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3D-microanatomy and histology of the hydrothermal vent gastropod *Lurifax vitreus* Warén & Bouchet, 2001 (Heterobranchia: Orbitestellidae) and comparisons with Ectobranchia

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Abstract Up to now, the internal anatomy of representatives of only two of three recent genera of the Orbitestellidae have been described. Herein, a species of the third genus, *Lurifax vitreus* from hydrothermal vent sites of the North Atlantic, is examined on the basis of semi-thin sections. Data on three-dimensional (3D)-anatomy and histology are provided in order to increase knowledge of the family. In addition, the original serial sections of *Orbitestella wareni* and *Microdiscula* cf. *subcanaliculata* of the original paper by Ponder (Journal of Molluscan Studies 56:515–532, 1990a) were reinvestigated and compared with *Lurifax vitreus*. Although *Lurifax vitreus* is significantly larger than the two former genera, it is again devoid of a gill but shows opponent ciliary stripes and a pallial tentacle. Most orbitestellid characters reflect plesiomorphic conditions among the Heterobranchia; autapomorphies of the family include an orthostrophic protoconch, massive jaws, a diagnostic radula type, paired posterior pedal glands with a common opening, and a pedally innervated copulatory organ. In agreement with actual molecular trees, our data suggest a placement of Orbitestellidae as basic Heterobranchia outside of Ectobranchia (Valvatoidea).

Keywords Heterobranchia · Orbitestellidae · Lurifax · 3D-reconstruction

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Introduction

Among basal heterobranch families, the overall marine Orbitestellidae Iredale, 1917 (= Microdisculidae Iredale & McMichael, 1962) are still controversial (for an overview of history and actual genera of these "allogastropod" or "heterostroph" clades see Hawe et al. 2013a). Iredale (1917) introduced Orbitestella and the Orbitestellidae (type species: Cyclostrema bastowi Gatliff, 1906) and characterised the group.

Orbitestellidae currently comprises four recent genera: (1) *Orbitestella* Iredale, 1917, species of which occur mainly in shallow water under rocks (e.g. Ponder 1990a; Bosch et al. 1995); (2) *Microdiscula* Thiele, 1912 species in shallow water habitats of Australia and Antarctic deep water; (3) *Boschitestella* Moolenbeek, 1994, the shell morphology (soft parts unknown) of the latter resembles that of *Orbitestella* in such a detailed way that the justification of a separate genus appears at least doubtful (Warén and Bouchet 2001). (4) Warén and Bouchet (2001) introduced *Lurifax* monotypically by *Lurifax vitreus*. Later on, *Lurifax japonicus* Sasaki & Okutani, 2005 from the North-West Pacific was described. Another fossil species, *Lurifax goederti* Kiel, 2006, was found in Tertiary cold-seep carbonates (Kiel 2006). (5) The genus *Xylodiscula* Marshall, 1988 was originally classified among Orbitestellidae. However, more recent studies (Warén 1992; Warén and Bouchet 2001; Høisaeter and Johannessen 2002, also personal observations) placed the taxon in a family proper (Xylodisculidae) within the Ectobranchia Fischer,

1884 (= Valvatoidea Hannibal, 1912: We prefer Ectobranchia because of (1) priority; (2) rankless Ectobranchia appears more appropriate than Valvatoidea implying superfamily rank; and (3) Ectobranchia cannot be confused with Valvatida resp. Valvatacea Blake, 1987 (Echinodermata: Asteroidea).

Australian representatives of the genera *Orbitestella* and *Microdiscula* were investigated in detail by Ponder (1990a). Simone and Zelaya (2004) added anatomical data on another species from the Beagle Channel off Argentina, *Orbitestella patagonia* Simone & Zelaya, 2004. As outlined by Ponder (1967, 1990a) and Simone and Zelaya (2004) Orbitestellidae was placed previously in various groups (e.g. Trochoidea, Rissooidea). Based on his thorough investigations, Ponder (1990a, b, 1991) put this group among the "allogastropod" (i.e. basic) Heterobranchia within the Valvatoidea (= Ectobranchia). This classification has been accepted by various authors (e.g. Healy 1991, 1993; Bieler et al. 1998; Ponder and de Keyser 1998) on the basis of morphological characters. However, more recent molecular data shed doubt on the placement within the Ectobranchia and showed Orbitestellidae as a separate clade among basal Heterobranchia unrelated to Ectobranchia (e.g. Dinapoli and Klussmann-Kolb 2010; Jörger et al. 2010; Dinapoli et al. 2011; Göbbeler and Klussmann-Kolb 2011; Schrödl et al. 2011; Brenzinger et al. 2013a, b).

Lurifax vitreus Warén et Bouchet, 2001 (page 207, figs. 37c, 44e-g, 46c,d, 47a,b) is a deep water species found in both hydrothermal vent and cold seep communities of the Atlantic and Mediterranean Sea (Warén and Bouchet 2001; Smriglio and Mariottini 2002; Roy et al. 2004; Kiel 2006; Lartaud et al. 2011; Cuvelier et al. 2011; Giuste and Sbrana 2012). Warén and Bouchet (2001) noted that the shell is unusually tall-spired and up to three times larger than those of typical orbitestellids. The smooth protoconch is multispiral and orthostrophic (Warén & Bouchet, 2001: fig. 37c, d). In general, shell features resemble that of *Leptogyra* Bush, 1897 (Neomphalida; cf. Heß et al. 2008) or *Cyclostremiscus* Pilsbry & Olsson, 1945 (Caenogastropoda: Vitrinellidae)—this deception was eponymous for this species the Swedish term "Lurifax" equals "smart aleck"). The classification of *Lurifax* among the Orbitestellidae has, up to now, been based solely on the distinct radula depicted by SEM by Warén and Bouchet (2001: figs. 47a, b) and has been questioned by Kiel (2006) because of the orthostrophic protoconch.

Accordingly, we analysed the anatomy and histology of *Lurifax vitreus* to clear up its family assignment and to obtain a broader database of this family. Our anatomical data on *Lurifax* demonstrate its orbitestellid nature and thus provide a better insight into the precise relationships and character evolution of the Orbitestellidae and other basal heterobranch with special reference to the Ectobranchia. Eventually, these data sets should be merged with molecular data towards an integrative approach to solving the relationships of lower heterobranch families.

Materials and methods

Materials

The specimens examined were from the Swedish Museum of Natural History, Stockholm and were kindly provided by Dr. Anders Warén (SMNH - 43242). They were found at the Lucky Strike vent field (North Atlantic, Mid-Atlantic ridge (37º17'N, 032º17'W) at a depth of 1,620–1,720 m between 3 and 9 July 1998. The final section series will be again deposited in the SMNH.

Additionally, we borrowed the original sections made by Ponder (1990a) of *Orbitestella wareni* (Reg. No.: C.478469. 001), *Microdiscula charopa* (Reg. No.: C.158250.001), and *Microdiscula* cf. *subcanaliculata* (Reg. No.: C.158134.001) from the Australian Museum Sydney for reinvestigation.

Methods

We applied the now well-established method of computer-aided virtual 3D-reconstruction based on semi-thin sections of the soft body (see also Haszprunar et al. 2011; Hawe et al. 2013a). Images and analyses of hard structures were already provided by Warén and Bouchet (2001), thus we restricted our study to soft body analysis.

Mechanical operations

We followed Ruthensteiner (2008) to obtain serial semi-thin sections. Originally collected for DNA sequencing purposes, the sample was initially preserved in 80 % alcohol. After a descending alcohol series, two specimens were transferred to 1 % ascorbic acid solution overnight for decalcification. The sections were then dehydrated in an ascending acetone series and subsequently embedded in pure Epon A [Glycidether 100 and DDSA (dodecenylsuccinic anhydrid) in the proportions 31:50] and left in a 60 °C incubator overnight to polymerise.

Sections (1.5 μm thick) of the embedded specimens were cut with an MT-7000 ultra microtome. Sections were stained with methylene blue after Richardson et al. (1960). After drying and embedding in the same plastic mixture, sections were photographed with bright field illumination on an Olympus CX 41 and camera attachment (Olympus DP 25) using cellD (5.1), and stored as .tiff format. For detailed information about the mechanical operations see the treatment of species #2 and #3 in Hawe et al. (2013a).

Digital procedure

The resulting image stacks were pre-processed in Photoshop CS4, where images were reduced in size (pixels) and cleaned to improve the quality of volume rendering. The machined stack

data were read into the 3D-reconstruction program Amira® 5.2.1 and aligned. As described earlier (e.g. Haszprunar et al. 2011; Hawe et al. 2013a) the individual organs were labelled and converted to 3D surface models. These models were finally embedded in the pdf-version after Ruthensteiner and Heß (2008) and Ruthensteiner et al. (2010). For Fig. 4E the Volume Rendering function of Amira® was used, illuminating each voxel by its own imaginary light source (Handschuh et al. 2010). This technique was also used to gain a first a priori overview of the already aligned serial sections.

Likewise Amira® was used to measure surfaces and distances on 3D models. A survey of histological sections was done using Photoshop CS4.

Results

Soft body morphology and histology

The folded and slightly retracted foot lacks epipodial tentacles, the front end is bilobate; the right lobe appears considerably larger. The snout is also bilobate; the left one is dorsally bend backwards.

Two well defined cephalic tentacles are situated laterally at the head (Fig. 1a). A prominent eye can be detected at the basis of each tentacle (Figs. 1a and 2b).

Two pedal glands are situated in the dorsal portion of the foot. The posterior gland is divided into two symmetrical parts found at both sides of the buccal apparatus (Fig. 2b, c). The proximally separated ducts (Fig. 3g) unite anteriorly of the glands, and a common opening is situated in the middle part of the foot (Fig. 1a). Furthermore, an anterior pedal gland with a dorsally positioned, ciliated duct exists. A large group of calcium cells are present below the anterior pedal gland.

Rhogocytes with many dark granules can be found within the haemocoel and connective tissues in the foot and throughout the whole animal (Fig. 3e).

A simple penis with an external and densely ciliated sperm groove (Fig. 4d, e) is situated on the right anterior epipodial side of the foot and continues along the pedal margin (Fig. 1a, c). In the contracted condition, the base of the penis lies in front of the right cephalic tentacle and the copulatory organ is innervated from the right pedal ganglion (Fig. 4d). A cerebropleurally innervated pallial tentacle is present at the right junction of mantle cavity roof and foot; it is in parts strongly ciliated.

Mantle cavity

The mantle cavity extends far into the first whorl. To the very left there is a striking, heavily blue-stained mantle gland with quite big cells followed by the simple, slightly elongated and inconspicuous osphradium, which is partly ciliated. Behind,

and to the right, a well-established pigmented mantle gland is visible and fills almost the whole dorso-median part of the mantle roof (Fig. 2a, c). The kidney is embedded in the pallial roof and lies posterior and to the left of the pigmented mantle gland (Figs. 1a, d and 2d: PMO). The covering, very thin mantle epithelium is partly ciliated. At the posterior right side of the pigmented mantle gland, the glandular gonoduct reaches into the mantle cavity. The anus is situated posterior in between the kidney and the gonoduct (Fig. 2d), gathering all openings in a small zone in the following order (posterior to anterior): anus–nephropore–gonoduct. A dorsal ciliated ridge (Figs. 2c, cf. 3h), actually a dorsal posterior continuation of the right sided pallial tentacle and the following posterior ciliated fold, leads from anterior right to the left posterior side of the pallial roof. The posterior ciliated fold extends from the posterior end of the pallial tentacle via the right pallial cavity edge to the opening of the gonoduct. In the center of the pallial cavity, a trapezoidal groove is located between the pigmented mantle gland and the dorsal ciliated ridge. This partly ciliated groove leads from anterior right to the posterior left side (Fig. 2b). At the right anterior end of the mantle cavity, there is again a third pale-blue-stained pallial gland with smaller cells. A ventral ciliated band is also present (Fig. 2b, c: cf). The whole posterior part of the mantle cavity is covered by a uniform glandular epithelium, the huge cells of which are densely filled with small granulae (Figs. 2d and 3a: ge), the nuclei are situated in the centre of the cell. In between, two different types of single mucous cells can be seen (stained dark blue and non-stained, respectively) at irregular intervals.

Heart, circulatory- and excretory-system

A folded kidney is placed at the posterior left side of the mantle roof, its outer epithelia appear very thin and well supplied with haemolymph sinuses (Figs. 2d and 3a: k).The efferent kidney sinus is located to the left and slightly posterior and leads into a monotocardian heart surrounded by a pericardium (Fig. 3a). From the pericard a short but prominent and ciliated reno-pericardioduct leads into the adjacent left kidney (Fig. 3a: pcd). A dorsally situated excretory pore can be found just in front of the anus.

Genital tract

The structure of the hermaphroditic genital system is very simple. The hermaphroditic gland is divided into the inner ovary part and the outer testis part, each with their own epithelium (Fig. 4b: arrowheads; Fig. 5). Both mature sperm (Fig. 4b) and mature eggs (about 100 μm in diameter, Fig. 4b) with a high content of yolk can be found and all stages of gametogenesis are visible in each compartment. In each outer area immature, barely developed eggs/sperm are present, and gametogenesis takes place towards the inside. Both gonad
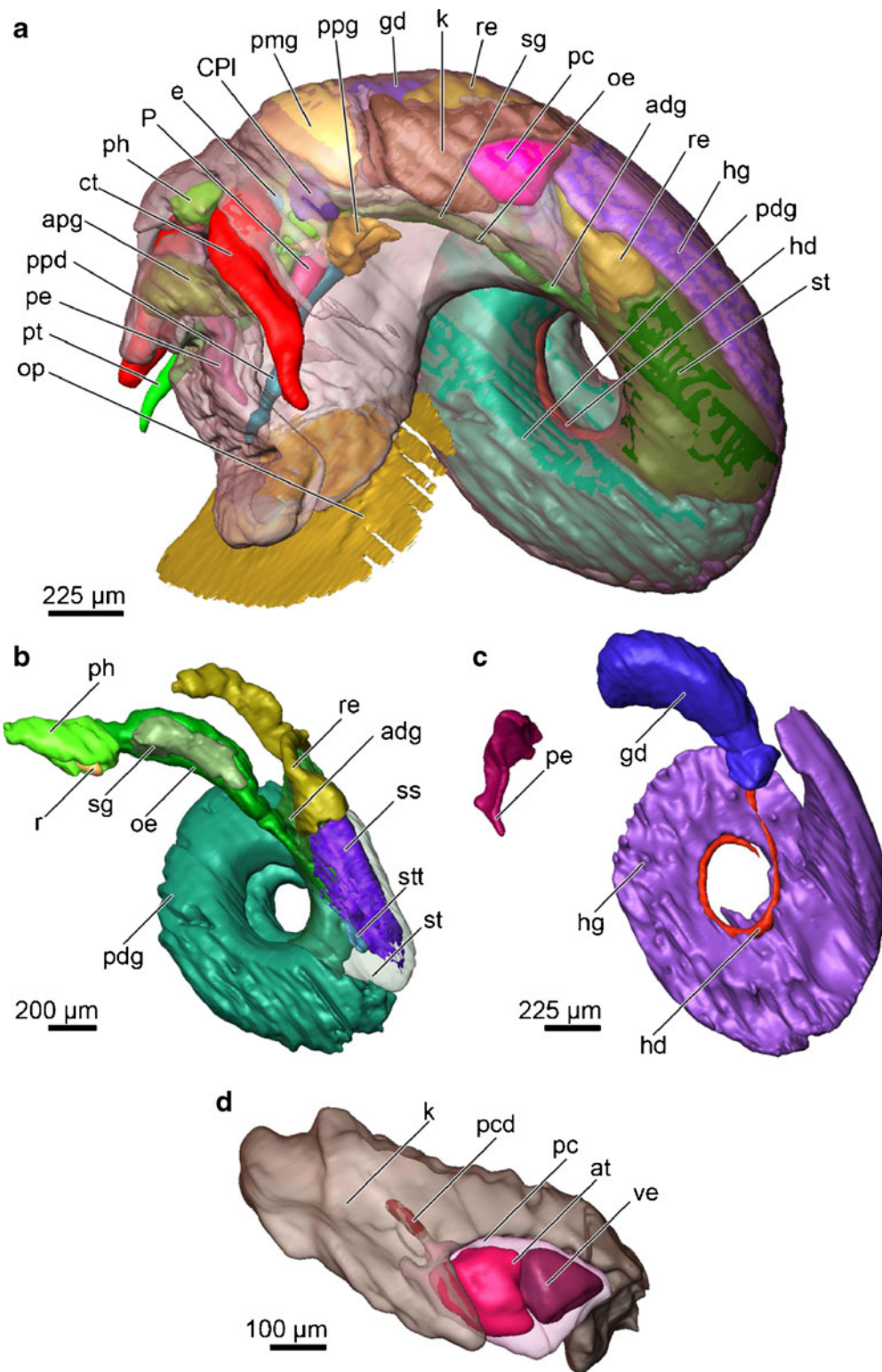
Fig. 1 a–**d** Interactive 3D-reconstructions of *Lurifax vitreus*. **a** All organs with transparent body surface. **b** Alimentary tract. **c** Hermaphroditic genital system. **d** Kidney, pericard and heart. *adg* Anterior digestive gland, *apg* anterior pedal gland, *at* atrium, *CPl* cerebro-pleural ganglia, *ct* cephalic tentacles, *e* eyes, *gd* gonoduct, *hd* hermaphroditic duct, *hg* hermaphroditic gland, *k* kidney, *oe* oesophagus, *op* operculum, *P* pedal ganglia, *pc* pericard, *pcd* pericardioduct, *pdg* posterior digestive gland, *pe* penis, *ph* pharynx, *pmg* pigmented mantle gland, *ppd* posterior pedal gland duct, *ppg* posterior pedal gland, *pt* pallial tentacle, *r* radula, *re* rectum, *sg* salivary glands, *ss* stomach shield, *st* stomach, *stt* stomach tooth, *ve* ventricle. An interactive version of this figure is attached in the Supplementary Material

Fig. 2 a–d Mantle cavity development to posterior. **a** 3D-reconstruction of mantle cavity and surrounding organs. **b** Anterior part of mantle cavity. **c** middle part of mantle cavity. **d** posterior part of mantle cavity. *at* atrium. *cf* Ventral ciliated fold, *cr* dorsal ciliated ridge, *e* eye, *gd* gonoduct (for separation of the different gland epithelia see Fig. 4), *ge* granular epithelium, *k* kidney, *mc* mantle cavity, *oe* oesophagus, *pc* pericard, *ph* pharynx, *pmg* pigmented mantle gland, *ppg* posterior pedal gland, *r* radula, *re* rectum, *sg* salivary glands, *st* stomach, *ve* ventricle, *vn* visceral nerve
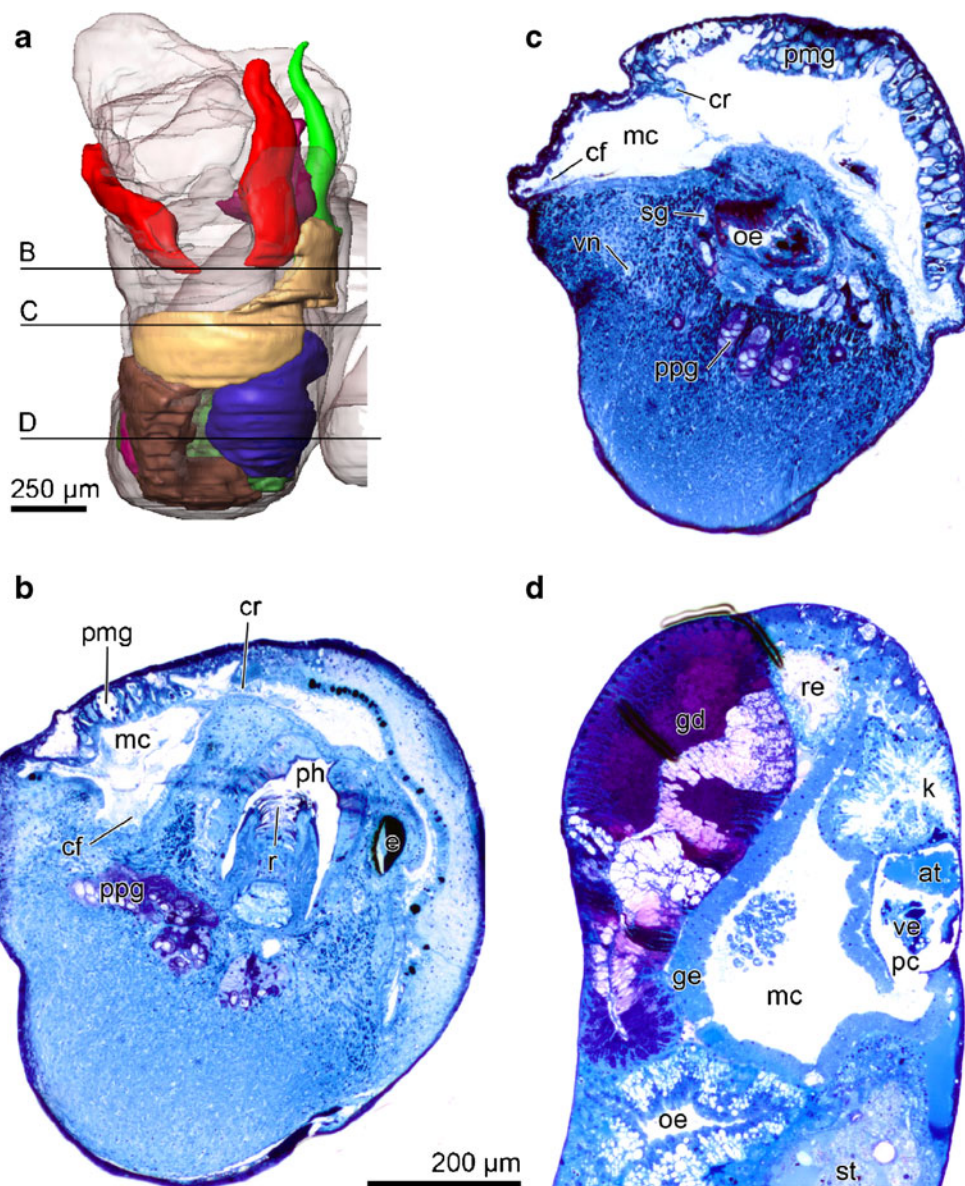
compartments fuse to a hybrid transition zone leading forwards at the inner first whorl. This tube extends forwards and expands to form the glandular gonoduct (Figs. 2d and 4c; 5). Here four different glandular epithelia can be differentiated according to staining and structure properties (Fig. 4c: I–IV; Fig. 5). A receptaculum seminis or other bags/pouches are not present.

The gonoduct leads further forwards until it reaches the right mantle cavity, where the gonopore is situated about 360 μm behind the penis at the line of the ventral ciliated fold (Fig. 2b, c).

Alimentary tract

The oral tube is lined with a thin cuticle. The following prominent jaws consist of multiple rows of tooth plates (Fig. 3b) and are provided with thick smooth muscles. The radula reaches deeply in the distal pharynx (Fig. 2b). Radula cartilages are lacking, instead a small muscular cushion is present. The paired salivary glands open into the pharynx on both sides at the level of the radula and extend along left and right side of the oesophagus with increasing volume in posterior direction (Fig. 3c, d).

The dorsal epithelium of the anterior oesophagus is highly glandular (stained violet with Richardson's reagent), whereas the ventral side is ciliated throughout (Fig. 3d). Pockets or food grooves are not present. The posterior oesophagus lacks glands or cilia. The large stomach (Fig. 3f) is coated on the entire inner surface with a thick cuticle forming a well-developed stomach tooth (about 170 μm in length) at the entrance of the stomach. Within the stomach, a mass of detritus can be seen. Two openings, which are separated by

Fig. 3 a–h Histological images of *L. vitreus*. **a** Heart and kidney. **b** Jaw. **c** Pharyngeal buccal complex. **d** Oesophageal complex. **e** Rhogocyte und surrounding calcium cells. **f** Stomach. **g** Right statocyst with several statocones, posterior pedal gland ducts and left pedal ganglion. **h** Dorsal mantle cavity with ciliated ridge (dorsal to the right). *at* Atrium, *Ca* calcium cells, *cr* dorsal ciliated ridge, *CPl/CPl'* right/left cerebropleural ganglia, *gd* gonoduct, *ge* granular epithelium, *j* jaw, *k* kidney, *mc* mantle cavity, *oe* oesophagus, *P* pedal ganglion, *pc* pericard, *pcd* pericardioduct, *ph* pharynx, *ppd* posterior pedal gland duct, *ppg* posterior pedal gland, *rh* rhogocyte, *sg* salivary glands, *sgd* salivary gland duct, *st* stomach, *sta* statocyst, *ste* stomach epithelia, *stt* stomach tooth. *Bars* **a**, **c**, **d** 100 μm; **b**, **f–h** 50 μm, **e** 20 μm
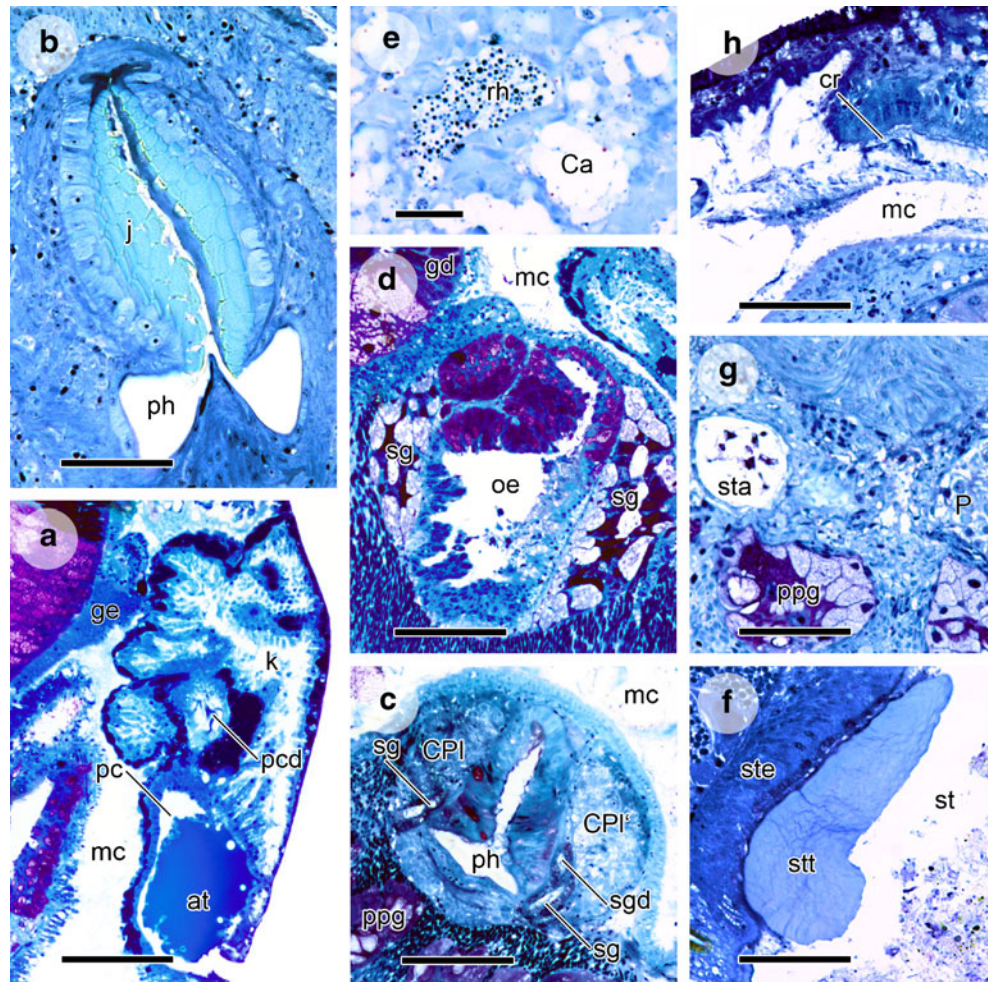
Fig. 4 a–e Histological images of the genital system of *L. vitreus*. **a** 3D-reconstruction of the genital system, view from left. **b** Hermaphroditic gland with border of testis and ovary compartments (*arrowheads*). **c** Gonoduct with four differently stained glandular epithelia. **d** Base of penis with sperm groove. **e** Penis depicted in volume rendering mode (see Materials and methods) showing the seminal groove (*dark red*). *I–IV* Different sorts of glandular epithelia, *eg* egg, *n* nucleus, *pe* penis, *pt* pallial tentacle, *sgr* sperm groove, *sp* sperm, ▶ border of ovary and testis compartments. *Bars* **b**, **d** 50 μm; **c** 100 μm
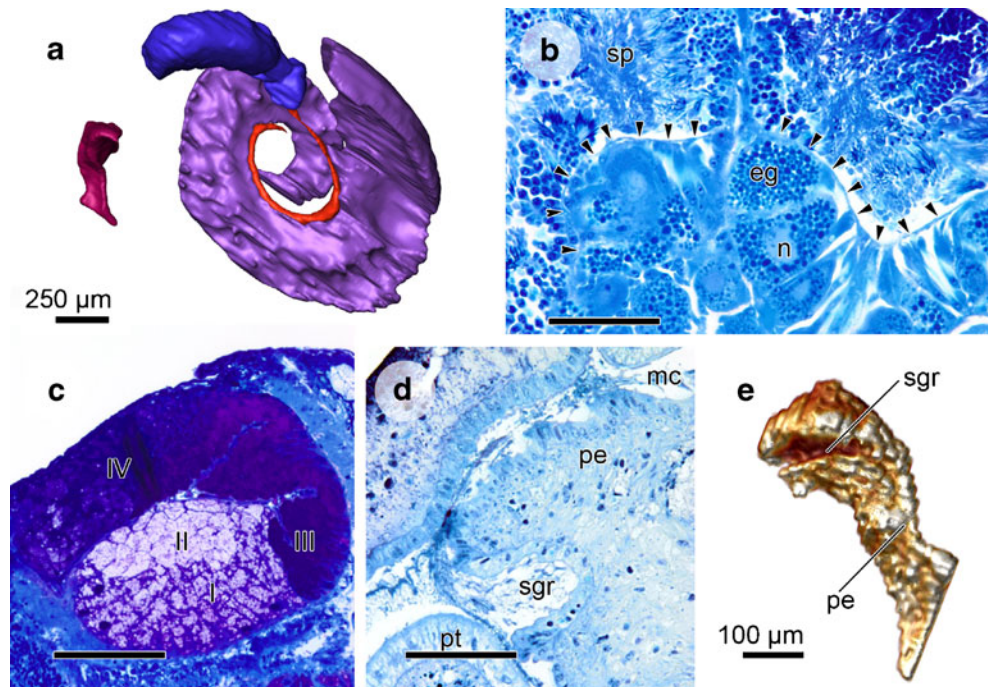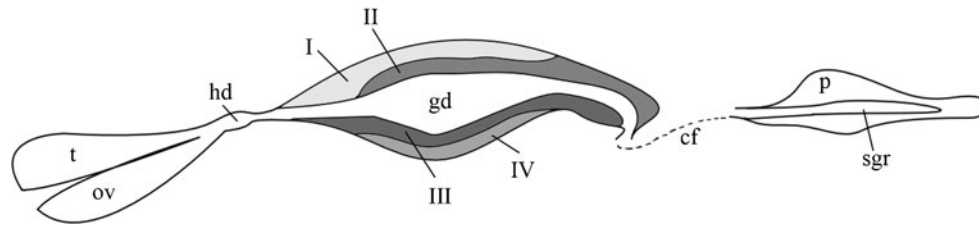
Fig. 5 Schematic representation of the genital system of *L. vitreus*. *I–IV* Different sorts of glandular epithelia, *cf* ciliated fold, *g* gonoduct, *hd* hermaphroditic duct, *ov* ovary part of hermaphroditic gland, *p* penis, *sgr* seminal groove, *t* testis part of hermaphroditic gland. Based on Fig. 9C in Ponder (1990a)

the opening of the oesophagus lead into the digestive glands—an anterior and a posterior gland. The voluminous posterior digestive gland fills together with the hermaphroditic gland the complete space of the rear coils. Next to the opening in the intestine, a small ciliated pouch can be seen, segregating an amorphous substance into the stomach, a true crystalline style could not be detected, however. The intestine is very short, non-ciliated and lacks any typhlosole. The following, very short rectum is in parts ciliated and opens just behind the PMO into the posterior region of the mantle cavity between the kidney and the oviduct (Fig. 2d).

Nervous system

The whole nervous system is highly concentrated. The cerebral ganglia are fused with the pleural ganglia on both sides and are located just behind the massive jaw apparatus. The cerebropleural ganglia are on level with the small buccal ganglia, being situated ventrally of the emerging oesophagus. The cephalic tentacle nerves are bifurcated shortly behind their offspring of the cerebral ganglia. The cerebro-pedal and pleuro-pedal connectives are very long. The pedal ganglia are located between the already separated ducts of the posterior pedal glands (on the ventral side) and the anterior pedal gland (on the dorsal side). In addition, from the right pedal ganglion a single penis nerve emerges (Fig. 4d) like the regular pedal nerves (anterior and posterior) supplying the foot.

The supraoesophageal ganglion is situated on the left side adjacent to the left cerebro-pleural ganglion. It is interconnected via a prominent connective running above the oesophagus with the right pleural ganglion, whereas a left zygoneury (a connection to the left pleural ganglion often occurring in caenogastropods) is not present. The suboesophageal ganglion is located immediately behind the right buccal ganglion—like the posterior part of the visceral loop and the visceral ganglion, the connective to the left pleural ganglion could not be detected. The elongated osphradial ganglion is situated beneath the osphradium on the left side of the mantle cavity roof; however, the connection to the supraoesophageal ganglion could not be detected either.

Sensory organs

The two highly pigmented, black eyes are situated lateral and just in front of the cerebropleural ganglia slightly beneath the surface epithelia and on one height with the basis of the cephalic tentacles (Fig. 2b). The more or less ovoid lens (stained dark blue) fills nearly the whole eye, leaving a small gap between lens and retina.

Large statocysts (about 50 μm in diameter) with several statocones inside are situated adjacent anterior and slightly outside the pedal ganglia (Fig. 3g: St). The osphradium with a slightly elongated osphradial ganglion is located at the left side of the mantle cavity roof. The dorsal tip of the whole osphradial structure is ciliated, closing the dorsal ciliated fold.

Discussion

Despite the exceptional hot-vent habitat there are very few differences—except shell shape and size—between *Lurifax* and the already studied (Ponder 1990a; Simone and Zelaya 2004) representatives of *Orbitestella* and *Microdiscula*. The stated differences between the orbitestellid genera are also based on a re-examination of the original section series from Ponder´s (1990a) work. The special characteristics of the orbitestellid shell (see Introduction) were discussed extensively by Warén and Bouchet (2001), thus this feature will not be further treated. Finally, we will briefly discuss the phylogenetic position of the Orbitestellidae among the basal Heterobranchia with special reference to Ectobranchia, Architectonicoidea and Omalogyrinidae.

Character discussion

Shell

Except *Lurifax* all other orbistellids (about 25 species have been formally described) have small (about 1 mm), nearly planispiral shells, which are often (*Orbitestella*, *Boschitestella*) heavily sculptured. Similar shells are present in taxa of the unrelated Skeneidae, Tornidae, Skeneopsidae,

Omalogyridae, or Glacidorbidae (e.g. Lima et al. 2011). The clear inclusion of *Lurifax* with a more globular and poorly sculptured shell in Orbitestellidae based on radula and soft part characteristics shows that shell morphology alone may be highly misleading in such small gastropod groups.

The multispiral, smooth protoconch of *Lurifax* is composed of an embryonic (protoconch I) and a larval (protoconch II) shell, and thus differs from the paucispiral ones of the remaining orbitestellids. Its specific structure reflects ancestral planktotrophy (multispiral) and actual non-planktic mode of development (smooth) (e.g. Jablonski and Lutz 1983; Nützel et al. 2006; Robertson 2012). The significant size difference between the large protoconch and the relatively small (100 μm) eggs found in the hermaphroditic gland, can be explained by subsequent growth of the larval shell after hatching.

Orthostrophic protoconchs in basal marine Heterobranchia are uncommon, but do also occur in Rissoellidae (e.g. Rodriguez-Babio and Thiriot-Quievreux 1974; Ponder and Yoo 1977; Rodriguez-Babio 1982) and Tjaernoeidae (Rodriguez-Babio and Thiriot-Quievreux 1974; Warén 1991; Rodriguez-Babio and Rubio 1993). Another example of an orthostrophic protoconch is *Hyalogyra* (in direct comparison to the heterostrophic shell situation found in Hyalogyrinidae) (Haszprunar et al. 2011).

Soft body morphology and histology

Except in size, the general morphology of the soft body of *Lurifax vitreus* is close to the observations made by Ponder (1990a) and Simone and Zelaya (2004) on *Orbitestella* and *Microdiscula*. Calcium cells in the foot are also found in the ectobranch Xylodisculidae (personal observation) and in Hyalogyrinidae, where they form a compact mass (Haszprunar et al. 2011)—these are present in the re-investigated specimens as well. The unique paired posterior pedal glands and their common duct and opening are present in all orbitestellids studied (Ponder 1990a; Simone and Zelaya 2004; this study).

As mentioned in the original description of Warén and Bouchet (2001), *Lurifax vitreus* has the unusual habit to automize the foot. However, we couldn't find any special adoptions for this in the histological semi-thin sections.

Mantle cavity

The presence of a contractile gill, which can be extended out of the mantle cavity, is diagnostic for all Ectobranchia (also *Xylodiscula planata*: Høisaeter and Johannessen 2002; *X. analoga*, personal observation). Concerning Orbitestellidae, it has been argued that the small size of *Orbitestella* and *Microdiscula* species (all about 1 mm) may have caused

secondary loss (in parallel to Omalogyridae or Rissoellidae). However, there are ectobranchs of similar size (e.g. *Valvata cristata* O.F. Müller, 1774) showing the ectobranch gill, whereas the much larger *Lurifax vitreus* (about 3 mm) again lacks a ventilator/respiratory gill like other Orbitestellidae. Accordingly, the lack of gill in Orbitestellidae probably is a genuine character of the family.

A prominent "hypobranchial-like mantle organ" (Ponder 1990a) or "pigmented mantle gland" (Simone and Zelaya 2004) has been described in Orbitestellidae. Similar glands have been described within the basal Heterobranchia are called "hypobranchial gland" (e.g. Fretter 1948; Haszprunar 1985b, c; Bäumler et al. 2008). Due to identical position and principal similar structure (glandular), we agree with Ponder (1990a, 1991) to assume homology of these glands for basal Heterobranchia. In addition, the prominent posterior glandular/granular mantle tissue is typical for all orbitestellids studied, whereas both glands are lacking in all investigated Ectobranchia (e.g. Bernard 1890; Ponder 1990b; Haszprunar et al. 2011; Hawe et al. 2013a; and unpublished observations). TEM investigations of this epithelium provided evidence for endosymbiotic bacteria in bacteriocytes (Hawe et al. 2013b).

Haszprunar (1988) and Ponder & Lindberg (1997) agreed in considering two opponent ciliated ridges as a synapomorphy for all basal Heterobranchia except Ectobranchia, where a secondary, densely ciliated gill and a pallial tentacle generate a water current within the mantle cavity and to expel the faeces.

Ponder (1990a: 522) stated a lack of ciliary ridges in *Orbitestella* and *Microdiscula*, but described "…a groove between the pallial gonoduct and the renal organ, this epithelium is thin and ciliated" (see also Ponder 1990a: fig. 8A). Our re-examination of the original sections revealed that this structure corresponds with the dorsal ciliated ridge and the partly ciliated groove aside mentioned above in *Lurifax*. Also Simone and Zelaya (2004: 161) mentioned "short, partially ciliary lobe dorsally located". Like Ponder (1990a) we assume expelling faeces as the primary function of these ciliary bands. Accordingly, we assume the two opponent (dorsal and ventral) ciliated tracts of Orbitestellidae as homologous to those found in Omalogyridae, Architectonicidae, Mathildidae, Rissoellidae, Acteonidae, and many euthyneuran Heterobranchia (e.g. Fretter 1948; Fretter and Graham 1954; Robertson 1985; Haszprunar 1985b, c, 1988; Ponder 1990a; Ponder & Lindberg 1997; Bäumler et al. 2008). In all cases such ciliary stripes subdivide the mantle cavity longitudinally into a left inhalant and a right exhalant chamber. In contrast, Murchisonellidae also have pallial tentacles and lack ciliary stripes within the mantle cavity (Warén 2013; Brenzinger et al. 2013a, b).

The posterior granulate and glandular mantle epithelium with endosymbiotic bacteria can also be found in the re-

investigated specimens of *Orbitestella* described by Ponder (1990a) (Hawe et al. 2013b).

Alimentary tract

Apomorphies of Orbitestellidae include the thick cuticular jaws and well developed jaw muscles, a character set that is present in all species studied (Ponder 1990a; Simone and Zelaya 2004; this study). The distinct radula type is one of the main autapomorphies of the Orbitestellidae. The single pair of marginal teeth, and the overall orbitestellid radula type was indeed the main argument for classifying *Lurifax* within this family by Warén and Bouchet (2001). The absence of true radula cartilages and the replacement by a muscular mass is a synapomorphy for Heterobranchia as a whole (e.g. Haszprunar 1985a, c, 1988; Salvini-Plawén & Haszprunar 1987; Ponder 1991; Ponder & Lindberg 1997).

In *Orbitestella* and *Microdiscula*, Ponder (1990a: 524 above right) described: "A pair of small salivary glands (Fig. 7; sg) run alongside the anterior part of the oesophagus, but are overshadowed by the pair of posterior pedal glands which follow it almost to the stomachs".

Also Simone and Zelaya (2004) described small salivary gland in *Orbitestella patagonia*. In contrast, *Lurifax* shows very prominent distal parts of the salivary glands lying along the oesophagus, and the histology of the salivary cells differs significantly in both colour and structure from the pedal gland cells. After re-examination of the original section slides of Ponder´s work we can confirm his observations—there are large differences in the organization of the posterior pedal gland volumes as well the salivary gland volumes between *Lurifax* and the specimens investigated by Ponder (1990a) and Simone and Zelaya (2004). We therefore assume the

dominant salivary glands as an apomorphy for *Lurifax* within the Orbitestellidae.

All orbitestellids studied have a straight anterior oesophagus without pockets but equipped with a very prominent dorsal gland (Ponder 1990a; this study).

In contrast to *Microdiscula* and *Orbitestella* (Ponder 1990a; Simone and Zelaya 2004), we could not detect a crystalline style proper within the stomach of *Lurifax vitreus*, yet a ciliated (style?) pocket and a cuticular gastric shield with a prominent stomach tooth is present. It remains unclear whether the amorphous substance reflects the crystalline style, which may be degraded due to fixation, or is a genuine character or the species or genus. Also the ectobranch Hyalogyrinidae show a wide variability within one family concerning inner stomach structures (Haszprunar et al. 2011). Haszprunar (1988), Ponder & Lindberg (1997), and Strong (2003) all assumed the gastric shield and style sac as plesiomorphies for gastropods. Among the Heterobranchia, the presence of a gastric shield and style sac is restricted to Orbitestellidae (except Lurifax) and most Ectobranchia reflecting their basic position within Heterobranchia (Haszprunar 1988; Ponder & Lindberg 1997; Haszprunar et al. 2011).

The position of the digestive gland (both parts) is identical in all orbitestellids studied (Ponder 1990a; Simone and Zelaya 2004; this study). A paired (anterior and posterior) digestive gland can be found in all investigated ectobranch species, whereas the digestive gland is unpaired in Mathildidae (Haszprunar 1985c), Architectonicidae (Haszprunar 1985b), and Omalogyrinidae (Bäumler et al. 2008; Fretter 1948), and Rissoellidae (Fretter 1948).

The short intestine of *Orbitestella* and *Microdiscula* is equipped with a typhlosole (Ponder 1990a) as it is the case in most valvatid species and cornirostrids (Bernard 1890; Cleland 1954; Rath 1986, 1988; Ponder 1990b; Hawe et al.

Table 1 Comparison of orbitestellid and ectobranch characters concerning autapomorphies of both taxa

	Orbitestellidae	Ectobranchia (=Valvatoidea)
Penis	Epipodial, pedally innervated (right sided)	If present, cephalic innervated, behind right cephalic tentacle
Pallial tentacle	− [b]	+
Ciliated ridges	+ [b]	−
Protoconch	Orthostrophic [a]	Hyperstrophic (except Valvatidae)
Jaws	Extremely massive [a]	Small
Posterior granular/glandular epithelium	+	−
Extendable gill	−	+
Cerebropleural–buccal connectives leading through the buccal protractor	−	+ [b]
Posterior pedal glands	Paired [a] with single conduct and opening	If present, single gland
Radula	Taenioglossate, always one pair of lateral teeth [a]	Rhipidoglossate [b]/ taenioglossate with different morphes

[a] Already proposed by Ponder (1990a)—for a better understanding, ectobranch characters are also listed for direct comparison

[b] Probably plesiomorphic for basal Heterobranchia

2013a) but not in Hyalogyrinidae (Haszprunar et al. 2011) and Xylodisculidae (personal observation). A typhlosole has not been found in *Lurifax*; however, this character may be masked due to the high filling level of the intestine (Rath 1986, 1988).

Heart, circulatory- and excretory system

The position of the kidney in the pallial roof, where it also acts as an (additional) respiratory organ, is typical for Heterobranchia (Haszprunar 1985a, 1988; Ponder 1990a, b, 1991; Ponder & Lindberg 1997). All Heterobranchia (except certain heart-less taxa) show a monotocardian heart.

Genital system

All investigated Orbitestellidae (Ponder 1990a; Simone and Zelaya 2004; this study) show a quite simple, monaulic, hermaphroditic genital system (Fig. 5), but sexual conditions obviously differ between species: *Orbitestella* and *Microdiscula* are protandric or simultaneous hermaphrodites with a large vesicular seminalis (Ponder 1990a; Simone and Zelaya 2004). Possible protandry could not be confirmed for *L. vitreus*, since juveniles could not be studied. The presence of both female and male germ cells in various stages of development suggests continuous reproduction probably correlated with the hydrothermal vent habitat of *Lurifax vitreus*.

The division of the hermaphroditic gonad in an ovary lobe and a testis lobe in Lurifax is also present in other basal Heterobranchia as the Hyalogyrinidae (Haszprunar et al. 2011), Valvatidae (Hawe et al. 2013a), Cornirostridae (Ponder 1991), Omalogyrinidae (Fretter 1948; Bäumler et al. 2008) and Architectonicidae, where even a nearly complete separation of the male and female duct systems exists (Haszprunar 1985b). A vesicula seminalis for the storage of autosperm could not be detected. Contrary to Ponder (1990a) and Simone and Zelaya (2004), who described two main types of epithelia (prostate and pallial oviduct gland), four types of glandular epithelia within the gonoduct could be differentiated in *Lurifax*, but in both cases the function(s) of the various mucous cell types remain obscure. Nevertheless, the whole genital system can be seen as very simple (e.g. no brood pouches, receptacle apparatus, separation of female and male ducts), which directly contrasts Ectobranchia (e.g. Rath 1986, 1988; Ponder 1990b; Haszprunar et al. 2011; Hawe et al. 2013a) and all other basal Heterobranchia so far studied (see review in Haszprunar 1985a), which have a much more complicated genital apparatus (except Rhodopidae, cf. Brenzinger et al. 2011, 2013a, b).

At first glance the presence of a prominent penis as found in Orbitestellidae is shared with all Valvatidae and Cornirostridae, and other basal Heterobranchia such as Rissoellidae or Acteonidae. However, Architectonicidae, Mathildidae, and Omalogyridae lack a penis at all, and Hyalogyrinidae show only a weak copulatory organ. In contrast to the copulatory organs of the other families, the penis of *L. vitreus* is innervated by the right pedal ganglion. Accordingly, this copulatory organ is a pedal structure and therefore should be considered as an independent novelty within the basal Heterobranchia. Simone and Zelaya (2004) could not detect the genital pore or a copulatory organ. In *Orbitestella* and *Microdiscula*, Ponder (1990a) described a cerebrally innervated penis resting at the lateral head posterior of the right cephalic tentacle (as in all other basal heterobranch taxa with copulatory organ). However, a reinvestigation of his serial sections of *Orbitestella wareni* and *Microdiscula* cf. *subcanaliculata* revealed that there is a pedal penis as well. Accordingly, a pedal penis combined with an outer seminal groove is considered as a synapomorphy of Orbitestellidae. The size and high amount of yolk found within the (possibly not fully mature) eggs as well as the smooth protoconch suggest a non-planktotrophic and possibly intracapsular mode of larval development for *Lurifax vitreus*. Considering this and the hydrothermal vent habitat of the species, conspecifity of the Atlantic and Mediterranean specimens of *Lurifax vitreus* (Smriglio and Mariottini 2002) appears at least doubtful, since determination of the latter is based on a single empty shell. Further studies on soft body and molecular analyses are required to check the status of Atlantic and Mediterranean populations.

Nervous system and sense organs

There are no differences in the central nervous system between the studied orbitestellids (Ponder 1990a; Simone and Zelaya 2004; this study). Fusion of cerebral and pleural ganglia is common among basal heterobranchs. Bifurcated tentacle nerves are typical for basal heterobranchs, whereas the conditions vary among euthyneuran taxa (Haszprunar 1985a, b, c, 1988; Ponder 1991; Huber 1993; Haszprunar et al. 2011; Hawe et al. 2013a).

The additional nerves leading alongside the oesophagus found in Architectonicidae (Haszprunar 1985b) and Valvatidae (Hawe et al. 2013a) could not be found in *Lurifax* or in the other orbitestellids re-examined. The cerebrobuccal connectives do not lead to within the buccal protractors as it is the case in most other basal Heterobranchia including Ectobranchia (Haszprunar 1985b, 1987, 1988; Haszprunar et al. 2011; Hawe et al. 2013a).

The presence of well-developed eyes with pigment in *Lurifax vitreus* is astonishing for an inhabitant of deep-water hydrothermal vents. Since also the larvae probably do not reach the photic zone, it is likely that the colonisation of this deep-water habitat happened quite recently in evolution.

Statocyst conditions differ between the orbitestellids: *Lurifax vitreus* shows several statocones within each statocyst, whereas both *Orbitestella* and *Microdiscula* species possess one single statolith (Ponder 1990a; Simone and Zelaya 2004;

personal observation). All Ectobranchia and Omalogyridae also have one statolith (Ponder 1990b, 1991; Bäumler et al. 2008; Haszprunar et al. 2011; Hawe et al. 2013a), whereas Architectonicidae and Mathildidae (Haszprunar 1985b, c) and most euthyneurans have statocones. However, at least euthyneuran veligers show the statolith condition (e.g. Chia et al. 1981; Wiederhold et al. 1990), accordingly simple heterochrony may easily change the adult type from statoconia to statoliths.

Systematic considerations

Placement of Lurifax among Orbitestellidae and monophyly of the family

Aside from non-planispiral and poorly sculptured shell, the larger size, the multispiral protoconch, and the hydrothermal vent habitat, only a few anatomical features differ between *Lurifax* and the remaining Orbitestellidae investigated by Ponder (1990a) and Simone and Zelaya (2004). Thus, *Lurifax* has significantly larger salivary glands and therefore smaller posterior pedal glands than its shallow water relatives. Otherwise, the orbitestellid anatomy appears very uniform. However, including the present work, only 5 of about 25 species have been studied concerning anatomy, and also molecular data are currently restricted to two species, thus statements about detailed internal systematics would be premature.

Based on the previous studies and the present work, the following features are considered as synapomorphies of Orbitestellidae reflecting the monophyly of this taxon (Table 1):

Placement of Orbitestellidae among basal Heterobranchia

The phylogenetic tree of the basal, non-euthyneuran Heterobranchia is still doubtful. There is general agreement that these "Heterostropha" Fischer, 1885 or "Allogastropoda" Haszprunar, 1985 are "a paraphyletic holding vessel for problematic taxa" (Bieler 1992:326) currently including Ectobranchia (Valvatoidea), Orbitestellidae, Architectonicoidea (Mathildidae, Architectonicidae, Omalogyridae: Haszprunar 1985a, 1988), Murchisonellidae (Ebalidae), Aclididae (*Aclis*, *Graphis* and *Larochella*), Cimidae, Rhodopemorpha (*Rhodope* and *Helminthope*), Tjaernoeidae, Rissoellidae, and Acteonoidea, whereas (contrary to previous ideas) the Pyramidelloidea (Amathinidae, Pyramidellidae) are to be placed among the Euthyneura (Jörger et al. 2010; Wilson et al. 2010; Dinapoli and Klussmann-Kolb 2010; Dinapoli et al. 2011; Göbbeler and Klussmann-Kolb 2011; Schrödl et al. 2011).

Due to various morphological similarities, Ponder (1990a, b) classified the Orbitestellidae within the Ectobranchia—a

view also supported by sperm fine structure (Healy 1990, 1993). However, already Ponder (1990a: 527) remarked that the characters shared by Orbitestellidae and Valvatidae are "probably plesiomorphic" (for Heterobranchia) and later on (Ponder 1991:22) considered Orbitestellidae as "rather distantly related to Cornirostridae and Valvatidae". Bieler et al. (1998: 318) again noted remarkable differences between the Orbitestellidae and the remaining taxa in "…the still ill-defined Valvatoidea". Recent molecular analyses always show the Orbitestellidae separated from the Ectobranchia (e.g. Dinapoli and Klussmann-Kolb 2010; Jörger et al. 2010; Schrödl et al. 2011) and our anatomical data support this view. Similarities between Ectobranchia and Orbitestellidae such as the pallial tentacle, the pallially located kidney, the lack of radular cartilages, the long and tubular salivary glands, the gastric shield or sperm details (Healy 1990, 1993) are all plesiomorphies of Heterobranchia, whereas the shared copulatory organ is considered as analogy due to different innervation (cerebral in Ectobranchia, pedal in Orbitestellidae). Moreover, Orbitestellidae share apomorphic characters with non-ectobranch Heterobranchia, namely the presence of opponent ciliary tracts. We conclude that Orbitestellidae cannot be included in Ectobranchia, but is an early heterobranch offshoot proper and that Ectobranchia is confirmed as the first subclade of Heterobranchia (Haszprunar et al. 2011; Brenzinger et al. 2013a, b).

Further phylogenetic assumptions and the degree of relationship to the remaining taxa of non-euthyneuran Heterobranchia (see above) will require careful analysis of morphology and molecules. The story will continue.

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