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ORIGINAL ARTICLE

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Prey capture and digestion in the carnivorous sponge *Asbestopluma hypogea* (Porifera: Demospongiae)

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Abstract *Asbestopluma hypogea* (Porifera) is a carnivorous species that belongs to the deep-sea taxon Cladorhizidae but lives in littoral caves and can be raised easily in an aquarium. It passively captures its prey by means of filaments covered with hook-like spicules. Various invertebrate species provided with setae or thin appendages are able to be captured, although minute crustaceans up to 8 mm long are the most suitable prey. Transmission electron microscopy observations have been made during the digestion process. The prey is engulfed in a few hours by the sponge cells, which migrate from the whole body towards the prey and concentrate around it. A primary extracellular digestion possibly involving the activity of sponge cells, autolysis of the prey and bacterial action results in the breaking down of the prey body. Fragments of the prey, including connective cells and muscles, are then phagocytosed and digested by archaeocytes and bacteriocytes. The whole process takes 8–10 days for a large prey. This unique feeding habit implies the capture and digestion of a macro-prey without any digestive cavity. It would appear to be an adaptation to life in deep-sea oligotrophic environments. Carnivorous sponges provide actual evidence, through a functional example, that a transition is possible from the filter-feeder poriferan body plan towards a different organizational plan through loss of the aquiferous system, a transition that has been hypothesized for the early evolution of Metazoa.

Keywords Porifera · Carnivory · *Asbestopluma* · Feeding process · Ultrastructure · Deep-sea · Evolution of Metazoa

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Introduction

Multicellular animals almost universally feed by means of a digestive tract or a digestive cavity. Apart from some parasites directly living at the expense of their host, the only exceptions are the Pogonophores (deep-sea animals relying on symbiotic chemoautotrophy and whose larvae have a temporary digestive tract) and two groups of microphagous organisms relying on intracellular digestion, the minor Placozoa and, most importantly, sponges (Porifera). Poriferans have a unique body plan as filter-feeders, with a very simple cellular organization and without any organ or specialized tissue. They intracellularly digest particles such as bacteria, micro-algae or colloids (Reiswig 1971) that are captured by means of a complex aquiferous system partially lined by flagellate cells provided with a collar of microvilli, the choanocytes. However, it has been shown recently that a carnivorous macrophage habit evolved in the deep sea poriferan taxon Cladorhizidae (Demospongiae) (Vacelet and Boury-Esnault 1995, 1996). This adaptation to the deep-sea condition of extreme oligotrophy and unpredictable food supply may be seen as a remarkable modification of the poriferan organization.

Contrary to representatives of Hexactinellida, other typical deep-sea poriferans that adapted to the deep-sea oligotrophic environment by optimizing their system of circulation and filtration of water (Perez 1996), these aberrant cladorhizid poriferans have reduced or even lost both the aquiferous system and the choanocyte cells diagnostic of Porifera, and are no longer filter-feeders. They preserve, however, a skeletal and cellular organization typical of poriferans, with siliceous spicules (mycalostyles and chelae; see Hajdu and Vacelet 2002) similar to those of Demospongiae from the taxon Poecilosclerida, and highly mobile, poorly differentiated cells. They are thus indisputable members of Porifera, although not covered by

the present conventional definition of the taxon (Bergquist 1978; Hooper and van Soest 2002). These animals feed on macroscopic prey that is captured and digested without any digestive cavity or specialized organ, a unique case in metazoans.

In the cladorhizid species, prey digestion occurs within animals that are composed of a matrix and slightly differentiated, very mobile cells not grouped into specialized tissues or organs, thus representing a typical poriferan organization despite the lack of an aquiferous system. This implies the intervention of a unique mechanism, by which the cells would individually feed by phagocytosis, after responding in a coordinated way to attraction by the prey and by ensuring its primary degradation, very likely through enzymatic action. This change in the conventional sponge organization could be of pivotal interest in the understanding of the early evolution of Metazoa. There is some evidence that all non-poriferan metazoans evolved from a sponge-like organization (Borchiellini et al. 2001; Collins and Valentine 2001; Medina et al. 2001). If this were true, the poriferan system of water circulation must have been lost during this evolution (Dewel 2000). Carnivorous sponges provide evidence, through a functional example, that such a loss can occur along with an altered mode of nutrition, resulting in an organization able to perform carnivorous feeding.

We present here the morphological and cytological phenomena accompanying prey capture and digestion in the species *Asbestopluma hypogea* Vacelet and Boury-Esnault, 1996, a species that lives in littoral caves of the Mediterranean and can easily be held under laboratory conditions. This species closely resembles by its shape and anatomy the deepest known sponge, *Asbestopluma occidentalis* (Lambe, 1883), which has been collected at a depth of 8,840 m (Koltun 1970) and which most likely has the same feeding habit. It is thus a very representative example of the trophic adaptations of abyssal or even hadal sponges.

Material and methods

Experimental animals

The study has been performed on the Cladorhizidae *A. hypogea*. The specimens were collected by scuba diving in the 3PP cave near La Ciotat on the Mediterranean coast of France (Vacelet et al. 1994), 18–20 m depth, 15–20 m from the cave entrance, where the seawater temperature shows only slight seasonal variations (from 13 to 16.5°C). The animals were collected by detaching fragments from the rocky cave wall and transferred to 1.5- to 3-l tanks in the laboratory, taking care to avoid increase in temperature above 16°C during transfer. They were kept in a dark chamber at a temperature of 13°C, with a biweekly or monthly change of seawater, carried out with care in order to avoid entanglement of the sponge filaments which are very sensitive to water movements. Bubbling, which also causes the filaments to entangle, proved to be unnecessary. The animals are able to live and reproduce for several years under these conditions, with a monthly or bimonthly feeding with living nauplii of the brine shrimp *Artemia* sp., although they proved able to sustain longer periods without food.

Feeding experiments

After collection, the animals were kept for 2 weeks without food in order to avoid interference with their natural food in the cave and to let them fully expand their filaments, which were temporarily entangled after collection. Specimens with a stalk 8–14 mm high and a body 4–8 mm long were then fed with living nauplii of *Artemia* sp. Three feeding series were carried out for 7, 8, and 11 days with nauplii 22, 13, and 28 days old, respectively, and from 1 to 5 mm long. After feeding, one specimen was fixed every day and processed for light and electron microscopy. Other specimens were also fed with a variety of other organisms considered as potential prey, without histological study: live or deep-frozen Mysidae *Hemimysis speluncola* Ledoyer, 1963 and *H. margalefi* Alcaraz et al., 1986 (Crustacea), trochophore larvae of *Malacoceros fuliginosus* (Claparède, 1870) (Annelida), small unidentified specimens of pycnogonids, ophiuroids and young fishes (Teleostei), pure culture of cells of *Agmenellum* sp. (Cyanobacteria) and *Tetraselmis striata* Butcher (Prasinophyceae), and deep-frozen mosquito larvae available on the market as food for aquarium fishes (Zolux). Trapping efficiency for various live crustaceans was also tested on formalin-killed or glutaraldehyde-killed sponge specimens.

Microscopy

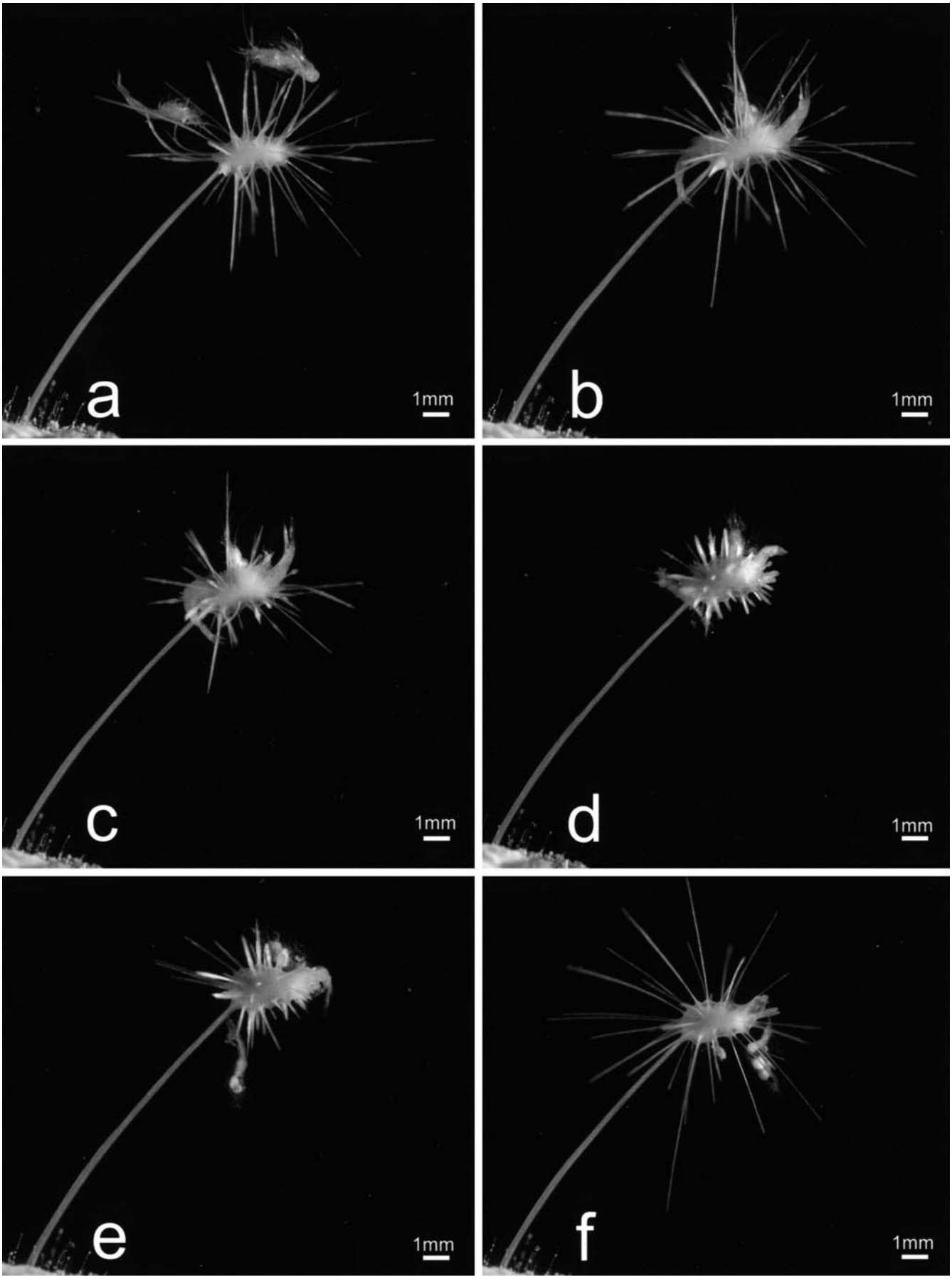
The sponges were fixed in 2.5% glutaraldehyde in a buffer composed of 0.4 M sodium cacodylate/sea water (1/1) for approximately 20 h, followed by postfixation in 2% osmium tetroxide in seawater for 2 h and desilicification in 5% hydrofluoric acid for 30 min. The specimens were embedded in Araldite for light and transmission electron microscopy (TEM). Semithin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Zeiss EM 912 electron microscope. Scanning electron microscopy (SEM) was performed on specimens critical-point dried, sputter-coated with gold–palladium and observed under a Hitachi S 570 microscope.

Results

Structure

Artemia salina (Linné, 1758), *H. speluncola* and *H. margalefi* are trapped by their setae in the dense cover of the hook-like microsclere spicules present on the surface of the filaments laterally arising from the poriferan body (Figs. 1, 2, 3a–c). The filaments, 30–60 in number, with an axis of longitudinally arranged megasclere spicules, are up to 10 mm long and 50–80 μm in diameter in a sponge of adult size under natural conditions in the cave (stalk 12–14 mm long, body up to 8 mm long and 1.2 mm in diameter). They could be slightly longer in specimens starving in an aquarium for several weeks. The microsclere spicules (Fig. 3c–e) are palmate anisochelae, 9–13 μm in length. They are anchored in the filament by

Fig. 1a–f Time-lapse photographs of the capture and digestion of two deep-frozen mysid shrimps *Hemimysis speluncola* by the carnivorous sponge *Asbestopluma hypogea*. **a** t=0, capture of two mysids. **b** t=14 h, beginning of engulfment. **c** t=26 h, process of engulfment; with shortening of filaments. **d** t=3.5 days, with short filaments and prey completely engulfed. **e** t=5 days, rejection of the carapace of one mysid and new growth of filaments. **f** t=8 days, rejection of the second carapace; the filaments have resumed their normal length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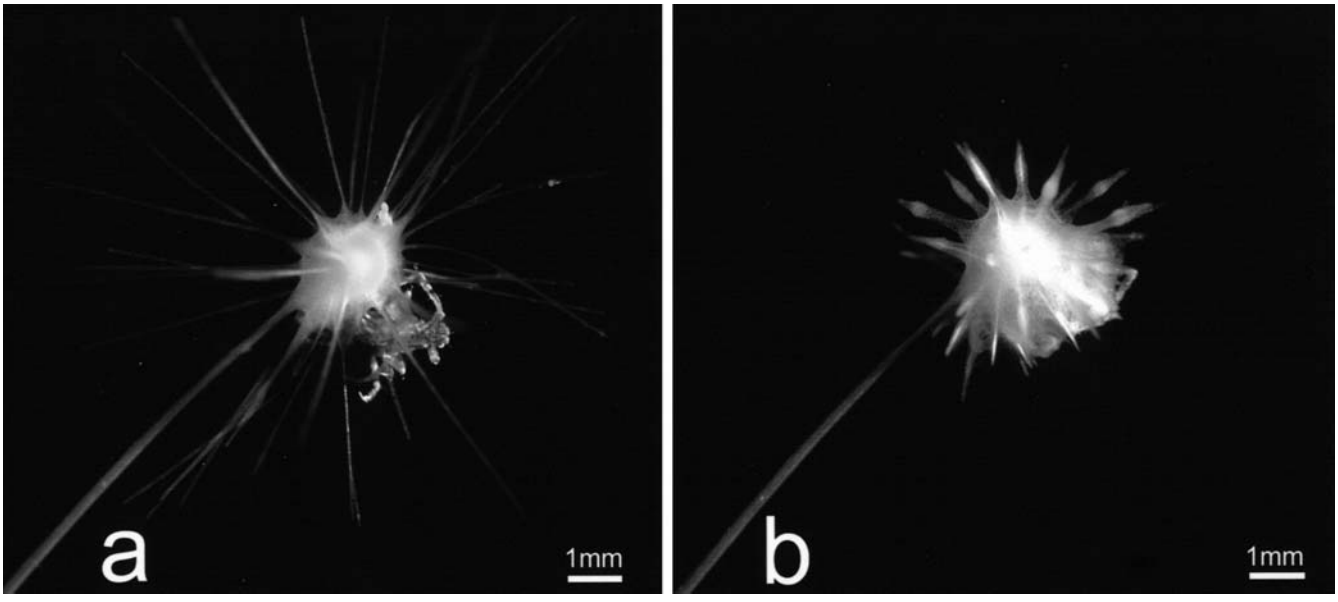


Fig. 2a, b Time-lapse photographs of the capture and digestion of a pycnogonid. **a** t=0, capture. **b** t=2.5 days, prey engulfed except one leg; cells of the filaments migrating towards the body

their small end composed of three denticulate basal teeth, with a hook-like protruding distal tooth, 5–6 μm long, making an angle of approximately 40° with the shaft (Fig. 3d). A fascicle of collagen fibrils, running through a hole between the three basal teeth, strengthens the anchoring of the spicule in the underlying mesohyl. The protruding part of the spicule is lined with a thin layer of cytoplasm from the sclerocyte in which the spicule is enclosed, with an elongate nucleus lining the shaft (see Vacelet and Boury-Esnault 1996).

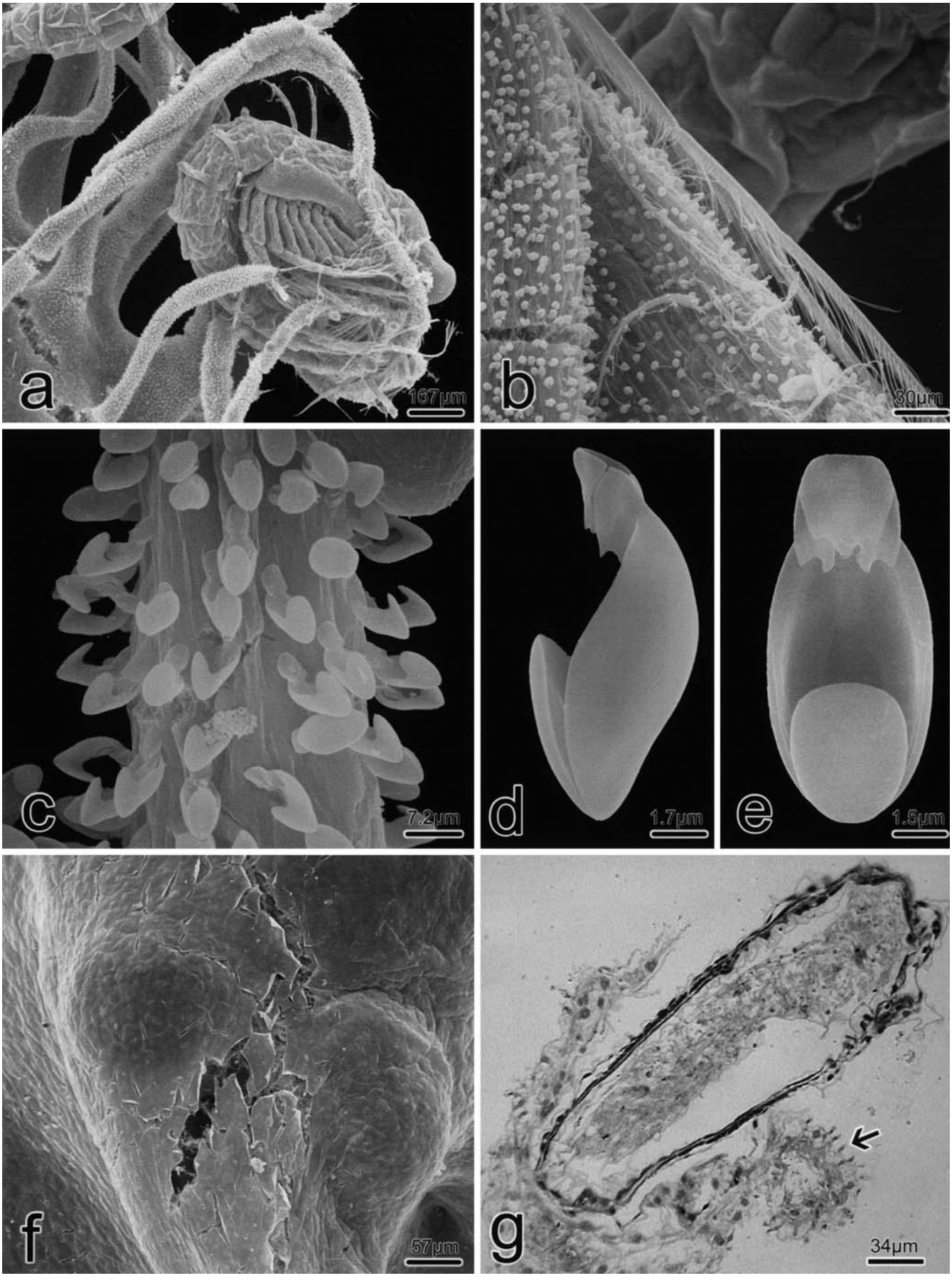
Prey capture of natural and artificial diet

The setae or thin appendages of the prey are caught within a maximally 2.5-μm-wide space, situated between the tooth of the large end and the shaft of the spicule. The spicules are preferentially arranged on the filament, with the hook-like large tooth turned towards the body. This arrangement strengthens the hold by the spicules when the prey pulls away from the sponge in an attempt to escape. When a prey is artificially pulled out, several anisochelae remain attached to its setae. The cover of spicules of the filaments is absent from the remaining sponge body. Regression stages in which the sponge is reduced to a peduncle and a round body devoid of filament are unable to catch prey.

The small prey such as nauplii of *A. salina* or small copepods are usually trapped and engulfed by a single filament. After being caught by one or more filaments, the larger prey, which most often vigorously struggle for several hours, become entangled in a larger number of filaments. The prey dies after a few hours, probably by asphyxia after partial engulfment by the sponge cells. Toxic action from the sponge cannot be excluded, although it appears rather unlikely given the time during which the struggling prey remains alive (2–3 h for *Artemia* nauplii and up to 6 h for a large mysid. There is no evidence for toxins nor for any attraction of the prey and *A. hypogea* is not bioluminescent.

In the cave environment, the most important natural prey appears to consist of different, up to 2-mm-long representatives of the crustacean taxa Copepoda, Isopoda and Ostracoda, which have been observed frequently in various stages of engulfment on the filaments of sponges after collection from the cave. The natural diet also includes small polychaete worms (Phyllodocidae and others) which have been observed trapped on several specimens. Large populations of the mysids *H. speluncola* or *H. margalefi* occurring in the cave and possibly performing circadian migrations as in other caves (Passelaigue and Bourdillon 1986) are potential prey of larger size, which are readily eaten in the aquarium. However, *A. hypogea* individuals that actually do indicate digestion of large prey by having their filaments retracted and by reorganizing their body are uncommon in the cave population. This observation leads us to suggest that the main natural diet consists of tiny crustaceans rather than relatively large prey.

Fig. 3a–g Scanning electron microscopy. **a** Capture of a living mysid, entangled in several filaments, t=15 min. **b** Detail of mysid setae caught in the anisochelae microscleres of the filament, t=15 min. **c** Detail of a filament showing the cover of hook-like anisochelae (sponge body towards the *top*). **d**, **e** Side and face view of anisochelae. **f** Head and two eyes of a mysid covered by sponge cells, t=16 h. **g** Light microscopy, semithin desilicified section showing part of an *Artemia* nauplius in the process of being covered by sponge cells; note the section of a filament with sclerocytes of anisochelae (*arrow*), t=12 h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Artificial feeding in the laboratory confirms that the most efficiently trapped prey are crustaceans, whose setae appear particularly well suited to be caught securely in the microsclere cover of the sponge filaments (Fig. 3b). A sponge of adult size (body 8 mm long) is able to simultaneously capture more than 20 newly hatched nauplii of *A. salina* or up to three 5- to 8-mm-long individuals of the tested Mysidacea. Other small invertebrates provided with setae or very thin appendages, such as adults or larvae of polychaetes, pycnogonids (Fig. 2a, b) and small ophiuroids, are suitable prey, although the capture is less successful than with crustaceans. This prey, especially polychaete larvae, more frequently succeed in escaping, probably because of the absence or reduced number of setae, appendages too large to pass between the shaft and the tooth of the anisochelae, and good swimming ability. Individuals of both mysid species that exceed 8 mm in length are strong enough to escape, either by breaking the filaments or by pulling out the hooked microscleres. After contact with the sponge and an escape from the trap, these large mysids always show a strong reaction, with abrupt, rapid swimming for a few seconds. *Asbestopluma hypogea* is also able to engulf and digest deep-frozen mysids or mosquito larvae. The engulfment of such dead prey, however, takes longer, due to the fact that they are not entangled in as many filaments as struggling prey (Fig. 1), but the further digestion process is the same as with living prey. Ciliates, flagellates or small individuals of Polycladida (Plathelminthes) that inhabit the experimental aquarium were not captured, and have frequently been observed safely gliding or swimming along the sponge filaments. Pure culture of Cyanobacteria or Prasinophycea, or young fishes even of an appropriate size, were not captured. Occasionally, leaf fragments of the Magnoliophyta *Posidonia oceanica* (L.) Delile were observed temporarily adhering to the filaments, but they were never engulfed.

Although the capture appears to be entirely passive, with the number of involved filaments depending on the size and the struggling activity of the prey, formalin-killed or glutaraldehyde-killed sponge specimens have a very reduced trapping efficiency for living prey, possibly indicating an adhesive role of the live cytoplasmic lining of the spicule.

After capture, morphological changes, more or less extensive according to the size and number of the prey, are observed in the sponge (Figs. 1, 2; video in Electronic Supplementary Material). The response is clearly linked to the size of the prey. A small prey is usually engulfed by a single filament, which becomes shorter and thicker, so that the prey eventually becomes enclosed in the lacunae lining the edge of the sponge body, producing only a local rearrangement, whereas a mysid as large as the sponge body produces the complete loss of filaments and dramatic changes in the body shape (Figs. 1, 2) due to cell migrations. Large prey entangled in several filaments causes a swelling of the body and a shortening of all filaments, which may completely disappear, including those that are not in direct contact with the prey. Shortening of filaments is most probably due to migration of cells towards the body, where the prey is engulfed. Swellings due to accumulation of cells in the middle part of the filaments are often visible (Fig. 2b). The spicule and spongin axis of the filaments are eliminated, with free megasclere spicules protruding for a while at the tip of the regressing filaments. The mature spermatic cysts which are often abundant on the filaments (Vacelet and Boury-Esnault 1996) are also probably released, even though not directly observed. These changes in filament morphology differ from those observed when the filaments are accidentally entangled in each other or when debris is deposited on their surface. In such cases, the filaments disentangle or become free of debris within a few hours, without notably shortening.

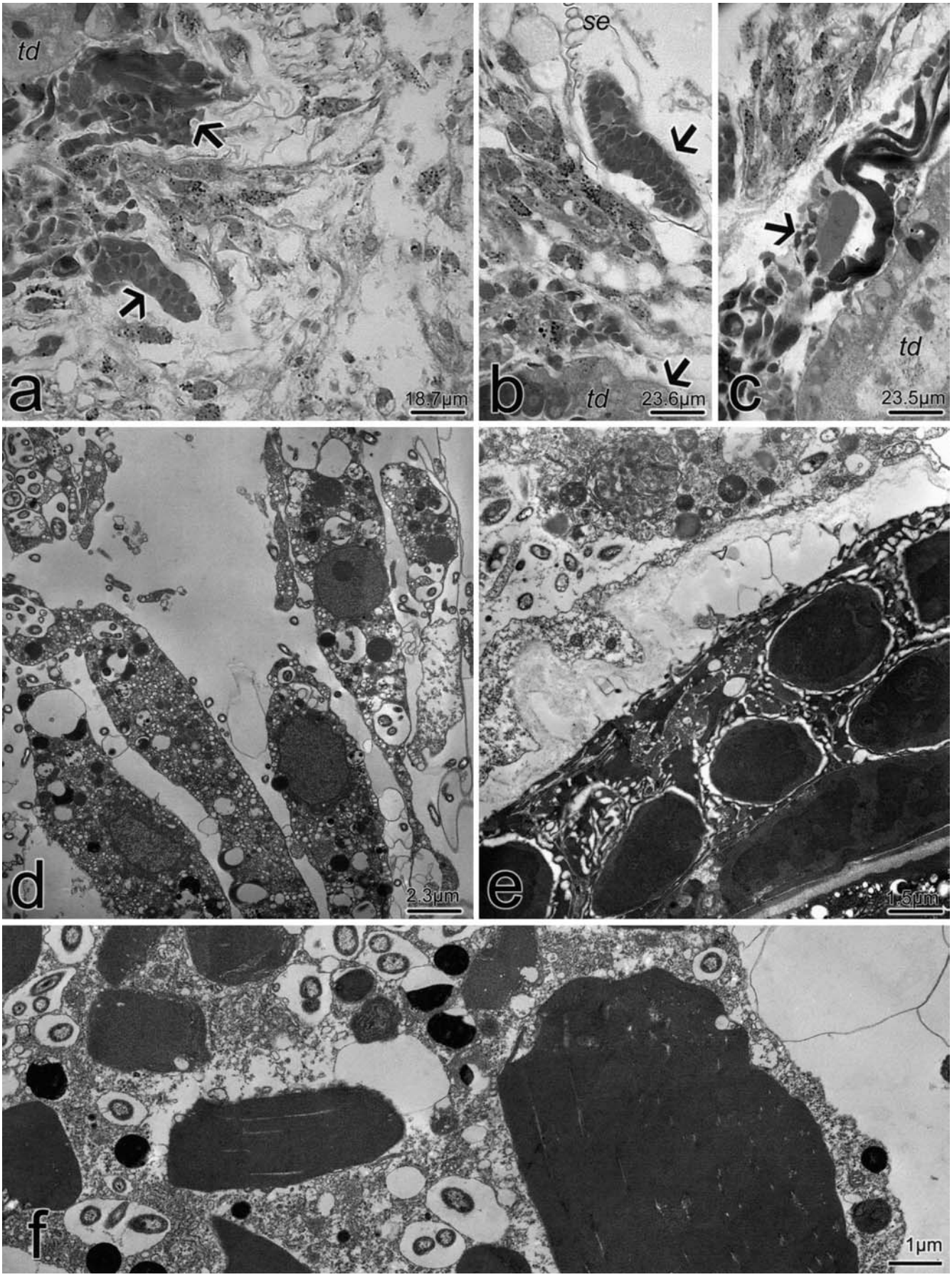
Digestion

Digestion occurs in three steps. In the first 12–24 h, the prey is engulfed by a covering of pinacocytes. Secondly, the prey is surrounded by various sponge cells and undergoes extracellular degradation, resulting in the breaking down of its body and the release of its elements in the sponge tissue. Thirdly, the fragments of the prey body are phagocytosed and intracellularly digested by the sponge cells. During this last step, part of the crustacean is rejected, while the sponge begins to elongate new filaments. The duration of the whole process varies with the size and type of the prey. The mean time for resuming a resting morphology after feeding on a 5- to 6-mm-long *A. salina* nauplius is approximately 8–10 days. When fed with cave mysids several millimetres in size, the apparently indigestible protective carapace is rejected after 5–8 days, and the sponge has resumed its normal morphology 10 days after prey capture (Fig. 1; video in Electronic Supplementary Material).

Engulfment and extracellular degradation of the prey

The engulfment of the prey is achieved by a veil of migrating elongate cells, which have the same characters as the pinacocytes covering the sponge body. The veil is devoid of anisochelae, and the sclerocytes enclosing anisochelae, which are numerous at the filament surface, do not play a role in the engulfment of the prey (Fig. 3f), with the exception of the very front line (Fig. 3g). Small prey is thus included in the lacunose space of the super-

Fig. 4a–f Prey *Artemia* nauplius, t=24 h. **a–c** Light microscopy, semithin desilicified sections. Degradation of the crustacean (*arrows*), including digestive tract (*td*) and setae (*se*), invaded by stellate sponge cells containing small dense granules. **d–e** Transmission electron microscopy. **d** Stellate bacteriocytes I with dense granules. **e** Contact between sponge cells (*upper left*) and crustacean body (*bottom right*) with connective cells. **f** Bacteriocyte I containing muscle fragments and dense granules, t=2 days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ficial zone that is well developed in starving specimens (Vacelet and Boury-Esnault 1996).

During the first hours after capture, the prey is surrounded by a pinacocyte layer (Fig. 3g) made by the existing exopinacoderm and by the migrating pinacocytes of the engulfing veil. The internalized pinacocyte layer rapidly dedifferentiates and disappears in a few hours, leaving the prey in direct contact with the intercellular matrix. In the following stage, large stellate sponge cells (archaeocytes and bacteriocytes) concentrate around the prey, and lacunae develop in the other parts of the body. This concentration of cells around the prey delimits a distinct area in the body, with a higher concentration of cells forming a kind of cyst around the prey. Between 12 to 24 h after capture, depending on the size of the prey, the prey is in the process of degradation, displaying the first signs of cuticle disruption. The cells forming a cyst around the prey are archaeocytes and mostly bacteriocytes I (Vacelet and Boury-Esnault 1996), i.e. elongate and more or less stellate cells with large vacuoles containing symbiotic bacteria similar to those occurring extracellularly in the mesohyl. These bacteriocytes I surrounding the prey are unusually large, up to 30 μm long with a nucleolate nucleus up to 6 μm in diameter, and they extend numerous pseudopodia (Fig. 4a–d, f). Their cytoplasm contains a high number of dense granules, 0.6–1 μm in diameter, which are only occasionally found in the bacteriocytes of the starving sponge. Extracellular symbiotic bacteria similar to the normal microflora observed both extracellularly and within the bacteriocytes are unusually numerous around the prey. At day 1, the crustacean outer cuticle is locally disrupted, with archaeocytes, bacteriocytes and bacteria infiltrating the body (Fig. 4a–c). Some parts of the crustacean, however, appear more resistant, such as the gills, thin appendages and setae (Fig. 4b). The digestive tract is never invaded by the sponge cells, although it begins to degrade at day 1, most likely under action of its own digestive enzymes. The first stage of this degradation is shown by a disorganization of the microvilli of the intestinal epithelium, followed by the degradation of the connective cells and a remarkable proliferation of bacteria inside the lumen (Fig. 5a, f). The basal lamina externally surrounding the intestinal epithelium is not degraded. No sponge cells have been observed inside the digestive tract, which is eliminated on day 3–4. The bacteria present up to day 3 in a dense aggregation in the lumen of the degenerating digestive tract belong to a single morphotype (rod-like cells, 1.4/0.4–0.6 μm, with a well-developed nuclear area, frequently in division process) which differs from the symbiotic bacteria found intra- or extracellularly in the sponge tissue. They likely belong to the prey's own intestinal microflora, present in small numbers in the digestive tract of the living crustacean and proliferating after its death. Another type of bacteria morphologically different from the normal symbiotic bacteria has been observed in one specimen at day 2 (Fig. 5e). They are filamentous, flagellum-like cells, 0.2 μm maximum width, abundant in the space between sponge cells and the crustacean body. They are also probably bacteria of foreign origin that multiply on contact with the prey.

Intracellular digestion

From day 2 to day 7, after disruption of the crustacean body, identifiable elements from the prey are phagocytosed by archaeocytes and bacteriocytes I which contain a large number of inclusions, 1–10 μm in diameter. In the early stage, most of these phagosomes are clearly identifiable as of crustacean origin. They contain fragments of connective tissue cells, well characterized by their mitochondria and endoplasmic reticulum being different from those of all sponge cells, and muscles of indisputable crustacean origin (Figs. 4f, 5b–d, 6b). An archaeocyte or a bacteriocyte may contain fragments of the same kind or of various kinds. In bacteriocytes, the crustacean fragments are sometimes included in the vacuoles containing symbiotic bacteria. The number of symbiotic bacteria in the bacteriocytes that contain phagosomes increases during the first stages of intracellular digestion, and dividing bacteria are more frequently seen than usual. Bacteriocytes II (Fig. 6b) do not play any role in phagocytosis.

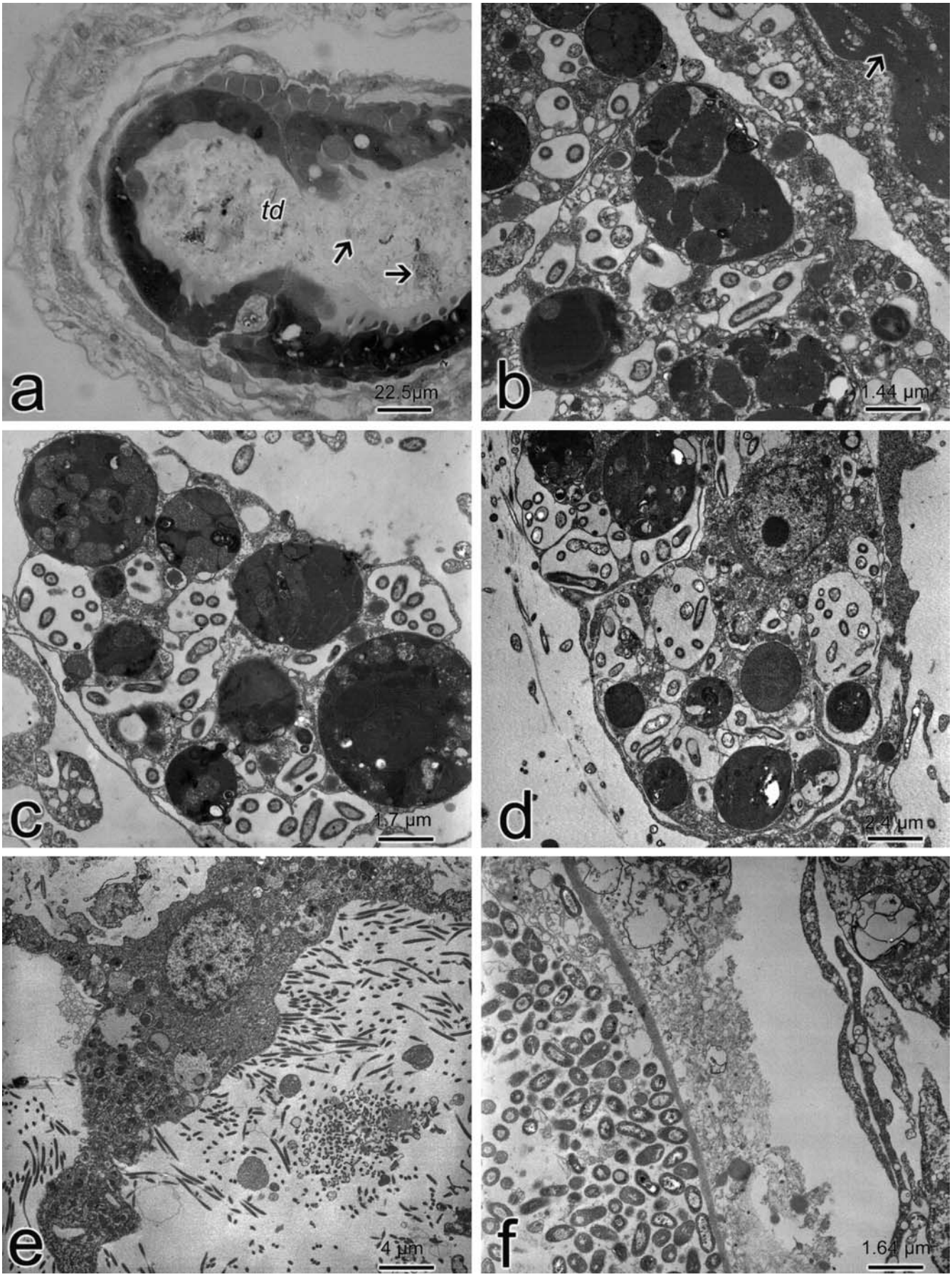
During the final stages of intracellular digestion, the crustacean structures are no longer identifiable, and most of the archaeocytes and bacteriocytes contain many phagosomes and lipidic inclusions (Fig. 6a). At this stage, the vacuoles containing symbiotic bacteria are often open, with release of bacteria from bacteriocytes I in the mesohyl.

Sponges regularly fed by nauplii or adults of *A. salina* exhibit an orange colour in the central part of their body, probably due to the accumulation of a beta-carotene. This colour is never observed in the natural population or in specimens fed in the aquarium by mysids, whose red colour disappears within a few hours.

Excretion

Some of the crustacean remnants that are apparently not digested are surrounded by sponge cells of the pinacocyte type and then released outside the sponge at day 3–5. These structures are chiefly fragments of the outer cuticle and of the gills (Fig. 6c, d). The digestive tract of brine shrimp nauplii, although considerably degraded either by autolysis or by the action of the bacteria that proliferate in its lumen, seems to be always released without further degradation. In the case of large mysids 5–8 mm in length

Fig. 5a–f Prey *Artemia* nauplius. **a** Light microscopy, semithin desilicified section, t=36 h. Crustacean muscles and digestive tract (*td*) with bacterial proliferation (*arrows*) in the lumen, surrounded by sponge cells. **b–f** Transmission electron microscopy. **b**, **c** Bacteriocytes containing phagosomes, including muscle fragments (*arrow*), t=2 days. **d** Bacteriocytes containing phagosomes, t=5 days. **e** Rod-like bacteria in between sponge cell and prey, t=2 days. **f** Proliferation of bacteria in the digestive tract, t=3 days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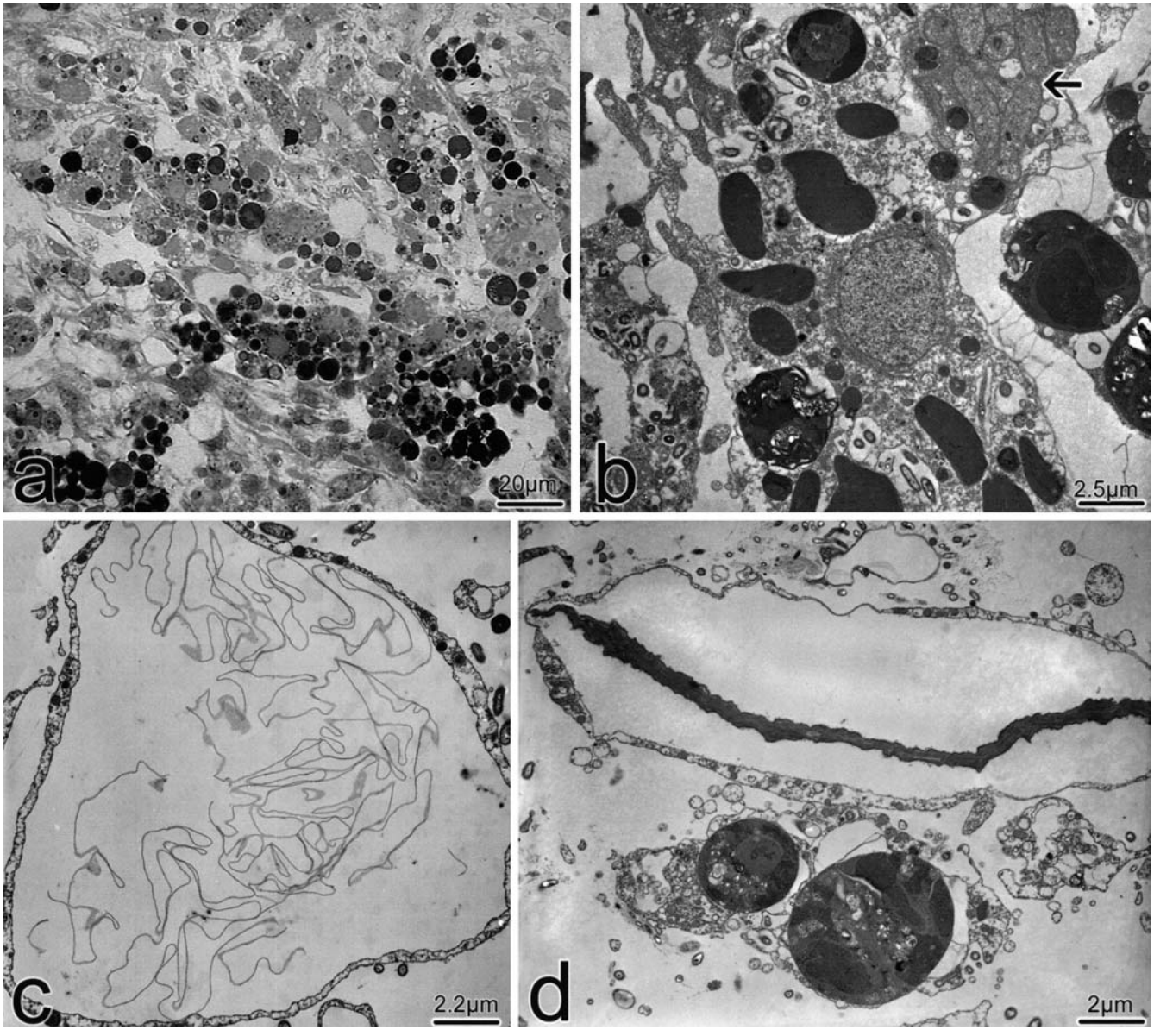

Fig. 6a–d Prey *Artemia* nauplius. **a** Light microscopy, semithin desilicified section. Sponge cells containing vitelline inclusions, t=4 days. **b–d** Transmission electron microscopy. **b** Vitelline inclusions in bacteriocyte I; note the bacteriocyte II (*arrow*). **c**, **d** Fragments of crustacean cuticle enclosed in a sponge cell; **c**=2 days. **d**=3 days

that are covered by a protective carapace, the whole carapace is rejected approximately on day 5–8 (Fig. 1e, f).

Discussion

The present observations clearly indicate that capture and digestion of various invertebrates actually is a feeding process by which the sponge derives its energy. At least parts of the prey are digested and actually used by the sponge. This is clearly shown by the presence in the sponge cells of peculiar crustacean structures in the first stages of intracellular digestion, and of a large number of lipidic droplets and reserve inclusions at the end of the process. The digestive process is highly specialized, in contrast to occasional encystment of foreign organisms that are eventually included in a defensive granuloma (Tuzet and Paris 1964; Cheng et al. 1968; Connes et al. 1971), or to accidental trapping of fishes in the oscula (Vooren 1973) which has been described in 'normal' filter-feeding sponges and does not contribute to their feeding process, contrary to what was believed by Aristotle for fish trapping (Aristotle 350 B.C.). Although the TEM technique used in this work does not allow in all cases clear identification of the origin of the phagocytosed material, there is indisputable evidence that many phagosomes come from crustacean elements. Muscle fragments and crustacean connective tissue cells, which are easily recognizable in TEM and could not be of sponge origin, are particularly numerous in the early stages of phago-

somes. The final step of the digestion of the prey is thus strictly intracellular, with degradation of the crustacean fragments in phagosomes of poorly differentiated cells. This is the usual process of intracellular digestion in poriferans (review in Simpson 1984), in which wandering cells of the mesohyl, mostly archaeocytes, digest particles that have been captured by choanocytes and also frequently phagocytose cell debris and other cells or symbiotic prokaryotes (Schmidt 1970; Willenz and Van de Vyver 1984; Hahn-Keser and Stockem 1997, 1998). This is thus a normal sponge behaviour pattern, the peculiarities being that the phagocytosed elements derive from a disrupted macro-prey, instead of small particles collected from the water filtered through an aquiferous system. The intracellular digestion appears similar to that observed in another carnivorous sponge, *Chondrocladia gigantea* (Hansen, 1885), in which cells of the archaeocyte type containing fragments of crustacean muscles have been reported (Kübler and Barthel 1999).

The capture involves special filaments covered by microsclere spicules similar to those of the poecilosclerid Mycalidae, to which the genus *Asbestopluma* is evidently allied (Hajdu and Vacelet 2002). These microscleres, which in other poriferans have no known function and are generally distributed without any order in the tissue, have a well-defined arrangement here, resulting in high efficiency in the capture of bristle-like crustacean setae. The filament structure is well designed for capture of prey with setae, mostly crustaceans, but various other prey can also be captured. Although the filaments superficially resemble the tentacles of cnidarian polyps, their mode of action is definitely different. They act passively, being devoid of muscles and nervous system, and they have no toxic structures such as cnidocytes. The trap is fully mechanical and does not rely on an adhesive substance as in many carnivorous plants.

The stages preceding intracellular digestion are unique not only for poriferans but also for metazoans. The captured prey are swallowed without any digestive cavity by engulfment first by pinacocytes and then by cells migrating from another part of the sponge body that encircle the prey, forming a kind of cyst in which the prey is broken down. Our morphological data could not indicate the precise mechanism of this primary degradation of the prey. Mere autolysis of the dead prey certainly occurs, in part involving the action of non-symbiotic microorganisms, as indicated by the proliferation of bacteria in the lumen of the digestive tract, or of rod-like bacteria morphologically different from the symbiotic bacteria in the sponge tissue near the prey. However, direct action of the sponge cells and possibly also of symbiotic bacteria is likely, given the short time needed for complete degradation of the prey. TEM observations indicate that there is no close contact between the crustacean and the sponge cells at this stage, suggesting secretion of extracellular enzymes inside the cyst, including chitinase responsible for the disruption of the crustacean cuticle. However, exoenzymes have not been evidenced in filter-feeding sponges (Cotte 1903; Schmidt 1970) and chitinases have been reported in sponges only in culture of microorganisms possibly associated with freshwater sponges (Ivanova et al. 1993). A bacterial origin of these enzymes cannot presently be excluded. Further studies including histochemical demonstration of degradative enzymes are needed to elucidate this question. If cells of the carnivorous sponge were actually secreting digestive exoenzymes in the cyst formed around the prey, this cyst could be considered as an equivalent of a digestive cavity, although temporary and formed according to a mechanism very different from that found in other metazoans.

The whole feeding process that ends in intracellular digestion is quite different from the process occurring in the other metazoan phylum devoid of a digestive cavity, the Placozoa. *Trichoplax adhaerens* Schulze, 1883 feeds by covering protozoans with the ventral surface, where cells, including gland cells absent from *A. hypogea*, produce digestive exoenzymes and absorb the resulting nutrients. Although there is no gut, the ventral surface may form, by contraction, a transient 'digestive bag' where predigestion may take place more efficiently, a behaviour pattern which has been considered as the first step of an archenteron (Grell and Ruthmann 1991).

From an ethological and ecological point of view, *A. hypogea* appears to be a highly effective 'sit-and-wait predator', very efficient in the capture of small crustaceans with minimal energy consumption. This efficiency and the ability to withstand long periods of starvation during which the filaments become thinner and longer, is well suited to the oligotrophic deep-sea environments where the carnivorous cladorhizid poriferans live, an environment in which other filter-feeders such as tunicates (Monniot and Monniot 1975, 1991; Monniot 1984) have developed a macrophagous carnivorous habit. The cavernicolous sponge of the present study, which most probably also lives in the bathyal zone of the Mediterranean, is highly successful in a relatively food-poor cave where it maintains a large population. However, the sponge does not thrive in the deepest reaches of the 120-m-long cave and is mostly abundant in the first 60 m, where crustaceans making circadian migrations are presumably more abundant. This feeding habit, however, is well suited only to very quiet environments, as water movements cause the filaments to temporarily entangle, reducing trapping efficiency. Such quiet environments are found in the 3PP cave, where water circulation inside the trapped cold water mass is very low with currents less than 1 cm/s, or more generally at great depths.

Although devoid of the conventional diagnostic characters of the Porifera, aquiferous system and choanocytes, and having developed special features for capture, engulfment and extracellular breaking down of macroscopic prey, this animal shows fundamental characteristics of poriferans in the use of nutriments, i.e. intracellular digestion by highly mobile cells acting individually although certainly responding to common signals. This confirms its sponge nature, already evident from the similarity of the spicule complement with a common family of non-carnivorous poriferans. This is evidence

that a fully functional metazoan with a macrophagous habit could derive from the filter-feeding sponge body plan, which constitutes an actual example of the possibility of transition from sponge to non-sponge metazoans that has been hypothesized in the early evolution of Metazoa (Dewel 2000). However, a functional digestive cavity similar to that of cnidarians is not achieved.

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