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Umbraculum umbraculum (Lightfoot, 1786) (Gastropoda, Opisthobranchia, Tylodinoidea) and the synonymy of *U. mediterraneum* (Lamarck, 1812)

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Abstract – Specimens of *Umbraculum* Schumacher, 1817 from the North Atlantic (Great Meteor Bank and Azores), the Mediterranean Sea and from the southwest Pacific (Australia, Solomon Ids, New Zealand) were investigated anatomically and histologically to clarify whether *U. mediterraneum* (Lamarck, 1812) and *U. umbraculum* (Lightfoot, 1786) are two distinguishable species or only one. Additional molecular analyses of one specimen each from Australia, the Azores and the Great Meteor Bank were performed, using 18S rRNA, 16S rRNA and COI genes. The morphological and molecular investigations give no evidence supporting their separation into two species. We therefore consider *U. mediterraneum* to be a junior synonym of *U. umbraculum*, and follow the assumptions of Burn (1959) that there is only one species of *Umbraculum*, with a wide spread distribution in tropical and warm temperate waters.

INTRODUCTION

Umbraculum Schumacher, 1817 (see Valdés 2001; Willan and Burn 2003) is the sole genus of the Umbraculidae, a family of the small opisthobranch group, the Tylodinoidea. Formerly, the names Umbraculoidea, or Umbraculomorpha were used for this small taxon, but more recently, the name Tylodinoidea has taken precedence (see Willan 1998; Wägele and Willan 2000; Willan and Burn 2003). The Tylodinoidea and the Pleurobranchoidea have usually been united in the Order Notaspidea (e.g. Willan 1998), but Schmekel (1985) suspected that the Notaspidea was a paraphyletic grouping, a view which was supported by Wägele and Willan (2000) and subsequently confirmed by molecular analyses (Wägele *et al.* 2003, Vonnemann *et al.* 2005, Wägele and Klussmann-Kolb 2005).

The genus *Umbraculum* Schumacher, 1817 is known from temperate and tropical seas, in many parts of the world, and species names have been given to shells from various geographic regions, for example *Umbraculum mediterraneum* (Lamarck, 1812) for material from the Mediterranean Sea, *Umbraculum plicatulum* (v. Martens, 1881) for Caribbean and west Atlantic specimens, and *Umbraculum umbraculum* (Lightfoot, 1786) and *Umbraculum sinicum* (Gmelin, 1793) for Indo-west Pacific specimens. A compilation of many of the early names can be found in Pilsbry (1896). Although most authors agree that there are too many species names, no consensus has been reached on just how many are valid. Burn (1959) considered that there is only one valid species (*U. sinicum*), but Marcus and Marcus (1967) and Marcus (1985) considered *U. plicatulum* von Martens, 1881 to be a valid name for the west Atlantic populations. Guangyu (1981) described a new species (*U. pulchrum*) from China and accepted the validity of five other species (*U. umbraculum, U. mediterraneum, U. plicatulum, U. pictum* (Bergh, 1905), and *U. ovalis* (Carpenter, 1856). Thompson (1970) distinguished one Atlantic and Mediterranean species (*U. mediterraneum*) and one Indo-west Pacific species (*U. sinicum*). More recently, Willan (1998: 979) stated that "The majority of opisthobranch systematists *maintain* that there is only a single species in the family, *Umbraculum umbraculum*, with a cosmopolitan distribution throughout tropical and warm temperate Indo-west Pacific seas...". This continuing exchange of opinions, without new information clearly shows that a re-investigation of specimens with different techniques is warranted.

During a German expedition with the Research Vessel *Meteor* to the Eastern Atlantic, West to the Canary Islands, many specimens of the genus *Umbraculum* were dredged on top of the under water plateau Great Meteor Bank. Problems in assigning the material to either of the two most accepted species (*U. umbraculum* and *U.*

mediterraneum) gave the impetus for a thorough analysis of specimens from the North Atlantic (Great Meteor Bank and Azores), the Mediterranean Sea and from the southwest Pacific (Australia, Solomon Ids, New Zealand) by anatomical and histological means to see what differences, if any, could be found. Additional molecular analyses of one specimen each from Australia, the Azores and the Great Meteor Bank were performed, using 18S rRNA, 16S rRNA and COI genes.

MATERIAL AND METHODS

Specimens studied

Meteor Bank (North Atlantic): 29 specimens collected by Nils Brenke and Gabi Strieso (Bochum) during a German expedition of *RV Meteor* at the Meteor Bank with an epibenthic sledge. Preserved in formalin/seawater, or 96 % alcohol. Length between 25 and 34 mm. 9 specimens. Stat. 481, 29° 51.7 N, 28°21.1 W, depth 347–370 m 7 Sept. 1998; 14 specimens, Stat. 486, 29° 45.7N 28°22.9 W, depth 298–310 m 8 Sept. 1998; 4 specimens, Stat. 487, 29° 54.1 N, 28°22.6 W, depth 314–326 m. 8 Sept. 1998; 2 specimens, Stat. 496, 29° 56.1 N, 28°37.6 W, depth 299–310 m 10 Sept. 1998; Three specimens of station 481 are deposited in the Zoological Collection of the Senckenberg Museum Frankfurt (SMF: 323036/2, SMF: 323037). No photos or description of living animals are available. Preserved specimens are figured in Figure 1C–E. **Azores** (North Atlantic): 1 specimen Varadouro, Faial (Funchal/Madeira), Oct. 2001. Coll: Peter Wirtz, preserved length: 14 mm. **Greece** (Chalkidike, Mediterranean Sea): 1 specimen, between Sithonia and Athos, Northern Greece, 25 m on sandy bottom, Aug. 1980. Coll: H. Wägele, preserved length: 100 mm (Figure 1A). **Australia** (New South Wales): 1 specimen, Shellharbour 15 Jan. 2002. Coll: A. Klussmann-Kolb, preserved length: 35 mm, preserved in formalin/seawater, a small piece of the foot in 96% alcohol. (Figure 1B); 2 specimens, Bare Is, Botany Bay, Sydney, NSW. 33º59.7'S, 151º13.8'E. 8 m, 24 Nov 1981, Coll: G. Avern, preserved length: 68 and 63 mm, AM C132149; 1 specimen, off Wreck Bay, NSW. 35º13'–35º12'S, 150º40'–150º44'E. 40–62 m, FRV "Kapala". 11 Feb 1993. Coll: K. J. Graham, preserved length: 83 mm, AM C305009; 1 specimen, Off Wollongong, NSW, 35°13'S, 150°37'E, 20 Oct 1993, preserved length ca. 100mm, AM C305010.1 specimen, off Tathra, NSW. 36º35'–36º43'S, 150º8'–150º11'E. 100–117m. FRV "Kapala". 18 May 1994, Stn: K94-11-5/08, Coll: K. J. Graham, preserved length: 100 mm, AM C305011; 1 specimen. Lord Howe Island, 31° 32.979 S, 159° 3.76 E, Sept 1962, preserved length 62 mm, AM C352520.2 specimens, Woody Head, NSW. 29º22'S, 153º22.5'E, 4 Nov 1963, Coll: F. Ros, preserved length: 59 and 60 mm, AM C352561 (Figure 1G); 1 specimen, Bare Is, Botany Bay, Sydney, 33º59.7'S, 151º13.8'E, Sponge.1967. Coll: N. Coleman, preserved length: 67mm. AM C352562; (Figure 1H); 1 specimen, off Green Cape, NSW. 37º12'–37º15'S, 150º21'E. 330 m, FRV "Kapala". Engel Trawl, 24 Oct 1979, Stn: K79-17-03, Coll: K. J .Graham, preserved length: 55 mm, AM C352563 (Figure 1F). **New Zealand**: 1 specimen, Piercy Is, Bay of Ids, New Zealand, 10 m in sponge. May 1976. Coll: N. Coleman, preserved length: 25 mm. AM C431437. **Solomon Islands**: 1 specimen, Trenchs Creek, Kakambona, Solomon Ids, 4 Jan 1984. Coll: I. Knight, preserved length: 35 mm. AM C141928.

Accesion numbers (NCBI)

Azores: DQ256205 (16S), DQ256202 (COI)
Meteorbank: DQ256204 (16S), DQ256201 (COI)
Australia: DQ256203 (16S), DQ256200 (COI).

Morphological investigations

Five specimens from the Meteor Bank, one from Greece and two from Australia were investigated by macroscopy. Three specimens from the Meteor Bank, and one specimen from the Azores were embedded in hydroxyethylmethacrylate (for technical description see Wägele 1997) for serial sectioning (2 µm). Slides were stained with toluidine blue. Morphology of the cuticular structures was investigated with a scanning electron microscope. Twelve specimens from the collections of the Australian Museum, Sydney (AM C) were studied externally, after the shell was removed, and two were dissected to compare the reproductive system.

Molecular investigations

One specimen each (or part of it) from Shellharbour (New South Wales, Australia), from the Azores and from the Great Meteor Bank (Atlantic Ocean) were analyzed. Sequences from *Tylodina perversa* (AY345024 and AF249809) and *Aplysia oculifera* (AF192302), taken from GeneBank were used as outgroups.

DNA Extraction, Amplification and Sequencing

Genomic DNA was extracted from alcohol-preserved specimens by means of the *Blood and Tissue-Kit* (Qiagen), guided by the enclosed protocol. The amplification of the complete 18S rRNA gene-region by PCR (Saiki *et al.*, 1988) was performed with primers developed by Trisha Spears (pers. comm.): forward (18A1), 5′-CCT A(CT)C TGG TTG ATC CTG CCA GT-3′ and reverse (1800), 5′-GAT CCT TCC GCA GGT TCA CCT ACG-3′. The partial 16S rRNA gene was amplified by 16a-3: 5′-CGC CTG TTT ATC AAA AAC AT-3′ and 16b-3: 5′-CCG GTC TGA ACT CAG

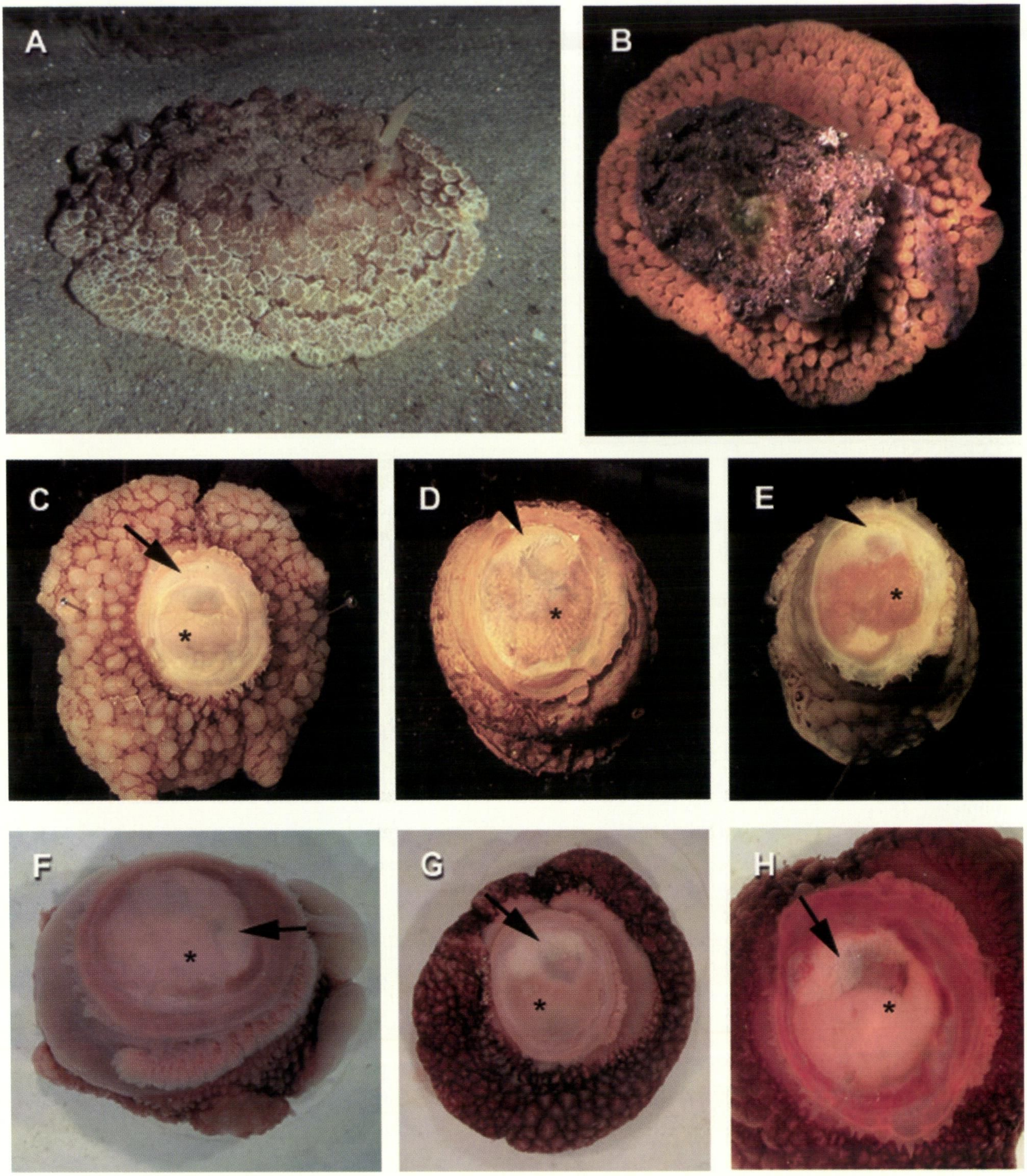

Figure 1 *Umbraculum umbraculum*, external morphology. A, Living animal from Chalkidike (Greece, Mediterranean Sea), in a depth of about 20 m; B, Living specimen from Shell Harbour (NSW, Australia), photo by courtesy of A. Klussmann-Kolb; C, preserved animal from Greece; D, Preserved specimen from Meteor Bank with shell removed, E, Preserved specimen from Meteor Bank with shell removed, F–H, Preserved specimens from collection of the Australian Museum with shell removed; F, Preserved specimen C352563a; G, Preserved specimen C352561-07a; H, Preserved specimen C352562a. Arrow indicates mantle gland, star indicates kidney.

ATC ATG T-3′ (Simon *et al*. 1994) and the COI gene by HCO: 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ and LCO: 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ (Boore and Brown 1994).The PCR was carried out in the thermal cycler *Progene* (Techne) under the following conditions: 95°C for 4 min, followed by 38 cycles of 30s at 94°C, 30s at 52,5°C, 2.5 min at 72°C and a final extension at 72°C for 10 min. Each PCR reaction mix (50 µl) contained 5 ml of 10′PCR buffer (Qiagen), 10 ml of *Q-Solution*

(Qiagen and Eppendorf), 5 ml of dNTP-mix (2 mM per dNTP), 0.5 ml of each primer, 0.3 ml of *Taq* polymerase (Qiagen), 0.25 to 3.0 ml of genomic DNA and 21.55 to 25.7 ml H_2O.

After purification of the PCR products by means of the *QIAquick PCR Purification Kit* (Qiagen), the 18S and 16S rRNA genes and the COI gene were sequenced directly by the chain-termination method (Sanger *et al.*, 1977) with the *Thermo Sequenase fluorescent labelled primer cycle sequencing Kit* (Amersham) on the automated sequencers *4000* and *4000IR*^{2} (Licor). Both strands of the genes were sequenced using the primers from the PCR and additional internal primers for the 18S rRNA gene.

Sequence assembly and alignment

The partial 18S rRNA gene sequences and the two strands of the 16S rRNA and COI genes were assembled and proof-read by means of AlignIR (LI-COR Biosciences). Sequences were aligned with CLUSTAL X (Thompson *et al.*, 1997) using the default parameters.

MORPHOLOGICAL DESCRIPTIONS

Animals from Meteor Bank

External morphology

Preserved length 25–34 mm, width 20–27 mm. Shell length 22–27 mm, width 15–19 mm; shell patelliform, apex subcentral, slightly to the left and backwards; protoconch present, coiled sinistrally. Shell calcareous, rather strong, colour white, in one animal with a more yellowish margin. Periostracum present, rather smooth and without algae (Figure 2A). Body roundish, truncate in front; foot rather high, surface with smooth tubercles, in some specimens more flat, in others rather prominent (Figure 1D–E). Mantle edge two-folded. Upper fold smooth, lower fold with tentacular processes or only zigzag folded (Figure 1D–E). Rhinophores greatly distorted through contraction in preserved animal. Eyes lying at the base of the rhinophores (Figure 2B). Oral lobes around ventrally positioned mouth present in all specimens, but withdrawn to a variable extent. Penis consisting of an elongate lobe, sometimes lying rather prominently between rhinophores and exterior oral lobes, but sometimes difficult to distinguish. Genital opening to the right of the base of the penis. Gill occupying the anterior and right part of the mantle groove beneath the mantle, running anteriorly from the left side of the head down the right side to the anus. Anterior part of gill completely attached to the mantle groove, each gill plume recognisable as an individual tripinnate gill. From midway down the gill, on the right side, gill has a typical smooth raphe with gill plumes on both sides. (Figure 2C, upper arrow). Posterior part of this typical plicatidium free (Figure 2C, lower arrow). Anus opens behind the end of the gill (Figure 2C). Osphradium not found.

Anatomy

Digestive system. Mouth opens ventrally, leading into a short oral tube. Pharyngeal bulb roundish, very muscular. Anterior part, including the labial disc, covered by a cuticular lining. No armament present. Colour of radula yellowish. Formula: 110–130 x 550–800.0.550–800. Hook-shaped laterals rather uniform in shape and size (Figure 3A, B). One anomaly occurring in every row was a single bicuspid lateral. Salivary glands flocculent, lobes located mainly around the pharynx, with many small efferent ducts uniting into one large and long duct. This efferent duct passing through the nerve ring and opening into the pharynx without any bulbous structures. Anterior oesophagus leaves pharynx postero-dorsally, passing through the nerve ring, widening slightly, before running to the posterior oesophagus, the crop (Figure 2C). Cuticle absent in anterior part of oesophagus. Entrance to crop demarcated by strong longitudinal folds with vertical ridges, covered by a strong cuticle. Cuticular lining consisting of rodlets (Figure 3C, D). Stomach very small. Only one entrance into digestive gland present, this opening lying next to opening of intestine. Intestine originating dorsally, forming a flow through system with stomach and oesophagus, running first to the left ventral side, then bending dorsally and to the right side, before entering the anal papilla (Figure 2C). No cuticular structures present in stomach or intestine. The latter without a typhlosole. Digestive gland covering stomach and proximal intestine. Contents of digestive tract consisting of soft material as well as sponge spicules, Foraminifera (Figure 3D), bryozoan fragments, and unidentifiable hard structures.

Genital system. Gonad lying in front of, as well as on top of, the anterior digestive gland. Gonoduct very thin, looping from the left to the right side, widening into a large folded ampulla. From the ampulla, the narrow postampullar gonoduct runs alongside the mucus gland before entering a common duct (pallial gonoduct of Marcus and Marcus 1967), which lies in the lateral body wall (Figure 2D). Vaginal duct opens into pallial gonoduct next to opening of postampullar gonoduct (Figure 2D, x). Vaginal duct elongate, leading back to large spherical bursa copulatrix. Receptaculum seminis, large, elongate, opening into vaginal duct through short duct. Albumen, membrane and mucus gland present, entering the pallial gonoduct next to the opening of the postampullar gonoduct and vaginal duct. Pallial gonoduct mostly enclosed in lateral body wall, leading to the mediofrontal notch and opening to

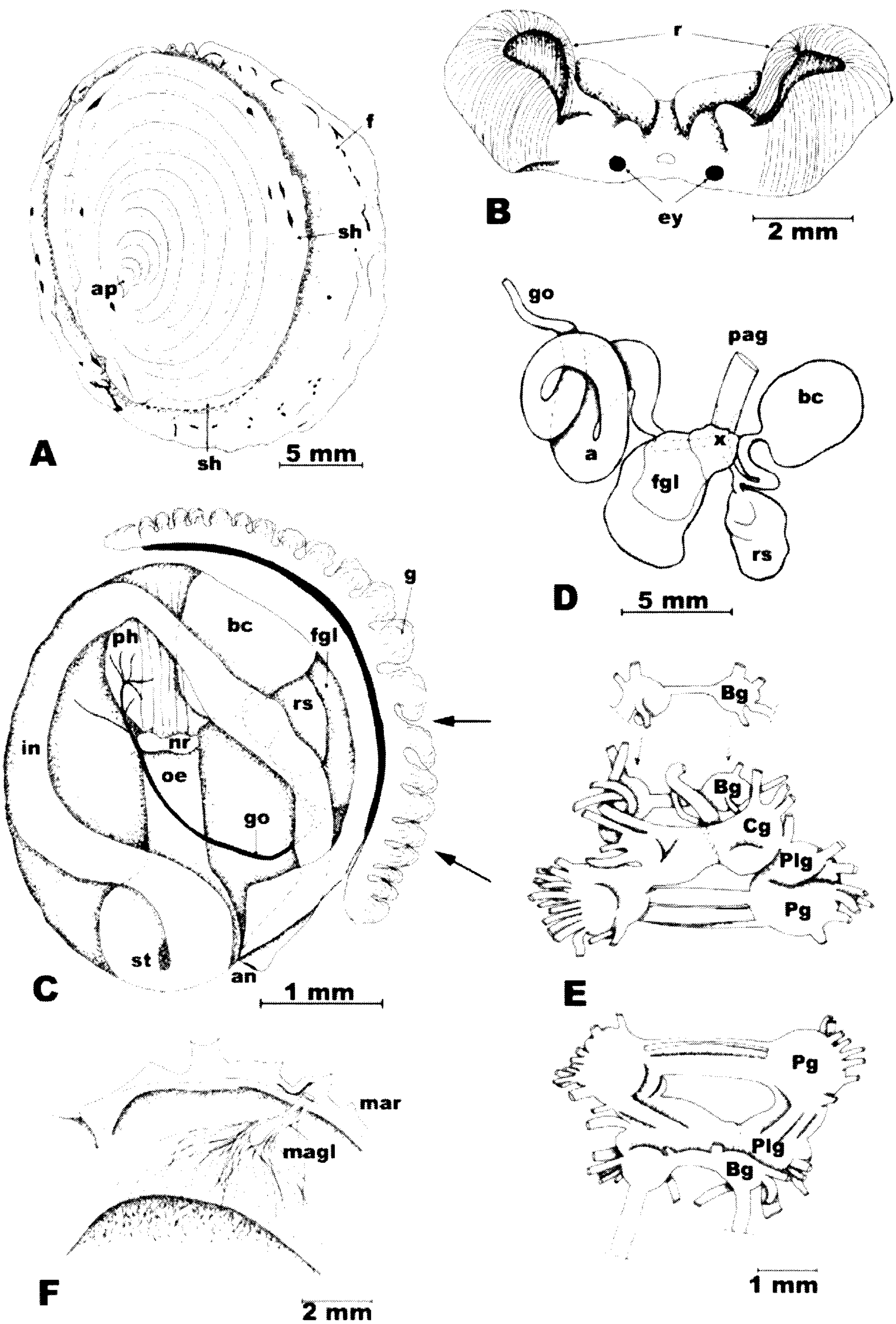

Figure 2 *Umbraculum umbraculum*, anatomy of animal from Meteor Bank; A, Preserved specimen, with shell in situ; B, rhinophores of preserved animal seen from in front; C, schematic outline of digestive tract; D, Genital system (dorsal view) with organs separated for general view; E, Nervous system from ventral and dorsal side; F, Mantle gland opening into anterior mantle rim. Abbreviations: a ampulla, an anus, ap apex, bc bursa copulatrix, Bg buccal ganglion, Cg cerebral ganglion, ey eyes, f foot, fgl female gland, g gill, go gonoduct, in intestine, magl mantle gland, mar mantle rim, nr nerve ring, oe oesophagus, pag pallial gonoduct, Pg pedal ganglion, ph pharynx, Plg pleural ganglion, r rhinophores, rs receptaculum seminis, sh shell, st stomach, x junction of postampullar duct, nidamental glands and vaginal duct, transition into pallial gonoduct

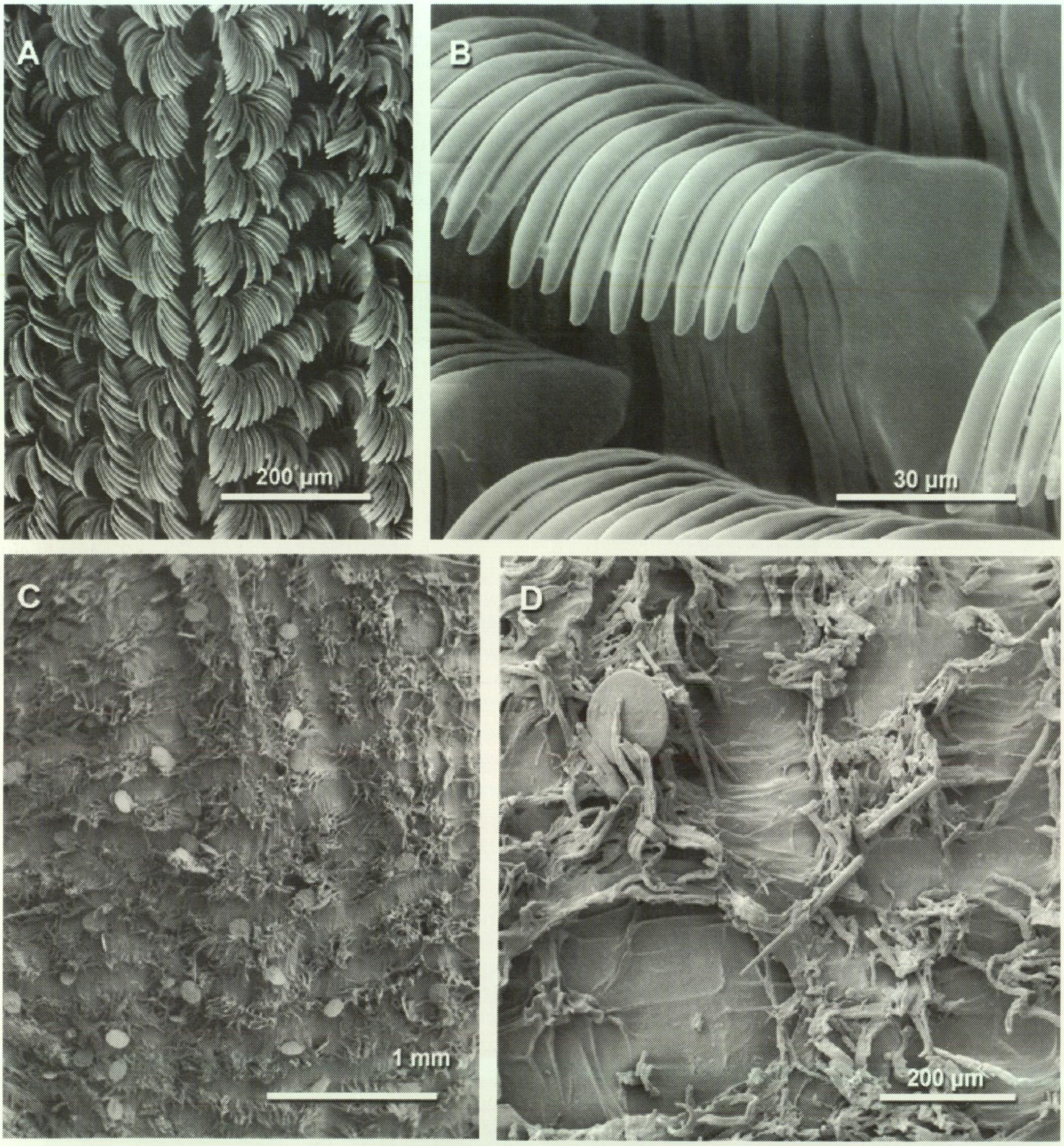

Figure 3 *Umbraculum umbraculum*, hard structures in digestive tract; A, Radula (part) of a specimen from Meteor Bank; B, Some laterals of same radula as in A; C, Cuticular layer of posterior ventral oesophagus; D, Detail of cuticular layer with spines and probably a foraminiferan; E, Apex of shell from Greek specimen; F, Detail of cuticular rodlets in posterior oesophagus from Greek specimen; G, Detail of cuticular rodlets in posterior oesophagus from Australian specimen.

the right of penial papilla. Pallial gonoduct is a common duct, with an internal fold separating it into 2 distinct tubes. Penial papilla is a folded lobe with an open sperm groove inside the lobe. No separate vas deferens or prostate gland present.

Nervous system. Nerve ring encircling oesophagus a short distance behind the pharynx (Figure 2C). Connectives between cerebral and pleural ganglia, as well as cerebral and pedal ganglia distinct but very short (Figure 2E). Thick pedal and thin parapedal comissures closely attached to each other by connective tissue. Visceral loop short, similar in length to pedal and parapedal commissures. No separate ganglia recognizable, but some ganglionic swellings on the right side next to the right pleural ganglion visible. Connectives to buccal ganglia of similar length as pedal and parapedal commissures. Radula nerve not united into one nerve.

Other organ systems. Large mantle gland present in the anterior part of the dorsal mantle, opening at the antero-frontal mantle edge, slightly to the right side (Figure 1D, E, 2F). U-shaped kidney closely attached to dorsal notum wall, composed of many tubules. Renopericardial duct and nephroproct not found. Heart lying in the anterior part of visceral cavity, orientated vertically. Ventricle muscular, lying to left of thin walled auricle. Connection of heart to the gill where plicatidium transforms into single gill plumes (Figure 2C upper arrow).

Histology

Tubercles without subepithelial glandular cells. Epithelial cells glandular, vacuoles staining homogenously light violet (Figure 4I). Gill (Figure 4A) heavily folded, epithelium near raphe with glandular cells. Epithelium between gill and mantle rim with high glandular cells, filled with dark violet staining granula. This glandular stripe visible along the whole gill. Lip cuticle smooth, without any traces of armament. Salivary glands lobular, cells with violet staining contents (Figure 4E). Anterior part of oesophagus in cross section round, inner part highly folded, without any cuticular lining or glandular cells. (Figure 4D). Cuticular layer and rodlets in posterior oesophagus (crop) very prominent (Figure 4F).

Gonad comprising spermatogonia and ripe sperm, as well as oogonians. Ampulla filled with sperm and oocytes (Figure 4G). Bursa copulatrix with apocrin secreting cells. Receptaculum seminis with underlying layer of muscles. Sperm is not orientated towards the walls. Pallial gonoduct in cross section with a fold, dividing the duct incompletely into two separate areas. No glandular tissue present, only ciliated cells. Nidamental glands composed of three distinct areas. Cells of all three areas high and columnar. Cells of capsule gland with small bluish stained granules, cells of membrane gland with bright red staining contents, sometimes granular, sometimes with a more homogenous contents. Mucous gland with dark violet staining granules. One area here composed of rather empty cells and pyknotic nuclei. Cells of kidney with large vacuoles and globular inclusions (Figure 4C).

Tubules of mantle gland embedded in notum muscle tissue. Glandular tubules only present in mantle tissue, never reaching into the visceral cavity. Ends of tubes narrow, formed by small cuboidal glandular cells with violet granules. Proximal parts, leading to the external opening, wider and formed by small non-glandular cuboidal cells. Contents of cells staining homogenously light violet.

Specimen from Azores

Histology

Epithelial cells glandular, vacuoles staining homogenously dark violet (Figure 4 H). Tubercles without any subepithelial glands. Rhinophores in sections clearly divided in two parts. Outer part with more and higher lamellae filling the interior space. Rhinophoral epithelium with a few small glandular cells containing tiny vacuoles with violet stained contents. Few muscles present. Inner part of rhinophores with fewer lamellae and nearly no glandular cells, underlain with an extremely thick muscle layer containing thick packages of especially longitudinal muscles. Anterior pharynx and labial disc with cuticle and without armament. Oesophagus with ciliated cells in anterior part, but starting in the posterior third with a cuticular layer and the rodlet like structures. Stomach and intestine with a folded and ciliated epithelium, no cuticle or glandular cells present.

Gonad not mature. Only male follicles present with spermatogonia in different stages. Gonad present mainly in front of the digestive gland, but not in the rear. U-shaped pallial duct running in lateral notum tissue, leading to the exterior above mouth, next to penis. No glandular epithelium present, only ciliated grooves. Bursa copulatrix with a very thin and apocrin-secreting epithelium, receptaculum seminis with a thick muscle layer and highly folded, but interior epithelium rather secretory. No sperm attached to the epithelium could be found. Nephrocardial duct funnel opening into pericardium, lying on the right side near to the gills, with long cilia.

Eyes composed of a pigment cup and a spherical lens.

Only few glandular tubules of mantle gland present in anterior part of mantle fold. Larger collecting tubes not present. Shell gland formed by high columnar cells, staining violet. From there small tubules running into the zigzag mantle rim.

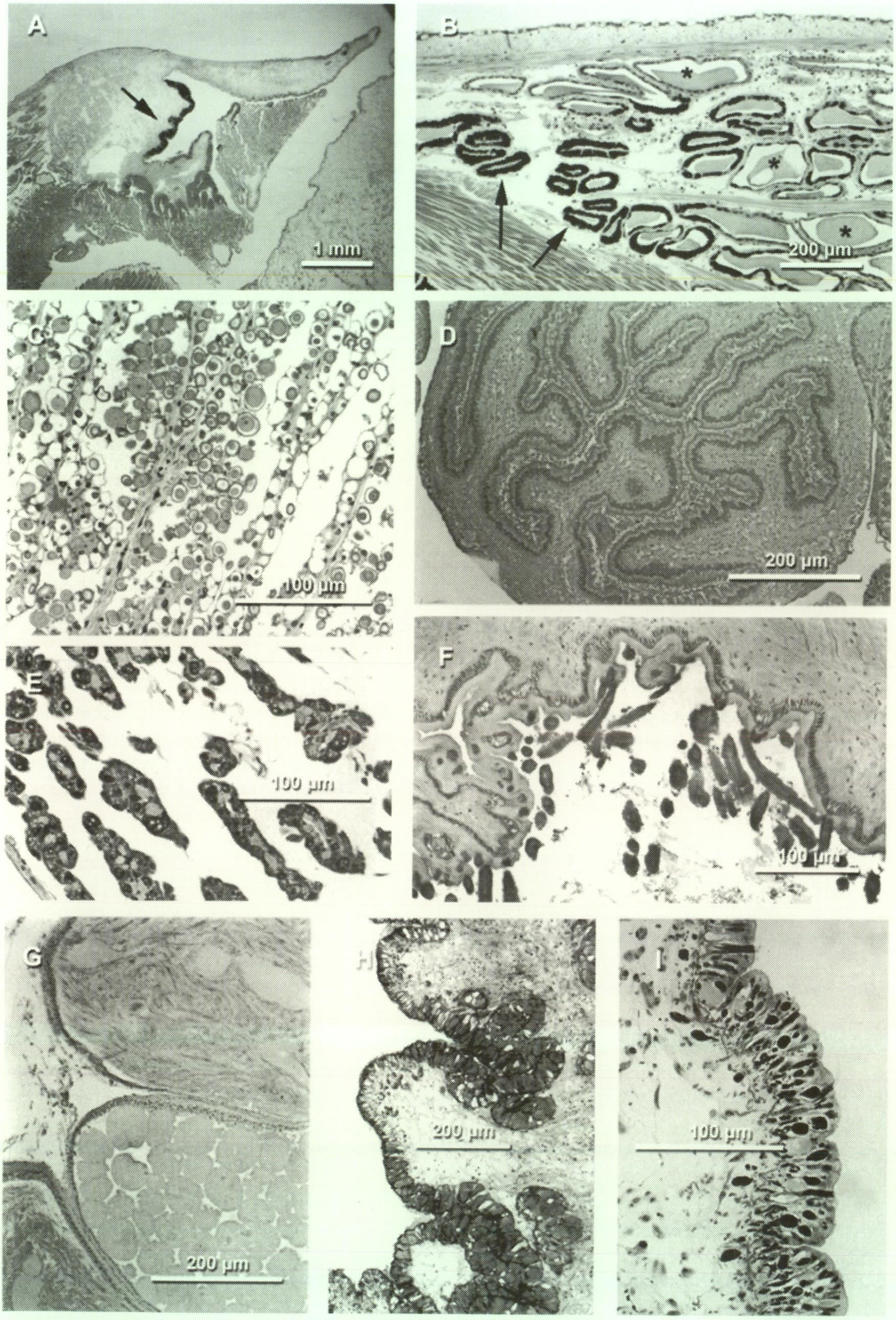

Figure 4 *Umbraculum umbraculum*, histological details of specimens from Meteor Bank and Azores; A, Gill plume. Note the dark glandular stripe between the gills and the mantle rim, as well as the glandular epithelium at the base of the gill lamellae (arrow); B, Mantle gland with different sections. Proximal tubules with dark stained epithelium (arrows), distal tubules with asterisks; C, Detail of excretory system. Note the inclusions in the large vacuoles of excretory cells, as well as in the lumen of the kidney; D, Cross section of anterior oesophagus; E, Salivary glandular tubules; F, Cross section of posterior oesophagus with cuticular layer and rodlets; G, Ampulla with sperm and eggs; H, Glandular epithelium of tubercle, specimen from Azores; I, Glandular epithelium of tubercle, specimen from Meteor Bank.

Specimen from Greece

External Morphology

Colour of living animal greenish to yellow (Figure 1A). Tubercles at the base whitish. White lines are running to the distal parts of the tubercles. Shell of the living animal covered thickly by algae. Rhinophores consisting of a rolled, retractile tubular tentacle, with an exterior basal swelling.

Preserved length (Figure 1C) 100 mm, breadth 85 mm, height 55 mm. Length of shell 73 mm, width 55 mm. Shell of same shape and structure as those from Meteor Bank, sinistrally coiled protoconch present (Figure 3E). In preserved specimens, body roundish, truncate in front, surface with prominent tubercles, which seem to have shrunken due to preservation. Penis large, in cross section U-shaped, with a distinct seminal groove lying between the two flaps and leading to the tip of penis. Anus ending behind the gill on a very prominent papilla.

Anatomy

Digestive tract. Position of oesophagus, stomach and intestine the same as described above. Oesophagus leaving the pharynx in a dorsal, slightly posterior position. In the beginning oesophagus rather thin, with a highly folded inner wall, then widening into a long ventrally lying sac-like part, which enters the stomach in the back of the visceral cavity. Sac-like part with cuticular rodlets (Figure 3F). One entrance from stomach into the digestive gland. The intestine without typhlosole.

Genital system. Genital system identical to that of the Meteor Bank specimens, except for a small pouch at the position where ampulla, vaginal duct and nidamental glands fuse to the common pallial duct (Figure 2 D, position marked with an x). Penis conical but highly contracted, with a deep furrow. Open seminal groove on the ventral side in the deep furrow of penis, running up to its tip.

Nervous system. Similar to the specimens from the Great Meteor Bank. The rhinophores (both parts) are innervated by one nerve from the cerebral ganglion. Optic nerve running very close to that rhinophoral nerve, before separating and running to the basal lying eyes.

Position of kidney the same as in specimens described above.

Other organ systems. Position of mantle gland anterior, in dorsal notum tissue. Many ducts leading to anterior notal edge, fusing there. Probably only one opening present. No further difference could be seen compared to the animals from the Great Meteor Bank.

Specimen from Australia (Shellharbour, NSW)

External morphology

Length of live animal 35 mm, width 30 mm, height 27 mm. Shell length 26 mm, width 21 mm, similar to specimens from Meteor Bank, but periostracum covered with algae, except of apex. Colour of living animal yellowish to brown (Figure 1B). Yellowish lines are running to the distal parts of the tubercles. Shell of the living animal with a thick cover of algae. Body roundish, truncate in front, surface papillate. Tubercles more prominent than in the specimens from the Meteor Bank. All other characters similar to the other investigated specimens.

Anatomy

Digestive tract. Pharynx similar to other investigated specimens. Ducts of lobular salivary glands very long, opening into buccal bulb next to transition into oesophagus. Position of oesophagus, stomach, opening into digestive gland and loops of intestine similar as in other specimens described above. Posterior part of oesophagus covered by cuticle with rodlets (Figure 3G). There is a single opening of the digestive gland into the stomach, at the same position as in other specimens, but unlike other specimens reported here, the main duct quickly divides into two separate ducts, one leading directly into the left side of the digestive gland, the other to the anterior right side.

Contents of digestive tract: soft material, as well as many smaller spicules, probably of sponges, many calcareous pieces and parts of a tube probably of a serpulid worm, spines of a sea urchin.

Nervous system. Nerve ring located around the oesophagus just behind the pharynx. Ganglia very concentrated. Annexed ganglia to the right pleural ganglia hardly discernible. All commissures very short. Subcerebral commissure present, closely annexed to the pedal commissure. Statocyst lying between pedal and pleural ganglion. Connectives to buccal ganglia rather short, radular nerves not fused.

Genital system. The gonad lies posteriorly on top of the digestive gland, but also extends anteriorly. The rest of the genital system is similar to other specimens. No prostatic areas were found. In this animal the open seminal groove in the penis is very distinct.

Other organ systems. Mantle gland could not be found in macro-preparation. Kidney of tubular to lamellate structure, lying dorsally underneath dorsal mantle epithelium, but not covering the anterior part with pericardium.

Specimens from the collection of the Australian Museum

Specimens in the Australian Museum collections were examined to confirm anatomical features. The reproductive system of one specimen (AM C305010) matched that of Atlantic specimens and had a small pouch, as in the Greek specimen, at the junction of the ampulla, vaginal duct and nidamental glands. The shell was removed from 15 specimens to confirm the presence of the mantle gland and extent of the kidney. In all but a few specimens, the mantle gland was clearly visible. In those that the gland could not be detected it is possible that the fixation and preservation history of the specimens may be the cause. The gland was more difficult to detect in alcohol fixed and preserved specimens, and neither the gland nor the kidney was visible in one example (AM C352520). In some lots the gland could be seen in one specimen but not another (AM C352561, C132149), but as these specimens were of similar size the absence could not be related to size. In another specimen the gland was visible but not the kidney (AM C305011) suggesting that as in nudibranchs, the inability to visually detect the presence of a gland should not be considered absolute proof of its absence.

MOLECULAR INVESTIGATIONS

Additionally to the morphological investigations molecular analyses were performed to reveal potential synapomorphies on the genetic level. Specimens from the Atlantic and the Pacific were included. To increase the reliability of the results, different genes (18S rDNA, 16S rDNA, COI) with different substitution rates were used.

Results

The complete 18S rRNA genes of all three specimens showed sequence lengths of 1789 nucleotides. They showed no differences at all.

The analyses of the partial 16S rRNA gene (477 Bp) revealed differences at two positions. Unfortunately, *Tylodina perversa* was not available as an outgroup, because it was not possible to obtain a PCR-product of this species. Alternatively, *Aplysia oculifera* was taken as the outgroup because molecular analyses (Vonnemann *et al.* 2005, Grande *et al.* 2004) suggested a close relationship of Tylodinoidea to Anaspidea and Cephalaspidea s.str. Comparison between the ingroup and the outgroup revealed that the two corresponding homologue nucleotides between the sequences of the specimens from Australia and the Meteor Bank are characters inherited from the ancestor, whereas the differing nucleotides of the specimen from the Azores represent mutations.

The cytochrome c oxidase subunit 1 sequences (length 649–650) clearly separate the three investigated *Umbraculum* specimens from the outgroup *Tylodina perversa* by 110 apomorphic positions. The three *Umbraculum* specimens differ in 12 positions, all representing the third codon position (Figure 5, positions indicated at stemline with plesiomorphic situation deduced by outgroup comparison). The amino acid sequence is the same in all three specimens of *Umbraculum*. Figure 5 indicates the derived and trivial positions mapped on the tree.

DISCUSSION

The anatomy of *Umbraculum*, from the Mediterranean Sea, was described in detail by Moquin-Tandon (1870) and Vayssière (1885). Marcus (1985) described Western Atlantic specimens, identified as *Umbraculum plicatulum* (von Martens, 1881), and Willan (1987, 1998) summarized our knowledge of its anatomy. Our investigation confirms many features, but also broadens our knowledge of the anatomy of this peculiar genus. Few authors have mentioned the

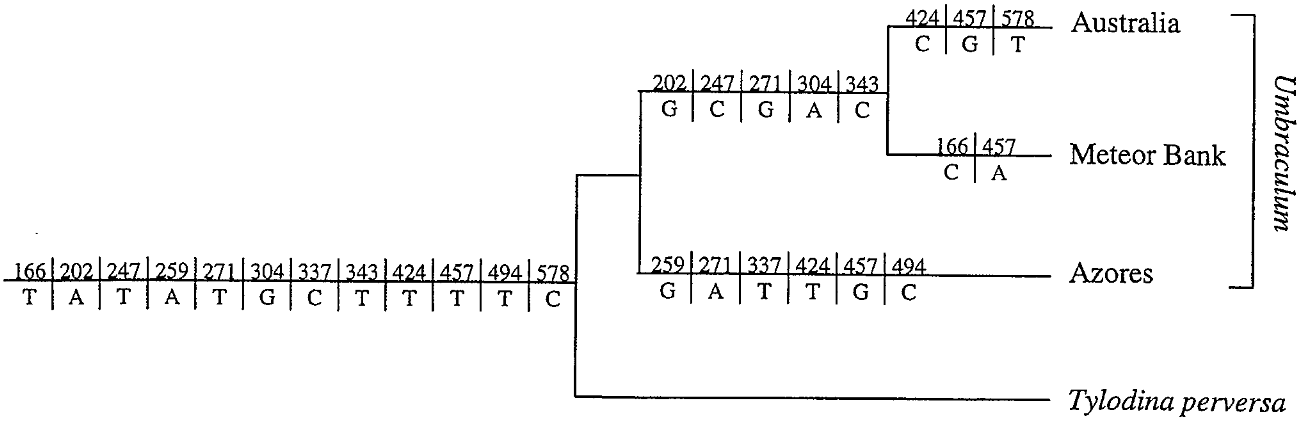

Figure 5 Maximum parsimony based tree of the COI gene of the three investigated *Umbraculum* specimens from the three different localities. Outgroup is represented by *Tylodina perversa*. Length of alignment 649 to 650 bp. Numbers above the branch line indicate position of nucleotide, letters below name the nucleotide. Positions on stemline (left) represent the plesiomorphic situation of those nucleotides, which show an apomorphic situation for at least in one *Umbraculum* specimen.

very conspicuous dorsal mantle gland, which can comprise one-third of the dorsal mantle tissue. Vayssière (1885) described the gland in a specimen from the Mediterranean Sea, but he confused it partly with the kidney, which lies alongside, and can easily be confused with it.. "...cette glande est constituée par une multitude de vésicules pyriformes, ayant parfois près de 1 millimetre chacune..." (Vayssière 1885: 137). This description of the contents of the gland clearly refers to the vesicles observed in the kidney (Figure 4C), although he correctly describes the ducts of the mantle gland. Moquin-Tandon (1870) also misinterpreted the kidney and the gland, which he called as a whole "Corps de Bojanus" (Moquin-Tandon 1870: 66). He described two ducts from the gland, one as the excretory duct on the right side, and the other (being the duct of the mantle gland) at the anterodorsal side of the mantle rim. We do not know the function of this gland. Its position is very similar to the dorsal mantle gland observed in *Tylodina*, but in *Tylodina*, there are many ducts leading from the mantle gland, and the morphology and histology of the gland differs considerably to that of *Umbraculum* (Wägele and Klussmann-Kolb, 2005). Nevertheless, the same position suggests a homology. The presence of a mantle gland was confirmed for nearly all investigated specimens, although in some, the gland seems to be very small. Actually in the juvenile specimen from the Azores, the gland was so small, that it was only found after a thorough investigation of the slides. The Australian specimen (Shellharbour) examined more thoroughly seemed to lack a mantle gland, but it probably was also too small to detect by macroscopical investigation. An external investigation of several specimens from the Australian Museum also revealed differences in the size of the gland. Interestingly, the Australian Museum material suggested that absence of the gland may be an artefact of preservation as in a preliminary survey, the gland could not be detected in specimens preserved in ethanol. However in 10% formalin preserved Australian specimens, seven clearly showed the gland. Two that showed no sign of the gland were collected with specimens that did, suggesting that our inability to easily detect the gland may be more significant then its apparent absence in some animals.

The gill on the right side is very similar to the pleurobranch gill, with a raphe and gill plumes on both sides. The posterior part of the gill is free, similar to that observed in *Bathyberthella antarctica* (Wägele and Willan, 1994). As in pleurobranchs, the entrance into the heart is very close to the beginning of this gill. The single gill plumes can be interpreted as a secondary elongation of the gill anteriorly. This assumption is strengthened by the finding of Cattaneo-Vietti (1986), that these gills are absent in juvenile animals. In preserved animals, the structure of the rhinophores is difficult to interpret because of the great degree of contraction. From observations and photographs of living animals, and histological examination, the rhinophore can be divided into two parts. There is the upper, enrolled tubular section which is capable of considerable extension, and a swollen basal part which is solid, but has a pit, or cavity, on the outer side which is connected to the upper tubular section by a shallow groove (see Rudman 2001; Akbatur 2002; Adams 2003). The bipartitioning of the rhinophore into a broad base and a narrower enrolled upper region is unique to *Umbraculum* and the closely related *Tylodina.*

A cuticle in the oral cavity is described by Marcus (1985). It seems probable that she meant the labial disc which demarcates the beginning of the pharynx. Both are covered with an unarmed cuticle in all specimens investigated here. The oral tube shows no cuticle. Marcus (1985) interpreted the distal oesophagus as a cuticularized stomach, but the true stomach is delineated by the entrance of the digestive gland and the exit of the intestine. This part shows no cuticle. Marcus (1985: Figure 12) clearly shows the enlarged posterior oesophagus and the small stomach, into which the digestive gland opens. The size of the animals investigated here (maximum 100 mm) is not the maximum length that can be obtained by this species with Thompson (1970) reporting a specimen of 160 mm, and Willan (1998) one of 280 mm. Nevertheless, nearly all of the investigated specimens showed a large gonad with granular appearance, indicating oocytes in the follicles. Only the small animal from the Azores (14 mm) had an immature gonad, whereas the other histologically investigated specimen (30 mm) had oogonians in the gonad, and ripe oocytes were detected in the ampulla, indicating that it was due to spawn when captured and preserved. Additionally, the mucous gland showed an area of empty cells, indicating, that the animal has already spawned at least once. According to unpublished results of the first author on histology of many opisthobranchs, it is evident, that most opisthobranchs are able to copulate and spawn long before they reach their final size.

The genital system in *Umbraculum* is unique within the Opisthobranchia in having its genital opening above the mouth, and an additional pallial gonoduct, embedded in the pallial wall, which runs a short distance from the genital opening, back along the right side of the body, before entering the body cavity to connect with the internal organs of the reproductive system. Marcus (1985) used the term 'pallial oviduct', but as this duct also serves as an autosperm duct, 'oviduct' is inappropriate. 'Pallial gonoduct', as used by Marcus and Marcus (1967), is preferred. In general terms the pallial gonoduct in *Umbraculum* can be considered a forward extension of the genital vestibule found in some monaulic cephalaspideans such as the Aglajidae (see Rudman 1974). The vestibule is a place into which the nidamental glands, the bursa copulatrix and receptaculum seminalis all open, and a place where eggs and endogenous sperm leave the system, and exogenous sperm enter. In *Umbraculum,* the vestibule is effectively lengthened so that the genital opening is moved far forward on the body, and the long external sperm groove, usually present in Cephalaspidea s.str. (see Mikkelsen 1996) is considerably shortened. As with the aglajids, there is no prostate gland associated with the internal reproductive system, in fact in *Umbraculum,* no prostate gland tissue was identified.

Our description of the nervous system agrees with that of Moquin-Tandon (1870: Plate F, 4 and 5). As he shows, the distance between the two buccal ganglia may vary, but we never observed them to be really close together.

There are a few minor morphological differences between the specimens from the Meteor Bank, the Mediterranean and the one specimen from Australia. These anatomical differences (entrances of digestive gland, position of gonad) are interpreted here as intraspecific variability, since a certain variability also occurred in the dissected specimens from the Meteor Bank. No information is available on specimens of the Meteor Bank while alive, therefore colouration is not known for these animals. In literature (e.g., Smriglio *et al.* 1990, Menezes 1991, Hartley 1964, Sidois 1996) and from our own investigations, the tubercles are described as homogenously coloured, or with longitudinal white stripes or lines. According to Thompson (1970: 176) Mediterranean specimens were "drab brown", whereas the Australian specimens had a "greenish tinge". Analysing all available pictures of living animals around the world (Rudman 1999a, b), no correlation between geographic distribution and colouration of the tubercles (greenish, brown, with or without white stripes) could be detected. Also other features, mentioned by Thompson (1970), e.g., that other organisms are attached to the shell, the shape of the peripheral tubercles, or the length of the lateral mantle processes ("peripheral tentacles") are not related to location.

Although the 18S gene is rather conservative and used for analysing deeper nodes, it was considered worth investigating in the light of finding long insertions in several opisthobranch groups, and the high variability and therefore high phylogenetic contents in these insertions (see Wägele *et al.* 2003). The lack of any differences between the three 18S rDNA sequences from different localities (Meteor Bank, Azores and Australia) gives evidence that the three sequences belong to the same species. The difference of only two positions within the analysed 16S rDNA is very low (0.42% sequence divergence) even for intraspecific variability. In the sequences used by Wägele *et al* 2003, *Austrodoris kerguelenensis* (Bergh, 1884) showed an intraspecific variability of about 1.5%, whereas the sequence divergence to a close relative, *Archidoris pseudoargus* (Rapp, 1827) is more than 10%.

Sequence divergence within the investigated three specimens from the three different localities is highest in the COI gene. But here, sequence divergence is still less than 2% and therefore does not indicate separate species. The analyses of the COI gene indicated a higher similarity between the specimens from the Great Meteor Bank and from Australia than between the two North Atlantic ones (Azores and Meteor Bank) (Figure 5). This is remarkable because of the huge distance between Australia and the North Atlantic in comparison to about 500 km between Meteor Bank and the Azores. There are no currents known that could prevent genetic exchange between the two localities in the North Atlantic, they belong to the same faunal community.

According to the results on anatomy, histology and three different genes, we consider the specimens investigated here as members of one species, *Umbraculum umbraculum* (Lightfoot, 1786). Although we have investigated specimens from only a few localities, evidence is high that all known *Umbraculum* specimens belong to one species, an assumption already expressed by Burn in 1959.

These findings and the availability of data on living animals from literature and in the internet allow us to reconsider other *Umbraculum* species, which have been discussed as valid in the last 40 years:

Specimens of *Umbraculum* from eastern Australia were identified by Thompson (1970) as *Umbraculum sinicum* (Gmelin, 1783). The author distinguished this species from *U. mediterraneum* because of the lack of algal growth on the shell, the colour, which he considered more greenish in the Australian specimens, the different shapes of the tubercles, and the elaboration of the pallial marginal tentacular processes. None of these differences are consistent in eastern Australian populations, and are part of the variability found in populations worldwide.

Marcus (1985) identified specimens from the Caribbean as *Umbraculum plicatulum* (von Martens, 1881). She described their anatomy and noted she had specimens of *U. mediterraneum* from the Mediterranean for comparison, but gave no separate information on the Mediterranean specimens, and did not mention any differences she considered to exist between these species.

Lin Guangyu (1981) gave no distinguishing features in his description of *Umbraculum pulchrum* but he did say that this species, with 'about 300 teeth in a half row' is the largest number of teeth ever reported for a species in this genus. However Marcus (1985 – for *U. plicatulum*) described 1200 teeth per half row in a specimen from Florida (length 95 mm), and O'Donoghue (1929) reported a specimen of *U. sinicum* with a half-row count of about 500. The specimens described here from the Meteor Bank show a range between 550 to 800, suggesting tooth number is variable. It is also extremely difficult to count the number of teeth accurately, many authors resorting to describing them as 'many' or using ∞.

On the basis of our investigations on several specimens from different geographical areas and with different methods, evidence is high that there is only one species of *Umbraculum*, with a wide distribution in tropical and temperate waters. It has not been recorded from the North Atlantic, eastern North Pacific, or polar waters. But a re-investigation of material from other areas, e.g. the Caribbean, with morphological and molecular means is warranted. We have listed below the major names which have been used, even if briefly, in the last 150 years, with type locality and nature of the description. Pilsbry (1896: 176–190) provides an extensive review of earlier names for this species, and the nomenclatural status of the genus has recently been reviewed (Valdés, 2001).

Umbraculum umbraculum (Lightfoot, 1786) (locality? -assumed IWP - shell)
Umbraculum sinicum (Gmelin, 1791) (locality? - assumed IWP - shell)
Umbraculum mediterraneum (Lamarck, 1819) (Mediterranean - shell)
Umbraculum indicum (Lamarck, 1819) (Indian Ocean - shell)
Umbraculum ovalis (Carpenter, 1856c) Bay of Panama, West America - shell only)
Umbraculum bermudense (Moerch, 1875) (Bermuda - drawing of animal)
Umbraculum plicatulum (von Martens, 1881) (Cuba - shell)
Umbraculum botanicum Hedley, 1923 (eastern Australia - shell, external animal)
Umbraculum pulchrum Lin Guangyu, 1981 (China - shell, external animal, radula)

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