Chapter 5 - Diversity and classification of species found

5.1 Introduction: parasite diversity and epidemiology

In this chapter, the species of parasite found in saigas and livestock during the present study are presented, along with their host ranges, and overall patterns of diversity in different hosts. The classification of parasites found in saigas is then reviewed in the light of recent advances in nematode systematics. Inconsistencies in the description of *Marshallagia* spp. in the literature caused particular difficulties in the diagnosis of abomasal nematode infections, which were addressed through morphometric analysis of the specimens found.

The accurate identification of parasite species is fundamental to the study of parasite distribution between hosts, and justifies the careful approach to species classification used in this chapter (Viney and Read, 2002). Differences between superficially similar parasites in host preference, developmental requirements and tolerances, and pathogenicity could critically affect observed patterns of infection and disease. The importance of diversity within classified parasite types to the dynamics of parasitism in multiple host systems is discussed, especially in terms of perceived host specificity and transmission between host species.

The chapter ends by considering what presence-absence data in the present study can tell us about parasite transmission between saigas and domestic livestock in Kazakhstan, and, further, what role the measurement of parasite genetic diversity might play in refining our understanding of parasite transmission at the wildlife-livestock boundary in general.

5.2 Overview of parasites found

5.2.1 Species

Parasites found in saigas, sheep and goats are listed in Table 5.1. Collectively, all species have previously been found in these hosts (see chapter 3). *M. schikhobalovi* Altaev, 1953, of which two male specimens were found in saigas in Betpak-Dala, has been found previously in Kazakhstan in sheep but not in saigas.

Larval cestodes were found in saigas attached to the mesentery and mesocolon, and identified as *Taenia hydatigena*. No hydatid cysts (larval *Echinococcus granulosus*) were found in the 144 sets of lungs and 50 livers examined, though a previous expedition to Betpak-Dala in 1996 did find this species in saigas (P. Torgerson, unpublished data). No *post mortem* examination of livestock for metacestodes was undertaken in the present study, but sheep and dogs in Kazakhstan are known to be commonly infected with *Echinococcus granulosus*, especially in the South (Shaikenov *et al*, 1999, Torgerson *et al*, 2002).

No trematodes were found in saigas, or in livestock. Several trematode species are known to occur in small ruminants in Kazakhstan (Kuznetsov and Dikov, 1979), but would not have been found using these methods. No trematode eggs were found in 40 samples of saiga faeces tested for them, and no adult flukes in 50 livers. Nematodes in the airways or lungs, or pathological changes caused by them, were not found on gross examination of 144 sets of saiga lungs.

5.2.2 Diversity

Including eggs found in the faeces, and the division of *Marshallagia* into three species (see later), the total number of gastrointestinal helminths found was 28, of which 15 were found in saigas, 20 in sheep, and 14 in goats. In past studies of saiga parasites, the number of species found has tended to be higher in larger samples (Fig. 5.1). If this is true across host species, saigas seem to have a poorer helminth fauna than sympatric domestic ruminants, since in this study fewer parasite species were recovered from a larger sample of saigas compared with sheep. Further evidence of a relatively impoverished fauna in saigas comes from the absence of lungworms and liver flukes from saigas, but not from sheep, in past studies (see chapter 3). The mean gastrointestinal nematode species richness per individual host in this study is tabulated for different host populations in Table 5.2. In the abomasum, saigas had 9 species, one more than sheep, but the mean number of species found in individuals was lower (1.5 c.f. 2.7 in Betpak-Dala samples), and the total sample size much higher (133 c.f. 25).

Table 5.1. Gastrointestinal helminth parasites found in saigas and livestock in Kazakhstan. Sample sizes are given in chapter 4. Asterisks (*) indicate that infection was inferred from eggs in the faeces. Other parasites found were: *Taenia hydatigena* larvae in saigas, *Dictyocaulus* sp. larvae in sheep and goats, and coccidial oocysts (*Eimeria* spp.) in all host species. *Haemonchus* and *Marshallagia* were initially identified to genus level only, and each counted as a single species, due to complications described later in this chapter. Ab = abomasum, SI = small intestine, LI = large intestine.

Species	Saigas			Sheep			Goats		
	Ab	SI	LI	Ab	SI	LI	Ab	SI	LI
Cestodes									
Avitellina centripunctata		+			+				
*Moniezia benedeni**					+			+	
M. expansa		+			+			+	
Thyzaniezia giardi					+			+	
Nematodes									
Haemonchus sp.				+			+		
Marshallagia spp.	+	+		+	+		+		
Nematodirella longissimespiculata		+							
Nematodirus abnormalis					+			+	
N. archari	+								
N. dogieli	+	+							
N. filicollis					+				
N. gazellae	+	+							
N. oiratianus	+	+		+	+		+	+	
N. spathiger	+				+			+	
Ostertagia ostertagi				+					
Parabronema skrjabini	+			+			+		
Skrjabinema ovis			+						
*Strongyloides papillosus**					+			+	
Teladorsagia circumcincta	+			+			+		
*Toxocara vitulorum**					+				
Trichostrongylus axei				+			+		
T. colubriformis	+			+	+			+	
T. probolorus					+				
Trichuris ovis									+
T. skrjabini						+			

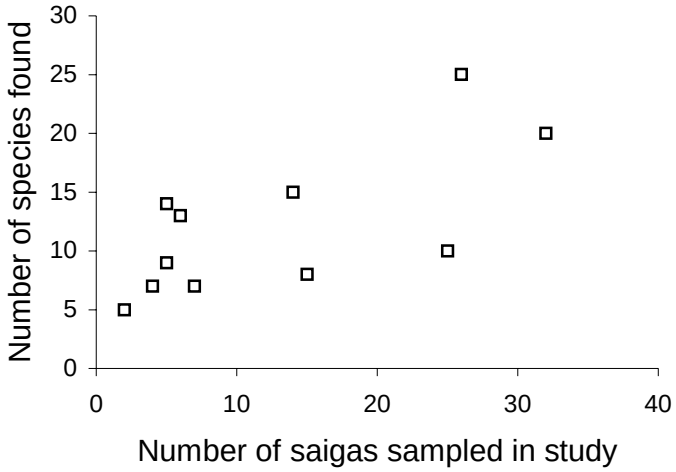


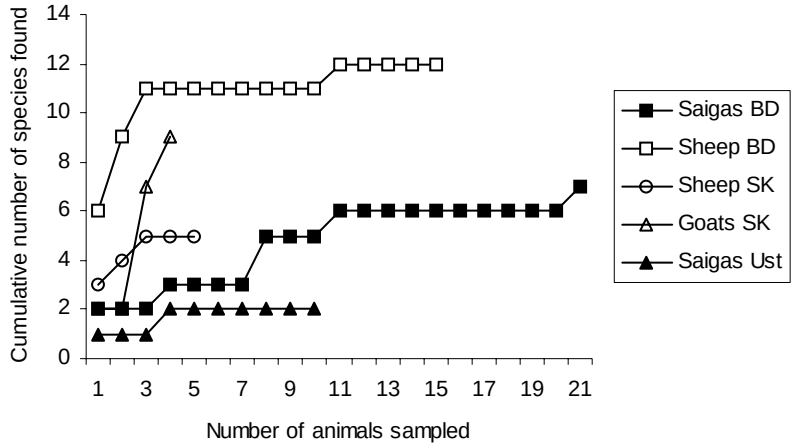
Figure 5.1. The number of species of gastrointestinal nematode found in saigas as a function of the number of animals sampled. Data from Sokolov and Lavrov (1956), Radionov (1973*a*), Scholl *et al* (1979), Berkinbaev (1992) and Shaikenov *et al* (1999). The present study found 15 species in 144 saigas, but fewer species were recognised (see text).

Table 5.2. The total species richness of nematodes in the abomasum and small intestine of saigas, sheep and goats in Kazakhstan, and mean species richness per individual animal. *Haemonchus* and *Marshallagia* were each counted as a single species. The median number of species in each host sample was significantly different in both anatomical sites (Kruskal-Wallis test, Chi-square 22.3, 4df, $p<0.001$; and Chi-square 23.9, $p<0.001$, in abomasum and in combined abomasum and small intestine, respectively). BD = Betpak-Dala; Ust = Ustiurt; SK = southern Kazakhstan (south of the BD saiga range); sp. = number of species; sd = standard deviation; n = number of animals sampled.

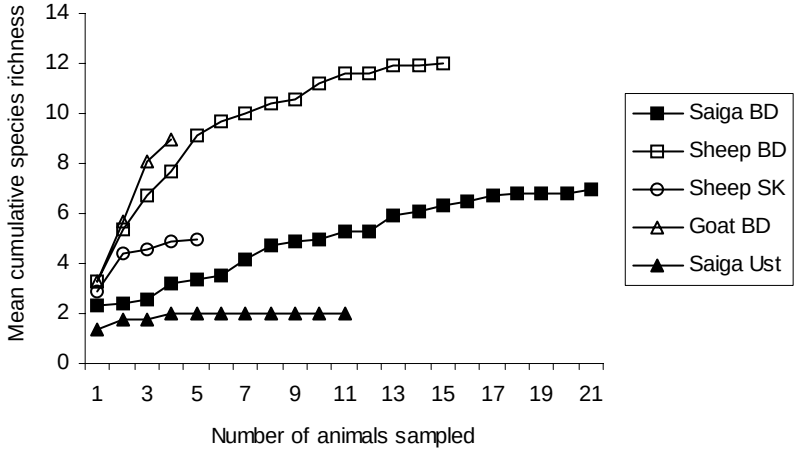
Population	Abomasum and Small Intestine			Abomasum only		
	Total sp.	Mean sp. (sd)	n	Total sp.	Mean sp. (sd)	n
Saiga BD	7	2.1 (0.83)	21	9	1.5 (0.74)	133
Saiga Ust	2	1.1 (0.30)	11	2	1.1 (0.30)	11
Sheep BD	12	4.1 (2.0)	15	8	2.7 (1.5)	23
Sheep SK	5	3.0 (1.6)	5	4	2.0 (1.2)	5
Goat BD	9	3.8 (3.3)	4	2	3.5 (3.1)	4
All	16			13		

Uneven sample size is known to lead to bias in measures of species diversity (Rosenzweig, 1999). The effect of sample size on observed parasite diversity here was investigated more closely by plotting cumulative species richness against the number of animals consecutively examined (Fig. 5.2*a*). The order in which individuals were sampled may affect the form of this distribution: to correct for this, the order of individuals was remixed randomly, then reversed, and the cumulative species richness recalculated in each case. The process was repeated five times, to generate ten randomly resampled series: the average of the ten series are plotted as cumulative species richness curves in Fig. 5.2*b*. It is clear that in all species the observed parasite

diversity, as measured by cumulative species richness, increases non-linearly with sample size. However, the rate of increase and number of parasite species at a given sample size were higher in domestic livestock than in saigas, and lowest in saigas from the Ustiurt range. Parasite diversity in goats, which initially appeared to be lower than in sheep in Betpak-Dala (see Table 5.2), showed an initial increase with sample size very similar to that in sheep, and sheep in Betpak-Dala carried more parasite species than sheep further south, irrespective of sample size.



(a) In the order sampled.



(b) Using simulated resampling (see text).

Figure 5.2. The cumulative number of nematode species found in the abomasum and small intestine of different ruminant populations in Kazakhstan, as more individuals are randomly sampled (see text). BD = Betpak-Dala; Ust = Ustiurt; SK = South of the Betpak-Dala saiga range.

The relative diversity of abomasal nematodes in each host species was investigated further using simulated resampling. Five individuals at a time were selected at random from each sample, and the total number of abomasal nematode species found in the five animals counted. *Marshallagia* and *Haemonchus* were again each assumed to be represented by a single species. Fifty sub-samples were taken from each population, and the frequency distribution of abomasal species richness plotted (Fig. 5.3). Despite the approximately equal number of species found in total in saigas and sheep in Betpak-Dala, consistently fewer were found in sub-samples from saigas ($n_1=n_2=50$, Mann-Whitney U=40.5, Z= -8.52, $p<0.001$), indicating that the higher total sample size in saigas is responsible for the high total diversity observed. Saigas in Ustiurt appeared to carry a lower number of abomasal nematode species irrespective of sample size.

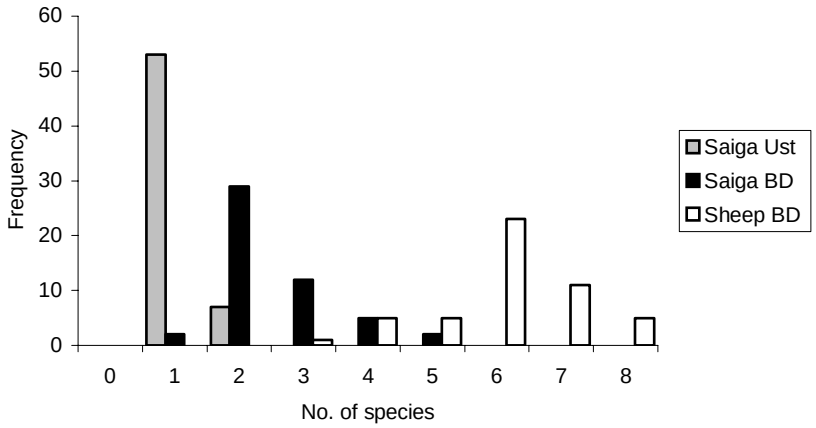


Figure 5.3. Abomasal nematode species richness in 50 samples of five individual animals drawn at random from saigas and sheep sampled in Ustiurt (Ust) and Betpak-Dala (BD). The number of species is the total number in five hosts. Total numbers sampled were: Saiga Ust = 11; Saiga BD = 133; Sheep BD (Chu) = 23. Nine species of nematode were found in total in saigas in BD, 8 in sheep in BD, and 2 in saigas in Ustiurt. Goats in BD carried 8 species, and sheep to the south of BD 4, but in both cases the number of animals sampled (4 and 5 respectively) made simulation redundant.

Measures of diversity other than species richness depend also on parasite distribution between hosts, and relative abundance. The likelihood of finding a parasite species in a given sample itself depends on parasite distribution and test sensitivity. Both issues are discussed in chapter 6.

5.2.3 Host range

The pattern of parasite distribution among hosts agrees broadly with that previously observed in Kazakhstan, and discussed in chapter 3. Among the nematodes, the genera *Marshallagia, Parabronema, Teladorsagia* and *Trichostrongylus* are present in all three host species. Within the moleinids, *Nematodirus oiratianus* and *Nematodirus spathiger* are similarly generalist, while *Nematodirella longissimespiculata*, *Nematodirus archari, Nematodirus dogieli* and *Nematodirus gazellae* appear to be restricted to saigas, and *Nematodirus abnormalis* and *Nematodirus filicollis* to domestic ruminants. While overlap in the host range of these species has been observed in the past, here they appear to be restricted to the hosts that are perceived in the Russian literature to be their primary hosts.

Both *Haemonchus* sp. and *Trichostrongylus axei* generally show little host specificity within ruminants in Kazakhstan (Boev *et al*, 1962), as elsewhere (Soulsby, 1982), but are absent here in saigas. Other species found in saigas in the past, and found in this study in livestock but not in saigas are *Trichostrongylus probolorus*, *Ostertagia ostertagi, Trichuris ovis* and *Trichuris skrjabini*. The generalist species that are present in saigas at a high enough abundance to be detected include *Nematodirus oiratianus* and *Parabronema skrjabini*, both of which are relatively resilient to arid conditions (see Fig. 3.6). *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, also relatively tolerant of desiccation among the trichostrongylids (Waller and Donald, 1970; Pandey *et al*, 1993), are also present.

The parasite species found in saigas in the present study, therefore, have all been associated previously either with a degree of specificity for saigas, or an ability to survive well in dry conditions. Species alleged in the past to spill over from sheep to saiga populations are in this instance absent from the saiga sample, and saiga species alleged to spill over into sheep are similarly absent from the sheep sample. This is at first sight consistent with decreased contact between host species. Observed presence or absence, however, is associated with parasite abundance as well as sample size, and the hypothesis of decreased contact will be addressed again after a consideration of parasite abundance in each population.

5.3 Classification of trichostrongylid species

5.3.1 Trichostrongylid systematics

The parasite species found in saigas in the past have already been listed in Table 3.1. Nomenclature and species classification in the Russian literature, however, have been inconsistent in taking account of ongoing developments in parasite systematics in the wider scientific community. The classification of the trichostrongylids, in particular, has been the subject of several reviews in recent decades, while parasitological studies in the Soviet Union and former Soviet countries have tended to follow the scheme of Skrjabin *et al* (1954). Many parasites described in this work have since been absorbed by other workers into new or pre-existing taxa. Thus, Skrjabin *et al* (1954) considered the superfamily Trichostrongyloidea to contain 429 species in 89 genera, while Levine (1980) recognises only some 225 species and 31 genera.

Confusion in trichostrongylid classification is particularly marked in the sub-family Ostertagiinae, in which description of species based on mutant specimens has been widespread, and new species have often been placed in unverified genera (Durette-Desset, 1989). In addition, classification in the past has proceeded with little thought given to phylogeny (Durette-Desset, 1982), with the result that the systematics of the group, and evolutionary relationships within it and with other related groups, remain far from resolved. Within the trichostrongylids, work is well under way to rectify this (Hoberg and Lichtenfels, 1994; Durette-Desset *et al*, 1999), though a unified view of systematics and phylogeny within the Trichostrongyloidea as a whole is still some way off (Hoberg *et al*, 2001).

Further advances in trichostrongylid classification have come with the recognition of polymorphy within the Ostertagiinae, morphological evidence for which is now strong (Dróżdż, 1995), and supported by molecular genetics (Zarlenga *et al*, 1998). In some but not all species of the group, there appears to be a dominant, major morph, and a less abundant minor morph that has characteristically thickened spicules. Genera that were erected to accommodate these forms are therefore absorbed into those of the major morphs (Dróżdż, 1995), leading to a reduction in the overall number of genera and species recognised in the Ostertagiinae.

As a result of this ongoing work, many of the parasite species listed in both saigas and domestic livestock in Kazakhstan may not correspond with those now generally accepted. It is important that species are universally recognised, so that diagnosis is consistent and biologically meaningful. In spite of the many unresolved issues in trichostrongylid systematics, there is a strong case for reviewing the species list of parasites of saigas. This is attempted in the next section.

5.3.2 Taxonomy of saiga nematodes

Changes to the names of nematode species found in saigas in Kazakhstan, proposed in the light of recent developments in taxonomy, are listed in Table 5.3. In some cases, names are simply changed for the sake of consistency with the western literature (e.g. *Trichuris ovis*), or placed in the more universally agreed genus (*Teladorsagia circumcincta, Ostertagia orloffi*). Polymorphy in male worms in the Subfamily Ostertagiinae accounts for the absorption of both *Ostertagiella (Grossispiculagia) occidentalis* and *O. trifida* into *Marshallagia marshalli* (Durette-Desset, 1982; Lichtenfels and Hoberg, 1994). The Family Molineidae awaits review (Lichtenfels *et al*, 1997); *Nematodirus* and *Nematodirella* species therefore retain their original classifications.

Table 5.3. Reclassification of saiga nematodes according to updated criteria in the literature (see text).

Species	**Comment**	**Reference**
Setaria labiatopapillosa Alecsandrini, 1838	Synonym of *S. cervi* Dujardin, 1845	Levine, 1980
Trichostrongylus skrjabini Kalantarjan, 1928	Synonym of *T. colubriformis*	Levine, 1980
Ostertagiella occidentalis Ransom, 1907 (Synonym *Ostertagia (Grossispiculagia) occidentalis*)	Minor morph of *Marshallagia marshalli*	Durette-Desset, 1982, confirmed by Lichtenfels and Hoberg, 1993.
Ostertagiella circumcincta (Stadelman, 1894) Ransom 1907	Becomes *Teladorsagia circumcincta*	Gibbons and Khalil, 1982
Ostertagiella orloffi Sankin, 1930	Becomes *Ostertagia orloffi*	Levine, 1980; Gibbons and Khalil, 1982
Ostertagiella trifida (Guille, Marotel, Panisset, 1911) Andreeva, 1957	Synonym of *O. occidentalis*, hence minor morph of *M. marshalli*	Lichtenfels and Hoberg, 1993
Ostertagiella trifurcata (Ransom, 1907) Andreeva, 1957	Minor morph of *Teladorsagia circumcincta*	Gasnier et al, 1993; Lichtenfels and Hoberg, 1993
Skrjabinagia lyrata Sjöberg, 1926	Becomes *O. lyrata*, which is the minor morph of *Ostertagia ostertagi* Stiles, 1892	Gasnier et al, 1993; Drozdz, 1995; Zarlenga et al, 1998
Trichocephalus ovis Abildgaard, 1795	Becomes *Trichuris ovis* (Abildgaard, 1795) Smith, 1908	Levine, 1980

Reclassification according to these criteria reduces the list of gastrointestinal nematodes of saigas in Kazakhstan from 32 to 26 species, with four further name changes. The proposed changes in classification have been incorporated retrospectively into Tables 3.2 and 5.1, and the preceding analyses in section 5.2. Classification of species within the genera *Haemonchus* and *Marshallagia* is problematic, and is discussed further.

5.3.3 Cryptic species and diversity within parasite taxa

Reviews of parasite taxa have frequently reduced species to synonymy, as discussed above. However, recognised species may equally conceal groups of morphologically indistinguishable (i.e. cryptic) species. Thus, *Haemonchus placei* has been shown to be distinct from *H. contortus* (Blouin *et al*, 1997), with which it was long considered synonymous (Gibbons, 1979). Lichtenfels *et al* (1997) further suggested that both *Teladorsagia circumcincta* and *Marshallagia marshalli*, with their wide host and geographical ranges, might also in fact consist of assemblages of cryptic species.

The description of diversity within parasite taxa is important to epidemiology if parasite sub-types differ in biological characteristics such as infectivity or pathogenicity for a particular host, or the conditions necessary for the development and survival of free-living stages. Traditional concepts and definitions of species may be poorly applicable to parasites (Kunz, 2002), while a significant proportion of total genetic variability may be found within parasite species and populations, rather than between them (Blouin *et al*, 1995). Genetic studies may be well placed to quantify this variation, but cannot easily subdivide or ascribe biological meaning to it. Morphological studies, meanwhile, have found little difficulty in dividing phenotypic diversity into manageable groups, but at the price of error and false synonymy.

Concealed variations within and between parasite species may be particularly important in the saiga-livestock-trichostrongylid system. Contact between host species is common, but only some parasites can be transmitted between them (see chapter 3). Cryptic species that differ in host specificity may be falsely ascribed a wide host range, while phenotypically divergent forms of the same species may give the illusion of different species with a common distribution. Small bionomic differences between parasite sub-types sharing a multi-host, environmentally variable

habitat may lead to differential utilisation of host resources and the external environment, and reinforce variations in life history without necessarily leading to large morphological differences or even reproductive isolation. Such variation may be important in determining the distribution of parasites among saigas and livestock, and, more generally, in other systems that feature contact between wildlife and livestock.

Elucidating the role of parasite diversity in the epidemiology of parasitism must rely first on its identification, description and appropriate subdivision. Morphological description still prevails in trichostrongylid identification, and many species can be identified only by examining the secondary sexual characteristics of the male, in particular the bursa and spicules (Levine, 1980). Molecular approaches show promise, but are still in their infancy, and cannot yet rival the ease, economy and large comparative database of morphology (Lichtenfels *et al*, 1997). Close and careful examination of phenotypic variation can in many cases identify discontinuities in groups of closely related sympatric species (e.g. Jacquiet *et al*, 1997), and relate them to important biological differences (Jacquiet *et al*, 1995*a*).

The ecological and epidemiological importance of parasite diversity is discussed further in section 5.6. In the present study, particular difficulties were encountered in identifying nematodes of the genera *Marshallagia* and *Haemonchus*, of which several species have been reported to be sympatric in ruminants in Kazakhstan (Boev *et al*, 1962). *Marshallagia* was recovered from both saigas and domestic ruminants, while *Haemonchus* was absent in saigas. A closer look at the morphology of *Marshallagia* specimens isolated from saigas and livestock is necessary in an effort to answer the following questions:

- Are *Marshallagia* nematodes in saigas and livestock drawn from a single, morphologically consistent group, or are they a collection of different and distinguishable sub-types?
- If different, do the observed differences in morphology correspond to species described in the Soviet and international literature? Which of the many characters described in the literature are most useful in differentiating species?
- Might different species or sub-types of *Marshallagia* differ in their inherent ability, or ecological tendency, to infect different host species? In other words, is phenotypic variation in *Marshallagia* uniformly distributed between hosts?

5.4 Morphometric analysis of *Marshallagia* in saigas, sheep and goats

5.4.1 Distinctness of species in the literature

Skrjabin *et al* (1954) recognise five species of *Marshallagia*: *M. marshalli* (Ransom, 1907) Orloff, 1933, *M. mongolica* Schumakovich, 1938, *M. schikhobalovi* Altaev, 1953, *M. orientalis* (Bhalerao, 1932) Travassos, 1937, and *M. brevispiculum* Mönnig, 1940, as well as one species, *Ostertagia (Grossispiculagia) occidentalis* Ransom, 1907, which has subsequently been renamed *M. occidentalis* and confirmed to be the minor morph of *M. marshalli* (Durette-Desset, 1982; Hoberg and Lichtenfels, 1994). All except *M. orientalis* and *M. brevispiculum* have been found in ruminants in Kazakhstan. An additional 10 species have since been described, of which 9 have been confirmed (Durette-Desset, 1989), and two – *M. dentispicularis* Asadov, 1954, and *M. schumakovitshi* Kadyrov, 1959 - found in sheep in Kazakhstan (Kuznetsov and Dikov, 1979). Five species and their minor morphs may therefore be sympatric in ruminants in this country.

A schematic diagram illustrating the characters used in the differentiation of *Marshallagia* species is presented in Fig. 5.4. *M. schikhobalovi* can be identified by its dorsal rib (the dorsal ray of the bursa), which is not deeply cleft, in contrast to the other species, while *M. occidentalis* has massive and heavily chitinised spicules, and is also easily recognisable. The description of *M. schumakovitschi* is not available; however, photomicrographs in Dróżdż (1995) suggest that its spicules may be superficially similar to those of *M. mongolica*, but smaller. *M. marshalli* and *M. mongolica* are more difficult to distinguish. Key differences cited in Skrjabin *et al* (1954) are summarised in Table 5.4, with differences in spicule morphology being most consistent. Irgashev (1973) disagrees, suggesting that the point of bifurcation of the dorsal rib of the bursa is more reliable than spicule morphology in distinguishing between the two species.

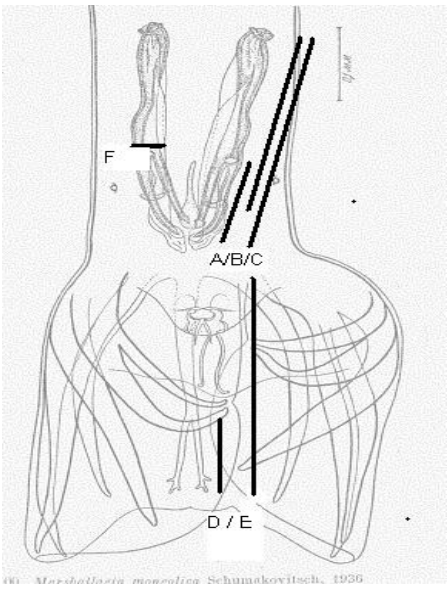


Figure 5.4. Diagram of the bursa of a male* Marshallagia *sp. (after Skrjabin *et al*, 1954). Labels A-F represent measures used in identification (see later).

Table 5.4. Differences between* Marshallagia *species as described in Skrjabin *et al* (1954, 1960).

Character	***M. marshalli***	***M. mongolica***	***M. schikhobalovi***
Point at which spicules split into three branches	Posterior quarter of spicule	Posterior third of spicule	Posterior sixth of spicule
Dorsal and medial branches of spicules	Of approximately equal length, and almost as long as lateroventral branch	Dorsal branch is shorter than the others	Of approximately equal length
Dorsal rib of bursa	Trunk at least 200µm long, and total length 280-400 µm	Trunk only 200µm long, and total length 310 µm	Total length around 235µm, ends in two very short (11µm) branches
Gubernaculum	Absent	Weakly-chitinised gubernaculum present	Absent

Despite the apparently clear-cut differences between *M. marshalli* and *M. mongolica* in the Russian literature, the range of key characters can overlap between species (Table 5.5). Even clear-cut differences may not be consistent. Patterns of branching of the dorsal ray can take a wide range of forms (Andreeva, 1957), and the original description of *M. schikhobalovi*, based primarily on this one character (Altaev, 1953), may in fact just represent a mutation of *M. marshalli* or *M. mongolica* (R. Lomakin, Russian Academy of Sciences Institute of Parasitology, Moscow, pers. comm.). The presence of a weakly-chitinised gubernaculum in *M. mongolica* is also of questionable consistency. In the present study, a clearly recognisable gubernaculum was identified in just one specimen. It is possible that weak chitinisation was faded to invisibility by the clearing agent lactophenol; this and other clearing agents are widely used in

parasitology, and characteristics unstable in it would, in any case, undermine the utility of this character in identification.

Table 5.5. Overlap in key measurements of* Marshallagia *spp. males. Measures are illustrated in Fig. 5.4. Sources: 1. Skrjabin *et al* (1954, 1960); 2. Overall range given by various sources cited in Irgashev (1973); 3. Measurements of Irgashev(1973), n=40 specimens; 4. Trach (1986).

Species	Worm length (mm)	Spicule length (μm)	Dorsal rib (ray) length (μm)	Dorsal rib bifurcation, from end (μm)	Source
M. marshalli	10-13	240-280	215-250	65-70	1
	7-15	210-315	210-400	50-87	2
	7-14	220-280	140-300	36-84	3
M. mongolica	11.5-14	260-281	310	110	1
	7-15	270-315	-	-	4

The host ranges of *Marshallagia* spp. are catalogued by Skrjabin *et al* (1954), and extended by more recent studies (e.g. Hoberg *et al*, 2001). The source of the specimens on which Skrjabin's descriptions are based is not, however, stated. Host-specific phenotypic variation could therefore confound identification based on these keys, and deserves investigation. Host-induced differences in morphology might lead to mistaken description of new species, and misguided notions of host specificity.

5.4.2 Morphological variation in Marshallagia

A total of 265 male specimens of *Marshallagia* recovered from the abomasa of 105 individual saigas, sheep and goats in the present study were measured in an attempt to identify them, and in particular to differentiate between *M. marshalli* and *M. mongolica*. The measurements used were based on key characteristics in Skrjabin *et al* (1954; refer also to Fig. 5.4), and are listed in Table 5.6. Specimens in which all characters were visible were selected for measurement. Those not measured were generally poorly positioned on the slide, and there is no reason to suppose bias in selection. Each specimen was first subjectively identified on the basis of keys and illustrations in Skrjabin *et al* (1954), Andreeva (1957) and Trach (1986), and then measured using a calibrated ocular micrometer. Worms were not straightened before being measured for length, and some additonal error is expected in this result.

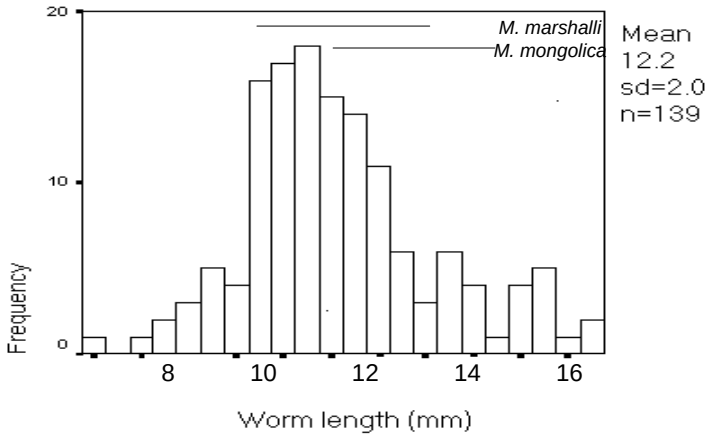
Table 5.6. ***Measurements taken from adult male nematodes for morphometric analysis of* Marshallagia *populations in saigas.*** Refer to Fig. 5.4 for an illustration of the measurements. The measures listed correspond to the following labels on Figure 5.4: 2=C; 3=F; 4=(C-A)/C; 5=B/C; 6=E; 7=(E-D)/E.

Measure	Name	Description	Units
1	Worm length	Total length	mm
2	Spicule length	Straight length end to end	µm
3	Spicule width	Width just proximal to branching point	µm
4	Spicule branch	Point at which spicules split into three branches, as a proportion of total spicule length	Ratio
5	Dorsal branch	Length of dorsal branch of spicule, as a proportion of total spicule length	Ratio
6	Dorsal rib (ray)	Straight length	µm
7	Dorsal rib (ray) bifurcation	Point at which dorsal rib bifurcates, as a proportion of dorsal rib length	Ratio

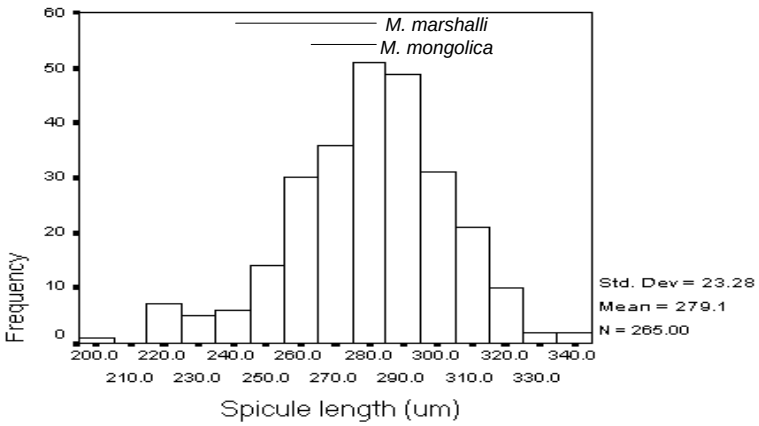
Frequency distributions of each measurement on the whole sample, with sample sizes, are shown in Fig. 5.5. All distributions are approximately Normal. Exclusion of worms identified as *M. occidentalis* and *M. schikhobalovi* successfully removed outliers. Unimodality in most of the plots suggests poor discriminatory ability between groups included in the sample. There is a suggestion of bimodality in the length of the dorsal branch of the spicule when measured against total spicule length; however, there is no clear separation that could be used as the basis of accurate and exclusive species identification.

Individual measurements are therefore ineffective in distinguishing consistently between *M. marshalli* and *M. mongolica* as described in the existing literature. Characters were compared together, using multivariate analysis, to see if combinations of characters could effectively separate groups. In the first analysis, 59 measured specimens of 3 putative species and the minor morph *M. occidentalis* were included. Factors that explained the maximum variance in the sample were extracted using principal components analysis (Fowler *et al*, 1998; Factor Analysis command in SPSS). Axes were kept orthogonal during rotation using the Varimax method with Kaiser normalisation (Kinnear and Gray, 2000), and only those components with an eigenvalue greater than unity were retained. The components that contributed significantly to overall variance were used to calculate factor scores for each measured specimen (Regression method, SPSS), and these were plotted on axes representing the first three principal components. These plots are reproduced in Fig. 5.6. Data points are labelled by supposed species; groupings were assessed by eye.

(a) Worm length



(b) Spicule length



(c) Spicule width

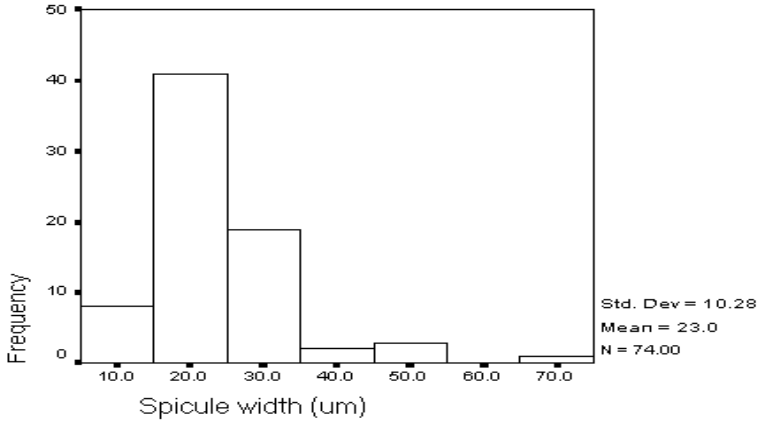
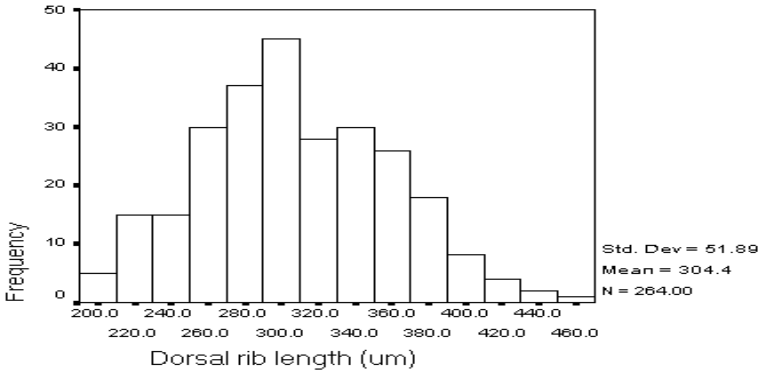


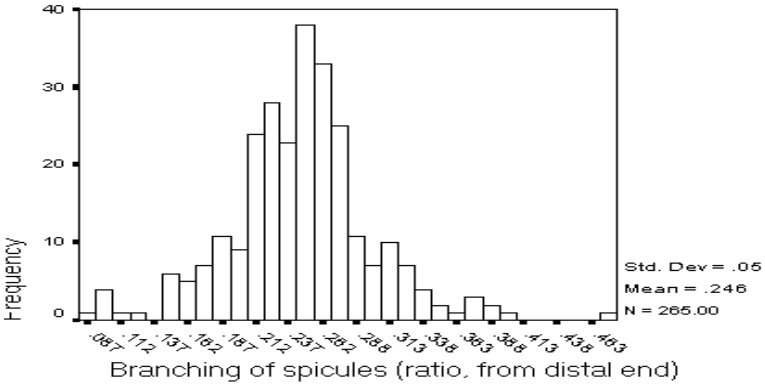
Figure 5.5. Frequency distributions of characters measured in 265 male* Marshallagia *specimens from saigas and domestic ruminants in Kazakhstan. um = µm. Summary statistics are printed to the right of the plots.

Bars mark ranges of measurements cited in Skrjabin *et al* (1954) for each species.

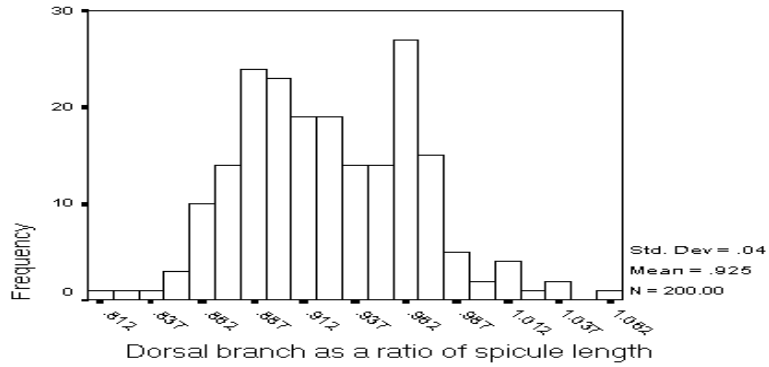
(d) Length of the dorsal rib of the bursa ***Figure 5.5 continued.***



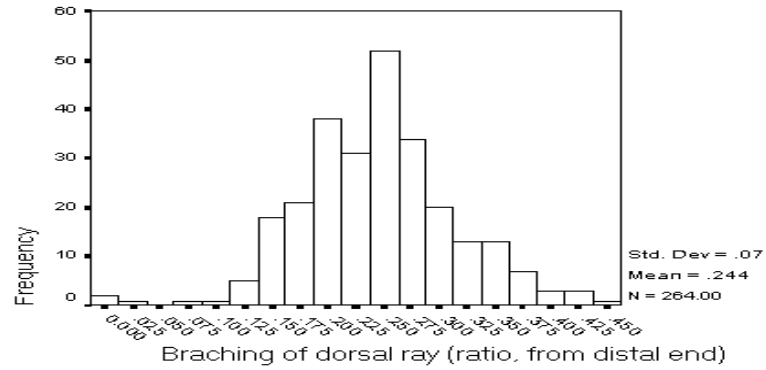
(e) Point of branching of spicules



(f) Ratio of dorsal branch to spicule length



(g) Point of branching of the dorsal rib



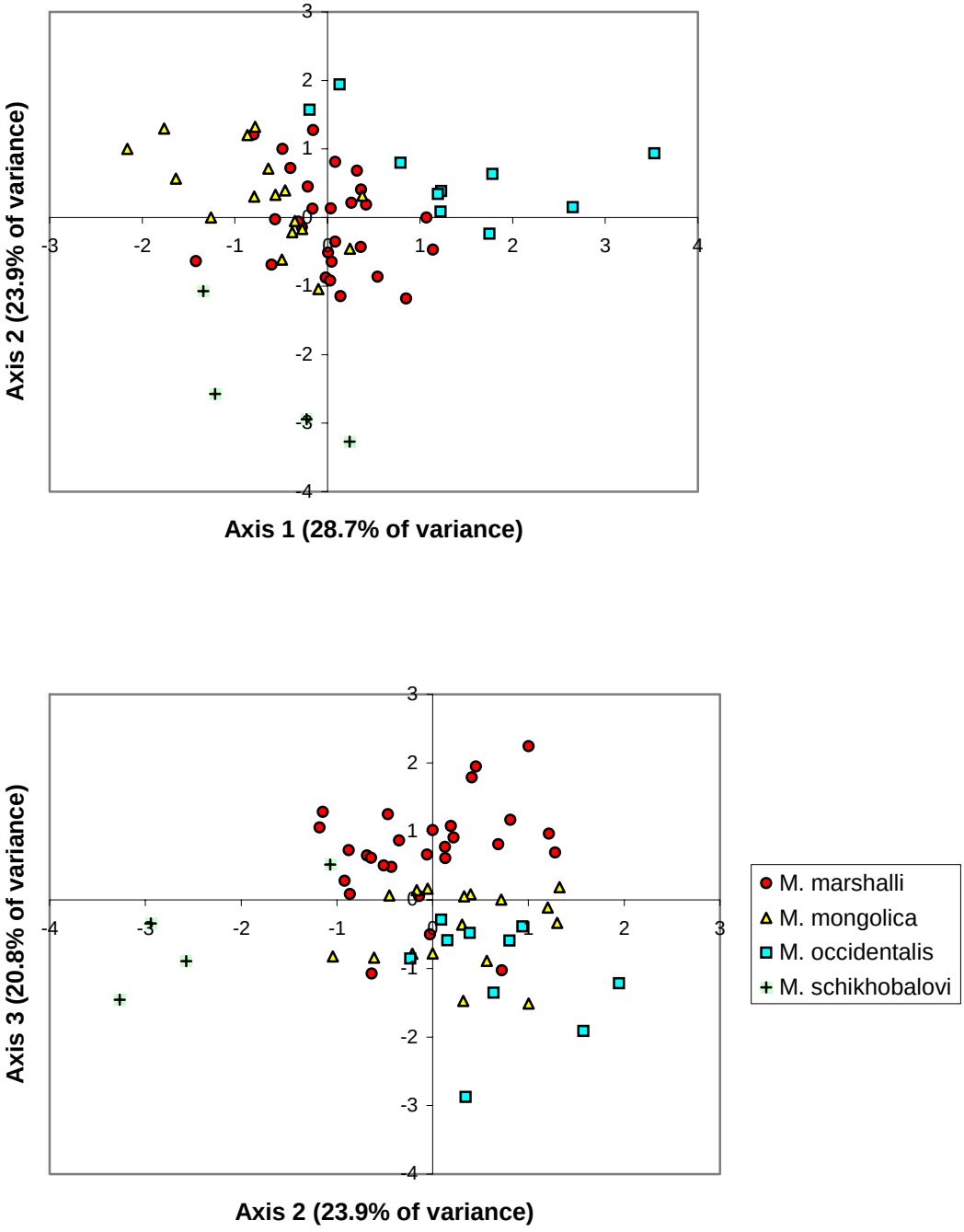


Figure 5.6. Principal components analysis of the morphology of 59 male* Marshallagia *specimens from saigas, sheep and goats in Kazakhstan. See text for methods and discussion.

The three principal components together accounted for 73.4% of the total variance. Component loadings are listed in Table 5.7: the first component was dominated by measures of overall worm and spicule size; the second by branching points of the dorsal rib and spicules; and the third by dorsal branch length and spicule morphology. A second, smaller, analysis excluding *M. schikhobalovi* and *M. occidentalis*, and using equal numbers of *M. marshalli* and *M. mongolica,* extracted two significant principal components, together accounting for 58.2% of the total variance (Table 5.8). Plots, not shown, produced similar groupings to Fig. 5.6, with some separation of groups but considerable overlap.

Table 5.7. Relative loadings of significant principal components (PC) in the morphometric analysis of 59* Marshallagia *specimens. Rotation method was varimax with Kaiser normalization; rotation converged in 7 iterations. The measures contributing most to each component are in bold text.

Measure	Name	PC 1	PC 2	PC 3
1	Worm length	**0.693**	-0.051	0.093
2	Spicule length	**0.880**	-0.239	0.021
3	Spicule width	**0.711**	0.338	-0.339
4	Spicule branch	0.226	**-0.784**	0.253
5	Dorsal branch	0.424	0.448	**-0.659**
6	Dorsal rib	0.120	0.141	**0.882**
7	Dorsal rib bifurcation	0.058	**0.814**	0.229

Table 5.8. Relative loadings of significant principal components (PC) in the morphometric analysis of 32* Marshallagia *specimens (16 identified as *M. marshalli* and 16 as *M. mongolica*). See text.

Measure	Name	PC 1	PC 2
1	Worm length	0.723	-0.250
2	Spicule length	0.787	0.380
3	Spicule width	0.008	0.726
4	Spicule branch	0.624	0.525
5	Dorsal branch	-0.729	-0.137
6	Dorsal rib	0.628	0.047
7	Dorsal rib bifurcation	-0.087	-0.760

Analysis of principal components confirmed the separation of the minor morph, *M. occidentalis,* from the rest of the genus, and the distinct morphology of worms identified as *M. schikhobalovi*. Among other specimens, there did appear to be some separation, especially along axis 3. However, this was by no means complete, confirming that the morphological characters used in the species descriptions and subsequent keys are not completely reliable at distinguishing between *M. marshalli* and *M. mongolica*. These two species may in fact form a single group, with

morphological variation continuous within it. There was also no evidence for the presence of other morphologically distinct species of *Marshallagia* in the sample.

5.4.3 Discriminating between species

The ambiguous morphological separation between *M. marshalli* and *M. mongolica* is not sufficient grounds to discount their existence as two distinct species. Past studies attribute significant ecological differences to them (see chapter 3), and some attempt must therefore be made to distinguish between the species in the present study.

The single character that is most clearly different between *M. marshalli* and *M. mongolica* on subjective inspection (Skrjabin *et* al, 1954) and on multivariate analysis (above) is the ratio of the length of the dorsal branch of the spicule to total spicule length. Specimens in the non-overlapping region of Fig. 5.6 can be recognised subjectively as having relatively very long or very short dorsal branches. For intermediate specimens, measures of spicule and bursa morphology may not be completely reliable in identification, but they could nevertheless have some predicitive value in assigning group membership. This was put to the test using discriminant function analysis (Fowler *et al*, 1998), which has proved useful elsewhere in distinguishing between sympatric and morphologically similar *Haemonchus* species (Jacquiet *et al*, 1997).

Discriminant analysis was performed using the Classify/Discriminant function in SPSS (Kinnear and Gray, 2000). The measurements of the 59 specimens in the first principal components analysis subjectively classified as *M. marshalli* or *M. mongolica* were compared using univariate ANOVA, after plotting individual variables to check for extreme outliers. Four measures were found to differ significantly between species at the $p<0.05$ level (Table 5.9). A canonical discriminant function was computed using the stepwise method, and was highly significant in distinguishing between groups (Wilk's lambda 0.531, Exact $F=24.7$ with 2 and 56 d.f., $p<0.001$). The measures retained were dorsal rib length (Dorsrib) and dorsal branch ratio (DBr). The discriminant function (DF) coefficients were:

$$DF = (0.015 \times \text{Dorsrib}) + (26.032 \times \text{DBr}) - 28.725 \qquad (5.1)$$

Individual worms with a negative value for DF were predicted to be *M. mongolica*, and those with a positive value *M. marshalli*.

Table 5.9. Univariate ANOVA of measurements of 59 male specimens of* Marshallagia marshalli *and* M. mongolica*, computed as part of the discriminant analysis described in the text. Measurement names are defined in Table 5.6. Significance is calculated on 1 and 57 degrees of freedom. Measures significant at the 0.05 level are in bold type.

Measure	Wilk's Lambda	F	Significance
Worm length	0.995	0.272	0.604
Spicule length	0.895	6.711	**0.012**
Spicle width	1.000	0.005	0.941
Dorsal branch	0.811	13.28	**0.001**
Spicule branch	0.887	7.254	**0.009**
Dorsal rib	0.605	37.24	**<0.001**
Dorsal rib bifurcation	0.960	2.376	0.129

Using the discriminant function, 83.1% of the original grouped cases were classified correctly, with *M. mongolica* identified more consistently than *M. marshalli* (Table 5.10). Separation between specimens was good but incomplete (Fig. 5.7). A second discriminant function was computed with all variables entering the analysis together rather than stepwise: this function classified 86.4% of original grouped cases correctly, a small improvement in accuracy for the additional effort of measuring 7 rather than 2 characters. Worm length is particularly time consuming to measure, and in this study took as long to complete as all the other measurements combined. The simpler two-character discriminant function was therefore preferred.

***Table 5.10. Success of the discriminant function in distinguishing between 59 specimens identified as* Marshallagia marshalli *and* M. mongolica.** Overall, 83.1% of original grouped cases were classified correctly.

Actual	Predicted	
	M. marshalli	*M. mongolica*
M. marshalli	32	9
M. mongolica	1	17

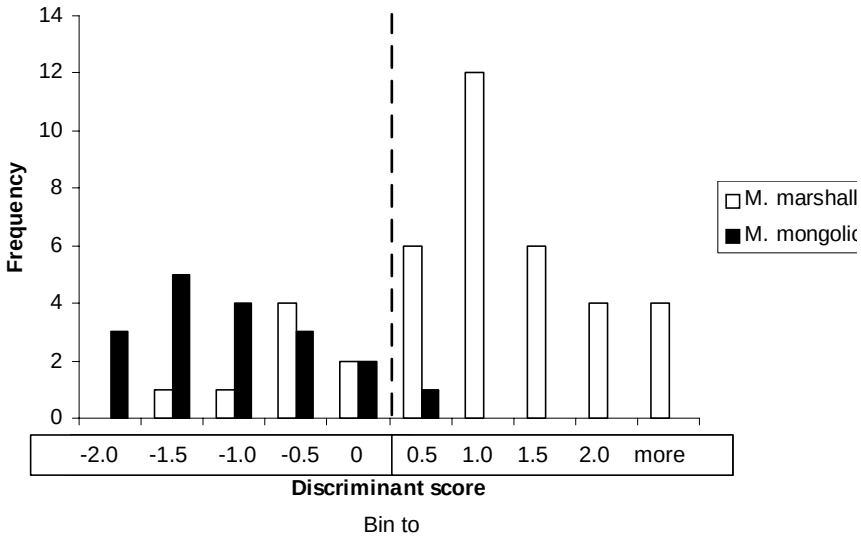


Figure 5.7. Separation of 59 male specimens of* Marshallagia *using a discriminant function (see text). The legend refers to identification based on subjective assessment of morphology, while the discriminant score is based on objective morphological analysis. A score of <0 predicts *M. mongolica*, and >0 *M. marshalli*.

The discriminant function was used as the final step in a key to identify *Marshallagia* spp. in the present study (Table 5.11). Of 758 specimens, 124 could not be identified subjectively (i.e. dorsal branch was of intermediate length): 66 of these were predicted to be *M. marshalli*, and 58 *M. mongolica*.

Table 5.11. A simplified key for the identification of* Marshallagia *species in saigas in Kazakhstan. See text.

1	Bursa elongated, with no discernable bifurcation, and dorsal rib longer than 200µm	*Marshallagia*
2	Dorsal rib of bursa unbranched; ends in two short (c. 11µm) offshoots	*M. schikhobalovi*
2	Spicules massive and characteristically thickened, and longer than 300µm	*Marshallagia,* minor morph
3	Dorsal branch of spicule clearly ends far short of end of spicule	*M. mongolica*
4	Dorsal branch of spicule reaches to or nearly to end of spicule	*M. marshalli*
5	Dorsal branch of spicule of uncertain length: measure length of dorsal spicule branch (A), total spicule length (B), and length of dorsal rib of bursa (C). Calculate: DF = (0.015 x C) + (26.032 x B/A) – 28.725	6
6	DF > 0	*M. marshalli*
7	DF < 0	*M. mongolica*

5.4.4 Vulvar morphotypes

The cuticle of the peri-vulvar region of trichostrongylids can show various forms of adornment, including large flaps of squared, rounded or linguiform shape, and small cytoplasmic knobs lateral or medial to the vulva (Levine, 1980). These morphological characters have been cited as species characteristics within the Haemonchiinae (Skrjabin *et al*, 1954), and ratios of different vulvar morphotypes have been shown to vary considerably within as well as between *Haemonchus* species (Humbert and Cabaret, 1995; Jacquiet *et al*, 1995*a*). Das and Whitlock (1960) used this as evidence for active speciation in the genus *Haemonchus*, while other workers have argued that vulvar morphotype is determined variously by the age of the infection and reproductive output of the female (Daskalov, 1972*a*; Daskalov, 1972*b*), or host resistance (Le Jambre and Ratcliffe, 1976).

Despite the doubts over the extent to which vulvar morphology is genetically determined, morphotype ratios have been used to identify nematode populations as belonging to a given species. In Saharan Africa, Jacquiet *et al* (1995*a*) found that the proportion of different vulvar morphotypes differed significantly between infected camels, cattle, sheep and goats, and attributed the difference to the unequal distribution of three *Haemonchus* species among these hosts. The value of peri-vulvar morphology in species identification appears to be relatively unexplored outside *Haemonchus*; however, a similar variety of forms has been described in *Ostertagia* (Michel *et al*, 1972) and *Marshallagia* (Trach, 1986).

In the present samples, 3 main vulvar morphotypes were observed in female *Marshallagia*, differing in the presence and shape of the main vulvar flap. Smaller knob-like embellishments were also seen. The morphotypes in the present study are categorised in Table 5.12. The lack of previous published data, and the common occurrence of apparently mixed infections of *Marshallagia marshalli* and *M. mongolica*, made it impossible to attribute particular female morphotypes to particular species. Frequencies of occurrence of the different morphotypes are, however, compared in different hosts in section 5.4.6.

Table 5.12. Peri-vulvar cuticular morphotypes observed in female* Marshallagia *specimens from saigas, sheep and goats. Fifteen combinations are theoretically possible: 12 were observed, but 'additional embellishments' occurred in only 14% of cases. Structures are similar in apearance to those described in *Ostertagia* (e.g. Michel *et al*, 1972). Trach (1986) described variation in vulvar morphology in *Marshallagia marshalli* in sheep in the Ukraine (Trach, 1986), and his categories are included for comparison. Cat = Category.

Cat	Name	Description	Trach category
Main cuticular flap			
A	Smooth	No visible flap.	*Forma typica*
B	Knob	Small, rounded cuticular structure, may be transversely striated, proximal to vulva and 40-150 µm long.	*Forma monolamellata* and *forma pseudovinus*
C	Flap	Large flap, 200-400 µm long. May be squared, rounded or linguiform.	*Forma ovinus*
Additional cuticular embellishments			
1	Smooth	None.	Not described
2	Medial knob	As 'B', but in addition to other structure.	
3	Lateral knob	As 'B', but located laterally, i.e. on the opposite side to main flap.	

5.4.5 Overlap in Marshallagia *distribution between hosts*

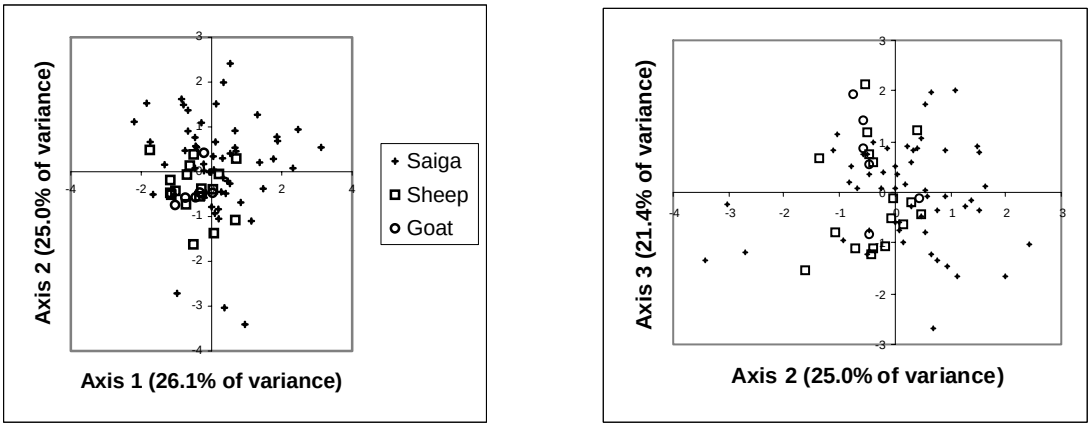
The distribution of putative species of *Marshallagia* between hosts, and the possibility of host-specific phenotypic variation, were examined, again using principal components analysis. The analysis was carried out as described in section 5.3.1: on this occasion some specimens that had been fully measured but not identified subjectively were included, bringing the sample size to 78. Specimens were marked according to host of origin rather than supposed *Marshallagia* species. Fifty-five specimens were from saigas, 17 from sheep, and 6 from goats. Plots of the first three principal components are presented in Fig. 5.8, and may be compared with those in Fig. 5.6.

There is no evidence for detectable host-species induced variation in *Marshallagia* morphology, nor that any of the morphological groups of male *Marshallagia* are restricted to particular host species (with the exception of *M. schikhobalovi*, which was rare and found only in saigas). However, it is possible that different *Marshallagia* types, whether or not they are truly species, occur with different frequency in each host.

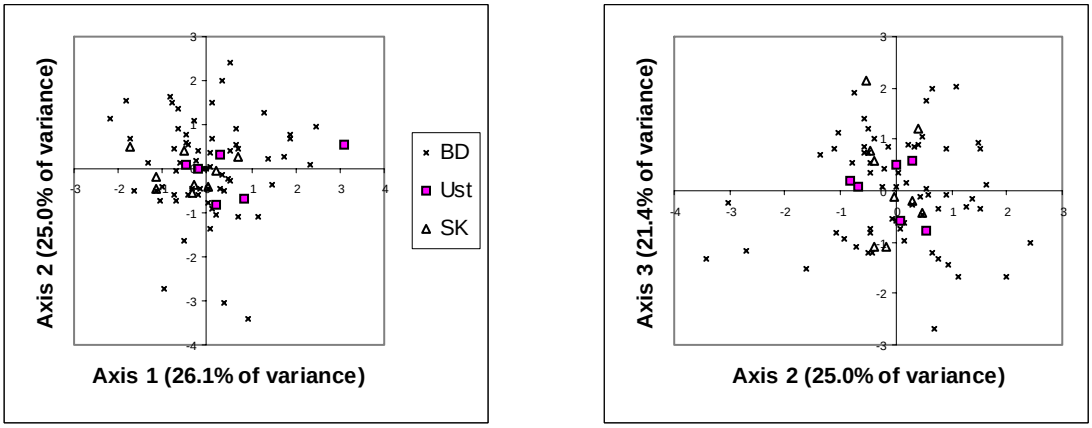
Specimens labelled according to their geographical region of origin, using the same principal components analysis, are also plotted in Fig. 5.8. The morphology of *Marshallagia* does not appear to be distinctive in any region, as specimens from Betpak-Dala, Ustiurt, and the area to the south of the Betpak-Dala saiga range are indistinguishable on the basis of measured characters.

Figure 5.8. Principal components analysis of the morphology of 78 male specimens of Marshallagia by host species and region of origin. BD = Betpak-Dala, Ust = Ustiurt, SK = Southern Kazakhstan, i.e. the area to the south of the Betpak-Dala saiga range. No clear groupings are evident.

(a) By host species.



(b) By region of origin.



5.4.6 Relative frequency in different hosts

A total of 758 male *Marshallagia* specimens which had been extracted and mounted semi-permanently onto slides were identified using the key in Table 5.11, and the relative frequency of different species calculated for each host population. These are presented in Table 5.13. *M. schikhobalovi* was found only in saigas in Betpak-Dala, and was excluded.

Table 5.13. Frequency of* Marshallagia *species in 758 preserved male specimens from different hosts and locations in Kazakhstan. Worms from two sheep from Almaty were included in the Taraz (southern Kazakhstan) sample. BD = Betpak-Dala.

Host	Location	*M. marshalli*	*M. mongolica*	*M. occidentalis*	*M. schikhobalovi*	Total
Saiga	BD	222	243	28	4	497
	Ustiurt	48	17	9	-	74
Sheep/goat	BD	95	46	14	-	155
	Taraz	25	7	-	-	32
Total		390	313	51	4	758

The frequencies of remaining species, together with the minor morph, were compared between host populations in a 4 x 3 contingency table using the Chi-square test (SPSS: Kinnear and Gray, 2000), and found to differ significantly between host populations (n=754, Chi-square 42.7, 6d.f., $p<0.001$). Considering *Marshallagia marshalli* and *M. mongolica* alone, however, and comparing host populations separately, no significant difference was found in the apparent species composition of *Marshallagia* populations in domestic ruminants inside the saiga range, and those in Taraz, to the south and outside the saiga range (n=173, Chi-square 1.42, 1df, $p=0.234$). *M. mongolica* was, however, more frequent in mounted samples from saigas in Betpak-Dala than in either sympatric sheep (n=606, Chi-square 16.7, 1df, $p<0.001$) or saigas in Ustiurt (n=530, Chi-square 15.6, 1df, $p<0.001$).

Table 5.14. Relative frequencies of vulvar morphotypes in 339 female* Marshallagia *specimens from saigas, sheep and goats in Kazakhstan. Morphotypes were described in Table 5.12, and the relative frequencies observed by Trach (1964) in sheep in the Ukraine are included for comparison. Worms from two sheep from Almaty are included in the Taraz sample. Actual frequencies observed are given, with percent of the total in brackets. Frequencies differed significantly (see text).

Host	Location	Vulvar morphotype						
		A		B		C		Total
Saiga	Betpak-Dala	50	(32)	44	(28)	63	(40)	157
	Ustiurt	1	(10)	5	(50)	4	(40)	10
Sheep	Betpak-Dala	13	(9)	27	(18)	109	(73)	149
	Taraz	4	(24)	-		13	(76)	17
Goat	Betpak-Dala	-		1	(17)	5	(83)	6
Sheep	Ukraine (Trach, 1984)	340	(68)	35	(7)	125	(25)	500
Total (Kazakhstan only)		68	(20)	77	(23)	194	(57)	339

The relative frequencies of vulvar morphotypes were also compared between host populations using 339 mounted female worms: results are presented in Table 5.14, and compared with previous published findings in *Marshallagia*. Numbers of worms in samples from saigas in Ustiurt and from sheep outside the saiga range were low, and were excluded from further analysis. Analysis of remaining data revealed that vulvar morphotype frequencies differed very significantly between saigas and domestic ruminants in the saiga range (n=306, Chi-square 37.9, 2 d.f., $p<0.001$). Saigas carried relatively more smooth forms, while sheep and goats together had more worms with well-developed cuticular flaps. Trach (1964), by contrast, found that smooth forms were dominant in sheep in the Ukraine, and the relative frequency of different forms differed significantly from that in sheep in Kazakhstan (n=657, Chi-square 115, 2df, $p<0.001$).

5.5 Identification of other helminths found

5.5.1 Trichostrongylids

Compared with *Marshallagia*, identification of other trichostrongylids was straightforward. The three *Trichostrongylus* species found were easily identified on the basis of spicule morphology; female *T. colubriformis* could also be distinguished by their relatively long ovejector apparatus (Skrjabin *et al*, 1954; Levine, 1980).

A closer examination of *Haemonchus* specimens was justified by the recorded occurrence of two species in Kazakhstan (*H. contortus* and *H. longistipes*, Kuznetsov and Dikov, 1979), and by the possible presence of *H. placei*, which was not recognised by Skrjabin *et al* (1954). Different species of *Haemonchus* are morphologically similar, and their occurrence in the same area has complicated identification in other studies (Jacquiet *et al*, 1995*a*). Relatively few specimens of *Haemonchus* were found in the present study, all in domestic ruminants. Seven mounted specimens from goats and four from sheep, were measured (Table 5.15), and compared using a discriminant function based on spicule morphology (Jacquiet *et al*, 1997). The results are in Table 5.16.

Table 5.15. Morphology of 11 male* Haemonchus *worms from 4 sheep and 2 goats in the Chu region of Kazakhstan. Mean measurements are given in μm (except for worm length, in mm), with standard deviations in brackets: spicule length is straight length end to end, and hook measurements straight distance from distal ends of spicules. DF(y1) and DF(y2) refer to mean co-ordinates on the discriminant function axes given in Jacquiet *et al* (1997).

Host	n	worms	Worm length	Spicule length	Right hook	Left hook	Gub	DF(y1)	DF(y2)
Sheep	4	4	21.1 (0.9)	489(14)	49 (2.4)	26 (3.2)	242(15)	1.09	2.63
Goats	2	7	18.9 (2.0)	456(18)	49 (4.3)	25 (2.9)	263(19)	0.75	0.67

Table 5.16. The* Haemonchus *specimens from Table 5.15, grouped by species, as predicted by the discriminant function of Jacquiet *et al* (1997). Species identification relied on axis y1: a value <0.63 indicated *H. contortus*, and >0.63 *H. placei*. Mean measurements are followed by standard deviation in brackets. See Table 5.15 for explanations.

Parasite	No. of hosts infected	No. of specimens measured	Spicule length	Right hook	Left hook	DF(y1)	DF(y2)
H. contortus	3	4	464 (28)	45 (2.0)	23 (0.5)	-0.01	1.83
H. placei	4	7	472 (20)	51 (2.1)	27 (2.8)	1.38	1.38

Male specimens of *Haemonchus* were on average larger in all measured dimensions than those found in small ruminants in Mauritania by Jacquiet *et al* (1997). Using their discriminant function, 7 of the 11 worms were classified as *H. placei*, including 3 of 4 specimens from sheep. This is surprising, as *H. contortus* is cited as the only species of importance found in sheep in Kazakhstan (Karabaev, 1973). The sample size in the present study is small, but so too is measurement error, and this is unlikely to cause misidentification. *Haemonchus contortus* populations in Kazakhstan and in Mauritania may differ in morphology due to strain differences, or host-induced phenotypic variation. Worm burdens in Mauritania were generally much higher than

in Kazakhstan (Jacquiet *et al*, 1995*a*), and host resistance has been shown to reduce adult worm size in trichostrongylids (Wallace *et al*, 1995; Stear *et al*, 1997), though there is no evidence for effects on spicule morphology. It is possible that apparent *H. placei* are in fact *H. tataricus* Evranova, 1940. This species occurs in sheep in the former Soviet Union, and has relatively long spicules (460-496μm; Skrjabin *et al*, 1954), but it has not been recorded from Kazakhstan, and bursal morphology in the specimens found appears in any case closer to *H. contortus / H. placei*. Finally, it could be that *H. placei* is in fact present in sheep and goats in Kazakhstan, and more important than previously supposed.

5.5.2 Other nematodes

Nematodirus species were identified by morphology of the spicule tips, and in some cases disposition of the bursal rays, using the keys already mentioned. Identification was straightforward: *N. gazellae*, dominant in saigas, was easily recognisable by its unique chitinised spur on the medial aspect of the fused area of the spicules (Skrjabin *et al*, 1954). *Nematodirella* species were differentiated by measuring the total spicule length: all specimens fell into the range for *N. longissimespiculata*, and outside the range for other species found previously in Kazakhstan. *Trichuris* species were identified on the basis of the spicule sheath, which shows a gradual expansion in *T. skrjabini*, and a bulb-like ending in *T. ovis* (Levine, 1980).

5.6 Discussion

5.6.1 Classification of diversity in saiga trichostrongylids

The results of multivariate analysis of morphology in male *Marshallagia* specimens from saigas seem to confirm that several distinct forms exist. These appear to correspond more or less to species described in the literature, though not all key characteristics are in fact useful in diagnosis. The separation of *M. marshalli* and *M. mongolica* using principal components analysis, however, is not only ambiguous, but may be illusory. Without labelling subjectively identified species in Fig. 5.6, there would be no obvious grouping. There is certainly a risk of circularity in using this technique to confirm morphological differences between species categorised on the

basis of the same morphological traits. The evidence for a distinct difference between *Marshallagia* species would be strengthened by morphological examination of specimens independently identified on different characteristics. Unfortunately, no such collection of specimens was available.

Direct measurement of genetic diversity within *Marshallagia*, and its correlation with visible morphological traits, would be an obvious step forward. The techniques to proceed with this work are available, and have already been used to compare closely related trichostrongylid species (Zarlenga *et al*, 1998). Eventually, a combined approach using both molecular and morphological approaches is likely to lead to great advances in trichostrongylid systematics and phylogeny (Lichtenfels *et al*, 1997). In the meantime, however, morphology is likely to remain the method of choice for the identification of parasite species. Not only is the comparative database for morphology much larger than for molecular studies, but the facilities, expertise and funding needed for such studies are unlikely to be routinely available outside the most developed countries for some time. Investigation of problems of parasite transmission between wildlife and livestock in poorer or more remote areas need not await their arrival. Jacquiet *et al* (1997) demonstrated that morphometric analysis can be useful in the diagnosis of closely related sympatric trichostrongylid species, and its use could be extended to the description of variation within known taxa.

Morphological characters not included in the present study may improve the ability of morphometric analysis to capture diversity in this group. The structure of the synlophe has been shown to vary consistently within as well as between trichostrongylid genera (Durette-Desset, 1989; Durette-Desset and Cabaret, 1994). Its description in *Marshallagia* species other than *M. marshalli* has the potential to resolve some of the difficulties outlined above. It is also worth noting that *M. occidentalis* (morph of *M. marshalli*) is the only minor morph to have been described in the genus, even though every species should have its own minor morph (Dróżdż, 1995). Contrary to expectation (Dróżdż, 1995), the abundance of minor morphs in individual hosts in the present study showed no correlation with that of dominant *Marshallagia* forms (data not presented). This may be because many infections contained both *M. marshalli* and *M. mongolica*, and different minor morphs were not distinguished. However, the factors determining the relative abundance of each morph

in polymorphic trichostrongylid species are wholly unknown, and this parameter may yet be shown to have biological relevance (Suarez *et al*, 1995).

If male morphology promises to have continued use in ordering diversity in *Marshallagia*, peri-vulvar morphology appears to have less potential. The differences observed in vulvar morphotype ratios between populations in different host species in this study found little agreement with results from morphological analysis of male specimens. Moreover, a mixture of species was probably present, confounding observed ratios of vulvar morphtype. Correlation between peri-vulvar morphology and genetic make-up, again using molecular techniques, may reveal it to be a useful phenotypic marker in the future. At present, however, there is no reliable evidence to support its use in either diagnosis or population studies of *Marshallagia*, in contrast with the situation in *Haemonchus* (Jacquiet *et al*, 1997).

A definitive classification of the diversity present within the genus *Marshallagia* in saigas, therefore, must await both a review of the overall systematics of the group, and a combined morphological and molecular study of parasites in this host. The former is under way (E. Hoberg, pers.comm.), as an extension of previous work on the phylogeny and systematics of the trichostrongylids (Durette-Desset, 1985; Hoberg and Lichtenfels, 1994; Durette-Desset *et al*, 1999). A study of diversity in *Marshallagia* of saigas at the molecular level, meanwhile, would be very useful if it were able to correlate this diversity with both morphological markers and biological traits such as host and environmental preferences. The resulting refinement in the diagnosis of helminthosis in the region would also justify the considerable logistical hurdles of such a study. For the time being, keys based on the morphology of species described in the literature will continue to serve as the main method of species identification. The simplified key presented in Fig. 5.11 is likely to prove useful in the rapid diagnosis of *Marshallagia* infections in saigas and domestic ruminants in Kazakhstan, and should minimise confusion between *M. marshalli* and *M. mongolica* in particular. Care is recommended in case species not included in the key are present.

The difficulties experienced in identifying *Haemonchus* species in this study also warrant further attention, if only because previous studies in Kazakhstan have failed to differentiate between *H. contortus* and *H. placei*. The potential epidemiological differences between these species are such that their differential diagnosis should be

routine in parasitological surveys (Lichtenfels *et al*, 1997), and this is recommended in future studies. Again, observed morphology did not correspond perfectly with published data from other parts of the world, and the possibility of local variations remains.

5.6.2. Parasite diversity and interspecific transmission

- *Species richness and overlap in faunas*

As species richness was shown to increase with sample size in the present study, so too apparent host specificity may be an illusion stemming from an inability to find a particular parasite in a given sample size. Chance absence of a parasite from the larger sample of two host species is less likely than absence from the smaller sample, and so the apparent restriction of *Ostertagia ostertagi* to sheep, for instance (n=30 abomasa), is more convincing than the apparent restriction of *Nematodirus gazellae* to saigas (n=144). Nevertheless, apparent patterns of host preference in Table 5.1 agree well with those in previous surveys (see chapter 3).

Some generalist species, previously found in saigas, were absent in this study, but present in sheep (see section 5.1.3). This may be due to decreased parasite prevalence in saigas since previous studies, or to generally low prevalence and an inadequate sample size. If these species are not able to maintain populations in saigas alone, and most infection of saigas originates from livestock, we might expect a decrease in contact between saigas and livestock to reduce the chances of finding them in saigas. However, there are many other possible explanations, including decreased density of saigas or sheep, or unfavourable climatic conditions that either affect transmission to saigas more than to livestock, or drive parasite populations in saigas down to undetectable levels because of lower starting abundance. Again, these questions cannot be answered using presence/absence data alone, but require analysis of relative parasite abundance now, and within host species now and in the past. The relationships between measured prevalence, abundance, sample size and test sensitivity are explored further in chapter 6.

Overall parasite diversity does appear to be lower in saigas than in sheep and goats irrespective of sample size, but again this may be confounded by differences in parasite abundance between host species. Trichostrongylid diversity is held to be

lower in central Kazakhstan, which is relatively arid (Kuznetsov and Dikov, 1979). Ustiurt is even more arid, and this, as well as less contact between saigas and livestock, might explain low diversity in this population. Sheep in Betpak-Dala carried more parasite species than sheep further South, in spite of previously noted high parasite diversity in the South of the country (Denisova, 1976). However, sheep within the saiga range are more likely to graze extensively, and may therefore have greater opportunity to visit diverse microhabitats and acquire many parasite species, irrespective of contact with wildlife (Boev *et al*, 1962; Poulin, 1998).

Confounding factors therefore prevent us from drawing firm conclusions regarding host range and interspecific transmission of parasites in saigas and domestic ruminants on the basis of presence-absence data alone. Nevertheless, most gastrointestinal nematode species found on this occasion in saigas have been found before in situations where there is little contact with livestock, such as on Barsa-Kel'mes island (Scholl *et al*, 1979), while all the species in sheep in Betpak-Dala have also been found in sheep outside the saiga range (Kuznetsov and Dikov, 1979). Evidence for potential interspecific transmission exists in that several parasite species are shared in this study, and all have been found in both host species in the past. The question of whether interspecific transmission actually occurs, however, will depend on whether shared parasites do actually comprise continuous gene pools across host species.

- *Diversity within parasite species*

Principal components analysis did not reveal any clear overall differences in the morphology of *Marshallagia* males between host species or populations in different areas. The relative frequency of different forms of *Marshallagia*, however, did vary, with *M. mongolica* most common in saigas in Betpak-Dala. This agrees with the existing literature, which suggests that saigas act as a reservoir host for *M. mongolica*, and are the principal source of infection for sheep (Radionov, 1973*a,b*). *M. marshalli*, meanwhile, is seen primarily as a parasite of sheep, which can be transmitted to saigas (Berkinbaev, 1992). The presence of *M. mongolica* in sheep outside the saiga range, and that of *M. marshalli* in the more isolated Ustiurt saiga population, however, seems to suggest that transmission between host species is not necessary for the persistence of either parasite in either host. Both *M. marshalli* and *M. mongolica* were found in saigas on Barsa-Kel'mes island by Scholl *et al* (1979).

It has also been demonstrated, however, that the morphological distinction between *M. marshalli* and *M. mongolica* is unclear. To some extent, apparent host specificity in parasites may be a function of the ease with which species can be recognised. Conversely, as already discussed, apparently generalist taxa may conceal cryptic species with more specific host or environmental preferences. Thus, in the present study, *Nematodirus* species were easily distinguished on the basis of spicule morphology, and the moleinids in general are held to be relatively host-specific in wild ruminants in North America (Hoberg *et al*, 2001). Oxyurid nematodes are also held to be highly host specific (Dunn, 1978), although *Skrjabinema ovis* has been found in a number of ruminant species worldwide (Levine, 1980), and in both saigas and domestic livestock in Kazakhstan (Boev *et al*, 1962). Levine (1980) pointed out that *Skrjabinema* eggs are larger in reindeers than in other host species, despite few distinguishing features in adult worms. Morphometric and/or genetic studies in *Skrjabinema* might reveal its apparently broad host range to be an illusion caused by morphological similarity between species.

Attributing host ranges to any of the parasite taxa found in saigas, therefore, depends on accurate diagnosis of species. Classification using existing keys may underestimate the underlying diversity in parasite taxa, particularly in the Ostertagiinae. The evaluation of such diversity may be a necessary part of future studies of the epidemiology of parasitism in multiple host species, including assessment of likely transmission risks at the wildlife-livestock boundary.

Among parasites of ruminants in Kazakhstan, there is evidence for distinct host preferences among the moleinids. Other species, including *Marshallagia*, cannot be said to show absolute host specificity, and differences in their abundance may be related to weight of exposure rather than to inherent susceptibility. Dividing *Marshallagia* specimens found in the present study into *M. marshalli* and *M. mongolica* alters the observed patterns of distribution of these parasites between hosts (see chapter 6). For the time being, it must be assumed that *Marshallagia* species in Kazakhstan are mutually transmissible between saigas and domestic ruminants.

- *Diversity in multiple host systems*

The results of this and previous studies suggest that a large number of gastrointestinal nematode species exist in ruminants in Kazakhstan, and are able to infect several different host species. The existence of multiple congeneric parasite species with overlapping anatomical and host ranges in the same geographical area, however, appears to confound expectations that closely related sympatric species should show niche separation (Pianka, 2000). Sympatric speciation should be associated with a break in gene flow, while ecological and physiological adaptation to a particular host species usually comes at the price of decreased ability to infect other hosts (Inglis, 1965). Where many closely related host species coexist, however, and adaptation to one host is unlikely to rule out exposure of other hosts to infective stages, a broad host range may itself confer a selective advantage. Such a situation could exist in trichostrongylids of ruminants (Poulin, 1998): 'accidental' ingestion of infective stages by hosts other than the primary host may favour persistence of the parasite population even if R_0 in these secondary hosts is low (see chapter 2). Natural selection might then favour parasites whose host range is broad, especially where infection of the primary host is unpredictable due to fluctuations in climate or host presence. *Haemonchus* species in Mauritania appear to use different host species, which are available at different times of year, to survive unfavourable environmental conditions during the dry season (Jacquiet *et al*, 1995*a*,*b*; 1996; 1998). *Marshallagia* species in Kazakhstan may similarly rely on broad host specificity to achieve maximum exploitation of host resources that vary widely in time and space, and survive in a harsh environment that provides poor shelter for a reservoir population of free-living stages.

The interaction between host preference and environmental conditions in shaping parasite life history strategy might also explain the adaptive significance of high levels of intraspecific genetic diversity in parasite populations. Limited gene flow between parasite infrapopulations might lead to the situation where many sub-types of parasites exist within a species, each differing slightly in their host and ecological preferences, but none dominating because of variation in host availability and environmental conditions. Host movement may further favour maintenance of parasite genetic diversity through the reintroduction of locally lost alleles (Lively and Apanius, 1995). Parasites may themselves encourage genetic diversity in their hosts (Gulland *et al*, 1993), and a high level of genetic diversity in both parasite and host

populations may then be important in stabilising the host-parasite relationship (Read *et al*, 1995).

A general understanding of patterns of host use in multiple host systems is likely to be some way off. Theoretical approaches are as yet largely unsupported by evidence from the field (Wilson *et al*, 2002; Grenfell *et al*, 2002). While the importance of the interaction between genetic diversity in hosts and parasites in determining the outcome of infection is well recognised in microparasites (Thrusfield, 1995), the large amount of diversity within macroparasite taxa is only now coming to light (Blouin *et al*, 1995; Anderson *et al*, 1998). Undoubtedly, the prediction of patterns of host specificity within and between parasite groups requires a better understanding of the phenotypic consequences of this diversity, and of host-parasite co-evolution (Hoberg and Lichtenfels, 1994; Hoberg *et al*, 1999; 2001). However, in the short term, more field data on the determinants of parasites' host and environmental preferences are needed, for instance to predict the likely fate of introductions of exotic parasites. Parasites have been shown to vary within species in their environmental requirements and tolerances (e.g., for *Haemonchus*, see Crofton *et al*, 1965; Le Jambre and Whitlock, 1976), and the flexibility afforded by such diversity will be central to new as well as existing problems of control, such as the effects of global climate change both on the geographical distribution of parasites, and the population dynamics of species within their existing ranges.

The results presented in this chapter illustrate that the distribution of ostensibly conspecific parasite populations between sympatric host species may not be all that it seems. Relating host preferences to underlying genetic diversity could lead to key advances in our understanding of parasite behaviour in multiple host systems. In the meantime, surveys of such systems should ally sound identification of parasite species with careful examination and comparison of parasite specimens recovered from different hosts. A critical approach to the inference of host specificity from the apparent presence of conspecific parasites in sympatric wildlife and livestock is needed if attempts to control interspecific transmission are to be based on sound diagnosis. This is equally true of systems that have been well studied in the past, since concealed diversity in the parasites found may not have been recognised previously.

5.6.3 Conclusions and further work

- Saigas on the whole carried a less diverse parasite fauna than either domestic sheep or goats, though many species of gastrointestinal nematode were shared. Sheep within the saiga range carried more species than those outside the range: this does not, however, necessarily imply parasite transmission from saigas.
- Observed species diversity was affected by sample size, and this should be borne in mind when comparing data between studies.
- *Marshallagia* specimens recovered from saigas and domestic ruminants in Kazakhstan were superficially similar morphologically, but were shown to vary discontinuously in some measured characters, though the lack of definite separation complicated diagnosis. Differences corresponded roughly to species described in the literature, but not all key characters were consistent or equally useful. Pending a definitive systematic review of the genus, described species should be considered valid. A simplified key, including a discriminant function based on morphology of the bursa and spicules, was devised, and should facilitate rapid identification of *Marshallagia* specimens in future saiga studies.
- A review of diversity within the genus *Marshallagia* is needed, and should aim to demonstrate consistent differences between described species, and pairing of major and minor morphs. Comparison of the synlophe may prove useful for this. Ultimately, however, molecular measurement of genetic diversity, and correlation with morphological variation, is needed so that future epidemiological studies have a sound diagnostic and taxonomic basis.
- Published descriptions of *Haemonchus* species matched specimens found in sheep and goats in southern Kazakhstan only imperfectly. Few specimens were recovered, however, and further work is needed for definitive identification.
- Several nematode species were found only in saigas, others only in domestic ruminants, and some in both. *Marshallagia marshalli* and *M. mongolica* were both found in saigas and livestock. Presence-absence data provide only a poor guide to host specificity, however, and detailed consideration of interspecific transmission of parasites between saigas and livestock requires comparison of parasite abundance between host species. This is undertaken in later chapters.