Taxonomical study of predatory and plant-parasitic mites associated with South African Solanaceae

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PROJECT COLLABORATION

This project is part of a collaborative project (**Table 1**) supported by three universities [Ankara University (**AU**) and Uludağ University (**UU**) in Turkey, and North-West University (**NWU**) in South Africa] and two institutes [French National Institute for Agricultural Research (**INRA**), the Centre for Biology and Management of Populations (**CBGP**) in Montpellier France, and Agricultural Research Council, Plant Protection Research Institute (**ARC-PPRI**) in Roodeplaat South Africa] that will enable integration of all discrimination methods. This exchange project will enable the building of capacity through the preparation of young scientists about identification of common plant parasitic and predatory mite species using **new** (**molecular** and **SEM**) together with **classical** methods. The proposed exchange programme is considered to be an opportunity of developing new discriminating tools for pest and predatory mite species with knowledge transfer among European (Turkish and French) and South African scientists. This project carries out a clear application and enables the transfer of expertise from science into practice.

Full Title of Collaborative Project: Detection and analysis of inter- and intra-specific variability of common pest and predatory mites using new molecular and imaging tools.

Table 1. Partner List

Partner Number	Partner Name	Partner Short Name	Country
1 Co-ordinator	Ankara University, Plant Protection Department	AU	Turkey
2 Partner	Uludağ University, Plant Protection Department	UU	Turkey
3 Partner	French National Institute for Agricultural Research, the Centre for Biological and Management of Populations	INRA	France
4 Partner	Agricultural Research Council, Plant Protection Research Institute	ARC	South Africa
5 Partner	North-West University	NWU	South Africa

ABSTRACT

Plant-feeding mites represent major pests in agriculture that are of importance to crops world-wide, as large populations of mites reduce the quality and quantity of yields. Alternatives to the use of pesticides are needed due to their negative effects and bio-control agents (predatory mites) remain advantages as they suppress spider mites and other plant pests. This study aims to determine species status of plant-feeding and predatory mites on plants of the family Solanaceae and to apply morphological and molecular data to determine phylogenetic relationships among economically important Phytoseiidae, Stigmaeidae and Tetranychidae. The material for this study was collected through plant beating and specimens were preserved in 75% and 96% ethanol respectively and mounted in Heinz's PVA medium on microscope slides. A survey was conducted during peak seasons to provide enough samples of pest and predatory species. Morphological analysis was performed and initial results indicate that 94% of the species identified were parasitic and 6% were predatory, which led to a predator:prey ratio of 1:17, where *Tetranychus evansi* Baker & Pritchard had the highest frequency of appearance. A modified Qiagen DNeasy tissue kit extraction protocol was used and Polymerase Chain Reaction was performed to amplify ribosomal ITS and mitochondrial *COI* gene fragments. The nucleotide sequence of a 700-bp fragment for ITS was determined by direct sequencing as well as for a 700-bp and 800-bp fragments for *COI*. The resulting data included 4 isolates that corresponded morphologically and molecularly with Phytoseiidae and 10 with Stigmaeidae. The phylogenetic trees agreed with the morphological data. For species that lack morphological descriptions in GenBank and are not placed within expected clades, one has to accept the possibility of miss identification and highlights the need to combine morphological and molecular approaches to guarantee solid species diagnosis. Ultimately, Solanaceae contain various parasitic mites, but predators seem low in numbers. This could be problamatic in finding effective bio-control agents.

Key words: Spider Mites, Predaceous Mites, Species identification, Tetranychidae, Phytoseidae, Stigmaeidae, Internal Transcribed Spacers (ITS), Cytocrome *c* Oxidase I (COI), Phylogenetic relationships.

OPSOMMING

Plantvoedende myte verteenwoordig plae in landbou wat belangrik is vir gewasse wêreldwyd, aangesien myte die kwaliteit en kwantiteit van die opbrengs kan verlaag. Alternatiewe vir plaagdoders is nodig as gevolg van hul negatiewe invloede; en biologiese beheermetodes (predatoriese myte) bly voordelig aangesien hulle spinmyte en ander peste onderdruk. Die studie se doelwit was om die status van plantvoedende en predatoriese spesies op Solanaceae vas te stel en morfologiese en molekulêre data te gebruik om filogenetiese verwantskappe tussen ekonomies belangrike Phytoseiidae, Stigmaeidae en Tetranychidae te bepaal. Die studiemateriaal is versamel deur plantklopping. Monsters is bewaar in 75% en 96% etanol onderskeidelik en gemonteer in Heinz se PVA-medium op mikroskoopplaatjies. Opnames is tydens piekseisoene gedoen om te verseker dat genoeg parasitiese en roofmyte versamel word. Morfologiese analises dui aan dat 94% van die geïdentifiseerde spesies parasities was en 6% predatories. Die bévinding het gelei tot 'n roofmyt:plaag verhouding van 1:17, waarvan *Tetranychus evansi* Baker & Pritchard die hoogste voorkomsfrekwensie gehad het. 'n Aangepaste ekstraheringsmetode met die Qiagen DNeasy weefselstel is gebruik en daarna die polimerase kettingreaksie om die ribosomale ITS en die mitochondriale *COI* geenfragmente te isoleer en te vermeerder. Die nukleotiedvolgorde van ’n 700-bp fragment van die ITS geenfragment is bepaal deur direkte volgordebepaling en 700-bp en 800-bp vir die *COI* geenfragment. Die gevolglike data sluit 4 isolate wat morfologies en molekulêr ooreengestem het met die Phytoseiidae en 10 met die Stigmaeidae in. Die filogenetiese bome het ooreengestem met die morfologiese data. Spesies gelys in GenBank, waarvan geen morfologiese beskrywings by gedoen is nie en nie in verwagte klades geplaas kan word nie, is moontlik misgeïdentifiseer en dit beklemtoon die noodsaaklikheid om morfologiese en molekulêre benaderings te kombineer om korrekte spesiesdiagnoses te verseker. Solanaceae bevat 'n verskeidenheid plantvoedende myte, maar predatore is grotendeels afwesig. Dit kan problematies wees vir die soektog na effektiewe biologiese beheermetodes.

Sleutelwoorde: Spinmyte, Predatoriese myte, Identifisering van spesies, Tetranychidae, Phytoseidae, Stigmaeidae, Interne getranskribeerde gaping DNS (ITS), Sitochroom *c* oksidase I (*COI*), Filogenetiese verwantskappe.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS....i
PROJECT COLLABORATION....ii
ABSTRACT....iii
OPSOMMING....iv
LIST OF TABLES....vii
LIST OF FIGURES....viii
CHAPTER 1: INTRODUCTION....1
1.1 Introduction to mites....2
1.2 Aims and Objectives....4
1.3 Hypothesis....5
CHAPTER 2: LITERATURE REVIEW....6
2.1 Introduction to Acari and their relationship with other Arachnida....7
2.2 Order Prostigmata....11
2.3 Order Mesostigmata. 21
2.4 Mites as plant parasites....31
2.5 Adaptation to Predation and the need for biological control....36
2.6 Mite taxonomy and identification....40
2.7 Introduction into molecular phylogeny of Acari....45
2.8 Mite genetic structure....46
2.9 Gene flow, speciation and molecular divergence....50
2.10 DNA barcoding....52
SECTION A: MORPHOLOGICAL REVIEW....54
CHAPTER 3: MATERIALS and METHODS....55
3.1 Collecting and mounting methods....56
3.2 Ethics statement....56
3.3 General handling of plant inhabiting mites....56
3.4 Collecting in the field....57
3.5 Mounting....57
3.6 Labelling....58

TABLE OF CONTENTS (Continued)

3.7 Mites mounted on slides....58
3.8 Biological sample preparation for SEM....58
3.9 Identification keys....59
CHAPTER 4: RESULTS and DISCUSSIONS....60
4.1 Collection sites....61
4.2 Species identification based on light microscopy....63
4.3 Morphological comparison between the two major pest mites based on SEM work....82
4.4 Morphological review of predatory species....94
SECTION B: MOLECULAR REVIEW....136
CHAPTER 5: MATERIALS and METHODS....137
5.1 Molecular based identification....138
5.2 DNA extraction....138
5.3 Amount of DNA....140
5.4 PCR amplification and sequencing....141
5.5 DNA sequences and sequence analysis....143
5.6 Sequence retrieval and dataset....143
5.7 Sequence and phylogenetic analysis....146
CHAPTER 6: RESULTS and DISCUSSIONS....148
6.1 RESULTS....149
6.1.1 DNA extraxtion, quantity and quality....149
6.1.2 PCR amplification and sequencing....150
6.1.3 Sequencing analyses....152
6.1.4 Phylogenetic analyses of the Phytoseiidae isolates....155
6.1.5 Phylogenetic analyses of the Stigmaeidae isolates....161
6.2 DISCUSSION....165
6.2.1 DNA extraction....165
6.2.2 PCR and sequencing analyses....166
6.2.3 Phylogenetic analyses....167
CHAPTER 7: CONCLUSION....171
CHAPTER 8: REFERENCES....174

LIST OF TABLES

Table 1 Partner List.....ii
Table 2.1 A comparison between extant Arthropoda's frontal segmental composition.....7
Table 2.2 Mite classification.....8
Table 2.3 The subclass Acari with its ordinal classification.....10
Table 2.4 Orders of Plant Mites.....41
Table 4.1 Recording of the presence or absence of parasitic and predatory families on solanaceous species.....63
Table 4.2 Parasitic/Predaceous mite infestation and density.....66
Table 4.3 Pest species found on Solanaceae.....69
Table 4.4 Differences between two major pests found in the family Tetranychidae.....75
Table 4.5 Characteristics of predatory mite species found on Solanaceae during survey.....79
Table 5.1 Mites identified for the extraction of DNA.....138
Table 5.2 PCR and sequencing primers used to obtain nuclear ribosomal ITS and mitochondrial *COI* sequences of mites during this survey.....141
Table 5.3 Specimens and species considered and their accession numbers in the NCBI GenBank.....144
Table 6.1 Quantity and quality of (ng/mite) of DNA extracts of Phytoseiidae, Tetranychidae and Tydeidae.....149
Table 6.2 Similarity results of the amplified sequences.....153

LIST OF FIGURES

Figure 2.1 External structures of a generalized prostigmatic mite in dorsal view.....11
Figure 2.2 External structures of a generalized prostigmatic mite in ventral view.....11
Figure 2.3 Gnathosoma of prostigmatic mite.....12
Figure 2.4 Side view of gnathosoma.....12
Figure 2.5 Chelicera.....12
Figure 2.6 Anterior part of the idiosoma.....13
Figure 2.7 Dorsal view of tydeid mite (Prostigmata: Tydeidae).....18
Figure 2.8 Ventral view (left) and dorsal view (right) of tetranychid mite (Prostigmata: Tetranychidae.....19
Figure 2.9 Tibia and tarsus I of a tetranychid mite (Prostigmata: Tetranychidae).....20
Figure 2.10 Dorsal view of stigmaeid mite (Prostigmata: Stigmaeidae).....20
Figure 2.11 Diagrammatic representation of a gamasine mite in ventral view.....23
Figure 2.12 Diagrammatic representation of the gnathosoma of a mesostigmatic mite.....24
Figure 2.13 Ventral view of the gnathosoma of a dermassoid mite.....24
Figure 2.14 Venter of the chelicera of a gamasine mite.....25
Figure 2.15 Life cycle of mites.....35
Figure 2.16 Life cycle of spider mites.....35
Figure 2.17 Dorso-ventral view of mite female to reveal general features of mites.....42
Figure 2.18 Schematic representation of ribosomal genes containing the areas targeted by the ITS primer.....49
Figure 4.1 Sampling locations throughout survey.....61
Figure 4.2 Sampling area in the North-West Province.....62
Figure 4.3 Sampling area in the North-West Province.....62
Figure 4.4 Sampling area in the Gauteng Province.....62
Figure 4.5 Sampling area in the Gauteng Province.....62
Figure 4.6 Sampling area in the Gauteng Province.....62
Figure 4.7 Sampling area in Kwa-Zulu Natal.....62
Figure 4.8 Comparative percentages of the abundance of species.....68
Figure 4.9 Dorsal view of *T. evansi* female.....83
Figure 4.10 Dorsal view of *T. urticae* Koch female.....83
Figure 4.11 *T. evansi* dorsum of opisthosoma of female showing striation patterns.....83

LIST OF FIGURES (Continued)

Figure 4.12. *T. urticae* dorsum of opisthosoma of female showing striation patterns.....83
Figure 4.13 Anterior view of *T. evansi* female.....84
Figure 4.14 Anterior view of *T. urticae* female.....84
Figure 4.15 *T. evansi* left pair of eyes.....84
Figure 4.16 *T. urticae* left pair of eyes.....84
Figure 4.17 *T. evansi* palptarus of female.....85
Figure 4.18 *T. urticae* palptarus of female.....85
Figure 4.19 *T. evansi* extruded stylophore showing the peritreme.....86
Figure 4.20 *T. urticae* extruded stylophore showing the peritreme.....86
Figure 4.21 *T. evansi* cheliceral stylets/chelicerae.....87
Figure 4.22 *T. urticae* cheliceral stylets/chelicerae.....87
Figure 4.23 *T. evansi* tarsal appendages of tarsus I of female.....87
Figure 4.24 *T. urticae* tarsal appendages of tarsus I of female.....87
Figure 4.25 *T. evansi* tibia and tarsus I of female.....88
Figure 4.26 *T. urticae* tibia and tarsus I of female.....88
Figure 4.27 *T. evansi* aedeagus of male.....89
Figure 4.28 *T. urticae* aedeagus of male.....89
Figure 4.29 Dorsal view of *T. evansi* male (left) and female (right) to show the difference between the sexes.....89
Figure 4.30 Female *Tetranychus* sp. (Acari: Tetranychidae) on *Solanum* sp. (Solanaceae) leaf.....90
Figure 4.31 *Tetranychus* sp. on leaf casting its old exoskeleton.....90
Figure 4.32 *Tetranychus* sp. hatching from egg.....90
Figure 4.33 *Tetranychus* sp. (Acari: Tetranychidae) on leaf next to a dust particle (corner right).....91
Figure 4.34 Microhabitat of spider mite colony showing spider mites (*Tetranychus* sp.) and their eggs on a *Solanum* sp. leaf.....91
Figure 4.35 Linear palps of Tenuipalpidae sp.....92
Figure 4.36 Tenuipalpidae palps.....92
Figure 4.37 Female genital aperature "transverse" of tenuipalpid mite, often covered by a plate.....93

LIST OF FIGURES (Continued)

Figure 4.38 Predatory mites (Mesostigmata: Phytoseiidae).....93
Figure 4.39 Predatory mite (Prostigmata: Tydeidae).....94
Figure 4.40 Differences between three subfamilies of the family Phytoseiidae.....95
Figure 4.41 *Amblyseius (Amblyseius) pretoriaensis* Ueckermann & Loots excoriate ventral view.....98
Figure 4.42 Dorsal view of *Amblyseius (Amblyseius) pretoriaensis* female.....100
Figure 4.43 Ventral view of *Amblyseius (Amblyseius) pretoriaensis* female.....100
Figure 4.44 Veriations in spermatheca of *Amblyseius (Amblyseius) pretoriaensis* female..101
Figure 4.45 Chelicera of *Amblyseius (Amblyseius) pretoriaensis* female.....102
Figure 4.46 Hypostome of *Amblyseius (Amblyseius) pretoriaensis* female.....102
Figure 4.47 Anterior margin of tectum of *Amblyseius (Amblyseius) pretoriaensis* female..103
Figure 4.48 Tarsus I of *Amblyseius (Amblyseius) pretoriaensis* female.....103
Figure 4.49 Genu II of *Amblyseius (Amblyseius) pretoriaensis* female.....104
Figure 4.50 Genu and tibia II of *Amblyseius (Amblyseius) pretoriaensis* female.....104
Figure 4.51 Leg IV of *Amblyseius (Amblyseius) pretoriaensis* female.....105
Figure 4.52 Dorsal view of *Amblyseius (Amblyseius) pretoriaensis* deutonymph.....105
Figure 4.53 Ventral view of *Amblyseius (Amblyseius) pretoriaensis* deutonymph.....106
Figure 4.54 *Typhlodromus (Anthoseius) microbullatus* Van der Merwe excoriate dorsal view.....109
Figure 4.55 *Typhlodromus (Anthoseius) microbullatus* excoriate ventral view.....109
Figure 4.56 Dorsal view of *T. (A.) microbullatus* Van der Merwe deutonymph.....110
Figure 4.57 Ventral view of *T. (A.) microbullatus* Van der Merwe deutonymph.....111
Figure 4.58 Leg IV of *T. (A.) microbullatus* Van der Merwe deutonymph.....111
Figure 4.59 Dorsal view of *T. (A.) microbullatus* protonymph.....112
Figure 4.60 Ventral view of *T. (A.) microbullatus* protonymph.....113
Figure 4.61 1 – Empodium of *Mullederia neomaculata* (Meyer and Ryke) female; 2 – Dorsal reticulate pattern of *Mullederia centrata* Meyer female; 3 – Ventral view of subcapitulum of *Mullederia centrata* Meyer female; and 4 – Empodium of *Mullederia centrata* Meyer female.....117
Figure 4.62 Aedeagus of *Cheylostigmaeus oudemansi* (Meyer and Ryke) male.....118
Figure 4.63 Chelicerae and related structures of *Cheylostigmaeus* sp.....118

LIST OF FIGURES (Continued)

Figure 4.64 Palp and ambulacral structures of *Cheylostigmaeus* sp.....118
Figure 4.65 6 – Dorsal view of *Zetziella buxi* Ueckermann & Meyer female; 7 – Ventral view of *Zetziella buxi* Ueckermann & Meyer female; 8 – Palpus of *Zetziella buxi* Ueckermann & Meyer female; 9 – Leg I of *Zetziella buxi* Ueckermann & Meyer female; 10 – Leg II of *Zetziella buxi* Ueckermann & Meyer female; 11 – Leg III of *Zetziella buxi* Ueckermann & Meyer female.....119
Figure 4.66 *Stigmaeius* sp. (Acari: Stigmaeidae) female: 247 – Dorsal view, 248 – Reticulation pattern of dorsal shield; 249 – Dorsal body seta; 250 – Ventral view; 251 – Palpus; 252 – Leg I; 253 – Leg II; 254 – Leg III; 255 – Leg IV.....120
Figure 4.67 Dorsal view of *Eustigmaeus spathatus* Ueckermann & Meyer female; 93 – Dorsal reticulate pattern; 94 – Dorsal seta; 95 – Ventral view of *E. spathatus* Ueckermann & Meyer female; 96 – Subcapitulum; 97 – Palpus; 98 to 99 – Empodia; 100 – Leg I; 101 – Leg II; 102 – Leg II; and 103 – Leg IV.....121
Figure 4.68 75 – Dorsal view of *Prostigmaeus vrystaatensis* Ueckermann & Meyer female; 76 – Dorsal reticulate pattern of female; 77 – Dorsal setae of female; 78 – Ventral view of female; 79 – Palpus of female; 80 – Empodium of female; 81 – Leg I of female; 82 – Leg II of female; 83 – Leg III of female; 84 – Leg IV of female; 85 – Tarsus I of male; 86 – Tarsus II of male; 87 - Tarsus III of male; 88 – Tarsus IV of male; 89 – Dorsal view of anal opening of male; 90 – Aedeagus.....122
Figure 4.69 138 - Dorsal view of *Ledermuelleriopsis terrulenta* Ueckermann & Meyer female; 139 - Dorsal reticulate pattern of female; 140 - Setae *ae* of female; 141 - Setae *e* of female; 142 to 143 - Setae *he* of female; 144 - Ventral view of female; 145 - Palpus of female; 146 - Leg I of female; 147 - Leg II of female; 148 - Leg III of female; 149 - Leg IV of female; 150 - Dorsal view of anogenital area of male.....123
Figure 4.70 151 - Ventral view of anogenital area of *Ledermuelleriopsis terrulenta* male, 152 - Tarsus I of male; 153 - Tarsus II of male; 154 - Tarsus III of male; 155 - Tarsus IV of male.....124
Figure 4.71 *Parastogmaeus capensis* (Meyer) empodium of female.....124
Figure 4.72 *Pilonychiopus tutus* Meyer, anogenital area of nymph.....125

LIST OF FIGURES (Continued)

Figure 4.73 158 - Dorsal view of *Eryngiopus parsimilis* Ueckermann & Meyer, female; 159 - Dorsal seta; 160 - Ventral view; 161 - Palpus; 162 - Leg I; 163 - Leg II; 164 - Leg III; 165 - Leg IV.....126
Figure 4.74 External structure of tydeid mite (Prostigmata: Tydeidae).....127
Figure 4.75 Scanning electron micrograph of Tydeidae sp.....128
Figure 4.76 Dorsal view of *Brachytydeus* sp. showing genital appearance.....129
Figure 4.77 Dorsal view of anterior part of aspidosoma of *Brachytydeus* sp.....130
Figure 4.78 Ventral striation between setae *mt* of *Brachytydeus* sp.....130
Figure 4.79 Dorsal fragment of *Brachytydeus* sp. with *f1* and *h1*.....131
Figure 4.80 Bothridial seta *bo* of *Brachytydeus* sp.....131
Figure 4.81 Dorsal rosette of *Brachytydeus* sp.....131
Figure 4.82 Dorsal striae of *Brachytydeus* sp. with tubercles.....132
Figure 4.83 Lyrifissure *ia* of *Brachytydeus* sp.....132
Figure 4.84 Lyrifissure *im* of *Brachytydeus* sp.....132
Figure 4.85 Cheliceral stilleto of *Brachytydeus* sp.....133
Figure 4.86 Palpal tibia and tarsus (right, dorsally) of *Brachytydeus* sp.....133
Figure 4.87 Coxal organ *cg* and seta *ic* of *Brachytydeus* sp.....134
Figure 4.88 Tarsus II, fragment with solenidion ωII of *Brachytydeus* sp.....134
Figure 5.1 Gradient of the PCR amplification of the nuclear ribosomal ITS for Phytoseiidae sp. 1 (Isolate 1) ran on a 1% agarose gel. Left to right: Lane 1 - GeneRuler; Lane 2 - 49.4°C; Lane 3 - 50.8°C; Lane 4 - 52.5°C; Lane 5 - 53.8°C.....142
Figure 6.1 PCR amplification of nuclear ribosomal ITS fragment run on a 1% agarose gel. Left to right: Lane 1 - GeneRuler 1kb DNA Ladder; Lane 2 - *T. (A.) microbullatus* - Isolate 2; Lane 3 - Phytoseiidae sp. 1 - Isolate 1; Lane 4 - Phytoseiidae sp. 2 - Isolate 3; Lane 5 - *Tetranychus evansi* - Isolate 5; Lane 6 - *Amblyseius pretoriaensis* - Isolate 6; Lane 7 - *Tetranychus* sp. - Isolate 4; Lane 8 - *Brachytydeus* sp. - Isolate 8.....151
Figure 6.2 PCR amplification of the *COI* gene fragment using C1J1718 and 773 *COI* primers, run on a 1% agarose gel Left to right: Lane 1 - Ladder; Lane 2 - *T. (A.) microbullatus* - Isolate 2; Lane 3 - Phytoseiidae sp. 1 - Isolate 1; Lane 4 - Phytoseiidae sp. 2 - Isolate 3; Lane 5 - *Tetranychus evansi* - Isolate 5; Lane 6 - *Amblyseius pretoriaensis* - Isolate 6; Lane 7 - *Tetranychus evansi* - Isolate 7; Lane 8 - *Brachytydeus* sp. - Isolate 8.....152

LIST OF FIGURES (Continued)

Figure 6.3 The evolutionary history of the Phytoseiidae based on the ITS gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.....156
Figure 6.4. The evolutionary history of the Phytoseiidae based on the ITS gene was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.....157
Figure 6.5 The evolutionary history of the Phytoseiidae based on the *COI* gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.....159
Figure 6.6 The evolutionary history of the Phytoseiidae based on the *COI* gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.....160
Figure 6.7 The evolutionary history of the Stigmaeidae based on the ITS gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.....161
Figure 6.8 The evolutionary history of the Stigmaeidae based on the ITS gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale,

LIST OF FIGURES (Continued)

with branch lengths in the same units as those of the evolutionary distances used to infeR the phylogenetic tree…….....162

Figure 6.9 The evolutionary history of the Stigmaeidae based on the *COI* gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.....163

Figure 6.10 The evolutionary history of the Stigmaeidae based on the *COI* gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.....164

CHAPTER 1: INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION TO MITES

Mites are extremely small organisms, usually less than 1mm long and this makes it difficult and sometimes even impossible to see them with the naked eye (Craemer *et al.*, 1998). They occur world-wide and are able to inhabit almost every environment and habitat that supports life (Craemer *et al.*, 1998). Mites can be parasitic, predatory, or even saprophagous, and prefer a wide variety of substances to feed on (Jeppson *et al.,* 1975). They are found feeding on fungi, parasitizing animals, insects and humans (parasites that feed on blood or tissue fluid of vertebrates or invertebrates), feeding on the decaying leaves of higher plants or feeding on living plant tissue, living in salt and fresh water, living in soil and on organic material of all kinds, and living on stored and processed products. Mites are placed within the subclass Acari in the animal kingdom. This subclass belongs to the class Arachnida, which contain all the eight-legged animals. Acari are the only subclass of the Arachnida that contains species with pest status and that are regarded as economically important (Craemer *et al.*, 1998).

The influence of plant-feeding (parasitic) mite populations in agriculture has become more pronounced in the past few decades. These tiny organisms are now considered to be an international pest. The large amount of changes in agricultural practices can cause mite populations to either increase or decrease in a local or regional area (Jeppson *et al.,* 1975). Unfortunately, due to commercial fields repeatedly making use of pesticides that kill non-target organisms, such as mite predators, it has led to predatory mite populations decreasing and parasitic mite populations increasing (Nauen *et al.*, 2001; Kim *et al.*, 2004). Therefore, agriculturists should be more aware that the relationships between organisms are constantly changing (Jeppson *et al.,* 1975).

The plant environment in agriculture, and in some forest areas, has drastically been changed by man. Many of these changes are obvious, but unfortunately some are not, such as the equivalent changes that take place among the arthropod complex, that is, the parasites, predators, and competitors. The intensity of mite populations may be altered as a result of gradual changes in cultivar plantings. Mites that do accompany a crop that is grown in a new area may become a major pest due to the lack of predators. Some of the enemies and competitors of these mites are left behind, and those that are transferred with the host may not

always be able to survive this new habitat. When the area is planted with a monoculture, it provides extensive food supply for a mite pest and limits the reservoir of predaceous mites, mite enemies and competitors. Large areas of plantings therefore increase the difficulty of employing effective pest management strategies (Jeppson *et al.,* 1975).

The reproductive capacity of many plant parasitic mites can destroy or seriously reduce plant growth or crop production if mortalities produced by adverse weather, climate, biological enemies, or man's intervention is absent. However, chemicals applied to crops for pest control may even provide a more favourable environment for development of some phytophagous mites. This is due to broad spectrum insecticides being destructive to predators of phytophagous mites, and some plant-feeding mites building up resistance to these chemicals. This has, no doubt, been a major contribution to the general increase of certain tetranychid mites worldwide (Jeppson *et al.,* 1975).

The Chelicerata is one of the largest groups of predominantly terrestrial animals and arthropods. Among these economically significant chelicerates, we find plant parasitic (e.g., spider mites: Tetranychidae) and animal parasites (ticks: Ixodidae) (Nauen *et al.,* 2001; Kim *et al.,* 2004). The majority of plant-feeding mites belong to the suborder Prostigmata. The adaptations of phytophagous forms are mainly associated with their feeding organs, although some may also contain an adaptive life cycle. The most highly specialized plant feeders are the Tetranychidae, with fused cheliceral bases to form an eversible stylophore and movable digits that are drawn out into flagelliform stylets that are used to pierce the epidermis of the host (Jeppson *et al.,* 1975). *Tetranychus urticae* Koch is a cosmopolitan phytophagous mite that is considered to be the most polyphagous pest species among spider mites. Studies on population genetics that make use of molecular markers, such as microsatellites, have proven to be extremely informative to address the questions about the structure of a population, the phylogeography and host preferences (Sabater-Muñoz *et al.,* 2012).

Morphological characters were traditionally used to determine the systematics of this group of organisms, however, these are not always easy to observe and many variations occur (Navajas *et al.,* 1992). The absence of voucher specimens is one of the main problems taxonomists face when analysing data. Voucher specimens are the most important currency in taxonomy, not only for molecular studies, but for morphological studies as well and are used in diagnostic, phylogenetic and phylogeographic analyses (Tixier *et al*., 2010). On the other

hand, distinguishing between species can be difficult due to morphological similarities. Various species require both sexes to make precise determinations (Li *et al.,* 2010). This gap is addressed by using both morphological comparisons and DNA sequence analysis (Young *et al.,* 2012). It is due to this reason that a molecular review is so advantages. Unfortunately, molecular studies on mite predators are quite new and DNA sequences of various species are absent from the GenBank. Nevertheless, molecular analysis used in taxonomic classification provides a solid foundation for phylogenetic hypothesis. By comparing sequences such as the small subunits of ribosomal RNAs or their genes, one is able to compare closely and distinct taxa (Navajas *et al.,* 1992).

Why Solanaceae?

Solanaceae was chosen as a focus group because this family includes a wide variety of commercial crops such as tomatoes, potatoes, eggplants, peppers, etc. These crops are prone to disease, pests and plagues.

1.2 AIMS and OBJECTIVES

The aims of the study are:

1 to collect phytophagous and predatory mite species on Solanaceae genera;

2 to discriminate between these species on a morphological basis as well as a molecular level;

3 to determine the phylogenetic relationship of the collected species;

4 to identify predatory species which can possibly act as biological control agents for spider mites.

The objectives of the project are:

1 to discriminate species through the use of SEM, based on external morphology;

2 to discriminate species by using a light microscope based on external morphology and determine the male and female features for identification purposes;

3 to discriminate between species and verify the identity of these samples via DNA sequencing;

4 to discriminate between species based on their phylogenetic relationship among each other.

1.3 HYPOTHESIS

Hypothesis 1: Endemic solanaceous crops are inhabited by various parasitic and predatory mites.

Hypothesis 2: Endemic Solanaceae species host beneficial bio-control agents (predatory mites).

CHAPTER 2: LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION TO ACARI AND THEIR RELATIONSHIP WITH OTHER ARACHNIDA.

Extant members of the ARTHROPODA are made up of three major assemblages: CHELICERATA (Xiphosura, Arachnida and Pycnogonida), CRUSTACEA and UNIRAMIA (Onychophora, Myriapoda and Hexapoda). The phylogenetic relationship between these assemblages is problematic. It is believed that the assemblages have evolved independently with 'arthropodization' occurring more than once in the group's history. Others think of Arthropoda as being monophyletic and bring together the Crustacea; Myriapoda and Hexapoda within the taxon Mandibulata. **Table 2.1** below reveals the differences between various assemblages through the progress of anterior differentiation. Conditions in cheliceral differences are apparent from that of Crustacea-Uniramia, as Crustacea-Uniramia lack chelicerae (Evans, 1989).

Table 2.1 A comparison between extant Arthropoda's frontal segmental composition (Evans, 1989).

Onychophora	Chelicerata	Crustacea	Myriapoda	Hexapoda
Antenna (Mouth)	[Precheliceral]	[Pre-antennulary]	[Pre-antennal]	[Pre-antennal]
Jaws	Chelicerae (Mouth)	Antennae I	Antennae	Antennae
Slime papillae	Pedipalps infront of leg I *	Antennae II (Mouth)	[Premandibular] (Mouth)	[Premandibular] (Mouth)
Legs I	Legs I and II *	Mandibles	Mandibles	Mandibles
Legs II	Legs II and III *	Maxillae I	Maxillae I	Maxillae I
Legs III	Legs III or V	Maxillae II	Maxillae II or Collum **	Maxillae II
Legs IV	Legs IV or VI*	Legs I or Maxillipeds	Legs I	Legs I
* Xiphosura				
** Diplopoda and Pauropoda				
[ ] Embryonic with or without transient limbs				

Three main lineages from the CHELICERATA are: (1) the Agalaspidida (no living relatives), (2) the Merostomata and (3) the Arachnida. The basic division of the CHELICERATA are

made up of four superorders: MEROSTOMATA (Xiphosura, Synxiphosura), HOLACTINOCHITINOSI (Eurypterida, Scorpiones, Uropygi), ACTINOCHAETA (Palpigradi, Solifugae, Acariformes, Schizopeltida and Pseudoscopriones) and ACTINODERMA (Amblypygi, Araneae, Ricinulei, Parasitiformes, Opiliones, Opilioacarina, Anthracomarti [extinct]). The Acariformes and Palpigradi fall under the taxon EPIMERATA, whereas the Parasitiformes and Notostigmata are included in the CRYPTOGNOMAE along with Ricinulei (**Table 2.2**) (Evans, 1989).

Table 2.2 Mite classification

Phylum: **ARTHROPODA**	
Subphylum: Chelicerata	
Class: Arachnida	
Subclass: Acari	
Superorder: Parasitiformes (=Anactinotrichina)	**Superorder:** Acariformes (=Actinotrichida)
Order: Mesostigmata	**Order:** Trombidiformes
	Suborder: Prostigmata

The Acari are considered to be comprised of three major groups of taxa with equal status, namely the Anactinotrichida, the Actinotrichida and the Opilioacarida (**Table 2.3**) (Evans, 1989).

Acari are thought of as monophyletic on the basis of one characteristic - that they contain a gnathosoma, represented by the two body segments. They are related closest to the Ricinulei, since they share a similar post-embryonic developmental characteristic with their sister group by containing a hexapod larva and three octopod nymphal instars. Combined they form the taxon Acarinomorpha. The Opiliones is considered to be an outgroup of the Acarinomorpha and both are included in the higher taxon Cryptoperculata. A gnathosoma on the other hand, is not only restricted to acarines but can also be found in Ricinulei. Below follows four synapomorphies that support the sister groups Acari and Ricinulei (Evans, 1989):

1. "A hexapod larva and three octopod nymphal instars"

This characteristic is used as the sole synapomorphic characteristic that groups the Acari and Ricinulei (Evans, 1989).

2. "A movable gnathosoma, separated by a circumcapitular suture from the idiosoma"

When a terrestrial arachnid adopts a predatory mode of life, a pre-oral cavity or floor is formed either by the 'labium' that develops as a sternal thickening between the palpcoxae in the embryo (e.g. Araneae) or the palpcoxae is enlarged or approximated, as well as their endites with part of or the complete obliteration of the 'labium'. The palpcoxae is not only fused in the mid-line that forms a compact subcheliceral unit in Acari and Ricinulei but also occurs in some Uropygi (Thelyphonida). However, the fused palpcoxae have restricted movement in the Uropygi. The gnathosoma is thought of as a 'unique constructed modification' (Evans, 1989).

3. "A roughened, scaly or denticulate labrum above the mouth"

This synapomorphy (possible automorphy) is based on insufficient knowledge of the labrum's form in other groups of arachnids and is of doubtful significance. Mesostigmata's processes of the labrum (whether they are present or whether they lack them) can be related to different feeding habits. Thus, if a similar type of labrum occurs in Acarinomorpha, it can be a consequence of functional processes (Evans, 1989).

4. "Trochanter of legs III and IV divided into 2 articulating segments"

This synapomorphy is considered to be 'speculative in a transformed series' and only exists in the tritonymphal stage of Acari and in adults of the Opilioacarida (Notostigmata). A similar division occurs in the trochanters of Solifugae that derived independently from that of the Acarinomorpha (Evans, 1989).

It is clear that the synapomorphies listed above would need further study to establish their character states (Evans, 1989).

Arachnids contains two defining features; (i) the cheliceral mouthparts which act as forceplike feeding organs, and (ii) they lack antennae. However, mites differ from other arachnids in that they partly lack abdominal segmentation. These chelicerae can be modified in some species or reduced in others. Most of the plant-feeding mites, such as Tetranychidae mites, contain modified needle-like chelicerae (Jeppson *et al.,* 1975).

Table 2.3 The subclass Acari with its ordinal classification

Subclass ACARI	
Superorder ANACTINOTRICHIDA	**Superorder ACTINOTRICHIDA**
Division Opilioacariformes	**Division Sarcoptiformes**
Order Notostigmata	Order Astigmata
	Order Cryptostigmata (plus Endeostigmata, in part)
Division Parasitiformes	**Division Trombidiformes**
Order Holothyrida	Order Prostigmata (plus Sphaerolichida)
Order Metastigmata	
Order Mesostigmata	

Van der Hammen (1973) describes Acari to be diphyletic in origin. He describes the Anactinotichida to consist of four orders: Opilioacarida (Notostigmata), Holothyrida (Tetrastigmata), Gamaside (Mesostigmata) and Ixodida (Metastigmata). He also described four orders in the Actinotrichida: Actinedida (Prostigmata, in part), Oribatida (Cryptostigmata), Acaridida (Astigmata) and Tarsonemida (Prostigmata, in part). The Opilioacarida is recognised as a sister group of Parasitiformes (=Mesostigmata-Metastigmata-Holothyrida assemblage) within the Anactinotrichida and thus receives an equal status of taxonomy compared to Parasitiformes. The Anactinotrichida and Actinotrichida are considered to be closer related to each other than any other group of Arachnida (Evans, 1989).

On the basis of a phylogenetic analysis of the taxon, the Actinotrichida can be divided into two assemblages, the Sarcoptiformes (Astigmata, Cryptostigmata and Endeostigmata [excluding the families Sphaerolichidae and Lordalychidae]) and (2) the Trombidiformes (Prostigmata, Sphaerolichida [Spaerolichidae] and Lordalychidae) (Evans, 1989).

Below are 14 apomorphic characteristics that describe Parasitiformes (Evans, 1989):

1. Parasitiformes species lost their dorsosejugal suture and effecement of their primary division between pro- and opisthosoma.
2. They contain one pair of stigmata, situated in region of leg III or IV.
3. The lateral lips of the supcapitulum are fimbriated and are often reduced into attenuated laciniae (secondarily reduced in certain parasitic taxa).
4. Palpal apotele sub-basal and paraxial on tarsus.
5. Tarsi of all the legs contain secondary, non-articulated divisions (basitarsal rings).
6. A gnathosomal tectum forms a supracheliceral vault.
7. Tarsi II to IV with a intercalary sclerite primitively contains 2 setae.
8. Effacement of external evidence of , and reduction in the number of, opisthosomal segments to approximately 10.
9. There is a reduction in the number of opisthosomal lyrifissures of fundamental,

disignatable pairs of larva.
10. The pretarsal setae are reduced to 1 pair on legs I to IV.
11. Paired sternapophyses are fused into a single tristosternum.
12. The trochanters of the legs (legs III and IV) are not divided.
13. The acrotarsus on legs II to IV are absent.
14. The lateral eyes are either reduced to 1 poorly developed pair or they are absent.

2.2 ORDER PROSTIGMATA

Prostigmata (**Figure 2.1** and **2.2**) are extremely heterogeneous and adults range from 100µm to 16mm. The chelicerae (**Figure 2.3** to **2.5**) may be chelate-dentate with 1-2 dorsal setae (occasionally neotrichous) but they usually lack setae, with their fixed digits being reduced in size and the movable digits are edentate and can be styliform. The Tragardh's organs are absent on the fixed digits and the chelicerae do not have associated cheliceral sheaths. The chelicerae can either be fused together or fused with the infracapitulum. Some species may carry 1-3 pairs of adorsal setae on their infracapitulum and a number of subcapitular setae although rutella are only present in a few families and they lack labiogenal sutures.

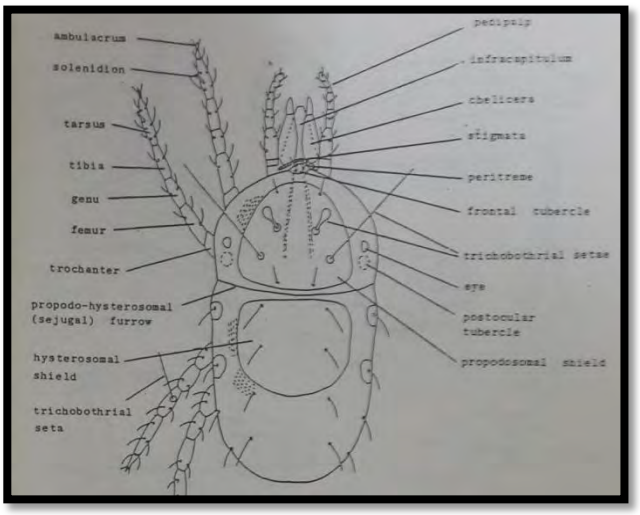

Figure 2.1 External structures of a generalized prostigmatic mite in dorsal view (MacFarlane, s.a.)

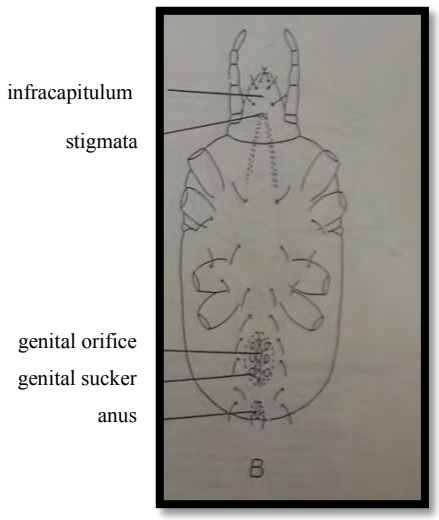

Figure 2.2 External structures of a generalized prostigmatic mite in ventral view (MacFarlane, s.a.)

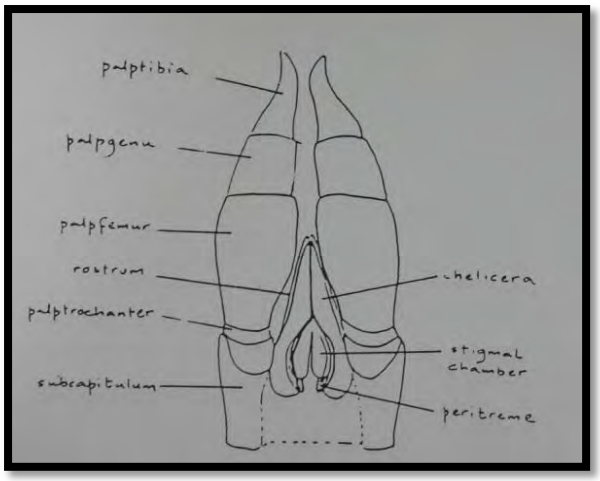

Figure 2.3 Gnathosoma of prostigmatic mite (MacFarlane, s.a.)

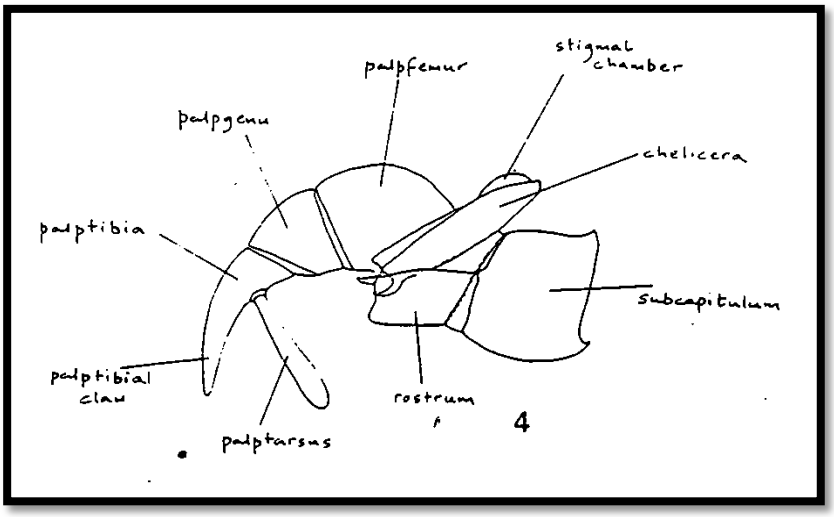

Figure 2.4 Side view of gnathosoma (MacFarlane, s.a.)

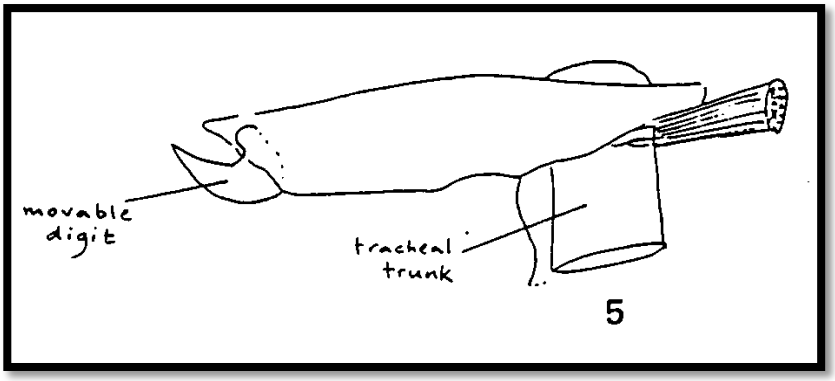

Figure 2.5 Chelicera (MacFarlane, s.a.)

Prostigmata usually contain a palpal supracoxal spine and various families posses a superficial or closed podocephalic canal. The majority of the Prostigmatic families' stigmata (situated between cheliceral bases) often opens the tracheal system with associated peritrimes. On the other hand, the tracheae (only present in females) of one major group, opens as a result of a pair of widely spaced stigmata at the anterior end of the propodosoma. Pedipalps vary in form and size, are one to five-segmented and often raptorial or forming a rostrum-like support for the styliform chelicerae. Prostigmatic mites lack a palpal apotele but a claw-like spur(s) frequently occurs dorsodistally on the palptibia and the palptarsus usually carry a single solenidion. The tritosternum or hypognathal groove is absent from the ventral groove. It is common to find that the idiosoma is poorly solerotized, with or without plating and the propodosoma usually contains 3-6 dorsal setae of which1-2 pairs may be trichobothria (elongated setae) (**Figure 2.6**) (MacFarlane, s.a.).

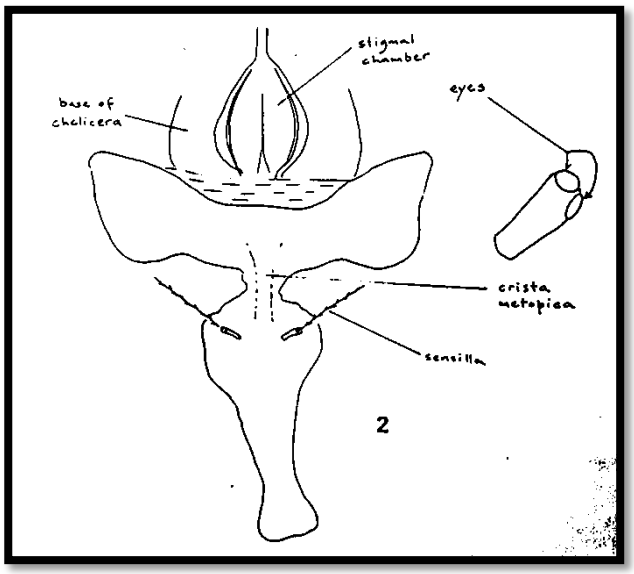

Figure 2.6 Anterior part of the idiosoma (MacFarlane, s.a.)

The trichobothria will not be located posterolaterally if there is only one pair present. There are a few families where the hysterosoma contains a holotrichous acariform chaetotaxy of 16 pairs but it is generally reduced although in some families there is pronounced neotrichy. A maximum of six pairs of capules or lyrifissures can be present but there are usually only four pairs. It is common to find that the idiosoma is ovoid but they are elongated or fusiform in a

few families. The genital orifice is longitudinal, usually at the posterior end of the opisthosoma and a pair of genital valves close the orifice. An ovipositor can either be present in prostigmatic mites or they can lack the presence of one. These mites generally bear 3 pairs of genital papillae, when present, occasionally 2 pairs, but in many water species they can be numerous. Latero-abdominal glands are absent in Prostigmata (MacFarlane, s.a.).

The leg coxae can either be plate-like or entirely fused with the venter of the idiosoma. Larvae, in families that have genital papillae in later stages, contain Claparede's organs between coxae I and II. The trochanter is the first movable segment and the basifemora and telofemora form the femora. The number of segments that occur can be reduced in some families. The leg apotele (ambulacrum) usually carry two lateral claws (which are known as the 'true claws') with/without a median element, the empodium, which can be claw-like, pad-like, bell-shaped or rayed and is frequently in the form of paired crotchet-like 'tenent' hair. Tenent hairs or simple pilosity can be found on lateral claws. Prostigmatic mites can contain bacilliform or setiform solenidia on the genua, tibiae and tarsi, but very long tapering solenidiae are never present at the distal end of the tibiae. In some families a tactile (true) seta and a solenidion are in close association and form a 'duplex seta'. The eupathidia (hollow 'true' setae) may be distally positioned on the tarsi but in a few families it can be more widely distributed. The tibia and tarsi may bear trichobothria and in various families the number of pairs of legs in one/both sexes/immature stages may be reduced (MacFarlane, s.a.).

Various families have no evidence of anamorphosis, segment PS remaining paraproctal throughout the life-cycle, while other families contain evidence of the addition of 1-3 post larval segments (AD, AN and PA). The amount of active immature stages, elattostases and calyptostases vary greatly within the order from a full developmental cycle comprising a calyptostatic prelarva and active larval, proto-, deuto- and tritonymphal stages to a condition where the whole life-cycle takes place within the female and the female then gives birth to adult males and females. Prostigmatic mites consist of free-living fungivorous, phytophagous, saprophagous and predaceous forms as well as gall-forming plant feeders and parasites and associates of arthropods and vertebrates. Various superfamilies are aquatic during either the whole or most of the life cycle and one family has predominantly marine species (MacFarlane, s.a.).

KEY TO PROSTIGMATA (ADULTS) FAMILIES (MacFarlane, s.a.)

1. Idiosoma fusiform and annulated with either two pairs of legs or four pairs of extremely short stumpy legs.....DEMODICOIDEA & ERIOPHYOIDEA
- If idiosoma fusiform, then not annulated and with four pairs of legs of more normal length.....2

2. Gnathosoma with small indistinctly segmented pedipalps and minute cheliceral stylets or regressive; dorsum of isiosoma covered with a series of shield, usually overlapping in the female; coxal sternites delineated by prominent apodemes; ambulacra usually with membranous pulvilla at least on legs II and III (Tarsonemina).....Tarsonemoidea & Pyemotoidea
- Gnathosoma usually with prominent four- to five-segmented pedipalps; shield on the dorsum, if present, not overlapping; coxal sternites usually at least partially delineated superficially; ambulacral pulvilli, where present, usually claw-like, pad-like or with tenet hairs.....3

3. Idiosoma and legs densely covered with setae; idiosoma with one or two pairs of eyes, sessile or stalked, and one or two pairs of trichobothria usually situated within areae sensilligerae with an associated crista metapiea; palptibia with distal claw.....PARASITENGONA
- Idiosoma not densely covered with setae; eyes, if present, never stalked; trichobothria where present without associated areae sensilligerae and crista metapiea.....4

4. Very characteristic yellow mites, the idiosoma completely armoured dorsally and ventrally; an anterior median and a pair of lateral eyes and two pairs of branched trichobothrial setae present; chelicerae independent, chelate; pedipalps without thumb-claw (Labidistommatoidea).....LABIDOSTOMMATIDAE
- Without this combination of characters.....5

5. Two pairs of trichobothrial setae present on the propodosoma; infracapitulum elongate; pedipalps prominent, raptorial, but without thumb-claw; pedipalptarsus either claw-like distally or terminating in two long setae; chelicerae independent, each with a long basal part and small digit; peritremes absent (Bdelloidea).....BDELLOIDEA
- Propodosoma with one pair of trichobothrial setae or none; if two pairs present then either pedipalps are not raptorial or conspicuous peritremes are present.....6

6. Chelicerae independent, each with a long slender basal part and a short movable digit; peritremes conspicuous; pedipalp tibia with one or three distal claws.....ANYSTOIDEA
- Chelicerae not of this type.....7

7. Basal parts of chelicerae not distinct from the infracapitulum; movable digits styliform; no idiosomal trichobothria present.....CHEYLETOIDEA
- Chelicerae free, fixed or coalesced with each other into a stylophore but always distinct from infracapitulum.....8

8. Basal parts of the chelicerae coalesced to form a stylophore; movable digits very long, styliform, and strongly recurved upwards basally; peritremes run over the antero-dorsal surface of the idiosoma; no idiosomal trichobothria or genital suckers

(Tetranychoidea).....9
- If chelicerae form a stylophore, then stylets shorter and nor recurved basally.....10

9. Pedipalps robust, five-segmented, with tibial claws; one of the distal pedipalptarsal setae modified as a spinneret. Spider mites.....**TETRANYCHIDAE**
- Pedipalps slender, one- to five-segmented, without a tibial claw or spinneret. False spider mites.....**TENUIPALPIDAE**

10. Empodium characteristic, consisting of one to five pairs of divergent tenent hairs, sessile or arising from a median process; pedipalp tibia usually with a distal claw; basal parts of chelicerae independent, fused or coalesced into a stylophore; peritremes present or absent; no idiosomal trichobothria or genital sucker (Rapgignathoidea).....11
- Empodium, when present, not of this type; pedipalp tibia without a distal claw; chelicerae independent or fused; peritremes never present; idiosomal trichobothria and genital suckers present or absent.....EUPODOIDEA

11. Idiosoma entirely covered dorsally and laterally with reticulated shield without transverse sutures and with a prominent anterior hood beneath which the gnathosoma can be retracted.....CRYPTOGNATHIDAE
- Dorsal idiosomal shields variously developed, if one shield completely covering idiosoma is present, then wihtout a hood under which gnathosoma can be retracted.....12

12. Idiodoma covered dorsally with a single hemispherical shield; a pair of conspicuous eyes present behind which is a narrow incomplete transverse groove which opens internally to a pair of large sacs or tubes. Aquatic or semiaquatic....HOMOCALIGIDAE
- Idiosoma not covered with a single hemispherical shield; without such a respiratory system.....13

13. Legs slender, stilt-like, all much longer than the body; setae on drosum of idiosoma and most leg setae, stout, barbed or serrate and set on tubercles.....CAMEROBIIDAE
- Legs never much longer than the body.....14

14. Pedipalptarsus carried in a pedant position below the tibial claw; if claw small, then tarsus not longer than the tibia; empodial tenent hairs arise from a median process.....15
- Pedipalp tarsus elongate; claw of pedipalp tibia small or absent; empodium with two pairs of tenent hairs which do not arise from a median process.....EUPALOPSELLIDAE

15. Chelicerae coalesced into a stylophore over which the peritremes run; dorsal plating absent or feeble.....CALIGONELLIDAE
- Chelicerae independent, fused or form a stylohpore; peritremes, when present, not running over dorsal surface of the chelicerae; dorsal plating often extensive.....16

16. Chelicerae form a stylophore, peritremes run laterally over the membrane between gnathosoma and idiosoma; coxae II and III constiguous.....RAPHIGNATHIDAE
- Chelicerae usually independent, occasionally fused or stylifore-like; peritremes usually absent; coxae II and III not contiguous..... **STIGMAEIDAE**

Cohort EUPODINA

The chelicerae of members of this cohort are chelate or the fixed digit is either reduced or absent and the movable digit can be elongated or styliform (they are usually separated but in some Tydeoidae species they are fused). The pedipalps can be raptorial but not always and lack a tibial claw and are four- to five-segmented (fewer in some parasitic Tydeoidae and Halacaroidea). The rutella is absent and the stigmata are located at the base of the chelicerae. Peritremes are never present and the podocephalic canal is superficial or can be internal. Eupodina mites posses one or two pairs of prodorsal trichobothria. The sclerotisation of the idiosoma is weak or has dorsal propodosomal and hysterosomal plates and the dorsosejugal furrow can either be present or they may lack the presence of it. Usually no addition of setae occur after the PS series in most families but AD (and AN) are added in some Bdellidae. Eugenital setae can be present in some families and absent in others and they contain 2-3 pairs of genital papillae, except for some Tydeoidae and Halacaroidae. The femora of the legs are regularly subdivided. The apoteles of the legs vary and usually bear claw-like lateral (true) claws but the empodium can be pad-like, claw-like rayed or even absent. The sperm is usually transferred from males to females by the spermaphores but direct insemination can take place in the families Cunaxidae and Halacaroidea. There are prelarval homeomorphic larva and three nymphal stages in the life-cycle of Eupodina mites, except in some parasitic Tydeoidea (MacFarlane, s.a.).

Families:
BDELLOIDEA: Bdellidae, Cunaxidae
EUPODIODEA: Eupodidae (incl. Penthaleidae and Strandtmanniidae), Penthalodidae, Rhagidiidae
HALACAROIDEA: Halacaridae (marine)
TYDEOIDEA: Ereynetidae, **Tydeidae** (**Figure 2.7**)

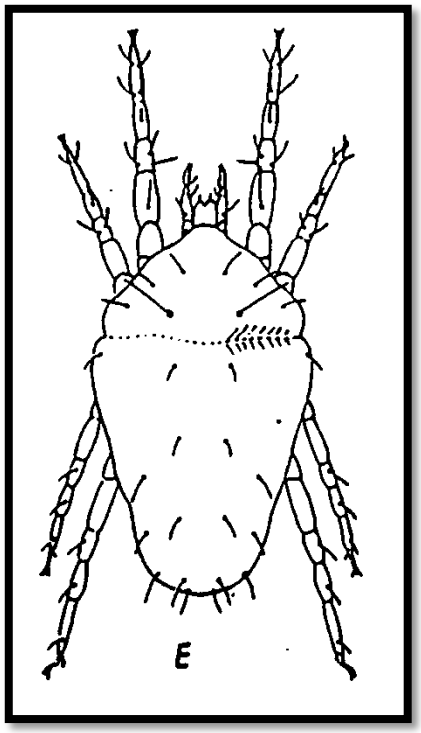

Figure 2.7 Dorsal view of tydeid mite (Prostigmata: Tydeidae) (MacFarlane, s.a.)

Cohort RAPHIGNATHAE

It is not unusual to find that the chelicerae of members of this cohort are fused together and on some occasions it's also fused to the infracapitulum. The fixed digit is reduced by a great amount and contains no setae and the movable digit is styliform. The pedipalps are made-up of five-segments and various families contain a tibial claw, whereas others such as Tenuipalpidae, Eriophyoidea and some Demodicoidea lack a tibial claw, and the number of segments are occasionally greatly reduced in Tenuipalpidae and Demodicidae. The palps may form a rostrum to offer support to the slender cheliceral stylets (Tetranychidae, Eriophyidae and Demodicidae). Some families bear stigmata at the cheliceral bases, generally with associated peritremes. When the podocephalic canal is detected, it is usually superficial. Raphignathae mites lack the propodosomal trichobothria and a frontal tubercle and the idiosomal chaetotaxy is generally extremely reduced. These mites also lack genital papillae and eugenital setae and the presence of anamorphosis is not evident. The femora of the legs are not subdivided (**Figure 2.9**). Families that are parasitic will often show a reduced number of leg segments and/or modifications for grasping hairs. The two pairs of legs on the posterior end of the mites are reduced or not present in some Harpyrhynchidae. The transfer of sperm from males to females are direct due to the male's terminal or dorsal aedeagus. Some members of the family Tetranychidae are parthenogenetic. A prelarval, homeomorphic larval,

and two nymphal stages can occur in the life cycle; but unlike other species, three nymphal stages will never occur (MacFarlane, s.a.).

Families:
RAPHIGNATHOIDEA: Caligonellidae, Camerobiidae (=Neophyllobiidae), Cryptognathidae, Eupalopsellidae, Homocaligidae, Raphignathidae, **Stigmaeidae** (incl. Barbutiidae) (**Figure 2.10**)
CHEYLETOIDEA: Cheyletidae, Cheyletiellidae
DEMODICOIDEA: Demodicidae, Harpyrhynchidae, Myobiidae, Psorergatidae, Syringophilidae
TETRANYCHOIDEA: **Tetranychidae** (**Figure 2.8**), Tenuipalpidae
ERIOPHYOIDEA: Eriophyidae, Diptilomiopidae (=Rhyncaphytoptidae), Phytoptidae (=Sierraphytoptidae)

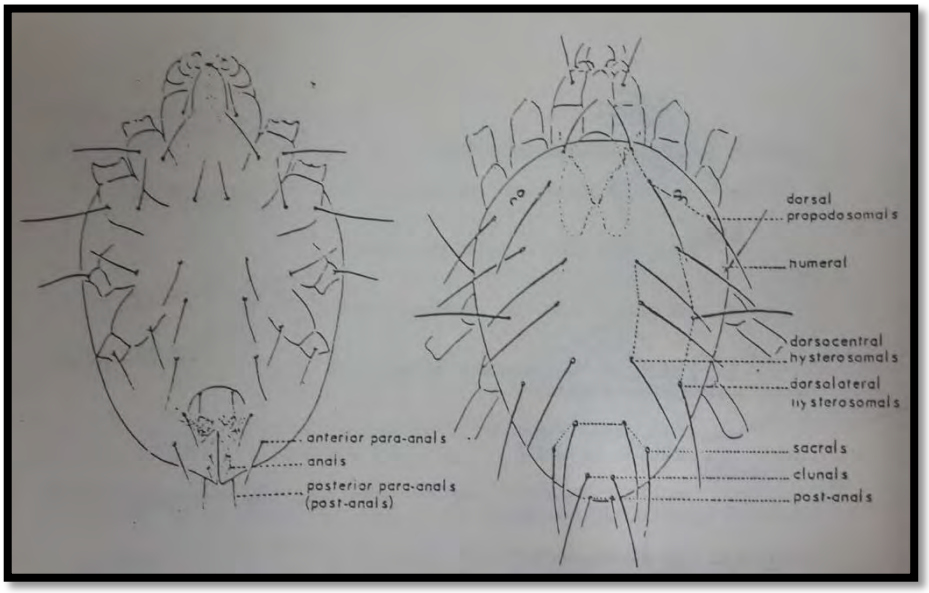

Figure 2.8 Ventral view (left) and dorsal view (right) of tetranychid mite (Prostigmata: Tetranychidae) (MacFarlane, s.a.)

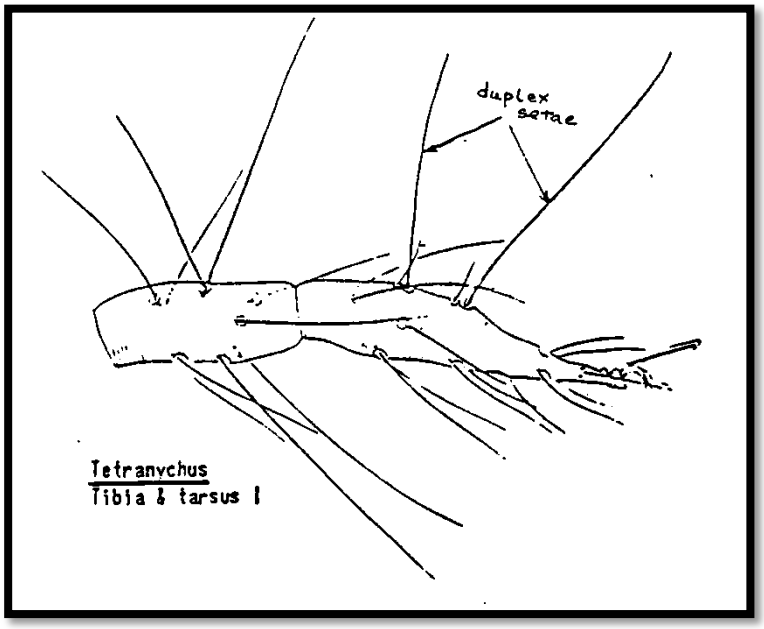

Figure 2.9 Tibia and tarsus I of a tetranychid mite (Prostigmata: Tetranychidae) (MacFarlane, s.a.)

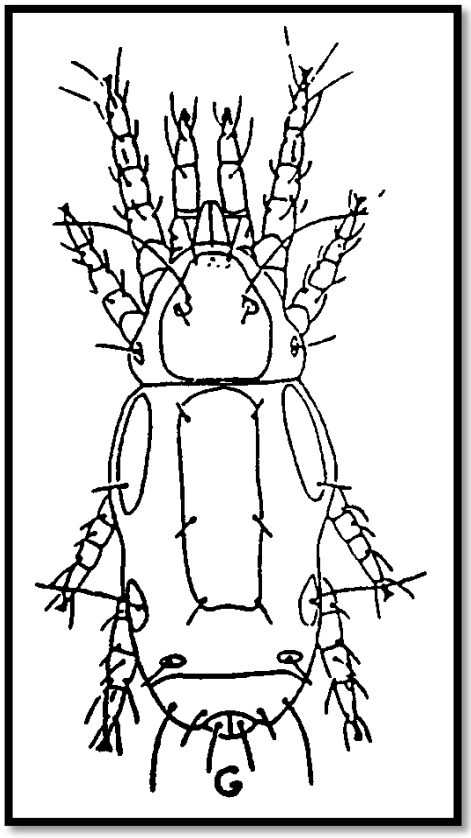

Figure 2.10 Dorsal view of stigmaeid mite (Prostigmata: Stigmaeidae) (MacFarlane, s.a.)

Stigmaeids inhabit plant and soil and feed on tetranychid mites, tenuipalpids and eriophyids. Stigmaeid mites, especially *Agistemus* sp. Summers and *Zetzellia* sp. Oudemans, are considered to be the second most important predators of spider mites, after phytoseiid mites (Kheradmand *et. al.*, 2007).

2.3 ORDER MESOSTIGMATA

Mites that belong to the order Mesostigmata vary in size from 200 to 2 000µm. Some of the smaller mesostigmatic forms are pale and weakly sclerotized but the idiosoma is usually only partially covered by a number of chestnut-brown shields. The idiosoma is seldom divided into regions and the tubular gnathosoma is movably articulated to it and lies in a camerostome. The three segmented chelicerae are usually chelate-denate but are subject to considerable modification in specialized parasitic species. A male's movable digit of its chelicerae may contain a spermadactyl or spermatotreme. On the chelicera's main body, there is a dorsal seta and two lyrifissures. The pedipalps usually bear five segments and an ambulacrum (apotele) that is represented by a two to four-tined claw-like structure at the inner basal angle of the tarsus. The venter of the gnathosoma contains a maximum of four pairs of setae and the external malae carry horn-like corniculi and they possess a gnathotectum (Evans, 1989).

All mesostigmatic mites bear an unpaired tritosternum, except most of the highly specialized parasitic species and are usually provided with either a pair of laciniae or a divided laciniae. Postembryonic developmental stages contain a differentiated sternal shield. However, this is absent in some larval stages. The genital orifice is in the form of a transverse slit and is situated in the intercoxal area. This opening is protected by one, three or four shields in the female and only by one or two shields in the male. The anus is subterminal and normally surrounded by a sclerotized shield and each anal valve can contain a maximum of one seta in the developmental stages. The coxae movement is articulated to the idiosoma and the legs contain six segments, excluding the ambulacrum. However, leg I is usually sensory and the ambulacrum may be absent. It is not unusual to find false segmentation of the femur and tarsus by lyrifissures. The first leg and occasionally the fourth can be crassate and spurred in

males and in some groups the legs may be completely drawn back into deep cavaties in the idiosoma. Leg II can also carry spurs in some parasitic species, such as Laelapidae, *Androlaelaps* ea (Evans, 1989).

These mites contain a pair of stigmata that's situated laterally or dorso-laterally in the region of coxae II-IV. The peritreme extends anteriorly as a slender channel except in larvae, where a respiratory system is absent, and certain endoparasites (Evans, 1989).

Most of the mesostigmatic mites are free-living in soil and decaying organic matter. Various species are adapted structurally and biologically for a parasitic life-style on vertebrates and invertebrates. Mesostigmata are cosmopolitan in distribution (Evans, 1989).

Below follows the morphology of mesostigmatic mites. It is not exhaustive and is used here only to introduce structural features which are used to classify and identify taxa.

The body of mesostigmata is divided into two major regions: the **gnathosoma**, this region of the mite's body is a small anterior feeding/trophic-sensory region, and is movably articulated to a larger sac-like **idiosoma** that carries the ambulatory appendages (**Figure 2.11**). The gnathosoma lays in the camerostome (an antero-ventral cavity situated in the idiosoma) and is largely formed by the appendages and sternal elements of the pedipalpal and cheliceral segments. These segments' tergal elements are thought to be incorporated into the idiosoma's anterior region and it is unclear whether or not a pre-cheliceral segment is represented in the gnathosoma. The idiosoma is divided into an anterior podosoma, carrying the amulatory appendages, and a posterior opisthosoma. The dorsal surface of the podosoma is termed the podonotum, and the dorsal and ventral surfaces of the opisthosoma is termed opisthonotum and opisthogaster, respectively (Evans, 1989).

External morphology of gnathosoma

The gnathosoma's (**Figure 2.12** and **2.13**) skeletal structure is formed by the walls of the palps' coxae (basal segments) which extend dorsally to surround the chelicerae and ventrally to combine sternal elements. The sclerotised tube that is formed in this way is referred to the gnathosomatic base or basis gnathosomatica. The mesial walls of the palpcoxae, situated dorsally to the pharynx, is connected by a shelf-like subcheliceral plate that divides the gnathosomatic cavity into two regions, namely the dorsal cheliceral region and the ventral

pharyngeo-hypostomatic region and provides attachment sites for the labral muscles and certain pharyngeal dilator muscles. The hypognatham is then formed by the same cheliceral plate and the pharyngeo-hypostomatic region of the gnathosoma. A deep V and U shaped pre-oral trough divides the subcheliceral plate (that's situated anteriorly to the oral opening) down the middle, and whose walls are formed by the anterior extension of the ventro-lateral walls of the pharynx. A lobe-like process, termed the labrum, lays within the pre-oral trough and exemplifies the anterior extension of the dorsal wall of the pharynx. The supra-labral process lies dorsally to the labrum and is seem as a solid central extension to the subcheliceral plate (Evans, 1989).

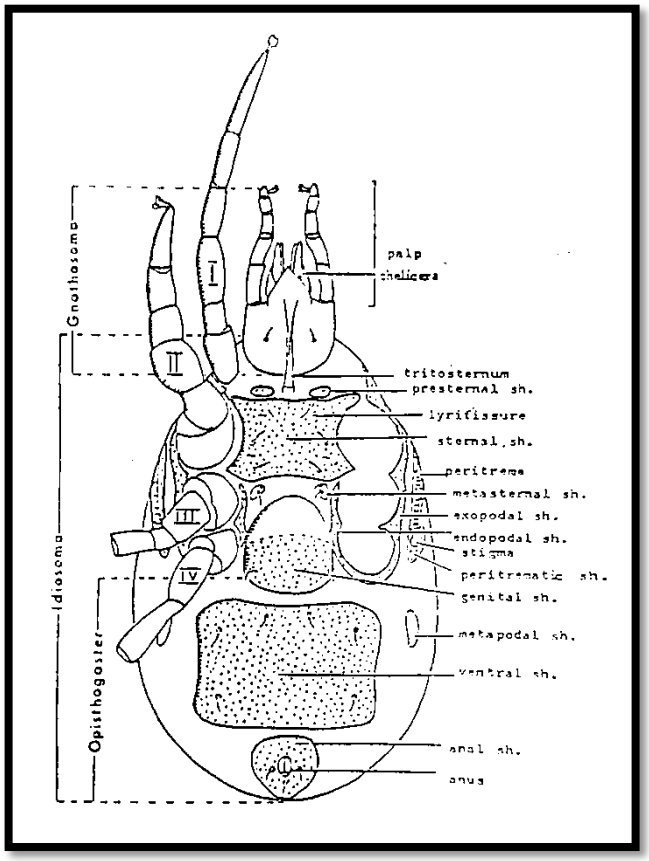

Figure 2.11 Diagrammatic representation of a gamasine mite in ventral view (Evans, 1989)

Arising from the subcheliceral plate near the origin of the pre-oral trough, to some extent anterior and dorso-lateral to the origin of the labrum, is a pair of variously shaped processes referred to as paralabra. Anterior to the oral opening lays the hypostome, a hypognathum that is produced into a beak-like structure, and supports the pre-oral trough. On either side of the trough the hypostome is divided into two lobes termed the internal mala (mala interna) and the external mala (mala externa). The internal malae terminate in simple or complex

hypostomatic processes and each mala typically carry a horn-like process that may be of setal origin and is referred to as the corniculus (Evans, 1989).

The hypostome can be somewhat or completely protected dorsally by a simple or detailed extension of the roof of the gnathosomatic base referred to as the gnathotectum. The venter of the hypognathum is supplied with a central hypognathal groove which is generally provided with rows of hypognathal denticles. The gnathosomatic base bears a pair of palpcoxal setae in the nymphal and adult stages while the hypostome carries two pairs of setae, namely the anterior hypostomatic and external posterior hypostomatic setae in larva and three pairs in the nymph and adults by the addition of the internal posterior hypostomatic setae, to the larval complement at the protonymphal stage (Evans, 1989).

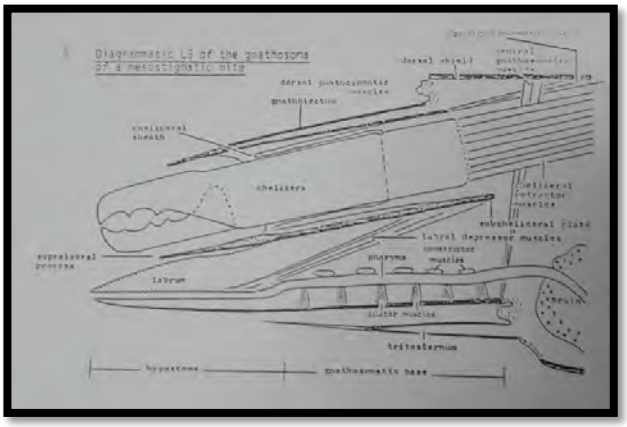

Figure 2.12 Diagrammatic representation of the gnathosoma of a mesostigmatic mite (Evans, 1989)

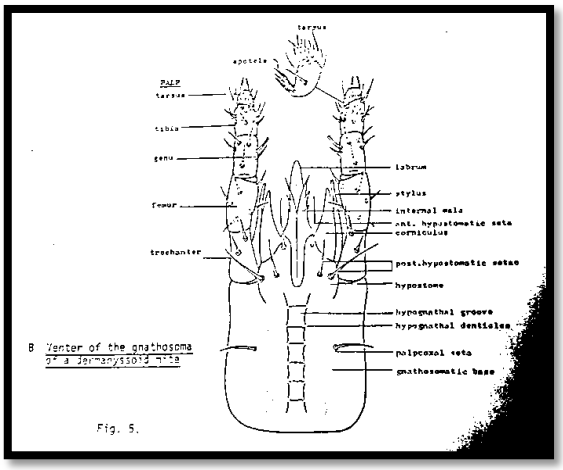

Figure 2.13 Ventral view of the gnathosoma of a dermassoid mite (Evans, 1989)

Chelicerae

The paired pre-oral chelicerae lies in the same area as the gnathosoma and is bounded dorsally and laterally by the mesial walls of the palpcoxae and ventrally by the cheliceral plate. These chelicerae are connected to a sclerotized framework, the gnathothecum, by the cheliceral sheath. In the posterior area the sheaths are constant with the walls of the gnathothecum and fuse in the middle to form the gnathothecal septum. The cheliceral body is divided into three regions known as articles: a basal article (I) to which the retractor muscles are attached, a middle article (II) movably articulated with the basal article and forms the fixed digit distally, and the movable digit (III) articulated ventrally with the middle article so that it opposes the fixed digit (**Figure 2.14**) (Evans, 1989).

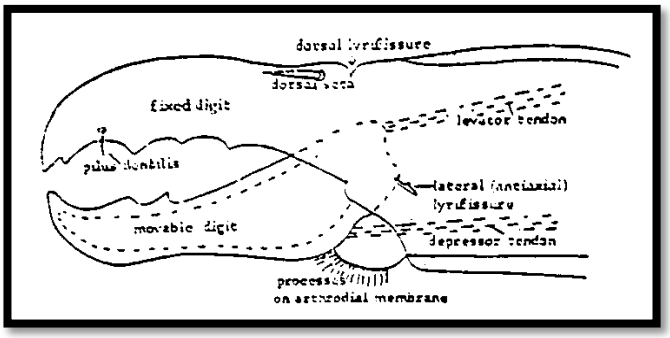

Figure 2.14 Venter of the chelicera of a gamasine mite (Evans, 1989)

The external (antiaxial) face of the chelicera in the region of the digits can carry two setae and two lyrifissures (mechano-receptors). A dorsal seta and its associated lyrifissure are located dorsally at the point of origin of the fixed digit. A second lyrifissure is normally present externally in close proximity to the condylar process of article II, and a specialised seta (by chance a chemoreceptor), the pilus dentilis (or cheliseta), in the distal half of the fixed digit. The pilus dentilis is shortened on the other hand is shortened (Evans, 1989).

Palps

The free-limb of each palp (or pedipalp) extends beyond the gnathosomatic base as a six segmented appendage of which the distal segment, the ambulacrum or apotele, is defined by an entire, two, three or four-tined movableclaw-like structure located at the inner basal paraxia angle of the tarsus. The proximal segment of the free-limb is composed by the

trochanter and is followed by the femur, genu, tibia and tarsus. Each free segment of the palp, aside from the apotele in all instars and the trochanter in the larva, is provided with tactile setae which displays considerable diversity in form (Evans, 1989).

External morphology of Idiosoma

The shape of the idiosoma in the majority of mesostigmatic mites have one of four shapes, namely, oval, ovate, subovate or sperical (Evans, 1989).

Dorsum of Idiosoma

Sclerotization: Dorsal sclerotization can be thought to comprise three basic elemens; a podonotal shield covering the anterior area of the idiosoma, a pygidial shield restricted to the posterior region of the idiosoma, and, between these two shields, two or more (seldom one) mesonotal shields or scutella. The opisthonotal shield is formed by a fused pygidial and mesonotal shields, whereas the holodorsal shield is formed by the entire podonotal and opisthonotal elements being fused. A schizodorsal shield results in the middle region due to the fusion of the podonotal and opisthonotal shield, where the unfused region is represented by lateral incisions (Evans, 1989).

Chaetotaxy and Porotaxy: The idiosmal dorsal surface is generally provided with a distinct pattern of setae in all post-embryonic and developmental stages and the nature of the chaetotaxy is commonly used as a taxonomic criterion in the Gamasina. The Sellnick System describes four paired longitudinal setal rows or series on the body dorsum and these rows are designated dorsocentral (j and J), mediolateral (z and Z), lateral (s and S) and marginal (r and R) (Evans, 1989).

The larva generally contains 10 pairs of setae on both the podonotal (j1, j3-j6, z2, z4, z5, s4 and s6) and the opisthonotal (J2-J5, Z3-Z5 and S3-S5) regions. The protonymphs bear 5 setal pairs extra on the podonotum (j2, s5, r3, r3 and r5) and the same amount to the opisthonotum (J1, Z1, Z2, S2 and R1). Setae z1, z3, z5, s1-s3, r1, r4 and r6 can be added to the protonymphal stages that complement the podonotum of the deuteronymph and S1, R2-R7 and the UR series on the opsithonotum. The deutonymphal number of setae are withheld in adults (Evans, 1989).

Venter of the Idiosoma

Intercoxal region: The appearance of setae in phytoseiid mites (Mesostigmata: Phytoseiidae) are delayed until they reach the deutonymphal stage. The process (setal suppression) that caused this hypotrichy has led to paedomorphosis. The intercoxal region of the deutonymph is usually occupied by a well-defined intercoxal shield that carries sternal setae 1-4; st. 4 is referred to as the metasternal setae and appears for the first time at the deutonymhal stage. The adult on the other hand is characterized by the appearance of the external opening that leads to the reproductive system within, seldom posterior to, the area of the intercoxae. The genital orifice in females is formed in a transverse slit round about the same level as the anterior margins of coxae IV while in the opening in males are presternal or within the sternogenital shield between coxae II and III (Evans, 1989).

Podal-peritrematic region: The coxae of the second to fourth legs are inserted in the acetabula of the podal shield which can be in a holopodal shield or endo- and exo-podal shield's form. Endopodal shields of coxae II and III in adults are normally fused with the sternal or sternogenital shields. The stigma is usually located dorsal to the exopodal shield between legs III and IV on either side of the idiosoma of the nymphs and adults (Evans, 1989).

Opisthogastric region: The anus is located subterminally, seldom termanilly, within an area of the opisthogaster that is shlerotized. In almost all immature instars of the Gamasina this sclerotized region forms a distinct anal shield which, characteristically, carries three setae comprising paired adanal setae and an unpaired postanal seta. The anal opening is covered by two very small shields that hardly ever carry a single euanal seta (Evans, 1989).

Methods of Insemination

Mesostigmatic mites reproduce through two main mehtods of insemination in females, namely, tocospermy and podospermy. In the topospermic form the spermatophores are transfered from the male's genital orifice to that of the female's either through the use of the chelicerae (by holding the neck of the spermatophores between the cheliceral shaft or by passing the neck of the spermatophore through a foremen [spermatotreme] in the movable digit) or by using the palps and legs I. Podospermic insemination takes place in the majority of the Gamasina. In the podospermic form the the sperm material enters the female's body through the solenostome (external opening). This external opening is seen as a sperm access

system (spermatheca) and is directly connected to the ovary. The male carries the spermatophores from his chelicerae to the solenostome region, where he introduces the spermatophores to the solenostome by using a spermadactyl (Evans, 1989).

The sperm access system consists of a tubulus (often with taenidia) that leads from the solenostome into a sperm maturation pouch referred to as the sacculus by way of a horn-like projection of the sacculus, the ramus. Gamasima mites contain two main types of sperm access systems, the phytoseiid and laelapid types. In the phytoseiid types, the organ is composed of paired structures with no connection between the sacculi and two seperate sperm ducts (Evans, 1989).

Parthenogenesis

Mesostigmatic mites can reproduce by means of arrhennotoky (haploid males from unfertilised eggs and diploid females from eggs receiving spermatozoa) and thelytoky (females are mainly born from unfertilised eggs, males absent or rare, if present, mites do not mate). Arrhenotoky is quite common in Phytoseiidae and is seen as a predominant form of reproduction. Phytoseiid females of the genera *Amblyseius* and *Typhlodromus*, will produce both haploid females and diploid males. However, mating is required in this form of reproduction for oviposition. This occurance is thought of as pseudo-arrehenotoky and icludes the elimination of a set of chromosomes in the embryos of the male line (Evans, 1989).

Post-embryonic developmental stages

Mesostigmatic mites contain a hexapod larva, two octopod nymphal stages (protonymph and deutonymph) and the adult stages (male and female) in their post-embryonic developmental cycle (Evans, 1989).

KEY TO POST-EMBRYONIC STAGES (Evans, 1989)

1. With six legs; hypostome with two pairs of setae*; palptrochanter with no setae; stigmata absent.....LARVA
- With eight legs; hypostoma with three pairs of setae; palptrochanter with one or two setae; stigmata present……...2

2. Intercoxal region without genital orifice, usually with entire intercoxal shield.....3
- Intercoxal region with genital orifice in the form of a transverse slit protected by one or more shields.....4

3. Palptrochanter typically with single ventral setae.....PROTONYMPH
- Palptrochanter typically with two ventral setae.....DEUTONYMPH

4. Entire intercoxal shield with small subcircular gential orifice protected by one or two small shields located near or at the anteriormargin of the shield or between coxae II - IV; movable digit of the chelicera with or without spermadactyl; leg II may be provided with spurs (hypertrophied setae) on one or more segments.....MALE
- Sclerotization of the intercoxal region typically comprising discrete sternal and genital elements or an entire intercoxal shield surrounding a large genital shield. Chelicera without spermatodactyl.....FEMALE

* Cuticular processes that are not of setal origin are excluded in the chaetotaxy.

Classification

Mites beloging to the order Mesostigmata have a maximum of 4 pairs of setae (excluding the cuniculi) on the venter of the subcapitulum. The tritosternum is usualy present in mesostigmatic mites and contains a distinctive base and 1-2 selulose laciniae (these structures are either reduced or completely absent in some parasitic taxa). The anal valves in adults are nude, but some can include only one pair of setae. The bases of the chelicerae are enclosed by a sclerotized ring and these rings usually contain a prejecting epistome (Lindquist *et. al.,* 2009). The order Mesostigmata constitutes three suborders: Monogynaspida, Sejida and Trigynaspida (Lindquist *et. al.,* 2009). The Antennophorina cohorts are equal to Camin and Gorirossi's concept of the **suborder TRIGYNASPIDA**, where the remainder of the cohorts will fall into the **suborder MONOGYNASPIDA** (Evans, 1989).

KEY TO THE SUBORDERS OF MESOSTIGMATA (Lindquist *et al.,* 2009)

1. Oviporus covered by a single plate (epigynal shield) with 0-1 pair of setae (they rarely contain 4 or 5 setae); tarsus of leg IV of deutonymphs and adult with a maximum of 18 setae, lacking setae *av4* and *pv4* and ventral intercalary sclerite....**MONOGYNASPIDA**
- Oviporus covered by a single large shield (epigynal shield) usually bearing 6 or more setae (rarely 2 or 4 setae in some *Sejus*) or by a complex of 2-4 genital shields or their remnants; tarsus of leg IV of deutonymphs and adults with a minimum of 20 setae, setae *av4* and *pv4* present (the paedomorphic milliperde-associated NEOTENOGYNIIDAE lack these setae, they are minute in some groups, e.g., PROMEGISTIDAE), usually on a ventral intercalary sclerite between the basitarsus and telotarsus.....2

2. Oviporus covered by a single large shield (epigynal shield) bearing 6 (rarely 2-4) to many setae and often notched or excavated anteriorly; chelicerae without excrescences.....SEJIDA
- Oviporus covered by 2-4 shields (2 latigynals, a mesogynal, and rarely a sternogynal shield) that may be united ir reduced; mesogynal shield nude, usually subtriangular but

often reduced or insensibly fused to other elements, latigynal shields each with one or more setae, free or fused posteriorly to ventral elements and/or medially to each other; movable cheliceral digit with medial or terminal dendritic, brushlike, or filamentous ventral excrescences.....TRIGYNASPIDA

COHORT GAMASINA

The classification of the Gamasina is in a state of constant change (Evans, 1989). It is placed within the suborder Monogynaspida (Lindquist *et at.,* 2009). On the basis of secondary sexual characteristics (various characteristics have features prominently in all major classifications since Berlese in 1892) the cohort can be divided intro three divisions (Evans, 1989):

1. Division DERMANYSSIDES: Marked sexual dimorphism in the chelicerae, male contain a spermatodactyl; presternal genital orifice in males; insemination of female by way of spermathecal apparatus (Michael's organ) opening at the base of legs III or on occasion near coxae IV (Evans, 1989).
2. Division PARASITIDES: Sexual dimorphism in chelicerae less marked, movable digit of male with spermatotreme; genital orifice of male presternal; insemination of female through the use of the genital orifice (Evans, 1989).
3. Division EPICRIIDES: Little sexual dimorphism of chelicerae or sexual dimorphism absent; male's genital orifice in sternal shield situated between coxae III; insemination of female through genital orifice (Evans, 1989).

Adults of the division Dermanyssides may have 3 ventral setae on tibia I. Two or four ventral setae can occur on this segment, but it is not often. However, the Phytoseioidea (Phytoseiidae) are less acceptable in this division and ignore various features of the external morphology of the adult stages (Evans, 1989).

Family PHYTOSEIIDAE Berlese (1816)

Phytoseiid mites inhabits a wide variety of plants including trees, shrubs and grasses where they prey, especially on, tetranychoid and eriophyoid mites. In the absence of living prey various species can survive and reproduce by feeding on fungal spores, pollen and plant tissue. Species of the genera *Amblyseius* and *Typhlodromus* are very important in the control of the red spider mites in Orchards. The potential economic importance of members of this family has lead to a great number of taxonomic work on this group. Phytoseiids can be

classified into two main approaches; the one groups genera into a plethora of generic taxa on the basis of minor differences in the dorsal idiosomatic chaetotaxy and the other in which only a few genera are recognised with the various chaetotactic variants being included in species-groups within the appropriate genus (Evans, 1989).

KEY TO THE SUBFAMILIES OF THE FAMILY PHYTOSEIIDAE

The division of the subfamilies are based on the patterns of the so-called "antero-lateral setae: (j3, z2, z3, z4, s4 and s6) of the podonotal area of the dorsal shield. Four different patterns are visible as follow (Evans, 1989):

1. Six pairs of antero-lateral setae present.....Phytoseiinae Berl.
- Four or five pairs of antero-lateral setae.....2

2. Four pairs (j3, z2, s4, s4) present.....Amblyseiinae Berl.
- Five pairs present.....3

3. Setae z3 present in addition to j3, z2, z4, z4.....Chantiinae Baker & Pritchard
- Setae z3 absent but j3, z2, z4, s4, s6 present.....Cydnodromellinae

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2.4 MITES AS PLANT PARASITES

Plant parasitic mites represent major pests in agriculture that are of critical importance to ornamentals and cultivated crops throughout the world (Nauen *et al.,* 2001; Kim *et al.,* 2004). Mites are very old parasites and in various cases date back around the same time as their hosts. Mites that chose a parasitic lifestyle arose from free-living mites. However, in most of these cases the parasitic form will be strongly modified and it is therefore not possible to reattach them to existing groups of free-living mites. In the process of adapting to a parasitic lifestyle, two different phenomena, completely independent of each other, are involved: namely construction and regression (Fain, 1989).

The constructive characters are characterized by the existing organs or the formation of new structures. When choosing a parasitic life, these specialized structures are considered to be

secondary adaptations. In contrast to constructive adaptation, degenerative or regressive phenomena are characterized by the progressive disappearance of various external structures such as the claws, shields, certain segments of the legs and palps, the chaetotaxy, etc. The chaetotaxy is an important component when studying mites. Some genera are distinguished by their characteristic setal pattern and other species can be identified on their chaetotaxy alone. This nomenclature simplifies the process of describing and comparing species (Fain, 1989).

Harmful mite species feed on and damage plants that are regarded as important to man (agricultural crops and ornamental plants) (Craemer *et al.*, 1998). These crops include tomatoes, berries, eggplants, peppers, tomatillos, and potatoes, all of which are part of the plant family Solanaceae. This plant family also includes tobacco (Nauen *et al.*, 2001; Kim *et al.*, 2004). They also feed on and damage fungi utilized by man (mushrooms), and parasitize humans and their domesticated farm and house animals. They have also been known to infest stored products such as grain, stored bulbs or processed products like cheese and bacon, and serve as a host or carrier (vector) of pathogenic organisms and transmit them to humans, their domesticated animals, and plants. Solanaceous crops are prone to several diseases and mite pests may have severe adverse affects on the production of these crops (Craemer *et al.,* 1998).

The emergence of crop pests challenges the biosecurity of plants. Spider mites (family: Tetranychidae) have emerged as a threat to solanaceous crops in Africa, with invasions characterized by their ability to deliver high reproductive outputs and to withstand a wide range in temperature. In the case of invasive arthropods, mites and other insects have caused annual damage of about $16 billion in the United States alone (Boubou *et al.,* 2010). One can only imagine what the worldwide estimate would be.

The spider mite family includes the most injurious plant-feeding mites. Some infest a wide range of host plants, whereas others prefer a specific host (Ben-David *et al.,* 2007). There are 26 Tetranychoidea species that are regarded as pests in southern Africa, three of which are regarded as serious invasive solanacious crop pests. These pest mites are: *Bryobia neopraetiosa* Meyer (brown lucerne mite), *Bryobia praetiosa* Koch (brown clover mite), *Bryobia rubrioculus* (Scheuten) (brown fruit tree mite or bryobia mite), *Eotetranychus falcatus* Meyer & Rodrigues (cotton green mite), *Eotetranychus lewisi* (McGregor) (poinsettia spider mite), *Eutetranychus orientalis* (Klein) (Lowveld citrus mite or Oriental

mite), *Meyernychus emeticae* (Meyer) (Angolan citrus mite), *Mononychellus tanajoa* (Bondar) (cassava green mite), *Mononychellus progresivus* Doreste (cassava green mite), *Oligonychus coffeae* (Nietner) (red tea mite), *Oligonychus mangiferus* (Rahman & Sapra) (mango red mite), *Panonychus citri* (McGregor) (citrus red mite), *Panonychus ulmi* (Koch) (European red mite), *Petrobia latens* (Müller) (brown wheat mite or onion mite), *Schizotetranychus asparagi* (Oudemans) (asparagus mite), *Tetranychina harti* (Ewing) (oxalis mite), *Tetranychus amicus* Meyer & Rodrigues (African spider mite or banana mite), ***Tetranychus evansi* Baker & Pritchard (tobacco spider mite - invasive solanacious crop pest)**, *Tetranychus hydrangeae* Baker & Pritchard (hydrangea spider mite), *Tetranychus lombardinii* Baker & Pritchard (crimson spider mite), ***Tetranychus ludeni* (dark-red spider mite or red-legged spider mite - invasive solanacious crop pest)**, *Tetranychus neocaledonicus* André (vegetable spider mite), *Tetranychus rooyenae* Meyer (cotton spider mite), *Tetranychus turkestani* Ugarov & Nikolski (Turkish spider mite), ***Tetranychus urticae* Koch (common spider mite or two-spotted spider mite - invasive solanacious crop pest)** and *Tetranychus zambezianus* Meyer & Rodrigues (ARC – PPRI, 2014).

Spider mites are able to disperse rapidly to exploit new feeding sites, and in the process, damaging agricultural and horticultural crops and causing economic loss (Ben-David *et al.,* 2007).

Plant-feeding mites that cause damage to plants are widely distributed as a result of man transporting their hosts, especially those that are valuable for ornamental and agricultural purposes. Several mite species of the family Tetranychidae will either move to the edge of the leaf or lower themselves from the host plant via silk threads. The slightest breeze will release the mite from the host and the thread serves as a balloon or parachute to carry the mite a considerable distance. Unfavourable host conditions and favourable weather stimulates mass "ballooning" (Jeppson *et al.,* 1975).

The biology of these pestiferus spider mites are characterized by a short generation time, high fecundity, rapid dispersal, effective exploitation of new feeding sites and a rapid development of resistance to acaricides (Ben-David, 2008). Population build-up of spider mites are often associated with weather that is dry or hot conditions. Optimal conditions (± 27°C) allows spider mites to hatch in as little as 3 days. They will become sexually mature in only 5 days and females can lay up to 20 eggs per day. Their lifespan is as little as 2 to 4 weeks and

allows them to lay hundreds of eggs. Due to their short lifespan, reproduction rate is high and mite populations are able to adapt quickly to resist pesticides. Chemical control methods can therefore become ineffective over a prolonged period if the same pesticide is used repeatedly (Fasulo and Denmark, 2009).

The cheliceral stylets of spider mites form a needle like piercing-sucking mouthpart that penetrates the plant tissue and allows them to suck out the content and cause mesophyll cells to collapse. This causes leaf area used for photosynthesis to decrease (Ben-David, 2008). When plants are under severe attack, the leaves and fruit will show a necrotic surface and defoliate, causing growth to cease. Ultimately the plant will wilt or dies off. Some spider mites are able to produce a fine webbing that they spin on plant parts and may, in some cases of sever attack, cover the plant completely, increasing the damage (Jeppson *et al.,* 1975). As soon as mites produce large, effective feeding populations, we are able to see the spotty yellowing and curling in leaves that reduce the quality of yields (Nauen *et al.*, 2001).

Spider mite species are able to develop large populations in a very short time span (Creamer *et al.,* 1998). Mites are known to be polyvoltine (producing several generations a year) (Stoeckli *et al.,* 2012) and reproduction includes ovipari (laying eggs), ovivivipari (eggs hatch into females), parthenogenesis (reproduction without fertilization), and arhenotoky (production of males from unfertilized eggs). Females do not need to be fertilised to produce offspring. Therefore biological control remains an important mean of control. Mites are able to duplicate at quite a rapid pace and can therefore be found in very high numbers. It is possible to find thousands of mites in a single plant gall or soil sample, as they are able to increase in number within a short period of time and some are even able to double their population size within 3-4 days. With an arrhenotokus mode of reproduction (females being diploid and haploid males are able to develop from unfertilized eggs), a small number of founding mites or even a single female can initiate a new mite colony that can build up rapidly as a result of several typical 'r-selected' traits (Boubou *et al.,* 2010).

Climate change is likely to complicate the impact of these pests even further. This is caused by a lengthening of the growing season in mid- and high latitudes of the southern hemisphere that has been reported as a clear indicator of the responses by mites to a warmer climate. Spider mite species have been regarded as a harmful invasive species for the past decade. These species (especially *Tetranychus evansi*) have become one of the most important dry

season pests to occur on tomatoes in Africa and can cause yield loss of up to 90% in South-East Africa and West Africa (Boubou *et al.,* 2010).

A maximum of six stages are found in a mite's life cycle (**Figure 2.15**), namely: egg, larva, protonymph, deutonymph, tritonymph, and adult. However, it may vary within mite taxa (Craemer *et al.*, 1998). At a certain stage of the life cycle (depending on the species) mites go into diapause. Diapause allows mites to adapt to their surrounding environment, as this is a period during which growth or development is suspended and physiological activity is diminished in response to adverse environmental conditions. Diapauses can occur when it is induced by environmental conditions and in some species is an obligatory part of the life cycle, where mites may be stimulated by certain stages by cold weather, extremely dry weather or rain. This ensures that mite populations are able to survive when e.g. a rain storm washes the rest of the population away (Danilevskii, 1965; Tauber *et al*., 1986).

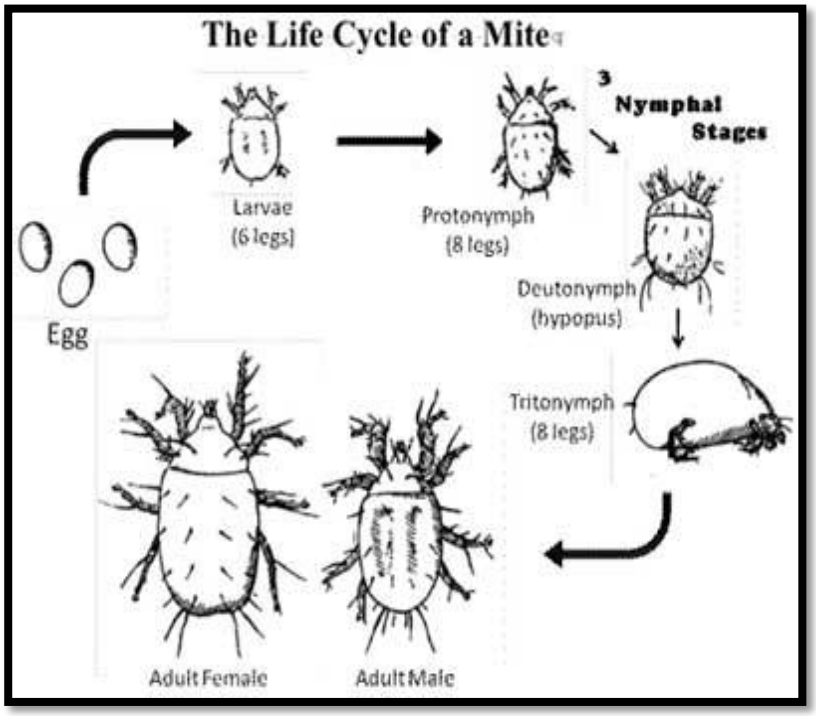

Figure 2.15 Life cycle of mites (Anon 1, 2011)

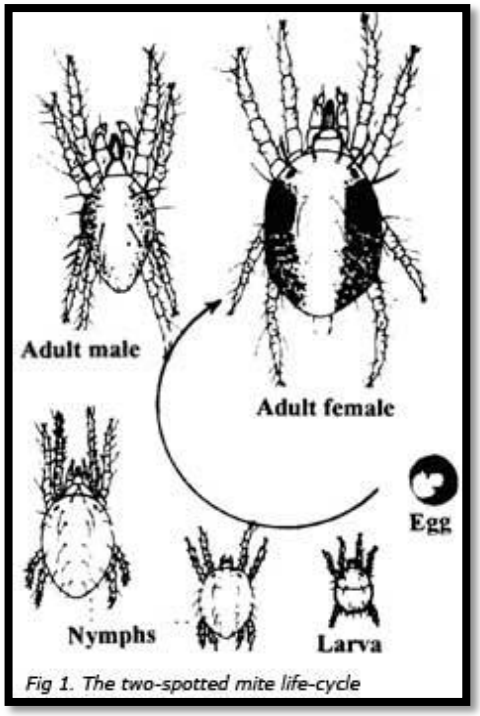

Figure 2.16 Life cycle of spider mites (Anon, 2010)

The life cycle of spider mites differ from that of other taxa. As mentioned, mites contain six stages, but spider mites lack the fifth stage, the tritonymphal stage, as is evident in **Figure 2.16**. These mites contain only the nymphal stages, protonymph and deutonymph, and these deutonymphs then turn into female/male adults, thus shortening the period it takes from egg stage to egg stage.

2.5 ADAPTATION TO PREDATION and THE NEED FOR BIOLOGICAL CONTROL

Mites can play a positive role in agriculture as well. Predators and their importance in agricultural systems have been ignored for a long time. Today, farmers are becoming more aware of their importance, due to the fact that spider mite species have become more resistant to certain pesticides. (Craemer *et al.*, 1998).

Commercial fields have been repeatedly making use of broad spectrum insecticide due to plant-parasitic mites' high reproductive potential and numerous annual generations, but widespread use of pesticides causes serious ecological problems. These pesticides kill not only plant feeding mites, but non-target organisms as well, including insects and mites that prey on these pests, and they tend to threaten human health. There are various effects pesticides can have on the health of humans; this however, depends on the type of pesticide. Some pesticides, such as organophosphates and carbamates, will affect the nervous system, where others will only irritate the skin or eyes. One of the major threats of pesticides includes residual chemicals remaining on crops, which can lead to evolution of resistance to these chemicals among spider mite populations. The high level residues of pesticides in fruit and vegetable products may pose a significant problem in terms of trade and food safety. Researchers have noted that there is an increased frequency of resistance (among tetranychid mites) to current existing pesticides, even if they contain different chemical structures (Nauen *et al.*, 2001; Kim *et al.*, 2004). The increased consumption of synthetic pesticides has led to a number of problems developing. Besides the fact that insects and mites become resistant to pesticide, some chemicals are unable to break down but accumulate in the food chain and cause environmental pollution and an ecological imbalance (Craemer *et al.*, 1998).

Acarologists are now investigating *Bacillus thuringiensis* (*Bt*) toxin expressed by transgenic crops, as studies have shown that they do not have a negative effect on *Tetranychus urticae*. In fact, these recent studies show that *T. urticae* prefer to feed on transgenic plants that express *Bt* over control plants, where mite females even laid significantly more eggs on Bt-leaves than on control plants (Grbic *et al.*, 2008). Alternatives to the use of pesticides are needed due to their negative effects, and biological control agents (predatory mites) provide one of the best options.

We can make use of two types of biological control methods. The first is natural biological control, where these predators are usually found in a natural ecosystem. These predatory organisms keep the population levels of the plant feeding mites at a natural level. As soon as these predatory agents are able to suppress the potential pest organism to an acceptable level, we then term them as being a natural biological control agent. We can also apply these predatory agents into nature and they are then known as induced or classical biological control agents (Craemer *et al.*, 1998).

Species from predatory families can be relatively small, 300-600µm long, and their colour ranges from white, light brown, red to dark brown, these mites feed on tetranychidae, eriophyids, acarids, tarsonemids, tenuipalpids, tydeids, pollen, honey-dew, plant juices, trips, crawlers of scale insects and eggs of citrus moths. Some species can act as facultative predators (Ueckermann and Loots, 1988).

The potential of facultative predators for biological pest control has been neglected over time due to the risk that crop feeding can cause damage economically and predation has been overlooked when compared to plant feeding. However, there are some advantages to facultative predators in the use of biological control. Management programs should therefore minimize their risks and maximize their benefits (Capinera, 2008).

There are two main advantages for using facultative predators in biological control. Firstly, they possess the capacity to establish themselves early in the crop field and thus prevent pests from building up large numbers within a population. For annual crops that have to be re-colonised every season, this trait is extremely positive to be found in bio-control agents. Secondly, it is possible for them to feed on plants when prey densities are low, thus preventing predators from migrating or even extinction. Unfortunately, there are some disadvantages to facultative predators that act as biological control as they do cause damage

to crops when feeding on plants, such as pollen-feeding phytoseiid mites (Acari: Phytoseiidae). These risks are depended on a number of factors such as (1) the amount of plant feeding vs. prey feeding, (2) the type of species it chooses as its host-plant and cultivar, (3) the feeding time on plants and crop phenology, and (4) the tissue fed on by predators. The relationship between the two types of feeding will be negative when predators choose to switch between plant and prey as alternative sources due to nutrients obtained from plants corresponding to those derived from prey. If predators prefer prey to plants, phytophagy will only occur when prey sources become scarce. It is therefore difficult in biological control (Capinera, 2008).

Pollen-feeding mites, such as some phytoseiid mites for example, or mites that feed on extra floral nectar will rarely, cause high crop damage, but damage can be severe when the plant part preferred by facultative predators are fruit, or moderate or even nil if leaves are preferred (Capinera, 2008).

Generalist predators on the other hand have a variety of positive traits, e.g., they are able to respond quick to a sudden increase in pest populations, high dispersal capacity, aggregative responses to patched prey distribution, they have the ability to survive at low target-prey density in essence, for adaptation to changing environments and prey densities, such as those found on crops that are planted annually (Capinera, 2008).

When choosing prey, it is noted that some species of predators, such as *Amblyseius teke* Pritchard and Baker (Acari: Phytoseiidae), are attracted by *Tetranychus* (Acari: Tetranychidae) species webbing, whereas other species such as *A. potentillae* Garman for example, prefer prey species that produces less webbing. Some species suppress prey that aggregates effectively. Pollen-feeding facultative predators often reproduce better on pollen than they would on spider mites and trips, but they are able to suppress tetranychid mites best when pollen is also available (Ueckermann and Loots, 1988).

Mites belonging to the family Phytoseiidae are probably the most effective and widespread predators of plant-feeding mites. They have a worldwide distribution, from the arctic to the tropics and one species or another has adapted to its surroundings (Jeppson *et al.,* 1975). Most predaceous mites found in the Phyotseiidae family are used as biological control agents to control pest mite populations and prevent or minimize damage to crops.

Only females that have mated will overwinter in temperate climates. Just as it is with plant-parasitic mites, the winter mortality for predaceous mites may be high, but this mortality is influenced by temperature extremes and whether or not there is an availability of protected places. These predatory mites are sometimes killed by early frost before they are able to reach protective winter quarters, although some species may survive temperatures of -29 to -31°C. Conditions that are considered to be unfavourable for predaceous mites are sometimes favourable for parasitic mites (Jeppson *et al.,* 1975).

Predaceous mites have diverse feeding habits; some are strict carnivores, while others prefer plant or plant-derived foods such as pollen and nectar. Some of these mite predators are particularly specific as to the mite species upon which they feed, and some strongly prefer webbing plant-feeding mites. Others prefer species that live in colonies rather than those distributed over the leaf surfaces. Some predaceous species are known to feed on species with a general distribution, as they are hindered by the webbing produced by spider mites (Jeppson *et al.,* 1975).

The number of mites a predator requires determines how effective it can regulate a plant-feeding population. Not all phytoseiid predators of plant-parasitic mites are completely dependent on mites for their food, and some require alternative food for maximum reproduction. The presence of various alternative available food in the field may have an important impact on the predator-prey interaction. In order to keep prey at low population levels, predators must (i) be able to search out and capture their prey, and (ii) be adapted to the type of habitat where their preferred prey lives. The external morphology of a plant may have an influence on the activities of predaceous mites and their resulting effectiveness. Sometimes the habits, distribution and activities of predaceous mites do not coincide with the most common parasitic mite prey (Jeppson *et al.,* 1975).

Some predacious species deposits most of their eggs on the undersides of downward curled leaves. This provides maximum protection against adverse weather, but, because these leaves often contain no prey, the newly hatched progeny are not ideally located to obtain food unless there is a high density of prey. However, some phytoseiids adapt their habits and activities to coincide their prey availability (Jeppson *et al.,* 1975).

There are several known predaceous mites among the family Stigmaeidae. This family is usually not able to keep tetranychid mites in check by itself, but it is occasionally able to

maintain populations at low densities. These predators assist other predators in regulating populations of important agricultural pest mites (Jeppson *et al.,* 1975).

Predaceous mites need some of the following characteristics in order to be effective pest predators (Jeppson *et al.,* 1975):

1. short developmental period - shorter than the developmental time of its prey;
2. should contain high reproductive potentials;
3. should be able to consume large amounts of prey or be able to survive on only a few;
4. should prefer the same host plant as prey;
5. searching capacity at low densities should be effective;
6. should prefer the same micro-habitat as prey;
7. season cycle should correspond with that of their prey;
8. should be able to tolerate extreme weather conditions as well as their prey does; and
9. should be able to tolerate pesticides.

No predator possesses all of these qualifications, but each contains some of these characteristics. Various predaceous mites have a shorter life cycle than their prey, equivalent reproduction potential, good searching capacity, and the ability to survive on a small amount of prey. This is especially true for mites feeding on other food sourced, but most phytoseiid mites are limited in the amount of prey they can consume (Jeppson *et al.,* 1975).

2.6 MITE TAXONOMY and IDENTIFICATION

In order to improve effective mite control, the key factor is new diagnostic methods to distinguish between different species or species that are closely related or sister species. These methods includes the use of taxonomy, new techniques [molecular and scanning electron microscopy (SEM)], and coordination of the studies between mite and host relationship and predator-prey associations. The involvement of morphological descriptions, make use of direct observations of phenotypic differences between mites. Although this technique has widely been used in acarology, it may not be as useful to discriminate between species that are morphologically very close (sibling species). Furthermore, intraspecific variability of these characteristics can, in some cases, complicate the determination of taxa.

Acarologists have noted over the years that intra-specific variation in species, such as tetranychids and phytoseiids, have resulted in numerous synonymous species. For taxonomic purposes, the original descriptions or systematic keys are used to establish species identification. For mites we make use of external morphological characteristics, such as dorsal chaetotaxy, setae of legs, reticulation pattern, shapes of spermatheaca, structure of spermadactyl and aedeagus, as well as the size and shape of dorsal setae. Misidentification occurs when there is a lack of consistency in these characters for mite species (IRSES, 2011).

Their body segments of the idiosoma, are fused into one, usually oval or a rounded unit. It is common to find mites with eight legs, but mite larvae bears six legs and some mite groups only have four legs as an extreme adaptation to parasitism in the microhabitat they live in. Through this we can distinguish them from insects containing six legs. They also do not bear antennae or wings as insects do (Craemer *et al.*, 1998).

Mites can be divided into seven orders, according to the position and presence/absence of their stigmata and peritremes (breathing apparatus). **Table 2.4** below indicates the four orders to which mites on plants belong (Craemer *et al.*, 1998):

Table 2.4 Orders of Plant Mites (Creamer *et al.,* 1998)

	Mesostigmata	**Sarcoptiformes (Cohort: Astigmata)**	**Oribatei**	**Trombidiformes (Sub-order: Prostigmata)**
Stigmata and Peritremes	Stigmata and peritremes present next to coxae of legs II-IX	Stigmata and peritremes absent, breathing through skin	Stigmata and peritremes at the base of leg I and II or opening on legs I and III	Stigmata and peritremes on anterior part of podosoma between chelicerae
Including species	Includes mostly predators of parasitic mites (e.g. Phytoseiidae)	Includes saprophytes, fungivores, free-living and parasitic mites	Includes mites feeding on rotting plant material and fungi	Includes plant-feeding mites (families Tetranychidae, Tarsonemidae and Eriophydae); predatory mites and animal parasitic mites

The gnathosoma resembles the head of the mite (**Figure 2.17**) only in that the mouthparts are appended to it. The brain is situated in the idiosoma and is behind the gnathosoma rather than in it. The eyes are located dorsal or dorsolateral on the propodosoma. A paired chelicerae, which is generally three-segments, are found above the mouth. The chelicerae and pedipalps on the gnathosoma form the organs for which food is acquired. The palps may act as simple sensory structures that are equipped with chemosensory or thigmotropic hairs that aid in food location. In some mites they are modified into a grasping or piercing raptorial organ (Jeppson *et al.,* 1975).

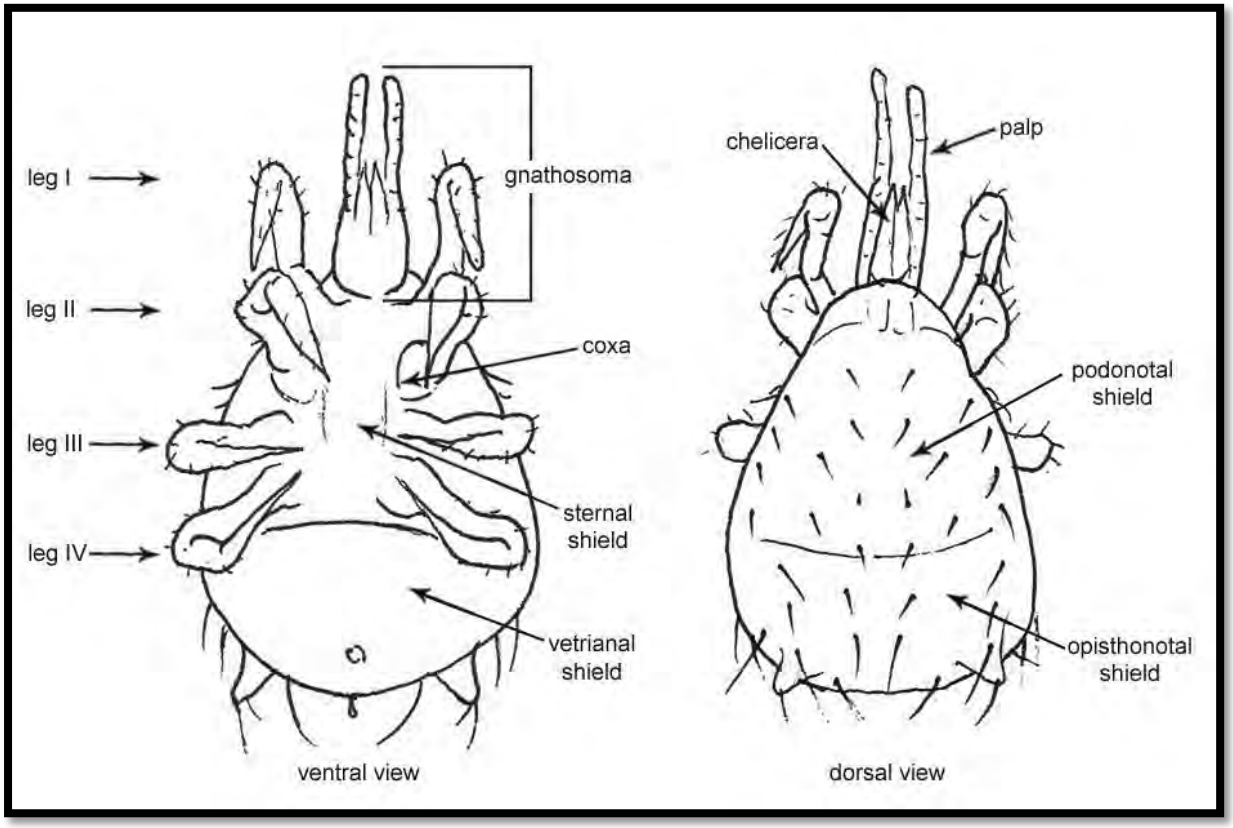

Figure 2.17 Dorso-ventral view of mite female to reveal general features of mites (Anon, s.a.)

The chelicerae may vary in structure, but they are never primarily sensory. The terminal third segment of the chelicera is generally modified into a digit that is movable and opposes the fixed distal portion of the second segment. These opposed digits, also known as chelae, are edentate or toothed for grasping or grinding. The chelicera may be attenuate or elongate in

some parasitic or plant-feeding groups, and serves as a piercing organ. It is not uncommon to find the fixed digit to be reduced on predacious or phytophagous species. The movable digit, developed into a styliform piercing structure, accompanies this reduction (Jeppson *et al.,* 1975).

Mites that belong to the family Tenuipalpidae, is generally referred to as false spider mites or flat mites, and they are primarily plant feeders. They are rarely detected by observers, due to their small size and sluggish activity; yet they may be found on most perennial plants. These mites contain a relatively long life cycle and a population will therefore increase slowly. This allows predators to prevent these mites from developing injurious populations (populations that damage plants and causes yield loss) (Jeppson *et al.,* 1975).

Mites belonging to the family Tydeidae are common fungi eaters. They also feed on decaying material, but some species feed on mites, whereas others may even cause damage to plants (Jeppson *et al.,* 1975).

The family Phytoseiidae are common predators that act as biological control agents in controlling mite pests on crops around the world (Kanouh *et al.,* 2010 and Okassa *et al.,* 2012). This family contains three sub-families and 84 genera, and is essentially defined by their idiosomal chaetotactic patterns (Kanouh *et al.,* 2010). The success of biological control depends greatly on the accurate identification of these predatory mite species and whether or not a certain species will aid in the control of a pest species. Unlike plant-feeding species, such as Tetranychidae, where males are also required to verify a species, species diagnostics of predatory species such as phytoseiid mites, are essentially based on the morphological characters of females, especially leg and dorsal chaetotaxy (especially setal length), spermatheca shape and cheliceral dentition. Thus, when only immature stages and/or males are collected, their identification becomes difficult and is sometimes even poorly supported. Molecular tools could be of great help to overcome these difficulties, as it is assumed that molecular sequences are identical for considered life stages (Okassa *et al.,* 2012).

For pest species, correct identification is of paramount importance for quarantine and management purposes: the development of biological and other control strategies (Ben-David, 2008).

The reproduction potentials, prey consumption capacities, and prey preferences of predators differ from species to species. The correct identification of collected predatory species is thus of utmost importance before a predator can be registered as a biological control agent (IRSES, 2011). The possibility may always arise that different species could contaminate a colony during mass-rearing, which could lead to unintended release of unwanted species into the field and could result to a failure in applying the correct dose of the intended species (Jayaprakash and Hoy, 2009). This is a critical component of any control effort with reference to plant-feeding mites, due to the amount of gene flow between populations; as they determine the diffusion risk of agronomically important genetic traits, e.g., virulence, insecticide resistance, host plant specificity, etc. However, it has been recorded in previous reports that reproductive incompatibility occurs within spider mite species such as *Tetranychus urticae* Koch, *Panonychus citri* (McGregor), and *P. mori* (Yokoyama), depend on geographical divergence (Osakabe and Sakagami, 1993). On the other hand, the family Tetranychidae is an invasive species and the recent introduction of *T. evansi* from South America has created novel problems in agriculture worldwide (Escudero and Ferragut, 2005). The transport of pest species to new biogeographic regions increase annually, and knowledge of the species geographic variation patterns can be very useful in tracing accidental pest introductions (Grbic *et al.*, 2008).

Identification and delineation of species in the family Tetranychidae has been debated over the past few decades. About 1200 different species have been described within the family, many of which are agronomically important. *Tetranychus urticae* Kock, 1836 and *Tetranychus kanzawai* Krishida, 1927 are two common major agricultural pest species that have been well studied due to their worldwide distribution. However, morphological identification of tetranychid species are difficult, as a number of potential diagnostic characters are limited (partly due to the small size of mites) and key traits are even able to exhibit large phenotypic plasticity. Many species are therefore unable to be distinguished on the basis of external morphology. In some species, females alone can be used to identify the species, whereas others require a microscopic examination of the shape of the aedeagus (part of the male genitalia) (Ros and Breeuwer, 2007).

Besides species identity, molecular methods are also able to determine the origin of invading species (Ben-David *et al.,* 2007) and are able to reveal close genetic relationships among some members of the spider mites. DNA-based identification holds several advantages over

traditional morphological methods and one does not need comprehensive knowledge of the morphology (Li *et al.,* 2010). However, this has lead to sequences being wrongfully deposited into the GenBank. It is important to combine a molecular study with a morphological study to prevent this from happening.

The identification of mites can be simplified by accumulating a large number of mite sequences, especially from genes such as the mitochondrial *cytochrome oxidase I* (*COI*) and the transcribed spacer region (ITS1 and ITS2) of nuclear ribosomal DNA that acts as a scaffold of a molecular method. In terms of identification purposes, this would however facilitate the use of species-specific sequences as DNA "biological barcodes" (Ben-David *et al.,* 2007).

Mite varieties maintained in gene banks are much more useful in predator breeding if they have been well characterized for important agricultural traits. For example, if farms suddenly face an outbreak of pesticide resistant spider mites, they search for potential predators in the gene bank. If predators in the gene bank have already been characterized for resistance, then farms and researchers do not need to conduct large-scale experiments to identify resistant genotypes.

Furthermore, the correct identification of spider mites and predatory mites present is a determining factor for implementing adequate management strategies.

There is thus a need for a detailed study of common plant parasitic and predatory mite species, especially sibling species and cosmopolitan species, which involve both molecular markers and the comprehensive morphological review through the use of SEM.

2.7 INTRODUCTION INTO MOLECULAR PHYLOGENY OF ACARI

Applying molecular techniques to the study of mites grants new understanding into the population structure and taxonomic relationships (Ramadan *et al*., 2004) and provide a solid foundation for constructing a phylogenetic hypotheses (Navajas *et al*., 1992). These applied methods are somewhat similar and effectively facilitated the identification of species that are

difficult to classify taxonomically, leading to an understanding in population structure and clarifies phylogenetic relationships among species (Ramadan *et al*., 2004).

For classification purposes, we mainly use morphological descriptions. However, molecular techniques are advantageous in that they allow for the potential to study DNA at individual base-pairs and indicate the direct way to measure and quantify the genetic variation within and between species (Ramadan *et al.,* 2004).

The introduction of molecular biological techniques, especially DNA amplification through the use of polymerase chain reaction and DNA sequencing, brought forth reasonable costs of applying molecular markers based on nucleotide sequences and made them more available (Dabert, 2006). DNA fragments that are amplified by polymerase chain reaction (PCR) can be examined for polymorphisms by acquiring the nucleotide sequence that grants access to the details of the differentiations in DNA (Ramadan *et al.,* 2004). Molecular markers have proven to be very useful in systematics and evolutionary acarology. DNA sequences have shown to be successful in studying the phylogenetics of mesostigmatid, sarcoptiform, and trombidiform mites. We have now reached the point where using molecular data in taxonomy and the genetic structure of a population has become a standard. Most of the mite DNA sequence data in the GenBank is Expressed Sequence Tags (ESTs) and represent genes that are expressed by mites, especially the species *Dermatophagoides farinae* and *Blomia tropicalis* (Dabert, 2006).

Up to present time, most acarological phylogenetic studies have placed their focus on groups of mites that hold economic importance such as the Tetranychoidea and the Phytoseiidae. The wide variety of mites makes them ideal for these types of studies as they represent unique opportunities to test various evolutionary hypotheses (Cruickshank, 2002).

2.8 MITE GENETIC STRUCTURE

As in other metazoans, mites have a circular mtDNA genome that contains about 18 000 genes. Of these 18 000 genes, 15 000 are used to make proteins. One major non-coding region occurs that is thought to play a role in the initiation of transcription or replication (or

both) of the DNA molecule. Their genome consists of 90 megabases [90 million base pairs of DNA bases (A, C, G and T)] (Navajas *et al.,* 2002). This is known to be the smallest arthropod genome that has been sequenced.

The ribosomal DNA (rDNA) region in eukaryotes consists of three highly conserved regions (18S rDNA, 5.8S rDNA and 28S rDNA) and two rapidly evolving regions, the internal transcribed spacer regions (ITS1 is located between 18S rDNA and 5.8S rDNA and ITS2 is between the 5.8S rDNA and 28S rDNA) (Ben-David, 2008).

Mesostigmatic mites tend to be free-living predators with a short lifespan and this will results in a haplodiploid genetic structure. Despite the short lifespan of Trombidiformes members, they do show great diversity in their genetic systems (Young *et al.,* 2012).

Mites are the most abundant and diverse group of arthropods, but it is rarely seen that they are targeted for detailed biodiversity studies due to constraints in their taxonomy (Li *et al.,* 2010). It is due to this reason that a molecular review is so advantageous. Unfortunately, molecular studies on mites are quite new and DNA sequences of various species are absent from the GenBank.

Morphological characters were previously used to determine the systematics of this group of organisms, however, these are not always easy to observe and many vary. Nevertheless, molecular analysis used in taxonomic classification provides a solid foundation for phylogenetic hypothesis. By comparing sequences of small subunits of ribosomal RNAs or their genes, one is able to compare closely and distinct taxa. PCR and chain termination sequencing of the *ITS* gene was used as the first step in the molecular study of the phylogenetic relationships of the mites to assess the level of variability among species (Navajas *et al.,* 1992) followed by the *COI* gene.

Molecular methods have revolutionised acarological systematics and are increasingly being applied to mites (Li *et al.,* 2010). DNA sequencing has become more important in systematics and routine identification (e.g., barcode initiative), and this will contribute in the future to address a large palette of questions. It is therefore crucial that associated sequences of wrongful identified species do not contaminate the valuable resource that the databases represent (de Mendonça *et al.,* 2011).

It is important to distinguish between DNA taxonomy and DNA barcoding. DNA taxonomy uses the evolutionary species concept to circumscript and delineates species, whereas DNA barcoding aims to identify and pre-define species and does not necessarily address species delineation per se. DNA taxonomy may serve as a database for DNA barcodes as it is based on one to several mitochondrial and nuclear DNA regions. DNA taxonomy acts as an offshoot of phylogenetics where we can investigate the evolutionary relationship between different mite species (Ros and Breeuwer, 2007).

Using DNA sequences in phylogenetics

When thinking about molecular phylogenetics, we assume that the differences in the nucleotide sequence between genome pairs reveal how long these two genomes share a common ancestor. Without question, sequences of DNA molecules provide the most circumstantial set of data for phylogenetic studies. However, the problem arises when deciding which fragment of which genome one should compare. Deciding on a molecular marker in a particular analysis is the main issue to address due to the rate of substitution of the sequence fragment. If the rate being studied is inappropriate for the level of divergence, it can cause misleading results or data (Dabert, 2006).

Keep in mind

When choosing a molecular marker, it is best to choose one that is present as a single copy in the haploid genome. The reason for this is that multiple-copy genes in sequences from different individuals in the phylogeny can be from different (paralogous) copies of this particular gene. However, multiple-copy genes can be advantageous if all sequenced copies are the same and the copy sequenced does not matter. These genes are usually easier to amplify and can lead to a higher yield. The two most frequently used groups of genes in phylogenetics that fall in this category are the nuclear ribosomal genes and the mitochondrial genes (Cruickshank, 2002). The mitochondrial genome, which is a singular copy, prohibits paralogous copies of genes (Dabert, 2006). Cells contain a large number of mitochondrial genes, but due to the population bottleneck effect, sequences in these copies are the same (Cruickshank, 2002). The nuclear mtDNA (including copies of mitochondrial genes) in Eukaryotic genomes, including arthropods, are transported into the nuclear genome (Dabert, 2006). Even if the ribosomal genes are more difficult to align, they are more likely to contain informative sites than protein-coding genes. If one removes the unaligned regions from the

ribosomal gene, it is still left with more informative sites than present in a protein-coding gene made up of the same length (Cruickshank, 2002).

Internal transcribed spacers (ITS1 and ITS2)

Spacer regions in the nuclear rDNA array can be used to form a conclusion on the phylogeny among closely related taxa that branched off from its ancestor in the last 50 Mya. Due to its rapid evolution, variation in these regions can be used for species and strain identification. During PCR amplification, conserved flanking regions of the 18S and 28S rDNAs assist the ITS1 and ITS2 sequence (**Figure 2.18**). However, a high substitution rate can cause the usefulness of these spacers to become unfavourable due to intraspecific or even intraindividual diversity of sequences. Studies on the ITS gene become more laborious and expensive due to this diversity that is caused by the amplified ITS fragment's need to be cloned in plasmids and then sequenced individually. Despite problems that may arise, the ITS sequence has been used successfully in various Acarological phylogenetic problems, especially in determining species status and studying the history of a species (Dabert, 2006).

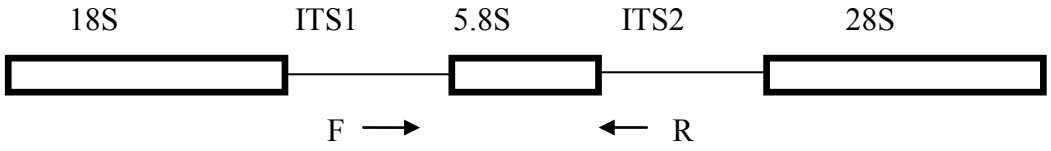

Figure 2.18 Schematic representation of ribosomal genes containing the areas targeted by the ITS primer.

In 1992, Navajas *et al.* published a paper where they found that when pairs of the nucleotide positions are compared, the substitution rate within genera was low and allows for good alignment (Ramadan *et al.*, 2004). For the research study done by Navajas *et al.* (1992) they made use of primers in the 5.8S and 28S rDNA gene to amplify the ITS2 region in a variety of Tetranychidae species and revealed the relevance of examining phylogenetic relationships within genera, but that differences between genera were to vast to allow for correct alignment of sequences. They also found that *Tetranychus* species are morphologically very close, but a significant amount of genetic divergence occured within the Tetranychidae family. For this study they made use of a combination of sequences between the ITS2 sequence and *COI* to

investigate intraspecific variations within *Tetranychus urticae* species and concluded that species-wide homogeneity of the ITS2 sequence occured, but the *COI* gene lead to extensive polymorphism. By adding more taxa to the study, Navajas *et al.* found that it lead to very low levels of variation at the ITS2 locus in this particular species (Cruickshank, 2002).

In the same study they constructed a phylogenetic tree of the genus *Tetranychus* where they reached the conclusion that the morphology is in agreement with the phylogeny. Aside from the aedeagus in males that may differ in shape, species appear to be closely related and present little sequence divergence. Even if a clear distinction can be made between the aedeagus shape of different species, they found it evident that species of the genus *Tetranychus* are morphologically and molecularly close (Navajas *et al.,* 1992).

Mitochondrial rDNA and protein-coding genes

The whole mtDNA molecule is involved in coding genes, excluding the control region (a long non-coding sequence) comprised of regulatory elements for the replication of mitochondrial genomes. Of these 37 genes, only a few have been sequenced for mite studies (this includes fragments that code for *cytochrome oxidase* subunits). Furthermore, the large rRNA subunits and the *cytochrome oxidase I* gene fragments (*COI* – more than 500 fragmentary sequences) are the markers used most often for deducing relationships at low and intermediate levels (Dabert, 2006). The *COI* and ITS2 gene has a similar range of uses, but the *COI* gene has a higher evolutionary rate than the ITS2 gene. Regardless of the high supstitution rate of this gene, its effectiveness is not limited to closely related species. Another advantage for using the *COI* gene is when taking a deeper level phylogeny approach, where this gene eliminates the hypervariable third codon position from the analysis (Cruickshank, 2002).

2.9 GENE FLOW, SPECIATION and MOLECULAR DIVERGENCE

Gene flow between various populations of the same species is important. The exchange of genes will determine the homogeneity of that species. A considerable amount of interest has occurred in mitochondrial DNA (mtDNA) in evolutionary studies. Mites form part of a

highly diverse group, containing 388 families, and including species of agronomic and medical importance. However, little is known about the organization and mode of evolution of this diverse group's mtDNA (Navajas *et al.,* 1996). Data collected during this study will be used to infer the mitochondrial genetic code in mites and reveal variations that occur in their mtDNA sequence composition. Inferring phylogenetic hypothesis based solely on morphological characters can be difficult due to convergent evolution. Molecular systematics can contribute to this area, whereas molecular markers allow taxonomic questions in acarology to be answered. In addition to phylogenetic analysis, biogeographic analysis provides a context for phylogenetic and evolutionary scenarios (Kanouh *et al.,* 2010).

The study of mites' population genetic structure appears as a powerful approach to estimate gene flow among mites infesting different plants in the agro-ecosystem. Different molecular techniques, such as microsatellite markers isolated in mite species and the sequence of mitochondrial DNA gene coding for *cytochrome c oxidase I* (*COI*) are used in mites to study inter- and intraspecific variation among populations. Microsatellite markers are chosen in this ecological study due to their high polymorphism. The use of acaricides in commercial fields would result in a genetic bottleneck in the founder population and reduce the population's genetic variability (Sabater-Muñoz *et al.,* 2012).

Intraspecific diversity is common in tetranychid mites, and is shown by the differences in both morphological characteristics and behaviour towards an environmental factor or in enzymatic systems. However, haplo-diploid sex determination (the most common reproduction system in Tetranychidae species) contains particular implications in regard to the maintenance of genetic diversity. In recent years, species diversity has become much easier to characterise through the evolution of genetic variability by using nucleotide sequences of DNA. This has made it possible to determine the status of studied taxa (Navajas *et al.,* 1994).

2.10 DNA BARCODING

We make use of DNA barcoding to identify and assign unknown specimens to species that have been described before and enhance new species discovered by using a threshold of sequence divergence (Ros and Breeuwer, 2007). Identification and discovery of mites has been accelerated by DNA barcoding and by analysing sequence variation in base pair segments of the *COI* and *ITS* gene (Young *et al.,* 2012) but with the increase in the amount of sequences being deposited in public DNA sequence databases, concern about taxonomical misidentification of specimens used to obtain these sequences is also growing (de Mendonça *et al.,* 2011). Nevertheless, DNA barcoding has been successful in various mite families and reveals that intraspecific sequence variation is consistently low, as little as one percent, while interspecific divergence can exceed 2% (Young *et al.,* 2012). This reveals the importance of combining a molecular study with a morphological study. DNA barcoding is a method proposed to classify mites to species level using a fragment of mitochondrial DNA, usually form the 5' region of *cytochrome oxidase subunit I* (*COI*) (Li *et al.,* 2010; Ros and Breeuwer, 2007). *COI* is well suited for population and species identification due to its high degree of polymorphism and is used to delineate tetranychid species. The ITS2 (internal transcribed spacers 2) sequence is also used to distinguish between mite species, while the ITS1 sequence is used less often (Li *et al.,* 2010).

The use of DNA barcodes (short DNA sequences from a standardized region of the genome) acts as a tool to facilitate species identification and discovery. The second internal transcribed spacers of nuclear ribosomal DNA (rDNA-ITS2) barcodes effectively discriminate among mite species. The barcode sequence of each species allows them to be distinguished from all other species and forms distinct, non-overlapping monophyletic groups in the maximum-parsimony tree (Ben-David *et al.,* 2007).

Due to various taxonomists questioning a number of mite families, the congruence in patterns of sequence variation across different taxonomic lineages allows the use of DNA barcodes to explore biodiversity in families that lack a well-developed taxonomic framework. DNA barcoding is becoming a standard practice in assessing diversity of poorly known groups as it allows biodiversity to be quantified and is reproducible (Young *et al.,* 2012).

DNA barcoding classifies mites to species level through the use of a mitochondrial DNA fragment, usually from the 5' region of *COI*. *COI* is ideal for population or species

identification due to its high degree of polymorphism. Several studies have used the *COI* region to delineate mite species. The ITS sequence is also used to distinguish species (Li *et al.,* 2010) but it is used less frequent.

In this paper, species are described using both morphological and genetic data (based on ITS and *COI* sequences) which allows phylogenetic comparisons and permits a more accurate determination of the systematic position within the genus.

SECTION A: MORPHOLOGICAL REVIEW

CHAPTER 3: MATERIALS and METHODS

A combined approach based on morphological and molecular data was undertaken to ensure that species identified and their attributed DNA sequence is reliable. Section A (CHAPTER 3 and 4) of this study will thus focus on taxonomy of mites and include morphological reviews. Section B (CHAPTER 5 and 6) will contain the molecular review and also follow a systematic approach.

SECTION A: MORPHOLOGICAL REVIEW

CHAPTER 3: MATERIALS and METHODS

3.1 COLLECTING and MOUNTING METHODS

Mites are very small and for identification purposes they usually need to be mounted on slides. We therefore used special techniques to collect and preserve them. A survey was conducted during peak seasons (September 2012 to April 2013) to provide enough samples of both pest and predatory mites. Morphological analyses were performed on male and female body shapes. Specimens were collected from 11 sites in 3 provinces. Collected provinces include KwaZulu-Natal, Gauteng, and North-West (**Figure 4.1** and **Table 4.1**).

3.2 ETHICS STATEMENTS

No permits were required for above mentioned collections as collection did not involve the sampling of endangered or protected species. However, permission was required for some sample locations as they were on privately owned properties.

3.3 GENERAL HANDLING OF PLANT INHABITING MITES

During all the methods, the mites were collected and manipulated with a fine paintbrush and were preserved and collected in plastic vials with 75% and 96% ethyl-alcohol. Seventy five

percent ethanol was used for light microscopy and mites were then carried over to 96% ethanol for molecular work.

3.4 COLLECTING IN THE FIELD

For the collecting of samples, the following is needed: 10x-hand lens, 75% and 96% ethyl-alcohol, pruning-shear, size 0 camel-hair paintbrush, plastic vials, marker (a pencil), labels and small glass vials.

For identification of mites in the field, a 10x-hand lens is essential, but it is necessary to identify them in a laboratory with a stereomicroscope. Leaves are cut off of different parts of the plant; three at the top, three in the middle, and three from the bottom. Plant material was shaken in larger bottles containing 75% ethyl-alcohol, to wash the mites off the material.

3.5 MOUNTING

In the laboratory specimens in each analytical unit were sorted into families under the stereomicroscope and predatory specimens were selected for sequence analysis. The selected mites were then transferred to smaller glass vials containing 96% ethanol for three to twelve months at -20°C to preserve the DNA.

Mites excluded from sequence analysis were left in ethyl-alcohol for at least 10 days before they were mounted in PVA medium (mounting and clearing medium). Both males and females were collected and mounted. The females were mounted dorso-ventrally and the males laterally so that the aedeagus can be seen in profile.

The mites were mounted in PVA medium as follows: (i) a drop of PVA medium was placed in the middle of a clean slide, (ii) a mite was then transferred to the drop with the paint brush and manoeuvred till it was lying in the middle of the drop, (iii) a cover slip was held on its

edge on the side of the drop, touching the PVA medium. It was then lowered gently onto the drop containing the specimen, and (iv) after all the mites were mounted, they were allowed to dry on a heat plate at 35 °C for 24 hours. As soon as they were dried, the slides were ringed with entellan to make them permanent.

3.6 LABELLING

Mites that were collected for this taxonomic study were labelled. All available information pertaining to a specimen was recorded on the labels attached to the specimens. Specimens that were processed were also labelled, to prevent collection data from being lost. The following information accompanied the batch of collected mites; host plant, locality, collector and a description of the symptoms and collection method (if possible).

3.7 MITES MOUNTED ON SLIDES

Slide mounts were stacked back-to-back with cardboard spacers separating the slides, using blank slides on either side for bracing and finally taped together to form a sturdy unit. These slides were then packed in specially made, commercially available boxes after they were identified.

3.8 BIOLOGICAL SAMPLE PREPARATION FOR SEM

(Mites on plant material)

(i) Firstly the material was fixed in 70% ethanol for 2-8 hours, (ii) it was then dehydrated in an ethanol series, 80%, 90%, and x2 100% for 15 – 30 min. During this process, samples are

not exposed to air. (iii) The samples were then critically point dried (CPD), and (iv) were then mounted on SEM stubs with double-sided carbon tape.

3.9 IDENTIFICATION KEYS

For taxonomic purposes, the original descriptions or systematic keys were used to establish species identification. For section A we made use of external morphological characteristics, such as dorsal chaetotaxy, setae of legs, reticulation pattern, shapes of spermatheaca, structure of spermadactyl and aedeagus, as well as the size and shape of dorsal setae. Type specimens from the ARC Roodeplaat, Pretoria were used to verify identification of mite species.

Identification keys used:

1. Fourteen apomorphic characteristics that describe Parasitiformes (Evans, 1989);
2. Key to Prostigmata (adults) families (MacFarlane, s.a.);
3. Key to post-embryonic stages (Evans, 1989);
4. Key to the suborders of Mesostigmata (Lindquist *et al.,* 2009);
5. Key to the subfamilies of the family Phytoseiidae (Evans, 1989);
6. Key to the genera of the family Phytoseiidae (Ueckermann, 1982);
7. Key to the species of the subgenus *Amblyseius* (Ueckermann and Loots, 1988);
8. Key to the species of the subgenus *Anthoseius* (Ueckermann and Loots, 1988);
9. Key to the stages of Stigmaeidae (Fan and Zheng, 2005); and
10. Key to the African genera of the Stigmaeidae (Meyer and Ueckermann, 1989).

CHAPTER 4: RESULTS and DISCUSSIONS

CHAPTER 4: RESULTS and DISCUSSIONS

4.1 COLLECTION SITES

Figure 4.1 shows collection sites containing mite populations of different species. These locations exclude sampling sites and only take an area into consideration. Thus, the amount of sampling may vary per locations as the size of the location may vary. Various samples per site will form one group so that these sampling sites as a whole are taken into account and pinpointed as one location. **Figure 4.2** to **4.7** below shows different sampling sites were samples were collected for this study.

Figure 4.1 Sampling locations througout survey.

Figure 4.2 Sampling area in the North-West Province

Figure 4.3 Sampling area in the North-West Province

Figure 4.4 Sampling area in the Gauteng Province

Figure 4.5 Sampling area in the Gauteng Province

Figure 4.6 Sampling area in the Gauteng Province

Figure 4.7 Sampling area in KwaZulu Natal

4.2 SPECIES IDENTIFIED BASED ON LIGHT MICROSCOPY

Mites were collected from 38 solanaceous species in three provinces (Gauteng, North-West and KwaZulu Natal). **Table 4.1** below presents species diversity of the parasitic and predaceous community in the study, and their distribution on different solanaceous crops.

Table 4.1 Recording of the presence or absence of parasitic and predatory families on solanaceous species.

Plant nr.	Solanaceae species	Altitude	Grid Reference	Province	Parasitic families present	Predatory families present	Sampler / Name of Collector
1	*Solanum viarum*	47 m	30°57.086' S 030°17.428' E	Southbroom, KwaZulu Natal	-	Phytoseiidae	Candice Ceustermans
2	*Solanum linnaeanum*	51m	30°57.632' S 030°16.770' E	KwaZulu Natal	Tetranychidae Tenuipalpidae Oribatidae	Phytoseiidae Stigmaeidae	Candice Ceustermans
3	*Solanum retroflexum*	52 m	30°57.632' S 030°16.770' E	KwaZulu Natal	Tetranychidae Tenuipalpidae	Tydeidae	Candice Ceustermans
4	*Solanum elaeagnifolium*	1268	25°36.289' S 028°25.730' E	Rinkhalsweg, Roodeplaat, Gauteng	Tetranychidae Oribatidae	-	Candice Ceustermans
5	*Solanum lichtensteinii*	1281 m	25°36.754' S 028°25.782' E	Roodeplaat, Gauteng	Tetranychidae	Stigmaeidae	Candice Ceustermans
6	*Solanum tomentosum var. coccineum*	1279 m	25°36.736' S 028°25.814' E	Roodeplaat, Gauteng	Tetranychidae	-	Candice Ceustermans
7	*Solanum lycopersicum*	1285 m	25°36.762' S 028°25.800' E	Roodeplaat, Gauteng	Tetranychidae	-	Candice Ceustermans
8	*Solanum lichtensteinii*	1122 m	25°22.836' S 028°24.404' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
9	*Datura ferox*	1105 m	25°22.828' S 028°24.399' E	De Wagendrift, Gauteng	Tetranychidae	Phytoseiidae Tydeidae	Candice Ceustermans
10	*Solanum panduriforme*	1129 m	25°23.125' S 028°24.103' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
11	*Solanum elaeagnifolium*	1132 m	25°23.129' S 028°24.104' E	De Wagendrift, Gauteng	Tetranychidae Oribatidae	-	Candice Ceustermans

12	*Datura ferox*	1132 m	25°23.139' S 028°24.103' E	De Wagendrift, Gauteng	Tetranychidae Oribatidae	-	Candice Ceustermans
13	*Solanum elaeagnifolium*	1129 m	25°23.122' S 028°24.106' E	De Wagendrift, Gauteng	Tetranychidae Oribatidae	-	Candice Ceustermans
14	*Solanum linnaeanum*	1140 m	25°23.152' S 028°23.711' E	De Wagendrift, Gauteng	Tetranychidae	Phytoseiidae	Candice Ceustermans
15	*Solanum lichtensteinii*	1145 m	25°23.154' S 028°23.648' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
16	*Datura ferox*	1322 m	25°43.422' S 028°14.441' E	Pretoria, Gauteng	Tetranychidae Tenuipalpidae	-	Candice Ceustermans
17	*Solanum elaegnifolium*	1323m	25°43.398' S 028°14.425' E	Pretoria, Gauteng	Tetranychidae	-	Candice Ceustermans
18	*Datura inoxia*	1439 m	26°17.281' S 027°09.023' E	North-West Province	Tetranychidae	-	Candice Ceustermans
19	*Datura stramonium*	1528 m	26°43.573' S 027°05.141' E	North-West Province	Tetranychidae	-	Candice Ceustermans
20	*Physalis viscosa*	1531 m	26°28.216' S 026°57.076' E	North-West Province	Tetranychidae	-	Candice Ceustermans
21	*Datura stramonium*	1355 m	26°44.300' S 027°04.131' E	Potchefstroom North-West Province	Tetranychidae	Erythraeidae	Candice Ceustermans
22	*Datura stramonium*	1346 m	26°44.198' S 027°03.968' E	Potchefstroom North-West Province	Tetranychidae	Tydeidae	Candice Ceustermans
23	*Datura ferox*	1309 m	26°49.193' S 027°03.718' E	North-West Province	Tetranychidae	-	Candice Ceustermans
24	*Datura ferox*	1314 m	26°49.235' S 027°03.378' E	North-West Province	Tetranychidae	Erythraeidae	Candice Ceustermans
25	*Datura ferox*	1326 m	26°49.335' S 027°03.814' E	North-West Province	Tetranychidae	-	Candice Ceustermans
26	*Solanum elaeagnifolium*	1393 m	26°34.934' S 027°06.581' E	North-West Province	-	Tydeidae	Candice Ceustermans

27	*Solanum lichtensteinii*	1386 m	26°34.989' S 027°06.480' E	North-West Province	Tetranychidae	Stigmaeidae	Candice Ceustermans
28	*Datura ferox*	1387 m	26°34.976' S 027°06.441' E	North-West Province	-	Iolinidae	Candice Ceustermans
29	*Solanum elaeagnifolium*	1275 m	26°18.442' S 027°50.053' E	Lenasia, Gauteng	Tetranychidae	-	Candice Ceustermans
30	*Solanum lichtensteinii*	1278 m	25°36.725' S 028°25.814' E	Rinkhalsweg, Roodeplaat, Gauteng	Tetranychidae	-	Candice Ceustermans
31	*Solanum nigrum*	1264 m	25°36.724' S 028°25.815' E	Rinkhalsweg, Roodeplaat, Gauteng	Tetranychidae	-	Candice Ceustermans
32	*Solanum tomentosum var. coccineum*	1279 m	25°36.764' S 028°25.781' E	Roodeplaat, Gauteng	Tetranychidae	-	Candice Ceustermans
33	*Solanum nigrum*	1281 m	25°36.777' S 028°25.807' E	Roodeplaat, Gauteng	Tetranychidae	Stigmaeidae	Candice Ceustermans
34	*Solanum sodomaeum*	1053 m	25°22.712' S 028°24.191' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
35	*Solanum elaeagnifolium*	1107 m	25°22.711' S 028°24.224' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
36	*Solanum lichtensteinii*	1115 m	25°22.821' S 028°24.355' E	De Wagendrift, Gauteng	Tetranychidae	Stigmaeidae	Candice Ceustermans
37	*Solanum lichtensteinii*	1106 m	25°22.836' S 028°24.406' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans
38	*Solanum panduriformeh*	1102 m	25°22.923' S 028°24.354' E	De Wagendrift, Gauteng	Tetranychidae	-	Candice Ceustermans

*** Collection sites were discarded where mites were completely absent**

The Hypotheses focuses on the suitability of the predators found during the study. Besides addressing the hypotheses, the study also determines the mites present on these crops based on quantitative data and ultimately strives to make a contribution towards mite management strategies.

A survey was conducted during peak seasons to provide enough samples of both pest and predatory mites. From these sample, 1 115 mites were identified, where 1 053 mites were plant-feeding mites and 62 were predatory.

We usually find that plants are infested by adult females or deutonymphs that are blown in from nearby crops, weeds or even other infested plants. This is visible from the samples collected, as plants closer to each other were more likely to contain parasitic and predatory mites of the same species.

Round about nine leaves were selected from 38 random solanaceous plants, where three leaves were taken from the top, three from the middle and three from the bottom of each plant. An infestation grading scale or a leaf damage index was used to determine the level and intensity of a particular mite infestation. The number of species and species richness differed considerably between parasitic and predatory mites (**Figure 4.8** Comparison of the total number of species identified showed a higher number of parasitic mites than predaceous species. After the mites were identified, a numerical value was assigned on the amount of parasitic and predatory mites per plant. Parasitic families included Tetranychidae, Oribatidae (Saprophytes), and Tenuipalpidae. Predatory families include Phytoseiidae, Tydeidae and Stigmaeidae. The tetranychid mites and the eriophyid mites were the most frequently occurring mite pests found on these crop plants (**Table 4.2**). However, samples found from the family Eriophyidae were discarded, due to them not being a focus group for this study. All samples, except collections 1, 26 and 28, were infested by spider mites. The densities among mite populations varied among infested areas. Differences in infestation levels and densities occur due to differences in the climatic conditions of the regions and the sampling period.

Table 4.2 Parasitic/Predaceous mite infestation and density

Mite diversity	Density	NM/20%	NM	RA (%)
PLANT-FEEDING MITES				
Tetranychidae *Tetranychus evansi* *Tetranychus urticae*	32 15	178 21	890 105	84 % 10 %

Tenuipalpidae	16	10	48	5 %
Oribatidae	2	2	10	1 %
PREDACEOUS MITES				
Phytoseiidae Phytoseiidae sp. 1 Phytoseiidae sp. 2 *Amblyseius pretoriaensis* *Typhlodromus microbullatus*	1 1 1 1	1 1 1 1	1 1 1 1	2 % 2 % 2 % 2 %
Stigmaeidae	5	5	23	37 %
Tydeidae Tydeidae sp. *Brachytydeus* sp.	9 4	6 1	28 4	44 % 6 %
Erythraeidae	1	1	2	3 %
Iolinidae	1	1	1	2 %

Density = ±mites found per plant (includes only the plants they were present on)

NM/20% = number (abundance) of mites identified per 20% quantity

NM = total number (abundance) of mites

RA = relative abundance

% of the mites species identified = parasitic

% of the mites species identified = predatory

Predator:Prey ratio = $^{\text{Number of predators found on plants}}/_{\text{Number of parasites found on leaves}}$

Predator/Prey ratio = 1/17

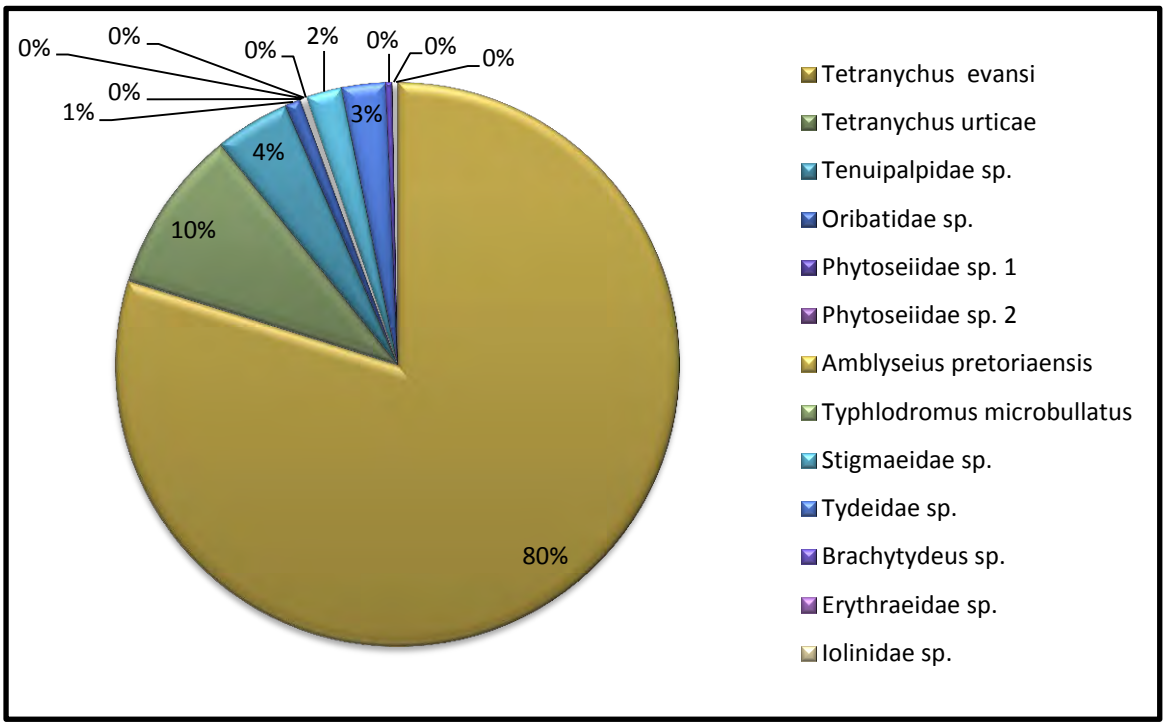

Figure 4.8 Comparative percentages of the abundance of species

Morphological analysis on the quantity of mites reveal a predator:prey ratio of 1:17, where about 94 % of the species identified were plant-feeding species and 6 % were predatory mites.

Four different species of parasitic mites were found during the survey on different host plants of the family Solanaceae, namely: *Tetranychus evansi, T. urticae,* Tenuipalpidae sp. and Oribatidae sp. These mites occurred on both the edge and inside of the crop and were found in patches or with wide distribution all over the crop. As can be seen in **Table 4.2**, *Tetranychus evansi* was the most abundant species from all the samples, with the highest frequency of appearance, reaching a relative abundance of 84 %, followed by *Tetranychus urticae* that had the second highest relative abundance (10 %), which is a significant lower amount. The high comparative occurrence of *Tetranychus evansi* in the survey suggests that this species is the main responsible pest for crop yield loss. This species seems to be very competitive and tends to replace other mite species that cohabit with it. Throughout the survey only 6 % of the mites identified were predaceous species. This shows the scarcity or non occurrence of predaceous mites that could regulate these parasitic mite populations. It is

also very important to note that these low densities may have been influenced by the month (season) in which the data was collected in the field, but yet it still does not seem to have an effect on *T. evansi* as it did on the other parasitic/predatory mites. The community structure and abundance of these predaceous and parasitic mites will differ from that of chemically treated or untreated plants. Low population densities of certain phytophagous mites may have been the result of predation. Furthermore, the disruption of predatory mites could be responsible for an increase in phytophagous mite abundance.

As crops grow throughout the season, the leaf surface will grow along with it. This will allow more space and food resources for plant-feeding mites. Therefore, as the leaf surface increases, the volume of biological predators needs to increase at the same time to give better coverage.

It is important to distinguish between species to determine their pest status and what control measures to take. The spider mite family, Tetranychidae, contains 70 genera and 1275 species and includes a variety of agricultural pests (Navajas *et al.,* 1992 and Hoy, 2011). **Table 4.3** below shows the differences between plant-feeding families found on solanaceous crops during this survey, where **Table 4.4** shows differences between two major pests found in the Tetranychidae family only.

Table 4.3 Pest Species found on Solanaceae

	Tetranychidae	Oribatidae	Tenuipalpidae
Common Name	Spider mites	Moss mites/Beetle mites	Flat mites/False spider mites
General Morphology	Small, usually less than 1 mm long Egg- or oval-shaped body Body segments fused into one unit, with no demarcation between cephalothorax and abdomen.	Species identification is rendered more difficult due to the great number of species (Bulanova-Zachvatkina, 1967). These mites are very small (0.2mm) and grow up to a millimetre long. Colour may vary from gender and stages in life	Mites are small and flat, reddish in colour and slow moving. Contain stylet-like chelicerae that are strongly recurved proximally and arise within an eversible stylophore.

General Morphology	First 2 body segments (including chelicerae and pedipalpi) form a separate movable gnathosoma (mouth region) Rest of the body is known as the idiosoma, consisting of the prodorsum and opisthosoma Eyes are present Chelicerae are stylet-like, retractable into a stylophore (fused chelicerae bases) Pedipalp distally with a thumb-claw complex consisting of a tibial claw and tarsus Adult and nymphal stages = 4 pairs of legs, larvae = 3 pairs of legs Legs consist of 6 leg segments = coxa, trochanter, femur, genu, tibia, and tarsus Tarsi distally with true claws/claws are pad-like Genital and anal opening are posterior on the ventral side Stigmata and peritremes (for breathing) anterior	cycle (Hoy, 2008). Mites are enclosed in a sclerotized integument. Their sexual, anal, and oral opening is covered with opercula (Bulanova-Zachvatkina, 1967). These mites are unique in the nearly complete absence of external structural characteristics differentiating males and females. Males can be distinguished from females either by the presence of a greatly enlarged seta on tarsus I or the interval between the anal and genital opening, or even both (Newell, 1956).	Has a simple palpus, which lacks a claw on the penultimate segment. Palpus segmentation is often reduced. The dorsum's chaetotaxi is of considerable importance. The amount of palpal segments and the number of setae both dorsal and ventral are often variable in species that are congeneric (Meyer, 1979). Tarsal claws with tenent hairs. Body flattened, reddish in colour. Somewhat sessile behaviour and slow moving. First pair of legs shorter than the body (Meyer, 1981). The propodosoma bears 3 pairs of setae. The hysterosoma has from 1-3 pairs of dorsocentrals, one pair of humeral and 5-7 pairs that are dorsolateral.

General Morphology	(in front) on prodorsum Shades of red, brown and green are found (depending on species present and stage in life cycle) Regarded as some of the most important pests on agricultural crops (Craemer *et al.*, 1998). Chelicerae bases fused to form a reversible stylophore with long slender recurved chelae. Peritremes ending simple or anastomosing distally, arising from base of stylophore Duplex setae present or absent on tarsi of legs I and II Tarsal claw and empodia pad-like/claw-like, empodium with/without tenent hairs Palpal tibia with a string claw forming a thumb-claw complex with palptarsus Female ovipositor on opisthosomal venter characteristically wrinkled		Can either contain 1-4 pairs of dorso-sublateral hysterosomals, or they may be absent. Metapodosomal venter bears 2 pairs of medioventral setae, but in different species they may vary from one to several pairs. A distinct ventral and genital plate may be present/absent (Meyer, 1979)

General Morphology	Male = aedeagus caudally, protrusible, variously shaped and important for species identification Female: the shape of the lobes of the dorsal striae is important in the identification of species (Craemer *et al.*, 1998)		
Biology	Life span = 13 to 32 days During this time, females can lay 100 to 150 eggs; 300 eggs under optimal conditions Life cycle from egg to adult = 9 to 14 days Population size can double in one/two days Optimal conditions = mild to warm weather with little to no rain They survive cold temperatures as resting females on the ground, under leaves and in cracks Larval stage emerges from egg and is 6-legged All subsequent stages have 8 legs Larval stage is followed by the proto- and	Oribatid mites are a group of mites that live in the soil. These soil mites are more abundant than any other group of mites that resides in soil. Females can lay eggs without being fertilized. They live in the top layer of soil, leaf litter, or other debris, but they can also be found on moss, lichen, and other plants that are low. These mites are interparous with adults living for a long time. They are known for their low metabolic rates, slow development and low fecundity. Developmental cycles consists of 2 morphologically and biologically distinct	Feed on leaves of plants, usually on the lower surfaces near the midrib or veins. Some species feed on plant bark, others on floral heads or under leaf sheaths, sheaths of grasses and most specialized form galls. Known to be parthenogenetic with thelytokous reproduction Males occur in low frequencies. Females = diploid (2n = 4 chromosomes) Males = haploid (n = 2 chromosomes) Other species = males and females diploid. Four stages in life cycle that are regarded as active stages (i.e., larva, protonumph,

Biology	deutonymphal stages and finally the adult stage A quiescent or resting stage occurs between each moult Males can be distinguished from females by their slender bodies that are pointed at the rear, and by their proportionately longer legs It is common to see males produced from unfertilized eggs Factors that influence population growth = climate, agricultural practices, predators, pesticide application and host plants (Craemer *et al.*, 1998)	stages (Bulanova-Zachvatkina, 1967). They have six active **instars*** = prelarva, larva, 3 nymphal stages, and adult (Hoy, 2008). Stages from larvae (after prelarva) to adult feed on a wide variety of material, including living and dead plant material. Some may be predatory, but they are not known for being parasitic even if they prey on living plants (Baker and Wharton, 1952). Presexual forms (larvae and nymphs) = soft integuments; marked by cutaneous respiration; inhabit soil's top layers. Sexual mature individuals = armoured and breathe through tracheae; inhabit soil surface and forest litter. The life cycle from egg to adult; lasts between 30 and 75 days (Bulanova-Zachvatkina, 1967). ***instar** = developmental stage of arthropods, between each moult, until	deutonymph, adult). A quiescent developmental (chrysalis) stage occurs between each active stage that is sessile, but physiologically active. Eggs and chrysalis stages are glued to the plant; however, motile stages are also difficult to remove. The rate of development is strongly influenced by temperature and host plant (Childers and Rodrigues, 2011).
Biology			

		they reach sexual maturity.	
Dispersal	Dispersed by wind Mites spins threads attaching one end to a twig or leaf Ballooning = females, which are attached to the other end of the thread, hangs 50-150 mm above the substrate and remains suspended, waiting for a breeze to break the thread Dispersal in this manner occurs due to a shortage of food or as a result of overpopulation Can be spread passively over both short and long distances by = irrigation and flood water, dust storms, insects, farm equipment, clothing, footwear, farm produce and containers, and planting stock Some species are readily spread in dried and cut flowers, leaves, bulbs and tubers of plants (Craemer *et al.*, 1998)	Oribatid mites are unable to burrow through soil and thus rely on larger animals to make tunnels so that they are able to move around (Hoy, 2008). They are able to migrate vertically during fluctuations of moisture and temperature (Bulanova-Zachvatkina, 1967).	The dispersal capacity of these mites through crawling is relatively limited (Alves *et al.*, 2005). Dispersal occurs mainly through the wind. Alternatives include animals and humans come in contact with mites or the transportation of plants or plant material containing mites (Kane *et al.*, 2004).
	Spins silk threads which anchors them to plants Use threads to anchor	Do not spin silk threads.	Do not produce webbing.

Silk production	eggs to plants Silk threads protect them from enemies and pesticide application In severe infestations, host plants may be completely covered in silk webbing (Craemer *et al.*, 1998)		

Table 4.4 Differences between the two major pests found in the family Tetranychidae

	Tetranychus evansi	*Tetranychus urticae*
Common Name	Tobacco spider mite	Two-spotted spider mite
Distribution and Hosts	Widespread in southern Africa Also occurs in Mauritius and North and South America Attacks tomato and other solanaceous plants such as potatoes, brinjals, gooseberries, peppers and sweet potatoes (Craemer *et al.*, 1998)	Distributed world-wide; very important pest species in the family Tetranychidae Has been recorded on more than 200 host plants throughout Africa It is a pest of deciduous fruit, ornamentals and other field crops, but is also known to attack strawberries, gooseberries and most vegetables Able to develop strain resistance to pesticides (Craemer *et al.*, 1998)
Field diagnosis	Females are oval and up to 0.5 mm long Adult females = orange-red and reddish legs Adult males = straw- to orange-	Oval females up to 0.5 mm long Adult females = straw-coloured/green with a dark blotch on each side with pale to yellow legs Adult male = smaller than females,

Field diagnosis	coloured Immature stages and eggs resemble those of the dark-red spider mite (Craemer *et al.*, 1998)	straw-coloured Larvae and nymphs = usually straw-coloured, but sometimes vary from light orange to yellow or reddish green, with a dark spot on each side Eggs = pearly-pink, light red or ivory-white and spherical (Craemer *et al.*, 1998)
Distinguishing characters	Proximal tactile setae of tarsus I are more or less in a line with the proximal duplex setae Female empodium I bears a small spur; the dorsal striae of the female bear semicircular lobes Males have mediodorsal spurs on empodia I and II which are smaller than the proximoventral spur; empodia III and IV have a small mediodorsal spur each and 3 pairs of proximoventral hairs; the small knob of the aedeagus forms an obtuse angle with the axis of the shaft; the knob bears a small anterior projection and a relatively longer, acute and somewhat deflexed, posterior projection (Craemer *et al.*, 1998)	Males = aedeagal knob is small, about one-fourth the length of the dorsal margin of the shaft; the knob's axis is parallel to/forms a small angle with the shaft axis; the knob's dorsal margin and the development of the anterior and posterior projections may differ; the empodium I bears a strong mediodorsal spur, that is about one-third the length of the 2 proximoventral spurs; the second empodium is provided with 3 pairs of proximoventral hairs and a strong mediodorsal spur Females = striae bear lobes that vary from triangular to broadly semicircular (Craemer *et al.*, 1998)
Damage	Mites prefer the lower surface of leaves, but severe infestations occur on both leaf surfaces as well as on stems As the population increases, mites are able to cover plants completely with webbing Damage first appears as stipples that later give the leaf a silvery or yellowish	These mites prefer to feed on the lower leaf surfaces, but as the population increases, they spread over the entire plant Chlorotic stipples are first noted on the leaves, followed by large areas turning yellow and leaves become convex The leaves may even become bronzed

Damage	appearance Eventually the leaves turn entirely yellow or brown and die off Plants are killed rapidly by these mites (Craemer *et al*, 1998)	in severe infestations, dry out and drop off During severe infestations, fine webbing is clearly visible (Craemer *et al.*, 1998)
Biology	Oviposition sites that are preferred are on the lower leaf surface at the junction of veins. Oviposition is increased by high humidity. Oviposition begins a day after emergence and females already reach their maximum egg-laying capacity on the fourth day. They are able to ovipost 30 eggs per day at this time, after which the rate of oviposition decreases. The incubation period of the eggs is 3 to 4 days at 23°C and 49-50% relative humidity. The developmental stages take 4 to 5 days, leading to a life cycle as short as 9 to 12 days. Fertilized females live 13 to 32 days, whereas unfertilized females live 27 to 39 days. Reproduction throughout the year results in 24 to 30 generations per year (Craemer *et al.*, 1998).	Optimal developing temperatures lie between 26°C and 30°C, and they flourish at relatively low humidities. Eggs hatch after 3 to 5 days in optimal conditions. Larvae = orange, turns green after feeding. After 2 to 3 days, larvae undergo a short quiescent period and moult to protonymph. After 2 to 3 days, the protonymph undergoes a quiescent period and moults into a deutonymph. After 2 days, the adult will emerge from the deutonymph's quiescent period. Under favourable conditions the life cycle (from egg to adult) is about 10 to 14 days. Extremely high humidities and low temperature can lead to diapauses; in warm conditions, the mites are able to reproduce throughout winter and about 20 generations a year can occur in the field.
Biology		Twenty-four hours after emergence, the female can start laying eggs and can

		lay up to 100-150 eggs in 20-30 days (Craemer *et al.*, 1998).
Predators	No predators are known (Craemer *et al.*, 1998).	**Potential Predators:** **Anystidae** *Anystis baccarum* *Chaussieria venustissima* **Cunaxidae** *Rubroscirus rarus* **Eupalopsellidae** *Eupalopsellus sellnicki* **Erythraeidae** *Balaustium graminum* *Abrolophus bipilum* **Phytoseiidae** *Amblyseius addoensis* *A. citri* *A. pretoriaensis* *A. teke* *A. tutsi* *Phytoseiulus longipes* *P. persimilis* **Stigmaeidae** *Agistemus africanus* **Tydeidae** *Lorryia relhaniae* *Tydeus reticoxus* (Craemer *et al.*, 1998)

The correct identification of predatory mites present is a determining factor for implementing adequate management strategies. Predaceous mites found on solanaceous crops are presented in **Table 4.5** Here we can see the taxonomic differences between these mite families.

Phytoseiidae are the most common and largest distributed predator family. They are known to prey on all stages of spider mites. Specific biological predator species are more effective at

different stages of spider mite populations and population densities. Some predators prefer the larval and nymph stage better than they do adults, where others are known to be more aggressive and are better at controlling higher population densities. One of the main concerns about predaceous mites is their low efficacy at low temperatures. This could be a possible reason for the low numbers in predators in some samples or even the absence in others. It might have been that the collection months were too late and that peak seasons for parasitic mites (in which collection took place) differ from that of predatory mites. Some predaceous species, such as Phytoseiids, are not effective in very dry warm conditions and need a relative humidity of at least 60-70% and temperatures of 20-30°C according to LEAF (Anon 2, 2012). This could have caused their absence during collection months that took place at a later stage and the presence of other predatory species.

In addition, the fact that some predaceous and parasitic mites were present in small numbers of samples could be related to their low population densities and it cannot be stated with confidence that these species have a restricted geographical distribution.

Table 4.5 Characteristics of predatory mite species found on Solanaceae during survey

	Phytoseiidae	Stigmaeidae	Tydeidae
Field diagnosis	Body is sclerotized and pyriform (Tixier *et al.*, 2012). Elongate - oval body = 0.2 - 0.6 mm in length. Colour = varies from white, light brown, dark brown to red (Craemer *et al.*, 1998).	Body elongate or oval (Craemer *et al.*, 1998). Colour = Orange, yellow, greenish or reddish (de Arruda Filho and de Moreas, 2003).	Body is oval in shape (**Figure 4.39**). Colour = White, yellow, green, orange to black (Darbemamieh *et al.*, 2010). Size = $^{1}/_{5}$ of a millimetre (Zhang *et al.*, 2001).
Distinguishing characters	Dorsal shield has 12-22 pairs of setae (maximum of 22 pairs of setae) (**Figure 4.38**)	Dorsal surface almost nude or covered with 1-16 shields (Craemer *et al.*, 1998). Frequent occurrence of	Palpal thumb-claw complex is absent. Prodorsum has 1 pair of trichobothria.

Distinguishing characters	Dorsal shield entire. Tibia I with 4 dorsal setae and 2 ventral setae. Sternal shield of female venter bears 2-3 pairs of setae, the genital shield 1 pair and the ventro-anal shield bears 0-4 pairs of setae. Sternal and genital shield of male venter fused (sterno-genital shield) with genital opening on anterior margin of shield, ventro-anal shield enlarged and free/fused with peritrenatal shields. Female spermatheca opens between leg III and IV. Male spermadactyl situated on chelicerae (Craemer *et al.,* 1998).	dorsal shield, but lacks ventral shield (de Arruda Filho and de Moreas, 2003). Cheliceral bases free, particularly fused/fused, but not with hypostome; peritremes usually present on prodorsum (Craemer *et al.,* 1998). Chelicera has stylet-like movable digits (de Arruda Filho and de Moreas, 2003). Palptibia with normal claw, with a seta-like or claw-like accessory claw at its base. Palptarsus with terminal eupathidium which may be a simple spine, bi- or tridentated on an erect tod or median knob (Craemer *et al.*, 1998). Coxae I and II separate from coxae III and IV and has a contiguous genital and anal opening (de Arruda Filho and de Moreas, 2003).	Palp simple and 4-segmented (Craemer *et al.*, 1998). Fixed cheliceral digits are reduced. Cheliceral bases are fused. Cheliceral digits are short, needle-like and movable (Zhang *et al.,* 2001). Dorsally = striated/reticulated idiosoma. Ventrally = striated idiosoma (Darbemamieh *et al.,* 2010).
Biology	Free living predators. Developmental cycle = 5 stages (egg, hexapode larva, protonymph, deutonymph and adult		Life cycle = 6 stages (egg, larva, protonymph, deutonymph, tritonymph and adult). Developmental time (egg

Biology	female/male (Tixier *et al.*, 1998). Resting stage is absent (Craemer *et al.*, 1998). Eggs = translucide and oval (Tixier *et al.*, 2012). Developmental duration = less than a week to 36-56 days or even longer (Craemer *et al.*, 1998). Developmental temperature ranges between 10°-30°C (25°C optimum). Fecundity = 0.1 to 4.5 eggs per day (mating is necessary to lay eggs) (Tixier *et al.*,1998) and ±40 -60 eggs per female during their life (Craemer *et al.*, 1998). Reproduction = pseudo-arrhenotoky (Tixier *et al.*, 2012). Some phytoseiids are able to reproduce parthenogenetically, but males are rare/absent and unfertilized eggs produce females (Craemer *et al.*, 1998).		to adult) = takes a few days to a couple of weeks at ambient temperatures (Zhang *et al.*, 2001).
Habitat	Females overwinter in temperate climates, underneath bark, dead	Found in bark of trees, on/in soil, grass, leaf, mulch, lichen, wood	Lives in soil, humus, moss, lichens, fungi, grass, on trees (found

Habitat	leaves and buds (Tixier *et al.*, 2012). Inhabits ornamental and cultivated plants, and orchards (Kaźmierczak and Lewandowski, 2006). Found living on leaves of various deciduous tree and shrub species (Kabíček, 2008).	boring beetles, crevices in rock and leaf cavities, and a few are known to be parasitic on phlebotomine flies (Nazari *et al.*, 2012).	living in bark, on leaves and fruit), straw and hay, in stored products, and in the nests of birds, mammals and stingless bees (O'Connor and Kimov, 2012)
Prey species	Prey on tetranychids (spider mites), eriophyids, tenuipalpids (false spider mites), acarids (stored product mites), tarsonemids and tydeids. They also feed on pollen, crawlers of scale insects, trips, honey-dew, plant juices and eggs of insects and mites (Craemer *et al.*, 1998).	Prey on tetranychids, tenuipalpids and eriophyids (Kheradmand *et al.*, 2007). Also feeds on eggs and immature stages of spider mites and eriophyids, and immature stages of scale (Nazari *et al.*, 2012).	Preys on adult eriophyid mites and tenuipalpid mites and their eggs. Feeds on pollen, leaf tissue and fungus (Hessein and Perring, 1986).

4.3 MORPHOLOGICAL COMPARISON BETWEEN THE TWO MAJOR PEST MITES BASED ON SEM WORK

The specimens examined using SEM have generated a number of insight into the ecology and basic biology of these species, and has also enhanced our understanding of the morphology of these mites.

Figure 4.9 compares the dorsal view for a *T. evansi* female to a *T. urticae* female (**Figure 4.10**). These two species may look as if though they have significantly little differences between them to someone who is unfamiliar with these species, which could lead to misidentification (Meyer, 1987). The exoskeleton of all mite species are complex, containing setae, pores, ridges and folds (Jeppson *et al.,* 1975) which can be seen in **Figure 4.11** and **4.12**.

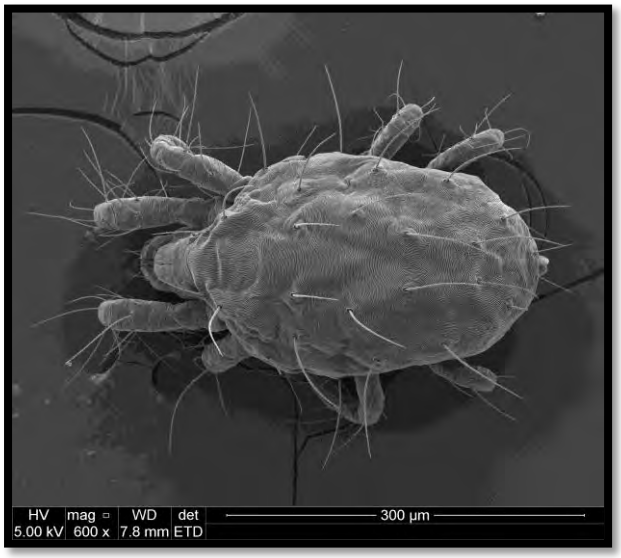

Figure 4.9 Dorsal view of *T. evansi* female

Figure 4.10 Dorsal view of *T. urticae* Koch female

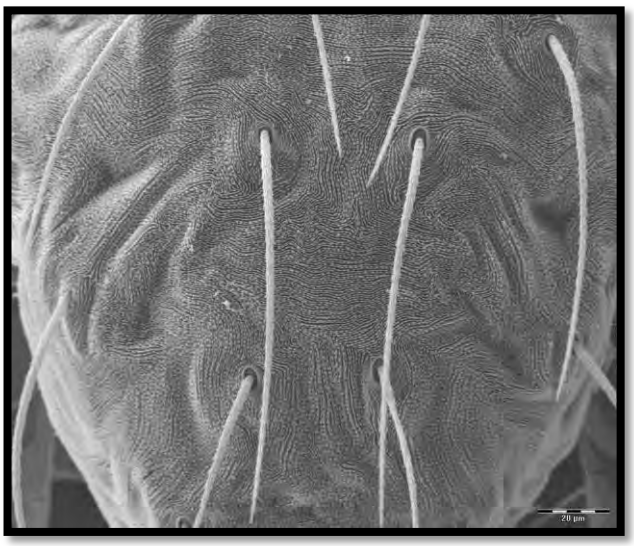

Figure 4.11 *T. evansi* dorsum of opisthosoma of female showing striation patterns

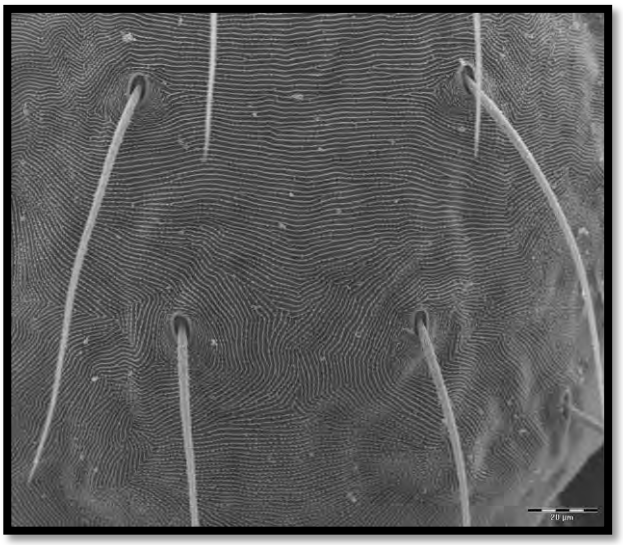

Figure 4.12 *T. urticae* dorsum of opisthosoma of female showing striation patterns

Figure 4.13 and **4.14** shows the anterior view of *Tetranychus* females. **Figure 4.15** and **4.16** shows the left pair of ites. These mites contain 4 eyes, two on each side.

Figure 4.13 Anterior view of *T. evansi* female

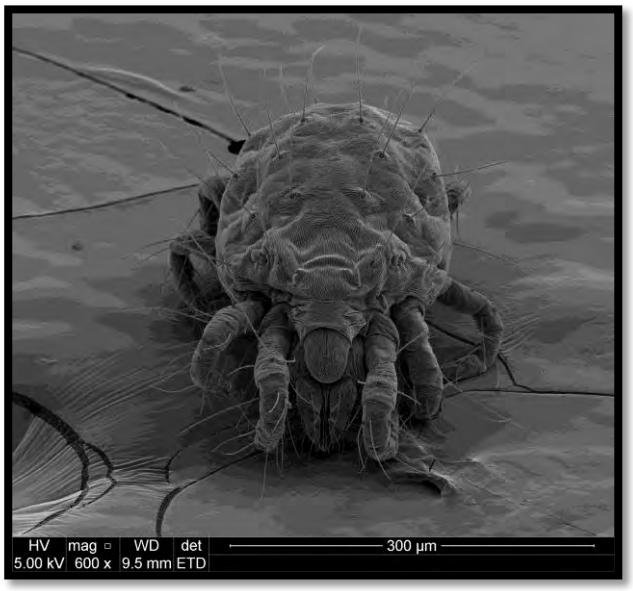

Figure 4.14 Anterior view of *T. urticae* female

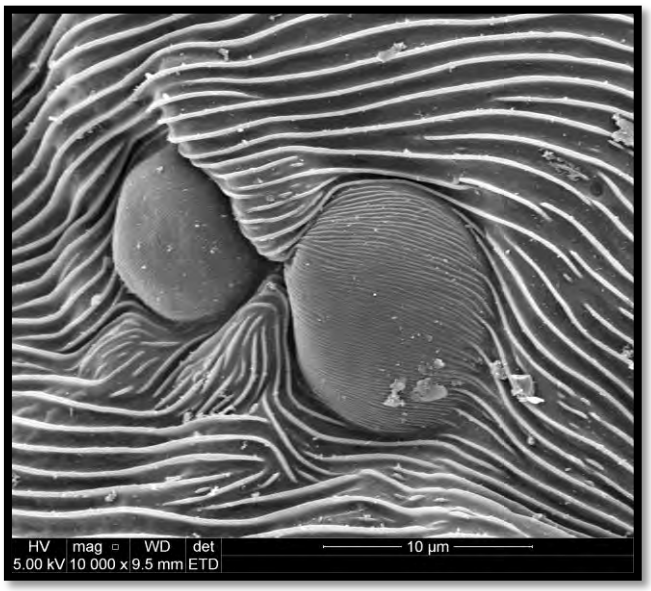

Figure 4.15 *T. evansi* left pair of eyes

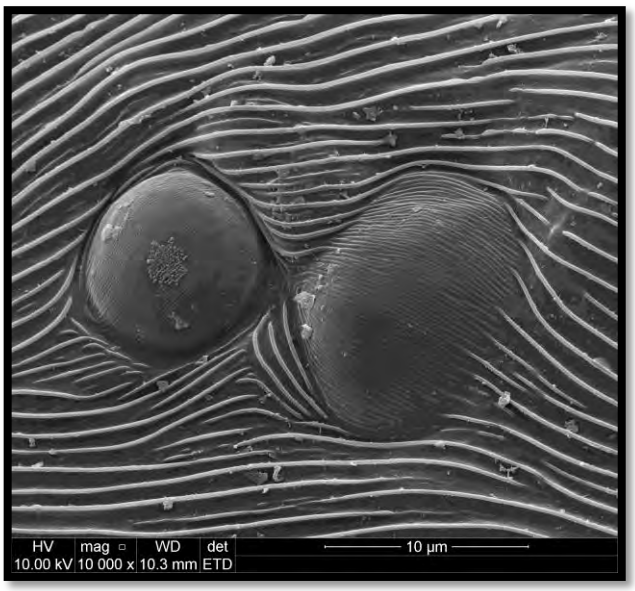

Figure 4.16 *T. urticae* left pair of eyes

Figure 4.17 and **4.18** reveals the ventral view of the rostral and palpal apices. The palpi are well developed and contain 4-5 segments ending in a thumb-claw. The palptarsal thumb bears 7 setiform structures; 3 setae, one solenidion, and 3 eupathidia (one of the eupathidia is the spinneret, from which a silken substance is extruded in the form of a single strand). Unlike spiders, the spinneret of spider mites is situated in the capitulum (head), on the gnathosoma. These silk strands are used as pathways over foliage, as a dispersal mechanism, protecting them against predators and can serve to a limit as a protection mechanism against pesticides (Meyer, 1987).

Chelicerae bases fused to form a reversible stylophore with long slender recurved chelae. Peritremes end distally as hook shaped (**Figure 4.19** and **4.20**).

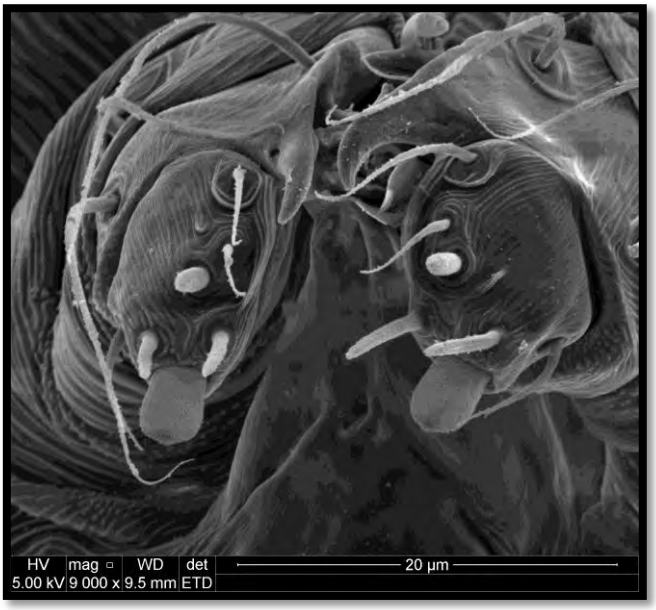

Figure 4.17 *T. evansi* palptarsus of female

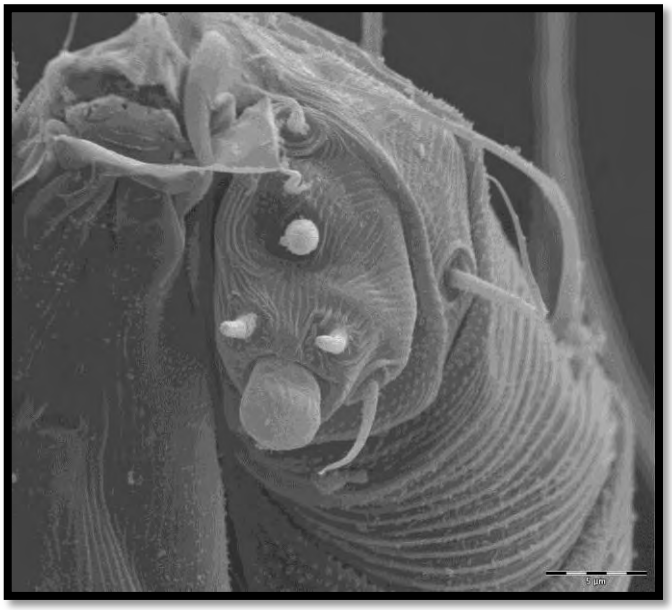

Figure 4.18 *T. urticae* palptarsus of female

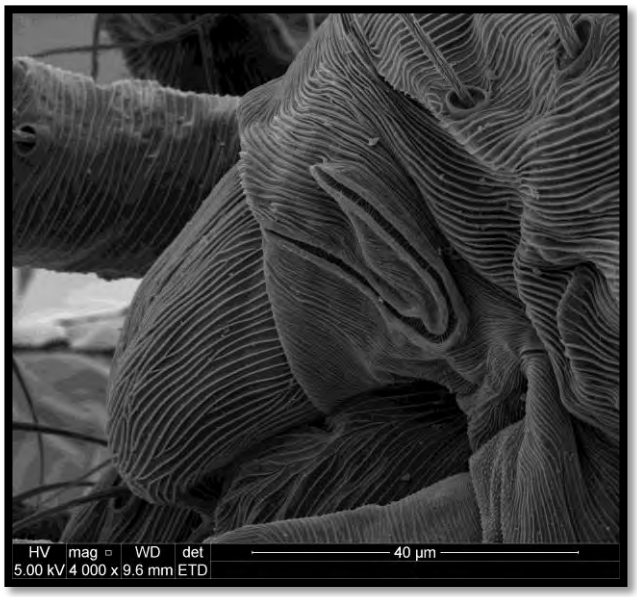

Figure 4.19 *T. evansi* extruded stylophore showing the peritreme

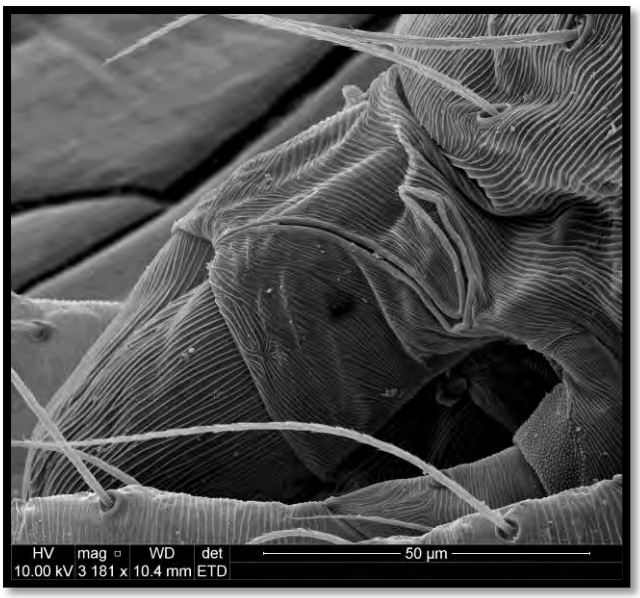

Figure 4.20 *T. urticae* extruded stylophore showing the peritreme

Figure 4.21 and **4.22** shows the cheliceral stylets. These long slender, grooved stylets are the moving digits of the chelicerae. The cheliceral bases are consolidated to form the stylophore which is retractable and extrudable. The two stylets fit together to form a food channel. These mites are parenchyma-sucking mites and aquire food by penetration of the leaf with their stylets and suck out the cell contents (Meyer, 1987).

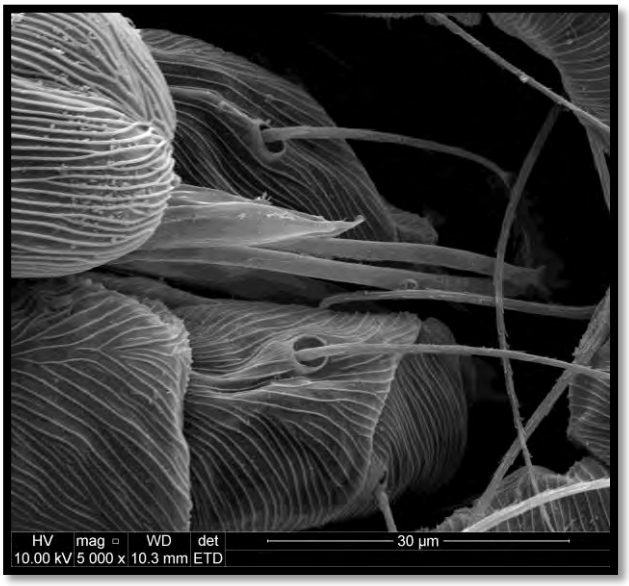

Figure 4.21 *T. evansi* chelicaral stylets/ chelicerae

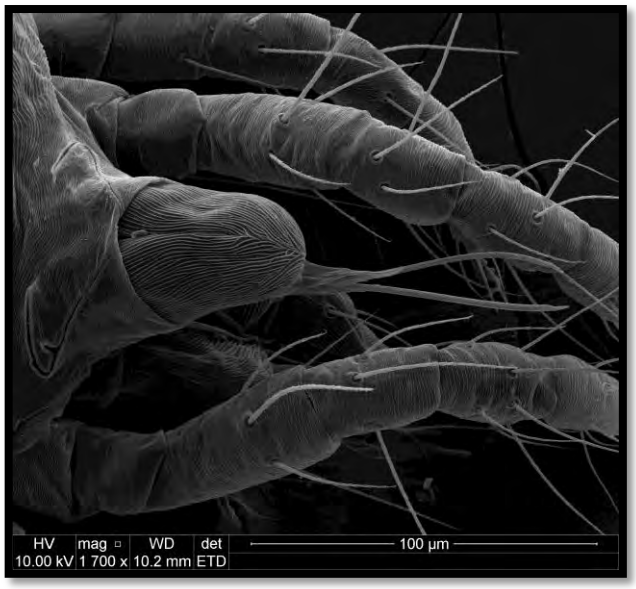

Figure 4.22 *T. urticae* cheliceral stylets/ chelicerae

Figure 4.23 and **4.24** shows the tarsal appendages of tarsus I. Each leg ends in a pretarsus and is structured to make mobility on webbing comfortable and easy (Meyer, 1987).

Figure 4.23 *T. evansi* tarsal appendages of tarsus I of female

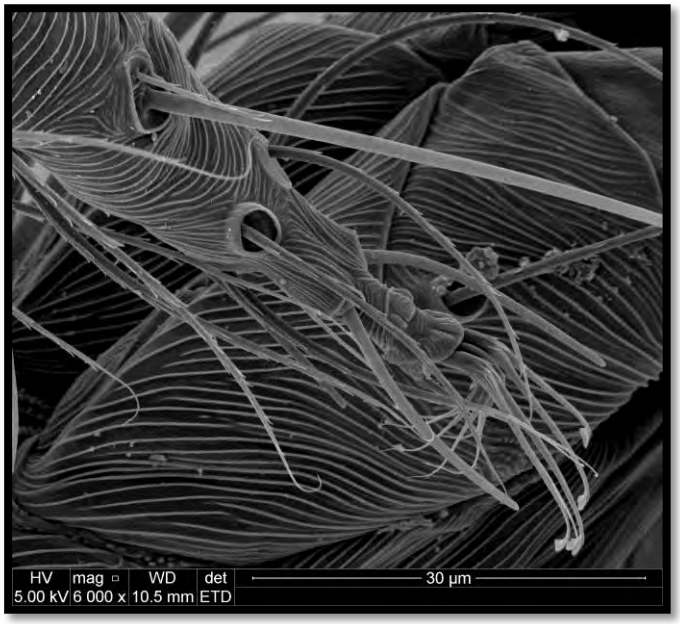

Figure 4.24 *T. urticae* tarsal appendages of tarsus I of female

Figure 4.25 and **4.26** shows the tibia and tarsus I. Tarsus I of *T. evansi* has the proximal pair of duplex setae more or less in a line with the 4 tactile setae and empodium I is provided with a minute mediodorsal spur (Meyer, 1987).

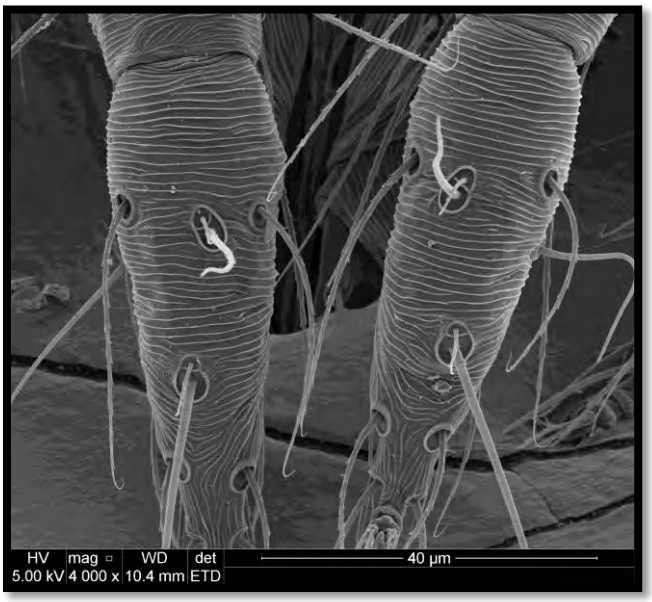

Figure 4.25 *T. evansi* tibia and tarsus I of female

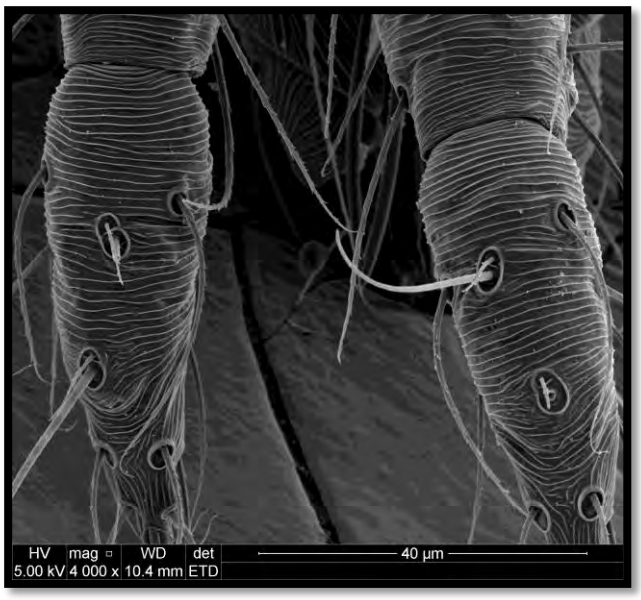

Figure 4.26 *T. urticae* tibia and tarsus I of female

Figure 4.27 shows the aedeagus of *T. evansi*. The male (**Figure 4.29**) contains a shaft of the aedeagus and it is slender and curves dorsad. The axis of the knob forms a strong angle with that of the shaft. The knob is relatively small and is provided with a small anterior angulation and a relatively longer acute and has a deflexed posterior projection. **Figure 4.28** on the other hand shows the aedeagus of *T. urticae*. This axis of the aedeagus knob is parallel to the axis of the shaft. The knob is only $^{1}/_{5}$ as long as the dorsal margin of the shaft and the anterior projection of the knob tend to be more acute (Meyer, 1987).

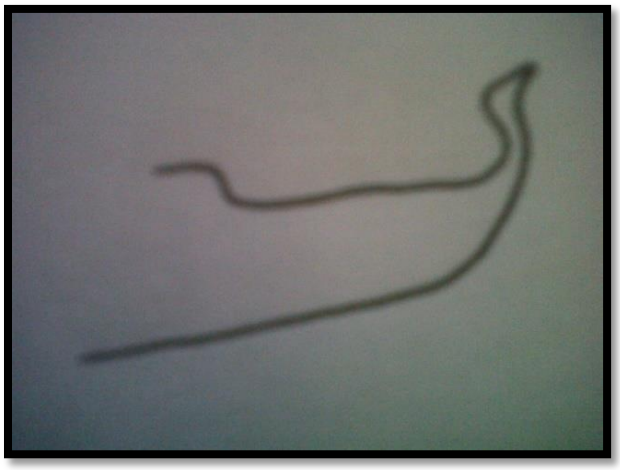

Figure 4.27 *T. evansi* aedeagus of male

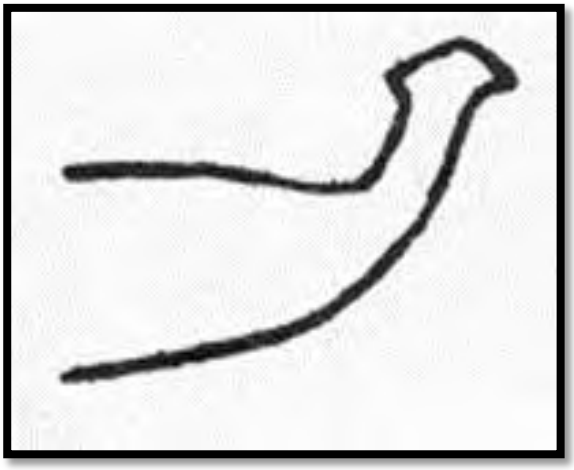

Figure 4.28 *T. urticae* aedeagus of male (Prince, 2000)

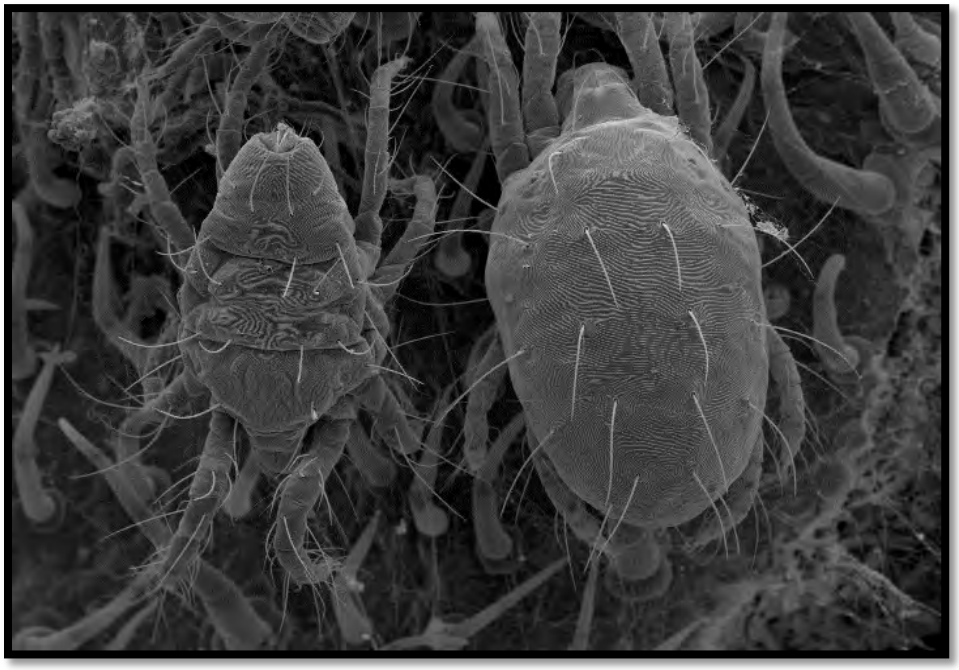

Figure 4.29 Dorsal view of *T. evansi* male (left) and female (right) to show the different body shapes between the sexes

Figure 4.30 shows a SEM image of a spider mite which is less than a millimetre long. The image reveals the complex microhabitat of this extremely small pest. This microhabitat is ideal for webbing and reproduction (laying eggs) due to the shape and amount of trigomes on the leaf surface. These trigomes, along with webbing, is an ideal shield against predators and pesticides.

Figure 4.30 Female *Tetranychus* sp. (Acari: Tetranychidae) on *Solanum* sp. (Solanaceae) leaf

In **Figure 4.31** we see a spider mite crawling out of its old exoskeleton. Mites will develop a whole new set of setae along with their new exoskeleton. In the right hand corner we see a spider mite egg. This shows the enormous size of eggs towards females and it is extraordinary to think about the amount of eggs a female will lay in her life time, considering the size of an egg (**Figure 4.32**).

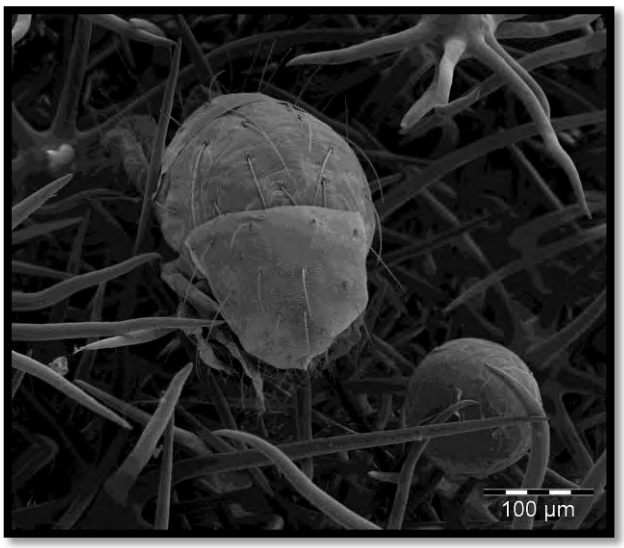

Figure 4.31 *Tetranychus* sp. on leaf casting its old exoskeleton

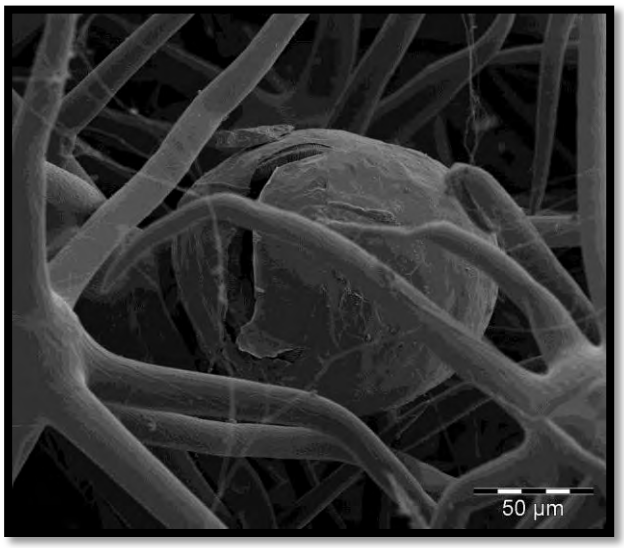

Figure 4.32 *Tetranychus* sp. hatching from egg

Speaking of size, in **Figure 4.33** a spider mite alongside a dust particle observed. Again, this gives us an idea of how small these pests are and why they are sometimes undetected when first inhabiting a plant.

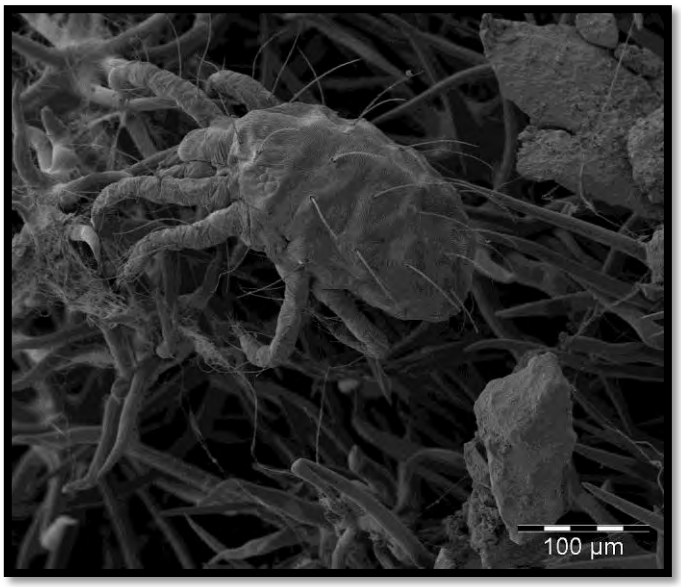

Figure 4.33 *Tetranychus* sp. (Acari: Tetranychidae) on leaf next to a dust particle (corner, right)

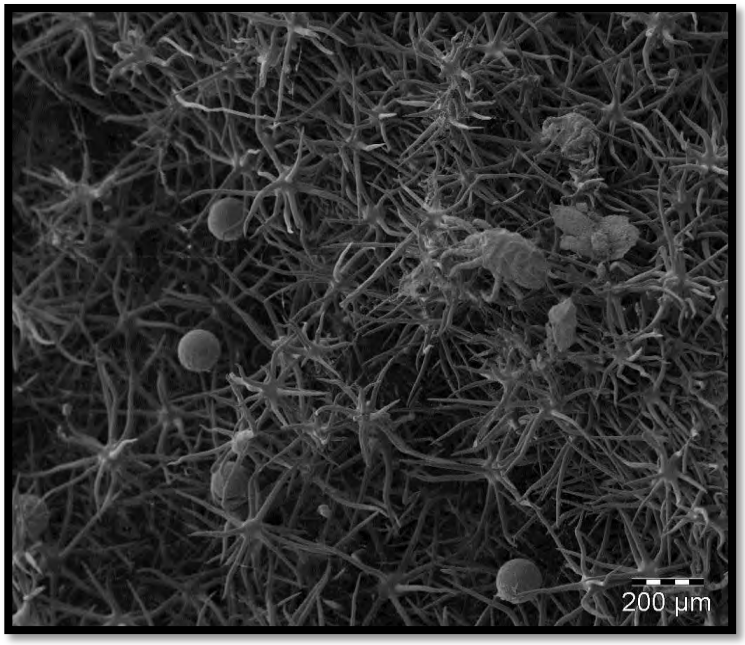

Figure 4.34 shows spider mites inhabiting a *Solanum* sp. plant, surrounded by their eggs and trigomes of the leaf. They will inhabit the leaves, stems and fruits of the plant.

In **Figure 4.35** and **4.36** we see the linear palps of the false spider mites, flat mites, Tenuipalpidae. Tenuipalpid mites have palpal tibia without spine like setae and the palpal segments are often reduced. **Figure 4.37** shows the "transverse´aperature.

Figure 4.35 Linear palps of Tenuipalpidae sp. (Welbourn, s.a.)

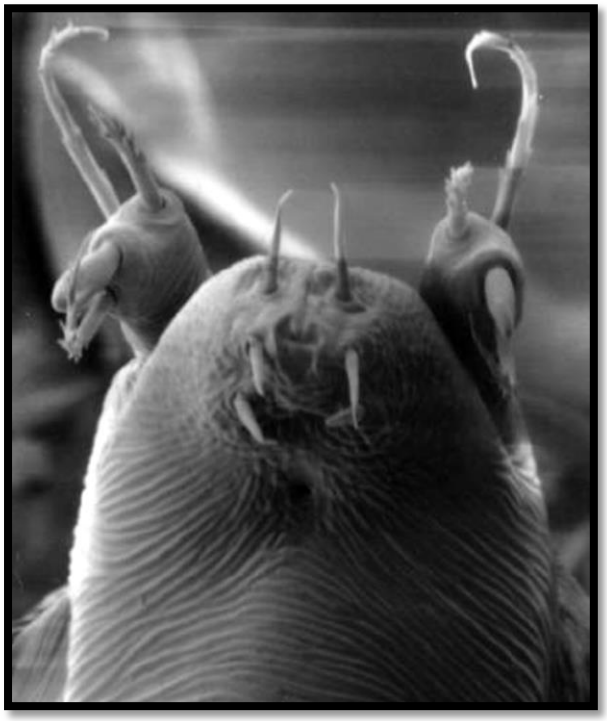

Figure 4.36 Tenuipalpidae palps (Welbourn, s.a.)

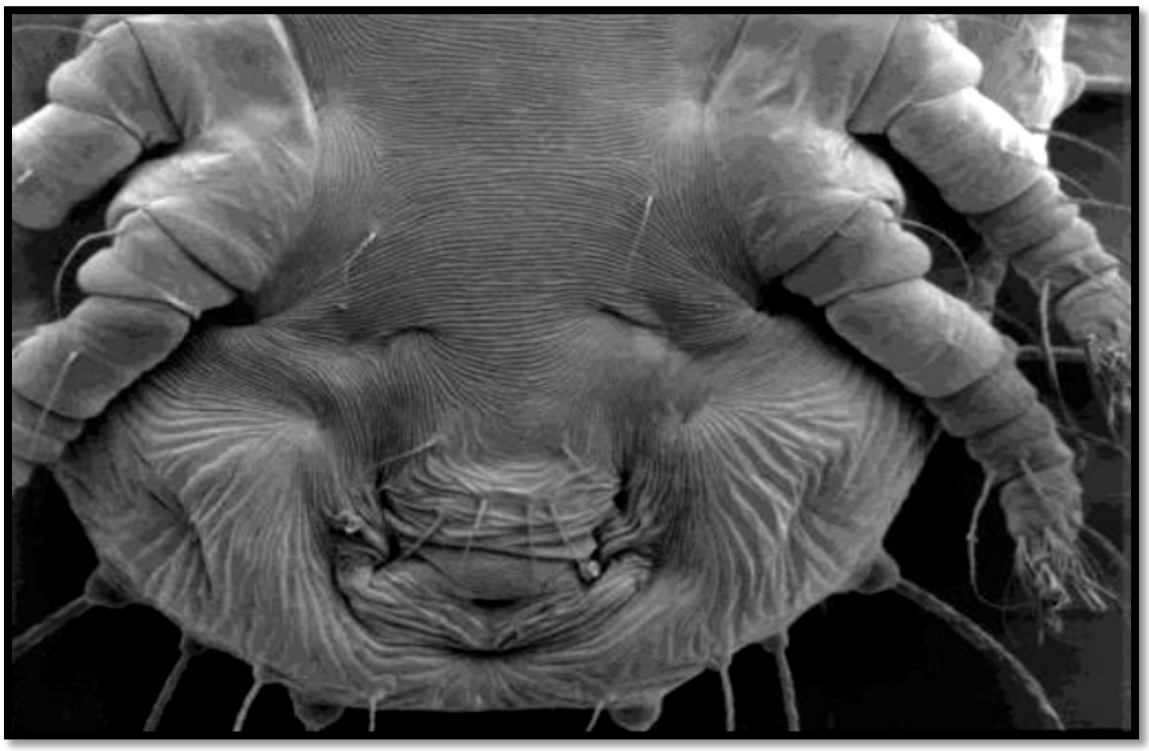

Figure 4.37 Female genital aperature "transverse" of tenuipalpid mite, often covered by a plate (Welbourn, s.a.)

Figure 4.38 Predatory mites (Mesostigmata: Phytoseiidae) (Welbourne, s.a.)

Figure 4.39 Predatory mite (Prostigmata: Tydeidae) (Wikipedia, 2005)

4.4 MORPHOLOGICAL REVIEW OF PREDATORY SPECIES

FAMILY PHYTOSEIIDAE

The predatory Phytoseiidae can be divided into three subfamilies; Amblyseiindae, Typhlodromindae and Phytoseiindae (**Figure 4.40**). For this study we will be focussing on the Amblyseiinae and the Typglodrominae.

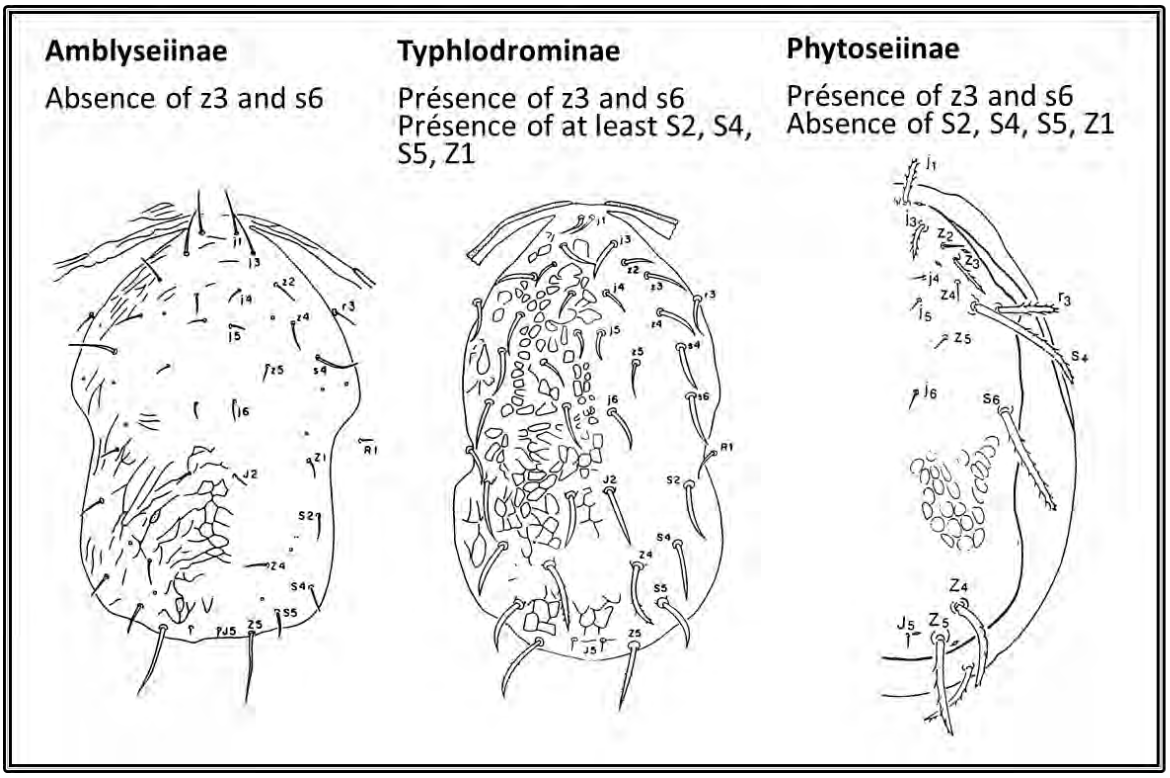

Figure 4.40 Differences between three subfamilies of the family Phytoseiidae (Tixier *et al.*, 2012)

KEY TO THE GENERA OF THE FAMILY PHYTOSEIIDAE (Ueckermann, 1982)

1. Dorsal plate undevided.....2

- Dorsal plate devided .....*Macroseius* Chant, Denmark & Baker

2. Proscutum with four prolateral setae (j3, z2, z4 and s4).....5

- Proscutum with five to six prolateral setae (j3, z2, z4, s4 and/or s6).....3

3. Gnathosoma same length as leg I.....*Gigagnathus* and *Paragigagnathus*

- Gnathosoma shorter than leg I.....4

4. Ventro-anal plate with one to three pairs of setae; setae r3 and R1 or r3 only on dorsal plate, R1 can be absent.....*Chantia* and *Phytoseius*

- Ventro-anal plate with three to four pairs of setae; setae r3 and R1 are always present, located on the interscutal membrane.....*Typhlodromus* Scheuten

5. Dorsal interscutal membrane sclerotized.....*Iphiseius* Berlese

- Normal dorsal interscutal membrane.....6

6. Ventro-anal plate present.....*Amblyseius* Berlese

- Anal plate present.....*Phytoseiulus* Evans

SUBFAMILY AMBLYSEIINAE

GENUS *AMBLYSEIUS*

SUBGENUS *AMBLYSEIUS* BERLESE

Species from this subgenus carry 16 or 17 pairs of setae on their dorsal shields with 4 pairs of prolateral setae (j3, z2, z4 and s4) and 5 pairs of postlateral setae (Z1, S2, S4, S5 and Z5), the interscutal membrane holds 2 pairs of setae (however, setae R1 can be placed on the dorsal shield), the sternal and ventro-anal shields carry 2-3 pairs of setae, the ventral shields are enlarged in some species, the number of setae may vary from 2-5 pairs on the opsithogastric cuticle, macrosetae can be present on legs I-IV with 0-7 on leg IV and genu II and III can carry 7-9 and 6-7 setae respectively (Ueckermann and Loots, 1988).

A. (A.) MESSOR GROUP

Members of this group only carry 16 pairs of setae on their dorsal shield and they lack setae J2 (Ueckermann and Loots, 1988).

A. (A.) OBTUSUS GROUP

Species from this group have setae j1, j3, s4, Z4 and Z5 that are much longer than setae (Ueckermann and Loots, 1988).

KEY TO THE SPECIES OF THE SUBGENUS *AMBLYSEIUS* (Ueckermann and Loots, 1988)

1. Setae J2 absent.....*messor*-group

- Setae J2 present.....2

2. Setae j1, j3, s4, Z4 and Z5 much longer than the rest of the dorsal body setae which are very short; setae S2, z2 and z4 may be relatively long, but not longer than setae j3; setae Z4 and Z5 are usually very long and whip-like.....*obtusus*-group.....3

3. Leg IV with 3 macrosetae.....4

- Leg IV with 4 macrosetae.....*A. (A.) ovalitectus* Van der Merwe

4. Setae z2 and z4 subequal to equal in length or setae z2 clearly longer than z4.....5

- Setae z4 clearly longer than setae z2.....*A. (A.) nemorivagus* Athias-Henroit and *A. (A.) comatus* Ueckermann & Loots

5. Setae z2 and z4 subequal to or equal in length, setae z2 much shorter than distances between their bases and those of setae z4.....6

- Setae z2 clearly longer than z4, setae z2 equal to or longer than distances between their bases and those of z4.....*A. (A.) foenalis* Berlese and *A. (A.) obtusus* Koch

6. Ventro-anal shield anteriorly narrower tha across anal opening.... *A. (A.) neolargoensis* Van Der Merwe

- Ventro-anal shield anteriorly as broad as or broader than across anal opening.....7

7. Setae Z5 shorter than half length of dorsal shield.....*A. (A.) bufortus* Ueckermann & Loots

- Setae Z5 very long, longer than half the length of dorsal shield.....8

8. Setae Z5 shorter than dorsal shield.....9

- Setae Z5 longer than dorsal shield.....*A. (A.) solus* Mattysse & Denmark

9. Cervix of spermatheca cup-shaped, bell-shaped, tube-shaped or funnel-shaped.....10

- Cervix distally tube-like or very slender and proximally dome-shaped or strongly flared.....*A. (A.) hamizensis* Athias-Henriot and *A. (A.) anomalus* Van der Merwe

10. Cervix of spermatheca cup-shaped, bell-shaped or funnel-shaped.....11

- Cervix tube-like.....*A. (A.) tamatavensis* Blommers

11. Cervix of spermatheca cup-shaped or bell-shaped.....12

- Cervix funnel-shaped.....*A. (A.) boina* Blommers

12. Ventro-anal shield with pre-anal pores widely spaced, posteroir to setae JV2.....*A. (A.) meridionalis* (Berlese)

- Pre-anal pores closer together, almost between setae JV2.....***A. (A.) pretoriaensis* Ueckermann & Loots** (**Figure 4.41**)

Amblyseius (*Amblyseius*) *pretoriaensis* Ueckermann and Loots

Figure 4.41. *Amblyseius(Amblyseius) pretoriaensis* excoriate ventral view

Amblyseius (Amblyseius) pretoriaensis bears a resemblance to *A. (A.) januaricus* Wainstein and Vartapetov, *A. (A.) perlongisetus* Berlese and *A. (A.) kaguya* Ehara. Based on the description Chant did of *A. (A.) perlongisetus* in 1957, it differs from *A. (A.) pretoriaensis* in that the ventro-anal shield is devoid of pre-anal pores and also has equally long setae Z4 and Z5. According to Gonzalez and Schuster's (1962) and Collyer's (1982) redescriptions mentioned in Ueckermann and Loots (1988), *A. (A.) perlongisetus* differs from *A. (A.) pretoriaensis* by having the longest macroseta on tibia IV and by having a spermatheca that is differently shaped. *A. (A.) pretoriaensis* differs from *A. (A.) kaguya* in that *A. (A.) kaguya* contains setae Z4 that are shorter than half the length of setae Z5, setae JV2 on the ventro-anal shield are situated closer together and they also have macrosetae on basitarsus and tibia IV which are not equally lengthed. *A. (A.) pretoriaensis* can be distinguished from *A. (A.) januaricus* by setae Z4 that's equal to or longer than half the length of setae Z5, the pre-anal pores on the ventro-anal shield are almost between setae JV2 and lastly has macrosetae on barsitarsus and tibia IV that are equal in length (Ueckermann and Loots, 1988).

Female. The measurements of the holotype with deviations in the paratypes in parentheses: Length of dorsal shield, 359 (339-385) µm; width, 209 (216 - 270) µm; leg I, 359 (339-408) µm; leg IV, 407 (337-439) µm; length of ventro-anal shield, 121 (116-134) µm; width, 87 (82-97) µm; sternal shield's length is 92 µm (77-92); width, 93 (88-108) µm; width of genital shield, 78 (77-86) µm; setae, j1, 31 (23-26) µm; j3, 54 (42-57) µm; j4, j5, j6, z2, z4 and z5, 5-6 µm; J5, Z1, S2, S4 and S5, 8 µm; s4, 94 (82-116) µm; Z4, 131 (120-146) µm; Z5, 236 (223-296) µm; r3, 20 (17-23) µm; R1, 15 (11) µm; JV5, 100 (77-105) µm; macrosetae: basitarsus IV, 85 (72-92) µm; tibia III, 31 (31-39) µm; tibia IV, 85 (40-52) µm; genu II, 35 (31-39) µm; genu III, 46 (40-52) µm and genu IV, 108 (102-123) µm (Ueckermann and Loots, 1988).

Dorsum (Figure 4.42). The dorsal shield is oval in shape and contains 17 pairs of setae, dorsomedian rugose patches and 11 pairs of pores. Setae j1, j3, s4, Z4 and Z5 are either long to very long in length. The remaining setae are short. Setae Z4 and Z5 are also somewhat serrated. Setae r3 and R1 are located on the interscutal membrane (Ueckermann and Loots, 1988).

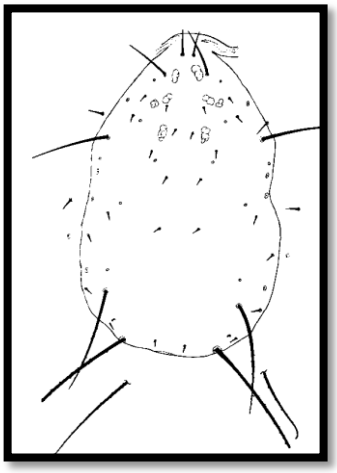

Figure 4.42 Dorsal view of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

The peritrematal shields are fused together with the dorsal shield on the anterior end and the peritremes reach anterolateral to setae j1 (Ueckermann and Loots, 1988).

Venter (Figure 4.43). The sternal shield carries 3 pairs of setae and 2 pairs of pores. The third pair of pores and fourth pair of setae are located on tiny metasternal shields (Ueckermann and Loots, 1988).

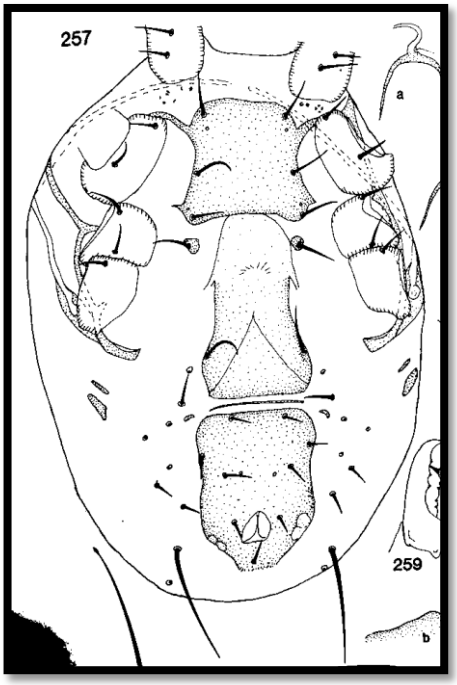

Figure 4.43 Ventral view of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

It is common to find a genital shield within this family. The ventro-anal shield is longer than it is broad and carries 3 pairs of pre-anal setae and 2 pores almost between setae JV2. The pre-anal setae can be found anterior to the middle of the anal opening. Four pairs of setae are situated on the opisthogastric membrane. Six pairs of small platelets and 2 pairs of metapodal shields are also located on the opisthogastric membrane. Setae JV5 are extremely long. A long slim platelet can be seen between the genital and ventro-anal shields (Ueckermann and Loots, 1988).

Spermatheca (Figure 4.44). The atrium is small in size and somewhat bulbous. The major duct is a slim tube and the cervix is cup-shaped and can differ as evident in **Figure 4.44** (Ueckermann and Loots, 1988).

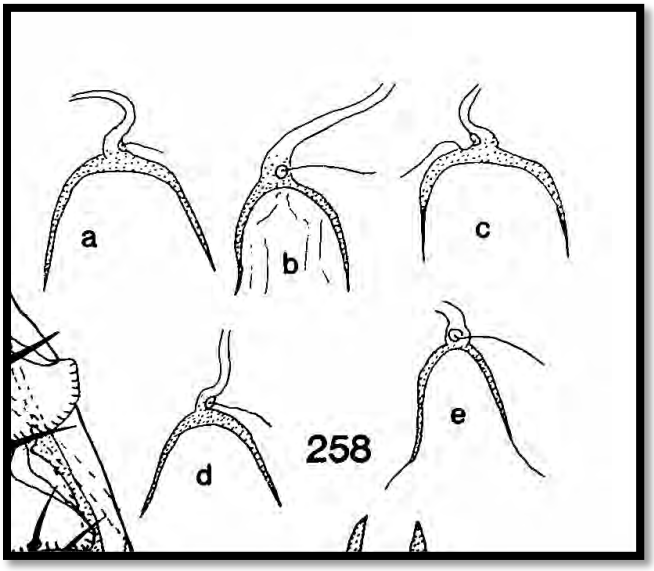

Figure 4.44 Variations in spermatheca of *Amblyseius (Amblyseius) pretoriaensis* females (Ueckermann and Loots, 1988)

Gnathosoma (Figure 4.45-4.47). The fixed cheliceral digit contains 7 - 9 small teeth and a pilus dentilis and the movable digits include 3 teeth (**Figure 4.45**). The malae internae are rounded on the lateral ends (**Figure 4.46**). As evident from **Figures 4.47**, the anterior margin of the tectum can vary (Ueckermann and Loots, 1988).

Figure 4.45. Chelicera of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

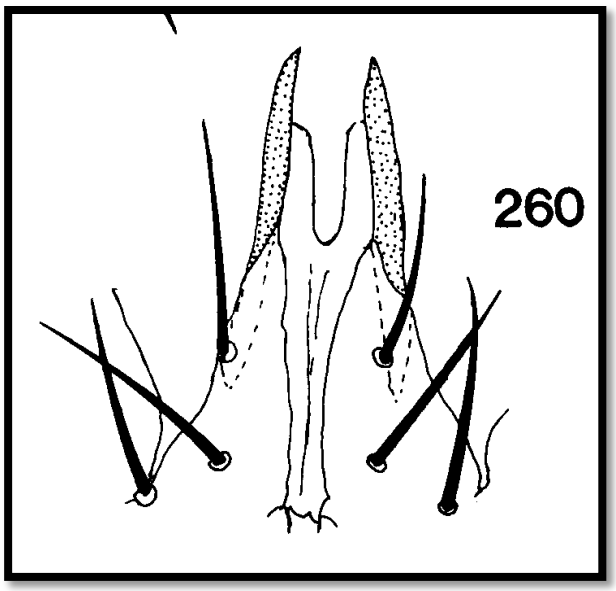

Figure 4.46. Hypostome of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

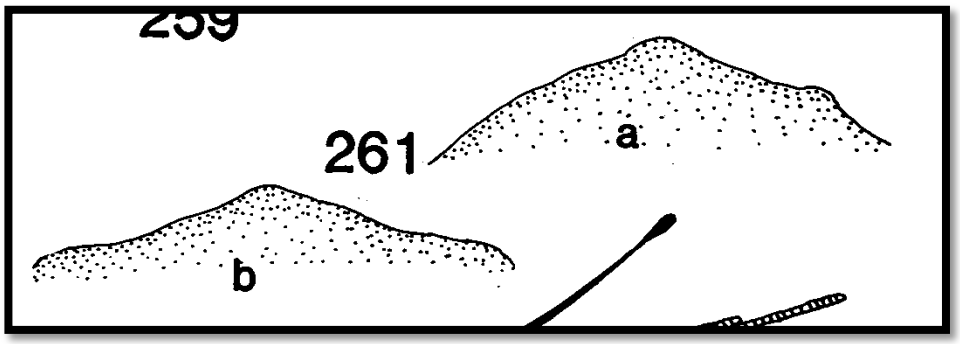

Figure 4.47 Anterior margin of tectum of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

Legs (Figure 4.48-4.51). The chaetotaxy of the legs are normal and tarsus I has 7 sensory setae that are located distally. One can find macrosetae on genu II, tibia and genu III and basitarsus, tibia and genu IV. The macrosetae on the fourth leg are slightly knobbed on the distal end and are long in length (Ueckermann and Loots, 1988).

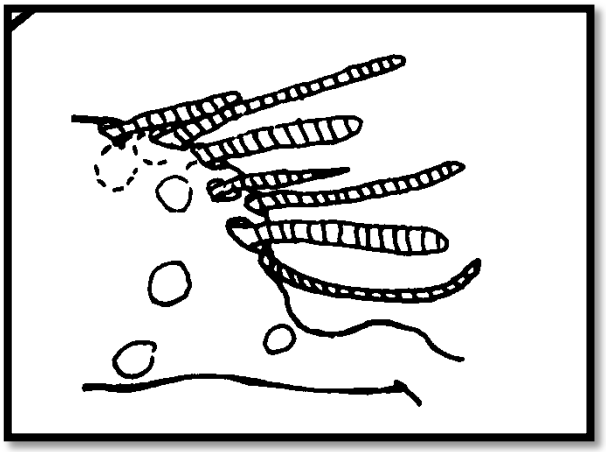

Figure 4.48. Tarsus I of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

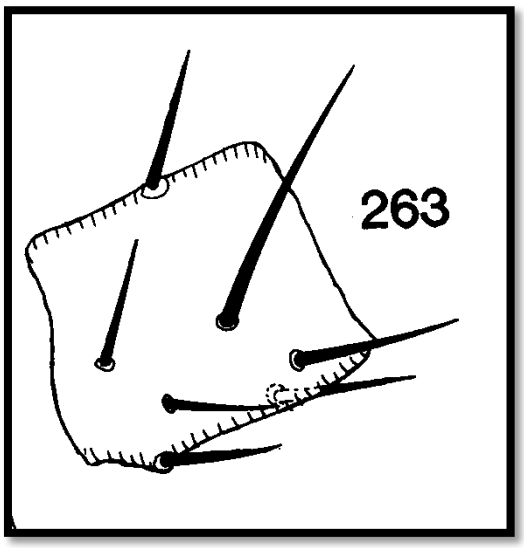

Figure 4.49 Genu II of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

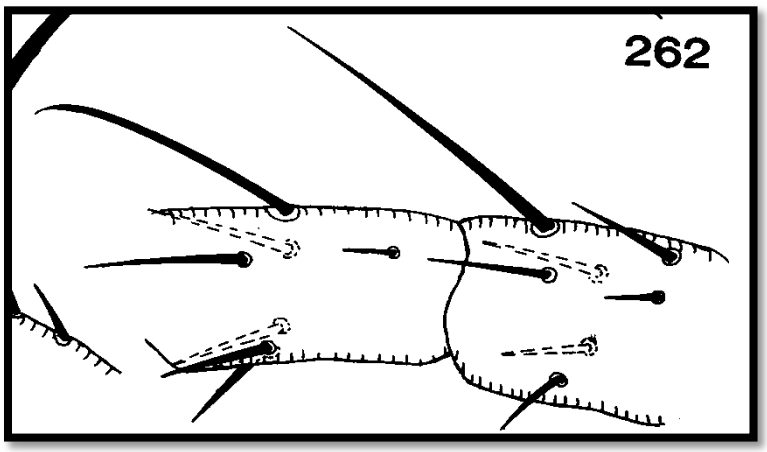

Figure 4.50 Genu and tibia II of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

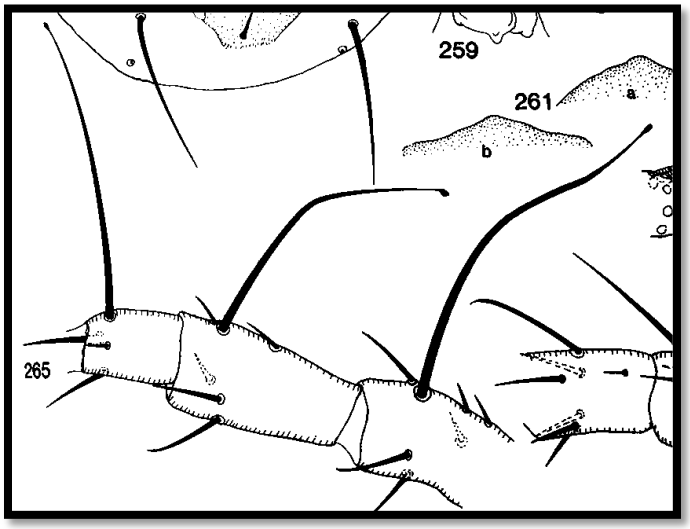

Figure 4.51 Leg IV of *Amblyseius (Amblyseius) pretoriaensis* female (Ueckermann and Loots, 1988)

Deutonymph (Figure 4.52-4.53). Dimensions: The length of the dorsal sheild is 227 µm; the width is 154 µm; leg I, 328 µm; leg IV, 339 µm; the length of the anal shield is 42 µm; the width is 54 µm; setae j1 is 23 µm; j3, 42 µm; j4z j5z j6 J2, J5, z2, z4, Z1, S2, S4, S5 and z6 is 6-8 µm; s4, 80 µm; Z4, 100 µm; Z5, 69 µm; JV5, 69 µm; macrosetae, basitarsus IV, 69 µm; tibia III, 34 µm; tibia IV, 72 µm; genu II, 32 µm; genu III, 46 µm and genu IV, 92 µm. The chaetotaxy of the dorsal shield is similar to that of the female. The shield contains six pairs of pores (Ueckermann and Loots, 1988).

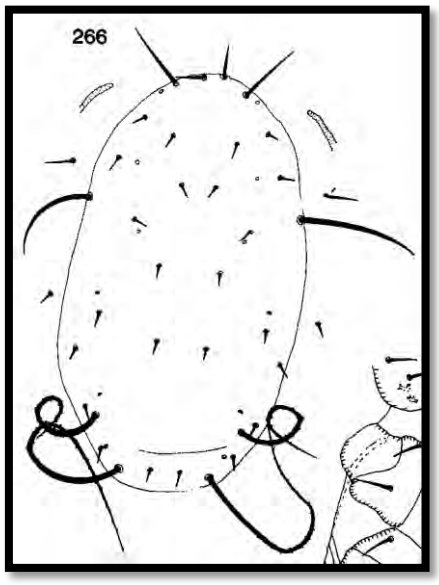

Figure 4.52. Dorsal view of *Amblyseius (Amblyseius) pretoriaensis* deutonymph (Ueckermann and Loots, 1988)

The sternal shield contains 4 pairs of setae and 2 pairs of pores. The genital setae can be found between coxae IV on the cuticle. The opisthogastric cuticle carries 7 pairs of setae, 2 pre-anal pores, 3 pairs of small platelets and an anal shield. Deutonymphs contain very long JV5 setae. The gnathosoma and legs are similar to that of females (Ueckermann and Loots, 1988).

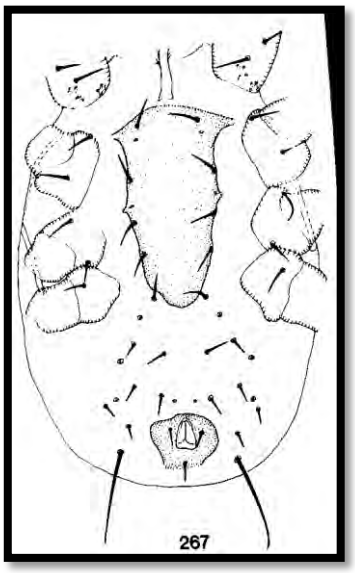

Figure 4.53 Ventral view of *Amblyseius (Amblyseius) pretoriaensis* deutonymph (Ueckermann and Loots, 1988)

SUBFAMILY TYPHLODROMINAE

SUBGENUS ANTHOSEIUS DE LEON

Mites from this subgenus are defined by 10 pairs of lateral body setae, six pairs of these 10 pairs of setae are anterior to the level of the R1 setae (j3, z2, z3, z4, s4 and s6). Anthoseius species have short thick legs and have 3 instead of 4 pairs of pre-anal setae on the ventro-anal shield (Ueckermann and Loots, 1988).

Species from this group contain setae z6 and 2, 3 or 4 pairs of pre-anal setae on the ventro-anal shield (Ueckermann and Loots, 1988).

Typhlodromus Anthoseius De Leon

These mites lack setae z6 and the presence of 3 pairs of pre-anal setae on the ventro-anal shield (Ueckermann and Loots, 1988).

KEY TO THE SPECIES OF THE SUBGENUS *ANTHOSEIUS* (Ueckermann and Loots, 1988)

1. Setae z6 present.....*Paraseiulus* Muma

- Setae z6 absent.....2

2. Ventro-anal shield with 4 pairs of pre-anal setae...rhenanus group.....*rhenanus* group.....3

- Ventro-anal shield with 3 pairs of pre-anal setae...barkeri group.....*barkeri* group

- Ventral-anal shield with 2 pairs of pre-anal setae.....*T. (A.) ilicis* (Athias - Henroit) (ilicis group)

3. Setae S4 and S5 equal or subequal in length.....4

- Setae S5 clearly shorter than S4.....*T. (A.) religiousus* Ueckermann & Loots

4. Setae Z5 pointed.....*T. (A.) capparidis* Van Der Merwe, *T. (A.) georgicus* Weinstain and *T. (A.) oasis* El Badry

- Setae Z5 knobbed or blunt distally.....5

5. Setae Z4 knobbed.....*T. (A.) bullatus* Van Der Merwe, *T. (A.) grastis spec. nov.* and *T. (A.) terrulentis* Van Der Merwe

- Setae Z4 pointed.....6

6. Genu II bears 8 setae.....*T. (A.) johannae* Ueckermann & Loots

- Genu II bears 7 setae.....7

7. Genu III bears 7 setae.....8

- Genu III bears 6 setae.....*T. (A.) aproxys* Van der Merwe

8. Most dorsal body setae smooth.....9

- All dorsal body setae serrated, except for setae J5.....*T. (A.)michaeli* Ueckermann & Loots

9. Leg IV with one macrosetae.....10

- Leg IV with 3 macroseta.....*T. (A.) umbraculus* spec. nov.

10. Macroseta on leg IV pointed.....*T. (A.) februs* Van der Merwe

- Macroseta on leg IV knobbed.....11

11. Cervix of spermatheca short and bell-shaped.....12

- Cervix long and tube-like.....*T. (A.) gardeniae* Schultz

12. Sternal shield with 2 pairs of setae.....***T. (A.) microbullatus* Van der Merwe** (**Figure 4.54-4.55**)

- Sternal shield with 3 pairs of setae.....*T. (A.) gouaniae* Schicha

Typhlodromus (Anthoseius) microbullatus Van der Merwe

Figure 4.54 *Typhlodromus (Anthoseius) microbullatus* Van Der Merwe excoriate dorsal view

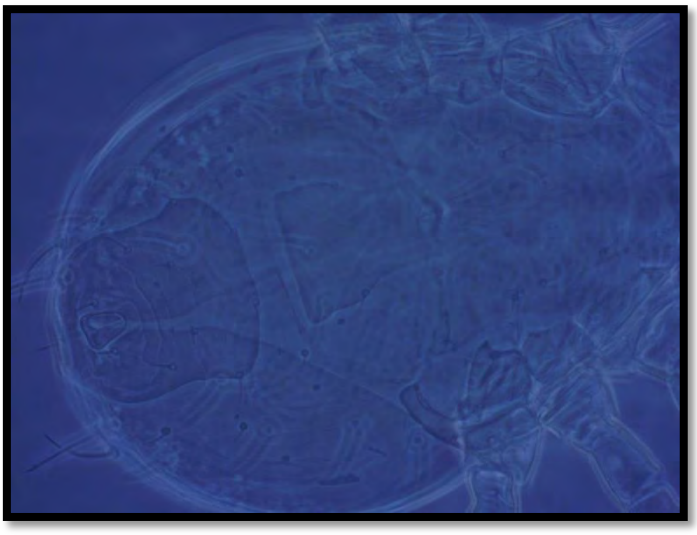

Figure 4.55 *Typhlodromus (Anthoseius) microbullatus* excoriate ventral view

Even though this species differ from other species, it is closely related to *T. (A.) apoxys* Van der Merwe and *T. (A.) saevus* Van der Merwe, as described below. It differs in that one or more knobbed setae on genu and tibia IV are present, setae Z5 is pointed distally rather than knobbed and setae JV5 is knobbed instead of pointed (Ueckermann and Loots, 1988).

Male. *T. microbullatus* males are similar to *T. (A.) saevus* Van der Merwe males. The only difference is that the macroseta on basitarsus IV is knobbed distally (Ueckermann and Loots, 1988).

Deutonymph. Dimensions: Dorsal shield's length, 202µm; width, 95µm; leg I, 202µm; leg IV, 202µm; anal shield's length, 32µm; width, 38µm; sternal shiel'd length, 85µm; width, 44µm; setae j1, j6 and z2, 14µm; j3, j4, j5, z3 and z5, 13µm; J2 and z4, 17µm; J5, 6µm; s4, 19µm; s6, 21µm; S2 and S4, 22µm; S5, 20µm; Z4, 25µm; Z5, 28µm; r3, R1 and JV5, 16µm; macroseta on basitarsus IV, 25µm (Ueckermann and Loots, 1988).

Dorsum (Figure 4.56). The dorsal shield, except for the medio-lateral incisions, bears a resemblance to that of the female. Setae S5, Z4 and Z5 are knobbed where z4 and z5 are serrated. The preitrematal shields are absent and peritremes reach the z2 setae's level (Ueckermann and Loots, 1988).

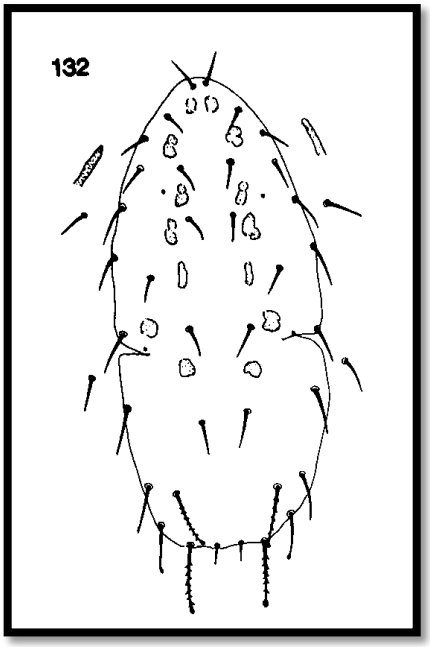

Figure 4.56 Dorsal view of *T. (A.) microbullatus* Van der Merwe deutonymph (Ueckermann and Loots, 1988)

Venter (Figure 4.57). The sternal shield carries 4 pairs of setae and 2 pairs of pores. The gential setae can be found on the cuticle between coxae IX. The opisthogastric cuticle carries 5 pairs of setae with setae JV5 being knobbed and longer than the others (Ueckermann and Loots, 1988).

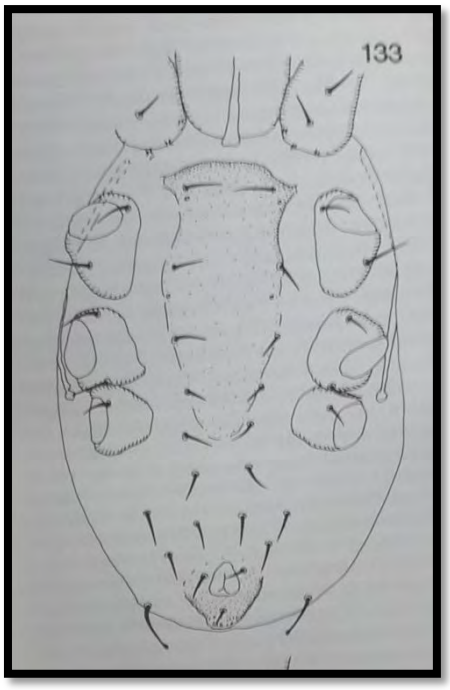

Figure 4.57 Ventral view of *T. (A.) microbullatus* Van der Merwe deutonymph (Ueckermann and Loots, 1988)

Leg (Figure 4.58) The deutonymph's chaetotaxy of its leg is almost identical to that of females. The basitarsus IV also carries a short knobbed seta besides the long knobbed macroseta. The tibia and the IV genu are provided with 3 and 4 knobbed setae accordingly (Ueckermann and Loots, 1988).

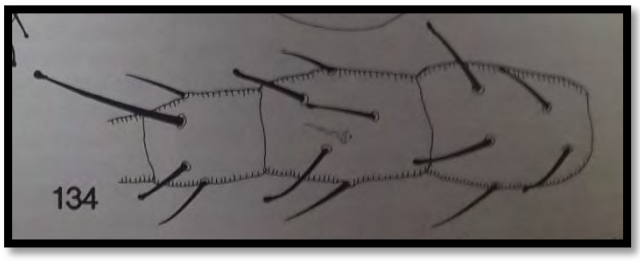

Figure 4.58 Leg IV of *T. (A.) microbullatus* Van der Merwe deutonymph (Ueckermann and Loots, 1988)

Protonymph (Figure 4.59-4.60). Dimensions: Podonotal shield's length, 113-115µm; width, 79-88µm; opisthonotal shield's length, 35µm; width, 54-57µm; anal shield's length, 25µm; leg I, 188-189µm; leg IV, 176-189µm; sternal shield's length, 85µm; width, 44µm; setae j1, j3, j6, J2, z4, R1, z2, s6 and S5, 13-14µm; JV5, j4 and j5, 9µm; J5, 6µm; s4, 16-17µm; z5, 11µm;z4, 21-22µm;Z5, 20-25µm; macroseta on basitarsus IV, 22-23µm (Ueckermann and Loots, 1988).

The idiosoma is dorsally coated with 2 shield, the anterior podonotal shield and the posterior apistonotal shield. The anterior podonotal shield bears 9 pairs of setae and 2 pairs of pores. All the setae on the opisthonotal shield are knobbed, with the exception of setae J5 and setae Z4 and Z5 are serrated. Setae r3, R1, s6, S2 and J2 are placed on the interscutal membrane and carry small distal knobs. The J2 setae are also serrated (Ueckermann and Loots, 1988).

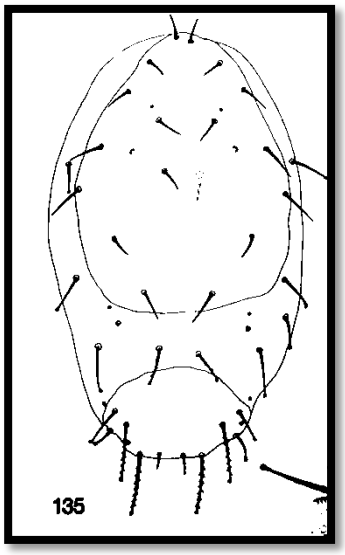

Figure 4.59 Dorsal view of *T. (A.) microbullatus* protonymph (Ueckermann and Loots, 1988)

The sternal shield is illegibly outlined and carries 3 pairs of setae and 2 pairs of setae and 2 pairs of pores. The opisthogastric cuticle carries 4 pairs of setae and 2 pre-anal pores. They lack peritrematal shields and peritremes are very short. The chaetotaxy of the legs are similar to the species *T. (A.) querellus* spec. nov. (Ueckermann and Loots, 1988).

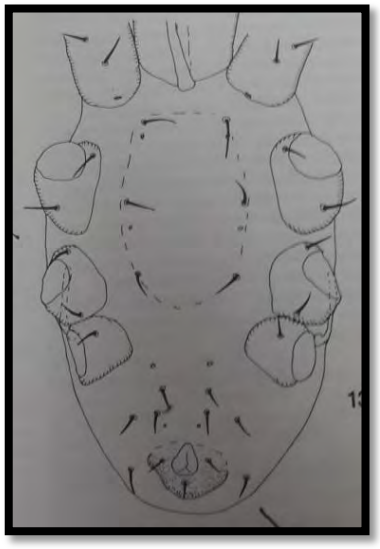

Figure 4.60 Ventral view of *T. (A.) microbullatus* protonymph (Ueckermann and Loots, 1988)

FAMILY STIGMAEIDAE OUEDEMANS

The family Stigmaeidae falls under the superfamily Raphignathoidea. It consists of a large cosmopolitan froup of genera which are identifiable by their distinctive configuration of the dorsal shield (Ueckermann and Meyer, 1987).

Type-genus: *Stigmaeus* Koch, 1836 (Meyer and Ueckermann, 1989).

The family Stigmaeidae can be distinguished by combining the follow number of characters: the dorsum is almost nude or completely covered by 2 shields or partly covered by 3 or more shields; the dorsal body setae can range from 12 to 14 pairs; the chelicerae are usually free, but they can be partly fused in some genera; a palpal thumb-claw is present and consists of a tibial claw with a seta-like or claw-like accessory claw at its base and a palptarsus; the thermal eupathidium on the palptarsus is in the form of a single spine, a bidentate, tridentate or 4 eupathidia; the genital and anal vestibule is covered by a single pair of anogenital covers (Meyer and Ueckermann, 1989).

Female. The idiosoma in Stigmaeidae females are round, oval or elongate when viewed dorsally. The gnathosoma is projected anterior to the prodorsum, while the chelicerae are

seperate or basally conjunct, conical. Species in this family lack a peritreme and their palps are stout, where the palptarsi are rarely elongate, the tibial claw is prominent and rarely less than 1/2 lengths of the palptarsus. The palptarsus contains 4 eupathidia, 3 of them (ul'ζ, ul"ζ, and sulζ) are often basally fused. The counts of the setae (excluding the solenidia and eupathidia) from the palpcoxa to the palptarsus are as follow: 1*elcp*, 0, 1-3, 1-2, 3+1 claw, 4. The subcapitulum is not elongated and includes 2 pairs (rarely 1 pair) of subcapitular setae. The prodorsum contains 2 pairs of vertical setae and 1-2 pairs of scapular setae. Eyes can be either present or absent in species included in this family. *pod* can also be either present or absent. The *c*-series, *d*-series, e-series on the dorsal hysterosoma contain 2 pairs of setae (rarely 1 pair), where the *f*-series include only 1 pair of setae and the *h*-series include 2, but rarely 3 pairs of setae. The seperation between coxae II and III is quite obvious. The ventral setae *4a* are rarely absent and the ventral opisthosoma contain 1-5 pairs of aggenital setae, where the genital and anal valves are fused or contiguous. These gential valves include 1-3 pairs of setae and the anal valves 3 pairs of pseudanal setae. Leg tarsal claws are present and nude and it is rare to find that they are absent. The empodial axis bears 3 shafts (rarely 2), where each shaft produces 1 pair of tenent hairs. Tarsi are not stalked. The counts of the solenidia on genu I-IV are as follow: 1, 0-1, 0, 0; on tibiae I-IV: 1-2, 1, 1, 0-1; and on tarsi I-IV: 1, 1, 1, 0-1. The counts of setae on legs I-IV are: coxae (excluding *1a*, *3a* and *4a*) 1-2 + 1*elcp*, 1-2, 1-2, 0-2; trochanters 1, 1, 1-2, 0-1; femora 4-6, 4-6, 2-3, 1-3, genua 1-5, 0-4, 0-3, 0-3; tibiae 5, 5, 5, 4-5; tarsi 9-14, 8-9. 6-7, 6-8 (Fan and Zhang, 2005).

Male. The hysterosoma in males are somewhat tapered and setae ps_1 and ps_2 are reduced and peg-like. The genital and anal openings are fused and they lack genital setae. An aedeagus is present and additional solenidia (ω_2) is at least present on tarsi I-II (Fan and Zhang, 2005).

Deutonymph. These stages are similar to adults but the lack genital folds and setae in both sexes and an aedeagus in males (Fan and Zhang, 2005).

Protonymph. Protonymphs contain only 1 pair of subcapitular setae and the ventral setae *4a* and genital setae are absent. They include fewer setae in the aggenital area and on segments of the legs than the deutonymphs and adults (Fan and Zhang, 2005).

Larva. Stigmaeidae larva lack subcapitular setae, ventral setae *4a*, genital and aggenital setae. They also do not contain a IV leg and have fewer setae on their leg segments than

protonymphs. A setal complex (similar to duplex setae in Tetranychidae) are present on leg I (Fan and Zhang, 2005).

KEY TO THE STAGES OF STIGMAEIDAE (Fan and Zhang, 2005).

1. With 4 pairs of legs; coxae II and III each with 1-2 setae; with 1-2 pairs of subcapitular setae.....2

- With 3 pairs pf legs; coxae II and III wihtout setae; without subcapitular setae.....larva

2. With 2 pairs of subcapitular setae (except *Mediolata,* palptarsi elongate); ventral setae *4a* rarely absent.....3

- With 1 pair of subcapitular setae; ventral setae *4a* absent.....protonymph

3. Genital folds and setae absent in female; males without aedeagus, trochanter IV nude.....deutonymph
- Genital folds present; genital setae present in female (*Agistemus, Eryngiopus, Mediolata, Neilstigmaeus, Parastigmeus, Pilonychiopus, Primagistemus, Prostigmaeus, Pseudostigmaeus, Scutastigmaeus, Stigmaeus, Storchia, Summersiella* and *Zetziella*) or absent (*Caligohumus, Cheylostigmaeus, Eustigmaeus, Ledermuelleriopsis, Makilingeria, Mendanaia, Mullederia, Mullederiopsis, Paravillersia, Postumius, Villersia* and *Villersiella*) in female; males with an aedeagus; trochanter IV often with 1 seta.....(adult).....4

4. Without aedeagus; tarsi I-II each with one solenidion.....female

- With aedeagus; tarsi I-II each with 2 solenidia.....male

KEY TO THE AFRICAN GENERA OF THE STIGMAEIDAE (Meyer and Ueckermann, 1989).

1. Opisthosoma completely or partly covered with 1 or more shields; 2 or more setae on at least one of the shields lying anterior to suranal shield.....2

- Opisthosoma (excluding suranal shields) without shields; small shields may be present at the bases of the setae but none bears more than 1 seta.....9

2. Prodorsal and opisthosomal shields not fused.....3

- Prodorsal and opisthosomal shields fused.....*Mullederia* Wood

3. Chelicerae free.....4

- Chelicerae partly fused.....*Cheylostigmaeus* Willmann

4. Dorsum covered with 9 to 16 shields.....5

- Dorsum covered with less than 9 shields.....6

5 Maximum number of setae on median opisthosomal shields 4 pairs; median shields occasionally represented by 1-3 piars of smaller shields; dorsal setae e_{1-2} never on seperate shields.....*Zetzellia* Oudemans

- Maximum number of setae on median shield 3 pairs; opisthosomal shields comprise of either 1 large median shield or 1 large median shield and 2 smaller median zonal shields; setae e_{1-2} invariably occur on different shields.....*Stigmaeus* Koch

6. Prodorsum with 4 pairs of setae, c_2 present.....7

- Prodorsum with 3 pairs of setae, c_2 absent; median shield bears 5 pairs of setae.....*Agistemus*

7. Dorsum alsmost completely covered by 3 to 4 shields (excluding humeral shields); terminal eupathidium on palptarsus tridentate.....8

- Dorsal shields reduced; prodorsal and median opisthosomal shields confined to midportion of the body; palptarsus with 4 eupathidia.....*Prostigmaeus* Kuznetzov

8. Dorsum almost completely covered by 3 shields (prodorsal, opisthosomal and suranal shield).....*Eustigmaeus* Berlese

- Dorsum almost completely covered by 4 shields (opisthosomal shields divided transversely).....*Ledermuelleriopsis* Willmann

9. Tarsi with membranous arolium.....10

- Tarsi without arolium.....11

10. Claws present; arolium between claws.....*Parastigmaeus* Kuznetzov

- Claws absent.....*Pilonychiopus* Meyer

11. Palptarsus with 4 eupathidia distally.....*Storchia* Oudemans

- Terminal eupathidium on palptarsus either bidentate/simple spine.....*Eryngiopus* Summers

GENUS *MULLEDERIA* WOOD

Type-species: *Mullederia arborea* Wood, 1964

Mullederia can be characterised by the dorsum which is covered by a fused prodorsal and opisthosomal shield, a posteroventral suranal shield and by 12 pairs of dorsal body setae. They do not contain the dorsal setae e_1. The terminal eupadidium on the palptarsus is tridentate and the anogenital area includes 1 or 2 pairs of aggenital and 3 pairs of anogenital setae. The empodium is made up of a membranous pad which may bear a rod with 2 or 3 pairs of raylets and arises beneath 2 claws which may be present (**Figure 4.61)** (Meyer and Ueckermann, 1989).

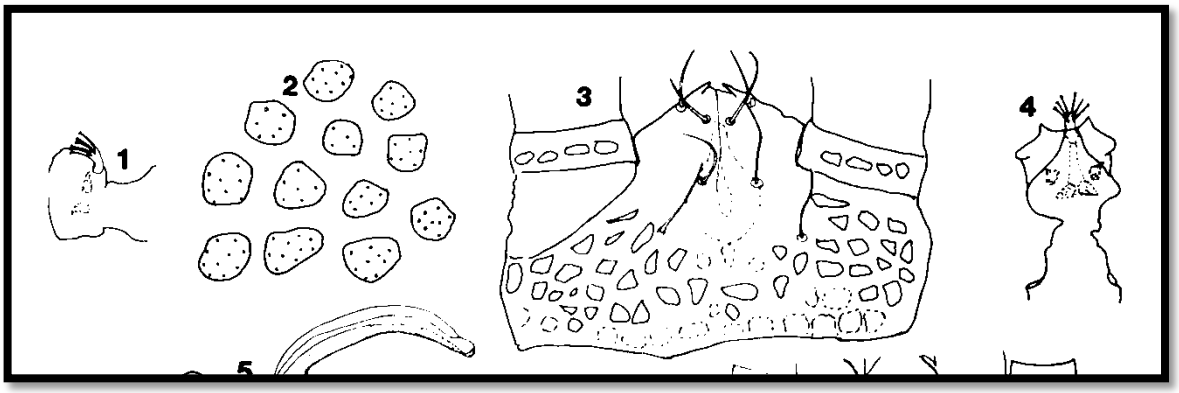

Figure 4.61 1 - Empodium of *Mullederia neomaculata* (Meyer and Ryke) female; 2 - Dorsal reticulate pattern of *Mullederia centrata* Meyer female; 3 - Ventral view of subcapitulum of *Mullederia centrata* Meyer female; and 4 - Empodium of *Mullederia centrata* Meyer female (Ueckermann and Meyer, 1987)

GENUS *CHEYLOSTIGMAEUS* WILLMANN

Type-species: *Cheylostigmaeus grandiceps* Willmann, 1951.

Cheylostigmaeus differs from other genera in the family Stigmaeidae due to its partly fused chelicerae (**Figure 4.63**). The dorsum of the idiosoma is covered with a prodorsal and opisthosomal shield which does not overlap the side-wall of the body. The suranal shield is generally in a posteroventral position (Meyer and Ueckermann, 1989). Below is the aedeagus of a male (**Figure 4.62**) and the palp and ambulacral of *Vheylostigmaeus* sp. (**Figure 4.64**).

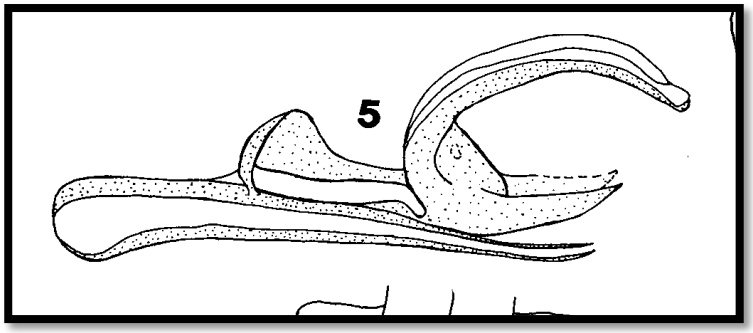

Figure 4.62 Aedeagus of *Cheylostigmaeus oudemansi* (Meyer and Ryke) male (Ueckermann and Meyer, 1987)

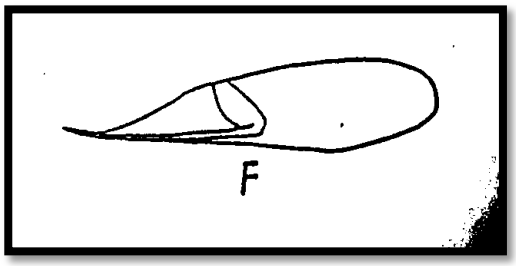

Figure 4.63 Chelicerae and related structures of *Cheylostigmaeus* sp. (MacFarlane, s.a.)

Figure 4.64 Palp and ambulacral structures of *Cheylostigmaeus* sp. (MacFarlande, s.a.)

GENUS *ZETZELLIA* OUDEMANS

Type-species: *Zetzellia methlagli* Oudemans, 1927

This genus is characterised by its prodorsal shield that contains 3 pairs of setae and setae c_2 that can be on seperate plates (**Figure 4.64**). Setae c_3 is located on small platelets. The median opisthosomal shield is either undivided or devided longitudinally into 2 or more sections, carrying c_1, d_1 and e_{1-2}. Setae d_2 and f_1 are situated on seperate plates. The suranal shield covers the posterior end of the opisthosoma and bears setae h_{1-2} (Meyer and Ueckermann, 1989).

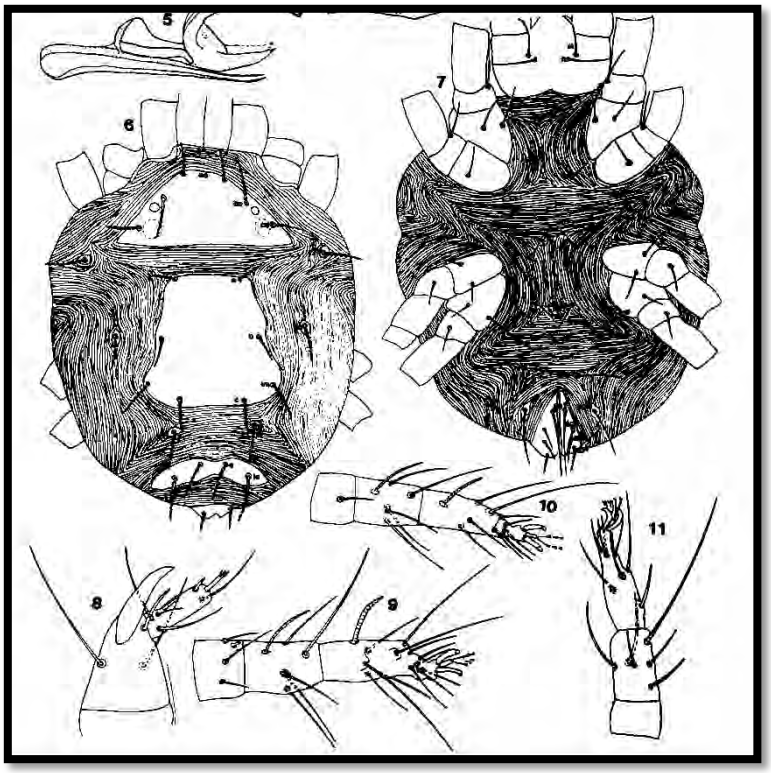

Figure 4.65 6 - Dorsal view of *Zetzellia buxi* Ueckermann & Meyer female; 7 - Ventral view of *Zetzellia buxi* Ueckermann & Meyer female; 8 - Palpus of *Zetzellia buxi* Ueckermann & Meyer female; 9 - Leg I of *Zetzellia buxi* Ueckermann & Meyer female; 10 - Leg II of *Zetzellia buxi* Ueckermann & Meyer female; 11 - Leg III of *Zetzellia buxi* Ueckermann & Meyer female (Ueckermann and Meyer, 1987)

GENUS *STIGMAEUS* KOCH

Type-species: *Stigmaeus cruentus* Koch, 1836

This genus can be characterised by the fact that it bears 4 pairs of setae on the prodorsum, either on a single large shield or on large median and a pair of small lateral shields (**Figure 4.66**). The opisthosomal shields consist of an unpaired median (which carries a maximum of 3 pairs of setae), an unpaired or paired suranal shield and 3 to 5 pairs of small shields (humerals, marginals, median zonals, lateral zonals and intercalaries); the latter platelets surround the median shield (Meyer and Ueckermann, 1989).

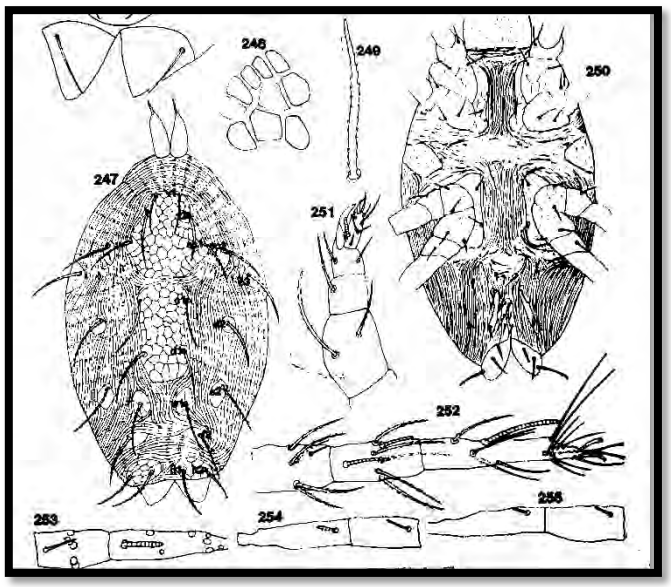

Figure 4.66 *Stigmaeus* sp. (Acari: Stigmaeidae) female: 247 - Dorsal view; 248 - Reticulation pattern of dorsal shield; 249 - Dorsal body seta; 250 - Ventral view; 251 - Palpus; 252 - Leg I; 253 - Leg II; 254 - Leg III; 255 - Leg IV (Ueckermann and Meyer, 1987)

GENUS *AGISTEMUS* SUMMERS

Type-species: *Caligonus terminalis* Quale, 1912

According to Gonzalez, 1965 (in Ueckermann and Meyer, 1987), *Agistemus* evolved from the *Zetzellia maori* Gonzalez group of species due to the ontogeny of the dorsal shield that is similar in *A. striolatus* Gonzalez and *Z. mali* Ewing (Ueckermann and Meyer, 1987). *Agistemus* includes species that bear 2 paired and 3 unpaired dorsal shields with the prodorsal

shield carrying 3 pairs of setae and the median opisthosomal shield 5 pairs (Meyer and Ueckermann, 1989).

GENUS *EUSTIGMAEUS* BERLESE

Type-species: *Stigmaeus kermesinus* Koch

The dorsum of the idiosoma in *Eustigmaeus* is almost completely covered by 3 shields (the prodosal, opisthosomal and suranal shield) and shields are usually ornamented (**Figure 4.67**). Setae c_3 is situated on the shields and dispalced to a ventrolateral position. The anogential area includes 1 to 3 pairs of aggenital setae and 3 pairs of anogenital setae (Meyer and Ueckermann, 1989).

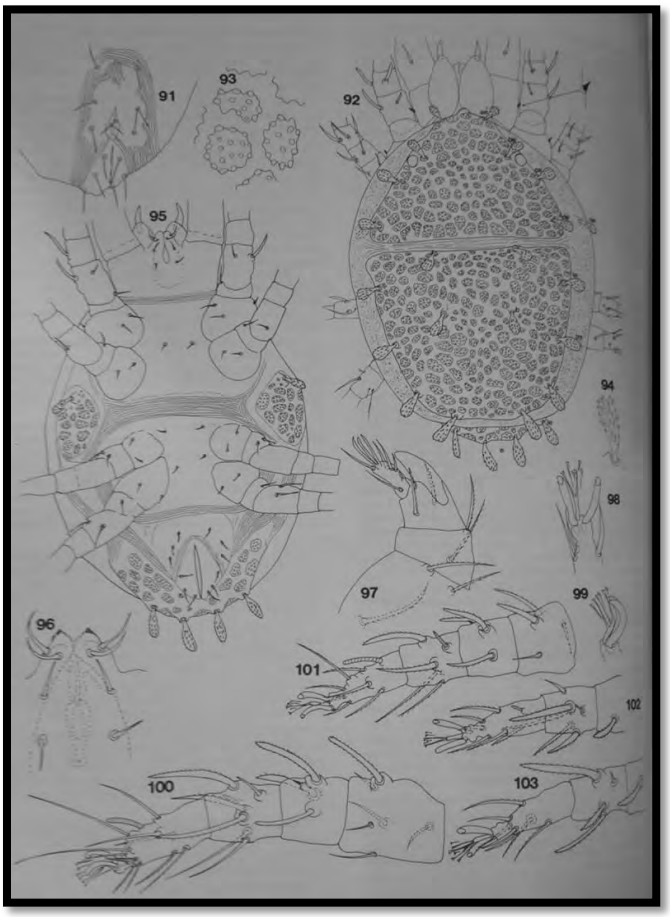

Figure 4.67 92 - Dorsal view of *Eustigmaeus spathatus* Ueckermann & Meyer, female; 93 - Dorsal reticulate pattern; 94 - Dorsal seta; 95 - Ventral view of *E. spathatus* Ueckermann & Meyer, female; 96 - Subcapitulum; 97 - Palpus; 98 to 99 - Empodia; 100 - Leg I; 101 - Leg II; 102 - Leg III; and 103 - Leg IV (Ueckermann and Meyer, 1987)

GENUS *PROSTIGMAEUS* KUZNETSOV

Type-species: *Prostigmaeus tauricus* Kuznetsov

This genus is characterised by its elongated and somewhat spinde-shaped idiosoma (**Figure 4.68**). The prodorsum contians 4 pairs of setae; The prodorsal and median opisthosomal shields are reduced and confined to the median part of the body and carries 3 and 2 pairs of setae respectively. Setae e_1 and f_1 are located on 4 platelets. The suranal sheild can either be divided or complete and bears 3 pairs of setae. The palptarsus contain a cluster of 4 distal eupathidia and the empodium on the leg tarsi are rod-like and bear 2 pairs of capitate raylets (Meyer and Ueckermann, 1989).

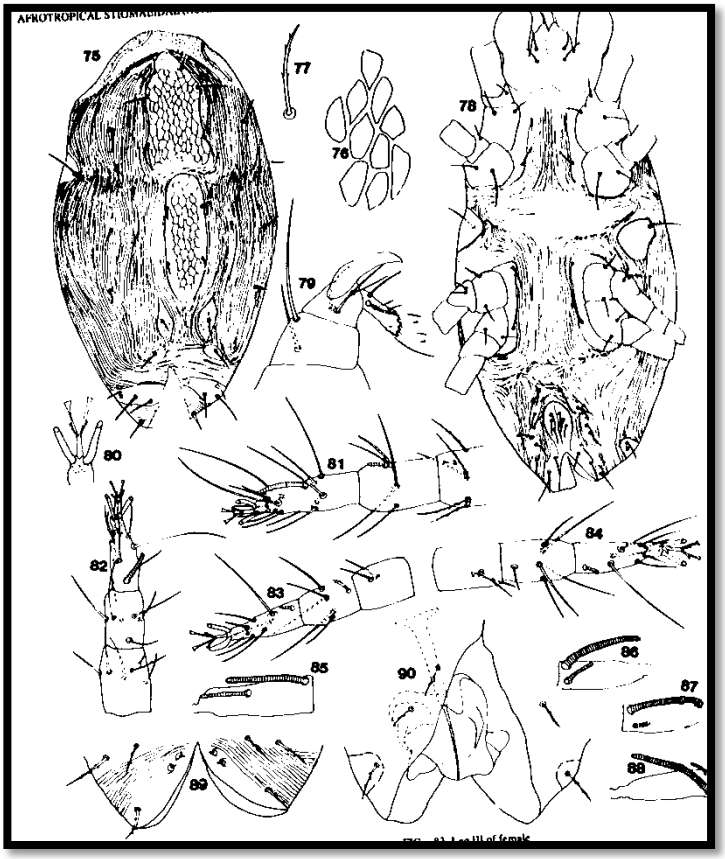

Figure 4.68 75 - Dorsal view of *Prostigmaeus vrystaatensis* Ueckermann & Meyer female; 76 - Dorsal reticulate pattern of female; 77 - Dorsal setae of female; 78 - Ventral view of female; 79 - Palpus of female; 80 - Empodium of female; 81 - Leg I of female; 82 - Leg II of female; 83 - Leg III of female; 84 - Leg IV of female; 85 Tarsus I of male; 86 - Tarsus II of male; 87 - Tarsus III of male; 88 - Tarsus IV of male; 89 - Dorsal view of anal opening of male; 90 - Aedeagus (Ueckermann and Meyer, 1987)

GENUS *LEDERMUELLERIOPSIS* WILLMANN

Type-species: *Ledermuelleriopsis triscutata* Willmann, 1951

The dorsum of *Ledermuelleriopsis* is almost completely covered by 4 shields (**Figure 4.69-4.70**). The opisthosomal shield is divided transversely (Meyer and Ueckermann, 1989).

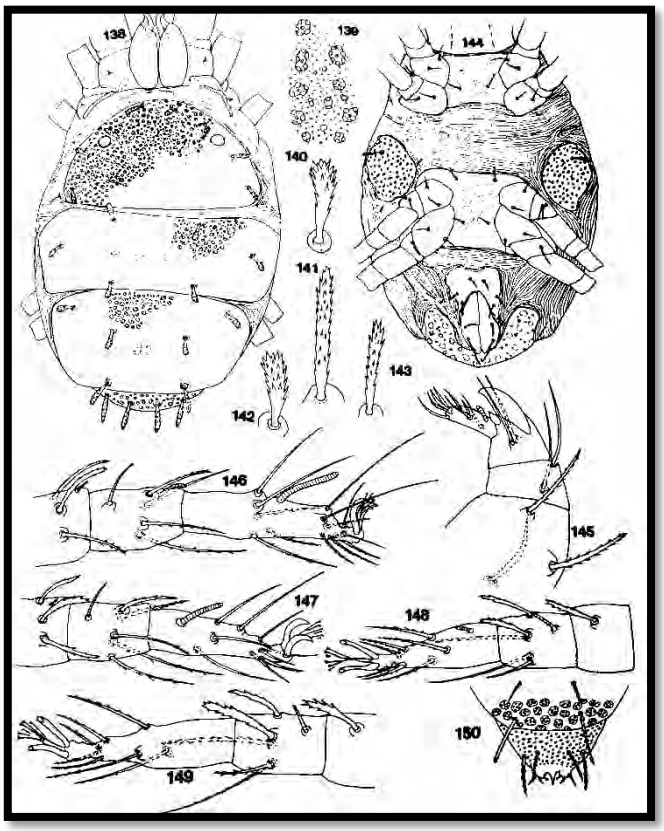

Figure 4.69 138 - Dorsal view of *Ledermuelleriopsis terrulenta* Ueckermann & Meyer female; 139 - Dorsal reticulate pattern of female; 140 - Setae *ae* of female; 141 - Setae *e* of female; 142 to 143 - Setae *he* of female; 144 - Ventral view of female; 145 - Palpus of female; 146 - Leg I of female; 147 - Leg II of female; 148 - Leg III of female; 149 - Leg IV of female; 150 - Dorsal view of anogenital area of male (Ueckermann and Meyer, 1987)

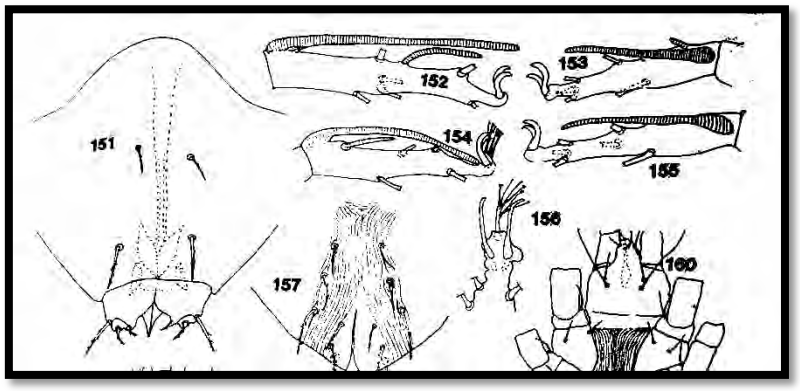

Figure 4.70 151 - Ventral view of anogenital area of *Ledermuelleriopsis terrulenta* male; 152 - Tarsus I of male; 153 - Tarsus II of male; 154 - Tarsus III of male; 155 - Tarsus IV of male (Ueckermann and Meyer, 1987)

GENUS *PARASTIGMAEUS* KUZNETSOV

Type-species: *Pseudostigmaeus capensis* Meyer, 1969

The prodorsal shield includes 2 pairs of setae (v_2 and sc_1). Setae sc_2 is located on small platelets. The opisthosomal shields are absent and setae are situated on small platelets. The suranal shield is present, whilst the endopodal shields are absent. The empodial rod has 3 pairs of capitate raylets and is borne distally on an arolium (**Figure 4.71**) (Meyer and Ueckermann, 1989).

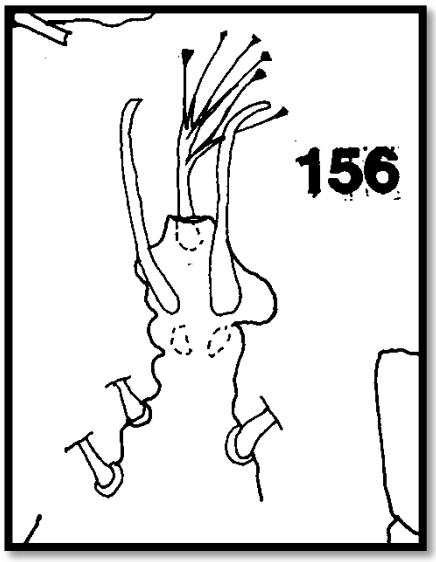

Figure 4.71 156 - *Parastigmaeus capensis* (Meyer) empodium of female (Ueckermann and Meyer, 1987)

GENUS *PILONYCHIOPUS* MEYER

Type-species: *Pilonychiopus cliffortus* Meyer, 1969

This genus is characterised by the dorsal plating being almost absent, except for a shield-like area medially on the prodorsum. The opisthosoma contains or is without a spindle-shaped median shield-like area. The suranal shield is rather well sclerotized and the drosum of the idiosoma bears 12 to 13 setae (**Figure 4.72**). Claws are absent on the legs and the empodial rod contains 3 pairs of raylets that are borne distally on a balloon-shape arolium (Meyer and Ueckermann, 1989).

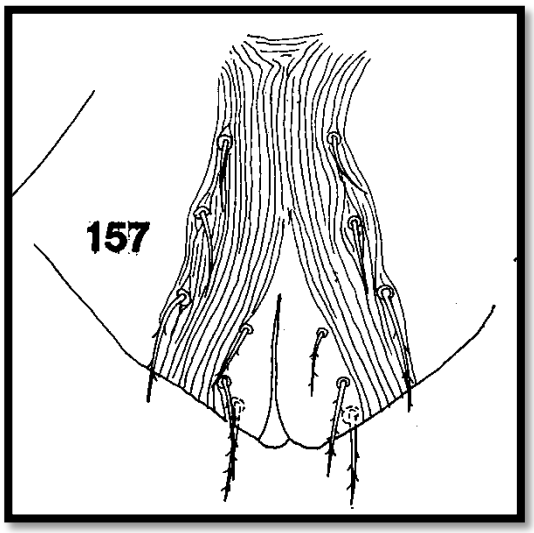

Figure 4.72 157 - *Pilonychiopus tutus* Meyer, anogenital area of nympha (Ueckermann and Meyer, 1987)

GENUS *STORCHIA* OUDEMANS

Type-species: *Caligonus robustus* Berlese, 1885

Storchia is defined by a dorsum with 13 to 14 pairs of setae (setae h_3 can be either absent or present). The dorsal plating is confined to an elongated prodorsal shield, a suranal shield that is divided and individual platelets around the bases of most of the dorsal body setae. The anogenital area contains 4 pairs of aggenital setae and 6 pairs of anogenital setae. The palptarsus includes 4 terminal eupathidia (Meyer and Ueckermann, 1989).

GENUS *ERYNGIOPUS* SUMMERS

Type-species: *Eryngiopus gracilis* Summers, 1964

This genus is defined by its elongated idiosoma and by containing dorsal plating that is almost obsolete (**Figure 4.73**). The shields are restricted to small, raised areas medially on the prodorsum and suranal shields. The posterior subcapitular setae and some ventral body setae are very long and the terminal eupathidium on the palptarsus can be either bifid or simple (Meyer and Ueckermann, 1989).

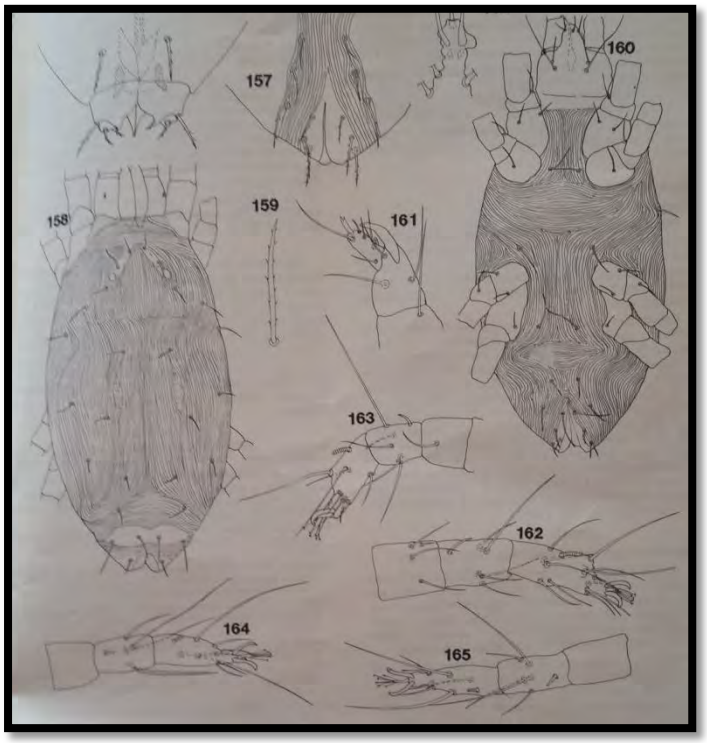

Figure 4.73 158 - Dorsal view of *Eryngiopus parsimilis* Ueckermann & Meyer, female; 159 - Dorsal seta; 160 - Ventral view; 161 - Palpus; 162 - Leg I; 163 - Leg II; 164 - Leg III; 165 - Leg IV (Ueckermann and Meyer, 1987)

FAMILY TYDEIDAE (OUTGROUP) KRAMER

Mites in the family Tydeidae are Prostigmatic mites and this family includes 7 subfamilies, over 50 genera and round about 500 described species across the globe (Zhang, *et. al.,* 2001).

These are extremely small mites, with an average body length of about one-fifth of a millimetre. This mite family belongs to the superfamily Tydeoidea, which differs from related mites by their reduced fixed cheliceral digits, fused cheliceral bases and short, needle-like moveable cheliceral digits. The family Tydeidae can be divided into six subfamilies: Meyerellinae, Australotydeinae, Tydeinae, Pretydeinae, Tydaeoinae and Pronematinae (Zhang, *et. al.,* 2001).

Unfortunately, due to high density of striae (**Figure 4.75**) and their difficult shape of setae (**Figure 4.74**), biologists and acarologists find it difficult to determine the species correctly (Darbemamieh, *et. al.,* 2010).

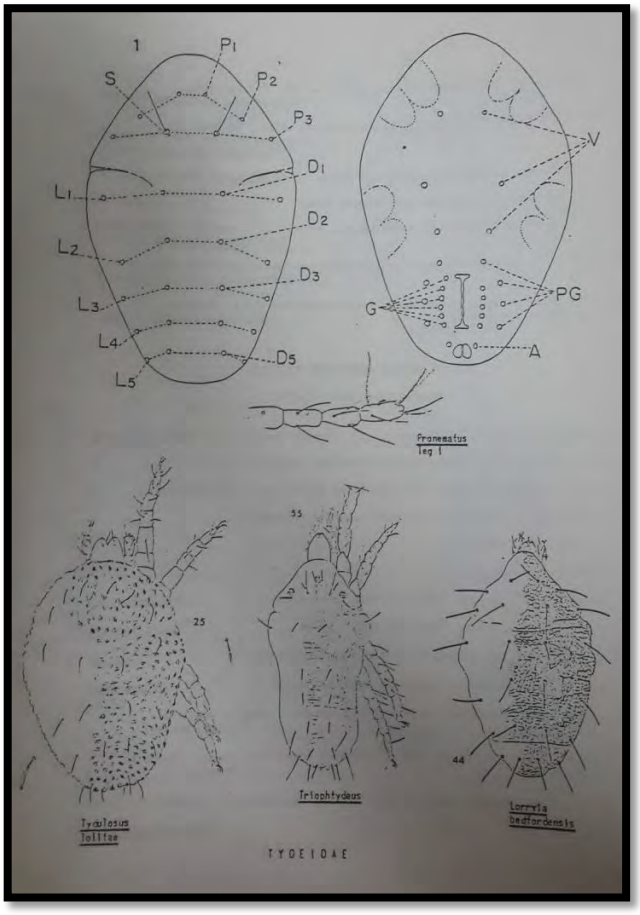

Figure 4.74 External structure of tydeid mites (Prostigmata: Tydeidae) (MacFarlane, s.a.)

Figure 4.75 Scanning electron micrograph of Tydeidae sp. (Wikipedia, 2014)

Brachytydeus sp. Thor

Brachytydeus sp. belongs to the "nobila group" of tydeid species. These mites are known to have relativly large and widened bodies with strong ornamentation, long, broad and rounded distlly palpal eupathidium and lurifissures *im* that are located antero-medially to setae e1. They are also characterised by the arrangement of their dorsal striations (**Figure 4.76** and **4.78**), which represents subtype "Biparalorryia-incerta". *Brachytydeus* sp. can also be distinguished ventrally between the metasternal setae where the striae will form a "V" or "U" - pattern. The dorsal body setae are moderately to strongly serated (Kaźmierski, 2009).

Idiosoma (**Figure 4.79-4.83).** These mites contain a broad-oval body and are dark green in colour. The dorsal ornamentation is located laterally and slightly posteriorly to setae c1 and one pair of rosettes are distinctly visible. These rosettes indicate the place of the internal sigillae (place where muscles are inserted). Reticulations are limited to the front of the aspidosoma in these species (**Figure 4.77**). A few single meshes occur ventrally on the end of

the opisthosoma. The tubercles on the striae are in the shape of a slightly flattened hemisphere and the reticulum contin the tubercles and "Y" - shaped cross-ties. It is unsure whether all *Brachytydeus* species have eyes. The bothridial setae are whip-like and are more than three times longer than the normal dorsal setae. These whip-like setae are narrowly-lanceolate, subtly curved, pointed and strongly serrated. Setae ps1 are more or less the same shape, but they are slightly finer and located ventrally. Lyrifissure *ia* lies posterior to setae c2 at the distance that's longer than $^1/_3$ of the c2 - e1 sector, and medially to the c2 - e1 line. Lyrifissure *im* lies in the same longitudinal row with lyrifissure *ia* and is medially and slightly anterior to e1. The ventral side of this species is more subtly striated. The striae form an obtuse "V - U" - pattern. Two genital pores are present in this species (Kaźmierski, 2009).

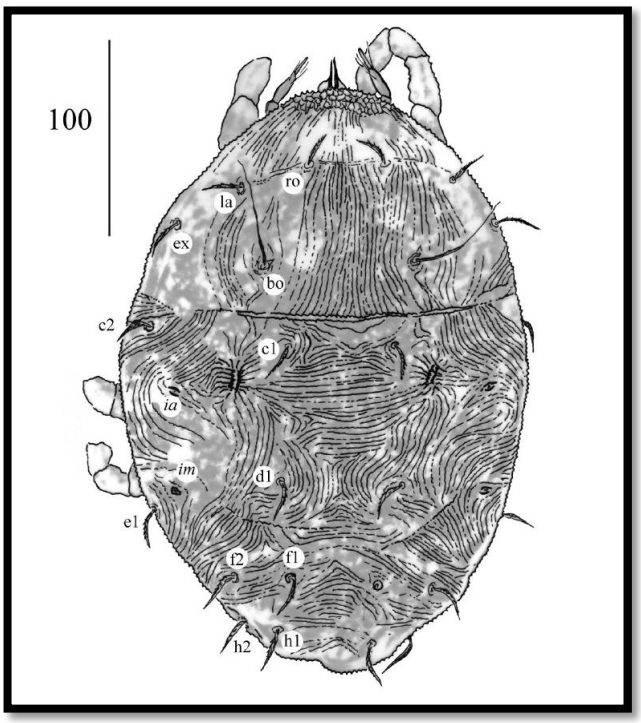

Figure 4.76 Dorsal view of *Brachytydeus* sp. showing genital appearance (Kaźmierski, 2009)

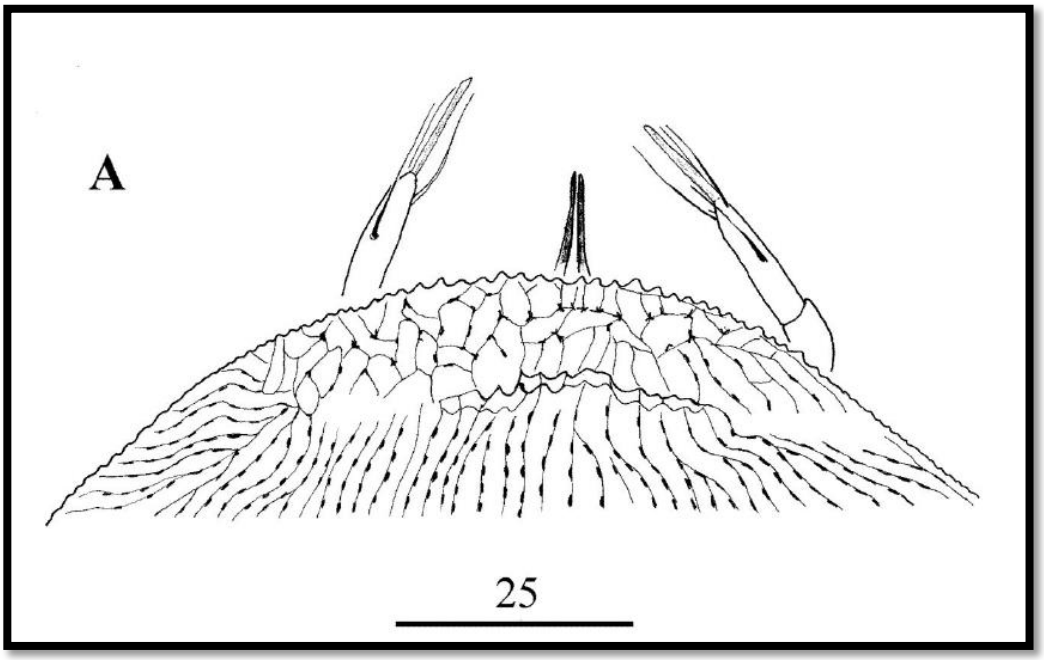

Figure 4.77 Dorsal view of anterior part of aspidosoma of *Brachytydeus* sp. (Kaźmierski, 2009)

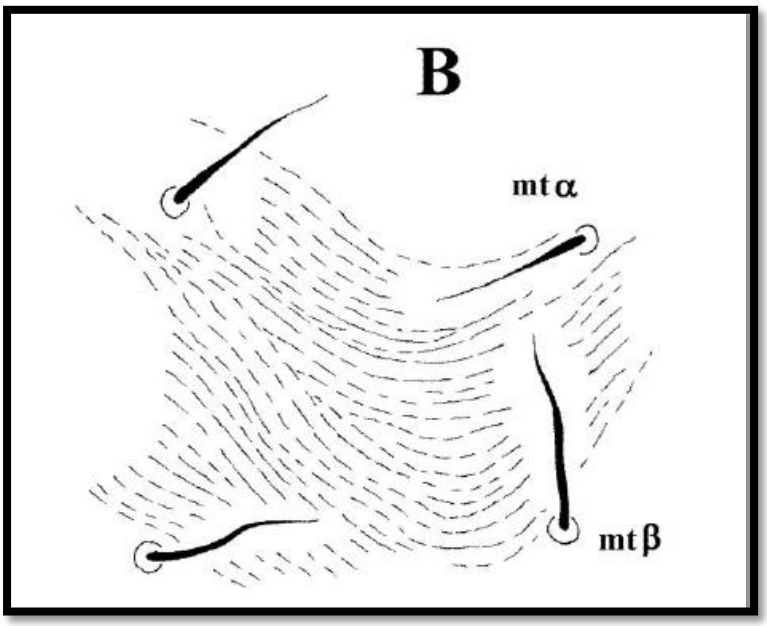

Figure 4.78 Ventral striation between setae *mt* of *Brachytydeus* sp. (Kaźmierski, 2009)

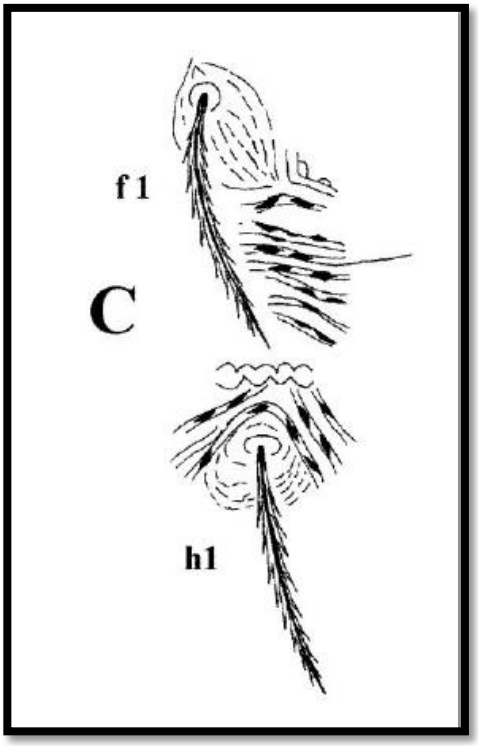

Figure 4.79 Dorsal fragment of *Brachytydeus* sp. with f1 and h1 (Kaźmierski, 2009)

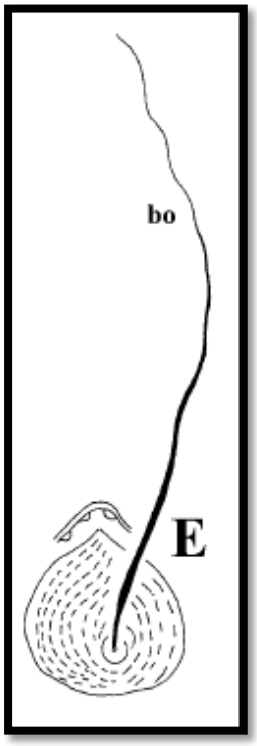

Figure 4.80 Bothridial seta *bo* of *Brachytydeus* sp. (Kaźmierski, 2009)

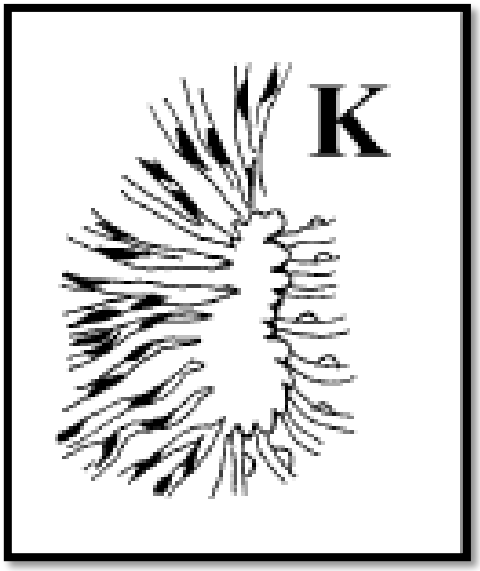

Figure 4.81 Dorsal rosette of *Brachytydeus* sp. (Kaźmierski, 2009)

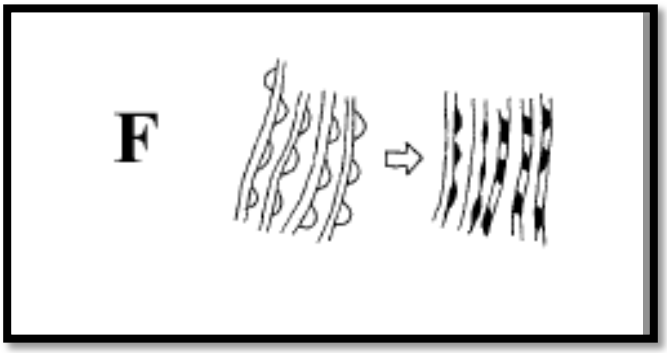

Figure 4.82 Dorsal striae of *Brachytydeus* sp. with tubercles (Kaźmierski, 2009)

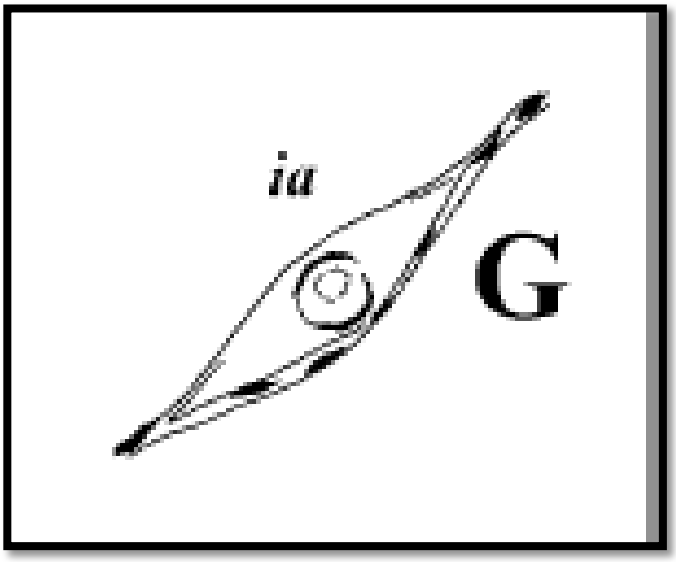

Figure 4.83 Lyrifissure *ia* of *Brachytydeus* sp. (Kaźmierski, 2009)

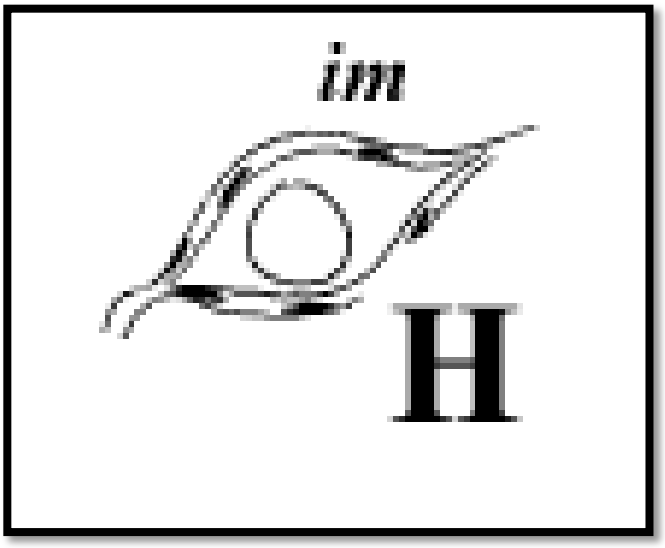

Figure 4.84 Lyrifissure *im* of *Brachytydeus* sp. (Kaźmierski, 2009)

Gnathosoma. The gnathosoma is hidden under the aspidosoma and only the ends of the stilettos and the palptarsi can be seen from above. The cheliceral stilettos (**Figure 4.85**) are longer than the palpal tarsus (**Figure 4.85**), but they are shorter than the palpal tarsus that's combined with its terminal eupathidium (pζ). The terminal eupathidium is stick-like, thick, straight, rounded dorsally (character of the "nobila" species group) and long, but not as long in length as the palptarsus. The palptarsal setae are more or less equally lengthed with their segments, but none are forked distally. Seta I is the longest among them, whereas 1 is the shortest. It is unclear whether the vestigial setula ba is present in this species. The characteristical measurements are as follow: stilettos - 21µm, palpal femurogenu – 23/11µm, df - 23µm, dg - 16µm, t' - 16µm, t" - 6µm, palptarsus – 15/5µm and (pζ) - 12µm (Kaźmierski, 2009).

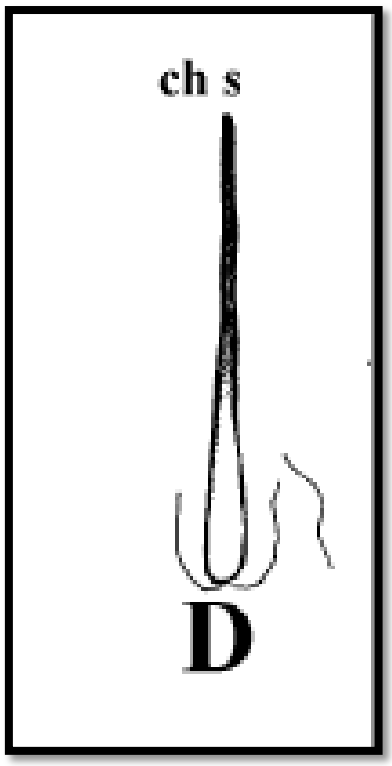

Figure 4.85 Cheliceral stiletto of *Brachytydeus* sp. (Kaźmierski, 2009)

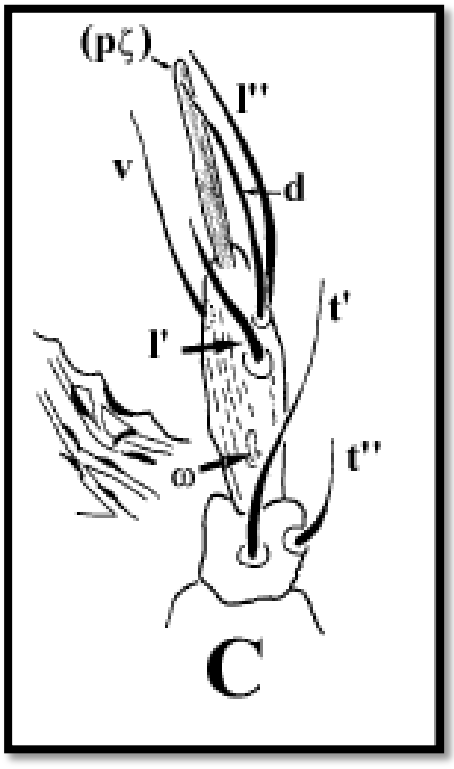

Figure 4.86 Palpal tibia and tarsus (right, dorsally) of *Brachytydeus* sp. (Kaźmierski, 2009)

Legs (Figure 4.87-4.88). The coxal organ of *Brachytydeus* species is oval. These species contain an epideral formula of 3-1-4-2, with a chaetotaxy (from tarsus to trochanter) as

follow: leg I (8-3+1-3-3-1), leg II (6-2-2-3-0), leg III (5-2-1-2-1) and leg IV (5-2-1-1-0). The formulus k" (4 long) is broadened distaly and contain three teeth, whereas the ωII solenidion is only 2 long. The empodial hook (*om*) is present in this species (Kaźmierski, 2009).

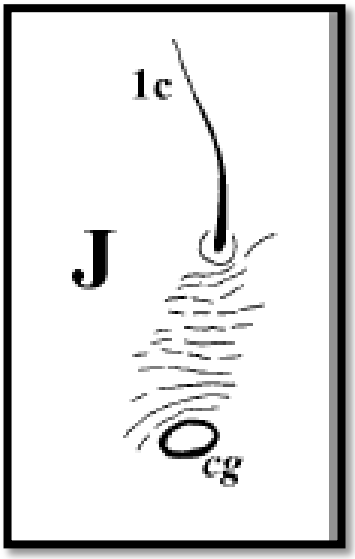

Figure 4.87 Coxal organ *cg* and seta 1c of *Brachytydeus* sp. (Kaźmierski, 2009)

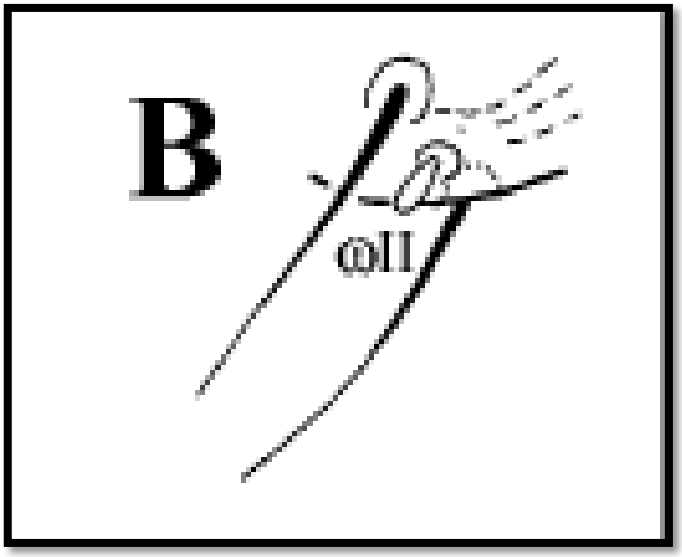

Figure 4.88 Tarsus II, fragment with solenidion ωII of *Brachytydeus* sp. (Kaźmierski, 2009)

Females. *Brachytydeus* females contain the same characters as listed above with the exception of the genital region, which is slightly bigger. The progential aperture leads to the progenital chamber and is shaped similarly to a recumbert letter H. These species contain 6 pairs of genital setae (*ge*) and four pairs of adgenita setae (*ag*) (genital organotaxy: 0-6-4) (Kaźmierski, 2009).

Males. Males are similar to females. However, the genital region does differ and a longitudinal progenital aperture and fair pairs of short and bushy eugenital setae (*eu*) are present. They have an average body length of about 334µm and a width of 242µm. The genital organotaxy is as follow: 4-6-4 and adults contain three pairs of sigillae (Kaźmierski, 2009).

Protonymph. Protonymphs differ from tritonymphs in their size (118/130µm), their genital organotaxy (a single pore, genical chaetotaxy 0-1), by their epidermal formula (3-1-3-0), their

nude trochanter I (lack of seta *tr*) and by the chaetotaxy of their leg IV (5-0-0-0-0) (Kaźmierski, 2009).

Larvae. These stages contain no genitals, have only six legs and nude trochanters. The epidermal has a formula of 3-1-2. The chaetotaxy of the legs has a formula of: I (8-3+1-3-3-0), II (6-2-2-3-1) and III (5-2-1-2-0). Tarsus I contains a double anabasis and the aspidosomal reticulum is inconspicuous. The "caudal" setae (*f, h*) are stronger than other setae and are broader in comparison to them. Their body size ranges from 140-145µm in length and 102-114µm in width (Kaźmierski, 2009).

SECTION B: MOLECULAR REVIEW

CHAPTER 5: MATERIALS and METHODS

SECTION B: MOLECULAR REVIEW

CHAPTER 5: MATERIALS and METHODS

5.1 MOLECULAR BASED IDENTIFICATION

Mites were collected as discussed in chapter 3, section 3.3 - 3.5. Selected mites' PCR products of the *COI* fragment and ITS region were sequenced and these sequences were cross-referenced with accessions in the GenBank database to confirm the identity of the species, and further used in phylogenetic analysis. After the DNA was extracted, mite cadavers were used to identify specimens to species level (**Table 5.1**) and compared to sequences obtained from international databses.

5.2 DNA EXTRACTION

The whole cell DNA was extracted from frozen tissue stored in 96% ethanol at -20°C.

The DNA extraction protocol from the spider mite team at the French National Institute of Agricultural Research (INRA) in Montpellier France, was followed during this study. The total genomic DNA from each specimen was extracted by using the Qiagen DNeasy tissue kit (Qiagen, Whitehead Scientific, South Africa), according to the DNA extraction protocol "Purification of Total DNA from Animal Blood or Cells" (Spin-Column Protocol) as adapted by Tixier *et al.* (2010) and de Medonça *et al.* (2011).

Table 5.1 Mites identified for the extraction of DNA

Mite Species	Isolate
Phytoseiidae	
Phytoseiidae sp. 1	Isolate 1
Typhlodromus microbullatus	Isolate 2

Phytoseiidae sp. 2	Isolate 3
Amblyseius pretoriaensis	Isolate 6
Tetranychidae	
Tetranychus sp.	Isolate 4
Tetranychus evansi	Isolate 5
Tetranychus evansi	Isolate 7
Tydeidae	
Brachytydeus sp.	Isolate 8
Stigmaeidae	
Stigmaeidae sp. 3	Stigmaeidae sp. Isolate 3
Stigmaeidae sp. 4	Stigmaeidae sp. Isolate 4
Stigmaeidae sp. 5	Stigmaeidae sp. Isolate 5
Stigmaeidae sp. 6	Stigmaeidae sp. Isolate 6
Stigmaeidae sp. 7	Stigmaeidae sp. Isolate 7
Stigmaeidae sp. 10	Stigmaeidae sp. Isolate 10
Stigmaeidae sp. 11	Stigmaeidae sp. Isolate 11
Stigmaeidae sp. 12	Stigmaeidae sp. Isolate 12
Stigmaeidae sp. 13	Stigmaeidae sp. Isolate 13
Stigmaeidae sp. 15	Stigmaeidae sp. Isolate 15

A single air dried mite was added to a 1.5 ml microcentrifuge tube containing lysis buffer (90 µl Phosphate-Buffered Saline – 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.4), 10 µl Proteinase K and 100 µl Buffer AL (Qiagen). Tubes were then mixed

by vortexing and centrifuged for 1 min at 8 000 rpm and incubated in a water bath at 56°C for 16 hours.

Hundred microliters of ethanol 100 % was added to the sample. The mixture was then transferred with a pipette into the DNeasy mini spin column and centrifuged for 1 min at 8 000 rpm. The collection tube and the flow-through were discarded and the spin column was kept. DNeasy column was placed in a new 2 ml collection tube and washed with 250 µl AW1 buffer and then removed by centrifuging for 1 min at 8 000 rpm. The collection tube was discarded and a second wash was performed by using 250 µl buffer AW2, followed by a centrifugation of 3 min at 13 000 rpm.

MiliQ d'H_2O was placed directly onto the DNeasy membrane (50 µl) to elute DNA and incubated for three min, after which it was centrifuged for 1 min at 8 000 rpm.

DNA was stored at -20°C until PCR amplification and sequencing took place.

At the end of the DNA extraction procedure, the column membrane was cut from the spin column and placed under the stereo-microscope. The mite's cadaver was collected from the membrane with a fine paintbrush. The mite is usually located on the top of the column, around the edge. The mite was then mounted on a slide in PVA medium and then observed under the microscope to determine the species of the specimen.

5.3 AMOUNT OF DNA

The total amount of DNA was assessed by using a spectrophotometer (NanoDrop ND 1 000). The maximum absorbance of DNA is at 260 nm; whereas protein absorbs light the strongest at 280 nm. The A_{260}/A_{280} ratio would therefore provide a clarity on the purity of the DNA, where values of 1,8-2,0 would suggest that the DNA is "clean".

5.4 PCR AMPLIFICATION and SEQUENCING

Two target DNA fragments were amplified and sequenced per single mite, namely: the nuclear ribosomal region spanning the ITS1, 5.8S and ITS2 region (about 700 bp) and a fragment of the mitochondrial *COI* gene (about 700-bp for the second *COI* gene and 800-bp for the first - **Table 5.2**).

The PCR reactions were performed in a volume of 25 µl. Each 25 µl volume per tube contained 3 µl of template DNA for the ITS/*COI* gene fragments, 12.5 µl KAPA MasterMix (ThermoScientific, South Africa), 1 µl forward primer (0.4 µM), 1 µl reverse primer (0.4 µM) primer and 7.5 µl ultrapure water.

The primers that were used to amplify the different gene fragments are listed in **Table 5.2**.

Failed amplification reactions from the *COI* primers C1J1718 and 773 were further processed by using the *COI* primers *COI*2F and *COI*2R to obtain PCR products.

Table 5.2 PCR and sequencing primers used to obtain nuclear ribosomal ITS and mitochondrial *COI* sequences of mites during this survey.

Fragment	Length (bp)	Position	Primer	Reference	Primers
ITS	700 700	3' of 18S 5' of 28S	18S 28SC	Medonça *et al.*, 2011	5'-AGA GGA AGT AAA AGT CGT AAC AAG-3' 5'-ATA TGC TTA AAT TCA GCG GG-3'
COI	800 800	*COI* *COI*	C1J1718 773	Medonça *et al.*, 2011	5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3' 5'-TAC AGC TCC TAT AGA TAA AAC-3'
COI	700 700	*COI* *COI*	*COI*2F *COI*2R	Ashton et al., 2008	5'-TTY GAY CCI DYI GGR GGA GGA GAT CC-3' 5'-GGR TAR TCW GAR TAW CGN CGW GGT AT-3'

Before the final amplification was done for sequencing purposes, PCR temperature gradients were run for both the ITS and *COI* primers to determine the optimal annealing temperature for all isolates. A gradient was run for Phytoseiidae sp. 1 (Isolate 1) (**Figure 5.1**),

Typhlodromus microbullatus (Isolate 2), *Tetranychus evansi* (Isolate 5), *Brachytydeus* sp. (Isolate 8), Stigmaeidae sp. 5 (Stigmaeidae sp. 5 Isolate 5), Stigmaeidae sp. 12 (Stigmaeidae sp. 12 Isolate 12) and Stigmaeidae sp. 15 (Stigmaeidae sp. 15 Isolate 15). The melting temperature for the ITS forward primer was 52.6°C and for the reverse primer 50.6°C. For the annealing temperature, a gradient was run between 48-55°C, after which an annealing temperature of 51°C was decided on (**Figure 5.1**). The melting temperature for the C1J1718 *COI* forward primer was 54.2°C and for the 773 *COI* reverse primer 46.3°C. A gradient was run between 46-55°C, after which annealing temperature of 46°C was decided on. The melting temperature for the *COI*2F *COI* forward primer was 65.5°C and for the *COI*2R *COI* reverse primer 57.6°C. A gradient was run between 56-63°C, after which annealing temperature of 57°C was decided on.

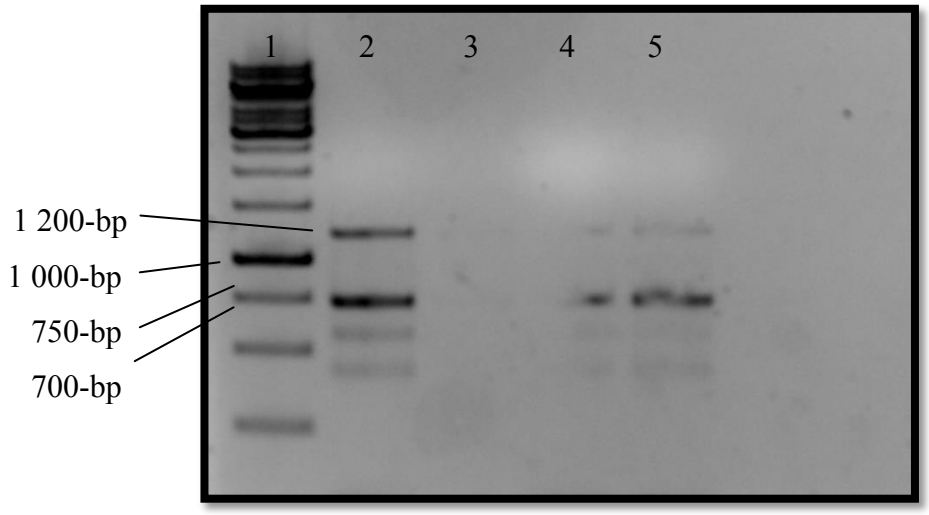

Figure 5.1 Gradient of the PCR amplification of the nuclear ribosomal ITS for Phytoseiidae sp. 1 (Isolate 1) ran on a 1% agarose gel. Left to right: Lane 1 - GeneRuler; Lane 2 - 49.4°C; Lane 3 - 50.8°C; Lane 4 - 52.5°C; Lane 5 - 53.8°C.

Samples were denatured at 94°C for 3 min and the thermal cycle was carried out for 35 cycles of 1 min denaturation at 94°C, annealed for 1 min at 51°C (ITS) and 46/57°C (*COI*), extended for 1 min 30 sec at 72°C and a final cycle where the extending step was 2 min at 72°C. PCR products (Amplified DNA) were visualized using 25 µl of the PCR reaction on a

1% agarose gel saturated with ethidium bromide in 1x TAE buffer (45 mM Tris base, 45 mM Boric acid and 1 mM EDTA, pH 8.0) (migration of 45 – 90 min at 90 volts).

The Gene Ruler DNA size marker 1kb DNA Ladder: 250 to 10 000bp were used to estimate the sizes of the amplified fragments.

Most sequencing templates were fragments from a single round of amplification. Those that provided too little product from a single amplification were reamplified at either a higher or a lower annealing temperature.

The amplification products were excised from the gel and sent to the University of Stellenbosch for purification and sequencing.

5.5 DNA SEQUENCES and SEQUENCE ANALYSIS

Sequencing reactions were carried out by the Central Analytical Facilities of the University of Stellenbosch in South Africa. By using the NucleoFast Purification System (obtained from Seperations), they allowed for post-PCR purification to take place. Sequencing was carried out with the BigDye Terminator V1.3 (Applied Biosystems), after which electrophoresis was performed on the 3730xl DNA Analyser (Applied Biosystems). The sequences were then analyzed and trimmed through the use of Sequencing Analysis V5.3.1 (Applied Biosystems). DNA fragments were sequenced in both directions and the phylogenetic relationships were estimated based on both ITS and *COI* sequences analyses.

5.6 SEQUENCE RETRIEVAL and DATASET

The studied dataset was constructed with the sequences generated during this study as well as the ITS and *COI* sequences of Phytoseiidae, Stigmaeidae and Tetranychidae that were available at National Centre for Biotechnology Information (NCBI) Bethesda, Maryland,

United States of America (accession numbers **Table 5.3**). Only sequence data that were closely related to the species identified during this study were added and considered in phylogenetic trees due to the scarcity of available data. The possible outgroups (where applicable) are also listed in **Table 5.3.** Sequences of the fragments of the different isolates from this study were compared to those deposited in the databases to determine sequence similarity.

Various ITS and *COI* sequences obtained during this study were identical or similar to several sequences already present in the GenBank dataset. Unfortunately, due to scarsity of sequencing data, it made it difficult to find GenBank accession numbers for the ITS or *COI* genes of some mites that were selected for phylogenetic comparisons.

Table 5.3 Species considered and their accession numbers in the NCBI GenBank

	GenBank accessions ITS	**GenBank accessions *COI***
Acarus farris	AY525591.1	GQ864338.1
Acarus siro	AY525583.1	-
Amblyseius andersoni	HQ404823.1	GU565316.1
Amblyseius deleoni	GU128958.1	-
Amblyseius herbicolus	KF219660.1	JX080329.1
Amblyseius largoensis A	KF219654.1	JX080348.1
Amblyseius largoensis B	KF219655.1	-
Eriophyes insidiosus	AJ251694.1	-
Eriophyes pyri	-	EU254715.1
Eustigmaeus sp.	-	JX837620.1
Eustigmaeus sp.	-	JX837688.1
Iphiseius degenerans	-	FM210120.1

Neoseiulus agrestis	HQ404814.1	-
Neoseiulus barkeri	HQ404813.1	-
Neoseiulus californicus A	-	AB500131.1
Neoseiulus californicus B	-	FM210117.1
Neoseiulus fallucis	-	JX080310.1
Neoseiulus idaeus	JF776276.1	-
Neoseiulus imbricatus	-	JX080312.1
Neoseiulus longilaterus	HQ404828.1	-
Neoseiulus womersleyi A	HQ404820.1	AB500132.1
Neoseiulus womersleyi B	-	AB500135.1
Phytoseiulus fragariae	GU591662.1	-
Phytoseiulus longipes	GU591679.1	-
Phytoseiulus macropilis	GU591660.1	-
Phytoseiulus persimilis A	GU591667.1	FM210193.1
Phytoseiulus persimilis B	HQ404818.1	FM210192.1
Phytoseiulus persimilis C	-	FM210191.1
Stigmaeopsis celarius A	FJ515683.1	-
Stigmaeopsis celarius B	JF774174.1	-
Stigmaeopsis miscanthi	JF774177.1	-
Stigmaeopsis nanjingensis	FJ515684.1	-
Tetranychus cinnabarinus	-	DQ437568.1
Tetranychus evansi	AB735996.1	FJ440678.1
Tetranychus kanzawai	-	AY044642.1

Tetranychus ludeni	JX497786.1	-
Tetranychus neocaledonicus	JX497787.1	JX075251.1
Tetranychus urticae	KF544955.1	AB066469.1
Tetranychus viennensis	JQ438841.1	-
Typhlodromus exhilaratus A	JF279155.1	JF279183.1
Typhlodromus exhilaratus B	-	JF279182.1
Typhlodromus phialatus A	JN793515.1	-
Typhlodromus phialatus B	HQ404829.1	-
Typhlodromus pyri A	JF279153.1	JF279181.1
Typhlodromus pyri B	JF279139.1	JF279163.1
Typhlodromus pyri C	-	JF279167.1
Uropoda orbicularis	-	JN992104.1

5.7 SEQUENCE and PHYLOGENETIC ANALYSIS

Sequences verification and alignment were done unambiguously with BioEdit and MEGA version 6 (Tamura *et al.,* 2013), using the following setting during alignment: gap open cost (15), gap extension cost (6.66) and end gap cost (as any other). Alignments were physically verified and equivocal bases were corrected by visual inspection.

The ITS and both *COI* matrices were analysed separately using MEGA version 6 (Tamura *et al.,* 2013). Due to the use of two different *COI* primer combinations, sequences will differ in length and in position on the *COI* region.

A distance method, Neighbour-Joining (Saitou and Nei, 1987), as well as a model based approach, Maximum Parsimony, was used. Neighbour-Joining was performed using the Jukes-Cantor model (Jukes and Cantor, 1969) and are in the units on the number of base

substitutions per site. The Maximum Parsimony trees were acquired by using the Tree-Bisection-Regrafting (TBR) algorithm (Nei and Kumar, 2000) with search level 1 in which the primary trees were obtained by adding sequences randomly (10 replicates). Codon positions that were taken into account for both models were the 1^{st} + 2^{nd} + 3^{rd} + Noncoding positions. The gap open penalties (GOP) were 15, the gap extension penalties (GEP) were 6.66 and the transition weight was 0.5 (Tamura *et al.,* 2013). Bootstrap analysis of 1 000 replicates were conducted to establish the internal support (Felsenstein, 1985). A bootstrap with a percentage of 80% or higher is considered to be a high bootstrap, but if a bootstrap support is 50% or higher, it is thought of as moderate. As soon as a bootstrap support is lower than 50%, it is considered to be a very low bootstrap support. The bootstrap consensus trees of the Neighbour-Joining and Maximum Parsimony are reported next to the branches.

CHAPTER 6: RESULTS and DISCUSSIONS

CHAPTER 6: RESULTS and DISCUSSIONS

6.1 RESULTS

A total of 1 115 mites were collected (**Table 4.2**) from 12 sites (**Figure 4.1**) (38 different hosts - solanaceous crops: **Table 4.1**) in Gauteng, KwaZulu Natal and the North-West Province. Ribosomal ITS and mitochondrial *COI* sequences were retrieved for 18 mites (4 Phytoseiidae isolates, 10 Stigmaeidae isolates, 3 Tetranychidae isolates and 1 Tydeidae isolate).

6.1.1 DNA EXTRACTION, QUANTITY AND QUALITY

Good quality total genomic DNA could be isolated from a single female specimen (**Table 6.1**). The amount of DNA isolated per specimen was adequate enough to run more than one PCR reaction (**Table 6.1**).

Table 6.1 Quantity and quality of (ng/mite) of DNA extracts of Phytoseiidae, Tetranychidae and Tydeidae

	Mite Species	DNA amount in ng/mite	DNA amount in ng/µl	$OD_{260/280}$
Phytoseiidae				
Isolate 1	Phytoseiidae sp. 1	280	5.6	1.87
Isolate 2	*T. microbullatus*	165	3.3	1.91
Isolate 3	Phytoseiidae sp. 2	180	3.6	1.26
Isolate 6	*A. pretoriaensis*	155	3.1	2.74
Tetranychidae				
Isolate 4	*Tetranychus* sp.	370	7.4	1.52
Isolate 5	*T. evansi*	170	3.4	1.07
Isolate 7	*T. evansi*	1305	26.1	1.57

Tydeidae				
Isolate 8	*Brachytydeus* sp.	160	3.2	1.29

The cadavers were successfully retained after DNA extraction as was seen in chapter 4, **Figures 4.41**, **4.54** and **4.55**. However, this could only be done for isolates 2 (*T. microbullatus*) and 6 (*A. pretoriaensis*) which belonged to the Phytoseiidae and for isolate 4 (*Tetranychus* sp.), 5 (*T. evansi*) and 7 (*T. evansi*), which belonged to the Tetranychidae.

6.1.2 PCR AMPLIFICATION AND SEQUENCING

Two target DNA fragments were amplified and sequenced per single mite, namely: the nuclear ribosomal region spanning the ITS1, 5.8S and ITS2 region (about 700 bp) and a fragment of the mitochondrial *COI* gene (about 800 bp).

Figure 6.1 presents an electrophoresis gel with the amplified nuclear ribosomal ITS fragments for Phytoseiidae and Tetranychidae. As evident in **Figures 6.1,** for the Phytoseiidae sp. 1 (Isolate 1) the ITS primers always amplified two fragments. Even amplified at different annealing temperatures, one fragment had an approximate size of 1 200-bp and the other a size of 700-bp.

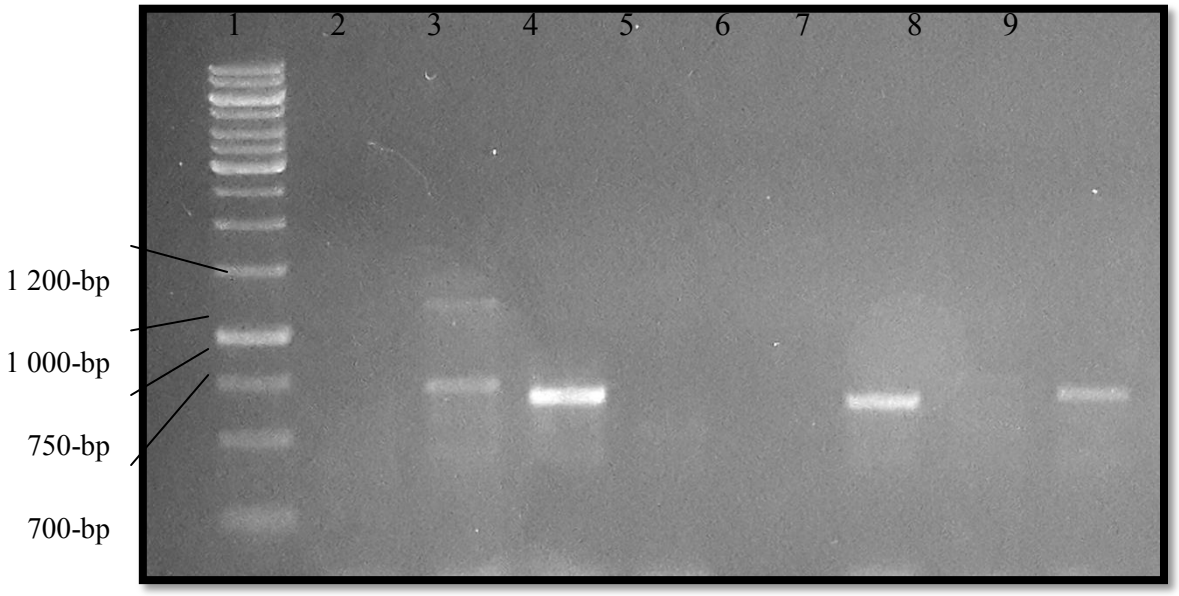

Figure 6.1 PCR amplification of nuclear ribosomal ITS fragment run on a 1% agarose gel. Left to right: Lane 1 - GeneRuler 1kb DNA Ladder; Lane 2 - *T. (A.) microbullatus* - Isolate 2; Lane 3 - Phytoseiidae sp. 1 - Isolate 1; Lane 4 - Phytoseiidae sp. 2 - Isolate 3; Lane 5 - *Tetranychus evansi* - Isolate 5; Lane 6 - *Amblyseius pretoriaensis* - Isolate 6; Lane 7 - *Tetranychus* sp. - Isolate 4; Lane 8 - *Brachytydeus* sp. - Isolate 8.

These universal DNA primers *COI*2F and *COI*2R only amplified a ±700-bp region of the mitochondrial *cytochrome oxidase subunit I* gene, compared to the ±800-bp region of the C1J1718 and 773 *COI* primers (**Figure 6.2**).

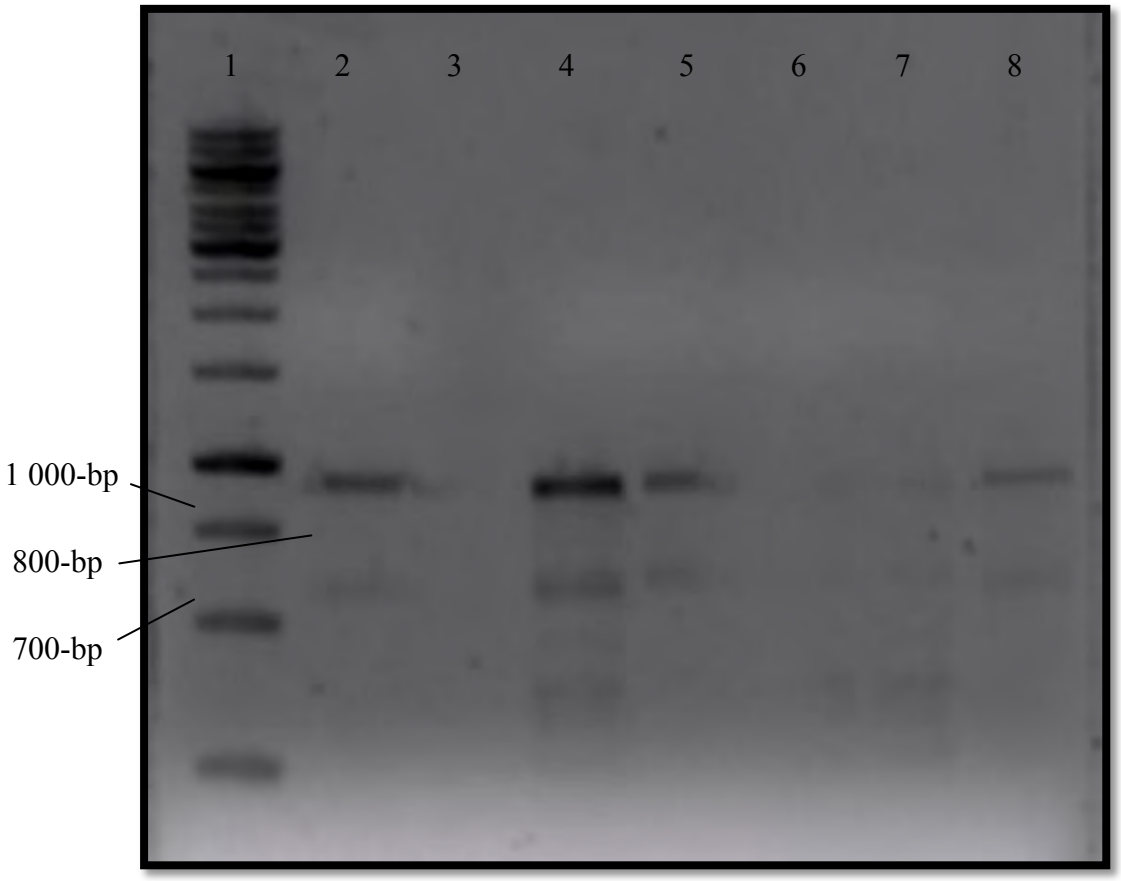

Figure 6.2 PCR amplification of the *COI* gene fragment using C1J1718 and 773 *COI* primers, run on a 1% agarose gel Left to right: Lane 1 - Ladder; Lane 2 - *T. (A.) microbullatus* - Isolate 2; Lane 3 - Phytoseiidae sp. 1 - Isolate 1; Lane 4 - Phytoseiidae sp. 2 - Isolate 3; Lane 5 - *Tetranychus evansi* - Isolate 5; Lane 6 - *Amblyseius pretoriaensis* - Isolate 6; Lane 7 - *Tetranychus evansi* - Isolate 7; Lane 8 - *Brachytydeus* sp. - Isolate 8.

6.1.3 SEQUENCING ANALYSES

Most templates used for sequencing were fragments obtained from a single round of amplification. Homology searches on GenBank using BLASTn (Altschul *et al,*. 1997), of the amplified sequences of both the ITS fragment and the *COI* gene fragment revealed the

following similarity results: **Table 6.2**. Various ITS and *COI* sequences obtained were identical to several sequences already present in the GenBank database. Some sequenced fragments could however not be matched to any other sequences in the database.

Table 6.2 Similarity results of the amplified sequences

Internal Transcribed Spacer					
Isolate	Organism and accession number	Percent Similarity	E-value	Query Cover	Bit Score
Phytoseiidae					
Isolate 1	*Typhlodromus pyri* (JF279152.1)	77%	1e-72	66%	273
Isolate 2	*Typhlodromus phialatus* (GU565315.1)	88%	1e-147	59%	522
Isolate 3	*Neoseiulus cucumerus* (GU966582.1)	90%	0.0	99%	823
Isolate 6	*Neoseiulus cucumerus* (JN020168.1)	82%	0.25	5%	35.6
Tetranychidae					
Isolate 4	Tetranychus viennensis (JQ438841.1)	92%	8e-66	41%	250
Isolate 5	*Tetranychus evansi* (AB738755.1)	86%	0.0	93%	1085
Isolate 7	*Tetranychus evansi* (AB738755.1)	82%	2e-24	16%	113
Tydeidae					
Isolate 8	-	-	-	-	-
Stigmaeidae					
Stigmaeidae Isolate 3	-	-	-	-	-
Stigmaeidae Isolate 4	Stigmaeidae sp. (JX838290.1)	93%	3.7	2%	22.9
Stigmaeidae Isolate 5	Stigmaeidae sp. (JX838290.1)	93%	3.6	2%	22.9
Stigmaeidae Isolate 6	Stigmaeidae sp. (JX836791.1)	100%	1.1	2%	24.7
Stigmaeidae Isolate 10	-	-	-	-	-
Stigmaeidae Isolate 11	Stigmaeidae sp. (JX838185.1)	100%	4.9	1%	22.9
Stigmaeidae Isolate 12	Stigmaeidae sp. (JX838290.1)	93%	3.6	2%	22.9
Stigmaeidae Isolate 13	Stigmaeidae sp. (JX838290.1)	93%	3.7	2%	22.9
Stigmaeidae Isolate 15	Stigmaeidae sp. (JX834417.1)	89%	2.5	1%	24.7
Cytochrome c Oxidase I					
Isolate	Organism and accession number	Percent Similarity	E-value	Query Cover	Bit Score

Phytoseiidae					
Isolate 1	*Typhlodromus pyri* (FM179372.1)	81%	8e-164	67%	576
Isolate 2	*Typhlodromus pyri* (FM179372.1)	82%	1e-170	68%	598
Isolate 3	*Neoseiulus womersleyi* (AB500134.1)	76%	1e-126	72%	452
Tetranychidae					
Isolate 4	*Tetranychus evansi* (AB981216.1)	74%	5e-95	64%	343
Isolate 5	*Tetranychus evansi* (FJ440678.1)	97%	0.0	94%	1442
Isolate 7	*Tetranychus evansi* (FJ440678.1)	96%	0.0	92%	1407
Tydeidae					
Isolate 8	-	-	-	-	-
Stigmaeidae					
Stigmaeidae Isolate 5	*Eustigmaeus sp.* (JX837688.1)	100%	4.7	1%	22.9
Stigmaeidae Isolate 7	Stigmaeidae sp. (JX838290.1)	90%	0.11	4%	28.3
Stigmaeidae Isolate 12	Stigmaeidae sp. (JX838181.1)	100%	8.9	3%	21.1
Stigmaeidae Isolate 15	*Eustigmaeus sp.* (JX837688.1)	88%	4.7	2%	22.9

On average for all taxa, the nucleotide frequencies for rDNA ITS of Phytoseiidae was A = 27.4%, T/U = 30.9%, G = 22.2% and C = 19.5%. The AT content was 58.3%. The nucleotide composition for the *COI* gene of Phytoseiidae was A = 29.6%, T/U = 38.0%, G = 17.0% and C = 15.4%. The AT content was 67.6%. The nucleotide composition for the rDNA ITS of Stigmaedae was A = 28.7%, T/U = 29.5%, G = 20.5% and C = 21.3%. The AT content was 58.2%. The nucleotide composition for the *COI* gene of Stigmaeidae was A = 32.0%, T/U = 40.8%, G = 12.7% and C = 14.5%. The AT content was 72.8%.

6.1.4 PHYLOGENETIC ANALYSIS OF THE PHYTOSEIIDAE ISOLATES

The evolutionary relationships for both the ITS and *COI* gene fragments were inferred using both a distance matrix approach, Neighnour-Joining (NJ) and a model approach, Maximum Parsimony (MP). A total of 25 ITS sequences and 17 *COI* sequences were retrieved from GenBank, as listed in chapter 5 **Table 5.3**. All of these accessions together with the 14 sequences [4 - Phytoseiidae (Isolate 1, 2, 3 and 6) and 10 - Stigmaeidae (Stigmaeidae Isolate 3, 4, 5, 6, 7, 10, 11, 12, 13 and 15)] obtained during this study served to create a dataset of 39 ITS sequences (25 - Phytoseiidae and 13 Stigmaeidae) and 24 *COI* sequences (18 - Phytoseiidae and 6 - Stigmaeidae).

The ITS dataset for the Phytoseiidae was the most informative and included 20 GenBank accessions of *P. persimilis, P. macropilis, P. fragariae, P. longipes, A. herbicolus, A. largoensis, A. deleoni, A. andersoni, N. longilaterus, N. idaeus, N. womersleyi, N. agrestis, N. barkeri, T. phialatus, T. exhilaratus* and *T. pyri.*

The Maximum Parsimony (MP) method was used in **Figure 6.3** to infer the phylogenetic relationships using the sequence of the ITS fragment. Based on evolutionary evidence, the genus *Typhlodromus* is considered to be older and therefore the trees were rooted with this genus. The tree was rooted with *T. pyri*. The cladogram reveals the most parsimonious tree with a length of 385. The consistency index was 0.635862, with the retention index being 0.778151 and the composite index 0.525778 (0.494797) for all sites (including the parsimony-informative sites in parentheses). The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates) are displayed next to the branches. The analysis includes 24 nucleotide sequences. A total of 385 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

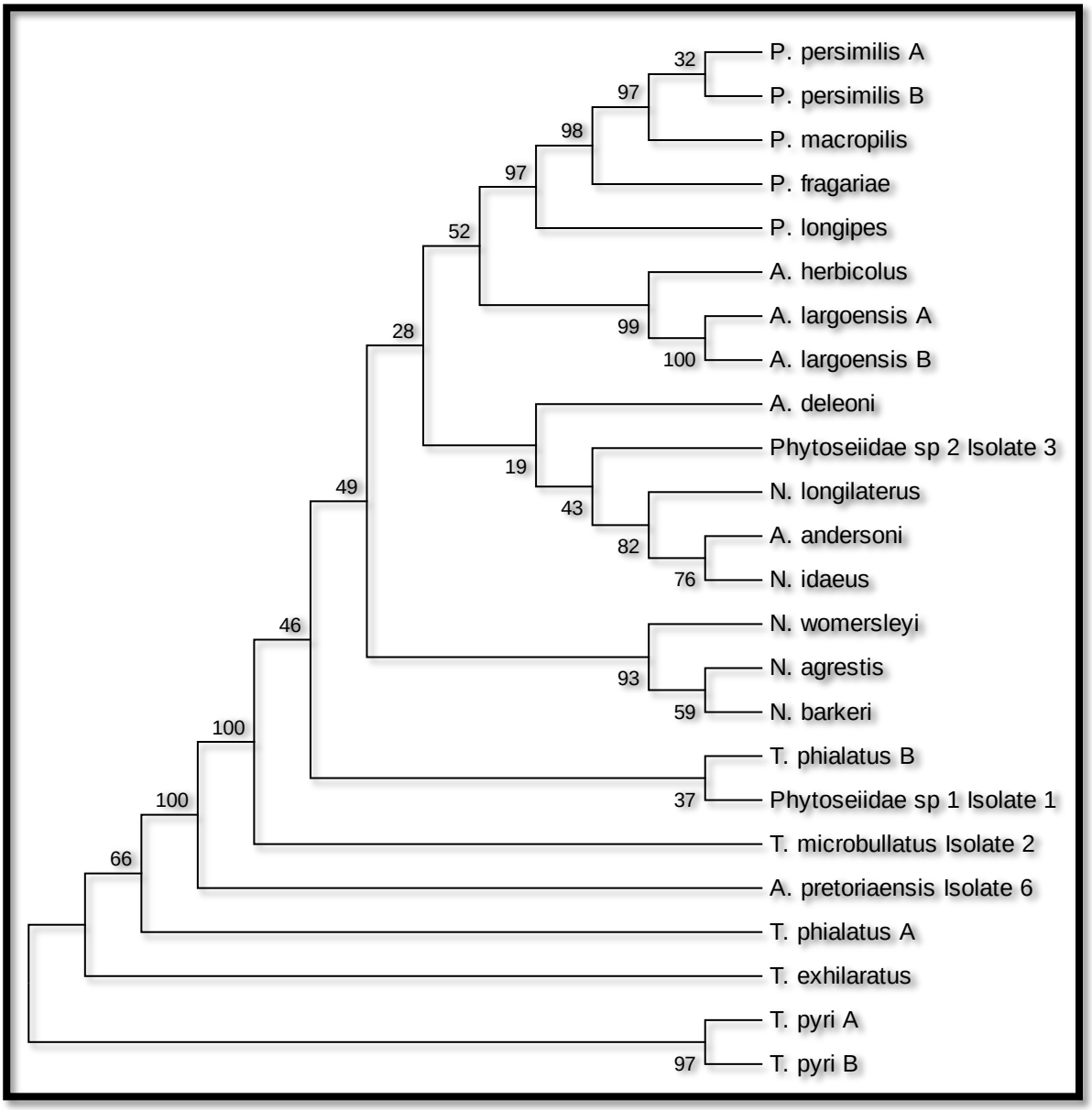

Figure 6.3 The evolutionary history of the Phytoseiidae based on the ITS gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

The Neighbour-Joining (NJ) method was then used (**Figure 6.4)** to infer the evolutionary history. The figure reveals the most favourable tree with the sum of branch length = 2.82079950. The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates), are displayed next to the branches. The tree is drawn to scale, with branch lengths containing the same units as the evolutionary distances that were used to infer the phylogenetic tree. Twenty four nucleotide sequences were included in the analysis. A total of 371 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

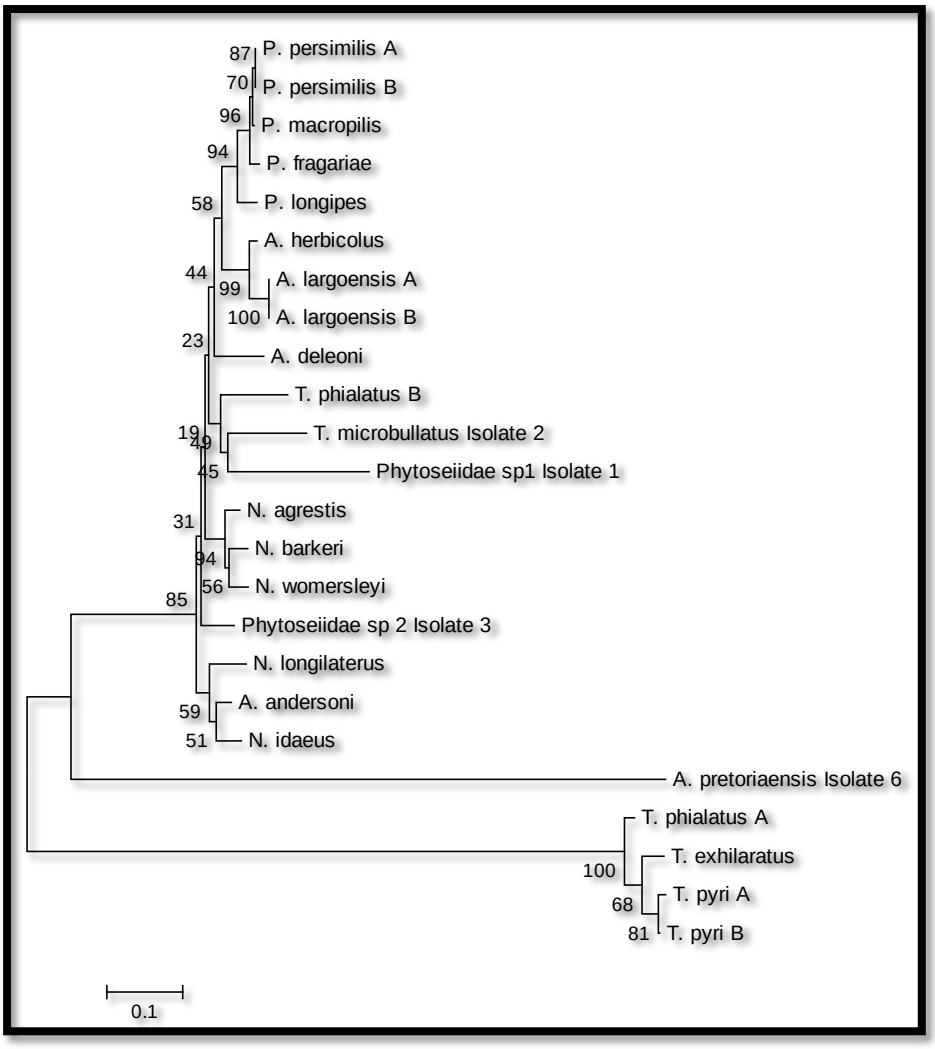

Figure 6.4. The evolutionary history of the Phytoseiidae based on the ITS gene was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000

replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

The phylogenetic grouping of species corresponds with that of their morphology identification and the clustering of species is similar between the MP and NJ analysis of the ITS gene fragment. In both cases the *Typhlodromus* forms a separate clade with high bootstrap support (97-100%). The taxon isolate 6 (*A pretoriaensis*) group within this clade in both approaches used. All the Phytoseiidae taxa containing the genera *Phytoseiulus, Neoseiulus* and *Amblyseius* form a separate clade with low bootstrap support (66%) to distinguish between them for the MP tree and a high bootstrap support (85%) for the NJ tree.

The *COI* dataset included 15 GenBank accessions of *N. californicus, N. fallacis, N. imbricatus, A. herbivolus, A. largoensis, A. andersoni, P. persimilis, I. degenerans, T. exhilaratus* and *T. pyri* for both the *COI* MP and NJ trees.

The MP method was used in **Figure 6.5** to infer the evolutionary history. The figure reveals the most parsimonious tree with a length of 637. The consistency index was 0.649329, with the retention index being 0.787817 and the composite index 0.529334 (0.511552) for all sites, including the parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates) are displayed next to the branches. The analysis includes 14 nucleotide sequences. A total of 285 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

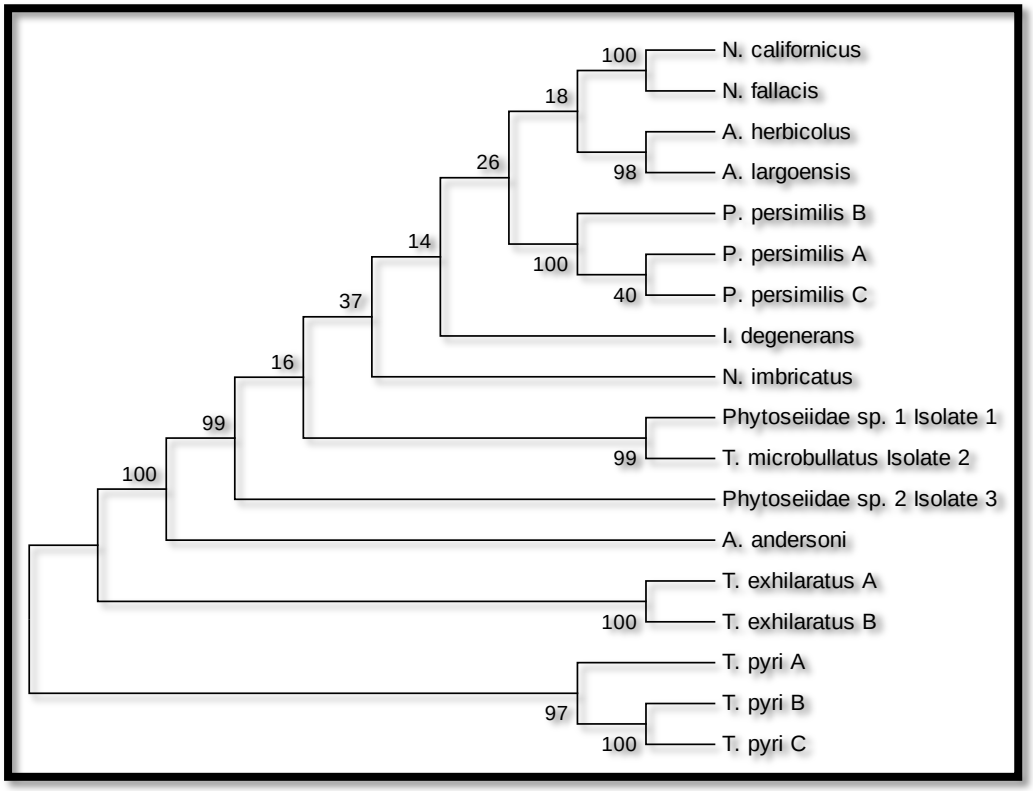

Figure 6.5 The evolutionary history of the Phytoseiidae based on the *COI* gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

The NJ method was used in **Figure 6.6** to infer the evolutionary history. The figure reveals the most favorable tree with the sum of branch length = 2.77590973. The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates), are displayed next to the branches. The tree is drawn to scale, with branch lengths containing the same units as the evolutionary distances that were used to infer the phylogenetic tree. Eighteen nucleotide sequences were included in the analysis. A total of 285 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

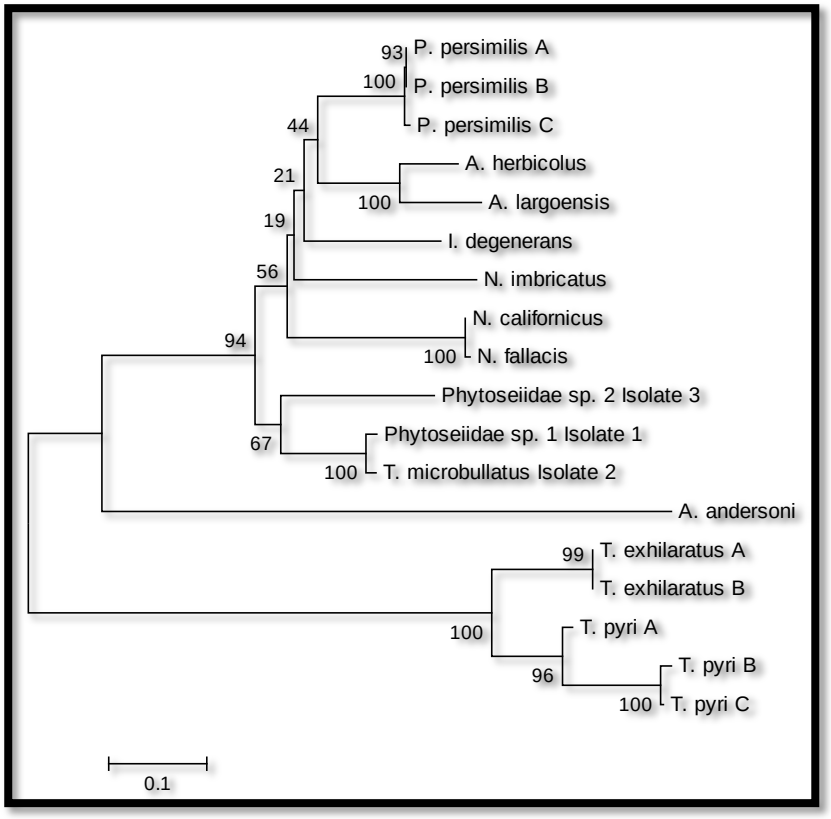

Figure 6.6 The evolutionary history of the Phytoseiidae based on the *COI* gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

Similar results can be observed when using the *COI* sequence data to infer the phylogeny of the dataset than what was observed with the ITS. In both cases the taxa *Typhlodromus* forms a separate clade with high bootstrap support (97 - 100%), while most of the Phytoseiidae taxa containing the genera *Phytoseiulus, Neoseiulus* and *Amblyseius* form a separate clade with very high bootstrap support (94 - 100%) to distinguish between them. According to the *COI* analysis isolate 3 forms a separate taxon from the other clades supported by a high bootstrap value (99 and 94% respectively.)

6.1.5 PHYLOGENETIC ANALYSES OF THE STIGMAEIDAE ISOLATES

The ITS dataset included 4 GenBank accessions of *Stigmaeopsis nanjingensis, S. celarius* and *S. miscanthi* for both the ITS MP and NJ tree

The MP method was used in **Figure 6.7** to infer the evolutionary history. The figure reveals the #1 out of 10 most parsimonious trees with a length of 370. The consistency index was 0.888218, with the retention index being 0.941270 and the composite index 0.847143 (0.836052) for all sites, including the parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates) are displayed next to the branches. The analysis includes 13 nucleotide sequences. A total of 255 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

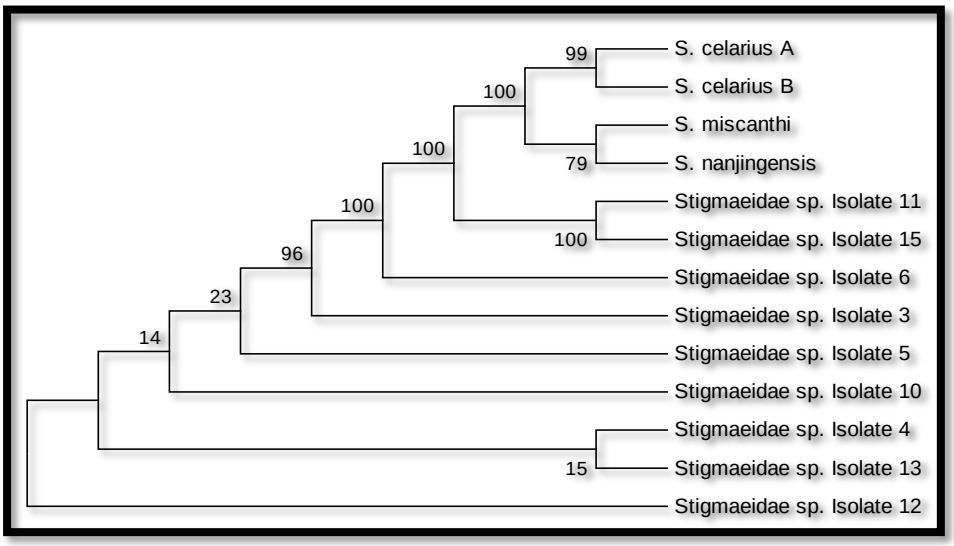

Figure 6.7 The evolutionary history of the Stigmaeidae based on the ITS gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

The NJ method was used in **Figure 6.8** to infer the evolutionary history. The figure reveals the most favourable tree with the sum of branch length = 2.32682229. The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates), are displayed next to the branches. The tree is drawn to scale, with branch lengths containing the same units as the evolutionary distances that were used to infer the phylogenetic tree. Thirteen nucleotide sequences were included in the analysis. A total of 255 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

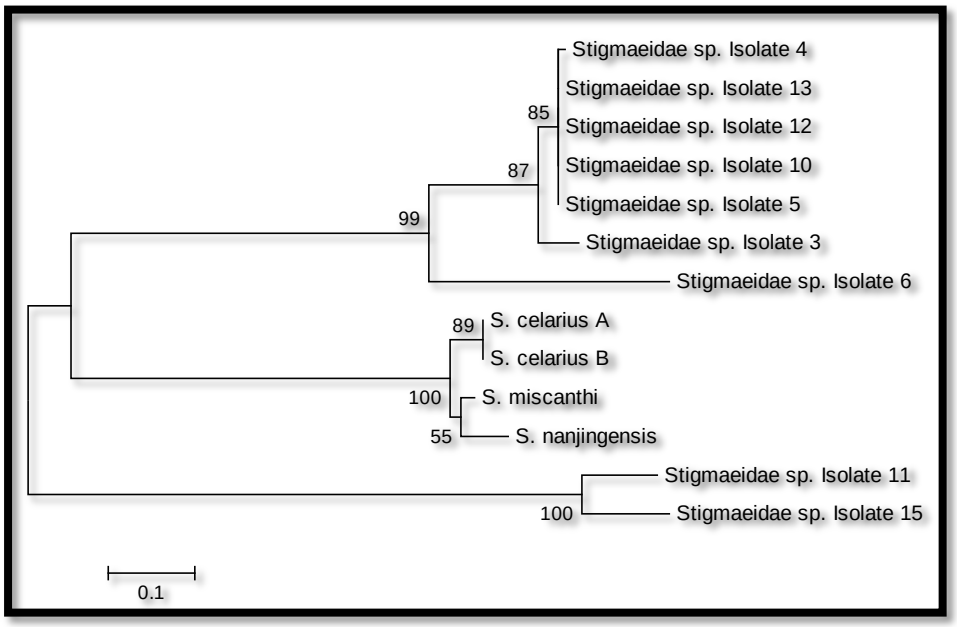

Figure 6.8 The evolutionary history of the Stigmaeidae based on the ITS gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

The resulting 13 ITS aligned sequences were used to produce a MP and NJ trees, forming three clades for the Stigmaeidae dataset with high bootstrap support (96 - 100%) in both

cases. Two clades that clearly differ from the other clades consist out of isolates 3, 4, 5, 6, 10, 12 and 13. Within this clade isolates 3 and 6 appears to not have a consistent grouping when analyzed with different methods. In both cases isolates 11 and 15 form a well-supported separate clade closer to *Stigmaeopsis*.

The *COI* dataset included 2 GenBank accessions of *Eustigmaeus* for both the *COI* MP and NJ trees. Unfortunately both ITS and *COI* for all the Stigmaeidae sequences could not be obtained and phylogenetic trees for the *COI* gene included only 2 Stigmaeidae species from GenBank and 4 sequences retrieved during this study. The *COI* gene for *Stigmaeopsis* that was used in the ITS MP and NJ tree was not available in GenBank and therefore *Eustigmaeus* was used.

The MP method was used in **Figure 6.9** to infer the evolutionary history. Above mentioned figure reveals the most parsimonious tree with a length of 269. The consistency index was 0.909091, with the retention index being 0.891892 and the composite index 0.825580 (0.810811) for all sites, including the parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates) are displayed next to the branches. The analysis includes 6 nucleotide sequences. A total of 273 positions were used in the final dataset (Purdom *et. al.,* 2000 and Tamura *et. al.,* 2013).

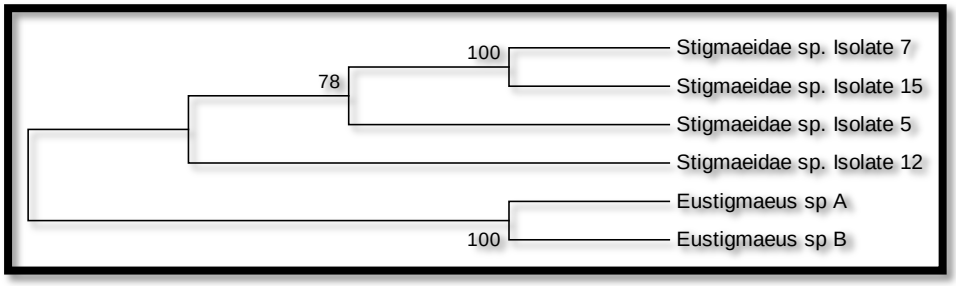

Figure 6.9 The evolutionary history of the Stigmaeidae based on the *COI* gene fragment was inferred by using the Maximum Parsimony method based on the Tree-Bisection-Regrafting (TBR) algorithm. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

The NJ method was used in **Figure 6.10** to infer the evolutionary history. The figure reveals the most favourable tree with the sum of branch length = 1.43065958. The percentage of replicate trees in which the associated taxa were clustered together through the use of the bootstrap test (1 000 replicates), are displayed next to the branches. The tree is drawn to scale, with branch lengths containing the same units as the evolutionary distances that were used to infer the phylogenetic tree. Six nucleotide sequences were included in the analysis. A total of 273 positions were used in the final dataset (Purdom *et. al.*, 2000 and Tamura *et. al.*, 2013).

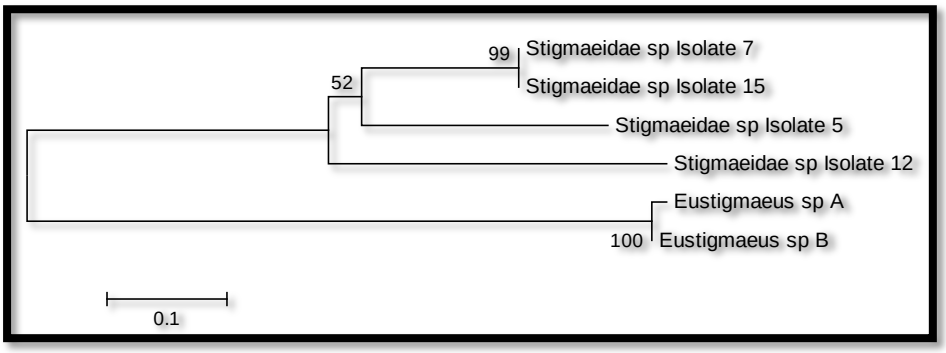

Figure 6.10 The evolutionary history of the Stigmaeidae based on the *COI* gene fragment was inferred using the Neighbor-Joining method based on the Jukes-Cantor method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

For the *COI* gene, *Eustigmaeus* sp. acts as an ancestral clade (100% bootstrap), indicating that the Stigmaeidae isolates from this study might have evolved from this genus. Isolates 5 and 12, group separate from isolates 7 and 15, with medium bootstrap support in both cases. Isolates 5 and 12 also grouped separately from isolate 15 in the ITS analysis.

6.2 DISCUSSION

In order to improve effective mite control a key factor is to investigate new diagnostic methods in order to distinguish between different species or species that are closely related or sister species. These methods include the use of taxonomy, biotechnology and scanning electron microscopy (SEM), as well as coordination of the studies between mite and host relationship and predator-prey associations. For taxonomical purposes, the original descriptions or systematic keys are used to establish species identification through external morphological characteristics and genetic traits. Although this technique has widely been used in acarology, it may not be as useful to discriminate between species that are morphologically very close (sibling species) (IRSES, 2011). Therefore, DNA sequencing has become more important in systematics and routine identification in mite species (e.g., barcode initiative), and this will contribute in the future to address a large palette of questions.

During this study two well-known gene fragments were investigated, namely the internal transcribed spacer (*ITS*) region of the 18S–5.8S–26S nuclear ribosomal cistron and the mitochondrial cytochrome oxidase I (*COI*) gene fragments. We base our conclusions on both the Neighbour- Joining Method (Jukes and Cantor, 1969) and the Maximum Parsimony methods (Farris *et al.,* 1970; Fitch *et al.,* 1971). The Maximum Parsimony approach is a non-paramatric statistical character-based method for constructing phylogeny, while Neighbour Joining is a clustering method using a distance matrix.

6.2.1 DNA EXTRACTION

During this study total genomic DNA was isolated from a single mite from every group or genera identified. The quantity and quality of DNA is not reported often in the literature. Jeyaprakash and Hoy, (2009) established that the amount of genomic DNA in a Phytoseiidae female varied between 109 and 200 ng, with the quality ($OD_{260/280}$) ranging from 1.3 to 8.8. Jeyaprakash and Hoy (2009) made use of a GuSCN lysis buffer and quantitative real-time PCR and ultimately developed a high-fidelity qRT-PCR protocol by using flow cytometry.

Konakandla *et al.* (*In* Okassa *et al.,* 2012) recorded that the amount of DNA in a *Phytoseiulus persimilis* female species was 1.3 ± 0.7 ng. They also made use of the DNAeasy tissue extraction kit and made some changes to the protocol according to the different life stages of mites used. Comparing results, we can conclude that the adapted Qiagen DNA extraction protocol used for this study is much more efficient than the one proposed by the latter authors (lysis buffer (GuSCN) and DNA isolation on silica matrix) and seems to lead to much better quality and quantity of DNA from the Phytoseiidae DNA extracts.

In 2012 Okassa *et al.* completed a study where they found that the amount of DNA extracted was related to the size of the mite, and that the OD260/280 value was higher in females than in eggs and larvae. The amount of DNA extracted from males, however were similar to that of deutonymphs, but this is possibly caused by these two life stages being similar in size. On the other hand, protonymphs contain a lower OD260/280 value than deutonymphs. Furthermore, the size of the DNA sequences from all the life stages was identical for all the markers used (Okassa *et. al*., 2012).

The adapted DNA isolation protocol has also allowed for mite cadavers to be retrieved, prepared and identified after DNA extraction. It was thus possible to identify and extract DNA from Phytoseiidae mites and to preserve the cadavers as voucher specimen to identify morphological characteristics. This was due to the hard exoskeleton of Phytoseiidae mites. Unfortunately, this protocol was not as successful for Stigmeidae mites, as they contain soft bodies, making it difficult to preserve cadavers as cadavers were broken down along with DNA extraction. In 2010, Tixier *et al.,* did a study on Phytoseiidae mites where they question the adequacy of this protocol for other mite families and stated that additional investigations are required.

6.2.2 PCR AND SEQUENCE ANALYSES

For bacteria a species is generally defined as a group of strains if they share approximately 70% or greater DNA-DNA relatedness and 5˚C `Tm and if their phenotypic characteristics agree with this definition (Wayne *et al*., 1987). During this study the isolates 1, 2, 3, 4, 5, 6,

7, Stigmaeidae Isolate 4, 5, 6, 7, 11, 12, 13 and 15 shared 74% - 100% sequence similarity with species on the Genbank database (**Table 6.2**). Some isolates (isolate 8, Stigmaeidae Isolate 3 and Stigmaeidae Isolate 10) however did not have similarity with any sequence on the database.

The amplified nuclear ribosomal ITS fragments for the Phytoseiidae sp. 1 (Isolate 1) always exhibited two bands on the agarose gel. When sequenced, the 1 200-bp fragment was similar to that of *Tetranychus evansi* and the 700-fragment was similar to that of several other but only Phytoseiidae species. Morphologically it is placed within the family Phytoseiidae and it is still unclear why the amplified nuclear ribosomal ITS fragments of this species constantly produces two sized band. One can speculate that, since the Phytoseiidae isolate feeds on the *Tetranychus* species, the 1 200 bpDNA fragment most probably originates from the prey's DNA. De Mendonça *et al.*, (2011) reports on a 1 200 bp fragment obtained for *Tetranychus evansi* using the same ITS primers as used in this study.

6.2.3 PHYLOGENETIC ANALYSES

The phylogenetic grouping of species based on the DNA sequence analyses corresponds with that of their phylogenetic grouping based on morphology and the clustering of species is similar between Phytoseiidae's ITS and *COI* gene fragment analyses. The subfamily Amblyseiinae includes the genera *Amblyseius, Neoseiulus* and *Phytoseiulus*. *Typhlodromus* is classified under the subfamily Typhlodrominae. As evident in **Figures 6.3** and **6.4**, the Phytoseiidae genera are branched off from the Typhlodrominae, showing a clear distinction between the two subfamilies. Sequences obtained from GenBank as being *Phytoseiulus, Amblyseius, Neoseiulus* and *Typhlodromus* are distributed in distinct clades in the phylogenetic trees.

The species *Amblyseius andersoni* described by C. Perez-Sayas, T. Pina, J. A. Jacas and M. A. Hurtado in 2010 did not cluster in the expected clade. No morphological description accompanied the publication in GenBank and one can't help but wonder if this species might have been wrongfully identified. In both the MP and NJ trees for the internal transcribed

spacer (ITS), *A. andersoni* is placed within the clade clustering with the genus *Neoseiulus.* Although phylogenetically close, they differ morphologically. *Amblyseius* falls within the obtusus group, containing setae j1, j3, s4, Z4 and Z5 that are much longer than the rest of the dorsal body setae (Ueckermann and Loots, 1988). *Neoseiulus* is characterized by its 19 dorsal idiosomal and interscutal setae (Tixier *et al.,* 2013), whereas *Amblyseius* only has 16 to 17 pairs (Ueckermann and Loots, 1988). The same goes for the ITS sequence of *A. deleoni*.

Amblyseius pretoriaensis (Isolate 6) on both the ITS MP and NJ trees was not clustered in the expected clade. Despite its location in these trees, the cadaver in **Figure 4.41** reveals that it is impossible for this genus to be classified within the subfamily Typhlodrominae due to its long setae and macro setae on leg IV. These species also contain macrosetae on leg II and III that are evident that it belongs to the genus *Amblyseius.* Setae j1, j3, s4, Z4 and Z5 are extremely long and they lack setae z3 and s6. These setae are not only absent in this genus, but the subfamily Amblyseiinae as a whole. Most species in the subfamily Typhlodrominae contain setae z3 and s6 making it possible to distinguish between these two sufamilies (Ueckermann and Loots, 1988).

In the MP and NJ tree for the *COI* gene analyses, it is excluded from other clades, branching off from *Neoseiulus* and *Amblyseius*. One is able to accept its positioning due to high bootstrap support. *A. andersoni* is placed in the subfamily Amblyseiinae. When looking at the ITS trees, even if positioned wrong, it still lies within the subfamily Amblyseiinae.

However, for the *COI* gene it is grouped with species belonging to the subfamily Typhlodrominae. Why it is placed in this subfamily is unclear, as they differ greatly when it comes to their morphology. The subgenus *Anthoseius* (subfamily: Typhlodrominae, including the genus *Typhlodromus*) is defined by its 10 pairs of lateral dorsal body setae of which 5 pairs are positoned anterior to the level of setae R1. They contain 6 pairs of prolateral setae (j3, z2, z3, z4, s4 and s6) and 4 pairs of postlateral setae (S2, S4, S5 and Z5). The subgenus *Amblyseius* (subfamily: Amblyseiinae) contains 16 to 17 pairs of dorsal body setae. They include 4 pairs of prolateral setae (j3, z2, z4 and s4) and 5 pairs of postlateral setae (Z1, S2, S4, S5 and Z5) (Ueckermann and Loots, 1988).

Typhlodromus pyri A branches off from *T. pyri* B and C. According to Tixier *et al.* (2013), these differences could be as a result of intraspecific variations.

The genus *Iphiseius* Berlese is considered to be unique. *Iphiseius* is characterized by its six pairs of dorsal setae on the dorsal shield, two pairs of median setae and five pairs of postlateral setae. What makes it unique is its sclerotized dorsal interscutal membrane. It bears two pairs of scapular setae on this membrane. *I. degenerans* has minute setae on its dorsal shield, except for the first dorsal and caudolateral setae which are short. The ventri-anal shield is divided into a ventral and an anal shield for both males and females (Van der Merwe, 1968). Branching off and being placed in its own group phylogenetically corresponds with its morphology, due to its sclerotized dorsal interscutal membrane.

The PCR results are consistent with section A that's based on the morphology of these mite species. Phylogenetic relationship analysis of *Typholodromus* and *Amblyseius* with other members of the family Phytoseiidae based on rDNA sequences give better insight into discrimination between species.

The resulting 13 ITS aligned sequences (255-bp) were used to produce a Maximum Parsimony (MP) and Neighbour-Joining (NJ) tree, forming four clades for the Stigmaeidae. The evolutionary relationships (using NJ method) for the ITS (**Figure 6.8)** and *COI* gene (**Figure 6.10)** of Stigmaeidae produced similar trees (excluding differences in bootstrap percentages). However, the sum of branch length is much higher for ITS (2.32682229) than that of the *COI* gene (1.43065958). This shows that the amount of changes within the NJ tree for ITS had a greater number of character changes on the tree. This parameter is minimized in parsimony, but still allows for the ITS MP tree to have a longer length of 370, compared to the 269 tree length of the *COI* gene

Both Phytoseiidae ITS and *COI* Maximum Parsimony trees have a lower consistency index (CI) than that of Stigmaeidae. The CI ranges from 1.0 (no homoplasy) to 0.0. This indicates that the amount of homoplasy is greater within Phytoseiidae than in Stigmaeidae mites. The rate of homoplasy within Stigmaeidae fall within an acceptable to excellent (meaning low to barely no homoplasy) range (>0.8 - >0.9). Homoplasy within Phytoseiidae is questionable, >0.6 (meaning more changes took place).

The appearance of polyphyly of some species forms as a result of misidentification of some of the accessions. Altogether, this suggests that several ITS and *COI* sequences deposited in GenBank were erroneously named due to misidentification of mite species. Taking these

concerns into account, it is not possible to make a definite conclusion regarding the identity of these sequences.

CHAPTER 7: CONCLUSION

CHAPTER 7: CONCLUSION

A large number of plant-feeding and predatory mites were collected from Solanaceae genera. Unfortunately, when comparing the quantity of spider mites found during the survey (94%) to the amount of predators (6%), we can conclude that the predators were less abundant on wild solanaceous plants where spider mites thrived. In some cases species were no longer present in the locality from where they were originally collected and that rendered it difficult to collect more species for further investigations. Species identified included *T. evansi, T. urticae,* Tenuipalpidae-, Oribatidae species, *A. pretoriaensis, T. microbullatus,* 2 Phytoseiidae species, Stigmaeidae-, Tydeidae, Erythraeidae- and Iolinidae species. *Tetranychus evansi* was the most abundant species from all the samples, with the highest frequency of appearance, reaching a relative abundance of 84 %. According to Creamer *et al.* (1998) no predators for this species is known. Despite the fact that Solanaceae contain various parasitic mites, absentee of predatory species could be problematic in finding effective bio-control agents, especially for *T. evansi*. Thus, both hypotheses can only be accepted partially, as solanaceous crops contained various parasitic mites but predatory mites were less active and further studies will have to be done to determine whether predatory species found during the survey will be effective in controlling spider mite populations.

Despite the diversity and abundance of spider mites and other mite species, they are rarely included in biodiversity assessments due to taxonomic barriers. It is clear that it is difficult to identify mites morphologically, as many key traits exhibit large phenotypic plasticity and lack suitable characters to identify them with. Therefore a combination of three methods were used for this study. These three methods were: light microscopy, which still remains the most available method, easy to perform, and inexpensive method but is less sensitive; SEM, which provides detailed morphological information but is a costly procedure; and third is through the use of phylogenetic relationships by using a non-destructive molecular method which provides data for molecular and morphological evolution. This new, non-destructive method for isolating DNA allowed for the description of species along with their morphological data. This was due to the identification of species before DNA extraction and the retrieval of cadavers after DNA extraction. Applying these molecular techniques to the identification of mites is very important for their taxonomy and opens new insight and facilitates in-depth studies for this Acari group on a morphological and molecular level.

Mite sequences in the GenBank database is currently very low (998 sequences, 47 species) compared to the known species diversity (2 238 species) and little are trustworthy (Tixier *et al.,* 2012). The misleading species assignment of sequences in a group of mite species that are closely related, as evident in this study for species such as *A. andersoni* and *A. deleoni*, it reveals the need for an accurate determination of species identity which should be supervised by specialists. While the molecular approach appears promising to solve disagreements within taxa, it rises from the present study by including both nuclear ITS and mitochondrial *COI* sequences, that specimens assessed in recent literature were incorrectly identified. As can be seen in the phylogenetic trees in chapter 6.1, invalidity of taxa occurs in GenBank. Even if some of the ITS or *COI* sequences were misidentified, some may also reflect the large variation of these genes within species. This raises concern as to what sequences are reliable, but also emphasises the importance of combining molecular and morphological identification as done in this study. Overall, when looking at the similarities of BLAST sequences and the phylogenetic relationships amongst species, we can conclude that molecular data corresponded with morphological descriptions of species in this study.

CHAPTER 8: REFERENCES

CHAPTER 9: REFERENCES

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