Acarologia
Open Science in Acarology

Notes on the embryological development of the *Brevipalpus yothersi* (Acari: Tenuipalpidae)

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Original research

ABSTRACT

Mites of the *Brevipalpus* genus are economically important due to their role as vectors of plant viruses. Among them, *B. yothersi* is well studied because it is the recognized main vector of the citrus leprosis virus C (CiLV-C), the causal agent of citrus leprosis, which is one of the most damaging viral diseases in the citrus crops. However, studies of several aspects of the biology of *B. yothersi* are still scarce, including its embryology. We performed a descriptive embryonic development study of *B. yothersi*, utilizing light and scanning electron microscope to obtain biological information of this particular phytovirus vector. Distinct events were recorded, such as centering of the nucleus, mitotic divisions for primitive body formation and appendages, as well as the presence of channels inside the egg and their possible association with the respiratory tract. Here we provide the first notes about embryonic development of this important mite vector in the Americas.

Keywords phytophagous mite; vector-mite; embryology; citrus leprosis disease

Introduction

Flat mites in the genus *Brevipalpus* Donnadieu (Tenuipalpidae) have a worldwide distribution with more than 300 plant species described as their host (Beard *et al.* 2015). Some *Brevipalpus* species are notorious by harboring *Cardinium* symbiont and to be only organism within the Metazoa that exists as female haploid state (Weeks *et al.* 2001) and there are ability to transmit plant viruses to important crops such as orchids and others ornamental plants, coffee, passion fruit and citrus (Childers and Rodrigues 2011; Dietzgen *et al.* 2018).

Among these species, there is *Brevipalpus yothersi* Baker that is the main vector of citrus leprosis (CL) disease (Bassanezi *et al.* 2019). CL severely affects the citrus industry production and has a multi-etiological viral cause (Freitas-Astúa *et al.* 2018). Nevertheless, citrus leprosis virus C (CiLV-C; family *Kitaviridae*; genus *Cilevirus*) and *B. yothersi* are the prevalent complex (virus-vector) present in citrus orchards of Latin America (Andrade *et al.* 2018; Bassanezi *et al.* 2019; Chabi-Jesus *et al.* 2021). Despite the importance of *B. yothersi* and the improvement in microscopy in the last years, studies on biology and embryological development are scarce.

Actually, embryology of the mites is difficult due to adhesion of the eggs to the substrate, and removal causes rupture due to the diminutive size, besides the impermeability of eggshell to fixatives (Thomas and Telford 1999; Laumann *et al.* 2010a). However, embryological development studies were successfully performed with mites from Tetranychidae and Eriophyoidea family/superfamily (Gotoh *et al.* 1994; Dearden *et al.* 2002; Chetverikov and Desnitskiy 2016) suggesting that it is possible to advance with information to the peculiar flat mites. *Tetranychus*

Received 25 August 2021
Accepted 23 January 2022
Published 26 January 2022

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Academic editor
Navia, Denise

https://doi.org/10.24349/0gr3-3a6b

ISSN 0044-586X (print)
ISSN 2107-7207 (electronic)

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urticae studies have focused embryology to phylogeny, through knowledge of genes responsible to embryo segmentation and germ-cell specification, and gene silencing studies (Dearden *et al.* 2002, 2003; Khila and Grbic 2007). Here, due to the current importance of the *B. yothersi* as biological system and viral vector, the main goal of this study was to provide information about embryonic development using light microscopy and scanning electron microscopy.

Adult females of *B. yothersi* isoline were transferred from orange fruits to arenas mounted on square Petri dishes (120 x 120 mm) containing jack bean leaves [*Canavalia ensiformis* (L.) DC] surrounded by moistened cotton. These arenas were observed hourly using the stereoscopic microscope, looking for the oviposition to collect the eggs and maintained under temperature 25 °C ± 5 °C and relative humidity 60% ± 10%. To observe the early stages of the development, freshly laid eggs were transferred to microscope slides containing one drop of mineral oil (liquid paraffin) and observed for seven hours. Embryos older than 20 hours after oviposition were immersed in Hoyer's medium on the microscope slide. These samples were examined in a Leica DM750 light microscope (LM) (Leica Microsystems, Co., Wetzlar, Germany).

Three hundred eggs of *B. yothersi* were fixed in Carnoy's solution for 30 minutes, washed twice in 90% ethanol for 10 minutes and transferred to 0.1M pH 7.4 phosphate buffer solution. The eggs were dehydrated in a graded series of acetone (30, 50, 70, 80, and 100% for 10 minutes), then critical point dried with liquid CO_2 (Baltec CPD 030), and transferred to aluminum stubs and sputter-coated with gold (Baltec SCD 050). Examinations were made using a tabletop Phenom scanning electron microscope (SEM).

Results and discussion

Brevipalpus yothersi embryological development

The *B. yothersi* embryological development was observed for seven days under experimental conditions and the eggs were easily visible by LM (Figure 1). Eggs collected soon after oviposition were slightly elongated and elliptical in shape (average of 108 µm length x 70 µm width) (Haramoto 1969), with light orange coloration, and nuclei that appeared red.

At the earliest cleavage stages, about one-hour post-laying, the nucleus occupied the center of the egg (Figure 1b). Two hours after oviposition, the first division began with the separation of the nucleus into two blastomeres of the same size and shape (Figure 1c) divided by a thin membrane. At three hours post-oviposition, an equal cleavage resulted in the total division of blastomeres into four cells (Figure 1d). Five hours after, two new divisions occurred with the formation of the eight and 16-cells stages (Figure 1e, f). After, a large number of cells were observed, suggesting the occurrence of three more cleavages, reaching a total of about 128 blastomeres (Figure 1g), following the formation of a cluster of cells, the morula.

In a period of 20 to 24 hours after egg-laying, the cells migrated and agglomerated in the peripheral region of the egg forming the blastula (Figure 1h). After 48 hours, the formation of the germinal disc (periblastula) in the ventral region was observed (Figure 1i). The periblastula started to stretch and then becomes flattened (Figure 1j). After three days, the embryo was laterally inside the egg, initiating the formation of the primitive body (Figure 1k), where it was visible the formation of metamers (Figure 1l). From the fourth to the fifth day, the formation of primordial appendages was evident (Figure 1m). On the sixth post-oviposition day, the dark-colored ocelli formation was initiated (Figure 1n), being more visible on the seventh day before the larvae hatched (Figure 1o), and ending with hatching of a hexapod larvae (Figure 1p). This pattern is according to other arachnids that only acquire the fourth pair of legs after their larvae undergo moults for the subsequent developmental stage (Dearden *et al.* 2002; Sharma 2018).

The analysis of the *B. yothersi* eggs in LM allowed the description of the different embryological stages. To our best knowledge, this report is the first embryological description of false spider mites and demonstrated that the use of the liquid paraffin was essential to observe

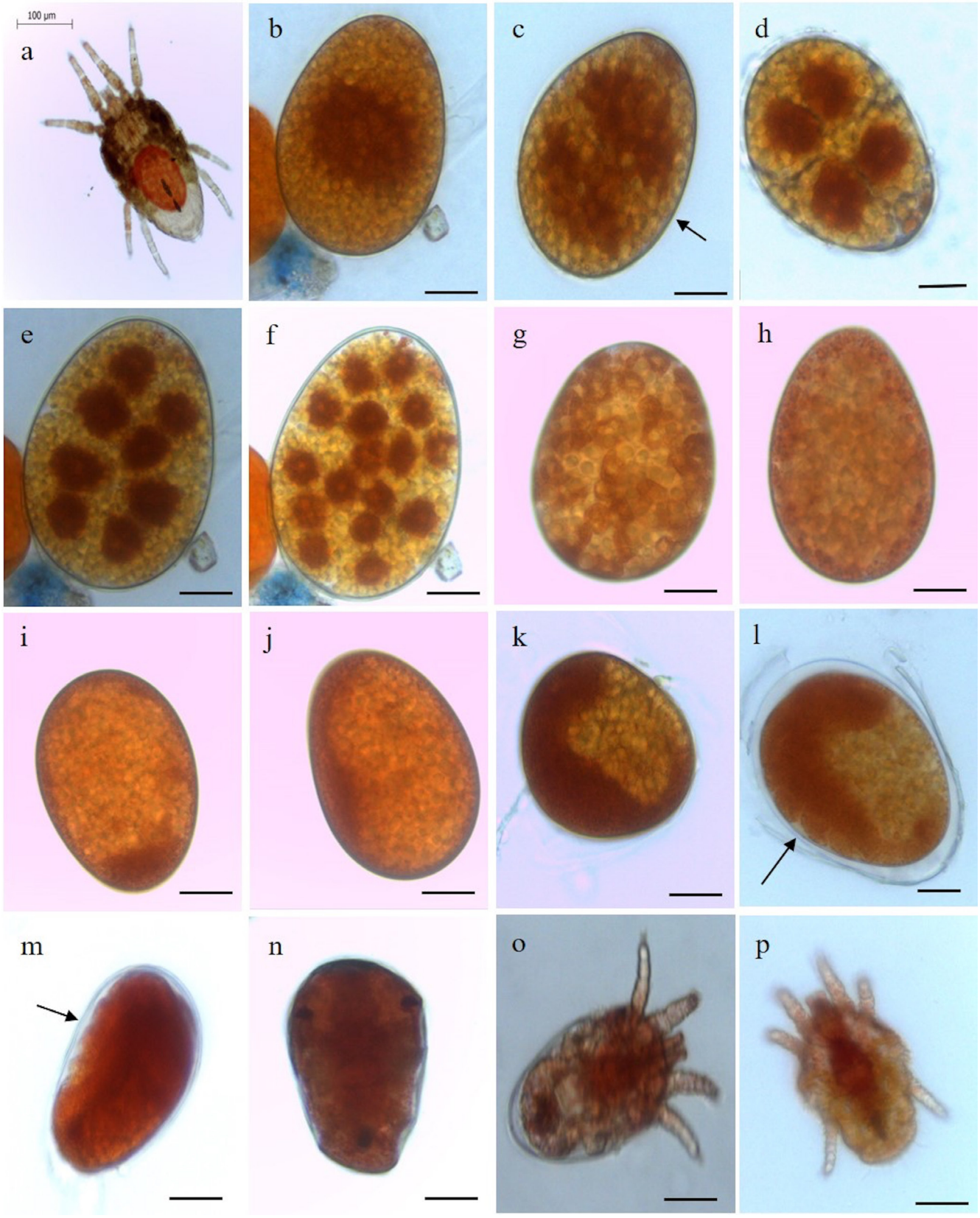


Figure 1 Development timeline of the *Brevipalpus yothersi*: (a) – *B. yothersi* female; (b) – Uncleaved egg: nucleus in the center of the egg; (c) – Two-cell stage embryo divided by visible membrane (arrow); (d) – Second cleavage: four-cell stage; (e) – Eight-cell stage; (f) – 16-cell stage; (g) – 128-cell stage; (h) – Migration of cells to the peripheral region: blastula formation; (i) – Visible germinal disc in the ventral region; (j) – Distension of the germinal disc on the side of the egg; (k) – Primitive body formation; (l) – Formation of metamers (arrow); (m) – Primordial appendages (arrow); (n) – Initiation of ocelli formation, more evident before (o) – hatching of the egg with emerging larva; (p) — Hexapod larvae seven days after oviposition. Scale bar length (a) 100 µm (magnification of 20x) and (b-p) 50 µm (magnification of 40x).

the first hours of the false spider mite development, since it enables us to monitor the sequence of embryonic divisions and to establish the exact period it occurred.

The eggs structure of the *B. yothersi* is a centrolecithal type, where the nucleus occupies a central position (Figure 1b), as described in *Ornithodoros moubata* Murray, an Argasidae tick (Aeschlimann and Hess 1984). Here, the cleavage observed was a superficial type, similar to descriptions made on Prostigmata mites (Gotoh *et al.* 1994; Dearden *et al.* 2002), but differs from previous proposals that holoblastic cleavage was a standard feature of Acari (Laumann *et al.* 2010b). However, embryological studies of the Eriophyoidea superfamily have reported that their cleavage is meroblastic type, with the nuclei founded in a single region, whereas the other regions of the egg contains only the yolk (Chetverikov and Desnitskiy 2016). These authors observed that nucleus division occurs at the egg periphery, a peculiarity of the embryological development of the Eriophyoidea family and differing from the other organisms of the Acari group. It particularly raises a possible hypothesis that a lineage of eriophyid mites could have diverged from the primitive Acari and transitioned from a superficial cleavage to a total cleavage and thereby acquiring it unusual meroblastic pattern.

The first cleavages of *B. yothersi* were similar to the mite species of the *Panonychus* genus (Tetranychidae) as reported by Gotoh *et al.* (1994). As illustrated and described in the 18 stages, noting that the interval between the first cell divisions was about one hour each and that from 100 blastomeres it was difficult to distinguish them. After observation of the cellular stage composed of 128-cells, the formation of the morula occurred, as visualized in *B. yothersi*.

The development of the *B. yothersi* embryos was slow compared to the tenuipalpid *Dolichotetranychus coco* Flechtmann & Fernando, maintained at a temperature of 25 °C ± 1 °C, where the formation of the first pair of legs and morphogenesis occurred after 35 and 47 hours, respectively (Santhosh *et al.* 2009). Here, the initial formation of the primitive body occurred on the third day after oviposition. Studies have reported that temperature variations and host plants affect the life cycle and embryonic development of different species of the mites (Haramoto 1969; Chiavegato 1986; Teodoro and Reis 2006; Santhosh *et al.* 2009). Thus, to avoid any possible influence, during egg analysis, the environmental conditions were controlled.

The chelicerates have some conserved characteristics during embryonic development. First, the prosoma is emerged followed by the development of the appendices, which appear as small buds of tissue (Sharma 2018) observed between the third and fifth days of the development of *B. yothersi* embryos. Other conserved characteristics were the migration from the mouth to a terminal region below the chelicerae and the formation of the ocelli (Sharma 2018). The total development time observed in *B. yothersi* was seven days (from the first cleavage to larvae hatching), differing from that observed in the *T. urticae* model mite, which has a complete embryonic development in 39 hours (25 °C ± 1 °C) and completing the life cycle in less than seven days (Rao *et al.* 1996; Dearden *et al.* 2002; Grbic *et al.* 2007; Khila and Grbic 2007).

Brevipalpus yothersi egg morphology

Details of external eggs morphology could be followed under SEM. Transverse streaks in the outer layer of the eggs called carenas (Rodrigues and Machado 1999) (Figure 2a) and a fold-like structure in the equatorial region of the egg (Figure 2b). An external globular structure were observed in studies by Rodrigues and Machado (1999). However, it was observed only in eggs with a more advanced development time but morphologically different from the one found in our study.

Eggshells frequently broke during fixation in Carnoy's solution, allowing for observation of the internal structure of eggs. Additionally, channels similar to small perforations were seen extending between the shell and intermediate lamella (Figure 3). These channels may be associated with the respiratory tract of the embryo. Such structures were also observed by Dittrich (1971) in studies with *T. urticae,* and they have been shown to give mites protection against desiccation and gas exchange.

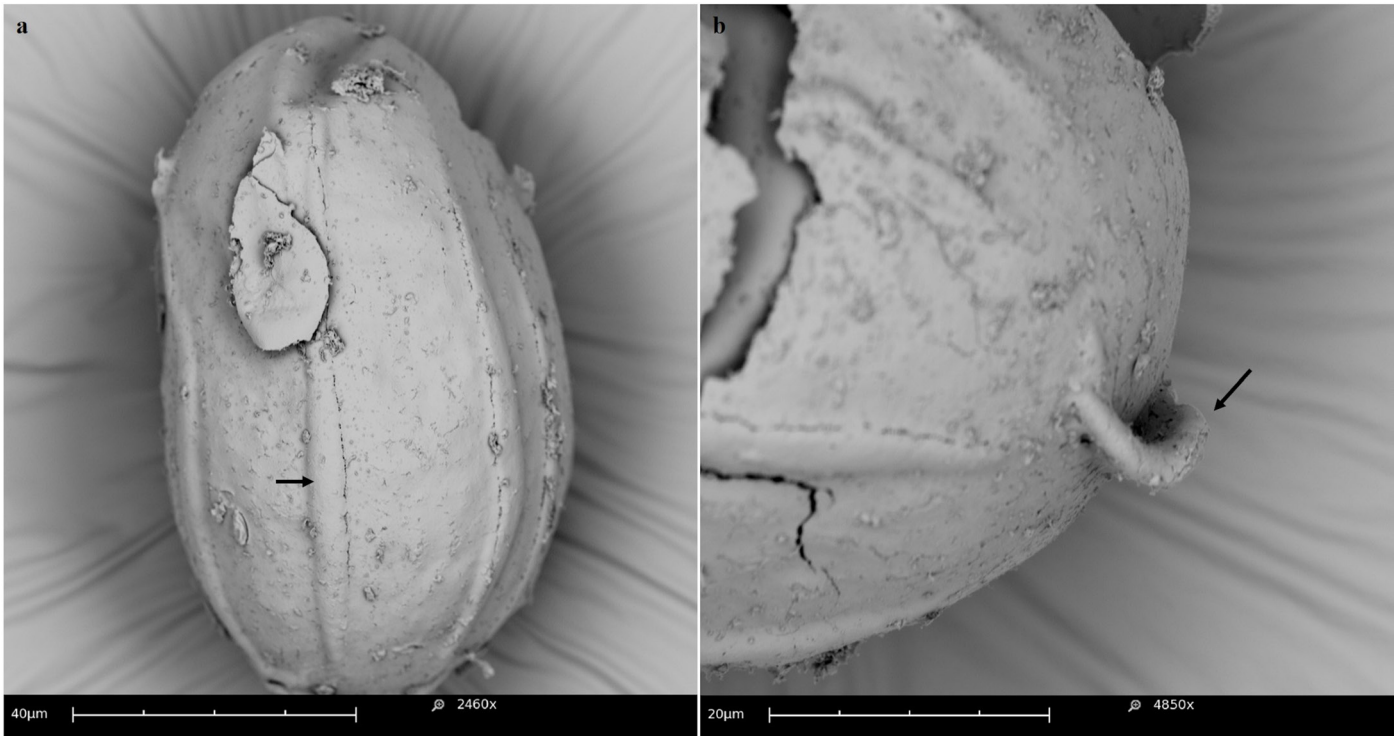


Figure 2 External morphology of the *B. yothersi* eggs: (a) – transverse streaks or carinae (arrow) and (b) – fold-like structure (arrow). Scale bar length (a) 40 µm and (b) 20 µm.

The *B. yothersi* mite has a peculiar form of reproduction in the animal kingdom, harboring *Cardinium* symbiont and resulting in an entirely haploid species (Weeks *et al.* 2001). These peculiarities making it an extremely interesting organism for genetic and biological studies. Its anatomy and post-embryonic biology have been explored, but the present work pioneered to show of the embryo formation and development stages. This information is essential for *B. yothersi* as biology preliminary knowledge and it also provides us with the visualization of the possible respiratory apparatus in embryos, which was associated to specific morphological adaptation of Tetranychidae. Further studies can be complemented by other robust techniques such as immunolabeling and confocal microscopy.

Conclusion

This study its first to provides timeline data of the *B. yothersi* embryological development. Also, it contributed to the establishment of appropriate protocols to *B. yothersi* embryological investigations, using light and scanning electron microscopy.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2009/13959-9; 2011/13869-0; 2013/07369-0; 2014/08458-9; 2016/21749-8). We are grateful to Acarology laboratory members for helpful discussions.



Figure 3 Internal analysis of the *B. yothersi* eggs: small perforations with a possible association with the respiratory system of mites. Scale bar length 20 µm.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TES, MAN, BAC, EWK. The first draft of the manuscript was written by TES and VMN, and all authors commented on previous versions of the manuscript. Funding acquisition: VMN, TES, EWK. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflicts of interest.

References

Aeschlimann A., Hess E. 1984. What is our current knowledge of acarine embryology? Acarology, 6: 90-99.

Andrade D.J.; Lorençon J.R.; Siqueira D.S.; Novelli V.M.; Bassanezi R.B. 2018. Space-time variability of citrus leprosis as strategic planning for crop management. Pest. Manag. Sci., 74: 1798-1803. https://doi.org/10.1002/ps.4877

Bassanezi R.B.; Czermainski A.B.C.; Laranjeira F.F.; Moreira A.S.; Ribeiro Junior. P.J.; Krainski E.T.; Amorim L. 2019. Spatial patterns of the *Citrus leprosis virus* and its associated mite vector in systems without intervention. Plant Pathol.; 68: 85-93. https://doi.org/10.1111/ppa.12930

Beard J.J., Ochoa R., Braswell W.E., Bauchan G.R. 2015. *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae) - a closer look. Zootaxa, 3944. https://doi.org/10.11646/zootaxa.3944.1.1

Chabi-Jesus C.; Ramos-González P.L.; Postclam-Barro M.; Fontenele R.S.; Harakava R.; Bassanezi R.B.; Moreira A.S.; Kitajima E.W.; Varsani A.; Freitas-Astúa J. 2021. Molecular epidemiology of citrus leprosis virus C: a new viral lineage and phylodynamic of the main viral subpopulations in the Americas. Front. Microbiol., 12: 641252. https://doi.org/10.3389/fmicb.2021.641252

Chetverikov P.E., Desnitskiy A.G. 2016. A study of embryonic development in eriophyoid mites (Acariformes, Eriophyoidea) with the use of the fluorochrome DAPI and confocal microscopy. Exp. Appl. Acarol., 68: 97-111. https://doi.org/10.1007/s10493-015-9982-4

Chiavegato L.G. 1986. Biologia do ácaro *Brevipalpus phoenicis* em citros. Brasília: Pesqui. Agropecu. Brasil., 21: 813-816.

Childers C.C., Rodrigues J.C.V. 2011. An overview of *Brevipalpus* mites (Acari: Tenuipalpidae) and the plant viruses they transmit. Zoosymposia, 6: 180-192. https://doi.org/10.11646/zoosymposia.6.1.28

Dearden P.K., Donly C., Grbic M. 2002. Expression of pair-rule gene homologues in a chelicerate: early patterning of the tow-spotted spider mite *Tetranychus urticae*. Development, 129: 5461-5472. https://doi.org/10.1242/dev.00099

Dearden P., Grbic M., Donly C. 2003. Vasa expression and germ-cell specification in the spider mite *Tetranychus urticae*. Dev. Genes Evol., 212: 599-603. https://doi.org/10.1007/s00427-002-0280-x

Dietzgen R.G., Freitas-Astúa J., Chabi-Jesus C., Ramos-González P.L., Goodin M.M., Kondo H., Tassi A.D., Kitajima E.W. 2018. Dichorhaviruses in their host plants and mite vectors. Adv. Virus Res., 102: 119-148. https://doi.org/10.1016/bs.aivir.2018.06.001

Dittrich V. 1971. Electron-microscopic studies of the respiratory mechanism of spider mite eggs. Ann. Entomol. Soc. Am., 64: 1134-1143. https://doi.org/10.1093/aesa/64.5.1134

Freitas-Astúa J., Ramos-González P.L., Arena G.D., Tassi A.D., Kitajima E.W. 2018. *Brevipalpus*-transmitted viruses: parallelism beyond a common vector or convergent evolution of distantly related pathogens? Curr. Opin. Virol., 33: 66-73. https://doi.org/10.1016/j.coviro.2018.07.010

Gotoh T., Kamoto T., Hatakeyama M., Gomi K. 1994. Embryonic development and diapause stage in *Panonychus* mites (Acari: Tetranychidae). Appl. Entomol. Zool., 29: 507-515. https://doi.org/10.1303/aez.29.507

Grbic M., Khila A., Lee K.-Z., Bjelica A., Grbic V., Whistlecraft J., Verdon L., Navajas M., Nagy L. 2007. Mity model: *Tetranychus urticae*, a candidate for chelicerate model organism. BioEssays, 29: 489-496. https://doi.org/10.1002/bies.20564

Haramoto F.H. 1969. Biology and control of *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae). 68th ed. Technical Bulletin. Honolulu: University of Hawaii, pp. 63.

Khila A., Grbic M. 2007. Gene silencing in the spider mite *Tetranychus urticae*: dsRNA and siRNA parental silencing of the Distal-less gene. Dev. Genes Evol., 217: 241-251. https://doi.org/10.1007/s00427-007-0132-9

Laumann M., Norton R.A., Heethoff M. 2010a. Acarine embryology: inconsistencies, artificial results and misinterpretations. Soil Organisms, 82: 217-235.

Laumann M., Bergmann P., Norton R.A., Heethoff M. 2010b. First cleavages, preblastula and blastula in the parthenogenetic mite *Archegozetes longisetosus* (Acari, Oribatida) indicate holoblastic rather than superficial cleavage. Arthropod Struct. Dev., 39: 276-286. https://doi.org/10.1016/j.asd.2010.02.003

Rao P.P., Praslicka J., Sutakova G. 1996. Effect of temperature and rearing method on development and fecundity of *Tetranychus urticae* (Acarina, Tetranychidae). Biologia, 51: 509-516.

Rodrigues J.C.V., Machado M.A. 1999. Notes on a probable respiratory apparatus in eggs of *Brevipalpus phoenicis* (Acari: Tenuipalpidae). Int. J. Acarol., 25: 231-234. https://doi.org/10.1080/01647959908684157

Santhosh P.P., Haq M.A., Ramani N. 2009. Biological studies of coconut infesting mite - *Dolichotetranychus cocos*. Advances in Environmental Biology, 3: 263-268.

Sharma P.P. 2018. Chelicerates. Curr. Biol., 28: R774-R778. https://doi.org/10.1016/j.cub.2018.05.036

Teodoro A.V., Reis P.R. 2006. Reproductive performance of the mite *Brevipalpus phoenicis* (Geijskes, 1939) on citrus and coffee, using life table parameters. Braz. J. Biol., 66: 899-905. https://doi.org/10.1590/S1519-69842006000500016

Thomas R.H., Telford M.J. 1999. Appendage development in embryos of the oribatid mite *Archegozeles longisetosus* (Acari, Oribatei, Trhypochthoniidae). Acta Zool., 80: 193-200. https://doi.org/10.1046/j.1463-6395.1999.00016.x

Weeks A.R., Marec F., Breeuwer J.A.J. 2001. A mite species that consists entirely of haploid females. Science, 292: 2479-2483. https://doi.org/10.1126/science.1060411