J.H., Riley, J., Self, J.T. (1981): A revision of the taxonomy of the blunt-hooked Raillietiella, pentastomid parasites of African, South-East-Asian and Indonesian lizards, with a description of a new species. Syst Parasitol., 3: 103 – 207

Ali, J.H., Riley, J. (1983): Experimental life-cycle studies of Raillietiella gehyrae Bovien 1927 and Raillietiella frenatus Ali, Riley and Self 1981: pentastomid parasites of geckos utilizing insects as intermediate hosts. Parasitology, 86(1): 147 – 160

Almeida, W.O., Freire, E.M.X., Lopes, S.G. (2008a): A new species of pentastomid infecting Tropidurus hispidus (Squamata: Tropiduridae) from Caatinga in Northeastern Brazil. Braz. J. Biol., 68(1): 199 – 203. DOI: 10.1590/S1519-69842008000100029

Almeida, W.O., Santana, G.G., Vieira, W.L.S., Wanderley, I.C., Freire, E.M.X., Vasconcelos, A. (2008b): Pentastomid, Raillietiella mottae Almeida, Freire and Lopes, 2008, infecting lizards in an area of caatinga, northeast, Brazil. Braz. J. Biol., 68(2): 631 – 637. DOI: 10.1590/S1519-69842008000200028

Almeida, W.O., Santana, G.G., Vieira, W.L.S., Wanderley, I.C., Ribeiro, S.C. (2009): Rates of pulmonary infection by pentastomids in lizards species from a restinga habitat in northeastern Brazil. Braz. J. Biol., 69(1): 197 – 200. DOI: 10.1590/S1519-69842009000100026

Anjos, L.A., Almeida, W.O., Vasconcelos, A., Freire, E.M.X., Rocha, C.F.D. (2008): Pentastomids infecting an invader lizard, Hemidactylus mabouia (Gekkonidae) in Northeastern Brazil. Braz. J. Biol., 69(3): 611 – 615. DOI: 10.1590/S1519-69842008000300019

Banajia, A.A., James, J.L., Riley, J. (1976): Some observations on egg production and uro-reinfection of Reighardia sternae (Diesing, 1864), a pentastomid parasite of the herring gull. Parasitology, 72(1): 81 – 91

Bosch, H. (1986): Experimental life-cycle studies of Raillietiella Sambon, 1910 (Pentastomida, Cephalobaenidae) the 4th stage larva is infective for the definitive host. Parasitol. Res., 72: 673 – 680

Brito, S.V., Corso, G., Almeida, A., Ferreira, F.S., Almeida, W.O., Anjos, L.A., Mesquita, D.O., Vasconcelos, A. (2014): Phylogeny and micro-habitats utilized by lizards determines the composition of their endoparasites in the semiarid Caatinga of Northeast Brazil. Parasitol. Res., 113(11): 3963 – 3972. DOI: 10.1007/s00436-014-0601-z

Christoffersen, M.L., De Assis, J.E. (2015): Class Eupentastomida Waloszek, Repetski & Maas, 2006. In: Klein, J.C.V.V., Charmantier-Daures, M., Schram, F.R. (Eds) Treatise on zoology-Anatomy, taxonomy, biology. The Crustacea. Volume 5. Leiden, Netherlands: Brill Publishers, pp 5 – 75

Cooney, J.D., Gehrs, C.W. (1980): Effects of varying food concentration on reproduction in Diaptomus clavipes Schacht. Am. Midl. Nat., 104(1): 63 – 69. DOI: 10.2307/2424959

Daniels, R.R., Beltran, S., Poulin, R., Lagrue, C. (2013): Do parasites adopt different strategies in different intermediate hosts? Host size, not host species, influences Coitocacemum parvum (Trematoda) life history strategy, size and egg production. Parasitology, 140(2): 275 – 283. DOI: 10.1017/S0031182012001564

Dias, E.J.R., Vicembradic, D., Rocha, C.F.D. (2005): Endoparasites infecting two species of whiptail lizards (Cnemidophorus abatesenis and C. ocellifer; Teiidae) in a restinga habitat of northeastern Brazil. Herpetol. J., 15: 133 – 137

Esslinger, J.H. (1962): Development of Porocephalus crotali (Humboldt, 1808) (Pentastomida) in experimental intermediate hosts. J. Parasitol., 48(3): 452 – 456. DOI: 10.2307/3275214

Esslinger, J.H. (1968): Morphology of the egg and larva Raillietiella furcocerca (Pentastomida) from a Colombian snake (Celia cilia). J. Parasitol., 54(2): 411 – 416. DOI: 10.2307/3276962

Fain, A. (1961): Les Pentastomides de l’Afrique Centrale [Pentastomids of Central Africa]. Ann. Musee. Roy. Afr. Centr. Ser. 8 Sci. Zool., 92: 1 – 115 (In French)

Fain, A. (1964): Observations sur le cycle évolutif du genre Raillietiella (Pentastomida) [Observations on the life cycle of the genus Raillietiella (Pentastomida)]. Bull. Acad. R. de Belg., 50(5): 1036 – 1060 (In French)

Gretillat, S., Brygoo, E.R. (1959): Raillietiella chamaeleonis n. sp. première espèce de Cephalobaenidae (Pentastomida) signalée a Madagascar [Raillietiella chamaeleonis n. sp. first species of Cephalobaenidae (Pentastomida) reported in Madagascar]. Ann. Parasitol. Hum. Comp., 34(1 – 2): 112 – 120. DOI: 10.1051/parasite/1959341112 (In French)

Heymons, R. (1926): Beiträge zur Kenntnis der Gattung Raillietiella Samb [Contributions to the knowledge of the genus Raillietiella Samb]. Zool. Anz., 67: 45 – 56 (In German)

Heymons, R. (1935): Pentastomida. In: Bronns Klassen und Ordnungen des Tierreiches. Volume 5, pp. 1 – 268

Keegan, H.L. (1943): Observations on the Pentastomid, Kiricephalus coarctatus (Diesing) Sambon. Trans. Am. Microscop. Soc., 62(2): 194 – 199. DOI: 10.2307/2322921

Kelehearn, C., Spratt, D.M., Dubey, S., Brown, G.P., Shine, R. (2011): Using combined morphological, allometric and molecular approaches to identify species of the genus Raillietiella (Pentastomida). PloS one, 6(9), e24936. DOI: 10.1371/journal.pone.0024936.

Lavoipierre, M.M.J., Rajamanickam, C. (1973): Experimental studies on the life cycle of a lizard pentastomid. J. Med. Entomol., 10(3): 301 – 302. DOI: 10.1093/jmedent/10.3.301

Lima, V.F., Brito, S.V., Araujo Filho, J.A., Teles, D.A., Ribeiro, S.C., Teixeira, A.A.M., Pereira, A.M.A., Almeida, W.O. (2018): Raillietiella mottae (Pentastomida: Raillietiellidae) parasitizing four species of Gekkota lizards (Gekkonidae and Phyllodactylidae) in the Brazilian Caatinga. Helminthologia, 55(2): 140 – 145. DOI: 10.2478/helm-2018-0006
Nicoli, R.M. (1963): Phylogénèse et systématique le phylum des Pentastomida [Phylogenesis and systematics of the phylum Pentastomida]. *Ann. Parasitol. Hum. Comp.*, 38(3): 483 – 516. DOI: 10.1051/parasite/1963383483 (In French)

Nicoli, R.M., Nicoli, J. (1966): Biologie des pentastomides [Biology of the pentastomids]. *Ann. Parasitol. Hum. Comp.*, 41(3): 77 – 255 (In French)

Noc, F. (1923): Sur l’embryon acariforme et les states larvaires des Linguatulides [On the acariform embryo and the larval states of the Linguatulids]. *Bull. Soc. Pathol. Exot.*, 16: 340 – 346. DOI: 10.1051/parasite/1966413255 (In French)

Pare, J.A. (2008): An overview of pentastomiasis in reptiles and other vertebrates. *J. Exot. Pet. Med.*, 17(4): 285 – 294. DOI: 10.1053/j.jepm.2008.07.005

Pence, D.B., Canceris, A.G. (1973): Observations on the Pentastome Raillietiella gehydro Bovien, 1927 (Cephalobaenida: Cephalobaenidae) from Mabuya homalocephala in Kenya. *Parasitol. Res.*, 41: 1 – 10

R Core Team. (2011): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rego, A.A. (1983): Pentastomídeos de répteis do Brasil: Revisão dos Cephalobaenidae [Pentastomids of reptiles from Brazil: Review of Cephalobaenidae]. *Mem. Inst. Oswaldo Cruz*, 78(4): 399 – 411. DOI: 10.1590/S0074-02761983000400003 (In Portuguese)

Riley, J. (1983): Recent advances in our understanding of pentastomid reproductive biology. *Parasitology*, 86(4): 59 – 83. DOI: 10.1017/S0031182000050848

Riley, J. (1986): The biology of pentastomids. *Adv. Parasitol.*, 25: 45 – 128. DOI: 10.1016/S0065-308X(08)60342-5

Riley, J., OXAS, J.L., Gilbert, M. (2003): Raillietiella trachea n. sp., a pentastomid from the trachea of an oriental white-backed vulture Gyps bengalensis taken in Pakistan, with speculation about its life-cycle. *Syst. Parasitol.*, 56: 155 – 161

Sambon, L.W. (1922): A synopsis of the family Linguatulidae. *J. Trop. Med. Hyg.*, 25(12): 188 – 206, 391 – 428

Self, J.T. (2009): Pentastomida: Tongue worms. In: Roberts, L.S., Janovy, J. (Eds). Gerald D. Shimidt & Larry S. Robert’s *Foundations of Parasitology* - McGraw-Hill, New York, p. 561-568

Sheather, A. L. (1923): The detection of intestinal protozoa and mange parasites by a flotation technique. *J. Comp. Pathol.*, 36(4): 266 – 275

Silva, E.G., Santos, M.E.P., Brito, S.V., Almeida, W.O., Ribeiro, S.C. (2019): Raillietiella mottae (Pentastomida: Raillietiellidae) infecting Ameiva ameiva (Squamata: Teiidae) in Araripe Plateau, Northeast Brazil. *Braz. J. Biol.*, 79(1): 100 – 103. DOI: 10.1590/1519-6984.178609

Sousa, J.G.G., Ribeiro, S.C., Roberto, I.J., Teles, D.A., Almeida, W.O. (2010): Ocorrência de pentastomídeos (Metameria: Ecdysozoa) no lagarto Phyllopezus pollicaris (Spix, 1825) [Occurrence of the pentastomids (Metameria: Ecdysozoa) in the lizard Phyllopezus pollicaris (Spix, 1825)]. *Cad. Cult. Ciênc.*, 2(2): 64 – 71 (In Portuguese)

Stiles, C. W. (1891): Bau und Entwicklungsgeschichte von Pentastomum probosciudem Rud. Und Pentastomum subcyllindricum Dies [Construction and history of Pentastomum probosciudem Rud. and Pentastomum subcyllindricum Dies.]. *Z. wiss. Zool. Abt. A.*, 52: 85 – 157 (In German)

Vrcibradic, D., Rocha, C.F.D., Bursey, C.D., Vicente, J.J. (2002): Helminth communities of two sympatric skinks (Mabuya agilis and Mabuya macrorhyncha) from two ‘restinga’ habitats in southeastern Brazil. *J. Helminthol.*, 76(4): 355 – 361. DOI: 10.1079/JOH2002134

Winch, J.M., Riley, J. (1985): Experimental studies on the life-cycle of Raillietiella gigliolii (Pentastomida: Cephalobaenidae) in the South American worm-lizard *Amphisbaena alba*: a unique interaction involving two insects. *Parasitology*, 91(3): 471 – 481. DOI: 10.1017/S0031182000062715