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Morphological identification of adult male *Haemonchus* species in goats from Thailand and Lao PDR

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Abstract. *Haemonchus* spp. or barber's pole worms are one of the most highly pathogenic nematodes of ruminants causing economic losses in livestock worldwide. The current study was a first attempt to identify *Haemonchus* spp. from goats in Thailand and Lao PDR. Utilizing the inexpensive tools of the discriminant function (DF) combined with synlophe patterns is fundamental for understanding their epidemiological aspects. In total, 255 randomly chosen adult male *Haemonchus* worms from goats in various areas in each country were identified individually. For both these countries, about 94% based on the DF values, and 99%, 98%, and 97% based on synlophe patterns in the region of the esophageal intestinal junction (EI), 4 mm from the anterior end, and at both these positions, respectively, were identified as *H. contortus*. Other identified specimens defined as *H. placei* and hybrids as well as unclassified species based on synlophe patterns were proved using polymerase chain reaction (PCR); this also included some randomly chosen *H. contortus* by DF and synlophe patterns. All those specimens were confirmed as *H. contortus* being strongly supported by some genetic evidences and UPGMA analysis. Thus, it was assumed that all specimens in the current study were *H. contortus*. The morphological differences of this predominant species (*H. contortus*) in goats between the two countries were: body length, gubernaculum length, and left spicule barb length, while almost all characters of male worms individually measured appeared to overlap, mostly in *H. contortus* and *H. placei*, which may lead to misclassification. Therefore, using the DF along with synlophe patterns can assist in increasing the accuracy of *Haemonchus* spp. identification from goats in some areas where funding is limited, particularly in Lao PDR. The present results revealed that synlophe patterns in the EI region seemed to be promising for the identification of *Haemonchus* spp., while molecular techniques are also required to address ambiguous identification with some specimens.

INTRODUCTION

Haemonchus spp. commonly recognized as barber's pole worm, cause haemonchosis and are found in the abomasa of large and small ruminants worldwide (Lichtenfels & Pilitt, 2000; Achi *et al*., 2003). Two major species have the greatest veterinary importance, namely *Haemonchus contortus* and *Haemonchus placei*. *H. contortus* is normally found in goats and sheep while *H. placei* is known to be a parasite of cattle (Lichtenfels *et al*., 1994). *H. contortus* is the most pathogenic of the common nematodes, particularly in young animals (Hoberg *et al*., 2004) and its blood-feeding behavior results in severe anemia and weight loss, with death eventually occurring in cases of heavy infection (Besier *et al*., 2016). These outcomes lead to a reduction in animal production and increase production costs, causing huge economic losses worldwide (Stevenson *et al*., 1995; Waller & Chandrawathani, 2005; Wang *et al*., 2017; Sallé *et al*., 2019). For example, in endemic areas of Australia, the mean annual cost due to *H. contortus* causing ewe death accounted for AUS 11/head, including treatment and cost of diagnosis (Besier *et al*., 2016). In Thailand and Lao PDR, goat

production is currently increasing, mostly due to smallholders as a means of generating income for local people, where haemonchosis in small ruminants such as goats caused by *Haemonchus* spp. including *H. contortus* seems to be a regular problem since the main strategies for parasite control are probably not effective as a consequence of drug resistance (Waller *et al*., 2004; Waller & Chandrawathani, 2005; Pralomka & Boonsanit, 2012; Sallé *et al*., 2019) and the ability of parasites that can adapt to variable environments in temperate and tropical climates (Troell *et al*., 2006). Although the losses due to the impacts of this parasite have not been officially recorded in both countries, this parasitic infection in Thailand (Laosutthipong & Eardmusic, 2019) and Lao PDR (Sato *et al*., 2014) has also been continually associated with a high infection rate (86.67% and 69%, respectively).

An accurate identification of *Haemonchu*s spp. distributed in goats will help to understand their epidemiological picture, current status, and can also be applied to support effective parasite control (Jacquiet *et al*., 1995; Silva *et al*., 2015). Using a traditional method such as stool examination to examine the parasite's egg is of limited value, since the morphology of their eggs is somewhat similar to that of other strongyle nematodes and hence is difficult to differentiate (Valderrabano *et al*., 2002) while identification of the infective larval stage via the fecal culture technique is useful for species identification, but is time-consuming and should be performed by scientific experts (Waller *et al*., 2004; Besier *et al*., 2016). A discriminant function (DF) combining the measurements of adult male *Hamonchus* spp. based on spicule length and spicule barb length is a rapid, easy and reliable tool (Achi *et al*., 2003; Amarante, 2011; Gharamah *et al*., 2011) that is commonly used to identified the three main *Haemonchus* spp. (*H. contortus*, *H. placei* and *H. similis*) occurring in naturally infected goats and sheep (Le Jambre & Whitlock, 1968; Jacquiet *et al*., 1995; Achi *et al*., 2003; Kumsa *et al*., 2008; Gharamah *et al*., 2011; Santos *et al*., 2014; Vadlejch *et al*., 2014) while synlophe or cuticular ridge patterns presenting on the body of parasite are one of the most useful characters used for identifying male and female *Haemonchus* spp. (Lichtenfels *et al*., 1994; Lichtenfels & Pilitt, 2000) and also has been applied for the identification of *Haemonchus* spp. in various areas, namely the United States (Lichtenfels *et al*., 1986), Malaysia (Rahman & Abd Hamid, 2007; Gharamah *et al*., 2011), and Brazil (Silva *et al*., 2015). Moreover, in some situations when the spicule measurements have overlapping values between *H. placei* and *H. contortu*s, the synlophe patterns can facilitate identification (Amarante, 2011). Molecular methods of which polymerase chain reaction (PCR) is one of the most advanced techniques are more rapid, and have high sensitivity and specificity for identifying worms with high certainty, including *Haemonchus* spp. (Roeber *et al*., 2011). However, the high cost of such techniques is a disadvantage (Comes *et al*., 1996; Achi *et al*., 2003; Gharamah *et al*., 2011), and these techniques might not be suitable for routine use, particularly in some developing countries where budgets are limited.

This was the first study to attempt to apply useful traditional methods as a low cost diagnosis based on morphological characters to identify adult male *Haemonchus* spp. in goats obtained from Thailand and neighboring Lao PDR, where advanced technologies such as PCR and also financial support are still restricted while haemonchosis in goats in both countries seems to be seriously affecting animal health and decreasing goat production. Thus, the aim of this research was to identify adult male *Haemonchus* spp. in infected goats collected from different areas in Thailand and Lao PDR by using a discriminant function (DF) combined with cuticular ridge patterns. PCR using the ITS2 region, a low intra-variation of ribosomal DNA (rDNA) was used to identify and confirm *Haemonchus* spp. in some cases (Stevenson *et al*., 1995; Gasser & Newton, 2000) with consideration of some basic genetic and phylogenetic data. Moreover, measurements of common morphological characters of predominant *Haemonchus* spp. were recorded and

compared, consisting of: total body length, cervical papillae, esophagus length, right and left spicule lengths, right and left spicule barb lengths and gubernaculum length. The significance of the distribution of known species of *Haemonchus* spp. in each country from this research will lead to improved strategies for parasite control and a future prevention program.

MATERIALS AND METHODS

Study area, animal hosts, and parasites

Adult male *Haemonchus* spp. were collected from the abomasa of goats in different local slaughterhouses in different geographical zones of Thailand and Lao PDR during the period from June to July 2019 (Figure 1). Most goats included in this research in Thailand were mixed breed while almost all animals in Lao PDR were native goats. Goats showed some possible clinical signs such as poor overall body condition, emaciation and loss of hair or rough hair coat were selected primarily for this investigation. Three different provinces of each country were chosen based on geographical distance apart (more than 500 km and 250 km in Thailand and Lao PDR, respectively) with high animal populations including there was scanty information of *Haemonchus* spp. in goats. In Thailand, the three provinces selected in the northern, central, and southern regions were Chiang Rai (19°54'30.89"N and 99°49'57.00"E), Chai Nat (15°11'3.60"N and 100°07'17.40"E) and Nakhon Sri Thammarat (8°26'6.59"N and 99°57'28.19"E), respectively. For Lao PDR, Luang Prabang province (19°53'21.3756"N and 102°8'0.4308"E) Savannakhet province (16°32'59.99"N and 104°44'59.99"E) and Champasak province (15°6'37.8252"N and 105°49'2.2476"E) located in the northern, central, and southern regions, respectively, were chosen.

Thirty and 68 infected goats from Thailand and Lao PDR were detected from 63 and 103 goats, respectively. The collected adult male worms from the abomasa were collected with modifications to Soulsby (1982) and MAFF (1971). The abomasa were opened and the contents inside were sieved and washed carefully with tap water. Then, the parasites were carefully collected and placed in a clean petri dish containing 0.85% normal saline. Adult male parasites obtained from individual hosts were washed three times with this physiological saline prior to fixing in 70% ethanol and storing at -20°C for further study.

Morphological characterization and species identification

Individual adult males of *Haemonchus* spp. were randomly selected from infected individual hosts in each province of Thailand and Lao PDR, with 255 adult male worms (85 worms/province) in each country being used. First, the posterior end of each worm was cut above the bursa to measure the spicule length (right: RSL and left: LSL), the tip to the hook of right and left spicule barbs (right: THr and left: THl), and the gubernaculum (GL) (Figure 2). Finally, synlophe patterns were cut in cross section at two main positions, namely the esophageal-intestinal junction (EI) and 4 mm from the anterior end. All measurements such as cervical papillae, esophagus length, and etc. were carried out under light microscopy at 40x/50x, 100x and 400x magnification (Olympus Corporation, Tokyo, Japan; Carl Zeiss Microscopy GmbH, Jena, Germany) except for the body length which was measured using stereomicroscopy (Olympus Corporation, Tokyo, Japan).

The discriminant function (DF) described by Jacquiet *et al.* (1997) and Achi *et al.* (2003) comprising three main parameters of male worms-TL (mean), THr, and THl was calculated using the formula:

$$DF = 0.0016\,TL + 0.128\,THr + 0.152\,THl - 9.97$$

Identification of *Haemonchus* spp. based on the DF value was classified into three categories: DF < 0.63 = *H. contortus*; 0.63 < DF < 3 = *H. placei*; DF > 4 = *H. similis*.

Observations of synlophe patterns in all adult male worms of *Haemonchus* spp. from both countries were conducted according to the procedure described by Lichtenfels *et al.* (1994) and Vongnady *et al.* (2020). Briefly, all specimens were cut on cross section at the level of the EI region and 4 mm from the

Figure 1. Sampling locations of *Haemonchus* spp. in goats from Thailand and Lao PDR.

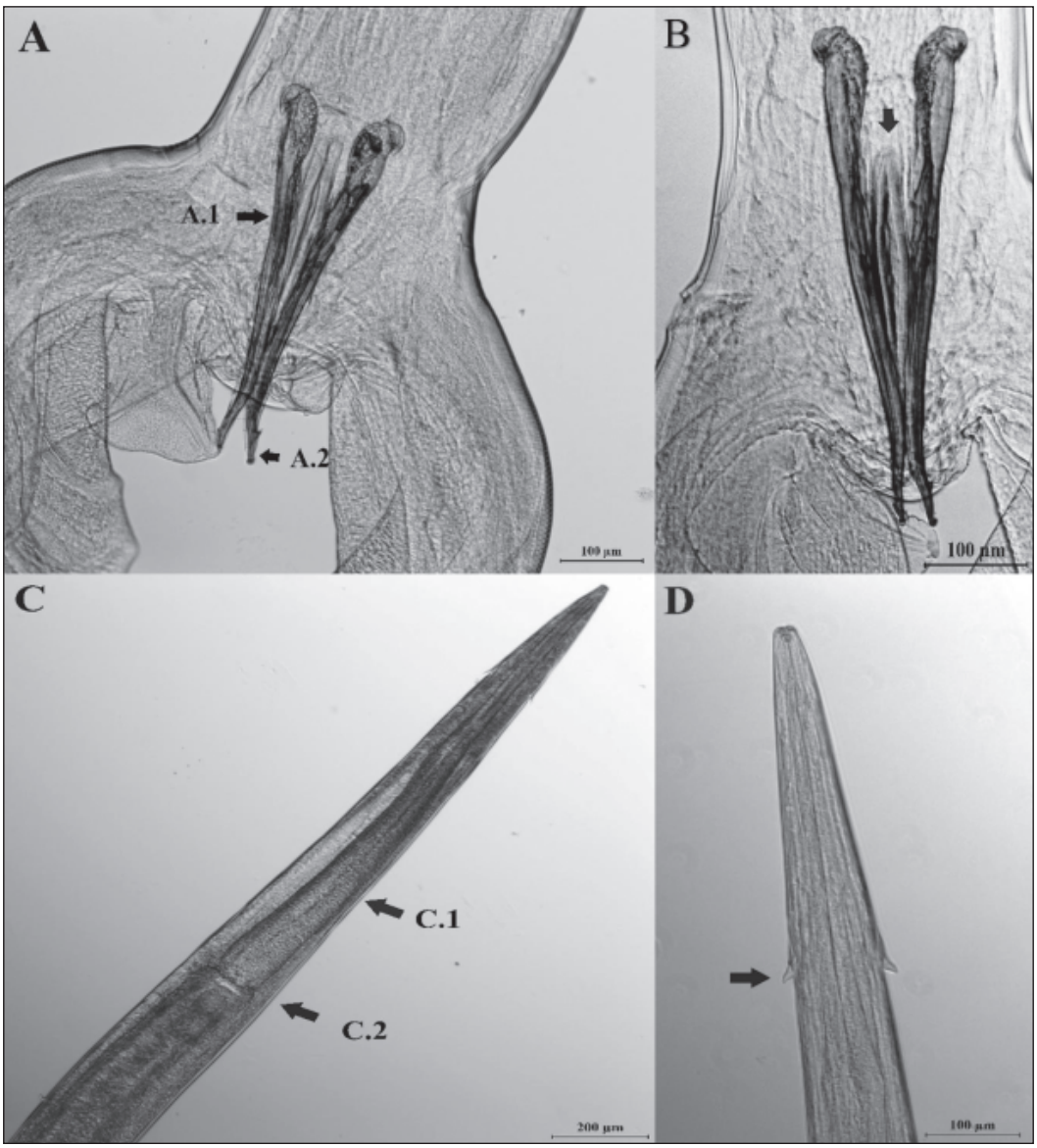

Figure 2. Some morphological characters of adult male *Haemonchus* spp. in goats from Thailand and Lao PDR used for species identification: (A) A.1, right/left spicules; A.2, right/left spicule barbs; (B) gubernaculum; (C) C.1, esophagus; C.2, esophageal intestinal junction; (D) cervical papillae.

anterior end using a freehand cut with a shape razor blade. These cross sections were mounted in glycerin jelly and left about 5 min before viewing and counting the number of cuticular ridges under a light microscope at 100x and 400x magnification. Identification of *Haemonchus* spp. using the synlophe patterns was based on three main researcher references, with the synlophe patterns at the EI region of *H. contortus*, *H. placei* and *H. similis* having 30, 34 and 34 ridges, respectively, (Lichtenfels *et al.*, 1994; Silva *et al.*, 2015) while at 4 mm, there were 30 ridges for each of *H. placei* and *H. similis* (Lichtenfels *et al.*, 1994) as well as *H. contortus* having 25 ridges (Lichtenfels *et al.*, 1994) and 24 and 26 ridges (Rahman & Abd Hamid, 2007).

Molecular identification

DNA extraction

Only 24 and 26 adult male worms from Thailand (Table 2) and Lao PDR (Table 3), respectively, were used for DNA isolation. These specimens derived from *H. placei* and hybrids that were identified using the DF, including unclassified species based on synlophe patterns at both positions as well as four worms in Thailand and eight worms in Lao PDR that were identified as *H. contortus* using the DF value and synlophe patterns. The rest of the parasite body from morphological characterization was extracted for genomic DNA using a DNA commercial tissue kit GF-1 Tissue DNA Extraction Kit (Vivantis Technologies Sdn, Bhd, Selangor Darul Ehsan, Malaysia). The procedure was carried out according to the manufacturer's instructions. Briefly, the remaining parts of each whole male worm were transferred to a 1.5 mL microcentrifuge tube. The parasite tissues were mechanically ground and with 250 µL of lysis buffer, and then 20 µL of proteinase K with 12 µL of lysis enhancer were added. The homogeneous mixture was incubated at 65°C for 90 min. After added binding buffer, this homogeneous mixture was incubated at 65°C for 10 min. The purified DNA binding on the membrane was eluted in elution buffer. The concentration of eluted DNA was measured using the NanoDrop procedure (Thermo Fisher Scientific, Wilmington, USA) and stored at -20°C until used.

PCR amplification and DNA sequencing

The ribosomal DNA internal transcribed spacer 2 (ITS2) region was amplified with specific primer sets described by Stevenson *et al*. (1995). PCR reaction reported by Vongnady *et al.* (2020) was prepared in a total volume of 20 µL containing 10X Taq Buffer with $(NH4)_2SO_4$, 5mM of each dNTPs, 25mM $MgCl_2$, 10µM of each primer, 1.25U/µL of Taq DNA polymerase (Thermo Fisher Scientific, Wilmington, USA) and 2 µL of DNA template. Negative (Water for molecular biology; AmpliChem GmbH-An ITW Company, Darmstadt, Germany) and positive samples (DNA sample of *H. contortus*) were added in each PCR run. The PCR cycling program (Thermal cycler; SensoQuest GmbH, Göttingen, Germany) with 30 cycles followed Gharamah *et al.* (2012), Mangkit *et al.* (2014) and Vongnady *et al.* (2020). In each case, 5 µL of each PCR product was added on 1% agarose gel stained with FluoroVue™ Nucleic Acid Gel Stain (SMOBIO Technology, Inc, Hsinchu City, Taiwan). The PCR products were run on 0.5xTBE buffer using electrophoresis (Hoefer, Inc., Massachusetts, USA) for 45 min at 80 V, and then the PCR products (321 bp) were compared with the DNA molecular marker (100 bp ladders) (Vivantis Technologies Sdn, Bhd, Selangor Darul Ehsan, Malaysia) and photographed using a gel documentation system (Omega Fluor and Omega Fluor Plus systems, Aplegen Inc., California, USA). All PCR products were purified and sequenced in both directions by Macrogen, Korea.

Data analysis

All randomly selected male *Haemonchus* spp. from goats distributed in three provinces each for Thailand and Lao PDR were identified as *H. contortus* based on the DF and synlophe patterns together with species confirmation from molecular analysis in some specimens. Morphological measurements and relevant data obtained from each morphological character of *H. contortus* were stored in a Microsoft Excel spreadsheet and some simple descriptive statistics were calculated. Most data were analyzed using R Studio version 3.6.1 to compare each character of *H. contortus* between two countries. Since all data were not normally distributed, the Wilcoxon Signed-Rank test, a non-parametric statistic was used. Significant difference was tested using a p-value less than 0.05. The ITS2 sequences at 231 bp were aligned and blasted via the MEGA program version 7 (Kumar *et al.*, 2016) while all DNA sequences from each country were grouped into genotypes. The polymorphisms of all genotypes in each country were compared with referent sequences of *H. contortus* (Accession number: X78803) and *H. placei* (Accession numbers: X78812, KF364623 and MH481601) in previous reports, and also the percentage

identity of each genotype was determined using the BioEdit package (Hall, 1999). Phylogenetic analysis via the MEGA program version 7 of *H. contortus* genotypes from Thailand and Lao PDR including another sequence of *H. contortus* from various hosts in different countries (Accession numbers: AB908961: Lao PDR; EU086378, EU084691: United States; HQ389229: Iran; JX869066: the Czech Republic; JX901156: Tunisia; KJ724250, KJ724315: Pakistan; KP101363, KP101364: Thailand; KP090288, KP090290: Egypt; KC415117, KY305780: China; KF364629, KC632567, X78803: Australia; LC360146, LC360147: Bangladesh; MH481574, MH481598: Ghana) used the unweighted pair group method with arithmetic mean (UPGMA) method with a bootstrap of 1000 replications based on the Kimura-2 parameter model and was constructed by comparison with outgroups (*H. placei*).

RESULTS

Adult male *Haemonchus* spp. used in the current study were collected from the abomasa of goats in Thailand and Lao PDR. The proportion of *Haemonchus* spp. in goats accounted for 47.62% (30/63) and 66.02% (68/103) in the three provinces sampled in each of Thailand and Lao PDR, respectively. All 255 adult male worms (85 worms/province) were utilized for species identification based on the discriminant function (DF) and synlophe patterns (esophageal intestinal junction, EI and 4 mm from anterior end). The DF value and synlophe patterns in Thailand showed that 93.73% (239/255) of adult worms were identified as *H. contortus* and 5.88% (15/255) of male worms were classified as *H. placei*, while only one worm was a hybrid (DF=0.63) (Table 1). The cuticular ridges of *H. contortus* in the EI region revealed that 99.22% (253/255) of specimens were *H. contortus* presenting 30 ridges, while 0.78% (2/255) of specimens were unidentified (2 worms showing 29 ridges). At 4 mm from the anterior end, the synlophe patterns revealed that 98.43% (251/255) of worms showed 26 ridges and were recognized as *H. contortus* while 1.57% (4/255) of male worms were unidentified species having 27 ridges (Figure 3) and using both positions, 97.65% (249/255) of worms were *H. contortus* (Table 1). In Lao PDR, the DF value determined that 94.12% (240/255) of adult worms were identified as *H. contortus* and 5.10% (13/255) of *Haemonchus* specimens were *H. placei*, while 2 worms were identified as hybrid (DF=0.63) (Table 1). The cuticular ridge patterns (Table 1) in the EI region showed that 99.22% (253/255) of specimens were *H. contortus* showing 30 ridges, with 0.78% (2/255) of worms not identified (2 worms had 29 ridges) while at 4 mm from the anterior end, the synlophe patterns indicated 97.65% (249/255) were *H. contortus* (showing 26 ridges) and 2.35% (6/255) of worms were unknown species, showing 27 ridges (Figure 3), and examination at both positions identified 96.86% (247/255) of specimens as *H. contortus*. However, some specimens from both countries identified as *H. placei* (DF>0.63) and hybrids (DF=0.63) based on the DF results (Figure 4), including unclassified species based on cuticular patterns at both positions, were confirmed using the PCR method and sequencing (Tables 2 and 3). Then, all sequences that were blasted for a preliminary check with the *Haemonchus* spp. in the GenBank database were classified as *H. contortus*.

In total, 24 and 26 adult male *H. contortus* mentioned earlier in goats from Thailand (Table 2) and Lao PDR (Table 3) respectively, were confirmed using PCR based on ITS2 with specific bands at 321 bp. A 231 bp of ITS2 sequences of *H. contortus* was used, and then was defined to six and ten distinct genotypes from original sequences in Thailand (Accession numbers: MT682914-MT682919) and Lao PDR (Accession numbers: MT682957-MT682966), respectively (Table 5). The sequence identity of genotypes derived from the *H. contortus* population ranged from 98.20 to 99.50% homology in each country. All genotypes in both countries were compared with reference sequences of *H. contortus* (Accession numbers: X78803, EU084691, KP101363) and the results indicated that sequence

Table 1. Identification of adult male *Haemonchus* spp. in goats from Thailand and Lao PDR based on the DF and synlophe patterns

Country	Province	No. of worms	Identification based on DF and synlophe patterns								
			DF			EI		4mm		EI/4mm	
			Hc(%)	Hp(%)	Hybrid(%)	Hc(%)	NP(%)	Hc(%)	NP(%)	Hc(%)	NP(%)
Thailand	Chiang Rai	85	81	4	0	84	1	85	0	84	1
	Chai Nat	85	79	6	0	84	1	84	1	83	2
	Nakhon Sri Thammarat	85	79	5	1	85	0	82	3	82	3
Total		**255**	**239(93.73)**	**15(5.88)**	**1(0.39)**	**253(99.22)**	**2(0.78)**	**251(98.43)**	**4(1.57)**	**249(97.65)**	**6(2.35)**
Lao PDR	Luangpabang	85	79	5	1	84	1	83	2	82	3
	Savannakhet	85	81	4	0	84	1	84	1	83	2
	Champasak	85	80	4	1	85	0	82	3	82	3
Total		**255**	**240(94.12)**	**13(5.10)**	**2(0.78)**	**253(99.22)**	**2(0.78)**	**249(97.65)**	**6(2.35)**	**247(96.86)**	**8(3.14)**

Abbreviations: DF, discriminant function; Hc, *H. contortus*; Hp, *H. placei*; NP, non-proved species; EI, esophageal-intestinal junction; 4 mm, region at 4 mm from the anterior end.

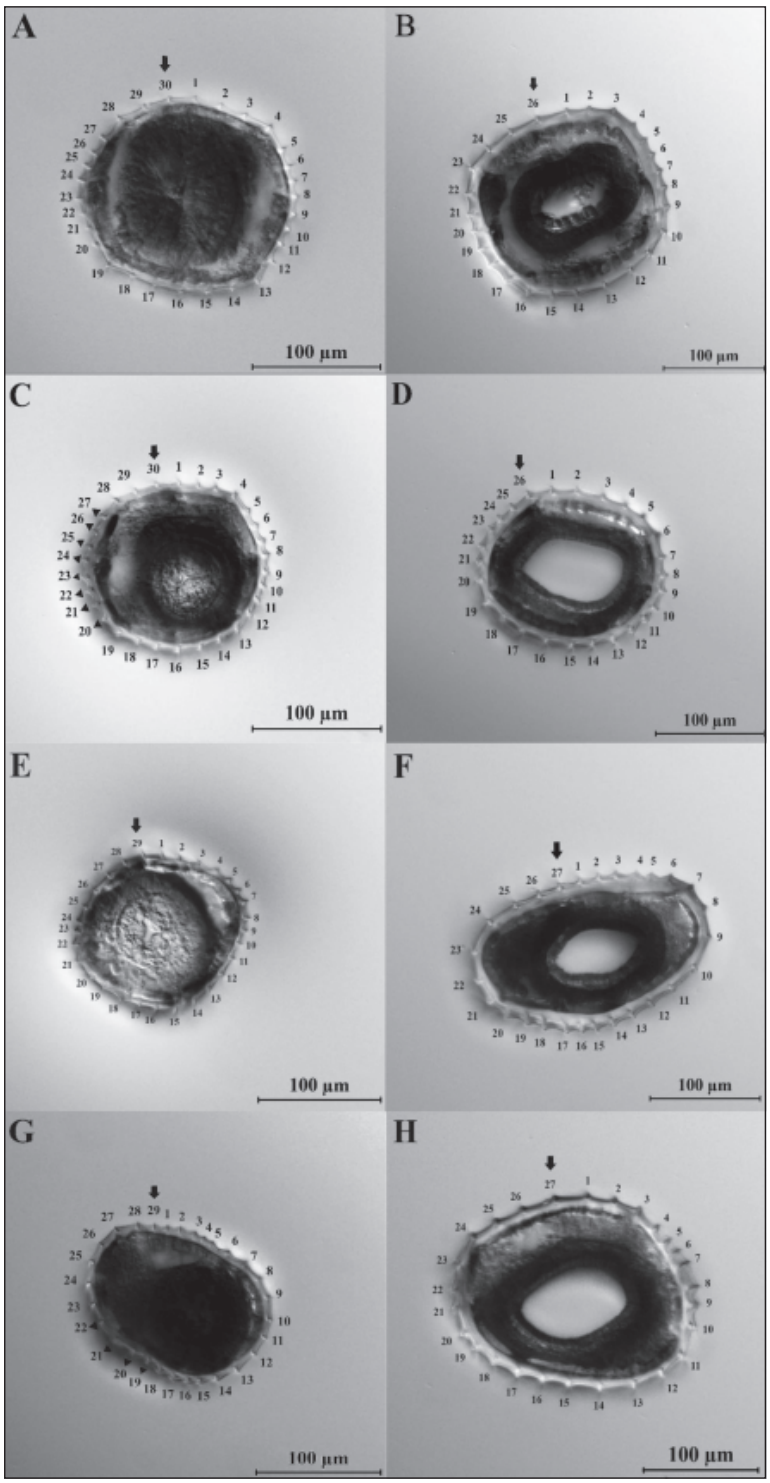

Figure 3. Synlophe patterns (cross section) of *H. contortus* in Thailand; (A) at esophageal intestinal junction, showing 30 ridges; (B) 4 mm region from the anterior end, showing 26 ridges; (E) esophageal intestinal junction, showing 29 ridges; (F) 4 mm region from the anterior end, showing 27 ridges, in Lao PDR; (C) esophageal intestinal junction, showing 30 ridges; (D) 4 mm region from the anterior end, showing 26 ridges; (G) esophageal intestinal junction, showing 29 ridges; (H) 4 mm region from the anterior end, showing 27 ridges.

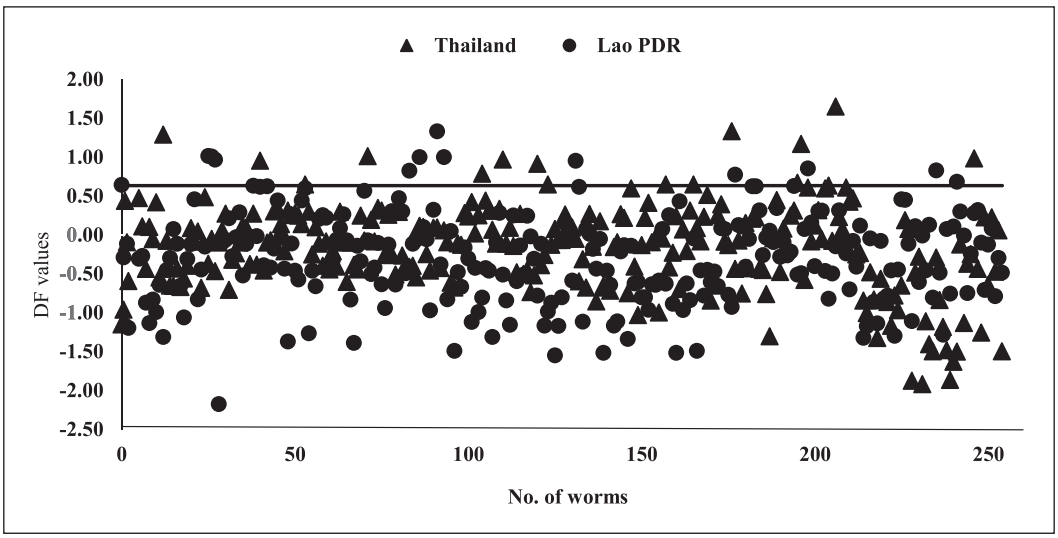

Figure 4. The value of discriminant function (DF) of individual adult male *Haemonchus* spp. in goats from Thailand and Lao PDR (dark black line, DF = 0.63).

identities ranged from 98.20 to 100% homology in Thailand and from 97.80 to 100% homology in Lao PDR. In addition, these genotypes for each country were compared with the outgroup *H. placei* (Accession numbers: X78812, KF364623, MH481601). This comparison showed 96.10–98.70% homology (Table 4). Moreover, the multiple alignments of six genotypes of *H. contortus* from Thailand compared with two sequence references (Accession numbers: X78803, KP101363) revealed six variable nucleotide positions: 10, 18, 21, 22, 123, and 196. Only two mutations (two: T<->C) were transition while four mutations were transversion (one: C<->A; two: T<->A; one: C<->G). In Lao PDR, the nucleotide polymorphism of the ten genotypes presented eight point mutations at positions 4, 10, 18, 21, 22, 55, 123 and 196. Five transversions (two: C<->A; two: T<->A; one: C<->G) and three transitions (three: T<->C) were detected. In addition, three main positions of genetic variations between *H. contortus* and *H. placei* was detected at positions 24, 205, and 219 (Table 5). The phylogenetic tree based on UPGMA analyzed with *H. contortus* sequences from different hosts in several countries revealed that all genotypes of *H. contortus* from Thailand and Lao PDR were grouped into two main groups (Figure 5), and distinctly differed from the three reference sequences of *H. placei* (Accession numbers: X78812, KF364623, MH481601) with a mean sequence identity of 97.09% homology. Group 1 comprised two genotypes from Thailand and three genotypes from Lao PDR as well as two sequences from Ghana. Group 2 was composed of four genotypes from Thailand and seven genotypes from Lao PDR which shared the same cluster with *H. contortus* from Egypt, China, Australia, Lao PDR, Thailand, Iran, Bangladesh, the Czech Republic, the United States, Pakistan, and Tunisia with a mean sequence identity of 98.77% homology.

Morphometric data obtained from the 255 adult male *H. contortus* goats in the two countries were measured and compared. According to the Wilcoxon signed-rank test used, there were no significant differences in the mean and median length of cervical papillae, esophagus length, spicule length (right/left), spicule barb length (right), the synlophe patterns in the EI region (mean= 29.99 ridges, median=30 ridges) and 4 mm from the anterior end (mean=26.01 ridges for Thailand and 26.02 ridges for Lao PDR, median=26 ridges) ($p>0.05$). However, there were significant differences in the body length, gubernaculum, and spicule barb length (left) between the *H. contortus*

Table 2. Species identification and genotypes of some adult male *Haemonchus* spp. in goats from Thailand based on morphological and molecular techniques

Genotype	No. of worms	Province	Sample code	RSL (µm)	LSL (µm)	THr (µm)	THl (µm)	Species identification					Molecular confirmation	
								DF		Synlophe			PCR(ITS2)	Sequencing
								-		EI	4 mm			
HCMTNS1N17	10	CR	HCMTCR3N9	460.00	460.00	52.50	25.00	1.29	Hp	30.00	26.00	Hc	+	Hc
		CR	HCMTCR3N37	450.00	450.00	50.00	25.00	0.95	Hp	30.00	26.00	Hc	+	Hc
		CR	HCMTCR7N2	430.00	430.00	45.00	22.50	-0.10	Hc	30.00	26.00	Hc	+	Hc
		CR	HCMTCR9N2	490.00	485.00	50.00	25.00	1.01	Hp	30.00	26.00	Hc	+	Hc
		CN	HCMTCN6N4	460.00	460.00	50.00	25.00	0.97	Hp	30.00	26.00	Hc	+	Hc
		CN	HCMTCN9N1	460.00	460.00	47.50	25.00	0.65	Hp	30.00	26.00	Hc	+	Hc
		NS	HCMTNS1N17	410.00	420.00	40.00	22.50	-0.77	Hc	30.00	26.00	Hc	+	Hc
		NS	HCMTNS2N1	460.00	460.00	45.00	27.25	0.67	Hp	30.00	26.00	Hc	+	Hc
		NS	HCMTNS4N16	390.00	400.00	37.50	17.50	-1.88	Hc	30.00	27.00	NP	+	Hc
		NS	HCMTNS5N3	450.00	450.00	48.75	26.25	0.98	Hp	30.00	26.00	Hc	+	Hc
HCMTCN3N2	8	CR	HCMTCR4N12	460.00	460.00	47.50	25.00	0.65	Hp	30.00	26.00	Hc	+	Hc
		CR	HCMTCR5N2	480.00	475.00	47.50	22.50	0.29	Hc	30.00	26.00	Hc	+	Hc
		CN	HCMTCN3N2	410.00	415.00	45.00	22.50	-0.13	Hc	29.00	26.00	NP	+	Hc
		CN	HCMTCN7N1	425.00	425.00	50.00	25.00	0.91	Hp	30.00	27.00	NP	+	Hc
		CN	HCMTCN15N9	460.00	460.00	47.50	25.00	0.65	Hp	30.00	26.00	Hc	+	Hc
		NS	HCMTNS3N8	450.00	450.00	47.50	25.00	0.63	hybrid	30.00	26.00	Hc	+	Hc
		NS	HCMTNS3N10	450.00	445.00	52.50	27.50	1.65	Hp	30.00	27.00	NP	+	Hc
		NS	HCMTNS7N1	410.00	410.00	45.00	23.75	0.06	Hc	30.00	27.00	NP	+	Hc
HCMTCN5N2	3	CN	HCMTCN5N2	410.00	405.00	46.25	27.50	0.78	Hp	30.00	26.00	Hc	+	Hc
		CN	HCMTCN14N9	435.00	430.00	45.00	22.50	-0.10	Hc	30.00	26.00	Hc	+	Hc
		NS	HCMTNS1N7	450.00	450.00	50.00	27.50	1.33	Hp	30.00	26.00	Hc	+	Hc
HCMTCN15N1	1	CN	HCMTCN15N1	460.00	460.00	47.50	25.00	0.65	Hp	30.00	26.00	Hc	+	Hc
HCMTCR2N1	1	CN	HCMTCR2N1	410.00	410.00	40.00	20.00	-1.15	Hc	29.00	26.00	NP	+	Hc
HCMTNS2N2	1	NS	HCMTNS2N2	465.00	465.00	50.00	26.25	1.16	Hp	30.00	26.00	Hc	+	Hc

Abbreviations: RSL, right spicule length; LSL, left spicule length; THr, tip-to-hook of right spicule; THl, tip-to-hook of left spicule; DF, discriminant function; EI, esophageal-intestinal junction; 4 mm, region at 4 mm from the anterior end; PCR, Polymerase chain reaction; CR, Chiang Rai; CN, Chai Nat; NS, Nakhon Sri Thammarat.

Table 3. Species identification and genotypes of some adult male *Haemonchus* spp. in goats from Lao PDR based on morphological and molecular techniques

Genotype	No. of worms	Province	Sample code	RSL (µm)	LSL (µm)	THr (µm)	THl (µm)	Species identification					Molecular confirmation	
								DF		Synlophe			PCR(ITS2)	Sequencing
								-		EI	4 mm			
HCMLCH3N1	10	LP	HCMLLP1N1	455.00	460.00	47.50	25.00	0.64	Hp	30.00	26.00	Hc	+	Hc
		LP	HCMLLP5N2	490.00	480.00	50.00	25.00	1.01	Hp	29.00	26.00	NP	+	Hc
		LP	HCMLLP5N3	460.00	460.00	50.00	25.00	0.97	Hp	30.00	27.00	NP	+	Hc
		LP	HCMLLP6N7	450.00	450.00	47.50	25.00	0.63	hybrid	30.00	26.00	Hc	+	Hc
		LP	HCMLLP7N7	475.00	460.00	45.00	22.50	-0.04	Hc	30.00	26.00	Hc	+	Hc
		CH	HCMLCH3N1	455.00	460.00	50.00	23.75	0.77	Hp	30.00	27.00	NP	+	Hc
		CH	HCMLCH5N3	470.00	470.00	45.00	22.50	-0.04	Hc	30.00	27.00	NP	+	Hc
		CH	HCMLCH11N1	470.00	470.00	47.50	26.25	0.85	Hp	30.00	26.00	Hc	+	Hc
		CH	HCMLCH17N2	445.00	435.00	46.25	25.00	0.45	Hc	30.00	27.00	NP	+	Hc
		CH	HCMLCH18N7	455.00	455.00	47.50	26.25	0.83	Hp	30.00	26.00	Hc	+	Hc
HCMLSV3N1	6	SV	HCMLSV3N1	450.00	450.00	50.00	27.50	1.33	Hp	30.00	26.00	Hc	+	Hc
		SV	HCMLSV5N2	420.00	425.00	46.25	22.50	0.05	Hc	30.00	26.00	Hc	+	Hc
		SV	HCMLSV11N1	430.00	435.00	45.00	25.00	0.28	Hc	30.00	26.00	Hc	+	Hc
		SV	HCMLSV18N1	450.00	450.00	50.00	25.00	0.95	Hp	30.00	27.00	NP	+	Hc
		CH	HCMLCH12N1	480.00	475.00	45.00	20.00	-0.41	Hc	30.00	26.00	Hc	+	Hc
		CH	HCMLCH19N1	445.00	445.00	45.00	27.50	0.68	Hp	30.00	26.00	Hc	+	Hc
HCMLCH1N1	2	CH	HCMLCH1N1	450.00	450.00	43.75	20.00	-0.61	Hc	30.00	26.00	Hc	+	Hc
		CH	HCMLCH9N1	450.00	450.00	47.50	25.00	0.63	hybrid	30.00	26.00	Hc	+	Hc
HCMLLP13N3	2	LP	HCMLLP13N3	455.00	460.00	47.50	22.50	0.26	Hc	30.00	26.00	Hc	+	Hc
		SV	HCMLSV1N2	440.00	445.00	47.50	27.50	1.00	Hp	30.00	26.00	Hc	+	Hc
HCMLLP5N1	1	LP	HCMLLP5N1	450.00	455.00	47.50	27.50	1.01	Hp	30.00	26.00	Hc	+	Hc
HCMLLP18N1	1	LP	HCMLLP18N1	445.00	440.00	45.00	25.00	0.30	Hc	30.00	26.00	Hc	+	Hc
HCMLLP19N1	1	LP	HCMLLP19N1	455.00	450.00	47.50	26.25	0.82	Hp	30.00	27.00	NP	+	Hc
HCMLSV4N1	1	SV	HCMLSV4N1	445.00	440.00	47.50	27.50	1.00	Hp	30.00	26.00	Hc	+	Hc
HCMLSV18N2	1	SV	HCMLSV18N2	440.00	440.00	47.50	25.00	0.61	Hc	29.00	26.00	NP	+	Hc
HCMLSV15N3	1	SV	HCMLSV15N3	400.00	400.00	40.00	17.50	-1.55	Hc	30.00	26.00	Hc	+	Hc

Abbreviations: RSL, right spicule length; LSL, left spicule length; THr, tip-to-hook of right spicule; THl, tip-to-hook of left spicule; DF, discriminant function; EI, esophageal-intestinal junction; 4 mm, region at 4 mm from the anterior end; PCR, Polymerase chain reaction; LP, Luangpabang; SV, Savannakhet; CH, Champasak.

Table 4. Pairwise identities (%) between six Thai genotypes and ten Laos genotypes of adult male *H. contortus* in goats compared with sequence references belonging to *H. contortus* and *H. placei*

No.	Sample code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	HCMTNS1N17	-																					
2	HCMTCN3N2	99.5	-																				
3	HCMTCN5N2	99.5	99.5	-																			
4	HCMTCN15N1	98.7	98.2	98.2	-																		
5	HCMTCR2N1	99.1	98.7	98.7	98.2	-																	
6	HCMTNS2N2	99.1	99.5	99.1	97.8	99.1	-																
7	HCMLCH3N1	99.5	100	99.5	98.2	98.7	99.5	-															
8	HCMLSV3N1	100	99.5	99.5	98.7	99.1	99.1	99.5	-														
9	HCMLCH1N1	99.5	99.5	100	98.2	98.7	99.1	99.5	99.5	-													
10	HCMLLP13N3	99.1	99.5	99.1	97.8	99.1	100	99.5	99.1	99.1	-												
11	HCMLLP5N1	99.5	99.1	99.1	98.7	98.7	98.7	99.1	99.5	99.1	98.7	-											
12	HCMLLP18N1	98.7	99.1	98.7	97.4	98.7	99.5	99.1	98.7	98.7	99.5	98.2	-										
13	HCMLLP19N1	99.5	99.1	99.1	99.1	99.1	98.7	99.1	99.5	99.1	98.7	99.1	98.2	-									
14	HCMLSV4N1	99.5	99.1	99.1	98.2	98.7	98.7	99.1	99.5	99.1	98.7	99.1	98.2	99.1	-								
15	HCMLSV18N2	99.1	98.7	98.7	98.7	98.2	98.2	98.7	99.1	98.7	98.2	98.7	97.8	98.7	99.5	-							
16	HCMLSV15N3	98.7	98.2	98.2	98.7	99.5	98.7	98.2	98.7	98.2	98.7	98.2	98.2	98.7	98.2	98.7	-						
17	X78803	98.7	99.1	98.7	98.2	98.2	98.7	99.1	98.7	98.7	98.7	98.2	98.2	99.1	98.2	97.8	97.8	-					
18	EU084691	99.5	100	99.5	98.2	98.7	99.5	100	99.5	99.5	99.5	99.1	99.1	99.1	99.1	98.7	98.2	99.1	-				
19	KP101363	100	99.5	99.5	98.7	99.1	99.1	99.5	100	99.5	99.1	99.5	98.7	99.5	99.5	99.1	98.7	98.7	99.5	-			
20	X78812	97.4	97.8	97.4	96.9	96.9	97.4	97.8	97.4	97.4	97.4	96.9	96.9	97.8	96.9	96.5	96.5	98.7	97.8	97.4	-		
21	KF364623	96.9	97.4	96.9	96.5	96.5	96.9	97.4	96.9	96.9	96.9	96.5	96.5	97.4	96.5	96.1	96.1	98.2	97.4	96.9	99.5	-	
22	MH481601	96.9	97.4	96.9	96.5	96.5	96.9	97.4	96.9	96.9	96.9	96.5	96.5	97.4	96.5	96.1	96.1	98.2	97.4	96.9	99.5	100	-

Note: 1-6, *H. contortus* from Thailand; 7-16, *H. contortus* from Lao PDR; 17-19, *H. contortus* references; 20-22, *H. placei* references.

Table 5. Nucleotide polymorphisms and distribution of six Thai genotypes and ten Laos genotypes of *H. contortus* in goats compared with sequence references of *H. contortus* and *H. placei*

No.	Genotypes	Variable positions												Number of *H. contortus*							
		4	10	18	21	22	24	55	65	123	196	205	219	CR	CN	NS	Total	LP	SV	CH	Total
1	X78803	C	C	T	C	T	A	C	A	C	A	G	A	-	-	-	-	-	-	-	-
2	KP101363	.	.	.	G	.	.	.	.	T	T	.	.	-	-	-	-	-	-	-	-
3	HCMTNS1N17 (MT682914)	.	.	.	G	.	.	.	.	T	T	.	.	4	2	4	10	-	-	-	-
4	HCMTCN3N2 (MT682915)	.	.	.	.	.	.	.	.	T	T	.	.	2	3	3	8	-	-	-	-
5	HCMTCN5N2 (MT682916)	.	.	.	S	.	.	.	.	T	T	.	.	0	2	1	3	-	-	-	-
6	HCMTCN15N1 (MT682917)	.	.	W	G	Y	.	.	.	T	.	.	.	0	1	0	1	-	-	-	-
7	HCMTCR2N1 (MT682918)	.	A	.	G	.	.	.	.	T	W	.	.	1	0	0	1	-	-	-	-
8	HCMTNS2N2 (MT682919)	.	A	.	.	.	.	.	.	T	T	.	.	0	0	1	1	-	-	-	-
9	HCMLCH3N1 (MT682957)	.	.	.	.	.	.	.	.	T	T	.	.	-	-	-	-	5	0	5	10
10	HCMLSV3N1 (MT682958)	.	.	.	G	.	.	.	.	T	T	.	.	-	-	-	-	0	4	2	6
11	HCMLCH1N1 (MT682959)	.	.	.	S	.	.	.	.	T	T	.	.	-	-	-	-	0	0	2	2
12	HCMLLP13N3 (MT682960)	.	A	.	.	.	.	.	.	T	T	.	.	-	-	-	-	1	1	0	2
13	HCMLLP5N1 (MT682961)	.	.	.	G	C	.	.	.	T	T	.	.	-	-	-	-	1	0	0	1
14	HCMLLP18N1 (MT682962)	A	A	.	.	.	.	.	.	T	T	.	.	-	-	-	-	1	0	0	1
15	HCMLLP19N1 (MT682963)	.	.	.	G	.	.	.	.	T	.	.	.	-	-	-	-	1	0	0	1
16	HCMLSV4N1 (MT682964)	.	.	.	G	.	.	T	.	T	T	.	.	-	-	-	-	0	1	0	1
17	HCMLSV18N2 (MT682965)	.	.	W	G	.	.	T	.	T	T	.	.	-	-	-	-	0	1	0	1
18	HCMLSV15N3 (MT682966)	.	A	W	G	.	.	.	.	T	W	.	.	-	-	-	-	0	1	0	1
19	X78812	.	.	.	.	.	G	.	.	.	.	A	G	-	-	-	-	-	-	-	-
20	KF364623	.	.	.	.	.	G	.	T	.	.	A	G	-	-	-	-	-	-	-	-
21	MH481601	.	.	.	.	.	G	.	T	.	.	A	G	-	-	-		-	-	-	-
	Total													**7**	**8**	**9**	**24**	**9**	**8**	**9**	**26**

Note: (.), represents similar positions; 1-2, *H. contortus* references; 3-8, *H. contortus* from Thailand; 9-18, *H. contortus* from Lao PDR; 19-21, *H. placei* references.

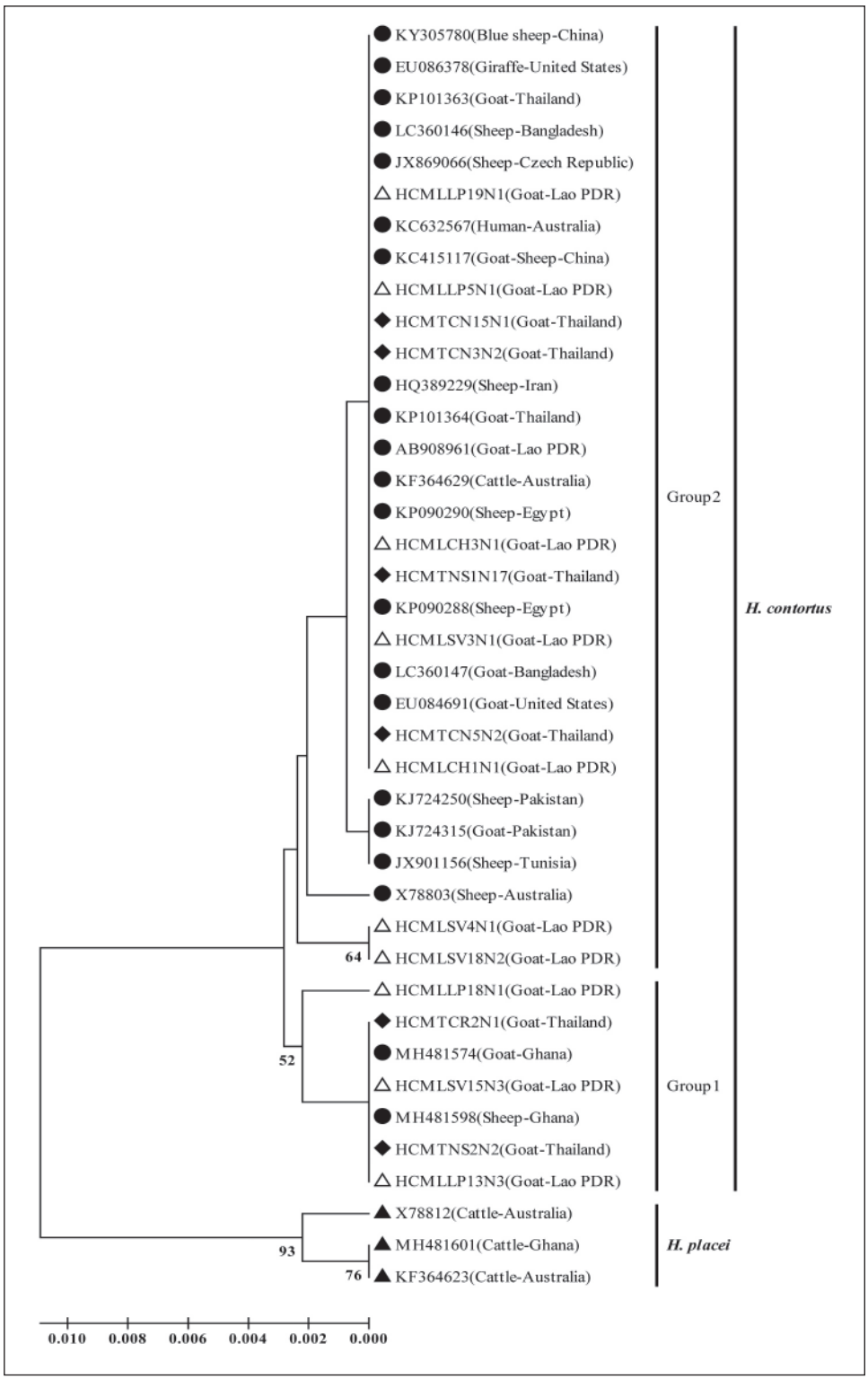

Figure 5. UPGMA analysis of *H. contortus* genotypes in goats from Thailand (six genotypes) and Lao PDR (ten genotypes) based on ITS2 comparison with sequences of *H. contortus* from different hosts in several countries together with *H. placei* (Accession numbers: X78812, KF364623, MH481601) as outgroups.

Table 6. Morphological comparisons of adult male *H. contortus* in goats between Thailand and Lao PDR

Characters	Country	N	Mean	SD	Median	Min-Max	W	p-value
Body length	Thailand	255.00	15.51	1.20	15.50	(12.50-18.00)	27587.00	0.003*
(mm)	Lao PDR	255.00	15.24	1.32	15.00	(12.50-19.50)		
Cervical papillae	Thailand	255.00	398.71	31.01	400.00	(310-470)	32624.00	0.947
(µm)	Lao PDR	255.00	399.75	25.89	400.00	(330-465)		
Esophagus length	Thailand	255.00	1295.98	107.86	1300.00	(1025-2375)	33094.00	0.727
(µm)	Lao PDR	255.00	1305.18	98.94	1300.00	(1050-1600)		
Gubernaculum	Thailand	255.00	214.53	11.40	210.00	(190-250)	39145.00	0.000*
(µm)	Lao PDR	255.00	219.31	13.73	220.00	(190-260)		
Spicule length	Thailand	255.00	430.08	21.18	430.00	(370-490)	30799.00	0.301
(right), (µm)	Lao PDR	255.00	428.59	21.17	425.00	(375-490)		
Spicule length	Thailand	255.00	428.96	21.11	430.00	(370-485)	30884.00	0.326
(left), (µm)	Lao PDR	255.00	427.53	21.10	425.00	(380-480)		
Spicule barb	Thailand	255.00	44.22	2.74	45.00	(36.25-52.50)	29616.00	0.076
length (right), (µm)	Lao PDR	255.00	43.79	2.68	43.75	(37.50-50.00)		
Spicule barb	Thailand	255.00	22.74	1.78	22.50	(15.00-27.50)	27218.00	0.001*
length (left), (µm)	Lao PDR	255.00	22.24	2.15	22.50	(15.00-27.50)		
Synlophe: EI	Thailand	255.00	29.99	0.09	30.00	(29-30)	32513.00	0.999
	Lao PDR	255.00	29.99	0.09	30.00	(29-30)		
Synlophe: 4 mm	Thailand	255.00	26.01	0.14	26.00	(25-27)	32643.00	0.790
	Lao PDR	255.00	26.02	0.27	26.00	(25-27)		

Abbreviations: N, number of worms; SD, standard deviation; Min, minimum; Max, maximum; W, Wilcoxon signed-rank test; EI, esophagus intestinal junction; 4 mm, region at 4 mm from the anterior end.
*Difference is statistically significant at $p<0.05$.

populations from Thailand and Lao PDR. The mean/median body length and left spicule barb length of this parasite from Thailand (mean=15.51 mm, median=15.50 mm; mean=22.74 µm, respectively) were slightly longer than those observed in *H. contortus* from Lao PDR (mean=15.24 mm, median=15 mm; mean=22.24 µm, respectively) while the gubernaculum of *H. contortus* in Thailand (mean=214.53 µm, median=210 µm was shorter than in *H. contortus* from Lao PDR (mean=219.31 µm median=220 µm) ($p < 0.05$) (Table 6).

DISCUSSION

Haemonchus spp. are blood sucking nematodes that cause a serious health problem for small ruminant production worldwide, particularly *H. contortus* that has been recovered from many parts of the world (Achi *et al.*, 2003; Kumsa *et al.*, 2008; Gharamah *et al.*, 2012; Akkari *et al.*, 2013; Yin *et al.*, 2013; Shen *et al.*, 2017; Kandil *et al.*, 2017; Dey *et al.*, 2019). Similarly, goats in Thailand and Lao PDR are now being raised in greater numbers, especially in smallholder form. Haemonchosis in goats caused by *Haemonchus* spp. is a continuous threaten with the current infection rate being 47.62% in Thailand and 66.02% in Lao PDR. Previously, postmortem and coproculture examination in Thailand and Lao PDR recorded high infection, accounting for 86.67% (26/30) (Laosutthipong & Eardmusic, 2019) and 69% (Sato *et al.*, 2014), respectively. This occurrence of *Haemonchus* spp. in these two tropical countries might be a consequence of suitable environmental

conditions such as moisture and temperature to facilitate the complete life cycle as well as these parasites being well adapted to survive in unfavorable conditions such as drought and high temperature. Moreover, they also can develop rapidly against many types of anthelmintic drugs (Besier *et al*., 2016; Emery *et al*., 2016).

The discriminant function (DF) described by Jacquiet *et al*. (1997) and synlophe patterns described by Lichtenfels *et al*. (1994) were mainly applied to identify *Haemonchus* spp. in naturally infected goats in Thailand and Lao PDR. DF values based on the spicule and spicule barb lengths have been widely and successfully used to identify *Haemonchus* spp. (Achi *et al*., 2003; Gharamah *et al*., 2011; Santos *et al*., 2014; Vadlejch *et al*., 2014; Sambodo *et al*., 2018) since this is a rapidly and easily performed method (Jacquiet *et al*.,1997). Our findings also revealed that DF was able to identify a predominant species, *H. contortus* both in Thailand and Lao PDR, accounting for about 94%. Only small proportions of specimens in Thailand (5.88%) and Lao PDR (5.10%) were classified as *H. placei* and hybrids (Table 1). However, all *H. placei* and hybrids were confirmed using PCR as *H. contortus*. Similarly, Vadlejch *et al*. (2014) revealed that three individual male *H. placei* obtained from sheep identified by DF were proved as *H. contortus* using molecular techniques, and they suggested that the DF was not able to provide accurate identification of male *Haemonchus* spp. to the species level, while Santos *et al*. (2014) also found species misidentification of adult male *Haemonchus* spp. in sheep. These findings indicated that misidentification based on the DF is not uncommon, and other forms of morphological examination are required.

The synlophe patterns described by Lichtenfels *et al*. (1994) were also applied in the current research and proved to be one of the most useful diagnostic morphological characters to distinguish *Haemonchus* spp. (Lichtenfels *et al*., 1986; Lichtenfels *et al*., 1994; Vadlejch *et al*., 2014). Using synlophe patterns based on the number of ridges either at the esophageal intestinal junction (EI) or 4 mm from the anterior end or from both positions of *Haemonchus* spp. in goats from Thailand and Lao PDR, about 97-99% were identified as *H. contortus*, showing 30 ridges and 26 ridges, respectively, while synlophe patterns in the EI region seemed to be more reliable resulting in 99% of *H. contortus* being identified. These results were based on Lichtenfels *et al*. (1994) and Silva *et al*. (2015) who reported the position in the EI region had 30 ridges and Rahman & Abd Humid (2007) reported that the region at 4 mm from the anterior end had 26 ridges in goats. However, 24 ridges for *H. contortus* at position 4 mm from the anterior end were also recorded in sheep (Rahman & Abd Humid, 2007) while Lichtenfels *et al*. (1994) reported 25 ridges; thus the synlophe patterns at this position of *H. contortus* varied somewhat between 24, 25, and 26 ridges. This variation in the cuticular ridge patterns of male *H. contortus* also occurred at similar positions in both countries; for example, in the EI region, the synlophe patterns presented 29 ridges, while variations in ridge number at 4 mm from the anterior end showed only 27 ridges in both countries. A closely related species, *H. placei* including *H. similis* showed synlophe patterns in the EI region for both these species having 34 ridges (Lichtenfels *et al*., 1994; Silva *et al*., 2015) and synlophe patterns at 4 mm from the anterior end of *H. placei* and *H. similis* presented 30 ridges (Lichtenfels *et al*., 1994). Therefore, our observations of varied ridge numbers for *H. contortus* at both positions were not consistent with the synlophe references regarding *H. placei* and *H. similis*.

However, all synlophe pattern variations mentioned above were properly resolved using PCR, and then identified as *H. contortus*, and we assumed that all 255 male *Haemonchus* spp. in each country were *H. contortus*. Therefore, the number of possible cuticular ridge patterns of *H. contortus* in the present study from both countries ranged from 29 to 30 ridges in the EI region and from 26 to 27 ridges at 4 mm from the anterior end, while the mean/median ridge numbers at both positions between two countries were not significantly different ($p>0.05$) (Table 6). These variable cuticular ridges in the current

study might occur naturally depending on many factors such as hosts and the area of study, as Rahman & Abd Humid (2007) reported synlophe patterns in the region 4 mm from the anterior end in goats and sheep in Malaysia with 26 and 24 ridges, respectively.

Molecular diagnosis based on the ITS2 region is commonly used as a molecular marker for specific identification of strongylid worms because of its low intra-variation (Heise *et al*., 1999; Gasser & Newton, 2000). According to our genetic data analysis as well as the phylogenetic tree based on UPGMA, all proved specimens (including four and eight randomly selected *H. contortus* from Thailand and Lao PDR, respectively identified clearly by their DF and synlophe patterns) from Thailand (Table 2) and Lao PDR (Table 3) were classified as a mono species, *H. contortus*. To provide accuracy for the identification of this species, some supporting evidences demonstrated that *H. contortus* was differentiated from a closely relate species, *H. placei* by the different nucleotide bases at positions 24, 205 and 219. These three positions were only found in *H. placei* and never presented in *H. contortus*, which was in agreement with previous reports in Bangladesh (Dey *et al*., 2019), Thailand (Mangkit *et al*., 2014), and the Czech Republic (Vadlejch *et al*., 2014). Moreover, the nucleotide difference at position 24 also is known as a restriction site of *Bfa*I of *H. placei*, being can be used as a diagnostic marker to differentiate *H. placei* from *H. contortus* (Stevension *et al*., 1995). In addition, the mean sequences identity of *H. contortus* in goats between the two countries showed 98.97% homology, compared with 97% homology for *H. placei* in Thailand and 96.86% homology in Lao PDR. The UPGMA method on a global scale is well supported and this method in the current study demonstrated that all genotypes of *H. contortus* from Thailand and Lao PDR were grouped in the same clade with *H. contortus* from various hosts in different countries with 98.77% homology, and were also clearly distinguished from *H. placei* with 97.09% homology on average. These features are consistent with some reports in Thailand (Mangkit *et al*., 2014; Laosutthipong & Eardmusic, 2019) and in Bangladesh (Dey *et al*., 2019). Interestingly, we also found that there were some genotypes of *H. contortus* that shared their genetic information between the two countries that might have been a consequence of infected host movement through Thailand-Laos border crossing points from the past and ongoing in the present. Therefore, based on the DF combined with synlophe patterns and supporting genetic data analysis using the UPGMA method, we assumed that all selected randomly specimens obtained from goats in Thailand and Lao PDR were the highly pathogenic nematode, *H. contortus*.

Significant morphological differences of *H. contortus* between Thailand and Lao PDR in the recent study were found in the body length, gubernaculum, and the left spicule barb length (Table 6). These differences might be associated with some specific factors, namely the area of study, diet, age of host, and parasite intensity (Kuchai *et al*., 2012) including the effect of fixative agent (ethanol) on the body of the parasite (Jacquite *et al*., 1997). Our findings indicated that the mean body length and other structure lengths of male *H. contortus* from both countries were varied somewhat compared with the findings of other researchers in previous investigations depending on the location of the study and the hosts, such as in ruminants in North America (Lichtenfels *et al*.,1994), in goats and sheep in Malaysia (Rahman & Abd Humid, 2007; Gharamah *et al*., 2011; Gharamah *et al*., 2014), in sheep in Egypt (Badawy *et al*., 2015), in goats in Indonesia (Sambodo *et al*., 2018), and in sheep in Brazil (Santos *et al*., 2014). However, most researchers recommended using the spicule and barb lengths as some of the most useful morphological characters for classifying *Haemonchus* spp. in ruminants (Jacquiet *et al*., 1997; Achi *et al*., 2003; Kumsa *et al*., 2008; Gharamah *et al*., 2011; Gharamah *et al*., 2014). In fact, our observations based on these characters of some individual *H. contortus* from Thailand and Lao PDR were not all within the ranges for *H. contortus* described by Lichtenfels *et al*. (1994), and some specimens

also overlapped in length with other species except for the spicule and barb lengths of *H. similis*, a parasite of cattle, that were distinguished clearly. In contrast, considering the mean lengths of the right and left spicule and barbs of *H. contortus* in the current study, there were minor differences from the mean length of the spicule (425 µm) and right (42 µm)/left (22 µm) barbs of *H. contortus* as referenced by Lichtenfels *et al.* (1994). As mentioned above, overlapping measurements of spicule and barb lengths including other structure lengths based on the individual worms in the current study were also consistent with some researchers, namely Lichtenfels *et al.* (1994), Santos *et al.* (2014), and Vadlejch *et al.* (2014), especially for the two main species, *H. contortus* and *H. placei*. Our study suggested that the use of these spicule and barb lengths may influence species identification and lead to misclassification in some samples. This is in agreement with Silva *et al.* (2015) who revealed that overlapping measurements occurred in the body length of some female worms, making this parameter are less valuable for identifying *Haemonchus* spp. However, the current observations revealed that applying spicule and barb lengths via the DF formula might be a better approach, while synlophe patterns and possibly molecular methods should be combined.

CONCLUSION

The present study was the first morphological identification of adult male *Haemonchus* spp. in goats raised in different regions of Thailand and Lao PDR using the discriminant function (DF) and synlophe patterns at the esophageal intestinal junction (EI) and at 4 mm from the anterior end. This research revealed the DF and synlophe patterns to be useful tools, particularly the synlophe patterns for identifying *Haemonchus* spp. and further subclassification to *H. contortus*. The synlophe patterns in the EI region seemed to be more promising than at 4 mm from the anterior end for identifying *Haemonchus* spp. Therefore, the use of the DF combined with synlophe patterns increased the accuracy of identifying *Haemonchus* spp. using a low-cost diagnostic device, and also provided invaluable epidemiological data on these parasites of goats in Thailand and Lao PDR, where advanced techniques and large budgets are not available in endemic areas. However, in some specimens, molecular techniques are also required.

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Ethics approval
This research was approved by the Kasetsart University Institutional Animal Care and Committee Use with the approval no. ACKU62-VTN-006, Kasetsart University, Thailand.

Conflict of interests
The authors declare no conflict of interest.

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