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Eriophyoid Mite of the Genus *Trisetacus* Reported on *Juniperus excelsa* in Lebanon

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ABSTRACT

We report for the first time the occurrence of *Trisetacus* acari Eriophyoid parasite infesting *Juniperus excelsa* seeds in Lebanon. The specimen studied belong most probably to the species *T. kirghizorum* (Shevchenko 1962) living on *J. semiglobosa* in the Kirghiz mountains. We suggest that *Trisetacus* spp. found in the seed cones of Kirghiz junipers as well as *J. excelsa* comprise a complex of cryptic species that might be distinguished using molecular markers. It is necessary to revise the group cupressi in the genus *Trisetacus* and an integrated phylogenetic study of the mites belonging to this genus could elucidate the patterns of eriophyoid mites evolution on conifers and could help in a better resolution of the Juniper phylogeny.

Keywords: Acari; Eriophyoidea; *Trisetacus*; Cryptic Species; *Juniperus*; Lebanon

1. Introduction

In Lebanon there are four Juniper species: *J. excelsa* (M.-Bieb., 1800), *J. foetidissima* (Willd., 1806), *J. oxycedrus* (L., 1753), and *J. drupacea* (Labill., 1791). The most common species is *J. excelsa*, covering around 11 318 hectares. It constitutes 9.93% of the total wooded area of the country and 26% of the total conifer forest cover. *J. excelsa* woodlands in Lebanon are not well protected and most of the populations suffer from a low regeneration rate [1]. The parasites associated with juniper species in the world are highly diversified and some are considered as pests causing important damages [2]. Despite the importance of parasitic attack on the forest health, no investigation has been made until today to identify and monitor the parasites of the juniper trees in Lebanon.

During our field work on *Juniperus excelsa* we have noticed the infestation of the seeds by a phytophageous acari, an Eriophyoid mite from the genus *Trisetacus* Keifer (1952). We report the occurrence of this parasite in Lebanon for the first time. We have observed this mite in the Qammoua mixed forest (34˚29'N, 36˚15'E) between 1400 m and 1800 m altitude, in Afqa juniper woodland (34˚4'N, 35˚54'E) between 1200 and 1800 m and in Danniyeh juniper woodland (34˚23'N, 36˚05'E) at 1600 m altitude. The highest level of infestation was found in the Qammoua forest with a very high percentage of infested trees with almost 90% of their cones deteriorated for three years round between 2006 and 2009.

The infestation symptoms are very clear, with seeds having an abnormal pointed end that is visible outside of the cone berry. Ripe infested seed cones shatter. Inside the cone, seeds are hypertrophic and the seeds tissues form a series of desiccated lamellas (**Figure 1**).

We have conducted a microscopic observation of pro-

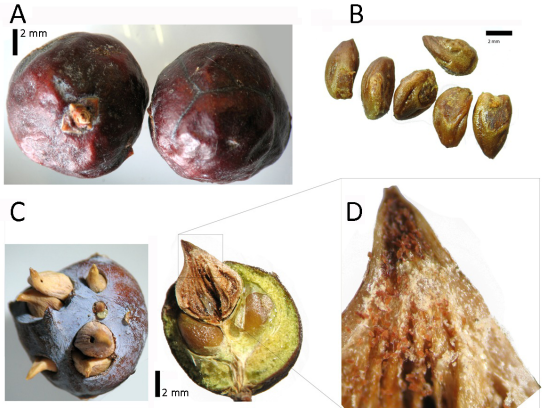

Figure 1. Healthy *J. excelsa* cones (A) and seeds (B); infested *J. excelsa* cones (C) and enlarged view of the infested seed (D) by *Trisetacus kirghizorum*.

togyne and deutogyne female and male specimens collected in the Qammoua forest. *Trisetacus* was first found and studied in *J. excelsa* seeds by A. P. de Millo in 1968 near Pljuchevoi in Crimea (Ukraine) [3]. In 1987, this mite was found on the same host in Perkalsky Arboretum (Pjatigorsk Prov., Russia) by I. G. Bagnjuk (S. I. Sukhareva and I. G. Bagnjuk pers. comm. December 2012). In 2010, they were registered in Nikitsky Botanical Garden (Yalta, Crimea, Ukraine) by Ph. E. Chetverikov.

2. Material and Methods

The material from Lebanon was collected by B. Douaihy between November 2007 and April 2010 in the Qammoua forest. Mites were collected from the infested opened seeds in vials containing 95% alcohol. After filtration, the mites were mounted in Hoyer's medium [4].

The specimens were first examined under a phase-contrast microscope (Primo Star Zeiss-Magnification: Primo plan Achromat 40×). Confocal laser scanning microscopy (CLSM) acquisition was carried out using protocol by Chetverikov (2012) [5] in the Center of Microscopy and Microanalysis of Saint-Petersburg State University (RUSSIA). The acquisitions were done using a Leica TCS SP2 spectral confocal and multiphoton system microscope with objectives 63 × N.A. 1.4-0.60 Oil lBL HCX PL APO and 40 × N.A. 1.25-0.75 Oil CS HCX PL APO at an excitation wavelength of 405 nm, and an emission wavelength range of 415 - 750 nm, at 20% intensity. The acquisition resolution was 1024 × 1024 pixels, speed of scanning 400, frame average 2, level of gain 600 - 700 and zoom factor 1.5 - 2.0. The digital images of the confocal stacks were processed using Fiji-win32-20091014 Open Source Image Processing Package to obtain maximum intensity and were checked for brightness and contrast correction with Photoshop CS2 version 9.0 (Adobe Systems Inc.).

We compared our data with measurements taken on the mites from Ukraine by A. P. De Millo as well as with *Trisetacus kirghisorum* [6].

3. Results

The microscopic observations (**Figures 2-4**) show that the *Trisetacus* specimens collected on *Juniperus excelsa* from Lebanon and Ukraine, and on *J. semiglobosa* were globally very similar and most probably belong to the same species *Trisetacus kirghizorum* that is morphologically close to *T. quadrisetus* (Thomas 1890) found on *J. communis* and *J. thurifera*.

4. Discussion and Conclusion

Mites of the genus *Trisetacus* that live on junipers, comprise a complex of about 12 closely related species from the group cupressi [7], and have been recorded in North America, Europe, Kirghizia and China [8]. Three of these 12 species [*T. sonare* (Narematsu & Kakizaki, 1940), *T. ramosus* (Hodgkiss, 1918), *T. cailloli* (Cotte, 1924)] have been inadequately described and the validity of their taxonomy needs to be demonstrated [7]. Half of the remaining 8 species live only on junipers, whereas

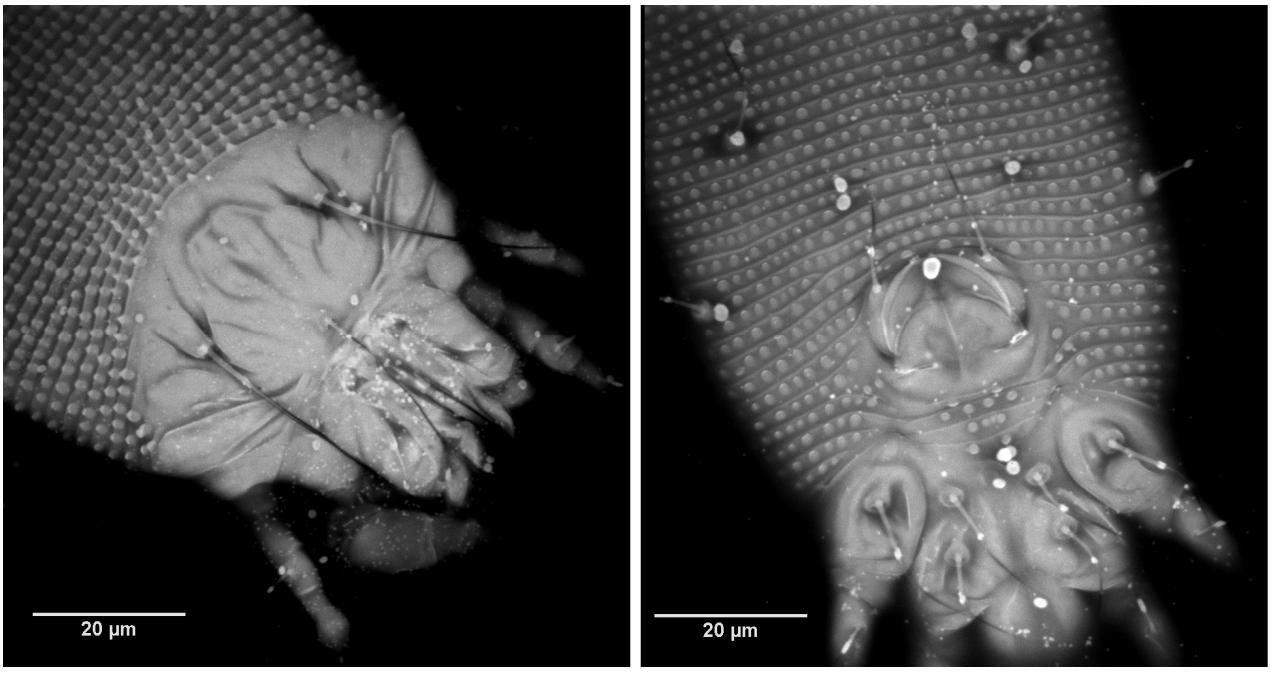

Figure 2. CLSM images of *Trisetacus kirghisorum* Shevchenko 1962 deutogyne female from seeds of *Juniperus excelsa*. Left—prodorsal shield; right—coxogenital area.

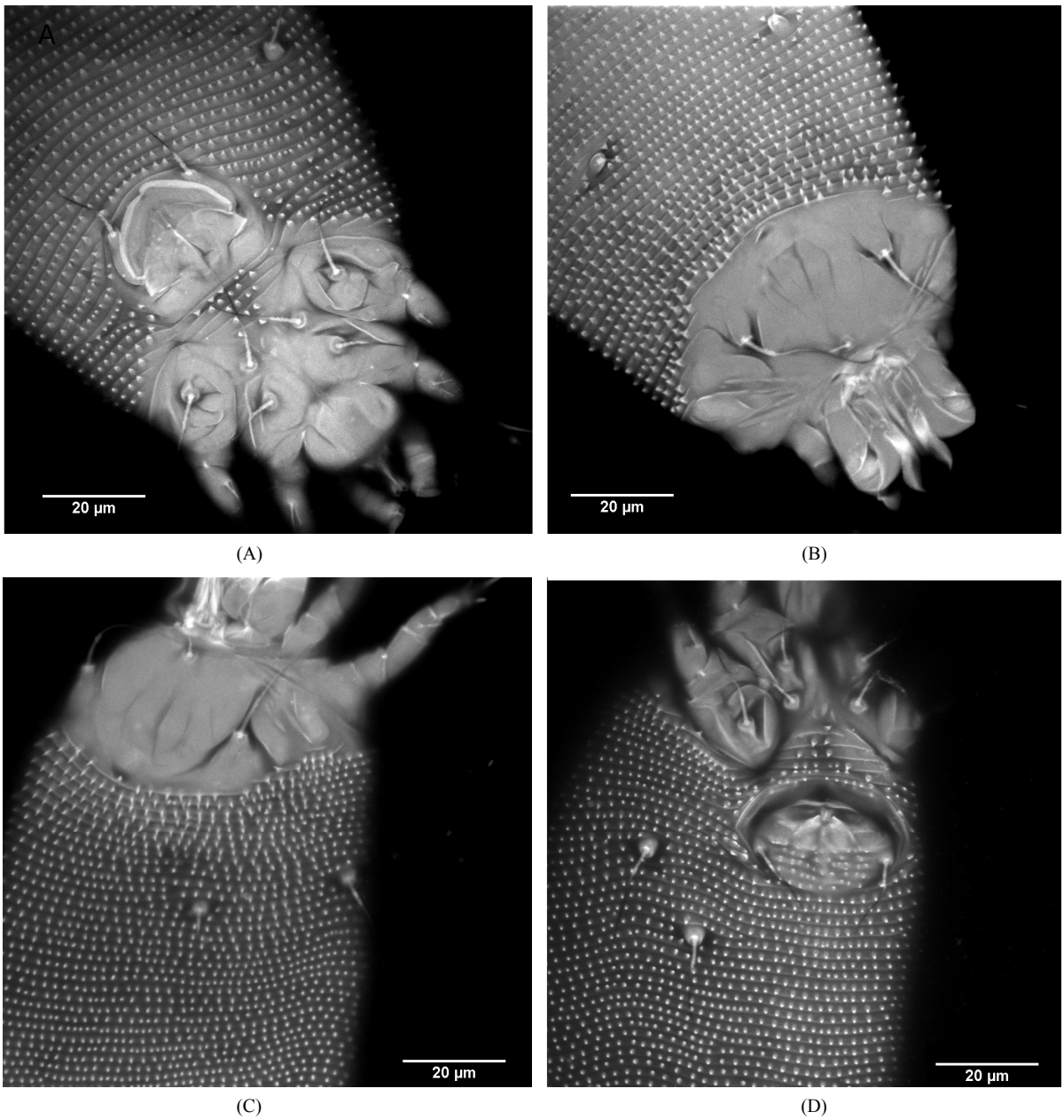

Figure 3. CLSM images of *Trisetacus kirghisorum* Shevchenko 1962 protogyne female (A, B) and male (C, D) from seeds of *J. excelsa* in Lebanon. A, C—prodorsal shield; B, D—coxogenital area.

the other 4 species (*T. chamaecypari*, *T. cupressi*, *T. distinctus*, *T. juniperinus*) were found on junipers and other conifers (e.g. *Chamaecyparis*, *Cupressus*, *Thuja* and *Cedrus*). Most of these 12 species live inside cones causing deformations, shriveling, and eventually destroy the seeds; except the vagrant mite *T. distinctus* and the hidden-living mite *T. juniperinus*, which form galls at the base of needles [8].

Mites from the seed cones of Crimean and Kirghiz junipers were studied from 1961 to 1968 by V. G. Shevchenko and A. P. De-Millo [3]. They found morphologically similar populations of 4 mites, *Trisetacus* spp. on different species of mountainous junipers. V. G. Shevchenko considered them to be separate monophagous species, because in all of their experiments, 95% - 100% of the mites transferred from one species of juniper to another died. Moreover, slight differences in the allometric growth of the prodorsal shield, opisthosoma, gnathosoma and legs were discovered in the course of their investigations of their postembryonic development. But the clear morphological criteria needed to distinguish these mites are absent. We suggest that mites of the genus *Trisetacus*, found in the seed cones of Kirghiz junipers as well as in the seed cones of *Juniperus excelsa* in Ukraine and

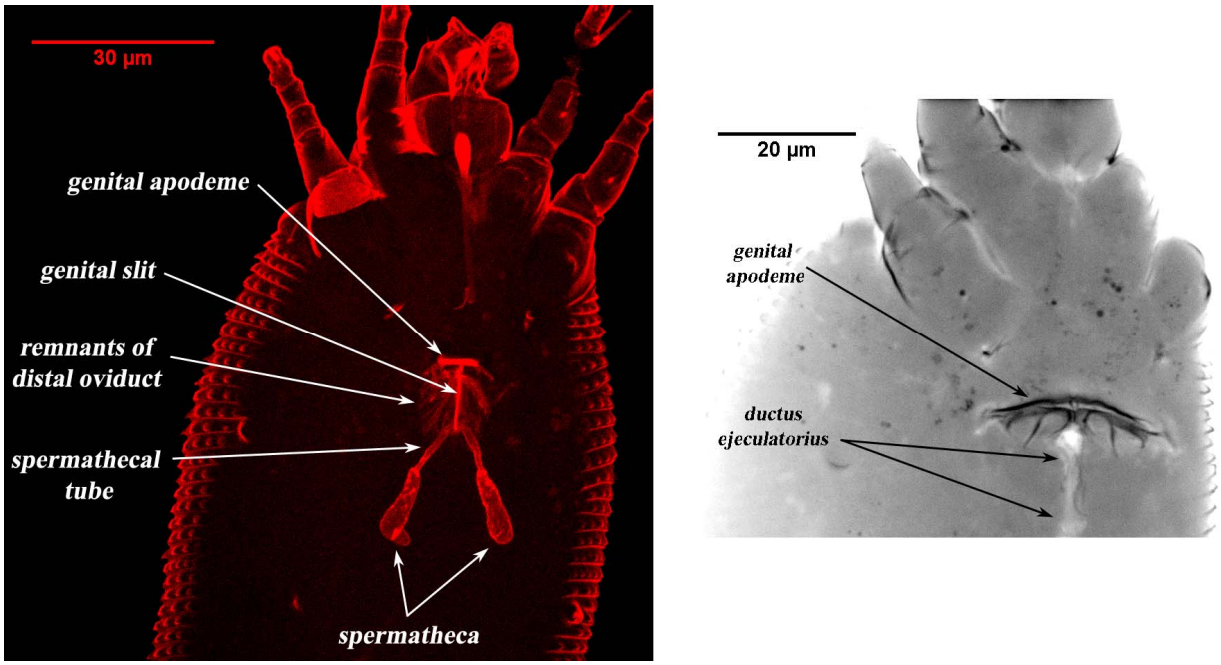

Figure 4. CLSM images of *Trisetacus kirghisorum* Shevchenko 1962 internal female (left) and male (right) genitalia from Lebanon. Note: The left figure is black and white inverted CLSM image.

Lebanon comprise a complex of cryptic species that might be distinguished on the basis of their DNA sequences. A similar conclusion was reached by Smith (1984, p. 1165) during his study on *Trisetacus* mites from North American Cupressaceae trees.

Therefore, it is necessary to revise the group cupressi in the genus *Trisetacus*, and then undertake a revision of the whole genus based on a comparison of morphological characteristics and DNA sequences. Such a revision should include the use of DNA barcoding techniques based on the CO1 gene [9]. Considering the primitive character of the genus *Trisetacus*, an integrated study of the mites belonging to this genus could elucidate the patterns of eriophyoid mites evolution on conifers. Moreover a phylogenetic study of *Trisetacus* species living on vicariant Juniper species like *J. excelsa*, *J. thurifera* and *J. semiglobosa* could help in a better resolution of the Juniper phylogeny.

Eriophyoid mites have a very small body size, with a mean length of 200 µm. Thus many species are frequently not detected and seem to be non-harmful to the host plant. On the opposite to leaf and wood parasites, seed parasites do not affect the tree health and do not put their host life at risk. Nevertheless those parasites, especially in the case of tree species suffering from low regeneration rate and a high percentage of empty seeds like *Juniperus excelsa* [1] can cause a significant reduction in their viable seeds percentage. It is the case of *T. kirghisorum* that was considered a serious limiting factor in the regeneration of *J. semiglobosa* woodlands in central Asia where the infestation have reached 90% of the seeds [6]. The mean number of filled seeds per cone of *J. thurifera* in the high mountains of Morocco and French Pyrenees was reduced to 0.13 because of *Trisetacus* infestation [10]. The infestation level of *T. quadrisetus* in *J. communis* in France reached 25% [2].

Therefore, studies are needed to be made in order to evaluate the consequences of the *Trisetacus* infestation on the regeneration capacity of *Juniperus excelsa* stands in Lebanon. It is important to establish a continuous observation protocol in order to better understand the evolutio n of the infestation each year and assess the need for an intervention to reduce this mite attacks. The control of cone parasites like *Trisetacus* spp. is very hard. The use of chemical treatments can be inefficient since those parasites stay most of their life cycle hidden. It is very important to study the life cycle of those parasites in order to detect their emergence periods when they are exposed. Mechanical treatments could be considered like an intensive collection of infested seeds.

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REFERENCES

[1] B. Douaihy, G. Restoux, N. Machon and M. Bou Dagher-Kharrat, "Ecological Characterization of the *Juniperus excelsa* Stands in Lebanon," in Press.

[2] A. Roques, "Pests of Conifer Cones and Seeds in France," French National Institute for Agricultural Research, Paris, 1983.

[3] A. P. De Millo, "Eriophyoid Mites (Acariformes, Eriophyoidea)—Pests of Junipers (Systematics, Biology, Phylogeny)," Ph.D. Thesis, Leningrad University (Now Saint-Petersburg State University), Leningrad, 1968.

[4] J. W. Amrine Jr. and D. C. M. Manson, "Preparation, Mounting and Descriptive Study of Eriophyoid Mites," In: E. E. Lindquist, M. W. Sabelis and J. Bruin, Eds., *Eriophyoid Mites*: *Their Biology*, *Natural Enemies and Control* (*World Crop Pests*), Elsevier Science B.V., Amsterdam, 1996, pp. 383-396.

[5] P. E. Chetverikov, "Confocal Laser Scanning Microscopy Technique for the Study of Internal Genitalia and External Morphology of Eriophyoid Mites (Acari: Eriophyoidea)," *Zootaxa*, Vol. 3453, 2012, pp. 56-68.

[6] V. G. Shevchenko, "New Four Legged Mite—*Trisetacus kirghisorum* sp. n. (Acarina: Eriophyidae)—The Pest of Seeds of Asian Mountain Juniper," *Proceeedings of Kirghyz Forest Experiment Station*, Vol. 3, 1962, pp. 299-305.

[7] I. M. Smith, "Review of Species of *Trisetacus* (Acari: Eriophyoidea) from North America, with Comments on All Nominate Taxa in the Genus," *Canadian Entomologist*, Vol. 116, No. 9, 1984, pp. 1157-1211. doi:10.4039/Ent116119-2.

[8] J. Amrine and E. de Lillo, Personal Database of Eriophyoids.

[9] P. E. Chetverikov, F. Beaulieu, T. Cvrković, B. Vidović and R. U. Petanović, "Oziella Sibirica (Acari: Eriophyoidea: Phytoptidae), a New Eriophyoid Mite Species Described Using Confocal Microscopy, COI Barcoding and 3D Surface Reconstruction," *Zootaxa*, Vol. 3560, 2012, pp. 41-60.

[10] M. A. El Alaoui El Fels, A. Roques and A. Boumezzough, "The Arthropods Related to Cones and Seeds of Incense-Juniper, *Juniperusthurifera* L., in Atlas Mountains of Morocco," *Ecologia Mediterranea*, Vol. 25, No. 1, 1999, pp. 95-110.

