

MORPHOLOGICAL AND BIOLOGICAL STUDY OF *RHABDIAS* SPP. (NEMATODA) FROM AFRICAN CHAMELEONS WITH DESCRIPTION OF A NEW SPECIES

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Summary:

Lung-dwelling females of *Rhabdias* (Rhabdiasidae), and possible migrating ceolomic young females were searched for in 46 chameleons, belonging to seven species. *Rhabdias chamaeleonis*, the single species identified to date in Africa, was found in *Chamaeleo (Trioceros) johnstoni* and *C. (T.) hoehnelii*, and redescribed; the sizes of the buccal capsule and oesophagus were stable compared to the length of the female parasites, which varied from 6 mm to 22 mm in length. A second species, *R. jarki* n. sp., was identified from one *C. (T.) johnstoni*; it differed from *R. chamaeleonis* in the shape of anterior region, the longer and slender oesophagus (ratio bulb diameter-body diameter at that level about 1/5 instead of 1/2), the arrangement of the head papillae, the shape of the buccal capsule, and the anatomy of the genital apparatus: one of the ovaries (the anterior or posterior one according to the specimen) had a band of small cells, among larger oocytes of the synapsis zone, which were likely to generate the spermatozoa present in the oviducts, whereas these two elements were absent from *R. chamaeleonis*. The parasitic females of *R. jarki* thus appeared to be hermaphroditic, whereas those of *R. chamaeleonis* appeared to be parthenogenetic. The free living phase of these *Rhabdias* species was heterogonic. The infective larva of *R. chamaeleonis* was 360-590 µm long, unmotile and at third stage inside the maternal cuticle. The free-living male of *R. jarki* was described. The numerous infective larvae recovered from cultures of unidentified *Rhabdias* were all in maternal cuticle (one larva/female instead of two as in *R. gemellipara* from *Calumma parsonii*, from Madagascar) and two kinds of larvae were identified, *R. chamaeleonis* and larvae 700-900 µm long provisionally identified to *R. jarki*. The behaviour of infective larvae and a few successful infections of insects suggest that, in the field, insect transport hosts are involved in transmission and in preventing infective larvae from drying out.

KEY WORDS : Rhabdiasidae, lung, chameleons, morphology, parthenogeny, hermaphroditism, free-living cycle, transmission, transport host.

Résumé : ÉTUDE MORPHOLOGIQUE ET BIOLOGIQUE DE *RHABDIAS* SPP. (NEMATODA) DE CAMÉLÉONS AFRICAINS ET DESCRIPTION D'UNE NOUVELLE ESPÈCE

Une recherche de *Rhabdias* est effectuée chez des caméléons (46 spécimens, sept espèces). *R. chamaeleonis*, seule espèce connue jusqu'à ce jour en Afrique, est récoltée chez *Chamaeleo (Trioceros) johnstoni* et *C. (T.) hoehnelii*, et redécrite; les tailles de la capsule buccale et de l'œsophage sont stables contrairement à la longueur des femelles qui varie de 6 mm à 22 mm. Une seconde espèce, *R. jarki* n. sp., est identifiée chez un des *C. (T.) johnstoni*; elle est distincte de *R. chamaeleonis* par la forme de la région antérieure du corps, l'œsophage plus long et plus grêle (rapport des diamètres du bulbe et du corps à ce niveau égal à 1/5 au lieu de 1/2), la position des papilles de la tête, la capsule buccale, ainsi que par l'anatomie de l'appareil génital: un des ovaires (ovaire antérieur ou postérieur selon les spécimens) a une bande de petites cellules parmi les larges ovocytes de la "zone synapsis", probablement à l'origine des spermatozoïdes qui sont présents dans les oviductes; ces deux éléments sont absents chez *R. chamaeleonis*. On peut en conclure que les femelles parasites de *R. jarki* sont hermaphrodites alors que celles de *R. chamaeleonis* sont parthénogénétiques. La phase libre de ces espèces est hétérogonique. Les larves infestantes de *R. chamaeleonis* sont longues de 360-590 µm, immobiles et au troisième stade dans la cuticule maternelle. Le mâle libre de *R. jarki* est décrit. Les nombreuses larves infestantes obtenues avec des cultures de *rhabdias* non identifiés sont dans la cuticule maternelle (une larve/femelle au lieu de deux chez *R. gemellipara* de *Calumma parsonii*, à Madagascar) et deux sortes de larves sont identifiées, *R. chamaeleonis* et des larves longues de 700-900 µm, attribuées à *R. jarki*. Le comportement des larves infestantes et quelques infections positives d'insectes suggèrent que, dans la nature, des insectes hôtes de transport interviennent dans la transmission, empêchant la dessiccation des larves infestantes.

MOTS CLÉS : Rhabdiasidae, poumons, caméléons, morphologie, parthénogenèse, hermaphroditisme, phase libre, transmission, hôte de transport.

INTRODUCTION

Species of *Rhabdias* Stiles & Hassall, 1905 (Rhabdiasidae, see Anderson & Bain, 1982), are lung nematodes with a complex and varied biology.

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Closely related to non-parasitic rhabditids, they have a free-living phase which is heterogonic (Leuckart, 1865; Mecznikow, 1865; Baker, 1979a; Kuzmin, 2000) or, less often, homogonic (Railliet, 1899; Goodey, 1924a; Kuzmin, 1999). Infective larvae are transmitted via the subcutaneous route when the hosts are amphibians (Fuelleborn, 1928; Schaake, 1931), but presumably *per os* for parasites of reptiles, with eventual acquisition of transport hosts as strongly suggested by Fuelleborn (1928) and Goodey (1924a). The parasitic phase is represented by females only and this raises

Total	n	Chameleon species	Origin	Coll. number	Sex	Source	Date import	Date necropsy	n rhabdias**	<i>Rhabdias</i> species	Remarks
1	1	<i>C. (T.) jobnstoni</i>	Burundi	310 HS	?	Import	25. 2. 02	8. 3. 02	0		
2	2	<i>id.</i>	<i>id.</i>	311 HS	M	<i>id.</i>	<i>id.</i>	8. 3. 02	1	<i>chamaeleonis</i>	
3	3	<i>id.</i>	<i>id.</i>	312 HS	M	<i>id.</i>	<i>id.</i>	<i>id.</i>	1	<i>chamaeleonis</i>	
4	4	<i>id.</i>	<i>id.</i>	313 HS	M juv	<i>id.</i>	<i>id.</i>	<i>id.</i>	1 juvenile	?	
5	5	<i>id.</i>	<i>id.</i>	316 HS	F	<i>id.</i>	<i>id.</i>	14. 3. 02	18	<i>chamaeleonis</i>	
6	6	<i>id.</i>	<i>id.</i>	317 HS	M	<i>id.</i>	<i>id.</i>	15. 3. 02	5	<i>jarki</i> n. sp.	1 perforating worm
7	7	<i>id.</i>	<i>id.</i>	318 HS	M juv	<i>id.</i>	<i>id.</i>	<i>id.</i>	0		
8	8	<i>id.</i>	<i>id.</i>	320 HS	M	<i>id.</i>	<i>id.</i>	29. 3. 02	2	?	
9	9	<i>id.</i>	<i>id.</i>	322 HS	M	<i>id.</i>	<i>id.</i>	2. 4. 02	3+3	?	Decaying host
10	10	<i>id.</i>	<i>id.</i>	323 HS	F pregnant	<i>id.</i>	<i>id.</i>	2. 4. 02	2	?	Decaying host
11	11	<i>id.</i>	<i>id.</i>	324 HS	M juv	<i>id.</i>	<i>id.</i>	2. 4. 02	1	?	Decaying host
12	12	<i>id.</i>	<i>id.</i>	325 HS	F juv	<i>id.</i>	<i>id.</i>	2. 4. 02	1+1	?	Decaying host
13	13	<i>id.</i>	<i>id.</i>	326 HS	M juv	<i>id.</i>	<i>id.</i>	2. 4. 02	2	?	Decaying host
14	14	<i>id.</i>	<i>id.</i>	327 HS	M	<i>id.</i>	<i>id.</i>	2. 4. 02	0		
15	15	<i>id.</i>	<i>id.</i>	331 HS	F	<i>id.</i>	<i>id.</i>	5. 4. 02	3+1	?	
16	16	<i>id.</i>	<i>id.</i>	332 HS	M juv	<i>id.</i>	<i>id.</i>	5. 4. 02	4+1	?	
17	17	<i>id.</i>	<i>id.</i>	333 HS	?	<i>id.</i>	<i>id.</i>	5. 4. 02	4 decayed	?	Decaying host
18	18	<i>id.</i>	<i>id.</i>	335 HS	?	<i>id.</i>	<i>id.</i>	12. 4. 02	larvae, eggs	?	
19	19	<i>id.</i>	<i>id.</i>	*336 HS	M juv	<i>id.</i>	<i>id.</i>	12. 4. 02	exp	*	
20	20	<i>id.</i>	<i>id.</i>	337 HS	F	<i>id.</i>	<i>id.</i>	12. 4. 02	0		
21	21	<i>id.</i>	<i>id.</i>	*341 HS	M	<i>id.</i>	<i>id.</i>	13. 4. 02	exp	*	
22	22	<i>id.</i>	<i>id.</i>	342 HS	F	<i>id.</i>	<i>id.</i>	15. 4. 02	11+11	<i>chamaeleonis</i>	
23	23	<i>id.</i>	<i>id.</i>	*344 HS	F	<i>id.</i>	<i>id.</i>	13. 5. 02	4+1	? other species	
24	1	<i>C. (T.) boebnelii</i>	?	392 HS	M juv	Seizure	?	29. 06. 02	1-0	<i>chamaeleonis</i>	Lung anthracosis
25	2	<i>id.</i>	?	431 HS	M juv	<i>id.</i>	?	24. 12. 02	0		Frozen
26	3	<i>id.</i>	?	432 HS	F juv	<i>id.</i>	?	26. 12. 02	0		Frozen
27	4	<i>id.</i>	?	434 HS	juv	<i>id.</i>	?	26. 12. 02	4+5+4 trachea	<i>chamaeleonis</i>	Frozen
28	1	<i>C. (T.) jacksonii</i>	?	394 HS	M juv	<i>id.</i>	?	9. 7. 02	0		Euthanized
29	2	<i>id.</i>	?	395 HS	M juv	<i>id.</i>	?	<i>id.</i>	0		Euthanized
30	3	<i>id.</i>	?	433 HS	M juv	<i>id.</i>	?	26. 12. 02	0		Frozen
31	1	<i>C. (C.) senegalensis</i>	Togo	330 HS	M juv	Import	1. 04. 02	4. 4. 02	0		
42	2-12	<i>id.</i>	<i>id.</i>	401-411 HS	diverse	Import	1. 9. 02	5. 9. 02	0		
43	1	<i>C. (C.) gracilis</i>	Togo	430 HS	?	Import	15. 12. 02	23. 12. 02	0		
44	2	<i>id.</i>	<i>id.</i>	436 HS	M	Import	<i>id.</i>	23. 1. 03	0		Lung inflammation
45	1	<i>C. (C.) chamaeleo</i>	?	391 HS	F	Seizure		29. 06. 02	0		
46	1	<i>Furcifer oustaleti</i>	Madagascar	321 HS	?	Import	25. 03. 02	2. 04. 02	0		

Coll: collection. * Experimental infection. ** Right and left lungs, when precised.

Table I. – Studied chameleonids and *Rhabdias* infection.

the question of their mode of reproduction. The parasitic females are generally said to be protandrous hermaphrodites (Baker, 1979a; Anderson, 2000), but in some species, and according to some authors, they may be hermaphroditic during their whole parasitic life (Schleip, 1911; Goodey, 1924a & b) or, presumably, parthenogenetic (Chabaud *et al.*, 1961) like a species of the closely related genus *Entomelas* Travassos, 1930 (*cf.* Seurat, 1920).

The material examined here was collected from chameleons from Africa. In this part of the Ethiopian Region, *Rhabdias* has been seldom studied, as is clear from a synthesis on the nematode parasites of batrachians and reptiles published by Baker (1987a), and a few other publications (Baker, 1987b; Kuzmin, 2001). Among batrachians, *R. bdellophis* Baylis, 1929, has been described from Tanganyika, *R. collaris* Baker, 1987 from Tanzania, and *R. africanus* Kuzmin, 2001 from South Africa; *R. bufonis* (Schranck, 1788), not restricted to Africa, was recorded from Yemen by Kuntz & Myers (1968) (in Baker, 1987a).

Among reptiles, *Rhabdias* spp. have been reported from South African snakes (Fantham & Porter, 1950; Hering-Hagenbeck & Boomker, 2000; Hering-Hagenbeck, 2001); they were identified as *R. fuscovenosa* (Railliet, 1899), which has a wide geographical distribution. Another species, restricted to Africa, *R. chamaeleonis* (Skrjabin, 1916), was described from chameleons; the host species was not identified and its geographical origin was rather vague (British East Africa). Later, other specimens recovered from diverse chameleonids in East Africa were identified as *R. chamaeleonis* (*cf.* Baylis, 1937).

From Madagascar, also part of the Ethiopian Region, a species of *Rhabdias* has been described from a chameleon, *R. gemellipara* Chabaud, Petter & Brygoo, 1961. These authors also published the first biological data on rhabdias from chameleonids (Chabaud *et al.*, 1961): the parasitic female was considered as parthenogenetic; the free-living females, after mating, produced two larvae each which developed by matricidal endotoky.

We had the opportunity to examine a large number of African chameleonids belonging to several species. Many specimens were infected with *Rhabdias*. We studied them with three objectives: to determine the specific diversity and the mode of reproduction of the parasitic females, by using morphological analysis, and transmission mode, involving attempts to infect other chameleons, insects and molluscs.

MATERIAL AND METHODS

The chameleons (Table D) were either legally imported into France by a dealer, who gave dying or recently dead specimens of five spe-

cies to our laboratory, or were seized by the French customs authorities and deposited in the Muséum National d'Histoire Naturelle, Paris (two species). The animals were examined at the time of their arrival in France or following a variable period of captivity (Table D). In the dealer's shop each species was kept in isolation. The majority of the seized animals were frozen before reaching the Muséum.

At necropsy, the coelomic cavity was flushed with saline in order to recover any young migrating nematodes (Baker, 1979a & b). Lungs were isolated by sectioning the anterior part of the trachea, then inflated with air with the aid of a pipette. Lesions or any anomalies of the lungs were noted and, eventually, histological analysis was performed. The number and position of the worms were noted (Table I, Fig. 1A) and they were then removed for study. The digestive tract was dissected out to be examined for *Rhabdias* eggs and larvae, and for any other nematodes.

To obtain the free-living stages, cultures were made *in vitro* in distilled water, to which a few small pieces of charcoal had been added to prevent bacterial pollution, in petri dishes 5 to 7 cm in diameter. Either entire gravid female worms or their mid-sections only were placed in the medium and teased to free eggs and hatching larvae. When only part of the female was used, the extremities were fixed for further morphological study; each petri dish contained only a part of a single female. Cultures were maintained at room temperature, 17-22° C, and observed almost daily.

Morphological study of parasitic females was made on living specimens or after fixation in hot 70 % alcohol and clearing in lactophenol; all fixed specimens were examined. A few immature and mature females were dissected to study the anatomy of the genital apparatus. The length and external diameter of the buccal cavity were measured. Free-living stages were studied alive and after fixation in 5 % formalin with 3 % acetic acid.

Transmission trials were made as follows. Infective larvae were either placed in contact with a chameleon and with small terrestrial molluscs (very young unidentified snails and slugs, collected from suburban gardens), or known numbers were forcibly fed to chameleons, and laboratory-bred locusts (*Schistocerca gregaria*) and crickets (*Acheta domestica*). The recipient experimental animals were dissected in saline at various times following exposure or feeding.

Chameleons are named according to the internet site EMBL <http://www.embl-heidelberg.de/-uetz/livingReptiles.html>, consulted on 14. 03. 2003. *Rhabdias* species are named according to Baker (1987a) and Kuzmin (1999, 2001) and his site <http://www.rhabdias.kiev.ua/ref.htm>.

RESULTS

The 46 chameleons studied belong to seven species (Table I): *Chamaeleo (Trioceros) johnstoni* Boulenger, 1901, *C. (T.) boehnelii* Steindachner, 1891, *C. (T.) jacksoni* Boulenger, 1896, *C. (C.) senegalensis* Daudin, 1802, *C. (C.) gracilis* Hallowell, 1844, and *C. (C.) chamaeleo* (Linnaeus, 1758), all from Africa, and one *Furcifer oustaleti* (Mocquard, 1894) from Madagascar. Two species were parasitized, *C. (T.) johnstoni*, not previously reported to harbour rhab-

diasids, and *C. (T.) boehnelii*; both live in mountains of Central and/or East Africa (Branch, 1998; internet site EMBL). *R. chamaeleonis* was identified in both host species, but a second, new species was also found in *C. (T.) johnstoni*. The existence of two species was discovered during the investigation, so that many *in vitro* cultures, but not all, were made with unidentified materials.

Pulmonary lesions were noted twice at necropsy (Table I); anthracosis was identified in one specimen (392 HS), and an inflammatory reaction in the other

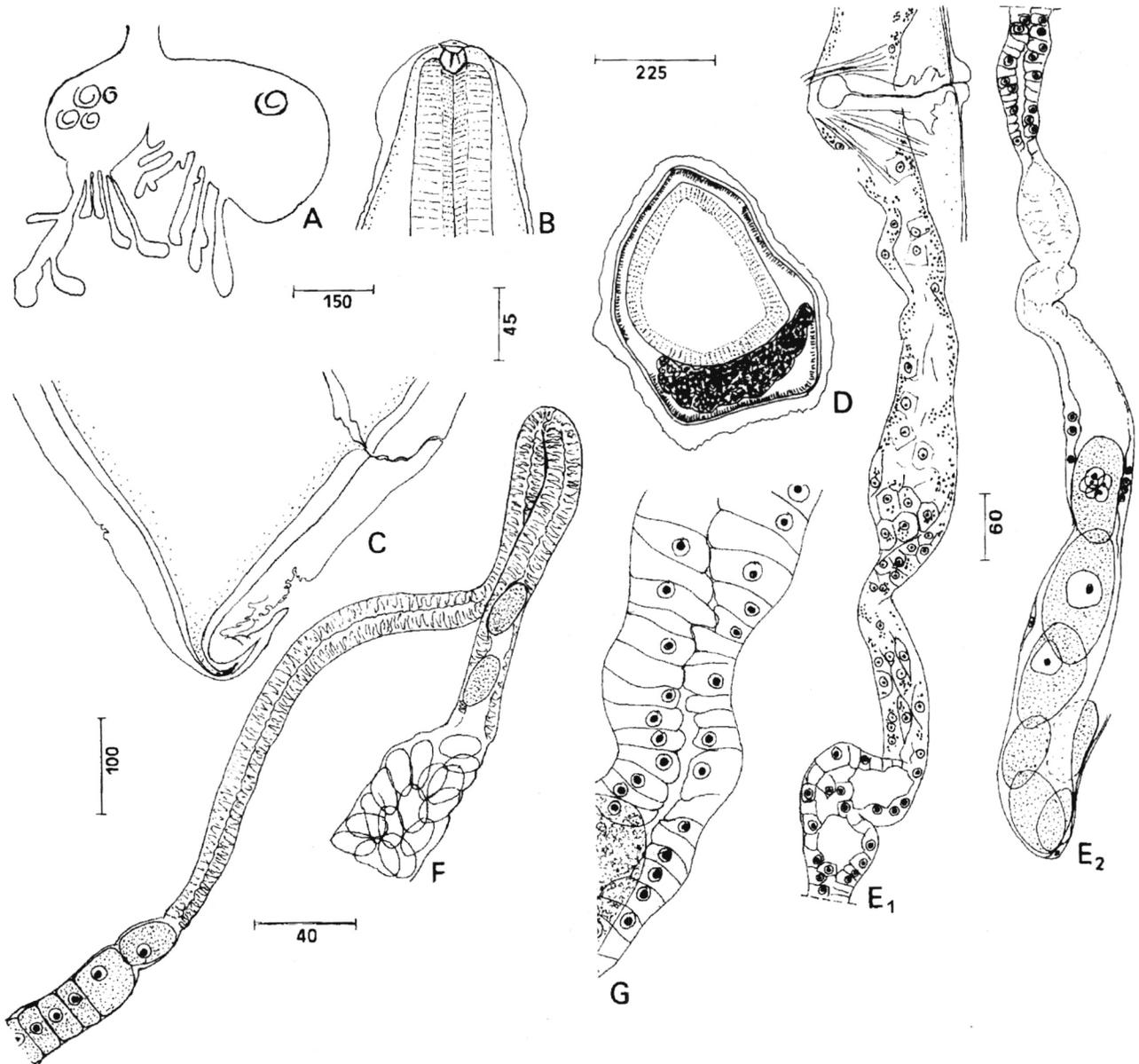


Fig. 1. – *Rhabdias* spp. from *C. (T.) johnstoni*, parasitic females. A. Position of worms in air-inflated lungs of a chameleon. B. Head of immature female *Rhabdias* sp. 313 HS. C. Tail of female *Rhabdias* sp. 344 HS, lateral view. D to G, *R. chamaeleonis* 416 HS. D. Transverse section of a mature female, near mid-body. E. Posterior genital organs of immature female, body 6.4 mm long, from vulva to ovary (in two parts 1 & 2); note absence of spermatozoa in the oviduct lumen and the egg with four nuclei at the proximal end of ovary (drawn at high magnification: objective $\times 50$, then reduced). F. Oviduct of a dissected female. G. Same female, detail. Scale bars: B, 100 μm ; C, 45 μm ; D, 225 μm ; E, 60 μm ; F, 150 μm ; G, 40 μm . For A, total lung width is 4 cm.

one but no adult or larval parasite was found (436 HS).

A few of the fixed parasitic females were impossible to identify. They were recovered from two *C. (T.) johnstoni*. The first specimen (313 HS) was a very young female 4.6 mm long and 240 µm wide, with vulva opened at mid-body and a juvenile buccal capsule (Fig. 1B); this tiny female, found alive in the stomach, was probably a recent infection. The second specimen (344 HS) was one of the five rhabdias recovered from the chameleon 344 HS, the other four having been kept for the cultures (not successful); it was a mature pulmonary female 23.6 mm long; only the posterior region was in good condition and the tail differed from that of all other recovered specimens, being short and comparatively very wide at the anus (200/180 µm), with a ventrally folded extremity (Fig. 1C). This worm suggests greater diversity of the rhabdiasids of African chameleons than was previously thought.

We present a morphological description of the females of the two identified species, followed by a taxonomic discussion, then a description of the free-living stages of each species and, in the last section, the results of transmission experiments.

RHABDIAS CHAMAELEONIS (SKRJABIN, 1916)

From *Chamaeleo (Trioceros) johnstoni* Boulenger, 1901, collected in the Kibira forest (3° 30' S, 29° 3' E) about 1,700 metres altitude, in the commune of Musigati (chief town Bubanza), Burundi, in January 2002. Specimens: 14 gravid and three immature females 316 HS (plus one female kept to study the developmental cycle); 22 gravid females, 342 HS; one gravid female 311HS, one gravid female 312 HS.

From *C. (T.) boehnelii* Steindachner, 1891. Unknown geographical origin but this species lives in mountains of Kenya and Uganda (Branch, 1998). Specimens: one gravid female 392 HS, 13 gravid females 434 HS.

All specimens are deposited in the Muséum National d'Histoire Naturelle, Paris.

The redescription of the parasitic females (Table II, Figs 1, 2, 3) is based on the two larger batches of specimens recovered from *C. (T.) johnstoni* (Table I): 316 HS, 17 females at different stages of maturation; and 342 HS, 22 females of which the extremities only were fixed (mid-body used for cultures); their body length is unknown; all were fully gravid.

Body bent dorsally, becoming coiled in gravid females. Body length from 5.7 to 22.85 mm (316 HS), maximum width at mid-body from 215 to 1250 µm; females over 11.6 mm all contained eggs. Cuticular vesicle present, of variable development but always thicker near the body extremities; beginning of the vesicle at level of the buccal capsule; caudal point inside the vesicle.

<i>Rhabdias</i> species	<i>chamaeleonis</i>		<i>C. (T.) boehnelii</i>		<i>chamaeleonis</i>		<i>jarki n. sp.</i>		<i>gemellipara</i>		<i>? gemellipara</i>	
	Authors	Host	<i>C. (T.) johnstoni</i>	<i>C. (T.) johnstoni</i>	Skrijabin, 1916	Chab., 1961	Chab., 1961	Chab., 1961	Chab., 1961	Chab., 1962	Chab., 1962	Chab., 1962
Geographical origin	Burundi	Brit. East Africa	Burundi	?	Brit. East Africa	Madagascar	Madagascar	Madagascar	Madagascar	Madagascar	Madagascar	Madagascar
MNHN collection number	316 HS	392 HS	311 HS	392 HS	434 HS	317 HS	317 HS	317 HS	317 HS	317 HS	317 HS	317 HS
n worms	9*	22	1	1	13	3	3	3	3	3	3	3
Length mm	5.7-6.4	17.2-22.85	21.9	22	20-28	11.8-12.3	11.8-12.3	11.8-12.3	11.8-12.3	11.8-12.3	11.8-12.3	11.8-12.3
Width at mid body	215-270	660-1250	1150	1000	700-975	500-600	500-600	500-600	500-600	500-600	500-600	500-600
Bucc. caps. length	20-25	25-30	30	20	18-23	13-16	13-16	13-16	13-16	13-16	13-16	13-16
Bucc. caps. ext. diam	30-38	42-45	45	35	32-45	40-42	40-42	40-42	40-42	40-42	40-42	40-42
Oesophagus length mm	700-800	800-965	845	1030	960-1170	1380-1500	1380-1500	1380-1500	1380-1500	1380-1500	1380-1500	1380-1500
Oesophagus width	70-80	75-85	75	70	90-110	45-52	45-52	45-52	45-52	45-52	45-52	45-52
Bulb diameter	125	140-170	180	170	145-180	100-110	100-110	100-110	100-110	100-110	100-110	100-110
Width at end of oesophagus	210-215	300-375	325	460	255-320	475-600	475-600	475-600	475-600	475-600	475-600	475-600
Tail	105-255	300-370	320	300	250-385	225-350	225-350	225-350	225-350	225-350	225-350	225-350
Width at anus	105-190	150-210	180	150	135-190	110-150	110-150	110-150	110-150	110-150	110-150	110-150

juv: juvenile; * middle-sized gravid; ** measured on original figures. Chab., 1961: Chabaud, Brygoo, Petter. Chab., 1962: Chabaud & Brygoo.

Table II. - *Rhabdias* spp. from chameleons. Major distinctive specific characters of parasitic females.

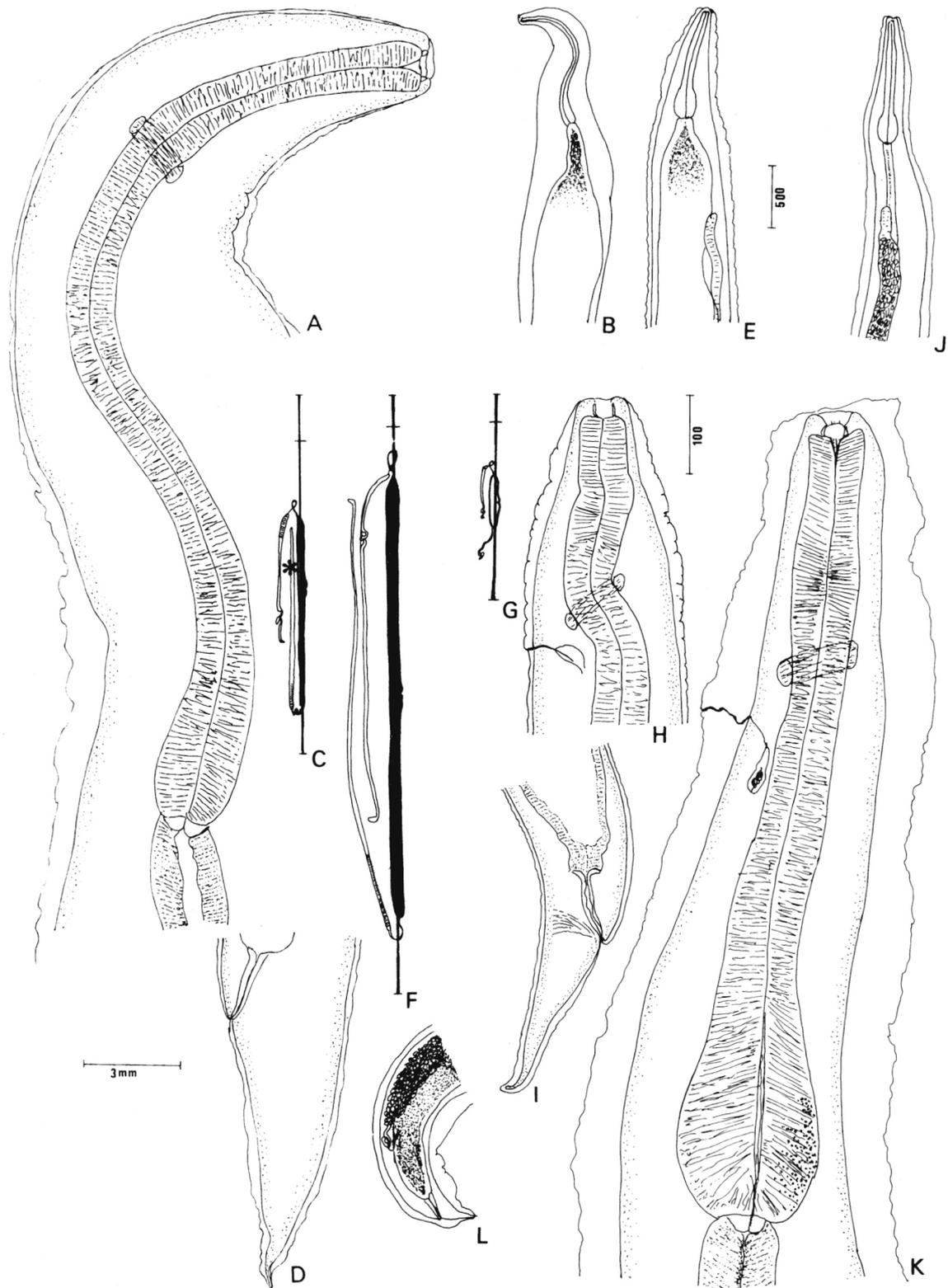


Fig. 2. – *Rhabdias jarki* n. sp. and *R. chamaeleonis*. A to D, *R. jarki*, female holotype. A. Oesophagus. B. Habitus of anterior region. C. Schematic representation of oesophagus and genital organs of this worm, 12.1 mm long (*: position of the band of small cells in the synopsis zone). D. Tail, left lateral view. E to L, *R. chamaeleonis*, E to I, specimens 416 HS from *C. (T.) johnstoni*. E. Habitus of anterior region. F. Schematic representation of oesophagus and genital organs in a mature female, 19 mm long. G. *Idem*, immature female, body 6.4 mm long (uterus empty). H. Same female, anterior region, left lateral view. I. Tail of this female, right lateral view. J to L, specimen 434 HS, from *C. (T.) boehnelii*. J. Habitus of anterior region. K. Oesophageal region. L. Posterior region, left lateral view. Scale bars: B, E, J, L, 500 µm; C, F, G, 3 mm; others, 100 µm.

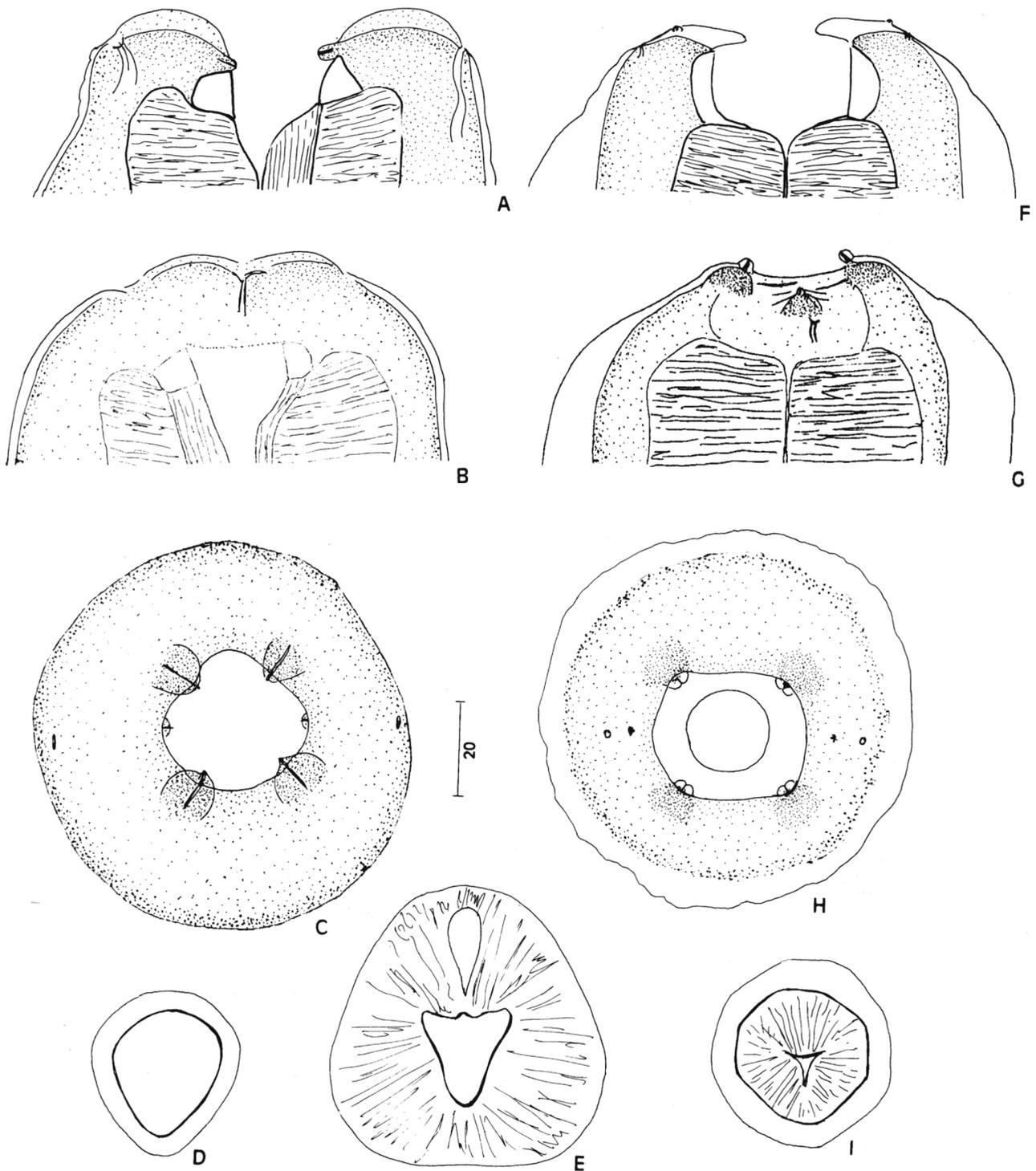


Fig. 3. – Head of *Rhabdias* spp. A to E, *R. jarki* n. sp. A. Median view. B. Lateral view. C. Apical view. D. Optical transverse section of buccal capsule. E. Apex of oesophagus with ductal end of dorsal oesophageal gland. F to I, *R. chamaeleonis*. F. Median view. G. Lateral view. H. Apical view. I. Optical transverse sections of buccal capsule and oesophageal lumen. Scale bar: 20 μ m.

Head (Fig. 3 F-I): four submedian papillae, each with salient cuticular apex, a cuticular fold joining them; very small lateral papillae, posterior to the submedian ones and thus far from the buccal aperture; amphids close to the lateral papillae. Round mouth, slightly posterior to the submedian papillae and surrounded by a cuticular velum. Buccal cavity sub-octagonal in transverse section. Buccal capsule with a wall, 20-30 μm long and external diameter from 30-50 μm , its dimensions hardly differing between small and large worms (Table II), but tending to be narrower when deep in the apex of the oesophagus. Nerve ring 230-405 μm from apex and excretory pore 310-390 μm from apex; no long excretory cells identified.

Oesophagus (Table II) thick, 65-85 μm at mid-length, with large bulb 125-180 μm wide, its diameter about half the body width at that level; apex of oesophagus generally flat, sometimes concave and forming a "shoulder" surrounding the buccal capsule, corpus slightly dilated. Length of oesophagus rather similar regardless of the body size, as seen in specimens 316 HS: 700 to 965 μm long. However, differences can arise due to the fixation procedure: the oesophagus was slightly longer in specimens 342 HS (anterior part only fixed), in which all the specimens had straight oesophagi, unlike those of 316 HS in which the anterior third was curved. Intestinal diameter rapidly increasing posteriad. Tail dorsally curved at level of anus in half of the gravid females but barely curved in others; straight in young females and in one specimen 13 mm long; tail 165 to 390 μm long, > 260 μm in all gravid females; width at anus not exceeding two-thirds of tail length. Very numerous eggs; embryonated eggs near vulva, 110-140 long/55-85 μm wide. Hatching larvae from uteri and lungs, 460-520/30-35 μm .

Vulva at mid-body or slightly posterior. The genital tract (Figs 1F, G & 2F) was larger in the larger parasitic females: ovaries were five times longer in gravid specimens than in immature ones, oviducts twice as long with much larger (2-3 fold) cells. In a dissected mature female, anterior ovary 10,000 μm long, its apex 3,680 μm posterior to vulva and 5,950 μm from the caudal point; anterior oviduct 1,600 μm long, its anterior curve 1,800 μm from apex. Posterior ovary 14,100 μm long, beginning 3,500 μm from head; posterior oviduct 1,250 μm long, its posterior curve 1,750 μm from the caudal point. Structure of anterior and posterior ovaries similar: regular increase in size of oocytes; ovary 100-150 μm thick near oviducts, with piles of rectangular ovulae at this level. Structure of oviducts also similar, their regular wall composed of cells 30-100 μm high and 15-25 μm wide. No seminal receptacle or spermatozoa identified. In a second dissected female, the size and anatomy of the ovaries and oviducts were similar.

The genital tracts of three young females measuring 5.7 to 6.4 mm (316 HS) were examined (Figs 1E &

2G); these females presented three stages of maturation: one had neither ovulae in the oviducts nor uterine eggs, one had ovulae in the oviducts and empty uteri, and one had nine developing eggs in the anterior uterus. In one specimen the posterior ovary was abnormally directed backwards (Fig. 2G). In all three, the shorter and thinner ovaries, maximum 18-25 μm thick, had only a few elongated ovulae near the oviducts (no piles of rectangular ovulae). The oviducts had two parts: that near the ovary with a thin irregular wall, its lumen with or without large female genital cells, some of them with a central group of a few nuclei (two females), interpreted as eggs at the beginning of division (Fig. 1E₂); the other part, near the uterus, with a wall of regular cells, 18-20/8-10 μm . No spermatozoa were identified in any oviduct.

• Taxonomic discussion

The description of *R. chamaeleonis* is brief (Skrjabin, 1916) but the characters and measurements, some of them estimated by us from the original Figure 75, agree with our specimens: curved habitus, large body size (24 mm/1,000 μm), dorsally bent tail and cuticular vesicle; the buccal capsule 20-34 μm ; the oesophagus is thick (100 μm), with a thick bulb (185 μm), which corresponds to half the body width at that level (380 μm). The oesophagus is 1,225 μm long (one specimen), slightly longer than in our specimens; this single difference may result from differences in the techniques of fixation and measurement. Specimens 316 HS and 342 HS from *C. (T.) johnstoni* are thus identified as *R. chamaeleonis*. Females 311 and 312 HS have similar morphology and belong to this species.

The specimens found in *C. (T.) boehnelii*, 392 HS and 434 HS, had similar measurements (Table II) and shape (Fig. 2 J-L) to those from *C. (T.) johnstoni*, except that the beginning of intestine of all specimens did not widen (Fig. 2J). The buccal capsule was often deeper at its oesophageal apex, which formed a 'shoulder' instead being flat, and the corpus was a little more dilated. In the absence of any clear-cut differences, the specimens from *C. (T.) boehnelii* are also identified as *R. chamaeleonis*.

RHABDIAS JARKI N. SP.

Type host: *Chamaeleo (Trioceros) johnstoni* Boulenger, 1901, adult male, number 317 HS.

Type locality: Kibira forest (3° 30' S, 29° 3' E), commune of Musigati (chief town Bubanza), Burundi, captured in January 2002.

Type material: female holotype 317 HS number 2; two female paratypes 317 HS (a fourth and fifth females were used for life cycle studies).

Specimens deposited in the Muséum National d'Histoire Naturelle, Paris.

The five females were gravid. One had perforated the pulmonary wall and was extracted by teasing the pulmonary tissue which had adhered to the worm.

Body folded dorsally or S-shaped; anterior region spirally twisted or bent at a right angle, its width considerably increased and was 475-600 μm at level of the end of oesophagus (Fig. 2A, B). Body length from 11.8 to 12.3 mm, width at mid-body from 500 to 600 μm . Cuticular vesicle of body present, not thick, beginning posterior to the buccal capsule, 135 μm from apex; caudal point inside or outside the vesicle.

Head (Fig. 3 A-E): four protuberances which bear the voluminous four submedian papillae which have each a terminal transparent cuticular apex, crossed by the sensory nerves; the apices of the papillae are below the summit of the protuberances, at level of the mouth and each papilla apex is bent towards the buccal cavity and overhangs it. The two lateral papillae are placed on two smaller lateral protuberances; the apices of their nerves are at the same level as those of the submedian papillae, on the edge of the buccal aperture. The amphids, with conspicuous channels, are far from the mouth and open at the level of the apices of the lateral papillae. Mouth with four small lobes, placed between the submedian protuberances. Buccal capsule with wall more or less triangular in longitudinal optical section. Buccal cavity triangular in transverse section. The ductal end of the dorsal oesophageal gland could be identified.

Oesophagus 1,380-1,500 μm , slender, 45-52 μm wide at mid-length, enlarged where in contact with the buccal capsule; slightly dilated corpus; bulb diameter 100-110 μm and body width at this level 475-600 μm (Table II). Intestine increases in diameter. Nerve ring 300 μm from apex; excretory pore and cells not identified. Tail 225 to 350 μm long; no dorsal bend at level of anus. Embryonated eggs (in lungs) 125-138/70-80 μm . Genital tract of holotype (Fig. 2C): vulva at midbody; anterior ovary 3,800 μm long, its apex 3,800 μm from the caudal point; initially folded in the shape of a Figure 8; the size of ovogonia and ovocytes increases regularly until, about 1,500 μm from the beginning of the ovary, a distinct zone is identified in the synapsis zone, composed of very small cells which contrast with the large ovocytes situated just before and after this band. Anterior oviduct 800 μm long, curving 3,400 μm from head; its anatomy could not be determined in detail. Posterior ovary 5,500 μm long, its apex 4,100 μm from head; posterior oviduct 800 μm long, curved and bent at 1,300 μm from the caudal point; the part of the oviduct which is close to the uterus is easily identified with its wall of regular, high cells.

Genital tract of the dissected female (Figs 4 & 6E): vulva 7.45 mm from apex; anterior ovary 5,500 μm long, its apex 3,400 μm from the caudal extremity; anterior oviduct 1,350 μm long, with one curve; it comprises a short part near the ovary, the lumen of which

contains three ovulae, and then a part the lumen of which is filled with a mass of spermatozoa, each 7 μm in diameter; the part near the uterus has a wall of regular, high cells (Fig. 4G-I). Posterior ovary 6,000 μm long, its apex 3,800 μm from the head; a band of small cells 7 μm in diameter is interspersed between the large ovocytes, 25-35 μm in diameter; this band is 1,650 μm from the beginning of the ovary. Posterior oviduct bends twice 1,600 μm from the tail tip; the part near the ovary has an almost empty lumen, except for a few spermatozoa the part near the uterus has typical, high wall cells (Fig. 4C-F).

Measurements of holotype: 12.15 mm long, 500 μm maximum wide, buccal capsule 13/42 μm ; oesophagus 1,400 μm long and 52 μm wide, with bulb 110 μm wide; vulva 6,100 μm from apex; tail 350 μm long.

• Taxonomic discussion

Specimens 317 HS are distinct from *R. chamaeleonis* in several obvious characters: the anterior region becomes very wide at the end of oesophagus, almost as wide as at the mid-body, and has a bend at the level of the oesophagus; the oesophagus itself is longer and mainly thinner, with the ratio of bulb/body diameters (at bulb level) about 1/5 instead of 1/2; the buccal capsule is shorter, and the detailed head anatomy is also different: well defined protuberances, amphids more distant from mouth, velum absent, transverse section of buccal cavity triangular and not suboctagonal. In addition, there are important differences in the genital tract: *i*) a band of small cells among the large ovocytes of the synapsis zone of a gonad is only observed in the batch 317 HS; spermatozoa could be elaborated in these bands, as shown by several detailed studies on other species (Schleip, 1911; Boveri, 1911; Goodey, 1924a; Dreyfus, 1937); *ii*) seminal receptacles and spermatozoa were identified only in 317 HS. These differences suggest different modes of reproduction – hermaphroditism in the present specimens and parthenogenesis in *R. chamaeleonis*. Specimens 317 HS are distinct from the parasites of Magalasy chameleons (Table II). *R. gemellipara* was described by Chabaud *et al.* (1961) from *Calumma parsonii* (Cuvier, 1824), then another sample from *Ca. brevicornis* (Günther, 1879) was provisionally identified as this species by Chabaud & Brygoo (1962). The buccal capsule and oesophagus measurements of these specimens are very different (Table II) and it is improbable that these two samples belong to the same species; our observations of diverse developmental stages of parasitic females in *R. chamaeleonis* showed that the anterior region of the worm (buccal capsule, oesophagus) does not grow significantly compared to the whole length (Table II). The original material of *R. gemellipara* differs from our specimens because it is smaller, probably not fully mature, with small buccal capsule and oesophagus, but with a long tail. The large

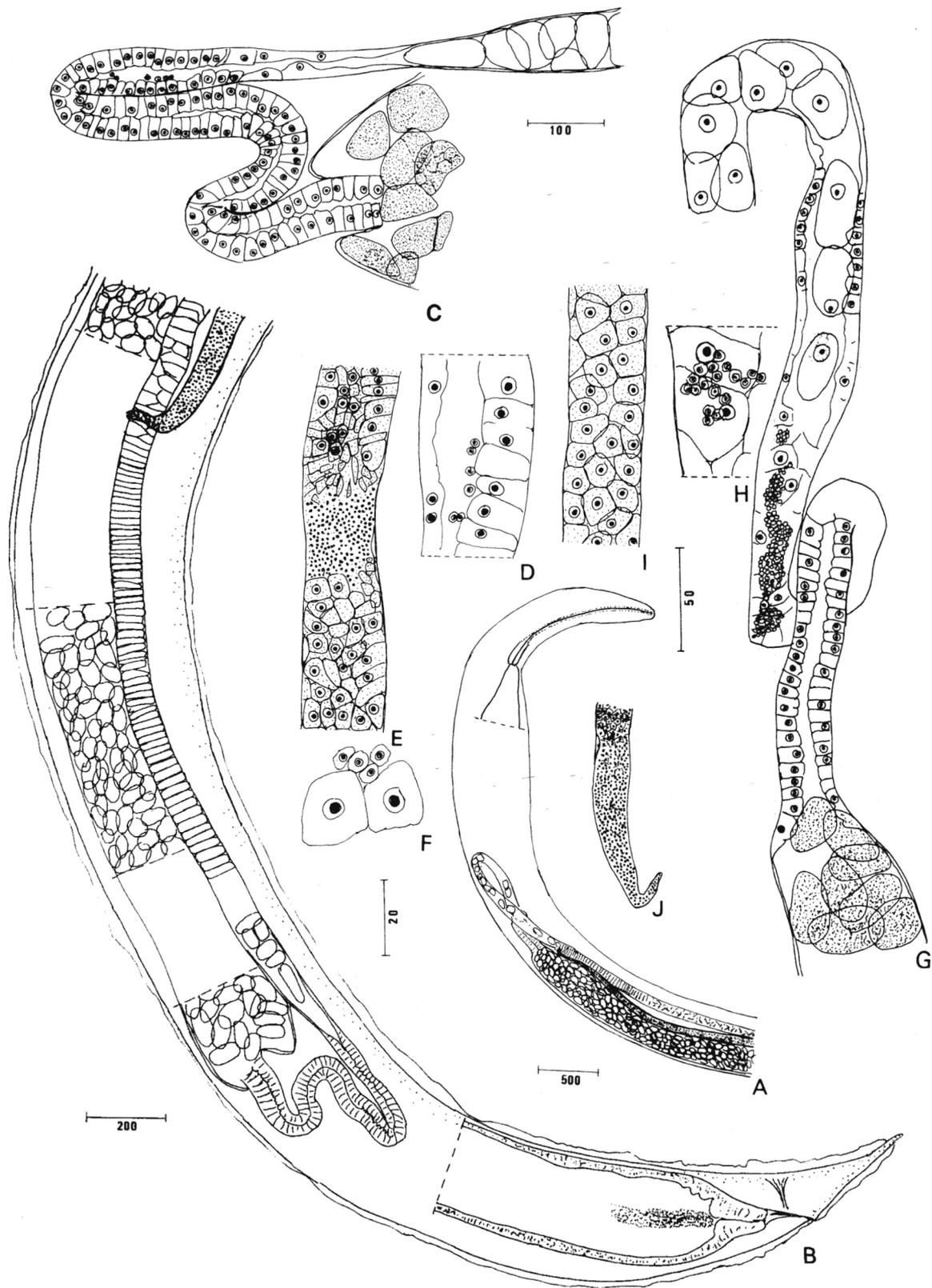


Fig. 4. – *R. jarki* n. sp., mature female paratype, 12.3 mm long. A. Anterior region with anterior oviduct and beginning of posterior ovary. B. Posterior region with posterior oviduct and beginning of anterior ovary with piled ovulae. C. End of posterior ovary, oviduct and beginning of uterus. D. Detail of this oviduct with six spermatozoa in the lumen. E. Synopsis zone of the posterior ovary 1,600 µm from its apex, with the transversal band of small genital cells contrasting with the voluminous ovocytes. F. Detail of this region. G. End of anterior ovary, oviduct with spermatozoa and beginning of uterus. H. Detail of the seminal vesicle wall and spermatozoa in the lumen. I. Anterior ovary, 1,600 µm from its apex, synopsis zone without small genital cells. J. Apex of anterior ovary. Scale bars: A, 500 µm; B, J, 200 µm; C, E, G, I, 100 µm; D, H, 50 µm; F, 20 µm.

specimens from *Ca. brevicornis* also differ from our material mainly by the great size of the buccal capsule (40-70 µm) and the long oesophagus (1,650 µm) (Chabaud & Brygoo, 1962).

The two species from other saurians differ from our specimens. *R. japalurae* Kuzmin, 2003, from agamids, has a more developed body vesicle with a swelling at level of oesophagus midlength, a greater ratio bulb diameter- body diameter (1/3, from Fig. 1C & E in Kuzmin, 2003, instead of 1/5), six small circumoral lips, a longer but narrower buccal capsule with thin wall. *R. anolis* Bursey, Goldberg & Tilford, 2003, from iguanids, is a small species with many distinct characters: no cuticular body vesicle, small buccal capsule (12/11 µm), short oesophagus (320 µm) with an anterior swelling, intestine wide at its beginning, tail much longer than wide, and two lateral caudal knobs (Bursey *et al.*, 2003). In addition undetermined rhabdias were mentioned in two previous reports: *i*) from *Anolis* spp., from Puerto Rico (Torres Ortiz, 1980); this material is likely close or similar to *R. anolis* parasitic in another *Anolis* species from Panama; *ii*) from *Japalura swinhonis* Günther, from Taiwan (Mang-hwa & Jun-yi, 1980); this japalura species was the second host of *R. japalurae* according to Kuzmin (2003) and was also from Taiwan.

The eight species from snakes differ from our material because they are very small, not exceeding 8 mm in length; these are *R. agkistrodonis* Sharpilo, 1976, redescribed by Kuzmin (1999), *R. elaphe* Sharpilo, 1976, close to the previous species, *R. eustreptos* (MacCallum, 1921) redescribed by Baker (1978), *R. fuscovenosa* (Railliet, 1899), redescribed by several authors (Goodey, 1924a; Chu, 1936a; Baker, 1978), *R. horigutii* (Yamaguti, 1943), *R. kurilensis* Sharpilo, 1976, *R. mar-*

inoi Kurochkin & Gus'kov, 1963, *R. vellardi* Pereira, 1928, *R. vibakari* Kuzmin, 1996.

Our specimens were also compared with the three species reported from batrachians in Africa. *R. bdellophis* is a very small species (5.1-5.6 mm) (Baylis, 1929); *R. bufonis* varies from 3.6 to 18.42 mm but the oesophagus is much shorter, 377-682 µm, than in our specimens (Hartwich, 1972); *R. africanus* also has a short oesophagus (Kuzmin, 2001).

Specimens 317 HS are a new species that we name *R. jarki* n. sp. to honour the Director of the Aenne and Feindt Foundation, Germany, which supported this work.

FREE-LIVING STAGES

- *Rhabdias chamaeleonis* from *C. (T.) johnstoni* and *C. (T.) boehnelii*

Twenty-two cultures were made using the 22 females of sample 342 HS. Numerous developing and moulting larvae and unfertilized females were observed; males were less frequent and were identified from the second day of culture. Recently fertilized females were not observed but the first gravid females were seen on the sixth day. Each contained one infective larva which itself was inside larval exuvium. An observation showed that the larval exuvium is composed of two larval moults: bacteria had partly destroyed the mother cuticle of one specimen, and the exposed larva, which was intact and alive, was easier to examine; two larval exuviae were identified at its extremities (Fig. 5N).

Morphology and measurements

The general anatomy (nerve ring, excretory pore, shape of oesophagus etc.) was like that of other *Rhabdias* species (Fig. 5 & Table III). The cephalic region

<i>Rhabdias</i> species	Male	Female	L3	n L3/F	Author
<i>chamaeleonis</i>	565/32/148 50/24/9	677/47/164 68	478/22/140 48	1	present study. 342 HS
<i>jarki</i> n. sp.	665/37/143 45/26/14	? ?	755/57/140 67.3	1	present study
<i>gemellipara</i>	810/32/165 62/32 & 17/x	1350/75/208 130	955/30/180 80	2	Chabaud <i>et al.</i> , 1961
<i>fuscovenosa</i>	homogonic		° 750/20/200 ° 66	×	Goodey, 1924
<i>agkistrodonis</i>	918/48/x 55/40/x	1144/65/139 84	* 847/31/170 88	1	Kuzmin, 1999
<i>elaphe</i>	768/41/113 48/36/x	1124/62/143 89	** 650/20/157 53	1	Kuzmin & Miskov, 1999
<i>vellardi</i>	720/34/150 50/35/12	1400/90/175 100	672/35/173 65	1	Pereira, 1928
<i>bufonis</i>	° 550/x/x x/x/x	° 650/54/? x	650/26/x x	2	Mecznikow, 1865

Upper values: length/width/oesophagus. Lower values: tail and, for male, /spicules/gubernaculum (means are reported, otherwise specified). ° Smaller dimensions or data obtained from Figures 5 & 6 of Goodey. * Homogonic infective larva not reported. ** Homo- and heterogonic infective larva are similar.

Table III. – Free living stages of *Rhabdias* spp. from reptiles and African amphibians.

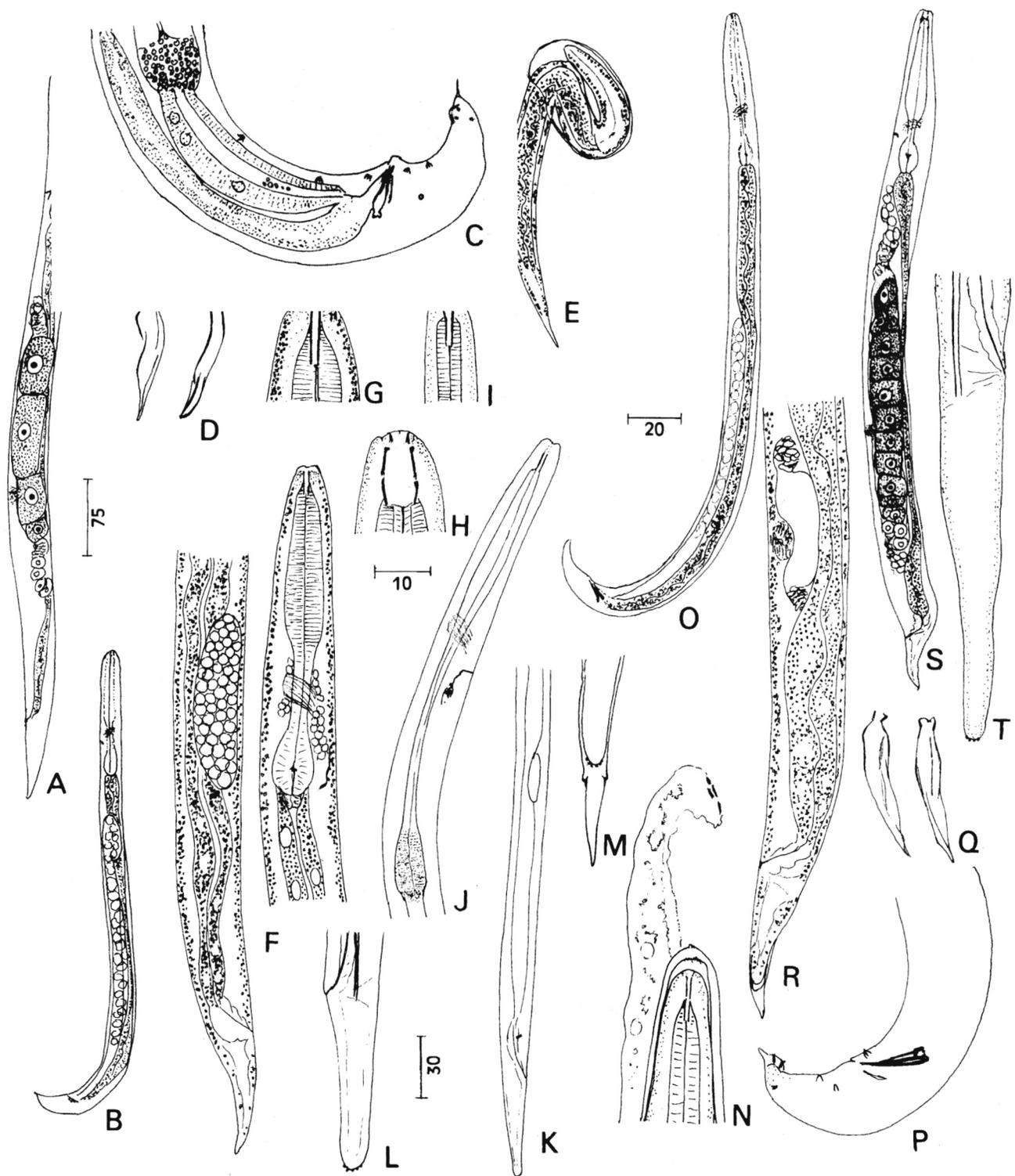


Fig. 5. – *Rhabdias* spp., free living stages. A to N, *R. chamaeleonis* (342 HS). A. Unfertilized female, left lateral view. B. Male, left lateral view. C. Tail of male, right lateral view. D. Left and right spicules. E. Embryonated egg from parasitic female, in culture. F. Hatching larva (in two parts). G. First stage larva, head. H. Female, head. I. Infective larva, head, lateral view. J. Exhsheathed infective larva, anterior region, left lateral view. K. *Idem*, posterior region, left lateral view. L. *Idem*, tail with beginning of right lateral ala. M. Tail extremity of infective larva within larval exuviae. N. Anterior end of infective larva with two larval exuviae, and maternal cuticle half destroyed by bacteria. O to Q, *R. jarki* n. sp. O. Male, left lateral view. P. Tail, left lateral view. Q. Left and right spicules. R to T, from unidentified parasitic females. R. Juvenile female. S. Unfertilized female 332 HS, with piled eggs. T. Tail of exsheathed infective larva 332 HS, provisionally identified as *R. jarki*. Scale bars: A, B, E, O, S, 75 µm; C, J, P, R, 20 µm; D, G, H, I, L, M, N, Q, T, 10 µm; F, K, 30 µm.

changed during development: stage 1 had an attenuated head, transparent and protuberant cuticular lips, each with a papilla, and a narrow buccal cavity; the adult stage had a wide buccal cavity; the infective larva had a depressed mouth, two median bosses, and a cylindrical or funnel-shaped buccal capsule (Fig. 5 G-I). The length of the buccal cavity and buccal capsule barely changed during development: buccal cavity 12 to 14 μm , buccal capsule 9 to 12 μm , composed of two segments in the female.

Male (342 HS, $n = 17$, from four cultures; Fig. 5B-D). 480-690 μm long and 28-45 μm wide; oesophagus 125-170 μm long, tail 45-58 μm long, regularly attenuated and often with remnants of larval cuticle attached and forming a transparent point; spicules similar in size, 17-31 μm (one abnormal specimen had shrunken spicules), gubernaculum 7-12 μm , usually about half the length of the spicules; subterminal short lateral alae; usually nine pairs of caudal papillae: three precloacal pairs, three pairs posterior to cloacal aperture (one pair lateral), three pairs anterior to tail tip (two pairs pedunculated on alae, one pair lateral). Anterior part of testis thick and curved; distance from testis curve to end of oesophagus equals twice the body width at this level.

Unfertilized females (342 HS; $n = 10$; Fig. 5A). 575-725 μm long, 40-50 μm wide, oesophagus 150-195 μm long, tail 60-75 μm long; two to four large ovulae in the uteri, usually 2-3, the larger ones in the anterior uterus. In young females the flexed ovaries and short oviducts are visible; they are later hidden by the expansion of the uteri.

Infective stage (342 HS; $n = 12$). Study of this stage is based on larvae from *in vitro* culture ($n = 9$) and exsheathed larvae from force-fed insects ($n = 3$; see below, transmission trials). Length with the maternal cuticle 550 to 720 μm ; without it, 360 to 590 μm (ranges from three parasitic females: 550-590; 360-432; 360-432; mean length $478.9 \pm 83.8 \mu\text{m}$); width 19-25 μm (mean $22.4 \pm 2.2 \mu\text{m}$); oesophagus 115-165 μm long, tail 35-63 μm long (mean $48 \pm 12 \mu\text{m}$). Maternal cuticle was smooth; the two larval exuviae had marked transverse striae and longitudinal crests, giving a chequered aspect (Fig. 6C); on the infective larva itself, only lateral alae were identified (Fig. 5L). Tail of larval exuvium pointed and with two notches in the posterior third (Fig. 5M); tail of infective stage with rounded extremity, decorated with 4-5 tiny buds (Fig. 5L).

No infective larvae were obtained from *C. (T.) boehnelii* 392 HS; the four males and four females studied had similar dimensions to those of 342 HS.

- *Rhabdias jarki* n. sp. from *C. (T.) johnstoni*

No infective larvae were obtained from the *in vitro* cultures of two females (317 HS). Molting larvae (day 2-3): 460-650 μm /25-40 μm . Females were rare (not mea-

sured). Two males were studied: 630 & 700 μm long, 40 & 35 μm wide, oesophagus 145 & 140 μm long, tail 40 & 49 μm long, tail extremity pointed; spicules similar 27 & 25 μm , gubernaculum 15 & 13 μm ; six pairs of caudal papillae identified: one precloacal pair, two pairs posterior to cloacal aperture (one pair lateral), two pairs near tail tip (one lateral pair, two pedunculated pairs on lateral alae) (Fig. 5P). Distance from testis curve to end of oesophagus is four times the body width at this level (Fig. 5O).

- *Rhabdias* spp.

Many *in vitro* cultures were prepared with unidentified rhabdias recovered from the *C. (T.) johnstoni*. Many infective larvae were obtained. All were inside the mother cuticle and had similar shapes and cuticular ornamentations; infective larval tail rounded with 4-5 cuticular buds. However, their sizes (maternal cuticle excluded) varied more than observed in 342 HS. In this respect the sample 332 HS was particularly interesting. Among the four *in vitro* larvae measured, three exceeded the maximum length of *R. chamaeleonis* being 700, 750 and 790 μm (without maternal cuticle). The sample 332 HS was also used to force-feed a chameleon (441 HS) which died two hours later (see below, transmission trial) and 18 exsheathed infective larvae were recovered; eight were 700-900 μm long, eight from 460 to 580 μm , like *R. chamaeleonis*, and two were close to the maximum length of this species, 620-630 μm . We have to conclude that the chameleon 332 HS harboured a mixed infection of two species at least; one was very probably *R. chamaeleonis*, the other could be *R. jarki* or an as yet unidentified species. We provisionally identified the large larvae as *R. jarki* n. sp.; their mean length is $755.4 \pm 61.4 \mu\text{m}$; their tail is 63-74 μm long with a round extremity, decorated with 4-5 buds (Fig. 5T).

We also noted that, of the two unfertilized females of 332 HS, one had 5-7 large ovulae instead of 2-4, and they were wider than long in this specimen (Fig. 5S). The measurements of the females were similar: 620 & 730 μm long, 60 & 63 μm wide, oesophagus 165 & 160 μm .

TRANSMISSION TRIALS

- Behaviour of infective larvae

Infective larvae remained on the bottom of the Petri dishes and did not move. If they were mechanically excited with a thin hair from a brush, they undulated and could migrate; the activity lasted a brief time, not exceeding five minutes. When the tail extremity or the foot of *C. (T.) johnstoni* was immersed in a small Petri dish containing infective larvae, these were neither excited nor attracted towards the animal's skin. When infective larvae were placed on a slide with a small

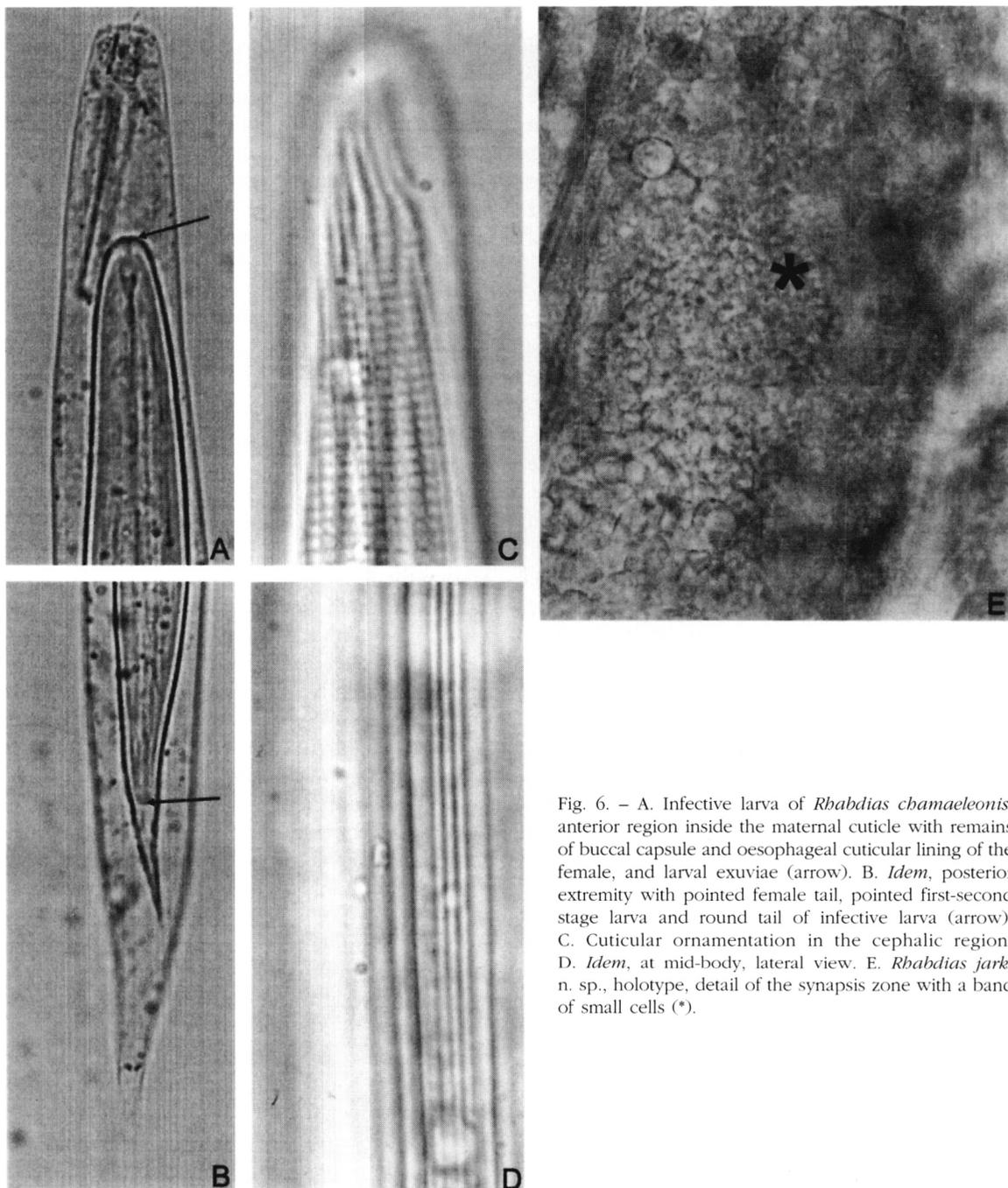


Fig. 6. – A. Infective larva of *Rhabdias chamaeleonis*, anterior region inside the maternal cuticle with remains of buccal capsule and oesophageal cuticular lining of the female, and larval exuviae (arrow). B. *Idem*, posterior extremity with pointed female tail, pointed first-second stage larva and round tail of infective larva (arrow). C. Cuticular ornamentation in the cephalic region. D. *Idem*, at mid-body, lateral view. E. *Rhabdias jarki* n. sp., holotype, detail of the synapsis zone with a band of small cells (*).

slug or snail they became motile but it seemed that this was passive movement induced by the displacement of the mollusc's foot.

Larvae survived a long time in the culture medium: those observed at three months were alive and still inside the maternal and larval cuticles. However, infective larvae were unable to resist desiccation.

- Attempts to infect chameleons with *Rhabdias* sp. and *R. chamaeleonis*

Two moribund *C. (T.) johnstoni* were force-fed. The first, 336 HS, received 20 larvae from 331 HS and 332

HS cultures of *Rhabdias* sp. It died four hours later and was necropsied 12 hours later; 11 larvae were recovered, all dead and inside their sheath; 10 were in the stomach (lumen, mucosa, wall), and one in the intestine. The second chameleon, 441 HS, received about 50 larvae from sample 332 HS; it died 2.5 hours later and was immediately dissected; 18 larvae were recovered, all from the stomach; they had lost the maternal and larval cuticles and were very motile.

One healthy *C. (T.) johnstoni* (344 HS) was force-fed with 50 larvae of *R. chamaeleonis* (sample 342 HS). It was killed 12 days later and thoroughly teased out but

no *Rhabdias* larva was recovered. At necropsy, it was found that 344 HS had a natural infection with *Rhabdias* sp., probably a third species (see above and Table I).

No larva was recovered in the liquid used to flush the coelomic cavity or from the lungs of all three animals.

- Attempts to infect terrestrial molluscs and insects with *R. chamaeleonis*

Eight crickets *A. domestica* and three locusts *S. gregaria* were force-ed with 25 to 50 *R. chamaeleonis* larvae (sample 342 HS). Crickets were dissected from day 1 to day 12 p.i.; two, dissected respectively two and five days p.i., had one larva each; both were alive and exsheathed and in the coelomic cavity. Locusts were dissected on D7-D8 p.i.; one had two larvae, one dead and still inside the sheaths and the other alive, motile, exsheathed; the location of these two larvae was not determined (they were found free in the Petri dish containing the gut).

No rhabdias larvae were recovered from one slug and two snails dissected from D1 to D5 following one hour contact with 10-13 infective larvae from sample 342 HS.

DISCUSSION

Rhabdias chamaeleonis has been reported by Baylis (1937) from several East African chameleons, *Bradypodion fischeri multituberculatus* Nieden, 1913, *C. (T.) bitaeniatus bitaeniatus* Fischer, 1884, *C. (T.) goetzi* (Tornier, 1899), *C. (T.) fuelleborni* Tornier, 1900, *C. (T.) tempeli* Tornier, 1889, and *C. (T.) boehnelii*. We identified *R. chamaeleonis* in this last species and in another *Trioceros*, *C. (T.) johnstoni*. However we have shown that at least one other species of *Rhabdias* is present in East African chameleons. Unexpectedly, the new species, *R. jarki* n. sp., was found in *C. (T.) johnstoni*, as well as the single unidentified female 344 HS.

The genital anatomy of the parasitic females of *R. chamaeleonis* and *R. jarki* differed, suggesting different modes of reproduction, parthenogenesis and hermaphroditism respectively. In *R. jarki* n. sp., a band of small cells in the synapsis zone was present on the anterior or the posterior ovaries, depending on the specimen; these small cells were thought to produce spermatozoa, like in some other rhabdias (Dreyfus, 1937). This suggested that the production of spermatozoa would alternate between the two ovaries, an hypothesis proposed by Schleip (1911) and Goodey (1924a) for some rhabdias of frogs and snakes.

The free-living cycle was elucidated for *R. chamaeleonis* and partially for *R. jarki*. Both species were heterogonic, like *R. gemellipara* in Madagascar (Chabaud

et al., 1961). The infective larvae of *R. chamaeleonis* developed with matricidal endotoky, each female producing a single larva, instead of two as in *R. gemellipara*. In addition, during this study, we had the opportunity to study the larval cuticle of the infective stage: two larval exuviae were present around the larva, showing that it is a third stage.

In the present study the infected chameleon species were those which live in cool and humid mountainous areas. This is consistent with the fact that the infective larvae of their rhabdias do not resist desiccation. They were able to survive in the coelomic cavity of insects, after having been ingested. However, a direct cycle would probably be successful, as demonstrated with *R. gemellipara* by Chabaud *et al.* (1961), following oral ingestion of infective larvae. Indeed we observed that they are rapidly exsheathed in the stomach of the recipient chameleon and become very motile. How they migrate to the lungs was not elucidated; no parasites were found in the coelomic cavity, unlike the species from amphibians (Baker, 1979b). Migrating larvae might be in the tissues and laceration could be necessary to release them, as in *R. fuscovenosa* (*cf.* Goodey, 1924a).

CONCLUSION

Rhabdias have a restricted distribution in reptiles which seems correlated to the constraints of humid habitats to complete their life cycles (Torres Ortiz, 1980). Several species have been identified in ophidians, mainly Colubridae, and a few Crotalidae and Viperidae. Several species have also been found in saurians, mainly chameleonids, and a few agamids and iguanids.

The parasites from ophidians are very small, with a short oesophagus and large excretory cells. The parasitic females are hermaphroditic, with a homogonic free-living cycle (Railliet, 1899; Goodey, 1924a; Baker, 1979a) which is obligatory, or facultative and associated with heterogony (Chu, 1936b; Kuzmin, 1999; Kuzmin & Miskov, 1999; Kuzmin, 2000). Their infective larvae migrate actively: Goodey (1924a) described the negative geotropism in *R. fuscovenosa*, where the infective larvae wave the anterior end, with the tail resting on soil particles. The behaviour suggests skin penetration of the host; however snakes cannot be infected that way (Chu, 1936b). A direct cycle succeeded experimentally (Chu, 1936b) but, in natural conditions, a vertebrate is likely needed as a transport host (Goodey, 1924a; Fuelleborn, 1928).

The rhabdias from chameleonids are large, with a long oesophagus and inconspicuous excretory cells. The parasitic females are hermaphroditic or parthenoge-

netic. The free-living cycle is heterogonic; the infective larva is nonmotile and can be transmitted orally. However it cannot resist desiccation and insects are required to complete the cycle in the natural environment.

The two species from Old World agamids and New World iguanids differ greatly from each other, the first resembling the *Rhabdias* spp. from chameleons. This is of particular interest since the origin of chameleons is controversial, arising either from agamids or from the Malagasy group of iguanids (Renous, 1982).

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