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New species of *Rhabdias* (Nematoda: Rhabdiasidae) from Afrotropical anurans, including molecular evidence and notes on biology

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Abstract: Despite the small sample size the diversity of *Rhabdias* Stiles et Hassall, 1905 from anurans in the Afrotropical region was found to be high. Four species were collected from four localities, one in South Africa, two on Cameroonese mountains and one in Madagascar: *Rhabdias picardiae* sp. n. from the bufonid *Amietophrynus gutturalis* (Power); *Rhabdias ohlerae* sp. n. and *Rhabdias tanyai* sp. n. from the arthroleptids *Leptopelis brevirostris* (Werner) and *Astylosternus rheophilus* Amiet, respectively; and *Rhabdias vencesi* sp. n. from the mantellid *Boophis madagascariensis* (Peters). Distinctive characters between these species are numerous and obvious, based on body size, shape and size of the buccal capsule, arrangement of head papillae, and shape and size of the oesophagus and intestinal apex. Molecular data based on 500 bp of 12S rDNA and 600 bp of *coxI* of three of the four species are presented. *Rhabdias vencesi* resembles *Rhabdias madagascariensis* Chabaud, Brygoo et Petter, 1961 from an African ptychadenid introduced on Madagascar, but differs in body size and head morphology. The remaining new species are clearly distinct from those previously known from Afrotropical anurans. Outside the Afrotropics, some *Rhabdias* species present characters similar to those observed in the new species, but they all differ in various other characters. No clear correlation was seen between *Rhabdias* species and families of anuran hosts in this region. However, the narrow buccal capsule seen in *Rhabdias* species from Afrotropical lissamphibians opposes them to the majority of *Rhabdias* parasitic in chamaeleonids. Furthermore, the infective larva of *R. vencesi* has a conical pointed tail, while those of *Rhabdias* from chameleons have a rounded tail tip ornated with a few buds.

Keywords: parasitic nematodes, *Rhabdias*, molecular characterisation, taxonomy, anurans, *Amietophrynus*, *Astylosternus*, *Boophis*, *Leptopelis*, Afrotropical region

Of the 39 species of *Rhabdias* Stiles et Hassall, 1905 parasitising anuran hosts (Baker 1987a, Bursey et al. 2003, Kuzmin et al. 2007, Martínez-Salazar and León-Règagnon 2007, Martínez-Salazar 2008, Kuzmin and Tkach 2009), only three have been described in the Afrotropical region, one from Ptychadenidae in Madagascar (Chabaud et al. 1961), another from Arthroleptidae in Tanzania (Baker 1987b) and a third from two members of Bufonidae in South Africa (Kuzmin 2001). Close to this region, in Egypt, *Rhabdias* was reported from a bufonid with a sub-Saharan distribution, the parasites being either assigned to a European species (Moravec et al. 1987), or left unnamed (Saad et al. 2009). A fifth *Rhabdias* species (Baylis 1929) is a parasite of Gymnophiona, a small group of lissamphibians.

Recently, renewed interest in the diversity of *Rhabdias* in the Afrotropical region led to detailed studies concentrating on hosts belonging to the Chamaeleonidae, a group of saurian hosts found to harbour a wide variety of lung worm species (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a, b).

During the past two years, lung worms were recovered from four species of Afrotropical anurans belonging to the Bufonidae, Arthroleptidae and Mantellidae. Morphological analysis of their *Rhabdias* fauna suggested the presence of a distinct and new species in each of the four hosts. In this paper, we give a morphological description of the new taxa and provide a molecular characterisation based on the 12S rDNA and mitochondrial cytochrome *c* oxidase subunit 1 (*coxI*) genes of three of the four species.

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In order to assess the mode of reproduction, the genital tracts of the parasitic females of each species were examined as in previous studies (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a, b). Free-living stages of one of the species were obtained through faecal culture and their development and morphology described. The aim of this study was not only to estimate *Rhabdias* diversity among lissamphibians in the Afrotropical region, but to collect additional information on species from this host group to elucidate their relationship with parasites from chamaeleonids.

MATERIALS AND METHODS

The hosts. The nomenclature of amphibian hosts follows that of Frost (2009). Frost et al. (2006) placed former members of the genus *Bufo* Laurenti (Bufonidae) in several genera, amongst others *Bufo*, *Rhinella* Fitzinger, *Amietophrynus* and *Duttaphrynus*, the latter two named by Frost et al. (2006); at the same time, several species were synonymized. In order to facilitate comparison with existing literature reports, the new and previous names are given here, as well as in the taxonomic discussions. *Bufo americanus* Holbrook is now listed as *Anaxyrus americanus* (Holbrook), *Bu. garmani* Meek, *Bu. gutturalis* Power, *Bu. regularis* Reuss and *Bu. maculatus* Hallowell have been placed into the genus *Amietophrynus*, and *Bu. melanostictus* Schneider is now listed as *Du. melanostictus* (Schneider) (Frost 2009). Kloss (1971) recorded '*Bufo typhonius* (L.)' from Belém, Pará, Brazil, as the type host of *R. androgyna* Kloss, 1971, but Frost et al. (2006) have subsequently split the *Bu. typhonius* complex into several genera and species. Given the geographic distribution of Kloss' (1971) specimens, it would seem most likely that his material belonged to what is currently known as *Rh. margaritifera* (Laurenti), a common South American toad occurring throughout Amazonian South America and eastern Panama (Frost 2009). Similarly, Travassos (1926) described *R. fülleborni* Travassos, 1926 from '*Bufo marinus* L.' from Brazil. However, several subspecies of the *Bu. marinus* complex have been synonymized and placed into different species within the genus *Rhinella*. Since Travassos (1926) listed Linnaeus as host authority, it appears most likely that he was referring to a host now listed as *Rh. marina* (Linnaeus) by Frost (2009). To avoid confusion, host genera are abbreviated using the first two letters of the generic name.

In September 2008, the lungs of five *Am. gutturalis* from South Africa were examined for the presence of *Rhabdias* by one of the authors (K.J.; no permit required). Several worms were recovered, fixed and stored in absolute ethanol. Two *Boophis madagascariensis* (Peters) (Mantellidae), imports from Madagascar, were necropsied; worms were fixed in hot 70% ethanol, except for two that were transferred directly into absolute ethanol for molecular analysis.

The remaining material was collected in Cameroon by N. L.-V. and I.I., while collaborating on a biodiversity project in this region (Lhermitte-Vallarino et al. 2008). Two arthroleptid species were captured on two distinct mountains in the Cameroonese volcanic chain, a single *Leptopelis brevirostris* (Werner) from Mount Cameroon and a single *Astylosternus rheophilus* Amiet from Mount Oku. Each frog harboured a single lung worm. The specimen from *As. rheophilus* was drawn alive under a stereomicroscope. Subsequently, a part of its mid-section was removed and fixed directly in absolute ethanol for molecular analysis. Both the anterior and posterior parts were fixed in hot 70% ethanol. The collection and exportation of specimens had been authorized by the Cameroon Ministry of Forests and Fauna. Hosts from Cameroon and Madagascar are kept in the National Collection of the Muséum National d'Histoire Naturelle (MNHN), Paris. Anurans from Cameroon were identified by A.M. Ohler, MNHN.

Morphological analysis. Specimens were cleared in lactophenol and observed under a compound microscope equipped with a drawing tube. Measurements were taken from drawings and are given in micrometres unless otherwise stated. Width of buccal capsule corresponds to its maximum external diameter, and buccal capsule length excludes the more or less distinct granulous posterior segment as described in Lhermitte-Vallarino et al. (2009b). Based on previous studies (Tkach et al. 2006, Lhermitte-Vallarino et al. 2008, Martínez-Salazar 2008), four ratios are provided: percentage of oesophagus length and tail length of total body length (TBL), distance from apex to vulva as percentage of TBL, and buccal capsule ratio (buccal capsule length/buccal capsule width). Two measurements regarding body width are given, the first indicating body width excluding the vesicle followed by the one including it in parentheses. Presented in the text is the type-series range of measurements for each morphological character. Individual measurements of the holotype and paratypes are listed in Tables 1–3. Ovaries were examined for the presence of a male gamete production area, named testis zone, according to Runey et al. (1978).

Molecular analysis. Specimens analysed in this study were stored in accordance with procedures specified in the Biorepositories initiative (http://www.biorepositories.org) and belong to the collection identified as 'zpl' of Milano Bicocca institution (MIB). For molecular analysis freshly recovered worms were transferred directly into absolute ethanol and kept refrigerated at 4°C: one paratype, and mid-section of another paratype 52 YU from *Am. gutturalis* (specimen vouchers MIB:zpl:01516 and MIB:zpl:01517); two fragments of one paratype 97 NL from *Bo. madagascariensis* (specimen vouchers MIB:zpl:00226 and MIB:zpl:00227), two fragments of a second paratype 97 NL (specimen voucher MIB:zpl:00228 and MIB:zpl:00229) and an additional female 99 NL (specimen voucher MIB:zpl:00230); mid-section of holotype 74 NL from *As. rheophilus* (specimen voucher MIB:zpl:00190). DNA extracts were prepared using 5 PRIME, ArchivePure DNA Purification Kit. *Rhabdias* species *coxI* amplification and sequencing were obtained following Folmer et al. (1994) using the primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'); 12S rDNA sequences were generated following Casiraghi et al. (2004) using the primer pair 12SF (5'-GTTCCAGAATAATCGGCTA-3') and 12SdegR (5'-ATTGACGGATGRTTTGTACC-3'). PCRs were performed in a volume of 20 µl under the following final conditions: 1X buffer including 2.5 mM $MgCl_2$ (MasterTaq kit, Eppendorf™), 0.2 mM of each dNTP, 1 µM of each forward and reverse primers and 1U of DNA polymerase (MasterTaq kit, Eppendorf™). The amplicons obtained are approximately 600 and 500 bp long, respectively. PCR products were gel-purified using the 5 PRIME, GelElute Extraction Kit and sequenced directly using ABI technology. Sequences were checked by eye with

BioEdit sequence alignment editor (version 7.0.5; Hall 1999) using GenBank sequences as reference sequences (FM179476–FM179479; FN395318–FN395323) and unambiguously aligned using ClustalX (Thompson et al. 1997). The *Rhabdias* species sequences are deposited in the EMBL Data Library under the following accession numbers: *R. picardiae* sp. n. 12S rDNA FN434093–FN434094, *coxI* FN434095–FN434096; *R. vencesi* sp. n. 12S rDNA FN434097–FN434101, *coxI* FN434102–FN434106; *R. tanyai* sp. n. 12S rDNA FN434097–FN434101, *coxI* FN434102–FN434106. The alignments were analysed using distance matrix method (i.e. neighbour joining). Nucleotide distances have been calculated using MEGA 4.0 (Tamura et al. 2007) – options: nucleotide, Kimura 2-parameter (K2P), complete deletion, standard error computation by bootstrapping 500 replicates.

***Rhabdias* biology.** For life-cycle studies of *R. vencesi*, four petri dish cultures were set up as detailed in Lhermitte-Vallarino and Bain (2004), using material collected from *Bo. madagascariensis* (99 NL) and distilled water with active charcoal as culture medium. Cultures were kept at room temperature and checked regularly for developing stages for several days. Two cultures were made from eggs released from a female *in vitro*, one from larvae recovered from the lungs and one from faecal larvae extracted from the rectum of the host. Only the latter yielded developing worms.

RESULTS

Rhabdiasidae Railliet, 1916

Rhabdias picardiae sp. n. Junker, Lhermitte-Vallarino et Bain Figs. 1A–F, 3A–F, Table 1

Small worms with variable dorsal bend. Body 8.0–8.35 mm long, 460(530)–580(600) wide at mid-length. Cuticular vesicle with irregular folds and indentations along entire length of body, including tip of tail (Fig. 1C, D); in median view, vesicle attached laterally to inner layer of cuticle via conspicuous fibres associated with subcuticular pores (Fig. 1B); at anterior extremity more or less pronounced inflation of cuticle extending posteriorly to approximately level of oesophageal-intestinal junction; vesicle less conspicuous in mid-section and again more pronounced in tail-region. Lateral chords thick, filling body cavity in anterior region (Fig. 1B, E).

Head with six papillae in apical view (Fig. 3C, D). Four submedian papillae arranged in square, anterior to lemon-shaped oral opening (Fig. 3D); each papilla with salient sensillum and slightly protruding base. Two very small lateral papillae posterior to submedian papillae level. Amphidian canals barely visible. Cuticular velum limiting oral opening, its lumenal edge drawn out into a sharp tip in longitudinal section (Fig. 3A, B).

Lumen of buccal cavity round in transverse section (Fig. 3E), triangular at oesophageal junction. Vestibulum short, 5–7 long. Buccal capsule wall triangular in longitudinal section, 7–10 long and 23–25 wide, with granulous posterior segment where it joins oesophagus; buccal capsule ratio 0.3–0.4; inner edge of buccal capsule convex, and slightly serrated in some specimens (Fig. 3A); outer edge with more or less distinct internal refringent zone; anterior apex meets velum in a pointed tip (Fig. 3A, B). Oesophagus without shoulders, its anterior edge rarely reaching mid-level of buccal capsule. Oesophagus 690–790 long (8.6–9.9% of TBL), slender, of equal width along anterior 2/3 of its length, then gradually widening into slim bulb; width at oesophagus mid-length 60–70, bulb 95–130 wide (Fig. 1B). Body width at bulb 160(180)–300(360). Nerve ring observed in two specimens, at 235 and 250 from anterior extremity. Excretory pore not identified, obscured by cells of lateral chord (Fig. 1B). Apex of intestine wider than bulb at oesophageal-intestinal junction; intestine occupying entire width of specimen anteriorly (Fig. 1A); often filled with dark contents.

Genital tract amphidelphic; genital bend in ovaries (Fig. 1C, F); anterior genital bend at 1375–2150 from anterior extremity, posterior genital bend at 490–600 from posterior extremity. Oviducts and proximal end of ovaries folded upon themselves. In a single specimen, uterus obscuring posterior genital bend and extending posteriad past anus, ending at 265 from tip of tail. Male gametes with light-reflecting nuclei seen in anterior and/or posterior oviduct of several specimens (Fig. 1F). Vulva without salient lips, slit-like, at 4535–5050 from head (55–62% of TBL). Uterus thin-walled, filled with numerous eggs, most of which contain first-stage larvae. Mature eggs 100–120 long and 60–69 wide (n = 10).

Tail 270–350 long, reaching 3.5–3.8% of TBL, tapering quickly to rounded tip. Body width at anus 120(240)–225(440). Caudal vesicle voluminous with irregular indentations; terminal, elliptical to lance-shaped segment surrounding tip of tail. Tip of tail with patch of fine granular material in 5/7 specimens (Fig. 1D).

Type host: *Amietophrynus gutturalis* (Power) (= *Bufo gutturalis* Power) (Bufonidae), 52 YU.

Type locality: Pretoria (25.66S, 28.15E), Gauteng Province, South Africa. Collection date: 01 September 2008.

Site of infection: Lungs.

Prevalence and intensity: 2/5 hosts infected with five (52 YU) and three (53 YU) parasites, respectively.

Type material: 52YU. Deposited in the MNHN collection: holotype female, one entire paratype female, two paratype females with anterior and posterior parts. One paratype female and mid-section of another used for molecular analysis; specimen vouchers MIB:zpl:01516 and MIB:zpl:01517. One paratype (N-938) in the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic. EMBL Data Library accession numbers: 12S rDNA FN434093–FN434094, *coxI* FN434095–FN434096.

Additional material: 53YU. Deposited in the MNHN collection.

Etymology: The species is named after Dr. Jackie A. Picard, who kindly made the hosts available to us.

Table 1. Morphometric characters of *Rhabdias picardiae* sp. n. from *Amietophrynus gutturalis* (Power) (Bufonidae) in South Africa. All measurements in micrometres unless otherwise indicated.

	Holotype	Paratypes					
MNHN collection	52 YU	52 YU	52 YU	52 YU	53 YU	53 YU	53 YU
Specimen	2	1	3	4	1	2	3
Length (mm)	8.15	8.0	–	–	8.0	8.35	–
Width at mid-body (with vesicle)	460 (530)	470 (515)	–	–	510 (580)	580 (600)	–
Vestibulum depth	7	5	6	–	6	5	6
Buccal capsule length	10	8	10	9	7	8	8
Buccal capsule max. external diameter	23	24	23	25	24	23	24
Buccal capsule ratio	0.4	0.3	0.4	0.4	0.3	0.3	0.3
Oesophagus length	720	700	710	690	790	720	–
Oesophagus width at mid-length	60	60	60	70	60	70	–
Bulb diameter	105	95	100	95	120	130	115
Body width at bulb (with vesicle)	170 (195)	160 (180)	280 (325)	265 (300)	205 (320)	300 (360)	240 (310)
Head to vulva	5050	4900	–	–	4535	4575	–
Anterior genital bend – head	2150	2000	–	1700	2075	1375	–
Posterior genital bend – tip of tail	600	540	490	–	600	265[a]	–
Tail length	300	280	270	350	300	300	–
Width at anus (with vesicle)	120 (240)	120 (290)	225 (440)	170 (220)	160 (250)	170 (250)	–
Apex to nerve ring	–	–	250	–	235	–	–
Oesophagus length as % of body length	8.8	8.8	–	–	9.9	8.6	–
Head to vulva as % of body length	62.0	61.3	–	–	56.7	55.0	–
Tail length as % of body length	3.7	3.5	–	–	3.8	3.6	–

[a]Uterus obscures posterior genital bend (pgb) and extends further posteriad than pgb.

Remarks. The first species to be compared with the present material are the four *Rhabdias* recorded from Afrotropical anurans (Table 3): *R. africanus* Kuzmin, 2001, type host *Am. maculatus* (Hallowell) (= *Bu. maculatus*), South Africa; *R. bufonis* (Schrank, 1788), assigned by Moravec et al. (1987) to lung worms recovered from *Am. regularis* (Reuss) (= *Bu. regularis*) in Egypt; *R. collaris* Baker, 1987, type host *Le. vermiculatus* (Boulanger) (Arthroleptidae) in Tanzania; and *R. madagascariensis* Chabaud, Brygoo et Petter, 1961, type host *Ptychadena mascareniensis* (Duméril et Bibron, 1841) [= *Rana* (*Ptychadena*) *mascareniensis*] (Ptychadenidae) in Madagascar.

Rhabdias africanus has a longer body (12.45–19.8 vs. 8–8.35), relatively shorter oesophagus (3.5–4.7% vs. 8.6–9.9%), twice longer buccal capsule (15–20 vs. 7–10) and two lateral pseudolabia (Kuzmin 2001), which we, however, consider simple swellings at the level of the amphids.

Rhabdias bufonis is in the following referred to as *R. bufonis* sensu Moravec, Baruš et Ryšavý, 1987, because the parasites from *Am. regularis*, a host with a strictly Afrotropical distribution (Frost 2009), differ from the redescriptions of Travassos (1930) and Hartwich (1972). The latter had been based on specimens recovered from European bufonids and ranids, as was the original description (not available). The main distinctive character is the size of the buccal capsule, which is of diagnostic importance (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008); irrespective of body size, it is 15 long and 21 wide in specimens from Egypt (Moravec et al. 1987) vs. 9–12 long and 8–10 wide in European specimens (Hartwich 1972). *Rhabdias bufonis* sensu Moravec, Baruš et Ryšavý, 1987 has a longer buccal capsule than *R. picardiae*, a shorter oesophagus (510 vs. 690–790) and a longer body (13.02 mm vs. 8.35 mm).

Rhabdias collaris has similar structures attaching the vesicle to the body, but its head is conspicuously thickened by peripheral muscle bundles, its oesophagus is shorter and shaped differently (Baker 1987b).

Rhabdias madagascariensis is shorter, 3.55 mm long, the oesophagus is club-shaped with a distinct dilatation anterior to nerve ring and the intestinal apex is narrower than the base of the oesophagus (Chabaud et al. 1961).

Rhabdias bdellophis Baylis, 1929 was described from the caeciliid *Scolecomorphus vittatus* (Boulanger) (= *Bdellophis vittatus*) (Gymnophiona), in Tanzania. It is distinct from *R. picardiae* in the "very vaguely defined buccal capsule", the about three times longer tail and slightly larger body (Baylis 1929) (Table 3).

Compared to the *Rhabdias* species from anuran hosts world-wide, *R. picardiae* is close to *R. americanus* Baker, 1978 from *Anaxyrus americanus* (Holbrook) (= *Bu. americanus)* (Bufonidae) in Canada in that the oesophagus is 770–870 long with a slightly inflated bulb, and its tail is entirely enclosed in the cuticular vesicle (Baker 1978, Kuzmin et al. 2003). However, *R. americanus* is distinct in having a thin tail (length/width ratio about 6 vs. 1–2.25), longer body (10.7–14.1 mm), well-developed lateral pseudolabia, a narrower buccal cavity (12–15 vs. 23–25), a much deeper vestibulum and a thicker vesicle (Baker 1978).

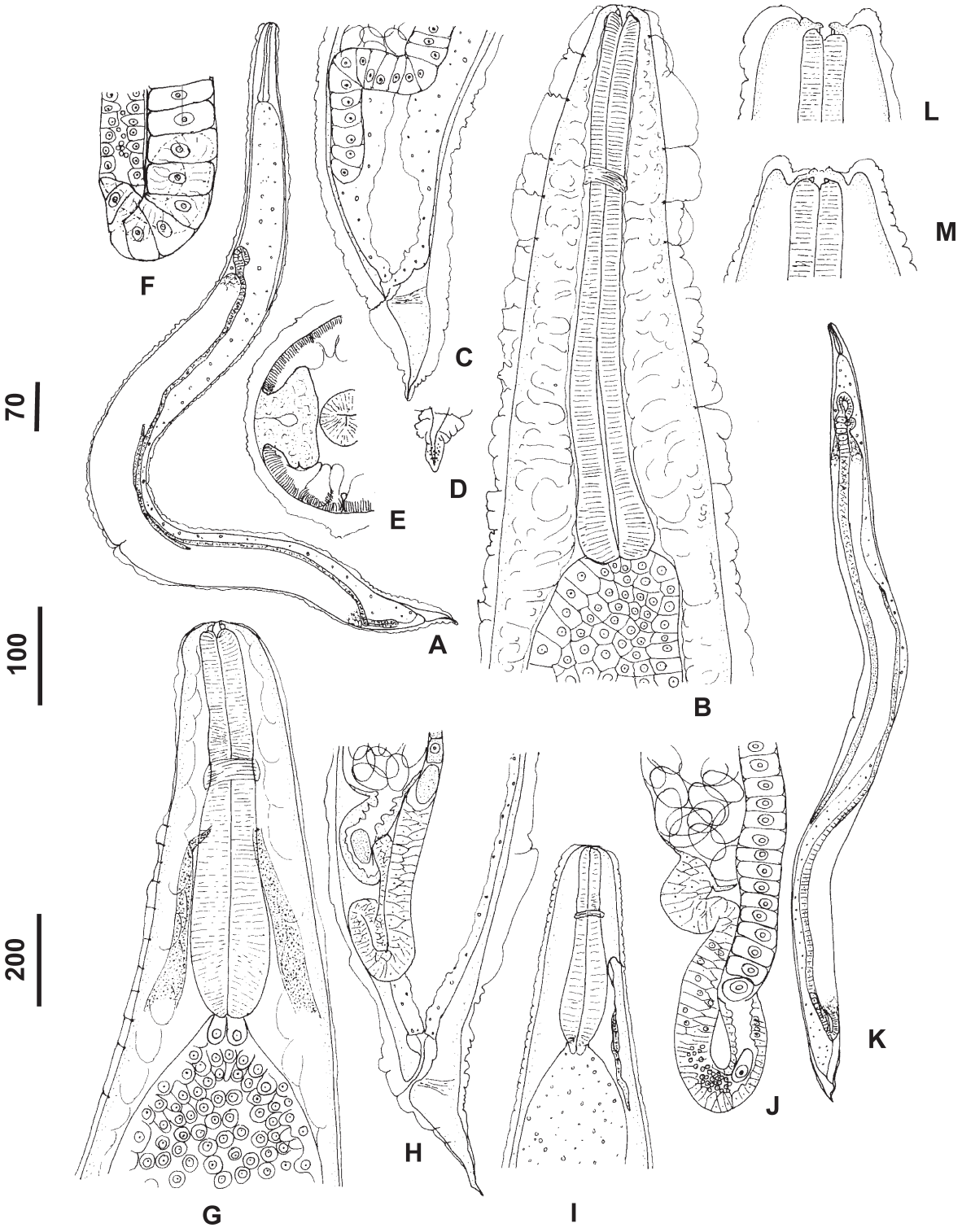

Fig. 1. A–F. *Rhabdias picardiae* sp. n. from *Amietophrynus gutturalis* in South Africa. **A –** habitus, left lateral view, holotype; **B –** anterior region, median view; **C –** posterior region, left lateral view; **D –** caudal extremity; **E –** transverse section at level of oesophagus, half body representation showing one large lateral chord; **F –** bend of terminal ovary and a part of oviduct with spermatozoa in the lumen, remainder of oviduct tightly coiled and hidden behind ovary. **G–M.** *Rhabdias vencesi* sp. n. from *Boophis madagascariensis* in Madagascar. **G –** anterior region, median view, with proximal part of excretory glands; **H –** posterior region, left lateral view; **I –** anterior region of another specimen, before fixation, with excretory glands and pore, lateral right view; **J –** end of posterior ovary, oviduct with ovulae and spermatozoa-like cells, and beginning of uterus; **K –** habitus, left lateral view; **L**, **M** – head of two females, more or less invaginated. Scale bars: A, body length 8.15 mm; B, D, G = 100 µm; C, H, I, J = 200 µm; E, F, L, M = 70 µm; K, body length 12 mm.

Table 2. Morphometric characters of *Rhabdias vencesi* sp. n. from *Boophis madagascariensis* (Peters) (Mantellidae) in Madagascar. All measurements in micrometres unless otherwise indicated.

	Holotype	Paratypes								Additional material		
MNHN collection	97 NL	97 NL	97 NL	97 NL	97 NL	97 NL	97 NL	97 NL	97 NL	99 NL	99 NL	99 NL
Specimen	8	1	2	3	4	5	6	7	9	1	2	3
Length (mm)	12.0	11.4	13.2	12.1	13.1	11.8	9.9	11.3	–	9.5	10.5	9.3
Width at mid-body (with vesicle)	600 (630)	680	620	600	700	770 (810)	680 (700)	640	–	570 (600)	630 (650)	560 (580)
Vestibulum depth	6	10	–	6	–	6	–	5	–	10	12	6
Buccal capsule length	6	10	–	9	6	6	7	7	6	6	6	5
Buccal capsule max. external diameter	20	20	–	22	20	19	20	21	20	22	22	19
Buccal capsule ratio	0.3	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3
Oesophagus length	525	550	520	550	550	570	540	530	550	500	495	540
Oesophagus width at mid-length	80	80	–	80	80	85	80	80	80	72	72	80
Bulb diameter	120	120	110	100	120	110	120	120	120	100	95	110
Body width at bulb (with vesicle)	270 (290)	230	210 (240)	250 (280)	270 (290)	300 (325)	260 (280)	250 (270)	340 (370)	240 (280)	250 (300)	220 (250)
Head to vulva	6150	6400	6400	6490	6650	6000	4850	5500	–	5050	5350	4600
Anterior genital bend – head	1150	620	1000	1130	940	1030	650	930	1100	930	1250	620
Posterior genital bend – tip of tail	1130	–	1130	1070	–	1060	1080	900	–	700	1075	1100
Tail length	340	315	320	260	300	305	350	290	–	230	375	240
Width at anus (with vesicle)	140 (180)	140 (320)	120 (170)	140 (160)	150 (170)	110 (140)	150 (220)	130 (155)	–	120 (155)	135 (145)	100 (130)
Apex to nerve ring	200	200	–	185	190	180	180	205	–	195	210	190
Oesophagus length as % of body length	4.4	4.8	4.0	4.5	4.2	4.8	5.5	4.7	–	5.3	4.7	5.8
Head to vulva as % of body length	51.5	56.0	48.7	53.7	50.8	50.8	49.2	48.7	–	53.2	51	49.5
Tail length as % of body length	2.8	2.8	2.4	2.1	2.3	2.6	3.5	2.6	–	2.4	3.6	2.6

Rhabdias vencesi sp. n. Junker, Lhermitte-Vallarino et Bain Figs. 1G–M, 3G–K, Table 2

Body more or less straight or slightly bent dorsally, 9.5–13.2 mm long. Width at mid-body 560(580)–770(810). Cuticle: vesicle more conspicuous in cephalic region, with internal transverse striae that are spaced at irregular intervals of approximately 20–30 and 10–20 in two specimens measured; thin or indistinct along body, but slightly more prominent in tail region.

Head with six papillae in apical view; four large, mammilate submedian and two smaller lateral papillae, each with terminal transparent cuticular apex and sensory sensillum; lateral papillae slightly posterior, submedian papillae directly adjacent to oral opening; amphids open on external surface of lateral papillae (Fig. 3H, I). Oral opening dorso-ventrally flattened, oval to spindle-shaped; velum absent. Vestibulum 5–12 long, difficult to discern in specimens with more or less invaginated head, resulting in wide range of measurements. Buccal capsule obscured by thickened apex in some invaginated specimens; no posterior segment identified; length 5–10, width 19–22, roughly triangular in longitudinal section, round in apical view; lumen cylindrical. Buccal capsule ratio 0.3–0.5. Oesophagus with shoulders, its apex extending anteriorly to mid-level or apex of buccal capsule; stout, club-shaped, 495–570 long, reaching 4.0–5.8% of TBL (Fig. 1G). Oesophagus width at mid-length 72–85, gradually widening to maximum width of 95–120 at bulb. Body-width at bulb level 210(240)–340(370). Slight dilatation of oesophagus immediately prior to nerve ring. Nerve ring at 180–210 from head. Excretory pore observed in one specimen before fixation (Fig.1I); anterior part of excretory glands identified (Fig. 1G); at 205 from anterior extremity. Apex of intestine bell-shaped, narrow at level of oesophageal-intestinal junction, but rapidly widening posteriorly to fill almost entire width of body (Fig. 1I); anterior intestinal wall thick, with conspicuous, densely crowded, large pear-shaped cells (Fig. 1G); cells take on a more conventional shape at approximately level of anterior genital bend. Posterior part of intestine usually filled with dark brown contents.

Genital tract amphidelphic, posterior and anterior genital bend at level of muscular oviduct (Fig. 1H, J); anterior genital bend at 620–1250 from head; posterior genital bend at 700–1130 from tip of tail. Oviducts and proximal end of ovaries folded upon themselves. Numerous small, globular cells, identified as male gametes, seen in lumen of anterior oviduct of five specimens, in which this character was studied (Fig. 1J); unusual non-light reflecting nuclei of spermatozoa suggest they are degenerating; same cells present in posterior oviduct of one of these specimens, but posterior oviduct of remaining four specimens obscured by intestinal contents. Vulva slit-like, inconspicuous, without salient lips, situated in mid-region of body at 4600–6650 from head or 48.7–56% of TBL. Apex of ovaries far overlapping level of vulva. Uterus sac-like, thin-walled, filled with numerous eggs, the majority of which contain first-stage larvae. Mature eggs 110–148 long and 56–72 wide (n = 10).

Tail conical, tapering gradually to slightly rounded tip. Tail length 230–375 or 2.1–3.6% of TBL. Caudal vesi-

cle with conspicuous inflation at anus, body width at that level 100(130)–150(220). Tip of tail without vesicle.

Type host: *Boophis madagascariensis* (Peters) (Mantellidae).

Type locality: Import from Madagascar; exact locality unknown. Collection date: 28 February 2008.

Site of infection: Lungs.

Prevalence and intensity: Type host specimen (97NL) harbouring 11 females, and a second host (99NL) harbouring 4 females.

Type material: 97NL. Deposited in the MNHN collection: female holotype, eight paratype females. Two fragments of two paratypes each used for molecular analysis; specimen vouchers MIB:zpl:00226, MIB:zpl:00227, MIB:zpl:00228 and MIB:zpl:00229 respectively. One paratype (N-939) in the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic. EMBL Data Library accession numbers: 12S rDNA FN434097–FN434101, *coxI* FN434102–FN434106.

Additional material: 99NL. Deposited in the MNHN collection: three females. One female used for molecular analysis (specimen voucher MIB:zpl:00230).

Etymology: The new species is named after Dr. Miguel Vences in recognition of his significant contribution to our knowledge of the amphibian fauna of Madagascar.

Remarks. *Rhabdias vencesi* most closely resembles *R. madagascariensis*. In both species the oesophagus is club-shaped with an anterior dilatation prior to the nerve ring, and the apex of the intestine is narrow. However, *R. madagascariensis* is readily distinguished by its smaller body and the proportionally longer oesophagus (3.55 vs. 9.3–13.2 mm, and 7.6 vs. 4.0–5.8% of TBL) and the anterior part of the intestine, which remains slender (Chabaud et al. 1961), whereas that of *R. vencesi* expands rapidly. Its mouth is round as opposed to dorso-ventrally flattened as in *R. vencesi.*

The remaining Afrotropical species are equally distinct (Table 3). *Rhabdias africanus* differs in having two distinct swellings at the level of the amphids, papillae that overhang the oral opening giving the mouth an octagonal shape, a longer buccal capsule (15–20 vs. 5–10) and a slender oesophageal bulb (65–80 vs. 95–120) (Kuzmin 2001). While being of similar length (9–12 vs. 5–10), the buccal capsule ratio of *R. bufonis* sensu Moravec, Baruš et Ryšavý, 1987 is greater than that of *R. vencesi* (0.7 vs. 0.3–0.5) and the intestinal apex is broader than the oesophageal base (Moravec et al. 1987). *Rhabdias collaris* has a conspicuous cephalic swelling, a round mouth and a distinctly longer vestibulum (42 vs. 5–12) (Baker 1987b). *Rhabdias picardiae* is shorter, has a lemon-shaped mouth opening, small lateral papillae and a buccal capsule with a distinct posterior segment. Furthermore, its oesophagus is long and narrow (8.6–9.9 vs. 4.0–5.8% of TBL) and the intestinal apex is wider than the oesophagus at the oesophageal-intestinal junction. *Rhabdias bdellophis* is distinct in having a shorter body and tail, a weakly developed buccal capsule and a relatively longer oesophagus (7.8–8.0 vs. 4.0–5.8% of TBL).

When compared to *Rhabdias* species from anuran hosts world-wide, an arrangement of head papillae with well-developed, salient lateral papillae as seen in *R. vencesi* is also present in *R. joaquinensi*s Ingles, 1935 from a ranid in California, *Rana aurora* Baird et Girard (Kuzmin et al. 2003). This species is distinct, however, in having an attenuated anterior body half, a postequatorial vulva and a cylindrical oesophagus (Kuzmin et al. 2003).

Rhabdias ohlerae sp. n. Junker, Lhermitte-Vallarino et Bain Figs. 2A–F, 3L, Q, Table 3

Body small, 6.5 mm long, dorsally bent, tapering at both ends; width at mid-body 380(420). Thin vesicle present along entire length of body, including tip of tail; difficult to discern in some places. Anterior extremity tapering rapidly from level of anterior genital bend anteriad, terminating in distinct dilatation that is set off from body by slight constriction (Fig. 2D, C); cephalic dilatation 90 long, 70 wide. Mouth opening limited by cuticular velum. Vestibulum 5 deep. Buccal capsule 11 long and 16 wide; ratio 0.7; posterior segment forming distinct ridges, where it rests upon oesophagus (Fig. 3L); lumen of buccal capsule funnel-shaped. Oesophagus club-shaped, 450 long (6.9% of TBL), with shoulders. Apex of oesophagus not reaching anterior border of buccal capsule (Fig. 3L). Anterior dilatation of oesophagus present at 100 from head, 41 wide, anterior to nerve ring (Fig. 2D). Subsequently, gradual increase in oesophagus width from 35 at mid-length to maximum of 55 at indistinct posterior bulb. Nerve ring and excretory pore at 150 and 190 from apex, respectively. Apex of intestine with a shallow depression to accommodate base of oesophagus, which is narrower than intestinal apex at junction (Fig. 2D). Intestine thick-walled, its cells forming a distinct giraffe pattern with narrow interstitial spaces (Fig. 2D); taking up entire width of body anteriorly, but narrowing at approximately level of anterior genital bend; filled with dark brown contents posteriorly.

Genital tract amphidelphic. Anterior genital bend in ovary, 850 from head; posterior genital bend in oviduct (Fig. 2F), 675 from tip of tail. Oviducts and proximal end of ovaries folded upon themselves. Ovary apices just overlapping vulva. Narrow band (30–50 wide) of small, poorly defined and irregularly arranged cells in posterior ovary interpreted as testis zone (Fig. 2E). Vulva without salient lips, at 3120 from head (48% of TBL). Uterus thin-walled, sac-like, filled with numerous eggs, many of which contain first-stage larvae. Mature eggs 104–120 long by 58–65 wide (n = 10).

Tail conical, 275 long, reaching 4.2% of TBL. Width at anus 140 (190). Distinct postanal swelling of body in lateral view (Fig. 2B). Caudal vesicle slightly more con-

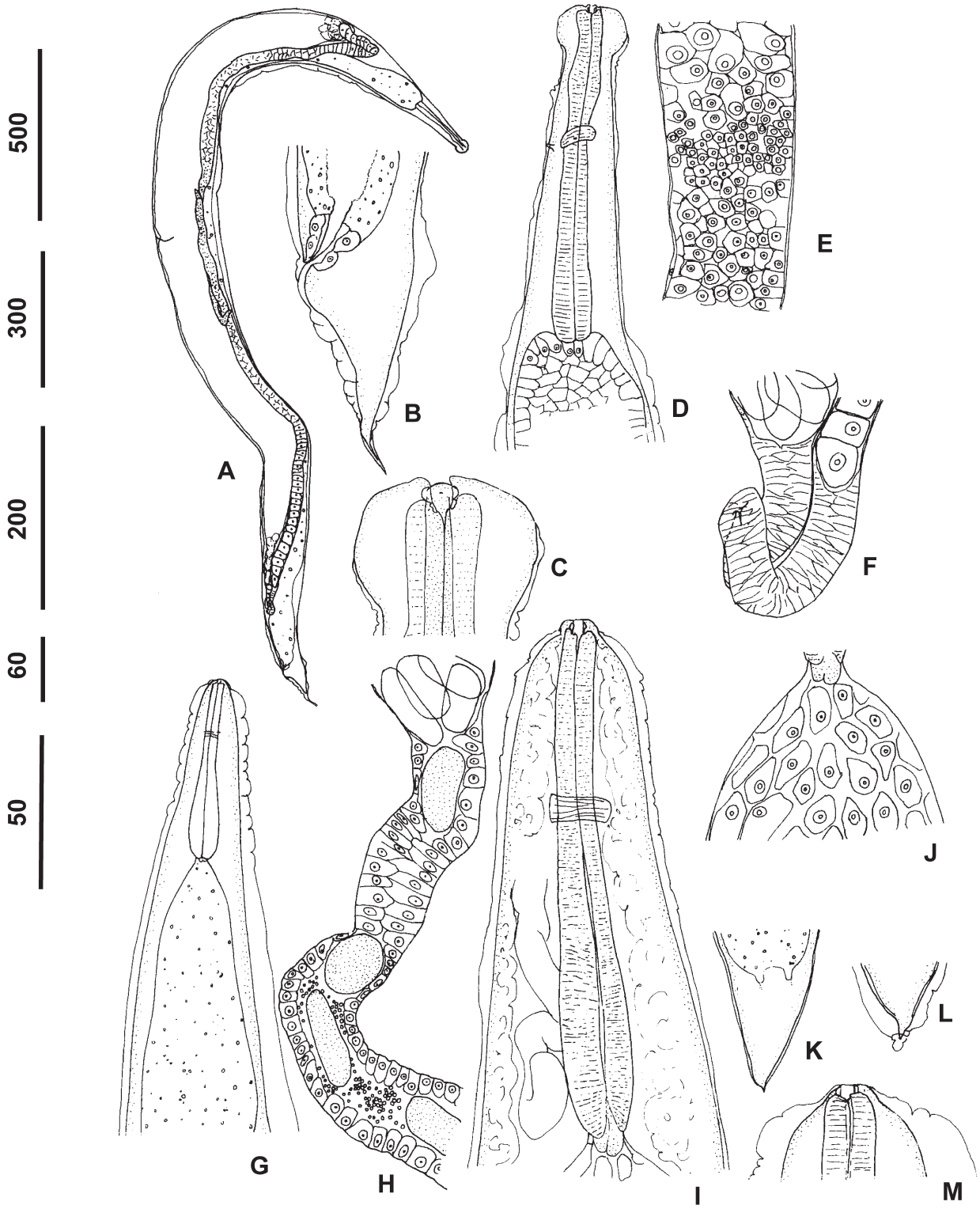

Fig. 2. A–F. *Rhabdias ohlerae* sp. n. from *Leptopelis brevirostris* in Cameroon. **A –** habitus, left lateral view; **B –** posterior region, left lateral view; **C –** head, lateral view; **D –** anterior region, left lateral view; **E –** proximal part of male area in ovary (top of drawing indicates direction of ovary apex); **F –** end of posterior ovary, oviduct and beginning of uterus. **G–M.** *Rhabdias tanyai* sp. n. from *Astylosternus rheophilus* in Cameroon. **G –** anterior region, before fixation; **H –** oviduct with ovulae and, possibly degenerate, spermatozoa up to beginning of uterus; **I –** oesophageal region with one of the excretory cells, dorso-ventral view; **J –** apex of intestine; **K –** posterior region with end of intestine and rectum, ventro-dorsal view (anus not identified); **L –** caudal extremity, ventro-dorsal view; **M –** anterior extremity, before fixation. Scale bars: A, body length 6.5 mm; B, D, F, H, I, J = 200 µm; C, E, L = 50 µm; G = 500 µm; K = 300 µm; M = 60 µm.

spicuous than along body, with irregular folds. Tip of tail 50 long, demarcated from rest of tail by sharp bend, enclosed in thin vesicle.

Type host: *Leptopelis brevirostris* (Werner) (Arthroleptidae).

Type locality: Petit Mont, Etinde Summit, Mount Cameroon, Cameroon (4.06N, 9.12E). Collection date: 28 April 2007.

Site of infection: Lungs.

Prevalence and intensity: A single specimen from a single host.

Type material: Holotype female: 26NL. Deposited in the MNHN collection.

Etymology: The species is named after Prof. Anne-Marie Ohler, for her contribution to the taxonomy of Asiatic amphibians and her assistance with the identification of some of the hosts examined herein.

Remarks. The distinct cephalic dilatation readily distinguishes *R. ohlerae* from its congeners in the Afrotropical region, excepting *R. collaris*, as well as from the majority of *Rhabdias* species from anuran hosts worldwide. While in *R. collaris* the cephalic inflation consists of two lobe-like, divergent structures, which open a wide, funnel-shaped vestibulum of considerable depth between them (42, measured on drawing) (Baker 1987b), that of *R. ohlerae* is round, with a small oral opening that leads into a short vestibulum (5 deep).

From anurans world-wide, the two other species with cephalic dilatations that are not based on vesicular inflation only are *R. androgyna* Kloss, 1971 from a Brazilian host identified as *Bu. typhonius* (L.) by Kloss (1971), but most likely *Rh. margaritifera* (Laurenti) (Frost 2009), and *R. sphaerocephala* Goodey, 1924 from the toad *Bu. bufo* (Linnaeus) (= *Bu. vulgaris*) in England, with additional details on head morphology given by Kuzmin et al. (2007). *Rhabdias androgyna*, as illustrated by Kloss (1971), is distinguished from the new species by a thicker body vesicle, deep vestibulum and longer body as well as oesophagus (9–13 mm and 577–618, respectively).

Rhabdias ohlerae most closely resembles *R. sphaerocephala*, being similar in body length (6.5 mm vs. 6–6.5 mm) and oesophagus length (450 or 6.9% of TBL vs. 430–450 or 6.6–7.5% of TBL) (Goodey 1924a; calculations based on minimum and maximum values). Further similarities are the shape of the oesophagus, including the anterior swelling situated just posterior to the cephalic dilatation, and the apex of the intestine, which encompasses the base of the oesophagus. However, the cephalic dilatation of *R. sphaerocephala* is distinctly wider than that of *R. ohlerae* (115–120 vs. 70; both sets of measurements excluding vesicle), the vesicle is thicker and the tail is shorter in *R. sphaerocephala* (190–220 or 2.9–3.4 % of TBL vs. 275 or 4.2 % of TBL) (Goodey 1924a; calculations based on minimum and maximum values).

Rhabdias tanyai sp. n. Junker, Lhermitte-Vallarino et Bain Figs. 2G–M, 3M, Table 3

Body with slight dorsal bend (Table 3), length >13.9 mm (length of anterior plus posterior fragment). Thin vesicle present along entire length of body, including tip of tail. Buccal capsule and oesophageal apex forming a flat-topped projection at cephalic end in fixed specimen; the same projection observed prior to fixation, but less pronounced (Figs. 2 G, I, M, 3M). Buccal capsule 12 long and 23 wide, with a ratio of 0.5; posterior segment not resting on but rather inserted into the oesophageal apices (Fig. 3M); anterior and posterior segments form shoe sole-shape; lumen of buccal capsule cylindrical. Oesophagus club-shaped, with shoulders, but apex not reaching mid-level of buccal capsule; 650 long, reaching a maximum of 4.7% of TBL when taking TBL as the sum of the anterior and posterior fragment; oesophagus width at mid-length 60, gradually increasing to maximum of 95 at bulb. Body width at bulb 270 (290). Nerve ring at 235 from apex, excretory pore not observed, but a long excretory gland identified. Apex of intestine bell-shaped, narrow at oesophageal-intestinal junction, but rapidly widening to take up almost entire width of body (Fig. 2G). Intestine thick walled, its cells arranged in a giraffe pattern with wide interstitial spaces (Fig. 2J); filled with dark brown contents posteriorly; terminating in two short, peg-like extensions.

Genital tract amphidelphic. Removal of mid-section made measurements impossible. Vulva absent from both fragments. It is difficult to assess if the following observations are typical features or result from specimen manipulation. Anterior oviduct-uterus junction observed, with uterus extending considerable distance anteriad from junction. Anterior genital bend not seen, but uterus clearly anterior-most portion of anterior genital tract. Ovaries disposed in generous loops across width of body, containing ovulae arranged in transverse bands of 5–6 each. Testis zone identified in posterior ovary. Lumen of anterior oviduct containing similar but smaller cells representing spermatozoa, possibly degenerate (Fig. 2H). Uterus thin-walled, sac-like, filled with numerous eggs, many of which contain first-stage larvae. Mature eggs 96–105 long by 50–62 wide (n = 5).

Tail conical, with short, stubby tip, 275 long, reaching a maximum of 2.0% of TBL, when taking TBL as the sum of the anterior and posterior fragments. Tip of tail enclosed in vesicle.

Type host: *Astylosternus rheophilus* Amiet (Arthroleptidae).

Type locality: Forest below Oku village (6.22N, 10.52E), Mount Oku, Cameroon. Collection date: 10 May 2007.

Site of infection: Lungs.

Prevalence and intensity: A single female from a single host.

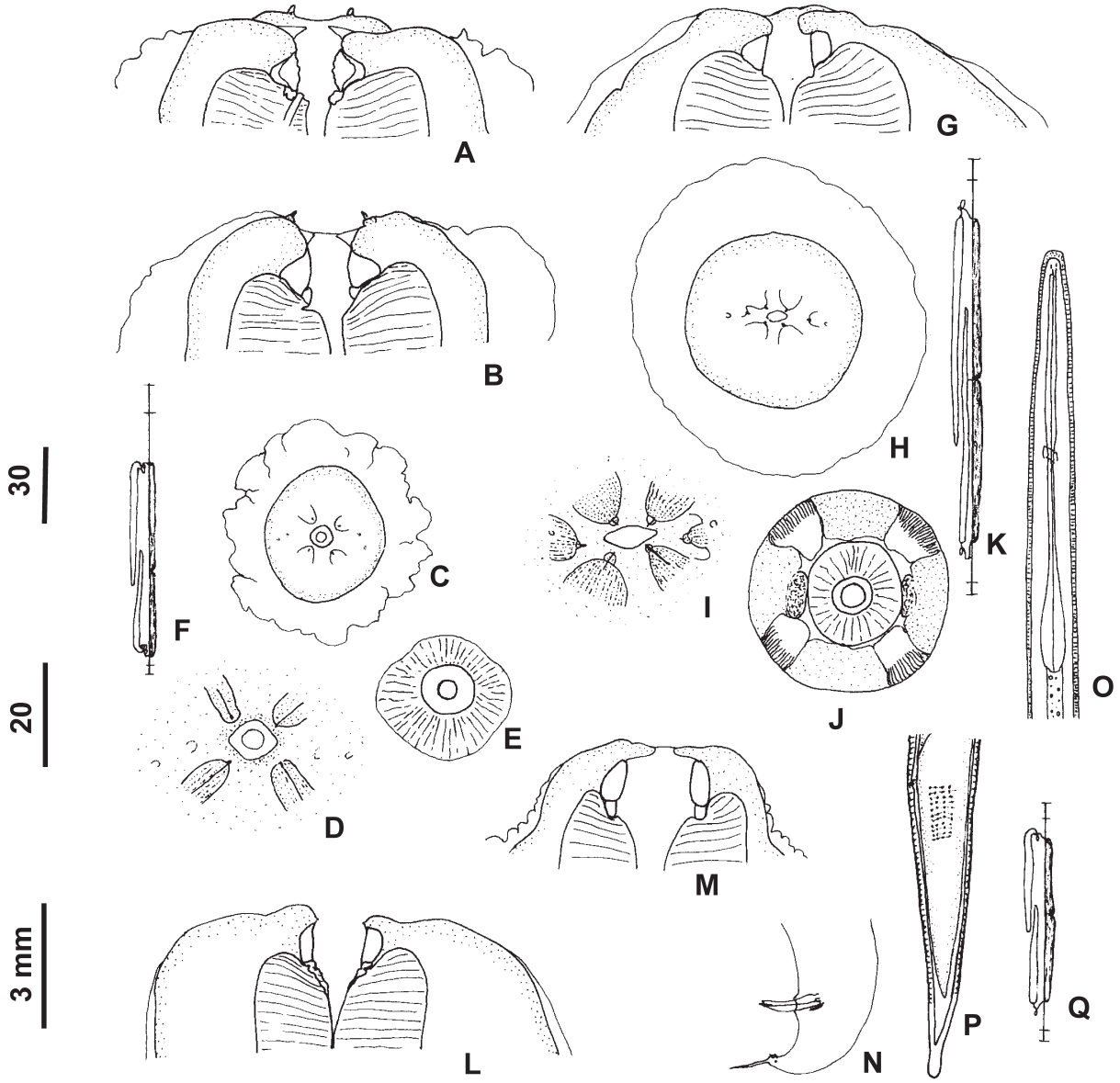

Fig. 3. A–F. *Rhabdias picardiae* sp. n. **A –** head, dorso-ventral view; **B –** head, lateral view (another specimen); **C–E –** frontal view; **C –** head; **D –** sensory papillae and mouth; **E –** buccal capsule and apex of oesophagus, transverse optical section; **F –** diagrammatic representation of holotype. **G–K.** *Rhabdias vencesi* sp. n. **G –** head, subdorso-ventral view; **H**, **I –** frontal view; **H –** head; **I –** sensory papillae and mouth; **J –** buccal capsule, apex of oesophagus, and the particular lateral structures along the oesophagus, transverse optical section; **K –** diagrammatic representation of holotype. **L.** *Rhabdias ohlerae* sp. n., buccal cavity and capsule, lateral view. **M.** *Rhabdias tanyai* sp. n., buccal cavity and capsule, dorso-ventral view. **N–P.** Free-living stages of *R. vencesi* sp. n. **N –** male, posterior region, left lateral view; **O**, **P –** infective larva, anterior and posterior region, right and left lateral view, respectively. **Q.** *Rhabdias ohlerae* sp. n., diagrammatic representation of holotype. Scale bars: A, B, D, E, G, I, L, M, P = 20 μm; C, H, J, N, O = 30 μm; F, K, Q = 3 mm.

Type material: 74NL; a single worm recovered. Deposited in the MNHN collection: posterior and anterior part of female holotype. Mid-section removed for molecular analysis; specimen voucher MIB:zpl:00190. EMBL Data Library accession numbers: 12S rDNA FN434097–FN434101, *coxI* FN434102–FN434106.

Etymology: The new species is named after Dr. Vincent Tanya, our colleague in Cameroon (EU projects on onchocerciasis), and in recognition of his administrative help concerning the field trip.

Remarks. *Rhabdias tanyai* can be distinguished from species hitherto described from Malagasy and African hosts (Table 3) by the cephalic projection, the shoe sole-shape of its buccal capsule and the way the posterior segment of the capsule is inserted into the oesophagus.

The only other *Rhabdias* species from anuran hosts with an anterior cephalic projection similar to that seen in *R. tanyai* is *R. brachylaimus* (von Linstow, 1903), originally described by von Linstow (1903) as *Angiostomum brachylaimus* from *Du. melanostictus* (Schneider) (= *Bu.*

Table 3. Morphometric characters of *Rhabdias tanyai* sp. n., *Rhabdias ohlerae* sp. n. and other *Rhabdias* from Afrotropical lissamphibian hosts. Host nomenclature according to Frost (2009). All measurements in micrometres unless otherwise indicated.

Host family	Arthroleptidae			Bufonidae			Mantellidae	Ptychadenidae	Caeciliidae
Host genus	*Astylosternus*	*Leptopelis*		*Amietophrynus*			*Boophis*	*Ptychadena*	*Scolecomorphus*
Host species	*rheophilus*	*brevirostris*	*vermiculatus*	*garmani & maculatus*	*gutturalis*	*regularis*	*madagascariensis*	*mascareniensis*	*vittatus*
Host locality	Cameroon	Cameroon	Tanzania	South Africa	South Africa	Egypt	Madagascar	Madagascar	Tanzania
Rhabdias species	*tanyai* sp. n.	*ohlerae* sp. n.	*collaris*	*africanus*	*picardiae* sp. n.	*bufonis*[b]	*vencesi* sp. n.	*madagascariensis*	*bdellophis*
MNHN collection and specimen number (this paper) or reference	26 NL 1	74 NL 1	Baker 1987	Kuzmin 2001	52 YU and 53 YU	Moravec et al. 1987	97 NL	Chabaud et al. 1961	Baylis 1929
Specimen number	Holotype	Holotype	n = 6	n = 9	n = 7	n = 10[d]	n = 12	n = 1	n = 2
Length (mm)	> 13.9[a]	6.5	9.1–10.2	12.45–19.8	8.0–8.35	2.99–13.02	9.5–13.2	3.55	5.1–5.6
Width at mid-body (with vesicle)	800	380	–	300–450	460–580	163–476	560–770	150	350–420
Vestibulum depth	4	5	42*	16*	5–7	–	5–12	–	–
Buccal capsule length	12	11	11*	15–20	7–10	15	5–10	1.5*	Vague
Buccal capsule max. external diameter	23	16	17*	20–23	23–25	21	19–22	8.5*	Vague
Buccal capsule ratio	0.5	0.7	0.6*	0.7[c]	0.3–0.4	0.7	0.3–0.5	–	–
Oesophagus length	650	450	480–540	570–710	690–790	288–510	495–570	270	400–450
Oesophagus width at mid-length	60	35	–	–	60–70	–	72–85	–	–
Bulb diameter	95	55	–	65–80	95–130	57–72	95–120	–	90
Body width at bulb (with vesicle)	270	125	–	–	160–300	–	210–340	–	–
Tail length	300	275	370–430	250–400	270–350	144–420	230–375	125	125–155
Width at anus (with vesicle)	–	140	–	–	120–225	–	100–150	–	–
Apex to nerve ring	235	150	190–245	–	235–250	168–240	180–210	130	160–170
Oesophagus length as % of body length	max. 4.7[a]	6.9	5.6[c]	3.5–4.7	8.6–9.9	–	4.0–5.8	7.6	7.8–8.0
Head to vulva as % of body length	–	48	56[c]	46.8–49.7	55–62	± equatorial	48.7–56	52	–
Tail length as % of body length	max. 2.2[a]	4.2	4.1[c]	1.6–3.2	3.5–3.8	–	2.1–3.6	3.5	2.5–2.8

[a] Based on body length being the sum of the anterior and posterior fragments; [b] sensu Moravec et al. 1987; [c] calculations based on measuremnets of holotype; [d] includes very young and fully gravid females; * calculated from drawings.

melanostictus) in Thailand and subsequently by Yuen (1965) from lissamphibians in Malaysia. Based on von Linstow's (1903) description, *R. brachylaimus* is, however, considerably shorter than *R. tanyai* (8.8 mm vs. >13.9 mm) and its oesophagus and tail are longer, reaching 6.7% and 4% of TBL, respectively. While specimens of *R. brachylaimus* described by Yuen (1965) are comparable to *R. tanyai* in body length, their oesophagus ranges from 270 to 370 in length only, compared to 650.

Molecular analyses

Taking an integrated taxonomical approach based on both morphological characterisation and classical DNA barcoding (Ferri et al. 2009), DNA sequences of three of the four species described herein were analysed. Comparison of the 12S rDNA and *coxI* sequences of *R. picardiae*, *R. tanyai* and *R. vencesi* confirmed their validity.

Data obtained, based on K2P distance matrix, showed a low nucleotide intraspecific distance both for *R. vencesi*. (12S rDNA 0.2% and *coxI* 0.2%) and *R. picardiae* (12S rDNA 0.3% and the sequences were identical in *coxI*), for which more than a single specimen could be analysed. Moreover, our results confirmed placement of the three species in three distinct Molecular Taxonomic Units (MOTU). In fact, the nucleotide interspecific distances (K2P) for the two molecular markers 12S rDNA and *coxI*, respectively, are: 6.7% (standard error 1.4%) and 9.9% (standard error 1.7%) between *R. picardiae* and *R. vencesi*; 5.8% (standard error 1.4%) and 13.2% (standard error 1.2%) between *R. picardiae* and *R. tanyai;* and 7.9% (standard error 1.6%) and 16.2% (standard error 1.7%) between *R. vencesi* and *R. tanyai*.

Free-living stages of *Rhabdias vencesi*

Descriptions of free-living stages of *R. vencesi* are based on material obtained from cultured larvae collected from the rectum of host 99 NL. Male and female adults were observed after 3 days, and several motile infective larvae were detected 7 days after the culture was started. Soon a high mortality rate was observed due to bacterial colonies.

Female: not measured, not drawn. Male (n = 1) (Fig. 3N): body length 578, with short lateral alae; tail 53 long, including a tail filament of 9 length; spicules 26 and 28, gubernaculum 13 long. Caudal papillae not studied in detail, but two subterminal pairs conspicuous.

Infective third-stage larva (n = 1) (Fig. 3O, P): enclosed in exuvial sheath, with checkered ornamentation; posterior part of sheath regularly attenuated, without subterminal indentation and bosses; tip of sheath blunt. Tail of larva tapering to conical, sharply pointed tip. Measurements exclude sheath: 575 long, 20 wide; nerve ring at 80 from head; buccal cavity funnel-shaped; buccal capsule 11 deep, 7 wide, composed of two segments; oesophagus 175 long; tail 58 long; genital anlage 50 long, situated at mid-length of intestine.

DISCUSSION

The *Rhabdias* fauna of Afrotropical anurans appears diverse and to date, including the present paper, eight species have been reported from four families of anuran hosts (Table 3). Neither these lungworms nor their hosts have been recorded from any of the other zoogeographic regions.

In the new species, the shape and structure of the buccal capsule is diverse; it can comprise a single segment as in *R. vencesi*, or two more or less distinct ones as in the remaining three species; its wall can be simple, or double as in *R. picardiae*. The mouth is subsphaerical or dorsoventrally flattened (*R. picardiae* and *R. vencesi*, respectively), and the lateral head papillae may be developed or reduced. These characters cannot be compared with many other species, but buccal capsule size, shape and size of the oesopahgus, body size and the configuration of the apex of intestine, to name a few, can be used for species differentiation (Kuzmin et al. 2003, Tkach et al. 2006). The latter are equally useful when distinguishing parasites from chamaeleonids (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a, b).

At present, the *Rhabdias* fauna of Afrotropical anurans presents the following picture. Three species, *R. bufonis* sensu Moravec, Baruš et Ryšavý, 1987, *R. africanus*, and *R. picardiae*, parasitise hosts of the family Bufonidae, represented by a single genus, *Amietophrynus* (formerly included in *Bufo*), which is limited to sub-Saharan Africa (Frost 2009). All four host species occur in savanna habitat and their distribution overlaps to varying degrees, with *Am. gutturalis* having the widest and *Am. maculatus* the narrowest geographic range (Passmore and Carruthers 1979). Several comments can be made when considering the *Rhabdias* species and host families in the Afrotropics.

In bufonids, while *R. picardiae* and *R. bufonis* sensu Moravec, Baruš et Ryšavý, 1987 are distinctly different (Table 3), their hosts are morphologically very close. Molecular evidence, however, suggests that the two bufonids separated some 5 to 6 million years ago (Channing and Howell 2006). The two South African species, *R. picardiae* and *R. africanus*, are morphologically no closer to each other than they are to *R. bufonis* sensu Moravec, Baruš et Ryšavý, 1987. In fact, with respect to relative oesophagus length, *R. africanus* and *R. picardiae* occupy opposite extremes of the wide range seen in this character in anuran hosts. The host of *R. picardiae* was collected in the Central Bushveld bioregion, whereas the two hosts of *R. africanus* were collected in the South African Lowveld Bushveld in the Kruger National Park (Mucina and Rutherford 2006). However, the three species from African bufonids share a common trait regarding oesophagus morphology: the bulb is not very distinct, and the anterior dilatation, which is relatively common in *Rhabdias* from other bufonids (Goodey 1924a, Yuen 1965, Kloss 1971, Tkach et al. 2006, Martínez-Salazar and León-Règagnon

2007), is absent. In addition, none of the species from African bufonids have well-developed pseudolabia. Contrary to this, pseudolabia are present in several *Rhabdias* from bufonids in the Nearctic (Baker 1978), Neotropic (Kuzmin et al. 2007, Martínez-Salazar and León-Règagnon 2007) and Oriental region (Lu 1934).

A further three species occur in Arthroleptidae (Table 3), with *R. collaris* and *R. ohlerae* parasitising *Leptopelis*, and *R. tanyai* using *Astylosternus*. Both genera are restricted to sub-Saharan Africa. In Cameroon, *R. ohlerae* and *R. tanyai* are morphologically very dissimilar (Fig. 2). This is not surprising considering that their respective hosts, *Le. brevirostris* and *As. rheophilus*, were collected on two isolated mountains of the Cameroonese volcanic chain, where endemicity is high in plants as well as animals (Letouzey 1985, Amiet 1987, Vivien 1991, Maley 1996, Küper et al. 2004). Interestingly, both *R. collaris* and *R. ohlerae*, collected from allopatric forest species of the genus *Leptopelis*, have cephalic dilatations and are close in other aspects of their morphology as well. Since *R. androgyna* (Kloss 1971) and *R. sphaerocephala* (Goodey 1924a) from Bufonidae in Brazil and Europe, respectively, possess similar dilatations without sharing any further resemblances, it is difficult to judge whether cephalic inflations indicate a common ancestry or are the result of convergent development preventing expulsion from the host's lungs (Baker 1987b).

A single species, *R. vencesi*, was recovered from Mantellidae (*Boophis*), a family restricted to Madagascar and Mayotte (Frost 2009). In the same region, *R. madagascariensis* was described from *Ptychadena*. The family Ptychadenidae was historically restricted to sub-Saharan Africa, but the genus *Ptychadena* has subsequently been introduced on Madagascar, the Seychelles and the Mascarene Islands. Both Malagasy *Rhabdias* species are closely related, suggesting speciation through host-switching, a process that occurred in many groups of nematode parasites (Durette-Desset et al. 1985, Guerrero et al. 2002).

All *Rhabdias* species from anuran hosts described herein differ from the large species of *Rhabdias* in African and Malagasy chameleons in the size of the buccal capsule. In *R. chamaeleonis* (Skrjabin, 1916), *R. jarki* Lhermitte-Vallarino et Bain, 2004, *R. cristati* Lhermitte-Vallarino et Bain, 2008, *R. okuensis* Lhermitte-Vallarino et Bain, 2008, *R. brevicorne* Lhermitte-Vallarino, Junker et Bain, 2009, *R. mariauxi* Lhermitte-Vallarino et Bain, 2009, *R. nasutum* Lhermitte-Vallarino, Junker et Bain, 2009, *R. rabetafikae* Lhermitte-Vallarino, Junker et Bain, 2009 and *R. rhampholeonis* Lhermitte-Vallarino et Bain, 2009 the buccal capsule is distinctly wider (≥35) than that seen in *Rhabdias* from anurans in the Afrotropical region (Table 3). Contrary to this, the buccal capsule of the two small species from chameleons, *R. gemellipara* Chabaud, Brygoo et Petter, 1961 and *Rhabdias* sp. of Lhermitte-Vallarino et al. (2009), is narrower (≤17) than that of the new taxa described herein (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a, b), excepting *R. ohlerae*, which is easily distinguished by its dilated anterior end.

The four *Rhabdias* species described herein share a number of biological characters with their congeners from other lissamphibian and saurian hosts, such as hermaphrodism in the parasitic females. Male gametes were positively identified in the oviducts of *R. picardiae* and testis zone in the posterior ovary of *R. tanyai*, while cells interpreted as such were seen in the oviduct of *R. vencesi* and in the posterior ovary of *R. ohlerae*. Contrary to Baker (1979), however, who observed protandry in *R. americanus* and *R. ranae*, this was not seen in the present material. Rather spermatozoa seem to be produced intermittently during the females' entire life, as in some *Rhabdias* from other lissamphibian (Travassos 1926, Chu 1936) and chameleon hosts (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a, b). Oviduct structure was uniform in the four new species, consisting of high, narrow cells as in *Rhabdias* species from Chamaeleonidae. No seminal receptacle as described by Baker (1979) in oviducts of young adults of *R. americanus* was identified.

Additional biological traits shared by *R. vencesi*, for which part of the life history could be studied, and *Rhabdias* from other lissamphibians and saurians, include heterogonic free-living males and females. Compared to species from chamaeleonids, the male caudal filament is almost twice as long (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a).

Two morphological characters of the infective stage are of interest: the ornamentation of the sheath and the shape of the tail extremity of the larva. *Rhabdias vencesi* shares the checkered sheath of its infective larva with congeners from chamaeleonids (Lhermitte-Vallarino et al. 2004, 2005, 2008, 2009a), *R. fuscovenosa* from a snake (Goodey 1924b) and infective larvae of other Rhabdiasidae, such as *Entomelas entomelas* (Dujardin, 1845) (Seurat 1920), *Pneumonema tiliquae* Johnston, 1916 (Ballantyne 1991) and *Chabirenia cayennensis* Lhermitte-Vallarino, Bain, Bertani, Voza, Attout et Gaucher, 2005 (Lhermitte-Vallarino et al. 2005). The ornamentation of the sheath is easily missed. Its description in several genera and species of Rhabdiasidae suggests that it is a character of Rhabdiasidae.

The tail extremity of the infective larva of *R. vencesi*, the first lung worm from Mantellidae, is pointed. In four of five species from bufonid and ranid anurans, for which this character is sufficiently described or illustrated, the tail extremity is blunt (Yuen 1965, Baker 1979), but pointed in the remaining one (Travassos 1926). It is also blunt in species from ophidians (Kuzmin 1999, Kuzmin and Miskov 1999). The bifid tail reported by Chu (1936) is the result of confusion with infective larvae of *Strongyloides*

Grassi, 1879. The four recently studied *Rhabdias* species from chamaeleonids present a particular morphology in that the tail is blunt and carries a small number of buds (Lhermitte-Vallarino et al. 2004, 2005, 2008, 2009a).

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