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New Species of *Kalicephalus* (Nematoda: Diaphanocephalidae) from a Snake, *Oxyrhabdium leporinum*, on Luzon Island, Philippines

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ABSTRACT: *Kalicephalus burseyi* n. sp. (Nematoda: Diaphanocephalidae) is described from a snake, *Oxyrhabdium leporinum*, on Luzon Island, Philippines. Unequal spicule length places the new species in the subgenus *Kalicephaloides*, which also includes *Kalicephaloides bungari*, *Kalicephaloides longispicularis*, and *Kalicephaloides alatospiculus*. *Kalicephalus burseyi* n. sp. has shorter spicules than *K. longispicularis* and longer spicules than in *K. alatospiculus* and *K. bungari*. The new species also differs from the other three species in details of the copulatory bursa morphology and some other features. The new species is the ninth member of the genus reported from the Philippines and the first *Kalicephalus* species found in *O. leporinum*.

KEY WORDS: *Kalicephalus burseyi*, Nematoda, Diaphanocephalidae, *Oxyrhabdium leporinum*, Luzon Island, Philippines.

Species of the genus *Kalicephalus* Molin, 1861 (Nematoda: Diaphanocephalidae) are primarily parasites of the esophagus, intestine, and stomach of snakes and, more rarely, lizards. Yamaguti (1961) listed 49 species and 1 subspecies in the genus. In his revision of the genus, Schad (1962) synonymized many species and described 5 new species. As a result of this revision, the number of species in the genus was reduced to 23, and they were arranged into 6 groups according to the morphologies of the buccal capsule, uterus, spicules, and bursa. Lichtenfels (1980) raised these 6 groups to the level of subgenera. Baker (1987) gave a comprehensive list of synonyms, subspecies, and hosts for 30 species in the 6 subgenera of *Kalicephalus* and noted that 1 species could not be assigned to a subgenus. Since 1987, 1 additional species has been described from water monitors in China (Zhang et al., 2011), bringing the total number of species in the genus to 32.

Examination of material collected by one of the authors (V.V.T.) in the course of a biodiversity survey of terrestrial vertebrates and their parasites in Aurora Province, Luzon Island, Philippines, has revealed the presence of a new species of *Kalicephalus*, among other parasites. This new species was found in the banded Philippine burrowing snake, *Oxyrhabdium leporinum* (Günther, 1858). The new species is described herein and differentiated from morphologically similar species. To the best of our

knowledge this is the first *Kalicephalus* species reported from *O. leporinum*.

MATERIALS AND METHODS

Twelve specimens of the new *Kalicephalus* species were collected from *O. leporinum* caught by hand on 22 June 2009, in a forest in Aurora Province, Luzon Island, Philippines (see taxonomic summary for locality details). The snake was necropsied immediately after being killed with 20% aqueous chloratone. Live nematodes recovered from the intestine were rinsed in saline, killed with hot 70% ethanol, and preserved in 70% ethanol. Before examination with light microscopy the nematodes were cleared in phenol/glycerine solution (ratio 2:1). Drawings were made with the aid of a drawing tube. Photomicrographs were made using a Zeiss Axio Imager M1 light microscope equipped with differential interference contrast microscopy optics. All measurements in the text are in micrometers unless otherwise stated.

Type material was deposited in the parasite collection of the Harold W. Manter Laboratory (HWML) of the University of Nebraska, Lincoln, Nebraska, U.S.A. Types were deposited at HWML with the understanding that representative types will ultimately be repatriated to collections in the Philippines. The host was deposited at the University of Kansas Natural History Museum, Lawrence, Kansas, U.S.A.

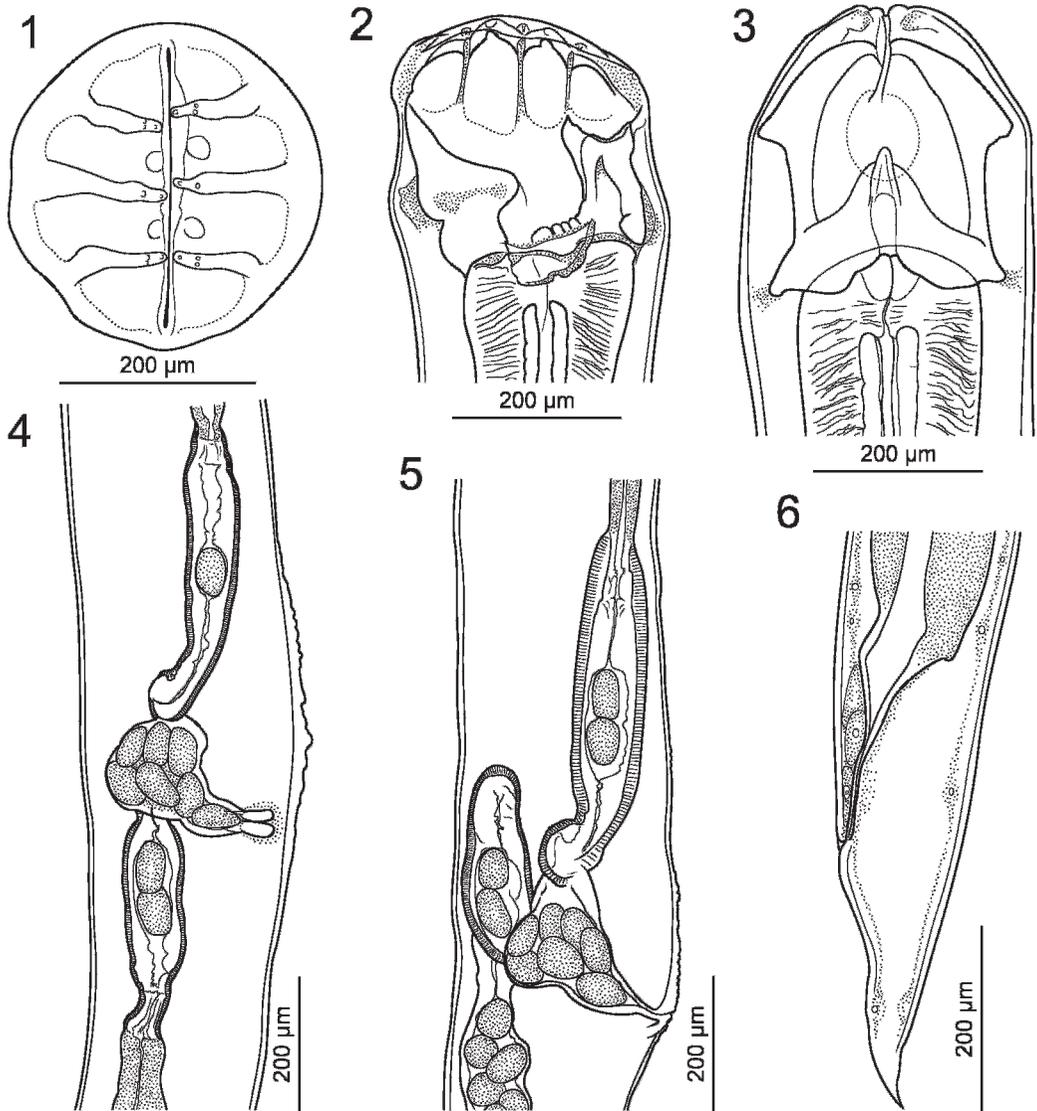
DESCRIPTION

Kalicephalus burseyi n. sp. (Figs. 1–20)

Diagnosis

Based on 4 complete (holotype and 3 paratypes) and 2 incomplete (1 anterior part and 1 damaged in

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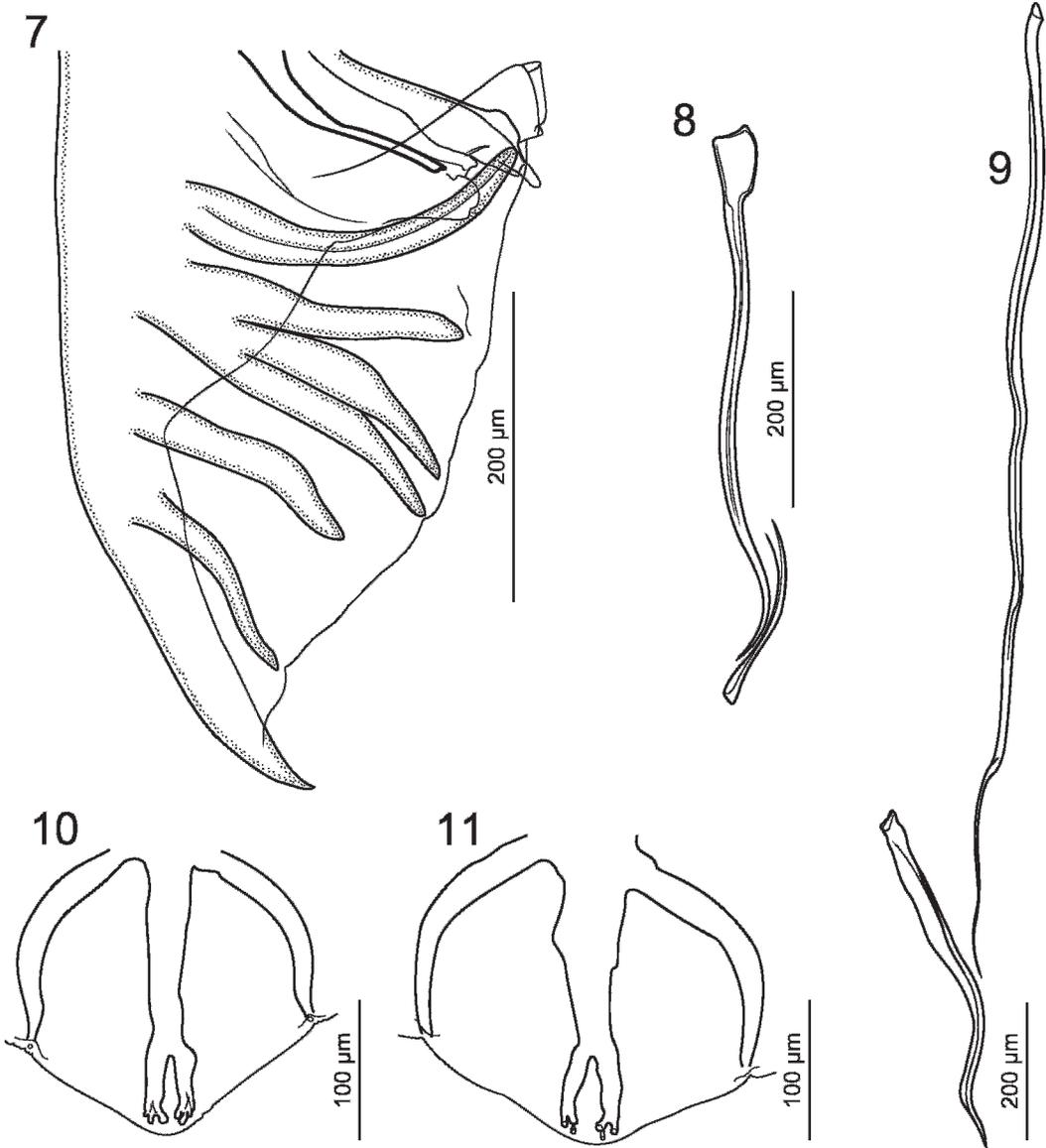


Figures 1–6. *Kalicephalus burseyi* n. sp. **1.** Apical view of anterior end (male). **2.** Lateral view of anterior end (male, holotype). **3.** Dorsal view of anterior end (female allotype). **4.** Vulva, vagina and ovjectors in smaller female paratype, ventrolateral view. **5.** Same structures in larger female paratype, lateral view. **6.** Tail end of female, lateral view (paratype).

posterior portion) male specimens and 6 complete female specimens (allotype and 5 paratypes).

General: Anterior half of body almost uniform in width; in lateral view, width of anterior end often somewhat larger than width of body at midlength. Posterior half of body tapering. Body cuticle finely transversely striated, with no inflations. Anterior edge slightly tilted dorsally: dorsal edge of buccal capsule somewhat shorter than ventral edge (Figs. 2, 15).

Anterior plates of buccal capsule valves thick, each with 3 longitudinal grooves in which parenchymatous bands situated (Fig. 18). Anterior chitinous ridges each with two rounded projections situated between lateral and submedian papillae (Figs. 1, 2). Posterior plates (Fig. 19) comparatively thin in anterior part, thickened posteriorly. On ventral side, valves joined by rounded step-shaped connection. On dorsal side, tooth-like dorsal gutter present (Fig. 4). Entrance to esophagus rounded in apical view (Fig. 20), cup-

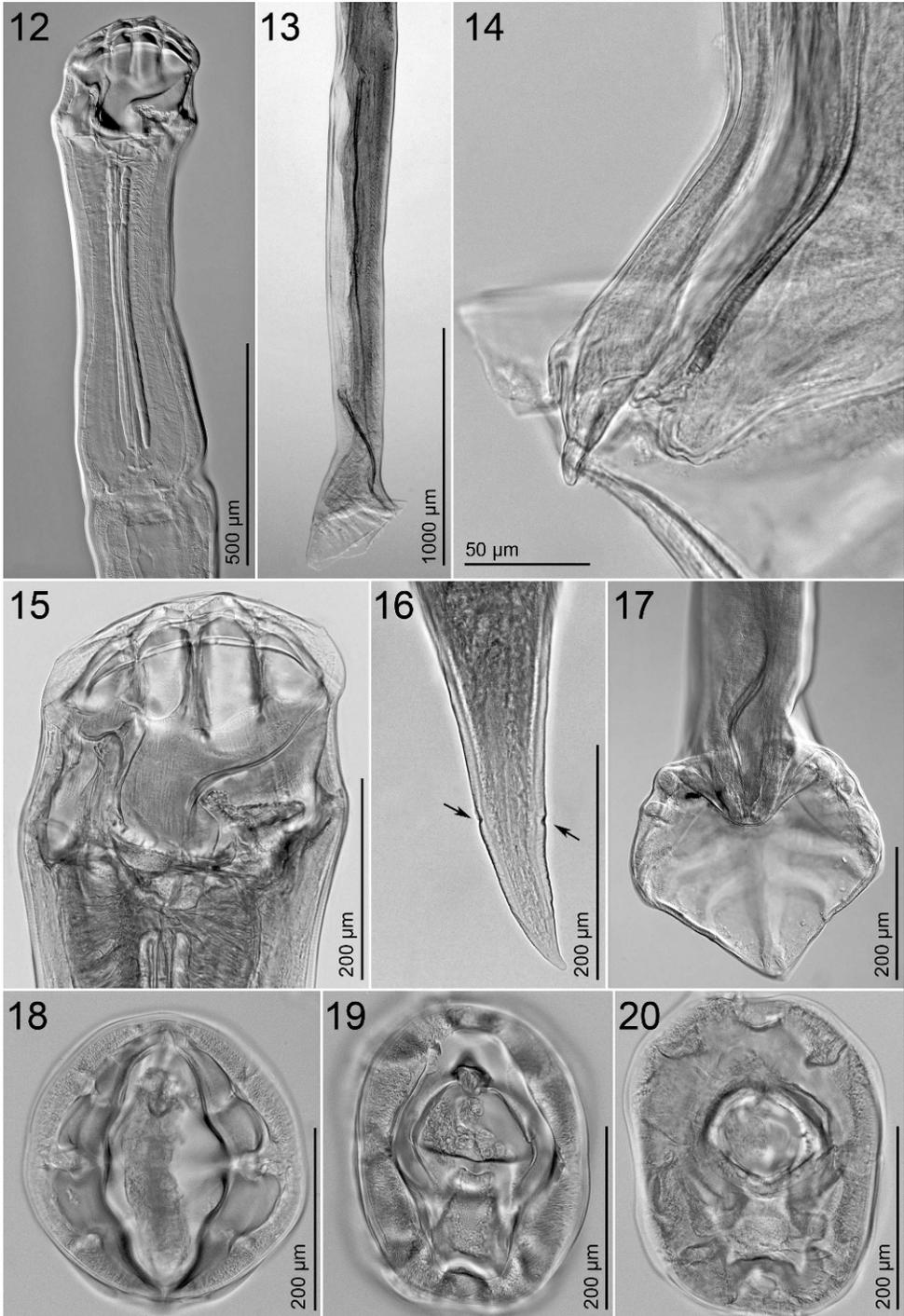


Figures 7–11. *Kalicephalus burseyi* n. sp. **7.** Caudal end of male, lateral view (holotype). **8.** Right spicule and gubernaculum, left lateral view (holotype). **9.** Spicules, ventral view (holotype). **10.** Dorsal ray of bursa, ventral view (holotype). **11.** Dorsal ray of bursa, dorsal view (paratype).

shaped in lateral view, with sclerotized walls. Esophagus club-shaped, muscular, with prominent egg-shaped bulb (Fig. 12). Lumen of esophagus thick-walled, except for its anterior-most part. Nerve ring surrounding esophagus at border between its anterior and middle thirds. Excretory pore situated slightly posterior to nerve ring. Deirids minute, rounded, situated at level of excretory pore. Excretory duct thin, short. Excretory glands spindle-shaped or

with rounded posterior part, with posterior ends situated at level of esophageal-intestinal junction. Intestine wide, thick-walled, its anterior part as wide as or slightly narrower than esophageal bulb (Fig. 12).

Males: Measurements of holotype are followed by ranges of all male types in parentheses. Body length 7.37 (5.82–7.80) mm. Body width at midlength 262 (219–349). Width of anterior end in lateral view 322



Figures 12–20. *Kalicephalus burseyi* n. sp. **12.** Anterior part of body, lateral view (paratype). **13.** Posterior part of body, lateral view (paratype). **14.** Genial cone, lateral view (holotype). **15.** Anterior end, lateral view (male paratype). **16.** Tail end of female, ventral view (paratype), arrows indicate phasmids. **17.** Posterior part of male, ventral view (paratype). **18–20.** Optical sections of buccal capsule, apical view. **18.** Anterior to dorsal gutter. **19.** Level of dorsal gutter. **20.** Entrance to esophagus.

(319–367). Buccal capsule depth 262 (262–296). Esophagus 708 (681–867) long or 9.6 (9.6–11.7) % of body length. Width of esophagus 198 (177–207) at anterior end, 135 (100–146) at midlength; maximum width of bulb 231 (178–245). Nerve ring and excretory pore at 228 (149–236) and 372 (236–372) from anterior end of esophagus, correspondingly.

Testis long, forming 2 bends in anterior part. Spicules dramatically dissimilar in size (Fig. 9). Left spicule thin, usually straight, sometimes undulated, 1.53 (1.0–1.55) mm long. Right spicule 542 (468–542) long, wider in anterior part, its posterior portion curved ventrally (Fig. 8). Both spicules finely transversely striated, with widened anterior ends, attenuated posterior parts and sharpened posterior ends. Gubernaculum 208 (164–208) long. Genital cone prominent, its tip rounded in ventral view (Figs. 14, 17). Bursa typical of the genus (Fig. 7). Ray 1 indistinct, rays 2 and 3 joined, rays 4, 5, and 6 situated close to each other, rays 7 and 8 separated; end of ray 7 not reaching edge of bursa. Dorsal ray of type I of Schad (1962) (Figs. 10, 11, 17), bifurcated at about beginning of its posterior third. Each of two branches of dorsal ray bears 3 short terminal branches, of which one is comparatively smaller and directed ventrally. Phasmids situated at about mid-distance between base of dorsal ray and its bifurcation.

Females: Measurements of allotype are followed by ranges of all female types in parentheses. Larger than males, body 8.5 (7.9–10.5) mm long, 340 (302–432) wide at midlength. Anterior end 343–403 wide in lateral view (not measured in allotype). Buccal capsule 320 (315–354) deep. Esophagus 900 (830–935) long or 10.6 (8.3–11.0) % of body length. Width of esophagus 230 (203–258) at anterior end, 170 (123–183) at midlength; maximum width of bulb 290 (239–330). Nerve ring and excretory pore situated at 330 (217–330) and 317–410 (not measured in allotype) from anterior end of esophagus.

Vulva postequatorial, situated at 5.82 (5.32–6.35) from anterior end (68.5 [60.2–68.5] % of general length). Body cuticle anterior to vulva with “jammed” surface covered with numerous folds (Figs. 4, 5). Slight elevation of body wall present around vulva. Distal part of vagina with thick sclerotized walls. Genital system generally amphidelphic. Posterior ovijector, however, transposed anteriorly in larger (older) females, forming several bends anterior to level of vulva (Fig. 5). In smaller females, posterior ovijector straight, situated posterior to level of vulva (Fig. 4). Anterior ovijector straight, with small

transverse bend close to vagina. Uteri tubular, elongated, thin-walled, filled with numerous eggs. Sclerotized valve present in lumen of each genital tube between oviduct and uterus. Both ovaries directed anteriorly. Anterior ovary forming several coils around intestine in anterior part of body. Eggs in ovijector and vagina in morula stage (Figs. 4, 5). Eggs measured in ovijectors and uteri closest to ovijectors 56–70 long and 34–43 wide ($n = 15$). Egg shell, when visible, 11–21 longer and 5–15 wider than egg. Tail conical, gradually tapering, with sharpened or rounded tip (Fig. 6). Phasmids situated posterior to midlength of tail (Fig. 16). Tail length 280 (247–330) or 3.3 (2.5–3.9) % of body length.

Taxonomic summary

Type host: *Oxyrhabdium leporinum* (Duméril, Bibron, and Duméril, 1854) (Reptilia: Squamata: Lamprophiidae). Host specimen: KUMNH 323386.

Infection: 12 specimens (6 males and 6 females) found in one *O. leporinum*.

Type locality: Barangay Lipimental, Municipality of San Luis, Aurora Province, Luzon Island, Philippines; 543 meters above sea level; 15°39'14"N; 122°11'10"E.

Specimens deposited: The type series consists of 12 fully mature specimens, 6 males (2 incomplete) and 6 females. Holotype: HWML, collection no. 68049 (labeled: ex. *Oxyrhabdium leporinum*, Barangay Lipimental, Municipality of San Luis, Aurora Province, Luzon Island, Philippines, 22 June 2009, coll. V. Tkach). Allotype: HWML, collection no. 68049 (labeled identically). Paratypes: HWML, collection no. 68050 (labeled identically).

Etymology: The species is named after Dr. Charles R. Burse (Pennsylvania State University, Shenango Campus, Sharon, Pennsylvania) in recognition of his many contributions to the parasitology of amphibians and reptiles.

REMARKS AND DISCUSSION

Species of *Kalicephalus* with unequal spicules were placed in the subgenus *Kalicephaloides* Yeh, 1956, by Lichtenfels (1980). This subgenus included *Kalicephalus bungari* (MacCallum, 1918) (synonyms: *Kalicephalus minutus* [Baylis and Daubney, 1922] and *Kalicephalus naiae* Maplestone, 1931), *Kalicephalus longispicularis* Schad, 1962, and *Kalicephalus alatospiculus* (Oshmarin and Demshin,

Table 1. *Kalicephalus* species, their hosts, and distribution in the Philippines. Localities are provided as mentioned in the references column.

Species	Hosts	Localities	References
<i>K. willeyi</i>	Philippine cobra, <i>Naja philippinensis</i> Palawan spitting cobra, <i>Naja naja mirolepis</i>	Alabang, Rizal Province, Luzon Terabanan, Palawan	Tubangui and Masiluñgan, 1937 Schmidt and Kuntz, 1972
<i>K. brachycephalus</i>	Mangrove snake, <i>Boiga dendrophila</i>	Terabanan, Palawan	Schmidt and Kuntz, 1972
<i>K. costatus</i>	Mangrove snake, <i>Boiga dendrophila</i>	Terabanan and Puerto Princessa, Palawan	Schmidt and Kuntz, 1972
<i>K. megacephalus</i> (?)	Water monitor, <i>Varanus salvator</i>	Terabanan, Palawan	Schmidt and Kuntz, 1972
<i>K. novaebritanniae</i>	Specklebelly keelback snake, <i>Rhabdophis chrysargos</i>	Terabanan, Palawan	Schmidt and Kuntz, 1972
<i>K. posterovulvus</i>	Bronze tree snake, <i>Dendrelaphis caudolineatus</i> Malayan banded wolf snake, <i>Lycodon subcinctus</i>	Terabanan and Puerto Princessa, Palawan	Schmidt and Kuntz, 1972
<i>K. sinensis</i>	Philippine ratsnake, <i>Coelognathus erythrurus</i>	Terabanan, Palawan	Schmidt and Kuntz, 1972
<i>K. viperae chungkingensis</i>	Brahminy blind snake, <i>Ramphotyphlops braminus</i>	near former Clark Air Force Base (15°20'N; 120°50'E), Bulacan Province, Luzon	Goldberg et al., 2005
<i>K. burseyi</i> n. sp.	Banded Philippine burrowing snake, <i>Oxyrhabdium leporinum</i>	Municipality of San Luis, Aurora Province, Luzon (15°40'N; 121°31'E)	This study

1972). Both the right spicule (0.91 to 1.19 mm) and left spicule (2.28 to 3.02 mm) of *K. longispicularis* are much longer than the corresponding spicules of *K. burseyi* n. sp. In addition, the terminal branches of the dorsal ray of *K. longispicularis* are of the type III pattern of Schad (1962), while the branches of the dorsal ray of *K. burseyi* n. sp. are of type I (Figs. 10, 11, 17). The branches of the dorsal ray of *K. alatospiculus* and *K. bungari* are also of type I (MacCallum, 1918; Oshmarin and Demshin, 1972), but the right spicule and left spicule of both species are considerably shorter than those of *K. burseyi* n. sp. The buccal capsules in both sexes of *K. burseyi* n. sp. are larger than in *K. bungari*, and the esophagus is longer in both sexes. In *K. alatospiculus*, the externo-dorsal and dorsal rays are illustrated as having a common base, while the bases are well separated in *K. burseyi* n. sp. (Fig. 7).

To the best of our knowledge, the new species is the first *Kalicephalus* recorded from the banded Philippine burrowing snake, *O. leporinum*. Eight species of *Kalicephalus* have been reported from reptiles in the Philippines to date (Table 1). Schad (1962) suggested that the distribution of *K. longispicularis* Schad, 1962, which was described from the reticulated python, *Python reticulatus* (Schneider, 1801) from the National Zoo (Washington, D.C., USA), should match the distribution of its definitive host, and thus it is likely to be distributed in the Philippines. However, parasites frequently have geographic distributions that are much smaller than that of their hosts (Nuismer et al., 2003). Considering

the very broad geographic distribution of the reticulated python and the fact that *K. longispicularis* was not reported from nature in the Philippines, this species cannot be included in the list of *Kalicephalus* known from the country. Thus, *K. burseyi* n. sp. is the ninth species of the genus reported from the country and the third species found on the northernmost island, Luzon, along with *Kalicephalus willeyi* Linstow, 1904 and *Kalicephalus viperae chungkingensis* Hsu, 1934 (Tubangui and Masiluñgan, 1937; Schad, 1962; Goldberg et al., 2005; Table 1).

Currently, there is little helminthological data for the majority of reptile species in the Philippines. Moreover, the majority of the islands in the archipelago have not been surveyed for endoparasites of reptiles. Considering the very diverse herpetofauna of the Philippines, the large number of islands, and the fact that 7 species of *Kalicephalus* have been found on Palawan Island alone, we anticipate that future studies will reveal additional species and information about the geographic distribution of *Kalicephalus* in the Philippines.

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