Research and Reviews in Parasitology, 53 (3-4): 97-102 (1993)

Editorial Fontalba, S.A.
Printed in Spain

LONGIBUCCA CATESBEIANAE N. SP. (NEMATODA: CYLINDROCORPORIDAE), A GASTROINTESTINAL PARASITE OF THE BULLFROG *RANA CATESBEIANA* SHAW, 1802 IN BRAZIL

F.L. DE SOUZA JUNIOR, P. DE TOLEDO ARTIGAS & M. LATERÇA MARTINS

Departamento de Parasitologia, Instituto de Ciências Biomédicas da Universidade de São Paulo, Avenida Professor Lineu Prestes nº 1374, Edifício Biomédicas II, São Paulo, Brazil

Received 3 June 1992; accepted 17 June 1993

REFERENCE: SOUZA JUNIOR (F.L. DE), TOLEDO ARTIGAS (P. DE) & LATERÇA MARTINS (M.), 1993.— *Longibucca catesbeianae* n. sp. (Nematoda: Cylindrocorporidae), a gastrointestinal parasite of the bullfrog (*Rana catesbeiana* Shaw, 1802) in Brazil. *Research and Reviews in Parasitology*, 53 (3-4): 97-102.

ABSTRACT: A new species of nematode of the genus *Longibucca* Chitwood, 1933 (Cylindrocorporidae) is described. The worms were obtained from the stomach and gut of bullfrogs (*Rana catesbeiana* Shaw, 1802). The specimens of anuran hosts were obtained from a frog farm in the city of Ubatuba, in the Southeast of São Paulo State, Brazil. The name *Longibucca catesbeianae* n. sp. is proposed for the new species, found invading stomach tissues only. Comparative morphobiometric data between this and other species of the same genus are presented.

KEY WORDS: *Longibucca catesbeianae* n. sp., Nematoda, Cylindrocorporidae, gastrointestinal parasite, bullfrog, *Rana catesbeiana*, Brazil.

INTRODUCTION

Nematodes of the family Cylindrocorporidae Goodey, 1939, were obtained from male and female adults of the species *Rana catesbeiana* Shaw, 1802 raised on a frog farm in the Southeast State of São Paulo, Brazil. Among the Cylindrocorporidae it is possible to find species which are free-living and saprophagous, and others that live as parasites of vertebrates such as amphibians, reptiles and mammals (CHITWOOD & CHITWOOD, 1977; ANDERSON & BAIN, 1982).

According to YAMAGUTI (1961) the genus *Longibucca* Chitwood, 1933 is the only one known that parasites vertebrates. The species of *Longibucca* are small worms with narrow lateral alae along the body and a round oral opening, surrounded by six discrete lips, each one with papillae disposed in circle and six others in a second, external circle (CHITWOOD, 1933; MCINTOSH & CHITWOOD, 1934; ELSEA, 1953; CHITWOOD & CHITWOOD, 1977). The genus includes the type species *Longibucca vivipara* Chitwood, 1933, from a reptilian, *Longibucca eptesica* Elsea, 1953 and *Longibucca lasiura* McIntosh et Chitwood, 1933, from bats (ANDERSON & BAIN, 1982). Our purposes in this paper are: 1) to describe a nematode found in the stomach and gut of amphibians and propose the name *Longibucca catesbeianae* n. sp. for it; 2) to compare its morphobiometric data with those of other known species of the same genus.

MATERIAL AND METHODS

The anurans utilized in this study came from a commercial frog farm which has operated intensively since 1982 in the city of Ubatuba, São Paulo, Brazil, producing *R. catesbeiana*. Parasites were taken from frogs collected among those in confinement and in others found in external areas or surroundings of the frog farm. A total of 679 confined specimens of *R. catesbeiana* were examined. Of these, 528 were tadpoles, 30 subadults and 121 adults. Species found outside the frog farm included: *R. catesbeiana* (18 adults); *Leptodactylus ocellatus* (Linnaeus, 1758) (32 adults); *Hyla albomarginata* Spix, 1824 (34 adults); *Hyla faber* Wied, 1824 (9 adults); *Bufo ictericus* Spix, 1824 (3 adults); *Bufo crucifer* Wied, 1821 (5 adults); *Phrynohyas mesophaea* Hensel, 1867 (2 adults) and *Hyla geographica* Spix, 1824 (107 tadpoles).

Tadpoles were kept in 20 l aquaria with dechlorinated and filtered running water until necropsies were perfomed. Subadults and adults of *R. catesbeiana* were kept in 50 l aquaria with a maximum of six animals per aquarium. Others were maintained isolated in small, individual aquaria. In the course of this experiment, tadpoles were fed with an adequate food mix whereas the young and adult forms received newborn mice.

The nematodes obtained through necropsies were fixed in hot AFA and preserved in a solution of 95% ethanol (70 GL) and 5% glycerin. Before being examined, the worms were cleared in Amann lactophenol. Camara lucida was used for drawings. The study *en face* was done according to the method of CHITWOOD & WEHR (1934). The histopathological examinations were done on the stomach and intestines of a heavily parasited bullfrog. The tissue sections were stained as usual with Haematoxylin-eosin.

RESULTS

A total of 889 amphibians were necropsied. Of these, 635 were tadpoles, 30 subadults and 224 adults. Only 67 (7,53%) adult specimens of *R. catesbeiana* examined were contaminated with *L. catesbeianae* n. sp. Of these, 54 (80,59%) were from the confinement while 13 (19,40%) were taken from the external and surrounding areas of the frog farm.

Nematodes were mainly in the stomach of frogs and in great number, usually over 2500 worms. In one case we counted 10726 worms in a frog captured outside the farm: 9680 in the stomach and the remaining 596 in the gut. The highest number of nematodes collected in frogs from the interior of the farm was 8826 (8630 in the stomach and 196 in the gut). A few frogs showed a low

Species	*L. catesbeianae* n. sp.	*L. vivipara*	*L. lasiura*	*L. eptesica*
Total length	545-700 (595,68)	500-550	660-740	485-647
Maximum width	10-14 (12,61)	10-12	22-28	20-32
Stoma length	50-57 (54,00)	55-60	40-44	—
Corpus length	11-25 (21,83)	18-20	22-32	—
Corpus width	4-6 (5,05)	4	7-8	—
Isthmus length	4-10 (8,35)	22	—	—
Isthmus width	2-4 (2,83)	2	—	—
Pseudobulb length	8-11 (9,48)	16	—	—
Pseudobulb width	5-8 (6,14)	4	—	—
Oesophageal length	31-44 (39,78)	—	46-51	56-60
Base of oesophagus[a]	85-105 (95,00)	115,7-122,7	97-120	—
Nerve ring[a]	75-85 (80,00)	70-75	68-78	—
Excretory pore[a]	95-105 (99,16)	65-75	100-105	—
Gonads[a]	260-350 (293,05)	224	300-400	—
Spicules	12-20 (15,85)	13-17	29-31* 22-25**	26-29
Gubernaculum	5-10 (6,85)***	4,5	16-20	22-25***
Cloaca[b]	65-90 (78,80)	—	78-93	—

Table 1.- - Comparative measures (μm) of 15 male specimens of *Longibucca catesbeianae* n. sp. collected from *Rana catesbeiana* Shaw, 1802, with other species of the genus *Longibucca* Chitwood, 1933. ( )=mean; a=distance from anterior extremity; b=distance from cloaca to the end of the tail; *=large spicule; **=small spicule; ***=measure taken along the inner margin of gubernaculum.

Species	*L. catesbeianae* n. sp.	*L. vivipara*	*L. lasiura*	*L. eptesica*
Total length	520-707 (631,04)	580-630	680-840	710-825
Maximum width	13-26 (19,50)	18-20	30-44	28-44
Stoma length	45-70 (54,42)	50-64	47-50	45-53
Corpus length	11-27 (20,00)	20-25	22-32	—
Corpus width	3-6 (4,83)	3,5-4	7-8	—
Isthmus length	4-12 (8,27)	18-20	—	—
Istmus width	2-4 (3,14)	1,5-2	—	—
Pseudobulb length	7-13 (10,07)	15-18	—	—
Pseudobulb width	5-8 (6,39)	5-6	- -	—
Oesophageal length	20-45 (38,07)	- -	56-60	46-51
Base of oesophagus[a]	72-100 (91,00)	107-131,5	97-120	—
Nerve ring[a]	60-90 (76,71)	85-97	68-78	—
Excretory pore[a]	75-105 (92,16)	80-92	100-105	78-92
Gonads[a]	90-200 (135,52)	160-200	200-260	—
Vulva[a]	425-573 (523,55)	480-520	560-690	—
Anus[b]	19-39 (28,95)	—	50-52	—
Tail[c]	62-100 (79,87)	- -	70-87	—
Total length of larvae	175-245 (210,68)	—	- -	194-470

Table 2.- - Comparative measures (μm) of 23 female adults and total lenght of 10 larvae of *Longibucca catesbeianae* n. sp. collected from *Rana catesbeiana* Shaw, 1802, with other species of the genus *Longibucca* Chitwood, 1933. ( )=mean; a=distance from anterior extremity; b =distance from vulva to anus; c=distance from anus to tail extremity.

contamination rate, with parasite counts under 100. In one of the frogs from the confinement, only 64 worms were found, in the stomach. On the other hand, the lowest number of nematodes counted in a frog captured outside the farm was 75 (72 in the stomach).

Inside the stomach the parasites were found within a large mass of mucus, which was practically absent in animals free from parasites or with a small number of worms. In histopathological examination of a bullfrog stomach in which 8630 nematodes had been counted, severe lesions of the epithelium were not observed. A large number of worms was found within the villi, but a few specimens were found in the glandular lumen. In some cases a mild inflammatory reaction, eosinophilic in nature, was observed around the worm-containing capsule (Fig. 8). No worms were observed to invade intestinal tissues. The number of worms collected in each specimen showed no relation to host sex. Both sexes are equally parasited.

Longibucca catesbeianae n. sp.

(Tables 1 and 2; Fig. 1-4)

Host: adults of *Rana catesbeiana* Shaw, 1802.
Parasited organ: stomach.
Locality: Itamambuca Beach, Ubatuba, São Paulo, Brazil.

Type-specimens: Helminth collection of the Museu de Zoologia da Universidade de São Paulo, Brazil, Holotype MZUSP No. 5754, Paratypes MZUSP No. 5755.

Description

A small cylindrical nematode with slender ends and maximum width at middle body. The cuticle is delicately striated by transversal rings which begin at a short distance from the anterior end and continue along the body becoming progressively indistinct towards the tail end. Narrow lateral alae can be seen along the body. It has a central, round oral opening with indistinct lips. Four peri-oral papillae (2 ventrolateral and 2 dorsolateral) surround the mouth in a circular disposition. The amphids are punctiform, lateral and hardly visible. The stoma is very long and uniform in diameter, except for a small thickening at the oesophagus juncture; short and cylindrical cheilostome; the esophastome is slightly thickened and has a funnel shape. The oesophagus is clearly divided into three portions: a cylindrical corpus, a narrow isthmus and an ovoid pseudobulb. The excretory pore is located slightly below the level of the juncture between pseudobulb and gut.

Male: The body length varies from 545 to 700 μm, with widths from 10 to 14 μm. The tail end is flected ventrally, ending in a long filament from 65 to 90 μm in length. Straight testicle. Two falciform spicules with equal lengths varying from 12,5 to 20,0 μm. Each spicule presents a curved lamina with a prominent manubrium and ventral tubercule; there is a membraneous winglike vellum extending from the more salient part of the tubercule up to the distal third of the lamina. A claw-shaped gubernaculum is present; it is in a posterodorsal position in relation to the distal portion of the spicules and well curved dorsally, with prominent edges in the distal end. Four pairs of genital papillae can be found: one pair is precloacal, the other three are postcloacal. The phasmids are ventral and punctiform and can be located between the cloacal opening and the first pair of postcloacal genital papillae.

Female: Body length varying from 520 to 707 μm, showing widths from 13 to 26 μm. A long slender tail varying from 62 to 100 μm in length can be found; it presents an antepudendum genital tube and sole ovary (monovarial) originating in the surroundings of the excretory pore. The uterus ends in a short cylindrical vagina uterina flected

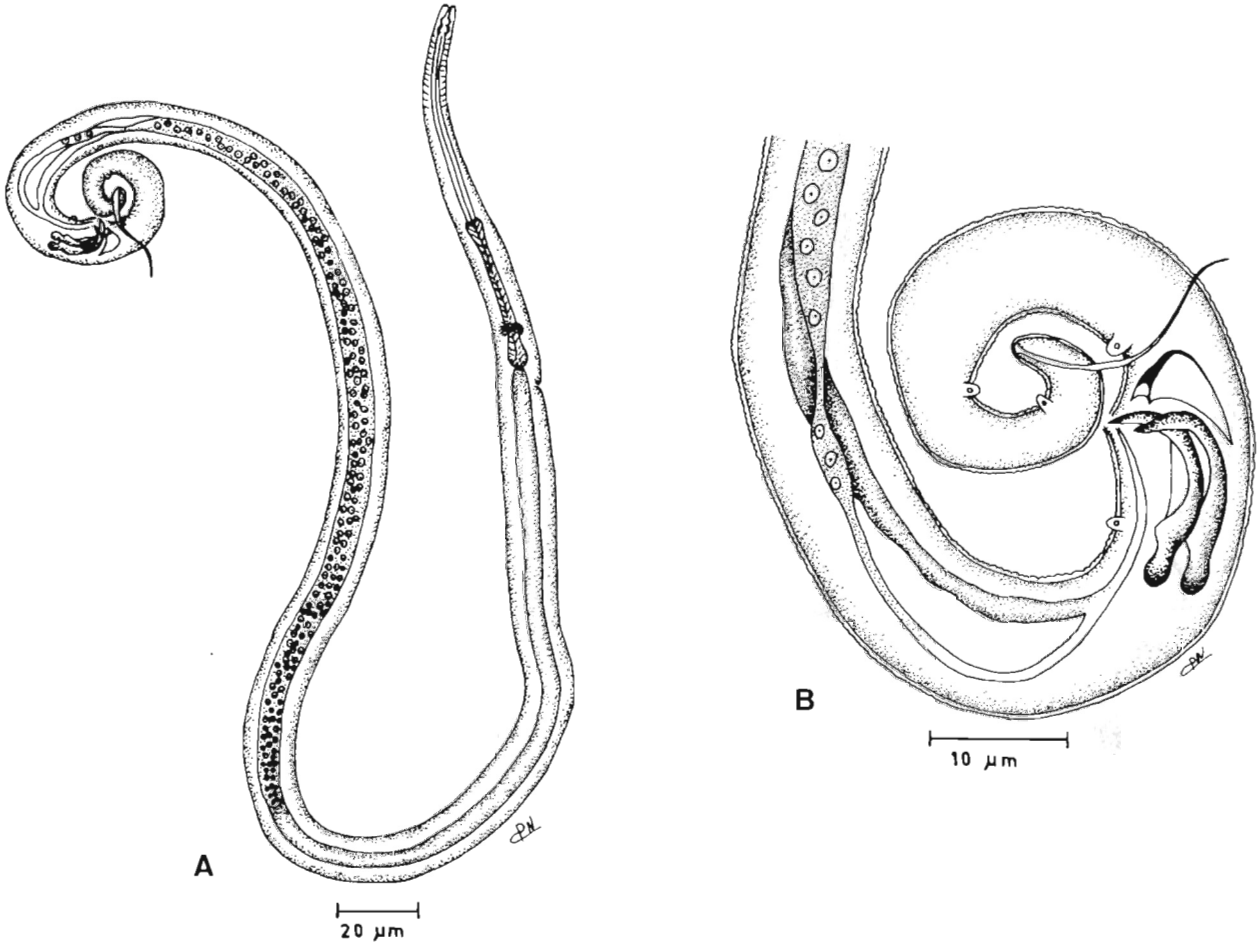

Fig. 1.— Male of *Longibucca catesbeianae* n. sp.: A) entire worm, lateral view; B) caudal extremity, lateral view.

towards the anterior end in such a way that the contour of its posterior limits is below the vulva. There is a small, narrow, vagina vera. In the juncture of this vagina vera with the vagina uterina a very small vestigial uterus, facing the anterior end, can be found. Viviparous.

DISCUSSION

The genus *Longibucca* was created by CHITWOOD (1933). At that time, the author described *L. vivipara* found in the stomach of *Pseudoboa cloelia* [at the moment *Clelia clelia* (Daudin, 1803)], a South American species of snake. MCINTOSH & CHITWOOD (1934) wrote about nematodes obtained from the intestine of *Lasiura borealis* [=*Lasiurus borealis* (Muller, 1776)] (Chiroptera) captured in Washington D.C., U.S.A. This new species, named *Longibucca lasiura*, was also reported to be found in the stomach of *Eptesicus fuscus fuscus* (Beauvois, 1796) (Chiroptera) as mentioned by the same authors in an appendix of their paper. This same year, TROMBA & SMITH (1934) reported the finding of *L. lasiura* in the stomach of two different species of Chiroptera: *Corynorhinus rafinesquii rafinesquii* (=*Plecotus rafinesquii* Lesson, 1827) and *Myotis lucifugus lucifugus* (Le Conte, 1827), also collected in the USA. Almost 20 years later, in 1953, the third species of *Longibucca* was found by ELSEA (1953) in the stomach of the Virginia bat, *Eptesicus fuscus fuscus*. On this occasion the author claims to have found apparently similar organisms inside the stomach of two other species of bats: *Myotis lucifugus lucifugus* and *Pipistrellus subflavus* (Cuvier, 1832). *Longibucca eptesica* was proposed for these helminths collected from American bats. More recently, DIER & CARR (1990) vaguely mentioned the findings of *Longibucca* sp. in a terrestrian turtle, *Rhinoclemmys pulcherrima pulcherrima* (Gray, 1855) captured in Acapulco, Mexico.

The name of the genus of these nematodes was pro-

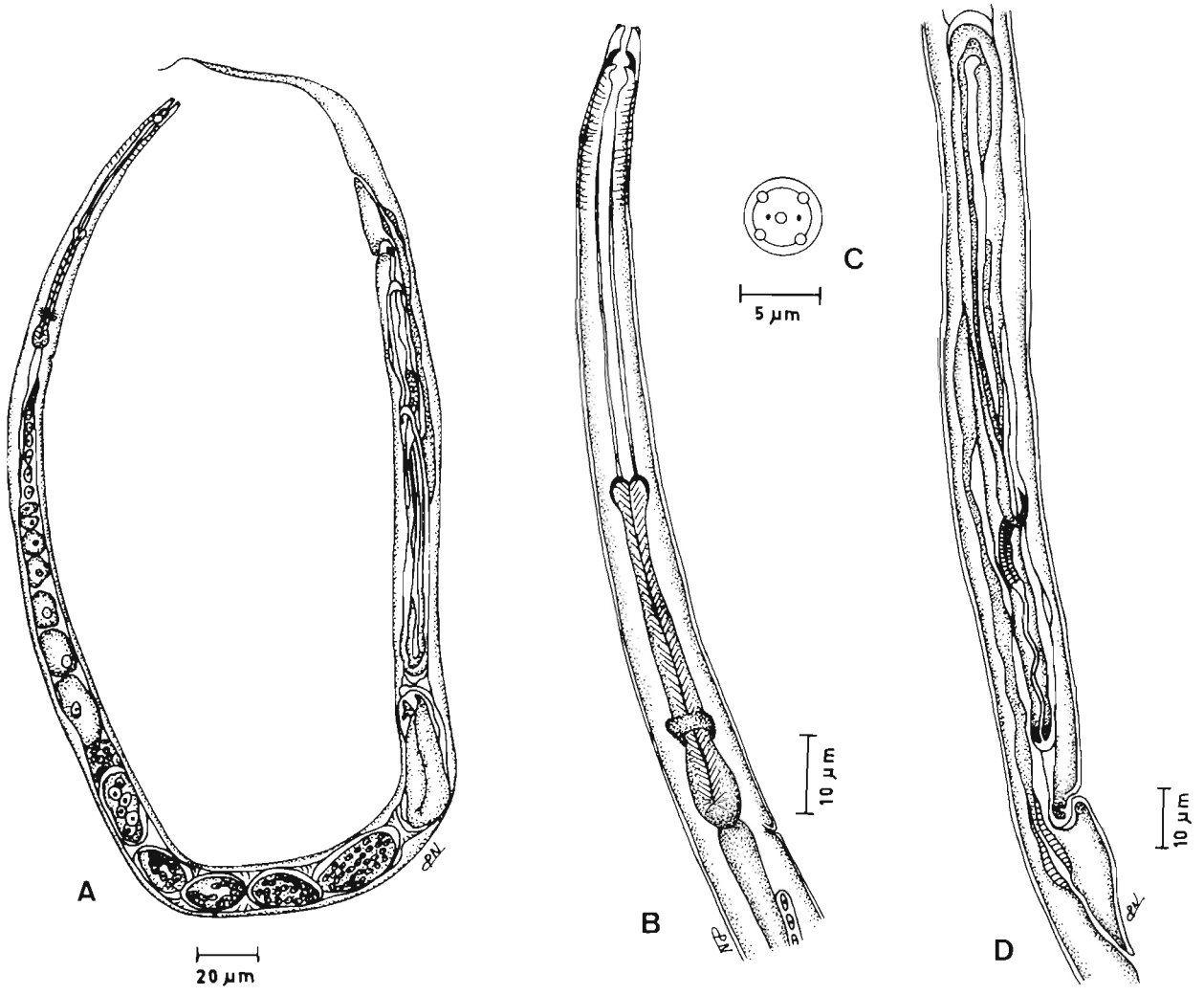

Fig. 2.— Female of *Longibucca catesbeianae* n. sp.: A) entire worm, lateral view; B) anterior extremity, lateral view; C) cephalic extremity, *en face* view; D) caudal extremity, lateral view.

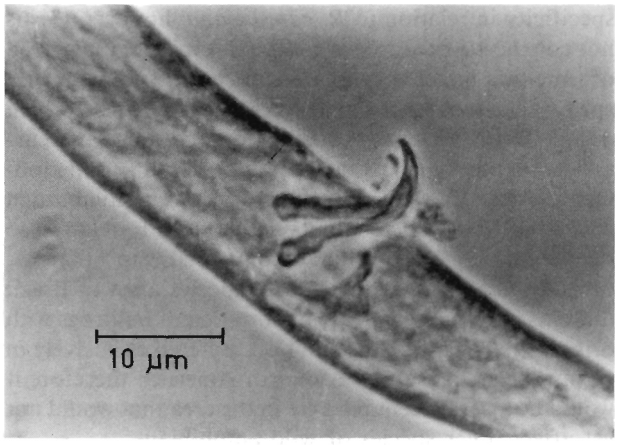

Fig. 3.— Male of *Longibucca catesbeianae* n. sp.: caudal extremity with everted spicules, lateral view.

posed for the extremely long stoma found in males and in females. Its length is greater than that of the oesophagus itself. In the new species there is a slight thickness at the level of the oesophagus-gut juncture. This thickening is also cited in the species previously described (CHITWOOD, 1933; MCINTOSH & CHITWOOD, 1934; ELSEA, 1953). The oesophagus is distinctively divided into three portions: corpus, a reduced isthmus and an ovoid pseudobulb; its total length is smaller than that mentioned for other species. This difference is observable if one compares the distances between the oesophagus base and the oral opening in *L. catesbeianae* n. sp., *L. vivipara* and *L. lasiura*.

The gut is a tube-like organ barely visible in females due to the unusual development of the genitals. In males, the intestine is perfectly visible up to the middle of the nematode's body, where the testicle begins.

Like in *L. eptesica*, the females show a muscular thickening at the end portion of the gut which, according to ELSEA (1953), serves as a rectal sphincter.

As in other *Longibucca*, gonads in both sexes are no more than a tubeform structure. In males, the testicle runs from the anterior portion of the body up to 260-350 µm. It is not flected in the proximal portion as in other species. The tail grows slender, usually flecting ventrally as in *L. vivipara*, quite different from *L. lasiura* and *L. eptesica* in which this distal portion is cone-shaped and slightly wide. Spicules are falciform and symmetrical as in *L. eptesica* and *L. vivipara*, though of different dimensions. Only four pairs of ventral genital papillae are found and none of the subdorsal ones were found, differing from the others species in which five or six pairs of papillae are described. For *L. lasiura* a fifth pair postcloacal and subdorsal is mentioned, while for *L. vivipara* and *L. eptesica* a sixth postcloacal and subdorsal pair can be found.

The females have one sole tubeform gonad, exceptionally well-developed, occupying considerable dimensions in relation to the worm's total length, reaching as far as the excretory pore in its anterior portion. In other species the ovary presents a flexion in the proximal portion; this has not been found in *L. catesbeianae* n. sp. females. Such anatomical features may well explain the greater length of the female gonad, which in some specimens reaches up to 90 µm from the oral portion of the nematode. Considerably greater measures are mentioned for others species. The ovary cells destined to form eggs are arranged in a single line beginning at a small germinative zone. They fill the organ's inner space, growing in size until the formation of a conglomerate of 6-10 round cells. In *L. eptesica*, according to ELSEA (1953), this structure is composed of 15-20 cells responsible for the egg shell formation. Indeed, in *L. catesbeianae* n. sp. only after this does egg development take place, with the simultaneous development of the egg shell. The egg shell grows adjusting itself to the development of the embryo up to L1 stage larvae located in the uterus distal portion. The simultaneous finding of two apparentely developed larvae in the uterus has been described for *L. lasiura* and *L. eptesica*. This was never the case for *L. catesbeianae* n. sp. We never found more than one completely developed larva inside the uterus. The length of ten of these uterine larvae were measured. The numbers obtained differ from those mentioned for *L. eptesica* (ELSEA, 1953). The vestigial uterus was first found in *Longibucca* by CHITWOOD (1933) when describing the genus and *L. vivipara* from the stomach of *C. clelia*. In that paper the author, though refering to a «posterior uterus rudimentary», described the genus as prodelphic and of «uteri divergent». The presence of a second nonfunctional uterus was also noted by ELSEA (1953) in *L. eptesica*. The reproductive organ in female specimens of *L. catesbeianae* n. sp. is therefore, of antepudendum type, according to MAGGENTI (1981), who thus named all

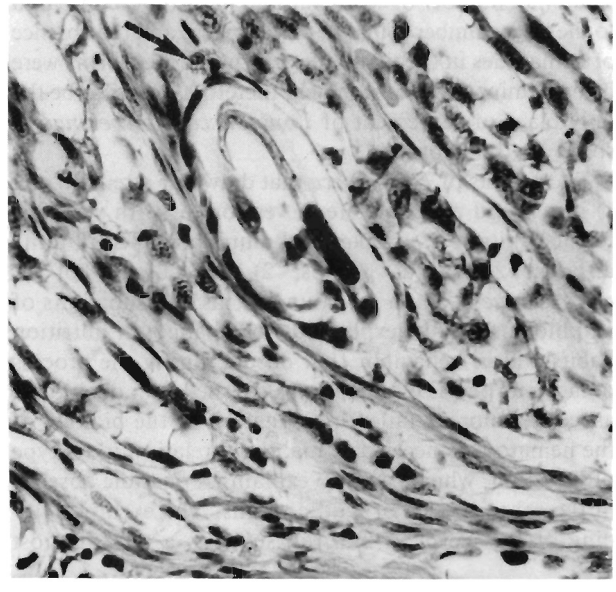

Fig. 4.— Section of stomach of *Rana catesbeiana* Shaw, 1802, showing *Longibucca catesbeianae* n. sp. in mucosa and surrounded by a mild cellular reaction. Arrow indicates an eosinophilous (400 x).

nematodes in which «the genital tube proceeds anterior from the vulva».

Parasitic lesions were not always observed in the animals parasited by *Longibucca*. DYER & CARR (1990) did not observe abnormalities in the stomach of *Rhinoclemmys p. pulcherrima* harbouring *Longibucca* sp. On the other hand, CHITWOOD (1933) demonstrated the presence of worm larvae and adults lodged chiefly in the mucosa and submucosa of the stomach of *C. clelia*. Some worms were surrounded by a tissue which showed a slight cellular reaction. MCINTOSH & CHITWOOD (1934) pointed out that in the stomach of bats the worms are found inside a layer of mucus and desquamated epithelium which contained lymphocytes and polymorphonuclears. Some of the worms were lodged in glands and in the interglandular tissue. Nevertheless, the authors pointed out that «the tissue of the stomach showed no marked pathological reaction». ELSEA (1953), upon examining the stomach of *P. subflavus* infected with *L. eptesica*, has also noticed the presence of nematodes in a small amount of mucus-like material which seemed to contain cellular fragments. The only pathological alteration found by this author was a slight surface erosion of the gastric mucosa. The presence of worms in the gastric glands was considered «merely fortuitous».

Such findings are similar to those of other authors, although *L. catesbeianae* n. sp. was never found in the submucosa of the bullfrog. Although we have found numerous worms deeply situated in the gastric tissues, we did not notice, confirming the observations of MCINTOSH & CHITWOOD (1934), severe lesions of the gastric epithelium. The finding of some worms inside the glandular lumen is occasional. The small inflammatory reaction produced by a large worm population suggests the existence of a good equilibrium in host-parasite relations. The small number of worms found in the gut, compared to the large number found in the stomach, and the absence of nematodes in the tissue suggest that those worms were being eliminated. The stomach, therefore, seems to be the only place of settlement of *Longibucca catesbeianae* n. sp. in the bullfrogs.

The aspects of *Longibucca* that draw our attention are: 1) it is found as a parasite in vertebrates with very different habits (bats, snakes and amphibians) from quite different zoological groups; 2) the absence of *L. catesbeianae* n. sp. in the young forms and subadults of amphibians may be explainable by changes in nutrition habits and in behaviour that occurs during the process of metamorphosis in the anurans. To shed light upon these aspects, a more detailed investigation of the biology of the nematode is necessary, mainly in relation to its type of infection, which can also explain its possible absence in the other species of wild anurans. The exclusive finding of the worm in bullfrog suggests a parasite-host specificity in relation to *R. catesbeiana*; however, this is not conclusive because we could not evaluate the period of time that infected frogs were in contact with the wild species. This contact could have been occurring for a decade, since the farm was set up, or it could have occurred more recently. This fact does not permit, at the moment, a detailed critical analysis of the parasitic burden in the bullfrogs captured outside the farm in relation to the confined ones. Our findings suggest that *L. catesbeianae* n. sp. seems to be a species alien to Brazil that was introduced with the importation of bullfrogs with original geographical distribution centered exclusively in certain areas in the east of North America; therefore it would be a very recent parasite in the area that would not yet affect other species of wild amphibians.

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. M.T.U. Rodrigues for identifying the anurans and sharing his knowledge of amphibians with us.

REFERENCES

ANDERSON (R.C.) & BAIN (O.), 1982.— Keys to genera of the Superfamilies Rhabditoidea, Dioctophymatoidea, Trichinelloidea and Muspiceoidea. In: *CIH Keys to the Nematode Parasites of Vertebrates* (R.C. Anderson, A.G. Chabaud & S. Willmott edit.), Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, 9: 1-26.

CHITWOOD (B.G.), 1933.— On some nematodes of the superfamily Rhabditoidea and their status as parasites of reptiles and amphibians. *Journal of the Washington Academy of Science*, 23: 508-520.

CHITWOOD (B.G.) & WEHR (E.E.), 1934.— The value of cephalic structures as characters in nematode classification, with special reference to the Spiruroidea. *Zeitschrift für Parasitenkunde*, 7: 273-335.

CHITWOOD (B.G.) & CHITWOOD (M.G.), 1977.— *Introduction to Nematology*. University Park Press, Baltimore, 334 pp.

DYER (W.G.) & CARR (J.L.), 1990.— Some Ascaridid, Spirurid, and Rhabditid nematodes of Neotropical turtle genus *Rhinoclemmys* in Mexico and South America. *Journal of Parasitology*, 76: 259-262.

ELSEA (J.R.), 1953.— Observations on the morphology and biology of *Longibucca eptesica* n. sp. (Nematoda: Cylindrocorporidae) parasitic in the Bat. *Proceedings of the Helminthological Society of Washington*, 20: 65-76.

MAGGENTI (A.R.), 1981.— *General Nematology*. Springer-Verlag, New York, 372 pp.

MCINTOSH (A.) & CHITWOOD (B.G.), 1934.— A new nematode, *Longibucca lasiura* n. sp. (Rhabditoidea, Cylindrocorporidae) from a bat. *Parasitology*, 26: 138-140.

TROMBA (F.G.) & SMITH (W.N.), 1934.— *Longibucca lasiura* McIntosh et Chitwood, 1934; new host records. *Proceedings of the Helminthological Society of Washington*, 19: 126.

YAMAGUTI (S.), 1961.— *The Nematodes of Vertebrates*. Volume III, Part I. Interscience Publishers Inc., New York, 679 pp.