REVISTA ECUATORIANA DE CIENCIA, TECNOLOGÍA E INNOVACIÓN EN SALUD PÚBLICA
CÓDIGO ISSN 2588-0551
DOI: https://doi.org/10.31790/inspilip.v6i1.267

Original article

Parasitism of *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in immature stages and adults of *Aedes aegypti* (Diptera: Culicidae) from Ecuador.

Parasitismo de Ascogregarina culicis (Apicomplexa: Lecudinidae) en estadios inmaduros y adultos de Aedes aegypti (Diptera: Culicidae) de Ecuador.

Open access

Citatión

Carrazco A , Ponce P, Lagos P, Coloma J, Varsovia C. Parasitism of *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in immature stages and adults of *Aedes aegypti* (Diptera: Culicidae) from Ecuador. Scientific Magazine **INSPILIP. 2022; 6 (1).**

Andrés Carrazco Montalvo MSc. [a] , acarrazco@inspi.gob.ec
Patricio Ponce Ph.D. [a], wponce@inspi.gob.ec
Paola Lagos MSc. [a] , jlagos@inspi.gob.ec
Josefina Coloma Ph.D. [b] , colomaj@berkeley.edu
Varsovia Cevallos, Ph.D. [a] , vcevallos@inspi.gob.ec

a. National Institute of Public Health Research; National Reference Center for Genomics, Sequencing and Bioinformatics (GENSBIO), Quito 170136, Ecuador
b. School of Public Health and Vaccinology, University of California, Berkeley, CA, USA, 94720-7360.

Correspondence: Varsovia Cevallos, Ph.D. **Email:** varsoviacevallos@yahoo.com; Tel.: +593999197839

Identification of the responsibility and contribution of the authors: (Conceptualization, V.C., and P.P.; methodology, V.C., A.C.-M., P.P., and P.L.; software, A.C.-M.; validation, A.C.-M.; formal analysis, A.C.-M.; investigation, V.C., A.C.-M., P.P., and P.L.; resources, V.C., P.P., and J.C.; data curation, A.C.-M.; writing—original draft preparation, A.C.-M, and P.L.; writing—review & editing, V.C., A.C.-M., P.P., and J.C.; visualization, A.C.-M., and P.L.; supervision, V.C., P.P., and J.C.; project administration, V.C., P.P, and J.C.; funding acquisition, V.C. and P.P. All authors have read and agreed to the published version of the manuscript.

Date of admission: 02/12/2021. **Approval date:** 31/03/2022. **Publication date:** 05/05/2022.

Abstract

Objectives: *Aedes aegypti* and Ae. albopictus transmit several medically important arboviruses to humans. These mosquitoes can carry gregarine protozoan parasites that invade the midgut epithelial cells, where they complete their life cycles. There are no reports of the presence of this parasite associated with *Aedes* species in Ecuador. This study aimed to identify the presence of *Ascogregarina* in naturally infected *Ae. aegypti* and Ae. albopictus that were collected in several localities of Ecuador. Methods: The Ascogregarine parasite was identified morphologically by microscopy and by sequencing using a ssrRNA conserved region. Results: We present the first record of the protozoan *Ascogregarina culicis* infecting *Aedes aegypti* from Ecuador. The parasite was found in 30 adult and immature mosquitoes out of 260 individuals. Conclusion: This study presents tools to identify the parasite and highlights the importance of studying the parasitism of mosquitoes for biological control and their possible role in arbovirus transmission.

Keywords: Parasites. Culicidae. *Aedes*. Apicomplexa. Entomology.

Resumen

Objetivos: *Aedes aegypti* Linnaeus 1762 y *Ae. albopictus* (Skuse, 1894) transmiten varios arbovirus de importancia médica a los seres humanos. Estos mosquitos pueden portar parásitos protozoarios gregarinos que invaden las células epiteliales del intestino medio, donde completan sus ciclos de vida. No existen reportes de la presencia de este parásito asociado a especies del género *Aedes* en Ecuador. Este estudio tuvo como objetivo identificar la presencia de *Ascogregarina* en *Ae. aegypti* y *Ae. albopictus* recolectados en varias localidades del Ecuador. Métodos: El parásito *Ascogregarina* fue identificado por microscopía y por secuenciación utilizando una región conservada de ARNss. Resultados: Presentamos el primer registro del protozoo *Ascogregarina culicis* infectando naturalmente *Aedes aegypti* en Ecuador. El parásito se encontró en 30 individuos adultos e inmaduros de 260 mosquitos analizados. Conclusión: Este estudio presenta herramientas para identificar el parásito y destaca la importancia de estudiar el parasitismo de la microbiota de los mosquitos, y su uso potencial en el control biológico y su posible papel en la transmisión del arbovirus a los humanos.

Palabras clave: Parásitos. Culicidae. *Aedes*. Apicomplexa. Entomología.

Introduction

Aedes aegypti (Linnaeus, 1762) and *Ae. albopictus* (Skuse, 1894) are responsible for transmitting several arboviruses of human importance such as Zika, Dengue, Yellow fever, and Chikungunya, among others [1].

These mosquitoes can naturally harbor protozoan parasites of the genus *Ascogregarina* (Apicomplexa: Lecudinidae). *Ascogregarina culicis* (Ross, 1898) has been identified in *Ae. aegypti* [2-4] in urban and suburban habitats [5] and *Ascogregarina* taiwanensis have been recorded in Ae. *albopictus*.

Aedes aegypti is widely distributed in tropical and subtropical areas of continental Ecuador and the Galápagos Islands, while *Aedes albopictus* (Skuse, 1894) was reported for the first time in Guayaquil, Ecuador in 2017 [6].

The parasite *Ascogregarina* oocysts are ingested by the first stage larvae and released as sporozoites, infecting the gut epithelial cells, and progressing inside the midgut cell as trophozoites. Once the ecdysone hormone (20-hydroxyecdysone) levels in mosquito metamorphosis increase, the trophozoites migrate to the Malpighian tubules, changing into sexual gametes [3].

The gametocyte contains hundreds of oocysts during the pupal stage and is released into the environment after the metamorphosis of pupae into adults. The transmission of these parasites to other mosquitoes occurs when oocysts released by defecation of infected larvae are ingested by co-habitat larvae.

The life cycle of the parasites seems to be completely adapted to the mosquito life cycle, suggesting strong parasite-host coevolution [3].

In Brazil, the presence of *Ascogregarina* taiwanensis and *Ascogregarina culicis* has been reported in larvae of *Ae. albopictus* and *Ae. aegypti*, respectively, with similar mean infection intensities in both species of mosquito [7, 8]

The role of the midgut microbiota of *Ae. aegypti* may contribute to understanding its population dynamics and its vectorial capacity of viral diseases [9].

There are no reports of the presence of this parasite associated with *Aedes* species in Ecuador. This study aimed to identify the presence of *Ascogregarina* in naturally infected *Ae. aegypti* and *Ae. albopictus* that were collected in several localities of Ecuador.

Materials and Methods

Collection of specimens

Immature individuals were collected in plastic containers and transported to the laboratory for adult emergence. Adults were collected using a Prokopack aspirator and stored individually in 1.5 ml tubes. The geographical coordinates of each collection location were recorded.

Immature stages and adults of *Aedes aegypti* and *Aedes albopictus* were collected in urban and rural localities from several tropical and/or subtropical areas in Ecuador (Table 1).

A total of 164 adults *Ae. aegypti* were collected in Esmeraldas, Manta, Guayaquil, Babahoyo, Chone, Portoviejo, Cumanda, Machala (Pacific coast); Santo Domingo de los Tsáchilas (Andean lowlands); Tena, Nueva Loja (Lago Agrio), Francisco de Orellana, Puyo (Amazon Basin) and Santa Cruz (Galápagos) in 2014. We collected 40 larvae, 15 pupae, and 40 adults of *Ae. aegypti* in Borbón (Pacific coast) and the same number of samples of *Ae. albopictus* in Cachaco (Pacific Coast) in 2020 (Table 1).

Voucher samples were deposited at the National Collection of Arthropod Vectors of the "Instituto Nacional de Investigación en Salud Pública" (INSPI) under the operating patent 018-2019-FAU-DPAP-MA.

Table 1. *Collection sites of Aedes aegypti and Aedes albopictus individuals in tropical and subtropical regions of Ecuador.*

Aedes aegypti						
Region	**Province**	**Locality**	**Latitude**	**Longitude**	**Collection year**	**Number of specimens**
Pacific coast	Esmeraldas	Esmeraldas	0.9592	-79.6539	2014	23
	Manabí	Manta	-0.9621	-80.7127	2014	8
	Guayas	Guayaquil	-2.1961	-79.8862	2014	68
	Los Ríos	Babahoyo	-1.8021	-79.5344	2014	5
	Manabí	Chone	-0.6981	-80.0936	2014	8
	Manabí	Portoviejo	-1.0545	-80.4544	2014	5
	Chimborazo	Cumanda	-2.2050	-79.1356	2014	2
	El Oro	Machala	-3.2586	-79.9605	2014	10
	Esmeraldas	Borbón	1.0883	-78.9889	2020	95
Western Andean slopes	Santo Domingo de los Tsáchilas	Santo Domingo	-0.2530	-79.1753	2014	6
Amazon basin	Napo	Tena	-0.9938	-77.8128	2014	1
	Sucumbios	Lago Agrio	0.0860	-76.8952	2014	14
	Orellana	Francisco de Orellana	-0.4664	-76.9871	2014	2
	Pastaza	Puyo	-1.4836	-78.0025	2014	4
Galápagos	Galápagos	Santa Cruz	-17.7862	-63.1811	2014	9

Aedes albopictus						
Region	Province	Locality	Latitude	Longitude	Collection year	Number of specimens
Pacific coast	Imbabura	Cachaco	0.8333	-78.4666	2020	95

Parasite morphological identification

All mosquitoes were dissected by cutting off the last abdominal segments. The intestines and Malpighian tubules were removed and placed on a slide and stained for 30 seconds with Lugol's solution. The identification of *Ascogregarina* at each stage was performed and photographed using an optical microscope (ZEISS), using 10x, 40x, and 100x magnification as necessary.

DNA extraction, PCR, and sequencing

Each mosquito was frozen in liquid nitrogen and then macerated using a micropistile. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, #69506). Manufacturer directions were followed with modifications: samples were lysed while shaking overnight at 56°C and genomic DNA was eluted in 100 µL of Buffer AE.

The PCR amplification of the small subunit from the ribosomal RNA (ssrRNA) was performed using the primers rPLU5 (5'-CCT GTT GTT GCC TTA AAC TTC-3') and rPLU6 (5' TTA AAA TTG TTG CAG TTA AAA CG-3') and following the protocol [10].

The PCR amplicons obtained by RPLU primers were Sanger sequenced in both directions at Macrogen services (Seoul, South Korea).

The obtained sequences were analyzed using the BLAST database (Basic Local Alignment Search Tool – NCBI – NIH) to confirm the identity of the *Ascogregarina* genus. For genetic analysis, sequence alignment and cleaning were performed using Geneious Prime 2019.0.4.17 [11].

The parasite identification was established by BLAST in the NCBI repository.

Results

The microscopy and molecular analysis showed the infection by *Ascogregarina* in 30 individuals *Ae. aegypti* out of the 260 individuals analyzed. Three stages of *Ascogregarina culicis* were identified in mosquito immature stages. Larvae had trophozoites located in the extracellular tissue of the intestine (Figure 1a).

While the pupae had gametocytes of the parasite located in the Malpighian tubules (Figure 1b), the adults presented oocysts located also in the Malpighian tubules (Figure 1c).

The parasite was not found in *Ae. albopictus* individuals analyzed. In the molecular analysis, 17 amplicons had a molecular weight of 693 bp, the expected size of *Ascogregarina*.

The BLAST result indicated 100% identity and query cover for *Ascogregarina culicis* (GenBank: KU238663.1, KU238664.1, KU238665.1).

Ascogregarina culicis was detected in *Ae. aegypti* from five localities in Ecuador: Santa Cruz (Galápagos), Esmeraldas, Borbón, Manta and Guayaquil (Pacific coast) (Figure 2).

Aedes aegypti infected with *Ascogregarina culicis* were collected during the dry, rainy, and transition seasons. Mosquito infection rates varied from 11% to 18%, with the highest infection observed in mosquitoes from Guayaquil. We present a summary of the individuals collected and the infection rate (Table 2).

***Figure 1.** Ascogregarina culicis found in different stages of Aedes aegypti.*
***a.** Trophozoite in the fourth larvae stage in the mid-gut of the larvae.*
***b.** Gametocytes in the Malpighian tubules of pupae.*
***c.** Oocyst in the Malpighian tubules of adults.*

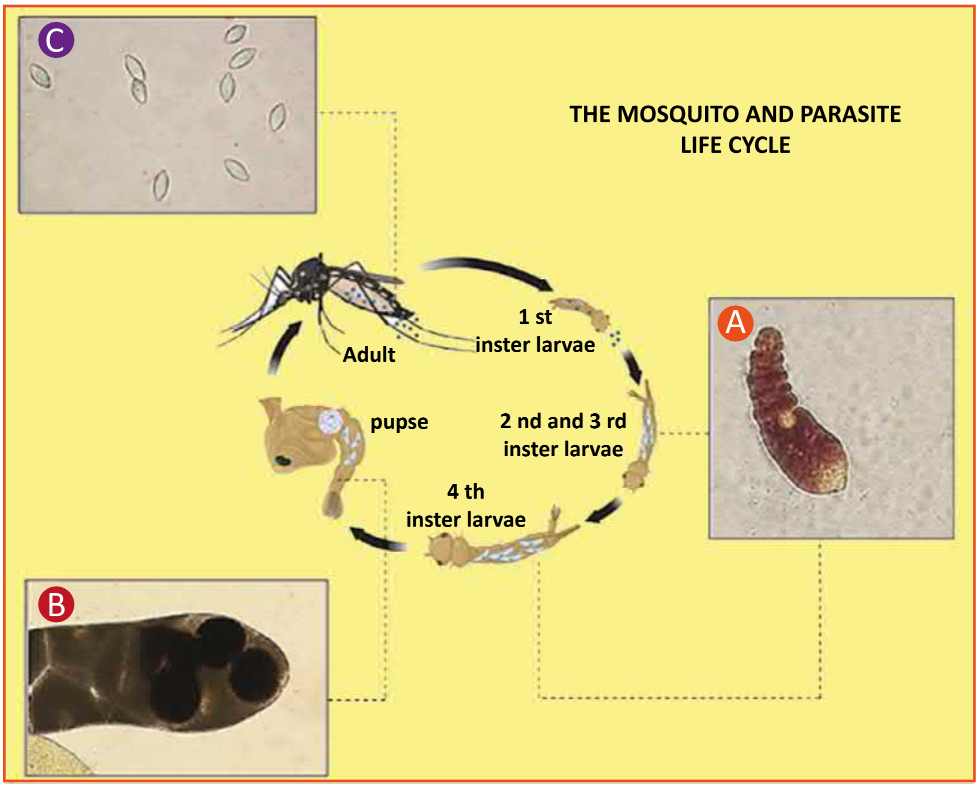

***Figure 2.** Map of distribution of Ascogregarina culicis in Aedes aegypti in Ecuador.*
This shows positive and negative samples.

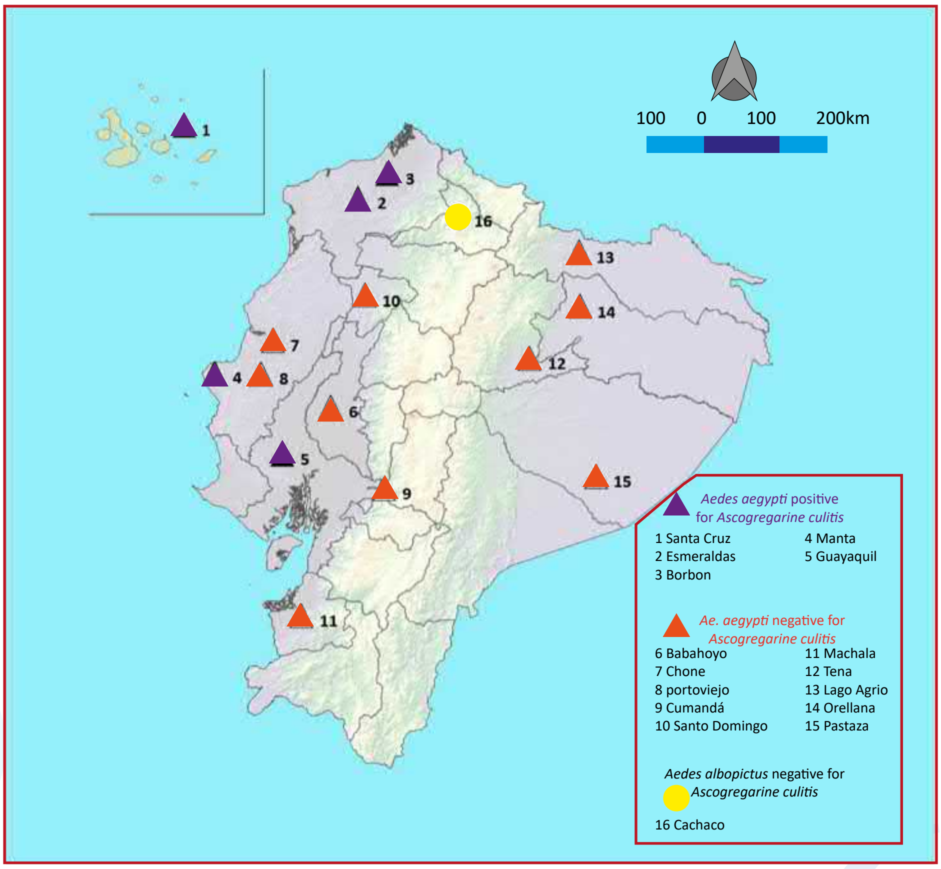

Table 2. *Aedes aegypti with the presence of Ascogregarina culicis identified by microscopy and PCR*

Locality	Collection date - Season	Parasite positive mosquitoes	Infection rate
Esmeraldas	01/09/2014 Dry 16/06/2014 Transition	2 1	3/23 (13%)
Guayaquil	17/03/2014 31/03/2014 Rainy 19/05/2014 Rainy 09/06/2014 Transition	1 2 3 6	12/68 (18%)
Manta	23/06/2014 Transition	1	1/8 (13%)
Santa Cruz	24/08/2014 Dry	1	1/9 (11%)
Borbón	10/03/2020 Rainy	13	13/95 (14%)
Total		**30**	**30/260 (12 %)**

Discussion

This is the first report of *Ascogregarina culicis* infecting *Aedes aegypti* in Ecuador. Previous studies have reported *Ae. aegypti* infected with this parasite in Colombia, Argentina, and Brazil [4, 7, 8, 12, 13].

The pathogenicity of *Ascogregarina* on the larval development of *Ae. aegypti* has contrasting reports. Some authors have reported higher mortality and shorter larval development [14, 15], while others have indicated no effect of the parasite on the larval development, size, mortality, or adult emergence [16]

Nevertheless, the possibility of using *Ascogregarina* as a biocontrol agent has been suggested, mainly because recent studies showed that under specific conditions (high dosage infection, crowding, non-natural infection, and low amount of nutrients), the mortality rate of mosquito larvae is increased [17-19].

Ascogregarina culicis and *Ascogregarina* taiwanensis [20] have been proposed as biological control of *Ae. aegypti* and Ae. *albopictus*, respectively [18].

In this study, *Ascogregarina culicis* was recorded during the rainy, dry, and transition seasons in a wide range of areas in Ecuadorian Coastal and insular regions. This has been previously reported in other studies [13], where the abundance of *Ascogregarina* parasite is correlated with high numbers of *Aedes* mosquitoes as its main reservoirs. Additionally, the molecular analysis of the parasite showed polymorphism in a sample from Esmeraldas that indicates genetic variability [21].

Ascogregarina parasites were not detected in samples from the Amazon region, this may be due to the low number of individuals analyzed.

Specific identification of *Ascogregarina* using morphological approaches is a complicated task because *Ascogregarina culicis* and *Ascogregarina* taiwanensis share similar sizes and morphology. The use of molecular tools has facilitated the identification process, reducing the subjectivity of morphological techniques [4].

For this analysis, a protocol initially designed to identify Plasmodium was used [10], since Plasmodium and *Ascogregarina* belong to the same phylum. We used 18S rRNA as a molecular marker for identification.

The taxonomy and classification studies of gregarines still present difficulties because microscopy data are not as reliable and molecular data are fragmentary [22].

The possible negative effects of *Ascogregarina* parasites on *Aedes* fitness and larvae progression to adult stages have sparked interest in the study of this naturally infecting parasite.

There may be a relationship between the presence of *Ascogregarina* and arboviral infections, mainly because the mosquito midgut epithelium cells are used by *Ascogregarina* sporozoites and viruses to complete the parasite life cycle or complete replication, respectively.

Few studies have investigated the effect of *Ascogregarina* on pathogen transmission by mosquitoes. A study by Mourya et al. [23] suggested that the Chikungunya virus could be vertically transmitted via *Ascogregarina* oocysts in *Ae. aegypti*. On the other hand, a recent study by Thongsripong and Wesson [24] concluded that *Ascogregarina culicis* infection does not influence the body size and dengue virus infection in *Ae. aegypti*.

Aedes albopictus analyzed in our study came from the Cachaco locality and all the specimens were negative for *Ascogregarina* species. Several articles report *Ae. albopictus* infected by *Ascogregarina* taiwanensis [7,25,26], and it has been suggested that *Ae. albopictus* has a competitive advantage in the absence of parasitism [27].

Although there are contrasting criteria among authors regarding the potential of *Ascogregarina* as mosquito biocontrol and as an arboviral prevention agent, our findings highlight the importance of understanding host-parasite interactions and their role in viral replication and transmission.

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgments: We thank Joan Ponce, Francesca Ponce, and Stephany Villota, for revising the manuscript.

Funding: This work was supported by the National Institutes of Health – National Institute of Allergy and Infectious Diseases NIH-NIAID Grant: Co-circulation on Zika and Dengue under environmental change and urbanization: a longitudinal study in Esmeraldas, Ecuador; A2CARES U01 AI151788-01 (NIH NIAID); and the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) [PIC-12-INHMT-002].

Data Availability Statement: Sequences used in this study are deposited in Genbank under the accession numbers KU238663.1, KU238664.1, KU238665.1).

References

1. Crawford, J. E., Alves, J. M., Palmer, W. J., Day, J. P., Sylla, M., Ramasamy, R., Surendran, S. N., Black, W. C., Pain, A., Jiggins, F. M., 2017. Population genomics reveals that an anthropophilic population of *Aedes aegypti* mosquitoes in West Africa recently gave rise to American and Asian populations of this major disease vector. BMC Biol, 15, 16, https://doi.org/10.1186/s12915-017-0351-0

2. Beier, J. C., Craig, G. B., 1985. Gregarine parasites of mosquitoes: In Integrated mosquito control methodologies. Volume 2, M. Laird (ed.). Academic, London, U.K., 167–184.

3. Lantova, L., Volf, P., 2014. Mosquito and Sand Fly Gregarines of the Genus *Ascogregarina* and Psychodiella (Apicomplexa: Eugregarinorida, Aseptatorina) – Overview of Their Taxonomy, Life Cycle, Host Specificity, and Pathogenicity. Infect. Genet. Evol. 28. Elsevier B.V.: 616–27, https://doi.org/10.1016/j.meegid.2014.04.021

4. Morales, M. E., Ocampo, C. B., Cadena, H., Copeland, C. S., Termini, M., Wesson, D. M., 2005. Differential identification of *Ascogregarina* species (Apicomplexa: Lecudinidae) in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by polymerase chain reaction. J. Parasitol. 91 (6): 1352–56, https://doi.org/10.1645/GE-442R.1

5. Thongsripong, P., Chandler, J. A., Green, A. B., Kittayapong, P., Wilcox, B. A., Kapan, D. D., Bennett, S. N., 2017. Mosquito vector-associated microbiota: Metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. Ecol Evol. 27;8(2):1352-1368, https://doi.org/10.1002/ece3.3676

6. Ponce, P., Morales, D., Argoti, A., Cevallos, V. E., 2017. First Report of *Aedes* (Stegomyia) *albopictus* (Skuse) (Diptera: Culicidae), the Asian Tiger Mosquito, in Ecuador. Journal of Medical Entomology, no. November (October): 1–2, https://doi.org/10.1093/jme/tjx165

7. Passos, R. A., Tadei, W. P., 2008. Parasitism of *Ascogregarina* taiwanensis and *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in larvae of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) from Manaus, Amazon Region, Brazil. J. Invertebr. Pathol. 97 (3): 230–36, https://doi.org/10.1016/j.jip.2007.09.008

8. Prophiro, J. S., Pereira, T. N., de Oliveira, J. G., Dandolini, G. W., Navarro da Silva, M. A., Santos da Silva, O., 2017. *Ascogregarina* taiwanensis infection in *Aedes aegypti* and *Aedes albopictus* in Santa Catarina, South Brazil. Rev. Soc. Bras. Med. Trop. 50 (2): 235–38, https://doi.org/10.1590/0037-8682-0048-2016

9. Yadav, K. K., Bora, A., Datta, S., Chandel, K., Gogoi, H. K., Prasad, G. B. K. S., Veer, V., 2015. Molecular Characterization of Midgut Microbiota of *Aedes albopictus* and *Aedes aegypti* from Arunachal Pradesh, India. Parasites & Vectors. 8 (1) : 641. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-015-1252-0

10. Snounou, G., Viriyakosol, S., Zhu, X. P., Jarra, W., Pinheiro, L., do Rosario V. E., Thaithong, S., Brown, K. N., 1993. High Sensitivity of Detection of Human Malaria Parasites by the Use of Nested Polymerase Chain Reaction. Mol Biochem Para-sitol. 61 (2): 315–20. Available at: https://doi.org/10.1016/0166-6851(93)90077-B

11. Geneious Prime 2019.0.4. Available at: http://www.geneious.com

12. Albicócco, A. P., Vezzani, D., 2009. Further Study on *Ascogregarina culicis* in Temperate Argentina: Prevalence and In-tensity in *Aedes aegypti* Larvae and Pupae. J. Invertebr. Pathol. 101 (3). Elsevier Inc.: 210–14. https://doi.org/10.1016/j.jip.2009.05.003

13. Vezzani, D., Wisnivesky, C., 2006. Prevalence and Seasonality of *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in Natural Populations of *Aedes aegypti* (Diptera: Culicidae) from Temperate Argentina. J. Invertebr. Pathol. 91 (3): 183–87, http://dx.doi.org/10.1016/j.jip.2005.12.001.20

14. Beier, J. C., 1983. Effects of Gregarine Parasites on the Development of Dirofilaria Immitis in *Aedes* triseriatus (Diptera: Culicidae). J Med Entomol 20 (1): 70–75. https://doi.org/10.1093/jmedent/20.1.70

15. Reyes-Villanueva, F., Becnel, J. J., Butler, J. F., 2003. Susceptibility of *Aedes aegypti* and *Aedes albopictus* larvae to *Ascogregarina culicis* and *Ascogregarina* taiwanensis (Apicomplexa: Lecudinidae). Florida. J Invertebr Pathol. 84(1):47-53. https://doi.org/10.1016/S0022-2011(03)00119-8

16. McCray, E. M., Fay, R. W., Schoof, H. F., 1970. The Bionomics of Lankesteria *Culicis* and *Aedes Aegypti*. J. Invertebr. Pathol. 16 (1): 42–53, https://doi.org/10.1016/0022-2011(70)90203-X

17. Fellous, S., Koella, J. C., 2010. Cost of Co-Infection Controlled by Infectious Dose Combinations and Food Availability. Oecologia 162 (4): 935–40, https://doi.org/10.1007/s00442-009-1535-2

18. Tseng, M., 2007. Ascogregarine Parasites as Possible Biocontrol Agents of Mosquitoes. JAMCA. 23 (sup 2): 30–34. https://doi.org/10.2987/8756-971X(2007)23[30:APAPBA]2.0.CO;2

19. Soghigian, J., Livdahl, T., 2017. Differential Response to Mosquito Host Sex and Parasite Dosage Suggest Mixed Dispersal Strategies in the Parasite *Ascogregarina* taiwanensis. Edited by Olle Terenius. PLOS ONE 12 (9): e0184573, https://doi.org/10.1371/journal.pone.0184573

20. Lien, S., Levine, N., 1980. Three new species of Ascocystis (Apicomplexa, Lecudinidae) from Mosquitoes. J Protozool, 27(2): 147-151. https://doi.org/10.1111/j.1550-7408.1980.tb04669.x

21. Parimittr, P., Chareonviriyaphap, T., Bangs, M., Arunyawat, U., 2018. Genetic variation of *Aedes aegypti* mosquitoes across Thailand based on nuclear DNA sequences. AGNR: Vol.52: 596-602. https://doi.org/10.1016/j.anres.2018.11.021

22. Simdyanov, T.G.; Guillou, L.; Diakin, A.Y.; Mikhailov, K.V.; Schrevel, J.; Aleoshin, V.V. 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 2017, 5, e3354. https://doi.org/10.7717/peerj.3354

23. Mourya, D. T., Singh, D. K., Yadav, P., Gokhale, M. D., Barde, P. V., Narayan, N. B., Thakare, J. P., Mishra, A. V., Shouche, Y. S., 2003. Role of Gregarine Parasite *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in the Maintenance of Chikungunya Virus in Vector Mosquito. J. Eukaryot. Microbiol. 50 (5): 379–82, https://doi.org/10.1111/j.1550-7408.2003.tb00153.x

24. Thongsripong, P., Wesson, D. M., 2021. Effects of *Ascogregarina culicis* (Eugregarinorida: Lecudinidae) on Mosquito Size and Dengue Virus Infection in *Aedes aegypti* (Diptera: Culicidae), Journal of Medical Entomology, Volume 58, Issue 3: 1442–1447, https://doi.org/10.1093/jme/tjaa280

25. Chen, W.J., 1999. The life cycle of *Ascogregarina* taiwanensis (Apicom- plexa: Lecudinidae). Parasitol. Today 15 (4), 153–156. https://doi.org/10.1016/S0169-4758(99)01418-0

26. Chen, W.J., Chow, C.Y., Wu, S.T., 1997. Ultrastructure of infection, development and gametocyst formation of *Ascogregarina* taiwanensis (Apicomplexa: Lecudinidae) in its mosquito host, *Aedes albopictus* (Diptera: Culicidae). J. Euk. Microbiol. 44 (2), 101–108. https://doi.org/10.1111/j.1550-7408.1997.tb05945.x

27. Stump, E., Childs, L.M., Walker, M., 2021. Parasitism of *Aedes albopictus* by *Ascogregarina* taiwanensis lowers its competitive ability against *Aedes* triseriatus. Parasites Vectors 14, 79. https://doi.org/10.1186/s13071-021-04581-0