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Article

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

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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; Tavoosi 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 °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 °C ± 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 °C ± 2.0 and 56 ± 10% RH, respectively.

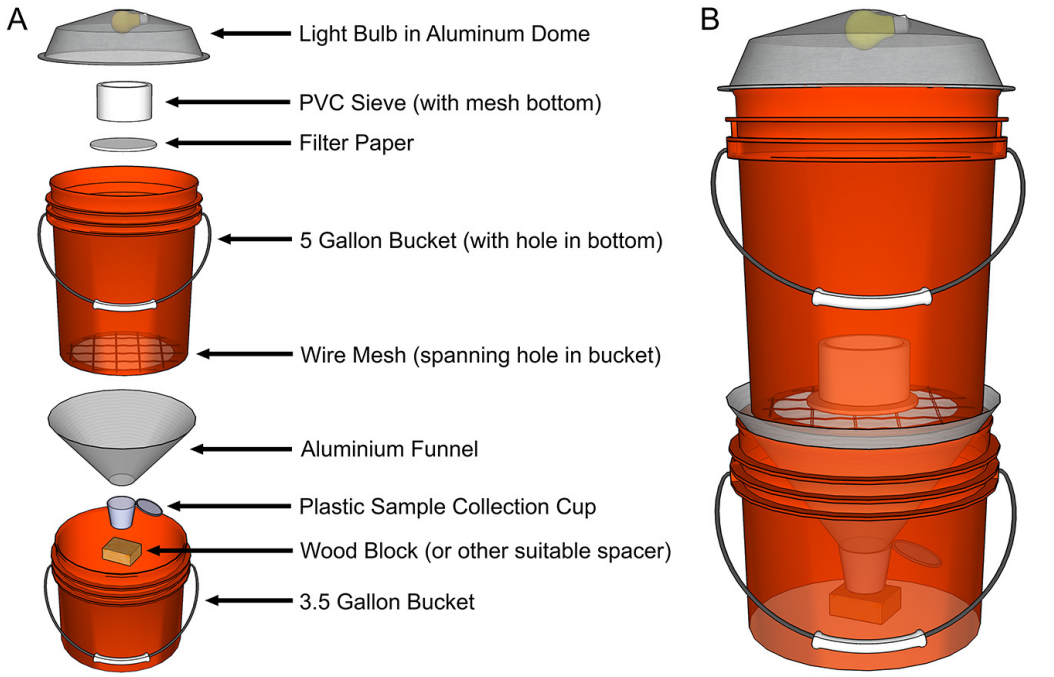

FIGURE 1. Galen-Berlese Funnel: a modified Berlese funnel for the collection of soil mites at optimal temperature and humidity levels utilizing off-the-shelf components, a key feature being a mild heat source which is sufficiently separated from the sample to prevent overheating and killing the mites 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 ± 0.1 C°, 70 ± 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 uL 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 Ratnasignham 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), Hrúzová and Fenďa (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 °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 °C for 10–15 minutes. Following etching, the temperature inside the chamber was lowered below -130 °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 °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 (± SE) species richness of Mesostigmata and mean sample (± 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 ± 1.3 a	5.5 ± 2.3 a	3 ± 1.8 a
Abundance of Mesostigmata immatures	1.9 ± 0.7 a	2.4 ± 0.9 a	1 ± 0.6 a
Total abundance of Mesostigmata	5.8 ± 1.9 a	8.4 ± 3.5 a	4.2 ± 2.4 a
Number of Mesostigmata species	1.3 ± 0.4 a	1.8 ± 0.3 a	1 ± 0 a
Abundance of *Lasioseius youcefi*	2 ± 1.1 a	2 ± 1.1 a	2.3 ± 1.7 a
Abundance of Oribatida [excluding Astigmatina] immatures	2.8 ± 1.1 a	11.8 ± 5.5 a	5.5 ± 3.4 a
Abundance of total Oribatida [excluding Astigmatina]	10 ± 2.1 a	19.7 ± 5.3 a	10.8 ± 3.9 a
Abundance of total Astigmatina	48.5 ± 32.6 a	42 ± 22.7 a	19.4 ± 9.7 a

TABLE 3. Mean sample (± 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 ± 3.7 a	6.2 ± 2 a	4.6 ± 1.9 a
Abundance of Mesostigmata immatures	2.8 ± 1.2 a	4.1 ± 1.4 a	1.4 ± 0.4 a
Total abundance of Mesostigmata	14.2 ± 5.2 a	10.8 ± 3.2 a	6.3 ± 2.3 a
Abundance of Oribatida [excluding Astigmatina] immatures	16.7 ± 7.3 a	11.8 ± 2.7 a	11 ± 3.8 a
Abundance of total Oribatida [excluding Astigmatina]	22.8 ± 8.7 a	17.8 ± 2.8 a	15.5 ± 4 a
Abundance of total Astigmatina	125.1 ± 59.9 a	83.1 ± 30.2 a	9.8 ± 6.2 a
Abundance of fungal feeder nematodes	2400 ± 823.9 a	1021.7 ± 279.8 a	1316.7 ± 258.7 a
Abundance of bacterial feeder nematodes	23200 ± 7974 a	8710 ± 1541.2 ab	5076.7 ± 1043.2 b
Abundance of plant parasitic nematodes	271.7 ± 149.7 a	166.7 ± 91.6 a	433.3 ± 258 a

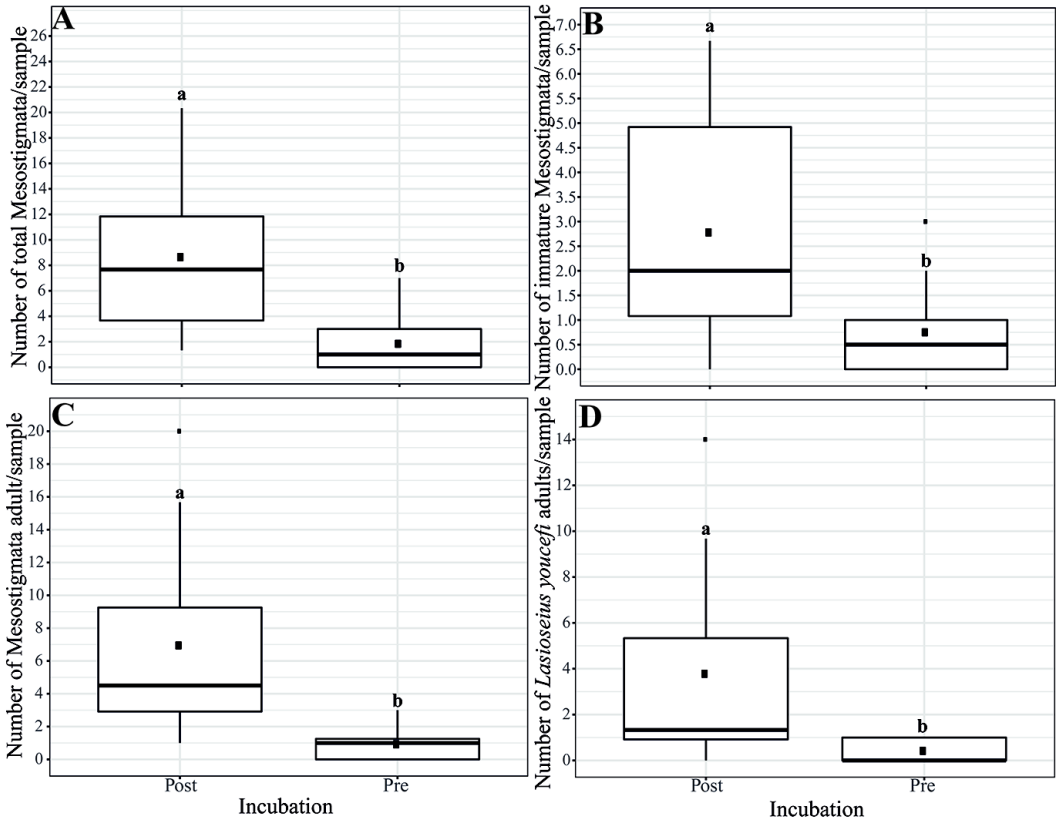

FIGURE 2. Mean number of individuals (± SE) extracted before (Pre) and after (Post) incubation of samples collected in three farming systems (no till, organic and till). (A) Total soil Mesostigmata mites. (B) Immatures of soil Mesostigmata mites. (C) Adults of soil Mesostigmata mites. (D) Females of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

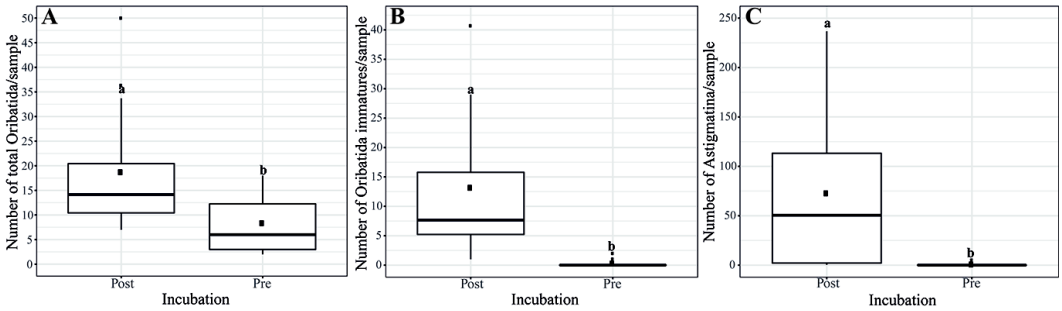

FIGURE 3. Mean number of individuals (± SE) extracted before (Pre) and after incubation of samples collected in three farming systems (no till, organic and till). (A) Total soil Oribatida (excluding Astigmatina) mites. (B) Immatures of soil Oribatida mites. (C) Total soil Astigmatina.

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 (χ^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 (± 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 ± 1.9 a	9.9 ± 3.3 a	5.3 ± 2.6 a
Abundance of Mesostigmata immature	2.5 ± 1.4 a	4.3 ± 1.2 a	1.6 ± 0.7 a
Total abundance of Mesostigmata	9 ± 3 a	15.3 ± 4.9 a	6.9 ± 3 a
Abundance of Oribatida [excluding Astigmatina] immatures	5.2 ± 1.8 b	23.3 ± 6.8 a	11 ± 3.3 ab
Abundance of total Oribatida [excluding Astigmatina]	10.3 ± 2.3 a	28.6 ± 8 a	17.2 ± 4.1 a
Abundance of total Astigmatina	97 ± 50.6 a	83.4 ± 46.2 a	37.5 ± 17 a
Abundance of fungal feeder nematodes	966.7 ± 447.1 a	1516.7 ± 485.8 a	2255 ± 638.6 a
Abundance of bacterial feeder nematodes	10526.7 ± 3350.6 a	10566.7 ± 2911.8 a	15893.3 ± 7894.8 a
Abundance of plant parasitic nematodes	505 ± 264.9 a	300 ± 140.3 a	66.7 ± 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 faming 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 *Scheloribates* sp. nr. *pallidulus* 3 in all three faming 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 6. Summary of Mesostigmata species and their abundance in soil samples collected from fields with different farming systems (Till, No-Till and Organic treatments), including values for pre- and post-incubation treatment. N-T: No-Till farming system; O: Organic farming system; T: Till farming system; A: Alfalfa amendment; B: Bean amendment; C: Control (without amendment).

Life stage	Family	Species	Pre-incubation			Post-incubation									Total
			N-T	O	T	N-T			O			T			
						A	B	C	A	B	C	A	B	C	
Adult	Ascidae	*Asca garmani* Hurlbutt, 1963†											1	1	2
		Gamasellodes n.sp.*ǂ†§				1	4	5	9	3	24			1	47
		Gamasellodes sp.1*†							1		1				2
		Gamasellodes sp.2*							1						1
		Neojordensia levis (Oudemans & Voigts, 1904)*ǂ†§										1			1
	Blattisociidae	*Lasioseius youcefi* Athias-Henriot, 1959 *†§	2	2	1	29	6	7	31	7	5	33	15	3	141
		Unidentified ●			1										1
		Lasioseius sp. 1	1		1										2
	Laelapidae	*Gaeolaelaps* sp. 1*†						1	1		2	1	5	1	11
		Pseudoparasitus sp. 1						1							1
		Stratiolaelaps scimitus (Womersley, 1956)					1								1
		Unidentified ●		1											1
	Macrochelidae	*Macrocheles kekensis* Kontschán, 2018*ǂ†§									1				1
	Ologamasidae	*Gamasiphis* sp. 1				1							1		2
		Unidentified ●*†	1					1	1						3
	Parasitidae	Cycetogamasus *diviortus* (Athias-Henriot, 1967)ǂ§		1		1	10		9	21	1				43
		Immature	5	2	2	4	22	4	27	16	8	3	11	5	109
		Unidentified●	1	0	1	5	5	0	11	0	4	0	1	0	28
		Total	10	6	6	41	48	19	91	47	46	38	34	11	397

* BIN new to BOLD; ǂ Taxon new to BOLD
† BIN new to GenBank; § Taxon new to GenBank
● Specimens could not be identified to family or genus because important characters were damaged or observed.

Cycetogamasus diviortus (Athias-Henriot, 1967)

Pergamasus diviortus Athias-Henriot, 1967: 23

Parasitus (*Neogamasus*) *diviortus*.—Tichomirov, 1971: 804; Tichomirov, 1977: 75, 77; Holzmann, 1969: 11, 20 (Synonymy with *Eugamasus insignis* Holzmann, 1969); Karg, 1971: 428, 439 (Synonymy with *Parasitus insignis*); Hyatt, 1980: 279 (Synonymy with *P. insignis*).

Cycetogamasus diviortus.—Athias-Henriot, 1980: 285, 289, 290, 294; Hennessey & Farrier, 1989: 9.

Specimens examined: 17 females and 10 males from soil of the Beltsville Agricultural Research Center (BARC), Maryland, USA (all of them with barcode compliant sequences, six females and two females also with LTSEM images).

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	*Galumna jacoti* Wharton, 1938*⸸†§			x
	Galumna minuta (Ewing, 1909)*⸸†§	x		
	Galumna virginiensis Jacot, 1929*⸸†§	x	x	x
	Unidentified●	x		
Oppiidae	*Oppiella nova* (Oudemans, 1902)		x	
Oribatulidae	*Zygoribatula undulata* Berlese, 1916*⸸†§		x	
Scheloribatidae	*Scheloribates* sp. nr. *pallidulus* 1 (Koch, 1841)*†	x		
	Scheloribates sp. nr. *pallidulus* 2 (Koch, 1841)*†		x	
	Scheloribates sp. nr. *pallidulus* 3 (Koch, 1841)*†	x	x	x
	Scheloribates sp. nr. *pallidulus* 4 (Koch, 1841)*†		x	x
Tectocepheidae	*Tectocepheus sarekensis* Trägårdh, 1910*†	x		
	Tectocepheus velatus (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, *h*3 about in longitudinal line with *h*1 and transversal of *h*2 (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).

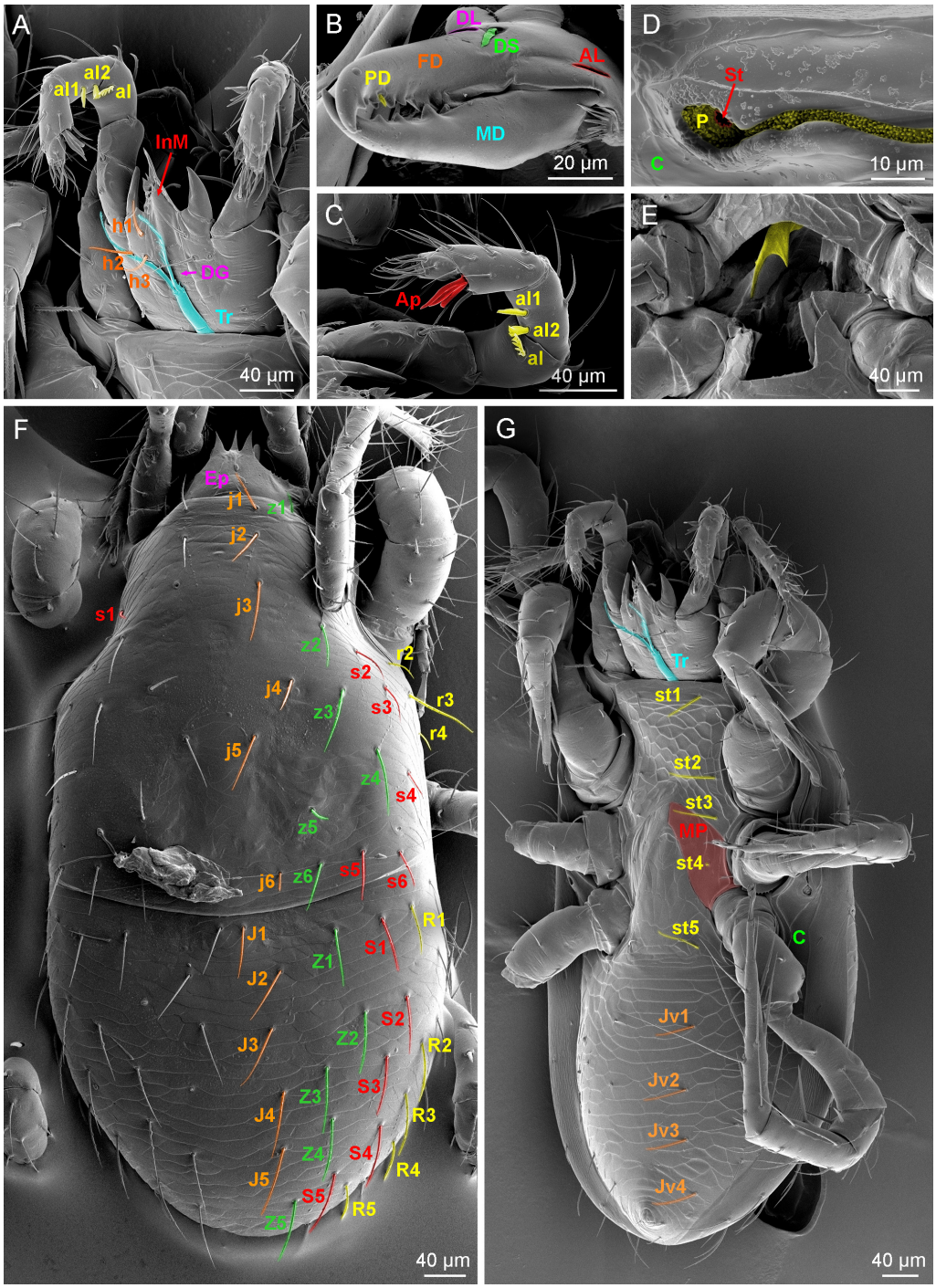

FIGURE 4. *Cycetogamasus diviortus* (Athias-Henriot, 1967) female, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Ventral view of gnathosoma. (B) Antiaxial view of chelicera. (C) Palp. (D) Posterior view of peritreme and stigma. (E) Anterior part of endogynum with two spinate processes (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.

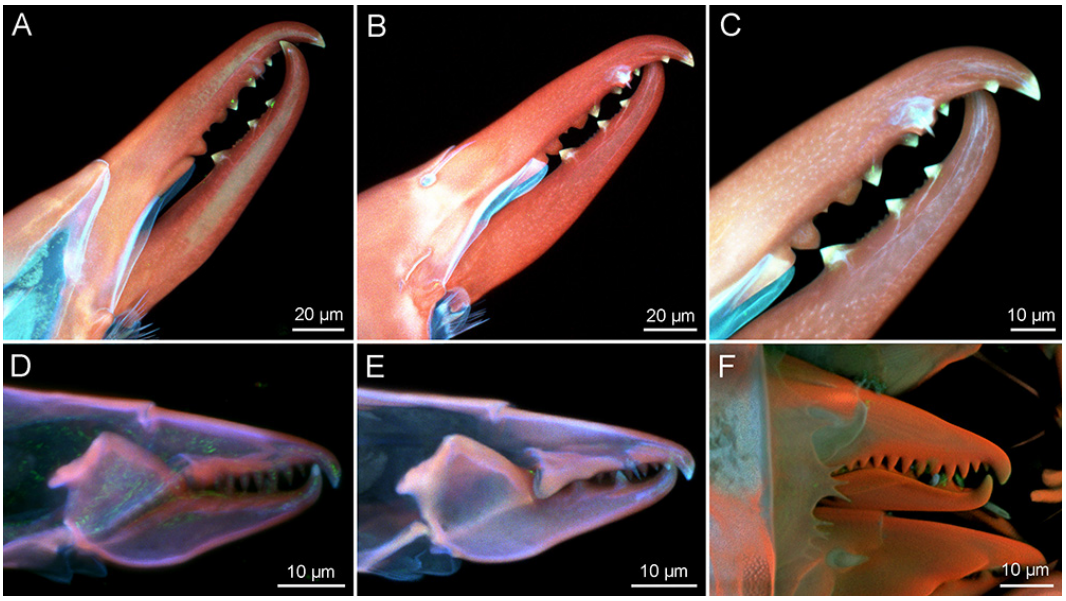

FIGURE 5. Confocal Laser Scanning Microscopy of the chelicera. (A-C) *Cycetogamasus diviortus* female (innervation white-blue with dendritic terminations green at the tip of the digits, the teeth, and the pilus dentilis), (A) adaxial profile, (B) abaxial profile, (C) abaxial profile. (D–F) *Lasioseius 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 (*al1*, *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; *h*3 about in longitudinal line with *h*1, mesad and slightly posterior to *h*2 (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).

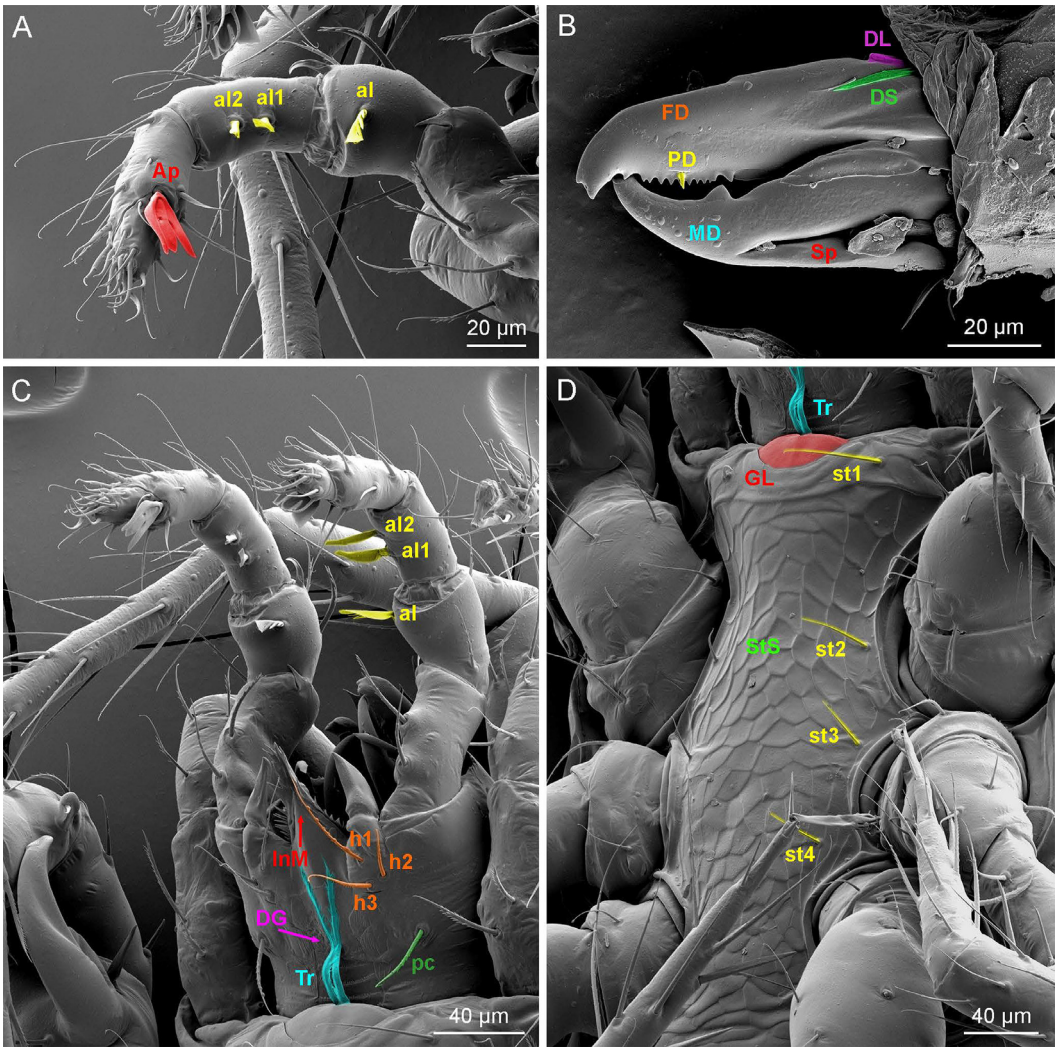

FIGURE 6. *Cycetogamasus diviortus (Athias-Henriot, 1967)* male, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Palp. (B) Antiaxial view of chelicera. (C) Ventral view of gnathosoma. (D) Anterior view of ventral idiosoma. Ap = apotele, FD = fixed digit, MD = movable digit, DL = dorsal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

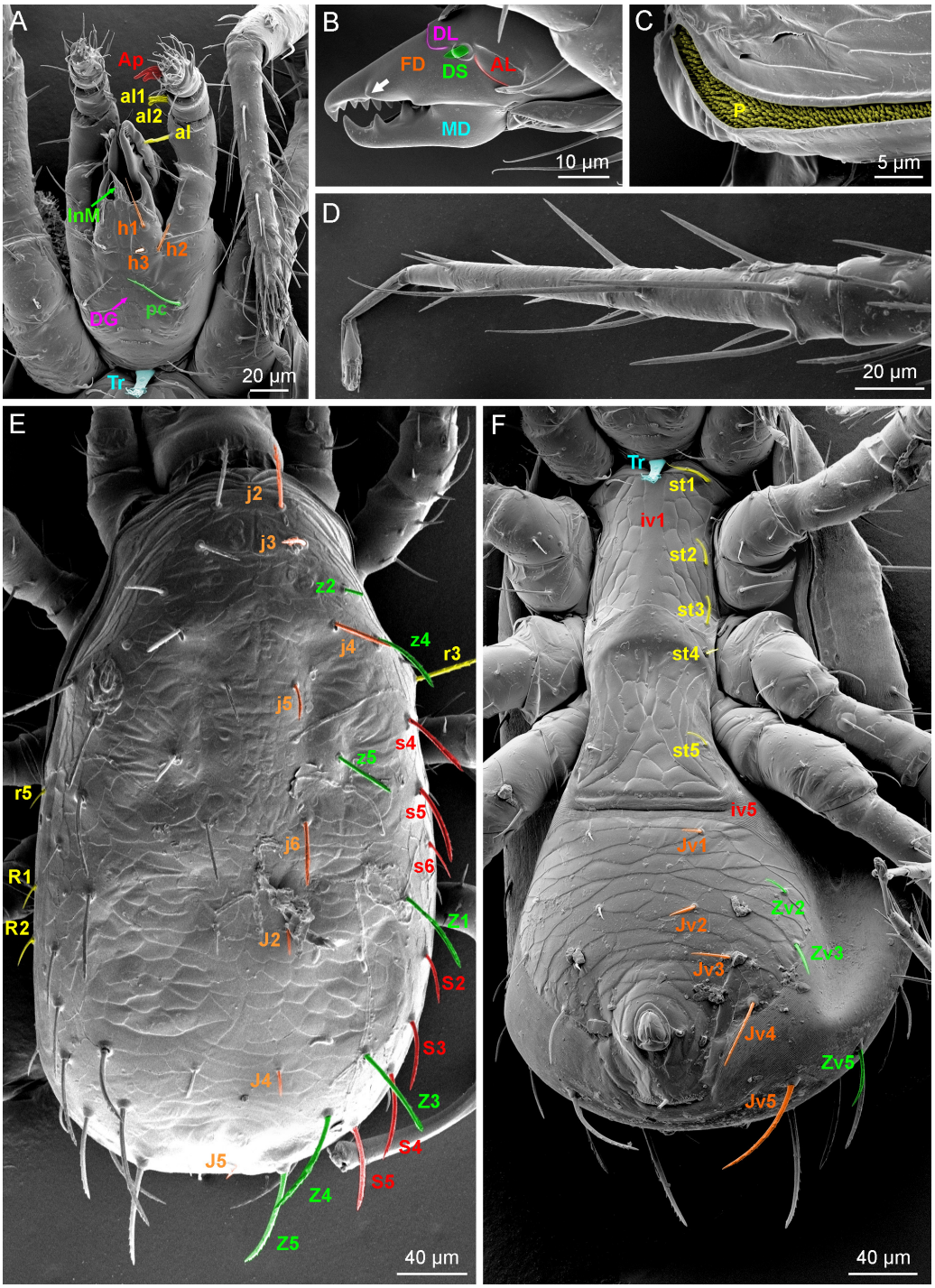

FIGURE 7. *Lasioseius youcefi* Athias-Henriot (1959) female, Low Temperature Scanning Electron Microscope (LTSEM) images. (A) Ventral view of gnathosoma. (B) Antiaxial view of chelicera. (C) Anterior part of peritreme. (D) Tarsus IV. (E) Dorsal idiosoma. (F) Ventral idiosoma. Ap = apotele, InM = internal 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 *al*2 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, *h*3 about in longitudinal line with *h*1 and mesad and slightly posteriad of *h*2, 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 *j*2, 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 *j*2 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 (*ad*2), basitarsus (*pd*3) and telotarsus (*pd*2) (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 bacteriovorus 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 (≤ 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 cetoniin 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).

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