How to destroy a snail? - Problems and solutions working with histological samples of micro-scaled gastropods

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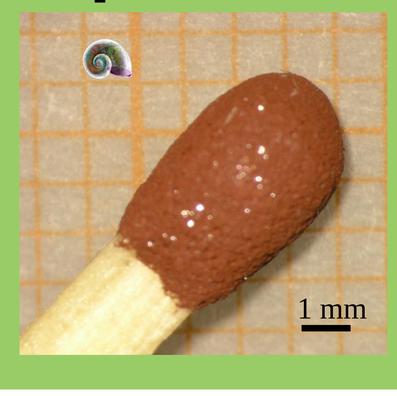
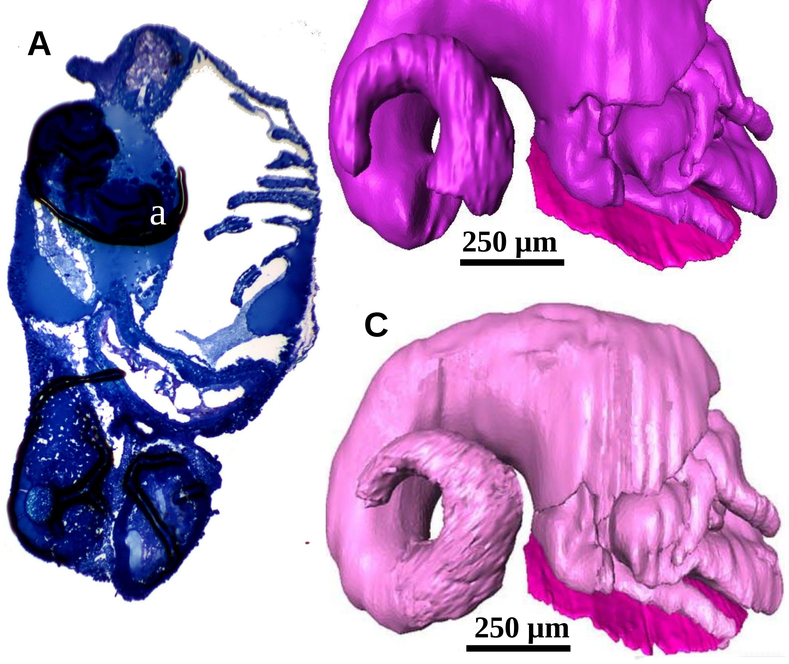


Introduction

Morphological and anatomical data are crucial to shed some light onto the polyphyletic assemblage of skeneimorph vetigastropods. Skeneimorph gastropods (Mollusca: Vetigastropoda) are small (diameter 1-5 mm) marine snails from shallow waters down to abyssal depths (e.g. hot vents, natural wood fall). To gain those data sets a lot of working steps are needed and multiple problems might occur: The material has to be plastic embedded, serial sectioned with diamond or glass knifes, stained and sealed. Even before that point the samples can be damaged massively, e.g. by freeze-storing or due to coagulation of the blood.

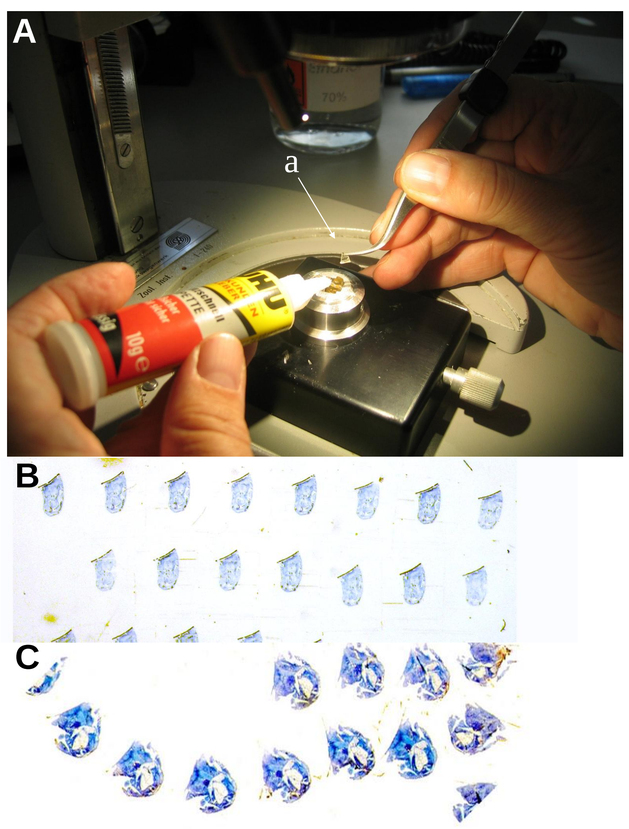
Results

Embedding:



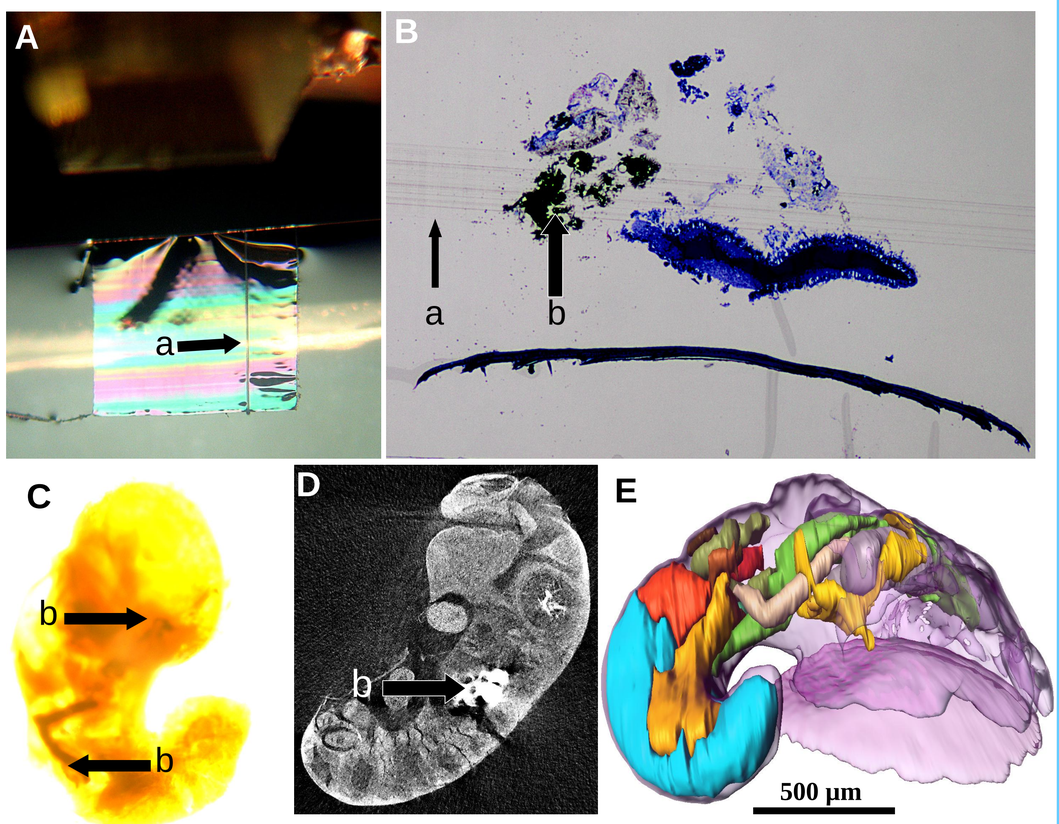
A: Plastic embedding of complete animals, especially when larger than 2.5 mm, needs an extended retention time during drying in the rising acetone/alcohol set, otherwise the plastic will not penetrate the sample constantly (**a**); high air humidity also can impede a constant penetration. **B**: Delicate details like the whorls of a sniail can break easily (**C**: original state reconstructed in AMIRA) Embedding need an effort of a lot of drying an washing steeps so small nets can be very useful here to avoid loosing you samples. [**A**: *Leucorhina caledonica*; **B,C**: *Ventsia tricarinata*;]

Trimming:



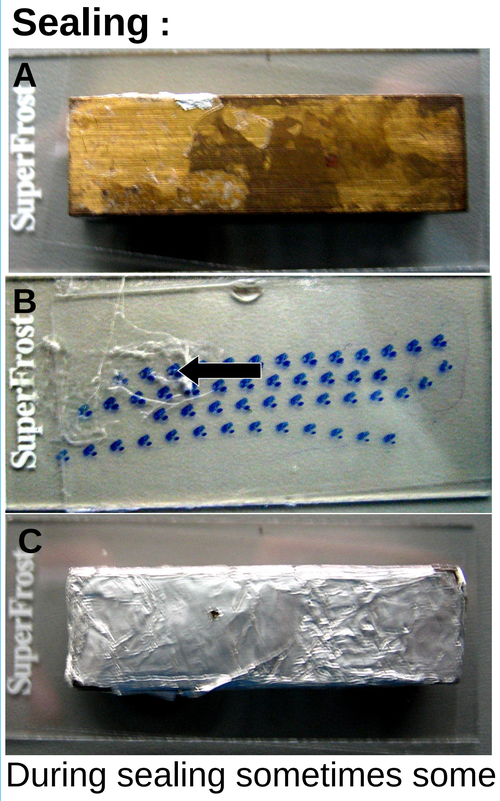
Trimming a fast but important step influence directly on the quality of the sections. The block should be a pyramidal as small as possible to get straight section series (**B**), otherwise the sections curl (**C**). **A**: Rarely the specimen breaks of while trimmed (**a**), but can be glued with superglue. [**B:** *Skenea serpuloides*; **C**: *Dikoleps nitens*]

Mineral particles vs diamond knife:



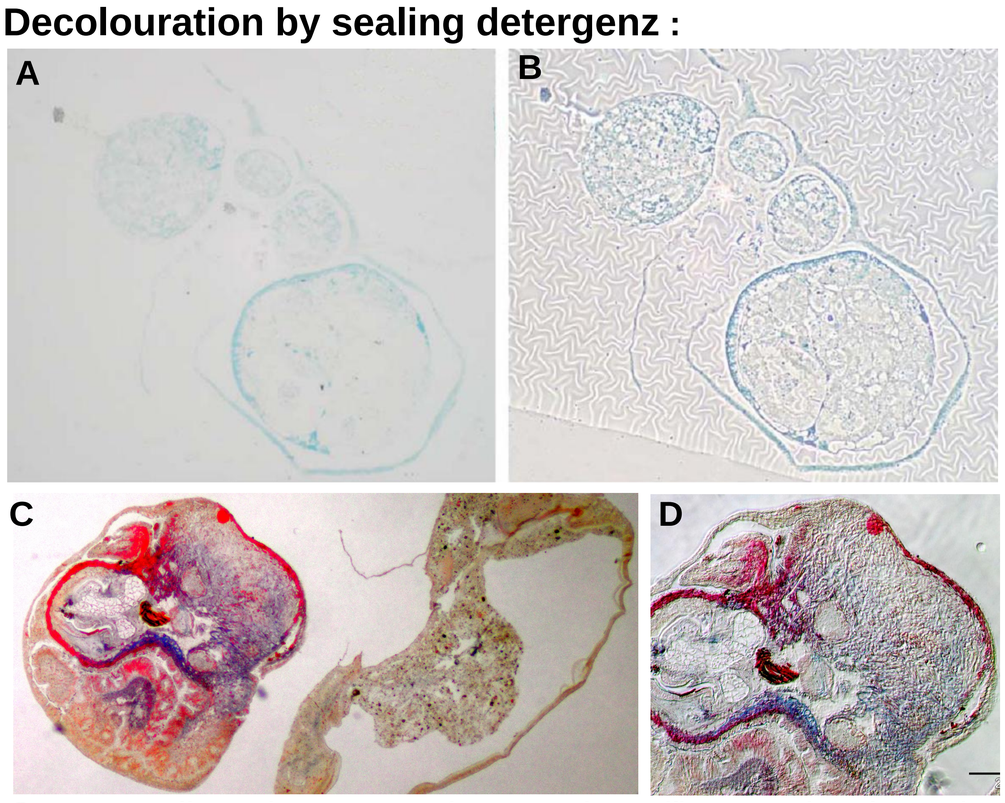
Mineral particles and solid gut/intestine content are a big problem when serial sectioning is performed. **A**: Artefact (**a**) on a section, caused by a break of in the diamond knife. **B**: Artefact (**a**) of a damaged glass knife, caused by stones in the mantle cavity (**b**). Some particles are already visible in the embedded animal (**C**). When not ex-changed regularly, glass knifes blunt, producing broken sections. MicroCT images, combined with 3D reconstruction, are a solution for such problematic specimens (**D**,**E**).
[**B-E**: *Protolira valvatoides*]

Sealing :



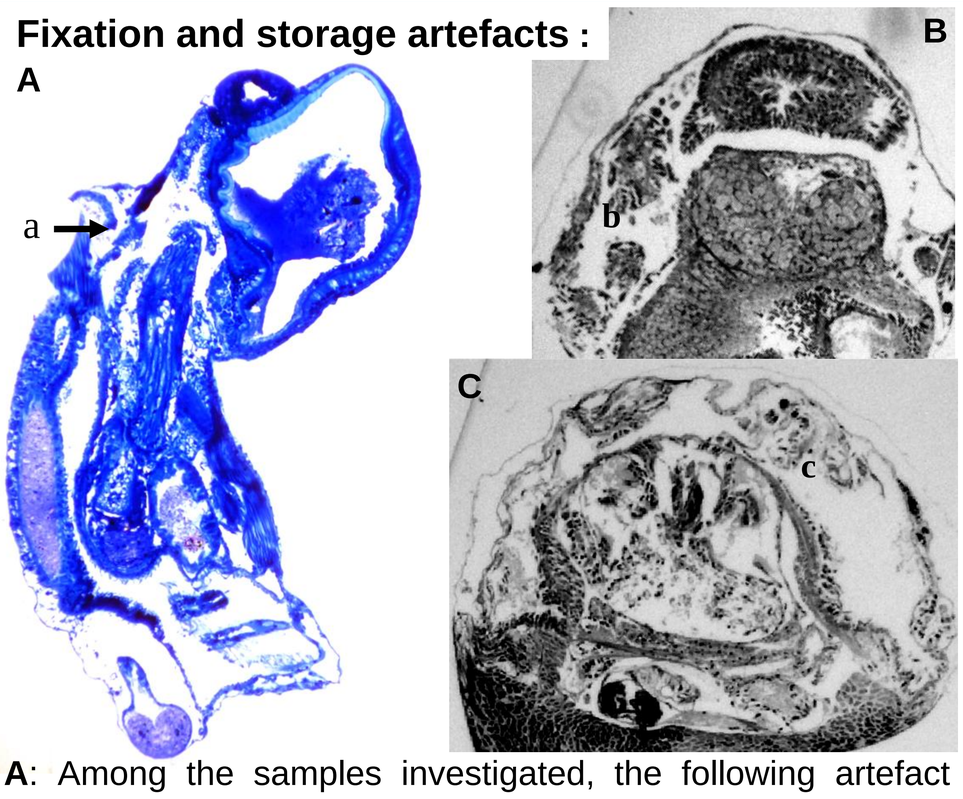
During sealing sometimes some detergent can soak between cover glass and balance weight (**A**); when removing the cover glass can break (**B**). Covering the weight with aluminium foil solve this problem (**C**). [Turbinid snail]

Decolouration by sealing detergenz :



Some sealing detergents (e.g. cedar oil) can decolour the stained samples. Picture **A** is a decoloured section stained with Richardson; **C** shows a 5 µm section stained originally with “Kernechtrot / sudan black” (both regular bright field light microscopy). Phase contrast light microscopy (**B**,**D**) can help to reach more information out of the sections. [**A,B**: *Xenoskenea* pellucida, **C,D**: *Carenzia carinata*]

Fixation and storage artefacts :



A: Among the samples investigated, the following artefact occasionally occurs: When the animals are anesthetized and fixed, the blood coagulates and expands, resulting in an “exploded” aspect of the heart (**a**). **B,C**: Some samples were deep freeze storage before but into a proper liquid fixative, so epithelial structures, e.g. tentacle survace (**b**), ctenidium (**c**), were badly damaged. [**A**: *Wanganella fissura*; **B,C**: *Bathyxylophila excelsa*]

Conclusions

Investigating animal with a body smaller than 2 mm, non-invastigative methods like microCT do not give satisfying resolutions. Semithin sectioning is the only method to investigate the histology of such small animals. While a lot of preparation steps are needed small helping devices like the proper glue, small nets or a piece of aluminium foil can solve quite some problems.

Acknowledgements

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