

## Discriminant analysis as a method for the numerical evaluation of taxonomic characters in male trichurid nematodes

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### Abstract

Samples of males of *Trichuris ovis* (Abildgaard, 1795), *T. skrjabini* Baskakov, 1924, *T. sylvilagi* Tiner, 1950, *T. suis* (Schrank, 1788) and *T. trichiura* (L., 1771) were compared on the basis of 15 morphometric characters. An analysis of their variability, correlations and a discriminant analysis yielded information on the diagnostic value of these characters and on the best combination of characters necessary for the successful differentiation of the species studied. The morphological similarity of the species studied was also assessed. The length and width of the spicule and body length proved to be the best diagnostic characters for the trichurid males studied. They permitted fairly reliable differentiation of the species *T. ovis*, *T. skrjabini* and *T. sylvilagi*. The species which exhibited the greatest similarity, *T. suis* and *T. trichiura*, were frequently misidentified using these features only but could be readily differentiated using seven characters.

### Introduction

Helminths represent a group of animals marked by high phenotypic variability. Reliable qualitative differential characters are often missing in cases where there are numerous closely related species, or at least they have not been determined, thus regularly causing taxonomic problems. Many species of the genus *Trichuris* Roederer, 1761 are morphologically well-defined; however, some of them can hardly be differentiated from one another. In such cases, the main identification criterion is their host species, e.g. *T. suis* (Schrank, 1788) from pigs and *T. trichiura* (L., 1771) from humans (Beer, 1976). Moreover, the morphological evaluation of numerous population samples often shows that the ranges of most or of all the characters in the species compared overlap and that no hiatuses exist (Gagarin, 1974).

In the present study we demonstrate how to

evaluate such morphometric characters, how to combine them and their possible use in the taxonomy of five trichurid species.

### Materials and methods

Measurements were made from sets of 50 adult males of five trichurid species with the aid of a drawing tube and map measurer. The following material was studied:

*Trichuris ovis* (Abildgaard, 1795) (OVI)

*Hosts:* Ten sheep *Ovis aries* harboured 22–71 worms.

*Locality:* Tomášovce, the Slovak Republic.

*Samples measured:* Five randomly selected adult males per host individual (n = 50).

*Trichuris skrjabini* Baskakov, 1924 (SKR)

*Hosts:* Ten sheep *Ovis aries* harboured 15–88 worms.

*Locality:* Spišský Štvrtok, the Slovak Republic.

*Samples measured:* Five randomly selected adult males per host individual (n = 50).

*Trichuris sylvilagi* Tiner, 1950 (SYL)

*Hosts:* Ten hares *Lepus europeus* harboured 11–55 worms.

*Locality:* Kolárovo, the Slovak Republic.

*Samples measured:* Five randomly selected adult males per host individual (n = 50).

Helminths of these three species are deposited in the museum collection of Parasitological Institute SAS in Košice.

*Trichuris suis* (Schrank, 1788) (SUI)

*Hosts:* Six yearling pigs *Sus scrofa* f. dom. harboured 45–132 worms.

*Locality:* Pardubice, the Czech Republic.

*Samples measured:* Eight randomly selected adult males per host individual from 5 pigs and 10 males from the 6th pig (n = 50).

*Trichuris trichiura* (L., 1771) (TRI)

*Hosts:* Two men *Homo sapiens* harboured 65 and 81 worms.

*Locality:* Havana, Cuba.

*Samples measured:* Twenty-five randomly selected adult males per host individual (n = 50).

Helminths of the last two species are in the collection of Prof. Hynek Lýsek, D.SC., Medical Faculty of Palacký University, Olomouc, the Czech Republic.

The selection of *Trichuris* spp. was influenced by their availability in sufficient numbers. We intentionally used species of a very similar morphology (*T. suis* and *T. trichiura*), as well as of a very distinct morphology (*T. ovis* and *T. skrjabini*).

The following morphometric features (some are

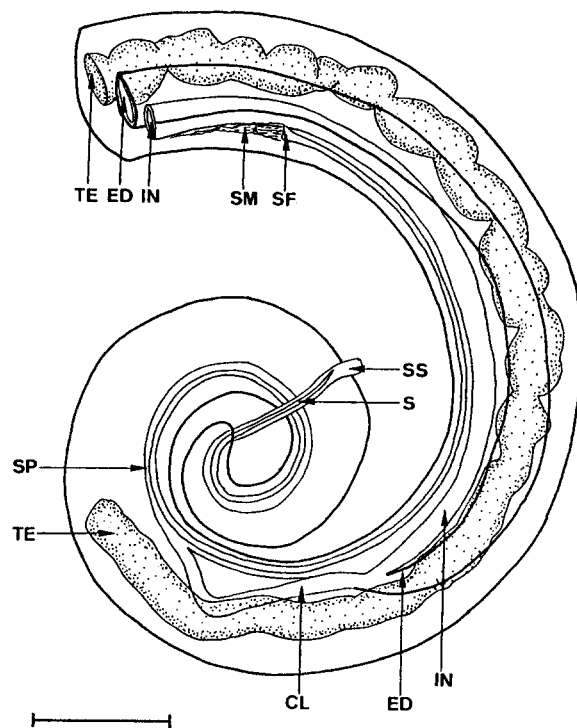


Fig. 1. Posterior end of male *Trichuris ovis*. Abbreviations: CL, cloaca; ED, ejaculatory duct; IN, intestine; S, spicule; SF, spicule shaft; SM, spicule retractor muscle; SP, spicule pouch; SS, spicule sheath; TE, testis. Scale-bar: 1 mm.

illustrated in Fig. 1) were measured in each specimen:

1. Total body length (BL).
2. Anterior body length (AL).
3. Anterior body width (AW).
4. Maximum posterior body width (PW).
5. Middle body width in place of oesophago-intestinal junction (MW).
6. Distance between cephalic extremity and anterior end of bacillary band (CBB).
7. Bacillary band length (BBL).
8. Spicule length (SL).
9. Spicule shaft width (SW).
10. Spicule sheath length (SSL).
11. Spicule sheath width in basal region (SSW).
12. Maximum width of spicular sheath (MSSW).
13. Cloaca length (CL).
14. Length of ejaculatory duct (EDL).

15. Distance between posterior end of testis and caudal extremity of body (TE).

The basic statistics (mean values, standard errors, coefficients of variation and correlation coefficients) were calculated for every species. The significance of correlation coefficients was tested at  $P < 0.01$  using Lawley's approximation. The existence of hiatuses between species were evaluated using the coefficient of difference

$$\text{C.D.} = \frac{\bar{X}_A - \bar{X}_B}{\text{S.D.}_A + \text{S.D.}_B},$$

where  $\bar{X}_A$  and  $\bar{X}_B$  are the means of evaluated features and  $\text{S.D.}_A$  and  $\text{S.D.}_B$  are their standard deviations. The difference of two species in relation to a particular character was considered as the hiatus, when the C.D. was higher than 1.75. The value represents the 96% non-overlap, i.e. 96% of individuals of species A did not overlap 96% of species B in the evaluated character (Mayr *et al.*, 1953).

Multivariate discriminant analysis (Manly, 1986) was used in this study as follows: each pre-specified trichurid male was characterised by a complex of 15 measured values. On the basis of these quantitative characters the discriminant functions were computed for each species. Then a theoretical reclassification of individuals was made independently of the original identification. Some of the worms with extreme values in terms of their characters corresponded more closely to the theoretical characteristics of another species and were classified incorrectly. The rule holds that the more the reclassification corresponds with the original identification, the more the species differ from each other.

The stepwise discriminant functions were calculated as a combination of the minimum number of characters necessary to best separate the species samples. The criterion of the quality of the classification procedure based on the selected characters is the probability of correct classification.

The discriminant analysis also permits the calculation of Mahalanobis generalised distances  $D^2$ , which represent the similarity among the different

species analysed. The lower the value of  $D^2$ , the more similar the sets are. The squares of  $D^2$  can be shown graphically. Three dimensions are required to express these relationships properly, but it is possible to draw a two-dimensional figure as a projection of a three-dimensional image onto a flat surface.

The data were processed at the Biometric Laboratory of The Department of Biophysics and Nuclear Medicine of the Medical Faculty of Palacký University in Olomouc (the Czech Republic).

## Results

The basic morphometric characteristics of *Trichuris* spp. males and a survey of differences in these groups are presented in Tables I and II.

Most characters of *T. trichiura* varied to a lesser degree than those of the other 4 species. The variability of the lengths (BL, AL), the widths of the body (AW, PW and MW) and the length of the spicule (SL) tended to be the lowest amongst all the characters in all of the species measured. Conversely, the features of the spicule sheath (SSL, SSW and MSSW) exhibited the highest variance, being determined by the degree of sheath evagination from the body (Tables I, II).

Hiatuses were ascertained in the length and width of spicule (SL, SW). They permitted differentiation of *T. skrjabini*, with its short, thin spicule, *T. suis* and *T. trichiura*, with their moderately long, thick spicule, *T. sylvilagi*, with its long, thin spicule, and *T. ovis*, with its long, thick spicule (Table II).

No hiatus was found in any character between *T. suis* and *T. trichiura*. They both differed, however, from other species in the length of the ejaculatory duct (EDL) and from *T. skrjabini* in the length of the cloaca (CL) (Table II).

A correlation coefficient analysis showed a strong correlation between the total length of the body (BL) and the length of the anterior body (AL), as well as between the width of the posterior (PW) and the middle (MW) of body in all of the species studied. Other groups of characters correlated in most of the species studied included

Table 1. Variability of the morphometric characters of *Trichuris* spp. males. Measurements in millimetres.

Character	<i>T. ovis</i> (n = 50)			<i>T. skrjabini</i> (n = 50)			<i>T. sylvilagi</i> (N = 50)			<i>T. suis</i> (n = 50)			<i>T. trichiura</i> (n = 50)		
	Mean ± S.D.	Range	CV (%)	Mean ± S.D.	Range	CV (%)	Mean ± S.D.	Range	CV (%)	Mean ± S.D.	Range	CV (%)	Mean ± S.D.	Range	CV (%)
1. (BL)*	60.91 ± 7.96	(46.8–88.6)	13.1	48.48 ± 4.91	(36.7–59.5)	10.1	38.70 ± 3.85	(25.9–44.2)	9.9	35.03 ± 3.49	(28.7–42.2)	10.0	35.92 ± 2.26	(31.4–41.1)	6.3
2. (AW)	40.28 ± 6.34	(28.1–66.6)	15.7	33.13 ± 3.80	(25.6–42.5)	11.5	22.44 ± 2.54	(13.3–25.8)	11.3	22.54 ± 2.22	(17.3–27.1)	9.9	22.43 ± 1.76	(18.2–26.3)	7.9
3. (AL)	0.16 ± 0.02	(0.14–0.23)	12.5	0.15 ± 0.01	(0.14–0.18)	7.4	0.18 ± 0.01	(0.15–0.21)	7.5	0.18 ± 0.01	(0.15–0.20)	6.8	0.14 ± 0.01	(0.12–0.17)	7.6
4. (PW)	0.72 ± 0.10	(0.50–1.03)	14.4	0.60 ± 0.06	(0.45–0.71)	10.4	0.70 ± 0.05	(0.57–0.82)	7.7	0.66 ± 0.08	(0.42–0.82)	11.8	0.60 ± 0.04	(0.54–0.76)	6.8
5. (MW)	0.32 ± 0.04	(0.23–0.44)	12.5	0.29 ± 0.03	(0.23–0.38)	11.1	0.30 ± 0.04	(0.23–0.26)	12.9	0.31 ± 0.03	(0.24–0.38)	11.0	0.41 ± 0.04	(0.30–0.48)	9.7
6. (CBB)	0.70 ± 0.17	(0.34–0.96)	24.9	0.63 ± 0.10	(0.42–0.87)	15.9	0.68 ± 0.09	(0.35–0.82)	12.9	0.63 ± 0.09	(0.42–0.87)	13.6	0.55 ± 0.11	(0.38–1.02)	19.8
7. (BBL)	1.38 ± 0.25	(0.85–1.79)	18.1	2.05 ± 0.35	(1.02–2.71)	17.0	1.89 ± 0.20	(1.55–2.42)	10.7	1.54 ± 0.34	(1.09–2.47)	22.9	1.41 ± 0.22	(0.77–1.84)	15.9
8. (SL)	6.12 ± 0.58	(5.10–8.58)	9.4	1.12 ± 0.11	(0.85–1.30)	9.8	7.17 ± 0.92	(3.96–9.12)	12.8	2.09 ± 0.17	(1.66–2.45)	8.1	2.54 ± 0.16	(2.23–2.96)	6.1
9. (SW)	0.09 ± 0.03	(0.06–0.21)	30.2	0.02 ± 0.01	(0.01–0.04)	31.3	0.03 ± 0.01	(0.01–0.04)	28.4	0.07 ± 0.01	(0.04–0.10)	18.7	0.06 ± 0.01	(0.04–0.09)	19.2
10. (SSL)	1.53 ± 0.64	(0.54–4.32)	42.2	0.09 ± 0.05	(0.00–0.26)	56.0	0.48 ± 0.28	(0.00–1.27)	62.1	0.22 ± 0.12	(0.03–0.57)	55.3	0.25 ± 0.16	(0.00–0.66)	63.7
11. (SSW)	0.07 ± 0.01	(0.04–0.10)	17.0	0.03 ± 0.01	(0.00–0.05)	38.5	0.02 ± 0.01	(0.00–0.03)	44.3	0.07 ± 0.02	(0.04–0.12)	24.4	0.05 ± 0.02	(0.00–0.08)	40.9
12. (MSSW)	0.12 ± 0.05	(0.06–0.23)	43.7	0.04 ± 0.02	(0.00–0.08)	39.9	0.05 ± 0.02	(0.00–0.12)	55.4	0.15 ± 0.11	(0.05–0.60)	74.5	0.06 ± 0.03	(0.00–0.18)	54.2
13. (CL)	1.95 ± 0.53	(1.06–3.02)	27.1	0.75 ± 0.18	(0.42–1.21)	24.6	1.81 ± 0.36	(1.09–2.72)	19.6	1.90 ± 0.36	(1.45–2.36)	12.3	1.98 ± 0.23	(1.51–2.30)	11.7
14. (EDL)	8.29 ± 1.36	(5.80–12.2)	16.4	8.69 ± 1.22	(6.49–10.7)	14.1	8.06 ± 1.23	(4.62–10.8)	15.3	3.21 ± 0.40	(2.60–4.14)	12.5	3.13 ± 0.39	(2.26–4.44)	12.4
15. (TE)	3.68 ± 1.13	(1.51–6.25)	30.6	1.88 ± 0.43	(1.00–2.72)	23.0	2.65 ± 0.68	(0.85–3.84)	25.8	3.23 ± 0.38	(2.48–4.08)	11.8	4.27 ± 0.39	(3.44–5.10)	9.1

\*For abbreviation see "Material and methods".

the length of body (BL) and various features of the reproductive system, such as the cloaca, ejaculatory duct and testis (CL, EDL and TE). The length (SSL) and both widths of the spicular sheath (SSW and MSSW) were also highly correlated.

The criterion for the judgement of the quality of the discrimination procedure is the probability (or percentage) of the classification being correct (Table III). The discrimination of males of the five trichurid species based on the set of 15 characters yielded an unambiguous result; all species differed from one another. The number of characters used for a subsequent analysis was reduced by eliminating the characters with a high variability and strong correlation. Four characters were analysed separately: total body length (BL); anterior body width (AW); spicule length (SL); and spicule shaft width (SW). The three best characters (SL, SW and BL) were used for a subsequent analysis. This combination of characters was sufficient to discriminate the species *T. ovis*, *T. skrjabini* and *T. sylvilagi*. However, 100% discrimination of the morphologically similar *T. suis* and *T. trichiura* was only possible using all 7 characters (Table III).

The great similarity of *T. suis* and *T. trichiura* was also confirmed by a graphic representation of the relative distances (Mahalanobis) between the species studied (Fig. 2). Another similar pair of species were *T. ovis* and *T. sylvilagi*. Conversely, the greatest differences were observed between males of *T. sylvilagi* and the other three species, *T. skrjabini*, *T. suis* and *T. trichiura*.

## Discussion

In this study morphometric variability was investigated in populations of five species of the genus *Trichuris*. Previously, Gagarin (1972) analysed the morphometric characters of thirteen trichurid species. He reported a considerable degree of variability, particularly in the characters relating to the reproductive system. Also, Knight (1972, 1984) and Hinks & Thomas (1974) studied the variability of *T. skrjabini* or *T. ovis* from various

Table II. A survey of morphological differences between *Trichuris ovis* (OVI), *T. skrjabini* (SKR), *T. sylvilagi* (SYL), *T. suis* (SUI) and *T. trichiura* (TRI) males.

Characters	Hiatuses between species*	Range of CV (%) in all species
Total and anterior body length (BL, AL)	–	6.3–15.7
Anterior, posterior and middle body width (AW, PW, MW)	–	6.8–14.4
Bacillary band (CBB, BBL)	–	10.7–24.9
Spicule length (SL)	SKR/SUI,TRI/OVI,SYL	6.1–12.8
Spicule width (SW)	SKR,SYL/TRI,SUI,OVI	18.7–31.3
Spicule sheath length (SSL)	SKR/OVI	42.2–63.7
Spicule sheath width (SSW, SW)	–	17.0–74.5
Cloaca (CL)	SKR/SUI,TRI	11.7–27.1
Ejaculatory duct (EDL)	SUI,TRI/SYL,OVI,SKR	12.4–16.4
Testis to posterior extremity (TE)	SKR/TRI	9.1–30.6

\*Comma indicates no hiatuses in the group of species; slash indicates hiatuses. Smaller character values are on the left, higher on the right side of the slash.

geographical regions and host species. Their measurements correspond well with our findings.

Rickard & Bishop (1991) produced a key to seven whipworm species from ruminants in North America, including *T. ovis* and *T. skrjabini*. These authors showed that some trichurid species can be characterised by unique qualitative characters, e.g. the males of *T. tenuis* can be recognised by the shape of the cloaca. The diagnostic characters of males of the other six species are the length of the spicule, the spicule shaft diameter and the length of the ejaculatory duct, which, especially the first two, also proved to be good characters in the present study.

The species *T. suis* and *T. trichiura* have long been a taxonomic problem: they are difficult to distinguish morphologically, being considered by some authors as synonyms (Sondak, 1948) and by others as valid species (Skrjabin *et al.*, 1957). Beer (1976) revealed differences in the sizes of their eggs and infective first stage larvae and in the rate of larval development. On the other hand, successful cross-infections were achieved in man with eggs of *T. suis* and in pigs with eggs of *T. trichiura*. Nevertheless, Beer suggested that the two parasites should be retained as separate species.

Using the scanning electron microscope, Tenora *et al.* (1992) found one pair of caudal papillae in males of *T. trichiura* which is absent in *T. suis* from different hosts and localities. The fact remains, however, that the similarity of the

species, based on morphometric features, is very high and no hiatuses were found between them. Only the combination of seven metric characters, as indicated in this study, permits us to distinguish the trichurids from pig to man.

As reported by Simpson *et al.* (1971), the coefficient of variation (CV) is very useful as a guide to the selection of characters that vary relatively little within a taxonomic group and are, therefore, useful for taxonomic comparisons. In case of trichurids, such characters include the length of the spicule and body, the width of the body, and the length of the ejaculatory duct. In these cases CV is <15%, which appears to be the limit for a relatively low degree of variability in this animal group. Most of the characters of *T. trichiura* exhibited lower CV values than those of the other four species, but this may be caused by the smaller number of *T. trichiura* hosts available and thus a smaller host influence on parasite variability.

Macko (1983, 1985) considered that the existence of hiatuses in the morphological characters between populations indicates the validity of species as well as their reproductive isolation. Hiatuses in our material were confirmed in only a few characters, namely in the spicule length and width, among the species *T. skrjabini*, the *T. suis/T. trichiura* group, *T. ovis* and *T. sylvilagi*. From this it can be inferred that the spicule is the best diagnostic character of trichurid males. Nevertheless, characteristics of the spicule are not adequate on their own to distinguish all species of

Table III. Classification matrix of *Trichuris* spp. males based on discriminant analysis.

Group	Percentage of cases classified into group					Average % correctly classified
	OVI	SKR	SYL	SUI	TRI	
Discrimination based on all 15 characters						
OVI*	<b>100</b>					
SKR		<b>100</b>				
SYL			<b>100</b>			100.0
SUI				<b>100</b>		
TRI					<b>100</b>	
Discrimination based on character BL*						
OVI	<b>82</b>	18**				
SKR	10	<b>82</b>	6		2	
SYL		22	<b>42</b>	30	6	56.4
SUI			24	<b>56</b>	20	
TRI			30	50	<b>20</b>	
Discrimination based on character AW						
OVI	<b>38</b>	30	6	16	10	
SKR	18	<b>54</b>		2	26	
SYL	16	4	<b>36</b>	44		43.2
SUI	28	4	20	<b>48</b>		
TRI	2	42			<b>56</b>	
Discrimination based on character SL						
OVI	<b>88</b>		12			
SKR		<b>100</b>				
SYL	8		<b>90</b>		2	92.4
SUI		2		<b>94</b>	4	
TRI				10	<b>90</b>	
Discrimination based on character SW						
OVI	<b>60</b>			24	16	
SKR		<b>68</b>	32			
SYL		32	<b>68</b>			59.4
SUI	24			<b>40</b>	36	
TRI	8		10	20	<b>62</b>	
Discrimination based on characters BL, SL and SW						
OVI	<b>100</b>					
SKR		<b>100</b>				
SYL			<b>98</b>		2	93.6
SUI				<b>82</b>	18	
TRI				12	<b>88</b>	
Discrimination based on characters BL, AL, AW, PW, MW, SL and SW						
OVI	<b>100</b>					
SKR		<b>100</b>				
SYL			<b>98</b>		2	99.6
SUI				<b>100</b>		
TRI					<b>100</b>	

\*For abbreviations see "Material and methods" and Fig. 2.

\*\*Numbers not in bold represent percentage of misclassified individuals of the species, e.g. 18% of *T. ovis* (OVI) were recognized by discriminant analysis as *T. skrajabini* (SKR), 10% of *T. skrajabini* were recognised as *T. ovis* and 6% as *T. sylvilagi*, and so on.

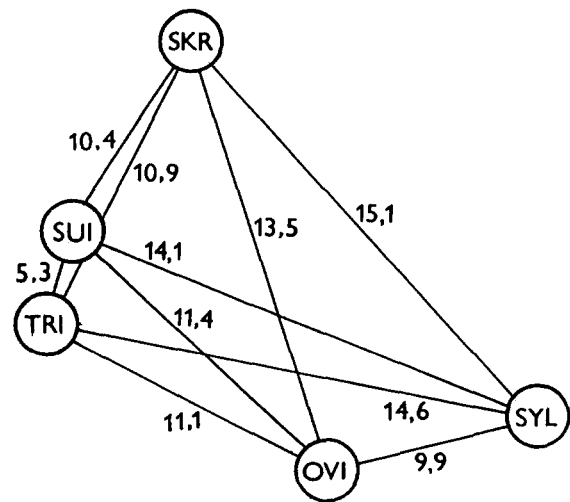


Fig. 2. Diagram of the similarity distances of trichurid males. Numbers represent a square of Mahalanobis  $D^2$  statistics. Abbreviations: OVI, *Trichuris ovis*; SKR, *T. skrajabini*; SUI, *T. suis*; SYL, *T. sylvilagi*; TRI, *T. trichiura*.

*Trichuris*. This conclusion was confirmed by the discriminant analysis, which permits the selection of further informative criteria, such as the length and width of the body.

In helminthology, discriminant analysis has rarely been used. Lýsek *et al.* (1975) used it to show that total egg length is the most reliable character for distinguishing *Trichuris trichiura* and *T. suis* eggs, and Georgi & McCulloch (1989) distinguished strongylid eggs recovered during faecal examination. Bray & des Clers (1992) discriminated the worms of five morphs of the *Lepidapedon elongatum* species-complex (Digenea) from different fishes. As in the case of our results, Bray & des Clers showed the advantages of the discrimination analysis for the evaluation of morphometric similarity (or differences) at the infraspecific and specific levels.

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