

## REAPPRAISAL OF THE SPECIFIC STATUS OF *Rhabdias* (NEMATODA: RHABDIASOIDEA) FROM MALAGASY CHAMELEONS IN THE PARIS MUSEUM COLLECTION

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### Summary:

To date *Rhabdias gemellipara* is the only species described from Malagasy chameleons, but heterogeneity of the material had been suspected. 11 samples of *Rhabdias* parasites present in the Paris Natural History Museum collection were examined. The size and shape of the buccal capsule, shape and length of the oesophagus, shape of the apical region of the intestine, extent of the genital tract and structure of the cuticular vesicle led to the distinction of five species. *Rhabdias rabetafikae* n. sp. parasitises *Calumma cucullatum* in the northeastern region (Cap Machoual). In the eastern region, *R. nasutum* n. sp. is parasitic in *C. nasutum*, and *R. brevicorne* n. sp. in *C. brevicorne*. All three species are similar in size to the African species parasitic in chameleons from which they can be distinguished by several characters. The small species, *R. gemellipara*, type host *C. parsonii* from the eastern region, was also found in *C. brevicorne* from the same geographic region. In the central region, *Rhabdias* sp., equally collected from *C. brevicorne*, is distinguished from *R. gemellipara* by a laterally flattened buccal capsule. All Malagasy species are hermaphrodites. Throughout the worm's life spermatozooids are formed intermittently in a band of cells situated at 1·2 mm from the extremity of the ovaries; they migrate in the ovaries and accumulate in the oviducts.

**KEY WORDS :** *Rhabdias*, new species, hermaphrodisim, chameleons, Madagascar.

**Résumé :** RÉVISION DU STATUT SPÉCIFIQUE DES *Rhabdias* (NEMATODA : RHABDIASOIDEA) PARASITES DE CAMÉLÉONS MALGACHES EN COLLECTION AU MUSÉUM NATIONAL D'HISTOIRE NATURELLE DE PARIS

*Rhabdias gemellipara* était le seul taxon reconnu chez les caméléons malgaches mais une hétérogénéité était soupçonnée. Les 11 lots de parasites présents dans les collections du MNHN-Paris ont été examinés. Les taille et forme des capsules buccales, forme et longueur de l'œsophage, forme de la région apicale de l'intestin, extension de l'appareil génital et structure de la vésicule cuticulaire du corps conduisent à distinguer cinq espèces : *R. rabetafikae* n. sp., parasite de *Calumma cucullatum* dans la Région Nord-Est (Cap Machoual), *R. nasutum* n. sp. parasite de *C. nasutum* et *R. brevicorne* n. sp. parasite de *C. brevicorne*, les deux dans la Région Est. Ces trois espèces sont grandes comme les espèces africaines parasites de caméléons dont elles sont distinctes par plusieurs caractères. La petite espèce, *R. gemellipara*, dont l'hôte-type est *C. parsonii* en Région Est est retrouvée dans la même région chez *C. brevicorne*. Dans la Région Centre, *Rhabdias* sp., récolté aussi chez *C. brevicorne*, est distinct de *R. gemellipara* par la capsule buccale aplatie latéralement. Toutes ces espèces sont hermaphrodites ; les spermatozoïdes sont formés pendant toute la vie des femelles mais de façon intermittente dans une bande de cellules située à 1·2 mm de l'extrémité des ovaires ; ils migrent dans les ovaires et s'accumulent dans les oviductes.

**MOTS CLÉS :** *Rhabdias*, nouvelles espèces, hermaphrodisme, caméléons, Madagascar.

## INTRODUCTION

Chamaeleonidae are one of a few groups of saurians of which the lungs are frequently parasitized by *Rhabdias* (Nematoda: Rhabdiasoidea). The majority of these lizards originate from Africa or Madagascar, and a single *Rhabdias* species has been reported from each of the two regions, namely *R. chamaeleonis* (Skrjabin, 1916) in Africa and *R. gemellipara* Chabaud, Brygoo & Petter, 1961 in Madagascar. While some progress has been made on the diversity of *Rhabdias* in Africa (Lhermitte-Vallarino & Bain, 2004 ; Lhermitte *et al.*, 2008), the situation in Madagascar appears complex.

Several samples of *Rhabdias* from Madagascar are deposited in the collection of the Muséum National d'Histoire Naturelle, Paris (Natural History Museum, MNHN): the type material of *R. gemellipara* recovered from a single *C. parsonii* (Cuvier, 1824), composed of one gravid female, remarkably small at 8 mm body length (Chabaud *et al.*, 1961); several "giant" specimens, 20–30 mm long, recovered from a single *Calumma brevicorne* (Günther, 1879) and identified as *R. gemellipara* by Chabaud & Brygoo (1962); small and large *Rhabdias* collected from several species of *Calumma* Gray 1865, one *Brookesia* Gray, 1865 (Chamaeleonidae) and one *Zonosaurus* Boulenger, 1887 (Gerrhosauridae), all identified as *R. gemellipara* by Caballero (1968). Kuzmin (2003) studied these collections, but only one sample containing giant specimens was found at the time (348 Q, Cap Machoual). He observed that a single specimen from *Zonosaurus* differed from typical *R. gemellipara* and speculated that it was either a host or age variation, or a different species, but thought the

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material insufficient to draw a final conclusion. Irrespective of the host, Kuzmin (2003) identified small specimens as *R. gemellipara*, and large specimens as belonging to the African species *R. chamaeleonis*, the then single known large species. However, in 2004, Lhermitte & Bain redescribed *R. chamaeleonis* and defined the characters which differentiate this species from the Malagasy material.

We examined both small and large *Rhabdias* from Madagascar present in the MNHN collection. The particularities of parasites from *Zonosaurus* reported by Kuzmin (2003) were confirmed. Similarly, *Brookesia* was found to harbour *Rhabdias* distinct from previously described species. These results are not included herein, but form part of an on-going study. In the present paper we propose to distinguish five species of *Rhabdias* parasitic in *Calumma* spp. These taxonomic hypotheses are supported by our previous studies on African material which demonstrated *i*) the validity of several diagnostic characters in addition to those commonly used, namely head ornamentation, shape of oesophageal-intestinal junction and extension of the genital tract in gravid females, and *ii*) that size and shape of buccal capsule as well as oesophagus vary little between small juvenile and large gravid females of a given species (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino *et al.*, 2008).

Since the parasitic generation of *Rhabdias* is exclusively female, its mode of reproduction has generated detailed analyses in the past. Hermaphroditism was demonstrated in several species from anurans and snakes, reviewed by Anderson (2000). In chameleons, both hermaphroditism and parthenogenesis were suggested. During the course of this study, examination of the female genital tract brought evidence that all *Rhabdias* spp. from Malagasy chameleons are hermaphroditic, including *R. gemellipara*, contrary to previous reports of Chabaud *et al.* (1961). As suggested by Lhermitte & Bain (2004) and Lhermitte *et al.* (2008), spermatozoa originate from a band of small cells in the ovaries. Spermatozoa production is intermittent.

## MATERIAL AND METHODS

All Malagasy specimens examined had been deposited in the MNHN collection. They were fixed in hot 70 % ethanol and stored in 70 % ethanol. A thorough search of parasite and host files enabled us to find the samples of small and giant *Rhabdias*, excepting one sample loaned 30 years ago (Table I). The natural hosts of these specimens belong to four chameleon species: *Calumma parsonii*, *C. brevicorne*, *C. cucullatum* (Gray, 1831) and *C. nasutum* (Duméril

Collection	Host species	Geographical origin			Field <i>Rhabdias</i> number	Field host number	Reference	Notes of Chabaud	n <i>Rhabdias</i> at present (past)
		Locality	District	S-Pref/Region					
11 G	<i>C. parsonii</i>	Mantadia	Périnet	A/East	1.4.61	ND	1		1 type
50 G	<i>F. lateralis</i>	Exp. infection	-	-	-	-	1		5 (5)
471 F	<i>C. brevicorne</i>	-	-	-	Bry 377	C 202*	2 & 3	n. sp.?	3 (ND)
481 F	<i>C. brevicorne</i>	Fiherenana	Fiherenana	A/East	194.61	C204	2 & 3		1 (3)
486 F	<i>C. brevicorne</i>	Fiherenana	Fiherenana	A/East	146.61	C202*	2 & 3		3 (ND)
496 F	<i>C. brevicorne</i>	Ampamaherana	Fandredava	C/Centre	103.62-13.3	C265	2 & 3		loaned 1968 (5)
498 F	<i>C. brevicorne</i>	Ambavaniasy	Périnet	A/East	10.II.62	C292	2 & 3	1 giant	1 (1)
501 F	<i>C. brevicorne</i>	Périnet	Périnet	A/East	II.62	C261	2 & 3		5 (6)
514 F	<i>C. brevicorne</i>	Ifanadiana	Ifanadiana	B/East	97.62-22.2	C316	2 & 3	1 giant	1 (1)
653 G	<i>C. brevicorne</i>	Ampamaherana	Fandredava	C/centre	163/64	ND	4		14 (ND)
474 F	<i>C. nasutum</i>	Ambavaniasy	Périnet	A/East	163/61	C226	-	n. sp.?	2 (ND)
348 Q	<i>C. cucullatum</i>	-	Cap Machoual	D/North-East	-	-	5		6 (ND)
719 G	<i>Br. ebenau</i>	-	Ampijorao	North-West	14/65	C761	4		1 (ND)
487 G	<i>Z. madag.</i>	Mahabo	-	-	Bry 70/64	-	4		ND
496 G	<i>Z. madag.</i>	Mahabo	-	-	Bry 45/64	-	4		lost
503 G	<i>Z. madag.</i>	Mahabo	-	East	Bry 35/64	-	4		4 (ND)
514 G	<i>Z. madag.</i>	Mahabo	-	East	Bry 12/64	-	4/5**		1 (ND)

*C. Calumma; F. Furcifer; Br. Brookesia; Z. madag. Zonosaurus madagascariensis.*

S-Pref: sub-prefecture. A: Moramanga; B: Ifanadiana; C: Fianaranstoa; D: Cap Machoual.

ND: not determined.

Field Rhabdias number: sometimes reflects date of worm recovery.

\*References: 1, Chabaud, Brygaa & Petter, 1961; 2, Chabaud & Brygaa, 1962; 3, Brygaa, 1963; 4, Caballero, 1968; 5, Kuzmin, 2003.

\*\* Two hosts with the same number (original error). \*\* not *R. gemellipara* according to Kuzmin, 2003.

Table I. – Malagasy specimens of *Rhabdias* recovered from chameleonids and other lizards, in the collection of the Muséum National d'Histoire Naturelle, Paris, and identified as *R. gemellipara* Chabaud, Brygaa & Petter, 1961.

& Bibron, 1836). They were captured in the eastern and central region of Madagascar, except *C. cucullatum* from Cap Machoual in the northeast. One sample was an experimental infection of *Furcifer lateralis* (Gray, 1831) captured in the Tananarive area (Chabaud *et al.*, 1961) with larvae from the female holotype of *R. gemellipara*.

Worms were cleared in lactophenol and studied under a Wild microscope equipped with a drawing tube, as described by Lhermitte & Bain (2004). Measurements were made on drawings and expressed in  $\mu\text{m}$ , except for body length and distance from head to vulva which are in millimetres. Where necessary, two measurements for body width are included, the first without vesicle and the second, in brackets, including it. Head papillae and shape of mouth opening were studied in apical view if sufficient specimens were available. Using a razor blade, the head was severed under a stereomicroscope and oriented under the microscope. The buccal capsule was described including shape, length and maximum external diameter; in one sample the buccal capsule appeared laterally flattened and its diameter was measured in lateral as well as dorso-ventral view; the buccal capsule ratio is defined as length/external diameter. In the Malagasy specimens the buccal capsule possesses a small oblique posterior part, more or less distinct and granulous, 3-4 long, which was not included in the length. The apex of the

oesophagus has "shoulders" when its external edge is anterior to the bottom of the buccal capsule. Bulb diameter and body width at that level were measured and the bulb ratio calculated. Several observations regarding the oesophageal-intestinal junction were recorded: diameter of intestine equal or greater than bulb basis; shape of anterior part of intestine, cylindrical or widening, expressed as the ratio of two intestine measurements: width of intestine 1 mm posterior to oesophageal-intestinal junction/width at junction. The extension of the genital tract is defined by the distance of anterior bend to head and posterior bend to tip of tail, as well as by the distance anterior ovary apex to head and posterior ovary apex to head. Vulva ratio is distance head to vulva/body length. As in previous studies (Lhermitte & Bain, 2004; Lhermitte *et al.*, 2008), a schematic representation of the female anatomy of the three new species is given. Length of caudal point was measured when distinct. Ovaries and oviducts were examined for production or storage of spermatozoa.

## RESULTS

All specimens of all samples in the MNHN collection (Table I) were examined, and large and small specimens were never found in the same

MNHN collection	348 Q 1, Holotype	348 Q 2	348 Q 3	348 Q 4	348 Q 5, anterior*	348 Q 6, anterior	348 Q 7, posterior	348 Q 8, posterior
Specimen								
Length mm	22.8	25.8	26.33	23.05	ND	ND	ND	ND
Width at mid-body (with vesicle)	900 (950)	800 (850)	1,150 (ND)	820 (860)	ND	ND	950 (1,050)	1,110 (1,200)
Buccal capsule length	13	12	12	12	ND	13	ND	ND
Buccal capsule external diameter	45	43	42	48	ND	42	ND	ND
Oeso. shoulders. Acute: +. Round: -	+	$\pm$	+	$\pm$	ND	$\pm$	ND	ND
Oesophagus length	1,450	1,500	1,390	1,350	1,400	1,500	ND	ND
Oesophagus width at mid-length	150	140	140	135	ND	140	ND	ND
Bulb diameter	225	230	240	210	225	230	ND	ND
Body width at bulb level (with vesicle)	425 (520)	490 (570)	520 (630)	400 (450)	600 (720)	550 (650)	ND	ND
Intestine width at 1mm / at apex	1.5	1.8	2.5	2.8	1.3	2	ND	ND
Vulva mm	12.3	14.2	14.6	13.5	ND	ND	ND	ND
Anterior genital bend-head	4,260	4,800	4,960	3,560	3,350	ND	ND	ND
Posterior genital bend-tip tail	3,250	3,800	ND	1,810 ?	ND	ND	3,700	3,625
Anterior ovary apex-head	15.8	17.47	ND	16	ND	ND	ND	5.1 post vulva
Posterior ovary apex-head	6,550	8,740	ND	9,700	ND	8,200	ND	ND
Tail	310	420	300	300	ND	ND	500	320
Width at anus (with vesicle)	165 (260)	230 (340)	155 (280)	135 (215)	ND	ND	240 (365)	175 (310)
Tail shape	conical	conical	conical	conical	ND	ND	conical	conical
Caudal point	85	110	110	80	ND	ND	120	75
Nerve ring	320	320	290	300	ND	290	ND	ND
Excretory pore	400	430	ND	365	ND	335	ND	ND
Habitus	Dors bow	Dors bow	Dors bow	Dors bow	ND	ND	Dors bow	Dors bow
Vulva ratio: head-vulva / body length	0.53	0.55	0.55	0.58	ND	ND	ND	ND

ND: not determined.

\* Female without head. Dors bow: dorsally bent bow.

Table II. - *Rhabdias rabetafikae* n. sp. from *Calumma cucullatum*, from Cap Machoual, northeastern region, Madagascar: holotype and paratypes.

sample. Large specimens were recovered from three species of *Calumma* and differed according to host. Small specimens belonged to two morphological types, one identical to *R. gemellipara* as re-examined in this study, the other distinct by several characters. All new species described herein are distinct from *R. japalurae* Kuzmin, 2003, host-type *Japalura swinhonis* Günther, as this species from an Oriental agamid has a large vesicle and its buccal capsule, oesophagus and body are much smaller or much greater than those of the large and small specimens, respectively. Taxonomic differentiation regarding *R. japalurae* is not repeated in the individual taxonomic discussions below.

In all specimens the structure of the lateral chords was peculiar in that the syncytium is divided with drop-shaped lines, more or less regular, giving the appearance of cellular organisation (Fig. 2G). Similar observations were made in African *Rhabdias* from chameleonids (personal observation) and it is probably a general feature of the genus (see Fig. 9 in Baker, 1978).

#### *RHABDIAS RABETAFIKAE* N. SP.

Type host: *Calumma cucullatum* (Gray, 1831).

Location in host: lungs.

Type locality: Cap Machoual, northeastern region, Madagascar.

Collection date: 23/11/1967.

Type specimens: female holotype 348 Q 1, two complete female paratypes, two anterior and two posterior parts of female paratypes 348 Q, all deposited in the MNHN collection.

Prevalence: not determined (one dissected host specimen reported).

Etymology: dedicated to Dr Lydia Rabetafika, our parasitologist colleague in Madagascar, University of Antananarivo.

Body large (Table II; Figs 1B,E, 2C, D, 3A-F, 4C-G,D).

Habitus: body dorsally bent, bow- or a question mark-shaped. Vesicle: extending from head to tip of tail, more prominent in anterior (100 wide) and posterior

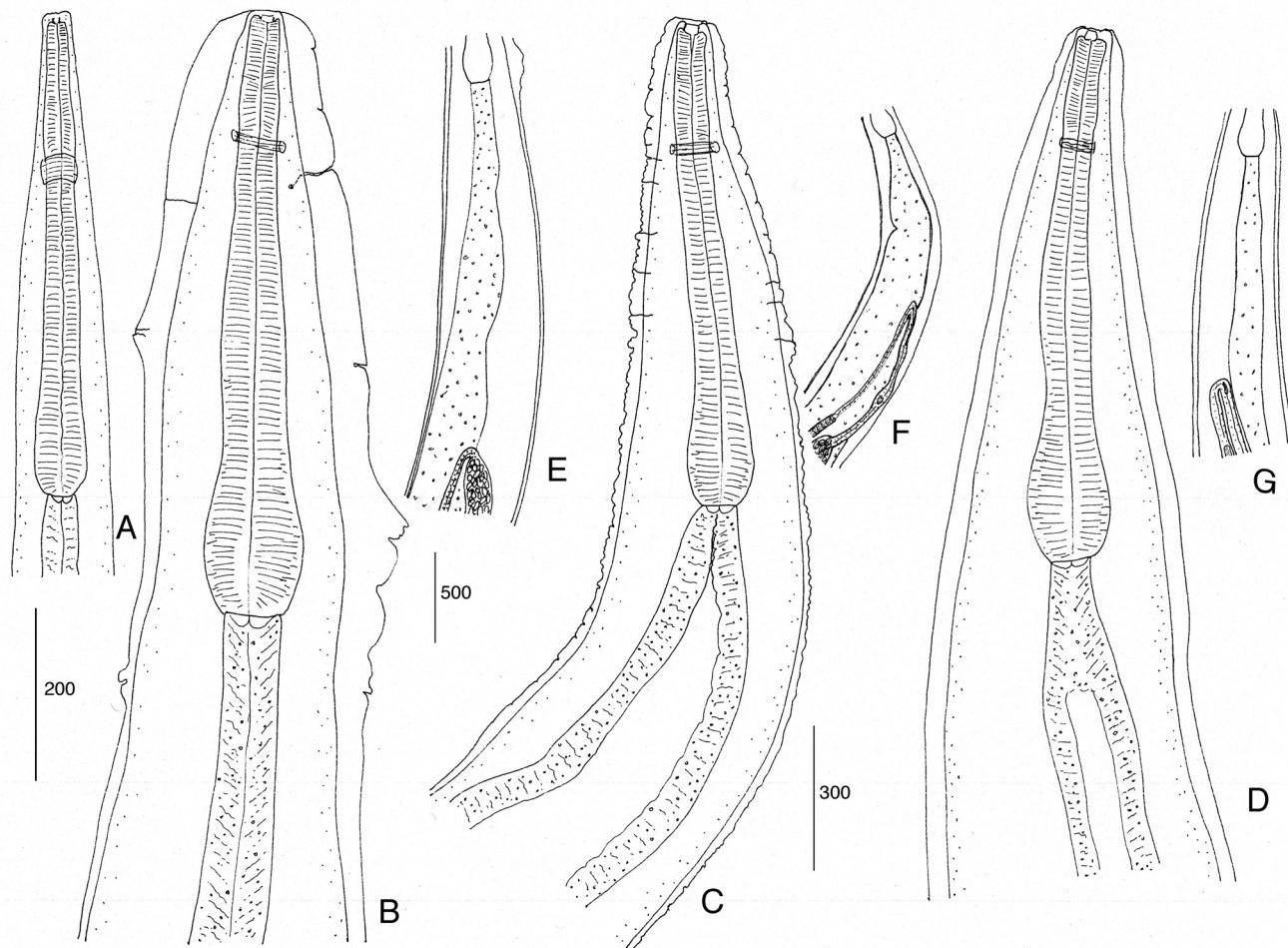


Fig. 1. – *Rhabdias* spp. from *Calumma* spp. A-D. Anterior region of females. A. *R. gemellipara*, holotype. B. *R. rabetafikae* n. sp., holotype. C. *R. nasutum* n. sp., holotype. D. *R. brevicorne* n. sp., holotype. E-G. Apex of intestine of *R. rabetafikae* (holotype), *R. nasutum* (paratype) and *R. brevicorne* (holotype), respectively. Scales in  $\mu\text{m}$ : A, 200; B, C, D, 300; E, F, G, 500.

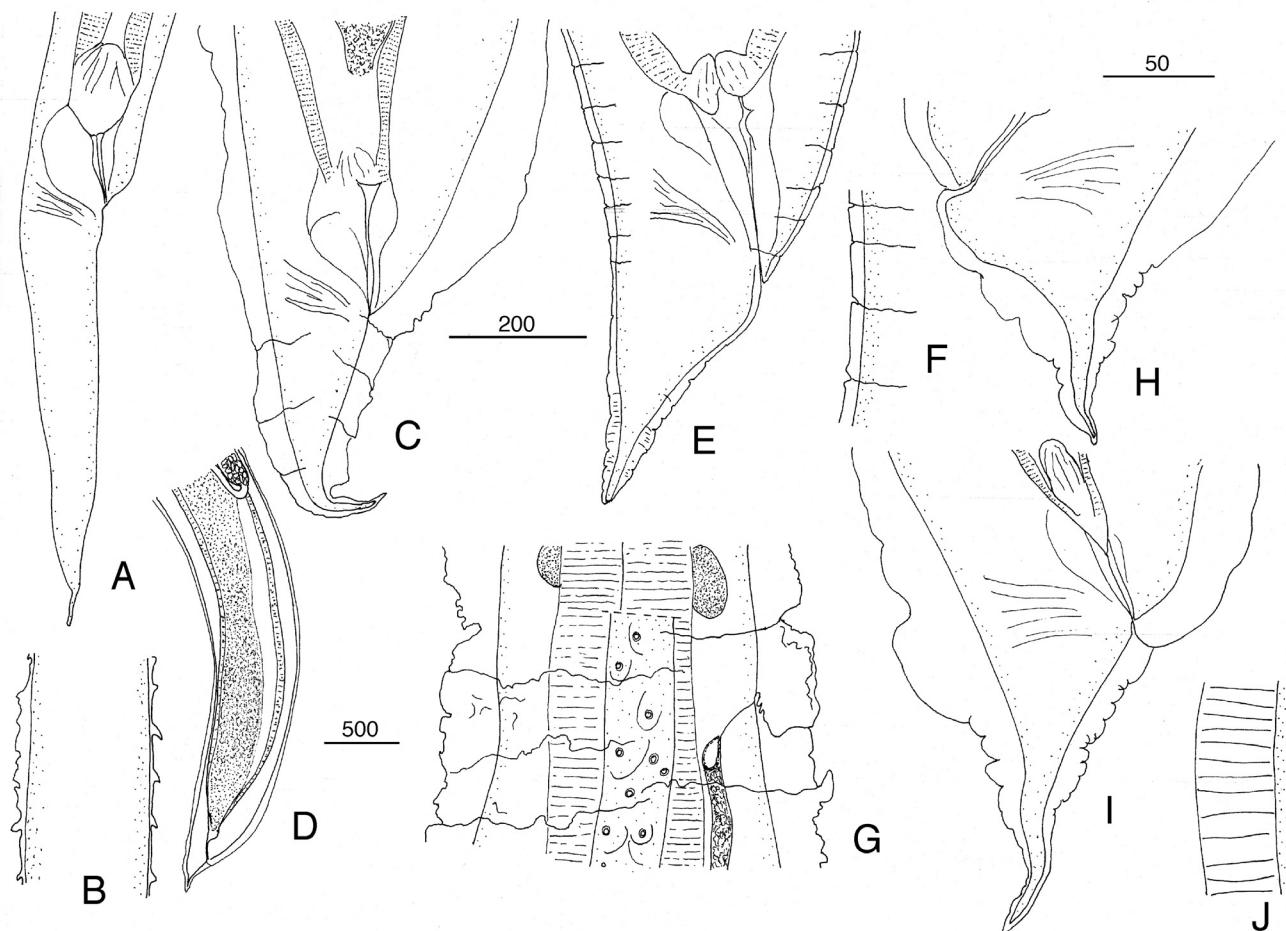


Fig. 2. – *Rhabdias* spp. from *Calumma* spp., posterior region of females and structure of body vesicle. A. *R. gemellipara*, experimental infection, tail, right lateral view. B. Vesicle, experimental infection, dorso-ventral view. C, D. *R. rabetafikae* n. sp., two paratypes, tail and posterior region respectively, right lateral view. E-G. *R. nasutum* n. sp., paratype. E. Tail, right lateral view. F. Vesicle, posterior region. G. Region of excretory pore and detail of lateral chord. H-J. *R. brevicorne* n. sp. H. Tail, left lateral view, holotype. I. Tail, right lateral view, 514 F. J. Vesicle, holotype. Scales in µm: A, B, F, G, J, 50; C, E, H, I, 200; D, 500.

region; transverse furrows 100-400 apart in oesophageal region, 50-150 in posterior region. Head: no lips; protuberances of four large papillae and two smaller amphids, all linked with a cuticular lining which is hexagonal and slightly flattened dorso-ventrally; amphidial pores anterior to papilla apices; each papilla with salient apex; no lateral papillae identified. Mouth opening depressed, round and lined with cuticular membrane (velum). Buccal capsule: short, diameter large; in transverse optical section, quadrangular posterior to the velum, then round; wall thick and well cuticularized; buccal capsule ratio 0.25-0.30; posterior oblique granulous segment 3-4 long. Oesophagus: thick; at apex, lumen lined with rugosities; round to acute shoulders; slight inflation anterior to nerve ring; posteriorly, progressive increase of diameter to 135-150 at mid-length; slight constriction before bulb; ratio bulb/body width 0.37-0.52. Intestine: apical diameter equals bulb at base; proximal part, an elongated thin

pyramid; prerectal constriction present in 5/6 specimens. Tail conical, straight, exceptionally bent dorsally (one specimen); long terminal point, surrounded with narrow to wide vesicle; point bent ventrally in two specimens. Genital tract: anterior and posterior bend at oviduct-uterus junction (one exception with bend at mid-oviduct); anterior bend far from head (3,350-4,960); posterior bend far from tip of tail (3,250-3,800) except in one specimen (1,810); ovocytes variable in shape, very elongated locally (125/35); oviducts 1,000-1,200 long, not coiled. Vulva ratio 0.53-0.58. Production area of male gametes in ovaries observed in five specimens, either present in both or one of the ovaries, anterior or posterior. A male gamete production area was 100-290 long and 1,000-2,000 from ovary apex. Migration of spermatozoa along ovaries was observed in three specimens, in anterior or posterior ovary. Spermatozoa were identified in the oviduct of one female (Fig. 4C-G; Table IV).

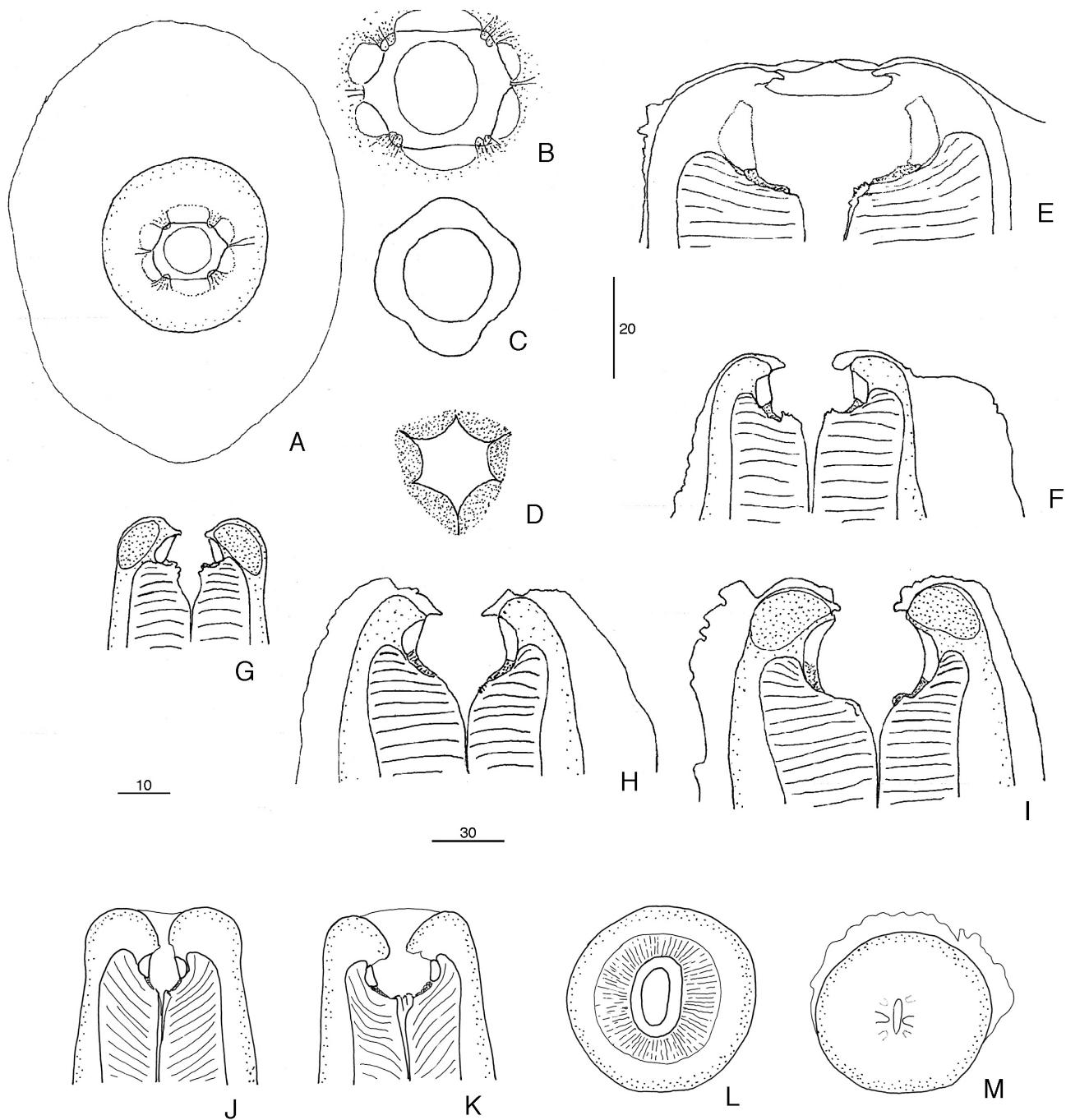


Fig. 3. — *Rhabdias* spp. from *Calumma* spp., head and buccal capsule. A-F. *R. rabetafikae* n. sp. A-D. Apical view, paratypes. A. Head and vesicle. B. The four cephalic papillae and amphids lined with a cuticular crest, and the round buccal velum. C. Detail of velum and, posteriorly, the larger quadrangular apex of buccal capsule. D. Posterior granulous segment of buccal capsule at junction with oesophagus. E. External lateral view and buccal capsule. F. Buccal capsule and velum, optical sagittal section. G-I. Other *Rhabdias* species, optical sagittal section. G. *R. gemellipara*, holotype. H. *R. nasutum* n. sp., holotype. I. *R. brevicorne* n. sp., 514 F. J-M. *Rhabdias* sp. 653 G. J. Lateral view. K. Dorso-ventral view. L, M. In front view, at level of buccal capsule and surface head, respectively. Scales in  $\mu\text{m}$ : A, F, H, I, 30; B, C, D, G, 20; J, K, L, M, 10.

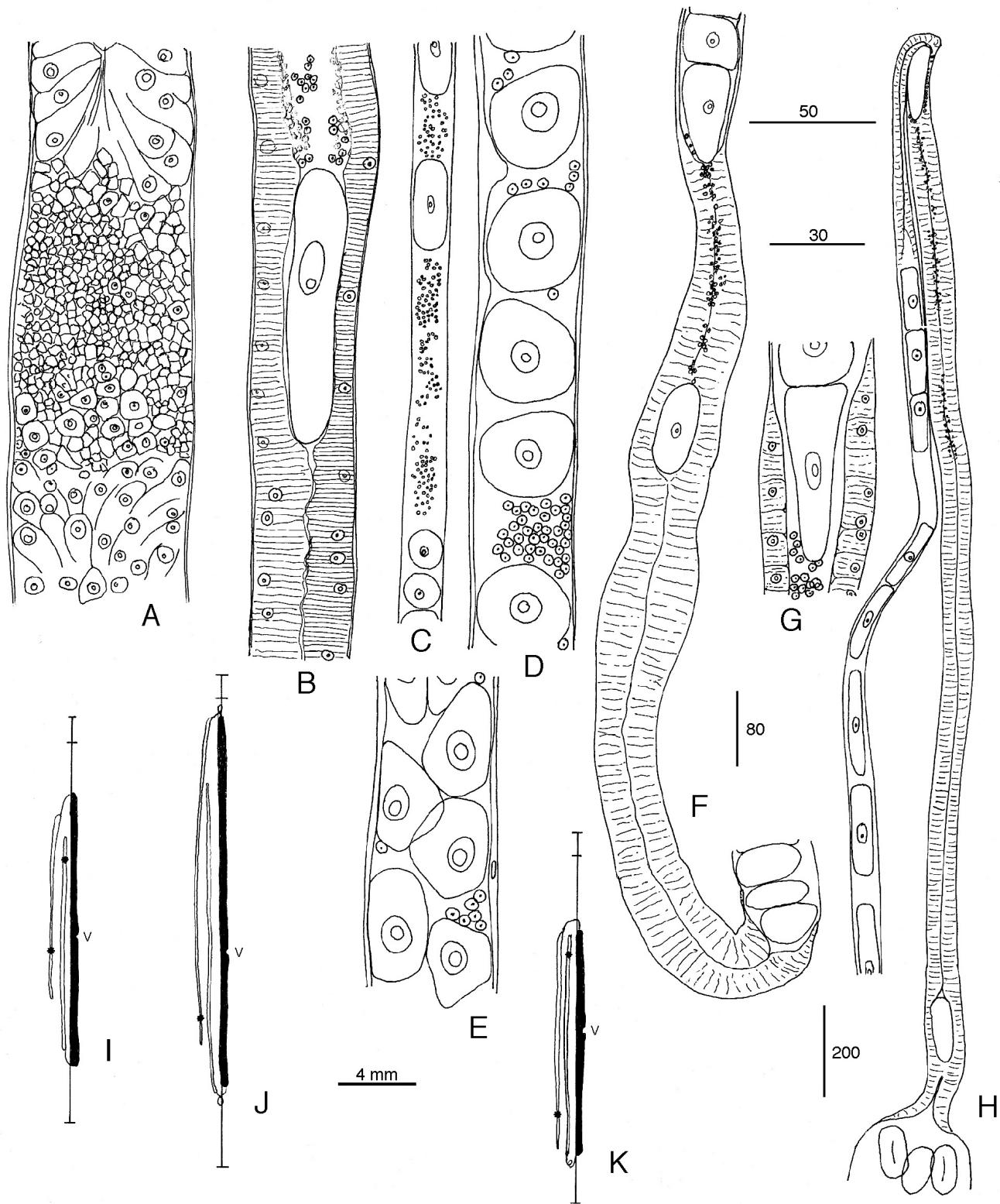


Fig. 4. – *Rhabdias* spp. from *Calumma* spp. A-H. Female genital tracts. A, B. *R. gemellipara*, holotype, male area of ovary and oviduct with spermatozoa, respectively. C-G. *R. rabetafikae* n. sp. C, D, E. Spermatozoa between ovulae, from posterior to more anterior parts of ovary, holotype. F. Posterior oviduct with spermatozoa, paratype. G. Detail of ovary-oviduct junction with ovulae and spermatozoa, another paratype. H. *R. brevicorne* n. sp., anterior oviduct with spermatozoa and junction with ovary and uterus, respectively, holotype. I-K. Schematic representation of females and extension of genital tract. I. *R. rabetafikae* n. sp. J. *R. brevicorne* n. sp. K. *R. nasutum* n. sp. v: Vulva. \*: Position of male area in ovary. Scales in  $\mu\text{m}$ , unless otherwise indicated: A, B, D, E, 50; C, F, 80; G, 30; H, 200; I, J, K, 4 mm.

## Taxonomic discussion

This material differs from *R. gemellipara* in a much wider buccal capsule (42-48 vs 14-15) with a smaller ratio (0.25-0.30 vs 0.46). It resembles the *Rhabdias* parasites of African chameleons, *R. chamaeleonis* and *R. jarki* Lhermitte-Vallarino & Bain, 2004 from Burundi and the two species recently described from Cameroon, *R. okuensis* Lhermitte-Vallarino & Bain, 2008 and *R. cristi* Lhermitte-Vallarino et Bain, 2008. Two of them, *R. jarki* and *R. okuensis*, have a short and wide buccal capsule and an oesophagus approximately 1,280-1,500 long, like the present specimens. However, the following main characters distinguish the African species: in *R. jarki*, oesophagus and bulb twice as thin (45-50 vs 135-150 and 100-110 vs 210-240, respectively), thin anterior vesicle, mouth with four small lobes and six papillae not lined with a crest ; in *R. okuensis*, oesophagus and bulb more slender (75-95 and 110-165), large anterior vesicle, crest joining the cephalic papillae in circular fashion, instead of hexagonally and slightly flattened dorso-ventrally (Lhermitte-Vallarino & Bain, 2004; Lhermitte-Vallarino & Bain, 2008).

The material from *Calumma cucullatum*, in the northeastern region (Cap Machoual) represents a new

species, for which we propose the name *Rhabdias rabetafikae*.

*RHADIAS NASUTUM* N. SP.

Type host: *Calumma nasutum* (Duméril et Bibron, 1836).

Location in host: lungs.

Type locality: Ambavianasy, Péritet District, Maramanga subprefecture, eastern region, Madagascar.

Collection date: 1961 (capture number 163/61).

Type specimens: female holotype 474 F 1, one female paratype 474 F, both deposited in the MNHN collection.

Prevalence: not determined (one dissected host specimen reported).

Body large (Table III; Figs 1C, F, 2E-G, 3H, 4K). Habitus: a dorsally twice coiled spiral. Vesicle: extending from head to tail, thicker in anterior region, less at tail, approximately five thick in mid-region; surface irregularly sinuous; in addition, transverse external furrows, usually approximately 50 apart. Head (no apical view): mouth depressed, lined with a cuticular velum; buccal cavity slightly wider at bottom; buccal capsule: large, walls moderately thick, ratio 0.31 & 0.38; pos-

<i>Rhabdias</i> species	<i>nasutum</i> n. sp.		<i>brevicorne</i> n. sp.	<i>brevicorne</i> n. sp.
Host species	<i>Calumma nasutum</i> Ambavianasy, Péritet		<i>Calumma brevicorne</i> Ambavianasy, Péritet	<i>Calumma brevicorne</i> Ifanadiana, Ifanadiana
Geographical origin	474 F Paratype	474 F1 Holotype	498 F Holotype	514 F
MNHN collection				
Specimen				
Length mm	19.8	20.5	27.6	34.5
Width at mid-body (with vesicle)	700 (706)	980 (986)	1,250 (1,310)	1,200 (1,280)
Buccal capsule length	16	15	17	20
Buccal capsule external diameter	42	48	40	55
Oeso. shoulders. Acute: +. Round: -	+ -	-	+ -	+ -
Oesophagus length	1,130	1,200	1,280	1,330
Oesophagus width at mid-length	90	105	120	100
Bulb diameter	140	150	200	150
Body width at bulb level (with vesicle)	210 (260)	420 (450)	440 (540)	410 (520)
Intestine width at 1 mm / at apex	4	5	2	2.6
Vulva mm	10.7	10.9	15.5	ND
Anterior genital bend-head	2,500	2,350	1,700	ND
Posterior genital bend-tip tail	1,300	1,950	3,500	ND
Anterior ovary apex-head	14,650	15,860	20,800	ND
Posterior ovary apex-head	5,750	5,200	6,000	ND
Tail	320	380	305	390
Width at anus (with vesicle)	180 (190)	200 (210)	270 (350)	195 (340)
Tail shape	Straight conical	Straight conical	Straight funnel	Straight funnel
Caudal point	Not defined	Not defined	50-150	85-200
Nerve ring	320	310	340	ND
Excretory pore	380	ND	ND	ND
Habitus	Helicoidal × 2	Helicoidal × 2	G	Circle and half
Vulva ratio: head-vulva / body length	0.54	0.53	0.56	ND
Structure of vesicle	Transverse furrows	Transverse furrows	Internal striae	Internal striae

ND: not determined.

Table III. – *Rhabdias nasutum* n. sp. and *R. brevicorne* n. sp. from *Calumma nasutum* and *Calumma brevicorne*, respectively, from the eastern region, Madagascar.

Collection number	Rhabdias identification	Anterior		Posterior		Migrating spermatozoa	Specimen number	Remarks
		Ovary	Oviduct	Ovary	Oviduct			
348 Q	<i>rabetafikae</i> n. sp.	290 (1,400)*	ND	0	+	Posterior ovary	1	Holotype
		120 (2,000)	0	135 (1,160)	+	0	2	
		0	0	0	0	0	3	Old
		0	+	140 (1,350)	+	Anterior ovary	4	
		ND	ND	120 (ND)	+	Posterior ovary	7	Posterior
		ND	ND	100 (ND)	+	0	8	Posterior
474 F	<i>nasutum</i> n. sp.	70 (1,900)	+	85 (1,000)	+	0	1	
		170 (1,350)	+	0	+	0	2	Holotype
498 F	<i>brevicorne</i> n. sp.	125 (1,750)	+	ND	+	0	1	Holotype
11G	<i>gemellipara</i>	100 (700)	+	0	+	0	1	Holotype
50 G	exp., 4.2	0	+	0	+	0	1	26 W f
		5	0	+	75 (780)	+	0	2
		5	0	+	0	+	3	28 W m
		4.2	0	+	0	+	4	27 W m
		3.4	0	0	0	0	5	19 W f, 2 L
		6.7	0	+	85 (1,050)	+	0	1
471 F		6	60 (700)	+	0	+	0	40 W m/f
		6.3	70 (700)	+	0	+	3	48 W m/f
		6.1	0	+	0	+	1	50 W, 2 L
481 F		6	30 (850)	+	40 (1,000)	+	0	43 W m
		6	65 (950)	+	0	+	2	43 W m
501 F		5.9	20-10*** (450)	+	0	+	Both ovaries	3
		6.4	0	+	0	+	0	40 W m
		6.2	0	+	50 (1,250)	+	0	2
		6.4	0	+	0	+	3	48 W m
		6.3	0	+	70 (1,900)	+	0	4
		6.2	0	+	0	+	5	34 W m
653 G**	<i>Rhabdias</i> sp. 6.8	0	+	0	+	0	2	19 W m
		6.7	15-25*** (1,300)	+	0	+	Both ovaries	3
		6.8	0	+	0	+	0	15 W m
		6.8	0	+	22-28 *** (1,000)	+	Posterior ovary	7
								12 W m

\* First number: length of the male genital region; number in brackets: distance male region to ovary apex. \*\*: Four arbitrarily chosen specimens.

\*\*\* Diameters of the syncytial structures containing spermatozoa type nuclei.

All specimens are gravid. W: eggs. m: morula type. f: folded embryo. L: larva. Worm length is reported for the small specimens. exp: expérimental infection. 653 G: 4 specimens taken as examples.

Table IV. – Male region and gametes in parasitic females of *Rhabdias* spp. from Malagasy chameleons.

terior oblique granulous segment present. Oesophagus: anterior part similar to *R. rabetafikae* n. sp.; constant diameter posterior to nerve ring; bulb slightly inflated; bulb ratio 0.66 & 0.35. Intestine: apical diameter equals bulb at base; proximal part, a short pyramid; no constriction in prerectal region. Tail straight, conical, gradually attenuated. Genital tract: anterior and posterior bend at mid-length of oviduct, anterior bend 2,350-2,300 from head, posterior bend 1,300-1,950 from tip of tail; oviducts not coiled; in ovaries mature ovocytes variable in shape, very elongated locally. Vulva ratio 0.54 & 0.53. Production area of male gametes in both specimens, in both and a single ovary, respectively; spermatozoa in oviducts (Table IV).

#### Taxonomic discussion

This material differs from *R. rabetafikae* n. sp. in that the buccal capsule is longer and its wall thinner, the

oesophagus is more slender (90-105 vs 135-150 at mid-length) and the body vesicle less developed with more numerous and regular transverse furrows. It differs from *R. gemellipara* in the larger buccal capsule (15-16/42-48 vs 7/15). It is distinct from African species: *R. jarki* and *R. okuensis* have a shorter and wider buccal capsule; in *R. cristati* the intestine apex is wider than the bulb; *R. chamaeleonis*, redescribed by Lhermitte-Vallarino & Bain (2004), has a longer buccal capsule (25-30 vs 15-16), a vesicle without furrows, a more conspicuous bulb, and a more anterior genital bend. We conclude that the specimens from *C. nasutum* represent a new species which we name *R. nasutum*.

#### *RHABDIAS BREVICORNE* N. SP.

Type host: *Calumma brevicorne* (Günther, 1879). Location in host: lungs.

Type locality: Ambavianasy, Périnet, Moramanga, eastern region, Madagascar.

Collection date: 10/02/1962.

Type specimen: 498 F, female holotype, in the MNHN collection.

Prevalence: not determined (one dissected host specimen reported).

Other specimen: 514 F, one female; Ifanadiana, Ifanadiana district, Ifanadiana subprefecture, eastern region, Madagascar.

Body very large (Table III; Fig. 1D, G, 2H-J, 3I, 4H, J). Habitus: dorsally coiled, G-shaped or one-and-half circle. Vesicle: extending from head to tail, smooth surface, but regular internal transverse striae 10 apart. Head: internal circular dense tissular structure surrounding the mouth (cephalic pads); mouth depressed, lined with cuticular velum. Buccal cavity slightly constricted at apex; buccal capsule: large, walls moderately thick and concave; buccal ratio 0.40 & 0.36; posterior oblique granulous segment. Oesophagus: similar to previous species anterior to nerve ring, slightly inflated at mid-length and constricted before bulb; bulb ratio 0.51 & 0.36. Intestine: apical diameter equals bulb at base; proximal part cylindrical; no constriction in prerectal region. Tail straight, posterior half thinner, funnel-shaped, long terminal point. Genital tract of holotype: anterior and posterior bend at first third of oviducts, anterior bend not far from head (1,700), but posterior bend far from tip of tail (3,500); oviducts not coiled; in ovaries mature ovocytes variable in shape, very elongated locally. Production area of male gametes in anterior ovary (Table IV). Female 514 F has a very black intestine and it was not possible to study the genital tract.

#### Taxonomic discussion

The material from *C. brevicorne* has a buccal capsule similar to that of *R. nasutum* n. sp., but differs from the latter by several characters: vesicle smooth with internal regular striae, oesophagus inflated at mid-length, intestine anterior part almost cylindrical, tail funnel-shaped, genital tract extends further anteriorly (1,700 to apex vs 2,350-2,500) and less posteriorly (3,500 to tip of tail vs 1,300-1,950). It differs from *R. rabetafikae*, *R. jarki* and *R. okuensis* in the shape of the buccal capsule, and from *R. gemellipara* in the buccal capsule size. It differs from *R. cristati* in a shorter buccal capsule (17-20 vs 30-42) and a wider intestinal apex, and from *R. chamaeleonis* in a longer oesophagus (1,280-1,330 vs 700-965) with a more conspicuous bulb, a vesicle without internal striae and a conical tail. We thus propose a new species, named *R. brevicorne*.

*R. gemellipara* Chabaud, Brygou & Petter, 1961 from *Calumma parsonii*

The type locality is Mantadia, Périnet district (11G); *Furcifer lateralis* trapped at Tananarive was used as experimental host (50G) (Figs 1A, 2A,B, 3G; Table V) Holotype: vesicle lost; head with cephalic pads; buccal capsule not flattened, almost cylindrical with reduced

<i>Rhabdias</i> species	<i>gemellipara</i>	<i>gemellipara</i>	<i>gemellipara</i>	<i>gemellipara</i>	<i>gemellipara</i>	<i>gemellipara</i>	<i>sp.</i>
Host species	<i>Calumma parsonii</i>	<i>Frucifer lateralis</i>		<i>Calumma brevicorne</i>			<i>Calumma brevicorne</i>
Geographic origin	Mantadia		Experimental infection	ND	<i>Fiheranana</i>	Périnet	Frandsdava
MNHN collection	11 G	50 G	471 F	481 F	486 F	501 F	653 G
Specimen	Holotype	1-5	1-3	1	1-3	1-5	1-14
Length (mm)	7.7	3.4-5.1	6-6.7	6.1	5.9-6	6.2-6.4	6.1-7.5
Width at mid-body	200	62-150	140-150	175	130-135	150-180	140-180
Buccal capsule length	7	6-7	5	5	5-6	5-7	5-6
Buccal capsule external diameter	15	14-15	14-17	16	16-17	15-17	16-17/9-12*
Oesophagus length	580	395-495	560-590	520	540-570	520-570	535-600
Oesophagus width at mid-length	42	38-48	40-45	45	40	42-45	35-43
Bulb diameter	65	56-78	65-75	75	65-75	70-75	60-70
Body width at bulb level	120	75-110	95-105	110	95-110	100-110	85-130
Vulva (mm)	3.9	1.8-2.5	3.2-3.6	3.5	3.2	2.7-3.4	3.2-3.9
Anterior genital bend-head	1,500	850-1,200	1,470-1,725	1,350	1,400-1,620	1,320-1,560	1,130-1,790
Posterior genital bend-tip tail	1,100	560-880	850-910	520	520-870	820-870	700-1,180
Tail	268	165-220	260-270	295	230-270	220-240	200-250
Width at anus (in lateral view)	80	32-55	45-50	50	45-50	40-50	40-50
Nerve ring	180	175-200	195-205	200	180-200	190-230	185-245
Number of eggs at morula stage	37	19-42	40	50	40-43	34-48	12-28

Individual values or range of measurements are reported. \* Lateral/dorso-ventral diameter.

Table V. – *Rhabdias gemellipara* from *Calumma parsonii*, *Furcifer lateralis* and *Calumma brevicorne* in the eastern region, Madagascar and *Rhabdias* sp. from *Calumma brevicorne* in the central region, Madagascar.

diameter anteriorly; apex of oesophageal lumen lined with rugosities. Other characters are as described by Chabaud *et al.* (1961). Production area of male gametes and spermatozoa were identified (Table IV; Fig. 4 A, B). Five specimens from one-month old experimental infection: habitus straight; body vesicle absent in two specimens, thin in remaining three with distant, loose folds, as described for holotype by Chabaud *et al.* (1961). Morphology similar to holotype, particularly the cylindrical buccal capsule, as drawn in apical view by Chabaud *et al.* (1961), with anterior diameter generally smaller, cephalic pads present in all four observed specimens and rugosities at apex of oesophageal lumen. Excretory cell often identified, 600-650 long. Tail straight with thin point. All five females with divided eggs, the smallest (3.4 mm long) with only 19 eggs but one larva, the others (4.22-5.07 mm long) with 26-42 divided eggs and no larvae. Production area of male gametes in ovaries observed in one female, and spermatozoa in oviducts of four females (Table IV). Two stages of the male area were identified: the numerous cells of the band were jointed together as usually observed (Fig. 4A); or cells were few, with irregular spaces between them, suggesting termination of a period of spermatozoa production.

#### Small specimens of *Rhabdias* spp. from *Calumma brevicorne*

Body about 6-7 mm long in all five samples (Table V), but two distinct morphological types can be distinguished. Samples from the East of Madagascar are similar to *R. gemellipara*: 481 F, 486 F, both from Fiherana, and 501 F from Périnet, as well as 471 F with unknown origin. In particular they share the internal circular dense tissular cephalic pads, more conspicuous in lateral than in median view, and a cylindrical buccal capsule with decreased anterior diameter; number of divided eggs ranged from 34 to 50, and two larvae were present in a single female (481 F). In each sample, a production area of male gametes was observed, and in two specimens spematozoa were seen between this area and the oviducts (Table IV).

Sample 653 G from the central region, composed of 14 specimens, was different (Fig. 3J, M): buccal capsule laterally flattened (except in one specimen) and more distant from apex (approximately 10 µm). In front view amphids identified; four papillae difficult to observe, tentatively illustrated (Fig. 3G). Rounded shoulders of oesophageal apex enveloping entire length of buccal capsule, either terminating at its apex or extending slightly beyond; cephalic pads absent (Fig. 3J-L). Despite these specimens being slightly longer than those in the above four groups, they had few divided eggs (12-28) and no larvae. In specimens of 653 G the male area, when present, was small, composed of one or two syncytial structures, 15-25 µm in diameter, contain-

ning a dozen of nuclei similar to those of spermatozoa. In addition, spermatozoa were observed along the ovaries (Table IV).

Sample 653 G is obviously distinct from *R. gemellipara* and other Malagasy species, but because the females are not fully gravid, we do not assign a specific name to them.

## DISCUSSION

We consider the large specimens from Malagasy chameleons distinct from *R. gemellipara*, host type *C. parsonii* from the eastern region, because in African *Rhabdias* the size of the buccal capsule in a given species remained constant despite an important growth in body length (Table VI). In *R. gemellipara* and the large specimens examined herein (3.4-8 and 19-34 mm, respectively), the buccal capsules differ hugely in size. None of the samples in the MNHN collection had a mixed infection with small and large specimens.

Not all large specimens were identical. The buccal capsule of *R. rabetafikae* n. sp. from *C. cucullatum* in the northeastern region of Madagascar has a particular shape and it is obviously a distinct species. While the material was recovered from a single chameleon only, it harboured five specimens, all of which were similar. The material is poor for *R. nasutum* n. sp. from *C. nasutum* and *R. brevicorne* n. sp. from *C. brevicorne*, both from the eastern region. However, we observed distinct differences regarding the vesicle, buccal capsule and shape of the oesophagus, apex of the intestine as well as the tail, and thus, they cannot be placed in the same taxon. The small specimens appeared to be heterogeneous as well. The four samples from the eastern region are similar amongst each other and share important diagnostic characters with *R. gemellipara*. We therefore assign them to the latter. Sample 653 G from the central region is distinct from *R. gemellipara* in that cephalic pads are absent and the buccal capsule is laterally flattened. Moreover, inspite of similar or slightly longer body length their stage of maturation is retarded (Tables IV & V). Despite these distinct characteristics, we report this material as *Rhabdias* sp. until fully gravid females can be observed.

In Africa the four species of *Rhabdias* from chamaeleonids are large worms. This study suggests that in Madagascar small and large species of *Rhabdias* exist in chameleons. The ontogeny of small and large species seems to differ. Specimens of *R. gemellipara* obtained through experimental infection are very small ( $\leq 5.4$  mm) but are at an advanced stage of maturation, as evidenced by the presence of divided eggs and even larvae. In contrast, in *R. chamaeleonis* from Burundi three young females, 5.7-6.4 mm long, had no

<i>Rhabdias</i> species	Body length	Buccal capsule		References
		Length	External diameter	
<i>chamaeleonis</i>	5.7-6.4	20-25	30-38	Lhermitte & Bain, 2004
	12-13.5	21-30	38-50	
	17.22-22.85	25-30	42-45	
<i>okuensis</i>	3.9	8	38	Lhermitte <i>et al.</i> , 2008
	4.5	7	38	
	5.5	9	38	
	15.8	10	35	
	18.15	10	45	
<i>cristati</i>	7.6	30	58	Lhermitte <i>et al.</i> , 2008
	12.5	38	61	
	15	35	62	
<i>gemellipara</i> type Experimental	8	7	15	Chabaud <i>et al.</i> , 1961
	3.4-5	6	14	
<i>rabetafikae</i> n. sp.	22.8-26.33	11-13	49-49	Present study
<i>nasutum</i> n. sp.	19.8	22	42	Present study
<i>idem</i>	20.56	20	48	
<i>brevicorne</i> n. sp.	27.6	22	42	Present study
<i>idem</i>	34.5	30	40	
<i>gemellipara</i> 481 F <i>gemellipara</i> 486 F <i>gemellipara</i> 501 F	5.9-6.7	5-6	14-17	Present study
	5.92	6	16	
	6.4	7	15	
sp. 653 G	6.7-6.8	4-5	13-17	Present study

Grey: young/small specimens.

Table VI. – Body length and size of buccal capsule in young/small (grey background) and main from African and Malagasy chameleonids.

ovulae, few ovulae and nine ovulae in the oviducts, respectively, and no eggs were seen in the uteri (Lhermitte-Vallarino & Bain, 2004). Two young *R. okuensis* females from Cameroon, 4 mm long, had no eggs in the uteri, and three eggs were found in the uterus of a third female, 6 mm long (Lhermitte-Vallarino *et al.*, 2008).

Concerning the biology of the parasitic females of Malagasy *Rhabdias* spp., hermaphroditism appears to be the mode of reproduction since each sample contained at least one specimen with spermatozoa. In both large and small females, a band of small cells was observed in either one or both ovaries at a distance of approximately one mm from the ovary apex (Table IV). We had previously observed bands of small cells in the ovaries of *Rhabdias* spp. from Africa and suggested that they might produce spermatozoa. We give here proof thereof: spermatozoa which were interspersed between the large oocytes in some ovaries indicate that these small cells are indeed areas of male gamete production (Fig. 4). Male gametes were found in young and old specimens and are produced throughout the females' life span. In less gravid females (653 G) and a few others (486 F), the production area was not a band, but a few small syncytial structures, as observed in young specimens of *R. okuensis* (Lhermitte *et al.*,

2008). The period of production of a band is discontinuous. Both stages of active spermatozoa production and regression of the male area, could be distinguished. Generally the production of spermatozoa was not synchronized in the anterior and posterior ovaries.

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