

# New morphological data on *Cucullanus pinnai pinnai* (Nematoda) parasitizing *Pimelodus maculatus* (Pimelodidae) in southeastern Brazil

Novos dados morfológicos de *Cucullanus pinnai pinnai* (Nematoda) parasito de *Pimelodus maculatus* (Pimelodidae) do Sudeste do Brasil

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## Abstract

This paper describes the morphology of *Cucullanus pinnai pinnai* parasitizing *Pimelodus maculatus* in the Guandu River, Brazil, based on differential interference contrast (DIC) microscopy and scanning electron microscopy (SEM), providing new morphological data about this species of parasite. Nematodes were collected between May and October 2012 from specimens of *Pimelodus maculatus* in the Guandu River ( $22^{\circ}48'2''S$ ,  $43^{\circ}37'35''W$ ), in the state of Rio de Janeiro, Brazil. Some characteristics of specimens of *Cucullanus* in this study fall within the range of morphological variations of previously studied *C. pinnai pinnai*. Most of the specimens studied here had excretory pore and deirids located at the posterior end of the oesophagus, a feature not recorded in previous studies of this species. In addition, the size of the gubernaculum was larger than the other specimens previously studied. The SEM and DIC analyses of *C. pinnai* revealed several morphological details of the cephalic region and the tail papillae. With regard to the polymorphism of *C. pinnai*, morphological and genetic studies of this cucullanid nematode are needed, involving large numbers of host species and a wide geographical distribution.

**Keywords:** *Cucullanus pinnai pinnai*, fish parasite, Pimelodidae, morphological data, Guandu River, Brazil.

## Resumo

O presente estudo descreve a morfologia de *Cucullanus pinnai pinnai*, parasito de *Pimelodus maculatus* do Rio Guandu, RJ, Brasil, utilizando recursos de contraste diferencial por interferência (DIC) e microscopia eletrônica de varredura (MEV), fornecendo novos dados morfológicos dessa espécie de *Cucullanus*. Os nematoides foram coletados em *Pimelodus maculatus*, entre maio e outubro de 2012, no Rio Guandu ( $22^{\circ}48'2''S$ ,  $43^{\circ}37'35''W$ ), Estado do Rio de Janeiro, Brasil. Algumas características dos espécimes de *Cucullanus* estudados estão de acordo com a amplitude de variação morfológica de *C. pinnai pinnai* previamente estudados. A posição do poro exretor e deirídeos nos *C. pinnai pinnai* estudados, é posterior ao final do esôfago na maioria dos espécimes, e essa característica não foi relatada previamente nesta espécie. O tamanho do gubernáculo é maior do que em outros espécimes de *C. pinnai pinnai* previamente estudados. As análises MEV e DIC demonstraram detalhes morfológicos da regiãocefálica e as papilas caudais dessa espécie. Em relação ao polimorfismo de *C. pinnai pinnai*, ao grande número de hospedeiros e à ampla distribuição geográfica desse cucullanídeo, será necessário um estudo que combine características genéticas e morfológicas desse parasito, com o objetivo de verificar possíveis novas espécies, especificidade de hospedeiros e localidades.

**Palavras-chave:** *Cucullanus pinnai pinnai*, parasito de peixes, Pimelodidae, dados morfológicos, Rio Guandu, Brasil.

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*Cucullanus* Müller, 1777 (Nematoda: Seuratoidea) comprises a large number of species that parasitize a great variety of freshwater and marine fish (MORAVEC, 2013). Twelve valid species of *Cucullanus* have been reported in freshwater fishes from Brazil (LUQUE et al., 2011).

*Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928, is the species of this genus with the largest range of host species in Brazil, and hence the one with the widest geographical distribution. Luque et al. (2011) listed a total of 17 nominal host species parasitized by this cucullanid species, distributed in Brazil's central-west, southeast and southern regions. In other countries of the neotropics, *C. pinnai* has been reported in fishes from Argentina by Hamann (1985) and from Paraguay by Petter (1995).

*Pimelodus maculatus* Lacépède, 1803 (Siluriformes, Pimelodidae), commonly known in Brazil as "mandi amarelo" or "bagre pintado," is distributed in several South American countries (FROESE & PAULY, 2014), and has been reported in Brazil in rivers ranging from the Amazon basin to the country's southernmost region (GODOY, 1987). In Brazil, this host species is parasitized by several nematode species, but to date, *C. pinnai pinnai* is the only valid species of this genus reported in *P. maculatus* (MORAVEC, 1998; LUQUE et al., 2011).

Morphological studies of *C. pinnai pinnai* by Moravec et al. (1993, 1997) indicate that this nematode species has a polymorphism relative to the position of the excretory pore and deirids, and the arrangement of caudal papillae in males. However, none of the previous morphological studies of this cucullanid species (TRAVASSOS et al., 1928; PETTER, 1995; MORAVEC et al., 1993, 1997) have characterized it based on differential interference contrast (DIC) microscopy and scanning electron microscopy (SEM) analyses. Therefore, the purpose of this study was to describe the specimens of *C. pinnai pinnai* collected from *P. maculatus* in the Guandu River, Brazil, using DIC and SEM methods and showing new morphological data.

Were studied nematodes collected in the intestine of 50 specimens of *Pimelodus maculatus*, collected between May and October 2012 in Guandu River ( $22^{\circ}48'2''S$ ,  $43^{\circ}37'35''W$ ), in the municipality of Seropédica, State of Rio de Janeiro, Brazil. The hosts were identified according to Britski et al. (1999).

The nematodes were fixed and preserved in formalin 5%. For identification and light microscopy morphometric studies, the nematodes were cleared in Amman's Lactophenol (1: 1: 2: 1 – phenol: lactic acid : glycerin: water). Measurements are given as ranges in micrometers ( $\mu m$ ), with the mean in parentheses. Photomicrographs were made using a compound Olympus BX51 light microscope equipped with Nomarski Differential Interference Contrast (DIC) optics. For scanning electron microscopy (SEM) some specimens were fixed in 1% modified Karnowsky (2% paraformaldehyde and 2.5% glutaraldehyde); postfixed in OsO<sub>4</sub>; dehydrated through a graded ethanol series; dried with CO<sub>2</sub>; coated with gold; and examined in a Quanta 200 FEI SEM, operating at 10 kV, at Center of Microscopy at the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil (<http://www.microscopia.ufmg.br>).

Identification of the parasites follows Moravec (1998) and Anderson et al. (2009). Voucher specimens are deposited in the

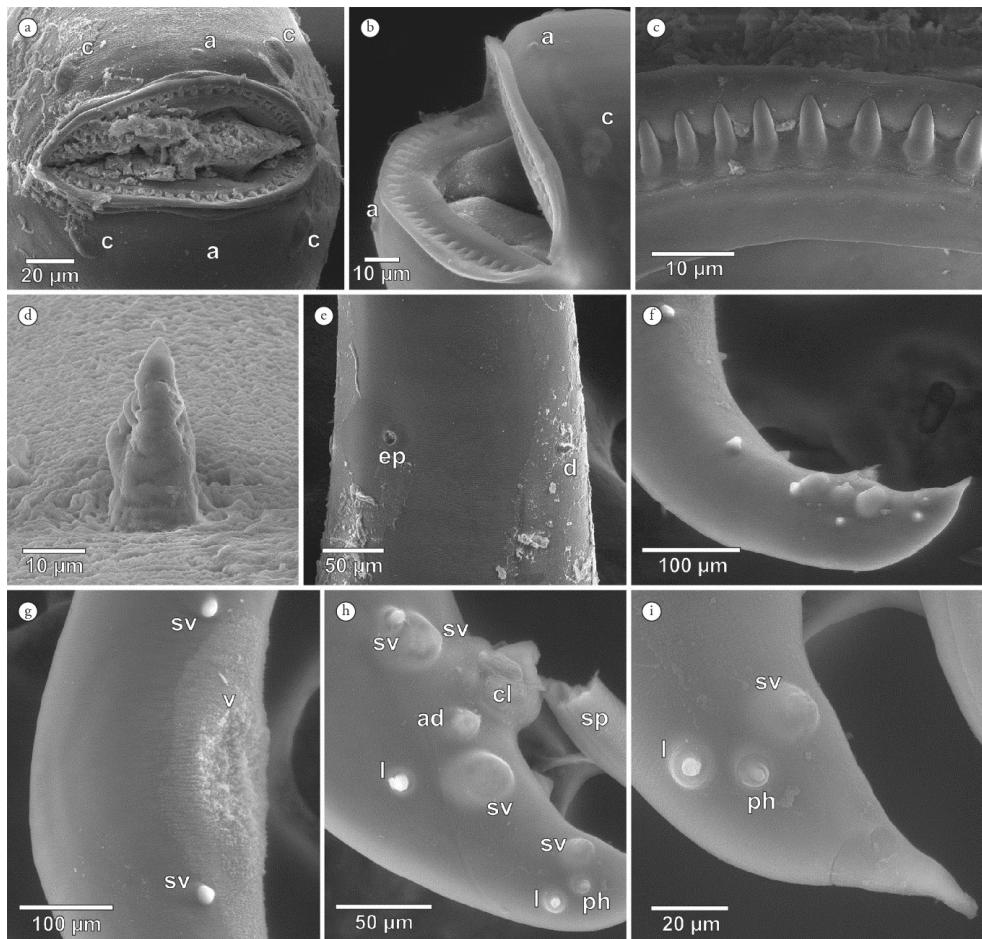
Instituto Oswaldo Cruz Helminthological Collection (CHIOC), Rio de Janeiro, Brazil.

## *Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928

**General:** Medium-sized nematodes. Body elongate. Slightly transversely striated cuticle. Cephalic end rounded, dorsoventrally expanded. Buccal opening dorsoventrally elongated, surrounded by narrow membranous ala (collarette) armed with approximately 60 minute triangular basal teeth (Figures 1a-c). Cephalic extremity with 2 pairs of submedian cephalic papillae (Figures 2b, 1a, b) and one pair of lateral amphids present. Oesophagus muscular expanded at ends, forming oesophastome (Figures 2a-c), opening into intestine through large valve. Nerve ring surrounding oesophagus at end of first third. Deirids spine-like (Figure 1d), and excretory pore at same level or posterior to oesophagus-intestinal junction (Figure 2d). Excretory pore weakly visible, slightly anterior or at the same level to deirids (Figures 2d, 1e). Both sexes have conical tail with pointed tip (Figures 2e, 1h, f, i).

**Male** (eight specimens measured): Length of body 6,651–10,526 (8,291); width at level of base of oesophagus 209–324 (281). Length of entire oesophagus 448–896 (759) long, representing 6.7–8.5 (7.9)% of entire body length. Maximum width of posterior part of oesophagus 142–308 (227). Distance of nerve ring from anterior end 231–402 (325). Deirids 225–975 (700) from anterior end (Figure 2c). Excretory pore 518–896 (733) from anterior end. Posterior region of body curved ventrally, with well developed precloacal sucker (Figure 1g) located 611–1,122 (871) from tip of tail. Cloacal region not protruded. Spicules equal, 400–713 (506) long, with pointed distal ends, representing 6–6.8 (6.1)% of body length. Gubernaculum spoon-shaped and well sclerotized (Figure 2f), 163–396 (265) long. Posterior region with 10 pairs of papillae, and 1 pair of papilla-like phasmids. Five pairs of precloacal subventral papillae; the first pair well anterior to ventral sucker border; the second somewhat posterior to ventral sucker (Figure 1g); the third approximately in mid-way between second pair of papillae and cloaca; and fourth anterior to fifth, which is close to the cloacal aperture (Figure 1h). One pair of adcloacal subventral papillae (Figure 1h). Four pairs of postcloacal papillae; first pair lateral slightly posterior to adcloacal papillae; second pair subventral immediately posterior to adcloacal papillae; third pair subventral, at the same level as the fourth pair of postcloacal, which is lateral (Figures 1h, i). One pair of small lateral papilla-like phasmids slightly posterior and between to third and fourth pairs of postcloacal papillae (Figure 1h, i). Tail 195–291 (241) long (Figures 2e, f, 1h, i).

**Female** (nine specimens measured): Length of body 6,833–12,586 (9,845), maximum width at level of the base of oesophagus 153–424 (294). Length of entire oesophagus 682–970 (898), representing 7.7–10 (9.1)% of entire body length. Maximum width of posterior part of oesophagus 162–191. Distance of nerve ring from anterior end 258–462 (350). Deirids 337–998 (693) from anterior end. Excretory pore 295–1,037 (724) from anterior end. Vulva slightly elevated (Figure 2g), postequatorial, 5,028–5,630 (5,211) from anterior end, representing 44.7–73.6% of body length. Ovijector



**Figure 1.** *Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928 scanning electron micrographs. (a) Anterior end of male, showing amphid and cephalic papillae, apical view, (b) Anterior end of male, showing amphid and cephalic papillae, ventral view, (c) teeth of the cephalic collarette, lateral view, (d) deirid, lateral view, (e) relative position of excretory pore and deirid, latero-ventral view, (f) posterior region of male, latero-ventral view, (g) Posterior end, showing sucker and first and second pair of precloacal papillae, lateral view, (h) cloacal region, showing adcloacal papilla, lateral papillae, papilla-like phasmid, spicule and subventral papillae, lateral view (i) tip of tail of male, showing lateral papillae, papilla-like phasmid and subventral papillae, lateral view. Abbreviations: a, amphid; ad, adcloacal papilla; c, cephalic papilla; ep, excretory pore; d, deirid; l, lateral papilla; ph, papilla-like phasmid; sp, spicule; sv, subventral papilla; v, ventral sucker.

directed anteriorly from vulva. Uteri amphidelphic. Eggs numerous, oval in shape, 61-69 (64) (n=30) length, 30-35 (33) (n=30) width. A pair of lateral papilla-like phasmids present between anus and tip of tail (Figure 2I). Tail 170-379 (297) long.

**Host:** *Pimelodus maculatus* Lacepède, 1803 (Pimelodidae, Siluriformes)

**Site of infection:** Intestine

**Prevalence:** 68% (50 host examined, 34 hosts infected)

**Mean intensity:** 3.17

**Mean abundance:** 2.16

**Localization:** Guandu River, Seropédica, State of Rio de Janeiro, Brazil (22°48'2"S, 43°37'35"W)

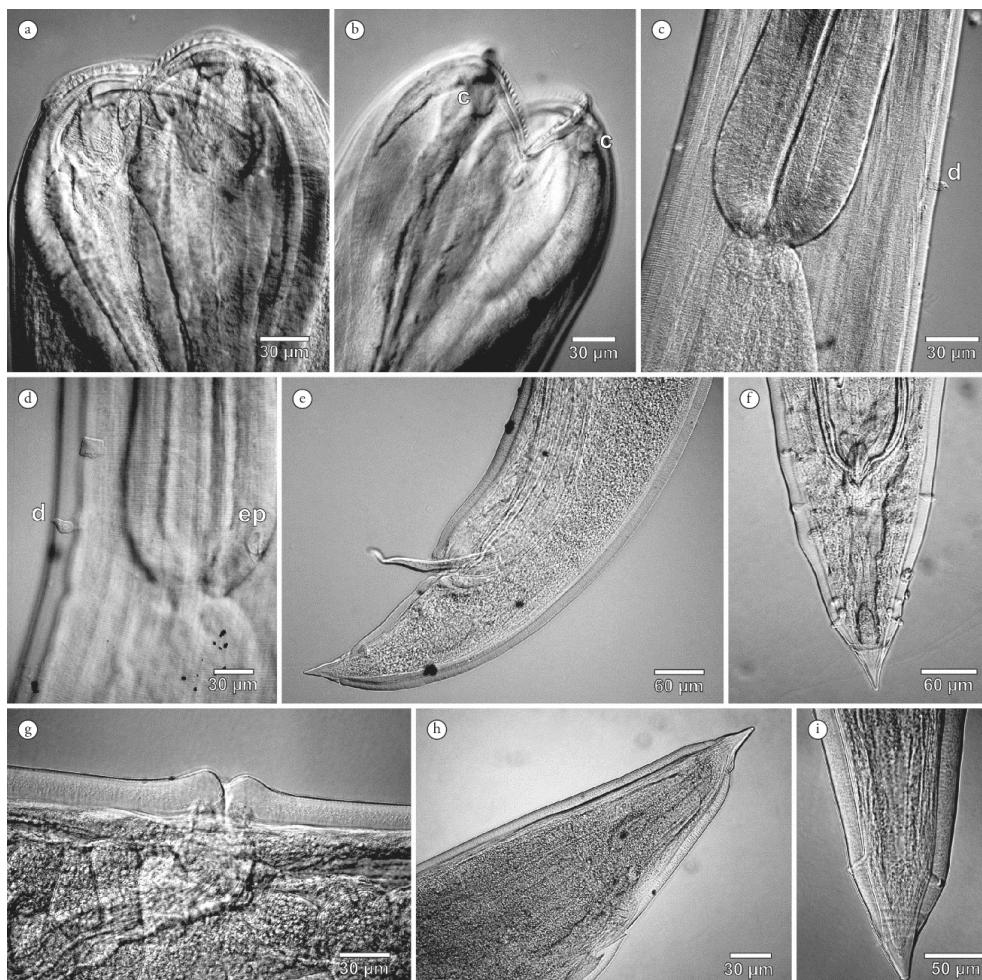
**Voucher specimens:** CHIOC No 36.732

*Cucullanus pinnai* Travassos Artigas & Pereira, 1928 was described from specimens collected from *Synodontis clarias* (Linnaeus, 1758) [= *Pimelodus clarias* (Linnaeus, 1758)] from Mogi Guaçu River, at the Cachoeira de Emas, in the municipality of Pirassununga, state of São Paulo, Brazil (TRAVASSOS et al., 1928). This description was based in only one male and an unspecified number of female

specimens; whose nerve ring, deirids and excretory pore positions and the presence of male gubernaculum were not reported.

Moravec et al. (1993) redescribed this species from specimens parasitizing *Pimelodus ornatus* Kner, 1858 and *Ageneiosus militaris* (Valenciennes, 1835) [= *Ageneiosus valenciennesi* (Bleeker, 1864)] from the Paraná River at locality of Guaíra, in the state of Paraná, Brazil. The authors' report provided the first description of the position of nerve ring, deirids and excretory pore in *C. pinnai*, and the presence of gubernaculum in males. However, these authors emphasized variations in the size of spicules and in the arrangement of postcloacal papillae in males of this species (MORAVEC et al., 1997).

In a subsequent studies with nematodes parasitizing fish in the Paraná River basin in Brazil, Moravec et al. (1997) collected specimens of *C. pinnai* from several species of Siluriformes fish, and proposed two subspecies for *C. pinnai*, namely: *C. pinnai pinnai* Travassos Artigas & Pereira, 1928; parasitic in fish of genera *Pimelodus* and *Pimelodella*; and *C. pinnai pterodorasi* Moravec, Kohn & Fernandes, 1997 parasitizing *Pterodoras granulosus*



**Figure 2.** *Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928 differential Interference Contrast light micrographs. (a) Anterior end of male, latero ventral view, (b) Anterior end of male, ventral view, (c) region of end of oesophagus, ventral view, (d) relative position of deirid and excretory pore, ventral view, (e) posterior region of male, lateral view, (f) tail of male, ventral view, (g) region of vulva of female, lateral view, (h) tail of female, lateral view, (i) tail of female, ventral view. Abbreviations: c, cephalic papilla; d, deirid; ep, excretory pore.

(Valenciennes, 1821) (Doradidae). According to these authors, the two subspecies differ with respect to the morphology of oesophastome and the position of their nerve ring.

Some morphometric and morphological features of the specimens of *Cucullanus* studied here are according to the range of morphological variations of *C. pinnai* reported by Hamann (1985) in fish from Argentina, Moravec et al. (1993) in fish from Brazil, and by Petter (1995) in fish from Paraguay; and the subspecies *C. pinnai pinnai* studied by Moravec et al. (1997) in fish from Brazil (Tables 1 and 2). However, in those studies, the authors reported that the excretory pore and deirids are anterior or near to the end of oesophagus, whereas we observed that these two structures are posterior to the end of oesophagus in most of specimens of *C. pinnai pinnai* of current study. Another difference observed between *C. pinnai pinnai* studied here and the specimens studied by Moravec et al. (1993, 1997) is the size of gubernaculum, which is two or three-fold larger in the specimens of *C. pinnai* of our study than in the specimens studied by Moravec et al. (1993, 1997). Hamann (1985) reports the presence of gubernaculum in *C. pinnai* from Argentina, however not provides the measurement of this structure.

A SEM analysis of *C. pinnai* was made for the first time in this study. This analysis revealed several morphological details of the cephalic end in this species, such as the number of teeth in the cephalic collarette; the shape of deirids; and the precise position of the cephalic papillae and amphids. The morphology of the tail of male was also analyzed by SEM to confirm the correct number and distribution of adcloacal and postcloacal papillae, corroborating the morphological descriptions made by Moravec et al. (1993, 1997).

With respect to the polymorphism of *C. pinnai*, given the large number of its host species, which include *Pimelodus albicans*, *P. maculatus*, *Paulicea luetkeni*, *Pimelodella gracilis*, *Pseudoplatystoma corruscans*, *Pseudopimelodus mangurus*, *Luciopimelodus pati*, *Megalonema platanum*, *Synodontis clarias*, *Steindachneridion parahybae* (all Pimelodidae), *Ageneiosus militaris* (Ageniosidae), and *Loricaria* sp. (Loricariidae) (all Siluriformes) (MORAVEC, 1998), and the wide geographical distribution for this cucullanid, it is reasonable to assume that the known subspecies are separate species. However, confirmation of this assumption will require an extensive collection of new specimens of *C. pinnai* from several host species and from different Neotropical river basins.

**Table 1.** Comparative measurements (in µm) of adult male specimens of *Cucullanus pinnai pinnai* from different host species of Argentina, Brazil and Paraguay.

Host species	Current Study		Travassos et al. (1928)		Hamann (1985)		Moravec et al. (1993)		Petter (1995)		Moravec et al. (1997)	
	<i>Pimelodus maculatus</i>	<i>Synodontis clarias</i> (= <i>Pimelodus clarias</i> ) (type host)	<i>Luciopimelodus pati</i>	<i>Pseudoplatystoma corruscans</i>	<i>Pimelodus albicans</i>	<i>Pimelodus claritas</i>	<i>Pimelodus ornatus</i>	<i>Ageniosus militaris</i>	<i>Pseudoplatystoma corruscans</i>	<i>Megalonema platannum</i>	<i>Pimelodus maculatus</i>	<i>Loricaria sp.</i>
Number of nematodes measured	8	1	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	2	1	12	6	3
Total body length	6,651-10,526	8,500	6,000-11,000	9,000-11,000	6,000-8,000	4,000-8,000	5,070	8,000	3,000-4,000	2,450-5,600	6,010-11,880	4,200
Body width	209-324	300	160-350	180-230	270-300	160-300	258-272	204	-	-	326-435	177
Oesophagus length	448-896	680-850	510-800	600-1,000	500-720	600-800	789	789	390-600	350-725	680-979	544
Nerve ring	231-402	-	-	-	-	-	313	299	180-250	140-250	286-313	204
Excretory pore	518-896	-	-	-	-	-	558	-	-	-	544	408
Deirids	225-975	-	-	-	-	-	639	585	350-450	320-575	680-966	405
Spicules	400-713	570	430-610	420-550	420-550	381-681	345	250-370	115-470	408-585	598	
Gubernaculum	136-396	-	-	-	-	-	63-69	63	-	-	60-75	63
Tail	195-291	200	150-260	200-250	150-180	150-200	114-192	190	-	-	190-286	109
Locality	Guandu River, Seropédica, Rio de Janeiro (Brazil)	Mogi-Guaçu River, Piras-sununga (type locality), São Paulo (Brazil)	Paraná River, Corrientes (Argentina)		Paraná River, Guairá, Paraná (Brazil)		Paraná River, Province of Itapúa (Paraguay)		Reservoir of Itaipu, Guaira, Paraná (Brazil)		Reservoir of Itaipu, Guaira, Paraná (Brazil)	

**Table 2.** Comparative measurements (in µm) of adult female specimens of *Cucullanus pinnai* from different host species of Brazil and Argentina.

Host species	Current Study	Travassos et al. (1928)		Hamann (1985)		Moravec et al. (1993)		Moravec et al. (1997)			
		<i>Pimelodus maculatus</i>	<i>Synodontis clarias</i> (= <i>Pimelodus clarias</i> ) (type host)	<i>Luciopimelodus pati</i>	<i>Pseudoplatystoma coruscans</i>	<i>Pimelodus albicans</i>	<i>Pimelodus clarinus</i>	<i>Pimelodella gracilis</i>	<i>Pimelodus maculatus</i>	<i>Paulicea luerkeni</i>	<i>Loricaria sp.</i>
Number of nematodes measured	9	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified	1	1	3	2
Total body length	6,833-12,586	5,900-9,800	6,000-13,000	9,000-17,000	8,000-16,500	5,000-7,000	9,070	7,630	6,460-12,310	5,300-5,940	5,070
Body width	153-424	280-300	250-400	300-500	410-500	320-410	367	299	272-394	231-272	245
Oesophagus length	682-970	680-850	600-1,500	600-1,200	600-1,100	600-850	898	857	694-952	598-707	612
Nerve ring	258-462	-	-	-	-	-	313	326	258-354	122-136	231
Excretory pore	295-1,037	-	-	-	-	-	680	653	476-925	517-639	449
Deirids	337-998	-	-	-	-	-	762	666	517-911	530-585	462
Vulva to posterior end	1805-6,956	2,100-3,900	2,000-6,000	3,000-5,500	2,000-4,000	2,000-3,000	3,750	3,350	2,830-4,990	2,420-2,590	2,680
Tail	170-390	240-450	220-400	220-450	240-400	240-400	258	231	286-381	204-272	204
Eggs (length × width)	61-69 × 30-35	45-54 × 27-29	40-75 × 31-50	40-75 × 31-50	40-75 × 31-50	40-75 × 31-50	42-51 × 30-33	-	45-63 × 30-42	-	54-60 × 39
Locality	Guandu River, Serté-pédica, Rio de Janeiro (Brazil)	Mogi-Guaçu River, Pirassununga (type locality), São Paulo (Brazil)	Paraná River, Corrientes (Argentina)	Paraná River, Paraná (Brazil)	Paraná River, Guaíra, Paraná (Brazil)	Paraná River, Guaíra, Paraná (Brazil)	Paraná River, Itaipu, Paraná (Brazil)	Reservoir of Paraná River, Itaipu, Paraná (Brazil)	Reservoir of Paraná River, Foz do Iguaçu, Paraná (Brazil)	Reservoir of Paraná River, Foz do Iguaçu, Paraná (Brazil)	Reservoir of Paraná River, Itaipu, Paraná (Brazil)

These specimens must be properly prepared for a combining morphological and genetic studies aimed to verifying potential new species, host and river basin specificity, and the phylogenetic relationships of *C. pinnae* with other species of *Cucullanus* parasitic in Neotropical fish.

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