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Confocal microscopy applied to water mite taxonomy with the description of a new genus of Axonopsinae (Acari, Parasitengona, Hydrachnidia) from Central America

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Abstract

Vagabundia sci **n. gen. n. sp.** of the subfamily Axonopsinae is proposed and described. Confocal Laser Scanning Microscopy, not previously applied to water mite taxonomy, allowed the acquisition and posterior processing of clean optical slices. The new species is compared to other mites that have been described as '*Axonopsella*-like'. *Vagabundia sci* **n. sp.** is named after the Science Citation Index, a sociological tool that, as explained in the text, has done more harm than good to the population of taxonomists.

Key words: *Vagabundia* **n. gen.**, interstitial water mite, confocal optical slicing

Introduction

Taxonomy demonstrates that observation is not a passive process and that the old adage 'an image is worth a thousand words' does not always ring true. Words help to focus the observation and observation is aided by images. However, observation and images cannot substitute for one another.

In previous publications (Valdecasas *et al.* 1997, 2001; Valdecasas & Camacho 2005), we have highlighted different microscopy and computer imaging techniques to aid in the interpretation of taxa including Extended Depth of Focus (EDF) images and Environmental Scanning Electron Microscopy (ESEM). To our knowledge, water mite taxonomy has never been examined with Confocal Laser Scanning Microscopy (CLSM), a not so recent technique that has been used sporadically as an aid in the taxonomy of copepods, tardigrades and insects (Galassi 1997; Rusel *et al*. 2001; Klaus *et al*. 2003).

As an application of CLSM, we describe in this publication a new genus and species of the subfamily Axonopsinae, one of the four subfamilies ascribed to the Family Aturidae (Acari, Hydrachnidia, Parasitengona).

To date, 37 genera have been described within the subfamily Axonopsinae, many of them in groups without clear taxonomic rank such as '*Axonopsella*-like', '*Axonopsis*-like'mites, etc., that share a certain appearance and series of characters but await a full revision for proper taxonomic placement (Cook 1974). The *Axonopsella*-like mites include the following eight genera: *Axonopsella*, *Polyaxonopsella*, *Submiraxona*, *Miraxona*, *Miraxonides*, *Neoalbia*, *Stygalbiella*, and *Adelaxonopsella* with a wide distribution in the Neotropical and Australasian realms.

Samples were taken from different streams and rivers during an intensive inventory of the epi- and hypogean environments of the island of Coiba Panama. The Río Escondido has provided a diversified fauna that we are describing in consecutive publications (Valdecasas 2001, 2008). A single specimen with characteristics of the *Axonopsella*-like mites found in Karaman-Chappuis samples is used to describe *Vagabundia* sci n. gen n. sp.

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Following materials and methods, we provide a brief summary of previous imaging techniques used for water mite taxonomy followed by a short commentary on CLSM in its application to water mite taxonomy; secondly, the new genus and species from Coiba Island, Panama is presented with the help of CLSM and compared to bright field EDF imaging.

Material and methods

Mites were collected in Karaman-Chappuis samples in Río Escondido a very short stream, 4 km long, on the island of Coiba. Panama. Mites were sorted and preserved in Koenike's fluid. Preliminary dissection, drawing and study were done in Koenike fluid under a stereoscopic microscope and a Zeiss Axiolab bright field microscope from which the preliminary EDF images were obtained. CLSM imaging of dissected parts transferred to glicerine jelly on three slides was obtained with a Leica TCS-SP2 confocal microscope with N PLAN L 20x NA 0.40 and HCX PL APO CS 40x NA 1.25 objectives. Image processing was done primarily with ImageJ research free image software (http://rsb.info.nih.gov/ij/). The dissected parts were reunited in a single slide in glicerine jelly and sealed.

Terminology used for morphological description follows that of Cook (1974). The holotype is deposited in the Hydrachnidia collection of the Museo Nacional de Ciencias Naturales, Madrid. All measurements are in µm. For a detailed description of Coiba Island biology, see Castroviejo (1997).

Imaging in water mite taxonomy

Previous work on water mite taxonomy has involved a) traditional hand drawing; b) analog photography associated with bright field and other light microscopy systems; and c) Electronic Scanning Microscopy. As discussed in Valdecasas & Camacho (2005), realism, resolution and portrayal of spatial relationships are the three main conditions for efficient iconic taxonomic representation. All of the techniques mentioned above can achieve these conditions, although the time and expenses involved vary. For instance, a high level of realism can be obtained in hand drawing but at the cost of much time and skill. Light microscopy can achieve excellent resolution of small structures but at the cost of losing spatial relationships. The main problem of ESEM is its inability to portray subepidermal structures. Although not an answer to all of these problems, CLSM complements and in some aspects overcomes some of the limitations of the techniques mentioned above.

Although the idea of a confocal microscope dates back to the 1960s, it was not in common use until the last 15 years. Devoted initially to imaging cell corpuscles and microorganisms, one of the first applications to taxonomy was in the study of copepods (Galassi, 1997). Subsequent applications range from insects to tardigrades to name a few (Schawaroch *et al.* 2005; Russel *et al.* 2001, Petrov 2007). However, its potential as a research tool for taxonomy has yet to be properly acknowledged; a recent multi-author contribution on 'imaging for taxonomy' failed to even mention it as a possible tool (Hauser *et al.* 2005).

The main characteristic of CLSM is its ability to capture clean optical slices that are visually equivalent to histological slices with no blurring above or below the optical slice (see Conchello & Littman 2005, for a summary of the physics of the technique and Klaus *et al.* 2003 for a discussion of the CLSM application in visualization of insect morphology, in which visualization challenges are very similar to those encountered in water mites). Basically, a very small area of the sample under study is illuminated with the light of an appropriate laser that will reflect a fluorescence that passes through a small pinhole. The pinhole only allows the passage of the light of the focused area under illumination discarding any additional light produced from any surrounding area. The whole sample is scanned sequentially and a complete image of an XY section of the object is thus constructed. The slicing is analogous to the traditional sectioning of histological microtomes. Only structures that have fluorescent material at the height Z under focus are represented in the image, all oth-

ers being black. Successive optical slices can be captured and a specific distance in Z is covered. The optical slices can then be further processed to obtain partial or complete confocal extended depth-of-focus images. An optimal interval in the Z axis between successive optical slices will potentially allow the treatment of the whole stack as a solid volume and will rotate it to obtain different angular views. The CLSM is limited to the capacity of the illuminating laser to penetrate into the object and the capacity of the material under study to fluoresce under the excitation of the laser, a characteristic to be decided on empirical grounds.

Systematic account

Family Aturidae Thor, 1900

Subfamily Axonopsinae Viets,1920

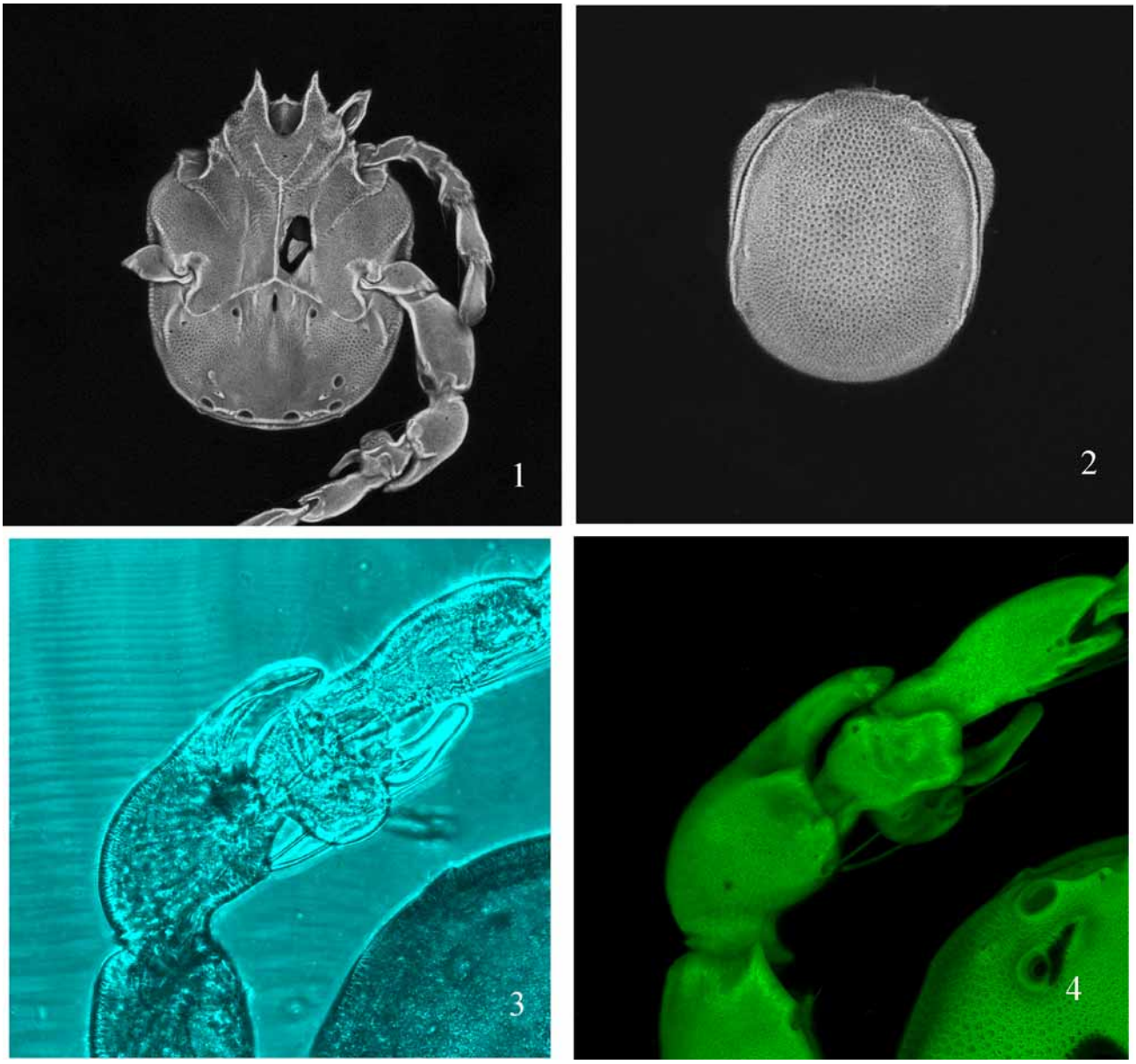
Vagabundia n. gen.

Diagnosis. With the characteristics of the family and subfamily as described by Cook (1974). *Vagabundia* n. gen. belongs to the '*Axonopsella*-like' group of water mites (Cook, op.cit). Body flattened. Dorsal and ventral shields present, not fused. Capitulum separated from the coxae. Coxae extending far beyond anterior end of body. Tips of first pair of coxae pointed. Suture line between first and second coxae meeting and continuing posteriorly as a long median suture line between the other coxae. Fourth coxae with marked posterior suture and with rounded projection that partially covers the insertion of the IV-Leg. Other coxae suture lines incomplete. A ridge present on each side extending anterolaterally and posterolaterally from processes in the IV-coxae. Genital field obliterated. Gonopore opening small and located near middle of body. Four pair of genital acetabula. Gonopore subterminal. Two pairs of small glandularia placed close together laterally near posterior end of fourth coxae. Capitulum with a long pointed rostrum. Setae on capitulum small. Sexual dimorphism in distal segments and claw of II-Leg, and third and fourth segments of IV-Leg male.

Vagabundia sci n. sp.

Figs 1–10

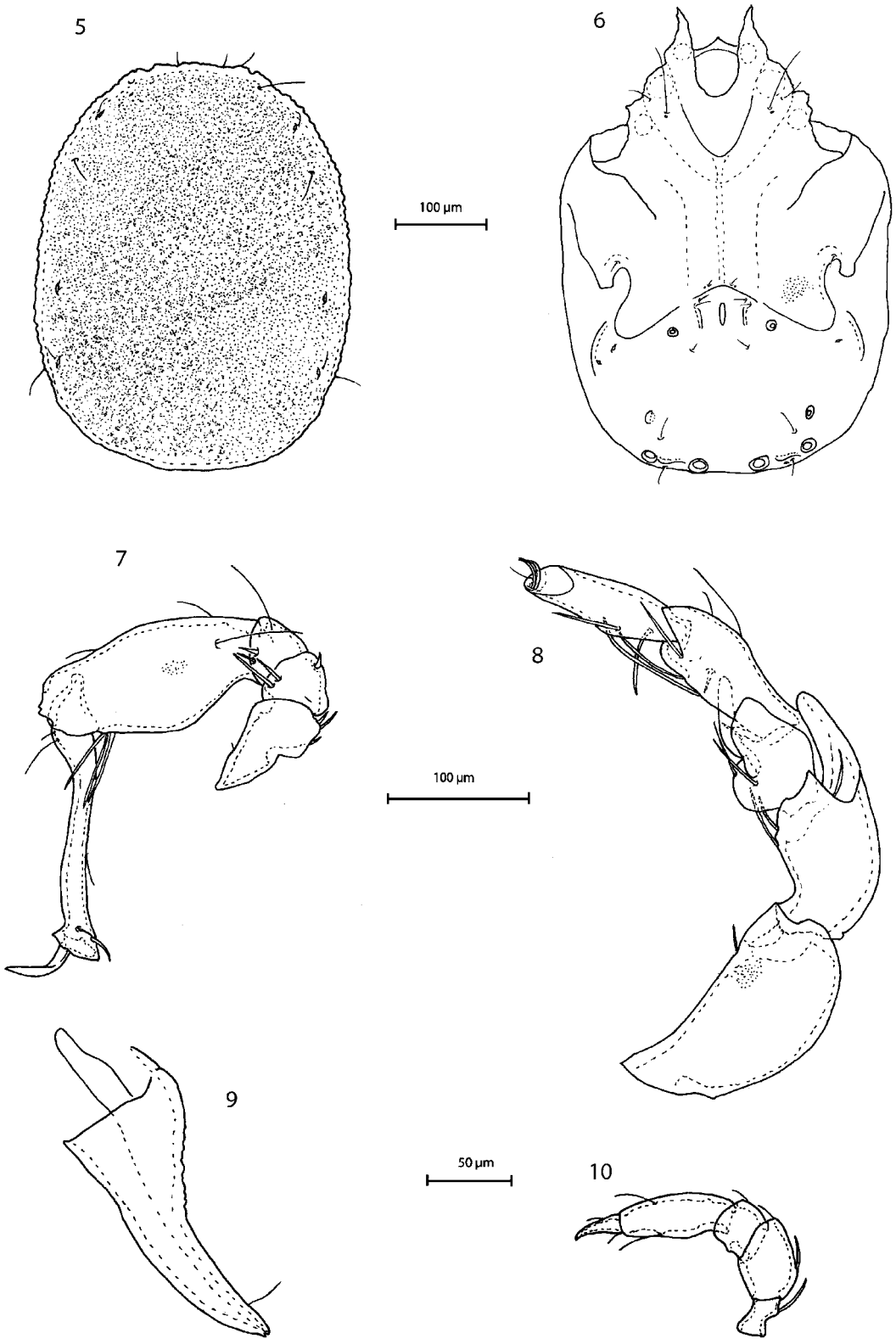
Male. Body rectangular. Length of dorsal shield 440, width 335. Three pairs of glandularia on the dorsal shield with postocularia located close to the first pair. Length of the ventral shield up to the tip of the first coxae 510, width 350. First three pairs of coxae projecting beyond the anterior end of the ventral shield (Figs. 1, 6). First coxae pointed, the second and third more or less rounded. Suture line between first and second coxae converging and continues in a straight line up to the posterior line of the fourth coxa. Suture line between second and third as well as third and fourth coxae incomplete. A longitudinal ridge at both sides and parallel to median suture line. Rounded projections covering the insertions of the fourth legs. A ridge present on each side extending anterolaterally and posterolaterally from processes in the IV-coxae. Genital field obliterated. There are four pairs of genital acetabula, one acetabula on the right side is reduced. Two pairs of small glandularia placed close together laterally near posterior end of fourth coxae. One of these glandularia on left side is reduced. Palps with a single dorsal seta on P-II and one ventral seta on P-IV (Fig. 10). Dorsal length of palp segments: P-I: 28; P-II 49; P-III 22; P-IV 72; P-V 31. Length of capitulum: 188. Length of chelicera: 225. II-Leg with morphological dimorphism of distal segments (Fig. 7). Length of dorsal segments of second leg: II-Leg-2: 35; II-Leg-3: 45; II-Leg-4: 45; II-Leg-5: 165; II-Leg-6: 195. IV-Leg with strong dimorphism in the third and fourth segments (Figs 3, 8). Length of dorsal segments of fourth leg: IV-Leg- 2: 150; IV-Leg-3: 185; IV-Leg-4: 68; IV-Leg-5: 145; IV-Leg-6: 112.



FIGURES 1–4. *Vagabundia sci* **n. sp.** (holotype). 1, ventral shield, CLSM; 2, dorsal shield, CLSM; 3, fourth leg EDF with bright field microscopy; 4, fourth leg EDF with confocal slicing.

Type material. Holotype: Male, 8-viii-1994, Rio Escondido, immediately above waterfall, Coiba Island, Panama. Dissected and on a permanent slides embedded in glycerine jelly.

Etymology. *Vagabundia* comes from the Spanish word 'vagabundo' that means 'wanderer'. It is a feminine substantive; s*ci* refers to Science Citation Index. We pointed out some time ago (Valdecasas *et al*. 2000) that the popularity of the Science Citation Index (SCI) as a measure of 'good' science has been damaging to basic taxonomic work. Despite statements to the contrary that SCI is not adequate to evaluate taxonomic production (Krell 2000), it is used routinely to evaluate taxonomists and prioritize research grant proposals. As with everything in life, SCI had a beginning and will have an end. Before it becomes history, I dedicate this species to this sociological tool that has done more harm than good to taxonomic work and the basic study of biodiversity. Young biologists avoid the 'taxonomic trap' or becoming taxonomic specialists (Agnarsson & Kuntner 2007) due to the low citation rate of strictly discovery-oriented and interpretative taxonomic publications. Lack of recognition of the value of these publications, makes it difficult for authors to obtain grants or stable professional positions.



FIGURES 5–10. *Vagabundia sci* **n. sp.** (holotype). 5, dorsal shield; 6, ventral shield; 7, II-Leg 2-6; 8, IV-Leg 2-6; 9, capitulum, lateral view; 10, right palp, lateral view.

Habitat. Interstitial habitat as sampled by the Karaman-Chappuis method.

Discussion.

On the morphology of the Axonopsella-like mites

Axonopsella-like mites belong to the subfamily Axonopsinae, having entire dorsal shields that may or may not be fused. As reviewed by Cook (1974), the body is dorsal-ventrally flattened and the eyes lie beneath the integument. The coxae are fused with the ventral shield and the capitulum is not fused with the coxae. Tips of the first three pairs of coxae are rounded or, rarely, somewhat pointed. The fourth coxae have large pointed or rounded projections partially covering the insertion of IV-legs. Genital acetabula vary from four pairs to several. The gonopore is terminal and wide in females, subterminal or ventral in males. Suture line of fourth coxae of females are more or less developed. Setae on capitulum are typically small. Sexual dimorphisms in IV-leg-4 is present in males. Male II-leg can be with or without sexual dimorphism.

Discussion of the eight genera described is limited by the fact that some of them are known only from the female specimens, and some species are temporarily assigned to a genus pending male description, and therefore subject to revision. *Vagabundia sci* n.sp known only from the male contributes to this temporary lack of definition of the group '*Axonopsella*-like' mites. Table 1 summarizes the main characteristics differentiating species of the genera, while taking the limitations mentioned into account. *Adelaxonopsella*, although not considered by Cook as *Axonopsella*-like, 'seems to have some affinities' (Cook, 1974: 329-330) with this group of species, and is included in the table for the sake of completeness. The long pointed capitulum, the dimorphism of the II-Leg and especially the IV-Leg are characters that clearly differentiate *Vagabundia* n. gen. from all other genera.

TABLE 1. Main morphological differences among the eight previously described '*Axonopsella*-like' mites and *Vagabundia* **n. gen.**

	Vagabundia **n. gen**.	*Axonopsella*	*Polyaxonopsella*	*Submiraxona*
Kown from	Male	Male and female	Female	Male and female
Coxae extending beyond anterior end of body	Yes	No	No	No (or only tips of 1rst coxae)
Pairs of genital acetabula	4	4	6	5 to many
Male II-Leg dimorphism	Yes	Yes	-	No
Male IV-Leg dimorphism	Yes	Yes	-	Yes
Tips of 1st coxae	Pointed	Rounded	Rounded	Rounded
Tips of 2nd and 3rd coxae	Rounded	Rounded	Rounded	Rounded
Rostrum of capitulum	Long and pointed	Bluntly	Bluntly	Bluntly

continued.

	Miraxona	*Miraxonides*	*Neoalbia*	*Stygalbiella*	*Adelaxonopsella*
Kown from	Male and female	Male and female	Female	Male and female	Female
Coxae extending beyond anterior end of body	1rst coxae extends slightly	No	Yes	Extends slightly	Yes
Pairs of genital acetabula	8 or more	4	6 or 8	4	4
Male II-Leg dimorphism	Yes	No	-	Yes	-
Male IV-Leg dimorphism	Yes	Yes	-	Yes	-
Tips of 1st coxae	Rounded	Rounded	Rounded	Rounded	Pointed
Tips of 2nd and 3rd coxae	Rounded	Rounded	Rounded	Rounded	Pointed
Rostrum of capitulum	Bluntly	Bluntly	Bluntly	Bluntly	Bluntly

The adequacy of describing a new genus and species from a single specimen is debatable, although this is not an uncommon practice. Many taxa have only parts of their body or incomplete specimens available for

study. What is an accepted practice in paleontology should be equally accepted in neontology. This is not to say that taxa should be described from single specimens if it can be avoided, but if the only specimen available differs enough from currently described species, there is no compelling reason that this information should not be presented to fellow specialists.

On CLSM

The specimen on which this interpretation is based had been in Koenike´s fluid for over 10 years, and no special treatment was applied before the confocal study. It was embedded in glycerine jelly prior to the microscopy study, as explained in Material and Methods section. The CLSM provides images with realism similar to that provided by the ESEM, and the processing of the slices for EDF are much more efficient in CLSM than in bright field microscopy. Figures 3 and 4 are provided to show that based on optical slicing images taken of the same specimen at the same time with the same microscope and objective, the resolution and discrimination of CLSM is far superior to that of the bright field EDF. This opens a potential avenue for a renewed study of the type specimens and other material of water mites that are stored in microscopic slides in museum and zoological collections around the world (see a partial list in www.watermite.org), something that cannot be said for ESEM. If it is shown that those mites respond to an exciting laser, then it would be possible to restudy structures and morphologies to obtain a more realistic 3D view of the animal.

Although stacks of images taken with CLSM are amenable to rotation as a solid volume in the three-space axis and, as such, are able to provide angles of view not possible with the microscope, it demands high memory and processing capacity from computer systems. An alternative to imaging structures that may not be visible in the 3D composition is to process partial EDF for sets of optical slices. Figure 11 provides one set of bright field microscopy and CLSM EDF taken with 100 consecutive optical slices of the fourth leg. It can be seen that CLSM better resolves structures that are collinear in the Z axis than bright field microscopy. For example, see the square structure overhanging the strong boomerang shape in Fig 11. In this sense, it is also important to check for structures that are shaded through EDF processing, and a correct drawing could complement the 2D summary image. CLSM seems to be, as well, the adequate technique to avoid the problem mentioned by Cook (1986: 211):'to be able to see many of the ventral structures…it was necessary to remove the dorsal shield (which is generally fused with the ventral shield) destroying or at least distorting the relationships of these posterior structures to each other.'

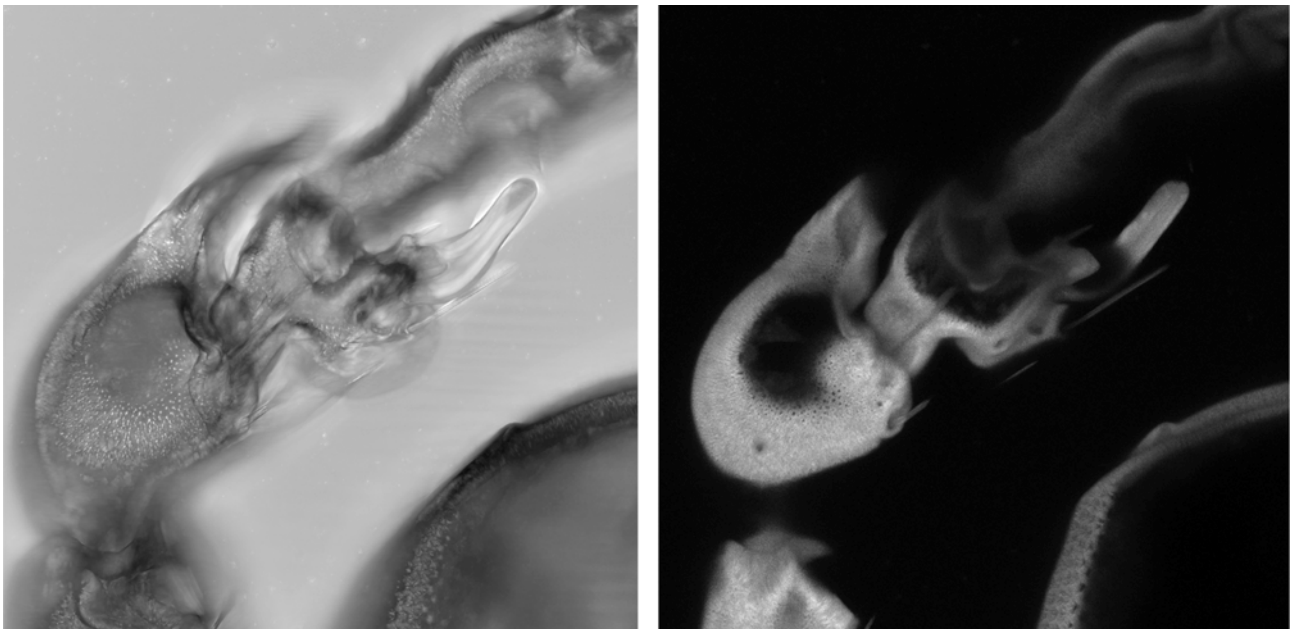


FIGURE 11. Bright field microscopy (left) and confocal (right) EDF images built with set of 100 consecutive optical slices. Step between optical slices 0.2 µm.

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