Scanning Electron Microscopy as a promising tool for rhabdocoel systematics

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AIM

The morphology and dimensions of the **sclerotised parts of the male copulatory organ** are among the most important character traits for species identification and description of **rhabdocoel flatworms** (Platyhelminthes). Usually, these structures are examined by light microscopy, which can typically reach a maximum magnification power of 1000x. However, Scanning Electron Microscopy (SEM) offers a magnification up to 1.000.000x, enabling visualisation of the finest morphological details. This may reveal taxonomically relevant intricacies that otherwise remain undetected. Nevertheless, this tool has not yet been employed in rhabdocoel systematics.
Hence, the aim of this study is twofold:

- To develop a method for isolating and visualising rhabdocoel sclerotised parts with SEM
- To present initial visualisations from a first phylogenetically diverse sample of rhabdocoel representatives

METHODS

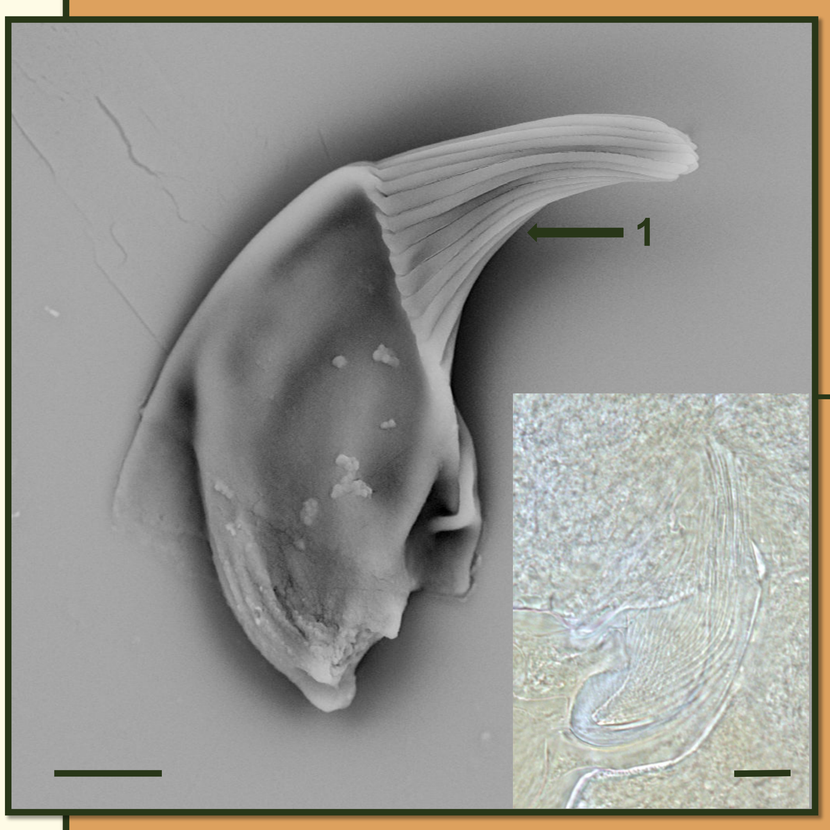
This method was developed using the protocol outlined by Fannes et al.(1) as a starting point. A range of specimens representing different lineages of Rhabdocoela were used to develop this method. Several morphotypes(2) of the cryptic species complex *Gyratrix hermaphroditus* Ehrenberg, 1831 were also included, as SEM may be particularly useful for studying and differentiating morphologically similar species.

The method is relatively straightforward:

1. Specimen digestion using proteinase K:TNES buffer (1:1)
2. Isolation of the sclerotised structure with a fine needle
3. Air-drying of the sample at room temperature
4. Sputter coating the sample with gold
5. Visualisation of the sample with SEM

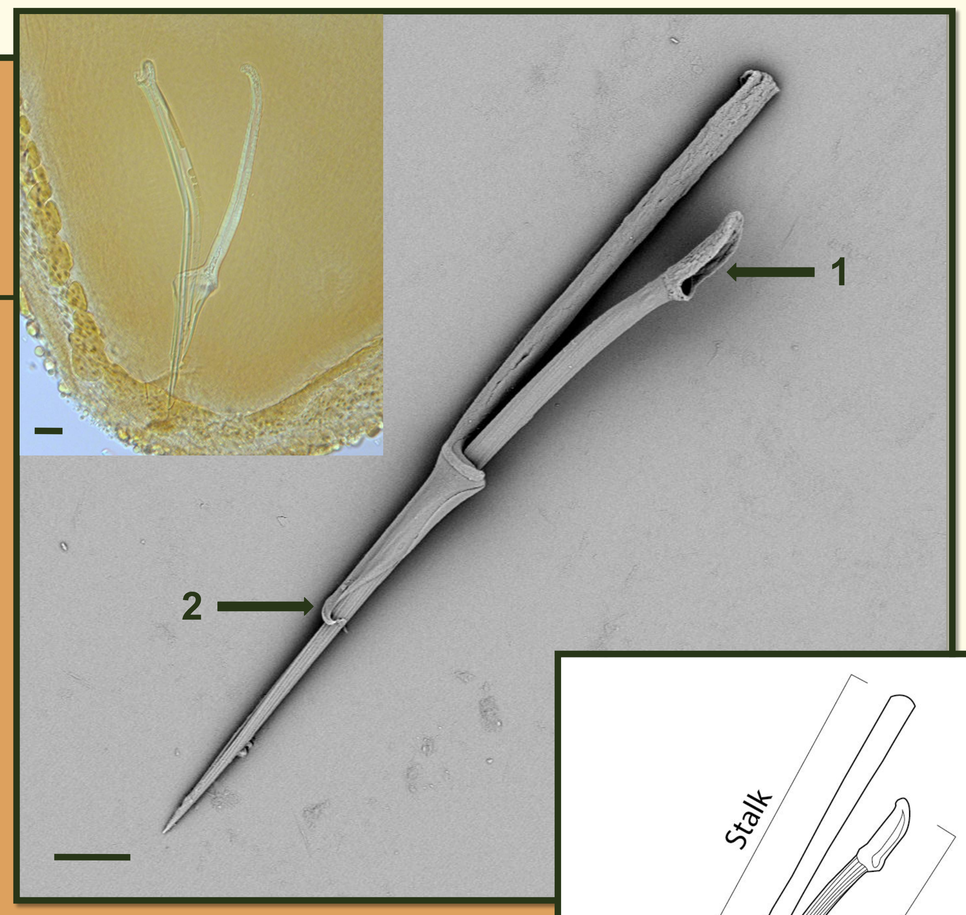
RESULTS

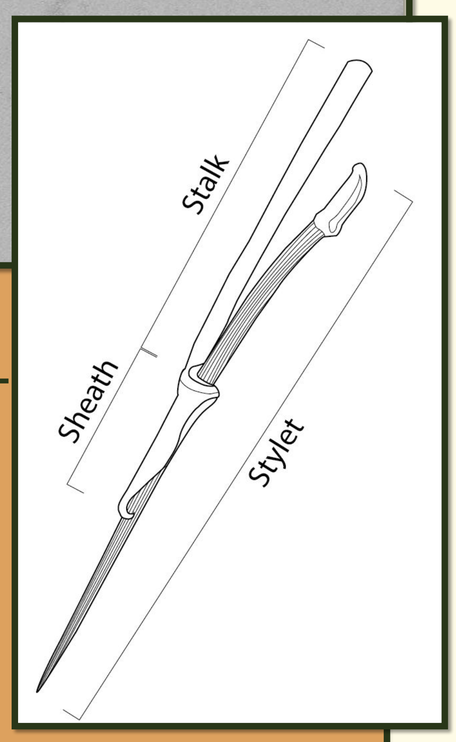
The method presented here has proven to be successful in isolating and visualising the sclerotised structures of our test samples. The protocol can be completed within two days, and is cost-effective overall. Light microscopy images are included to compare the two techniques. Scale bars of presented images are 10 µm.



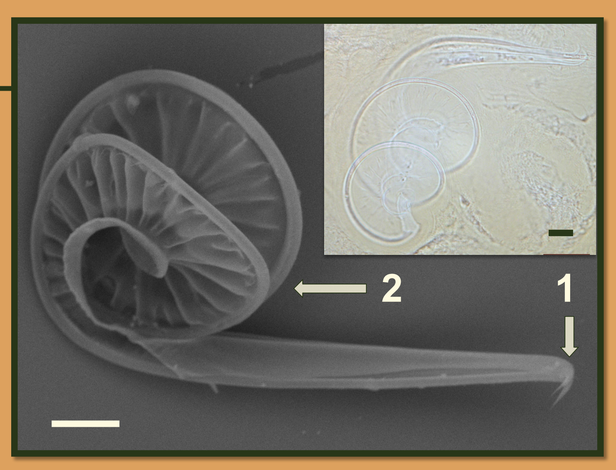
SEM image of the sclerotised copulatory structure of ***Proschizorhynchus martinezi*** Gobert, Reygel, Artois, 2017 (‘Schizorhynchidae’, Schizorhynchia). Individual ridges of the ‘cirrus sheath’ can be distinguished (1).

SEM image displaying the intact sclerotised part of the copulatory organ of ***G. hermaphroditus*** (Polycystididae, ‘Eukalyptorhynchia’), with the stylet, sheath, and stalk clearly visible.
Very small morphological structures can be discerned in the copulatory organ, including the taxonomically important(2) ‘stylet flag’ (1) and ‘hook’ (2), which are typically challenging to observe with light microscopy (Pers. Obs.).

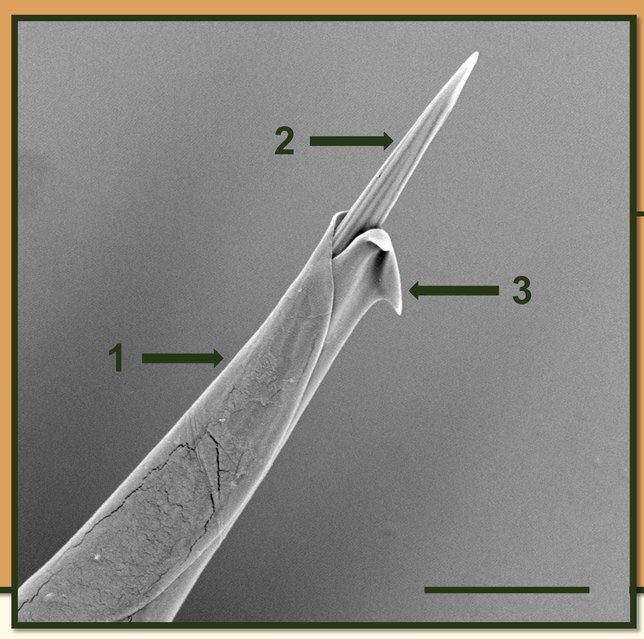




Schematic overview of the sclerotised part of the male copulatory organ of ***G. hermaphroditus.***



SEM image of the sclerotised, coiled stylet of ***Trigonostomum setigerum*** Schmidt, 1852 (Trigonostomidae, Dalytyphloplanida).
The distal hook (1) and stylet spires (2) are apparent.



Detailed SEM image of the copulatory sheath (1) and stylet (2) of ***G. hermaphroditus***.
The ‘hook’ (3) on the sheath is cleary visible.

CONCLUSIONS

In this study, we introduce a straightforward, rapid, and relatively inexpensive method for visualising sclerotised structures in rhabdocoel flatworms using Scanning Electron Microscopy. Its application enables an in-depth examination of the morphological intricacies that may remain hidden or subject to interpretation under light microscopy. We are optimistic that this protocol will prove valuable in yielding fresh taxonomic insights for this group, facilitating the identification or morphologically similar species, and thereby aiding in the resolution of cryptic species complexes.

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(1) Fannes, W., M.P.M. Vanhove, T. Huyse, and G. Paladini, *A scanning electron microscope technique for studying the sclerites of Cichlidogyrus.* Parasitology Research, 2015. **114**(5): p. 2031-2034.
(2) Tessens, B., et al., *Is ‘everything everywhere’? Unprecedented cryptic diversity in the cosmopolitan flatworm Gyratrix hermaphroditus.* Zoologica Scripta, 2021. **50**(6): p. 837-851.