Displacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* (Acari: Ixodidae) in the Eastern Cape Province, South Africa

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Abstract The objective of the study was to establish to what extent the native tick species *Rhipicephalus decoloratus* had been displaced by the invasive introduced tick, *Rhipicephalus microplus* at two communally grazed areas in the Eastern Cape Province, South Africa. To this end ticks were collected monthly from 5 cattle over a period of 2 years and from 10 drag-samples of the vegetation over a period of one year at each locality. Whereas 10 years previously only *R. decoloratus* and no *R. microplus* had been recorded in the vicinity of the two sites, *R. microplus* now comprised the bulk of collections at both. Furthermore, significantly greater numbers of *R. microplus* were collected from cattle at both localities during the 2nd year of the survey than during the 1st ($P<0.05$ and $P<0.01$). In addition to 83 instances of intraspecific coupling, there were 17 instances of *R. microplus* males coupled with *R. decoloratus* females. Collections made from cattle and goats on 2 farms close to the study sites revealed that *R. microplus* was present on both host species and that it significantly outnumbered *R. decoloratus* on one of the farms ($P<0.001$). *R. decoloratus* and *R. microplus* larvae as well as larvae exhibiting characteristics of both species were collected from the vegetation.

Keywords: cattle, *Rhipicephalus decoloratus*, *Rhipicephalus microplus*, displacement, South Africa

Introduction

Globally, ticks are considered the most important external parasites of livestock, and they, and the diseases they transmit, represent a severe constraint to successful stock farming in many countries (Jongejan and Uilenberg 2004). Their impact is usually more severe in developing, resource-limited countries than in the developed world (De Castro 1997). In the Eastern Cape Province, South Africa, ticks and tick-borne diseases and their control are considered a major challenge for most small scale cattle farmers (Masika et al. 1997). There are approximately 3.1 million beef cattle in this province, and these comprise nearly a quarter of the total cattle population of South Africa (Anon. 2008), and it is estimated that more than 65% of these cattle are farmed in communally grazed areas (Anon. 2003). Moreover, the geographical distributions of practically all the economically important tick species that infest livestock in South Africa, include the communally grazed areas of this province (Howell et al. 1978; Horak et al. 2009).

In addition to the threat of infestation by indigenous ticks and the diseases they transmit, cattle in several countries on the African continent are put at risk by the introduction and spread of the Pantropical blue tick, *Rhipicephalus microplus*. This tick was recorded in the southern coastal belt of South Africa as early as 1908 (Howard 1908), but its actual geographical distribution in this country was only mapped several decades later (Howell et al. 1978). By 2009 Horak et al. (2009) noted that *R. microplus* was the dominant species in the eastern regions of the Eastern Cape Province, compared to the earlier dominance of the indigenous African blue tick *Rhipicephalus decoloratus*.

In neighbouring and other countries in Africa Mason and Norval (1980) stated that there was little doubt that *R. microplus* had been introduced into Zimbabwe from Mozambique and that it had subsequently spread westwards along the Zambezi Valley escarpment and southwards towards the Harare (Salisbury) district. Lynen et al. (2008) documented the expansion of its distribution in Tanzania at the expense of *R. decoloratus* compared to that recorded for these ticks by Yeoman and Walker (1967) many years earlier. The first record of *R. microplus* in West Africa is that of Madder et al. (2007), who reported its presence in Ivory Coast. By 2008 it had almost completely displaced the various indigenous *Rhipicephalus* (*Boophilus*) spp. on farms around the village where it had first been detected (Madder et al. 2011). In 2008, a new focus of invasion was detected in the Department of Mono in south-western Benin, West Africa and by 2011 *R. microplus* had invaded the southern half of that country (Madder et al. 2012; De Clercq et al. 2012). A survey conducted in southern Mozambique, bordering the Kruger National Park in South Africa, in which park only *R. decoloratus* is present (Horak et al. 1992; 2003), yielded only *R. microplus* and no *R. decoloratus* from cattle and goats sampled at 30 dip-tanks (Horak et al. 2009).

The aims of the present investigation were to establish to what extent the invasive species *R. microplus* had displaced the native *R. decoloratus* in two inland, communally grazed areas to the west of the city of East London in the Eastern Cape Province, South Africa.

Materials and methods

Study localities (Figure 1A, B)

The Ncerha communal grazing area (33° 04'S; 27° 34'E; alt. 197 m) lies within the Albany Coastal Belt (Mucina and Rutherford 2006). The most common grass species here are *Cynodon dactylon, Digitaria eriantha, Eragrostis plana*, *Sporobolus fimbriatus, Themeda triandra* and *Paspalum dilatatum*, while common trees include *Acacia karoo, Diospyros simmii, Maytetus heterophylle, Grewia occidentalis* and *Scutia myrtina*. The highest mean atmospheric temperatures are recorded in January and February (26˚C) and the lowest in June (13˚C). Average annual rainfall varies between approximately 900 mm in the hot wet season and 450 mm in the cool dry season. The spring months tend to be windy while least wind is recorded from January to March.

The Majali communal grazing area (32° 44'S; 27° 31'E, alt. 609 m) is located in Bhisho Thornveld (Mucina and Rutherford 2006). The most common grass species are *Eragrostis plana*, *Sporobolus africanus* and *Themeda triandra*, while common trees include *Acacia karoo, Ehretia rigida, Lippie javanica* and *Scutia myrtina*. The climate varies between a hot-wet season and a cool dry season. The highest mean temperature is recorded in February (26˚C) and the lowest in June (7˚C). Average annual rainfall fluctuates between approximately 900 mm in the hot wet season and 500 mm in the cool dry season.

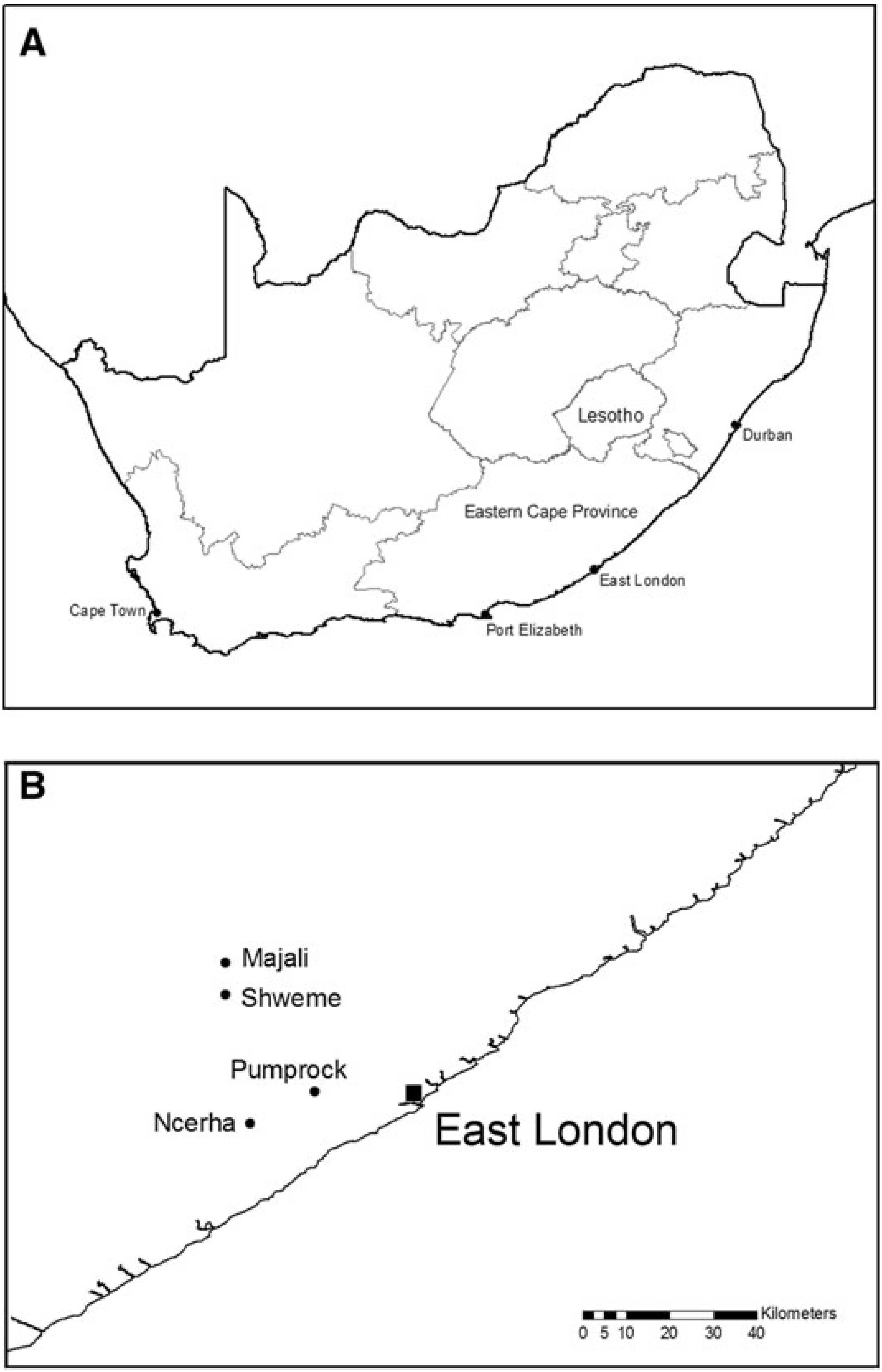

Fig. 1 Four localities in the Eastern Cape Province, South Africa, at which ticks were collected

Cattle ticks

Adult ticks were collected monthly at both localities from February 2010 until January 2012 from 5 healthy, approximately 2 year-old cattle that had visible tick infestations. Animals were selected from a pool of five different owners. However, the 5 animals varied from month to month due to the nature of the communal grazing set up. Ticks were collected mainly from one side of the animal, including half the head and one ear, but also from the whole of the upper perineum and tail brush. Ticks from each animal were stored in 70% ethanol in internally labelled vials for later identification and counting. From February 2011 to January 2012 the numbers of male *R. decoloratus* or male *R. microplus* coupled with female ticks were recorded, as were the species of the female ticks with which the males had coupled. Coupling of male ticks with engorged nymphs was also recorded.

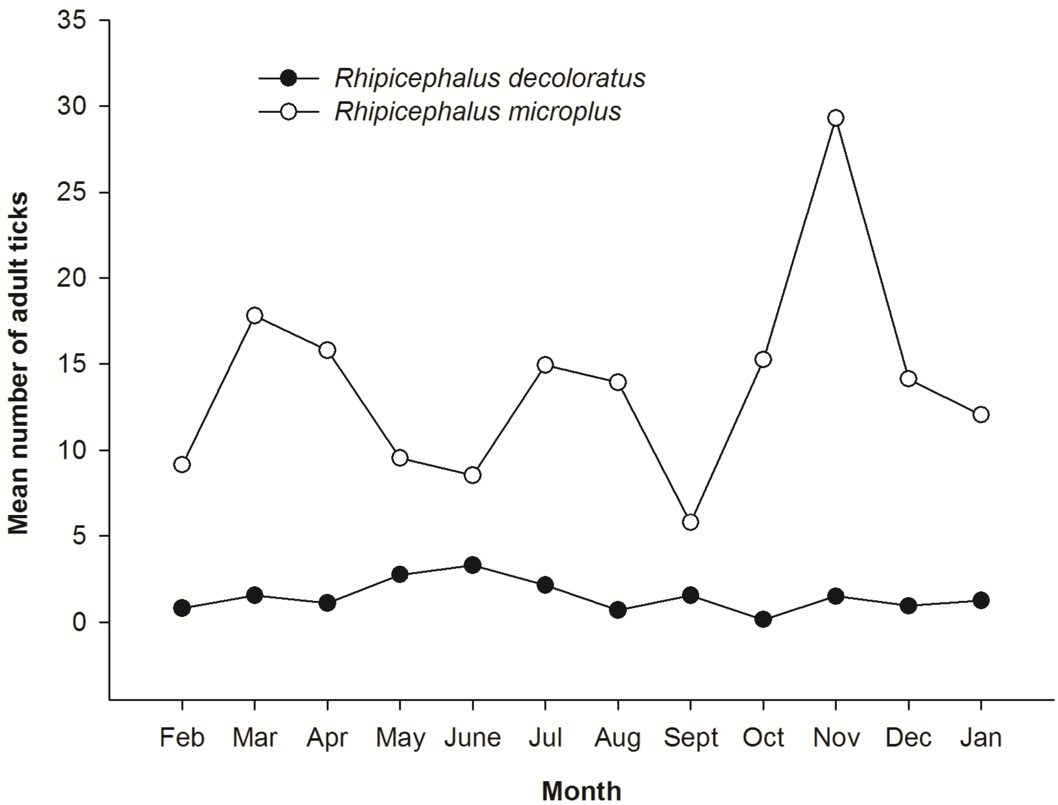

Fig. 2 Seasonal abundance of the adults of R. decoloratus and R. microplus on cattle at two communally grazed localities in the East London District of the Eastern Cape Province, South Africa (years and sites combined)

Because of the proximity of the study sites to each other, and the effect that regular plunge-dipping in the formamidine compound, amitraz, probably had on tick numbers, the mean monthly numbers of adult ticks from both localities and over the 2 years of the study, have been separately combined for each species in order to strengthen any pattern of seasonal abundance that may emerge and to enhance the graphic presentation of results (Figure 2).

Questing ticks

Rhipicephalus (*Boophilus*) spp. larvae questing for hosts on vegetation in the communal grazing areas were collected by drag-sampling as described by Nyangiwe et al. (2011). Ten replicate 100m long drags, approximately 50m apart, were performed monthly for a period of 12 months (February 2011 – January 2012) at both localities. After each drag all ticks on the flannel strips were collected by means of fine-tipped forceps and stored in 70% ethanol in internally labeled vials for later identification and counting. Once the larvae present in each of the drag-samples had been identified and counted they were decanted with the alcohol in which they had been preserved into a single large vessel reserved for each of the localities. Larvae were identified using the descriptions provided by Arthur and Londt (1973) for *R. decoloratus*, Londt and Arthur (1975) for *R. microplus*, and Gothe (1967) for the larvae of both species. To avoid confusion with other *Rhipicephalus* species the subgenus name, *Boophilus* has been included whenever reference is made to these ticks at the generic but not at the specific level.

It was only after all the questing larvae had been identified, counted and decanted into a single vessel for each locality that it was realized that the *R.* (*Boophilus*) spp. group of larvae apparently comprised three entities. These consisted of larvae that were typically *R. decoloratus* or *R. microplus* and larvae that exhibited characters intermediate between the two species. The palps of the latter group of larvae were often upright instead of sloping inwards as for *R. microplus*, the palps were sometimes longer than those of *R. decloratus* or *R. microplus*, but shorter than the palps of *Rhipicephalus evertsi evertsi*. The scutum of some larvae, that in all other aspects resembled *R. microplus*, was longer and more angular and similar in appearance to that of *R. decoloratus*, and the idiosoma of some of these larvae was oval and not circular as in the case of questing *R. decoloratus* and *R. microplus* larvae.

During the identification of ticks in the individual drag samples the atypical larvae had unfortunately been assigned to either *R. decoloratus* or *R. microplu*s depending on their most prominent characteristics. This was done because at the time it had seemed almost impossible that there could be a third *R.*

(*Boophilus*) species in South Africa, in which only *R. decoloratus* and *R. microplus* are known to be present. In an attempt to rectify this mistake 300 *R.* (*Boophilus*) spp. larvae from each of the vessels reserved for the two localities were re-examined and identified to the best of our ability. The proportions of these larvae belonging to the three groups were used to allocate the remainder of the larvae collected from the vegetation to one of the groups. This procedure made it impossible to separately determine the seasonality of the larvae in the three groups and they have been combined as *R.* (*Boophilus*) spp., and their seasonality graphically illustrated (Figure 3).

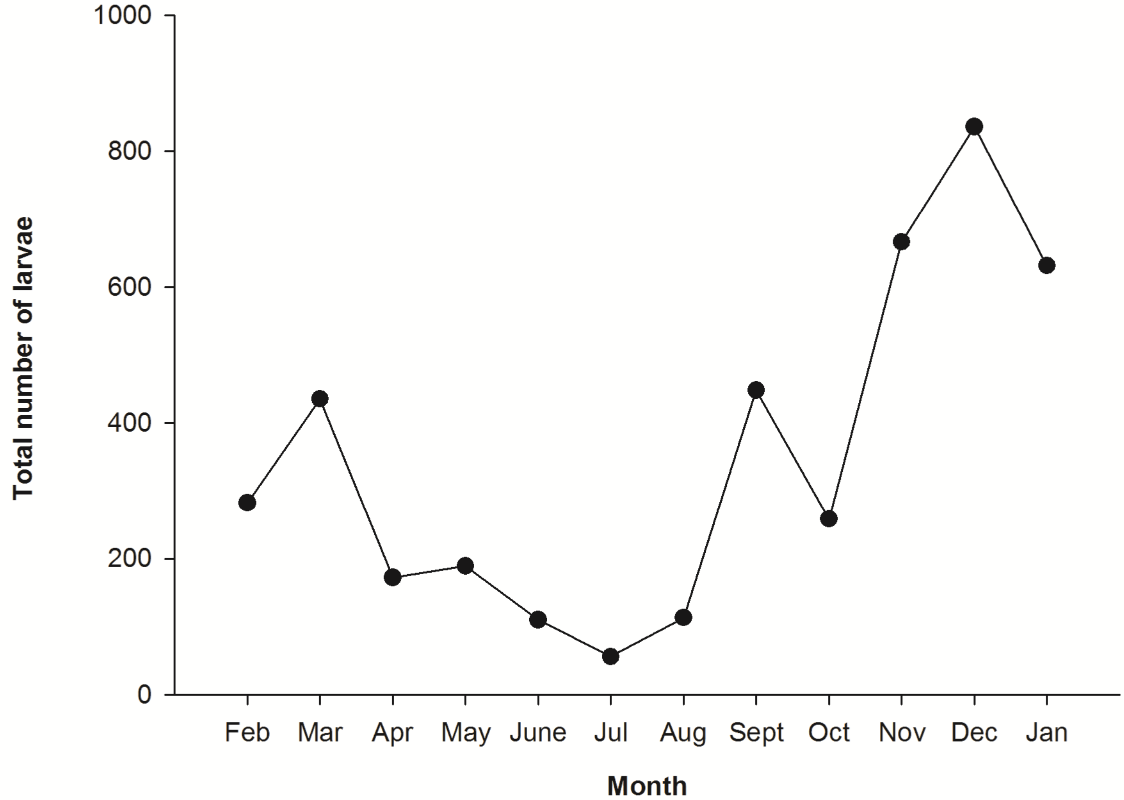

Fig. 3 Seasonal abundance of larvae in the three entities constituting the Rhipicephalus (Boophilus) group combined on vegetation at two communally grazed localities in the East London District of the Eastern Cape Province, South Africa (sites combined)

Additional collections

Ticks were collected during March and again during September 2011 from five cattle and from five goats on two privately owned farms. One of these farms, 'Pumprock' (33° 00' S, 27° 42' E, alt 280 m) was close to the Ncerha communal grazing area, while the other, 'Shweme' (32° 48' S, 27° 31' E, alt. 522 m) was close to the Majali communal grazing area (Figure 1A, B). These collections were made in order to compare the proportional representations of *R. decoloratus* and *R. microplus* on privately owned farms with those on the two communally grazed areas.

Table 1. Total number of ticks in collections (n=120) from cattle and from the vegetation (n=120) within the Ncerha and Mjali communal grazing areas

Locality and tick species	Collections from cattle (n=120)				Vegetation collections (n=120)	
	Males	Females	Total	Number positive	Larvae	Number positive
Ncerha communal grazing area						
Rhipicephalus decoloratus	52	207	259	69	252	
Rhipicephalus microplus	425	751	1176	89	603	} 114
Rhipicephalus (*Boophilus*) sp.	0	0	0	0	524	
Majali communal grazing area						
Rhipicephalus decoloratus	12	66	78	27	378	
Rhipicephalus microplus	578	1379	1957	109	1151	} 116
Rhipicephalus (*Boophilus*) sp.	0	0	0	0	1322	

Results

The total numbers of adult *R. decoloratus* and *R. microplus* collected from cattle and the numbers of larvae of the *R.* (*Boophilus*) spp. grouping collected from the vegetation at the Ncerha and Majali communal grazing areas are summarized in Table 1. At both localities *R. microplus* was the dominant species on cattle, while a substantial number of *R. decoloratus* adults were also collected at Ncerha, but not at Majali. More larvae of the three entities within the *R.* (*Boophilus*) spp. grouping were collected from vegetation at Majali than at Ncerha.

The mean numbers of adult ticks collected from cattle between February 2010 and January 2012 and of *R.* (*Boophilus*) spp. larvae collected from the vegetation at Ncerha and Majali between February 2011 and January 2012 are summarized in Table 2. Significantly more adult *R. decoloratus* (W=35.5, P<0.01) were collected per month from cattle at Ncerha than at Majali, whereas significantly more adult *R.*

Table 2. Mean number of adult ticks (±SE) collected per month from cattle over two years and mean number of larvae (±SE) collected per month from vegetation over a single year at Nchera and Majali. Wilcoxon signed rank test statistics (*W*) for paired monthly differences in the abundance of ticks between sites and associated P-values are presented.

Tick species	Instar	Ncerha	Majali	Test statistic	P-value
Cattle (5 animals per month per site, February 2010 to January 2012)					
Rhipicephalus decoloratus	Adult	10.79 ± 2.19[ac]	3.25 ± 1.09[ad]	35.5	P<0.01
Rhipicephalus microplus	Adult	49.00 ± 13.63[bc]	81.54 ± 11.33[bd]	42.0	P<0.01
Vegetation (10 drag samples per month, per site, February 2011 to January 2012)					
Rhipicephalus (*Boophilus*) spp.	Larvae	57.46 ± 11.40	118.79 ± 30.09	23.0	P=0.209

Values with the same superscript are significantly different

[a and b] = P<0.01

[c and d] = P<0.001

microplus were collected per month from cattle at Majali than at Ncerha (Wilcoxon sign rank tes, *W*= 42.0, P<0.01). In addition, more *R. microplus* than *R. decoloratus* were collected from cattle at both Ncerha (*W*=10.0, P<0.001) and Majali (*W*=2.0, P<0.001). There was no significant difference between the numbers of *R*. (*Boophilus*) spp. larvae collected at the two sites.

A comparison of the mean number of ticks collected from cattle per month per year is summarized in Table 3. The numbers of *R. decoloratus* collected monthly during the 1st year of the study were not significantly different from those collected in the 2nd year at either locality. Significantly more *R. microplus* were collected in the second year of the study at both Ncerha (*W*=7.0, P<0.05) and Majali (*W*=3.0, P<0.01) than in the first year.

Table 3. Mean number of adult ticks (±SE) collected monthly from February 2010 and 2011 to January 2011 and 2012 respectively from 5 cattle per site per year at Nchera and Majali. Wilcoxon signed rank test statistics (*W*) for paired monthly differences in the abundance of ticks between sites and associated P-values are presented. (significant differences are presented in bold).

Site	Tick species	Feb'10-Jan'11	Feb'11-Jan'12	Test statistic	P-value
Ncerha	*Rhipicephalus decoloratus*	7.42 ± 2.23	14.17 ± 3.61	17.5	P=1.378
	Rhipicephalus microplus	22.08 ± 5.79[a]	75.92 ± 24.72[a]	7.0	P<0.05
Majali	*Rhipicephalus decoloratus*	4.50 ± 1.82	2.00 ± 1.82	14.0	P=0.169
	Rhipicephalus microplus	45.67 ± 14.07[b]	117.42 ± 14.07[b]	3.0	P<0.01

Values with the same superscript are significantly different

Combining the findings at the two localities the observations on coupling and cross-coupling of *R. decoloratus* and *R. microplus* can be summarized as follows. There were 12 instances of *R. decoloratus* males coupled with *R. decoloratus* females, 71 instances of *R. microplus* males coupled with females of the same species, and 17 instances of *R. microplus* males coupled with *R. decoloratus* females. No couplings between *R. decoloratus* males and *R. microplus* females were observed. Furthermore, 13 male *R. microplus* were found attached adjacent to and clasping engorged nymphs. Upon dissecting 10 of these nymphs, 9 were going to moult to female ticks and the gender of the 10th could not be determined.

The seasonal abundances of adult *R. decoloratus* and *R. microplus* are depicted in Figure 2, and that of larvae in the three entities constituting the *R.* (*Boophilus*) spp. grouping combined in Figure 3. No clear pattern of seasonal abundance for adult *R. decoloratus* was evident, and although adults of *R. microplus* appeared to peak in November, this was largely driven by a substantial number of ticks (165) collected from a single animal. Most larvae of the three entities in the *R.* (*Boophilus*) spp. grouping were present in March and from September to January, while few were collected from June to August.

Table 4. Ticks collected during March and during September 2011 from five cattle and five goats on the farms 'Pumprock' and 'Shweme' close to the Ncerha and Mjali communal grazing areas (see text for two sample Z-test statistics and associated P-values).

Tick species	Total numbers of ticks collected							
	Collections from cattle (n=10)				Collections from goats (n=10)			
	Males	Females	Total	No. positive	Males	Females	Total	No. positive
	Pumprock' farm							
Rhipicephalus decoloratus	2	3	5^{a}	2	0	2	2^{b}	2
Rhipicephalus microplus	108	65	173^{ae}	10	73	36	109^{be}	5
	'Shweme' farm							
Rhipicephalus decoloratus	27	83	110^{cd}	10	0	6	6^{d}	2
Rhipicephalus microplus	16	27	43^{cf}	7	5	1	6^{f}	3

Values with the same superscript are significantly different (P< 0.001)

The numbers of adult *R. decoloratus* and *R. microplus* collected from cattle and goats on the farm 'Pumprock', close to the Ncerha community and on the farm 'Shweme', close to the Majali community are summarized in Table 4. *R. microplus* was significantly more abundant than *R. decoloratus* on cattle (Z=-14.43, P<0.001) and goats (Z=-11.14, P<0.001) at 'Pumprock', while more *R. decoloratus* than *R. microplus* were present on cattle (Z=6.045, P<0.001), but not goats, at 'Swheme'. The numbers of *R. decoloratus* collected from cattle and goats at 'Pumprock' did not differ significantly, most likely because of the few ticks collected there, whereas significantly more *R. decoloratus* were recovered from cattle than from goats at 'Swheme' (Z=10.18, P<0.001). More *R. microplus* were collected from cattle than from goats at both sites ('Pumprock', Z=4.26, P<0.001; 'Swheme', Z=5.40, P<0.001).

Discussion

The adults of *R. decoloratus* and *R. microplus* collected from cattle could easily be distinguished from each other on their characteristic morphological features, and no aberrations in these features were evident. Questing larvae typical of *R.* (*Boophilus*) *decoloratus* and *R.* (*Boophilus*) *microplus* could also be identified. However, as mentioned above, a large number of *R.* (*Boophilus*) sp. larvae displayed features characteristic of both species. Consequently, these larvae were designated as belonging to the *R.* (*Boophilus*) spp. group and could represent hybrids between the two species. Hybrids between *Rhicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus* have successfully been reared to adults by Davey and Hilburn (1991). The adults were, however, sterile. It would require laboratory experimentation, outside the scope of this investigation, to determine the viability of the so-called hybrids presently encountered.

Two studies, in which an attempt was made to collect all ticks from kudus (*Tragelaphus strepsiceros*) and impalas (*Aepyceros melampus*), shot and processed specifically for this purpose, revealed that for every *R. decoloratus* female there are at least two males, five to six nymphs and 10 to 12 larvae on the same animal (Horak et al. 1992, 2003). There is no reason to believe that this pattern should be otherwise for *R. microplus*. Consequently, the number of male ticks of both species collected in the present study should have been at least twice that of the number of females. The fact that the number of females recovered outnumbered the number of males suggests that the collections were by no means exhaustive. In addition, when sampling living animals it is virtually impossible to collect all adult ticks of these species from the numerous sites to which they attach. Consequently the numbers of adult *R. decoloratus* and *R. microplus* collected from cattle at both localities do not represent the true burdens of these ticks. Despite this drawback it is evident that considerably more adult *R. microplus* than adult *R. decoloratus* were recovered at both communal grazing areas.

Based on collections of ticks made from cattle over the preceding decades, Howell et al. (1978) mapped the geographical distributions *R. decoloratus* and *R. microplus* in South Africa. At the time, *R. decoloratus* occupied almost the entire eastern region of the Eastern Cape Province while the distribution of *R. microplus* was patchy and discontinuous. A few years later, Baker et al. (1981) and Baker (1982) mapped a considerably more extensive distribution for *R. microplus* in the eastern region of the Eastern Cape Province. During surveys conducted in 2004 and 2005 it became obvious that the distribution patterns of the two species in this region had reversed and that *R. microplus* was now the dominant species (Horak et al. 2009). The latter authors also suggested that *R. microplus* was in the process of displacing *R. decoloratus*. In contrast Nyangiwe et al. (2011) reported that although questing larvae of *R. microplus* outnumbered those of *R. decoloratus* on the vegetation of an experimental farm in Döhne Sourveld in the eastern region of the Eastern Cape Province, the ratio between the two species remained more or less stable over a period of 5 years.

Until the fairly recent past *R. decoloratus* seemingly remained the only species present to the immediate west of the city of East London, Eastern Cape Province. Rechav (1982), who collected ticks from cattle in the coastal region approximately 20 km to the south-west of East London, recovered large numbers of *R. decoloratus*, but no *R. microplus*. Furthermore, a survey conducted by Horak (1999) along the coast approximately 150 km to the south-west of East London yielded a small number of *R. decoloratus*, but also no *R. microplus*. While Mekonnen et al. (2002, 2003), who conducted acaricide resistance studies on ticks collected from cattle in the immediate vicinity of the Nchera and Majali communally grazed areas, also recorded only *R. decoloratus* and no *R. microplus*.

In the light of the present results it would seem that *R. microplus* is a recent introduction into the region immediately to the west of East London and that, as is the case in the more eastern region of the Eastern Cape Province, it is in the process of superseding *R. decoloratus*. Domestic cattle are the most efficient hosts of *R. microplus* (Mason and Norval 1980). Goats play a lesser, but still significant role (Nyangiwe and Horak 2007; De Matos et al. 2009), and as demonstrated on the farms 'Pumprock' and 'Shweme' in this study. It is thus on cattle and possibly to a lesser extent on goats, purchased in localities where *R. microplus* is present, that it has been introduced into the region. However, where in the past displacement was a seemingly slow process, its pace now appears to have accelerated, an observation similar to that reported by Tonnenson et al. (2004) in Limpopo Province, South Africa and Madder et al. (2011) in the Ivory Coast and De Clercq et al. (2012) in Benin.

Judging by the significant increase in numbers at both Majali and Ncerha during the second year of the survey compared to the first, it is also possible that *R. microplus* was being selected for resistance against Amitraz, the acaricide in use at both localities. This eventuality would further enhance its potential as an invasive species. The most recently published results of acaricide resistance studies in the eastern region of the Eastern Cape Province would seem to support this possibility. Acaride resistance tests conducted on *R. decoloratus* on two communally grazed areas close to Ncerha and Majali revealed no resistance to amitraz (Mekonnen et al. 2002), while tests conducted against *R. microplus* at 45 communally-grazed localities to the east of East London revealed emerging resistance to amitraz at two localities and resistance at a third (Ntondini et al. 2008). The possibility thus exists that *R. decoloratus* populations were being suppressed by the application of amitraz while the acaricide-resistant component of the *R. microplus* populations continued to flourish.

The relative abundance of *R. microplus*, in comparison to that of *R. decoloratus* on the farm 'Pumprock', implies that it is successfully displacing *R. decoloratus* also at this locality. Although *R. decoloratus* outnumbered *R. microplus* on the farm 'Shweme', future tick collections are likely to reveal encroachment by *R. microplus* also here.

Horak et al. (2009) commented fairly extensively on the likely reasons for the displacement of *R. decoloratus* by *R. microplus*. One of them being that *R. microplus* males, because of their slightly shorter life cycle than that of *R. decoloratus*, coupled with the fact that there are always more male ticks than female ticks (Horak et al. 1992, 2003), would not only mate with conspecific females but excess males would mate with those of *R. decoloratus*. Seventeen such couplings were observed in the present study, as were 13 incidents where *R. microplus* males were attached to engorged nymphs, which when dissected almost invariably proved to be the precursors of female ticks. The variations in morphological characters observed in larvae collected from the vegetation and grouped as *R.* (*Boophilus*) spp., with some of these characters seen as gradations between *R. decoloratus* and *R. microplus*, suggest that the eggs resulting from cross-matings may not always be sterile and that hybridization may indeed be possible. No adult hybrids exhibiting taxonomic features of both species were, however, collected.

The slightly shorter parasitic portion of the life cycle of *R. microplus* (Arthur and Londt 1973; Londt and Arthur 1975), coupled with the fact that within the eastern region of the Eastern Cape Province *R. microplus* larvae are present on vegetation during winter while those of *R. decoloratus* almost disappear (Nyangiwe et al. 2011), may result in *R. microplus* completing one more life cycle per year than *R. decoloratus*. This would also enhance its chances of displacing *R. decoloratus*.

Displacement of an indigenous species by a foreign species has implications for biodiversity. When, however this displacement occurs between tick species, there may also be serious implications for the transmission of disease. The indigenous tick *R. decoloratus* is the vector of *Babesia bigemina*, the causative organism of African redwater in cattle, while the invasive *R. microplus* transmits not only *B. bigemina* but also the more virulent *Babesia bovis*, the causative organism of Asiatic redwater in cattle (De Vos et al. 2004). The spread of *R. microplus* in South Africa has in several instances been accompanied by outbreaks of Asiatic redwater in regions in which only African redwater was recorded in the past. Such outbreaks are likely to increase as the tick expands its distribution range.

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