Received: 8 May 2021 | Revised: 18 June 2021 | Accepted: 25 August 2021

DOI: 10.1002/jemt.23929

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Extended depth of focus ultraviolet imaging compared with laser scanning confocal microscopy for the study of micro-Arthropoda surface texture, with the description of a new species of *Brachypodopsis* (Acari: Hydrachnidia)

Antonio G. Valdecasas

Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Correspondence
Antonio G. Valdecasas, Museo Nacional de Ciencias Naturales, CSIC, c/José Gutiérrez Abascal, 2, 28006 Madrid, Spain.
Email: valdeca@mncn.csic.es

Review Editor: Alberto Diaspro

Abstract

Visualization and representation are two processes at the core of basic biodiversity studies. Visualization involves the examination, sorting, and evaluation of similarities and differences among specimens by specialists who then assign them to the same or different species. It is a cognitive process. Representing involves transmitting the knowledge obtained in the first step to others, usually specialists of the group under study, generally through written descriptions aided by representative drawings and/or images. In this work, I describe a new species of water mite, *Brachypodopsis guillermoi* n. sp. (Acari, Hydrachnidia), from the island of Coiba off the Pacific coast of Panama, using both laser scanning confocal microscopy and extended depth of focus microscopy with visible (wavelength: 400–700 nm) and ultraviolet (wavelength: 365 nm) light. A comparison of the surface texture representation obtained from these imaging methods suggests that extended depth of focus ultraviolet microscopy can be a cost-effective alternative to laser scanning confocal microscopy for the description of exoskeletal features of micro-arthropods.

KEYWORDS
cost-effective alternative, Hydrachnidia, technique comparison, UV light, water mites

1 | INTRODUCTION

Species taxonomic information traditionally includes written descriptions and drawings and/or images that depict representative structures. Both of these approaches are complementary and required. Drawings may be carried out by eye or helped with optical apparatus such as drawing tubes attached to microscopes. Besides drawings, an extensive array of imaging techniques can be used, from cameras attached to bright field microscopes to more sophisticated laser scanning confocal microscopes (LSCM) (Valdecasas, 2008) or electron microscopes, either classical or environmental (Valdecasas & Camacho, 2005). In addition, the use of CT-scanning techniques, which allow for the 3D rendering of structures, has become almost routine for new species descriptions (Landschoff, Komai, du Plessis, Gouws, & Griffiths, 2018, and references therein). Using images can be a complex process as it involves many procedures, beginning from specimen preparation to image acquisition and image processing, with some methodologies better suited for some organisms than for others. The substantial difference in the cost, accessibility, and maintenance of

apparatus for different techniques is also a major consideration for their use in species descriptions.

The highly diverse phylum Arthropoda delimits those organisms that have an external exoskeleton. The exoskeleton has been the primary basis for the discovery, diagnosis, and taxonomy of thousands of species in the classes Insecta, Arachnida, and Crustacea, and other minor groups within Arthropoda. Three main characteristics have been used for the taxonomic description of arthropod species: structure shape, color, and texture. The exoskeleton of many arthropods is autofluorescent, a property that can be used to produce clear, high-resolution images by LSCM that are suitable for taxonomic purposes (Chetverikov, 2012; Valdecasas, 2008). Ultraviolet (UV) light can also be used to produce fluorescent images that provide details on body shape and texture (Haug et al., 2011; Lawrence, 1954; Marek, 2017). The combination of UV illumination with extended depth of focus (EDF) imaging algorithms (Valdecasas, Marshall, Becerra, & Terrero, 2001; Zalevsky, 2010) expands the possibilities to produce even more realistic images of organisms under study than UV illumination alone.

In this work, I use LSCM and EDF (with visible and UV light) to describe a new species of water mite belonging to the genus *Brachypodopsis* and compare the applicability of these two imaging techniques for the presentation of diagnostic features in micro-arthropods. The new species was collected from the island of Coiba of the Pacific coast of Panama. This island previously served as a penitentiary, and owing to its special status, 70% of its primary forest has been preserved (Castroviejo, 1997). Coiba is now part of a national park that was declared a World Heritage Site by UNESCO (UNESCO, 2005).

2 | MATERIAL AND METHODS

Water mites were sampled using a triangular net with a 250 μm mesh size. Animals were sorted and stored in Koenike's fluid (10% Glacial Acetic Acid, 45% Glycerine and 45% water) (Cook, 1974).

Whole specimens were first studied and imaged by LSCM with a Leica TCS SPE microscope (Leica Microsistemas S.L.U., L'Hospitalet de Llobregat, Spain). EDF images of the specimens were then acquired with standard LED and UV illumination using the following equipment: a Sony a7R III digital camera fitted with a Sony 70-200 mmm f/2.8 lens (maximum resolution: 7,952 × 5,304 pixels) and either a 10× or 20× Mitutoyo objective with 0.28 and 0.42 N.A., respectively. The camera and lens were supported on a Wemacro (Ultramacro Ltd, Wickhambrook United Kingdom) automatic stacking rail with a minimum displacement step of 1 μm. Visible light was provided by a Volador (Bright star consulting e. K., Bad Pyrmont, Germany) flashlight equipped with a 4*CREE XM-L2 (U4) LED bulb, and ultraviolet light, by a Thorlabs (Thorlabs GmbH, Dachau/München, Germany) mid-power collimated 365-nm LED light source. Figure 1 shows the general setup of the equipment used for EDF imaging.

The LSCM stacks were processed with Fiji/ImageJ ver 1.53h and Amira ver 5.4.3. The EDF visible and UV light images were processed with Helicon Focus ver. 7.6.4 Pro.

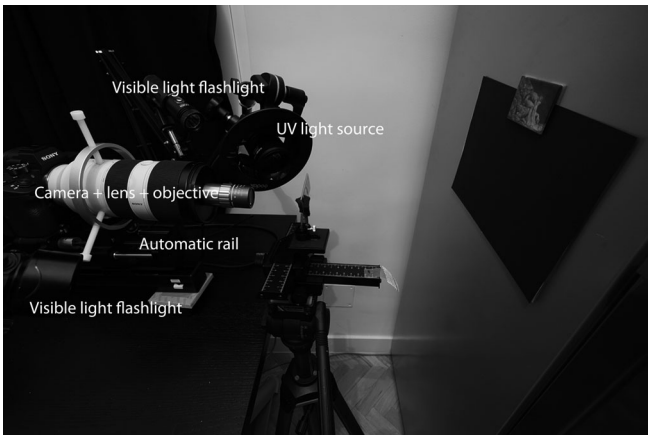

FIGURE 1 Setup used for the acquisition of extended depth of focus (EDF) images under visible or UV illumination

Palps were carefully dissected from each specimen using tungsten needles (World Precision Instruments [World Precision Instruments Inc, Sarasota, Florida]) and then placed on a permanent slide in glycerin jelly. To construct the slides, a Thermo Shandon (Thermo Fisher Scientific Inc, Alcobendas, Spain) 24 × 40 mm, 1.5 mm thick coverslip supported on a metal slide with an open circle in the middle was used instead of a standard glass slide; a 18 × 18 mm, 0.13–0.16 mm thick coverslip was placed over the palps. This arrangement allows both sides of the slide to be viewed under equal optical conditions (Valdecasas, 2008). The same equipment used for the image acquisition of whole specimens was used to image the palps.

3 | RESULTS

Family: Aturidae Thor, 1900.

Subfamily: Axonopsinae K. Viets, 1929.

Genus: *Brachypodopsis* Piersig, 1903.

For a modern morphological delimitation of the genus, see Smit (2020).

Brachypodopsis guillermoi n. sp. (Figures 2–6).

Holotype male, Negro River, Coiba Island, Panama (7°21′52″N, 81°44′52″W). Kicking sample from gravel bottom. August 19, 1994. Collection number: MNCN 20.02/19862.

3.1 | Diagnostic characters

Brachypodopsis guillermoi n. sp. resembles *Paraxonopsis stolli* (Viets, 1978) in the general shape of the body and the structure of the palp. However, *P. stolli* is larger in body size than *B. guillermoi* n. sp. (length and width: respectively, 368 and 300 μm in *P. stolli* compared with 316 and 284 μm in the new species). The external margin of coxae I and II is step shaped in *B. guillermoi*, and almost rectilinear in *P. stolli*. The discrepancy in the number of acetabula between the two sides of the genital area (2 on the left, 3 on the right) is likely due to an anomaly in development.

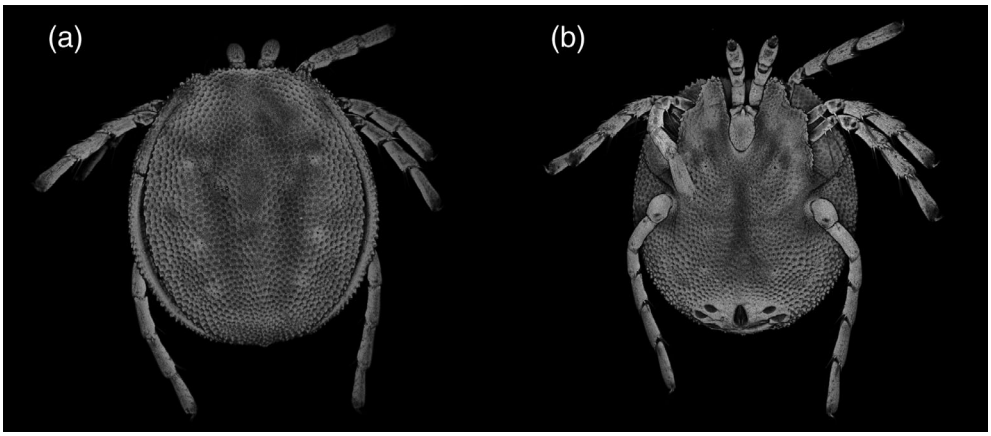

FIGURE 2 *Brachypodopsis guillermoi* n. sp. Laser scanning confocal microscopy. (a) Dorsal view; (b) ventral view

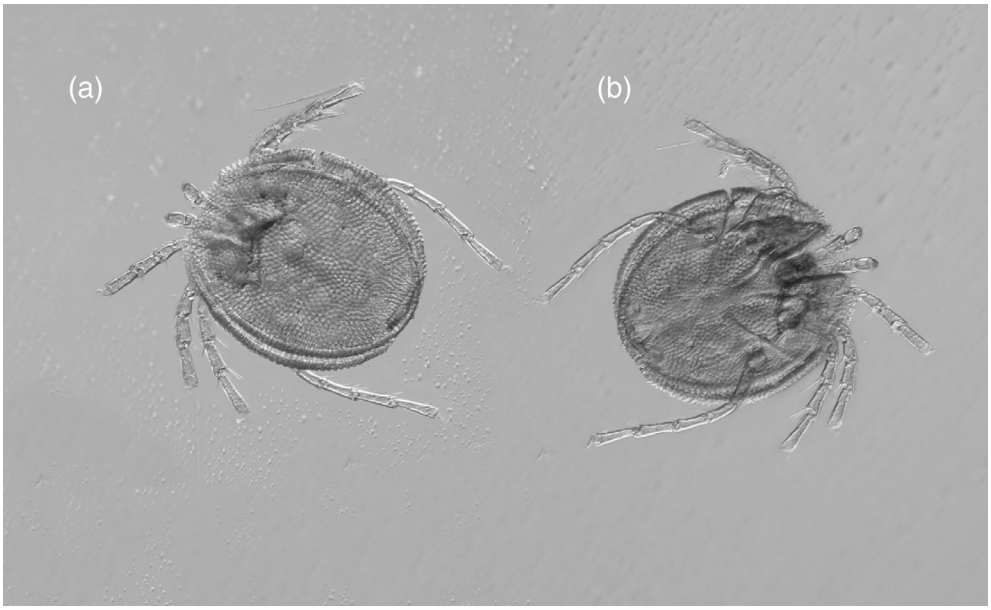

FIGURE 3 *Brachypodopsis guillermoi* n. sp. EDF with visible light. (a) Dorsal view; (b) ventral view

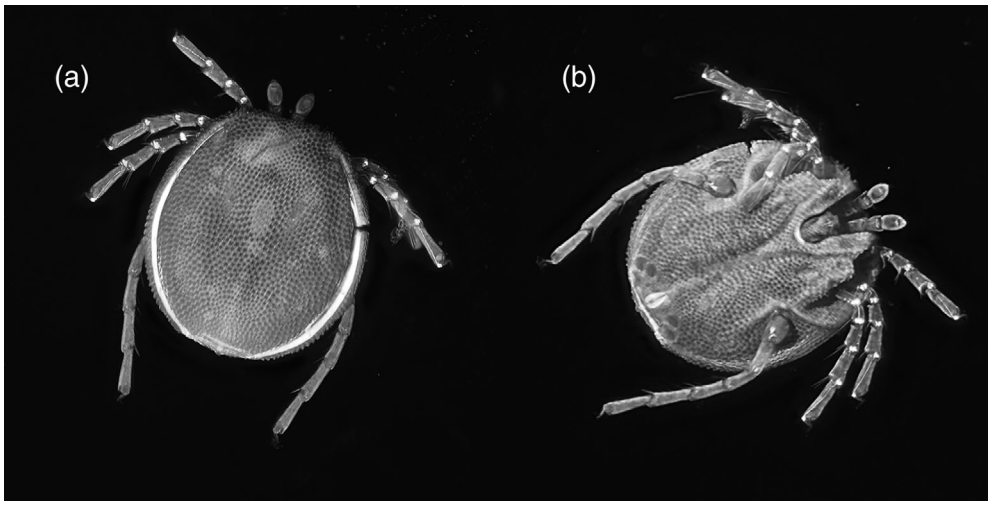

FIGURE 4 *Brachypodopsis guillermoi* n. sp. EDF with UV light. (a) Dorsal view; (b) ventral view

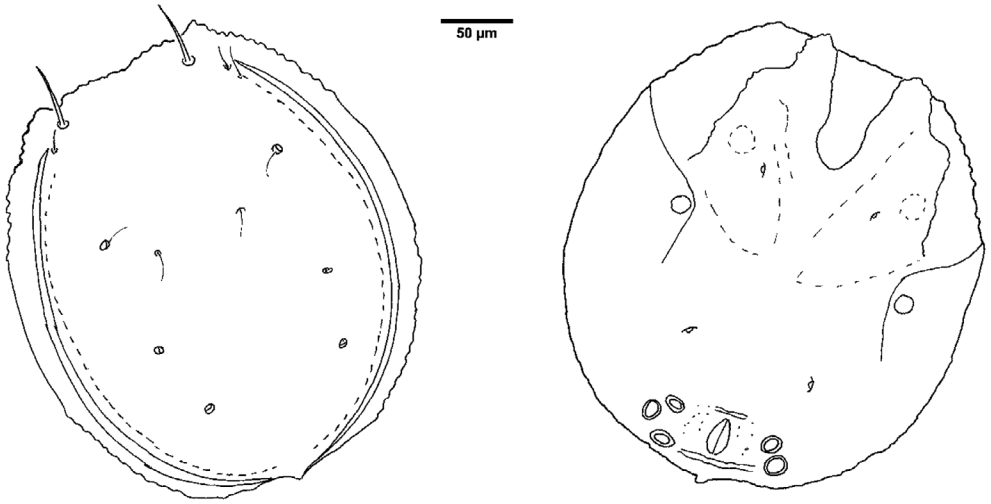

FIGURE 5 *Brachypodopsis guillermoi* n. sp. Schematic drawing. (a) Dorsal view; (b) ventral view

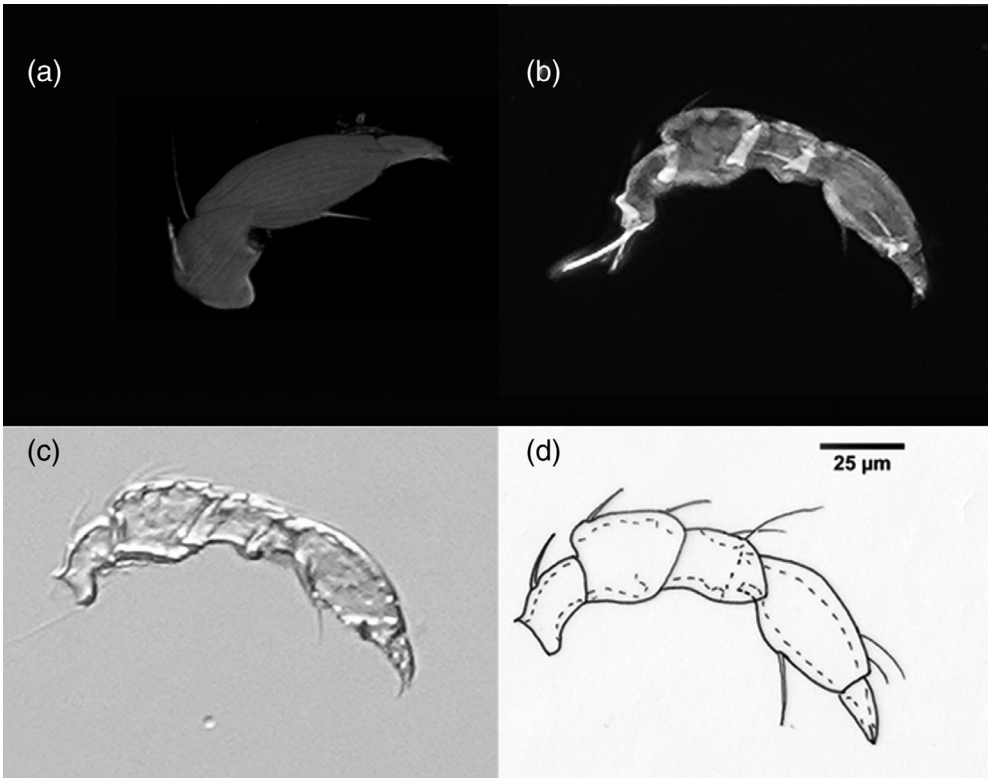

FIGURE 6 Palp of *Brachypodopsis guillermoi* n. sp. (a) Laser scanning confocal microscopy, three last segments; (b) EDF with UV light; (c) EDF with visible light; (d) schematic drawing of the palp

3.2 | Description

Color not registered before fixation. Anteriorly truncated rounded body shape. No glandularia located posteriorly between dorsal and ventral shield. Dorsal shield fused anteriorly with ventral shield. Total length of dorsal shield from the edge front to the level of the excretory pore, 326 µm; maximum width, 251 µm. Three pairs of glandularia along the longitudinal axis. The coxae do not exceed the dorsal shield. Total length of the ventral shield from the anterior tip of coxae I to the posterior margin, 316 µm; maximum width, 284 µm (Figures 2a, 3a, 4a, and 5a).

Anterior border of coxae I and II step sized. Length of gnathosoma bay, 93 µm; maximum width at the end of coxae I, 60 µm. A pair of glandularia between the insertions of IV Leg and the genital area. Two or three acetabula adjacent to the genital aperture. Maximum distance between outer borders of acetabula, 117 µm. Length of genital aperture, 27 µm (Figures 2b, 3b, 4b, and 5b).

Palp segment P-I with a dorsal seta in a medial position; two dorsal setae on P-II; two setae and a triangular hyaline projection on P-III. Three setae on P-IV, two dorsal and one ventral, with the ventral one positioned on a bulge; no setae on P-V. Dorsal length of palp segments (P-I to P-V): 25, 38, 21, 50, and 22 µm (Figure 6a–d).

No sexual differentiation of leg segments. One swimming setae on IV-Leg-4, and two on IV-Leg-5.

3.3 | Etymology

The new species is dedicated to my brother Guillermo for his support of my research, especially during a time when I found it extremely difficult to obtain grants for basic taxonomic research in Spain.

3.4 | Remarks

I assign this species to the genus *Brachypodopsis*. In a revision of the subfamily Axonopsinae (Smith, Cook, & Gerecke, 2015; see also Gerecke, Gledhill, Pešić, & Smit, 2016 for some contradictory statement about glandularia on the dorsal shield), several previous subgenera of *Axonopsis* were elevated in rank to independent genera. Of those, *Paraxonopsis* and *Brachypodopsis* remain poorly defined as some diagnostic characters overlap between the two genera. For instance, *Paraxonopsis* is delimited as having three or four genital acetabula and two, or rarely one, glandularia between the insertions of the fourth legs and the genital area. *Brachypodopsis* may also have three or four genital acetabula and one glandularia between the insertions of the fourth legs and the genital area. The main character that appears to distinguish the two genera is the tendency of species of *Paraxonopsis* to develop cauda, which are lacking in those of *Brachypodopsis*. In this sense and following the identification diagnostic key of Smit (2020), both *B. guillermoi* n. sp. and *P. stolli* (Viets, 1978) key to *Brachypodopsis as* cauda are not observed in either species. I think that this uncertainty will be easily resolved once molecular data are available for species of both genera.

TABLE 1 Some characteristics of laser scanning confocal microscopy (LSCM), extended depth of focus (EDF) with UV or visible light

	Confocal	Ultraviolet	Visible
Surface texture rendering	√	√	—
3D reconstruction	√	—	—
Color	—	—	√
Cost	High	Low	Low
Maintenance	Expensive	Inexpensive	Inexpensive

4 | DISCUSSION

4.1 | On visualization and imaging in taxonomy

Taxonomic descriptions are statements of a comparative nature that establish a set of features delimiting one species from another (Nixon & Wheeler, 1990). To transmit this information, written descriptions are usually aided by drawings and/or images. Drawings, besides those aspiring to represent an artistic rendering, are usually schematized and adapted to communicate with specialists of the organisms under study.

Since the invention of photography, images have supplemented the information content of drawings. Advances in photography have paralleled those in microscopic techniques and instruments, making it easier to study organisms or their parts, especially those that require magnification. With the advent of digital photography and the development of new microscopic techniques, the rendering of diagnostic features in a realistic context is closely approaching. Nevertheless, even with highly sophisticated techniques such as micro-CT scans, drawings that complement images (either separate to or superimposed on images) are sometimes still needed for a complete and highly informative rendering of a structure (Landschoff et al., 2018).

Although around 18,000 new species of plants and animals are described per year, this effort may not be enough to help counteract the high number of species that become extinct. Part of the reason why more species are not described each year is due to the low academic status of the field of taxonomy and the low turnover rate of taxonomists (the so-called "taxonomic impediment"; de Carvalho et al., 2005). In this sense, a realistic imaging of organisms can achieve the following objectives: (a) improve the transmission of knowledge of diagnostic characters to current and future specialists; (b) facilitate a greater appreciation of organisms by the general public; and (c) encourage new generations to embark on taxonomic studies. One problem is that informative and attractive visual presentation of small organisms may require expensive instruments. In this study, I conclude that EDF with UV illumination offers a budget friendly alternative to

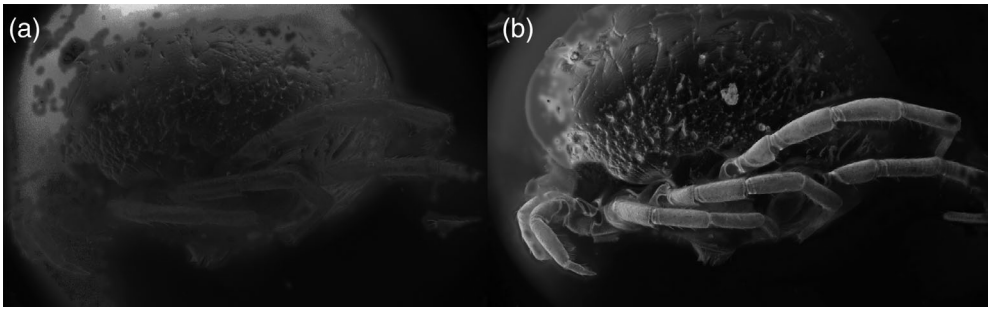

FIGURE 7 *Hydrachna skorikowi* (Piersig, 1900) (a) EDF with visible light; (b) EDF with UV light

LSCM, particularly for surface texture rendering. A comparison of the imaging techniques used in this study is shown in Table 1.

UV radiation reflected back as visible light is called fluorescence, and it is well-known that a variety of organisms are autofluorescent under UV light, although the functional or selective value of this fluorescence has only been demonstrated in a few of them (Johnsen, 2012). UV light is also routinely used in forensic applications given that "UV light does not penetrate even very thin layers of materials, making surface topology more apparent" (Richards, 2010). A good example of this fact is shown in Figure 7a,b, which shows a specimen of the water mite *Hydrachna skorikowi* (Piersig, 1900). In this specimen, more diagnostic features can be clearly discerned using EDF with UV illumination than with visible light. Notably, for EDF image acquisition, aside from the illumination source, all of the other mechanical, optical, and software elements are the same for imaging with visible or UV light, making it easy to use the advantages of both for species descriptions.

ACKNOWLEDGMENTS

Melinda Modrell corrected the English of an earlier version of this manuscript, and Miguel Angel Alonso Zarazaga helped with nomenclatorial issues.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Antonio G. Valdecasas https://orcid.org/0000-0002-5665-4906

REFERENCES

Castroviejo, S. (1997). *Flora y Fauna del Parque Nacional de Coiba (Panamá)* (534 pp.). Madrid, Spain: AECI.

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.

Cook, D. R. (1974). *Water mite genera and subgenera* (Vol. 21, 860 pp.). U.S.A: Memories American Entomological Institute.

de Carvalho, M. R., Bockmann, F. A., Amorim, D. S., de Vivo, M., de Toledo-Piza, M., Menezes, N. A., … Nelson, G. (2005). Revisiting the taxonomic impediment. *Science*, *307*, 353.

Gerecke, R., Gledhill, T., Pešić, V., & Smit, H. (2016). In R. Gerecke (Ed.), *Süßwasserfauna von Mitteleuropa*, *Chelicerata: Acari III* (Vol. 7/2-3, pp. 1–429). Heidelberg, Germany: Springer. https://doi.org/10.1007/978-3-8274-2689-5_1

Haug, J. T., Haug, C., Kutschera, V., Mayer, G., Maas, A., Liebau, S., … Waloszek, D. (2011). Autofluorescence imaging, an excellent tool for comparative morphology. *Journal of Microscopy*, *244*, 259–272.

Johnsen, S. (2012). *The optics of life. A biologist's guide to light in nature*. U.S.A: Princeton University Press.

Landschoff, J., Komai, T., du Plessis, A., Gouws, G., & Griffiths, C. L. (2018). MicroCT imaging applied to description of a new species of *Pagurus* Fabricius, 1775 (Crustacea: Decapoda: Anomura: Paguridae), with selection of three-dimensional type data. *PLoS One*, *13*(9), e0203107. https://doi.org/10.1371/journal.pone.0203107

Lawrence, R. F. (1954). Fluorescence in Arthopoda. *Journal Entomological Society South Africa*, *17*, 167–170.

Marek, P. (2017). Ultraviolet-induced fluorescent imaging for millipede taxonomy. *Research Ideas and Outcomes*, *3*, e14850. https://doi.org/10.3897/rio.3.e14850

Nixon, K. C., & Wheeler, Q. D. (1990). An amplification of the phylogenetic species concept. *Cladistics*, *6*, 211–223.

Richards, A. (2010). *Reflected ultraviolet imaging for forensics applications*. Retrieved from http://www.ultravioletcameras.com/pdf/Reflected_UV_Imaging_for_Forensics_V3.pdf

Smit, H. (2020). Water mites of the world. *Monografieën van der Nederlandse Entomologische Vereniging*, *12*, 1–774.

Smith, I. M., Cook, D. R., & Gerecke, R. (2015). Revision of the status of some genus-level water mite taxa in the families Pionidae Thor, 1900, Aturidae Thor, 1900, and Nudomideopsidae Smith, 1990 (Acari: Hydrachnidiae). *Zootaxa*, *3919*(1), 111–156.

UNESCO. (2005). Coiba National Park and its Special Zone of Marine Protection. Retrieved from https://whc.unesco.org/en/list/1138/

Valdecasas, A. G. (2008). Confocal microscopy applied to water mite taxonomy with the description of a new genus of Axonopsinae (Acari, Parasitengona, Hydrachnidia) from Central America. *Zootaxa*, *1820*, 41–48.

Valdecasas, A. G., & Camacho, A. I. (2005). On the environmental scanning electron microscope (ESEM) for taxonomic purposes. *Invertebrate Biology*, *124*(1), 67–74.

Valdecasas, A. G., Marshall, D., Becerra, J. M., & Terrero, J. J. (2001). On the extended depth of focus algorithms for bright field microscopy. *Micron*, *32*, 559–569.

Viets, K. O. (1978). Neue und seltene Wassermilben (Hydrachnellae, Acari) aus Guatemala – Teil IV. *Acarology*, *19*, 463–483.

Zalevsky, Z. (2010). Extended depth of focus imaging: A review. *SPIE Reviews*, *1*(1), 018001. https://doi.org/10.1117/6.0000001

How to cite this article: Valdecasas, A. G. (2021). Extended depth of focus ultraviolet imaging compared with laser scanning confocal microscopy for the study of micro-Arthropoda surface texture, with the description of a new species of *Brachypodopsis* (Acari: Hydrachnidia). *Microscopy Research and Technique*, 1–6. https://doi.org/10.1002/jemt.23929