Morphology of an endosymbiotic bivalve, *Entovalva nhatrangensis* (Bristow, Berland, Schander & Vo, 2010) (Galeommatoidea)

J. LÜTZEN[1], B. BERLAND[2], & G.A.BRISTOW[2*]

[1]Biological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark
[2]Department of Biology, University of Bergen, N-5020 Bergen, Norway
*Corresponding author, Email: glenn.bristow@bio.uib.no

Abstract

We describe the morphology of *Entovalva nhatrangensis* Bristow, Berland, Schander & Vo, 2010, an endosymbiontic bivalve living in the oesophagus of *Holothuria spinifera* and *H. leucospilota* in Vietnam. The delicate shells are entirely internalized. The body is very small compared to the foot, which is dorso-ventrally flattened and contains the digestive diverticula and the fertile parts of the gonads. Even though the gills are small, they probably serve in collecting suspended matter, and in addition, the species clearly feeds on benthic diatoms, which it probably sorts out from the contents of the host's gut. The species is a protandric hermaphrodite. Most males have a total length of 1.5–3.0 mm and above that size start changing sex to become females, which may attain a total length of nearly nine mm. Sperm is transferred in spermatophores with a solid wall produced by glands within the male siphon. One to three spermatophores are placed on the gills of females and the ova become fertilized as they pass from the genital pores to the siphon, where they are brooded until released as D-larvae.

Key words: functional anatomy, hermaphroditism, protandry, spermatophore, *Holothuria spinifera*, *Holothuria leucospilota*, Bivalvia, Heterodonta

Introduction

Sea cucumbers are frequent hosts of symbiotic bivalves. Most such bivalves, considered ectocommensals, are byssally attached to, or move freely upon, the skin of burrowing hosts, which provide shelter and ensure a constant flow of water rich in oxygen and particulate matter through the burrow. Two bivalve genera, *Entovalva* Voltzkow, 1890, and *Cycladoconcha* Spärck, 1931, are outstanding in that all five described species are endosymbionts in holothurians. In addition they have completely internalized shells and relatively small gills. The species show a tendency to displace a smaller or larger part of the gonad and digestive diverticula into the foot, resulting in a diminution of the visceral mass. Ample material of a recently described species, *Entovalva nhatrangensis* Bristow, Berland, Schander & Vo, 2010, has allowed us to study the morphology and reproduction of a representative of the genus in more detail.

Materials and methods

The hosts [*Holothuria spinifera* (Théel, 1886) and *H. leucospilota* (Brandt, 1835)] were collected by scuba divers at approximately 3–8 m in Nha Trang Bay, Vietnam (c 12°12'N, 109°13'E). The sediment was fine coral sand with a small amount of organic debris. Both host species are surface detritus feeders and are mainly found under coral ledges during the day and actively feed at night.

They were generally held overnight in running sea water by the divers and then delivered to the Research Institute for Aquaculture No. 3 (RIA 3), Nha Trang, the following morning. Generally 6–8 hosts were collected at one time. At RIA 3 they were held in running seawater until dissection, which took place within 24 hours of their arrival.

Entovalva nhatrangensis was never found outside its hosts. Within the host it was found only in the oesophagus. Twenty two of 23 (95%) *H. spinifera* were found to contain the bivalves with the number in each individual host ranging from 1–167 (average intensity 84.5). Only one of 30 (3.3%) *H. leucospilota* contained a small number (5) of bivalves.

Further information on the hosts, localities, type material deposition, etc., can be found in Bristow *et al.* (2010).

Bivalves used for microanatomical purposes were preserved in 4% formaldehyde in seawater. Many specimens of all sizes were stained in modified carmalum, cleared in benzyl-alcohol, and mounted in Canada balsam (Berland 2005). Eighteen bivalves were embedded in Araldite® M and the entire body or parts of it cut into 2-μm thick serial sections were stained with toluidine blue. Sections of a few specimens of *Entovalva lessonothuriae* Kato, 1998, prepared in the same way, were examined for comparison. Because the shell is internal it is often difficult to see and measure, and therefore sizes to the nearest 0.1 mm are given as total length (TL) from the tip of the foot to the tip of the siphon. This is possible because the foot in this species is only slightly retractable and consequently has a more or less constant length.

Results

External anatomy

The bivalve body consists of an elongate foot, a rather

short and thick visceral portion and a large exhalant siphon (Fig. 1A). As in other species of *Entovalva*, right and left halves of the mantle extend above and beyond the shell and fuse dorsally, resulting in the entire shell being completely and permanently enclosed (Figs 5, 6). The two mantle halves also fuse along the ventral midline, leaving a minute anteriorly directed inhalant opening through which the foot protrudes. Posteriorly the mantle is extended to form a bell-shaped siphon, almost as spacious as the body and opening by way of a very small circular exhalant aperture. Although in sections the exterior of the foot and mantle shows many wrinkles, there are neither papillae nor pallial tentacles. The mantle is covered everywhere by an unciliated epithelium

which seems to bear a very delicate cuticle. The colour in life is white or slightly off-white with a yellowish tinge.

The shell is very thin, whitish but semi-translucent, moderately inflated and distinctly inequilateral, in that the umbo is placed well behind the middle (Fig. 1B). The dark interior ligament is short, and visible through the transparent shell. The prodisscoconch II forms a rounded cup. The length of prodisscoconch II varies between 240 and 270 µm, while prodisscoconch I (measured from the D-shaped larvae) is about 90 µm in length.
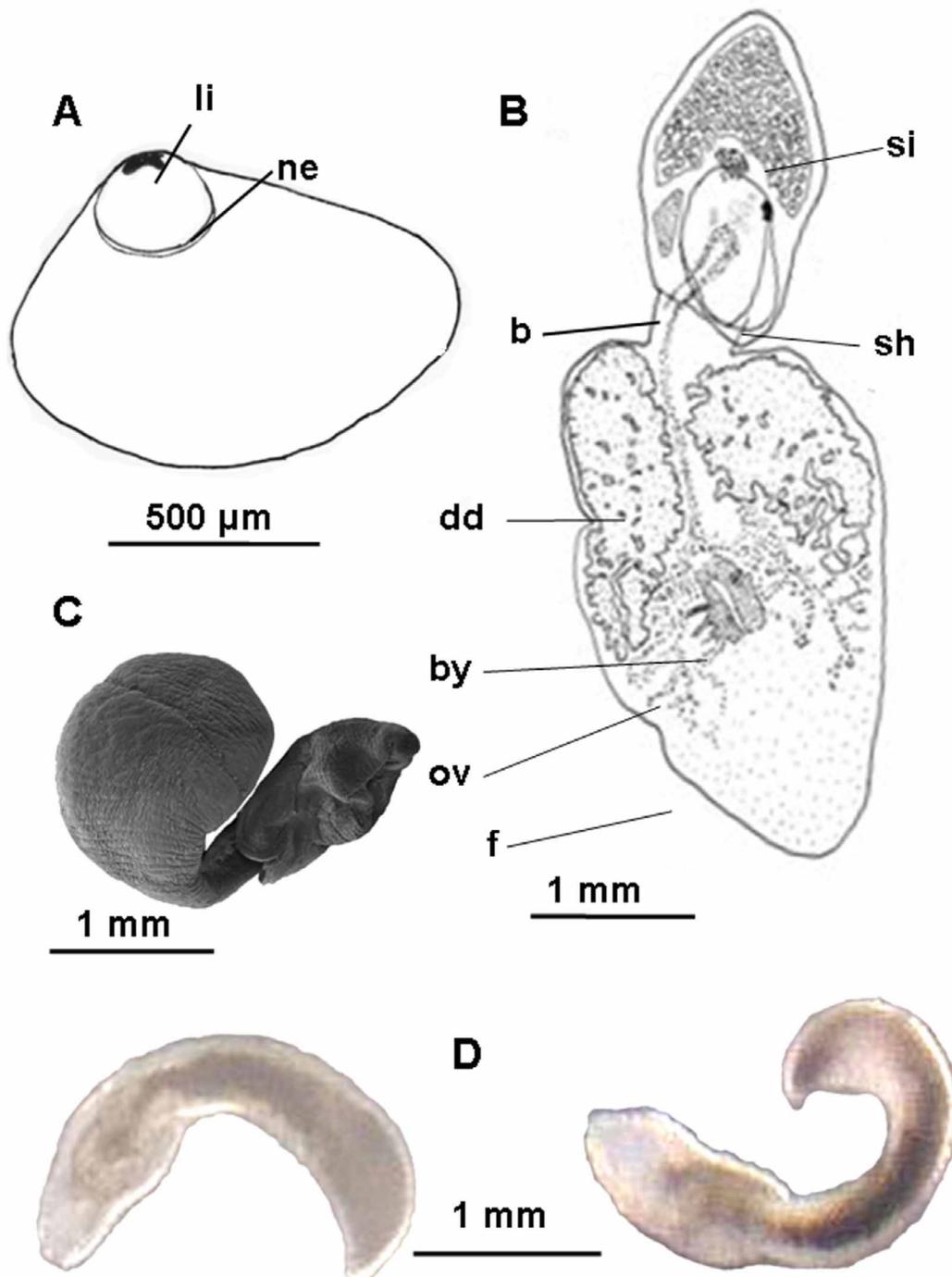
FIGURE 1. *Entovalva nhatrangensis*. **A.** Right valve of a female. **B.** Drawing of entire mature female. **C.** SEM of a large individual. **D.** Digitial photographs of living small specimens. Abbreviations: b, body; by, byssus gland; dd, digestive diverticula; f, foot; li, ligament; ne, prodisscoconch II; ov, ovary; si, siphon filled with embryos; sh, shell.

The foot protrudes from the anteriorly directed inhalant mantle opening (Figs 1A, 2). By far the major part of the foot lies permanently outside the shell and body and cannot be drawn into the small mantle cavity. The foot is dorso-ventrally flattened, tongue-shaped, anteriorly bluntly pointed, broadest near the middle, and with a posterior narrow, stalk-shaped connection with the body. Although it is bilaterally symmetrical, the stalk is permanently twisted in such a way that the ventral foot area in many specimens is turned towards, and often makes contact with, the host's esophageal wall. The foot is from 1.5 to twice as long as the combined body and siphon (Fig. 1A) with a maximum length and breadth of 5.0 x 3.7 mm (foot) and 3.9 mm (length of body and siphon).
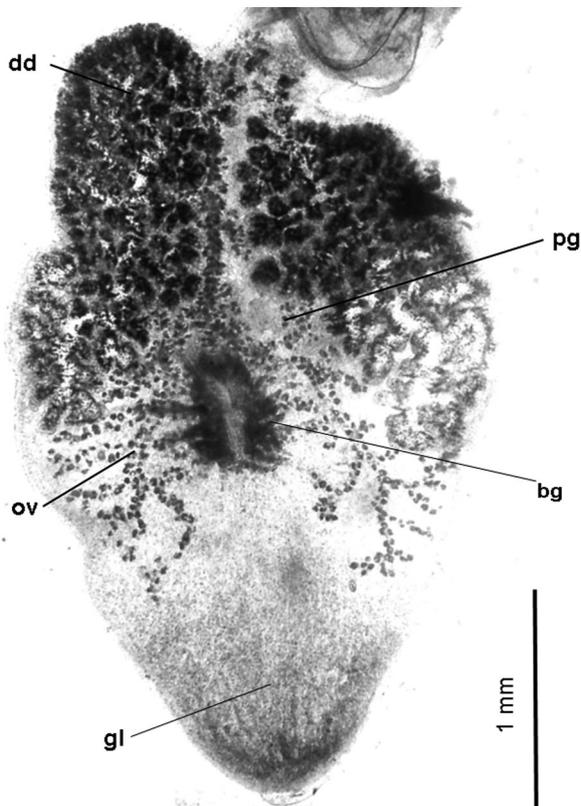
FIGURE 2. *Entovalva nhatrangensis*. Photo of the foot of a living female seen from the ventral side. Abbreviations: by, byssus gland; dd, digestive diverticula; gl, gland cell complex in distal part; ov, ovary; pg, pedal ganglion.

In live and preserved mounted specimens stained with carmalum, most of the organs within the foot are visible (Fig. 2). There is a branching system of yellowish or yellow-brownish digestive diverticula occupying most of the lateral parts of each side. All the diverticula ultimately empty into two prominent ducts which traverse the slender foot stalk to enter the body. The major parts of the gonads are displaced into the foot where they form a profusely branching system of minor tubules, all arranged in a single plane, and occupying the distal half of the foot, except for its tip. All the gonadial tubules collect into a single sinuous tube which, following the foot's midline, runs anteriorly through the foot

and foot stalk and into the body. The digestive diverticula and gonads partially overlap each other. Near the centre of the foot, in an area not occupied by digestive diverticula or gonads, a small opaque body represents the fused pedal ganglia. The byssus apparatus lies slightly anterior to the ganglia, distinguished as a short oblong body, which stains darkly because of the many glands associated with it. It includes many globular gland cells with darkly staining granules and a long slender neck which discharges into the byssus groove and byssus gland (Fig. 3). The byssal groove is short, deep and lined by a cuboidal epithelium with short cilia. In medium-sized females the byssal groove is 350–400 µm long and up to 200 µm deep. Posteriorly it continues into a small subdivided byssus gland, the individual crypts of which collectively produce a single short but fairly thick byssus thread (Fig. 3B). In mounted preparations it could sometimes be seen protruding from the byssus opening. One bivalve had the end of the thread inserted into a small piece of tissue that had been torn off (Fig. 3B), possibly originating from the host's oesophagus. The few muscles associated with the byssus apparatus are local and spread into the subepithelial muscle sheet.

The epidermis of the foot consists of cuboidal epithelium underlain by a very thin sheet of muscle fibers. The epithelium covering the entire distal half of the foot's ventral side, from the distal part to about the level of the pedal ganglion, is heavily ciliated while all other surfaces are unciliated (Figs 3, 4A). The presence of a fortified epithelial surface suggests that the non-ciliated epithelia are covered with a very delicate cuticle, but this could not be confirmed. The interior of the distal part of the foot is supplied with numerous globular gland cells, which stain metachromatically with toluidine blue and open on the ciliated ventral surface near the tip (Figs 2, 4B).

Mantle cavity and gills (Fig. 5)

Because of the reduced size of the body, the mantle cavity is correspondingly small. This also applies to the suprabranchial chamber which may be why the ova do not develop here, but largely behind it in the interior of the siphon.

The gills are represented by the inner demibranch only but have been further reduced in that there are filaments only on the descending lamella while the ascending lamella is a narrow non-respiratory membrane fused with the visceral mass (Fig. 5A). There are at least 25 gill filaments in large females. Transverse sections of the filaments show that there are paired tracts of lateral cilia, a tract of short frontal cilia, and, between the two, tufts of latero-frontal cilia (Fig. 5B). A ciliated tract which probably functions as a food groove runs along the ventral margin of each gill. The anterior tip of the gill is in contact with the labial palps. Each of the two palps consists of two flattened lobes that face each other (Fig. 5A). The opposing tall epithelia of the lobes are heavily ciliated and have an uneven surface, but the usual arrangement of regularly spaced ridges and grooves seen in other bivalves seems to be absent.
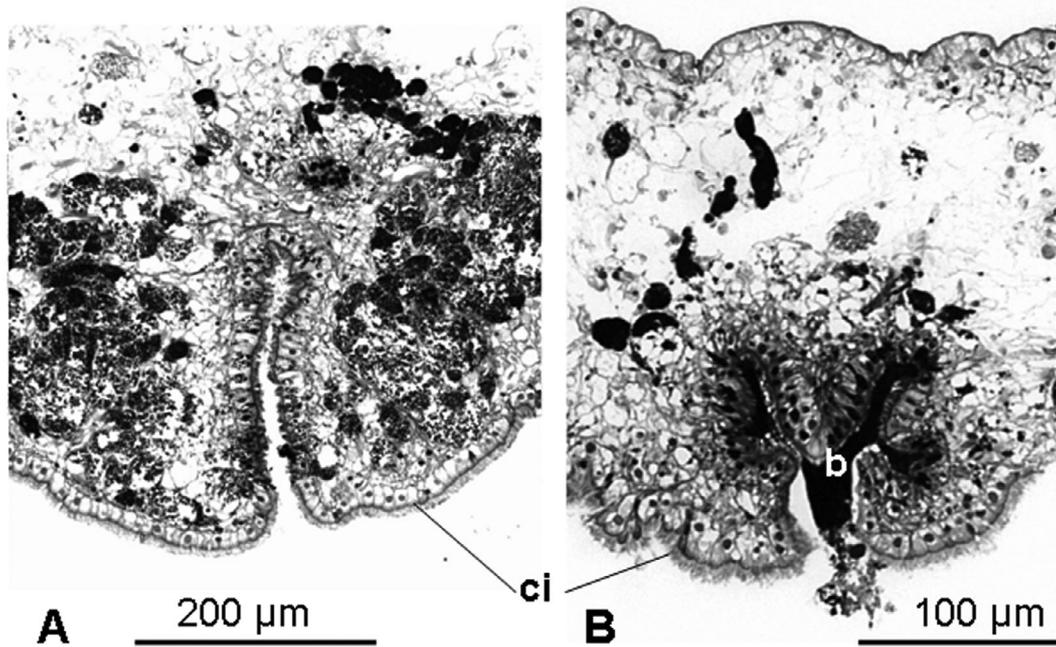
FIGURE 3. *Entovalva nhatrangensis,* female. Transverse section through the byssal groove (**A**) and opening of the byssus gland (**B**) with a short byssus (b) attached to a piece of tissue (of host oesophagus ?). Abbreviation: ci, ciliated epithelium of ventral side of foot. Toluidine stained 2-µm thick Araldite® M sections.
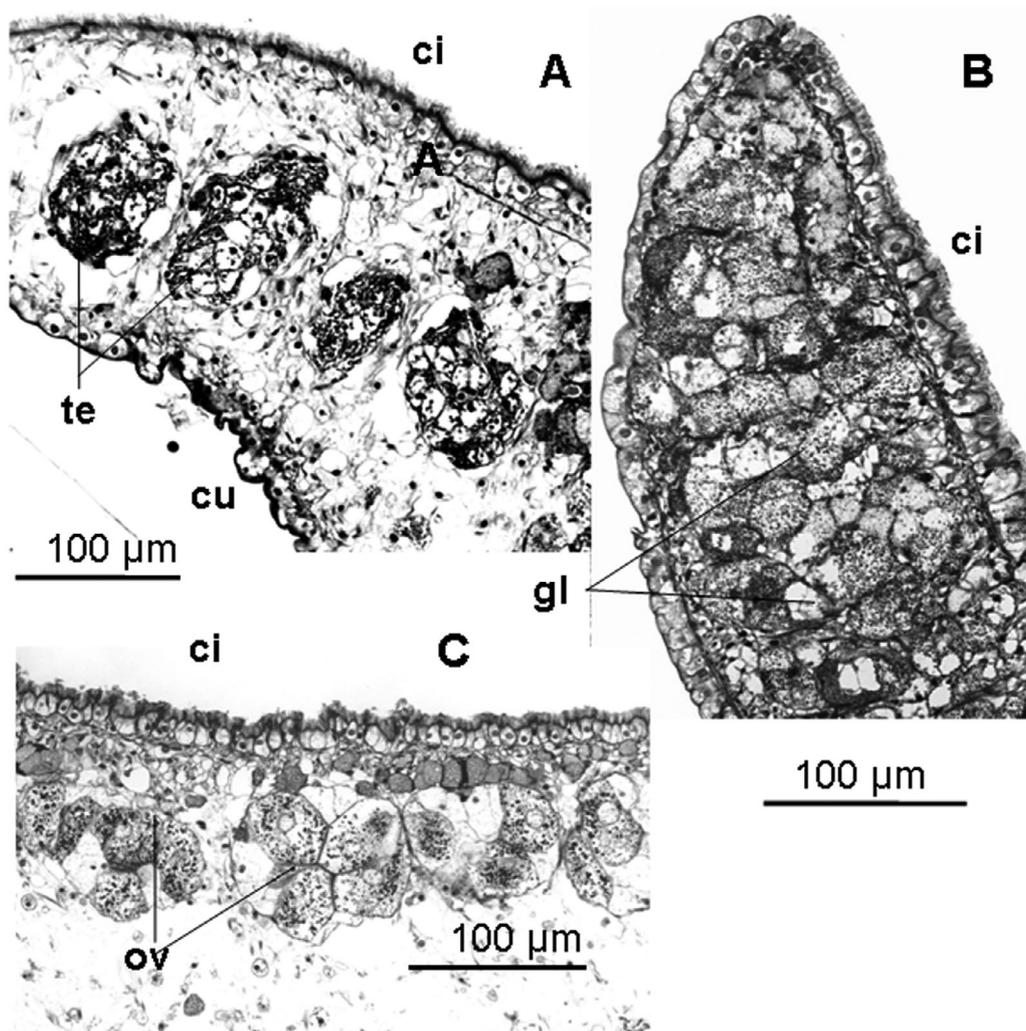
FIGURE 4. *Entovalva nhatrangensis*. Transverse sections through foot of a 2.0 mm long male (**A**), and two females (**B, C**). Abbreviations: ci, ciliated epithelium of ventral side; cu, cuticularized epithelium of dorsal side; gl, gland cells (within tip of foot); ov, ovarial tubules; te, testis tubules with spermatozoa and 'nurse cells'. Toluidine stained 2-µm thick Araldite® M sections.
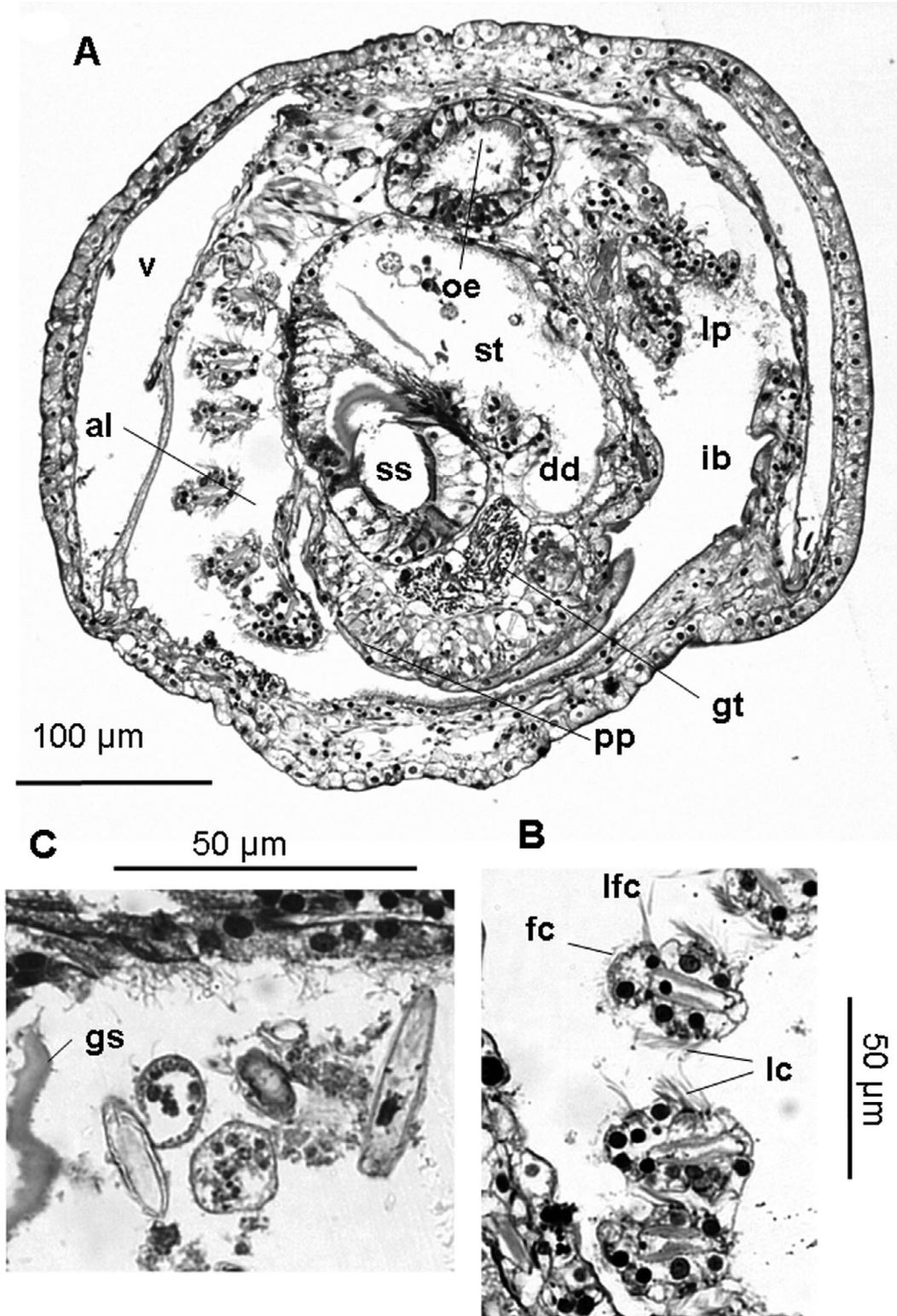
FIGURE 5. *Entovalva nhatrangensis*. **A.** Transverese section through the body of a male level with the oesophagus (oe) and anterior part of stomach (st). **B.** Transverse sections of the gill filaments. **C.** Diatoms and other unicellular algae in the stomach. Abbreviations: al, ascending gill lamella; dd, one of two main ducts from digestive diverticula opening into stomach; fc, frontal cilia; gs, gastric shield; gt, gonadial tube of male; ib, infrabranchial chamber; lc, lateral cilia; lfc, latero-frontal cilia; lp, basis of labial palp; pp, posterior pedal retractor; ss, style sac; v. valve (dissolved). Toluidine stained 2-µm thick Araldite® M sections.

The siphon

    The epithelium lining the interior consists of tall and short cells irregularly mixed together (Fig. 8A). Most of them are unciliated, but a few carry a small tuft of short flagellae. In siphons that have become distended, most of the cells become stretched out. A few and very scattered muscle fibers underlie both the interior and exterior epithelia of the siphon and, when contracted, may reduce the diameter of the siphon.

In each of five males in which the siphon was sectioned, two longitudinal tracts of tall gland cells stretch from near the base of the siphon towards its tip (Figs 6C, D, 8B).
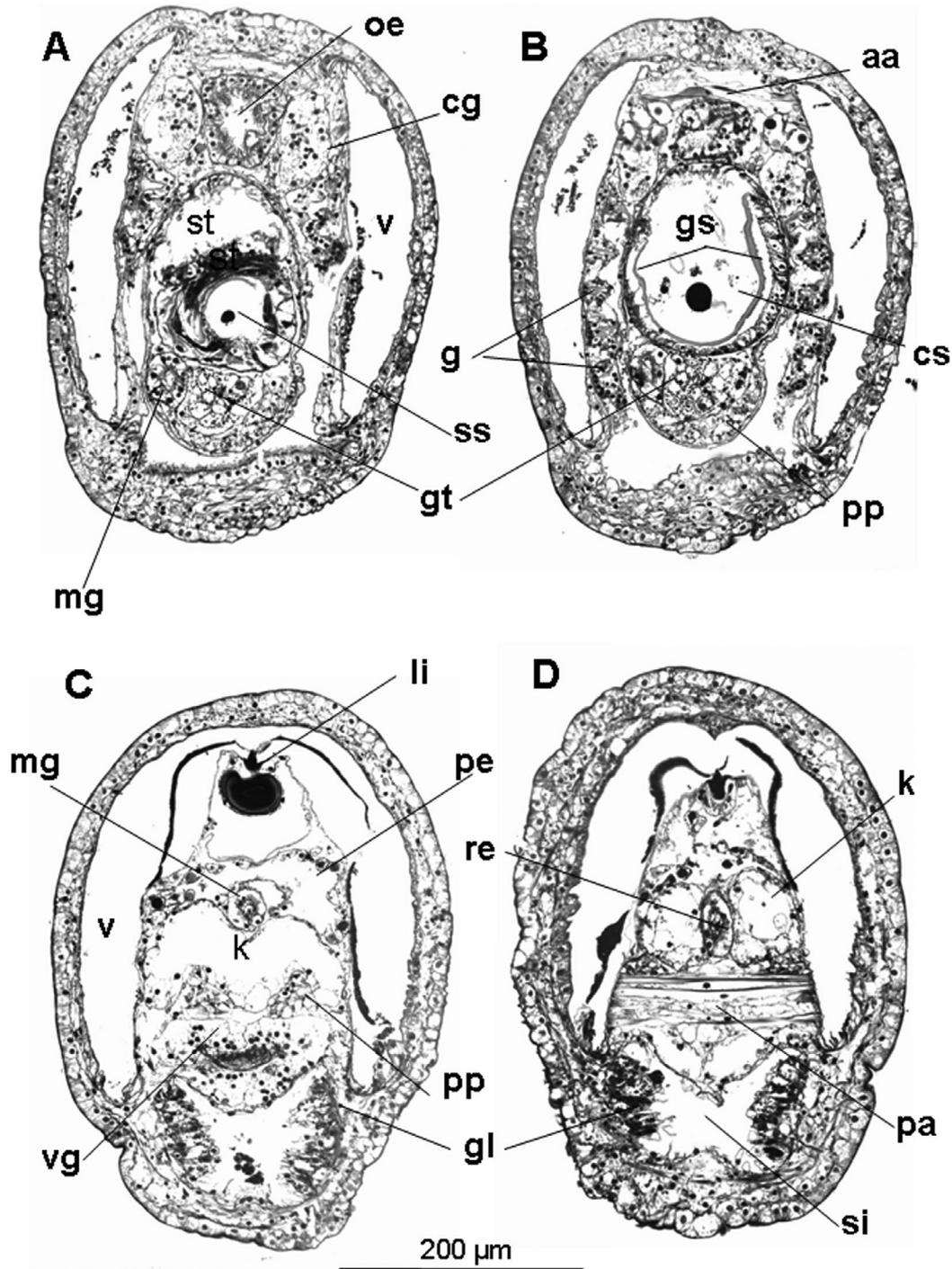
FIGURE 6. *Entovalva nhatrangensis*, four consecutive transverse sections through the body of a male level with the oesophagus (**A**), the anterior adductor muscle (**B**), the ligament (**C**), and the posterior adductor muscle (**D**). Abbreviations: aa, anterior adductor muscle; cg, cerebro-pleural ganglion; cs, crystal style; g, gill; gl, glandular tracts believed to produce wall of spermatophore; gs, gastric shield; gt, gonadial tube with spermatozoa and „nurse cells"; k, kidney; li, ligament; mg, midgut; oe, oesophagus; pa, posterior adductor muscle; pe, pericardium; pp, posterior pedal retractor, re, rectum; si, siphon (basis); ss, style sac; st, stomach; v, valve (dissolved); vg, visceral ganglia. Toluidine stained 2-µm thick Araldite® M sections.

Although separate from each other, these tracts are close together in the ventral part of the siphon. The gland cells are club-shaped, much taller (40–50 µm) than the ordinary surrounding epithelium and packed with small and large spherical granules which stain intensely with toluidine blue. In a 3.0 mm long bivalve preserved when in the process of sex reversal, these glands were still present, but definitely reduced compared with their condition in males. These glandular tracts were absent in the siphons of six sectioned females (TL 3.0–5.4 mm).

Musculature

The two adductor muscles are almost equal in size. The posterior one is much closer to the small ligament than the anterior one, and is also more distant from the hinge line (Fig. 6). Neither anterior nor posterior byssal retractors could be found, while the paired posterior pedal retractors are inserted dorsal to the posterior adductor. They run forward between the kidneys and the visceral ganglia (Fig. 6C, D) and after having fused take a more superficial course along the ventral side of the foot (Figs 5A, 6A, B). Level with the stalk of the foot, the pedal retractors are no longer recognizable as discrete muscles as their fibers spread diffusely into the subepithelial layer of the tongue-shaped part of the foot.

Alimentary system

The mouth is a simple pore opening at the base of the two labial palps. It is never prolonged into a snout as in some other galeommatoideans. It leads into a ciliated and moderately longitudinally folded oesophagus (Figs 5A, 6A, B) which opens into the dorsal ciliated part of the stomach. No obvious musculature is associated with the oesophagus. The oviform stomach is more or less circular in transverse sections (Figs 6A, B, 7). Its antero-ventral part has a thick gastric shield while the dorsal and posterior parts are uniformly and heavily ciliated, but particular sorting areas could not be identified. Benthic diatoms of many sizes and unspecified unicellular algae occurred in the stomach (Fig. 5C) and also in the infrabranchial chamber.
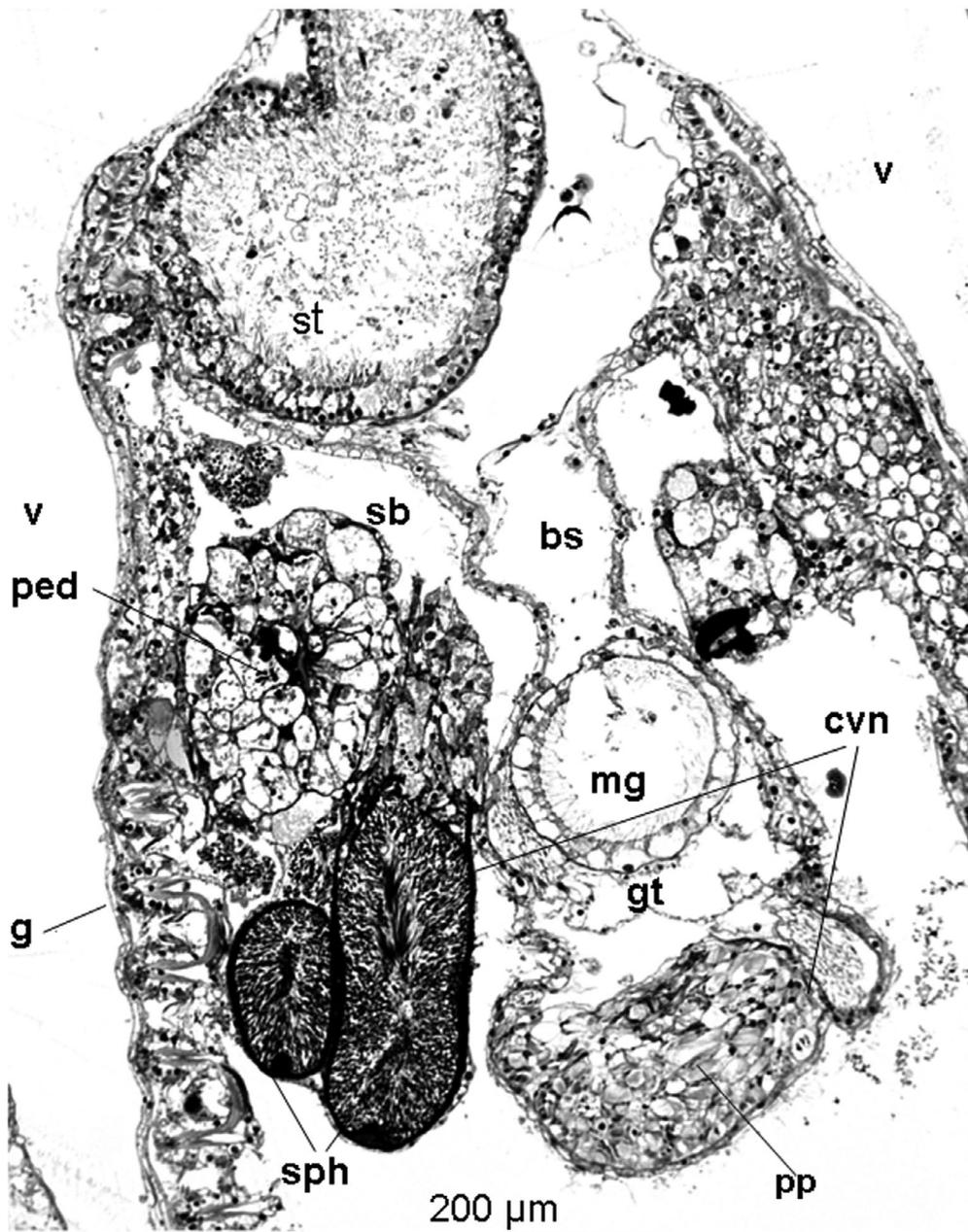
FIGURE 7. *Entovalva nhatrangensis*, female. Transverse section through the posterior part of the body. Abbreviations: bs, blood sinus; cvn, cerebro-visceral nerve; g, gill; gt, gonadial tube (empty); mg, midgut;: ped, peduncle swollen into a lump of cells; pp, posterior pedal retractor; sb, suprabranchial chamber; sph, two spermatophores attached to peduncle; st, stomach; v, valve (dissolved). Toluidine stained 2-µm thick Araldite® M sections.

Having traversed the base of the foot, the two main ducts of the digestive diverticula fuse immediately in front of the stomach before entering its anterior part (Fig. 5A). In addition to the normal digestive cells of the acini involved in absorption, phagocytosis and food storage there are many minute pyramidal cells distinguishable by their very darkly staining cytoplasm. The acini open into many small collecting ducts with an unspecialized cuboidal epithelium which ultimately fuse with the two wide main ducts described above (see description of foot). No ciliation was seen in any parts of the digestive diverticula.

The style sac is half as long as the stomach and contains a crystalline style which projects into the anterior part of the stomach (Fig. 6B). It is a conical structure which lies in the base of the foot with its long axis along that of the foot and its pointed tip directed forward. It is lined by a uniform columnar ciliated epithelium except for a longitudinal typhlosole (Figs 5A, 6A). The style sac opens into the foremost part of the stomach level with the common duct of the digestive diverticula. Shortly behind this point the narrow, ciliated intestine leaves the stomach. It runs forward, then loops backward under the stomach, accompanying the gonadial tube for its entire length (Figs 6A, B, 7). The rectum, distinguished from the intestine by its much shorter cilia, runs posteriorly to the anus between the pericardium (dorsally) and the kidney (ventrally)(Fig. 6C, D).
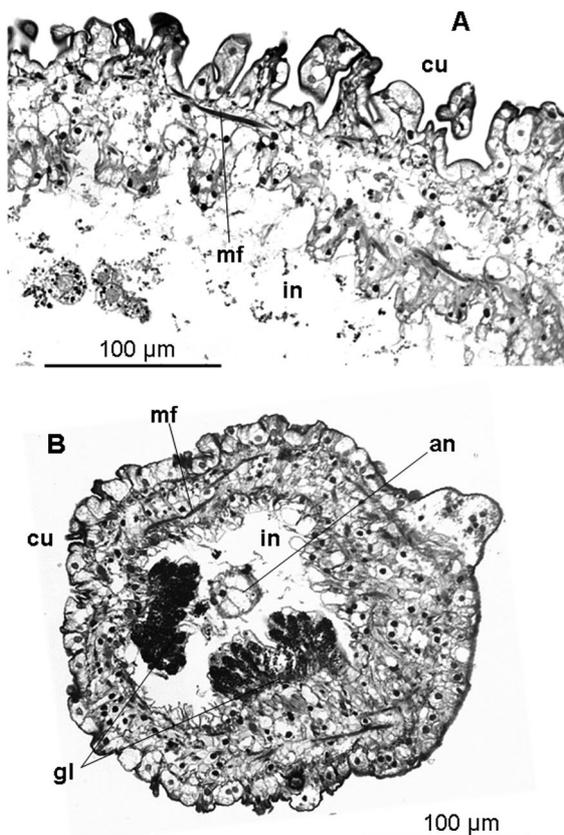
FIGURE 8. *Entovalva nhatrangensis.* A. Transverse section through the wall of the siphon of a female. B. Transverse section through the siphon of a male. Abbreviations: an, anus; cu, cuticularized epithelium; gl, glandular tracts believed to produce wall of spermatophores; in, interior of siphon, mf, muscle fiber. Toluidine stained 2-μm thick Araldite® M sections.

Reno-pericardial system (Fig. 6C, D)

The kidney consists of left and right sacs at each side of the rectum which anteriorly fuse to a single sac. The pericardium is penetrated by the rectum and contains a heart with a single ventricle and two auricles.

Nervous system

Paired pleuro-cerebral ganglia lie at each side of the oesophagus (Fig. 6A) and connect with the two fused visceral ganglia (Fig. 6C) via paired cerebro-visceral nerves (Fig. 7). The latter are placed level with the ligament and beneath the posterior adductor ventrally in the hindmost part of the visceral mass. Very long paired cerebro-pedal connectives traverse the part of the foot posterior to the two fused pedal ganglia. Two statocysts, each containing a single spherical statolith, are included superficially into the fused pedal ganglia.

Reproductive system

The general layout of the male and female reproductive systems is the same. The part of the gonad in which oo- or spermatogenesis takes place is a ramified system of gonadial tubules lying exclusively in the foot (Fig. 2). All of them ultimately unite posteriorly in the foot to form a single, wider, gonadial tube which runs backwards along, and ventral to, the paired main ducts of the digestive diverticula. Having entered the body, the gonadial tube, whether male or female, continues backwards to be constricted between the stomach or gut and the pedal retractors (Figs 5A, 6B, C, 7). Posteriorly, it expands into a slightly more spacious sac lying in the rear part of the body behind the stomach. Two small paired invaginations of the epithelium of the visceral mass at this level may indicate where the genital openings will appear in the spawning animal. Along the course of the gonadial tube and its terminal expansion the walls are lined with a squamous to cuboidal unciliated epithelium consisting of lightly staining cells with small nuclei. Sperm occurred along the length of the gonadial tube in males, while the tube was empty in the few sectioned females. However, this is assumed to be where the mature ova accumulate before oviposition.

Germ cells are produced only in the pedal part in both sexes. Spermatogenesis occurred in all male specimens examined. Twenty of these males had a total body length (TL) of 1.5 mm to 3.0 mm and three other male specimens had a TL of 4.1, 4.1, and 4.4 mm. Two of the latter specimens carried spermatophores in the gills. The sex of a 2.9 mm long specimen could not be determined, as no germ cells were identified in the gonad. While in most of the smaller males the testicular tubules were thick and swollen with sperm (Fig. 4A), in males above 2.5 mm, sperm were generally few and scattered but sometimes present in the body part of the gonad. All bivalves above 5.3 mm in TL were females with oocytes of different sizes in the ovary (Fig. 4C), and with/without spermatophores and/or embryos. The smallest female recorded was a 3.0 mm long bivalve with very small oocytes in the ovary and sperm from an earlier male phase in the gonadial tube. Two other small

females (TL 3.8 and 4.3 mm) were sexually mature as they had embryos (and spermatophores) in the brood chamber. Since some of these specimens were mounted preparations the exact histological state of the pedal gonad could not be checked.

There is only one type of sperm cell but 'nurse cells' are also found in the testes. The filamentous spermatozoa have heads 5.5–6.3 µm long which taper anteriorly. In the pedal part of the testes, as well as everywhere in the gonadial tube, the spermatozoa often lie scattered between loose groups of polygonal to spherical 'nurse cells', 10–12 µm across, and with a light cytoplasm and a central nucleus. These cells were absent from the ovary. The oocytes protrude into the lumen of the ovarial tubules and, compared to other galeommatoideans, have an unusually light cytoplasm with relatively few yolk granules (Fig. 4C). Maximum oocyte size was not recorded, but young embryos measured 70–80 µm in diameter.

Because of their solid walls, spermatophores could often be seen through the body in mounted specimens. One or two (or in one case three) spermatophores were present in several of the females (Fig. 7). Each spermatophore is suspended from one of two paired outgrowths hanging down from the posterior roof of the suprabranchial chamber. Before a spermatophore has been attached, this outgrowth is a slender, c 175 µm long peduncle made up of a single layer of epithelial cells arranged back to back. After a spermatophore has been received and attached, the peduncle increases in volume and the part of it nearest the spermatophore swells into a lump with tightly packed cells (Fig. 7). The spermatophore is bell- or barrel-shaped with a small terminal pore for the escape of the sperm, and, opposite to this, a wide opening which is plugged by peduncular tissue.

The wall of the capsule is quite thick and solid, except for the part opposite the terminal pore which is thinner and often intricately folded into the tissue of the peduncle. This tissue may invade the adjacent part of the interior of the spermatophore and thereby come into intimate contact with the sperm. A continuation of the peduncular tissue eventually covers the exterior surface of the spermatophore capsule as a thin unicellular sheet save for the terminal pore. Within the spermatophore capsule, sperm are arranged with their heads towards the wall and their filamentous tails occupying the interior.

The very hindmost part of the suprabranchial chamber, and in particular, the interior of the adjoining siphon, serves as a brood chamber where the eggs, embryos and larvae will be brooded to the D-shaped larval stage (prodissoconch I), at about 90 µm in length.

Discussion

Entovalva belongs to a small assemblage of probably closely related (Kato 1998; Lützen *et al.* 2005) galeommatoidean genera (*Devonia* Winckworth, 1930, *Cycladoconcha* Spärck, 1931, *Anisodevonia* Kato, 1998, and *Austrodevonia*

Middelfart & Craig, 2004). Middelfart and Craig (2004) have made a useful compilation of the animals and shells of representative species. The shell is thin and completely or incompletely enclosed within the mantle. The foot is a prominent structure which, in species of *Devonia* and *Entovalva,* is so large that it can no longer be retracted into the mantle cavity. The gills, always represented by the inner demibranch only, may protrude into the spacious and often bell-shaped exhalant siphon. The decisive autapomorphy for representative species of four of the five genera is, however, that they all produce and transfer spermatophores with a solid wall and of a type not known in other galeommatoideans, let alone other bivalves (Lützen *et al.* 2005; present paper). Spermatophores have not been described from *Austrodevonia*, but probably because their existence was not known at the time and no animals were sectioned (Middelfart and Craig 2004). The taxonomic position of the five genera within the superfamily is not known, but inclusion in the family Galeommatidae, as proposed by Morton and Scott (1989) was not supported by Lützen *et al.* (2005), who did not resolve the question but pointed to a strong affinity to other galeommatoideans and particularly to the Kellidae or Montacutidae.

The foot

The original description of *E. nhatrangensis* contained a number of minor inaccuracies which can now be corrected. One is the often cited, but erroneous, statement that the foot in *Entovalva* and *Anisodevonia* is bilaterally compressed. Sections through the foot of the present species indisputably show the statocysts, pedal ganglia, the two cerebro-pedal connectives, the digestive diverticula including its two main ducts, and the branched gonad are symmetrically arranged with respect to a mid plane in which lie the byssus apparatus and the unpaired gonadial duct. The apparent bilateral condition of the foot occurs through a twisting of the base of the foot whereby the anatomically ventral ('right') side is turned towards the host's oesophagus (Kato 1998; Bristow *et al.* 2010). The ciliation of this side may aid in creeping along the oesophageal wall and the byssus emerging from the byssus gland is ideally placed for attaching to it. The byssus apparatus of *Entovalva mirabilis* Voeltzkow, 1890 was wrongly considered a sucker for attachment by Voeltzkow (1890). In *E. nhatrangensis*, although of moderate size, the byssal apparatus is of a normal layout for galeommatoidean bivalves and produces a single byssus thread which is apparently used to attach the animal to the wall of the oesophagus of the host or to other objects taken in by the host as observed by Schepman and Nierstarsz (1913). *E. mirabilis* and *E. lessonothuriae* Kato, 1998 can creep with the aid of the foot (Voeltzkow 1890; Kato 1998) while the motility of *E. nhatrangensis* is apparently more limited (Bristow *et al.* 2010).

In *Anisodevonia*, *E. mirabilis* and *E. lessonothuriae* the ovary and digestive diverticula protrude into the basal and middle part of the foot (Voeltzkow 1890; Schepman and Nierstarsz 1913; Lützen *et al.* 2005), while the testis is mainly restricted to the body. In the present species all the digestive diverticula and the major parts of the protandric

hermaphroditic gonad occupy the main part of the foot, and this has resulted in its expansion and the ensuing diminution of the body.

Feeding

Bristow *et al*. (2010) suggested the possibility that *E. nhatrangensis* might be parasitic and speculated that the folded surface of the foot and body could absorb substances from the host. However, comparing sections of the skin with sections of many other galeommatoidean bivalves, we must conclude that the surface is not particularly heavily folded in the present species. Moreover, the surface of the mantle and most parts of the foot are provided with a very delicate cuticle, a structure not compatible with an absorptive surface. Malard (1903) suspected *Devonia perrieri* (Malard, 1903) pulled host tissue into the byssus gland for ingestion, but this was doubted by Schepman and Nierstrasz (1913) and Ohshima (1931). Voeltzkow (1890) also considered *E. mirabilis* to be a parasite, but in spite of this observed that it fed on organic matter, such as diatoms engulfed by its host. Re-examination of our sections of a male *E. lessonothuriae* showed diatoms in the stomach, and diatoms were present, although not many, in the stomach and mantle cavity in several specimens of *E. nhatrangensis*. They were also often absent but this may be because they had been digested while the bivalves were kept for up to 48 hrs in the laboratory prior to fixation. Probably all species of *Entovalva* select benthic diatoms (and other organic particles of same size) from the bottom material ingested by the host. It is possible that food particles such as diatoms become attached to the glandular tip of the foot and, possibly by bending of the foot, are brought to the mantle opening and perhaps manipulated by the labial palps. If the ciliated ventral side of the foot has no locomotory function, it may aid in transportation of food particles. The gill area is small in *E. nhatrangensis*, but since the gill filaments have the same structure as in filter-feeding bivalves and there is a ctenidial food groove, it is probably able to supplement feeding on diatoms by collecting small suspended particles on the gills. *Austrodevonia sharnae* Middelfart & Craig, 2004, which has relatively large gills, is definitely known to be a suspension feeder (Middelfart and Craig 2004).

Reproduction

The gonad of galeommatoideans consists of a single body lying in the visceral mass. In males, it is often more or less completely subdivided into a large anterior portion in which spermatogenesis takes place and a less spacious posterior portion where the mature sperm are stored until they are released. The present species differs from this model in that a third, middle part, the gonadial tube, has been inserted between the other two, and that the normally undivided ovary has been similarly divided into three parts. The germ cells have been produced in the gonad lying in the foot, and the sole function of the gonadial tube is to convey them backwards for storage in the rear part of the gonad, close to the genital orifices. A similar construction is not seen in other species of *Entovalva*, although in some of them

part of the ovary has invaded the foot. In *E. nhatrangensis*, the gonadial modification is necessary because the entire reproductive productive part of the gonad has been relegated to the foot.

Entovalva mirabilis and *E. lessonothuriae* are both believed to be protandric hermaphrodites as the males are always smaller than the females. However, specimens in the process of sex reversal have not been found in any of these species. In *E. nhatrangensis* males are also smaller (TL: 1.5–4.4 mm) than females (TL: 3.0–7.5 mm) although there is some overlap. As none of 20 bivalves <= 3.0 mm were juvenile females, clearly a sex reversal takes place in this species, occurring probably at a TL of 3.0 to 4.4 mm. One specimen registered as a female (TL 3.0 mm) was in the process of changing from male to female.

The head of the filamentous spermatozoa is closely similar in shape and size to the type of sperm known from four other species of *Devonia*, *Anisodevonia* and *Entovalva* (Lützen *et al*. 2005). The abundance of 'nurse cells' throughout the testis, and their absence in the ovary, suggests that they play a role in the maintenance of the sperm cells. Similar cells were present in *Anisodevonia ohshimai* (Kawahara, 1942), but absent in *E. lessonothuriae* (Lützen *et al*. 2005).

Although sometimes erroneously construed as seminal receptacles or vesiculae seminales (Schepman and Nierstrasz 1913; Spärck 1931), these structures are actually true spermatophores and occur in representatives of all four genera as they do in the present species, while they are absent from all other bivalves. All galeommatoideans are brooders which require that sperm in one way or another get access to the ctenidial or siphonal brood chamber. In many species this happens by bulk transfer of sperm within gelatinous 'sperm balls'; according to Mann (1984) a spermatophore must have a solid outer wall, so these do not qualify as spermatophores. Lützen *et al*. (2005) suggested that the spermatophores of *Entovalva*, *Devonia* and *Ansiodevonia* are manufactured within the siphon. The ample material of male *E. nhatrangensis* shows that sperm produced in the part of the testis located in the foot are transferred to, and stored in, the hindmost part of the testis lying in the body. Two tracts of tall gland cells lining the interior of the siphon in males are absent in the females. In all probability the spermatophore capsule is formed from the secretion of these glands when, following the release of sperm into the siphon, it becomes spread out along its inner wall. Moulding of the spermatophores within the siphon would explain why it has a large basal opening (representing the base of the siphon), and a minute terminal pore for sperm release (representing the small siphonal aperture).

The spermatophores are always attached to the gills in the hindmost part of the suprabranchial chamber which communicates directly with the siphon. How the placement of the spermatophore takes place is open to speculation, but evidently the males, always small compared to the females, have to enter through the female's siphonal aperture to get access to the siphon's interior, and then to the gill.

Embryonic development to the D-shaped larval stage takes place in the siphon as in *E. mirabilis* (Voeltzkow 1890). Voeltzkow observed how the progeny vigorously rotated within the siphon, just as is the case in the allied *Anisodevonia ohshimai* (see Lützen *et al*. 2005). This may be by the combined action of the cilia in the interior epithelium and currents generated by the gills. How the larvae are released into the water is not known for the present or other species of *Entovalva*. It is possible, as suggested by Voeltzkow (1890) and Kato (1998), that the larvae are able to pass freely along the entire length of the host gut to be expelled together with the faeces. From the considerable size difference in the shell of the D-shaped larva and prodisscoconch II a planktotrophic phase of some length is inferred. The easy accessibility of *E. nhatrangensis* means that further studies could well resolve some of the remaining problems related to the feeding, reproductive and larval biology of this species.

Acknowledgements

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