



# Morphological and Molecular Characterization of *Toxocara apodemi* (Nematoda: Ascarididae) from Striped Field Mice, *Apodemus agrarius*, in Korea

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**Abstract:** Adult ascarid worms from the field mice, *Apodemus agrarius*, were observed with a light and scanning electron microscope, and molecularly analyzed with 18S rRNA gene. In the scanning electron microscope, 3 prominent labia were present in the anterior end of male and female worms, but the interlabia and gubernaculum were absent. Scanning electron micrographs showed cervical alae as vestigial organs that looked like a slightly uplifted superficial sewing stitch. Total 6 pairs of post-cloacal papillae were observed on the tail of the male worms. The tail of female worms was blunt and conical shape with a spine-like structure, mucron. The eggs were sub-globular, coated with the albuminous layer and 73 by 82  $\mu\text{m}$  in average size. The superficial pits of *T. apodemi* egg (mean  $8.6 \times 6.7 \mu\text{m}$ ) are obviously bigger than those of *Toxocara* spp. The partial sequence of 18S rRNA showed the sequence homology of *Toxocara canis* (99.6%), *Toxocara cati* (99.4%), *Toxascaris leonina* (99.4%), and *Toxocara vitulorum* (99.2%). Conclusively, it was confirmed that ascarid nematodes, *Toxocara apodemi*, recovered from striped field mice in Korea are taxonomically conspecific relationship with genus *Toxocara* and genetic divergence from other *Toxocara* species.

**Key words:** *Apodemus agrarius*, *Toxocara apodemi*, molecular characterization, scanning electron microscopy, Korea

## INTRODUCTION

The 2 genera, *Toxocara* and *Neoascaris*, of the superfamily Ascaridoidea have a close morphological relationship, the genetic and morphological differentiations between 2 genera incompletely remain yet [1]. The subfamily Toxocarinae is morphologically characterized by a ventriculus without appendices and the absence of gubernaculum. Additionally, the members in the genus *Toxocara* does not have the intestinal cecum and interlabia [2]. And then in the species identification of the genus *Toxocara*, some morphological characteristics, i.e., the shape of cervical alae in the cross-sectional samples, the spicule length and lip structure act as the differential keys [3]. Ac-

cording to the morphological characteristics of the genus *Toxocara* reported by Warren [1], this nematode group with genus *Toxascaris* are differentiated from genus *Ascaris* by the arrow-shape head, and they are separated each other by the egg shape. The eggs of *Toxocara* spp. have corrugated shells but those of *Toxascaris* have smooth shells.

More than 28 species including some synonyms have been reported in the genus *Toxocara* from a variety of host animals [3-13]. Most of these parasitize in terrestrial mammals except for *T. pearsei*, which is a parasite of snapping shrimp, *Synalpheus brooksi* [3,14]. At least 5 species, i.e., *T. canis* from canids, *T. cati* from felids, *T. vitulorum* from ruminants, *T. pteropodis* from bats and *T. malaysiensis* from cats, are known to be the zoonotic agents [4,5]. Another species of *Toxocara* have been reported in bats (*T. cynomycterides*), canids (*T. marginata*), carnivores (*T. alienata*, *T. anakumae*, *T. herpestum*, *T. paradoxura*, *T. sprenti*, *T. suricattae*, *T. tanuki* and *T. vajrasthirae*), elephants (*T. elephantis*), felids (*T. canarisi*, *T. genettae* and *T. lyncis*), hippopotamus (*T. hippopotami* and *T. vincenti*), ruminant (*T. manza-*

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*diensis* and *T. warren*), and rodents (*T. apodemi*, *T. indica* and *T. mackerrasae*) respectively.

Among the toxocarid nematodes, *T. canis* and *T. cati* are most commonly found from the intestines of dogs and cats worldwide [15,16]. A variety of *Toxocara* species is commonly found in livestock, but their available scientific informations are not enough, especially about a toxocarid from wild field mice, *Apodemus agrarius*. *Toxocara apodemi* was first reported from the intestine of the wood mouse, *Apodemus peninsulae* from the Central National Forest, Bupyeong-ri, Namyangju-si, Gyeonggi-do, Korea [13]. This nematode was reported as a new species of the genus *Neoascaris*, and named as *Neoascaris apodemi* by Olsen in 1957. Later, this species of nematode was found in the small intestine of striped field mice in China and emended as *T. apodemi* [6], although it was more or less small than *N. apodemi* [13].

Traditionally, *Toxocara* spp. have been identified and classified based on their morphological features and host species [1-3]. Also, DNA sequencing is recently used with identification for *Toxocara* species [17]. Nevertheless, there is no reports for identification of *T. apodemi* by scanning electron microscopy (SEM) study and genetic information. The present study provides the first SEM data about the morphology of *T. apodemi* and its genetic characterization using 18S rRNA analysis.

## MATERIALS AND METHODS

### Examined animals and morphological examination

A total of 70 wild rodents (56 *A. agrarius*, 4 *A. peninsulae*, 8 *Mus musculus*, and 2 *Rattus norvegicus*) were captured at 7 sites (Yeongdeok-gun, Uljin-gun and Sangju-si in Gyeongsangbuk-do, Hapcheon-gun in Gyeongsangnam-do, Yeongwol-gun in Gangwon-do, Buan-gun, and Iksan-si in Jeollabuk-do) of Korea between March and September 2019 (IACUC No. SNU-190524-2). Adult parasites were collected from the intestines after necropsy. The ascarid eggs were isolated from the uterus of a female worm and observed under a phase-contrast microscope. For the SEM of eggs, the eggs dehydrated in graded series of ethyl alcohol were sprinkled on the single side of double faced adhesive tapes, dried in a CO<sub>2</sub> critical point dryer, and coated with gold. For turning transparent, the worms were placed in lacto-phenol solution (20 ml glycerin, 10 ml lactic acid, 10 ml phenol, and 10 ml distilled water) for 24 hr and observed under a phase-contrast microscope. For the SEM study, the transparented worms were washed 5 times with 0.2

M cacodylate buffer (pH 7.3), fixed in 2.5% glutaraldehyde, and subjected to post-fixation treatment with 1% osmium tetroxide at 4°C. The specimens of worm were dehydrated in graded series of ethyl alcohol, dried in a CO<sub>2</sub> critical point dryer (CPD 030, BAL-TEC, Los Angeles, California, USA), coated with gold, and finally examined by SEM (XL30 ESEM TMP, Philips, Praha, Czech Republic) at 15 kV.

### Sequence and phylogenetic analyses

Genomic DNA was extracted from 6 parasites using the DNeasy® Blood and Tissue Kit (Qiagen, Alameda, California, USA) according to the manufacturer's instructions. Based on the common 18S ribosomal RNA gene sequences of *T. canis* (JN256977 and U94382), primers were designed using the online tool Primer3 Input (version 0.4.0, <http://bioinfo.ut.ee/primer3-0.4.0/>). The sequences of the primers used to detect the genus *Toxocara* were as follows: 5'-GCTAATACATGCACCAAAGC-3' (forward primer) and 5'-GATCACGGAGGATTTTCAAC-3' (reverse primer). The primer sets were designed to amplify an amplicon of 1,374 bp and were variable for all sequences aligned. PCR was performed using a MyCycler Personal Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA) using EmeraldAmp GT PCR Master Mix (Takara, Shiga, Japan) with 1 µl of DNA under standard conditions of 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1.5 min for 30 cycles and additional extension at 72°C for 10 min. The PCR products were visualized via electrophoresis on 1.2% agarose gel, and then purified using the QIAquick PCR Purification Kit (Qiagen). PCR amplicons were sequenced using the ABI Prism Big Dye terminator v. 3.0 ready reaction cycle sequencing kits (Applied Biosystems, Foster City, CA, USA) with the sequencing primer (5'-CCCTC-CATTTGCATGTTG-3'). After trimming the nonspecific sequencing data, the 1,351 bp sequences were aligned using Clustal Omega program (Clustal O). A phylogenetic tree based on sequence analysis was constructed by the neighbor-joining (NJ) method using the program BLAST tree. The NJ method was based on a guide tree as pairwise and multiple alignment parameters [18].

## RESULTS

### Samples collected

Total 9 ascarid nematode worms (4 males, 4 females, and 1 juvenile) were harvested in the small intestines of 5 *A. agrarius*

(8.9%), which were captured in Yeongdeok-gun and Ulljin-gun, Gyeongsangbuk-do, and Iksan-si, Jeollabuk-do, Korea.

### Morphology of both sexes

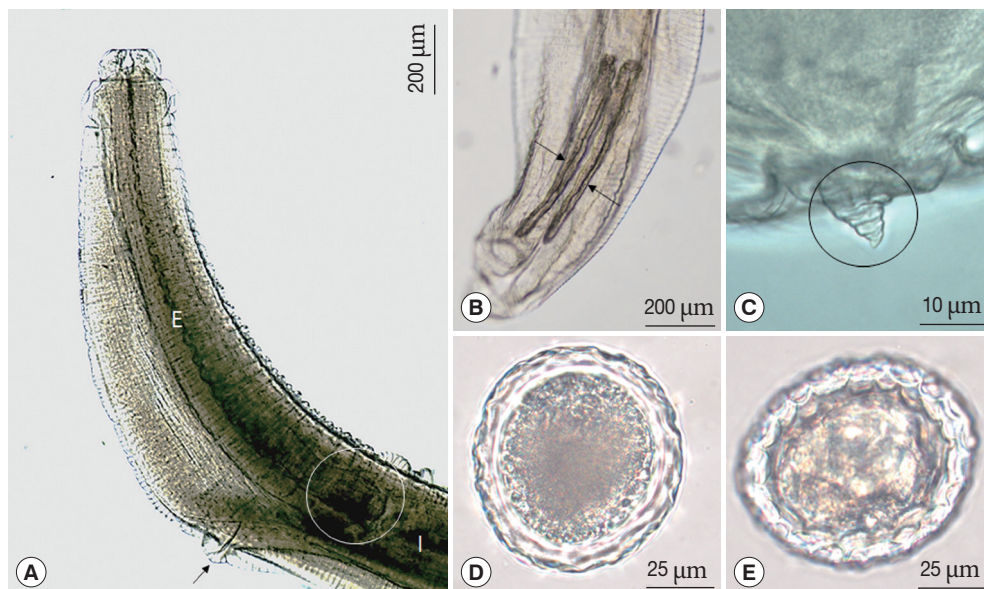
The ventriculus lacked an appendix (Fig. 1A) and the cervical alae were the vestigial organs that looked like an uplifted sewing stitch on the proximal portion of labia and an simple sewing stitch on the distal portion of labia (Fig. 2A, D, E, F).

Figs. 2 and 3 (female) and Fig. 4 (male) show the SEM images illustrating the characteristic features of the adult worm. In adults of both the sexes, the anterior end had 3 prominent labia, which are characteristic feature of the ascarid nematodes; 1 dorsal and 2 subventral labia, surrounding the oral aperture with a median groove running longitudinally along its inner length; the interlabia was absent (Fig. 2A-C). The labia of both sexes were similar in shape, structure, and organizations, except that they were larger in the females. Three lips had 4 relatively large papillae. The dorsal labium had 2 large papillae at the dorsolateral portion, while each subventral labium had a large extemolateral papilla and an amphid (Fig. 2A, B). The amphids, lateral to the extemolateral papillae, appeared as small domed areas with a central pore (Fig. 2B). The conspicuous and strong dentigerous ridges were present on all labia (Fig. 2A-C). The lips had a superficial median notch

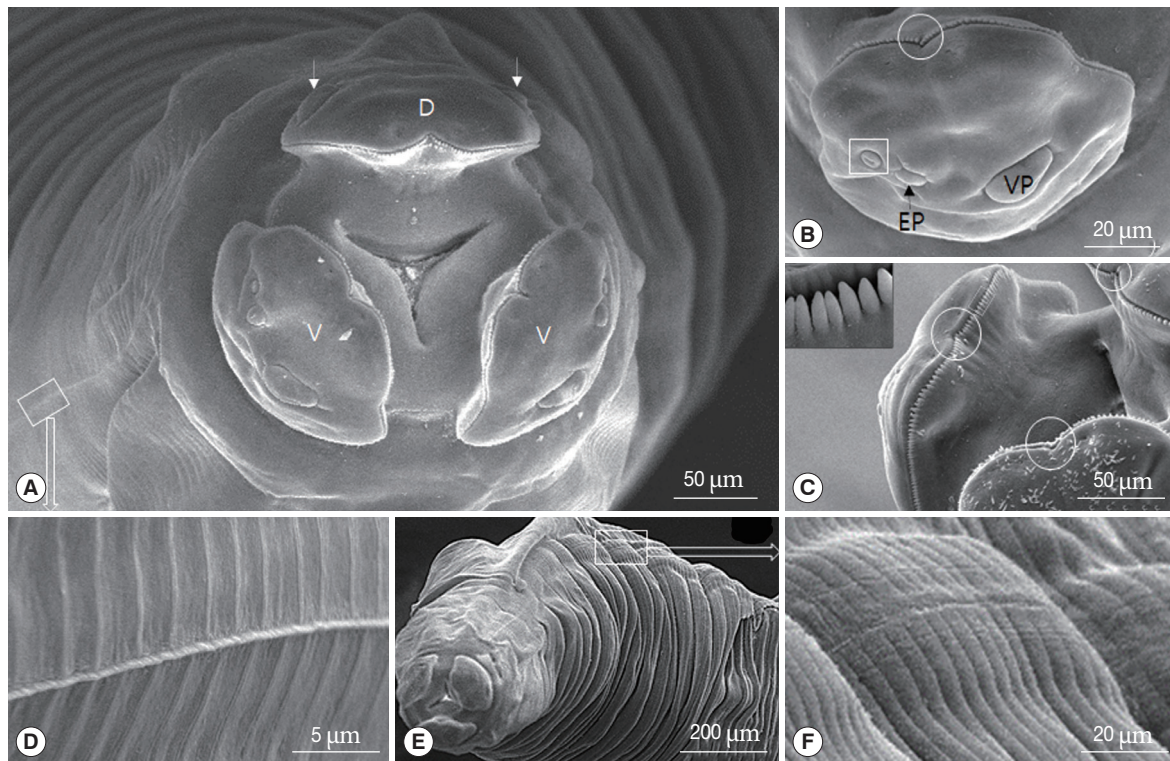
lined with the denticles of the dentigerous ridge (Fig. 2B, C). The strong denticles were distributed consistently and was particularly evident in *en face* view (Fig. 2B, C). The triradiate oral aperture was located at the base of the stoma (Fig. 2A) and the esophagus was claviform. The posterior end of the esophagus was separated from the rest of the esophagus in form of a well-defined muscular bulb formed by a conspicuous constriction. The cuticle was finely cross striated throughout the body (Figs. 2D, E, and 3A).

### Morphology of female

Adult females measured 61 and 67 mm in length and 2.15 and 2.61 mm in width at mid-body. The esophagus was 2.85-3.25 mm long and 0.42-0.51 mm at maximum width. The esophageal bulb measured 0.45-0.50 mm long and 0.42-0.50 mm wide. The ventriculus lacked an appendix (Fig. 1A). The excretory pore was represented by a slit which is situated at the ventriculus (Fig. 1A). The vulva was located about 3/10 of body length from the anterior end (for example, if the body were 52 mm long, the vulva opened 15.2 mm from the anterior end). The phasmid pore was located laterally behind the cloaca (Fig. 3A). The tail of the female slightly was blunt and bent to the ventral side. The tail was terminated in a conical shape with a retractile spine-like structure (Figs. 1C, 3B). Eggs



**Fig. 1.** Light micrographs of *Toxocara apodemi*. (A) Anterior end of female with ventriculus (circle) and excretory pore (arrow). (B) Posterior end of male with 2 spicules (arrows). (C) Posterior end of female. Note the blunt tail terminating in a conical shape with a retractile spine-like structure (circle). (D, E) Egg. Note outer surface of the egg was marked with a network of ridges. E, esophagus; I, intestine.



**Fig. 2.** Scanning electron micrographs of female *Toxocara apodemi*, anterior end. (A) Frontal view showing one dorsal labium, 2 subventral labia and oral aperture. Dorsal papillae (arrow head). (B) Subventral labium. Amphid (square), subventral papilla and externo-lateral papilla. Superficial median notch (circle). (C) Inner surface of dorsal labium and superficial median notch (circles). Strong tooth (square). (D) Cervical ala, which is located on the proximal labium, appear like a vestigial organ. (E, F) Cervical ala which is located on the distal labium. D, dorsal labium; EP, externo-lateral papilla; V, subventral labium; VP, subventral papilla.

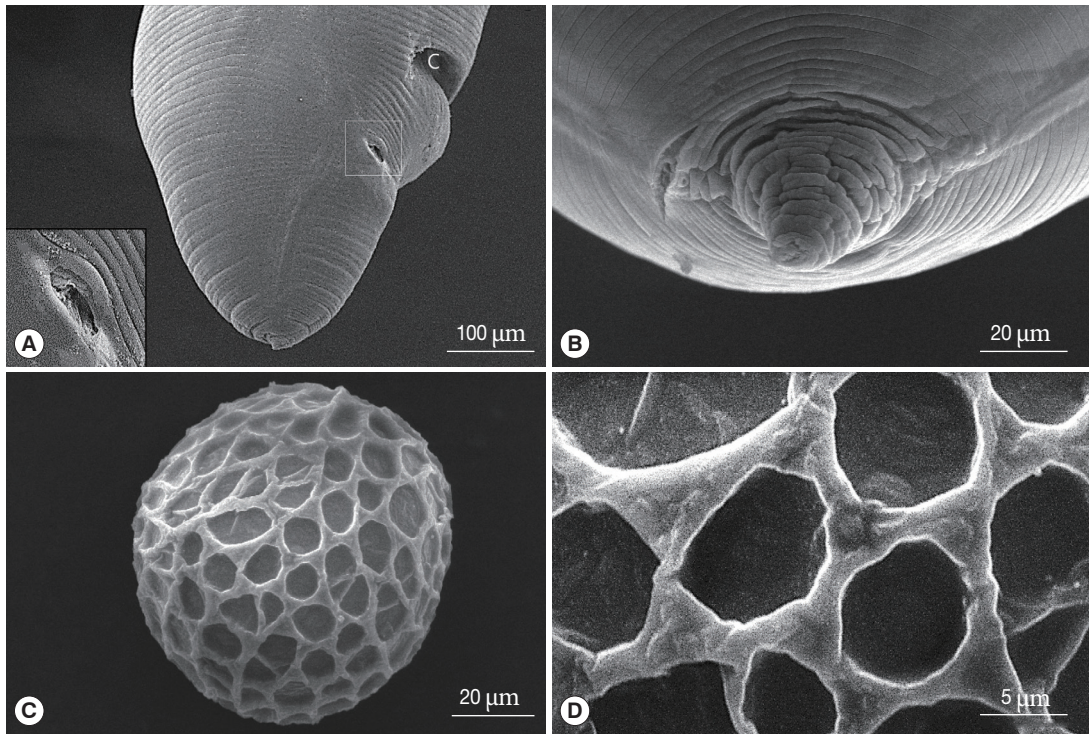
were oval and measured 73-82  $\mu\text{m}$  (mean 78.8  $\mu\text{m}$ ,  $n=10$ ). The outer surface of eggs was coarsely pitted and had a mosaic pattern formed by a network of ridges (Fig. 1D, E). SEM findings revealed that the surface of the egg was covered by web-like albumin and consists of numerous pits surrounded by elevated albumin (Fig. 3C, D). The mosaic pits were measured 7.3-12.8  $\mu\text{m}$   $\times$  5.7-7.3  $\mu\text{m}$  (mean 8.6  $\times$  6.7  $\mu\text{m}$ ).

#### Morphology of male

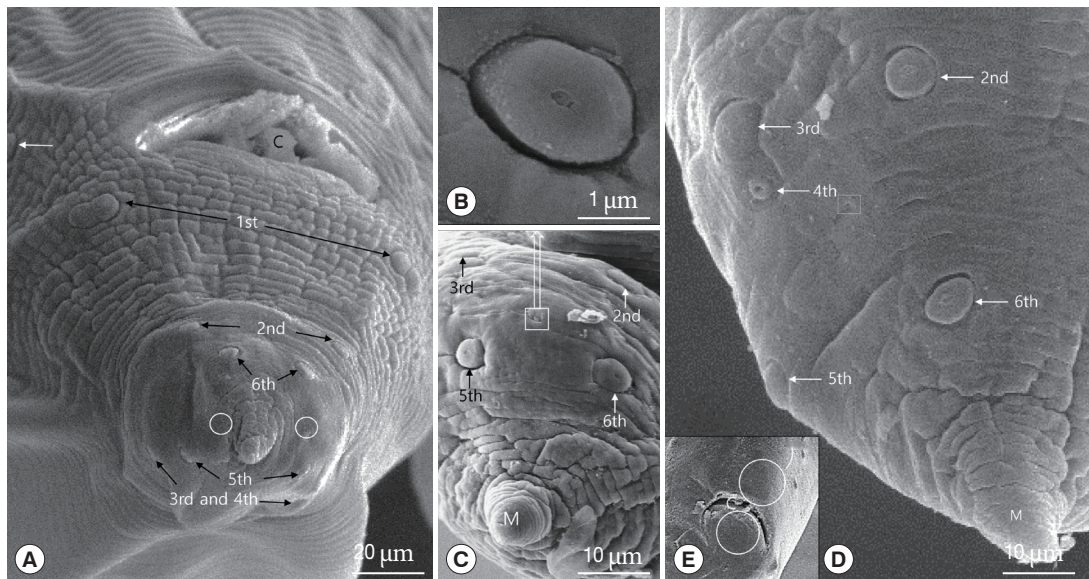
Adult males measured 47-52 mm (mean 50.7 mm) in length and 0.9-1.4 mm in width at mid-body. The esophagus was 2.4-2.6 mm long and 0.26-0.45 mm at the maximum width. The tail was 0.17-0.27 mm long and the gubernaculum was absent (Fig. 1B). The spicules were subequal and were 0.45-0.53 mm long (Fig. 1B). The caudal end was ventrally curved, displaying 12-14 pairs of proximal pre-cloacal papillae arranged in subventral lines (Fig. 4A). The subventral lines were arranged as double parallel lines. Pre- and post-medial

cloacal papillae were not observed (Fig. 4A, E). Postcloacal papillae were numbered from the cloacal opening posteriorly.

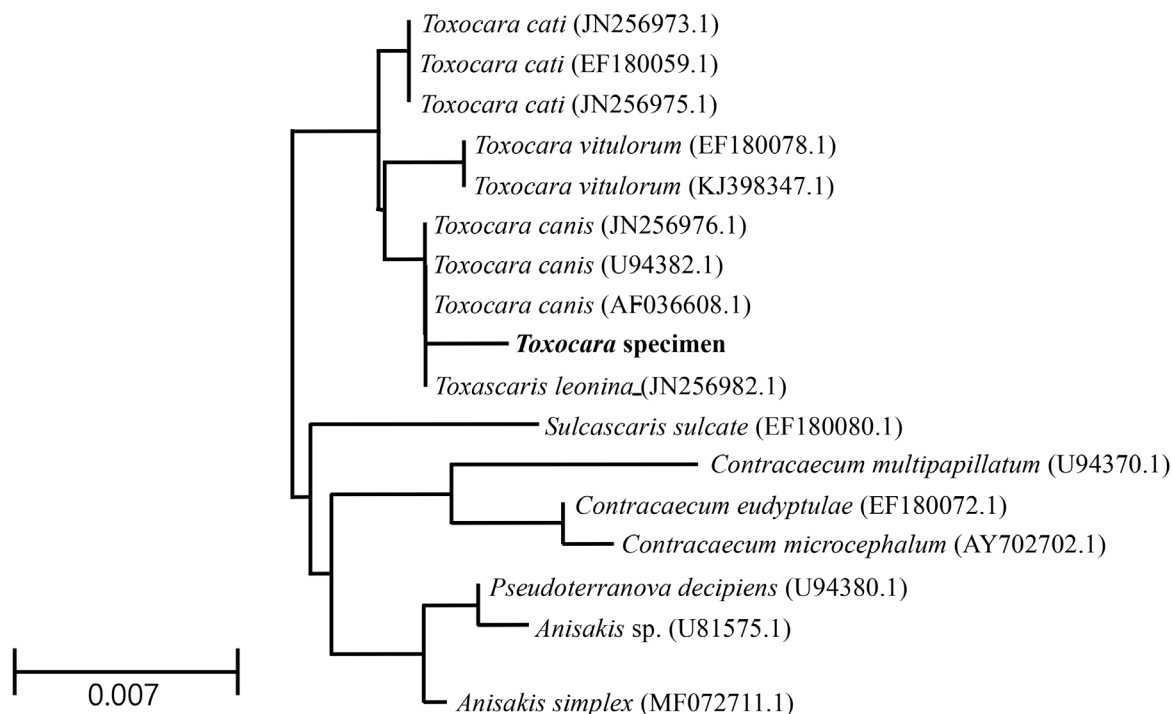
There were 6 pairs of postcloacal papillae: 3 pairs of postcloacal latero-ventral papillae (1st, 2nd, 6th pairs) and 3 pairs of postcloacal latero-dorsal (3rd, 4th, 5th pairs) (Fig. 4A, C, D). The papillae were distributed as follows: the 1st pair of postcloacal latero-ventral papillae, which were doubled, was located on each side behind the cloacal opening and the 2nd pair was located right behind them. The 3rd, 4th, and 5th pairs of postcloacal latero-dorsal papillae were located latero-dorsally beyond the cross lateral line. Further, the 3rd and 4th postcloacal latero-dorsal papillae were closely associated with each other, and the 4th pair was smaller than other papillae (Fig. 4D). The phasmid, which was much smaller than the papillae (< 3  $\mu\text{m}$  in diameter), was located laterally between the 4th and 5th pairs of postcloacal latero-ventral papillae (Fig. 4D). Like the rest of the body, the distal end of the tail formed a conical appendage that was transversely striated and terminated in a mucron (Fig. 4A, C, D).



**Fig. 3.** Scanning electron micrographs of female *Toxocara apodemi*. (A) Lateral view of the posterior end. C, cloaca Phasmid pore highlighted in an insert. (B) Ventral view of the posterior end. Terminal spike. (C) Egg. Note the characteristic superficial pits. (D) Magnified surface of egg. Note the elevated albumin coat.



**Fig. 4.** Scanning electron micrographs of male *Toxocara apodemi*, posterior end. (A) Ventro-frontal view, a pre-cloacal papillae (white arrow) and post-cloacal papillae (numbered). The 1st post-cloacal papillae are doubled. The 1st, 2nd, and 6th post-cloacal papillae located latero-ventrally. The 3rd, 4th, and 5th post-cloacal papillae located latero-dorsally. Phasmid pores (circles) lateral in position. (B) Phasmid. (C) Latero-frontal view. The 2nd to 5th post-cloacal papillae and phasmid. The phasmid is small and lateral in position. (D) Lateral view. Positions of papillae and phasmid pores (square). (E) Ventral view. Note that the pre-cloacal and post-cloacal median papillae in circles are not observed. C, cloaca; M, mucron.



**Fig. 5.** A phylogenetic tree (Neighbor Joining) based on 18S rRNA sequences.

#### Molecular characteristics and phylogenetic position

The genotype and phylogenetic relationship with other *Toxocara* species were determined using molecular characterization of the 18S rRNA gene. The sequencing information was registered into GenBank and received the accession number (MT460393.1) for *Toxocara* specimens. The partial sequences (1,351 bp) of the *Toxocara* specimens contained 2 unique gaps apart from sequences of genus *Toxocara*, including *T. canis*, *T. cati*, *T. vitulorum*, and *Toxascaris leonina*. The partial sequences also had 3 to 8 variable sites compared to aligned sequences. Results of the partial sequences showed the sequence homology of the 18S rRNA gene to that of *T. canis* (99.6%), *T. cati* (99.4%), *Ta. leonina* (99.4%), and *T. vitulorum* (99.2%) (Fig. 5). After the sequence of *Toxocara* specimens was compared with several genes, the sequence data were inferred using the NJ method (Fig. 5). Interestingly, the partial sequences of the 18S rRNA gene of *Toxocara* specimens showed high similarity to those of the parasite *Sulcascaris sulcate* (98.4%, EF180080.1), *Contracaecum multipapillatum* (98.3%, U94370.1), *Contracaecum eudypulae* (98.0%, EF180072.1), and *Contracaecum microcephalum* (98.0%, AY702702.1).

#### DISCUSSION

The subfamily Toxocarinae is characterized by the presence of a ventriculus without appendices and the absence of interlabia and gubernaculum [2]. In this study, the nematodes did not have intestinal cecum, interlabia, appendices of ventriculus, or gubernaculum. Also, the parasites were isolated in *A. agrarius* captured from 3 areas (Uljin-gun and Yongduk-gun in Geongbuk-do and Iksan-city in Chonbuk-do). The *Toxocara* spp. of Muridae include *T. apodemi* and *T. mackerrasae* [1,13]. They differ in their selection of hosts; the *T. mackerrasae* infects *Rattus fuscipes* and *Hydromys chrysogaster*, while *T. apodemi* infects *A. peninsulae* and *A. agrarius*. *T. apodemi* was reported to infect *A. peninsulae* and *A. agrarius* in China [6,9,13]. Currently, in Korea, *T. apodemi* infects *A. peninsulae* and *A. agrarius*.

The diagnostic keys for *Toxocara* created by Warren [1] indicate that the external prolongations of labial pulp in *T. apodemi* are symmetrical and rounded at the anterior border. The terminal spike on the tail of the female is present in *T. apodemi* but not in *T. mackerrasae*. The spicules of *T. apodemi* are 0.55 mm long in males 35.0 mm and 0.65 mm long in 35 to 36 mm long. The measurements of spicules of other species, including *T. mackerrasae* (0.65 mm), *T. tanuki* (3.0-3.5 mm), *T.*

*cati* (1.71-1.90 mm), *T. canis* (0.91-0.97 mm), *T. pteropodis* (0.58-0.73 mm), *T. vincenti* (0.68 mm), *T. paradoxum* (2.71-3.12 mm), *T. pearsei* (1.2-1.32 mm), *T. malaysiensis* (right 0.46-0.68 mm and left 0.48-0.76 mm), *T. lynx* (1.3-1.8 mm), and *T. hippopotami* (0.83-1.01 mm) have been reported [1,3,8,11]. In this study, the spicules were subequal and measured 0.45-0.53 mm long. The spicules were smaller than that of other species but closer to *T. apodemi* (0.45-0.54 mm) [13].

The 3 labia in this study were present as in ascaridoid nematodes and the denticles had a consistent distribution and a triangular outline of labium; however, *T. malaysiensis* has a deep median notch lined with denticles of the dentigerous ridge, which is absent in the most toxocarids, such as *T. canis*, *T. cati*, and *T. tanuki* [1,11]. In this study, the specimen also has strong denticles on the dentigerous ridge and a superficial median notch on each lip.

Gibbons et al. [3] reported a new species, *T. malaysiensis*, based on the following features: presence of the esophagus with a ventriculus without an appendix, the absence of an intestinal cecum, prominent dentigerous ridges on each of the 3 lips, a V-shaped supporting bar in the cervical alae, male with sub-equal, alate spicules, female with vulva opening in the anterior half of the body, and sub-globular eggs with a thick pitted shell. *Toxocara* spp., including *T. canis*, *T. cati*, *T. hippopotami*, *T. lynx*, *T. malaysiensis*, *T. pteropodis*, *T. tanuki*, and *T. vincenti*, have cervical alae [3,6,8], while *T. indica* and *T. apodemi* do not have cervical alae [12,13]. *T. indica* differs from all the known species of the genus *Toxocara* in having subequal spicules and the absence of cervical alae, except *T. alienata* and *T. apodemi*. *T. indica* resembles *T. alienata* in the absence of cervical alae [12]. Asakawa et al. and Olsen [6,13] reported that *T. apodemi* lacks cervical alae. However, our SEM images showed cervical alae as vestigial organs that looked like a slightly uplifted superficial sewing stitch.

The major and minor axes of *T. canis* eggs measure 71.6-91.2  $\mu\text{m}$  by 63.4-79.0  $\mu\text{m}$ , *T. cati* eggs measure 63.7-88.1  $\mu\text{m}$  by 53.3-73.3  $\mu\text{m}$ , and *T. malaysiensis* eggs measure 60-68  $\mu\text{m}$  by 68-76  $\mu\text{m}$  [3,19]. In our study, the eggs measured 73-82  $\mu\text{m}$ . Although the eggs observed in this study were smaller than the *T. canis* eggs and bigger than *T. malaysiensis* eggs, other significant differences were not observed. SEM images showed that the *Toxocara* eggs with an elevated albuminous coat were similar in shape to those of other members of the genus *Toxocara* [19]. Further, the surface of the egg observed in our study was coarsely pitted and covered with elevated albumin, similar to

that of other toxocarids [1].

The tail of the male has a curved posterior end that distinguishes it from the straight-tailed female. The posterior end of the female is terminating in a conical shape with a retractile spine-like structure in *T. apodemi* and *T. indica*, but that of other species (i.e., *T. malaysiensis*, *T. lynx*, *T. tanuki*) are conical [1,3,8,11,12]. Similar to *T. indica*, the sampled individuals had 6 pairs of postcloacal papillae, as opposed to the 5 pairs observed in *T. tanuki* and *T. vitulorum* [11,12]. Further, we observed that the posterior end of the female had a conical, retractile spine-like structure, which is a characteristic feature of *T. apodemi* [1].

Fagerholm [20] reported that the precloacal median papilla in the males of genus *Toxocara* was simple and surrounded by a cuticle of bifid appearance. Sanmartín et al. [21] also indicated that the precloacal median papilla was highly variable in the genus *Toxocara*, but the cuticle of bifid appearance in *T. genettae* was not observed. In *T. apodemi*, Olsen [13] described that 12 to 14 pairs of pre-anal (precloacal) papillae and 6 pairs of post-anal (postcloacal) papillae are present, the posterior 3 pairs of the post-anal papillae being located on the lob-like terminal portion of the tail. The 3th, 4th, and 6th pairs of these papillae are laterally located, while the 2nd and 5th pairs are sub-ventral, the 2nd pair consisting of double papillae. The 1st pair is also on the ventral surface, but much closer to the lateral margin [13]. In this study, the 1st pair of postcloacal papilla numbered by Olsen [13] was located on the ventrolateral portion in front of cloaca, i.e., the papilla was precloacal papilla. Therefore, we numbered the 1st pair of postcloacal papilla from the double papillae, which was numbered the 2nd pair by Olsen [13], as the 1st pair of postcloacal papilla. Especially, the 3rd and 4th postcloacal papillae were closely associated with each other, and the 4th pair of post cloacal papillae were smaller than other papillae. The 4th pair of this study was not described by Olsen [13].

The precloacal median papilla and a single postcloacal median papilla were found in *T. tanuki* [11]. However, like *T. indica* [12], we did not observe the precloacal and postcloacal median papillae in the present study.

Sanmartín et al. [21] discussed that the eggs of *T. genettae* are similar in shape and surface structure to those of other members of the genus, and their size (64-74  $\times$  50-54  $\mu\text{m}$ ) is similar to that of *T. cati*, and measurements of the superficial pits of egg (4.8  $\times$  3.2  $\mu\text{m}$ ) are similar to those of *T. canis*, but slightly smaller. In this study, the size of egg (73-82  $\mu\text{m}$ ) and the su-

perforial pits of egg (mean  $8.6 \times 6.7 \mu\text{m}$ ) are obviously bigger than those of *T. genettae*, *T. cati* and *T. canis*.

For the identification of parasites, the 18S rRNA gene was analyzed as it is highly conserved and contains variable regions flanked by more conserved regions [18,22]. Although partial sequences of the 18S rRNA gene of *T. apodemi* were similar to those of genus *Toxocara*, they were not perfectly matched with other members of *Toxocara* and a few unique variable regions were observed. Since there are not many sequences in the *Toxocara* clade, the morphology and gene of *T. apodemi* strongly support that this parasite is close to the *Toxocara* clade.

In conclusion, this is the first study to characterize *T. apodemi* using morphological features, SEM, and sequencing analysis. In SEM images, the cervical alae of *T. apodemi* was showed as the vestigial organs that looked like a slightly uplifted superficial sewing stitch. Also, we firstly found out the smaller 4th pair of postcloacal papilla, which is closely associated with 3rd papilla. In particular, the 3rd, 4th, and 5th pairs of postcloacal papilla is situated on the latero-dorsal surface than on the latero-ventral surface. The eggs of *T. apodemi* are easily distinguished from those of other *Toxocara* species by the bigger superficial pits. Although the partial 18S rRNA sequence of *T. apodemi* showed high homology of other *Toxocara* species, we found that the sequence of *T. apodemi* is not totally matched with those of other *Toxocara* species. It was confirmed that ascarid nematodes, *T. apodemi*, recovered from striped field mice in Korea are taxonomically conspecific relationship with genus *Toxocara* and genetic divergence from other *Toxocara* species.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

## REFERENCES

1. Warren EG. Studies on the morphology and taxonomy of the

- genera *Toxocara* Stiles, 1905 and *Neoascaris* Travassos, 1927. *Zool Anz* 1970; 185: 393-442.
2. Hartwich G, Anderson RC, Chabaud AG, Willmott S. *CIH Keys to the Nematode Parasites of Vertebrates*. Farnham Royal, UK. Commonwealth Agricultural Bureaux. 1974, pp 1-15.
3. Gibbons LM, Jacobs DE, Sani RA. *Toxocara malaysiensis* n. sp. (Nematoda: Ascaridoidea) from the domestic cat (*Felis catus* Linnaeus, 1758). *J Parasitol* 2001; 87: 660-665.
4. Poulsen CS, Skov S, Yoshida A, Skallerup P, Maruyama H, Thamsborg SM, Nejsum P. Differential serodiagnostics of *Toxocara canis* and *Toxocara cati*--is it possible? *Parasite Immunol* 2015; 37: 204-207.
5. Le TH, Anh NTL, Nguyen KT, Nguyen NTB, Thuy DTT, Gasser RB. *Toxocara malaysiensis* infection in domestic cats in Vietnam--an emerging zoonotic issue? *Infect Genet Evol* 2016; 37: 94-98.
6. Asakawa M, Li JE, Guo AH, Yang XY, Huhebateer, Liu ZL, Liu Y, Cao XM, Chen KY. A new host and locality record for *Toxocara apodemi* (Olsen, 1957) (Nematoda: Ascarididae) from striped field mice, *Apodemus agrarius* (Pallas) (Rodentia: Murinae) in Changsha, China. *J Rakuno Gakuen Univ Nt Sci* 1994; 19: 193-196.
7. Li K, Lan Y, Luo H, Zhang H, Liu D, Zhang L, Gui R, Wang L, Shahzad M, Sizhu S, Li J, Chamba Y. Prevalence, associated risk factors, and phylogenetic analysis of *Toxocara vitulorum* infection in yaks on the Qinghai Tibetan Plateau, China. *Korean J Parasitol* 2016; 54: 645-652.
8. Macchioni G. A new species, *Toxocara lynx*, in the caracal (*Lynx caracal*). *Parassitologia* 1999; 41: 529-532.
9. Warren EG. Two new species of *Toxocara* from viverrid hosts. *Parasitology* 1972; 65: 179-187.
10. Liu D. *Toxocara*. In Liu D ed, *Handbook of Foodborne Diseases*. Boca Raton, USA. CRC Press 2018.
11. Alexander U, Lim CW, Kim B, Hong EJ, Kim HC, Park BK. Morphological and molecular characterization of *Toxocara tanuki* (Nematoda: Ascaridae) from Korean raccoon dog, *Nyctereutes procyonoides koreensis*. *Korean J Parasitol* 2018; 56: 567-575.
12. Naidu TSV. Two new ascarid nematodes from vertebrate hosts from India. *Folia Parasitol* 1981; 28: 327-334.
13. Olsen LS. A new species of *Neoascaris* (Nematoda) from a Korean wood mouse. *Trans Am Microsc Soc* 1957; 76: 205-208.
14. Hartwich G. Key to genera of the Ascaridoidea. In Anderson RC, Chabaud AG, Willmott S eds, *CIH Keys to the Nematode Parasites of Vertebrates*, No. 2. Farnham Royal, UK. Commonwealth Agricultural Bureaux. 1974, pp 1-83.
15. Moreira GM, Telmo Pde L, Mendonça M, Moreira AN, McBride AJ, Scaini CJ, Conceição FR. Human toxocarriasis: current advances in diagnostics, treatment, and interventions. *Trends Parasitol* 2014; 30: 456-464.
16. Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels R. How common is human toxocarriasis? Towards standardizing our knowledge. *Trends Parasitol* 2009; 25: 182-188.
17. Chen J, Zhou DH, Nisbet AJ, Xu MJ, Haung SY, Li MW, Wang CR, Zhu XQ. Advances in molecular identification, taxonomy,



- genetic variation and diagnosis of *Toxocara* spp. *Infect Genet Evol* 2012; 12: 1344-1348.
18. Hong EJ, Sim C, Chae JS, Kim HC, Park J, Choi KS, Yu DH, Yoo JG, Park BK. A horsehair worm, *Gordius* sp. (Nematomorpha: Gordiida), passed in a canine feces. *Korean J Parasitol* 2015; 53: 719-724.
  19. Uga S, Matsuo J, Kimura D, Rai SK, Koshino Y, Igarashi K. Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. *Vet Parasitol* 2000; 92: 287-294.
  20. Fagerholm HP. Systematic implications of male caudal morphology in ascaridoid nematode parasites. *Syst Parasitol* 1991; 19: 215-229.
  21. Sanmartín ML, Alvarez E, Gijón Botella H, Iglesias R, Estévez J, López-Román R. A scanning electron microscope study of *Toxocara genette* Warren, 1972 (Ascaridae), with data on morphometric variation. *Folia Parasitol* 1992; 39: 355-367.
  22. Choi TI, Hong EJ, Ryu SY, Sim C, Chae JS, Kim HC, Park J, Choi KS, Yu DH, Yoo JG, Park BK. Detection and identification of *Sarcocystis cruzi* (Protozoa: Apicomplexa) by molecular and ultrastructural studies in naturally infected Korean cattle (*Bos taurus coreanae*) from Daejeon, Korea. *Korean J Parasitol* 2018; 56: 121-127.

