Research Article

Morphometrical and Molecular Characterization of *Oesophagostomum columbianum* (Chabertiidae: Oesophagostominae) and *Haemonchus contortus* (Trichostrongylidae: Haemonchinae) Isolated from Goat (*Capra hircus*) in Sylhet, Bangladesh

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This study was aimed at describing two (2) intestinal nematodes from naturally infected native breed of goats (*Capra hircus*) in Bangladesh, identified as *Oesophagostomum columbianum* (Curtice, 1890) Stossich 1899 and *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898. The identification was made based on morphometric features and was confirmed by amplifying internal transcribed spacer (ITS) and cytochrome *c* oxidase (*cox1*) gene. Well-developed lateral alae, distinct cervical papillae anteriorly to esophageal expansion, and male spicule length (0.73-0.79 mm, *n* = 2) were characteristically observed in *O. columbianum*. At the same time, male spicule length (0.40-0.46 mm, *n* = 2) and position of female vulvar flap (4.30-4.54 mm from posterior end, *n* = 3) were observed in *H. contortus*. DNA sequence homology of the ITS and *cox1* gene of both specimens revealed the same results, showing similarity to the GenBank sequences of *O. columbianum* (GenBank No. KC715827; JX188470) and *H. contortus* (GenBank No. KJ724377; HQ389229). Phylogenetic analysis computed by maximum livelihood (ML) from the ITS nucleotide sequences revealed that the *O. columbianum* and *H. contortus* isolates identified in this study were clustered in the same clade with isolates from China and Iran, respectively. This study, for the first time, illustrates the characteristics of *O. columbianum* and *H. contortus* in Bangladesh, combining both morphological and molecular data. The universal primer-based polymerase chain reaction (PCR) protocol could be an economical and efficient option for researchers from poor resource settings for precise identification of nematodes. The information generated in this study may contribute to formulating effective control strategies against these nematodes.

1. Introduction

Infection with gastrointestinal nematodes (GINs) is considered one of the significant problems causing considerable economic losses in ruminant farming. Over 150 species of internal and external parasites have been reported to infect goats and sheep worldwide [1, 2]. *Oesophagostomum* or nodular worm is a parasitic nematode of the large intestine.
belonging to the family Chabertiidae (Popova, 1952) and is one of the most common and widely distributed nematodes of livestock and wild ruminants [3]. Oesophagostomum columbianum (Curtice, 1890), O. asperum (Koehler, 1930), O. venulosum (Rudolphi, 1809), and O. kansuensis (Hsiung et Kung, 1955) are the dominant species; in most cases, animals get infected through contaminated foods, water, or soil [2]. Oesophagostomum columbianum has been cited as the primary causal agent of nodular enteritis, responsible for decrease productivity in sheep and goats around the world, for instance, in Kashmir Valley, India, [2] and Ethiopia [4]. Mature worms of these species inhabit the mucosa of the host’s digestive tract and suck blood that leads to pernicious anaemia and significant weight loss [1]. Penetration of the mucosa of the intestine by larvae can cause severe diarrhoea with black-green faeces containing mucus and blood. On the other hand, Haemonchus or barber’s pole worm is a parasitic nematode belonging to the family Trichostrongylidae (Leiper, 1908; Leiper 1912) and is a blood-sucking nematode that inhabits in the abomasum of small ruminants worldwide. Haemonchus contortus (Rudolphi, 1803) Cobb, 1898; H. placei (Place, 1893) Ransom, 1911; and H. similis (Travassos, 1914) are reported as the most pathogenic nematodes of sheep, cattle, and goats worldwide, causing significant production losses [5]. These nematodes are found mostly in tropical regions and also reported to occur increasingly in subtropical areas [6, 7]. These nematodes live in the digestive tract of sheep and goat; adult worms suck blood from the intestinal mucosa and cause anaemia, oedema, diarrhoea, and even death [8]. Economic losses are encountered in terms of production and body weight loss, direct medication costs, and mortality-related loss.

Small ruminant, especially goat, has become an important farming system in Bangladesh for a long time. However, to date, very few studies on ruminant parasitism have been conducted for the identification of GINs in this area. While several studies reported the high prevalence of O. columbianum and H. contortus in Bangladesh from sheep and goat based on coprological approaches [6, 9, 10], explanation of key identification criteria and genetic analysis for those nematodes were lacking. The conventional coprological methods include morphology-based identification of eggs or larvae that are cumbersome and difficult to distinguish from other species with similar morphological structures or minor morphological variations. A multidisciplinary approach, including both morphological and DNA-based molecular techniques, should provide a more reliable means of identification [11]. Ribosomal DNA (rDNA) genes and their related spacer regions and mitochondrial DNA (mtDNA) provide useful information for the development of diagnostic probes or species identification makers in this regard. While molecular methods are the gold standard for the precise identification of nematode species, identification based on the morphometry is cost-effective than that based on molecular techniques. Designing species-specific primer for each species and current state-of-the-art molecular diagnostic tools is costly and complex, particularly in limited resource settings. Although we emphasize on DNA-based diagnostic, several issues are still relevant, and understanding financial barrier and solutions like the broadest access must be considered. Therefore, the current study was aimed at characterizing two common nematode species, O. columbianum and H. contortus, isolated from the intestine of the indigenous goats based on morphometric and economic PCR protocol. In our study, for the first time, we characterized adult O. columbianum and H. contortus in Sylhet, Bangladesh, combined with morphometry and sequence analysis. Findings of the study may contribute to a better understanding of the morphological traits and identification of these nematodes, especially in the Indian subcontinent where GINs have a worrying role in small ruminant farming.

2. Materials and Methods

2.1. Specimens and Morphological Analysis. Naturally infected adult nematodes were obtained from the abomasum of the large intestine of Black Bengal goats (Capra hircus) from local abattoirs in Sylhet, Bangladesh (geographically located at 24.89°N 91.88°E), in January 2020. The Black Bengal goat is an indigenous goat breed and found all over Bangladesh. Collected nematodes were washed in 0.9% saline, fixed into 70% ethanol and 10% neutral-buffered formalin, and brought to the Department of Parasitology, Chungbuk National University, Republic of Korea, for further studies. Parasite materials (PRB001197 and PRB001198) used in this study were stored in the International Parasite Resource Bank (iPRB), Republic of Korea. For morphological observation, the worms were placed in glycerine alcohol solution (90 ml 70% ethanol and 10 ml glacial glycerine) for 24 hr until they become transparent, then mounted with glycerin jelly (10 g gelatin, 500 ml glycerine, 10 g phenol, and 60 ml distilled water). Observations and measurements were conducted under a light microscope (Olympus BX-53, Tokyo, Japan) with an ocular micrometer. The following measurements (in millimeter) were taken: body length, body width, esophageal length, esophageal width, length of spicule, length of gubernaculum, distance from the vulva to posterior end, and length of tail. Buccal cavity and vulvar flap morphology were also used to make species identification.

2.2. DNA Extraction. DNA extraction was done from ethanol-preserved samples following a previous protocol [12]. Collected adult worms were washed 3 times in PBS before DNA extraction. Total genome DNA from individual worm was extracted by using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany; Cat Nos. 69504 and 69506). Except the elution step, where distilled water was used instead of elution buffer and was repeated twice, the remaining of the DNA extraction was performed according to the manufacturer’s protocol. The concentration and purity of DNA were measured (NanoDrop Spectrophotometer, Thermo Fisher Scientific Solutions Co., Ltd., Korea), and stored at −20°C until required for PCR.

2.3. PCR Amplification and DNA Sequencing. The rDNA region spanning ITS region (ITS1, 5.8S, and ITS2) and a region within mitochondrial cytochrome c oxidase subunit I (cox1) were amplified and sequenced by cycle sequencing.
The target regions were amplified by the primer set (Table 1) previously described by Hu et al. [13] and Jacquiet et al. [14]. The PCR reactions were performed in a Kyratec PCR Thermal Cycler (Queensland, Australia). The volume of mixture was similar for both primer pairs and was carried out in a final reaction mixture containing 25 μl, including 1 μl of each primer (10 pmol), 1 μl of generic DNA, 6 μl of 5X PCR Master Mix (ELPIS biotech, South Korea), and 16 μl of distilled water. Negative control was applied in each run. PCR condition for coxl was 94°C for 3 min; (94°C 45 sec ; 48°C 1 min; 72°C for 1 min) Þ 35; and 72°C 10 min. PCR condition for ITS was 95°C for 10 min; (95°C 45 sec; 55°C 50 sec; 72°C for 50 sec) Þ 30; and 72°C 5 min. When amplifications did not work adequately, the annealing temperature was changed and adjusted. The PCR products were run on a 1.5% agarose gel and visualized using a UV transilluminator. DNA sequencing was performed by a company (Cosmogenetech, Daejeon, Korea). Cycle sequencing was performed using a BigDye terminator kit (version 3.1, Applied Biosystems, Foster City, California, USA). The reaction products were directly sequenced using a DNA sequencer (ABI3730XL, Applied Biosystems).

2.4. Sequencing and Phylogenetic Analysis. The obtained sequences were assembled with Geneious program 9.0 (Biomer, Auckland, New Zealand) [15]. Sequences were aligned using ClustalW multiple alignment implanted MEGA7 [16, 17]. Both alignments were trimmed to the length of the shortest sequence. Sequencing analysis was carried out by BLAST algorithms and databases from the National Center for Biotechnology Information database. Phylogenetic trees were constructed based on ITS region of the newly obtained sequences, and selected reference sequences available in GenBank, using maximum likelihood (ML) algorithms with bootstrap values calculated using 1000 replicates. The multiple alignments were performed with the program Muscle [18], and substitution model (T92+G) was chosen according to the Modeltest using MEGA7. To describe the best substitution patterns, the lowest BIC (Bayesian Information Criterion) scores were considered.

3. Results and Discussion

In this study, adult worm specimens were precisely characterized by morphological features and DNA analysis, based on both nuclear ITS and coxl gene. We choose these markers due to their high interspecific levels of variability and the availability of universal primers.

3.1. Oesophagostomum Columbianum (Curtice, 1890) Stossich 1889. Description of worms was based on 3 female and 2 fully mature worms as whole-mounted specimens (Figure 1; Table 2). The descriptions are as follows: medium-sized worms with sexual dimorphism, males being smaller and thinner than females; body straight, tapering at both ends with a transversally striated cuticle; anterior end curved dorsally into a hook; well-developed lateral alae extending nearly the entire length of the body, and surrounded by a well-demarcated cylindrical and sub-globular oral collar; mouth has ring-like projection leading to a small buccal capsule, surrounded by leaf-like structures which constitute the leaf-crown; buccal capsule surrounded by external corona radiata and the internal corona radiata (Figure 1(a)); internal corona radiata present with numerous elements; cephalic vesicle located just before the middle of the esophagus and demarcated by the cervical groove (Figure 1(b)); well-developed esophagus, club-shaped, shrinks immediately behind the esophageal duct, and dilates gradually to the posterior end.

The following are descriptions for the male: total body length 12.3–13.1 [12.7]; maximum width 0.29–0.37 [0.33]; length of esophagus 0.76–0.78 [0.77]; maximum width of esophagus 0.09–0.11 [0.10]; length of buccal capsule 0.05, depth of mouth capsule 0.09. Copulatory bursa symmetrical, bell-shaped, with ventrolateral lobes; dorsal ray broad at origin, 0.15–0.19 [0.17] in length, arising from a common dorsal trunk and divided into two terminal branches, each of which gives a short lateral stem; ventral rays end quite near to the border of the lateral lobe; spicules equal, slender, alate with blunt tips, 0.73–0.79 [0.75] long; gubernaculum elongate, margins irregular, length 0.09–0.13 [0.11] (Figure 1(d)).

The following are descriptions for the female: total body length 15.6–16.8 [16.2]; maximum width 0.30–0.38 [0.34]; length of esophagus 0.77–0.81 [0.79], width of the esophagus 0.1–0.12 [0.11]; length of mouth capsule 0.05, depth of mouth capsule 0.10; tail straight and conical; distance of anus from posterior end of body 0.31–0.33 [0.32]; vulva elliptical, slightly pronounced, anterior to the anus and opens 0.71–0.83 [0.77] from posterior end (Figure 1(c)).

Our specimens were identified as O. columbianum as well as differentiated from the other Oesophagostomum species considering host and characteristics of buccal capsule with other cephalic and cervical structures, spicule, and gubernacular length and position of the vulva and anus. The morphology and morphometry of the present Oesophagostomum specimens were identical to those of O. columbianum documented previously [19–23]. According to Railliet and Henry [20], one or more of the following characteristics such as the number of elements in corona radiata, position of cervical papillae, and structure of male bursa seem to be the most appropriate generic features of Oesophagostomum. In our specimens, cervical papillae were observed anterior to esophageal expansion which is consistent with the description. This species was discussed in some detail by Goodey [22] where he stated that the structure of anterior parts and spicule length of this genus are so crucial to the systematics and are the key to species differentiation. The male spicule length of O. columbianum is varying from 0.75 to 0.80, and cephalic vesicle is not distinct. Other Oesophagostomum species of small ruminants like O. asperum has well-defined cephalic vesicles and somewhat longer spicules. Furthermore, Goodey [22] and Zhao et al. [24] reported well-developed lateral alae in O. columbianum, while other Oesophagostomum of small ruminants has no lateral alae. Therefore, all of these criteria evidently indicate our specimens as O. columbianum.
Table 1: Primer sets used, with nucleotide sequence, target region, species, and size of amplicon.

| Primers          | Target region | Species       | Amplicon size (bp) |
|------------------|---------------|---------------|--------------------|
| NC5: 5′-gtaggtgaacctgcggaaggatcatt-3′ | ITS (rDNA)    | *O. columbianum* | 782                |
| NC2: 5′-taggtctttctcgcctcgt-3′       |               | *H. contortus*  | 789                |
| JB3: 5′-cttttgggctatctgagttat-3′    | *cox1* (mtDNA)| *O. columbianum*| 397                |
| JB4.5: 5′-taaagaaagaacataatgaaaatg-3′ |               | *H. contortus*  | 383                |

Figure 1: *Oesophagostomum columbianum*: (a, b) anterior end showing corona radiata and esophagus; (c) posterior end of female showing vulva and anus; (d) bursa of male showing dorsal rays and spicules.

Table 2: Comparative measurement (in mm) of present *Oesophagostomum columbianum* isolated and those previously recorded.

| Body parts                     | Present specimen | Goodey (1924) | Ransom (1911) | Soota (1981) |
|--------------------------------|------------------|---------------|---------------|--------------|
| Male                           |                  |               |               |              |
| Body length                    | 12.3-13.1        | 12-14         | 12-16         | 9-14         |
| Body width                     | 0.29-0.37        | 0.23-0.40     | —             | —            |
| Length of the esophagus        | 0.76-0.78        | —             | —             | 0.6-0.8      |
| Length of spicules             | 0.73-0.79        | 0.75-0.80     | 0.75-0.85     | 0.7-0.8      |
| Length of the gubernaculum     | 0.09-0.13        | 0.10-0.15     | 0.1           | —            |
| Female                         |                  |               |               |              |
| Body length                    | 15.6-16.8        | 15-18         | 14-18         | 12-16        |
| Body width                     | 0.30-0.38        | 0.30-0.5      | —             | —            |
| Length of the esophagus        | 0.77-0.81        | —             | —             | 0.7-0.8      |
| Distance of the vulva from posterior end of the body | 0.71-0.83 | 0.75-0.80 | 0.9-1.0 | 0.71-1.03 |
| Distance of the anus from posterior end of the body | 0.31-0.33 | 0.5-0.6 | 0.3 | 0.31-0.42 |
Due to limited resources, designing and applying specific primers for individual helminth species are difficult for researchers from poor-resource countries. To address this issue, we choose universal primer set instead of species-specific primers, to validate the capacity of universal primers and to identify nematodes. rDNA and cox1 markers have also been used to identify successfully nematode species in Strongyloides [11]. In our study, the cox1 sequence identities of Oesophagostomum species ranged from 96.2% to 96.9%, when compared with reference sequences from GenBank databases using BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/). Hebert et al. [25] described that the percentage of sequence divergences at the cox1 gene for the same generic species of nematodes estimated falling in a particular range is 4.8%. The sequence of Bangladesh-origin specimen is most closely related to but distinct from Chinese isolate O. columbianum from sheep (GenBank No. KC715827), indicating that the parasite has acquired genetic changes after entering into the new host (goat) and new location (Bangladesh). In Bangladesh, various host species such as sheep, goats, cattle, and buffaloes share similar grazing fields; thus, breaking down the host barrier and moving parasites from one host to another are a common occurrence [26]. While several studies mentioned that universal primer-based cox1 gene sequences have limitation to identify nematode in species-level previously, most of the studies have been conducted based on the rDNA, particularly from the ITS region [24, 27]. Sequence analysis of this genetic marker provides an intent and specific means of species identification. In this study, the ITS (ITS1-5.8S-ITS2) sequences of Oesophagostomum species showed a similarity of 98.4% to 98.8% with previously published O. columbianum sequences and showed to be closer to Chinese isolate O. columbianum from sheep (GenBank No. JX188470). These differences may be associated with the geographical origins and animal husbandry system. Application of additional genetic markers may be a good option to verify the species identification; however, it should be verified by morphological observation. Also, very few sequences targeting ITS1-5.8S-ITS2 region of rDNA are currently available in the NCBI database.

To determine the taxonomic positions, a genetic tree of O. columbianum among other members of the genus Oesophagostomum was built using the ML method (Figure 2). The tree was reconstructed utilizing ITS sequences and Bunostomum trigonocephalum (GenBank No. KC998804) was used as the outgroup. The phylogenetic data revealed that O. columbianum isolated in Sylhet, Bangladesh, was closely related to the Chinese isolate. Because of commercial relationship between Bangladesh and China, as well as neighboring countries (India, Myanmar, and Pakistan), it might be possible that the origin of O. columbianum had been found in China. On the tree, our specimen is positioned in the sister clade of two nodule worms, O. dentatum and O. quadrispinus-latum. The DNA sequence results reported herein were consistent with previous studies of Dorris et al., Zhao et al., and Newton et al. [11, 24, 28].

3.2. Haemonchus contortus (Rudolphi, 1803) Cobb, 1898. Description of worms was based on 3 female and 2 fully mature male as whole-mounted specimens (Figure 3; Table 3). Filiform- (cylindrical) shaped worms are relatively small-sized, tapering towards the anterior end in males and both ends in females. Posterior tip of females showed a “barber pole,” while males appeared to expand in a copulatory bursa (Figure 3(c)). No morphological differences were observed in the anterior structures of male and female; both had a small buccal capsule and a long esophagus.

The following are descriptions for the male: total body length 18.8-20.4 [19.6]; maximum width 0.31-0.43 [0.36]; length of esophagus 1.55-1.67 [1.61]; maximum width of esophagus 0.11-0.15 [0.13]; shoulder region showed a pair of wedge-shaped cervical papillae (Figure 3(a)); distance of cervical papillae from anterior end of body 0.37-0.45 [0.41]. Copulatory bursa asymmetrical with two distinct lobes; lateral lobe containing an inverted Y-shaped dorsal ray (bifurcated); lateral rays arise from a common trunk, and ventral rays are fused proximally and separated dorsally; paired spicules, equal, 0.40-0.46 [0.43] long; gubernaculum elongate, length 0.19-0.23 [0.21].

The following are descriptions for the female: total body length 26.8-27.4 [27.1]; maximum width 0.39-0.47 [0.43]; length of esophagus 1.88-1.96 [1.92], width of the esophagus 0.09-0.15 [0.12]; distance of cervical papillae from anterior end of body 0.45-0.51 [0.48]; tail thin, straight, and pointed (Figure 3(d)); distance of the anus from posterior end of the body 0.46-0.54 [0.50]; vulva located in the posterior third of the body and covered by a prominent knob-shaped vulvar flap (Figure 3(b)) and opens 4.30-4.54 [4.42] from posterior end.

The morphological observation and morphometric features of the present specimens were consistent with the description of the genus Haemonchus and were identical to those of H. contortus documented previously [5, 21, 29–31]. H. contortus is one of the nematodes of both domestic and wild mammals, most commonly encountered all over the world, and variation in the different measurements can, therefore, be expected. According to the characteristics of the gastrointestinal Trichostrongyloidae described by Daska-lov [31] and Santiago [5], vulvar flap of female, bifurcated dorsal rays of male, and the egg size can be considered appropriate parameters in the identification and distinguishing of genus Haemonchus from other GINs. However, intragenus morphological differentiation between various Haemonchus species is arbitrary; many variations and combination of characteristics make it difficult in precise identification. Though several studies have showed the differences between H. placei and H. contortus, there is still some argument concerning their identities. Taylor et al. [32] stated that H. contortus and H. placei are the single species and H. contortus with only strain adaptation for domestic ruminant. However, this statement was proven inconsistent by do Amarante [33] where he mentioned substantial morphological, biological, and genetic evidence of the existence of both species. Also, Lichtenfels et al. [5] distinguished H. contortus from H. similis and H. placei based on spicule structure and length and vulvar structure and tail length. According to his description, the mean spicule length for H. contortus is around 0.38-0.42, whereas the average spicule length for H.
placei and H. similis is 4.3-5.1 and 3.0-3.8, respectively. The mean tail length for H. contortus is around 0.25-0.53, whereas the average spicule length for H. placei and H. similis is 0.37-0.72 and 0.13-0.27, respectively. The spicule length (0.40-0.46) and tail length (0.46-0.54) of our specimen were consistent with the distinctive characteristics of H. contortus given by Lichtenfels et al. [5].

Molecular identification of H. contortus was examined by sequence differences in the cox1 and ITS regions with other Haemonchus species including H. placei and H. similis. The cox1 sequence identities of Bangladesh-origin Haemonchus species ranged from 96.8% to 97.3%, showing the highest homology to the H. contortus isolate from goat in Pakistan (GenBank No. KJ724377). On the other hand, within ITS1-5.8S-ITS2 sequences, identities ranged from 97.7% to 99%, showing the highest homology to the H. contortus isolate from sheep in Iran (GenBank No. HQ389229). Meshgi et al. [34] revealed the similar observation and reported no molecular difference between H. contortus from sheep and goat isolates. To determine the taxonomic positions of the present specimen, a phylogenetic tree among members of the genus Haemonchus was constructed using ML, based on the ITS sequences (Figure 4), with Trichostrongylus axei (GenBank No. MN845163) as the outgroup. The phylogenetic data revealed that the Bangladesh origin H. contortus showed similarity with Iranian isolate. In such a scenario, the reason is the introduction of the parasite population by imported animals of the same origin as there are evidences of direct animal movement between Bangladesh and Iran through the neighboring countries, especially during the

![Figure 2: Phylogenetic relationships of present specimen (O. columbianum) with other members of Oesophagostominae reconstructed by ML method based on the ITS sequences. Bootstrap values are shown above branches. The scale bar represents 0.10% divergence.](image)

![Figure 3: Haemonchus contortus: (a) anterior end showing cervical papillae; (b) knobbed vulvar flap of female; (c) bursa of male showing dorsal rays and spicules; (d) posterior end of female showing the anus and tail.](image)
Since precise identification and molecular analysis, which parasitized indigenous goat, tortus based on morphological properties, morphometry, record for these species. The present is close to our study area, which thereby forms a new locality Meghalaya [23, 37]. The geographical location of that areas are widespread and have been documented from different geographic settings and hosts will be helpful. Findings of our study might have a significant implication for the epidemiology, taxonomy, and population dynamics, as well as for the management and control of these nematodes.

4. Conclusions

We identified Oesophagostomum columbianum and Haemonchus contortus from the goat in Bangladesh. To our knowledge, for the first time, we obtained DNA sequences of O. columbianum in Bangladesh and H. contortus in Sylhet, Bangladesh. Findings of our study indicated a high specificity for identifying nematodes. Also, the universal primers, if protocol is accurately designed, could give precise results and could be an economic option for the researchers from poor-resource settings. However, to describe genetic diversity in more detail, additional observation of specimens from different geographic settings and hosts will be helpful. Findings of our study might have a significant implication for the epidemiology, taxonomy, and population dynamics, as well as for the management and control of these nematodes.

Table 3: Comparative measurement (in mm) of present Haemonchus contortus isolated and those previously recorded.

| Body parts                              | Present specimen | Santiago (1968) | Ransom (1911) | Lichtenfels et al., (1994) |
|-----------------------------------------|------------------|-----------------|---------------|----------------------------|
| Male                                    |                  |                 |               |                            |
| Body length                             | 18.8-20.4        | 14-17           | 10-20         | 11.0-17.0                  |
| Maximum thickness                       | 0.31-0.43        | 0.199-0.265     | 0.40          |                            |
| Length of the esophagus                 | 1.55-1.67        | 1.444-1.743     | 1.5           | 1.09-1.55                  |
| Cervical papillae                       | 0.37-0.45        | —               |              | 0.27-0.46                  |
| Length of spicules                      | 0.40-0.46        | 0.398-0.448     | 0.30-0.50     | 0.38-0.47                  |
| Length of gubernaculum                  | 0.19-0.23        | 0.199-0.349     | 0.20          | 0.19-0.25                  |
| Female                                  |                  |                 |               |                            |
| Body length                             | 26.8-27.4        | 20-27           | 18-30         | 14.8-27.2                  |
| Body thickness                          | 0.39-0.47        | 0.215-0.332     | 0.50          | —                          |
| Length of the esophagus                 | 1.88-1.96        | 1.162-1.662     | —             | 1.15-1.66                  |
| Cervical papillae                       | 0.45-0.51        | —               |              | 0.24-0.48                  |
| Distance of the vulva from posterior end of the body | 4.30-4.54        | 3.81-5.31       | 3-4.5         | 3.01-4.90                  |
| Distance of the anus from posterior end of the body | 0.46-0.54        | 0.415-0.513     | 0.40-0.63     | 0.25-0.53                  |

Figure 4: Phylogenetic relationships of present specimen (H. contortus) with other members of Haemonchidae reconstructed by the ML method based on the ITS sequences and numbers at the branch nodes indicate percentage bootstrap support for 1000 replicates.
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