Morphology, genetic characterization and molecular phylogeny of pinworm Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae) from the endangered Tibetan antelope Pantholops hodgsonii (Abel) (Artiodactyla: Bovidae)

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Research

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Abstract

Background The Tibetan antelope Pantholops hodgsonii (Abel) (Artiodactyla: Bovidae) is an endangered and endemic species of mammal to the Qinghai-Tibetan Plateau. Parasites and their caused parasitic diseases are considered to be important threats in the conservation of the Tibetan antelope. However, our present knowledge of the composition of the parasites from the Tibetan antelope remains limited. Methods Large numbers of nematode parasites were collected from a dead Tibetan antelope. The morphology of these nematode specimens were observed using light and scanning electron microscopy. The nuclear and mitochondrial DNA sequences [i.e. small ribosomal DNA (18S), large ribosomal DNA (28S), internal transcribed spacer (ITS) and cytochrome c oxidase subunit 1 (cox 1)] were amplified and sequenced for molecular identification. Moreover, phylogenetic analyses were performed using maximum likelihood (ML) inference based on 28S and 18S + 28S + cox 1 sequence data, respectively, in order to clarify the systematic status of these nematodes. Results Integrated morphological and genetic evidence reveals these nematode specimens to be a new species of pinworm Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae). There is no intraspecific nucleotide variation found between different individuals of S. longicaudatum sp. n. in the partial 18S, 28S, ITS and cox 1 sequences. However, the high level of nucleotide divergence was revealed between the new species and its congeners in 28S (8.36%) and ITS (20.3–23.7%) regions, respectively. Molecular phylogenetic results supported that the genus Skrjabinema should belong to the subfamily Oxyurinae (Oxyuroidea: Oxyuridae), instead of the subfamily Syphaciidae or Skrjabinemiinae in the traditional classification, which formed a sister relationship to the genus Oxyuris . Conclusions A new species of pinworm Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae) was described. Skrjabinema longicaudatum sp. n. represents the first species of Oxyurida (pinworm) and the fourth nematode species reported from the Tibetan antelope. Our results contribute to our knowledge on the species diversity of parasites from the Tibetan antelope, and also clarified the systematic position of the genus Skrjabinema .

Background

The Tibetan antelope *Pantholops hodgsonii* (Abel) (Artiodactyla: Bovidae) is an endangered and endemic species of mammal to the Qinghai-Tibetan Plateau. The population of the Tibetan antelope has been severely dropped, because of the loss and fragmentation of its habitat and commercial poaching [1, 2]. The latest estimate of the global population of the Tibetan antelope is 100 000–150 000 mature individuals (https://www.iucnredlist.org/species/15967/50192544). This species is listed as “Near Threatened” in the IUCN Red List of Threatened Species™ and also listed as a Class I (Endangered in China) National Protected Wild Animal Species in China.

Parasites and their caused parasitic diseases are considered to be important threats in wildlife conservation, because they can potentially impair the health of wildlife, decrease fitness, cause population declines and even contribute to local extinction [3–7]. Parasites are also significant pathogens of the Tibetan antelope [1, 2]. To date, there have been 17 species of ectoparasites and endoparasites

reported from the Tibetan antelope, including 5 species of oestrid and hippoboscid flies, 7 species of protozoan, 2 species of tapeworms, 3 species of nematodes [1, 2, 8–12].

In the present study, some nematode specimens of pinworm (Oxyurida) were collected from the digest tract of the Tibetan antelope, which were identified morphologically as a new species of the genus *Skrjabinema* (Oxyurida: Oxyuridae) using light and scanning electron microscopy. The nuclear and mitochondrial DNA sequences [i.e. small ribosomal DNA (18S), large ribosomal DNA (28S), internal transcribed spacer (ITS) and cytochrome c oxidase subunit 1 (*cox*1)] were also amplified and sequenced for molecular identification of this new species. Moreover, in order to clarify the systematic status of the genus *Skrjabinema*, phylogenetic analyses were performed using maximum likelihood (ML) inference based on 28S and 18S + 28S + *cox*1 sequence data, respectively.

Methods

Parasite collection

A Tibetan antelope died naturally in the Hohxil National Nature Reserve, Qinghai Province, China. The digestive tract of this died Tibetan antelope was sent to the Key Laboratory of Adaptation and Evolution of Plateau Biota (AEPB), Northwest Institute of Plateau Biology, Chinese Academy of Sciences for examination of parasites. Large numbers of nematode parasites were isolated from the caecum and colon. Specimens were fixed and stored in in 5% glycerine plus 70% ethyl alcohol (EtOH) until study.

Morphological observation

For light microscopical studies, nematodes were cleared in lactophenol. Drawings were made using a Nikon microscope drawing attachment. For scanning electron microscopy (SEM), the anterior and posterior part of specimens were re-fixed in 4% formaldehyde solution, post-fixed in 1% OsO4, dehydrated via an ethanol series and acetone, and then critical point dried. Samples 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 micrometers (μm) unless otherwise stated.

Molecular procedures

Three female specimens were randomly chose 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 18S-F (5'-CGCGAATRGCTCATTACAACAGC-3') and the reverse primer 18S-R (5'-GGGCGGTATCTGATCGCC-3') [13]. 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') [14]. The ITS-1 region of nuclear rDNA was amplified by PCR using the forward primer SS1 (5'-GTTTCCGTAGGTGAACCTGCG-3') and the reverse primer SS2R (5'-AGTGCTCAATGTGTCTGCAA-3'). The ITS-2 region of nuclear rDNA was amplified by PCR using the forward primer NC13 (5'-

ATCGATGAAGAACGCAGC-3') and the reverse primer NC2 (reverse: 5'-TTAGTTTCTTTTCCTCCGCT-3') [15]. The partial *cox*1 region was amplified by PCR using the forward primer *cox*1F2020 (5'-GAG TAC TAA TCA TAA GGA TAT TGG-3') and the reverse primer *cox*1R2020 (5'-ACA TAA ACY TCA GGA TGA CCA-3'), both designed by ourself. The cycling conditions were as described previously [16]. PCR products were checked on GoldView-stained 1.5% agarose gels and purified with Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing was carried out using a Dye Deoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems, California, USA) and an automated sequencer (ABI-PRISM 377). Sequences were aligned using ClustalW2. 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).

Phylogenetic analyses

Phylogenetic trees were constructed using maximum likelihood (ML) inference with MEGA X based on the partial 28S and 18S + 28S + *cox*1 sequence data, respectively. *Pseudonymus spirotheca* (Oxyurida: Thelastomatoidea: Pseudonymidae) was treated as the outgroup. The ingroup includes the representatives of the Oxyuridae with the 28S and 18S + 28S + *cox*1 sequence data available in the GenBank database. We used a built-in function in the software MEGA X to select a best-fitting substitution model for the present sequences according to the Bayesian information criterion. The K2 (Kimura 2-parameter) + G model for the 28S sequence data, and the HKY (Hasegawa-Kishino-Yano) + G + I model for the 18S + 28S + *cox*1 sequence data were identified as the optimal nucleotide substitution model, respectively. Reliabilities for ML trees were tested using 1000 bootstrap replications, and bootstrap values exceeding 80% were showed in the phylogenetic trees.

Results

Description of Skrjabinema longicaudatum **sp. n. (**Figs. 1–3**)**

Small-sized, whitish nematodes. Body cylindrical, maximum width at slightly posterior to mid-body. Cephalic vesicle indistinct in both sexes (Fig. 1A, C). Lateral alae present in both sexes (Figs. 1C and 2A, C, D). Sexual dimorphism prominent in cephalic structure (Figs. 1B and 3A, B, D). Cuticle with remarkable transverse annulations in the anterior part of body (Fig. 2D, E). Somatic papillae absent. Buccal cavity very small, without cuticular tooth or other ornament. Oesophagus divided into short pharynx, cylindrical corpus, indistinct isthmus and remarkable posterior bulb with valves (Fig. 1A). Nerve ring situated at about 1/4 of total oesophageal length (Fig. 1A). Excretory pore located in a depression, far distance posterior to oesophago-intestinal junction (Figs. 1A and 2A, B). Deirids not observed.

Male (based on 3 mature specimens): Body 1.92–2.85 (2.38) mm long; maximum width 141–151 (146). Oral aperture simple, triradiate, surrounded by three small, more or less triangular lips with small apical median notch (Fig. 3D). Interlabia or interlabial projection absent (Fig. 3D). Oesophagus 400–454 (427) in total length, representing 15.7–20.9 (17.6) of body length; pharynx + corpus + isthmus 255–308 (280) long, size of bulb 146 × 132. Nerve ring 170–200 (185) and excretory pore 760–890 (828) from cephalic

extremity, respectively. Lateral alae narrow, extending from about level of nerve ring to anterior region of cloaca (Fig. 1C). Posterior end of body distinctly curved ventrally (Fig. 1H). Spicule single, pointed at distal end, 74–81 (76.7) long, representing 3.15–3.85 (3.43) % of body length (Fig. 1E, G, H). Gubernaculum small, well sclerotized, about 48 long (Fig. 1G, H). Caudal papillae large, three pairs in total, arranged as follows: one pair precloacal, one pair paracloacal and one pair postcloacal (Fig. 1G). Preventral, median finger-like protuberance present (Fig. 1H). Tail 33 long, ending in a short finger-like tip (Fig. 1G, H). Phasmids present slightly posterior to cloaca.

Female (based on 10 mature specimens)

Body 9.92–12.1 (11.1) mm long; maximum width 396–574 (475). Cephalic extremity with three anchor-shaped lips, each lip with two triangular lateral lobes not attached to cephalic rim (Figs. 1B and 3A–C). Interlabia digitiform, between lateral lobes of lips (Figs. 1B and 3A, B). Four large cephalic papillae and two small amphidial pores present (Figs. 1B and 3A, B). Oesophagus 832–881 (853) in total length, representing 6.98–8.78 (7.75) % of body length; pharynx + corpus + isthmus 634–703 (671) long, size of bulb 168–188 (181) × 139–188 (161). Nerve ring 198–248 (225) and excretory pore 1.24–1.92 (1.77) mm from cephalic extremity, respectively. Lateral alae extending from long distance posterior to base of cephalic extremity and ending at about middle of tail (Fig. 2A, C, D, F, H). Vulva transverse slit, very small, with rudimental vulval lips under SEM observation, 2.97–3.37 (3.16) mm from cephalic extremity, representing 25.1–31.1% (28.7%) of body length (Figs. 1D and 3E). Egg asymmetrical, flattened at one side, embryonated or nonembryonated, thick-shelled, with smooth surface, 40–59 (51) × 20–40 (30) (n = 20) (Figs. 1I; 2I and 3F). Cloaca with small precloacal lip (Fig. 2G). Tail slender, very long, 2.63–3.13 (2.90) mm, with pointed tip, representing 23.0–27.7 (26.3) % of body length (Figs. 1F and 3F). Phasmids not observed.

Taxonomic summary

Type host: Tibetan antelope *Pantholops hodgsonii* (Abel) (Artiodactyla: Bovidae: Caprinae).

Type locality

Hoh Xil Nature Reserve near Wudaoliang (35°26'N, 93°17'E), Qinghai Province, China.

Site of infection

Caecum and colon.

Prevalence and intensity of infection

A single Tibetan antelope examined with intensity 124 worms.

Type specimens: Holotype: male (HBNU–N-2020M001L), allotype: female (HBNU–N-2020M002L), paratypes: 9 females (HBNU–N-2020M003L) deposited in College of Life Sciences, Hebei Normal University, Hebei Province, China. paratypes: 2 males and 100 females (KLAEPB No.019001) deposited in

Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Qinghai Province, China.

Etymology

The specific epithet is derived from a combination of the Latin words *longus*- (long) and *caudatum*- (cauda), and refers to the unusually long tail in the female of the new species.

Genetic characterization

Partial 18S region

Three 18S sequences of *S. longicaudatum* sp. n. obtained herein are all 678 bp in length and represent one genotype. There are two species of *Skrjabinema* with 18S sequence registered in GenBank, namely *S. kamosika* (AB699690) and *Skrjabinema* sp. (EF180060). Pairwise comparison of 18S sequences between *S. longicaudatum* sp. n. and the two species of *Skrjabinema* displayed 0.29–1.18% nucleotide divergence. The 18S sequences of *S. longicaudatum* sp. n. are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov).

Partial ITS region

Three ITS sequences of *S. longicaudatum* sp. n. obtained herein are all 1079 bp in length and represent one genotype. There are two species of *Skrjabinema* with ITS sequence registered in GenBank, namely *S. kamosika* (AB699691) and *Skrjabinema* sp. (AB367796). Pairwise comparison of ITS sequences between *S. longicaudatum* sp. n. and the other two species of *Skrjabinema* displayed 20.3–23.7% nucleotide divergence. The ITS sequences of *S. longicaudatum* sp. n. are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov).

Partial 28S region

Three 28S sequences of *S. longicaudatum* sp. n. obtained herein are all 819 bp in length and represent one genotype. There is only one species of *Skrjabinema*, namely *S. ovis* (KY990019) with 28S sequence registered in GenBank. Pairwise comparison of ITS sequences between *S. longicaudatum* sp. n. and *S. ovis* displayed 8.36% nucleotide divergence. The 28S sequences of *S. longicaudatum* sp. n. are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov).

Partial cox1 region

Three *cox*1 sequences of *S. longicaudatum* sp. n. obtained herein is 405 bp in length. There is no species of *Skrjabinema* with *cox*1 sequence registered in GenBank. The *cox*1 sequence of *S. longicaudatum* sp. n. are deposited in the GenBank database (http://www.ncbi.nlm. nih.gov).

Phylogenetic analyses (Figs. 4, 5)

Phylogenetic results constructed based on the partial 28S sequence data showed the representatives of the family Oxyuridae were divided into three monophyletic clades. The clade I includes members of the genera *Syphacia*, *Passalurus*, *Syphatineria*, *Syphabulea* and *Rauschtineria*, representing the subfamily Syphaciinae. The clade II contains species of the genera *Oxyuris* and *Skrjabinema*, representing the subfamily Oxyurinae. The clade III includes species of the genus *Trypanoxyuris*, representing the subfamily Enterobiinae (Fig. 4). *Skrjabinema longicaudatum* sp. n. displayed a sister relationship to *S. ovis*.

Phylogenetic tree constructed based on the 18S + 28S + *cox*1 sequence data had similar topology to the phylogenetic results using the partial 28S sequence data, representatives of the Oxyuridae also divided into three monophyletic clades (Fig. 5). Species of *Trypanoxyuris* and *Enterobius* formed the clade I, representing the subfamily Enterobiinae. The members of *Syphabulea* and *Syphacia* grouped together (clade II), belonging the subfamily Enterobiinae. The clade III includes representatives of *Oxyuris* and *Skrjabinema*, representing the subfamily Oxyurinae. *Skrjabinema longicaudatum* sp. n. clustered together with *S. kamosika*.

Discussion

The genus *Skrjabinema* Werestschagin, 1926 (Oxyuridea: Oxyuridea) currently includes 10 nominal species reported from various ruminants worldwide, namely *S. alata* Mönnig, 1932, *S. africana* Mönnig, 1932, *S. caprae* Schad, 1959, *S. chubuki* Gagarin & Sapozhnikov, 1968, *S. kamosika* Hasegawa, Sato, Suzuki & Kaneshiro, 2012, *S. ovis* (Skrjabin, 1915), *S. parva* Dikmans, 1942, *S. rupicaprae* Böhm & Gebauer, 1930, *S. skrjabini* Gagarin & Sapozhnikov, 1968 and *S. tarandi* Skrjabin & Mizkewitsch, 1930 [17–23]. However, some of these species have not been sufficiently well described, especially the details of cephalic structure.

Skrjabinema ovis is the type species of this genus, which has been widely reported from goat and sheep in Asia, Europe, America and Australia [21]. This species was recorded from *Capra aegagrus hircus* (Linnaeus), *Ovis aries* Linnaeus and *Procapra przewalskii* Büchner in China [24]. The new species is different from *S. ovis* by the absence of cephalic vesicle in female (*vs* the presence of remarkable cephalic vesicle in the female in *S. ovis*), slightly shorter spicule (0.074–0.081 mm long in the new species *vs* 0.09–0.12 mm long in *S. ovis*) and longer gubernaculum (0.048 mm long in *S. longicaudatum* sp. n. *vs* 0.019–0.025 mm long in the latter). *Skrjabinema longicaudatum* sp. n. has the spicule without dilated proximal end, longer gubernaculum (0.019–0.025 mm long) and the caudal alae ending at about level of half of tail length in female, which is different from that of *S. parva* (the proximal end of spicule extended into goblet-shaped, gubernaculum 0.01–0.016 mm long and caudal alae ending at level close to tail tip in female). The absence of sub-interlabial projections in the cephalic end in the male, the new species can be easily distinguished from *S. kamosika*, *S. tarandi* and *S. caprae* (the presence of sub-interlabial projections in the cephalic end in the male). Moreover, the caudal alae of female in *S. tarandi* and *S. caprae* are very long (ending near to the tail tip *vs* caudal alae ending at about half of tail length in the female of *S. longicaudatum*). *Skrjabinema longicaudatum* sp. n. differs from *S. rupicaprae* by having

larger body size of male (1.92–2.85 mm long *vs* 1.54–1.79 mm long in *S. rupicaprae*), relatively shorter spicule (spicule representing 3.15–3.85% of body length in the former *vs* representing 4.47–5.20% of body length in the latter) and slightly longer gubernaculum (0.048 mm long in the new species *vs* 0.025 mm long in *S. rupicaprae*). The new species can be distinguished from *S. chubuki* and *S. skrjabini* by having different morphology of lips in female (triangular lateral lobes of lip small, not attached to the cephalic rim in *S. longicaudatum* sp. n. *vs* triangular lateral lobes of lip very large, attached to the cephalic rim in the latter two species).

Mönnig (1932) [17] described *S. alata* and *S. africana* based on only female specimens in South Africa. Both of species are differentiated from the new species by distinctly smaller body size of female (4.61–5.80 mm long in *S. alata* and *S. africana vs* 9.92–12.1 mm long in *S. longicaudatum* sp. n.). In addition, *S. africana* is different from *S. longicaudatum* sp. n. by having the caudal alae of female ending very near to the tail tip (*vs* caudal alae ending at about level of half of tail length in *S. longicaudatum*). *Skrjabinema alata* differs from the new species by having relatively longer oesophagus (oesophagus representing 12.8–14.3% of body length in *S. alata vs* representing 6.98–8.78% of body length in *S. longicaudatum*). Moreover, *S. longicaudatum* sp. n. can be easily distinguished from all its congeners by the unusually long tail in female (tail 2.63–3.13 mm, representing 23.0–27.7% of body length *vs* not over 1.60 mm, representing 6.68–20.6% of body length in the other species of *Skrjabinema*).

It is difficult to identify and discriminate the pinworms only using traditional methods due to their extraordinary morphological similarity and sometimes the male worms unavailable [25]. The molecular approaches have been employed for identification and discrimination of pinworms in some previous studies [23, 25–31]. However, to date, the genetic data of pinworms available in the GenBank database remains limited, which has hindered the further studies of DNA-based taxonomy, population genetics and phylogenetics of this group of nematode parasites.

In the present study, we amplified and sequenced the partial 18S, 28S, ITS and *cox*1 sequences of our specimens for future use in the molecular identification of this new species. There is no intraspecific nucleotide variation found between different individuals of *S. longicaudatum* sp. n. in the partial 18S, 28S, ITS and *cox*1 sequences. However, the high level of nucleotide divergence was revealed between the new species and its congeners in 28S (8.36%) and ITS (20.3–23.7%) regions, respectively. The more slowly evolving 18S gene may be not suitable for species identification of *Skrjabinema*, because very low level of interspecific nucleotide variation detected between different species of *Skrjabinema* (0.29–1.18%). However, the 18S gene could be chose to provide resolution at higher taxonomic ranks. It is the first time to report the *cox*1 sequence of *Skrjabinema* species.

The systematic position of *Skrjabinema* is still under debate. Skrjabin et al. (1960) [21] placed this genus into the subfamily Syphaciinae Railliet, 1916 in Syphaciidae Skrjabin and Schikhobalova, 1951. Erkulov & Moldopiyazova (1975) [32] proposed a new subfamily Skrjabinemiinae for the genera *Skrjabinema* and *Citellina*. Hugot (1981) [33] degraded the family Syphaciidae to a subfamily in Oxyuridae and refused the validity of Skrjabinemiinae. The present phylogenetic analyses using the partial 28S and 18S + 28S +

*cox*1 sequence data, respectively, both supported the genus *Skrjabinema* to be a member of the subfamily Oxyurinae and has a sister relationship with the genus *Oxyuris*, which agreed well with the recent molecular phylogenetic results [25].

Our present knowledge of the composition of the nematode parasites in the Tibetan antelope remains limited. As far as we know, only three species of nematodes have been recorded from the Tibetan antelope, including *Nematodirus* sp., *Marshallagia mongolica* Schumakoviech, 1938 and *M. marshalli* (Ransom,1907) (Rhabditida: Strongyloidea) [9–11]. *Skrjabinema longicaudatum* sp. n. represents the first species of Oxyurida (pinworm) and the fourth nematode species reported from the Tibetan antelope.

Conclusions

A new species of pinworm *Skrjabinema longicaudatum* sp. n. (Oxyurida: Oxyuridae) was described using light and scanning electron microscopy, based on specimens collected from the endangered Tibetan antelope. *Skrjabinema longicaudatum* sp. n. represents the first species of Oxyurida (pinworm) and the fourth nematode species reported from the Tibetan antelope. The nuclear and mitochondrial DNA sequences (i.e. 18S, 28S, ITS and *cox*1) were amplified and sequenced for molecular identification of this new species. Phylogenetic analyses using maximum likelihood (ML) inference based on 28S and 18S + 28S + *cox*1 sequence data, respectively, both supported that the genus *Skrjabinema* should belong to the subfamily Oxyurinae (Oxyuroidea: Oxyuridae), instead of the subfamily Syphaciidae or Skrjabinemiinae in the traditional classification, which formed a sister relationship to the genus *Oxyuris*. Our results contributed to the knowledge on the species diversity of parasites from the Tibetan antelope, provided useful genetic data for molecular identification and phylogeny of Oxyuridae, and also clarified clarified the systematic position of the genus *Skrjabinema*.

Declarations

Availability of data and materials

The nuclear and mitochondrial DNA sequences of *Skrjabinema longicaudatum* sp. n. obtained in this study were deposited in the GenBank database. Type specimens of the new species were deposited in College of Life Sciences, Hebei Normal University, Hebei Province, and Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Qinghai Province, China.

Ethics approval and consent to participate

This study was conducted under the protocol of the Ethical Commission of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences and Hebei Normal University. All applicable institutional, national and international guidelines for the protection and use of animals were followed.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

All authors contributed to the study design. Yi-Fan Cao and Dang-Wei Zhou, Shi-Long Chen carried out sample collection. Liang Li, Hui-Xia Chen, Dang-Wei Zhou, Yi-Fan Cao and Yang Li identified the nematode specimens, analyzed morphological and genetic data. Liang Li and Dang-Wei Zhou conducted the phylogenetic analyses and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

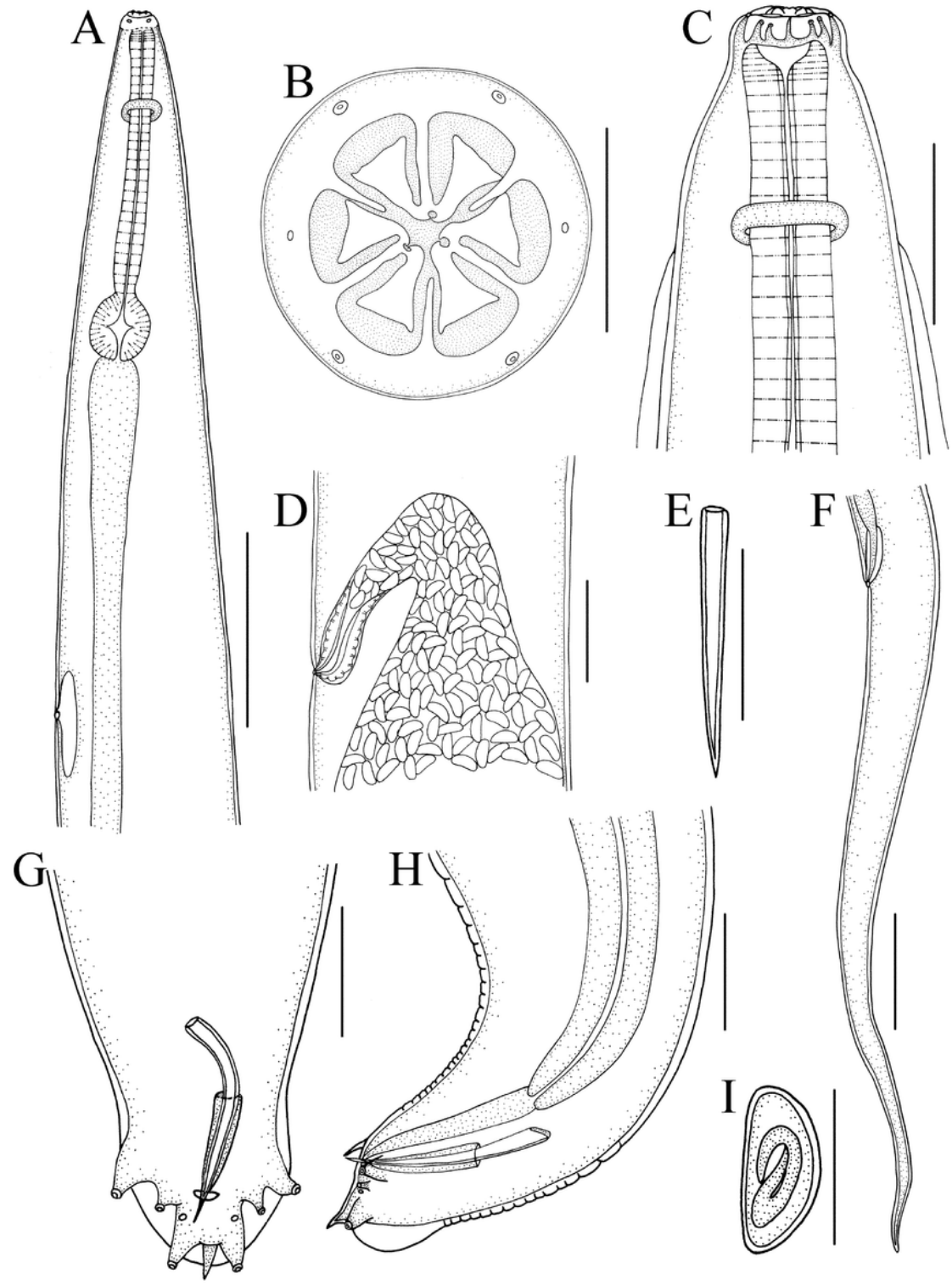

Figure 1

Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae) from from the endangered Tibetan antelope Pantholops hodgsonii (Abel) (Artiodactyla: Bovidae) in China. A, anterior part of female, lateral view; B, cephalic end of female, apical view; C, anterior part of male, dorsal view; D, region of vulva, lateral view; E, spicule; F, posterior end of female, lateral view; G, posterior end of male, ventral view; H, posterior end of male, lateral view; I, egg. Scale bars: A, F = 500 μm; B, E, G, H, I = 50 μm; C = 100 μm; D = 200 μm.

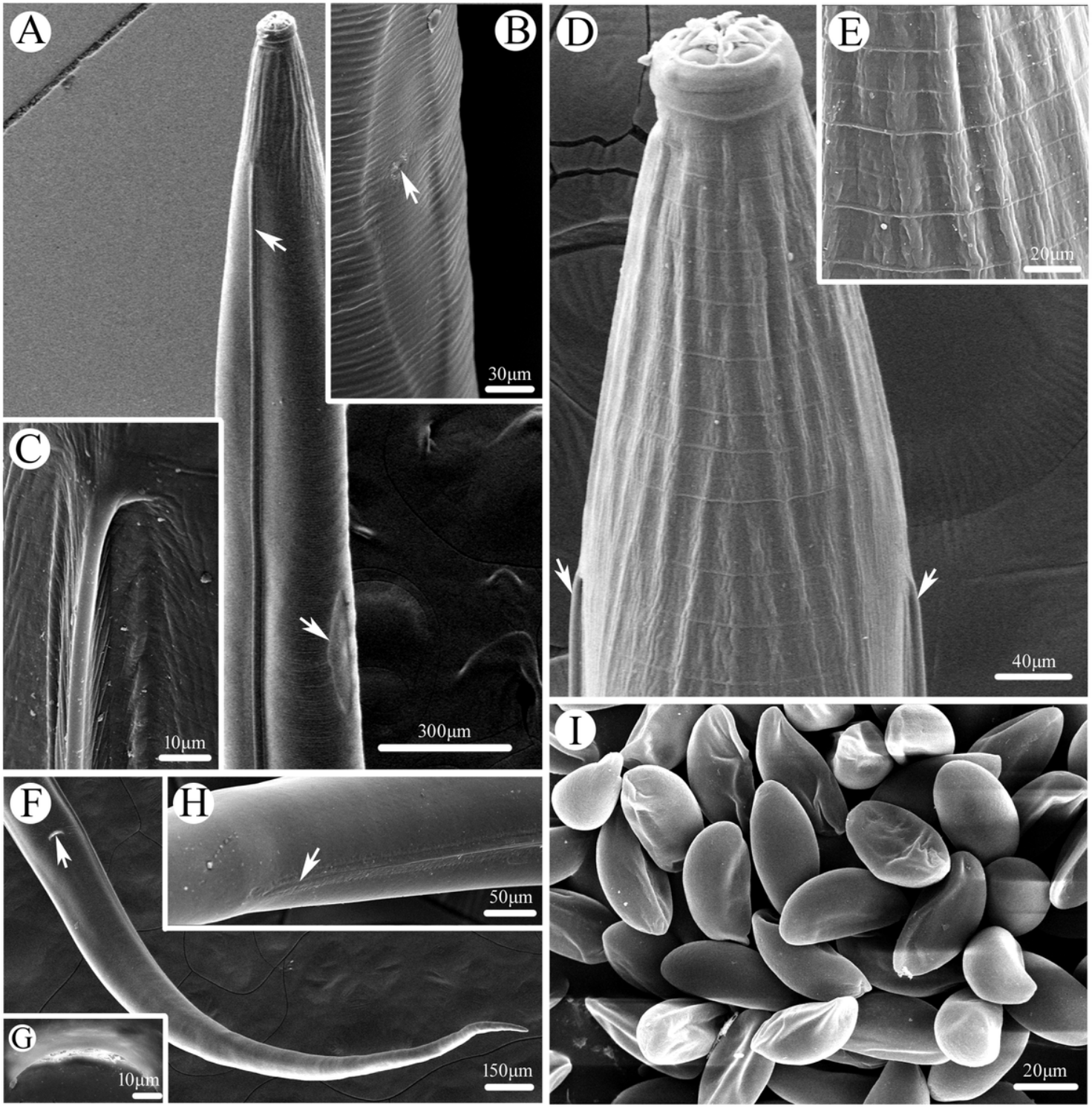

Figure 2

Scanning electron micrographs of Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae) from from the endangered Tibetan antelope Pantholops hodgsonii (Abel) (Artiodactyla: Bovidae) in China. A, anterior part of female (lateral ala and depressed region around excretory pore arrowed), lateral view; B, magnified image of depressed region and excretory pore (excretory pore arrowed); C, magnified image of the original position of lateral ala; D, anterior part of female (lateral alae arrowed), dorsal view; E, magnified image of transverse annulations in the anterior part of body; F, posterior end of female (cloaca

arrowed), ventral view; G, magnified image of cloaca; H, magnified image of the ending position of caudal ala; I, magnified image of eggs in uterus in different views.

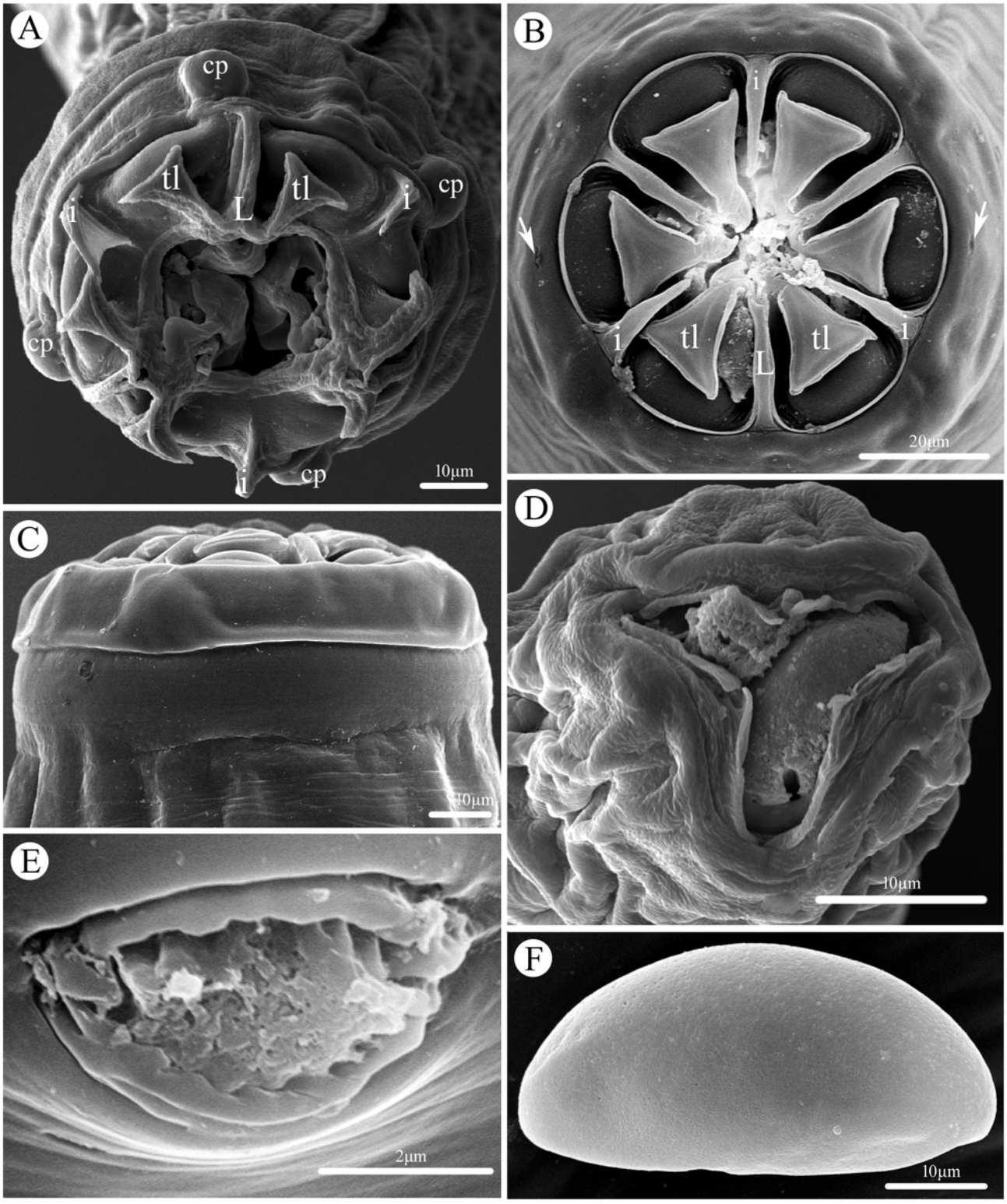

Figure 3

Scanning electron micrographs of Skrjabinema longicaudatum sp. n. (Oxyurida: Oxyuridae) from from the endangered Tibetan antelope Pantholops hodgsonii (Abel) (Artiodactyla: Bovidae) in China. A, cephalic end of female, sub-apical view; B, cephalic end of female (amphidial pores arrowed), apical view;

C, cephalic end of female, lateral view; D, cephalic end of male, apical view; E, magnified image of vulva; F, magnified image of egg, lateral view. Abbreviation: L, lip; i, interlabia; tl, triangular lateral lobes of lip; cp, large cephalic papillae.

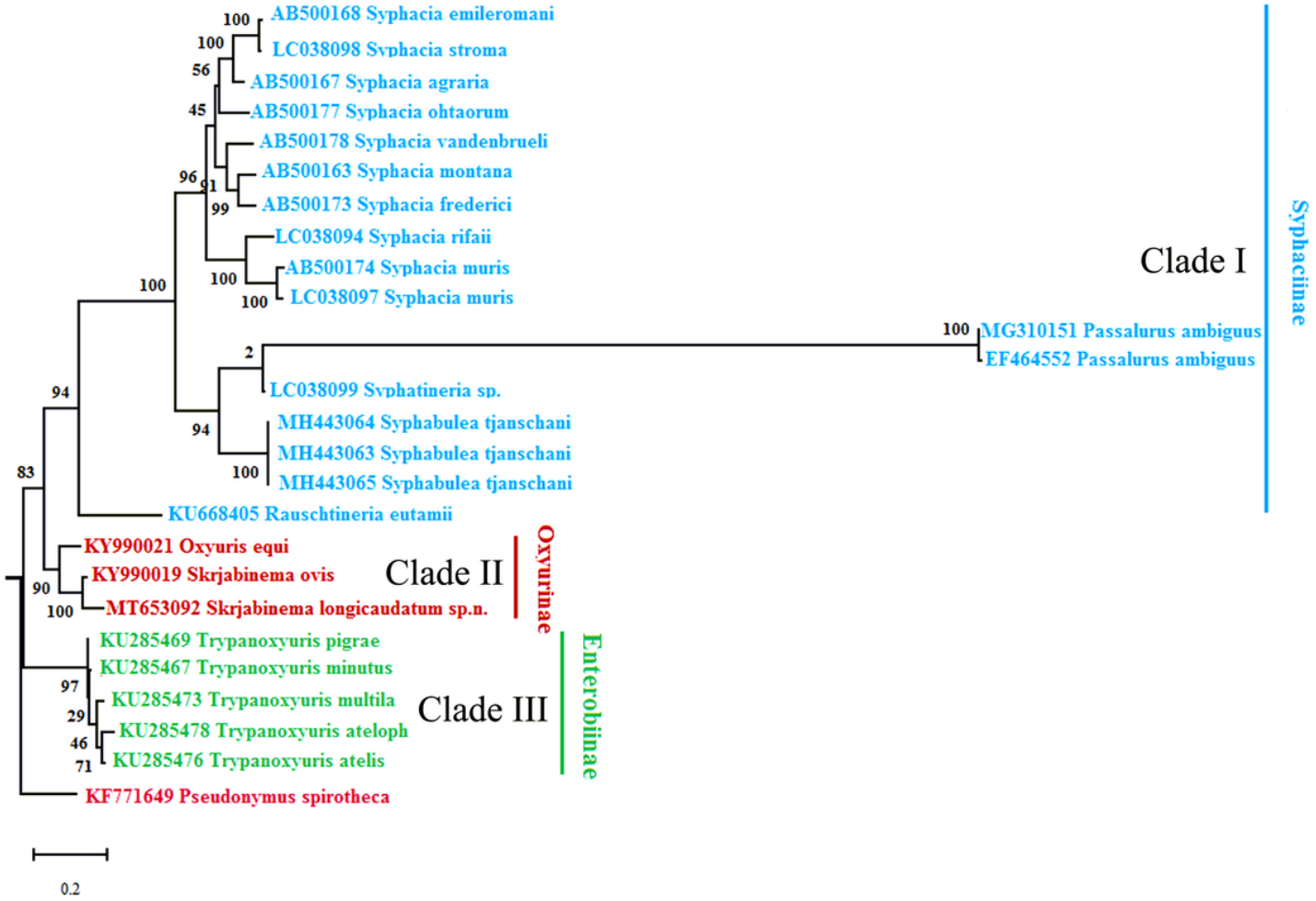

Figure 4

Maximum likelihood (ML) tree constructed from the partial 28S gene data showing the phylogenetic relationships of representatives of the family Oxyuridae. Pseudonymus spirotheca (Oxyurida: Pseudonymidae) was chosen as the outgroup.

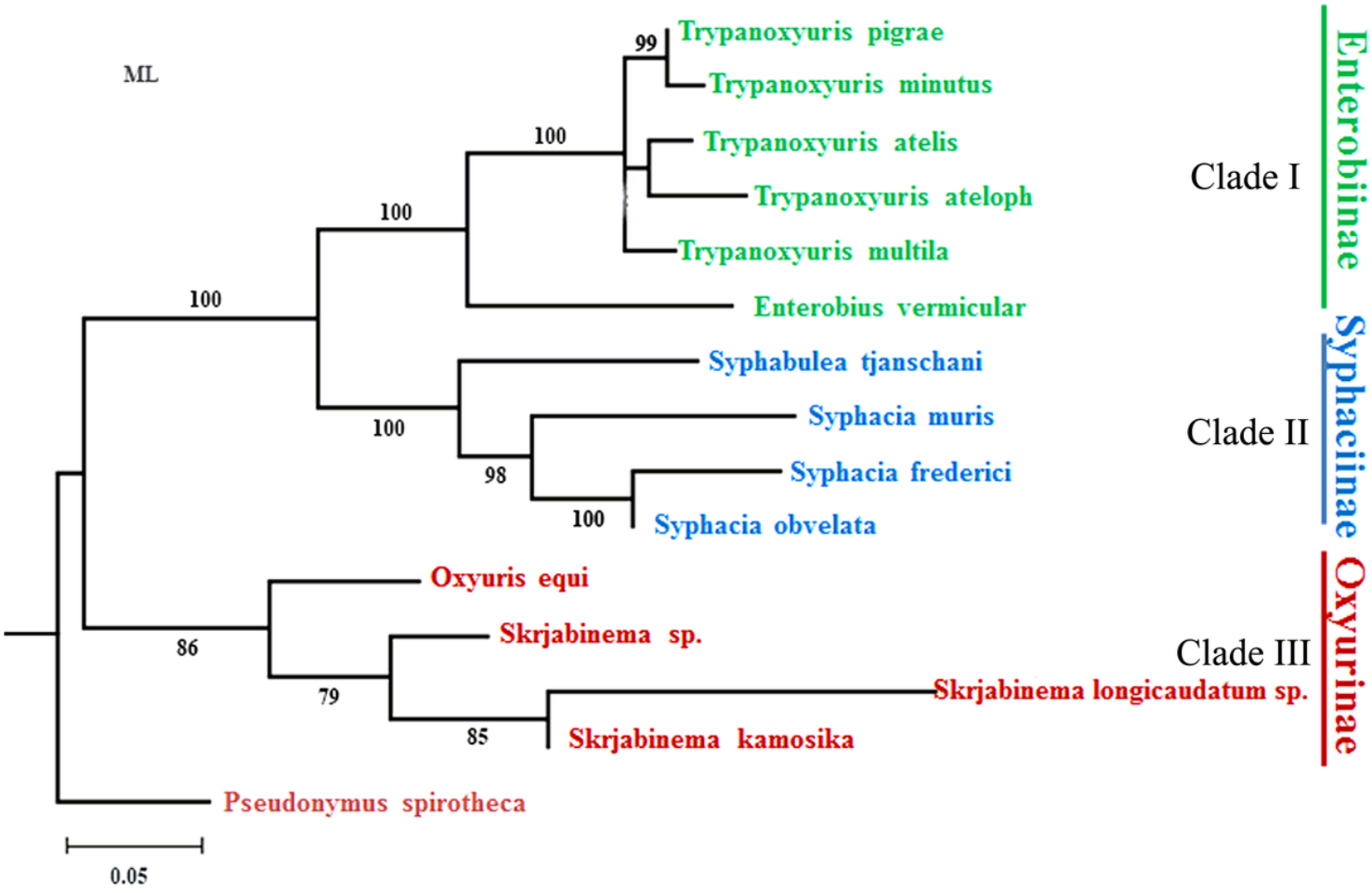

Figure 5

Maximum likelihood (ML) tree constructed from the partial 18+28S+cox1 gene data showing the phylogenetic relationships of representatives of the family Oxyuridae. Pseudonymus spirotheca (Oxyurida: Pseudonymidae) was chosen as the outgroup.