A new species of *Rhabdias* Stiles et Hassall, 1905 (Nematoda: *Rhabdiasidae*) from *Blommersia domerguei* (Guibé) (Amphibia: Mantellidae) in Madagascar

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Abstract: *Rhabdias blommersiae* sp. n. (Nematoda: *Rhabdiasidae*) is described from the lungs of Domergue’s Madagascar frog, *Blommersia domerguei* (Guibé) (Amphibia: Mantellidae), in Madagascar. The new species differs from congeners parasitizing amphibians in having a smaller body and buccal capsule, six equal lips, large excretory glands of unequal length and a posteriorly inflated body vesicle. A combination of characters distinguishes it from Afromalagasy species of *Rhabdias* Stiles et Hassall, 1905. *Rhabdias blommersiae* is the third species of the genus described from amphibians in Madagascar. Close similarities in the number and shape of circumoral structures in two *Rhabdias* species described from mantellid hosts in Madagascar suggest a close relationship and common origin of the two species, with subsequent adaptation to separate hosts within the Mantellidae.

Keywords: lung nematodes, *Rhabdias blommersiae* sp. n., anurans, *Blommersia*, Afrotopics, morphology

Lung nematodes of the genus *Rhabdias* Stiles et Hassall, 1905 parasite amphibian and reptilian hosts worldwide. To date, more than 80 species, of which approximately 50 in amphibians, have been assigned to this genus (Kuzmin and Tkach 2012). A total of eight *Rhabdias* species have been described from amphibian hosts in the Afrotropics, *R. bdellophis* Baylis, 1929 from a caecilian and seven species from anurans: *R. africanus* Kuzmin, 2001; *R. col laris* Baker, 1987; *R. madagascariensis* Chabaud, Brygoo et Petter, 1961; *R. oholaeae* Junker, Lhermitte-Vallarino et Bain, 2010; *R. picardiae* Junker, Lhermitte-Vallarino et Bain, 2010; *R. tanyai* Junker, Lhermitte-Vallarino et Bain, 2010 and *R. vencesi* Junker, Lhermitte-Vallarino et Bain, 2010 (see Baylis 1929, Chabaud et al. 1961, Baker 1987, Kuzmin 2001, Junker et al. 2010).

Two of the latter, *R. madagascariensis* from *Pty chadena* (syn. *Rana*) *mascareniensis* (Dumérl et Bibron) (Ptychadenidae) and *R. vencesi* from *Boophis madagascar iensis* (Peters) (Mantellidae), originate from Madagascar (Chabaud et al. 1961, Junker et al. 2010).

The diversity of *Rhabdias* species reported from amphibians in the Afrotropical realm is presently lower than that in the Neotropical realm (13 known species) or Palaeartic realm (14 known species). However, recent investigations indicate this to be the result of a lack of data rather than a reflection of the actual status of the genus. In fact, recent data obtained in this region (Camerooneese Volcanic range, South Africa, Madagascar), although still scant, suggest a high diversity of *Rhabdias* in amphibian as well as reptilian hosts (Lhermitte-Vallarino and Bain 2004, Lhermitte-Vallarino et al. 2008, 2009a,b, 2010a,b, Junker et al. 2010).

In this study, two specimens of *Rhabdias* recovered from a second mantellid host, the Domergue’s Madagascar frog, *Blommersia domerguei* (Guibé), were examined. Based on their morphology, these specimens were readily assigned to the genus *Rhabdias*. However, they clearly differed from all presently known species within the genus and are herein described as belonging to a new species, *Rhabdias blommersiae*. Their morphological characters and affinities to their Afrotropical congeners from amphibian hosts are discussed.

MATERIALS AND METHODS

The two specimens described below were part of a collection of helminths from amphibians of one of the authors (LdP; PL050118H1 and PL050118H2) and had been collected from *Blommersia domerguei* (LdP; AL050118A3) during an un-
related study in Madagascar. They are now deposited in the collection of zooparasitic nematodes of the Muséum National d’Histoire Naturelle, Paris, France (MNHN), accession number 352 YU, as holotype and paratype, respectively. The specimens had been fixed and stored in 70% ethanol. For morphological studies, they were cleared in lactophenol and examined under a Wild compound microscope equipped with a drawing tube.

Measurements were taken from drawings and are given in micrometres unless otherwise specified. Cephalic and buccal structures (velum, vestibule, buccal cavity, buccal capsule) were identified and measured as described in Lhermitte-Vallarino et al. (2010a). In accordance with earlier publications, four ratios are provided (Tkach et al. 2006, Martínez-Salazar and Léon-Régagnon 2007, Lhermitte-Vallarino et al. 2008). The genital tract was studied in detail to identify spermatooza and the testis zone (Runey et al. 1978), since these biological characters may be of interest to our understanding of the evolution of Rhabdias. A holotype specimen of the closely related species *R. cariensis* (MNHN E188) was used for comparative studies. In this specimen the head is absent. Nomenclature of amphibian hosts follows Frost (2013).

**RESULTS**

*Rhabdias blommersiae* sp. n. Figs. 1–8

**Description** (based on two gravid individuals; measurements are given for the holotype, followed by the paratype in parentheses). Relatively small species. Body length 3.6 (3.2) mm, maximum width at mid-body 185 (180). Body tapering towards both extremities; anterior end rounded, posterior end attenuated (Fig. 6). Body cuticle slightly inflated in anterior part, thin at mid-body, distinctly inflated in posterior part, with deep irregular transverse folds (Fig. 8).

Oral opening small, round (Fig. 2). Six circumoral lips, approximately equal in shape and size, each bearing a small papilla. Amphids indistinct. All lips distant from mouth, but lateral ones at somewhat larger distance from anterior end of oesophagus, slightly anterior to its bend at distal part of ovaries. Proximal ends of ovaries slightly overlapping at level of vulva (Fig. 7). Testis zone and spermatooza could not be identified in ovaries. Oviducts short and straight.

Tail short, conical, 147 (142) long, i.e. 4.1% (4.4%) of body length. Caudal vesicle inflated, completely enclosing tip of tail, invaginated on tail tip (Fig. 5) in both specimens.

**Type host**: Domergue’s Madagascar frog, *Blommersia domergui* (Guibé) (Amphibia: Mantellidae), host (field No. AL05011813) collected on 18 January 2005.

**Type locality**: Madagascar, Special Reserve Ambobitandy, 125 km north of Antananarivo (18°09'59"S; 47°17'00"E). Altitude 1 448–1 662 m (Stephenson et al. 1994).

**Site in host**: Lungs.

**Prevalence and intensity of infection**: One of five examined host specimens harboured two *R. blommersiae* specimens.

**Type material**: Holotype and one paratype deposited in the MNHN collection (accession number 352 YU).

**Etymology**: This species is named after its type host genus.

**Remarks**. In size, oesophagus and tail length, distance from the apex to the vulva and egg size, *R. blommersiae* sp. n. resembles its sympatric congener *R. madagascariensis*, particularly the holotype described by Chabaud et al. (1961). However, the anterior end of *R. madagascariensis* is truncated and the circumoral lips and buccal capsule are indistinct, its oesophagus possesses a prominent dilation just anterior to the nerve ring, and the excretory glands are similar in length, 400 µm and 350 µm, respectively (Chabaud et al. 1961). In addition, a prominent excretory sinus is absent in *R. madagascariensis*, the intestine is narrower than the oesophageal bulb, and the body vesicle is inconspicuous in the posterior part of the body and does not reach the tip of the tail (Chabaud et al. 1961; own observations).

The shape of the anterior end, oesophagus and body vesicle, as well as the presence of six small lips, approach *R. blommersiae* to the second species from Malagasy am-
Figs. 1–5. *Rhabdias blommersiae* sp. n. from *Blommersia domerguei* (Guibé) in Madagascar. **Fig. 1.** Anterior end, lateral view (holotype). **Fig. 2.** Anterior end, apical view (paratype). **Fig. 3.** Anterior end, apical view, optical section at level of buccal capsule at mid-depth (paratype). **Fig. 4.** Anterior part, lateral view (holotype). **Fig. 5.** Posterior end, lateral view (holotype).

A further six *Rhabdias* species have been described from amphibia on the African continent: *Rhabdias bdellophis* from the caeciliid *Scolecomorphus vittatus* (Boulenger) (syn. *Bdellophis vittatus*), and the remaining five from anurans. The former differs from *R. blommersiae* in being slightly larger (5.1–5.6 mm vs 3.2–3.6 mm) and having a smaller oesophagus and tail ratio (7.8–8.0 and 2.5–2.8% vs 9.2–9.6 and 4.1–4.4%, respectively). Its buccal capsule is described as ‘vague’ (Baylis 1929), as opposed to a small but distinct buccal capsule in *R. blommersiae*. 

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phibians, *R. vencesi*. The latter is, however, much larger (9.5–13.2 mm long), its buccal capsule is about twice as wide as that of *R. blommersiae* (19–22 µm vs 11–12 µm); an anterior dilation is present in the oesophagus and the posterior part of the tail is enclosed in a thin, non-inflated vesicle (Junker et al. 2010).

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20 µm | 100 µm
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20 µm | 100 µm
100 µm

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The longer body and a conspicuous cephalic dilation readily distinguish *R. collaris* from *Leptopelis vermicultatus* (Boulenger) (Arthroleptidae) in Tanzania from the new species. This also holds true for *R. ohlerae* from *L. brevirostris* (Werner) (Arthroleptidae) in Cameroon (see Baker 1987, Junker et al. 2010). The remaining four species are also larger in size. In addition, in *R. tanyai* from *Astylosternus rheophilus* Amiet (Arthroleptidae) in Cameroon, a short, flat-topped anterior projection encloses the buccal capsule and the oesophagus ratio reaches a maximum of 4.7% (Junker et al. 2010).

The following two species are parasitic in Bufonidae in South Africa: *R. africanus* from *Amietophrynus maculatus* (Hallowell) differs from *R. blommersiae* in having a wider buccal capsule (20–23 μm) and a shorter oesophagus ratio (3.5–4.7% vs 9.2–9.6%) (Kuzmin 2001); *R. picardiae* from *A. gutturalis* (Power) can be distinguished by its wider buccal capsule (23–25 μm), elongated oesophagus and its post-equatorial vulva (Junker et al. 2010).

We thus conclude that the specimens from *B. domerguei* in Madagascar represent a new species for which we propose the name *R. blommersiae*.

**DISCUSSION**

The amphibian fauna of Madagascar is considered one of the world’s most important ‘hot spots’ for amphibian diversity, with more than 240 species described, the majority of which are endemic and many of which are characterized by small geographic ranges (Andreone et al. 2008, 2010, Vences et al. 2010). Its long isolation from other land masses, together with its large size and varied topography, are considered some of the main contributing factors to its faunal diversity (Fisher 1996). Despite this astounding host diversity, only three *Rhabdias* species from Malagasy amphibians have been studied to date.

Two of these, *R. vencesi* and *R. blommersiae*, have been described from mantellid hosts that are endemic to Madagascar. *Blommersia domerguei* occurs on the plateau of central Madagascar and its eastern slopes, where it has been recorded from 900 to 2000 m above sea level. Its geographic range partially overlaps with that of *Boophis madagascariensis*, which is found from sea level up to altitudes of 1700 m and inhabits numerous localities in eastern and central Madagascar. Of the three amphibian hosts of *Rhabdias* in Madagascar, *Ptychadena mascareniensis* has the widest geographic distribution, being found throughout Madagascar, at altitudes ranging from sea level to over 2000 m (Nussbaum and Vallan 2004, Vallan and Glaw 2004, Rödel et al. 2009); it also has a wide distribution in sub-Saharan Africa and molecular evidence suggests that it reached Madagascar through transoceanic dispersal long before human settlement (Vences et al. 2004, Frost 2013).
Because of the relative geographic isolation of Madagascar hosts and, consequently, their parasites, one might expect to find characters that unite the three Rhabdias species from Malagasy amphibians and oppose them to other congeners. Whereas specimens from Madagascar share a stout, distinctly club-shaped oesophagus, this is not a characteristic restricted to them, but is present in numerous other Rhabdias species from amphibians, including newts and salamanders, worldwide (Baker 1987, Goodey 1929, Kuzmin et al. 2001, 2003, Kuzmin 2005, Martinez-Salazar 2008). Oesophagus shape in general does not seem to relate to either geographic origin or host phylogeny.

A further character shared by all three Rhabdias species from Malagasy amphibians are conspicuous excre- tory glands, but they have also been observed in Rhabdias species from amphibians from other geographic regions, e.g. R. alabialis Kuzmin, Tkach et Brooks, 2007 from Costa Rica, R. bicornis Lu, 1934 and R. globocephala Kung et Wu, 1945 from China (Lu 1934, Kuzmin 2005, Kuzmin et al. 2007).

Similarly, small-sized species of Rhabdias (body length of gravid individuals < 5 mm) do not seem to form a separate group of related species within the genus Rhabdias in amphibian hosts either. Rhabdias blommersiae differs from species that are of similar size, R. australiensis Bursey et Goldberg, 1945 from China (Wu & Kuzmin, 1945), but is also supported by their specificity to hosts of the genus Rana Linnaeus, as well as their geographic distribution in the Americas. No lips or papillae were reported in the description of R. australiensis (one specimen available; Moravey and Sey 1990).

The number, shape and position of circumoral structures are equally similar in two of the species from Malagasy, R. vencesi and R. blommersiae. Their specificity to frogs of the family Mantellidae, as additional character, may indicate a close relationship and common origin. In this case, speciation within Rhabdias might have occurred as a result of adaptation to separate hosts within the Mantellidae. Given that the diversity of potential amphibian hosts in Madagascar is very high, it is likely that the diversity of Rhabdias species parasitizing Malagasy amphibians is distinctly higher than currently reflected in the literature.

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