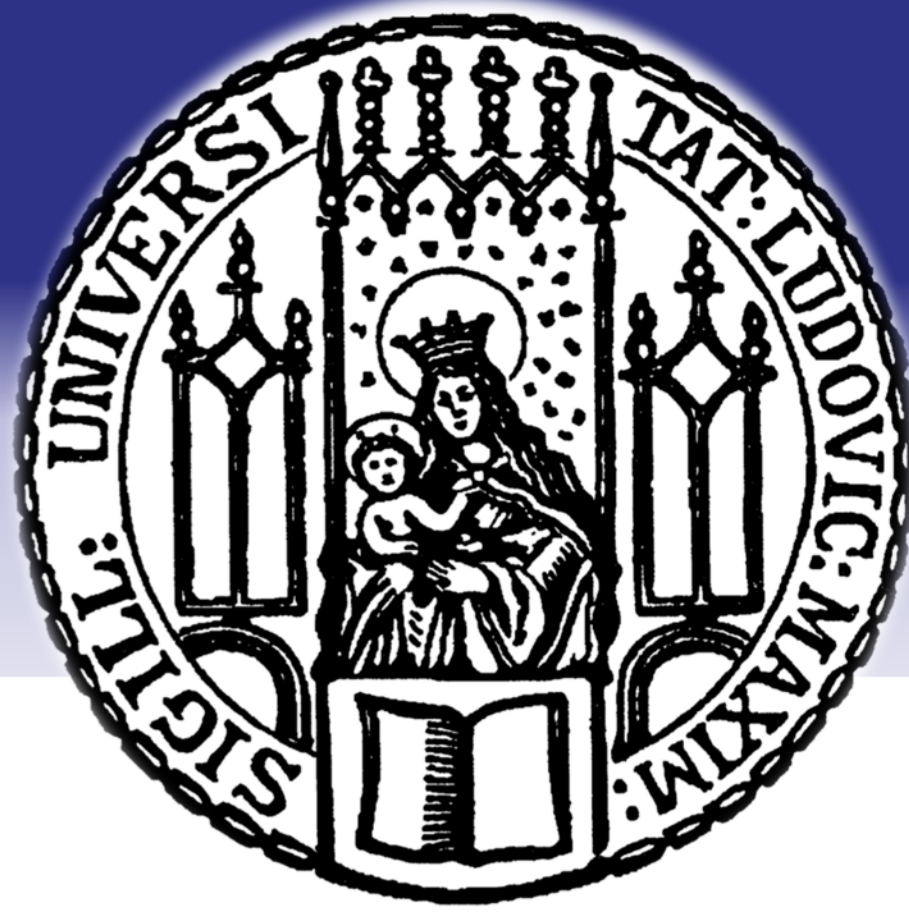


Endosymbiotic bacteria in the hot-vent gastropod *Lurifax vitreus* (Heterobranchia: Orbitestellidae)

Andreas Hawe, Heidemarie Gensler, Gerhard Haszprunar

Biozentrum der LMU München, Großhaderner Str. 2, 82152 Planegg, Germany

A.Hawe@gmx.de



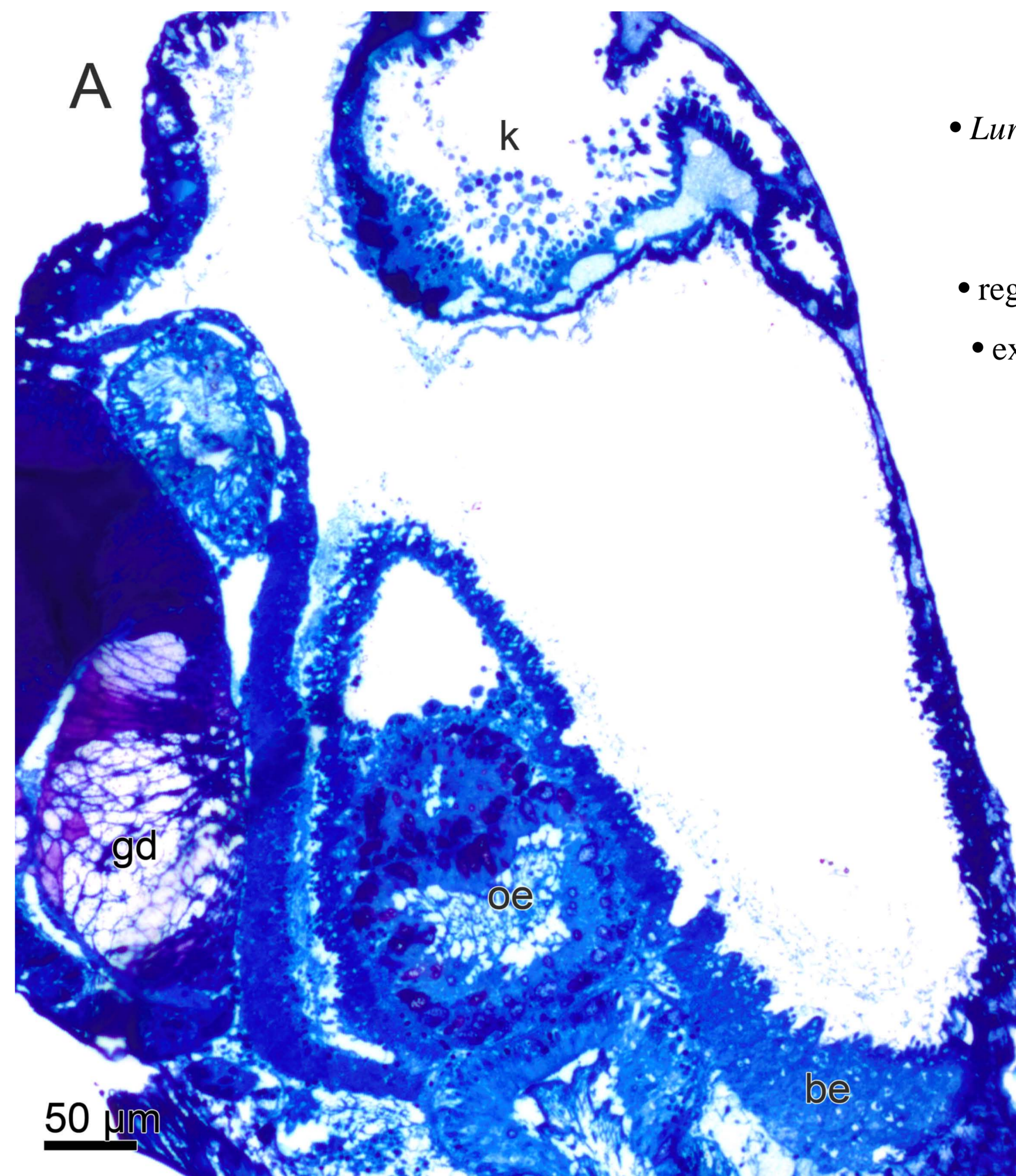
LMU

BioZentrum

Systematische Zoologie
AG Prof. Haszprunar

Introduction

Molluscs of chemosynthetic habitats (e.g. hot-vents, cold seeps, whale or wood falls) frequently harbor ecto- or endosymbiotic bacteria, which use hydrogen sulfide or methane as energy source. Among the Gastropoda so-called “bacteriocytes” (tissues with intracellular bacteria) have been demonstrated in Peltospiridae (Neomphalida), Lepetelloidea (Vetigastropoda) or Provannidae (Loxosomatoidea: Caenogastropoda), but not yet in higher gastropods (Heterobranchia). As part of our studies on basal heterobranch families we also examined the Orbitestellidae on the basis of semi-thin section series and computer-aided 3D-reconstructions. We noticed an atypical “granular-glandular” epithelium in the posterior mantle cavity within *Lurifax vitreus* (Fig. A: be). We examined this special epithelium thereafter by means of ultra-thin sections and TEM. We also examined other genera of orbitestellids (*Orbitestella wareni*, *Microdiscula charopa*, *M. vanhoeffeni*) by regular light microscopy (mostly provided by Winston Ponder).

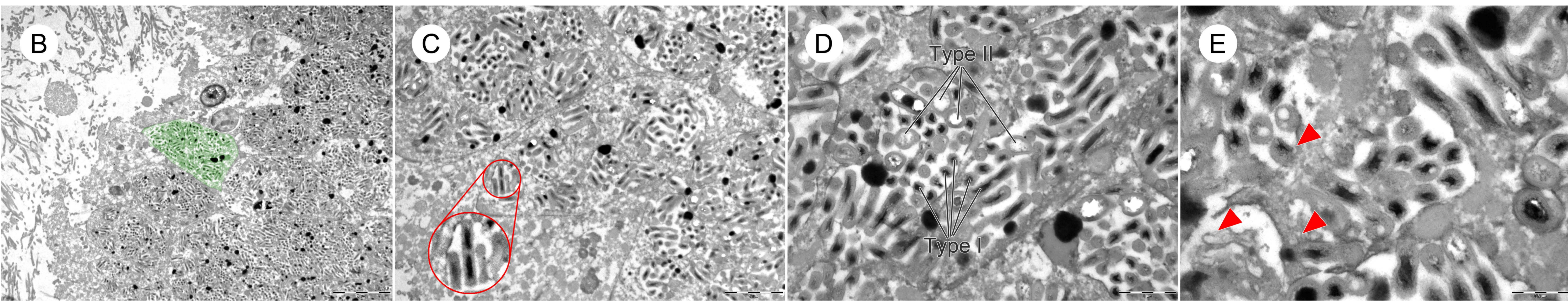


Methods

- *Lurifax vitreus* (originally fixed in 70 % ethanol) destined for 3D reconstruction
- decalcification in ascorbic acid (2 %)
- regular treatment for TEM (but no osmification)
- examination with a FEI Morgagni transmission electron microscope (TEM) at 80 kV

Figure Legend

- A:** Histological semi-thin section of the ROI of *Lurifax vitreus*.
- B-E:** TEM pictures of bacteriocytes.
- F:** Surface rendering and **G:** volume rendering of the bacteriocyte epithelium, located in the posterior mantle cavity.
- H:** Histological semi-thin section of *Orbitestella wareni* (original sections made by Ponder).



Results

- pallial epithelium covering parts of the gonoduct, oesophagus, stomach and rectum but not kidney (Figs. B, F, G)

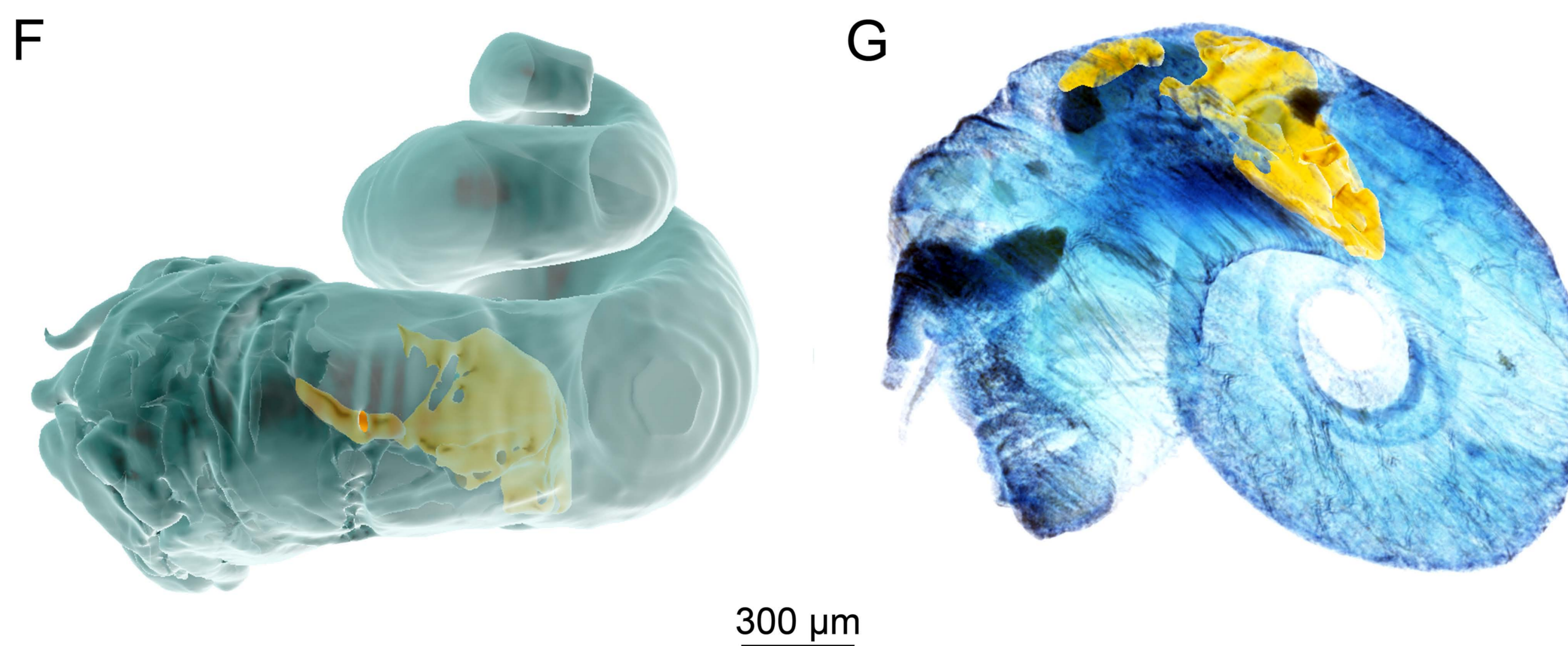
- bacteria enclosed in all vacuoles (Figs. C, D)

- bacteria appear rod-shaped or palisade-like (depending most likely on section plane)

- two morphotypes distinguishable, both have thick walls (Fig. D)

- different stages of cleavage (insert, Fig. C) and degeneration (arrowheads, Fig. E) visible

- *Orbitestella wareni* shows very similar epithelium (Fig. H), in contrast to both *Microdiscula* species with regular pallial cavity



Functional aspects

- Bioluminescence and direct digestive support are unlikely due to the position in the posterior mantle cavity.

- Reducing (hydrogen sulphide or methane) habitat suggests bacterial chemo-autotrophy.

- *O. wareni* may live in sulphide-rich microhabitat (original found “beneath rocks at low tide” (Ponder 1990: 515)) – otherwise interpretation of the bacteriocyte epithelium occurrence is difficult.

- Non-reduced digestive system reflects limited physiological importance of symbiosis, yet the considerable size of bacteriocytes supports the opposite.

- Further conclusions and insights require several prerequisites:

- 1) Better preserved material for cytological details of bacteriocytes.
- 2) Ultra-structural, molecular and immunocytochemical methods must be applied in order to determine and characterize the housed bacteria.
- 3) Investigations on bacterial transfer during reproduction are necessary.

Abbreviations: mc: mantle cavity, be: bacteriocyte epithelium, gd: gonoduct, k: kidney, oe: oesophagus.

Acknowledgements: We are grateful to Anders Warén who send us the original specimens of *Lurifax vitreus*. We are deeply indebted to Winston Ponder for providing us his semi-thin sections of *Orbitestella wareni*.