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# The use of extended depth of field for taxonomic data mobilisation via internet – Verhoeff's gonopod preparations as a pilot study

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In the framework of the project "centres of excellence of innovative data mobilisation" of GBIF-D (the Global Biodiversity Information Facility – Germany; BMBF grant 01LI01001 B), the node invertebrates II uses several digitalisation units to produce extended depth of field photos of micropreparations at high resolution, and combines them with online databases available from the GBIF portal to establish complete taxonomic information systems, e. g. GloMyrIS. Exemplified by Carl Wilhelm Verhoeff's gonopod preparations of Diplopoda, we give a survey of the technical procedures, the results and their significance for taxonomic revisions and data exchange among myriapodologists.

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## Introduction

The collection of Carl Wilhelm Verhoeff (1867–1945), one of the world's most influential myriapodologists, is a true challenge: Verhoeff described about 2000 taxa, published 670 papers (Verhoeff & Mauermayer 1962), and left a legacy of c. 10 000 specimens to the Bavarian State Collection of Zoology (ZSM) (with a lot further specimens housed in the museums in Berlin, Vienna, Hamburg and others). Many of the scientific names Verhoeff attributed remain equivocal, taxon specific sets of characters are incompletely described, and the descriptions are difficult to understand, since they focus mainly on the male genital apparatus (the gonopods). Moreover, type designation is often chaotic, specimen labelling unclear, and connections between description, ethanol stored bodies, and microscope slides of the male genitalia are hard to trace. Finally, for many taxa (species, subspecies and "varieties") it is not clear if they represent real, natural entities or artificial constructs. Hence a thorough revision of Verhoeff's collection – and the taxa described, including modern techniques of analysis, is needed, but only at the beginning (e. g. Djursvoll 2008, Pilz et al. 2008, Shear 1987, Shelley et al. 2005, Stoev 2009, Stoev & Enghoff 2008, Spelda 2001, 2008).

The necessary large scale taxonomic revision can only be achieved on an international level by myriapodologists specialised in different myriapod subgroups. The key to such a revision – and the core of the Verhoeff collection, are the gonopod micropreparations.

The advantages of internet accessible taxonomic information as it is now available via the GBIF portal are thus obvious. But in the current phase of including digitalized picture information on the internet, we seek to go further by producing high resolution extended depth of field photos of Verhoeff's gonopod preparations that allow myriapodologists to directly access the primary information and basis of the old taxonomic descriptions.

Since about a decade, Computer software such as Automontage (www.syncroscopy.com/syncros-

copy/am.asp), CombineZ (www.hadleyweb.pwp.blueyonder.co.uk/) and Helicon Focus (www.heliconsoft.com/heliconfocus.html) make it possible to produce extended focus and even stereo photographs of gonopods at high resolution which clearly display their structure and spatial arrangement. This in turn allows gonopod redescriptions at much greater levels of detail than those given in Verhoeff's original publications.

For the requested mass digitalization of the gonopod micropreparations we have established a new microscope unit at the ZSM including a computer unit that allows to generate photo stacks of gonopods at a resolution of 2580 × 1944 pixels per image and to calculate extended depth of field photos that can be added to our already existing datasets. The microscope is specially adapted for microslides, i. e. high resolution at high working distance, that can be used for all kind of three-dimensional preparations of myriapods and other Arthropoda (the same technique has been applied to water mites in Goldschmidt & Melzer (2011)). In this paper we survey the technical procedure that hopefully will bring us one step further to myriapod cybertaxonomy and online determination aids.

Our work is embedded in the framework of activities of the node invertebrates II of GBIF-Germany (Global Biodiversity Information Facility), funded between 2002 and 2005 (German Federal Ministry of Education and Research, grant 01LI0205), and again since October 2010 (GBIF-D, "centres of excellence of innovative data mobilisation"; BMBF grant 01LI01001 B, a cooperation between the nodes "invertebrates II" and "mycology" and the IT centre of the SNSB; Melzer et al. 2011, www.gbif.de/evertebrata2, www.snsb.info/).

The GloMyrIS subproject of the node "invertebrates II" (Spelda 2005, Melzer et al. 2011), offers an open-access, online database of Verhoeff's millipede material housed in museums in Germany, which is available via the GBIF portal and Systax (www.biologie.uni-ulm.de/systax/; see also e. g. Moritz & Fischer 1974, Weidner 1960 for printed catalogues of type material in the collections of Berlin and Hamburg), to facilitate taxonomic research on Myriapoda. Altogether c. 13000 datasets relating specifically to Verhoeff material, and thousands of data entries on taxa, media and links between species and the relevant literature are currently available (Spelda 2005). In particular, Verhoeff's gonopod micropreparations (2052 of Chilopoda, 3924 of Diplopoda) housed at the ZSM are documented including microphotos, as well as object- and labelscans.

## Methodology and results

Micropreparations are analysed on a Leica DM5000B light microscope using conventional bright field illumination and the following objectives (in brackets: magnification/numerical aperture): HXC PL Fluotar (1.6×/0.05), N PLAN (2.5x/0.07), HCX PL Fluotar (5×/0.15), N Plan (10x/0.30) and N Plan (20×/0.35). A survey of the microscope settings is given in Table 1 in order to optimize brightness, contrast, optical depth of field and other parameters.

Stacks of photos are generated with a Jenoptik ProgRes Speed XT core 5 digital camera and ProgRes Capture pro v. 2.8.0 computer programme. Afterwards extended depth of field pictures are calculated with Helicon Focus 5.1 using algorithm B and settings "Full resolution (100 %), radius 15, grading 2". Examples of photos from picture stacks are given in Fig. 1A–C, and of computer generated extended depth of field pictures in Fig. 1D–F and 2. Moreover we applied a high pass filter in Photoshop CS vs. 6.0.1 in Fig. 1D–F, which considerably improves acuity and contrast of minute structures, e. g. setae and cuticular ornamentation (compare Fig. 1E left and right).

As we expect fast improvement of image processing programs in the future we save our TIFF picture stacks on a server. This will allow us to recalculate them when better programs with improved algorithms will be available (see chapter "discussion" for further details).

According the experience we made with this project in the last months, our detailed protocol can be used not only by expert myriapodologists and microscopists, but also by advanced students after a relatively short training period, which is of importance since mass digitalisation of many specimens in one step, as is done, e. g., by D-Scan (this issue) is not possible here, but every microslide has to be edited separately. With c. 6000 micropreparations housed at ZSM, this leads to estimated 70000 single photos that have to be taken.

Currently approx. 900 stacks which equals approx.

**Table 1.** Miscoscope and camera settings for different magnifications and applications ("nec" – Latin for "especially not" – stresses values outside the optimum).

| Objective | Exposure time [ms] | Intensity | Aperture | Working distance [mm] |
|---|---|---|---|---|
| 1.6× | 12 | 048 | 10 (nec 5, 15) | >12 |
| 2.5× | 12 | 043 | 15 (nec 10, 20) | 11.2 |
| 5× | 12 | 115–054 | 10–20 | 12 |
| 10× | 12 | 046 | (2)–5 | 11 |
| 20× | 12 | 087 | (2)–5 for high contrast, 10 for discrimination | 6.9 |
| 40× | 12 | 107 | 10 | 3.3–1.9 |

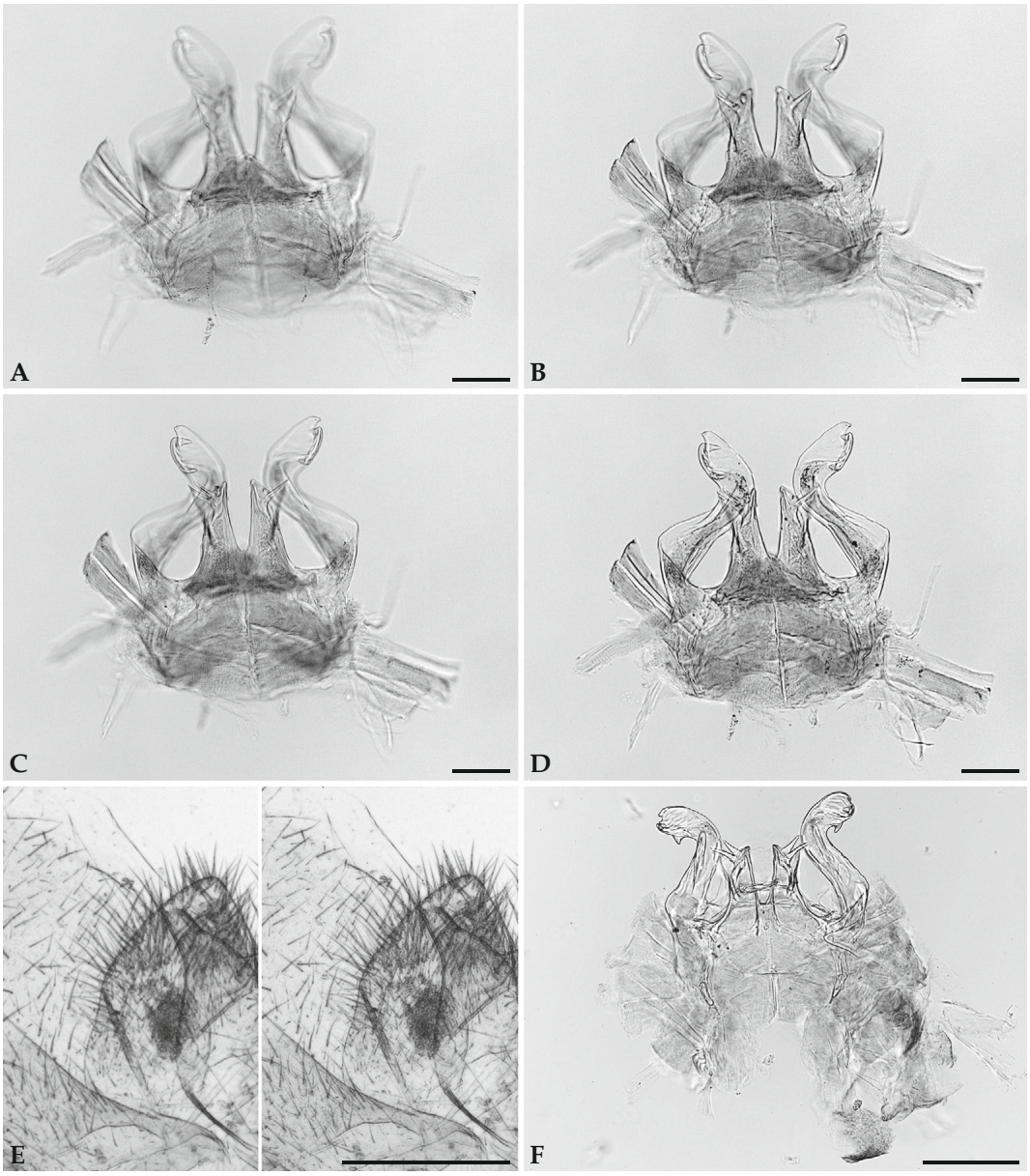


**Fig. 1. A-D.** Verhoeff's classical "flat" micropreparations and their computer aided imaging. Bars = 100 µm. **A-C.** Selected photos from stack of 12 photos of the anterior gonopods of *Ochogona brentana* (Verhoeff, 1927). **D.** Extended depth of field picture of same stack generated with Helicon Focus. **E.** Extended depth of field pictures of details of telopodit of an undescribed sphaerotherid from the Verhoeff collection. Left: normal extended focus picture; right: same, after application of high pass filter (diameter 2,7 pix); note drastic improvement of acuity of minute structures. **F.** Modern, "deep" micropreparation of anterior gonopods of *Ochogona brentana* (Verhoeff, 1927). Bar = 200 µm.

13 500 mediafiles are available, including many Sphaerotheriida, Glomerida and Chordeumatida of the Verhoeff collection. Examples for Verhoeff's original "flat" gonopod preparations where gonopods were flattened during the mounting process, are given in Fig. 1A–D and Fig. 2, for modern "deep" preparations in hollow slides retaining the gonopod's 3-dimensionality and steric arrangement of structures, in Fig. 1F.

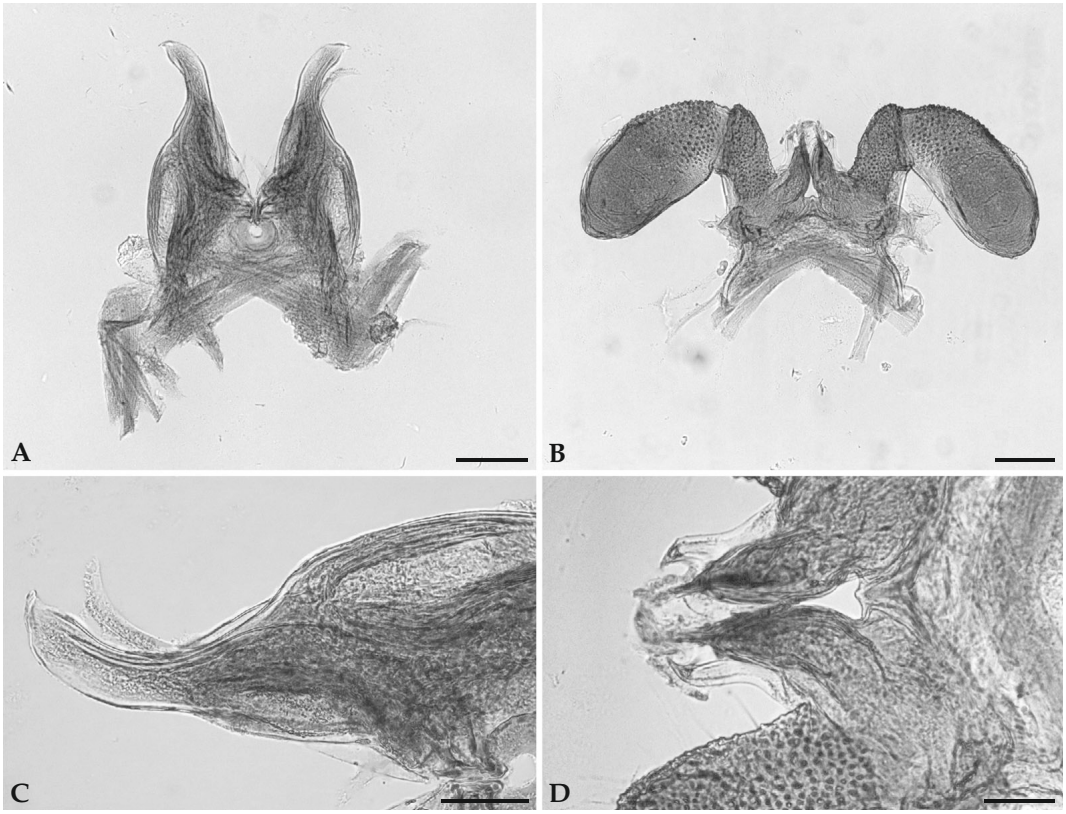


**Fig. 2.** Extended depth of field photos of anterior (**A,C**) and posterior gonopods (**B,D**) of syntype of *Japanosoma scabrum* Verhoeff 1914. **A,B.** Overviews of Verhoeff's micropreparations. **C,D.** Details of telopodite and colpocoxite, respectively. Bars = 100 µm (A,B) and 50 µm (C,D).

As can be seen in Fig. 1, three dimensionality, general architecture of gonopods and their species-specific structures relevant for determination and taxonomic revisions are preserved and depicted in great detail and resolution.

## Conclusions and outlook

In traditional species descriptions in myriapods (and also other arthropod lineages) line drawings are used as standard method for depicting the species specific sets of characters. With this method, the main weakness of photography can be compensated, viz. the limited depth of field. However, also drawings have their weaknesses: First, regardless of the masterhood of the drawings, a subjective element is unavoidable; second, the drawings and their requirements have drastically changed with time and with the amount of known species resulting in the fact that older drawings are often outdated after some time. The quality of drawings often changed from paper to paper in the same author, and sometimes late papers are of lower accuracy than early ones, as can be seen by comparing e. g. Attems (1898) with Attems (1951). In contrast Verhoeff's drawings have always been at a high standard but have been based nearly exclusively on micropreparations. The problem was excellently described by Shear (1987): "Because he lacked access to a dissecting microscope during the early decades of his work, Verhoeff usually mounted his material on slides after pulling it apart as best he could. As a result, his interpretations of the ways in which structures fit together in the whole animal are sometimes mistaken, but his illustrations of gonopods are often highly detailed and usually accurate". The case of *Japanosoma scabrum* Verhoeff 1914 (Fig. 2) gives a good impression how different the same micropreparations have been interpreted by Verhoeff (1914) himself and Shear (1987). While only Verhoeff (1914) depicted a metazonite, Shear (1987) depicted several details of the gonopods in detail and also the antenna and eyepatch of a

dissected female syntype from the Berlin museum (ZMB 12852, see Moritz & Fischer 1974). While in Verhoeff's (1914) time an overview of the gonopods (Figs 2A–B) was absolutely sufficient, the discovery of many closely related genera forced Shear (1987) to examine taxonomically important gonopod parts (Figs 2C–D) in detail.

To sum up, a method combining the depth of field of a drawing with the objectivity of a photo would be a remedy to these problems. We believe that in our project we get a step closer to this aim, and will provide valuable primary information on Verhoeff's gonopod preparations to myriapodologists worldwide for their taxonomic work, but also for future automatic determination aids based on pictorial information.

Nevertheless this method also still has its weaknesses. First, the present extended focus software solutions have difficulties to detect which structures are above or below in transparent preparations, as they extract them from the criterion of maximum contrast. The detection of the correct layer is a special challenge in old micropreparations, which have been pressed during their development. It has to be admitted, that these layers have even been mistaken in the drawings of the earlier authors. It is very probable that new algorithms, which construct a 3D-model, will overcome the layer problem. But in many cases new preparations without pressure are needed to show the correct conformation of genital structures. Another weakness is also connected with the layer problem: If we have structures in different layers which completely cover each other, there is no way to define how to extract a deeper layer. It is only possible to detect the top layer. But maybe even this problem can be resolved with 3D-reconstructions by an algorithm which subtracts layer by layer.

Beside these problems the objective display of photographic images allows the search for structures which have been overlooked by earlier authors. Especially if only outlines are needed, a photographic display of transparent structures is in most cases superior to any drawing and in non-transparent objects the results of extended focus software are technically mature to be superior in every case (see e.g. Golovatch et al. 2004). But as we need different drawings for finer structures, also different images have to be taken for that purpose, because the physical limits of optical systems are impossible to overcome.

The second aspect of our mass digitalisation project is to use modern information technology to crosslink our computer generated high resolution pictures with our data on the Verhoeff collection that are already available via the GBIF portal as a common platform for biodiversity information on myriapods. With the new element of modern imaging and image analysis techniques primary information for taxonomists can be combined further with internet available biodiversity data to provide a complete information systems on taxa (see also, e.g. Döring & Behrendsohn 2007, Mayo et al. 2008, Zauner 2009).

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