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Seuratascaris schmackeri sp. nov. (Nematoda: Ascarididae) from the Chinese Frog *Odorrana schmackeri* Boettger, 1892 (Amphibia: Anura) Based on Morphological and Molecular Evidence

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ABSTRACT

Here, we examined the sequences of the internal transcribed spacer1 (ITS1) region, the partial small ribosomal RNA gene (18S), and mitochondrial cytochrome-*c* oxidase subunit 1 (COI) genes of *Seuratascaris schmackeri* sp. nov., a member of ascaridoids, exhibiting characteristics of Ascaridoidea *sensu* Chabaud (1965) identified through microscopy. This new species was collected from the small intestine of the Chinese frog species *Odorrana schmackeri* Boettger, 1892, acquired from four regions of Anhui Province, southeastern China. To our knowledge, one species of the *Seuratascaris* genus, namely *Seuratascaris numidica* (Seurat, 1917) Sprent 1985, has so far been recorded. The morphology of *S. schmackeri* sp. nov. differs from that of *S. numidica* and exhibit a few unique characteristics, including more denticles in the lip, shorter intestinal caecum, longer spicular, more caudal papillae, and pre-, ad-, and post-cloacal caudal papillae pairs in the ratio of 3: 1: 6-7. BLAST analyses of the COI sequences show 59.31% nucleotide divergence with *Seuratascaris numidica* (Seurat, 1917) (GenBank acc. no. MG434691 and MG434692). Through morphological and molecular characterization of *S. schmackeri* sp. nov., we generated new data on the *Seuratascaris* genus, providing a crucial scientific basis for future studies on the genus.

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INTRODUCTION

A new genus, *Seuratascaris* Sprent, 1985 initially described as *Porrocaecum numidicum,* was discovered from a frog species, *Pelophylax saharicus* (syn. *Rana saharicus*), collected in Algeria by Seurat (1917). Remarkable morphological variability was noted among the specimen isolated from various frogs and toads in different geographical locations worldwide, including Mediterranean specimens and South East Asia specimens (Sprent, 1985). Sprent also considered that the species identified by Le Van Hoa (1960), namely *numidicum* Seurat, 1917, *ranae* Gupta, 1959, *cacopi* Chatterji, 1936, and *Amplicaecum communis* Yuen, 1963, belong to the genus of *Seuratascaris* and are merged to a single species. The type species, *S. numidica*. *Seuratascaris,* a parasite of amphibians, is uniquely distinguished from other ascarids by intestinal caecum, presence or absence of interlabial, the absence of an oesophageal appendix, and lateral alae, the sort and number of caudle papillae, and a distinctive blunt end of an immature *Seuratascaris*.

Recently, Chen *et al*. (2018) provided the morphological characterization and molecular evidence of *S. numidica* from the frog, *Hoplobatrachus chinensis* (Osbeck, 1765), in China. However, due to a lack of genetic data, Chen and colleagues could not confirm whether *S. numidica* is a single species or not. Wang *et al*. (1981) had reported *Angusticaecum wuyiensis*, characterized by longer

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intestinal caecum, precolocal (5-6 pairs), postcolocal (4 pairs), large preanal central papilla, from *Rana schmackeri* Boettger,1892 (Syn. *Odorrana schmackeri* Boettger, 1892) in Wuyi Mountain, Fujian Province, China. According to Chen *et al*. (2018), *A. wuyiensis* Wang, 1981 is a novel synonym of *Seuratascaris numidica*.

The present study morphologically recognized several Ascarididae nematodes collected from the intestine of *Odorrana schmackeri* in Anhui Province, China, as species in the genus *Seuratascaris*. Detailed evidence of molecular characterization of the ITS, COI, and 18S regions of the present specimen suggests a new species, designated as *Seuratascaris schmackeri* sp. nov.

MATERIALS AND METHODS

Collection of nematode samples and morphological observation

Eighty-eight frogs, *Odorrana schmackeri*, collected between June and July 2018 in the south of Anhui province, China, were examined for nematodes. Frogs were sampled from the following locations: Zhanghe (30°6'N, 118°47'E, n=22) in Jixi County, Yiwanling (30°23'N, 118°43'E, n=21), in Jingde County, Taoling (30°31'N, 118°38'E, n=13) in Jing County, Fuxi (30°4'N, 118°9'E, n=32) in Huangshan city.

Nematodes isolated from the intestine of frogs were selected and immediately immersed in 60 °C water to keep their body stretched. The worms were then fixed in 70% alcohol and disposed of gradually, awaiting subsequent experiments. The fixed nematodes were transparentized with lactic acid, phenol, and glycerin and placed under a light microscope (Olympus BX51FL-DIC) to examine the internal anatomy structure.

The morphological characteristics of the nematode body surface were assessed by a scanning electron microscope (SEM). Briefly, two females and two male specimens were pre-fixed in 3.0% glutaraldehyde, later fixed in 1% OsO_4, and processed via gradient alcohol dehydration. The specimens were gold-coated and examined by Gemini SEM 300 (Carl Zeiss, Germany) at the accelerating voltage of 20KV. The diagram was made with the aid of a Nikon mi-manually depending on the light microscopy images. Measurements were recorded in millimeters (mm) unless diversely verbalized. Voucher specimens were stored in the Medical Parasitology Department of Wannan Medical College in Anhui Province of China.

DNA extraction and sequencing analyses

Three specimens were selected randomly for sequencing of ITS, COI, and 18S regions. Briefly, genomic DNA of the helminth was extracted using a TIANamp Genomic DNA Kit (TianGen Biotech, Beijing, China) following the manufacturer's instructions. The target regions were amplified by polymerase chain reaction (PCR) using the following primers pairs: ITS1 (Gasser *et al*., 1999); COI (Folmer *et al*., 1994); 18S (Floyd *et al*., 2005). The cycling conditions were as described by Li *et al*. (2016). The amplified products were electrophoresed on GoldView-stained 1.5% agarose gels and purified with the Column PCR Product Purification Kit (Sangon Biotech, Shanghai, China). The amplicons were sent to Sangon Biotech (Shanghai) Co. Ltd. for clone sequencing (ABI 3730, USA). Sequencing for every swatch was fulfilled for both lines. The sequences were strung out by the DNAMAN software (Lynnon Corporation, Canada) and altered in manual-acting, excluding primers, respectively. Next, the resulting sequences were compared (using the algorithm) to the available sequences in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) using the BLAST program. All sequences of the new nematode species have been deposited in the GeneBank database (http://www.ncbi.nlm.nih.gov).

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RESULTS

(Figs. 1-3)

Seuratascaris schmackeri sp. nov.

General

These are medium-length thin worms. The bodies of nematodes treated with alcohol appear white, non-transparent, and cylindrical. One-third of the middle body is the widest part. The females are larger than males. The cuticle is striped breadthwise and interspersed with slight elevation on occasion. The cephaliced features comprise three conspicuous quadrate lips with dentigerous ridges, one in dorsal position and two in latero-ventral position, broader rather than long. Whereas the dorsal lip possesses two larger external papillae, each of two latter lips have one larger papilla in ventro-lateral position and a smaller single latero-ventral papilla together with the amphidial pore in the lateral position. Interlabial is absent. Highly developed shorter postlabial grooves are found on the bottom of two neighboring lips, tied together by cuticular interlacement. The inside surface of the edged lips is jagged; the dentations are finespun, slim but protuberant, with approximately 60-70 and 90-100 denticles on each lip in female and male, respectively. Isthmus is wide (attached to the body), ventriculus, and lacks the ventricular appendix. The intestinal caecum is shorter. The intestine membrane is cast into characteristic transverse folds that give the internal surface an irised curl aspect. These spines

give a herring-bone appearance of the external body layer in the side of view. The rectal gland is present. The tail is round and blunt with a small tip in two sexes. Phasmids are absent.

Male (n=3. Units of measurement in mm)

Length 5.81-12.30, maximum breadth 0.23- 0.34, head diameter 0.05- 0.08. Dorsal lip length 0.049-0.061, maximum width 0.061-0.800, interlabia absent, inconspicuous postlabial grooves (Figs. 2A, 3A), 0.014 (22.95% of max. width) in a 5.81 specimen. Pulp is not deeply divided anteriorly. Oesophagal length 1.25, shorter intestinal caecum (Fig. 1A), left 0.116 (9.3% of the length of the oesophagus), right 0.119 (9.5% of the length of the oesophagus), respectively. Pharynx and never ring from the anterior extremity of the body 0.10 and 0.45, respectively. Caudal alae are well-developed, which project 10-11 pairs of pedunculated papillae, of which 3 pairs are precloacal, 1 pair ad-cloacal, and 6-7 pairs post-cloacal, big median papillae in front of cloacal (Fig. 2B, E, F). The two spicules are equal (Fig. 2C), relatively strong, and sharpened near the tip. One of the spicules has a C-shaped wing membrane and half-wraps the other (Fig. 2C), 0.532-0.719 long. Gubernaculum is absent. The tail is blunt, 0.364-0.610 long, with a small tip.

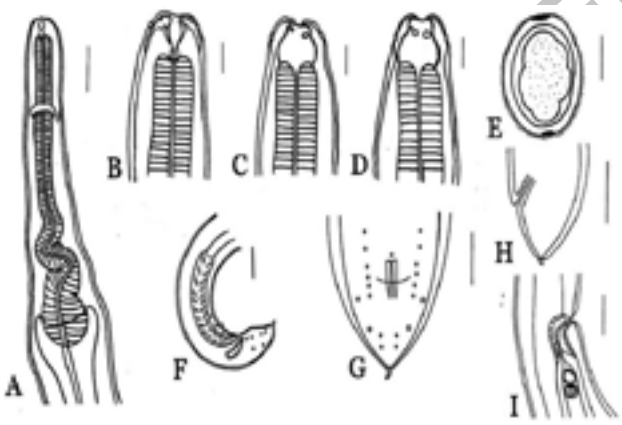

Fig. 1. *Seuratascaris schmackeri* sp. nov. from *Odorrana schmackeri* in China. (A) anterior part of male, laterial view, showing oesphagus, nerve-ring, excretory pore, intestinal caecum. (B) anterior part of male, laterial view, showing interlabial and postlabial groove. (C) anterior part of male, dorsal view, showing dorsal lip with two pipillars. (D) anterior part of female, lateral-ventral view, showing lateral-ventral lip with one papillae and amphid. (E) egg. (F) posterior end of male, lateral view. (G) posterior end of male, ventral view. (H) posterior end of female, lateral view. (I) region of Vulva, lateral view. Scale bars: A= 200µm; B, C, D= 500µm; E= 50µm; F, G, H = 200µm; I = 300µm.

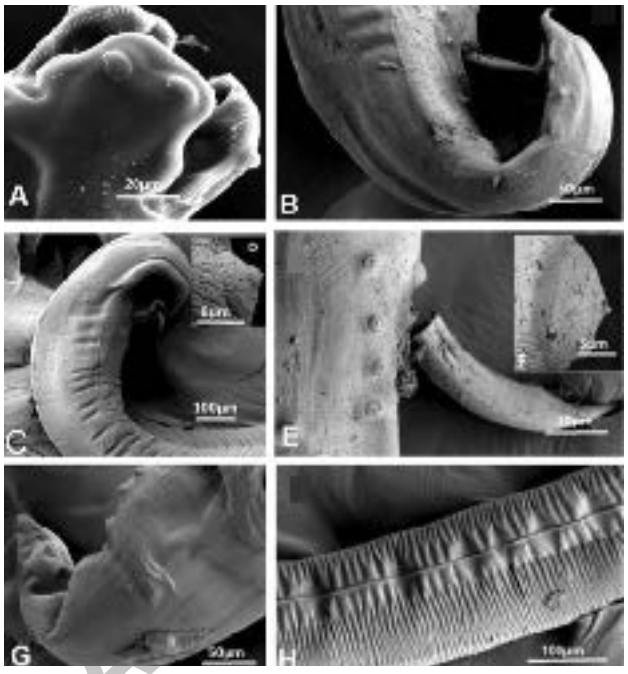

Fig. 2. Scanning eletron micrographs of *Seuratascaris schmackeri* sp. nov. from *Odorrana schmackeri* in China, male. (A) cephalic extremity, dorsal view, dorsal lip with two papillae, subventral lip with denticles, showing interlabial and postlabial groove. (B) tail extremity, laterial view, spicule. (C) posterior end, lateral view, precloacle adcloacle papillae and herringbone cuticle apperance. (D) image of precloacle papillae. (E) cloacle region, lateral view, adcloacle papillae, a big median papillae in front of cloacle. (F) image of median papillae in front of cloacle, lateral view. (G) posterior end, ventral view, postcloacle papillae. (H) middle of body, lateral view, lateral alae.

Female (n=4, Units of measurements in mm)

Length 25.33-30.90, maximum breadth 0.70-0.74, head diameter 0.11-0.19. Latero-ventral lip length 0.093, maximum width 0.12, postlabial grooves 0.031 long (26.25% of maximum width) in a 26.84 species. Length of the oesophagus and intestinal caecum 5.23-6.89 and 0.182-0.199 (2.6-3.5% of the length of the oesophagus), respectively. Never ring from the anterior extremity of the body 1.36-1.94. Vulva at anterior half of the body, 7.74-8.94 from the anterior end and offered with two prominent lips one anterior and the other posterior. The vagina is long and muscular, running obliquely posteriorly in the body across the dorsal side of the body cavity. Uteri are long and parallel, running posteriorly. The origin of ovarian coils is a little anterior to the level of the anus. The tail is blunt, 0.127-0.224 long, with a pointed tip. The anus, 0.192-0.203 from the posterior end (Fig. 3C-D). The vagina is muscular and runs towards the posterior extremity, joining the common uterus formed by two uteri.

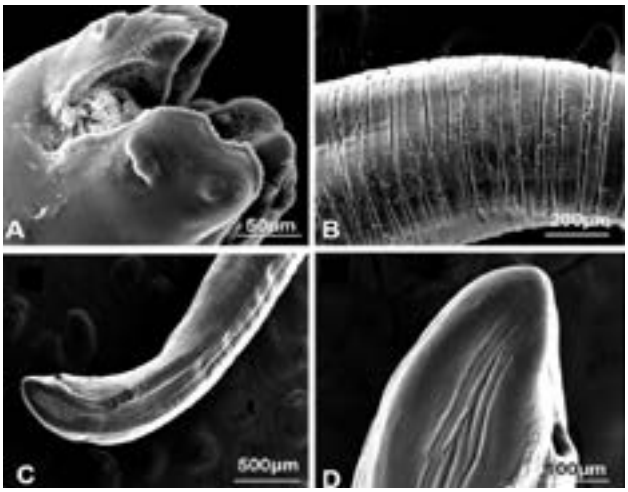

Fig. 3. Scanning eletron micrographs of *Seuratascaris schmackeri* sp. nov. from *Odorrana schmackeri* in China, female. (A) cephalic extremity, lateral view, lateral-ventral lip with one oval papillae and one small round papillae and amphid, lip with denticles, showing interlabial and postlabial groove. (B) middle of body, lateral view. (C) posterior end, lateral view, anus. (D) posterior end, lateral view.

Egg (n=10, Units of measurement in mm)

Oval, eggshell with three layers: The inner membrane is extremely thin, the middle layer is thicker, concave egg covers at both ends or one end of the egg, and the outermost layer comprises proteins with uneven surface and fine particles, 0.134-0.143×0.083-0.086.

Taxonomic summary

Geographical location - Jing County, Fuxi (30°4' N, 118°9' E), Huangshan city, Zhanghe (30°6' N, 118°47' E), Jixi County, Yiwanling (30°23' N, 118°43' E), Jingde County, Taoling (30°31' N, 118°38' E), Anhui Province, China.

Host: *Odorrana schmackeri* Boettger, 1892, Class Amphibia, Order Anura.

Site of infection: Small intestine.

Level of infection: Total rate 11.4 % (10/88), the average and scope of infection intensity 0.58 (0.00, 11.00).

Specimens: Holotype, male (SS2018001M); allotype, female (SS2018001F); paratypes, males (SS2018002M) and females (SS2018002F). Specimens are deposited in the Medical Parasitology Department of Wannan Medical College, Wuhu, Anhui province, China.

Etymology: Based on the name of the host.

Remarks

Morphological evidence

The genus *Seuratascaris,* ranging from small to medium-sized ascaridoids with characteristics of Ascaridoidea *sensu* Chabaud (1965), was described to possess several other unique characteristics. Lips are slightly narrower than the body, with a wide isthmus (attachment to body) and dentigerous ridge all-round margin. Anterior margin has a cleft in pulp not deeply divided anteriorly. The median lobe is absent. The Interlabial region forms an interlabial ridge of variable form, cervical alae, ventriculus, and lacks a gubernaculum. Excretory pore and cervical papillae are slightly behind the nerve ring. The excretory nucleus is relatively large, situated on the left side in the commissural part of the excretory cell. The excretory system is bilateral. Intestinal caecum and rectal glands are present, male with relatively short, stout spicules, ventral cuticular precloacal ornamentation not present, female with vulva anterior to middle of body, uterus didelphic, opisthodelphic, parasites of old world anurans (Sprent, 1985).

S. schmackeri sp. nov. possesses characteristics of the genus *Seuratascaris,* including three dentigerous ridges around lips, wide isthmus, intestinal caecum present, gubernaculum absent, with large median papillae in front of the cloaca, and stout spicules. *Seuratascaris* was considered to comprise type species, *S. numidica*, manifesting polymorphism regarding the form of interlabial region and the number of precloacal papillae (Sprent, 1985). *S. schmackeri* sp. nov. poses unique morphological characteristics different from *S. numidica*, including shorter intestinal caecum (2.6-9.5% of oesophagus length vs 43-58% and 68.4–71.1%) (Sprent, 1985; Chen *et al*., 2018), longer stout spicules (0.532-0.719 vs 0.14-0.34 and 0.15-0.17) (Sprent, 1985; Chen *et al*., 2018) and different pairs of caudle papillae (pre-: ad-: post cloacal papillae = 3: 1: 6-7 vs 2-3, 3-5, 5-6: 0: 3-4 and 4: 0: 4-5) (Sprent, 1985; Chen *et al*., 2018).

Seuratascaris numidica (Seurat, 1917), from *Hoplobatrachus chinensis* (Osbeck) (Anura: Dicroglossidae), is the first record of the *Seuratascaris* genus in China (Chen *et al*., 2018). *Seuratascaris schmackeri* sp. nov. is the second record of the genus *Seuratascaris* in China; notably, the first record of this genus infected a Ranidae frog, *O. schmackeri*.

Molecular evidence

Sequences recovered from three different *S. schmackeri* sp. nov. specimens were identical (100% homology) for 18S rDNA, ITS and COI gene amplified fragments. Excluding primers, the18S rDNA, ITS, and COI genes of *S. schmackeri* sp. nov. were 885, 763, and 655 base pairs long and deposited in the GenBank database (accession nos. MN120312, MT434777, and MN120313, respectively).

No species of *Seuratascaris* and Angusticaecinae with

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18S sequences are available in GenBank. A comparison of the 18S sequences of *S. schmackeri* sp. nov. with the species of Ascarididae, *Baylisascaris ailuri* (JN256991), *B. schroederi* (JN256992), *B. transfuga* (JN256988), *Porrocaecum angusticolle* (EU004820), *P. reticulatum* (MF072700), *Parascaris equorum* (U94378), *Ascaris suum* (MN558962), and *A.* sp. (JN256985) in GenBank show 5.98–6.31% nucleotide divergence.

One other species of *Seuratascaris* and no Angusticaecinae with ITS sequences is available in GenBank (*Seuratascaris numidica,* MG434689, MG434690). Pairwise comparison between *S. schmackeri* sp. nov. and *S. numidica* show 3.82% nucleotide divergence.

One other species of *Seuratascaris* and no Angusticaecinae with COI sequences is available in GenBank (*Seuratascaris numidica,* MG434691, MG434692). Pairwise comparison between *S. schmackeri* sp. nov. and *S. numidica* show 59.31% nucleotide divergence. A comparison of the COI sequences of *S. schmackeri* with the species of Ascarididae, *Ascaris lumbricoides* (AP017677, KY368757, KY368759, 045803), *A.* sp. (MH059555, KC839986), *A. suum* (KY045800), *A. ovis* (KU522453), *Parascaris equorum* (MF678786), and *P. univalens* (KM216010, KM067271) in GenBank show 14.16–15.29% nucleotide divergence.

DISCUSSION

The genus *Seuratascaris* Sprent, 1985 is discriminated from other genera of Ascaridoidea, including *Amplicaecum* Seurat, 1917, *Orneoascaris* Skrjabin, 1916, *Angusticaecum* Baylis, 1920, *Ophidascaris* Baylis, 1921 and *Freitasascaris* Sprent, 1983 by posterior borders of lips with wider isthmus (attachment to body) and an interlaboratory area characterized by a variable form of an interlaboratory ridge. *Angusticaecum numidicum* Seurat, 1917 found in anuran amphibians was re-classified to *Amplicaecum* Baylis, 1920 by Chabaud and Campana-Rouget (1955), subsequently to *Orneoascaris* Skrjabin, 1916 by Le van Hoa (1960) and finally to *Seuratascaris* Sprent, 1985 by Sprent (1985). *S. numidica* is a specialized parasitic nematode species of amphibians only (Sprent, 1985).

Species collected from frogs and toads in France and South East Asia (Mediterranean, Bali, North Borneo, Malaya, Burma, West Irian New Guinea, and Queensland) by Sprent (1985) were merged to a single species, *Seuratascaris numidica* (Seurat, 1917).

Seuratascaris spp. has multifarious anuran hosts. Unlike *Orneoascaris* spp., *Seuratascaris* spp. cannot extend their scope to reptiles. Literature evidence shows that *S. numidica* was recovered from the digestive tract of frogs in South East Asia, including stomach of the brackish water frog, *Rana cancrivora* Gravenhorst, 1829 from the Philippines (Bursey *et al*., 2003), *Sylvirana supragrisea* (Anura: Ranidae) from Papua New Guinea (Bursey *et al*., 2008), the Mao-Son frog, *Hylarana maosonensis* Bourret, 1937 from Vietnam (Bursey and Goldberg, 2011), *Hylarana waliesa,* from Papua New Guinea (Goldberg *et al*., 2013), intestine and stomach of the frog, *Hoplobatrachus tigerinus,* from India (Sou and Bursey, 2017), stomach and small intestine of Ranid frogs, *Chalcorana labialis*, *Hylarana erythraea*, and *Pulchrana banjarana,* all from Southeast Asia (Goldberg *et al*., 2017a), stomach of the toad, *Phrynoidis asper,* from Peninsular Malaysia (Goldberg *et al*., 2017b), gastrointestine of the frog, *Hoplobatrachus chinensis,* from Chnia (Chen *et al*., 2018), intestine of the frog, *Limnonectes macrocephalus,* from Philippines (Goldberg *et al*., 2019).

Seuratascaris schmackeri sp. nov. is discriminated from *S. numidica* by a shorter intestinal caecum and two equal longer spicules. While the Asian specimens reported by Sprent (1985) possessed paracloacal double papillae, the newly described species has one simple paracloacal papillae. Sprent (1985) and Chen *et al*. (2018) found that the intestine caecum length of *S. numidica* is not more than 60% and 68.4%-71.7% of oesophageal length, respectively, whereas that of *S. schmackeri* sp. nov.is only 2.6%-9.5%. The denticles on each lip of *S. schmackeri* sp. nov. are fine and slim but prominent, with approximately 60-70 and 90-100 denticles in females and males, respectively, differing from 64-76 denticles on each lip of *S. numidica* (Chen *et al*., 2018).

Molecular analysis of the specimens of *S. schmackeri* sp. nov. shows no nucleotide variation in the18S, ITS, and COI sequences. A comparison of ITS and COI sequences of *S. schmackeri* sp. nov. to those of *S. numidica* Seurat, 1917 in GenBank demonstrate 3.82% and 59.31% nucleotide divergence, respectively.

CONCLUSIONS

Morphological and genetic identification of *Seuratascaris schmackeri* sp. nov. from the small intestine of *O. schmackeri* in China is reported. The difference of morphological characteristics and the divergence of 18S, ITS, and COI between *S. schmackeri* sp. nov. and *S. numidica* Seurat, 1917 suggest that the presently described specimen is a new species of the genus *Seuratascaris*. *S. schmackeri* sp. nov. is the second species of this genus recovered worldwide.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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