Biodiversity assessment of tetranychid mites in Kenya and the conservation hotspots of Tanzania.

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Dedication

This work is dedicated to my late mother Grace; who saw the start but not the finish of this journey. May God rest her soul in eternal peace!

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Abstract

The aims of this study were to develop a detailed record of the tetranychid mites of Kenya and Tanzania, to assess the diversity of tetranychid mites in the east African biodiversity hotspots and to determine female characters that can be used to identify the species of the economically important *Tetranychus* species found in these countries. The genetic diversity of the most abundant *Tetranychus* species (*Tetranychus evansi* Baker & Pritchard) was also assessed.

The Tetranychidae (Acari) contain some of the most important pest species of phytophagous mites worldwide. Out of the almost 1,300 species in this family, 256 species are known to occur in Africa. Before this study, ten species had been reported from Kenya and only three in Tanzania. The genus *Tetranychus* to which most of the pest species belongs to, can only be identified to species level by the use of the male aedeagus that is often difficult to visualize.

The natural habitat, the Eastern Arc Mountains and East African Coastal Forests in Kenya and Tanzania is recognized as biodiversity hotspots but prior to his study, information on Tetranychidae in these hotspots was lacking. Thus, no information on the natural mite fauna composition was available.

In Kenya, 18 tetranychid mite species from various plant hosts have been recorded. Four of these species belong to the subfamily Bryobiinae and the other 14 to the subfamily Tetranychinae. Eight of the mite species identified belong to the genera *Bryobia*, *Petrobia*, *Peltanobi*a, *Paraplonobia*, *Duplanychus*, *Eutetranychus* and *Mixonychus* and are being reported for the first time in Kenya while the other ten had already been reported before. For Tanzania, six species belonging to the genera *Tetranychus*, *Eutetranychus* and *Mixonychus* are being reported for the first time from Tanzania and other three had been reported before. A list of these species, their brief descriptions as well as a key for identification is provided. A redescription of *Peltanobia erasmusi* including previously undescribed male characters is given.

Schizotetranychus kwalensis sp. nov. from Kenya and *Brevinychus meshacki* from Tanzania were collected on *Omorcarpum kirkii* (Fabaceae) from Matuga, Kwale district, Kenya and *Philonoptera eriocalyx* (Fabaceae) from Sangasanga, Mvomero district, Tanzania respectively and described. Revised keys of *Brevinychus* and of the African species of *Schizotetranychus* are also provided.

Tetranychus evansi Baker & Pritchard ranked highest in abundance amongst all the tetranychid mites collected. It was found in four out of five fragments of the hotspot, and it survives in a wide range of altitudes from as low as 123 m to 1655 m. Molecular examination of *T. evansi* collected from Kenya and Tanzania and on different host plants revealed an identical DNA sequence of the mitochondrial COI fragment and 19 identical microsatellite alleles suggesting a single introduction of this species to this part of East Africa.

Female characters of four *Tetranychus* species found in Kenya were explored using the scanning electron microscope. Differences in the distances between the duplex setae of species belonging to the *desertorum* group (*Tetranychus evansi* Baker & Pritchard and *Tetranychus ludeni* Zacher) and those grouped by Flechtmann and Knihinicki (2002) under group 9 (*Tetranychus neocaledonicus* Andre and *Tetranychus urticae* Koch) were observed. The dorsal striae of *T. evansi*, *T. neocaledonicus* and *T. urticae* have semicircular lobes whereas those on the dorsal striae of *T. ludeni* are triangular.

Key words: Taxonomy, Tetranychidae, Spider mites, *Tetranychus evansi*, *Peltanobia erasmusi*, *Brevinychus, Schizotetranychus*, Eastern Arc Mountains and Coastal Forests of Tanzania/Kenya hotspots.

Opsomming

Die doel met hierdie studie was om ‘n volledige lys van spesies van die familie Tetranychidae vir Kenia en Tanzanië saam te stel, die diversiteit van die Tetranychidae in die Oos-Afrikaanse brandpunte vas te stel en om te bepaal of daar wyfie-kenmerke is wat gebruik kan word om spesies van die ekonomies-belangrike genus *Tetranychus* te identifiseer. Die genetiese verskeidenheid/diversiteit van die mees oorvloedige *Tetranychus*-spesie (*Tetranychus evansi* Baker & Pritchard) was ook bepaal.

Die familie Tetranychidae (Acari) bevat van die belangrikste plantparasitiese spesies wêreldwyd. Van die byna 1 300 spesies in die familie, kom 256 in Afrika voor. Voor hierdie studie was slegs 10 spesies uit Kenia bekend en drie uit Tanzanië. Spesies van die genus *Tetranychus*, waaraan die meeste van die plaag-spesies behoort, kan slegs geïdentifiseer word op grond van die manlike aedeagus wat nie altyd duidelik waarneembaar is nie. Die natuurlike habitat, die Oostelike Bergreeks en die Oos-Afrikaanse kuswoude in Kenia en Tanzanië is erkende biodiversiteitsbrandpunte, maar voor die studie het inligting oor die Tetranychidae in die brandpunte ontbreek.

In Kenia is 18 Tetranychidae spesies van verskeie gasheerplante aangeteken. Vier van die spesies behoort tot die subfamilie Bryobinae en die ander 14 tot die subfamilie Tetranychinae. Agt van die spesies behoort tot die genera *Bryobia*, *Petrobia*, *Peltanobi*a, *Paraplonobia*, *Duplanychus*, *Eutetranychus* en *Mixonychus* en word vir die eerste keer aangeteken in Kenia terwyl die ander tien reeds voorheen gerapporteer was. Vir Tanzanië word ses spesies van die genera *Tetranychus*, *Eutetranychus* en *Mixonychus* vir die eerste keer aangeteken terwyl drie spesies voorheen bekend was. ‘n Lys van die spesies met ‘n kort beskrywing van elk en ‘n sleutel tot die spesies word gegee asook ‘n herbeskrywing van *Peltanobia erasmusi* wat ‘n eerste beskrywing van die mannetjie insluit.

Schizotetranychus kwalensis sp. nov. van Kenia en *Brevinychus meshacki* sp. nov. van Tanzanië word beskryf**.** Hulle is versamel van *Omorcarpum kirkii* (Fabaceae) van Matuga, Kwale distrik, Kenia en *Philonoptera eriocalyx* (Fabaceae) van Sangasanga, Mvomero distrik, Tanzanië, respektiewelik. Sleutels tot die Afrika-spesies van *Brevinychus* en *Schizotetranychus* word ook gegee.

Tetranychus evansi Baker & Pritchard was die dominante van al die spinmyt-spesies wat versamel is. Dit het in vier van die vyf fragmente van die brandpunte voorgekom en is aanpasbaar by ‘n wye reeks hoogtes bo seevlak, van so laag as 123 m tot 1655 m. ‘n Molekulere ondersoek van *T. evansi*, versamel in Kenia en Tanzanië van verskeie gasheerplante, het ‘n identiese DNA-opeenvolging van die mitochondriale COI-fragment en 19 identiese microsatelliet-allele opgelewer, wat dui op ‘n enkele invoering van die spesie in hierdie deel van Oos-Afrika.

Wyfiekenmerke van vier *Tetranychus*-spesies van Kenia is ondersoek met behulp van ‘n skandeer-elektronmikroskoop. Verskille is gevind in die afstande tussen die dupleksetas op tarsus I van spesies van die *desertorum*-groep (*Tetranychus evansi* Baker & Pritchard en *Tetranychus ludeni* Zacher) en die van groep 9 (*Tetranychus neocaledonicus* Andre en *Tetranychus urticae* Koch). Lobbe van die dorsaalstrias van *T. evansi*, *T. neocaledonicus* and *T. urticae* is halfrond terwyl die van *T. ludeni* driehoekig is.

Sleutelwoorde: Tetranychidae, *Tetranychus evansi*, *Peltanobia erasmusi, Brevinychus meshacki, Schizotetranychus kwalensis,* Oostelike Bergreeks, Kuswoude, Tanzanië, Kenia, brandpunte

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CHAPTER 1: INTRODUCTION

1.1 General introduction

The Tetranychidae, known as spider mites, comprise a large group of plant feeding mites that rank among the most important pests of the Acari of agricultural crops. Damages have been reported in many economically important crops which include ornamentals, fruits, food and horticultural crops (Jeppson *et al*., 1975). Spider mites generally prefer the underside of plant leaves and take up cell content from the leaves. Damage first appears as stipples that later develop into yellowish and silver appearance. High infestation rates mostly occur under dry and hot conditions (Smith Meyer, 1996; Varela *et al*., 2003), thus Kenya and most of Africa is an ideal environment for these mites.

The "Spider Mites Web" database (Migeon & Dorkeld, 2006-2011) lists 1,272 species that belong to Tetranychidae. From Africa, 39 genera and 357 species of tetranychid mites have been recorded (Migeon & Dorkeld, 2006). Out of these, 219 species have been recorded from South Africa, compared to a range from none to 56 species for each of the remaining African countries with ten species reported from Kenya, five from Uganda and only three from Tanzania. Out of the ten species recorded in Kenya, six, (*Eutetranychus orientalis* Klein, *Mononychellus progresivus* Doreste, *Oligonychus coffeae* Nietner, *Oligonychus gossypi* Zacher, *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch), are well known pests of cultivated crops and three (*Tetranychus neocaledonicus* Andre, *Tetranychus ludeni* Zacher and *Tetranychus lombardiini* Baker & Pritchard) are also known to attack crops. It is only *Schizotetranychus spiculus* Baker & Pritchard which is not known to feed on cultivated plants.

All three species reported from Tanzania, *Mononychellus progresivus* Doreste, *Oligonychus coffeae* (Nietner) and *Oligonychus gossypii* (Zacher) are well known pests of cultivated plants. In contrast, in South Africa many species have been reported from wild plants. The large discrepancy in information available for South Africa viz-a-viz other African countries most probably does not reflect factual differences in biodiversity but is more probably caused by lack of taxonomic expertise in large parts of Africa and a lack of collecting in natural habitats.

1.2 Taxonomic history of Tetranychidae

The Tetranychidae was established by Donnadieu in 1875. A comprehensive treatment of this family was done by Murray in 1877 and in 1913; additions to its description were made by Antonio Berlese who recognized the importance of the empodium of the tarsus as a taxonomic character. Ewing (1913) described several spider mites species and for the first time used the male genitalia for species diagnosis. Boudreaux (1956) included the use of the shape of the dorsal integumentary lobes in the diamond shaped area between the third and the fourth dorsal central setae on female opisthosoma for species separation in Tetranychidae. Pritchard and Baker in 1955 and Wainstein in 1960 conducted other, major studies involving higher classifications of tetranychids.

The family Tetranychidae has two subfamilies, Bryobiinae and Tetranychinae, which have three tribes each with 35 genera in the Bryobiinae and 36 in the Tetranychinae (Bolland *et al*., 1998). Members of Tetranychidae are characterized by having their stylophore reversible, with long slender whiplike movable chelicerae; the peritremes simple or anastomosing distally, arising from the base of the stylophore; duplex setae are usually present on tarsus I and II; the tenent hairs of the ambulacra are present; tarsal claws and empodia either padlike or clawlike; the palpal tibia forms a clawlike complex with the palpal tarsus. The female genitalia are wrinkled. The male aedeagus in members of Tetranychinae is variously shaped and species specific (Baker & Tuttle, 1994).

The number of described tetranychid species remained stable for 75 years, until their economic importance in agriculture became more evident. Then the number of known species increased significantly: from 102 species in 1950 (McGregor, 1950), to 1,189 species in 1996 (Bolland *et al*., 1998) to 1,250 species in 2006 (Migeon & Dorkeld, 2006).

In Africa, the number of tetranychid species reported ranges from 219 from South Africa to one species in some countries as represented in Table 1.1. For many countries there are no reports at all.

1.3 Tetranychids of economic importance

Members of several tetranychid genera are widely referred to as spider mites and are known worldwide as important agricultural pests. These include *Panonychus,*

Bryobia, Amphitetranychus, Eutetranychus, *Eotetranychus, Petrobia* (Baker & Tuttle, 1994), *Oligonychus*, *Tetranychus*, *Schizotetranychus*, (Jeppson *et al*., 1975) and *Mononychellus* (Yaninek & Herren, 1988). Many studies have focused on the genus *Tetranychus* due to the fact that they are cosmopolitant and attack a wide range of cultivated crops (Jeppson *et al*. 1975).

Members of *Tetranychus* are recognized on account of a single pair of para-anal setae, empodia split into three pairs of proximoventral hairs, (2 pairs in one case); empodia may possess mediodorsal spurs, shorter than proximoventral hairs, male empodium I usually bearing tridigitate spurs and the aedeagus bends dorsally (Smith Meyer, 1987). Species identification in this genus has heavily relied on the male aedeagus and attempts to use female characters have only resulted in assembling the species of this genus into nine groups of similar females without clearly discriminating the species (Flechtmann & Knihinicki, 2002). Problems with species separation of *Tetranychus* mites have been reported, for example the case of *T. urticae* Koch and *T. cinnabarinus* Boisduval (Boudreaux, 1956). The separation of these species was supported by some studies (Zhang & Jacobson, 2000) but rejected by others (Smith Meyer, 1996). Debates on the specific status of *T. urticae* and *T. cinnabarinus* have never ended since the first revision of the Tetranychidae by Pritchard and Baker (1955) who listed 43 synonyms under *T. telarius* = *T. urticae*. More evidence seems to suggest that *T. cinnabarinus* is a species, or at least a subspecies, derived from *T. urticae* and it may be hypothesized that speciation is still in progress (Hance *et al*., 1998; Sugasawa *et al*., 2002). Zhang and Jacobson (2000) were convinced that the character differences were sufficient to show that *T. urticae* and *T. cinnabarinus* are separate species.

In practice, the proper identification of a species is essential, as each species has different requirements and niches and similar looking species might not both be pests. So, not surprisingly misidentification of tetranychid mites in the past have led to wrong control approaches as the case of *T. evansi* in Southern Africa (Knapp *et al*., 2003). The establishment of clear characters for identification of both sexes was therefore an essential part of this work.

Tetranychus evansi is an important invasive species in Africa and Southern Europe and has also been reported from Asia. It is believed to have originated from South America (Gutierrez and Etienne, 1986). On continental Africa, it was first recorded on tobacco in Zimbabwe in 1979 (Blair, 1983) from where it presumably spread to other parts of Africa. It was recorded in Kenya for the first time in 2001 on *Lycopersicon esculentum* in Mwea, Central Kenya (Knapp *et al*., 2003). Reports from several parts of Southern Europe have been published too (Ferreira & Carmona, 1995; Ferragut & Escudero, 1999; Migeon, 2005; Castagnoli *et al*., 2006; Tsagkarakou *et al*., 2007).

Tetranychus evansi is considered the most important dry season pest on tomato cultures in East and Southern Africa (Varela *et al*., 2003). Beyond tomatoes, it is found in eggplants and African black nightshades which are important indigenous leafy vegetables in several countries in Africa (Dhellot *et al*., 2006).

1.4 Molecular markers in acarology

Invasive alien species pose a huge problem to modern day agriculture and many species threaten the livelihoods of millions of people, e.g. the invasive fruitfly *Bactrocera invadens* (Drews *et al*., 2005) (Diptera: Tephritidae) devastates the mango production in Kenya and elsewhere. Despite these massive effects, the colonizing populations of invasive species are often only a few individuals (Elton, 1958) inadvertently introduced with trade. On a population level, random selection of a small number of individuals from the whole genetic diversity causes random genetic drift known as founder effect (Lande & Barrowclough, 1987; Tsutsui *et al*., 2000). The founders carry only a fraction of the gene pool of the whole species and diversify from there. Thus, a reduction of genetic variability is a common feature of invasive species and introductions in general (Lande & Barrowclough, 1987; Roderick & Navajas, 2003; Solignac *et al*., 2005). In some cases, however, genetic variability of invasive populations may be higher than predicted by genetic drift, such as when the invasion phenomenon leads to the presence of different fixed haplotypes in diverse geographical regions (Gasparich *et al*., 1997) or when multiple invasions stem from different regions with fixed haplotypes (Stepien *et al*., 2002; Kolbe *et al*., 2004). Therefore, the introduction events leave traces in the genome of the invasive alien

species and therefore a valuable approach to the study of introductions and routes of invasive species involves the use of molecular markers (Solignac *et al*., 2005).

During the last two decades, polymerase chain reaction (PCR, Saiki *et al*., 1988) based approaches have become a very popular tool in acarological systematics. A number of molecular techniques have been employed to study genetic diversity in mites and these include rDNA sequencing (genes), mtDNA sequencing, PCR restriction fragments length polymorphism (PCR-RFLP), microsatellites, direct amplification of length polymorphism (DALP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and allozymes. Most commonly used genetic regions are mitochondrial DNA (Cytochrome oxidase genes) or markers of the nuclear ribosomal DNA (internal transcribed spacers, ITS) (Navajas & Fenton, 2000).

Moreover, recent studies have shown that microsatellite markers can be faithfully extended beyond population genetics and can be used for studying phylogenetic relationships of closely related species with fewer loci than previously assumed (Schlotterer, 2001).

1.5 The East African biodiversity hotspots

The Eastern Arc and Coastal forests of Tanzania and Kenya are one among the 25 biodiversity hotspots for conservation priorities (Myers, 2000). The Eastern Arc Mountains stretch for 900 km from the Makambako gap, southwest of the Udzungwa mountains in southern Tanzania to the Taita Hills in south-coastal Kenya (Fig. 1.1) (GEF, 2002). They comprise a chain of 12 mountain blocks, from south to north: Mahenge, Udzungwa, Rubeho, Uluguru, Ukaragu, South and North Nguru, Nguu, East Usambara, West Usambara, North Pare, South Pare and Taita Hills.

The area defined by the coastal forests of Tanzania and Kenya within the hotspot includes intervening, non-forest habitats between the forest patches. Although the main biodiversity is concentrated in the forests, there are a significant number of endemic species (especially plants) found in non-forested habitats.

In Kenya, the Northern Zanzibar-Inhambane Coastal Forest Mosaic is mostly confined to a narrow coastal strip except along the Tana River where it extends inland to include the forests of the lower Tana River (the northern-most of which occur within the Tana Primate National Reserve). In Tanzania, the Mosaic runs from the

northern border to the southern border along the coast, contracting in the Rufiji Delta region. There are also some outlying forests located up to ca. 300 km inland at the base of a few of the Eastern Arc Mountains (Udzungwa, Mahenge, Uluguru and Nguru) (WWF-US 2003). Most coastal forests are found between 0-50 m and 300-500 m altitude; although in Tanzania they extend up to 1040 m (Burgess *et al*., 2000). Rainfall ranges between 500 mm/year (northern Kenya and southern Tanzania) and 2000 mm/year (Pemba) (Clarke, 2000). There are two rainy seasons (long-rain season, April-June; short-rain season, November-December) in the north, but only one (April-June) in the south. The two largest coastal forests are both in Kenya (Arabuko-Sokoke, ca. area 370 km^2 and Shimba Hills, ca area 63 km^2).

Much of the habitat mosaic has been converted to subsistence agriculture, interrupted by plantations and human settlements, including the large cities of Mombasa and Dar es Salaam (populations of more than 700,000 and 3 million, respectively). Therefore, the agricultural production landscape is actually within the hotspot and has significant effects via pesticides and land use change on the natural vegetation. The latter might also serve as reservoir or refuge for pest species and their predators alike, and must be sampled to provide a full picture of mite biodiversity.

Since little to no information on the acarifauna of this region is available, which would give a more thorough view on its natural composition and as a possible reservoir for the pests in the non-crop seasons, collection was done at random in several places in these hotspots.

1.6 Objectives

The objective of this study was to assess the diversity of tetranychid mites in Kenya and conservation hotspots in Tanzania taking into consideration the different fragments of the hotspot and cultivated and non-cultivated areas.

These objectives are reported in the following chapters:

i. Re-description of *Peltanobia erasmusi* Meyer (Acari: Tetranychidae) using males;

ii. Description of two new spider mites species (Acari: Tetranychidae) from Kenya and Tanzania;
iii. Using scanning electron microscopy to differientiate between species of the genus *Tetranychus* using female characters;
iv. Establish species list for the diversity of tetranychid mites in Kenya and Tanzania and the Eastern African biodiversity hotspots.

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Table 1.1: Tetranychid species reported in Africa compiled from Migeon and Dorkeld (2006). For all other countries no records were available

Country	**No. of species recorded**
Algeria	14
Angola	10
Benin	4
Burkina Faso	1
Burundi	1
Cameroon	13
Central Africa Republic	2
Chad	2
Congo Brazaville	8
Congo (DRC, formerly Zaire)	25
Egypt	41
Ethiopia	5
Gabon	1
Ghana	1
Guinea	1
Kenya	10
Liberia	1
Libya	5
Madagascar	56
Malawi	23
Mali	4
Mauritania	3
Morocco	12
Mozambique	20
Namibia	9
Niger	2
Nigeria	21
New Guinea	12
Rwanda	2
Senegal	9
Sierra Leone	2
Somalia	1
South Africa	219
Sudan	5
Swaziland	1
Tanzania	3
Togo	3
Tonga	4
Trinidad and Tobago	12
Tunisia	12
Uganda	5
Zambia	6
Zimbabwe	31

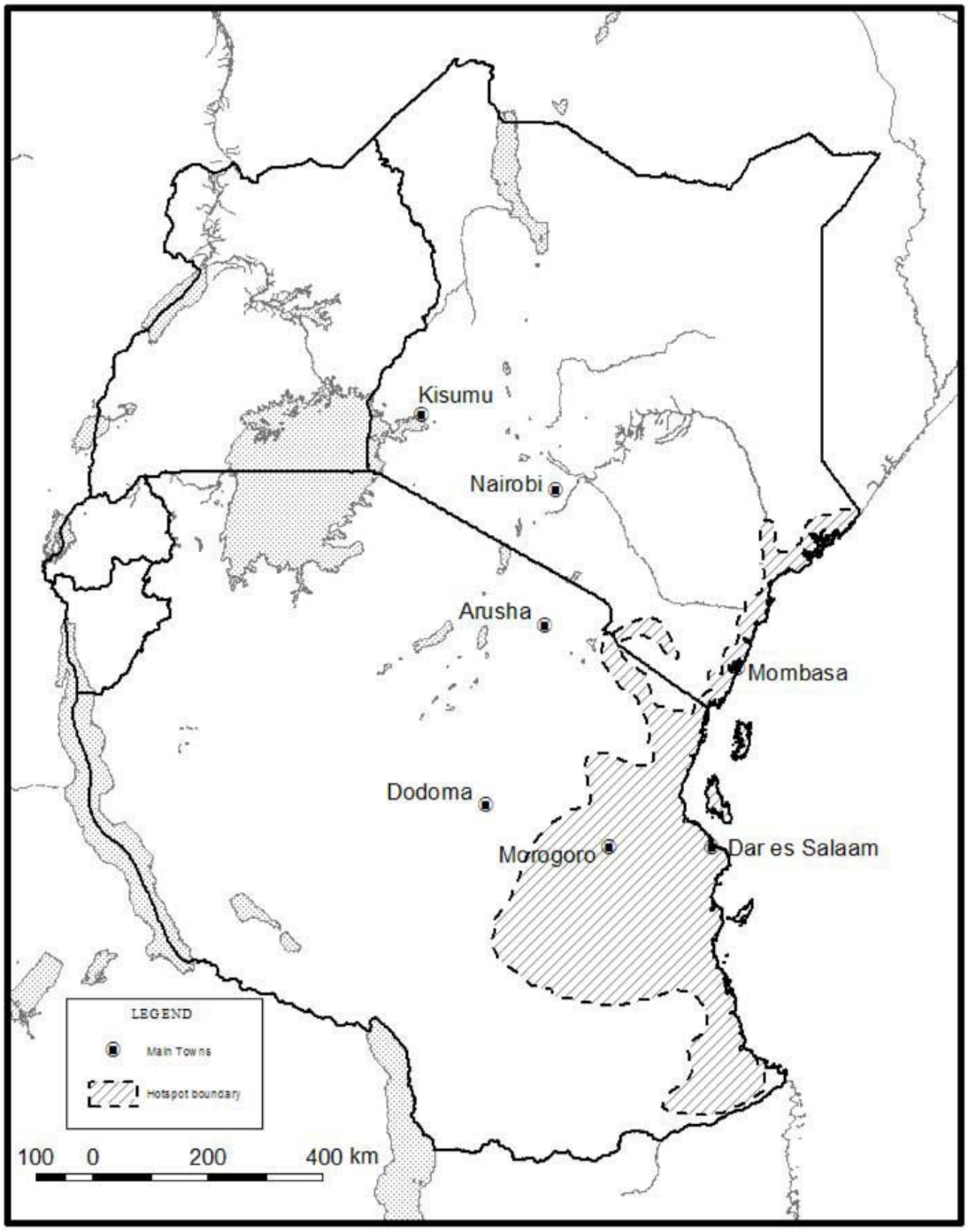

Figure 1.1: Location of the Eastern Arc Mountain and Coastal Forests hotspot.

CHAPTER 2: THE TETRANYCHID MITES OF KENYA AND TANZANIA WITH A RE-DESCRIPTION OF *PELTANOBIA ERASMUSI* MEYER BASED ON MALES.

2.1 Abstract

Eighteen tetranychid mite species from various plant hosts in Kenya and nine species from Tanzania have been recorded. Four species of these belong to the subfamily Bryobiinae and the other 14 belong to the subfamily Tetranychinae. Eight of the mite species identified belong to the genera *Bryobia*, *Petrobia*, *Peltanobi*a, *Paraplonobia*, *Duplanychus*, *Eutetranychus* and *Mixonychus* and are being reported for the first time in Kenya while the other ten had been reported before. Six species belonging to the genera *Tetranychus*, *Eutetranychus* and *Mixonychus* are being reported for the first time from Tanzania and the other three had been reported before. A list of these species, their brief descriptions as well as a key for identification is provided and a redescription of *Peltanobia erasmusi* Meyer (Acari: Tetranychidae) to include male characters that were not included in earlier descriptions, is given.

2.2 Introduction

The family Tetranychidae is one of the most important families of the Acari because many members are serious pests of agricultural crops. This family comprises a large group of about 1,250 phytophagous species (Migeon & Dorkeld, 2006) and they damage mainly ornamentals and horticultural crops. An earlier record of spider mites from Kenya was given in the world's catalogue of Tetranychidae by Bolland et al. (1998), which listed nine spider mite species from Kenya, and a more recent database 'The Spider web' (Migeon & Dorkeld, 2006, accessed in January 2008) which is a compilation of findings from many authors lists ten mite species. These earlier records include *Eutetranychus orientalis* (Klein), *Mononychellus progresivus* (Doreste), *Oligonychus coffeae* (Nietner), *Oligonychus gossypi* (Zacher), *Schizotetranychus spiculus* (Baker & Pritchard), *Tetranychus evansi* (Baker & Pritchard), *Tetranychus urticae* (Koch), *Tetranychus neocaledonicus* (Andre), *Tetranychus ludeni* (Zacher) and *Tetranychus lombardiini* (Baker & Pritchard). The latest record was that of *Tetranychus evansi* Baker and Pritchard in 2001 (Knapp *et al*., 2003). An earlier record of spider mites from Tanzania lists three tetranychid mite species from this country namely *Mononychellus progresivus* Doreste, *Oligonychus*

coffeae Nietner and *Oligonychus gossypi* Zacher (Migeon & Dorkeld, 2006). Apart from *Schizotetranychus spiculus* Baker & Pritchard, the rest of the species reported earlier from Kenya and Tanzania are well known pests of cultivated plants but in a country like South Africa, many species have been recorded from natural vegetation.
This study gives a report of tetranychid mites collected from September 2005 to March 2007 and from February 2008 to March 2008 in Kenya and Tanzania respectively together with data on the host plants and place of collection. In addition, tetranychids reported before by different authors are also included and remarks on their first report added. A taxonomic key to identify these species is also provided and illustrations of *Peltanobia erasmusi* Meyer males are included.

2.3 Materials and Methods

Mites were collected from Malindi, Kwale and Taita districts of Coastal Province, Kiambu, Kirinyaga and Nyeri districts in Central Province, Baringo, Laikipia and Nakuru districts in Rift Valley Province, Makueni and Machakos districts of Eastern Province, Bungoma, Busia and Mumias districts for Western Province, Migori, Kisumu and Suba districts in Nyanza Province and Nairobi province for Kenya. In Tanzania, mites were collected from Arusha, Lushoto and Muheza areas of the Usambara Mountains and Morogoro and Mvomero districts of the Uluguru Mountains. Spider mites on randomly selected host plants were collected from the stated areas in both cultivated fields and natural vegetation. Mites were collected from points which were at least 5 km apart and this distance was measured by the use of a GPS. The area name and the GPS co-ordinates of each sampling site were taken, the host plant of the mites and the kind of damage on the host plant noted, if any. In cases where the plant species was not known, some plant parts which include leaves, flowers and fruits were taken, pressed carefully and taken to the laboratory where they were mounted in a herbarium and prepared for identification by a qualified botany technician from the University of Nairobi, Botany department.

The sampling procedure entailed collecting infested leaves in situations where spider mite damage symptoms were visible or beating of the plant and collecting mites from a beating plate placed beneath the plant in cases where damage is not easily visible and when dealing with large plants. When sampling from cultivated crops, weeds and wild plants near farmer fields, leaves were first examined to ensure they have mites

using a hand lens. The leaf samples were put in brown paper bags, then placed in a cool box and transported to the laboratory at ICIPE. In cases where the beating method was used, individual mites were directly handpicked from the plate using a fine hair brush and put into vials containing 70% ethanol and taken to the laboratory.

2.3.1 Preparation of specimens and mites identification

In the laboratory, a good number of males and females from each of the sites sampled were picked under a stereo microscope with a fine brush and transferred into small vials containing 70% ethanol where they remained for ten days for the purpose of clearing to remove internal tissues (Craemer et al., 1998). After ten days, the mites preserved in 70% ethanol were mounted for identification. Polyvinyl alcohol (PVA) mountant was used and this was preferred in spider mites where the dorsal lobes are of taxonomic importance. PVA was prepared using the formula given below as described by Boudreaux and Dosse (1963):

10g Polyvinyl alcohol; 40-60 cc distilled water; 35 cc lactic acid (85-92%); 10 cc glycerine; 20 cc phenol-water solution; 1.5% 100g chloral hydrate.

Water was added to the PVA powder in a large beaker, stirring constantly, the mixture being heated in a water bath to just below boiling. To the PVA-water mixture, lactic acid was added and stirred for a few minutes. Glycerine was added and stirred again until smooth. The mixture was cooled to lukewarm and the chloral hydrate previously dissolved in the phenol solution added and thoroughly stirred. The mixture was then filtered in a suction funnel through a filter paper.

A small drop of polyvinyl alcohol (PVA) was placed in the middle of a clean slide. A specimen was transferred to the drop using a brush and manoeuvred to ensure the females lie dorsally and the males laterally to view the aedeagus. A cover slip was placed over the specimen by holding it on its edge on the side of the drop touching the PVA medium then gently lowering it onto the drop. The mounted slides were allowed to dry in an oven at 40°C for 24 hours (Craemer *et al*., 1998).

Identification was carried out under X400 to X1000 magnification with a Leica DMLB phase contrast compound microscope (Leica Microsystems, Wetzlar, Germany). The mites were identified up to species level using the shape of the male aedeagus and the position of the duplex setae as the distinguishing characteristics as described by Craemer *et al*. (1998) and Meyer (1987), and the shape of the dorsal

lobes and the variations in the number of the setae on tibia I in the female (Zhang & Jacobson, 2000). Other features that were used for identification include the distances between bases of the solenidia in the two duplex setae, the ratio between the length of setae and the distance to next setae, length and shape of the setae, distances between bases of genital setae and the ratio between length of subcapitular setae and the distance between their bases (Zhang & Jacobson, 2000).

The terminology used for the body seta is according to Lindquist (1985) and the style of description follows that of Smith Meyer (1987). World distribution of each species is according to Migeon and Dorkeld (2006). Host plants and their scientific names are also given.

2.4 Results and Discussion

2.4.1 Details of the species collected

2.4.1.1 *Bryobia* Koch, 1836 (Bryobiinae: Bryobiini)

Adult members of *Bryobia* have 4 pairs of setae on the prodorsum, first two pairs set on prominent prodorsal lobes; 12 pairs of setae are located on the opisthosoma; fourth pair of dorsocentral setae (*f1*) marginal; peritreme ends either simply or in anastomosis. Empodia on tarsi pad-like and with tenent hairs.

Bryobia praetiosa Koch, 1836

Bryobia graminum Shrank, 1781; *Bryobia macrotibialis* Mathys, 1962

Tarsus I very long, more than twice as long as other tarsi. Empodium of adults with one pair of tenent hairs. Solenidia and tactile setae on tarsi III and IV forming duplex sets, genu II with 6 setae. It has five pairs of genitoanal setae and one pair of pregenital setae. Tarsus I with two pairs of duplex setae and tarsus II has one pair. The peritreme ends in a protruding sausage-like structure (anastomosis) four times longer than broad. Body measurements are as follows: Body length: 550 µm, width 350 µm. Leg I is 590 µm long and leg II is 240 µm long.

Leg chaetotaxy as follows: genua 8-6-6-5; femora 21-11-5-(4/3); coxae 2-1-1-1.

Solenidia on tarsus III and IV associated with tactile setae to form duplex setae. Leg I slightly longer than total body length.

Specimens examined: Four females collected on *Chloris gayana* (Poaceae) in Ngarenyiro, Laikipia district (N00°04.971'; E036°55.956') and three larvae collected

on *Sida schimperiana* (Malvaceae) from Kitengela, Kajiado district (S01°32.319'; E036°56.497'), Kenya.

Remarks: This species has a world-wide distribution and occurs on a wide range of low lying host plants belonging to a wide range of families (Migeon & Dorkeld, 2006). Previous records of this species on members of the family Poaceae which include wheat, rye and barley were reported in Arizona (Tuttle & Baker, 1964). In this study, the specimens were collected in savanna grasslands. The species is dark red in colour in the field.

2.4.1.2 *Paraplonobia* (*Anaplonobia*) Tuttle & Baker, 1964 (Bryobiinae: Hystrichonychini)

Prodorsum with three pairs of setae; opisthosoma with ten pairs of dorsal setae; prodorsum without lobes over gnathosoma; opisthosoma without plates; coxal formula not exceeding 4-3-2-2 and dorsal setae not set on strong tubercles.

Paraplonobia (Anaplonobia) prosopis Tuttle & Baker, 1964

Aplonobia prosopis Tuttle & Baker, 1964; *Neopetrobia prosopis* Smith Meyer, 1987

The peritremes end in anastomosis with a network of cell-like structures. Prodorsal and opisthosomal setae are strongly serrate and sub-spatulate with *e2, f1, f2* and *h1* set on small tubercles. Setae well separated from each other, almost equidistant between all dorsocentrals, dorsal setae sub-equal in length. Dorsal striations faint, longitudinal on prodorsum and transverse on hysterosoma and without lobes. Leg setae strong, lanceolate and serrated. Legs are shorter than the body.

Leg chaetotaxy as follows: tarsi 13(2)-10(1)-9-8; tibiae 9-7-8-7; genua 4-4-3-2; femora 5-4-3-3; coxae 2-2-1-1.

Specimens examined: Four females collected on *Prosopis juliflora* (Fabaceae) from Marigat, Baringo district (N00°28.907'; E036°03.230'), Kenya.

Remarks: *P. prosopis* Tuttle & Baker was first described from *Prosopis juliflora* in Arizona (USA) and the second record of this species is from Mexico (Tuttle & Baker, 1964). This is the first record of this species in the Afrotropical region on *P. juliflora* which is an invasive plant species in Kenya after being introduced as a land reclamation plant in the semi-arid parts of the country. The males of this species are not known. In the field, this species is dark red in colour.

2.4.1.3 *Peltanobia* Smith Meyer, 1974 (Bryobiinae: Hystrichonychiini)

Prodorsum with three pairs of setae and opisthosoma with ten pairs of dorsal setae. The first four pairs of dorsocentrals *c1*, *d1*, e*1* and *f1* are located on cushion-like sclerites.

Peltanobia erasmusi Smith Meyer 1974

The first description of this species was based on females only (Figure 2.1) as follows.

Female: Dorsum provided with 13 pairs of broadly spatulate, serrate setae, which are located on prominent tubercles; three pairs present on propodosoma and ten pairs on hysterosoma; four of five pairs of dorsocentral hysterosomal setae located on four oblong cushion-like plates, which have rounded corners; Setae *c3* situated in line with three pairs of dorsolateral setae *c2*, *d2* and *e2*, *f2* and fifth pair of dorsocentrals all located marginally; dorsal setae subequal and shorter than distances between bases of consecutive setae except for fifth pair of hysterosomals, which are the longest. Striae on integument absent.

Stylophore relatively broad and acuminate anteriorly; peritreme branches distally and forms a horn-like complex structure protruding above prodosoma. Palptarsus bears one solenidion and six additional setae. Tarsus I with two pairs of duplex setae, tibia I with one pair.

2.4.1.4 Re-description of *Peltanobia erasmusi* Smith Meyer - using male characters (Figs. 2.2-2.3).

Types: One holotype male and two paratype males, from Rongai, Nakuru.

Dimensions: Length of body (including gnathosoma) in micrometers (µm) (612-625) 618.5; body width (310-322) 316

Gnathosoma: Palp tarsus thick three times as long and wide (Fig. 2.2C).

Dorsum: Peritremes anastomosing and protrude as horn-like structures above prodorsum as in the female (Fig. 2.2A and B). Dorsal plates faintly visible in males compared to the females which have distinct cushion-like plates.

Dorsal setae short and spatulate with lengths as follows (in µm); *ve* (24-25) 24.5; *sci* (22-23) 22.5; *sce* (26-28) 27; *c1*(17-19) 18; *c2* (25-31) 28; *c3* (28-30)29; *d1* (18-21)

19.5; *d2* (28-29) 28.5; *e1* (17-24) 20.5; *e2* (31-42) 36.5; *f1* (23-25) 24; *f2* (35-39) 37; *h1* (36-45) 40.5.

Legs: Chaetotaxy as follows: tarsi 15+2-15+1-14-14; tibiae 15+2-9-8-8; genua 5-5-5-5; femora 9-6-4-4; trochanters 1-0-1-1; coxae 2-2-1-1.

Leg lengths (in μm): tibiotarsus I (232); tibiotarsus II (145); tibiotarsus III (157); tibiotarsus IV (189) (Figure 3).

Male diagnosis: Males smaller in size compared to females, with a more elongated and narrow body. Aedeagus long and straight protruding at posterior end and its shaft narrows distally to a sharp tip. Aedeagus bears a sheath attached to it (Fig. 2.2A and D). It has 5 pairs of genito-anal setae. Para-anals (*h2* and *h3*) considerably small in size, lanceolate and are borne on strong tubercles.

Specimens examined: Three females and three males collected from *Cynodon dactylon* (Poaceae) in Rongai, Nakuru district (S00°09.033'; E035°50.749'), Kenya.

Remarks: This species was first described from grass in Umfolozi Game Reserve, South Africa and has also been reported from Zimbabwe on *Commelina* sp. and *Ipomaea magnusiana* (Smith Meyer, 1987). The specimens used in this description were collected on grass from an open grazing patch. In the field, this species is dark red in colour, appear round in shape and larger compared to other spider mites species collected. Damage symptoms are not clearly visible on the host plants.

2.4.1.5 *Petrobia* Ewing, 1909 (Bryobiinae: Petrobiini)

It has three pairs of prodorsal setae and ten pairs of opisthosomal setae all borne on prominent tubercles, peritreme ending simply with a bulb-like structure. Empodium curved distally and has two rows of ventrally directed tenent hairs. With three pairs of anal and three pairs of para-anal setae. Tarsus I has two pairs of duplex setae

Petrobia (Tetranychina) harti Ewing, 1909

Female: Dorsal body setae long, slender, spiculate, on prominent tubercles and much longer than distances between bases of consecutive rows of setae; *f1* closer together than other dorso-centrals. Prodorsum between *ve* and *sci* punctate, opisthosoma with transverse striae which bear lobes.

Males: Dorsal setae much shorter and borne on weak tubercles, legs I and IV very long, more than twice length of body but legs II and III of ordinary length i.e. as long as the body. Aedeagus slightly curved, narrowing caudally to tip.

Leg chaetotaxy: tarsi 13(2)-10(1)-9-8; tibiae 9-7-8-7; genua 4-4-3-2; femora 5-4-3-3; coxae 2-2-1-1.

Specimens examined: Eight females and five males collected on *Oxalis compressa* (Oxalidaceae) from Runda, Kiambu district (S01º13.470'; E036º48.050'), Kenya.

Remarks: This species has a worldwide distribution and has been reported from a wide range of hosts. Many weed species of the genus *Oxalis* have been recorded to host this species. From Africa, the earlier reports of this species are from Egypt, Southern Africa and the Indian Ocean islands of Madagascar and Mauritius. They are bright red and their long legs and setae are conspicuous even under a hand lens.

2.4.1.6 *Duplanychus* Smith Meyer, 1974 (Tetranychinae: Eurytetranychini)

True claws pad-like with tenent hairs, empodium rudimentary. Prodorsum has three pairs of setae, opisthosoma with ten pairs of setae, two pairs of anals and two pairs of para-anals. All setae set on strong tubercles. First pair of dorsocentral setae (*c1*) contiguous, fourth pair of dorsocentrals (*f1*) more widely spaced than other dorsocentrals; fourth pair of dorsolaterals (*f2*) conspicuously smaller and shorter than rest of dorsal setae; dorsum is punctulate.

Duplanychus sanctiluciae Smith Meyer, 1974

This species is characterized by dorsal body setae which are setose and expanded distally. Peritreme ends in a simple bulb. Palp tarsus small, twice as long as broad with a teat-like structure at tip. Setae sci much longer than seta ve and sce, at least more than twice their lengths. Setae *c3, e2* and *f2* very short, less than half lengths of corresponding dorsocentrals.

Leg chaeotaxy: The leg chaetotaxy of this species can vary within specimens, even amongst type specimens. Tarsi 10(1)+2-9(1)-8(1)-8(1); tibiae 8(2)-7(1)-7-7;
genua 5-5-3-2; femora 8(1)-7-4-4; coxae 2-2-1-1.

Specimens examined: Kenya: Eight females collected from *Grewia plagiophylla* (Tiliaceae) and one female from *Anacardium occidentale* (Anacardiaceae) both from Gede, Malindi district (S03°20.132'; E040°00.779').

Remarks: This species was first reported and described from South Africa on *Scutia mytrina* (Rhamnaceae) and *Grewia caffra* (Tiliaceae) and this is the first report of this species outside South Africa. In the field they are grayish green in colour.

2.4.1.7 *Eutetranychus* Banks, 1917 (Tetranychinae: Eurytetranychini)

Empodia absent or rudimentary. Three pairs of propodosomal setae and ten pairs of hysterosomal setae mostly set on tubercles; dorsocentral setae in normal position; peritremes simple or slightly expanded distally; two pairs of anal and two pairs of para-anal setae. Tarsus I and II without characteristic duplex setae but with loosely associated setae probably homologous, but alveoli not coalesced. Legs of males relatively longer than those of females.

Eutetranychus africanus Baker & Pritchard, 1960.
Anychus africanus Tucker, 1926
Dorsal setae of this species serrate, sub-spatulate borne on tubercles. Dorsocentral setae *c1, d1, e1* and *f1* half length of corresponding dorso-laterals *c2, d2, e2* and *f2* which are long and slender. Body measurements are 500 μm long and 360 μm wide.
Leg chaetotaxy as follows: tarsi 15(2)-13(1)-10(1)-10(1); tibiae 9(1)-6-6-7;
genua 5-5-2-2; femora 8-6-3-1; coxae 2-2-1-1. The bent portion of the aedeagus is almost of equal length to the dorsal margin of the shaft (Fig. 2.4A).
Specimens examined: Kenya: Five females from *Citrus sinensis* (Rutaceae) in Shimba Hills, Kwale district (S04°20.121'; E039°28.877'); eight females and one male on *Harrisonia abyssinica* (Simaroubaceae) from Muhaka, Kwale district (S04°10.611'; E039°26.852') and 13 specimens on *Cadiaeum variegatum* (Euphorbiaceae) from Shimba Hills, Kwale (S04°20.913'; E039°19.688').
Tanzania: Four females from *Maeopsis eminii* (Rhanaceae) in Amani, Muheza district (S05°06.127'; E038°37.638') and two specimens on *Cadiaeum variegatum* from Matombo, Morogoro district (S07°03.385'; E037°45.737').
Remarks: This species is distributed in the Afrotropical, Australasian and Oriental regions and has been recorded on oranges, lemons, frangipani and a variety of other host plants. It was first described on oranges, lemons (Rutaceae) and frangipani (Apocynaceae) from South Africa (Tucker, 1926). In the field this species appear dull grey in colour.

Eutetranychus carinae Smith Meyer, 1974

Anychus orientalis Klein, 1936

This species has sub-spatulate setae, dorsolaterals and *h1* set on very weak tubercles, dorsocentrals *c1, d1, e1* and *f1* not set on tubercles. Solenidion of loosely associated setae on tarsus I about two thirds length of proximal tactile seta whereas that of tarsus II is same length or slightly longer than proximal tactile seta. Dorsal striations faint compared to other two species of *Eutetranychus* examined. The male aedeagus has its bent portion longer than the dorsal margin of the shaft (Fig. 2.4B)

Leg chaetotaxy as follows: tarsi 12(1)-10(1)-8(1)-8(1); tibiae 9(1)-6-5-6;
genua 5-5-2-2; femora 8-6-2-1; coxae 2-2-1-1.

Specimens examined: Three females and one male collected on *Ficus burkei* (Moraceae) from Alupe area, Busia district (N00°29.870'; E034°07.732') and two females collected on *Ricinus communis* (Euphorbiaceae) from Marigat, Baringo district (N00°28.132'; E036°00.906'), Kenya .

Remarks: This species was first recorded on *Ficus* sp. and *Morus* sp. from South Africa (Smith Meyer, 1974). It has been reported from several *Ficus* species and it seems to show preference for the members of the Moraceae. This species has previously been reported from South Africa only and thus its distribution records are still very limited. They are grey in colour.

Eutetranychus orientalis Klein, 1936

This species is characterized as follows: Striae on prodorsum longitudinal and tuberculate; striation pattern between second (*d1*) and third pairs of dorsocentral setae (*e1*) vary from longitudinal to V-shaped; 13 pairs of dorsocentral setae set on tubercles and vary in length and shape; dorsolateral setae (*c2*, *d2*, *e2* and *f2*) long and lanceolate, subspatulate or broadly spatulate with dorsocentral setae (*c1*, *d1*, *e1*, *f1* and *h1*) short and spatulate, lanceolate or subspatulate.

The leg chaetotaxy as follows: tibiae 10-6-6-7; genua 5-5-2-2; femora 8-6-3-1;
coxae 2-1-1-1.

Specimens examined: Kenya: Three males and five females collected on *Citrus limon* (Rutaceae) from Baringo district (N00º29.132'; E036º00.906'); five males and eight females on orange *Citrus sinensis* (Rutaceae) from Makueni district (S01°50.188';

E037°38.166'). More specimens of this species were collected on *Citrus paradisi* (Rutaceae) from Kilifi and *Melia azadarach* (Meliaceae) from Kisumu.

Tanzania: Thirty six specimens collected on *Citrus limon* and twenty three specimens from *Citrus sinensis* both from Kwabada, Muheza district (S05°20.238'; E038°45.092).

Remarks: This species can be separated from the closely related *E. africanus* by the presence of only a single seta on coxa II and an aedeagus whose bent portion is longer than the dorsal margin of the shaft (Figure 2.4C). It has a wide distribution in the Afrotropical, Australasian and Palearctic regions as a pest of citrus. It has also been reported on members of other host families. In Kenya, this species was previously recorded on *Citrus* sp. (Rutaceae) from Thika district, Kenya (Smith Meyer, 1987) and in this study it was collected from citrus trees in most of the areas where sampling was done. They are grey in colour and in cases of severe infestation; the plant appears dull in colour as though covered by a grey layer of soot.

2.4.1.8 *Mixonychus* Ryke & Meyer, 1960 (Tetranychinae: Tetranychini)

This genus has a claw-like empodium which is devoid of proximoventral hairs and much longer than pads of true claws, true claws pad-like with tenent hairs, dorsal integument in this genus appears reticulate due to clustering of striae, has ten pairs of opisthosomal setae present on dorsum, has two pairs of para-anal setae, peritreme ends in a simple bulb, duplex seta on tarsus I distal and approximate.

Mixonychus acaciae Ryke & Meyer, 1960

Distinctive of this species are female dorsal opisthosomal setae which are set on tubercles, spiculate, do not taper, longer than half the distances to next row of setae. Fourth pair of dorsocentrals (*f1*) nearer to each other than members of other three pairs of dorsocentrals.

Leg chaetotaxy as follows: tarsi 10(2)-9(1)-7-7; tibiae 9-5-5-5; genua 5-5-3-3; femora 7-6-3-2; coxae 2-2-1-1.

Specimens examined: Kenya: Nine females collected from *Acacia nilotica* (Fabaceae) from Machakos district (S01º25.137'; E037º00.953') and two females on *Acacia* sp. from Baringo district (N00°30.590'; E035°38.766').

Tanzania: 15 females collected on *Prosopis chilensis* (Fabaceae) from Sangasanga area, Mvomero district (S06°55.249'; E037°30.074').

Remarks: This species has previously been reported in the Southern Africa region only. It was first described on *Acacia karoo* from South Africa (Ryke & Meyer, 1960) and has subsequently been collected on several species of *Acacia.* Although it is known to occur on hosts from other plants families, it seems to exhibit preference for the family Fabaceae. It is however found in small numbers and thus do not seem to cause any serious threat to the thorn trees. They are reddish brown in colour.

2.4.1.9 *Mononychellus* Wainstein, 1960 (Tetranychinae: Tetranychini)

This genus has two pairs of anal setae and two pairs of para-anal setae, striae on opisthosoma variable usually with prominent lobes, dorsal body setae strongly serrate and borne on small tubercles, tarsus I with two sets of distal and adjacent duplex setae, empodium padlike, split distally into three hairs.

Mononychellus progresivus Doreste, 1981

Female characterized by first to third pairs of dorsocentral setae (*c1*, *d1* and *e1*) which are progressively longer towards rear, first pair about half as long as distances to bases of second pair; dorsal body setae generally long, setose and tapering but somewhat widened. Dorsal striations bear rounded lobes with basal spots. Aedeagus somewhat straight, narrowing distally to a relatively slender neck before ending in small angulations with anterior ventral angulation being acute and the distal dorsal one being very slightly curved.

Leg chaetotaxy as follows: tarsi 13+2-12+1-10-9; tibiae 9(1)-7-6-6; genua 5-5-4-3; femora 10-7-4-3; coxae 2-2-1-1.

Specimens examined: Three females and one male on *Manihot esculenta* (Euphorbiaceae) from Kabarnet, Baringo district (N00°27.785'; E035°45.722'), Kenya.

Remarks: An earlier record of this species in Kenya and Tanzania was reported by Girling *et al*. (1978) as *Mononychellus tanajoa* (Bondar) and later Guitterez (1987) reported that all the species from Africa earlier reported as *Mononychellus tanajoa* were in fact *Mononychellus progresivus*. The host plant and specific location where this species was collected from in Kenya was not specified by the authors. This

species is widespread in the tropics where *Manihot esculenta* is cultivated. It is likely that the pest spread with the spread of this crop to many tropical countries. This is one of the most important arthropod pests of *Manihot esculenta* and is amongst the spider mite species that are host specific since all the reports of this pest are from *Manihot* sp as a host plant. There have however been numerous debates on the identity of this species occurring in Africa with some authors insisting that the species that occur in cassava fields all over Africa is *Mononychellus tanajoa* (Bondar) and thus most publications that deal with its control and economic importance refer to *Mononychellus tanajoa* which has been successfully controlled using the phytoseiid mite *Typhlodromalus aripo* (De Leon). However, comparing the features of the specimens I have with the description given by Smith Meyer (1987) together with the paper by Guitterez (1987) show that our specimens correspond to the description of *Mononychellus progressivus*.

2.4.1.10 *Oligonychus* Berlese, 1886 (Tetranychinae: Tetranychini)

With a single pair of para-anal setae, empodium well developed and clawlike, with proximoventral hairs, body setae usually not set on tubercles. Two pairs of duplex setae on tarsus I distal and approximate.

Oligonychus coffeae Pritchard & Baker, 1955.
Acarus coffeae, Nietner, 1861
Peritreme ends in a bulb. Aedeagus distally bends ventrad at a right angle to shaft axis and gradually narrows to a slender truncate tip; male palptarsus with a tiny terminal sensillum; male tarsus bears three tactile setae and two solenidia proximal to duplex setae; empodia provided with five pairs of proximoventral hairs; serrate dorsal body setae of female longer than distances between consecutive setae.
Specimens examined: Kenya: Four females and two males on *Mangifera indica* (Anacardiaceae) from Bungoma district (N00°25.425'; E034°30.225').
Tanzania: Four females and two males collected on *Manihot sp*. from Matombo, Morogoro district (S07°03.385'; E037°45.737').
Remarks: This species has a world-wide distribution and is found on a wide range of host plants. It was first collected on *Coffeae arabica* (Rubiaceae) from Sri Lanka (Nietner, 1861). The first record of this species in Kenya was on *Anacardium*

occidentale (Anacardiacea) from Matuga, Kwale district (Baker & Pritchard, 1960) and its first record in Tanzania appeared in a distribution of pests map without host and locality specifications (CAB, 1963). They are dark red in colour.

Oligonychus gossypii Pritchard & Baker, 1955
Paratetranychus gossypii Zacher, 1921
Peritreme of this species ends with a small curve. Dorsal setae long and lanceolate extending beyond bases of next row of setae. Male aedeagus narrows distally and curves dorsad at about a right angle; distally, aedeagus has a large knob bearing a small anterior projection and a long, undulate posterior projection; tip directed ventrad; female opisthosoma with longitudinal striae between members of third (*e1*) and fourth (*f1*) pairs of dorsocentral setae; a diamond shaped structure present between these two pairs of setae. Leg chaetotaxy as follows: tarsi 13(1)+2-8+1-8-8/7; tibiae 10-7-6-7; genua 5-5-4-4; femora 10-6-4-4; foxae 2-2-1-1.
Specimens examined: Four males and six females collected on *Haplocoelum inoploeum* (Sapindaceae) from Malindi district (S03°11.53'; E039°55.25').
Remark: This species was first described on *Gossypium* sp. from Togo (Zacher, 1921). This species is widely distributed in the Afrotropics and Neotropics and it has a wide host range. In Africa, it is considered as a pest of cassava. An earlier record of this species in Kenya and Tanzania was by Nyiira (1982) on an unspecified host plant and location.

2.4.1.11 *Schizotetranychus* Trägårdh, 1915 (Tetranychinae: Tetranychidae)

This genus has 2 pairs of para-anal setae (h2 and h3), duplex setae of tarsus I distal and approximate, empodia strong, claw-like, split and with appendant hairs. Peritreme is mostly simple.

Schizotetranychus spiculus Baker & Pritchard, 1960
Females of this species have short dorsal body setae which are broader at base, tapering distally and subequal in length, half length of longitudinal intervals between them. Longitudinal striae extend to first pair of dorsocentral setae (*c1*); striae between first and second pairs of dorsocentral setae transverse, between second and third pairs

form a V pattern; fourth pair of dorsocentrals *f1* situated further apart than other three pairs of dorsocentrals.

Remarks: This species was described on *Citrus* sp. (Rutaceae) from Kaloleni, Mombasa district; by Baker & Pritchard (1960). It has been reported on *Murraya koenigii* (Rutaceae) from India (Karuppuchamy & Mohanasundaram, 1987). No specimens were collected in this study.

2.4.1.12 *Tetranychus* Dufour 1832 (Tetranychinae: Tetranychini)

Has a single pair of para-anal setae, duplex setae of tarsus I widely separated, dividing it into three more or less equal parts, empodia split into three pairs of proximoventral hairs, male empodia may possess medio-dorsal spur, body setae not set on tubercles.

Tetranychus evansi Baker & Pritchard, 1960

Male of *T. evansi* has a slender aedeagal shaft that curves dorsad; axis of knob forms a strong angle with shaft axis; knob small but bearing a small anterior projection and a relatively longer acute and somewhat deflexed posterior projection (Figure 2.5A). Female tarsus I with proximal pair of duplex setae more or less in a line with tactile setae and empodium I with a minute mediodorsal spur.

Specimens examined: Many specimens have been examined all collected on solanaceous plants from Kenya which include *Lycopersicon esculentum* from Kajiado, Migori, Suba, Nakuru, Machakos, Kwale, Makueni and Taita districts; *Solanum incanum* from Mwea; *Solanum nigrum* from Baringo, Kiambu and Taita; *Solanum melongena* from Kibwezi and Machakos and *Solanum tuberosum* from Sagana, Nyeri district.

Tanzania: Many specimens were collected on *Solanum aethiopicum*, *Lycopersicon esculentum*, and *Datura stramonium* (all Solanaceae) from Tengeru, Arusha district (S03°22.371'; E036°48.233'); *Solanum tuberosum* from Lushoto district (S04°45.206'; E038°20.497') and *Solanum aethiopicum* from Mukuyuni, Morogoro district (S06°55.249'; E037°30.074').

Remarks: This species was first described from Mauritius on *Lycopersicon esculentum* (Baker & Pritchard, 1960) and is considered an invasive in Africa, Europe and parts of Asia. It has a worldwide occurrence but is not considered as a serious pest in the country of origin which is believed to be Brazil. However, in introduced

places, it posses a threat to commercial production of solanaceous crops especially in greenhouse conditions and warmer climates. This species has been reported on a wide variety of host plants but it shows preference for the plants from the family Solanaceae. An earlier report of this species was on *Lycopersicon esculentum* (Solanaceae) from Kirinyaga district (Knapp *et al*., 2003) and is currently the most important pest in tomato production in Kenya being wide spread in the major tomato growing areas. Biological control experiments of this pest using the phytoseiid mite *Phytoseiulus longipes* (Evans) show high levels of control in the laboratory (Ferrero *et al*., 2007). This species is brick-red to dark orange in colour when observed in the field.

Tetranychus lombardinii Baker & Pritchard, 1960

Aedeagus curves dorsad, forming a knob about one fourth to one fifth length of dorsal margin of shaft; knob and shaft axes almost parallel; posterior projection small and acute; anterior projection rounded (Figure 2.5B) male empodium I with two proximoventral spurs and a minute mediodorsal spur; empodium II with three pairs of proximoventral hairs and a tiny mediodorsal spur; female striae between third (*e1*) and fourth (*f1*) pairs of dorsocentral setae longitudinal, a diamond-shaped figure formed between these setae; dorsal lobes narrow, triangular, mostly taller than broad and well separated at their bases; in some specimens ventral lobes weak or absent but usually low, semicircular, broad and extend from genital opening to near gnathosoma; lobes on prodorsum very broad and hardly more than an occasional incision of striae.

Specimens examined: Five males and several females collected on *Morus* sp. (Moraceae) from ICIPE compound, Nairobi (S01°13.140'; E036°53.440'), Kenya.

Remark: This species has been reported from the Tropics, Australia and Oriental Indian regions. It has a wide host range and occurs as a pest of several agricultural crops (Smith Meyer, 1974). The first record of this species in Kenya was on *Spinacia oleraceae* (Amaranthaceae) from Ruiru, Thika district (Baker & Pritchard, 1960). It appears uniformly red in colour in the field.

Tetranychus ludeni Zacher, 1913

Aedeagus bends dorsad; aedeagal knob without a posterior projection, anterior projection small and acuminate; knob and shaft axis parallel (Figure 2.5C). Proximal pair of duplex setae on tarsus I almost in a straight line with tactile setae.

Specimens examined: Kenya: Many individuals available collected on *Physalis heterophylla* (Solanaceae) growing in a flower bed in Kangemi, Nairobi (S01°15.984'; E036°45.810') and on *Bidens pilosa* (Asteraceae) in Runda (S01°13.470'; E036°48.050') Nairobi.

Tanzania: Twenty specimens collected on *Vigna unguiculata* from Tengeru, Arusha district (S03°22.371'; E036°48.233').

Remarks: This species is widespread world-wide and occurs on a wide range of host plants. It was first described on *Cucurbita* sp. and *Salvia splendens* from Germany (Zacher, 1913). Its first report in Kenya was on *Chrysanthemum* sp. (Asteraceae) from an unknown locality (Jeppson et al., 1975).

Tetranychus neocaledonicus André, 1933.

Distinctive of this species is shape of male aedegus that curves dorsad and both anterior and posterior projections are rounded and knob bears a small indent medially (Figure 2.5D). The female tarsus I with proximal pair of duplex setae distal to proximal tactile setae.

Specimens examined: Five males and several females collected on *Manihot esculenta* (Euphorbiaceae) from Shimba Hills, Kwale district (S04°21.818; E039°19.490'), Kenya.

Remarks: This species was first described on *Gossypium* sp. from New Caledonia (Ándre, 1933) and is widely distributed in both the Afrotropics and Neotropics, USA and Palearctic region on a wide range of host plants (Migeon & Dorkeld, 2006). In Kenya, the first record of this species was on *Ricinus communis* (Euphorbiaceae) from Nairobi (Baker & Pritchard, 1960).

Tetranychus urticae Koch, 1836

Knob of male aedeagus always small, axis of knob parallel to shaft axis or forms a small angle with shaft axis; dorsal margin of knob and development of anterior and posterior projections may vary, but in most cases they are similar (Figure 2.5E); male empodium I with strong medio-dorsal spur; about one third the length of the two proximoventral spurs; empodium II consists of three pairs of proximoventral hairs and a strong mediodorsal spur.

Specimens examined: From many hosts all over Kenya: *Lycopersicon esculentum* (Solanaceae), *Phaseolus vulgaris* L. (Fabaceae), *Zea mays* L. (Poaceae), *Amaranthus*

hybridus L. (Amaranthaceae), *Carica papaya* (Caricaceae), *Galinsoga parviflora* (Asteraceae), *Desmodium* sp. (Fabaceae), *Bidens pilosa* (Asteraceae), *Cucurbita pepo* (Cucurbitaceae), *Citrullus lanatus* (Cucurbitaceae), *Lactuca sativa* (Asteraceae), *Rosa* sp. (Rosaceae), *Passiflora edulis* (Passifloraceae), *Helianthus annuus* (Asteraceae), *Euphorbia* sp. (Euphorbiaceae), *Tradescantia fluminensis* (Commelinaceae), *Citrus* sp. (Rutaceae), *Brassica* sp. (Brassicaceae), *Lantana camara* (Verbenaceae), *Datura stramonium* (Solanaceae), *Pisum sativum* (Fabaceae), *Dianthus caryophyllus* (Caryophyllaceae).

Tanzania: Fourty two specimens collected on *Hibiscus rosa-chinensis* from Moshi district.

Remark: *Tetranychus urticae* was first described on *Glyine max* and *Urtica* sp. from Germany (Koch, 1836). It is one of the most cosmopolitan spider mite species with a wide host range. It is considered a pest of many crops and is the most studied spider mite species with a high rate of pesticide resistance reported on this species by many authors. In Kenya, it is a major menace in cut flower production especially in *Rosa* sp. Its first record in Kenya was on *Allium ampeloprasum* (Alliaceae) from Machakos district and on *Lathyrus odorata* (Fabaceae) from Nairobi (Baker & Pritchard, 1960).

2.4.2 Key to the tetranychid mite species of Kenya and Tanzania

1	Empodium with tenent hairs; female with three pairs of anal setae, males with five pairs of genito-anal setae	Bryobiinae … 2
	Empodium without tenent hairs or empodium absent	Tetranychinae…7
2	Empodium pad-like	3
	Empodium claw-like	Petrobiini……. 6
3	True claws claw-like	Bryobiini …..…. *4*
	True claws pad-like	Hystrichonychini.. 5
4	Prodorsum with setiferous lobes and 4 pairs of setae, fourth pair of dorsocentrals f1 marginal. The first 2 pairs of setae set on anterior lobes with broad bases almost triangular	*Bryobia praetiosa* Koch

5	First four pairs of dorsocentrals located on oblong cushion-like plates peritreme ends in a horn-like shape above rostrum	*Peltanobia erasmusi* Meyer
	Dorsal setae not set on tubercles, empodial claw bearing two rows of tenent hairs. Peritremes anastomosing, first pair of dorsocentrals (c1) half the distances to bases of next setae	*Paraplonobia prosopis* Tuttle and Baker
6	Opisthosoma with 10 pairs of dorsal body setae, tarsus I with 2 sets of duplex setae. All dorsal setae set on strong tubercles, dorsum striate, empodium claw bent well beyond the end of pad of true claw	*Petrobia harti* Ewing
7	Empodium rudimentary or absent, tarsus I may have one set of duplex setae or duplex setae may be absent	Eurytetranychini…8
	Empodium pad-like or claw-like, duplex setae on tarsus I and II	Tetranychini…11
8	Presence of duplex setae in tarsus I, all setae thick, serrate, long and set on strong tubercles, first pair of dorsocentrals c1 contiguous. Dorsal setae expanded distally	*Duplanychus sanctiluciae* Meyer
	Tarsi I and II devoid of duplex setae, seta spatulate and shorter than distances between consecutive seta, setae not contiguous	*Eutetranychus*…… 9
9	Coxa II with 1 seta	*Eutetranychus orientalis* Klein
	Coxa II with 2 setae	10
10	Tibia III with 6 setae, femur III with 3 setae	*Eutetranychus africanus* Tucker
	Tibia III with 5 setae, femur III with 2 setae	*Eutetranychus carinae* Meyer
11	Empodium claw-like	12
	Empodium pad like ending in a tuft of hairs	15
12	Empodium claw-like devoid of proximoventral hairs, Dorsum reticulate due to striae clustered into lumps, dorsal setae serrate, moderately long and set on small tubercles	*Mixonychus acaciae* Ryke and Meyer
	Empodium claw-like with proximoventral hairs	13
13	Empodium split bilaterally into two claw-like structures with appendant hairs, Fourth pair of dorsocentral setae f1 further apart than the other three pairs of dorsocentrals, striae between d1 and e1 form a V pattern	*Schizotetranychus spiculus* Baker and Pritchard

	Empodium entire, not split and having appendant hairs	*Oligonychus* …14
14	Aedeagus bent dorsad at a right angle, distally with a large knob bearing a small anterior projection and a long undulate posterior projection, tip directed ventrad	*Oligonychus gossypii* Zacher
	Aedeagus bends ventrad at a right angle to the shaft axis, gradually narrows to a slender truncate tip	*Oligonychus coffeae* Nietner
15	With 2 pairs of para-anal setae, striae on the opisthosoma with lobes. [b] Tibia II with 7 tactile setae, aedeagus turns ventrad and has distal angulations	*Mononychellus progresivus* Doreste
	With 1 pair of para-anal setae	*Tetranychus*… 16
16	Tarsus I with the proximal pair of duplex setae distal from the nearest tactile setae	17
	Tarsus I with the proximal pair of duplex setae almost in line with the near tactile setae	19
17	Male aedeagus knob with anterior and posterior projections	18
	Male aedeagus knob without posterior and anterior projection and has a small indent medially	*Tetranychus neocaledonicus* André
18	Aedeagus with small knob, the knob axis almost parallel to shaft axis, with a posterior projection slightly longer than that of the anterior projection	*Tetranychus lombardiini* Baker & Pritchard
	Male aedeagus knob with small posterior and anterior uniform projections, axis of knob parallel to shaft axis	*Tetranychus urticae* Koch
19	Aedeagal shaft curves dorsad; axis of knob forms a strong angle with shaft axis; knob small bearing a small anterior projection and a relatively longer acute and slightly deflexed posterior projection	*Tetranychus evansi* Baker & Pritchard
	Aedeagus bends dorsad; aedeagal knob without a posterior projection, anterior projection small and acuminate; knob and shaft axis parallel	*Tetranychus ludeni* Zacher

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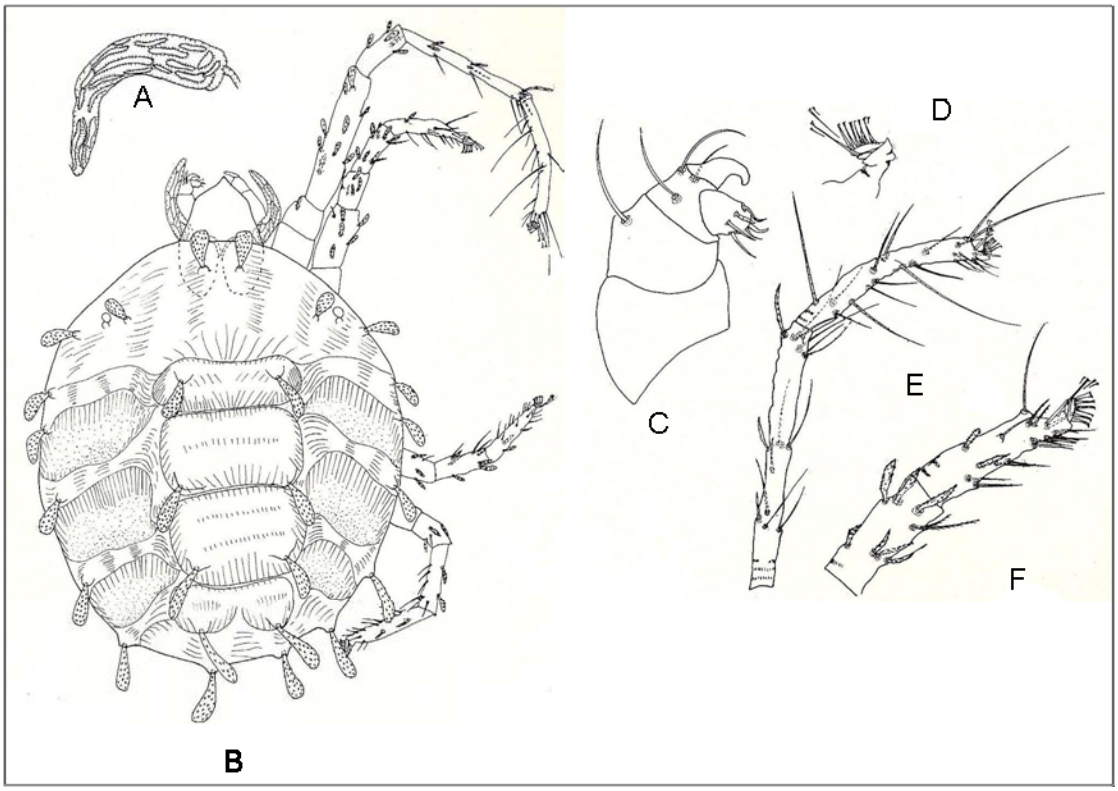

Figure 2.1: *Peltanobia erasmusi* Smith Meyer female (from Smith Meyer 1974, with permission). (A) peritreme, (B) dorsum, (C) palp-tarsus, (D) empodium, (E) tarsus and tibia I, (F) tarsus and tibia II.

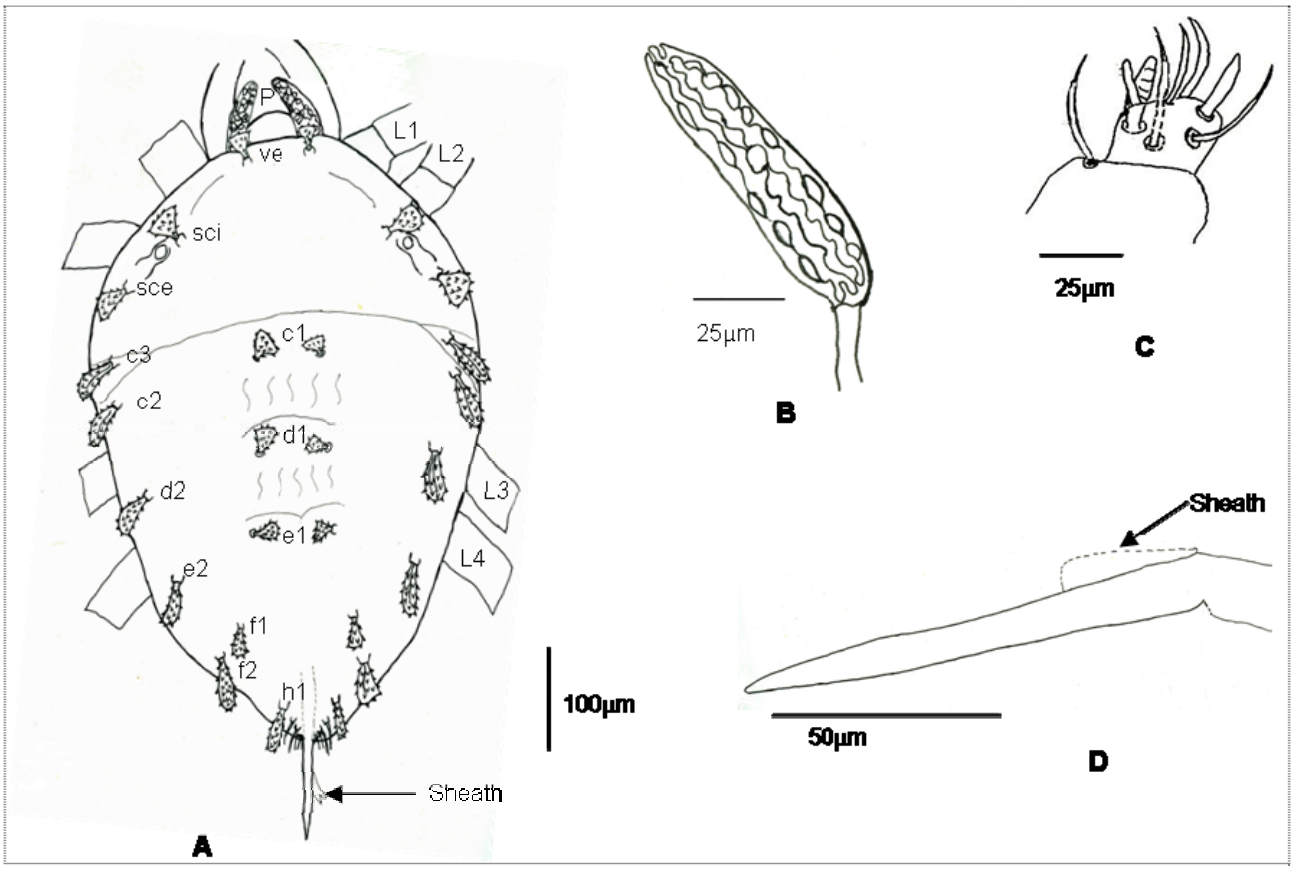

Figure 2.2: *Peltanobia erasmusi* Smith Meyer, male: (A) dorsum (B) peritreme (C) distal segment of the palp tarsus (D) male aedeagus.

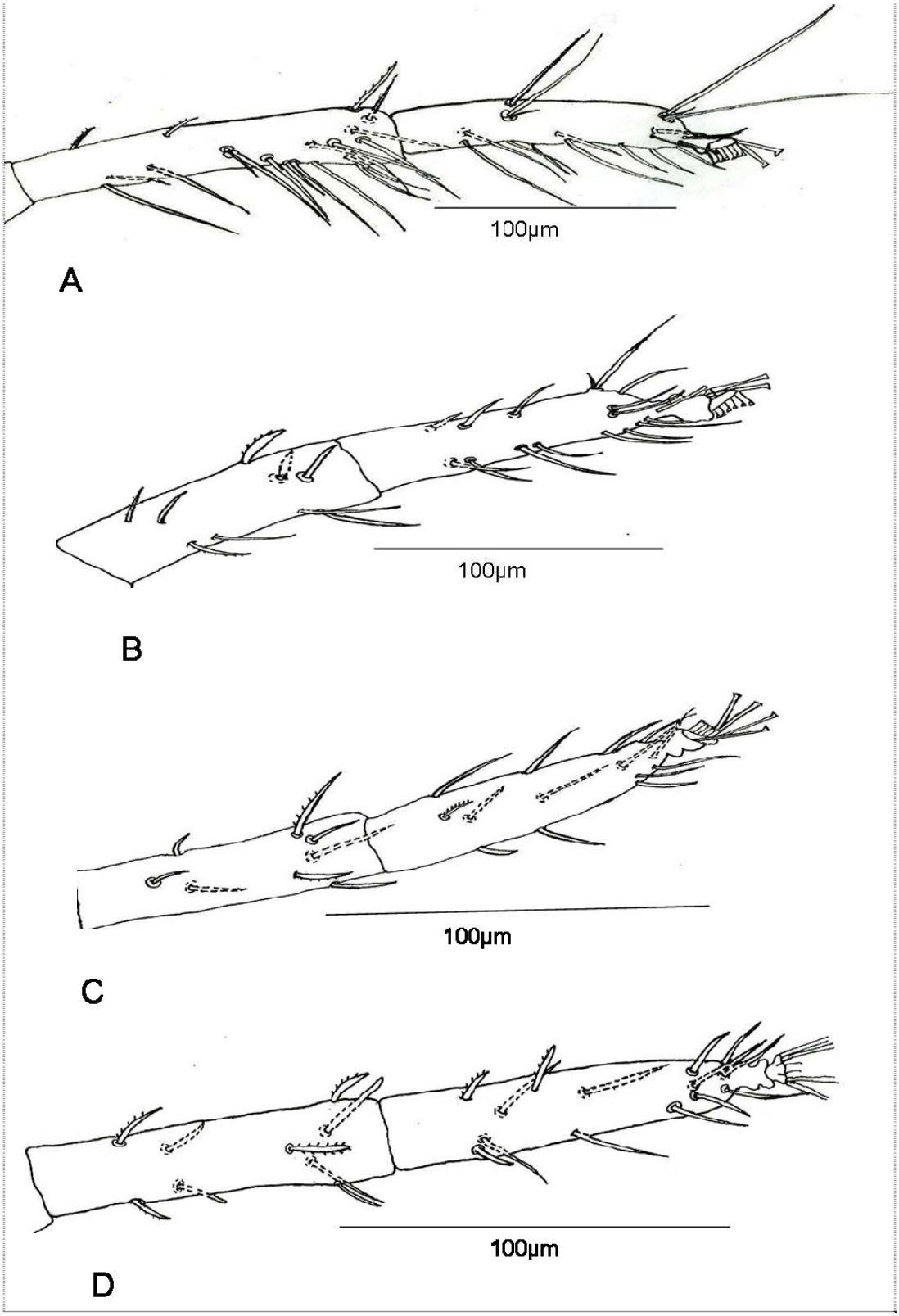

Figure 2.3: *Peltanobia erasmusi* Smith Meyer male (A) tarsus and tibia leg I, (B) tarsus and tibia leg II, (C) tarsus and tibia leg III, (D) tarsus and tibia leg IV.

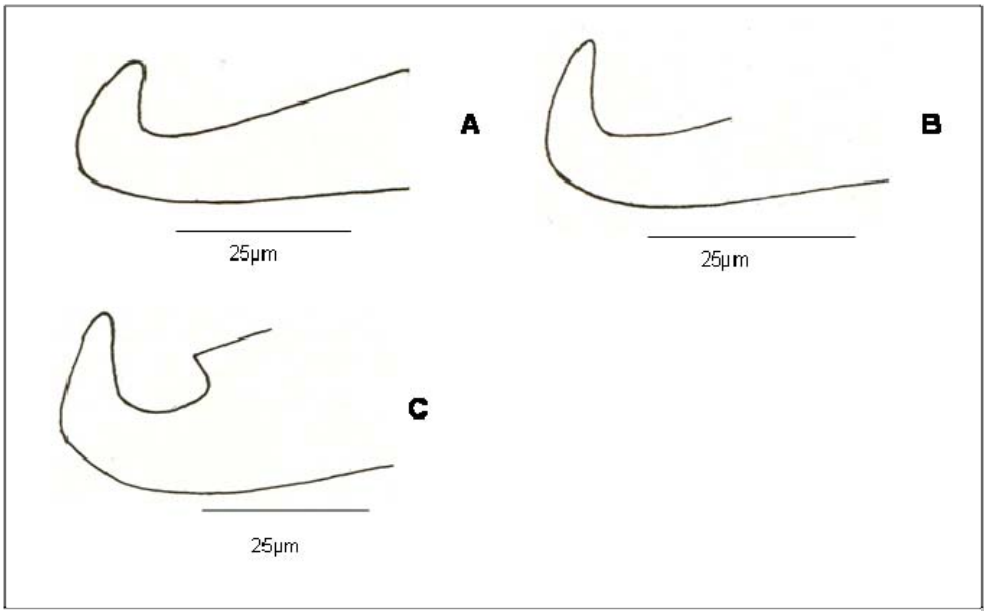

Figure 2.4: *Eutetranychus* sp. aedeagi (from Meyer, 1974 with modifications): (A) *E. africanus* aedeagus, (B) *E. carinae* aedeagus, (C) *E. orientalis* aedeagus.

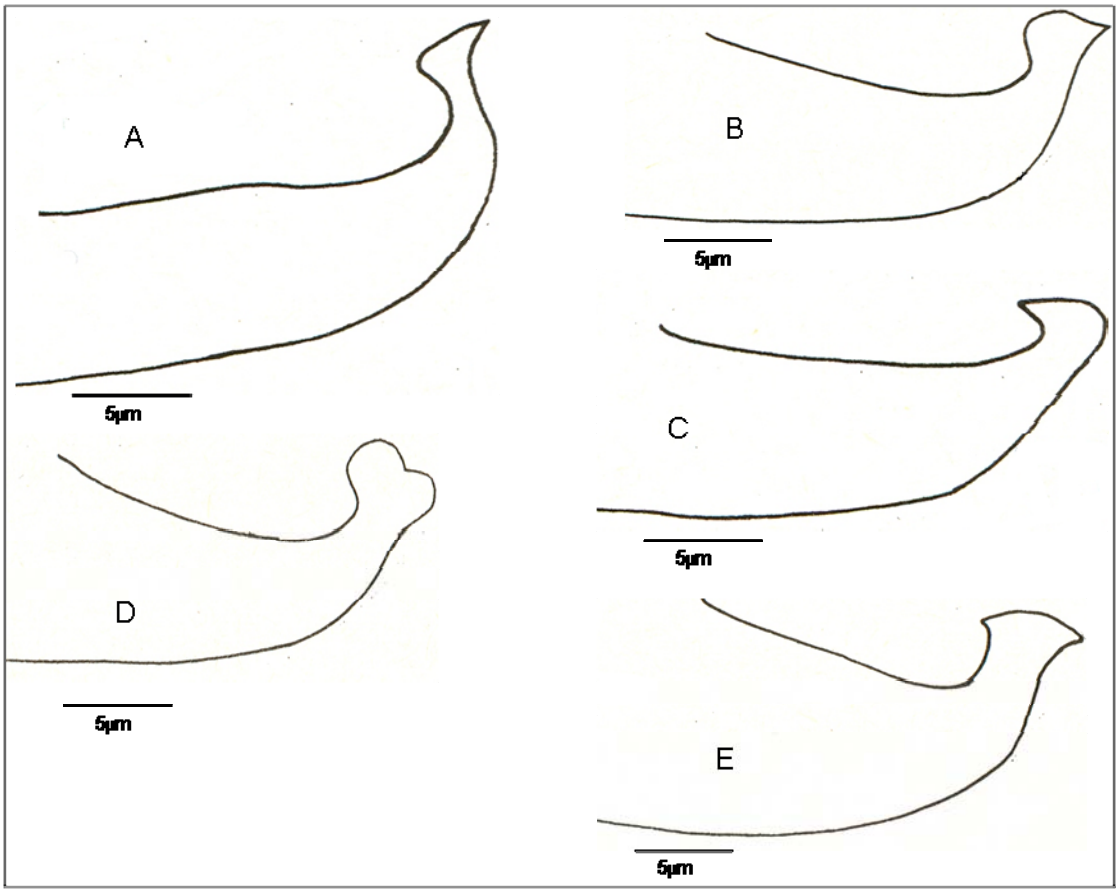

Figure 2.5: *Tetranychus* sp. aedeagi modified after Smith Meyer, 1974 (with permission): (A) *T. evansi*, (B) *T. lombardiini,* (C) *T. ludeni*, (D) *T. neocaledonicus,* (E) *T. urticae*.

CHAPTER 3: DESCRIPTION OF *BREVINYCHUS MESHACKI* FROM KENYA AND *SCHIZOTETRANYCHUS SP. NOV*. FROM TANZANIA

3.1 Abstract

Two new spider mite species *Brevinychus meshacki* Toroitich and Ueckermann sp. nov. from Tanzania and *Schizotetranychus kwalensis* sp. nov. from Kenya are described and illustrated**.** They were collected on *Lonchocarpus eriocalyx* (Fabaceae) from Sangasanga, Mvomero district, Tanzania and *Omorcarpum kirkii* (Fabaceae) from Matuga, Kwale district, Kenya respectively. Revised keys of *Brevinychus* Smith Meyer and of the African species of *Schizotetranychus* Trägårdh are also provided.

3.2 Introduction

Smith Meyer (1974) erected the genus *Brevinychus* in the subfamily Tetranychinae based on two species *Brevinychus mbandu* and *Brevinychus parvulus* and designated *B. mbandu* as the type species. The type material of this species was collected on *Philonoptera violacea* (Klotzsch) Schrire (Fabaceae: Papillionoideae) mostly in and around the Kruger National Park in South Africa. Type material of *Brevinychus parvulus* was collected on *Neorautanenia* sp. (Fabaceae) and *Diospyros zombensis* (B.L. Burtt) F. White (Ebenaceae) in Malawi. This genus is small, consisting of only three known species and its economic importance is not yet known.

The new *Brevinychus* species is based on specimens collected on *Lonchocarpus eriocalyx* (Papilionaceae) growing by the roadside at Sangasanga area, Mvomero district in Tanzania (S06°55.249'; E037°30.074').

Schizotetranychus Trägårdh in the subfamily Tetranychinae, was defined based on the description of *Tetranychus schizopus* by Trägårdh in 1915. Around 115 species of this genus have been described from all over the world with 20 species occurring in Africa. One species *Schizotetranychus spiculus* Baker & Pritchard was described from Kenya and was the only species from this genus reported from Kenya before this study (Migeon & Dorkeld, 2006). Species of economic importance include: *Schizotetranychus asparagi* Oudemans which is a pests of Asparagaceae and *Ananas* sp. (Smith Meyer, 1996), *Schizotetranychus baltazari* Rimando reported as pest of citrus from Taiwan, *Schizotetranychus hindustanicus* Hirst on citrus in India, *Schizotetranychus oryzae* Rossi de Simons on rice in Argentina, Brazil, Colombia,

and Texas (US) (Jeppson *et al.,* 1975) and three species *Schizotetranychus nanjingensis* Ma & Yuan, *Schizotetranychus bambusae* Reck and *Schizotetranychus tenuinidus* Zhang & Zhang on bamboo in China (Zhang *et al*., 2000).

The specimens on which the description of the new *Schizotetranychus* species is based on were collected on *Ormocarpum kirkii* (Fabaceae) growing by the roadside in Matuga area, Kwale district (S04°11.356'; E039°31.808'), Kenya.

3.3 Materials and methods

Mites were collected using the beating method whereby a 40 cm diameter enamel beating plate was placed under the plant and the foliage and twigs were beaten to dislodge the mites. The mites which fell on the plate were picked using a fine hair brush and directly put in vials containing 70% ethanol. The specimens were left in 70% ethanol for ten days to clear then were later examined under a Leica MZ8 (Leica Microsystems, Wetzlar, Germany) dissecting microscope. They were then mounted in polyvinyl alcohol (PVA) medium on glass microscope slides for identification and description. Drawings were made under a Zeiss Axioskope (Carl Zeiss Ltd, Jena, Germany) phase-contrast compound microscope using a drawing tube. Setal notations used are according to Lindquist (1985). Body measurements were taken under the microscope directly connected to a computer using Olympus soft imaging system (Soft Imaging Systems, Münster, Germany) and are given in micrometers (µm). The measurements given are based on the holotype followed by the range of paratype measurements in parenthesis.

The leg setal counts include solenidia but duplex setae are indicated in brackets. Holotypes and paratypes are deposited in the Biosystematics Support Unit collection (BSU) collection, ICIPE – African Insect Science for Food and Health, Nairobi, Kenya and other paratypes have been deposited in the Arachnida Collection of the Biosystematic Programme, ARC-PPRI, Pretoria, South Africa.

3.4 Results

3.4.1 Genus *Brevinychus* Smith Meyer, 1974

Type-species: *Brevinychus mbandu* Smith Meyer

This genus closely resembles *Mixonychus* Ryke & Meyer in that it has a claw-like empodium which lacks proximoventral hairs, true claws pad-like provided with tenent

hairs, duplex setae on tarsus I are distal and approximate, dorsally it has three pairs of propodosomal setae (*ve, Sci, Sce*) and ten pairs of opisthosomal setae (*c1, c2, c3, d1, d2, e1, e2, f1, f2, h1*) with most of the body setae borne on tubercles. Ventrally, it has two pairs of anal and two pairs of para-anal setae

It can be differentiated from *Mixonychus* Ryke & Meyer by the empodial claw which is very short, about the same length as the pads of true claws whereas that of *Mixonychus* is much longer than pads of true claws, more than half the length of tenent hairs. The opisthosoma of this genus is punctate whereas that of *Mixonychus* is reticulate.

3.4.1.1 Brevinychus meshacki Toroitich & Ueckermann sp. nov. (Figs. 3.1 and 3.2)

Types - Holotype female, Tanzania, Mvomero district, by the roadside in Sangasanga area (S06°55.249'; E037°30.074') on *Philonoptera eriocalyx* tree, Date: 16th February 2008; Collector: Faith Toroitich; three female paratypes and one paratype nymph, same data as holotype deposited in ICIPE – Biosystematics Unit, Kenya. Three female paratypes, same data as holotype, were deposited in the mite collection of the Biosystematics Programme of ARC-PPRI, South Africa. The following description is based on the holotype and three adult female specimens (N=4)

***Description* – Female**- Length of body (including gnathosoma) in µm: 370 (370-395), width 267(262-272)

Dorsum: Body punctuate except for a large reticulate pattern located centrally between the *Sci* pair of setae of the propodosoma and extending posteriorly almost to the *c1* setae (Figure 3.1A). The peritreme is simple and ends with a terminal bulb (Figure 3.1B) and the dorsal body setae are serrate except setae *e1* and *f1* which are spatulate (Fig. 3.1A) with the following lengths in µm : *ve* 42 (40-42), *Sci* 72 (61-72), *Sce* 46(43-46), *c1* 19(12-24), *c2* 59(59-69), *c3* 49(42-49), *d1* 56(53-58), *d2* 60(54-60), *e1* 7(7-9), *e2* 58(54-60), *f1* 8(8- 9), *f2* 44(44-48), *h1* 39(37-40).

***Venter*:** Spermatheca oval shaped with a long narrow tube which fades out near the area between leg III and IV (Fig. 3.1E). Ventral setae are slender and smooth with lengths (in µm) as follows: *ag* 37.5 (30-42.5), *g1* 20 (20-22.5), *g2* 22.5(22.5-25), *ps1* 12(12-15), *ps2* 15 (12.5-17.5), *h2* 17.5(15-22.5), *h3* 25(22.5-25)

Gnathosoma: Palpi 5-segmented, palp tarsus with relatively thick terminal sensillum, 1.5 times as long as broad (Fig 3.1C).

Legs: Empodium very short and claw-like (Fig. 3.1D)

Chaetotaxy: tarsi: 14(2)-11(1)-11-11; tibiae: 9-5-6-4; genua: 5-5-3-2; femora: 5-5-5-2; trochanters: 1-1-1-1; coxae: 2-2-1-1.

Leg lengths in µm: Leg I 310 (300-325), Leg II 255 (250-255), Leg III 275 (250-275) and Leg IV 290 (275-290). Tibiotarsi (Fig. 2): I (115), II (139), III (153) and IV (136).

Protonymph: The protonymph is similar to the adult female in shape and dorsal setation but has fewer leg setae: tarsi: 13(1)-10-10-10; tibiae: 7-5-4-3; genua: 3-3-1-1; femora: 3-3-2-2; trochanters: 1-1-1-1; coxae: 2-2-1-1.

Diagnosis: This species can be recognized by the first, third and fourth pairs of dorsocentral setae (*c1*, *e1* and *f1*) (Fig. 3.1) which are much shorter than the dorsolateral setae; only the second dorsocentral setae (*d1*) is of similar length to the dorsolateral setae; and the central region of the propodosoma is distinctly reticulate. In *B. mbandu,* the central region of the prodorsum is distinctly reticulate, as in *B. meshacki*, but both *e1* and *f1* are shorter than the dorsolateral setae (Fig. 3.3A) and in *B. parvulus*, all of the dorsocentrals (*c1, d1, e1* and *f1*) are shorter than the dorsolateral setae (Fig. 3.3B) and the propodosoma is entirely punctuate.

Etymology: The species is named after Dr. Meshack Obonyo, husband of the first author for his much valued encouragement and support.

3.4.1.2 Brevinychus mbandu Smith Meyer, 1974

This species is recognized by having only the third (*e1*) and fourth (*f1*) pairs of dorsocentral setae much shorter than other dorsal body setae and the body punctuate with a large reticulate area on the centre of the propodosoma (Fig. 3). Leg chaetotaxy as follows: coxae 2-2-1-1; femora 6-5-4-3; genua 5-5-3-2; tibiae 8/9(1)-6-5-5; tarsi 13(1)+2dupl – 12+1dupl-10(1)-10(1).

3.4.1.3 Brevinychus parvulus Smith Meyer, 1974

This species is distinctive in having all four pairs of dorsocentral setae (*c1, d1, e1* and *f1*) minute and the remainder of the dorsal setae relatively long, and the entire dorsum punctate (Fig. 4). Leg chaetotaxy is as follows: coxae 2-2-1-1; femora 8-6-3-2; genua 5-5-3-2; tibiae 8/9(1)-5-5-5; tarsi 11(1)+2dupl – 12+1dupl-10(1)-10(1).

3.4.1.4 Key to the species of *Brevinychus* females (males unknown)

1	Dorsocentral setae (*c1, d1, e1* and *f1*) subequal in length, all much shorter than the dorsolateral setae, propodosoma entirely punctuate	*Brevinychus parvulus* Smith Meyer
	Dorsocentral setae of varying lengths, with atleast one pair similar in length to the dorsolateral setae; propodosoma with a large central reticulate area	2
2	Opisthosoma with third and fourth pairs of dorsocentral setae (*e1* and *f1*) much shorter than the dorsolateral setae (*c1* and *d1* long)	*Brevinychus mbandu* SmithMeyer
	Opisthosoma with the first, third and fourth pairs of dorsocentrals (*c1, e1* and *f1*) much shorter than the dorsolateral setae, (only *d1* long)	*Brevinychus meshacki* Toroitich & Ueckermann **sp. nov.**

3.4.2 Genus *Schizotetranychus* Trägårdh, 1915

Type species: *Tetranychus schizopus* Trägårdh

The empodium of this genus is claw-like and two-pronged with appendant hairs. It has three pairs of propodosomal setae and ten pairs of opisthosomal setae; two pairs of para-anal setae and two pairs of anal setae. The duplex setae on tarsus I are distal and approximate.

3.4.2.1 *Schizotetranychus kwalensis* sp. nov.(unpublished)

Types: Holotype female and three paratype females collected from Kwale.

Descriptions based on the holotype female and three paratype females.

Dimensions: Length of body (including gnathosoma) in micrometers (µm): 474(474-506), Breadth (width) 284(284-308)

Dorsum: Dorsal body setae short, tapering distally and are subequal in length, about half the length of the longitudinal intervals between them. Striae between all dorsocentral setae (*c1–f1*) are transverse, fourth pair of dorsocentrals *f1* is situated a little closer than other 3 pairs of dorsocentrals (Fig. 3.4A).

The peritreme ends simple with a terminal bulb (Fig. 3.4E). Dorsal body setae are lanceolate and slightly barbed (Fig. 3.4C) with lengths in micrometers (µm): *ve* 36(36-37), *sci* 46(46-49), *sce* 44(44-48), *c1* 31(29-31), *c2* 34(34-36), *c3* 48(47-50), *d1* 29(29-31), *d2* 38(38-39), *e1* 32(32-33), *e2* 40(39-41), *f1* 35(35-36), *f2* 41(40-44), *h1* 42(41-42).

Venter: The striations in the pre-genital area are transverse.

Gnathosoma: Stylophore convex mediodistally (Fig 3.4A), palp tarsus with terminal sensillum thick, twice as long as broad (Fig 3.4B).

Legs: empodium two pronged claw (Fig. 3.4D)

Chaetotaxy: tarsi: 10(2)-10(1)-8-8; tibiae: 8-5-5-5; genua: 5-5-3-3; femora: 8-5-3-2; trochanters: 1-1-1-1; coxae: 2-2-1-1.

Length of tibio-tarsi: I (75), II (68), III (67) and IV (78) (Fig 3.5);

Diagnosis: This species is close to *Schizotetranychus umtaliensis* Smith Meyer having a simple peritreme, short, serrate opisthosomal setae, opisthosomal dorsal striations being transverse and stylophore convex mediodistally but differs from it by having female opisthosomal setae about half the length of distances between bases of consecutive setae and setae *sci* and *sce* subequal in length, *ve* shortest as well as seven tactile setae on tibia I and a solenidion whereas *S. umtaliensis* has female opisthosomal setae about a third the length of distances between bases of consecutive setae, setae *sce* longer than *ve* and *sci*; tibia I with six tactile setae and a solenidion

Etymology: The species name *kwalensis* is derived from the district Kwale where this species was found.

3.4.2.2 Key to the African species of *Schizotetranychus* Trägårdh

1	Female prodorsum with longitudinal striae	2
	Female prodorsum with a reticulate pattern medially	*S. reticulatus* Baker & Pritchard
2	Dorsal opisthosomal setae shorter than distances between consecutive setae	3
	All or most dorsal opisthosomal setae as long as or longer than distances between consecutive setae	10
3	Opisthosoma with fourth pair of dorsocentral setae (*f1*) considerably further apart than second pair of dorsocentral setae (*d1*)	4
	Opisthosoma with fourth pair of dorsocentral setae (*f1*) not further apart than second pair of dorsocentral setae (*d1*)	5
4	Opisthosoma with transverse striae between first *(c1*) and second (*d1,)* pairs of dorsocentral setae	*S. spiculus* Baker & Pritchard

	Opisthosoma with longitudinal striae between first (*c1*) and second (*d1*,) pairs of dorsocentral setae	*S. sacrales* Baker & Pritchard
5	Peritreme simple	6
	Peritreme ends in a rounded loop; aedeagus gradually tapering and forming a sigmoid distal part, without a distinct knob	*S. pennamontanus* Meyer
6	Stylophore convex mediodistally	7
	Stylophore cleft mediodistally; aedeagal shaft broad at base but narrowing distally, distal part about as long as shaft	*S. rhodanus Baker &* Pritchard
7	Striae on opisthosoma generally transverse, opisthosomal setae sparsely to densely setose	8
	Striae irregular between setae *c1 – e1*; opisthosomal setae awl-shaped and almost smooth; aedeagal shaft short and broad, distal part much shorter than shaft	S. *gausus* Baker & Pritchard
8	Setal formula of femora 8-5-3-2	9
	Setal formula of femora 9-7-4-3 or 9-7-4-4	11
9	Female with opisthosomal setae about a third the length of distances between bases of consecutive setae, setae *sce* longer than *ve* and *sci*; tibia I with six tactile setae and a solenidion	*S. umtaliensis* Smith Meyer
	Female with opisthosomal setae about half the length of distances between bases of consecutive setae, setae *sci* and *sce* subequal in length, *ve* shortest; tibia I with seven tactile setae and a solenidion	***Schizotetranychus kwalensis* sp. nov.**
10	Aedeagus bent ventrad; female terminal sensillum on palptarsus slightly longer than broad; setal formula of tibiae 10(1)-7-5-5	*S. asparagi* Oudemans
	Aedeagus bent dorsad; female terminal sensillum on palptarsus about twice as long as broad; setal formula of tibiae 10(1)-7-6-6 or 7	*S. protectus* Smith Meyer
11	Opisthosoma with the first three pairs of dorsocentral setae (*c1*, *d1* and *el* nearly or about as long as intervals between consecutive setae	12
	Opisthosoma with the first three pairs of dorsocentral setae (*c1*, *d1* and *el)* longer than intervals between consecutive setae	13
12	Aedeagus with distal part evenly tapering, sigmoid; peritreme hooked distally	*S. australis* Gutierrez
	Aedeagus with small, acute anterior projection on distal part, forming a knob; peritreme straight distally,	*S. dalbergiqe* Smith Meyer

	ending in a simple bulb	
13	Aedeagus with distal part evenly tapering	14
	Aedeagus with small, acute anterior projection on distal part forming a knob, shaft very long; dorsal striae of female with tiny, narrow dorsal lobes sharply pointed at their tips	*S. tephrosiae* Gutierrez
14	Aedeagal shaft nearly straight; distal end of aedeagus abruptly narrowed and drawn out like an elongated triangle	*S. triquetrus* Smith Meyer
	Aedeagal shaft bent ventrad or dorsad	15
15	Aedeagus bent dorsad	16
	Aedeagus bent ventrad forming a sigmoid, caudally directed distal end	*S. sayedi* Attiah
16	Aedeagus with distal part much shorter than dorsal margin of shaft	17
	Aedeagus with distal part about as long as or longer than dorsal margin of shaft	19
17	Aedeagus with dorsal margin of shaft concave; peritreme retrorse distally; tibia I with seven tactile setae	*S. arcuatus* Smith Meyer
	Aedeagus with dorsal margin straight; peritreme dilated into a bulb or chamber	18
18	Striae anterior to female genital flap transverse; tibia III with five tactile setae	*S. nesbitti* Smith Meyer
	Striae anterior to female genital flap longitudinal; tibia III with four tactile setae	*S. tuttlei* Zaher, Gomaa & EI-Enany
19	Aedeagus with distal part sigmoid; terminal sensillum on male palptarsus rudimentary	*S. miyatahus* Smith Meyer
	Aedeagus with distal part nearly straight; terminal sensillum on male palptarsus well developed	20
20.	Aedeagus broadly curved dorsad at an obtuse angle; distal curved part about as long as dorsal margin of shaft	*S. filifolius* Smith Meyer
	Aedeagus bent dorsad at about a right angle; distal curved part longer than dorsal margin of shaft	*S. setariae* Smith Meyer

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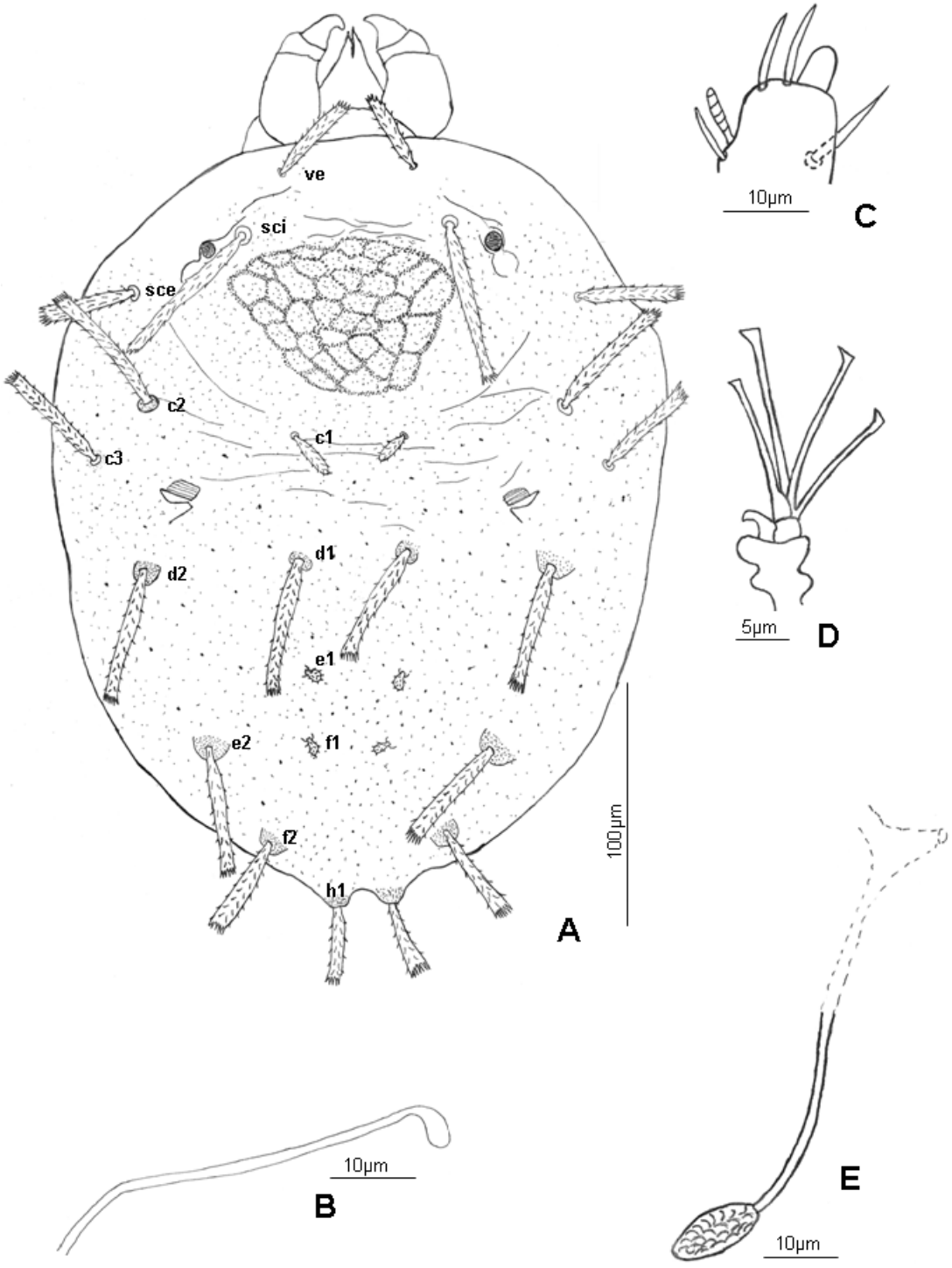

Figure 3.1: *Brevinychus meshacki* sp. nov. female. (A) dorsum, (B) peritreme, (C) palpus, (D) empodium, (E) spermatheca.

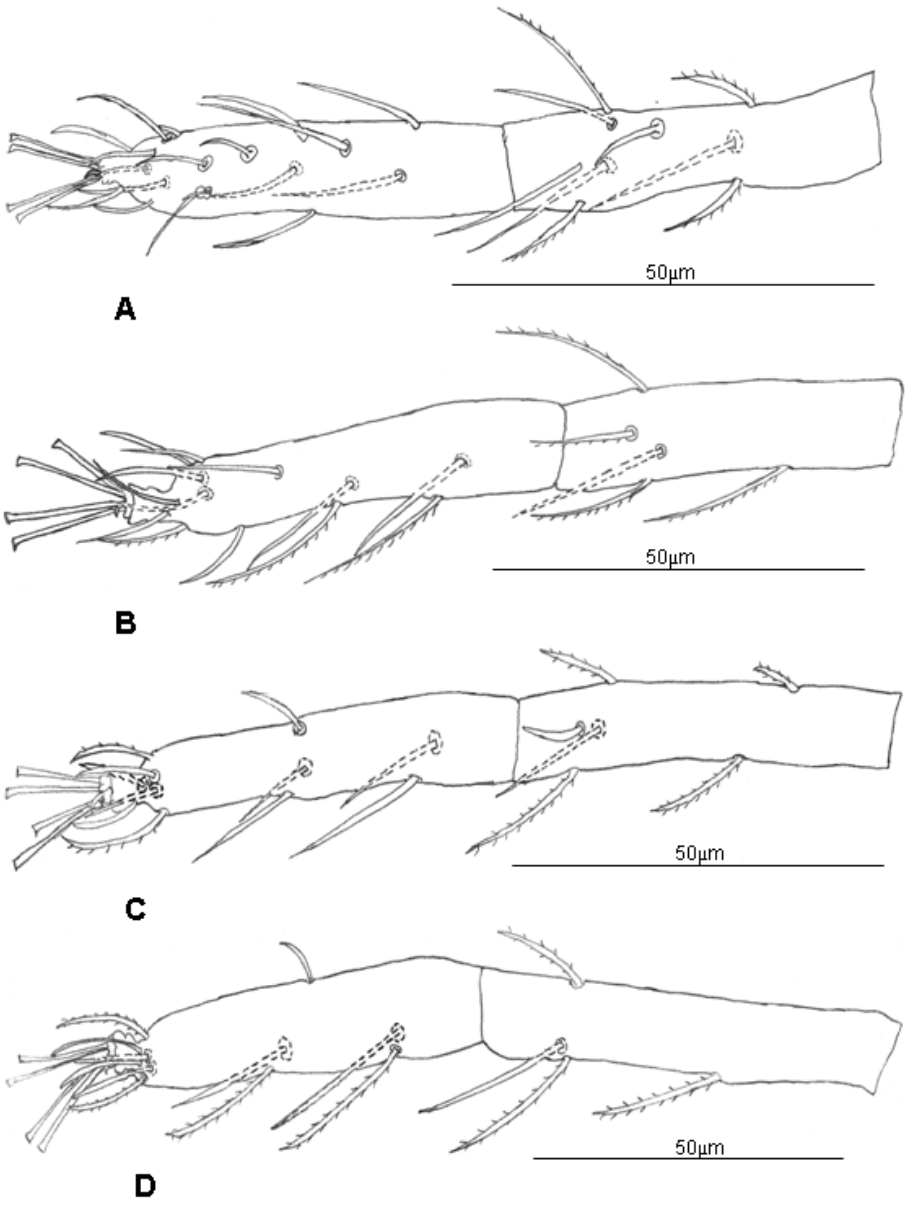

Figure 3.2: *Brevinychus meshacki* sp. nov. female legs: (A) tibiotarsus I, (B) tibiotarsus II, (C) tibiotarsus III, (D) tibiotarsus IV.

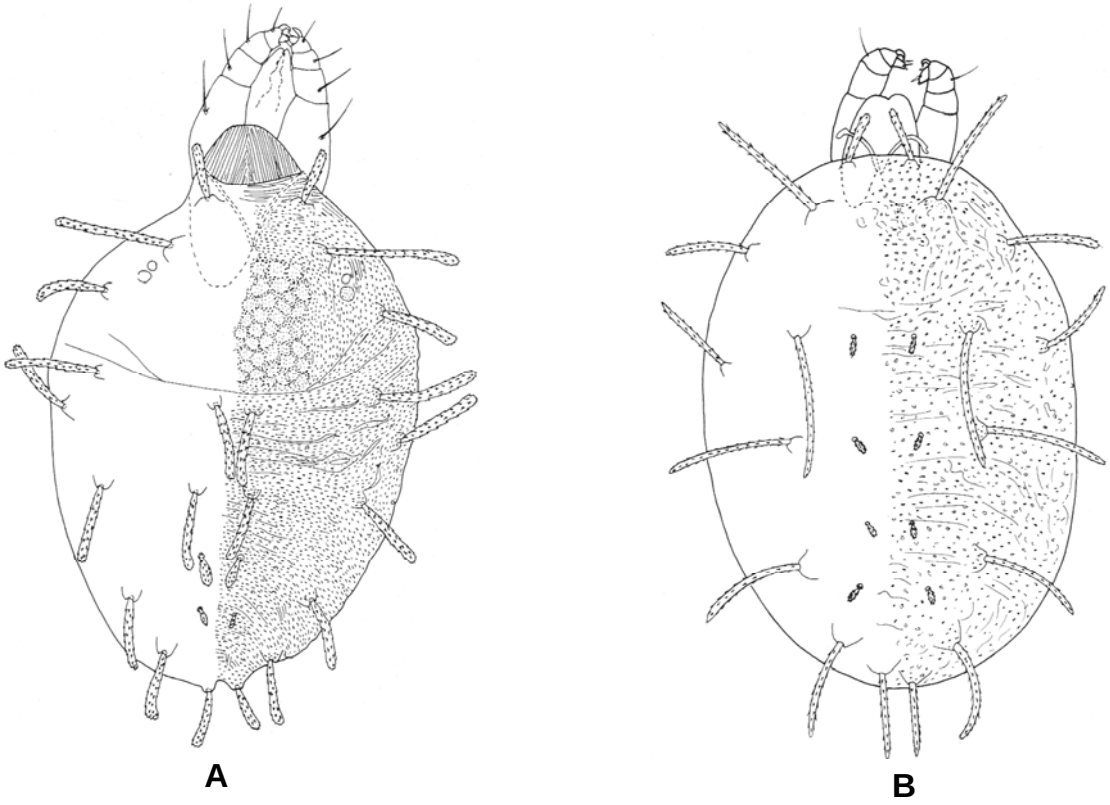

Figure 3.3: (A) *Brevinychus mbandu*, Smith Meyer (B) *Brevinychus parvulus* Smith Meyer (Adapted from Smith Meyer, 1974 with permission).

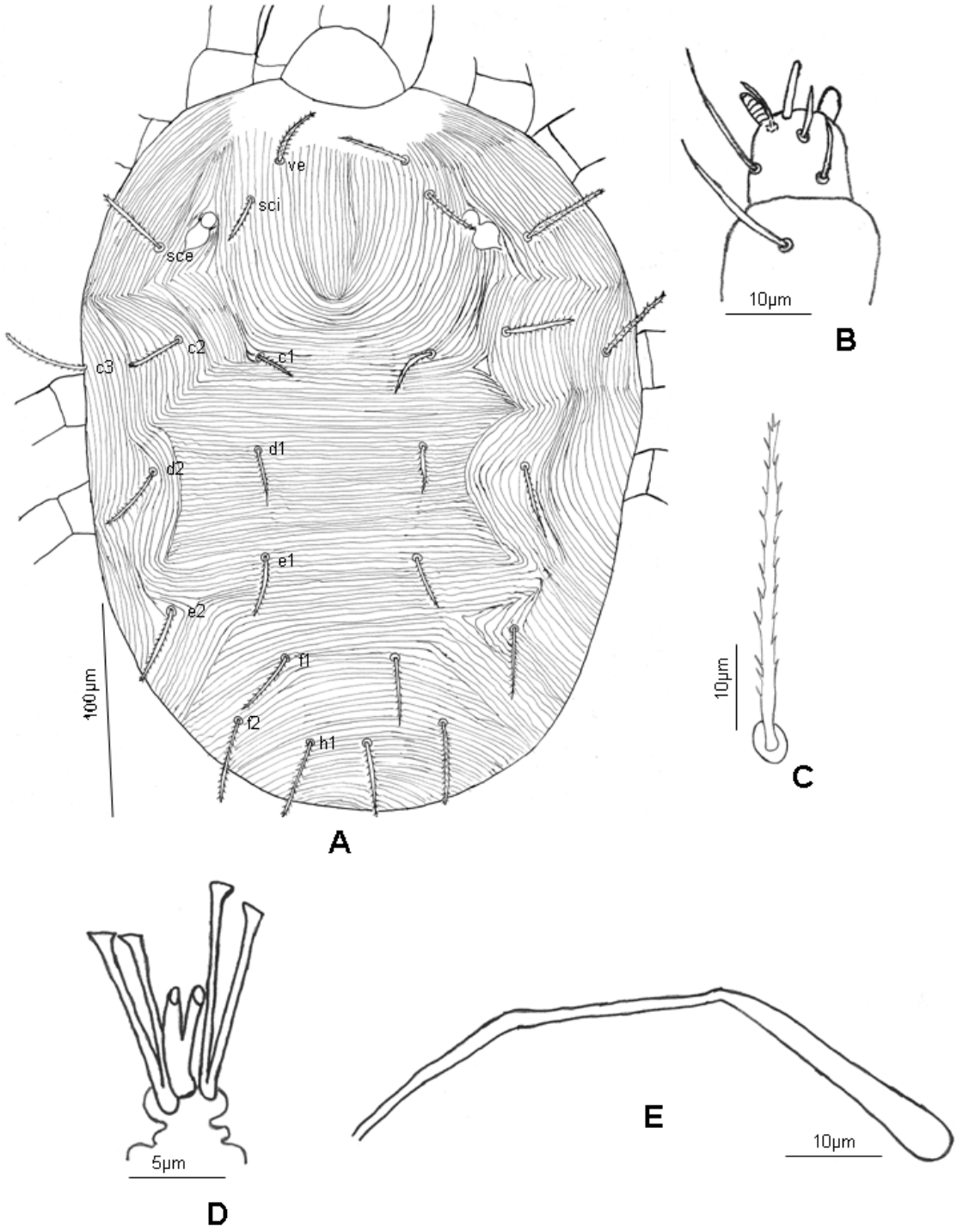

Figure 3.4: *Schizotetranychus kwalensis* sp. nov. female: (A) dorsum, (B) palpus, (C) dorsal setae *sce*, (D) empodium, (E) peritreme.

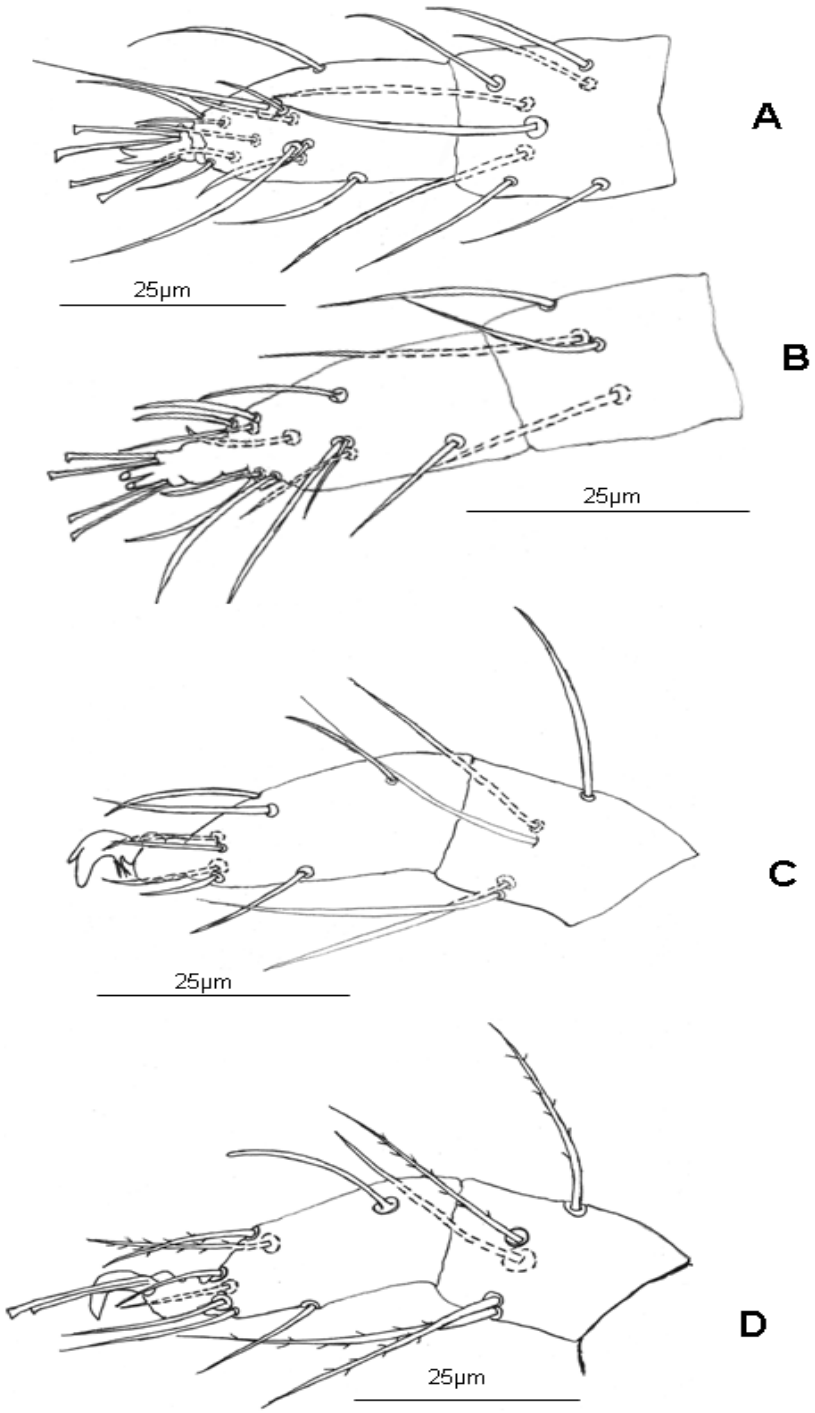

Figure 3.5: *Schizotetranychus kwalensis* sp. nov. female. (A) Lateral view of tibiotarsus I, (B) tibiotarsus II, (C) tibiotarsus III, (D) tibiotarsus IV.

CHAPTER 4: SPECIES IDENTIFICATION OF FEMALE *TETRANYCHUS* USING SCANNING ELECTRON MICROSCOPY.

4.1 Abstract

Female characters of four *Tetranychus* species found in Kenya were explored using scanning electron microscopy. Among the characters examined are the distances between the duplex setae and the shape of the lobes on dorsal striae. *Tetranychus evansi* Baker and Pritchard and *Tetranychus ludeni* Zacher had their two sets of duplex setae further apart with the proximal pair being almost in line with the nearest tactile setae whereas those of *Tetranychus neocaledonicus* Andre and *Tetranychus urticae* Koch were closer to each other with the proximal pair being distal to the nearest tactile setae. The dorsal striae of *T. evansi*, *T. neocaledonicus* and *T. urticae* have semicircular lobes whereas those on the dorsal striae of *T. ludeni* are triangular and the striations in the pregenital area are transverse in *T. neocaledonicus* and longitudinal in *T. evansi*, *T. ludeni* and *T. urticae.*

4.2 Introduction

Members of the spider mite genus *Tetranychus* Dufour are some of the most economically important species of the Tetranychidae. They can inflict severe damage on agricultural and horticultural crops that often lead to economic losses (Jeppson *et al*., 1975). This genus comprises of 121 species worldwide but only five species are known to occur in Kenya (Migeon & Dorkeld, 2006; Toroitich *et al*., 2009).

The diagnostic characters for *Tetranychus* were discussed by Meyer (1987) and include the possession of a single pair of para-anal setae; empodia split into 3 pairs of proximoventral hairs (with the exception of *Tetranychus fijiensis* Hirst where these are split into 2 pairs); mediodorsal spurs often present on the empodia (shorter than proximoventral hairs); in males, tridigitate spurs are usually borne on empodium I; male aedeagus curved dorsad and the appearance of integumentary striae are also of significance.

The shape of the male aedeagus has been the major character in species determination in *Tetranychus* (Smith Meyer, 1987; Baker & Tuttle, 1994). However, the precise determination of species based on the male aedeagus alone is sometimes not accurate enough and the use of morphological characters of both sexes becomes necessary. An

example is *Tetranychus evansi* Baker & Pritchard which was initially misidentified as *Tetranychus marianae* McGregor by Silva (1954) because of the similarity of the shape of the aedeagus of the two species. A subsequent and more detailed examination of the chaetotaxy on the legs of female mites allowed the two species to be distinguished. The proximal pair of duplex setae on female tarsus I provided clarification (Baker & Pritchard, 1960; de Moraes *et al.*, 1987).

Moreover, the male aedeagus is a difficult structure to accurately examine due to the specialized mounting technique it requires: the specimen has to be placed in a lateral position before a cover slip is lowered and should remain in that position. Furthermore, minimal variations in the shape of the aedeagus, such as angulations and projections of the knob, may determine a decision for or against a species. In this genus, the male: female ratio in a fertilized population is usually 1:3 (Helle & Sabelis, 1985) and this means that the chances of randomly selecting a male is three times less than that of selecting a female. This is further complicated by the fact that males are a significantly smaller than females and paler in colour, thus reducing chances of finding and collecting males especially by the untrained eye.

Flechtmann and Knihinicki (2002) developed identification keys based on female characters of *Tetranychus* and placed species of this genus into nine phenetic groups that do not necessarily have phylogenetic significance.

In Kenya, five *Tetranychus* species have been reported namely *Tetranychus evansi* Baker & Pritchard, *Tetranychus lombardiini* Baker & Pritchard, *Tetranychus ludeni* Zacher, *Tetranychus neocaledonicus* Andre and *Tetranychus urticae* Koch.

The purpose of this study was to explore the possibility of using more female characters for species determination of members of the *Tetranychus* found in Kenya using scanning electron microscopy.

4.3 Materials and Methods

4.3.1 Collection of the mites

The spider mites used in this study include: *Tetranychus evansi*, collected on *Solanum incanum* from Mwea, Kirinyaga district (S00°39.208'; E037°21.750'), *Tetranychus ludeni* collected on *Physalis heterophyla* from Kangemi, Nairobi (S01°15.984'; E 036°45.810'), *Tetranychus neocaledonicus* collected on *Manihot esculentum* from Shimba Hills, Kwale (S 04°21.818'; E 039°19.490') and *Tetranychus urticae*

collected on *Lycopersicon esculentum* from Mwea, Kirinyaga (S 00°39.208'; E 037°21.750'). Twenty females and ten males each of the species listed above were collected with the help of a fine hair brush and put in absolute ethanol. They were then transported to the electron microscopy laboratory of the North West University Potchefstroom campus, South Africa, where the SEM images were taken.

4.3.2 Scanning electron microscopy

Five best preserved adult mites per species were selected for scanning electron microscopy. The mites were removed from absolute ethanol, dehydrated in an acetone series (70, 80, 90, 100%) for 15 minutes at each concentration, critical-point dried, mounted on stubs with double-sided carbon tape and coated with a 25 nm layer of gold/palladium in a sputter coater. Specimens were studied with a Phillips XL30 DX-4i scanning electron microscope operating at 5–10 kV.

The characters examined are: The shape of the dorsal lobes, the shape of dorsal striations between the dorsocentral setae *d1* and *e1*, the position of the proximal duplex setae relative to the nearest tactile setae, the shape of the striations on the female pre-genital area and genital flap and the shape of the female pretarsus of leg I.

4.4 Results

Dorsal lobes: There were variations in the appearances of the dorsal lobes of the different species of spider mites examined. *Tetranychus urticae* lobes appeared faint and the dorsal striae widely spaced (Fig. 4.1D) compared to those of the other species. There were no marked differences between the dorsal lobes of *Tetranychus neocaledonicus* (Fig. 4.1C) and those of *Tetranychus evansi* (Fig. 4.1A).

The dorsal lobes of *Tetranychus ludeni* (Fig. 4.1B) appeared distinct from those of the other species, they appeared narrower at the tip, almost with a triangular appearance compared to those of *T. neocaledonicus* and *T. evansi* which were circular shaped and with a cup-like impression on the lobes (Figs 4.1A and C).

Duplex setae: The distance between the two pairs of duplex setae shorter in *T. urticae* and *T. neocaledonicus* (Figs 4.2 C and D) compared to that between the duplex setae of *T. evansi* and *T. ludeni* (Figs 4.2 A and B). The proximal pair of duplex setae on tarsus I is distal to the nearest tactile setae in *T. urticae* and *T. neocaledonicus* (Figs.

4.2 C and D) whereas the proximal pair of duplex setae is almost in horizontal line with the nearest tactile setae in *T. evansi* and *T. ludeni* (Figs. 4.2 A and B).

Dorsal striae: The striae between the dorsocentral setae d1 longitudinal in *T. evansi* and *T. ludeni* (Figs 4.3 A and B) whereas those of *T. neocaledonicus* and *T. urticae* are transverse (Figs 4.3 C and D).

Pre-genital striations: The striae of *T. neocaledonicus* appear transverse whereas those of the other examined species *T. evansi, T. urticae* and *T. ludeni* are longitudinal (Fig. 4.4).

Shape of leg I pretarsi: There were no observable differences in the shape of the pretarsi of female leg I (Fig. 4.5).

Palp-tarsi: There were no observable differences in the shape and size of the palp tarsi (Fig. 4.6).

4.5 Discussion

Inferring from the scanning electron microscope images, the spider mites divide into two groups. *Tetranychus evansi* and *T. ludeni* group together, the only difference between them being the dorsal lobes that have pointed, almost triangular, tips for *T. ludeni* as opposed to those of *T. evansi* and *T. neocaledonicus* that are circular at the tip and cup-shaped. *Tetranychus evansi* and *T. ludeni* had earlier been grouped together in the *desertorum* group proposed by Pritchard & Baker (1955). Flechtmann & Knihinicki (2002) also put these species in *Tetranychus* group 5 based on having all four proximal tactile setae in line with proximal pair of duplex setae on tarsus I and dorsomedian empodial spur tiny or absent.

Tetranychus neocaledonicus and *T. urticae* group together and their only differences lie in the dorsal lobes which appear weaker in *T. urticae* but strong and cup-shaped in *T. neocaledonicus* and the pre-genital striations which appear longitudinal in *T. urticae* but transverse in *T. neocaledonicus*. These species belong to group 9 according to Flechtmann & Knihinicki (2002) which comprise many species of economic importance.

Moreover, differences in the distance between the duplex setae of the *desertorum* group (*T. evansi* and *T. ludeni*) and group nine mites (*T. neocaledonicus* and *T. urticae*) were observed. Differences in the shape of the dorsal striations between setae d1 of the desertorum group and group nine mites were noted. Based on the female characters studied here, it is possible to separate between species of *Tetranychus*

based on females. However, the differences are subtle and often times hard to visualize under the ordinary microscope but these differences are visible with SEMs.

In conclusion, female characters can be used to separate *Tetranychus* mites to some degree, but not all the species can be identified to species level based on female characters. The groupings of the species according to Flechtmann & Knihinicki (2002) were confirmed by this study, but additional differences between groups 5 and 9 that were not included in earlier keys were noted. It was possible to differentiate between the species of *Tetranychus* but since the differences are very subtle, males may still be required to be able to determine the particular species.

4.6 References

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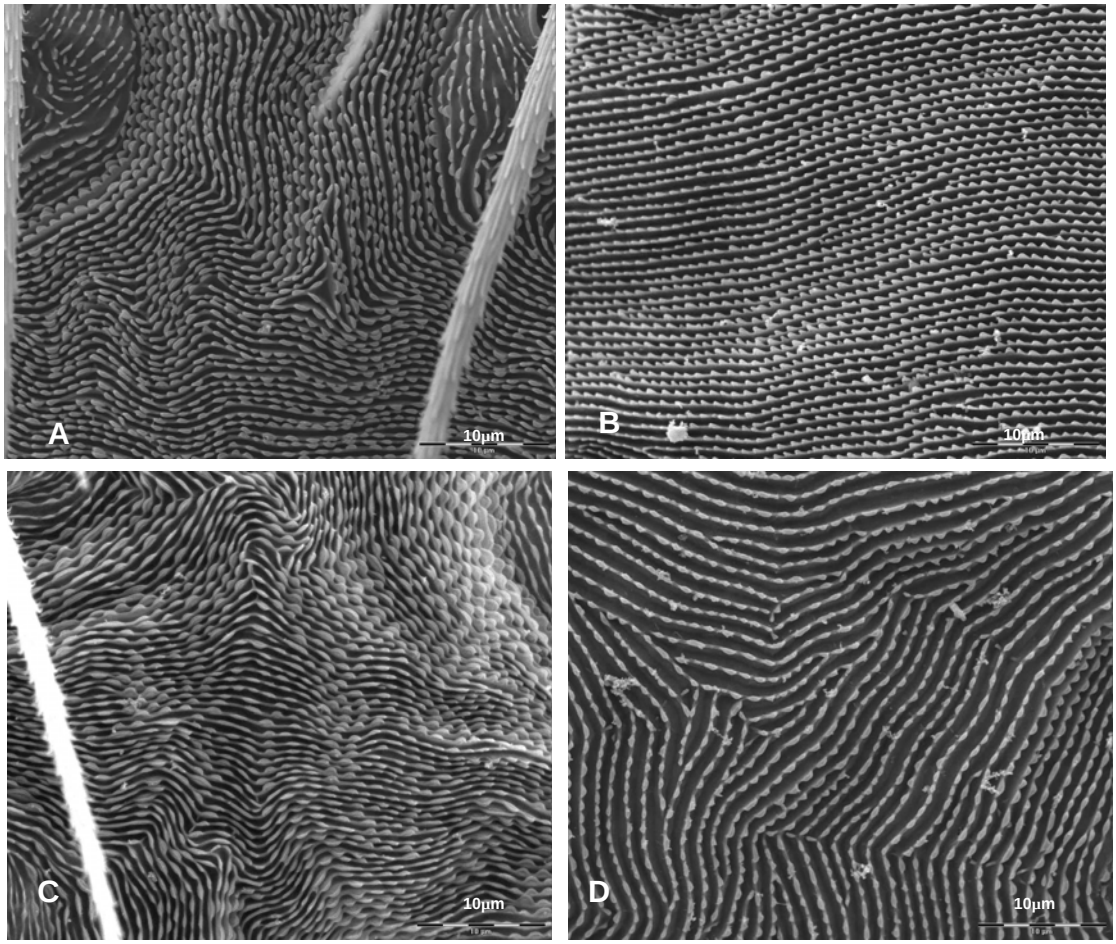

Figure 4.1: *Tetranychus.* Dorsal lobes. (A) *T. evansi*, (B) *T. ludeni*; (C) *T. neocaledonicus*; (D) *T. urticae.*

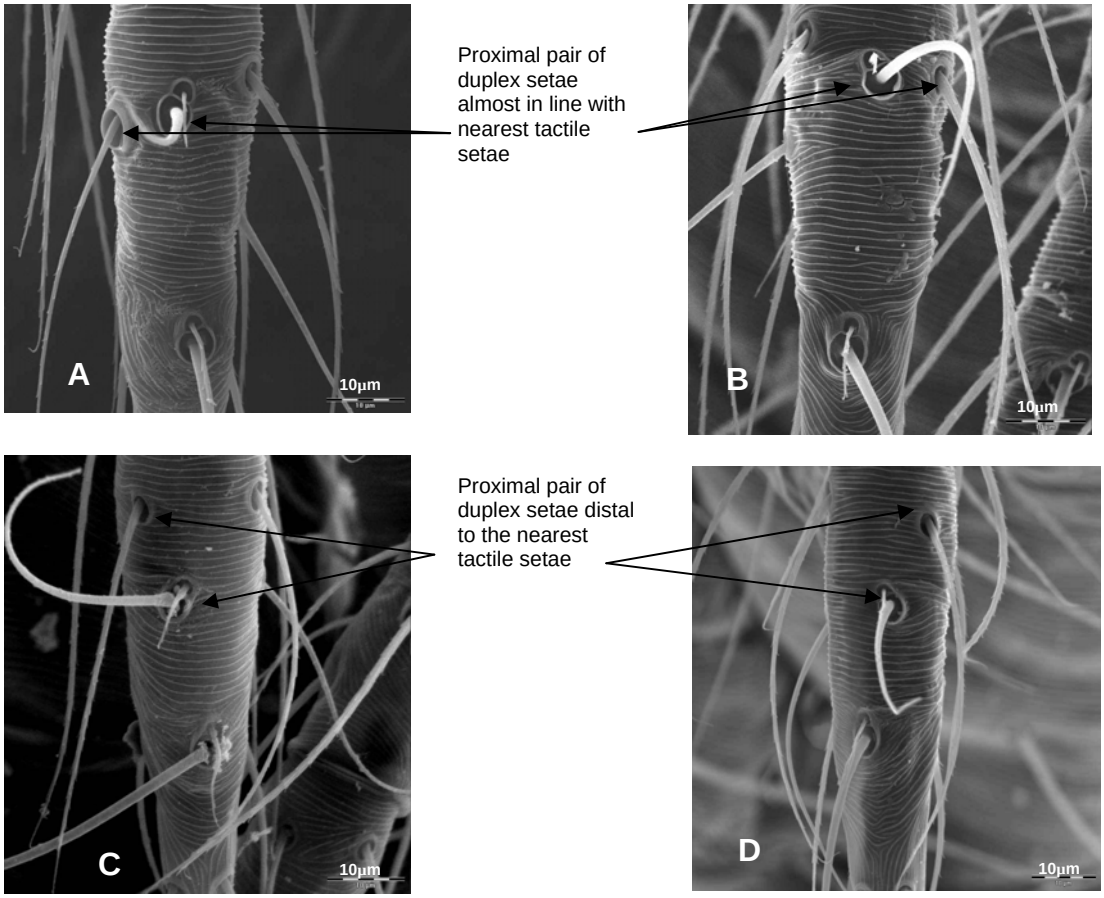

Figure 4.2: Duplex setae of tarsus I in *Tetranychus.* (A) *T. evansi*, (B) *T. ludeni*; (C) *T. neocaledonicus*; (D) *T. urticae*.

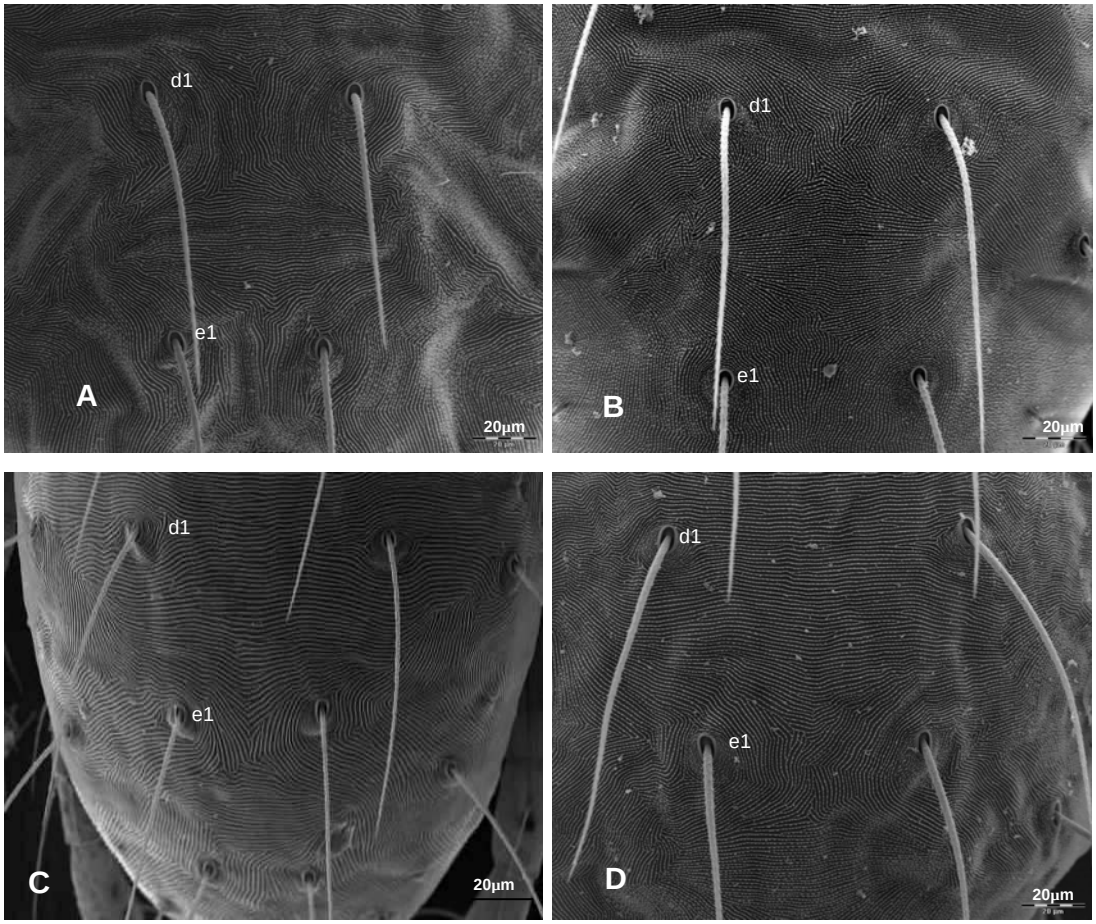

Figure 4.3: *Tetranychus*. Dorsal striations between the dorsocentral setae d1 and e1 (A) *T. evansi*, (B) *T. ludeni*; (C) *T. neocaledonicus*; (D) *T. urticae*.

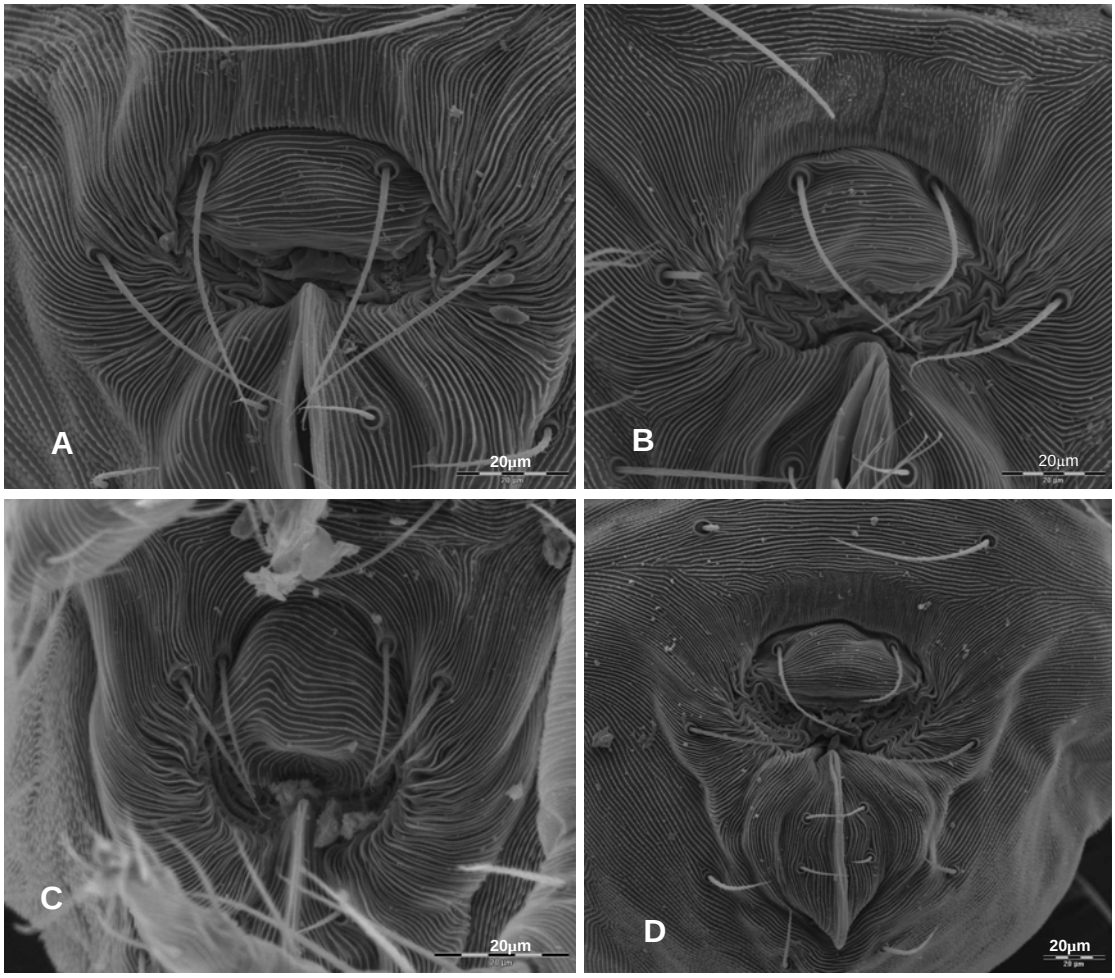

Figure 4.4: *Tetranychus.* Striations in the pregenital area. (A) *T. evansi*, (B) *T. ludeni*, (C) *T. neocaledonicus*, (D) *T. urticae*.

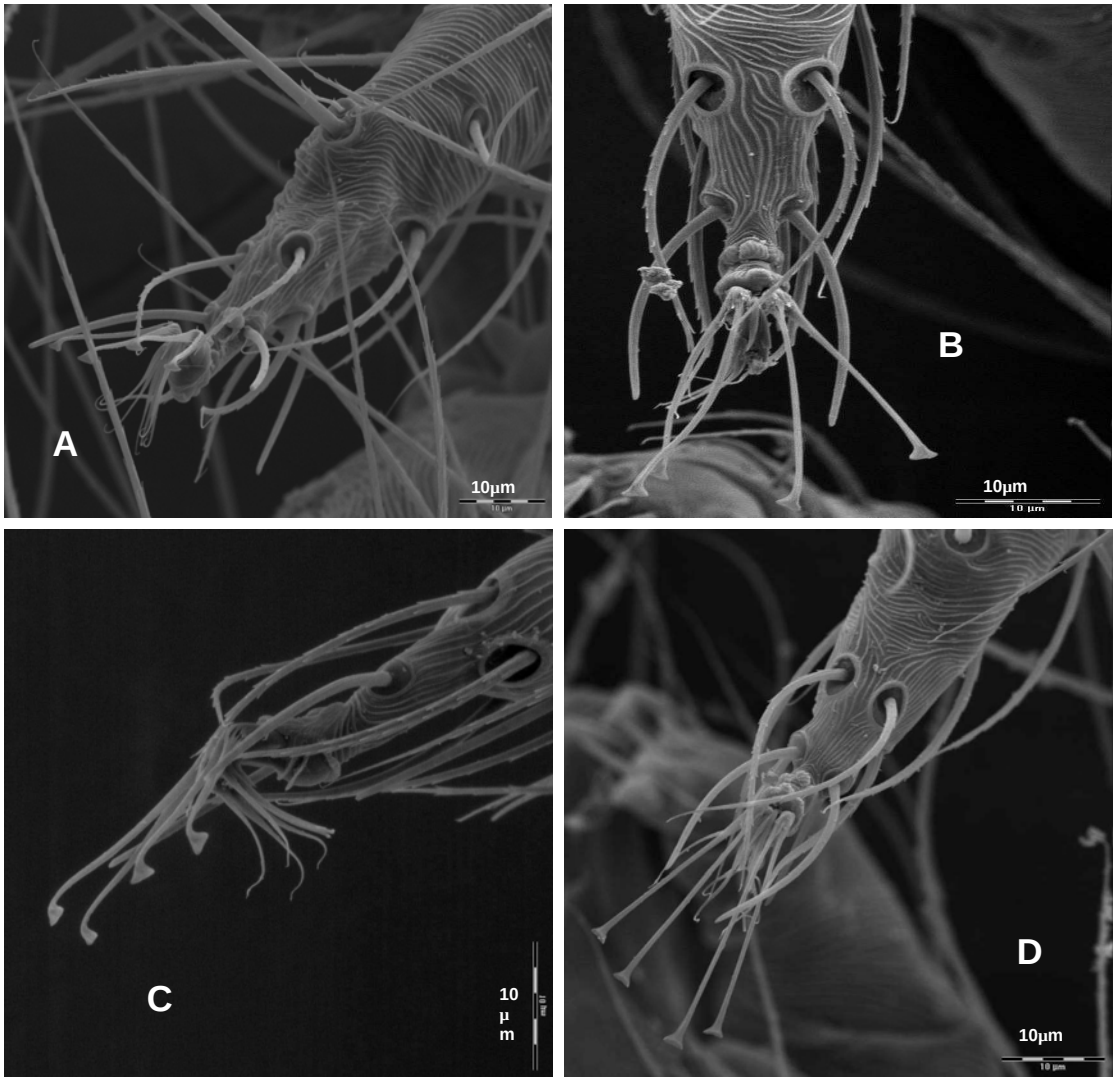

Figure 4.5: *Tetranychus.* Female pretarsi. (A) *T. evansi,* (B) *T. ludeni,* (C) *T. neocaledonicus,* (D) *T. urticae*.

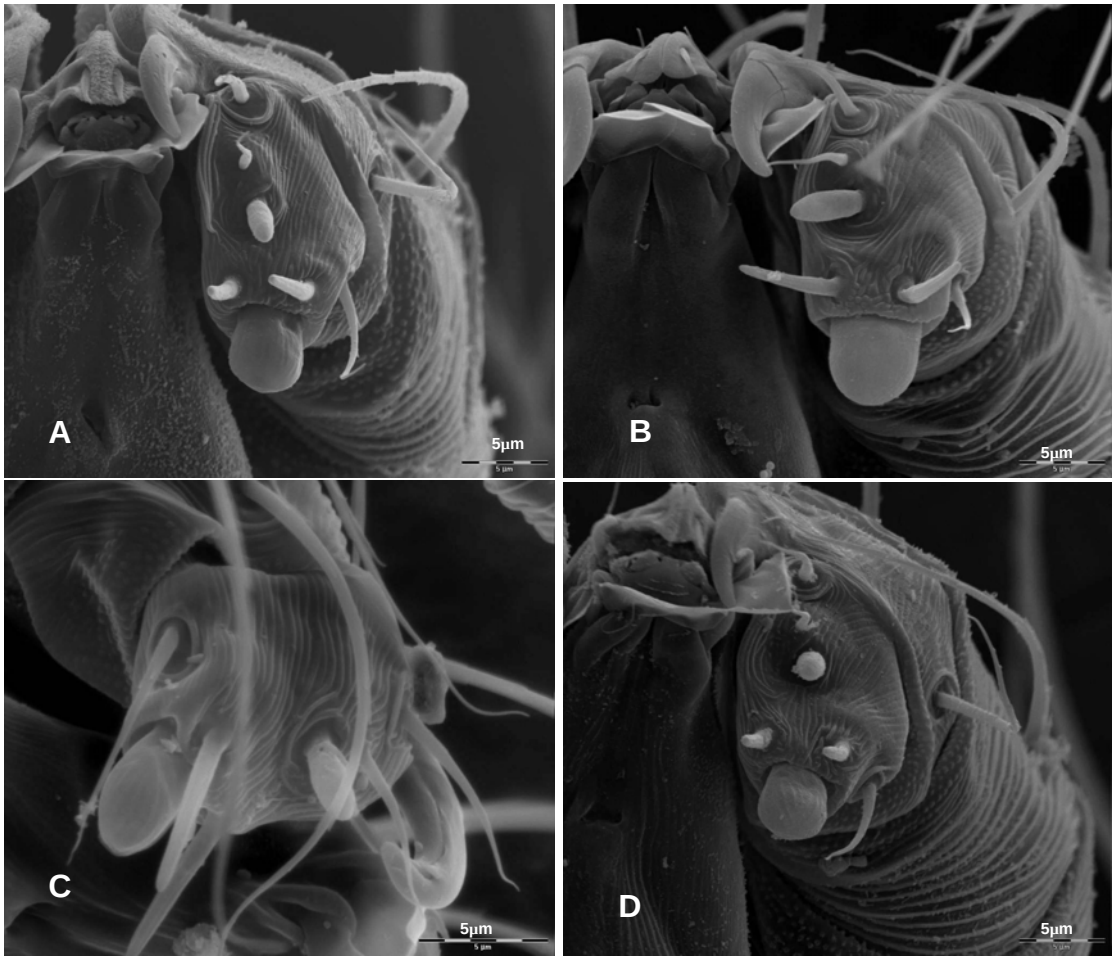

Figure 4.6: *Tetranychus.* Female palptarsi. (A) *T. evansi,* (B) *T. ludeni,* (C) *T. neocaledonicus,* (D) *T. urticae*.

CHAPTER 5: DIVERSITY OF TETRANYCHID MITES IN THE EASTERN AFRICAN BIODIVERSITY HOTSPOTS

5.1 Abstract

The diversity of plant inhabiting mites in the Eastern Arc Mountains and Coastal Forest Mosaic hotspots was evaluated. Species diversity in five fragments of this hotspot was compared. Diversity and abundance in cultivated habitats versus natural habitats were assessed and genetic diversity of the tetranychid species that ranked highest in abundance in these hotspots was evaluated. Species richness was found to be higher in natural habitats whereas species abundance was higher in cultivated habitats. The highest species diversity was observed in Arabuko Sokoke forest whereas diversity was lowest in Taita Hills.

Tetranychus evansi Baker & Pritchard (Acari: Tetranychidae) ranked highest in abundance amongst all the tetranychid species collected. The species was present in four out of the five fragments of the hotspot, and it survived in a wide range of altitudes from as low as 123 m to 1655 m. Molecular examination of *T. evansi* collected from Kenya and Tanzania and on different host plants revealed identical DNA sequences of the mitochondrial COI fragment and 19 microsatellite loci suggesting a single introduction of this species to East Africa.

5.2 Introduction

Definitions of biodiversity range in scope from "the number of different species in the some location" (Schwarz *et al*., 1976) to "all the diversity and variability in nature" (Spelleberg and Hardes, 1992) and "the variety of life and its processes" (De Long, 1996). Biodiversity studies usually encompass three domains: genetic diversity, species diversity and ecosystem diversity in a hierarchical order (Newmark, 2002).

The Easten Arc and Coastal forests of Tanzania and Kenya biodiversity hotspot (EACF) is home to 1,500 plant and 121 vertebrate species, which are endemic to this region (Myers *et al.*, 2000). This hotspot faces threats such as commercial cultivation of vegetables, which are sold in the domestic markets in urban centres like Dar es Salaam, Arusha, Mombasa and Nairobi, and from the growing of cardamom and other spices under forest cover. These activities result in forest clearance and the destruction of its undergrowth as well as pollution as a result of excessive use of agrochemical inputs, i.e fertilizers and pesticides. However, the livelihoods of many

of the people living in and around the hotspot strongly depend on this vegetable production. Many vegetables are ideal host plants for spider mites.

Apart from their agricultural importance, spider mites are a neglected taxon within the biodiversity of the hotspot, so little is known on their natural habitats and life history. Knowledge of the mite fauna in cultivated and uncultivated areas of the hotspot does not only contribute to understanding the biodiversity of the hotspot but also helps to design environmentally sound control strategies for spider mites damaging vegetables and therefore improving the livelihoods of the locals. Some spider mites also have earned the status of invasive alien species, e.g. the red spider mite *Tetranychus evansi* Baker and Pritchard, believed to have originated from South America (Gutierrez and Etienne, 1986). This pest was first recorded in continental Africa on tobacco in Zimbabwe in 1979 (Blair, 1983) from where it is believed to have spread to other parts of Africa. It was recorded in Kenya for the first time in 2001 on *Lycopersicon esculentum* in Mwea, Central Kenya (Knapp *et al*., 2003). It has also been reported in several regions of Southern Europe (Ferreira & Carmona, 1995; Ferragut & Escudero, 1999; Migeon, 2005; Castagnoli *et al*., 2006; Tsagkarakou *et al*., 2007). In Kenya, *Tetranychus evansi* has been reported on tomatoes from all the main tomato growing areas (Toroitich *et al*., 2009) and is considered the most important dry season pest on tomato production (Varela *et al.,* 2003). Control strategies for this pest are under investigation and, in particular, efforts to find a suitable biological control agent. To this end, in-depth understanding of the pest's biology is paramount. Previous studies on *T. evansi* show that this species can be effectively controlled by a phytoseiid species *Phytoseiules longipes* from Brazil (Furtado *et al*., 2007) whereas the same predator from South Africa was not effective for the control of this species (de Moraes & McMurty, 1985). This could be due to differences in the prey or the predator.

One valuable approach to the study of sources and introduction routes of invasive arthropods uses molecular markers (Solignac *et al.,* 2005). Evolutionary studies on mites have already surveyed the mitochondrial *Cytochrome Oxidase* subunit I (COI) gene and nuclear marker ITS region (Navajas & Fenton, 2000).

Moreover, recent studies have shown that microsatellite markers can be faithfully interpreted beyond population genetics and be used for studying phylogenetic

relationships of closely related species with fewer loci than previously assumed (Schlotterer, 2001).

This study was therefore designed to assess the species diversity of tetranychid mites in the EACF hotspot and to document the species diversity, identify the most important species and assess the genetic diversity of this species using mitochondrial COI and known microsatellite markers.

5.3 Materials and methods

5.3.1 Mites collection sites

The study covered five fragments of the East African Conservation hotspots namely Usambara and Uluguru mountains in Tanzania, and Taita Hills, Shimba Hills and Arabuko Sokoke forest in Kenya.

In Eastern Usambara, two regions were chosen, Kwa Bada location, Muheza district which represents fruit orchard and farmlands in the lowland altitude areas and higher altitude Amani nature reserve and the farms within the forested area.

In the Uluguru mountains area, sampling was done in the higher regions of Mgeta – Nyandira where temperate fruits and vegetables are grown and in the lower and warmer side of Matombo area where citrus fruit orchard are widespread and some vegetable production is done.

In Taita Hills, sampling was done in Chawea forest and the vegetable farms surrounding it. In Shimba Hills, sampling was done in the farms and natural vegetation within its environs and in Arabuko Sokoke, sampling was done in farms and natural vegetation around the forest (Fig. 5.1). Mites were collected from within forested areas where possible, forest margins and from farms surrounding the forested areas. The area name and the GPS coordinates of each sampling site were taken as well as the host plant of the mites.

5.3.2 Mite collection technique

Spider mites from randomly selected host plants were collected. A beating plate (tray) was placed under the plant and the plant is beaten with a stick. This ensured that in case few mites were present on the plant causing little or no symptoms, they do fall on the plate and were then picked using a fine brush and put directly into 70% ethanol in

small vials. In cases where symptoms were obvious, some leaves were picked and placed in paper bags and kept in a cooler box with ice cubes for transportation to the laboratory at ICIPE for analysis.

5.3.3 Preparation of specimen and mites identification

In the laboratory, the mites were left in the 70% ethanol for ten days for the purpose of clearing to dissolve the internal tissues (Craemer et al., 1998). An additional sample of the mites were preserved in 95% ethanol and kept in the fridge at 4°C for molecular studies. After ten days, the mites preserved in 70% ethanol were mounted in PVA mountant for identification. Identification was carried out under high power magnification of a Leica DMLB phase contrast microscope (Leitz Microsystems Germany). The mites were identified to species level where possible using the shape of the male aedeagus and the position of the duplex setae as the distinguishing characteristics as described by Craemer *et al*. (1998) and Meyer (1987), and the shape of the dorsal lobes and the variations in the number of the setae on tibia I in the female (Zhang & Jacobson, 2000). Other features that were used for identification include the distances between bases of the solenidia in the two duplex setae, the ratio between the length of setae and the distance to setae, length of the setae, distances between bases of genital setae and the ratio between length of subcapitular setae and the distance between their bases (Zhang & Jacobson, 2000).

The species richness, abundance and diversity between the different hotspots and habitats was determined using Biodiversity R statistical package (Kindt & Coe, 2005)

5.3.4 Collection of mites for molecular studies

The most commonly found tetranychid species for vegetable farmers around the EACF was identified as *Tetranychus evansi* and samples were taken for molecular studies. *Tetranychus evansi* populations were collected from seven localities in Kenya and three localities in Tanzania on a number of host plants from between November 2007 and April 2008. All the samples were collected from farms and the areas surrounding farms. From each sampling unit, one infested leaf was picked per plant for 50 plants. In situations, like for *Solanum incanum* from Mwea and *Solanum tuberosum* from Lushoto where the plants were less than fifty, one leaf was picked from different parts of the few plants sampled i.e. top, middle and bottom sections of

the same plant. The leaf samples were put in brown paper bags, kept in a cooler box with ice and taken to the laboratory at ICIPE where one female per leaf was picked and put in absolute alcohol in 2 ml vials. These were labelled and kept in the freezer at -20°C; a sample of fifty females per population was preserved. The origin and host plants details of the specimen analysed are provided in Table 5.3.

5.3.5. Molecular analysis of *Tetranychus evansi* from Kenya and Tanzania

This work was carried out at the Centre de Biologie Gestation Population (CBGP), Baillarguet, Montpellier, France. Total DNA was extracted from a whole, single female specimen using the DNeasy tissue kit (Qiagen, USA) following the Spin Column protocol for purification of total DNA from animal blood and cells. Manufacturer's instructions were modified for extraction of small mites as described by Navia *et al*. (2005). The main steps of the Qiagen protocol were followed except that all volumes were reduced by half: A single mite specimen was placed in an individual tube with a drop of 100% ethanol prior to DNA extraction. Excess ethanol was carefully removed from the tube and 90µl of PBS, 10µl of Proteinase K and 100µl of Buffer Al (Qiagen) were added. The tubes were homogenized by vortexing and placed in a water bath at 70°C for approximately two hours. The sample was then centrifuged for 3 minutes at 1300 revolutions per minute (rpm). Hundred microlitres of 100% ethanol was added in the sample and homogenized by vortexing. The mixture was deposited on a column DNeasy placed in a 2ml collection tube (provided with the kit). This was centrifuged for 1 min at 8000 rpm, the supernatant and collection tube were discarded and column placed in a new collection tube. Two hundred and fifty microlitres of Tampon AW1 (Qiagen) was added in the column, centrifuged for 1 minute at 8000 rpm and the supernatant and collection tube were discarded. The column was placed in a new collection tube, 250 µl of Tampon AW2 (Qiagen) was added, centrifuged for 3 minutes at 13000 rpm to dry the DNeasy membrane and the supernatant and collection tube were discarded.

The column was placed in a new collection tube and traces of buffer trapped on the column ring were eliminated with the aid of a cone P10. The tubes were centrifuged a new for 3 minutes at 13000 rpm, column was placed in another collection tube and 50µl ultra pure distilled water was put on the membrane to elute the DNA. This was left on ice for 10 minutes and centrifuged for 1 minute at 8000 rpm. The last step was

repeated by pipetting the solution obtained back onto the membrane to improve the quality of DNA obtained. This solution was used as template for PCR reactions.
Polymerase chain reaction (PCR) was used to amplify the 950 bp fragment of the mitochondrial gene *Cytochrome Oxidase I* (COI) using C1J1718 and CIOREVA primers (Table 5.2). The amplification reactions were carried out in a 50 µl volume containing 2 units of *Taq* polymerase, 1x enzyme buffer, 35pmoles of each primer, 5nmoles of dNTP, 75nmoles of $MgCl_2$ and 2 µl DNA as matrix in ITS2 amplification and 4 µl DNA for COI amplification. An initial denaturation step of 94ºC for 3 mins was followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 51ºC for 1 min and extension at 72ºC for 1 min 30s and a final elongation of 10min at 72ºC.

Microsatellites analysis was done using samples of five populations from Kenya namely KenES (Shimba Hills, *Lycopersicon esculentum*), KenW (Sindo, *Lycopersicon esculentum*), Ken-A3 (Athi-River, *Solanum melongena*), Ken-A4 (Athi-River, *Solanum scabrum)* and Ken-A5 (Athi-River, *Lycopersicon esculentum*) and two populations from Tanzania, TZE (Morogoro, *Solanum aethiopicum*) and TZ (Arusha, *Solanum scabrum*). These populations also represented mites collected on different host plants and from regions with the farthest geographical separation. Samples were initially denatured at 95ºC for 15 mins, and PCR was carried out for 40 cycles of 30s denaturation at 94ºC, 1 min 30s annealing at 57ºC and 1 min extension at 72ºC and a final elongation of 30min at 60ºC.
An average of 22 individuals per population was amplified using the microsatellite primers in table 5.3.
COI sequences obtained were edited using the Seqscape statistical package and were aligned using CLUSTAL W (Thompson *et al*., 1994).

5.4 Results

5.4.1 Plant inhabiting mite species diversity within the EACF hotspot

The total number of plant inhabiting mite species collected from different locations in the EACF varied. The Usambara Mountains recorded the largest number belonging to a total of nine families, whereas Shimba Hills had the least number of species, belonging to three families (Table 5.4).
Species belonging to the Eupalopsellidae and Eupodidae were collected from the Usambara Mts only, whereas those of Camerobiidae were collected from the Uluguru

Mountains only (Table 5.4). Mites belonging to the family Tetranychidae were collected from all hotspots. A total of nine tetranychid species (Table 5.4) were collected from the EAM and EACF. Out of these, four (*Eutetranychus orientalis, Eutetranychus africanus, Mixonychus acaciae* and *Tetranychus evansi*) are new records for Tanzania and one *Brevinychus meshacki* sp. nov. being new to science whereas two (*Duplanychus santiluciae* and *Eutetranychus africanus)* are new records for Kenya. Most tetranychids were collected from low altitude areas under 900m except three instances where *Tetranychus evansi* was collected at altitudes of 1376 m in Taita Hills upto 1655 m in Western Usambara.

Species richness of plant inhabiting mites as well as tetranychid mites was higher in natural habitat than in cultivated habitats (Figures 5.2 and 5.3) but species abundance was higher in cultivated habitats than in natural habitats (Figures 5.4 and 5.5).

Species diversity of plant inhabiting mites encountered was highest in Arabuko Sokoke and lowest in Shimba Hills (Table 5.5) whereas the diversity of tetranychid mites was highest in Shimba Hills and lowest in Taita Hills (Table 5.6).

In tetranychid mites rank abundance, *Tetranychus evansi* ranked as the highest in overall abundance accounting for 79.5% of all the tetranychid mites counted whereas *Duplanychus sanctiluciae* ranked the lowest accounting for an insignificant percentage less than 0.1% of all the (Table 5.7)

Eutetranychus orientalis were collected mainly in citrus orchards in low altitude areas of Usambara Mts and Shimba Hills but in numbers below economic threshold level. The damage was not visible and farmers did not complain about any losses caused by this pest. On the contrary, *T. evansi* was collected in four out of the five hotspots sampled and in all cases, it was found in very high numbers with an abundance of 1601 mites counted per plant. They were found attacking mainly vegetable farms and causing high losses even whole plant deaths.

5.4.2 Genetic diversity of *Tetranychus evansi* from Kenya and Tanzania

COI mitochondrial data:

From the 16 *T. evansi* populations analyzed, 32 COI sequences were obtained; 20 for Kenya and 12 for Tanzania. The sequences obtained were identical to each other. The sequence was 868 base pairs as follows:

TTTAATAATC AATACTATAG ATTTATGTTT TCCACGGATT AATAATATGA GTTTTTGATTTTTAATCCCT
TCACTTATAT TAATAATTAG AGCATCAATA AAAGGAGTTA TAAATGGGGTAGGATGAACA ATATATCCTC
CATTAACTTC AATTCAATAC
TTCATATCATCTTCAATTGAAATAATAATTTTTTCATTACATATTGCAGGAATTTCTTCAATTGCAAGATCAATTAA
TTTTATTTCAACT ATTTTATTAA TAAAAAATAA AAATTATTTT TTAAGAAATA TAACATTATTTACTTTATCT
ATTTTAATTA CTACATTTCT TCTTCTATTA GCTTTACCAG TTTTAGCAGGAGCTGTAACA ATAATTTTGA
TAGATCGAAA TTTTAATACT
TCTTTTTTTGACCCAAGAGGTGGAGGAGATCCTATTTTATATCAACATTTATTTTGATTTTTTGGGCACCCAGAAGT
TTATATTTTAATT TTACCAGGAT TTGGTATAAT TTCACATATT ATTAGATATA ATTTAGGAAAAAAAGAAGTT
TTTGGAAAGA TTGGAATAAT ATTTGCTATA ATATCAATTG GTTTATTAGGATTTATTGTT TGAGCACATC
ATATATTTAC AGTGGGGATA GATGTAGATA CACGAGCTTA TTTTACAGCA GCTACAATAA TTATTGCTAT
TCCAACAGGA ATTAAAATTT
TTAGATGATTTACTACTATCTTAAATTCACACATTAATTTTAGTATCTCAATATACTGATCTATAGGATTTTTAATTA
TA TTTTCAATTG GTGGATTTAC AGGAATTGTA GCTTCAAATT CATGTTTAGA TATTAATTTA CATGACACTT
ATTATATT

Furthermore, no sequence variations between mites from different host plants were observed.

Microsatellites data:

16 microsatellite loci were amplified for the six populations of *Tetranychus evansi* analyzed with a total of 115 individuals analyzed. Identical alleles were obtained for all the loci as shown in Table 5.8.

5.5 Discussion

Results show that tetranychid mites are found in a wide range of habitats in the hotspot but their diversity and abundance varied depending on the habitat. Species diversity was higher in natural habitats compared to the cultivated habitats whereas tetranychid abundance was greater in cultivated habitats. Species of *Tetranychus* were found in farms and in very high densities and the monoculture probably ensured constant supply of host plants for the mites. The absence of significant populations of predatory mites that could serve as biological control agents of *Tetranychus* in the farms could be due the monoculture or use of pesticides by farmers although this fact was not established in this study.. Furthermore, spider mites preferred warmer areas with lower altitudes except the case of *Tetranychus evansi* which was found even in higher altitude areas of Western Usambara (1655m) and Taita Hills at 1436 m and 1376 m. This could be attributed to the fact that *T. evansi* displays a high intrinsic rate of increase within a broad temperature spectrum compared to other members of the family as reported by Bonato (1999). Furthermore, *T. evansi* being an invasive species in Africa and parts of Europe (Boubou *et al*, 2010; Migeon *et al*., 2009; Toroitich *et al*., 2008) could be the reason for this occurrence since several studies to date have shown that founder effects and bottlenecks are not an obstacle for invasion success (Solignac *et al.,* 2005). In addition, plasticity in life history traits seems to be

important for the successful expansion of an invasive species (Chen *et al*., 2006; Valiente *et al*., 2010; Wang *et al*., 2005).

Molecular studies indicated that *Tetranychus evansi* in Kenya and Tanzania probably came from a single ancestry due to the high level of genetic similarity observed. It is probable that there was a single source of introduction of this species from the Southern African region and the species spread northwards to other countries (Keizer & Zuurbier, 1999) as the sequence obtained here is similar to the one from South African populations (Boubou *et al*., 2010). This is also supported by the following reports for Africa, which show a northwards trend (with all the caution of inconsistent sampling and incorrect identifications): Zimbabwe in 1979 (Blair, 1983), Reunion Island (Gutierrez & Etienne, 1986), South Africa (Smith Meyer, 1996), Congo (Bonato, 1999), Kenya (Knapp *et al.,* 2003), Tanzania (Toroitich *et al*., 2008). Such a case of a single introduction colonising a whole country has been reported for the invasive Argentine fire ant in New Zealand (Corin *et al*., 2007).

Furthermore, studies have shown that the reduction of genetic variability is a common feature of invasive species and introductions in general (Lande & Barrowclough, 1987; Roderick & Navajas, 2003; Solignac *et al.,* 2005). Colonizing populations of invasive species are usually founded by only a few individuals (Elton, 1958), causing random genetic drift known as founder effect (Lande & Barrowclough, 1987; Tsutsui *et al*., 2000).

Host plants did not affect the genetic composition of *T. evansi*. A similar trend was observed by Ros and Breeuwer (2007) who found no correlation between COI divergence and associated host plant species in *Tetranychus urticae* collected from plants belonging to 121 plant families. This aspect is important for pest management as this may allow the use of the similar management approaches on different host plants taking into consideration local adaptation facilitated by isolated genetic pools (for example pesticide resistance and host-plant adaptations) that can make certain populations of a pest more or less harmful to agriculture (Carbonelle *et al.,* 2007).

In conclusion, species diversity and richness was found to be high in natural undisturbed habitats but species abundance was generally low due to the presence of natural enemies whereas monoculture encourages the proliferation of pest species as well as reduction in species diversity. Habitat fragmentation leads to a reduction in biodiversity whereas genetic variability in invasive species is very low due to founder effect.

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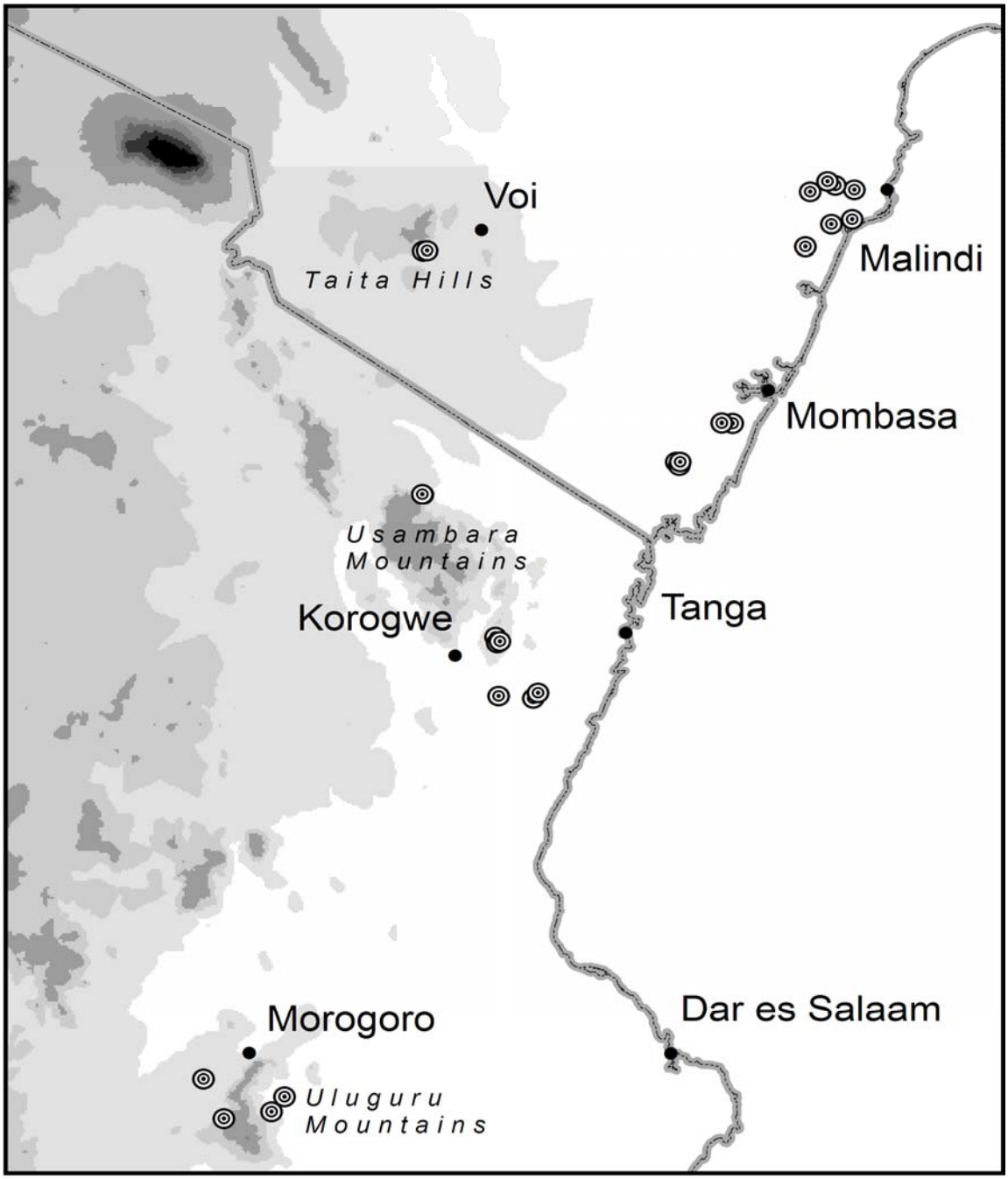

Figure 5.1: Map of EACF hotspots showing areas where mite collection was done.

Table 5.1: *Tetranychus evansi* collection sites.

Collection date	GPS	Elevation	Location	District	Host plant	Laboratory number
06/11/2007	S00°39.208'; E037°21.750'	1189	Mwea	Kirinyaga	*Solanum incanum*	K2
19/11/2007	S00°30.953'; E034°09.902'	1130	Sindo	Suba	*Lycopersicon esculentum*	K13
20/11/2007	S01°01.302'; E034°03.349'	1148	Muhuru-Bay	Migori	*Lycopersicon esculentum*	K15
28/11/2007	S01°25.137'; E037°00.953'	1489	Athi-River	Machakos	*Lycopersicon esculentum*	K4
28/11/2007	S01°23.460'; E037°02.552'	1503	Athi-River	Machakos	*Solanum melongena*	K3
03/12/2007	S01°23.460'; E037°02.552'	1503	Athi-River	Machakos	*Solanum scabrum*	K5
15/02/2008	S03°22.371'; E036°48.233'	1256	Tengeru	Arusha	*Solanum aethiopicum*	K10
15/02/2008	S03°22.371'; E036°48.233'	1256	Tengeru	Arusha	*Lycopersicon esculentum*	K7
15/02/2008	S03°22.371'; E036°48.233'	1256	Tengeru	Arusha	*Solanum scabrum*	K16
15/02/2008	S03°22.371'; E036°48.233'	1256	Tengeru	Arusha	*Datura stramonium*	K14
19/02/2008	S02°22.371'; E038°05.171'		Kibwezi	Makueni	*Lycopersicon esculentum*	K1
28/02/2008	S04°45.206'; E038°20.497'	1601	Baga	Lushoto	*Solanum tuberosum*	K12
20/02/2008	S04°20.846'; E039°18.808'	123	Shimba Hills	Kwale	*Lycopersicon esculentum*	K8
05/03/2008	S06°55.249'; E037°30.074'	591	Mukuyuni	Morogoro	*Solanum aethiopicum*	K9
06/04/2008	S03°28.242'; E038°20.577'	1436	Chawia	Taita	*Lycopersicon esculentum*	K6
06/04/2008	S03°28.187'; E038°21.554'	1376	Chawia	Taita	*Solanum scabrum*	K11

Table 5.2: PCR primers used for COI.

Primer name	Reference	Primer sequence	Annealing temperature (ºC)
C1J1718	Simon *et al*. 1994	5'-GGAGGATTTGGAAATTGATTAGTTCC-3'	51
COIREVA	Gotoh *et al*., 2009	5'-GATAAAACGTAATGAAAATGAGCTAC-3'	51

Table 5.3: PCR primers for microsatellites analysis (From Navajas *et.al*. unpublished.)

Primer name	Amount of template DNA used
7M1(C1+C46+C38+C22+C18+C23+C21)	2µl
C5F+C14Rb	2µl
C45	4µl
7M2(C9+C48+C40+C43+C34+C29)	2µl
C44	2µl
C4	2µl

Table 5.4: Table of plant inhabiting mites found in the EAM and EACF.

Hotspot	**Mites family**	**Species**	**Habitat**
Usambara	Ascidae	*Ascus sp.*	Natural
		Lasioseius sp.	Natural
	Bdelloidea	*Bdella* sp.	Natural
	Cheyletidae	*Chelacaropsis* sp.	Cultivated
		Hemicheyletia sp.	Natural
	Eupalopsellidae	*Exorthis caudata*	Cultivated
	Eupodidae	*Eupodes sigmoensis*	Natural
	Phytoseiidae	*Propriosiopsis* sp.	Natural
		Amblyseius sundi	Cultivated
		Amblyseius herbicolus	Natural
		Typhlodromus crassus	Natural
		Typhlodromus transvaalensis	Cultivated
	Stigmaeidae	*Agistemus* sp.	Natural
	Tetranychidae	*Eutetranychus africanus*	Natural
		Eutetranychus orientalis	Cultivated
		Oligonychus sp	Cultivated
		Tetranychus evansi	Cultivated
	Tydeidae	*Brachytydeus* sp.	Natural
Uluguru	Anystidae	*Anystis* sp.	Natural
	Camerobiidae	*Neophyllobius* sp	Natural
	Tetranychidae	*Brevinychus meshacki* sp.nov	Natural
		Eutetranychus africanus	Cultivated
		Mixonychus acaciae	Natural
		Oligonychus coffeae	Natural
		Oligonychus sp.	Cultivated
		Tetranychus evansi	Cultivated
	Tydeidae	*Tydeus grabousi*	Natural
		Tydeus grabousi	Cultivated
		Brachytydeus curiosa	Cultivated
		Brachytydeus sp.	Natural
Shimba Hills	Cheyletidae	*Hemicheyletia* sp.	Natural
	Tetranychidae	*Eutetranychus africanus*	Cultivated
		Eutetranychus africanus	Natural
		Eutetranychus orientalis	Cultivated
		Oligonychus sp	Natural
		Tetranychus evansi	Cultivated
		Tetranychus neocaledonicus	Cultivated
Arabuko Sokoke	Bdellidae	*Bdella* sp.	Cultivated
	Cunaxidae	*Cunaxa* sp.	Natural
	Phytoseiidae	*Ueckermannseius* sp.	Natural
	Stigmaeidae	*Agistemus* sp.	Natural
		Eustigmeius sp.	Cultivated
	Tenuipalpidae	*Raoiella indica*	Natural
		Raoiella macfarlanei	Natural
		Tenuipalpus sp.	Natural
	Tetranychidae	*Duplanychus sanctiluciae*	Natural
		Oligonychus gossypii	Natural
		Oligonychus sp.	Cultivated
		Tetranychus sp.	Cultivated
Taita Hills	Ascidae	*Lasioseius* sp.	Natural
	Phytoseiidae	*Amblyseius largoensis*	Natural
		Typhlodromus sp	Natural
	Tetranychidae	*Tetranychus evansi*	Cultivated

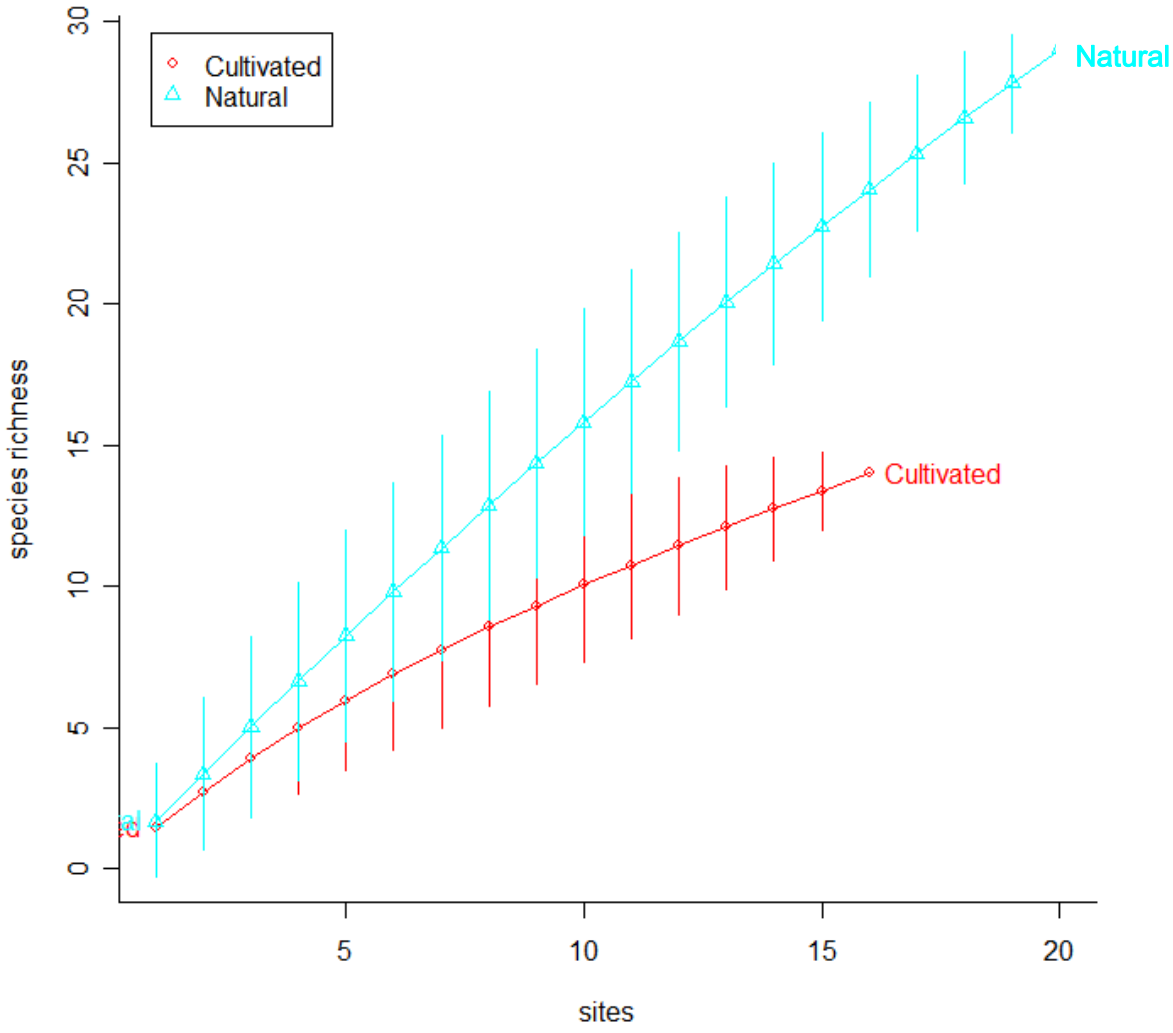

Figure 5.2: Graph showing species richness of all plant inhabiting mites between two natural and cultivated habitats in the East African Coastal Forests.

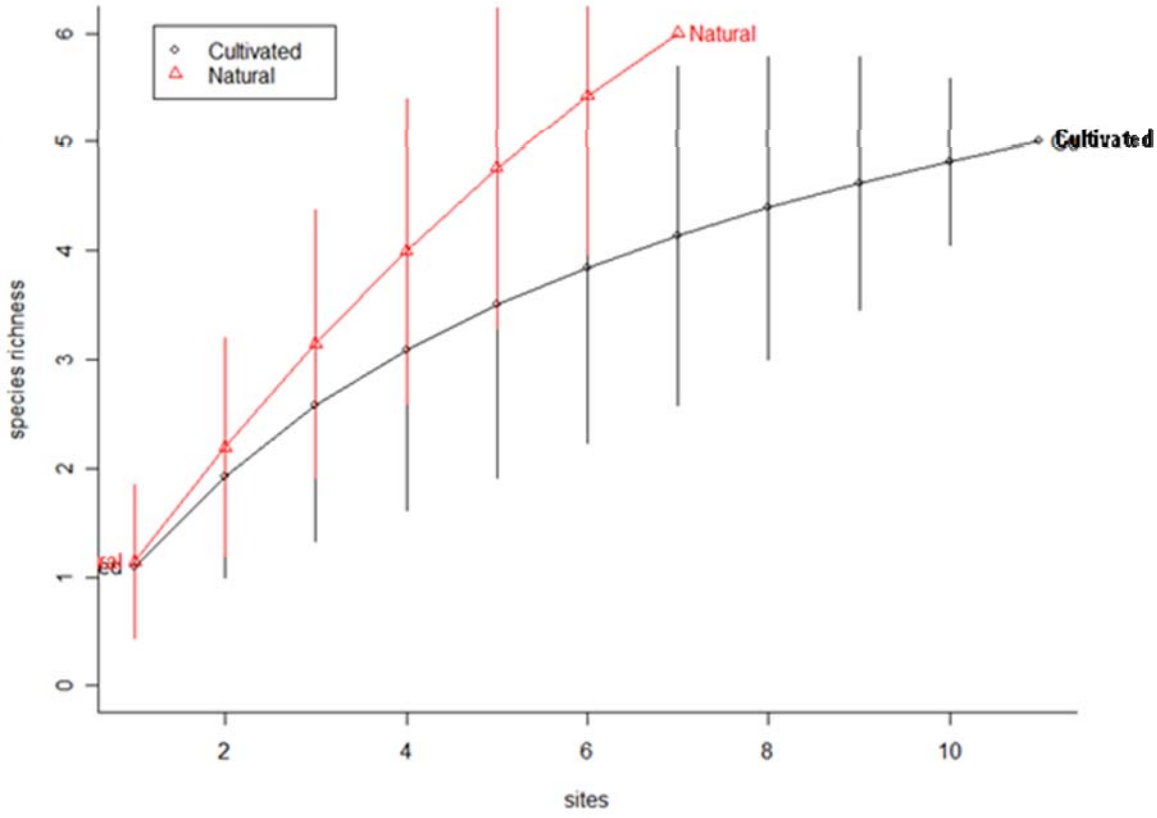

Figure 5.3: Graph showing the differences in tetranychid mites' species richness between cultivated and natural habitats in the East African Coastal Forests.

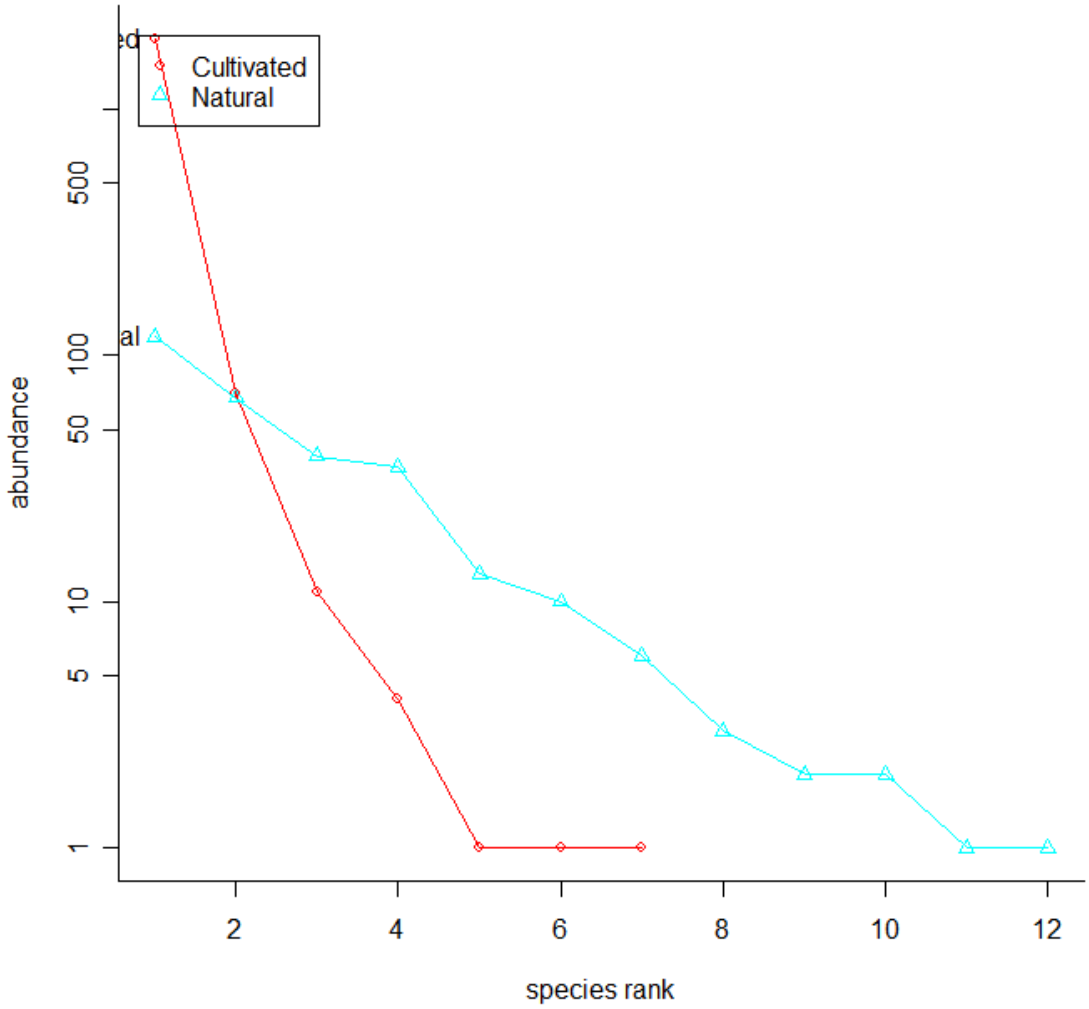

Figure 5.4: Graph showing the differences in plant inhabiting mites' species abundance between cultivated and natural habitats in the East African Coastal Forests.

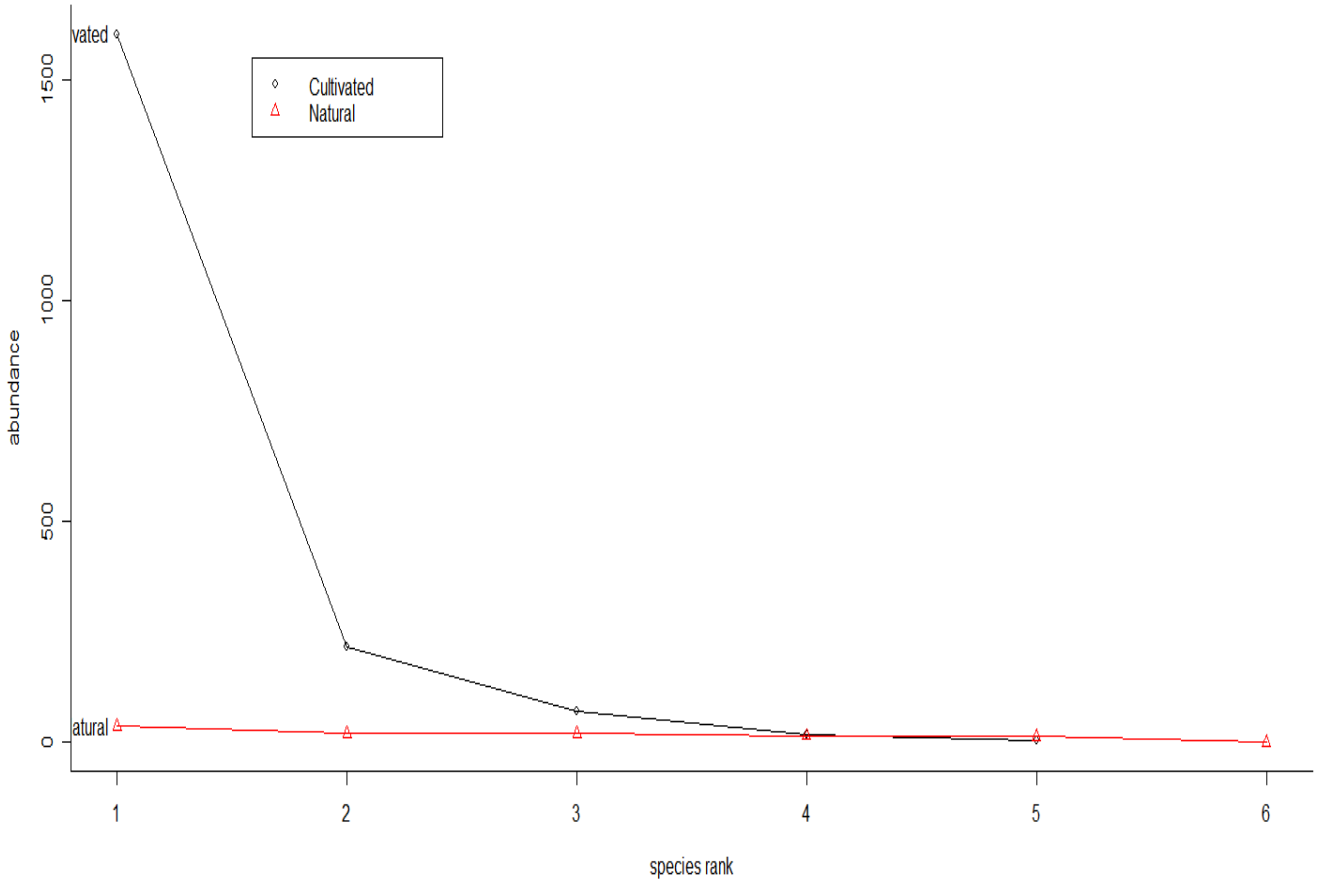

Figure 5.5: Graph showing the differences in species abundance of tetranychid mites between cultivated and natural habitats in the East African Coastal Forests.

Table 5.5 Diversity of all plant inhabiting mites in the different hotspots of the EACFM.

Hotspot	**N**	**Shannon**	**Simpson**	**Inverse Simpson**
Arabuko Sokoke	10	1.233	0.642	2.793
Shimba Hills	10	0.000	0.000	1.000
Taita Hills	10	0.123	0.046	1.048
Uluguru	10	0.584	0.366	1.577
Usambara	10	0.455	0.178	1.216

Table 5.6 Diversity of tetranychid mites in the different hotspots of the EACFM.

Hotspot	**N**	**Shannon**	**Simpson**	**Inverse Simpson**
Arabuko Sokoke	10	0.124	0.053	1.056
Shimba Hills	10	0.968	0.553	2.236
Taita Hills	10	0.000	0.000	1.000
Uluguru	10	0.510	0.227	1.294
Usambara	10	0.507	0.257	1.346

Table 5.7 Table of the rank abundance of the different tetranychid species in the EACFM.

Species	**Rank**	**Abundance**	**Logabundance**	**Proportion**
Tetranychus evansi	1	1601	3.2	79.5
Tetranychus neocaledonicus	2	216	2.3	10.7
Eutetranychus orientalis	3	72	1.9	3.6
Oligonychus gossypi	4	36	1.6	1.8
Eutetranychus africanus	5	29	1.5	1.4
Revinychus meshacki	6	21	1.3	1.0
Oligonychus sp.	7	20	1.3	1.0
Mixonychus acaciae	8	15	1.2	0.7
Oligonychus coffeae	9	4	0.6	0.2
Duplanychus sanctiluciae	10	1	0.0	0.0

Table 5.8: Distribution of allele frequencies of analysed *Tetranychus evansi* samples using microsatellite markers

Locus evaTC1-F5 = C1	Population	Alleles			Number of genes						
		118	124	126							
	KenES	1	0	0	24						
	KenW	1	0	0	20						
	TZ	1	0	0	42						
	TZE	1	0	0	38						
	Ken-A3	1	0	0	40						
	Ken-A5	1	0	0	34						
	Ken-A4	1	0	0	32						
Locus evaATCT1-H4 = C18	Population	Alleles		Number of genes							
		170	188								
	KenES	1	0	24							
	KenW	1	0	20							
	TZ	1	0	42							
	TZE	1	0	38							
	Ken-A3	1	0	40							
	Ken-A5	1	0	34							
	Ken-A4	1	0	28							
Locus evaTC1-A12 = C21	Population	Alleles									Number of genes
		211	213	215	219	221	228	230	232	239	
	KenES	0	0	0	0	0	0	1	0	0	24
	KenW	0	0	0	0	0	0	1	0	0	12
	TZ	0	0	0	0	0	0	1	0	0	42
	TZE	0	0	0	0	0	0	1	0	0	32
	Ken-A3	0	0	0	0	0	0	1	0	0	40
	Ken-A5	0	0	0	0	0	0	1	0	0	34

Table 5.8 (continued)…….

	Ken-A4	0	0	0	0	0	0	1	0	0	30
Locus evaTC1-H4 = C22	Population	Alleles		Number of genes							
		173	175								
	KenES	1	0	24							
	KenW	1	0	20							
	TZ	1	0	42							
	TZE	1	0	38							
	Ken-A3	1	0	40							
	Ken-A5	1	0	34							
	Ken-A4	1	0	30							
Locus evaTC5-E6 =C23	Population	Alleles		Number of genes							
		299	302								
	KenES	0	1	22							
	KenW	0	1	18							
	TZ	0	1	40							
	TZE	0	1	34							
	Ken-A3	0	1	40							
	Ken-A5	0	1	32							
	Ken-A4	0	1	30							
Locus evaTC2-G2 = C38	Population	Alleles									Number of genes
		178	180	182	184	186	188	190	196	198	
	KenES	0	0	0	0	0	0	0	1	0	22
	KenW	0	0	0	0	0	0	0	0.95	0.05	20
	TZ	0	0	0	0	0	0	0	0.976	0.024	42
	TZE	0	0	0	0	0	0	0	1	0	38
	Ken-A3	0	0	0	0	0	0	0	1	0	40

Table 5.8 (continued)

	Ken-A5	0	0	0	0	0	0	0	0.912	0.088	34
	Ken-A4	0	0	0	0	0	0	0	0.846	0.154	26
Locus evaTC2-A8 =C46	Population	Alleles					Number of genes				
		279	281	283	285	287					
	KenES	0	0	1	0	0	20				
	KenW	0	0	1	0	0	18				
	TZ	0	0	1	0	0	40				
	TZE	0	0	1	0	0	36				
	Ken-A3	0	0	1	0	0	36				
	Ken-A5	0	0	1	0	0	28				
	Ken-A4	0	0	1	0	0	22				
Locus evaTG2-A7 = C5F	Population	Allèles		Number of genes							
		283	285								
	KenES	0	1	24							
	KenW	0	1	16							
	TZ	0	1	42							
	TZE	0	1	36							
	Ken-A3	0	1	40							
	Ken-A5	0	1	32							
	Ken-A4	0	1	30							
Locus evaTC2-A2 = C29	Population	Alleles							Number of genes		
		167	169	170	172	174	176	180			
	KenES	0	0	0	1	0	0	0	22		
	KenW	0	0	0	1	0	0	0	20		
	TZ	0	0	0	1	0	0	0	42		
	TZE	0	0	0	1	0	0	0	38		

	Ken-A3	0	0	0	1	0	0	0	40			
	Ken-A5	0	0	0	1	0	0	0	34			
	Ken-A4	0	0	0	1	0	0	0	28			
Locus evaTC5-E3 = C34	Population	Alleles					Number of genes					
		300	305	308	314	316						
	KenES	0	0	0	0	1	24					
	KenW	0	0	0	0.056	0.944	18					
	TZ	0	0	0	0	1	40					
	TZE	0	0	0	0	1	34					
	Ken-A3	0	0	0	0	1	40					
	Ken-A5	0	0	0	0	1	34					
	Ken-A4	0	0	0	0	1	30					
Locus evaTG1-D9 = C4	Population	Alleles										Number of genes
		253	255	259	263	264	265	267	269	275	277	
	KenES	0	0	0	0.958	0	0.042	0	0	0	0	24
	KenW	0	0	0	1	0	0	0	0	0	0	18
	TZ	0	0	0	1	0	0	0	0	0	0	38
	TZE	0	0	0	1	0	0	0	0	0	0	38
	Ken-A3	0	0	0	1	0	0	0	0	0	0	40
	Ken-A5	0	0	0	1	0	0	0	0	0	0	34
	Ken-A4	0	0	0	1	0	0	0	0	0	0	26
Locus evaTC3-D3 = C40	Population	Alleles					Number of genes					
		105	116	118	122	124						
	KenES	0	0	1	0	0	24					
	KenW	0	0	1	0	0	18					
	TZ	0	0	1	0	0	42					
	TZE	0	0	1	0	0	38					
	Ken-A3	0	0	1	0	0	40					

Table 5.8 (continued)

	Ken-A5	0	0	1	0	0	34					
	Ken-A4	0	0	1	0	0	32					
Locus evaTC1-A8 =C43	Population	Alleles							Number of genes			
		229	234	236	240	242	243	246				
	KenES	0	0	0	1	0	0	0	24			
	KenW	0	0	0	1	0	0	0	20			
	TZ	0	0	0	1	0	0	0	40			
	TZE	0	0	0	1	0	0	0	38			
	Ken-A3	0	0	0	1	0	0	0	40			
	Ken-A5	0	0	0	1	0	0	0	34			
	Ken-A4	0	0	0	1	0	0	0	28			
Locus evaTC1-G11 = C44	Population	Allèles							Number of genes			
		299	301	318	324	326	328	334				
	KenES	1	0	0	0	0	0	0	24			
	KenW	1	0	0	0	0	0	0	16			
	TZ	1	0	0	0	0	0	0	40			
	TZE	1	0	0	0	0	0	0	38			
	Ken-A3	1	0	0	0	0	0	0	38			
	Ken-A5	1	0	0	0	0	0	0	32			
	Ken-A4	1	0	0	0	0	0	0	26			
Locus evaTC5-D2 = C48	Population	Alleles			Number of genes							
		225	227	229								
	KenES	0	1	0	20							
	KenW	0	1	0	20							
	TZ	0	1	0	42							
	TZE	0	1	0	38							

Table 5.8 (continued)

	Ken-A3	0	1	0	40					
	Ken-A5	0	1	0	34					
	Ken-A4	0	1	0	30					
Locus evaATCT2-G11= C9	Population	Alleles				Number of genes				
		121	127	130	146					
	KenES	0	1	0	0	24				
	KenW	0	1	0	0	20				
	TZ	0	1	0	0	42				
	TZE	0	1	0	0	38				
	Ken-A3	0	1	0	0	40				
	Ken-A5	0	1	0	0	34				
	Ken-A4	0	1	0	0	32				

Figure 5.6: An eggplant (*Solanum aethiopicum*) field in Mukuyuni area in Uluguru mountains environ heavily invested with *Tetranychus evansi*.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

The mites collection was done to determine the first species list of Tetranychidae in Kenya and Tanzania as well as determine the habitat use effects on tetranychid species composition in five fragments of the Eastern African biodiversity hotspot. Since agricultural systems are often discontinuous, i.e. the crop plant is not available through out the year but only in wet seasons, the pest needs a natural plant to overcome the seasons. In fact, Tetranychid mites' host range proved to be significantly wider than was known before this study. This includes *Oligonychus gossypii* on *Haplocoelum inoploeum, Bryobia praetiosa* on *Chloris gayana*, *Duplanychus sanctiluciae* on *Anacardium occidentale* among others. These species were mainly collected from plants outside the cultivated crops range that had not been previously sampled before this study. This highlights the fact that with more collections, more tetranychid mites may be reported and new species described, and so increasing the surprisingly low number of species found outside South Africa, which undoubtedly is a clear sampling artefact. The two new species described during this study *Brevinychus meshacki* sp. nov. and *Schizotetranychus kwalensis* sp. nov. were both collected on uncultivated plants.

Quite a number of tetranychid species were found in cultivated crops, notably, mites from the genus *Tetranychus* were mostly found in vegetable farms with the exception of *Tetranychus neocaledonicus* which was found in large densities on cassava crops. This observation is consistent with earlier reports that this genus is one of the most important pests in agriculture (Smith Meyer, 1996). Members of *Eutetranychus* were abundant on citrus trees in all the areas sampled and on some non-cultivated plants. The species *Euteranychus orientalis* was particularly abundant in citrus orchards and this species has been recorded all over the Afrotropical region on citrus trees. However, the economic importance of this species has not been given as much attention as *Eutetranychus banksi* which occurs in the USA (Baker & Tuttle, 1994). Due to the abundant nature of *E. orientalis* and its potential as a citrus pest, biological studies for this species need to be carried out, examining its life cycle and biological control agents. *Mononychellus progressivus* was exclusively found on cassava plants from this study and previous records, indicating a specialisation to this plant. This pest (possibly of South American origin, as is cassava) is also widespread in the

tropics causing major economic losses on this important staple food in tropical Africa (Girling, *et al*., 1978, Nyiira, *et al*., 1982,). *Oligonychus coffeae* was found on mango trees but in very low numbers such that no damage symptoms were visible. The origin of this species is unclear.

Quite apart from its economic significance in agriculture, no identifcation key(s) for all life stages of the species in the genus *Tetranychus* has been developed. Inferring from the scanning electron microscope images, the spider mites divide into two groups. *Tetranychus evansi* and *T. ludeni* group together, the only difference between them being the dorsal lobes that have pointed, almost triangular tips for *T. ludeni* as opposed to those of *T. evansi* and *T. neocaledonicus* that are circular at the tip and cup shaped. These species had earlier been grouped together in the *desertorum* group proposed by Pritchard and Baker (1955). Flechtmann and Knihinicki (2002) also put these species in *Tetranychus* group 5 based on having all four proximal tactile setae in line with proximal pair of duplex setae on tarsus I and dorsomedian empodial spur tiny or absent.

Tetranychus neocaledonicus and *T. urticae* group together and their only differences lie in the dorsal lobes which appear faint in *T. urticae* but strong and cup-shaped in *T. neocaledonicus* and the pre-genital striations which appear longitudinal in *T. urticae* but transverse in *T. neocaledonicus*. These species belong to group 9 according to Flechtmann and Knihinicki (2002) which comprise many species of economic importance. Moreover, differences in the distance between the duplex setae of the *desertorum* group (*T. evansi* and *T. ludeni*) and group nine mites (*T. neocaledonicus* and *T. urticae*) were observed.

Based on these observations, it can be concluded that female characters can be used to separate between species of *Tetranychus* to some degree although the differences are subtle and often times hard to visualize and may not be sufficient to allow accurate identification of species, thus until more characters are identified, males may still be needed to confirm species identity in this genus. The groupings of the species according to Flechtmann and Knihinicki (2002) were confirmed by this study based on the study of members of group 5 and 9, and some additional differences between groups 5 and 9 that were not included in earlier keys were noted.

The diverse and fragmented nature of the habitats in the East African Arc and Coastal Forests hotspot allows for a great diversity of tetranychid species but their diversity and abundance varied significantly depending on the specific habitat. Species diversity was higher in natural habitats compared to the cultivated habitats whereas tetranychid mites' abundance was greater in cultivated habitats. Species of *Tetranychus* were found on farms in very high densities and the monoculture probably ensured constant supply of host plants for the mites.

The potential biocontrol agents, the predatory mites appears to have a different habitat preference or altitudinal range than that of *Tetranychus.* Few members of Stigmaeidae and Cheyletidae were found in the lower altitude areas but in very few numbers in farms. This could be attributed to the fact that most invertebrate natural enemies prefer complex structured natural habitats (Langelloto & Denno, 2004). Phytoseiid mites preferred the cooler and higher altitude areas as was observed in this study whereas the tetranychid mites preferred warmer areas with lower altitudes except the case of *Tetranychus evansi* which was found even in higher altitude areas of Western Usambara (1655m) and Taita Hills at 1436 m and 1376 m. This could be attributed to the fact that *T. evansi* displays a high intrinsic rate of increase within a broad range of temperatures compared to other members of the family as reported by Bonato (1999). Furthermore, *T. evansi* being an invasive species in Africa, Europe and parts of Asia(Boubou *et al*., 2010; Migeon *et al*., 2009; Toroitich *et al*., 2008) has experienced the founder effect, i.e. a reduction in the gene pool, which could reduce adaptability of species to new environment since a great number of alleles are missing. However, several studies to date have shown that founder effects and bottlenecks are not an obstacle for invasion success (Solignac *et al.,* 2005) and the great number of invasive alien species confirms these studies.

The interesting gap in the altitudinal range in predator and prey might increase further with climate change since the cooler regions will decrease and move down further in altidude moving potential biocontrol agents further away from its potential prey *Tetranychus*. The latter possibly follows the warmer temperature to high altitudes threatening crops in these regions, which were so far uninfested.

Before this study, it was unclear how many times *Tetranychus evansi* was introduced in Kenya and Tanzania. There could be several independent introductions for each or in each country, or just one for the whole region, distributed through formal and

informal trade in the region, possibly by wind as well. Molecular studies carried out here indicate that *Tetranychus evansi* in Kenya and Tanzania probably came from a single ancestry due to the high level of genetic similarity observed. Hence, it is probable that there was a single source of introduction of this species to this part of East Africa. Despite founder and bottle neck effects, cases of a single introduction leading to colonisation of a whole country has been reported before, e.g. for the invasive Argentine fire ant in New Zealand (Corin *et al*., 2007).

Furthermore, studies have shown that the reduction of genetic variability is a common feature of invasive species and introductions in general (Lande & Barrowclough, 1987; Roderick & Navajas, 2003; Solignac *et al*., 2005). From the samples analyzed, host plant effect on the genetic diversity of *T. evansi* was not observed. A similar trend was observed by Ros and Breeuwer (2007) who found no correlation between COI divergence and associated host plant species in *Tetranychus urticae* collected from plants belonging to 121 plant families. This aspect is important for pest management strategies as this probably allows the use of the same management approaches for the control of this mite on different host plants However, local adaptation facilitated by isolated genetic pools (for example pesticide resistance and host-plant adaptations) are some of the traits that can make certain populations of a pest more or less harmful to agriculture (Carbonelle *et al*., 2007).

In conclusion, the expected outcomes of mite species and other arthropods in agricultural and natural systems are confirmed. Species diversity and richness is high in natural and undisturbed habitats but species abundance is generally low due to the presence of natural enemies whereas monoculture encourages the proliferation of pest species as well as reduction in species diversity. The genetic variability in invasive species is very low due to founder effect, but this does not always hamper distribution. Moreover, the availability of food and the lack of predators and other natural enemies present in the home range but not in the new range can make the species more harmful to agriculture in the regions of its introduction than in the regions of origin.

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