

RHABDIAS (NEMATODA: RHABDIASIDAE) FROM CHAMAELEONIDAE (SAURIA): TWO NEW SPECIES FROM *TRIOCEROS ELLIOTI* IN EAST AFRICA AND ONE FROM *BROOKESIA SUPERCILIARIS* IN MADAGASCAR

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Summary:

Rhabdias casiraghii n. sp. and *R. kibiraensis* n. sp. are described from *Trioceros ellioti* from Burundi and co-infection was observed in one of the host specimens. Distinctive characters between these species are, among others, the mouth and buccal capsule in front view, both of which are round in the former and laterally flattened in the latter species. Both species have a complete set of submedian head papillae (three in each submedian axis) as observed in the fourth stage larva of *R. americanus* from anurans. This primitive character opposes them to other species parasitic in Chamaeleonidae that have a single papilla per axis. The third species is the first described from the primitive chamaeleonid genus *Brookesia*; *R. brygooi* n. sp. from *B. superciliaris* from Madagascar can be distinguished from other *Rhabdias* in Chamaeleonidae by the small diameter of its mouth and buccal capsule. In this character, it resembles parasites from anurans. However, its infective larva has a rounded caudal extremity ornated with buds, as described in species of *Rhabdias* parasitic in Chamaeleonidae.

KEY WORDS: biology, chameleons, 12S rDNA, infective larva, morphology, *Rhabdias brygooi* n. sp., *Rhabdias casiraghii* n. sp., *Rhabdias kibiraensis* n. sp.

Résumé : RHABDIAS (NEMATODA : RHABDIASIDAE) PARASITES DE CHAMAELEONIDAE (SAURIA) : DEUX NOUVELLES ESPÈCES CHEZ *TRIOCEROS ELLIOTI* EN AFRIQUE ORIENTALE ET UNE CHEZ *BROOKESIA SUPERCILIARIS* À MADAGASCAR

Rhabdias casiraghii n. sp. et *R. kibiraensis* n. sp. sont décrits chez *Trioceros ellioti* au Burundi, et la co-infection a été observée chez un spécimen hôte. Les caractères distinctifs entre les deux espèces sont, entre autres, la bouche et la capsule buccale qui, en vue apicale, sont soit rondes soit aplaties latéralement. Les deux espèces ont un nombre complet de papilles submédianes (trois dans chaque axe submédian), comme chez le quatrième stade larvaire de *R. americanus*. Ce caractère primitif les oppose aux autres parasites de Chamaeleonidae qui n'ont qu'une papille dans chaque axe. La troisième espèce est la première décrite chez le genre primitif de caméléonidé *Brookesia*; *R. brygooi* n. sp. chez *B. superciliaris* à Madagascar est distinct des autres espèces parasites de Chamaeleonidae par le petit diamètre de la bouche et de la capsule buccale; par ce caractère, cette espèce ressemble aux parasites d'anoures. Cependant, sa larve infectante a une extrémité caudale arrondie et ornée de quelques petits bourgeons, comme cela est décrit chez les espèces de *Rhabdias* parasites de Chamaeleonidae.

MOTS CLÉS : biologie, caméléon, 12S rDNA, larve infectante, morphologie, *Rhabdias brygooi* n. sp., *Rhabdias casiraghii* n. sp., *Rhabdias kibiraensis* n. sp.

INTRODUCTION

Historically, only two species of *Rhabdias* Stiles & Hassal, 1905 had been reported from the lungs of chamaeleonids, *R. chamaeleonis* (Skrjabin, 1916) from Africa and *R. gemellipara* Chabaud, Brygoo & Petter, 1961 from Madagascar. In recent years,

however, several species of *Rhabdias* have been described from the Afrotropic region (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino *et al.*, 2008; 2009a, b). In Africa, *R. jarki* Lhermitte-Vallarino & Bain, 2004 originates from mountains in Burundi, the same region *R. chamaeleonis* was described from; *R. okuensis* Lhermitte-Vallarino & Bain, 2008, *R. cristati* Lhermitte-Vallarino & Bain, 2008, as well as *R. rhampoleonis* Lhermitte-Vallarino & Bain, 2009, were found in the Cameroonese volcanic chain. *Rhabdias mariauxi* Lhermitte-Vallarino & Bain, 2009 originates from Tanzania. The two last species parasitise members of the so-called leaf chameleons and were found in a single species each of *Rhampoleon* Günther, 1874 and a species of *Rieppeleon* Matthee, Tilbury & Townsend, 2004. The remaining *Rhabdias* species using chameleon hosts in Africa were recovered from *Trioceros* Swainson, 1839, a former subgenus of *Chamaeleo* Laurenti, 1768, recently elevated to generic level (Tilbury & Tolley, 2009). In Madagascar, the species described recently are

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R. rabetafikae Lhermitte-Vallarino, Junker & Bain, 2009, *R. brevicorne* Lhermitte-Vallarino, Junker & Bain, 2009, *R. nasutum* Lhermitte-Vallarino, Junker & Bain, 2009, and *Rhabdias* sp. Lhermitte-Vallarino, Junker & Bain, 2009, all of which parasitise the Malagasy chamaeleonid genus *Calumma* Gray, 1865.

The three new species described herein are reported from hosts that had not previously been investigated, namely *Trioceros ellioti* Günther, 1895 from East Africa and *Brookesia superciliaris* (Kuhl, 1820) from Madagascar. *Trioceros* and *Brookesia* occupy different positions in the monophyletic Chamaeleonidae. *Trioceros* exhibits many derived characters, whereas *Brookesia* Gray, 1864 takes up a basal position in phylogenies constructed using classical as well as molecular analyses (Klaver & Böhme, 1986; Tilbury & Tolley, 2009).

An integrative taxonomic approach combining morphology and molecular analysis was possible for one of the new *Rhabdias* spp., of which specimens were recovered alive. Free-living stages of the *Rhabdias* species parasitising *B. superciliaris*, which was maintained in captivity for about a month, could be obtained for further comparative studies.

The purpose of this paper was not only to describe the new species and in doing so to increase our knowledge on the diversity of *Rhabdias* in general, but also to highlight the following points: (i) a single host specimen may harbour more than one *Rhabdias* species at a time; (ii) a primitive pattern of head papillae with four submedian groups of three sensillae each is present in the two species from *T. ellioti*. This is in contrast with species from previously studied chamaeleonids, where a single papilla was identified in each submedian position; (iii) the small mouth and small buccal capsule diameter sets the parasite from *Brookesia* apart from other *Rhabdias* species of similar body width parasitic in Chamaeleonidae; such a character is, however, shared with the great majority of species from anurans (Martínez-Salazar & León-Règagnon, 2007).

MATERIALS AND METHODS

The specimens of *T. ellioti* from Burundi were legally imported into France in 2003 by a dealer, who donated dying or recently dead specimens to the Muséum National d'Histoire Naturelle (MNHN), Paris, France. The specimens of *B. superciliaris* were imported into France by the same dealer in January 2008, subsequently acquired by the MNHN research unit of R. B. and identified by the latter. Their exact geographic origin is unknown, but the species occurs in the Eastern and North-Eastern regions of Madagascar (Glaw & Vences, 2007). Specimens of *B. superciliaris* were kept alive for several weeks, their fresh faeces

collected and observed for the presence of *Rhabdias* first-stage larvae. Ultimately, carcasses were fixed in 10 % formalin for identification and comparative anatomical studies.

Generic names of chamaeleonids follow Tilbury & Tolley (2009). Authority names of the new species are Lhermitte-Vallarino, Barbuto & Bain.

MORPHOLOGICAL ANALYSIS

Worms were fixed and stored in 70 % ethanol and cleared in lactophenol for identification. Morphological studies and measurements were performed as described in previous studies (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino *et al.*, 2008; 2009a, b). Where necessary, two values are listed for buccal capsule length: length of anterior segment and, in brackets, total length including the posterior segment (Fig. 1); two buccal capsule ratios were calculated: length of anterior segment/external diameter, and total length of buccal capsule/external diameter. Measurements are given in micrometres, unless otherwise stated. Ovaries were examined for the presence of a testis zone (Runey *et al.*, 1978; Junker *et al.*, 2010).

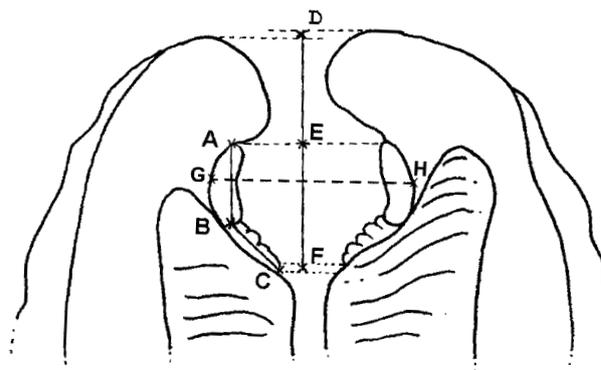


Fig. 1. – Schematic representation of anterior extremity of *Rhabdias*. Distance AB, length of anterior segment of buccal capsule; distance BC, length of posterior segment of buccal capsule; distance EF, total length of buccal capsule (= depth); distance GH, maximum width of buccal capsule (= external diameter); distance DE, vestibulum depth.

MOLECULAR ANALYSIS

Biological material analysed in this study was stored according to procedures specified in the Biorepositories initiative (<http://www.biorepositories.org>) and belongs to the collection identified as 'zpl' of Milano Bicocca institution (MIB). For molecular analysis, freshly recovered worms were transferred directly into absolute ethanol and stored at 4 °C. DNA extract from one specimen (MIB:zpl:00233) was prepared using the 5 PRIME, ArchivePure DNA Purification Kit. The 12S rDNA gene sequence was generated following Casiraghi *et al.* (2004). The sequence obtained is about 500 bp long. PCR products were gel-purified using the 5 PRIME,

GelElute Extraction Kit, and directly sequenced using ABI technology. The obtained sequence has been deposited in the EMBL Data Library under the accession number FN 557014.

STUDIES ON THE BIOLOGY

Life-cycle studies of *Rhabdias* from *B. superciliaris* were based on petri dish cultures of faeces in distilled water to which active charcoal had been added (Lhermitte-Vallarino & Bain, 2004). Cultures were kept at approximately room temperature and checked regularly for developing stages for several days.

RESULTS

Two new species were identified from *T. ellioti* and co-infection occurred in one of two host specimens. A single new species was described from *B. superciliaris*.

RHABDIAS CASIRAGHII N. SP. LHERMITTE-VALLARINO, BARBUTO & BAIN
(Figs 2, 3A-D; Table 1)

Type host: *Trioceros ellioti* Günther, 1895, a gravid female 39 CE; collected in June 2003.

Type locality: Kibira forest, at about 1,700 metres of altitude, Commune of Musigati, Bubanza Province, Burundi (3° 30' S, 29° 3' E).

Location in host: lungs.

Type material: seven worms recovered. Female holotype 39 CE; six female paratypes 39 CE. All deposited in the MNHN collection of zooparasitic nematodes.

Additional material: a single female 210 CE, recovered from a *T. ellioti* from the same geographic area (this *T. ellioti* specimen concurrently infected with a second species of *Rhabdias*).

Etymology: the species is dedicated to our colleague Dr M. Casiraghi, Università degli Studi Milano Bicocca, Italy.

Morphology

Body dorsally bent and posterior region spirally coiled (Fig. 2B); in three specimens, including holotype, body constriction present at level of anterior bend of genital tract (Fig. 2C). Vesicle present along entire body; more pronounced from head to end of oesophagus; in this region, approximately five to eight, deep transverse furrows; in lateral view, furrows attached to body by particular structures; structures apparently consist of a vacuole equipped with a single pore (Fig. 2F); pores aligned on edge of lateral hypodermal chord; pores often associated with secretory products. In posterior region, vesicle well developed, without additional

structures. Excretory pore posterior to nerve ring; excretory chamber and wide excretory channels containing irregular masses of a coagulated substance (Fig. 2K).

Head (Fig. 3A-D): six well-developed papillae arranged in a circle, their stems crossing the thick transparent cuticle: (i) four bulging submedian papillae, each with thick recurrent stem and transparent nipple with salient sensilla, situated anterior to velum of mouth aperture; in addition, two bright points visible on bulging part of each submedian papilla, these interpreted as externolabial and cephalic papillae (Fig. 3B); (ii) Two slightly thinner lateral papillae, each with salient sensilla. Amphids not identified. Mouth slightly depressed; velum subsphaerical, with two slight lateral notches (Fig. 3C). Buccal cavity rather flat, cup-shaped in longitudinal view (Fig. 3A); buccal capsule thick-walled, suboctagonal posterior to velum in frontal view (Fig. 3C) then round (Fig. 3D); posterior segment present, but separation from anterior segment more or less distinct (Fig. 3A). Oesophagus: shoulders present, glandular secretions seen in dorsal one; muscular fibres conspicuous anterior to nerve ring; posterior to nerve ring, shape cylindrical, with or without slight inflation at mid-length; bulb shape varies depending on worm orientation: oval in lateral view, rhomboidal in median view, with fibres running from sides of bulb to lateral chords (Fig. 2D, E). Intestine: apex narrower than bulb diameter, long anterior part almost cylindrical; intestine pale and thick, two thirds of body width.

Genital tract: anterior bend at level of oviduct-ovary junction, its distance to head rather constant; posterior bend at variable distance from tail tip; oviducts tightly coiled to straight. In some specimens, ovulae reaching 400 in length near oviduct (Fig. 2A, C). Testis zone observed in one specimen, 500 long and 1,000 from ovary apex. Spermatozoa identified along ovaries between large ovocytes and ovulae (Fig. 2J), and in oviducts of almost all specimens (6/7); spermatozoa also found in distal part of uteri of some specimens (Fig. 2A). In all females, uteri thin, half filled, predominantly with hatched larvae; a few larvated eggs present in distal part of uteri and, in posterior part, a few ovulae and developing eggs. Pseudocoelomocytes identified, 120 × 90 (Fig. 2A, G, H): an anterior group of three to four cells and a posterior group of two to three cells, each near oviduct region. Vulva at mid-length. Tail relatively thin compared to vesicle; conical, usually straight; caudal tip straight or bent dorsally.

Taxonomic discussion

With the exception of two small species from Madagascar, *R. gemellipara* and *Rhabdias* sp., *Rhabdias* species from chamaeleonids are large (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino *et al.*, 2008; 2009a, b). The present

specimens resemble these large species in having a long (22-28 mm), rather thick (660-850) body combined with a wide buccal capsule (38-44). However, *R. chamaeleonis*, *R. cristati* and *R. rhampoleonis* are distinct in having a longer buccal capsule (≥ 20 vs 12). The remaining six species have a short buccal capsule, its anterior segment being 12-18 long; these are *R. jarki*, *R. okuensis*, *R. rabetafikae*, *R. nasutum*, *R. brevicorne* and *R. mariauxi* (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino *et al.*, 2008; 2009a, b). Their differences from the present material are outlined below.

Rhabdias jarki and *R. okuensis*, from two species of *Trioceros* in East and West Africa, respectively, have a thick-walled buccal capsule that is subtriangular in longitudinal section and lacks a posterior segment. Moreover, their oesophagus is longer (1,300-1,500), while their body is shorter (11-12 and 15-18 mm, respectively). The following three species are parasitic in *Calumma* spp. from Madagascar. *Rhabdias rabetafikae* has a buccal cavity that is cylindrical rather than cup-shaped, and the walls of its buccal capsule are thicker. Furthermore, its oesophagus is wider (135-150 at mid-length vs 65-90), as well as longer (1,350-1,500). The caudal vesicle of *R. nasutum* is thin, and its intestine widens rapidly from its apex onwards. *Rhabdias brevicorne* has a thicker body, cephalic pads, and a higher buccal capsule ratio (0.42 vs 0.23-0.31). In addition, the shape of its tail differs in being funnel-shaped, with an abruptly narrowing posterior half. *Rhabdias mariauxi* from *Rieppeleon brevicaudatus* (Matschies, 1892) in Tanzania has a dorso-ventrally flattened mouth and buccal capsule, a shorter body (13-15 mm) and shorter oesophagus (800-920).

Furthermore, all species from chamaeleonids can be distinguished from our material by the reduced number of submedian head papillae and the non-rhomboidal shape of their bulb.

We conclude that the material recovered from *T. ellioti* from Burundi represents a new species, for which we propose the name *R. casiraghii*.

One of four females collected from an additional *T. ellioti* (210 CE) from Kibira forest was similar to the present material in the size and shape of its buccal capsule (posterior segment distinct) and all other measurements. It was therefore assigned to *R. casiraghii* as well.

RHABDIAS KIBIRAENSIS N. SP. LHERMITTE-VALLARINO, BARBUTO & BAIN
(Figs 3E-H; 4; Table II)

Type host: *Trioceros ellioti* Günther, 1895, adult male 210 CE; collected in June 2003.

Type locality: Kibira forest, at about 1,700 metres of altitude, Commune of Musigati, Bubanza Province, Burundi (3° 30' S, 29° 3' E).

Location in host: lungs.

Type material: three worms recovered. Female holotype 210 CE; two female paratypes 210 CE. Deposited in MNHN collection of zooparasitic nematodes.

Etymology: the new species is named in reference to the type locality.

Morphology

Posterior region bent; body slightly thicker in posterior third (Fig. 4B). Vesicle: thick along entire body; no structures of attachment observed. Head: four large submedian lobes, hunched over laterally flattened vestibulum (Fig. 3E, F); each of the four submedian lobes (or lips) with deep terminal sensilla (Fig. 3F) and two papillae; papillae identified by their bright nerves; no papillae identified on two lateral lobes; lateral lobes with almost bifid internal edge; an additional small dorsal lobe present (Fig. 3F). Vestibulum deep. Velum not identified. Buccal cavity delineated by anterior segment, nearly tubular in longitudinal view (Fig. 3E); subtriangular and laterally flattened in frontal view (Fig. 3G), but triangular and slightly flattened laterally at level of posterior segment (Fig. 3H). Buccal capsule: composed of thick external wall and bright, thin internal layer; extending anteriorly from well-delineated and transversely striated posterior segment (Fig. 3E, G). Oesophagus: acute shoulders; grossly cylindrical with slight constriction at level of nerve ring; posteriorly, progressive increase in diameter to form a chianti bottle-shaped bulb, its maximum diameter near base. Intestine: apex slightly wider than base of bulb, rapidly increasing in diameter.

Genital tract: although specimens are well-preserved, ovaries largely indiscernable, locally ghostly (the same was observed in the *R. casiraghii* n. sp. female 210 CE from the same host); oviducts equally transparent, slightly more distinct near uterus only; genital bend at oviduct-uterus junction (Fig. 4E); uteri filled with larvated eggs, except near oviducts (twenty or so divided eggs counted at each extremity). No spermatozoa identified. Vulva slightly posterior to mid-body. Tail conical, straight, its tip straight or bent ventrally; refringent fine granules present in vesicle surrounding caudal tip (Fig. 4C, D).

Taxonomic discussion

Rhabdias kibiraensis n. sp. and *R. casiraghii* n. sp. share the arrangement of their head sensory organs in four submedian groups of three papillae each. However, *R. kibiraensis* n. sp. differs in having a buccal capsule with a distinct posterior segment and a particularly structured wall that consists of a thin internal and a thick external layer. Furthermore, both its mouth and buccal capsule, are laterally flattened.

In having a body that is moderately wide (510-600) and 11.40-13.35 mm long, the present specimens resemble

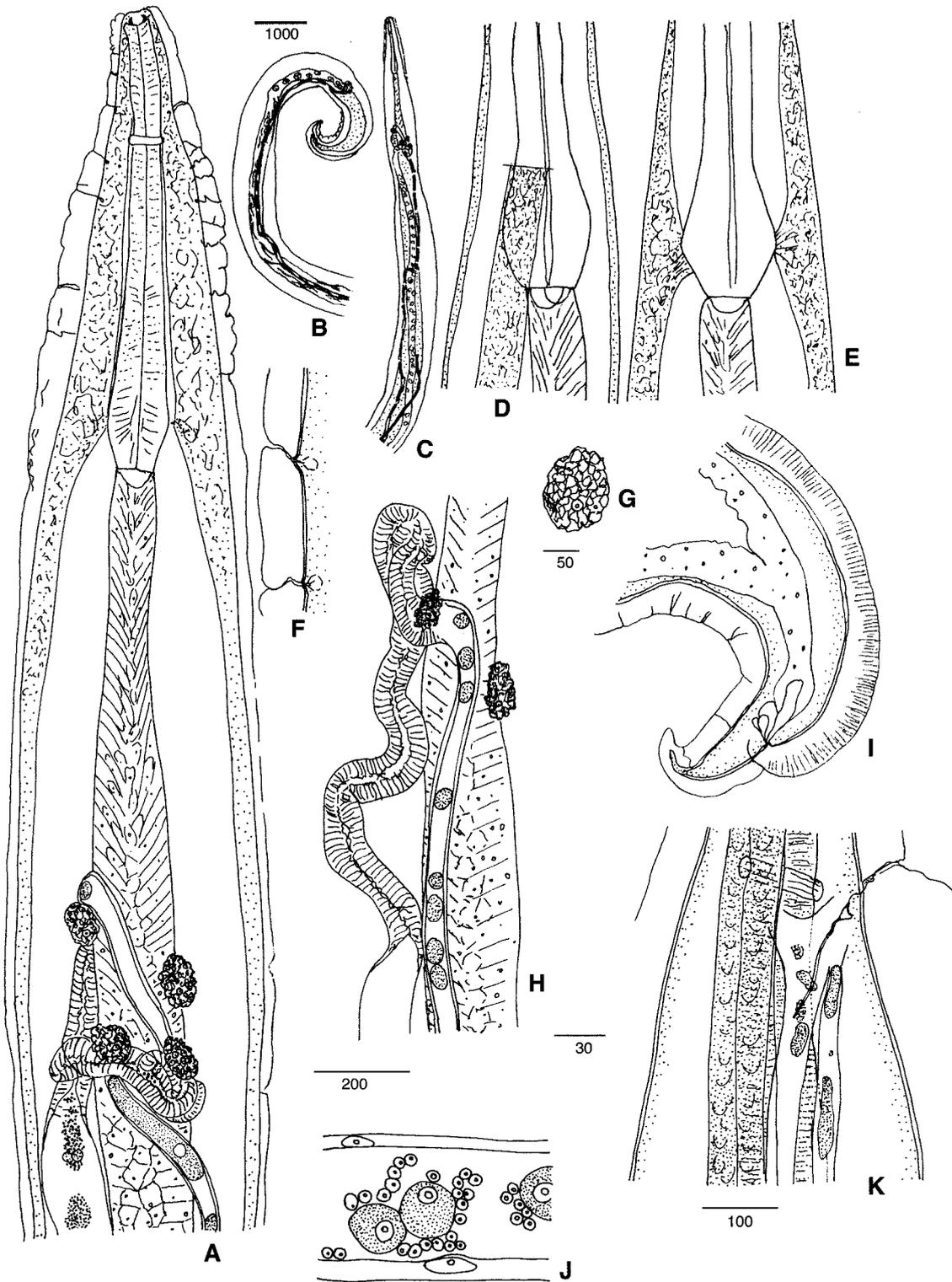


Fig. 2. – *Rhabdias casiraghii* n. sp. from *Trioceros ellioti*. A. Anterior region, dorso-ventral view, from head to genital tract bend showing ovary with elongated ovulae, tightly coiled oviduct and beginning of uterus; spermatozoa present in the two last parts; four coelomocytes present (holotype). B. Coiled habitus of posterior region (holotype). C. Habitus of anterior region, with body constriction posterior to genital bend level (holotype). D & E. Different shape of bulb in lateral and median view. F. Lateral pores at base of folds of anterior vesicle. G. A pseudocoelomocyte with central nucleus and cytoplasm filled with irregular granules. H. Anterior bend of genital tract, and ovary with few round ovulae; two of the four pseudocoelomocytes figured. I. Tail, right lateral view (holotype). J. Posterior ovary with spermatozoa and ovulae. K. Excretory cell and ducts, nerve ring and lateral chord, right lateral view. Scales in μm : A, D, E, H, I, 200; B, C, 1,000; F, G, 50; J, 300; K, 100.

Specimen	MNHN collection						39 CE						210 CE		
	Holotype	Paratype 1	Paratype 2	Paratype 3	Paratype 4	Paratype 5	Paratype 6	Range							
Length (mm)	22.25	25.65	26.30	26.22	25.7	28.2	26.25	22.25-28.2							
Width at mid-body (with vesicle)	800 (860)	850 (970)	800 (810)	800 (800)	800 (800)	800 (830)	660 (680)	660-850 (680-970)							850 (ND)
Vestibulum depth (vesicle excluded)	10	12	12	7	9	10	8	7-12							ND
Buccal capsule length: anterior segment (total)	12 (14)	12 (14)	12 (14)	12 (14)	10 (16)	12 (14)	10 (15)	10-12 (14-16)							12 (17)
Buccal capsule external diameter	38	42	44	43	43	40	40	38-44							42
Oesophagus length	1,055	1,200	1,170	1,155	1,110	1,175	1,110	1,055-1,200							1,220
Oesophagus width	65	78	80	85	90	75	80	65-90							110
Bulb diameter	150	190	180	210	190	180	180	150-210							165
Body width at bulb (with vesicle)	475 (570)	500 (750)	375 (500)	410 (500)	380 (700)	385 (518)	425 (500)	375-500 (500-750)							385 (410)
Vulva - Head (mm)	11.32	13.55	13.13	13.40	13.31	14.80	13.15	11.32-14.80							15.95
Anterior genital bend - head	2,150	2,000	2,100	2,825	2,100	3,100	2,100	2,100-3,100							2,500
Posterior genital bend - tip tail	1,350	1,500	2,900	2,700	1,300	2,150	1,680	1,300-2,900							2,100
Tail length	240	235	230	400	260	250	250	230-400							340
Width at anus (with vesicle)	95 (260)	100 (275)	120 (230)	145 (285)	130 (260)	180 (300)	100 (250)	95-180 (230-300)							180
Length of tail tip	65	90	70	85	55	90	100	55-100							ND
Nerve ring	285	285	290	275	290	315	300	275-315							320
Excretory pore	330	325	365	ND	340	380	ND	325-380							380
Habitus	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral							Spiral
Ratio buccal capsule length / diameter	0.37	0.29	0.27	0.27	0.23	0.30	0.25	0.23-0.31							0.28
Ratio buccal capsule total length / diameter	0.36	0.33	0.31	0.32	0.37	0.35	0.37	0.31-0.37							0.40
Ratio bulb width / body width at oesophagus end	0.31	0.38	0.48	0.51	0.50	0.52	0.42	0.31-0.52							0.23
Ratio bulb width / oesophagus width	2.30	2.43	2.25	2.47	2.11	2.4	2.25	2.11-2.47							1.50
Ratio head - vulva / body length	0.51	0.53	0.50	0.51	0.52	0.46	0.50	0.46-0.53							0.52
Bulb shape	rhomboidal	rhomboidal	rhomboidal	rhomboidal	rhomboidal	rhomboidal	rhomboidal	-							ND
Apex of intestine	thin	thin	thin	thin	thin	thin	thin	-							thin
Tail shape	s	s	d	s	s	s	s	-							s
Tail tip	d	s	s	s	d	d	d	-							s

ND: not determined; d: bent dorsally; s: straight; v: bent ventrally.

Table 1. – *Rhabdias casiraghi* n. sp. from *Tritoceros ellioti* Günther, 1895 from Burundi. All measurements in micrometres unless otherwise stated.

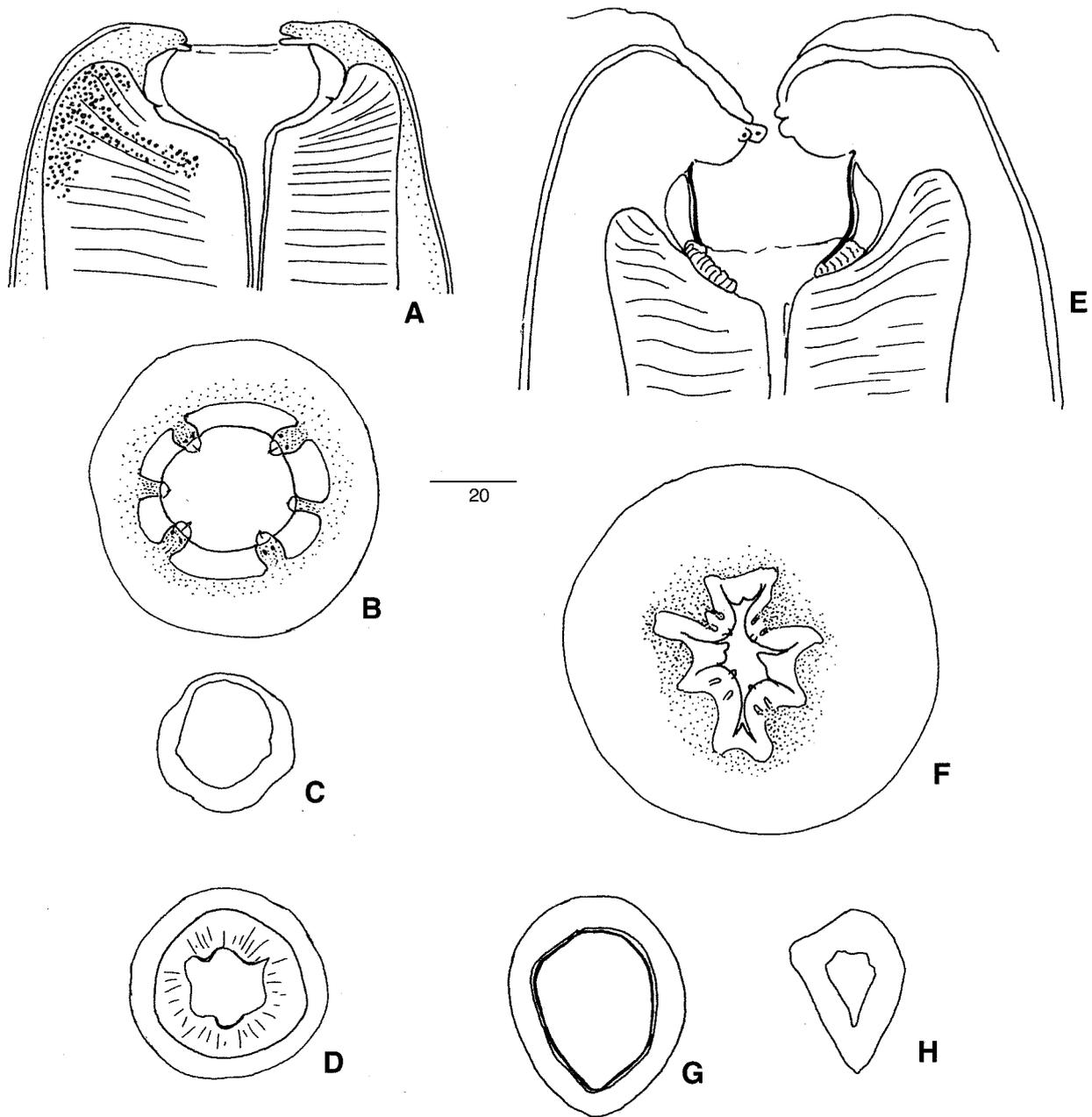


Fig. 3. – Head of *Rhabdias* n. spp. from *Trioceros ellioti*. A-D. *R. casiraghii* n. sp., a paratype. A. Right lateral view. B. Head papillae, apical view (velum not represented). C. Internal delination of velum with two lateral notches (inner line) and apex of buccal capsule (outer line), in front view. D. Transverse section of buccal capsule and, posteriorly, junction with oesophagus. E-H. *R. kibiraensis* n. sp., a paratype. E. Head, right lateral view. F. Head papillae, apical view. G. Buccal capsule, optical transverse section. H. Posterior segment, en face view. Scales in μm : A-H, 20.

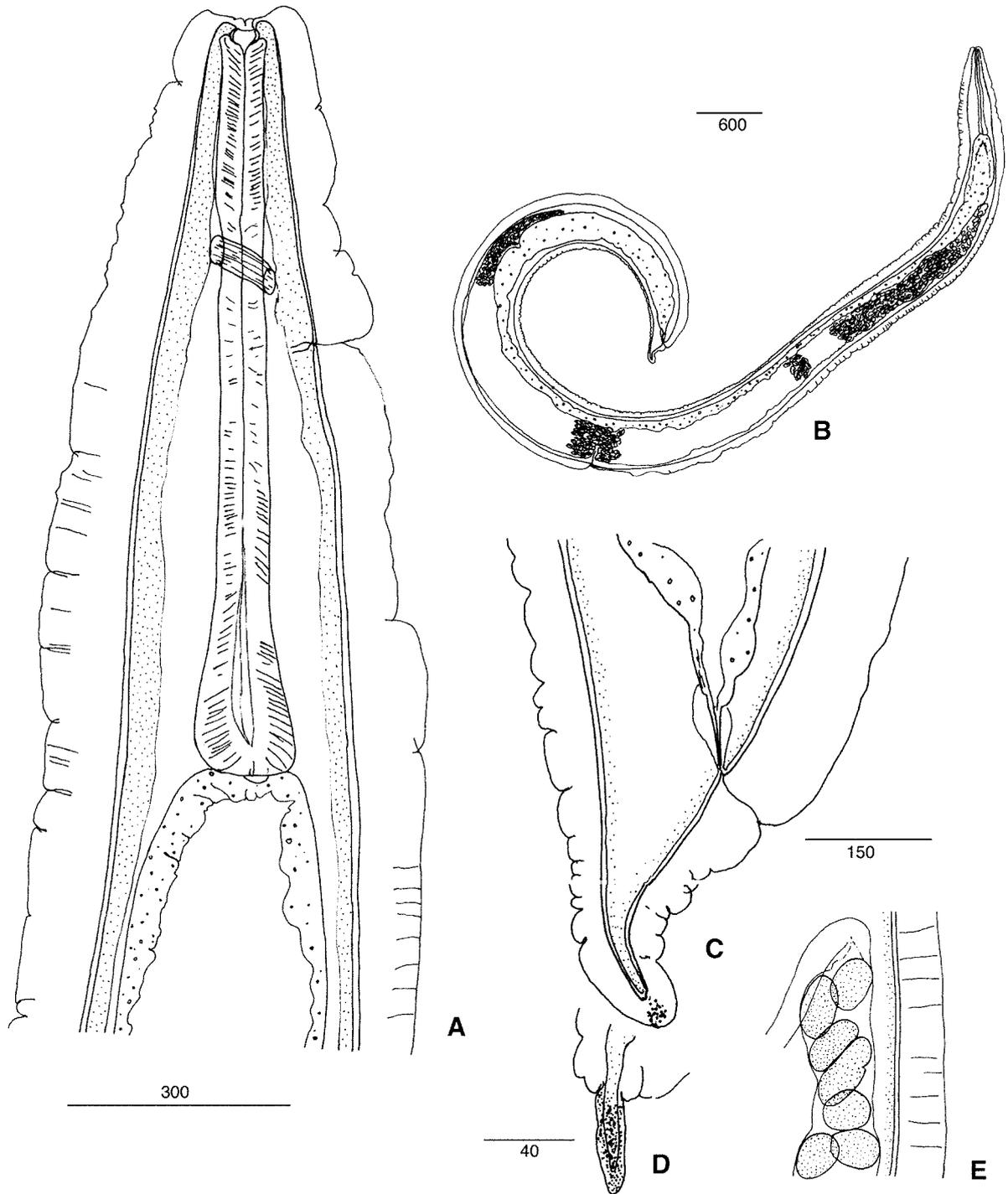


Fig. 4. – *Rhabdias kibiraensis* n. sp. from *Trioceros ellioti*, from Burundi. A. Anterior region, right lateral view (holotype). B. Habitus, right lateral view (holotype). C. Tail, right lateral view (holotype). D. Caudal extremity with refringent granules (a paratype). E. Anterior genital bend with transparent oviduct and beginning of uterus (body vesicle on right) (holotype). Scales in μm : A, E, 300; B, 600; C, 150; D, 40.

	<i>R. kibiraensis</i> n. sp.			<i>R. brygooi</i> n. sp.		
	210 CE	210 CE	214 CE	214 CE	214 CE	217 CE
MNHN collection number						
Specimen	Holotype	Paratype 1	Paratype 2	Holotype fixed	Holotype alive	Paratype fixed, except*
Length (mm)	12.30	11.40	13.35	13.8	9.6	8.65*
Width at mid-body (with vesicle)	540 (580)	510 (530)	600 (690)	480 (500)	600	410 (450)
Vestibulum depth (vesicle excluded)	21	23	15	15	ND	24
Buccal capsule length: anterior segment (total)	15 (22)	18 (26)	15 (22)	14 (20)	ND	14 (18)
Buccal capsule external diameter	41	44	42	25	ND	28
Oesophagus length	1,035	1,03	1,03	820	850	800
Oesophagus width at mid-length	65	80	62	100	ND	100
Bulb diameter	150	150	150	160	ND	180
Body width at bulb (with vesicle)	325 (520)	340 (430)	300 (490)	250 (275)	ND	280 (315)
Vulva - Head (mm)	7.00	6.30	7.07	7	5.15	4.45*
Anterior genital bend - head	2,380	2,480	1,750	1,750	ND	ND
Posterior genital bend - tip tail	1,600	2,200	2,250	1,600	ND	800
Tail length	300	270	270	340 (360)	400	250
Width at anus (with vesicle)	140 (200)	130 (210)	155 (280)	150 (165)	120 (150)	100 (130)
Length of tail tip	43	40	65	45	40	35
Nerve ring	325	350	340	280	ND	270
Excretory pore	400	415	440	ND	ND	350
Habitus, expressed as letter	J	J	J	I	S	C
Ratio buccal capsule length / diameter	0.36	0.40	0.35	0.56	ND	0.50
Ratio buccal capsule total length / diameter	0.53	0.59	0.52	0.80	ND	0.64
Ratio bulb width / body width at oesophagus end	0.46	0.44	0.44	0.64	ND	0.64
Ratio bulb width / oesophagus width	2.30	1.87	2.41	1.60	ND	1.80
Ratio head - vulva / body length	0.57	0.55	0.53	0.50	ND	0.51
Bulb shape	chianti bottle	chianti bottle	chianti bottle	oval	ND	oval
Apex of intestine	thick	thick	thick	thin	thin	thin
Tail shape	s	s	s	s	s	v
Tail tip	s	v	v	s	s	s

ND: not determined; s: straight; v: bent ventrally.

Table II. - *Rhabdias kibiraensis* n. sp. from *Trioceros ellioti* Günther, 1895 from Burundi, and *R. brygooi* n. sp. from *Brookesia superciliana* (Kuhl, 1820) from Madagascar. All measurements in micrometres unless otherwise stated.

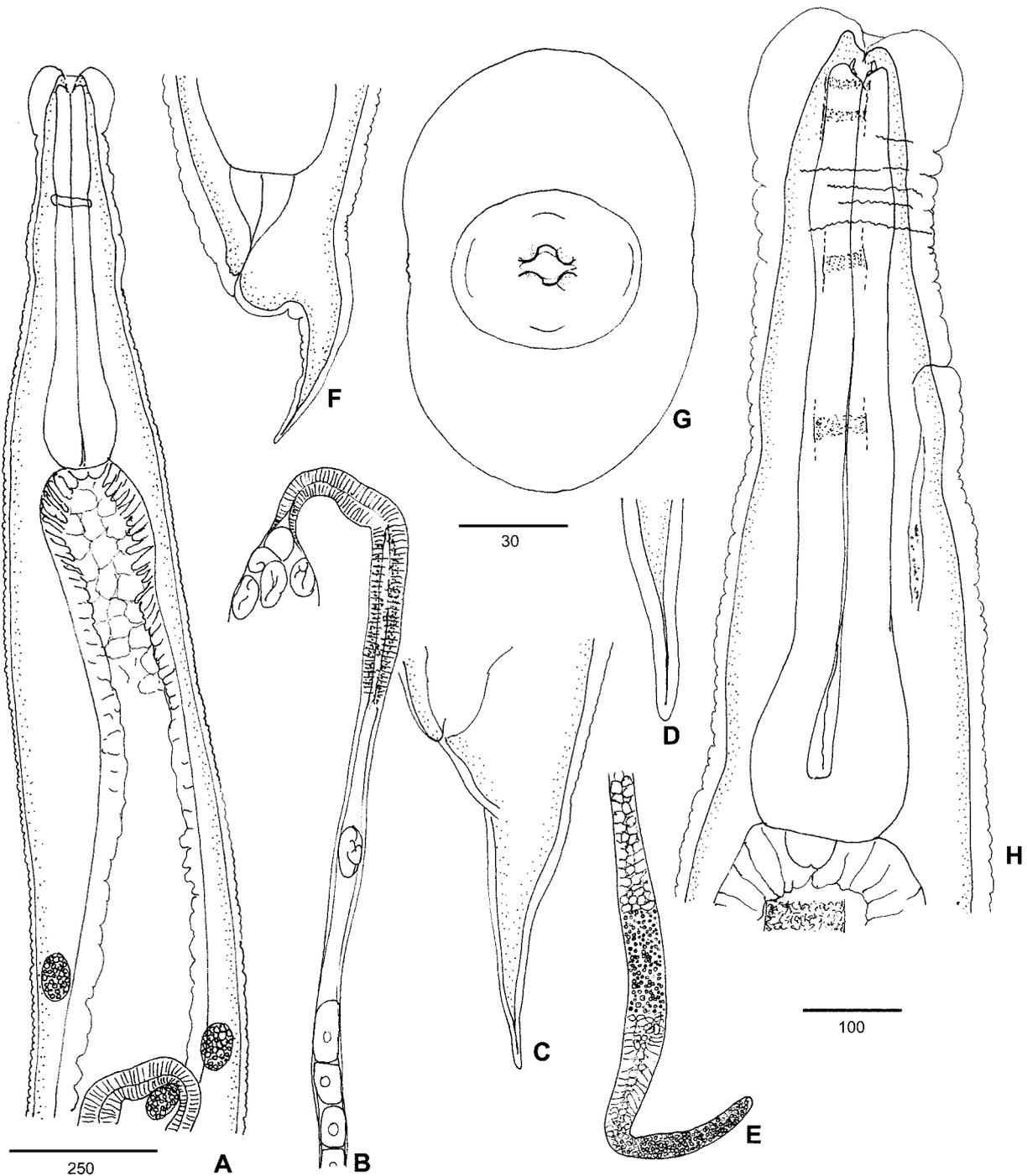


Fig. 5. – *Rhabdias brygooi* n. sp. from *Brookesia superciliaris*, from Madagascar. A-E. Holotype. A. Anterior region with pseudocoelomocytes and anterior bend of genital tract, dorso-ventral view. B. Anterior bend of genital tract with end of ovary, oviduct with a larva and spermatozoa, and beginning of uterus. C. Tail, left lateral view. D. Tail point. E. Testis zone in anterior ovary. F-H. Paratype. F. Caudal region, left lateral view. G. Head, in front view. H. Anterior region, right lateral view (lateral chord reported in five places from head to intestine level). Scales in μm : A, B, 250; C, E, F, H, 100; D, G, 30.

R. jarki collected at the same locality (Lhermitte-Vallarino & Bain, 2004). However, *R. jarki* has several distinct characters: a thin vesicle; the protuberances that bear the head papillae are well spaced from one another; the buccal capsule is not flattened laterally; the buccal capsule wall is more or less triangular in longitudinal optical section and a distinct posterior segment is absent. Furthermore, the oesophagus is longer (1,380-1,500 *vs* 1,030-1,035) and its bulb is not chianti bottle-shaped. Of all other species of *Rhabdias* parasitic in chamaeleonids only one, *Rhabdias* sp. from *Calumma brevicorne* in Madagascar, has a laterally flattened mouth. Specimens of *Rhabdias* sp. were not fully mature and measured 6-7 mm in length. They were smaller than the present specimens with respect to all other measurements as well. While having a distinct posterior segment like *R. kibiraensis* n. sp., the wall of the anterior segment of the buccal capsule of *Rhabdias* sp. has a simple structure (Lhermitte-Vallarino *et al.*, 2009b). We consider the specimens from *T. ellioti* from Burundi a new species, for which we propose the name *R. kibiraensis*.

RHABDIAS BRYGOOI N. SP. LHERMITTE-VALLARINO, BARBUTO & BAIN (Figs. 5 & 6; Table II)

Type host: *Brookesia superciliaris* (Kuhl, 1820). Collection date unknown.

Type locality: unknown; distribution of host species ranges from East to North East Region of Madagascar (Glaw & Vences, 2007).

Location in host: lungs.

Type material: two worms recovered. Female holotype 214 CE. One paratype 214 CE. Deposited in MNHN collection of zooparasitic nematodes.

Additional material: a single female 217 CE from *B. superciliaris* (same dealer). Median part used for molecular analysis (voucher specimen MIB:Zpl:00233).

Etymology: the species is dedicated to Prof. E.R. Brygoo for his exceptional contribution to our knowledge of Malagasy chameleons and their parasites.

Morphology

Both type specimens were drawn before and after fixation in 70 % ethanol. Following fixation, the holotype was thinner and distinctly longer than would have normally been expected. Living worms moved slightly and an error pertaining to body length, but not body width, when measuring the unfixed specimen cannot be excluded. Both sets of measurements are reported in Table II. Prior to fixation, the paratype was sectioned at mid-body and a small part was removed for molecular studies; this somewhat disturbed the alignment of the genital tract.

S-shaped. Vesicle conspicuously dilated in cephalic part, thin along body. Head: surface marked with radial folds of transparent vesicle; a cephalic plateau with two lateral

and two median rims (Fig. 6A), as well as sensory organs, visible beneath. Four submedian papillae, each with a tiny terminal sensillum (Fig. 6C); in addition, a single refringent spot present in each of the two subdorsal papilla-bearing formations (Fig. 6B); no lateral papillae; amphids not observed. Vestibulum and mouth flattened dorso-ventrally, vestibulum netting needle-shaped (Figs. 5G; 6A, B, C); buccal cavity subtriangular, flattened dorso-ventrally. Mouth and buccal cavity with small diameter (maximum 25 and 28, respectively, in dorso-ventral view). Anterior and posterior segment of buccal capsule well delineated; internal aspect of anterior segment with longitudinal crests (Fig. 6D, F, G). Oesophagus relatively thick, with swelling anterior to nerve ring; a second swelling present or not at mid-length (Fig. 5A, H); bulb oval. Intestine: apex slightly wider than bulb diameter; no rapid increase in diameter.

Genital tract: ovaries not overlapping; in holotype, a testis zone in anterior ovary, 115 long, 290 from ovary apex; spermatozoa in oviducts; genital bend at end of oviducts near uteri; uteri filled with larvae, some of them found in oviducts (Fig. 5B). Pseudocoelomocytes identified in anterior region, at level of oviduct (Fig. 5A). Tail conical and straight (Fig. 5C) or bent ventrally at end of anterior third (Fig. 5F); caudal tip thin and straight.

Taxonomic discussion

Rhabdias mariauxi is the only *Rhabdias* from chamaeleonids with a dorso-ventrally flattened mouth and a buccal capsule similar to the present material, but its body is wider (700-740 *vs* 410-480) and its mouth has a greater maximum diameter (40-45 *vs* 25-28).

The two small *Rhabdias* species, *R. gemellipara* and *Rhabdias* sp., are distinct in having a round and laterally flattened buccal capsule, respectively (Chabaud *et al.*, 1961; Lhermitte-Vallarino *et al.*, 2009b).

All large *Rhabdias* species share, amongst other characters, a wide buccal capsule (diameter \geq 35) that distinguishes them from the present specimens.

None of the *Rhabdias* species previously described from chameleons present a cephalic dilatation that is based on an inflation of the vesicle only. A similar character has, however, been observed in congeners from anuran hosts, namely *Rhabdias alabialis* Kuzmin, Tkach & Brooks, 2007 from a bufonid in Central America and *Rhabdias globocephala* Kung & Wu, 1945 from a racophorid in the Eastern Palaearctic. *Rhabdias alabialis* and *R. globocephala* redescribed by Kuzmin (2005) from another host are readily distinguished from our material by their much narrower buccal capsule (external diameter 10-15 and 12-17, respectively *vs* 25-28). Furthermore, despite being similar in body length, *R. alabialis* has a shorter oesophagus (340-445 *vs* 800-865), and *R. globocephala* has six circumoral lips, whereas only four submedian papillae are present in the current specimens (Kuzmin, 2005; Kuzmin *et al.*, 2007).

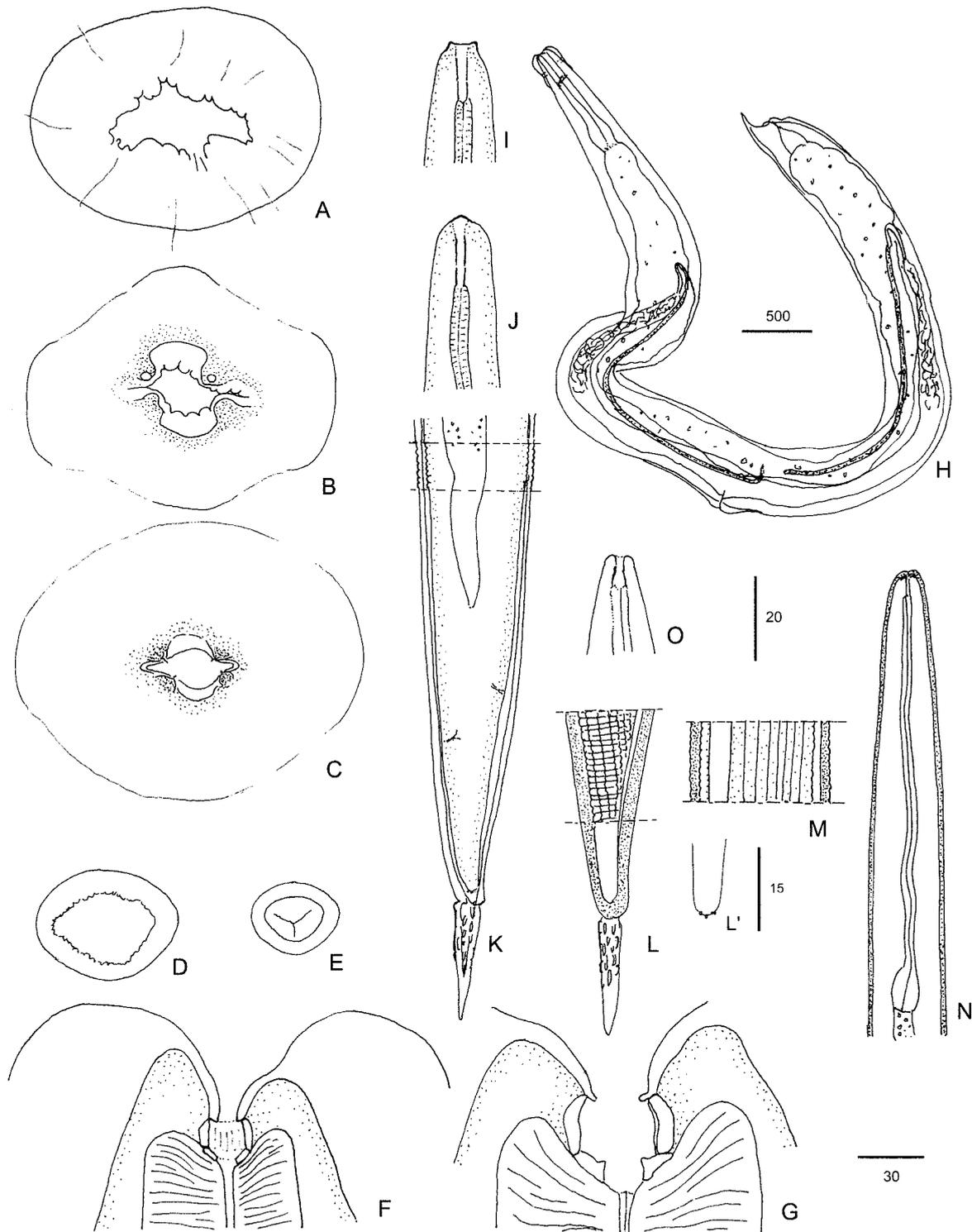


Fig. 6. – *Rhabdias brygooi* n. sp. from *Brookesia superciliaris*, from Madagascar. A-E. Head, front view of the paratype. A. Superficial view, with radiated folds of the vesicle. B. Below, at level of the submedian papillae and vestibulum. C. More posteriorly, at level of the submedian sensilla, and netting needle-shaped velum. D. Buccal capsule, anterior segment. E. Buccal capsule, posterior segment and lumen of oesophagus. F & G. Buccal capsule of the paratype and holotype, respectively. H. Habitus of the paratype, before fixation, left lateral view. I-N. Infective larva. I & J. Anterior extremity of exsheathed larva, lateral and dorso-ventral view, respectively. K. Posterior end of larva in the sheath, ventral view (note the buds on tail extremity). L. Caudal extremity of the sheath, external ornamentation (shown between the dotted lines and on caudal point), ventral view. L'. Tail extremity of larva, rounded with few buds. M. Mid-body of larva in the sheath (densely dotted), showing longitudinal crests of the larva itself (spaces between them dotted) and lateral chord (not dotted, on left). N. Anterior region of larva in the sheath. O. Anterior extremity of a free-living female. Scales in μm : A-E, G, I-O, 20; F, N, 30; H, 500; L', 15.

The material from *B. superciliaris* in Madagascar represents a hitherto undescribed taxon, for which we suggest the name *R. brygooi*.

The buccal capsule of the female specimen recovered from a second *B. superciliaris*, 217 CE, was completely obscured by brownish tissue, but because it was similar in general morphology and measurements corresponded well, it was assigned to *R. brygooi* n. sp. as well.

Molecular analyses

Using an integrated taxonomic approach based on morphological characterisation, as well as on DNA barcoding (Ferri *et al.* 2009), the new species was also characterized by 12S rDNA sequence analysis.

Data obtained, based on K2P distance matrix, confirmed that the new species is a new MOTU, different from other *Rhabdias* species, for which molecular data on 12S rDNA is available, i.e. *R. okuensis*, *R. mariauxi*, *R. rhampboleonis* (Lhermitte-Vallarino *et al.*, 2008; 2009a). In fact, the nucleotide interspecific distances (K2P) between *R. brygooi* and the three *Rhabdias* species previously mentioned are: 5.3 % (standard error 1.2 %) with *R. mariauxi*; 4.5 % (standard error 1.1 %) with *R. okuensis*; and 6.6 % (standard error 1.3 %) with *R. rhampboleonis*.

Free-living phase of *R. brygooi* n. sp.

The complete development of free-living stages of *R. brygooi* n. sp. was studied based on faecal cultures from *B. superciliaris* 217 CE. Males and females developed and each female produced a single infective larva through matricidal endotoky.

Male (n = 1). Body 745 long, 30 wide; oesophagus 135 long; testis bent 280 from apex; tail, spicules and gubernaculum 55, 23 & ND long, respectively.

Infective larva (n = 7). Sheath: thick with checkered ornamentation (Fig. 6L), anterior part rounded (Fig. 6N), posterior part with a subterminal constriction. Larva: head with a dorsal and ventral elevation (Fig. 6I, J); cuticle ornated with longitudinal crests (Fig. 6M); buccal capsule composed of an anterior and posterior segment, apparently situated anterior to oesophagus apex; tail extremity rounded, ornated with a few buds (Fig. 6L').

Body 633-920 long, 26-29 wide; buccal capsule 9-11 long; nerve ring 100 from head; oesophagus 160-180 long; tail 53-65 long; caudal point of sheath 22-24 long.

GENERAL DISCUSSION

Pseudocoelomocytes are large cells thought to be involved in the immune response of nematodes as e.g. scavenger cells (see Tahseen, 2009). They had previously been described in some *Rhabdias*

species from ophidian (Kuzmin & Tkach, 2008) and anuran hosts (Baker, 1979; Kuzmin, 2005), but their presence in *R. casiraghii* n. sp. and *R. kibiraensis* n. sp. constitutes the first report of pseudocoelomocytes in *Rhabdias* from chamaeleonids. As found in other nematodes, their number and location were more or less constant within species.

In possessing aligned lateral pores that open into the internal layer of the cuticle, the body vesicle of *R. casiraghii* n. sp. is the most complex that we have observed to date. A similar arrangement of ducts leading to hypodermal glands was noted in several *Rhabdias* species from anurans (Baker, 1987; Singh & Ratnamala, 1977a, b): *R. collaris* Baker, 1987 in Tanzania, *R. shorrti* (Singh & Ratnamala, 1977) and *R. thapari* (Singh & Ratnamala, 1977) in India. Similar structures were also described by Ballantyne (1991) in another rhabdiasid genus, *Pneumonema* Johnston, 1916, which has no body vesicle but spines. Features more commonly associated with the vesicle are transverse furrows and, in some species, fibres of attachment to the internal cuticular layer; the latter were observed in *R. okuensis* from *Trioceros* in Cameroon and *R. picardiae* from a bufonid in South Africa (Lhermitte-Vallarino *et al.*, 2008; Junker *et al.*, 2010). The role of the body vesicle is as yet undetermined; it is suggested that this complex apparatus allows the parasites to resist the variation in pressure caused by the rhythmic contraction of the host's lungs. Indeed, lung metastrongylids also have a vesicle.

For the first time evidence is presented that a single host specimen can harbour concurrent infections of more than one species of *Rhabdias*. This is, however, not surprising amongst nematodes as there are numerous examples of congeneric co-parasitism amongst intestinal trichostongylids (Boomker *et al.*, 1989; Chabaud & Durette-Dessset, 1978) and also amongst filarioids (Bain *et al.*, 1979; Uni *et al.*, 2001). Co-infection with congeneric species has been explained by isolation of host populations allowing divergence of the ancestral parasite species; when host populations joined again, derived forms were confronted with each other and partly eliminated (Chabaud & Durette-Dessset, 1978). This hypothesis might be applicable to the parasites of *T. ellioti* as well, because its distribution range and its capacity to adapt to a variety of habitats are greater than in many other chamaeleons (Necas, 2004).

Indeed, the two species co-infecting *Trioceros ellioti*, *R. casiraghii* n. sp. and *R. kibiraensis* n. sp., share the primitive arrangement of their head sensillae: an internal circle of four submedian recurrent sensilla, and an outer circle of four pairs of sensilla. We interpret the latter as being the externo-labial and cephalic papillae. Such a pattern has been described by Baker (1979) in young parasitic adults of *R. americanus* Baker, 1978, from a North American bufonid.

The same primitive disposition was not identified in *R. brygooi* n. sp. from *B. superciliaris* in Madagascar. However, its morphology shows an interesting character; excepting the two small species *R. gemellipara* and *Rhabdias* sp., the buccal capsule diameter of *R. brygooi* n. sp. is by far the smallest amongst *Rhabdias* from chameleons. Hence, the possibility that *R. brygooi* n. sp. is a capture from species parasitic in Malagasy anurans cannot be excluded. To date, a single species, *R. madagascariensis* Chadaud, Brygoo & Petter, 1961 from *Ptychadena mascareniensis* (Duméril & Bibron, 1841) [= *Rana (Ptychadena) mascareniensis*] (Ranidae), has been reported in Madagascar. However, a further species parasitic in Mantellidae has been described by Junker *et al.* (2010), and a closer investigation would in all likelihood reveal *Rhabdias* to be more diversified in this host group as well.

A second possibility is that the parasite from *Brookesia*, a genus thought to have diverged early (Klaver & Böhme, 1986; Tilbury & Tolley, 2009), represents the primitive state of the lineage of *Rhabdias*, which evolved in the monophyletic family Chamaeleonidae. Arguments that support this second hypothesis are the length of its oesophagus (820-900) although an oesophagus length of ≥ 800 is not exclusive to chamaeleonids, and several characters of the infective larva, such as a rounded tail tip with buds and the subterminal indentation and bulges that are present on the larval sheath (Fig. 6K, L).

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