

A NEW SPECIES OF *RHABDIAS* (NEMATODA: RHABDIASIDAE) FROM AGAMID LIZARDS ON LUZON ISLAND, PHILIPPINES

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ABSTRACT: *Rhabdias odilebaini* n. sp. is described on the basis of specimens found in the lungs of 2 species of agamid lizards: the Philippine flying lizard *Draco spilopterus* and the marbled bloodsucker *Bronchocela marmorata*. Specimens were collected in Aurora Province, Luzon Island, Philippines. The new species of *Rhabdias* is characterized by presence of 4 submedian lips, inconspicuous lateral lips, rounded cross-shaped oral opening, and tail end bent dorsally. This species is morphologically distinct from other *Rhabdias* spp. that parasitize reptilian and amphibian hosts, including 3 other species known to parasitize lizards of the Agamidae.

Rhabdias Stiles et Hassall, 1905, includes approximately 70 species of nematodes parasitic in amphibians and reptiles worldwide (Kuzmin and Tkach, 2002–2011). To date, lizards of the Agamidae Spix, 1825, were known to host only 3 *Rhabdias* species, namely, *Rhabdias japalurae* Kuzmin, 2003, described from 2 species of japalures in southern Japan and Taiwan, *Rhabdias singaporensis* Bursey, Hoong et Goldberg, 2012, from *Calotes versicolor* (Daudin, 1802) in Singapore, and *Rhabdias mcguirei* Tkach, Kuzmin et Brown, 2011, from a flying lizard *Draco spilopterus* (Wiegmann, 1834) in the Philippines (Kuzmin, 2003; Tkach et al., 2011; Bursey et al., 2012). Sixteen other *Rhabdias* species have been described from related lizard families within the Iguania suborder, namely, the Chameleonidae and Polychrotidae in tropical Africa, Madagascar, and Central America (Bursey et al., 2003; Lhermitte-Vallarino and Bain, 2004; Martínez-Salazar, 2006; Bursey et al., 2007; Lhermitte-Vallarino et al., 2008; Lhermitte-Vallarino et al., 2009; Lhermitte-Vallarino et al., 2010). Although *Rhabdias* spp. have been documented primarily from tropical lizards, a recently described species (*Rhabdias lacertae* Moravec, 2010) was found in lacertid lizard (suborder Lacertilia) in the temperate zone of Europe (Moravec, 2010).

In 2009 we conducted a biological survey of terrestrial vertebrates and their parasites in Aurora Province, Luzon Island, Philippines. Initial examination of the parasitic nematodes from agamid lizards revealed a new species (*R. mcguirei* from *D. spilopterus*) that was described by Tkach et al. (2011). Examination of additional material from this survey has revealed another new species of *Rhabdias* from 2 agamid lizards, i.e., *D. spilopterus* and the marbled bloodsucker *Bronchocela marmorata* Gray, 1845. Although the new species and *R. mcguirei* both parasitize *D. spilopterus*, these 2 species of *Rhabdias* are clearly morphologically distinct. The new species is described herein and differentiated from morphologically similar species.

MATERIALS AND METHODS

Lizards were collected by hand or using pitfall traps in several collecting sites in the Aurora Province, Luzon Island, Philippines, during an expedition from 21 May to 1 July 2009. Lizards were necropsied immediately after being killed with 20% aqueous chloratone. Live nematodes recovered from the lungs were rinsed in saline, killed with hot 70% ethanol, and

preserved in 70% ethanol. Before examination using light microscopy, nematodes were cleared in phenol/glycerine solution (ratio 2:1). Drawings were made with aid of a drawing tube. All measurements in the text are in micrometers unless otherwise stated.

Type specimens were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, Nebraska, and the parasite collection at the College of Veterinary Medicine, University of the Philippines–Los Banos, Los Banos, Philippines. The reptilian specimens have been deposited in the University of Kansas Museum of Natural History (KUH), Lawrence, Kansas.

DESCRIPTION

Rhabdias odilebaini n. sp.

(Figs. 1–5)

Diagnosis: Based on 4 specimens, i.e., 1 holotype and 3 paratypes; measurements of the holotype are followed by ranges for the whole series in parentheses: Comparatively large *Rhabdias* species. Body length 14.9 (10.3–14.9) mm, width at midlength 560 (490–600). Anterior extremity rounded, posterior extremity tapering. Inflation of body cuticle (body vesicle) more pronounced on anterior and posterior parts of body. In anterior part, body vesicle swollen, rounded on anterior end and becoming thinner posteriorly (Fig. 1). In caudal region, vesicle tapering posteriorly, covering tail tip (Fig. 5). Lateral pores inconspicuous under light microscope. Four submedian lips present close to oral opening (Fig. 3). Each lip with small papilla on inner side. Lateral lips and amphids inconspicuous. Oral opening rounded, cross-shaped, laterally elongated, leading to narrow vestibulum about 18 long (Fig. 2). Buccal capsule oval-shaped in apical view (Fig. 4), slightly elongated laterally. Anterior segment of buccal capsule barrel-shaped in lateral view; posterior segment wide and shallow, funnel-shaped (Fig. 2). Outer diameter of buccal capsule 25 (25–25), total length of both segments 15 (15–17). Esophagus club-shaped, occupying about half of body width in anterior part of body (Fig. 1). Width of esophagus 57 (55–62) in anterior part, 57 (52–57) at midlength. Dilation of muscular part of esophagus conspicuous, 67 (65–72) wide. Posterior bulb egg-shaped (Fig. 1), 115 (102–115) wide. Nerve ring situated at 240 (220–280) from anterior end of esophagus (26.1% [23.5–28.3%] of esophagus length). Length of esophagus 920 (880–980), or 6.2% (6.7–8.5%) of body length. Excretory pore and excretory duct not observed in studied specimens. Intestine narrow at esophageal-intestinal junction (Fig. 1), then widening posteriorly and occupying whole internal body space anterior to loop of anterior genital tube. Posterior part of intestine wide, thin-walled, filled with dark contents.

Genital system amphidelphic, typical of the genus. Vulva at 8.2 (5.65–8.2) mm from anterior end, slightly postequatorial (55.0% [54.4–55.0%] of body length). Lips of vulva indistinct. Vagina short, transverse, lined with thin cuticle. Uteri thin-walled, filled with numerous eggs containing first-stage larvae. Egg size 112–137 × 55–65 (n = 10, measured in uteri of holotype).

Caudal region conical. Rectum almost tubular, lined with thick cuticle. Tail 490 (360–500) long, or 3.3% (3.1–3.5%) of body length. Tail tip rounded. Posterior part of tail bent to dorsal side in all studied specimens (Fig. 5). Phasmids situated posterior to midlength of tail.

Taxonomic summary

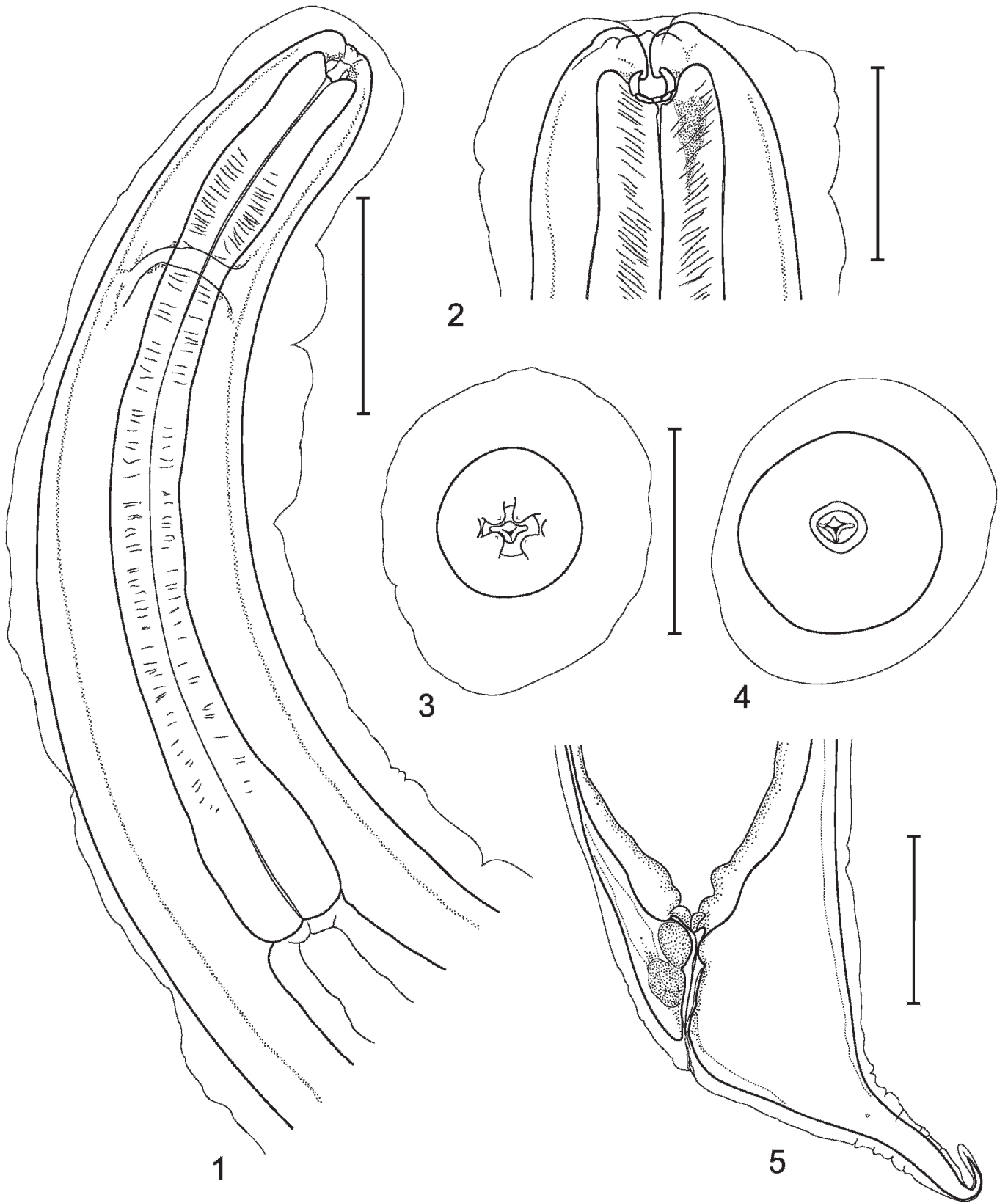
Type host: *Draco spilopterus* (Wiegmann, 1834) (Reptilia: Sauria: Agamidae).

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FIGURES 1–5. *Rhabdias odilebaini* n. sp. (1) Anterior part of body, lateral view. (2) Head end, lateral view. (3) Head end, apical view. (4) Head end, apical view, optical section at level of buccal capsule mid-depth. (5) Tail end, lateral view. Scale bars: 1, 5 = 0.2 mm; 2–4 = 0.1 mm.

Other hosts: *Bronchocela marmorata* Gray 1845 (Reptilia: Sauria: Agamidae).

Infection: Found in 3 of 7 examined *D. spilopterus* and in 1 of 6 examined *B. marmorata*. Each infected host specimen contained only 1 nematode.

Type locality: Aurora Memorial National Park, Sitio Dimani, Barangay Villa Aurora, Municipality of Maria Aurora, Aurora Province, Luzon Island, Philippines, 500 m (a.s.l.), 15.680°N, 121.336°E.

Other localities: Aurora Memorial National Park, Sitio Dimani, Barangay Villa Aurora, Municipality of Maria Aurora, Aurora Province, Luzon Island, Philippines, 500 m (a.s.l.), 15.685°N, 121.341°E; Barangay Zabali, Municipality of Baler, Aurora Province, Luzon Island, Philippines, 75 m (a.s.l.), 15.742°N, 121.576°E.

Specimens deposited: The type series consists of 4 fully mature specimens. Holotype: HWML, collection no. 67081 (labeled: ex. *Draco spilopterus*, Aurora Memorial National Park, Sitio Dimani, Barangay Villa Aurora, Municipality of Maria Aurora, Aurora Province, Luzon Island, Philippines, 28 May 2009, coll. V. Tkach), host specimen KUH 323057. Paratypes: HWML, collection no. 67082 (labeled: ex. *Draco spilopterus*, Aurora Memorial National Park, Sitio Dimani, Barangay Villa Aurora, Municipality of Maria Aurora, Aurora Province, Luzon Island, Philippines, 22 May 2009, coll. V. Tkach), host specimen KUH 32305. Two paratypes were deposited in the parasite collection at the College of Veterinary Medicine, University of the Philippines–Los Banos. One of them was labeled: ex. *Bronchocela marmorata*, Barangay Zabali, Municipality of Baler, Aurora Province, Luzon Island, Philippines, 8 June 2009, coll. V. Tkach, host specimen KUH 323048; the second specimen was labeled ex. *Draco spilopterus*, Barangay Zabali, Municipality of Baler, Aurora Province, Luzon Island, Philippines, 8 June 2009, coll. V. Tkach, host specimen KUH 323072.

Etymology: The species is named after Dr. Odile Bain (Museum National d'Histoire Naturelle, Paris, France) for her tremendous contribution to our knowledge of zooparasitic nematodes and, particularly, *Rhabdias* species from reptiles.

Remarks

The relatively large body size, presence of numerous eggs in uteri, and fully developed larvae in the eggs are characters that *R. odilebaini* shares with other *Rhabdias* species known from lizards of the Agamidae, Polychrotidae, and Chamaeleonidae, as well as some species parasitizing amphibians. The arrangement of circumoral structures (submedian lips overhanging edge of oral opening, lateral lips inconspicuous) of *R. odilebaini* resembles those of *Rhabdias leonae* Martinez-Salazar, 2006, *Rhabdias mariauxi* Lhermitte-Vallarino and Bain, 2009, *Rhabdias kibiraensis* Lhermitte-Vallarino, Barbuto and Bain, 2010, *Rhabdias brygooi* Lhermitte-Vallarino, Barbuto and Bain, 2010, *Rhabdias singaporensis* Bursey, Hoong, and Goldberg 2012 (Bursey et al., 2012) (all parasitic in reptiles), and *Rhabdias africanus* Kuzmin, 2001 and *Rhabdias joaquinensis* Ingles, 1935 (both parasitic in amphibians).

Rhabdias odilebaini is morphologically most similar to *R. brygooi* from a chamaeleonid lizard *Brookesia superciliaris* (Kuhl, 1820) in Madagascar. The 2 species are similar in body size, length of esophagus and tail, shape of oral opening, number and position of lips, and structure of the buccal capsule. *Rhabdias odilebaini* differs from *R. brygooi* by the shape of anterior part of intestine, which is narrow at the esophageal-intestinal junction and widening posteriorly in *R. odilebaini*, whereas in *R. brygooi* it is wide at esophageal-intestinal junction without sharp increase in diameter (Lhermitte-Vallarino et al., 2010). Internal surface of anterior segment of the buccal capsule bears longitudinal crests in *R. brygooi* (Lhermitte-Vallarino et al., 2010), whereas in *R. odilebaini* it is smooth. The esophagus is markedly wider in *R. brygooi*; its width at midlength is 75–100 and posterior bulb is 125–180 wide (Lhermitte-Vallarino et al., 2010), whereas in *R. odilebaini* the esophagus width at midlength is 52–55 and posterior bulb is 102–115.

The head-end morphology (shape and position of circumoral structures and structure of the buccal capsule) of *R. odilebaini* is also similar to 2 other species described from chamaeleonid lizards in Africa, *R. mariauxi* and *R. kibiraensis*. The new species differs from *R. mariauxi* in having smaller buccal capsule (15–17 × 25 in *R. odilebaini* vs 17–18 × 40–48 in *R. mariauxi*) and tail tip enclosed in body vesicle (not enclosed in *R. mariauxi*). In addition, the anterior part of intestine in *R. odilebaini* is

narrower than esophageal bulb, whereas in *R. mariauxi* it is wider than esophageal bulb (Lhermitte-Vallarino et al., 2009).

Rhabdias odilebaini differs from *R. kibiraensis* by having a smaller buccal capsule (15–17 × 25 in *R. odilebaini* vs 22–26 × 41–44 in *R. kibiraensis*) and differently shaped esophageal bulb (egg-shaped in *R. odilebaini* vs broad-based in *R. kibiraensis*). Additionally, the oral opening in *R. odilebaini* is laterally elongated, whereas in *R. kibiraensis* the oral opening is dorsoventrally elongated (Lhermitte-Vallarino et al., 2010).

Rhabdias odilebaini differs from *R. leonae* described from *Norops megapholidotus* Smith, 1933, in Mexico (Martinez-Salazar, 2006) in having a wider buccal capsule (25 in *R. odilebaini* vs 11–19 in *R. leonae*), longer esophagus (880–980 or 6.7–8.5% of body length in *R. odilebaini* vs 670–751 or 4.7–5.89% of body length in *R. leonae*), and longer tail (360–500 or 3.1–3.5% of body length in *R. odilebaini* vs 217–325 or 1.84–2.77% of body length in *R. leonae*). The 2 species are also separated geographically.

Rhabdias odilebaini differs from *R. singaporensis* recently described from *C. versicolor* (Daudin, 1802) in Singapore (Bursey et al., 2012) by the structure of buccal capsule consisting of anterior and posterior segments, larger buccal capsule (25 wide in *R. odilebaini* vs 12–20 in *R. singaporensis*), larger diameter of esophagus (65–72 wide in muscular part and 102–115 maximum width in *R. odilebaini* vs 46–55 and 70–104, correspondingly, in *R. singaporensis*), and longer esophagus (880–980 in *R. odilebaini* vs 497–689 in *R. singaporensis*) and tail (360–500 in *R. odilebaini* vs 122–348 in *R. singaporensis*). These differences are particularly meaningful considering that *R. singaporensis* is very similar in size or even a somewhat longer species (10.2–17.9 mm) than *R. odilebaini* (10.3–14.9 mm). The posterior half of the tail in *R. odilebaini* is narrower and tapers more gradually than that in *R. singaporensis* (Bursey et al., 2012).

Rhabdias odilebaini differs from *R. joaquinensis* (parasite of frogs in North America) and *R. africanus* (parasite of toads in Africa) by the absence of lateral pseudolabia and the buccal capsule consisting of anterior and posterior segments. Additionally, *R. odilebaini* is different from *R. joaquinensis* in the body shape, and the size and position of the vulva, which is situated closer to the middle of body in *R. odilebaini*. The new species has a wider buccal capsule, which is 25 in diameter in the new species vs 10–12 in diameter in *R. joaquinensis* (Kuzmin et al., 2003). In contrast to the new species, *R. africanus* possesses lateral pseudolabia and has comparatively wider and shorter vestibulum of the stoma, shorter esophagus (575–710 in *R. africanus* vs 880–980 in *R. odilebaini*), and narrower esophageal bulb (65–80 in *R. africanus* vs 102–115 in *R. odilebaini*). The esophagus in *R. africanus* lacks prominent dilation of the muscular part (Kuzmin, 2001), which is present in the new species. All 3 species are separated geographically.

Rhabdias odilebaini differs from *R. japalurae* Kuzmin, 2003, known from agamid lizards in Japan and Taiwan (Kuzmin, 2003) by the shape of oral opening (laterally elongated in *R. odilebaini*, round in *R. japalurae*), absence of body cuticle inflation at level of the posterior part of esophagus, longer vestibulum of the stoma, and presence of anterior and posterior segments in the buccal capsule.

Rhabdias odilebaini differs from *R. mcguirei* recently described from agamid lizards in Philippines (Tkach et al., 2011) by the shape of oral opening (rounded cross-shaped in *R. odilebaini* vs round in *R. mcguirei*), size of buccal capsule (15–17 × 25 in *R. odilebaini* vs 17–22 × 37–40 in *R. mcguirei*), and longer tail (360–500 in *R. odilebaini* vs 290–360 in *R. mcguirei*), which is bent dorsally in *R. odilebaini* and straight in *R. mcguirei*.

DISCUSSION

In the present study, *R. odilebaini* was found in 2 host species, *D. spilopterus* and *B. marmorata*. Another *Rhabdias* species, *R. mcguirei*, was also recently described from *D. spilopterus* from the same region of Luzon Island. It is not unusual for single host species to be parasitized by 2 different species of rhabdiasid nematodes in the same region. For instance, European common toads *Bufo bufo* (Linnaeus, 1758) are known to host *Rhabdias bufonis* (Schrank, 1788), *Rhabdias rubrovenosa* (Schneider, 1866), and *Rhabdias sphaerocephala* Goodey, 1924 (Hartwich, 1975); cane toads *Bufo marinus* (Linnaeus, 1758) host *Rhabdias fulleborni* Travassos, 1926, *Rhabdias pseudosphaerocephala* Kuzmin, Tkach

et Brooks, 2007, and *Rhabdias alabialis* Kuzmin, Tkach et Brooks, 2007 (Kloss, 1974; Kuzmin et al., 2007). Single individuals of the European slow-worm *Anguis fragilis* Linnaeus, 1758, are known to simultaneously harbor 2 closely related species of *Entomelas* nematodes (*Entomelas entomelas* [Dujardin, 1845] and *Entomelas dujardini* [Maupas, 1915]). The European glass lizard *Pseudopus apodus* (Pallas, 1775) can simultaneously harbor 2 other *Entomelas* species: *Entomelas ophisauri* (Kreis, 1940) and *Entomelas kazakhstanica* Sharpilo et Vakker, 1972 (Sharpilo, 1976; unpubl. obs.). Interestingly, we found both *R. mcguirei* and *R. odilebaini* in a single individual of *D. spilopterus*. To the best of our knowledge, discovery of 2 *Rhabdias* species in *D. spilopterus* in Philippines is the first case of this kind in lizards in Asia.

Species of *Rhabdias* that are parasites of amphibians are usually rather strictly host-specific (Tkach et al., 2006); however, we know little about the host specificity of rhabdiasids parasitizing lizards. In our study, *R. odilebaini* was found in 2 different host species. It was found most frequently in *D. spilopterus* (3/7 hosts infested) and less frequently in *B. marmorata* (1/6 hosts infested). These 2 lizard species are syntopic and are both frequently found in the crowns of coconut trees, which may provide opportunities for the exchange of these parasites; therefore, it is possible that *B. marmorata* is an accidental host, and that *R. odilebaini* typically complete their life cycle in *D. spilopterus*. However, we did not observe any significant morphological differences in specimens of *R. odilebaini* from both lizard species; moreover, the presence of reproductive adults in both host species suggests that *R. odilebaini* may be able to exploit both of these host species on a regular basis. The sample sizes in our study are too small to draw robust conclusions about the host specificity of *R. odilebaini*. Presumably additional studies of helminths in lizards from the Philippines will yield more robust data on the host specificity of this, and other, *Rhabdias* spp.

Most *Rhabdias* spp. parasitizing lizards (except for *R. lacertae*) are morphologically similar to each other. All these species are mid- to large-sized rhabdiasids, with prominent inflation of the cuticle on at least some parts of the body, a nerve ring situated in the anterior half of the esophagus, and with numerous eggs in the uteri. Moreover, the majority of morphological features found in species from lizards are also typical of *Rhabdias* spp. from amphibians. This fact suggests close relationships of *Rhabdias* spp. parasitizing amphibians and lizards. It remains unclear, however, whether colonization of lizards with *Rhabdias* species was a single evolutionary event or a result of multiple host-switching events. Obtaining molecular data from a broad range of rhabdiasids will be crucial in improving our understanding of the relationships between *Rhabdias* spp. parasitizing lizards and those parasitizing amphibian hosts.

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