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Research paper

Morphological and genetic characterization of *Syphabulea tjanschani* (Ablasov, 1962) (Nematoda: Oxyuridae), with phylogenetic position of *Syphabulea* in Oxyuridae

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ABSTRACT

Pinworms are important parasitic nematodes in animals and humans, and many species are of medical and veterinary importance. The genus *Syphabulea* is a poorly known group of pinworms. The systematic position of *Syphabulea* in Oxyuridae remains unclear. Moreover, there is still a paucity of detailed information on some morphological aspects of the type species *S. tjanschani* (Ablasov, 1962). In the present study, the detailed morphology of *S. tjanschani* was studied using light microscopy and, for the first time, scanning electron microscopy. The results revealed three traits useful for the characterization of the species including: cuticular depression around excretory pore, non-prominent labial teeth and number of adhesive ridges in three ventral mammelons. The ribosomal [small ribosomal DNA (18S), internal transcribed spacer (ITS) and large ribosomal DNA (28S)] and mitochondrial [cytochrome *c* oxidase subunit 1 (*cox*1)] target regions of *S. tjanschani* were also firstly amplified and sequenced for future use in the molecular identification of this poorly known species. In addition, in order to investigate the systematic position of *Syphabulea* in Oxyuridae, the phylogenetic analyses of representatives of the Oxyuridae were performed using maximum likelihood (ML) and maximum parsimony (MP) methods based on 18S, 28S and 18S + 28S + *cox*1 sequence data, respectively. The phylogenetic results based on different sequence data all supported the genus *Syphabulea* to be a member of the subfamily Syphaciinae. The phylogenetic analysis based on 28S sequence data also showed *Syphabulea* as sister to the genus *Syphatineria*. Our present study represents the first attempt to resolve the systematic position of the genus *Syphabulea* using phylogenetic analyses based on molecular data.

1. Introduction

Pinworms are important parasitic nematodes in animals and humans, and many species are of medical and veterinary importance (Skrjabin et al., 1960). The genus *Syphabulea* is a poorly known group of pinworms. Gubanov (1964) established *Syphabulea* as a subgenus of *Syphacia*. Hugot (1981) firstly raised it to full generic status and considered that *Syphabulea* may be closely related to the genus *Syphatineria* based on the morphological characters, geographical distribution and host types (Hugot, 1988). According to Hugot (1981) and Hugot and Feliu (1990), *Syphabulea* can be easily distinguished from *Syphatineria* by having three ventral cuticular mamelons in male and eggs with large operculum occupying large middle part of convex side of shell (*vs* the presence of only two ventral cuticular mamelons in male and eggs with small operculum at one end in *Syphatineria*). However, the phylogenetic position of *Syphabulea* in Oxyuridae remains unclear.

The genus *Syphabulea* currently includes 10 species, which commonly occur in the digestive tract of various sciuroid rodents (Price, 1928; Cameron, 1932; Li, 1933; Ablasov, 1962; Gubanov, 1964; Schmidt and Kuntz, 1968; Quentin and Krishnasamy, 1975; Hugot, 1981; Hugot and Feliu, 1990). According to Hugot and Feliu (1990), *S. tjanschani* (Ablasov, 1962) is the type species of *Syphabulea*, mainly distributed in in China and USSR (Kirgizstan and Siberia) (*S. sobolevi* Gubanov, 1964 was treated as a synonym of *S. tjanschani* in this study). Although the morphology of *S. tjanschani* has been studied by Li (1933), Ablasov (1962) and Gubanov (1964), respectively, there is still a paucity of detailed information on some morphological aspects of this species.

The Chinese rock squirrel *Sciurotamias davidianus* (Milne-Edwards) (Rodentia: Sciuridae) is an endemic species to China. During a recent

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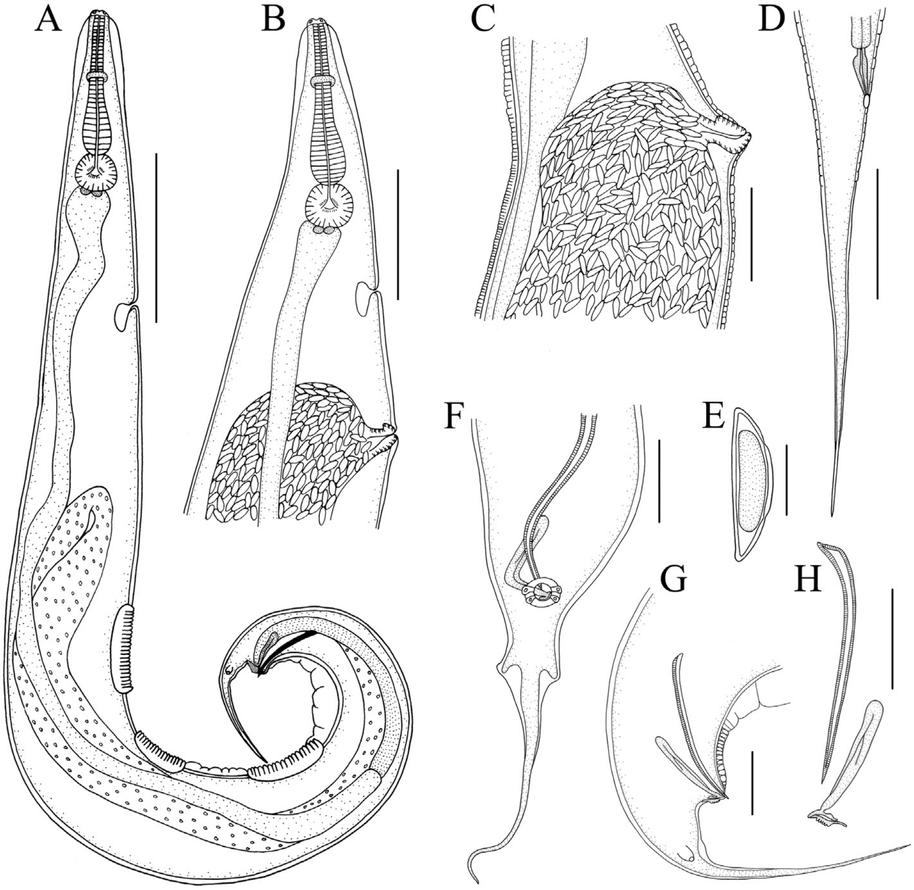

Fig. 1. *Syphabulea tjanschani* (Nematoda: Oxyuridae) collected from the Chinese rock squirrel *Sciurotamias davidianus* (Rodentia: Sciuridae) in China. A, adult male, lateral view; B, anterior part of female, lateral view; C, region of vulva, lateral view; D, tail of female, lateral view; E, egg, lateral view; F, posterior end of male, ventral view; G, posterior end of male, lateral view; H, Spicule and gubernaculum. Scale bars: A, B, D = 500 μm; C = 300 μm; E = 50 μm; F, G, H = 100 μm.

parasitological survey of the rodents in Hebei Province, China, numbers of *Syphabulea* nematodes were collected from the large intestine of *S. davidianus*, which were identified morphologically as *S. tjanschani*. The detailed morphology of this species was studied using light microscopy and, for the first time, scanning electron microscopy. Specimens of *S. tjanschani* were also characterized using molecular methods by sequencing and analyzing the ribosomal [small ribosomal DNA (18S), internal transcribed spacer (ITS) and large ribosomal DNA (28S)] and mitochondrial [cytochrome *c* oxidase subunit 1 (*cox*1)] target regions, respectively, for future use in the molecular identification of this poorly known species. Moreover, the phylogenetic analyses of oxyurid nematodes based on 18S, 28S, and 18S + 28S + *cox*1 sequence data, respectively, were performed to determine the phylogenetic position of *Syphabulea* in Oxyuridae.

2. Materials and methods

2.1. Light and scanning electron microscopy

One dead Chinese rock squirrel *S. davidianus* was dissected for parasites, that was collected from Xiling Mountain (40°04′–40°16′N, 115°19′–115°33′E), Hebei Province, China. Numbers of nematodes were collected from the large intestine of *S. davidianus* and washed in physiological saline, then fixed and stored in 80% ethanol until studied. For light microscopical studies, nematodes were cleared in lactophenol. Drawings were made with the aid of a Nikon microscope drawing attachment. For scanning electron microscopy (SEM), specimens were fixed in 4% formaldehyde solution, post-fixed in 1% OsO4, dehydrated *via* an ethanol series and acetone, and then critical point dried. The specimens were coated with gold and examined using a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 20 kV. Measurements (the range, followed by the mean in parentheses) are given in micrometres unless otherwise stated. Voucher specimens were deposited in College of Life Sciences, Hebei Normal University, Hebei Province, China.

2.2. Molecular procedures

The midbody region of three specimens were randomly selected for molecular analysis. Genomic DNA from each sample was extracted using a Column Genomic DNA Isolation Kit (Shanghai Sangon, China) according to the manufacturer's instructions. The partial 18S region was amplified by polymerase chain reaction (PCR) using the forward primer 18SF (5′-CGCGAATRGCTCATTACAACAGC-3′) and the reverse primer 18SR (5′-GGGCGGTATCTGATCGCC-3′) (Floyd et al., 2005). The partial 28S region was amplified by PCR using the forward primer 28S-F (5′-AGCGGAGGAAAAGAAACTAA-3′) and the reverse primer 28S-R (5′-ATCCGTGTTTCAAGACGGG-3′) (Nadler and Hudspeth, 1998). The ITS-1 region was amplified by PCR using the forward primer SS1 (5′-GTTTCCGTAGGTGAACCTGCG-3′) and the reverse primer SS2R (5′-AGTGCTCAATGTGTCTGCAA-3′); the ITS-2 region was amplified by PCR using the forward primer NC13 (5′-ATCGATGAAGAACGCAGC-3′) and the reverse primer NC2 (reverse: 5′-TTAGTTTCTTTTCCTCCGCT-3′) (Zhu et al., 2000). The partial *cox*1 region was amplified by PCR using the forward primer COF (5′-AGTTCTTTGGGTCATCCTGGTAG-3′) and the reverse primer COR (5′-TAGGCACAGCAATAATCATAGTAGC-3′) designed by the authors of the present study. The cycling conditions were as described previously (Li et al., 2018). PCR products were checked on GoldView-stained 1.5% agarose gels and purified with Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing for each sample was carried out for both strands. Sequences were aligned using ClustalW2 and adjusted manually. The DNA sequences obtained herein were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology

Information (NCBI) database (http://www.ncbi.nlm.nih.gov). The 18S, 28S, ITS and *cox*1 sequences of *S. tjanschani* are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov).

2.3. Phylogenetic analyses

Phylogenetic trees for the 18S, 28S and 18S + 28S + *cox*1 sequence data were inferred using both maximum likelihood (ML) and maximum parsimony (MP), as implemented in MEGA 7 (Kumar et al., 2016). *Pseudonymus spirotheca* belonging to the family Pseudonymidae (Thelastomatoidea) was selected as the outgroup. The K2 (Kimura 2-parameter) + I model for 18S sequences, the GTR (General Time Reversible) + G model for 28S and HKY (Hasegawa-Kishino-Yano) + G model for 18S + 28S + *cox*1 sequence data were identified as optimal nucleotide substitution model, respectively. Reliabilities for both ML and MP trees were tested using 1000 bootstrap replications and nodes with bootstrap values exceeding 70% were considered well supported (Hillis and Bull, 1993).

3. Results

3.1. Description of Syphabulea tjanschani (Ablasov, 1962) (Figs. 1–3)

Small-sized, whitish nematodes. Cuticle with fine transverse striations. Maximum width of body at slightly posterior to mid-body (Fig. 1A). Cephalic extremity with three small triangular lips, more or less equal in size; two large papillae and one median amphid on each lateroventral lip, labial teeth not prominent (Figs. 2A, 3A, B). Cephalic vesicle conspicuous in both sexes (Fig. 1A, B). Cervical alae absent. Oesophagus consisting of indistinct pharynx, cylindrical corpus and terminating in conspicuous valved bulb (isthmus absent) (Fig. 1A, B). Nerve ring suited at about 1/3 of oesophageal length (Fig. 1A, B), excretory pore located in a depression, some distance posterior to oesophageal bulb (Figs. 1A, B, 3F). Deirids not observed. Tail of both sexes very long, with pointed tip (Figs. 1D, F, G, 3E).

3.1.1. Male (based on 8 mature specimens)

Body 3.05–5.10 (3.75) mm long; maximum width 360–520 (424). Cephalic vesicle 210–300 (250) long, 20–40 (30) wide (Fig. 1A). Oesophagus 520–650 (561) mm in total length, representing 12.8–17.1 (15.1) % of body length; pharynx 20–40 (28) long; corpus 390–490 (421) long, maximum width 50–70 (60); size of posterior bulb 90–130 (113) long × 90–140 (123) wide. Nerve ring and excretory pore170–200 (185) and 760–890 (835), respectively, from anterior extremity (Fig. 1A). Posterior end of body distinctly curved ventrally. Three ventral mamelons present, almost equidistant, anterior (1st) mamelon with approximately 16 ridges, largest in size, 210–500 (286) long × 138–208 (179) wide; middle (2nd) mamelon with approximately 12 ridges, smallest in size, 140–240 (176) long × 118–180 (162) wide; posterior (3rd) mamelon with approximately 14 ridges, 170–320 (246) long × 126–196 (174) wide (Figs. 1A, 2C, D, E, G); posterior edge of each mamelon 795–2000 (1138) (1st), 630–1500 (983) (2nd) and 215–850 (542) (3rd) long from cloaca, respectively (Fig. 1A). Spicules single, pointed at distal end, 180–260 (213) long,

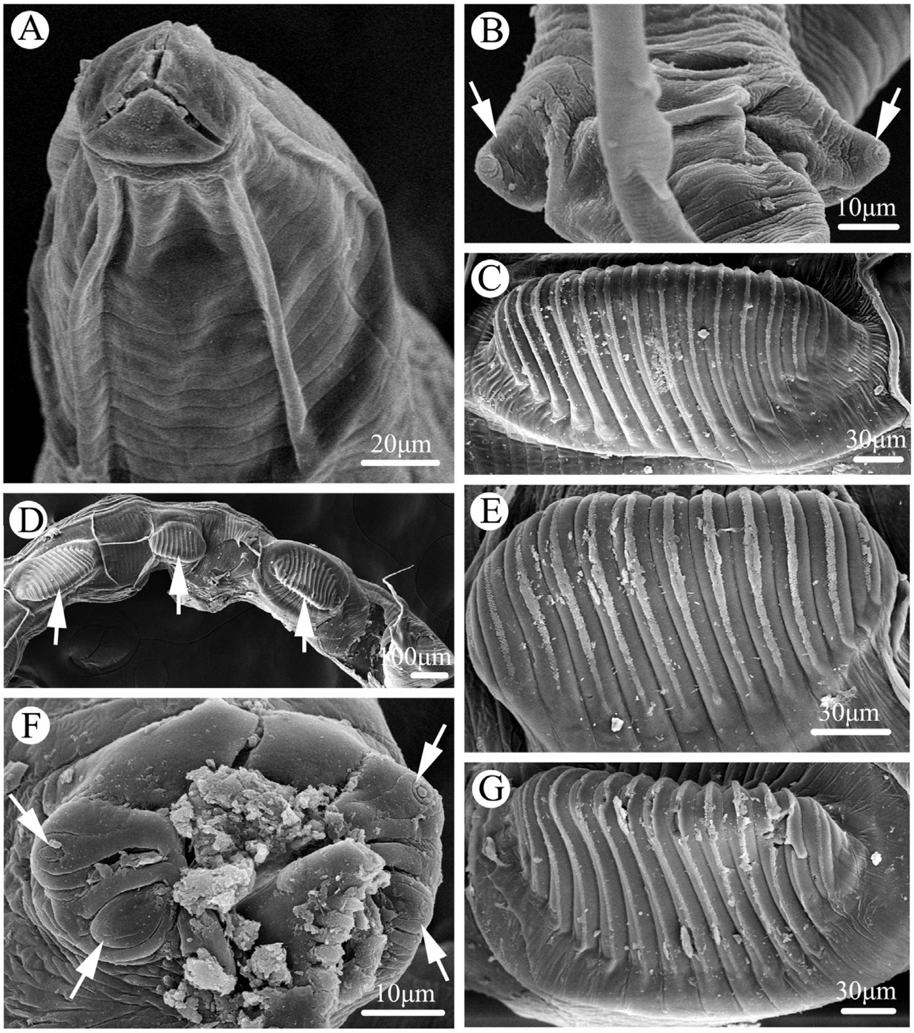

Fig. 2. Scanning electron micrographs of *Syphabulea tjanschani* (Nematoda: Oxyuridae) collected from the Chinese rock squirrel *Sciurotamias davidianus* (Rodentia: Sciuridae) in China. A, anterior part of male, apical view; B, magnified image of large, pedunculated postcloacal papillae (arrowed), ventral view; C, magnified image of first mamelon with approximately 16 ridges, ventral view; D, distribution of three mamelons (arrowed) in male, ventral view; E, magnified image of second mamelon with approximately 12 ridges, ventral view; F, magnified image of cloacal region (two pairs of paracloacal papillae arrowed), ventral view; G, magnified image of third mamelon with approximately 14 ridges, ventral view.

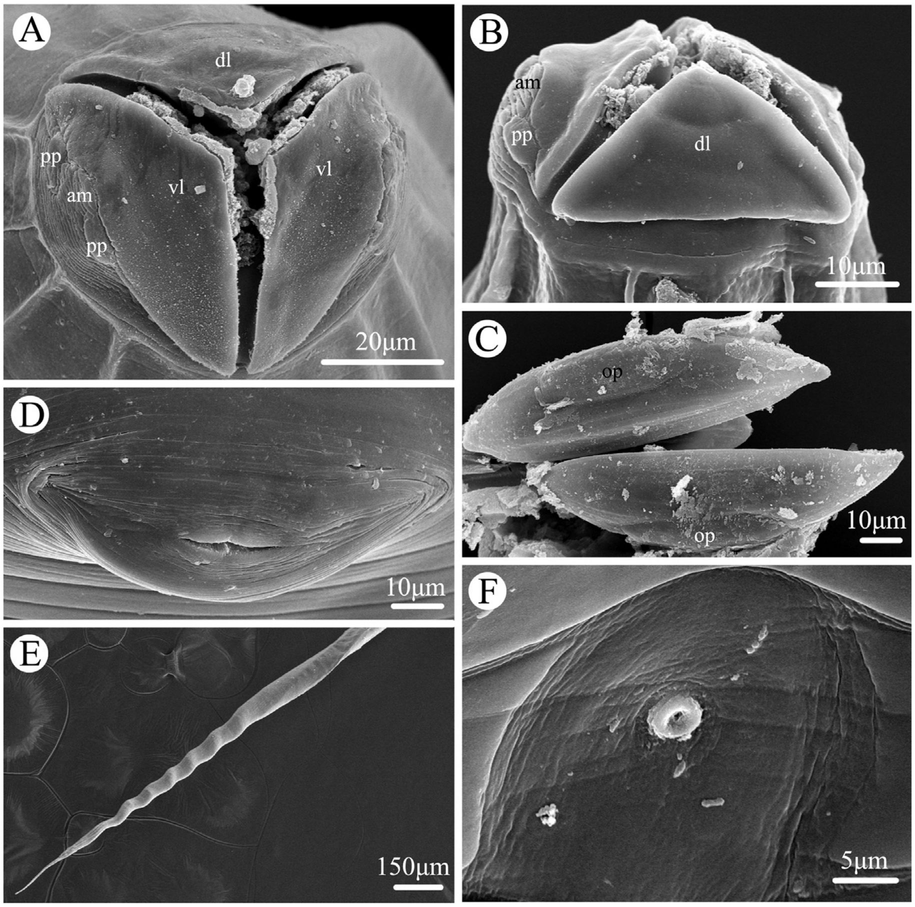

Fig. 3. Scanning electron micrographs of *Syphabulea tjanschani* (Nematoda: Oxyuridae) collected from the Chinese rock squirrel *Sciurotamias davidianus* (Rodentia: Sciuridae) in China. A, cephalic extremity of female, apical view; B, cephalic extremity of female, lateral view; C, magnified image of eggs; D, magnified image of vulva; E, tail tip of female; F, magnified image of excretory pore. Abbreviations: dl, dorsal lip; vl, ventro-lateral lip; am, median amphid; pp., cephalic papillae; op, operculum of egg.

representing 4.82–6.56 (5.73) % of body length (Fig. 1F–H). Gubernaculum well sclerotized, 110–150 (126) long, accessory piece of gubernaculum with ornamentation (Fig. 1G, H). Caudal papillae three pairs in total, arranged as follows: two pairs paracloacal and one pair postcloacal (Figs. 1F, 2F); postcloacal papillae large, pedunculate (Figs. 1F, G, 2B). Tail 300–500 (384) long, with pointed tip (Fig. 1A, F, G). Phasmids not observed.

3.1.2. Female (based on 10 mature specimens)

Body 7.56–9.40 (8.62) mm long; maximum width 620–880 (748). Oesophagus 720–790 (755) mm in total length, representing 8.09–10.2 (8.79) % of body length; pharynx 30–40 (34) long; corpus 550–590 (572) long, maximum width 60–80 (71); size of posterior bulb 120–160 (148) long × 170–180 (178) wide. Nerve ring and excretory pore 190–270 (216) and 740–1240 (1036) respectively, from anterior extremity (Fig. 1B). Vulva usually protruded in fully gravid specimens, 1.16–1.86 (1.50) mm from anterior extremity, representing 13.3–21.6 (17.5) % of body length (Figs. 1B, C, 3D). Eggs asymmetrical, flattened at one side, nonembryonated, thin-shelled, 70–110 (98) × 20–30 (29) in size (n = 20), large operculum present, occupying large middle part of convex side of shell (Figs. 1E, 3C). Tail 1.54–2.60 (2.11) long, with pointed tip (Fig. 1D, 3E). Phasmids not observed.

3.1.3. Host and locality of present material

Chinese rock squirrel *Sciurotamias davidianus* (Milne-Edwards) (Rodentia: Sciuridae); Xiling Mountain, Hebei Province, China.

3.1.4. Site in host

Large intestine.

3.1.5. Intensity

Single squirrel examined with 26 nematodes.

3.1.6. Voucher specimens

8 males and 18 females (HBNU–M2018004L).

3.2. Genetic characterization

3.2.1. Partial 18S region

Three partial 18S sequences of *S. tjanschani* with length 1639 bp were obtained herein. All the sequences are identical. There is no species of *Syphabulea* with 18S sequence data registered in GenBank. Pairwise comparison between *S. tjanschani* and the other species of Oxyuridae with 18S sequences available in GenBank (including JF934731, KY462826, KU285457, KU285462, KU285460, KU285456, KU285453, JF934730, EF180079, EF180075, EF180066, EF180062, EF180061, EF180060, KU180664, AB699690), showed 7.65% (*Syphacia obvelata* KY462826) to 18.7% (*Wellcomia siamensis* EF180079) nucleotide differences. The partial 18S sequences of *S. tjanschani* are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers MH443060-MH443062).

3.2.2. Partial 28S region

Three partial 28S sequences of *S. tjanschani* with length 785 bp were obtained herein. All the sequences are identical. There is no species of *Syphabulea* with 28S sequence data registered in GenBank. Pairwise comparison between *S. tjanschani* and the other species of Oxyuridae with 28S sequences available in GenBank (including AB500163, AB500167, AB500168, AB500173, AB500174, AB500176-500178, LC038094, LC038097-038099, KU668405, MG310151, EF464552, KY990019, KY990021, KU285478, KU285476, KU285473, KU285469, KU285467), showed 19.1% (*Syphatineria* sp. LC038099) to 57.8% (*Passalurus ambiguus* MG310151) nucleotide differences. The partial 28S sequences of *S. tjanschani* are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov) (accession numbers MH443063-MH443065).

3.2.3. Partial ITS region

Three partial ITS sequences of *S. tjanschani* with length 874 bp were obtained herein. All the sequences are identical. There is no species of *Syphabulea* with ITS sequence data registered in GenBank. Pairwise comparison between *S. tjanschani* and the other species of Oxyuridae with ITS sequences available in GenBank (including EU263106, KT853017, MF142456, MF142444), all showed over 40.0% nucleotide differences. The ITS sequences of *S. tjanschani* are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov) (accession numbers MH443066-MH443068).

3.2.4. Partial cox1 region

Three partial *cox*1 sequences of *S. tjanschani* with length 499 bp were obtained herein. All the sequences are identical. There is no species of *Syphabulea* with *cox*1 sequence data registered in GenBank. Pairwise comparison between *S. tjanschani* and the other species of Oxyuridae with *cox*1 sequences available in GenBank (including AB626880, HM204812, MF142430, KU285485, KT875320, KU285498, KU285497, KU285494, KU285488, AB626879, AB626860, AB626859, MF142429, MF142428, AF263482, GQ332427, KT879302, LC038087, NC_027190, KJ768611, FR687979, FR687972, FR687968, AB254450), showed 8.85% (*Syphacia obvelata* MF142430) to 23.6% (*Syphacia rifaii* LC038087) nucleotide differences. The *cox*1 sequences of *S. tjanschani* are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov) (accession numbers MH497374-MH497376).

3.3. Phylogenetic analyses

Phylogenetic trees constructed from the 28S sequence data using ML and MP methods had almost identical topology, only with small difference in clade support (Fig. 4). The representatives of the Oxyuridae were divided into three distinct clades (subfamilies): Enterobiinae (including *Trypanoxyuris atelophora*, *T. atelis*, *T. multilabiatus*, *T. pigrae* and *T. minutus*), Oxyurinae (including *Oxyuris equi* and *Skrjabinema ovis*) and Syphaciinae (including *Passalurus ambiguus*, *Syphatineria* sp., *Syphabulea tjanschani*, *Rauschtineria eutamii* and *Syphacia* spp.). All of the three clades are monophyletic, with strong support in both ML and MP trees (Fig. 4). In the subfamily Syphaciinae, the genus *Syphabulea* formed a sister relationship to *Syphatineria*.

Phylogenetic trees constructed from the 18S sequence data using ML and MP methods had similar topology (Fig. 5), but both are different from the phylogenetic trees from 28S sequence data. The monophyly of the subfamily Oxyurinae (including *Oxyuris* and *Skrjabinema*) was well supported only in ML tree. The representatives of the Enterobiinae (including *Trypanoxyuris*, *Lemuricola*, *Enterobius* and *Pongobius*), grouped together with species of *Passalurus* (belonging to Syphaciinae) with weak support value in both ML and MP trees. *Syphabulea* showed the sister relationship to the genus *Syphacia* in MP tree, but *S. tjanschani* nested in species of *Syphacia* in ML tree.

Phylogenetic trees constructed from the 18S + 28S + *cox*1 sequence data using ML and MP methods also had similar topology (Fig. 6), which are similar to the phylogenetic results from 28S sequence data. The monophyly of the representatives of Oxyurinae (including *Oxyuris* and *Skrjabinema*), Enterobiinae (including *Trypanoxyuris* and *Enterobius*) and Syphaciinae (including *Syphabulea* and *Syphacia*) was well supported.

4. Discussion

Li (1933), Ablasov (1962), Petrov and Bayanov (1962) and Gubanov (1964) reported this species from *Sciurus vulgaris* (L.) and *Sciurotamias davidianus* (Milne-Edwards) in China and USSR (Kirgizstan and Siberia), respectively. The morphology and measurements of the present specimens are almost identical to the previous description of *S. tjanschani* by Li (1933), Ablasov (1962) and Gubanov (1964), including the body size, the morphology and lengths of oesophagus, spicule, gubernaculum and tail, the number and arrangement of the caudal papillae, the size and distribution of mamelons, the position and morphology of vulva and the size of eggs (Table 1). Therefore, we considered our material to be conspecific with *S. tjanschani*. Although the present material and some of Li's (1933) specimens are both collected from *S. davidianus* in China, there are remarkable morphometric differences between our male specimens and Li's (1933) material, especially in the lengths of body and oesophagus, and the ratio of spicule to body length (Table 1). In contrast, the morphometrics of our specimens are more similar to Gubanov's (1964) material, which were collected from *S. vulgaris* in Siberia. The measurements of Li's (1933) material are almost identical to Ablasov's (1962) specimens collected from *S. vulgaris* in Kirgizstan. However, we considered that all the morphometric differences among the different material should be treated as intraspecific variability,

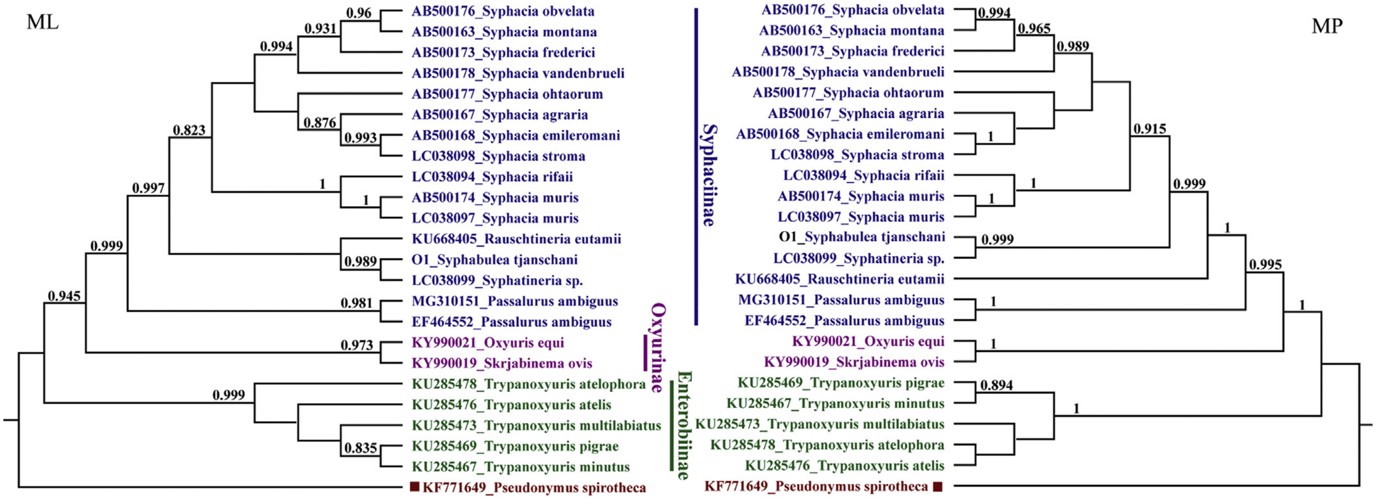

Fig. 4. Maximum likelihood (ML) and Neighbour-Joining (NJ) trees based on the 28S sequence data showing the phylogenetic relationships of representatives of Oxyuridae. *Pseudonymus spirotheca* belonging to the family Pseudonymidae (Thelastomatoidea) was selected as the outgroup. Bootstrap values exceeding 70% were displayed.

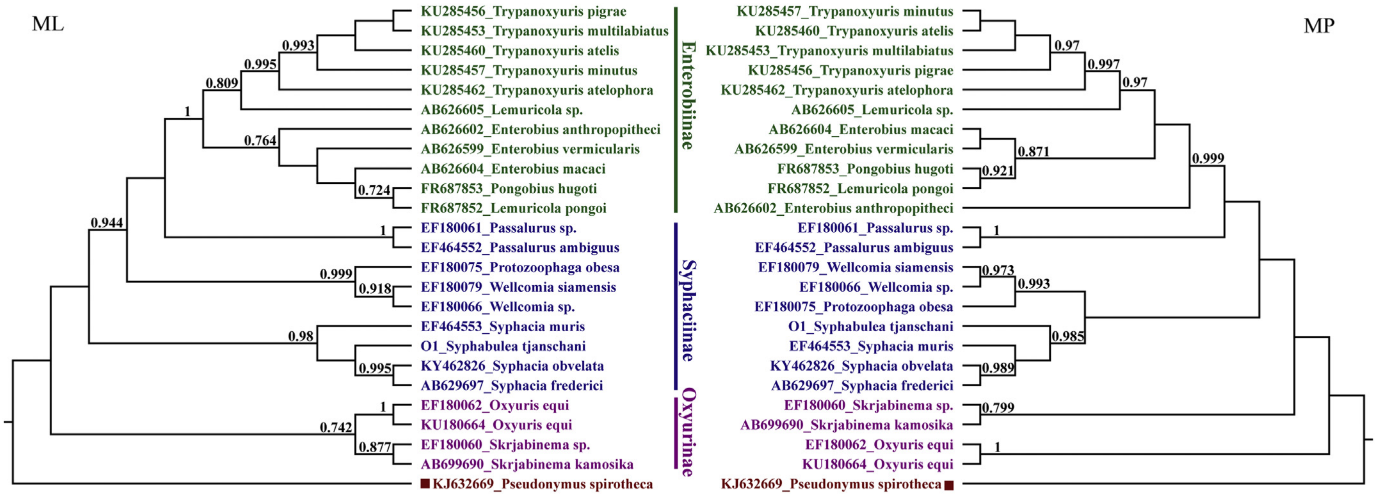

Fig. 5. Maximum likelihood (ML) and Neighbour-Joining (NJ) trees based on the 18S sequence data showing the phylogenetic relationships of representatives of Oxyuridae. *Pseudonymus spirotheca* belonging to the family Pseudonymidae (Thelastomatoidea) was selected as the outgroup. Bootstrap values exceeding 70% were displayed.

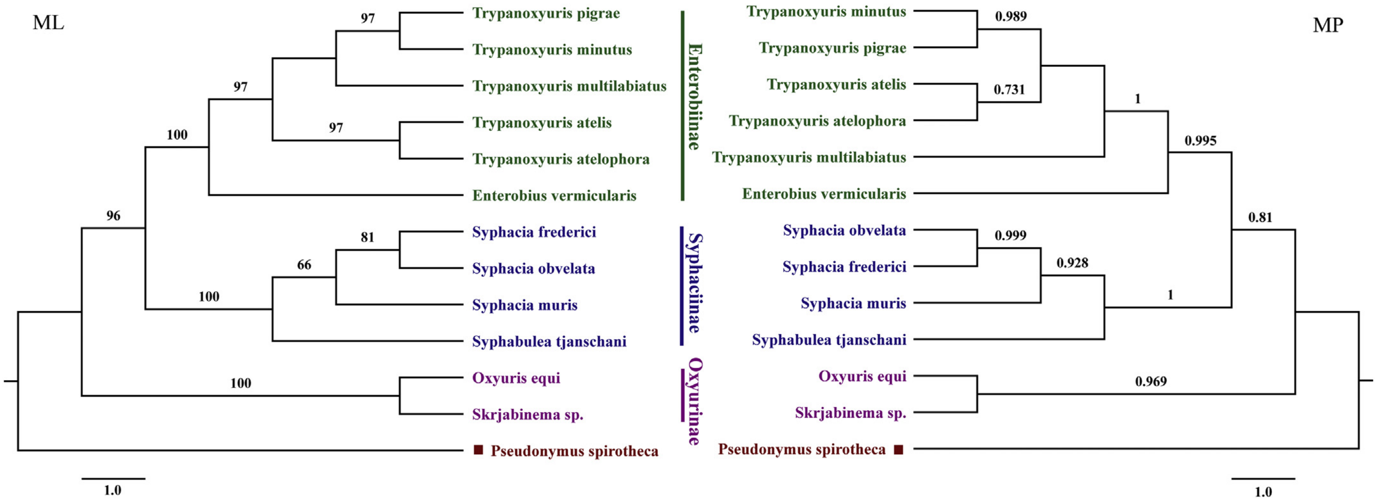

Fig. 6. Maximum likelihood (ML) and Neighbour-Joining (NJ) trees based on the 18S + 28S + *cox*1 sequence data showing the phylogenetic relationships of representatives of Oxyuridae. *Pseudonymus spirotheca* belonging to the family Pseudonymidae (Thelastomatoidea) was selected as the outgroup. Bootstrap values exceeding 70% were displayed.

probably resulted by intensity of infection or geographical localities.

The present study provided the first data on the detailed structure of *S. tjanschani* using scanning electron microscopy (SEM). All the previous studies did not state the detailed morphology of mamelons in *S. tjanschani* (Li, 1933; Ablasov, 1962; Petrov and Bayanov, 1962; Gubanov, 1964). Our SEM observation revealed the three ventral mamelons with different number of cuticular ridges on the surface (first mamelon with approximately 16 ridges, second mamelon with approximately 12 ridges, third mamelon with approximately 14 ridges) (Fig. 2C, E, G). The detailed morphology of excretory pore has been proved to be of taxonomic significance in *Syphabulea* by Quentin and Krishnasamy (1975) and Hugot and Feliu (1990). However, many species of *Syphabulea* lacked of the detailed information of this character when they were originally described. Our present study clearly showed the excretory pore of *S. tjanschani* located in a depression, which is different from that in *S. magnispiculoides* (Quentin and Krishnasamy, 1975), *S. sarawakensis* (Quentin and Krishnasamy, 1975) and *S. mascomai* Hugot and Feliu, 1990, but similar to that in *S. schmidti* (Quentin and Krishnasamy, 1975). According to Hugot (1981), the cephalic structures are critical to discriminating species of *Syphabulea*, but the cephalic structures of *S. tjanschani* has not been adequately described in the previous studies. Our SEM observations conformed the labial teeth not prominent and the presence of two large papillae and one median amphid on each lateroventral lip (Fig. 3A, B). In addition, the detailed morphology of caudal papillae and eggs of *S. tjanschani* have also been reported for the first time in this study.

In the genus *Syphabulea*, *S. tjanschani* differs from *S. magnispiculoides*, *S. schmidti*, *S. sarawakensis*, *S. coli* (Schmidt and Kuntz, 1968), *S. critesi* (Schmidt and Kuntz, 1968) and *S. magnispicula* (Schmidt and Kuntz, 1968) by having different arrangement of ventral mamelons in male (three mamelons almost equidistant in *S. tjanschani vs* first and second mamelons very close to each other in the latter six species), slightly protruded vagina (*vs* vagina distinctly protruded in the latter six species) and different morphology of accessory piece of gubernaculum. *Syphabulea tjanschani* can be easily distinguished from *S. thompsoni* (Price, 1928) and *S. maseri* Hugot, 1981 by having different arrangement of caudal papillae (absence of precloacal papillae in *S. tjanschani vs* presence of one pair of precloacal papillae in *S. thompsoni* and *S. maseri*) and smaller operculum of egg. In addition, *S. thompsoni* and *S. maseri* have remarkable lateral alae (without lateral alae in *S. tjanschani*). Hugot and Feliu (1990) reported *S. mascomai* from *S. vulgaris* in Spain. The morphology and measurements of *S. mascomai* are very

Table 1

Morphometric comparisons of *Syphabulea tjanschani* (Ablasov, 1962) collected from the Chinese rock squirrel *Sciurotamias davidianus* (Milne-Edwards) (Rodentia: Sciuridae) with previous descriptions (measurements in millimeters).

	Present study		Ablasov (1962)		Gubanov (1964)		Li (1933)	
Characteristics	Male (*n* = 8)	Female (*n* = 10)	Male (n = ?)	Female (n = ?)	Male (n = ?)	Female (n = ?)	Male (*n* = 4)	Female (n = 4)
BL	3.05–5.10	7.56–9.40	2.50–2.74	7.44–8.10	3.60–3.74	8.30	2.65–2.92	7.80–8.25
OL	0.42–0.52	0.59–0.63	0.36	0.52–0.61	0.36–0.46	0.62	0.32–0.38	0.55–0.60
SB	0.09–0.13 × 0.09–0.14	0.12–0.16 × 0.17–0.18	0.10 × 0.10	0.13–0.15 × 0.13–0.15	0.11–0.13 × 0.11–0.13	0.14 × 0.14	0.10–0.12 × 0.10–0.12	0.14–0.15 × 0.14–0.15
NL	0.17–0.20	0.19–0.27	0.13	0.20–0.22	0.15–0.17	0.19	0.12–0.15	0.20–0.21
EL	0.76–0.89	0.74–1.24	0.50	0.90–0.92	0.61–0.65	1.24	0.52–0.55	0.91–0.93
TL	0.30–0.50	1.54–2.60	0.34	1.28	0.35–0.39	2.85	0.35–0.40	1.4–1.7
SL	0.18–0.26	–	0.20	–	0.19–0.20	–	0.21–0.24	–
SL/BL	4.82–6.56%	–	7.30%	–	5.76–5.81%	–	7.92–8.22%	–
GL	0.11–0.15	–	0.10	–	0.11–0.13	–	0.10–0.13	–
NCP	0–2-1	–	0–2-1	–	0–2-1	–	0-2-1	–
SM1	0.21–0.50	–	?	–	?	–	0.20–0.30	–
SM2	0.14–0.24	–	?	–	?	–	0.12–0.16	–
SM3	0.17–0.32	–	?	–	?	–	0.20–0.24	–
DMC1	0. 80–2.00	–	?	–	1.78–1.81	–	0.85–0.95	–
DMC2	0.63–1.50	–	?	–	1.31–1.34	–	0.60–0.65	–
DMC3	0.22–0.85	–	?	–	0.84–0.91	–	0.30–0.32	–
DVC	–	1.16–1.86	–	1.28	–	1.40	–	1.45–1.60
DVC/BL	–	13.3–21.6%	–	17.2%	–	16.9%	–	18.6–19.4%
ES	–	0.07–0.11 × 0.02–0.03	–	0.09 × 0.03	–	0.10 × 0.03	–	0.09–0.10 × 0.03–0.035
Hosts	*Sciurotamias davidianus*		*Sciurus vulgaris*		*Sciurus vulgaris*		*Sciurus vulgaris, Sciurotamias davidianus*	
localities	China		USSR		USSR		China	

Abbreviations: BL–length of body, OL (not including bulb)–length of oesophagus, SB–size of bulb, NL–length of nerve ring to cephalic end, EL–length of excretory pore to cephalic end, SL–length of spicule, GL–length of gubernaculum, NCP–numbers and arrangement of cloacal papillae, SM1–length of first mamelon, SM2–length of second mamelon, SM3–length of third mamelon, DMC1-3–distance of posterior edge of each mamelon to cloaca, ES–size of eggs, TL–length of tail, DVC–distance of vulva to cephalic end.

similar to *S. tjanschani*. We can distinguish the two species by the different size of body and eggs (body size: male 2.65–5.10 mm, female 7.45–9.40 mm in *S. tjanschani vs* male 2.62 mm, female 4.59 mm in *S. mascomai*; egg size: 0.07–0.11 × 0.02–0.03 mm in *S. tjanschani vs* 0.14 × 0.05 mm in *S. mascomai*).

It is not easy to identify and differentiate the pinworms only using morphological characters because of their small body size, the extraordinary morphological similarity and sometimes the lack of male worms (male worms die after mating). Recently, some previous studies have proved that it is very useful to utilize the molecular sequence data (*i.e.* 18S, 28S, ITS and *cox*1) for species identification (Nakano et al., 2006; Parel et al., 2008; Hasegawa et al., 2012; Koehler et al., 2014; Solórzano-García et al., 2015, 2016). However, to date, only a minority of oxyurid species can be obtained for molecular study. In the present study, we firstly amplified and sequenced the partial 18S, 28S, ITS and *cox*1 sequences of *S. tjanschani* for future use in the molecular identification of this poorly known species. There is no intraspecific genetic divergence detected between different individuals of *S. tjanschani* in the partial 18S, 28S, ITS and *cox*1 sequences, but the high level of genetic divergence was found between *S. tjanschani* and the other oxyurid species in all the four molecular markers (7.65–18.7% in 18S, 19.1–57.8 in 28S, over 40.0% in ITS, 8.57–23.0% in *cox*1). The level of genetic divergence in *cox*1 region between *Syphabulea* and the other genera of Oxyuridae is similar to that reported in the previous study (13.0–17.8% in *cox*1 region between *Trypanoxyuris* and the other genera of Oxyuridae), but much higher in 18S region (only 4.1–5.3% in 18S region between *Trypanoxyuris* and the other genera of Oxyuridae) (Solórzano-García et al., 2016).

Hugot (1981) raised the subgenus *Syphabulea* of *Syphacia* to full generic status mainly based on morphological characters and host types. However, the present phylogenetic study using 18S sequence data well supported the generic status of *Syphabulea* only in the MP tree, because *S. tjanschani* nested in the representatives of *Syphacia* in the ML tree. In contrast, the phylogenetic results based on 28S and 18S + 28S + *cox*1 sequence data, respectively, showed *Syphabulea* to be a separate genus, that formed a sister relationship to *Syphatineria* or *Syphacia*. The present results are congruent with the previous phylogenetic studies using morphological characters by Hugot (1988) and Hugot et al. (2013). According to Hugot (1988) Hugot et al. (1996) and Adamson (1989), the Oxyuridae were divided into three subfamilies, including Oxyurinae, Enterobiinae and Syphaciinae, and the subfamily Syphaciinae also includes several tribes (*i.e.* Syphaciini, Acanthoxyurini, Higertiini, Passalurini and Protozoophagini). Our present phylogenetic analyses using 28S and 18S + 28S + *cox*1 sequence data sequence data supported the monophyly of Oxyurinae, Enterobiinae and Syphaciinae. The results are largely in agreement with the previous hypotheses (Hugot, 1988; Adamson, 1989; Hugot et al., 1996). Our present study represents the first attempt to resolve the systematic position of the genus *Syphabulea* using phylogenetic analyses based on molecular data.

5. Conclusion

The genus *Syphabulea* is a poorly known group of pinworms. Our present study redescribed the type species *S. tjanschani* using light microscopy and, for the first time, scanning electron microscopy. Some previously unreported morphological features of taxonomic significance (*i.e.* lips, excretory pore, mamelons, caudal papillae and egg) were revealed by our SEM observations. The 18S, ITS, 28S, *cox*1 sequence data of *S. tjanschani* were also firstly amplified and sequenced for future use in the molecular identification. In addition, our phylogenetic results based on 18S, 28S and 18S + 28S + *cox*1 sequence data showed the genus *Syphabulea* to be a member of the subfamily Syphaciinae. The phylogenetic analyses based on 28S and 18S + 28S + *cox*1 sequence data supported that *Syphabulea* represented a separate genus, which formed a sister relationship to *Syphatineria* or *Syphacia*. However, a more rigorous phylogenetic study with broader representation of *Syphabulea* and Oxyuridae is required to confirm the validity of this genus.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study was conducted according to the Hebei Normal University Experiments in Animals Policy and was approved by the Animal Ethics Committee of Hebei Normal University as complying with the Animal Protection Law of the People's Republic of China.

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