Bug's Eye View

Brian J. Ford & Debbie J. Stokes

Many of the earliest published microscopical works were of arthropod pests. *Pulex* the flea and *Pediculus* the louse featured as large engravings in Robert Hooke's great work *Micrographia* (1665) and served greatly to increase the general interest shown in the book.

The bed bug was less frequently pictured. Although it has been closely associated with the human host for thousands of years (these insects have been found in excavations from Ancient Egypt, for example) it did not become such a popular subject for microscopists. Present-day domestic populations of bed bugs are set to increase, and it is instructive to consider how the anatomical features of these insects can be addressed by present-day investigators. We briefly present three approaches to the microscopical investigation of the bed bug using transmitted light, incident light and secondary electron imaging.

Bed Bugs

The common bed bug *Cimex lectularius* is one of 75 species in the family *Cimicidae* within the order *Heteroptera: Hemiptera,* the true bugs. In the north-eastern United States the bat bug *C. pilosellus* is also widespread in human habitations, while *C. hemipterus* is a tropical species that is found in homes in Florida and elsewhere. Other warm-blooded hosts have parasitic genera of their own. *Cymexopsis* infests swifts and *Oeciacus* feeds on martins and swallows. *Haematosiphon inodora* is a pest of poultry that has also been known to feed on human blood.

These arthropods obtain blood from the peripheral circulation by sucking at night. The proboscis is a complex structure, with a pointed stylet that pierces the epidermis contained within a cylindrical sheath through which blood is drawn from pooled blood from the penetrated capillaries. Like other blood-sucking insects that inject an anticoagulant prior to ingesting blood, *Cimex* introduces a flush of saliva at the moment of piercing the host epidermis. Curiously, unlike fleas and lice, *Cimex* does not transmit disease. It often produces a temporary irritation or wheal, but there are no records of bed bugs transmitting human blood-borne infections.

Appearance

Preparations of insects are routinely made by clearing the body contents with sodium hydroxide NaOH and mounting in a permanent medium (traditionally Canada balsam). This technique well preserves the microanatomy of the chitinous exoskeleton and allows us to visualise appendages and surface hairs. Adult insects, however, lose much of their intrinsically interesting features in this preparation process.

Cimex is a case in point. Its thorax and abdomen are lustrous and waxy, with characteristic sculptured bands that convey a distinctive appearance. The flattened permanent preparation examined with transmitted illumination with which microscopists are familiar bears little resemblance to the living insect. Although fine surface details can be well resolved, the configuration of the entire insect cannot be deduced and observers who have studied microscope slides of the cleared exoskeleton would not readily recognise the insect in life.

The adult *C. lectularius* is a dusky brown insect approximately 4.5 mm in length and has a flattened appearance which changes dramatically when it feeds. The bugs can engorge themselves with human blood in 5 minutes and the body then becomes spheroidal and deep red in colour, often being described as having the appearance of 'animated blood drops'. At this stage they are easily inadvertently crushed by the host and it is brownish spots on bed linen – the crushed remains of adult bed bugs – that are often the first signs of infestation.

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Microscopy

Bench microscopes are rarely fitted with low-power objectives and it is at modest magnifications – 20x or 35x, for example – that such specimens are initially best examined. Observations of bed bugs can be well made with a hand lens, and even toy digital microscopes (like the QX microscopes made by Intel and Digital Blue) give satisfactory images.

Rather than using transmitted illumination, incident light should be employed when examining the fresh or living insects. In this case a Prior 45 mm objective magnifying 2.7x seemed an ideal lens to

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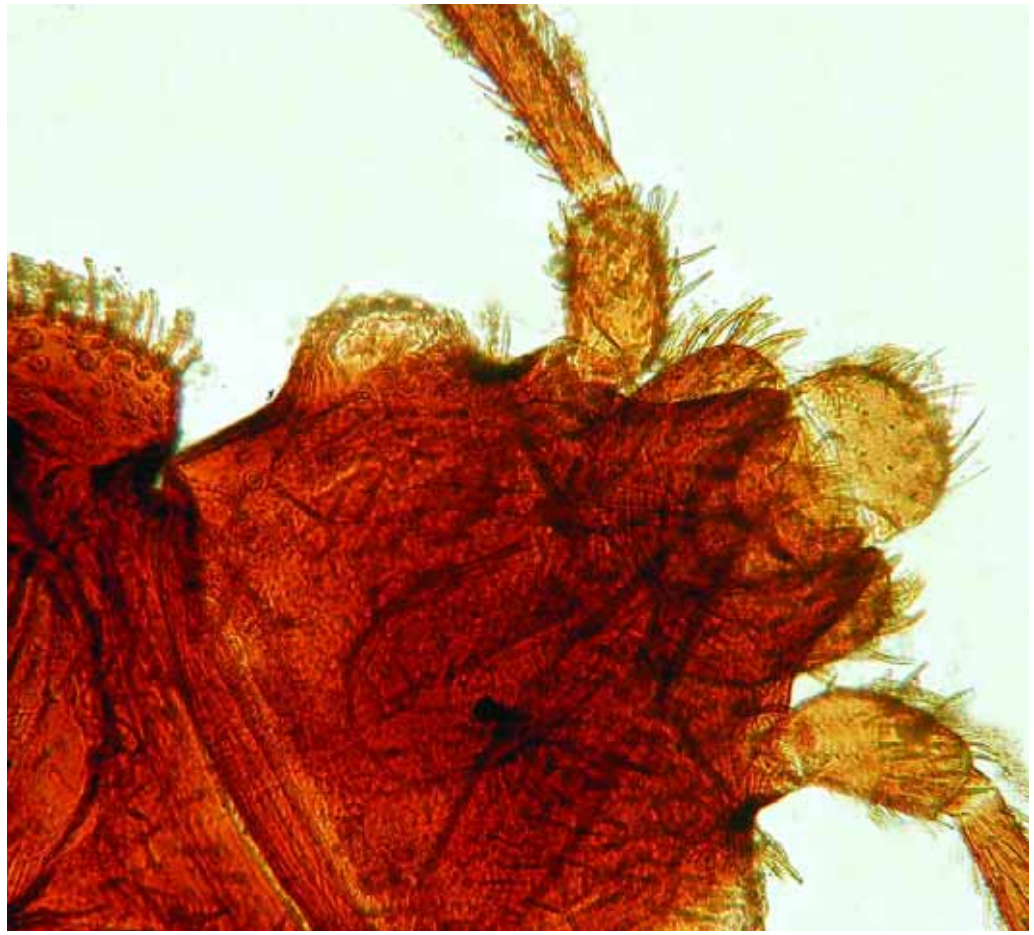

Fig.1. Adult bed bug, Cimex, exoskeleton cleared in sodium hydroxide and mounted. Fine details of chitinous appendages and hair-like projections are well displayed, though there is little impression of the insect as a whole specimen (120x).

provide good images of the entire insect but the working distance proved to be too great for the maximum stage drop on the available Olympus BH microscope. The lenses chosen for microscopy of the anatomy of living insects were the Olympus Plan 10/0.25 and an Olympus Plan 4/0.10. Incident light was provided through daylight-corrected LEDs.

Correlated microscopy of cleared and living specimens (Figures 1 and 2, respectively) immediately shows that the use of incident light on whole insects gives a very different impression to transmitted-light microscopy of cleared permanent preparations. Vivid though the appearance may be, it is ultimately compromised both by the resolution limits of the optics and – more importantly – by the restricted depth of focus. In practice, differential focussing on several planes enables the microscopist to select in-focus areas using image-editing software. Their superposition allows us to derive a satisfactorily focused image of the entire

Fig. 2.VGA image of a living Cimex adult produced by the Digital Blue QX5 microscope. Resolution is reduced, but incident light gives a vivid impression of the surface configuration and the lustrous sculpturing of the living insect (50x).

organ, thus circumventing the restricted focal depth of the original images.

Limits to resolution cannot be similarly addressed at this level. Modern instruments such as confocal scanning laser microscopy can offer remarkably improved resolution. An alternative means of addressing the problem is the use of scanning electron microscopy (SEM). Variable-pressure electron microscopy can even allow us to examine living insects and obtain detailed studies of surface anatomy, and in this research we have utilised an environmental SEM (ESEM), which can operate in the presence of water vapour in the specimen

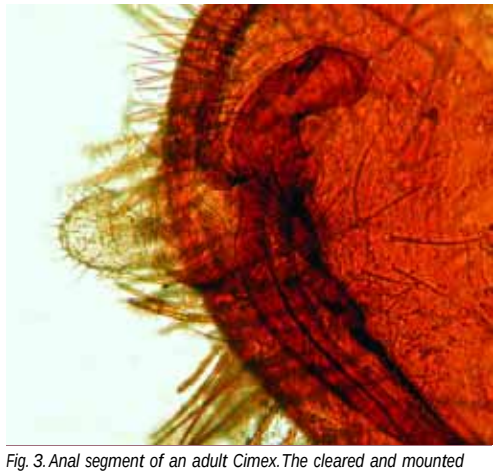

Fig. 3. Anal segment of an adult Cimex. The cleared and mounted specimen gives good images of chitinous sculpturing and fine detail on an anal palp projecting to the left (320x).

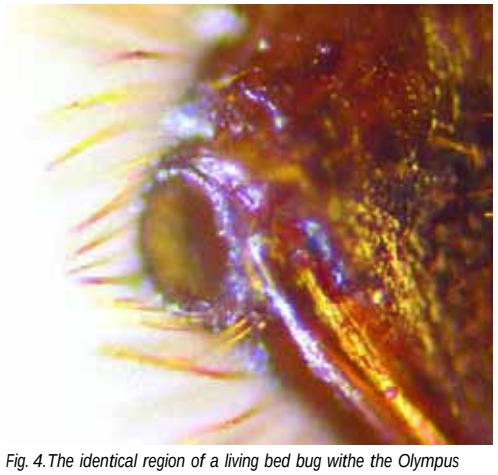

Fig. 4. The identical region of a living bed bug withe the Olympus Plan 4/0.10 objective gives a clear impression of the glistening exoskeleton and the structures associated with the anal aperture. This image was assembled from three digital micrographs taken at successive focal planes. Resolution of the hairs in this optical image is reduced (320x).

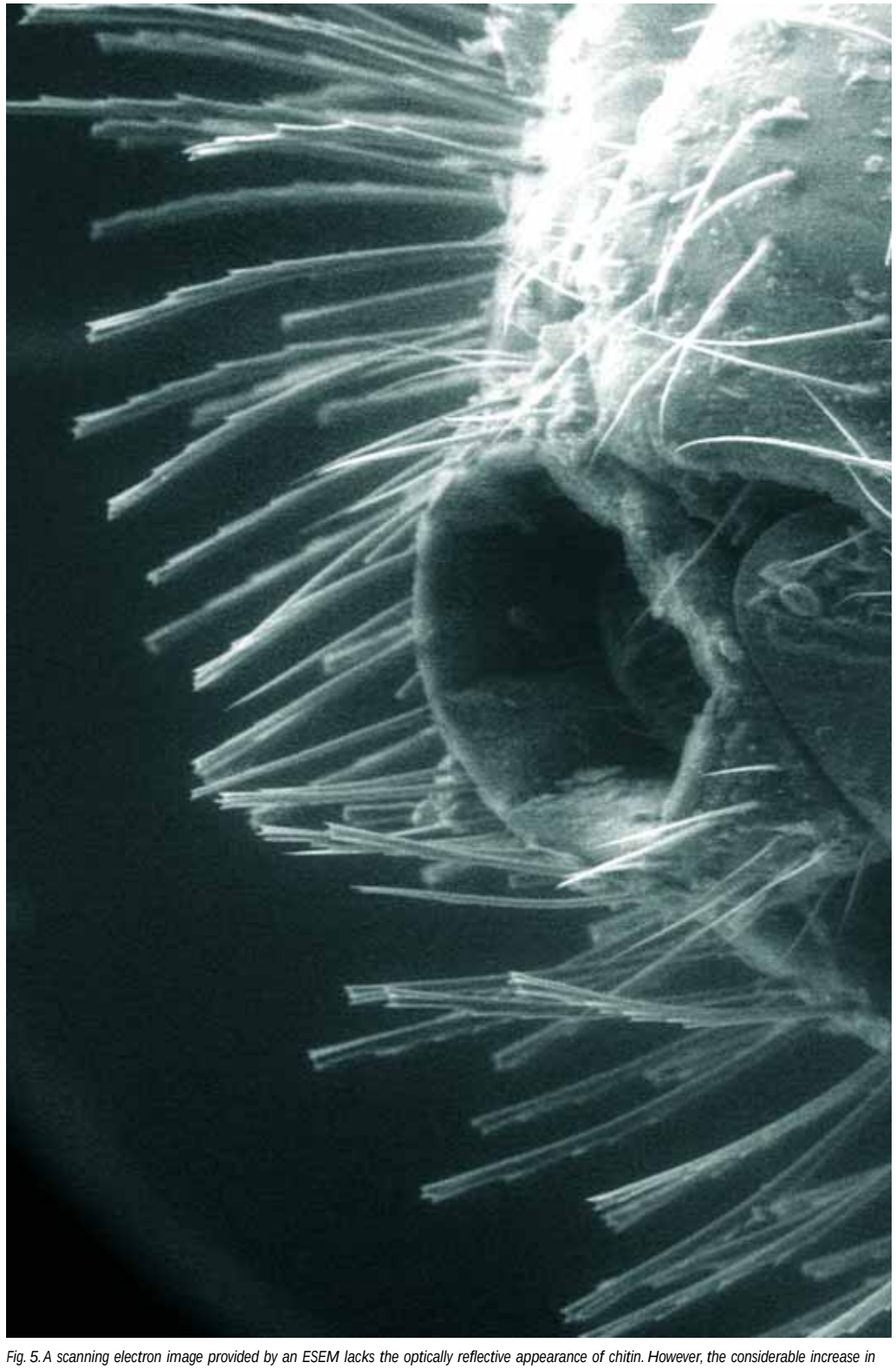

Fig. 5. A scanning electron image provided by an ESEM lacks the optically reflective appearance of chitin. However, the considerable increase in resolution and depth of field reveals copious fine structural details (800x). Imaged in water vapour with p ~ 2000 Pa (15 torr), T = 24°C, relative humidity ~ 75%.

Fig. 6. The basal component of a sensory appendage is adequately displayed in the cleared specimen. Note the compound eye (top left) just visible in this field of view. Studies of chitinous hairs are possible up to the limits of optical resolution (300x).

chamber. Although typical gas pressures, on the order of 10^2 – 10^3 Pa, are somewhat lower than atmospheric pressure (10^5 Pa), these are within the limits that *Cimex* and other arthropoda can survive. Crucially, water vapour in the specimen chamber can be maintained at levels corresponding to those found in the ambient atmosphere. As testimony to this, we noted that live insects restrained on adhesive tape were found to be moving after removal from the specimen chamber of an ESEM, allowing one to be confident that the morphology of living insects is well represented by this technique.

Additionally, a non-living specimen can quickly accumulate detritus on its surface, potentially obscuring features of interest; hence it is advantageous to examine live specimens whenever possible. Careful control of electron optics and detection allows one to obtain vivid electron micrographs of the external structure. Figures 3, 4 and 5 offer, respectively, a comparison between transmitted light, incident light and secondary electron ESEM imaging of a similar region of the adult *Cimex*.

Figures 6, 7 and 8 demonstrate how each aspect of this correlated approach allows us to gain an overall impression of the colours, textures and structure of the bug. Microscopy of the compound eye is an excellent case in point. The cleared specimen reveals the organ well enough (Figure 9), but there is no impression given of the appearance in life. Bed bugs have eyes that are near-black in colour – morphologically reminiscent of a blackberry – and only incident light microscopy can

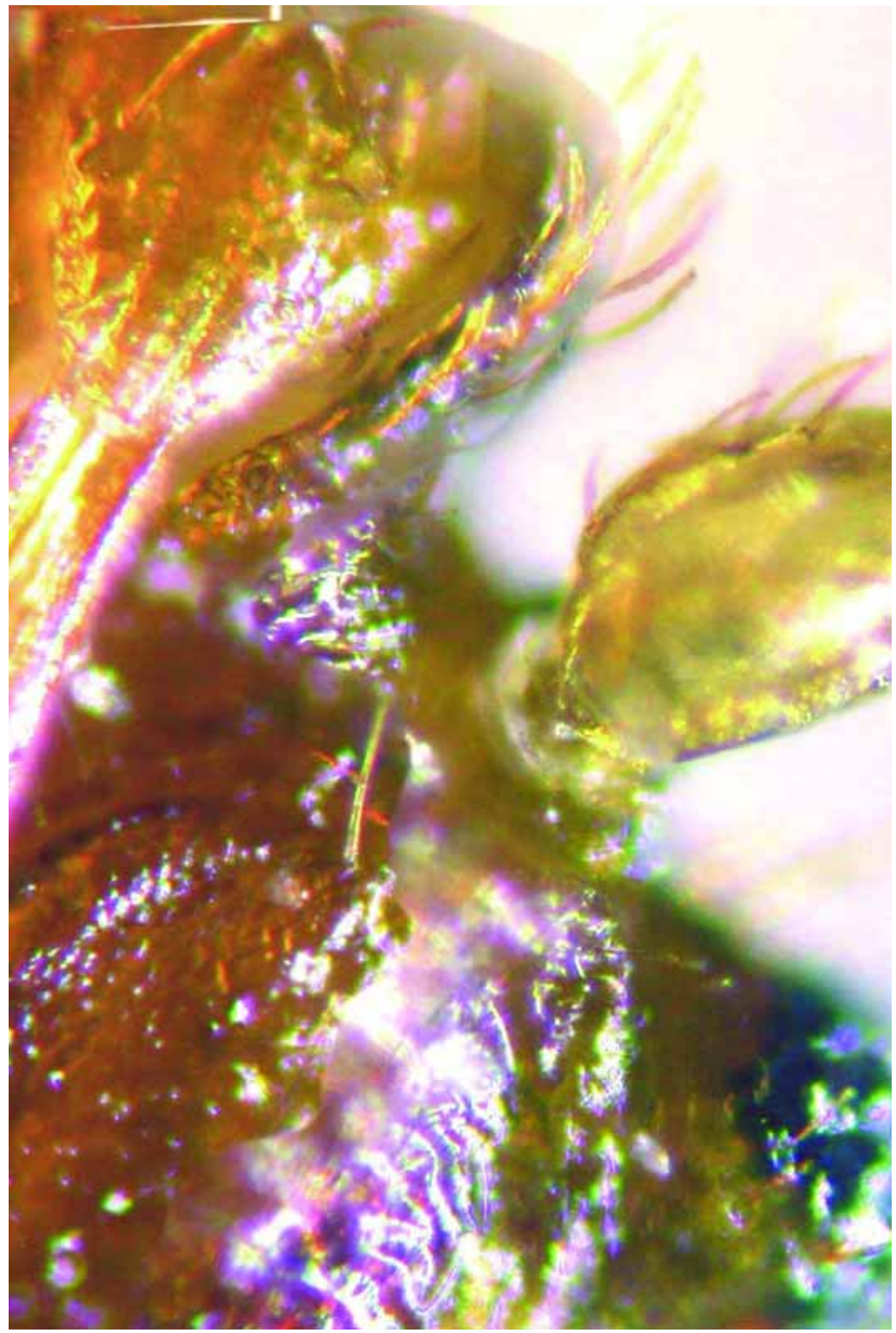

Fig. 7. This incident-light image with the Prior Plan 10/0.25 objective reveals the bed bug to have an attractive and lustrous hue. Limits both to depth of field and resolution are apparent, yet viewed alongside the previous image a more realistic impression of the bug is obtained (480x).

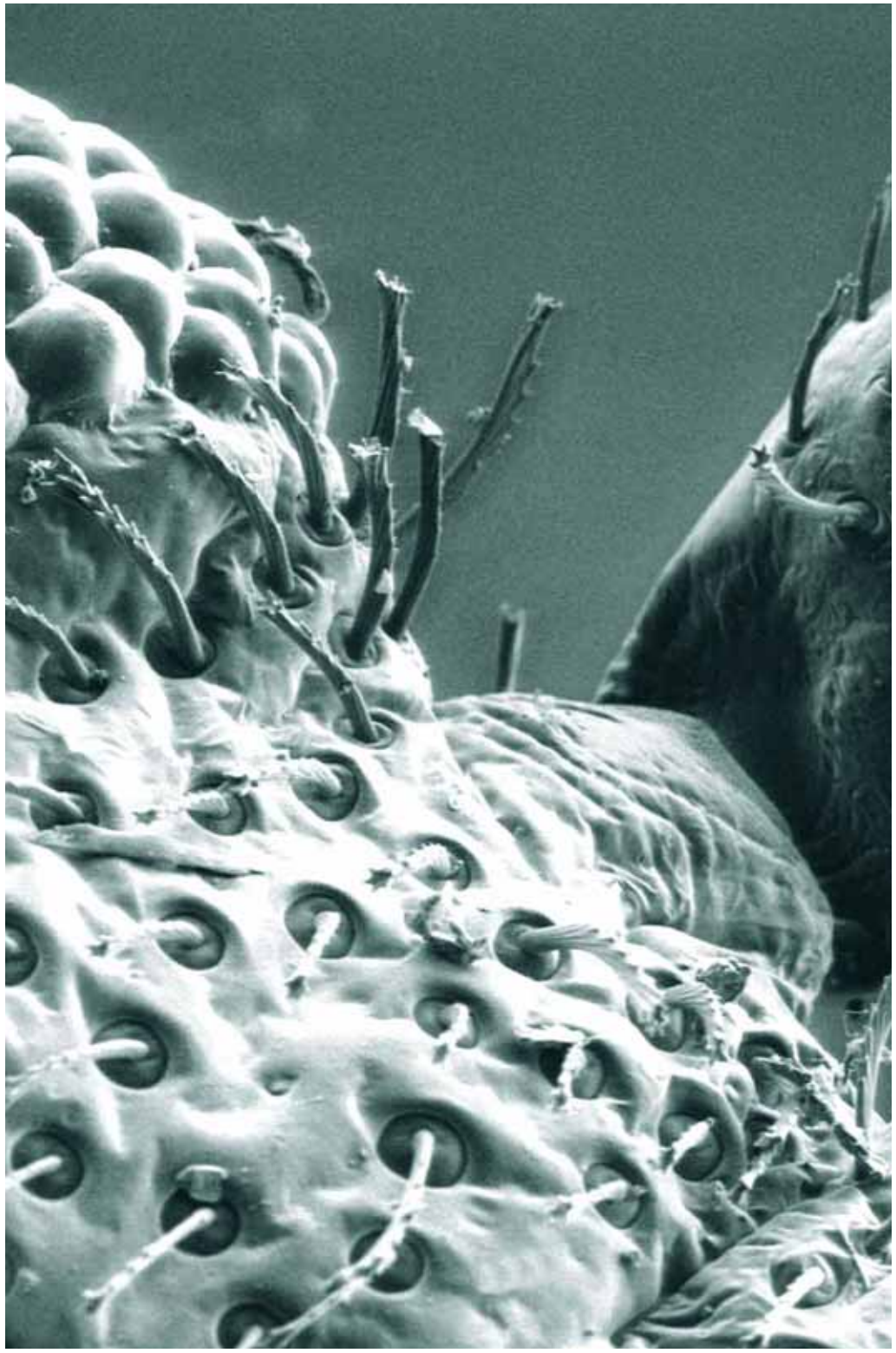

Fig. 8. The greater focal depth and increased resolution of the ESEM image allow one to study the fine details of structure like those around the compound eye (upper left) and the sensory hairs themselves. Such images are best viewed as correlated optical micrographs if the nature of Cimex is to be properly understood (540x). Imaged in water vapour with p ~ 2000 Pa (15 torr), T = 24°C, relative humidity ~ 75%.

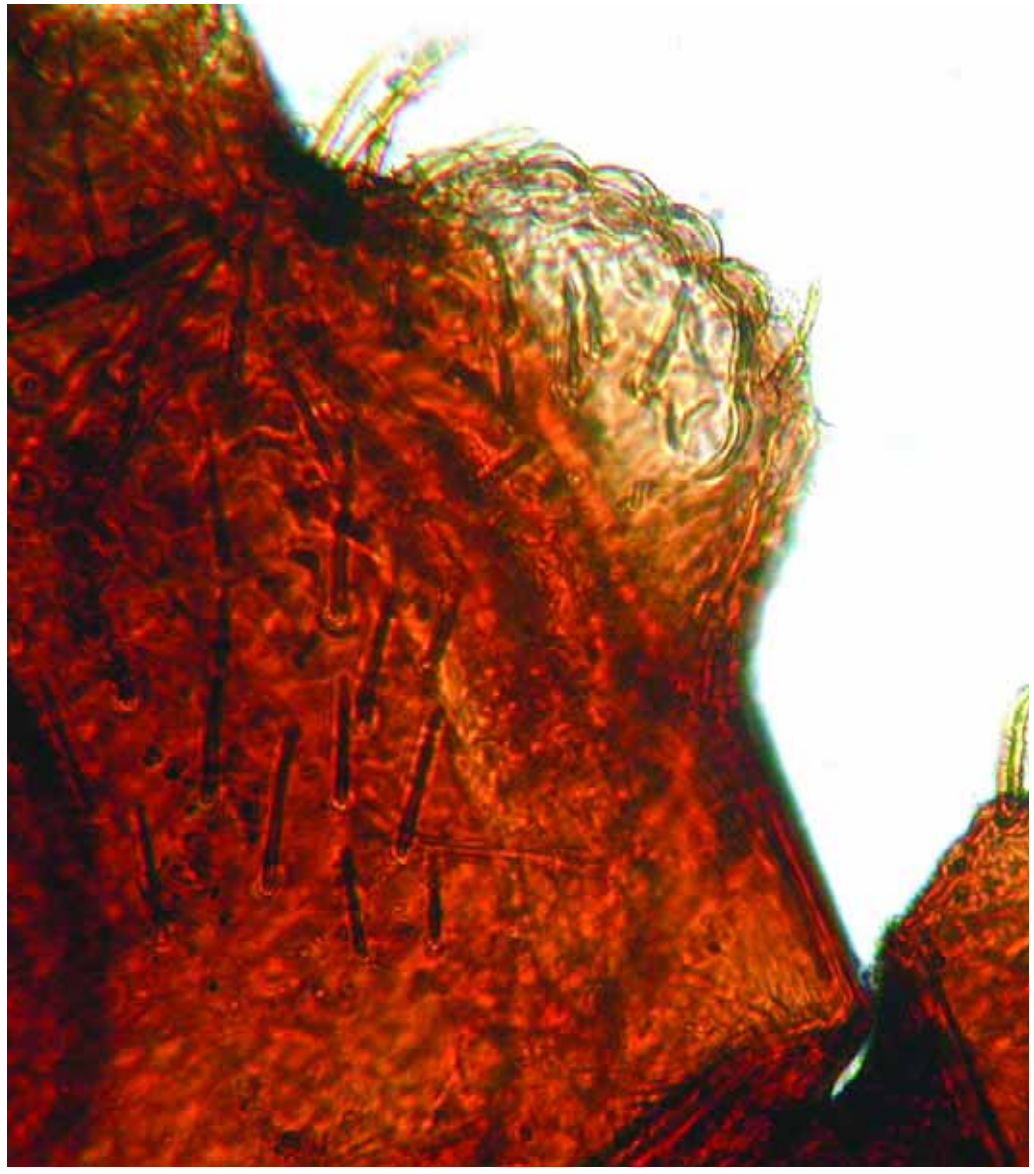

Fig. 9. Sensory hairs projecting from the thorax of the insect carapace are well displayed in the cleared Cimex specimen. Although refractive ocelli comprising the translucent compound eye are visible (above), the eye itself is apparent only as a relatively insignificant structure (300x).

adequately display this (Figure 10). Meanwhile, the surface-sensitive secondary electron image (Figure 11) shows very clearly the detailed topography of the eye and surrounding areas.

Conclusion

Incident light microscopy is conventionally confined to areas such as geology and metallurgy, being little used for biological specimens. Although scanning electron microscopes can reveal extraordinary amounts of structural detail in arthropoda, it is clear that incident light is the only method that fully reveals the subtlety of colour and surface appearance of insects as they are in life. Even low-power digital microscopes can provide useful information in revealing the aesthetic properties of insects such as *Cimex*, the bed bug.

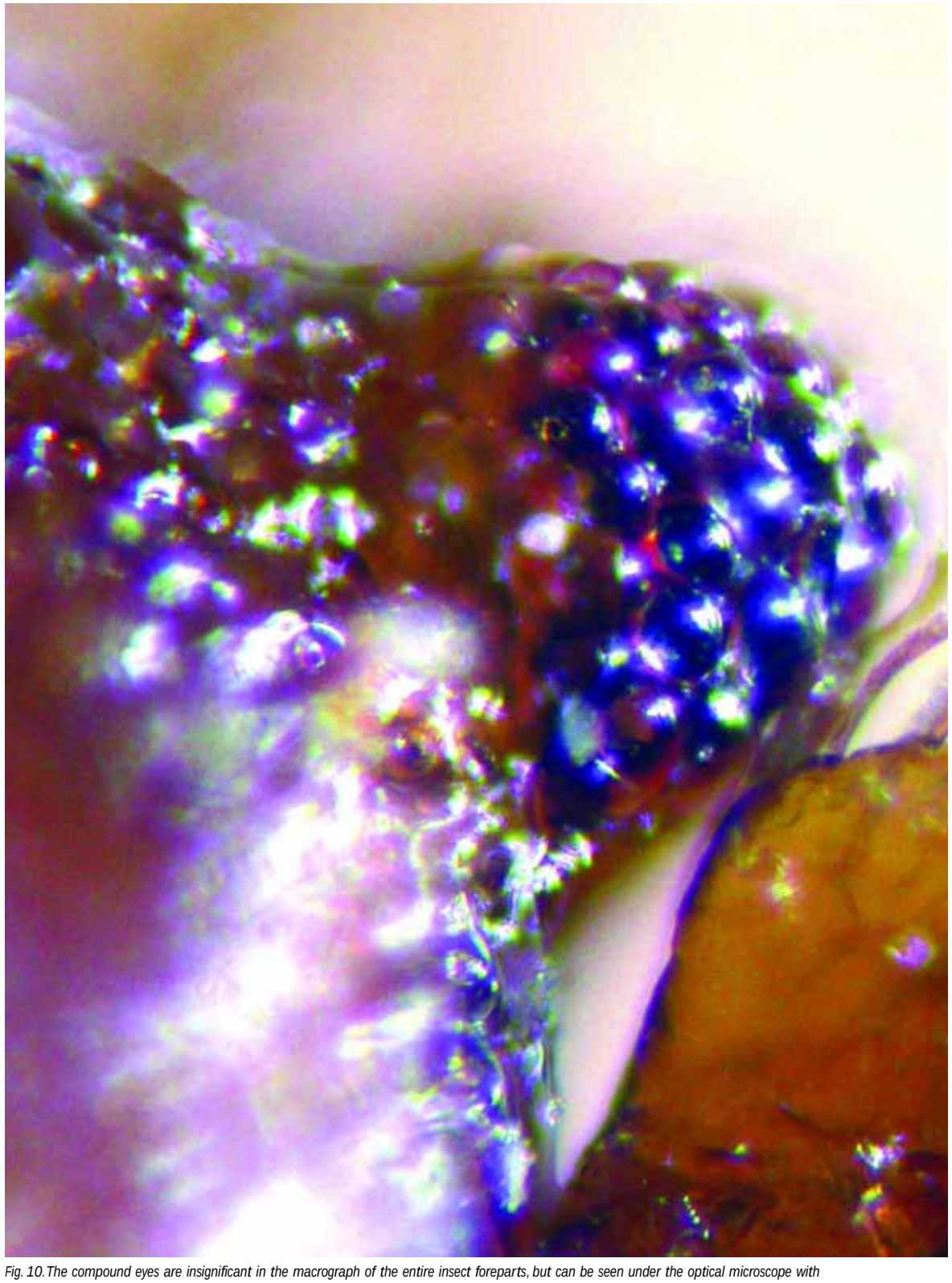

Fig. 10. The compound eyes are insignificant in the macrograph of the entire insect foreparts, but can be seen under the optical microscope with incident light to be rich in colour. The reflective chitinous lenses and highly pigmented ocelli present a striking appearance in the living bug (500x).

Fig. 11. ESEM offers this striking study of the entire eye structure. Surrounding sensory hairs are well characterised in this micrograph of the living adult Cimex. The three methods of microscopy reveal disparate aspects of the eye structure (300x). Imaged in water vapour with p ~ 2000 Pa (15 torr), T = 24°C, relative humidity ~ 75%.

Fig.12. An adult bed-bug, Cimex lectularius. These obligate parasites are set to become increasingly common in recent years (15x).

Fig.13. The authors: Dr Debbie Stokes and Professor Brian J Ford at the Cavendish Laboratory, Cambridge.

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Field of views were unavailable at the time of going to press. These will be provided in the next issue of infocus for reference, and are available online at www.rms.org.uk/infocus.

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