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Use of stereophotography in insect science – methods and applications

Robert STURM

A b s t r a c t : Since the 1960s professional stereophotography has found numerous fields of application in various disciplines of the natural sciences. Recently, this optical technique has advanced to a standard method with regard to the comprehensive description of superficial structures and the measurement of spatial extensions of diverse physical, chemical or biological objects. Classical stereophotography is founded upon the generation of so-called stereo-pairs, which require imaging of the item of interest from two different perspectives. More recent stereoscopic techniques, whatsoever, allow the production of three-dimensional images after computation of an object depth map of a single photograph. The present contribution deals with the documentation of various applications of stereophotography in entomological research. Furthermore, the scientific potential of this optical method is subjected to an intense debate.

K e y w o r d s : Stereoscopy, light microscopy, electron microscopy, macrophotography, stereo-pair, object depth mapping.

Introduction

In the past decades, stereophotography has attracted increased attention in the research community and has thereby advanced to an optical standard procedure in various scientific disciplines. In natural sciences stereoscopic techniques have to be regarded as indispensable in the meantime, because they are routinely used for the detailed analysis of spatial object extensions, complex morphological items and specific superficial structures (STURM 2008, 2009, 2015, 2016a, 2017a). In numerous biological research fields stereoscopic photography already disposes of a long-term prosperity; besides a continuous confrontation of the three-dimensional imaging method with new scientific problems also an increasing transfer of the technique towards the microscopic level can be recognized (RAAP & CYPIONKA 2011, STURM 2016b, 2017b). In chemistry and physics stereophotography is still evaluated as marginal method, although the procedure strongly supports the pedagogic and visual access to these disciplines (STURM 2016a, 2017a).

In entomological research three-dimensional visualization methods have been only applied in specific cases hitherto (e.g., GOLDSTEIN et al. 2003, STURM 2016a, 2017a). This circumstance can be traced back to several reasons: (1) Entomological research covering the distribution of single insect species or diverse ecological and ethological problems does not require detailed images of the animals themselves, but increasingly sets its focus on the colonized habitats and related environmental conditions. (2) Due to their strongly reduced body size numerous insects evade the resolving power of an ordinary stereo-camera (camera with two objectives). Therefore, the production of three-dimensional photographs requires a much higher technical effort (macro-photography, microscopic imaging). (3) Publications on stereoscopic topics in insect science are quite rare in both the German-speaking and English-speaking region, so that most entomologists do not possess the necessary knowledge for the computer-aided production of simple 3D effects (CYPIONKA et al. 2016, SCHEIDEL 2009, RAAP & CYPIONKA 2011). However, in systematic and structural research it is only a question of time, until three-dimensional visualization has got its way as standardized optical method.

The present contribution pursues two main goals: In the first part stereoscopic methods summarized by STURM (2016a, 2017a) are subjected to a detailed description. Besides the production of classical stereo-pairs, which show the interesting objects from two different perspectives, also a computer-aided procedure generating the spatial impression from a single photograph is mentioned (CYPIONKA et al. 2016). The second part deals with various application examples of stereophotography and three-dimensional imaging in insect science. Finally, an answer should be given to the question, to what extent future problems of entomological research can be solved with the help of stereoscopic techniques.

Material and Methods

Production of stereoscopic images on the macroscopic scale

For the generation of three-dimensional photographs of macroscopic objects, usage of a stereo-camera (e.g., Aiptek iS2) with the possibility of close-ups is highly recommended. If an ordinary camera with single objective is used for the photographic work, production of the stereo-pairs should be carried out according to the guide-lines of the German Stereoscopic Association (SCHEIDEL 2009, STURM 2016a). Based on these recommendations the object has to be recorded from two positions, which are characterized by a small horizontal displacement. Thereby, exposure time and depth of focus have to be kept constant, and the so-called vertical parallax has to be minimized as much as possible. As an alternative to the transposition of the camera along a virtual horizontal line also its movement along a virtual arc of a circle by 5-10° is permitted. Here, enhanced consideration has to be shown for so-called trapezoid errors (SCHEIDEL 2009, STURM 2016b). Single stereo-pairs, which have been produced according to the methods described above, can be transformed into red-green anaglyphs by using appropriate computer programs (e.g., Adobe Photoshop, Anaglyph-Maker, Picolay). Such anaglyphic images have to be viewed with respective red-green glasses.

Production of stereoscopic images on the microscopic scale

On the microscopic level generation of three-dimensional images turns out to be a little more complex than on the macroscopic scale. The two basic principles for the achievement of spatial effects are summarized in Fig. 1a and 1b. In reflected-light microscopy the object can be photographed from two slightly displaced positions. Alternatively, imaging of the interesting item can take place before and after a little rotation of the structure (Fig. 1a). Here, it is also of high importance that all settings of the camera and lighting are kept constant in order to avoid subsequent image corrections (STURM 2016a, STURM 2016b). In the case of transmitted-light microscopy the classical method of object recording from two different perspectives is supplemented by another procedure, where the transparent item is photographed under permanent change of the focus point (Fig. 1b). As a result of this optical recording technique an image stack is produced, which traces the different positions of the focus point and can be again transformed into a red-green anaglyph (STURM 2016a, STURM 2017b). In scanning electron microscopy the researcher is referred to the classical method of stereoscopic image production (see above). Modern microscopes offer the possibility of an infinitely variable inclination of the sample holder, due to which two optimal positions of the object with minimal changes of depth of focus and lighting can be found rather easily.

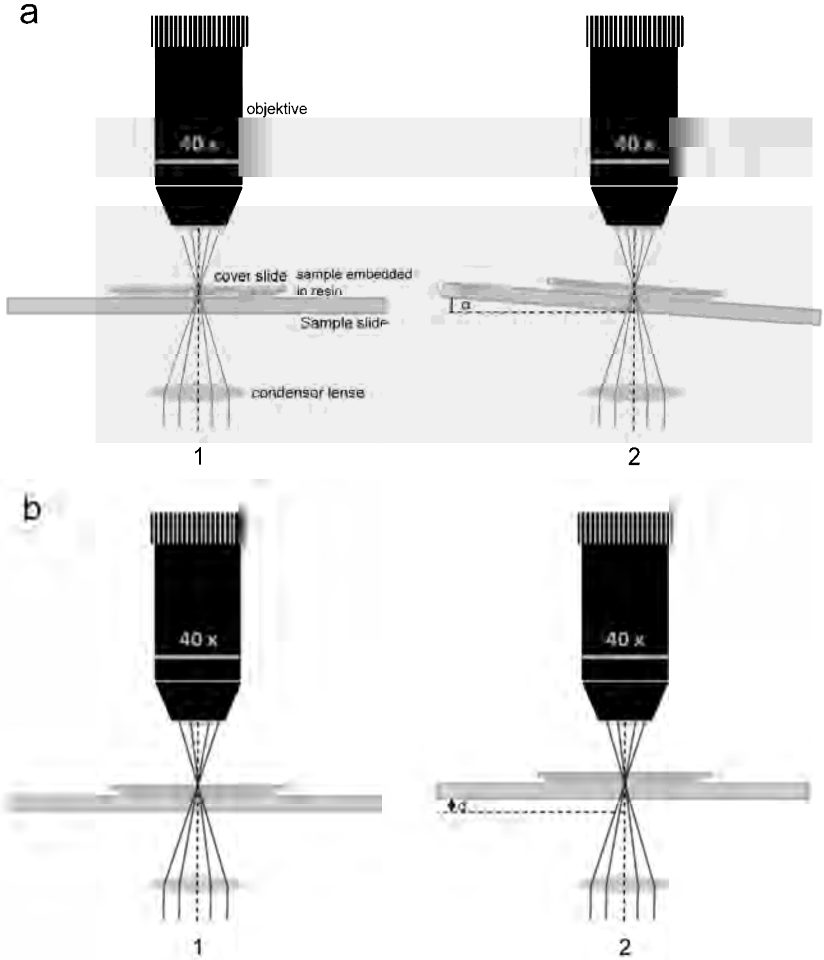

Fig. 1: Methods for the generation of stereo-pairs in microscopy (STURM 2016a, STURM 2016b): (a) procedure with slight rotation of the object by an angle α; (b) displacement of the focus plane by the value d and resulting production of an image stack.

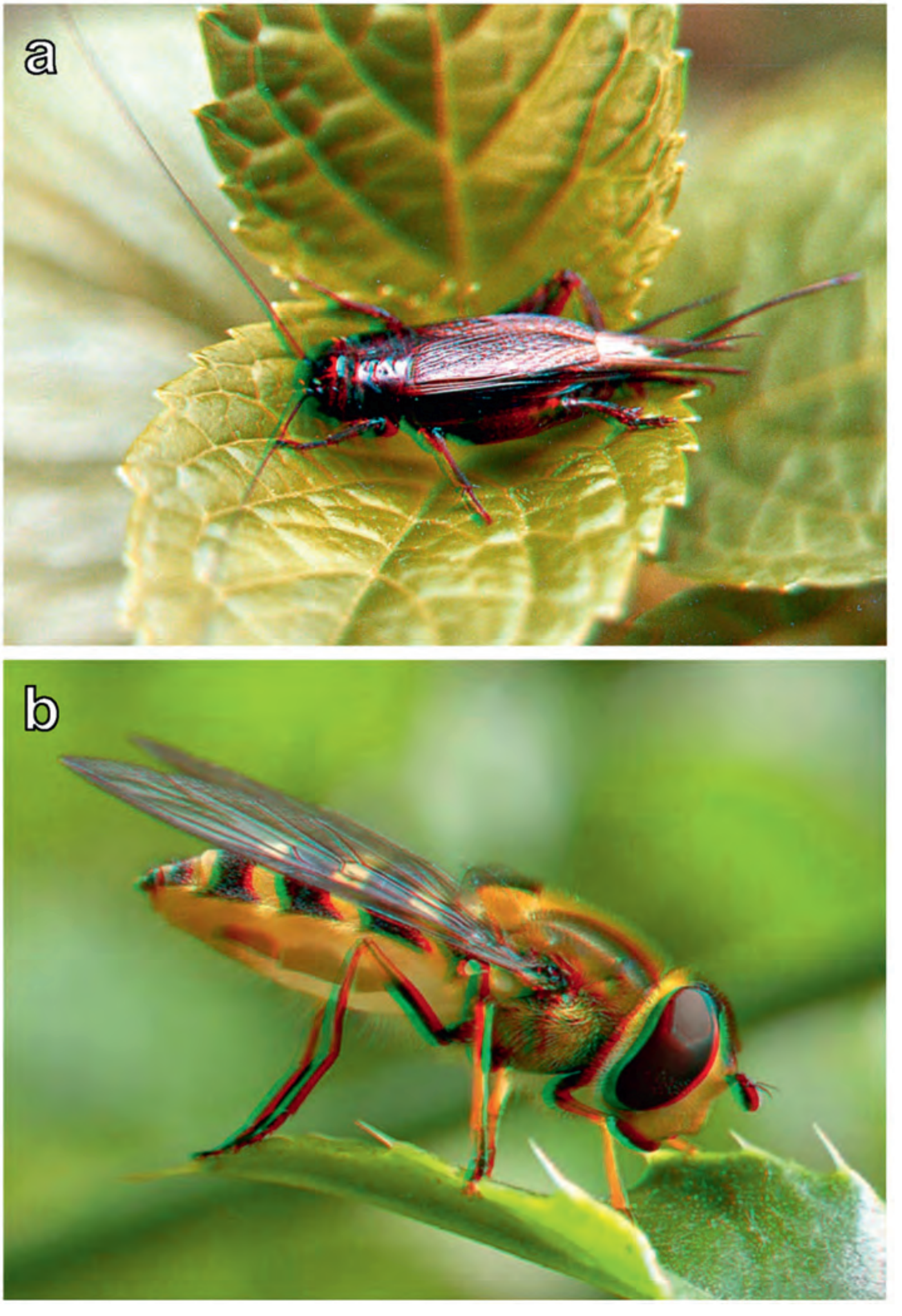

Fig. 2: Stereoscopic macro-photographs (red-green anaglyphs) of different insects: (**a**) Australian field cricket; (**b**) hoverfly. All images have to be watched by using red-green glasses.

3D images obtained from a single photograph

Ordinary photographs of an arbitrary object can be subsequently furnished with a spatial effect by using appropriate computer programs such as Picolay, Adobe Photoshop or Blender. Generation of the three-dimensional impression is founded upon the assumption that macro- and micro-photographic images show a decline of brightness and sharpness with increasing spatial depth. Or with other words: Structures being depicted in the foreground of a photograph appear brighter and sharper than those being placed in the background. This logical circumstance can be used for the production of a brightness- or sharpness-related depth map of the image and for the assignment of a single frame to each specific colour code. As a consequence of that, an image stack is generated, which can be again transformed into a stereo-pair or a red-green anaglyph with the help of respective computer software.

The subsequent addition of spatial effects to a single photograph indeed includes several steps of extrapolation and rendering, which may result in a significant falsification of the object shape in extreme cases. Respective distortions of the photographed structures especially occur in animated image sequences, so that the three-dimensional impression may strongly recede into the background. Therefore, it is highly recommended to use only small modifications of the perspective, which are computed on the basis of the depth map described above.

Results

On both the macroscopic and microscopic level stereophotography strongly supports the generation of remarkable three-dimensional effects. Spatial imaging of single insects (Fig. 2) especially pursues the intention of an appropriate visual presentation of the animals. In addition, it also may serve for the estimation of proportions between the depicted organism on the one side and the direct vicinity (e.g., leaf) on the other. In the case of close-ups of the insect head stereoscopic effects may be used for a reliable presentation of the whole plasticity being typical for the masticatory apparatus, the antennae and the sensory hairs. Here, it is partly possible to obtain a more detailed picture with regard to the functionality of the respective organs.

Whilst the macroscopic 3D image primarily serves for representative purposes, application of stereoscopic techniques in micro-photography pursues some additional goals. As exhibited in Fig. 3, spatial arrangement of single organs can be submitted to a closer exploration with the help of this method. The stereo-pair shows the opened reproduction tract of the female Australian field cricket with all its essential components (ovaries, spermatheca, accessory glands). Based on these images positions and proportions of single structures may be subjected to a more detailed scientific documentation. In addition, the photographs allow a better insight into the complexity of the neural and tracheal networks. Depth and structure of single parts of given organs (e.g., terminal capitula of the female accessory glands, Fig. 4) can be remarkably pronounced by using the stereoscopic effect. This partly results in the generation of superelevated structures, which serve for a better understanding of the organs and dispose of a certain potential for the solution of scientific problems.

Fig. 3: Microscopic 3D image of the opened abdomen of the female Australian field cricket (image width: 5 mm).

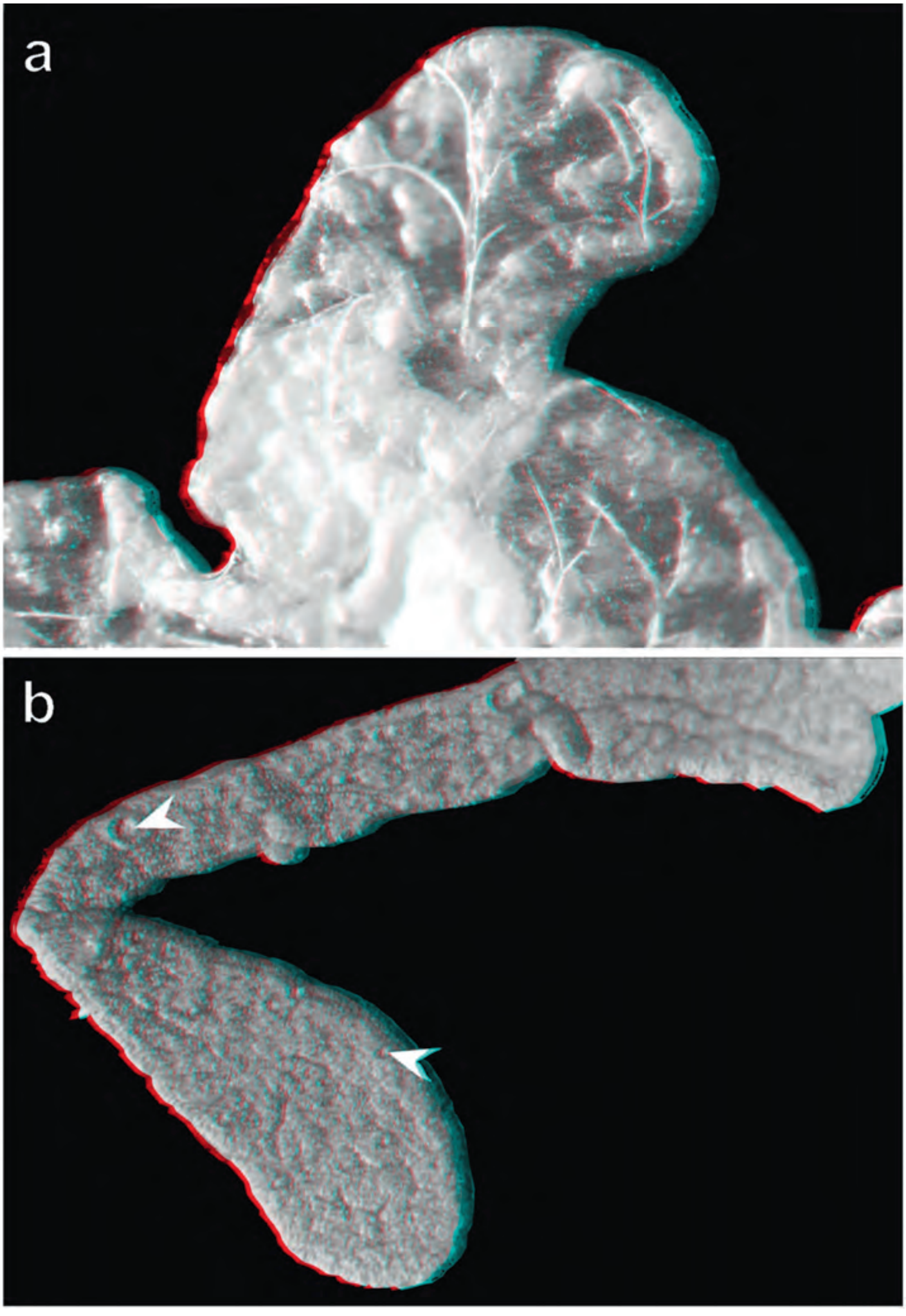

Fig. 4: Light-microscopic 3D images of the terminal sections of the female accessory glands in the Mediterranean field cricket (**a**) and Australian field cricket (**b**) (image widths: 2 mm).

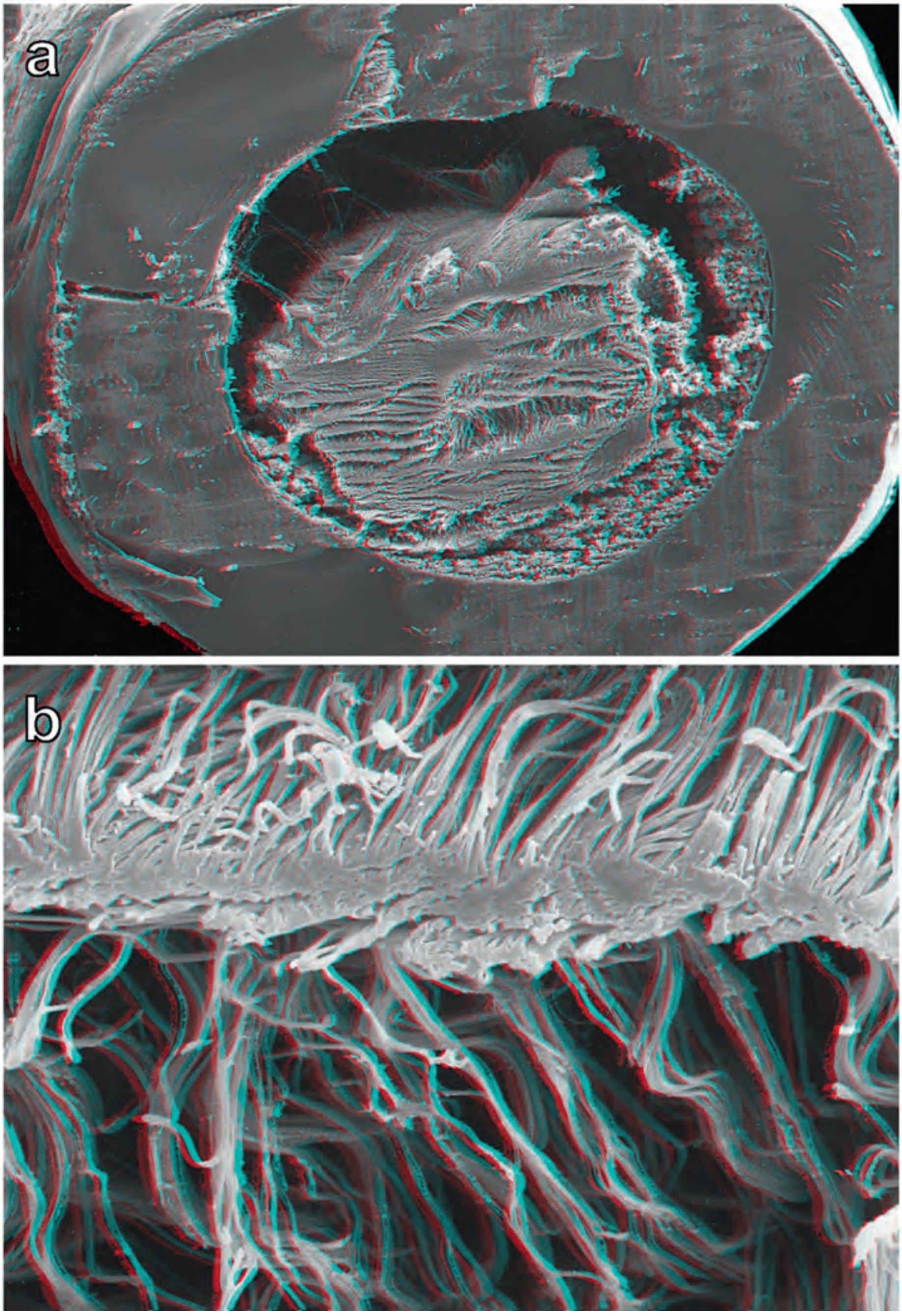

Fig. 5: Electron-microscopic 3D images of the spermatophore (**a**) and single spermatozoa (**b**) Australian field cricket (image widths: a: 0.5 mm; b: 0.05 mm).

In scanning electron microscopy with its detailed structural analysis three-dimensional imaging has its most spectacular and, from a scientific perspective, most essential field of application. Especially this microscopic discipline has an outstanding requirement for spatial effects, because numerous structures can be only documented satisfactory under addition of this specific information. This among other becomes true for the spermatophore of the male Australian field cricket with its regularly arranged spermatozoa (Fig. 5). The stereoscopic image not only allows the analysis of the spatial arrangement of single structural elements, but also enables the exact measurement of these components. This application of the 3D image in morphometry will be discussed in future studies.

Discussion and Conclusions

As could be demonstrated by means of some illustrative examples from entomological research, application of simple methods already results in the production of impressive three-dimensional effects of the depicted objects. Spatial visualization is not at all restricted to the macroscopic scale, but may also produce a respective effect in light and electron microscopy. Application of stereoscopic techniques in malacology (STURM 2011, 2017a) and palaeontology (STURM 2009, 2016a) has among other resulted in the finding that the 3D image offers a remarkable support with regard to the interpretation of superficial structures or the estimation of structural proportions. As already mentioned elsewhere (STURM 2016a), advantages of stereoscopic photography can among other take effect in the determination of insect species, which is often founded upon respective structural attributes. Since insect science exhibits an enhanced need for efficient methods of species determination, the related potential of 3D imaging may be outlined more in detail in future investigations.

The present contribution makes clear that spatial visualization techniques applied to macro-photography above all serve for purposes of presentation. The scientific value of stereoscopic images surely calls for a practical evaluation. Another situation is given for the field of micro-photography, where three-dimensional photography can contribute to a significant improvement of the understanding of specific structures and their functions. Exploration of the exact spatial arrangement of single structural components often results in a complete picture with regard to the functionality of the respective organ (RAAP & CYPIONKA 2011, STURM 2016a, 2016b). Thereby, the spatial impression obtained from direct microscopic viewing of the object is transferred on the photographic image and is stored there permanently (SCHEIDEL 2009, STURM 2016a).

Besides entomological research itself also any teaching related to insect science can benefit from the stereophotography of larger and smaller objects. This starts with the conception of presentations on the basis of the three-dimensional visualization method, which probably awakens increased interest for the discipline among disciples and students. The use of optical devices for an appropriate viewing of the 3D images does not represent a imperative necessity in computer-aided presentations anymore, because the spatial impression can be proposed by using animated image sequences in the meantime (RAAP & CYPIONKA 2011, STURM 2017c). To what extent entomology disposes of the potential for the creation of three-dimensional picture-books and lexica, can be only evaluated after the permanent establishment of stereoscopic methods in this scientific discipline.

The hypotheses outlined in this contribution lead to the conclusion that entomological research may expect a significant intensification of three-dimensional photography in near future. Although the scientific value of a great multitude of 3D images cannot be supported unequivocally, such photographs have to be evaluated as highly innovative in presentation concerns. Also the pedagogical effect of the 3D effect has been already brought into play from multiple sources and should represent the content of respective investigations.

Zusammenfassung

Verwendung der Stereofotografie in der Entomologie − Methoden und Anwendungen.

Seit den 1960er Jahren hat die professionelle Stereofotografie zahlreiche Anwendungsfelder in verschiedenen naturwissenschaftlichen Disziplinen gefunden. In jüngerer Vergangenheit ist diese optische Technik zu einer Standardmethode bezüglich der ausführlichen Beschreibung von oberflächlichen Strukturen und der Vermessung räumlicher Ausdehnungen von physikalischen, chemischen und biologischen Objekten avanciert. Die klassische Stereofotografie gründet auf der Herstellung sogenannter Stereopaare, welche die Aufnahme des Gegenstandes aus zwei verschiedenen Perspektiven erfordern. Aktuellere stereoskopische Techniken ermöglichen dagegen die Produktion dreidimensionaler Bilder nach Computer-unterstützter Generierung der Objekttiefenkarte einer einzelnen Fotografie. Der vorliegende Beitrag beschäftigt sich mit unterschiedlichen Anwendungsmöglichkeiten der Stereofotografie in der entomologischen Forschung. In weiterer Folge wird das mögliche wissenschaftliche Potenzial dieser optischen Methode einer intensiven Debatte zugeführt.

References

CYPIONKA H., VÖLCKER E. & M. ROHDE (2016): Stacking-Pragramm PICOLAY — Erzeugung virtueller 3D-Bilder mit jedem Lichtmikroskop oder REM. — BIOspektrum **22**: 143-145.

GOLDSTEIN J.I., NEWBURY D.E., JOY D.C., LYMAN C.E., ECHLIN P., LITSHIN E., SAWYER L. & J.R. MICHAEL (2003): Scanning Electron Microscopy and X-ray Microanalysis. — 378 pp., New York (Springer).

RAAP E. & H. CYPIONKA (2011): Vom Bilderstapel in die dritte Dimension: 3D-Mikroaufnahmen mit PICOLAY. — Mikrokosmos **100**: 140-144.

SCHEIDEL A.J. (2009): Stereoskopie in Bild und Video – Möglichkeiten, Anwendungen und Grenzen des räumlichen Sehens. — 156 pp., Mainz (Universität Mainz).

STURM R. (2009): 3D photography of fossils: Ammonites from the Northern Limestone Alps in Austria. — Dep. Mag. **18**: 10-13.

STURM R. (2011): 3D photographs of fossil gastropods from the ancient Paratethys ocean. — Dep. Mag. **24**: 12-15.

STURM R. (2015): Die Stereofotografie biologischer Objekte. — BIUZ **45**: 52-55.

STURM R. (2016a): Stereoskopie in Mathematik und Naturwissenschaften. — 127 pp., Göttingen (Cuvillier-Verlag).

STURM R. (2016b): Die Stereofotografie und ihre Nutzung zur Klärung wissenschaftlicher Fragestellungen. — Mikroskopie **3**: 86-100.

STURM R. (2017a): Stereoskopische Methoden in Mathematik und Naturwissenschaften. — Naturw. Rundsch. **70**: 168-174.

STURM R. (2017b): Stereoscopic Photography in Transmitted Light Microscopy. — Microsc. Today **27**: 47-49.

STURM R. (2017c): Cricket embryos in 3D. — Micscape Magazine, June 2017.

Anschrift des Verfassers: MMMMMMag. Dr. Robert STURM
Brunnleitenweg 41, A-5061 Elsbethen, Austria
E-mail: sturm_rob@hotmail.com