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EDITORIAL

Acarological Studies: A new forum for the publication of acarological works

Salih DOĞAN

Department of Biology, Faculty of Sciences and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey
e-mail: salihdogan@erzincan.edu.tr

Acarological Studies (*AS*) is a peer-reviewed, international, scientific journal which aims to promote research worldwide in acarology. *AS* is published by the online hosting service, JournalPark, which facilitates faster publication, greater visibility, increased access and comprehensive archiving.

This July 2019 issue of *Acarological Studies* serves as the Congress Proceedings for the 15th International Congress of Acarology (XV ICA) which was held from 2 to 8 September, 2018 in Antalya, Turkey. The proceedings include 13 papers accepted for publication after review by at least two reviewers and linguistic editing. I sincerely thank the numerous acarologists who reviewed the submitted papers and ultimately, the accepted papers. I am sure that these articles will be widely read and referenced and also help stimulate studies on the wonderful, diverse world of acarology.

Acarological Studies, which provides full, open access, started life at the beginning of this year. This July issue completes the first year of issues of *AS*. During its first year, *AS* will have been published twice on-line and have included 22 papers spanning 175 pages in total. *AS* is expected to give a new impetus to acarology (Doğan, 2019) and be an important new platform for acarological research results. With your continuing support, *AS* will, step by step, reach that objective with each new issue.

Doğan (2019) stated that there are 16 journals on acarology. Six of these, including the journal *Acarines* of the Egyptian Society of Acarology, are not currently active. Dr Hany El-Kawas and Dr Mohamed W. Negm have just informed me that *Acarines* was published until 2018 and still active although on the web page of the journal the last publication was in 2014.

In addition, Dr. Karl-Heinz Schmidt advised me that there were some other short-lived journals dealing with acarology, or at least acarology in part. One of those, *Acari - Blätter für* Milbenkunde, was published by Max Sellnick at irregular intervals between 1923 and 1944. Another early journal was *Lansania*. It was published in Japan under the editorship of Kyukichi Kishida. *Lansania* published original papers on arachnology and zoology that covered a wide variety of animal taxa, including Acari, between 1929 and 1941 (Tennent et al., 2008).

The main purpose of journals is to officially disseminate scientific information via recognized scientific forums (Lancaster and Smith 1978; Shokraneh et al., 2012). Most scientific and scholarly journal procedures are based on some form of peer review or editorial refereeing to validate and improve the quality of the manuscript.

I am grateful to all editors, members of the advisory board and reviewers for improving the quality of *Acarological Studies*. Thanks for the great special efforts to this journal by Drs Adem Keskin (Tokat Gaziosmanpaşa University, Tokat, Turkey) and Naci Bayrak (Provincial Directorate of National Education, Erzurum, Turkey). In the near future *AS* will be indexed and abstracted in the citation indexes and platforms covering the world's top tier international and regional journals.

I would like to take this opportunity to remind you that in order to shorten the journal's evaluation process, it is important for authors to note the following steps. Firstly, please thoroughly check your manuscript several times and then have it checked by a native speaker of English (if relevant). Then, after carefully reading and applying the journal's rules, including formatting, submit your manuscript.

In conclusion, I am sure that the papers in this issue of *Acarological Studies* will prove interesting and informative. Please note that the next issue of *AS* will be published in early 2020. I am very much looking forward to seeing your manuscript submitted for inclusion in that issue.

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The editor thanks Drs Karl-Heinz Schmidt (Hintere Dorfstraße 29, 02791 Oderwitz, Germany), Mohamed W. Negm (Faculty of Agriculture, Ibaraki University, Japan) and Hany El-Kawas (Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt), who were kind enough to draw to his attention a number of journals dealing with the Acari. I am grateful to Drs Sebahat Ozman-Sullivan (Ondokuz Mayıs University, Samsun, Turkey) and Gregory T. Sullivan (The University of Queensland, Brisbane, Australia) for reading and commenting on this editorial.

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REVIEW

An overview of the XV International Congress of Acarology

Sebahat K. OZMAN-SULLIVAN [1,3], **Gregory T. SULLIVAN**[2]

[1] *Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, 55139 Samsun, Turkey*
[2] *The University of Queensland, School of Earth and Environmental Sciences, 4072 Brisbane, Australia*
[3] Corresponding author: sozman@omu.edu.tr

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ABSTRACT: The XV International Congress of Acarology (XV ICA) was held in Antalya, Turkey between 2 and 8 September, 2018. The theme of the congress was "The Acari: very small but impossible to deny!". The congress addressed all aspects of fundamental, agricultural, medical and veterinary acarology and related topics. There were 271 participants from 44 countries across six continents. In total, there were 279 presentations, namely 188 oral presentations and 91 poster presentations, in 21 sections. The congress had a four day scientific program, one day for the technical and social tours, and a one day post-congress tour.

Keywords: Mite, tick, congress, XV ICA 2018, Antalya, Turkey.

INTRODUCTION

The International Congress of Acarology (ICA) has been conducted six times in Europe, four times in North America, two times in Asia and once in both South America and Australia/Oceania. The first congress (I ICA) was held at Fort Collins, Colorado, USA in 1963 with 146 participants representing 23 countries (Woolley, 1964; Flechtmann, 2011), and XIV ICA was held in Kyoto, Japan in 2014 (Gotoh and Taylor, 2016).

The most recent congress, XV ICA (http://www.acarology.org/ica/ica2018/), was held from 2 to 8 September, 2018 in Antalya, Turkey, fifty five years after the first congress in the USA. The XV ICA congress venue was the magnificent Mediterranean beachside resort, Swandor Hotels & Resorts Topkapi Palace, which was built in a similar architectural style to the Ottoman Topkapi Palace in Istanbul. The congress covered all aspects of fundamental, agricultural, medical and veterinary acarology and related topics. The congress theme was, "The Acari: very small but impossible to deny!", and a tulip-shaped mite was designed and adopted as the congress logo because the tulip is a cultural symbol of Turkey. The congress bag was blue to represent the colour of the Mediterrenean Sea. The congress abstract book and photos can be found on the congress website; the abstract book also includes a list of participants and their e-mail addresses (http://www.acarology.org/ica/ica2018/files/ICA_2018_Abstract_Book.pdf).

CONGRESS PARTICIPANTS

The XV ICA Organizing Committee was delighted to have 271 participants from 6 continents and 44 countries (Fig. 1). The age of participants, which ranged from students in their 20s to veteran acarologists in their 90s, speaks volumes to the power of ICA to inspire acarologists and bring them together every four years. The participants at XV ICA were from Australia (1), Austria (3), Bangladesh (1), Belgium (4), Brazil (8), Canada (4), China (20), Colombia (2), Costa Rica (1), Cyprus (1), Czech Republic (2), Finland (2), France (3), Georgia (5), Germany (3), Greece (2), Hungary (1), India (10), Iran (8), Iraq (1), Israel (4), Italy (5), Japan (17), Lithuania (3), Mexico (3), New Zealand (2), Pakistan (2), Philippines (1), Poland (10), Portugal (7), Romania (3), Russia (9), Saudi Arabia (5), Serbia (6), South Africa (5), South Korea (1), Spain (6), Sri Lanka (2), Thailand (3), The Netherlands (8), Tunisia (1), Turkey (69), United Kingdom (2) and United States of America (15).

There were 381 participants from 44 countries at XIII ICA in Brazil, including 173 from Brazil (Moraes and Proctor, 2011), and 310 participants from 44 countries at XIV ICA in Japan (Gotoh and Taylor, 2016), including 112 from Japan (Masahiro Osakabe - XIV ICA Secretary-general; pers. comm., 26.07.2019). That means the last three congresses have all been attended by about 200 participants from non-host countries.

CONGRESS PROGRAM

The seven days of the congress included a registration day, a four day scientific program, one day for the technical and social tours, and a one day post-congress tour.

Registration

Registration for the congress started on Sunday, 2 September, 2018 and continued during the congress.

Opening ceremony

On the morning of Monday, 3 September, Sebahat K. Ozman-Sullivan, President of the XV ICA 2018, officially welcomed participants to the congress. Ferit Turanli, President of the Entomological Society of Turkey, then gave a short history of entomological and acarological research in Turkey. Peter Schausberger, General

Figure 1. Group photograph of the participants at the XV International Congress of Acarology, Antalya, Turkey.

Turkey. Peter Schausberger, General Secretary of the Executive Committee of the ICA, then presented a moving tribute to dear colleagues, Maurice W. Sabelis, Philippe Lebrun, Gerd Alberti, James A. McMurtry, Gwylim O. Evans, Jean Louis Camicas, James H. Oliver, Jaroslav Smrz and Donald A. Griffiths, who had passed away since XIV ICA 2014 in Kyoto. Zhi-Qiang Zhang, President of the Systematic and Applied Acarology Society, then announced the winners of the inaugural James Allen McMurtry Award (Zhang, 2018a,b). The two winners of the $5,000 awards were Gerald W. Krantz of the USA and Evert E. Lindquist of Canada for their outstanding contributions to acarology over a long period (Walter and Halliday, 2018; Beaulieu and Behan-Pelletier, 2018) (Fig. 2).

Recognition of sponsors

After the opening ceremony and between the presentations of the first two keynote speakers, Maria Navajas and Kosta Y. Mumcuoglu, representatives of the sponsors were invited to the stage to receive their commemorative plaques by Gregory T. Sullivan, the Scientific Secretary and Master of Ceremonies for all congress proceedings. The sponsors, whose contributions were pivotal to the staging of the congress, were Biobest, Koruma Tarim, Leica, Agrobio, Arslanturk Ltd., Dogal Tarim, Koppert, Bayer, Ecaser, Karimex and SG Koleoglu Ltd.

Overview of presentations

The congress comprehensively addressed all aspects of acarology under 21 different sections. In total, there were 279 presentations, namely 188 oral and 91 poster presentations. The oral presentations commenced on the afternoon of Monday, 3 September.

Oral presentations

The oral program included six keynote presentations, three invited presentations, five symposium-specific lectures and the presentations at the 4 symposia, in addition to the presentations in the regular sections.

The six keynote speakers were Maria Navajas (France), Kosta Y. Mumcuoglu (Israel), Peter Schausberger (Austria), James W. Amrine (USA), Edward A. Ueckermann (South Africa) and Hans Klompen (USA).

The three invited speakers in the Biodiversity section were Olga L. Makarova (Russia), Frederic Beaulieu (Canada) and Fabio Akashi Hernandes (Brazil). The five symposium-specific lectures were given by Michael L. Levin (USA), Lidia Chitimia-Dobler (Germany), Derya Yapar (Turkey), Ayse Semra Gureser (Turkey) and Ali Kemal Erenler (Turkey).

Four symposia in total were conducted on Monday 3, Tuesday 4 and Thursday 6, September. They were: ‘Parasitic and free living mites of medical and veterinary

Figure 2. Sebahat K. Ozman-Sullivan, President of the XV International Congress of Acaralogy (XV ICA) (right), accepted the James A. McMurtry Award on behalf of G. W. Krantz from Zhi-Qiang Zhang, President of the Systematic & Applied Acarology Society and XVI ICA (left), during the opening ceremony at the XV ICA in Antalya, Turkey.

importance', which was organized by Kosta Y. Mumcuoglu; 'Ticks and tick-borne diseases' was organized by Kosta Y. Mumcuoglu and Michael L. Levin; 'Invasive species and biosecurity: Understanding and preventing mite bioinvasions' was organized by Maria Navajas and Denise Navia; and 'Mite-plant interactions: From responses to tolerance/resistance' was organized by Maria Pappas, Raul A. Sperotto and Merijn Kant.

Overall, there were 38 sessions of oral presentations under the 21 sections in three halls over the four days of the scientific program. The 21 sections in alphabetical order were Agricultural acarology, Alternative pesticides, Aquatic acarology, Biodiversity, Biological control, Chemical control and resistance, Dispersal of mites, Ecology and behaviour of mites, Evolution and phylogeny, Integrated pest management, Mite-plant interactions, Mites of medical importance, Mites of veterinary importance, Population dynamics, Soil acarology, Taxonomy and systematics, Tick biology and physiology, Tick epidemiology and systematics, Ticks and tick-borne diseases in Turkey, Ticks and tick-borne diseases worldwide and Understanding and preventing mite bioinvasions.

The medical and veterinary acarology sections deserve special mention. About 35 presenters participated in these sections and collectively they made an outstanding contribution to the congress via their eight sessions, which constituted more than 20% of all sessions. The next busiest sections were Biological control (5), Mite bioinvasions (4), Taxonomy and systematics (3), Agricultural acarology (3) and Mite-plant interactions (3).

Poster presentations

The 91 posters were presented on Tuesday, 4 September and Thursday, 6 September in the poster hall. The posters, which covered a broad spectrum of acarological pursuits, generated considerable interest and discussion.

Technical and social tours

The technical and social tours were conducted on Wednesday, 5 September. The morning technical tour commenced at the Antalya 2016 International Expo Center, which was built for an international horticultural exposition. It was followed by an excellent video presentation about the Batı Akdeniz (Western Mediterranean) Agricultural Research Institute by the manager Abdullah Unlu, and then a tour of its glasshouses, propagation facilities and citrus arboretum. The afternoon social tour included the amazingly well preserved Aspendos antique theatre, a Roman aqueduct, and the Temple of Apollo at the ancient city of Side on the beautiful Mediterranean coastline.

Closing ceremony

The closing ceremony was conducted on the afternoon of Friday, 7 September. The congress president Sebahat K. Ozman-Sullivan gave her closing address and then presented plaques to the six keynote speakers during the General Assembly. The Organising Committee of XV ICA created six awards from congress sponsorships for the best oral and poster presentations by students. The six winners were selected by the Scientific Committee members who attended the congress. The six keynote speakers presented the awards for the best three student oral presentations to Sophie Le Hesran (The Netherlands), Alicja Laska (Poland) and Gongyu Lin (Canada), who each received 500 euros, and for the best three student poster presentations to Svetlana S. Paponova (Russia), Natalia Szudarek-Trepto (Poland) and Yusuke Hasegawa (Japan), who each received 250 euros (Fig. 3).

Peter Schausberger then announced the new membership of the Executive Committee of the ICA. He also proposed that deceased ICA Honorary Members become Eternal Members of the ICA. The proposal was unanimously accepted by the General Assembly. Peter Schausberger also announced that there had been three excellent bids to host XVI ICA 2022, namely Auckland, New Zealand; Montpellier, France; and Dublin, Ireland. He then revealed that after due consideration of the relative merits of the three bids by the Executive Committee of the ICA, the New Zealand bid had been selected.

Figure 3. Keynote speakers and student award winners at the XV International Congress of Acarology, Antalya, Turkey.

Zhi-Qiang Zhang, President of the XVI ICA, on behalf of the successful New Zealand bid team, gave a presentation on Auckland and the congress venue, The University of Auckland, and invited all XV ICA participants to attend. Short presentations on future events were then given by Andreas Walzer on the IOBC/WPRS - Working Group "Integrated Control of Plant-Feeding Mites" in Vienna, Austria in September, 2019; by Enrico de Lillo on the European Acarology Congress in Bari, Italy in July, 2020; and by Maria Pappas on the European Congress of Entomology in Hersonissos, Crete, Greece, in 2022. Denise Navia then gave a presentation on the III Latin American Congress of Acarology, which was held from July 29 to August 02, 2018 in Pirenópolis, Goiás, Brazil. Congress president Sebahat K. Ozman-Sullivan then thanked everyone for making XV ICA 2018 a memorable event. She then drew the final curtain on the congress by encouraging all acarologists to meet in Auckland in 2022.

Miscellaneous issues

Business of the Executive Committee of the ICA. The meeting of the Executive Committee (EC) of the International Congress of Acarology (ICA) on 6 September was attended by Peter Schausberger (General Secretary - Austria), Sebahat K. Ozman-Sullivan (Turkey), Mohammed Haq (India), Kimiko Okabe (Japan), Rosta Zemek (Czech Republic) and Xiao-Yue Hong (China). First term members Mohammed Haq, Raphael de Campos Castilho (Brazil), Ronald Ochoa (USA), Rosta Zemek and Kimiko Okabe will remain on the EC until ICA 2022. The outgoing second term members were Peter Schausberger, Farid Faraji (The Netherlands), Rachid Hanna (Cameroon), Xiao-Yue Hong and Sebahat K. Ozman-Sullivan but due to her role as past president of ICA 2018, she remains on the EC as an ex-officio member until ICA 2022. Kimiko Okabe became the new general secretary of the EC-ICA and Rosta Zemek became its new treasurer.

The EC also selected new members for the five open positions. The inductees were Ali Reza Saboori (Iran), Enrico de Lillo (Italy), Frederic Beaulieu (Canada), Jingze Liu (China) and Marie-Stephane Tixier (France). The EC also selected Gilberto de Moraes (Brazil), Jun-ichi Aoki (Japan)and Mohammed Haq (India) as new Honorary members to join the nine on-going Honorary Members who are Carlos H. W. Flechtmann (Brazil), David R. Cook (USA), Evert E. Lindquist (Canada), Gerald W. Krantz (USA), Michel Bertrand (France), Reinhard Schuster (Austria), Ting-Huan Wen (China), Vikram Prasad (USA), and Yuri Balashov (Russia) who passed away in 2012 (Filippova, 2013).

Social activities

The welcome reception was held on Monday, 3 September on the seafront in the garden of the Kubbe bar of the congress venue. The cocktails were accompanied by a musical trio who were followed by the dance troupe, Seyyah, which performed a series of high energy, unforgettable traditional folk dances.

The gala dinner was staged on Thursday, 6 September on a boat and it was accompanied by music and dancing. The splendid coastline of Antalya was visible as the boat headed to the spectacular Duden Waterfall which spills into the sea, an impressive sight that is artificially lit at night.

The accompanying persons' tours were three half-day tours, as follows: Monday 3 September - Visit to the ancient city of Perge; Tuesday 4 September - A half day tour to the waterfalls in the area - Kurşunlu Waterfall, Aksu River and Duden Waterfalls; and Thursday 6 September – The destination was Antalya, a city that offers many cultural and natural delights. The tour visited the historic old town of the Ottoman Empire and its historic harbour, and also the shopping bazaar.

The post congress tour was staged on Saturday, 8 September. It was a day full of great natural beauty and fascinating history in one of the most beautiful parts of Turkey. The tour group visited the ruined city of Myra (Demre) with its impressive Lycian tombs carved from rock. The group then viewed the ancient amphitheater and the Church of St Nicholas. That was followed by an enjoyable boat trip on the sea from Theimussa (Ucagiz) to the ancient sunken city of Simena on picturesque Kekova Island (home of the sun) where there was a break for swimming and snorkelling. A fascinating Lycian necropolis of chest type tombs spread out along the coastline at Theimussa. Beneath the turquoise waters along the northern shore of Kekova Island lie the remains of the city of Simena, which was ravaged by earthquakes around two thousand years ago.

CONCLUDING STATEMENT

On behalf of the Organizing Committee, we greatly appreciate the opportunity to organize XV ICA 2018 in Antalya, Turkey. We believe XV ICA achieved its objective of facilitating the coming together of researchers, students and representatives of institutions worldwide to discuss their latest research results, topical issues and new directions in research. We were very pleased to receive considerable positive feedback about the congress, including that of Zhang (2018b). We trust that you'll want to experience the ICA environment again and that you are already thinking about the next congress. We therefore look forward to meeting with all our acarological friends and colleagues at XVI ICA 2022 in Auckland, New Zealand.

Acknowledgements

On behalf of the XV ICA 2018 Organizing Committee, we thank: the 11 sponsors; the 15 supporting organisations that included international academic societies and universities; the Executive Committee of the ICA for their support; Zhi-Qiang Zhang for hosting the congress website under the domain http://www.acarology.org/ica/; the Scientific Committee members for reviewing the congress abstracts; symposia organisers; keynote speakers; invited speakers; symposium-specific lecture presenters; section chairs; all participants, including Ting-huan Wen and Uri Gerson, for their enthusiasm and making a special effort to attend the congress; Kosta Y. Mumcuoglu for organizing the medical and veterinary acarology group; Abdullah Unlu for organizing the technical tour; Ozer Akbasli for finding a number of sponsors; Tetsuo Gotoh and Owen Seeman for their contributions; David E. Walter, Ronald Ochoa, Kosta Y. Mumcuoglu, Sevgi Sevsay, Salih Doğan, Sebahat K. Ozman-Sullivan and Mustafa Acici who gave permission for their images to be used on the home page of the congress website; Bilkon Travel Agency for facilitating the congress; Hakki Bayindir from Bilkon for managing the congress website; Mujdat Tos who designed the congress logo; and Gregory T. Sullivan for creating the congress theme. We also sincerely thank all those other people who contributed to the success of the congress.

"The Acari: very small but impossible to deny!"

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RESEARCH ARTICLE

Alternative control agents of the dried fruit mite, *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) on dried apricots

Vefa TURGU [1], **Nabi Alper KUMRAL** [2,3]

[1] *Department of Plant Protection, Institute of Natural and Applied Science, Bursa Uludağ University, Bursa, Turkey*
[2] *Department of Plant Protection, Faculty of Agriculture, Bursa Uludağ University, Bursa, Turkey*
[3] Corresponding author: akumral@uludag.edu.tr

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ABSTRACT: Turkey is the biggest dried apricot producer and exporter of the world. *Carpoglyphus lactis* (L.) (Astigmata: Carpoglyphidae) is the most common species on dried apricots. When *C. lactis* feeds on the fruit sugar of the dried apricot, the mite accelerates the development of mould fungi and causes unwanted flavours and odours, due to its digestive products, secretions and dead bodies. These contaminants cause skin diseases and some disorders of the digestive system in humans. Fumigants such as methyl bromide and phosphine are used for the control of this harmful species all over the world. However, methyl bromide has been restricted in many countries including Turkey, because the chemical depletes ozone layer and causes acute deaths in humans. Biological studies have shown that reducing the amounts both of oxygen and humidity negatively affects the population development of *C. lactis*. Based on this evidence, the effects of different physical agents, namely ferric oxide and ozone gas (oxygen depleters), and calcium chloride and silica gel (humidity reducers) to packaged dried apricot infested with *C. lactis* were studied. The lethal dose concentrations and lethal times of each product were determined by probit analysis. In this study, the female mites were successful controlled with all of the physical agents. A death rate of the females of 99% was most rapidly obtained with ozone gas treatment, followed by ferric oxide. Ozone gas was particularly toxic, causing a significantly high level of mortality after application at 44.4 mg/L (LT_{99}= 39 hours). When ferric oxide was applied a dose of 9000 mg/L volume and higher doses, it killed all the female mites within 3 days. Above the dose of calcium chloride at 3000 mg /L killed all of the females within 81 hours. Lastly, the silica gel affected mites over a longer period i.e., two weeks and at a higher dosage (56000 mg/L volume).

Keywords: *Carpoglyphus lactis*, control, desiccants, dried apricot, oxygen absorbers, ozone.

INTRODUCTION

The dried fruit mite, *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) is one of the main pest mite of dried fruits (Hughes, 1976; Özer and Toros, 1978). This stored-product pest mite is found generally on food substances containing sugars from which bacterial activity gives rise to lower fatty acids such as lactic, acetic and succinic acids (Hughes, 1976). This mite was the first found in İzmir on dried fruits such as apricots, figs and raisins with 53.3% infestation rate (Özer and Toros, 1978; Genç and Özar, 1986). After this study, *C. lactis* was found as the most abundant mite in 69% of the total mites in stored dried apricots in Malatya, Elazığ and İzmir province of Turkey (Çobanoğlu et al., 2004).

Turkey is one of the world's leading producers and exporter of dried apricot with the first rank (Anonymous, 2017a; 2017b; 2017c). After sun-drying, the apricot fruits could be safe in terms of microbial activity providing intermediate moisture due to below certain levels of water content. The loss in fruit moisture content of dried apricots result in the accumulation of sugars as crystals first on the surface and then within the fruit tissue. Consequently, *C. lactis* feeds by the fruit sugar of the dried apricot and then mold fungi develop on digestive products, secretions, dead bodies of the mite the dried fruits. These occur unwanted flavour and odours. Infestation of *C. lactis* on dried apricots also causes skin diseases, some disorders in the digestive system and allergy on humans (Hughes, 1976, Özer and Toros, 1978; Genç and Özar, 1986; Özer et al., 1989; Turanlı, 2003; Çobanoğlu et al., 1996; Aksoy et al., 2004; Öztekin et al., 2007; Güldalı and Çobanoğlu, 2010; Hubert et al, 2011; 2015).

Single common control method of *C. lactis* is fumigation with some chemicals such as methyl bromide and phosphine. In recent years, alternatively some chemicals, such as carbonyl sulphite, sulphuryl fluoride, cyfluthrin and iodomethane are applied in the control of this mite (Ferizli et al., 2004; Meyvacı and Fatih, 2007; Aksoy et al., 2008; Şen et al., 2009). But these compounds cause some problems: (1) Human health: they cause acute deaths in personals, (2) Environmental problem: destruction of ozone layer (methyl bromide), (3) Safety problem: corrosive and inflammable in buildings and transporters (phosphine), (4) Ecological problem: they are broad spectrum pesticides, eradicates non-target organisms, (5) Application problem: fumigation difficulties and slow effects (phosphine), (6) Resistance problem: resistance development in some mite population. Since methyl bromide has got thinning effect of the ozone layer, its use is restricted in many countries implemented the Kyoto Protocol (Emekçi et al., 2004; Ferizli et al., 2004). MeBr has been gone to be phased out since 2007 according to the Methyl Bromide Action Plan of the Turkish Government (Desmarchelier, 1998, Anonymous, 2017d). For this reason, there is a need for new environmental friendly control methods.

Astigmat stored-product pest mites which rely solely on cutaneous respiration, are thus weakly sclerotized and vulnerable to desiccation (Collins, 2006). Development and/or reproduction of *C. lactis* varies depend on temperature, humidity and oxygen levels. The adult's life span reaches maximum level at 18°C and 65% relative humidity and the most fecundity was observed at 28°C and 80% humidity conditions. *C. lactis* may die in low humidity and at high temperatures (Cunnington, 1985; Emekçi and Toros, 1989; Bell and Conyers, 2002; Güldalı and Çobanoğlu, 2010). Previous biological studies have shown that reducing amounts of oxygen and/or humidity was negative affected development of *C. lactis* (Longshu et al., 1992; Ferizli and Emekçi, 2000; Collins, 2006; Şen et al., 2009; Aksoy et al., 2012). Based on this evidence, different physical agents could be affected on *C. lactis.* For example, ozone gas depletes oxygen and is oxidative agent; ferric oxide, calcium chloride and silica gel are humidity reducers and ferric oxide is also oxygen absorbent (Yıldırım et al., 2018). Until now, some previous studies about increasing carbon dioxide or decreasing humidity have given quite successful results (Emekçi et al., 2004; Wang et al., 2008; Işıkber et al., 2015). But, there is no study about the effects of ferric oxide, ozone gas, calcium chloride and silica gel against *C. lactis.* The aims of this study were to determine the lethal concentrations (LC_{50}, LC_{90} and LC_{99}) and lethal times (LT_{50}, LT_{90} and LT_{99}) of these physical agents by probit analysis in laboratory conditions during 2017-2018 years.

MATERIAL AND METHODS

Mite culture

This study performed at Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, Toxicology and Acarology Laboratory in climate chambers during 2017-18. Commercial dried apricots were obtained from a market. The apricot fruits were sterilized with ethyl alcohol (70%). Also, all experimental materials and apricot fruits were dried and sterilized under an UV-chamber. *Carpoglyphus lactis* population was collected from dried apricots a traditional market of Bursa province (Turkey). The species was identified based on morphological characters with a light microscopy (Hughes, 1976). For mass rearing of mite population on dried apricots, plastic boxes contain dried apricots placed on moistened cotton were used. The mite colony was mass reared in a separate insectarium with a 16-h light (at 27 ± 1◦C and 60 ± 5% RH) / 8-h dark (23 ± 1◦C and 60 ± 5% RH) cycle. Light was provided with white lamps.

Bioassays

Lethal concentration (LC_{50}, LC_{90}, LC_{99}) and lethal time (LT_{50}, LT_{90}, LT_{99}) of ozone gas (O_3), ferric oxide, silica gel and calcium chloride for *C. lactis* were calculated by probit analysis method using results of modified fumigation bioassay (Simon, 2014). In O_3 lethal concentration bioassays, dried apricot fruits infested with 50 same age female mites were putted in tulle packets placed in 250 cc glass Erlen jars. The jars with valve system were sealed and their airs were vacuumed by a pump. After, the different doses of O_3 (mg/m^3) were applied with an Ozone generator. Non-ozonation Erlen jars were used as control. In ferric oxide bioassays, single dose ready ferric oxide packagings (3.5 gr) were used, but these putted in different volume glass jars (100, 250, 500, 1000 etc.). Dried apricot fruits infested with 50 female mites putted in a tulle packet were placed to the jars. Then, the packages were quickly thrown into these jars, because the material absorbs quickly ambient oxygen when vacuumed ferric oxide packagings are opened. The jars without ferric oxide were used as control. Similar method was used for Silica gel (2 gr/package) and calcium chloride (2 gr/package). At least, five or more different doses of each agent were applied. The treatments were carried out three replicates with 50 female mites. Live mites/insects were counted at each 24h. They were considered alive if they that were able to walk at least one body length when probed with a paintbrush while viewed under a stereomicroscope. Mortality data obtained from bioassays were corrected using the Abbott formula (Abbott, 1925). The numbers of alive and dead mites were subjected to probit analysis (SPSS 23. version) to determine LC values for each agents (Simon, 2014). In lethal time bioassay, same methods were used, but LC_{90} value of each agent was applied at least seven times (hours).

RESULTS

The toxicities of four physically agents namely O_3, ferric oxide, calcium chloride and silica gel to *C. lactis* females were examined by the modified fumigation method and the probit analysis results are summarized in Table 1. All physically agents killed females of *C. lactis* in different doses and times. Based on slope value, the O_3 gas response of *C. lactis* females was heterogeneous (the slope of the curve was significantly not steeper) and reflected more variability among individuals. Forty-eight hours after O_3 gas application, its median lethal dose was found as 16.04 mg/L for *C. lactis* females. The highest lethal doses, LC_{90} and LC_{99}, were calculated as 30.49 and 42.28, respectively. The confidence limits of all this lethal doses were very narrow. In ferric oxide bioassays, sixty hours after application of the agent on dried apricot fruits, the median lethal dose of ferric oxide was 3232 mg/L for *C. lactis* females. The highest doses, LC_{90} and LC_{99}, were estimated as 9145 and 21351mg/L, respectively. Different to previous agent response, ferric oxide response of *C. lactis* females was more homogeneous ($b>2$). Seventy-two hours after calcium chloride application, the median lethal dose of calcium chloride was found as 1052 mg/L for *C. lactis* females. The highest doses, LC_{90} and LC_{99}, were calculated as 3044 and 4670 mg/L, respectively. Similar to O_3 gas response of *C. lactis* females, the mite response was heterogeneous ($b<2$) but reflected less variability among individuals. In silica gel bioassays, 240 hours after silica gel application, its median dose was 35063 mg/L.

Table 1. The probit analysis and lethal concentration results of O_3, ferric oxide, calcium chloride and silica gel for *Carpoglyphus lactis*.

		O_3	Ferric oxide	Calcium chloride	Silica gel
Number of experimental dose		7	8	12	7
Time (hours)		48	60	72	240
Death rate of control individuals (%)		3.4	6.0	10.23	4.0
Slope±SE		0.089±0.05	2.84±0.173	0.643±0.042	2.105±0.137
LC_{50} (mg/L)		16.04	3232	1052	35063
95% confidental limits	Highest	13.9	1734	341	31828
	Lowest	18.1	5311	2055	39406
LC_{90} (mg/L)		30.5	9145	3044	56407
95% confidental limits	Highest	27.7	5522	2046	49924
	Lowest	34.2	30340	6803	66742
LC_{99} (mg/L)		42.3	21352	4670	73808
95% confidental limits	Highest	38.01	10477	3110	64094
	Lowest	48.2	170170	11000	89611
X^2		23.7	232.23	487.98	27.154
Probability		0.03	<0.01	<0.01	0.012

The highest lethal doses, LC_{90} and LC_{99}, were estimated as 56407 and 73808 mg/L, respectively. But, the response of *C. lactis* females to silica gel was more homogeneous (b>2).

The lethal times of LC_{90} values for four physically agents to *C. lactis* females were examined by the modified fumigation method and the probit analysis results are summarized in Table 2. The median lethal times (LT_{50}) of LC_{90} values for O_3, ferric oxide, calcium chloride and silica gel were found as 13.43, 48.70, 38.48 and 219.7 hours, respectively. The lethal times (LT_{90} and LT_{99}) killed 90% and 99% of the mite population were estimated as 27.54 and 39.04; 62.16 and 73.13; 62.09 and 81.05; 332.3 and 424.2 hours; for O_3, ferric oxide, calcium chloride and silica gel, respectively.

DISCUSSION

This study is the first report of the control for *C. lactis* females on dried apricot to tested physical agents namely O_3 gas, ferric oxide, calcium chloride and silica gel. The results obtained from this work showed that the females of *C. lactis* can be affected from all of physical agents depending on dose and time. The low dose of O_3 gas killed 90 percent of *C. lactis* females within two days. Until now, there has no attempt about control of *C. lactis* females with O_3 gas. But, our findings are consistent with other studies that O_3 gas reported to be effective against harmful insect species at high concentrations and short exposure times (Öztekin et al., 2007; Işıkber et al., 2015). Previous studies demonstrated that ozone is an unstable triathomic form of oxygen and the oxygen atoms in this triple atom structure spontaneously decompose to form hydroxyl radicals and other free radicals or to contact with oxidizable surfaces (Perez et al., 1999). In accordance with our findings, these properties of ozone can provide to prevent bacterial, yeast and mold growth and insect infestations during storage (Xu, 1999).

In addition, both humidity reducer and oxygen absorbent agent, ferric oxide, killed 90 percent of *C. lactis* females at nearly 9000 mg/L dose within two and half days. The findings obtained from this study are the first evidences on control of *C. lactis* females with ferric oxide. In accordance with our result, it was reported that ferric oxide is effective on insects in the same phylum (Arthropod) as mites (Janjarasskul and Suppakul, 2018). Previous studies showed that some metal or metal oxides show antibiological properties and act as an oxygen and/or moisture barrier for some bioagents. For this purpose, the some metals such as zinc oxide, silica, titanium dioxide, aluminum oxide and iron oxide are used commercially in food packages (Janjarasskul and Suppakul, 2018). Confirming our results, because of the effects of oxygen-binding of iron oxides, they are commonly used in packaging of fruits as well as many other foods and feeds for control of microorganism and insect infestations and color, odor and ransity changes of fruits (Kavas and Kavas, 2001; Çelik and Tümer, 2016; Janjarasskul and Suppakul, 2018; Yıldırım et al., 2018).In this study, nearly 9000 mg/L dose of the other water binding agent, calcium chloride, killed 90% of *C. lactis* females within three days. Compared to ferric oxide, the agent showed effect on *C. lactis* females at less dose and longest time. But, just the higher dose of silica gel compared to other desiccants killed 90% of *C. lactis* female population approximately after two weeks.

Table 2. The probit analysis and lethal time results of O_3, ferric oxide, calcium chloride and silica gel for *Carpoglyphus lactis*.

		O_3	Ferric oxide	Calcium chloride	Silica gel
Number of experimental time		14	7	10	6
Death rate of control individuals (%)		9.33	6.00	10.23	4
Slope±SE		0.091±0.04	0.095±0.006	0.055±0.03	0.11±0.001
LT_{50} (hours)		13.43	48.70	38.84	219.7
95% confidental limits	Highest	11.30	43.04	35.96	200.2
	Lowest	15.97	55.46	42.04	247.1
LT_{90} (hours)		27.54	62.16	62.09	332.3
95% confidental limits	Highest	23.52	55.41	57.39	294.4
	Lowest	34.13	78.95	68.13	394.8
LT_{99} (hours)		39.04	73.13	81.05	424.2
95% confidental limits	Highest	32.78	63.39	74.22	368.3
	Lowest	49.63	100.21	90.05	518.2
X^2		233.09	150.41	26.65	45.71
Probability		<0.01	<0.01	<0.01	<0.01

Desiccants such as calcium chloride or silica gel are commonly used to manage humidity in the food packaging headspace. These desiccants are commonly placed into packages in the form of sachets, microporous bags, or are integrated in pads so that they inhibit microbial development (Yıldırım et al., 2018). It was reported that their absorption capacity depends on its water vapour sorption isotherm (Sängerlaubet al., 2013). Differences between the absorption capacities of calcium chloride and silica gel may be caused differences in their lethal doses and lethal times for *C. lactis* females. Confirming our argument, Singh et al. (2016) showed that some desiccants such as $CaCl_2$, KCl, and sorbitol, have fast-absorbing capacity with moisture holding capacity (0.91 ± 0.01 [g H_2O/g] in 120 h) compared other water binding agents. As a result, our study showed that some moisture-absorbing agents could control *C. lactis* females at varying doses and times. In this study, the importance of moisture control in combating acarid mites has been shown. Consisting with our study, Solomon (1966) revealed that compared with larger arthropods, the acarid mites have high rates of uptake or loss of water as the percent of weight, but low rates per unit area of its surface. Such arthropods, with a high surface/volume ratio, and living at humidity down to about 62% RH, need a relatively impervious cuticle. Previous laboratory and field studies demonstrated that some inert materials (e.g. silica gel) leading to death through desiccation have proved effective as grain protectants against to some astigmat mites *Acarus siro* L., *Lepidoglyphus destructor* (Schrank), *Tyrophagus longior* (Gervais) and *Tyrophagus putrescentiae* (Schrank) (Collins, 2006).

As a conclusion, O_3 gas agent has the fastest effect following by ferric oxide, calcium chloride and silica gel, respectively, based on lethal time results. But, there are some disadvantages of the agents in control of *C. lactis*. For example, O_3 can be degraded quickly to O_2 and O^- atoms. In that reason, the effect of O_3 gas is instantaneous and non-persistent (Güzel-Seydim et al., 2004). On the other hand, ferric oxide, calcium chloride, silica gel agents could be used only within small volume food packages. The effect of silica gel is very slow. Short term application of O_3 could be suitable for control of the mite in storages. Ferric oxide and calcium chloride could be controlled *C. lactis* during long time. Also, a combined control strategy can be applied against *C. lactis*. Firstly, O_3 gas could be used for fumigation application for 2 days in the storage of dried apricot. After, the dried apricot can be packaged with ferric-oxide or calcium chloride until eating up by consumers. In the future, these combined applications should be tested in producer conditions for reflecting the practice. However, the effects of the agents on microbial load and fruit quantitative should be investigated.

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RESEARCH ARTICLE

A species being worthy of its name: Intraspecific variations on the gnathosomal characters in topotypic heteromorphic males of *Cheylostigmaeus variatus* (Acari: Stigmaeidae)

Salih DOĞAN [1,3], **Sibel DOĞAN** [1] **Qing-Hai FAN**[2]

[1] *Department of Biology, Faculty of Sciences and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey*
[2] *Plant Health and Environment Laboratory, Ministry for Primary Industries, Auckland, New Zealand*
[3] Corresponding author: salihdogan@erzincan.edu.tr

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ABSTRACT: *Cheylostigmaeus variatus* (Acari: Stigmaeidae) is only known from Turkey. It has been called *variatus*, due to showing variations in the shapes of lamellae and dorsal cheliceral surfaces in the heteromorphic males. During the present work, we found five topotypic heteromorphic male specimens among mite specimens in the grassy soil and moss collected from the type locality, Ekşisu Marsh in Erzincan province, Turkey. These topotype specimens of the species including holotype and paratype were examined. During the examination, we observed variations in the shapes of lamellae, dorsal cheliceral surfaces and apophyses on the internal and external surfaces of palp femur. We consider that the differences observed in the heteromorphic male specimens are intraspecific variations, that it is true to its epithet name and that there is not enough evidences to justify a new species. This hypothesis may be supported by the rearing of a population of the species or molecular evidence.

Keywords: Gnathosoma, heteromorphic, intraspecific, mite, morphology, variation.

Zoobank: http://zoobank.org/93C2C7C6-A25C-44B7-9539-55697DC2A021

INTRODUCTION

Stigmaeidae is the most widespread and abundant family in the superfamily Raphignathoidea. Most of stigmaeid species have been considered as free-living predators (Fan and Flechtmann, 2015; Fan et al., 2016). The family comprises 34 genera (Fan et al., 2016). By now, 11 genera of Stigmaeidae have been recorded from Turkey; one of them is *Cheylostigmaeus* Willmann. Members of this genus are found in all regions of the world except Antarctic (Fan and Zhang, 2005; Fan et al., 2016). By now, 34 species of the genus are known in the world (Doğan et al., 2015b; Fan et al., 2016), and 7 of which were reported from Turkey (Erman et al., 2007; Doğan, 2007, 2019; Doğan et al., 2015b; Bingül et al., 2016; Doğan and Bingül, 2017).

Distinguishing characters of the species in the genus *Cheylostigmaeus* such as the structure of the gnathosoma are mainly based on males. In this genus, the gnathosoma may show sexual dimorphism unlike other members of the family. Polymorphism may occur in males of this genus. The homeomorphic males resemble the females in the absence of lamellae on subcapitulum. The lamellae and/or the palpal apophyses are characteristic in heteromorphic males.

In the present work, morphological variations in distinguishing characters in five topotypic heteromorphic male specimens are identified in *Cheylostigmaeus variatus* Doğan, Dilkaraoğlu and Fan. Asymmetric and numerical variations in the body setae occurring in some adult females and males in some species of *Eustigmaeus*, *Stigmaeus* and *Storchia* were previously known (Akyol, 2011; Dilkaraoğlu et al., 2016a; Bingül et al., 2017a, 2017b; Bingül and Doğan, 2017; Koç and Poyraz Tınartaş, 2017). The variations in the shapes of lamellar projections, verrucae on dorsal surfaces of chelicerae and structures of apophyses on palpal segments in these specimens are noted here. With this study, we aimed to provide new data on variations observed in *C. variatus*.

MATERIAL AND METHODS

The mite specimens were extracted using Berlese-Tullgren funnels, cleared in 60% lactic acid and mounted on microscopic slides in Hoyer's medium under a Leica EZ4 stereo microscope using the standard method (Walter and Krantz, 2009). The specimens were examined and photos were taken with an Olympus BX63 microscope. Topotype specimens were coded as T1-T5.

RESULTS

In this study, we found five topotypic heteromorphic male specimens of *Cheylostigmaeus variatus* among mite specimens in the grassy soil and moss collected from the type locality, Ekşisu Marsh (Erzincan province, Turkey). These topotype specimens of the species including holotype and paratype were examined. Differential features of gnathosoma in the type specimens were noted. During the examination, we observed variations in the shapes of lamellae, dorsal cheliceral surfaces and apophyses on the internal and external surfaces of palp femur (Figs 1-6).

The lamella wing-like and lamellar projections bear four minor cusps on outer margin in holotype (Figs 1A, 4A) and are incised in paratype male (Figs 1B, 4B). The lamella is incised in one topotype (T1) as in paratype (Figs 1C, 4C). In the other topotype specimens, lamellae are not wing-like. In one topotype (T2), the left lamellar

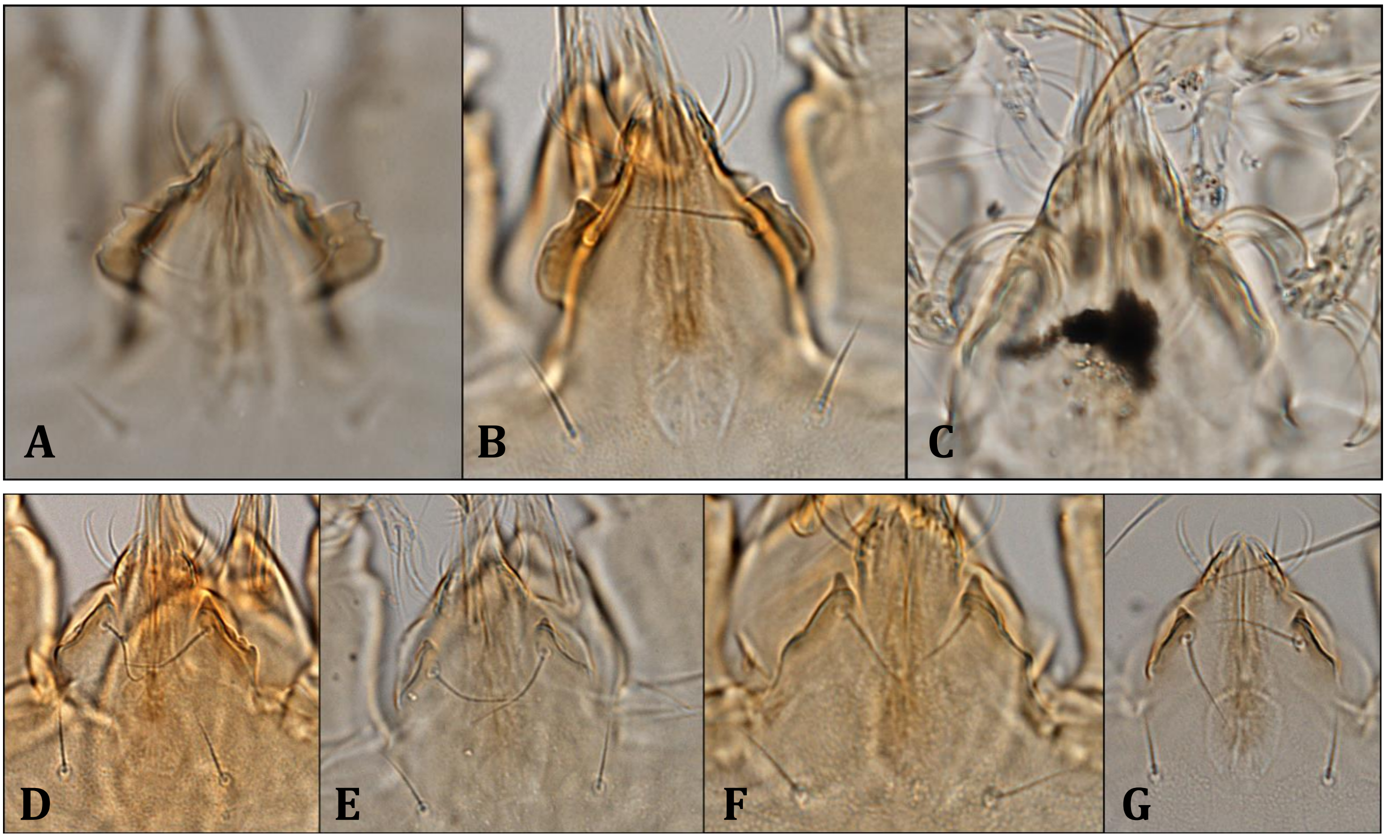

Figure 1. *Cheylostigmaeus variatus* (heteromorphic male) – Variations in the shapes of lamellar projections: A) Holotype, B) Paratype, C-G) T1-T5, respectively.

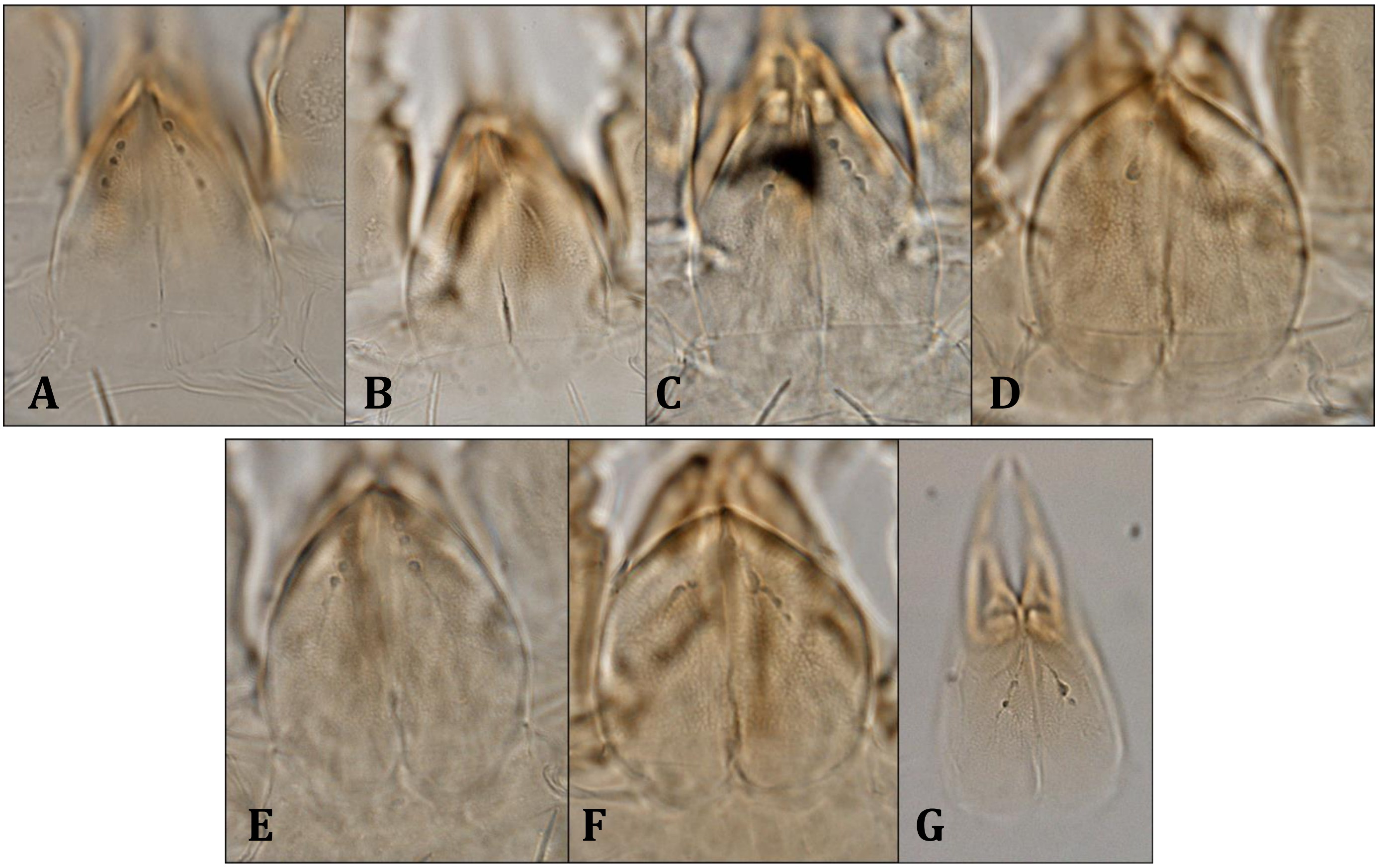

Figure 2. *Cheylostigmaeus variatus* (heteromorphic male) – Variations on dorsal cheliceral surfaces: A) Holotype, B Paratype, C-G) T1-T5, respectively.

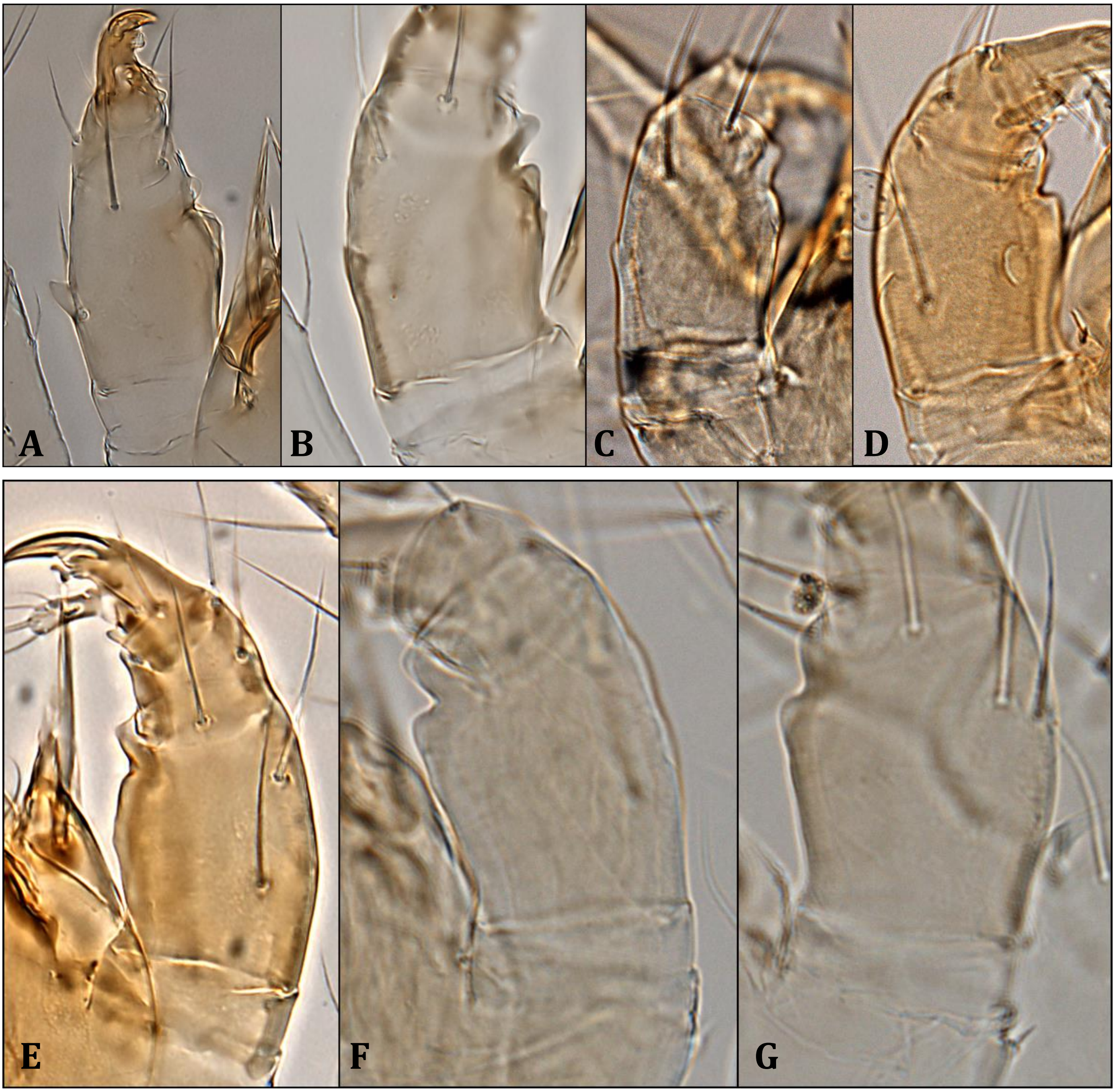

Figure 3. *Cheylostigmaeus variatus* (heteromorphic male) – Variations on the internal and external surfaces of palp femur: A) Holotype, B) Paratype, C) T1, D) T4, E-G) T2, T3 and T5, respectively.

projection with three minor cusps on outer margin while not bearing cusps on the right side (Figs 1D, 4D). Lamellae in the others (T3-T5) are similar in shape, namely pointy ear-like (Figs 1E-G, 4E-G).

Chelicerae dorsally with four pairs of verrucae in holotype (Figs 2A, 5A), sclerotic ridges in paratype (Figs 2B, 5B), and in the topotypes (T1-T5) dorsal cheliceral surfaces as shown in Figure 2 (C-G) and Figure 5 (C-G).

The external surface of femur with one spiky apophysis and its internal surface with three apophyses in holotype and paratype (Figs 3A, B, 6A, B). In two topotypes (T1, T4) apophysis in the external surface of femur are smaller than those of paratype and holotype and blunt-ended, and their internal surfaces with two apophyses (Figs 3C, D, 6C, F). In three topotypes (T2, T3, T5) apophysis in the external surface of femur is absent, and its internal surface with two apophyses (Figs 3E-G, 6D, E, G).

DISCUSSION

Cheylostigmaeus variatus has been described from Turkey by Doğan et al. (2015b), and presently only known from Turkey. The name of this species, *variatus*, refers to the variation in the shapes of lamellae and dorsal cheliceral surfaces in the males. We consider that the differences observed in the heteromorphic male specimens of the species are intraspecific variations, that this species is worthy of its name and that there is not enough evidences

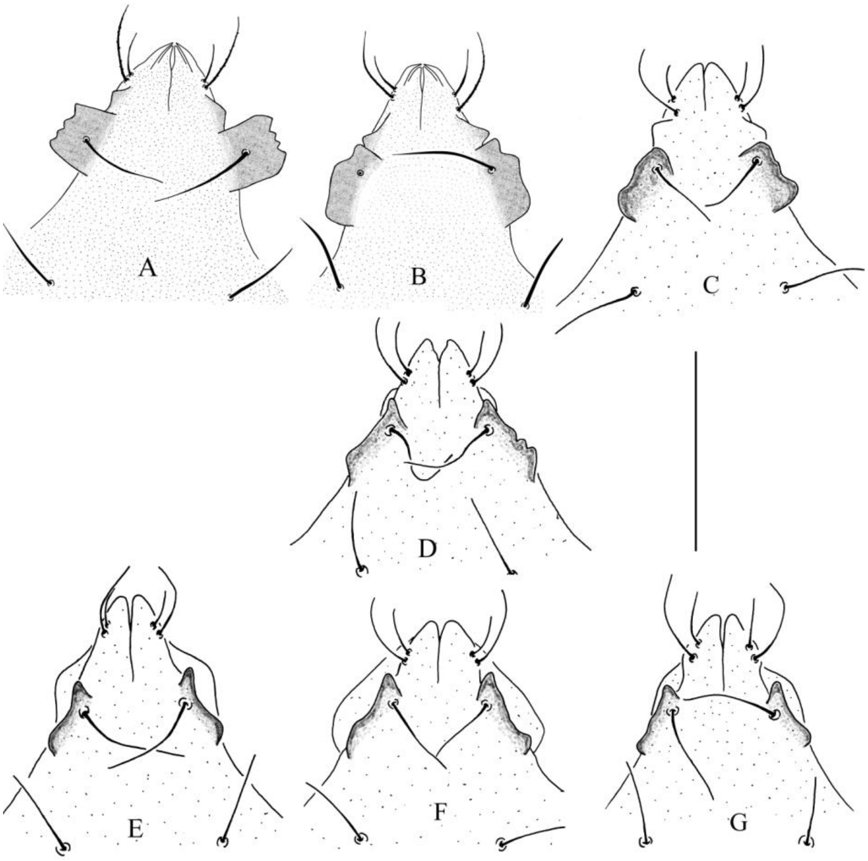

Figure 4. *Cheylostigmaeus variatus* (heteromorphic male) – Variations in the shapes of lamellar projections: A) Holotype, B) Paratype, C-G) T1-T5, respectively. Scale bar 50 µm.

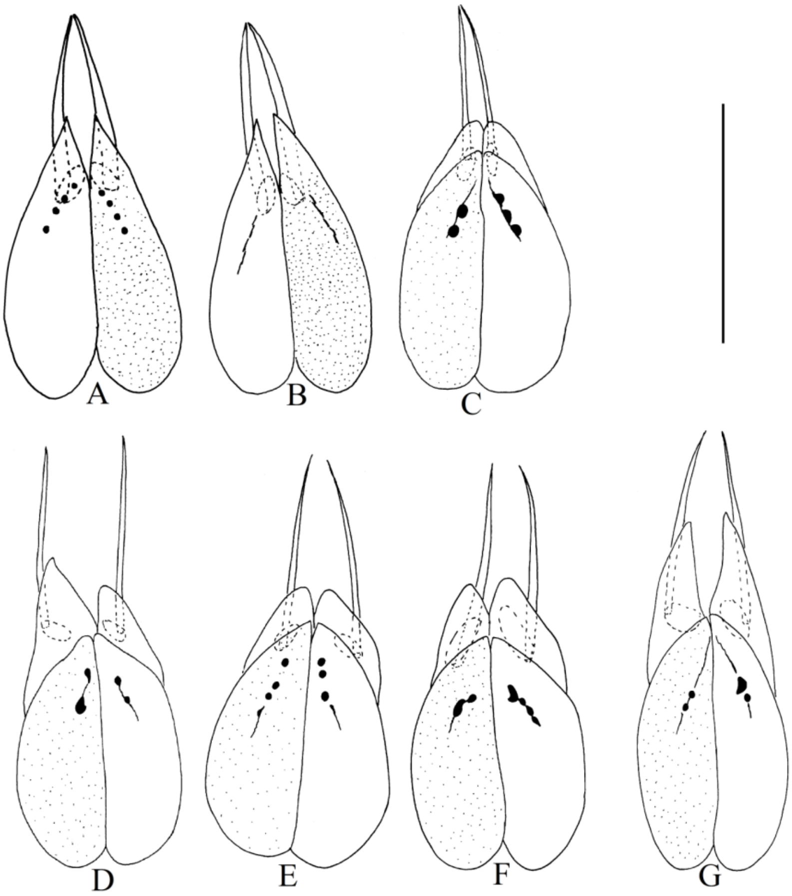

Figure 5. *Cheylostigmaeus variatus* (heteromorphic male) – Variations on dorsal cheliceral surfaces: A) Holotype, B) Paratype, C-G) T1-T5, respectively. Scale bar 100 µm.

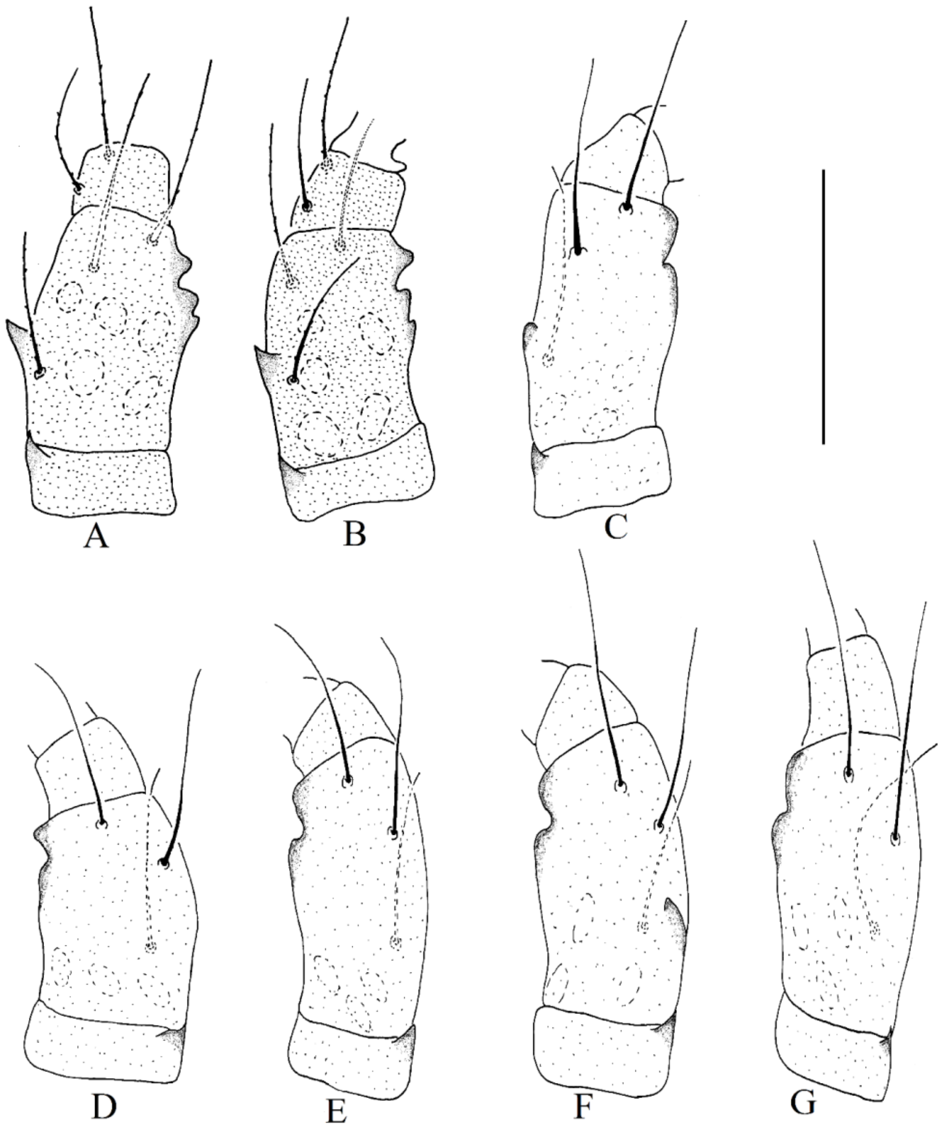

Figure 6. *Cheylostigmaeus variatus* (heteromorphic male) – Variations on the internal and external surfaces of palp femur: A) Holotype, B) Paratype, C-G) T1-T5, respectively. Scale bar 100 µm.

to justify a new species. This hypothesis cannot be further clarified until a population of *C. variatus* is reared or its molecular evidence is provided.

The mites show a variety of morphological variations, as in other organisms (Bingül et al., 2018). Intraspecific variation may be problem for species identification, and may lead to taxonomic confusion. The adaptations of organisms to different environmental conditions may cause the genetic differentiation and intraspecific variation (Bingül et al., 2017b).

In spite of many biological, faunistic and taxonomic works on stigmaeid mites, studies on their morphological variations remain limited (Akyol, 2011; Doğan et al., 2015a, 2015b, 2016, 2017; Dilkaraoğlu et al., 2016a, 2016b; Bingül and Doğan, 2017; Bingül et al., 2017a, 2017b; Koç and Poyraz Tınartaş, 2017), and external variations in the genus *Cheylostigmaeus* have not been reported yet except *C. variatus* Doğan, Dilkaraoğlu and Fan. This study will help to better explain the variations in morphological characteristics of *C. variatus*.

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RESEARCH ARTICLE

Seasonal distribution and damage potential of *Raoiella indica* (Hirst) (Acari: Tenuipalpidae) on areca palms of Kerala, India

Prabheena PRABHAKARAN [1,2], **Ramani NERAVATHU** [1]

[1] *Division of Acarology, Department of Zoology, University of Calicut, Kerala, India*
[2] Corresponding author: p.prabheena@gmail.com

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ABSTRACT: Tenuipalpid mites represent an important group of Acari characterized by polyphagous habit on a wide variety of crops. Among the injurious species of tenuipalpids, *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae), the Red Palm Mite (RPM), is a serious pest on a variety of plants. In the present study, infestation and distribution of the species on *Areca catechu* L. (Arecales: Arecaceae), an important plantation crop of Kerala (India), were studied. The leaf sampling was carried out once in a month in Kozhikode and Malappuram districts of North Kerala between 2011 and 2013. Both uninfested and infested leaf samples were subjected to observation under a stereo zoom microscope (MVNSZ-450) to collect data on the population density of the mite. Feeding impact of the RPM on the areca palm was assessed through quantitative estimation of the total contents of nitrogen, phenolic compounds and chlorophyll, and free proline. Results of population studies revealed peak population density of RPM during April-May months. A positive correlation was recorded between mite population and temperature and a negative correlation between mite population and relative humidity. The percentage losses of 'total nitrogen' and 'total chlorophyll contents were estimated as 51-53% and 59-60% respectively in RPM infested leaves. The percentage increases recorded in the proline and phenolic compound contents of mite infested leaf samples were 182-198% and 90-92%, respectively, thereby revealing the stress induced by the RPM to areca palms. Data obtained on the changes induced by RPM on chlorophyll, proline, phenolic compound and total nitrogen contents were found significant at 0.05 level, when subjected to statistical analysis following t-test, thereby establishing the pest status of RPM on *A. catechu*.

Keywords: *Areca catechu*, proline, *Raoiella indica*, total chlorophyll, total nitrogen, total phenolic compound.

INTRODUCTION

Mites and ticks constitute an important and most diverse group of arachnids and they are noticeably less known than the other groups of arthropods such as spiders. The present work is focused on an important species of phytophagous Acari, *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae) which feeds voraciously by sucking the cell sap of the host plant, *Areca catechu*. The Red Palm Mite (RPM), *R. indica* causes severe damage to plants belonging to the family Arecaceae, especially to coconut (*Cocos nucifera* L.), and also to bananas, *Musa acuminata* (Musaceae) and some other plant families (Flechtmann and Etienne, 2004, 2005; Etienne and Flechtmann, 2006). It is the first mite species in which feeding was observed through stomata of its host plants (Ochoa et al., 2011). Significantly, higher population densities of RPM have been recorded on areca palm, with peak population in April/May in Karnataka, India (Yadavbabu and Manjunatha, 2007). This study was in line with the present study. However, despite of the high incidence and wide distribution trend of RPM on varied economic crops of Kerala, only very scanty information (on only population dynamics) was available on this mite in South India (Taylor et al., 2011). Hence the present study has been carried out to procure data on seasonal population density and the severity of damage induced by *R. indica* on an important economic crop of Kerala, viz. *A. catechu* by making qualitative and quantitative assessment of damage potential induced by *R. indica* in Kozhikode and Malappuram districts of Kerala between 2011 and 2013.

Goal of the present study was to determine the seasonal population density of *Raoiella indica* on areca palms of two districts of North Kerala. And also the present work was taken up to understand the severity of damage induced by *R. indica* on host plant *Areca catechu* by estimating biochemical components such as chlorophyll, nitrogen, phenol and proline.

MATERIAL AND METHODS

Mite rearing in the laboratory

The mites were cultured in the laboratory condition for observation on the feeding habits of mites. Rearing of different life stages of mites were maintained on the host plant leaves in the laboratory condition and the leaves upon damage were replaced with fresh ones. Each culture set was maintained with two duplicates to confirm the observation and each culture set consisted of 1-3 mature leaf discs, kept in Petri dishes lined with a moistened cotton pads to maintain the water content of leaves up to maximum number of days. Along with experimental set up, stock cultures of the mites were also maintained in the laboratory on the areca leaf lets collected from the field to ensure constant supply of life stages.

The seasonal population density of *Raoiella indica* on *Areca catechu*

Field sampling of mite infested leaves was carried out from areca plantations in North Kerala where palms belonging to six to seven years of age were cultivated in separate gardens. Sampling was conducted for a three year period, from 2011 to 2013, from plants cultivated in 10 localities of Kozhikode and Malappuram districts of North Kerala. During the study period, field surveys were undertaken once in a month in order to procure temporal data on mite infestation. Total of 10 areca gardens were used for sampling of areca leaves. In each garden, young palms, not taller than 3 meters were selected randomly and three fronds (leaf of the palm) were sampled (bottom, middle and top). On each frond, five leaflets were removed from the region close to the rachis. Leaflets were transported to the laboratory for subsequent microscopic observation, to record the presence of *R. indica* and its life stages*.* The number of different life stages of the mite present on individual leaflets of bottom, middle and top fronds was counted separately under a stereo zoom microscope at fortnightly intervals. The mites were carefully mounted on a slide and these slide mounted specimens were identified under the high power of a Carl Zeis research microscope with the help of keys, literatures and then it was confirmed with the help of an expert. No other phytophagous inhabitants could be noticed during field sampling. The injurious status of the mite was analyzed through repeated field studies by collecting mite infested and uninfested (control) leaves and recording damage symptoms such as chlorotic spots, yellowing, greyish brown patches etc. The injurious status of the mite infestation was determined by analyzing how much area of the leaf lamina was infested by the mites. The leaves with more than half of the laminar surface showing visible symptoms of mite infestation were categorized as severely infested.

Qualitative assessment of damage potential

Qualitative assessment of damage potential of *R. indica* was made by conducting regular observation on the feeding activities of the various life stages and recording the damage symptoms induced on the host plant during progressive feeding. Qualitative assessment of feeding was made simultaneously through repeated field cum laboratory studies. Field collected mite infested and uninfested samples of host leaves were subjected to microscopic examination in order to record the nature of incidence, severity of infestation, population density, distribution pattern, damage symptoms induced on the leaves etc. Results of field studies were confirmed through simultaneous microscopic observation of leaves maintained in stock cultures in the laboratory. Observation on individual leaf was continued to record the qualitative difference between the experimental and control leaves and also to gather knowledge on the damage potential of the mite. Renewal of damaged/decayed leaf discs was made in every 2 weeks and the observations were continued with fresh leaves.

Quantitative assessment of damage potential

Quantitative assessment of damage potential of RPM on leaves of *A. catechu* was carried out through biochemical analysis of various parameters such as chlorophyll a, chlorophyll b, total chlorophyll, phenolic compound, free proline and total nitrogen contents. For rating the damage, field sampling was done in April-May months, in this period the active number of mites inhabited on the single leaf let were 50 -70 and these number of mites were quite similar in all infested sample leaves during this period.

Estimation of Chlorophyll loss

The chlorophyll contents of mite infested and uninfested (control) leaf samples were estimated following the method of Arnon (1949). The exuviae, eggs, life stages and fecal matter of mites were carefully removed with the help of camel hair brush under a stereomicroscope from the infested leaves before subjecting these for chlorophyll analysis. Chlorophyll was extracted from 2g of the infested and uninfested leaf sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5minutes. The extraction was repeated until the residue became colorless. The absorbance of the solution was recorded in a Shimadzu UV-VIS spectrophotometer (Model UV – 1601) at 645 nm, 663 nm and 750 nm against the solvent blank of 80% acetone, for chlorophyll a, b and total chlorophyll.

Chlorophyll **a** (µg/ml) = [12.69 (A_{663}-A_{750})-2.69 (A_{645}-A_{750})/ Dry weight] x Volume

Chlorophyll **b** (µg/ml) = [22.9 (A_{645}-A_{750})-4.68 (A_{663}-A_{750})/ Dry weight] x Volume

Total chlorophyll (µg/ml) = [20.12 (A_{645}-A_{750}) + 8.02 (A_{663}-A_{750})/ Dry weight of Sample] x Volume

A = Absorbence

Estimation of Nitrogen

Estimation of total nitrogen content present in mite infested and uninfested leaf sample was made by following the method of Kjeldahl (1883) from Interfield Laboratories Pvt. Ltd., Cochin, Kerala.

The amount of nitrogen was calculated as follows:

Nitrogen (%) = (A-B) X C/D X E/(FXG) X 100

A = Titer value for digested sample, ml

B = Titer value for blank, ml

C = Nitrogen equivalent of ammonium sulphate, mg

D = Titer value for ammonium sulphate, ml

E = Volume of digested sample, ml

F = Volume of sample taken for distillation, ml

G = Sample weight, mg

Estimation of Proline

Free proline contents present in the mite infested and uninfested samples were estimated following the method of Bates et al. (1973). Other intrusive materials were also presumably removed by absorption to the protein sulphosalicylic acid complex. The extracted proline was made to react with ninhydrin in acidic conditions (pH 1) to form the chromophore (pink color) and read at 520 nm. Two hundred (200 mg) milligrams of mite infested and uninfested fresh leaf samples were weighed separately and homogenized in 10 ml of 3% (w/v) aqueous sulfosalicylic acid using a clear glass mortar and pestle. The homogenate was filtered through Whatman No. 2 filter paper. From the filtrate, 2 ml aliquot was taken and mixed with 2ml glacial acetic acid and 2ml acid ninhydrin. The tubes with mixture were heated in a boiling water bath for 1 hour and then the reaction was terminated by placing the tubes in an ice -bath. For color development, 4.0 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then, the pink colored toluene layer was separated and brought to room temperature. The color intensity of the solution was measured at 520 nm using toluene as reagent blank in a Spectrophotometer. L. Proline was used as the standard. The amount of proline in the test sample was calculated from the standard curve (mg/g tissue).

Estimation of Phenolic Compounds

The response of plants to mite attack in terms of concentration of total phenolic compound content of each extract was determined, following Folin-Ciocalteu's colorimetric method, based on oxidation-reduction reaction method (Waterhouse, 2003). The sample (0.5 g) was homogenized in 10 times volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipetted out and the volume in each tube was made up to 3.0ml with distilled water. Folin-Ciocalteau's Reagent (0.5 ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer against a reagent.

RESULTS

Results of the present study clearly revealed the seasonal incidence and injurious status of *R. indica* on areca palms cultivated the Kozhikode and Malappuram districts of North Kerala. Population buildup of the mite was found initiated during April to May months of the years 2011-2013, in the field in which a rise in temperature was experienced. The mite population attained the peak level in April i.e. it approximately 100-120 active and inactive stages of mites per leaflet and subsequently showed a decline from the last week of May and this declining trend was continued till December. During the study, the lowest population density of the mite could be recorded during the months of October, November and December. The population density of the mite was high during the first week of March and continued up to the first week of May with the peak formation in April-May. A decline in population could be observed from June onwards. The number of mites was found to vary with respect to the leaflets of individual leaf frond. High degree of mite incidence was noted on the bottom frond leaflet when compared to the top and middle frond leaflets (Tables 9, 10). Very low population density of the mite could be recorded during the monsoon and winter seasons. In all examined cases, infestation of RPM was found confined to the lower surface of leaflets and often the number of mites recovered from a single leaflet ranged from 70-100 during the months of April and May (Tables 9, 10). All the life stages of the mite were predominantly red in color, while the adult females often exhibited dark colored areas on the body (Fig. 1 A). Feeding activity of large numbers of the various life stages of the mite imparted the development of localized yellow coloration on the leaf lamina (Figs 1 B-D). On progressive feeding, these yellow patches coalesced to form bronze colored areas, thereby leading to the drying up of leaves.

During heavy infestation (more than half of the leaf lamina showing chlorotic spots, yellow patches and greyish brown areas etc.), simultaneous and repeated feeding resulted in the formation of irregular greyish brown patches on the leaf surface. The symptoms could be easily perceived by the bronzed appearance of the leaves. Results of quantitative studies clearly established that infestation by *R. indica* on areca palms could induce the drastic alterations in chlorophyll a, b and total chlorophyll, proline, phenolics and total nitrogen. The amount of chlorophyll presents in the mite infested and uninfested areca leaf samples on estimation revealed a drastic decline in both 'a', 'b' and total chlorophyll pigments. The mean amounts of chlorophyll 'a', 'b' and total chlorophyll in the infested leaf samples recorded during the study were 0.47±0.01, 0.72±0.01 and 1.180±0.009 were mg/gm tissue respectively (Table 1). This showed that the mite infested leaf samples had a loss of 62.21±0.63% of Chlorophyll 'a' pigment when compared to the uninfested leaves of areca. The amount of chlorophyll 'b' pigments recorded in the mite infested and uninfested areca leaves were 1.71±0.02 and 0.72±0.01 mg respectively which showed a loss of 57.67±0.73% chlorophyll 'b' owing to infestation by RPM. The percent loss of total chlorophyll in mite infested leaf samples recorded during the study were 59.884±0.375% tissue, respectively (Figs 2-4).

Similar to chlorophyll 'a' and 'b' pigments, RPM infestation was also found to lead to loss in nitrogen content. The mite infestation was found to induce 51.92±0.72% loss of total nitrogen content in areca leaves (Table 2, Fig. 5). The amount of proline in the mite infested samples showed an increase than that of uninfested leaves (Table 3, Fig. 7). The mean proline content of mite infested leaf sample recorded during the study was about 3 times than that of the uninfested sample which could be accounted to 190.8%. Similar to proline content, the phenolic compound content of the areca leaves also showed an increase owing to infestation by the RPM. The uninfested and infested areca leaves contained 1.40±0.01 and 2.65±0.02 mg of phenolics/g tissue, respectively (Table 4,

Table 1. Quantitative difference in chlorophyll pigments induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

Chlorophyll	**S. No.**	**Chlorophyll (mg/g tissue)**		**Loss in chlorophyll**	**% chlorophyll loss**
		Uninfested	Infested		
Chlorophyll a	1	1.31	0.52	0.79	60.30
	2	1.23	0.45	0.78	63.41
	3	1.22	0.47	0.75	61.48
	4	1.22	0.46	0.76	62.30
	5	1.12	0.41	0.71	63.39
	6	1.41	0.40	1.01	71.63
	7	1.10	0.43	0.67	60.91
	8	1.08	0.56	0.52	48.15
	9	1.30	0.51	0.79	60.77
	10	1.49	0.45	1.04	69.80
Mean±SEM		1.25±0.01	0.47±0.01	0.78±0.02	62.21±0.63
Chlorophyll b	1	1.90	0.63	1.27	66.84
	2	1.69	0.68	1.01	59.62
	3	1.88	0.75	1.13	59.93
	4	1.67	0.73	0.94	56.56
	5	1.46	0.70	0.76	52.39
	6	1.69	0.73	0.96	56.88
	7	1.80	0.63	1.17	65.14
	8	1.80	0.64	1.16	64.58
	9	1.39	0.80	0.59	42.91
	10	1.85	0.89	0.96	51.83
Mean±SEM		1.71±0.02	0.72±0.01	1.00±0.02	57.67±0.73
Total Chlorophyll	1	3.201	1.145	2.055	64.210
	2	2.912	1.127	1.785	61.299
	3	3.091	1.221	1.870	60.500
	4	2.883	1.187	1.697	58.840
	5	2.581	1.106	1.475	57.137
	6	3.092	1.121	1.971	63.740
	7	2.885	1.056	1.830	63.412
	8	2.865	1.196	1.669	58.261
	9	2.689	1.299	1.390	51.691
	10	3.335	1.343	1.993	59.747
Mean±SEM		2.953±0.023	1.180±0.009	1.773±0.022	59.884±0.375

Fig. 6). The per cent increase in phenolic compound content due to *R. indica* was recorded as 91.18±1.77%. These data when subjected to statistical analysis (t-test) were found significant at 0.05 levels (Tables 5-8).

DISCUSSION

Areca palms are widely cultivated throughout Kerala and the present observation forms the first report on the incidence of Red Palm Mite on areca nut in Kozhikode and Malappuram districts of North Kerala (Prabheena and Ramani, 2014). The results of the present study enabled to record infestation by *R. indica* as one of the major problems in the areca nut plantations of North Kerala. Temperature is a main abiotic factor for poikilothermic insects (Parmesan, 2006) and changes in surrounding temperature regimes would certainly influence the development rates, voltinism and survival of insects and subsequently act upon size, density and genetic composition of populations, as well as the extent of host plant exploitation (Bale et al., 2002). In the present study also, temperature and relative humidity of the habitat were found to exert a profound influence in determining the population size of the red palm mite.

Rainfall generally exerts a negative impact on the population density of the insect and mite pests on various crops through mechanical action. A lowest rate of incidence was recorded for the spider mite pest, *Oligonychus ilicis* in rainy season (Pallini et al., 1992). Similarly, a decrease in the phytoseiid predatory mite population was recorded in citrus plantation as a function of rainfall (Reis et al., 2000). Results of the present study helped to confirm the above findings by recording a decline in RPM population in areca plantations under the influence of rainfall in Kerala, as the number of mites encountered during this

Table 2. Quantitative difference in nitrogen contents induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

S.No.	Nitrogen (mg/g tissue)		Nitrogen loss in mg	% loss
	Uninfested	Infested		
1	22.90	12.20	10.70	46.72
2	22.00	13.90	8.10	36.82
3	24.30	9.50	14.80	60.91
4	22.30	11.77	10.53	47.22
5	25.70	10.90	14.80	57.59
6	24.70	9.50	15.20	61.54
7	26.60	11.70	14.90	56.02
8	22.10	11.31	10.79	48.82
9	19.40	8.90	10.50	54.12
10	23.14	11.69	11.45	49.48
MEAN±SEM	23.31±0.20	11.14±0.14	12.18±0.24	51.92±0.72

Table 3. Quantitative difference in proline contents induced by the feeding activity of *R. indica* on *Areca catechu*.

S. No.	Free proline (mg/g tissue)		Raise in Proline	% Raise in Proline
	Uninfested	Infested		
1	0.483	0.986	0.503	104.14
2	0.576	1.407	0.831	144.27
3	0.345	1.048	0.703	203.77
4	0.379	1.310	0.931	245.65
5	0.428	1.441	1.013	236.68
6	0.462	1.172	0.71	153.68
7	0.317	1.124	0.807	254.57
8	0.297	1.324	1.027	345.79
9	0.359	0.959	0.6	167.13
10	0.510	1.290	0.78	152.94
Mean±SEM	0.416±0.01	1.21±0.02	0.791±0.02	190.8±7.10

Table 4. Quantitative difference in phenol content induced by the feeding activity of *Raoiella indica* on *Areca catechu*.

S. No.	Phenolic compounds (mg/g tissue)		Raise in Phenolics	% Raise in Phenolics
	Uninfested	Infested		
1	1.446	2.768	1.322	91.42
2	1.353	2.772	1.419	104.88
3	1.195	2.348	1.153	103.00
4	1.465	2.605	1.141	77.82
5	1.473	2.336	0.863	58.59
6	1.295	2.754	1.459	112.66
7	1.349	2.625	1.276	94.59
8	1.281	2.601	1.320	103.04
9	1.488	2.490	1.002	67.34
10	1.614	3.192	1.578	97.77
Mean±SEM	1.40±0.01	2.65±0.02	1.25±0.02	91.18±1.77

Table 5. Statistical analysis using t-test – Chlorophyll.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df	
									Lower	Upper
Chlorophyll a	Equal Variance assumed	6.210	.023	17.352	18	.000	.78200	.04507	.68732	.87668
	Equal Variance not assumed			17.352	11.543	.000	.78200	.04507	.68337	.88063
Chlorophyll b	Equal Variance assumed	3.92	.061	16.458	18	.000	.99500	.06046	.86798	1.12202
	Equal Variance not assumed			16.458	12.893	.000	.99500	.06046	.86428	1.1257
Total Chlorophyll	Equal Variance assumed	6.707	.018	22.811	18	.000	1.773	.07774	1.6099	1.9366
	Equal Variance not assumed			22.811	11.646	.000	1.773	.0774	1.6033	1.9432

Table 6. Statistical analysis using t-test – Nitrogen.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	T	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df	
									Lower	Upper
Nitrogen	Equal Varriance assumed	.936	.346	14.993	18	.000	12.17700	.81221	10.4706	13.883
	Equal Varriance not assumed			14.993	16.344	.000	12.17700	.81221	10.4581	13.896

Table 7. Statistical analysis using t-test – Proline.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df	
									Lower	Upper
Proline	Equal Varriance assumed	7.037	.016	-12.77	18	.000	-.79050	.06187	-.92048	-.66052
	Equal Varriance not assumed			-12.77	13.636	.000	-.79050	.06187	-.92352	-.65748

Table 8. Statistical analysis using t-test – Phenol.

		Levene's Test for Equality of Variances		t-test for Equality means						
		F	Sig	t	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	95% Confidence Interval of the df Lower	Upper
Phenol	Equal Varriance assumed	1.944	.180	-14.303	18	.000	-1.25320	.08762	-1.4373	-1.0692
	Equal Varriance not assumed			-14.303	13.198	.000	-1.25320	.08762	-1.4422	-1.06420

Table 9. Sesonal population density of *Raoiella indica* on areca palms of Kozhikode and Malappuram districts in 2011-2012.

Month of sampling 2011 April – 2012 March	Average Temp (°C)/ Average RH (%)	Mean number of different life stages of *Raoiella indica* mites/leaf let								
		Bottom frond			Middle frond			Top frond		
		Egg	Immature stages	Adult	Egg	Immature stages	Adult	Egg	Immature stages	Adult
April	29/70	48	28	26	49.8	30	28.8	54	33	27.6
May	34/65	33	18	9	15.6	11.4	12	31.8	8.34	11.4
June	29/75	12.6	12	8.4	9.6	12	12.6	12	12.9	10.8
July	27/80	3	2.4	3	2.4	1.5	3	3.9	3.6	4.2
August	27/85	6	1.2	.6	.3	.3	.6	1.2	.6	1.5
September	28/80	1	2	1	3	1	1	1	1	1
October	27/85	2	1	2	1	.5	1	1	2	1
November	26/85	.5	1	1	.5	1	.5	.5	1	1
December	25/85	2	2	5	1	2.5	2	1.5	3	2.5
January	29/80	16	12	12	8	8	6	12	10	4
February	28/75	14	20	20	20	18	16	18	12	10
March	29/75	33	18.6	13.5	30	20.4	18.9	21.6	12	11.4

season was comparatively low, 5-10 active life stages of the mites per leaflet.

The population density of the RPM could be observed as the maximum during April-May and then it followed a declining trend to reach moderate and scanty levels during November-March and June-October periods respectively. This is in accordance with the earlier reports (Yadavbabu and Manjunatha, 2007) on *R. indica* which showed the peak population of the mite from March - to the first week of May and then a decline in population from June onwards. Feeding activity of the RPM was found to induce development of localized yellow patches on the leaflets of areca palm. Stunted growth and withering of leaves in RPM infested palms were already reported in South Indian conditions (Puttarudraiah and Channabasavanna, 1956).

Apart from the physical damage, the feeding activity of *R. indica* was found to induce alterations in the biochemical constituents of the host plant, *A. catechu*. Among plants, chlorophyll pigments are responsible for absorbing light energy from the sun and therefore, chlorophyll content

Table 10. Sesonal population density of *Raoiella indica* on areca palms of Kozhikode and Malappuram districts in 2012-2013.

Month of sampling 2012 April – 2013 March	Average Temp (°C)/Average RH (%)	Mean number of different life stages of *Raoiella indica* mites/leaf let								
		Bottom frond			**Middle frond**			**Top frond**		
		Egg	**Immature stages**	**Adult**	**Egg**	**Immature stages**	**Adult**	**Egg**	**Immature stages**	**Adult**
April	30/75	58.8	39.9	29.1	57.9	34.2	32.4	60.9	33.3	32.7
May	34/65	36	22.5	12.6	33.3	24.6	15	34.5	19.2	15.6
June	28/70	16.5	15	14.7	15.9	13.5	17.1	14.4	15.6	13.2
July	27/75	5.1	3.6	5.4	6.3	4.5	8.1	4.8	6.6	4.5
August	27/80	2.7	3.3	2.1	3	2.1	2.4	8.4	2.4	2.1
September	30/75	4	3	1	2	2	1	1	2	1
October	28/80	1.5	2	2.5	1.5	2	2.5	1	3	1
November	27/85	1	1	2.5	1	1.5	1	1	1.5	2
December	25/85	2	3	3	1.5	3	2	2	2.5	4
January	27/80	12	15	8	10	8	8	10	14	6
February	29/80	20	16	12	14	12	10	18	10	10
March	29/78	37.8	24	20.4	30.3	28.5	23.4	30.6	28.5	17.7

is considered as a key experimental parameter in plant biology and agronomy (Lamb et al., 2012). Feeding activity of RPM caused significant reduction in the chlorophyll contents of their host plants thereby supporting earlier findings (Ghoshal, 2013; Prabheena and Ramani, 2013).

Nitrogen is one of the vital elements in plants and which plays a key role in chlorophyll production and forms part of the various proteins that have major roles in many metabolic processes associated with plant growth (Sinfield et al., 2010). Leaves of areca plants infested with *R. indica* disclosed significant reduction in the total nitrogen content. Similar results were reported by earlier workers (Ghoshal et al., 2005) also by accounting the percent loss of nitrogen in *Corchorus capsularis* L. (Malvaceae) owing to feeding by *Polyphagotarsonemus latus*.

The amino acid, proline is believed to be a compatible solute and the accumulation of proline was noticed in plants, produced when unfavourable conditions prevailed (Aspinall and Paleg, 1981). During the microbial infection, the proline contents of certain plants become raised many folds in sensitive and resistant cultivators (Raj et al., 1983; Gupta, 2001). In the present investigation, the amount of proline was found increased significantly on the host plant of RPM thereby supporting the earlier records on the impact of mite feeding on tomato (Kielkiewicz, 2005) and bean (Farouk and Osman, 2012). Phenolic compound plays an important role in plant defence mechanism and an increased concentration of phenolics was observed in plants infested with fungi (Senthil et al., 2010). In the present study, results of phenol estimation in mite infested and control leaves of various host plants clearly revealed the elevation of phenol concentration in the mite infested leaves.

The results of the study disclosed that the temperature and relative humidity of the habitat exert a major role in determining the population size of the *R. indica*. The heavy loss of biochemical components such as chlorophyll contents and nitrogen as evidenced by the present study revealed the potential of *R. indica* to affect adversely the general health of the host plant *A. catechu*, thereby reduction in the growth rate of the host plant. This study enabled to record *R. indica* as one of the major mite species infesting areca nut plantations of Kerala, and which warrants the urgent attention to formulate appropriate regulatory measures to be adopted to check the population rise of this notorious pest and safeguard the areca plantations of Kerala in future.

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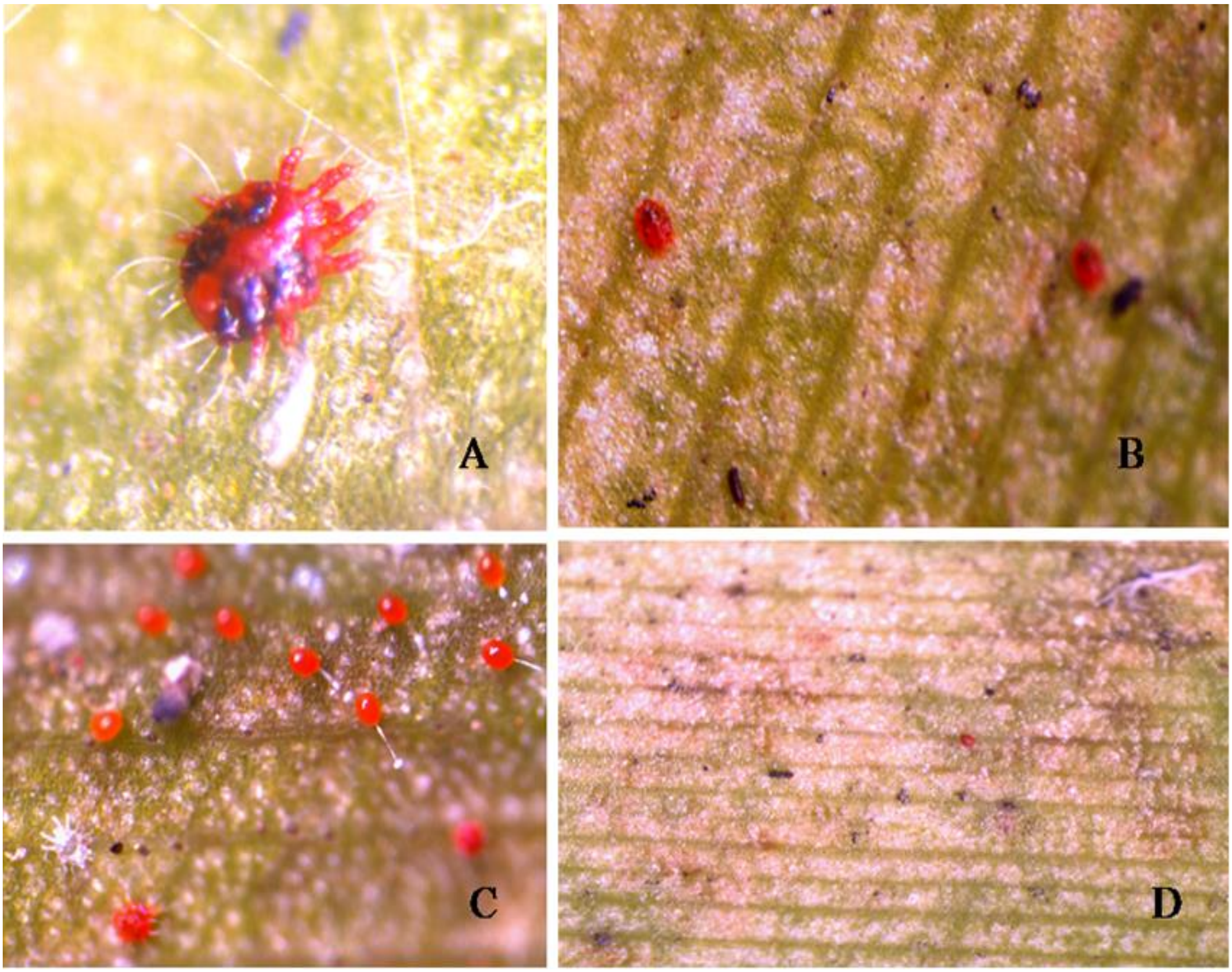

Figure 1. A) Adult female of *Raoiella indica* on areca leaf, B) Infested leaf lamina with *R. indica*, C) Eggs and larva of *R. indica*, D) Heavily infested leaf.

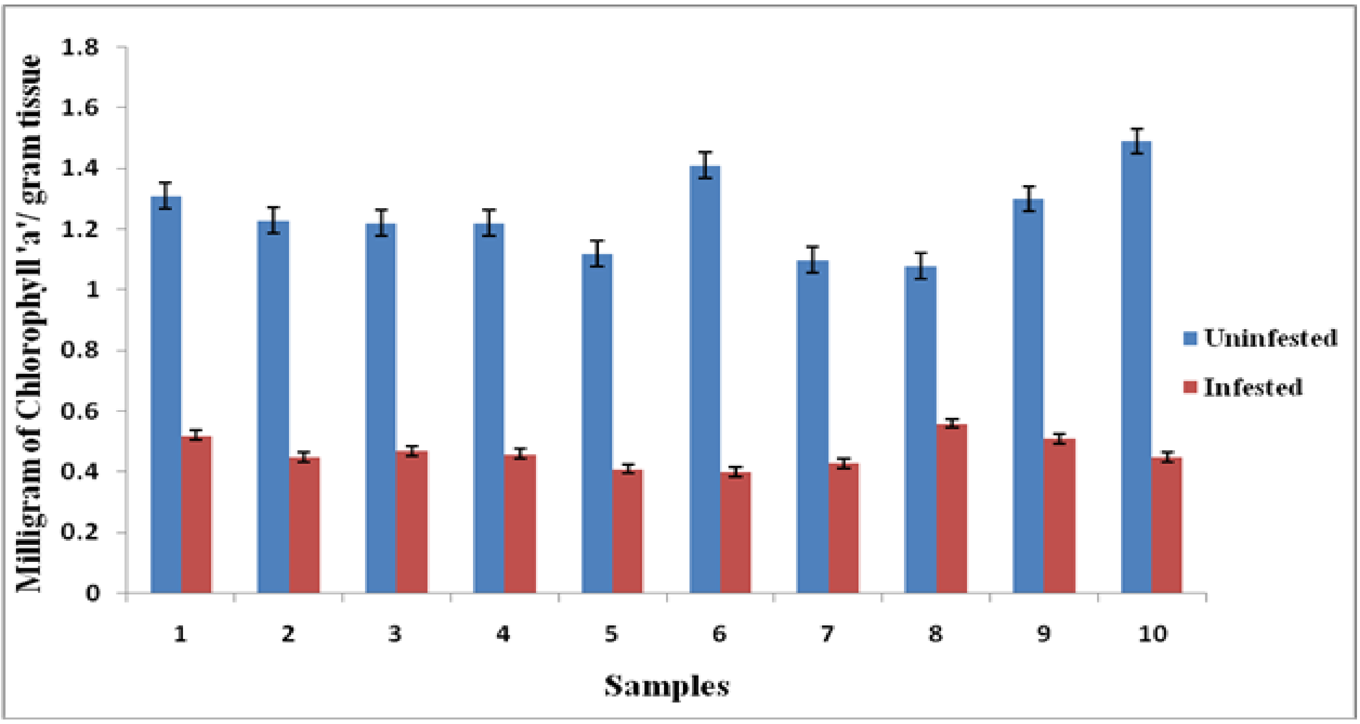

Figure 2. Amount of chlorophyll ‘a’ in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

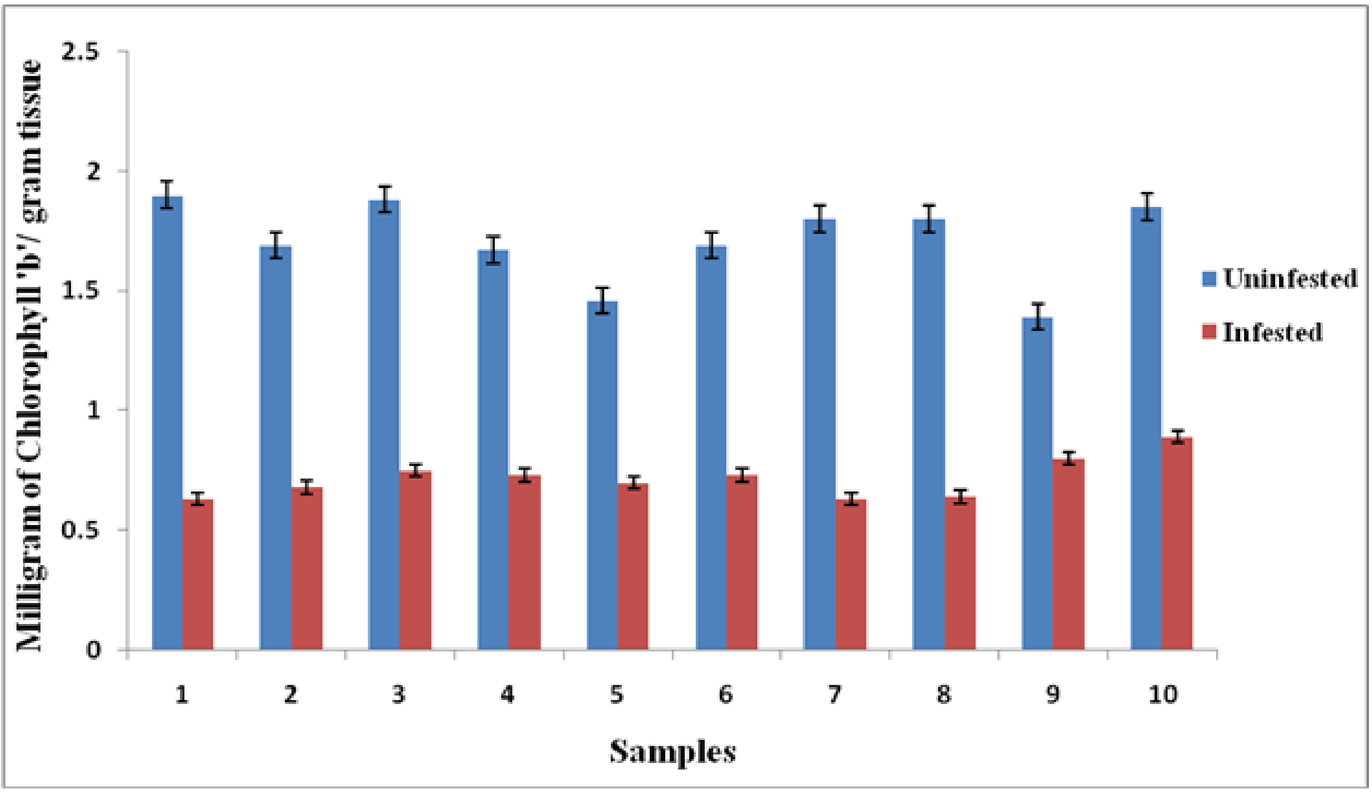

Figure 3. Amount of chlorophyll 'b' in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

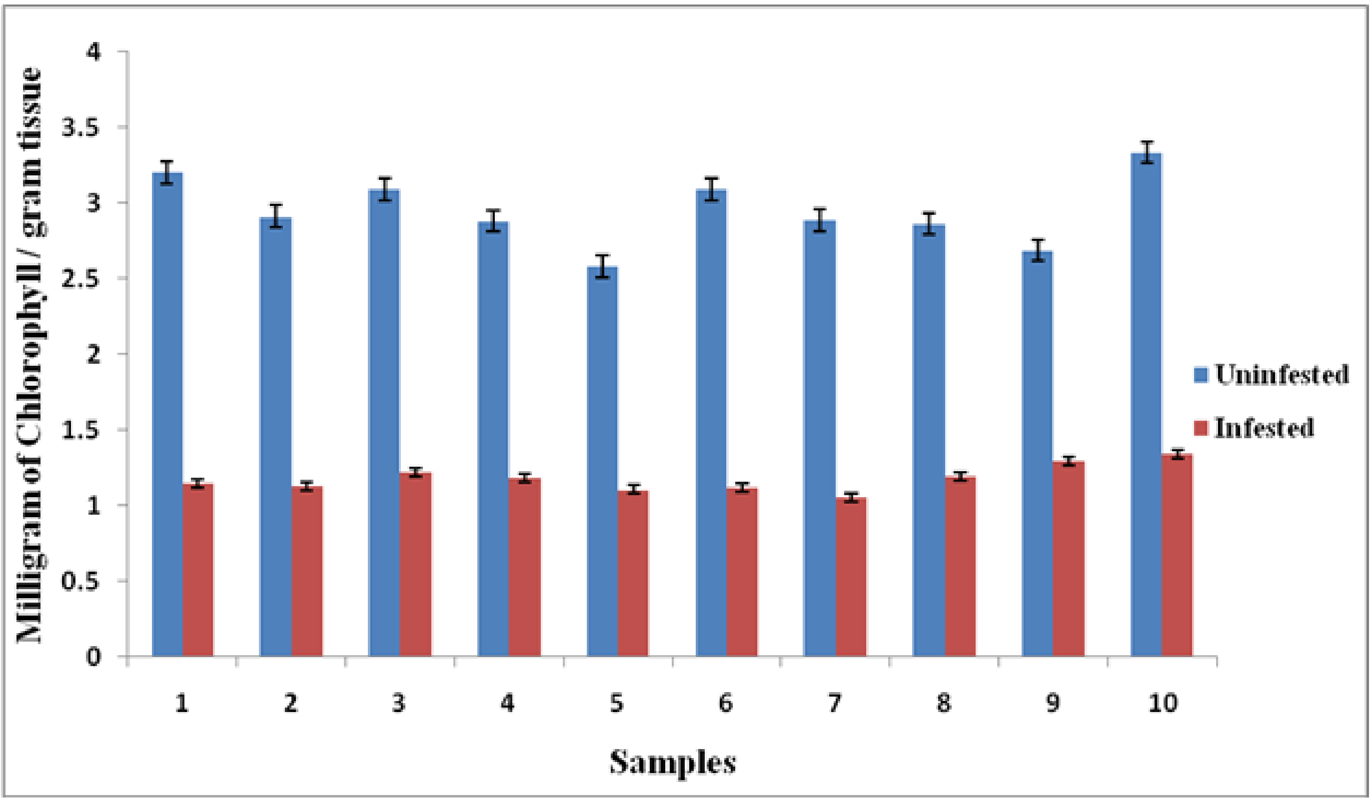

Figure 4. Amount of total chlorophyll in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

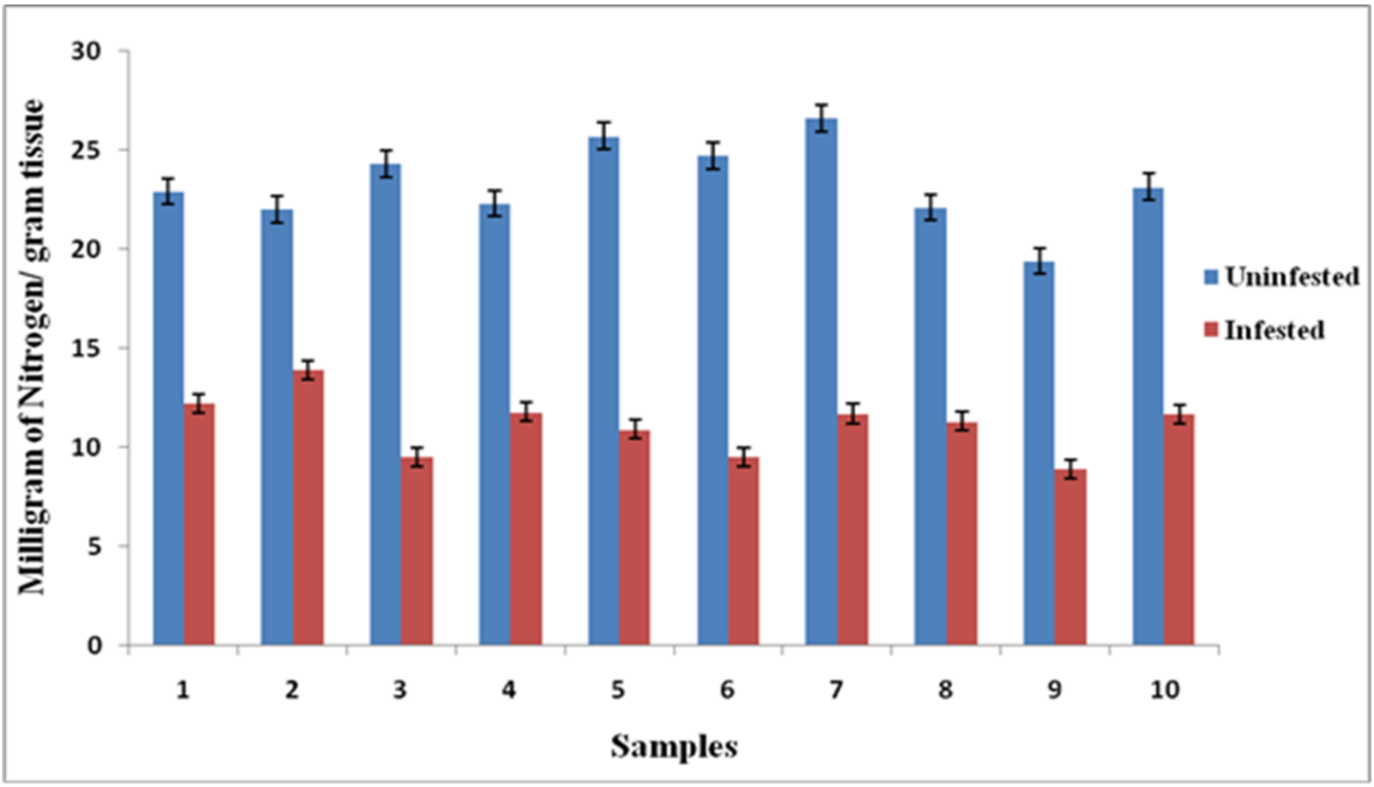

Figure 5. Amount of nitrogen in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

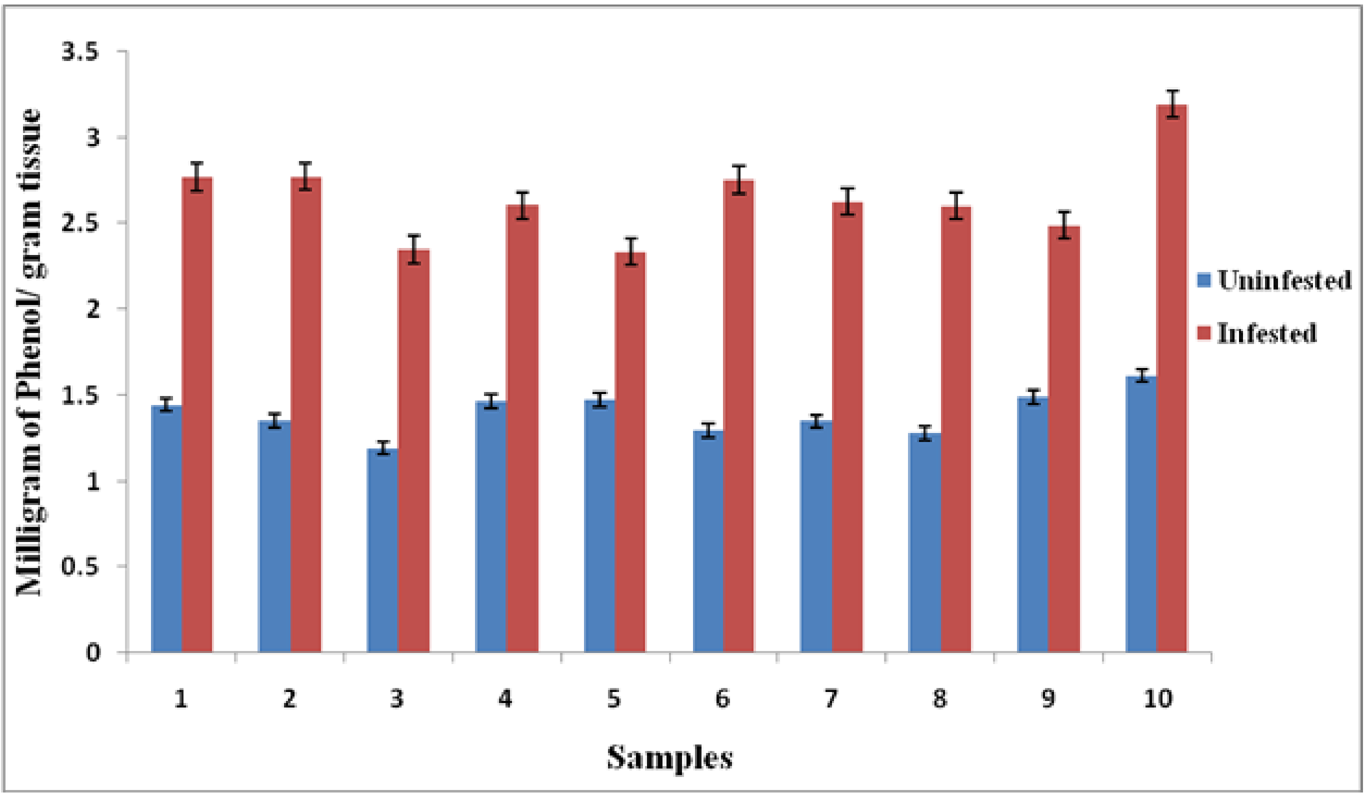

Figure 6. Amount of phenol in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

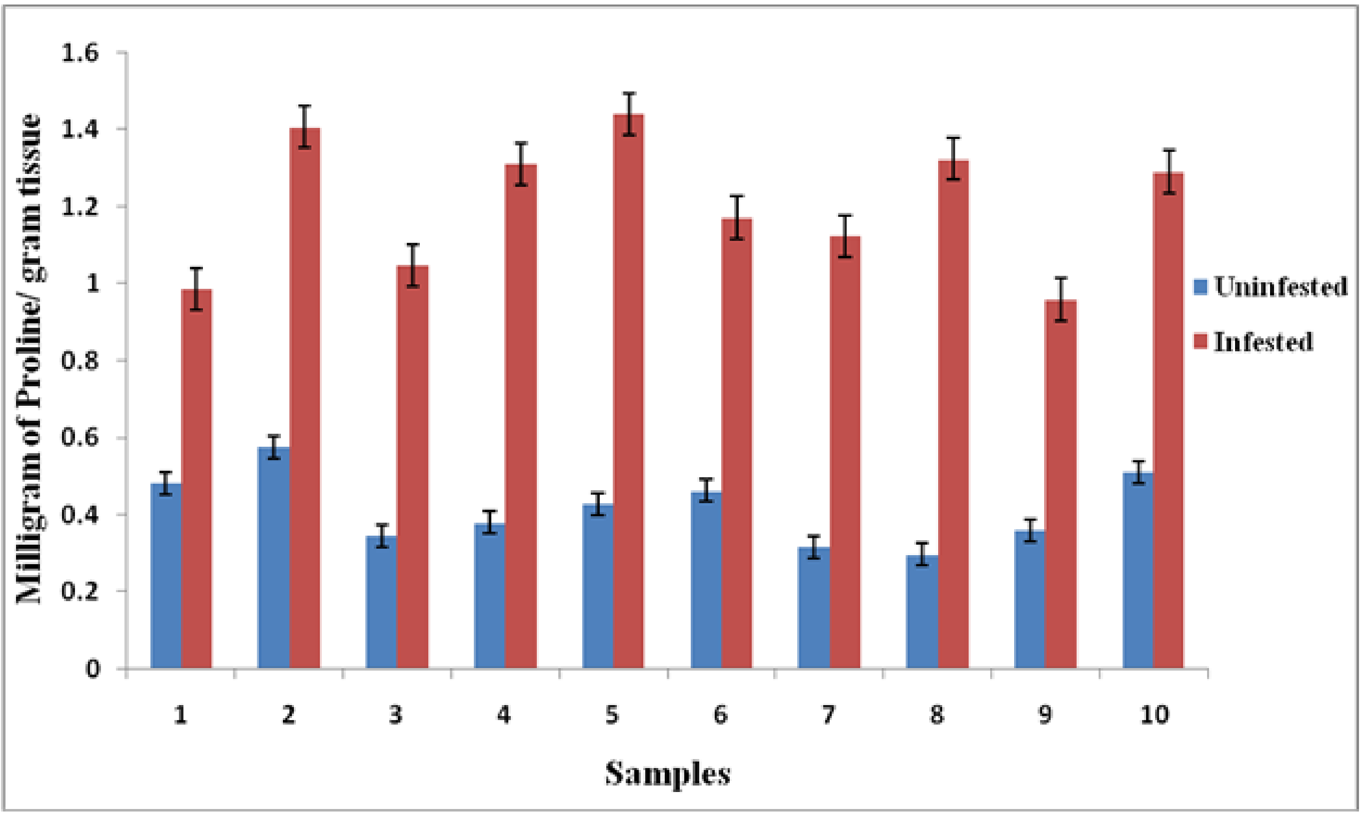

Figure 7. Amount of proline in *Raoiella indica* infested and uninfested *Areca catechu* leaf sample.

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Feeding impact of *Cisaberoptus kenyae* Keifer (Acari: Eriophyidae) on photosynthetic efficiency and biochemical parameters of *Mangifera indica* L.

Ramani NERAVATHU

Division of Acarology, Department of Zoology, University of Calicut, Kerala, India
e-mail: drnramani@gmail.com

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ABSTRACT: *Cisaberoptus kenyae* Keifer, the mango leaf coating mite is a widely distributed eriophyid species in the tropics, infesting all varieties of mango trees. The mite produces a white colored leaf coating on the adaxial surface of mango leaves, which extends to the entire leaf lamina in severely infested leaves. The present paper discusses the feeding impact of the mite on the photosynthetic pigments, and other biochemical parameters of mango leaves. Biochemical studies enabled to record a significant decrease in the chlorophyll and carotenoid pigments in mite infested mango leaves when compared to the uninfested leaves. The percentage loss in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was observed to be 65.80-84.64, 76.57-95.29, 68.69-87.13 and 39.31-56.75 respectively. Analysis of photosynthetic efficiency by measuring chlorophyll fluorescence (F_v/F_m) using the Handy PEA Hansatech Instrument pt. Ltd, UK revealed a decreased value, falling in the range of 25.15-28.43%. Mite infestation induced a significant loss in total carbohydrate also (57.86-80.58%) when estimated through Anthrone's method. Contrary to the above, a significant increase was observed in the proline and total phenol concentrations in mite infested leaves. An increase from 2.9578±0.36 to 5.2611± 0.61 µg/g was observed in the concentration of proline in mite infested leaf tissue. Total phenol content showed an increase from 55.14± 1.72 to 81.16± 0.85 mg/g in mite infested leaves. Results showed a statistical significance ($p<0.05$), confirming that infestation by *C. kenyae* induced severe stress, leading to enhanced production of defense compounds like proline and total phenol, thereby adversely affecting the photosynthetic efficiency and primary metabolite production of the host, *M. indica*.

Keywords: Carotenoids, chlorophyll, *Cisaberoptus kenyae*, *Mangifera indica*, proline.

INTRODUCTION

Eriophyids represent a highly host specific group of phytophagous mites, infesting almost all categories of economic crops and inducing diverse forms of plant abnormalities. A good number of these mites are designated as notorious pests of fruit crops. *Mangifera indica* is prone to attack by a variety of pests, comprised mainly of insects and mites. Of the major group of mite pests attacking mango foliage, *Cisaberoptus kenyae* of the family Aberoptidae has been recognized as a common species which induces a prominent leaf coating on mango leaves (Keifer, 1966). The species is commonly called as the mango mite of the tropics and it enjoys a wide distribution in all mango cultivated regions. The mite initiates the leaf coating as a white powdery substance secreted at the leaf petiole and which gradually extends as silken strands across the laminar area on the adaxial surface. When fully formed, the leaf coating appears as an ashy white colored membrane on the upper surface and in severely infested leaves, the entire leaf lamina is covered by the membrane. Under high magnification, the membrane appears as a web formed by crude silken strands. The mite lives gregariously under the web which affords protection to its various life stages viz. the egg, nymph I, quiescent stage I, nymph II, quiescent stage II, adult male and adult females (protogyne and deutogyne).

Cisaberoptus kenyae was known as a silver blotch mite (Sternlicht and Goldenberg, 1976), it causes damage in the epidermal tissue that leads to necrosis and subsequent secretion of a milky white substance from the midrib or veins. The leaf coating produced by the mite was found to cause leaf decline, but without inducing any mechanical damage (Hassan and Keifer, 1978). Mite infestations were found relatively higher on majority of the mango trees in India, especially in South India where all varieties showed the presence of leaf coating. The level of infestation and population density of *C. kenyae* had a direct correlation with the varietal difference of mango trees as evidenced through population studies carried out in Kerala on five mango varieties (Ramani and Haq, 1989). Detailed studies were undertaken on the incidence of the mite and its natural enemies (Ramani, 1991). Developmental parameters of the mite were also elucidated on mango leaves from Kerala (Ramani and Haq, 1991). Studies on monthly fluctuations in the population density of the mite, susceptibility of common varieties of mangoes to mite infestation as well as per cent of leaf area coated by the mite on different mango varieties etc. were performed in North India at Varanasi, Uttar Pradesh (Rai et al., 1993). Investigation on seasonal incidence and control measures using chemical and botanical acaricides against *C. kenyae* infesting mango cultivars such as Neelum, Bangalora, Malgoa, Rumani and Sappattai was carried out in Tamil Nadu, South India (Umapathy and Rajendran, 1999). Population studies of the species were carried out on mango trees in Egypt also, for a period of two years (Al-Azzazy, 2005). Detailed studies were undertaken on the seasonal distribution pattern and population density of the mite on two mango varieties such as Hindi and Alphonso cultivars in Egypt (Abou-Awad et al., 2009), the authors collected data also on the control aspects of the mite employing natural enemies like the predatory mites as well as acaricides. The effect of feeding activity of the mite on the macro and micronutrient levels of mango leaves was studied also in Egypt (Abou-Awad et al., 2012).

A present study was undertaken to gather knowledge on the extent of damage induced by the mite on the physiological parameters of the host, such as the cellular damages, photosynthetic pigment concentration and photosynthetic efficiency, and other biochemical components like total carbohydrate, phenol and proline contents.

MATERIAL AND METHODS

Collection of leaf samples for biochemical estimation

Leaf samples required for biochemical estimation were collected randomly from the mango variety, 'Rumani' cultivated in the premises of the Department of Zoology, University of Calicut. Both the control and experimental leaves were collected from the same host plant. The leaves were collected randomly from different heights of the tree, the uninfested leaves were treated as control while mite infested leaves showing the presence of leaf coating and harboring a population range of 88-144 mites/ cm^2 area were considered for the experiments.

Qualitative estimation of feeding damage

Assessment of visual damage symptoms

Visible symptoms of damage induced by the mite on the foliage of the mango variety, *M. indica rumani* were traced based on the presence, nature and extent of leaf coating and other associated symptoms developed on the leaves.

Elucidation of cellular levels of leaf damage

Anatomical features of uninfested and mite infested leaves of *M. indica* were studied by examining stained sections and comparing the cellular characteristics of both samples. For making sections of uninfested and infested leaf tissues, the leaf samples collected from the host plant were brought to the laboratory and thoroughly cleaned with distilled water for further processing. Thin sections of mite infested and uninfested leaves were made using a sharp blade, then were placed in separate cavity blocks. Sections of both leaf samples were dehydrated in alcohol series and stained in safranin. After proper staining, the sections were destined in distilled water and then on microscope slides using glycerin. The mounted slides were examined using a microscope to observe the cellular features for making a ready comparison of the cellular damage induced by the mite. Appropriate photographs were taken using a microscope (Labomed Lx 400) and presented.

Quantitative estimation of damage

Estimation of photosynthetic pigments

Concentration of photosynthetic pigments like chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids present in the uninfested and mite infested leaf tissues was estimated using the method of Arnon (1949) and Coombs et al. (1985). One gram of the fresh leaf tissue, representing the uninfested and mite infested tissues separately, was cut into small pieces and placed into separate specimen bottles (for control and experimental samples) containing 10 ml of absolute ethanol and stored in the dark for two weeks. One ml of the filtered extract was then diluted with 6 ml of absolute ethanol and the absorbance of the chlorophyll solution was measured using a UV-spectrophotometer (Shimadzu) at 645, 663, and 470 nm (at 750 nm also for making correction for impurities) against the solvent blank (absolute ethanol). The amounts of chlorophylls and carotenoids present in the mite infested and uninfested leaf samples were calculated and expressed in mg/g fresh weight of the leaf tissue, using the formula of Arnon (1949), as given below. The experiment was repeated 9 times to get concordant results. The data were statistically analysed using SPSS Statistics (IBM version 19) and the values were expressed as Mean ± SEM.

- mg chlorophyll a = $[12.7\ (A663- A750) - 2.69\ (A645- A750)] \times V/\ (W\times1000)$
- mg chlorophyll b = $[22.9\ (A645- A750) - 4.68\ (A663- A750)] \times V/\ (W\times1000)$
- mg total chlorophyll = $[20.2\ (A645- A750) + 8.02\ (A663A750)] \times V/\ (W\times1000)$
- mg carotenoids = $[1000\ (A470) + 3.27\ \{(\text{chlorophyll a}) - (\text{chlorophyll b})\}] \times V/\ (W\times229\times1000)$
- Where A is the absorbance, V is the volume and W is the fresh weight of tissue extracted (g).
- Based on the above equations, calculations were made and the data were presented in µg/g tissue.

Measurement of photosynthetic efficiency

Photosynthetic efficiency of the mite infested and uninfested mango leaves was analysed with the help of a portable fluorescence monitoring system (Handy PEA, Hansatech Ltd., Vorfolk, UK) by measuring chlorophyll fluorescence. Prior to the fluorescence measurements, a circular surface of the upper face of the leaves of both the control and experimental categories was dark adapted for 15-20 minutes using the dark adaptation clips. Data on general parameters like F_0 (minimum/initial fluorescence), Fm (maximum fluorescence), Fv (variable fluorescence) etc. were recorded separately for uninfested and mite infested leaves. The values of Fv/Fm (where $Fv = Fm - F_0$), a parameter commonly known as maximum quantum yield of primary photochemistry or maximal electron transport rate (ETR) of PS II of both uninfested and infested leaves were recorded separately. The data were statistically analysed using independent-sample's T-test, following SPSS version 16.0.

Estimation of total carbohydrates

The amount of total carbohydrates present in mite infested and uninfested mango leaf samples was estimated using Anthrone's method (Hedge and Hofreiter, 1962). The leaf samples were thoroughly cleaned, a 500 mg of each leaf sample (control and experimental separately) was homogenized with 5 ml of 2.5N HCl. The homogenate was then kept in hot water bath for about 3 hours and then each sample was made up to 25 ml and centrifuged at 2000 rpm for about 20 minutes. 0.1 ml of each supernatant was taken in a test tube and made up to 1.0 ml with distilled water. To each test tube, 4.0 ml of cold Anthrone's reagent was added and heated for 8 minutes and then cooled to room temperature. The dark green color developed was read in a UV-spectrophotometer at 630 nm against glucose as standard. The amount of total carbohydrates present in each leaf sample was calculated separately for mite infested and uninfested samples following the equation given below:

$$\text{Amount of carbohydrates present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of the test sample}} \times 100$$

Estimation of total phenol

The concentration of total phenol present in mite infested and uninfested mango leaves was estimated using the method of Malick and Singh (1980). 1.0 g each of thoroughly cleaned leaf sample was ground in a mortar with 10 ml of 80% ethanol and then was centrifuged at 10,000 rpm. To the residue, 5.0 ml of 80% ethanol was added, ground and centrifuged. The supernatant was evaporated to dryness and to the dried residue, 5.0 ml of distilled water was added and mixed well. From the above solution, 1.0 ml was pipetted out and to which 2.0 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added. To the above mixture, 20% solution of sodium carbonate was added and kept in a water bath for 1 minute, cooled to room temperature and then the OD of the solution was read at 650 nm in a UV-spectrophotometer against tannic acid used as standard for the reaction. The total phenol content of the leaves was estimated following the equation given below and expressed in µg phenol/g sample.

$$\text{Concentration of sample} = \frac{\text{Standard concentration} \times \text{Sample OD}}{\text{OD of standard}} \times \frac{5 \times 1}{1\ \text{gm}}$$

Estimation of proline

The feeding impact of *C. kenyae* on the production of stress amino acids, like proline in mango leaves, was assessed using the method of Bates et al. (1973). The experiment was conducted by homogenizing 0.5 g of thoroughly washed leaf samples (mite infested and uninfested samples separately) in 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through a glass fibre filter and to 2.0 ml of the filtrate, 2.0 ml of glacial acetic acid and 2.0 ml of acid ninhydrin reagent were added. The solution was heated for one hour in a hot water bath and the reaction was terminated by keeping the test tubes in an ice bath for 5 minutes. To each of the ice-cold test tube, 4.0 ml of toluene was added and stirred well for 20 seconds. The separated toluene layer was taken out and read at 520 nm in a UV- spectrophotometer (Shimadzu). The concentration of proline was determined from the standard curve and calculations were made following the equation given below and expressed on a fresh weight basis.

$$\mu\text{g moles per gm tissue} = \frac{\mu\text{g proline ml} \times \text{ml toluene}}{115.5} \times \frac{5}{\text{gm sample}}$$

RESULTS

Results of field observations revealed infestation by *C. kenyae* on almost the mango trees grown/cultivated in the Calicut University Campus and adjacent localities, as evidenced through the presence of ashy-white coating developed on the upper surface of leaf lamina (Figs 1A,B). The newly sprouted leaves were found devoid of mite infestation. The mite was found to induce the development of a white powdery substance, initially on the leaf petioles of *M. indica*. Subsequently, with the population growth and progressive feeding activity of the life stages of the mite, the infested leaves developed ashy –white colored silken strands which gradually became hardened as a silver colored membrane, covering the entire leaf lamina including the mid rib and veins (Fig. 1C). All life stages of the mite such as the egg, nymphal stages, quiescent stages and adult (male and females) were found to enjoy a secluded habitat underneath the leaf coating (Figs 1D-F) and the areas covered by the leaf coating turned into brown-black colored. All the active life stages sucked out the plant sap, resulting in the color change, loss of vigor, browning, blackening, drying up and defoliation. Bronzing and necrosis were also common on the adaxial leaf surface where the foliar surface completely turned into black colored and quite often became dry.

The population density of the mite showed variation and the maximum population size was evident during the summer months. During the present study, leaf samples with a population density of 88-144 mites/cm^2 were considered for biochemical estimations. Heavy mite infestation was common during the summer months. However, during monsoon season, the leaf coatings were very closely adhered to the leaf surface and relatively low populations of *C. kenyae* were observed on wet leaves. Severely infested leaves appeared brown-black colored, especially under the leaf coating, which often turned to dried patches along the mid rib and side veins.

Results of histological studies disclosed reduced number of chloroplasts in the palisade and spongy tissues of the mesophyll in mite infested leaves. The cell size in the spongy layer of mesophyll tissue was also found reduced. The control leaf showed tightly packed, elongate cells with dense chloroplast in the palisade layer (Figs 2A-C), while the mite infested leaf showed loosely arranged and less elongate palisade cells with scanty amount of chloroplast or quite often with a total absence of chloroplast also. The cuticle and upper epidermis of mite infested leaf were disrupted occasionally, and the underlying palisade

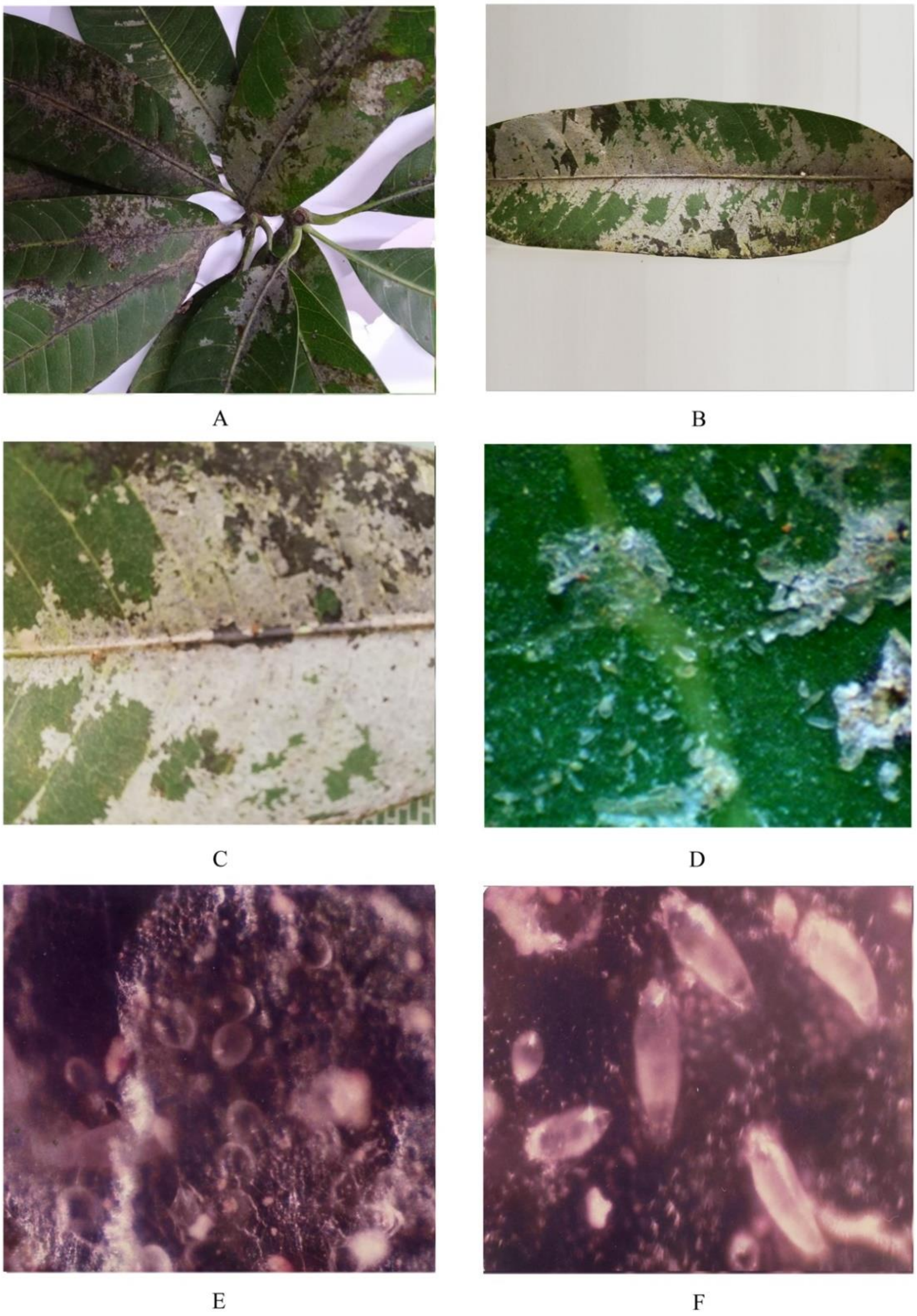

Figure 1. A) Twig of *Mangifera indica* L. showing infestation by *Cisaberoptus kenyae* Keifer, B) A single leaf showing the ashy white coating, C) Damage on the adaxial surface including midrib, D) A colony of *Cisaberoptus kenyae*, showing the different life stages exposed after removing leaf coating, E) An enlarged view of eggs laid under the leaf coating, F) Enlarged view of different life stages.

tissue also presented brown colorations and symptoms of drying up of leaves (Fig. 2D). Spongy layer of mesophyll showed very low amount of chloroplast and often appeared shrunken and irregular with larger intercellular spaces (Figs 2E,F).

Results of biochemical estimation of photosynthetic pigments revealed a significant reduction ($p<0.05$) in the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid pigments in mite infested mango leaves. As presented in Table 1, the mean concentrations of chlorophyll a, b and total carotenoids in mite infested mango leaves were 872.017 μg/g, 438.89 μg/g and 1426.66 μg/g respectively when compared to 3519.07 μg/g, 3119.79 μg/g and 6456.65 μg/g of the uninfested mango leaves. Thus, the percentage loss was 75.22±9.42, 85.93±9.36 and 77.91±9.22 in chlorophyll a, chlorophyll b and total chlorophyll respectively (Table 1), which showed a significant ($p<0.05$). Similarly, a significant reduction ($p<0.05$) in the carotenoid pigments also was recorded during the study which was accounted to 2125.72 μg/g in mite infested leaves against 4090.62 μg/g in uninfested leaves (Table 1), thereby resulting in a percentage loss of 48.03 ±8.72 ($p<0.05$) (Table 1).

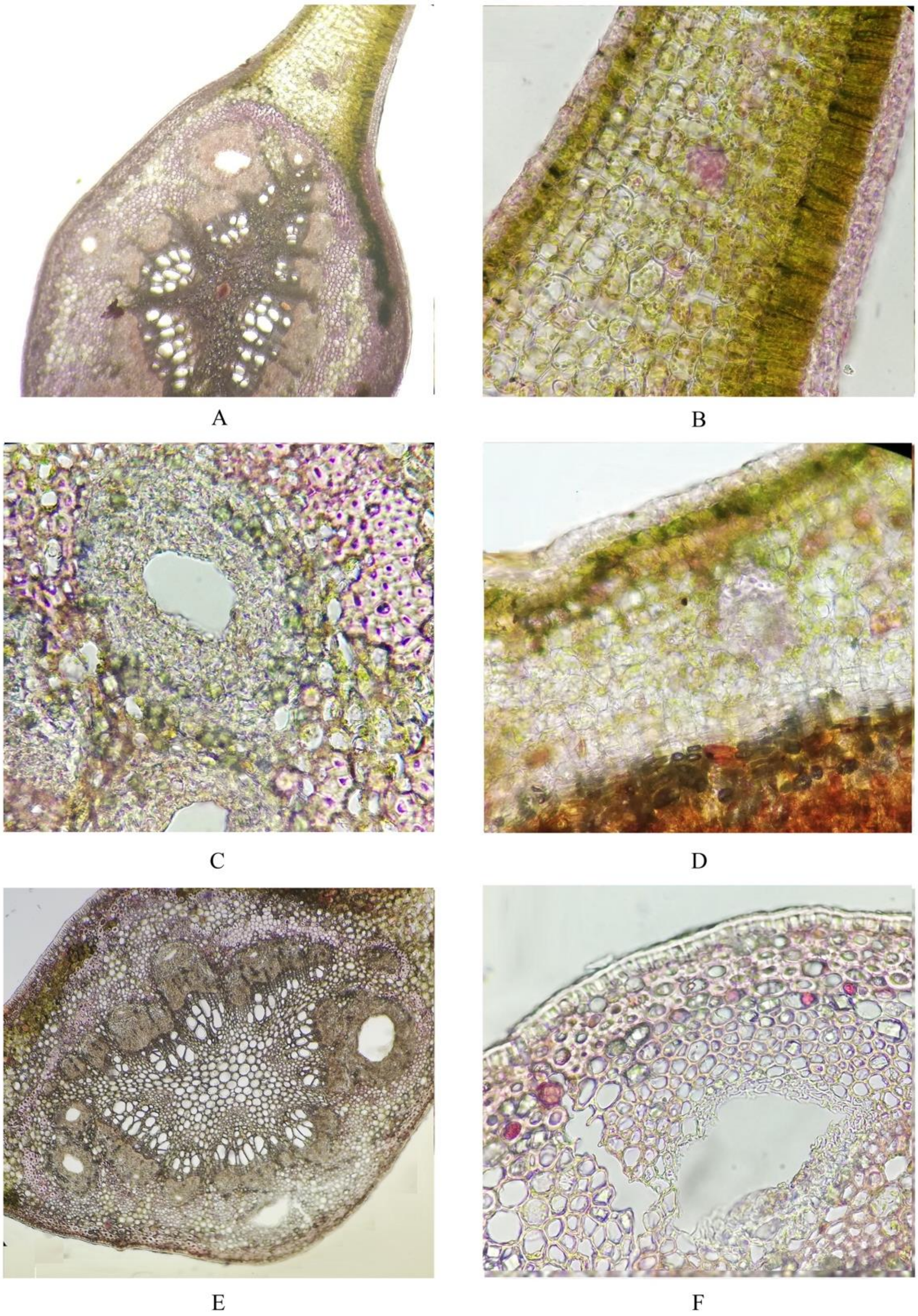

Figure 2. A-C) Uninfested mango leaves showing the normal densely packed, chloroplast rich palisade tissue, spongy mesophyll and vascular bundles, D-F) Mite infested mango leaves showing drying symptoms on cuticle, epidermis and palisade tissue, reduced number of chloroplast, irregular & shrunken spongy mesophyll tissue with larger intercellular spaces.

When the leaf chlorophyll fluorescence parameters such as F_0 (minimum/initial fluorescence), Fm (maximum fluorescence), Fv (variable fluorescence) etc, which were measured separately for uninfested and mite infested mango leaves, a reduction was observed in the Fv/Fm values in mite infested leaves (0.6188±0.0133) when compared to those of uninfested leaves (0.8457±0.012) (Table 2). The percentage loss in chlorophyll fluorescence was recorded as 25.15-28.43 (Table 2) owing to infestation by *C. kenyae*.

A significant reduction ($p<0.05$) in total carbohydrate was also observed during the study in mite infested mango leaves. The mean concentration of total carbohydrate in uninfested leaves was 85.48±0.88 mg/g while it was 26.31 ±0.87mg/g. (Table 3) in mite infested leaves, which could account for a mean percentage loss of 69. 22±11.36. Unlike the depleting effect on various biochemical parameters like the photosynthetic pigments and total carbohydrate, feeding activity of *C. kenyae* enhanced production of stress factors like proline and phenolic compounds as observed during the study (Table 3). Mite infested leaves

Table 1. Changes in the concentration of photosynthetic pigments (µg/gm tissue) in the leaves of *Mangifera indica* L. due to feeding impact of *Cisaberoptus kenyae* K. (Mean ±SEM)*

Photosynthetic pigments	Concentration in uninfested mango leaf tissue (µg/g)	Concentration in mite infested mango leaf tissue (µg/g)	Loss in concentration of pigments (µg/g)	Per cent loss of pigments
Chlorophyll a	3519.07±39.27 a	872.02±2.26 b	2647.05±37.1	75.22±9.42
Chlorophyll b	3119.79±51.94 a	438.89±3.28 b	2680.9±48.66	85.93 ±9.36
Total Chlorophyll	6456.65±61.47 a	1426.66±4.77 b	5029.99±56.7	77.91 ±9.22
Total Carotenoids	4090.62±8.52 a	2125.72±1.09 b	1964.91±7.43	48.03 ±8.72

*Different letters in each row indicate significant differences - $p<0.05$.

Table 2. Changes in the values of F_v/F_m and photosynthetic efficiency induced by the feeding activity of *Cisaberoptus kenyae* K. in the leaves of *Mangifera indica* L. (Mean ±SEM)*

F_v/F_m value in uninfested mango leaf tissue	Fv/Fm value in mite infested mango leaf tissue	Reduction in Fv/Fm value	Per cent loss in photosynthetic efficiency
0.8456±0.01158 a	0.6188±0.01304 b	0.2268±0.00146	26.79 ±1.64

*Different letters in each row indicate significant differences - $p<0.05$.

Table 3. Changes in the concentration of various biochemical constituents induced by the feeding activity of *Cisaberoptus kenyae* K. in the leaves of *Mangifera indica* L. (Mean±SEM)*

Biochemical parameters	Concentration in uninfested mango leaf tissue (µg/g)	Concentration in mite infested mango leaf tissue (µg/g)	Loss/increase in concentration (µg/g)	Per cent loss/increase in concentration
Total carbohydrates	85.48± 0.88 a	26.31±0.87 b	-59.17±0.01	33±11.36
Total Phenol	55.14±1.72 a	81.16±0.85 b	+26.02±0.87	47.18 ±5.06
Proline	2.96±0.36 a	5.26±0.61 b	+2.68±0.25	77.70 ±6.94

*Different letters in each row indicate significant differences - $p<0.05$.

showed a mean percentage increase of 47.18 ±5.06 in phenolic compounds when compared to that of the uninfested leaves (Table 3). A significant increase was observed in the concentration of proline also in mite infested leaves. Results of the study disclosed a mean concentration of 2.96±0.36 µg/g of proline in uninfested leaves whereas mite infested leaves showed an enhanced level, reaching a mean value of 5. 26±0.61 µg/g which could account to a mean percentage increase of 77.7±6.94 (Table 3).

DISCUSSION

Cisaberoptus kenyae was designated as the mango leaf coating and webbing mite or blotch leaf miner mite (Keifer, 1966) infesting under the epidermis of the upper surface of young leaves, raising the epidermal cells, and inducing the development of curling and browning spots on the undersides of mango leaves (Abou-Awad et al., 2009). In the present study, infestation of C. kenyae was readily identified under field conditions by the presence of ashy-white membranous coating on the adaxial surface of mango leaves. However, no curling or spotting symptoms were noticed on mite infested leaves. All life stages of the mite were found to colonize the upper leaf surface, enjoying the microhabitat available underneath the leaf coating secreted by the mite and the upper cuticle. Leaves of all mango varieties examined showed mite infestation, though population differences could be observed depending upon varietal difference (Ramani and Haq, 1988; Abou-Awad et al., 2009).

Feeding activity of eriophyid mites induces diverse types of toxemic and other non-distortive changes in their host plants (Oldfield, 1996), leading to the development of symptoms like galling, russeting, browning, bronzing, silvering, chlorotic spots, reddening and so on (Craemer et al., 1996; Rancic et al., 2006; Petanovic and Kielkiewicz, 2010a,b). Visible symptoms of such infestation could be associated with discolorations of plant organs, brown scarification, necrosis and destruction of buds, witches broom effect, distortion of veins, chlorotic spots, mottled appearance, presence of spotting on abaxial surface, rapid cell death and collapse of lower epidermal and mesophyll layers, development of lesions, failure of nutritive cell formation, premature defoliation etc. (Jeppson et al., 1975; Westphal, 1992; Cullen and Briese, 2001). In the present study, *C. kenyae* was found to induce damage symptoms such as brown to black coloration developed underneath the leaf coating and drying symptoms on the foliar surface including the midrib and other veins. In severely infested cases, the entire leaf lamina was found to be covered by the coating with the upper surface completely turned black. Development of such brown-black colorations and subsequent drying up of leaves is a clear indication of chlorophyll loss resulted from the progressive feeding activity of the mite through leaf sap drainage. Further, the leaf coating on the upper leaf surface would hinder penetration of sunlight through the epidermal layer, which is the most essential factor for photosynthetic activity. Thus, the combined effect of chlorophyll destruction and prevention of direct entry of sunlight would impose an adverse impact on the photosynthetic efficiency of the host, *M. indica.*

Results of histological studies indicated that mite feeding would lead to a reduction in the chloroplast in the palisade and spongy tissues of the mesophyll. Additionally, the size of spongy cells also got reduced. Mite infested leaf sections showed the presence of larger intercellular spaces and often the inner mesophyll layer appeared shrunken and irregular. The cuticle and upper epidermis of mite infested leaf often showed signs of disruption and underlying palisade tissue became brown colored. This is a clear indication of drying up of leaves due to the removal of plant sap and the resulting water loss induced by the feeding activity of the mite. Thus the present study seems to support the earlier findings on similar cellular deformities leading to disruption of cuticle, punctured and collapsed epidermal cells, and reduced number of spongy and palisade parenchyma cells in mite infested leaves (Park and Lee, 2002; Sangeetha et al., 2011).

The photosynthetic efficiency of plants to a large extent is determined by the chlorophyll content of the leaves (Lahai et al., 2003; Netondo et al., 2004). Feeding by *C. kenyae* was found to induce significant reduction in photosynthetic pigments, resulting in respective per cent loss of 75.22±9.42, 85.93±9.36, 77.91±9.22 and 48.03±8.72 in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, thereby supporting earlier reports made on spider mites (Puchalska, 2006; Sangeetha and Ramani, 2011a-c), false spider mites (Prabheena and Ramani, 2013, 2014) and eriophyid mites (Nasareen et al., 2012). Chlorophyll a and b pigments in the leaf chloroplasts serve vital functions in photosynthesis, being instrumental in absorbing solar radiation, and through resonance transfer, channel the resulting excitation to the reaction centres, and thereby promoting release of electrons and setting up the photochemical process. Chlorophyll a is most essential for oxygenic conversion of light energy to the stored chemical energy (Gropper et al., 2009). Carotenoids also would help to channel photons unabsorbed by chlorophylls to the reaction centre for photosynthesis (Niyogi, 1999) and thus function as light-harvesting pigments. Hence, a significant reduction in the chlorophylls and carotenoids by *C. kenyae* would definitely impair the photosynthetic activity, leading to substantial decline in the vigor and biomass of *M. indica.* Thus the results of the study corroborate with the earlier findings made on another eriophyid species, *Acalitus hibisci* on its host, *Hibiscus vitifolius* where it induced a loss of 18.05% and 39.01%, respectively in chlorophyll a and b pigments (Chakrabarti et al., 1999). However, contradictory to the present finding on the reduced carotenoid concentration induced by *C. kenyae*, the feeding of *A. hibisci* enhanced production of carotenoids by 59.8%.

Feeding activity of *C. kenyae* was also found to reduce photosynthetic efficiency of mango trees as evidenced during the study, based on measurement of chlorophyll fluorescence. A percentage loss of 25.15-28.43 was recorded in the chlorophyll fluorescence owing to infestation by *C. kenyae.* Being a very rapid, nondestructive, and early indicator of stress, chlorophyll fluorescence has been employed as a widely used parameter to evaluate impact of varied factors on photosynthetic efficiency of plants, even in the absence of visible symptoms (Iatrou et al., 1995). Emission of chlorophyll fluorescence is often negatively correlated with photosynthetic efficiency (Pereira et al., 2000) and *Fv/Fm* values are taken as an index to assess the potential quantum yield of PS II and thus the physiological state of photosynthetic apparatus of plants (Maxwell and Johnson, 2000; Pereira et al., 2000). Generally, *Fv/Fm* values are constant, falling in the range of 0.778 to 0.860 for healthy plants and reduced *Fv/Fm* values indicate severe abiotic or biotic stress conditions of host plants (Kawashima and Nakatani, 1998; Pospíšil et al., 1998; Schansker et al., 2005) which in turn would lead to a drastic reduction in chlorophyll content (Morales et al., 1991). A significant reduction by about 20% is suggestive of disturbance of PSII (Berova et al., 2007), which could have resulted from the destruction of photosynthetic pigments and feeding stress induced by herbivory. In the present study, a decrease of > 20% was recorded in the *Fv/Fm* value, thereby indicating a reduction in the photosynthetic efficiency, resulted from a decrease in chlorophyll content, owing to mite feeding. Various authors have established a close linkage between the chlorophyll reduction and the reduced photosynthetic activity of mite infested plants (Bondada et al., 1995; Haile and Higley, 2003). The feeding activity of arthropods is known to damage xylem or phloem (Welter, 1989), and which in turn often would affect other physiological functions of host plants like transport of water and sugars, stomatal aperture etc. and hence would lead to a decline in the photosynthesis of remaining leaf tissues also (Nabity et al., 2009). The sucking activity of *C. kenyae* has led to structural degeneration of chloroplast in the palisade and spongy layers of mesophyll tissue and which would have an adverse impact on the neighbouring tissues also, thereby culminating in significant loss in photosynthetic pigments and photosynthetic efficiency of the host as observed during the present study. Added to these, the leaf coating produced by the mite would hamper the entry of sunlight on the leaf surface and which in turn would aggravate loss in photosynthetic efficiency.

Sap sucking arthropods like the mites are known to induce a significant alteration in various metabolic products and such significant changes were induced by *C. kenyae* also in *M. indica.* Dependence of phytophagous mites on minerals and other phytochemical components of host plants to meet their nutritional and reproductive needs has been well established (Al-Azzazy, 2012). *C. kenyae* was also shown to induce a significant reduction in various macro and micronutrients in two mango cultivars studied in Egypt (Abou-Awad et al., 2012). Furthermore, the present data confirmed that feeding activity of *C. kenyae* not only would affect the micro and macronutrient contents but also the photosynthetic pigment concentration, photosynthetic efficiency as well as the total carbohydrate content of its host, *M. indica.* In addition, it would also induce biotic stress, leading to enhanced production of stress factors like proline and phenols.

Herbivory is known to generate diverse types of defensive responses in plants which often would lead to a reduction in the expression of photosynthesis related genes (Kessler and Baldwin, 2002). Several such defensive compounds serve as biocides against herbivores and their production would destroy the normal photosynthetic or homeostatic functioning of plants (Zangerl et al., 2002). Phenolic compounds are produced as a part of defense-related system, which are designated as widely distributed secondary plant products to counteract stress conditions (Harborne, 1980). Phenol offers resistance to diseases and pests in plants and enhanced production of phenolics in economically important plants during pest attack was recorded and it was concluded that the increase in total phenols induced resistance in hosts against herbivory (Ananthakrishnan et al., 1992). Plants rapidly synthesise phenolic compounds and often polymerize them in the cell walls in order to defense against infection (Matern and Kneusel, 1988). Certain phenolic compounds serve to precipitate plant proteins so as to convert them as indigestible to herbivores. Mango leaves infested by *C. kenyae* showed a mean percentage increase of 47.18±5.06 in phenolic compounds when compared to that of the uninfested leaves, thereby supporting the earlier finding that the plant enhanced production of phenolic compounds to defense herbivory.

Proline, one of the basic amino acids is produced by plants to defend against herbivory which affords protection to cellular structure and cytoplasmic enzymes (Serrano and Gaxiola, 1994) by storing nitrogen and carbon sources. Proline plays a critical role in protecting plants under stress condition (Kuznetzov et al., 1999) as it moves between tissues, and promotes detoxification of reactive oxygen species and stabilization of cell membranes (Kavi Kishor et al., 2005). The highly enhanced levels of proline in mango leaves infested by *C. kenyae* as observed during the current study could be considered as a defense mechanism developed by the plant against the feeding stress induced by the mite. Degradation of photosynthetic pigments, destruction of chloroplast, reduction in chlorophyll fluorescence and net photosynthetic rate generally indicate the stress conditions faced by plants (Bounfour et al., 2002; Anitha and Ramani, 2016). The results of the present study strongly support the above by clearly establishing the extent of damage induced by the leaf coating mite by way of inducing depletion of photosynthetic pigments, decline of photosynthetic efficiency of the plant, decrease in total carbohydrate and by stimulating the production of defensive substances like proline and phenolic compounds.

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RESEARCH ARTICLE

First record of *Cheletonella* (Acariformes: Cheyletidae) in Poland, with comments on other member of the genus

Salih DOĞAN [1,3], Sibel DOĞAN [1], Joanna MĄKOL[2]

[1] *Department of Biology, Faculty of Sciences and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey*
[2] *Department of Zoology and Ecology, Faculty of Biology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland*
[3] Corresponding author: salihdogan@erzincan.edu.tr

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ABSTRACT: Members of the family Cheyletidae are generally free-living predators, and have a worldwide distribution. The genus *Cheletonella* Womersley within the family Cheyletidae comprises four species. This study presents the first record of the *Cheletonella* and *C. vespertilionis* Womersley for the fauna of Poland, and is based on mite specimens found in litter and detritus samples collected from the foot of wall of a tunnel used as shelter by bats, and found in litter and soil samples located close to the bat boxes in the city park. *Cheletonella summersi* Chatterjee and Gupta is considered here as a *species inquirenda.* It is also provided an updated list of cheyletid mites recorded from Poland.

Keywords: Acari, *Cheletonella*, Cheyletidae, fauna, *species inquirenda.*

Zoobank: http://zoobank.org/CC44E52E-646B-4E58-B6C8-88DEDC9E8904

INTRODUCTION

The family Cheyletidae with around 500 described species in 77 genera (Fuangarworn and Lekprayoon, 2010; Doğan et al., 2011; Zhang et al., 2011; Negm and Mesbah, 2014; Bochkov and Abramov, 2016; Salarzehi et al., 2018, 2019) contains mainly free-living predators, and has a worldwide distribution. The genus *Cheletonella* Womersley is recognized within the family by the following traits: its body ovoid, a single shield on the propodosoma, eyes absent, palp tarsus with two comb-like setae and two sickle-like setae, palp claw with basal teeth, and all legs with claws.

Mites of the family Cheyletidae in Poland are still poorly known. A total of 16 species of the family have been recorded from Poland until present (Table 1). The current paper aimed to contribute to the knowledge on mite diversity in Poland, and it presents new distribution data on *Cheletonella vespertilionis* Womersley. The present finding of *C. vespertilionis*, based on the specimens collected in Nowogród Bobrzański and Wrocław, constitutes a new country record. The identity of *Cheletonella summersi* Chatterjee and Gupta is doubtful, and it is considered here as a *species inquirenda*.

MATERIAL AND METHODS

Mite specimens were extracted in Berlese-Tullgren funnels from litter and soil samples collected in the city park (Wrocław, Poland) and samples of litter and detritus taken from the foot of tunnel wall (Nowogród Bobrzański, Poland) in 2010. The specimens were mounted on microscopic slides in Hoyer's medium using by the standard method (Walter and Krantz, 2009). The material is deposited in the Acarology Laboratory of Erzincan Binali Yıldırım University, Erzincan, Turkey. Measurements were taken in micrometers (µm) using Leica Application Suite (LAS) Software Version 3.8. Mean values are followed by the range (given in parentheses). Body length measurements represent the distance between the base of the gnathosoma and the posterior part of the idiosoma, width was measured at the broadest point of the idiosoma. Leg length was measured from the tip of the claws to the trochanter base. Dorsal setal and leg setal designations follow Kethley (1990) and Grandjean (1944), respectively.

RESULTS

Genus: *Cheletonella* Womersley

Species: *Cheletonella vespertilionis* Womersley

Female

Body ovoid, length (including gnathosoma) 556 (478-600), width 306 (246-339).

Length of gnathosoma 180 (169-193), width 145 (139-148) (Fig. 1). Rostrum pointed, with two pairs of adoral setae ($or_{1,2}$). Longitudinal apodeme on midventral line of subcapitulum, between one pair of subcapitular setae (*m*). Protegmen conical, dorsal surface with faint broken striae. Tegmen with broken striae and small punctations scattered over its surface. Peritremes with nine chambers on each side (Fig. 2A). Palps short and thick. Palp tarsus with two comb-like and two sickle-like setae. Palp tibia striated, bearing one dorsal, one ventral and one inner ventral acicular setae, palp claw bearing three basal teeth

Table 1. Cheyletid mite species hitherto recorded from Poland

Species	Related references
Acaropsellina docta (Berlese, 1886)	Hagstrum et al. (2013)
Acaropsellina sollers (Kuzin, 1940)	Hagstrum et al. (2013)
Cheletonella vespertilionis Womersley, 1941	Current paper
Cheyletia papillifera Volgin, 1955	Solarz (1989), Skoracki (2008)
Cheyletiella blakei Smiley, 1970	Kaźmierski and Magowski (2008)
Cheyletiella yasguri Smiley, 1965	Kaźmierski and Magowski (2008)
Cheyletiella parasitivorax (Mégnin, 1878)	Kaźmierski and Magowski (2008)
Cheyletus eruditus Schrank, 1781	Chmielewski (1971, 1991), Skoracki (2008), OConnor and Klimov (2012)
Cheyletus portentosus Koch and Berendt, 1854	Koch and Berendt (1854)
Cheyletus trouessarti Oudemans, 1902	Haitlinger (1982), Fain and Bochkov (2001b), Skoracki (2008)
Cheletopsis basilica Oudemans, 1904	Bochkov et al. (2002), Skoracki (2008)
Cheletopsis daberti Kivganov and Bochkov, 1994	Bochkov et al. (2002), Skoracki (2008)
Cheletopsis impavida Oudemans, 1904	Bochkov et al. (2002), Skoracki (2008)
Cheletopsis mariae Mironov, Bochkov and Chirov, 1991	Bochkov et al. (2002), Skoracki (2008)
Cheletopsis norneri (Poppe, 1888)	Bochkov et al. (2002), Skoracki (2008)
Eucheyletia flabellifera (Michael, 1878)	Haitlinger (1982), Fain and Bochkov (2001a), Skoracki (2008), Hagstrum et al. (2013)
Ornithocheyletia dubinini Volgin, 1964	Skoracki et al. (2004), Skoracki (2008)

(Fig. 2B). Palp genu short, with outer ventral acicular seta and one dorsal seta similar in form to dorsal body setae. Outwardly bulged femur striated, elbow-like in the middle of segments, with one dorsal seta similar in form to dorsal body setae, and two ventral acicular setae.

Dorsum (Figs 3-4) with 15 pairs of fan-like setae including humerals, dorsal body setae homeomorphic. Dorsal idiosoma covered by only one prodorsal shield, sculptured like that of stylophore, with four pairs of setae (Fig. 3). Eyes absent. Venter striated; intercoxal setae *1a, 3a* and *4a* piliform. Anogenital setae three pairs ($ag_{1\text{-}3}$), two pairs of genital setae ($g_{1\text{-}2}$) and three pairs of pseudanal setae ($ps_{1\text{-}3}$), $ps_{1\text{-}2}$ bifurcate, ps_3 smooth (Fig. 5).

Leg I 304 (283-325), leg II 254 (209-283), leg III 271 (234-297), leg IV 314 (297-323). Chaetotaxy of leg segments as follows: coxae 2–1–2–2, trochanters 1–1–2–1, femora 2–2–2–1, genua 2(+1κ)–2–2–2, tibiae 4(+1φ)–4–4–4, tarsi 9(+1 ω)–8(+1 ω)–7–7.

Figure 1. *Cheletonella vespertilionis* Womersley (Female) – Gnathosoma in dorsal view.

Figure 2. *Cheletonella vespertilionis* Womersley (Female) – A) Stylophore, B) Right palpal tibia and tarsus in dorsal view.

Male

Length of body (including gnathosoma) 472 (463-482), width 245 (237-263). Length of gnathosoma 167 (165-169), width 135 (128-140). Leg I 307 (300-318), leg II 217 (209-225), leg III 245 (240-254), leg IV 284 (273-297).

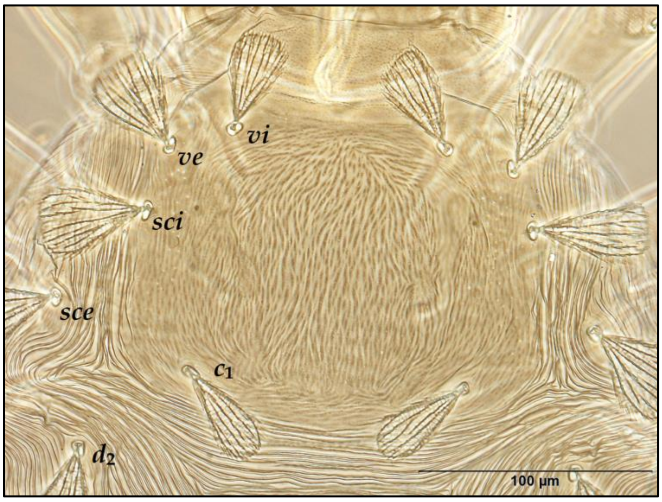

Figure 3. *Cheletonella vespertilionis* Womersley (Female) – Prodorsal region.

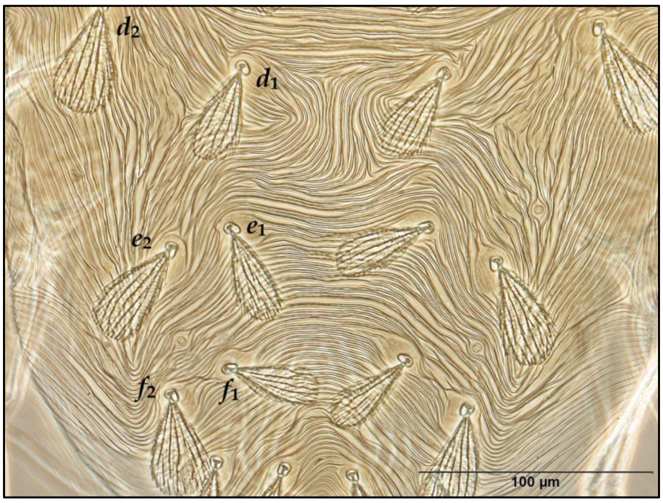

Figure 4. *Cheletonella vespertilionis* Womersley (Female) – Dorsum of hysterosoma.

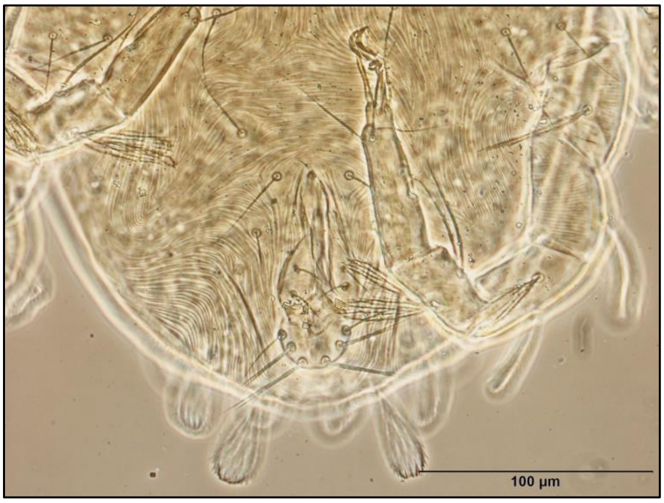

Figure 5. *Cheletonella vespertilionis* Womersley (Female) – Anogenital region.

Resembles female in general appearance, but: dorsal body setae 13 pairs, each tibiae II-IV bearing extra one dorsal solenidion and each tarsi II-IV bearing extra one ventral solenidion, anogenital shields situated posteriorly (Fig. 6)

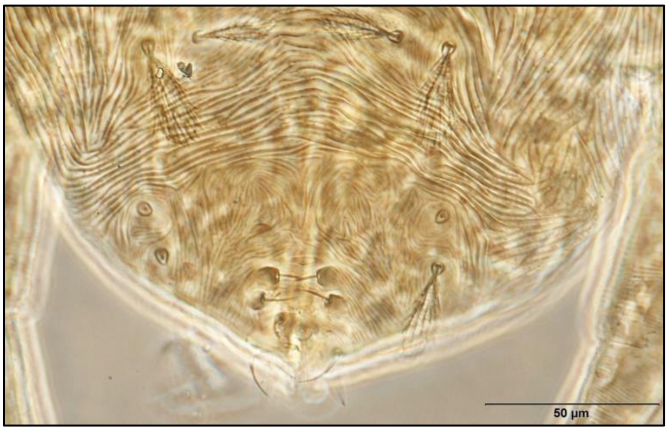

Figure 6. *Cheletonella vespertilionis* Womersley (Male) – Anogenital region.

Material examined

Poland: close to the entrance of Mopkowy Tunnel, Nowogród Bobrzański, May 2010, 3 ♀♀, from litter and detritus samples, 51°48' N 15°13' E (the seconds were not given as there were few collecting sites), 81 m a.s.l., coll. D. Łupicki. Poland: Szczytnicki Park, Wrocław, May 2010, 20 ♀♀ and 14 ♂♂, from litter and soil samples, 51°06'52''N 17°04'51''E, 120 m a.s.l., coll. the students research group.

Distribution

Algeria, Armenia, Australia, Belgium, Brazil, Egypt, Iran, Malaysia, Tajikistan, Ukraine, USA (Womersley, 1941; Baker, 1949; Volgin, 1955, 1969; Summers and Price, 1970; Fain and Nadchatram, 1980; Gerson, 1994; Halliday, 1998; Fain and Bochkov, 2001a; da Silva Ezequiel et al., 2001; Webster and Whitaker, Jr., 2005; Whitaker, Jr. et al., 2009; Doğan et al., 2011; Negm and Mesbah, 2014; Salarzehi et al., 2018) and Poland (current paper).

DISCUSSION

Cheletonella vespertilionis Womersley was collected in birds' nest, in bumblebees' nest, on gray hamster, on bats, in guano of some bats, in forest soil, some kind of orchard soil, and in house dust (Doğan et al., 2011; Negm and Mesbah, 2014). The Polish specimens were found in litter, close to the entrance of Mopkowy Tunnel [an area protected within the Natura 2000 network (Centrepiece of EU Nature and Biodiversity Policy)] used by bats as a shelter but also in litter and soil samples located close to (underneath) the bat boxes in the city park in Wrocław. Therefore, in both cases the affinity of the species to microhabitats occupied by bats cannot be excluded. *Cheletonella vespertilionis* is newly recorded species for the Polish fauna. This is the first reported occurrence of the genus *Cheletonella* Womersley from Poland.

Polish specimens closely resemble the type and other known specimens of *Cheletonella vespertilionis* in general features, and their body sizes are within the range of the size variation (Womersley, 1941; Volgin, 1969; Summers and Price, 1970; Fain and Bochkov, 2001a).

By now seven species have been described in this genus: *Cheletonella caucasica* Volgin, *C. hoffmannae* Smiley, *C. iraniensis* Salerzehi, Hajizadeh and Ueckermann, *C. juglandis* Xia, Zhu and Ye, *C. pilosa* Tseng, *C. summersi* Chatterjee and Gupta and *C. vespertilionis* Womersley (Table 2). Gerson et al. (1999) suggested that *C. pilosa* and *C. juglandis* should be placed elsewhere by having the M-shaped peritremes, lanceolate-barbed dorsal setae and ultralong humerals, dissimilar to the dorsal ones. *Cheletonella caucasica* was considered as synonym of *C. vespertilionis* by Fain and Bochkov (2001a). Also *C. juglandis* was regarded a *species inquirenda* by the same authors as based on teleonymph stage (Fain and Bochkov, 2001a).

Cheletonella summersi represented by a single male specimen was inadequately described by Gupta (2002). Anogenital region in the original figures provided by Gupta (2002) was absent, and the author referred to the female in the figure caption. The identity of this species is doubtful, and it is considered here as a *species inquirenda* (Table 2). Further examination based on more specimens is necessary in order to ascertain the species identity.

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Table 2. Species list of the genus *Cheletonella* Womersley

Cheletonella hoffmannae Smiley, 1996: 241 [USA (Indiana)]
Cheletonella iraniensis Salerzehi, Hajizadeh and Ueckermann, 2019: 189 [Iran]
Cheletonella pilosa Tseng, 1977: 240 [Taiwan]
Cheletonella vespertilionis Womersley, 1941: 61 [Australia, by monotypy] TYPE-SPECIES [=*Cheletonella caucasica* Volgin, 1955: 168]
SPECIES INQUIRENDAE
Cheletonella juglandis Xia, Zhu and Ye, 1999: 150 [China]
Cheletonella summersi Chatterjee and Gupta, 2002 *in*: Gupta, 2002: 46 [India]

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RESEARCH ARTICLE

Potential of oribatid mites in biodegradation and mineralization for enhancing plant productivity

Mohamed Abdul HAQ

Division of Acarology, Department of Zoology, University of Calicut, Kerala, 673 635, India
e-mail: haqzas@yahoo.co.in

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ABSTRACT: The degradation of litter is an essential process of the soil ecosystem leading to nutrient cycling and is mediated by a heterogeneous group of soil organisms. Oribatid mites represent one of the predominant agents of litter biodegradation in the soil. The ubiquitous presence and extensive diversity of this group of mites make them integral to the process of mineralization of litter in almost all types of soil ecosystems. However, an overall assessment of the mineralization potential of different groups of oribatid mites depicts the relative advantage of lower groups of oribatids, namely the lohmannoid and phthiracaroid members, in the degradation of leafy and woody elements of litter. Degradation of such complex materials primarily necessitates additional qualities such as strong and well developed oral and holding appendages, and the presence of the necessary enteric microflora and associated enzymes, for on-going degradation. In-depth field and laboratory studies of two representative species of the above two groups of mites, viz. *Atropacarus* (*Hoplophorella*) *chaliyamensis* Haq and Xavier, 2005 and *Heptacarus hirsutus* Wallwork, 1964, with the vegetable crop *Vigna unguiculata*, clearly demonstrated that these species make a remarkable contribution to the process of nutrient cycling. The combined feeding activity of these two species on the woody elements of litter was found to enhance the release of nitrogen, phosphorous and potassium, as evidenced by the increased concentration of these minerals in fecal pellets. However, a decrease in the levels of calcium and magnesium was observed in the fecal pellets. The decrease in calcium may be accounted for by its immediate requirement in body maintenance. The impact of oriculture on plant productivity was evidenced through increased plant growth, higher yield and better quality of the pods produced by the treated plants.

Keywords: Oribatid mites, biodegradation, nutrient cycling, fertility, plant productivity, oriculture.

INTRODUCTION

Oribatid mites constitute one of the suborders of Acari and are well known for their taxonomic diversity, wide geographic distribution and adaptive radiation in terms of habitats. Subias (2018) compiled a list of more than 11,000 species of oribatid mites described so far. These mites enjoy cosmopolitan distribution with varying abundance across different geographic realms (Gergócs and Hufnagel, 2015). They are predominant in soil ecosystems but have invaded many of the terrestrial habitats and even aquatic habitats (Maraun et al., 2007; Schatz and Behan-Pelletier, 2008; Norton and Behan-Pelletier, 2009; Murvanidze et al., 2016; Behan-Pelletier and Norton, 2016). The majority of the soil oribatids feed on plant litter, fungi, and algae (Behan-Pelletier and Norton, 2016). Varied food preference has been noticed within the litter feeding oribatid mites in the soil and has been investigated extensively for a better understanding of the extent of contribution of these mites to the functioning of the edaphic community (Macfadyen, 1961; Haq, 1994).

Oribatid mites exhibit profound diversity in the soil ecosystem. They have invaded a wide range of microhabitats and adopted an array of nutritional habits. The items of food they ingest range from bacteria, algae, and fungi to leafy and woody materials in the plant litter and to the wastes of living animals and dead remains of animals. Accordingly, they have been grouped into three major feeding guilds, namely the microphytophages, macrophytophages and panphytophages (Schuster, 1956; Hartenstein, 1962). Luxton (1972) further sub-categorized them into a still narrower range of feeding guilds and assigned many more categories to accommodate marginal species showing deviations from the major food and feeding habits.

Jacot's findings (1930, 1936, 1939) on the nutritional habits of oribatid mites were instrumental to the assumption that some species of these mites are specific in their food requirements. Furthermore, Forsslund (1938), Gourbiere et al. (1985) and Lions and Gourbiere (1988) showed that some species of these mites specifically feed on fungal hyphae and spores whereas others live mainly on leaf and needle litter. Harding and Stuttard (1974) stated that the food spectrum of oribatid mites includes algae, lichens, mosses, and pollen. Riha (1951) showed the affinity of oribatid mites for dead collembolans and worms. Rockett and Woodring (1966), Muraoka and Ishibashi (1976), Rockett (1980) and Stefaniak and Seniczak (1981) reported feeding of food items mentioned in the above categories by various specious of oribatid mites. Accordingly, mites have been grouped into three major feeding guilds, namely the microphytophages, macrophytophages and panphytophages. However, these guilds are not sufficient to accommodate many other feeding habits observed among them. There has been tremendous progress in the knowledge of feeding guilds in oribatid mites over the years. Zinkler (1971), Luxton (1972, 1979) and Urbasek and Stary (1994) have contributed knowledge on the enzyme activity of these mites. Haq (1984) demonstrated the role of microbes in the nutrition of *Heptacarus hirsutus* and Haq (1987) reported on the biodegradation of cellulose in the gut of the same species. This has provided greater insight into the possible relationships between the functional aspects of feeding trends of mites and their ability to biodegrade plant structural polysaccharides. The feeding guilds of oribatid mites are related to the carbohydrase activities of these mites (Siepel and Dijkman 1993). Additional aspects of nutritional biology were reviewed later (Schneider et al. 2004). The functional attributes of oribatid mites in the soil ecosystem were reviewed by Haq (1994, 1996), and the contribution of these mites towards the degradation of plant litter and nutrient recycling (Fig. 1) and maintenance of soil fertility were identified as major roles being played by these mites in the soil (Haq, 2016).

Among the Oribatida, members of the lower taxa like the lohmannoid and phthiracaroid groups have been observed to be more competent in the process of degradation of the residues of higher plants in the litter and the mineralization process through a series of studies on different aspects of the nutritional biology of various groups of oribatid mites (Haq and Prabhoo, 1976; Haq, 1976, 1982, 1992, 1994, 1996, 2007a; Haq and Konikkara, 1988; Haq and Xavier, 2005). The current study was carried out to analyze the practical impacts of these groups of mites in biodegradation, mineralization, nutrient cycling and the enhancement of soil fertility. Two species of mites, namely *Atropacarus* (*Hoplophorella*) *chaliyamensis* Haq and Xavier (2005) representing Phthiracaridae, and *H. hirsutus* Wallwork, 1964 representing Lohmanniidae were selected as the model organisms for the current study. The ability of these two species for wood degradation has been repeatedly established through earlier studies of the author (Haq, 1987, 2016). Specifically, the current work is undertaken to determine the enhancement of soil fertility by these mites in the growth and yield of the vegetable plant, *Vigna unguiculata*.

MATERIAL AND METHODS

Sampling, extraction and identification of mites

The mites for the current study, *A.* (*H.*) *chaliyamensis* of the family Phthiracaridae and *H. hirsutus* belonging to the family Lohmanniidae, were collected from 4 different locations on Beypore and Chaliyam beaches (Figs 2-3), near Calicut University Campus, Malappuram District, Kerala, India. These two species of mites were abundantly available on driftwood, logs, leaf and woody litter samples on the beach shore (Figs 4-6). The extraction of mites from the above litter samples were carried out via the technique of Berlese's Funnel Apparatus (1905) and the Open Brass Funnel Apparatus designed by Haq and Ramani (2002) (Fig. 7). The former species was erected as a new species by Haq and Xavier (2005) and the latter species were identified by comparison with the characters given in the original description provided by Wallwork (1964). No other specific terminologies have been used here.

Nutritional Biology

Analysis of gut content

Live individuals of the selected species of mites were collected from the beaches and subjected for gut content analysis following the protocol described in Haq and Prabhoo (1976) and Haq (1982, 2007b).

Analysis of laboratory feeding

Live mites collected from the beaches were reared in the laboratory in plastic culture vessels and their feeding preferences were screened, as described by Haq and Pabhoo (1976) and Haq (2007a).

Analysis of the morphology of mouthparts

The mouthparts of the mites were dissected from collected specimens, processed and examined according to the methods illustrated by the author (Haq, 2007a).

Analysis of gut enzymes

A qualitative profile of the enzyme contents of the mites was determined according to the protocol described by the author (Haq, 1984, 1987, 2007a).

Analysis of gut microbiota

The gut microbiota of the mites was investigated by inoculating the freshly deposited fecal material of the mites in basic culture media for the bacteria and fungi as described by Haq (1984, 2007a) and Haq and Konikkara (1988).

Biomass Reduction

A known weight of driftwood pieces obtained from the two seashores was offered to 10 adult mites of both *A.* (*H.*) *chaliyamensis* and *H. hirsutus* in separate laboratory culture vessels and the mites were kept under observation at standard laboratory conditions for one month, providing the same quantity of the same food material on completion of feeding on each batch of food material offered. The number of days utilized for completion of every batch of food and the final mass of fecal pellets generated from each batch of food were recorded. The average reduction in the quantity of biomass observed was calculated from the data obtained.

Nutrient Analysis

In order to assess the nutrient turnover effected through the feeding activity of the mites on plant litter, the levels of nitrogen, phosphorous, potassium, calcium, and magnesium were estimated in decomposing wood samples and fecal pellets produced by the mites after

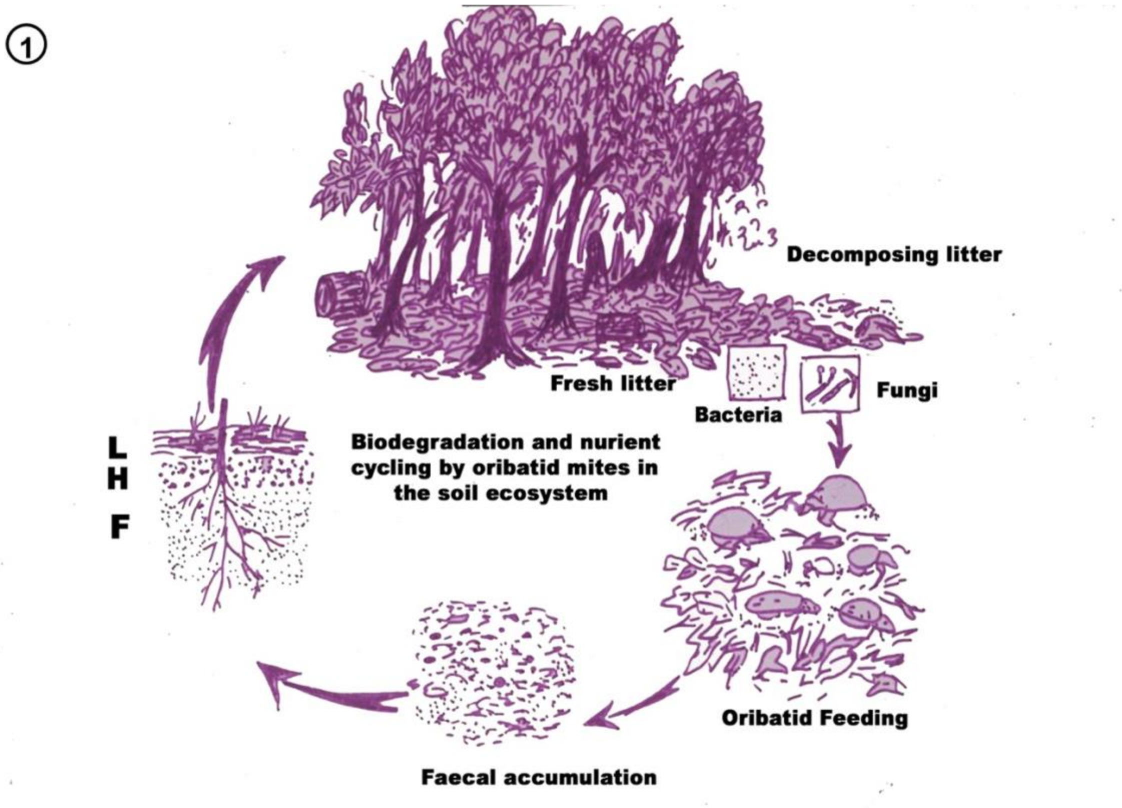

Figure 1. Nutrient cycling.

Figure 2. Beypore Beach with plant litter accumulation on shore.

Figure 3. Chaliyam beach with drift wood and litter accumulation.

Figure 4. Logs of drift wood – A,C) Chaliyam beach, B,D) Beypore Beach.

Figure 5. Samples of various stages of decomposing litter (A-D) used as source for extraction of the mites.

Figure 6. Degraded leafy and woody litter maintained in the field and the laboratory (A-D) for mass culturing of the mites.

Figure 7. Open Brass Funnel Apparatus used for extraction of mites (Haq and Ramani 2002).

Figure 8. Field trial plot of oriculture with *Vigna unguiculata* plants, A) Experimental and control plants after two weeks of growth, B) Experimental and control plants after four weeks of growth, C) Experimental and control plants after eight weeks of growth, D) Experimental plants with matured pods.

feeding, as per the methods elaborated by Haq (1996, 2007a).

Breeding Biology

The mites were reared in individual culture chambers with a Plaster of Paris-charcoal base. They were offered driftwood pieces, as this food was ideal for supporting the development of these mites (Haq, 1987). The cultures were maintained at 27±2°C and relative humidity of 80-82%. The cultures were monitored daily for detection of spermatophores and eggs. As and when the eggs were found, the pieces of wood with eggs were transferred to specially prepared culture vessels comprised of glass rings fixed on a Plaster of Paris-charcoal base in a Petri dish. Cover slips were used as lids for the individual culture cells within the Petri dish. Life history studies of the mites and their individual stages of development were undertaken with their preferred foods and monitored using appropriate microscopes.

RESUTLS

Nutritional profiles

The laboratory feeding experiment showed similar nutritional profiles for both species, including gut content analysis (Table 1). In general, the phthiracarid, *A.* (*H.*) *chaliyamensis* showed a preference towards the woody tissues of higher plants, except for occasional feeding on leaf litter. *A.* (*H.*) *chaliyamensis* showed a wood boring habit (Figs 10 C, 11 D), as the adults often tunnelled into the wood pieces offered as food and laid eggs inside the tunnels. The lohmanniid mite, *H. hirsutus* exhibited a general preference towards high cellulose content litter like the driftwood from the beach and midribs and the veins of dried leaves. The larvae and nymphs often remained within the tunnels (Figs 14 A,B) which were often filled with the fecal material of the mite. The feeding activity was initiated with the soft mesophyll tissue, followed by the veins and the midrib region. This type of feeding often resulted in the appearance of skeletonized leaves in the cultures. When offered the dried roots of *Calotropis gigantea*, the mites bored holes into the root tissues and created tunnels in the woody parts of the roots. Macrophytophagous phthiracarid mites primarily depend on woody tissues and hence are designated as xylophages. The chelicerae of *A.* (*H.*) *chaliyamensis* were broad and stout with denticulate body (Fig. 16 C). They possess well developed and highly sclerotized chelae. The movable and fixed digits of the chelicerae carried 4-5 teeth to help cut the hard wood pieces while feeding on them. The rutellar dendites bear dorsal concavities called vestibules, to accommodate large wood pieces for the masticatory action of the chelicerae of *A.* (*H.*) *chaliyamensis*. The chelicerae of *H. hirsutus* were less broad, elongated or with round base (Fig. 16 D). The movable digit had 3-4 teeth and the fixed digit had 2-3. This helped in the cutting of food particles. The rutella had a broader distal end and a narrow proximal end bearing 3-4 notches distally. It appeared flat, more or less triangular in shape and strongly sclerotized.

Gut enzyme profile

Qualitative analysis of the gut enzymes of the mites is presented in Table 1. The carbohydrate digesting enzymes, maltase, cellulase and cellobiase, indicated their ability to digest simple as well as complex carbohydrates.

Gut microbiome

Microbial assay of the fecal materials of the mites confirmed the occurrence of different forms of bacteria in the gut, as indicated in Table 1. Both the mite species harbored Gram+ and Gram- bacteria in their gut, with microbial diversity greater in *H. hirsutus*.

Biomass reduction

Estimates of total biomass reduction in samples of selected litter items offered to the mites in the laboratory cultures are presented in Table 2. The percentage reduction in the biomass by *A.* (*H.*) *chaliyamensis* was greater.

Nutrient analysis

Quantitative changes in the concentration of N, P and K as a result of feeding by *A.* (*H.*) *chaliyamensis* and *H. hirsutus* are presented in Table 3. All three macronutrients increased in quantity after feeding and digestion. Therefore, feeding on and digestion of litter released the nutrients bound in the plant tissues.

Breeding Biology

Atropacarus (*Hoplophorella*) *chaliyamensis*

Adult females of *A.* (*H.*) *chaliyamensis* (Fig. 10 A) oviposited mostly inside the wood and rarely among the fecal pellets (Figs 10 B,D). The eggs were inserted into the wood channels and pits (Fig. 11 D) created by their feeding activities. The eggs were mostly solitary (Fig. 12 E) and seldom found in clusters but always within the area of their fecal material. The incubation period ranged from 4-5 days (Table 4). On the third day of incubation, a tiny black patch appeared within the egg shell. This patch darkened and became prominent afterwards. This was followed by splitting of the egg along the patch, releasing the larva. Soon after hatching, the larvae remained immobile for about two hours and afterwards showed signs of feeding and remained concealed within the wood piece. The larvae excavated the wood during their feeding activity. The active period of larva ranged from 4-5 days (Table 4). The physical activities of the larvae became restricted towards the end of this period. The larvae gradually became inactive, swollen in appearance, sluggish in habit and stopped feeding. Then they became immobile when entering the first quiescent phase. At the end of the 3-4 days of quiescent period (Table 4) the process of moulting lasted 2-3 hours. At the beginning of this process, a vertical slit appeared on the notogaster and increased in size. After about 15-30 minutes, the prodorsum came out. Up and down movements of the body resulted in the casting off of the exuvium.

The newly moulted nymph remained stationary for some time near the exuvium; after that, it moved away in search

Table 1. Evaluation of nutrition profiles of oribatid mites

Species	Lab Feeding	Gut content	Enzyme Profile	Gut Microbiome	Oviposition	Feeding category
A. (*H.*) *chaliyamensis*	Fungi/Algae: No Leaf: Yes Wood: Yes	Fungi/Algae:No Leaf: Yes Wood: Yes	Maltase Cellulose Cellobiase	Gram+: 3 Gram –: 2 Fungi: 0	Wood Dried roots	Macrophytophage: Xylophage
H. hirsutus	Fungi/Algae: No Leaf: Yes Wood: Yes	Fungi/Algae: No Leaf: Yes Wood: No	Maltase Cellulose Cellobiase	Gram+: 1 Gram –: 6 Fungi: 0	Wood Dried roots Leaf	Macrophytophage: Xylophage

Table 2. Biomass reduction of litter samples by feeding of oribatid mites under laboratory conditions

Mite species	Initial weight (mg)	Final weight (fecal pellets)* (mg)	% reduction of biomass*	No. of days utilized*
A. (*H.*) *chaliyamensis*#	10	9.12	8.8±0.421	2.2±0.105
H. hirsutus#	10	9.25	7.5±0.312	2.3±0.213

#Food offered: Drift wood

*Average of 5 triplicate samples

Table 3. Estimation of nutrient release from litter by feeding of oribatid mites

Nutrients analysed	% Increase	% Decrease
Nitrogen (N)	0.48 ±0.03	NA
Phosphorus (P)	0.06±0.12	NA
Potassium (K)	0.05±0.01	NA
Calcium (Ca)	NA	0.30±0.04
Magnesium (Mg)	NA	0.21±0.02

*Average of 5 triplicate samples

of food. The protonymph continued to be active for about 14-15 days and then entered the second quiescent phase which lasted for 3-4 days (Table 4).

Moulting of the second quiescent phase released the deutonymph. These nymphs excavated the wood during their feeding activity which resulted in the formation of irregular tunnels within the wood. The activity of the nymph could be easily distinguished by their fecal pellets, especially around the tunnels. The active period of the deutonymph ranged from 17-18 days after which it became quiescent. The third quiescent phase extended up to 4-5 days (Table 4) and terminated with the emergence of the tritonymph.

The tritonymph had an active period of 19-20 days, at the end of which it entered into the fourth quiescent phase. This lasted for 6-7 days and was followed by the emergence of the adult (Table 4). The newly moulted adults appeared pale yellow in colour with a light-pinkish tinge. The colour changed to wheat-yellow on the fourth or fifth day after emergence. Adults often wandered outside the wood tunnels (Figs 10 A, 11 A,B) whereas the immatures remained mostly inside (Figs 10 C, 11 C,D). The results of this study indicated that the development of *A.* (*H.*) *chaliyamensis* from egg to adult is completed within 81-89 days (Table 4).

Heptacarus hirsutus

The development of *H. hirsutus* (Figs 13 A,B) involved egg, larva and three nymphal stages interrupted by four quiescent stages between the successive larval stages to the adult stage. Gravid females of *H. hirsutus* generally carried 1 or 2 eggs at a time, which were visible through the integument under microscope. The female deposited solitary eggs on food material like leaf litter packed with fecal pellets. Most of the time the eggs were burried in tunnels bored into the wood. A longitudinal section of the wood tissue revealed the presence of all the life stages, well protected inside the tunnels (Figs 14 A,B). The number of eggs laid by a female under laboratory conditions varied between 6 and 8. However, the egg laying capacity of the adult female increased to 9-16 within one year on retaining the same culture conditions, including the rearing vessels. The incubation period ranged from 22-26 days (Table 5). About 13 days after oviposition, a conical projection appeared towards the animal pole of the egg. Gradually the colour of the egg turned light brown. Prior to hatching, a crescentic area developed along the antero-median portion of the egg, which appeared to be thin. This area gradually got stretched out due to the pressure exerted by the developing larva. As a result, the stretched area ruptured a little ahead of the middle and the slit extended in both directions. The larva stretched out its legs and crawled out through the slit. The egg case after emergence of the larva can be visible for 2-3 days with the half opened slit portion facing upward. The larva which emerged out of the egg appeared very sluggish. It continued to remain in the tunnel bored into the wood. Immediately after emergence, the larva remained motionless for about half an hour. This inactive period represented the hardening period. Then it gradually became active and started feeding on the woody tissue. Continued feeding by the larva led to the extension of the feeding tunnel. As feeding progressed, the tunnel became packed with fecal pellets and the larva subsequently selected an adjacent region of the wood for further feeding. This active period of larval life continued for about 16-18 days (Table 5). By this time, it had increased in size and attained a light brown colour. After the active period, it entered into the quiescent phase for 9-12 days (Table 5). During this time, it suspended all life activities and remained motionless. The quiescent phase ended with moulting. The slow moulting process required 2-4 hours. The body during this phase became slightly swollen and translucent. The postero-lateral region of the notogaster developed a few weakened areas on either side along which narrow slits appeared. Through one of these slits the last pair of legs protruded. The slit became extended in both directions due to the pressure exerted by the emerging individual. The moulting individual gradually came out with a backward thrust of its body. This helped to anchor the last pair of legs of the larva to the ground which allowed backward movement of the body and forward movement of the front pairs of legs simultaneously to push and release the moulting skin forward. Step by step the process finally resulted in the emergence of the protonymph from the larval quiescent stage. The process of moulting remained more or less the same in all the successive stages. Moulting of the first quiescent phase released the protonymph.

Unlike most of the other species of oribatid mites, *H. hirsutus* can multiply in large numbers in woody logs, particularly of marine nature. Availability of this preferred food in large quantities encourages the mite to bore deeply into the wood, creating numerous channels that accommodate sufficient colonies for further feeding activities. Feeding by the protonymph was more active. The individuals fed on the woody tissue for about 19-22 days, followed by the second quiescent phase which lasted for 9-11 days (Table 5). Moulting of the second quiescent phase gave rise to the deutonymph. The deutonymph continued to be active for 22-24 days, feeding voraciously on the wood after which it entered the third quiescent phase. The deutonymphal quiescence period lasted for 10-12 days (Table 5). It underwent moulting as in earlier phases, emerging as the tritonymph. The tritonymph, the largest among all nymphal stages, had an active life of 22-26 days. Active feeding by the tritonymph extended the tunnel deeper into the wood (Figs 14 A,B). There was active participation of the various stages of *H. hirsutus* in feeding and the final production of eggs by the matured females (Figs 14 C,D) in laboratory cultures. The fourth quiescent phase extended for 10-12 days (Table 5). The adult emerged after the moulting of the fourth quiescent phase. It appeared brown in colour and produced large numbers of fecal pellets (Fig.15 D). The total development from the egg to the adult stage ranged between 141-154 days for *H. hirsutus* (Table 5).

Field trial of soil fertility enhancement

The impact of oribatid mite feeding on plant materials enabled decomposition of these components and the increase in soil fertility was assessed by using a field experiment on *V. unguiculata* (Figs 8. A-D). Table 6 illustrates the influence of oribatid mite feeding on vegetative growth of the plant with reference to the increase in length of the plant. The rate of increase in plant length was much higher in the plants grown with oribatid mite exposure than the control. The length of pods was another parameter compared in the field trials. Table 7 shows the difference in pod length of the experimental and control plants. The average difference in pod length of the two sets of plants varied between 1.7 and 2 cm. Apart from this, feeding of preferential food items encourages better life activities rendering sufficient encouragement for reproductive potential. Such activity may increase the rate of degradation. This in turn could increase the rate of soil mineralisation also (Figs 9. A-H).

DISCUSSION

Soil is a unique and complex entity supporting the growth and development of all floral and faunal communities. It provides a holistic platform for the life activities of innumerable organisms including autotrophs and heterotrophs. The soil ecosystem acts as a reservoir for many materials, including old and dead components of flora and fauna being deposited continuously. These

Table 4. Postembryonic development of *A.* (*H.*) *chaliyamensis* under laboratory conditions

No	E	L	IQ	PN	IIQ	DN	IIIQ	TN	IVQ	Total
1	4	11	3	14	3	17	5	19	6	82
2	4	12	4	15	4	18	5	20	6	88
3	5	12	4	15	4	18	5	20	6	89
4	4	11	3	14	3	17	4	19	6	81
5	4	11	3	14	3	17	4	19	6	81
6	5	12	4	15	3	18	5	20	7	89
7	4	11	3	14	3	17	4	19	6	81
8	4	11	3	14	3	17	4	19	6	81
9	4	11	3	14	3	17	4	19	6	81
10	4	11	3	14	3	17	4	19	6	81
Range	4-5	11- 12	3-4	14-15	3-4	17-18	4-5	19-20	6-7	81-89

E: Egg, L: Larva, I-IVQ: Quiescent periods, PN: Protonymph, DN: Deutonymph, TN: Tritonymph

Table 5. Postembryonic development of *H. hirsutus* under laboratory conditions

No	E	L	IQ	PN	IIQ	DN	IIIQ	TN	IVQ	Total
1	22	16	9	19	9	22	10	26	11	144
2	23	16	9	19	9	22	10	22	11	141
3	23	16	9	19	10	24	10	22	10	146
4	26	16	11	21	9	22	12	25	10	152
5	23	18	9	19	11	24	12	26	12	154
6	23	18	9	19	11	22	10	22	11	145
7	25	16	12	22	9	22	10	22	11	149
8	23	17	12	21	10	22	10	24	11	150
9	23	16	9	19	9	23	10	26	10	145
10	22	16	9	19	9	22	12	22	11	142
Range	22-26	16-18	9-12	19-22	9-11	22-24	10-12	22-26	10-12	141-154

E: Egg, L: Larva, I-IVQ: Quiescent periods, PN: Protonymph, DN: Deutonymph, TN: Tritonymph

Table 6. Comparison of the length of *Vigna unguiculata* plants (in cm) grown under oriculture with control plants (up to 60 days age)

Age of plants in days	Control	Experimental	Difference in length (in cm)
10	55.2	62.6	7.4
20	61.0	96.8	35.0
30	72. 8	162.4	89.6
40	88.9	220.6	131.7
50	104.6	296.2	191.6
60	154. 2	324. 5	170.3

*Average of 10 plants

Table 7. Comparison of pod length (in cm of *Vigna unguiculata* in oriculture and control trials

No.	Experiment I	Control I	Difference in length	Experiment II	Control II	Difference in length
1	24	22	2	23	22	1
2	25	20	5	24	21	3
3	23	23	0	25	20	5
4	24	22	2	24	22	2
5	22	21	1	25	22	3
6	24	20	4	22	19	3
7	24	23	1	23	21	2
8	23	22	1	24	22	2
9	22	22	0	22	22	0
10	24	23	1	24	24	0
Average	23.5	21.8	1.7	23.6	21.6	2.0

structural elements of living organisms are a rich source of nutrients but in a bound condition. Therefore, release of the bound nutrients and energy in the waste materials of living organisms is crucial for the maintenance of the ecosystem.

This essential need of the ecosystem is being fulfilled by the collective efforts of the detritivorous faunal components, particularly the arthropod community inhabiting the soil ecosystem. Arachnids have been identified as the most important mediators of the process of decomposition in the soil (Tadros, 1976).

Oribatid mites represent one of the active links in the decomposer food web by playing multiple roles in the decomposition process. They are the only group among the arachnid members that are contributing to the soil structure (Norton, 1985).

The microscopic size and lower energy requirements of *A.* (*H.*) *chaliyamensis* and *H. hirsutus* make their contribution to the process of decomposition less recognizable than that of other arthropods and annelids but this seeming disadvantage is compensated by their abundance, diversity and adaptability (Tadros, 1976; Wallwork, 1976; Mitchell and Parkinson, 1976; Petersen, 1982a,b; Petersen and Luxton, 1982; Haq, 1994; 2007a). The unique adaptive strategies of oribatid mites have helped them in the invasion and colonization of many habitats, including those which are generally not suitable, as in the case of the Arctic region (Behan-Pelletier and Hill, 1978). Surprisingly, the mites in the present study, viz. *A.* (*H.*) *chaliyamensis* and *H. hirsutus* have acqired considerable environmental adaptability, even as marine or littoral zone inhabitants. Therefore, the contribution of this acarine group to the process of nutrient cycling in the soil ecosystem cannot be overlooked.

Three major feeding guilds have been identified among the oribatid mites, based on the type of food materials they prefer within the soil habitat (Schuster, 1956; Hartenstein 1962; Luxton, 1972; Haq, 1994, 1996). Of these, microphytophages represent the group with direct involvement in the litter decomposition process while the macrophytophages facilitate the process indirectly and panphytophages play a dual role, both direct and indirect (Haq, 1987). Materials of higher plant origin like dried leaves, twigs and decaying wood and roots constitute the principal food items of macrophytophages (Luxton, 1972; Behan-Pelletier and Hill, 1978; Haq, 1982; Haq and Ramani, 1991; Ramani and Haq, 1991; Haq, 1994, 2007a, 2016). The macrophytophagous oribatid mites are armored with strong, well-developed mouth parts for the trituration of woody materials. The rate of consumption is determined by several factors such as the nutrient content of the materials, nutritional requirements of the species concerned and also food processing and assimilatory efficiencies of the mite species (Haq, 2016). The nutritive value of the three principal categories of food materials in the decomposition food web decreases in the order of fungi > foliage material > woody elements (Slansky and Scriber, 1985). Nitrogen is an important nutritional requirement for all organisms. The organic matter of plant origin is poor in nitrogen in comparison with fungi. The nitrogen content of 36.2g of wood has been equated to that of 1g of fungal spores (Merrill and Cowling, 1966). The proportion of digestion among the detritivores is 6-35% against that of 42-97% in the case of the fungal feeders (Berrie, 1976; Cummins and Klug, 1979). This shows relatively poor processing ability of the former. In addition, the macrophytophagous oribatid mites have poor food assimilation efficiency that ranges between 10-15% against that of the 50-65% assimilatory efficiency of the microphytophages (Luxton, 1972). The dependence of these mites on food items of low nutrient content, coupled with their poor digestive and assimilatory powers, demands the consumption of enormous quantities of food. This is a positive factor from the ecological point of view as the contribution of these mites towards biodegradation of litter in the soil ecosystem will be always higher when compared to other feeding guilds of oribatids (Haq, 1994).

Oribatid mites exhibit wide ranging nutritional habits within the soil ecosystem (Schuster, 1956; Hartensten, 1962; Woodring, 1963; Shereef, 1970; Luxton, 1972; Haq and Prabhoo, 1976; Haq, 1982, 1994, 1996, Maraun et al., 1998). This imposes selection of preferred microhabitats and distribution pattern for individual categories of these mites so as to ensure the availability of surplus food for each species. In the current study, collection localities of

Figure 9. Oribatid mites and their feeding on decaying wood and leaf litter – A) *A*. (*H*.) *chaliyamensis* initiating feeding on decaying wood piece offered as food, B) Cut opened wood piece showing feeding galleries of *H. hirsutus*, C) Feeding galleries of *H. hirsutus* filled with fecal pellets, D) Mass of fecal pellets formed after feeding on leaf litter by *H. hirsutus*, E) Skeletonized leaf, F) Eggs of *H. hirsutus* in laboratory cultures, G) Immatures of *H. hirsutus* feeding on leaf tissue, H) *A.* (*H.*) *chaliyamensis* colonizing a piece of dried root offered for feeding in the laboratory culture.

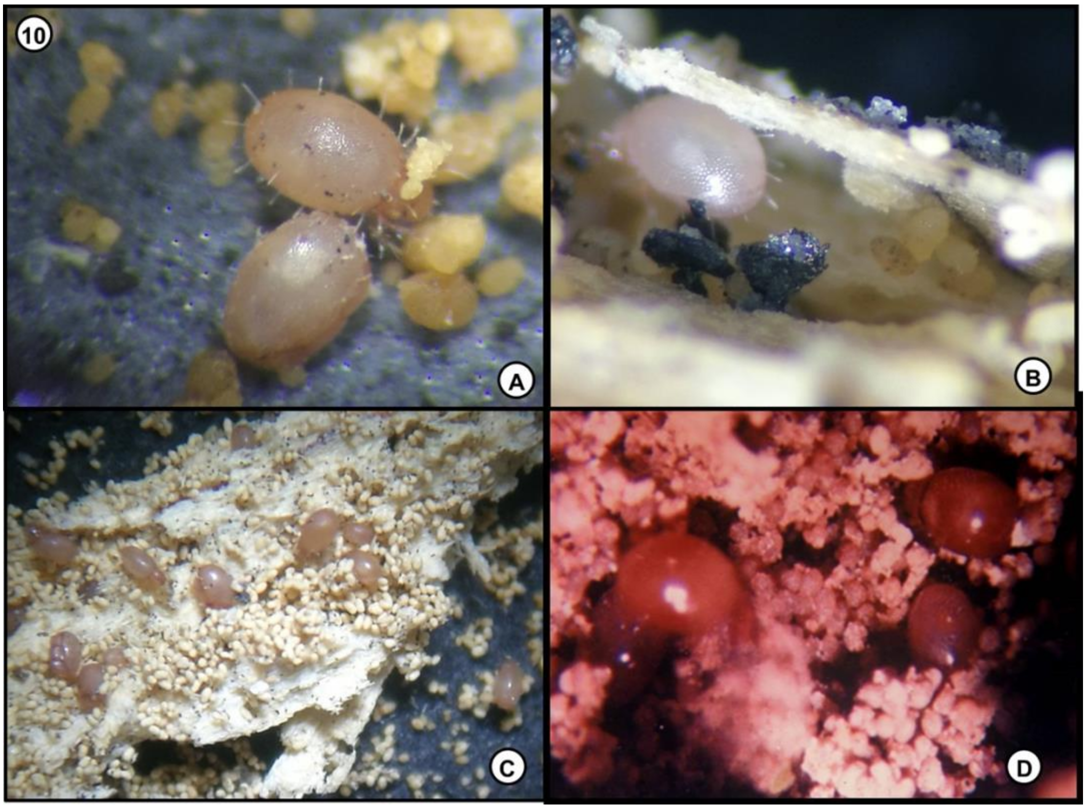

Figure 10. Degradation of wood pieces by *A*. (*H*.) *chaliyamensis* – A) Active adults, B) Formation of channel in the wood by vigorous feeding, C) Trituration of wood by the mite, D) Fecal mass produced by larvae and nymphs after feeding.

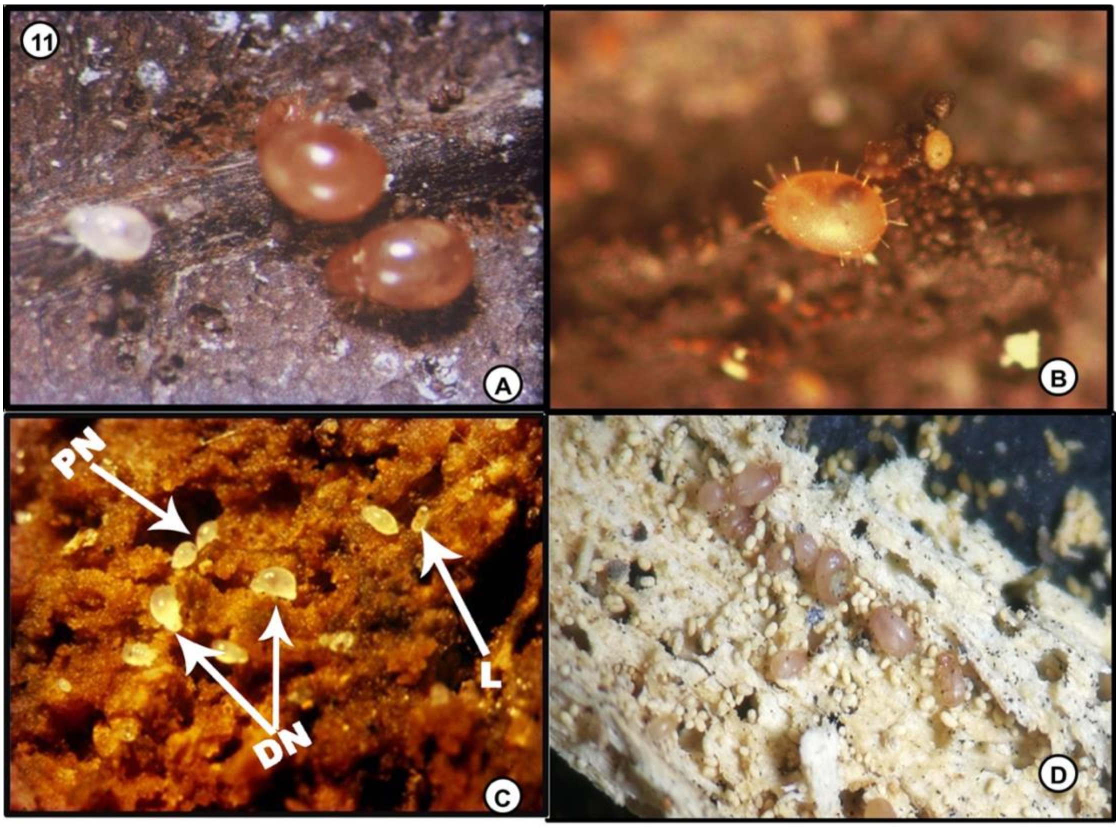

Figure 11. A) Continued feeding on bark tissue by *A.* (*H.*)*. chaliyamensis*, B) Production of fecal pellets after feeding on bark tissue,, C-D) Larval and nymphal stages of *A.* (*H.*) *chaliyamensis* from the channels and pits created by the feeding activity (L: Larva, PN: Protonymph, DN: Deutonymph).

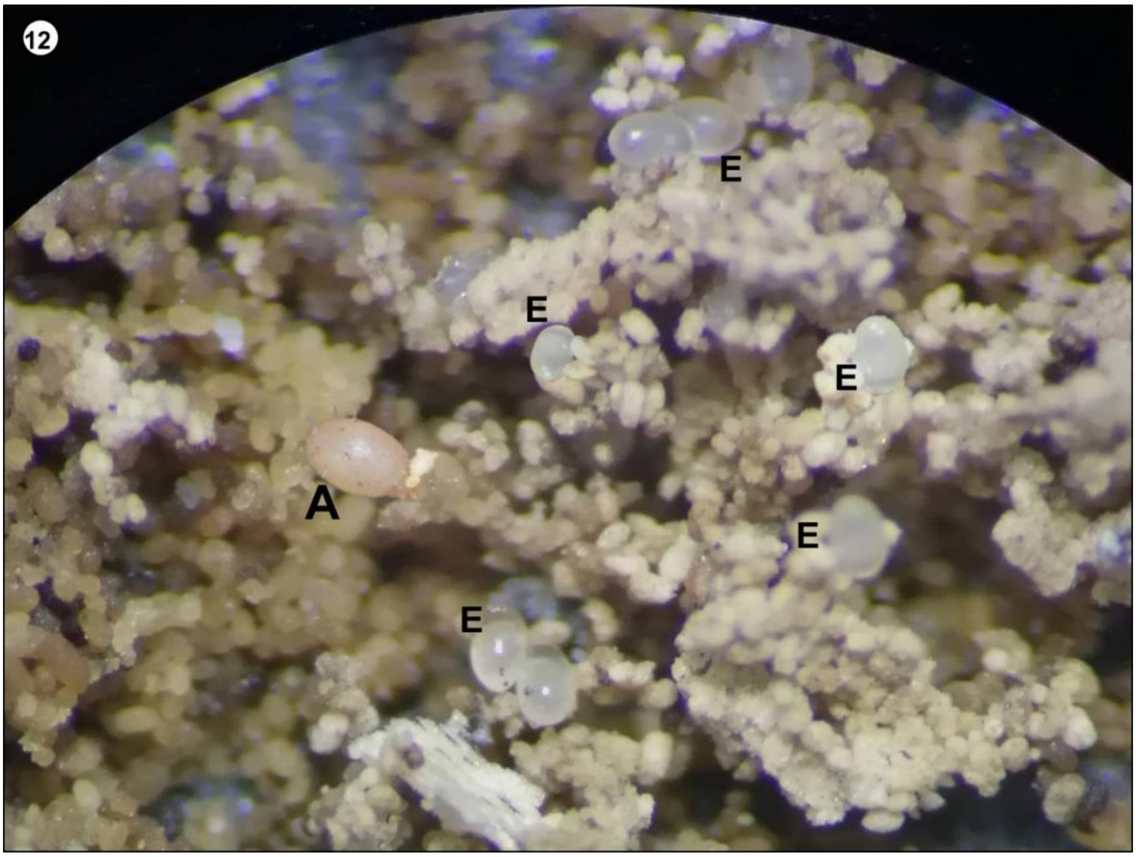

Figure 12. Eggs of *A.* (*H.*) *chaliyamensis* laid among fecal pellets in the laboratory (A: Active adult of *A.* (*H.*) *chaliyamensis*, E: Eggs).

Figure 13. Active adults of *H. hirsutus* (A-B) among fecal mass they produced in laboratory culture.

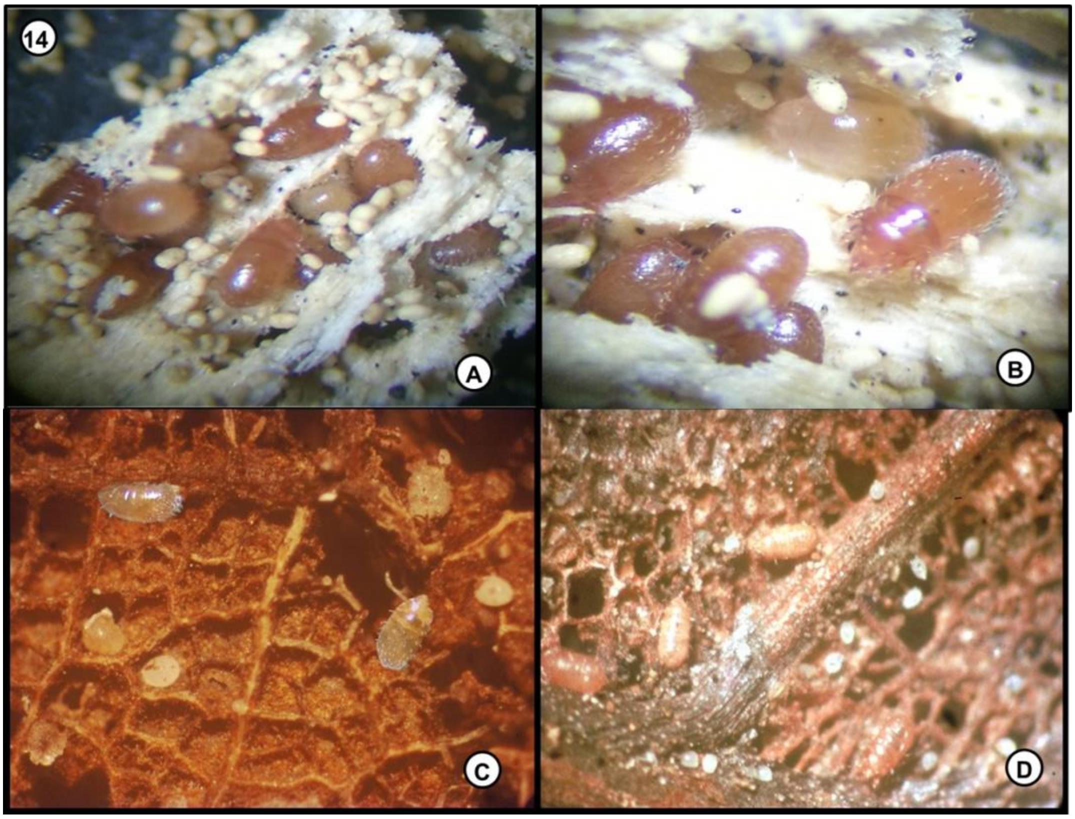

Figure 14. Wood and leaf litter feeding by *H. hirsutus* in the laboratory–A,B) Channels (A) and holes (B) produced in wood pieces by the feeding activities of *H. hirsutus* (Note the presence of larval and nymphal instars packed in pits and channels of the wood pieces given for feeding), C) Adults with eggs on softened leaf lamina offered as food, D) Eggs packed between veinlets in laboratory cultures.

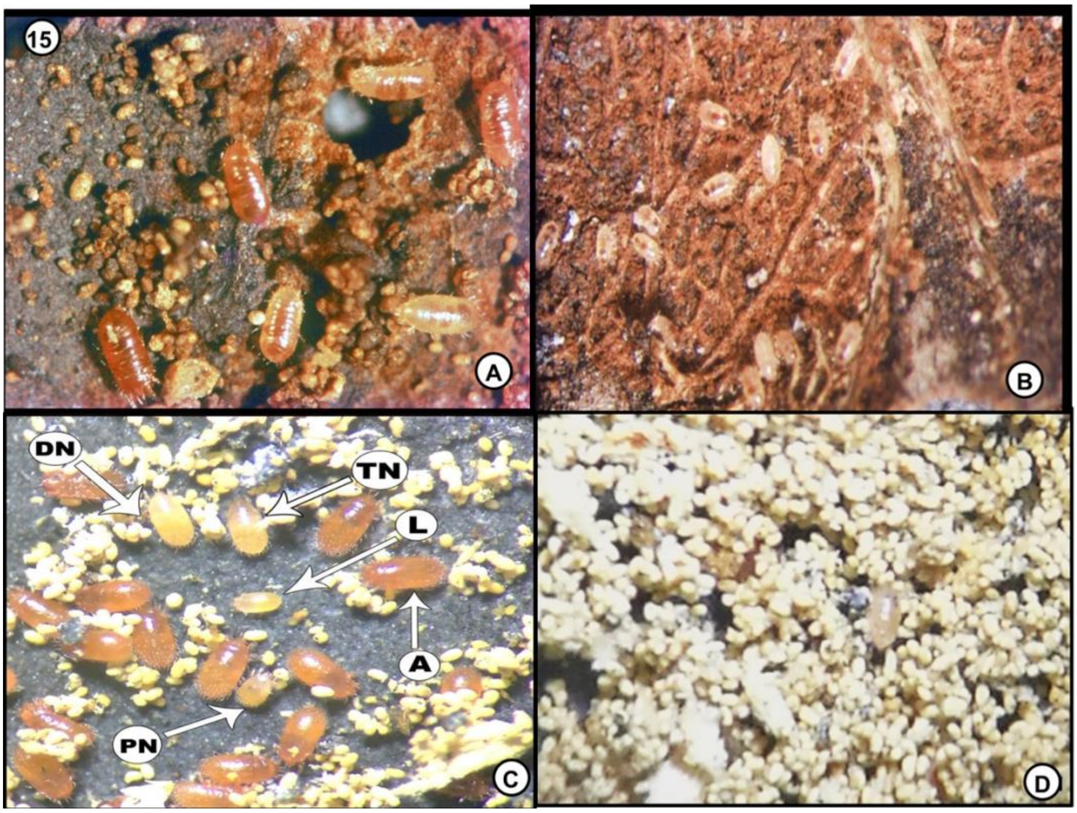

Figure 15. A) Other members of lomanniid mites cultured for similar studies, B) Active participation of various stages of *H. hirsutus* during feeding on leaf tissue and producing eggs, C) A: Adult, L: Larva, PN: Protonymph, DN: Deutonymph, TN: Tritonymph, D) Mass production of fecal pellets after feeding on wood by *H. hirsutus*.

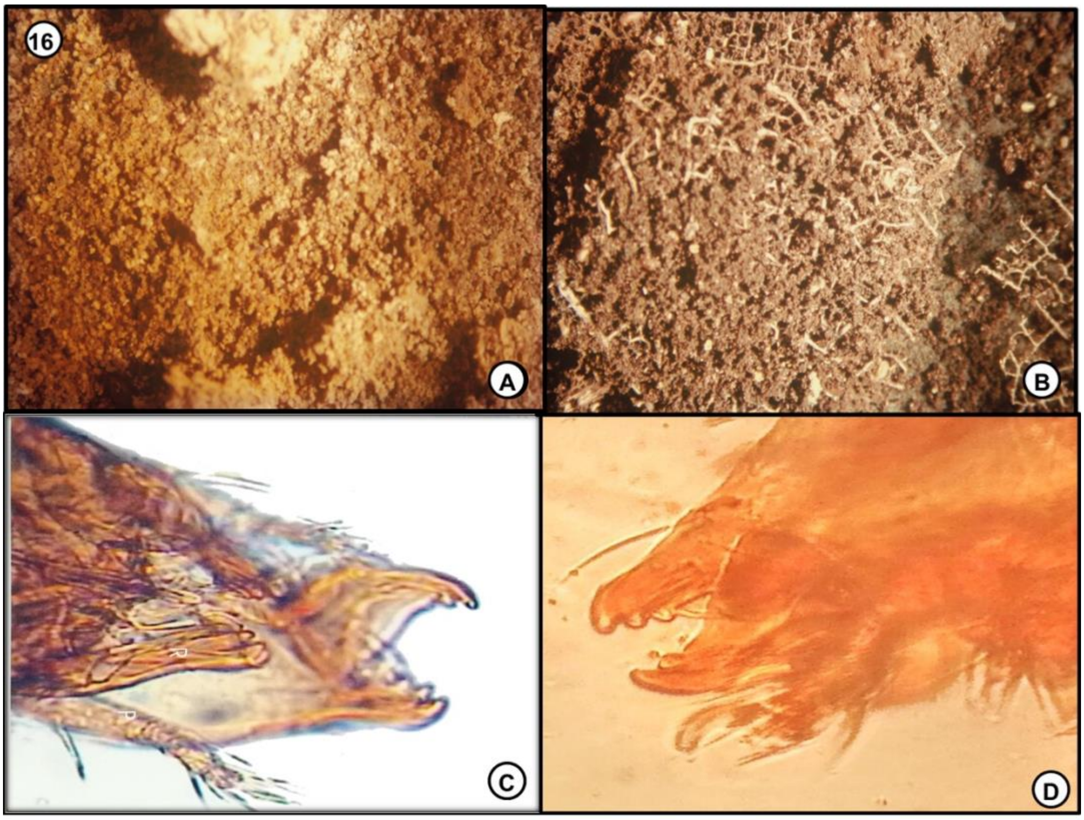

Figure 16. A) Mass production of feces by *A. (H.) chaliyamensis* after feeding on wood under laboratory conditions, B) Mass production of fecal material by *H.hirsutus* after simulatneous feeding on wood and leaf litter, C) Chelicera of *A.* (*H.*) *chaliyamensis*, D) Chelicera of *H. hirsutus*.

the mites comprised of secondary forest ecosystem, grassland, agriculture and monoculture land and beach soil, presenting varied abiotic and biotic factors. Three species of mites, namely *A. longisetosus, O. kuhnelti* and *S. minuta* exhibited their presence across all the four localities having distinct environmental conditions, thereby presenting the eurytypic distribution pattern referred by Wallwork (1976) for species of *Oppia* and *Scheloribates*. Further investigation on feeding habits of these mites have indicated the prevalence of panphytophagy among them. This feeding habit may be owed to their eurytypic distribution as hypothesized, based on various research (Luxton, 1972; Harding and Stuttard, 1974; Behan-Pelletier and Hill, 1978).

The classic nutritional habits assigned for the soil oribatids, namely macrophytophages, panphytophages and microphytophages (Schuster, 1956; Hartenstein, 1962; Luxton, 1972) could be observed among the mite species investigated in this study as well. Macrophytophagy was the dominant food habit among the mites screened during the current study. This trend was recorded among 68% of the species, followed by panphytophagy (19%) and microphytophagy (13%). Although the basic organisation and pattern of the gnathal structures are the same in *A.* (*H.*) *chaliyamensis* and *H. hirsutus*, certain variations of these structures were obvious and can be related to their food and feeding habits. The presence of the vestibule on the rutella observed in *A.* (*H.*) *chaliyamensis* can be related to the xylophagous feeding of the species, as suggested by Woolley (1967). The prominent and strong teeth on the chelicerae of this species resembled the teeth reported among other species of this genus (Dinsdale 1974). The stenarthric infracapitulum with its highly reduced mentum allows maximum rutellar mobility laterally. Dinsdale (1974) noted that the rutellae normally envelop the retracted chelicerae but during feeding the chelicerae are protruded and the rutellae diverge as a result of the associated deformation of the infracapitulum. Furthermore, Grandjean (1957) suggested that during the retraction of the chelicerae the rutellae converge to scrape their outer surfaces and the resulting particles are collected by the mouth. The structure and organization of the mouthparts of *A.* (*H.*) *chaliyamensis* has provided evidence for its ability to triturate comparatively large and stout wood pieces very effectively, involving the application of the maximum power of the rutellae. The involvement of hemolymph pressure and anal and adanal musculature in the defecation process of oribatid mites has been reported (Heethoff and Norton, 2009) and matches with the anatomy of the macrophytophages.

In the current study, both the species of mite showed a preference for the drift wood collected and showed similar feeding activity, with tunnelling into the wood and laying eggs within the tunnels. The wood feeding tendency of *H. hirsutus* is exceptional among the lohmanniid members but was reported by the author earlier for this species (Haq, 2007a). Gut enzyme analysis was used as a complementary assay to confirm the feeding habits of oribatid mites in the laboratory experiments. In a similar study on multiple groups of oribatid mites, Haq and Konikkara (1988) and Haq (2007a) discussed the enzyme profiles of the macrophytophages, panphytophages and microphytophages, and reported that the macrophytophages possess the three carbohydrases, cellulase, cellobiase and maltase. This has been demonstrated again in the current study, confirming the macrophytophage identity of *A.* (*H.*) *chaliyamensis* and *H. hirsutus*.

The occurrence of symbiotic microorganisms in the gut of oribatid mites and their role in the digestion by the latter was reported by Haq and Konikkara (1988) who hypothesized that the microbes were the sources of the enzymes detected in the gut of these mites. *In vitro* cellulose digestion by gut microbes from oribatid mites was reported by the author later (Haq, 2007a). During the current study, diverse microbial colonies were isolated from the gut contents and fecal pellets of both the species of mites, indicating their role in the digestion of the cellulose rich food consumed by these mites. The presence of carbohydrases among a wide range of oribatid mite species and determination of their feeding guilds based on their enzyme profile was reported with more narrow levels of categorization splitting the classic macrophytophage, panphtophage and microphytophage categories into 9 feeding guilds (Siepel and de Ruiter-Dijkman, 1993).

The contribution of oribatid mites to the biodegradation of soil organic matter at the functional level is undisputed and well-illustrated in many terrestrial ecosystems like tropical forest soil, temperate forest soil, grasslands, peat soil and even degraded ecosystems like mines. However, quantitative assessment of the extent of litter biomass turnover by these mites is meagre, though the concept was demonstrated with artificial food (i.e. filter paper) offered to *H. hirsutus* (Haq, 1987). Considering this information gap, the current study estimated the loss of biomass due to the feeding activity of two mite species under laboratory conditions. There was an average reduction of 8.8% and 7.5% in the mass of the wood tissue offered as food to *A.* (*H.*) *chaliyamensis* and *H. hirsutus*, respectively, within a period of 2 to 3 days. This type of study on more species is essential in assessing the contribution of these mites to the biodegradation process.

Chemical analysis conducted in the present study showed a general increase in the concentration of the three macronutrients tested. This has established the potential of oribatid mites in the enhancement of soil fertility through the enzymatic breakdown of litter components in their gut and subsequent release of nutrients to the environment. Information regarding this crucial role played by oribatid mites was provided by a number of earlier workers (Schuster, 1956; Wallwork, 1958; Hartenstein, 1962; Hayes, 1963; Berthet, 1964; Luxton, 1966; Kowal, 1969; Kowal and Crossley, 1971; Hammer, 1972) who stressed the bioprocessing ability of oribatid mites. The relevance of these mites in the recycling of a few essential nutrients like calcium and potassium has been brought to light (Cornaby et al., 1975; Gist and Crossley, 1975; Werner and Dindal, 1987). Norton (1985) reported that feeding by oribatid mites leads to an increase in the nitrogen content of organic litter. Haq (1996) conducted quantitative analysis of certain macro- and micronutrients in selected items of plant litter after its consumption by oribatid mites and reported a general increase in concentration of nitrogen and phosphorus in all the materials tested. Ramani and Haq (2001) showed that the feeding activity of *H. rimosus* and *Lohmannia* sp. increased the nutrient status of the litter of *Artocarpus integrifolia.* The same authors found that both macro- and micronutrient levels increased. These observations, in conjunction with the present findings, signify the involvement of oribatid mites in soil productivity.

The global distribution and diverse habitats occupied by oribatid mites when coupled with their varied feeding habits (Hayes, 1963; Luxton, 1966, 1972; Haq, 1976, 1982; Haq and Prabhoo, 1976; Behan-Pelletier and Hill, 1978, 1983; Ramani and Haq, 1991) affirm the significant role of these mites in the process of nutrient and energy cycling. The importance of rhizophagous (i.e. feeding on dead roots of plants) oribatid mites in soil aeration, drainage and clearing of dead mass of roots in the soil profile has been reported (Rogers, 1939; Ghilarov, 1971) and hence the significance of *A.* (*H.*) *chaliyamensis* and *H.hirsutus* needs special appraisal.

The ability of oribatid mites to degrade plant materials containing complex organic molecules, which they ingest as food, and the mode of achievement was an integral part of this study. Therefore, tracing the mechanisms involved in the hydrolysis of complex plant polysaccharides like cellulose, lignin and chitin was considered essential for ascertaining their impact in the process of decomposition (Hartenstein, 1962). This was evidenced by the production of large quantities of excrements rich in nutrients by *A.* (*H.*) *chaliyamensis* and *H. hirsutus* (Figs 16 A, B). The decrease observed in the cellulose content of *Quercus* leaf litter after digestion by oribatids like *Hermanniella* sp. and *Peloribates* sp. (Gasdorf and Goodnight, 1963) indicated the cellulolytic activities of this group of mites. Synthesis of the enzyme cellulase is rare among animals. Generally, this unusual capacity is possessed by microbiota. Very few organisms, including silverfish (Lasker and Giese, 1956) and a marine crustacean (Boyle and Mitchell, 1978), possess the ability to digest cellulose without the help of microbes (Waller and La Fage, 1987). Confirmation of this theoretical view was achieved through the enzyme assay of 14 species of oribatid mites (Luxton, 1972; Haq and Konikkara 1988, Haq, 2007a) which established the presence of several carbohydrase groups of enzymes in their intestines. Further evidence of the presence of enzymes like cellulase, carboxymethyl cellulase, xylanase and pectinase that are capable of breaking down plant structural polysaccharides has also been reported (Haq and Konikkara, 1988). Meanwhile, a contradictory result establishing the absence of cellulase activity and cellulolytic gut symbionts in the case of Phthiracarid members has also been provided (Dinsdale, 1974). The presence of cellulase, glucosidase, galactosidase, trehalase, raffinase and lactase in the gut of the xylophagous mite, *H. hirsutus,* was correlated with preferential wood feeding and also the ability to digest ingested fungal particles (Haq, 1984).

Obviously, the above information has clearly revealed the fact that large quantities of litter of higher plant origin supported a rich population of phthiracarid and lohmanniid mites. Therefore, further extention of field cum laboratory studies have been followed on two representative members of the above families. These two groups of mites have been identified as macrophytophages (Haq and Prabhoo, 1976; Behan-Pelletier and Hill, 1978; Haq, 1982, 1984, 1987, 1994, 1996; Haq and Konikkara, 1988). A majority of lohmanniid mites are phyllophagous, which restricts them to areas where leafy litter is available. However, the lohmaniid species of the current study, *H. hirsutus*, is an exception that prefers the woody tissues of higher plants, particularly driftwood and roots of *C. gigantea* in the beach ecosystem (Haq, 1987, 1994, 1996; Seniczak et al., 2018). Therefore, this species can be assigned to the subcategory of xylophages of the major feeding guild, macrophytophages. The members of the family Phthiracaridae are typically known for their wood feeding habit categorized as xylophagous (Haq, 2007a). The results of the gut enzyme profile and microbiome assay of these mites in the current study have provided evidence of their competency in the decomposition of the cellulose rich woody tissues of the higher plants. The actual role of these mites in mineralization and nutrient cycling has been established through the increased major macro- and micronutrient content of the partially digested woody tissue defecated by the mites, as reported for other oribatid mites (Haq, 2007a, 2016).

The current study recorded an increase in macronutrients in the woody litter from the collection sites after its digestion by the two species of mites studied. The trend in percentage increase of the macronutrients in the descending order in the experimental trials was N>P>K. However, the concentration of Ca and Mg decrease after digestion by the mites. The reduction in the Ca content may be attributed to the requirement of the nutrient by these mites. A similar study on the effect of the mite *Scheloribates moestus* Banks feeding on corn and oak litter reported an increase in the quantities of polysachharides and phenols in the fecal matter produced after feeding on corn litter but no change in polysaccharide and phenol contents in faecal pellets of those fed on oak litter, indicating the influence of the type of litter on the process of decomposition and turnover of nutrients associated with digestion (Wickings and Grandy 2011). Hence it appears that more investigations on nutrient conversion in the litter of same plant species by different species of mites and vice versa are required in order to elicit specific details of the nutrient release achieved through digestion by oribatid species during litter decomposition. The experimental evidence of nutrient enhancement of soil by oribatid mites and the potential scope of their use for enhancement of soil fertility using the okra plant as a model system (Haq, 2016) confirmed the nutrient enrichment of plant litter through decomposition by oribatid mites at a gross level.

Further studies carried out on the driftwood pieces, litter and roots of *C. gigantea* collected from Chaliyam and Beypore beaches supported the full growth and reproduction of *A.* (*H.*) *chaliyamensis* and *H. hirsutus* in laboratory cultures. This provides confirmatory evidence for their xylophagous habit, as breeding activity and persistence are the ultimate standards in assessing the food preference of oribatid mites (Haq 1984, 1994, 2007a). The total period of ontogeny ranged from 81-89 days for *A.* (*H.*) *chaliyamensis* and 141-154 days for *H. hirsutus* in the current study. The duration of ontogenic development of oribatid mites is highly dependent on the type of nutrition (Brückner et al., 2018). The development of certain species of mites can take more than one year (Schatz, 1985). The duration of ontogeny of both species studied in the present investigation is comparatively higher with reference to the species of oribatids in the tropics. The longer developmental period of the members of Phthiracaridae and Lohmaniidae appears to be advantageous to the ecosystem when viewed from the angle of litter degradation, as the feeding and efficiency of the nymphal stages of *H. hirsutus* have been rated as par with that of the adult (Haq, 1987). In considering the feeding habit, enzyme profile, microbiome of the gut and nutrient turnover efficiency of *A.* (*H.*) *chaliyamensis* and *H. hirsutus,* the current study has established their competency beyond doubt in the bioprocessing of plant litter and enhancing soil fertility. The longer developmental period of the mites complements their niche in the soil ecosystem. Therefore, these two species have fulfilled the purpose of a model system by demonstrating biodegradation, nutrient release and enhancement of soil fertility.

Extension of the laboratory feeding and breeding studies of the mites was made through a field trial on soil fertility enhancement using *V. unguiculata* as the model plant and *A.* (*H.*) *chaliyamensis* to validate the concept of “oriculture farming practice” (Haq, 2007a,b, 2016). Establishment of a population of mites involved in biodegradation is essential for oriculture farming. Biotic potential is an important determining factor for population growth of any species. The availability of preferred food in surplus is one of the key factors governing biotic potential in the environment. Both the oribatid species used in this study produced a large numbers of eggs and a large population in the laboratory cultures. This facilitated the tracing of their ontogeny in the laboratory, proving their ability to produce large populations when their preferred food is available in surplus.

The final phase of bioprocessing of organic residues in the soil is the release of previously bound nutrients in them in a form freely available for absorption by plants. The macro-, meso- and microfauna of the soil bring about this transformation through their synergistic activities, mostly linked to their nutrition and growth. The contribution of each category of soil fauna towards nutrient cycling is determined by the quantity, quality and composition of the nutrients released during their bioprocessing activities. This aspect is well demonstrated primarily by the preference for woody elements in the case of *A.* (*H.*) *chaliyamensis* and *H. hirsutus* and secondarily by leafy elements of the litter by *H. hirsutus.* Biodegradation ensures the availability of the essential nutrients in soil for plant growth. Timely absorption of optimal amounts of nutrients optimizes plant growth and productivity. Vegetative growth, flowering and seed production are considered the milestones in plant life and are used as performance indicators for the assessment of agricultural productivity. The present study used plant height and pod length as the measurable indices for assessing the productivity of the vegetable crop *V. unguiculata* for the evaluation of the impact of oriculture in soil fertility enhancement.

The current study has demonstrated that oriculture has a positive impact on the vegetative growth of the selected plant *V. unguiculata* through a clear enhancement in the rate of vegetative growth of the plant at different age levels, as envisaged by length of the twiners plant height and pod length as stated above. Oriculture influenced the productivity of the plants as well, with enhanced growth of the pods produced by the treated plants, as per the results of the present study. This is comparable to the outcomes of earlier small scale trials on the same plant where reduction in flowering time and increase in average biomass and number of pods produced by *V. unguiculata* was demonstrated. However, the lohmanniid mite *Meristacarus degradatus* was used as the test species for demonstrating oriculture (Haq, 2007b). The same author (2016) further confirmed the value of oriculture with okra plants by using five species of oribatid mites. Therefore, the potential of oribatid mites to contribute to soil fertility enhancement and organic farming through oriculture (Xavier and Haq, 2005; Haq, 2007a, b, 2016), the long awaited result aimed during the study has been evidenced. The diverse ecological niches occupied by oribatid mites and their ability to tolerate lower soil humidity levels when compared to earthworms make oriculture practice complementary to vermiculture in supporting sustainable agriculture.

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RESEARCH ARTICLE

Oribatid mites as potential predators of the root knot nematode, *Meloidogyne incognita*

Nithinya RAMAKRISHNAN [1,3], **Ramani NERAVATHU** [2]

[1] *Department of Zoology, Sree Narayana College, Kannur, Kerala, India*
[2] *Department of Zoology, University of Calicut, Kerala, India*
[3] Corresponding author: rnithinyasnc@gmail.com

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ABSTRACT: Oribatid mites, which represent the most dominant group among the soil mites, have considerable diversity in their feeding habits. Nematodes quite often serve as the most favoured prey choice for oribatids, a situation which could be better exploited in biocontrol strategies. Hence the present study was carried out to explore the predatory habits of selected species of oribatid mites on a notorious nematode pest, namely the root knot nematode, *Meloidogyne incognitia*, under laboratory conditions. The study was commenced by collecting soil samples from various localities of Kannur and Malappuram districts of Kerala, India in the period March, 2014 to February, 2015. Among the various species of oribatid mites extracted, three *Scheloribates* species, *viz. Scheloribates* (*Scheloribates*) *praeincisus* (Berlese, 1910), *Scheloribates fimbriatus africanus* Wallwork, 1964 and *Scheloribates* (*Scheloribates*) *latoincisus* Hammer (1973), were selected for studies of their predatory potential on the second stage juveniles (J2 juveniles) of *M. incognita.* During feeding experiments each of the three mite species was offered 20 J2 juveniles of *M. incognita* in petri dishes containing 2% sterilized water agar. Observations were made on the feeding behavior of the species, including rate of consumption, for comparative evaluation. The per day percentage consumption of J2 juveniles by *S.* (*S.*) *praeincisus*, *S. fimbriatus africanus* and *S.* (*S.*) *latoincisus* were 57.00 ± 7.3598, 60.00 ± 6.3738 and 57.00±5.9675, respectively. Statistical analysis following one way ANOVA and Turkey's HSD Post Hoc Test, showed no significant difference in the consumption rates among the species ($P = 0.452 \geq 0.05$). It is concluded that the three *Scheloribates* spp. have equivalent potential to suppress root knot nematode populations.

Keywords: Oribatid mites, predation, root knot nematode, *Scheloribates*, biocontrol.

INTRODUCTION

Soil is a complex and dynamic ecosystem that contains diverse floral and faunal communities. Faunal components are dominated by arthropods, followed by nematodes. Among the soil arthropods, mites especially oribatid mites, outnumber other mesofaunal elements (Petersen and Luxton, 1982). Oribatid mites have a critical role in the environment, including enhancement of porosity, aeration, mixing of soil and its many microbes, biodegradation of organic residues, nutrient cycling of soil, bioindication of soil health status and pollution, and biocontrol of pests, parasites and weeds (Krantz and Walter, 2009). The elimination of mites was reported to result in a 40% reduction in decomposition, suggesting that mites would affect the decomposition of buried litter by regulating the population size of bacterial grazers and cephalobid nematodes (Gan et al., 2014).

Plant parasitic nematodes feed on all parts of plants. In different ways with their specialized feeding apparatus called the stylet. Root knot nematodes of the genus *Meloidogyne* are the most notorious plant parasites, infesting about 2,000 plant species, including food crops, vegetables, fruit and ornamental plants in tropical, subtropical and temperate regions, causing approximately 5% loss of global crops (Sasser and Carter, 1985). For those *Meloidogyne* spp. that have host ranges that are large (total number of hosts) and broad (large number of plant families with species susceptible to the nematode), there are quite distinct differences in their overlapping host ranges (Perry et al., 2009). Infestation results in symptoms like chlorosis, distortion of root structure and gall formation, and reduced growth and yield. Severe infestation leads to complete deformation of the root system, hindering nutrient and water uptake, which ultimately leads to death of the plant. Populations of plant parasitic nematodes are kept in check by soil arthropods like soil mites, in undisturbed and agricultural ecosystems (Gulati, 2012).

During embryonic development species of *Meloidogyne* passes through four juvenile stages (J1-J4) before final transformation into adult. The first stage juvenile molts within the egg, embedded in the root of the host plant and hatches as a second stage juvenile. After hatching, the motile infective J2juvenile stage migrates through the soil and may re-enter the same host plant or enter the root of another suitable host plant (Eisenback and Triantaphyllou, 1991).

An early study on the biocontrol of nematodes was made by Linford and Oliveira (1938), they collected many biotas that were antagonistic to the root-knot nematode, *M. marioni* in Hawaiian soils. Their studies presumed six mites of undetermined taxonomic status to restrict nematodes. Since then, many nematophagous mites obtained from several acarine cohorts have been documented. Apart from the disciplinary disjunction between soil acarology and nematology, incorrect assumptions have delayed understanding of the strong interaction between mites and nematodes. It was generally believed that mites and nematodes rarely come in close proximity which would inhibit the evolution of complex relationships like predation to develop between them. Nematodes are slender and soft bodied, and they can enter very small soil-pore spaces unavailable to mostly broader and less flexible mites. However, many nematophagous mites are elongate, flexible, and capable of extruding their chelicerae a considerable distance into small pores (Walter and Proctor, 2013).

Nematophagy has been reported in various groups of mites like the Mesostigmata, Astigmata and Cryptostigmata (Rocket et al., 1966; Rocket, 1980; Koehler, 1997; Bilgrami, 1993; Al Rehiayani and Fouly, 2005; Olivera et al., 2007) but still represents a very poorly explored area of research. Hence, the present study was undertaken to evaluate the feeding and biocontrol potential of 3 species of mites belonging to the suborder Oribatida on one of the most notorious root knot nematodes, *M. incognita*, which induces root galls in the tomato (*Solanum lycospersicum)*.

MATERIAL AND METHODS

Methods adopted for obtaining root knot nematode

Twenty-five samples of nematode infected tomato roots were collected from each of two localities, namely Kannur and Malappuram districts of Kerala, India in the period March, 2014 to February, 2015. Roots were placed in polythene bags, sealed, labelled and then brought to for examination to the Soil Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India. Root knots were gently teased apart by using a needle to facilitate the collection of males and females. Females were used for identification based on the perineal pattern on the cuticle. J2 juveniles of *Meloidogyne incognita* were extracted with a Sieve-Petri dish setup, which consisted of a plastic sieve placed between two Petri dishes, with the lower Petri dish filled with water. The infested root samples were retained on the plastic sieve and left completely immersed in water overnight. J2 juveniles that hatched out into the water in the lower Petri dish were collected and used for the experiment.

Collection and culturing mites

Twenty soil samples were collected from two districts, Kannur and Malappuram, Kerala, India from March 2014 to February 2015. The samples were collected in polythene bags. Oribatid mites were extracted by using an open brass funnel apparatus (Haq and Ramani, 2002). Live specimens were isolated and then cultured on leaf, wood, moss and litter in cylindrical plastic vials (3.8 cm diameter, 4.5 cm height). The base of the culture vessel was filled to 2 cm depth with a solid mixture of plaster of Paris and charcoal (4:1 ratio). The mites were cultured at room temperature (31^0C, RH 74%).

Processing of mites for taxonomic studies

The mites for taxonomic studies were preserved in 70% alcohol and were dehydrated by passing through an alcohol series (90% and then absolute alcohol). When the mites were completely dehydrated, they were transferred to a clearing medium prepared by mixing lactic acid and alcohol (1:1 ratio). Based on sclerotization, the mites required a few days to several weeks or months for clearing. The cleared specimens were temporarily mounted in glycerine. Permanent slide mounts were prepared in Hoyer's medium. Glass bristles (0.5 mm thick) were added to mounting medium in order to prevent the specimen from being crushed under the weight of coverslip. Mounted specimens were identified by using the following keys (Balogh, 1965, 1972; Balogh and Mahunka, 1983; Balogh and Balogh, 1988, 1990, 2002; Subias, 2012 and edition updated in 2014).

Experimental design of preliminary feeding trials

Screening of mites with predatory potential was carried out in culture cells with a base made from a plaster of Paris – Charcoal mixture (4:1). Twenty J2 stages of *M. incognita* in 0.5 ml tap water were transferred separately to different culture cells. The water was soon absorbed by the Plaster of Paris –Charcoal mixture, leaving the nematodes exposed at the bottom of the culture cell. Two adult female mites of selected species, starved for 24hrs were then released into the cell. The feeding behavior displayed by the tested species of mites was checked under a Stereozoom microscope (Macro Vis, USA - Model No.MVNSZ-405). Behaviour of the mites was noted every hour for 6 hours, after which the number of the remaining J2juveniles was counted. Based on the results of this preliminary feeding study, mites with predatory potential were identified and considered for detailed experiments.

Conduct of feeding experiments

Experiments were conducted in Petri dishes (40 mm diameter and 10 mm deep) containing a 4 mm deep layer of 2% sterilized water agar at room temperature 31^{o}C-32^{o}C and RH 74-82%. Each treatment in every experiment had 4 replicates. The experiment was commenced with the starving for 24 hours of a single female mite (each of the three species) selected during the preliminary studies (day 1 of experiment).

Twenty J2 juveniles of *M. incognita* in 0.5 ml tap water were then added to the central part of the Petri dish. After the water drop had been completely absorbed by the agar, the starved female mite was placed in the Petri dish. Each Petri dish was covered with a piece of parafilm (day 2 of experiment).

The feeding response of adult mite on the nematodes was observed every two hours under a stereozoom microscope. After 24 hrs, the mite was removed and the Petri dishes were rinsed with distilled water three times to collect the remaining nematodes in a counting chamber and the number of nematodes was counted under stereozoom microscope (Macro Vis, USA - Model No.MVNSZ-405). The feeding experiments were carried out with mites that were 35 days old (all the *Scheloribates* spp. used here took a maximum of 33 days for development from egg to adult stage at room temperature 31°C -32°C and RH 74-82%).

The mites were then starved again for 24 hrs (day 3 of experiment) and then provided with nematodes on the fourth day. The experiment was repeated until the 10th day, with each day of nematode inoculation followed by a day of starvation.

Multiple-food choice preference test

The multiple-food choice test, was carried out in cylindrical plastic culture vessels (3.8 cm diameter and 4.5 cm height) and having a base of plaster of Paris - Charcoal mixture (4:1). Adult female mite (each of the three selected mite species) was starved for 48 hrs and placed precisely at the central portion, on the base of plastic vessel. Five live specimens each of *M. incognita* female, male and J2 juvenile were placed at equal distance from each other, at a distance of 1 cm from the mite at the centre. The experiment was carried out in 5 replicates. Feeding behaviour was observed for 1 hr and observations on feeding preference were noted.

Statistical Analysis

The experimental results are presented as mean ± SD. Statistical evaluation of data was done with one way ANOVA and Turkey's HSD Post Hoc Test. Results were considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The present study explored the predatory potential of three species of *Scheloribates* – *S.* (*S*). *praeincisus*, *S. fimbriatus africanus* and *S.* (*S.*) *latoincisus* on J2 juveniles of *M. incognita* (Figs 1A-C). Among the 3 selected *Scheloribates* species selected for study, the highest predation was exhibited by adults of *S. fimbriatus africanus* with a per day average feeding rate of 12.00±1.2748 (60 ± 6.3738%); followed by S. (*S.*) *latoincisus* 11.4 ±1.1937 (57 ±5.9675%) and *S.* (*S.*) *praeincisus*, 10.6±2.3822 (57.500±7.3598) (Fig. 2). The statistical analysis of data by one way ANOVA (with Turkey's HSD Post Hoc Test) revealed no significant difference in the per day feeding rate between the *Scheloribates* species on J2 juveniles of *M. incognita* ($p \geq 0.05$). Therefore, the three *Scheloribates* species appear to have equivalent potential to suppress root knot nematode populations.

In the present study, the per day average consumption rate of the three *Scheloribates* species (*S. fimbriatus africanus*, *S.* (*S.*) *latoincisus* and *S.* (*S.*) *praeincisus*), on J2 juveniles of *M. incognita* was 11.3. This value is less than the mean per day consumption rate (18.3± 0.8 per day) of J2 juveniles of *M. javanica* by adults of *Pergalumna* sp., an oribatid mite (Oliveira et al., 2007). This difference in feeding rate may be attributable to differences in the prey or predator species, or both, and the experimental conditions.

The current study revealed the feeding behavior of *Scheloribates* (Fig. 1D). The mites generally devoured the entire bodies of J2 juveniles but when disturbed the prey were left without completing consumption. Around 60-80 sec was required for complete consumption. While feeding, the mites exhibited up and down as well as side to side movements of the anterior part of the body. No regional specificity to the body of the prey was observed among the *Scheloribates* species; the mites actively moved around the experimental area and initiated feeding at the site of first contact with the prey body.

None of the three selected mites studied exhibited a preference for live adult females of *M. incognita* but fed on adult males of *M. incognita* offered during feeding trials (Fig. 3). The oribatid mites probably did not feed on *M. incognita* females because of the large, pear-shaped body (671 x 600 µm) of females. All the mites studied preferred the J2 stages of the nematode when compared with adults ($p \geq 0.05$). A similar preference for the juveniles compared to females of *Meloidogyne* was reported for predation by oribatid mites (Muraoka and Ishibashi, 1976).

Under natural conditions it would be rare for soil mites to directly encounter adults of *M. incognita* since soil mites are usually restricted to rhizhosphere, while *M. incognita* adults are found within the roots of plants. In this scenario, the biocontrol of *M. incognita* by oribatid mites like *Scheloribates* would appear unlikely, but the J2 juveniles of *M. incognita* hatch into the soil before infesting the root of the same plant or a neighboring host plant.

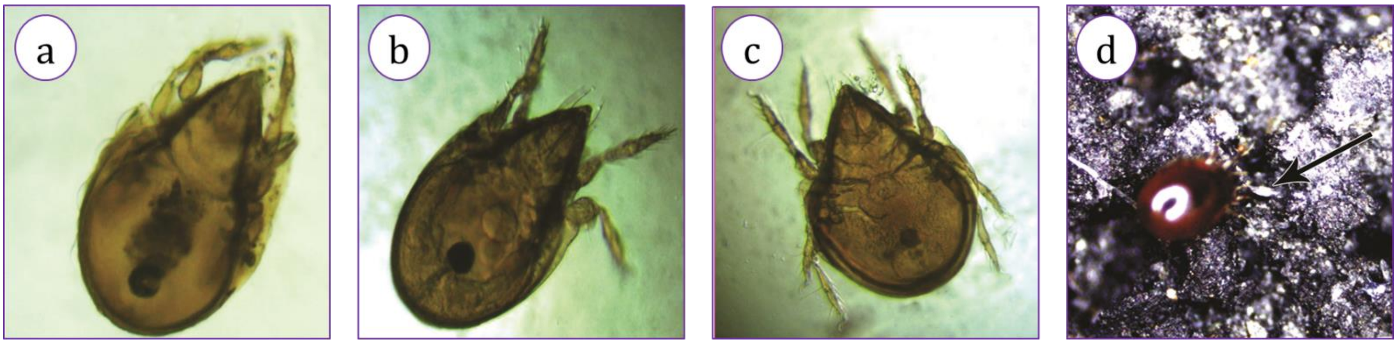

Figure 1. A) *Scheloribates* (*Scheloribates*) *praeincisus* (Berlese, 1910). B) *Scheloribates fimbriatus africanus* Wallwork, 1964. C) *Scheloribates* (*Scheloribates*) *latoincisus* Hammer, 1973. D) *Scheloribates* mite feeding on J2 juvenile of *M. incognita*.

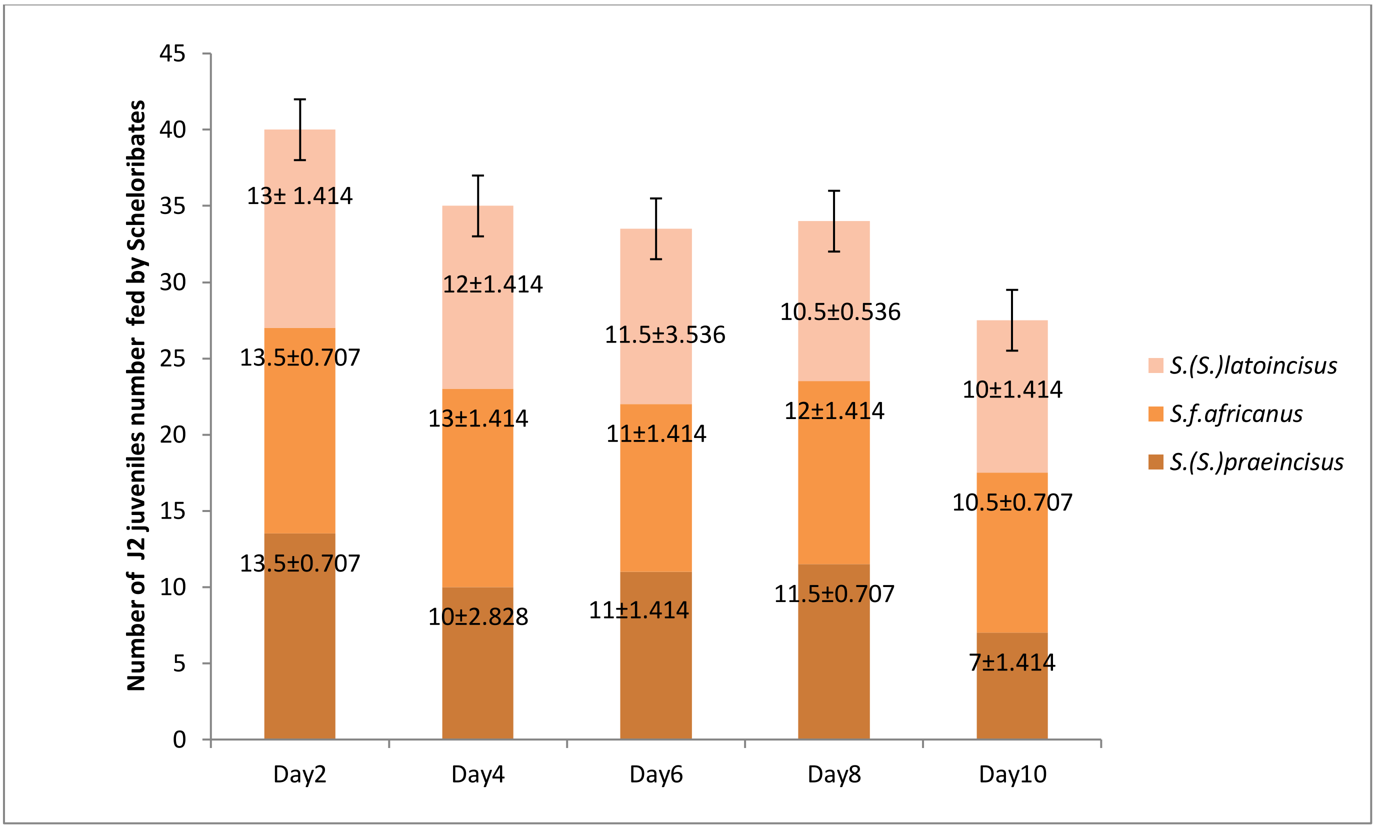

Figure 2. Mean rate of predation of J2's of *M. incognita* by *Scheloribates* species.

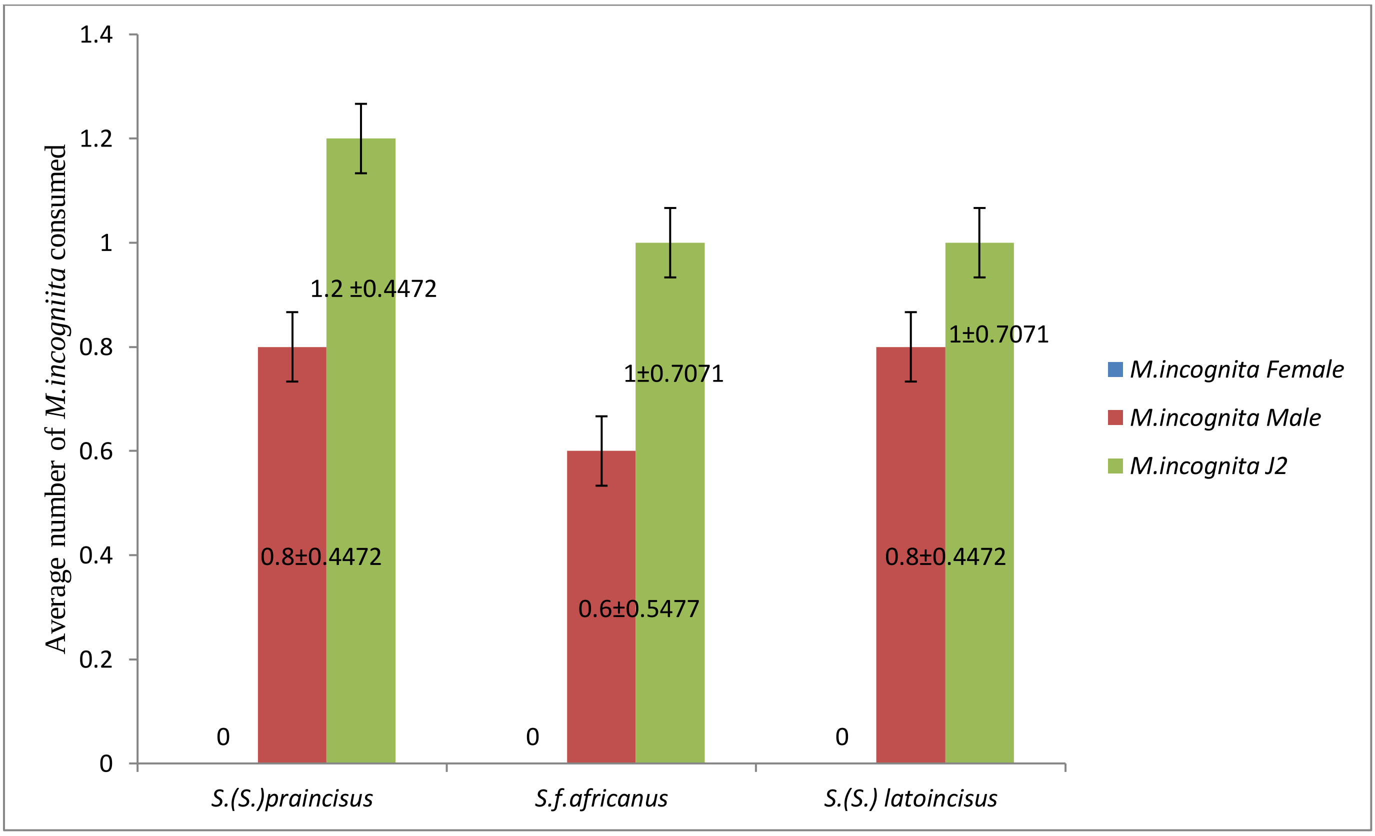

Figure 3. Feeding preferences of *Scheloribates* species on *Meloidogyne incognita*.

The *Scheloribates* mites in the soil could effectively predate on these juvenile nematodes. The predation by oribatid mites on J2 juveniles of *M. incognita* could not only decrease the nematode load in the soil but also reduce the spread to the next host. Moreover, the role of oribatid mites in biodegradation would enhance soil fertility (Haq, 2016). The results of the present study helped to establish three oribatid species as prospective biocontrol agents of *M. incognita*. More laboratory studies and field oriented studies are warranted to explore the potential of predatory mites for inclusion in integrated pest management (IPM) strategies. In addition, the potential of oribatid mites could be exploited not only for the reduction of numbers and spread of root knot nematodes but also for the enhancement of soil fertility and crop production.

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RESEARCH ARTICLE

Raphignathoidea (Acari: Trombidiformes) of Turkey: A review of progress on the systematics, with an updated checklist

Salih DOĞAN

Department of Biology, Faculty of Sciences and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey
e-mail: salihdogan@erzincan.edu.tr

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ABSTRACT: The present paper provides a historical review of the researches on the superfamily Raphignathoidea in Turkey, with an updated list of the species known from Turkey. Most species reported from this country have been published in a wide range of different journals, mainly by Turkish researchers and a few by their colleagues from other countries, so there was a need to combine them in one paper. To date, about 250 papers on the taxonomy, fauna, morphology, biology and other related fields have been published by 90 authors. Presently, Raphignathoidea is represented in Turkey by 217 species in 26 genera of 8 families. The recorded species richness of the Turkish Raphignathoidea is about 20% of the known world Raphignathoidea fauna.

Keywords: Checklist, faunistic, historical review, mite, Raphignathoidea, taxonomy, Turkey.

INTRODUCTION

The Raphignathoidea is a superfamily of the Acari, belonging to the mite suborder Prostigmata in the order Trombidiformes. This group currently contains 11 families, 8 of them have been recorded from Turkey: Barbutiidae, Caligonellidae, Camerobiidae, Cryptognathidae, Eupalopsellidae, Homocaligidae, Raphignathidae and Stigmaeidae (Doğan, 2007; Doğan and Dönel, 2009; Doğan et al., 2014b). The superfamily currently consists of around 1000 valid species in 66 genera (Fan and Zhang 2005, Zhang et al., 2011; Fan et al., 2016, 2019; Akyol, 2017).

Most species which are discovered from this country were published in scattered journals, mainly by the Turkish researchers and a few by their colleagues from other countries (Table 1). Up to date, this group is represented in Turkey by 217 species in 26 genera of 8 families (Table 2). The recorded species richness of the Turkish Raphignathoidea is about 20% of the world Raphignathoidea fauna. The aim of this paper is to provide an overview of researches on the Raphignathoidea of Turkey with an updated list of 217 raphignathoid species and some unidentified species in 9 genera, and to facilitate the future studies of the Raphignathoidea in Turkey.

Researches on raphignathoid mites in Turkey

Historical review

In Turkey, the first studies on this superfamily coincide with the early 1950s. These were agricultural studies related to the mite existence of Turkey in which new records were given. In this group, the first species *Zetzellia mali* (Ewing) was recorded by Düzgüneş (1963). It was known as the generic level in the past by the same author (Düzgüneş, 1954). Then, Özkan et al. (1988) listed the same species referring to these references. Özkan, who was basic contributor to the development of modern Acarology, also made a significant contribution to taxonomy of ticks and water mites in Turkey.

The taxonomic studies on this superfamily started mainly in the mid 1990s and these studies continue. During this time some dissertation studies on this group were written by Koç (1995), Doğan (2002), Kara (2005), Akyol (2007), Kazaklı (2008), Dönel (2010), Özçelik (2010), Somuncu (2012), Uluçay (2013), Karasu (2015), Aksoy (2016), Bingül (2016) and Dilkaraoğlu (2016).

Taxonomic and faunistic research

Taxonomic research on this superfamily was initiated by Dr. N. Ayyıldız and his students Dr. K. Koç and Dr. S. Doğan, and their co-workers published a series of studies from 1996 to 2019. Doğan authored or co-authored many papers and described many species from 2001 to present.

The series of taxonomic studies on the superfamily were published from Turkey mainly by: Koç and Ayyıldız in 1996-2000, Koç in 1999-2005, Doğan and Ayyıldız in 2001-2006, Doğan in 2003-2008, 2019, Doğan and Doğan in 2018-2019; Doğan et al. in 2003-2019, Akyol and Koç in 2006-2017, Dönel and Doğan in 2010-2013, Akyol in 2011-2019, Uluçay and Koç in 2013-2014, Uluçay in 2014-2015, Dilkaraoğlu et al. in 2014-2016, Bingül and Doğan in 2016-2017, Bingül et al. in 2016-2018, Akyol and Gül in 2018.

Doğan (2001-2019) and his collaborators described many species, named 66 species, and recorded 43 species from Turkey for the first time. Doğan and Dönel (2010) recognized Cryptofavognathus as a distinct genus under the family Cryptognathidae Oudemans.

Doğan et al. (2016a) and Bingül et al. (2016) revealed the raphignathoid mite diversity in peridomestic habitats of

Erzincan city center. They digitally mapped the urban mite dispersion areas in the city using GIS spatial analysis techniques based on the locations. Doğan et al. (2019d) showed distribution areas of raphignathoid mite species of Harşit Valley (Turkey) on the maps using by Geographic Information Technology (GIT). Akyol and Özdemir (2016) statistically evaluated the dispersion of raphignathoid mite in Aegean coast, and Akyol and Koç (2016a) evaluated biodiversity of these mites from the interior and the coastal Aegean region.

Ueckermann and Koç (2005) drew raphignathoid specialists' attention to the authorship of this superfamily, according to article 36 of the International Code of Zoological Nomenclature.

Some pure faunistic works from 2003 to present time were performed by Çobanoğlu et al. (2003), Gençer et al. (2005), Kasap and Çobanoğlu (2007), Göven et al. (2009), Akyol (2012a, 2017), Somuncu and Koç (2012b), Dilkaraoğlu et al. (2016g), Doğan et al. (2014c, 2015d, 2017e, 2018d) and Akyol and Koç (2017c).

Some taxonomic problems

Many species are still unknown and undescribed in Turkey. It is expected that the number of raphignathoid species in Turkey which inhabit different biotopes and climatic conditions will increase with comprehensive surveys.

There are some common problems in the taxonomy of this superfamily in Turkey. The first one, unfortunately, relationships between the Turkish acarologists and researchers suffer from a lack of communication. They work obliviously and separately from each other. Because of this situation, far from doing collective and comprehensive studies, they may will cause a taxonomic conflict sometimes. The striking one, some species have repeatedly been recorded under different papers. Another one is linguistic barrier. The Turkish researches are facing difficulties in communication with their colleagues who speak different languages.

Another problem, perhaps the most important one is that the specimens are missing or unavailable for further studies. Type specimens of a species are of great significance in nomenclature and taxonomy. If the concept of a new species is a hypothesis, then the future test of this hypothesis rests on the study of the primary type specimens. Therefore, they must be held in trust for science by the persons/institutions, which serve as repositories for primary type specimens (Liu et al., 2017). In Turkey many materials have been stored in the author's or their universities' collection in bad conditions. Unfortunately, there is no any natural historical museum or institution in Turkey for preserving of the type material and slide specimens used in publication. For depositing the specimen series in different collections in Turkey, professional institution must be urgently established. From now on, when there are multiple specimens, some can be sent to the depositories in different countries apart from our own country. This minimises the risk of loss of the specimens and facilitates access to them.

Catalogues and checklists

The mite lists of Turkey were previously published by Özkan et al. (1988, 1994). They mentioned only one raphignathoid species (*Zetzellia mali*) in the first list in 1988. Their second list in 1994 did not contain any raphignathoid taxa. Firstly, Doğan (2007) listed 112 raphignathoid species (including 2 undetermined species) in 6 families of the superfamily Raphignathoidea with distribution records and habitats in Turkey. Immediately after this first list, Erman et al. (2007) provided a supplement list of 291 Turkish mite taxa in the order Trombidiformes including Raphignathoidea. Then, Doğan (2008a) catalogued 52 species of the family Cryptognathidae Oudemans including distribution and habitat information of known cryptognathid species. Akyol (2013c) stated 152 species in 21 genera of 7 families, Akyol (2014b) enumerated 157 species in 22 genera of 7 families, and later Akyol et al. (2016) counted 174 species in 23 genera of 7 families in this superfamily in Turkey. The recent increase in the number of newly described and recorded species from Turkey has further exacerbated the urgency of an updated list on the superfamily.

Research on morphology, biology and related fields

In Turkey there are limited researches about ecology and distribution of soil mites (Ayyıldız, 1987; Koç, 1991; Koç and Ayyıldız, 1992; Ayyıldız and Koç, 1994; Dik et al., 1999; Önen, 2002; Soydan, 2002; Kahveci et al., 2006; Urhan et al., 2008; Koç and Somuncu, 2011; Önen and Koç, 2011; Bezci et al., 2017). Somuncu and Koç (2012a), Akyol and Koç (2016b), and Koç and Poyraz Tınartaş (2017a) surveyed seasonal distribution of raphignathoid mites in Afyonkarahisar province and two counties of İzmir and Manisa.

Koç (1999), Doğan et al. (2004) and Dönel et al. (2011) examined the morphological features of all developmental stages of some raphignathoid mites. Doğan and Ayyıldız (2003d), Doğan (2008a), Dönel and Doğan (2011a), Uluçay (2015a), Uluçay and Koç (2014b), Dilkaraoğlu et al. (2016e), Doğan et al. (2017c,f, 2018f,l) gave first descriptions of immature and male stages of some species.

Doğan et al. (2017a, 2018a) discovered pharate female of *Eustigmaeus segnis* (Koch) under its deutonymphal integument.

Koç and Akyol (2004, 2007), Doğan (2006b), Akyol (2010, 2011a, 2014a, 2018c), Doğan et al. (2015a,c, 2016b, 2017d, 2018b,c,j,k), Dilkaraoğlu et al. (2015d, 2016f), Koç and Poyraz Tınartaş (2017b), Bingül and Doğan (2017c) and Bingül et al. (2017a-d, 2018) mentioned some morphological variations and abnormalities in some members of this superfamily.

Kütük and his co-workers with their overseas cooperation studied biology and life table parameters of *Agistemus industani* Gonzalez, *A. cyprius* Gonzalez and *A.*

floridanus Gonzalez (Stigmaeidae) under laboratory conditions (Goldarazena et al., 2004).

Doğan et al. (2003b, 2008), Ocak et al. (2006, 2007) and Dönel et al. (2009-2010, 2012a) isolated external and internal fungi of raphignathoid mites and discussed the relationships between the isolated fungi and the mites. These studies were the primary works describing the mycoflora of raphignathoid mites. Most of fungi isolated from the mites are saprophytic and soilborn, only a few are entomopathogenic. The authors considered, on bases of their finding in the studies, that mites live in soil and decaying plant material, the setae on the mouthparts, leg setae and body setae are suitable for carrying fungus spores and some mites feed on them.

Karasu et al. (2015, 2018) determined infra-specific genetic variations based on RAPD-PCR method in *Eustigmaeus erciyesiensis* (Stigmaeidae). This work was a molecular-based study on *Eustigmaeus* species. Molecular approaches are currently among the most popular topics in life sciences and provide many opportunities in the solution of various problems. Karasu's work is important in terms of being the first study in this genus.

Checklist of the superfamily Raphignathoidea of Turkey

The Turkish raphignathoid species are arranged alphabetically within families and for each entrance the origin of reference to the published record is provided. Data from unpublished theses were not included here. In undetermined species list, the abbreviation ''spp.'' used as plural shows possibly distinct unspecified species of the same genus in multiple recordings. References perused for the compilation of this research work, have been listed alphabetically in the reference list.

The family with largest number of species was Stigmaeidae (54.3%), with 118 species in 11 genera. The second one was Camerobiidae (16.1%), with 35 species in 3 genera. The other Raphignathidae was the third (10.5%), represented by 23 species in one genus.

Family BARBUTIIDAE (2 species in 1 genus)

1. *Barbutia anguineus* (Berlese) – Doğan and Dönel, 2009
2. *Barbutia iranensis* Bagheri, Navaei and Ueckermann – Dilkaraoğlu et al., 2016d; Doğan et al., 2016c; Dönel Akgül, 2016, 2019

Family CALIGONELLIDAE (14 species in 3 genera)

1. *Caligonella humilis* (Koch) – Koç and Ayyıldız, 1996b; Doğan, 2003c, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Dönel and Doğan, 2012a; Somuncu and Koç, 2012a,b; Doğan et al., 2015d, 2017e, 2018d,h,j; Bingül et al., 2016; Akyol and Koç, 2016a; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a; Akyol and Gül, 2018b
2. *Caligonella haddadi* Bagheri and Maleki – Yamaç et al., 2019
3. *Caligonella urhani* Akyol – Akyol, 2018a
4. *Molothrognathus crucis* Summers and Schlinger – Doğan, 2003c, 2007; Erman et al., 2007
5. *Molothrognathus kamili* Doğan – Doğan, 2003c, 2007; Erman et al., 2007; Akyol, 2017; Akyol and Gül, 2018b
6. *Molothrognathus phytocolus* Meyer and Ueckermann – Akyol and Koç, 2012
7. *Molothrognathus terrulentus* Meyer and Ueckermann – Akyol and Koç, 2012; Bingül et al., 2016
8. *Molothrognathus venusta* (Khaustov and Kuznetsov) – Koç and Ayyıldız, 1997a; Doğan, 2003c, 2007; Erman et al., 2007; Doğan et al., 2015d

   [*Molothrognathus artvinensis* Koç and Ayyıldız – Koç and Ayyıldız, 1997a]
   Synonymised to *M. venusta* by Doğan (2003c).
9. *Neognathus eupalopus* Meyer and Ueckermann – Doğan and Doğan, 2019b
10. *Neognathus ozkani* Akyol and Koç – Akyol and Koç, 2012; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a
11. *Neognathus pusillus* Doğan and Doğan – Doğan and Doğan, 2019b
12. *Neognathus spectabilis* (Summers and Schlinger) – Doğan, 2003c, 2007; Doğan et al., 2003b; 2015d, 2017e, 2018d; Erman et al., 2007; Bingül et al., 2016
13. *Neognathus terrestris* (Summers and Schlinger) – Doğan and Ayyıldız, 2003b; Doğan, 2003c, 2007; Erman et al., 2007; Dönel, and Doğan, 2012a,c; Dönel et al., 2012a; Doğan et al., 2015d, 2017e, 2018d,h,j; Bingül et al., 2016; Akyol, 2017
14. *Neognathus ueckermanni* Bagheri, Doğan and Haddad – Dilkaraoğlu et al., 2014; 2015e; Doğan et al., 2018d

Family CAMEROBIIDAE (35 species in 3 genera)

1. *Camerobia turcicus* Koç and Kara – Koç and Kara, 2005; Doğan, 2007; Erman et al., 2007
2. *Neophyllobius afyonensis* Akyol and Koç – Akyol and Koç, 2006e; Doğan, 2007; Erman et al., 2007
3. *Neophyllobius askalensis* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003d; Doğan, 2007; Erman et al., 2007
4. *Neophyllobius atriplicis* Bolland – Koç, 2001; Doğan, 2007; Erman et al., 2007
5. *Neophyllobius ayvalıkensis* Akyol – Akyol, 2013d

[*Neophyllobius conocarpi* Bolland – Akyol, 2013a]

6. *Neophyllobius ayyildizi* Koç and Madanlar – Koç and Madanlar, 2002; Doğan, 2007; Erman et al., 2007
7. *Neophyllobius bolvadinensis* Akyol and Koç – Akyol and Koç, 2006c; Doğan, 2007; Erman et al., 2007
8. *Neophyllobius communis* Gerson – Koç, 1999; Doğan, 2007; Erman et al., 2007; Çobanoğlu and Yeşilayer, 2016; Akyol, 2017
9. *Neophyllobius demirsoyi* Akyol and Koç – Akyol and Koç, 2006c; Doğan, 2007; Erman et al., 2007
10. *Neophyllobius fani* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003d; Doğan, 2007; Erman et al., 2007
11. *Neophyllobius izmirensis* Akyol – Akyol, 2013d
12. *Neophyllobius karabagiensis* Akyol and Koç – Akyol and Koç, 2006a; Doğan, 2007; Erman et al., 2007; Akyol and Gül, 2018c
13. *Neophyllobius lachishensis* Bolland – Akyol and Koç, 2006c; Doğan, 2007; Erman et al., 2007; Somuncu and Koç, 2012a,b; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a; Akyol and Gül, 2018c
14. *Neophyllobius lamimani* McGregor – Akyol, 2012a,b; 2013b, 2017, 2018b
15. *Neophyllobius olurensis* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003d; Doğan, 2007; Erman et al., 2007
16. *Neophyllobius orhani* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003d; Doğan, 2007; Erman et al., 2007
17. *Neophyllobius parthenocissi* Bolland – Akyol, 2012a,b; 2017, 2018b
18. *Neophyllobius persiaensis* Khanjani and Ueckermann – Çobanoğlu and Yeşilayer, 2016
19. *Neophyllobius podocarpi* Bolland – Koç, 2005b; Doğan, 2007; Erman et al., 2007
20. *Neophyllobius populus* Akyol and Koç – Akyol and Koç, 2006a; Doğan, 2007; Erman et al., 2007; Akyol and Gül, 2018c
21. *Neophyllobius quercus* Uluçay and Koç – Uluçay and Koç, 2014b
22. *Neophyllobius sultanensis* Akyol and Koç – Akyol and Koç, 2006e; Doğan, 2007; Erman et al., 2007
23. *Neophyllobius turcicus* Koç and Ayyıldız – Koç and Ayyıldız, 1996a; Doğan, 2007; Erman et al., 2007
24. *Neophyllobius yunusi* Akyol and Koç – Akyol and Koç, 2006c; Doğan, 2007; Erman et al., 2007; Uluçay and Koç, 2014b; Akyol, 2017; Akyol and Gül, 2018c
25. *Tycherobius anatolicus* Uluçay, Koç and Akyol – Uluçay et al., 2016c
26. *Tycherobius bollandi* Ayyıldız and Doğan – Ayyıldız and Doğan, 2003; Doğan, 2007; Erman et al., 2007
27. *Tycherobius dazkiriensis* Akyol and Koç – Akyol and Koç, 2006a; Doğan, 2007; Erman et al., 2007
28. *Tycherobius farsiensis* Khanjani, Yazyanpanah, Ostovan and Fayaz – Akyol and Koç, 2017b; Koç and Poyraz Tınartaş, 2017a
29. *Tycherobius iranensis* Khanjani, Yazyanpanah, Ostovan and Fayaz – Uluçay et al., 2016c
30. *Tycherobius izmirensis* Akyol and Koç – Akyol and Koç, 2017b; Akyol and Gül, 2018c
31. *Tycherobius polonicus* Bolland – Koç, 2005b; Koç and Akyol, 2007; Doğan, 2007; Erman et al., 2007
32. *Tycherobius quercus* Bolland and Koç – Bolland and Koç, 2001; Doğan, 2007; Erman et al., 2007
33. *Tycherobius sahragardi* Khanjani, Hajizadeh, Hoseini and Jalili – Uluçay et al., 2016c
34. *Tycherobius stramenticola* Bolland – Koç and Ayyıldız, 1998a; Koç and Akyol, 2007; Doğan, 2007; Erman et al., 2007; Somuncu and Koç, 2012a,b; Koç and Poyraz Tınartaş, 2017a
35. *Tycherobius turcicus* Ayyıldız and Doğan – Ayyıldız and Doğan, 2003; Doğan, 2007; Erman et al., 2007

Family CRYPTOGNATHIDAE (18 species in 3 genera)

1. *Cryptognathus ayyildizi* Akyol and Koç – Akyol and Koç, 2010; Uluçay and Koç, 2013a
2. *Cryptognathus lagena* Kramer – Koç and Ayyıldız, 1998b; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Özçelik et al., 2010; Dönel and Doğan, 2008a, 2011b, 2012a; Dönel et al., 2012a; Uluçay and Koç, 2013a; Bingül et al., 2016; Akyol, 2017; Doğan et al., 2018e
3. *Cryptognathus luteolus* Summers and Chaudhri – Koç and Ayyıldız, 1998b; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2008a, 2011b; Ayyıldız et al., 2010; Dönel et al., 2012a
4. *Cryptognathus kutahyaensis* Uluçay and Koç – Uluçay and Koç, 2013a
5. *Cryptognathus ozkani* Doğan and Ayyıldız – Doğan and Ayyıldız, 2001, 2003d; Doğan et al.,

2003b; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2008a, 2011b; Dönel et al., 2012a

6. *Cryptofavognathus afyonensis* (Koç and Akyol) – Koç and Akyol, 2004; Doğan and Dönel, 2010

   [*Favognathus afyonensis* Koç and Akyol – Koç and Akyol, 2004; Doğan, 2007, 2008a; Erman et al., 2007]

7. *Cryptofavognathus anatolicus* Doğan and Dönel – Doğan and Dönel, 2010

8. *Favognathus acaciae* Doğan and Ayyıldız – Doğan and Ayyıldız, 2004a; Doğan, 2007, 2008a; Erman et al., 2007; Dönel and Doğan, 2011b, 2012a; Dönel et al., 2012a; Bingül et al., 2016

9. *Favognathus amygdalus* Doğan and Ayyıldız – Doğan and Ayyıldız, 2004a; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2008a, 2011b; Uluçay and Koç, 2013a; Akyol, 2017; Gül and Akyol, 2017a

10. *Favognathus bafranus* Doğan – Doğan, 2008a; Dönel and Doğan, 2011b

11. *Favognathus cucurbita* (Berlese) – Doğan and Ayyıldız, 2004a; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2008a, 2011b; Uluçay and Koç, 2013a; Doğan et al., 2015d, 2018e; Akyol, 2017; Gül and Akyol, 2017a

12. *Favognathus distortus* (Kuznetsov) – Doğan, 2008a; Dönel and Doğan, 2011b; Doğan et al., 2018e

13. *Favognathus erzurumensis* Doğan and Ayyıldız – Doğan and Ayyıldız, 2002; Doğan, 2007, 2008a; Erman et al., 2007

14. *Favognathus izmirensis* Akyol – Akyol, 2011b, 2012a; Akyol, 2017; Gül and Akyol, 2017a

15. *Favognathus kamili* Dönel and Doğan – Dönel and Doğan, 2011b; Uluçay and Koç, 2013a; Doğan et al., 2015d, 2018e; Bingül et al., 2016; Akyol, 2017; Gül and Akyol, 2017a

    [*Favognathus orbiculatus* (Livshitz) – Koç and Ayyıldız, 1999; Doğan et al., 2003b; Doğan and Ayyıldız, 2004a; Doğan, 2007; Erman et al., 2007]

16. *Favognathus luxtoni* Koç and Ayyıldız – Koç and Ayyıldız, 1999; Doğan, 2007, 2008a; Erman et al., 2007; Akyol, 2017

17. *Favognathus manisaensis* Akyol and Koç – Akyol and Koç, 2017a

18. *Favognathus turcicus* Koç and Ayyıldız – Koç and Ayyıldız, 1999; Doğan, 2007, 2008a; Erman et al., 2007

Family EUPALOSELLIDAE (6 species in 3 genera)

1. *Eupalopsellus deformatus* Fan – Dilkaraoğlu et al., 2016c; Doğan et al., 2017g

2. *Eupalopsellus olandicus* Sellnick – Doğan and Ayyıldız, 2004; Doğan, 2007; Erman et al., 2007

3. *Eupalopsellus prasadi* Bagheri and Khanjani – Kasap et al., 2013a,b

4. *Eupalopsellus rostridius* Summers – Doğan, 2006b, 2007; Erman et al., 2007; Dönel and Doğan 2010; Dilkaraoğlu et al., 2016e

5. *Eupalopsis maseriensis* (Canestrini and Fanzago) – Doğan, 2008b

6. *Saniosulus deliquus* Doğan, Bingül and Doğan – Doğan et al., 2019a

   [*Saniosulus longidius* Fan, Zhang and Liu – Bingül et al., 2016: Doğan et al., 2019a]

Family HOMOCALIGIDAE (1 species)

1. *Homocaligus crassipus* Fan – Doğan et al., 2014a,b

Family RAPHIGNATHIDAE (23 species in 1 genus)

1. *Raphignathus afyonensis* Akyol and Koç – Akyol and Koç, 2006b; Doğan, 2007; Erman et al., 2007

2. *Raphignathus atyeoi* Meyer and Ueckermann – Doğan, 2003b; Doğan, 2007; Erman et al., 2007

3. *Raphignathus bathursti* Meyer and Ryke – Akyol and Koç, 2010

4. *Raphignathus collegiatus* Atyeo, Baker and Crossley – Doğan and Ayyıldız, 2003e; Doğan, 2003b, 2007; Koç and Akyol, 2004; Erman et al., 2007; Bingül et al., 2016; Doğan et al., 2018g, 2019c

5. *Raphignathus emirdagiensis* Akyol and Koç – Akyol and Koç, 2006b; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2012a; Bingül et al., 2016

6. *Raphignathus ensipilosus* Meyer and Ueckermann – Akyol and Koç, 2006b; Doğan, 2007; Erman et al., 2007

7. *Raphignathus erzincanica* Doğan – Doğan, 2003b, 2007; Erman et al., 2007; Dilkaraoğlu et al., 2016a

8. *Raphignathus fani* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003e; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2011a; Dönel et al., 2012a; Bingül et al., 2016; Doğan et al., 2018g, 2019c

9. *Raphignathus giresuniensis* Doğan – Doğan, 2003b, 2007; Erman et al., 2007

10. *Raphignathus giselae* Meyer and Ueckermann – Akyol and Koç, 2006d, 2007; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2012a; Akyol, 2017

11. *Raphignathus gracilis* (Rack) – Koç and Ayyıldız, 1996c; Doğan, 2003b, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Somuncu and Koç, 2012a,b; Dönel and Doğan, 2012a; Dönel et al., 2012a; Yeşilayer and Çobanoğlu, 2013; Doğan et al., 2015d, 2018g,j, 2019c; Bingül et al., 2016, 2017b, 2018; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a

12. *Raphignathus hecmatanaensis* Khanjani and Ueckermann – Doğan, 2003b, 2007; Erman et al., 2007; Dönel and Doğan, 2012a; Akyol, 2014a, 2017, 2018c; Akyol and Koç, 2016a; Bingül et al., 2016, 2017b, 2018; Gül and Akyol, 2017b; Doğan et al., 2018g, 2019c

13. *Raphignathus karabagiensis* Akyol and Koç – Akyol and Koç, 2006b; Doğan, 2007; Erman et al., 2007

14. *Raphignathus kelkitensis* Dönel and Doğan – Dönel and Doğan, 2011a

15. *Raphignathus khorramabadensis* Bagheri – Uluçay et al., 2016b

16. *Raphignathus koseiensis* Dönel and Doğan – Dönel and Doğan, 2013b

17. *Raphignathus kuznetzovi* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003e; Doğan, 2007; Erman et al., 2007; Somuncu and Koç, 2012a,b; Dönel and Doğan, 2012a; Dönel et al., 2012a; Bingül et al., 2016, 2017b, 2018; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a; Doğan et al., 2018g, 2019c

    [*Raphignathus hirtellus* Athias and Henriot – Koç and Ayyıldız, 1996c; Ayyıldız et al., 2010].

18. *Raphignathus ozkani* Doğan – Doğan, 2006b; 2007; Erman et al., 2007

    [*Raphignathus domesticus* Shiba – Doğan and Ayyıldız, 2006].

19. *Raphignathus protaspus* Khanjani and Ueckermann – Akyol and Koç, 2006d, 2007; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2012a; Özçelik et al., 2010; Somuncu and Koç, 2012a,b; Gül and Akyol, 2017b; Koç and Poyraz Tınartaş, 2017a

20. *Raphignathus quadrigeminus* Dönel and Doğan – Dönel and Doğan, 2013b

21. *Raphignathus ueckermanni* Koç and Kara – Koç and Kara, 2005; Doğan, 2007; Erman et al., 2007; Somuncu and Koç, 2012a,b; Akyol, 2017; Gül and Akyol, 2017b; Koç and Poyraz Tınartaş, 2017a

22. *Raphignathus vahiti* Doğan – Doğan, 2003b, 2007; Erman et al., 2007; Dönel and Doğan, 2012a

23. *Raphignathus zhaoi* Hu, Jing and Liang – Akyol and Koç, 2006b,d, 2007; Doğan, 2007; Erman et al., 2007; Özçelik et al., 2010; Dönel and Doğan, 2012a; Dönel et al., 2012a; Gül and Akyol, 2017b

Family STIGMAEIDAE (118 species in 11 genera)

1. *Agistemus collyerae* Gonzalez-Rodriguez – Kasap et al., 2013a; Yeşilayer and Çobanoğlu, 2013

2. *Agistemus duzgunesae* Koç, Çobanoğlu and Madanlar – Koç et al., 2005; Doğan, 2007; Erman et al., 2007

3. *Agistemus terminalis* (Quayle) – Göven et al., 2009; Yeşilayer and Çobanoğlu, 2013; Kasap et al., 2013a

4. *Cheylostigmaeus californicus* Summers and Ehara – Akyol and Koç, 2008, 2010

5. *Cheylostigmaeus salinus* Evans – Dönel and Doğan, 2011c

6. *Cheylostigmaeus mirabilis* Wood – Bingül et al., 2016

7. *Cheylostigmaeus salmani* Koç – Koç, 2005a; Doğan, 2007; Erman et al., 2007; Akyol, 2017

8. *Cheylostigmaeus tarae* Khanjani – Bingül et al., 2016; Bingül and Doğan, 2017a,b

9. *Cheylostigmaeus urhani* Dönel and Doğan – Dönel and Doğan, 2011c; Doğan et al., 2015d; Bingül et al., 2016

10. *Cheylostigmaeus variatus* Doğan, Dilkaraoğlu and Fan – Doğan et al., 2015c, 2018i

11. *Eryngiopus anatolicus* Doğan, Dilkaraoğlu and Fan – Doğan et al., 2015b; Dilkaraoğlu et al., 2016g

12. *Eryngiopus coheni* Vacante and Gerson – Uluçay, 2015d

13. *Eryngiopus siculus* Vacante and Gerson – Akyazı et al., 2016

14. *Eryngiopus tauricus* Kuznetsov – Yeşilayer and Çobanoğlu, 2013

15. *Eustigmaeus absens* Doğan – Doğan, 2005, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Dilkaraoğlu et al., 2016g

16. *Eustigmaeus anauniensis* (Canestrini) – Koç and Ayyıldız, 2000; Doğan et al., 2003b, 2015d, 2018h,j; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Özçelik et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c; Dönel

et al., 2012a; Dilkaraoğlu et al., 2016g; Bingül et al., 2016; 2017c,d; Akyol and Koç, 2016a; Akyol, 2017; Akyol and Gül, 2017

17. *Eustigmaeus anophthalmus* Doğan, Dönel and Özçelik – Doğan et al., 2011

18. *Eustigmaeus bali* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003c; Doğan, 2007; Erman et al., 2007

19. *Eustigmaeus chilensis* (Chaudhri) – Koç and Ayyıldız, 2000; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010

20. *Eustigmaeus collarti* (Cooreman) – Doğan 2004c; 2006a, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Bingül et al., 2016; Doğan and Doğan, 2018

21. *Eustigmaeus dogani* Khanjani, Fayaz, Mirmoayedi and Ghaedi – Uluçay and Koç, 2013b, 2014a

22. *Eustigmaeus erciyesiensis* Doğan, Ayyıldız and Fan – Doğan et al., 2003a, 2004, 2015d; Doğan, 2005, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Dilkaraoğlu et al., 2016g; Karasu et al., 2015, 2018; Doğan and Doğan, 2018

23. *Eustigmaeus erzincanensis* Doğan – Doğan, 2005, 2007; Erman et al., 2007; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c; Doğan et al., 2015d, 2017f; Bingül et al., 2016; Dilkaraoğlu et al., 2016a,g

24. *Eustigmaeus erzurumensis* Doğan – Doğan, 2005, 2007; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c; Dönel et al., 2012a; Bingül et al., 2016; Dilkaraoğlu et al., 2016g

25. *Eustigmaeus jiangxiensis* Hu, Chen and Huang – Doğan, 2005, 2007; Erman et al., 2007; Özçelik et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c, 2012a; Dilkaraoğlu et al., 2016g; Bingül et al., 2016; 2017c,d; Doğan and Doğan, 2018

26. *Eustigmaeus johnstoni* Zhang and Gerson – Özbel et al., 1999; Doğan, 2007; Erman et al., 2007; Pekağırbaş et al., 2017

27. *Eustigmaeus kentingensis* Tseng – Doğan, 2005, 2007; Erman et al., 2007

28. *Eustigmaeus kermesinus* (Koch) – Koç and Ayyıldız, 2000; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010

29. *Eustigmaeus kulaensis* Akyol – Akyol, 2019a

30. *Eustigmaeus lacuna* (Summers) – Özçelik and Doğan, 2011; Dönel and Doğan, 2011c Dilkaraoğlu et al., 2016g

31. *Eustigmaeus pinnatus* (Kuznetsov)

[*Eustigmaeus ioanninensis* Kapaxidi and Papadoulis – Doğan et al., 2003a, 2015d; Doğan, 2007; Erman et al., 2007; Özçelik and Doğan, 2011; Bingül et al., 2016; 2017c,d; Akyol, 2017; Akyol and Gül, 2017]
Synonymised to *E. pinnatus* by Khaustov (2019).

32. *Eustigmaeus ottavii* (Berlese) – Koç and Ayyıldız, 2000; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010

33. *Eustigmaeus rhodomela* (Koch) – Dönel and Doğan, 2011c; Dönel et al., 2011; Dilkaraoğlu et al., 2016g

[*Eustigmaeus fani* Doğan – Doğan, 2005, 2007; Erman et al., 2007]
Synonymised to *E. rhodomela* by Faraji et al. (2007).

34. *Eustigmaeus schusteri* (Summers and Price) – Doğan, 2005, 2007; Erman et al., 2007

35. *Eustigmaeus sculptus* Doğan, Ayyıldız and Fan – Doğan et al., 2003a, 2015d; Doğan, 2007; Erman et al., 2007; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c; Dönel et al., 2012a; Dilkaraoğlu et al., 2016g; Bingül et al., 2016, 2017c,d; Akyol, 2017; Akyol and Gül, 2017; Doğan and Doğan, 2018

36. *Eustigmaeus segnis* (Koch) – Koç and Ayyıldız, 2000; Doğan, 2005, 2007; Ocak et al., 2006, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Özçelik et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c, 2012a; Somuncu and Koç, 2012a,b; Dönel et al., 2012a; Dilkaraoğlu et al., 2016g; Bingül et al., 2016, 2017c,d; Doğan et al., 2003b, 2015d, 2017a, 2018a,c,h; Akyol and Koç, 2016a; Akyol, 2017; Akyol and Gül, 2017; Doğan and Doğan, 2018

37. *Eustigmaeus setiferus* Bagheri, Saber, Ueckermann, Ghorbani and Bonab –Doğan et al., 2019d

38. *Eustigmaeus turcicus* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003c; Doğan, 2005, 2007; Erman et al., 2007; Özçelik et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c; Dönel et al., 2012a; Bingül et al., 2016; Dilkaraoğlu et al., 2016g; Akyol, 2017; Doğan et al., 2018f, 2019b

39. *Eustigmaeus vacuus* Doğan – Doğan et al., 2003b; Doğan, 2005, 2007; Erman et al., 2007; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c, 2012a

40. *Eustigmaeus varius* Dönel and Doğan – Dönel and Doğan, 2011c

41. *Ledermuelleriopsis aydinensis* Akyol and Gül – Akyol and Gül, 2019

42. *Ledermuelleriopsis ayyildizi* Doğan – Doğan, 2004b, 2007; Erman et al., 2007; Özçelik and

Doğan, 2011; Dönel and Doğan, 2011c; Dönel et al., 2012a; Doğan et al., 2015d, 2018h,j; Bingül et al., 2016; Dilkaraoğlu et al., 2016g; Akyol, 2017

43. *Ledermuelleriopsis bisetalis* Doğan – Doğan, 2004b, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Dilkaraoğlu et al., 2016g

44. *Ledermuelleriopsis giresuniensis* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003a; Doğan, 2007; Erman et al., 2007

45. *Ledermuelleriopsis indiscretus* Dönel and Doğan – Dönel and Doğan, 2011c

46. *Ledermuelleriopsis pulumosus* Willmann – Koç and Ayyıldız, 1997c; Doğan, 2007; Erman et al., 2007; Somuncu and Koç, 2012a,b; Doğan et al., 2015d; Bingül et al., 2016; Akyol, 2017; Koç and Poyraz Tınartaş, 2017a; Akyol and Gül, 2017

47. *Ledermuelleriopsis punctata* Soliman – Koç and Ayyıldız, 1997c; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010

48. *Ledermuelleriopsis rizeiensis* Doğan – Doğan, 2004a; Doğan, 2007; Erman et al., 2007; Dilkaraoğlu et al., 2016g

49. *Ledermuelleriopsis sezeki* Doğan – Doğan, 2004b, 2007; Erman et al., 2007; Özçelik and Doğan, 2011

50. *Ledermuelleriopsis tamariski* Maleki and Bagheri – Bingül and Doğan, 2016a,b; Bingül et al., 2016

51. *Ledermuelleriopsis toleratus* Kuznetsov – Doğan, 2004b, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Bingül et al., 2016; Dilkaraoğlu et al., 2016g

52. *Ledermuelleriopsis triscutata* Willmann – Koç and Ayyıldız, 1997c; Doğan, 2007; Erman et al., 2007

53. *Mediolata acus* (Summers) – Doğan, 2006b, 2007; Erman et al., 2007

54. *Mediolata chanti* Gonzalez-Rodriguez – Yeşilayer and Çobanoğlu, 2013

55. *Mediolata granaria* Gonzalez-Rodriguez – Dönel and Doğan, 2012b

56. *Mediolata obtecta* Dönel and Doğan – Dönel and Doğan, 2012b

57. *Mediolata ozkani* Dönel and Doğan – Dönel and Doğan, 2012b

58. *Mediolata petilus* Doğan and Ayyıldız – Doğan and Ayyıldız, 2004b; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2012a

59. *Mediolata pini* (Canestrini) – Dilkaraoğlu et al., 2016g; Bingül et al., 2016

60. *Mediolata turcica* Dönel and Doğan – Dönel and Doğan, 2012b

61. *Prostigmaeus integrius* Dönel and Doğan – Dönel and Doğan, 2011c

62. *Stigmaeus additicius* Dönel and Doğan – Dönel and Doğan, 2011c, 2012a; Doğan et al., 2015d

63. *Stigmaeus amasyanus* Dönel, Doğan, Sevsay and Bal – Dönel et al., 2012b

64. *Stigmaeus angustus* Dönel and Doğan – Dönel and Doğan, 2011c

65. *Stigmaeus ayyildizi* Dönel and Doğan – Dönel and Doğan, 2011c

66. *Stigmaeus communis* Doğan, Doğan and Erman – Doğan et al., 2017d

67. *Stigmaeus berwariensis* Uluçay – Uluçay, 2015d

68. *Stigmaeus bifurcus* Bingül, Doğan and Dilkaraoğlu – Bingül et al., 2017a

69. *Stigmaeus ceylani* Uluçay – Uluçay, 2015b

70. *Stigmaeus creber* Barilo – Uluçay, 2015c

71. *Stigmaeus dazkiriensis* Akyol and Koç – Akyol and Koç, 2007; Akyol and Gül, 2017

72. *Stigmaeus devlethanensis* Akyol and Koç – Akyol and Koç, 2007; Dönel and Doğan, 2011c; Doğan et al., 2015d; Dilkaraoğlu et al., 2015c, 2016g; Bingül et al., 2016

73. *Stigmaeus dogani* Akyol – Akyol, 2019b

74. *Stigmaeus elongatus* Berlese – Doğan, 2003a, 2007; Erman et al., 2007; Koç and Somuncu, 2012; Somuncu and Koç, 2012a,b; Doğan et al., 2015d, 2018b, 2019e; Bingül et al., 2016

75. *Stigmaeus erzincanus* Doğan, Bingül, Dilkaraoğlu and Fan – Doğan et al., 2015a, 2017c, 2018h; Bingül et al., 2016, 2017c,d; Dilkaraoğlu et al., 2016a

76. *Stigmaeus fidelis* Kuznetsov – Özçelik and Doğan, 2011; Dönel and Doğan, 2012a

77. *Stigmaeus fimus* Doğan, Doğan and Erman – Doğan et al., 2017d

78. *Stigmaeus furcatus* Dönel and Doğan – Dönel and Doğan, 2011c; Doğan and Doğan, 2019c

79. *Stigmaeus glabrisetus* Summers – Özçelik and Doğan, 2011; Dönel and Doğan, 2011c

80. *Stigmaeus harsitensis* Doğan, Doğan and Erman – Doğan et al., 2017d

81. *Stigmaeus hashtrudensis* Bagheri and Maleki – Uluçay, 2015c

82. *Stigmaeus indivisus* Doğan – Doğan, 2019

83. *Stigmaeus kamili* Doğan and Ayyıldız – Doğan and Ayyıldız, 2003f; Doğan, 2007; Erman et al., 2007

84. *Stigmaeus karabagiensis* Akyol and Koç – Akyol and Koç, 2007; Somuncu and Koç, 2012a,b

85. *Stigmaeus kelkitensis* Dönel and Doğan – Dönel and Doğan, 2011c; Dilkaraoğlu et al., 2016b,g

86. *Stigmaeus kumalariensis* Akyol and Koç – Akyol and Koç, 2007; Doğan et al., 2015d, 2018k, 2019f, Uluçay, 2015a

87. *Stigmaeus livschitzi* Kuznetsov – Dilkaraoğlu et al., 2016g; Doğan et al., 2017b

88. *Stigmaeus longiclipeatus* Doğan, Doğan and Erman – Doğan et al., 2017d

89. *Stigmaeus longipilis* (Canestrini) – Koç and Ayyıldız, 1997b; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2012a; Dilkaraoğlu et al., 2015d, 2016f,g

90. *Stigmaeus luxtoni* Wood – Doğan, 2003a, 2007; Erman et al., 2007; Özçelik et al., 2010; Bingül et al., 2016

    [*Stigmaeus turcica* Doğan – Doğan, 2003a, 2007; Erman et al., 2007]
    Synonymised to *S. luxtoni* by Faraji and Ueckermann (2006).

91. *Stigmaeus miandoabiensis* Bagheri and Zarei – Bingül et al., 2016, 2017a

92. *Stigmaeus obtectus* Summers – Koç and Ayyıldız, 1997b; Doğan, 2007; Erman et al., 2007; Ayyıldız et al., 2010

93. *Stigmaeus pilatus* Kuznetsov – Doğan and Ayyıldız, 2003f; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2011c, 2012a; Dönel et al., 2012a; Doğan et al., 2015d; Bingül et al., 2016; Dilkaraoğlu et al., 2016g; Akyol, 2017; Doğan and Doğan, 2018

94. *Stigmaeus planus* Kuznetsov – Koç and Ayyıldız, 1997b; Doğan, 2007; Erman et al., 2007; Dönel and Doğan, 2011c; Doğan et al., 2015d

95. *Stigmaeus pricei* Summers – Dilkaraoğlu et al., 2016g

96. *Stigmaeus pseudoparmatus* Doğan, Doğan and Erman – Doğan et al., 2017d

97. *Stigmaeus pulchellus* Kuznetsov – Uluçay, 2015a

98. *Stigmaeus sariensis* Bagheri – Uluçay et al., 2016a

99. *Stigmaeus scaber* Summers – Koç and Ayyıldız, 1997b; Doğan, 2007; Erman et al., 2007

100. *Stigmaeus seferihisarensis* Koç – Koç, 2005c; Doğan, 2007; Erman et al., 2007

101. *Stigmaeus siculus* (Berlese) – Doğan, 2006b, 2007; Erman et al., 2007; Dönel and Doğan, 2011c

     [*Stigmaeus scilus* [sic] (Berlese) – Dönel and Doğan, 2012a]

102. *Stigmaeus solidus* Kuznetsov – Dönel and Doğan, 2011c; Doğan et al., 2016b; Dilkaraoğlu et al., 2015f, 2016g; Bingül et al., 2017c,d

103. *Stigmaeus sphagneti* (Hull) – Doğan et al., 2014c, 2015d

104. *Stigmaeus tokatensis* Dönel, Doğan, Sevsay and Bal – Dönel et al., 2012b

105. *Stigmaeus tolstikovi* Khaustov – Doğan and Doğan, 2019a

106. *Stigmaeus urhani* Koç – Koç, 2005c; Doğan, 2007; Erman et al., 2007

107. *Stigmaeus uzunolukensis* Özçelik and Doğan – Özçelik and Doğan, 2011; Dilkaraoğlu et al., 2016g,h

108. *Storchia ardabiliensis* Safasadati, Khanjani, Razmjou and Doğan – Akyol, 2011a, 2012a, 2017; Koç and Somuncu, 2012; Somuncu and Koç, 2012a,b; Doğan et al., 2015d; Bingül et al., 2016

109. *Storchia hakkariensis* Uluçay – Uluçay, 2014

110. *Storchia hendersonae* Fan and Zhang – Dilkaraoğlu et al., 2015a,b; 2016g

111. *Storchia mehrvari* Bagheri and Gheblealivand – Koç and Poyraz Tınartaş, 2017a,b

112. *Storchia robusta* (Berlese) – Koç and Ayyıldız, 1997b; Doğan and Ayyıldız, 2003f; Doğan, 2007; Erman et al., 2007; Akyol, 2010, 2011a, 2017; Ayyıldız et al., 2010; Özçelik et al., 2010; Özçelik and Doğan, 2011; Dönel and Doğan, 2011c, 2012a; Somuncu and Koç, 2012a,b; Yeşilayer and Çobanoğlu, 2013; Doğan et al., 2015d; Dilkaraoğlu et al., 2016g; Bingül et al., 2016, 2017c,d; Koç and Poyraz Tınartaş, 2017a; Akyol and Gül, 2017

113. *Villersia sudetica* Willmann – Koç and Ayyıldız, 1997b; Doğan, 2007; Erman et al., 2007; Özçelik and Doğan, 2011

114. *Zetzellia crassirostris* (Leonardi) – Çobanoğlu and Kaźmierski, 1999

115. *Zetzellia erzincanica* Bingül and Doğan – Bingül and Doğan, 2017c

116. *Zetzellia kamili* Akyol and Gül – Akyol and Gül, 2018a

117. *Zetzellia mali* (Ewing) – Çobanoğlu and Kaźmierski, 1999; Akyazı and Ecevit, 2003; Çobanoğlu et al., 2003; Doğan, 2007; Kasap and Çobanoğlu, 2007; Kumral and Kovancı, 2007; Elma and Alaoğlu, 2008; Kasap et al., 2008, 2013a, 2014; Göven et al., 2009; Sağlam and Çobanoğlu, 2010; Dönel and Doğan, 2013a: Yeşilayer and Çobanoğlu, 2013; Satar et al., 2013; Çobanoğlu and Kumral, 2014; Kumral and Çobanoğlu, 2015a,b; Akyazı et al., 2017; Inak and Cobanoglu, 2018; Soysal and Akyazı, 2018; Altunç and Akyazı, 2019

     [*Mediolata mali* Ewing – Düzgüneş, 1963; Özkan et al., 1988]

118. *Zetzellia talhouki* Dosse – Koç and Madanlar, 1998; Doğan, 2007; Erman et al., 2007; Kasap et al., 2013a; Yeşilayer and Çobanoğlu, 2013; Akyazı et al., 2017

Undetermined species

1. *Agistemus* spp. – Özman and Çobanoğlu, 2001; Gençer et al., 2002, 2005; Çakmak and Akşit, 2003; Altunç and Akyazı, 2019

2. *Mediolata* spp. – Akyazı and Ecevit, 2003; Yanar and Ecevit, 2005, 2008; Çobanoğlu and Kumral, 2014

3. *Molothrognathus* sp. – Koç and Poyraz Tınartaş, 2017a

4. *Neognathus* sp. – Akyol and Gül, 2018b

5. *Neophyllobius* spp. – Doğan et al., 2015d; Akyazı et al., 2017; Koç and Poyraz Tınartaş, 2017a

6. *Raphignathus* sp. – Koç and Poyraz Tınartaş, 2017a

7. Stigmaeidae spp. – Bayram and Çobanoğlu, 2006

8. *Stigmaeus* spp. – Doğan et al., 2003b; Bayram and Çobanoğlu, 2006; Çobanoğlu, 2008

9. *Tycherobius* spp. – Akyol, 2017 (in immature stages); Koç and Poyraz Tınartaş, 2017a

10. *Zetzellia* sp. – Özman and Çobanoğlu, 2001; Satar et al., 2013

Table 1. A list of authors and their co-authors who published some raphignathoid papers on the taxonomy, fauna, morphology, biology and related fields in Turkey

A. Kaźmierski (Poland)	İ. Kasap	O. Erman
A. Özdemir	İ. Ocak	O.A. Kesik
A. Yarımbatman	İ. Uluçay	Ö. Alaoğlu
A. Yeşilayer	İ. Hasenekoğlu	Ö.F. Algur
B. Güven	İ.C. Balcıoğlu	Q.-H. Fan (New Zealand)
B. Kovancı	K. Koç	R. Akyazı
B. Polat	K.S. Coşkuncu	S. Adil
C. Uysal	M. Ada	R. Atlıhan
D. Akyol	M. Akyol	S. Çobanoğlu
D.A. Bal	M. Aykut	S. Doğan
D. Yanar	M. Bingül	S. Doğan (Dilkaraoğlu)
E. Ökten	M. Çankaya	S. Örtücü
E. Poyraz Tınartaş	M. Kara	S. Özçelik
E. Zeytun	M. Karakuş	S. Özensoy
E.A. Ueckermann (S. Africa)	M. Kuzucu	S. Pehlivan
F. Akkafa	M. Pekağırbaş	S. Satar
F. Akyazi	M. Soysal	S. Sevsay
F. Ersin	M. Ulukanlıgil	S. Somuncu
E. Polat	M.A. Göven	S. Töz
F. Faraji (The Netherlands)	M.B. Kaydan	S.Ç. Yamaç
F. Sezek	M.N. Aydoğan	S.K. Arserim
F.N. Elma	M.P. Gül	S.K. Özman
G. Dönel Akgül	M.S.Özgökçe	T. Akşit
H. Aksoy	M.Z. Alkan	Ş. Bayram
H. Eren	N. Ayyıldız	Ş. Kök
H. Kütük	N. Karasu	Ş. Uğurlu
H.D. Sağlam	N. Madanlar	Y. Özbel
H.H. Özbek	N.A. Kumral	Y.E. Altunç

H.R. Bolland (The Netherlands)	N.S. Gençer	Z. Düzgüneş
İ. Çakmak	O. Ecevit	Z. Soysal

Table 2. The number of raphignathoid mite species known from Turkey.

Superfamily RAPHIGNATHOIDEA
Family BARBUTIIDAE (1 genus, 2 species)
Genus *Barbutia* (2 species)
Family CALIGONELLIDAE (3 genera, 14 species)
Genus *Caligonella* (3 species)
Genus *Molothrognathus* (5 species)
Genus *Neognathus* (6 species)
Family CAMEROBIIDAE (3 genera, 35 species)
Genus *Camerobia* (1 species)
Genus *Neophyllobius* (23 species)
Genus *Tycherobius* (11 species)
Family CRYPTOGNATHIDAE (3 genera, 18 species)
Genus *Cryptognathus* (5 species)
Genus *Cryptofavognathus* (2 species)
Genus *Favognathus* (11 species)
Family EUPALOSELLIDAE (3 genera, 6 species)
Genus *Eupalopsellus* (4 species)
Genus *Eupalopsis* (1 species)
Genus *Saniosulus* (1 species)
Family HOMOCALIGIDAE (1 genus, 1 species)
Genus *Homocaligus* (1 species)
Family RAPHIGNATHIDAE (1 genus, 23 species)
Genus *Raphignathus* (23 species)
Family STIGMAEIDAE (11 genera, 118 species)
Genus *Agistemus* (3 species)
Genus *Cheylostigmaeus* (7 species)
Genus *Eryngiopus* (4 species)
Genus *Eustigmaeus* (26 species)
Genus *Ledermuelleriopsis* (12 species)
Genus *Mediolata* (8 species)
Genus *Prostigmaeus* (1 species)
Genus *Stigmaeus* (46 species)
Genus *Storchia* (5 species)
Genus *Villersia* (1 species)
Genus *Zetzellia* (5 species)

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RESEARCH ARTICLE

Acarine biodiversity associated with bark beetles in Mexico

M. Patricia CHAIRES-GRIJALVA [1], Edith G. ESTRADA-VENEGAS [1,3], Iván F. QUIROZ-IBÁÑEZ [1], Armando EQUIHUA-MARTÍNEZ [1], John C. MOSER [2†], Stacy R. BLOMQUIST [2]

[1] *Colegio de Postgraduados, Carretera México-Texcoco Km.36.5, Montecillo, Texcoco, Estado de México C.P.56230. México*
[2] *Southern Research Station, United States Department of Agriculture, Alexandria Forestry Center, 2500 Shreveport Hwy, 71360 Pineville, Louisiana, USA*
[3] Corresponding author: edith_ev@yahoo.com.mx

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ABSTRACT: The phloem of dying trees provides habitat for a large number of bark beetles and their associated mites. These mites depend on the scolitids for moving from one place to another, and directly or indirectly for their nutrition. In Mexico, there have been very few works on this topic. The first three studies in Mexico included isolated records of these associations, while the last three refer to new records for several states in the country. A total of 62 mites species were recorded in the present study. The most diverse order was Mesostigmata with 66% of the recorded species, followed by the suborder Prostigmata with 24% and the cohort Astigmatina with 10%. *Trichouropoda polytricha* (Vitzthum, 1923), *Proctolaelaps subcorticalis* (Lindquist, 1971), *Proctolaelaps dendroctoni* Lindquist and Hunter, 1965, *Schizosthetus lyriformis* (McGraw and Farrier, 1969) and *Dendrolaelaps neodisetus* (Hurlbutt, 1967) were the most common species associated with bark beetles in this study. *Dendroctonus frontalis* Lindquist and Hunter, 1965 is the bark beetle with the highest reported number of associated mites in Mexico and worldwide. Among the species mentioned in this study, there was an interesting range of feeding habitats and habits. The different associations among beetles and mites provide an interesting topic for future research.

Keywords: Coleoptera, *Dendroctonus*, logs, natural forest, phoretic, symbiosis

INTRODUCTION

Among the bark beetles species, there are symbiotic interactions, many of which are maintained through multiple factors, such as climate, predation and the use of resources. Mites are important symbiotic organisms associated with bark beetles. More than one hundred species of mites have been reported from bark beetles worldwide; some phoretic species are associated with adult beetles and other invertebrates on infested trees (Hofstetter et al., 2013; Lindquist, 1969). Many of these mites depend on bark beetles or other insects to be transported inside the trees (Moser, 1985, Moser et al., 2010). Other studies report other microorganisms such as protozoa and nematodes in these associations (Perotti and Braig, 2011). The galleries of bark beetles (Coleoptera: Curculionidae: Scolytinae) are located under the bark of trees, and all developmental stages live there but other arthropods and microorganisms live and feed there (Lindquist, 1975).

The bark beetles are host species for a diverse community of arthropods and microorganisms that are found subcortically, mainly mites. Lindquist (1975) stated that the associations between mites and other arthropods can be based on a number of factors, namely: 1) the presence of a stable habitat that guarantees the supply of food resources and the protection of both organisms; 2) specificity for the habitat, in which the mite shows preference for the habitat and not for the host; 3) the mites specificity on the host; 4) specificity of the mites for a site on the host; and 5) synchrony of the life cycle of the mite with the insect, the cycle of the first one being shorter than that of the host, and which represents an adapted state in which it remains until its biological cycle is synchronized with the host.

Several groups of mites are associated with these insects, forming dynamic, interspecific relationships. The relationships of mites and their hosts are defined by distinctive patterns, which have been shaped by long evolutionary processes, involving a succession of interactive responses, which resulted in different biorelationships (Hoffmann, 1988; Krantz and Walter, 2009). The importance of these relationships is that energy flows are created within the trophic networks and they contribute to the structuring of the ecosystem.

Mite communities that live in the galleries of bark beetles are not only associated with these insects but there are species that feed on fungi, leaf litter and other insects (Kaliszewski, 1993; Walter and Proctor, 1999). There is an extensive body of literature, especially related to *Dendroctonus frontalis* Zimmerman, 1868 (Kinn and Witcosky, 1978; Hofstetter, 2011; Hofstetter et al., 2013, Hofstetter et al., 2015), *D. rufipennis* Kirby, 1837 (Cardoza et al., 2008), *Ips typographus* (Linnaeus, 1758) (Takov et al., 2009), *Pityokteines* spp. (Pernek et al., 2008, 2012), and *Scolytus* spp. (Moser et al., 2010, but species associated with ambrosia beetles are just beginning to be studied. Mites are good examples of phoretic organisms; mites associated with insects are transported to new habitats, in which they each play different ecological roles.

The term phoresy (from the Greek *phora*: carry, have) defines one of the many kinds of association among animals (Trägårdh, 1943). Mites are the main examples. Farish and Axtell (1971) proposed a new definition: "Phoresy is a phenomenon in which an animal actively looks for and lives on the outer surface of another animal for a limited time, during which the linked animal (called a phoretic) stops feeding and development, presumably as a result of dispersal. This process is undoubtedly important in mites' evolution, which has followed different pathways in the Mesostigmata, Prostigmata and Astigmatina (Cross and Bohart, 1969). The families in which phoresy occurs are Macrochelidae, Parasitidae, Laelapidae, Ascidae, Eviphididae and some Uropodines, and some families from other groups, such as the Scutacaridae and Anoetidae (Athias-Binche, 1994; Reynolds et al., 2014)

The bark beetles galleries provide habitat for a large number of boring insects, mainly beetles and their associated mites. The best known mites are those associated with the genera *Dendroctonus*, *Ips*, *Tomicus*, *Hylesinus*, *Hylastes*, *Dryocoetes* and *Scolytus*, each having 15 to 20 species of associated mites, of which 10 or 12 can be common in scolitid habitat (Hofstetter and Moser, 2014). The relationship between the food webs in the galleries and on the bark beetles can be complicated. The feeding habits represented in galleries include fungivory, saprophagy (*Tarsonemus* spp., *Pygmephorus* spp., *Histiogaster spp.*, *Histiostoma* spp., among others), polyphagy and nematophagy, as well as specialized predation in the early stages of bark beetles (*Digamasellus* spp., *Lasioseius* spp.) or as monophagous or oligophagous parasites of bark beetles larvae (*Pyemotes* spp., *Paracarophenax* spp. and *Iponemus* spp.) (Lindquist, 1970; Cross and Moser, 1971).

These groups have different degrees of morphological and physiological adaptations. They are usually associated with the insect nymphal stage. However, little is known about the biology of phoretic mite and the host. The discovery of a phoretic relationship guides us to a better understanding of the ecological role of both species (Binns, 1972).

There are records of mites associated with scolytids from 15 countries. Of the total of 178 species of mites, 96 are included in the order Mesostigmata (52%), 55 in the suborder Prostigmata (32%), 14 in the suborder Oribatida (8%) and 13 in the cohort Astigmatina (8%) (Chaires-Grijalva et al., 2012). In Mexico, some studies have been carried out on mites associated with scolytid beetles, especially on conifers (Table 1) (Gispert, 1983; Hoffmann and López-Campos, 2000; Chaires-Grijalva, 2013, 2015; Quiroz-Ibáñez, 2016; Quiroz-Ibáñez et al., 2017). However, due to the difficulty in observing subcorticular mites, very little is known about their biology and behaviour. A common characteristic observed in the communities of scolytids is the diversity and the number of organisms present in the subcortical galleries. The main objective of the current study was to document the diversity of mites associated with different species of bark beetles, from 2000 to the present.

MATERIAL AND METHODS

Review of bark beetles collection. Visits were made to different collections of bark beetles and associated mites in Mexico and the USA, namely the Universidad Nacional Autónoma de México (UNAM) (National Collection of Mites), Escuela de Ciencias Biológicas of Instituto Politécnico Nacional (ENCB-IPN) (Mites collection ENCB-IPN), Universidad Autónoma Metropolitana (UAM), Universidad Autónoma Chapingo (UACh) (Acarology Collection), Colegio de Postgraduados (CP) Campus Montecillo (Acarology Collection), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Campus Experimental Pabellón (Entomology and Acarology Collection), and Entomology collection of Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas Forestales (CENID-COMEF). The United States Forest Service, Southern Research Station in Pineville, Louisiana (USDA - SF) (USDA - FS Forest Mite Collection) collection was also visited. In all of these collections, an exhaustive review of mite species associated with bark beetles.

Sampling. In addition, samples of bark and logs showing signs of damage by bark beetles (Scolytinae) were obtained from 24 states of Mexico from April 2007 to November 2017. The samples were from the following states: Aguascalientes, Baja California, Ciudad de México, Chiapas, Chihuahua, Coahuila, Colima, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo Leon, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tlaxcala, Veracruz and Zacatecas (Figure 1). The samples were transported in plastic bags and transferred to Forest Entomology Laboratory at Colegio de Postgraduados, for mite revision under a stereoscopic microscope. The logs were placed in emergence chambers to collect insect adults and then the mites were separated from them.

Figure 1. Sampling states for scolytins and associated mites in Mexico.

Bark beetle identifications. Bark beetles and associated arthropods were collected from bark and log samples with forceps and then placed in separate vials with ethanol. Mites attached to the collected bark beetles were removed and placed in a solution of lactic acid to clear. The specimens collected from the bark beetles were identified by using the keys of Cibrian et al. (1995) and Wood

(2007). The bark beetle identifications were corroborated by Dr. Armando Equihua-Martínez, a scolytin specialist in Mexico.

Mite identifications. Mites attached to the collected bark beetles were removed from each individual, placed in a solution of lactic acid to clear them, mounted on glass slides using Hoyer's medium and identified. In addition to the phoretic associates collected on bark beetles, mites and other organisms found in the bark beetle galleries were also collected and observed. During a visit to the laboratory of the Forest Service of the USA, the first author reviewed the world's largest collection of mites associated with bark beetles. The diagnostic characteristics of the species collected in this work were compared by means of two optical microscopes with phase contrast. The mites were identified by using the keys of Lindquist and Evans (1965), Lindquist and Hunter (1965), McGraw and Farrier (1969), Cross and Moser (1971), Lindquist (1971) and Karg (1993). The species were corroborated by Dr. John C. Moser. The mites were deposited in the personal collection of Dr. Edith G. Estrada-Venegas.

RESULTS

A total of 13,859 mites were collected, including 62 species of mites belonging to three orders, namely: Mesostigmata (41), Trombidiformes (suborder Prostigmata) (15) and Sarcoptiformes (suborder Oribatida, cohort Astigmatina) (6) (Figure 2). The number of species recorded until 1983 was 29; they were included in two orders (Mesostigmata, and Trombidiformes)(in Table 1). It should be noted that from 1983 to 2007 no new results were reported for Mexico.

From the samplings made since 2007, 37 are new records for Mexico (Table 2). Twenty four species (65%) belong to the order Mesostigmata of the species, 10 species (27%) to Trombidiformes (suborder Prostigmata) and three species (8%) to Sarcoptiformes (suborder Oribatida, cohort Astigmatina).

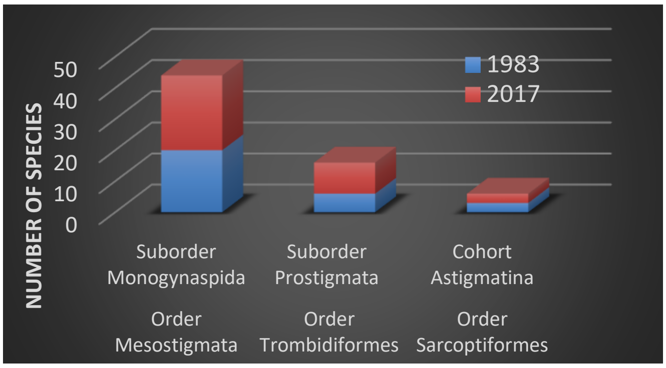

Figure 2. Mite orders associated with bark beetles.

The mesostigmatid mites were the most diverse, with 66% above of the recorded species. In this order are included nine families, as shown in Table 2 and Figure 3. The suborder Prostigmata contributed 24% above of species in three families and the cohort Astigmatina with two families contributed 10% of species. Within the populations collected, the females were most abundant (8415), followed by nymphs (5172). Ereynetidae and Acaridae were the most abundant families but the most diverse families were Tarsonemidae with eight species and Trematuridae with seven species. The most common species are *Trichouropoda polytricha, Proctolaelaps subcorticalis, Elattoma* sp. and *Histiogaster* sp. The best represented states of Mexico were Chihuahua, Jalisco and Veracruz.

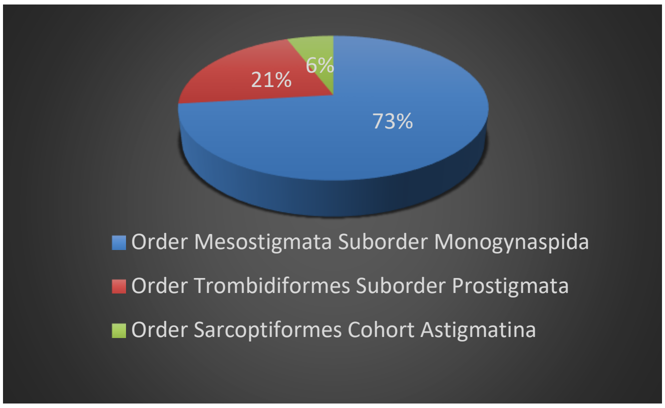

Figure 3. Percentage of species per suborder found in current samplings in Mexico.

DISCUSSION

A great diversity of mesostigmatid mites is associated with bark beetles, although there is no clear distinction between truly cortical species and those that exist on the forest floor (Beaulieu et al., 2006). However, many species of mites live exclusively in decaying wood and subcortically, environments in which they have complex relationships with bark beetles. These mites rely on subcortical insects for their dispersal and introduction into suitable habitats for survival (Szymkowiak et al., 2007)

Mite communities can be large and complex, with multiple food guilds and trophic levels (Lindquist, 1969, McGraw and Farrier, 1969). However, due to the difficulty in observing subcortical mites, very little is known about their biology and behaviour. A common characteristic observed in communities of scolytins are the diversity and the number of organisms present in the subcortical galleries.

There are four dominant groups in the galleries; bark beetles, mites, nematodes and fungi. Barras (1979) has described the subcortical ecosystem of the scolytins as a "supra-organism" due to the co-evolutionary relationships existing in it.

The greatest diversity of mite associations with other arthropods occurs in the Mesostigmata. These associations include occasional, facultative or obligatory phoresy for dispersal. The complexity of these associations suggests a long history of evolution between these mites and their hosts. These range from the synchronization of the life cycle of both species, morphological adaptations, special mechanisms of fixation, physiological adaptations such as resistance to dehydration, cessation of development and metabolic reduction and the absence of response to normal stimuli, such as food, mating behaviour, humidity and temperature (Szymkowiak et al., 2007; Walter and Proctor, 2013).

Table 1. Mite species reported associated with bark beetles in Mexico until 1983

Species	Animal host	Plant host	Reference
ORDER MESOSTIGMATA			
TREMATURIDAE			
Trichouropoda australis (Hirschmann, 1972)	*D. frontalis* *I. bonanseai*	*P. hartwegii*	Moser et al., 1974
Trichouropoda hirsuta (Hirschmann, 1972)	*D. frontalis*	Not recorded	Moser and Roton, 1971
Trichouropoda polytricha (Vitzthum, 1923)	*I. bonanseai* *I. typographus*	*P. hartwegii*	Moser et al., 1989
PARASITIDAE			
Schizosthetus lyriformis (McGraw and Farrier, 1969)	*D. frontalis*	*P. echinata* *P. taeda* *P. oocarpa* *P. contorta*	McGraw and Farrier, 1969; Moser et al., 1974; Gispert, 1983
DIGAMASELLIDAE			
Dendrolaelaps neodisetus (Hurlbutt, 1967)	*D. frontalis* *I. bonanseai*	*P. hartwegii*	Moser and Roton, 1971; Gispert, 1983; McGraw and Farrier, 1969
Dendrolaelaps quadrisetus (Berlese, 1920)	*D. frontalis* *I. calligraphus*	*P. virginiana* *P. echinata* *P. taeda*	Gispert, 1983
Dendrolaelaps sp.	*Ips bonanseai*	*P. hartwegii*	Gispert, 1983
MACROCHELIDAE			
Macrocheles boudreauxi (Krantz, 1965)	*D. frontalis*	*P. echinata* *P. taeda* *P. arizonica*	Moser and Roton, 1971
ASCIDAE			
Arctoseius cetratus (Sellnick, 1940)	*I. bonanseai*	*P. hartwegii*	Gispert, 1983
Arctoseius semicissus (Berlese, 1892)	*I. cribicollis*	*P. montezumae*	Gispert, 1983
Asca pini (Hurlbutt, 1963)	*I. bonanseai*	*P. hartwegii*	Gispert, 1983
Lasioseius corticeius (Lindquist, 1971)	*I. bonanseai*	*P. hartwegii*	Gispert, 1983
Lasioseius safroi (Ewing, 1920)	*D. frontalis*	*P. hartwegii*	Gispert, 1983
Proctogastrolaelaps libris (McGraw and Farrier, 1969)	*D. frontalis* *S. multistriatus*	*P. echinata*	McGraw and Farrier, 1969
Proctoalelaps dendroctoni (Lindquist and Hunter 1965)	*D. frontalis*	*P. contorta*	Lindquist, 1971; Moser et al., 1974; Gispert, 1983
Proctolaelaps hystricoides (Lindquist and Hunter, 1965)	*D. frontalis*	*P. montezumae*	Moser et al., 1974
Proctolaelaps hystrix (Vitzthum, 1923)	*D. frontalis* *D. rhizophagus*	*P. taeda* *P. arizonica* *P. montezumae*	Moser et al., 1974
Proctolaelaps subcorticalis (Lindquist, 1971)	*D. frontalis* *I. bonanseai*	*P. leiophylla*	McGraw and Farrier, 1969; Moser et al., 1974; Gispert, 1983
Gamasolaelaps subcorticalis (McGraw and Farrier, 1969)	*D. frontalis* *I. avulsus* *I. calligraphus* *I. lecontei*	*P. taeda* *P. oocarpa*	McGraw and Farrier, 1969
ORDER TROMBIDIFORMES			
SUBORDER PROSTIGMATA			
TARSONEMIDAE			
Tarsonemus endophloeus (Lindquist, 1969)	*D. frontalis*	*P. hartwegii*	Moser et al., 1974
Tarsonemus ips (Lindquist, 1969)	*D. frontalis*		Moser et al., 1974
Tarsonemus triarcus (Lindquist, 1969)	*I. bonanseai*	*P. hartwegii*	Gispert and Atkinson, 1982
Ereynetes scutulis (Hunter, 1964)	*D. frontalis*		Moser et al., 1974
Heterotarsonemus lindquisti (Smiley, 1969)	*D. frontalis*		Moser et al., 1974; Lindquist, 1969
Pyemotes sp.	*D. frontalis*		Moser et al., 1974
ORDER SARCOPTIFORMES			
COHORT ASTIGMATINA			
ACARIDAE			
Histiogaster arborsignis (Woodring, 1963)	*D. frontalis*		Moser et al., 1974
Histiogaster rotundus (Woodring, 1966)	*D. frontalis*		Moser et al., 1974
Tyrophagus putrescentiae (Schrank, 1781)	*D. frontalis*		Gispert and Atkinson, 1982

In the present study, mesostigmatid mites, contributed the highest number of species associated with bark beetles, mainly in 8 families. Ascidae and Digamasellidae contributed the most species, with the genera *Lasioseius*, *Proctolaelaps*, *Dendrolaelaps* and *Trichouropoda* frequently found in association with 11 bark beetle species, with *Dendroctonus frontalis* (Zimmerman, 1868) being the species with the highest number of mites species.

Moser (1975) reported that *Proctolaelaps dendroctoni*, *P. hystricoides*, *P. hystrix*, *Dendrolaelaps neocornutus*, *D. neodisetus*, *Macrocheles boudreauxi* and *Schizosthetus lyriformis* are natural enemies of *D. frontalis* in Louisiana in the United States of America, while the *Dendrolaelaps quadrisetus*, *Arctoseius cetratus*, *Asca pini*, *Lasioseius cortiseius*, *L. safroi*, *Proctolaelaps subcorticalis* and *Androlaelaps casalis* are only possible candidates as biological control agents (Chaires-Grijalva et al., 2013) However, the impact of these mites on bark beetles in Central America has never been evaluated.

The deutonymphs of the family Trematuridae in the genus *Trichouropoda* were the most abundant; this is the main stage for their dispersion (Bajerlein, 2013), trematurids mites grouped in the elytral decline and ventral surface. In other mesostigmatid families, females have been recorded as the main stages for dispersal and establishment on different hosts (Lindquist, 1969; Moser and Roton, 1971; Krantz and Walter, 2009), which is supported by the results obtained in this work. The phoretic relationships that mites have developed with the scolytins have facilitated specializations.

CONCLUSIONS

In this study, the Mesostigmata contributed 66% of the species, followed by the suborder Prostigmata with 24% and the cohort Astigmatina with 10%. *Trichouropoda polytricha*, *Proctolaelaps subcorticalis*, *Proctolaelaps dendroctoni*, *Schizosthetus lyriformis* and *Dendrolaelaps neodisetus* are the most commonly reported species associated with bark beetles and were reported in all the studies that have been carried out in Mexico. The great diversity is mainly due to the variety of food resources, as well as the great adaptability of the mites present. Among the species mentioned in this study there is an interesting range of feeding habits, as well as habitats and associations with different organisms.

Dendroctonus frontalis is the bark beetle with the highest number of associated mites in Mexico and worldwide (Chaires-Grijalva, 2013). As demonstrated in this study, there is a great diversity of mites associated with bark beetles; this is mainly due to the variety of food resources that bark beetle galleries provide, as well as the wide range of adapatations they have for the different environments in which they occur. Among the species mentioned in this study there is an interesting range of trophic habits, as well as habitats, and different associations that make these groups worthy of more study.

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Table 2. Species reported associated with bark beetles in Mexico from 2007 to 2017

Species	Mites collected			Animal host	Plant host
	♀	♂	Nymph		
ORDER MESOSTIGMATA					
SEJIDAE					
Sejus boliviensis Hirschmann and Kaczmarek, 1991	5	2	1	*D. frontalis*	*P. hartwegii*
NENTERIDAE					
Nenteria ca. *breviunguiculata* Willmann, 1949			9	*D. frontalis*	*P. hartwegii*
Nenteria sp.	1			*D. frontalis*	*P. teocote*
TRICHOUROPODIDAE					
Trichouropoda adjuncti Wisniewski and Hirschmann, 1988			54	*D. frontalis*	*P. hartwegii*
Trichouropoda australis Wisniewski and Hirschmann, 1988	9			*D. frontalis*	*P. hartwegii*
Trichouropoda hondurasae Hirschmann and Wisniewski, 1986			38	*D. frontalis*	*P. teocote*
Trichouropoda fallax Vitzthum, 1926	2		11	*D. frontalis*	*P. teocote*
Trichouropoda ovalis (C.L. Koch, 1839)			62	*D. rhizophagus*	*P. arizonica*
Trichouropoda polytricha (Vitzthum, 1923)	4	1	135	*I. bonanseai* *I. typographus*	*P. hartwegii*
Trichouropoda sp. 1			6	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 2			18	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 3			17	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 4			31	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 5			4	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 6			1	*D. frontalis*	*P. teocote* *P. hartwegii*
Trichouropoda sp. 7			4	*D. frontalis*	*P. teocote* *P. hartwegii*
PARASITIDAE					
Schizosthetus lyriformis (McGraw and Farrier, 1969)	10	1	30	*D. frontalis* *D. valens* *I. bonanseai* *I. calligraphus* *I. confusus* *I. grandicollis* *P. mexicanus*	*P. echinata* *P. taeda* *P. oocarpa* *P. contorta*
DIGAMASELLIDAE					
Dendrolaelaps neocornutus (Hurlbutt, 1967)	17	3	5	*D. frontalis* *D. rhizophagus*	*P. echinata* *P. arizonica*
Dendrolaelaps neodisetus (Hurlbutt, 1967)	17		9	*D. frontalis* *I. bonanseai*	*P. hartwegii*
Dendrolaelaps quadrisetus (Berlese, 1920)	8			*D. frontalis*	*P. hartwegii*
Dendrolaelaps sp.	2			*D. frontalis*	*P. oocarpa*
MACROCHELIDAE					
Macrocheles boudreauxi (Krantz, 1965)	6	1	1	*D. frontalis*	*P. arizonica* *P. echinata* *P. taeda*
Macrocheles sp.			11	*D. frontalis*	*P. arizonica*
ASCIDAE					
Arctoseius cetratus (Sellnick, 1940)	2			*I. bonanseai*	*P. hartwegii*
Arctoseius semicissus (Berlese, 1892)	5			*I. cribicollis*	*P. montezumae*
Asca pini (Hurlbutt, 1963)	8		2	*I. bonanseai*	*P. hartwegii*
Lasioseius corticeius	1		1	*I. bonanseai*	*P. hartwegii*
Lasioseius dentatus Fox, 1946	1			*D. frontalis*	*P. hartwegii*
Lasioseius imitans Berlese, 1910	10		4	*D. adjunctus*	*P. hartwegii*
Lasioseius safroi (Ewing, 1920)	29		8	*D. adjunctus* *D. frontalis* *I. bonanseai*	*P. hartwegii*
Lasioseius sp.	26		3	*D. frontalis*	*P. teocote*

Table 2. Species reported associated with bark beetles in Mexico from 2007 to 2017 (Continued)

Species	**Mites collected**			**Animal host**	**Plant host**
	♀	**♂**	**Nymph**		
	ORDER MESOSTIGMATA				
MELICHARIDAE					
Proctoalelaps dendroctoni (Lindquist and Hunter, 1965)	28	1	7	*D. frontalis* *D. ponderosae*	*P. contorta*
Proctolaelaps hystricoides (Lindquist and Hunter, 1965)	14		4	*D. frontalis*	*P. echinata* *P. montezumae* *P. taeda*
Proctolaelaps hystrix (Vitzthum, 1923)	17	3	5	*D. frontalis* *D. rhizophagus*	*P. arizonica* *P. montezumae* *P. taeda*
Proctolaelaps subcorticalis (Lindquist, 1971)	93	3	41	*D. frontalis* *D. mexicanus* *I. bonanseai* *I. cribicollis*	*P. leiophylla* *P. montezumae*
Proctolaelaps sp.	57			*S. schevyrewi*	*U. pumila*
LAELAPIDAE					
Androlaelaps casalis Berlese, 1887	1			*D. frontalis*	*P. teocote*
Hypoaspis (*Cosmolaelaps*) ca. *Vacua* Michael, 1891	26	2	8	*D. frontalis*	*P. teocote* *P. hartwegii*
	ORDER TROMBIDIFORMES				
SUBORDER PROSTIGMATA					
PYGMEPHORIDAE					
Elattoma sp.	7200			*I. calligraphus*	*P. chiapensis*
TARSONEMIDAE					
Tarsonemus ips (Lindquist, 1969)	6			*D. frontalis*	*P. teocote*
Tarsonemus krantzi (Smiley and Moser, 1974)	17			*D. frontalis*	*P. teocote*
Tarsonemus sp.1	2			*D. frontalis*	*P. teocote*
Tarsonemus sp.2	40	6		*D. frontalis*	*P. teocote*
Tarsonemus sp.3	26			*D. frontalis*	*P. teocote*
Tarsonemus sp.4	5	2		*D. frontalis*	*P. teocote*
Tarsonemus sp.5	2			*D. frontalis*	*P. teocote*
Tarsonemus sp.6	2			*D. frontalis*	*P. teocote*
Tarsonemus sp.7	2			*D. frontalis*	*P. teocote*
Tarsonemus sp.8	2			*D. frontalis*	*P. teocote*
EREYNETIDAE					
Ereynetes scutulis (Hunter, 1964)	10			*D. frontalis*	*P. teocote*
	ORDER SARCOPTIFORMES				
COHORT ASTIGMATINA					
ACARIDAE					
Histiogaster sp.	700	243	2374	*S. schevyrewi*	*U. pumila*
Cosmoglyphus sp.			1279	*S. schevyrewi*	*U. pumila*
HISTIOSTOMATIDAE					
Histiostoma varia (Woodring and Moser, 1970)			980	*I. calligraphus*	*P.chiapensis*

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RESEARCH ARTICLE

Population development of European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae) on apple orchards in Çanakkale, Turkey

İsmail KASAP [1,4], Şahin KÖK [1], Serkan PEHLİVAN [2], Gökhan BAŞTUĞ [3]

[1] *Department of Plant Protection, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[2] *Department of Plant Protection, Faculty of Agriculture, Çukurova University, Adana, Turkey*
[3] *Koruma Chlorine Alkali Products, Kocaeli, Turkey*
[4] Corresponding author: ikasap@comu.edu.tr

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ABSTRACT: The aim of this study was to determine the seasonal population dynamics of the European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae) on different apple varieties in apple orchards in Çanakkale, Turkey, during 2010-2011. Surveys were carried out weekly from April to November in sprayed and unsprayed orchards on cultivated Golden Delicious, Starking Delicious and Grany Smith apple varieties. The results of the study indicated that the population density of spider mites began to increase generally in early May, and reached the maximum level from mid June to late August. Afterwards, it preserved to late September in both years. During the both years, *P. ulmi* more prefered the Starking Delicious than Golden Delicious and Grany Smith apple varieties in the all orchards. In this period, the population densitiy of *P. ulmi* reached to maximum level with 115.9 and 75.3 mites/per leaf on the Starking Delicious apple variety in 2010 and 2011 years, respectively in sprayed orchards. In unsprayed orchard, spider mites remained at very low levels certainly because of the presence of *Typhlodromus athiasae* Porath and Swirski (Acari: Phytoseiidae) which was only one predator species on spider mites. On the other hand in sprayed orchards, although *Stethorus punctillum* (Col.: Coccinellidae) and *Zetzellia mali* (Acari: Stigmaeidae) were the most abundant predatory species on spider mites, they could not control the spider mites on both apple cultivar and years.

Keywords: Apple, Çanakkale, *Panonychus ulmi*, population dynamics, Tetranychidae

INTRODUCTION

Apple is one of the most common crops in the world, and also Turkey. About 3.032.164 tons of apple are produced per year in Turkey. Also, apple is one of the major fruit crop in the Çanakkale region of Turkey, where approximately 101.943 tons of apple including 3.36 % of Turkey's total apple production are produced per year in 41.767 ha area (Anonymous, 2017).

Although many arthropod species and diseases cause economic losses on apple areas, spider mites are one of the most important pests of apple production areas throughout the world. The European Red Mite, *Panonychus ulmi* Koch. (Acari: Tetranychidae) is the most serious species of these spider mites in apple areas. It can be damaged nearly all apple growing areas in both Çanakkale province and Turkey (Huffaker et al., 1970; McMurtry et al., 1970; Jeppson et al., 1975; Düzgüneş, 1977; Şekeroğlu, 1977; Yiğit and Uygun, 1982; Erkam and Gürkan, 1983; Çiftçi et al., 1985; Uygun et al., 1992; Karaca, 1994, Erol and Yaşar, 1996; İncekulak and Ecevit, 2002; Bulut and Madanlar, 2004; Kasap, 2004; Yanar and Ecevit, 2005; Kasap and Çobanoğlu, 2007). In many commercial apple orchards, the chemical applications are used for management of the European Red Mite, *P. ulmi* and other pest mites. The disruptive effects of these pesticides, especially due to development of resistance by pests, have led to greater reliance on natural enemies for their control in the Çanakkale region (Erol and Yaşar, 1996; Atlıhan et al., 2002; Yardım et al., 2002). However, as a result of the use of intensive pesticides, the elimination of natural enemies from the environment has been causing spider mites outbreaks. The effects of predatory mites on the seasonal abundance of spider mites have not been previously investigated in commercial apple orchards in Çanakkale.

In recent years, population densities and economical importance of spider mites has greatly increased in Çanakkale. However, population dynamics of the European red mite *P. ulmi* and their natural enemies in apple orchards has been poorly investigated. The present study was primarily designed to provide data on the population dynamics of the European red mite *P. ulmi* and their natural enemies in Çanakkale province of Turkey.

MATERIAL AND METHODS

Ten apple orchards (4 orchards in Bayramiç-Evciler, 2 orchards in Çanakkale-Kepez, 1 orchard in Çanakkale-Kepez and 3 orchards in Yapıldak Lepseki-Umurbey) were selected to determine the population dynamic of *Panonychus ulmi* on apple orchards (Starking Delicious, Golden Delicious and Grany Smith apple varieties) in the Çanakkale province during 2010 – 2011 years. In 2011, two apple orchards (1 orchard in Bayramiç-Evciler, (Golden Delicious, Starking Delicious apple varieties) 1 orchard in Çanakkale-Kepez and 1 orchard in Çanakkale-Umurbey) were added to these orchards. Of these orchards, Evciler 3-4 and Yapıldak, Umurbey 3 are completely without pesticides and other orchards, especially Kepez 1-2 and Evciler 1-2, are heavily sprayed with pesticides. The apple cultivars, Golden Delicious and Starking Delicious, were planted in a mosaic in these orchards, but the Grany Smith apple cultivar was also planted. The size of these gardens is about 20-80 decares, and the age of trees ranged between 8-20 years. Samplings were carried out from selected apple trees in every week from March to November for the both years. For sampling, 10 apple trees that the best represent of the orchard were determined for every apple orchards using in experiments and 10 leaves from every apple trees in these orchards were collected. In total, one hundred leaves of apple trees were collected from every apple orchards. Thee leaves were collected randomly from the periphery (1.2—2.3 m high) of the marked trees. The leaves were brought to the lab in plastic containers and stored at 4 ºC in the refrigerator. The *P. ulmi* on leaves collected from apple orchards were counted using the spider mite brushing machine. Firstly, the mites were brushed from the leaves with brushing machine onto a collecting plate and then all stages such as egg and motile stages of *P. ulmi* were counted under stereomicroscope (magnification 40 x) in the Acarology and Systematic Laboratory of Çanakkale Onsekiz Mart University within three days after collection from apple trees. Afterwards, the results obtained as total leaves were then rated to be individual per leaf (Henderson and McBurnie, 1943).

RESULTS

Seasonal fluctuations of the European Red Mite, *P. ulmi* varied in sprayed and unsprayed orchards throughout the study period (Fig 1). The population of *P. ulmi* reached the highest density in different period in different apple orchards of Çanakkale Province (Kepez, Evciler, Yapıldak and Umurbey) during 2010 and 2011 (Fig 1). As a result of counting in the Kepez 1 orchard, it was determined that population of *P. ulmi* showed an increase from May to October during 2010. In Kepez 1 orchard, this population reached the peak twice in the year with 49.9 and 48.1 mites per leaf on the Starking Delicious apple cultivar and 24.6 and 25.1 mites per leaf on the Golden Delicious apple cultivar and then continued to decrease until the end of the year. In 2011 year, *P. ulmi* population peaked with 66.8 and 53.6 mites per leaf on the Starking Delicious apple cultivar and 52.4 and 53.9 mites per leaf on the Golden Delicious apple cultivar. In the Kepez 2 orchard, population of *P. ulmi* reached to the peak twice both July and August during 2010 with 28.4 and 23.1 mites per leaf on the Starking Delicious apple cultivar and 15.2 mites per leaf on the Golden Delicious apple cultivar. But, in 2011 year, on Starking and Golden Delicious apple cultivars, the *P. ulmi* population had a lower peak with 19.4 and 4.0 mites per leaf respectively. However, it was determined that population density of *P. ulmi* in the Kepez 2 orchard lower than other orchard. In this period, Evciler 1 and 2 orchards, the population densities of *P. ulmi* reached the maximum level with 115.9 and 75.3 mites/per leaf on the Starking Delicious apple variety in 2010 and 2011 years, respectively in sprayed orchards. But, in the same orchards on Golden Delicious apple cultivars, the *P. ulmi* population had a lower peak with 69.6 and 14.8 mites per leaf, respectively.

From untreated orchards with pesticides, the Yapıldak 1, population of *P. ulmi* almost never observed on all apple varieties during both 2010 and 2011. In Kepez and Evciler orchards, population densities of *P. ulmi* were quite high level because of the fact that natural enemies were not effective due to intensive use of chemicals against *P. ulmi*. However, *Typhlodromus athiasae* (Acari: Phytoseiidae) which natural enemy of spider mites was effective against *P. ulmi* in Yapıldak 1 orchard where pesticides were not used. As a result of the present study, population density of *P. ulmi* showed an increase from April to October during both 2010 and 2011 in sprayed orchards. In these orchards, *Stethorus punctillum* (Coleoptera: Coccinellidae) and *Zetzellia mali* (Acari: Stigmaeidae) that is an important natural enemy of spider mites was effective on *P. ulmi* during both years.

DISCUSSION

This study strongly suggests that pesticide applications changed the population development and the pattern of dominance of spider mites in apple orchards. Also, pesticide application apparently resulted in an increase in *P. ulmi* populations in the two sprayed orchards and years on apple cultivars in Çanakkale. These results are in agreement with Amano and Chant (1990), Hardman et al. (1997), Van de Vrie (1985) and Yanar and Ecevit (2008). Kasap (2011) reported that the population densities of *P. ulmi* began to increase generally in early May, reached the maximum level from mid June to late August and maintained until late September in 2002 and 2003 years in Van province. He informed that the dominant species on Golden and Starking Delicious apple cultivars was *P. ulmi* in Van province. In addition, Kasap (2011) indicated that in unsprayed orchards, spider mites remained at very low levels because of the presence of predaceous mite *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) which was the only predator of spider mites. On the other hand, in sprayed orchards, although *S. punctillum* and *Z. mali* were the most abundant predatory species on spider mites. Also, Yanar & Ecevit (2008) reported that the dominant phytophagous mites in sprayed orchards in Tokat, Turkey, were *A. viennensis* and *P. ulmi*, whereas in unsprayed orchards they were *Eotetranychus uncatus* Garman (Tetranychidae) and *Cenopalpus pulcher* Can. and Fanz. (Tenuipalpidae). Predatory mites could able to control spider mites in no pesticide application orchards, but not in the sprayed orchards. İncekulak & Ecevit (2002) also reported that spider mites populations were suppressed by predators in unsprayed orchards in Amasya, Turkey. The results obtained in this study are consistent with the results of studies on the other spider mites and also *P. ulmi*.

The results of this study suggest that predatory phytoseiids may play a major role in the control of spider mites in the Çanakkale province of Turkey, if not killed by the use of pesticides.

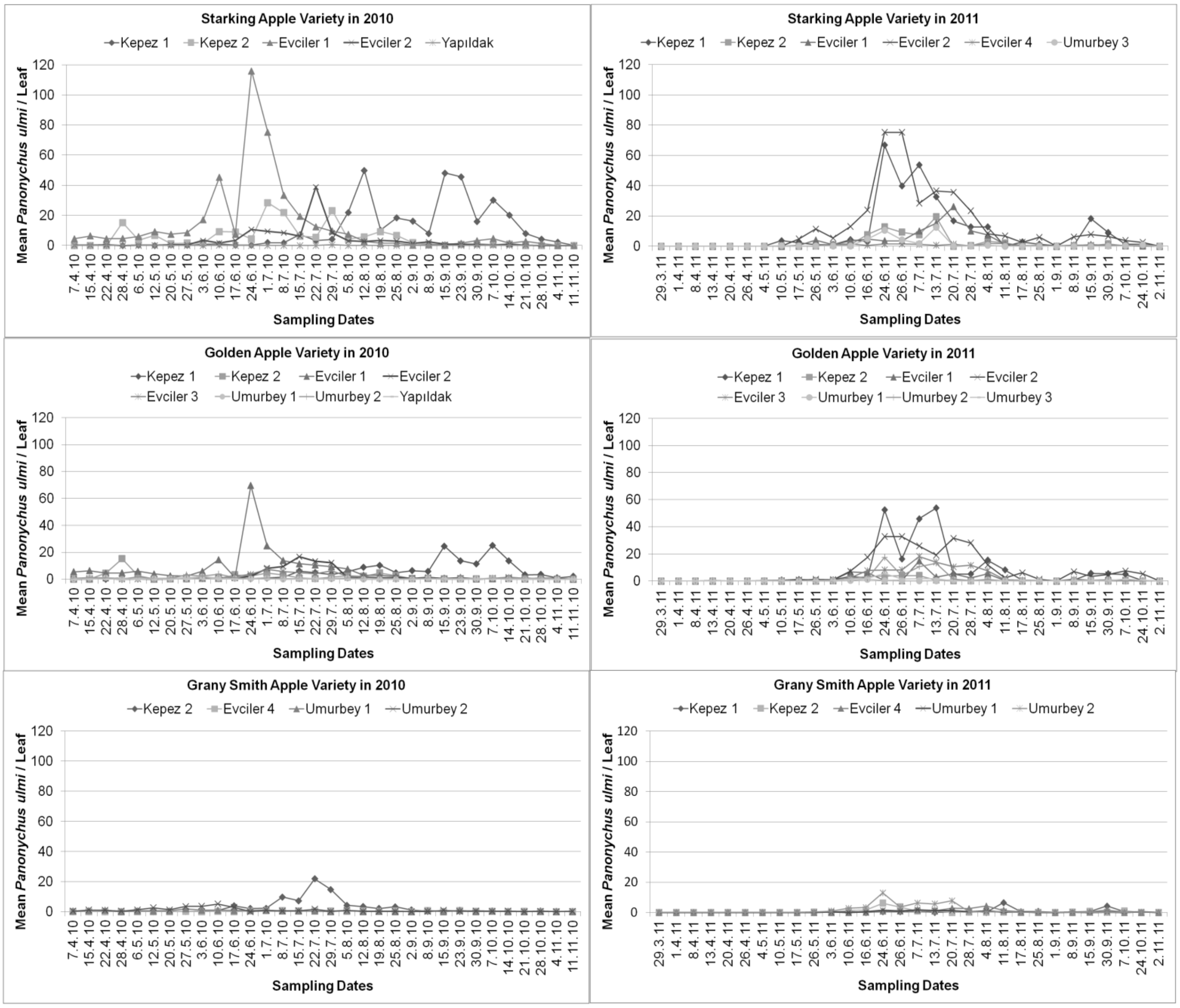

Figure 1. The population of *Panonychus ulmi* on different apple cultivars in 2010 and 2011

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RESEARCH ARTICLE

Population dynamics of the coconut mite, *Aceria guerreronis* Keifer in Kerala, India

Thalakkattil Raghavan SOBHA [1,3], **Mohamed Abdul HAQ** [2]

[1] *Department of Zoology, Farook College, Kozhikode, Kerala, 673632, India*
[2] *Division of Acarology, Department of Zoology, University of Calicut, Kerala, 673635, India*
[3] Corresponding author: sobharaghav@yahoo.co.in

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ABSTRACT: The history of the invasion of the coconut mite, *Aceria guerreronis* Keifer of Kerala, India dates back to the early 1990s and within no time the pest took a toll on coconut farming and dependent industries of this state. The coconut trees as well as the economy of Kerala suffered a serious setback over the years. The current study evaluated the population dynamics of the mite on Chowghat green dwarf variety of coconut which is highly susceptible to mite infestation. The survey of coconut mite population was carried out on the Calicut University campus and surrounding areas from 2016-2018. The population density of the mite was estimated by counting the number of mites in the meristematic zone of the infested nuts at biweekly intervals. A seasonal fluctuation in the population density was observed as a normal trend annually with higher density during January to May and lower density during the period of June to August. Furthermore, an overall decline in the population density was recorded during the rainy season of the study period.

Keywords: Chowghat green dwarf, *Aceria guerreronis*, economic loss, seasonal fluctuation.

INTRODUCTION

Coconut is grown in many parts of the world, but it is mainly cultivated in tropical countries, more specifically in Asia. It is well known as a versatile cash crop serving domestic, commercial and industrial purposes. The plant is widely accepted for its excessive usage in an array of human needs. What is amazing is that no part of the plant need be discarded as extravagant. Probably this is one of the reasons for its versatility. It is grown in 93 countries by about 11 million farmers on 12 million hectares across the world. However, Asia comes first in production by attaining over 80%.This is jointly shared by Indonesia, Philippines and India with a total of 73% with India in the third position in the world production of coconut (Adkins et al., 2006). The credibility maintained by India in world coconut production took a dive by the introduction of the coconut pest, *Aceria guerreronis* in 1998 (Sathiamma et al., 1998).

Aceria guerreronis is a serious threat and like many invasive agricultural pests it displays dramatic population growth, leading to serious outbreaks. Within a short span, it spread to new areas making threat to the whole of Kerala and neighbouring states (Haq, 1999a; Mohanasundaram et al., 1999; Muthiah et al., 2001). Substantial loss of yield in coconut production along with further invasion of the mite pest into new areas resulted in undue decline in production (Haq, 2011). Control practices though followed promptly with invasion, it remained unsuccessful to a certain extent (Arie et al., 2003; Rethinam, 2003). The net result was the current drop of 50% in coconut production, rendering a great threat to mainly coconut farmers in Kerala.

The invasion, rapid spread, outbreak and associated economic loss have jeopardized the economy of people of all sectors connected with coconut, (Haq, 2006). The dynamics of this species are determined by various factors like temperature, RH, rainfall and wind velocity. The factors favourable to repeated invasion, subsequent crop loss and further control measures need to be taken as the most important requirements (Haq et al., 2000; Haq and Sobha, 2009). As far as Southern India is concerned, Kerala deserves special attention in coconut crop productivity (Haq, 1999 b). The climatic barriers in Kerala when compared with other states are more lenient and frequent, particularly the rain. Rain fall in Kerala is connected with seasons and 2-3 cyclic rainfalls are normal for the state. *A. guerreronis* reaches high population densities at 35°C. Therefore, these high population densities occur from the end of March – May which also precedes the onset of a period of high rainfall in Kerala. The latter can cause a substantial drop in the mite numbers depending on the intensity of the rain. Distribution of *A. guerreronis* occurs mainly during dry spells and it is also during these spells that it is more severe and prevalent (Zuluaga and Sanchez, 1971; de Souza et al., 2012). Rainy season prevents all chances of transfer of the colonies to new areas. Normally matured mites will move to the periphery of the tepal from the meristematic area to be transferred by the direction of the wind. Rain fall very often hinders their transfer rendering them to remain on the periphery of the tepals as dead ones. Therefore, the colony may perish during rainy season and this depends on the intensity of the rain too. The frequency of rainfall showed a negative significant correlation and drought length showed a positive significant correlation with the population densities (Haq, 1999a; Aratchige et al., 2012; Sobha and Haq, 2015). However in other localities there was no clear relationship between coconut mite population and dry or wet weather (Mariau, 1977; Howard et al., 1990; Ramaraju et al., 2000). Studies on the effect of abiotic factors on the population dynamics of *A. guerreronis* play an important role in the evaluation of effective control measures. Therefore, the aim of the present study was to assess the influence of climatic factors like temperature, RH and rain fall on the population density of *A. guerreronis* at Calicut University and nearby areas. Future studies will concentrate more on the economic importance of this mite.

MATERIAL AND METHODS

Aceria guerreronis being so minute and its feeding and breeding sites are effectively protected by the foliage of the host plants, it necessitates special techniques for its collection. Added to this, the comparatively tall, very erect and inaccessible nature of the plant, *Cocos nucifera* further creates problems of greater magnitude for its collection. However, success of sampling to a greater extend depends on regular and timely collection of materials without interruption. The study was carried out from July 2016- July 2018. Coconut plants of the common Chowghat green dwarf variety with plants between 20-30 years old, growing adjacent to residential quarters and nearby houses in and around the Calicut University campus, were chosen for the mite population assessment. The study area comprises three locations namely Chettiyarmad, University campus and Kohinoor. Coconut plants growing in these locations have spread out bunches (Figs 1A, B) carrying buttons ranging from 10-16 in a rachilla. Clear infection of nuts can be visible when the nut reaches 4-8 weeks onwards (Figs 2, 4B). After 8 weeks the infected nuts show dried up and clear symptom (Fig. 3) and eventually they become cracked (Fig. 4A). The mean of collected buttons from each locality has been considered for final assessment of the population. Sampling was done biweekly between 6-8 am by removing the button along with some portion of rachis. In the laboratory, the tepals of each button were carefully removed, one by one (Fig. 5A). The infested area of the meristematic tissue of the button (Fig. 5B) was observed under a stereo microscope and the number of adult and nymphal stages of mites (Fig. 6) present per square centimetre area of affected meristematic tissue were counted directly for population estimation. Severely infected nuts may promote falling off of tender coconuts leaving the tepals tightly adhered to the rachis (Fig. 7). Arithmetic mean of monthly meteorological data on temperature, relative humidity, rainfall and wind velocity during the study period have been obtained from meteorological centre of CWRDM, Calicut was taken into consideration in the present study.

For statistical analysis first scatter diagrams has been plotted to check the relation between the variables viz., temperature, rainfall, relative humidity and wind velocity with mite population. Accordingly, a correlation matrix was prepared and is presented in the table. A multiple regression model has been fitted for predicting mite population with all the climatic variables.

Figure 1. Spread out bunches of coconut - A) Nuts of 1-2 weeks without infection, B) Nuts of 2-4 weeks with rare symptoms of infestation.

Figure 2. Nuts of 4-8 weeks showing clear symptoms of attack by *A. guerreronis*.

Figure 3. Nuts of above 8 weeks showing dried up symptoms of attack in most of them by *A. guerreronis*.

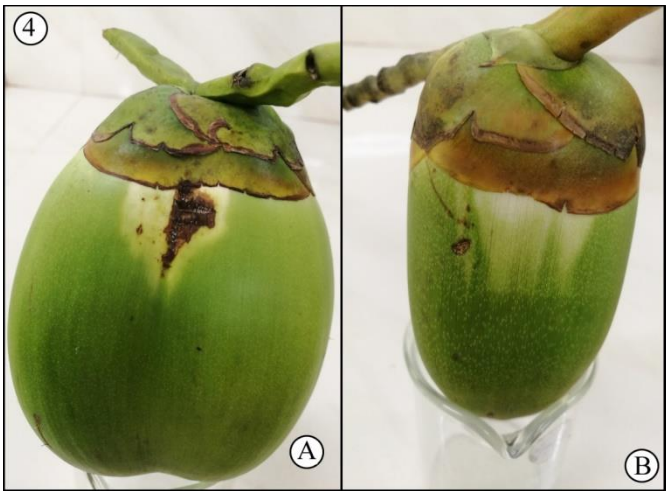

Figure 4. A) Nuts showing deep crack of infection after 12 weeks, B) Clear, broad and visible symptoms of mite infection below tepals.

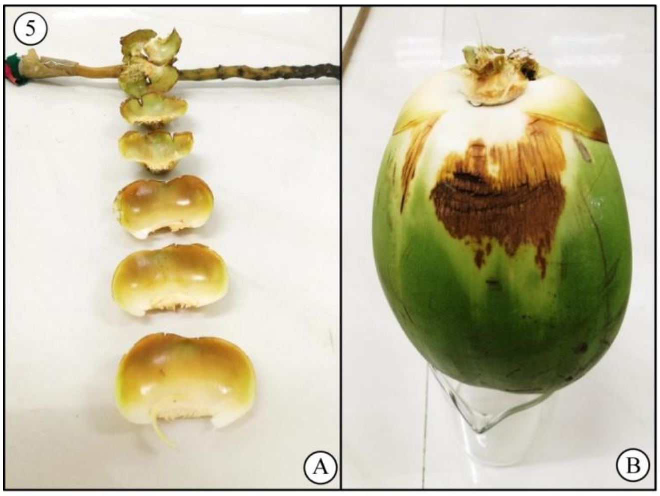

Figure 5. Removed tepals from meristematic area of the nuts for closer view of mite population - A) Two whorls of 6 tepals separated from the nut, B) Partly exposed meristematic area of the nut showing spread out symptom of attack.

Figure 6. Different life stages of *A. guerreronis* from the mite infected nut.

RESULTS

In the current study, sampling from the Chowghat green dwarf variety of coconut covering all seasons from 2016 - 2018 presented a varying degree of mite population. This trend considerably differs since the time of its invasion. The results of the study further revealed convincing evidence of population deterioration. All four climatic factors, namely temperature, RH, rainfall and wind velocity played a role in the fluctuation of the population. Temperature appeared to be the most striking along with rainfall and RH. Results of the study as shown in the correlation table also clearly indicate that among the 4 meteorological parameters, three of them, temperature, rainfall and RH have greater significance (Table 1).

Hence the effect of these variables on mite population was studied in detail with specified statistical analysis. The statistical study showed an overall performance of intermittent population fluctuation with respect to temperature and rain fall gradients.

Influence of temperature on mite population

The influence of temperature during the two-year survey disclosed a very convincing trend, namely, a successive increase in population from January to May for both the years (2017 and 2018) (Fig. 8). Further, it enabled strikingly to follow a receding pattern in population from higher levels to moderate and moderate to low levels in the succeeding months of June -October. In the years, 2016 and 2018, the impact of temperature on population density of the mite remained very striking, following a positive correlation trend. In 2016 to 2017, the population density of the mite showed a high degree of positive correlation (r=0.94) with temperature. During 2017-2018, the correlation between mite population and temperature was still higher (r=0.65). Considering all the climatic variables a multiple linear regression model is fitted for predicting the mite population. Eliminating the least significant variables using statistical techniques, we got the final regression model as Y= -1216.024 + 45.638 x X. where Y=mite population and X =temperature. Also, the R squared value is 0.871 which means that 87.1% of the variations in the response variable is explained by our linear model (Fig. 9). So from this regression equation we can predict the mite population for different known values of temperature. Predicted values from the above regression equation and observed values are plotted in Fig. 10. But when the temperature got raised above 35 ^{0}C, the population density of the mite decreased significantly.

Influence of rainfall on mite population

Despite the positive impact of temperature on the population density of the mite, rainfall exerted a negative impact namely a decline in population. The population density of the mite reached the minimum when rainfall reached its maximum. In 2016-2017 the population density of the mite showed a moderate degree of negative correlation with respect to rainfall. During 2017-2018 the degree of negative correlation between the mite population and the rainfall even increased.

Table. 1. Correlation between mite population and climatic variables

		Maximum Temperature	Maximum RH	Maximum Rainfall	Mite Population	Maximum Wind Velocity
Mite Population	**Pearson Correlation**	0.933	- 0.772	- 0.741	1	0.570
	Sig. (2-tailed)	0.000	0.000	0.000		0.003
	N	**25**	**25**	**25**	**25**	**25**

By a regression method we tried to analyse the effect of rainfall on mite population and got a linear relationship between the two variables. In 2016-2017 the correlation between rainfall and mite population with r = -0.67 .i.e. a moderate linear relationship can be seen (Fig. 11). Hence we fit the line of best fit (i.e. regression equation) which is of the form Y= -0.2976xX+349, where Y=mite population and X =rainfall. During 2017-2018 the correlation with rainfall and mite population with r= -0.76 i.e. much higher negative correlation can be noticed by comparing the r value of previous year (Fig. 12). Here also we fit the line of best fit which is of the form Y=-0.3158 xX+347.44. Hence from these two equations we can predict the mite population with corresponding values of rainfall during the years.

RH is also exerted a negative impact on the population. The population density of the mite reached the minimum when RH reached its maximum, though some exceptions were also observed. The population decreased drastically with respect to increase in rainfall coupled with prevalence of RH. The mite population followed a declining trend, even when the rainfall was very low, but the RH very high. Accordingly the mite could not revive its population density even when the temperature increased, leading to prevalence of the very low population in the field.

Figure 7. Intact tepals attached to the rachis after falling off highly infected tender coconuts.

DISCUSSION

The overall performance of *Aceria guerreronis* in the Chowghat green dwarf variety of coconut from 2016 to 2018 attempt to suggest that the plant is acquiring vigour to overcome the severe attack of the mite. Chowghat green dwarf variety of coconut is known to be very much amenable to attacks by *A. guerreronis* in Kerala (Haq, 2001), yet survival of this variety from such a strong and long-lasting impediment necessitates the developing of cultivars resistant against the attack of *A. guerreronis* and other mites. This is particularly important in view of the preference shown by the Indian and foreign tourists for coconut milk.

Population density of the coconut mite is greatly influenced by climate (Mariau, 1969; Otterbein, 1988). Among the climatic factors considered for the study, temperature, RH and rainfall have a stronger influence on population fluctuations in the study period. In a general observation it was found that the influence of all three parameters up to the optimum tolerance capacity is permissible for the survival of the mite species. But these climatic factors exert a combined influence at the verge of population regaining process of the mite in the field. This may probably hinder the harmony of life cycle, multiplication of generation and hence its population size. The mite attained a high population early in January, 2016 because of high temperature, very low RH and practically no rainfall. This tendency was also reported from Guerrero, Mexico (Mariau 1969), Brazil (Lawson-Balagbo et al., 2008; Reis et al., 2008) and Sri Lanka (Aratchige et al., 2012). The positive and negative influence of temperature and rainfall, respectively on this mite's population were already recorded for South Indian conditions (Pushpa and Nandihalli, 2009). The adverse effect of rainfall leading to a reduction in the population density of *Mononychellus tanajoa* (Bondar) and its predators in Africa was reported by Yaninek *et al.* (1989). However, contradictory reports also exist from Benin and the Ivory Coast supporting a negative relationship between the coconut mite population and temperature, with 5 times the population density of the mite in wet season oppose to the dry season (Julia and Mariau, 1979). *Aceria guerreronis* peaked during January, 2017 in the present study but decline again with a decrease in temperature in succeeding months of June, in the presence of higher RH and rainfall. The rainfall further increased during the monsoon period of June – July, resulting in lower temperatures, culminating in a further decline of the mite population. Afterwards, the temperature got up steadily, reaching higher gradients with a striking decrease in both RH and rainfall. The decline of mite population owing to rainfall and increase in RH observed during the present study contradicts the earlier findings (Otterbein, 1988) in Costa Rica that the

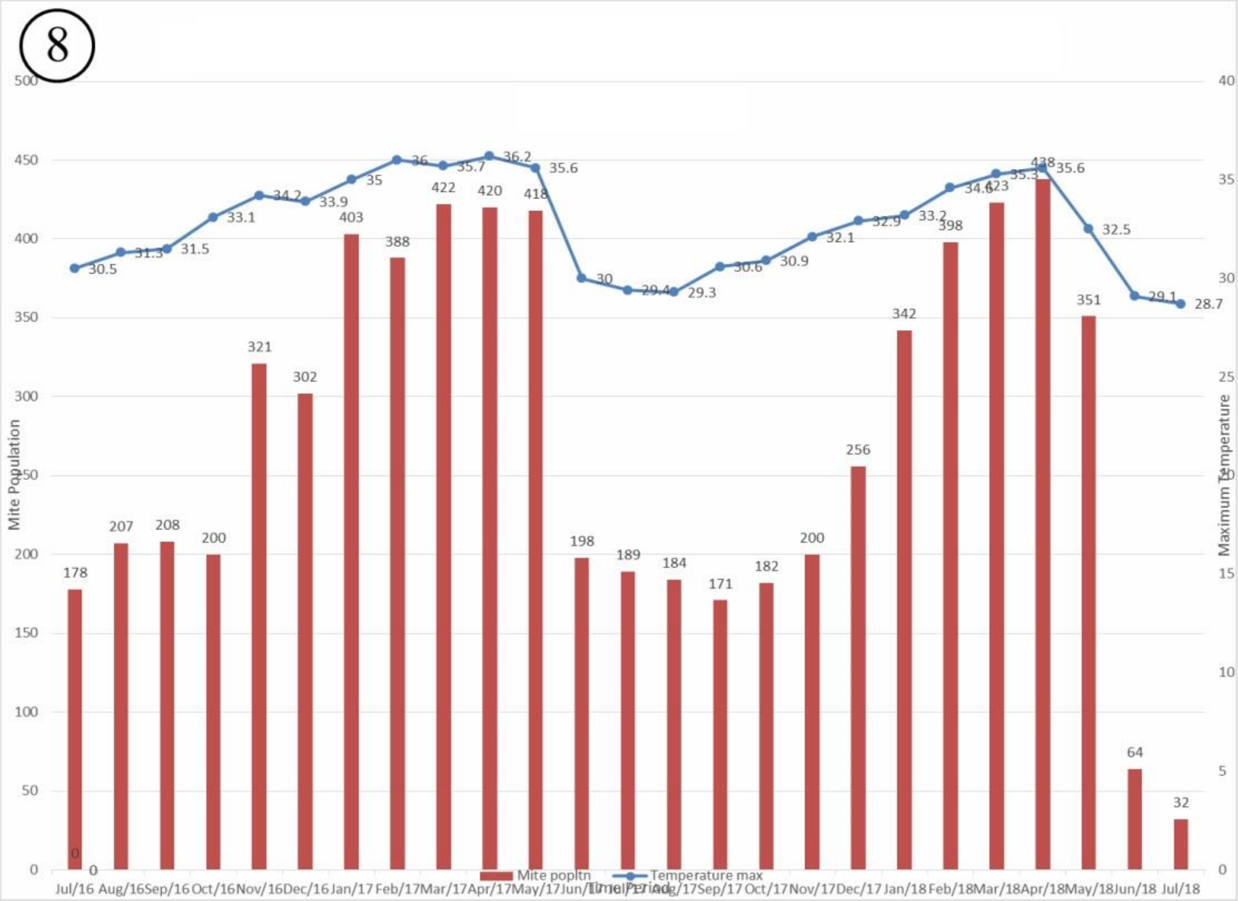

Figure 8. Influence of temperature on mite population during 2016-2018.

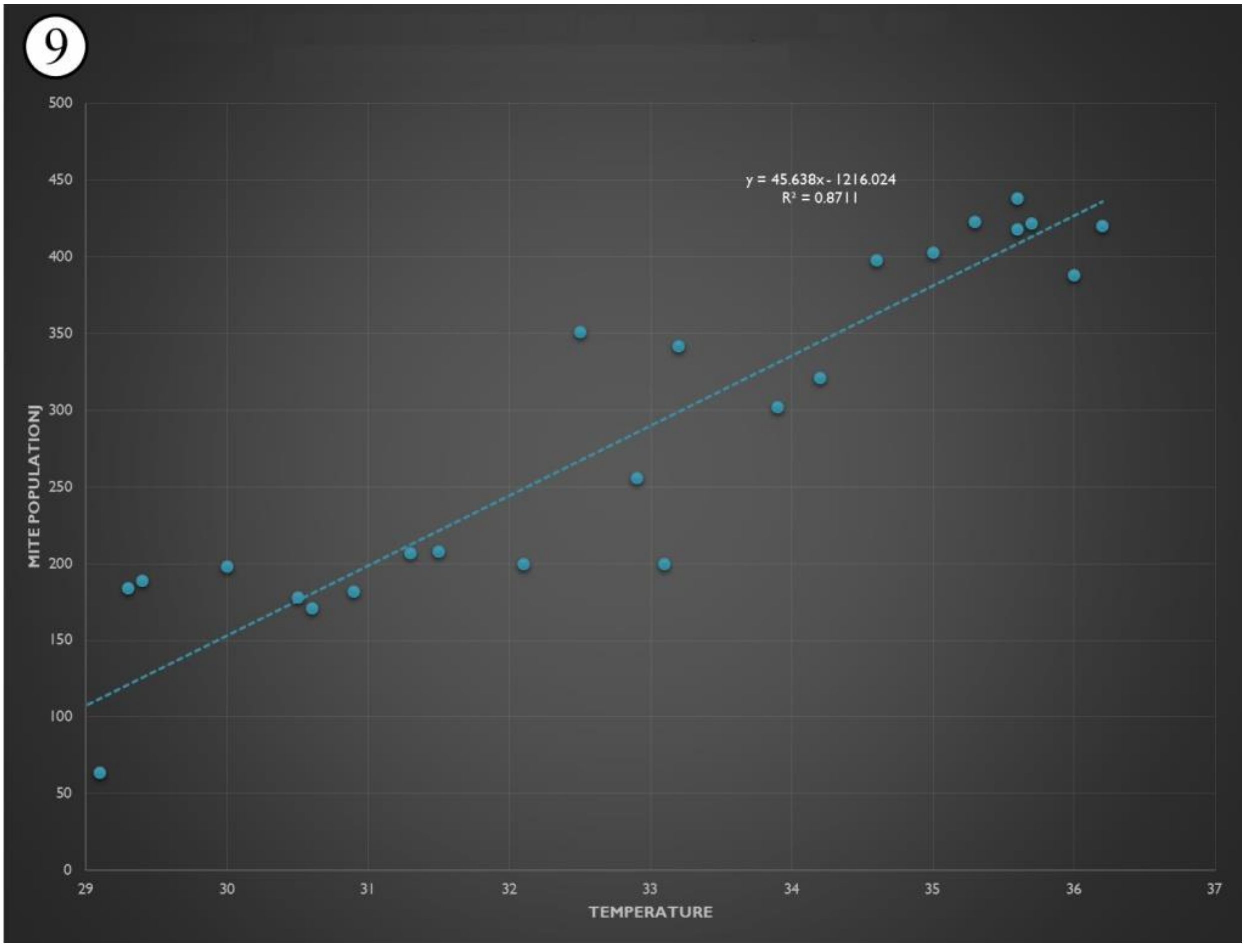

Figure 9. Multiple regression model for the mite population for the period of July 2016-2018.

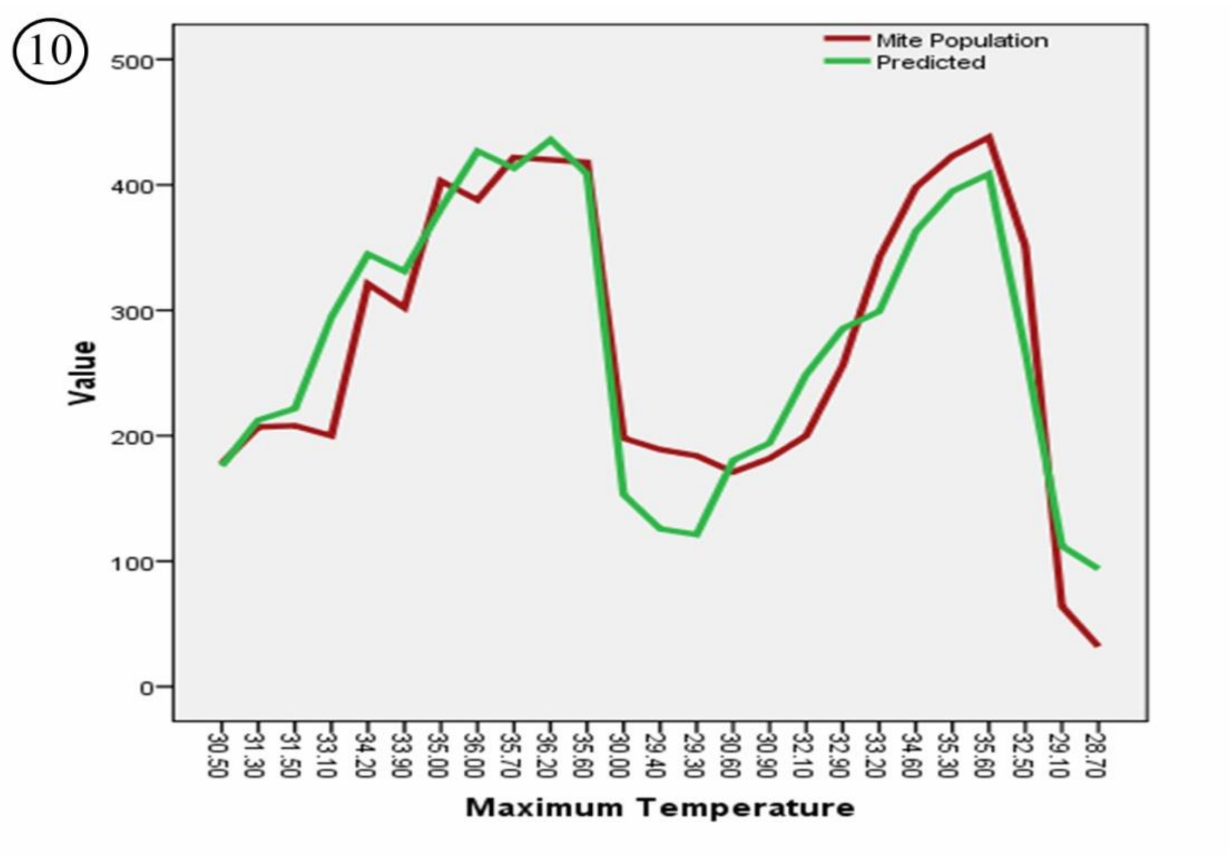

Figure 10. Comparison of mite population observed and predicted values with temperature.

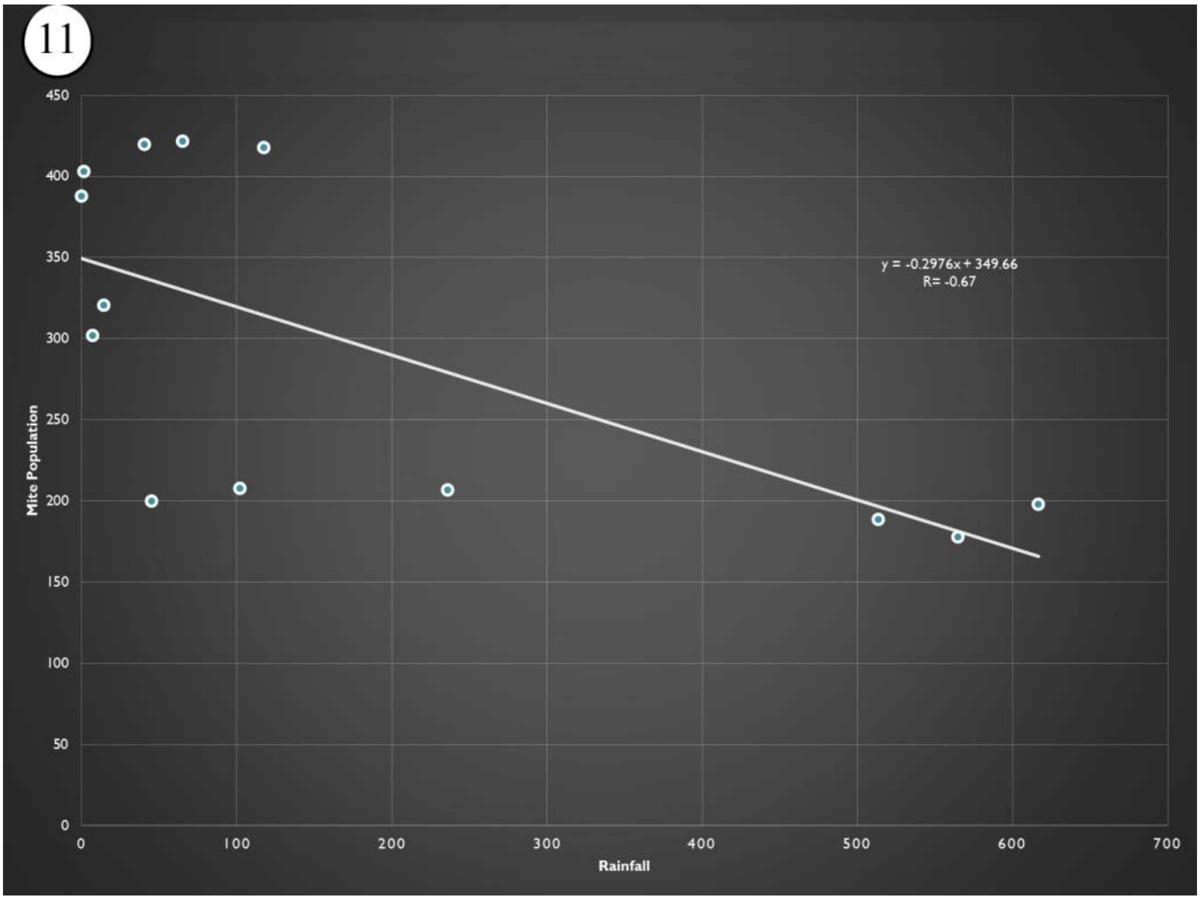

Figure 11. Influence of rainfall on mite population for the period of July 2016-July 2017.

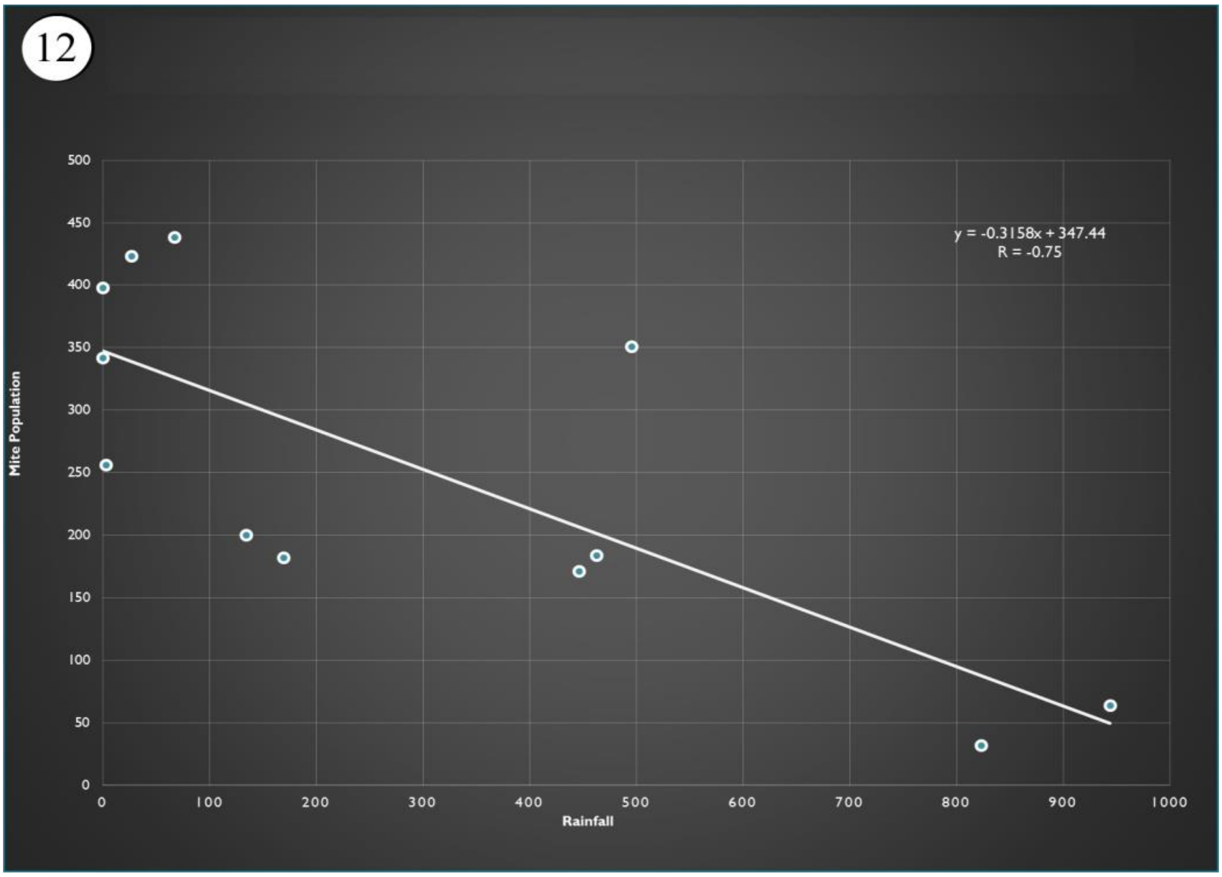

Figure 12. Influence of rainfall on mite population for the period of August 2017-July 2018.

greatest nut damage is caused by the mite in periods of frequent heavy rainfall and high humidity.

However, the mite population could not rise up to the original trend and level in population build up, attributing the influence of all the climatic factors as depicted in 2016. It is interesting to note that the population has attained the maximum level in January to May during 2017 and 2018 and decline again during the successive months, reaching to the minimum during the months of June to October of all the above said years. The climatic influence, particularly after March though proved favourable to population replenishment of the mite, the population could not be regained. This led to the suppression of population leading to its decline. Several such co-incidents intermittently in the fields might have operated to bring down the population of the mite. Therefore, individual and collective influence of all the above climatic factors in the population build-up of mite is well evidenced. The influence of "global warming" if any, experienced during the study period need to be assessed to get a clear picture on the influence of climatic factors. Prolonged temperature above 35 °C attributed to a supportive factor to our laboratory observation on the number of generations this mite could complete per year. (Haq, 2001; Sobha and Haq, 2011). Temperature above 35 °C was not found favourable for the completion of life cycle as usual. The duration of the developmental period of the mite extended double the time than normally required. The nymphal stages were not found feeding and ultimately were found dying. Very often, the newly emerged females were not found ovipositioning. This prompted to think that prevalence of temperature above 35 °C in the field has been instrumental to the population decline of the mite throughout Kerala.

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SHORT NOTE

Mites associated with ambrosia and bark beetles (Curculionidae: Scolytinae) in avocado orchards in Michoacan, Mexico

Estefanni N. SANDOVAL-CORNEJO[1], Edith G. ESTRADA-VENEGAS[1,3], Armando EQUIHUA-MARTÍNEZ[1], Jesús ROMERO-NÁPOLES [1], Dionicio ALVARADO-ROSALES[2]

[1] *Colegio de Postgraduados, Fitosanidad - Entomología y Acarología, Km 36-5 Carretera México Texcoco, Montecillo, 56230, Estado de México, México*
[2] *Fitosanidad - Fitopatología. Km 36-5 Carretera México Texcoco, Montecillo, 56230, Estado de México, México*
[3] Corresponding author: edith_ev@yahoo.com.mx

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ABSTRACT: Avocado is one of the most important crops in Mexico. To investigate mite species associated with ambrosia and bark beetles in avocado plantations, three study sites with different management and crop conditions were selected in the state of Michoacan. Orchards were checked for trunks infested with ambrosia and bark beetles during 2017. In the laboratory, avocado trunks were placed in emergence chambers, and insects and mites collected, mounted and identified. Four species of Scolytinae were found associated with avocado trunks; three ambrosia beetle species, namely *Monarthrum exornatum*, *M. frimbriaticorne* and *Dryocoetoides capucinus*, and one bark beetle, *Phloeocleptus plagiatus*. *Monarthrum fimbriaticorne* was the most common beetle found at the three sites. The seven mite species collected belonged to 6 genera, *Proctolaelaps, Trichouropoda, Mexecheles, Eutogenes*, *Elattoma* and *Tyrophagus. Elattoma abeskoun* was the most abundant species in this study. These results represent the first report in the country on mites associated with ambrosia and bark beetles in avocado orchards.

Keywords: *Persea americana*, Acari, Scolytinae, association.

Approximately 250 mite species have been reported as phorectic on insects; many of them transport microorganisms like bacteria, fungi and nematodes (Hofstetter et al., 2015). Mites are associated with a great number of Scolytinae, with studies on these interactions having been done mainly in forest ecosystems (Wirth et al., 2016); however, knowledge of these associations in agricultural systems and fruit trees is limited (Ahadiyat and Ostovan, 2006; Chaires-Grijalva, 2013).

Due to the scarce information available on the association between ambrosia and bark beetles and mites in fruit trees, and the economic importance of avocado (*Persea americana* Mill.) in Mexico, the objective of the present research was to study the mite species associated with ambrosia and bark beetles in three avocado orchards in Nuevo Parangaricutiro, Ziracuaretiro and Uruapan, Michoacan, Mexico.

The samples were collected from June to November, 2017 from three orchards located in the state of Michoacan, Mexico: “El Durazno” (19°22’30” N, 102°14’16” W, 2245 masl) in the municipality of Nuevo Parangaricutiro, “La Ziranda” (19°24’00” N, 101°54’56” W, 1304 masl) in the municipality of Ziracuaretiro and “La Piedra China” (19°21’19” N, 102°03’34” W, 1564 masl) in the municipality of Uruapan. Infested trunks were transported to the Entomology and Acarology Laboratory in Texcoco, where they were placed in emergence chambers. Emerging adult insects were collected and their phoretic mites separated from June to December, 2017. Specifically, the insects were carefully checked under a stereoscopic microscope to separate the mites fixed to the exoskeleton. Permanent mounting was done in Hoyer’s medium and then taxonomic determination was done by using specialized keys. The mite slides were placed in the personal collection of Dr. Estrada-Venegas.

A total of 659 mites were collected, of which 76.17% were found on *Phloeocleptus plagiatus* Wood, 13.96% on *Monarthrum exornatum* (Schedl), 9.40% on *Monarthrum fimbriaticorne* (Blandford) and 0.47% on *Dryocoetoides capucinus* (Eichhoff). The following mite species were identified: *Proctolaelaps bickleyi* (Bram) and *Proctolaelaps subcorticalis* Lindquist (Melicharidae); *Mexecheles virginiensis* (Baker) and *Eutogenes foxi* Baker (Cheyletidae); *Elattoma abeskoun* Rahiminejad and Hajiqanbar (Pygmephoridae); *Tyrophagus putrescentiae* (Schrank) (Acaridae) and *Trichouropoda* sp.1 (Trematuridae). Of these seven species, six of them (*P. bickleyi*, *P. subcorticalis*, *E. foxi*, *M. virginiensis, E. abeskoun* and *Trichouropoda* sp. 1) were associated with *P. plagiatus*, the same six species were associated with *M. exornatum*, three species (*P. bickleyi, T. putrescentiae* and *Trichouropoda* sp. 1) with *M. fimbriaticorne* and one (*P. bickleyi*) with *D. capucinus*.

The most abundant family was Pygmephoridae (*E. abeskoun)* with 61.45% of all the collected specimens, followed by Trematuridae with 26.25%, Melicharidae with 10.92% and Cheyletidae and Acaridae with 1.38%.

The species of phoretic mites collected in this study are new records of associations as phoretic forms with the ambrosia beetle species, *M. exornatum, M. fimbriaticorne* and *D. capucinus*, and with the bark beetle, *P. plagiatus*, in *P. americana* wood in the state of Michoacan, Mexico; and in the case of the species *M. virginiensis* and *E. abeskoun*, new records for Mexico.

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