Description of *Pseudocapillaria (Discocapillaria) trilobularis* n. sp. (Capillariidae) and redescription of *Heliconema alatum* (Majumdar, 1965) (Physalopteridae), two nematodes parasitising synbranchiform fishes in India

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Abstract One new and one already known species of nematodes are described based on specimens collected from synbranchiform fishes of the River Ganga, India, in 2021 and 2022. *Pseudocapillaria (Discocapillaria) trilobularis* n. sp. (Capillariidae) from the intestine of cuchia *Monopterus cuchia* (Hamilton) (Synbranchidae) is mainly characterised by the presence of the large ventral postcloacal lobe in the male, the spicule length 351 µm, eggs size 60–69 × 30–36 µm (with protruding polar plugs) and by the body length (male 10.50 mm, females 11.02–12.44 mm). It is the fourth species of this genus recorded from fishes in India. The species *Heliconema alatum* (Majumdar, 1965) (Physalopteridae) is resurrected. This nematode is redescribed from specimens collected from the intestine of zig-zag eel *Mastacembelus armatus* (Lacepède) (Mastacembelidae). The examination by SEM revealed some previously not reported morphological features in this nematode species, e.g., the presence of cephalic papillae or a lateroterminal depression and two inner flat dorsoventral teeth on each pseudolabium. Based on these findings, *H. monopteri* Moravec, Chaudhary & Singh, 2019 is considered a junior synonym of *H. alatum*.

Introduction

As recently pointed out by Moravec et al. (2022), regardless of the large number of mostly taxonomic and faunistic papers treating freshwater fish nematodes in South Asian countries including India (Sood 2017), knowledge of the real fauna of these parasites in this region is still scarce. This unsatisfactory situation is not only due to the fact that many species of fish nematodes in this region were poorly described or incorrectly identified, but also because there are
very many unsolved taxonomic problems in the respective nematode groups.

During parasitological examinations of some freshwater fishes in district Muzaffarnagar and Bijnor of Uttar Pradesh, India carried out in 2021 and 2022, adult nematode specimens were collected from the intestines of two species of synbranchiform fishes (Synbranchiformes), the cuchia Monopterus cuchia (Hamilton) (Synbranchidae) and the zig-zag eel Mastacembelus armatus (Lacepède) (Mastacembelidae). A closer examination of their morphology with the use of both light (LM) and scanning electron microscopy (SEM) showed that they belong to one new and one known but insufficiently described species. Results of their study are presented below. The results of molecular studies on these species will be published later.

Materials and methods

Fish were caught from district Muzaffarnagar (29.4727°N, 77.7085°E) and Bijnor (29.3833°N, 79.1833°E), Uttar Pradesh, India. The live nematodes obtained were washed in physiological saline and then fixed in hot 70% ethanol. For LM examination, they were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. Specimens used for SEM examination were postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. The fish nomenclature follows FishBase (Froese & Pauly, 2022).

Family Capillariidae Railliet, 1915

Pseudocapillaria Freitas, 1959

Type species: Pseudocapillaria tomentosa (Dujardin, 1843) Lomakin & Trofimenko, 1982.

Pseudocapillaria (Discocapillaria) trilobularis. (n. sp.)

Type-host: Monopterus cuchia (Hamilton) (Synbranchiformes, Synbranchidae), cuchia.

Type-locality: Fish market in district Muzaffarnagar (29.4727°N, 77.7085°E) (fish allegedly caught in the River Ganga), Uttar Pradesh, India (dates of collection 13–29.x. and 3.xii. 2021).

Type-material: Helminthological Collection of the Institute of Parasitology, BC CAS, České Budějovice, Czech Republic (holotype and allotype mounted on SEM stubs, plus 5 paratypes in vials, Cat. No. N–1270).

Prevalence and intensity: In 3 of 7 fish examined; 2–3 nematodes per fish.

Site in host: Intestine.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for Pseudocapillaria trilobularis n. sp. is urn:lsid:zoobank.org:act:D49EF3C4-383D-4ECB-94C9-G7CB933EDA0D.

Etymology: The specific name trilobularis (= three-lobular) is a Latin adjective and it relates to the three-lobed appearance of the male caudal end of this nematode.

Description

General Small, filiform nematodes with finely transversely striated cuticle (Fig. 2A, D). Anterior end of body narrow, rounded; cephalic papillae indistinct. Two inconspicuous lateral bacillary bands extending along almost whole body length (Fig. 1D). Muscular oesophagus long, narrow (Fig. 1A). Stichosome consisting of single row of about 35–45 elongate stichocytes without transverse annuli; 1–2 elongate stichocytes light in colour alternating with 1–2 short, darker (more granular) ones; nuclei of stichocytes large (Fig. 1B, C). Nerve ring encircling muscular oesophagus at approximately one fourth of its length. Two wing-like glandular cells present at level of oesophago-intestinal junction (Fig. 1C).

Male [Based on 1 specimen, holotype] Length of body 10.50 mm, maximum width 57. Width of lateral bacillary bands at region of posterior end of oesophagus 24. Length of entire oesophagus 5.48 mm, representing 52% of body length. Length of muscular oesophagus 246, of stichosome 5.39 mm; number of
stichocytes about 35. Nerve ring situated 90 from anterior extremity. Seminal vesicle elongate-oval, 96 long, 36 wide (Fig. 1F). Spicular canal absent. Spicule well sclerotized, smooth, 351 long (Fig. 1F). Proximal end of spicule markedly expanded, 15 wide, without lobular rim; width of middle part of spicule 6; distal end narrowed, rounded, 3 wide (Fig. 1J). Spicular sheath without spines; contracted inner surface of withdrawn sheath appearing as spirally coiled thread surrounding anterior half of spicule (Fig. 1F). Posterior end of body obtuse, provided with 2 large, round ventrolateral lobes 6 long and 9 wide in lateral view; posterior lip of cloaca elevated, forming conspicuously large ventral lobe subdivided by median, longitudinal slit-like depression into 2 lateral parts; each part probably provided with indistinct papilla (Figs. 1E, K, 2A–C). Width of caudal end in lateral view 27, length of tail 12.

Female [Based on 6 gravid specimens; measurements of allotype in parentheses; measurements of 1 non-gravid specimen in brackets] Length of body 11.02–12.44 (11.83) [10.31] mm, maximum width 75–84 (78) [72]. Width of lateral bacillary bands at region of posterior end of oesophagus 30–33 (30) [27].
Length of entire oesophagus 3.78–4.56 (3.75) [3.26] mm, representing 34–37% (34%) [36%] of body length. Length of muscular oesophagus 180–303 (303) [330], of stichosome 3.49–4.28 (3.78) [3.52]; number of stichocytes about 43–45 (43) [40]. Nerve ring situated 81–102 (87) [96] from anterior extremity. Vulva located 3.78–4.56 (4.08) [3.78] mm from anterior end of body, at 34–38% (34%) [37%] of body length, at 0 (0) [21] posterior to level of oesophago-intestinal junction. Vulval lips not protruding (Fig. 1C). Vagina short, muscular. Eggs arranged in single file in uterus. Eggs oval, mostly with slightly protruding polar plugs (Fig. 1G). Egg wall appearing as two-layered; inner layer thin, hyaline, outer layer thick, with rough net-like structure on surface (Fig. 1H). Size of eggs including polar plugs 60–69 × 30–36 (69 × 33) [-], thickness of egg wall 3 (3); polar plugs 4–6 (6) long and 6–9 (6–9) wide, their protruding parts 0–3 (3) high. Content of fully developed eggs uncleaved. Caudal end rounded, 30–33 (30) [33] wide in lateral view, anus subterminal (Figs. 1I, 2D); tail 7–9 (9) [9] long. Rectum formed by hyaline tube 99–138 (102) [105] long (Fig. 1I).

Remarks

Moravec (1982) proposed a classification system of capillariid nematodes with newly defined genera and, at present, this is widely used. According to this system, the capillariid genus *Pseudocapillaria* Freitas, 1959 includes parasites of all main classes of vertebrates (fishes, amphibians, reptiles, birds and

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**Fig. 2** *Pseudocapillaria (Discocapillaria) trilobularis* n.sp., scanning electron micrographs. A, B, Caudal end of male, sublateral and ventral views, respectively; C, Caudal end of male, apical view; D, Caudal end of female, sublateral view. **Abbreviations:** a, anus; b, ventrolateral caudal lobe; c, ventral caudal lobe with median slit-like depression.

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mammals). However, some species were assigned to this genus tentatively, so that subsequent detailed studies may result in establishing new genera for them (Moravec, 1982).

In his monograph, Moravec (2001a) listed 15 species of Pseudocapillaria as gastrointestinal parasites of freshwater, brackish-water and marine fishes, but one of them, *P. sphyræni* (Parukhin, 1971), was later transferred to the genus *lobocapillaria* Moravec & Beveridge, 2017 (see Moravec & Beveridge, 2017). An additional four new species of *Pseudocapillaria*, *P. novaecaledoniensis* Moravec & Justine, 2010, *P. nannupensis* Hobbs & Hassan, 2010, *P. moraveci* Iglesias, Centro, García & García-Estevez, 2013 and *P. bumpi* Svitin, Bullard, Dutton, Netherlands, Syrota, Verneau & du Preez, 2021, were described since and *Capillaria decapteri* was transferred to this genus as *P. decapteri* (Luo, 2001) (Luo, 2001; Hobbs & Hassan, 2010; Moravec & Justine, 2010; Iglesias et al., 2013; Svitin et al., 2021). Consequently, at present *Pseudocapillaria* comprises 19 valid species parasitic in fishes, nine of which being parasites of freshwater fishes.

Species of *Pseudocapillaria* from fishes are representatives of four subgenera: *Pseudocapillaria* Freitas, 1959, *Ichtiov capillaria* Moravec, 1982, *Indocapillaria* De & Maity, 1995 and *Discocapillaria* De & Maity, 1996 (see Moravec, 2001a). In possessing the posterior cloacal lip modified as a conspicuously large ventral lobe, *P. trilobularis* n. sp. belongs to the hitherto monotypic subgenus *Discocapillaria*. The type and the only species of this subgenus is *P. (D.) margolisi* De & Maity, 1996 described from the intestine of cypriniform fishes, *Pethia conchonius* (Hamilton) (Cyprinidae) (type-host), *Puntius sophore* (Hamilton) (Cyprinidae) (type-host), *Amblypharyngodon mola* (Hamilton) (Danionidae) in West Bengal, India (De & Maity, 1996).

Specimens of the present new species are much larger than those of *P. margolisi* (body length of male 10.50 mm vs 9.99–3.16 mm, of female 11.02–12.44 mm vs 3.38–6.83 mm) and their stichocytes are usually more numerous (35–45 vs 24–37). The spicule of *P. trilobularis* n. sp. is distinctly longer than that of *P. margolisi* (351 μm vs 85–295 μm) and its proximal end is smooth, without lobular rim (vs with distinct lobular rim). Moreover, the marked longitudinal depression on the male ventral lobe, which is present in *P. trilobularis* n. sp., was not observed in *P. margolisi*. However, it is necessary to remark that the latter species was studied only by LM, whereas details of the male caudal end of the new species were revealed by SEM. In our opinion, the above-mentioned morphometrical differences between these two forms, and also the fact that hosts of these nematodes belong to different fish orders (Synbranchiformes vs Cypriniformes), are sufficient to consider the specimens from *M. argus* to represent a new species.

In contrast to *P. margolisi* and *P. trilobularis* n. sp., both representatives of the subgenus *Discocapillaria*, all species belonging to other three subgenera of *Pseudocapillaria* have no ventral caudal lobe in the male. Many of them also differ from the new species in having the spicule distinctly shorter (less than 300 μm) or longer (exceeding 440 μm). Only one species of the subgenus *Pseudocapillaria*, *P. tomentosa* (Dujardin, 1843), and three species of the subgenus *Ichtiov capillaria*, *P. nannupensis*, *P. novaecaledoniensis* and *P. salvelini* (Polyansky, 1952), have the spicule length similar to that of *P. trilobularis* n. sp. However, *P. tomentosa*, a parasite mainly of Holarctic cypriniforms (Moravec, 2001a), differs from the new species in having the anterior end of spicule provided with a distinct lobular rim (vs lobular rim absent). In contrast to *P. trilobularis* n. sp., the two male ventrolateral caudal lobes of *P. nannupensis*, *P. novaecaledoniensis* and *P. salvelini* are connected between each other by a short dorsal cuticular membrane (vs dorsal cuticular membrane absent). Moreover, *P. nannupensis*, a parasite of the Australian freshwater catfish (*Hobbs & Hassan, 2010*), has smaller eggs (48–55 × 21–26 μm vs 60–69 × 30–36 μm) with non-protruding polar plugs, whereas *P. salvelini*, a parasite mainly of Holarctic salmonids (Moravec, 2001a), is characterised by the lobular anterior end of spicule (vs nonlobular). *Pseudocapillaria novaecaledoniensis*, a parasite of the marine perciform fish in New Caledonia (Moravec & Justine, 2010), also differs in smaller eggs (48–54 × 21–24 μm) and stichocytes subdivided into transverse annuli (vs stichocytes without annuli).

To date, besides the above-mentioned *P. margolisi*, an additional two species of *Pseudocapillaria* have been reported from freshwater fishes in India, both belonging to the nomenotypical subgenus *Pseudocapillaria*: *P. indica* Moravec, Razia Beevi, Radhakrishnan & Arthur, 1993, a parasite of *Channa gachua* (Hamilton) (Anabantiformes, Channidae) in Kerala (Moravec et al., 1993), and *P. lepidocephali* De &
Maity, 1994, a parasite of *Lepidocephalichthys guntea* (Hamilton) (Cobitidae, Cypriniformes) in West Bengal (De & Maity, 1994). The spicule of both these species is distinctly shorter than that of *P. trilobularis* **n. sp.** (240–282 μm and 104–161 μm, respectively, vs 351 μm). Consequently, at present, there are four known species of *Pseudocapillaria* parasitizing freshwater fishes in India.

*Pseudocapillaria trilobularis* **n. sp.** is the first Indian capillariid studied by SEM. Unfortunately, anterior body ends of the three specimens examined by this method proved not to be clean enough to make detailed observation of the cephalic structures. Nevertheless, it can be assumed that the general structure of the cephalic end of this nematode is similar to those in other capillariids, i.e., that there are present two small lateral lips bearing minute amphids and 12 cephalic papillae in two circles surrounding lips (e.g., Baruš et al., 1981; Moravec, 2001b; González-Solís et al., 2014; Moravec & Beveridge, 2017; Moravec & Barton 2018, 2019).

**Family Physalopteridae Railliet, 1893**

*Heliconema*

Type-species: *Heliconema heliconema* Travassos, 1919.

*Heliconema alatum* (Majumdar, 1965) De, 1988

Syns.: *Notopteroides alatae* Majumdar, 1965; *Pseudoproleptus armati* Sahay, Sinha & Sadhu, 1970; *Paraleptus komiyai* Sood, 1970; *Heliconema kherai* Gupta & Duggal, 1989; *H. monopteri* Moravec, Chaudhary & Singh, 2019.

**Host:** *Mastacembelus armatus* (Lacepède) (Synbranchiformes, Mastabembridae), zig-zag eel.

**Locality:** Fish market in Bijnor (29.3724°N, 78.1358°E) (fish allegedly caught in the River Ganga), Uttar Pradesh, India (dates of collection 4.xii.2021 and 6.i.2022).

**Voucher-material:** Helminthological Collection of the Institute of Parasitology, BC CAS, České Budějovice, Czech Republic, Cat. No. N–1265.

*Prevalence and intensity:* In 3 of 4 fish examined; 1–2 nematodes per fish.

**Site in host:** Intestine.

**Description**

**General** Medium sized, whitish nematodes with thick, transversely striated cuticle. Cephalic end rounded.

Cuticle in cephalic region inflated to form cephalic vesicle extending posteriorly to about level of deirids and anteriorly forming somewhat extended collar (Figs. 3A, 4A). Oral aperture dorsoventrally elongate, oval, rather large, surrounded by 2 massive, rounded lateral pseudolabia. Each pseudolabium bears 2 large submedian (dorsolateral and ventrolateral) cephalic papillae and oval lateroterminal depression filled with irregularly lobular mass; small lateral amphids situated between both cephalic papillae (Figs. 3A, 4B, D). Inner surface of each pseudolabium with triangular terminal lateral tooth (internoterminal tooth) situated immediately near inner border of cephalic depression and simple flat tooth at each dorsoventral extremity; no denticles present near terminal lateral teeth (Figs. 3A, 4B, D). Buccal cavity short. Oesophagus divided into short, narrow anterior muscular portion and much longer, wide glandular portion. Nerve ring encircles muscular oesophagus approximately at its middle or somewhat posterior to it. Small simple deirids situated just anterior to level of nerve ring (Fig. 3A). Excretory pore slightly anterior to anterior end of glandular oesophagus (Fig. 3A). Tail of both sexes with rounded tip.

**Male** [Based on 1 specimen] Length of body 24.68 mm, maximum width 462. Pseudolabia 36 long. Cephalic vesicle 231 long and 272 wide. Buccal cavity 30 long. Entire oesophagus 3.01 mm long, representing 12% of body length; muscular oesophagus 422 long and 68 wide; glandular oesophagus 2.58 mm long and 190 wide; length ratio of two parts of oesophagus 1:6.1. Nerve ring, deirids and excretory pore 313, 245 and 381 from anterior extremity, respectively. Caudal end spirally coiled, provided with lateral alae supported by 4 pairs of subventral pedunculate preanal papillae arranged in couples, and 4 single pairs of subventral postanal papillae, which are rather large and pedunculate; an additional 2 pairs of small postanal sessile papillae situated more ventrally slightly posterior to level of last subventral postanal pair (Figs. 3C, D, 4E). Pair of minute...
phasmids present posterior to posteriormost pair of papillae (Figs. 3C, 4E). Ventral precloacal surface with about 15 longitudinal tesselated ridges (area rugosa) (Fig. 4C). Spicules unequal in length, but equally broad (Fig. 3D); left spicule 552 long, right spicule 258 long; length ratio of spicules 1:2.14. Length of tail 272.

**Female** [Based on 2 non-gravid specimens; measurements of 1 incomplete gravid specimen, with missing anterior portion, in parentheses] Length of body 28.61–31.59 mm (body fragment 38.98 mm long), maximum width 585–598 (639). Pseudolabia 27–41 (-) long. Cephalic vesicle 272–313 (-) long and 313–367 (-) wide. Buccal cavity 41 (-) long. Entire oesophagus 4.26–4.68 (-) mm long, representing 15% of body length; muscular oesophagus 558–625 (-) long and 82 (-) wide; glandular oesophagus 3.70–4.05 (fragment 2.34) mm long and 204–231 (190) wide; length ratio of two parts of oesophagus 1:6.5–6.6 (-). Nerve ring, deirids and excretory pore 354–408 (-), 286–340 (-) and 476–585 (-) from anterior extremity, respectively. Vulva postequatorial, situated 19.27–19.88 mm from anterior end of body, at 63–67% of body length (vulva of incomplete gravid specimen at 18.46 mm from posterior extremity, i.e. somewhat postequatorial). Vulval lips not elevated. Vagina narrow, muscular, directed posteriorly from vulva. Uteri empty in nongravid specimens, those of gravid specimen containing numerous oval, thick-shelled, embryonated (larvated) eggs (Fig. 3E); eggs 42–45 × 27, with wall 4 thick. Tail short, 136–163 (190), with rounded tip; pair of small lateral phasmids situated near tail tip (Figs. 3B, 4F).

**Remarks**

This species was poorly described by Majumdar (1965) as *Notopteroidea alatae* from specimens collected from the intestine of *M. armatus* at Calcutta, India. Later Johnston & Khera (1967) synonymised *Notopteroidea* Chakravarty & Majumdar, 1962 with *Pseudoproleptus* Khera, 1955 (Cystidicolidae), to which they had transferred this species, renaming it *P. alatus*. Irrespective of the ICZN, Sahay et al. (1970) renamed the same species as *P. armati*. However, only De (1988), based on the re-examination of the deposited paratype specimens of *N. alatae*, showed that this species belongs, in fact, to *Heliconema*...
**Fig. 4** *Heliconema alatum* (Majumdar, 1965), scanning electron micrographs. A, Anterior end of male, dorsoventral view; B, Cephalic end of male, apical view (arrow indicates amphid); C, Posterior end of male, ventral view (arrows indicate preanal papillae); D, Region of pseudolabia, subapical view; E, Tail of male, ventral view (arrows indicate six postanal papillae and phasmid posterior to them); F, Tail of female, lateral view (arrow indicates phasmid). *Abbreviations*: a, cephalic papilla; b, submedian tooth; c, lateral tooth; d, lateroapical pseudolabial depression; e, cephalic hood; f, cloacal aperture; g, anus
Travassos, 1919 (Physalopteridae) and he synonymised it with *H. longissimum* (Ortlepp, 1922).

However, as explained by Moravec et al. (2019), the species *H. longissimum* was inadequately described from snakes in Australia and this name should only be used for the type specimens originally studied by Ortlepp (1922), until this species is redescribed in detail based on a newly collected topotypic material and the validity of *H. longissimum* is confirmed. Therefore, the records of *P. armatus* in India by Ogden (1969) and De et al. (1978).

The latter authors and De (1988) considered *Paraleptus komyai* Sood, 1970 from *M. armatus* in India to be identical with those studied by them and misidentified as *H. longissimum*. Moravec et al. (2019) designated the latter species as *H. kherai* Gupta & Duggal, 1989 (see Gupta & Duggal 1989), but, in accordance with the ICZN, *H. alatum* should be the valid name for this species, with *H. kherai* as its junior synonym.

*Heliconema alatum* was not previously studied by SEM and its most complete description based on LM was provided by De et al. (1978). However, some taxonomically important features, such as the cephalic papillae or the presence of a lateroterminal depression filled with irregularly lobular mass and two inner flat dorsoventral teeth on each pseudolabium, are reported here for the first time. The use of SEM made it also possible to establish the exact number and distribution of the male caudal papillae, which are otherwise difficult to study by LM, as well as the structure and numbers of ventral precloacal ridges, etc.

The measurements of the present nematodes agree well with those given for this species by De et al. (1978), except for the size of eggs, which were found to be somewhat larger (42–45 × 27 μm vs 37 × 22 μm) than reported by those authors (probably they measured less developed eggs).

Moravec et al. (2019) described *Heliconema monopteri* Moravec, Chaudhary et Singh, 2019 from the synbranchiform fish *Monopterus cuchia* (Hamilton) in India, differentiating it from *H. kherai* (= a synonym of *H. alatum*) from *M. armatus* mainly by a distinctly postequatorial vulva and the presence of the pseudolabial lateroterminal depressions. However, as confirmed in this study, pseudolabial depressions are also present in nematodes from *M. armatus* and the position of their vulva is also distinctly postequatorial (at 63–67% of the body length). Since there are no morphological or biometrical differences between these species and because their hosts belong to the same fish order (Synbranchiformes), we consider *H. monopteri* a junior synonym of *H. alatum*.

There is another nematode species, *Pseudoproleptus vestibulus* Khera, 1955, reported from *M. armatus* in India, which is morphometrically very similar to *H. alatum*, differing from it mainly in the presence of a considerably longer vestibule in addition to some details in the structure of the cephalic end. Even though *P. vestibulus* belongs to a different nematode family (Cystidicolidae) than *H. alatum* (Physalopteridae), because of its inadequate descriptions (Khera, 1955; Soota & Sarkar, 1980), both these species were probably confused between each other in the past Indian helminthological literature. A detailed redescription of *P. vestibulus*, based on newly collected specimens from its type host (*M. armatus*), is highly needed, especially because this nematode is the type species of the genus.

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Data Availability All samples used in this study have been deposited in the relevant curated, internationally recognised museum collection as outlined in this paper.

Declarations

Conflict of interest František Moravec is a member of the Editorial Board of Systematic Parasitology.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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