

# A new species of *Cruzia* (Ascaridida; Kathlanidae) parasitizing *Salvator merianae* (Squamata, Teiidae) from the Atlantic forest in Brazil

Uma nova espécie de *Cruzia* (Ascaridida; Kathlanidae) parasitando *Salvator merianae* (Squamata, Teiidae) de Mata Atlântica no Brasil

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## Abstract

*Cruzia lauroi* sp. nov. is described from *Salvator merianae* (Duméril & Bibron, 1839) (Squamata; Teiidae). The new species differs from all previously described species through several morphological characteristics: number of tooth like structures per row in the inner pharynx; and presence of unpaired papillae on the anterior border of the cloacal aperture. However, *Cruzia lauroi* sp. nov. is closest to *C. tentaculata* (Rudolphi, 1819), through having similar distribution of male caudal papillae, unpaired pre-cloacal papillae and females with an pre-equatorial vulva. *Cruzia lauroi* sp. nov. differs from *C. tentaculata* regarding smaller total body length of individuals, higher number of tooth like structures per row in the pharynx, greater size of diverticulum, smaller size of spicules and a more anterior vulva than in *C. tentaculata*; and the males do not have caudal alae. *Cruzia mazza*, *C. travassosia*, *C. mexicana* and *C. testudines* were considered to be *species inquirendae*, because their descriptions need more detailed taxonomic studies.

**Keywords:** *Cruzia lauroi* sp. nov., lizard, new species, *Salvator merianae*, Brazil.

## Resumo

*Cruzia lauroi* sp. nov. é uma nova espécie descrita em *Salvator merianae* (Duméril & Bibron, 1839) (Squamata; Teiidae). Essa nova espécie difere de todas as espécies descritas anteriormente por diferentes características morfológicas: pelo número de dentes por coluna longitudinal interna na faringe; pela presença de papila ímpar na borda anterior da cloaca. Entretanto, *Cruzia lauroi* sp. nov. possui similaridades com *C. tentaculata* (Rudolphi, 1819), por ter distribuição semelhante das papilas caudais dos machos, por possuir papila pré-cloacal ímpar e por possuir fêmeas com vulva pré-equatorial. *Cruzia lauroi* sp. nov. se difere de *C. tentaculata* em relação ao menor comprimento total do corpo dos indivíduos, pelo maior número de dentes por coluna longitudinal interna na faringe; pelo maior tamanho de divertículo; pelo menor tamanho de espículos; por possuir fêmeas com vulva mais próxima a extremidade anterior do que observado em fêmeas de *C. tentaculata*; e pelos

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machos não possuem asa caudal. *Cruzia mazza*, *C. travassosia*, *C. mexicana* e *C. testudines* foram consideradas *species inquirendae*, pelo fato de suas descrições necessitarem de maior detalhamento taxonômico.

**Palavras-chave:** *Cruzia lauroi* sp. nov., lagartos, nova espécie, *Salvator merianae*, Brasil.

## Introduction

Nematodes of the genus *Cruzia* Travassos, 1917, have been reported to inhabit the large intestine of amphibians, reptiles and, especially, mammals (Ruiz, 1947; Lent & Freitas, 1948; Ubelaker & Younus, 1965; Adnet et al., 2009). Until the current study, this genus included 12 nominal species that occur in North, Central and South America (Ruiz, 1947; Lent & Freitas, 1948; Wahid, 1964; Costa, 1965; Ubelaker & Younus, 1965; Adnet et al., 2009). However, some species of this genus need more detailed taxonomic studies and need to be re-evaluated.

In reptiles of the Neotropical region, three species of *Cruzia* have been reported: *Cruzia rudolphii* Ruiz, 1947; *C. tentaculata* (Rudolphi, 1819); and *Cruzia travassosia* Khalil & Vogelsang, 1932 (Ruiz, 1947; Lent & Freitas, 1948; Baker, 1987; Ávila et al., 2008; 2010; Ávila & Silva, 2010). However, most of these reports do not provide any morphological or morphometric data on the species. *Cruzia fulleborni* Khalil and Vogelsang, 1930, was reported by Ávila & Silva (2010) to be a parasite of *Tupinambis teguixin* (Linnaeus, 1758) (Squamata; Teiidae) (= *Salvator merianae*) from Argentina and Paraguay. It was transferred to the genus *Schizobucca* Schuurmans-Stekhoven Jr., 1950 (Ascaridida; Kathlanidae) by Schuurmans-Stekhoven (1950).

This study describes a new species of *Cruzia* parasitizing *Salvator merianae* (Duméril & Bibron, 1839) (Squamata; Teiidae), from the Atlantic forest biome region in the state of Minas Gerais, Brazil.

## Materials and Methods

The nematodes were collected from the large intestine of one specimen of *S. merianae* that was found dead in the municipality of Juiz de Fora (21°76' S; 43°21' W), state of Minas Gerais, Brazil. This lizard was identified based on Peters et al. (1986) and was deposited in the Coleção Herpetologica da Universidade Federal de Juiz de Fora – Repteis (CHUFJF – Repteis 1488), Universidade Federal de Juiz de Fora, state of Minas Gerais, Brazil.

Living parasites were fixed in 4% hot formalin and were kept in this for 15 days. After this period, they were conditioned in 70°GL ethanol for morphological studies. For species identification, 20 adult nematodes were separated for analysis. The specimens were clarified in Amann's lactophenol, mounted on temporary slides and analyzed under an Olympus BX-41 microscope equipped with drawing tube and micrometric ocular lens, in the *Laboratorio de Helmitos Parasitos de Vertebrados* of the *Instituto Oswaldo Cruz*, FIOCRUZ, Rio de Janeiro, Brazil. All measurements are given in micrometers as ranges.

The specimens were identified to genus level in accordance with Chabaud (1978). The identification key of species of *Cruzia* was elaborated from the original descriptions of the species of this genus or studies of revision of these species. Holotype, allotype and paratypes were deposited in the Helminthological Collection of the *Instituto Oswaldo Cruz* (CHIOC), FIOCRUZ, RJ, Brazil.

## Results

### Description

#### *Cruzia lauroi* sp. nov. (Figure 1)

**General morphology.** The parasites are long, straight and thick. The mouth is surrounded by three well-formed lips (Figure 1B). The shape of the lips is pyramidal, with triangular spaces left between each of them (Figure 1B). The dorsal lip has a pair of lateral papillae, and the two latero-ventral lips have only one papilla and one amphid on each (Figure 1B). One pair of tiny tooth like structures is located at the inner margin of each lip (Figure 1B). The pharynx is lined with a cuticle that has three columns with two parallel lines of tooth like structures each, that look like a saw, which project into the lumen (Figures 1A, B). The intestinal diverticulum extends anteriorly beyond the esophageal bulb (Figure 1A). Lateral alae are absent.

**Males** (Based on 10 adult specimens). Body elongated. Total body length 6.60–8.82 mm ( $7.41 \pm 0.98$  mm), maximum width 337–375 ( $362.5 \pm 35.94$ ). Pharynx 97–150 ( $125 \pm 26.46$ ) long. Esophagus 1.31–1.95 mm ( $1.63 \pm 0.29$  mm) long and 80–150 ( $117.5 \pm 31.22$ ) wide, bulb 200–310 ( $270 \pm 60.83$ ) long and 200–300 ( $253 \pm 50.33$ ) wide. Nerve ring 260–480 ( $375 \pm 90$ ) and excretory pore 770–930 ( $840 \pm 93.1$ ) from cephalic end. Intestinal diverticulum 550–850 ( $775 \pm 150$ ) long. Posterior region ventrally curved, becomes narrower posterior to cloacal aperture and almost cylindrical in the posterior third, ending as a conical tip shape (Figure 1C). Cloacal aperture 140–250 ( $197.5 \pm 45$ ) from posterior end. Caudal region shows 10 pairs of sessile papillae symmetrically ventro-laterally located, three large pre-cloacal papillae, three ad cloacal papillae and four post-cloacal papillae, and one unpaired papillae at the anterior border of cloacal aperture (Figures 1C-E). Gubernaculum with triangular shape (Figure 1D) 130–180 ( $155 \pm 20.82$ ) long. Spicules similar, long and robust (Figures 1C, D), 600–750 ( $648 \pm 63.06$ ) long.

**Females** (Based on 10 adult specimens). Body elongated. Total body length 8.21–9.75 mm ( $8.89 \pm 0.66$  mm), maximum width 370–450 ( $420 \pm 34.64$ ). Pharynx 140–200 ( $165 \pm 25.17$ ) long. Esophagus 1.56–2 mm ( $1.87 \pm 0.22$  mm) long and 120–150 ( $133 \pm 12.88$ ) wide, bulb 270–330 ( $302 \pm 25$ ) long and 280–330 ( $307.5 \pm 22.17$ ) wide. Nerve ring 260–420 ( $370 \pm 77.89$ ) and excretory pore 870–920 ( $955 \pm 55.1$ ) from cephalic end. Intestinal diverticulum 470–980 ( $725 \pm 294.45$ ) long. Vulva pre-cloacal, 1.71–2.17 mm ( $1.95 \pm 0.23$ ) from cephalic end. Eggs not observed, adult females, but with eggs not yet formed. Posterior end sharply conical (Figure 1F), tail 610–920 ( $730.5 \pm 130.48$ ) long.

### Taxonomic summary

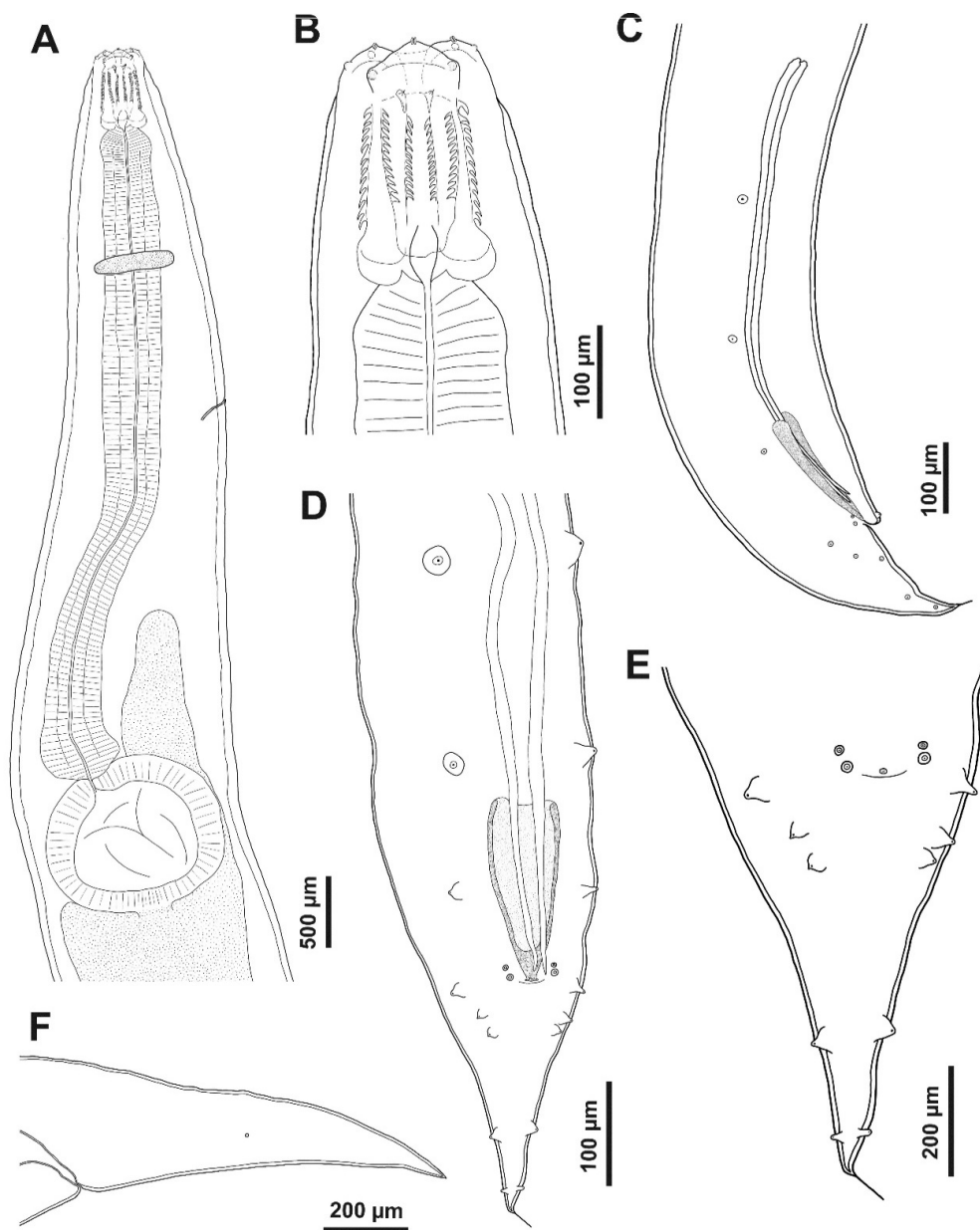
**Type-host:** *Salvator merianae* (Duméril & Bibron, 1839) (Squamata, Teiidae) (Argentine Black and White Tegu).

**Locality:** Municipality of Juiz de Fora (21°76'S, 43°21' W), state of Minas Gerais, Brazil.

**Site of infection:** Large intestine.

**Type-material:** Holotype male (CHIOC 38781a), allotype female (CHIOC 38781b), 2 male and 2 female paratypes (CHIOC 38781c).

**Etymology.** Specific name is in honor of Dr. Lauro Travassos from the *Instituto Oswaldo Cruz*, FIOCRUZ, Brazil, for his great contribution to the knowledge of Brazilian helminth biodiversity.



**Figure 1.** *Cruzia lauroi* sp. nov. (A) anterior region of body, male, lateral view; (B) cephalic end, male, lateral view; (C) posterior region, male, lateral view; (D) posterior region, male, latero-ventral view; (E) tail, male, latero-ventral view; (F) tail, female, lateral view.

### Discussion

The new species was assigned to the genus *Cruzia* because of the following morphological characteristics. The anterior end shows an oral aperture surrounded by three lips: on the dorsal lip, there is a pair of lateral papillae; and on the latero-ventral lips, one lateral papilla on each. There is one pair of tooth like structures located at the inner margin of each lip. The pharynx shows three columns with small tooth like structures, like a saw, which projects inwards and into the lumen. The intestinal diverticulum is prominent: it projects anteriorly and goes beyond the esophageal bulb. In males, the caudal region presents symmetrically located sessile papillae, a triangular gubernaculum and two spicules that are similar to each other and do not have a cloacal sucker.

Currently, nine species of *Cruzia* are considered to be valid (Table 1): six have been described from mammal hosts, two are parasitic in reptiles and one occurs in amphibians (Sprehn, 1932; Ruiz, 1947; Wolfgang, 1951; Crites, 1956; Wahid, 1964; Costa, 1965; Ubelaker & Younus, 1965; Guerrero, 1971; Adnet et al., 2009).

In different species of *Cruzia*, the number of teeth per row in the inner pharynx may be the main characteristic enabling species differentiation. *Cruzia lauroi* sp. nov. has 12 teeth in each row in the pharynx, which differs from *C. buckleyi* Wahid, 1964, and *C. empera* Guerrero, 1971, which have fewer teeth per row (Wahid, 1964; Guerrero, 1971). It also differs from *C. americana* Maplestone, 1930, *C. boliviana* Sprehn, 1932, *C. brasiliensis* Costa, 1965, and *C. rudolphii* Ruiz, 1947, which have considerably greater numbers of teeth per row than does *Cruzia lauroi* sp. nov. (Sprehn, 1932; Ruiz, 1947; Crites, 1956; Costa, 1965) (Table 1).

*Cruzia cameroni* Wolfgang, 1951, *C. tentaculata* (Rudolphi, 1819) and *C. tropidodipsi* Ubelaker & Younus, 1965, present variation in the number of teeth per row of the pharynx that is close to that of *Cruzia lauroi* sp. nov. (Wolfgang, 1951; Ubelaker & Younus, 1965; Adnet et al., 2009) (Table 1). However, *Cruzia lauroi* sp. nov. differs from *C. cameroni* and *C. tropidodipsi* in that these last two species do not have an unpaired papilla on the anterior border of the cloacal lip; and in relation to the position of the vulva, which in *Cruzia lauroi* sp. nov. is pre-cloacal (Table 1).

The species of the genus *Cruzia* in which the set of morphological and morphometric characteristics is closest to those of *Cruzia lauroi* sp. nov. is *C. tentaculata* (Rudolphi, 1819) (Table 1), which occurs mainly in mammals of the family Didelphidae (Travassos, 1917, 1922; Ruiz, 1947; Adnet et al., 2009). The latter species has also been recorded in *Tupinambis teguixin* (Linnaeus, 1758) (Squamata; Teiidae), and *Rhinella marina* (Linnaeus, 1758) (Anura; Bufonidae) (Ruiz, 1947).

*Cruzia lauroi* sp. nov. differs from *C. tentaculata* in that the individuals of the new species have shorter body length, higher number of toothlike structures per row of the pharynx, greater size of diverticulum in comparison with the size of the esophagus, smaller absolute size of spicules and a more anterior vulva than in *C. tentaculata*; and the males do not have caudal alae (Table 2).

**Table 1.** Species of *Cruzia* Travassos, 1917, taxonomic characters of species, type hosts and localities of species.

<i>Cruzia</i> spp.	Characters						Type host	Country	References
	Male			Female					
	Number of tooth-like structures	Spicule (µm)	Gubernaculum (µm)	Caudal papillae pre: ad: postcloacal	Unpaired Papillae	Vulva position			
<i>Cruzia americana</i> Maplestone, 1930	15–18	890–1000	140–160	3: 3: 5	No	Pre- equatorial	<i>Didelphis marsupialis</i> (Mammal)	USA	Crites (1956)
<i>Cruzia boliviana</i> Sprehn, 1932	16–18	880	180	3: 3: 4	Yes	Pre- equatorial	<i>Tolypeutes matacus</i> (= <i>T. conurus</i> ) and <i>Dasyops novemcinctus</i> (= <i>T. novemcinctus</i> ) (Mammal)	Paraguay and Bolivia	Sprehn (1932)
<i>Cruzia brasiliensis</i> Costa, 1965	17–22	848	219	3: 3: 5	Yes	Equatorial	<i>Sus scrofa</i> (Mammal)	Brazil	Costa (1965)
<i>Cruzia buckleyi</i> Wahid, 1964	6–7	1500–1700	260	3: 2: 4	Yes	Post-equatorial	<i>Callimico goeldii</i> (Mammal)	South America*	Wahid (1964)
<i>Cruzia cameroni</i> Wolfgang, 1951	13–17	1170–1270	180–195	3:3:4	No	Equatorial	<i>Didelphis marsupialis</i> (Mammal)	Trinidad	Wolfgang (1951)
<i>Cruzia empera</i> Guerrero, 1971	9	697–737	123–131	3: 3: 4	No	Equatorial	<i>Rhinella marina</i> (= <i>Bufo marinus</i> ) (frog)	Venezuela	Guerrero (1971)
<i>Cruzia lauroi</i> sp. nov.	12	600–750	130–180	3: 3: 4	Yes	Pre-equatorial	<i>Salvator merianae</i> (Lizard)	Brazil	Current study
<i>Cruzia rudolphi</i> Ruiz, 1947	16–18	490	130	3: 3: 4	Yes	Equatorial	<i>Erythrolamprus aesculapii</i> (Snake)	Brazil	Ruiz (1947)
<i>Cruzia tentaculata</i> (Rudolphi, 1819)	10	890–960	160–190	3: 3: 4	Yes	Pre- equatorial	<i>Didelphis aurita</i> (Mammal)	Brazil	Adnet et al. (2009)
<i>Cruzia tropidodipsi</i> Ubelaker and Younus, 1965	13–14	760–1210	150	3: 3: 4	No	Post- equatorial	<i>Tropidodipsas fasciata</i> (Snake)	Mexico	Ubelaker & Younus (1965)

\*Host from an unnamed country in South America, and inhabiting the Zoological Garden of London, UK.

**Table 2.** Comparative morphometrics of *Cruzia lauroi* sp. nov. and *Cruzia tentaculata* (Rudolphi, 1819).

	<i>Cruzia lauroi</i> sp. nov.		<i>Cruzia tentaculata</i> (Rudolphi, 1819)	
Hosts	<i>Salvator merianae</i> (Duméril & Bibron, 1839)		<i>Didelphis aurita</i> (Wied-Neuwied, 1826), <i>D. marsupialis</i> Linnaeus, 1758	
References	Current study		Adnet et al. (2009)	
Localities	Brazil		Brazil, Colombia	
	Females	Males	Females	Males
Body length (mm)	8.21–9.75	6.60–8.82	11.40–12.20	11–12.05
Body width	370–450	330–410	500–550	530–650
Pharynx length	140–200	90–150	170–260	170–210
Number of tooth-like structures in each row	12	12	10	10
Oesophagus length (mm)	1.56–2	1.31–1.95	1.59–2.16	2.07–2.32
Oesophagus width	120–150	80–95	90–140	100–130
Oesophagus bulb length	270–330	200–310	250–270	290–320
Oesophagus bulb width	280–330	200–300	250–320	270–330
Nerve ring	260–420	260–480	480–550	480–550
Excretory pore	920	770–930	1180–1300	1180–1300
Diverticulum length	470–980	550–850	390–450	370–420
Diverticulum - oesophagus ratio	40%	42%	20%	18%
Spicule length		600–750		890–960
Gubernaculum length		130–180		160–190
Number and position of caudal papillae		10 pairs (3 pre + 3 ad + 4 post) + 1 unpaired		10 pairs (3 pre + 3 ad + 4 post) + 1 unpaired
Vulva from anterior end (mm)	1.71–2.17 (Pre equatorial)		5.54–5.83 (Pre equatorial)	
Vulva - body length ratio	21%		48%	
Egg length	-		100–130	
Egg width	-		60	
Tail	610–920	140–250	690–1040	150–180



The most recent identification key for species of *Cruzia* was drafted by Ruiz (1947). However, some species were proposed after that study (*C. brasiliensis*, *C. buckleyi*, *C. cameroni*, *C. empera* and *C. tropidodipsi*); or were included in other genera, as was the case of *Pseudocruzia orientalis* (Maplestone, 1931), which was originally described as *C. orientalis* and was transferred to *Pseudocruzia* by Wolfgang (1953), and *Schizobucca fulleborni* (Khalil and Vogelsang, 1931), which was originally described as *C. fulleborni* and was transferred to *Schizobucca* by Schuurmans-Stekhoven Jr. (1950). Chabaud (1978) considered that the genera *Schizobucca* and *Pseudocruzia* were valid. Therefore, we present here the latest key to identification of valid species of *Cruzia*.

### Key to species of *Cruzia*

1. Male with less than 15 tooth-like structures in each columns of pharynx ..... 2
  - Male with 15 or more tooth-like structures in each columns of pharynx ..... 6
2. Absence of unpaired papilla on the anterior border of the cloaca ..... 3
  - Presence of unpaired papilla on the anterior border of the cloaca ..... 4
3. Spicules greater than 900  $\mu\text{m}$  ..... *Cruzia cameroni* Wolfgang, 1951
  - Spicules shorter than 900  $\mu\text{m}$  ..... *Cruzia empera* Guerrero, 1971
4. Vulva pre-equatorial ..... 5
  - Vulva post-equatorial ..... *Cruzia buckleyi* Wahid, 1964
5. Male without caudal alae. Spicules shorter than 800  $\mu\text{m}$ . Twelve tooth-like structures in each columns of pharynx ..... *Cruzia lauroi* sp. nov.
  - Male with small caudal alae. Spicules larger than 800  $\mu\text{m}$ . Ten tooth-like structures in each columns of pharynx ..... *Cruzia tentaculata* (Rudolphi, 1819)
6. Presence of unpaired papilla on the anterior border of the cloaca ..... 7
  - Absence of unpaired papilla on the anterior border of the cloaca ..... 8
7. Eleven pair of caudal papillae. Vulva pre-equatorial .....
  - ..... *Cruzia americana* Maplestone, 1930
  - Ten pair of caudal papillae. Vulva post-equatorial .....
    - ..... *Cruzia tropidodipsi* Ubelaker and Younus, 1965
8. Spicules greater than 600  $\mu\text{m}$  ..... 9
  - Spicules shorter than 600  $\mu\text{m}$  ..... *Cruzia rudolphi* Ruiz, 1947
9. Eleven pair of caudal papillae ..... *Cruzia brasiliensis* Costa, 1965
  - Ten pair of caudal papillae ..... *Cruzia boliviana* Sprehn, 1932

Only a few species of *Cruzia* have been reported from reptiles in South America (Ávila et al., 2010). Moreover, most of these records do not detail any morphological or morphometric characteristics of the parasites, which leaves doubt regarding their identification. *Cruzia tentaculata* was reported parasitizing *T. teguixin* in Uruguay but Lent & Freitas (1948) did not provide any taxonomic description of this species. The same situation occurred in relation to the records of *C. travassosia* in *S. merianae* from the Cerrado biome in Brazil, which was studied by Ávila et al. (2010); and in relation to *C. rudolphii* from *Hoplocercus spinosus* Fitzinger, 1843 (Squamata; Hoplocercidae) in the Brazilian Cerrado (Ávila et al., 2008). The study by Ruiz (1947) is the only one that has provided detailed taxonomic descriptions of the species of *Cruzia* parasitizing Brazilian hosts, including *C. rudolphii*, which is a parasite of *Erythrolamprus aesculapii* (Linnaeus, 1758) (Squamata; Colubridae) in Brazil. Therefore, it can be stated that further taxonomic studies on species of *Cruzia* in Brazilian reptiles are necessary, in order to understand the real dimensions of the biodiversity of the species of this genus in Brazilian reptiles.

Other species previously assigned to *Cruzia* deserve some comments. *C. mazza* and *C. travassosia*, which were found in mammals of the genus *Tolypeutes* Illiger, 1811 (Cingulata; Dasypodidae), in Argentina, are poorly described, because the number of



tooth like structures is unknown, the description needs more graphic representation of taxonomic structures, the information about type material is non-existent and further details on the type locality were not reported (Khalil & Vogelsang, 1932). In relation to *Cruzia mexicana*, a parasite from an unspecified species of lizard in Mexico, the number of tooth like structures in the pharynx was not informed and the information about type material is non-existent (Khalil, 1927). *Cruzia testudinis*, which occurs in *Terrapene carolina* (Linnaeus, 1758) (Testudines, Emydidae) in the USA, was poorly described, since the number of tooth like structures in the pharynx is unknown and the graphical representation of the species is non-existent (Hardwood, 1932). Therefore, we prefer to consider these species as *species inquirendae*.

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