

Anatomy and systematics of bathyphytophilid limpets (Mollusca, Archaeogastropoda) from the northeastern Pacific

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Accepted 28 September 1995

Haszprunar, G. & McLean, J. H. 1995. Anatomy and systematics of bathyphytophilid limpets (Mollusca, Archaeogastropoda) from the northeastern Pacific.—*Zool. Scr.* 25: 35–49.

Bathyphytophilus diegensis sp. n. is described on basis of shell and radula characters. The radula of another species of *Bathyphytophilus* is illustrated, but the species is not described since the shell is unknown. Both species feed on detached blades of the surfgrass *Phyllospadix* carried by turbidity currents into continental slope depths in the San Diego Trough. The anatomy of *B. diegensis* was investigated by means of semithin serial sectioning and graphic reconstruction. The shell is limpet-like; the protoconch resembles that of pseudococculinids and other lepetelloids. The radula is a distinctive, highly modified rhipidoglossate type with close similarities to the lepetellid radula. The anatomy falls well into the lepetelloid bauplan and is in general similar to that of Pseudococculinidae and Pyropeltidae. Apomorphic features are the presence of gill-leaflets at both sides of the pallial roof (shared with certain pseudococculinids), the lack of jaws, and in particular many enigmatic pouches (bacterial chambers?) which open into the posterior oesophagus. Autapomorphic characters of shell, radula and anatomy confirm the placement of *Bathyphytophilus* (with *Aenigmabonus*) in a distinct family, Bathyphytophilidae Moskalev, 1978. As revealed by a cladistic study, the Bathyphytophilidae should be classified within the Lepetelloidea close to the Lepetellidae, Pyropeltidae, and Pseudococculinidae. © 1996 The Norwegian Academy of Science and Letters.

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Introduction

Wolff (1976, 1979) studied the usage of plant remains as the food source for diverse groups of macrofaunal invertebrates in the Puerto Rico and Cayman Trenches. He figured (Wolff, 1976: 167, fig. 2K) some minute (2–3 mm) limpets obviously feeding on detached blades of the turtle grass, *Thalassia testudinum* (Hydrocharitaceae). This limpet species was subsequently described by Moskalev (1978) as *Bathyphytophilus caribaeus* in the new family Bathyphytophilidae. At the same time, Moskalev also added a second genus and species, *Aenigmabonus kurilokamtschaticus*, from the abyssal Kurile–Kamchatka Trench. Moskalev (1978) described shell, external morphology, and radula of these species and observed brooding in *B. caribaeus*.

In this paper, we first illustrate the bathyphytophilid radula with SEM and provide details on the internal anatomy of the genus. We describe a second species of *Bathyphytophilus*, *B. diegensis* sp. n., from continental slope depths in the San Diego Trough, eastern Pacific Ocean, and illustrate the radula of a third species, also from the San Diego Trough. The latter species is not named because the shell is unknown. We provide the first description of the anatomy of a bathyphytophilid limpet.

The anatomy of all other known families of the Cocculini-formia (Addisoniidae, Cocculinidae, Cocculinellidae, Osteopeltidae, Pseudococculinidae, Choristellidae; cf. McLean & Haszprunar, 1987; Haszprunar 1987a,c, 1988a,b, 1992) has been outlined in detail or given at least briefly (Bathysciadiidae, Lepetellidae; cf. Haszprunar 1988c, 1996). This paper provides the missing information on the solely known* remaining family for which anatomical information was previously lacking.

In order to clarify the systematic position of the Bathyphytophilidae within the Lepetelloidea, and the interrelationships of the remaining lepetelloidean families, a cladistic analysis was utilized.

Material and methods

External morphology and anatomy

Sources and localities for the two species of *Bathyphytophilus* are detailed in the systematic section that follows. All specimens are

* According to Warén (1991: 82) a new family might be necessary for *Pilus conica* (Verrill, 1884), for which the anatomy and radula are unknown.

deposited in the Los Angeles County Museum of Natural History (LACM).

In addition to standard photography, shells were examined with SEM. Radulae were examined using SEM, following extraction by dissolution of tissues with 10% NaOH at room temperature for 48 hr, washing in distilled water, drying from a drop of distilled water, and coating with gold palladium.

A single paratype specimen of *Bathyphtophilus diegensis* was prepared for semithin serial sectioning. The specimen was originally preserved in 70% ethanol, which resulted in a good fixation, although histological details cannot be given satisfactorily for all tissues (see results). After dehydration, the specimen was embedded in plastic (araldite) and serially sectioned (2 μm) with "Ralph-knives" according to the method of Smith & Tyler (1984). Monochromatic staining was done by Methylene Blue (Richardson *et al.*, 1960).

The plastic block was trimmed symmetrically, so that the edges of the sections could be used as reference lines. Before embedding, the specimen was photographed from dorsal and lateral perspectives, so that the projected contours could be used as reference lines in addition to the symmetry plane of paired structures such as pedal cords or shell muscle. For graphic reconstruction, each section was represented by a transverse line, and the structures and organs were projected onto a horizontal plane. After some jagged lines had been smoothed, the graphic reconstructions were used as illustrations with some shading and semischematic patterning added.

Cladistic analysis

Large agreement exists that the monophyly of the lepetelloidean families is generally well founded on autapomorphic conditions of radula and anatomy (Marshall 1983, 1986; McLean 1985, 1988; McLean & Haszprunar 1987; Haszprunar 1987a, c, 1988a, b, c, d, 1992, 1996; Dantart & Luque 1994). The only exception concerns the Pseudococculinidae, the status of which as a holophyletic versus a paraphyletic taxon is still open (Haszprunar 1988). Accordingly, we coded the two nominal subfamilies, Pseudococculininae and Caymanabysiinae, as separate taxa.

The cladistic analysis was done using PAUP 3.1.1. (Swofford 1993). Coding of characters (Tables II, III) followed the NEXUS-format of PAUP. Each character state was represented by a numeric value; unknown or questionable states were coded by '?'. Multistate characters were generally considered as unordered, all characters being equally weighted. Generally the 'heuristic' and 'branch and bound' options of PAUP 3.1.1. were applied to create the trees, and the ACCTRAN option was applied to optimize the character states. The trees were rooted by using the outgroup method.

During the analysis, it became increasingly clear that the topology of the trees heavily depends on the selected outgroup(s). Because a thorough discussion on the relationships of cocculiniform groups among the Gastropoda is beyond the scope of this contribution, we decided to use an artificial outgroup. By coding the respective characters with '?', we left open the controversial question of whether the lepetelloidean ancestor was a primary limpet (Haszprunar 1988d) or a coiled organism (Ponder & Lindberg 1995).

Systematics

Superfamily LEPETELLOIDEA Dall, 1892

Family BATHYPHTOPHILIDAE Moskalev, 1978

The family includes two monotypic genera: *Bathyphtophilus*, type species *B. caribaeus* from 5800–6500 m in the western Atlantic Cayman Trench, in which the protoconch was said to have "large pitted sculpture" and the radula five pairs of marginal teeth, and *Aenigmabonus*, type species, *A. kurilokamtschaticus* from 6160–6120 m in the northwest Pacific Kurile–Kamchatka Trench, in which the protoconch was said to have a "micropitted sculpture" and the radula 20 pairs of marginal teeth per row.

The new family was mainly justified on the food source of detached seagrasses transported to the deep sea and the corresponding radular distinctions. A study of the internal anatomy reported in this paper supports the family level distinction (see discussion part).

Genus *Bathyphtophilus* Moskalev, 1978

Description. Shell small, thin and fragile, elevated, apex at one-quarter length of shell from posterior margin, below highest elevation of shell; anterior slope broadly convex, posterior slope slightly concave; sculpture of fine concentric growth lines and faintly indicated radial striae. Protoconch with lateral indentation and flared lip, partially immersed in posterior slope; sculpture of numerous longitudinal rows of pits.

Radula with rachidian, four lateral elements (small pair of inner laterals, large second lateral here termed the pluricuspid, inner and outer basal elements) and three to five pairs of reduced marginals. Tooth rows markedly asymmetric, resulting from alternating positions on left and right sides of massive pluricuspid teeth. Rachidian broad, membranaceous, shaft broad, tip overhanging, overhang with single centrally placed, weakly projecting denticle, edge of overhang slanted down to right, corresponding to asymmetry derived from alignment of pluricuspid teeth; rachidian also with narrow lateral flanges, usually obstructed by pluricuspid teeth. Inner lateral teeth reduced, mostly hidden behind pluricuspid teeth, tips with single cusps. Pluricuspid with broad base; shaft massive, concave on inner side, outer edge continuous with overhang, evenly convex; entire tip projecting inwardly, tip with four cusps, an acutely tapered main cusp, strong lesser cusps on either side and a small nub-like outer cusp. Edge of ribbon bordered by flat-surfaced outer basal element, forming nearly straight outer edge. Inner basal plate exposed only near outer base of pluricuspid tooth. Marginal teeth three to five pairs, vestigial, appearing to arise between inner and outer basal plates below base of pluricuspid; shafts of marginals relatively short, strap-like, curved; tips finely digitate.

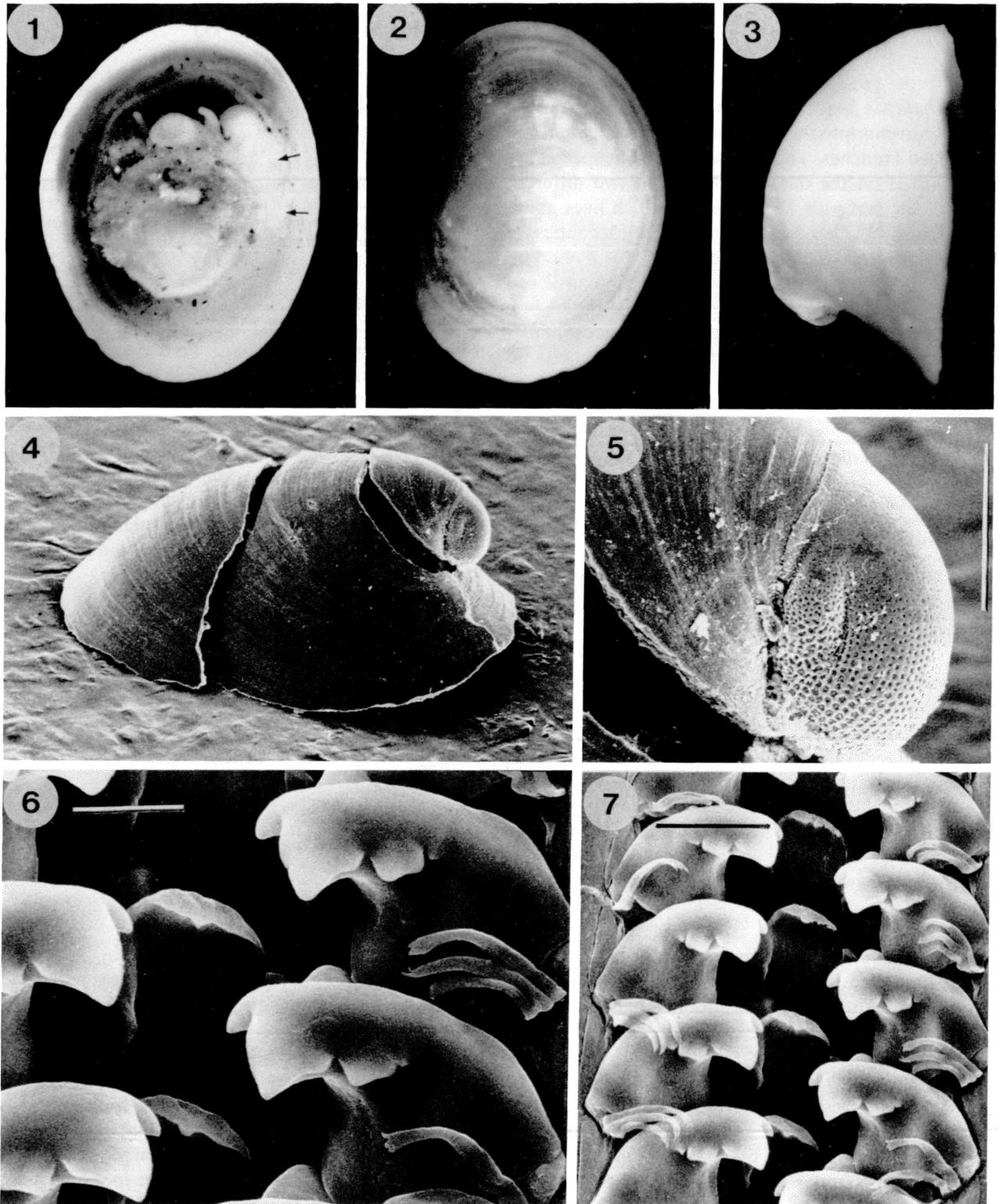
Snout broad, eyes and oral lappets lacking, single posterior pair of epipodial tentacles; smooth cephalic tentacles moderately long in preserved condition, right cephalic tentacle with ciliary band extending along neck to genital opening; shell muscle horseshoe-shaped, markedly asymmetrical. A single, reduced gill-leaflet at the left mantle roof, many well-developed gill-leaflets at right mantle roof and in right subpallial groove; gill-leaflets with sensory pockets but lacking skeletal rods. No hypobranchial gland; heart large and monotocardian. Left kidney small and pallial, large right kidney ramifies between viscera at left side. Testis and ovary separated, with a common hermaphroditic gonoduct; a urinogenital opening with the right kidney; broader. No jaws, no salivary glands (?) and two pairs of radular cartilages. Anterior oesophagus with dorsal food channel and lateral pouches, posterior oesophagus uniquely embedded in a mass of enigmatic pouches (bacterial chambers?). Stomach large, two digestive glands with food particles inside. Very long intestine shows several loops, rectum runs through ventricle. Nervous system streptoneurous and hypoathroid; osphradial epithelium well developed, statocysts contain several small statoconia.

Remarks. The radular description given above is based on the SEM micrographs of the two species treated here. Moskalev's (1978) radular description and drawing for

Bathyphytophilus caribaeus were based on polarizing and phase contrast microscopy of the type species. His drawing shows a larger inner basal plate that underlies the entire base of the pluricuspid tooth. The extent of that tooth is not directly confirmed by our SEM micrographs, but it may be that Moskalev's rendition is correct, the

process of drying the radula for SEM study resulting in shrinkage in our preparations.

Sizes of the limpets are similarly small in the three known species of *Bathyphytophilus*, although the width of the blades for each seagrass differs. The type species occurs on detached blades and rhizomes of the broad-



Figs 1-7. *Bathyphytophilus diegensis* sp. n. from San Diego Trough, 1.224 m.—1-3. Holotype, anterior at top, shell length 1.9 mm.—1. Ventral view of intact specimen, showing snout, projecting cephalic tentacles, foot sucker, and brooded eggs (arrows) in left subpallial cavity.—2. Dorsal view of shell.—3. Right lateral view of shell.—4-5. Paratype, SEM views of left side of shell showing protoconch; shell broken during mounting. Length 1.1 mm.—4. Left side showing protoconch.—5. Protoconch, scale bar = 100 μ m.—6-7. SEM views of radula of paratype.—6. Scale bar = 10 μ m.—7. Scale bar = 20 μ m.

Table 1. Dimensions (in mm) of type material of *Bathyphytophilus diegensis*

No.	Length	Width	Height	Remarks
1	1.9	1.5	1.0	Holotype
2	1.9	1.4	—	Sectioned, shell intact
3	1.7	1.2	—	Shell only
4	1.1	—	—	SEM, shell and protoconch
5	0.8	0.6	—	Shell broken
6	0.7	0.4	—	
7	0.6	0.5	—	

bladed turtle grass *Thalassia*, which lives in shallow water. *Thalassia* blades are commonly transported by turbidity currents into the hadal depth of the Cayman and Puerto Rico Trenches. The two eastern Pacific species are here reported on the detached blades of two different species of the surfgrass *Phyllospadix*, which lives abundantly along rocky shores in California. Type localities for each are close to the base of Coronado Canyon at continental shelf depth on the eastern flank of the San Diego Trough. Turbidity currents frequently occur in the submarine canyons along the Californian coast. Thus there is likely to be a steady supply of food for the bathyphytophilid limpets.

Bathyphytophilus diegensis sp. n. (Figs 1–7)

Holotype. LACM 2398.

Type locality. San Diego Trough at base of Coronado Canyon, off Tijuana, Baja California, Mexico (32°18.2'N, 117°29.8'W), 1224 m.

Paratypes. Six specimens collected along with partially decomposed surfgrass *Phyllospadix scouleri* Hooker (Zosteriaceae) from type locality, collected by Peter A. Jumars, Scripps Institution of Oceanography, 6 December 1971. Six specimens, including one section series, LACM 2399. No other specimens are known.

Description. Shell (Figs 1–5) small, thin, fragile, elevated, apex at one-quarter length of shell from posterior margin, below highest elevation of shell; outline of margin asymmetric, right side more convex than left; anterior slope broadly convex, posterior slope slightly concave; sculpture of fine concentric growth lines and faintly indicated radial striae. Protoconch length 200 µm, with lateral indentation on each side and flared lip, sculpture of numerous pit rows, pits more weakly developed near protoconch rim.

Dimensions. See Table I for measurements. Radula (Figs 6, 7) as described for the genus. Lateral flanks of rachidian and tips of inner laterals concealed behind pluricuspid; three pairs of marginal teeth.

Remarks. *Bathyphytophilus diegensis* differs from *B. caribaeus* in having less pronounced concentric sculpture (compare Moskalev 1978: fig. 7), and three pairs of marginal teeth rather than five pairs. Its marginal tooth count also differs from *Bathyphytophilus* sp. treated here, which also has five pairs. Asymmetry of the shell outline, which is apparent in Figs 1 and 2, was not mentioned for the type species, which was shown only in oblique view, but such asymmetry may prove to be a specific character.

As described for the type species by Moskalev (1978),

the shell of *B. diegensis* is extremely thin and fragile, as is evident from the breakage illustrated in Fig. 4.

Leaves of the preserved plant material are 2 mm wide and are nearly flat in cross-section. It is identified as *Phyllospadix scouleri* Hooker (see Abbott 1975). The leaves of the plant are thus much wider than the width of any of the limpet specimens.

Bathyphytophilus sp. (Figs 8–11)

Material examined. LACM 147454. One specimen, body only, radula removed for SEM preparation, preserved with original material of partially decomposed surfgrass *Phyllospadix torreyi* Watson (Zosteriaceae). San Diego Trough, off Tijuana, Baja California (32°23.5' to 18.8'N, 117°31.75' to 31.01'W), R/V *Melville*, 16 December 1969, 25 foot otter trawl, 1207–1234 m.

Description of radula (Figs 10, 11) supplemental to the detailed generic description: marginal teeth pairs five; inner lateral of left side of ribbon well-formed with large tip, not hidden behind pluricuspid; lateral flanges of rachidian well marked.

Remarks. The radula differs from that of *B. diegensis* in having five rather than three pairs of marginal teeth. Other radular differences may be due to size differences and relative shrinkage of the radula during drying, exposing the flanges of the rachidian and tips of the inner laterals to various degrees. The marginal tooth count is considered to be a generic character, although it is possible that the number of marginal teeth may vary intraspecifically and that the specimen is actually conspecific with *B. diegensis*. Until further material is available, it seems prudent not to name a new species in the absence of the shell.

Leaves of the preserved plant material are 1 mm wide and are elliptical in cross-section. The host plant is identified as *Phyllospadix torreyi* Watson (see Abbott 1975). In this case, the width of the body matches the width of the leaves of the host plant. This limpet occurred on the other of the two species of *Phyllospadix*, and it may be that the two limpet species are host specific.

Anatomy and histology of *Bathyphytophilus diegensis* sp. n.

External structures

For external morphology see the diagnosis part.

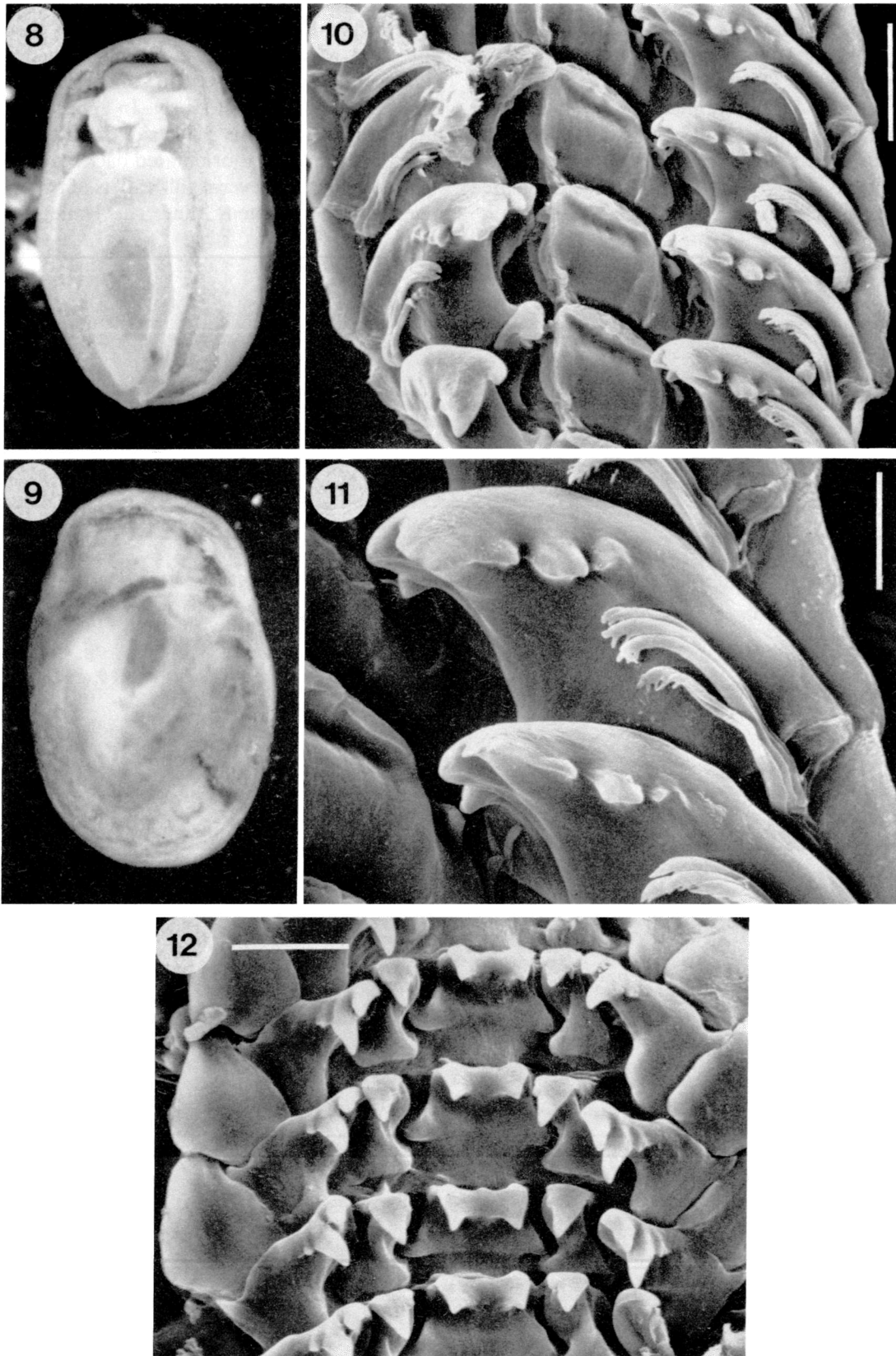
The foot sole shows two zones (Figs 8, 22). Centrally the epithelial cells are flat and lack cilia, the visible 'cuticula' of the cells is probably a microvillar border. This area is surrounded by a broad zone of elongated and densely ciliated cells. Laterally, the foot extends considerably; here the dorsal epithelium is composed of large, flat and glandular cells. A large, anterior pedal gland opens at the anterior edge of the foot sole; a distinct propodium is not present, however. Otherwise, many subepidermal glands are present, particularly laterally. A single pair of epipodial tentacles is present at the posterior end.

The mantle margin is quite simply structured. Special

sensory elements are represented by very small and short papillae; otherwise the mantle margin is smooth. The mantle sinus (i.e. a vessel lacking an endothelium) of the mantle is very wide (Fig. 16: *ms*). Gill-leaflets are present (see below).

Muscle system

The shell muscle is well developed and asymmetrically horseshoe-shaped (Fig. 13: *sm*). It is interpreted as paired because of the type of innervation (Haszprunar 1985).



Figs 8–12. Bathyphytophilidae and Lepetellidae.—8–11. *Bathyphytophilus* sp. from San Diego Trough, 1.207–1.234 m.—8–9. Intact body prior to removal of radula, length 1.5 mm.—8. Oblique ventral view; note the head without oral lappets and the two zones of the pedal sole.—9. Dorsal view.—10–11. SEM views of radula.—10. Scale bar = 20 μ m.—11. Scale bar = 10 μ m.—12. SEM view of radula of *Lepetella* sp. (730–750 m off Punta Topocalma, Chile, LACM 66–152), for comparison with bathyphytophilid radula. Scale bar = 20 μ m.

The shell muscle is a solid organ penetrated by nerves (to the mantle margin or to the epipodial tentacles) only. As usual, in limpets, the dorsoventral muscle fibres are intercrossed ventrally. The foot musculature is weakly developed (Fig. 18). In accordance with the somewhat asymmetrical shell (see *Diagnosis*), the paired head retractors of *B. diegensis* show an asymmetrical arrangement. The right retractor forms a hook at the anterior edge of the right shell muscle similar to conditions in many other lepetelloid (*Pyropelta*, *Cocculinella*, *Osteopelta*; see McLean & Haszprunar 1987; Haszprunar 1988a) or fissurelloid limpets. In contrast, the left head retractor has its insertion area much more centrally situated, while there is no connection with the insertion area of the left shell muscle (Figs. 13, 17: *hr*).

Mantle roof (Fig. 13)

The mantle cavity is very shallow in *B. diegensis*; to the left and right the 'cavity' is deeper than in the central region, which is occupied by the heart. Indeed, the central posterior border of the mantle roof is more anteriorly situated than the anterior end of the shell muscles. The anterior mantle roof contains numerous blood sinuses. The right mantle roof is mainly occupied by the left kidney and also the anus, while the urinogenital opening is positioned more posteriorly. In general, the arrangement of mantle organs reflects a 'detorted' condition in that the anal region is orientated backwards to the right.

B. diegensis has several gill-leaflets of two kinds. In the investigated specimen, nine gill leaflets occupy the

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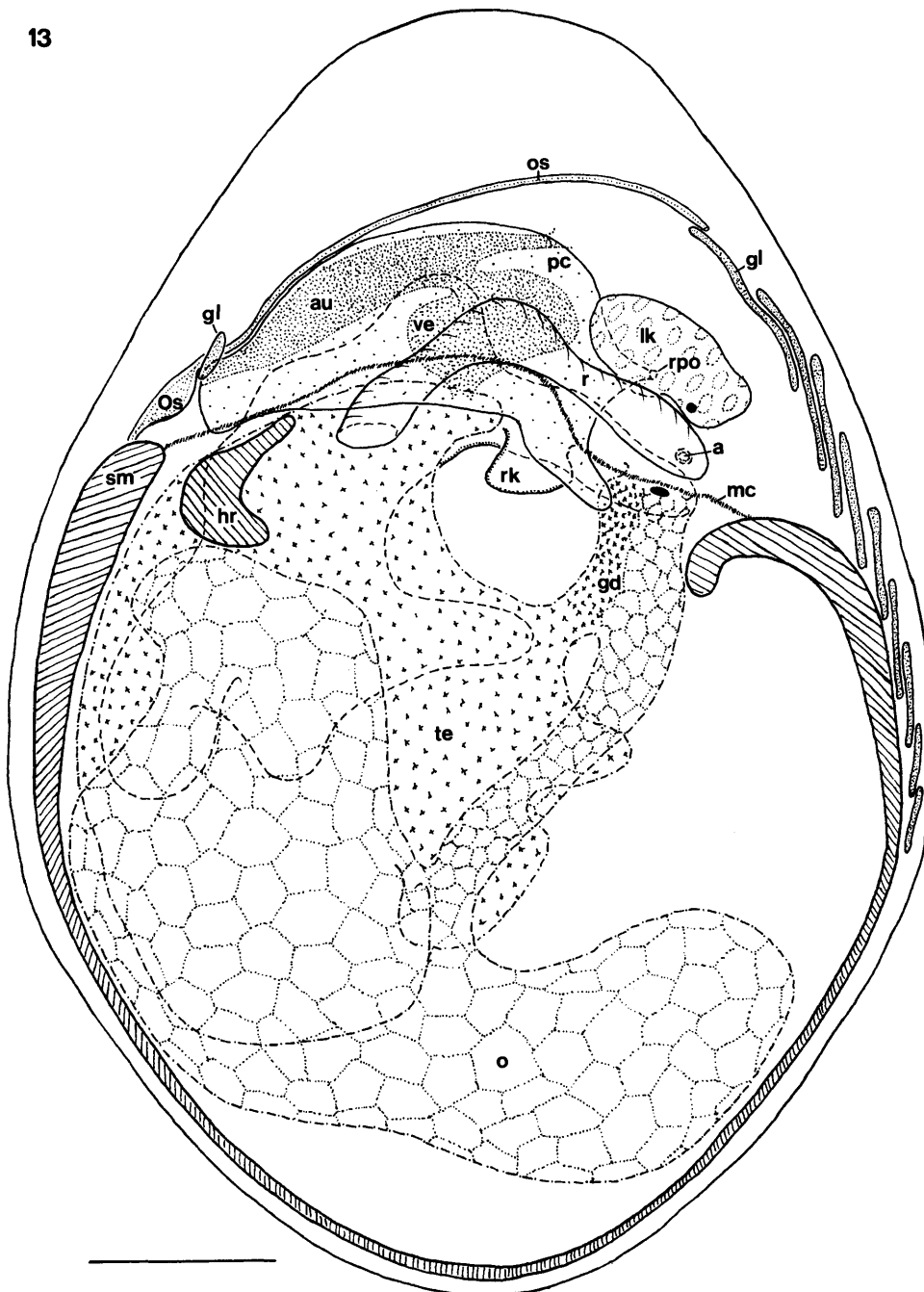


Fig. 13. *Bathiphytophilus diegensis* paratype. Mantle organs and coelomic system (dorsal view). *a* anus; *au* auricle; *gl* gill-leaflet; *gd* gonoduct; *hr* attachment zone of head retractor; *lk* left kidney; *mc* posterior end of mantle roof; *o* ovary; *Os* osphradial ganglion; *os* osphradial nerve; *pc* pericard; *r* rectum; *rk* right kidney; *rpo* renopericardial opening; *sm* attachment zone of shell muscle; *te* testis; *ve* ventricle. Scale bar: 200 μ m.

anterior right mantle roof and continue backwards into the right subpallial cavity. Posteriorly the leaflets become less and less distinct, and there is a continuum from typical gill-leaflets through simple folds to a non-differentiated epithelium covering the mantle sinus. A well-differentiated gill-leaflet is provided with a distinct efferent axis with dense and large cilia that are laterally situated (Figs 20, 21). The efferent axis also has a sensory pocket (bursicle), the short tube of which opens posteriorly and is heavily ciliated (Fig. 20: arrow). Three simple epithelial folds (respiratory), which lack sensory pockets, are situated at the outer left mantle roof adjacent to the mantle border (Fig. 17: *ml*). There is an additional, single gill-leaflet at the point of entrance of the mantle sinus into the auricle, immediately adjacent to the osphradial ganglion. This one has a well developed axis with dense ciliation and a sensory pocket, though it lacks a respiratory zone (Fig. 19).

An hypobranchial gland is lacking, but two glandular fields with very similar histology are found in front of the anterior ends of the shell muscles (see genital system).

Heart and excretory system

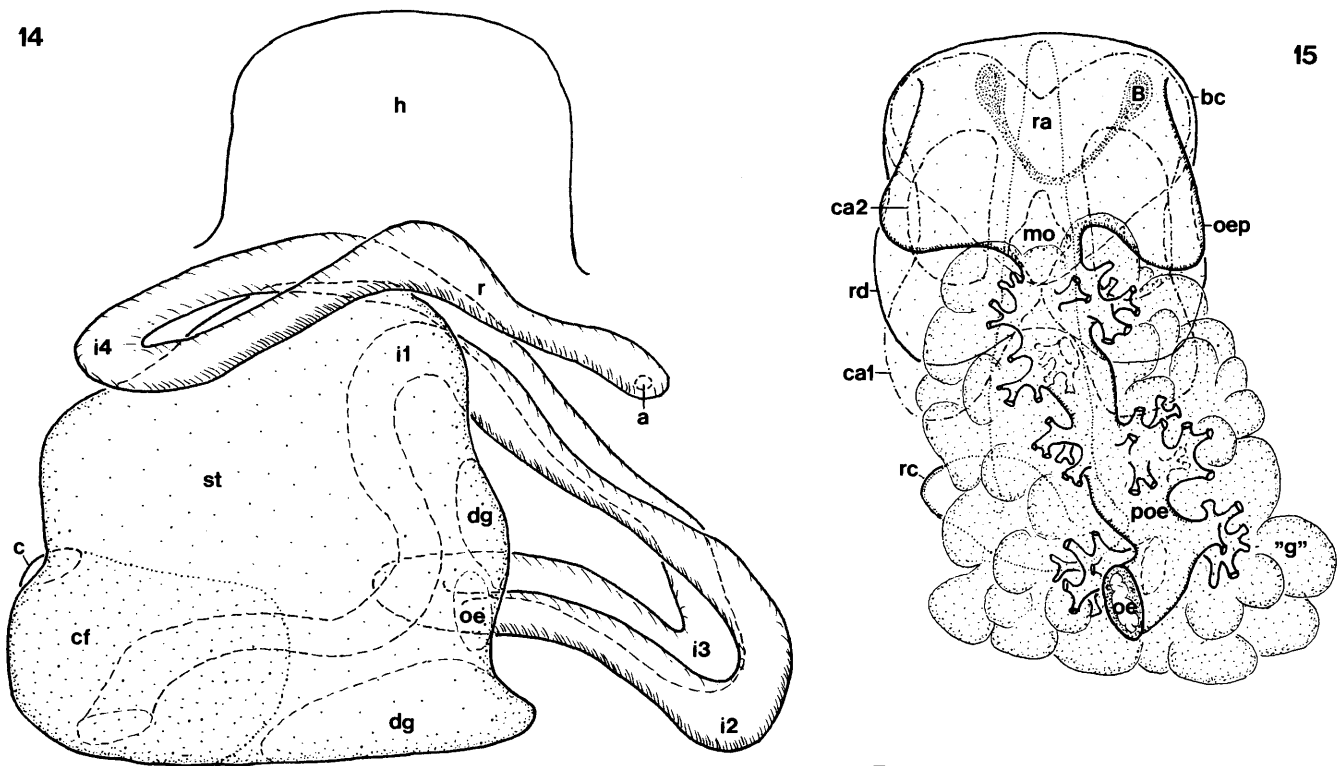
The large pericardium occupies the central and left mantle roof and includes a single, anteriorly left situated auricle and a ventricle, which is penetrated by the rectum (Figs 13, 16: *ve*). There is no pericardial gland, and the aorta is very short. The oxygenated blood from the gill-leaflets is collected by a mantle sinus, which fuses with numerous small sinuses of the anterior mantle roof before entering the auricle at the extreme right. In addition, a

second pass of blood coming from the left mantle sinus enters the auricle at the very left side.

Two kidneys are present (Fig. 13). The left one is rather small, occupies the right anterior pallial roof in front of the rectum, and is connected to the pericardium via a ventrally situated, short reno-pericardial duct. The opening of the left kidney is provided with a distinct sphincter. The right kidney differs entirely in histology from the left, and forms a system of large coelomic cavities occupying free space between the elements of the alimentary tract and gonad at the left side (Figs 17, 18: *rk*). It is not connected to the pericardium and has a common opening with the gonoduct.

Genital system (Fig. 13)

B. diegensis is a simultaneous hermaphrodite, the gonad of which is divided into testis and ovary. The testis is situated anteriorly left, while the ovary is placed dorsally and occupies the whole left posterior part of the body. All stages of ovo- and spermiogenesis are exhibited. Ripe eggs are large (up to 140 μm diameter), filled with many yolk vesicles and have a large nucleus. They are covered by a distinct, dark egg-layer (Fig. 23). Ripe sperm cells have filamentous heads. The proximal oviduct and proximal vas deferens are simple, ciliated tubes, which fuse after a short distance, with the hermaphroditic part of the gonoduct lacking accessory glands. A common urinogenital opening with the right kidney is situated at the right posterior end of the mantle roof. Ventrally, a small glandular field is present, and for a short distance, a ciliated groove can be detected. This is continued forwards as a band of ciliated and mucous cells. The ciliated



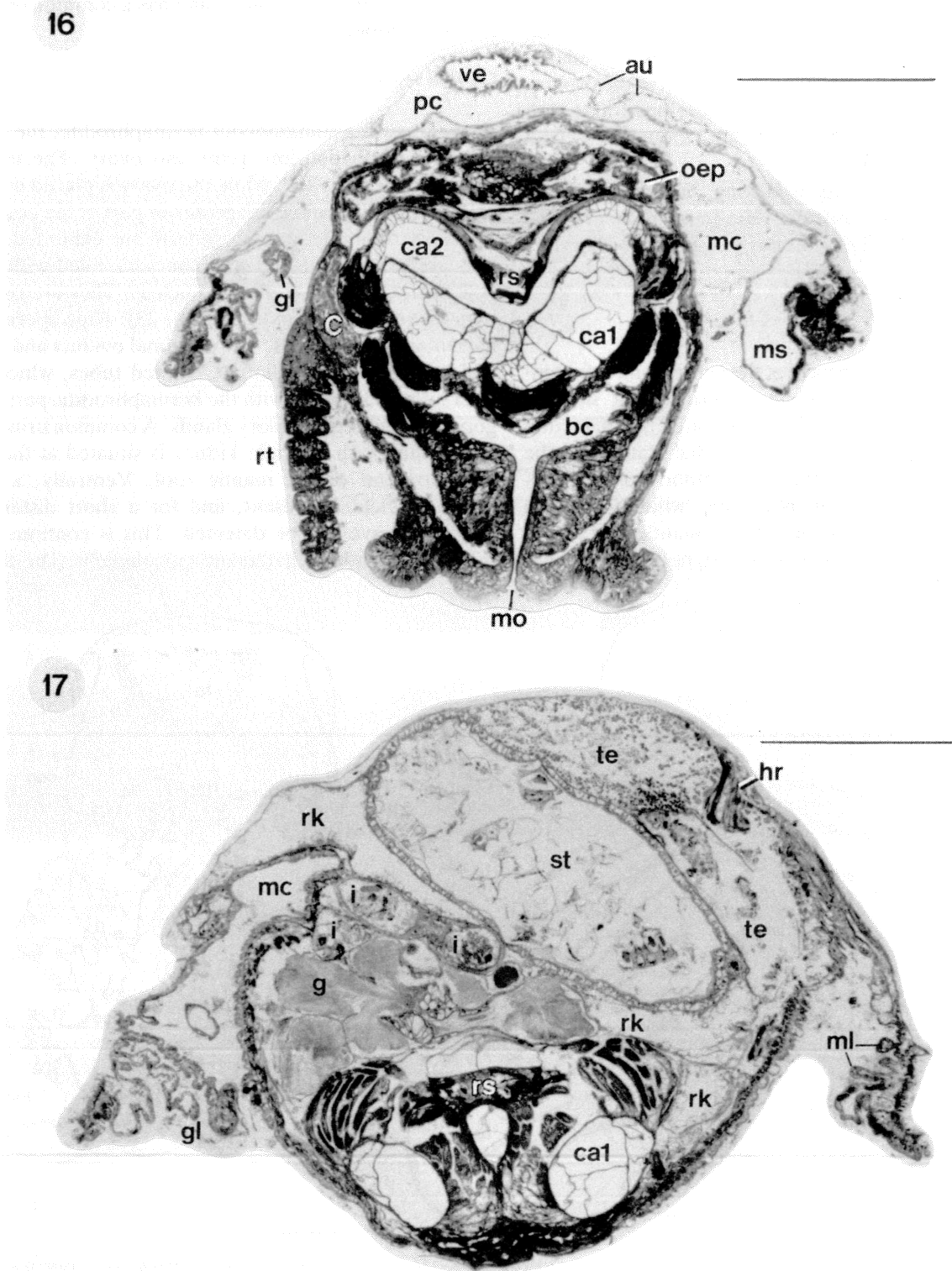
Figs 14–15. *Bathyphytophilus diegensis* paratype. Alimentary tract (dorsal view).—14. Head and posterior alimentary tract (stomach and intestine removed); the grape-like pouches are thought glassy). *a* anus; *B* buccal ganglion; *bc* buccal cavity; *c* caecum of stomach; *ca1,2* anterior and posterior cartilage; *cf* ciliary field of stomach; *dg* opening of digestive gland; *g* grape-like pouches (bacterial chambers); *h* head; *i1–4* course of intestine; *mo* mouth opening; *oe* opening of oesophagus into stomach; *oep* oesophageal pouches; *poe* posterior oesophagus; *r* rectum; *ra* radula; *rc* radular caecum; *rd* radular diverticulum; *st* stomach. Scale bar: 200 μm .

band reaches the outer wall of the right cephalic tentacle which is not otherwise specialized (Fig. 16: *rt*). There is no trace of a receptaculum. An additional glandular field, which is histologically very similar to that near the genital opening, is situated immediately in front of the anterior end of the left shell muscle.

Like many other lepetelloid limpets, *B. diegensis* broods its eggs in the pallial cavity (Fig. 1: arrow).

Alimentary tract (Figs 14, 15)

The mouth opening is ventrally situated, and its lining appears to be cuticularized (Fig. 16: *mo*). Jaws are lacking, and the short and narrow sublingual pouch is devoid of a subradular organ. Salivary glands could not be detected; however, the respective region was tangentially sectioned, so the salivary glands might not have been



Figs 16–18. *Bathyphtophilus diegensis* paratype no. 2, semithin cross-sections (frontal view, compare with Figs 13–15).—Fig. 16. Line of mouth opening.—Fig. 17. Line of attachment zone of left head retractor.