Zootaxa 3919 (1): 179–191
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3919.1.8
http://zoobank.org/urn:lsid:zoobank.org:pub:D2D46CBC-4201-4001-AECA-8E660611CD5A

Confocal microscopy refines generic concept of a problematic taxon: rediagnosis of the genus *Neoprothrix* and remarks on female anatomy of eriophyoids (Acari: Eriophyoidea)

PHILIPP E. CHETVERIKOV[1,2,4], ALEXEY G. DESNITSKIY[2] & DENISE NAVIA[3]

[1]*Zoological Institute, Russian Academy of Sciences, Universitetskaya Embankment 1, 199034 St. Petersburg, Russia*
[2]*Saint-Petersburg State University, Universitetskaya nab., 7/9, 199034, St. Petersburg, Russia*
[3]*Embrapa Recursos Genéticos e Biotecnologia, 70.770-900, Brasília, Brazil*
[4]*Corresponding author. E-mail: p.chetverikov@bio.spbu.ru*

Abstract

Due to the higher resolution, confocal microscopy (CLSM) can be applied to refine the origin of tiny structures of the autofluorescent exoskeletons of microarthropods (mites in particular) which are hard to visualize using traditional differential interference contract light microscopy (DIC LM) and phase contrast light microscopy (PC LM). Three-dimensional (3D) reconstructions of the prodorsal shield topography of eriophyoid mites using *Neoprothrix hibiscus* Reis and Navia as a model, suggest that the structures originally treated as paired setae *vi* are two internal rod-like apodemes. Based on this, the genus *Neoprothrix* is excluded from the subfamily Prothricinae Amrine and transferred to the subfamily Sierraphytoptinae Keifer. Observations on partially cleared specimens of *N. hibiscus* showed that remnants of the central nervous system, paired glands and developing oocytes can be visualized using DIC LM and CLSM methods. New high quality microscope images are provided of recently described "flower-shaped" structures and two main components of yolk inclusions of the mature eggs inside the oviduct.

Key words: arthropod reproductive anatomy, mite yolk, 3D reconstruction, Phytoptidae

Introduction

Prodorsal shield chaetom, shape of gnathosoma and anatomy of internal genitalia are the three key morphological characters used in the systematics of eriophyoid mites at the family level (Amrine *et al*. 2003). The prodorsal shield of contemporary eriophyoids bear from zero to five setae. A five-setous prodorsal shield is considered to be ancestral (Schevchenko *et al*. 1991). Among extant eriophyoids, only two early-derivative, conifer-associated eriophyoid genera, namely *Pentasetacus* Schliesske, 1985 and *Loboquintus* Chetverikov et Petanović, 2013, possess five prodorsal shield setae: they have paired *sc*, paired *ve* and a single *vi* (Chetverikov *et al.* 2013, 2014a). Some scientists have hypothesized that the basal ancestor of eriophyoids might have had six setae on the prodorsal shield and paired seta *vi* (Farkas 1965; Amrine *et al.* 2003). Six-setous eriophyoids have never been found, but mites with four setae (presumably paired *ve* and *vi*) on their anterior prodorsal shield margin have been described. They belong to the subfamily Prothricinae Amrine, 1996. Until 2014, this subfamily had comprised only one species, *Prothrix aboula* Keifer, 1965, described from an unidentified plant from the Philippine Islands. This is a tiny, differentially annulated, notably flattened and presumably vagrant mite "…apparently with short-stalked recurved spermathecae" (Keifer 1965, p.2). The homology of its two pairs of prodorsal shield setae has been uncertain: some authors treat them as paired *ve* and paired *vi* (Amrine *et al*. 2003, p. 9), whereas others consider them to be paired *ve* and anteriorly placed paired *sc* (Keifer 1965, p.2; Chetverikov *et al.* 2013, p.2, footnote 1). Shevchenko *et al.* (1991) argued that gradual reduction of the number of prodorsal shield setae had occurred during the evolution of eriophyoid mites. Reexamination of *P. aboula* using various microscopic tools and DNA based methods would potentially provide new important data for the phylogenetic analysis of Eriophyoidea, including testing the hypotheses on the evolution of the prodorsal chaetom as mentioned above. Unfortunately, samples of *P.*

Accepted by D. Knihinicki: 24 Dec. 2014; published: 16 Feb. 2015

lacking detailed line drawings and high-quality LM microphotographs. It is likely that the descriptions are based mainly on the six poor quality SEM images of deformed mites that were published by Huang (1992: Figs. 29–34, pp. 238 & 239). Only some diagnostic characters such as the pattern on the prodorsal shield, appearance of setae *ve* and *c1*, ventral opisthosomal setae and shape of the opisthosmal annuli are visible on the images. The two knobbed tarsal I solenidia were mistakenly mentioned in the diagnosis of genus *Neopropilus* (Huang 1992, p. 228: "…tarsus I with 2 knobbed solenidia and tarsus II with 1…") but in fact are probably tarsal solenidion ω I and tibia solenidion φ I.

Distribution. To date, *Neoprothrix hibiscus* has only been recorded from Brazil (Reis *et al.* 2014). However, the distribution of this species is likely to be more widespread since it had once been intercepted at the quarantine border in Sydney, Australia (coll. G. Goodyer) on *Hibsicus* cuttings which had allegedly been illegally smuggled into the country from Hawaii (via New Zealand) in January 1998. At the time, the only two specimens (one female and one male on a single microscope slide) were incorporated into the reference collection of the Agricultural Scientific Collections Unit, Orange NSW, Australia, where they remained in the "obscure" category for 16 years after tentatively being placed in the genus *Neopropilus*. Following the recent description of *Neoprothrix hibiscus* by Reis and Navia 2014 (see Reis *et al.* 2014), the intercepted specimens have since been identified as the same species but their true country of origin remains inconclusive (D. Knihinicki *pers. comm.* 24 October 2014).

Acknowledgements

We are grateful to Dr. S. Bolton (Ohio State University, USA), Prof. C.H.W. Flechtmann (Universidade de São Paulo, Brazil) and an anonymous reviewer for their valuable comments and linguistic corrections. Mite collection was supported by the National Council for Scientific and Technological Development (CNPq), Brazil (to DN). All other steps of the study were supported by Russian Science Foundation (RSCF grant #14-14-00621) to the first author.

References

Alberti, G. & Nuzzaci, G. (1996) Oogenesis and spermatogenesis. *In*: Lindquist, E.E., Sabelis, M.W. & Bruin, J. (Eds.), *Eriophyoid Mites: their Biology, Natural Enemies and Control.* World Crop Pests 6. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 151–167.

Amrine, J.W. Jr. (1996) *Keys to the World genera of the Eriophyoidea (Acari: Prostigmata).* Indira Publishing House, West Bloomfield, Michigan, USA, 186 pp.

Amrine, J.W. Jr. & Manson, D.C.M. (1996) Preparation, mounting and descriptive study of eriophyoid mites. *In*: Lindquist, E.E., Sabelis, M.W. & Bruin, J. (Eds.), *Eriophyoid Mites: their Biology, Natural Enemies and Control.* World Crop Pests 6. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 383–396.

Amrine, J.W. Jr., Stasny, T.A. & Flechtmann, C.H.W. (2003) *Revised Keys to World Genera of Eriophyoidea (Acari: Prostigmata)*. Indira Publishing House, West Bloomfield, Michigan, USA, 244 pp.

Baker, E.W., Kono, T., Amrine, J.W. Jr., Delfinado-Baker, M. & Stasny, T.A. (1996) *Eriophyoid Mites of the United States.* Indira Publishing House, West Bloomfield, Michigan, USA, 394 pp. + i–viii.

Chetverikov, P.E. (2012) Confocal laser scanning microscopy technique for the study of internal genitalia and external morphology of eriophyoid mites (Acari: Eriophyoidea). *Zootaxa*, 3453, 56–68.

Chetverikov, P.E. (2014a) Distal oviduct and genital chamber of eriophyoids (Acariformes, Eriophyoidea): refined terminology and remarks on CLSM technique for studying musculature of mites. *Experimental and Applied Acarology*, 64 (4), 407–428.

Chetverikov, P.E. (2014b) Comparative confocal microscopy of internal genitalia of phytoptine mites (Eriophyoidea, Phytoptidae): new generic diagnoses reflecting host-plant associations. *Experimental and Applied Acarology*, 62 (2), 129–160.

Chetverikov, P.E. (2014c) *What is the origin of the genital "flower-shaped" figures rarely observed in genital areas of eriophyoids (Acariformes, Eriophyoidea)?* Abstract book, XIV International Congress of Acarology, 13–18th July, 2014, Kyoto, Japan, pp. 62.

Chetverikov, P.E. & Sukhareva, S.I. (2009). A revision of the genus *Sierraphytoptus* Keifer 1939 (Eriophyoidea, Phytoptidae). *Zootaxa*, 2309, 30–42.

Chetverikov, P.E., Beaulieu, F., Cvrković, T., Vidović, B. & Petanović, R. (2012) *Oziella sibirica* (Eriophyoidea: Phytoptidae), a new eriophyoid mite species described using confocal microscopy and COI barcoding. *Zootaxa,* 3560, 41–60.

Chetverikov, P.E., Cvrkoviæ, T., Vidoviæ, B. & Petanović, R.U. (2013) Description of a new relict eriophyoid mite, *Loboquintus subsquamatus* n. gen. & n. sp. (Eriophyoidea, Phytoptidae, Pentasetacini) based on confocal microscopy, SEM, COI barcoding and novel CLSM anatomy of internal genitalia. *Experimental and Applied Acarology*, 61 (1), 1–30.

Chetverikov, P.E., Beaulieu, F., Beliavskaia, A.Y., Rautian, M.S. & Sukhareva, S.I. (2014a) Redescription of an early-derivative mite, *Pentasetacus araucariae* (Eriophyoidea, Phytoptidae), and new hypotheses on the eriophyoid reproductive anatomy. *Experimental and Applied Acarology*, 63, 123–125.

Chetverikov, P.E., Cvrković, T., Makunin, A., Sukhareva, S., Vidović, B. & Petanović, R. (2014b) *Basal divergence of Eriophyoidea (Acariformes, Eupodina) inferred from combined partial COI and 28S gene sequences and CLSM comparative genital anatomy*. Abstract book, XIV International Congress of Acarology, 13-18th, July, 2014, Kyoto, Japan, p. 62.

Chetverikov, P.E., Craemer, C. & Sukhareva, S.I. (2014c) CLSM anatomy of internal genitalia of *Mackiella reclinata* n. sp. and systematic remarks on eriophyoid mites from the tribe Mackiellini Keifer, 1946 (Eriophyoidea, Phytoptidae). *Zootaxa*, 3860 (3), 261–279.

Claxton, N.S., Fellers, T.J. & Davidson, M.W. (2005) *Laser scanning confocal microscopy. Department of Optical Microscopy and Digital Imaging, National High Magnetic Field Laboratory*, Florida State University, 37 pp. Available from: http://olympusfluoview.com/theory/LSCMIntro.pdf (accessed 25 July 2014)

de Lillo, E., Craemer, C., Amrine, J.W. Jr. & Nuzzaci, G. (2010) Recommended procedures and techniques for morphological studies of Eriophyoidea (Acari: Prostigmata). *Experimental and Applied Acarology*, 51, 283–307.

Dobrivojević, K. & Petanović, R. (1982) *Fundamentals of Acarology*. Slovo Ljubve Publishing, Belgrade (in Serbian), 284 pp.

Farkas, H.K. (1965) Some problems of eriophyid mites phylogeny (Acarina, Eriophyoidea). *Zeszyty Problemowe Posterow Nauk Rolniczych*, 65, 189–194.

Huang, K.-W. (1992) Some new eriophyoid mites from Taiwan (Acarina: Eriophyoidea). *Bulletin of the National Museum of Natural Science*, 3, 225–240.

Keifer, H.H. (1944) Eriophyid Studies III. *Bulletin of the California Department of Agriculture*, 33, 18–38.

Keifer, H.H. (1946) Eriophyid Studies III. *Bulletin of the California Department of Agriculture*, 35, 39–48.

Keifer, H.H. (1965) *Eriophyid Studies B-13*. California Department of Agriculture: Bureau of Entomology, 20 pp.

Kirejtshuk, A.G., Chetverikov, P.E. & Azar, D. (2014) Libanopsinae, new subfamily of the family Sphindidae (Coleoptera, Cucujoidea) from Lower Cretaceous Lebanese amber, with remarks on using confocal microscopy for the study of amber inclusions. *Cretaceous Research*, 52 (Part B), 461–479.
http://dx.doi.org/10.1016/j.cretres.2014.02.008

Lindquist, E.E. (1996) External anatomy and systematics. 1.1.1. External anatomy and notation of structures. *In*: Lindquist, E.E., Sabelis, M.W. & Bruin, J. (Eds.), *Eriophyoid Mites: their Biology, Natural Enemies and Control*. World Crop Pests 6. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 3–31.

Nuzzaci, G. & Alberti, G. (1996) Internal anatomy and physiology. *In*: Lindquist, E.E., Sabelis, M.W. & Bruin, J. (Eds.), *Eriophyoid Mites: their Biology, Natural Enemies and Control.* World Crop Pests 6. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 101–150.

Reis, A.C., Gondim, M.G.C. Jr., Flechtmann, C.H.W. & Navia, D. (2014) New eriophyoid mites (Acari: Prostigmata: Eriophyoidea) from cultivated plants from northeastern Brazil, including the second taxon in the Prothricinae. *Journal of Natural History*, 48 (19–20), 1135–1152.

Schliesske, J. (1985) Zur Verbrietung und Ökologie einer neunen ursprünglichen Gallmilbenart (Acari: Eriophyoidea) an *Araucaria araucana* (Molina) K. Koch. *Entomologische Mitteilungen zoologische Museum Hamburg*, 8, 97–106.

Shevchenko, V.G., Bagnyuk, I.G. & Sukhareva, S.I. (1991) A new family of Pentasetacidae (Acariformes, Tetrapodili) and its role in treatment of the origin and evolution of the group. *Zoologichesky Zhurnal*, 70 (5), 47–53.