

In memory of Gary Bauchan: Integrated taxonomy of soil mites in farming systems

Authors: Rueda-Ramírez, Diana, Carta, Lynn, Mowery, Joseph, Bauchan, Gary, Ochoa, Ronald, et al.

Source: Systematic and Applied Acarology, 27(2) : 181-208

Published By: Systematic and Applied Acarology Society

URL: <https://doi.org/10.11158/saa.27.2.3>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Article

In memory of Gary Bauchan: Integrated taxonomy of soil mites in farming systems

DIANA RUEDA-RAMÍREZ^{1*}, LYNN CARTA², JOSEPH MOWERY³, GARY BAUCHAN³, RONALD OCHOA⁴, MONICA YOUNG⁵, JANDIR CRUZ SANTOS⁵ & ERIC PALEVSKY⁶

¹ Humboldt Universität zu Berlin, Institute of Biology, Ecology, Philippstraße 13, 10115 Berlin, Germany. ruedadia@hu-berlin.de, dianaru@gmail.com, https://orcid.org/0000-0002-7642-2256

² Mycology and Nematology Genetic Diversity and Biology Laboratory, USDA-ARS, Beltsville, MD 20705. lynn.carta@ars.usda.gov

³ Electron and Confocal Microscopy Unit, USDA-ARS, Beltsville, MD 20705. joseph.mowery@usda.gov; Gary.Bauchan@ARS.USDA.GOV

⁴ Systematic Entomology Laboratory, USDA-ARS, Beltsville, MD 20705. ron.choa@usda.gov

⁵ Centre for Biodiversity Genomics, University of Guelph, Canada. myoung02@uoguelph.ca https://orcid.org/0000-0002-7388-8202; jandir_jc@hotmail.com https://orcid.org/0000-0001-6307-2472

⁶ Neve Ya'ar Research Center, Agricultural Research Organization (ARO), Israel. palevsky@volcani.agri.gov.il https://orcid.org/0000-0002-4958-8920

*Corresponding author

Abstract

Current and future legislation requiring the reduction of pesticides use, coupled with global initiatives for the promotion of soil health and conservation of soil biodiversity are creating opportunities for studies aimed at highlighting ecosystem services provided by functioning soil food webs in agricultural systems, including soil predatory mites. However, the key personnel for performing such studies are expert taxonomists, who are already spread very thin. To meet this demand, we propose an integrative approach where scientists (without expertise in taxonomy) play a significant role in supporting expert taxonomists. Soil samples were collected at the USDA ARS Farming Systems Project (FSP) at the Beltsville Agricultural Research Center, either incubated or not, followed by extraction of mites and nematodes. Improved modified Berlese funnels and an extraction protocol were utilized to improve sequencing success. Incubation dramatically enhanced the number of extracted individuals per sample whilst the daily freezing of extracted mites substantially improved the sequencing success rate compared to previous studies. Taken together, this led to the addition of records for eight Mesostigmata and ten Oribatida BINs to BOLD (Barcode of Life Datasystem). Fifteen species of Mesostigmata species were found, with three dominant species, *Cycetogamasus diviortus* (Athias-Henriot, 1967) (Parasitidae), *Lasioseius youcefi* Athias-Henriot 1959 (Blattisociidae) and a new species of *Gamasellodes* (Ascidae). LTSEM imaging followed by molecular identification contributed further details to the published descriptions of *C. diviortus* and *L. youcefi*. In line with our general aim, collecting, extracting, identification to morpho-species, sample preparation for DNA barcoding and uploading relevant information to BOLD was performed by trained personnel, but without taxonomic expertise. Whereas our skilled taxonomists focused on the morphological identifications using light microscopy, expanding on existing descriptions using LTSEM images and in a subsequent manuscript the description of a new species. We believe this division of tasks and labor will set the stage for further collaborative integrated studies between ecologists, biocontrol specialists and expert taxonomists for the identification, evaluation, and description of known and novel soil acarine biological control agents (BCAs).

Keywords: Acari, soil mites, Biocontrol agents, Parasitiformes

Introduction

Numerous generalist soil predatory mite species, belonging to the cohort Gamasina, have been identified and their feeding ecology evaluated to some extent under laboratory conditions (Carrillo *et al.* 2015). Studies to determine biocontrol efficacy of augmentative releases in greenhouses and outdoors have been conducted mostly with species that are commercially available (Gerson & Weintraub 2007). Such cosmopolitan species have been studied as potential bio-control agents (BCAs) of pests of similar taxa, but also for control of pests belonging to completely different groups. For examples, the laelapid species *Gaeolaelaps aculeifer* (Canestrini, 1884) and *Stratiolaelaps scimitus* (Womersley, 1956) (Mesostigmata: Laelapidae), have been considered as potential BCAs of various species of thrips pests of greenhouses (Berndt *et al.* 2004; Park *et al.* 2021; Rueda-Ramírez 2018; Rueda-Ramírez *et al.* 2021), citrus orchards (Navarro-Campos *et al.* 2020) and open fields (Castro-López & Martínez-Osorio 2021; Castro-López *et al.* 2021). Both have also been released for the control of shore flies (Diptera: Ephydriidae) (Vänninen & Koskula 2004) in greenhouses, fungus gnats (Diptera: Sciaridae) in green houses and mushroom cultivation (Freire *et al.* 2007; Acharya *et al.* 2019; Tavooosi Ajvad *et al.* 2020) and for phytonematodes (Salehi *et al.* 2014; Azevedo *et al.* 2020; Yang *et al.* 2020). Additionally, *G. aculeifer* was assessed as a BCA for the bulb mite *Rhizoglyphus robini* Claparède, 1869 (Astigmatina: Acaridae) under greenhouse and field conditions (Lesna *et al.* 2000).

In contrast to augmentative releases, comparatively few studies have investigated the conservation of soil predatory mites for the biological control of above and below ground pests. Astigmatina have recently been reported as factitious prey for rearing predatory mesostigmatic soil mites of the families Laelapidae and Rhodacaridae (Barbosa and Moraes 2016). They have also been used as alternative prey to conserve populations of *Macrocheles robustulus* (Berlese, 1904) (Mesostigmata: Macrochelidae) to enhance the biological control of sciarid flies (Grosman *et al.* 2011) as well as for an unidentified species of *Cosmolaelaps* (Mesostigmata: Laelapidae) for the control of the prepupae and pupae of the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) (Munoz Cardenas 2017). More recently, provisioning of the free-living nematode *Rhabditella axei* (Cobbold, 1884) to the soil predatory mites *Macrocheles embersoni* Azevedo, Castilho & Berto, 2017 (Mesostigmata: Macrochelidae) and, *S. scimitus* (Mesostigmata: Laelapidae) and *Parasitus bituberosus* Karg, 1972 (Mesostigmata: Parasitidae) enhanced the biological control of the house fly *Musca domestica* Linnaeus, 1758 (Diptera: Tachinidae) (Azevedo *et al.* 2019), the root knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) (Azevedo *et al.* 2020) and WFT (Rueda-Ramírez *et al.* 2019), respectively.

Yet, despite the available knowledge on soil predatory mites, most soil born pests are still controlled by applying pesticides to the soil (many being hazardous), mainly because of their low cost and lack of expertise needed to apply them. However, the days of these disruptive practices appear to be numbered with the growing public (Schaub *et al.* 2020) and scientific awareness of the negative impacts of pesticides on human and animal health, biodiversity and the environment (Mahmood *et al.* 2016). Substantial reductions in pesticide use are in fact major expected impacts in the recently launched EU Farm to Fork strategy (European Union 2021). Additionally, registrations of hazardous pesticides are being reviewed and accordingly banned by the respective authorities (European Food Safety Authority 2021; Food and Agriculture Organization 2021; United States Environmental Protection Agency 2021) resulting in fewer available pesticides to growers.

Whether augmentative releases of reared soil predators (Collier & Van Steenwyk 2004), or conservation of a natural assemblage of predators (Begg *et al.* 2017), will be utilized for the control of soil pests, the soil biotic and abiotic attributes must be considered as they can limit predation

efficacy. Compact agricultural soils (Batey 2009) lack structure, limiting the mobility and ability of predatory mites to forage for food (Erktan *et al.* 2020) and will either be too wet or too dry to support population development. Additionally, agricultural soils deficient in organic matter (Hijbeek *et al.* 2017) will lack the nutrients necessary for soil microbiota growth, the essential diet of free-living nematodes, which in turn are one of the main food sources of soil predatory mites (Walter 1988). Here too, similar to the concerns regarding hazardous pesticides described above, there is a growing change in public and scientific awareness with respect to soil health. Initiatives across the globe, including the EU Soil and Biodiversity Strategies, EU Soil Observatory, UN FAO Global Soil Partnership (GSP), the Intergovernmental Technical Panel on Soils (ITPS), together with the UN Convention on Biological Diversity (CBD) and the Global Soil Biodiversity Initiative (GSBI) are joining hands and setting the stage for better soil practices, including the promotion of soil conservation and soil biodiversity. These initiatives provide new opportunities for multidisciplinary studies aimed at showcasing the ecosystem services provided by functioning soil food webs in rehabilitated soils, including soil predatory mites. Soil mite diversity studies are the basis for understanding the interactions of these organisms, their roles in soil food webs and their potential as BCAs. These studies require methodologies that allow efficient collection, extraction, and accurate identification. During extraction, densities may be under estimated, as has been observed in the case of immature or less mobile stages (Kuenen *et al.* 2009; Lakly & Crossley 2000; Søvik & Leinaas 2002). Variations in the efficiency of funnel extraction methods have been found to be related to changes in soil moisture content and temperature (Macfadyen 1961). Therefore, manipulating parameters to accommodate mite development or motility, such as incubation of samples with soil amendments, can improve extraction efficacy of slow moving and rare species. In terms of accurate identification, mite taxonomists are essential, but such specialists are becoming increasingly scarce, making it very difficult for the identification of species in biodiversity and ecological studies. To meet this demand, mite taxonomists will need to collaborate with experts specialized in DNA barcoding.

The general objective of this study was to develop and provide methodologies for the integrated taxonomy of soil predatory mites, belonging to the cohort Gamasina, meant to encourage collaboration between expert mite taxonomists and scientists with expertise in extracting, identifying, DNA barcoding and conserving potential acarine BCAs. Accordingly, our specific objectives were to: 1) maximize number of extracted individuals from a soil sample for morphological and molecular identifications (ample specimens allows for the utilization of a number of integrative techniques and technologies); 2) improve the sequencing success rate of DNA barcoding and contribute sequences of expertly identified soil predatory mites to the Barcode of Life Database (BOLD); 3) to provide information on the species diversity of mites in farming systems; 4) to improve and enhance the descriptions of the dominant soil predatory mites using high resolution imaging.

Methods & materials

Collection, incubation and extraction of mites and nematodes

Sampling was conducted at the USDA ARS Farming Systems Project (FSP) at the Beltsville Agricultural Research Center (BARC), Maryland, USA, established in 1993. Soil samples were taken from the four replicates of the conventional till, no-till and organic treatments on April 30th 2019 (for latitude and longitude of the 12 sampling sites see Table 1).

Sampling was done in spring before tillage and planting. No winter cover crops were grown due to heavy rains in the fall of 2018. From each sampling site, using a core sampler, 36 cores of 5 cm

depth and 5 cm diameter were taken. Soil was removed from the cores, gently mixed in a 20 l bucket and then poured into a plastic bag (yielding approximately 3.5 l of soil per site). This large soil sample was used to determine abundance of mite species on the day of sampling (pre-incubation) and to demonstrate the effect of incubation with and without soil amendments on mite and nematode (sorted by functional groups) abundance.

TABLE 1. Site details including plot number, farming system project (FSP) treatment (no till, organic and till), block, and geospatial coordinates.

| Plot # | Block | FSP | Latitude ° | Longitude ° |
|--------|-------|---------|------------|-------------|
| 101 | 1 | Till | 39.029635 | -76.899249 |
| 103 | 1 | No-till | 39.02946 | -76.899258 |
| 110 | 1 | Organic | 39.029562 | -76.898248 |
| 213 | 2 | Till | 39.028026 | -76.899258 |
| 216 | 2 | No-till | 39.027749 | -76.899305 |
| 206 | 2 | Organic | 39.028658 | -76.899278 |
| 314 | 3 | Till | 39.029007 | -76.894693 |
| 305 | 3 | No-till | 39.029007 | -76.89573 |
| 308 | 3 | Organic | 39.029007 | -76.895367 |
| 406 | 4 | Till | 39.028100 | -76.895267 |
| 407 | 4 | No-till | 39.028100 | -76.895142 |
| 415 | 4 | Organic | 39.028089 | -76.894251 |

For mite extraction, soil (470 cc) was poured into a sieve 6 cm high and 10 cm in diameter. Sieves were made from cylinders cut as slices from a PVC pipe, with aluminum window screen (square holes 1.8 mm) welded to the cylinder bottom with a hotplate, to retain soil but allow mite movement for Berlese extraction. A new modified Berlese funnel (Fig. 1) was developed utilizing off-the-shelf components (Berlese trap-<https://www.Bioquip.com>, CA USA), herein named the Galen-Berlese funnel after Galen P. Dively who devised the configuration. Galen-Berlese funnels were set up in a ventilated room, at ambient temperature during the month of May 2019, at the University of Maryland, Beltsville Facility. The Galen-Berlese funnel optimizes temperature and humidity levels inside the apparatus by featuring a mild heat source, sufficiently separated from the sample to prevent overheating and killing the mites before they can escape down the funnel. A variable wattage light bulb (incandescent TM, 29 W, 380 lumens, Ecosmartinc.com, FL USA) was fitted inside the aluminum dome (Bioquip) and connected to a plug-in lamp dimmer (300 W incandescent, 120 VAC, Cat. No. TBL03, Leviton.com, China) which was adjusted daily to keep the topsoil temperature at a mean of 30 °C. The funnel was assembled with two standard 5-gallon construction buckets instead of expensive ring stands, making these affordable, easy to transport, and assemble. The lower bucket was cut to a height of 24 cm and served as a support structure for the 30.5 cm diameter aluminum funnel (Bioquip). A plastic sample cup (76 mm diameter, Snap-Seal™ No. 1730, Corning.com, USA) half filled with 95% ethanol was placed beneath the funnel and elevated using a wood block to ensure the cup directly touched the bottom of the funnel to reduce ethanol evaporation. Without this arrangement, ethanol evaporated rapidly, losing half the ethanol volume in 24 hours. A 20 cm diam. hole was cut in the bottom of the upper bucket and a sturdy wire mesh placed over the hole to support the sample. Soil samples containing mites were placed sitting atop a 12.5 cm diameter filter paper in the PVC sieve (described above), to retain debris while allowing mites to freely pass through. To prevent DNA degradation over the 10-day extraction

period, and to avoid processing daily samples of mites, we used two sets of collection cups that were rotated daily. While one set was placed in the funnel the other was stored at $-20\text{ }^{\circ}\text{C}$ for 24 hours. At the end of the 10-day period, mites were transferred with a pipette from the collection cups to 20 ml glass vials and stored in the freezer. Mean topsoil temperatures of all funnels monitored once daily at mid-morning over the 10-day period with an infrared thermometer was $30.2\text{ }^{\circ}\text{C} \pm 2.1$. Top soil temperature and humidity continuously monitored in one funnel with a data logger in the center of the room was $29.0\text{ }^{\circ}\text{C} \pm 2.0$ and $56 \pm 10\%$ RH, respectively.

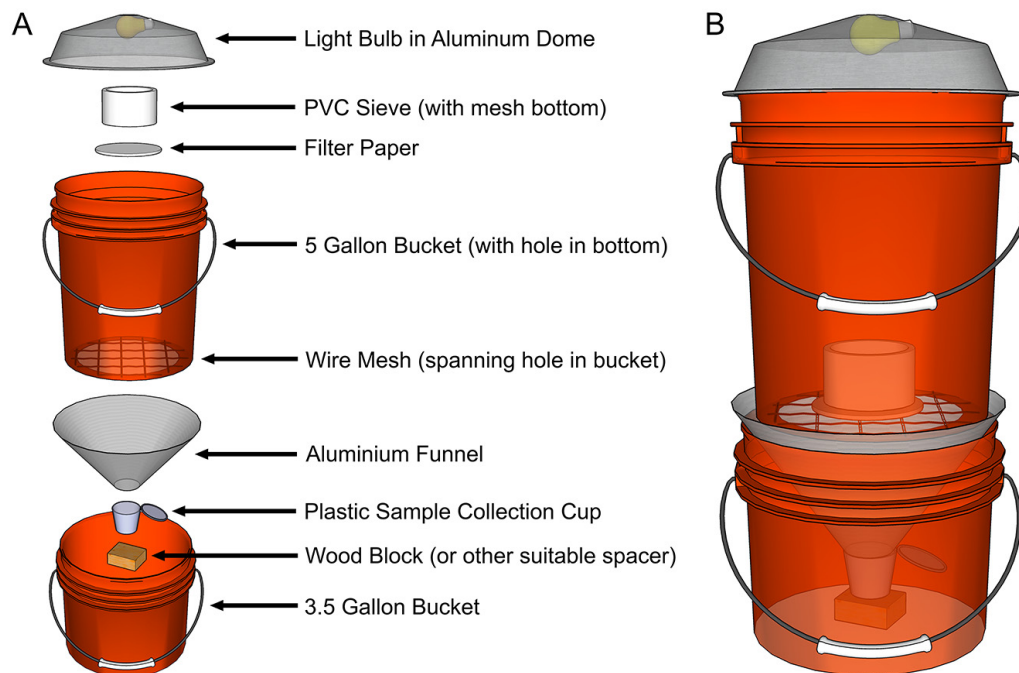


FIGURE 1. Galen-Berlese Funnel: a modified Berlese funnel for the collection of soil mites at optimal temperature and humidity levels utilizing off-the-shelf components, a key feature being a mild heat source which is sufficiently separated from the sample to prevent overheating and killing the mites before they can escape down the funnel. (A) Expanded view diagram. (B) A partially transparent view of the assembled apparatus.

For assessing the effect of incubation, soil samples were prepared in duplicate, destined for nematode or mite extraction. Sieves were half filled with soil, followed by 5 g of either organic green bean pods (Azevedo *et al.* 2019; Azevedo *et al.* 2020), fresh chopped alfalfa grown as a cover crop, or nothing as a negative control, which was then covered with soil, filling the sieve to the brim. Sieves were watered to run off and left to drain for 18 hours. To slow down water loss and to prevent soil from sifting out during the incubation period, sieves were covered with plastic wrap (Glad Press'n Seal, www. Glad.com, USA) and ventilated by puncturing with a nail, 1.8 mm in diameter, 10 punctures in the bottom and top surfaces. Then, sieves were weighed individually and placed in a climate chamber, arranged in a random block design for 17 days in darkness at $22.4 \pm 0.1\text{ }^{\circ}\text{C}$, $70 \pm 3\%$ RH. To compensate for water loss, we continued to monitor sieve weight twice a week and added water accordingly with a syringe, injecting the water into the soil. Water loss ranged between 1–1.5 ml per sieve per day. For the sieves allocated for nematode extraction in Baermann funnels we continued to add water throughout the incubation period, following which, nematodes were extracted from a sub-sample of 70 g of soil in Baermann funnels and were tallied to functional groups under a

light microscope. As we did not want samples to be too wet prior to the extraction in the Galen-Berlese funnels, we stopped adding water on day 12, five days before extraction. To prevent the soil from drying like a brick during the extraction, possibly hindering mite movement, the soil sample was removed, mixed, and poured back into the sieve and then placed on the filter paper in the funnel following the protocol above.

DNA barcoding of soil predatory mites

The specimens from each sample were imaged with a Zeiss motorized Axio Zoom V16 microscope, creating extended focused images. The specimens were assigned to morpho-species using the resultant image library, and morpho-species abundance was tallied for each sample. Up to five mites from each morpho-species and sample and all LTSM imaged mites (described below) were selected for molecular analysis. The specimens were arrayed into 96-well microplates (Eppendorf), containing 30 μ L of 95% ethanol in each well, leaving one empty well to serve as a negative control. Specimen details, including collection information, order level taxonomy, and associated images were uploaded to BOLD.

The plates were sent to the Canadian Centre for DNA barcoding (CCDB) at the University of Guelph in Ontario, Canada, for molecular analysis (<http://ccdb.ca>). They were sequenced with a cocktail (1:1 ratio) of LepF1/LepR1 (Hebert *et al.* 2003) and LCO1490/HCO2198 (Folmer *et al.* 1994) primers using standard protocols modified to allow voucher recovery following DNA extraction (Ivanova *et al.* 2007; Ivanova & Grainger 2007a; Ivanova & Grainger 2007b; Porco *et al.* 2010). The DNA extracts were stored in -80°C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net), and the vouchered specimens were retained in 96-well microplates with 95% ethanol for subsequent morphological preparations.

The sequences were assembled from forward and reverse chromatograms using CodonCode Aligner v. 4.2.7 and uploaded to BOLD. They were inspected for potential contamination or misidentification by examining their placement in a Neighbor-Joining tree and by querying each record against BOLD's complete reference library using the BOLD Identification Engine. Cases of suspected contamination were flagged and filtered from the reference library, and all remaining sequences meeting minimum quality criteria (≥ 500 base pairs, $< 1\%$ ambiguous nucleotides, free of contamination and stop codons) were assigned to molecular operational taxonomic units by BOLD using the Barcode Index Number system (BIN; Ratnasingham & Hebert 2013). Sequencing success rates were evaluated and compared across the major groups using the Pearson's chi-square test.

Following morphological identification (described below), the distribution of intra- and inter-specific divergences were examined using the 'Barcode Gap Analysis' tool on BOLD. The concordance between BINs and species were evaluated following methods in Ratnasingham and Hebert (2013), where species were categorized as matches (perfect correspondence between one species and one BIN), splits (one species is represented by more than one BIN), merges (two or more species are assigned to a single BIN), and mixtures (a combination of splits and merges). Species and BIN records were also cross referenced with BOLD and GenBank to assess the novelty of their contribution to the reference library.

Identification of soil mites

The mites were morphologically identified following DNA extraction. Mesostigmatic mites were mounted in Hoyer's medium and first identified to family, based on Lindquist *et al.* (2009). Adult females were identified to genera, based on Hyatt (1980), Krantz and Ainscough (1990), Hruzová and Fend'a (2018), and unpublished keys of the Acarology Summer Program (Soil Acarology, Ohio State University, 2014), and to species, based on the original and complementary

descriptions. Oribatids were sent to the specialist Roy Norton for identification. All specimens were deposited in the United States National Mite Collection, USDA-ARS, BARC.

High resolution imaging of the dominant soil mesostigmatic species

Specimens from three abundant morpho-species were subjected to low temperature scanning electron microscopy (LTSEM). Females and males, were removed from ethanol 95%, arranged on double sided carbon tape mounted on standard brass plates, six to eight specimens per plate, one to two plates per species and frozen in liquid nitrogen ($-198\text{ }^{\circ}\text{C}$). The brass plate, containing the frozen sample, was transferred to the Quorum PP2000 cryo transfer system (Quorum Technologies, East Sussex, UK) attached to an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Dallas, TX USA). The specimens were freeze etched inside the cryotransfer system to remove any surface contamination (condensed water vapor) by raising the temperature of the stage to $-90\text{ }^{\circ}\text{C}$ for 10–15 minutes. Following etching, the temperature inside the chamber was lowered below $-130\text{ }^{\circ}\text{C}$, and the specimens were coated with a 10 nm layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled ($-130\text{ }^{\circ}\text{C}$) cryostage in the SEM for observation. An accelerating voltage of 5 kV was used to view the specimens. Images were captured using a 4pi Analysis System (Durham, NC USA).

A Zeiss LSM710 confocal laser scanning microscopy (CLSM) system was utilized to capture three dimensional fluorescent images with an excitation wavelength of 405 nm, 488 nm, and 561 nm with a broad filter set to detect all emission wavelengths between 410–704 nm. The chelicerae were dissected from mites and mounted in glycerin between two coverslips. Images were captured using a Zeiss Axio Observer inverted microscope with a 63x 1.4NA Plan-Apochromat objective. Zeiss Zen 2012 Pro software was used to obtain 20-150 z-stack images which were processed into maximum intensity projection images and three-dimensional projection images. Nomenclature of idiosomal setae is based on Lindquist & Evans (1965). The number of teeth of each cheliceral digit does not include the apical 'hook'.

Statistical analysis

The variables used for comparison were the abundances of Mesostigmata adults, Mesostigmata immatures, total Mesostigmata, the most abundant Mesostigmata species, Oribatida immatures (excluding Astigmatina), total Oribatida (excluding Astigmatina), total Astigmatina, fungal feeder nematodes, bacterial feeder nematodes and plant parasitic nematodes. The term Oribatida throughout the text refers to Oribatida (excluding Astigmatina).

Effects of incubation and farming system on mite abundance

To evaluate the effect of incubating the soil sample on mites and nematodes, the mean data from samples incubated with and without plant amendments was calculated for each plot with each farm system, as the "plant amendment" factor was not a source of variation in the samples to which pre-incubation extraction was performed. In this way, a balanced design was obtained. Then a linear mixed effects model with farming system (three levels: no-till, conventional till and organic) and incubations (two levels: no incubation and incubation) as fixed factors and a random effect per plot was used. For the variables total abundance of Mesostigmata, abundance of immature Mesostigmata, total abundance of Oribatida, total abundance of Astigmatina and *Lasioseius* abundance, the model with a Gaussian distribution was fitted. For the variables abundance of immature Oribatida and abundance of Mesostigmata adults, the model with a Poisson distribution was adjusted.

Effect of plant amendment on mites and nematodes

To evaluate the effect of the amendment on mites and nematodes, data from the samples post-incubation was used. A linear mixed effects model with farming system (three levels: no-till, conventional till and organic) and plant amendment (3 levels: alfalfa, bean, control) as fixed factors and a random effect per plot was used. Due to the over-dispersion of the data, some variables were transformed (Zhang & Davidian 2001; Fitzmaurice *et al.* 2012). The variables total number of Oribatida, the total number of Astigmatina, and the total number of plant-parasitic nematodes were transformed with the mathematical expression $\log(x + 1.5)$. The nematode variables total number of fungal feeders' and total number of bacterial feeders were transformed with the mathematical expression $\sqrt[3]{x + 1.5}$.

All statistical analyses and figures were prepared in R 4.0.2 (R Core Team 2020) and the package lme4 (Bates *et al.* 2015).

Results

Incubation and extraction of mites and nematodes

Pre- and post-incubation abundance differed significantly in total Mesostigmata ($\chi^2_{1,47} = 11.93$, $P < 0.001$), Mesostigmata immatures ($\chi^2_{1,47} = 12.94$, $P < 0.001$), Mesostigmata adults ($\chi^2_{1,47} = 38.74$, $P < 0.001$), *Lasioseius youcefi* Athias-Henriot, 1959 ($\chi^2_{1,47} = 5.35$, $P = 0.02$) (Fig. 2), total Oribatida ($\chi^2_{1,47} = 7.18$, $P = 0.007$), Oribatida immatures ($\chi^2_{1,47} = 27.74$, $P < 0.001$), and total Astigmatina ($\chi^2_{1,47} = 9.6$, $P = 0.001$) (Fig. 3). In contrast the farming system had no effect on any mite groups considering pre- and post-incubation treatment together (Table 2).

Abundance among the different types of plant amendments differed significantly only in the bacterial feeder nematodes ($\chi^2_{2,35} = 9.21$, $P = 0.009$). Samples incubated with alfalfa had the highest abundance of bacterial feeder nematodes, which was significantly different from their abundance in samples incubated without plant amendments (control) ($P < 0.05$). For the other groups of mites and nematodes the plant amendment had no effect on the abundance ($P < 0.05$) considering the farming systems together (Table 3).

TABLE 2. Mean sample (\pm SE) species richness of Mesostigmata and mean sample (\pm SE) abundance of various mite groups (Mesostigmata, Oribatida [excluding Astigmatina] and Astigmatina) collected in three farming systems (no till, organic and till), extracted before (pre-) and after (post-) incubation. Significant differences (P values < 0.05) are indicated by different lower-case letters following each variable between columns (in the same row).

| | No-till | Organic | Till |
|--|-------------------|------------------|------------------|
| Abundance of Mesostigmata adults | 3.3 \pm 1.3 a | 5.5 \pm 2.3 a | 3 \pm 1.8 a |
| Abundance of Mesostigmata immatures | 1.9 \pm 0.7 a | 2.4 \pm 0.9 a | 1 \pm 0.6 a |
| Total abundance of Mesostigmata | 5.8 \pm 1.9 a | 8.4 \pm 3.5 a | 4.2 \pm 2.4 a |
| Number of Mesostigmata species | 1.3 \pm 0.4 a | 1.8 \pm 0.3 a | 1 \pm 0 a |
| Abundance of <i>Lasioseius youcefi</i> | 2 \pm 1.1 a | 2 \pm 1.1 a | 2.3 \pm 1.7 a |
| Abundance of Oribatida [excluding Astigmatina] immatures | 2.8 \pm 1.1 a | 11.8 \pm 5.5 a | 5.5 \pm 3.4 a |
| Abundance of total Oribatida [excluding Astigmatina] | 10 \pm 2.1 a | 19.7 \pm 5.3 a | 10.8 \pm 3.9 a |
| Abundance of total Astigmatina | 48.5 \pm 32.6 a | 42 \pm 22.7 a | 19.4 \pm 9.7 a |

TABLE 3. Mean sample (\pm SE) abundance of nematodes and various mite groups (Mesostigmata, Oribatida [excluding Astigmatina] and Astigmatina) after incubation with two plant amendments (alfalfa and bean) and without an amendment (control) collected in three farming systems (No-till, Organic and Till) together. Significant differences (P values < 0.05) are indicated by different lower-case letters following each variable between columns (in the same row).

| | Alfalfa | Bean | Control |
|--|---------------------|----------------------|-----------------------|
| Abundance of Mesostigmata adults | 10.1 \pm 3.7 a | 6.2 \pm 2 a | 4.6 \pm 1.9 a |
| Abundance of Mesostigmata immatures | 2.8 \pm 1.2 a | 4.1 \pm 1.4 a | 1.4 \pm 0.4 a |
| Total abundance of Mesostigmata | 14.2 \pm 5.2 a | 10.8 \pm 3.2 a | 6.3 \pm 2.3 a |
| Abundance of Oribatida [excluding Astigmatina] immatures | 16.7 \pm 7.3 a | 11.8 \pm 2.7 a | 11 \pm 3.8 a |
| Abundance of total Oribatida [excluding Astigmatina] | 22.8 \pm 8.7 a | 17.8 \pm 2.8 a | 15.5 \pm 4 a |
| Abundance of total Astigmatina | 125.1 \pm 59.9 a | 83.1 \pm 30.2 a | 9.8 \pm 6.2 a |
| Abundance of fungal feeder nematodes | 2400 \pm 823.9 a | 1021.7 \pm 279.8 a | 1316.7 \pm 258.7 a |
| Abundance of bacterial feeder nematodes | 23200 \pm 7974 a | 8710 \pm 1541.2 ab | 5076.7 \pm 1043.2 b |
| Abundance of plant parasitic nematodes | 271.7 \pm 149.7 a | 166.7 \pm 91.6 a | 433.3 \pm 258 a |

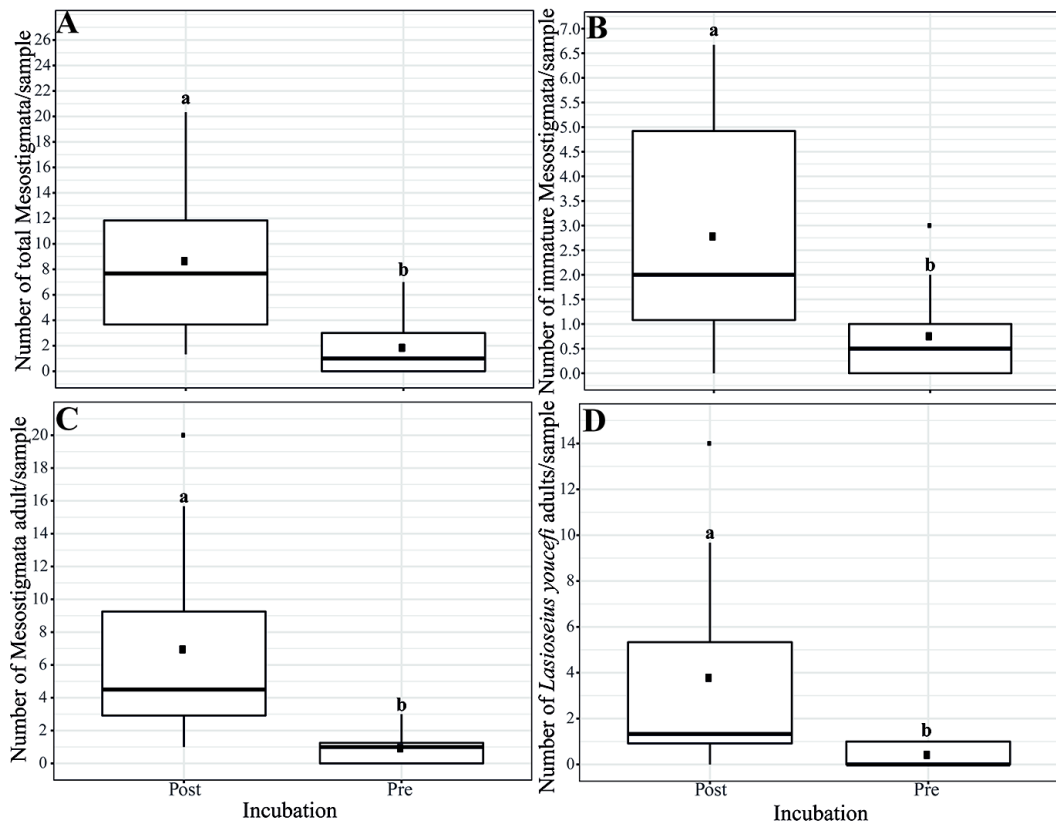


FIGURE 2. Mean number of individuals (\pm SE) extracted before (Pre) and after (Post) incubation of samples collected in three farming systems (no till, organic and till). (A) Total soil Mesostigmata mites. (B) Immatures of soil Mesostigmata mites. (C) Adults of soil Mesostigmata mites. (D) Females of *Lasioseius youcefi* (Mesostigmata: Blattisociidae).

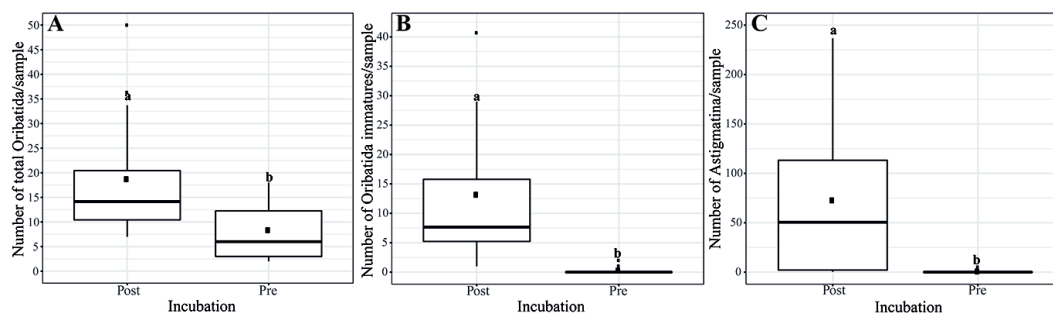


FIGURE 3. Mean number of individuals (\pm SE) extracted before (Pre) and after incubation of samples collected in three farming systems (no till, organic and till). (A) Total soil Oribatida (excluding Astigmatina) mites. (B) Immatures of soil Oribatida mites. (C) Total soil Astigmatina.

Taking into account only the samples that were incubated, the farming system affected the abundance of immature Oribatida ($\chi^2_{2,35} = 5.99$, $P = 0.04$) considering the plant amendment treatments together. The organic farming system had the highest abundance of immature Oribatida, while the No-till system had the lowest (Table 4). For the other groups of mites and nematodes the farming system had no effect on abundance ($P < 0.05$) considering the plant amendments and the control together.

DNA barcoding of soil mites

Barcode compliant sequences were recovered from 150 of the 201 specimens analyzed, with an overall sequencing success rate of 75%. Sequencing success was not significantly different ($\chi^2 = 2.0259$, $P = 0.15$) between the two main groups (Mesostigmata, Oribatida) evaluated (Table 5).

Minimum quality requirements for BIN assignment were met by 164 sequences which were assigned to 24 BINs in total ($x = 6.8$ specimens/BIN). Most of these BINs (18) were new to BOLD (75%) and 19 of them lacked a close match in GenBank (79%). Of the 24, 15 BINs (63%) were morphologically identified to the species level, while six (25%) were identified to genus, two (8%) to family, and one BIN was identified only to the order level (4%). Together these sequences represented 16 species, 14 genera, 11 families, and two orders.

TABLE 4. Mean sample (\pm EE) abundance of nematodes and various mite groups (Mesostigmata, Oribatida [excluding Astigmatina] and Astigmatina) collected in three farming systems (no till, organic and till) and extracted after incubation (only post-incubation). Significant differences (P values < 0.05) are indicated by different lower-case letters following each variable between columns (in the same row).

| | No-till | Organic | Till |
|--|------------------------|------------------------|------------------------|
| Abundance of Mesostigmata adults | 5.7 \pm 1.9 a | 9.9 \pm 3.3 a | 5.3 \pm 2.6 a |
| Abundance of Mesostigmata immature | 2.5 \pm 1.4 a | 4.3 \pm 1.2 a | 1.6 \pm 0.7 a |
| Total abundance of Mesostigmata | 9 \pm 3 a | 15.3 \pm 4.9 a | 6.9 \pm 3 a |
| Abundance of Oribatida [excluding Astigmatina] immatures | 5.2 \pm 1.8 b | 23.3 \pm 6.8 a | 11 \pm 3.3 ab |
| Abundance of total Oribatida [excluding Astigmatina] | 10.3 \pm 2.3 a | 28.6 \pm 8 a | 17.2 \pm 4.1 a |
| Abundance of total Astigmatina | 97 \pm 50.6 a | 83.4 \pm 46.2 a | 37.5 \pm 17 a |
| Abundance of fungal feeder nematodes | 966.7 \pm 447.1 a | 1516.7 \pm 485.8 a | 2255 \pm 638.6 a |
| Abundance of bacterial feeder nematodes | 10526.7 \pm 3350.6 a | 10566.7 \pm 2911.8 a | 15893.3 \pm 7894.8 a |
| Abundance of plant parasitic nematodes | 505 \pm 264.9 a | 300 \pm 140.3 a | 66.7 \pm 44.9 a |

TABLE 5. Summary of the total number of Mesostigmata and Oribatida [excluding Astigmatina] specimens extracted from the soil samples compared with the number of specimens analyzed through DNA barcoding and the resultant high-quality sequences.

| Taxon | Total number of specimens | No. of DNA barcoded specimens (% of total) | No. of specimens with barcode compliant sequences (% of sequenced) | No. of DNA barcoded specimens with LTSEM images | No. of specimens with LTSEM and barcode compliant sequence (% of sent) |
|-----------------------------------|---------------------------|--|--|---|--|
| Mesostigmata | 397 | 152 (38) | 116 (76) | 18 | 12 (67) |
| Oribatida [excluding Astigmatina] | 772 | 49 (6) | 34 (69) | 0 | 0 |
| Total | 1169 | 201 (17) | 150 (75) | | |

All 15 (100%) of the morphologically identified species perfectly corresponded with their BIN assignments, and no instances of BIN splits, mixtures, or mergers were detected. However, six (40%) of these species were represented by just a single sequence and were consequently excluded from the barcode gap analyses. Of the nine species with more than one representative, the average maximum intraspecific divergence was low (0.9% p-distance) and ranged from 0–1.7%. Conversely, the average minimum interspecific divergence was high (23.4% p-distance) and ranged from 19.2–27.5%.

Identification of soil mites

Mesostigmata

We found a total of 397 non-Uropodina Mesostigmata, of which 118 were found in the no-till system, 190 in the organic system, and 89 in the till system (Table 6). Of these mites, only 22 were obtained from the part of the samples that were extracted before incubation (pre-incubation). Incubation of the samples with the two plant amendments and the control allowed the extraction of 375 mites from the same samples.

We found a total of 16 mesostigmatic species through morphological and molecular techniques, belonging to six different families.

Blattisociidae was the dominant family pre- and post-incubation, in all the farming systems. Of this family, two species were found before (pre) incubation, while *Lasioseius youcefi* was the only species found in this family after (post-) incubation and dominated these samples.

Species of Ascidae and Parasitidae were also relatively abundant. Ascidae was composed of at least four species, most of which were found in the organic and no-till systems and only after incubation of the samples. *Cycetogamasus diviortus* (Athias-Henriot, 1967) was the only species of Parasitidae, found exclusively in the organic and no-till systems.

Individuals of *Gaeolaelaps* sp., *Stratiolaelaps scimitus* (Laelapidae) and *Macrocheles kekensis* (Macrochelidae), were extracted from the incubated samples (post-incubation).

Oribatida

We found a total of 13 oribatid species through morphological and molecular techniques, belonging to five different families (Table 7). Of these species, most derived from the organic and no-till systems (nine respectively). In the till system, we identified six species. We identified *Galumna virginiensis* and *Schelorbates* sp. nr. *pallidulus* 3 in all three farming systems.

High resolution imaging of the dominant soil Mesostigmata species

Using LTSEM and confocal high-resolution imaging along with molecular and morphological identifications (of the same specimens), we describe below characteristics not clearly observed or presented in previous descriptions of *Cycetogamasus diviortus* and *Lasioseius youcefi*. The description of *Gamasellodes* n. sp. among the most dominant species found in this study, utilizing the same integrated approach, is presented in Rueda-Ramírez *et al.* (2022, in this special issue).

TABLE 7. Oribatida (excluding Astigmatina) species in fields with different farming systems (Till, No-Till and Organic treatments) from soils samples, pre- and post-incubation combined.

| Family | Species | N-T | O | T |
|-----------------|---|-----|---|---|
| Galumnidae | <i>Galumna jacoti</i> Wharton, 1938*‡†§ | | | x |
| | <i>Galumna minuta</i> (Ewing, 1909)*‡†§ | x | | |
| | <i>Galumna virginiensis</i> Jacot, 1929*‡†§ | x | x | x |
| | Unidentified● | x | | |
| Oppiidae | <i>Oppiella nova</i> (Oudemans, 1902) | | x | |
| Oribatulidae | <i>Zygoribatula undulata</i> Berlese, 1916*‡†§ | | x | |
| Scheloribatidae | <i>Scheloribates</i> sp. nr. <i>pallidulus</i> 1 (Koch, 1841)*† | x | | |
| | <i>Scheloribates</i> sp. nr. <i>pallidulus</i> 2 (Koch, 1841)*† | | x | |
| | <i>Scheloribates</i> sp. nr. <i>pallidulus</i> 3 (Koch, 1841)*† | x | x | x |
| | <i>Scheloribates</i> sp. nr. <i>pallidulus</i> 4 (Koch, 1841)*† | | x | x |
| Tectocepheidae | <i>Tectocepheus sarekensis</i> Trägårdh, 1910*† | x | | |
| | <i>Tectocepheus velatus</i> (Michael, 1880)*† | x | x | |
| | Unidentified● | x | x | x |
| Unidentified | | x | x | x |

* BIN new to BOLD; ‡ Taxon new to BOLD

† BIN new to GenBank; § Taxon new to GenBank

N-T: No-Till farming system; O: Organic farming system; T: Till farming system

● Specimens could not be identified to family or genus because important characters were damaged or not observed.

Adult female (Figs. 4A–G). Morphological characterization added to (Athias-Henriot 1967) and re-description (Hennessey and Farrier 1989):

Gnathosoma. Deutosternal groove delimited by subparallel lateral lines, with ten transverse rows, the most distal row bimodal-like and others with 25–55 denticles (Fig. 4A). Corniculi entire horn-shaped, well separated from each other, subparallel (Fig. 4A). Fixed cheliceral digit with seven large teeth, the most proximal larger extending twice its height basally, and a small one between the fifth and sixth most distal teeth; movable cheliceral digit with three large teeth and a row of small teeth between them (Fig. 4B, Fig. 5A–C); the confocal images show innervation (white-blue) of the tips of the two digits of the chelicera, of four more distal teeth and the pilus dentilis of the fixed digit and the three larger teeth of the movable digit (dendritic terminations in green; Fig. 5A–C). Paraxial seta (*al*) on palpfemur divided into several branches and paraxial setae (*al1*, *al2*) on palpgenu spatulate and dilated laterally; palp apotele trifurcate (Fig. 4C). Anterior margin of epistome trifurcate (Fig. 4F). Internal malae separated medially, all margins fimbriate; hypostomal setae aciculate, *h3* about in longitudinal line with *h1* and transversal of *h2* (Fig. 4A).

Dorsal idiosoma. Podonotal shield almost reticulated throughout, except the median and lateral regions between setae *j3–j5*, and *z3–z4*. Opistonotal shield well-reticulated throughout (Fig. 4F).

Ventral idiosoma. Cingulum (Fig. 4G) and anterior part of endogynum with two spinate processes (Fig. 4E; colored in yellow).

Peritreme and peritrematic shield. Peritreme extending anteriorly to almost the level of *z1*, structure of the peritrematal wall consisting of fine villi (Fig. 4D), described by Witalinsky (1980) as cuticular minute spines for Parasitidae. Peritrematal shield fused to opisthogastric shield at the level of coxa IV (cingulum) (Fig. 4G).

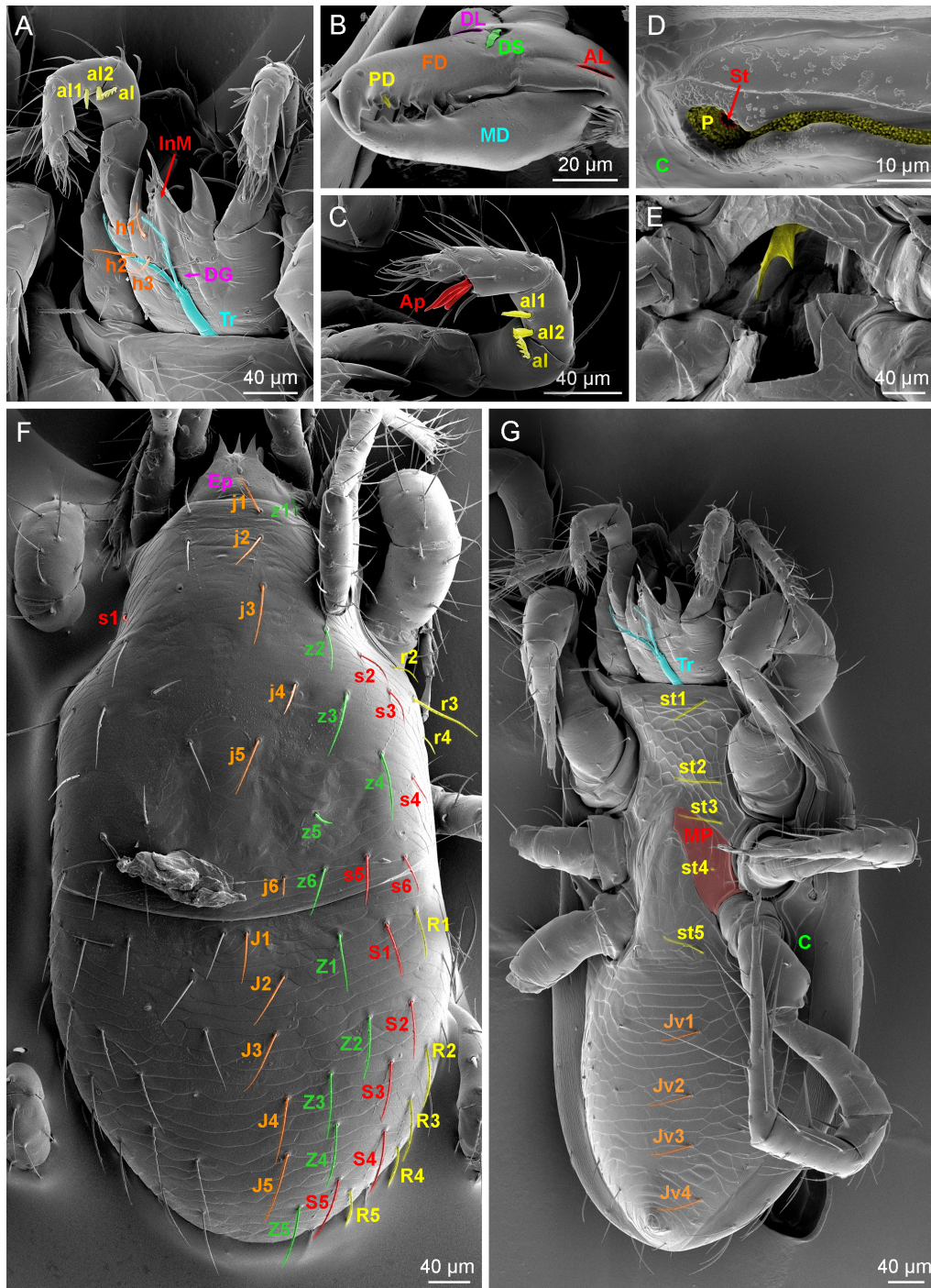


FIGURE 4. *Cytogamasus diviortus* (Athias-Henriot, 1967) female, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Ventral view of gnathosoma. (B) Antiaxial view of chelicera. (C) Palp. (D) Posterior view of peritreme and stigma. (E) Anterior part of endogynum with two spinate processes (colored in yellow). (F) Dorsal idiosoma. (G) Ventral idiosoma. InM = internal malae, DG = deutosternal groove, Tr = tritosternum, FD = fixed digit, MD = movable digit, DL = dorsal lyrifissure, DS = dorsal setae, AL = antiaxial lyrifissure, PD = pilus dentilis, Ap = apotele, C = cingulum, P = peritreme, Ep = Epistome, MP = metasternal plates.

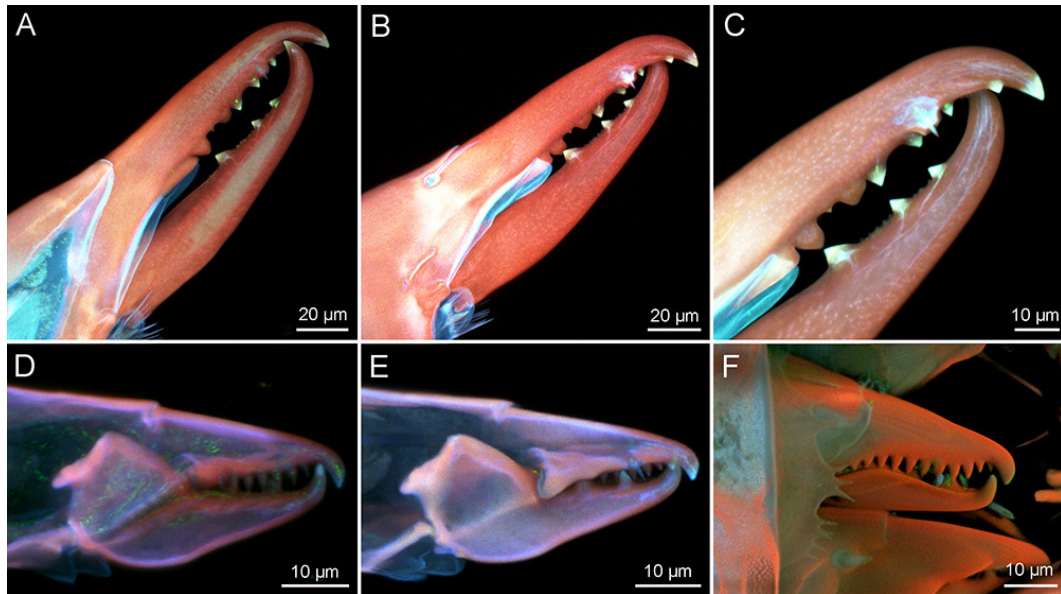


FIGURE 5. Confocal Laser Scanning Microscopy of the chelicera. (A–C) *Cycetogamasus diviortus* female (innervation white-blue with dendritic terminations green at the tip of the digits, the teeth, and the pilus dentilis), (A) adaxial profile, (B) abaxial profile, (C) abaxial profile. (D–F) *Lasioseius youcefi* female (innervation and dendritic terminations green blue), (D) adaxial profile, (E) abaxial profile, (F) Three-dimensional projection image.

Male (Figs. 6A–D). Morphological characterization added to the original description (Athias-Henriot 1967) and re-description (Hennessey & Farrier 1989):

Gnathosoma. Paraxial seta (*al*) on palpfemur divided into several branches and paraxial setae (*all*, *al2*) on palpgenu spatulate and dilated laterally; apotele trifurcated (Fig. 6A); fixed cheliceral digit with ten small teeth; movable cheliceral digit with a large tooth (Fig. 6B). Corniculi entire; internal malae separated medially, all margins fimbriate; hypostomal setae aciculate; *h3* about in longitudinal line with *h1*, mesad and slightly posterior to *h2* (Fig. 6C).

Ventral idiosoma. Tritosternal base covered by genital lamina (Fig. 6D).

***Lasioseius youcefi* Athias-Henriot, 1959**

Lasioseius youcefi Athias-Henriot, 1959: 177.

Lasioseius youcefi Hirschmann.—1962: 30; Bregetova, 1977: 178; Tseng, 1978: 124; 1982: 4; Walter & Lindquist, 1989: 2800; Farrier & Hennessey, 1993: 39; Karg, 1993: 254; Lee & Lee, 1998: 17; Kamali *et al.*, 2001: 8; Gwiazdowicz, 2007: 88; Christian & Karg, 2008: 69; Gwiazdowicz *et al.*, 2008: 38; Zhang & Fan, 2010: 284; Bai, 2013: 97; Eliaderani *et al.*, 2013: 130; Moraes *et al.*, 2015: 28.

Lasioseius (Criniacus) youcefi.—Karg, 1980: 364.

Lasioseius (Cuspiacus) youcefi.—Christian & Karg, 2006: 219.

Lasioseius mcgregori Chant, 1963: 276 (Synonymy with *L. youcefi* by Walter & Lindquist, 1989: 2800; Farrier & Hennessey, 1993: 39; Halliday, 2005: 21; Christian & Karg, 2006: 219).

Lasioseius mcgregori.—Lindquist, 1964: 242; Bregetova, 1977: 179; Kandil, 1980: 85; Gupta, 2003: 5.

Lasioseius (Criniacus) mcgregori.—Karg, 1980: 362.

Lasioseius (Lasioseius) paucisetosus Westerboer, 1963: 274 (Synonymy with *L. youcefi* by Bregetova, 1977: 178; Karg, 1980: 364; Walter & Lindquist, 1989: 2800; Farrier & Hennessey, 1993: 39; Halliday, 2005: 21; Christian & Karg, 2006: 219).

Lasioseius paucisetosus.—Karg, 1965: 206; 1971: 250.

Lasioseius paucisetosus Westerboer (in part).—Lapina, 1976: 72. Misidentification of *L. youcefi*, according to

Walter & Lindquist, 1989: 2800.

Lasioseius (Lasioseius) proteae Ryke, 1964: 343 (Synonymy of *L. youcefi* by Karg, 1980: 364; Walter & Lindquist, 1989: 2800; Farrier & Hennessey, 1993: 39; Halliday, 2005; Christian & Karg, 2006: 219).

Lasioseius lasiodactyli Ishikawa, 1969: 112 (Synonymy of *L. youcefi* by Christian & Karg, 2006: 219).

Lasioseius lasiodactyli.—Lee & Lee, 1998: 16.

Lasioseius peritremus Nasr & Abou-Awad, in Zaher, 1986: 70 (Synonymy of *L. youcefi* by Gwiazdowicz, 2007: 80).

Lasioseius peritremus.—Nasr & Abou-Awad, 1987a: 31; 1987b: 89; Fouly, 1997: 2.

Lasioseius (Crinidens) peritremus.—Christian & Karg, 2006: 152.

Specimens examined: 21 females from soil of the Beltsville Agricultural Research Center (BARC), Maryland, USA (11 with barcode compliant sequences, four of them also with LTSEM images and two with only LTSEM).

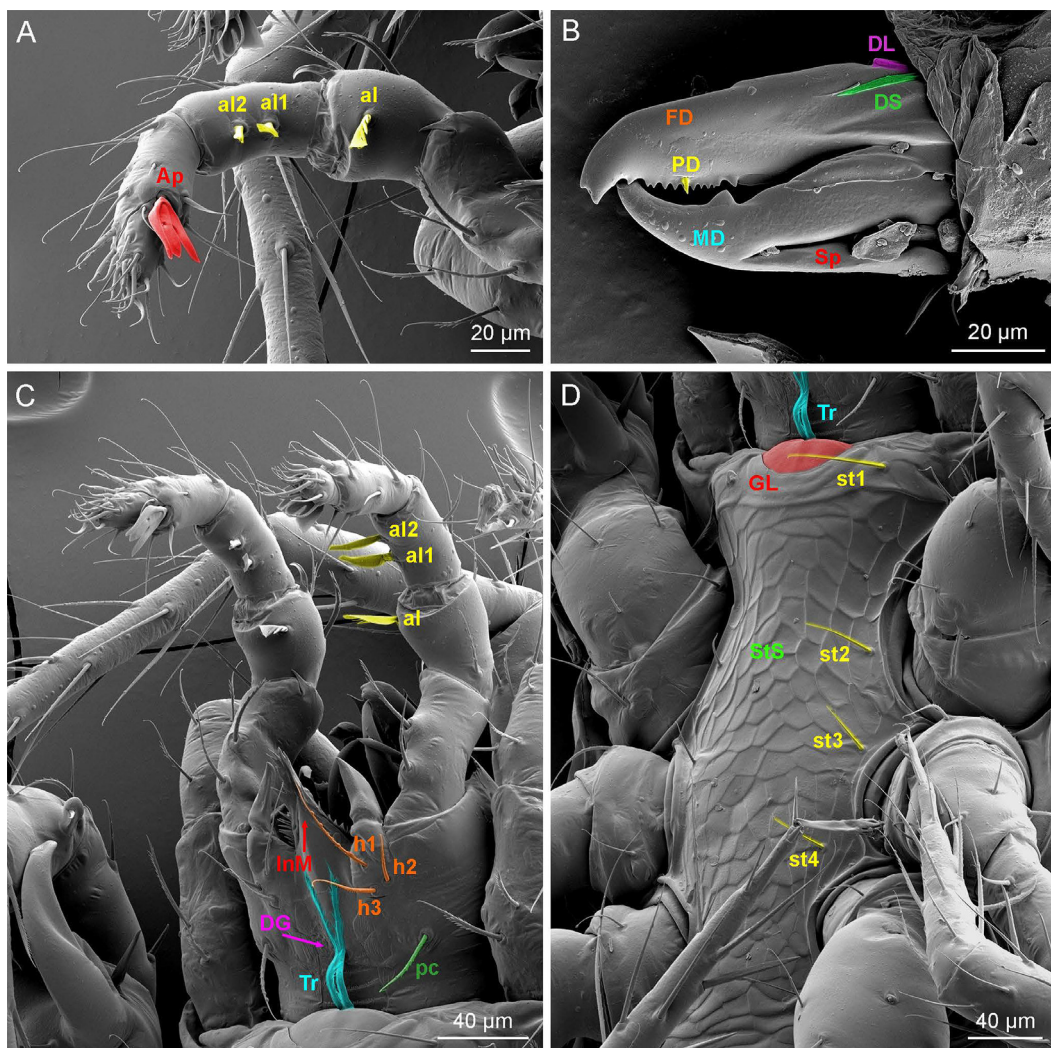


FIGURE 6. *Cytogamasus diviortus* (Athias-Henriot, 1967) male, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Palp. (B) Antiaxial view of chelicera. (C) Ventral view of gnathosoma. (D) Anterior view of ventral idiosoma. Ap = apotele, FD = fixed digit, MD = movable digit, DL = dorsal lyrifissure, DS = dorsal setae, PD = pilus dentilis, Sp = spermatotrema, InM = internal malae, DG = deutosternal groove, Tr = tritosternum, GL = genital lamina.

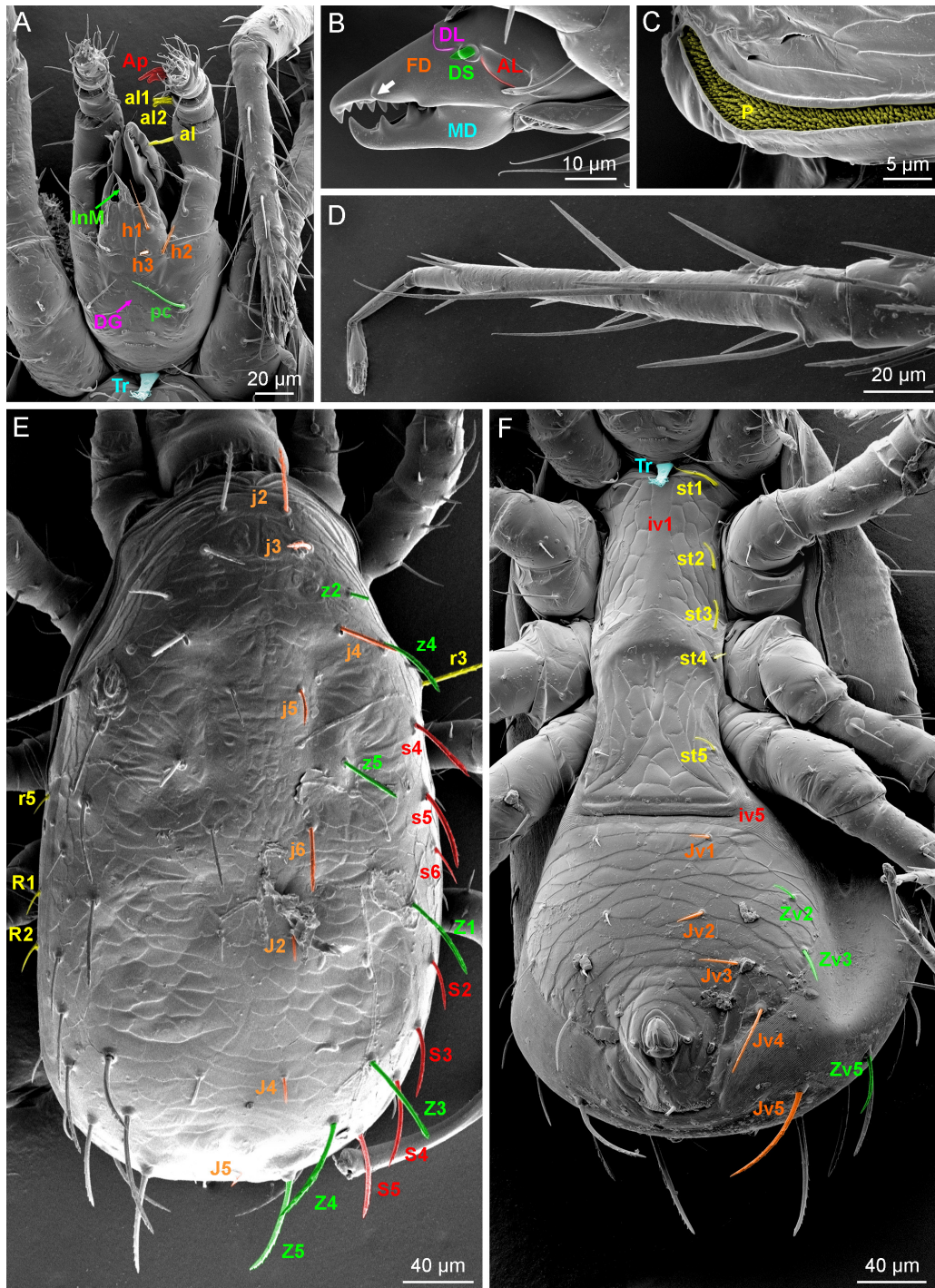


FIGURE 7. *Lasioseius youcefi* Athias-Henriot (1959) female, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Ventral view of gnathosoma. (B) Antiaxial view of chelicera. (C) Anterior part of peritreme. (D) Tarsus IV. (E) Dorsal idiosoma. (F) Ventral idiosoma. Ap = apotele, InM = internal malae, DG = deutosternal groove, Tr = tritosternum, FD = fixed digit, MD = movable digit, DL = dorsal lyrifissure, DS = dorsal setae, AL = antiaxial lyrifissure, P = peritreme.

Adult female (Figs. 7A–F). Although the characteristics of this species have been described in detail in previous descriptions and re-descriptions (Athias-Henriot 1959; Walter & Lindquist 1989; Moraes *et al.* 2015), the images allowed us to re-confirm the following characteristics:

Gnathosoma. Deutosternal groove delimited by subparallel lateral lines, except for the two most proximal rows; with seven transverse rows, the first (distal) to fifth with 7–10 denticles each; sixth and seventh (most proximal) with 15–20 denticles (Fig. 7A). General shape of chelicera typical of the phytoseioides group; especially evident is the antiaxial surface of the fixed cheliceral digit with its subterminal pointed process with a shallow depression near the base (Moraes *et al.* 2015; Fig. 7B; Figs. 5D–F); the confocal images show innervation of the tips of the two digits of the chelicera and of all the teeth of the movable and fixed digits (innervations and dendritic terminations in green-blue; Figs. 5D–F). Setae *al1* and *al2* of palpgenua and *al* of palpfemur spatulate; palp-apotele bifurcate; internal malae separated medially, all margins fimbriate; corniculi horn-shaped, well separated from each other, subparallel; hypostomal setae aciculate and smooth, *h3* about in longitudinal line with *h1* and mesad and slightly posteriad of *h2*, which is shorter.

Dorsal idiosoma. Holodorsal shield reticulate almost throughout, with 23 pairs of setae. Shapes of the setae as described by Moraes *et al.* (2015), except for *j2*, with intermediate length comparing other dorsal setae, lightly serrate and stout (Fig. 7E).

Ventral idiosoma. Sternal and epigynal shields reticulated, except for a smooth vertical midline in the sternal shield (Fig. 7F).

Peritreme. Peritreme extending well anteriorly to region between *j2* setae (Fig. 7E). The structure of the peritrematal wall consisting of fine villi (Fig. 7C), was described by Witalinsky (1980) as cuticular minute spines for Parasitidae.

Legs. Leg IV with three macrosetae on genu (*ad2*), basitarsus (*pd3*) and telotarsus (*pd2*) (Fig. 7D).

Notes. As described by Moraes *et al.* (2015), the reticulation showed a slight variation between the specimens collected in France and United Arab Emirates. In this case, the specimens are from another continent, which verifies the variations in the ornamentation strength within populations. Likewise, according to our observations, the ornamentation is more visible in the LTSEM images when compared with the same specimens mounted on slides (latter not shown).

Discussion

Incubation and extraction of mites and nematodes

In the present study, in three farming systems (no till, organic and conventional till) we evaluated the effect of incubation, with and without an organic amendment on soil predatory mite abundance and diversity, as well as on the abundance of free living nematodes sorted to functional group, compared to samples immediately processed (without incubation). Incubation is known as an effective method for enhancing abundances of bacteria that cannot be cultivated on standard microbiological media (Davis *et al.* 2005). However, this method as far as we know, has yet to be used for amplifying population abundances of predatory mites. Using our modified Galen-Berlese funnels, we succeeded in extracting mites from soil samples at relatively high densities, that were almost undetectable without incubation, as was the case for species of Ascidae. While alfalfa as a soil amendment significantly enhanced the extracted number of bacteriovorous nematodes compared to no amendment, it did not affect mite abundance, possibly because food was not a limiting factor as nematodes were also abundant in the control. As pesticides can negatively impact soil organisms in general, we expected the organic treatment to have a higher abundance of soil mites, but our findings showed equally low abundances across all three farming treatments, except for immatures

of Oribatida. This could be attributed to the early sampling date which was performed before tilling was conducted in the till and organic treatments and before conventional pesticides were applied in the till and no-till treatments. Additionally, extremely high precipitation in the preceding autumn and no winter cover-crop could have contributed to the overall low abundances. Treonis *et al.* (2018) conducted their sampling in the FSP plots in June 2014 during the wheat phase, where they found higher abundances of fungal feeders in the organic treatment. Further suggesting that our results may have differed if we would have sampled during the cropping season.

Although the Galen-Berlese funnels were previously used successfully for extracting arthropods from litter and soil (Dubey *et al.* 2020), it is named and described in detail for the first time in this study (Figure 1). While many studies have used modified Berlese funnels for the extraction of arthropods (for example André *et al.* 2002; Barberena-Arias *et al.* 2012; Dizlek *et al.* 2019; N'Dri *et al.* 2018), we believe there is merit in describing the Galen-Berlese funnel as it allows for standardization, since all parts and components are off the shelf items, relatively inexpensive and easily assembled. Maximizing extraction of adult life stages (including the completion of immature development) can be attributed to gradual desiccation, due to the low temperature emitted from the dimmed light source, the distance between the light bulb and soil sample and the soil moisture retained by the enclosing bucket. However, if the Berlese funnels are intended to determine population densities in the soil sample at the time the soil is sampled, this gradual process could be problematic because mites could reproduce during the extraction procedure. In fact, this was demonstrated by Knapp *et al.* (2018) by placing known numbers of *M. robustulus* and *G. aculeifer* adults with and without their respective prey in commercially available Berlese-Tullgren funnels (Burkard Scientific, Uxbridge, UK). For both predator species without prey, fewer adults were recovered and no immatures were found, whereas with prey, total abundance almost doubled, of which 50% were immatures.

In the present study, our system of using two sets of collection cups, rotated daily, one set in the funnels the other stored at -20°C for 24 hours, yielded a sequencing success of over 75%, which was substantially better than the 46% attained in a previous study (Young *et al.* 2019).

DNA barcoding of soil mites

Currently, parameterization of the DNA barcode reference library remains low for mites, limiting our ability to gain species-level identifications from barcode-based surveys of the fauna (Young *et al.* 2019). For example, BOLD's Taxonomy Browser indicates that for Mesostigmata, most BINs are attributed to Laelapidae (339) but just 38% of these BINs are identified to the species level. Conversely, more than 70% of Macrochelidae (74), Ologamasidae (39), and Blattisociidae (195) BINs have been identified to species. Of all the Mesostigmata families found in this study, BOLD contains 1,116 BINs of which just 458 (41%) are linked to species names. Species-level parameterization for Oribatida BINs is even lower. Opiidae, for example, has the highest sarcoptiform BIN richness (231) on BOLD and only 21% of these BINs are identified to species. The new records provided by our study increase the number of BINs correlated with reliable morphological identifications, serving as a reference for future studies in systematics, taxonomy, ecology, and biological control.

In total, 18 BINs and 8 species were new to BOLD's reference library; 19 BINs and 9 species were also novel records for GenBank. The four morphologically identified Mesostigmata species contributed to BOLD included *Gamasellodes* n. sp., *Neojordensia levis*, *Macrocheles kekensis* and *Cycetogamasus diviortus*. However, the DNA barcodes from our *Cycetogamasus diviortus* specimens closely matched ($\leq 2\%$ divergence) sequences already on BOLD, identified only as Parasitidae, but likely represent this species. Four species of Oribatida were also new to BOLD and included *Galumna jacoti*, *G. minuta*, *G. virginiensis* and *Zygoribatula undulata*.

Identification of soil mites

Of the Mesostigmata species, only *N. levis* (Santos *et al.* 2021) and *M. kekensis* (Kontschán 2018) correspond to new records for the United States of America (USA). *Neojordensia levis* had been previously reported in Europe and Asia, associated with moss, edaphic substrates, nests, grasses, and fungi (Santos *et al.* 2021). *Macrocheles kekensis* was recently described with specimens collected in a cetonin beetle in Hungary (Kontschán 2018). Among the other species new to BOLD, *Cycetogamasus diviortus* was described with specimens from France found in compost (Athias-Henriot 1967) but has subsequently been found in other countries in Europe, Asia and North America (Karg 1971; Hyatt 1980; Hennessey & Farrier 1989).

The other Mesostigmata species already represented on BOLD and reported in the USA correspond to widely distributed species. *Asca garmani* and *L. youcefi* are cosmopolitan species, found in several continents in various edaphic substrates, as well as in plants and nests (Moraes *et al.* 2015; Santos *et al.* 2021; Santos *et al.* 2018). *Stratiolaelaps scimitus*, despite being confused with *Stratiolaelaps miles* (Berlese 1892), is a well-known species used commercially as a biocontrol agent and known to be adventive in several countries (Walter & Campbell 2003).

High resolution imaging of the dominant soil Mesostigmata species

The use of high-resolution LTSEM images allowed the observation of previously described characteristics difficult to observe or distorted in mounted specimens (Oldfield *et al.* 1972; Witalinski 1980; Baker 1995; Otto 1999; Witalinski & Borsuk 2002). Dowling *et al.* (2010) demonstrated that LTSEM images are not only useful for the observation of difficult-to-see morphological features but also to allow tissue conservation for subsequent molecular studies, which we confirmed through post-imaging DNA barcode analysis for several specimens in this study.

This is the first study proposing a nomenclatural assignment of the dorsal setae for *Cycetogamasus diviortus*. The 3D images also enabled visualization of characteristics, such as the anterolateral setae of the palpgenu and palpfemur used for the identification of this group. Details in the structure of the peritrema and stigma allowed us to confirm the observations of these structures in other Parasitidae by Witalinski (1980). The 3D structure of the anterior spinate processes of the endogynium is also visualized by LTSEM for the first time in *C. diviortus*, placing this species within the type 1 endogynium as described by Witalinski & Borsuk (2002).

Morphological characteristics unique to *L. youcefi* have been described in detail in previous descriptions and re-descriptions of this species (Athias-Henriot 1959; Walter and Lindquist 1989; Moraes *et al.* 2015). However, the change in ornamentation caused not only by the slide mounting of the specimens, but also due to the variation between populations as described by Moraes *et al.* (2015) was evident. Changes in ornamentation in slide-mounted specimens compared to specimens photographed by LTSEM have been reported (Rezende *et al.* 2019; Welbourn *et al.* 2003). SEM photographs of the mouthparts of this species were previously taken and analyzed by Walter and Lindquist (1989). The confocal and LTSEM images taken in this study complement their work by confirming their observations and provide additional images of the entire specimens.

The confocal images of the two Mesostigmata species presented in this work allowed us to observe not only external morphological features of the chelicerae, but also the sensory innervation of both digits of the chelicerae. This innervation and dendritic terminations at the tip, teeth, and *pilus dentilis* has been reported before for different groups of mites (de Lillo & Aldini 1994; de Lillo *et al.* 1996; Alberti & Coons 1999; Alberti *et al.* 2011; de Lillo *et al.* 2001; Evans 1992; Nuzzaci & Di Palma 2002) and in Ricinulei (Talarico *et al.* 2007). In the present study, innervation with dendritic termination was only evident in some teeth in *C. diviortus*, whereas innervation with dendritic termination was observed in all teeth in *L. youcefi*. In both mites, there is innervation toward the tip

of both digits, which corresponds to the innervation of the apical pit sensillum reported previously (Evans 1992; de Lillo *et al.* 2001; Alberti *et al.* 2011).

Conclusion

Our general objective of this study was to improve methodologies for the integrated taxonomy of soil predatory mites. One of the main factors limiting accurate morphological and molecular identifications of soil predatory mites is the number of available specimens. Incubating samples is a relatively simple solution that dramatically enhanced the number of individuals extracted from samples. The daily freezing of Galen-Berlese extracted specimens (in 95% ethanol) substantially improved the sequencing success rate compared to previous studies. Finally, LTSEM imaging followed by molecular identification contributed further details to the published descriptions of *C. diviortus* and *L. youcefi*. In line with our general aim, the collecting, extracting, identification to morpho-species, sample preparation for DNA barcoding and uploading relevant information to BOLD was performed by scientists without expertise in taxonomy. Whereas our skilled taxonomists focused on the morphological identifications using light microscopy, expanding on existing descriptions using LTSEM images and in a subsequent manuscript the description of a new species (Rueda-Ramírez *et al.* 2022, in this special issue). We believe this division of tasks and labor will set the stage for further collaborative integrated studies between ecologists, biocontrol specialists and expert taxonomists for the identification, evaluation and description of known and novel soil acarine BCAs.

Data Accessibility

All specimen and sequence data are publicly accessible on BOLD through the DS-SPMFLN dataset (<https://doi.org/10.5883/DS-SPMFLN>). The sequences are also available on GenBank (OK072891 - OK073066).

Acknowledgements

This manuscript is dedicated to the memory of Dr. Gary Bauchan, our dear friend and colleague, who passed away from complications of Covid-19 in January 2021. Gary was the Director of the Electron & Confocal Microscopy Unit at the Beltsville Agriculture Research Center of the USDA since 2008, where he co-created many stunning images including the ones presented here. This work was an integral part of a Czech-Israeli Cooperative Scientific Research project titled ‘New biorational methods applied to control selected pests as an alternative to chemical pesticides to prevent contamination of soil and water resource’, supported by the Ministry of Science Technology & Space, State of Israel (project 3-13035) and by the Ministry of Education, Youth and Sports of the Czech Republic (project 8G15006). Our special thanks to Galen P. Dively from the Dept. of Entomology, University of Maryland, who devised the funnel configuration and graciously granted access to his funnels for the duration of the study and to Kevin Conover, University of Maryland, Beltsville Facility, who kindly provided the requested space and facilities to perform the extractions. Andrew Ulsamer, Margaret MacDonald and Shiguang Li for their technical support and to the USDA National Program and APHIS for support, assistance and consumables. We express our gratitude to Dr. Cavigelli, Dr. Maul and Ms. Tran for providing access and facilitating soil sampling

in the FSP plots. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. To David Walter, Gilberto J. de Moraes, Ilinca Juvara-Balş and Owen Seeman for their help in the confirmation of some characters and identifications of the mesostigmatic species. To Roy Norton for his help with the identification of Oribatida. To DFG Deutsche Forschungsgemeinschaft for the financial support to the first author.

References

- Acharya, R., Hwang, H.-S., Shim, J.-K., Yu, Y.-S. & Lee, K.-Y. (2019) Control efficacy of fungus gnat, *Bradysia impatiens*, enhanced by a combination of entomopathogenic nematodes and predatory mites. *Biological Control*, 138, 104071.
<https://doi.org/10.1016/j.biocontrol.2019.104071>
- Alberti, G. & Coons, L.B. (1999) Acari - Mites. In: Harrison, F.W. (Ed.) *Microscopic Anatomy of Invertebrates*. Vol. 8c. New York, Wiley-Liss, pp. 515–1265.
- Alberti, G., Heethoff, M., Norton, R.A., Schmelzle, S., Seniczak, A. & Seniczak, S. (2011) Fine structure of the gnathosoma of *Archeogozetes longisetus* Aoki (Acari: Oribatida, Trhypochthoniidae). *Journal of Morphology*, 272, 1025–1079.
<https://doi.org/10.1002/jmor.10971>
- André, H.M., Ducarme, X. & Lebrun, P. (2002) Soil biodiversity: myth, reality or conning? *Oikos*, 96, 3–24.
<https://doi.org/10.1034/j.1600-0706.2002.11216.x>
- Athias-Henriot, C. (1959) Phytoseiidae et Aceosejidae (Acarina, Gamasina) d'Algérie. III Contribution aux Aceosejinae. *Bulletin de la Société d'Histoire Naturelle de L'Afrique du Nord*, 50, 158–195.
- Athias-Henriot, C. (1967) Observations sur les *Pergamasus*. I. Sous-genre *Paragamasus* Hull, 1918 (Acariens Anactinotriches: Parasitidae). *Memoires du Museum National d'Histoire naturelle (Paris) (n.s.) Serie A, Zoologie*, 49, 1–198.
- Athias-Henriot, C. & Lee, E. (1980) Parasitidae nord-coréens (Parasitiformes): apparentés au genre *Neogamasus* Tikh., avec création de trois nouveaux genres. *Annales Historico-naturales Musei Nationalis Hungarici*, 72, 285–294.
- Azevedo, L., Leite, L., Chacon-Orozco, J., Moreira, M., Ferreira, M., Cano, L., Borges, V., Rueda-Ramírez, D., de Moraes, G. & Palevsky, E. (2019) Free living nematodes as alternative prey for soil predatory mites: An interdisciplinary case study of conservation biological control. *Biological Control*, 132, 128–134.
<https://doi.org/10.1016/j.biocontrol.2019.02.007>
- Azevedo, L.H., Moreira, M.F.P., Pereira, G.G., Borges, V., de Moraes, G.J., Inomoto, M.M., Vicente, M., de Siqueira Pinto, M., Peres, L.P., Rueda-Ramírez, D., Carta, L., Meyer, S.L.F., Mowery, J., Bauchan, G.R., Ochoa, R. & Palevsky, E. (2020) Combined releases of predatory mites and provisioning of free-living nematodes for the biological control of root-knot nematodes on 'Micro Tom tomato'. *Biological Control*, 146, 104280.
<https://doi.org/10.1016/j.biocontrol.2020.104280>
- Bai, X. (2013) Notes on the mesostigmatic mifs (sic) from Nigxia, China (Acari, Mesostigmata). *Acta Arachnologica Sinica*, 22, 96–109 [in Chinese].
- Baker, A.S. (1995) A redescription of *Halotydeus destructor* (Tucker) (Prostigmata: Penthalaeidae), with a survey of ontogenetic setal development in the superfamily Eupodoidea. *International Journal of Acarology*, 21, 261–282.
<https://doi.org/10.1080/01647959508684069>
- Barberena-Arias, M.F., González, G. & Cuevas, E. (2012) Quantifying variation of soil arthropods using different sampling protocols: is diversity affected. *Tropical Forest. InTech Online Publisher, www.intechopen.com*, 51–70.
- Barbosa, M.F.C. & Moraes, G.J. de (2016) Potential of astigmatid mites (Acari: Astigmatina) as prey for rearing edaphic predatory mites of the families Laelapidae and Rhodacaridae (Acari: Mesostigmata). *Experimental and Applied Acarology*, 69, 289–296.
<https://doi.org/10.1007/s10493-016-0043-4>
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of*

- Statistical Software*, 67, 1–48.
<https://doi.org/doi:10.18637/jss.v067.i01>
- Batey, T. (2009) Soil compaction and soil management – a review. *Soil Use and Management*, 25, 335–345.
<https://doi.org/10.1111/j.1475-2743.2009.00236.x>
- Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G.L., Mansion-Vaquie, A., Pell, J.K., Petit, S., Quesada, N., Ricci, B., Wratten, S.D. & Birch, A.N.E. (2017) A functional overview of conservation biological control. *Crop Protection*, 97, 145–158.
<https://doi.org/10.1016/j.cropro.2016.11.008>
- Berlese, A. (1892) Acari, Myriopoda et Scorpiones, hucusque in Italia reperta.
- Berndt, O., Poehling, H.M. & Meyhöfer, R. (2004) Predation capacity of two predatory laelapid mites on soil-dwelling thrips stages. *Entomologia Experimentalis et Applicata*, 112, 107–115.
<https://doi.org/10.1111/j.0013-8703.2004.00185.x>
- Bregetova, N. (1977) Family Aceosejidae Baker and Wharton, 1952 (sensu Evans, 1958). In: Ghilyarov, M. & Bregetova, N. (Eds.), *Key to the Soil-Inhabiting Mites. Mesostigmata*, Nauka, Leningrad, pp. 169–226. [in Russian]
- Carrillo, D., Peña, J.E. & de Moraes, G.J. (2015) *Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms*. Springer International Publishing, 328 pp.
- Castro-López, M.A. & Martínez-Osorio, J.W. (2021) *Allium cepa* L. responses when *Gaeolaelaps aculeifer* Canestrini and *Parasitus bituberosus* Karg are used to control *Thrips tabaci* Lindeman. *Revista Colombiana de Ciencias Hortícolas*, 15, e11001.
<https://doi.org/10.17584/rcch.2021v15i1.11001>
- Castro-López, M.A., Ramírez-Godoya, A., Osoriob, W.M. & Rueda-Ramírez, D. (2021) Predation and oviposition rates of *Gaeolaelaps aculeifer* and *Parasitus bituberosus* (Acari: Laelapidae and Parasitidae) on pre-pupae/pupae of *Thrips tabaci* (Thysanoptera: Thripidae). *Acarologia*, 61, 394–402.
<https://doi.org/10.24349/acarologia/20214438>
- Chant, D. (1963) The subfamily Blattisocinae Garman (= Aceosejinae Evans) (Acarina: Blattisocidae Garman) (= Aceosejidae Baker and Wharton) in North America, with descriptions of new species. *Canadian Journal of Zoology*, 41, 243–305.
<https://doi.org/10.1139/z63-025>
- Christian, A. & Karg, W. (2006) The predatory mite genus *Lasioseius* Berlese, 1916 (Acari, Gamasina). *Abhandlungen und Berichte des Naturkundemuseums Görlitz*, 77, 99–250.
- Christian, A. & Karg, W. (2008) A revised setal nomenclature based on ontogenetic and phylogenetic characters and universally applicable to the idiosoma of Gamasina (Acari, Parasitiformes). *Soil Organisms*, 80, 45–79.
- Collier, T. & Van Steenwyk, R. (2004) A critical evaluation of augmentative biological control. *Biological Control*, 31, 245–256.
<https://doi.org/10.1016/j.biocontrol.2004.05.001>
- Davis, K.E., Joseph, S.J. & Janssen, P.H. (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Applied and Environmental Microbiology*, 71, 826–834.
<https://doi.org/10.1128/AEM.71.2.826-834.2005>
- de Lillo, E. & Aldini, P. (1994) Contributo alla conoscenza delle parti boccali in femmine di *Typhlodromus exhilaratus* Ragusa (Acari: Phytoseiidae). In: Congresso nazionale italiano di Entomologia, Udine, pp. 287–294.
- de Lillo, E., Di Palma, A. & Nuzzaci, G. (2001) Morphological adaptations of mite chelicerae to different trophic activities (Acari). *Entomologica*, 35, 125–180.
- de Lillo, E., Nuzzaci, G. & Aldini, P. (1996) Fine morphology of the mouthpart sensilla in females of *Typhlodromus exhilaratus* Ragusa (Phytoseiidae). In: *Acarology*, I.X., Mitchell, R.D., Horn, D.J., Needham, G.R. & Welbourn, W.C. (Eds.), Ohio Biology Survey, Columbus, OH, USA, pp. 287–295.
- Dizlek, H., Karagoz, M., Faraji, F. & Cakmak, I. (2019) Mites in dried figs of Turkey: diversity, species composition and density. *Systematic & Applied Acarology*, 24, 992–997.
<https://doi.org/10.11158/saa.24.6.4>
- Dowling, A.P., Bauchan, G.R., Ochoa, R. & Beard, J.J. (2010) Scanning electron microscopy vouchers and genomic data from an individual specimen: maximizing the utility of delicate and rare specimens. *Acarologia*, 50, 479–485.
<https://doi.org/10.1051/acarologia/20101983>
- Dubey, A., Lewis, M.T., Dively, G.P. & Hamby, K.A. (2020) Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation. *Journal of Applied Ecology*, 57, 936–951.
<https://doi.org/10.1111/1365-2664.13595>

- Eliaderani, K.F., Nemati, A. & Kocheili, F. (2013) Some mesostigmatid mites from Iran with their world distribution. *Journal of Crop Protection*, 2, 127–138.
- Erktan, A., Or, D. & Scheu, S. (2020) The physical structure of soil: Determinant and consequence of trophic interactions. *Soil Biology and Biochemistry*, 148, 107876. <https://doi.org/10.1016/j.soilbio.2020.107876>
- European Food Safety Authority (2021) Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances and on the maximum residue level (MRL) application procedure.
- European Union (2021) From farm to fork. Available from https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal/actions-being-taken-eu/farm-fork_en (accessed 1 September 2021).
- Evans, G.O. (1992) *Principles of Acarology*. CAB international, pp. 563.
- Farrier, M. & Hennessey, M. (1993) *Soil-inhabiting and free-living Mesostigmata (Acari-Parasitiformes) from North America: An annotated checklist with bibliography and index*. North Carolina Agricultural Research Service Technical Bulletin, 302, 408 pp.
- Fitzmaurice, G.M., Laird, N.M. & Ware, J.H. (2012) *Applied Longitudinal Analysis*. John Wiley & Sons, 506 pp.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*, 3, 294–299.
- Food and Agriculture Organization (2021) Restrictions and bans. Available from <http://www.fao.org/pesticide-registration-toolkit/tool/page/pret/restrictions-and-bans> (accessed 20 May 2021).
- Fouly, A. (1997) Influence of different nourishment on the biology of *Lasioseius dentatus* (Fox), a new record from Egypt (Acari: Gamasida: Ascidae). *Egyptian Journal of Biological Pest Control*, 7, 1–6.
- Freire, R.A.P., de Moraes, G.J., Silva, E.S., Vaz, A.C. & de Campos Castilho, R. (2007) Biological control of *Bradyzia matogrossensis* (Diptera: Sciaridae) in mushroom cultivation with predatory mites. *Experimental and Applied Acarology*, 42, 87–93. <https://doi.org/10.1007/s10493-007-9075-0>
- Gerson, U. & Weintraub, P. (2007) Mites for control of pests in protected cultivation. *Pest Management Science*, 63, 658–676. <https://doi.org/10.1002/ps.1380>
- Grosman, A.H., Messelink, G.J. & Groot, E.B.D. (2011) Combined use of a mulch layer and the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese) enhance the biological control of sciarids in potted plants. *IOBC/WPRS Bulletin*, 68, 51–54.
- Gupta, S.K. (2003) A monograph on plant inhabiting predatory mites of India. Part II: Order: Mesostigmata. *Memoirs of the Zoological Survey of India*, 20, 1–185.
- Gwiazdowicz, D.J. (2007) *Ascidi mites (Acari, Mesostigmata) from selected forest ecosystems and microhabitats in Poland*. Wydawnictwo Akademii Rolniczej im. Augusta Cieszkowskiego w Poznaniu, 248 pp.
- Gwiazdowicz, D.J., Blaszkak, C. & Ehrnsberger, R. (2008) Die Milben in der Zoologischen Staatssammlung München. Teil 12. Gattungen: *Blattisocius* Keegan, 1944, *Hoploseius* Berlese, 1914, *Lasioseius* Berlese, 1916, *Platyseius* Berlese, 1916. *Spixiana*, 31, 35–39.
- Halliday, R. (2005) Predatory mites from crops and pastures in South Africa: Potential natural enemies of redlegged earth mite *Halotydeus destructor* (Acari: Pentheleidae). *Zootaxa*, 1079, 11–64. <https://doi.org/10.11646/zootaxa.1079.1.2>
- Hebert, P.D., Ratnasingham, S. & de Waard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences*, 270, S96–S99. <https://doi.org/10.1098/rsbl.2003.0025>
- Hennessey, M. & Farrier, M. (1989) Mites of family Parasitidae (Acari: Mesostigmata) inhabiting forest soils of North and South Carolina. *North Carolina Agricultural Research Service, Technical Bulletin*, 291, 1–78.
- Hijbeek, R., Cormont, A., Hazeu, G., Bechini, L., Zavattaro, L., Janssen, B., Werner, M., Schlatter, N., Guzmán, G., Bijttebier, J., Pronk, A.A., van Eupen, M. & van Ittersum, M.K. (2017) Do farmers perceive a deficiency of soil organic matter? A European and farm level analysis. *Ecological Indicators*, 83, 390–403. <https://doi.org/10.1016/j.ecolind.2017.08.023>
- Hirschmann, W. (1962) Gangsystematik der Parasitiformes. Teil 5. Gamasiden Rückenhaarbestimmungsstafeln von 260 *Typhlodromus*-Arten der Erde. Gänge, Chaetotaxie Porotaxie, Mundwerkzeuge von *Typhlodromus* und verwandten Gattungen von *Proctolaelaps*, *Melichares*, *Lasioseius*, *Iphidozercon*, *Sejus*, *Rhodacarellus*, *Rhodacarus*, *Gamasellus*, *Veigaia*, *Macrocheles ivanovi*. Erstversuch der Aufstellung eines Gangsystems der Gamasiden aufgrund der Gnathosomaunterseite. *Acarologie Schriftenreihe für Vergleichende Milbenkunde*, 5,

- Holzmann, C. (1969) Die Familie Parasitidae Oudemans [,] 1901 (eine systematische Studie aus dem Jahre 1955). *Acarologie. Schriftenreihe für Vergleichende Milbenkunde*, 13, 3–55.
- Hrúzová, K. & Fend'a, P. (2018) The family Parasitidae (Acari: Mesostigmata)-history, current problems and challenges. *Acarologia*, 58, 25–42.
<https://doi.org/10.24349/acarologia/20184280>
- Hyatt, K.H. (1980) Mites of the Subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles. *Bulletin of the British Museum of Natural History Zoology*, 38 237–378.
<https://doi.org/10.5962/p.12620>
- Ishikawa, K. (1969) Studies on the mesostigmatid mites in Japan. IV. Family Blattisociidae Garman. *Reports of Research Matsuyama Shinonome Junior College*, 4, 111–139.
- Ivanova, N., DeWaard, J. & Hebert, P. (2007) CCDB protocols, glass fiber plate DNA extraction. Available from ccdb.ca/site/wp-content/uploads/2016/09/CCDB_DNA_Extraction.pdf
- Ivanova, N. & Grainger, C. (2007a) CCDB protocols, sequencing. Available from ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Sequencing.pdf (accessed 12 March 2020).
- Ivanova, N. & Grainger, C. (2007b) COI amplification. Available from ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Amplification.pdf (accessed 12 March 2020).
- Kamali, K., Ostovan, H. & Atamehr, A. (2001) *A catalog of Mites and Ticks (Acari) of Iran*. Islamic Azad University Scientific Publication Center, Tehran, 196 pp.
- Kandil, M. (1980) Three new *Lasioseius* species from Hungary (Acari: Mesostigmata, Podocinidae). *Folia Entomologica Hungarica*, 41, 75–86.
- Karg, W. (1965) Larvalsystematische und phylogenetische Untersuchung sowie Revision des Systems der Gamasina Leach, 1915 (Acarina, Parasitiformes). *Mitteilungen aus dem Zoologischen Museum in Berlin*, 41, 193–340.
<https://doi.org/10.1002/mmnz.19650410207>
- Karg, W. (1971) Acari (Acarina), Milben: Unterordnung Anactinochaeta (Parasitiformes). Die freilebenden Gamasina (Gamasides), Raubmilben. *Die Tierwelt Deutschlands*, 59, 1–475.
- Karg, W. (1980) Die Raubmilbengattung *Lasioseius* Berlese, 1916. *Zoologische Jahrbücher Abteilung für Systematik, Ökologie und Geographie der Tiere*, 107, 344–367.
- Karg, W. (1993) Acari (Acarina), Milben Parasitiformes (Anactinochaeta), Cohors Gamasina Leach, Raubmilben. In: *Die Tierwelt Deutschlands und der angrenzenden Meeresgebiete nach ihren Merkmalen und ihrer Lebensweise*, 59. Überarbeitete Auflage, VEB Gustav Fischer Verlag, Jena, Stuttgart, New York, pp. 523
- Knapp, M., van Houten, Y., van Baal, E. & Groot, T. (2018) Use of predatory mites in commercial biocontrol: current status and future prospects. *Acarologia*, 58, 72–82.
<https://doi.org/10.24349/acarologia/20184275>
- Kontschán, J. (2018) *Macrocheles kekensis* sp. n., a new macrochelid mite associated with a centoniin beetle from Hungary (Acari, Mesostigmata). *ZooKeys*, 768, 97–104.
<https://doi.org/10.3897/zookeys.768.24460>
- Krantz, G. & Ainscough, B. (1990) Acarina: Mesostigmata (Gamasida) In: Dindal, D.L. (Ed.), *Soil Biology Guide*. New York, Wiley-Interscience, pp. 583–666.
- Kuenen, F.J., Venema, H., van Gestel, C.A. & Verhoef, H.A. (2009) Extracting soil microarthropods with olive oil: A novel mechanical extraction method for mesofauna from sandy soils. *European Journal of Soil Biology*, 45, 496–500.
<https://doi.org/10.1016/j.ejsobi.2009.07.001>
- Lakly, M.B. & Crossley, D.A., Jr. (2000) Tullgren extraction of soil mites (Acarina): Effect of refrigeration time on extraction efficiency. *Experimental and Applied Acarology*, 24, 135–140.
<https://doi.org/10.1023/A:1006416731804>
- Lapinia, I. (1976) Gamasoid mites of the family Aceosejidae Baker et Wharton, 1952 in the fauna of the Latvian SSR. *Latvijas entomologs*, 19, 65–90.
- Lee, S. & Lee, W. (1998) Four mites of the genus *Lasioseius* (Acari, Mesostigmata, Ascidae) from Korea. *Korean Journal of Systematic Zoology*, 14, 13–19.
- Lesna, I., Conijn, C.G.M., Sabelis, M.W. & van-Straalen, N.M. (2000) Biological control of the bulb mite, *Rhizoglyphus robini*, by the predatory mite, *Hypoaspis aculeifer*, on lilies: Predator-prey dynamics in the soil, under greenhouse and field conditions. *Biocontrol Science and Technology*, 10, 179–193.
<https://doi.org/10.1080/09583150029314>
- Lindquist, E. (1964) *A Revision of Mites of the Subfamily Blattisociinae (Acarina: Blattisociidae) in America North of Mexico*, p. 413 Vol. Ph.D. University of California, Berkeley.

- Lindquist, E.E. & Evans, G.O. (1965) Taxonomic concepts in the Ascidae, with a modified setal nomenclature for the idiosoma of the Gamasina (Acarina: Mesostigmata). *Memoirs of the Entomological Society of Canada*, 47, 1–64.
<https://doi.org/10.4039/entm9747fv>
- Lindquist, E., Krantz, G. & Walter, D. (2009) Order Mesostigmata. In: Krantz, G. & Walter, D. (Eds.), *A Manual of Acarology*. Lubbock, Texas, Texas Tech University Press, pp. 124–232.
- Macfadyen, A. (1961) Improved funnel-type extractors for soil arthropods. *The Journal of Animal Ecology*, 171–184.
<https://doi.org/10.2307/2120>
- Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A. & Hakeem, K.R. (2016) Effects of pesticides on environment. In: *Plant, Soil and Microbes*. Springer, pp. 253–269.
- Moraes, G.d., Abo-Shnaf, R.I., Perez-Madruga, Y., Sanchez, L., Karmakar, K. & Ho, C.-C. (2015) The *Lasioseius* phytoseioides species group (Acari: Blattisociidae): new characterisation, description of a new species, complementary notes on seven described species and a taxonomic key for the group. *Zootaxa*, 3980, 1–41.
<https://doi.org/https://doi.org/10.11646/zootaxa.3980.1.1>
- Munoz Cardenas, K.A. (2017) What lies beneath? Linking litter and canopy food webs to protect ornamental crops, p. 144. In: Department of Population Biology, Institute for Biodiversity and Ecosystem Dynamics. Vol. PhD. University of Amsterdam, Amsterdam, The Netherlands.
- N'Dri, J.K., Guéi, A.M., Hance, T., Yaco, J.G., Ahui, J.-L.D. & Andrac, H.M. (2018) From the efficiency of Berlese Tullgren funnel to the spatiotemporal variation of two uropodina genera, *Afrotrachytes* Kontschán, 2006 and *Trachyuropoda* Berlese, 1888 (Acari, Mesostigmata) in Côte d'Ivoire. *Journal of Advances in Biology*, 11, 2201–2217.
<https://doi.org/10.24297/jab.v11i1.7470>
- Nasr, A. & Abou-Awad, B. (1986) New species of the genus *Lasioseius* from Egypt (Acari: Ascidae). *Bulletin de la Société Entomologique d'Égypte*, 66, 85–96.
- Nasr, A. & Abou-Awad, B. (1987a) Description of some ascid mites from Egypt (Acari: Ascidae). *Acarologia*, 28, 27–35.
- Nasr, A.K. & Abou-Awad, B.A. (1987b) New species of the genus *Lasioseius* from Egypt (Acari: Ascidae). *Bulletin de la Société Entomologique d'Égypte*, 66, 85–96.
- Navarro-Campos, C., Beltrà, A., Calabuig, A., Garcia-Marí, F., Wäckers, F.L. & Pekas, A. (2020) Augmentative releases of the soil predatory mite *Gaeolaelaps aculeifer* reduce fruit damage caused by an invasive thrips in Mediterranean citrus. *Pest Management Science*, 76, 2500–2504.
<https://doi.org/10.1002/ps.5791>
- Nuzzaci, G. & Di Palma, A. (2002) Mouthparts of a tydeid mite: an ultrastructural and functional investigation. *Entomologica*, 36, 71–91.
- Oldfield, G., Newell, I. & Reed, D. (1972) Insemination of protogynes of *Aculus cornutus* from spermatophores and description of the sperm cell. *Annals of the Entomological Society of America*, 65, 1080–1084.
<https://doi.org/10.1093/aesa/65.5.1080>
- Otto, J. (1999) Revision of the genus *Erythracarus* Berlese (Acarina: Anystidae: Erythracarinae). *Journal of Natural History*, 33, 825–909.
<https://doi.org/10.1080/002229399300146>
- Park, J., Mostafiz, M.M., Hwang, H.-S., Jung, D.-O. & Lee, K.-Y. (2021) Comparison of the predation capacities of two soil-dwelling predatory mites, *Gaeolaelaps aculeifer* and *Stratiolaelaps scimitus* (Acari: Laelapidae), on three thrips species. *Journal of Asia-Pacific Entomology*, 24, 397–401.
<https://doi.org/10.1016/j.aspen.2021.01.009>
- Porco, D., Rougerie, R., Deharveng, L. & Hebert, P. (2010) Coupling non-destructive DNA extraction and voucher retrieval for small soft-bodied Arthropods in a high-throughput context: the example of Collembola. *Molecular Ecology Resources*, 10, 942–945.
<https://doi.org/10.1111/j.1755-0998.2010.2839.x>
- R Core Team (2020) R: A language and environment for statistical computing. Available from <http://www.R-project.org/> (accessed 2020)
- Ratnasingham, S. & Hebert, P.D. (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS One*, 8, e66213.
<https://doi.org/10.1371/journal.pone.0066213>
- Rezende, J., Bauchan, G., Lofego, A., Welbourn, W. & Ochoa, R. (2019) New findings on tarsonemid mites (Prostigmata: Tarsonemidae) under the LT-SEM (Low Temperature Scanning Electron Microscopy)—the case of gen-

- era *Daidalotarsonemus* and *Excelsotarsonemus*. In: Skvarla, M., Ochoa, R., Rodrigues, J. & Hutcheson, H. (Eds.) *Contemporary Acarology*. Cham, Switzerland, Springer Nature AG, pp. 49–66.
- Rueda-Ramírez, D. (2018) Soil mesostigmatid mites (Acari: Mesostigmata) inhabiting rose fields and neighboring vegetation in the Bogota plateau and their potential role as biological control agents of *Frankliniella occidentalis* (Insecta: Thysanoptera). In: *Entomology and Acarology* Vol. PhD. ESALQ- Universidade de São Paulo / Pontificia Universidad Javeriana, Piracicaba, SP, Brazil, p. 237.
- Rueda-Ramírez, D., Mowery, J., Bauchan, G., Ochoa, R., Young, M.R., Cruz, J. & Palevsky, E. (2022) In memory of Gary Bauchan: Utilizing an integrated taxonomy approach for the description of a new species of *Gamaselodes* (Mesostigmata: Ascidae). *Systematic & Applied Acarology*, 27(2), 165–180.
<https://doi.org/10.11158/saa.27.2.2>
- Rueda-Ramírez, D., Varela Ramírez, A., Ebratt Ravelo, E. & de Moraes, G.J. (2021) Edaphic mesostigmatid mites (Acari: Mesostigmata) and thrips (Insecta: Thysanoptera) in rose cultivation and secondary vegetation areas in the Bogotá plateau, Colombia. *International Journal of Acarology*, 47, 8–22.
<https://doi.org/10.1080/01647954.2020.1866666>
- Rueda-Ramírez, D., Rios-Malaver, D., Varela-Ramírez, A. & de Moraes, G.J. (2019) Biology and predation capacity of *Parasitus bituberosus* (Acari: Mesostigmata: Parasitidae) on *Frankliniella occidentalis* (Thysanoptera: Thripidae), and free-living nematodes as its complementary prey. *Pest Management Science*, 75, 1819–1830.
<https://doi.org/10.1002/ps.5326>
- Ryke, P. (1964) Acarina associated with Protea flowers in the Cape Province. *Journal of the Entomological Society of Southern Africa*, 26, 337–354.
- Salehi, A., Ostovan, H. & Modarresi, M. (2014) Evaluation of the efficiency of *Gaeolaelaps aculeifer* in control of plant parasitic nematode *Tylenchulus semipenetrans* under greenhouse conditions. *Journal of Entomology and Nematology*, 6, 150–153.
- Santos, J., Demite, P. & Moraes, G.d. (2021) Ascidae Database. Available from <http://www.lea.esalq.usp.br/acari/ascidae> (accessed 04 April 2021).
- Santos, J.C., Rueda-Ramírez, D., Demite, P.R. & De Moraes, G.J. (2018) Ascidae, Blattisociidae and Melicharidae (Acari: Mesostigmata): Zoogeographic analyses based on newly available databases. *Zootaxa*, 4377, 542–564.
<https://doi.org/10.11646/zootaxa.4377.4.4>
- Schaub, S., Huber, R. & Finger, R. (2020) Tracking societal concerns on pesticides—a Google Trends analysis. *Environmental Research Letters*, 15, 084049.
<https://doi.org/10.1088/1748-9326/ab9af5>
- Søvik, G. & Leinaas, H.P. (2002) Variation in extraction efficiency between juvenile and adult oribatid mites: *Ameronothrus lineatus* (Oribatida, Acari) in a Macfadyen high-gradient canister extractor. *Pedobiologia*, 46, 34–41.
<https://doi.org/10.1078/0031-4056-00111>
- Talarico, G., Palacios-Vargas, J.G. & Alberti, G. (2007) Taste while chewing? Sensory structures in the chelicerae of *Pseudocellus pearsei* (Chamberlin & Ivie, 1938)(Ricinulei, Arachnida). *Revista Ibérica de Aracnología*, 15, 47–53.
- Tavoosi Ajvad, F., Madadi, H., Michaud, J.P., Zafari, D. & Khanjani, M. (2020) Combined applications of an entomopathogenic fungus and a predatory mite to control fungus gnats (Diptera: Sciaridae) in mushroom production. *Biological Control*, 141, 104101.
<https://doi.org/10.1016/j.biocontrol.2019.104101>
- Tichomirov, S. (1977) Fam. Parasitidae Oudemans, 1901. In: Ghilarov, M.S. (Ed.) *Handbook for the Identification of SoilInhabiting Mites. Mesostigmata. I*. Zoological Institute of the Academy of Sciences, USSR, Nauka, Leningrad, pp. 55–107.
- Tichomirov, S. (1971) Genus *Parasitus* (Gamasoidea, Parasitidae). I. The subgenus *Neogamasus*. *Zoologicheskii Zhurnal*, 50, 803–816.
- Treonis, A.M., Unangst, S.K., Kepler, R.M., Buyer, J.S., Cavigelli, M.A., Mirsky, S.B. & Maul, J.E. (2018) Characterization of soil nematode communities in three cropping systems through morphological and DNA metabarcoding approaches. *Scientific Reports*, 8, 2004.
<https://doi.org/10.1038/s41598-018-20366-5>
- Tseng, Y. (1978) The mites family Ascidae of Taiwan (Acarna: Mesostigmata). Part I. Genus *Lasioseius* Berlese. *Plant Protection Bulletin*, 20, 117–132.
- Tseng, Y.H. (1982) *A vatalogue and bibliography of Acari of Taiwan (Arachnida: Acarina)*. Tainan Branch Office, Bureau of Commodity Inspection and Quarantine, Ministry of Economic Affairs, Tainan, 164 pp.
- United States Environmental Protection Agency. (2021) Reregistration and Other Review Programs Predating Pesticide Registration Review. Available from <https://www.epa.gov/pesticide-reevaluation/reregistration-and-other>

- review-programs-predating-pesticide-registration (accessed 20 May 2021).
- Vänninen, I. & Koskula, H. (2004) Biocontrol of the shore fly *Scatella tenuicosta* with *Hypoaspis miles* and *H. aculeifer* in peat pots. *BioControl*, 49, 137–152.
- Walter, D.E. (1988) Nematophagy by soil arthropods from the shortgrass steppe, Chihuahuan desert and Rocky Mountains of the central United States. *Agriculture, Ecosystems & Environment*, 24, 307–316.
[https://doi.org/10.1016/0167-8809\(88\)90074-6](https://doi.org/10.1016/0167-8809(88)90074-6)
- Walter, D.E. & Campbell, N.J. (2003) Exotic vs endemic biocontrol agents: would the real *Stratiolaelaps miles* (Berlese)(Acari: Mesostigmata: Laelapidae), please stand up? *Biological Control*, 26, 253–269.
[https://doi.org/10.1016/S1049-9644\(02\)00171-8](https://doi.org/10.1016/S1049-9644(02)00171-8)
- Walter, D.E. & Lindquist, E.E. (1989) Life history and behavior of mites in the genus *Lasioseius* (Acari: Mesostigmata: Ascidae) from grassland soils in Colorado, with taxonomic notes and description of a new species. *Canadian Journal of Zoology*, 67, 2797–2813.
<https://doi.org/10.1139/z89-396>
- Welbourn, W.C., Ochoa, R., Kane, E.C. & Erbe, E.F. (2003) Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. *Experimental and Applied Acarology*, 30, 107–133.
<https://doi.org/10.1023/B:APPA.0000006545.40017.a0>
- Westerboer, I. (1963) Die Familie Podocinidae Berlese 1916. In: Stammer, H.J. (Ed.) *Beiträge zur Systematik und Ökologie Mitteleuropäischer Acarina. Band II, Mesostigmata, Abschnitt IV*. Akademische Verlagsgesellschaft Geest & Portig K.–G., Leipzig, pp. 179–450.
- Witalinski, W. (1980) Fine structure of the respiratory system in mites from the family Parasitidae (Acari, Mesostigmata). *Acarologia*, 21, 330–339.
- Witalinski, W. & Borsuk, K. (2002) Endogynium types in Parasitidae as revealed by SEM (Acari: Gamasida: Parasitina). In: *Acarid Phylogeny and Evolution: Adaptation in Mites and Ticks*. Springer, 153–168.
https://doi.org/10.1007/978-94-017-0611-7_16
- Yang, S.-H., Wang, D., Chen, C., Xu, C.-L. & Xie, H. (2020) Evaluation of *Stratiolaelaps scimitus* (Acari: Laelapidae) for controlling the root-knot nematode, *Meloidogyne incognita* (Tylenchida: Heteroderidae). *Scientific Reports*, 10, 1–8.
<https://doi.org/10.1038/s41598-020-62643-2>
- Young, M.R., Moraza, M.L., Ueckermann, E., Heylen, D., Baardsen, L.F., Lima-Barbero, J.F., Gal, S., Gavish-Regev, E., Gottlieb, Y., Roy, L., Recht, E., El Adouzi, M. & Palevsky, E. (2019) Linking morphological and molecular taxonomy for the identification of poultry house, soil, and nest dwelling mites in the Western Palearctic. *Scientific Reports*, 9, 5784.
<https://doi.org/10.1038/s41598-019-41958-9>
- Zaher, M. (1986) *Predaceous & nonphytophagous mites in Egypt*. Cairo University, Faculty of Agriculture, Cairo, Egypt, 567 pp.
- Zhang, D. & Davidian, M. (2001) Linear mixed models with flexible distributions of random effects for longitudinal data. *Biometrics*, 57, 795–802.
<https://doi.org/10.1111/j.0006-341X.2001.00795.x>
- Zhang, Z.-Q. & Fan, Q.-H. (2010) Blattisociidae of China: a review, with a checklist. *Zoosymposia*, 4, 280–287.
<https://doi.org/10.11646/zoosymposia.4.1.18>

Submitted: 10 Sep. 2021; accepted by Antonella DiPalma: 21 Dec. 2021; published: 12 Jan. 2022