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A NEW TECHNIQUE FOR EXAMINING SURFACE MORPHOSCULPTURE OF SCORPIONS

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ABSTRACT. A new technique for examining the exomorphology of the scorpion epicuticle is described that utilizes the fluorescent property of scorpion cuticle. Fluorescence of the scorpion exoskeleton under longwave ultraviolet light is a well known property previously only utilized for the capture or observation of scorpions at night. Fluorescence is an energy emission that is analogous to the secondary electron emissions utilized in electron microscopy to provide information about surface detail. This new technique is fast, inexpensive and non-destructive, and provides an alternative means of documenting of surface macrosculpture for the description and identification of scorpion species.

Keywords: Fluorescence, scorpion, epicuticle, exomorphology, images

Among the many unique and unusual features that scorpions exhibit, arguably the most curious is their fluorescence on exposure to long wavelength UV (ultraviolet light). Fluorescence is an energy (light) emission that results from the excitation of electrons in certain compounds by light of specific wavelengths. Once excited by a photon, the electrons of these compounds almost immediately return to their previous energy state, and simultaneously a lower level energy emission (visible light) results.

Pavan (1954a) first demonstrated that UV light of wavelength 366.3 nm causes maximum fluorescence of scorpion epicuticle. Scorpion fluorescence has captivated the interest of researchers (Honetschlacher 1965; Lawrence 1954; Williams 1980) since it was revealed that they exhibit this curious phenomenon. Pavan (1954a, 1954b) found that the fluorescence emanates from the outermost layer of the cuticle, the epicuticle, but was unable to discover the fluorescent compound or compounds. More recently, Stachel and Stockwell (1999) isolated the fluorescent compound, β-carboline, from scorpion epicuticle. The intensity of scorpion fluorescence varies among species and the time elapsed since the last molt, and non-fluorescent scorpions are unknown (Stahnke 1972). Despite a reasonable understanding of the origins of the fluorescence in scorpions, there is still no consensus as to why this phenomenon exists. The fluorescent property has to date been exploited most successfully as a tool in their observation, detection or capture (Stahnke 1972). Scorpions are predominantly nocturnal and, equipped with a blacklight, a researcher can find many more specimens, as well as species, at night than is possible during the day with an equivalent searching effort (Honetschlacher 1965; Lamoral 1979; Sissom et al. 1990; Williams 1980).

Images of biological specimens made from SEM (scanning electron microscopes) predominantly utilize the detection of emission of SEs (secondary electrons) to form digital images. Secondary electrons result when a focused beam of electrons of sufficient acceleration voltage passes over a suitable (conductive) subject, typically of high molecular mass. High energy electrons from the primary beam displace loosely bound outer-orbital electrons in the subject, and SEs are emitted from the surface of the specimen. These SEs have a different voltage (energy) to that of the electron beam that scans the surface of the

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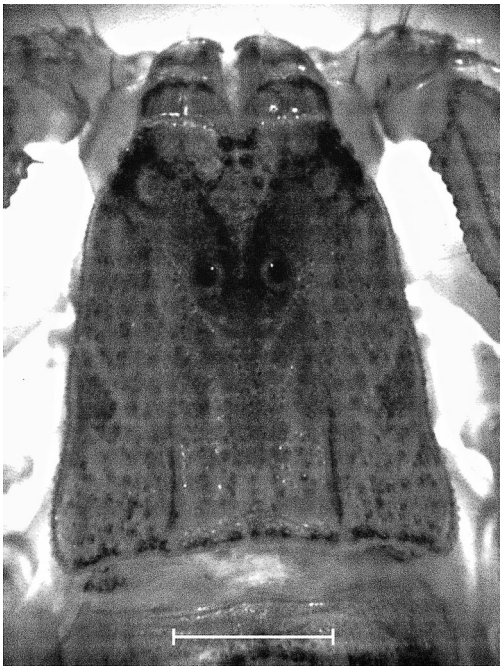

Figure 1.—Carapace of *Lychas* sp.1. from Australia (WAM; conventional image (scale bar = 1mm).

Figure 2.—Carapace of *Lychas* sp.1. from Australia, fluorescence image (scale bar = 1mm).

specimen and are principally utilized in the production of images of surface detail from electron microscopes. The production of the SE emission in an electron microscope is analogous to the light emission called fluorescence, and prompted the author to considered experimentation with imaging of cuticular surface detail from the fluorescence of scorpions. The surface sculpturing (granulations and carinae) of scorpions is frequently utilized in the identification of species (Lamoral 1979; Sissom 1990), however, this useful character can be difficult to examine as it is often obscured by complex color patterns lying beneath the cuticle. Images made using this fluorescence technique were recently published by Prendini (2003a, 2003b, 2003c) and further exemplify its usefulness.

METHODS

The specimens used to exemplify this technique are lodged in the Western Australian Museum: *Lychas* sp. 1 (ESV2255), *Lychas* sp.2 (T56392) and *Lychas variatus* (Thorell 1876) (WAM 97/1226); and the Queensland Museum: *Hemilychas alexandrinus* (Hirst 1911) (S58519). Images were taken with a Leica DC100 digital camera attached to a Leica MZ6 stereo dissection microscope, fitted with an iris diaphragm. Standard illumination was provided from a Leica light source. Ultraviolet illumination was provided from modified portable blacklight units normally utilized in the field detection and collection of scorpions. Each unit consisted of a portable 12V fluorescent light fixture, fitted with two black light tubes (National, FL8 BL-B), and powered by a 12V rechargeable lead-acid battery (Panasonic, LC-R127R2P). When fluorescence images were being taken, the two blacklights were placed on either side of the specimen. Specimens were imaged at night to minimize extraneous light. Conventional illumination was used, with the iris diaphragm fully constricted to provide maximum depth of field, to position and focus the image, after which the blacklights were switched on and all other sources of illumination (except computer monitor which was turned away from the microscope) were switched off. All images were taken with the slow imaging option of the DC100 software, owing to the lower intensity of the fluorescence, longer periods (up

Figure 3.—Mesosomal tergites of *Lychas* sp.2. from Australia, conventional image (scale bar = 1mm).

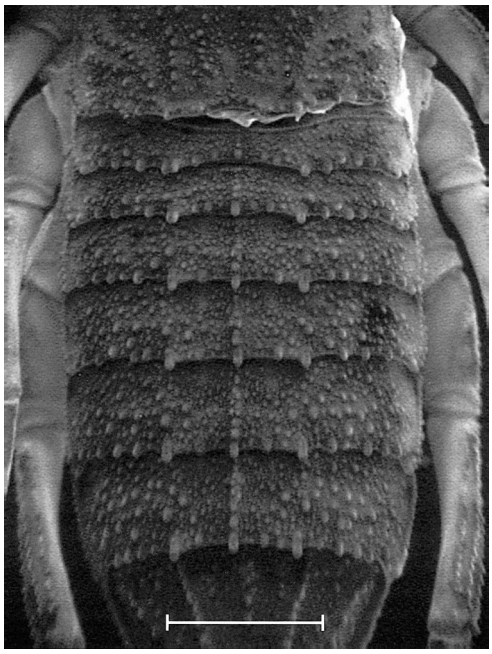

Figure 4.—Mesosomal tergites of *Lychas* sp.2. from Australian, fluorescence image (scale bar = 1mm).

to 10 seconds) were required per image capture.

For imaging, each specimen was placed into a small glass petri dish with enough 90–100% ethanol to just cover it. The clarity of the image deteriorated considerably as the depth of ethanol above the specimen increased. More dilute concentrations of ethanol were also trialed, however 70–80% mixtures developed a faint scum over the surface that decreased the clarity of the images.

Images of epicuticle fluorescence were in shades of blue, and these were converted to greyscale in the graphics editing package Corel™ Photo-Paint (version 7). Some images were also enhanced for publication by making minor improvements to levels of brightness and contrast, the same adjustments typically being conducted on images made using a SEM. The technique described in this contribution is exemplified using four different species of Australian buthids.

RESULTS

Fluorescence images (Figs. 2, 4, 6 & 8) reveal grey scale surface detail and structure of the external sculpturing of scorpion exoskeleton. Conventional imaging under ethanol almost completely obscures the surface sculpturing in images, (Figs. 1, 3, 5 & 7) but provides accurate documentation of color patterning. Surface detail revealed in the fluorescent images mimics those obtained from scanning electron microscopes except that setae and macrosetae are not revealed, and the depth of field is relatively shallow.

Figures 1 and 2 depict the carapace of *Lychas* sp. 1, an undescribed species from Australia. The specimen had become badly distorted during or following preservation (tissue displacement was evident beneath the cuticle). Consequently, under conventional illumination the carapace had a glassy semitransparent appearance, resulting from light reflecting from beneath the carapace. The fluorescent image reveals only the surface detail and this detail could accurately be compared with more intact specimens or similarly damaged specimens to facilitate the identification of more intact specimens of this species. Figures 3 and 4 depict the mesosomal tergites of *Lychas* sp. 2, another undescribed *Lychas* species from Australia. Figure 5 depicts the ornately patterned carapace of *Lychas variatus*

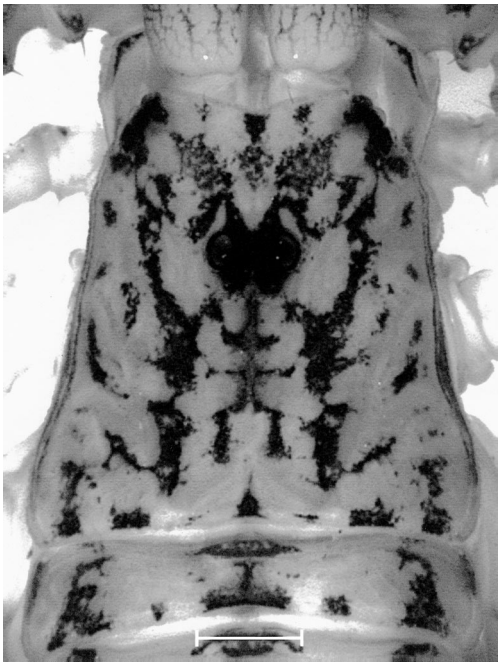

Figure 5.—Carapace of *Lychas variatus* (Thorell 1876), conventional image (scale bar = 1mm).

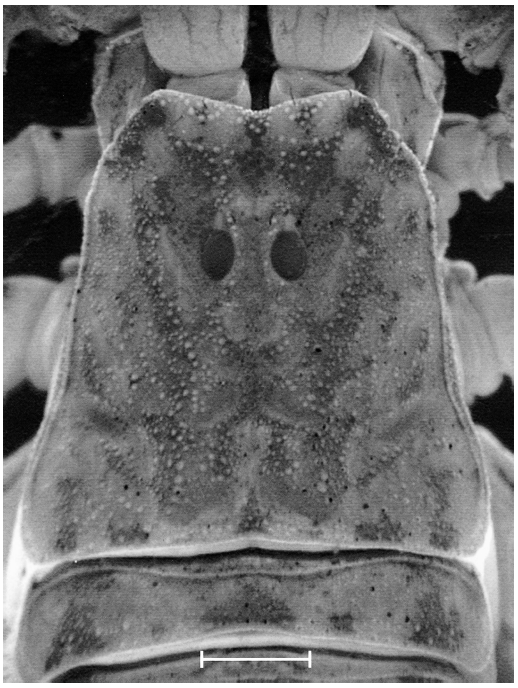

Figure 6.—Carapace of *L. variatus*, fluorescence image (scale bar = 1mm).

while the fluorescent image (Fig. 6) reveals the poorly sculptured surface. Figures 7 and 8 depict the lateral aspects of metasomal segment V of *Hemilychas alexandrine*, another Australian buthid. The finely reticulate pattern is seen in Fig. 7 using standard illumination, whereas punctated nature of this metasomal segment is revealed in the fluorescent image, Fig 8. In this case the fluorescent image revealed surface detail not associated with granulations, but with punctations.

DISCUSSION

The technique described here, for imaging scorpions under UV light provides images with detail similar to those taken with a scanning electron microscope. Unlike specimens examined in a conventional SEM, those from which the fluorescence images were made were not coated in conductive material and were taken under normal atmospheric conditions. Fluorescence imaging is a non-destructive technique that can be applied to type specimens. These images reveal information about the surface sculpturing of the cuticle that may otherwise be obscured or over-enhanced by subcuticular pigmentation. Images of scorpion fluorescence are proposed to augment line drawings for the documentation of surface sculpture in scorpions. This imaging protocol provides a much cheaper substitute for SEM. Using a digital camera, mounted to a dissection microscope, images can be taken as quickly or slowly as possible and adjustment of the specimen can be made directly and immediately. A particular advantage of this technique over SEM is the ability to manage very large specimens. Many scorpions are too large to be examined examined using SEM without dissecting the specimen before mounting the area of interest onto a stub. The fluorescent technique described here can be applied to large scorpions without dissecting the specimen.

Some drawbacks of this technique relate to the low depth of field experienced at high magnifications, and a slightly grainy appearance to the images. The relatively large size of scorpions, compared with most other chelicerates, implies that low depth of field issues are not likely to be experienced unless imaging the smallest of scorpions, or very small structures such as chelicerae and tarsi. An ad-

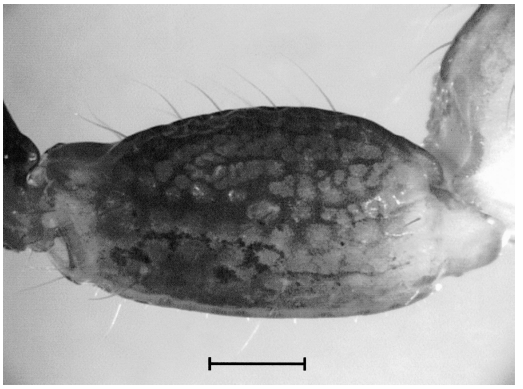

Figure 7.—Lateral aspect of metasoma V of *Hemilychas alexandrinus* (Hirst 1911), conventional image showing color patterning and some setation (scale bar = 1mm).

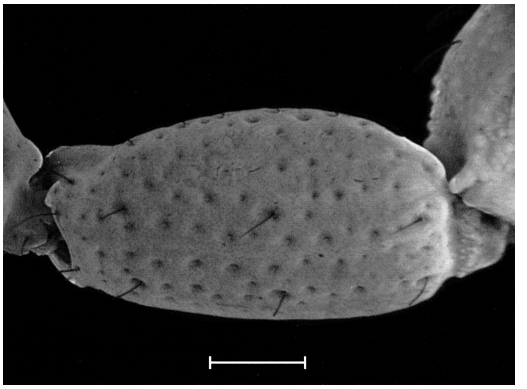

Figure 8.—Lateral aspect of metasoma V of *H. alexandrinus*: fluorescence image, showing surface punctations (scale bar = 1mm).

ditional drawback is the non-fluorescent nature of setae and macrosetae, making this technique unsuitable for investigation into chaetotaxy. Interestingly, the base of some setae and the areoles of trichobothria typically fluoresce more brightly than the surrounding surfaces and this property can assist in the location of trichobothria. The quality of digital images has improved considerably since this study was conducted, and smoother images are already characteristic of high resolution digital cameras. With the application of fluorescent stains and histological preparations, this technique may be applicable to other organisms that do not naturally fluoresce.

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LITERATURE CITED

Hirst, S. 1911. Descriptions of new scorpions. Annals and Magazine of Natural History (8) 8:462–473.

Honetschlacher, L.D. 1965. A new method for hunting scorpions. Turtox News 43:69–70.

Lamoral, B.H. 1979. The Scorpions of Namibia (Arachnida: Scorpionida). Annals of the Natal Museum 23:497–784.

Lawrence, R.F. 1954. Fluorescence in Arthropoda. Journal of the Entomological Society of Southern Africa 17:167–170.

Pavan, M. 1954a. Primi dati per la caraterizzazione della sostanza fluorescente nel tegumento degli scorpioni. Bolletino Societa Italiana Biologia Sperimentale 30:803–805.

Pavan, M. 1954b. Studi sugli scorpioni I, una nuova caratteristica tipica del tegumentodegli scorpioni. Italian Journal of Zoology 21:283–291.

Prendini, L. 2003a. Discovery of the male of *Parabuthus muelleri*, and implications for the phylogeny of *Parabuthus* (Scorpiones: Buthidae). American Museum Novitates 3408:1–24.

Prendini, L. 2003b. A new genus and species of bothriurid scorpion from the Brandberg Massif, Namibia, with a reanalysis of bothriurid phylogeny and a discussion of the phylogenetic position of *Lisposoma* Lawrence. Systematic Entomology 28:149–172.

Prendini, L. 2003c. Systematics and biogeography of the family Scorpionidae (Chelicerata: Scorpiones), with a discussion on phylogenetic methods. Invertebrate Systematics 17:185–259.

Sissom, W.D. 1990. Systematics, biogeography, and paleontology. *In* G. A. Polis (ed) Systematics, biogeography, and paleontology. Stanford University Press, Stanford, pp. 64–160.

Sissom, W.D., G.A. Polis and D.D. Watt. 1990. Field and Laboratory methods. *In* G. A. Polis (ed) Field and Laboratory methods. Stanford University Press, Stanford, pp. 445–461.

Stachel, S.L., S.A. Stockwell and D.L.V. Vranken. 1999. The fluorescence of scorpions and cataractogenesis. Chemistry and Biology 6:531–539.

Stahnke, H.L. 1972. UV light, a useful field tool. Bioscience 22:604–607.

Thorell, T. 1876. Etudes scorpiologiques. Atti Societe Italienne de Sciences Naturelles 19:75–272.

Williams, S.C. 1980. Scorpions of Baja California, Mexico, and adjacent islands. Occasional Papers of the California Academy of Sciences 135:1–127.

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