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RHABDIAS (NEMATODA, RHABDITOIDEA) FROM THE *MARINUS* GROUP OF *BUFO*. A STUDY OF SIBLING SPECIES

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CONTENTS

ABSTRACT

This work begins with some conisderations on the hosts; their lung nematodes are analyzed from the current point of view of the nematologists. For the analysis of sibling species quantitative characters and chromatophilous techniques were employed. After obtention and analysis of the free-living generation and to understand the degree of host-specificity and the range of variation of each parasitic species, cross-infestations in the hosts were made.

INTRODUCTION

This is an attempt at a better understanding of the evolution of *Rhabdias*, a nematode genus with a complex life cycle, whose dioic, free-living generation lives in excrement of animals that harbour the preceding generation as hermaphroditic parasites.

The parasitic generation depends entirely on the internal conditions offered by the hosts, from the moment the infective larvae penetrate the skin or are swallowed. These filariform larvae are not selective: they penetrate any kind of animal tissue, of endothermic or exothermic vertebrates, and even of invertebrates (indifferent histotropism, Brumpt, 1921, Kosuge, 1924). Researches by Fülleborn (1920 to 1928) show that if the host does not offer the infective larvae con-

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ditions to reach the lungs, they encyst in some other organ, mainly the kidneys, which they reach through the lymphatic circulation, immediately after penetration of the host's body through the skin. I have had the opportunity to observe the same fact during my work (Photos 1 and 2).

The free-living, dioic, and rhabditiform generation is exposed both to the external environment and to the physical and chemical conditions of the excrement. During this period of life, *Rhabdias* is coprophagous and behaves like the Rhabditidae, from which the Rhabdiasidae supposedly descend (Chitwood & McIntosh, 1934).

The complexity of the life cycle, and the different habits of the two generations lead to two important consequences:

i) The geographical distribution of the species of *Rhabdias* is not necessarily the same as that of the host, considering that the free-living generation is directly influenced by the environment; and,

ii) the fact that the larvae inhabit the circulatory system and lungs of the hosts probably involves greater specifity than that presented by coprophagous parasites living in the large intestine.

These two facts necessarily result in intraspecific morphological variation, which, up to now, has not been adequately analyzed by nematologists. Considering that hermaphroditism is a recent acquisition (Potts, 1908, 1910), I believe it to be one of the reasons which explains the morphological uniformity in the different species of *Rhabdias*. Species identification is very difficult, if not impossible, by the classical methods of nematology. This difficulty has been felt by many nematologists. Fotedar's (1965) opinion that the free-living generation of the different species would solve the problem, is not valid, because, as shall be seen later, free-living forms are also morphologically uniform, and undergo physiological variations, which, if analyzed in isolated samples, lead to erroneous conclusions.

In order to achieve a more detailed analysis of these parasites, it was necessary to find a group of hosts with an ample geographical distribution, and a relatively high infestation with *Rhabdias*. These characteristiscs were found in Bufonidae (Amphibia, Anura), more exactly in the *marinus* group of *Bufo* (Martin, 1972, or *Bufo* gr. *valliceps*, South American Section, cf. Tihen, 1962 A), of which 3 species occur in Brazil: *B. marinus* (with the subspecies *marinus*, *paracnemis*, and *ictericus*), *B. crucifer*, and *B. arenarum*. I have analyzed the qualitative and quantitative characters of their parasites, obtaining inconclusive results. Afterwards, the quantitative analysis was improved, by statistical methods which did not permit a significant grouping of the samples.

I was inclined to believe that I was dealing with a single species, when the free-living generations of a mixed infestation in the leptodactylid frog *Thoropa miliaris* lead me to further improve my laboratory technique.

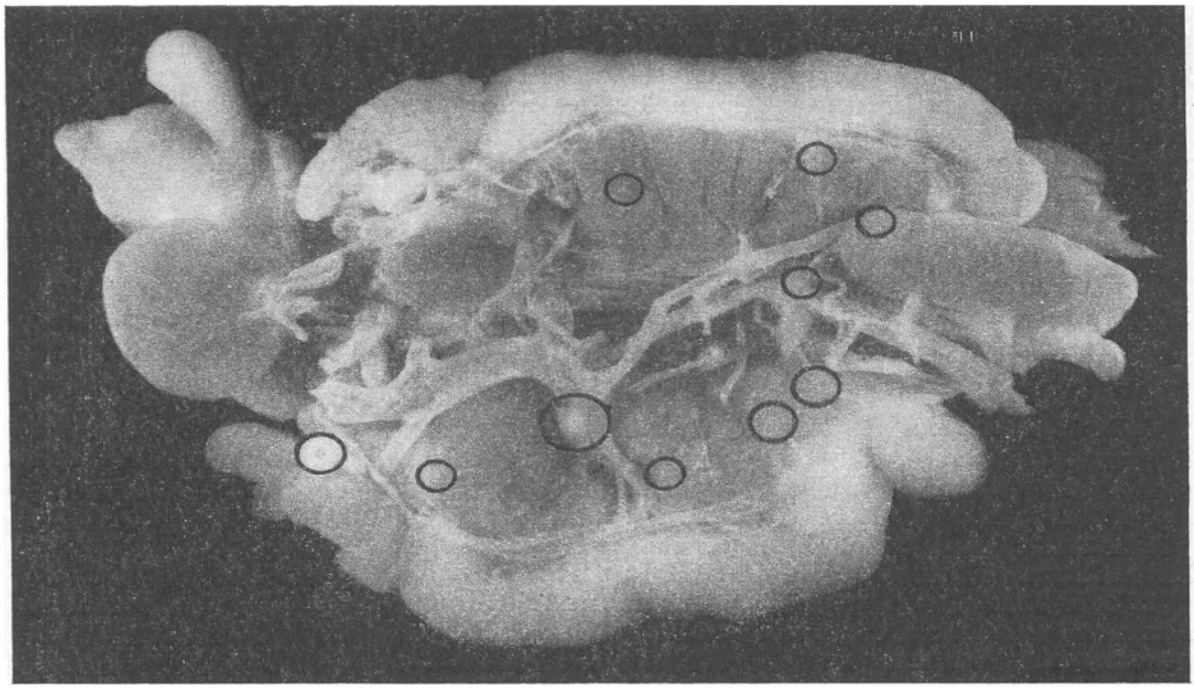


Photo 1. Urogenital system of *Bufo m. ictericus* showing encisted *R. hermaphrodita* larvae which did not reach the lungs of the host.

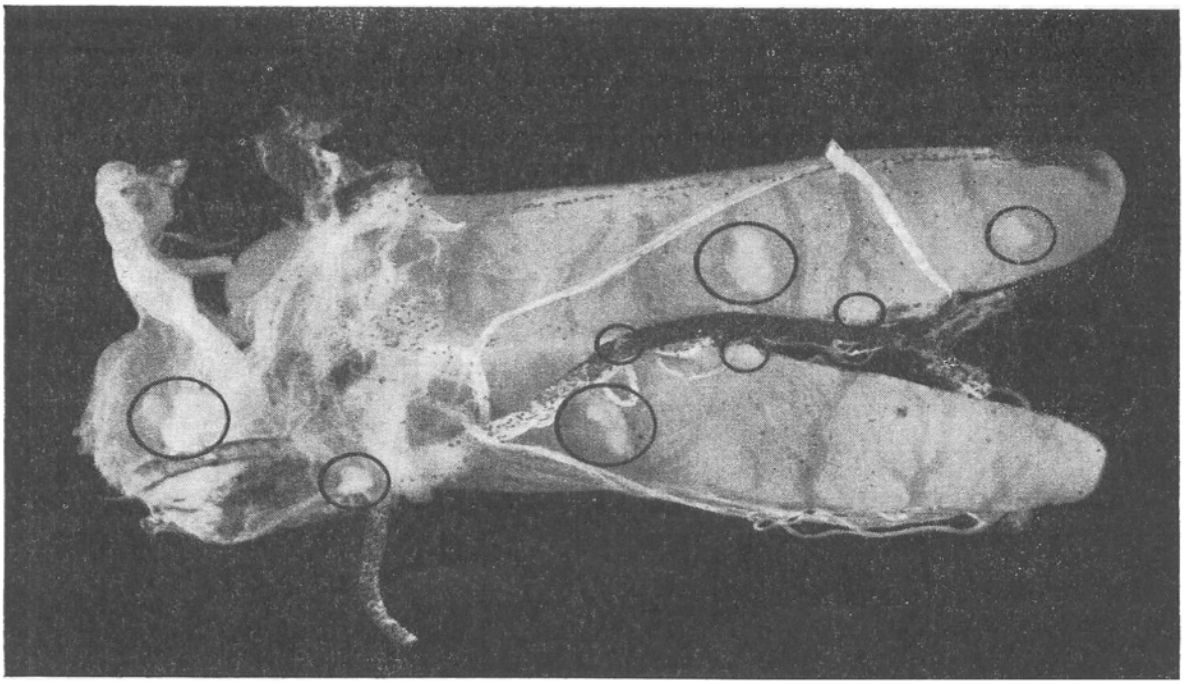


Photo 2. Urogenital system of *B. crucifer* showing encisted *R. fuelleborni* larvae which did not reach the lungs of the host.

Chromatophilic analyses permitted the recognition of 2 species of *Rhabdias* parasitizing this group of hosts: *R. fuelleborni* Travassos, 1926, and *R. hermaphrodita* Kloss, 1971. After the characterization of the species I could undertake a better analysis of their variation, by means of cross-infestations, using different species of hosts, or the same species of hosts from the same, or different localities.

MATERIAL

For this study I used helminthological materials from the "Museu de Zoologia da Universidade de São Paulo, Brasil" (MZUSP), and from the "Instituto Oswaldo Cruz, Rio de Janeiro" (IOC). Hosts and their localities are:

Bufo arenarum. Minas Gerais: Calciolândia and Congonhas do Campo (MZUSP). Paraguay: Asunción (IOC). Uruguay: Montevideo (IOC). *Bufo crucifer.* Espírito Santo: Sta. Teresa (MZUSP), Sooretama (IOC); Rio de Janeiro: Angra dos Reis (IOC); Guanabara (IOC); São Paulo: S. Paulo, Embu, Casa Grande, Sto. Antonio do Pinhal, Engenheiro Marsillac (MZUSP); Sta. Catarina: Novo Horizonte (MZUSP). *Bufo m. marinus.* Amazonas: Rio Preto da Eva (MZUSP), Manaus (IOC); Pará: Maicuru (IOC), Belém (MZUSP). *Bufo m. paracnemis.* Fernando de Noronha (IOC); Bahia: Salvador, Entre Rios, Euclides da Cunha, Serrinha (IOC); Ceará: Fortaleza (IOC); Minas Gerais: Belo Horizonte, Matosinhos, Pirapora (IOC), Lagoa Santa, Sabará (MZUSP); São Paulo: Emas (MZUSP); Mato Grosso: Salobra (IOC); Paraná: Guaíra (MZUSP). Paraguay: Asunción, Isla Valle, Luque, Trinidad, Chaco-í, Areguá, Ypacaray, Coronel Bogado, Remanso Castillo, Villarrica, Pto. Juan Barbero (IOC). *Bufo m. marinus* x *B. m. paracnemis.* Rondônia: Príncipe da Beira (MZUSP). *Bufo m. ictericus.* Minas Gerais: Caldas (IOC); Guanabara (IOC); Rio de Janeiro: Guapi-Mirim, Petrópolis, Teresópolis, Parati, Angra dos Reis, Coroa Grande, Itatiaia, Serra da Bocaina (IOC); São Paulo: S. Paulo, Pindamonhangaba, Bariri, Campinas (IOC), Casa Grande (MZUSP); Paraná: Curitiba, Piraquara (IOC); Sta. Catarina: Novo Horizonte (MZUSP); Rio Grande do Sul: Salvador do Sul, Caxias do Sul (MZUSP). *Thoropa miliaris.* Guanabara (IOC); Rio de Janeiro: Angra dos Reis (IOC); São Paulo: Casa Grande (MZUSP); Sta. Catarina: Novo Horizonte (MZUSP).

For the mapping of localities, were used the 1 : 1 000 000 charts published by the "Instituto Brasileiro de Geografia e Estatística" and by the American Geographical Society, and also the Gazetteers of the United States Board on Geographic Names.

To avoid repetitions of localities and hosts names, these will normally be abbreviated as follows:

a	*Bufo arenarum*
A	Asunción
AM	Amazonas
Ar	Areguá
B	Belém
BA	Bahia
BH	Belo Horizonte
c	*Bufo crucifer*
C	Caldas
Cb	Curitiba
CB	Coronel Bogado
CE	Ceará
Cg	Congonhas do Campo
CG	Casa Grande
Cí	Chaco-í
Cl	Calciolândia
Cr	Coroa Grande
CR	Costa Rica
CS	Caxias do Sul
E	Emas
Eb	Embu
ES	Espírito Santo
F	Fortaleza
FN	Fernando de Noronha
G	Guaíra
GB	Guanabara
GO	Goiás
i	*Bufo m. ictericus*
IB	Bermuda Islands
IV	Isla Valle
L	Luque
LS	Lagoa Santa
m	*Bufo m. marinus*
mxp	*B. m. marinus x B. m. paracnemis*
M	Montevideo
MG	Minas Gerais
Mn	Manaus
MT	Mato Grosso
NH	Novo Horizonte
p	*Bufo m. paracnemis*
P	Parati
PA	Pará
PB	Príncipe da Beira
Pd	Pindamonhangaba
PJB	Pto. Juan Barbero
PR	Paraná
RC	Remanso Castillo
RE	Ribeirão dos Enganos
RJ	Rio de Janeiro
RO	Rondônia
RPE	Rio Preto da Eva
RS	Rio Grande do Sul
RV	Rio Verde
S	Salobra
SB	Serra da Bocaina
SC	Sta. Catarina
SP	São Paulo
SS	Salvador do Sul
ST	Sta. Teresa
T	Teresópolis
Td	Tramandaí
Tm	*Thoropa miliaris*
Tr	Trinidad
VR	Villarrica
Y	Ypacaray

CONSIDERATIONS ON THE HOSTS

The different species groups of *Bufo* are characterized specially by their skull anatomy (Baldauf, 1959; Tihen, 1962 A; Martin, 1972). *B. crucifer*, *B. arenarum* and *B. marinus* are representatives of the *valliceps* group, according to Tihen, or of the Group II of Baldauf, the two groups containing the same species of *Bufo*. The most important characters used by these two authors to group the species were the types of occipital groove, the type of contact between the frontoparietal and prootic bones, the general aspect of the frontoparietals, the elevation of the skull, the type of jaw articulation, characteristics of the pelvic appendages, and some important internal skull characters. Martin (1972) split the few groups of Tihen and Baldauf in several others, based on characters considered of secondary value. Thus, he considered *B. arenarum* and *B. marinus* representatives of the *marinus* group (= South American Section of the *valliceps* group, cf. Tihen 1962 A), and *B. crucifer* alone representing the *crucifer* group (cf. Tihen 1962 A also from the South American Section). This splitting by Martin was based on the general shape of the skull, the moderately occluded suprapterygoid fenestra (in the *marinus* group it is nearly completely occluded), and slender limb bones. The anatomical separation of *B. crucifer* in a group of its own needs more detailed study. The studies

of karyotypes (Bogart, 1972) and the evidence from hybridization (Blair, 1972) indicate that *B. crucifer* is the oldest species of the South American *valliceps* group of Tihen, which is confirmed by the geographical distribution and ontogenetic characters. The secondary submetacentric constriction on the long arm of chromosome 1 represents an early dichotomy in *B. crucifer*, probably also responsible for the evolution of the line leading to *B. arenarum;* and the results of hybridization of *B. arenarum* with *B. valliceps* are fairly good (52.6%, 85.3%), in opposition to *B. crucifer* x *B. valliceps*, the offspring of which did not go beyond the larval stage.

In my opinion, the validity of separating or not *B. crucifer* from the group *marinus*, and the exact grouping of the anatomical characters need more complete and accurate study of many species of *Bufo*. In the chapter of conclusions about the parasitism with *Rhabdias* I shall comment again on this detail.

The *valliceps* group (= group II cf. Baldauf), is represented by 23 species: *alvarius, arenarum, canaliferus, cavifrons, coccifer, crucifer, debilis, empusus, gemmifer, granulosus, guentheri, macrocristatus, marinus, marmoreus, mazatlanensis, occidentalis, peltocephalus, perplexus, punctatus, quercicus, retiformis, typhonius*, and *valliceps*. Tihen included erroneously *B. blombergi* in this group, but some skeletons examined by myself have shown that this species represents the *haematiticus*, not *valliceps* group, as said by Martin (1972).

The geographical distribution of the *valliceps* group ranges from the "Monte" region (44°S) in Argentina (Hueck, 1966), to 38°N in the United States. In this group, *B. marinus* and *B. valliceps* are the two species which show more anatomical affinity to Group I of Baldauf *(terrestris, woodhousei, microscaphus, americanus, cognatus, hemiophrys,* and *houstonensis)*. Hybridization of *B. marinus* and *B. valliceps* with *B. cognatus* and *B. americanus* resulted in diploid hybrids in percentages varying between 20.6 and 92%, in opposition to a result between gastrula and 5.7% only, when crossing *B. cognatus* and *B. americanus* with other species of the *valliceps* group cf. Tihen (Blair, 1972).

Group I is distributed in North America, from 23° to 70°N. According to Baldauf, Group I gave origin to Group II, in disagreement with the results obtained by Bogart (1972) and Blair (1972). I shall not analyze this question as it does not concern the present work. Baldauf's analysis of the two groups also reveals that *B. valliceps* and *B. marinus* not only resemble Group I, but also *B. melanostictus* and *B. parietalis*, from Asia, whose skull anatomy was studied by L. S. Ramaswami (Baldauf, 1959). This opinion is confirmed by the results of hybridization of *B. melanostictus* with *B. valliceps* and *B. marinus*, with 29.7 and 81.9% of diploid hybrids. But *B. melanostictus* crosses with *B. crucifer* resulted in 22.2% of diploid hybrids, and *B. melanostictus* x *B. arenarum* in 43.7%. So one may conclude that not only *B. marinus*, but all South American Section of the *valliceps* group is in some way related to *B. melanostictus*.

Tihen (1962 A) divided the *valliceps* group in 3 sections: the South American Section (= *marinus* group + *crucifer* group of Martin, 1972),

the Mexican Section, and the Caribbean Section. The South American Section comprises *arenarum, marinus,* and *crucifer,* therefore having one representative (*marinus*) with closer anatomical affinity with Group I of Baldauf, and all of them being genetically linked to *melanostictus* of Asia. It is a Section with an ample geographical distribution, occurring from 44°S in Argentina, to Mexico-United States border, at about 30°N.

Three representatives of the South American Section occur in Brazil: *arenarum* (= *rufus*), *crucifer,* and *marinus,* the latter with the subspecies *m. marinus, m. paracnemis,* and *m. ictericus.*

B. marinus has the broadest distribution, from Mar del Plata to the Southern United States. It lives both in forests and in open country, the different ecological environments determining its subspecific characters. *Bufo m. paracnemis* occurs in open country; in the southern part of the Atlantic rain forest (Hueck, 1966) and in the subtropical forest (Hueck, 1966), lives *Bufo m. ictericus;* in the tropical rain forest occurs *Bufo m. marinus.* There are intergrading populations in the transition zones between "cerrados", "campos" and forests, and open areas resulting from the destruction of forests. *B. marinus* shows great variation in skull measurements.

B. arenarum is a form of the open formations. It occupies all the "Monte" region, the "Pampas", the dry forests from the Chaco and the Mesopotamic Park (Hueck, 1966), the southern coast of Brazil, "cerrados" in Minas Gerais, the northeastern "caatinga", and the enclaves of "campos" and "cerrados" within the subtropical and the *Araucaria* forests. *B. arenarum* has its range in Argentina and the southern part of Brazil, where it lives side by side with *Bufo m. paracnemis,* a typical open country subspecies. As *B. marinus, B. arenarum* also shows a great variation in skull measurements.

B. crucifer is a typical Atlantic rain forest and subtropical forest dweller, sympatric with *Bufo m. ictericus.* It can also be found in forests enclaves in "cerrados" and "caatingas". *B. crucifer* is a monotypic species, with apparently random variation in colour.

R. J. Williams (1956) studied and compared the anatomy and biochemistry of vertebrates, and concluded that there is always an intrinsic relation between them, the same occuring with the respective variations. Therefore, if the morphological variation of *B. marinus* and *B. arenarum* is considered, a relatively large variation in biochemical conditions may also be expected. It is difficult, if not impossible, to distinguish *B. marinus* and *B. arenarum* by their skeletons (Tihen, 1962 B, and personal observations). Having examined 8 skeletons of *B. arenarum* from 8 localities, and 114 individuals kept in alcohol, from 19 localities, and 18 skeletons of *B. marinus* from 9 localities and 2,906 individuals kept in alcohol, from 215 localities, I found consistent differences only in the granular glands: in spite of living in open country, the granular glands of *B. arenarum* never agglomerate to form the paracnemic and antebrachial glands observed in *Bufo m. paracnemis.* The parotids, formed by the concretion of granular glands, are oblong

in *B. arenarum*, and sub-triangular in *Bufo m. paracnemis*. *B. arenarum* resembles *Bufo m. ictericus*, but these two species never occur together, because the latter is a forest dweller.

The distribution of *Thoropa miliaris* overlaps that of *Bufo m. ictericus* and *B. crucifer*.

RHABDIAS FROM THE *MARINUS* GROUP OF *BUFO*

The following species of *Rhabdias* have been recorded from the *marinus* group of *Bufo*:

Rhabdias sphaerocephala Goodey, 1924

Originally described from *Bufo bufo* (L.) (= *B. vulgaris* Gthr.) from England. The next host reported was *B. marinus* from Mexico (Bravo H. & Caballero, 1940), Costa Rica (Brenes & Bravo H., 1959), Bermuda Islands (Williams, 1959 and 1960), and the Amazonian region (Kloss, 1971). *R. sphaerocephala* has also been found parasitizing *Bufo m. paracnemis* from open country in Central Brazil, and the transition zones between forests and "cerrados".

In the original description, measurements were: total length 6.0 to 6.5 mm; width 0.30 to 0.35 mm; vulva to anterior extremity 3.3 to 3.5 mm; width of anterior extremity, including cuticle 0.17 to 0.19 mm; width of anterior extremity without cuticle 0.115 to 0.120 mm; esophagus 0.43 to 0.45 mm; tail 0.19 to 0.22 mm. Characteristics of this species are: the globular anterior extremity, the well developed uteri, and the slight dilation near the anterior extremity of the esophagus. The last mentioned character led to the opinion that the parasites of *B. marinus* from Mexico, Costa Rica and Bermuda Islands should be referred to *R. sphaerocephala*, with the observation that the Middle American samples had a less developed esophagean dilation than those from England.

Measurements of the American individuals, in mm, were (Bravo H. & Caballero, 1940, and Brenes & Bravo H., 1959):

	Mexico	Costa Rica
length	6.240 — 7.058	6.704 — 11.600
width	0.331 — 0.390	0.416 — 0.480
cephalic width (without cut.)	0.066 — 0.082	0.075 — 0.093
cephalic width (with cut.)	0.112 — 0.123	0.105 — 0.131
length of buccal capsule	0.010 — 0.016	0.045 — 0.056
width of buccal capsule	0.010 — 0.012	0.019 — 0.022
length of esophagus	0.409 — 0.420	0.432 — 0.448
width of esophagus	0.078 — 0.090	0.075 — 0.082
tail	0.332 — 0.442	0.330 — 0.432
vulva to anterior extremity	3.802	3.680 — 6.240
length of eggs	0.098 — 0.106	0.094 — 0.112
width of eggs	0.053 — 0.061	0.056 — 0.060

This is not an ideal way of presenting measurements, since highest values of all variates are not always found in the largest individual. Nevertheless, measurements of the width of the anterior extremity without cuticle show that the American individuals have a narrower extremity than the European ones, a character also easily observed in the figures. Some authors do not accept that a same species occurs in different hosts and localities: ... "Die Parasiten werden vielfach mit europaeischen Arten verwechselt, was auch mit fast allen anderen Parasiten der brasilianischen Batrachier geschieht"... (Travassos, 1926); ... "il semble *a priori* peu vraisemblable, pour des nématodes d'amphibiens ou de reptiles, qu'une espèce anglaise, telle que *Rh. sphaerocephala* Goodey, 1924, puisse se trouver à Costa Rica"... (Chabaud, Brygoo & Petter, 1961).

In my previous paper on rhabdiasids of Brazilian *Bufo* (Kloss, 1971), I accepted the identity of the parasites of *Bufo m. marinus* and *Bufo m. paracnemis* with those of *B. bufo* from England, not only because *R. sphaerocephala* had previously been referred to as a parasite of Middle American *B. marinus,* but also because living animals examined showed the same globular anterior extremity as figured by Goodey in 1924. After fixation, this character was partially lost. Since measurements of my material agreed perfectly with those given by Goodey, Bravo H. & Caballero, and Brenes & Bravo H., I did not hesitate to identify the species as *R. sphaerocephala.* In the same paper, some data on the free-living generation were added: females bear 1 to 4 infective larvae each, depending on the local climate.

The relevant statistics of the linear regression of esophagus and tail length on total length of the hermaphroditic forms, are:

	n	b	a
esophagus	17	0.0155 ± 0.0031	0.2837 ± 0.0223
	20	0.0083 ± 0.0031	0.3753 ± 0.0331
	26	0.0136 ± 0.0028	0.3387 ± 0.0141
	60	0.0125 ± 0.0059	0.2904 ± 0.0412
	16	0.0254 ± 0.0098	0.1393 ± 0.1100
	62	0.0071 ± 0.0014	0.3974 ± 0.0100
	30	0.0144 ± 0.0017	0.3050 ± 0.0173
tail	17	0.0128 ± 0.0050	0.1581 ± 0.0360
	26	0.0070 ± 0.0026	0.1879 ± 0.0100

Rhabdias fuelleborni Travassos, 1926

This species was described on the assumption that parasites from different hosts and localities are different. Not even a differential diagnosis was given. The host was *B. marinus* from São Paulo, Brazil, and the measurements were: total length 10 to 12 mm; width without cuticle 0.47 to 0.48 mm; esophagus 0.45 to 0.50 x 0.10 mm; tail 0.37 to 0.42 mm; vulva to anus 5.0 to 5.7 mm. Travassos called the attention to the anterior extremity of the esophagus which shows a slight dilation which recalls the rhabditid origin of the parasite. Bravo

H. & Caballero (1940) called attention to the resemblance between *R. fuelleborni* and the form they called *R. sphaerocephala* from Mexican *B. marinus*. The anterior extremities of the two species are really identical, but *R. sphaerocephala* is shorter and has a weaker buccal capsule and has a post-equatorial vulva (in *R. fuelleborni* it is pre-equatorial). These authors preferred to consider the two species as different. The redescription of *R. sphaerocephala* from Costa Rica (Brenes & Bravo H., 1959) showed the little importance of the differences in length, since it included individuals as long as the original materials of *R. fuelleborni*.

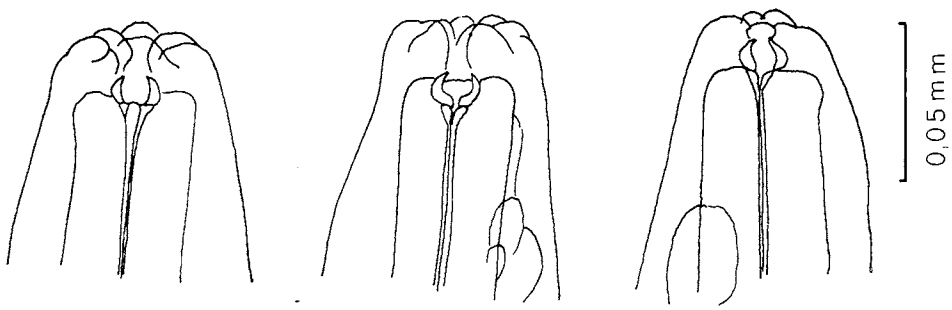


Fig. 1. Different positions of the stom of *R. fuelleborni*, which depend on the contractions of the mouth.

In my recent paper (Kloss, 1971) I gave the following differential diagnosis: *R. fuelleborni* differs from *R. sphaerocephala* by its slenderness, and by attaining a longer length. It also does not present the muscular cephalic dilation, and the females of the free-living generation bear 1 to 2 infective larvae.

The relevant statistics of the linear regression of esophagus and tail length on body length, are:

	n	b	a
esophagus	20	0.0211 ± 0.0078	0.3229 ± 0.0927
	19	0.0149 ± 0.0048	0.4693 ± 0.0608
	25	0.0152 ± 0.0055	0.4071 ± 0.0655
tail	20	0.0422 ± 0.0159	0.0503 ± 0.1894
	25	0.0349 ± 0.0128	0.1481 ± 0.1532

Rhabdias elegans Gutiérrez, 1945

A species described from *B. arenarum* from La Plata, Argentina. In the original description it was compared with *R. fuelleborni*, being found to differ in measurements: total length 4.55 to 9.50 mm; width 0.270 to 0.357 mm; buccal capsule 0.007 x 0.007 mm; esophagus 0.314 to 0.490 x 0.042 to 0.059 mm; tail 0.255 to 0.400 mm; vulva

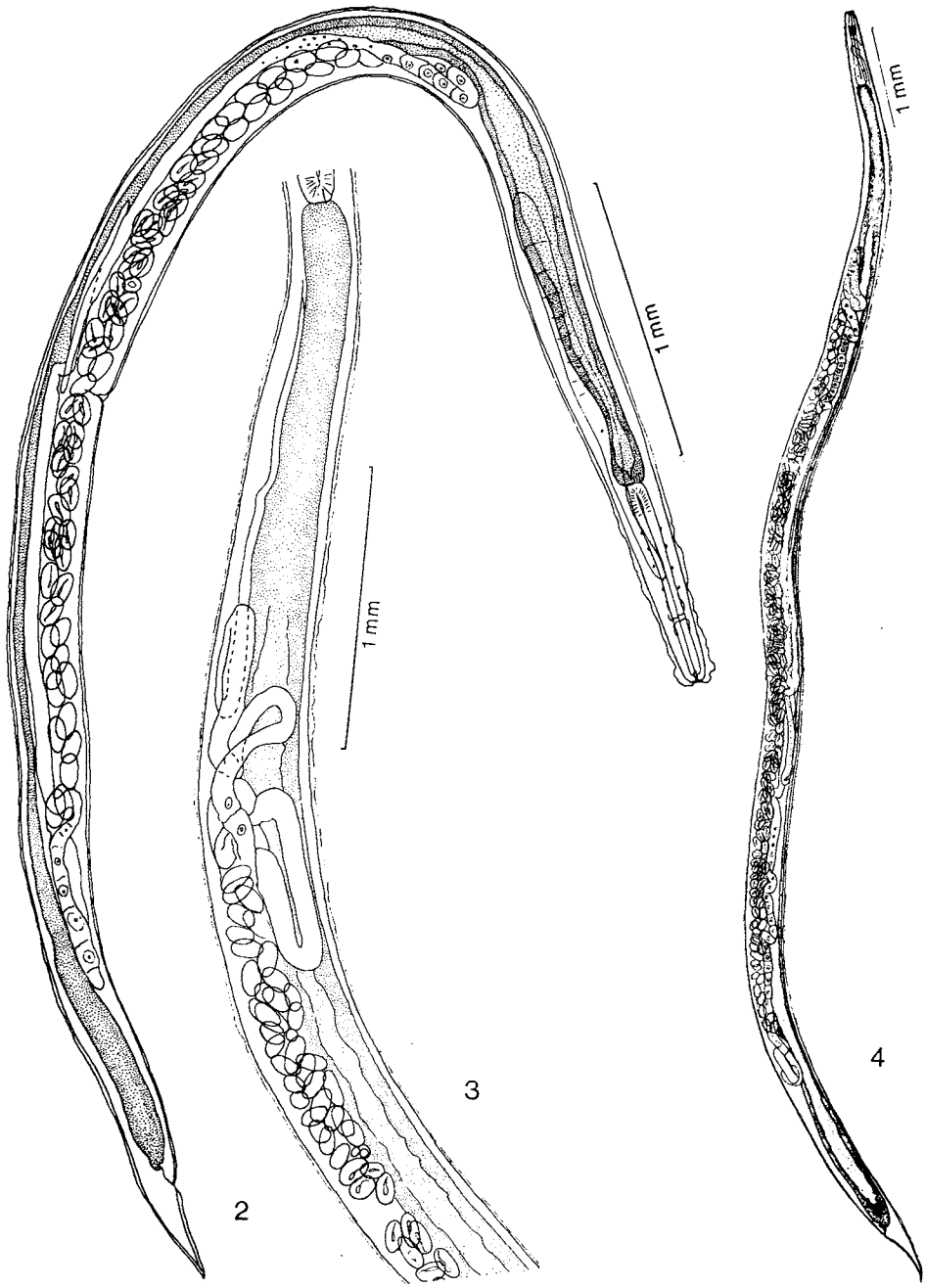


Fig. 2, *R. fuelleborni*. Fig. 3, Abnormality in the position of the anterior ovary of a *R. fuelleborni*. Fig. 4, *R. hermaphrodita*.

to caudal extremity 2.4 to 4.4 mm. Differences between the two species are in the buccal capsule, 0.007 x 0.009 mm (*sic*) for *R. elegans* and 0.009 x 0.009 mm for *R. fuelleborni*. The species described by Travassos is also longer, with a longer tail. Nevertheless, if proportions are analyzed, the two species match as already observed by Brenes & Bravo H. (1959). Even so, I (1971) preferred to consider *R. elegans* as a distinct species, because the individuals examined, in spite of having the same features as *R. fuelleborni*, never attained to total length of this species. Besides, *R. elegans* has a shorter tail; its proportions are similar to those of *R. sphaerocephala*, without the cephalic dilation. Females from the free-living generation bear 3 to 4 infective larvae. The relevant statistics of the linear regression of esophagus on body length, are:

	n	b	a
esophagus	12	0.0361 ± 0.0150	0.1498 ± 0.1442

Rhabdias hermaphrodita Kloss, 1971

A species found in the lungs of *B. crucifer*. It resembles *R. sphaerocephala*, but lacks the globular anterior extremity. The tail is shorter than that of *R. fuelleborni*. Measurements were presented individually, in tables:

	body length	esophagus	tail
CG	4.326 — 9.723	0.371 — 0.597	0.226 — 0.412
Eb	5.245 — 11.567	0.417 — 0.653	0.268 — 0.482
SP	4.077 — 7.663	0.268 — 0.385	0.268 — 0.375
ST	7.030 — 12.872	0.482 — 0.663	0.332 — 0.524

The relevant statistics of the linear regression of esophagus on body length, are:

	n	b	a
esophagus	7	0.0275 ± 0.0035	0.2892 ± 0.0400
	11	0.0338 ± 0.0076	0.2434 ± 0.0591
	28	0.0240 ± 0.0047	0.3269 ± 0.0435
tail	7	0.0261 ± 0.0109	0.1385 ± 0.1260

According to the description above, it may be immediately perceived that morphological differences among species of *Rhabdias*, qualitative and quantitative, are at least subtle. *R. sphaerocephala* and *R. fuelleborni* are the most characteristics, but *R. elegans* and *R. hermaphrodita* show intermediate characteristics, and would easily be determined erroneously, because the concepts "robust" and "slender" vary from author to author, and the samples are not always amenable to regression analysis.

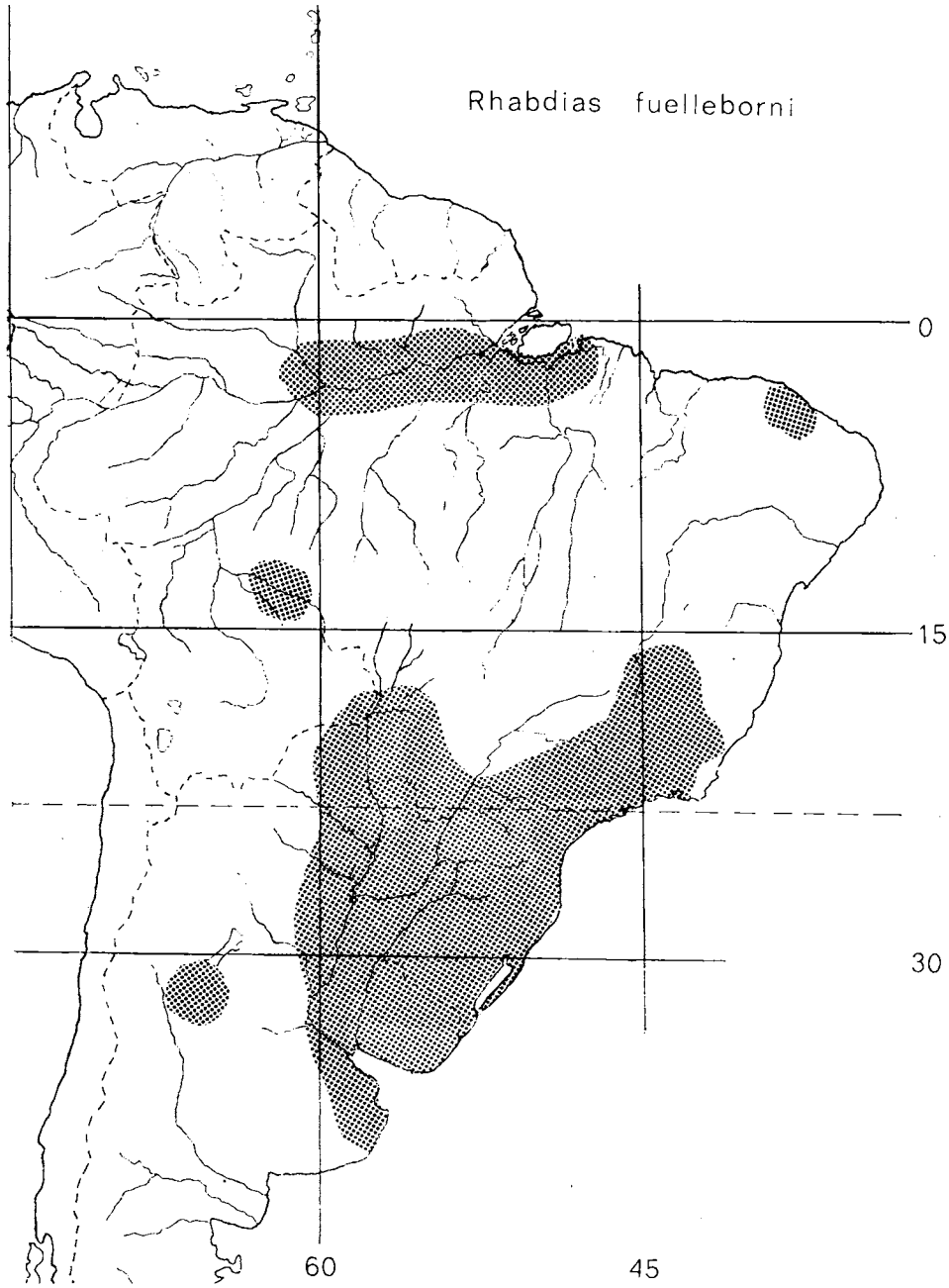
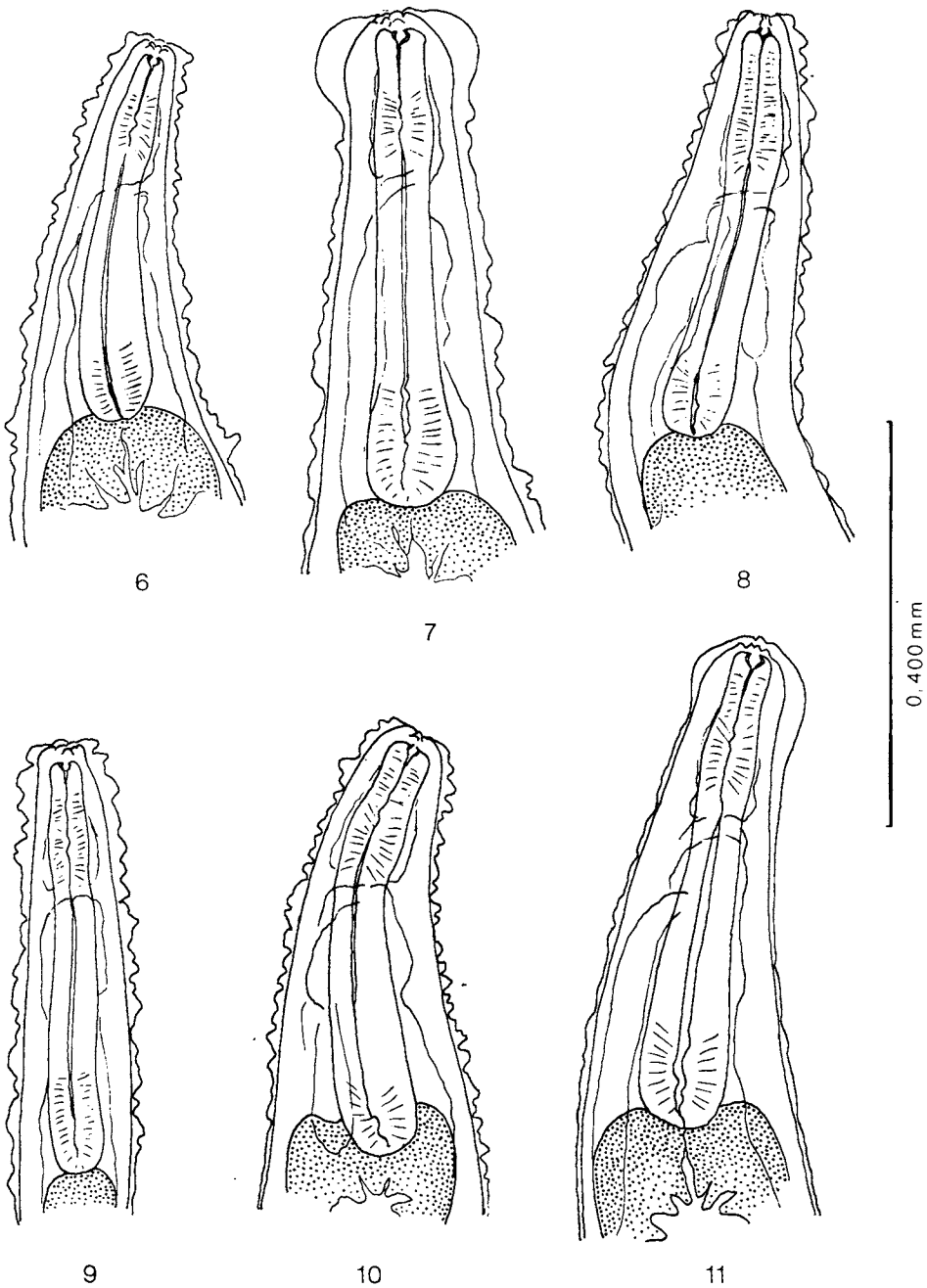
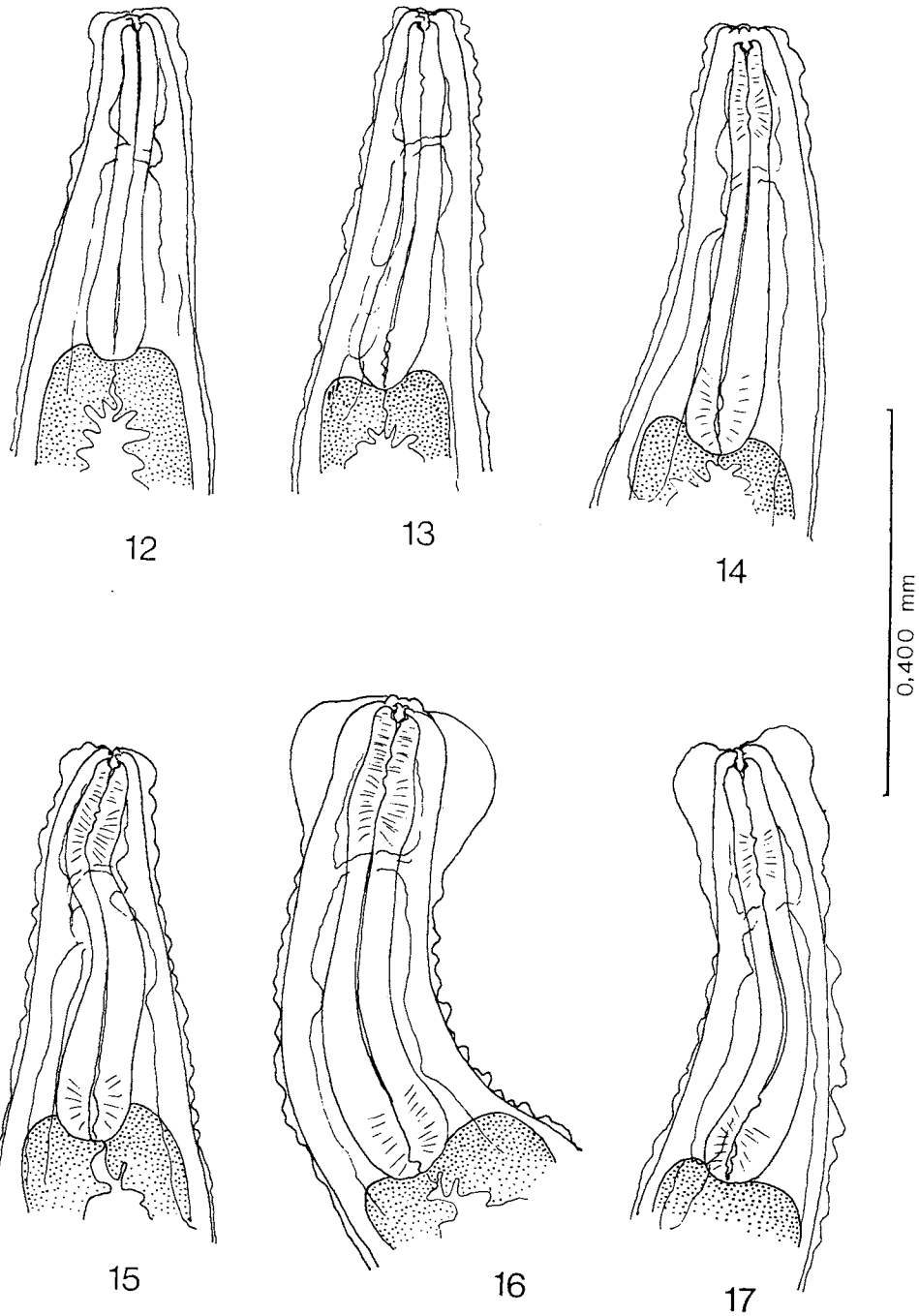


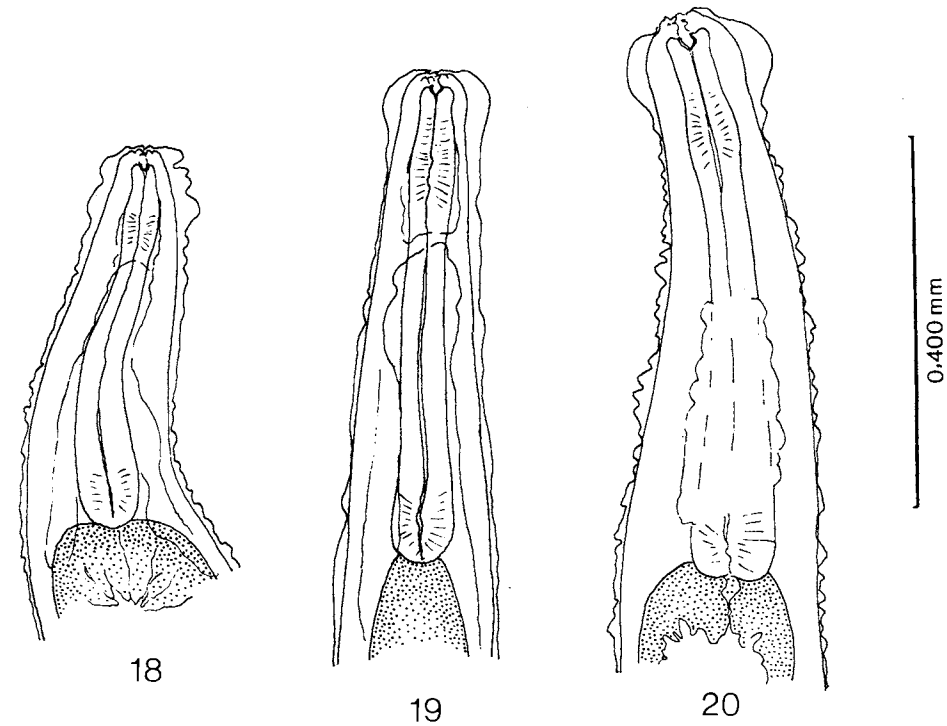
Fig. 5, References to the geographical distribution of *R. fuelleborni* in South America.



Variations of the anterior extremity of *R. fuelleborni* of *Bufo m. paracnemis*. Figs. 6, 7 Trinidad. Figs. 8, 9 Isla Valle. Figs. 10, 11 Coronel Bogado.



Variations of the anterior extremity of *R. fuelleborni* of *Bufo m. paracnemis*. Figs. 12, 13 Areguá. Fig. 14 Chaco-i. Figs. 15, 16 Ypacaray. Fig. 17 Luque.



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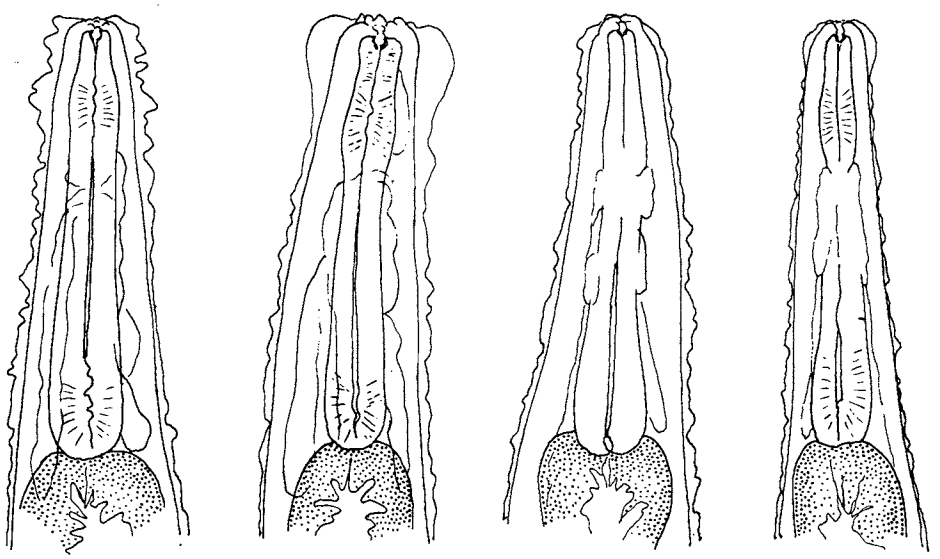
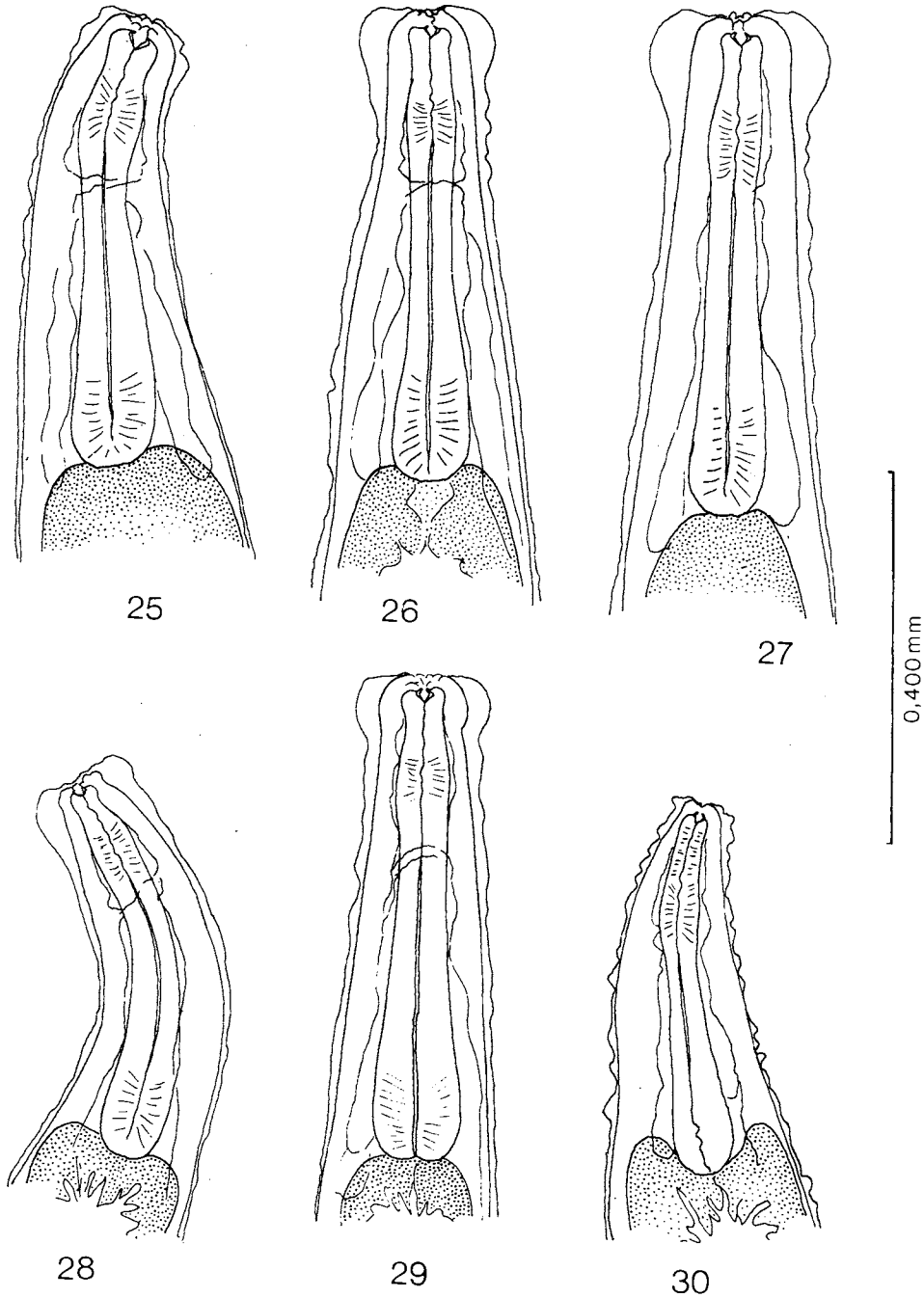
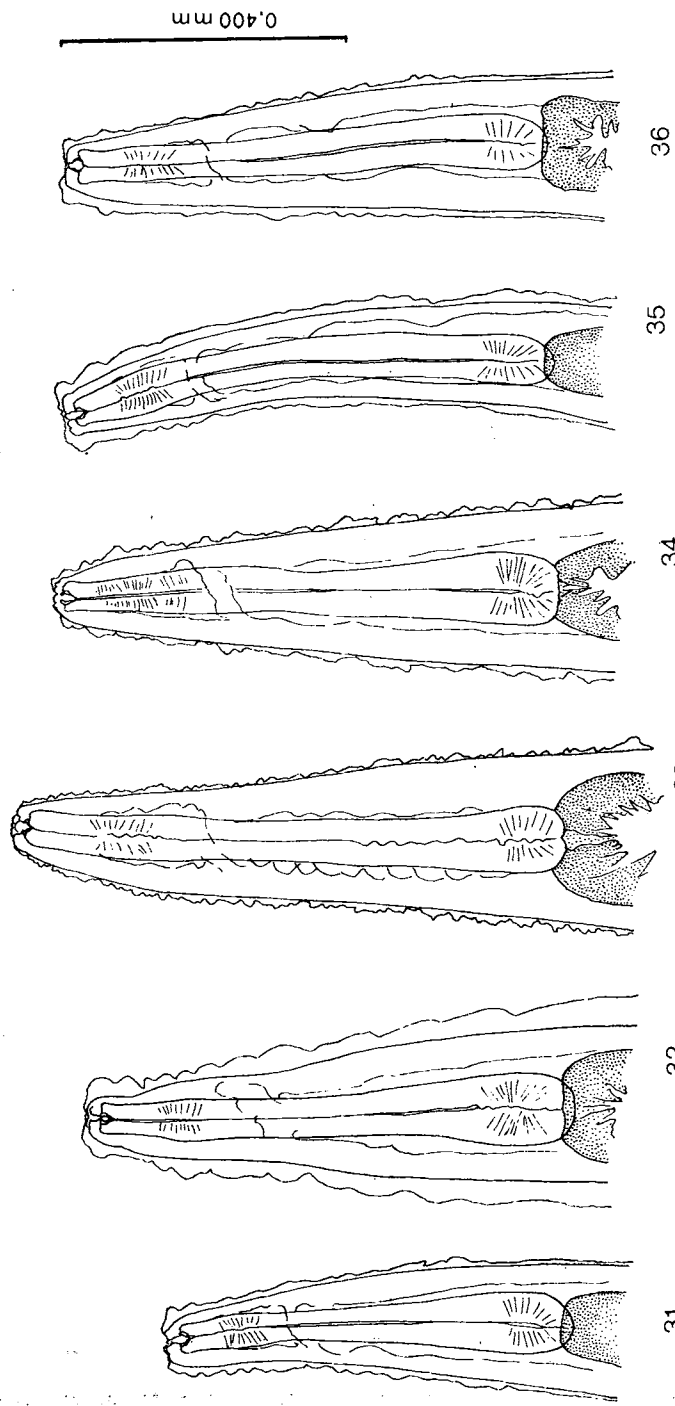


21 22 23 24

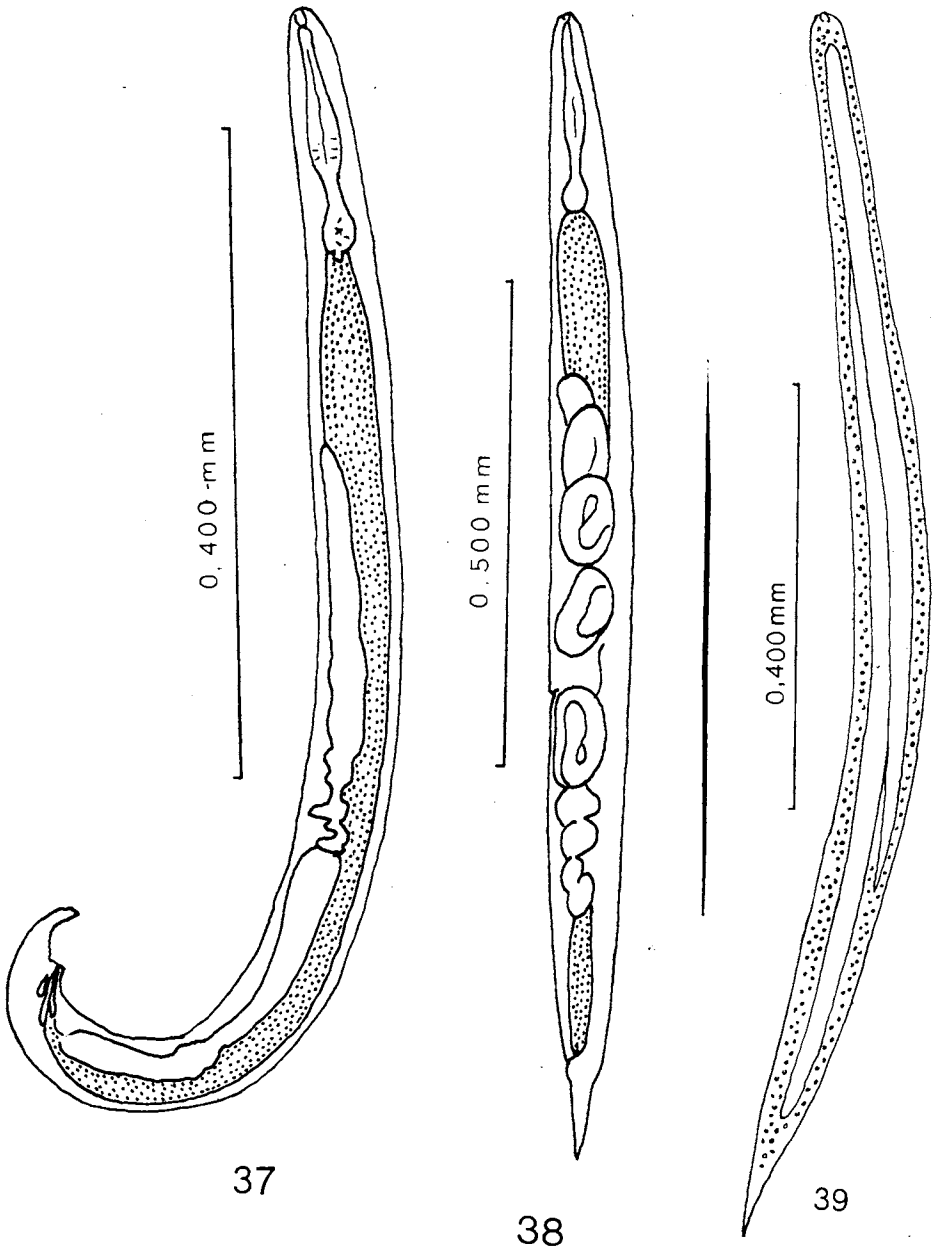
Variations of the anterior extremity of *R. fuelleborni* of *Bufo m. paracnemis* (figs.18-22), and *B. arenarum* (figs. 23, 24). Fig. 18 Villarrica. Fig. 19 Guaíra. Fig. 20 Emas. Fig. 21 B. Horizonte. Fig. 22 Lagoa Santa. Fig. 23 Congonhas do Campo. Fig. 24 Calciolândia.



Variations of the anterior extremity of *R. fuelleborni* of *Bufo m. marinus* (figs. 25-28), *B. m. marinus* x *B. m. paracnemis* (fig. 29), and *Bufo m. paracnemis* (fig. 30). Fig. 25 Belém. Fig. 26 Maicuru. Fig. 27 R. Preto da Eva. Fig. 28 Manaus. Fig. 29 Príncipe da Beira. Fig. 30 Remanso Castillo.



Variations of the anterior extremity of *R. fuelleborni* of *Bufo m. ictericus*. Fig. 31 Serra da Bocaina. Fig. 32 Rio de Janeiro. Fig. 33 Casa Grande. Fig. 34 Novo Horizonte. Fig. 35 Caxias do Sul. Fig. 36 Salvador do Sul.



Free-living generation of *R. fuelleborni*. Fig. 37 Male. Fig. 38 Female. Fig. 39 Female with infective larvae.

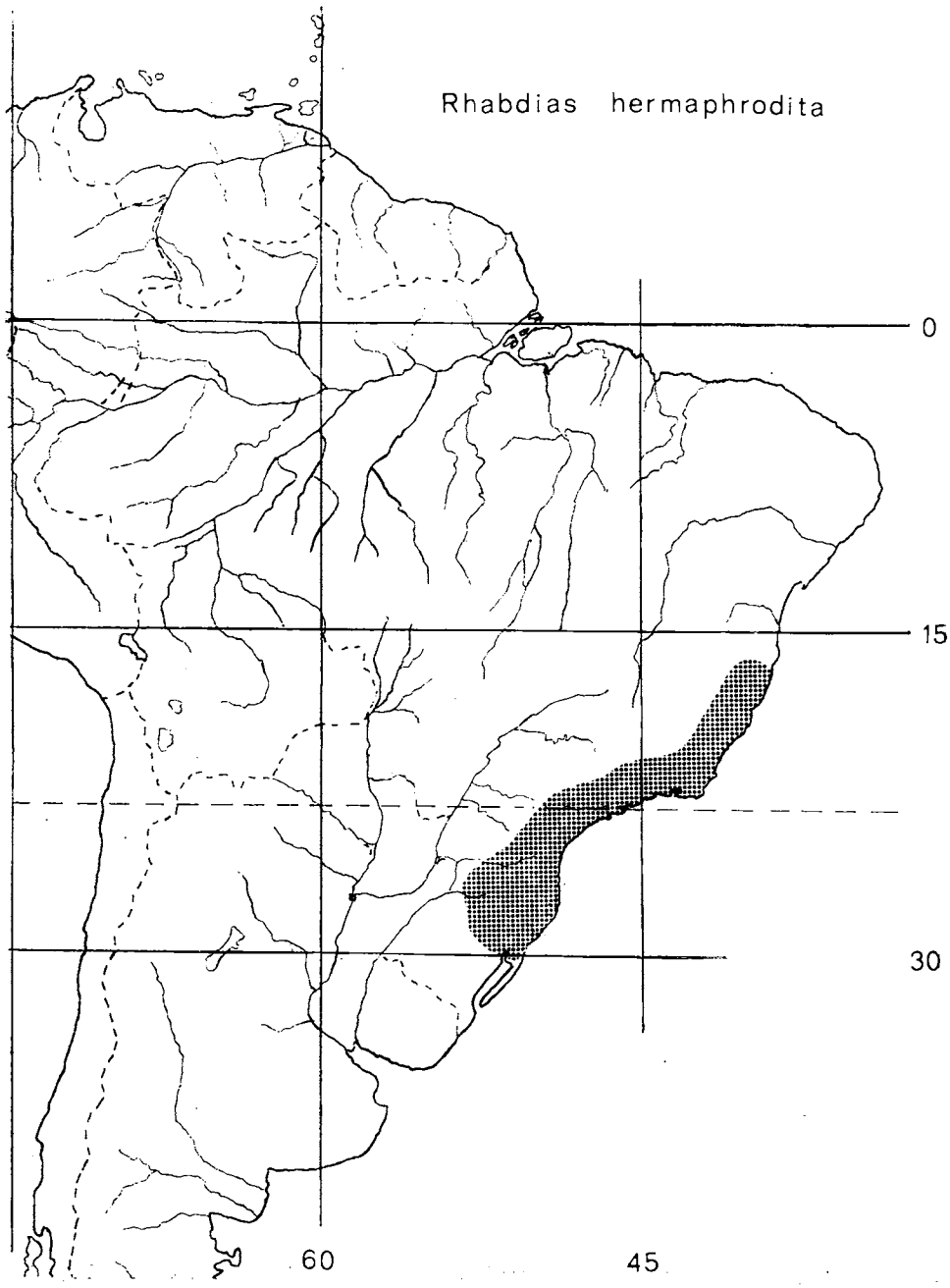
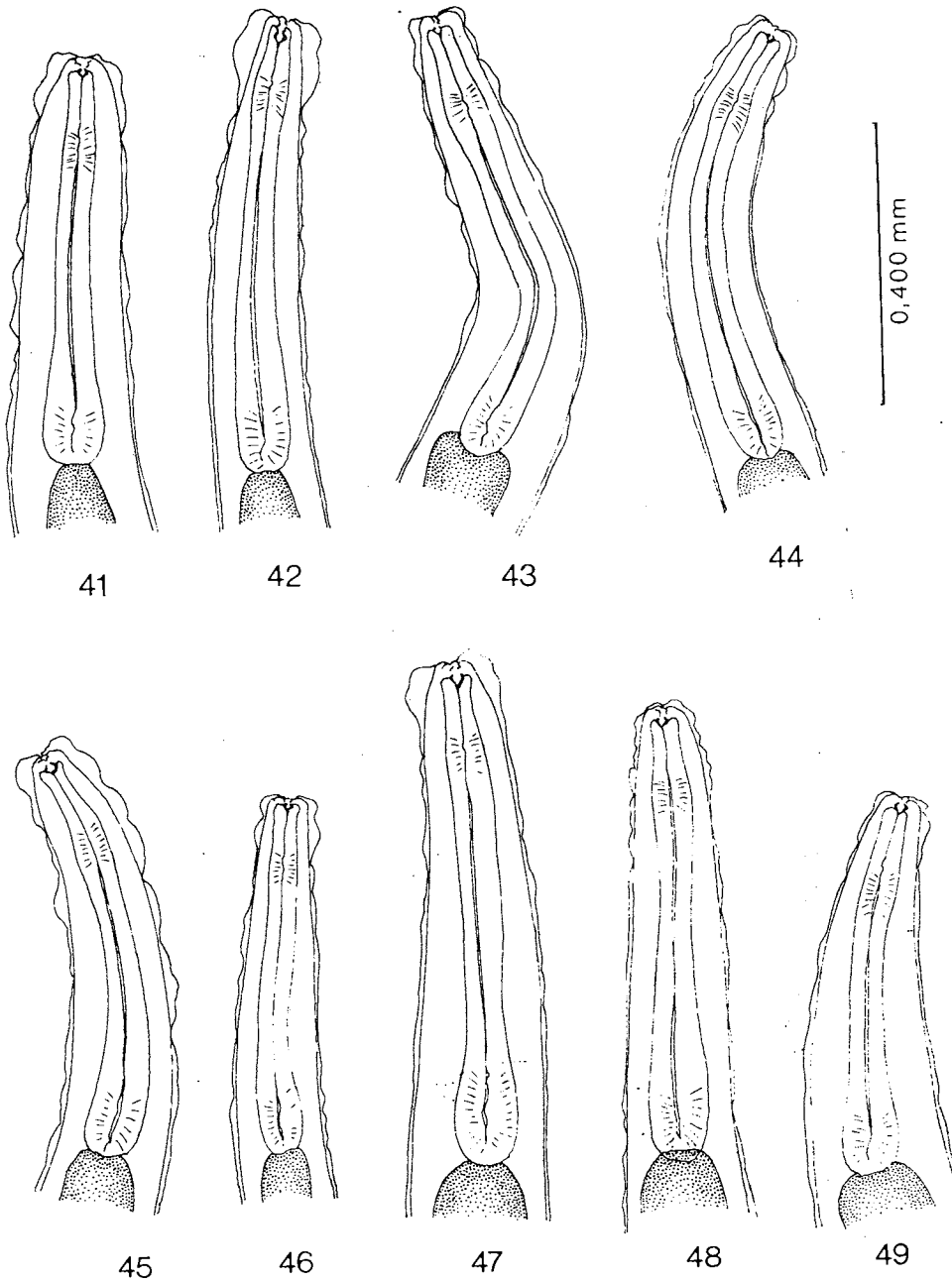
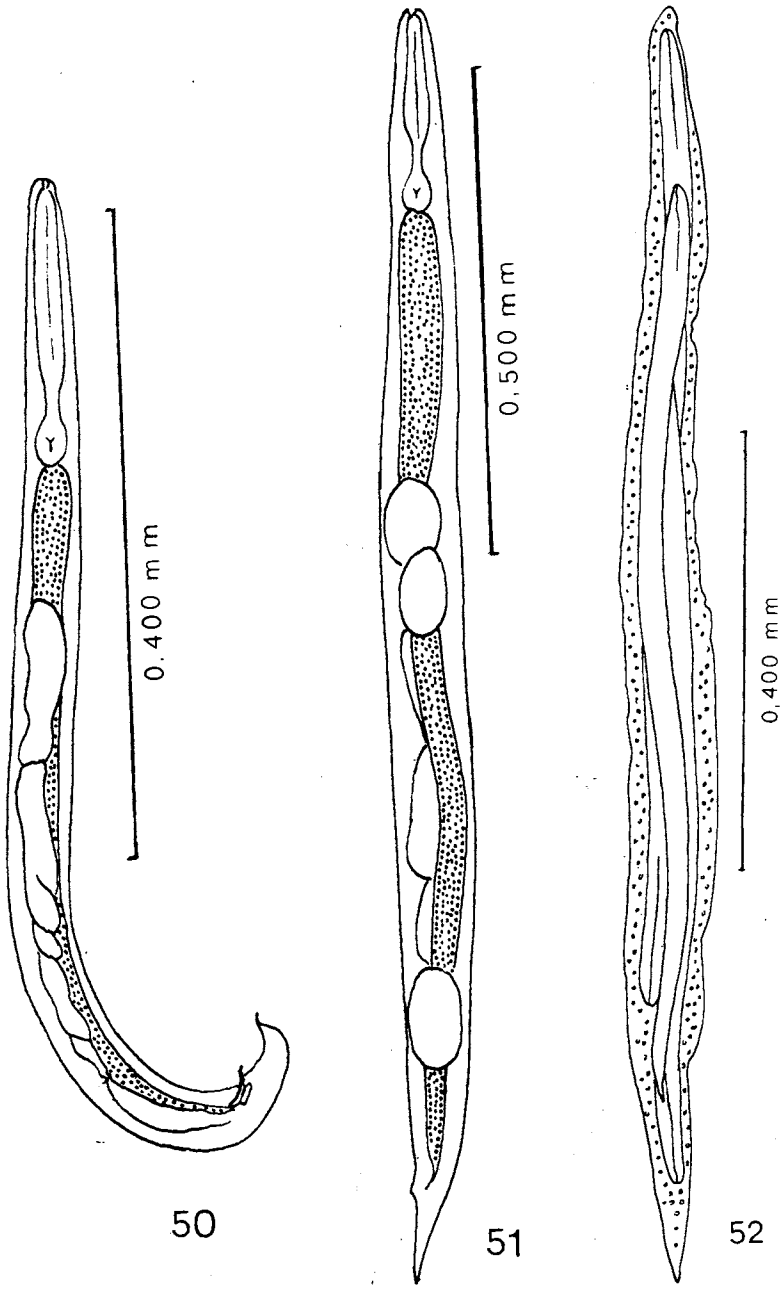


Fig. 40, References to the geographical distribution of *R. hermaphrodita* in South America.



Variations of the anterior extremity of *R. hermaphrodita* of *B. crucifer* (figs. 41, 42, 45, 47, 48), and *T. miliaris* (figs. 43, 44, 46, 49). Fig. 41 Sooretama. Fig. 42 Sta. Teresa. Fig. 43 Rio de Janeiro. Fig. 44 Angra dos Reis. Figs. 45, 46 Casa Grande. Fig. 47 Embu. Figs. 48, 49 Novo Horizonte.



Free-living generation of *R. hermaphrodita*. Fig. 50 Male. Fig. 51 Female. Fig. 52 Female with infective larvae.

STATISTICAL ANALYSES

Aiming at improving the systematic analysis of my materials, I tried grouping samples by their qualitative morphological characters, such as the cephalic dilation, and the presence or absence of the small dilation called the pseudo-bulb on the anterior end of the esophagus. However, there were samples which presented extreme variations of these characters, such as those from Trinidad, Coronel Bogado and Ypacaray (Paraguay) with individuals with both globular and pointed anterior extremities (figs. 10, 11, 14, 15, 19, 20), and great variation of the pseudobulb (figs. 6 to 36). This sort of analysis had to be abandonned.

Afterwards I tried statistical analyses of the measurable characters, the length of esophagus, and of the tail. The buccal capsule did not show any differences, and the position of the vulva, a character used by some authors, was found to be always on the middle third of the body, sometimes a little ahead, other times behind the middle of the body, depending on the pressure of the uteri full of eggs upon the ovijector

To determine the nature and extent, if any, of the relationship of size of organs with body length, I performed regression analyses, considering the body length as the independent variate, and the esophagus and tail lengths as dependent variates.

Not all samples did show significant regressions; in these cases I applied analysis of variance, using the method of Kramer and Duncan.

The proportion of infested individuals in the samples of *Bufo* submitted to autopsy was also investigated. The confidence limits (95%) of the percentages are drawn from the binomial distribution tables of J. R. Geigy, S. A. (1965). The significance of the differences was estimated by the chi-square distribution (Geigy's tables, 1965).

The abbreviations used, are:

a = regression constant
A = tail
b = regression coefficient
EF = esophagus
L = body length
n = number of individuals
ov.a. = anterior ovary
r = correlation coefficient

The analyses of the samples of *Rhabdias,* for each host, resulted in:

Bufo m. marinus

i) Esophagus:

The samples from Belém and Maicuru (PA), Rio Preto da Eva and Manaus (AM) (graph 1) were statistically analyzed. Only the

sample from Rio Preto da Eva did not show a regression significant at the 5% level. In the other 3 samples, the relevant statistics of the linear regression of esophagean length on body length, are:

	n	b	a
Manaus	26	0.0136 ± 0.0028	0.3387 ± 0.0141
Maicuru	20	0.0083 ± 0.0031	0.3753 ± 0.0331
Belém	17	0.0155 ± 0.0031	0.2837 ± 0.0223

Upon analysis these samples were found to be not homogeneous as to the regression constant. Kramer's test showed that Manaus and Belém differ significantly, and that Maicuru is intermediate.

Mapping the esophagean means, I obtained a mosaic distribution.

ii) Tail:

Only two samples showed significant regression of tail on body lengths:

	n	b	a
Manaus	26	0.0070 ± 0.0026	0.1879 ± 0.0100
Belém	17	0.0128 ± 0.0050	0.1581 ± 0.0360

These two lines do not differ significantly.

Considering the results above, the 4 samples of *Rhabdias* obtained from *Bufo m. marinus* may be said to represent a single species.

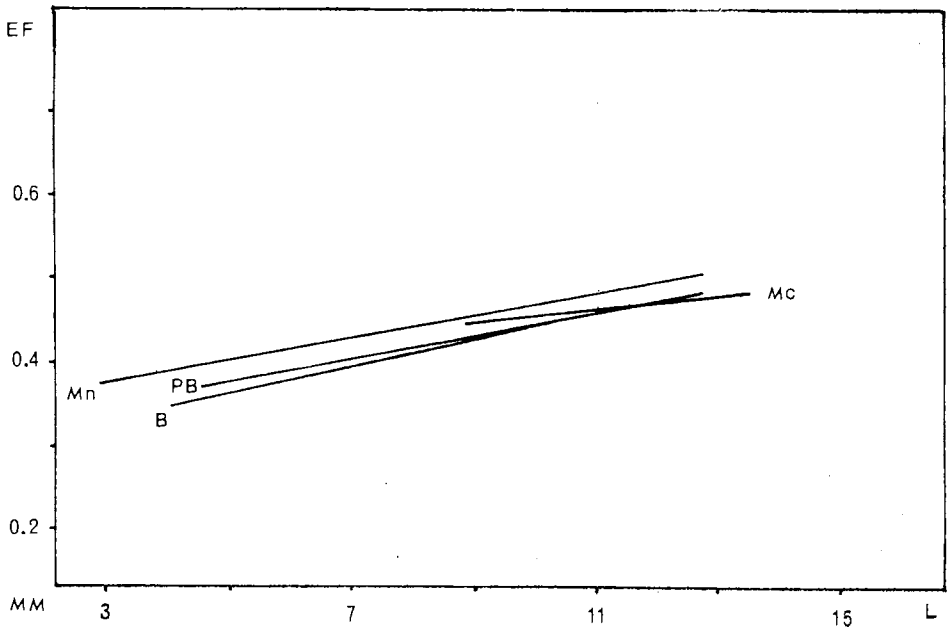
Bufo m. paracnemis

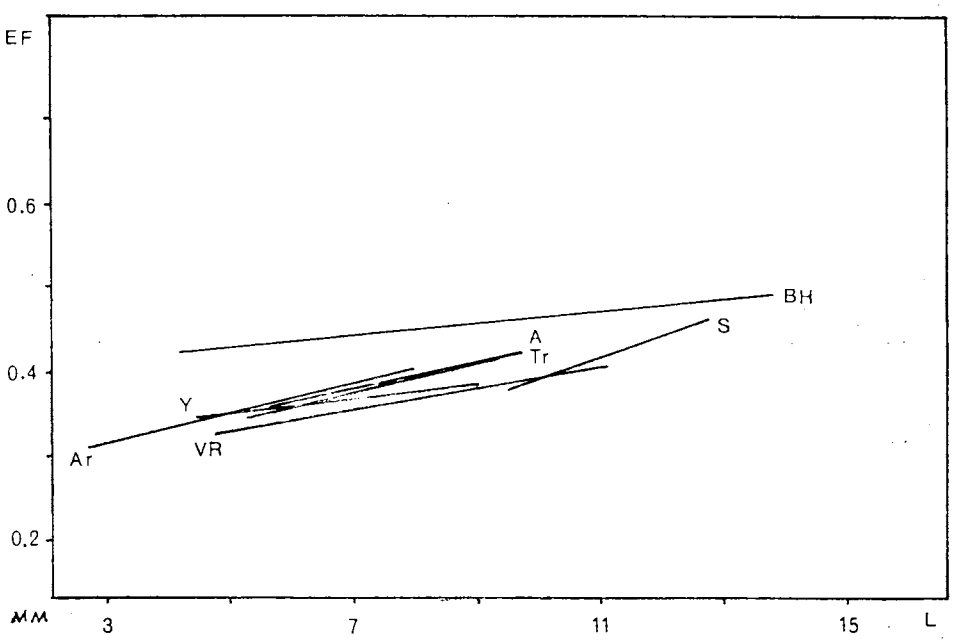
i) Esophagus:

The following samples of *Rhabdias* from this host, were statistically analyzed: Lagoa Santa and Belo Horizonte (MG), Emas (SP), Guaíra (PR), Salobra (MT), Coronel Bogado, Pto. Juan Barbero, Luque, Isla Valle, Chaco-í, Remanso Castillo, Asunción, Trinidad, Ypacaray, Areguá and Villarrica (Paraguay) (graph 2).

Most samples did not show correlation between esophagus and body lengths. The only ones in which the regressions were significant at the 5% level, were those from B. Horizonte, Salobra, Trinidad, Areguá, and Villarrica:

	n	b	a
B. Horizonte	62	0.0071 ± 0.0014	0.3974 ± 0.0100
Salobra	16	0.0254 ± 0.0098	0.1393 ± 0.1100
Asunción	20	0.0162 ± 0.0060	0.2700 ± 0.0446
Trinidad	19	0.0171 ± 0.0074	0.2551 ± 0.0539
Areguá	19	0.0170 ± 0.0052	0.2688 ± 0.0319
Villarrica	20	0.0123 ± 0.0053	0.2699 ± 0.0399





GRAPH 1. Regression lines of esophagean length on body length of *R. fuelleborni* of *Bufo m. marinus*. GRAPH 2. Regression lines of the esophagean length on body length of *R. fuelleborni* of *Bufo m. paracnemis*.

These regressions did not differ in the coefficients of regression, but the constants of regression were seen to differ significantly. Kramer's test separated on one side the samples from B. Horizonte and on the other those from southwestern Brazil and Paraguay.

The mapping of the means of the esophagean measurements resulted in a mosaic distribution.

ii) Tail:

No *Rhabdias* sample from *Bufo m. paracnemis* showed correlation between tail and body length. Analysis of variance resulted in a mosaic.

Bufo m. ictericus

i) Esophagus:

I analyzed the samples of *Rhabdias* from *Bufo m. ictericus* from Rio de Janeiro (GB), Serra da Bocaina, Teresópolis and Parati (RJ), Caldas (MG), Casa Grande (SP), Novo Horizonte (SC), Salvador do Sul and Caxias do Sul (RS) (graph 3). Only the samples from Rio de Janeiro, Novo Horizonte, Salvador do Sul, Caldas and Parati showed significant regressions:

	n	b	a
Rio de Janeiro	19	0.0149 ± 0.0048	0.4693 ± 0.0608
Novo Horizonte	20	0.0137 ± 0.0042	0.4657 ± 0.0564
Salvador do Sul	25	0.0152 ± 0.0055	0.4071 ± 0.0655
Caldas	20	0.0211 ± 0.0078	0.3229 ± 0.0927
Parati	20	0.0174 ± 0.0048	0.3305 ± 0.0349

The coefficients of regression are homogeneous, but the constants are not. Kramer's test resulted into a mosaic distribution.

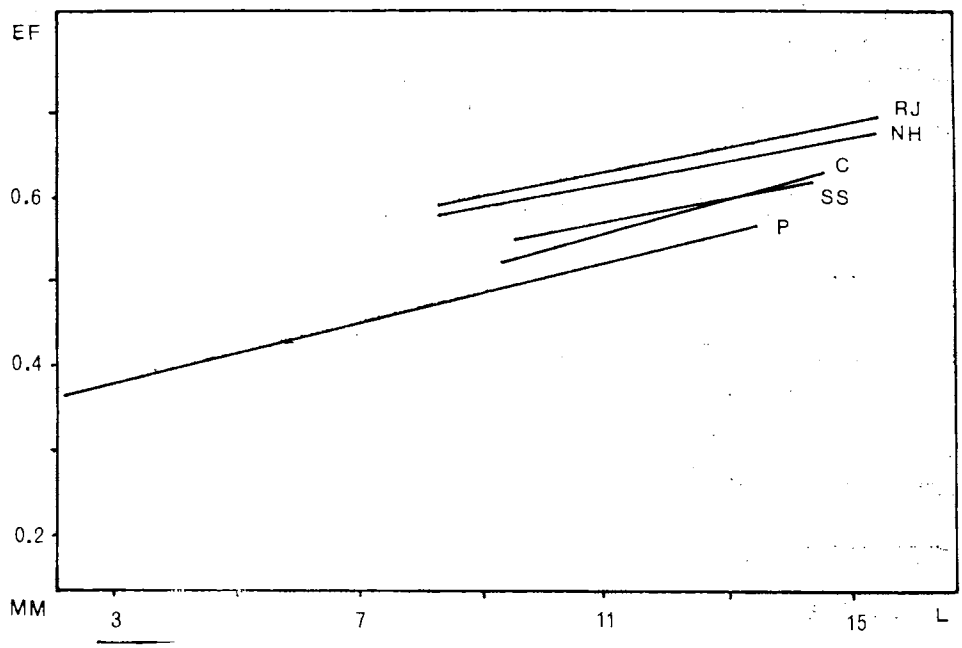
ii) Tail:

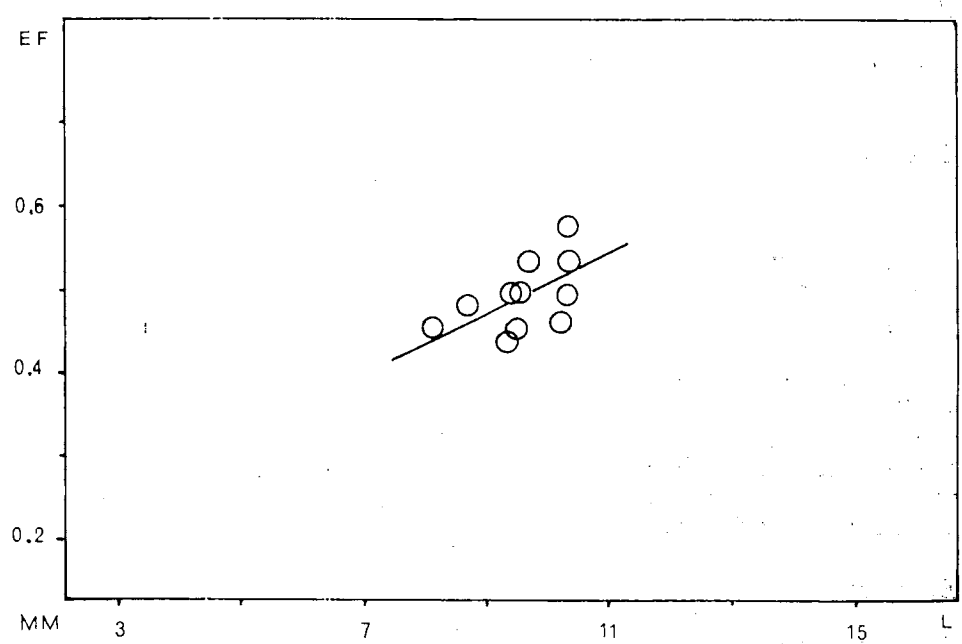
Only 4 samples showed a significant correlation coefficient between tail and body length:

	n	b	a
Novo Horizonte	20	0.0271 ± 0.0060	0.2240 ± 0.0769
Salvador do Sul	25	0.0349 ± 0.0128	0.1481 ± 0.1532
Caldas	20	0.0422 ± 0.0159	0.0503 ± 0.1894
Parati	20	0.0197 ± 0.0059	0.2967 ± 0.0412

The four samples are homogeneous.

From the analyses above, I believe that *Rhabdias* from *Bufo m. ictericus* represents a single species, some of the samples having larger individuals than others. Otherwise, the samples from *Bufo m. marinus* may be considered as identical to those from *Bufo m. ictericus*. Lung-





GRAPH 3. Regression lines of the esophagean length on body length of *R. fuellebornı* of *Bufo m. ictericus.* GRAPH 4. Regression line of the esophagean length on body length of *R. fuellebornı* of *B. arenarum* from Montevideo. n = 12 r = 0.5920 b = 0.0361 ± 0.0150 a = 0.1498 ± 0.1442

parasites of *Bufo m. paracnemis* can be separated in two groups by their constants of regression: (i) from B. Horizonte, and (ii) from Areguá, Asunción, Trinidad, Salobra, and Villarrica.

In order to compare all the samples obtained from *B. marinus*, the regression lines were reduced, as follows:

(i) Manaus, Maicuru, and Belém.

(ii) Salobra, Asunción, Areguá, Trinidad, and Villarrica.

(iii) Rio de Janeiro, Novo Horizonte, Salvador do Sul, Caldas, and Parati.

Comparing these 3 regression lines, I also included those from the samples from B. Horizonte (*Bufo m. paracnemis*) which separated from those of group (ii), and from Príncipe da Beira (*B. m. marinus* x *B. m. paracnemis*) the only sample of this host (graph 1).

These regression lines were seen to be heterogeneous as to the coefficients of regression. Kramer's test isolated the samples obtained from *Bufo m. ictericus* from the other subspecies of *B. marinus*.

Bufo arenarum

(i) Esophagus:

From the 4 samples analysed statistically, Asunción (Paraguay), Montevideo (Uruguay), Congonhas do Campo and Calciolândia (MG), only Montevideo showed a significant correlation between esophagus and body lengths (graph 4):

	n	b	a
Montevideo	12	0.0361 $\pm$ 0.0150	0.1498 $\pm$ 0.1442

ii) Tail:

Tail length of *Rhabdias* did not show any correlation with body length in any of the 4 samples.

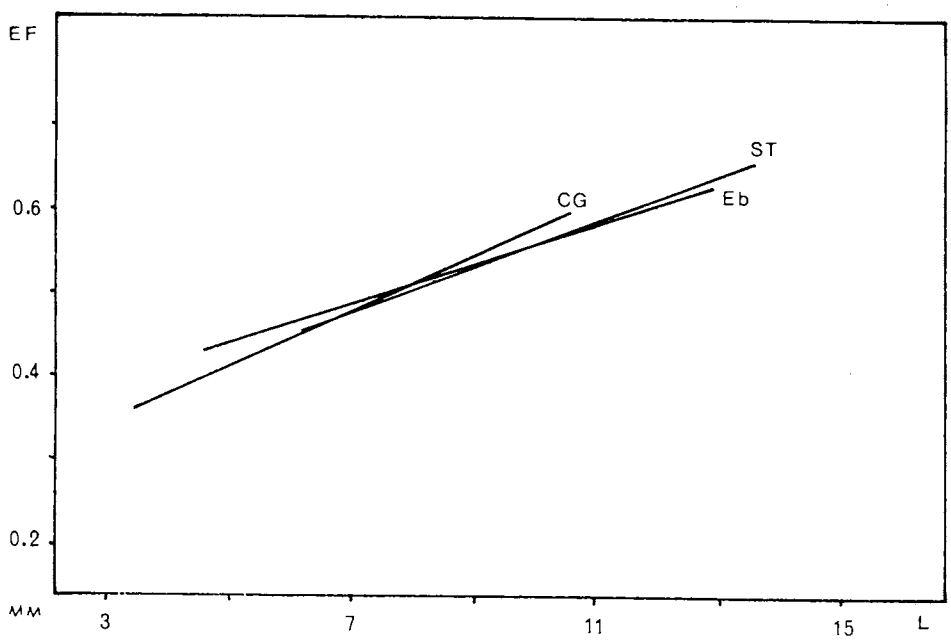
The regression of esophagean length on the body length of the Montevideo sample, compared with those from *Bufo m. marinus* x *B. m. paracnemis* from Príncipe da Beira, *Bufo m. paracnemis* from B. Horizonte, and the common regression lines obtained with the samples from *Bufo m. marinus*, *Bufo m. ictericus*, and *Bufo m. paracnemis*, did not show significant differences. With the inclusion of the Montevideo sample, the significant difference shown by the *Rhabdias* from *Bufo m. ictericus* disappeared.

Bufo crucifer

(i) Esophagus:

Four samples of rhabdiasids from *B. crucifer* were analyzed: Sta. Teresa (ES), Embu and Casa Grande (SP), and Novo Horizonte (SC). Only the sample from Novo Horizonte did not show a significant re-

gression. In the other 3 samples, the relevant statistics of the linear regression of esophagus on body length, were (graph 5):



GRAPH 5. Regression lines of the esophagean length on body length of *R. hermaphrodita* of *B. crucifer*.

	n	b	a
Sta. Teresa	7	0.0275 ± 0.0035	0.2892 ± 0.0400
Embu	28	0.0240 ± 0.0047	0.3269 ± 0.0435
Casa Grande	11	0.0338 ± 0.0076	0.2434 ± 0.0591

The 3 lines are homogeneous.

ii) Tail:

Only the measurements of the samples of *Rhabdias* from Sta. Teresa and Embu showed significant regressions:

	n	b	a
Sta. Teresa	28	0.0275 ± 0.0109	0.1385 ± 0.1260
Embu	7	0.0214 ± 0.0056	0.1774 ± 0.0529

The 2 lines do not differ significantly.

Finally, I included the common regression line obtained from the samples of *Rhabdias* of *B. crucifer* in the homogeneity test with the regression lines of all the other samples of *Rhabdias* of *Bufo* gr. *marinus*, finding no significant differences at the 5% level.

The statistical analyses of the measurable characters revealed no clear differentiation pattern of the organs analyzed. There is a perfect intergradation between the extreme values, without any geographical pattern, nor any grouping of hosts.

FREE-LIVING GENERATION

The next step undertaken to characterize the rhabdiasids was the obtention of the free-living generation. Fotedar (1965) called attention to the difficulty in determining species of *Rhabdias;* in his oppinion many of the names considered valid were probably synonyms; the solution would likely be found in the free-living generation.

Material and methods:

The culture methods were the most simple ones, using excrement of the host from which the hermaphroditic forms were obtained. After removal of coarse parts of the excrement, such as insect carcasses and wood fragments, a small portion was placed in a watch-glass and softened with water. One to three lacerated mature hermaphroditic individuals were placed in this culture medium, which was covered with a Petri dish large enough to keep some air inside and to avoid too rapid drying.

The hatching process lasted from 12 to 15 hours, depending on the room temperature. During cold weather, the cultures were heated during daytime with a light bulb. Males and numerous females lived in the excrements. 24 hours after hatching, the females were observed with eggs containing a larva; 50 hours after hatching, the filariform larvae started to lacerate the maternal cuticle. In all cultures, females always bore filariform larvae. While the dioic generation remained in the excrements, the phototropic filariform larvae migrated to the edge of the culture, and appeared in the surrounding, more diluted excrement.

I obtained the free-living generation of the parasites of the following hosts:

Bufo m. marinus from Rio Preto da Eva (AM), and Belém (PA). *Bufo m. paracnemis* from Emas (SP), and Sabará (MG). *B. m. marinus* x *B. m. paracnemis* from Príncipe da Beira (RO). *Bufo m. ictericus* from Casa Grande (SP), and from Salvador do Sul (RS). *B. arenarum* from Calciolândia and Congonhas do Campo (MG). *B. crucifer* from Casa Grande (SP). *Thoropa miliaris* also from Casa Grande (SP).

Previously free-living generations of Brazilian rhabdiasids were obtained by L. Travassos (1926), when he described *R. fuelleborni* of *B. marinus* from S. Paulo. J. Fahel (1952) recorded the same species of parasite from *Leptodactylus pentadactylus,* and also described the free-living generation, but the description and figures are reproductions of Travassos' work.

I was not able to find qualitative morphological differences in all the samples of free-living forms. Females from open country are a

little larger than those from forests, as was observed in descendants of lung-parasites of *Bufo m. paracnemis* from Emas, *B. arenarum* from Calciolândia (open country), *Bufo m. marinus* from Belém, and *Bufo m. ictericus* and *B. crucifer* from Casa Grande (forest). The body proportions fluctuate very much, but always keep a certain pattern, identical for all samples.

The number of infective larvae born by each female, is variable: of *Bufo m. marinus* from Rio Preto da Eva, always 1 larva; of *Bufo m. marinus* from Belém, 1 to 2 larvae; of *B. m. marinus* x *B. m. paracnemis* from Príncipe da Beira, 2 to 3 larvae; of *Bufo m. ictericus* from Casa Grande (Boracéia), always 2 larvae; the same host also from Casa Grande (dam) and from Salvador do Sul, 2 to 3 larvae. The parasites of *B. arenarum* from Calciolândia and Congonhas do Campo, bore 3 to 4 larvae; of *Bufo m. paracnemis* from Emas and Sabará, 3 to 4 larvae; and those from *B. crucifer* from Casa Grande (Boracéia), 3 to 4 larvae.

The infective larvae of the different samples did not differ among themselves. Very slender and active, they only showed the intestine and the contour of the esophagus.

The number of larvae produced per female may be under genetical control, but the variation probably depends on the external conditions. This can be observed in the samples of *Rhabdias* of *Bufo m. ictericus* from Casa Grande: in the Boracéia Biological Station, a more humid region, the free-living generation constantly bears 2 infective larvae, in proportion to those from near the dam, a drier region in the same forest, which bear 2 to 3 infective larvae. In the same Biological Station, where *Rhabdias* of *Bufo m. ictericus* always bear 2 infective larvae, per female, those of *B. crucifer* bear 3 to 4 larvae. This observation calls attention to the fact that in this region, the parasites of the two hosts are different.

CHROMATOPHILIC TECHNIQUES

Nematodes are usually stained by Semichon's acetocarmine, which stains the organs in different shades, varying from pink to red, making easier the analyses of specific differences.

I was inclined to believe that I was dealing with a single species of *Rhabdias* until the moment I found a massive infection of the leptodactylid *Thoropa miliaris* from Casa Grande, São Paulo. At a first look I apparently had 2 species of parasites, but I could not separate them morphologically. The females of the free-living generation of this massive infestation bore 2 or 4 infective larvae, no female bearing 3 larvae.

I started experiments with some stains trying to find some morphological character that would permit a recognition of the species. Alcoholic chlorocarmine, iron acetocarmine, borax carmine (Grenacher), iron haematoxylin (Weigert), acid fuchsin, picric acid, toluidine blue, cottonblue, and lactic acid orcein were tested.

Positive results were only obtained with a mixture of equal parts of Semichon's acetocarmine and alcoholic chlorocarmine, which evinced

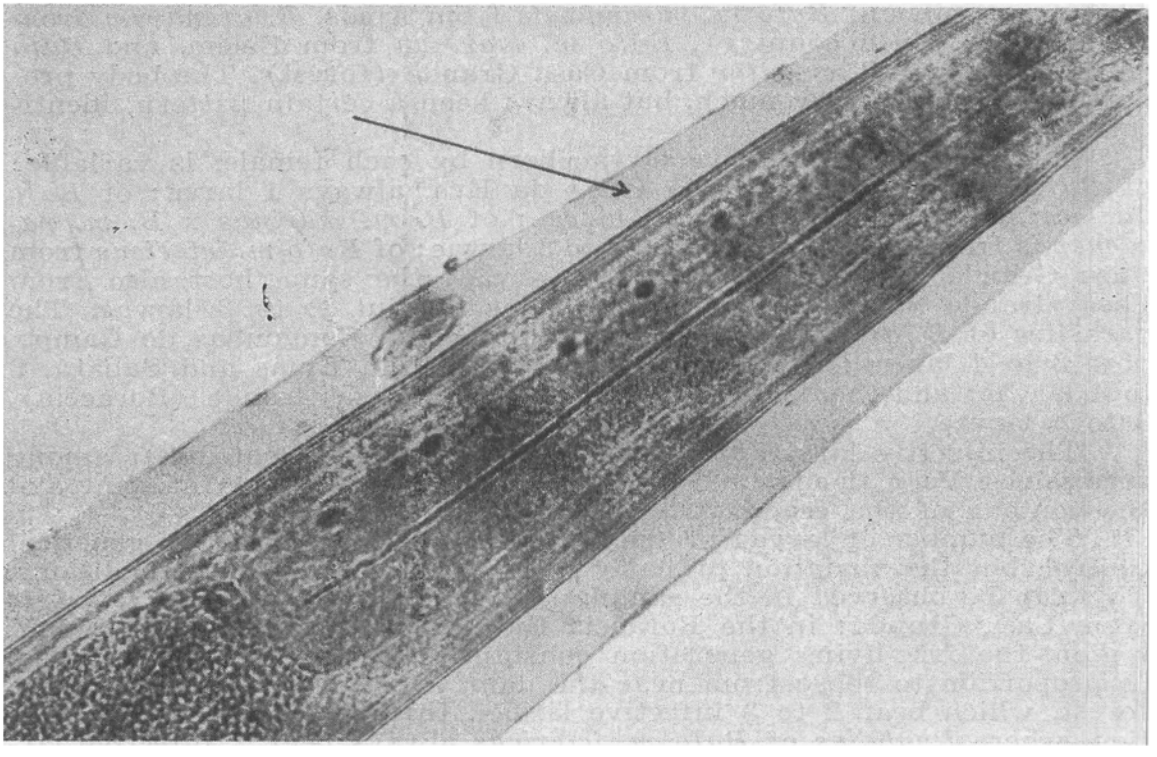


Photo 3. Anterior extremity of *R. hermaphrodita* showing the nuclei of the excretory system cells.

the nuclei of two longitudinal rows of cells that end at the level of the excretory pore. These were interpreted as cells of the excretory system.

One group of rhabdiasids from the massive infestation of *T. miliaris* had the nuclei of those cells completely stained in red, showing a regular distribution of the cells; the other group had the nuclei stained at the periphery only showing an alternate disposition of cells (Photos 3 and 4).

Staining tests were undertaken with rhabdiasids fixed and kept in acetoaldehyde. Since all the material of the helminthological collection of the Museu de Zoologia da Universidade de São Paulo and of the Instituto Oswaldo Cruz is fixed and kept in acetoaldehyde, all the samples of *Rhabdias,* not only from *Bufo* and *Thoropa,* but also those from *Leptodactylus,* were treated with this mixture of carmine.

The parasites of *Leptodactylus* did not react to the chemicals. The samples of rhabdiasids from *B. crucifer* and from single infestations of *T. miliaris* had the nuclei of their excretory system completely

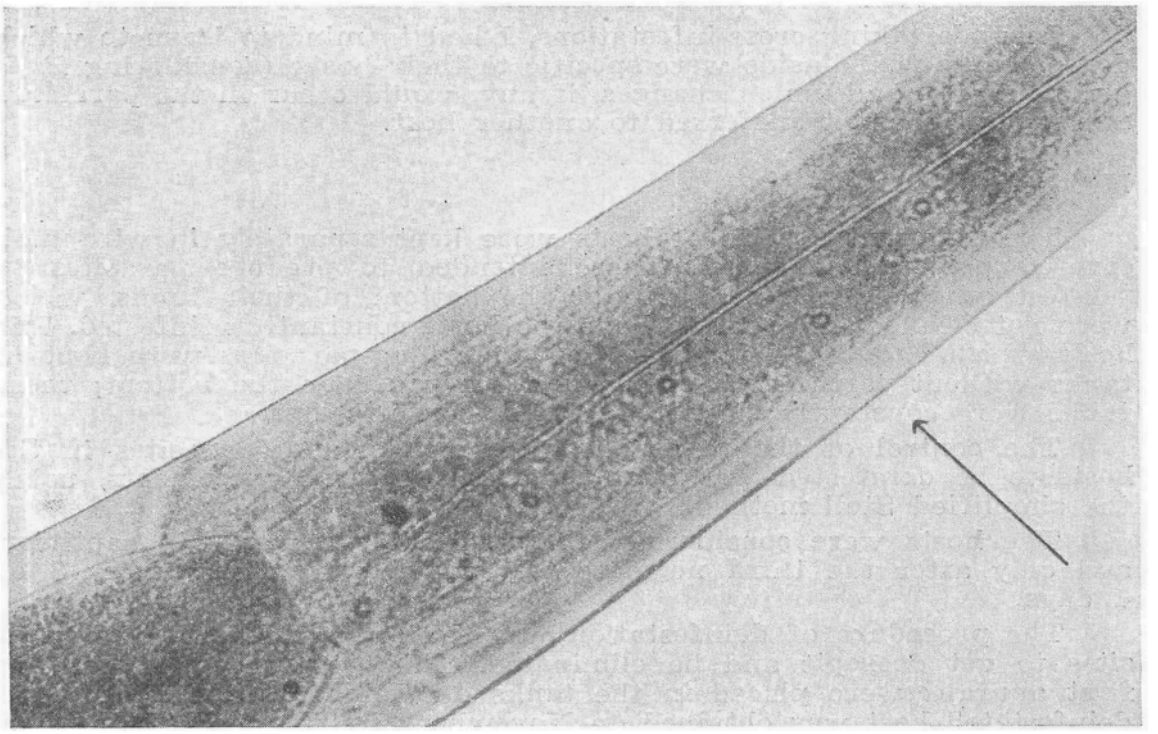


Photo 4. Anterior extremity of *R. fuelleborni* showing the stained periphery of the nuclei of the excretory system cells.

stained. Parasites from *Bufo m. marinus, Bufo m. paracnemis, B. m. marinus* x *B. m. paracnemis, Bufo m. ictericus* and *B. arenarum* had them only peripherically stained.

Considering that the first reference to *Rhabdias* of *B. marinus* from São Paulo was made under the name *R. fuelleborni* (Travassos, 1926), I considered those rhabdiasids with peripherically stained nuclei as representing this species. The first reference to lung parasites of *B. crucifer* was that of Kloss (1971), under the name *R. hermaphrodita;* samples with completely stained nuclei will therefore be referred to this species.

Plotting the measurements of the individuals from the mixed infestation on graphs, it became clear that the smaller individuals were *R. fuelleborni,* and the larger *R. hermaphrodita.* After this, new cultures were prepared to obtain the free-living generation; at this time, of the two species separately. Females of *R. fuelleborni* (the smaller hermaphroditic form) bore 2 infective larvae, and those of *R. hermaphrodita* (the larger hermaphroditic form), bore 4 infective larvae each.

CROSS-INFESTATIONS

In undertaking cross-infestations, I had in mind to learn to which degree these rhabdiasids were specific to their hosts (considering *Bufo* gr. *marinus*), and which changes, if any, would occur in the parasite's morphology when transferred to another host.

Methods:

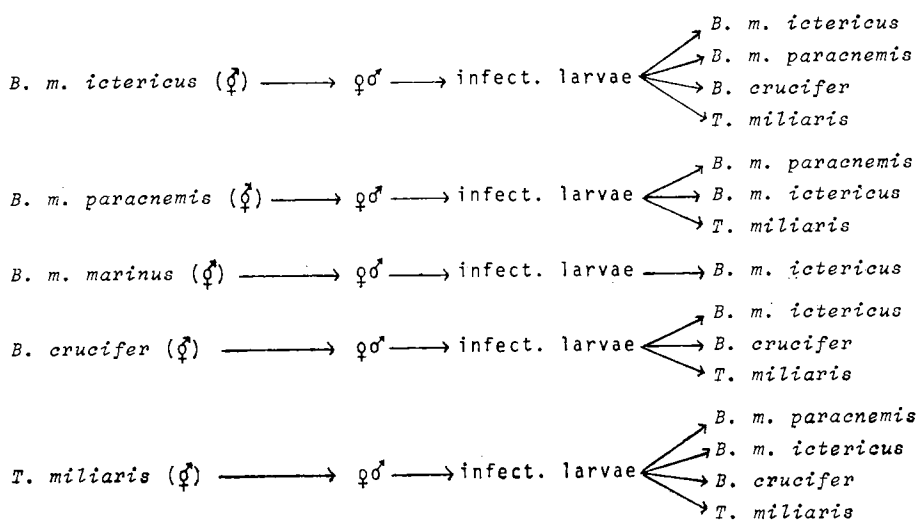
The different species of hosts were kept separately in wire-mesh covered polyethilene tanks. Those intended to produce parasites to reinfest other hosts, had earth at the bottom of their tanks, which were not cleaned, in order to have the hosts constantly reinfested. The hosts which had to be deinfested of their lung-parasites were kept in tanks without earth, with only wet filter-paper on the bottom; these tanks were constantly cleaned to avoid reinfestations.

The control of the presence or absence of lung-parasites in the hosts to be deinfested was done by examining the excrements, using the simplified Stoll method.

The hosts were considered deinfested after 3 weeks in captivity, and only after the third negative test which were made every 3 or 4 days.

The procedure of deinfestation was that of waiting for the parasites to get decrepit and be eliminated. This took a long time. The first anurans were placed in the tanks on October 13, and the first deinfestated host was obtained on November 30. The cross-infestations were ended on March 16, with some *Bufo m. ictericus* still eliminating rhabdiasids from the first day they were placed in the tank.

The following were the schemes of cross-infestations:



After the hosts were deinfested of their lung parasites, I started to get the free-living generation and infective larvae. The procedures of reinfestation were two:

(i) spreading a film of the culture liquid with infective larvae on the belly of the host; or

(ii) putting the watch-glass with culture into a glass just the size of the host which was put into it afterwards and kept there for half an hour or more.

The first method was better. In spite of only a few parasites reaching the lungs, this method was better than the second one, because the hosts usually destroyed the culture with urine.

After being reinfested, the hosts were washed and placed again in the tanks where they remained for 2-3 weeks before being autopsied.

The remaining specimens of the original sample from which the individuals for the free-living generation were removed, were fixed and mounted on slides for morphological and biometric analyses.

The samples obtained from the reinfestation were also fixed on slides to be compared with the original sample.

Results:

(i) *B. crucifer*, *T. miliaris* and *Bufo m. ictericus* from Casa Grande (SP), and *Bufo m. paracnemis* from Emas (SP) were reinfested with *R. fuelleborni* of *Bufo m. ictericus* from Casa Grande. There are two original samples (graphs 6 and 7) because *Bufo m. paracnemis* was reinfested after the first lot of hosts.

a) *B. crucifer*: no infective larvae reached the lungs.

b) *T. miliaris*: no infective larvae reached the lungs.

c) *Bufo m. ictericus* : a difference at the 5% level occurred in the constants of regression of esophagus length on body length. The coefficients of regression did not change significantly.

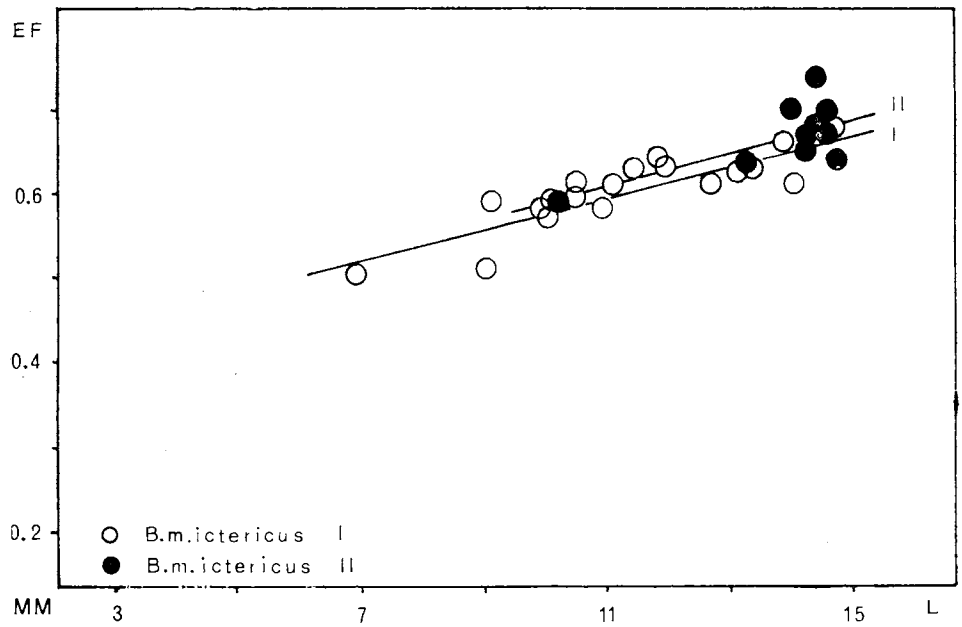
d) *Bufo m. paracnemis*: since the original sample did not show a significant correlation coefficient of esophagus length on body length, I had to compare the means. The differences of means of esophagus length between original and resultant samples was highly significant. The pseudobulb, less visible in the original sample, was well defined in the resultant sample, whose individuals had also a more or less globular anterior extremity, not observed in the original sample.

(ii) *Bufo m. ictericus* and *T. miliaris* from Casa Grande (SP), and *Bufo m. paracnemis* from Emas (SP) were reinfested with *R. fuelleborni* of *Bufo m. paracnemis* from Emas (SP) (graphs 8 and 9).

Results:

a) *T. miliaris*: the infective larvae did not reach the lungs.

b) *Bufo m. ictericus*: the bulb-like anterior extremity became reduced. The differences in the means of esophagus length between the original and the resultant samples were highly significant.



GRAPH 6. Regression lines of the esophagean length on body length of *R. fuelleborni* of *Bufo m. ictericus* (I) introduced into *Bufo m. ictericus* (II) from the same locality. (I) n = 20 r = 0.8507 b = 0.0183 ± 0.0026 a = 0.3943 0.0306. (II) n = 10 r = 0.7134 b = 0.0214 ± 0.0074 a = 0.3728 ± 0.1031

c) *Bufo m. paracnemis*: the reinfestation of this host with *R. fuelleborni* from another host of the same species, altered significantly the means of the esophagean length only. In all other characters the parasites did not change.

(iii) *Bufo m. ictericus* from Casa Grande (SP) were reinfested with *R. fuelleborni* of *Bufo m. marinus* from Belém (PA). *Bufo m. ictericus* was the last deinfested host species which remained in the tanks, when *Bufo m. marinus* was received from the north (graph 10).

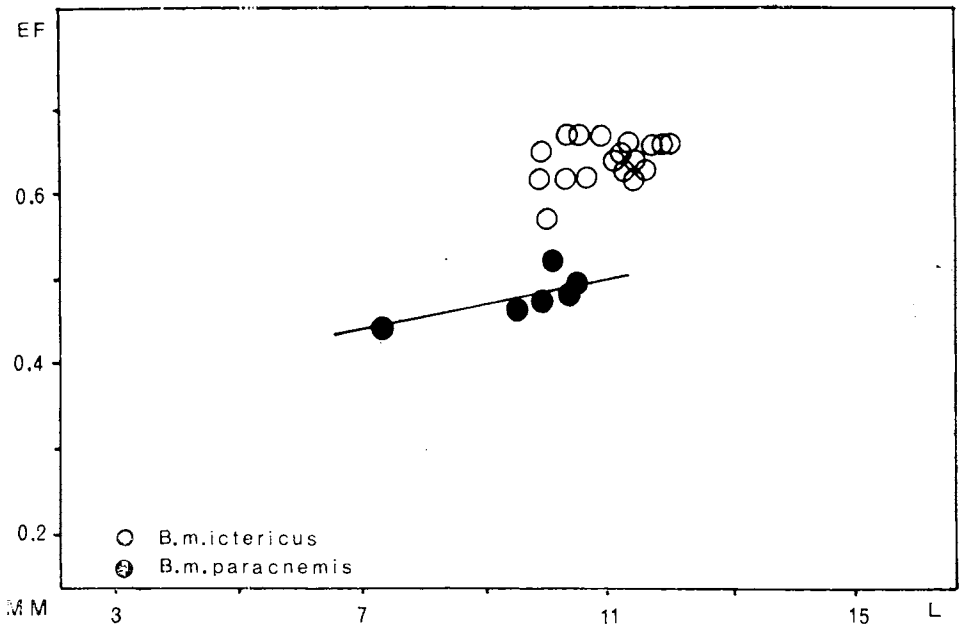
The resultant sample did not grow up completely, in spite of producing eggs. The mean esophagus length was significantly lower than that of the original sample. In *Bufo m. marinus* the parasites had a globular cephalic extremity, which continued to appear in the resultant generation reared in *Bufo m. ictericus*.

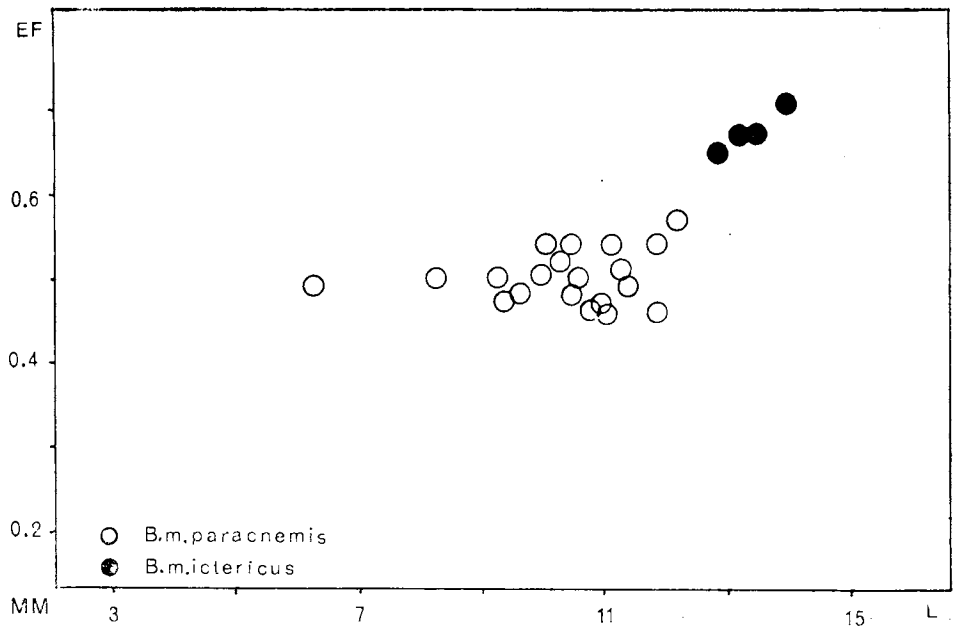
(iv) *Bufo m. ictericus*, *T. miliaris* and *B. crucifer* from Casa Grande (SP) were reinfested with *R. hermaphrodita* of *B. crucifer* from the same locality (graphs 11 and 12).

Results:

a) *Bufo m. ictericus*: the lungs of the host were not reinfested.

b) *T. miliaris*: the difference between the coefficients of regression of esophagus length on body length of the rhabdiasids from the original





GRAPH 7. Esophagean length on body length of *R. fuelleborni* of *Bufo m. ictericus* from Casa Grande (○) introduced into *Bufo m. paracnemis* from Emas (●). (○) n = 20 r = 0.3888. (●) n = 6 r = 0.7350 b = 0.0185 ± 0.0085 a = 0.2996 ± 0.0827.
GRAPH 8. Esophagean length on body length of *R. fuelleborni* of *Bufo m. paracnemis* from Emas (○) introduced into *Bufo m. ictericus* from Casa Grande (●). (○) n = 21 r = 0.2260. (●) n = 5 r = 0.9710 b = 0.0492 ± 0.0069 a = 0.0209 ± 0.0934.

sample and those from the resultant sample was not significant. The difference between the two constants of regression was significant.

c) *B. crucifer*: the means of esophagus length of the two samples did not differ significantly.

The comparison of the means of esophagus length of the three samples, original sample and the two resultant samples from *T. miliaris* and *B. crucifer*, did not show significant differences among them.

(v) *Bufo m. ictericus*, *B. crucifer* and *T. miliaris* from Casa Grande (SP), and *Bufo m. paracnemis* from Emas (SP), were reinfested with *R. hermaphrodita* of *T. miliaris* from Casa Grande (graphs 13 and 14).

Results:

a) *Bufo m. ictericus*: the infective larvae did not reach the lungs.

b) *Bufo m. paracnemis*: the infective larvae did not reach the lungs.

c) *B. crucifer*: the only difference observed in the resultant sample was the highly significant difference between the constant of regression of esophagus length on body length.

d) *T. miliaris*: the observation was identical to that with *B. crucifer*.

Conclusions from the cross-infestation tests:

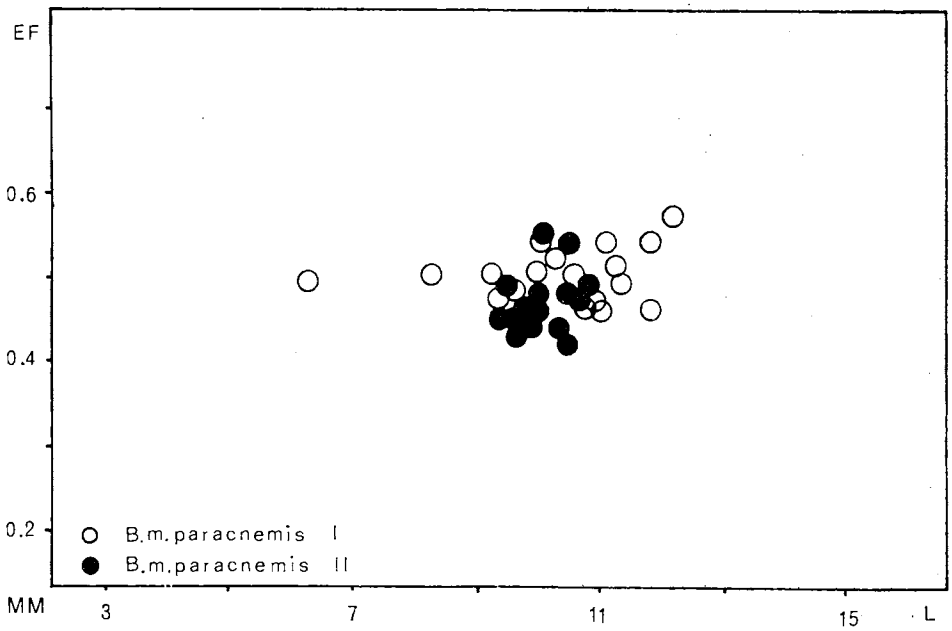
1) *R. fuelleborni* is apparently unable to adapt itself to *B. crucifer*. The 7 reinfestation trials of this host with the lung-parasites of *Bufo m. ictericus*, did not give any positive results. Even in nature, where the two hosts are sympatric, sometimes even syntopic, *R. fuelleborni* was never observed in association with *B. crucifer*.

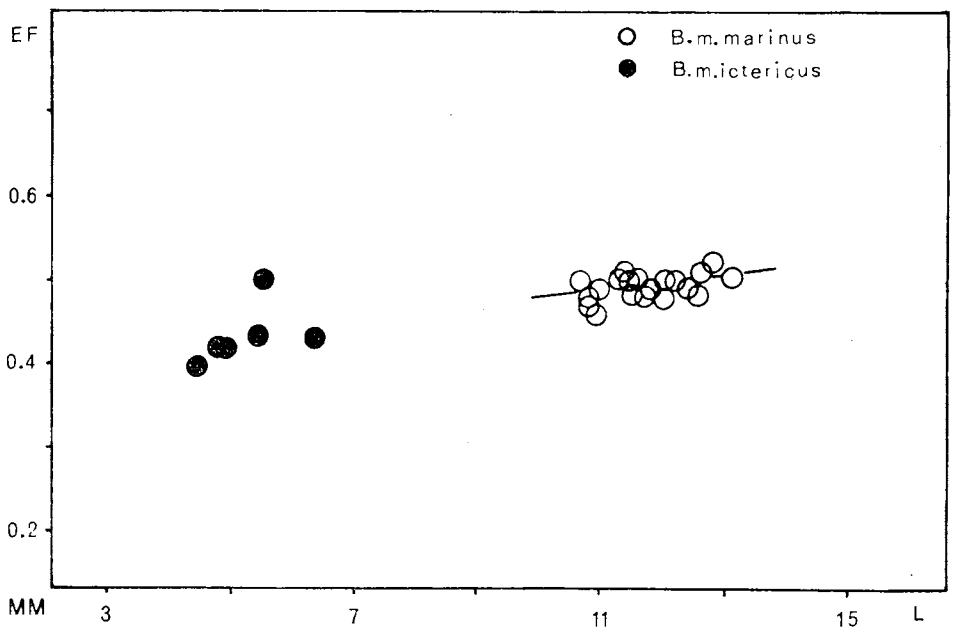
2) The possibilities of infestation of *T. miliaris* by *R. fuelleborni* probably depend on certain conditions which ceased to exist when the host was kept in captivity. No essay to infest *T. miliaris* with the parasites of *Bufo m. ictericus* and *Bufo m. paracnemis* had any result, in spite of the parasite being recovered from the lungs of this leptodactylid, together with *R. hermaphrodita*.

3) *R. hermaphrodita* probably has no conditions to reach the lungs of *Bufo m. ictericus* and *Bufo m. paracnemis*. In nature, this parasite was never found in *B. marinus*, and the results of laboratory tests to infest them were negative.

4) The results of reinfestation of *Bufo m. ictericus* with *R. fuelleborni* of *Bufo m. paracnemis* showed that the globular dilation of the cephalic extremity, and the pseudobulb at the anterior extremity of the esophagus are very unstable characters in the parasites of *Bufo m. paracnemis*. This has also been observed in *R. fuelleborni* from Paraguayan hosts (figs. 6-17).

The reinfestation of *Bufo m. ictericus* with the lung parasites of *Bufo m. marinus* revealed that those two morphological characters are





GRAPH 9. Esophagean length on body length of *R. fuelleborni* of *Bufo m. paracnemis* (I) introduced into *Bufo m. paracnemis* (II) from the same locality. (I) n = 20 r = 0.3888. (II) n = 17 r = 0.1804. GRAPH 10. Esophagean length on body length of *R. fuelleborni* of *Bufo m. marinus* from Belém (○) introduced into *Bufo m. ictericus* from Casa Grande (●). (○) n = 20 r = 0.4368 b = 0.0094 ± 0.0048 a = 0.3837 ± 0.0535. (●) n = 6 r = 0.5041.

more constant in *R. fuelleborni* of *Bufo m. marinus* than of *Bufo m. paracnemis*. When transferred to *Bufo m. ictericus*, the globular cephalic extremity and the pseudobulb remained as in the original sample.

5) The constant of regression of the esophagean length on body length of *Rhabdias* may change significantly among hosts of the same species and locality, according to the reinfestations of *Bufo m. ictericus* from Casa Grande with the parasites of another *Bufo m. ictericus* from the same locality, and of *T. miliaris* with the parasites of another *T. miliaris* from Casa Grande also.

6) The esophagus length of *R. fuelleborni* may vary significantly, independent of the host species. It was observed in the reinfestations of *Bufo m. ictericus* and *Bufo m. paracnemis* with the lung parasites of *Bufo m. paracnemis*, and the reinfestations of *Bufo m. ictericus* with the parasites of *Bufo m. marinus*.

The mean of the esophagus length may vary significantly in the same species of host, but still more so in different hosts.

7) *R. hermaphrodita* does not show qualitative morphological variations as observed in *R. fuelleborni*. Its variations are only quantitive. As observed in *R. fuelleborni*, the constant of regression of the esophagean length on body length may vary significantly, independent of the host, but this variation is less marked than in *R. fuelleborni*.

OBSERVATIONS ON MIXED INFESTATIONS

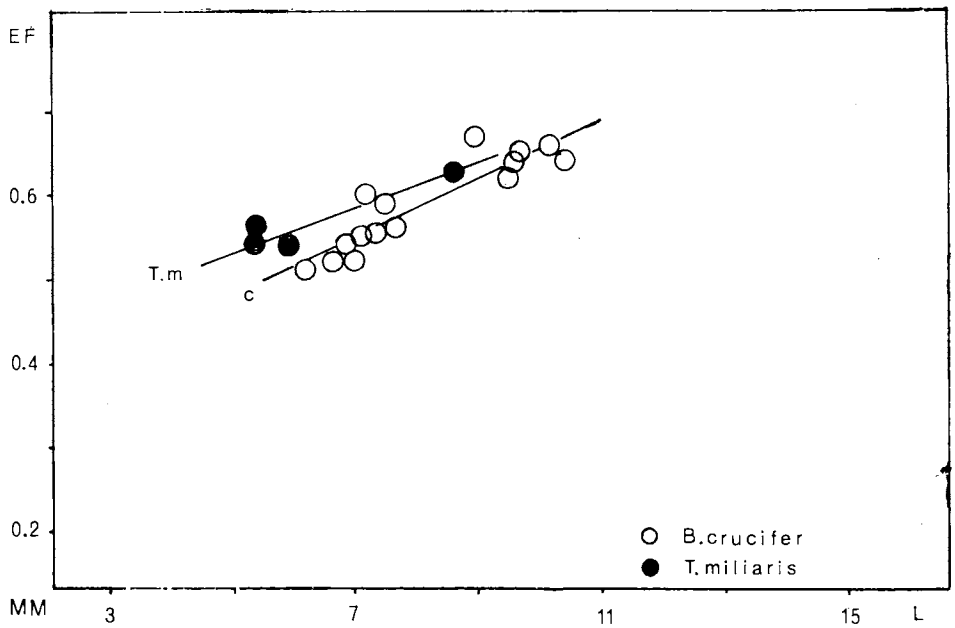
R. fuelleborni from mixed infestations of *T. miliaris* from Casa Grande (SP) showed a kind of inhibition (graphs 15 and 16).

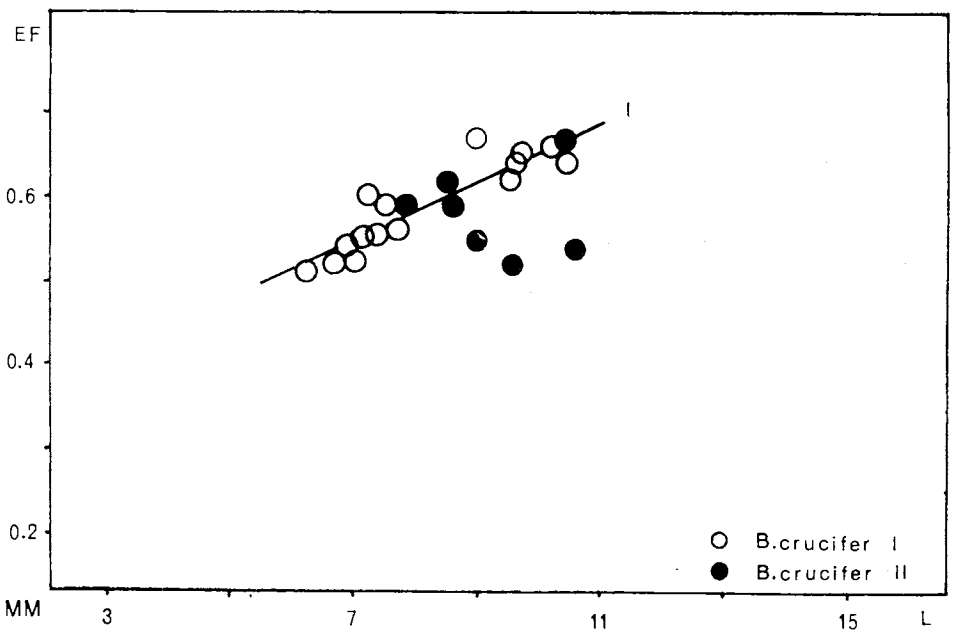
In this host, together with *R. hermaphrodita*, *R. fuelleborni* does not grow as much as when associated with *Bufo m. ictericus* from the same region. The tail, normally conic elongated, is simply conical, and the pseudobulb at the anterior extremity of the esophagus is more developed. While *R. hermaphrodita* starts to ovulate when attaining 6 mm of length, *R. fuelleborni* does it at 3 mm of body length.

If we compare the regression lines of esophagean length on body length of *R. hermaphrodita* from a mixed infestation with that from a single infestation in *T. miliaris*, we observe no significant differences, showing that the rhythm of growth in this parasite species is not altered when together with *R. fuelleborni*.

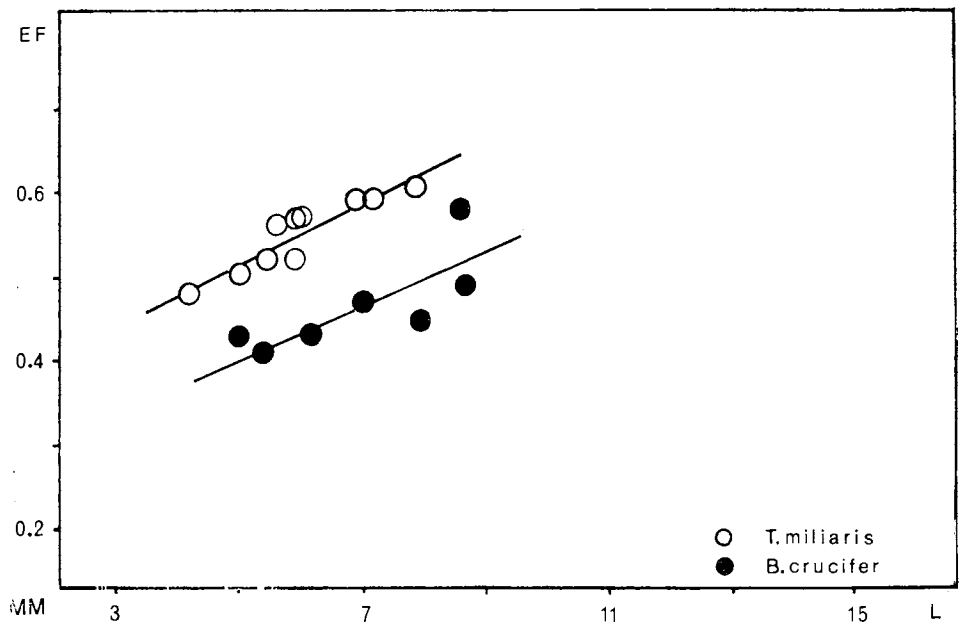
With *R. fuelleborni* it is different: its rhythm of growth is so altered, that the differences between the regression coefficients of the measures of the esophagus on body length of a mixed infestation and a single one, is highly significant.

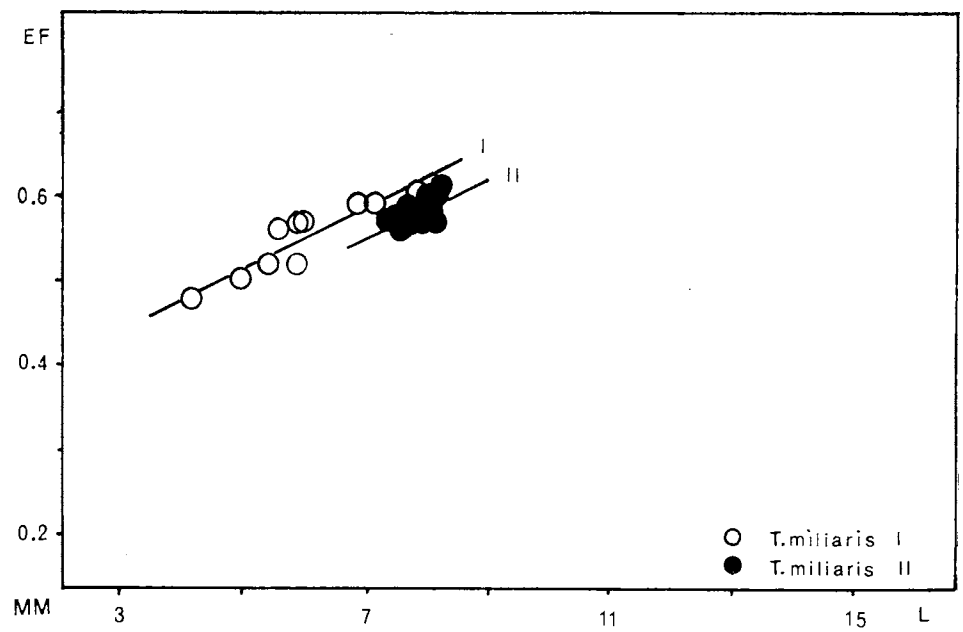
Bufo m. ictericus from the same locality (Casa Grande), were reinfested with infective larvae obtained from the very different *R. fuelleborni* of the mixed infestation of *T. miliaris*. In the *Bufo* host the parasites showed their normal feature, growing to 16 mm length, and the tail was conic elongated again. (Graphs 17 and 18, figs. 53-55).





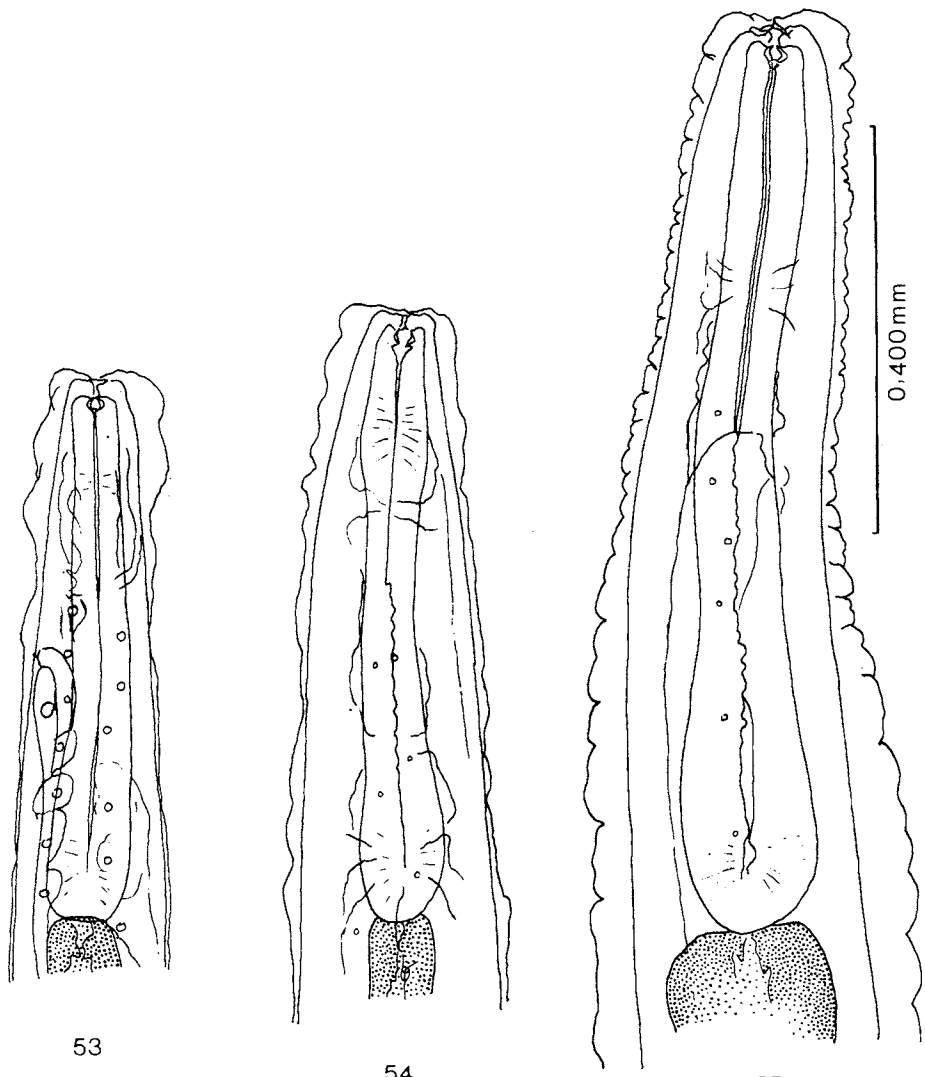
GRAPH 11. Regression lines of the esophagean length on body length of *R. hermaphrodita* of *B. crucifer* introduced into *T. miliaris* from the same locality. (○) n = 15 r = 0.9052 b = 0.0362 ± 0.0047 a = 0.2939 ± 0.0388. (●) n = 4 r = 0.9459 b = 0.0284 ± 0.0068 a = 0.3849 ± 0.0444. GRAPH 12. Esophagean length on body length of *R. hermaphrodita* of *B. crucifer* (I) introduced into *B. crucifer* (II) from the same locality. (○) n = 5 r = 0.9052 b = 0.0362 ± 0.0047 a = 0.2939 ± 0.0388. (●) n = 7 r = 0.0153.





GRAPH 13. Regression lines of the esophagean length on body length of *R. hermaphrodita* of *T. miliaris* introduced into *B. crucifer* from the same locality. (○) n = 10 r = 0.9366 b = 0.0367 ± 0.0048 a = 0.3311 ± 0.0294. (●) n = 7 r = 0.7938 b = 0.0304 ± 0.0104 a = 0.2522 ± 0.0741. GRAPH 14. Regression lines of the esophagean length on body length of *R. hermaphrodita* of *T. miliaris* (I) introduced into *T. miliaris* (II) from the same locality. (○) n = 10 r = 0.9366 b = 0.0367 ± 0.0048 a = 0.3311 ± 0.0294. (●) n = 12 r = 0.6078 b = 0.0370 ± 0.0153 a = 0.2879 ± 0.1201.

It appears that when together with *R. hermaphrodita, R. fuelleborni* does not reach full growth, behaving as very young individuals which start to produce eggs when their esophagus still represents 10% of the body length. The next generation of parasites, in *Bufo m. ictericus,* had the esophagus representing 5% of the body length again, and the pseudobulb practically disappeared.



Anterior extremity of *Rhabdias* of a mixed infestation of *T. miliaris.* Fig. 53 *R. hermaphrodita.* Fig. 54 *R. fuelleborni.* Fig. 55 Descendant of *R. fuelleborni* of a mixed infestation introduced in *Bufo m. ictericus.*

DISCUSSION AND CONCLUSIONS

A) The 2 species of *Rhabdias* of the *marinus* group of *Bufo* can only be characterized by their chromatic affinities. When fixed with acetoaldehyde and stained with a mixture of equal parts of Semichon's acetocarmine and chloridric carmine, the nuclei of the cells of the excretory system have their periphery stained and show an alternate arrangement in R. *fuelleborni;* those of *R. hermaphrodita* have the same nuclei completely stained, showing a regular disposition.

Morphological characters, qualitative and quantitative are extremely variable.

B) An evaluation of the relationships of *R. fuelleborni* and *R. hermaphrodita* with other *Rhabdias* depend on a revision of the group, using more refined methods, as kariology, sorology, electrophoresis, etc.

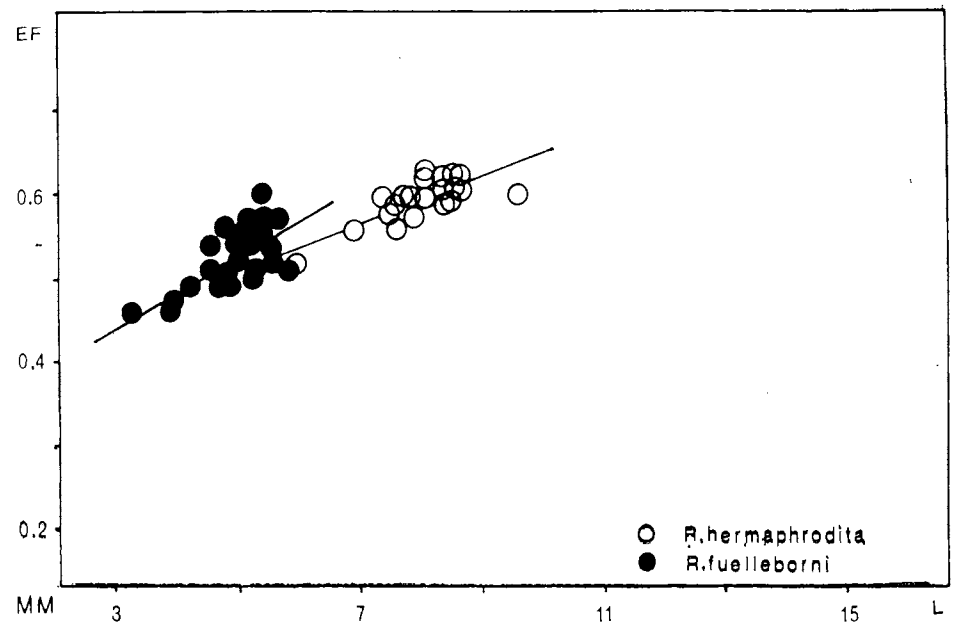
C) The number of infective larvae produced by one free-living female is problably under genetic control, but may vary, depending on the ecological conditions of the environment.

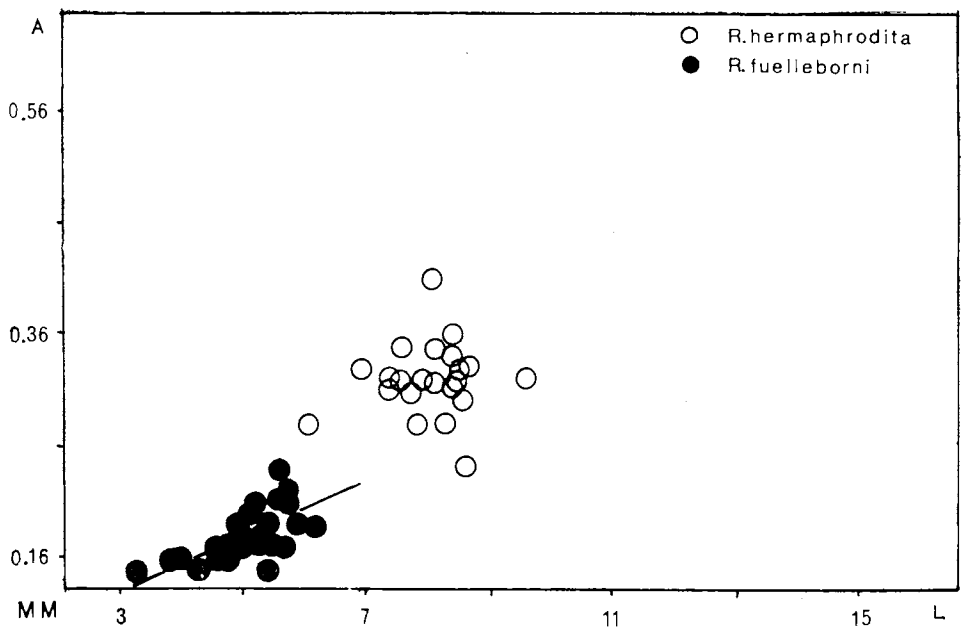
D) The only host species found with a mixed infestation of *Rhabdias* was *T. miliaris* from Casa Grande (SP). In these samples, *R. fuelleborni* always showed some inhibition, while *R. hermaphrodita* was unaltered. This inhibition, allied to the fact that never has been observed a single infestation of this host with *R. fuelleborni*, but with *R. hermaphrodita*, one may accept the second species of *Rhabdias* as being the normal lung parasite of *T. miliaris.*

E) The high 0_2 tension in the lungs, and the chemical composition of the hosts blood may be the main conditions which permit a parasitic life of *Rhabdias.* Remembering again R. J. Williams (1956), who studied the relations between the anatomy and the biochemistry of several vertebrates, we observe that the two hosts with a greater morphological variation (*B. marinus* and *B. arenarum*) are infested by a parasite (*R. fuelleborni*) which also shows a great morphological variation, besides a great adaptative capacity. On the other hand, *B. crucifer* which is restricted to the subtropical and Atlantic rain forest, has restricted morphological variation, and so have its lung parasites (*R. hermaphrodita*).

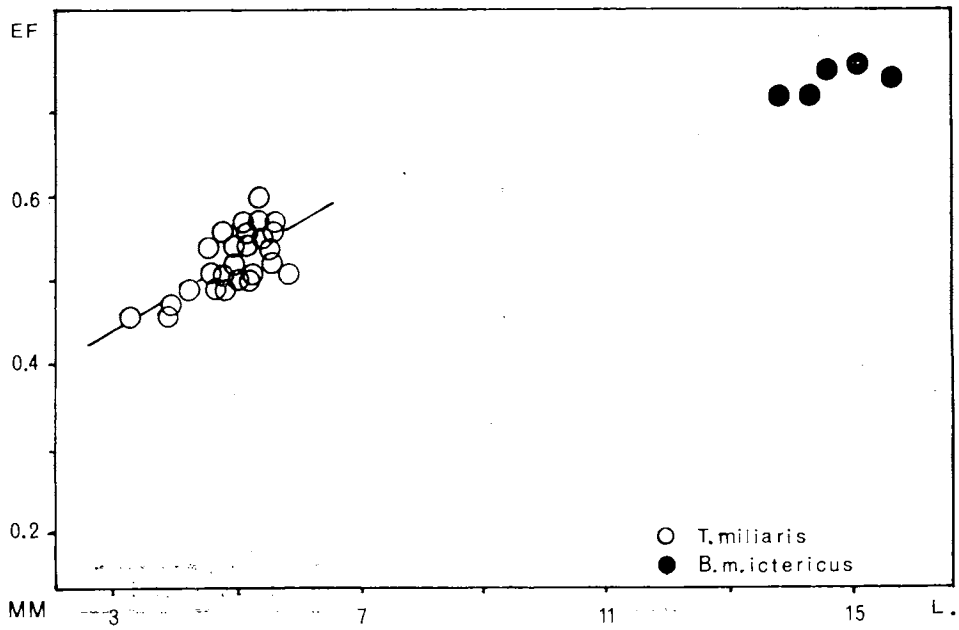
Based on these observations, one may conclude that there are eurixene *Rhabdias* species, with great capacity of adaptation and a wide distribution; and others which are stenoxene, with a reduced capacity to adapt themselves to other hosts, having consequently a restricted distribution.

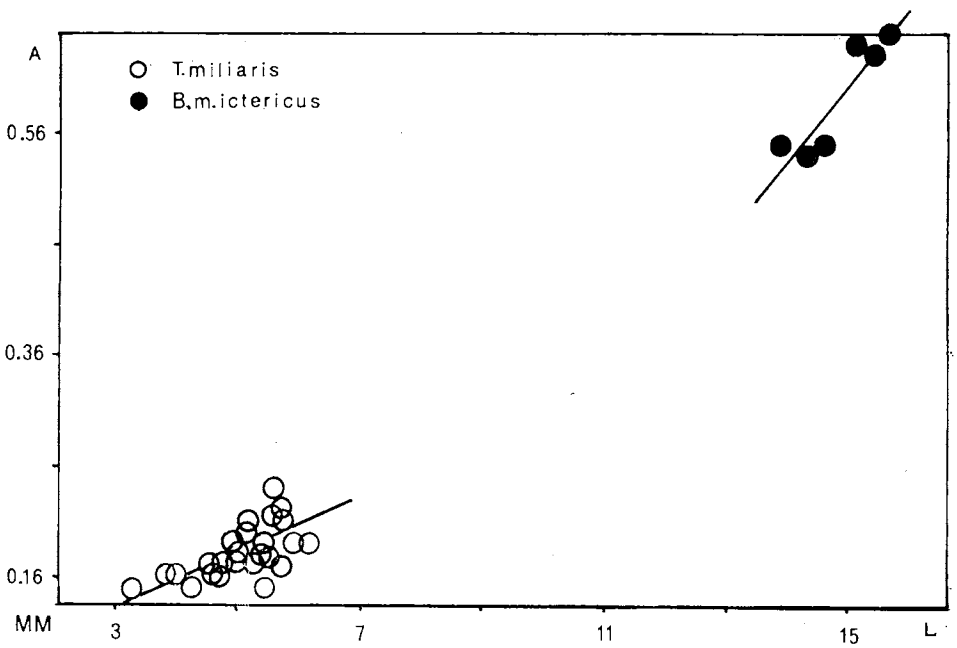
F) At Casa Grande (SP) the rate of infestation of *T. miliaris* and *B. crucifer* do not differ significantly, what may indicate that the two





GRAPH 15. Regression lines of the esophagean length on body length of *Rhabdias* of a mixed infestation of *T. miliaris*. *R. hermaphrodita* (○) n = 23 r = 0.7440 b = 0.0290 ± 0.0056 a = 0.3651 ± 0.0460. *R. fuelleborni* (●) n = 29 r = 0.7460 b = 0.0418 ± 0.0071 a = 0.3176 ± 0.0367. GRAPH 16. Tail length on body length of *Rhabdias* of a mixed infestation of *T. miliaris*. *R. hermaphrodita* (○) n = 23 r = 0.0978. *R. fuelleborni* (●) n = 29 r = 0.6410 b = 0.0237 ± 0.0054 a = 0.0622 ± 0.0280.





GRAPH 17. Esophagean length on body length of *R. fuelleborni* of a mixed infestation of *T. miliaris* introduced into *Bufo m. ictericus* from the same locality. (○) n = 29 r = 0.7460 b = 0.0418 ± 0.0071 a = 0.3176 ± 0.0367. (●) n = 6 r = 0.5842 GRAPH 18. Regression lines of the tail length on body length of *R. fuelleborni* of a mixed infestation of *T. miliaris* (○) introduced into *Bufo m. ictericus* (●) from the same locality. (○) n = 29 r = 0.6410 b = 0.0237 ± 0.0054 a = 0.0622 ± 0.0280. (●) n = 6 r = 0.8813 b = 0.0690 ± 0.0185 a = 0.4307 ± 0.2744.

hosts may be offering the same, or nearly the same conditions to *R. hermaphrodita.* The rate of infestation of *Bufo m. ictericus* from the same locality is significantly higher than either (graph 23). At this region *Bufo m. ictericus* walks around and reproduces itself at a time when temperature and humidity favoures the free-living generation of the lung parasites. At the same time *B. crucifer* remains retired, only appearing when *Bufo m. ictericus* disappears, i.e., when temperature and humidity are no longer favourable to the reproduction of the free-living generation of rhabdiasids (personal observations).

At Novo Horizonte (SC), a significant difference is observed in the rate of infestation of *B. crucifer* and *T. miliaris,* and not of *B. crucifer* and *Bufo m. ictericus,* which are syntopic and more numerous, reproducing at the same time during the year. In this case, the difference in percentages of infested hosts may be explained by the different location in the habitat: *Bufo m. ictericus* and *B. crucifer* were collected at nearly 450 m altitude, and *T. miliaris* at a cold cave at nearly 650 m altitude, at the beginning of the mountain slopes. The rates of infestation of *Thoropa* from Casa Grande and Novo Horizonte do not differ significantly.

It appears that the distribution of *R. hermaphrodita* is less extensive than that of one of its hosts (*B. crucifer*), which is found at lower latitudes. The number of infested *B. crucifer* starts to decrease from the State of Rio de Janeiro to the north: at Angra dos Reis (RJ) 7.14% of hosts were infested with *R. hermaphrodita,* and in the city of Rio de Janeiro (GB) 5.88%. At Ribeirão dos Enganos (ES) and in Salvador (BA), no *R. hermaphrodita* were found (graph 23). It will be necessary to confirm these data in relation to *T. miliaris,* the other host of *R. hermaphrodita,* whose distribution extends to the southern part of Minas Gerais and Espírito Santo.

Of course not only the parasitic form, but also the free-living generation of certain species of *Rhabdias,* has its own ecological requirements, so that the geographical distribution of the parasite may well be narrower than that of the host.

An apparently eurixene species of *Rhabdias* may have occasional barriers represented by locals climate which do not permit the survival of the free-living generation, or of the infective larvae, resulting into blank spaces in their distribution.

G) In Brazil and neighbouring countries, the only lung parasites of the *marinus* group of *Bufo,* including *B. crucifer,* are *R. fuelleborni* and *R. hermaphrodita. R. fuelleborni* is found infesting *B. marinus* and *B. arenarum,* and *R. hermaphrodita* infests *B. crucifer.* Neither of the two parasites infests the host of the other specie (Figs. 5 and 40).

Considering that *B. marinus* is anatomically close to *Bufo* of the Group I (Baldauf, 1959) which occur in North America, and to *B. melanostictus* and *B. parietalis* in Asia, it is to be expected that it may have some chemical affinities with some of the species also, as it may have to *B. arenarum.*

Considering also the great adaptive plasticity of *R. fuelleborni*, this parasite may probably have conditions to infest one or more species of *Bufo* which have some affinity to *B. marinus*. It may as well have already been referred to under some other name, or names, in North America and India. Only a revision of the genus *Rhabdias*, using better methods, may clear the real *status* of *R. fuelleborni* Travassos, 1926.

Colam (1971) discovered a difference between what he called *R. sphaerocephala* of *Bufo m. marinus* from Costa Rica and Jamaica, and *R. bufonis* of *Rana temporaria* from Europe. In *R. sphaerocephala sensu* Bravo H. & Caballero, the goldenbrown granules on the gastrodermic cells measure about 2 μm, and those in *R. bufonis*, about 0.5 μm. From this, it may be concluded that the lung parasite of *B. marinus* in Middle-America is not the same as that of the European ranid.

H) The host-specificity shown by *R. fuelleborni* infesting *B. marinus* and *B. arenarum*, and by *R. hermaphrodita* infesting *B. crucifer* only, suggest that the splitting of the South American Section of the *valliceps* group of *Bufo*, in groups *marinus* and *crucifer*, proposed by Martin (1972), may be right, not based on the anatomical characters analyzed by this author, but on some more important characters which still have to be found. The karyotypes of *B. marinus* and *B. arenarum* indicate that these two species of *Bufo* are more recent than *B. crucifer* (Bogart, 1972). The difference in time of existence may be also a factor of host-specificity, independent of the group of *Bufo* to which the species belongs. I am much more inclined to accept the second hypothesis, based on the existence of *R. fuelleborni* and *R. hermaphrodita* in *T. miliaris*.

I) Supposing that the nematodes whose reproductive organs normally accompany the growth of the body, have already acquired a biological balance in relation to the host, against those whose reproductive organs mature irregularly (graphs 19 to 22), it can be suggested that *R. fuelleborni* is better adapted to *B. marinus* and *B. arenarum* than to *T. miliaris*, and that R. *hermaphrodita* adapts itself better to *T. miliaris* than to *B. crucifer*.

The great adaptation capacity of *B. marinus*, and the variations observed in its skeleton measurements, suggest it to be a more recent species than *B. crucifer*, a species with more limited habitat and habits, with more stable morphology. *B. arenarum* which only differs visibly from *B. marinus* in the disposition of the granular glands, also shows an ample variation in skeleton measurements, which are identical to those of *B. marinus*, what may suggest that these two species evolved at about the same time.

If *B. crucifer* and *B. marinus* + *B. arenarum* had had the same ancestral, *B. crucifer* must have differentiated very much from it, representing a new habitat for rhabdiasids, occupied by *R. hermaphrodita*, a local species which already may have been infesting *T. miliaris*. *R. fuelleborni* still continued to infest the ancestral which may have

continued to exist at this time. When *B. marinus* and *B. arenarum* appeared, *R. fuelleborni* transferred to the new hosts which probably have more chemical affinity with their ancestral than *B. crucifer*.

Bogart (1972) is of the oppinion that *B. marinus* probably came from an ancestral showing a karyotype with only a secondary subtelocentric constriction on the short arm of chromosome 7, and that the other constriction, a submetacentric one on the short arm of chromosome 5, might have evolved recently.

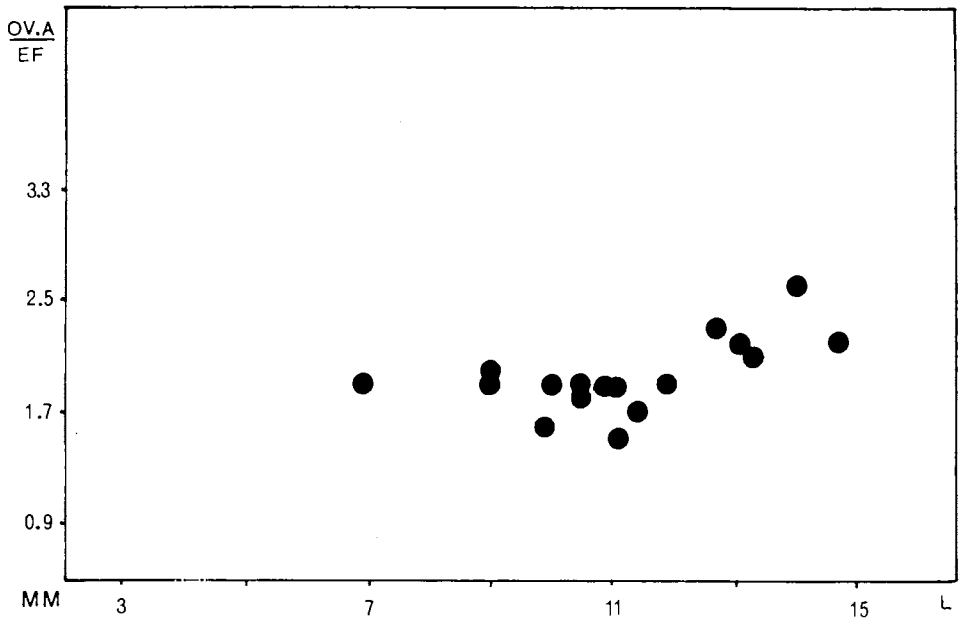
B. crucifer could have had an ancestral with also a secondary subtelocentric constriction on the short arm of chromosome 7, and a metacentric secondary constriction on the long arm of chromosome 1. The submetacentric secondary constriction also on the long arm of chromosome 1 represents an early dichotomy which was probably also responsible for the evolution of the line leading to *B. arenarum* which presents this same type of constriction on chromosome 1 besides the subtelocentric constriction on the short arm of chromosome 7. Besides these, *B. crucifer* presents other metacentric and subtelocentric constrictions which do not appear in *B. arenarum* and *B. marinus* either. I believe Bogart exagerates the importance of the distance between *B. marinus* and *B. crucifer* and *B. arenarum*. I prefer to believe that the karyotypes of the ancestor of *B. marinus* were much more complex than suggested by Bogart, and that *marinus* and *arenarum* followed the same evolutionary line which is, in a way, related to that of *B. crucifer*.

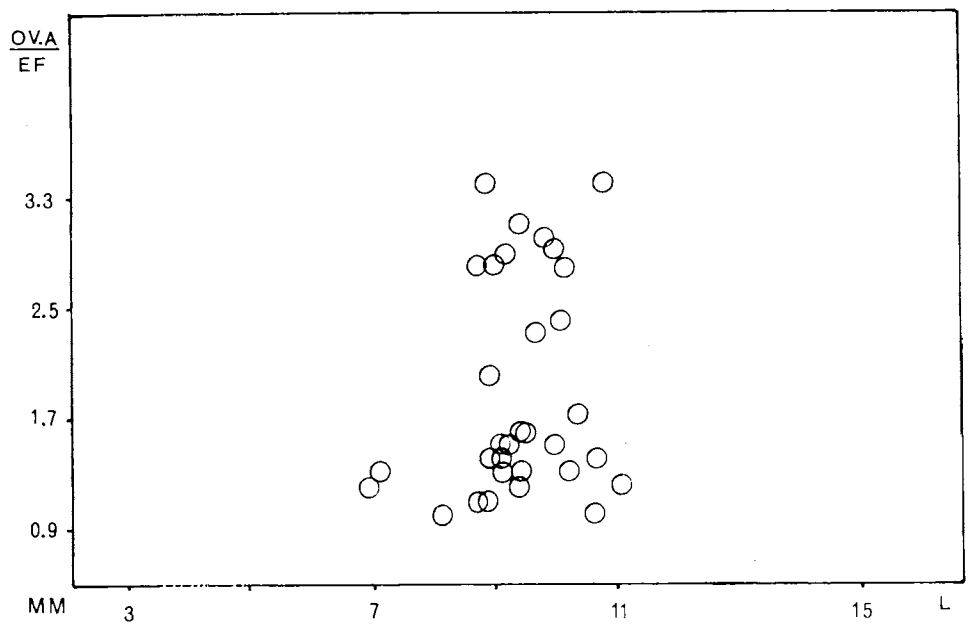
J) The eurixene species of *Rhabdias* ovulate late when infesting ideal hosts, and reduce the number of infective larvae when living in better climatic conditions.

In one of the extremities of the geographical distribution of *B. marinus*, we have the best host (*Bufo m. ictericus*), and on the other extremity, the most favourable climate (Amazon region). *Bufo m. paracnemis* and *B. arenarum* appear to represent less appropriate habitats, living in regions less appropriate for the survival of the free-living generation.

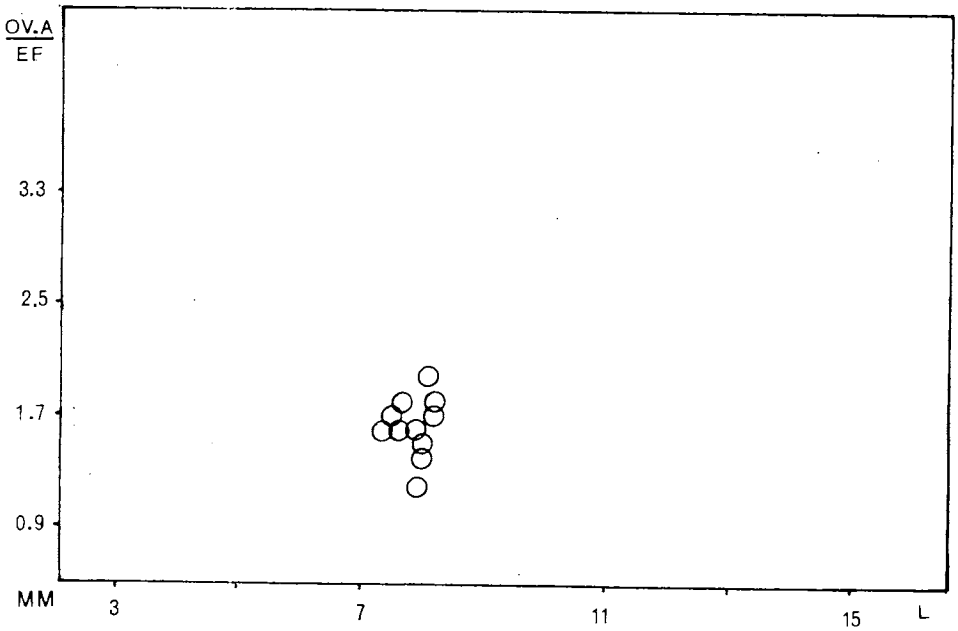
Remembering that the behaviour of the parasites of *Bufo m. marinus* and *Bufo m. paracnemis* is the same, i.e., they ovulate early, one may conclude that the physiological conditions of the two hosts are very much the same, differing from those of *Bufo m. ictericus* which lives in subtropical and Atlantic rain forest. The physiological conditions of *Bufo m. ictericus* probably are suffering under adaptations, as may be observed with the *Rhabdias* sample from Parati (RJ) whose behaviour is the same as that from *Bufo m. paracnemis* and *Bufo m. marinus*. This adaptation of its physiological conditions may indicate a relatively recent invasion of the subtropical and Atlantic forests by *B. marinus*.

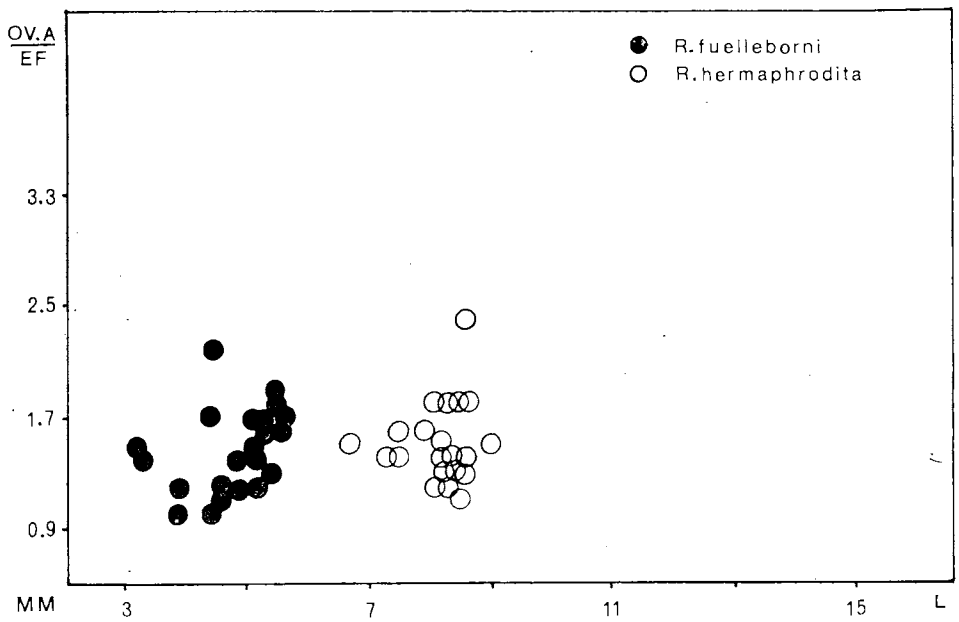
The cephalic dilation and the pseudobulb at the anterior extremity of the esophagus are not constant in the *Rhabdias* samples from *Bufo m. paracnemis*, especially in those from Paraguay. *Rhabdias* lose these characters, when transferred from *Bufo m. paracnemis* to *Bufo m.*



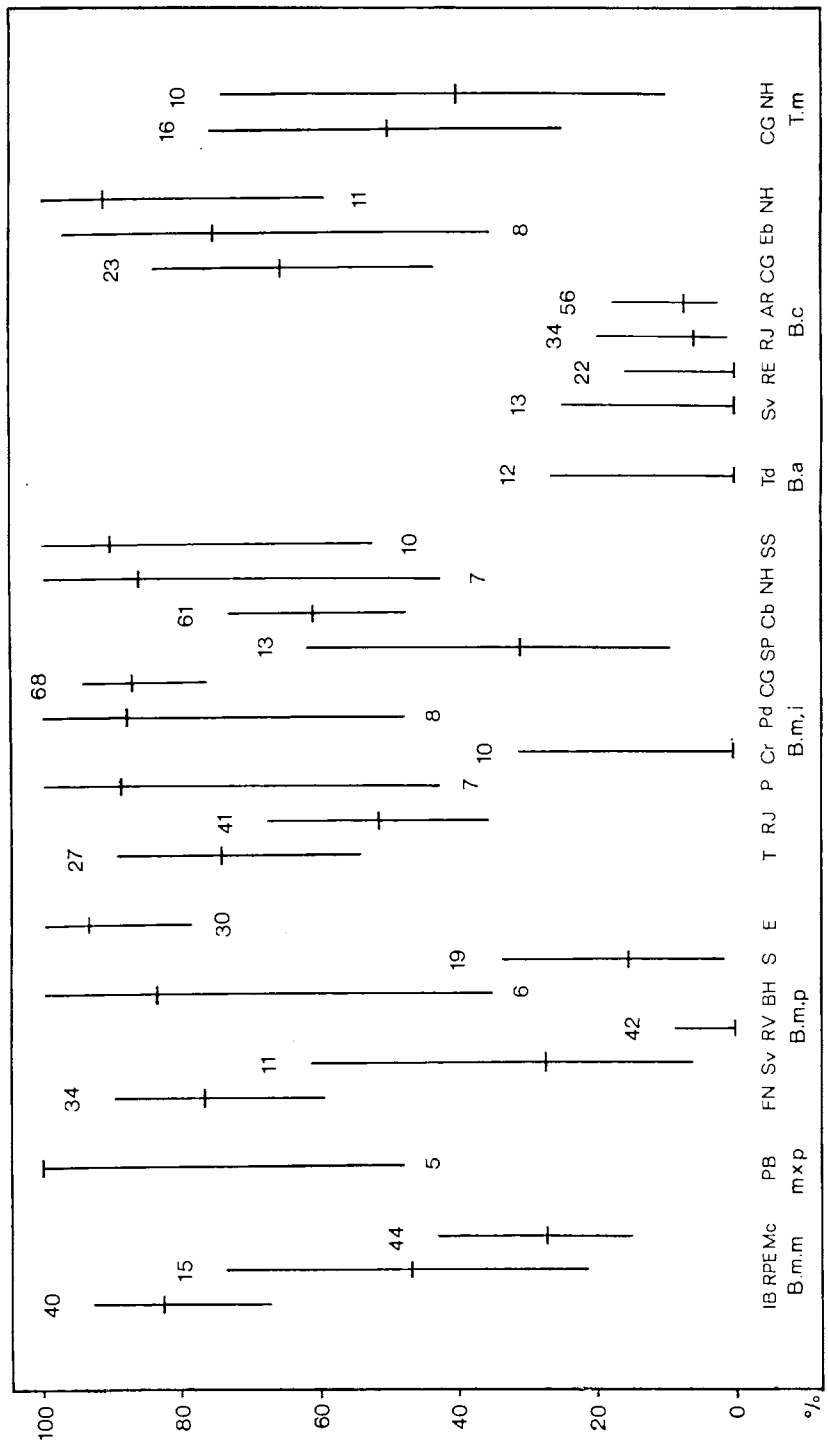


GRAPH 19. Distance between the extremity of the anterior ovary to base of esophagus (growth region) on body length of *R. fuelleborni* of *Bufo m. ictericus*.
GRAPH 20. Distance between the extremity of the anterior ovary to base of esophagus (growth region) on body length of *R. hermaphrodita* of *B. crucifer*.





GRAPH 21. Distance between the extremity of the anterior ovary to base of esophagus (growth region) on body length of *R. hermaphrodita* of *T. miliaris*.
GRAPH 22. Distance between the extremity of the anterior ovary to base of esophagus (growth region) on body length of *B. fuelleborni* (○) and *R. hermaphrodita* (●) of a mixed infestation of *T. miliaris*.



GRAPH 23. Percentages of hosts infected with *Rhabdias*.

ictericus. *Rhabdias* of *Bufo m. marinus*, with the same characters, maintain them when transferred to *Bufo m. ictericus*. If we also consider that the paracnemic glands are constant in *Bufo m. paracnemis*, and that they were completely lost by *Bufo m. marinus*, even when this host lives in open places like the "cerrados" in the Amazon region, and that *Bufo m. ictericus* may have very agglomerated granular glands on the posterior legs, so that they may easily be confounded with *Bufo m. paracnemis* (personal observation), one may believe that the invasion of the northern part of Brazil by *B. marinus* came from the south, and occurred earlier than the invasion of the southern forests. Certain characters of the hosts (paracnemic glands) as well as of their lung parasites (anterior dilation of the esophagus), have to be irreversible (host and *Rhabdias* from the north), what cannot be observed on hosts and *Rhabdias* from the southeastern part of Brazil.

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REFERENCES

BAER, J. G.

1930. Deux helminthes nouveaux, parasites de *Urocotyphlus oxyurus* (Gray), gymnophione de l'Inde méridionale. *Rev. suisse Zool. 37* (1): 43-52, 5 figs.

1951. *Ecology of animal parasites.* Univ. Illinois Press, Urbana. x + 224 pp., 162 figs.

BAIR, T. D.

1955. The oxygen consumption of *Rhabditis strongyloides* and other nematodes related to oxygen tension. *J. Parasit. 41* (6): 613-623.

BALDAUF, R. J.

1959. Morphological criteria and their use in showing bufonid phylogeny. *J. Morphol. 104:* 527-560, 13 figs.

BLAIR, W. F.

1972. Evidence from hybridization. *In:* W. F. Blair, *Evolution in the genus Bufo:* 196-232, 6 figs. Univ. Texas Press.

BOGART, J. P.

1972. Karyotypes. *In: Ibidem:* 171-195, 8 figs.

BOLK, L., E. GÖPPERT, E. KALLIUS & W. LUBOSCH (Edts.)

1933. *Handbuch der vergleichenden Anatomie der Wierbeltiere. VI.* Urban & Schwarzenberg, Berlin. xii + 854 pp., 629 figs., 5 pls.

1937. *Idem III Ibidem. xi* + 1018 pp., 981 figs.

BOVERI, T.

1911. Ueber das Verhalten der Geschlechtschromosomen bei Hermaphroditismus. Beobachtungen an *Rhabditis nigrovenosa. Verh. phys.-med. Ges. Würzb., (NS) 41* (5): 83-97, 19 figs.

BOVIEN, P.

1938. Some types of association between nematodes and insects. *Vidensk. Medd. dansk naturh. Foren. Kbh. 101:* 1-114, 31 figs.

BRAVO H. M. & E. C. CABALLERO

1940. Nematodos de los batracios de México. IV. *An. Inst. Biol. Univ. Méx. 11* (1): 239-247, pls.

BRENES, R. R. & M. BRAVO H.

1959. Helmintos de la República de Costa Rica. VIII. Nematoda 2. Algunos nemátodos de *Bufo marinus marinus* (L.) y algunas consideraciones sobre los generos *Oxysomatium* y *Aplectana. Rev. Biol. trop. 7* (1): 35-55, pls.

BRUMPT, E.

1921. Mode de pénétration des nématodes dans l'organism des mammifères, histotropisme et histodiagnostic. *C. R. Soc. Biol.*. Paris, *85* (2): 203-206.

BRYGOO, E. R.

1963. Contribution à la connaissance de la parasitologie des caméléons malgaches. *Ann. Parasit. hum. comp. 38* (2): 149-334, figs.

CHABAUD, A. G., E. R. BRYGOO & A. J. PETTER

1961. Descriptions et caractères biologiques de deux nouveaux *Rhabdias* malgaches. *Ibidem 36* (5-6): 752-763, 5 figs.

CHITWOOD, B. G. & M. B. CHITWOOD

1937. *An introduction to Nematology. I.* (1). Washington. 53 pp., 53 figs.

CHITWOOD, B. G. & A. MCINTOSH

1934. A new variety of *Alloionema* (Nematoda: Diplogasteridae), with a note on the genus. *Proc. helm. Soc. Wash. 1* (2): 37-38, fig. 13.

CHU, T. C.

1936. Studies on the life history of *Rhabdias fuscovenosa* var. *catanensis* (Rizzo, 1902). *J. Parasit. 22* (2): 140-160. 9 figs.

COLAM, J. B.

1971. Studies on the gut ultrastructure and digestive physiology in *Rhabdias bufonis* and *R. sphaerocephala* (Nematoda: Rhabditida). *Parasitology 62:* 247-258, 1 fig.. 4 pls.

DAVAINE, C. J.

1863. Sur un mode de dissémination des oeufs chez les entozoaires des voies respiratoires. *C. R. Soc. Biol.,* Paris (1862) *4* (3): 267-271.

DESSAUER, H. C.

1967. Molecular approach to snake taxonomy. *Herpetologica 23:* 148-155.

DESSAUER, H. C. & E. NEVO

1969. Geographic variation of blood and liver proteins in cricket frogs. *Biochem. Genetics 3:* 177-188, 8 figs.

DREYFUS, A.

1937a. Sobre o mecanismo de formação dos espermatozoides nas zonas testiculares da forma parasita de *Rhabdias fuelleborni* Trav. *Mem. Inst. Butantan 8* (1): 5-9, pl.

1937b. A espermatogênese nos machos da geração de vida livre de *Rhabdias fuelleborni* Trav. *Rev. Biol. Hyg.,* S. Paulo 8 (1): 10-18, pls.

1937c. Hermafroditismo alternante proterogínico em *Rhabdias fuelleborni* Trav. *Mem. Inst. Butantan 11:* 289-297, pls.

DUNCAN, D. B.

1970. Multiple comparison methods for comparing regression coefficients. *Biometrics 26* (1): 141-143.

FAHEL, J.

1952. Fauna helmitológica das "gias" de Salvador *Leptodactylus pentadactylus* (Laur.). *An. Acad. bras. Ci. 24* (4): 389-436, 9 pls., 40 figs.

FILIPJEV, I. N.

1934. The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithson. misc. Coll. 89* (6) 3216, 63 pp., 8 pls.

FISHER, R. A. & F. YATES

1948. *Statistical tables for biological, agricultural and medical research.* (3rd rev. Ed.). Oliver & Boyd, London. viii + 112 pp.

FOTEDAR, D. N.

1965. *Rhabdias bufonis* (Schrank, 1788) Stiles & Hassall, 1905 from the lungs of *Bufo viridis* in Kashmir and notes on some amphibian species of the genus. *Kashmir Sc. 2* (1-2): 20-26.

FÜLLEBORN, F.

1920a. Perkutane Infektion bei *Angiostomum nigrovenosum. Arch. Schiffs-u. Tropenhyg. 24* (6): 176.

1920b. Ueber die Anpassung der Nematoden an den Parasitismus und den Infektionsweg bei Askaris und anderen Fadenwuermern des Menschen. *Ibidem 24:* 340-347, fig.

1921. Ueber die Wanderung von Askaris- und anderen Nematodenlarven im Koerper, und intrauterine Askaris Infektion. (Abstr.). *Ibidem 25* (5): 146-149, figs.

1928. Ueber den Infektionsweg bei *Rhabdias bufonis (Rhabdonema nigrovenosa)* des Frosches nebst Versuchen ueber die Lymphzirkulation des letzteren. *Zbl. Bakt.* (1) *109* (7-8): 444-462, 3 figs., 1 pl.

GALLI-VALERIO, B.

1909. Notes de parasitologie et de technique parasitologique. *Ibidem* (1) *51* (5): 538-545, 2 figs.

GEIGY, J R., S. A. (Ed.)

1965. *Tablas cientificas.* Documenta Geigy. Basilea. 6th Ed., 783 pp.

GOODEY, T.

1924. Two new species of the nematode genus *Rhabdias. J. Helminth. 2* (5): 203-208, 11 figs.

GUTIÉRREZ, R. O.

1945. *Contribución al conocimiento de los nematodes parásitos de anfibios argentinos.* Tesis Mus. La Plata, n. 8, 37 pp., 24 figs.

HUECK, K.

1966. *Die Wälder Südamerikas.* Gustav Fischer Verl., Stuttgart. xviii + 422 pp., 253 figs.

HYMAN, L. H.

1951. *The invertebrates. 3* N. York, vii + 572 pp., 223 figs.

INGLIS, W. G.

1971. Speciation in parasitic nematodes. *In:* Dawes, B., *Advances in Parasitology. 9.* Academic Press, N. York: 185-223, 2 figs.

KLOSS, G. R.

1971. Alguns *Rhabdias* (Nematoda) de *Bufo* no Brasil. *Papeis avulsos Zool.,* S. Paulo, *24* (1): 1-52, 72 figs.

KOSUGE, I.

1924. Wie weit wirken bei dem Eindringen von *Strongyloides*-Larven und anderen parasitischen Nematoden in das Gewebe spezifische Reize ? *Arch. Schiffs-u. Tropenhyg. 28* (5): 179-187.

LEIPER, R. T.

1911. Demonstration of nematode parasites. *Proc. zool. Soc. Lond.*: 620-621.

LEUCKART, K. G. F. R.

1879. *Allgemeine Naturgeschichte der Parasiten.* Leipzig. x + 216 pp., 92 figs.
1886. *Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten.* Leipzig. 2d Ed. *1* (1), xxi + 1000 pp., 57 figs.

LINSTOW, O. F. B. v.

1882. Helminthologische Studien. *Arch. Naturgesch. 48* (1): 1-25, pls. 1-2, 29 figs.
1886. Helminthologische Beobachtungen. *Ibidem 52* (1): 113-138, pls. 6-9.
1890. Beitrag zur Kenntniss der Vogeltaenien, nebst Bemerkungen ueber neue und bekannten Helminthen. *Ibidem 56* (1): 171-188, pl. 10, 20 figs.

MARTIN, R. F.

1972. Evidence from osteology. *In:* W. F. Blair, *Evolution in the genus Bufo.* Univ. Texas Press: 37-70, 16 figs.

MAYR, E.

1969. *Animal species and evolution.* Harvard Univ. Press, Cambridge. 4th Ed. xiv + 797 pp., figs.

MCCUE, J. F. & R. E. THORSON

1964. Behaviour of parasitic stages in helminths in a *termal gradient. J. Parasit. 50* (1): 67-71.

MCCUTCHEON, F. H. & F. G. HALL

1937. Hemoglobin in the Amphibia. *J. cell. comp. Physiol. 9* (2): 191-197, 2 figs.

MECHNIKOV, E.

1865. Ueber die Entwickelung von *Ascaris nigrovenosa. Arch. Anat. Physiol.:* 409-420, pl. 10, 11 figs.

NEDKOFF, P.

1897. *Ueber die Metamorphose des Geshlechtsapparates bei Ascaris nigrovenosa.* Diss, Leipzig 34 pp., 1 pl., 10 figs.
1898. Ueber die Metamorphose des Geschlechtsapparates bei *Ascaris nigrovenosa* (Abstr.). *Zbl. Bakt.* (1) *24* (13): 506-507.

NOBLE, G. K.

1954. *The biology of the Amphibia. 2d Ed. Dover Publ., Inc.. Inc.. N. York. 577 pp.,* 174 figs.

ÖRLEY, L.

1886. *Die Rhabditiden und ihre medizinische Bedeutung.* Berlin. 84 pp., pls.

POTTS, F. A.

1908. Sexual phenomena in the free-living nematodes. *Proc. Camb. phil. Soc. 14* (4): 373-375.
1910. Notes on the free-living nematodes. I. The hermaphrodite species. *Quart. J. micr. Sci. 55* (3), n. s.: 433-483, 11 figs.

RAILLIET, *A.*

1899. Évolution sans hétérogonie d'un angiostome de la couleuvre à collier. *C. R. Acad. Sci.,* Paris *129* (26): 1271-1273.

REICHENBACH-KLINKE, H. & E. ELKAN

1965. *The principal diseases of lowver vertebrates.* London. xii + 600 pp., 397 figs.

ROGERS, W. P.

1957. An alternative approach to the study of host-parasite specifity. *In: Symp. Host specifity among parasites of vertebrates:* 309-311. Neuchâtel.

1962. *The nature of parasitism.* Academic Press, N. York. ix + 287 pp., 40 figs.

ROGERS, W. P. & R. I. SOMMERVILLE

1963. Infective stages of nematode parasites and its significance in parasitism. *In:* B. Dawes, *Advances in Parasitology.* I: 109-177, 4 figs. Academic Press. London.

ROMER, A. S.

1959. *The vertebrate body.* W. B. Saunders Co., Philadelphia. viii + 644 pp., 390 figs.

SCHAAKE, M.

1931. Infektionsmodus und Infektionsweg der *Rhabdias bufonis* Schrank (*Angiostomum nigrovenosum*) und die Metamorphose des Genitalapparates der hermaphroditischen Generation. *Z. Parasitenk. 3* (4): 517-648, 34 figs.

SCHNEIDER, A. F.

1866. *Monographie der Nemotoden.* Berlin. viii + 357 pp., 122 figs., 28 pls.

SEMENOV, V. D.

1929. Eine neue Nematodenart — *Rhabdias microoris* n. sp. — aus den Lungen von Amphibien. *Zbl. Bakt.* (1) *114* (7-8): 533-539, 4 figs.

SEURAT, L. G.

1920. *Histoire naturelle des nématodes de la Bérberie. I.* Alger. vi + 221 pp., 34 figs.

SKRJABIN, K. I., N. P. SCHIKHOBALOVA, A. A. SOBOLEV, A. A. PARAMAROV & V. E. SUDARIKOV

1954. Description of nematode parasites. IV. (Russian text). *Acad. Nauk SSSR.* Lab. Helminth. *4.* 927 pp., 165 figs.

STEBBINS, R. C. & J. H. HENDRICKSON

1959. Field studies of amphibian in Colombia, South America. *Univ. Calif. Publ. Zool. 56* (5): 496-540, 1 fig.

STEEL, R. G. D. & J. H. TORRIE

1960. *Principles and procedures of statistics.* McGraw Hill Book Co., Inc. N. York. xiv + 481 pp., figs.

STEKHOVEN JR., J. H. S.

1952. Nematodos parasitarios de anfíbios, pájaros y mamíferos de la República Argentina. *Acta zool. lilloana 10:* 315-400, figs.

STILES, C. W. & A. HASSALL

1905. The determination of generic types and a list of roundworms, with their original and type species. *Bull. U. S. Bur. anim. Ind. 79,* 150 pp.

STUART, L. C.

1951. The distributional implication of temperature tolerance and hemoglobin in the toads *Bufo marinus* (Linnaeus) and *Bufo bocourti* Brocchi. *Copeia* (3): 220-229, 3 figs.

TIHEN, J. A.

1962a. Osteological observations on New World *Bufo*. *Amer. Midl. Nat.* 67 (1): 157-183, 38 figs.

1962b. A review of New World fossil bufonids. *Ibidem 68:* 1-50, 62 figs.

TRAVASSOS, L.

1926. Entwicklung des *Rhabdias fuelleborni* n. sp. *Arch. Schiffs-u. Tropenhyg. 30* (9): 594-602, 21 figs.

1930. Pesquisas helmintológicas realizadas em Hamburgo. VII. *Mem. Inst. Osw. Cruz 24* (3): 161-181, pls. 51-57, 42 figs.

VANZOLINI, P. E.

1970. Zoologia sistemática, geografia e origem das espécies. *Inst. Geografia USP*, s. Teses e Monografias *3*. 56 pp., maps, graphs.

WALTON, A. C.

1929. Studies on some nematodes of North American frogs. I. *J. Parasit. 15* (4): 227-240, pls. 16-20, 43 figs.

1946. The nematode parasites of Bufoninae. II. *Ibidem 31*, suppl. (1945): 24.

WEDL, C.

1856. Ueber die Mundwerkzeuge von Nematoden. *S. B. Akad. Wiss. Wien* (3) *19* (1): 33-69, pls. 1-3, figs. 1-41.

WENZEL, R. L., V. J. TIPTON & A. KIEWLICZ

1966. The streblid batflies of Panama (Diptera Calypterae: Streblidae). *In:* Wenzel & Tipton Ed., *Ectoparasites of Panama:* 405-675, figs. 38-146, Field Mus. nat. Hist., Chicago.

WILLIAMS, R. J.

1956. *Biochemical individuality. The basis for the genetotrophic concept.* John Wiley & Sons, Inc, N. York. vii + 214 pp., 17 figs.

WILLIAMS, R. W.

1959. Some nematodes parasites of the tree frogs, toads, lizards, and land crabs of the Bermuda Islands. *J. Parasit. 45* (2): 239.

WILLIAMS, R. W. & E. (LONDON)

1960. Observations on the life history of *Rhabdias sphaerocephala* Goodey, 1924 from *Bufo marinus* L., in the Bermuda Islands. *J. Helminth. 34* (1-2): 93-98, pls.

YAMAGUTI, S.

1961. *Systema Helminthum. 3. Nematodes I.* London. 679 pp.

YUEN, P. H.

1965. Some studies on the taxonomy and development of some rhabdiasoid and cosmocercoid nematodes from Malayan amphibians. *Zool. Anz. 174* (4-5): 275-298, figs.

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