The soft body parts of freshwater bryozoans depicted by scanning electron microscopy

M.G. Walzl & E.R.Wöss

Abstract: For the first time, special scanning electron microscopical preparation techniques, i.e. chemical dehydration and air drying with hexamethyldisilacane, were used to study the Phylactolaemata. This approach depicted the growth form of colonies and the outer structures of zooids of *Plumatella casmiana* and *P. fungosa* three-dimensionally. The structures of cystids and polypids, for example the cystid wall, lophophore, gut, funiculus and the muscles, are represented using dissected zooids. Moreover, this technique revealed the structures of both asexual reproduction (the buds, the generation and germination of statoblasts) and the organs for sexual reproduction (testis, ovary, embryo sac, larva).

Key words: Phylactolaemata, *Plumatella*, anatomy, sexual propagation, statoblast formation.

Introduction

The phylum Bryozoa consists of three classes, the Phylactolaemata, Gymnolaemata and Stenolaemata (Ryland 1970). The vast majority of bryozoan taxa are marine, inhabiting depths from the intertidal to the abyssal. Freshwater bryozoans comprise a smaller number of about 60 species (Wood 1989) and can mostly be assigned to the class of phylactolaemates. This group exclusively inhabits freshwater, as do a smaller number of gymnolaemate species, all belonging to the order Ctenostomata. All freshwater bryozoans lack mineralized skeletons. This is in striking contrast to most bryozoans, which have skeletons made of calcite or, less frequently, aragonite. All Stenolaemata and the order Cheilostomata of the Gymnolaemata possess calcareous skeletons; they are among the most common groups of macrofossils found in the post-Cambrian marine fossil record (Taylor 2005). In total, 14.700 species have been described in the fossil record (Horowitz & Pachut 2000), and about 5.600 extant bryozoans are known (Todd 2000). The morphological features of bryozoan skeletons form the basis for classifications, and these features differ between the tubular stenolaemate bryozoans and the boxlike gymnolaemate cheilostomes. In most stenolaemate bryozoans, the exterior surface is insufficient to differentiate taxa, and taxonomic separation in that group relies heavily on characters visible in petrographic thin sections (Sandberg 1977, see also Ernst and Scholz et al. this volume). The frontal surfaces of cheilostome zooecia, however, are covered by membranes or by calcified walls which offer varying amounts of morphological details (Banta 1973; see also Bader & Schäfer, Novosel, and Vávra this volume). In the latter case, scanning electron microscopy (SEM) is broadly used to study bryozoan skeletons and has became the standard tool in taxonomic work for both palaeontologists and biologists. Even in ctenostomate bryozoans, where calcified skeletons are missing, the process of bioimmuration (the preservation of an organism by the skeletal overgrowth of a neighbouring encruster) allows the details of the zooids to be described using SEM techniques (Voigt 1966; Taylor 1990; Todd 1994).

In the phylactolaemate group, the soft-bodied zooecia offer less distinctive characters (Kraeplin 1887, 1892; Braem 1890; Hyman 1959; Lacourt 1968; Woollacott & Zimmer 1977; Mukai 1982). In

Denisia 16, zugleich Kataloge der OÖ. Landesmuseen Neue Serie 28 (2005), 49–58

contrast to marine species, where heterozooids such as avicularia and vibracularia are present (RYLAND & HAYWARD 1977; HAYWARD & RYLAND 1979), the uniform shape of the autozoids of phylactolaemate species has raised less interest for SEM studies. Ultrastructural investigations on spermatozoan structure and larva (FRANZÉN 1982, FRANZÉN & SENSENBAUGH 1983) sporadically involve SEM. Most ultrastructural work, however, has traditionally focused on statoblasts. These dormant bodies are excellently suited for SEM because their shell consists of a chitinised cuticula. They have been the target of numerous comparative morphological investigations (e.g. WIEBACH 1974; RAO & BUSHNELL 1979; MUKAI 1999; MUNDY 1980; GOETHALS et al. 1984; ODA & MUKAI 1985; GEIMER & MASSARD 1986; POURCHER & D'HONDT 1987; WOOD & WOOD 2000). In some cases, such as within the genus *Plumatella*, SEM has become the only reliable tool for species distinction (GEIMER & MASSARD 1987; WOOD 2001; TATICCHI & PIERONI 2005 and see also Taticchi et al. this volume).

Despite the great benefit of SEM, this standard method has rarely been used to study the soft-bodied outer and inner parts of the zooids (but see: MUKAI et al. 1997), probably due to the complexity of the preparation techniques required. This SEM study is an introduction for further ultrastructure work on reproduction in freshwater bryozoans. First results are presented in preparation techniques of soft-bodied inner and outer parts of the zooids of two species, *Plumatella fungosa* and *P. casmiana*. These species have already been examined with respect to the formation of sexual and asexual propagules and the reproductive cycle in freshwater bryozoans (WÖSS 2002).

Material and methods

Colonies of *Plumatella casmiana* were collected on 17.5.1992 from a pond at Laxenburg (Lower Austria) and colonies of *P. fungosa* on 7.7. and 3.9.1992 from a backwater of the Danube River at Bad Deutsch Altenburg (Lower Austria).

The colonies were transported in pondwater, along with the logs and twigs on which they grew, to the laboratory and left there undisturbed at least until most of the polypids had protruded. Then, with a pipette, a saturated aqueous solution of chloral hydrate ($Cl_3CCH(OH)_2$) was added dropwise and carefully to the water surface. The specifically heavier chloral hydrate solution sinks down to the colony and narcotizes the zooids, so that most of the polypides remain protuded. After 15 minutes, an equal volume of 1 % aqueous buffered formaldehyde solution with pH 7.2 (LILLIE 1954) was added. For definitive fixation the colonies were removed and immersed into a 4 % buffered formaldehyde solution. The colonies were stored in this medium until examination.

For further detailed SEM investigations, selected parts of colonies or single zooids were separated from the substratum using a sharp razor blade and transferred to distilled water to wash out excessive formaldehyde. The distilled water was changed 3 times after 15 minutes (important to avoid precipitation during the following processes). Afterwards, the samples were dehydrated chemically with acidified 2,2-dimethoxypropane (DMP) (MULLER & JACKS 1975). For acidification and activation, 1 ml 25 % HCL was added to 100 ml DMP shortly before use. For rapid dehydration, 1 part water in the sample vials was mixed with 3 parts DMP. A rapid cooling of the vials documented the endothermic chemical process that yields anhydrous methanol and acetone. After 20 minutes (although an overnight delay has no negative effect) the solution was replaced twice with water-free acetone, for 15 minutes in each case. The acetone was exchanged with HMDS (1,1,1,3,3,3-hexamethyldisilazane), the sample initially being immersed for 30 min in a 1:1 mixture of acetone and HMDS followed by 30 minutes in pure HMDS, and then air dried on filter paper under a fume hood (BRAY et al. 1993; NATION 1983). After drying, the samples were transferred individually on aluminiumstubs using a fine pencil. Single zooids or parts thereof were mounted with TEMPFIX-thermo glue (Neubauer Chemikalien company, Germany), and parts of colonies or groups of zooids were mounted using silver paste. All samples were then sputter coated with 40 nm of gold in a Agar B 7340 sputter coater.

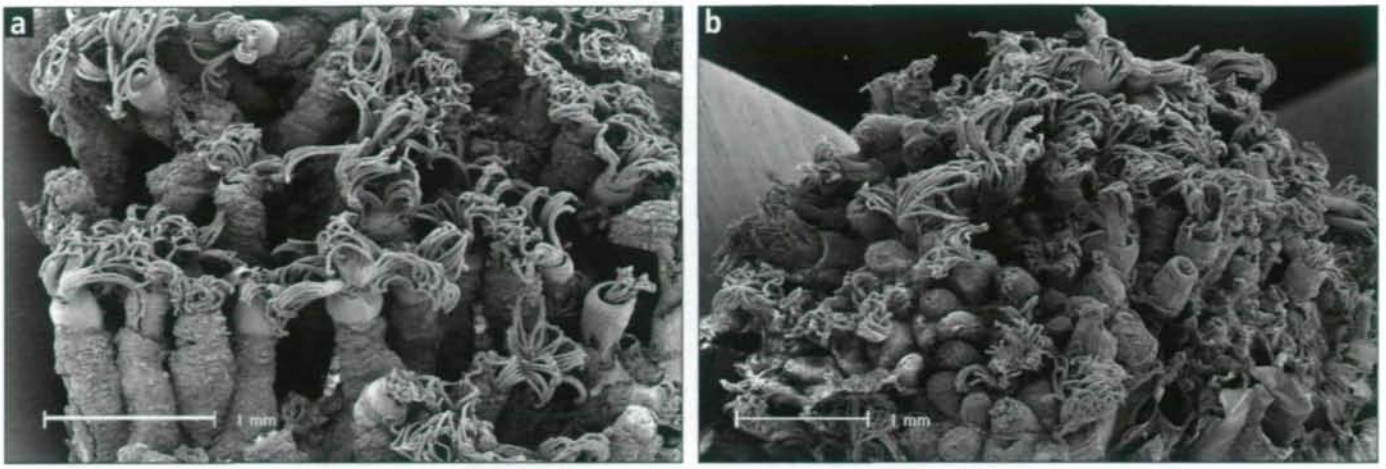


Fig. 1: Parts of colonies with many protruded polypides of *Plumatella casmiana* (**a**) and *P. fungosa* (**b**).

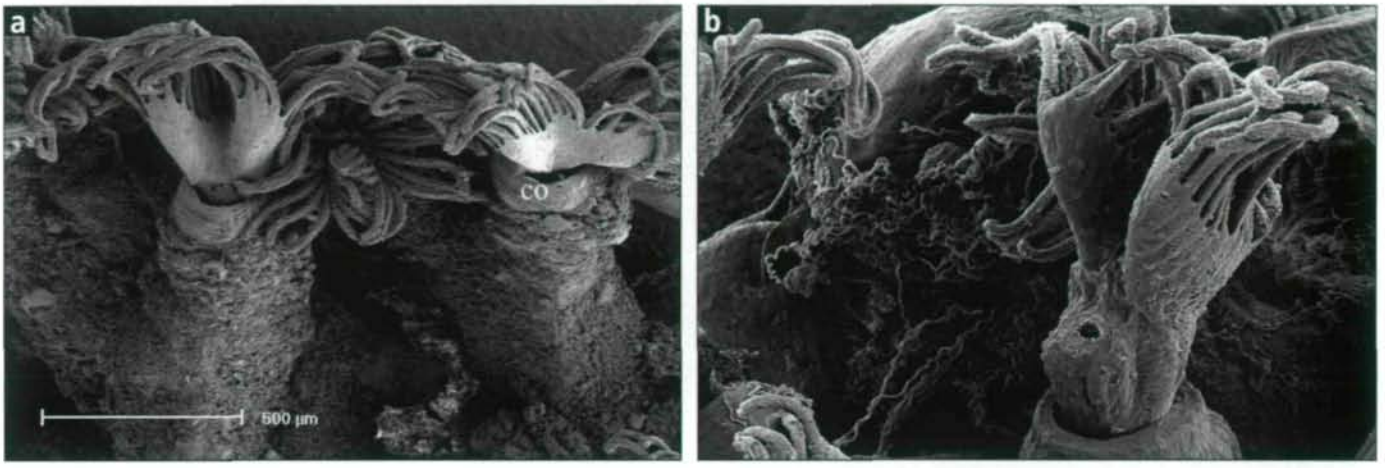


Fig. 2: Zooids with protruded polypides of *Plumatella casmiana* (**a**) with rough, incrusted outer cystid wall and collar region (co) and *P. fungosa* (**b**) with smooth outer surface of cystid wall.

Specimens were examined at 10 to 15 keV in a Philips XL20 scanning electron microscope and photographed digitally. In order to view the internal organs, zooids were opened with two tungsten needles that were sharpened by repeatedly inserting the needle tips into an aqueous potassium hydroxide solution under 6 volts of alternating current generated by a microscope transformer. The zooids were opened either in the phase of washing in water or after air drying.

Results and discussion

The colonies of *Plumatella casmiana* and *P. fungosa* are characterized by a different growth form. *Plumatella casmiana* shows an irregularly "knotty" arrangement of zooids, which are attached to the substrate in a sheetlike growth form; colonies of *P. fungosa*, however, are packed more densely, with regular fused zooids resulting in an erect and massive growth form (Fig. 1).

The fully grown monomorphic zooids of both species differ in size. *Plumatella casmiana* zooids are smaller, and the horseshoe-shaped lophophore therefore bears only 25-40 tentacles, whereas 40-60 tentacles are present in *P. fungosa* (Fig. 2). The single

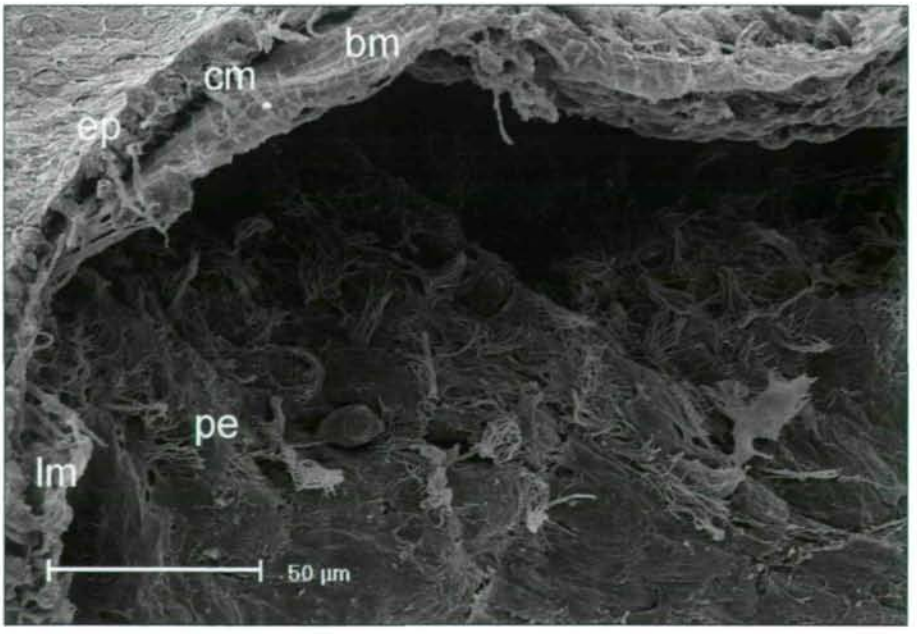


Fig. 3: *Plumatella fungosa* – endocyst body wall with epidermis (ep), circular muscles (cm), basement membrane (bm), longitudinal muscles (lm) and ciliated peritoneal cells (pe).

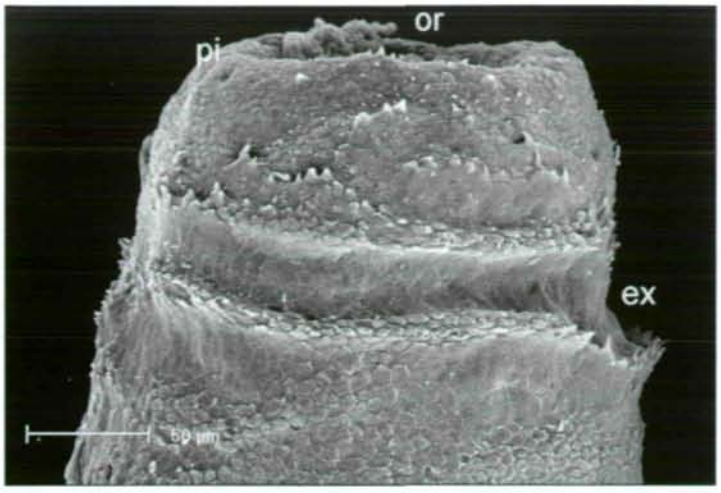


Fig. 4: *Plumatella fungosa* – endocyst body wall with pits (pi) arround the edge of the orificium (or) and rows of epidermal cells bearing extensions (ex).

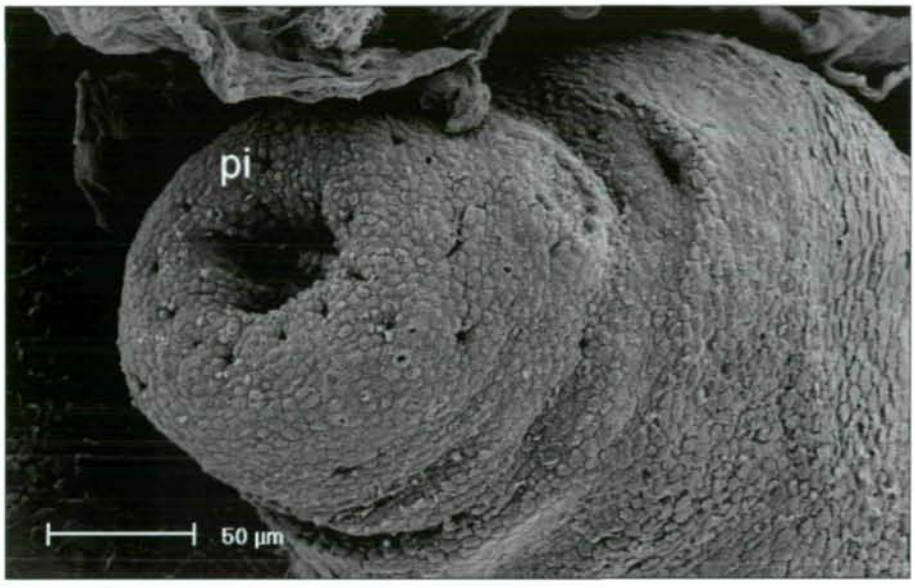


Fig. 5: *Plumatella fungosa* – young, newly budded zooid, polypide retracted, regularly distributed pits (pi).

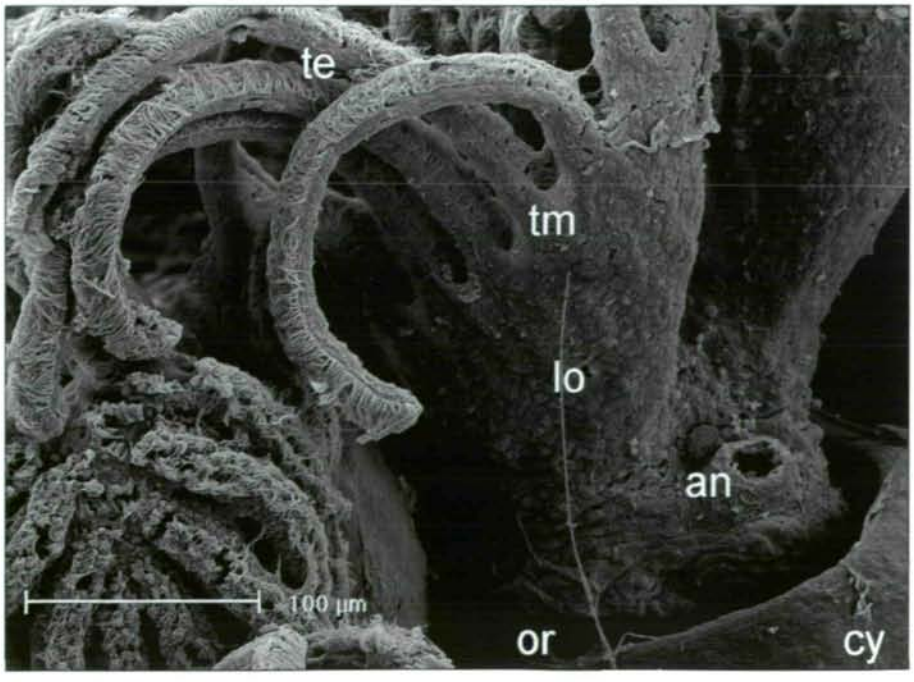


Fig. 6: *Plumatella casmiana*, dorsolateral view – polypide protruding from the orificium (or) of the cystid (cy) with the horseshoe-shaped lophophore (lo) and the anus (an) outside the lophophore. Ciliated tentacles (te) originate on the lophophore, and are interconnected at their base by an intertentacular membrane (tm).

zooids consist of two functional units, the cystid (with its protective cover and the budding region including the female reproductive organs) and the polypid, which mainly serves for nutrition and respiration.

All cystids of *P. fungosa* have a smooth outer surface, whereas those of *P. casmiana* have a rougher outer surface usually incrusted by microparticles and tend to form a keel. A conspicuous feature is that the anterior end of the *P. casmiana* cystid has no incrustations and shows a distinct collar region (Fig. 1a, 2a).

The body wall of the cystid consists of an outer non-living part and an inner living part, which ends with the outermost epidermis. The inner part of the body wall (endocyst) is not connected rigidly with the outer part of the body wall (ectocyst). Therefore, during preparation, the endocyst can be easily pulled out from the chitinuous ectocyst tube (sheath) to investigate the surface of the epidermis. The endocyst wall is multilayered, and its mesodermal part is separated from the ectodermal part by a basement membrane. On the outside of the basement membrane is the one-layered epidermis, with a circular muscle layer in between; on the inner side there is the peritoneal epithelium of the metacoel, underlain by a longitudinal muscle layer (Fig. 3). The innermost layer of the endocyst wall is the peritoneum of the metacoel. In certain areas of this coelothelium, groups of cells bear cilia for circulation of the coelomic fluid. Each cell has clearly visible cilia arranged in a row like the teeth of a comb (Fig. 3). This arrangement probably more effectively transports coelomic fluid. At some specimens, certain ectocyst-sheath-secreting epidermal cells bear conspicuous extensions, probably to anchor the endocyst to the ectocyst tube. In *P. fungosa* these epidermal extensions are sometimes arranged in series of rows (Fig. 4). The orifice of the cystid is sometimes surrounded by pits (Fig. 4), and especially the young zooids,

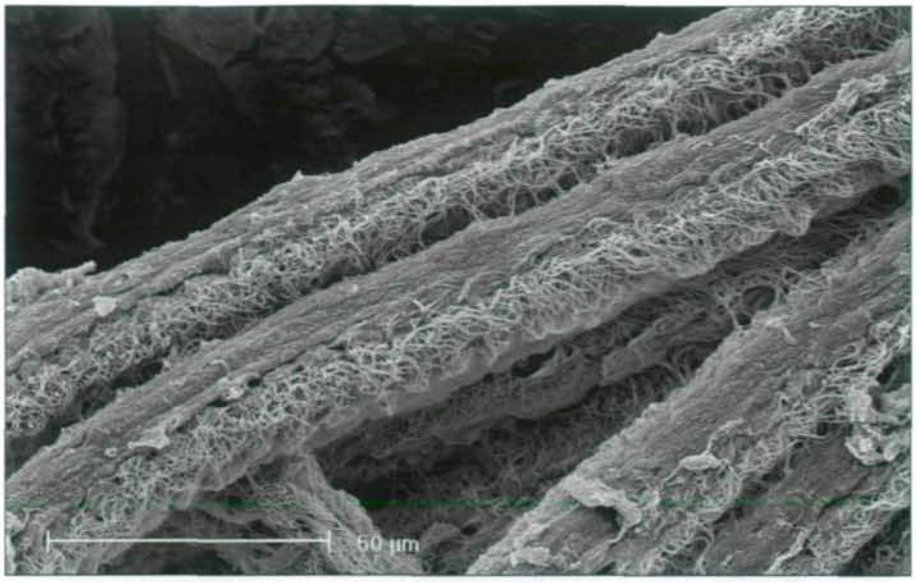


Fig. 7: *Plumatella fungosa* – tentacles with rows of multiciliated cells at their lateral and inner sides.

shortly after evagination, show a regular arrangement of these pits. These structures can be interpreted either as sensory pits or as vestibular pores (Fig. 5).

The polypide consists of the lophophore and the V-shaped gut, which is connected with the ventral cystid wall by a hollow peritoneal cord, the funiculus.

The lophophore bears tentacles interconnected at their bases by the intertentacular membrane, a fold of the lophophore (Fig. 6). Three rows of epidermal cells of the tentacles bear cilia directed laterally and to the inner side of the lophophore. The synchronized beating of the cilia generates a water current that transports particles toward the mouth (Fig. 7, 8). The mouth is situated centrally on the lophophore and is encircled by the tentacles. Dorsally, the mouth is overhung by a flap, the epistome, which can close the opening (Fig. 8). As opposed to the mouth opening, the anus lies outside the tentacle circle at the dorsally open side of the lophophore (Fig. 6).

The gut is divided into different parts – the pharynx, oesophagus, cardia, caecum and the intestine – and hangs into the coelomic cavity of the cystid. A large, paired muscle on either side of the gut extends across the metacoel. On one side, the muscle inserts in the ventro-lateral cystid wall, and on the other side at the base of the lophophore, i.e. at the tentacle sheath, with two smaller bundles also inserting directly on the gut (Fig. 9). The gut wall consists of the endothelium and two layers of muscles, an inner circular muscle layer and an outer longitudinal layer. At the bulged end of the V-shaped gut, the peritoneum of the gut continues to a hollow peritoneal cord, the funiculus (Fig. 10).

The funiculus runs across the metacoel and passes into the peritoneum of the cystid wall (Fig. 11). Statoblast formation begins at the insertion area of the border funiculus-

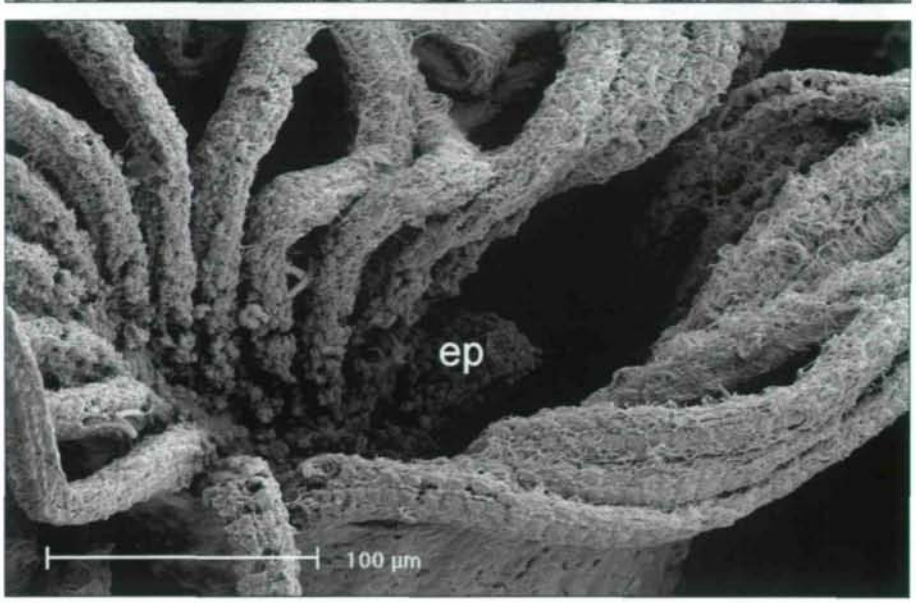


Fig. 8: *Plumatella fungosa* – horseshoe-shaped lophophore with tentacles; the mouth is located in the bend of the lophophore, overhung dorsally by the epistome (ep).

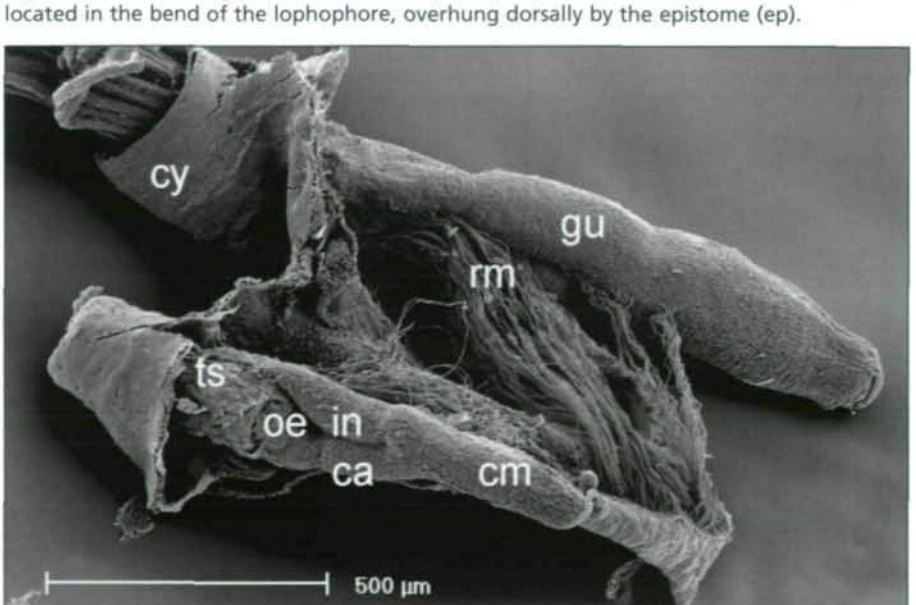


Fig. 9: *Plumatella fungosa* – two dissected zooids with opened cystids (cy). Gut (gu) and retractor muscles (rm), tentacle sheath (ts), oesophagus (oe) cardia (ca); caecum (cm), intestine (in).

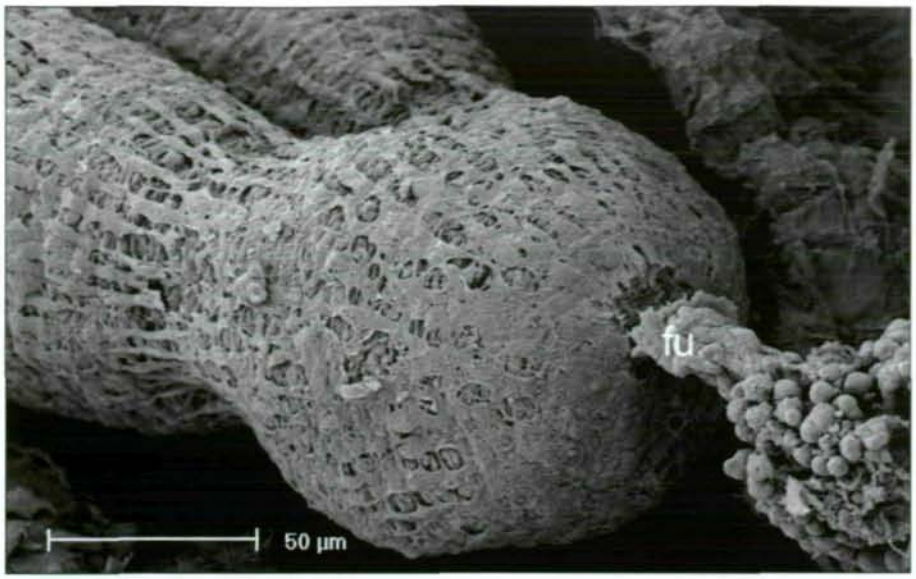


Fig. 10: *Plumatella fungosa* – tip of the V-shaped gut with funiculus (fu).

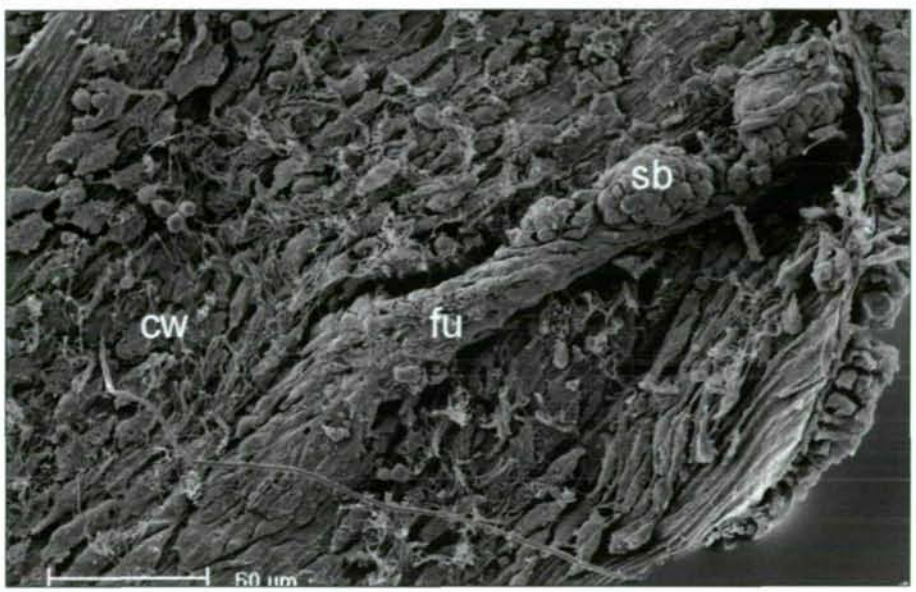


Fig. 11: *Plumatella fungosa* – funculus (fu) connected to the ventral inner side of the cystid wall (cw), with the first small buds of statoblasts (sb).

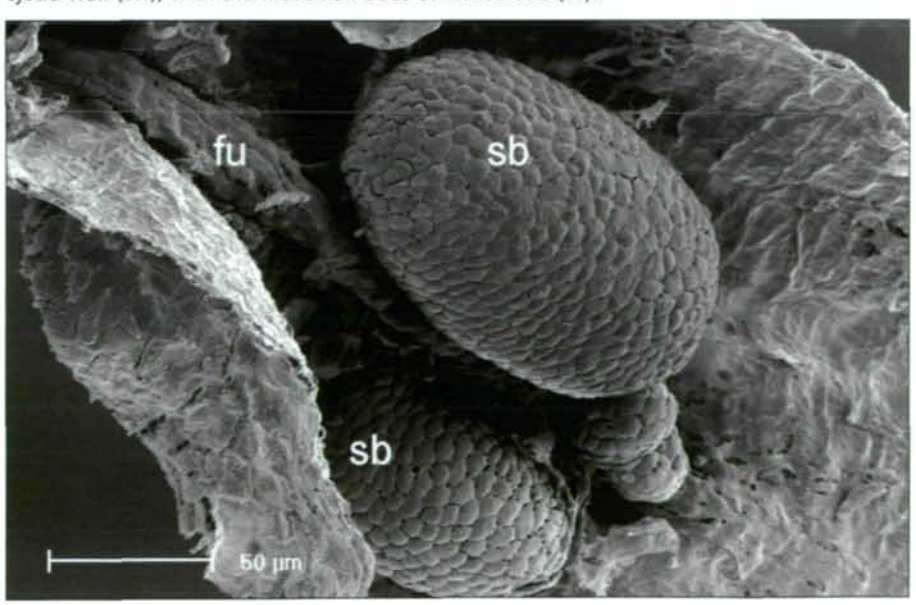


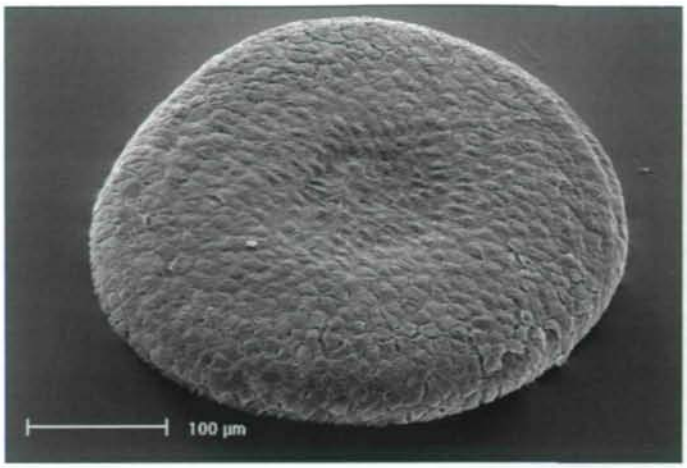
Fig. 12: *Plumatella fungosa* – growing statoblast (sb) bulging out of the funiculus (fu).

cystid wall (Fig. 11). During growth, statoblasts are transported toward the gut (Fig. 12) and, after having reached their final size, they detach from the funiculus and are free-floating in the coelomic fluid, moved by beating of the cilia of peritoneal cells. In this phase of development the statoblasts are still surrounded by the funculus epithelium and by the outer epidermal layer of the statoblast. Every statoblast floating in the coelomic fluid shows a different level of degeneration of the funiculus epithelium (Fig. 13). Statoblasts released by the zooid consist of two valves formed by the outer epidermal layer. Under favourable conditions, statoblasts germinate by opening of the valves at the equatorial suture (Fig. 14); the inner living ectodermal and mesodermal material then generates the primary zooid (Fig. 15).

The propagation of zooids via budding takes place at the ventral cystid wall and follows a strict pattern (JULLIEN 1885; BRAEM 1890; KRAEPLIN 1892; MARCUS 1925; CORI 1941). The main bud generated by the adult zooid is followed by an adventive bud situated between main bud and ovary (Fig. 16).

In temperate zones, sexual reproduction begins in late spring. In *P. casmiana*, the gonad formation is simultaneous (WÖSS 2002). The testis anlage originates at the upper part of the funiculus, close to the caecum; it then expands to cover the entire funiculus, even between the statoblasts (Fig. 17). Spermatogenesis is clearly visible along the different sections of the funiculus. Different zooids show the diverse stages, starting with a few large spermatogones, followed by spermatids and ripe long-tailed spermatozoa (Fig. 18). The ovary, a peritoneal sac of the cystid wall, is positioned between the budding zone and the orificium (Fig. 16, 19; see also d'Hondt this volume). The small ovary contains only a few eggs.

In ripe zooids, above the ovary, the cystid wall elongates as a second peritoneal sac, the so-called embryo sac (Fig. 16, 19, 20). Only one fertilized egg is stored in this em-



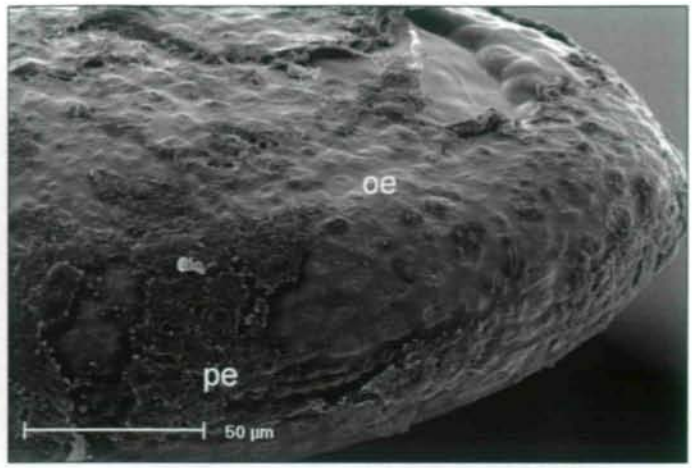


Fig. 13: Statoblasts of *Plumatella fungosa* (**a**) and *P. casmiana* (**b**) detatched from the funiculus and rotating within the fluid of the coelomic cavity, covered by the peritoneal layer (pe) of the funiculus and the outer epidermal layer (oe) of the statoblast.

bryo sac. It develops there into the so-called larva. After embryogenesis, the ciliated larva is released and swims with the aboral pole oriented forward (Fig. 21).

Future prospects

This paper is a preliminary study in terms of future work on classical questions in zooid anatomy and reproduction. Certain issues, e.g. in the field of sexual propagation, were already addressed by the earliest bryozoan workers but remain unsolved until today. The planned studies will focus on reproduction biology as well as on the anatomy of sensory organs.

To investigate details of gametogenesis, sperm release, fertilization and nidation of the egg, and embryogenesis, SEM will be used in combination with series of semithin sections and transmission electron microscopy. In asexual reproduction, the docu-

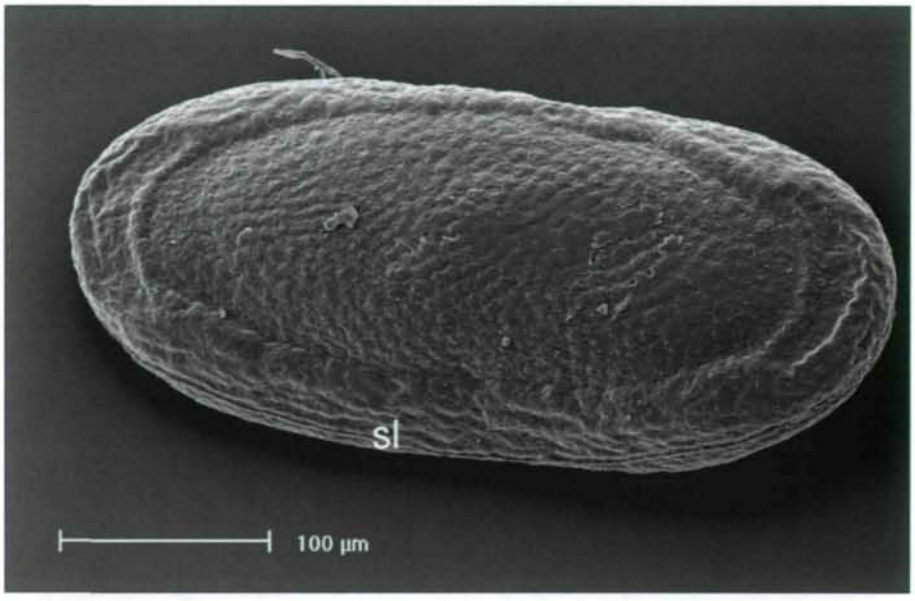


Fig. 14: *Plumatella casmiana* – mature statoblast, the two valves of the shell demarcated by the equatorial suture line (sl).

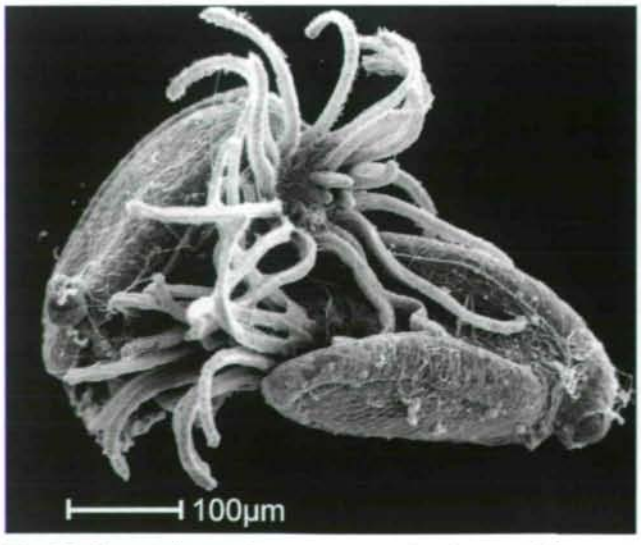


Fig. 15: *Plumatella casmiana* – two germinating statoblasts surrounded by their valves.

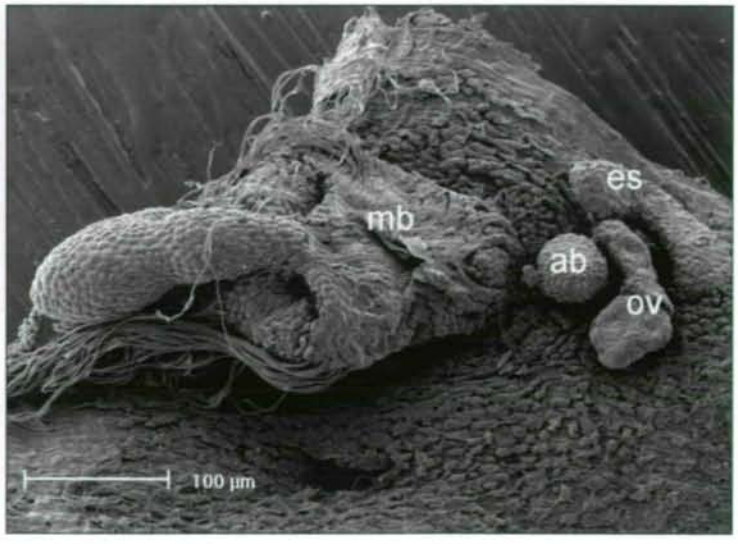


Fig. 16: *Plumatella fungosa* – inner side of ventral endocyst wall with main bud (mb), adventive bud (ab), ovary (ov) and embryo sac (es).

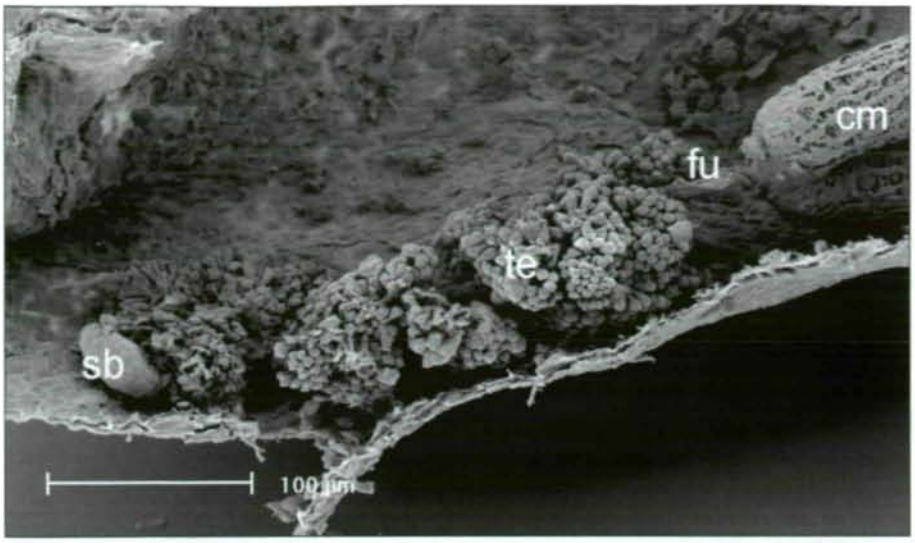


Fig. 17: *Plumatella fungosa* – opened endocyst with caecum (cm), funiculus (fu), testis (te) and statoblast (sb).

References

Banta W.C. (1973): The significance of areolae in cheilostome Bryozoa. — In: Larwood G.P. (Ed.): Living and Fossil Bryozoa. Acad. Press, New York: 209-219.

Bray D.F., Bagu J. & P. Koegler (1993): Comparison of hexamethyldisilazane (HMDS), Peldri II, and critical-point drying methods for scanning electron microscopy of biological specimens. — Microsc. Res. Techn. **26**: 489-495.

Braem F. (1890): Untersuchungen über die Bryozoen des süßen Wassers. — Biblioth. Zool. **2** (**6**): 1-134.

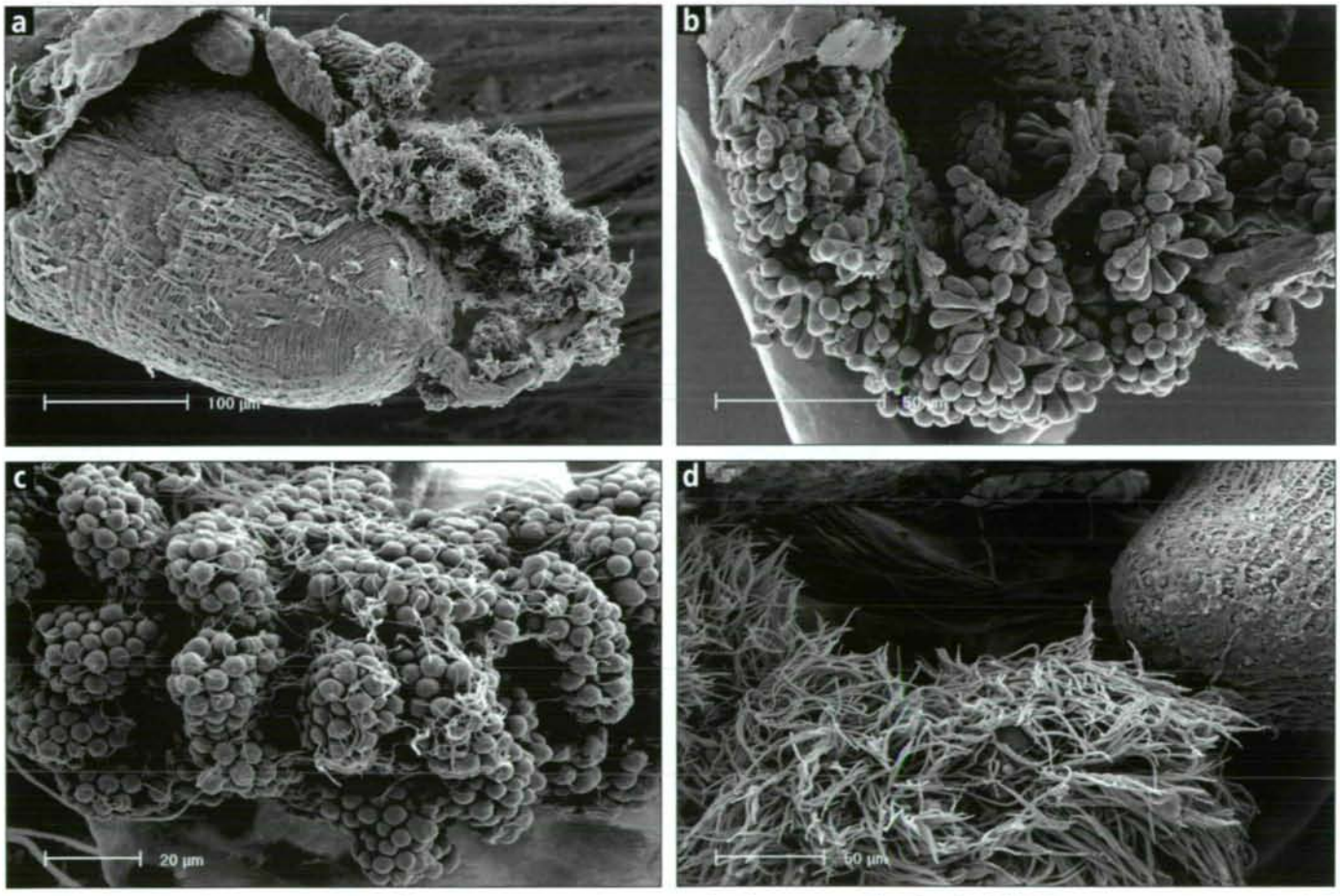


Fig. 18: *Plumatella fungosa* – overview of testis (**a**); spermatogonia (**b**); spermatids (**c**); ripe, long-tailed spermatozoa (**d**).

mentation will focus on the formation and release of the different kinds of statoblasts. Furthermore, studies will be initiated on the presence of sensory organs in the tentacles and the epistome.

Acknowledgments: Many thanks to Mag. Daniela Gruber, Cell Imaging and Ultrastructure Research Unit, University of Vienna, for scanning electron microscopy and digital photography assistance.

Cori C.J. (1941): Bryozoa. — In: Kükenthal W. & T. Krumbach (Eds.): Handbuch der Zoologie, Gustav Fischer, Stuttgart: 263-502.

Franzén Å. (1982): Ultrastructure of spermatids and spermatozoa in the fresh water bryozoan *Plumatella* (Bryozoa, Phylactolaemata). — J. Submicrosc. Cytol. **14** (2): 323-336.

Franzén Å. & T. Sensenbaugh (1983): Fine structure of the aoical plate in the larva of the freshwater bryozoan *Plumatella fungosa* (Pallas) (Bryozoa: Phylactolaemata). — Zoomorphology **102**: 87-98.

Fig. 19: *Plumatella fungosa* – tentacle sheath (ts) of polypide and endocyst (ec), turned inside out with main bud (mb), adventive bud (ab), ovary (ov) and embryo sac (es) and duplicature bands (db) between tentacle sheath and endocyst wall.

GEIMER G. & J.A. MASSARD (1986): Les bryozoaires du Grand-Duché de Luxembourg et des régions limitrophes. — Trav. sci. Mus. d'Hist. nat. Luxembourg **7**: 1-187.

GEIMER G. & J.A. MASSARD (1987): Note sur les caractères distinctifs de *Plumatella repens* (LINNÉ, 1758) et de *Plumatella fungosa* (PALLAS 1786) (Bryozoa, Phyalactolaemata). — Arch. l'Inst. Grand-Ducal Luxembourg, sect. Sci. nat., phys. math. (N.S.) **60**: 41-46

GOETHALS B., VOSS-FOUCART M.F. & G. GOFFINET (1984): Composition chimique et ultrastructure de l'ectocyste et de la coque des statoblastes de *Plumatella repens* et *Plumatella fungosa* (Bryozoaires Phylactolèmes). — Annales Sci. Nat., Zool., Paris 13e Série **6**: 197-206.

HAYWARD P.J. & J.S. RYLAND (1979): British ascophoran bryozoans. — In: KERMACK D.M. & R.S.K. BARNES (Eds.): Synopsis of the British Fauna (New Series) **14**: 1-312.

HOROWITZ A.S. & J.F. PACHUT (2000): The fossil record of bryozoan species diversity. — In: HERRERA CUBILLA A. & J.B.C. JACKSON (Eds.): Proc. 11th Intern. Bryozool. Assoc. Conf. Smithsonian Tropical Research Inst., Balboa, Republic of Panama: 245-248.

HYMAN L.H. (1959): The Invertebrates: Volume 5: Smaller Coelomate Groups. — McGraw-Hill Book Comp., New York, London, Toronto: 1-783.

JULLIEN J. (1885): Monographie des Bryozoaires d'eau douces. — Bull. Soc. Zool. France, Meulan **10**: 91-207.

KRAEPLIN K. (1887): Die Deutschen Süßwasserbryozoen. I. Anatomisch-systematischer Teil. — Abh. Naturw. Ver. Hamburg **10**: 1-168.

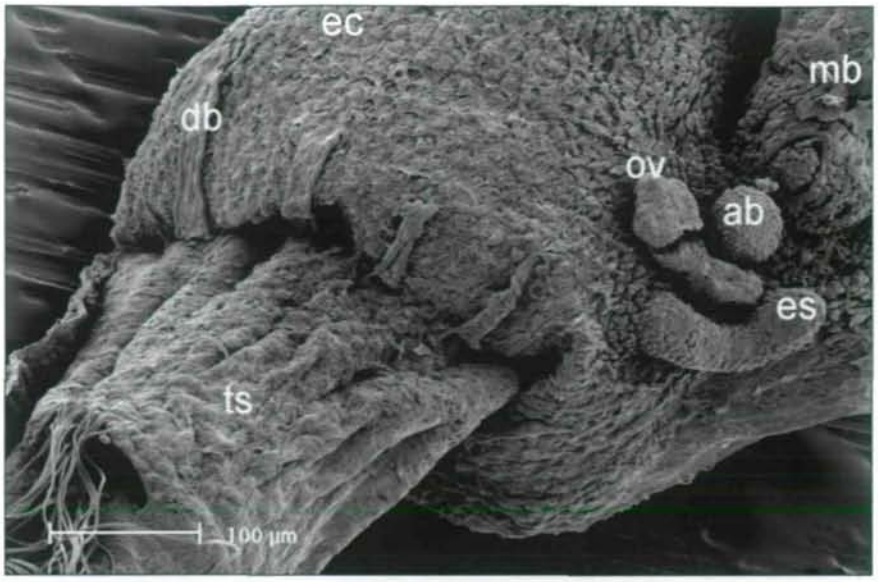
KRAEPLIN K. (1892): Die Deutschen Süßwasserbryozoen. II. Entwicklungsgeschichtlicher Teil. — Abh. Naturw. Ver. Hamburg **12**: 1-67.

LACOURT A.W. (1968): A monograph of the freshwater Bryozoa – Phylactolaemata. — Zool. Verh., Leiden **93**: 1-159.

LILLIE R.D. (1954): Histopathologic Technic and Practical Histochemistry. — The Blakiston Company, New York, Toronto: 1-501.

MARCUS E. (1925): Bryozoa. — In: SCHULZE P. (Ed.): Biologie der Tiere Deutschlands, Lieferung 14, Berlin: 1-46.

MUKAI H. (1982): Development of freshwater bryozoans (Phylactolaemata). — In: HARRISON F.W. & R.R. COWDEN (Eds.): Developmental Biology of Freshwater Invertebrates. Alan R. Liss Inc., New York: 535-576.



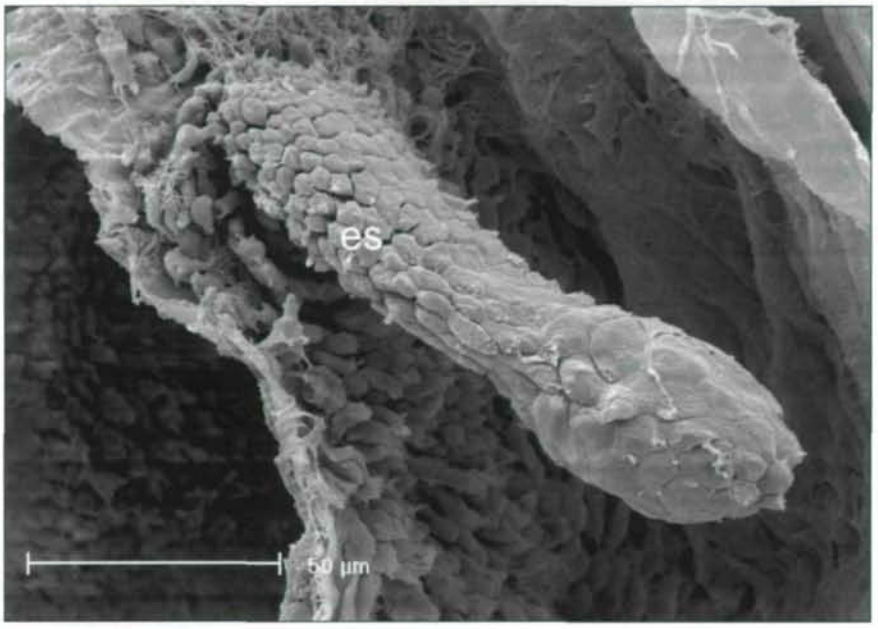


Fig. 20: *Plumatella fungosa* – embryo sac (es).

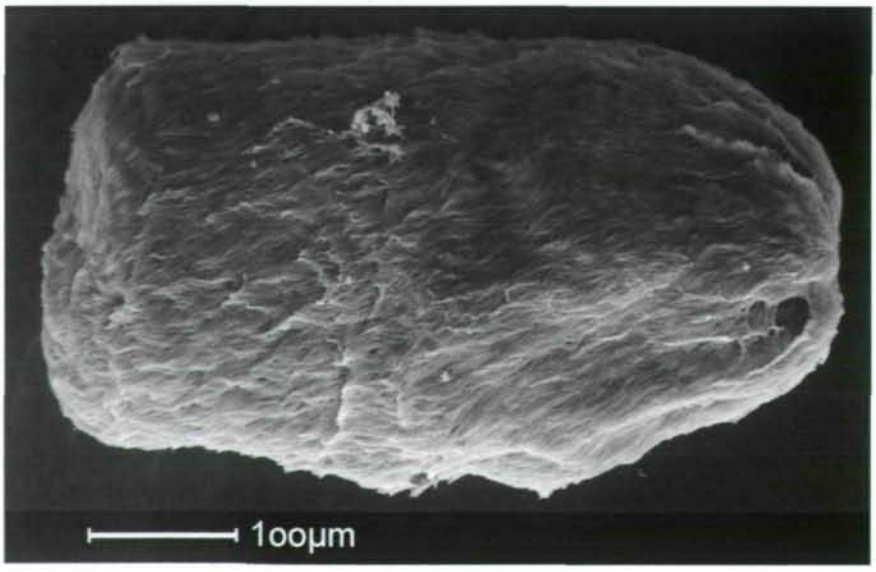


Fig. 21: *Plumatella fungosa* – ciliated larva.

MUKAI H., TERAKADO K. & C.G. REED (1997): Bryozoa. — In: HARRISON F.W. & R.M. WOOLLACOTT (Eds.): Microscopic Anatomy of Invertebrates **13**. Wiley-Liss, New York: 45–206.

MUKAI H. (1999): Comparative morphological studies on the statoblasts of lower phylactolaemate bryozoans, with discussion on the systematics of Phylactolaemata. — Science Rep. Fac. Educ., Hunma Univ. **46**: 51-91.

MULLER L.L. & T.J. JACKS (1975): Rapid chemical dehydration of samples for electron microscopic examinations. — J. Histochem. Cytochem. **23**(2): 107-110.

MUNDY S.P. (1980): Stereoscan stdies of Phylactolaemata bryozoan statoblasts including a key to the statoblasts of the British and European Phylactolaemata. — J. Zool. **912**: 511-530.

NATION J.L. (1983): A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. — Stain Tech. **58**: 347-351.

ODA S. & H. MUKAI (1985): Fine surface structure of the statoblasts of higher phylactolaemate bryozoans. — In: NIELSEN C. & G.P. LARWOOD (Eds.): Bryozoa: Ordovician to Recent. Olsen & Olsen, Fredensborg, Denmark: 234-244.

POURCHER A.-M. & J.-L. D'HONDT (1987): Étude ultrastructurale du sessoblaste et du flottoblaste chez *Plumatella fungosa* (PALLAS, 1768) (Bryozoaires, Phylactolaemates). — Annal. Sci. Nat., Zool., Paris 13e Série **8**: 209-216.

RAO K.S. & J.H. BUSHNELL (1979) : New Structures in Binding Designs of Freshwater Ectoprocta Dormant Bodies (Statoblasts). — Acta Zool. (Stockh.) **60**: 123-127.

RYLAND J.S. (1970): Bryozoans. — Hutchinson Univ. Library, London: 1-175.

RYLAND J.S. & P.J. HAYWARD (1977): British anascan bryozoans. — In: KERMACK D.M. (Ed.): Synopsis of the British Fauna (New Series) **10**: 1-187.

SANDBERG P.A. (1977): Ultrastructure, mineraloga, and development of bryozoans skeletons. — In: WOLLACOTT R.M. & R.L. ZIMMER (Eds.): Biology of Bryozoans. Acad. Press, New York, San Francisco, London 143-181.

TATICCHI M.I. & G. PIERONI (2005): Freshwater bryozoa of Italy. A survey of some species from the Italian bryozoan collection of A. Viganó with new records. — In MOYANO G.H.I., CANCINO J.M. & P.N. WYSE JACKSON (Eds.): Bryozoan Studies 2004. Proc. 13th Intern. Bryozool. Assoc. A.A. Balkema Publ., Leiden, London, New York, Philadelphia, Singapore: 317-327.

TAYLOR P.D. (1990): Preservation of soft-bodied and other organisms by bioimmuration - a review. — Palaeontology **33**: 19-35.

TAYLOR P.D. (2005): Bryozoans. — In: SELLEY R.C., COOKS L.R.M. & I.R. PLIMER (Eds.): Encyclopaedia of Geology **2**, Elsevier, Amsterdam: 310-320.

TODD J.A. (2000): The central role of ctenostomes in bryozoan phylogeny. — In: HERRERA CUBILLA A. & J.B.C. JACKSON (Eds.): Proc. 11th Intern. Bryozool. Assoc. Conf. Smithsonian Tropical Research Institute, Panama: 104-135.

TODD J.A. (1994): The role of bioimmuration in the exceptional preservation of fossil ctenostomates, including a new Jurassic species of *Buskia*. — In: HAYWARD P.J., RYLAND J.F. & P.D. TAYLOR (Eds.): Biology and Paleobiology of Bryozoans. Olsen & Olsen, Fredensbörg, Denmark: 187-192.

VOIGT E. (1966): Die Erhaltung vergänglicher Organismen durch Abformung infolge Inkrustation durch sessile Tiere. — Neues Jahrb. Geol. Palaeontol., Abh. **125**: 401-422.

WIEBACH F. (1974): Specific structures of sessoblasts (Bryozoa: Phylactolaemata). — Docum. Lab. Geol. Fac. Sci. Lyon H.S. **3** (fasc. 1): 149-154.

WOOLLACOTT R.M. & R.L. ZIMMER (1977): Biology of Bryozoans. — Acad. Press, New York, San Francisco London: 1-566.

WOOD T.S. (1989): Ectoproct bryozoans of Ohio. — Bull. Ohio Biol. Survey New Series (**2**): 1-70.

WOOD T.S. (2001): Bryozoans. — In: THORP J. & A. CORVICH (Eds.): Ecology and Classification of North American Freshwater Invertebrates, 2nd ed. Acad. Press, New York: 505-525.

WOOD T.S. & L.J. WOOD (2000): Statoblast morphology in historical specimens of phylactolaemate bryozoans. — In: HERRERA CUBILLA A. & J.B.C. JACKSON (Eds.): Proc. 11th Intern. Bryozool. Assoc. Conf. Smithsonian Tropical Research Institute, Panama: 421-430.

WÖSS E.R. (2002): The reproductive cycle of *Plumatella casmiana* (Phylactolaemata: Plumatellidae). — In: BUTLER C., SPENCER JONES M. & P. WYSE JACKSON (Eds.): Bryozoan Studies 2001. Proc. 12th Intern. Bryozool. Assoc. A.A. Balkema Publ., Rotterdam: 347-352.

Addresses of authors:

a.o. Univ.-Prof. Mag.
Dr. Manfred G. WALZL
Department of Theoretical Biology
University of Vienna
Althanstraße 14
A-1090 Vienna, Austria
E-Mail: manfred.walzl@univie.ac.at

Mag. Dr. Emmy R. WÖSS
(corresponding author)
Department of Freshwater Ecology
University of Vienna
Althanstraße 14
A-1090 Vienna, Austria
E-Mail: emmy.woess@univie.ac.at