The Journal of Eukaryotic Microbiology

ISOP Published by the International Society of Protistologists

Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

Morphology and Phylogenetic Position of Two New Gregarine Species (Apicomplexa: Eugregarinorida) Parasitizing the Lubber Grasshopper *Taeniopoda centurio* (Drury, 1770) (Insecta: Orthoptera: Romaleidae) in Mexico

Jorge Humberto Medina-Durán[a,b], Rosaura Mayén-Estrada[b], Ricardo Mariño-Pérez[c] & Hojun Song[c]

a Posgrado en Ciencias Biológicas, Edificio D, 1˚ piso, Circuito de Posgrados, Universidad Nacional Autónoma de México, Ciudad Universitaria, Z. C. 04510, Coyoacán, Ciudad de México, México

b Laboratorio de Protozoología, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Av. Universidad 3000, Circuito Exterior S/N, Ciudad Universitaria, 04510, Coyoacán, Ciudad de México, México

c Department of Entomology, Texas A&M University, 2475 TAMU, 77843-2475, College Station, Texas, USA

Keywords

Amoebogregarina taeniopoda; apicomplexan parasites; eugregarines; Hidalgo State; insect parasites; *Quadruspinospora mexicana*; *SSU* rDNA phylogeny.

Correspondence

J.H. Medina-Durán, Laboratorio de Protozoología, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Av. Universidad 3000, Circuito Exterior S/N, Ciudad Universitaria, 04510, Coyoacán, Ciudad de México, Mexico

Telephone number: +(52)56224924;

FAX number: +(525)56224828;

e-mail: jorgemedinad@ciencias.unam.mx

Received: 23 November 2018; revised 21 May 2019; accepted June 12, 2019.

doi:10.1111/jeu.12748

ABSTRACT

Eugregarines are understudied apicomplexan parasites of invertebrates inhabiting marine, freshwater, and terrestrial environments. Most currently known terrestrial eugregarines have been described parasitizing the gut from less than 1% of total insect diversity, with a high likelihood that the remaining insect species are infected. Eugregarine diversity in orthopterans (grasshoppers, locusts, katydids, and crickets) is still little known. We carried out a survey of the eugregarines parasitizing the Mexican lubber grasshopper, *Taeniopoda centurio*, an endemic species to the northwest of Mexico. We described two new eugregarine species from the gut of the host: *Amoebogregarina taeniopoda* n. sp. and *Quadruspinospora mexicana* n. sp. Both species are morphologically dissimilar in their life-cycle stages. Our SSU rDNA phylogenetic analysis showed that both species are phylogenetically distant to each other, even though they parasitize the same host. *Amoebogregarina taeniopoda* n. sp. clustered within the clade Gregarinoidea, being closely related to *Amoebogregarina nigra* from the grasshopper *Melanoplus differentialis*. *Quadruspinospora mexicana* n. sp. clustered within the clade Actinocephaloidea and grouped with *Prismatospora evansi*, a parasite from dragonfly naiads. *Amoebogregarina taeniopoda* n. sp. and *Q. mexicana* n. sp. represent the first record of eugregarines found to infect a species of the family Romaleidae.

GREGARINES are a group of unicellular eukaryotes that parasitize mainly the digestive tracts of invertebrates from marine, freshwater, and terrestrial habitats (Schrével and Desportes 2013). Even though gregarines are regarded as one of the most diverse and early branching groups of the Apicomplexa, their diversity is far from being completely known (Clopton 2002; Rueckert and Horák 2017; Schrével and Desportes 2013). Modern gregarine taxonomic arrangements are not fully understood because they are currently based on scarce molecular phylogenetic data mainly from small subunit rDNA (SSU rDNA), where its evolutionary rates are the most disparate of any eukaryote group (Cavalier-Smith 2014). In addition, recent molecular phylogenetic analyses based on available molecular data are unable to resolve many of the nodes that connect lineages of gregarines, complicating the establishment of their relationships (Cavalier-Smith 2014; Rueckert et al. 2015; Simdyanov et al. 2017). In the most recent classification of eukaryotes (Adl et al. 2019), three gregarine groups are recognized: archigregarines, eugregarines, and neogregarines. However, some other recent classification schemes based mainly on molecular data only recognize the archigregarines

and eugregarines, placing neogregarines within the eugregarines (Cavalier-Smith 2014; Simdyanov et al. 2017). So far, the phylogenetic relationships of the gregarines are very sensitive to taxon sampling, changes in the alignment, and models used, which leaves the deeper relationships amongst eugregarine clades still unresolved, therefore, also leaves the current organization of gregarine groups with a level of uncertainty (Simdyanov et al. 2017).

Eugregarines (Eugregarinorida Léger, 1900) are characterized by having large extracellular trophozoites morphologically different from the sporozoites. The trophozoites possess an attachment region, which is mostly absent in mature gamonts, known as the mucron in aseptate forms and the epimerite in septate ones. Movement typically occurs by gliding locomotion that is thought to be facilitated by longitudinal pellicular folds, 12 nm apical filaments, and cytoskeletal elements (Scherével and Desportes 2013; Simdyanov et al. 2017). Traditionally, eugregarines have been divided into two groups: aseptate and septate gregarines, based on the presence of a septum. However, recent phylogenetic analyses (Cavalier-Smith 2014; Rueckert et al. 2011; Simdyanov et al. 2017) have questioned if the septum is a reliable character for taxonomic division. In terrestrial habitats, eugregarines are frequent parasites in the gut of arthropods, with insects being the most common hosts infected. Levine (1988) mentioned that of the approximately 686,000 named species of insects at the time, there were only about 2,200 of them with reports of gregarines. By now, there are about one million described insect species (Stork 2018), but the number of insects with reports of eugregarines has remained almost the same.

The descriptions of eugregarines in insects are currently biased toward the species within Odonata, Coleoptera, and Diptera. In contrast, other common insect orders such as Orthoptera include fewer reports of their eugregarine parasites (Desportes 2013). Orthopterans (grasshoppers, locusts, katydids, and crickets) are a diverse group of insects with many economically important species due to their potential as a food source or as plagues in agricultural areas (Fontana et al. 2008). For example, species of the grasshopper genus *Sphenarium* are important elements in the diet of Mexican people since pre-Columbian times and, at the same time, some species are known to be serious crop pests in central Mexico of corn (*Zea mays*) and beans (*Phaseolus vulgaris*), both fundamental elements of the Mexican diet (Cerritos and Cano-Santanta 2008; Sanabria-Urbán et al. 2017). As an attempt to contribute to the knowledge of the diversity of eugregarines in orthopterans, we have carried out a survey of the eugregarines that parasitize the Mexican lubber grasshopper *Taeniopoda centurio* (Drury, 1770) (Romaleidae), which is a common species in the northeastern mountain ranges of Mexico. In some areas, an increase in individuals where food is not a limiting resource for the grasshoppers can cause plague, but can also provide utility as a food resource as well as be used for traditional medicine (De Jesús-Bonilla et al. 2017, 2019; Mariño-Pérez et al. 2011).

The main objective of this study is to describe two new gregarine species *Amoebogregarina taeniopoda* n. sp. and *Quadruspinospora mexicana* n. sp. parasitizing the gut of *T. centurio*. We studied the life cycle, morphology, ultrastructure, and phylogenetic position based on the SSU rDNA of the two new eugregarine species and compared their general morphology with closely related species. Our findings represent the first record of eugregarines for orthopterans from Mexico.

MATERIALS AND METHODS

Sample collection and isolation

Adults of the Mexican lubber grasshopper, *T. centurio* (Drury, 1770) (Orthoptera: Romaleidae), were collected manually or with a sweep net during the months of September–November 2017, obtaining 30 individuals each month, from croplands surrounded by cloud forest (20°59′32.99″N, 98°39′34.73″W) in the municipality of Tlanchinol, Hidalgo State, Mexico, at 1,526 masl. They were identified to species level using De Jesús-Bonilla et al. (2017). Specimens were transported alive and decapitated prior to dissection. After dissection, the alimentary canal of each host was removed with fine forceps into insect muscle solution (Belton and Grundfest 1962; Clopton and Lucarotti 1997), and the different life-cycle stages of the eugregarines were identified and isolated with a fine glass pipette under a Nikon SMZ800 (Nikon Corporation, Tokyo, Japan) stereomicroscope.

Light and scanning electron microscopy

Eugregarines were isolated with a fine glass pipette from a well dish and observed with a Nikon Labophot-2 differential interphase contrast (DIC) microscope with a Nikon Digital Sight DS2Mv (Nikon Corporation) adapted camera for live observation and microphotographic records of the cells. We measured the width and length of each compartment in trophozoites and gamonts, and the total width and length in gametocysts and oocysts. Morphological identification was based on Clopton (2002, 2004) and Desportes (2013), and we followed the taxonomic arrangement proposed by Simdyanov et al. (2017).

For Scanning Electron Microscopy (SEM), the digestive tracts of infected hosts were fixed in 2.5% glutaraldehyde 0.1 M Na-cacodylate buffer-0.1 M sucrose pH 7.4. Trophozoites, gamonts, and syzygies from each eugregarine species were released by gentle agitation of the gut tissue. Eugregarine cells were then isolated with fine glass pipettes and transferred to fresh fixative for 3 h, then cells were placed for 15 min in 0.1 M Na-cacodylate buffer without sucrose three times to remove the excess fixative, and stored in the same buffer for 24 h. Cells were dehydrated in a graded series of ethanol for 15 min at 10%, 30%, 40%, 50%, and 70%, and stored at 4 °C. Cells were transferred to absolute ethanol and critical point dried with CO_2. Cells were mounted with adhesive carbon tape on aluminum stubs and sputter-coated with gold. SEM stubs were observed with a Hitachi Model SUI510 (Hitachi Ltd., Tokyo, Japan) microscope. All SEM

images were presented on a black background using Adobe Photoshop CC (Adobe Systems Incorporated, San Jose, CA).

Parasitological observations

We calculated prevalence, defined as the percentage of the number of hosts infected with at least one cell of a parasite species; intensity, defined as the number of individuals of a particular parasite species in a single infected host; and mean intensity, defined as the average intensity of a particular species of parasite among the infected members of a particular host species (Bush et al. 1997). Prevalence of infection was calculated for the total number of hosts and sexes. Intensity of infection was calculated for the total number of infected hosts and sexes. Both prevalence and intensity values were estimated considering all life-cycle stages of the gregarines with the exception of the sporozoites because they were small and might be overlooked. Sex bias on the prevalence of the gregarines was calculated using Fisher Exact Test using R Studio 1.1.419 (RStudio Inc., Boston, MA).

DNA extraction and PCR amplification

For each of the two gregarine morphospecies, we isolated 100 cells (gamonts and syzygies). To avoid contamination of the isolates with a different gregarine species, the cells were pooled one by one based on its morphology with the help of a micropipette under a stereoscope Nikon SMZ800. Cells were then fixed with 100% ethanol and stored in 1.5 ml centrifuge tubes at −20 °C. Genomic DNA was extracted using the Gentra Puregene mouse tail kit (Qiagen, Valencia, CA). The quality and concentration of DNA extracts were measured using a DeNovix Spectrophotometer (Wilmington, DE). For PCR amplification of the SSU rDNA, two universal outside primers, F1 (5′-GCGCT ACCTGGTTGATCCTGCC-3′), and R1 (5′-GATCCTTCTGCA GGTTCACCTAC-3′) (Leander et al. 2003) were used in 50 μl PCR reactions using Invitrogen Platinum Taq Master Mix (ThermoFisher Scientific, Waltham, MA). The BioRad T100 thermal cycler was used for the PCR, and the following PCR protocol was used: initial denaturation at 94 °C for 2 min, followed by 34 cycles at 94 °C for 45 s, 45 °C for 45 s, and 72 °C for 2 min, and final extension at 72 °C for 5 min. Polymerase chain reaction products were cleaned with USB ExoSAP-IT PCR (Life Technologies Corporation, Carlsbad, CA) and then Sanger sequencing was performed at the University of Arizona Genetics Core using primers F1, R1, and the internal primers F2 (5′-AAGTCTGGTGCCAG-CAGCC-3′), R2 (5′-CGCAAGGCTGAAACTTAAA-3′), WSSU5-5 (5′-AACTTAAAGGAATTGACGGAAG-3′), and WSSU2-3 (5′-GCAAGTCTGGTGCCAGCAGCC-3′) (Clopton 2009; Rueckert et al. 2011). Raw sequence data were trimmed and contigs were assembled using Geneious 10 (Kearse et al. 2012; Biomatters Ltd, Auckland, New Zealand). Novel SSU rDNA sequences were initially analyzed using a BLAST search implemented in Geneious to confirm their identities as apicomplexan sequences. The two novel sequences of the SSU rDNA were deposited in GenBank under accession numbers MK181531 and MK181532.

Molecular phylogenetic analyses

The two sequences were aligned with other 83 apicomplexan and alveolate sequences downloaded from GenBank. Alignment was carried out in MUSCLE (Edgar 2004) using default parameters implemented in Geneious. The alignment was subsequently edited manually by eye to remove ambiguous gaps. The dinoflagellates *Prorocentrum micans* and *Hematodinium* sp. (GenBank accession numbers AJ415519.1 and AF286023) were used as outgroup taxa. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed with GTR + I + G model selected using JModelTest v.0.1.10 (Darriba et al. 2012). Maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE v. 8.2.9 (Stamatakis 2014) with 1,000 bootstrap replications. Bayesian inference was performed using MrBayes 3.2 (Ronquist et al. 2012) using default priors, with four runs each with chains of 10,000,000 generations, with trees sampled every 1,000 generations. We plotted the likelihood trace for each run to assess convergence in Tracer V1.6 (Rambaut et al. 2018), and discarded an average of 25% of each run as burn-in. The resulting tree topologies were visualized on Geneious. Both ML and BI analyses were performed via the CIPRES Science Gateway (Miller et al. 2011).

RESULTS

Morphological description of *Amoebogregarina taeniopoda* n. sp

Trophozoites, single gamonts, and gamonts in syzygy were isolated from the midgut, and gametocysts from the hind-gut of *T. centurio*. All stages were yellowish under the dissecting microscope. Trophozoites (Fig. 1A–C) possessed a shallowly elliptoid to depressed deltoid metamorphic epimerite, that is, the epimerite was retained and incorporated to the protomerite of the cell after the detachment from host gut epithelium (average length × width = 45.8 μm × 69.2 μm, range = 28–64 μm × 54–94 μm, n = 18) (Fig. 1A, B). The protomerite was panduriform to broadly panduriform, constrained at the apex (average length × width = 86.6 μm × 77.2 μm, range = 42.9–185.9 μm × 42.9–128.7 μm, n = 18), and its deutomerite was oblong or elliptoid (average length × width = 272 μm × 124.7 μm, range = 157.3–371.8 μm × 71.5–200.2 μm, n = 18). Gamonts (Fig. 1D) were similar in shape to the trophozoites, but the epimerite was absent or highly incorporated to the protomerite, and slightly bigger than the trophozoites (gamonts deutomerite: average length × width = 403.1 μm × 178.4 μm, range = 143–572 μm × 114.4–271.7 μm, n = 20; gamonts protomerite: average length × width = 121.5 μm × 115.4 μm; range = 42.9–271.7 μm × 71.5–185.9 μm, n = 20). The protomerite and deutomerite of the cells were divided by a distinct septum. The nucleus was spherical (diameter = 50.5 μm,

n = 18), and it was located from the middle to the lower portion of the deutomerite (Fig. 1C). Association of gamonts in syzygy was precocious, caudofrontal and biassociative (Fig. 1E). Trophozoites, gamonts, and gamonts in syzygy were stiff and capable of gliding movements. Gametocysts showed a hyaline epicyst and the dehiscence occurred through sporoducts (Fig. 1F), releasing doliform oocysts through monete chains (average length × width = 7.05 μm × 4.7 μm, range = 7–7.25 μm × 4.6–5.2 μm, n = 31) (Fig. 1F, G). The SEM micrographs showed that the whole cell presented longitudinal and parallel epicytic folds running along the longitudinal axis with exception of the epimerite (Fig. 1H–J). In the middle of the cell, the density of folds was 6–7 folds per micron. Morphometric data are shown in Table 1.

Morphological description of *Quadruspinospora mexicana* n. sp

Trophozoites and gamonts were observed from the midgut and gametocysts from the hindgut of *T. centurio*, all stages were whitish under the dissecting microscope. Trophozoites (Fig. 2A, B) possessed a subspherical rosette-shape epimerite with 7–12 digitiform process (Fig. 2A) (average length × width = 35.6 μm × 32 μm, range = 21–42.9 μm × 21.4–42.9 μm, n = 10). The protomerite of the cells were luniform (average length × width = 57.7 μm × 89.8 μm, range = 24.5–100.1 μm × 38.5–143 μm, n = 10), and the deutomerites were narrowly to very deeply obdeltoid, or narrowly obpyriform (average length × width = 257.3 μm × 124.2 μm; range = 150.2–429 μm × 80.5–164.4 μm, n = 10). Gamonts (Fig. 2C) were similar in shape to trophozoites but without the epimerite that is detached from the cell. The deutomerite of gamonts also could present a narrowly obtrullate shape and were considerably bigger than the trophozoites (deutomerite: average length × width = 655.4 μm × 239.6 μm; range = 243.1–1,186.9 μm × 93.1–471.9 μm, n = 27; protomerite: average length × width = 95.8 μm × 189.6 μm; range = 28.1–171.6 μm × 85.8–343.2 μm, n = 27). The protomerite and deutomerite of the cells were divided by a distinct septum. In all cases, the nucleus was spherical (diameter = 57.2 μm, n = 6), and it was located in the middle portion of the deutomerite (Fig. 2C). Both trophozoites and gamonts were solitary and gamonts in syzygy were not observed. Trophozoites and gamonts were flexible and capable of gliding movements. Gametocysts were spherical and showed a thick gelatinous epicyst and the dehiscence occurred by a simple rupture of the cyst releasing single doliform oocysts in mass with two spines in each pole (Fig. 2D) (average length × width = 8.8 μm × 5.1 μm; range = 7–10.5 μm × 4.6–5.2 μm, n = 30; average spines length = 26.6 μm, range = 21–35 μm, n = 30). The SEM micrographs (Fig. 2E–G) showed that the whole cell presented longitudinal epicytic folds arranged in waves running along the longitudinal axis (Fig. 2E and G). In the middle of the cell, the density of folds was five folds per micron. Morphometric data are shown in Table 2.

General parasitological observations

Of the 91 grasshoppers examined, 80 were coinfected with both eugregarine species (87.9%). Prevalence between sexes showed that 30 of 35 female (85.7%), and 50 of 56 males (89.2%) were coinfected, and no sex bias on the prevalence of gregarines was detected (Fisher's Exact test, P = 0.7434). For *A. taeniopoda* n. sp., a total of 78 of 91 (85.7%) specimens were infected. Prevalence differences between sexes showed that 28 of 35 female grasshoppers (80%), and 50 of 56 males (89.3%) were parasitized with *A. taeniopoda* n. sp. No sex bias was detected (Fisher's Exact test, P = 0.2347). In the case of *Q. mexicana* n. sp., 72 of 91 (79.1%) specimens were infected. Prevalence differences between sexes showed that 26 of 35 females (74.3%), and 46 of 56 (82.1%) were parasitized. No sex bias was detected (Fisher Exact test, P = 0.431).

The mean intensity of coinfection with both eugregarine species was 105 cells per host (range = 1–387 cells, n = 78). Female mean intensity was 113 cells per host (range = 1–381 cells, n = 28). Male mean intensity was 101 cells (range = 1–321 cells, n = 50). For *A. taeniopoda* n. sp., a mean intensity of 58 cells was found (range = 1–232 cells, n = 76). Female mean intensity was 57 cells (range = 2–232, n = 26). Male mean intensity was 58 cells (range = 1–200, n = 50). For *Q. mexicana* n. sp. the mean intensity was 56 cells per host (range = 1–191, n = 70). Female mean intensity was 70 cells (range = 1–191, n = 26). Male mean intensity was 48 cells (range = 1–160, n = 46).

Molecular phylogenetic position of *Amoebogregarina taeniopoda* n. sp. and *Quadruspinospora mexicana* n. sp

The partial SSU rDNA sequence from *A. taeniopoda* n. sp. (Genbank accession number MK181531) has a length of 1,764 bp and a G + C content of 45.5%, while SSU rDNA sequence from *Q. mexicana* n. sp. (Genbank accession number MK181532) has a length of 1,766 bp and a G + C content of 44.9%.

The resulting alignment was 1,466 bp in size. Both, BI and ML analyses recovered an almost identical tree topology, the only difference being that the BI tree showed a backbone polytomy. In general, BI and ML analyses of the 85-taxon alignment of SSU rDNA sequences yielded strong nodal supports for individual clades, but the deeper nodes of the tree were poorly resolved, and the two dinoflagellate sequences designated as the outgroup are on separate nodes (Fig. 3).

The clade comprising eugregarines showed low support in both ML and BI trees (42 maximum likelihood bootstrap [MLB], BI = 0.64 Bayesian posterior probability [BPP]). Three terrestrial eugregarine clades were recovered, and comprise members from the superfamilies Gregarinoidea, Actinocephaloidea, and Stylocephaloidea. In the terrestrial gregarine clade I (nodal support of 100 MLB, 1 BPP),

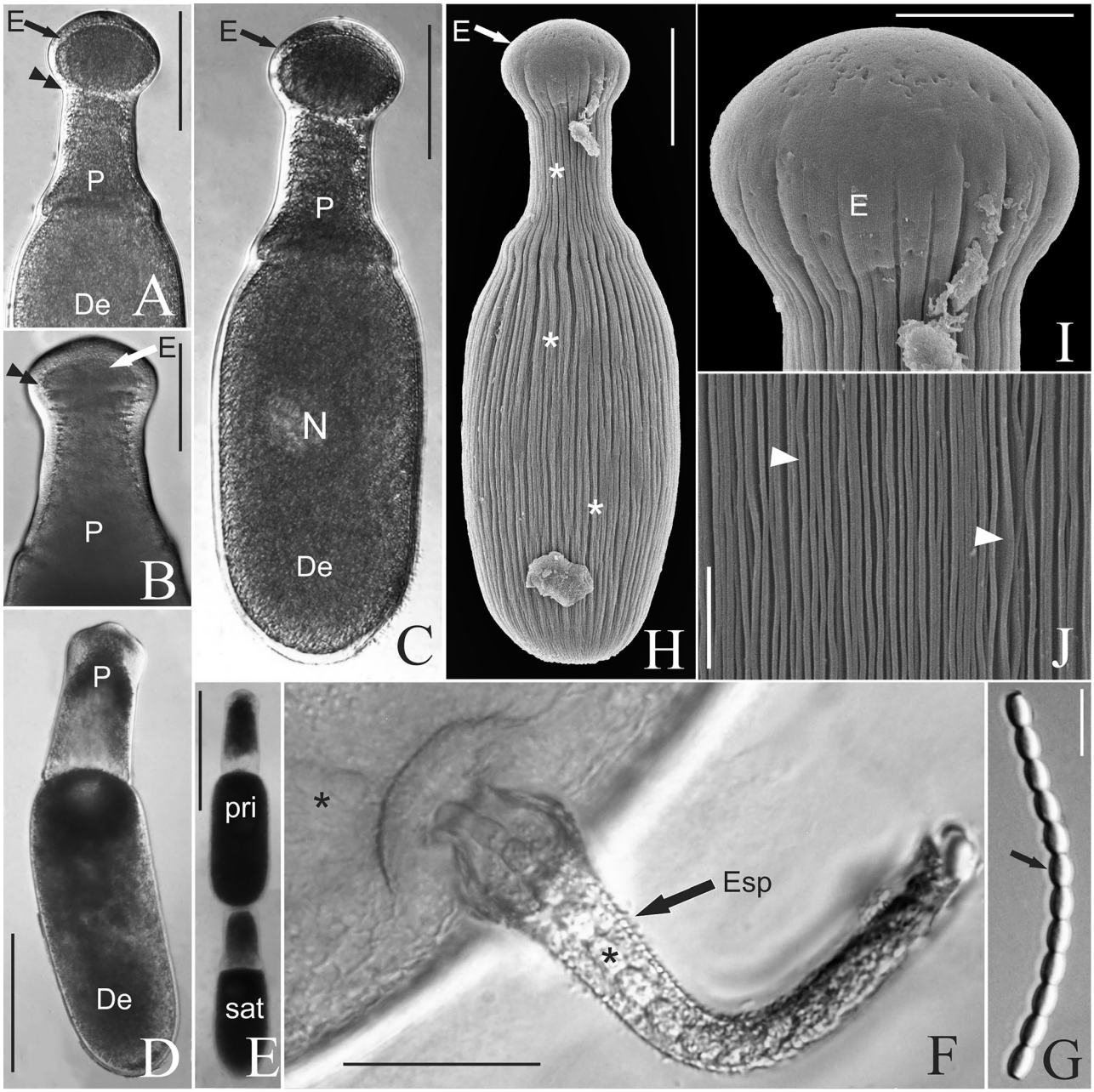

Figure 1 Differential interference contrast (DIC) light micrographs and scanning electron micrographs (SEM) showing the general morphology and surface ultrastructure of the life-cycle stages of *Amoebogregarina taeniopoda* n. sp. from the lubber grasshopper *Taeniopoda centurio* **A–C.** Trophozoites with three compartments: the epimerite (E), protomerite (P), and deutomerite (De). Process of assimilation of the epimerite in A and B is pointed with the double arrowhead. In C, the nucleus (N) is visible in the middle of the deutomerite. **D.** A gamont with the epimerite fully assimilated. The cell only retains the protomerite (p) and deutomerite (De). **E.** Association of gamonts in syzygy is caudofrontal and biassociative, conformed for a primite (pri) and a satellite (sat). **F.** Gametocyst dehiscence through sporoducts (Esp) releasing oocysts (asterisk). **G.** Doliform oocysts (arrow) released in monete chains. **H.** SEM of a trophozoite cell showing epicytic folds (asterisks) running longitudinally and parallel along the whole cell excluding the epimerite (E). **I.** Higher magnification SEM of the attachment zone of the epimerite (E). **J.** Higher magnification SEM of the longitudinal epicytic folds (arrowhead). Scale bars: A–C = 80 μm; D = 100 μm; E = 200 μm; F = 30 μm; G = 15 μm; H = 90 μm; I = 35 μm; J = 2 μm.

which included the eugregarines of superfamily Gregarinoidea, *A. taeniopoda* n. sp. formed sister to *Amoebogregarina nigra* with a strong nodal support (100 MLB, 1 BPP). In the terrestrial gregarine clade II (nodal support of 71 MLB, 0.7 BPP), comprised of gregarines of superfamily Actinocephaloidea, *Q. mexicana* n. sp. was

Table 1. Morphometric data of *Amoebogregarina taeniopoda* n. sp.

Characters[a]	Mean	SD	Min	Max	*n*
Trophozoites					
Deuteromerite length	272.0	47.3	157.3	371.8	18
Deuteromerite width	124.7	39.9	71.5	200.2	18
Protomerite length	86.6	36.8	42.9	185.9	18
Protomerite width	77.2	22.2	42.9	128.7	18
Epimerite length	45.8	10.5	28.6	64.3	18
Epimerite width	69.2	12.9	54.3	94.1	18
Nucleus	34.4	8.39	21.4	50.1	14
Gamonts					
Deuteromerite length	403.1	110.0	143	572	20
Deuteromerite width	178.3	47.73	114.4	271.7	20
Protomerite length	121.5	65.02	42.9	271.7	20
Protomerite width	115.4	34.04	71.5	185.9	20
Nucleus	50.5	13.7	28.6	71.5	18
Gametocysts					
Total length	422.6	18.1	400.4	443.3	7
Total width	413.6	23.7	386.1	443.3	7
Epicysts width	76.8	15.8	57.2	92.9	7
Oocyst					
Length	7.05	0.106	7	7.2	31
Width	4.7	0.13	4.6	5.2	31

Max = Maximum; Min = Minimum; *n* = Number of individuals; SD = Standard deviation.
[a]All measurements in μm.

recovered as sister to *Prismatospora evansi* with nodal support of 57 MLB and 0.87 BPP. Terrestrial gregarine clade III (nodal support of 100 MLB, 1 BPP) was only composed of the eugregarines belonging to superfamily Stylocephaloidea in which all are parasites of beetles (Fig. 3). In both ML and BI analyses, terrestrial eugregarines clade I nested within the group comprising aquatic eugregarines clade I (Cephaloidophoroidea), where all members parasitize crustaceans.

DISCUSSION

Recent descriptions of gregarines have been biased toward the marine species (Diakin et al. 2017; Iritani et al. 2017; Rueckert and Leander 2010; Rueckert et al. 2010, 2015; Wakeman and Leander 2013; Wakeman et al. 2018), in which the inclusion of molecular markers, in particular SSU rDNA sequences, has been very informative in the delineation of very closely related species (Wakeman and Leander 2013). On the other hand, terrestrial gregarines, in which the majority of species reported are from insects, have received less attention. Most of the descriptions of eugregarines from insects have been done mainly based on morphological characteristics by using light microscopy and line drawings (Bhatia and Setna 1924; Semans 1943; Théodorides et al. 1958, 1972, 1975). Also, in older works, the description of species was often based on more ambiguous traits (i.e. description of species based only in the shape of gamonts), contributing to an underestimation of eugregarine diversity. Only few works have recently included the descriptions of terrestrial eugregarines based on both morphological and molecular evidence (Clopton 2009; Lantová et al. 2010; Leander et al. 2003; Rueckert and Devetak 2017; Votýpka et al. 2009). The inclusion of molecular information in gregarines is important because it can discriminate different species from one another, especially those species that are prone to cryptic speciation and convergent evolution (Adl et al. 2007; Wakeman and Leander 2013). In the present study, we have included the SSU rDNA sequences combined with the host affinity, type locality, trophozoite morphology and ultrastructure, and morphology of other life-cycle stages to provide a comprehensive taxonomic description of the gregarines.

Taxonomic considerations for *Amoebogregarina taeniopoda* n. sp

Genus *Amoebogregarina* Kula and Clopton, 1999, was erected for gregarines in which the epimerite is assimilated into the protomerite after detachment from host gut epithelium. *Gregarina nigra* described from *Melanoplus differentialis* (Orthoptera: Acrididae) was established as the type species of the genus by Kula and Clopton (1999). Genus *Amoebogregarina* contains five species: *A. nigra* (Watson, 1915), *A. ampulla* (Lange and Cigliano, 2004), and *A. dhawanii* (Pushkala et al., 2000) which are exclusively parasites of orthopterans of families Acrididae and Pyrgomorphidae, while the two remaining species, *A. crenata* (Bathia and Setna, 1924) and *A. nymphaea* (Lipa and Triggiani, 1989), are parasites of coleopterans of family Chrysomelidae. For a detailed diagnosis of the genus and its species refer to Clopton (2002), Desportes (2013) and Kula and Clopton (1999). *Amoebogregarina* seems to be distributed worldwide, while *A. ampulla* and *A. dhawanii* are only reported in Argentina and India, respectively, *A. nigra* seems to have a wider distribution, reported from the United States, Papua New Guinea, Thailand, South Africa, Democratic Republic of the Congo, Ivory Coast and Senegal (Desportes 2013). However, most identifications of *A. nigra* are only based on vague descriptions and line drawing schemes (Seck and Toguebaye 1995; Semans 1943; Théodorides et al. 1972, 1975).

The only available gene sequence from this genus corresponds to the type species, *A. nigra*. In our molecular phylogenetic analysis, *A. taeniopoda* n. sp. formed a clade with the septate gregarines, *A. nigra*, several species of *Gregarina* infecting earwigs (Dermaptera), crickets (Orthoptera) and cockroaches (Blattodea), and species of genus *Leidyana* parasites of crickets. *Amoebogregarina taeniopoda* n. sp. was placed within the highly supported clade of terrestrial gregarines belonging to superfamily Gregarinoidea (Fig. 3). Although the SSU rDNA sequences of *A. taeniopoda* n. sp. and *A. nigra* are very similar to each other with a genetic distance of 1.3%, both lineages are morphologically quite different (Table 3). The main morphological traits that distinguish *A. taeniopoda* n. sp. from *A. nigra* are the shape of the epimerite and protomerite, and the size of the trophozoite cell. While for *A. nigra,* the shape of the epimerite is shallowly ovoid to transversely elliptoid, for *A. taeniopoda* n. sp. its epimerite

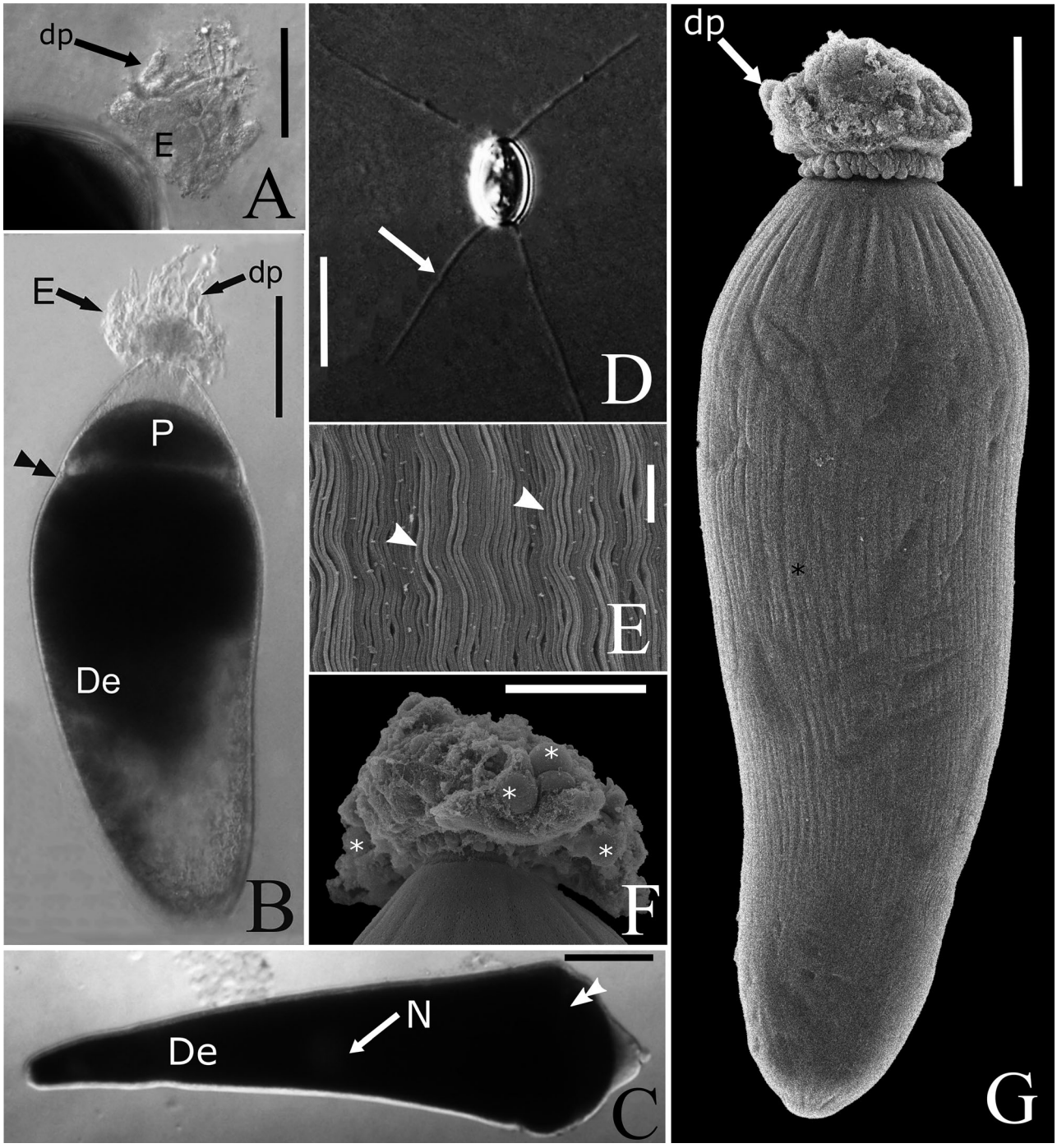

Figure 2 Differential interference contrast (DIC) light micrographs and scanning electron micrographs (SEM) showing the general morphology and surface ultrastructure of the life-cycle stages of *Quadruspinospora mexicana* n. sp. from the lubber grasshopper *Taeniopoda centurio* **A.** Epimerite (E) of a trophozoite showing digitiform process (dp). **B.** Trophozoites possess an epimerite (E) in the anterior part of the cell. The protomerite (P), and deutomerite (De) divided by a septum (double arrowhead). **C.** A gamont retaining only the deutomerite (De) and protomerite (P) divided by a septum (double arrowhead). The nucleus (N) is visible in the middle portion of the deutomerite. **D.** Doliform oocyst with two spines (arrow) in each pole. **E.** Higher magnification SEM of the longitudinal epicytic folds arranged in waves (arrowhead). **F.** Higher magnification with SEM of the attachment zone of the epimerite with digitiform process (asterisk). **G.** SEM of a trophozoite cell showing epimerite and a digitiform processes (dp). Scale bars: A = 35 μm; B = 60 μm; C = 100 μm; D = 10 μm; E = 3 μm; F = 25 μm; G = 35 μm.

Table 2. Morphometric data of *Quadruspinospora mexicana* n. sp.

Characters[a]	Mean	SD	Min	Max	*n*
Trophozoites					
Deuteromerite length	257.35	104.22	150.2	429	10
Deuteromerite width	124.23	28.6	80.5	164.4	10
Protomerite length	57.7	26.73	24.5	100.1	10
Protomerite width	89.83	37	38.5	143	10
Epimerite length	35.66	9.18	21	42.9	10
Epimerite width	32.06	8.82	21.4	42.9	10
Nucleus	34.18	6.12	28	42.9	10
Gamonts					
Deuteromerite length	655.41	286.51	243.1	1,186.9	27
Deuteromerite width	239.65	107.67	93.1	471.9	27
Protomerite length	95.83	39.56	28.1	171.6	27
Protomerite width	189.6	70.67	85.8	343.2	27
Nucleus	57.2	18.08	28.6	71.5	6
Gametocysts					
Total length	538.03	76.36	443.3	700.7	16
Total width	535.31	68.29	457.6	715	16
Epicysts width	170.7	31.53	114.4	214.5	16
Oocyst					
Length	8.8	0.66	7	10.5	30
Width	5.15	0.22	4.6	5.25	30
Spine length	26.65	4.63	21	35	30

Max = Maximum; Min = Minimum; *n* = Number of individuals; SD = Standard deviation.
[a]All measurements in μm.

is shallowly elliptoid to depressed deltoid. These differences make the length of the epimerite of *A. taeniopoda* n. sp. greater (mean length of 45.8 μm) in comparison to the epimerite of *A. nigra* (mean length of 37.9 μm). Conversely, the epimerite of *A. taeniopoda* n. sp. is narrower (mean width of 69.2 μm) than those of *A. nigra* (mean width of 81 μm). With respect to the protomerite shape, *A. nigra* possesses an oblong to transversely oblong protomerite, while *A. taeniopoda* n. sp. has a panduriform to broadly panduriform protomerite constrained at the apex. The complete morphological comparison of *A. taeniopoda* n. sp. with the other *Amoebogregarina* species parasitizing grasshoppers is also shown in Table 3. The morphological differences of both species, along with the distinctions in host utilization, and the distribution discrepancies are the traits justifying the establishment of the new species.

Taxonomic considerations for *Quadruspinospora mexicana* n. sp

Genus *Quadruspinospora* Sarkar and Chakravarty, 1969 is characterized for having solitary trophozoites with subspherical epimerites with digitiform processes and ellipsoidal or ovoid oocysts with long filament-like polar spines released by simple rupture of the gametocyst. *Quadruspinospora japonicus* (formerly described as *Coronoepimeritus japonicus* by Hoshide 1958) from *Locusta migratoria*, *Oedaleus infernalis*, *Oxya japonica,* and *O. velox* (Orthoptera: Acrididae) is the type species of the genus (Clopton 2002; Desportes 2013; Sarkar and Chakravarty 1969). Genus *Quadruspinospora* contains 14 species, all of which are exclusively parasites of orthopteran families, Acrididae, Pyrgomorphidae, and Tetrigidae (Desportes 2013; Yumnam and Mohilal 2017): *Q. aelopii* Sarkar and Chakravarty, 1969, *Q. attractomorphae* (Haldar and Chakraborty, 1978), *Q. chakravartyi* (Chakraborty and Haldar, 1974), *Q. dichotoma* Kundu and Haldar, 1983, *Q. indoaiolopii* Haldar and Chakraborty, 1976, *Q. megaspinosa* Haldar and Chakraborty, 1976, *Q. acridae* (Haldar and Chakraborty, 1979), *Q. adigitalis* Datta et al., 1990, *Q. caudata* Modak et al., 2008, *Q. hieroglyphae* Mandal and Ray, 2007, *Q. japonicus* Hoshide, 1958, *Q. cloptoni* Modak et al., 2008, *Q. platyepimerita* Datta et al., 1990 and *Q. oxyae* Yumnam and Mohilal, 2017. *Quadruspinospora* species have been only described from India and Japan, which means that *Q. mexicana* n. sp. represents the first record for this genus in the New World.

Until now, there has not been any available sequence of any *Quadruspinospora* species in any database such as GenBank. The establishment of *Q. mexicana* n. sp. within genus *Quadruspinospora* based on morphological evidence needs to be confirmed with the sequence of the type species. In our molecular phylogenetic analysis, the closest relatives of *Q. mexicana* n. sp. were both septate and aseptate gregarines including *Prismatospora evansi* infecting dragonflies (Odonata), *Monocystis agilis* a parasite of earthworms (Annelida), *Syncystis mirabilis* a neogregarine parasite of dragonflies, and *Gregarina ctenocephali* a parasite of fleas (Siphonaptera). The close relation of an aseptate gregarine (*M. agilis*) with other septate gregarines, including *Q. mexicana* n. sp., represents another example that supports the recent proposals of abolishing the division between septate and aseptate gregarines because it does not reflect accurate phylogenetic relationships (Cavalier-Smith 2014; Rueckert et al. 2011; Simdyanov et al. 2017). In the same way, the inclusion of neogregarines (*Ophryocystis elektroscirrha* and *S. mirabilis*) within a clade of eugregarines supports the idea that neogregarines should be incorporated into the Eugregarinorida (Simdyanov et al. 2017). *Quadruspinospora mexicana* n. sp. clustered within the clade of terrestrial gregarines belong to superfamily Actinocephaloidea (Fig. 3). The main morphological characteristics that distinguish *Q. mexicana* n. sp. from the other described species are the shape of the epimerite and the size of the gamonts (Table 4). *Quadruspinospora mexicana* n. sp. is the larger of the described species of *Quadruspinospora*, with a mean total length of 751.24 μm, while the rest of the species have average total lengths ranging from 146 to 489 μm. Comparison of the epimerite show that, for example, the epimerite of *Q. aleopii* is subspherical with 8–12 digitiform processes with a length of 15–20 μm, or in the case of *Q. acridii*, the epimerite is composed of 10–13 digitiform processes with a length of 10–30 μm. In contrast, the epimerite of *Q. mexicana* n. sp. is subspherical and rosette-shaped with 7–12 digitiform processes and is slightly larger with a length of 21 to almost 43 μm. As we have mentioned above, one of the more evident characteristics that

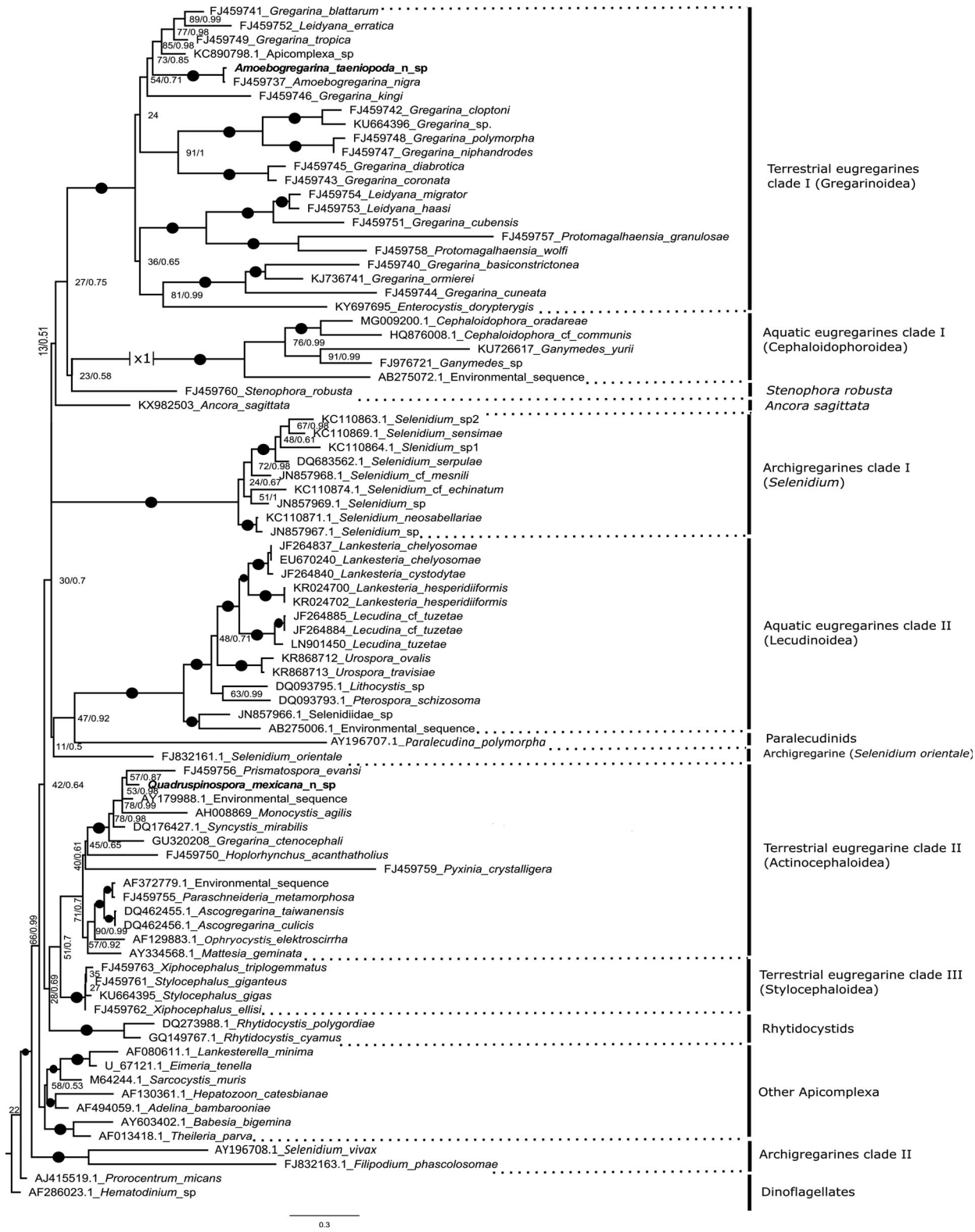

Figure 3 SSU rDNA phylogenetic tree derived from the Maximum Likelihood analysis from *Amoebogregarina taeniopoda* n. sp. and *Quadruspinospora mexicana* n. sp. plus 91 sequences of other gregarine taxa and dinoflagellates as outgroup taxa. The tree was inferred using GTR + I + G substitution model. The numbers on the nodes show the bootstrap values. Dots denote the nodes where support values were higher than 95%. The sequences of *Amoebogregarina taeniopoda* n. sp. and *Quadruspinospora mexicana* n. sp. are highlighted in bold.

Table 3. Morphological comparison of *A. taeniopoda* n. sp. with previous described species of *Amoebogregarina* in Orthoptera

	A. dhawanii	*A. ampulla*	*A. nigra*	*A. taeniopoda* n. sp.
Total length and width of the cell	465.5–910 × 119.7–235	280–584 × 104–296	252.7–814 × 66.5–320.5	200.2–843.7 × 114.4–271.7
Epimerite shape (length and width)	Simple, button-shaped (mean: 34 × 50)	Simple and globular (?)	Shallowly ovoid to transversely elliptoid (20–50.5 × 54.9–195.1)	Shallowly elliptoid to depressed deltoid (28.6–64.3 × 54.3–94.1)
Protomerite shape (length and width)	? (119.7–266 × 93.1–208)	Ovoid to panduriform* (96–192 × 72–160)	Oblong to transversely oblong (54.5–123.7 × 42–126.4)	Panduriform to broadly panduriform, constrained at the apex (42.9–185.9 × 42.9–128.7)
Deutomerite shape (length and width)	Cylindrical (319.2–715 × 119.7–235)	Obpanduriform to doliform* (180–464 × 104–296)	Narrowly ovate to square (190.6–458.9 × 67.8–393.7)	Oblong or eliptoid (143–572 × 114.4–271.7)
Gametocyst shape (length and width)	Round (208–338 in diameter)	Spherical (104–360 in diameter)	Oblate to transversely elliptic (318.7–488 × 216–348)	Nearly pherical (400.4–413.6 × 386.1–443.3)
Oocysts shape (length and width)	Barrel shaped or cylindrical (6 × 4)	Doliform (5.7 × 2.8)	Doliform (7.9–8.9 × 5–6.2)	Doliform (7–7.25 × 4.6–5.25)
Nucleus shape	Spherical	?	Circular, placement variable	Spherical, located from the middle to the lower portion of the deutomerite
Nucleus diameter	?	?	32.3–74.5 in diameter	28.6–71.5 in diameter
Host	*Atractomorpha crenulata* (Orthoptera: Pyrgomorphidae)	*Ronderosia bergi* (Orthoptera: Acrididae)	Various species of orthopterans of family Acrididae and Pyrgomorphidae	*Taeniopoda centurio* (Orthoptera: Romaleidae)
Localities	Tamil Nadu, India	Provinces of Misiones, Buenos Aires and San Luis, Argentina	America: USA; Africa: South Africa, Senegal, Ivory Coast, Congo; Asia: Thailand	Tlanchinol, Hidalgo, Mexico
Reference	Pushkala et al. (2000)	Lange and Cigliano (2004)	Kula and Clopton (1999) and Desportes (2013)	This study

Measurements in μm; ? data not available, * data from pictures on the respective paper.

distinguishes *Q. mexicana* n. sp. from the other described species is the total length and width of the cells, which is the larger species belonging to genus *Quadruspinospora*. *Quadruspinospora mexicana* n. sp. has a range of 271.2–1,358.5 μm length and 93.1–471.9 μm width, in contrast to *Q. aleopii* in which the length ranges from 92.5 to 290 μm, and the width spans from 40 to 125 μm. In the case of *Q. acridii*, the length of the cell goes from 70 to 720 μm and the width is not mentioned in the original description. The morphological and molecular evidence along with the new ultrastructural evidence, host utilization, and the distribution of the host justify the establishment of the new species.

In conclusion, this is the first report of *A taeniopoda* n. sp. and *Q. mexicana* n. sp. parasitizing a species of the family Romaleidae. Both species represent the first records of gregarines parasitizing grasshoppers from Mexico, and for *Q. mexicana* n. sp., this is the first report for the New World. The above findings show the gaps in the knowledge of eugregarine diversity in insects. For example, only 140 gregarine species reported for 450 of the almost 28,000 valid orthopteran species have been described, which represents only around 1.5% of the total orthopteran diversity (Cigliano et al. 2018; Desportes 2013). This data show the necessity of carrying out more research focused on the diversity, which will help to resolve gregarine systematics and elucidate its evolution.

TAXONOMIC SUMMARY

Phylum Apicomplexa Levine, 1970
Subphylum Sporozoa Leuckart, 1879
Class Gregarinomorpha Grassé, 1953
Order Eugregarinorida Léger, 1900
Superfamily Gregarinoidea Labbé, 1899
Family Gregarinidae Labbé, 1899
Genus *Amoebogregarina* Kula and Clopton, 1999

Amoebogregarina taeniopoda n. sp. Medina-Durán, Mayén-Estrada, Mariño-Pérez and Song.

Diagnosis. Trophozoites with a shallowly elliptoid to depressed deltoid metamorphic epimerite. Cell is divided into protomerite and deutomerite by a septum visible under optical microscope. Protomerite panduriform to broadly panduriform, constrained at the apex. Deutomerite oblong or elliptoid. Trophozoites on average 404.6 μm long and 124.7 μm wide. Gamonts were similar in shape to the trophozoites, but slightly bigger and with the epimerite absent or highly incorporated to the

Table 4. Morphological comparison of *Q. mexicana* n. sp. with previous described species of *Quadruspinospora*

	Q. aleopii	*Q. acridii*	*Q. oxyae*	*Q. mexicana* n. sp.
Total length and width of the cell	92.5–290 × 40–125	70–720 × ?	120–163.6 × 66.8–106.6	271.2–1,358.5 × 93.1–471.9
Epimerite shape (length and width)	Subspherical knob with 8–12 stumpy digitiform processes (15–20 × ?)	Subspherical knob with 10–13 digitiform processes (10–30 × ?)	Question mark like, with digitiform process (6.5–23.6 × ?)	Subspherical rosette-shape with 7–12 digitiform process (21–42.9 × 21.4–42.9)
Protomerite shape (length and width)	Hemispherical (15–65 × 35–100)	Hemispherical (15–130 × 30–310)	Hemispherical (27.5–47.1 × 40.1–92.9)	Luniform (24.5–171.6 × 38.5–343.2)
Deuteromerite shape (length and width)	Obpanduriform* (62.5–205 × 40–125)	Very deeply obdeltoid* (45–590 × ?)	Cylindro-conical (87.9–116.5 × 66.8–106.6)	Narrowly obdeltoid or narrowly obpyriform in trophozoites; narrowly obtrullate in gamonts (150.2–1,186.9 × 80.5–471.9)
Gametocyst shape (length and width)	Spherical to subspherical (530–590 in diameter)	Spherical to slightly oval (350–380 × 240)	Spherical (90–129.7 × 59.7–97.4)	Spherical (443.3–715 in diameter)
Oocysts shape (length and width)	Oval (8 × 4)	Oval (9 × 5)	Oval (11.5 × 7.2)	Doliform (7–10.5 × 4.6–5.2)
Spine length	36–38	30	19.5–21.5	21–35
Nucleus shape	Spherical	Oval or elliptical	Spherical	Spherical, located in the middle portion of the deuteromerite
Nucleus diameter	47	?	20.5–30.4	57.2
Host	*Aiolopus* sp. (misspelled *Aelopus* sp. in the original description) (Orthoptera: Acrididae)	*Acrida exaltata* (Orthoptera: Acrididae)	*Oxya hyla hyla* (Orthoptera: Acrididae)	*Taeniopoda centurio* (Orthoptera: Romaleidae)
Localities	West Bengal, India	West Bengal, India	Manipur, India	Tlanchinol, Hidalgo, Mexico
Reference	Sarkar and Chakravarty (1969)	Haldar and Chakraborty (1976)	Yumnam and Mohilal (2017)	This study

All measurements in μm; ? data not available, * data from pictures on the respective paper.

protomerite. Gamonts on average 524.6 μm long and 178.4 μm wide. Spherical nucleus located from the middle to the lower portion of the deutomerite with an average diameter of 50.5 μm. Association of gamonts in syzygy precocious, caudofrontal and biassociative. Gametocysts with a hyaline epicyst and dehiscence occuring through sporoducts. Doliform oocysts released through sporoducts in monete chains. Trophozoites, gamonts, and associations stiff and capable of gliding movements. Cell surface of trophozoites composed by longitudinal and parallel epicytic folds running along longitudinal axis of the whole cell excluding the epimerite. Density of folds 6–7 epicytic folds/micron. Cells are yellowish under stereoscope.

Remarks. Although *A. nigra* gene sequence is very similar to *A. taeniopoda* n. sp., morphological dissimilarities, as well as host species and distribution differences justify the establishment of this new taxon.

DNA sequence. SSU rDNA sequence GenBank accession number MK181531.

Type locality. Host in croplands surrounded by cloud forest (20°59′32.99″N, 98°39′34.73″W, 1,526 masl) in Tlanchinol municipality, Hidalgo, Mexico, (Sierra Madre Oriental Province, Neotropical Region (Morrone 2017).

Type habitat. Terrestrial.

Type host. *Taeniopoda centurio* (Drury, 1770) (Insecta, Orthoptera, Caelifera, Romaleidae).

Location in host. Trophozoites, gamonts, and associations in midgut and gastric cecum. Gametocysts in hindgut.

Type micrograph: Fig. 1C and H.

Hapantotype: Trophozoites, gamonts, and associations on SEM stubs with gold sputter-coat have been deposited at the Department of Life Sciences, National History Museum of London under the registration numbers NHMUK 2019.5.13.1, NHMUK 2019.5.13.2, NHMUK 2019.5.13.3, and NHMUK 2019.5.13.4.

Zoobank Registration LSID: urn:lsid:zoobank.org:act:D85F30D8-0EBA-4E7F-A202-00D18B5EF8F1

Etymology: The specific epithet, *taeniopoda*, refers to the genus of the type host.

Superfamily Actinocephaloidea Léger, 1892
Actinocephalidae Léger, 1892
Genus *Quadruspinospora* Sarkar and Chakravarty 1969

Quadruspinospora mexicana n. sp. Medina-Durán, Mayén-Estrada, Mariño-Pérez and Song.

Diagnosis. Trophozoites with a subspherical rosette-shape epimerite with 7–12 digitiform process. Cell is divided into protomerite and deutomerite by a septum visible under optical microscope. Protomerite luniform, and deutomerite was narrowly to very deeply obdeltoid, or narrowly obpyriform. Trophozoites on average 350.7 μm long and 124.2 μm wide. Gamonts were similar in shape than the trophozoites, but without the epimerite that is detached from the cell and considerably bigger. Gamonts on average 751.2 μm long and 239.6 μm wide. Spherical nucleus located in the middle portion of the deutomerite with an average diameter of 57.2 μm. Trophozoites and gamonts

solitaries and the presyzygial association was not observed. Gametocysts with a thick gelatinous epicyst and dehiscence occurring through simple rupture of the cyst. Doliform oocysts with two spines in each pole released in mass. Trophozoites and gamonts flexible and capable of gliding movements. Cell surface of the trophozoites composed by longitudinal epicytic folds arranged in waves running along longitudinal axis of the whole cell. Density of folds 5 epicytic folds/micron. Cells are whitish under stereoscope.

DNA sequence. SSU rDNA sequence GenBank accession number MK181532.

Type locality. Host in croplands surrounded by cloud forest (20°59′32.99″N, 98°39′34.73″W, 1,526 masl) in Tlanchinol municipality, Hidalgo, Mexico, (Sierra Madre Oriental Province, Neotropical Region (Morrone 2017).

Type habitat. Terrestrial.

Type host. *Taeniopoda centurio* (Drury, 1770) (Insecta, Orthoptera, Caelifera, Romaleidae).

Location in host. Trophozoites and gamonts in midgut and gastric cecum. Gametocysts in hindgut.

Type micrograph: Fig. 2B–E

Hapantotype: Trophozoites and gamonts on SEM stubs with gold sputter-coat have been deposited at the Department of Life Sciences, National History Museum of London under the registration numbers NHMUK 2019.5.13.5, NHMUK 2019.5.13.6, and NHMUK 2019.5.13.7.

Zoobank Registration LSID: urn:lsid:zoobank.org:act:C6B56953-EA90-4352-9E6A-A79E081FA5DD

Etymology: The specific epithet, *mexicana*, reflects the geographical distribution of the species and the type host in Mexico.

ACKNOWLEDGMENTS

This work was possible through funding from Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, and CONACyT for the grant to JHMD for Master's studies, Programa de Estímulos para Estudios de Posgrado (PAEP) for support to JHMD for a short research stay in Texas A&M University, USA, and IOS-1253493 to HS, the United States Department of Agriculture (Hatch Grant TEX0-1-6584 to HS) to support molecular data generation. We gratefully acknowledge one anonymous reviewer and Kevin Wakeman for their comments that improve substantially the manuscript. We are thankful to Santiago Medina-Jiménez for his help to locate and collect specimens, Biól. Margarita Reyes-Santos Laboratorio de Protozoología, UNAM, for her technical support, and MSc María Berenit Mendoza-Garfias Instituto de Biología, UNAM, for her technical assistance for SEM procedures and images of apicomplexan species. Publication of this article was supported by the R.P. Hall Fund.

LITERATURE CITED

Adl, S. M., Bass, D., Lane, C. E., Lukes, J., Schoch, C. L., Smirnov, A., Agatha, S., Berney, C., Brown, M. W., Burki, F., Cárdenas, P., Cepicka, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A. A., Hoppenrath, M., James, T. Y., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D. J. G., Lara, E., Gall, L. L., Lynn, D. H., Mann, D. G., Molera, R. M., Mitchell, E. A. D., Morrow, C., Park, J. S., Pawlowski, J. W., Powell, M. J., Richter, D. J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F. W., Cortes, G. T., Youssef, N., Zlatogursky, V. & Zhang, Q. 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. *J. Eukaryot. Microbiol.*, 66:4–119.

Adl, S. M., Leander, B. S., Simpson, A. G. B., Archibald, J. M., Anderson, O. R., Bass, D., Bowser, S. S., Brugerolle, G., Farmer, M. A., Karpov, S., Kolisko, M., Lane, C. E., Lodge, D. J., Mann, D. G., Meisterfeld, R., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Smirnov, A. V. & Spiegel, F. 2007. Diversity, nomenclature, and taxonomy of protists. *Syst. Biol.*, 56:684–689.

Belton, P. & Grundfest, H. 1962. Potassium activation and K spikes in muscle fibers of mealworm larva (*Tenebrio molitor*). *Am. J. Physiol.*, 203:588–594.

Bhatia, B. L. & Setna, S. 1924. On some new cephaline gregarines. *Parasitology*, 16(3):279–288.

Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, 83:575–583.

Cavalier-Smith, T. 2014. Gregarine site-heterogeneous 18S rDNA trees, revision of gregarine higher classification, and the evolutionary diversification of Sporozoa. *Eur. J. Protistol.*, 50:472–495.

Cerritos, R. & Cano-Santanta, Z. 2008. Harvesting grasshoppers *Sphenarium purpurascens* in Mexico for human consumption: a comparison with insecticidal control for managing pest outbreaks. *Crop Prot.*, 27:473–480.

Cigliano, M. M., Braun, H., Eades, D. C. & Otte, D. 2018. Orthoptera Species File. Version 5.0/5.0. [Retrieval date: 27th-July-2018]. http://Orthoptera.SpeciesFile.org

Clopton, R. E. 2002. Phylum Apicomplexa Levine, 1970: Order Eugregarinorida Léger, 1900. *In:* Lee, J. J., Leedale, G., Patterson, D. & Bradbury, P. C. (ed.). Illustrated Guide to the Protozoa, 2nd ed. Society of Protozoologists, Lawrence, KS. p. 205–288.

Clopton, R. E. 2004. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. *Comp. Parasitol.*, 71:130–140.

Clopton, R. E. 2009. Phylogenetic relationships, evolution, and systematic revision of the septate gregarines (Apicomplexa: Eugregarinorida: Septatorina). *Comp. Parasitol.*, 76:167–190.

Clopton, R. E. & Lucarotti, C. J. 1997. *Leidyana canadensis* n. sp. (Apicomplexa: Eugregarinida) from larval Eastern Hemlock Looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae). *J. Eukaryot. Microbiol.*, 44:383–387.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*, 9:772.

De Jesús-Bonilla, V. S., Barrientos-Lozano, L. & Zaldívar-Riverón, A. 2017. Sequence-based species delineation and molecular phylogenetics of the transitional Neartic-Neotropical grasshopper genus *Taeniopoda* (Orthoptera, Romaleidae). *Syst. Biodivers.*, 15:600–617.

De Jesús-Bonilla, V. S., Meza-Lázaro, R. N. & Zaldívar-Riverón, A. 2019. 3RAD-based systematics of the transitional Neartic-Neotropical lubber grasshopper genus *Taeniopoda* (Orthoptera: Romaleidae). *Mol. Phylogenet. Evol.*, 137:64–75.

Desportes, I. 2013. Systematics of terrestrial and fresh water gregarines. *In:* Desportes, I. & Schrével, J. (ed.), Treatise on

Zoology – Anatomy, Taxonomy, Biology: The Gregarines, the Early Branching Apicomplexa, 1st ed., Vol. 2. Brill, Leiden, Netherlands. p. 377–671.

Diakin, A., Wakeman, K. C. & Valigurová, A. 2017. Description of *Ganymedes yurii* sp. n. (Ganymedidae), a new gregarine species from the Antarctic amphipod *Gondogeneia* sp. (Crustacea). *J. Eukaryot. Microbiol.*, 64:56–66.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32:1792–1797.

Fontana, P., Buzzetti, F. M. & Mariño-Pérez, R. 2008. Grasshoppers, locusts, crickets and katydids of Mexico. WBA Books, Verona. p. 272.

Haldar, D. P. & Chakraborty, N. 1976. Observations on the morphology and life-history of three new species of cephaline gregarines (Protozoa: Sporozoa) from grasshoppers in West Bengal. *Proc. Zool. Soc. Calcutta*, 29:73–81.

Hoshide, H. 1958. Studies on the cephaline gregarines of Japan. II. 3. Description of the members belonging to the families Didymophyidae, Actinocephalidae, Acanthosporidae, Stylocephalidae, Dactylophoridae. *Bull. Fac. Educ. Yamaguchi Univ.*, 8:35–101.

Iritani, D., Wakeman, K. C. & Leander, B. S. 2017. Molecular phylogenetic positions of two new marine gregarines (Apicomplexa) – *Paralecudina anankea* n. sp. and *Lecudina caspera* n. sp. – from the intestine of *Lumbrineris inflata* (Polychaeta) show patterns of co-evolution. *J. Eukaryot. Microbiol.*, 65:211–219.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28:1647–1649.

Kula, R. R. & Clopton, R. E. 1999. *Amoebogregarina nigra* n. gen., n. comb. (Apicomplexa: Gregarinidae) from adult *Melanoplus differentialis* (Orthoptera: Acrididae) in South Nebraska. *J. Parasitol.*, 85:321–325.

Lange, C. E. & Cigliano, M. M. 2004. The life cycle of *Leidyana ampulla* sp. n. (Apicomplexa: Eugregarinorida: Leidyanidae) in the grasshopper *Ronderosia bergi* (Stal) (Orthoptera: Acrididae: Melanoplinae). *Acta Protozool.*, 43:81–87.

Lantová, L., Gosh, K., Svobodová, M., Braig, H. R., Rowton, E., Weina, P., Volf, P. & Votýpka, J. 2010. The life cycle and host specificity of *Psychodiella sergenti* n. sp. and *Ps. tobbi* n. sp. (Protozoa: Apicomplexa) in sand flies *Phlebotomus sergenti* and *Ph. tobbi* (Diptera: Psychodidae). *J. Invertebr. Pathol.*, 105:182–189.

Leander, B. S., Clopton, R. E. & Keeling, P. J. 2003. Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and β-tubulin. *Int. J. Syst. Evol. Microbiol.*, 53:354.

Levine, N. D. 1988. The protozoan phylum Apicomplexa. I. CRC Press, Inc., Boca Raton, FL. p. 203.

Mariño-Pérez, R., Fontana, P. & Buzzetti, F. M. 2011. Identificación de plagas de chapulín en el norte-centro de México. *In:* García-Gutiérrez, C. & Lozano-Gutiérrez, J. (ed.), Control biológico de plagas de chapulín en el norte-centro de México. Universidad Autónoma de Zacatecas, México. p. 35–55.

Miller, M. A., Holder, M. T., Vos, R., Mildford, P. R., Liebowitz, T., Chan, L., Hoover, P. & Warnow, T. 2011. The CIPRES Portals. CIPRESS http://www.phylo.org/ssub_sections/portal

Morrone, J. J. 2017. Neotropical biogeography: regionalization and evolution. CRC Press, Boca Raton, FL. p. 282.

Pushkala, K., Muthukumaravel, K., Johny, S., Muralirangan, M. C. & Sanjayan, K. P. 2000. Two new species of cephaline gregarines in grasshoppers (Insecta: Orthoptera) from Tamil Nadu, (India): *Retractocephalus dhawanii* sp. n. (Eugregarinida: Didymorphyidae) and *Hentschelia gilli* sp. n. (Eugregarinida: Dactylophoridae). *J. Orthop. Res.*, 9:57–66.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.*, 67:901–904.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Dearling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, 61:539–542.

Rueckert, S., Chantangsi, C. & Leander, B. S. 2010. Molecular systematics of marine gregarines (Apicomplexa) from Northeastern Pacific polychaetes and nemerteans, with descriptions of three novel species: *Lecudina phyllochaetopteri* sp. nov., *Difficilina tubulani* sp. nov. and *Difficilina paranemertis* sp. nov. *Int. J. Syst. Evol. Microbiol.*, 60:2681–2690.

Rueckert, S. & Devetak, D. 2017. Gregarines (Apicomplexa, Gregarinasina) in psocids (Insecta, Psocoptera) including a new species description and their potential use as pest control agents. *Eur. J. Protistol.*, 60:60–67.

Rueckert, S. & Horák, A. 2017. Archigregarines of the English Channel revisited: new molecular data on *Selenidium* species including early described and new species and the uncertainties of phylogenetic relationships. *PLoS ONE*, 12:e0187430.

Rueckert, S. & Leander, B. S. 2010. Description of *Trichotokara nothriae* n. gen. et sp. (Apicomplexa, Lecudinidae) – an intestinal gregarine of *Nothria conchylega* (Polychaeta, Onuphidae). *J. Invertebr. Pathol.*, 104:172–179.

Rueckert, S., Simdyanov, T. G., Aleoshin, V. V. & Leander, B. S. 2011. Identification of a divergent environmental DNA Sequence clade using the phylogeny of gregarine parasites (Apicomplexa) from crustacean hosts. *PLoS ONE*, 6:e18163.

Rueckert, S., Wakeman, K. C., Jenke-Kodama, H. & Leander, B. S. 2015. Molecular systematics of marine gregarine apicomplexans from Pacific tunicates, with descriptions of five novel species of *Lankesteria*. *Int. J. Syst. Evol. Microbiol.*, 65:2598–2614.

Sanabria-Urbán, S., Song, H., Oyama, K., González-Rodríguez, A. & Cueva-del-Castillo, R. 2017. Integrative taxonomy reveals cryptic diversity in neotropical grasshopper: taxonomy, phylogenetics, and evolution of the genus *Sphenarium* Charpentier, 1842 (Orthoptera: Pyrgomorphidae). *Zootaxa*, 4274(1):1–86.

Sarkar, A. & Chakravarty, M. 1969. Gregarines (Protozoa: Sporozoa) from insects. I. New cephaline gregarines of the family Actinocephalidae. *Proc. Zool. Soc. Calcutta*, 22:17–29.

Schrével, J. y. & Desportes, I. 2013. Introduction: gregarines among Apicomplexa. *In:* Desportes, I. & Schrével, J. (ed.), Treatise on Zoology – Anatomy, Taxonomy, Biology: The Gregarines, the Early Branching Apicomplexa, 1st ed, Vol. 1. Brill, Leiden. p. 7–21.

Seck, A. G. & Toguebaye, B. S. 1995. Etude taxonomique de quelques grégarines (Protozoa, Apicomplexa) parasites de criquets du Sénégal. Description d' *Actinocephalus pyrgomorphae* n. sp. *Bull. Inst. Fond. Afr. Noire Cheikh Anta Diop, Dakar, sér. A*, 48:37–47.

Semans, F. M. 1943. Protozoan parasites reported from the Orthoptera, with special reference to those of Ohio. IV. Classified list of the protozoan parasites of the Orthoptera of the world. Classes Mastigophora, Sarcodina, and Sporozoa. *Ohio J. Sci.*, 43:221–234.

Simdyanov, T. G., Guillou, L., Diakin, A. Y., Mikhailov, K. V., Schrével, J. & Aleoshin, V. V. 2017. A new view on the morphology and phylogeny of eugregarines suggested by the evidence from the gregarine *Ancora sagittata* (Leuckart, 1860) Labbé, 1899 (Apicomplexa: Eugregarinida). *PeerJ*, 5:e3354.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30:1312–1313.

Stork, N. E. 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Annu. Rev. Entomol.*, 63:31–45.

Théodorides, J., Desportes, I. & Jolivet, P. 1972. Grégarines de la Nouvelle-Guinée et des îles voisines. *Cah. Pac.*, 16:110–168.

Théodorides, J., Desportes, I. & Jolivet, P. 1975. Grégarines de la Thailande. *Ann. Parasitol. Hum. Comp.*, 50:145–159.

Théodorides, J., Ormières, R. & Jolivet, P. 1958. Eugrégarines parasites d'Orthoptéroïdes. *Explor. Parc. Nat. Albert, Bruxelles*, 2:3–44.

Votýpka, J., Lantová, L., Gosh, K., Braig, H. & Volf, P. 2009. Molecular characterization of gregarines from sand flies (Diptera: Psychodidae) and description of *Psychodiella* n. g. (Apicomplexa: Gregarinidae). *J. Eukaryot. Microbiol.*, 56:583–588.

Wakeman, K. C. & Leander, B. S. 2013. Molecular phylogeny of marine gregarine parasites (Apicomplexa) from tube-forming polychaetes (Sabellariidae, Cirratulidae, and Serpulidae), including descriptions of two new species of *Selenidium*. *J. Eukaryot. Microbiol.*, 60:514–525.

Wakeman, K. C., Yabuki, A., Fujikura, K., Tomikawa, K. & Horiguchi, T. 2018. Molecular phylogeny and surface morphology of *Thiriotia hyperdolphinae* n. sp. and *Cephaloidophora oradareae* n. sp. (Gregarinasina, Apicomplexa) isolated from a deep sea *Oradarea* sp. (Amphipoda) in the West Pacific. *J. Eukaryot. Microbiol.*, 65:372–381.

Yumnam, I. & Mohilal, N. 2017. A new species of *Quadruspinospora* Sarkar and Chakravarty, 1969 (Apicomplexa: Conoidasida) from Orthopteran insects of Manipur, India. *J. Parasit. Dis.*, 41:313–317.