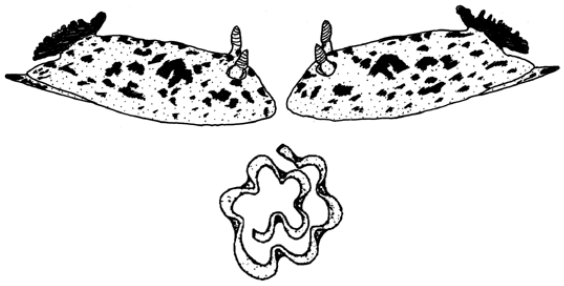
Comparative histology and ultrastructure of the nidamental glands and egg masses of the Opisthobranchia (Mollusca, Gastropoda): a functional and evolutionary approach



Dissertation

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Für meine Eltern,

die in mir die Begeisterung für die Wunder der Natur geweckt haben.

Hiermit versichere ich, dass ich die Arbeit selbständig verfasst, keine anderen Quellen und Hilfsmittel als die angegebenen benutzt und die Stellen der Arbeit, die anderen Werken dem Wortlaut oder Sinn nach entnommen sind, in jedem einzelnen Fall unter Angabe der Quelle als Entlehnung kenntlich gemacht habe.

Hattingen, 22. April 1999

Annette Klussmann-Kolb

Parts of this thesis have already been published, have been submitted or are in the preparation for publication:

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TABLE OF CONTENTS

ABSTRACT

The hermaphroditic opisthobranch gastropods possess complex reproductive systems with an immense array of different male and female structures. The nidamental glands are part of the female system. They form sac-like enlargements of the oviduct and are responsible for the formation of the gelatinous egg masses. While the fertilized ova pass through the various parts of the nidamental glandular system, they are enveloped by different layers of albumen and mucus which are successively added.

In the present study the nidamental glands of 32 species of opisthobranchs, belonging to the "Cephalaspidea s. l.", Anaspidea, Sacoglossa, Tylodinoidea, Pleurobranchoidea and Nudibranchia and of five species of the Pyramidelloidea, Pulmonata and Gymnomorpha have been investigated histologically, histochemically and ultrastructurally. The egg masses of nineteen opisthobranch taxa and one pulmonate species have also been studied with the same techniques.

Generally speaking, all species investigated possess three different glandular parts within the nidamental glandular system. In *Acteocina atrata*, *Acteon tornatilis*, *Philine alata*, *Pupa sulcata*, *Scaphander nobilis* and *Runcina adriatica* (belonging to the "Cephalaspidea s. l."), as well as in all Anaspidea and Sacoglossa an albumen gland is present at the proximal part of the oviduct. An albumen gland is also found in the pyramidelloid, pulmonate and gymnomorph species. In contrast, *Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis gardineri* (belonging to the "Cephalaspidea s. l.") and all Tylodinoidea, Pleurobranchoidea and Nudibranchia possess a proximally lying capsule gland at the same position. Following the albumen/capsule glands is always a membrane gland, and a distally lying mucous gland is also present in all species. In some species of all major taxa glandular tissue lines the distal oviduct.

The epithelia of all glandular parts have a typical arrangement of alternating glandular cells and supporting cells. While the glandular cells are mostly columnar to highly columnar and contain secretory products of various shapes, textures and histochemical staining properties, the supporting cells are slender, wedge-shaped and bear cilia of various lengths.

The albumen glands are saccular or tubular organs and contain secretory vesicles of relatively large size. The contents of these vesicles are often amorphous. They stain

basophilic and the secretory products seem to contain neutral polysaccharides and proteins.

The capsule glands are always tubular. The glandular cells are filled with secretory granules of generally smaller size than the albumen vesicles. The granules in the capsule gland are of homogeneous structure, ultrastructurally distinct substructures are usually visible. The staining properties are basophilic, the secretory products contain neutral polysaccharides and proteins.

The membrane glands are small, heavily convoluted tubes, often only distinguishable histologically. The glandular cells are generally shorter than in any other glandular part investigated, and contain mucus of various textures. The mucus stains acidophilic and is composed of acidic and sulphated mucopolysaccharides.

The mucous glands comprise the largest glandular areas within the nidamental glands. They are composed of widely coiled tubes. The glandular cells contain mucus of different structure, which is composed of acidic and sulphated mucopolysaccharides.

The alternating supporting cells usually bear short cilia in all glandular parts, except for the membrane gland, where the cilia are always very long.

Due to similar histological, histochemical and ultrastructural characteristics, the albumen and capsule glands are considered to be homologous glandular parts in all taxa investigated. The membrane glands are also homologous, as are the mucous glands.

The egg masses of opisthobranchs have a rather uniform structure. The zygotes (or ova and allosperm) are embedded in a viscous mass of albumen in those species possessing an albumen gland. Albumen filling the lumen between capsule and embryo is missing in the other species. Zygotes and albumen are enveloped by a mucoid membrane, forming distinct capsules. In the species, which possess a capsule gland and which lack albumen in the capsules, an additional, possibly albuminous inner capsule layer is present adjacent to the membrane. This inner capsule layer dissolves during intracapsular development, apparently ingested by the veliger in the capsule. The capsules themselves are surrounded by an inner mucous layer and embedded in a mucous matrix of varying consistency. Along the outside of the egg mass a multi-layered outer mucous cover is present. The various layers within the egg masses are considered to be homologous within all taxa investigated.

The formation of egg masses has been reconstructed directly from serial sections of nudibranch specimens preserved during the process of spawning. The different glandular

parts were found to have the following functions: capsule gland: secretion of inner capsule layer; membrane gland: formation of outer capsule layer (=membrane); mucous gland: successive secretion of inner mucous layer, mucous matrix and outer mucous cover.

The results gained from the present study suggest that a functional change has occurred within the evolution of the nidamental glands of the Opisthobranchia: in the common ancestor of the "derived Cephalaspidea" (*Haminoea*, *Chelidonura* and *Philinopsis*), Tylodinoidea, Pleurobranchoidea and Nudibranchia the albumen gland has changed into a capsule gland and consequently the albumen in the egg capsules has taken the form of a compact inner capsule layer. This event is believed to have taken place only once. The possession of a capsule gland and an inner capsule layer, respectively, are therefore considered to be synapomorphic characters of the monophylum comprising the "derived Cephalaspidea", Tylodinoidea, Pleurobranchoidea and Nudibranchia. The "Cephalaspidea s. l." are found to be paraphyletic.

When we no longer look at an organic being as a savage looks at a ship, as at something wholly beyond his comprehension; when we regard every production of nature as one which has had a history when we contemplate every complex structure and instinct as the summing up of many contrivances each useful to the possessor,...; when we thus view each organic being, how far more interesting, I speak from experience, will the study of natural history become.

Charles Darwin

1. INTRODUCTION

The Opisthobranchia, collectively known as the "seaslugs", comprise a very diverse group of gastropod molluscs. Among them some of the most bizarre and colourful marine invertebrates are found. To date about 3000 species have been described, but some thousands are still awaiting to be discovered (Richard Willan, pers. comm.). Within the taxon Opisthobranchia the "Cephalaspidea s. l." (nomenclature follows Mikkelsen 1996), Anaspidea, Sacoglossa, Tylodinoidea and Pleurobranchoidea (the nomenclature of these two taxa follows Wägele & Willan in press), the Nudibranchia and three less known groups (Thecosomata, Gymnosomata and Acochlidoidea) are united. The Opisthobranchia are mainly benthic organisms, occurring in almost every marine habitat from shallow waters to the deep sea. Not only because of their colourful appearance but also due to various, sometimes spectacular adaptations in their feeding behaviour, defence mechanisms and reproductive biology, they are of major interest to different fields of the biological sciences.

Although the Opisthobranchia show such a diversity in form and structure, they all have one thing in common: they are hermaphrodites and possess rather complex reproductive systems. Many functional problems occur when the same individual has to perform both sex roles, often more or less at the same time, since numerous species are known to be simultaneous hermaphrodites and reciprocal copulation occurs in many species. Thus, within the evolution, the Opisthobranchia have developed an immense array of hermaphroditic structures to meet these needs.

Our current knowledge about the reproductive systems of opisthobranchs is mainly based on anatomical studies (e. g. Guiart 1901; Pruvot-Fol 1960; Schmekel 1970; Sanders-Esser 1984). Ghiselin (1965) was the first to analyse comparatively the structure of the

reproductive systems within the major opisthobranch taxa on a functional base, mainly by collecting data from the literature but also by performing anatomical and histological investigations. He very well documented the possible evolutionary changes that have lead from a hypothetically monaulic (one common gonoduct for conducting the eggs, autosperm and the allosperm) ancestor to the diaulic and triaulic forms (two or three separate gonoducts, respectively), which can be observed in various opisthobranch groups today. Ghiselin believed that these changes have occurred independently more than once within the Opisthobranchia and always have led to an increase in the efficiency of the system. Regressive change from a diaulic or triaulic system back to a monaulic system did not happen according to him. Although Ghiselin´s study is still considered to represent a fundamental work about comparative functional morphology of the reproductive system within the Opisthobranchia, it only provides sparse information about the structure of the nidamental glands in the major opisthobranch taxa. The nidamental glands are sac-like enlargements of the oviduct and are responsible for the formation of the gelatinous egg masses. Ghiselin proposed a three parted nidamental glandular system to be present in the hypothetical ancestor of the Opisthobranchia. According to him the ancestor possessed an albumen gland at the proximal end of the spermoviduct, followed distally by a membrane gland, which led into a mucous gland further distally. This situation is believed to be present in all recent opisthobranch groups, except for the Pleurobranchoidea and Nudibranchia, which are supposed to have lost the albumen gland (Schmekel 1985). Information about the histology and ultrastructure of the nidamental glands in the various opisthobranch taxa are scattered throughout the literature (Cephalaspidea: Fretter & Graham 1954; Rudman 1971, 1972 a, b, c, 1974; Kress & Schmekel 1992; Anaspidea: Mazzarelli 1891; Eales 1921; Thompson & Bebbington 1969; Beeman 1970; Coggeshall 1972; Thomas 1975; Sacoglossa: Sanders-Esser 1984; Pleurobranchoidea: Wägele & Hain 1991; Nudibranchia: Schmekel 1971; Wägele 1989a), but extensive comparative studies of the histology and ultrastructure of the nidamental glands of all major opisthobranch taxa are lacking to date. Likewise, detailed comparative studies of the functional morphology of the nidamental glands within the Opisthobranchia have never been undertaken. The function of the glands has mainly been deduced from their location in the genital system in comparison with the structure of the various layers found in the egg masses. Only few studies actually deal with the passage and formation of the egg ribbon within the

nidamental glandular systems (e. g. Thompson 1961; Thompson & Bebbington 1969; Rudman 1971; Robles 1975).

The egg masses of opisthobranchs have been subject to many studies, but these were mainly of morphological (e. g. O'Donoghue 1922; Hurst 1967; Greene 1968; Fernandez-Ovies & Ortea 1981), physiological (e. g. Todd & Havenhand 1985; Havenhand & Todd 1988) or ecological nature (e. g. Thompson 1958b; Clark et al. 1979; Jone et al. 1996). Thompson (1967) increased our knowledge about developmental processes in opisthobranchs and presented a classification of developmental types. Information on the light microscopic structure and the ultrastructure of opisthobranch egg masses are sparse. Eyster (1986) presented a review of data about the ultrastructure of nudibranch egg capsules and Wägele (1989b; 1996) described the histology and ultrastructure of egg masses of Antarctic Pleurobranchoidea and Nudibranchia. Up to now, extensive comparative investigations of the egg masses of opisthobranchs are lacking and their fine structure has never been studied in comparison to the structure of the nidamental glands.

"...Reproductive morphology has been shown to be of primary importance for phylogenetic relationships (...). As opisthobranchs have been cited for their rampant parallel evolution (...), it is even more important to identify homology in structure and to determine where anatomical transformations have occurred monophyletically" (Gosliner 1994: 253).

The focus of this study are the histology and fine structure of the nidamental glands and egg masses of representatives of the major opisthobranch taxa and some outgroup taxa for comparison. Histochemical investigations serve to further characterize the substances which are produced in the different glandular parts and those substances which are present in the various layers of the egg masses. The aim of this study is to determine homologous parts and structures within the nidamental glands and egg masses, respectively. Secondly this study is aimed at a clarification of the function of the nidamental glands in the formation of the egg mass. In conclusion, a reconstruction of the possible evolution of the nidamental glandular system within the Opisthobranchia is proposed and the implications of the findings for phylogenetic studies are evaluated.

And whilst men agree to admire and magnify the false powers of the mind, and neglect or destroy those that might be rendered true, there is no other course left but with better assisstance to begin the work anew, and raise or rebuild the sciences, arts and all human knowledge from a firm and solid basis.

Francis Bacon

2. MATERIAL AND METHODS

2.1 Investigated species

In total 32 species of opisthobranchs and six species belonging to the Pyramidelloidea, Pulmonata and Gymnomorpha, which represent outgroup taxa, have been investigated. Tables 1 and 2 show the species examined with regards to the nidamental glandular system and the egg mass, respectively. For each species the number of specimens, the collection site, the size and the methods applied are given.

Table 1: Species investigated with regards to the nidamental glandular system

SPECIES	SPECI-MENS	COLLEC-TION SITE	LENGTH	HISTO-LOGY	HISTO-CHEM.	TEM*
"CEPHALASPIDEA s. l." (nomenclature after Mikkelsen 1996)						
Acteocina atrata[♦] Mikkelsen & Mikkelsen, 1984	2	Indian River Lagoon, USA	4 mm (fix*)	M*	-	-
Acteon tornatilis[♦] (Linné, 1758)	2	Irish Sea	7 mm (fix)	M, P*	+	-
Pupa sulcata[♦] (Gmelin, 1791)	1	Western Australia	8 mm (fix)	M	-	-
Philine alata[♦] Thiele, 1912	1	Antarctica	14 mm (fix)	M	+	-
Scaphander nobilis[♦⌘] Verrill, 1884	1	21°36,2 Min N; 18° 40,6 Min W; depth 2710m	33 mm (fix)	M	+	-
Runcina adriatica[♦⌘] Thompson, 1980	2	Corse, France	1.5 mm (fix)	M	-	-
Haminoea cymbalum (Quoy & Gaimard, 1835)	2	Dingo Beach, Australia	9 mm (alive)	M	+	-
Chelidonura inornata Baba, 1949	2	Keeper Reef, Australia	21, 23 mm (alive)	M, P	+	-

SPECIES	SPECI-MENS	COLLEC-TION SITE	LENGTH	HISTO-LOGY	HISTO-CHEM.	TEM
Philinopsis gardineri (Eliot, 1903)	2	Dingo Beach, Australia	20, 63 mm (alive)	M	-	+
ANASPIDEA						
Aplysia punctata (Cuvier, 1803)	3	Helgoland, Germany; Villefranche, France	18, 35, 63 mm (fix)	M	+	+
Bursatella leachii (de Blainville, 1817)	2	Townsville and Dingo Beach, Australia	78, 82 mm (alive)	M, P	+	-
Phyllaplysia taylori† Dall, 1900	1	Victoria, B. C., Canada	18 mm (fix)	M	+	-
Petalifera cf. *petalifera* (Rang, 1828)	1	Dingo Beach, Australia	6 mm (alive)	M	-	-
SACOGLOSSA						
Oxynoe viridis† (Pease, 1861)	1	Cottesloe Reef, Australia	15 mm (fix)	M	+	-
Elysia ornata (Swainson, 1840)	2	Dingo Beach, Australia	30, 35 mm (alive)	M, P	+	-
Elysia viridis (Montagu, 1810)	1	Helgoland, Germany	25 mm (alive)	M	+	-
Thuridilla hopei (Verany, 1853)	3	Roses, Spain	6, 8, 9 mm (fix)	M	+	+
TYLODINOIDEA (nomenclature after Wägele & Willan in press)						
Tylodina perversa† (Gmelin, 1790)	1	Chalkidike, Greece	18 mm (fix)	M	+	-
PLEUROBRANCHOIDEA (nomenclature after Wägele & Willan in press)						
Berthella stellata (Risso, 1826)	1	Townsville, Australia	8 mm (fix)	M	+	-
Euselenops luniceps (Cuvier, 1817)	1	Dingo Beach, Australia	110 mm (alive)	M	+	-
NUDIBRANCHIA- Anthobranchia (nomenclature after Wägele & Willan in press)						
Acanthodoris pilosa (Abildgaard, 1789)	2	Helgoland, Germany	20, 23 mm (alive)	M	+	+
Adalaria proxima (Alder & Hancock, 1854)	1	Sarzeau, France	16 mm (fix)	M	+	-

SPECIES	SPECI-MENS	COLLEC-TION SITE	LENGTH	HISTO-LOGY	HISTO-CHEM.	TEM
Chromodoris magnifica (Quoy & Gaimard, 1832)	3	Orpheus Island, Australia	40, 65, 70 mm (alive)	M, P	+	-
Dendrodoris nigra (Stimpson, 1855)	4	Dingo Beach; Magnetic Island, Australia	48-58 mm (alive)	M	-	vesti-bular gland
Onchidoris bilamellata (Linné, 1767)	1	Helgoland, Germany	25 mm (alive)	M	-	-
Polycera quadrilineata (Müller, 1776)	3	Helgoland, Germany	18, 20, 20 mm (alive)	M	+	+
NUDIBRANCHIA- Cladobranchia (nomenclature after Wägele & Willan in press)						
Lomanotus vermiformis Eliot, 1908	1	Dingo Beach, Australia	15 mm (alive)	M	+	-
Tritonia plebeia (Johnston, 1838)	2	Helgoland, Germany	13, 15 mm (alive)	M	+	+
Dermatobranchus semistriatus Baba, 1949	3	Balgal Beach, Australia	18, 20, 21 mm (alive)	M	+	+
Eubranchus exiguus (Alder & Hancock, 1848)	2	Helgoland, Germany	3 mm	M	-	+
Flabellina gracilis (Alder & Hancock, 1844)	1	Ostershelde, Netherlands	9 mm (fix)	M	+	+
GYMNOMORPHA						
Onchidella celtica (Cuvier, 1817)	2	Ile d´Eu, France	6 mm	M	-	-
PULMONATA						
Biomphalaria glabrata (Sars, 1818)	2	laboratory culture, RUB*	14 mm (fix, enrolled)	M	-	-
Laemodonta octanfracta[⌖⌘] (Jonas, 1845)	1	Oahu, Hawaii, USA	6 mm (fix)	M	-	-
Lymnaea stagnalis (Linné, 1758)	1	pond at RUB	18 mm (fix)	M	+	-
Pyramidelloidea						
Pyramidella sulcata[⌖] (Adams, 1854)	1	north-western Australia	25 mm (fix)	M	+	-

* Abbreviations: fix: length after fixation; Histochem: histochemistry; M: methacrylate histology; P: paraffin histology; RUB: Ruhr-University Bochum; TEM: Transmission Electron Microscopy; ⌘ sections stored at the Zoologische Staatssammlung München

Table 2: Species investigated with regards to the egg mass

SPECIES	COLLECTION SITE	HISTOLOGY	HISTO-CHEMISTRY	TEM
"CEPHALASPIDEA S. L."				
Acteocina atrata	Indian River Lagoon, USA	M, P	+	-
Haminoea cymbalum	Dingo Beach, Australia	M	-	-
Philinopsis cyanea Martens, 1879	Dingo Beach, Australia	M, P	-	-
Chelidonura inornata	Orpheus Island, Australia	M	-	-
ANASPIDEA				
Aplysia punctata	Helgoland, Germany	M, P	+	+
Phyllaplysia taylori[♦]	Bamfield, Vancouver Island , Canada	M, P	+	-
Bursatella leachii	Townsville, Australia	M	+	-
SACOGLOSSA				
Elysia ornata	Dingo Beach, Australia	M	+	+
NUDIBRANCHIA				
Acanthodoris pilosa	Helgoland, Germany	M, P	+	+
Adalaria proxima	Sarzeau, France	M	-	-
Chromodoris magnifica	Orpheus Island, Australia	M	+	+
Dendrodoris nigra	Magnetic Island, Australia	M	-	+
Onchidoris bilamellata	Helgoland, Germany	M	+	-
Polycera quadrilineata	Helgoland, Germany	M	+	+
Lomanotus vermiformis	Dingo Beach, Australia	M	+	-
Tritonia plebeia	Helgoland, Germany	M	+	+
Dermatobranchus semistriatus	Balgal Beach, Australia	M	+	+
Eubranchus exiguus	Helgoland, Germany	M	+	+
Flabellina gracilis	Osterhelde, Netherlands	+ (semi-thin epon resin sections)	-	+

SPECIES	COLLECTION SITE	HISTOLOGY	HISTO-CHEMISTRY	TEM
PULMONATA				
Radix peregra Müller, 1774	laboratory culture, University of Bielefeld	M	-	+

2.2 Methods

2.2.1 Collection of animals and egg masses

Except for the samples indicated by ⊕ in tables 1 and 2 all animals and egg masses have been collected and preserved by the author. The animals were collected in the intertidal or subtidal and most were kept in aerated aquaria overnight in the hope of egg laying. Only egg masses laid by identified specimens in the tanks or those which could be identified in the field without doubt were used in this study.

Detailed protocols of the following described methods can be found in the appendix.

2.2.2 Methacrylate Histology

For histological investigations animals and egg masses were preserved in 4-6% formalin in seawater after relaxation with $MgCl_2$ or methol flakes in seawater. When material was provided by colleagues it was confirmed by them that the animals had been preserved in 4-6% formalin in seawater. The egg mass of *Phyllaplysia taylori* had been preserved in Bouins solution (9g picric acid, 250 ml formaldehyde and 40 ml glacial acetic acid per litre). Specimens and egg masses were transferred to 70% ethanol at least 24 hours before further processing.

Shelled animals were treated with a solution of 10% formalin and 5% formic acid in seawater or freshwater, respectively, to decalcify the shell. Length of treatment depended on the thickness of the shell, varying from 30 minutes to more than 10 hours.

After dehydration in a graded ethanol series of increasing concentration, animals, dissected reproductive systems or pieces of egg masses were embedded in methacrylate

resin (Technovit 7100 by Kulzer). Serial sections (2.5 µm thick) were made for reconstruction of the organ systems and structures of interest. The sections were stained with toluidine blue, additional sections were also stained with several other stains for histochemical studies (see 2.2.4). Sections of the vestibular gland of *Dendrodoris nigra* were gram.stained according to Twort (see Bandcroft & Stevens).
The dried sections were mounted with Eukitt, Entellan or DPX.

2.2.3 Paraffin Histology

The same preservation methods as described in 2.2.2 apply to paraffin histology. The samples were dehydrated with TBA (tert-butanol) and embedded in paraplast. Sections of 5-12 µm were stained with Azan. The sections were mounted with Eukitt or Entellan.

2.2.4 Histochemistry

The following staining reactions have been applied to methacrylate (M) and to paraffin (P) sections respectively:

PERODIC ACID SCHIFF (PAS): M	indication of neutral and sulphated acidic mucopolysaccharides, muco- and glycoproteins
ALCIAN BLUE PH 1.0: M	indication of sulphated acidic mucopolysaccharides
ALCIAN BLUE PH 2.5: M	indication of acidic and sulphated mucopolysaccharides
BROMEPHENOL BLUE: M	indication of proteins
AZAN: P	differentiation of secretory products, which stain differently (blue or red)

The sections were studied using an Olympus BX 50 light microscope. Photographs of single sections were taken with an Olympus OM 2N and prints were computer edited using Adobe Photoshop 5.0 on a Macintosh.

2.2.5 Transmission Electron Microscopy

Small samples of the nidamental glandular system or egg mass were preserved in glutaraldehyde buffered in cacodylate buffer or seawater. Either NaCl or sucrose was added to the cacodylate buffer to keep the osmolarity equal to the animals environment. Postfixation was performed with OsO_4 buffered in cacodylate buffer or seawater. See the following list for the preservation methods applied:

1. 3% glutaraldehyde in cacodylate buffer with 5% sucrose; 2% OsO_4 in cacodylate buffer with 5% sucrose:

A) nidamental glands: *Acanthodoris pilosa*, *Polycera quadrilineata*, *Tritonia plebeia*

B) egg masses: *Acanthodoris pilosa*, *Polycera quadrilineata*, *Tritonia plebeia*, *Eubranchus exiguus*

2. 3% glutaraldehyde in cacodylate buffer with 0.9 % NaCl; 2% OsO_4 in cacodylate buffer with 0.9 % NaCl:

A) nidamental glands: *Aplysia punctata*, *Thuridilla hopei*, *Eubranchus exiguus*, *Flabellina gracilis*

B) egg masses: *Aplysia punctata*, *Acanthodoris pilosa*, *Polycera quadrilineata*, *Flabellina gracilis*

3. 3% glutaraldehyde in seawater; 1% OsO_4 in seawater:

A) nidamental glands: *Philinopsis gardineri*, *Dendrodoris nigra*, *Dermatobranchus semistriatus*

B) egg masses: *Elysia ornata*, *Chromodoris magnifica*, *Dendrodoris nigra*, *Dermatobranchus semistriatus*

4. 3% glutaraldehyde in cacodylate buffer; 2% OsO_4 in cacodylate buffer

A) egg mass: *Radix peregra*

Semi-thin sections (2.5 µm) were made using glass knives and stained with toluidine blue. Ultra-thin sections (70-95 nm) were cut with a Leica diatome knife and stained with uranyl acetate and lead citrate.

The sections were studied with a Hitachi H 500 TEM at the Universität Bielefeld and with a Zeiss EM 109 at the Ruhr-Universität Bochum. Photographs were edited with Adobe Photoshop on a Macintosh.

Generally speaking the processing of the membrane and mucous glands as well as the mucous structures in the egg masses was rather difficult. Fixation and/or embedding was often of poor quality, and consequently the sectioning, especially for TEM was difficult.

The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed.

Albert Einstein

3. RESULTS AND DISCUSSION

3.1 Terminology

a) ANTERIOR REPRODUCTIVE SYSTEM

The terminology of the different parts of the reproductive system in opisthobranchs is very inconsistent throughout the literature. Therefore the terms applied in this study will be explained in the following.

The terms used here are mainly based on those published by Ghiselin (1965), Beeman (1970), Thompson (1976), Hadfield & Switzer-Dunlap (1984) and Schmekel (1970, 1971, 1985). Ghiselin (1965) classified the genital systems of opisthobranchs based on the number of pallial gonoducts leading from the ampulla to the genital aperture(s). He used the terms monaulic for a system with just one common duct (spermoviduct) and diaulic and triaulic for systems with two and three ducts (vas deferens, oviduct and vaginal duct), respectively. For a schematic outline of hypothetical monaulic and triaulic genital systems see Figures 1A,B. In monaulic forms the autosperm is often transported in an open seminal groove leading from the aperture of the spermoviduct, sometimes referred to as the wide or large hermaphroditic duct (Thompson & Bebbington 1969; Painter et al. 1985, respectively), to the penis in the anterior region of the body near the head. Diaulic systems can either be androdiaulic, if the vas deferens is separated from the otherwise undivided pallial gonoduct, or oodiaulic, if the oviduct is separated. Often associated with the vas deferens are a glandular prostate and a muscular penis. Two different kinds of pouches can be attached to the vaginal duct, a proximal receptaculum seminis and a distal bursa copulatrix.

The oviduct is the most complex structure in the hermaphroditic system. It can be divided into the proximal and distal oviduct (proximal = near the gonad; distal = near the genital opening). The former is merely a ciliated non-glandular duct leading from the postampullary duct to the nidamental glands, which represent glandular enlargements of

the distal oviduct. In some species the proximal oviduct dilates to form a pouch-like structure, the fertilization chamber.

In the nidamental glandular system of the Opisthobranchia four parts can be distinguished on their histological base (from proximally to distally): an albumen or a capsule gland, a membrane and a mucous gland. A membrane and a mucous gland are always present, whereas the presence of an albumen or a capsule gland varies in the species. The membrane gland has been termed a winding gland in Anaspidea (e. g. Thompson & Bebbington 1969; Thompson 1976) or proximal mucous gland in Nudibranchia (Wägele 1989a). The distal oviduct which leads into a vestibule or just into the female genital aperture is normally non-glandular and ciliated. However in some species the duct can be lined by glandular tissue which I call adhesive region in accordance with Wägele (1989a) (translated after Schmekel 1971). In the Anaspidea almost the complete length of the oviducal channel of the spermoviduct is lined by glandular tissue. It is called here oviducal gland in accordance with Beeman (1977). In some species small glands are attached to the vestibulum or distal oviduct. They are termed vestibular or accessory glands here. In the literature (Beeman 1970, Thomas 1975) the vestibule in anaspids is termed an atrium. Attached to it is the atrial gland.

b) EGG MASS

Figures 25 and 26 give a general outline of the morphology of the egg masses investigated here.

The overall fine structure of opisthobranch egg masses is quite uniform and thus general terms can be applied to the different structures. Since histological investigations on egg masses are sparse, new terms have to be created for certain structures that have not yet been described. A schematic outline of a longitudinal section through a piece of an egg mass is given in Figure 1C.

Along its outer edge the egg mass is surrounded by an outer mucous cover, which is normally composed of distinct layers of mucus (see also Gibson et al. 1970, Wägele 1989b). Adjacent to the inner side of the outer mucous cover is a mucous matrix, in which the egg capsules are embedded. The capsule is formed by a single layered membrane (fig. 1Ca) or by a double layer, an outer membrane and an inner capsule layer (fig. 1Cb).

Hence, the term "capsule" is used here irrespective of the number of layers. The membranes themselves are surrounded by inner mucous layers. The egg capsules are arranged in tube-like structures in egg masses of higher complexity. These tubes are folded within the egg mass. The capsules contain one to several embryos and in some opisthobranchs (and pulmonates) also an albuminous fluid (fig. 1Ca).

In the following the nidamental glands of all species investigated are described. The major groups of opisthobranchs and the outgroups are discussed in separate chapters. At the beginning of each chapter a general description of the outline of the genital systems (except for separated male parts of the pallial gonoduct) is given for those species investigated here, followed by a detailed account of the histology and ultrastructure (if investigated) of the different parts of the nidamental glands in the various species. At the end of each chapter the findings are discussed with data gathered from the literature. Furthermore, the light-microscopic structure and the ultrastructure of the egg masses of 19 opisthobranch and one pulmonate species are described and compared to data in the literature. At the end of this ‘results and discussion’-chapter (chapter 3.10), histochemical staining properties of the nidamental glands and egg masses are presented and discussed.

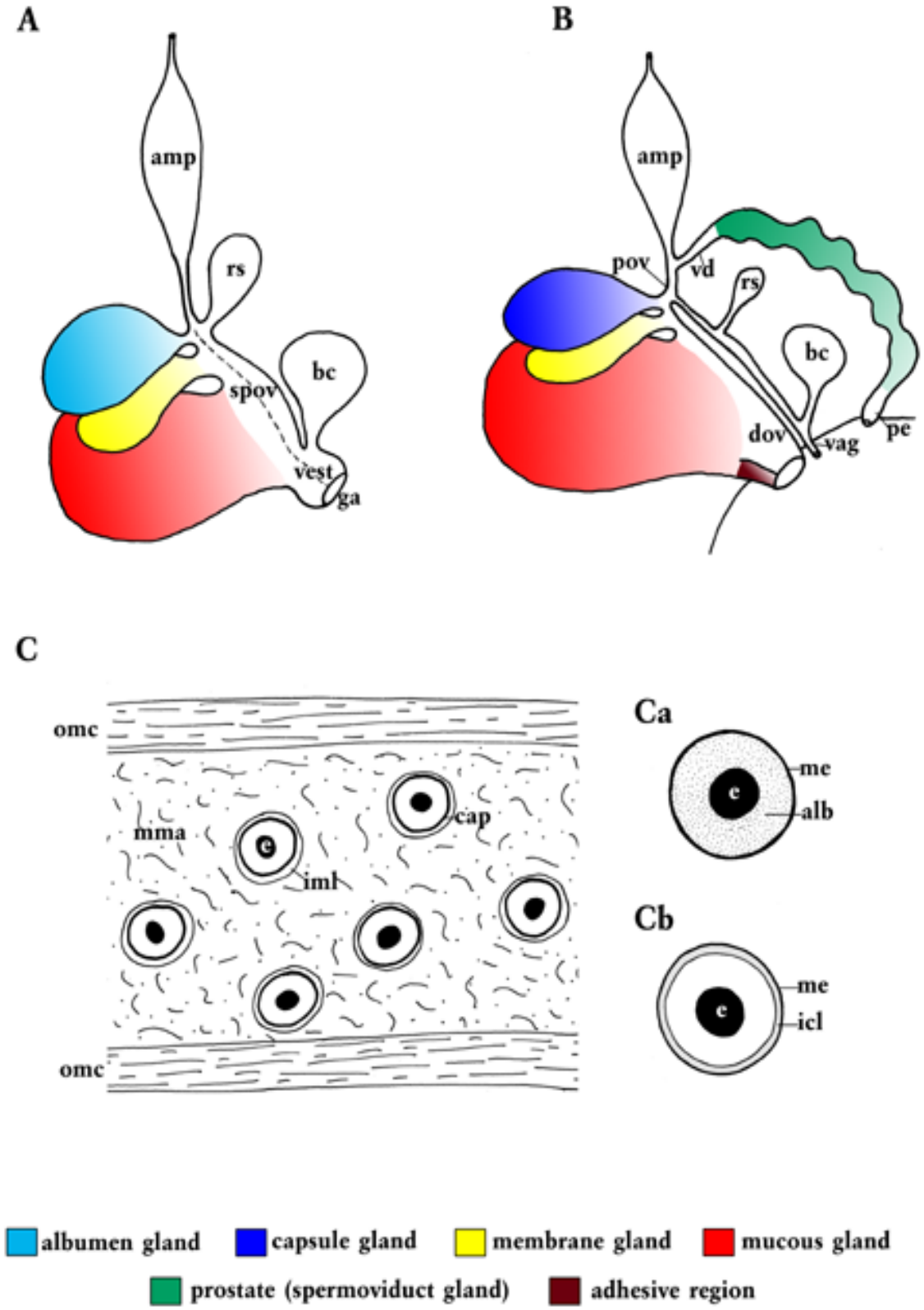

Figure 1: A: Schematic outline of the anterior reproductive systems of hypothetical monaulic (A) and triaulic (B) forms. C: Schematic outline of a longitudinal section through a hypothetical egg mass of an opisthobranch.
The colour coding for the various glandular parts of the anterior reproductive systems shown here also applies to the other figures of the same type.

3.2 The anterior reproductive systems of the "Cephalaspidea s. l."

For schematic outlines of the reproductive systems of the investigated "Cephalaspidea s. l." refer to Figure 2. Except for *Acteon tornatilis* and *Pupa sulcata*, which have a diaulic genital system, all other investigated "Cephalaspidea s. l." species show monaulic anterior reproductive systems with separated seminal grooves and penes.

The ampulla is a mainly tube-like structure, sometimes heavily folded, as for example in *Chelidonura inornata* and *Philinopsis gardineri*. The latter shows an unusually long and glandular postampullary duct, whereas in *Runcina adriatica* this duct is very short. The postampullary duct is glandular also in *Scaphander nobilis*. *Acteon tornatilis* and *Scaphander nobilis* are lacking a receptaculum seminis. A bursa copulatrix is missing in *Runcina adriatica*. All other species exhibit receptacula semines and bursae copulatrices. The latter always insert more distally than the receptacula at the spermoviduct.

Acteon tornatilis and *Pupa sulcata* are shown to have a large vas deferens, differentiated in a proximal glandular prostate and a distal non-glandular duct. The most distal part of the vas deferens in *Acteon tornatilis* could not be examined because of very bad histological sections.

The nidamental glandular mass is divided into three distinct parts in all species examined. Whereas the most proximal part can be referred to as an albumen gland in *Acteocina atrata, Acteon tornatilis, Pupa sulcata, Philine alata, Runcina adriatica* and *Scaphander nobilis*, *Haminoea cymbalum, Chelidonura inornata* and *Philinopsis gardineri* show a capsule gland there. All species have a membrane gland and a mucous gland. *Pupa sulcata, Philine alata* and *Scaphander nobilis* show specially differentiated glandular cells along the most distal part of the oviduct. In some species the hermaphroditic duct leads distally into a muscular vestibule, which itself discharges into the genital opening. No distinct vestibule could be found in *Acteocina atrata, Philine alata, Runcina adriatica* and *Chelidonura inornata*.

Comparative schematic drawings of the major cell types of the nidamental glands are shown in table 3. Unfortunately the tissue of the investigated specimens of *Acteocina atrata* was very badly preserved, thus the cellular appearance was difficult to interpret. Hence no schematic drawings of the histology of the glandular cells of this species could be made, but a general description of the cellular structures is still possible.

All staining properties stated in the following refer to toluidine blue staining. For other histochemical staining properties see table 9, page 129-131.

3.2.1 Histology of the nidamental glands:

ALBUMEN GLAND (table 3; fig. 3C, D): The albumen gland mostly exhibits a pouch-like or bulbous organ, which can consist of only few narrow folds, e .g in *Acteocina atrata* and *Pupa sulcata* or comprise large folds as in *Acteon tornatilis*, or be internally folded as in *Scaphander nobilis.* In *Runcina adriatica*, in contrast, the albumen gland is tubular. The albumen glands of *Acteon tornatilis*, *Scaphander nobilis* and *Runcina adriatica* are located in between the folds of the mucous glands (fig. 3C, D). In general the glandular epithelium is composed of columnar to highly columnar cells containing vesicles of different texture and sizes. The largest vesicles are found in *Acteocina atrata* (up to 8.5 µm in diameter). Only few vesicles are visible embedded in homogeneously blue staining cytoplasm. The vesicles stain blue and have a white rim. In *Acteon tornatilis* the vesicles are up to 3.0 µm in diameter and show a dark pink staining centre and a lighter staining rim. In *Philine alata* vesicles of round to elliptical shape are found (up to 7.5 µm in diameter). They are also composed of two differently staining substances: the centre of each vesicle stains light blue, whereas the rim stains dark blue. In the lumen of the gland whole vesicles are visible, which seem to dissolve as they move through the gland. *Scaphander nobilis* shows secretory vesicles of varying sizes (up to 7.0 µm in diameter) and homogeneous structure. They stain dark blue. In some cells the vesicles seem to fuse in the apical part of the cell. Very few interspersed cells have homogeneously pink staining contents in this species. The small vesicles of *Pupa sulcata* (up to 2.4 µm in diameter) are composed of dark blue-staining substances embedded in a light blue staining matrix. This texture is only visible with high magnifications. In the albumen gland of *Runcina adriatica* the secretory vesicles (up to 5.0 µm) have a homogeneous structure and stain dark blue. Except for *Pupa sulcata* the glandular cells of all species contain large nuclei located mostly in the basal part of the cell. In *Acteon tornatilis* the nuclei can also be found medially. In the latter and in *Scaphander nobilis* prominent nucleoli are visible. All species investigated show the typical arrangement of alternating glandular and supporting cells. The latter are small and slender and have a prominent triangular apex in *Philine alata* and *Runcina adriatica*. The supporting cells

generally bear short to moderately long cilia. In *Acteocina atrata* the supporting cells cannot be distinguished although cilia are visible. A small nucleus is found in the apical part of the supporting cells of all species except for *Pupa sulcata*.

The albumen gland shows a continuous transition to the following membrane gland in *Acteocina atrata*, *Acteon tornatilis* and *Pupa sulcata*. In *Philine alata* the two glands are connected by a small non-glandular duct, whereas the albumen gland of *Scaphander nobilis* discharges into the spermoviduct. *Runcina adriatica* presents a different situation: the albumen gland discharges into a dilation of the oviduct, which is considered to be a fertilization chamber. The membrane gland of *Runcina adriatica* also originates in this chamber.

CAPSULE GLAND (table 3; fig. 3A, B): Instead of an albumen gland *Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis gardineri* possess a capsule gland at the proximal part of the oviduct. This gland is always tubular in structure. In *Haminoea cymbalum* the capsule gland is coiled a few times, whereas in the other two species the pseudo two-layered epithelium forms pouch-like structures branching off from the central duct of the gland (fig. 3A, B). The glandular cells are prismatic (*Haminoea cymbalum*) to highly columnar (*Chelidonura inornata* and *Philinopsis gardineri*). The secretory granules are round and stain dark blue in all three species. The sizes of the granules vary: *Chelidonura inornata* possesses the largest granules (up to 3.5 µm in diameter), whereas in *Philinopsis gardineri* they are up to 1.25 µm in diameter. The size of the granules in *Haminoea cymbalum* is almost uniform and averages 2.0 µm in diameter. The nuclei are generally found in the basal parts of the glandular cells. They contain prominent nucleoli in *Chelidonura inornata* and *Philinopsis gardineri*. Glandular cells are always alternating with supporting cells. The latter have a triangularly shaped apex in *Chelidonura inornata* and *Philinopsis gardineri* and contain an apically lying nucleus in all three investigated species. The supporting cells bear extremely short cilia in *Haminoea cymbalum*, whereas in *Chelidonuta inornata* and *Philinopsis gardineri* the cilia are of moderate length. The capsule glands of the latter two species show a continuous transition to the membrane gland. In *Haminoea cymbalum*, in contrast, the capsule gland discharges into the spermoviduct.

MEMBRANE GLAND (table 3; fig. 3A): The membrane gland comprises the smallest glandular part within the nidamental glands. It is always tubular and consists of few

narrow coils (fig. 3A). The glandular cells have a prismatic (*Pupa sulcata*), columnar (*Philine alata*, *Runcina adriatica*, *Haminoea cymbalum*) to highly columnar shape (*Acteon tornatilis*, *Scaphander nobilis*, *Chelidonura inornata*, *Philinopsis gardineri*). The secretory contents of the glandular cells have various textures and staining properties. The mucus can take the form of small droplets, as in *Acteocina atrata*, *Acteon tornatilis*, *Pupa sulcata*, *Scaphander nobilis* and in the proximal part of the membrane gland of *Runcina adriatica*. In the distal part of the membrane gland of the latter species the mucous contents have the form of heterogeneous fibres packed into larger vesicles. Similar vesicles are also found in *Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis gardineri*. In the latter two species the mucus in the vesicles sometimes coagulates and forms droplets. In *Philine alata* the dark red staining, irregularly shaped mucous patches are embedded in homogeneously pink-staining mucus. In general, the staining properties of the glandular epithelium range from red to dark violet. Small nuclei are always located basally in the glandular cells. In *Acteon tornatilis*, *Philine alata* and *Philinopsis gardineri* the nuclei seem to be pycnotic. In the alternating supporting cells apically lying nuclei can be found in almost all species, except for *Acteocina atrata*, *Pupa sulcata* and *Haminoea cymbalum*. The supporting cells always bear very long cilia. They are not distinguishable in *Scaphander nobilis*, though. The membrane gland directly joins to the mucous gland in *Acteocina atrata*, *Pupa sulcata*, *Scaphander nobilis*, *Runcina adriatica* and *Haminoea cymbalum*. In *Acteon tornatilis*, *Philine alata*, *Chelidonura inornata* and *Philinopsis gardineri* the membrane gland discharges into the oviduct or spermoviduct, respectively.

MUCOUS GLAND (table 3; fig. 3D, E, F): The mucous gland is the largest glandular part of the nidamental glands. It generally comprises a large, widely coiled tube. In *Pupa sulcata* the proximal part of the mucous gland is a small, narrow tube, which dilates further distally to form the major part of the gland. In *Acteon tornatilis* and *Haminoea cymbalum* the proximal part of the mucous gland is narrowly coiled (fig. 3C), whereas the distal part is also widely coiled as in the other species (fig. 3E, F). The glandular cells are columnar (*Runcina adriatica*, *Haminoea cymbalum*) to highly columnar (all other species). The secretory contents have different texture and staining properties. The mucus can either be homogeneous (*Acteocina atrata*, *Pupa sulcata* in proximal part) or filamentous (*Acteocina atrata*, *Pupa sulcata* in the distal part, *Scaphander nobilis*, *Haminoea cymbalum*, *Chelidonura*

inornata). In other species it has the form of small droplets (*Philine alata*, *Scaphander nobilis*, *Runcina adriatica*) or the mucus is packed into larger vesicles which sometimes dissolve, thus filling the cells with homogeneous patches of mucus (*Acteon tornatilis*, *Runcina adriatica*, *Chelidonura inornata*). In *Philinopsis gardineri* the mucous contents in the vesicles sometimes coagulate and form compact droplets. Usually different cell types are present in the same species or even individual. The staining properties of the glandular cells of the mucous gland are generally acidophilic and range from light pink, to red and dark violet. Filamentous mucus generally stains light pink to light violet, whereas droplets and homogeneous mucous patches stain darker. The small nuclei are found basally, in *Acteon tornatilis*, *Pupa sulcata*, *Runcina adriatica* and *Philinopsis gardineri* they seem to be pycnotic. All species investigated have alternating glandular cells and small supporting cells. The latter bear short cilia and contain an apically lying nucleus, which is not visible in *Scaphander nobilis*. The supporting cells have a triangular apex in *Acteon tornatilis*, *Philinopsis gardineri*, *Pupa sulcata* and *Haminoea cymbalum* and partly cover the adjacent glandular cells in the latter two species. In most species the mucous gland discharges into the distal spermoviduct or oviduct, but in *Acteon tornatilis*, *Haminoea cymbalum* and *Philinopis gardineri* it connects distally to the vestibulum.

ADHESIVE REGION (table 3): The most distal part of the oviduct is lined by a glandular epithelium in *Pupa sulcata*, *Philine alata* and *Scaphander nobilis.* The glandular cells are highly columnar in *Pupa sulcata*, whereas *Philine alata* and *Scaphander nobilis* show prismatic glandular cells in the region. The secretory products have different structures. The mucous secretions can either have the form of droplets of different sizes, as can be found in some cells of all three species. In other cells the mucus is filamentous (*Philine alata* and *Scaphander nobilis*) or the droplets or mucous coagulations dissolve and form homogeneous patches (*Pupa sulcata*). The latter stain red, whereas the droplets stain dark violet. The mucus filaments stain lighter violet. The nuclei found in the glandular cells are small and located near the base of the cell. In *Scaphander nobilis* nuclei are not visible in those glandular cells, which contain mucous filaments or large small mucous droplets. Alternating supporting cells contain apically lying nuclei in *Scaphander nobilis*. In the other two species the nuclei are not visible in the supporting cells. These bear short cilia in all three species.

ACCESSORY GLAND AT VESTIBULUM: This small gland found in *Philinopsis gardineri* is follicular in shape and composed of columnar secretory cells containing numerous blue-staining, round granules. The nuclei are lying basally. Interspersed are undifferentiated cells. In between the glandular cells ciliated supporting cells are visible.

3.2.2 Ultrastructure of the nidamental glands:

The ultrastructure of parts of the nidamental glands has been investigated for *Philinopsis gardineri* only. Thus all following descriptions refer to this species.

CAPSULE GLAND: Ultrastructural investigation of the capsule gland of *Philinopsis gardineri* reveals that the granules found in the glandular cells have a homogeneous structure (fig. 18B). Their size varies, the largest vesicels found have a diameter of 1.25 µm. The granules almost completely fill the upper 2/3 of the cell. Further basally the number of mature granules decreases; instead few immature granules can be found. These have a slightly different structure to the mature ones: they have an electron-dense centre which is surrounded by coarse material forming a lighter rim. The nucleus is large and contains a very prominent nucleolus. Rough endoplasmic reticulum (ER) can be found especially around or near the nucleus. Further away from the nucleus only vesicular ER is found. Few Golgi complexes are present in the basal half of the cell. Spread throughout the cell are elongated mitochondria showing distinct cristae. The apical surface of the cell extends microvilli. These are present also at the apical membrane of the alternating supporting cells which also bear cilia having a typical 9+2 pattern in the shaft in all glands described. Apically the supporting cells are almost as broad as the glandular cells.

MEMBRANE GLAND: The mucous vesicles in the glandular cells of the membrane gland are small and contain loose mucous fibres. The nuclei are small and contain dense chromatin. They seem to be pycnotic. No Golgi complexes or endoplasmic reticulum could be found in the cells. The glandular cells are completely filled with mucous vesicles. The supporting cells bear extremely long cilia and apically contain numerous large mitochondria. The nuclei are also large and active.

Because of poor preservation of the tissue no data on the ultrastructure of the mucous gland could be gathered.

3.2.3 Discussion

With regards to the nidamental glandular system, the "Cephalaspidea s. l." do not present a uniform picture. This is due to the different glandular parts at the proximal part of the oviduct. Some species possess an albumen gland, some a capsule gland at the same position. While the albumen glands are mostly sac-like structures (except for *Runcina adriatica*), the capsule glands are always tubular. The types of granules or vesicles in the glandular cells of the two glands differ considerably: the albumen vesicles are mostly large, (size up to 8.5 µm), elliptically- to round-shaped and usually composed of differently staining substances, whereas the granules in the capsule glands are usually smaller (size up to 3.5 µm) and of homogeneous structure. They stain darker blue than the albumen vesicles (except for *Runcina adriatica*). All cephalaspids have a membrane and a mucous gland, both lined by glandular epithelium containing mucous substances. The mucous gland is always much larger than the membrane gland and not as narrowly coiled. Usually the supporting cells of the latter bear longer cilia than those of the mucous gland. Additionally to these two glands *Pupa sulcata*, *Philine alata* and *Scaphander nobilis* also show a specialized glandular tissue along the distal oviduct (adhesive region). The glandular cells in this region seem to represent mucous gland cells in all three species.

Although the information about the histology and ultrastructure of the nidamental glands in cephalaspids is sparse, some data are available from the literature to which the present findings can be compared. The differentiation between two different parts of mucous glands as presented in this study for all cephalaspidean species has already been described by Fretter & Graham (1954) in their anatomical and histological description of the reproductive system of *Acteon tornatilis*. They distinguished between an upper and a lower part of the mucous gland. The upper part of the mucous gland is connected with the albumen gland whereas the lower mucous gland lies more distally. Thus I assume that the upper mucous gland of Fretter & Graham is equivalent to the membrane gland described here whereas the lower mucous gland of Fretter & Graham accords with the mucous gland described in this study. Johansson (1954) distinguished between a posterior and an

anterior part of the mucous gland of *Acteon tornatilis*, but his description of the histology of the different parts is incomplete. Thus it is difficult to compare his findings to the present ones. Rudman (1972c) described a narrowly coiled anterior and a widely coiled posterior mucous gland in *Pupa kirki* (see figures 8A and B, page 60). According to him these two glands are not connected. A membrane gland, as has been found for *Pupa sulcata* in the present study, was not observed by Rudman in *Pupa kirki.* Since the absence of a membrane gland is a very unusual situation in opisthobranchs I assume that Rudman was incorrect and the anterior mucous gland described by him actually accords with the membrane gland described here.

In his morphological description of philinacean opisthobranchs Gosliner (1988) reported three glandular parts in the nidamental glands of different species of *Philine*. He distinguished between an albumen, a membrane and a mucous gland. This is confirmed by the present study.

Rudman (1971, 1972a and b, 1974) presented rather extensive anatomical and histological studies of the reproductive systems of different cephalaspidean genera. In his investigations of several species of *Haminoea*, *Chelidonura*, *Philinopsis* and *Melanochlamys* he always reported of an albumen-capsule gland complex opposed to the mucous gland. He described the cells of the albumen gland to contain secretory spherules, whereas the cells of the capsule gland do not contain distinct spherules. In all genera described by him there is always a direct connection between albumen and capsule gland, but whereas in *Philinopsis* only the capsule gland opens distally into the vestibule (Rudman 1972a), both glands open separately into the vestibule in *Chelidonura* (Rudman 1972b). From the histological descriptions presented by Rudman it seems that his albumen glands accord with the capsule glands described in the present study for *Chelidonura inornata* and *Philinopsis gardineri*, whereas his capsule glands are equivalent to the membrane glands described here. The histological description of the mucous gland of *Melanochlamys* (Rudman 1974), which according to the author is the same in *Chelidonura*, accords with my findings regarding the mucous gland of *Chelidonura inornata*. Specialised glandular regions of the distal oviduct as have been found in *Pupa sulcata*, *Philine alata* and *Scaphander nobilis* in the present investigation, have not been described previously in these or other cephalaspidean species.

The only ultrastructural investigation of the nidamental glands in a cephalaspidean species known to me has been undertaken by Kress & Schmekel (1992). The authors also differentiated between three histologically and ultrastructurally distinct parts in the gland mass of *Runcina coronata* and *Runcina ferruginea*. In these two species the albumen gland has a granular structure. The internal structures of the granules can vary from homogeneously light (as described in the present study for *Philinopsis gardineri*) to electron-dense with a mottled structure (as will be described later in this study in the capsule gland of *Tritonia plebeia*, see page 60). A third type of granule described for the two species of *Runcina* shows dark centres surrounded by less electron-dense material. This accords with the structure described for the vesicles in the albumen gland of *Acteon tornatilis* and *Acteocina atrata* in the present study. As can be seen from the description of Kress and Schmekel, the albumen gland of *Runcina* contains granule types typical for albumen glands of other cephalaspidean species as well as granule types that can be found in capsule glands for example of nudibranchs. With this information in mind it is difficult to decide whether the gland described here for *Runcina adriatica* is an albumen gland or rather a capsule gland. But since Kress & Schmekel (1992) give evidence for an albuminous fluid in the egg mass of *Runcina coronata* it is probable that the gland found in *Runcina adriatica* is an albumen gland (for a more detailed discussion of the homologies of the different glandular parts in opisthobranchs see chapter 4.2). In their description Kress and Schmekel also report of an egg-capsule gland forming a transitional duct between the albumen and the distal mucous gland. The egg-capsule gland consists of a small, folded tube, that is heavily ciliated. The glandular cells composing the capsule gland are reported to contain mucous substances. Thus I assume the capsule gland of *Runcina coronata* and *Runcina ferruginea* is conform to the membrane gland described in the present study for *Runcina adriatica*. The description by Kress & Schmekel of the mucous gland accords with the present findings of the mucous gland of *Runcina adriatica*.

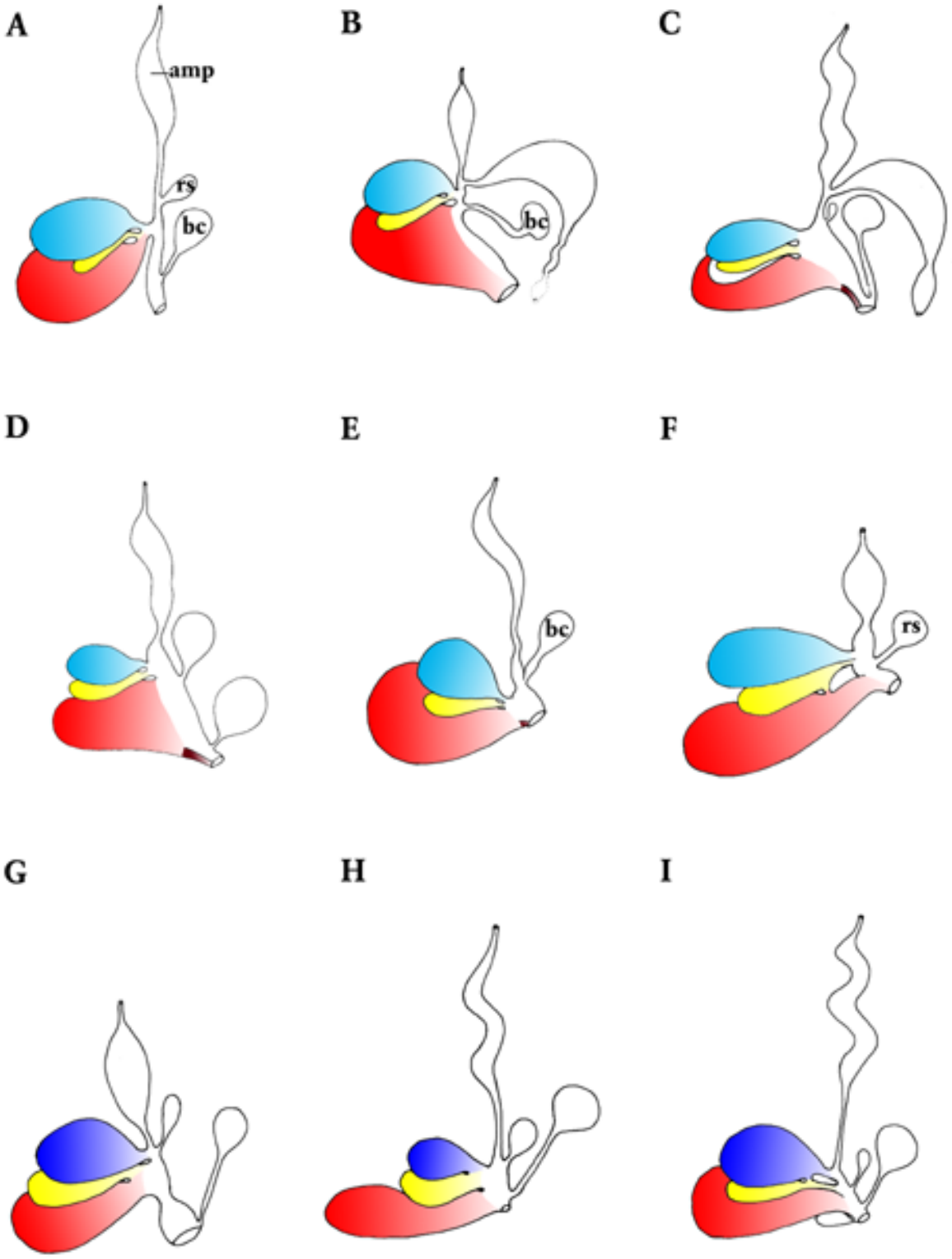

Figure 2: Schematic outlines of the anterior reproductive systems of several “Cephalaspidea s. l.”. A: *Acteocina atrata*; B: *Acteon tornatilis*; C: *Pupa sulcata*; D: *Philine alata*; E: *Scaphander nobilis*; F: *Runcina adriatica*; G: *Haminoea cymbalum*; H: *Chelidonura inornata*; I: *Philinopsis gardineri*.

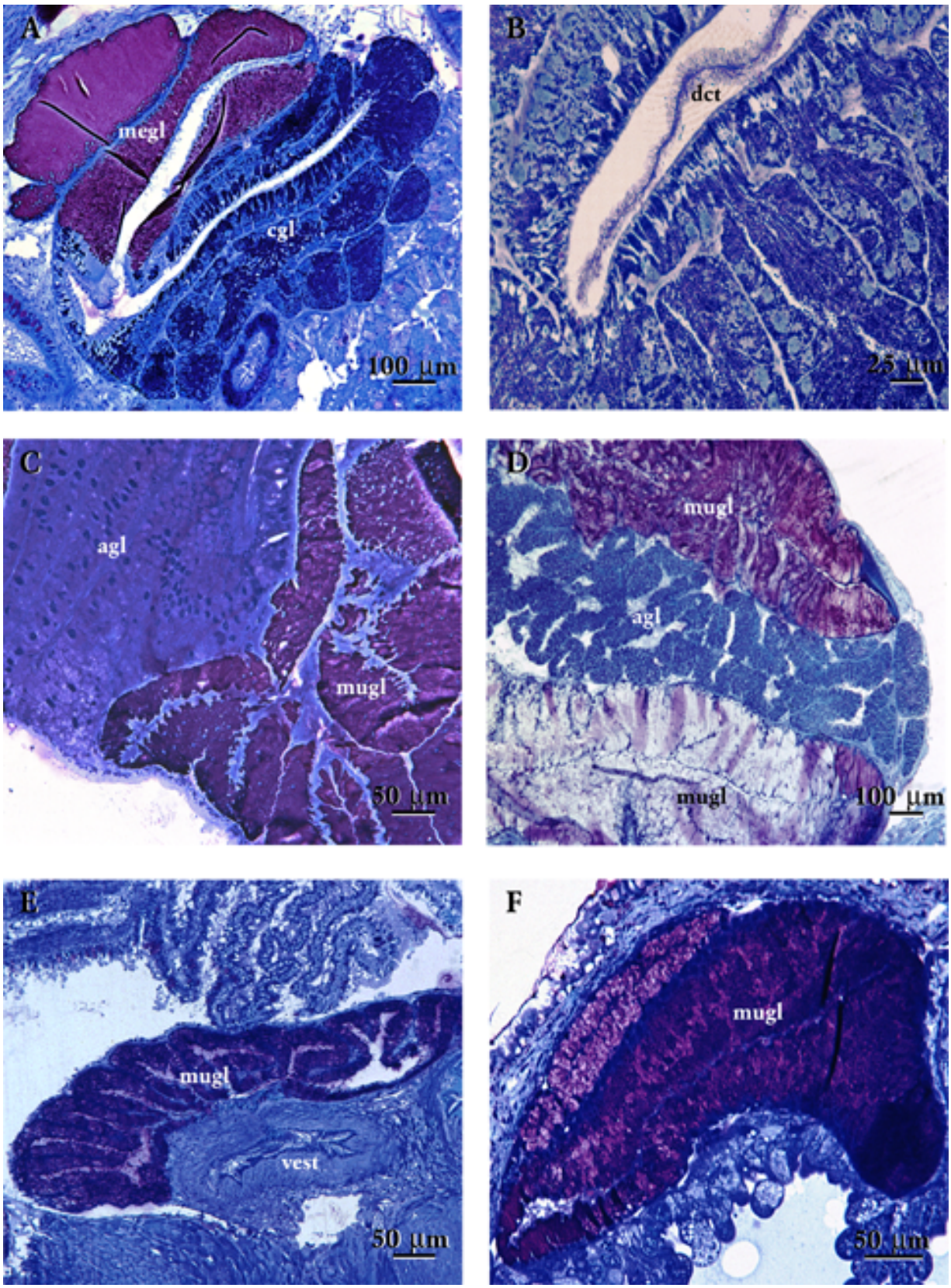

Figure 3: Histological sections through the nidamental glands of several "Cephalaspidea s. l.". A: *Chelidonura inornata* - capsule and membrane gland; B: *Philinopsis gardineri* - capsule gland; C: *Acteon tornatilis* - albumen and membrane gland; D: *Scaphander nobilis* - albumen and mucous gland; E: *Haminoea cymbalum* - mucous gland; F: *Runcina adriatica* - mucous gland.

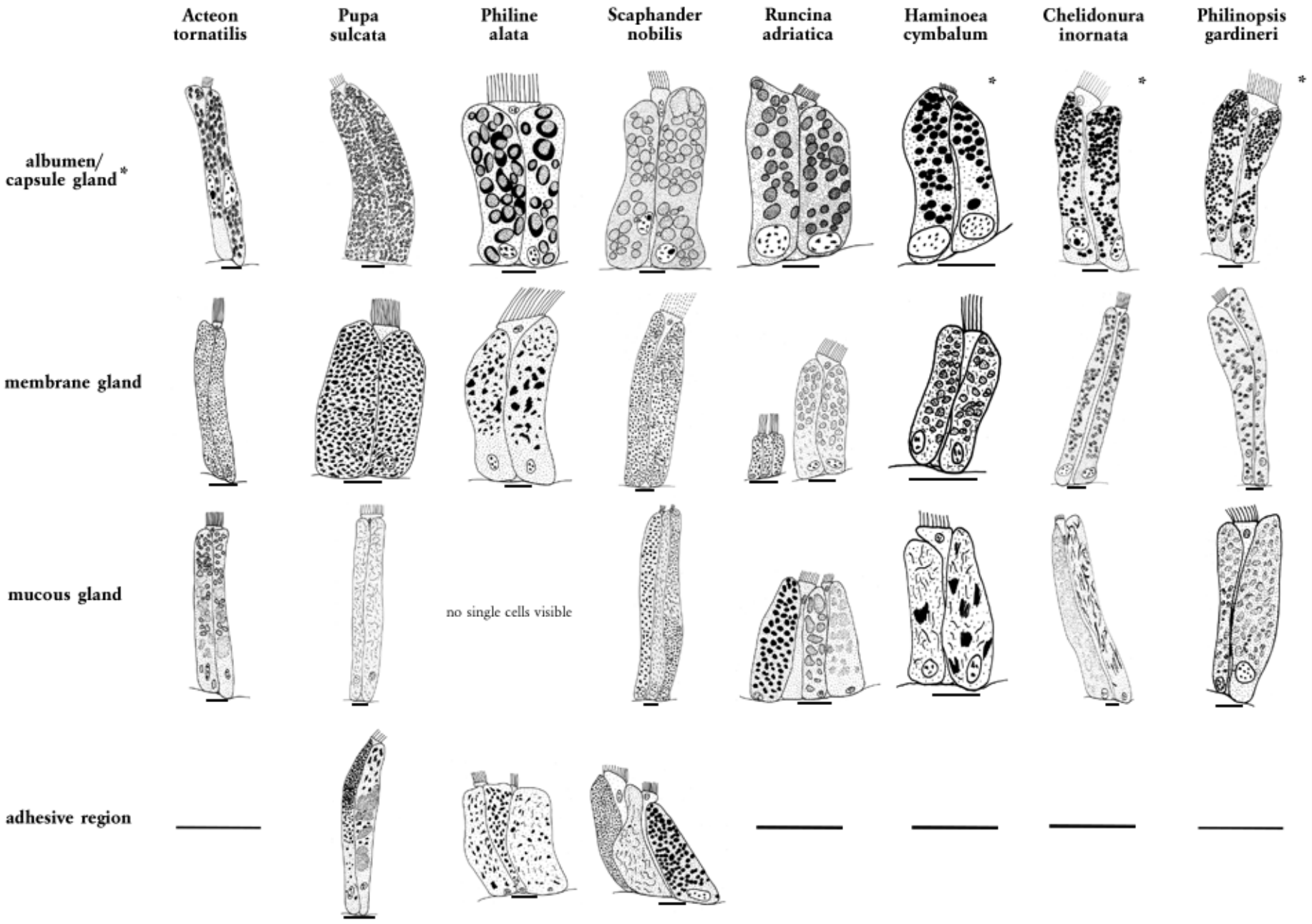

Table 3: Comparative schematic drawings of the major cell types of the nidamental glands of several "Cephalaspidea s. l."; scale bars = 10 µm.

3.3 The anterior reproductive system of the Anaspidea

For schematic outlines of the anterior reproductive systems of the Anaspidea refer to Figure 4. The Anaspidea studied here show monaulic reproductive systems. The nidamental glands are attached to the pallial spermoviduct. This latter duct is internally folded and bears three incompletely separated channels, namely the oviducal channel, the internal autospermal groove (see Thompson & Bebbington 1969; Thomas 1975) and the vaginal channel. The oviducal channel is always lined by glandular tissue (oviducal gland). In *Aplysia punctata* this glandular part of the oviducal channel is very large and forms a pouch. In *Bursatella leachii* it is also prominent, while in the other two species investigated this structure is confined to a smaller area. All species also show another type of glandular tissue related to the autospermal groove (spermoviduct gland). The ampulla is mostly tube-like. The postampullary duct forms a dilation in its distal region, here referred to as a fertilization chamber. This area is the central part of the system; all parts join here. The nidamental glands originate in the fertilization chamber, as do the spermoviduct and the oviducal glands. In *Aplysia punctata* and *Petalifera cf. petalifera* the duct leading to the recepataculum seminis also inserts at the fertilization chamber, whereas in *Bursatella leachii* and *Phyllaplysia taylori* it inserts further distally at the spermoviduct.

The nidamental glandular complex can be divided into three parts. Most proximally a large sac-like albumen gland is found. Adjacent to this is the membrane gland followed further distally by the large mucous gland. This latter gland always shows a continuous connection to the oviducal gland.

At the distal end of the spermoviduct a bursa copulatrix inserts, and in all species that were investigated there is an atrial gland attached to the atrium.

Comparative schematic drawings of the major cell types of the nidamental glands and the glandular spermoviduct are shown in table 4.

3.3.1 Histology of the nidamental glands:

ALBUMEN GLAND (table 4; fig. 6A, B): The albumen gland forms a sac-like organ in all anaspidean species investigated. Internally it builds secondary folds, which are very

obvious in *Aplysia punctata*, *Bursatella leachii* and *Phyllaplysia taylori* (fig. 6A) but less distinct in *Petalifera cf. petalifera* (fig. 6B). Supporting cells and glandular cells alternate. The glandular cells are broad to columnar, in *Phyllaplysia taylori* their apex is broader than the base. The glandular cells are filled with vesicles of round to elliptical shape which contain blue-staining secretions. In *Aplysia punctata* the secretions form dense patches, whereas the secretory contents of the vesicles in *Phyllaplysia taylori* are of heterogeneous, filamentous structure. *Bursatella leachii* shows two types of vesicles in the albumen gland: apically few round to elliptically shaped vesicles with heterogeneous contents can be found, whereas basally larger vesicles with homogeneous contents are present. The latter are probably pre-stages of the vesicles found in the apical part. The secretory vesicles of *Petalifera cf. petalifera* have amorphous contents. The size of the vesicles differs considerably within the species: in *Aplysia punctata* and *Bursatella leachii* they are up to 3.5 µm in diameter, *Phyllaplysia taylori* possesses vesicles of up to 10.0 µm and in *Petalifera cf. petalifera* the vesicles have a diameter of up to 13.5 µm. The staining properties are basophilic, the vesicles stain light to dark blue and they are embedded in light blue staining cytoplasm. Large nuclei are found in the basal part of the cell, in *Phyllaplysia taylori* distinct nucleoli are visible. The supporting cells are hardly visible (*Aplysia puncata*, *Petalifera cf. petalifera*) and bear short cilia or cannot be observed with low power microscopy at all (*Bursatella leachii*, *Phyllaplysia taylori*).

MEMBRANE GLAND (table 4; fig. 6B, C): In contrast to the large albumen gland, the membrane gland comprises a smaller glandular area and forms a tube-like structure. It is narrowly coiled in all species. Prismatic, stout to columnar glandular cells alternate with small, slender supporting cells. The glandular cells are filled with secretions of various form and texture: in *Aplysia punctata* the secretions have the shape of small vesicles, while in *Petalifera cf. petalifera* and *Phyllaplysia taylori* they take the form of small droplet-like vesicles. Since in *Bursatella leachii* the glandular epithelium is in a bad condition due to the fact that the animal had just spawned before preservation, filamentous secretions are scattered throughout the glandular cells. The mucous secretions stain light to dark pink (*Aplysia punctata*) (fig. 6C), red to pink (*Bursatella leachii*), dark red (*Petalifera cf. petalifera*) (fig. 6B) or dark pink (*Phyllaplysia taylori*). In all species small nuclei are found basally, in *Petalifera cf. petalifera* they seem to be pycnotic. The supporting cells contain apically lying nuclei in *Petalifera cf. petalifera* and *Bursatella leachii*, while in *Aplysia punctata* and *Phyllaplysia*

taylori no nuclei are visible within the supporting cells. The apices of the supporting cells have a triangular shape in all species and bear very conspicuous long cilia.

MUCOUS GLAND (table 4; fig. 6D): This is the largest organ in all four species. It is also sac-like and similarly folded internally (fig. 6D) as the albumen gland. Glandular cells and supporting cells alternate. The glandular cells are columnar (*Aplysia punctata*, *Bursatella leachii*) to highly columnar (*Petalifera cf. petalifera*) and contain mucous secretions of various structure. In *Phyllaplysia taylori* single cells are hardly visible. The mucous secretions can be of homogeneous structure (*Bursatella leachii*) or in the form of vesicles with homogeneous (*Petalifera cf. petalifera*, *Phyllaplysia taylori*) or heterogeneous contents (*Aplysia punctata*). The staining properties vary from red to violet. The small nuclei lie basally in the glandular cells and are seemingly pycnotic in *Aplysia punctata* and *Petalifera cf. petalifera*. In *Bursatella leachii* the nuclei are large and contain nucleoli. The supporting cells are small and appear wedge-shaped in *Bursatella leachii*. In *Phyllapslysia taylori* they are not visible with low power microscopy. They bear short cilia and contain an apically lying nucleus, which is not visible in *Petalifera cf. petalifera*.

3.3.2 Histology of the glandular tissue of the spermoviduct:

OVIDUCAL GLAND (table 4; fig. 5A-D; 6E): The oviducal gland lines the oviducal channel of the spermoviduct. In *Aplysia punctata* it forms secondary wide folds (fig. 6E). The glandular cells are stout (*Petalifera cf. petalifera*), prismatic (*Phyllaplysia taylori*) or highly columnar (*Aplysia punctata*, *Bursatella leachii*). They contain numerous small granules of round (*Aplysia punctata*, *Petalifera cf. petalifera*, *Phyllapslysia taylori*) or irregular shape (*Bursatella leachii*). The granules cluster in the apical halves of the cells, which is very conspicuous in *Phyllaplysia taylori*. They have acidophilic staining properties, staining dark blue to dark violet. The position of the mostly large nuclei is basally but can also be medially in *Phyllaplysia taylori.* The nuclei contain visible nucleoli in *Petalifera cf. petalifera*. The alternating supporting cells have apically a triangular shape in *Bursatella leachii* and *Aplysia punctata*, and they cover the apices of the glandular cells in the former species. In *Aplysia punctata* and *Bursatella leachii* the supporting cells bear long cilia, whereas in *Phyllaplysia taylori* the cilia are of moderate length and in *Petalifera cf. petalifera* short cilia are found. Apically lying nuclei are visible in the supporting cells of all species except for *Petalifera cf. petalifera.*

SPERMOVIDUCT GLAND (table 4; fig. 5A-D; 6E): The glandular tissue of the spermoviduct gland lines the autospermal groove of the spermoviduct. It is composed of alternating glandular and supporting cells. The glandular cells are columnar (*Petalifera cf. petalifera* and *Phyllaplysia taylori*) to highly columnar (*Aplysia punctata* and *Bursatella leachii*). They contain vesicles filled with substances staining light (*Aplysia punctata*) or dark blue (*Bursatella leachii*, *Petalifera cf. petalifera* and *Phyllaplysia taylori*). In *Phyllaplysia taylori* the vesicles seem to have alveolar substructures when examined with high magnification, whereas in *Bursatella leachii* the vesicles seem to have a light rim. Generally large nuclei are found in a basal position, in *Petalifera cf. petalifera* they can also be located medially. The supporting cells are very conspicuous and bear short to moderately long cilia. They overlap the apices of the adjacent glandular cells in *Phyllaplysia taylori*.

ATRIAL GLAND (table 4; fig. 6F): This gland is attached to the distal spermoviduct (atrium), near the genital opening. It has the shape of a narrowly coiled tube. The glandular cells are prismatic (*Aplysia punctata*, *Petalifera cf. petalifera*) or columnar (*Bursatella leachii*, *Phyllaplysia taylori*). They contain secretory vesicles of different sizes, structure and staining properties. Numerous small, round vesicles are found in *Aplysia punctata*. These vesicles are composed of a dense dark blue staining centre surrounded by a lighter rim. In *Bursatella leachii* different vesicle types are present: some vesicles stain dark grey or dark blue in the centre whereas the rim is staining lighter. Others appear to contain homogeneous contents staining light blue. On an average the vesicles are much larger than the one in *Aplysia punctata*. This is also true for the other two species, *Phyllaplysia taylori* and *Petalifera cf. petalifera*. Whereas in the former species the secretory vesicles are of round to elliptical shape they have irregular shapes in the latter species. In both species dark blue staining substances are embedded in a light blue staining matrix. Nuclei are generally found basally or medially, as in *Phyllaplysia taylori*, and contain prominent nucleoli in the latter species and in *Petalifera cf. petalifera*. The supporting cells, which alternate with the glandular cells in all species investigated, contain an apically lying nucleus, which is not visible in *Aplysia punctata* and *Bursatella leachii*, though. The cells bear short to moderately long cilia.

3.3.3 Ultrastructure of the nidamental glands:

The description of the ultrastructure of the nidamental glands is based only on investigation of *Aplysia punctata*.

ALBUMEN GLAND (fig. 7A-D): Embedded within the cytoplasm of the glandular cells are elliptically-shaped vesicles (size up to 3.5 µm in diameter). The number and size of the vesicles increases towards the apical part of the cell. The vesicles are membrane-coated and show contents of different structures: large areas within the vesicles are composed of electron-dense, homogeneous substances whereas other areas are of a loose, filamentous structure (fig. 7B, D). Within the electron-dense substance more compact, filamentous substructures are visible at high magnifications (see arrow in fig. 7B). It seems that the relative portion of the electron-dense substance is decreasing in vesicles further apically, but this cannot be proven quantitatively without morphometrical studies. Before the vesicles are ejected, the membrane seems to fuse with the cell membrane, and the amorphous contents of the vesicles are then poured into the lumen (see arrow in fig. 7C). Also in some areas whole albumen vesicles could be seen in the lumen of the membrane gland. These vesicles might result from dissolved cells. No active ejection of whole vesicles was observed.
In the basal part of the glandular cells a round nucleus is found. A nucleolus is sometimes visible. Around the nucleus prominent Golgi complexes and large areas of rough endoplasmic reticulum are present. Mitochondria are spread throughout the cell, their number decreasing towards the apical part. The apices of the glandular cells bear small microvilli, as do the alternating supporting cells which are small and wedge-shaped. The microvilli appear in between short cilia. In the apical part of the supporting cells the nucleus, few mitochondria, (possibly) glycogen and some lipid droplets can be found. A basal lamina underlies the epithelial cells on the basal side.

MEMBRANE GLAND (fig. 8A): Apically the glandular cells are filled with numerous densely packed, small vesicles, all containing a meshwork of heterogeneous mucous fibres. In some areas these vesicles seem to fuse into one large vesicle, and the mucous fibres seem to coagulate to form electron-dense mucous lumps. These condensed mucous lumps can easily break out of the sections when cut ultra-thin. The number and size of the

vesicles decrease towards the basal part of the cell, where only small vesicles are found, mostly with condensed contents. Basally large Golgi complexes can be found close to the small, electron-dense nucleus. Few mitochondria and vesicular endoplasmic reticulum are present. The basal cell membrane is forming a labyrinth adjacent to the basal lamina.

Because of poor preservation or embedding the mucous gland could not be investigated ultrastructurally.

3.3.4 Ultrastructure of the glandular tissue of the spermoviduct:

OVIDUCAL GLAND (fig. 8B, C, D): The highly columnar glandular cells of the oviducal gland contain numerous round granules in the apical part (fig. 8B). The size of the granules varies from approximately 0.3 to 1.7 µm in diameter. The size and number of the granules decrease towards the basal part of the cell. The membrane-coated granules have a very characteristic structure: within a homogeneous substance that is completely filling the granule, electron-dense lamellae-like substructures are present giving the granule a mitochondrion-like pattern (see insert in fig. 8B). When ejected the granule matrix seems to dissolve (see arrow in fig. 8B). Nascent granules with a still very amorphous shape are found in the centre of large Golgi areas in the basal part of the cell (fig. 8D). The nucleus is located basally and is very large. Endoplasmic reticulum is found in close proximity to the nucleus. The supporting cells have a mushroom-like appearance, the base is narrow and the apex broad. The apical surface of the supporting cells is bordered by numerous long cilia. In between the cilia microvilli are present. The supporting cells contain numerous mitochondria close to the ciliary rootlets. The elongate nuclei are located in the apical halves of the cells.

ATRIAL GLAND (fig. 8C): The vesicles found in the glandular cells of the atrial gland are up to 2.5 µm in diameter. They are round and have a characteristic structure: electron-dense centres are surrounded by less electron-dense material. The number of vesicles decreases towards the base of the cell, where a large nucleus that nearly always contains a prominent nucleolus, few Golgi complexes, rough endoplasmic reticulum and mitochondria are located. The supporting cells contain a small nucleus and numerous mitochondria in the apical part. The apex bears long cilia and microvilli in between those.

The spermoviduct gland has not been investigated ultrastructurally.

3.3.5 Discussion:

Ghiselin (1965) considered the reproductive systems of anaspids to be oodiaulic. I cannot agree with this, since the nidamental glands are merely attached to the spermoviduct and do not comprise a separated tube. In all four species the large glandular mass is separated into three distinct parts, namely the albumen, the membrane and the mucous gland. While albumen and mucous gland always exhibit sac-like structures, the membrane gland can be sac-like or tubular. The lining epithelia of the different glandular areas are very similar in all species investigated. In the albumen gland and the mucous gland the epithelium is internally folded. The albumen gland cells always contain large vesicles filled with amorphous blue-staining substances, whereas in the glandular cells of the mucous gland irregularly shaped vesicles containing red- to violet-staining mucous substances dominate. The glandular cells of the membrane gland contain either mucous vesicles or droplets. Their supporting cells bear much longer cilia than in the other glandular parts.

Additionally to the three nidamental glands, all anaspidean species studied possess two glandular regions in the spermoviduct with rather uniform histological appearances: the first part, the oviducal gland, in *Aplyia punctata* much larger than in the other species, always corresponds to the distal part of the mucous gland. The lining epithelium is very similar in all investigated species: the glandular cells are always filled with little granules staining from blue to dark violet. The second glandular part of the spermoviduct is the spermoviduct gland. Its glandular cells mostly contain larger vescicles that stain light to dark blue. Ghiselin (1965) and Thompson & Bebbington (1969) referred to the spermoviduct gland as a prostate. All species have an atrial gland discharging into the distal part of the spermoviduct. This gland is always tubular and narrowly coiled and the glandular cells contain relatively large vesicles of different texture.

Because of its large size and because it is sometimes quite abundant and easily accessible, *Aplysia* has long been a favourit object for anatomical studies of opisthobranchs. The increasing popularity of *Aplysia* in neurophysiological and other research areas in the last decades gave rise to quite a few histological and ultrastructural studies of these animals. Nevertheless, Beeman (1970) states in his description of the reproductive system of

Phyllaplsyia taylori: "No part of the anaspidean reproductive system has given rise to more confusion than the female gland mass" (page 7). This still holds true today.

One of the earliest detailed descriptions of the reproductive system of *Aplysia* was presented by Mazzarelli late last century (1891). He already distinguished three parts in the female gland mass of different species of *Aplysia*, among them *Aplysia punctata*: the albumen gland, the "porzione a gomitolo" and the nidamental gland. The latter accords with the mucous gland described for *Aplysia punctata* in the present study. Mazzarelli described the "porzione a gomitolo" as a coiled gland containing mucous substances. Eales (1921) and Thompson & Bebbington (1969) referred to it as the winding gland. It accords with the membrane gland described here. Beeman (1970) also called this part of the female gland mass in *Phyllaplysia taylori* a membrane gland, but also stated that this glandular part may just be a specialized part of the mucous gland. According to Mazzarelli (1891), Eales (1921) and Thompson & Bebbington (1969) the winding gland of *Aplysia punctata* discharges distally into the mucous gland. This could not be found in the present study of *A. punctata*. Here the membrane gland is shown to open into the fertilization chamber, from where the mucous gland originates as well. The histological descriptions of the winding gland, the mucous gland and the albumen gland in the previously mentioned publications are congruent with the findings of this study in *Aplysia punctata* and *Phyllaplysia taylori*. In contrast, Fretter & Hian (1984) reported in their comparative study of *Dolabella auricularia*, *Aplysia californica* and *A. punctata* of a vesicle found in the winding gland of all three species. They believed it to be a specialized part of the winding gland in which the eggs are being encapsulated. I could not find any structure in the membrane gland of *Aplysia punctata* or in any other region which would accord with the findings by Fretter and Hian. Thomas (1975) described the histology of the winding gland in *Bursatella leachii plei* differently than presented for *Bursatella leachii* here: glandular cells and ciliated cells are not alternating, but according to him the secretory cells of the winding gland are interspersed among the ciliated cells in varying abundance.

Coggeshall (1972) presented a detailed description of the ultrastructure of the nidamental glands in *Aplysia californica*. He distinguished four types of secretory cells in the albumen, winding and mucous gland. His description of the fine structure of the albumen granules is congruent with those described for *Aplysia punctata* here. Secretory vesicles in the membrane gland of this species similar to the metachromatic granules Coggeshall found in

the winding gland of *A. californica* are also described in this study. His description of the filamentous granules in the mucous gland cells accords with the fine structure of vesicles found in cells of the mucous gland of nudibranchs described later in this thesis (see pages 65, 67/68).

Much attention has been paid to the structure of the atrial gland of different species of *Aplysia*. This organ is supposed to produce a hormone responsible for egg laying (Kelner et al. 1984). Beard et al. (1982) and van Heumen et al. (1995) presented ultrastructural studies of the atrial gland of *Aplysia californica*. Their findings regarding the fine structure of the vesicles in the secretory cells of the atrial gland accord with the descriptions presented here for *Aplysia punctata*. Painter et al. (1985) investigated the anatomy and histology of the spermoviduct of three species of *Aplysia* with special regards to the atrial gland. They descibed four secretory regions in the spermoviduct, which they called secretory epithelium of the red hemiduct, secretory epithelium of the white hemiduct, atrial gland-like epithelium and accessory gland of the copulatory duct. The latter is congruent with the atrial gland described here for *Aplysia punctata*, whereas the secretory epithelium of the red hemiduct accords with the oviducal gland described in the present study. No equivalent to the secretory epithelium of the white hemiduct could be found in *Aplysia punctata*. A glandular oviducal channel was also described by Eales (1921) and Thompson & Bebbington (1969) for *Aplysia punctata*, by Beeman (1970) for *Phyllaplysia taylori* and by Thomas (1975) for *Bursatella leachii plei*. This gland is always shown to have a direct connection to the mucous gland as also presented for the four anaspids studied here. The ultrastructure of the vesicles in the oviducal gland of *Aplysia punctata* described here accords with the fine structure of the secretory granules found in the red hemiduct of *Aplysia californica* (Beard et al. 1982). A spermoviduct gland is described for *Aplysia punctata* (Thompson & Bebbington 1969), *Phyllaplysia taylori* (Beeman 1970) and *Bursatella leachii plei* (Thomas 1975).

The description of the reproductive system of *Petalifera* cf. *petalifera* given in the present study is the first for this species.

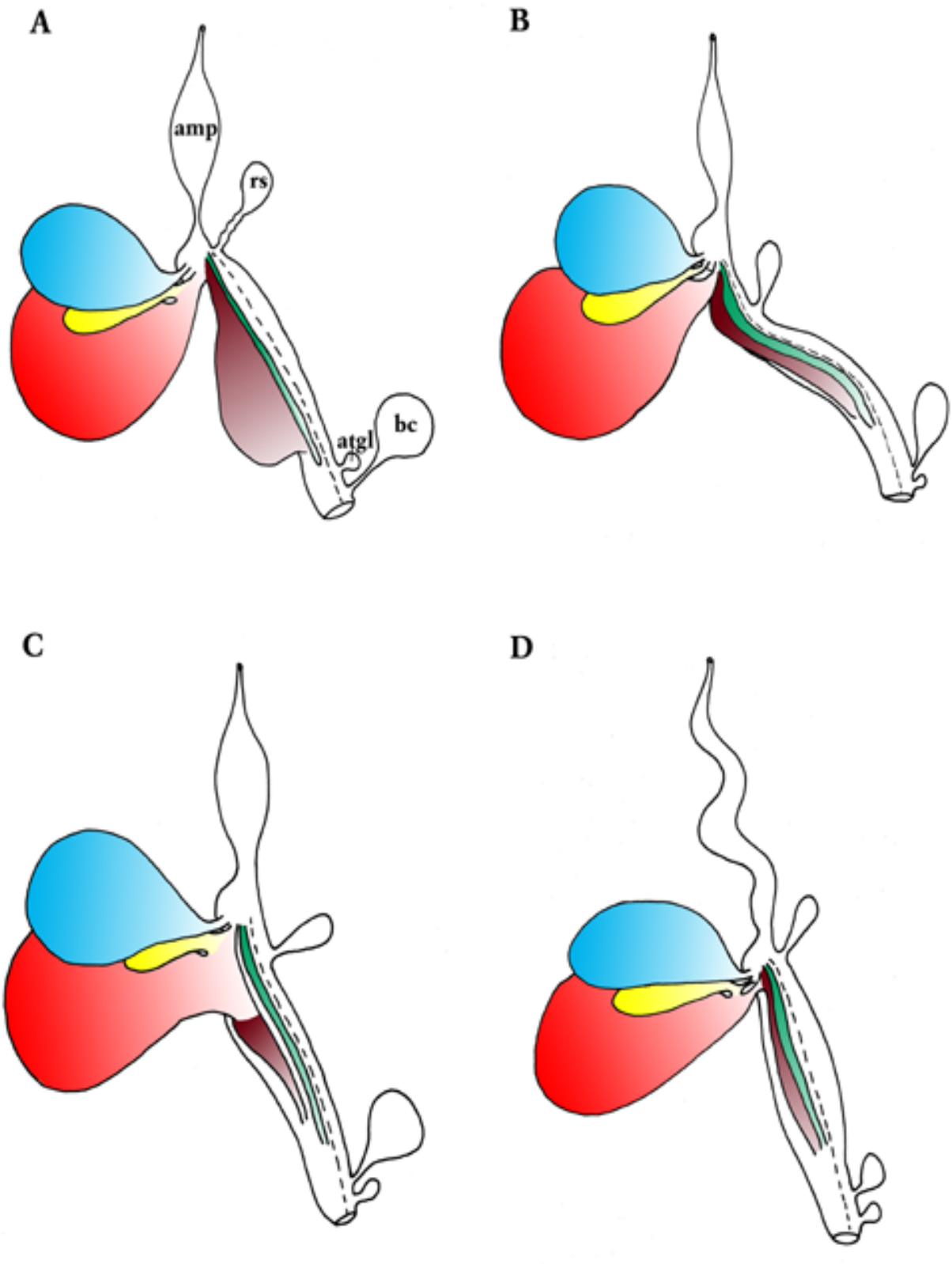

Figure 4: Schematic outlines of the anterior reproductive systems of several Anaspidea. A: *Aplysia punctata*; B: *Bursatella leachii*; C: *Phyllaplysia taylori*; D. *Petalifera cf. petalifera.*

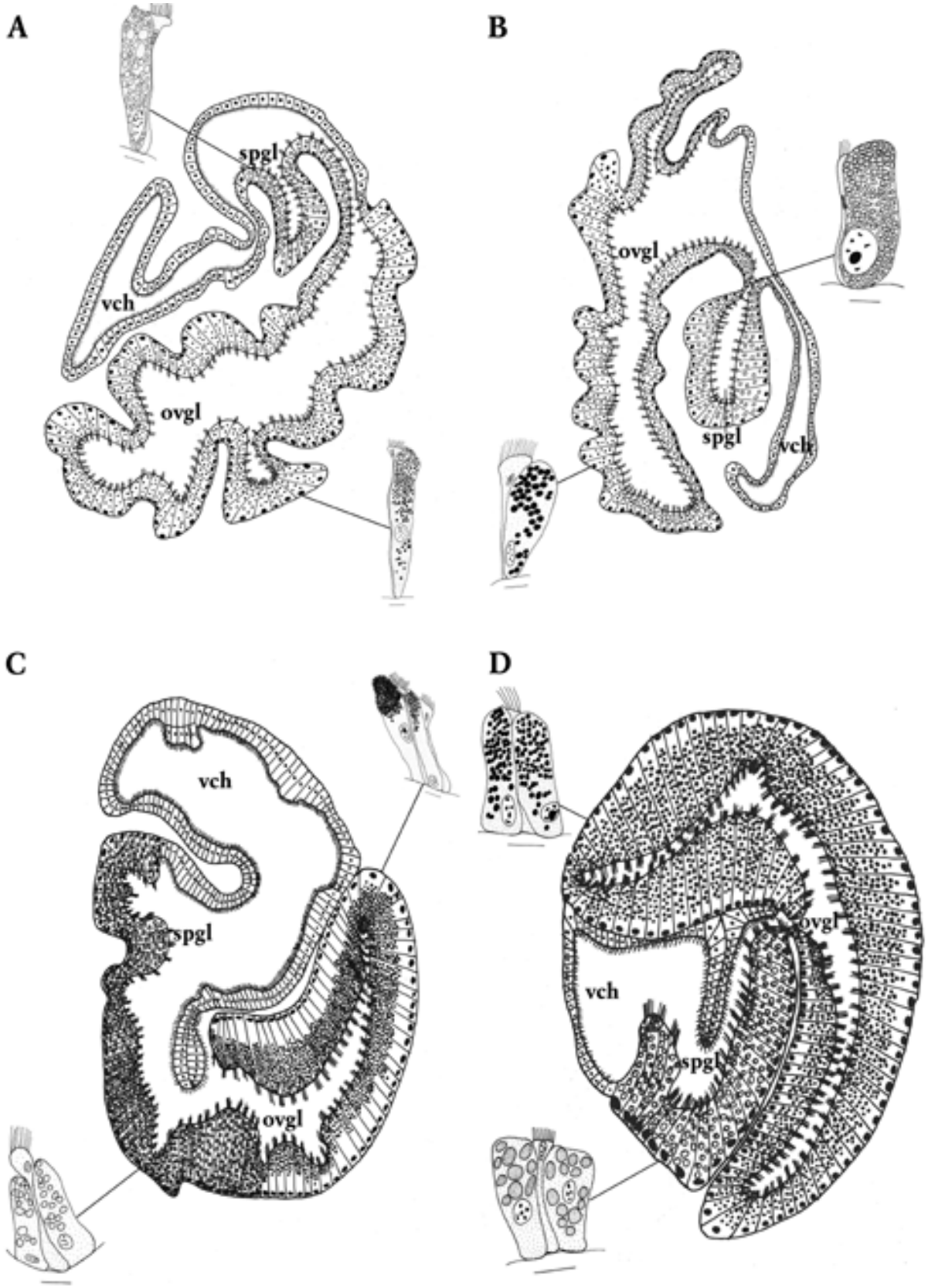

Figure 5: Schematic cross sections through the spermoviduct and schematic outlines of the major glandular cell types in the spermoviduct of several Anaspidea. A: *Aplysia punctata*; B: *Bursatella leachii*; C: *Phyllaplysia taylori;* D: *Petalifera cf. petalifera*; scale bars: 10 µm.

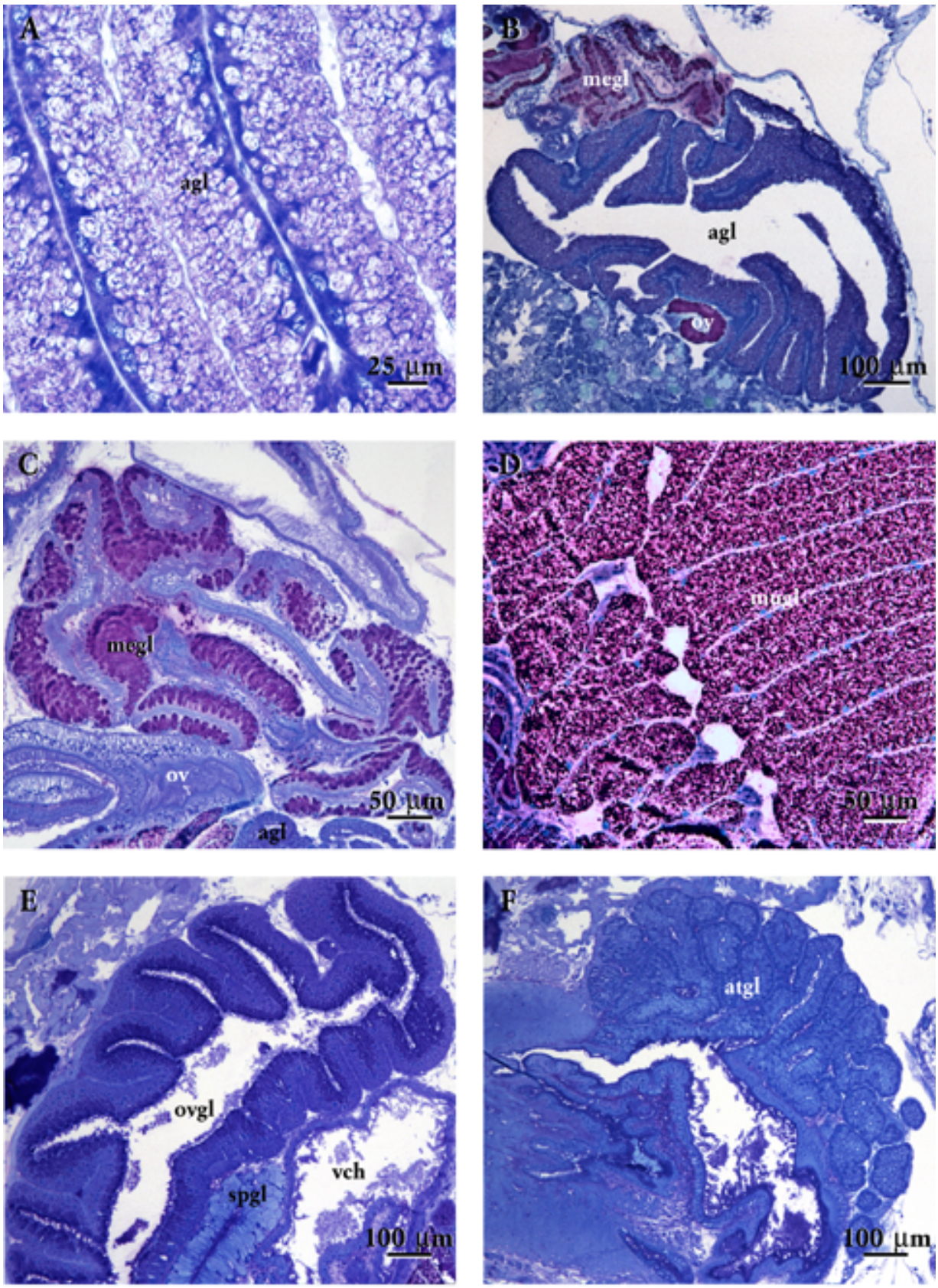

Figure 6: Histological sections through the nidamental glands and accessory glands at the oviduct of several Anaspidea. A: *Phyllaplysia taylori* - albumen gland; B: *Petalifera cf. petalifera* - albumen and membrane gland; C: *Aplysia punctata* - membrane gland: D: *Aplysia punctata* - mucous gland; E: *Aplysia punctata* - oviducal and spermoviduct gland; F: *Aplysia punctata* - atrial gland.

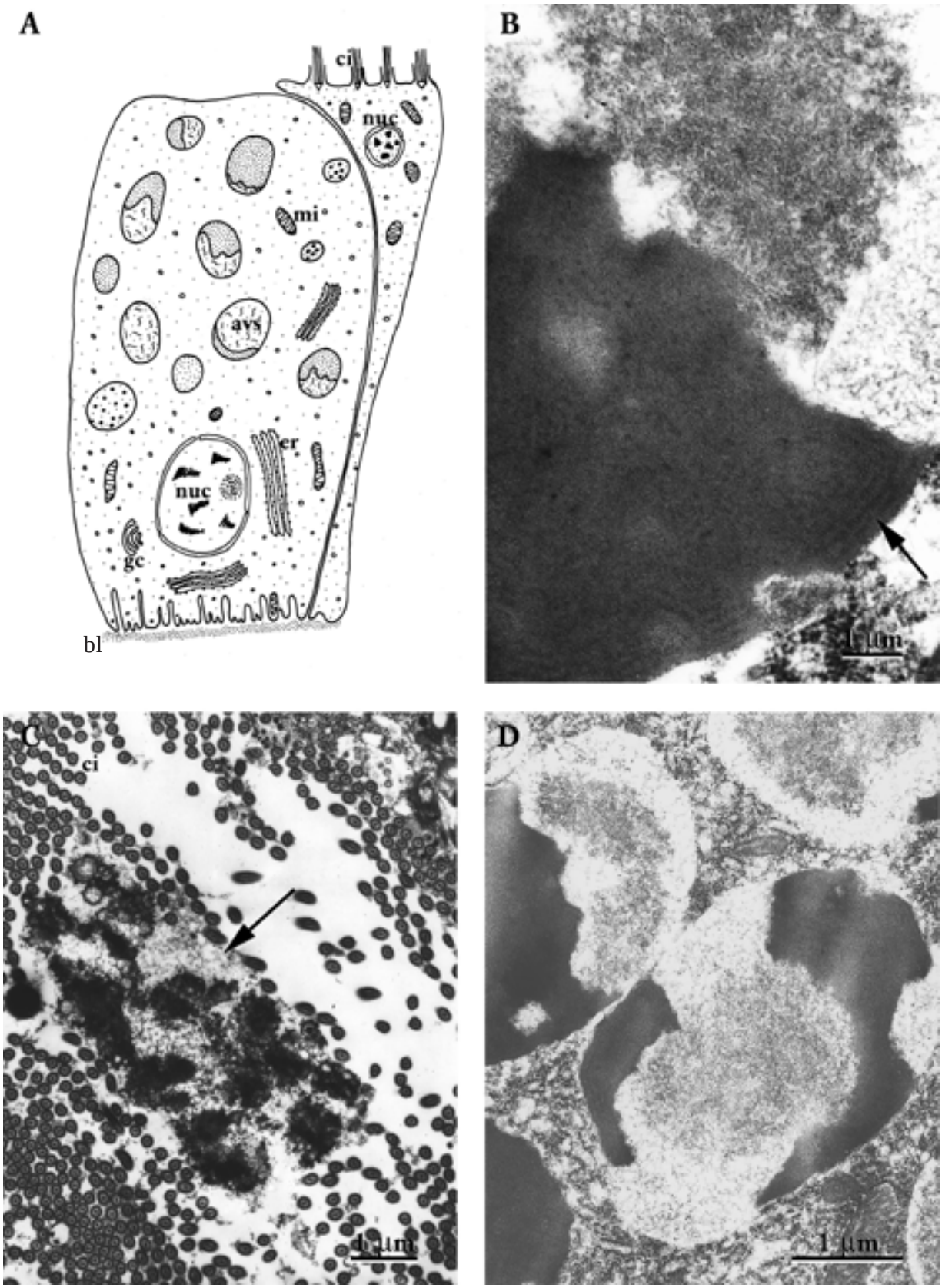

Figure 7: Ultrastructure of the albumen gland of *Aplysia punctata*. A: schematic outline of a glandular and a supporting cell; B, D: albumen vesicles (arrow in figure B indicates electron-dense substructures); C: amorphous albumen material in lumen of gland (arrow).

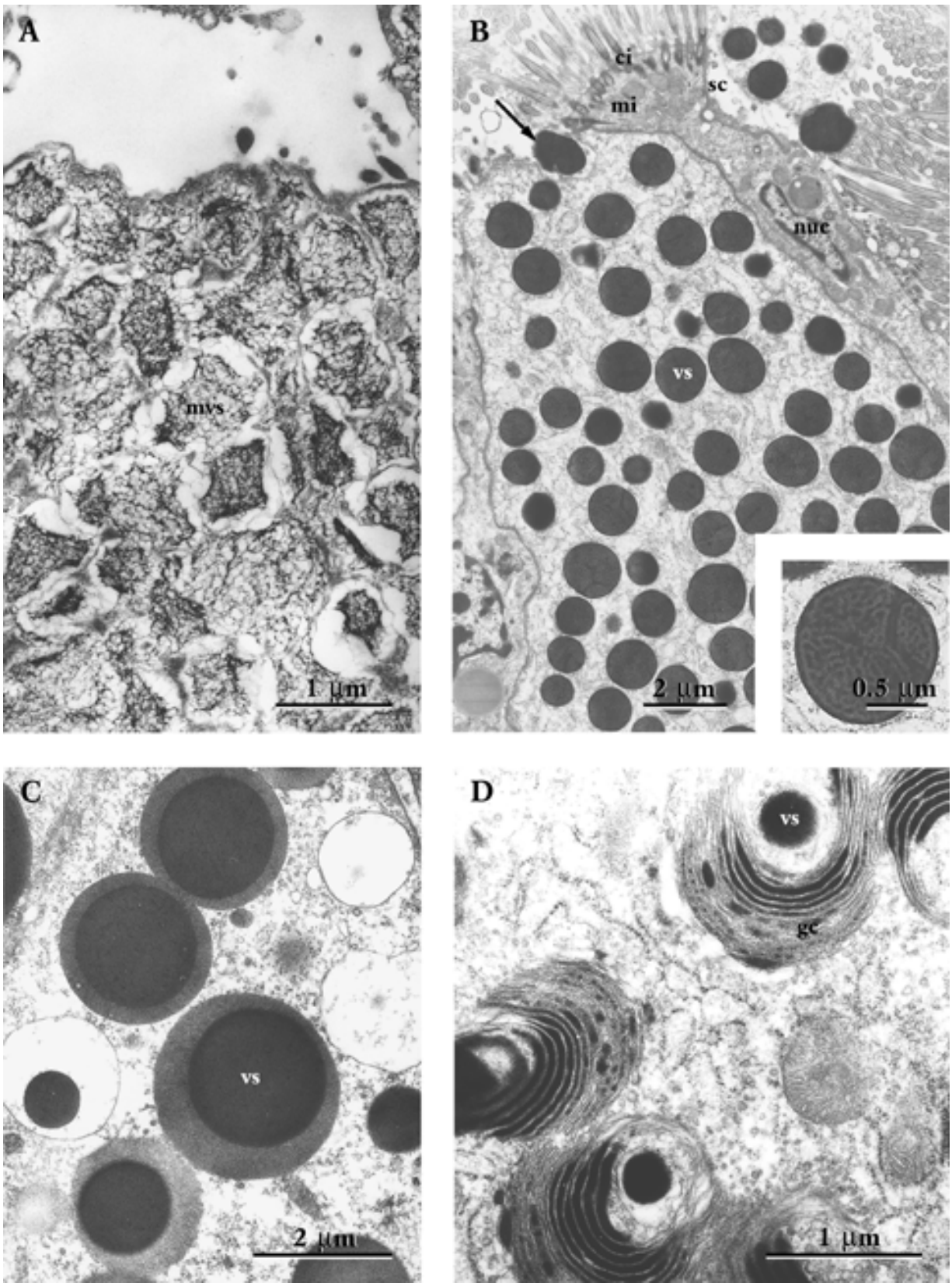

Figure 8: Ultrastructure of several glandular parts of the anterior reproductive system of *Aplysia punctata*. A: membrane gland; B, D: oviducal gland (arrow in B indicates ejection of secretory material); C: atrial gland.

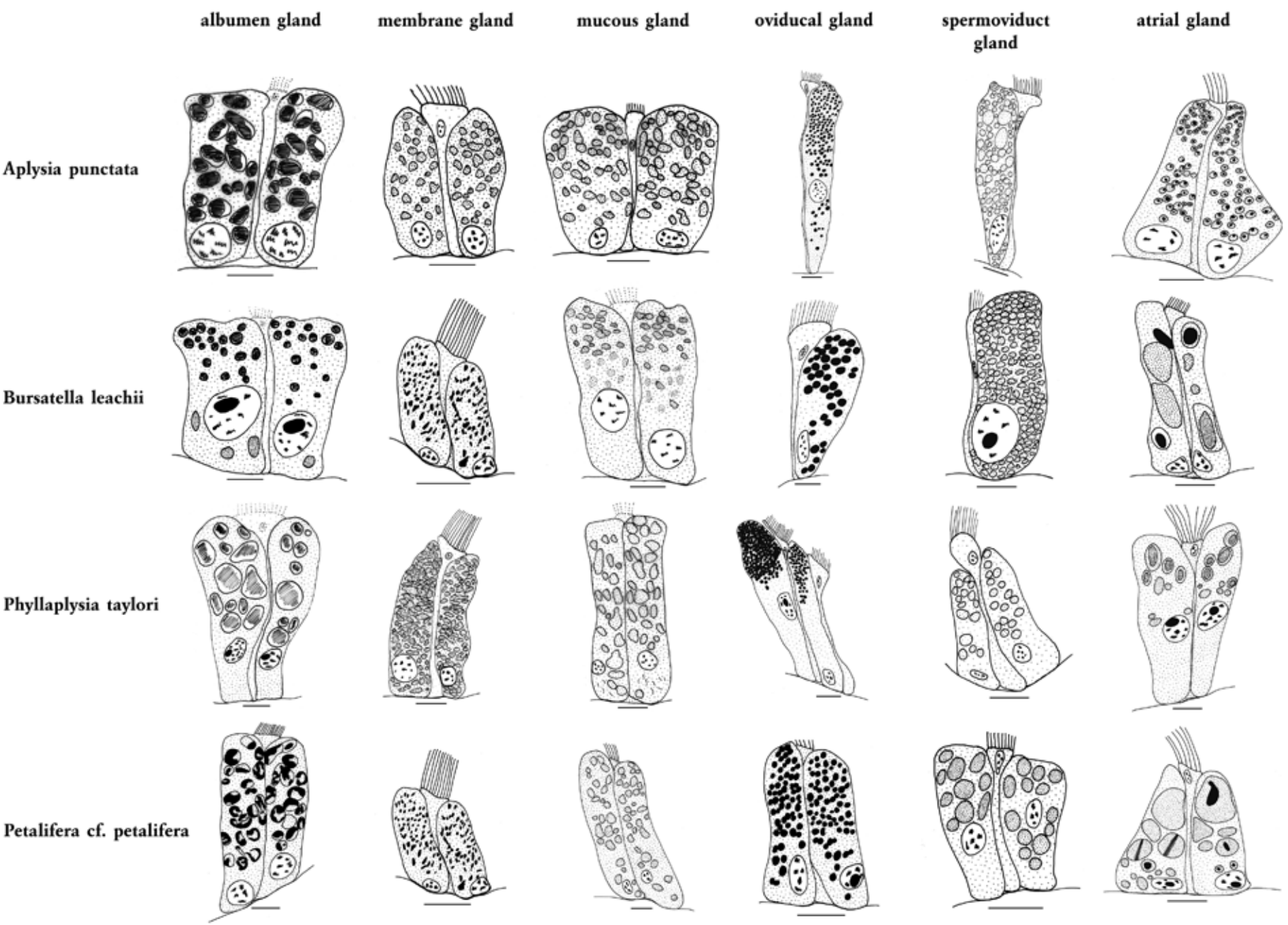

Table 4: Comparative schematic drawings of the major cell types of the nidamental glands and the glandular spermoviduct of several Anaspidea; scale bars = 10 µm.

3.4 The anterior reproductive systems of the Sacoglossa

The Sacoglossa show slightly different genital systems to the ones I have described earlier for the "Cephalaspidea s. l." and the Anaspidea. All species studied here have an androdiaulic system with the vas deferens and the accessory prostate gland separated (fig. 9A-C). A prominent muscular penis could be found in *Oxynoe viridis*and in the *Elysia* species. In the primitive species *Oxynoe viridis* (Jensen 1996) the prostate and the nidamental glands comprise a compact glandular mass located in the central body cavity, whereas the prostate and the albumen gland in more derived species such as *Elysia viridis, Elysia ornata* and *Thuridilla hopei* (Jensen 1996) are tubular and branched within the lateral parapodia. Only the mucous glands of the nidamental gland systems are compact and located in the central body cavity. Small ducts connect the follicles of the prostate and the albumen gland with the vas deferens and the oviduct, respectively.

The ampullae are tube-like (*Oxynoe viridis, Elysia ornata*) or bladder-like (*Elysia viridis, Thuridilla hopei*) structures. In *Oxynoe viridis* the vas deferens branches off the postampullary duct most proximally, whereas in the other species postampullary duct, vas deferens and oviduct meet at a "junction". In *Elysia* species and *Thuridilla hopei* the oviduct with its accessory glands has a rather complicated structure. Small ducts from the various glandular parts interconnect. For a better perspicuity the schematic drawings in Figure 9A-C are held rather simple.

Receptacula could not be found in any species. A bursa copulatrix always inserts distally at the oviduct or has a separate opening distally next to the genital aperture of the oviduct.

All studied sacoglossan species show three separate glandular parts in the nidamental gland systems as also described for the "Cephalaspidea s. l." and Anaspidea. Most proximally the albumen gland is located, forming follicular branches within the parapodia in *Elysia* and *Thuridilla hopei*. In *Oxynoe viridis* the membrane gland is set apart clearly from the mucous gland whereas in *Elysia* and *Thuridilla hopei* membrane and mucous glands are closely connected. In *Oxynoe viridis* the distal oviduct is lined by glandular tissue.

Comparative schematic drawings of the major cell types of the nidamental glands are shown in table 5. Since the cells of *Elysia* and *Thuridilla hopei* are very similar, the latter species is not presented in the table.

3.4.1 Histology of the nidamental glands:

ALBUMEN GLAND (table 5; fig. 10A, B): In *Oxynoe viridis* the albumen gland is a compact organ lying in the central body cavity and being composed of small tubules (fig. 10A). The broad, columnar glandular cells in this species contain small dark blue staining vesicles of round to elliptic shape and different size (up to 4.5 µm in diameter). A large round nucleus is lying basally. Nucleoli are sometimes visible. The supporting cells are small. They contain a centrally lying nucleus and bear moderately long cilia. At the distal end of the albumen gland the efferent duct leading to the following membrane gland is lined by a glandular epithelium slightly different from the albumen gland cells: the glandular cells are more slender and contain numerous small dark blue staining round granules (approximately 0.6 µm in diameter). The basally lying nuclei do not contain visible nucleoli. The alternating supporting cells are more prominent here than in the proximal albumen gland. They have a triangular apex containing a small nucleus and they bear long cilia. In the two *Elysia* species and in *Thuridilla hopei* the albumen gland is branched within the parapodia. In the latter species the tubules are smaller than in the former species. The glandular cells alternate with supporting cells. In *Elysia* and *Thuridilla hopei* the club shaped glandular cells are arranged around a small central duct into which they discharge. The ducts are connected to a larger efferent duct lined by columnar, non-glandular, ciliated cells. The glandular cells contain numerous large round to elliptically shaped secretory vesicles. In *Elysia viridis* the vesicles seem to be composed of a lighter staining centre surrounded by a darker staining rim. In *Thuridilla hopei* they stain homogeneously dark blue. A large nucleus containing a prominent nucleolus is found in the basal part of the glandular cells of *Thuridilla hopei*, whereas the glandular cells in *Elysia viridis* contain small nuclei without visible nucleoli.

MEMBRANE GLAND (table 5; fig. 10C): In *Oxynoe viridis* the membrane gland directly joins to the glandular epithelium of the efferent duct of the albumen gland described above. The membrane gland is narrowly coiled in this species, whereas it is lining the efferent duct of the mucous gland in its most proximal part in *Elysia viridis* and *Thuridilla hopei* (fig. 10C). The glandular cells are columnar in all three species. In *Oxynoe viridis* they contain homogeneous pink staining mucus in the apical 2/3 of the cell and irregularly formed mucous droplets basally. In *Elysia viridis* the mucus has the form of irregular

droplets staining dark pink, whereas in *Thuridilla hopei* heterogeneous mucous fibres staining pink are present. Pycnotic nuclei are found in all species, located in the basal halves of the glandular cells. The alternating supporting cells bear moderately long (*Oxynoe viridis*, *Thuridilla hopei*) or long cilia (*Elysia viridis*). A distinct nucleus could only be found in *Oxynoe viridis*, located apically in the supporting cells.

MUCOUS GLAND (table 5; fig. 10D, E): The mucous gland forms a large, widely coiled tube in *Oxynoe viridis* (fig. 10E) and comprises a two-folded tube in *Elysia viridis* (fig. 10D). In *Thuridilla hopei* it is separated into two distinct parts. Proximally the columnar glandular cells are arranged around a small central duct. This duct leads to the second more distal part which is tubular. The glandular cells alternate with supporting cells in all species investigated. In *Oxynoe viridis* the columnar glandular cells contain numerous small, irregularly formed vesicles filled with violet staining mucus. These vesicles are embedded in homogeneously violet staining cytoplasm. In *Elysia viridis* the glandular cells are not distinguishable singly. But violet staining mucous droplets embedded in homogeneously violet staining cytoplasm can be observed in the cells. The glandular cells in *Thuridilla hopei* have a slender columnar shape and contain mostly dark violet staining mucus, partly heterogeneous filamentous mucus. Nuclei are located basally, in some cells of *Oxynoe viridis* they can also be found medially. In *Elysia viridis* and *Thuridilla hopei* the nuclei seem to be pycnotic. Alternative supporting cells are visible in *Oxynoe viridis*. They bear short cilia and contain an apically lying nucleus.

ADHESIVE REGION (table 5; fig. 10F): A glandular tissue within the most distal part of the oviduct is only found in *Oxynoe viridis*. This part is very prominent. The glandular epithelium lines a large tubular organ. The glandular cells are broad basally and narrow slightly towards the apex. They contain numerous small irregularly shaped granules staining dark blue to violet. The granules partly show a darker staining rim. Large elliptically shaped nuclei are located in various positions. Nucleoli are always visible. The supporting cells are small and wedge-shaped and bear short to moderately long cilia. They contain an apically lying nucleus.

3.4.2 Ultrastructure of the nidamental glands:

The ultrastructure of the nidamental glands has only been investigated in *Thuridilla hopei*.

ALBUMEN GLAND (fig. 11A-C): The vesicles filling the glandular cells of the albumen gland of *Thuridilla hopei* are round-to elliptically-shaped and have different sizes (0.5 - 3.5 µm in diameter). They are composed of two distinct substances, one electron-dense substance showing a mottled pattern and a less electron-dense substance of homogeneous structure (fig. 11A, B, C). The relative amount of the more electron-dense substance seems to be higher in the vesicles than the less electron-dense substance. In small areas within the electron-dense substance the mottled pattern is not obvious but homogeneous patches are apparent. The vesicles are membrane-coated (see arrow in fig. 11C). In immature vesicles the electron-dense substance seems to be less dense than in mature vescicles. Neither discharging of the vesicles nor of the substances composing them could be observed. But a dissolution of the less electron-dense substance is visible in vesicles close to the apical cell membrane (see arrow in fig. 11B). A large active nucleus is located at the ablumenal end of the cell (fig. 11A). A nucleolus is almost always visible. Concentrated near the nucleus are prominent Golgi complexes and large areas of parallel cisternae of rough endoplasmic reticulum. The latter are also found embedded within the cytoplasm throughout the cell. Round to elongate mitochondria are also spread throughout the cell. The apical surface of the glandular cells projects microvilli into the lumen of the ductules into which they discharge. The glandular cells alternate with small columnar cells lining the central ductule. The non-glandular supporting cells bear short cilia. In between the cilia small microvilli are present. Elongate and round mitochondria as well as vesicles probably containing glycogen can be observed in the cells.

The membrane and mucous glands have not been infiltrated by the embedding media very well, thus the quality of the ultra-thin sections was very poor. Nevertheless few data could be gained for the ultrastructure of these two regions:

MEMBRANE GLAND: The glandular cells contain small vesicles which are densely packed. The vesicles are filled with dense mucous fibres, partly coagulating to compact mucous lumps. Cell membranes are hardly visible. The nuclei are small, and contain

electron-dense chromatin. The supporting cells bear long cilia and contain a small nucleus and mitochondria in the apical part of the cell.

MUCOUS GLAND (fig. 20B): The contents of the glandular cells are similar to those of the membrane gland, but the mucous vesicles are larger and contain less electron-dense and less coagulated mucous fibres. Basally a small nucleus with dense chromatin is visble as well as few Golgi complexes. Small supporting cells can be observed studded apically with short cilia. They contain mitochondria, a nucleus is not visible.

3.4.3 Discussion:

The differentiation between primitive and derived species of Sacoglossa follows Jensen's (1996) phylogenetic analysis of this taxon. In comparison to the opisthobranch taxa described above, the anterior reproductive systems of the Sacoglossa, especially the derived species, are more complex and show complicated structures. However, the separation of the nidamental glands into three distinct parts as in all opisthobranch species investigated could also be observed in the four sacoglossans studied here. The structure of these glandular parts differs within the four sacoglossans. The albumen glands of the two species of *Elysia* and *Thuridilla hopei* are branched tubules, which are spread throughout the parapodia almost along their total lengths (from the anterior body region to the posterior). This tubular arrangement is already apparent in *Oxynoe viridis,* but the tubules comprise a compact glandular mass and are not highly branched. The structure of the membrane gland varies considerably among the species. In *Oxynoe viridis* the membrane gland has a narrowly coiled tubular structure, whereas *Elysia* and *Thuridilla hopei* possess membrane glands which are not separated structures but rather line the proximal part of the efferent duct of the mucous glands. Nevertheless, their cellular structure is similar to the one found in *Oxynoe viridis*. The mucous glands in the Sacoglossa have the form of long tubes. In *Oxynoe viridis* the tube is large with a rather broad lumen, whereas *Elysia* and *Thuridilla hopei* have long narrow tubes with a central efferent duct. *Oxynoe viridis* shows a very prominent adhesive region lining the distal part of the oviduct. In *Elysia* and *Thuridilla hopei* the distal oviduct is not glandular.

Data on the histology and fine structure of the nidamental glands in the Sacoglossa are sparse. One very detailed analysis of seventeen sacoglossan species, among them *Oxynoe*

olivacea, *Elysia viridis* and *Thuridilla hopei*, has been presented by Sanders-Esser (1984). Her findings on the nidamental glands of these species are very similar to the ones described in the present study. Still, some differences are worth discussing: Sanders-Esser described the supporting cells in the albumen gland of *Elysia viridis* to be very broad at their apex, thus overlapping the apices of the glandular cells. This could neither be observed with low power microscopy in this species nor with TEM in *Thuridilla hopei* in the present investigation. The structure of the secretory granules as described here and by Sanders-Esser (1984) for *Elysia viridis* was also found in *Elysia maoria* by Reid (1964). Sanders-Esser (1984) gave evidence for the fine structure of the albumen fluid in the lumen of the gland of *Elysia viridis* which could not be observed in the present study. According to her, after the albumen has been discharged into the lumen of the gland, it can either have a homogeneous structure or the granule-like structure can persist until the eggs, coated by the albumen, have been laid. In *Calliphylla mediterranea* the granule structure of the coating albumen is also still apparent once the eggs have been laid into the surrounding seawater (Sanders-Esser 1984). The author did not distinguish between two different parts of mucous glands as presented for all species here, but she discussed the possibility that the first part of the glandular oviduct accords with the membrane gland of anaspids. This structure corresponds to the membrane gland described here (see discussion page 35). No information is available on the nidamental glands of *Oxynoe*, but Yamasu (1968) described the histology of the reproductive system of a closely related genus (Jensen 1996), namely *Julia*. According to Yamasu (1968) the albumen gland of *Julia japonica* is not ciliated, which does not correspond to the findings in *Oxynoe viridis*. The author found two different parts of mucous glands as presented here. He also called the proximal part a capsule or membrane gland. In his classification of the families of the Sacoglossa, Gascoigne (1985) distinguished three glandular parts in the female reproductive system, namely an albumen, a capsule and a large oviducal gland.

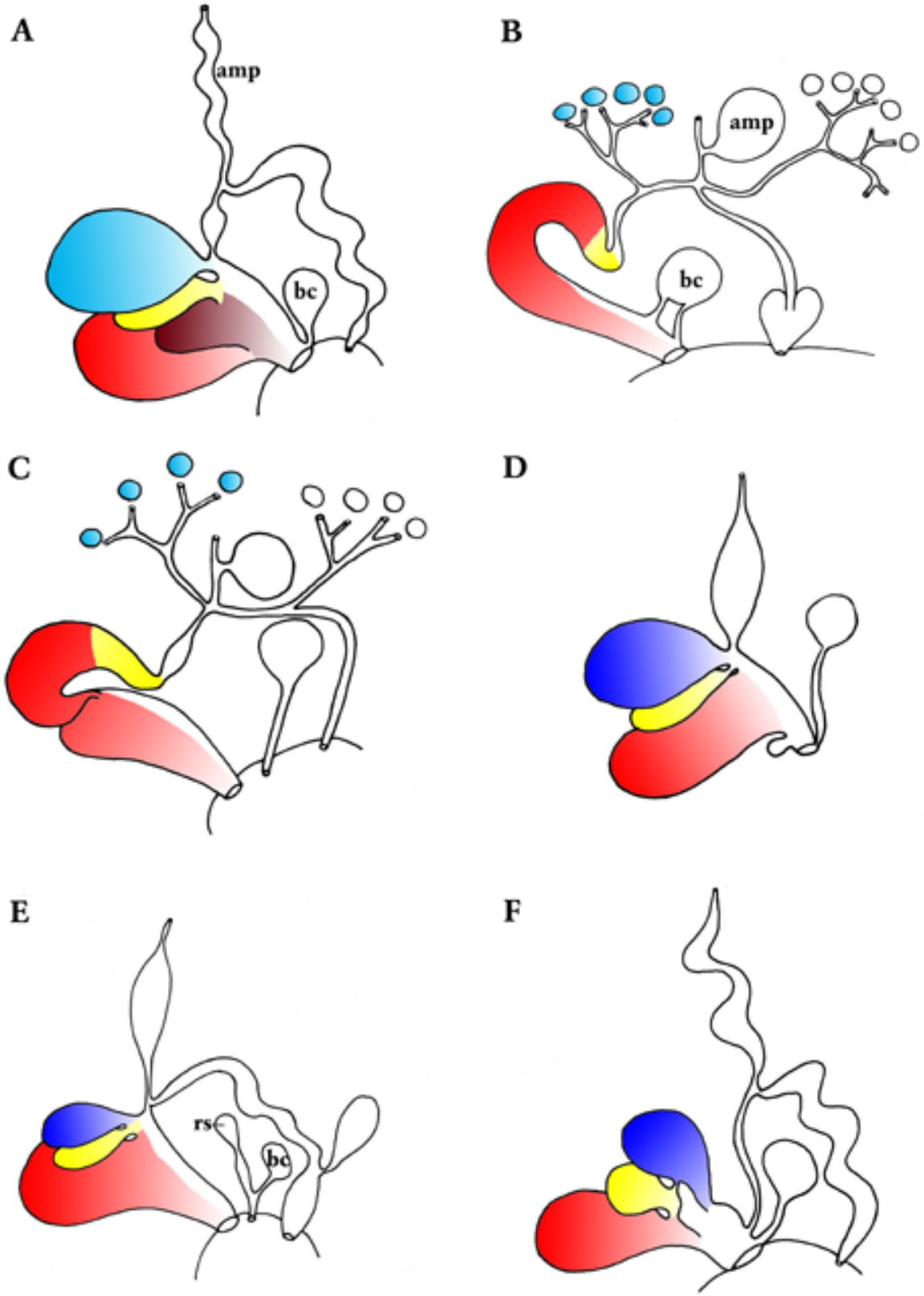

Figure 9: Schematic outlines of the anterior reproductive systems of several Sacoglossa, Tylodinoidea and Pleurobranchoidea. A: *Oxynoe viridis*; B: *Elysia viridis*; C: *Thuridilla hopei*; D: *Tylodina perversa*; E: *Berthella stellata*; F: *Euselenops luniceps*.

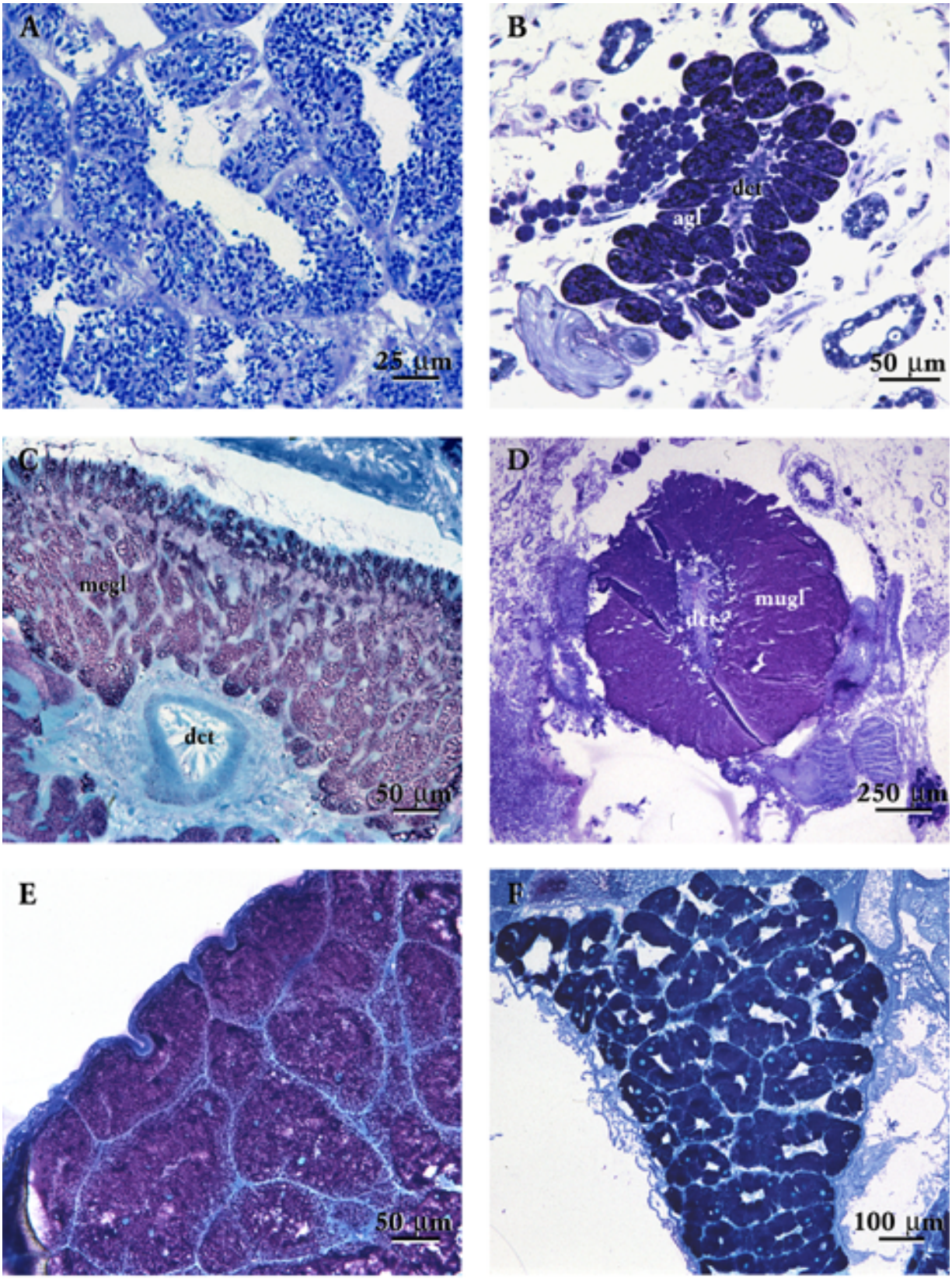

Figure 10: Histological sections through the nidamental glands of several Sacoglossa. A: *Oxynoe viridis* - albumen gland; B: *Elysia viridis* - albumen gland; C: *Thuridilla hopei* - membrane gland; D: *Elysia viridis* - mucous gland; E: *Oxynoe viridis* - mucous gland; F: *Oxynoe viridis* - adhesive region.

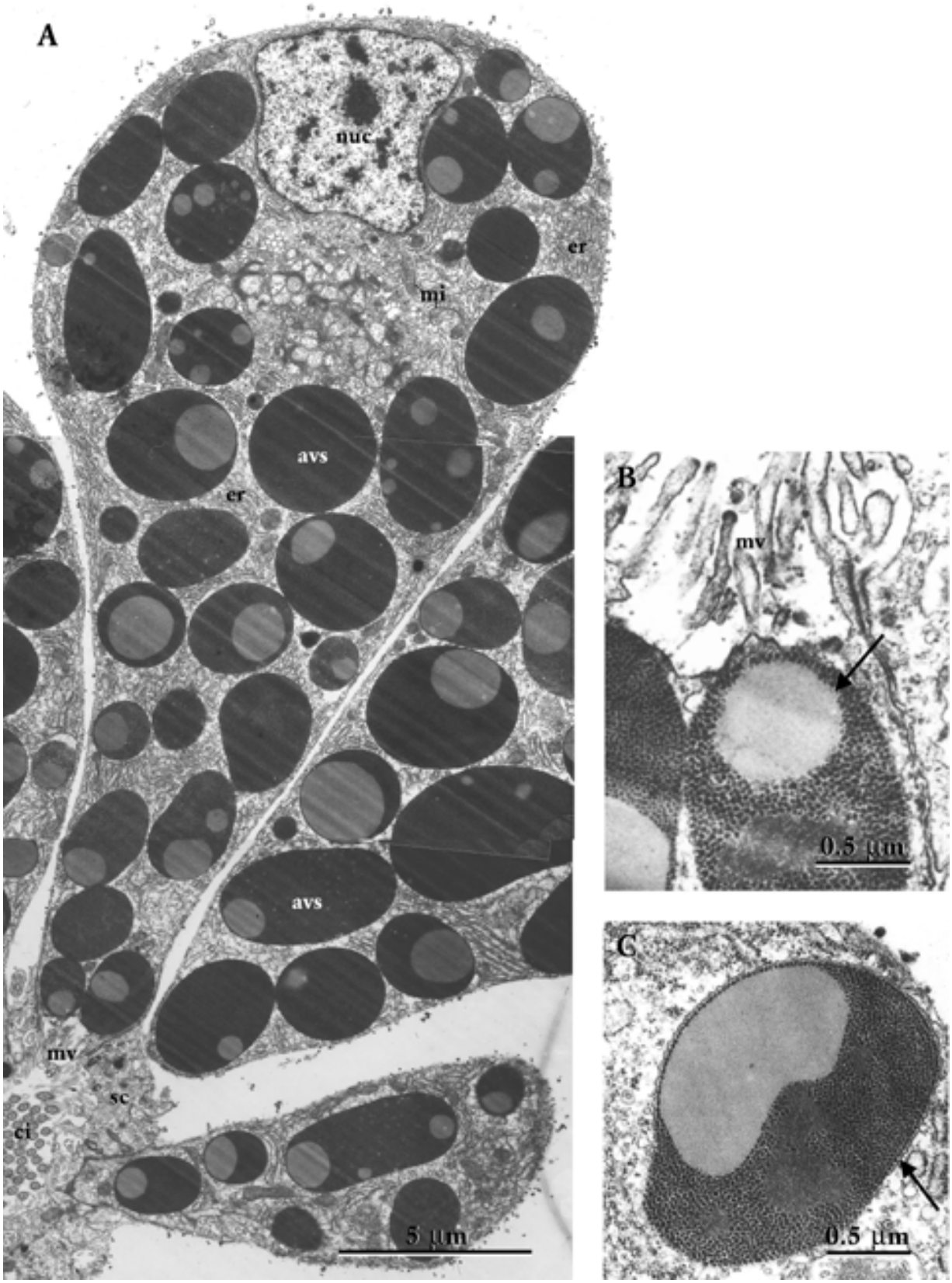

Figure 11: Ultrastructure of the albumen gland of *Thuridilla hopei*. A: whole glandular cell; B: dissolving secretory substances (see arrow) at lumenal tip of glandular cell; C: membrane-bound albumen vesicle (arrow indicates membrane).

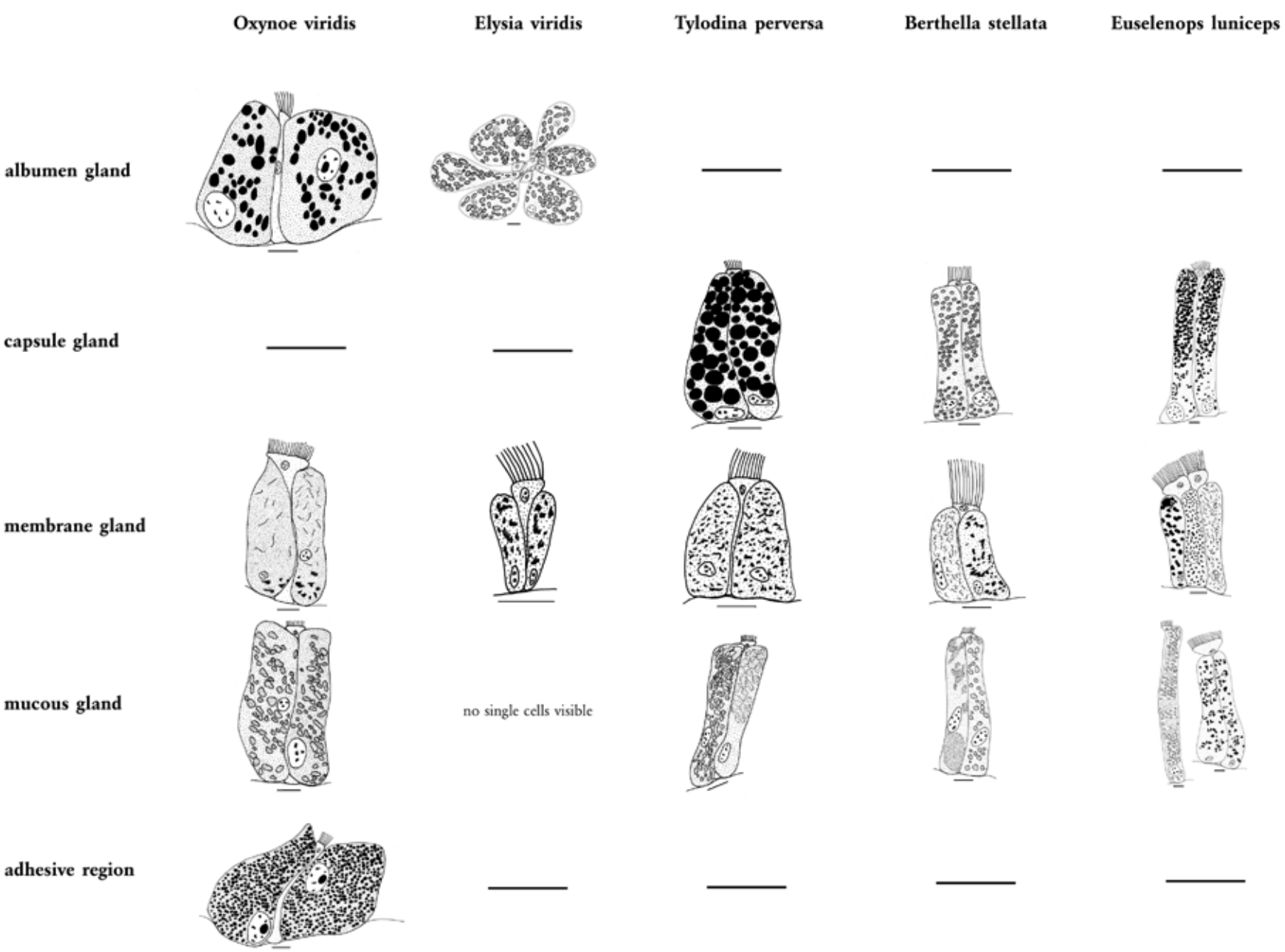

Table 5: Comparative schematic drawings of the major cell types of the nidamental glands of several Sacoglossa, Tylodinoidea and Pleurobranchoidea; scale bars = 10 µm.

3.5 The anterior reproductive systems of the Tylodinoidea

Only one tylodinid species has been investigated in the present study. *Tylodina perversa* possesses a monaulic genital system (fig. 9D). The distal male part of this system has not been investigated further. The ampulla is tube-like. The female part consists of a very short proximal oviduct, a large nidamental gland mass and an accessory gland at the vestibulum. The nidamental glands are composed of three distinct parts, namely a capsule gland, a membrane and a mucous gland. At the distal end of the oviduct a bursa copulatrix inserts. The receptaculum seminis is located proximally.

Comparative schematic drawings of the major cell types of the nidamental glands are shown in table 5.

3.5.1 Histology of the nidamental glands:

CAPSULE GLAND (table 5; fig. 12A): The capsule gland in *Tylodina perversa* is a large tubular organ with narrow coils (fig. 12A). Glandular cells and supporting cells alternate. The glandular cells are columnar to highly columnar and contain round dark blue-staining granules of up to 5.5 µm in diameter. At high magnification the granules seem to have fine substructures. The nuclei are small and located basally in the cells. Supporting cells are very small, hardly visible and bear short cilia.

MEMBRANE GLAND (table 5; fig. 12C): Capsule gland and membrane gland are connected by the distal oviduct. The membrane gland also exhibits a narrowly coiled tube (fig. 12C), but it is smaller than the capsule gland. The glandular cells are prismatic and filled with heterogeneous mucous masses, staining dark violet. The mucus partly forms droplets. Pycnotic nuclei are found in the basal parts of the cells. The supporting cells have a triangular apex which partly overlaps the glandular cells apically. The supporting cells contain an apically lying nucleus and bear long cilia (fig. 12C).

MUCOUS GLAND (table 5; fig. 12E): This large organ both originates and discharges into the distal spermoviduct. No direct connection to the previous membrane gland has been observed. The mucous gland is also tubular (fig. 12E), but it comprises wide coils in contrast to the membrane and the capsule gland. The glandular cells of the mucous gland

are highly columnar. Dark violet-staining mucous vesicles are embedded within homogeneously staining cytoplasm. The vesicles seem to dissolve in parts to form homogeneous mucous masses. Elliptical nuclei are located in the basal halves of the glandular cells. The alternating supporting cells are not as prominent as in the membrane gland and bear shorter cilia.

ACCESSORY GLAND OF THE DISTAL OVIDUCT: This small, follicular gland is composed of prismatic glandular cells containing grey- to pink-staining cytoplasm. Embedded within this cytoplasm are dark blue- to violet-staining small irregularly shaped secretory droplets.

3.5.2 Discussion

As has been described for some cephalaspidean species above, *Tylodina perversa* also possesses a capsule gland at the proximal part of the oviduct. The narrowly coiled tubular structure and the larger size of the granules is slightly different from the cephalaspidean species. The presence of a membrane gland and a mucous gland accords with all other species studied here. The histology of the accessory gland differs from the one described in *Philinopsis gardineri* and from the atrial gland of the Anaspidea as well as from the vestibular gland described below for *Dendrodoris nigra* (see chapter 3.7.1, page 64).
No data from the literature are available to which the present findings can be compared.

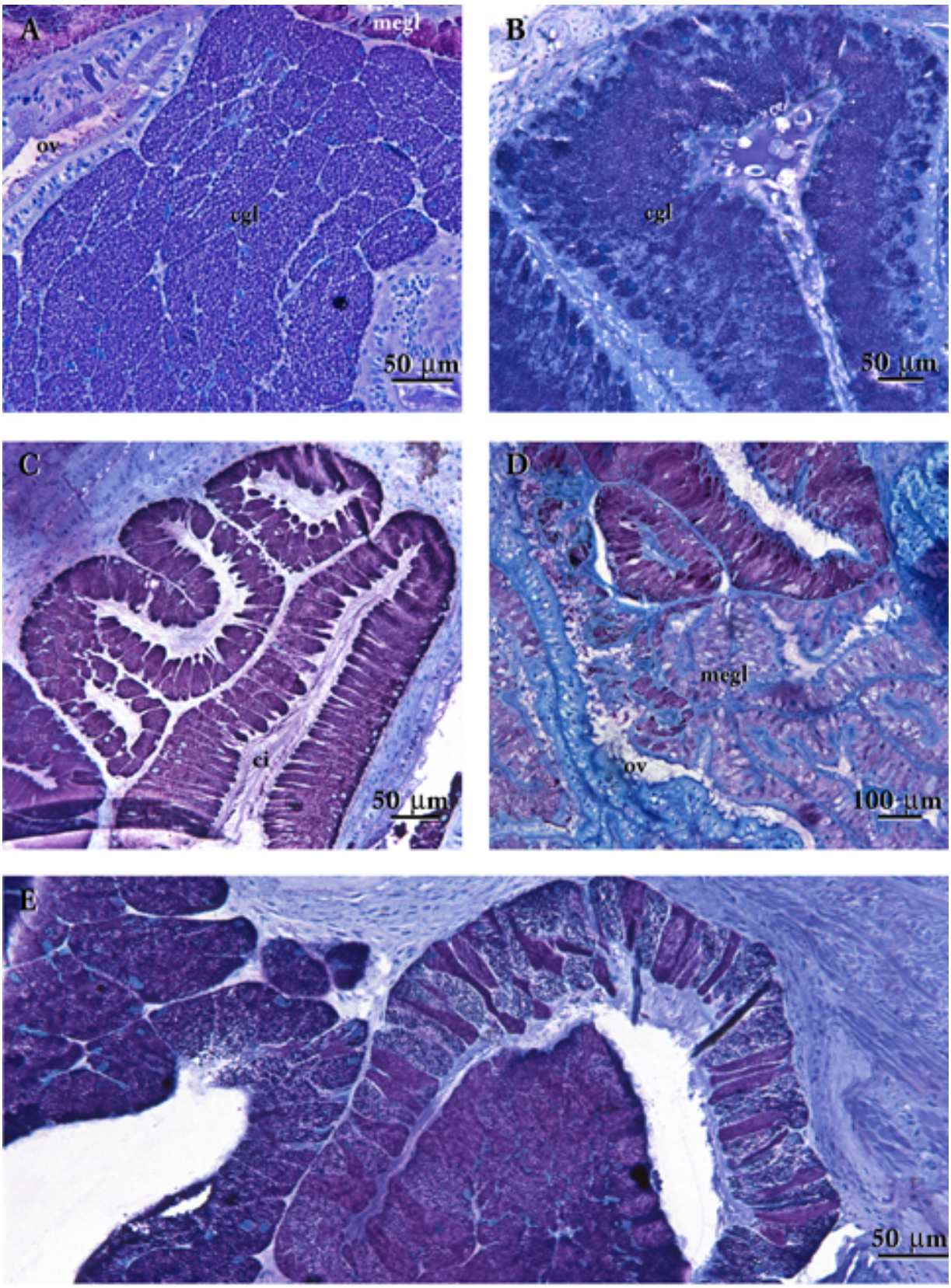

Figure 12: Histological sections through the nidamental glands of Tylodinoidea and Pleurobranchoidea. A: *Tylodina perversa* - capsule gland; B: *Euselenops luniceps* - capsule gland; C: *Tylodina perversa* - membrane gland; D: *Euselenops luniceps* - membrane gland; E: *Tylodina perversa* - mucous gland.

3.6 The anterior reproductive systems of the Pleurobranchoidea

Berthella stellata and *Euselenops luniceps* both possess androdiaulic reproductive systems (fig. 9E, F). While the ampulla in *Berthella stellata* is a straight tube it is heavily coiled in *Euselenops luniceps.* In both species the vas deferens is glandular in its proximal part forms a prostate, which branches off the postampullary duct most proximally. *Berthella stellata* possesses a penial gland attached to the proximal region of the muscular penis. In this species the proximal oviduct is short and soon discharges into the capsule gland. *Euselenops luniceps* presents a slightly different picture: the proximal oviduct is very long and joins distally to the vestibulum, to which the nidamental glands are also connected. Both species show three distinct parts in the nidamental gland system: a capsule, a membrane and a mucous gland. The latter is always the largest organ. In *Euselenops luniceps* the distal bursa copulatrix inserts at the vestibulum whereas in *Berthella stellata* bursa copulatrix and receptaculum seminis have a separate opening close to the opening of the oviduct. No receptaculum seminis could be found in *Euselenops luniceps.*

Comparative schematic drawings of the major cell types of the nidamental glands are shown in table 5.

3.6.1 Histology of the nidamental glands:

CAPSULE GLAND (table 5; fig. 12B): The capsule gland is tubular in both species, but in *Euselenops luniceps* it is narrowly coiled, whereas in *Berthella stellata* it comprises only few, slightly wider coils. The highly columnar glandular cells contain numerous round granules in both species, averaging 1.5 µm in diameter in *Berthella stellata* and up to 4.5 µm in *Euselenops luniceps* (fig. 12B). In the latter species the average size of the granules and their number are smaller in the basal part of the cell than in the apical part. The texture of the granules is homogeneous in both species, in *Berthella stellata* they stain light blue, whereas they have dark blue staining properties in *Euselenops luniceps.* Generally, large nuclei are found in the basal parts of the glandular cells. The alternating supporting cells are slender and have a triangular apex. They contain apically lying nuclei and bear short cilia. In *Euselenops luniceps* black pigments are visible at the apical rim of the supporting cells. The

capsule gland shows a continuous transition to the membrane gland in *Berthella stellata*, whereas in *Euselenops luniceps* a small ciliated duct connects both glands.

MEMBRANE GLAND (table 5; fig. 12D): The membrane gland is also tubular and narrowly coiled, as has been described for the capsule gland. The glandular cells are prismatic (*Berthella stellata*) to columnar (*Euselenops luniceps*) and contain mucus of different textures. In *Berthella stellata* the mucus has the form of heterogeneous fibres, partly coagulating to form droplets. The mucus stains dark red to violet in this species. In *Euselenops luniceps* the mucus is heterogeneous in the proximal part of the gland and forms distinct droplets of different sizes further distally. The staining properties are light pink to violet (fig. 12D). The small nuclei are found basally in both species. The supporting cells are very prominent in *Euselenops luniceps*, their triangular apices partly overlapping the apices of the adjacent glandular cells. In both species apically lying nuclei are found in the supporting cells which bear very long cilia, especially in *Berthella stellata*.

MUCOUS GLAND (table 5): This gland builds a large tubular organ. The tube is widely coiled having a relatively large lumen. The glandular epithelium is composed of alternating glandular and supporting cells. The glandular cells are highly columnar in shape. In *Berthella stellata* the mucous contents have the form of mucous vesicles of different sizes and shapes. But they can also dissolve or fuse, thus filling the cells with a homogeneous mass of mucus. The staining properties vary from red to violet. Relatively large nuclei are located medially to basally in the glandular cells. The alternating supporting cells are pigmented apically and bear short cilia. Nuclei are not visible in the cells. In *Euselenops luniceps* the glandular cells exhibit basically two cell types: the first type contains a heterogeneous mucous mass often forming compact droplets staining red to violet. The alternating supporting cells are prominent, have a triangular apex and overlap the glandular cells. This type of epithelium dominates in the proximal part of the gland. Further distally the glandular cells are more slender, more elongate and the mucous contents are in the form of small vesicles staining pink to light violet. The mucous vesicles sometimes fuse to build a homogeneous mass of mucus. The supporting cells in this glandular part are smaller and bear shorter cilia. In both glandular cell types the small nuclei are found basally, whereas in the supporting cells they are located apically.

3.6.2 Discussion

The two pleurobranchoid species investigated show quite similar reproductive systems with regards to their nidamental glands. Both have three distinct glandular parts clearly distinguishable by their characteristic histology: capsule gland, membrane gland and mucous gland. All three types of glands are tubular structures. While the capsule gland and the membrane gland are narrowly coiled and confined to a small area of the nidamental gland system the mucous gland is a very large organ in both species and always widely coiled. The relative size of the organs and the cells is different in both species due to very different body sizes (*Berthella stellata*: 8 mm fixed; *Euselenops luniceps*: 110 mm alive).

Only few anatomical data are available on the reproductive systems of pleurobranchoid species. Jensen (1994) described the anatomy of the anterior reproductive system of *Euselenops luniceps*. In a drawing of the reproductive system she distinguished between an albumen, a capsule and a mucous gland in the nidamental gland system, but she does not describe these parts any further. She did not mention the capsule gland in the describing text. Thus it is difficult to compare her findings to the present data. As far as I know, only one histological investigation of a pleurobranchoid reproductive system has been undertaken so far (Wägele & Hain 1991). The authors also described three glandular parts in the female glands of *Thomthompsonia antartica* (described as *T. spirobranchalis*), namely the capsule gland, the proximal and the distal mucous gland. From the position of the proximal mucous gland in this species I assume that it corresponds to the membrane gland described for the two species in the present study. The distal mucous gland of *Thomthompsonia antartica* accords with the mucous gland observed here.

3.7 The anterior reproductive systems of the Nudibranchia

All nudibranch species investigated show either androdiaulic or triaulic reproductive systems (fig. 13, 14). In the triaulic *Onchidoris bilamellata* the proximal oviduct branches off far distally thus giving the appearance of a pseudo-diaulic system. The ampulla is mostly tube-like and coiled. All species have glandular prostates either lining the proximal vas deferens or forming a separate gland attached to the vas deferens by a duct (*Eubranchus exiguus*). In the androdiaulic systems the receptaculum seminis can either branch off at the proximal end of the oviduct or it can open via a separate duct to the outside near the opening of the distal oviduct. *Lomanotus vermiformis* has two receptacula. A bursa copulatrix is always missing in androdiaulic forms. Triaulic species have both a bursa copulatrix (distally) and a receptaculum seminis (proximally). In some species investigated here the vaginal duct has a separate opening (*Acanthodoris pilosa, Adalaria proxima*), in others (e. g. *Chromodoris magnifica*, *Dendrodoris nigra*, *Onchidoris bilamellata* and *Polycera quadrilineata*) it discharges via the distal oviduct.

Usually the proximal oviduct is short and not differentiated from the postampullary duct. In *Acanthodoris pilosa* and *Chromodoris magnifica* though it forms a dilation or fertilization chamber. Both species were preserved during the process of spawning with eggs still in the lumina of the nidamental glands. *Onchidoris bilamellata* had probably just spawned before preservation. Thus large areas of the glands are worn out, the cells having no distinct structure. Still in few parts the histology of the glands is still apparent and could be reconstructed. The nidamental glands can always be divided into three distinct parts, a proximal capsule gland, a membrane gland and a mucous gland. All glandular parts are tubular structures, the mucous gland being the largest part in all species investigated. The distal oviduct is lined by a specialized glandular tissue (adhesive region) in all species except for *Onchidoris bilamellata*. Attached to the vestibulum of *Dendrodoris nigra* is a vestibular gland.

Comparative schematic drawings of the major cell types of the nidamental glands and the adhesive region are shown in table 6 (Anthobranchia) and table 7 (Cladobranchia).

3.7.1 Histology of the nidamental glands of the Anthobranchia:

CAPSULE GLAND (table 6, fig. 15A, B, C; 16A): In all anthobranch species investigated the capsule gland has the form of a narrowly coiled tube. In *Dendrodoris nigra* the coils are arranged extremely densely, thus the lumina are very small (fig. 15B). The glandular cells are columnar to highly columnar and contain distinct round granules of different sizes: the smallest granules are found in *Adalaria proxima* (up to 4.0 µm in diameter) and *Acanthodoris pilosa* (up to 1.3 µm). In *Polycera quadrilineata* the granules are up to 4.0 µm in diameter, whereas *Dendrodoris nigra* has slightly larger granules of up to 6.0 µm in diameter. The largest granules are found in *Chromodoris magnifica* (up to 9.0 µm). Generally the staining properties of the granules are blue to dark blue (fig. 15A, C). An exception is *Dendrodoris nigra*, which has granules staining light blue (fig. 15B) and having an even lighter rim. The granules are embedded in blue-staining cytoplasm in all species. They sometimes accumulate in the apical halves of the cell, but can also be found in fewer numbers further basally (*Adalaria proxima*, *Onchidoris bilamellata*) or be spread more or less evenly throughout the cell (*Chromodoris magnifica*, *Dendrodoris nigra*, *Polycera quadrilineata*). Generally a large nucleus is found basally in the glandular cells, showing a prominent nucleolus in *Adalaria proxima* and *Chromodoris magnifica*. The always alternating supporting cells are usually inconspicuous, bear short cilia and contain an apically lying nucleus, which is not visible in *Adalaria proxima*. In *Acanthodoris pilosa* eggs are not visible in the lumen of the capsule gland, whereas in *Onchidoris bilamellata* few single eggs can be observed and in *Chromodoris magnifica* numerous eggs are visible. In the latter species the eggs appear in groups, narrowly clustered and having no definite egg shape yet (fig. 16A). They are not covered by a capsule but amorphous material having the same staining properties as the granules of the capsule gland and the capsule of the egg mass can be found in the lumen of the gland (see arrow in fig. 16A). In *Onchidoris bilamellata* the eggs are not covered by secretory material either, but few blue staining secretions are visible in the lumen of the capsule gland.

Whereas in *Adalaria proxima* and *Onchidoris bilamellata* the capsule gland discharges into the distal oviduct, a small connecting duct between capsule and membrane gland is present in the other four anthobranch species.

MEMBRANE GLAND (table 6; fig. 15B; 16B): This glandular part is also narrowly coiled (fig. 15B) but generally comprises a smaller glandular area than the capsule gland. The glandular cells are prismatic to columnar. They contain mucus of various textures and staining properties. Heterogeneous mucous filaments dominate and are present in all species except for *Dendrodoris nigra*. In this species the mucus has the form of irregular droplets. Small mucous droplets are also partly present in the glandular cells of *Acanthodoris pilosa*. In *Onchidoris bilamellata* the mucous fibres sometimes coagulate and form larger homogeneous mucous patches. The staining properties vary from light pink or light violet for the mucous filaments to red or darker violet for the droplets. Nuclei are generally located in the basal halves of the glandular cells but can also occur in a medial position (*Chromodoris magnifica*). The alternating supporting cells are conspicuous in all species examined. They have triangular apices, which are very broad in *Onchidoris bilamellata* and *Polycera quadrilineata*. In the latter species the apices of the supporting cells partly overlap the apices of the adjacent glandular cells. The supporting cells always bear extremely long cilia and contain small apically lying nuclei.

In *Acanthodoris pilosa* few single eggs are visible in the lumen of the membrane gland. Proximally they are not covered by a capsule, but further distally a distinct capsule is visible, surrounding the eggs. Adjacent to the blue staining inner capsule layer is a violet staining thin mucous layer forming the membrane. In the lumen of the gland, secretion droplets of the same staining property as the membrane are visible. In *Chromodoris magnifica* numerous eggs are also visible within the lumen of the membrane gland, embedded in an amorphous blue-staining mass, looking like capsule material (fig. 16B). In the proximal parts of the gland the eggs are still not encapsulated by a distinct capsule, further distally they appear to be arranged in a line and the capsule material spreads around the individual eggs. No space is visible between eggs and capsule sheath. Also attached to the outer edge of the blue-staining inner capsule layer is a thin layer of violet-staining membrane. This mucous membrane seems to be secreted by the glandular cells of the membrane gland. No eggs are found in the membrane gland of *Onchidoris bilamellata*.

The membrane gland shows a continuous transition to the mucous gland in *Chromodoris magnifica*, *Dendrodoris nigra* and *Polycera quadrilineata*, whereas it discharges into the distal oviduct in the other three species.

MUCOUS GLAND (table 6; fig. 15C; 16C, D, E): This largest part of the nidamental glands can be divided into a narrowly coiled proximal part and a widely coiled distal part in *Acanthodoris pilosa*, *Adalaria proxima*, *Chromodoris magnifica* and *Dendrodoris nigra*. This division is not apparent in *Onchidoris bilamellata* and *Polycera quadrilineata* (fig. 15C). The shape of the glandular cells varies in the different parts of the mucous gland. In the proximal part the cells are shorter, having a columnar form, whereas further distally they are usually more slender and highly columnar. The mucous contents are also variable in the different gland parts. In *Onchidoris bilamellata* the mucus has the form of small vesicles embedded in heterogeneous, filamentous mucus throughout the mucous gland. Small violet staining mucous vesicles or homogeneous mucus is also found in *Polycera quadrilineata*. In contrast, the glandular cells in the proximal part of the mucous gland of *Adalaria proxima* contain large filamentous mucous patches, whereas the glandular cells further distally are filled with homogeneously violet staining mucus. In some areas the mucus is packed into small, distinct vesicles. *Dendrodoris nigra* has a different structure. In this species glandular cells in the proximal part of the mucous gland are filled with homogeneous mucus which is interspersed in between distinct mucous vesicles staining pink to violet. In the distal part the more slender glandular cells are filled with small dark violet staining mucous droplets, accumulating in the apical halves of the cells. In *Acanthodoris pilosa* the mucous contents in the glandular cells of the proximal part form large amorphous patches apically, discharging of this mucus is visible in some cells. Towards the basal parts of the cells the mucus has the form of small droplets. It stains dark violet. The eggs in the lumen of this glandular part are arranged in a line, encapsulated and surrounded by a membrane and an inner mucous layer (fig. 16C). Only a small space between inner capsule layer and eggs is visible. Further distally the glandular cells have similar contents. The egg capsules, with a distinct space between eggs and capsule, are embedded in a mucous matrix here and layers of mucus are added to the outer edge of the spawn mass (fig. 16D). In *Chromodoris magnifica* the glandular cells of the mucous gland are filled with mucous vesicles, which partly dissolve to form homogeneous mucous patches. The staining properties are violet. Eggs found in the proximal part of the mucous gland are encapsulated and first mucous secretions forming the inner mucous layers are secreted. Further distally the egg ribbon present in the lumen contains encapsulated eggs (the capsule consisting of two visible layers, one inner layer staining

blue and one thin outer membrane staining violet). A non-staining cavity is visble between eggs and inner capsule layer. The membranes are surrounded by a dark violet staining inner mucous layer and are embedded in a mucous matrix composed of mucous layers of different density staining light violet. Further distally, shortly before entering the distal oviduct, the egg ribbon shows a distinct outer mucous cover composed of few layers of mucus, all staining in different shades of violet (fig. 16E). The glandular cells of the most distal part of the gland secrete mucus added to the outer edge of the egg ribbon.
In all glandular parts of all investigated species small nuclei are found basally in the glandular cells. They seem to be pycnotic in the proximal part of the mucous gland of *Dendrodoris nigra* and in *Polycera quadrilineata*. A conspicuous nucleolus is visible in the nuclei of the mucous gland of *Acanthodoris pilosa*. Glandular cells are always alternating with small supporting cells. The latter are generally wedge-shaped and bear short cilia. They contain an apically lying nucleus, which is not visible in *Adalaria proxima* and in the distal glandular part of *Dendrodoris nigra*.

ADHESIVE REGION (table 6; fig. 16F): Except for *Onchidoris bilamellata* all species have a glandular adhesive region, lining the most distal part of the oviduct. In *Chromodoris magnifica* only one side of the oviduct is lined by the glandular epithelium. The glandular cells are always columnar, sometimes highly columnar (*Acanthodoris pilosa*). They contain mucous droplets of round (*Chromodoris magnifica, Dendrodoris nigra*) to irregular shape (*Acanthodoris pilosa*, *Adalaria proxima*, *Polycera quadrilineata*). In *Acanthodoris pilosa* and *Adalaria proxima* the droplets cluster in the apical part of the glandular cells. The size of the droplets also varies, *Acanthodoris pilosa* having the smallest and *Adalaria proxima* the largest. The staining properties are uniformly dark violet. Round to elliptical nuclei are found basally, sometimes also medially (*Chromodoris magnifica*). The alternating supporting cells are more conspicuous than in the mucous gland further proximally. They bear short to moderately long cilia. An apically lying nucleus is only visible in *Adalaria proxima* and *Dendrodoris nigra.* Part of the egg mass is also visible in this glandular part in *Chromodoris magnifica* (fig. 16F). No active secretion is visible but granular secretions are present in the lumen of the gland and also at the outer mucous layer of the egg ribbon. In *Acanthodoris pilosa* the egg ribbon visible in the adhesive region seems to have the same structure as in

the most distal part of the mucous gland. Secretion of mucous material which is added to the outer mucous cover of the spawn mass is still visible in the adhesive region.

VESTIBULAR GLAND (fig. 15D): *Dendrodoris nigra* possesses a small gland attached to the vestibulum by a small ciliated duct. Internally the gland is lined by a convoluted epithelium composed of cuboidal to prismatic cells. The cells are bordered by an extraordinary thick purple fringe which differs from all other epithelia lined by microvilli or cilia.

3.7.2 Ultrastructure of the nidamental glands of the Anthobranchia:

In *Acanthodoris pilosa* and *Polycera quadrilineata* the nidamental glands have been investigated with TEM. Additionally the vestibular gland of *Dendrodoris nigra* has been studied ultrastructurally.

CAPSULE GLAND (fig. 18A, F; 19A): The granules found in the glandular cells of the two antobranch species are composed of electron-dense, filamentous substructures, which are embedded in a less electron-dense matrix. The substructures have the form of concentric circles or labyrinthine lamellae. In *Acanthodoris pilosa* these substructures are very conspicuous, (fig. 18E) even at low magnifications, whereas in *Polycera quadrilineata* they can only be observed with higher magnifications (< 20.000 x). In the latter species more electron dense mature granules are found in between amorphous, less electron dense granules composed of concentric lamellae (fig. 19A). The latter comprise nascent granules. They are membrane-coated, as are the granules in *Acanthodoris pilosa*. In the mature granules of *Polycera quadrilineata* a membrane is not apparent. The number of granules is generally higher in the apical part of the cells than further basally. Ejection of secretory material could not be observed directly. Granules close to the apical cell membrane, before they are ejected, get an amorphous shape and the secretory material is ejected as an amorphous mass in, as has been observed in *Acanthodoris pilosa*. The apical cell membranes project microvilli into the lumen. In the basal halves of the cells Golgi complexes are visible. In *Polycera quadrilineata* electron-dense material similar to that of the secretory granules can be observed in the parallel cisternae of the Golgi complexes. A large nucleus is located basally in both species. Around the nuclei large areas of

endoplasmic reticulum are present, in *Polycera quadrilineata* ER is also spread throughout the rest of the cells. Generally, the ER can have the form of parallel cisternae or be vesicular. Mitochondria, with conspicuous cristae, are also scattered throughout the cells in both species, whereas lipid droplets are mainly found in the basal part of the glandular cells. The alternating supporting cells are studded with cilia apically, in between the cilia microvilli are also present. The cells contain an apically lying nucleus which is much smaller than the one in the glandular cells. A distinct basal lamina, underlining the epithelium, is only visible in *Acanthodoris pilosa*.

MEMBRANE GLAND (fig. 20A, C): The glandular cells of the membrane gland are filled with small vesicles containing mucous filaments. In the apical part of the cells the vesicles are densely packed and fuse to form larger conglomerations. The mucus in the vesicles consists of loosely packed fibres sometimes coagulating to compact mucous lumps (fig. 20C). These coagulations easily break out of the ultra-thin sections. Basally a small nucleus, containing dense chromatin, is present. Additionally few Golgi complexes and lipid droplets are found. Very few mitochondria are spread throughout the cell. Endoplasmic reticulum is not visible. The supporting cells contain apically numerous mitochondria and bear numerous extremely long cilia (fig. 20C) compared to those of the capsule and mucous glands. Nuclei are not visible in the supporting cells. In *Polycera quadrilineata* electron-dense, amorphous material can be observed in the lumen of the gland.

MUCOUS GLAND (fig. 21A, C): In *Acanthodoris pilosa* the mucous vesicles found in the mucous gland are large (often just one vesicle per cell), whereas in *Polycera quadrilineata* they are smaller. In the latter species sections have been taken from the more distal part of the mucous gland. The vesicles are generally filled with a meshwork of heterogeneous mucous filaments, in *Polycera quadrilineata* packed more densely than in *Acanthodoris pilosa*. The mucus never coagulates to compact clusters though, as has been observed in the membrane gland. Small nuclei are found basally, as well as Golgi complexes (fig. 21C). Nascent vesicles can be seen in the centre of the Golgi areas in *Polycera quadrilineata*. Few mitochondria, ribosomal vesicles and lipid droplets are also present randomly in the basal halves of the glandular cells (fig. 21C). The basal cell membrane has a labyrinthine

structure in *Polycera quadrilineata*. The supporting cells are not as conspicuous as in the membrane gland in any of the species. They bear short cilia. Nuclei are not visible.

VESTIBULAR GLAND (fig. 22): Ultrastructural investigations reveal that between the microvilli and also in the lumen of the gland many thousands of rod-shaped, gram-negative bacteria averaging 0.38 µm in diameter are present (fig. 22B, C). They are packed densely. The epithelial cells of the gland contain a centrally lying nucleus, a nucleolus is mostly visible (fig. 22A). Numerous vesicles of varying sizes from 0.5 - 3.0 µm can be found in the apical part. The contents of these vesicles are unknown. In the basal part of the cells Golgi complexes, endoplasmic reticulum and few other vesicles are located. Mitochondria are spread throughout the cells. A thick layer of connective tissue is underlying the epithelium of the gland.

3.7.3 Histology of the nidamental glands of the Cladobranchia:

CAPSULE GLAND (table 7; fig. 17A): The capsule gland also exhibits a coiled tube in the Cladobranchia, but the coils are not as narrow as in the Anthobranchia. This is especially apparent in *Lomanotus vermiformis*, where the coils are unusually wide (fig. 17A). The columnar to highly columnar glandular cells contain numerous small, dark blue staining granules of varying sizes (*Lomanotus vermiformis*: up to 3.5 µm in diameter; *Tritonia plebeia*: up to 2.5 µm; *Dermatobranchus semistriatus*: up to 3.0 µm; *Eubranchus exiguus*: up to 1.5 µm; *Flabellina gracilis*: up to 2.5 µm). The granules are usually embedded in blue staining cytoplasm and accumulate in the apical halves of the cells, but also occur in fewer numbers in the basal halves. In *Eubranchus exiguus* few larger granules (approximately 3.0 µm in diameter) are present in the basal halves of the cells. They stain light pink. The nuclei are generally located basally in the glandular cells. In *Lomanotus vermiformis*, *Tritonia plebeia* and *Flabellina gracilis* nucleoli are visible. The alternating supporting cells are small, have triangular apices, contain an apically lying nucleus and bear short cilia.
The capsule gland shows a continuous transition to the membrane gland in all species investigated, except for *Tritonia plebeia*, in which the capsule gland discharges into the distal oviduct.

MEMBRANE GLAND (table 7; fig. 17A): This small glandular area is narrowly coiled in all cladobranch species and comprises, in comparison to the anthobranchs, fewer coils. The glandular cells have a prismatic (*Tritonia plebeia*, *Eubranchus exiguus*), columnar (*Dermatobranchus semistriatus*, *Flabellina gracilis*) or highly columnar shape (*Lomanotus vermiformis*). They contain heterogeneous mucous fibres staining pink (fig. 17A). In *Lomanotus vermiformis* the fibres also coagulate to form small droplets staining darker. Larger mucous coagulations can also be observed in *Tritonia plebeia*, they occur in between the mucous fibres and have a slightly darker staining property than those. Small nuclei are found basally in all species, in *Lomanotus vermiformis*, *Tritonia plebeia*, *Eubranchus exiguus* and *Flabellina gracilis* they seem to be pycnotic. The alternating supporting cells are very conspicuous in the membrane glands of all species studied. They contain an apically lying nucleus and generally bear long cilia. In *Eubranchus exiguus* the cilia are only slightly longer than those in the capsule gland. The supporting cells of *Flabellina gracilis* are laterally distended at the apex and partly cover the apices of the adjacent glandular cells. The membrane gland discharges directly into the mucous gland in all cladobranch species investigated except for *Lomanotus vermiformis* and *Dermatobranchus semistriatus*. In these species it opens distally into the oviduct.

MUCOUS GLAND (table 7; fig. 17B, C): The mucous gland comprises a widely coiled tube in all species investigated. A differentiation into a more narrowly coiled proximal part and a widely coiled distal part, as has been described for most anthobranchs, could not be observed in any cladobranch species. The lining epithelium is always highly columnar. The glandular cells are filled with mucus of various textures and staining properties. In *Lomanotus vermiformis* the glandular cells contain densely packed irregular mucous vesicles staining dark pink in the proximal part of the gland. Further distally the cells are filled with heterogeneous mucous fibres staining violet. *Tritonia plebeia* possesses mucous vesicles of different sizes, filled with homogeneously pink to violet staining mucus throughout the whole length of the gland. In *Dermatobranchus semistriatus* the glandular cells mainly contain large mucous vesicles staining pink to violet (fig. 17C). In some parts the vesicles can dissolve, thus filling the cells with homogeneous mucus. This can also be observed in *Eubranchus exiguus*, but here the mucus stains red to violet. In other cells within the mucous gland of *Dermatobranchus semistriatus* the vesicles are filled with darker violet

staining mucus. *Flabellina gracilis* has glandular cells containing small mucous vesicles, large compact mucous droplets are heterogeneous mucous fibres. The staining properties vary form pink to violet (fig. 17B). In all of the species investigated a small nucleus is found lying basally in the glandular cells. In *Flabellina gracilis* some nuclei contain a prominent nucleolus. The nuclei found in *Lomanotus vermiformis*, *Dermatobranchus semistriatus* and *Eubranchus exiguus* seem to be pycnotic. The alternating supporting cells always contain an apically lying small nucleus and bear short cilia.

ADHESIVE REGION (table 7; fig. 17D): All cladobranch species, which have been studied, show a specialized glandular epithelium lining the distal oviduct. The columnar glandular cells are always filled with small round to irregularly shaped mucous droplets staining dark violet (fig. 17D). In *Lomanotus vermiformis* the droplets cluster in the apical parts of the cells, whereas in the other four species they are spread throughout the cells, but the number usually decreases further basally. The nuclei are always conspicuous and contain prominent nucleoli in *Eubranchus exiguus* and *Flabellina gracilis*. In the wedge-shaped supporting cells the nuclei are located apically. The cells bear short cilia.

3.7.4 Ultrastructure of the nidamental glands of the Cladobranchia

Except for *Lomanotus vermiformis* all other four cladobranch species have been investigated ultrastructurally.

CAPSULE GLAND (fig. 18A, C, D; 19B, C, D): The secretory granules in the glandular cells of the capsule generally have a round shape, but in *Flabellina gracilis* the granules are of an irregular form. This might be a preservation artefact because the animal had already spawned before preservation and a deformation of the granules could be possible. The granules are usually composed of an electron-dense matrix. In *Dermatobranchus semistriatus* the granules appear to be homogeneous (fig. 18C) similar to *Philinopsis gardineri* (fig. 18B), whereas in the other three species more electron-dense substructures are visible embedded in the matrix. These substructures have different shapes in the various species. In *Tritonia plebeia* they have a mottled pattern (fig. 19B), sometimes concentric circles are visible at the outer edge of the granules; in *Eubranchus exiguus* and *Flabellina gracilis* they appear dot-like. In the latter two species the substructures are only visible at higher magnifications (in

Eubranchus exiguus at >12.000 x, in *Flabellina gracilis* at >20.000 x). The granules are membrane-coated in all species investigated. But the membrane is more conspicuous in nascent granules than in mature ones. In *Tritonia plebeia* a distinct membrane coating the mature granules is only visible in those granules which occur close to the apical cell membrane. It seems, in cells actively secreting, that the vesicle membrane fuses with the cell membrane, which had bended inward forming a groove, before the secretory material is poured into the lumen. This could be observed in *Eubranchus exiguus* (fig. 18C) and *Flabellina gracilis*. Nascent granules are usually less electron-dense than mature ones. Generally both granule types are found in the same cell, the mature granules usually accumulating in the apical part of the cell, whereas the nascent granules appear further basally. In *Tritonia plebeia*, though, nascent and mature granules are also intermingled in the apical half of the cell (fig. 19B). A large nucleus is always found in the basal half of the glandular cell (fig. 19C). Prominent areas of rough endoplasmic reticulum are often present in close proximity to the nucleus, usually having the form of parallel cisternae or being vesicular (fig. 19C). ER can also be found scattered throughout the rest of the cell but in lower quantity. In the basal halves of the cells conspicuous Golgi complexes are found. Active secretion of secretory material at the distal ends of their cisternae could be observed in *Tritonia plebeia* (see arrow in fig. 19D), *Eubranchus exiguus* and *Flabellina gracilis*. No Golgi complexes are visible in *Dermatobranchus semistriatus.* Round to elongated mitochondria are spread throughout the glandular cells in all species studied. Additionally few lipid droplets could be found in *Eubranchus exiguus*, occurring basally. The alternating supporting cells are small and have a triangular apex containing a small nucleus and many mitochondria. The apical cell membrane is studded with cilia and in between those microvilli are present. The epithelium is underlined by a basal lamina in all species.

MEMBRANE GLAND (fig. 20A, D, E): The secretory vesicles in this gland generally fuse in the apical part of the glandular cell forming more indistinct vesicles which contain large patches of secretory material, whereas more basally they are more distinct and smaller. The secretions have the form of a meshwork of loose fibres filling the vesicles completely. In *Tritonia plebeia* and *Dermatobranchus semistriatus* coagulation of secretory material was observed forming compact mucus lumps (fig. 20D). These often break out of the ultra-thin sections. The contents of the vesicles are usually less dense in vesicles

occurring basally. In *Flabellina gracilis* coagulation of mucus is sparse throughout the whole cell. The apical cell membrane is lined by microvilli. During secretion, which was observed in *Flabellina gracilis* (fig. 20E), the vesicle membrane seems to fuse with the apical cell membrane and ruptures. The mucus is ejected as an amorphous mass. In the basal part of the cells small nuclei are found, always containing very dense chromatin. Golgi complexes and rough endoplasmic reticulum are present in close proximity to the nuclei. But they are not as prominent as in the capsule gland. Few mitochondria are also present close to the nuclei. The cell membrane is highly folded basally. The supporting cells are very conspicuous in the membrane gland. They contain even more mitochondria than the supporting cells in the capsule gland and bear much longer cilia (fig. 20D). In between the cilia small microvilli are found. A basal lamina underlines the epithelium.

MUCOUS GLAND (fig. 21A, B, D): The secretory vesicles in the mucous gland are usually larger than those found in the membrane gland. They are not as distinct. Often, the apical part of the glandular cell is completely filled with amorphous, filamentous mucus, never coagulating to compact structures. Further basally smaller vesicles are found, filled with less dense material, as has been observed for example in *Tirotnia plebeia* (fig 21B). The nuclei found in the glandular cells, are very small and contain electron-dense chromatin in all species investigated. They are probably pycnotic. Large Golgi complexes and mitochondria are found near the nuclei (fig. 21D). Rough endoplasmic reticulum is present in *Eubranchus exiguus* close to the Golgi complexes, but could not be found in the other species. The supporting cells are generally smaller in the mucous gland than in the membrane gland. They bear less and shorter cilia and the number of mitochondria is lower. In *Flabellina gracilis* the supporting cells are inconspicuous. The mucous gland of *Dermatobranchus semistriatus* has not been investigated ultrastructurally, because the embedding media had not infiltrated the tissue properly.

3.7.5 Discussion:

The nudibranch species examined in this study show quite uniformly structured nidamental glands. Three glandular parts are always present, a proximal capsule gland, a membrane gland and a mucous gland. The histology of these parts in the various taxa is similar although certain dissimilarities can be noted: the secretory granules in the capsule

gland have generally a greater size in the anthobranch species (up to 9 µm in diameter) than those of the cladobranchs (up to 3.5 µm in diameter). In *Chromodoris magnifica* and especially in *Dendrodoris nigra* they also stain much lighter.

Two distinct parts of the mucous gland can be distinguished in all anthobranchs except for *Onchidoris bilamellata* and *Polycera quadrilineata*: a narrowly coiled proximal part and a widely coiled distal part. The histology of the mucous gland differs considerably with regards to the structure of the secretory products in all species investigated. Different cell types can be found throughout the glands. These cell types may represent different functional stages and are therefore difficult to interpret. The distal oviduct is lined by glandular epithelium in all species except for *Onchidoris bilamellata*. Since the specimen investigated here had spawned just before preservation, the glandular tissue was in a considerably bad condition, which might be the reason why no glandular tissue was found in the distal oviduct. Investigation of another specimen could help to clarify this question.

The ultrastructure of the nidamental glands is also uniform in nudibranchs. The granules in the capsule glands are usually electron-dense and contain even more electron-dense substructures in all species investigated except for *Dermatobranchus semistriatus*, which possesses homogeneous granules. The mucous contents of the membrane gland are more compact than those of the mucous gland, and the supporting cells within the membrane gland bear longer cilia and contain more mitochondria than the supporting cells of the other two glands. This is uniform in all nudibranchs studied.

The presence of a vestibular gland containing possibly symbiotic bacteria, as has been found in *Dendrodoris nigra*, is unique within the gastropoda and has never been reported in any gastropod species before. The function of this gland and its bacteria is unkown. Presence of identically appearing bacteria in the egg masses of *Dendrodoris nigra* (see chapter 3.9.12, pages 108/109) suggests, that the bacteria might play a role in the egg laying process. For further details of the structure of the vestibular gland, the reader is referred to Klussmann-Kolb & Brodie (1999), where this data is discussed in a broader sense.

In contrast to the other opisthobranch taxa presented in this study quite a few studies on the histology of the nidamental glands have been undertaken in the Nudibranchia. Pohl (1905) presented a very detailed anatomical and histological description of the reproductive system of *Polycera quadrilineata* early this century. He distinguished between an

albumen gland ("Eiweißdrüse"), a capsule gland ("Schalendrüse") and a nidamental gland ("Nidamentaldrüse"). Pohl found two ducts emerging from the albumen gland, one leading to the capsule gland, the other joining the vestibulum distally. The latter could not be found in the present study. The nidamental gland, which accords with the mucous gland described for *Polycera quadrilineata* here, was separated by Pohl into different parts ("pars conjunctiva", "pars convoluta" and "pars constructa"). This separation has not been undertaken in the present study, but Pohl´s "pars convoluta" could accord with what has been found in other anthobranch species in this study to be the more narrowly coiled proximal part of the mucous gland. In *Polycera quadrilineata*, though, I could not find any such part.

Thompson (1961) published a histological investigation of the reproductive system of *Tritonia hombergi*. His descriptions do not correspond to the present findings of *Tritonia plebeia* in certain aspects. Thompson called the proximal glandular part an "albumen gland", a term he later exchanged with "capsule gland" in a paper on *Archidoris pseudoargus* (1966). The author did not distinguish between a membrane and a mucous gland. In his opinion the mucous gland has the structure of a large tube with a central lumen into and out of which numerous convoluted tubules open. This structure could not be found in *Tritonia plebeia*.

Wägele (1989a) distinguished between three different glands at the oviduct of Antarctic nudibranchs (*Austrodoris kerguelenensis*, *Aegires albus*, *Bathydoris clavigera*, *Bathydoris hodgsoni* and *Tritoniella belli*) for the first time. She called them capsule gland, proximal and distal mucous gland. Her description of the cellular structure of the proximal mucous gland is in accordance with what I have described for the different nudibranch species as the membrane gland. In the distal mucous gland (here corresponding to the mucous gland) Wägele described different cell types which she also thought may represent different functional stages. Another conformity between the data from Wägele and the present data is the fact that the granules in the capsule gland are larger in anthobranch species than in cladobranch nudibranchs. Futhermore, the author described a specialised glandular tissue near the distal opening of the oviduct in *Phyllidia pulitzeri* (Wägele 1985) and *Austrodoris kerguelenensis* (Wägele 1989a). She referred to this part as the "adhesive region" or "Kleberegion", a term which has also been used in the present study. A differentiation of three glandular parts within the nidamental glands of different species of *Armina* was

presented by Kolb (1998). In accordance with Wägele (1989a) the author distinguished between a capsule gland and a proximal and distal mucous gland in three species of this genus. Thorough histological investigations of two different species of *Flabellina* were presented by Schulze & Wägele (1998) and Schulze (1998). In *Flabellina affinis* and *Flabellina pedata* the authors distinguished between five glandular parts within the nidamental glandular mass, namely the capsule, the proximal and the distal mucous gland and two terminal parts. The latter have not been found here in *Flabellina gracilis*. Schulze & Wägele (1998) believed that the terminal part 1 accords with what Schmekel (1971) has described as the "Kleberegion" (=adhesive region). An adhesive region has also been described for *Flabellina gracilis* in the present study. Since the histological descriptions of the terminal part 1 in *Flabellina affinis* (Schulze & Wägele 1998) and the adhesive region in *Flabellina gracilis* (present study) differ considerably, it cannot be decided at this point in time whether they are homologous structures. Ultrastructural investigations might bring further clarification in this matter.

The first and only detailed comparative histological and ultrastructural study of nudibranch reproductive systems so far has been undertaken by Schmekel (1971) Many of her findings are still valid today and can be compared to the data gathered in the present study. In general, the data from Schmekel and my own data are very conform, but a few issues are debatable: Schmekel (1971) distinguished between two different parts in the nidamental glands of nudibranchs, the "Eiweissdrüse" (albumen gland), which she later calls "membrane gland" (1985) and the "Mukusdrüse" (mucous gland). The former corresponds to the capsule gland I have described for the various species here and is therefore not homologous to the membrane gland I have described for various species. Schmekel´s descriptions of the histology and ultrastructure of the different glandular parts were summaries based upon examinations of 29 species (histologically) and 13 species (ultrastructurally) of opisthobranchs. Schmekel described the secretory granules of the "Eiweissdrüse" to be composed of two different components as has been described here for most nudibranch species. In her opinion the form of the more electron-dense substructures are species-specific. She never found the granules to be of homogeneous structure, as I have observed in *Dermatobranchus semistriatus*. According to Schmekel the granules in the "Eiweissdrüse" are only membrane-coated when they are immature. This cannot be confirmed here since mature granules have been found to be bound by a

distinct membrane, which sometimes is very difficult to locate accurately, though. Schmekel found the secretory vesicles in the mucous gland always to be surrounded by a distinct membrane. This agrees with the present data. Lastly, according to Schmekel the vesicles never fuse in mature cells. However, I found fused vesicles in mature cells of the membrane as well as the mucous gland.

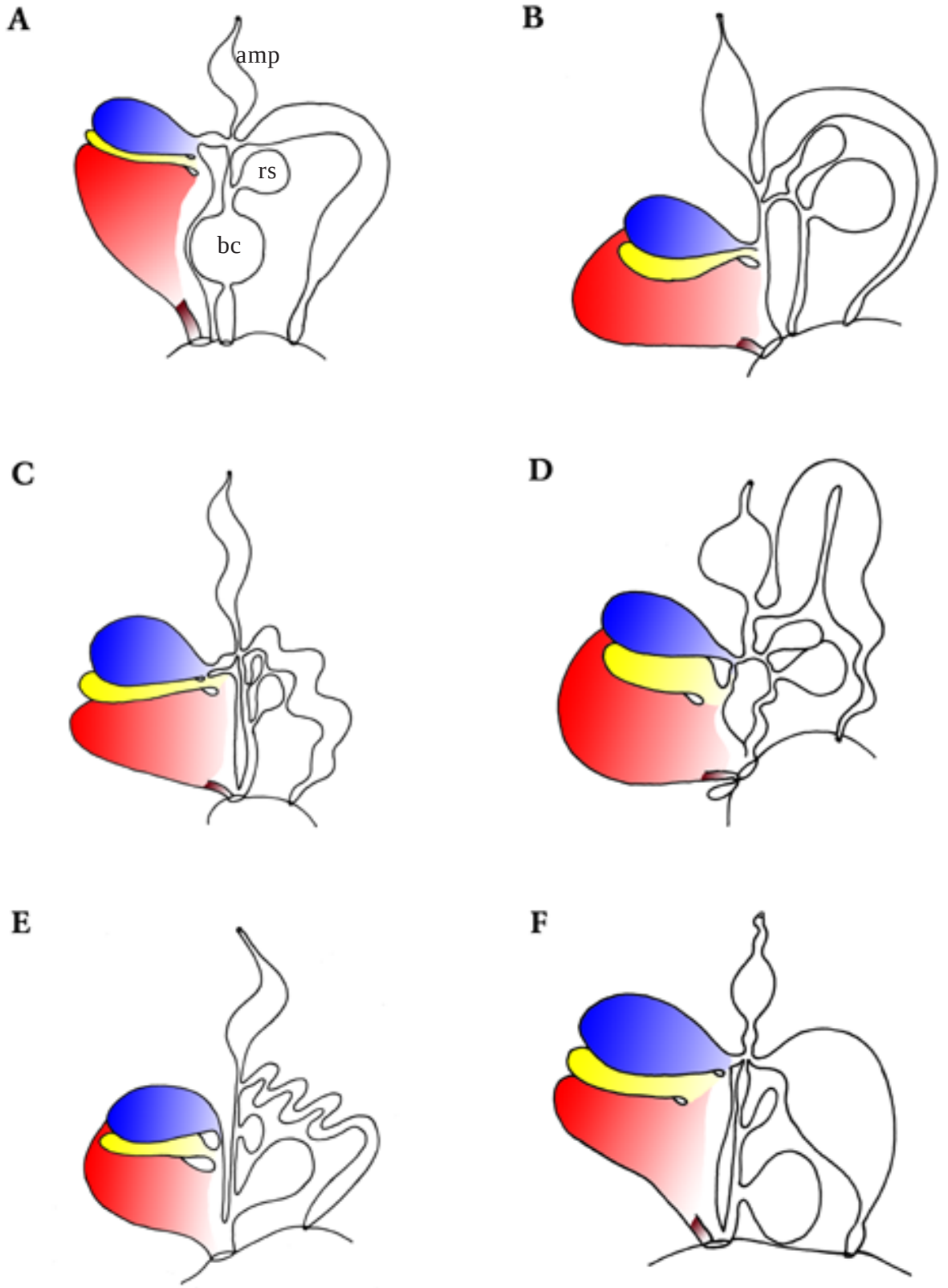

Figure 13: Schematic outlines of the anterior reproductive systems of several Anthobranchia (Nudibranchia). A: *Acanthodoris pilosa*; B: *Adalaria proxima*; C: *Chromodoris magnifica*; D: *Dendrodoris nigra*; E: *Onchidoris bilamellata*; F: *Polycera quadrilineata*.

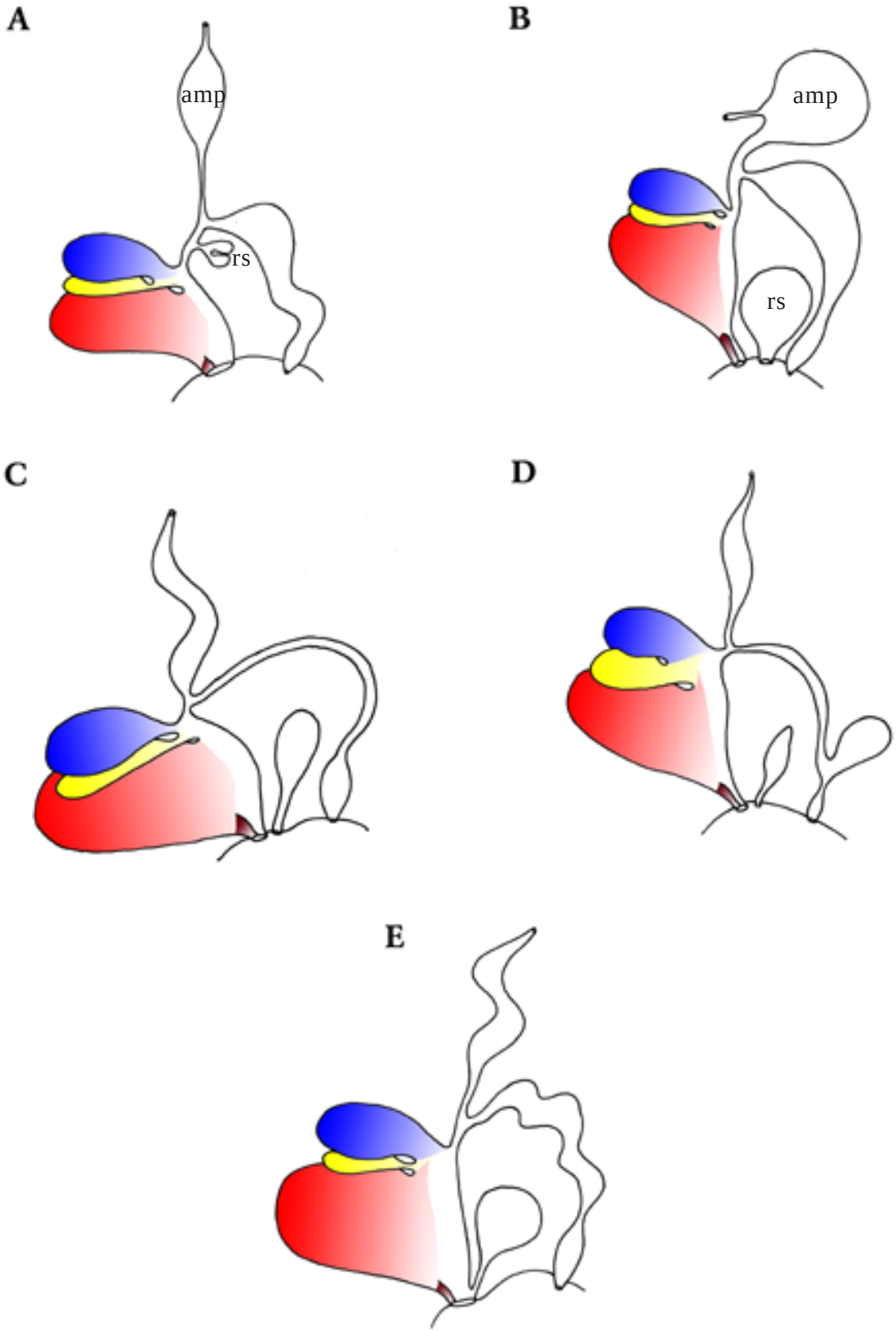

Figure 14: Schematic outlines of the anterior reproductive systems of several Cladobranchia (Nudibranchia). A: *Lomanotus vermiformis*; B: *Tritonia plebeia*; C: *Dermatobranchus semistriatus*; D: *Eubranchus exiguus*; E: *Flabellina gracilis*.

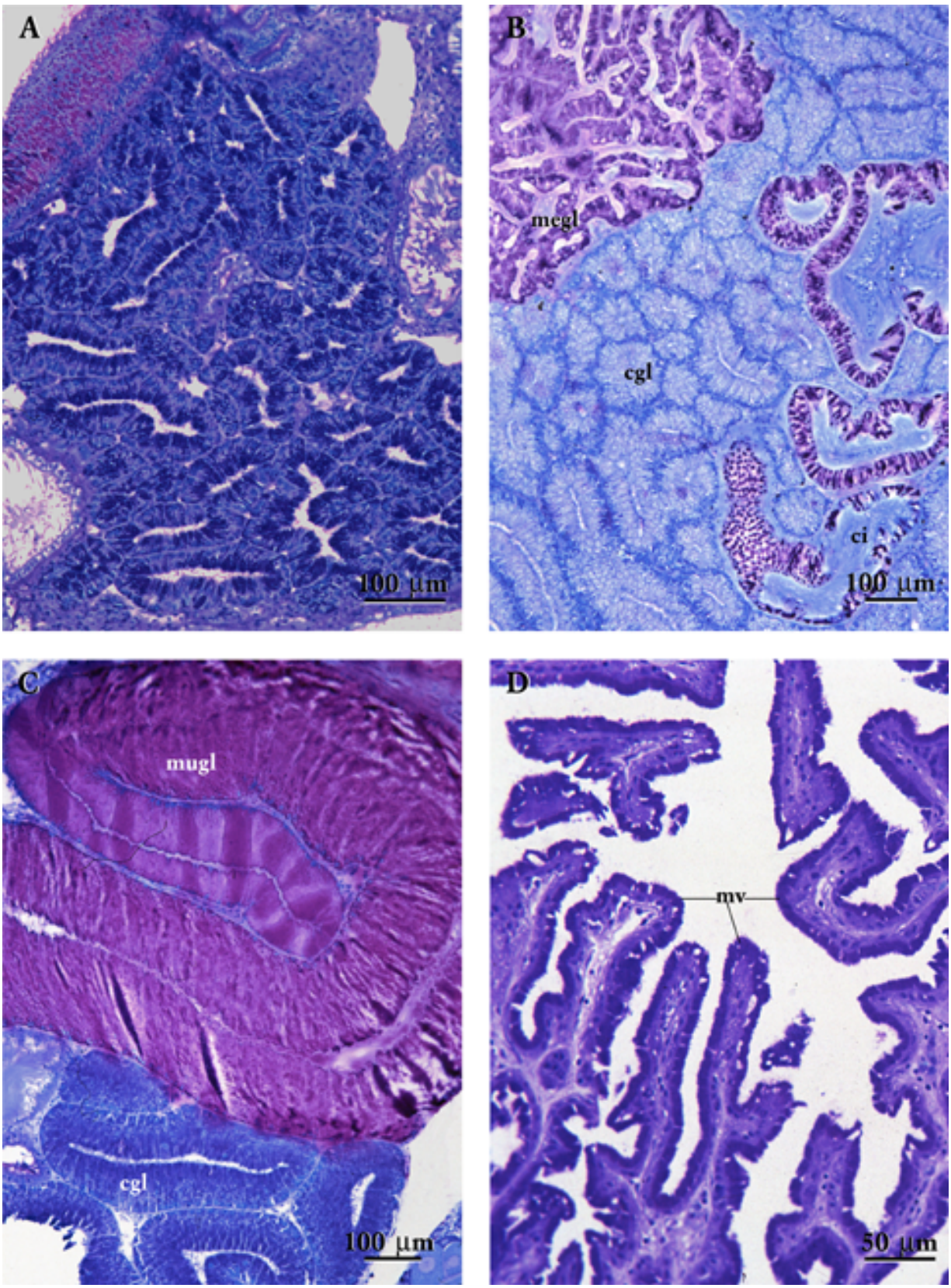

Figure 15: Histological sections through the nidamental glands and accessory gland of several Anthobranchia (Nudibranchia). A: *Adalaria proxima* - capsule gland; B: *Dendrodoris nigra* - capsule and membrane gland; C: *Polycera quadrilineata* - capsule and mucous gland; D: *Dendrodoris nigra* - vestibular gland.

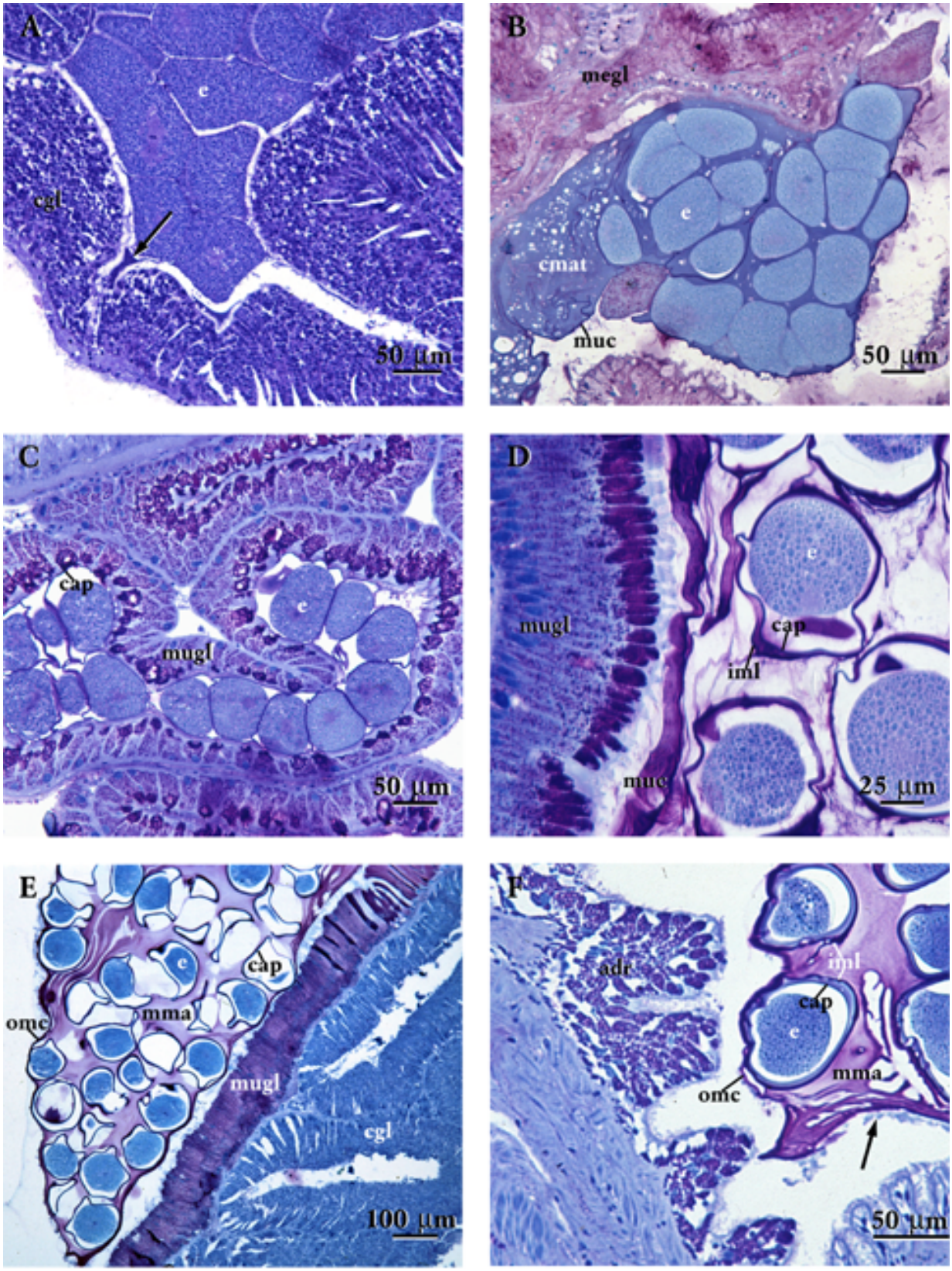

Figure 16: Histological sections through the nidamental glands of two anthobranch species preserved during spawning. A: *Chromodoris magnifica* - capsule gland (arrow indicates capsule material); B: *Chromodoris magnifica* - membrane gland; C: *Acanthodoris pilosa* - mucous gland (proximal part); D: *Acanthodoris pilosa* - mucous gland (distal part); E: *Chromodoris magnifica* - mucous gland (distal part); F: *Chromodoris magnifica* - adhesive region (arrow indicates secretory material).

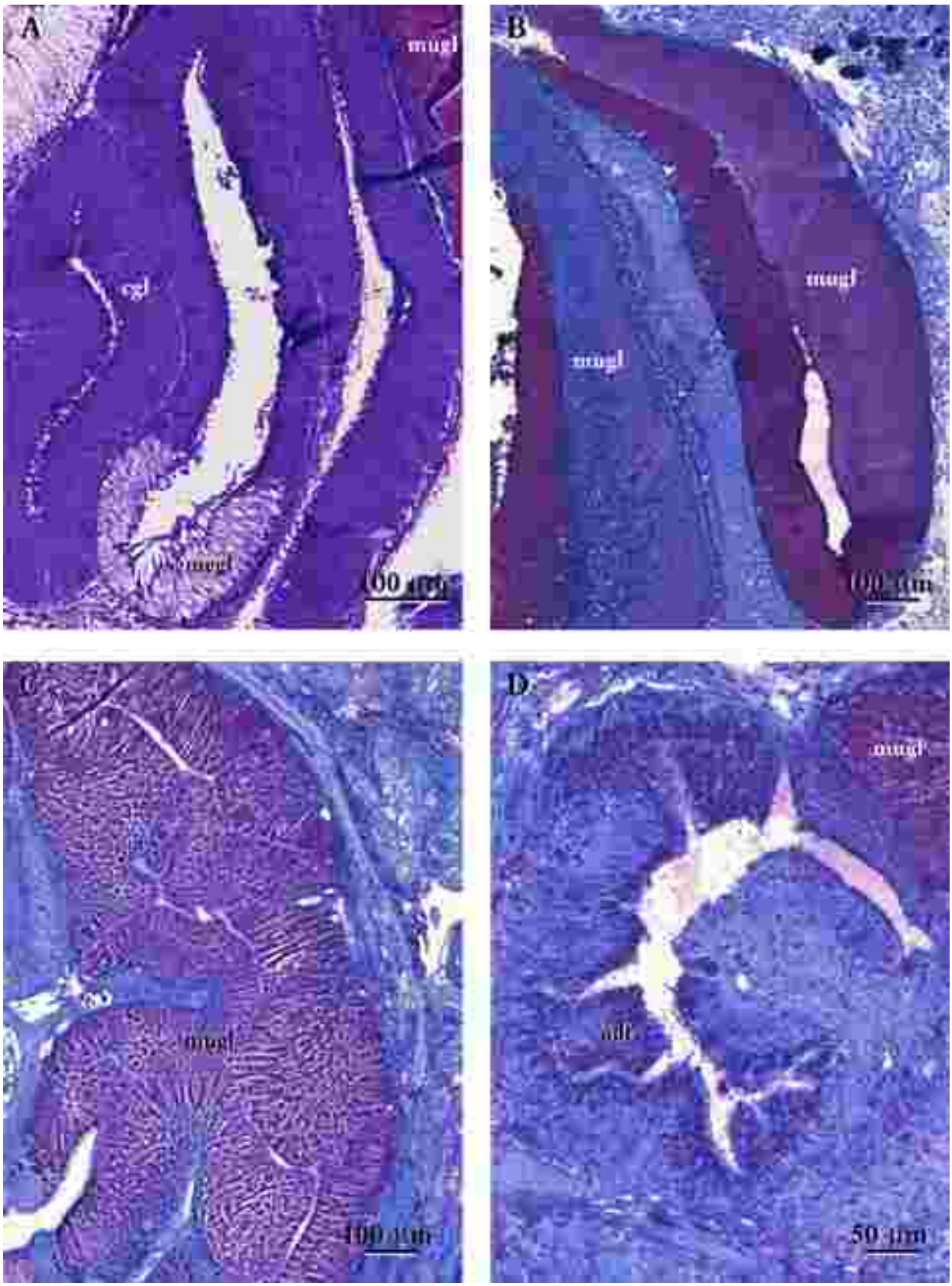

Figure 17: Histological sections through the nidamental glands of several Cladobranchia (Nudibranchia). A: *Lomanotus vermiformis* - capsule and membrane gland; B: *Flabellina gracilis* - mucous gland; C: *Dermatobranchus semistriatus* - mucous gland; D: *Dermatobranchus semistriatus* - adhesive region.

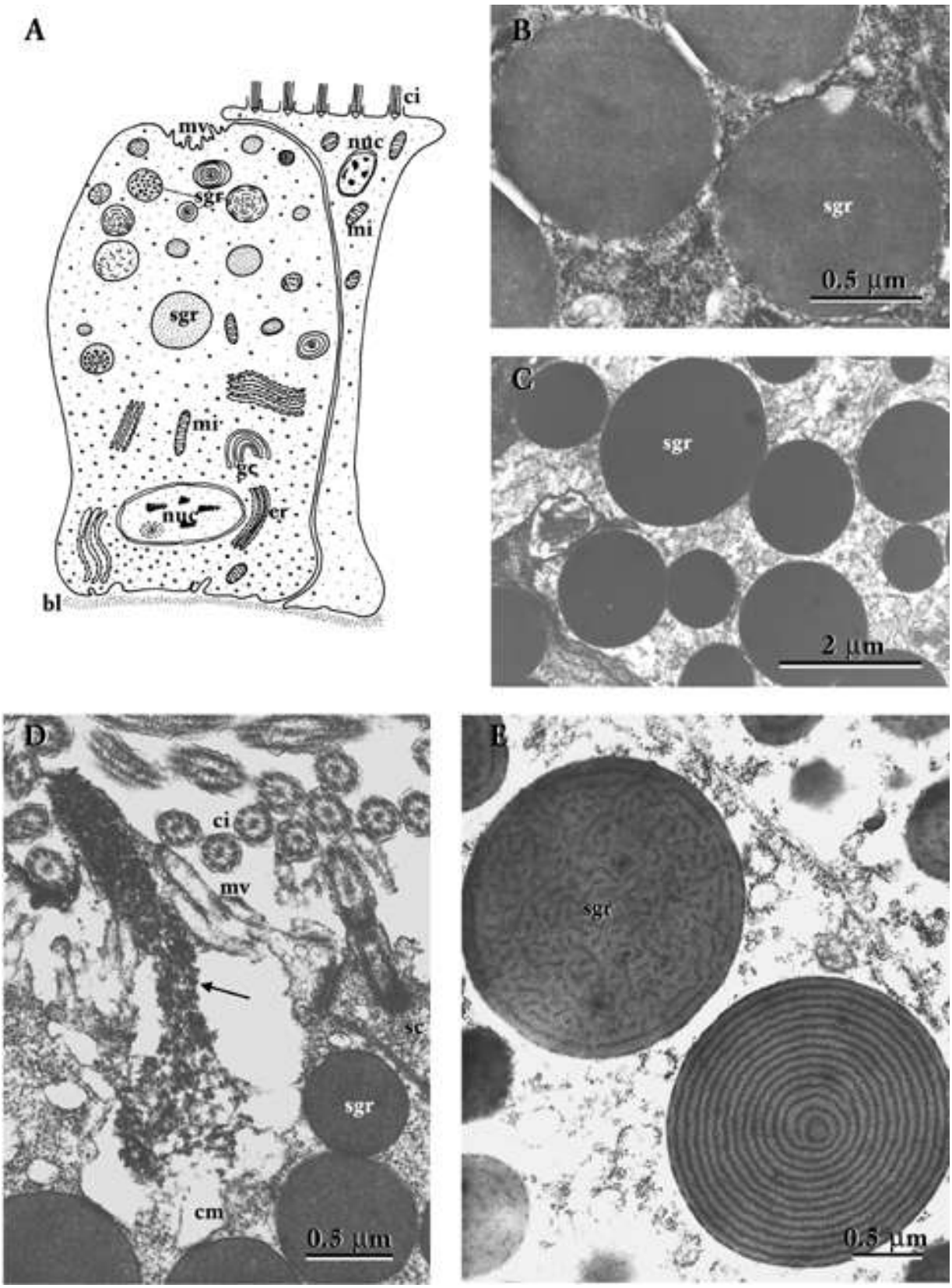

Figure 18: Ultrastructure of the capsule glands of several Nudibranchia and one "Cephalaspidea s. l.". A: Schematic outline of a glandular and a supporting cell; B: *Philinopsis gardineri* - secretory granules; C: *Dermatobranchus semistriatus* - secretory granules; D: *Eubranchus exiguus* - ejection of secretory material (see arrow); *Acanthodoris pilosa* - secretory granules.

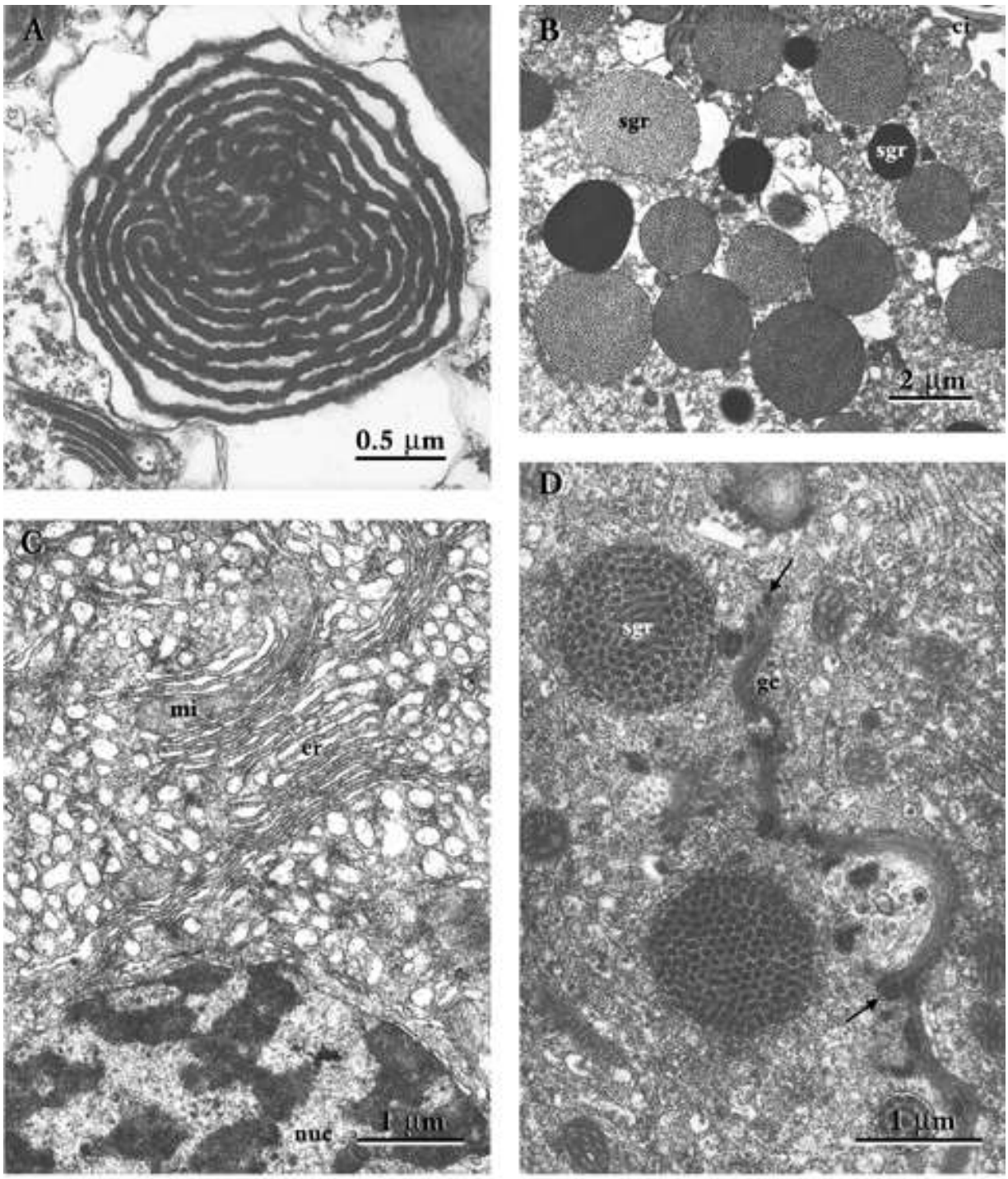

Figure 19: Ultrastructure of the capsule glands of several Nudibranchia. A: *Polycera quadrilineata* - nascent secretory granule; B: *Tritonia plebeia* - nascent and mature secretory granules; C: *Eubranchus exiguus* - nucleus and endoplasmic reticulum in basal part of glandular cell; D: *Tritonia plebeia* - Golgi complexes with areas of active secretion (see arrows) in basal part of glandular cell.

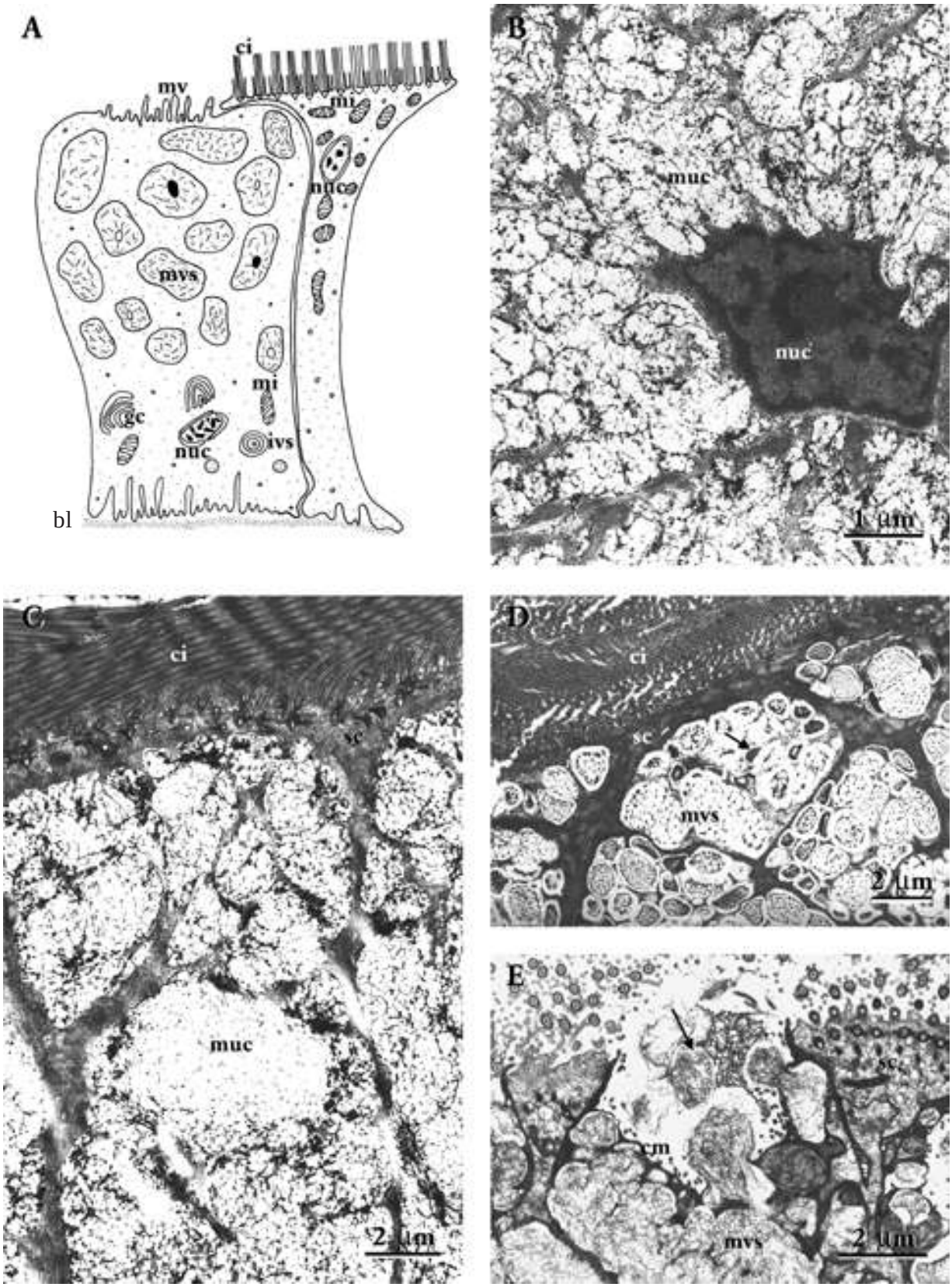

Figure 20: Ultrastructure of the membrane glands of several Nudibranchia and one Sacoglossa. A: Schematic outline of a glandular and a supporting cell; B: *Thuridilla hopei* - distal part of glandular cell; C: *Polycera quadrilineata* - apical part of epithelium; D: *Tritonia plebeia* - apical part of epithelium; D: *Flabellina gracilis* - apical part of epithelium (arrow indicates secretory material in gland lumen).

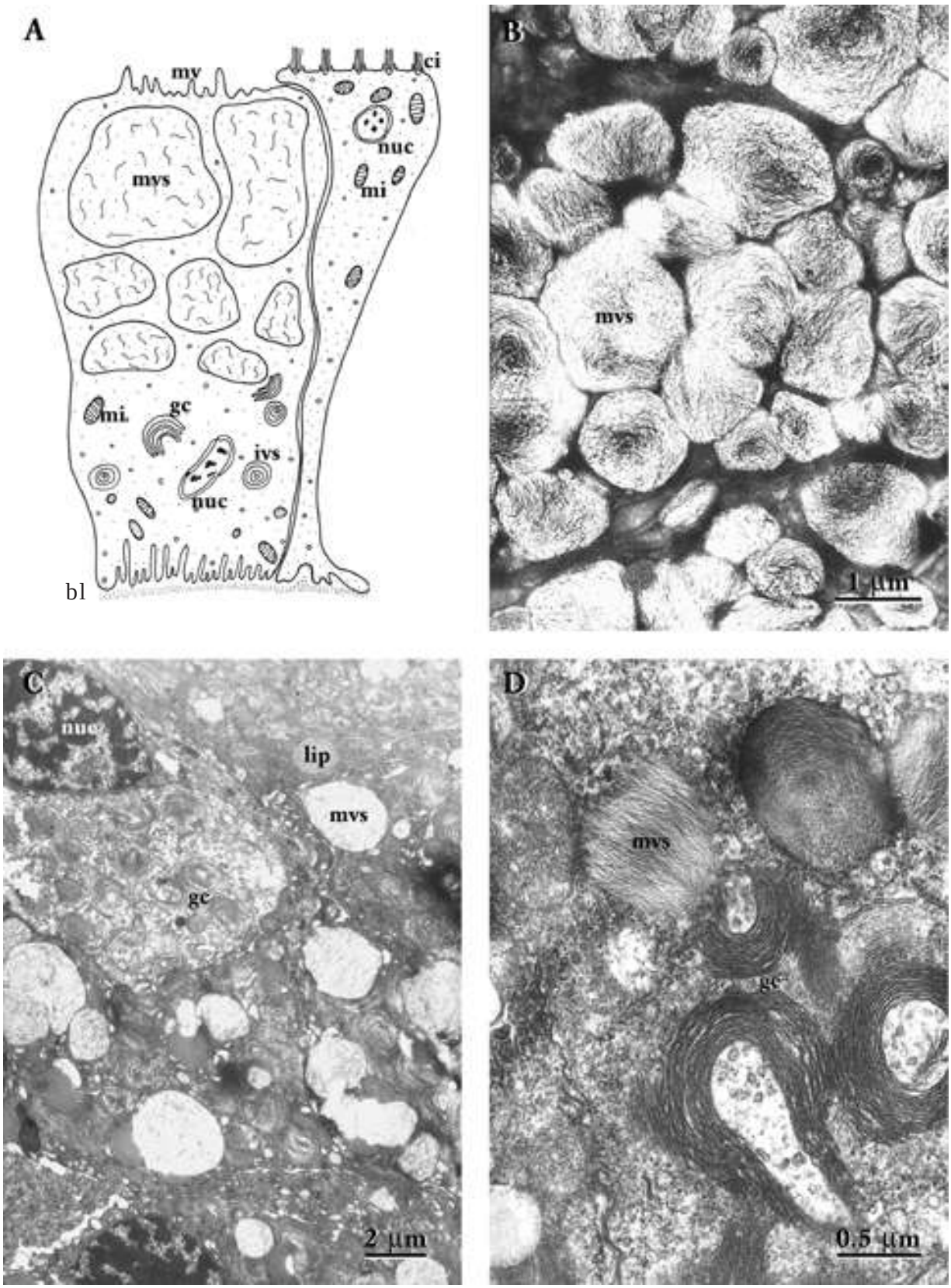

Figure 21: Ultrastructure of the mucous glands of several Nudibranchia. A: Schematic outline of a mucous gland cell and a supporting cell; B: *Tritonia plebeia* - mucous vesicles in mid part of glandular cell; C: *Acanthodoris pilosa* - basal part of glandular cell; D: *Tritonia plebeia* - Golgi complexes and nascent secretory vesicles in basal part of glandular cell.

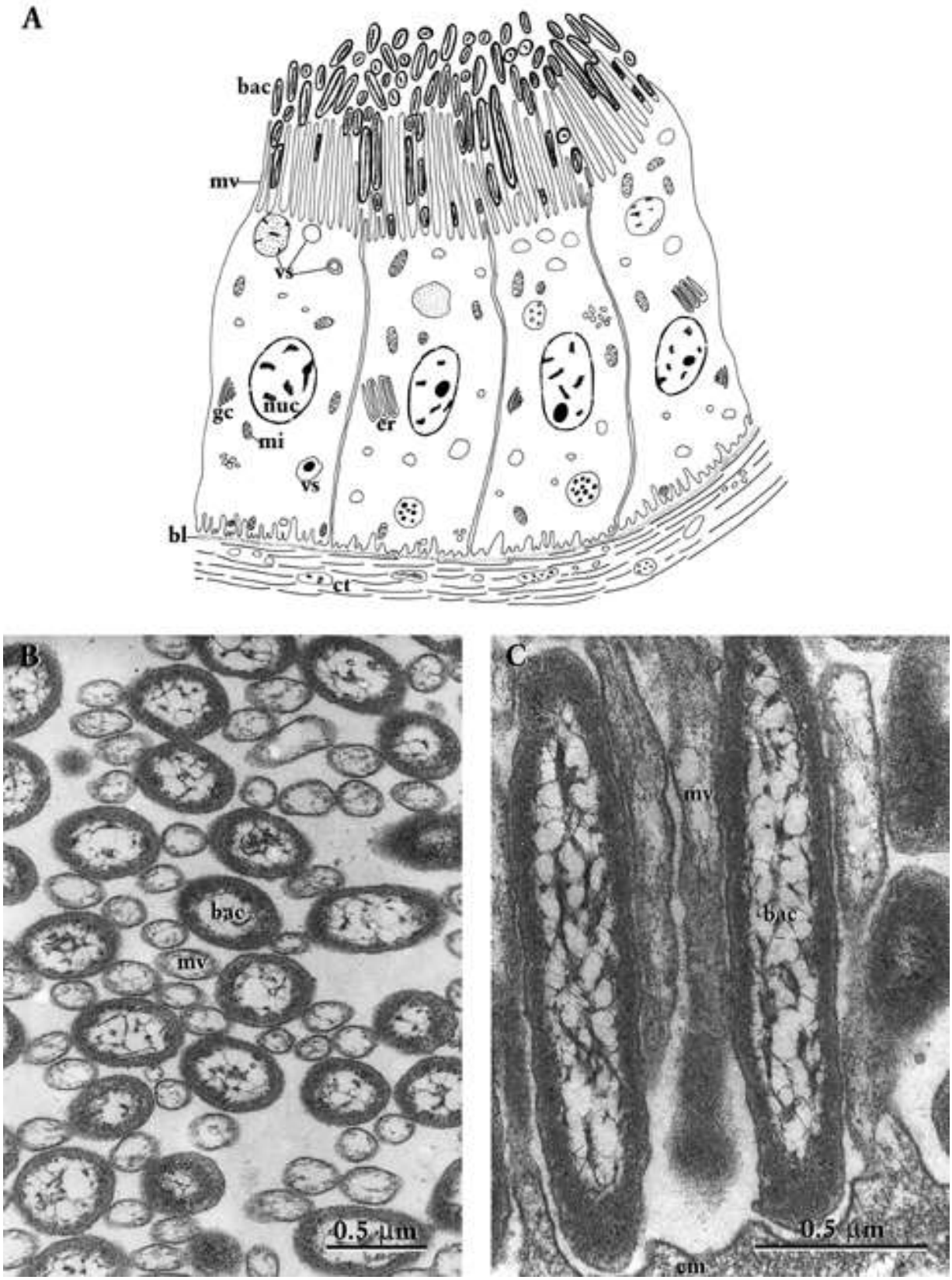

Figure 22: Ultrastructure of the vestibular gland of *Dendrodoris nigra*. A: Schematic outline of the epithelium; B: Cross section of bacteria in the gland lumen; C: Longitudinal section of bacteria in between apical microvilli.

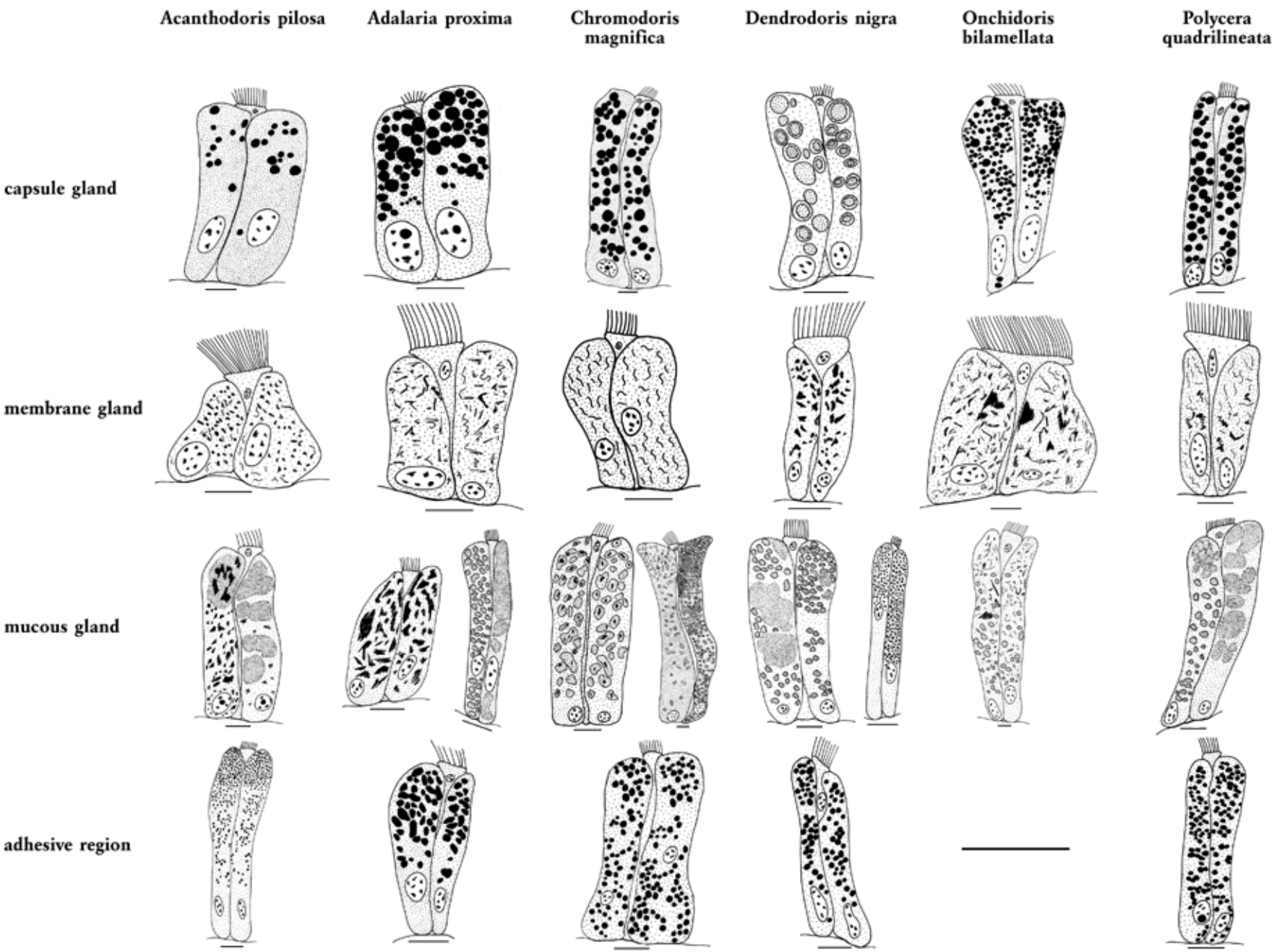

Table 6: Comparative schematic drawings of the major cell types of the nidamental glands of several Anthobranchia (Nudibranchia); scale bars = 10 µm.

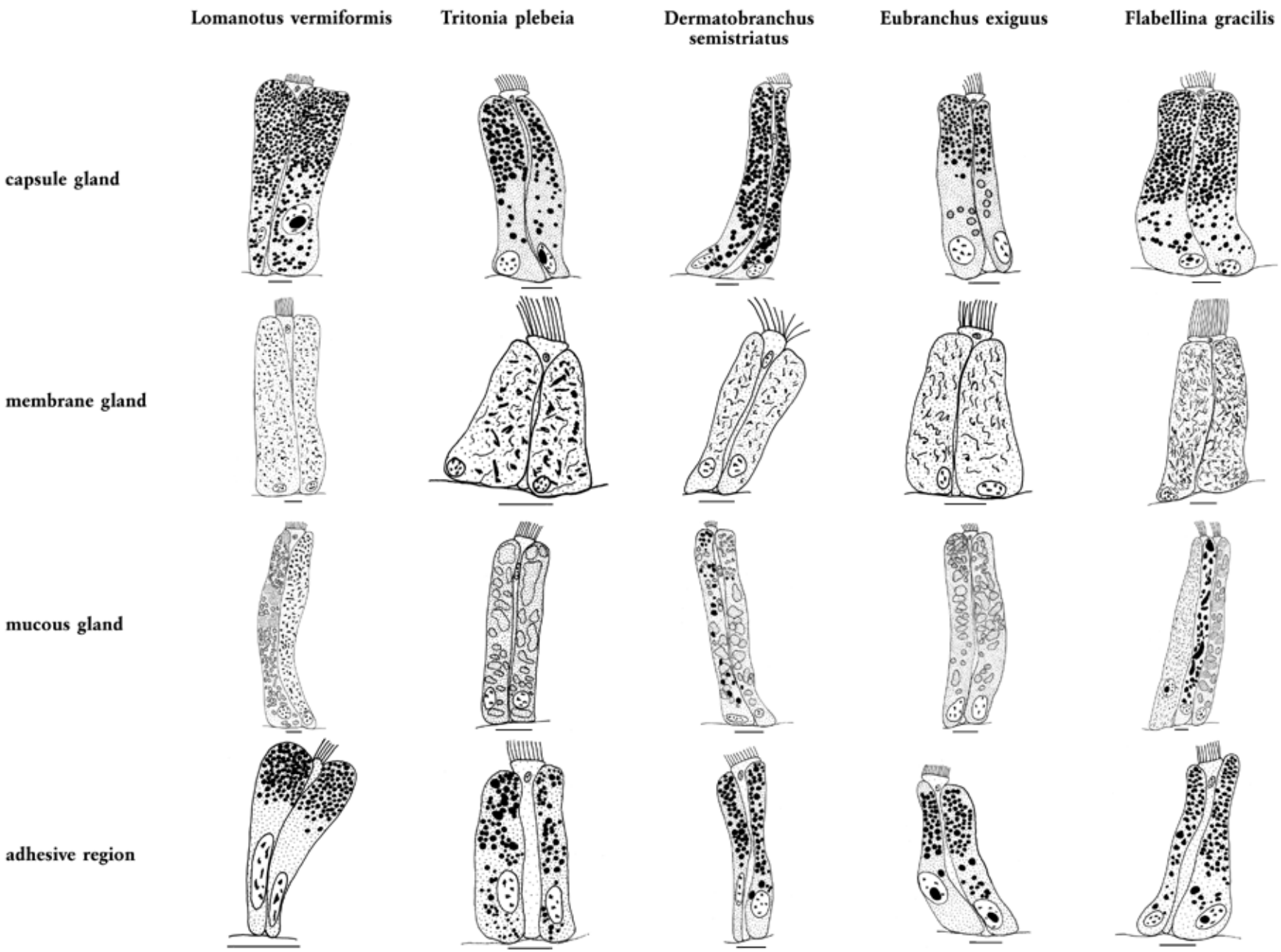

Table 7: Comparative schematic drawings of the major cell types of the nidamental glands of several Cladobranchia (Nudibranchia); scale bars = 10 µm.

3.8 The anterior reproductive systems of some Pyramidelloidea, Pulmonata and Gymnomorpha

The gastropod species investigated here belong to the Gymnomorpha (*Onchidella celtica*), the Pulmonata (*Biomphalaria glabrata*, *Laemodonta octanfracta* and *Lymnaea stagnalis*) and to the Pyramidelloidea (*Pyramidella sulcata*). For schematic outlines of the anterior reproductive systems refer to fig. 23. While *Pyramidella sulcata* and *Laemodonta octanfracta* exhibit monaulic reproductive systems the other three species have androdiaulic systems. In these species the vas deferens and accessory prostate glands are separated from the common oviduct and vagina. Because of incomplete sections the course of vas deferens and vaginal duct in *Lymnaea stagnalis* could not be followed and have therefore been omitted in the schematic drawings.

The ampullae are tube-like structures. In *Biomphalaria glabrata* the ampulla could not be reconstructed completely due to incomplete sections. Except for *Pyramidella sulcata*, which has a receptaculum seminis branching off the proximal spermoviduct, all other species do not possess receptacula but show bursae copulatrices discharging into the distal spermoviduct or distal oviduct respectively.

The nidamental gland mass is divided into three distinct parts (as has been described for all other gastropod species investigated here). Attached to the proximal partof the spermoviduct or oviduct respectively an albumen gland is found. This consists of two separated lobes in *Onchidella celtica* but comprises one compact glandular mass in the other species. Following the albumen gland is a membrane gland, which typically is a very small glandular part compared to the mucous gland. The latter comprises the largest part of the glandular system.

Comparative schematic drawings of the major cell types of the nidamental glands are shown in table 8.

3.8.1 Histology of the nidamental glands of *Pyramidella sulcata*

ALBUMEN GLAND (table 8): This organ is composed of densely packed tubules in *Pyramidella sulcata*. Highly columnar glandular cells are alternating with ciliated supporting cells. The glandular cells contain numerous light blue-staining secretory vesicles of

different shape (approximately up to 7.0 µm in diameter). Large, elliptically-shaped nuclei are located in a medial to basal position. The duct leading to the spermoviduct at the distal end of the albumen gland is lined by glandular cells containing smaller darker blue-staining secretory vesicles. The alternating supporting cells bear longer cilia here than those lying further proximally. They always contain an apically lying nucleus.

MEMBRANE GLAND (table 8): The membrane gland is lined by columnar glandular cells alternating with prominent supporting cells. The glandular cells are filled with small mucous droplets staining dark violet. Pycnotic nuclei are found basally. The supporting cells have apical extensions partly covering the apices of the adjacent glandular cells. They contain apical nuclei and bear long cilia.

MUCOUS GLAND (table 8): The mucous gland exhibits a large tube with a central lumen. The lining epithelium is folded internally. On the one side of the lumen the glandular cells contain dense heterogeneous mucous fibres staining pink to violet. Single cells cannot be observed. The other side consists of non-glandular ciliated cells. Single cells could not be observed in the glandular mass of the mucous gland. The mucous gland discharges directly into the genital aperture.

3.8.2 Histology of the nidamental glands of the Pulmonata

ALBUMEN GLAND (table 8; fig. 24A): The albumen glands of the pulmonate species investigated are tubular organs. The tubules are small and densely packed. The glandular cells are cuboidal in shape in *Laemodonata octanfracta* and columnar in *Biomphalaria glabrata* and *Lymnaea stagnalis*. They contain secretory vesicles of different sizes, shapes and structures. They are generally round to elliptically shaped and can get very large (in *Laemodonta octanfracta* up to 8.0 µm in diameter; in *Lymnaea stagnalis* up to 9.5 µm and in *Biomphalaria glabrata* up to 10.0 µm). The vesicles have amorphous contents in *Laemodonta octanfracta* and *Biomphalaria glabrata*, whereas they appear homogeneous in *Lymnaea stagnalis*. In *Laemodonta octanfracta* the vesicles have a similar appearance to those found in *Phyllaplysia taylori*: a heterogeneous centre is surrounded by a homogeneously lighter staining rim. *Biomphalaria glabrata* possesses vesicles which have light to dark staining contents of irregular shape. The general staining properties are light to dark blue; the

vesicles stain lightest in *Lymnaea stagnalis*. The nuclei found basally in the glandular cells are large and conspicuous in *Biomphalaria glabrata* and *Lymnaea stagnalis*. They contain prominent nucleoli. In *Laemodonta octanfracta* the nuclei are small and nucleoli are not visible. Alternating supporting cells are generally small and wedge-shaped. They bear short cilia. In *Lymnaea stagnalis* they have a triangular apex. An apically lying nucleus is only visible in the supporting cells of *Laemodonta octanfracta*. In the lumen of the glands of the latter species and of *Lymnaea stagnalis* blue staining secretions are visible. The albumen gland discharges via a small ciliated glandular (*Lymnaea stagnalis*) or non- glandular duct (*Biomphalaria glabrata*) into the oviduct. In *Laemodonta octanfracta* the albumen gland has a continuous transition to the membrane gland. At this transition small prismatic cells are present containing small round, dark blue staining granules. The supporting cells in this area are heavily ciliated.

MEMBRANE GLAND (table 8; fig. 24B,C): The membrane gland consists of few narrow coils in *Laemodonta octanfracta* (fig. 24B) and *Biomphalaria glabrata*, whereas it comprises a relatively large tube in *Lymnaea stagnalis* (fig. 24C). Here numerous glandular outpockings originate from a central duct. The glandular cells are bottle-shaped in *Laemodonta octanfracta*, and columnar in the other two species. The secretory contents are of heterogeneous structure (*Laemodonta octanfracta*) or in the form of small mucous droplets (*Biomphalaria glabrata* and *Lymnaea stagnalis*). The staining properties range from pink to red or violet. Small nuclei are generally located in the basal halves of the glandular cells. The conspicuous supporting cells are wedge-shaped in *Laemodonta octanfracta* and *Biomphalaria glabrata* and have a broad triangular apex in *Lymnaea stagnalis*. They bear long cilia. An apical nucleus is only visible in *Laemodonta octanfracta* and *Lymnaea stagnalis*. The membrane gland shows a continuous transition to the mucous gland in *Biomphalaria glabrata*, whereas in the other two species it discharges into the oviduct (*Lymnaea stagnalis*) or spermoviduct (*Laemodonta octanfracta*).

MUCOUS GLAND (table 8; fig. 24B, D): The mucous gland is tubular in all three pulmonate species investigated. In *Laemodonta octanfracta* it is internally folded, but the epithelium does not show any active glandular cells in the studied animal. The cells are almost empty and are hardly stained with toluidine blue (fig. 24B). Only few small dark blue staining droplets are found scattered throughout the cells and very few non-staining

vacuoles containing small secretion droplets occur basally. In *Biomphalaria glabrata* and *Lymnaea stagnalis* the mucous gland can be divided into two distinct parts. In the former species the proximal part forms a small tube and the distal part comprises a large, widely coiled glandular mass. The lumina are small throughout the gland. The glandular cells are highly columnar and filled with mucous vesicles of different sizes as well as filamentous mucus. The mucus stains violet in various shades. Interspersed are flame-like cells containing small, densely-packed secretory droplets which stain dark violet. This cell type is also found in *Lymnaea stagnalis* (see arrwow in fig. 24D). In this species the proximal part of the mucous gland is narrowly coiled. Lumina are hardly visible here. The highly columnar glandular cells are filled with densely packed secretory vesicles staining pink. Further distally the glandular epithelium forms large parallel lamellae composed of different cell types with different structured mucous contents (fig. 24D). In some cells the mucus forms densely packed vesicles mainly dissolved thus filling the cells with homogeneous mucous patches. The mucus stains violet here. Other cells contain heterogeneous mucous fibres staining light blue to grey. All glandular cells in all three species contain small nuclei located basally. The supporting cells are wedge-shaped and bear short cilia. Nuclei are not visible in the supporting cells.

ADHESIVE REGION (table 8, fig. 24B): An epithelium similar to the glandular epithelia described for the distal oviducts of various opisthobranch species is also found in *Laemodonta octanfracta* (fig. 24B). The glandular cells are prismatic and contain numerous small round secretory granules staining dark violet. They alternate with supporting cells bearing short cilia.

3.8.3 Histology of the nidamental glands of *Onchidella celtica*

ALBUMEN GLAND (table 8): In *Onchidella celtica* the albumen gland is composed of two separate lobes each discharging into the oviduct via a separate duct. The columnar glandular cells contain large round to elliptically-shaped secretory vescicles staining light to dark blue. In some vescicles small darker staining substructures are visible with higher magnification. The vesicles can get up to 10.0 µm in diameter. Large nuclei are found

basally, sometimes a prominent nucleolus is visible. In the alternating supporting cells the nuclei are located apically. Short cilia are present.

MEMBRANE GLAND (table 8): This glandular part is tubular and comprises narrow coils. The glandular cells are columnar, slender and have a narrow apex. They contain irregular, dark violet staining mucous droplets embedded in homogeneously violet-staining cytoplasm. Pycnotic nuclei are located basally. The supporting cells bear extremely long cilia and contain an apically lying nucleus.

MUCOUS GLAND (table 8): The tubular organ is widely coiled and has large lumina. The glandular epithelium consists of highly columnar cells filled with small mucous vesicles staining pink to dark violet. In cells where the vesicles have fused or are dissolved the cells are filled with homogeneous mucus. In the more proximal part of the mucous gland, where the glandular cells are usually filled with large dark violet staining mucous droplets, the supporting cells bear longer cilia than further distally. Nuclei are always pycnotic and found basally. The supporting cells have lateral extensions apically, partly covering the apices of the adjacent glandular cells. Nuclei lie apically.

3.8.4 Discussion

The Pyramidelloidea, Pulmonata and Gymnomorpha investigated show monaulic and diaulic genital systems. The general structure of the nidamental glandular system is the same as in the other species investigated: three glandular parts are present, an albumen gland, a membrane gland and a mucous gland. Additionally, *Laemodonta octanfracta* possesses glandular tissue in the distal oviduct.

The albumen gland is always a compact organ lined by a glandular epithelium that contains large secretory vesicles. Membrane gland and mucous gland can be differentiated according to their location, size and structure. The membrane gland is small and highly convoluted, whereas the distally lying mucous gland is a large, widely coiled organ. The glandular cells in the membrane gland are usually smaller than those of the mucous gland and the supporting cells bear longer cilia in the former. In the mucous gland different cell types occur with different structure and staining properties.

Histological and histochemical investigations of the reproductive system in pyramidelloids and euthyneurans have been undertaken already some decades ago. Different authors have described different glandular parts in the female part of the genital system of various species. Since the description of the most proximal glandular part (albumen gland) is very consistent throughout the literature, special attention is drawn here to the differentiation within the membrane and mucous glands.

Fretter & Graham (1949) gave a very detailed description of the anatomy and histology of the reproductive organs in pyramidellid species based on *Odostomia* and *Chrysallida*. They distinguished between an upper and a lower mucous gland, which appear to be identical to the membrane and the mucous gland, respectively, that I have found in *Pyramidella sulcata*. Different species of the genus *Laemodonta* are reported to possess a posterior and an anterior mucous gland (viewed in relation to the genital opening) (Martins 1996). From the descriptions by Martins it seems possible that his posterior mucous gland accords with what I have found to be the membrane gland in *Laemodonta octanfracta*. The anterior mucous gland corresponds with the mucous gland. Morton (1955) and Berry et al. (1967) also found a division into posterior and anterior mucous glands in other species of the Ellobiidae.

The pallial gland Martins (1996) described for *Laemodonta cubensis* was not found in *Laemodonta octanfracta*. Morton (1955) described a pallial gland in *Carychium* independently from the reproductive organs, whereas in *Laemodonta cubensis* this gland is shown to be attached to the genital aperture (Martins 1996). The function of this gland is unknown.

Since the studies of pulmonates have a long tradition, the terminology for different parts of the reproductive system differs slightly from what is used in other euthyneurans. Bretschneider (1948) divided the mucous gland mass in *Lymnaea stagnalis* into a proximal "pars contorta", a "glandula nidamentaria accessoria" and a "pars nidamentaria". Holm (1946) used the term "uterus" for the pars contorta in *Lymnaea stagnalis appressa*. The glandula nidamentaria accessoria of Bretschneider (1948) was later called "muciparous gland" by Plesch et al. (1971), whereas the authors termed the pars nidamentaria "oöthecal gland", respectively. When the findings of these authors (Holm 1946; Bretschneider 1948; Plesch et al. 1971) are compared to my data on *Lymnaea stagnalis* it seems probable that the pars contorta or uterus accords with the membrane gland I have described. A differentiation between a pars nidamentaria accessoria (muciparous gland) and a pars

nidamentaria (oöthecal gland) was not found by me. Nevertheless, according to the descriptions by Plesch et al. (1971) I assume that the muciparous gland complies with the proximal part of the mucous gland I have observed in *Lymnaea stagnalis*. Plesch et al. reported of twelve different cell types in the various glandular parts of the oviduct of *Lymnaea stagnalis*. Their description is based on an extensive histochemical investigation of these glands. A similar histochemical study was performed by de Jong Brink (1969) for *Biomphalaria glabrata*. She found ten cell types in the albumen, muciparous and oöthecal glands of this species. She also investigated the ultrastructure of the different cell types. The secretory vesicles de Jong Brink found in the albumen gland are very similar in their fine structure to the ones I have described for *Aplysia puntata*, and her results on the ultrastructure of the different cell types of the muciparous gland and the oöthecal gland correspond to my descriptions of the fine structure of the glandular cells in the membrane and mucous glands, respectively, in various opisthobranch species. Weiss & Wägele (1998) investigated the histology of the organ systems of various onchidiid species, among them *Onchidella celtica*. Their findings regarding the structure of the albumen gland agree with the present data, but the authors did not distinguish between a membrane gland and a mucous gland in the distal part of the oviduct of this species as has been shown here. Fretter (1943) described different glandular pouches to be present in *Onchidella celtica*, but she failed to differentiate these further with regards to their different histological resemblances.

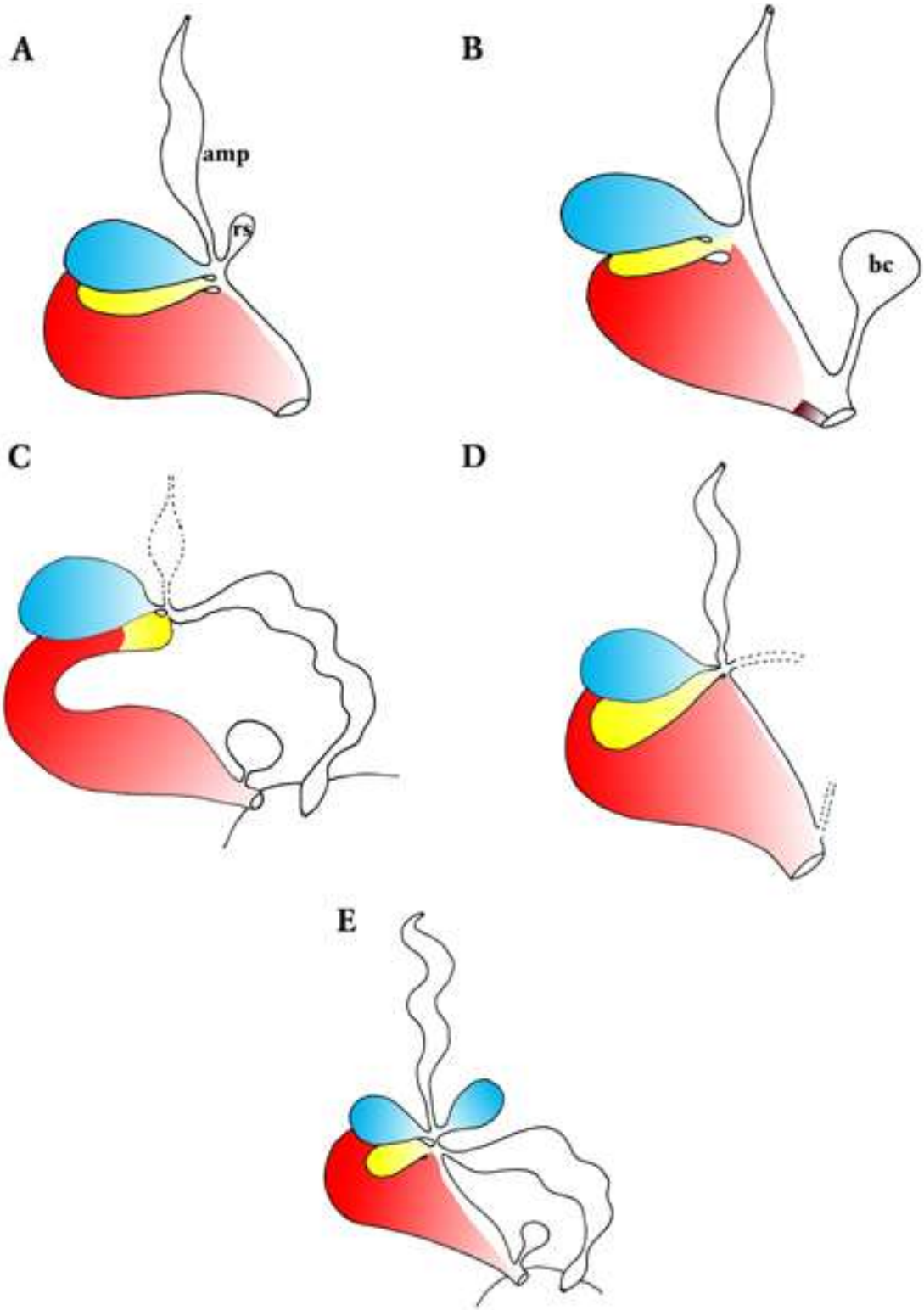

Figure 23: Schematic outlines of the anterior reproductive systems of several Pyramidelloidea, Pulmonata and Gymnomorpha. A: *Pyramidella sulcata*; B: *Laemodonta octanfracta*; C: *Biomphalaria glabrata*; D: *Lymnaea stagnalis*; E: *Onchidella celtica*.

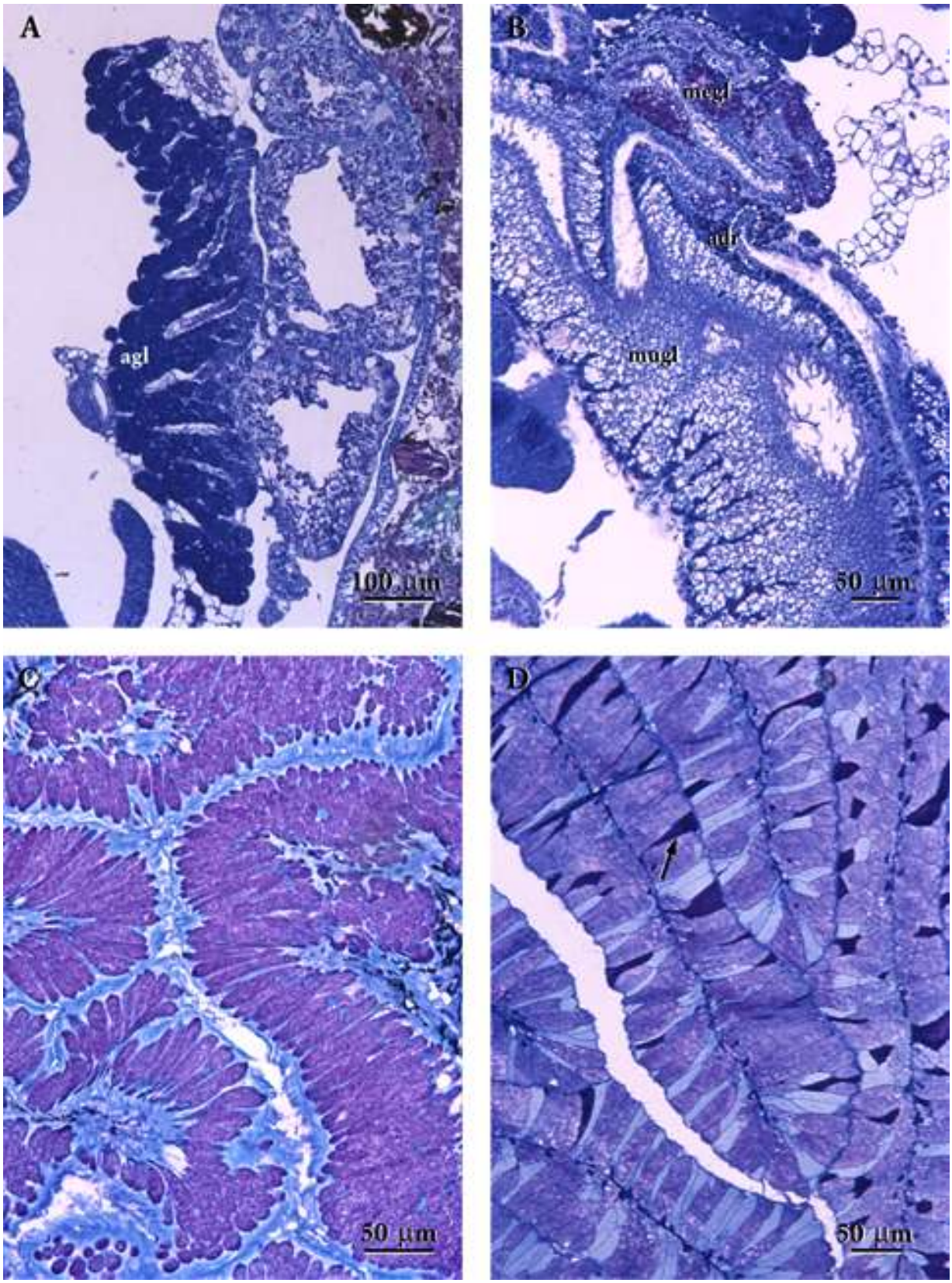

Figure 24: Histological sections through the nidamental glands of several Pulmonata. A: *Laemodonta octanfracta* - albumen gland; B: *Laemodonta octanfracta* - membrane gland, mucous gland and adhesive region; C: *Lymnaea stagnalis* - membrane gland; D: *Lymnaea stagnalis* - mucous gland.

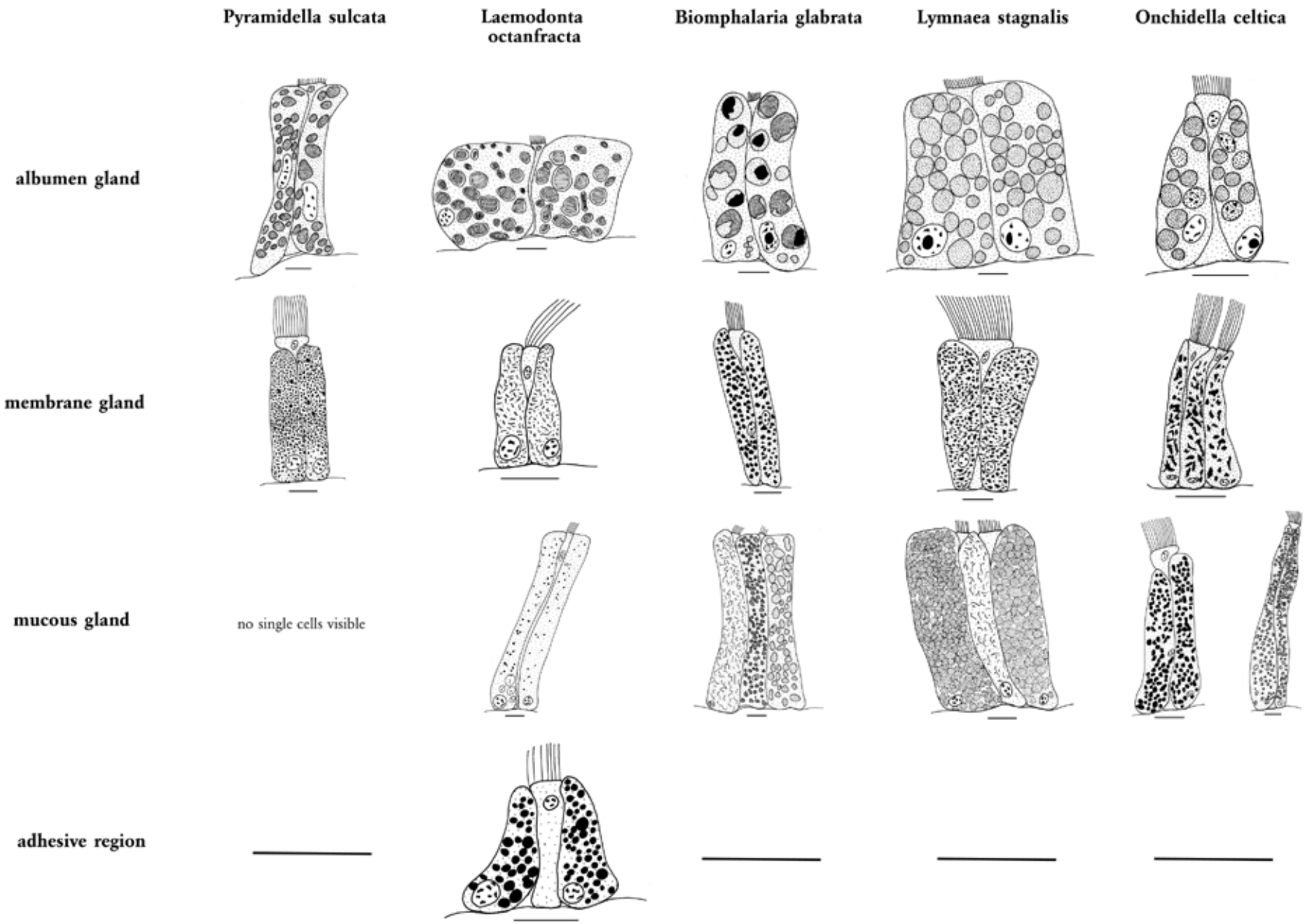

Table 8: Comparative schematic drawings of the major cell types of the nidamental glands of several Pyramidelloidea, Pulmonata and Gymnomorpha; scale bars = 10 µm.

3.9 The egg masses of several opisthobranch and one pulmonate species

The egg masses of nineteen opisthobranch species belonging to the ""Cephalaspidea s. l.", Anaspidea, Sacoglossa and Nudibranchia have been investigated. Additionally, one egg mass of *Radix peregra*, a pulmonate species, has been studied.

The overall fine structure of the egg masses is rather similar and complies with the description given in the 'terminology'-chapter (3.1, pages 13/14). Differences are apparent in the number of embryos per capsule, the number of capsules per egg mass (not further investigated in this study), the presence or absence of albumen within the capsules, the thickness of the capsule, the fine structure of the capsule and the density and fine structure of the mucous layers.

In most cases the developmental stages of the embryos were not known exactly. A rough division into "early-", "veliger-" and "pre-hatch-" stages will be applied instead. In early developmental stages the larvae have not yet developed distinct cilia. These are present in the veliger stage and the pre-hatch stage. The latter is characterized by partly dissolved mucous layers. Since the egg capsules have collapsed during the processing for methacrylate histology and TEM (dehydration), capsule volumina have not been estimated. The thickness of the capsules was measured on TEM-photos if possible, but for those species not investigated ultrastructurally, the thickness was estimated from semi-thin cross sections.

All staining properties mentioned in the following refer to toluidine blue staining. For other histochemical staining reactions of the various structures on the egg masses see table 10 (page 131).

3.9.1 *Acteocina atrata*

Two egg masses (early and pre-hatch stages) have been investigated with semi-thin resin sections. The egg mass of *Acteocina atrata* has the shape of a dew berry attached to a long fine strand (fig. 25A). It exhibits a globular mass, very delicate in structure.

Light-microscopic structure of the egg mass:

The embryos are encapsulated singly by a dark violet staining membrane (approximately 1 µm thick). The membrane has a homogeneous structure. Within the capsule a flocculent mass of blue-staining albumen, partly closely attached to the membrane and appearing more dense there, is present in the early developmental stage (fig. 27A). In the pre-hatch stage the amount of albumen has decreased, only few small patches are still visible. The veliger larvae in the "pre-hatch"-egg mass show distinct shells. Adjacent to the outside of the membrane is a compact, homogeneously violet-staining inner mucous layer. The capsules are irregularly arranged within the egg mass and embedded in a filamentous mucous matrix. This matrix stains light violet. Few compact, dark violet staining mucous strings are running through the matrix. Mucous droplets are also sometimes visible within the matrix. On the outside of the whole egg mass it is surrounded by an outer mucous cover. In the early developmental stage this mucous cover is not very distinct, it seems to have dissolved in parts. In contrast, the outer mucous cover appears very distinct in the egg mass at the pre-hatch-stage. It is obvious from sections of this egg mass that the outer mucous cover is composed of three distinct layers (fig. 27C): the outermost layer (I) is approximately 2 µm thick and stains dark violet. The same holds true for the innermost layer (III), which is more compact though. In between both layers a thick layer (II, approximately 25 µm), staining light violet and appearing less dense, is present. The fine strand, to which the globular egg mass is attached, is composed of homogeneous mucus staining violet.

3.9.2 *Haminoea cymbalum*

One egg mass was investigated histologically. Before fixation the egg mass was sausage-shaped (fig. 26B) and had a light green colour similar to the colour of the animals. The embryos were in the veliger-stage of development.

Light-microscopic structure of the egg mass:

Generally one embryo is encapsulated in a distinct capsule (fig. 28A). In few capsules two veligers can be observed, these are usually smaller than those found singly in the capsules. The space between embryo and capsule seems to be empty. No albumen is found. The

capsule is about 1 µm thick. Two capsule layers are visible, the outer membrane staining darker violet than the inner capsule layer (fig. 27B). Adjacent to the membrane a homogeneously pink staining inner mucous layer of different thickness is present. On longitudinal sections the egg capsules appear in tube-like structures, tied together by fine mucous strands running through the mucous matrix in which the capsules are embedded (fig. 28A). The mucous strands stain dark violet and seem to be compact structures, whereas the mucous matrix is loosely arranged and stains light violet. Fine filamentous structures are visible within the matrix, as well as small mucous droplets. The outer mucous cover is composed of three layers: small, compact innermost and outermost layers having a dark violet colour, and in between those a broader layer (approximately 8 - 15 µm thick), composed of fine densely-packed parallel mucous layers which are staining lighter. On the outside of the egg mass amorphous masses of unidentified substances and few unidentified organisms are present.

3.9.3 *Chelidonura inornata*

The egg mass studied histologically was laid in the laboratory. It displayed an elongate, sausage-shaped mass and had a very delicate structure. The spawn was attached to a fine strand of mucus (fig. 25B). The egg mass was preserved at an early developmental stage.

Light-microscopic structure of the egg mass:

The embryos are encapsulated singly. Albumen is not visible. The capsules are approximately 1.5 µm thick and stain dark blue. Only one distinct layer is visible. Surrounding each capsule is a small violet staining inner mucous layer. On longitudinal sections tube-like arrangements of the capsules can be found. The light violet staining matrix is more dense than in *Haminoea cymbalum*, numerous interweaving, fine mucous strands can be observed. These stain darker violet and are more compact than the surrounding matrix. An outer mucous cover is hardly differentiated from the matrix (fig. 27D). At the outer edge of the egg mass the mucous strands just seem to be packed more densely.

3.9.4 *Philinopsis cyanea*

This egg mass was also obtained from an animal kept overnight in an aerated tank. Its shape is very similar to the egg mass of *Chelidonura inornata* (fig. 25B). The egg mass was preserved at an early developmental stage.

Light-microscopic structure of the egg mass:

The histology of the egg mass of *Philinopsis cyanea* is very similar to that of *Chelidonura inornata*. In contrast to the singly encapsulated embryos of the latter, *Philinopsis cyanea* encapsulates 2 to 5 embryos in one capsule. The capsules are very thin and hardly visible. They stain dark blue to violet. Albumen is not found within the capsules. The light violet-staining matrix is more dense than in *Chelidonura inornata* and less mucous strands are embedded in the mucous matrix. An outer mucous cover is also not differentiated.

3.9.5 *Aplysia punctata*

Aplysia punctata produces an egg mass exhibiting a tangled mass composed of one heavily convoluted ribbon (fig. 25C). The colour of the living mass is pink to light violet. Within the spawn ribbon the egg capsules are densely packed and enveloped by several, partly very stiff mucous layers. One egg mass was investigated with light as well as transmission electron microscopy. The embryos in the piece of spawn mass investigated with TEM were in an early veliger stage (no distinct cilia developed yet), those studied light-microscopically in a later developmental stage (cilia visible)

Light-microscopic structure of the egg mass:

1 to 3 embryos are encapsulated in a thin capsule, composed of one membrane layer, which stains dark violet. In the space between embryo and capsule few granular substances are visible (fig. 29A) These stain blue. They are probably residues of albumen in the capsules. The capsules are embedded in a compact matrix staining violet. A tube-like arrangement of the capsules cannot be observed in the present. The matrix appears filamentous in parts, partly small vacuole-like structures are visible (fig. 29A). Within these small dark violet staining mucous droplets can be observed. The outer mucous cover is a very distinct structure (up to 80 µm thick) and can be easily differentiated from the matrix (fig. 29A). It is composed of several mucous layers of different structure. With light

microscopy at least six different layers are visible. The outermost layer is the smallest. It stains dark blue and has a fuzzy appearance. Adjacent parts of the egg string seem to be attached to each other such, that the two adjacent outermost layers of the outer mucous cover are hooked together. The second layer of the outer mucous cover is composed of filamentous parallel layers of mucus. It stains pink. Adjacent is the third layer composed of dark violet staining mucous, as in all following layers. In the third layer numerous vacuole-like structures, densely packed, are visible. They can also be observed in the following layers, but in decreasing numbers. In the fourth to sixth layer vertically arranged fibres are apparent. The outer mucous cover surrounds the whole ribbon on both sides and gives it a certain stiffness.

Ultrastructure of the egg mass:

The membrane is composed of one electron-dense layer (0.25 µm thick). Adjacent to the outside of the membrane is a delicate, filamentous mucous layer (fig. 31A). In the part of the egg mass investigated with TEM the albumen comprises a flocculent mass filling the space between inner capsule wall and embryo completely. The veliger larvae shows prominent cilia at its surface, microvilli also project into the lumen of the capsule. Few coated vesicles are visible subepidermally. The mucous matrix is not very distinct in the ultra-thin sections. When visible it displays a loose, filamentous structure, additionally containing few small, electron-dense mucous droplets. The outer mucous cover shows the differentiation in differently structured layers as has been observed already with light microscopy. The outermost layer is composed of flocculent mucus (fig. 32B). Along its outer edge bacteria are visible. A small electron-dense layer separates the outermost layer from the next. This layer contains large vacuole-like structures embedded in filamentous mucous (see asterisk in fig. 32B). The "vacuoles" contain small electron-dense droplets. Few smaller "vacuoles" are also found in the following layers which are more compact and composed of densely-packed parallel mucous fibres. The number of compact layers cannot be exactly determined since the single layers are hardly separated from each other.

3.9.6 *Bursatella leachii*

The egg mass of this anaspid species is very similar to that described for *Aplysia punctata*. It also comprises a tangled mass (fig. 25C). The embryos were in the early veliger stage when preserved.

Light-microscopic structure of the egg mass:

1 to 3 embryos are encapsulated by a dark violet staining membrane (fig. 28B) which is thicker than the membrane in the egg mass of *A. punctata* (approximately 1 µm thick). Within the capsules, surrounding the embryos, is an amorphous mass of blue staining albumen. The densely-packed capsules are embedded in a compact matrix having a meshwork-like structure. It stains violet. The outer mucous cover is composed of different layers (3-5), the outermost being less dense than the others. Vacuole-like structures, as have been described for *Aplysia punctata* are also visible in *Bursatella leachii*. The outer mucous cover can be up to 60 µm thick.

3.9.7 *Phyllaplysia taylori*

In contrast to the other two anaspid species the egg mass of *Phyllaplysia taylori* is a flat disc attached with one broad side to the substrate (in this case *Zostera*) (fig. 25E). The egg capsules are densely packed in up to five layers on top of each other. Surrounding the capsules is compact mucus. The embryos are in an early developmental stage.

Light-microscopic structure of the egg mass:

The embryos are embedded singly in a compact blue staining mass of albumen, encapsulated by relatively large capsules (fig. 29B). The latter are composed of a dark blue staining membrane which is 2 µm thick. Additionally to the albumen a small spherical structure staining the same as the albumen is visible in some capsules. With high magnification it seems that the membrane is composed of two layers, similar to the ones described for *Haminoea cymbalum*, but the outer layer does not belong to the membrane. It is part of the adjacent mucous matrix. The matrix surrounds the capsules with a small compact, dark violet staining mucous layer. In some parts, where the matrix has separated

from the membrane, the latter appears one-layered. The capsules are densely-packed in the lower half of the egg mass. The matrix is very dense and stains violet. Numerous small mucous filaments interweave the matrix giving it a meshwork appearance. The filaments are more densely-packed in the lower part of the egg mass (fig. 29B). The transition between mucous matrix and outer mucous cover is not distinct. At the upper edge of the spawn mass the mucous seems to be more compact and arranged in longitudinal mucous fibres. Along the outer edge a compact, homogeneously dark violet-staining mucous layer (approximately 0.7 - 3.5 µm thick) is present (fig. 29B). At the lower edge of the egg mass, where it is attached to *Zostera*, this layer as well as the longitudinal mucous fibres are missing.

3.9.8 *Elysia ornata*

The spawn ribbon of *Elysia ornata* is a small band, spirally coiled and attached to the substrate with its broad side (fig. 25D). The colour of the living egg mass is pale yellow. Pieces of one egg mass containing embryos at an early developmental stage were investigated with light and transmission electron microscopy.

Light-microscopic structure of the egg mass:

One embryo is found per capsule. Within the capsule the embryo is surrounded by a blue staining mass of albumen (fig. 28C). The capsules are packed densely in the spawn mass. They seem to be irregularly arranged (fig. 25D; 28C), tube-like organization was not observed. The membranes forming the capsules stain dark blue to violet. Surrounding the membrane is a small inner mucous layer, hardly visible, staining red to dark violet. The mucous matrix, in which the capsules are embedded, has partly a homogeneous, partly a filamentous structure. It stains pink. Since the capsules are packed very densely, the matrix is hardly visible between them. An outer mucous cover is structurally hardly distinguishable from the matrix. The mucous layers at the outer edge of the spawn mass are staining lighter pink than the matrix and a small outermost layer appears more compact and stains dark violet. It surrounds the egg mass on both sides.

Ultrastructure of the egg mass:

The membrane in the egg mass of *Elysia ornata* is composed of one homogeneous layer (fig. 31B), as has been described for *Aplysia punctata*. In *Elysia ornata* this layer measures 0.45 µm. The albumen, completely filling the space between membrane and embryo, has a dense, floccular appearance. Cilia or microvilli, projecting from the surface of the embryo, are not found, neither coated vesicles. The embryo is filled with large round to elliptically-shaped, electron-dense yolk vesicles. Closely attached to the outside of the membrane is a small, filamentous mucous layer which is less electron-dense than the membrane. It accords with the inner mucous layer observed with light microscopy. A coherent mucous matrix is not visible, only few mucous filaments and droplets, spread throughout the sections outside the capsules, are found. The outer mucous cover is distinct. It consists of four layers. The small outermost layer has a fuzzy structure. Adjacent to it is the broadest layer, composed of electron-dense mucous fibres arranged diagonally. The third layer is less electron-dense and consists of fine mucous filaments, irregularly arranged. In the innermost layer the mucous fibres are larger than those of the previous layer, but they are even less densely packed.

In contrast to the previous species described (except for *Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis cyanea*), the egg capsules of the following eleven species, which all belong to the Nudibranchia, do not contain any albumen.

3.9.9 *Acanthodoris pilosa*

The spawn ribbon of this species has the form of a broad band, spirally coiled and attached to the substrate (e. g. *Fucus*) with its small edge (fig. 26A). It is white-coloured. When viewed with a stereo-microscope, the egg capsules look like strings on a pearl within the surrounding mucous matrix. Additionally to the formation of the egg mass within the nidamental gland system, which has been described in chapter 3.7.1 (pages 60-64), two egg masses have been investigated: one at an early developmental stage with semi-thin methacrylate sections and TEM, and one at a pre-hatch stage with TEM.

Light-microscopic structure of the egg mass:

In *Acanthodoris pilosa* 1 to 2 embryos are encapsulated in one capsule. The capsule is composed of two layers, the inner one staining blue, the outer being thinner and staining dark violet. Adjacent to the outer membrane layer is a homogeneously violet-staining inner mucous layer of varying thickness (fig. 30A), enveloping the egg capsules, which are arranged in tube-like structures (fig. 28E). These seem to be coiled within the mucous matrix. The tubes are built by fine mucous strands running through the matrix in which the capsules are embedded. The matrix has a loose, filamentous structure and stains pink to light violet. Surrounding the whole egg mass is a distinct outer mucous cover. This consists of at least four layers (fig. 30A). The outermost layer is compact and dark violet-staining; the second layer comprises a band of densely arranged, longitudinal mucous fibres, which stain lighter violet; the third layer exhibits a small band of mucous fibres staining darker violet and the innermost layer has a more heterogeneous, filamentous appearance and is set off against the adjacent mucous matrix with a small, dark violet-staining mucous layer.

Ultrastructure of the egg mass:

As has been already observed with low power microscopy, the capsule is composed of two distinct layers. The membrane (0.07 µm thick at an early developmental stage) is more electron-dense than the inner capsule layer (1.27 µm) (fig. 31C). During intracapsular development of the embryos the inner capsule layer seems to dissolve, visible at the pre-hatch stage (fig. 31D). In some parts of the egg mass investigated the inner capsule does not form a uniform layer, but the capsule material has become an amorphous mass lying in between the outer membrane and the veliger. At the pre-hatch stage, the thickness of the inner layer has decreased by half (0.6 µm) while the outer membrane layer is still 0.06 µm thin. Microvilli are projecting from the epidermis of the veliger towards the dissolving egg capsule. Coated vesicles are visible subepidermally in the larva. Within the coated vesicles fine granular material, similar in appearance to the dissolving capsule material, can be observed. The space between capsule and embryo seems to be empty in the early developmental stage, except for few patches of fine granular material of unknown composition.

Closely attached to the outside of the membrane is fine mucous material, probably representing the inner mucous layer, which does not form a continuous layer around the capsules. The matrix has a loose filamentous structure. Small condensed mucous droplets are interspersed. The mucous strands within the matrix are composed of fibres of various consistencies. Most fibres are thin and arranged in parallel layers. On the outer edge of the strands the fibres are more compact and more electron-dense.
The outer mucous cover is composed of parallel mucous layers of different structure and density. The outermost edge has a "fuzzy" appearance and is closely attached to a layer (0.4 µm thick) of dense filamentous structure. Following inwards is a less electron-dense layer (0.8 µm thick) composed of fine heterogeneous mucous fibres. The next mucous layers are composed of electron-dense, longitudinal mucous fibres arranged in a loose pattern. The thickness of these layers varies considerably and cannot be estimated exactly. The transition to the mucous matrix is not distinct.

3.9.10 *Adalaria proxima*

The egg mass of *Adalaria proxima* has a similar appearance to the one described for *Acanthodoris pilosa* (fig. 26A). Its living colour is cream white. One egg mass at an early developmental stage has been investigated with semi-thin methacrylate sections.

Light-microscopic structure of the egg mass:

1 to 2 embryos per capsule are visible. The capsule (0.7 µm) stains dark blue. With higher magnifications two distinct layers are visible, the outer membrane staining darker than the inner layer. The capsules are arranged in tube-like structures coiled within the matrix. They are surrounded by partly broad, light pink-staining inner mucous layers. The matrix has a similar structure and staining property to the matrix in *Acanthodoris pilosa*, but is more dense. The outer mucous cover is differently structured on both sides of the egg mass. On one side it consists of 4 distinct layers (fig. 30B): the outermost layer is irregular and has a "fuzzy" appearance (I). It stains dark violet. Adjacent is a dense layer (II) which stains homogeneously pink and is composed of vertical mucous fibres. In the third layer (III) blue staining mucous fibres form an irregular meshwork. The innermost layer (IV) stains homogeneously light pink and has a similar structure to the second layer. It is separated

from the adjacent matrix by a compact mucous band. On the opposite side of the egg mass the third layer is missing.

3.9.11 *Chromodoris magnifica*

The spawn ribbon of *Chromodoris magnifica* has the typical dorid form of a spirally coiled band (fig. 26A). The living egg mass has a white colour. Additionally to the egg mass formation within the nidamental glands (see chapter 3.7.1, pages 60-64) one egg mass at the veliger stage has been investigated ultrastructurally.

Ultrastructure of the egg mass:

In *Chromodoris magnifica* the capsule, containing one embryo, consists of two distinct layers, as has been described for all other nudibranch species so far. The membrane layer is less electron-dense than the inner layer and thinner (approximately 0.2 µm versus 2.38 µm, respectively). While the membrane comprises a compact structure, the inner capsule layer seems to dissolve in parts (fig. 31E). This phenomenon has also been observed in *Acanthodoris pilosa*. Dissolving capsule material is visible in the capsules, surrounding the embryos. The epidermis of the embryos projects microvilli into the lumen of the capsule. In few areas, formation of coated vesicles can be observed at the surface of the epidermis (see arrow in fig. 31F). Subepidermally vesicles containing material of similar structure to the capsule material, are found (see arrow in fig. 31E). Closely attached to the membrane is fine heterogeneous mucous material, resembling parts of the inner mucous layer. In the ultra-thin sections this mucous layer is not very distinct. The mucous matrix has a loose, heterogeneous structure, comprising fine mucous strands and small droplets interspersed. The matrix is closely attached to the outer mucous cover which consists of three distinct layers. The outermost layer is 0.3 µm thick and is composed of dense, heterogeneous mucous fibres (fig. 32C). Adjacent is a layer of loosely arrange mucous fibres, more electron-dense than the previous. This layer is 0.75 µm thick, The innermost layer has a similar appearance to the outermost layer and is 0. 35 µm thick.

3.9.12 *Dendrodoris nigra*

Dendrodoris nigra produces egg ribbons of a cream colour similar in appearance to the ones described before for *Acanthodoris pilosa* and *Adalaria proxima* (fig. 26A). Three egg masses at advanced developmental stages (veliger) have been studied with light-microscopy. Pieces of a different egg mass, preserved at successive developmental stages (2 days, 4 days, 8 days) have been investigated with TEM.

Light-microscopic structure of the egg mass:

The structure of the egg ribbon is very similar to the ones described for the other dorid species, but in contrast to those the capsules are packed very densely within the mucous matrix. They are arranged in tube-like structures, which seem to be coiled within the spawn mass. The capsule stains dark violet and at the veliger-stage only one distinct layer is visible. The eggs are encapsulated singly. An inner mucous layer, surrounding the capsules, is hardly visible probably due to the density of the capsule arrangement. The dense, filamentous matrix partly contains small droplets. It stains light violet. An outer mucous cover is distinct and consists of at least three layers. The outermost layer is loose and contains large vacuole-like structures. It stains light blue. The second layer has a homogeneous appearance, longitudinal violet staining mucous fibres are visible. The innermost layer comprises a small and compact dark violet staining band.

Ultrastructure of the egg mass:

At an early developmental stage (2 days of intracapsular development), the capsule shows ultrastructurally two distinct layers. The membrane is more electron-dense and 0.08 µm thin, whereas the inner layer is 2 µm thick. No albumen is visible within the capsule. The membrane is surrounded by a small band of heterogeneous mucous fibres forming the inner mucous layer. The matrix has a loose, filamentous structure and forms a continuous transition to the outer mucous cover. The three layers of the latter, which have already been observed with low power microscopy, can also be observed ultrastructurally. Additionally, a layer of loosely arranged heterogeneous mucous fibres, which could not be distinguished with light-microscopy, is present at the outer edge. The vacuole-like

structures of the adjacent layer do not seem to have any contents. The total thickness of the outer mucous cover is approximately 10 µm.

Ultrastructural investigation showed that bacteria with an identical appearance to those observed in the vestibular gland of *Dendrodoris nigra* (see chapter 3.7.2, page 66) are also present in the mucous surrounding the egg capsules. In the 2 day old egg mass, the bacteria are located mainly in the outer mucous cover and only relatively few (< 2 μm^{-2}) are present in the mucous matrix. In the egg mass preserved after four days, the overall structure of the mass has not changed but the number of bacteria has increased (< 5 μm^{-2}) and the bacteria have spread throughout all the mucous layers. The bacteria have also penetrated to the outside of the capsules (fig. 33A) The capsule at this stage is still approximately 2 µm thick. On the eighth day of intracapsular development (shortly before hatching) many thousands of bacteria are present in the dissolving egg mass (5 - 10 μm^{-2}). The majority of bacteria are located in the mucous matrix and around the capsule (fig. 33B) which, in comparison to the developmental stages described before, has become very thin (0.08 µm). The ultrastructure of the capsule and the thickness suggest that this thin layer at the eighth day of development represents the membrane layer found in the earlier developmental stages. The inner capsule layer seems to have dissolved completely, similar to the phenomenon described for *Acanthodoris pilosa*. No residues of capsule material are visible within the capsule. No bacteria could be found within the capsule nor incorporated within the veliger larva.

3.9.13 *Onchidoris bilamellata*

Onchidoris bilamellata produces very similar egg ribbons to the already described anthobranchs. The white spawn mass comprises a broad band, attached to the substrate in a irregular coil (fig. 26A). One egg mass at an early intracapsular developmental stage, has been investigated with methacrylate histology.

Light-microscopic structure of the egg mass:

Up to five embryos can be found per capsule. The capsule stains dark blue. With low power microscopy only one distinct layer is visible (approximately 1.35 µm thick). Closely

attached to the capsule is a dark violet staining inner mucous layer. The capsules are arranged in tubes, surrounded by dark violet staining, fine mucous strands. The tubes themselves are coiled within in a loose, filamentous mucous matrix, staining pink. The outer mucous cover, which surrounds the whole egg mass, consists of three layers: the outermost layer comprises a compact mucous band, staining homogeneously dark violet. The adjacent layer stains light pink and has a heterogenous, filamentous structure. The third layer is composed of longitudinal mucous fibres staining violet. The outer mucous cover is approximately 5.2 µm thick.

3.9.14 *Polycera quadrilineata*

Polycera quadrilineata also lays egg masses of the typical dorid shape: small, white bands are attached to the substrate with the small edge in spiral coils (fig. 26A). One egg mass of an early developmental stage has been studied with light and electron microscopy. Additionally an egg mass of a late veliger stage has been investigated with TEM.

Light-microscopic structure of the egg mass:

In *Polycera quadrilineata* the embryos are encapsulated singly. The capsule stains dark blue, only one layer is visible with low power microscopy. The adjacent inner mucous layer is very small and dense and stains dark violet. Tubular arrangements of the capsules are visible. Thick mucous strands form the tubules in which the capsules are embedded. The tubes form coils within the surrounding matrix. The latter is loose and filamentous and stains light pink. The outer mucous cover is composed of three layers, the outermost being of a heterogeneous structure, whereas the two adjacent layers have a homogeneous appearance. The densely packed mucous fibres of the two inner layers are staining violet, while the outermost layer stains darker.

Ultrastructure of the egg mass:

Ultrastructural investigation reveals, that the capsule consists of two distinct layers at an early developmental stage. The outer membrane layer (0.06 µm) is more electron-dense than the inner capsule layer (0.25 - 0.3 µm) (fig. 33C). At the late veliger stage only one electron-dense capsule layer (0.05 - 0.06 µm thick) is present. It probably resembles the

membrane layer of the early developmental stage. At the late veliger stage fine granular material is present within the capsule, probably resulting from the dissolved inner capsule layer. This has also been described for *Acanthodoris pilosa*, *Chromodoris magnifica* and *Dendrodoris nigra*. The veliger projects microvilli and cilia into the lumen of the capsule. A distinct, compact inner mucous layer (0.4 µm) is visible at an early developmental stage (fig. 33C). The matrix has a loose filamentous structure at all stages. Small, electron-dense droplets are interspersed. Fine, compact mucous fibres are scattered throughout the matrix, forming tube-like structures. The outer mucous cover is composed of numerous densely packed mucous fibres of various structures arranged in parallel layers.

3.9.15 *Lomanotus vermiformis*

In contrast to the egg masses of the anthobranch species described before, the egg masses of the following species comprise small delicate ribbons, much smaller than those of the dorids. *Lomanotus vermiformis* places its white egg ribbons in form of a corkscrew (fig. 26C) around the hydrozoa on which the animals prey. The embryos of the investigated egg mass were at an early developmental stage when preserved.

Light-microscopic structure of the egg mass:

Each egg capsule contains one embryo (fig. 30C). The capsules are very thin (0.3 - 0.7 µm) and stains dark blue. The inner mucous layer is not distinguishable. The loose, filamentous mucous matrix is hardly visible. Fine mucous strands surround groups of capsules. The outer mucous cover is not as distinct as in the previous species described. Sometimes it forms a continuous transition of the inner mucous layers and cannot be differentiated from the latter. The outer mucous cover consists of three layers, two small, compact mucous layers staining dark violet enclosing a broader, more heterogeneous layer staining lighter.

3.9.16 *Tritonia plebeia*

Tritonia plebeia produces egg masses very similar in appearance to *Lomanotus vermiformis* (fig. 26C). The egg ribbon of the former was found on *Alcyonium*. One egg mass at the veliger stage was investigated with light and electron microscopy.

Light-microscopic structure of the egg mass:

One veliger is found per capsule. The capsules are loosely arranged. They are thin and stain dark blue. A dark violet staining inner mucous layer is closely attached and often hardly distinguishable from the capsule (fig. 28D). A matrix is not visible. The outer mucous cover is composed of at least four different layers of mucus. The outermost layer is heterogeneous and stains light blue. Adjacent is a small compact layer staining dark violet, followed by a broader layer of light pink staining heterogeneous mucous fibres. The innermost layer has a similar appearance to the second one.

Ultrastructure of the egg mass:

The embryos within the piece of egg mass investigated are at different developmental stages. Some have not yet developed cilia, whereas in others prominent cilia are visible (fig. 33D). Accordingly the capsules do not have a uniform structure. Those eggs with the less developed embryos show two-layered egg capsules similar to the ones I have described before for the other nudibranch species. The outer, more electron-dense membrane layer is 0.07 µm thick while the inner capsule layer measures 0.2 µm. Its inner edge has a rough, irregular structure. Capsules containing further developed veligers consist of one electron-dense layer of 0.07 µm (fig. 33D). This layer probably represents the membrane layer described before. The inner capsule layer has probably dissolved during intracapsular development. Few fine granular material, possibly residues of the dissolved inner capsule, is visible in the lumen of the capsule of the further developed larvae. Along the epidermis of the veligers prominent microvilli are found. A distinct inner mucous layer is not visible, but fine filamentous material can be observed closely attached to the outer capsule. The matrix is hardly distinguishable, only few fine filaments are scattered throughout the space between capsules and outer mucous cover. The different layers of the latter are distinct. The outermost layer consists of fine, loosely arranged mucous fibres. Adjacent are large, bubble-like structures partly containing heterogeneous structures (fig. 32E). Fine longitudinally mucous fibres compose the third layers. They are loosely packed, while in the fourth layer the mucous fibres are more densely arranged.

3.9.17 *Dermatobranchus semistriatus*

The spawn of *Dermatobranchus semistriatus* has the shape of a stout sausage. At the base a stiff mucous strand attaches the egg mass to the substrate (fig. 26B). The living spawn has a translucent white colour. One egg mass at an early developmental stage was investigated with methacrylate histology and another one at the veliger stage with TEM.

Light-microscopic structure of the egg mass:

One embryo is found per capsule. The capsules are irregularly arranged within the mucous matrix. The capsule is rather thin and with low power microscopy only one layer is visible. It stains blue to violet. Adjacent to the outside of the capsules is a small inner mucous layer staining dark violet. The matrix is filamentous and more dense than in the other cladobranch species described. Small dark violet staining mucous droplets and fine mucous strands are scattered throughout the light violet-staining matrix. The outer mucous cover is very distinct and much more compact than in the other nudibranch species. It has a similar appearance to the outer mucous cover described for *Aplysia punctata*. Numerous dark violet staining, longitudinal mucous fibres are densely packed to form the mucous cover, measuring up to 75 µm. The outermost edge of the mucous cover has a "fuzzy" structure.

Ultrastructure of the egg mass:

At higher magnifications two layers are visible composing the capsule. The outer membrane layer is more electron-dense than the inner layer and measures 0.1 µm whereas the latter has an extension of 0.7 µm. At its inner edge the inner capsule layer has an amorphous structure and seems to dissolve. Microvilli are projecting from the epithelial surface of the veliger into the capsule lumen, where coarse granular material, similar to the dissolving capsule material, is present. The inner mucous layer has a very loose, heterogeneous structure in the ultra-thin sections and forms a continuous band, surrounding the capsules.

The mucous matrix is hardly distinguishable. Few mucous strands can be observed in between the capsules and the outer mucous cover. The latter comprises various layers of longitudinal mucous fibres with irregularly arranged mucous fibres scattered throughout.

Along its outer edge the mucous cover contains large bubble-like structures. Within these small, spirally coiled, electron-dense structures are visible (see arrow in fig. 32 D).

3.9.18 *Eubranchus exiguus*

This small cladobranch species produces small sausage shaped egg masses (fig. 26B). They are delicate in structure and have a white colour. Egg masses with veligers at an advanced developmental stage have been observed with light and electron microscopy.

Light microscopic structure:

Only one embryo is found per capsule. The capsules are very thin and just one violet staining layer can be observed. The capsules show an irregular arrangement within the egg mass. A violet staining inner mucous layer, surrounding each capsule, is visible. The matrix is rather dense, small dark violet staining mucous droplets and fine mucous strands can be found scattered throughout. The outer mucous cover consists of three layers, one heterogeneous outermost layer staining light pink, a second layer of less dense mucous fibres having lighter staining properties and an inner layer of homogeneous, compact structure staining dark violet.

Ultrastructure of the egg mass:

Two layers can be seen to compose the capsules with higher magnifications (>30.000x). The outer membrane seems to have a similar electron density as the inner capsule layer, but is smaller (0.05 µm) than the latter (0.08 µm). At the inner side of the capsule the inner layer has a rather amorphous structure, it seems to be dissolving. Coarse floccular material is visible in the lumen of the capsule. Microvilli are projecting from the epidermis of the larva into the capsule lumen. Cilia are also visible. In general the inner mucous layer is closely attached to the outer membrane, but partly separates from the latter. It is composed of dense mucous fibres. The matrix has a loose, filamentous structure (fig. 33E) and shows a continuous transition to the outer mucous cover at the outer edge of the egg mass. The three layers of the outer mucous cover found with light microscopical

investigation can also be distinguished ultrastructurally. The outermost layer has a "fuzzy" appearance along its outer edge. It also contains large bubble-like structures with similar spirally coiled contents as described for *Dermatobranchus semistriatus*. Adjacent to this layer is a small layer composed of heterogeneously arranged mucous fibres. The innermost layer consists of densely packed heterogeneous mucous fibres. The extension of the outer mucous cover is not uniform since it has a wavy outline.

3.9.19 *Flabellina gracilis*

The small spirally coiled egg ribbon (fig. 26C) of *Flabellina gracilis*, similar in appearance to *Lomanotus vermiformis* and *Tritonia plebeia*, has a white colour. It is very delicate in structure and easily breaks apart when handled without care. The embryos of this egg mass were at an early developmental stage when the egg mass was preserved. Light microscopic and TEM investigations were performed on the same egg mass.

Light-microscopic structure of the egg mass:

The embryos are encapsulated singly by a small, dark blue-staining capsule. Only one layer is visible with low power microscopy. The embryo is filling the capsule almost completely. A dark violet-staining inner mucous layer surrounds groups of capsules, forming long bands within the egg mass. The matrix has a very loose, filamentous structure and stains light violet. The outer mucous cover comprises three distinct layers. The outermost layer (2.0 µm) consists of densely-packed heterogeneous mucous fibres staining light pink. The second layer is the broadest (approximately 18 µm). It is composed of fine, heterogeneous mucous fibres irregularly arranged. This layer stains light violet. The third and innermost layer (2.5 µm) is a compact mucous band of homogeneous structure.

Ultrastructure of the egg mass:

Ultrastructurally it is apparent that the capsule is composed of two layers. The outer membrane layer, more electron-dense than the inner layer, measures 0.1 µm. The inner capsule layer is of heterogeneous structure at its inner side, thus the thickness cannot be measured exactly. The capsule material seems to dissolve. Coarse, granular material is

visible within the capsule lumen. Adjacent to the outer capsule is a band of loose, heterogeneous mucous fibres. The matrix is composed of fine mucous strands. These are clearly composed of two layers of mucous fibres: the outer layer consists of compact fibres the inner layer of fine, parallel filaments. Scattered throughout the matrix are electron-dense mucous droplets. The three layers of the outer mucous cover, which have been observed already in the semi-thin sections with light microscopy can also be found ultrastructurally. The outermost layer contains bubble-like structures similar to the ones described for *Tritonia plebeia*. The other layers are composed of heterogeneous mucous fibres of different density and thickness. In general the innermost layer is more electron-dense than the other two layers.

In contrast to the nudibranch species described above the egg capsules of the following pulmonate contain albumen.

3.9.20 *Radix peregra*

The spawn mass of *Radix peregra* has the form of a stout sausage (fig. 26D) and has a whitish, creamy colour. The large egg capsules are embedded in thick layers of dense mucous fibres. One egg mass was examined light-microscopically as well as electron-microscopically. Due to poor fixation or poor penetration of resin, the embryos could not be investigated, thus their developmental stage is unknown.

Light-microscopic structure of the egg mass:

The capsules are relatively thin structures and enclose the embryo embedded in a dense mass of dark blue staining albumen. The capsule has the same staining property. Surrounding the capsules are layers of mucous fibres staining violet to light blue. The mucous layers are densely-packed. A mucous matrix is hardly visible. Enclosing the egg mass is a broad outer mucous cover consisting of three mucous layers (fig. 30D). The outermost layer is small and stains dark blue to violet. The broadest layer (approximately 85 µm thick) contains heterogeneous mucous fibres, loosely arranged and vacuole-like structures, scattered throughout. This middle layer stains light blue to violet. The innermost layer comprises a compact mucous band staining dark blue to violet. From this

innermost layer multi-layered mucous strands run through the egg mass and connect to the inner mucous layers surrounding the capsule.

Ultrastructure of the egg mass:

The capsule of *Radix peregra* is composed of one electron-dense membrane layer (approximately 1 µm thick). The albumen comprises a homogeneous, electron-dense mass enclosing the embryo. Surrounding the capsule are at least fifteen mucous layers of different texture and density (fig. 32A). Closely attached to the capsule is a filamentous layer, less electron-dense than the other layers. Even with high magnifications, a mucous matrix is not visible. The space between the inner mucous layers surrounding the capsule and the outer mucous cover seems to be empty. The outer mucous cover is less electron-dense than the inner mucous layers and consists of loosely arranged mucous fibres. Large, vacuole-like structures are visible in between the mucus. These vacuoles have no visible contents.

3.9.21 Discussion

The fine structure of the egg masses investigated here appears to be rather uniform within the various euthyneuran taxa. The spawn always exhibits a mucoid mass, enveloping the egg capsules which contain the larvae. In some opisthobranch taxa (some "Cephalaspidea s. l.", Anaspidea and Sacoglossa) and in the Pulmonata an albuminous fluid is present in the capsules. This albumen always comprises a blue staining mass which ultrastructurally appears flocculent. In these taxa the capsule consists of one membrane layer, whereas in other taxa, which also lack an albuminous fluid in their egg capsules (some "Cephalaspidea s. l.", Nudibranchia), two layers are visible (mainly at higher magnifications). Pleurobranchoidea also show two-layered capsules and lack albumen (personal observation on *Bathyberthella antarctica*; Wägele 1996). The thickness of the capsules shows considerable interspecific variation, *Eubranchus exiguus* having the thinnest (0.13 µm) and *Chromodoris magnifica* the thickest (2.52 µm) capsule. The thickness can also vary intraspecifically, depending on the developmental stage. This was observed in some nudibranch species (*Acanthodoris pilosa*, *Chromodoris magnifica*, *Dendrodoris nigra*); the inner capsule layer dissolves during intracapsular development and the veliger larvae appeared

to ingest the inner layer of the capsules. In almost all cases investigated the egg capsules are surrounded by an inner mucous layer, which can be very thin and hardly visible (for example in *Elysia ornata*) or very conspicuous (e. g. in *Acanthodoris pilosa*). While the arrangement of the capsules is rather irregular in taxa like some "Cephalaspidea s. l.", Anaspidea and Sacoglossa, the capsules are arranged in tube-like structures, regularly coiled within the egg ribbon in other taxa (some "Cephalaspidea s. l.", anthobranch Nudibranchia). A mucous matrix is almost always present. In some species it has a very dense structure (e. g. in the anaspidean species), in others it can be of loose composition and hardly visible (e. g. *Lomanotus vermiformis*). In *Radix peregra* no matrix was found. The outer mucous cover, surrounding the egg mass, is always multi-layered. In *Dermatobranchus semistriatus* and *Radix peregra* it is exceptionally thick and, in the former, consists of numerous mucous layers. The density and fine structure of the mucous layers comprising the outer mucous cover vary considerably within the species.

While the morphology of the egg masses of opisthobranchs (and other Euthyneura) has been subject to many studies (refer for example to O'Donoghue 1922; Hurst 1967; Greene 1968; Bandel 1976; Fernandez-Ovies & Ortea 1981 or Soliman 1987), information about the histology and fine structure of opisthobranch egg masses is sparse. A review of the data about the fine structure of nudibranch egg capsules was presented by Eyster (1986). Her ultrastructural investigations of egg capsules of various nudibranch species revealed the capsules to be composed of two layers, as has been demonstrated by me for *Haminoea cymbalum* and the nudibranch species. Eyster also found granular material in the capsule of some species, which she believed to be albumen. I doubt that this material is equivalent to the albumen found in other opisthobranch taxa. From Fig. 7 (page 209) of the paper by Eyster I rather assume that the granular material resembles residues of the inner capsule layer, similar to those I found in *Chromodoris magnifica* and *Acanthodoris pilosa*. Wägele (1989b, 1996) presented the first more detailed histological investigations of opisthobranch egg masses. Her results of the fine structure of the egg masses of various Antarctic Pleurobranchoidea and Nudibranchia are in agreement with my findings. She also found the egg capsules to be surrounded by various mucous layers of different fine structure. Ultrastructural investigations of the egg masses of *Austrodoris kerguelenensis* and *Archidoris pseudoargus* (Wägele 1989b) revealed that the egg capsule in these species is likewise composed of two distinct layers. Wägele observed the egg capsule

of *Austrodoris kerguelenensis* to be interspersed by large bubble-like structures. Embryonic tissue was found to penetrate into these bubbles and the capsule seemed to attentuate. This observation might describe a similar phenomenon as the dissolving inner capsule layer found in the present study in several nudibranch species. The egg capsules of the Antarctic species Wägele has described are extraordinarly thick compared to those of the species investigated here, as are the surrounding mucous layers. Wägele reported of bacteria in the mucous layers of *Austrodoris kerguelenesis* and *Tritoniella belli*. This agrees with the present findings of *Dendrodoris nigra*. Thompson (1958) described the egg mass of *Adalaria proxima*. He also found tube-like structures surrounding the egg capsules and forming spirals through the mucous mass. He referred to these layers as the secondary membrane and to the capsule enclosing the eggs as the primary egg case. Jensen (1996) reported of extra-capsular yolk in the egg mass of *Elysia ornata*. This cannot be confirmed by the present data. Bridges (1975) gave a detailed description of the larval development of *Phyllaplysia taylori*. In her paper she also described the egg mass of this species. According to her the membrane enclosing the embryo is composed of albumen (she called it albumen membrane). Bridges also found a spherical structure in the egg capsules and believed it had a nutritional function. Other information of the fine structure of opisthobranch egg masses is scattered throughout the literature (Reid 1964; Fretter & Bun Hian 1984; Kress & Schmekel 1992) but detailed comparative studies are lacking to date.

Plesch et al. (1971) described the fine structure of the egg mass of the pulmonate *Lymnaea stagnalis* which is similar to the one found in *Radix peregra*. The terminology they use for the various structures differs from that used here. According to the authors the eggs are surrounded by a "perivitelline fluid", which accords to the albumen found in *R. peregra*. Embryos and perivitelline fluid are encapsulated in a "membrana interna" to which is attached a "membrana externa". In contrast, in *Radix peregra* the membrane is composed of only one layer. Plesch et al. observed that the "eggs" (encapsulated embryos) of *Lymnaea stagnalis* are joined together by thin egg strings, which probably accord with the inner mucous layer I have described for *R. peregra*. The mucous matrix, found in the latter, is termed "tunica interna" by Plesch et al. in *Lymnaea stagnalis*. The outer mucous cover I have described for *Radix peregra* corresponds to the "tunica capsulis" or "oötheca" in *Lymnaea stagnalis* (Plesch et al. 1971).

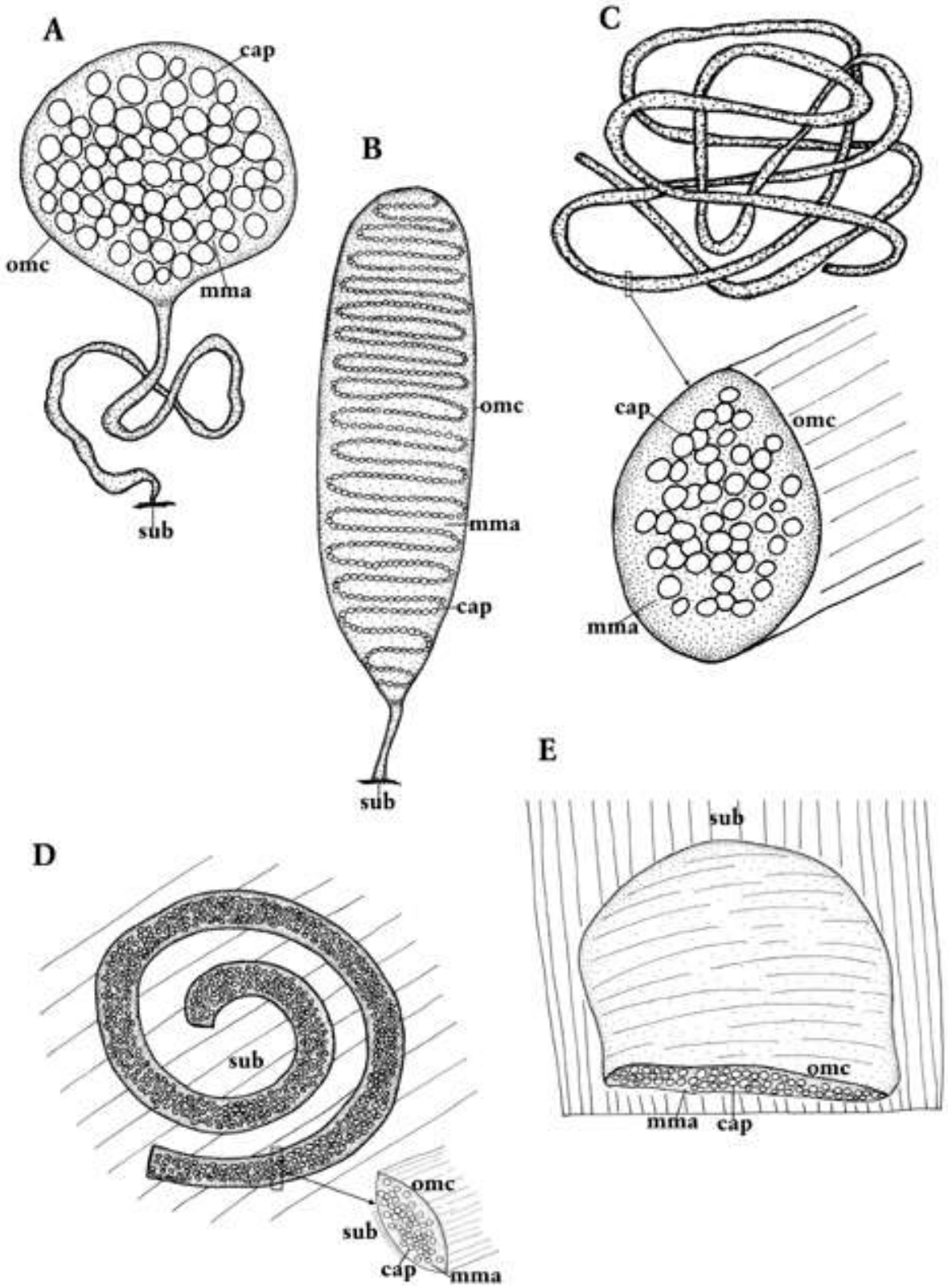

Figure 25: Half-schematic outlines of the morphology of the egg masses of several Opisthobranchia. A: *Acteocina atrata*; B: *Chelidonura inornata*, *Philinopsis cyanea*; C: *Aplysia punctata*; D: *Elysia ornata*; E: *Phyllaplysia taylori.*

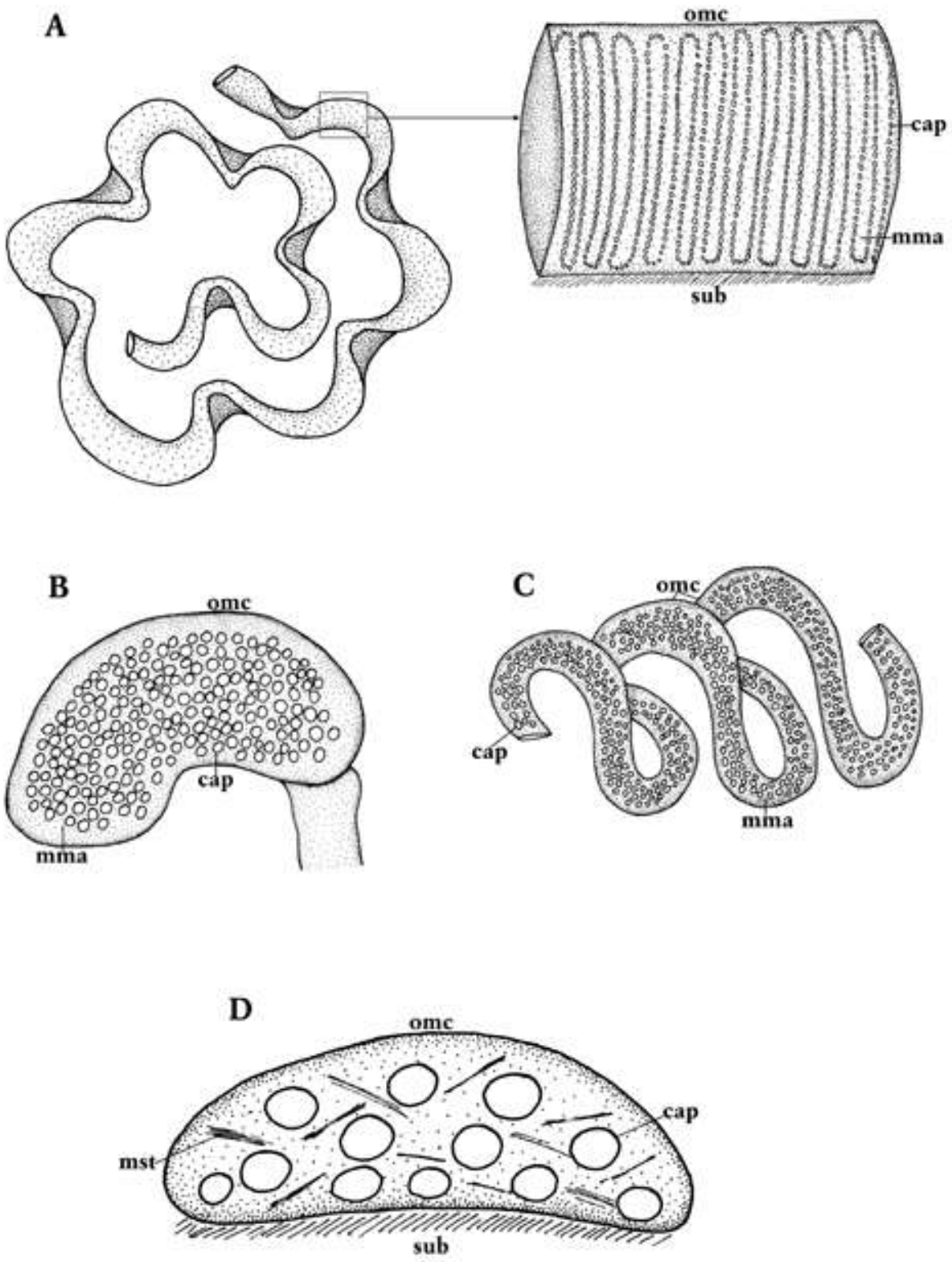

Figure 26: Half-schematic outlines of the morphology of the egg masses of several Euthyneura. A: *Acanthodoris pilosa, Adalaria proxima, Chromodoris magnifica, Dendrodoris nigra, Onchidoris bilamellata, Polycera quadrilineata*; B: *Haminoea cymbalum, Dermatobranchus semistriatus, Eubranchus exiguus*; C: *Tritonia plebeia, Flabellina gracilis*; D: *Radix peregra*.

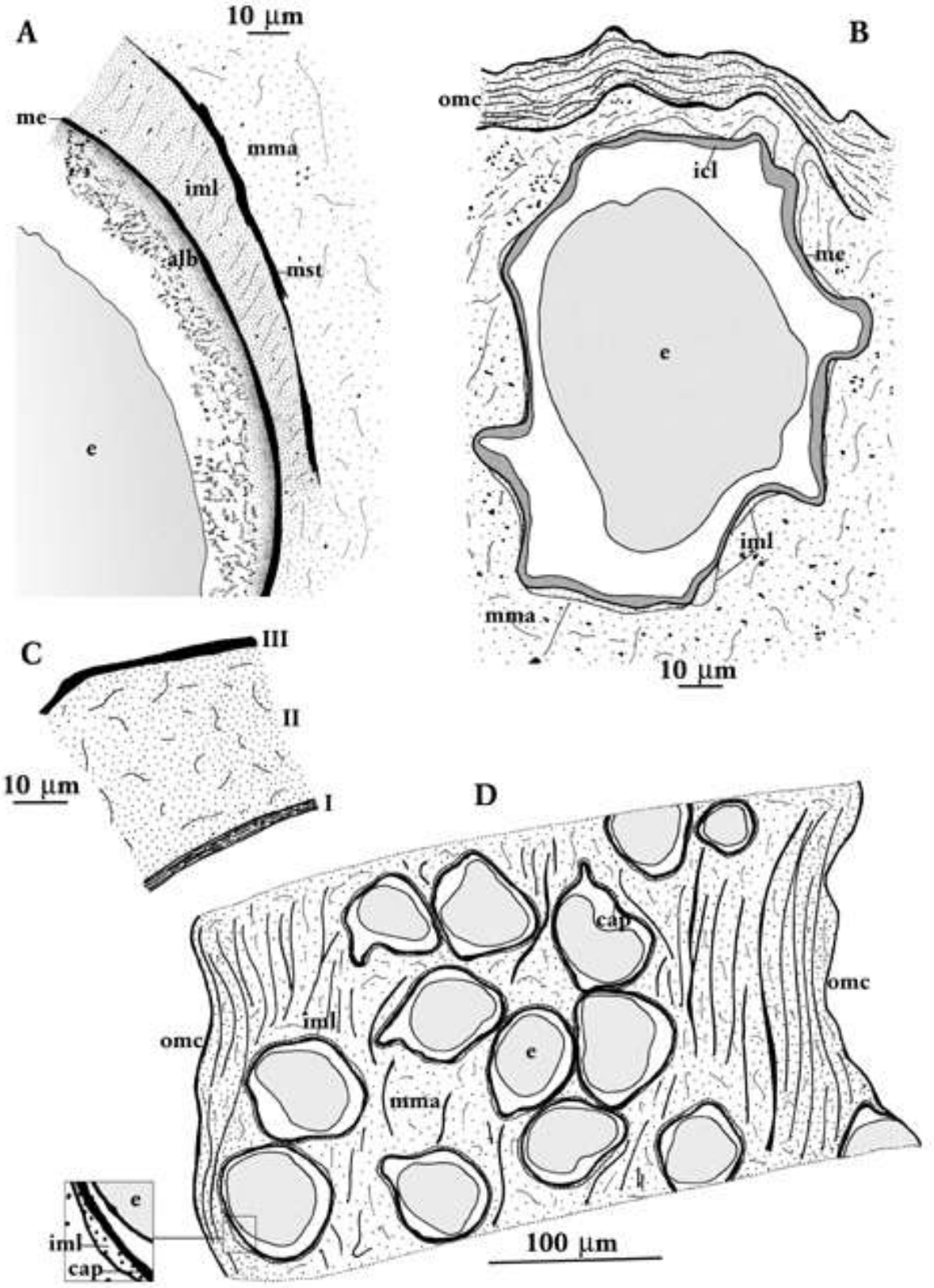

Figure 27: Schematic outlines of the light-microscopic structure of the egg mass of several "Cephalaspidea s. l.". A: *Acteocina atrata* - cross section through a piece of egg mass; B: *Haminoea cymbalum* - cross section through a piece of egg mass; C: *Acteocina atrata* - cross section through the outer mucous cover; D. *Chelidonura inornata* - cross section through a piece of egg mass.

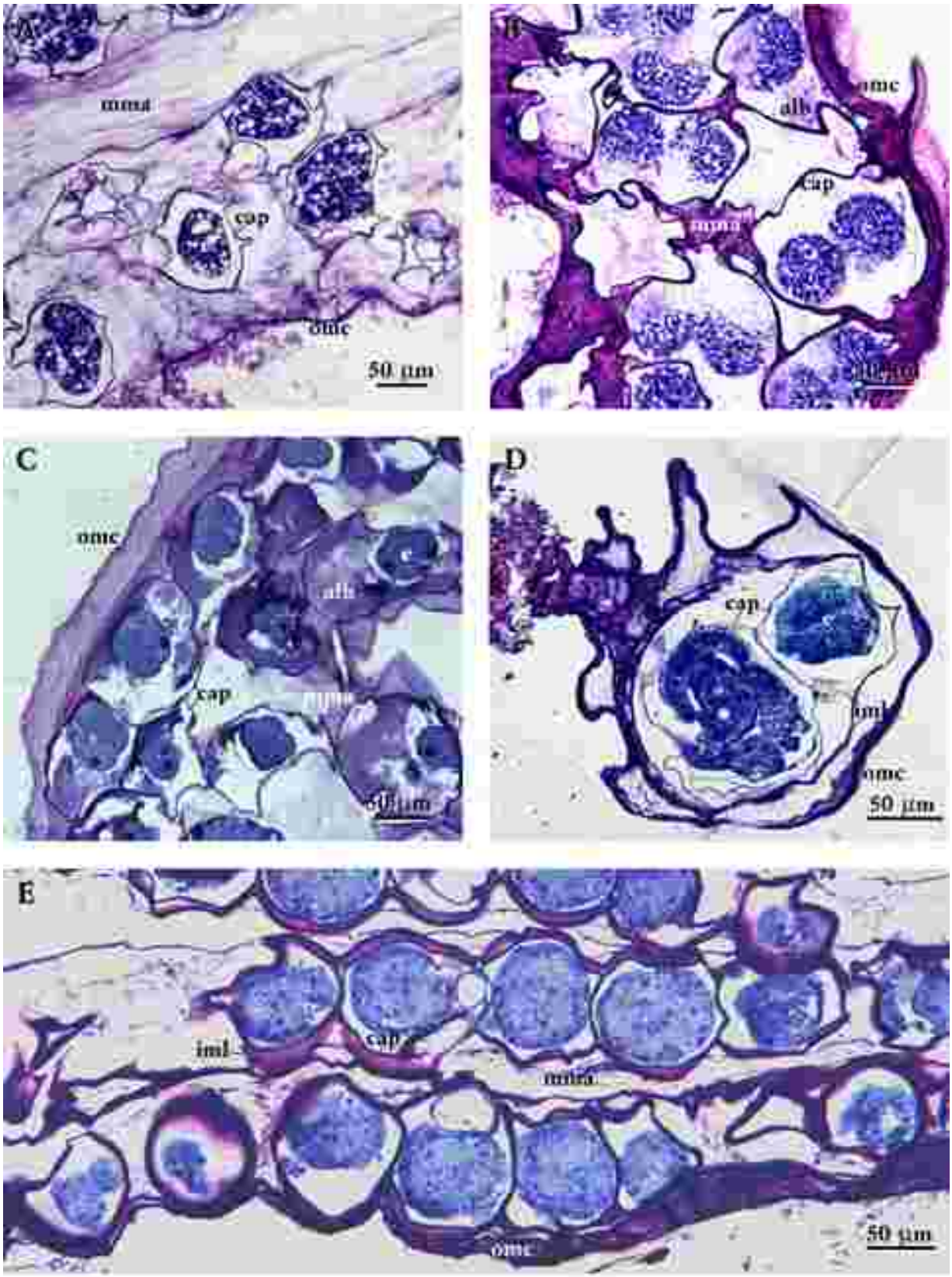

Figure 28: Histological sections of the egg masses of several Opisthobranchia. A: *Haminoea cymbalum* - longitudinal section; B: *Bursatella leachii* - longitudinal section; C: *Elysia ornata* - cross section; D: *Tritonia plebeia* - cross section; E: *Acanthodoris pilosa* - longitudinal section.

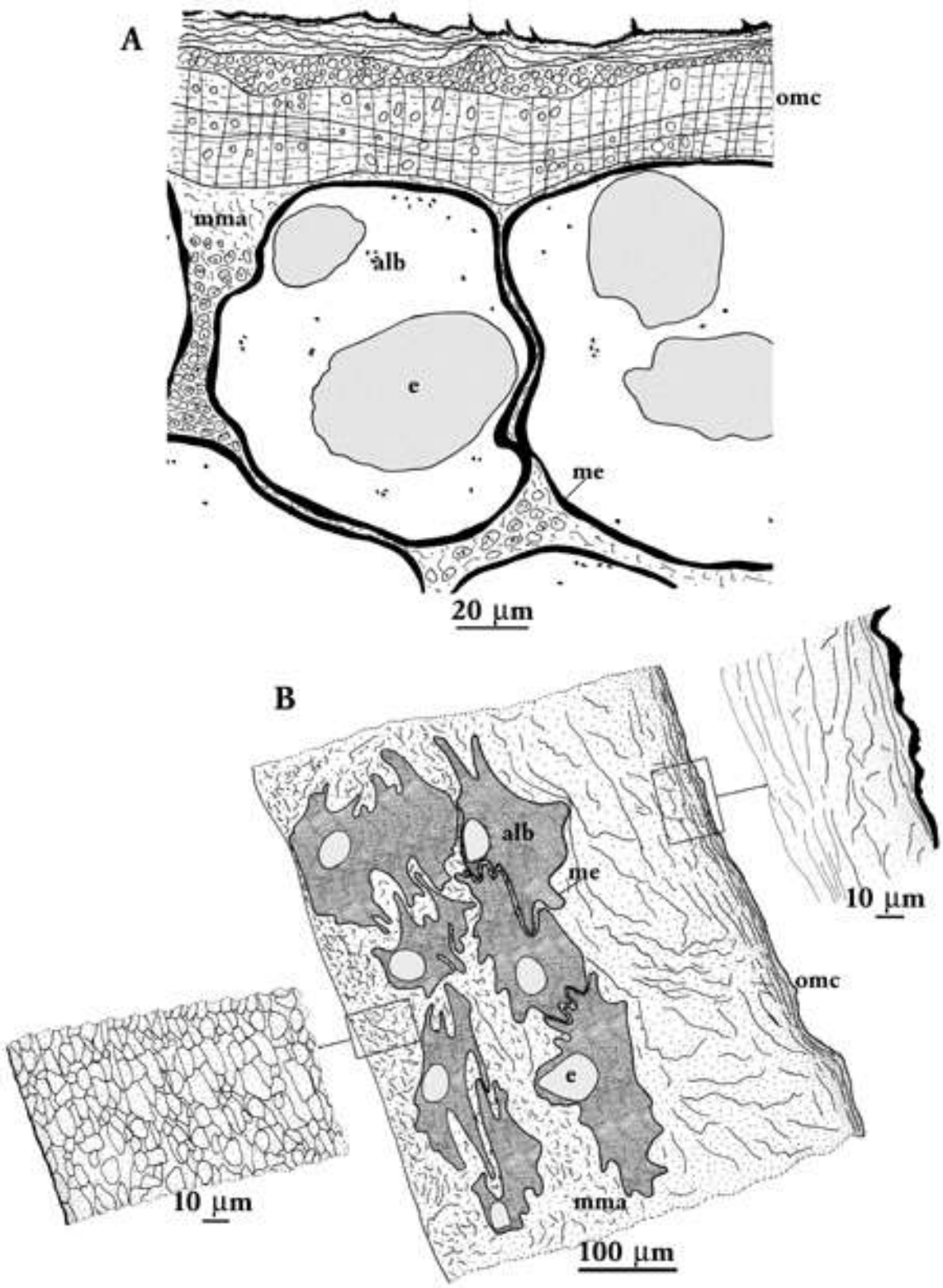

Figure 29: Schematic outlines of the light-microscopic structure of the egg masses of several Anaspidea. A: *Aplysia punctata* - cross section through a piece of egg mass; B: *Phyllaplysia taylori* - cross section through a piece of egg mass.

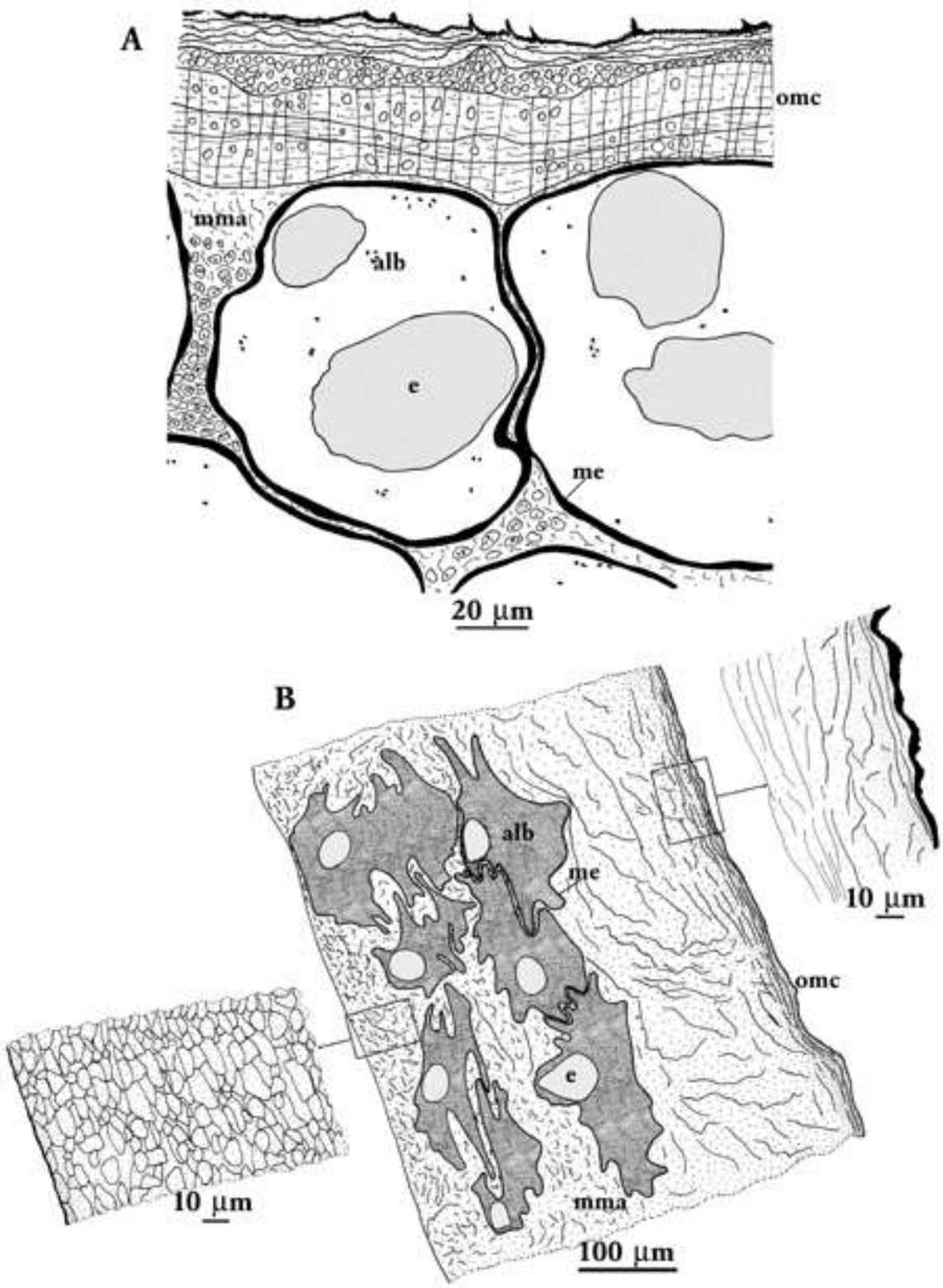

Figure 29: Schematic outlines of the light-microscopic structure of the egg masses of several Anaspidea. A: *Aplysia punctata* - cross section through a piece of egg mass; B: *Phyllaplysia taylori* - cross section through a piece of egg mass.

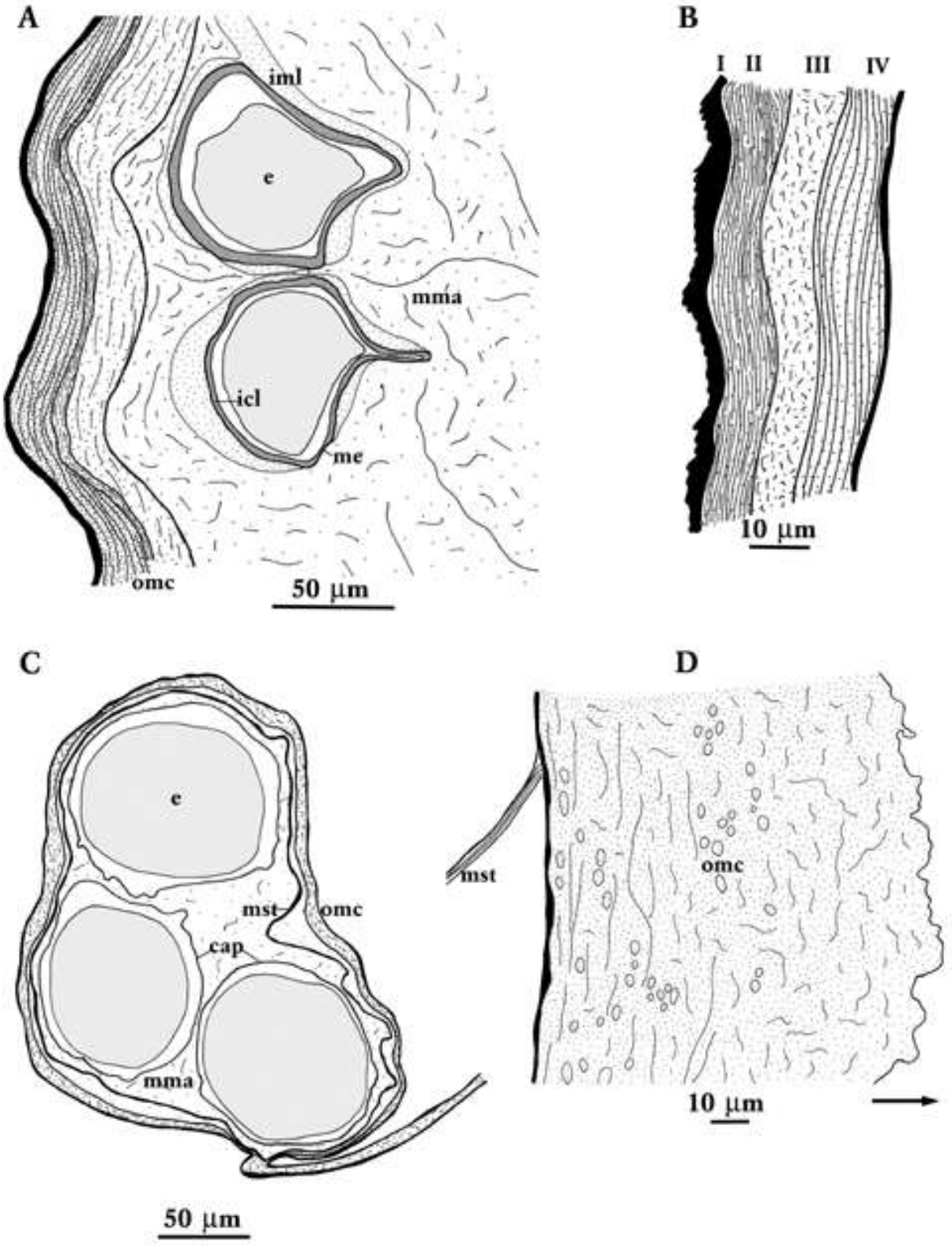

Figure 30: Schematic outlines of the light-microscopic structure of the egg masses of several Nudibranchia and one species of the Pulmonata. A: *Acanthodoris pilosa* - cross section through a piece of egg mass; B: *Adalaria proxima* - longitudinal section through the outer mucous cover; C: *Lomanotus vermiformis* - cross sections through a piece of egg mass; D: *Radix peregra* - longitudinal section through the outer mucous cover (arrow points towards the outside of the egg mass).

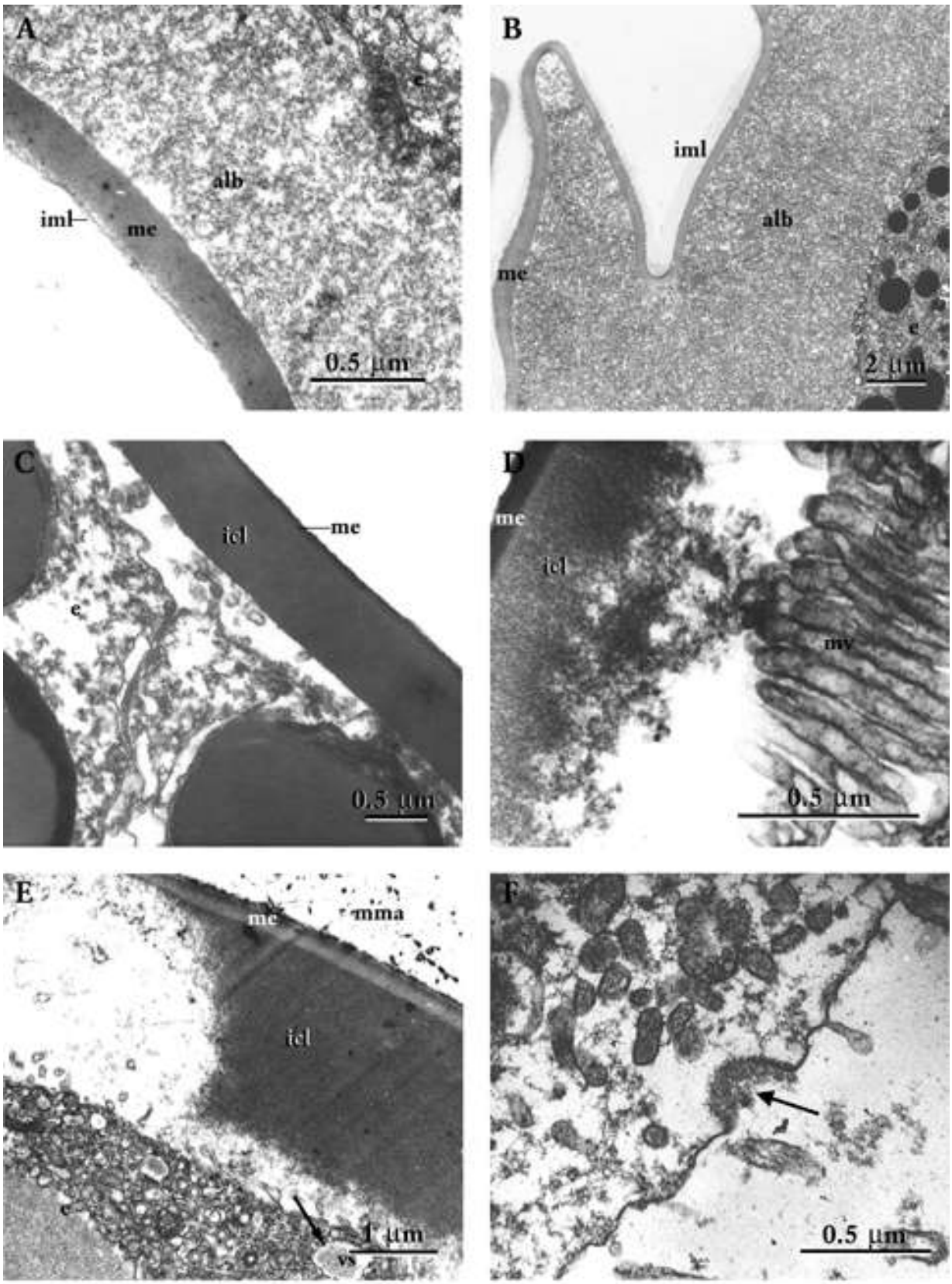

Figure 31: Ultrastructure of the egg masses of several Opisthobranchia. A: *Aplysia punctata* - cross section through part of an egg capsule; B: *Elysia ornata* - cross section through part of an egg capsule; C, D: *Acanthodoris pilosa* - cross sections through parts of egg capsules at an early (C) and a pre-hatch (D) developmental stage; E: *Chromodoris magnifica* - cross section through part of an egg capsule (arrow indicates supepidermal vesicles); F: *Chromodoris magnifica* - cross section through part of the embryo (arrow indicates coated vesicle).

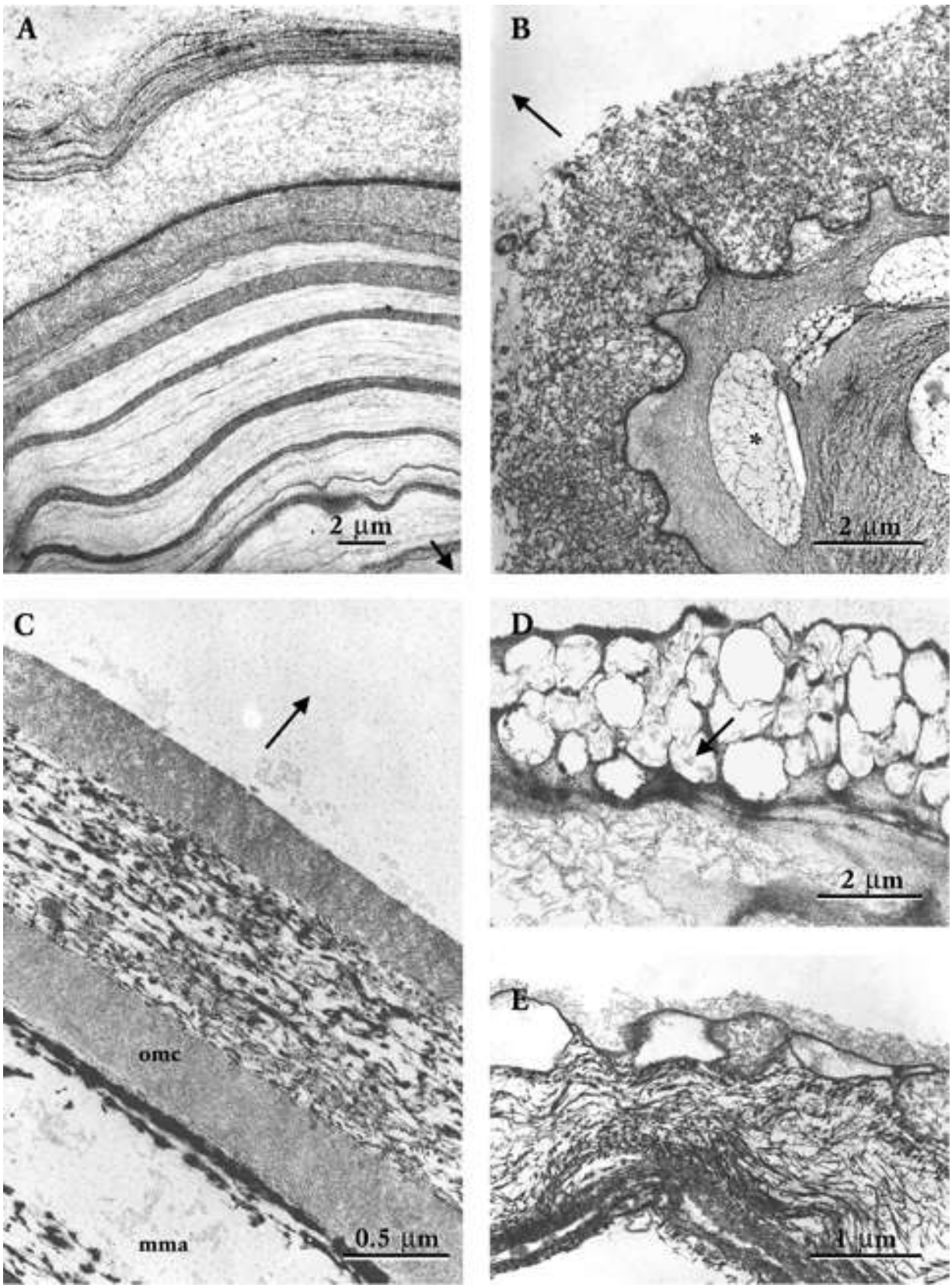

Figure 32: Ultrastructure of the mucous layers within the egg masses of several Opisthobranchia and one species of the Pulmonata. A: *Radix peregra* - inner mucous layers (arrow points towards the egg capsule); B: *Aplysia punctata* - part of outer mucous cover (arrow points towards the outside of egg mass, asterisk indicates "vacuole"-like structures); C: *Chromodoris magnifica* - outer mucous cover (arrow points towards the outside of egg mass); D: *Dermatobranchus semistriatus* - part of outer mucous cover (arrow indicates substructures in "vacuoles"); E: *Tritonia plebeia* - outer mucous cover.

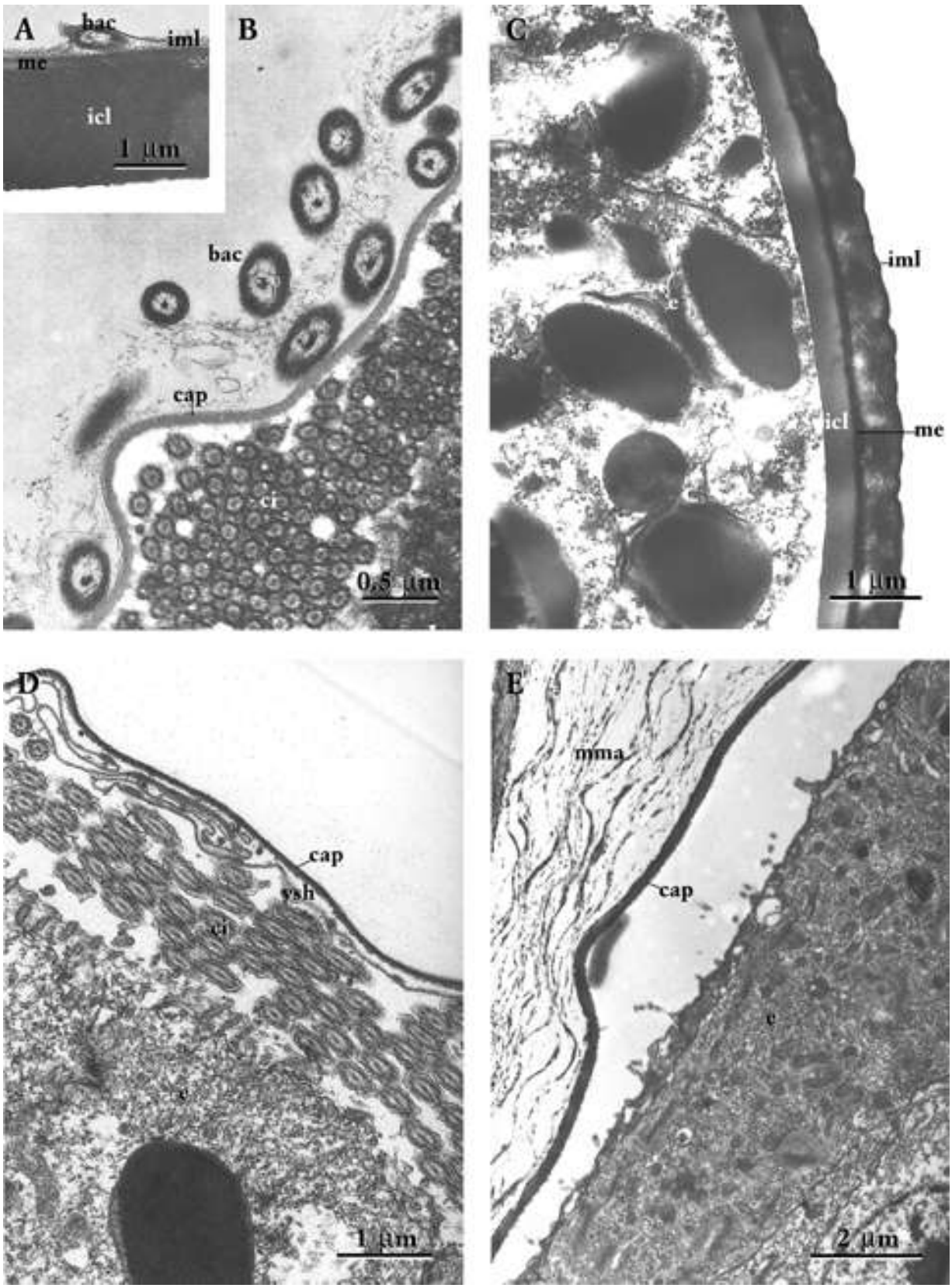

Figure 33: Ultrastructure of the egg masses of several Nudibranchia. A: *Dendrodoris nigra* - cross section through part of an egg capsule (4th day of development); B: *Dendrodoris nigra* - cross sections through part of an egg capsule with adjacent bacteria (8th day of development); C: *Polycera quadrilineata* - cross section through part of en egg capsule and adjacent inner mucous layer; D: *Tritonia plebeia* - cross section through part of an egg capsule; E: *Eubranchus exiguus* - cross section through part of an egg capsule and adjacent mucous matrix.

3.10 Histochemistry

Tables 9 and 10 present comparative histochemical staining properties of the different parts of the nidamental glands and egg masses of representative opisthobranch and outgroup taxa.

Table 9: Histochemistry of the nidamental glands of various opisthobranch, pulmonate and pyramidelloid taxa

SPECIES		ALCIAN PH 2.5	ALCIAN PH 1.0	AZAN	BPB	PAS	TOLUIDINE BLUE
Acteon tornatilis	albumen gland			blue	-	+	blue-pink
	membrane gland			red	-	-	violet
	mucous gland	+		-	-	++	red-violet
Scaphander nobilis	albumen gland	-			-	+	blue
	membrane gland						violet
	mucous gland	+			-	++	red-violet
Haminoea cymbalum	capsule gland					-	dark blue
	membrane gland					-	red
	mucous gland	+				-	violet
Chelidonura inornata	capsule gland			blue		+	dark blue
	membrane gland			-		-	violet
	mucous gland			-		-	violet
Philinopsis gardineri	capsule gland						dark blue
	membrane gland						pink
	mucous gland	+				-	violet
Aplysia punctata	albumen gland	-	-		-	++	blue
	membrane gland	+	++		-	+	violet-pink
	mucous gland	+	++			++	red-violet
	oviduct gland	-				+++	dark purple
	spermoviduct gland	-				-	blue-green
Bursatella leachii	albumen gland	-	-	blue	+	+	blue
	membrane gland	+	+	light blue	-	-	red
	mucous gland	++	+	-	-	++	violet
	oviduct gland	-	-	blue	-	+++	dark purple
	spermoviduct gland	-	-	pink	+	-	blue
Phyllaplysia taylori	albumen gland	-	-		+	-	blue
	membrane gland	-	+			-	light violet
	mucous gland	+	++		-	++	violet
	oviduct gland	-	-		-	+++	purple
	spermoviduct gland	-	-		-	-	blue
Oxynoe viridis	albumen gland				+	+	blue
	membrane gland					+	pink
	mucous gland	+			-	+++	violet
	adhesive region	+++			-	+++	dark purple
Elysia ornata	albumen gland			blue			darl blue
	membrane gland						violet
	mucous gland						pink
Elysia viridis	albumen gland		-		-	+++	dark blue
	membrane gland	+	-		-	+	violet-pink
	mucous gland	+++	+++		-	+++	violet

SPECIES		ALCIAN PH 2.5	ALCIAN PH 1.0	AZAN	BPB	PAS	TOLUIDINE BLUE
Thuridilla hopei	albumen gland				-	+++	blue
	membrane gland					++	dark violet
	mucous gland				-	+	pink
Tylodina perversa	capsule gland	-	-		-	-	blue
	membrane gland	-	-		-	-	violet
	mucous gland	+	+		-	+	purple
Berthella stellata	capsule gland	-			-	-	blue
	membrane gland	+				++	violet
	mucous gland	++			-	+++	red-violet
Euselenops luniceps	capsule gland	++			-	+++	blue
	membrane gland	+				+	pink
	mucous gland	+			-	++	violet
Adalaria proxima	capsule gland				-	+	blue
	membrane gland				-	-	pink
	mucous gland				-	+++	violet
Acanthodoris pilosa	capsule gland	-			-	+++	blue
	membrane gland				-	-	pink
	mucous gland		+		-	++	violet
Polycera quadrilineata	capsule gland	-			++	-	blue
	membrane gland	-			-	-	pink
	mucous gland	+	++		-		red-violet
Chromodoris magnifica	capsule gland	-	-	blue	+++	++	blue
	membrane gland	+	+		-	-	pink-violet
	mucous gland	++	+	light blue	-	+++	red-violet
Lomanotus vermiformis	capsule gland	-	-		+	+++	blue
	membrane gland				-	-	light pink
	mucous gland	++	+		-	++	red-violet
Tritonia plebeia	capsule gland	-			-	-	dark blue
	membrane gland	+			-	-	pink
	mucous gland	++				+	red-violet
Flabellina gracilis	capsule gland	-			-	++	blue
	membrane gland	+				+	pink
	mucous gland	+++			-	++	red-purple
Dermato-branchus semistriatus	capsule gland	-	-	blue	+	+++	dark blue
	membrane gland	+		-	-	-	pink
	mucous gland	++		light blue	-	+++	violet
Pyramidella sulcata	albumen gland	-			+++	++	blue
	membrane gland	+			-	+	violet
	mucous gland	++			-	+	violet
Lymnaea stagnalis	albumen gland				+	+++	dark blue
	membrane gland					+	red-violet
	mucous gland				-	++	pink-purple

-: not stained; +: weakly stained; ++: moderately stained; +++: brightly stained; blank space: no results; Bpb: bromephenol blue (this coding also applies to table 10, see following page)

Table 10: Histochemistry of the egg masses of various opisthobranch taxa

SPECIES		ALCIAN 2.5	ALCIAN 1.0	AZAN	BPB	PAS	TOLUIDINE BLUE
Acteocina atrata	albumen			blue	+++	+++	blue
	capsule	+		light blue	++	+++	violet
	mucous matrix	+		light blue	-	++	pink/violet
	outer mucous cover	+++		blue	-	+++	violet
Haminoea cymbalum	capsule					++	violet
	mucous matrix					+	light violet
	outer mucous cover					-	dark violet
Aplysia punctata	albumen		-	-	-	+	blue
	capsule		+	-	+	++	dark violet
	mucous matrix		+	-	-	+	violet
	outer mucous cover		+++	blue	-	+++	dark violet
Bursatella leachii	albumen	-			+	+++	blue
	capsule	-			+	-	dark violet
	mucous matrix	++			-	-	violet
	outer mucous cover	++			-	++	dark violet
Phyllaplysia taylori	albumen	-	-	blue	+	+	blue
	capsule	-	-	orange	+	-	blue
	mucous matrix	+	+	light blue	-	+	violet
	outer mucous cover	++	+	light blue	-	++	dark violet
Elysia ornata	albumen	-		blue	++	+++	blue
	capsule	-		light blue	+	++	violet
	mucous matrix	++		light blue	-	++	pink
	outer mucous cover	++			+	+	violet
Acanhtodoris pilosa	capsule	outer layer +	+	light blue	++	++	blue/violet
	mucous matrix	++	+	light blue	-	+	pink
	outer mucous cover	++	+		-	+++	dark violet
Dendrodoris nigra	capsule	outer layer +				+	dark violet
	mucous matrix	+				+	violet
	outer mucous cover	++				+++	dark blue/violet
Polycera quadrilineata	capsule	+				++	dark blue
	mucous matrix	++				+	light pink
	outer mucous cover	+++				+++	dark violet
Lomanotus vermiformis	capsule	-			+	+	blue/violet
	mucous matrix	-			-	-	
	outer mucous cover	++			-	++	violet
Tritonia plebeia	capsule	-	-		+		dark blue
	mucous matrix	-	-		-		
	outer mucous cover	++	++		-	+++	blue - violet
Dermato-branchus semistriatus	capsule	+			+	++	blue - violet
	mucous matrix	+			-	+	light violet
	outer mucous cover	+++			-	+++	dark violet

Since not all staining reactions could be applied to all species, the data gained here are rather incomplete. However, some general results are obvious.

The contrast in the staining reactions of the albumen - capsule glands versus the membrane and mucous glands is already apparent in the toluidine blue staining. While the former stain bluish, the latter always stain red to violet. This leads to the conclusion that the albumen and capsule glands contain basophilic muco-substances, while the membrane

and mucous glands produce acidophilic muco-substances. This is also apparent in the staining properties of the albumen fluid and capsules and the mucous layers in the egg masses, respectively. The PAS-positive staining reactions of the albumen and the capsule (and the albumen/capsule glands) point towards the presence of neutral polysaccharides. Albumen and capsule glands as well as the albumen and capsules of the egg mass show a positive reaction to bromephenol blue. Thus proteins are probably also present in the glands and egg mass structures. Neutral polysaccharides are also present in the membrane and mucous glands (mucous layers of the egg masses). Since the staining reactions of these structures with the Alcian stains (pH 1 and pH 2.5) are positive, a presence of acidic, sulphated mucopolysaccharides is suggested. Some conspicuous staining reactions are worth mentioning: In *Acteocina atrata* and *Aplysia punctata* the capsule is also stained by Alcian. The same holds true for the outer capsule layers of *Acanthodoris pilosa* and *Dendrodoris nigra* as well as the capsule of *Polycera quadrilineata* and *Dermatobranchus semistriatus*. In these cases the capsules seem to contain also acidic mucopolysaccharides, although the capsule glands of the nudibranchs mentioned do not produce these substances. This incongruence will be of importance later, when the functional morphology of the nidamental glands will be discussed (chapter 4.4). The capsule gland of *Euselenops luniceps* also shows a positive staining reaction to Alcian pH 2.5. In comparison to the other species this reaction is rather unusual and requires further investigation, especially of the egg masses of this specie.

It was hoped that the Azan reaction, as a tri-chrome staining sensitive to various glandular tissues, would shed more light on the differentiation between albumen and capsule glands. This was not achieved. The staining reactions of both glandular types to all stains (including Azan) are very similar, suggesting that similar substances are produced by these glands. In the egg masses the albumen fluid within the egg capsules as well as the capsules themselves also have similar staining.

3.10.1 Discussion

In general it needs to be mentioned that the data gained by the histochemical study are very incomplete and can only lead to preliminary conclusions. In the future more intensive studies with various different stains and possibly also with immuno-histochemical and

immuno-cytochemical methods need to be applied to further characterise the biochemical substances within the nidamental glands and the egg mass layers.

Histochemical studies on glandular parts of the reproductive systems as well as on egg masses are quite abundant among gastropods but are rather sparse in opithobranchs. Bayne (1968) presented a histochemical study of the egg masses of various pulmonate, one prosobranch and one opisthobranch (*Aplysia punctata*) species. His results on *Aplysia punctata* are congruent with my findings and seem to be conform with the other species Bayne investigated. He found neutral polysaccharides as well as proteins in the nutritive fluids and acidic, sulphated mucopolysaccharides in the supportive layers of the egg masses. Okotore et al. (1982) found a strong fluorescence after applying antibodies against galactans to the albumen gland of pulmonate snails. Bretschneider (1948), Grainger & Shillitoe (1952), Rangarao (1963), Bayne (1966), de Jong Brink (1969), Plesch et al. (1971) and Els (1978) reported of the presence of galactogen in the albumen glands of pulmonates. Bayne (1968) doubted that the neutral polysaccharide present in the albumen fluid of prosobranchs and opisthobranchs is also galactogen. Instead he speculated that in these taxa galactose and fucose might be present instead, which Horstmann (1959) reported to be present in the eggs (not further specified which parts of the eggs) of e. g. *Aplysia depilans* and *A. limacina*. Els (1978) stated that galactose and glucose function as precursors for galactogen (in pulmonates), but since galactose is not normally present in food the author assumed glucose to serve as the precursor for galactogen. The histochemical results on the mucous glands and mucous layers within the egg masses are very uniform throughout various gastropod groups. Plesch et al. (1971) presented an extensive histochemical study of the reproductive tract and egg mass of *Lymnaea stagnalis*; their findings correspond to the data I gained in my study. The authors found the mucous glands in the reproductive system as well as the mucous layers in the egg mass to contain acidic, sulphated mucopolysaccharides. These were also reported for equivalent structures in other pulmonate species (de Jong Brink 1969; Els 1978). Wägele´s (1989b) results on the histochemical staining reactions of the layers in egg masses of Antarctic nudibranchs accord with my own results.

We are approaching a new age of synthesis, when the testing of consilience is the greatest of all intellectual challenges. Philosophy, the contemplation of the unkown, is a shrinking dominion. We have the common goal of turning as much philosophy as possible into science.

Edward O. Wilson

4. CONCLUSIONS

4.1. The homology concept

Although the term `homology´ has been used in various sciences long before Darwin, today it is generally agreed that all aspects of the definition and analysis of homology (in comparative biological sciences) must be based on the principles of the evolutionary theory (Bock 1989). The identification of homologous structures is the first and fundamental step in the study of phylogenetic relationships (Osche 1973). With other words "it is a genuine scientific task to work out hypotheses on homology of structures. Moreover, homologies play a central role in the reconstruction of phylogeny" (Dohle 1989:355). Thus the first definition of homology, postulated by Owen in 1843, as "the same organ in different animals under every form and function" (cited after Bock 1989) has been given a more specific, phylogenetic context. Wagner (1989) and Roth (1984) call this the historical or phylogenetic homology concept, respectively. Both authors demand a more inclusive biological homology concept, which not only includes the continuity of descent from a common ancestor but also considers the correspondence between parts of the same organism, referred to as iterative homology or homonomy. Others, for example Bock (1989), reject the inclusion of iterative homology in the general homology concept. The most commonly used homology concept is that of common descent. Dohle (1989:355), for example, regards "corresponding or similar structures which can be traced back to a common origin... as homologous". In order to use homologies for phylogenetic analyses the condition must be satisfied that the homologous structures are hereditary (Osche 1973; Sudhaus 1980). A coincidence of similarity in structure has to be excluded, in order to properly identify homologues. Thus "homology can be inferred when the similarities of two structures are so complex that another explanation than a common origin can virtually be excluded" (Dohle 1989:355). The identification of homologous

structures is the most difficult and yet the most important task in phylogenetic studies. By comparing similar structures in different organisms it is generally agreed to use certain criteria for the explanation of homologies. Remane (1952) postulated three main criteria for homology: the criterion of position, the criterion of specific quality and the criterion of continuity. If two structures have the same position in a comparable system of likewise homologous structures, if they are of similar or identical structure or even fine-structure and if the change from one stage of the considered structure to the other stage can be traced back gradually by the presence of intermediate stages (phylogenetically or ontogenetically), these two considered structures are most probably homologous. By applying these criteria it is important to confirm that position and specific quality are not coupled (Dohle 1989), but coincidence of two homologues underlines the probability of homology of each feature (Rieger & Tyler 1979). To summarize Remane's criteria in one criterion: "The only valid empirical test of hypotheses about homologues is similarity of all kinds between homologous features" (Bock 1989:327). Thus correspondence in complexity of the structures is the fundamental criterion for the explanation of homology. In Bock´s opinion the complexity of the feature (structure) influences the degree of confidence in the particular homologue, which in his eyes is "more difficult and far more important than the determination of the homologues themselves" (page 341). However, comparative morphological investigation in phylogenetic analyses cannot end with the determination of homologues. Two structures cannot definitely be considered homologous until the probability of them being analogous is dismissed (Rieger & Tyler 1979).

4.2 Homologues in the nidamental gland system of the Opisthobranchia and outgroup taxa

Based on the data gained from the present study of the nidamental glands of different Opisthobranchia and outgroup taxa the homologies of the different glandular parts are discussed. The main criterion applied here to explain homology is corresponding complexity. The more complex the similarity of the considered structures/organs is, the more probable their homologies are. The estimation of complexity is based on gross morphological, histological, histochemical and ultrastructural properties. It is also tried to

exclude the possibility of a structure being analogous to another by discussing the probabilities of these structures to have evolved independently due to similar selective pressures.

For a better comparison the main characteristics of the nidamental glands in the major taxa of the Opisthobranchia and in the outgroup taxa are listed in the following table 11 (page 143/144). Data on ciliation refer to the supporting cells, all other data to the glandular cells.

As can be seen from the data presented in chapter 3 all opisthobranch species investigated as well as the outgroup taxa possess three distinct glandular parts in the nidamental glandular system. These are the albumen or alternatively the capsule gland at the proximal part of the oviduct, followed by the membrane gland and further distally the mucous gland. In some species the most distal portion of the oviduct (or the oviducal channel of the spermoviduct) is also lined by glandular epithelium (adhesive region or oviducal gland, respectively). The relative positions of these different glandular parts are always the same in all species investigated. Accessory glands of the vestibulum or oviduct occur randomly in few species. In the Anaspidea a spermoviduct gland lining the autospermal groove of the spermoviduct and an atrial gland, discharging into the distal part of the spermoviduct near the genital aperture, are present additionally to the nidamental glands mentioned before.

Common to all glands is the pattern of alternating glandular and ciliated supporting cells. The four glandular parts of the nidamental glandular system can be divided into two types of glands, based on their histochemical staining properties: on the one hand we find the basophilic albumen and capsule glands, on the other hand the acidophilic membrane and mucous glands and the acidophilic adhesive region (or oviducal gland). The secretory products of the albumen and capsule glands appear to contain neutral polysaccharides as well as proteins, whereas the membrane and mucous glands secrete neutral polysaccharides and acidic mucopolysaccharides. The structures of the secretory products in the two glandular types also differ considerably. The secretions of the albumen and capsule glands are packed into distinct vesicles or granules, whereas the secretions of the membrane and mucous glands take the form of either a homogeneous mass, heterogeneous fibres or irregular droplets.

If we compare the structure of the glands throughout the taxa investigated we find certain characteristics for each gland which are similar throughout the taxa.

The **albumen gland** can have the form of a sac or a tube. It discharges either into the oviduct or into a fertilization chamber, but never directly into the adjacent membrane gland. The secretory products are mostly packed in form of large round or elliptically-shaped vesicles and have a homogeneous or amorphous structure. In *Aplysia punctata* filamentous substructures are found within the electron-dense substance of the secretory vesicles (see fig. 8B). De Jong Brink (1969) also showed the albumen vesicles of *Biomphalaria glabrata* to have a similar fine structure as those found in *Aplysia punctata*. Additionally to the secretory products the glandular cells contain active nuclei and large areas of endoplasmic reticulum and Golgi complexes in the basal part of the cell, even if the cell is not actively secreting. This suggests that the glandular cells are merocrine, i. e. that they keep the capability to actively secrete throughout the span of reproductive activity of the animal. The supporting cells of the albumen gland bear short cilia (see tables 3-8).

The **capsule gland** is always tubular and mostly narrowly coiled. The secretory products take the form of distinct round to irregularly shaped granules, their size is generally smaller than the size of the albumen vesicles. The granules can be of homogeneous texture or the electron-dense granule matrix can contain even more electron-dense substructures. As has been described for the albumen gland, the capsule gland is also merocrine, the glandular cells containing large active nuclei, prominent ER and Golgi complexes. Therefore, similar to the albumen gland, the capability of secretion throughout the lifetime of the animal can be asumed (see also Schmekel 1971). The supporting cells in the capsule gland bear short cilia (compare tables 3-8). The gland discharges either into the distal oviduct or into the membrane gland.

The **membrane gland** is also tubular and often as narrowly coiled as the capsule gland. In contrast to the latter it comprises a smaller glandular area. The mostly heterogeneous secretory contents of the glandular cells are typically packed into small mucous vesicles and either form a dense meshwork of mucous filaments or compact mucous coagulation. ER cannot be found in the glandular cells, and the nuclei often have a pycnotic appearance. It seems as if the glandular cells of the membrane gland stop their production of secretory material once they have reached full maturity. It is noticeable that in contrast

to the other glandular parts the supporting cells of the membrane gland always bear very long cilia (compare tables 3-8).

The **mucous gland** can either be a continuous transition of the membrane gland or originate in the distal oviduct or spermoviduct, respectively. It comprises a large tube with mostly wide coils in contrast to the narrowly coiled membrane gland. The glandular cells of the mucous gland are generally higher than those of the membrane gland, but this character is not very sound since the height of cells also depends on the angle of sectioning. The secretions in the mucous gland can also take various textures, from homogeneous masses to heterogeneous filaments or irregular droplets. Ultrastructural investigation reveals that the secretory products of the mucous gland are packed into less distinct and generally larger mucous vesicles than those of the membrane gland. Compact coagulations of mucous fibres were never found in the mucous gland. The heterogeneity of the mucous texture in the various glandular cells of the mucous gland might be due to different functional stages of the cells (see also Schmekel 1971; Wägele 1989a). As will be discussed later the mucous gland produces different layers of mucus within the egg mass which have different fine structures (see chapter 4.4). The cilia of the supporting cells are generally shorter in the mucous gland than in the membrane gland (compare table 3-8). The glandular cells do not contain ER and mostly have very electron-dense or even pycnotic nuclei. As in the membrane gland it seems that the cells stop secretion once they are fully mature. This has also been observed by Schmekel (1971).

The **adhesive region** and the **oviducal gland** comprise mostly tubular structures or simply line a certain area of the tube-like distal oviduct. The glandular cells contain secretory granules or vesicles of different shape and size. Active nuclei (and in the oviducal gland of *Aplysia punctata* also prominent ER and Golgi complexes) are almost always found, suggesting that the cells are merocrine.

In anaspids two additional glandular areas can be found, which also show certain characteristic features. The **spermoviduct gland** always lines the autospermal groove of the spermoviduct. The glandular cells are filled with distinct vesicles showing basophilic staining properties. The supporting cells bear short cilia. The **atrial gland** is narrowly coiled and discharges via a small non-glandular duct into the distal spermoviduct. Its glandular cells contain large vesicles of homogeneous or amorphous texture. The supporting cells are studded with short cilia.

Based on the similar relative position within the nidamental glandular system, gross morphology, histological characters, ultrastructure and histochemical staining properties I assume that the albumen glands, the capsule glands, the membrane glands and the mucous glands comprise homologous glandular parts in the various taxa of the Opisthobranchia, Gymnomorpha, Pulmonata and Pyramidelloidea. To summarize, the main arguments that support the hypotheses for these homologies are:

1. **Identical position of the glandular parts within a (hypothesized) homologous glandular system.** The albumen gland and the capsule gland always occur at the most proximal end of the oviduct or spermoviduct, respectively. The next gland further distally is the membrane gland and the mucous gland occurs most distally.

2. **Similar histology and ultrastructure of the glandular epithelium.** The vesicles in the albumen gland are of amorphous structure, composed of substances of different densities (compare for example the texture of the vesicles in the albumen gland of *Aplysia punctata* (fig. 7D) presented here with the structure shown for the corresponding feature in *Biomphalaria glabrata* (de Jong Brink 1969). The texture of the granules in the capsule glands is either homogeneously electron-dense or the granules contain more electron-dense substructures. The texture of the mucous vesicles in the membrane glands are also similar throughout the taxa (dense meshwork or compact coagulations) and differ from the mucous vesicles in the mucous gland (loose meshwork). The supporting cells in the membrane glands always bear extremely long cilia in contrast to the other glandular parts. All glands show the same pattern of alternating glandular and supporting cells.

3. **The similar mode of secretion**. The albumen glands are merocrine, so are the capsule glands, whereas membrane and mucous glands stop secreting when the cells have reached full maturity.

4. **The similar histochemical staining properties**. Albumen glands and capsule glands are basophilic and contain neutral polysaccharides and

proteins; membrane and mucous glands are acidophilic and contain neutral polysaccharides and acidic mucopolysaccharides.

Generally speaking the features adduced here to support the hypotheses about homologous structures within the nidamental glandular system of Euthyneura and Pyramidelloidea show a high degree of complexity and therefore strengthen the confidence in these hypotheses. Nevertheless, one could argue against the position of the glands as a very sound argument for homology since a successive addition of the various secretions of the nidamental glands demands for the successive position of the glands within the system. However, the different layers within an egg mass do not necessarily have to be secreted by glandular tissues which show exactly this location within the organ systems of the animal. In order to exclude the possibility that the different nidamental glands have evolved independently within the studied taxa, we need to consider the selective pressures which have led to the evolution of these systems. The nidamental glands are responsible for the formation of a gelatinous egg mass which is laid down on suitable substrate in a marine (Opisthobranchia, Gymnomorpha, Pyramidealloidea) or freshwater (Pulmonata) habitat. Other taxa within the Mollusca, which have to meet the same requirements with regards to their reproduction, have developed different modes of egg mass production and thus different structures within the reproductive and other organ systems. For example, some bivalves (*Nucula delphinodonta* and *Turtonia minuta*) use secretions of the hypobranchial gland to secrete egg capsules surrounding the embryos (Fretter & Graham 1964). Many prosobranchs envelope their eggs and allosperm in one large, fibrous capsule (D´Asaro 1988), rather than in individual, thin, membranous egg capsules, as found in opisthobranchs. Various prosobranchs include additional nutritive nurse eggs within the egg capsules (Fretter & Graham 1964). These nurse eggs serve, additionally to the albumen, as a food source for the developing embryos. Mucous secretions can come from a variety of sources since glandular cells producing mucous are present in various organs or epithelia. Secretions for agglutination of eggs to form an egg mass come from the pedal sole in *Acmaea tessulata* and from the enlarged urogenitalpapilla in *Margarites lulicinus* and *Calliostoma zizyphinum*, all belonging to the archaeogastropods (Fretter & Graham 1964). According to the latter authors, the opisthobranch *Tethys* secretes a sticky substance from a fold of the upper lip and adds it to the egg mass, which

is then attached to the substrate. These few examples show that a variety of possibilities exist to realize the secretion of different layers to form an egg mass, in which the offspring can develop independently from the parents. Hence, it can be concluded that an analogous development of such similar structures as the nidamental glands in the studied gastropod taxa is less likely than homologies among these glands.

Based on the identical position and the similar histochemistry and ultrastructure it is further concluded that the albumen glands and capsule glands are homologous as the most proximal glandular parts in the nidamental glandular system, but that the albumen glands have undergone a functional change in the evolution of higher Opisthobranchia by forming the inner capsule layer (see also chapter 4.5). Further comparative ultrastructural, histochemical and biochemical studies would elucidate this question more. If, for example, the biochemical components of the albumen and the inner capsule layer (and the corresponding secretory products in the albumen and capsule glands) are the same (possibly galactogen or derivatives), a homology between these structures is even more probable.

The conclusions on the hypotheses about homologies of the various glandular parts within the nidamental glands finally dismiss the speculations about possible homologies of "membrane" or "winding glands" in Anaspidea and Sacoglossa with the "capsule gland" in Nudibranchia, as have been put forward by Sanders-Esser (1984), Hadfield & Switzer-Dunlap(1984) and Wägele (1987), respectively.

Whether the glandular tissue of the oviduct (adhesive region) and the oviducal gland are also homologous in the taxa described cannot be conclusively clarified at this point in time. Since the position of the glandular tissue varies considerably within the major superordinate taxa (compare for example the Anaspidea, the Nudibranchia and *Laemodonta*), it is also possible that the glandular tissue just resembles a specialised part of the mucous gland, having evolved independently in the major taxa. As will be disussed in the chapter on functional morphology (chapter 4.4), the glandular tissue of the distal oviduct and the oviducal gland probably secrete a sticky substance, attaching the strings of the egg ribbon to one another or to the substrate. Thus similar functional selective pressures might have led to the independent development of these glandular tissues in the various major superordinate taxa. Nevertheless, a homology of these glandular parts

within the superordinate taxa is possible (at least within the Anaspidea and Nudibranchia due to similar position, histological structure and histochemical staining properties).

The atrial glands and spermoviduct glands within the anaspidean taxa are most probably also homologous because of their congruous complex structure. Whether the spermoviduct glands of the anaspids are homologous with the prostate of other taxa (as has been suggested by Ghiselin 1965 and Thompson & Bebbington 1969) still has to be clarified by extensive comparative studies.

Table 11: Main characteristics of the nidamental glands in the major opisthobranch and outgroup taxa

TAXON	ALBUMEN GLAND	CAPSULE GLAND	MEMBRANE GLAND	MUCOUS GLAND	ADHESIVE REGION/OVI-DUCAL GLAND
"Cephalaspidea s. l."	sac-like, seldom tubular; distinct vesicles, mostly large and composed of different substances; active nuclei; short cilia; basophilic; containing neutral polysaccharides.	tubular; small granules of homogeneous texture; active nuclei; short cilia; basophilic; containing neutral polysaccharides.	tubular; narrow coils; mucous contents in form of homogeneous masses or small droplets; pycnotic nuclei; long cilia; acidophilic.	tubular; wide coils; mucous contents in form of homogeneous masses, heterogeneous fibres or droplets; pycnotic nuclei; short cilia; acidophilic; containing neutral polysaccharides and acid muco-polysaccharides.	few mucus cells containing homogeneous mucous masses, mucous fibres or droplets; pycnotic nuclei; short cilia; acidophilic.
Anaspidea	sac-like; internally folded; large vesicles containing heterogeneous or amorphous substances; active nuclei; inconspicuous supporting cells with short cilia; basophilic; containing proteins and neutral polysaccharides.		tubular; narrow coils; mucous contents in form of heterogeneous fibres forming coagulations or droplets; pycnotic nuclei; long cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; internally secondary folds; homogeneous or heterogeneous mucous contents or droplets; pycnotic nuclei; short cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	epithelium lines oviducal part of spermoviduct; small granules; active nuclei; moderately long cilia; acidophilic; containing neutral polysaccharides.
Sacoglossa	compact tubular or highly branched in the parapodia; large vesicles composed of substances of different density; active nuclei; short cilia; basophilic; containing neutral polysaccharides and proteins.		narrowly coiled tube or lining efferent duct of mucous gland in most proximal part; mucous contents in form of droplets; pycnotic nuclei; moderately long cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; mucous contents in form of homogeneous masses or heterogeneous filaments or droplets; pycnotic nuclei; short cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; irregularly shaped vesicles; active nuclei; moderately long cilia; acidophilic; containing neutral polysaccharides and acidic muco-poly-saccharides.
Tylodinoidea		tubular; narrow coils; granules with fine substructures; small nuclei; inconspicuous supporting cells; basophilic.	tubular; narrow coils; heterogeneous mucous filaments or droplets; pycnotic nuclei; long cilia; acidophilic.	tubular; wide coils; mucous contents in form of homogeneous masses or droplets; pycnotic nuclei; short cilia; acidophilic; containing neutral polysaccharides and acidic mucopolysaccharides.	

TAXON	ALBUMEN GLAND	CAPSULE GLAND	MEMBRANE GLAND	MUCOUS GLAND	ADHESIVE REGION/OVI-DUCAL GLAND
Pleurobranchoidea		tubular; narrow coils; granules of homogeneous structure; active nuclei; moderately long cilia; basophilic; containing neutral polysaccharides and possibly acidic mucopoly-saccharides.	tubular; narrow coils; mucous contents in form of homogeneous masses, heterogeneous fibres or droplets; pycnotic nuclei; long cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; wide coils; mucous contents in form of homogeneous masses, heterogeneous filaments or droplets; short cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	
Nudibranchia		tubular; mostly narrow coils; granules of homogeneous structure or with substructures; active nuclei; short cilia; basophilic; containing neutral polysaccharides and proteins.	tubular; narrow coils; mucous contents in form of heterogenous fibres or droplets; ultrastructurally mucus forms meshwork or coagulations; nuclei pycnotic (except when cells are actively secreting); long cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; wider coils; mucous contents in form of homogeneous masses, heterogeneous filaments or droplets; ultrastructurally mucous fibres are more loosely packed than in membrane gland; pycnotic nuclei; short cilia; acidophilic, containing neutral polysaccharides and acidic muco-polysaccharides.	small epithelium lining distal oviduct; round to irregularly shaped granules; active nuclei; moderately long cilia; acidophilic.
Outgroup taxa	densely tubular; large secretory vesicles with homogeneous or amorphous contents; active nuclei; short to moderately long cilia; basophilic; containing neutral polysaccharides and proteins.		tubular; narrow coils; moucus contents in form of heterogeneous fibres or droplets; pycnotic nuclei; long cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	tubular; wide coils or parallel folds; mucous contents in form of homogeneous masses, heterogeneous fibres or droplets; pycnotic nuclei; short cilia; acidophilic; containing neutral polysaccharides and acidic muco-polysaccharides.	lining distal oviduct almost completely; granules of homogeneous texture; short cilia; acidophilic.

4.3 Homologues in the egg masses of the Opisthobranchia and Pulmonata

As has been shown in chapter 3. 9 the egg masses of opisthobranchs and pulmonates are very uniform in their fine structure. Table 12 shows the main characteristics of the different structures in the studied taxa.

Table 12: Main characteristics of the different structures within the egg masses of the major opisthobranch taxa and the Pulmonata.

TAXON	ALBUMEN	CAPSULE	INNER MUCOUS LAYER	MUCOUS MATRIX	OUTER MUCOUS COVER
"Cephalaspidea s. l."	present in *Acteocina* - flocculent mass; basophilic	one compact layer in *Acteocina* and *Chelidonura*, acido-philic; two layers in *Haminoea;* inner layer basophilic, outer layer acidophilic.	homogeneous texture; acidophilic.	fine filamentous structure; droplets visible; mucus strands; acidophilic.	multi-layered; layers of various textures; acidophilic.
Anaspidea	flocculent or compact mass; basophilic.	one compact layer; acidophilic; in *Phyllaplysia* basophilic	filamentous to homogeneous in texture; acidophilic.	dense meshwork; acidophilic.	multi-layered; layers of various textures; acidophilic.
Sacoglossa	flocculent mass; basophilic.	one compact layer; acidophilic.	filamentous in texture; acidophilic.	partly homogeneous, partly filamentous structure; acidophilic.	multi-layered; layers of various textures; acidophilic.
Pleuro-branchoidea (pers. observ.; Wägele 1996)	absent	two layers; outer layer acodiphilic; inner layer basophilic	not distinguishable	dense meshwork	multi-layered; layers of various textures; acidophilic.
Nudibranchia	absent	two layers; outer layer more compact than inner layer, acidophilic; inner layer basophilic.	homogeneous, seldom heterogeneous in texture; acidophilic.	loose filamentous to dense structure; acidophilic.	multi-layered; layers of various textures; acidophilic.
Pulmonata	compact mass, basophilic.	one layer; acidophilic.	multi-layered; layers of various textures; basophilic and acidophilic.	only heterogeneous mucous strands visible; acidophilic.	multi-layered; layers of various textures; acidophilic.

The same criteria (except for criterion 3) as have been adduced for the homologies of the various nidamental glands are put forward here for the homologies of the various structures within the egg masses of opisthobranchs and pulmonates. Because in all studied taxa the general structure of the egg mass is very similar and because corresponding layers (with similar functions, see chapter 4.4) can be found in all species, I conclude that the

fine-structures of the egg masses, as they have been described here, namely the membranes, the inner capsule layers (in some "Cephalaspidea s. l."; Pleurobranchoidea and Nudibranchia), the inner mucous layer, the mucous matrix and the outer mucous cover, are homologous structures throughout the taxa. The albumen found in some "Cephalaspidea s. l.", Anaspidea, Sacoglossa and the Pulmonata is also considered to be homologous in these taxa.

Special attention has to be paid to the structure of the capsule. In some "Cephalaspidea s. l.", Anaspidea, Sacoglossa and in the Pulmonata the capsule is composed of one layer, namely the membrane, showing acidophilic staining properties. In Pleurobranchoidea (pers. observ.) and Nudibranchia the capsule is composed of two layers, an acidophilic outer layer (membrane) and a basophilic inner layer. Since the inner capsule layer has been shown to dissolve and probably is ingested by the larvae during intracapsular development (see chapters 3.9.9 and 3.9.11), I assume that this layer serves a nutritive function for the veliger similar to the albumen in pulmonate and other opisthobranch taxa. This is further supported by the likely homology of albumen and capsule gland (see previous chapter) and I conclude that the inner capsule layer in the egg masses of the "Cephalaspidea"-taxa *Haminoea*, *Chelidonura* and *Philinopsis*, as well as the Pleurobranchoidea and Nudibranchia is homologous to the albumen in the other "Cephalaspidea" (*Acteocina*), the Anaspidea, Sacoglossa and Pulmonata. The inner capsule layer is not visible until a distinct intracapsular space between the embryo and capsule is formed. This space occurs during egg movement through the mucous gland of the nidamental glandular system (compare chapter 3.7.1). It seems that here the capsule material, which has been deposited around the eggs, coagulates to a compact layer. Thus the inner capsule layer appears to be made from coagulated albumen, which Bayne (1966, 1967) has also found in the pulmonate *Agriolimax reticulatus* and de Jong Brink (1969) in *Biomphalaria glabrata*. Plesch et al. (1971) doubted that this is also true for *Lymnaea stagnalis*. The outer membrane layer in *Haminoea*, *Chelidonura*, *Philinopsis* and in the Pleurobranchoidea and Nudibranchia, in contrast, is homologous to the membranes in *Acteocina* and the Anaspidea and Sacoglossa as well as the pulmonates.

If this hypothesis is true, we would also expect, on further investigation, the Tylodinoidea (which posses a capsule gland) and other Pleurobranchoidea to possess two-layered egg capsules in which the inner layer is dissolving and ingested by the veliger. It could be

further expected to find always one membranous layer, which is not dissolved during intracapsular development, in all Cephalaspidea possessing an albumen gland and in the Anaspidea and Sacoglossa. However, exceptions are found in *Chelidonura inornata*, *Onchidoris bilamellata* and *Phyllaplysia taylori*. The former two species possess a capsule gland, but the egg capsules show to be composed of only one layer under the light microscope. Possibly the second layer is so thin that it cannot be detected with low power microscopy, or it has already been dissolved before the preservation of the egg masses. The basophilic membrane found in *Phyllaplysia taylori* also stands in contrast to the other findings. This anaspidean species would be expected to have an acidophilic capsule layer, but with AZAN staining the capsule of *Phyllaplysia taylori* stains bright orange, almost the same staining property as found for the yolk vesicles in the embryo. Since *Phyllaplysia taylori* is reported to have direct development (Bridges 1975), the membrane might contain some different nutritive substances, which possibly serve for the larvae as an additional food source just before hatching, when the capsules are dissolved. Further histochemical and biochemical studies might help to elucidate these findings. An analogous development of the various layers within the egg masses of the taxa investigated is considered less likely than homologous generation, due to the same reasons that have been stated in the previous chapter for the development of the nidamental glands. Formation of such similar structures of the egg mass, as has been described here for the various opisthobranch and pulmonate species, is not neccessarily compulsory because of similar selective pressures.

4.4 Aspects of functional morphology

a) NIDAMENTAL GLANDS:

The nidamental glands of the two nudibranch species *Acanthodoris pilosa* and *Chromodoris magnifica* have been preserved during the process of spawning. By studying serial sections of these organ systems, the passage of the eggs through the glands could be reconstructed. The findings gave first hints for the function of the various glandular parts in the formation of the egg mass. The data about the fine structure of the glands and different layers of the egg masses lent further support for the hypotheses about the functional morphology of the nidamental glands postulated in the following.

As has been described for *Chromodoris magnifica*, the eggs, which enter the oviduct through the ampulla and the postampullary duct, pass the first glandular part of the oviduct, namely the capsule gland, in groups. The absence of eggs in the capsule gland of *Acanthodoris pilosa* is probably due to the fact that the eggs had already passed through this gland before the animal had been preserved. The animal had already produced an almost complete spawn mass. Although capsule material is secreted in the capsule gland (see fig. 16A), the eggs are not encapsulated there. They merely pass the narrow coils of the gland and are transported through to the next glandular part, the membrane gland. The capsule material is also transported to the membrane gland, because eggs can be found embedded in capsular material within the lumen of this gland (see fig. 16B). In the membrane gland mucous substances are secreted and added to the mass of eggs and capsule material. Characteristic of the epithelium of the membrane gland is the heavy ciliation of the supporting cells. Additionally, a higher number of mitochondria was found in the supporting cells of this glandular part. I assume that these fine structural properties serve the same function: within the membrane gland, the eggs are rotated around their own axis (a very energy consuming process, for which many mitochondria are needed), while at the same time the capsule material is portioned and layed around the eggs. This capsule material forms the inner capsule layer of the pleurobranchoid and nudibranch egg capsules. Furthermore, secretions from the glandular cells of the membrane gland are added to the outside of the inner capsule layer, thus forming the membrane layer. As the encapsulated eggs enter the proximal part of the mucous gland they are enveloped in an inner mucous layer (see fig. 16C) and arranged in tube-like structures. The egg cordon passes through the mucous gland, where the various mucous layers (mucous matrix, outer mucus cover) are added successively (see fig. 16D, E). The coils of the mucous gland are much wider distally than those of the membrane gland and capsule gland, owing to the fact that the egg cordon becomes larger and broader as it moves through the glandular mass. In the most distal region of the mucous gland the egg mass forms a broad band, the egg capsules are arranged in tubes and the tubes are already coiled within the mucoid spawn band. The adhesive region (or the oviducal gland of anaspids, respectively) probably secretes an adhesive substance forming the outermost layer of the egg mass, which aids in attaching the spawn ribbon to the substrate or in sticking the various parts of the egg string together, so the whole ribbon forms a tangled mass (fig. 25C) (see

description of outermost mucous layer in the egg mass of *Aplysia punctata*). This is concluded from the fibrous or fuzzy fine structure of the outermost mucus layer in most egg masses. Taking into consideration that the oviducal gland secretes a sticky substance, it is also obvious why *Aplysia punctata* has such a large oviducal gland compared to *Phyllaplysia taylori*, for example (compare fig. 4A and C). The spawn mass in *Aplysia punctata* comprises a large tangled mass, for which much sticky substance is needed, so that the various parts of the ribbon stick to each other. *Phyllaplysia taylori*, in contrast, produces flat egg masses, which are attached to the substrate with one side only, thus less adhesive substance is necessary. The function of the albumen gland could not be studied directly, but by comparing the structure of the glands and the albumen in the corresponding egg masses, it appears that the albumen gland secretes the albuminous fluid found within the one layered egg capsules of the pulmonate, cephalaspidean (*Acteocina atrata*), anaspid and sacoglossan species. The eggs are said not to traverse the albumen gland (see for example Eales 1921; Thompson & Bebbington 1969; Beeman 1970; Robles 1975), but secretions are poured upon them as they pass the opening of the albumen gland.

Although only two species of nudibranchs have been investigated in more detail with regards to the functional morphology of the nidamental glands, it is deduced here, due to the similarity of structure of the glands and egg masses, that the general function of the nidamental glands in formation of the egg masses is the same in all Euthyneura. I conclude that the different parts of the nidamental glands have the following functions:

albumen gland: secretion of albumen fluid;

capsule gland: secretion of material for the inner capsule layer;

membrane gland: secretion of outer capsule material; encapsulation of eggs by rotation of the eggs within the lumen of the gland;

mucous gland: successive secretion and addition of the inner mucous layer, the mucus matrix and the outer mucus cover

adhesive region / oviducal gland: secretion of adhesive substance

The functions proposed here for the different parts of the nidamental glands show to be very consistent with statements found scattered throughout the literature. Table 13 (pages 152-154) summarizes the functions that have been addressed by different authors. Since

the terminology of the different glandular parts is very inconsistent throughout the literature, the terms applied by the cited authors have been put in italics.

The data gathered here from the literature show that the ideas about the functional morphology of the nidamental glands mostly confirm the hypotheses proposed in the present study. A few aberrations, however, need to be mentioned. Thompson and Bebbington (1969) doubt Ghiselin´s (1965) observations, that the capsule is formed in the "winding gland"(= membrane gland). The former authors stated that the capsule in *Aplysia* was not visible until the egg mass had entered the distal part of the mucous gland. The results of the present study support Ghiselin´s findings, that the membranous capsule is formed in the membrane gland, but it may be possible that the capsule sheath, which is very thin in various species, might not be distinct until adjacent layers of mucus have been secreted, and this might be the reason, why Thompson and Bebbington (1969) have not been able to see the capsule until the egg mass had travelled further through the glandular mass (see also Hadfield & Switzer-Dunlap 1984). Rudman (1972c) did not find a membrane around the eggs in *Pupa*. He also could not find a membrane gland (he called it capsule gland) in *Pupa kirki*. This is a very unusual condition and Rudman´s findings have to be doubted, but if he was correct, this correlation would also support the hypothesis that the membrane gland secretes the membrane. Robles (1975) observed the oviducal gland of *Bulla gouldiana*, which lines the oviducal channel of the spermoviduct to exhibit a transition between the membrane gland and the primary lobe of the mucous gland and stated that the oviducal gland secretes layers of mucus which are added to the egg capsules. From the findings of the present investigation I doubt that Robles´ reconstruction of the pathway of the oviducal gland is correct. The oviducal gland was never found to be continuous with the membrane gland, but rather originates in the fertilization chamber of the monaulic system (see for example *Aplysia punctata*) and is confluent with the distal part of the distal mucous gland. Eales (1921), Fretter (1943) and Beeman (1970) assumed that the oviducal gland of *Aplysia*, *Onchidella celtica* and *Phyllaplysia taylori*, respectively, might serve to lubricate the egg string. All other authors stated here assume an adhesive function of the gland, as has also been postulated in the present study. The function of the oviducal gland remains unclear and has to be submitted to further investigation. Based on her comparative ultrastructural investigations of the nidamental glands of different nudibranchs, Schmekel believes that the mucoid contents of the

vescicles in the mucous gland build the inner mucous layers in the egg mass while the membranes of the vesicles form the outer layers. I doubt that this is the case. It is rather probable that the successive parts ot the mucous gland form the different layers as the egg ribbon moves through the glandular mass.

If the hypotheses about the function of the different parts of the nidamental glands are consolidated by future studies, a uniform terminology, based on functional morphology, would be necessary in the future. I would suggest the following terms for the successive glandular parts (from most proximal to the most distal region):

albumen gland (if present), **capsule gland** (if present), **membrane gland**, **mucous gland** and **adhesive region**.

Table 13: Data about the functions of the nidamental glands in various euthyneuran and pyramidelloid taxa, gathered from the literature

TAXON	ALBUMEN GLAND	CAPSULE GLAND	MEMBRANE GLAND	MUCOUS GLAND	ADHESIVE REGION/OVI-DUCAL GLAND	REFERENCE
Opisthobranchia in general (based on investigation of *Dendrodoris albopunctata*)	***albumen gland*** (note that *Dendrodoris* does not posses an albumen gland!) - production of albumen		***membrane gland*** - secretion of membrane	***mucous gland*** - adding layers of mucus to membranes		Ghiselin 1965
"Cephalaspidea s. l." (*Haminoea*)		***albumen gland*** - production of albumen	***capsule gland*** - eggs are encapsulated; eggs are joined together by short string	***posterior mucus gland*** - egg string receives inner coat ***anterior mucus gland*** - eggs are surrounded by outer mucus coat		Rudman 1971
"Cephalaspidea s. l." (*Melanochlamys*)			***capsule gland*** - tissue probably produces capsule			Rudman 1972
"Cephalaspidea s. l." (*Bulla*)	***albumen gland*** -production of albumen		***membrane gland*** -first layer of mucus or capsule secreted	***mucous gland*** - forming of distinct egg string	***oviducal gland*** - further layers of mucus are added to capsule	Robles 1975
Anaspidea (*Aplysia*)	***albumen gland*** - providing eggs with coating of albumen		***convoluted (winding) portion of mucous gland*** - probably makes the shell (capsule)	***mucous gland*** - shells (capsules) are coated with mucus, and the balls are strung together to make a cylindrical egg string	***glandular oviduct*** -secretion of lubricating fluid	Eales 1921
Anaspidea (*Aplysia*)	***albumen gland*** - production of albumen		***winding gland*** -addition of first mucous secretions to stream of ova and sperm	***mucous gland*** - addition of jelly; in more distal parts egg capsules have been formed and a stratified coat around the egg stream is visible	***female channel of wide distal hermaphroditic duct*** - secretion of adhesive substance, facilitating attachment of spawn ribbon to substrate	Thompson & Bebbington 1969

TAXON	ALBUMEN GLAND	CAPSULE GLAND	MEMBRANE GLAND	MUCOUS GLAND	ADHESIVE REGION/OVI-DUCAL GLAND	REFERENCE
Anaspidea (*Phyllaplysia*)	***albumen gland*** - providing the ova with loose mush of albumen		***membrane gland*** - first sacculations smoothing albumen of ova; further distally wrapping eggs with membrane	***mucous gland*** - mucus is added in layers to rotating egg-string	***oviducal gland*** - egg string lubrication or treatment of egg string with bacteriostatic material	Beeman 1970
Anaspidea (*Dolabella*)			***winding gland*** - secretion of capsule material: formation of capsule in vesicle at apex of gland; secretion of outward passage provides mucous covering of capsules	***mucous gland*** - embedding of series of capsules in viscous secretions	***oviducal channel*** - produces the final tacky covering of the spawn	Fretter & Hian 1984
Sacoglossa (*Acteonia, Limapontia*)	***albumen gland*** -provides albumen fluid for the eggs		***shell gland***	***glandular oviduct*** divided into four regions: -1st: provision of roping secretions for eggs: 2nd: secretion of embedding mucus; 3rd: provision of rind mucus	***4th region of glandular oviduct*** - secretion of sticky mucus which assists in concealment of the spawn mass	Gascoigne 1956
Sacoglossa	***Eiweißdrüse*** -secretion of "Eiweiß" (albumen)		***faltiger Ovidukt; erster drüsiger Oviduktabschnitt*** - secretion of egg membrane			Sander-Esser 1984
Nudibranchia (*Archidoris, Tritonia*)		***albumen gland*** -secretion of primary egg cases	***mucous gland*** -addition of protective mucous			Thompson 1961; Thompson 1976
Nudibranchia (*Austrodoris, Bathydoris*)		***capsule gland*** -formation of capsule wall				Wägele 1996

TAXON	ALBUMEN GLAND	CAPSULE GLAND	MEMBRANE GLAND	MUCOUS GLAND	ADHESIVE REGION/OVI-DUCAL GLAND	REFERENCE
Gymnomorpha (*Onchidella*)	***albumen gland*** - secretion of albumen			***glandular pouches of oviduct*** (six pouches) -1st: acts as a fertilization chamber; 2nd 4th: secretion of inner layers of capsule wall; 5th-6th: secreton of outer capsule layers	***posterior part of oviduct*** - lubrication of egg mass; addition of thin mucoid covering	Fretter 1943
Pulmonata (*Lymnaea*)	***albumen gland*** - secretion of perivitelline fluid and material of inner capsule membrane		***pars contorta*** - secretion of outer capsule membrane	***glandula nidamentaria accessoria*** (proximal part): - secretion of tunica interna; ***glandula nidamentaria*** (distal part): - secretion of tunica capsulis		Bretschneider 1948
Pulmonata (Ellobiidae)			***posterior mucus gland*** - secretion of successive layers of egg capsule and probably some part of mucus of eggs			Morton 1955
Pulmonata (*Lymnaea*)	***albumen gland*** - secretion of perivitelline fluid		***pars contorta*** - *posterior* (proximally): production of membrana interna; - *anterior* (distally): membrana externa	***muciparous gland*** (proximal part): - secretion of tunica interna; ***oöthecal gland*** (distal part): - secretion of tunica externa		Plesch et al. 1971

b) EGG MASSES

The egg masses of the euthyneuran species investigated proved to have a very uniform structure. Certain layers occur successively within the egg mass and presumably serve different functions. Since the egg masses of euthyneurans are laid on the substrate and are then left alone by the parent, protective layers (for protection against mechanical damage, infection, predation and possibly osmotic stress, see Todd 1981) have to be provided for the developing offspring. Nutritive layers are neccessary to ascertain that enough energy is present for the embryos to finish intracapsular development.

The fertilized ova (or unfertilized ova and allosperm, see Thompson 1976; Eyster 1986) are enveloped by a mass of albumen in some opisthobranch and in pulmonate taxa. This albumen is assumed to serve a nutritive function (see for example also George & Jura 1958; Bayne 1968; Beeman 1977; Clark & Jensen 1981; Schmekel 1985). A similar function is proposed for the inner capsule layer found in the egg masses of some cephalaspidean (*Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis cyanea*), pleurobranchoid and nudibranch opisthobranchs. The coated vesicles observed in the epidermis of the veliger larvae of *Acanthodoris pilosa* and *Chromodoris magnifica* (see fig. 31F) suggest that receptor-mediated endocytosis of dissolved capsule material is taking place (compare Goldstein et al. 1979). Hence, the dissolved capsule material is probably ingested by the larvae and thus possibly provides nutrition. This could also explain the extraordinarly thick capsules of Antarctic opisthobranchs, Wägele (1989b, 1996) has found. These species have very long intracapsular development (up to 2 ½ or more years). Thus a thick capsule wall contains lots of capsule material which can be consumed during these long developmental times. A second function of the inner capsule layer might also be to provide additional stability. The outer membrane layer in *Haminoea cymbalum*, *Chelidonura inornata* and *Philinopsis cyanea* and in pleurobranchoid and nudibranch taxa, as well as the membrane in other "Cephalaspidea s. l." and in anaspidean and sacoglossan species (homologous structures, see chapter 4.3) serve to envelope the embryos and albumen or inner capsule layer, respectively. By enveloping the embryos, the membrane forms distinct, separated spaces within the egg masses, where the offspring can develop independently from each other. Schmekel (1971) reported of a fluid inside the capsules of nudibranchs, which she found to be not visible with Transmission Electron Microscopy.

She did not further specify the contents of this fluid. Eyster (1986) believed that the intracapasular fluid described by Schmekel, influences diffusional exhcange of gases for respiration and of wastes of the embryo in the capsule. No substances, aquivalent to the intracapsular fluid described by Schmekel, have been found in the egg masses investigated in the present study.

The inner mucous layer, which occurs adjacent to the membrane, envelops adjacent capsules, thus arranging them in a line, forming tube-like structures. Bretschneider (1948) assumed that the tunica interna in *Lymnaea stagnalis*, which corresponds to the inner mucus layer described here, protects the embryos against desiccation. The mucus matrix serves as a mass, in which the capules can be embedded, thus providing more stability for the whole egg mass. The outer mucous cover lends further stability to the egg mass and may also serve as a barrier for microorganisms to invade the egg mass. Bretschneider (1948) found the tunica externa in *Lymnaea stagnalis*, corresponding to the outer mucous cover, to contain protein. Thus he presumed it might serve as a first nutritive source for the hatching larvae, which he also observed to crawl on top of the mucous cover right after hatching. In most species the outermost layer of the outer mucous cover has an adhesive function, concealing the egg mass to the substrate. We might call this layer "adhesive layer" in the future. In summary, following terms can be used for the different structures in the egg masses:

albumen (if present), **inner capsule layer** (if present), **membrane** (the inner capsule layer and the membrane can be referred to as the **capsule)**, **inner mucous layer**, **mucous matrix, outer mucous cover**, **adhesive layer**.

4.5 Aspects of evolutionary change and phylogenetic implications

Based on the data presented in this study, especially considering the likely homologies of the various glandular parts within the nidamental glands and within the layers of the egg masses (see chapters 4.2 and 4.3), it is possible to reconstruct the ground pattern of the nidamental glands and its products in the Opisthobranchia. Moreover a possible scenario for evolutionary change of this organ system within the Opisthobranchia can be proposed. This leads to certain phylogenetic implications which will be discussed and compared to data gained from the literature.

Since the division of the nidamental glands into a proximally lying albumen gland, followed by a membrane gland and a distally lying mucous gland has been observed in the "Cephalaspidea s. l.", considered to comprise primitive taxa within the Opisthobranchia (Schmekel 1985; Mikkelsen 1993), as well as in the sister groups Pulmonata and Gymnomorpha (Haszprunar 1985), this configuration is proposed for the ground pattern of the hypothetical opisthobranch ancestor. This accords with the hypotheses of Ghiselin (1965) and Gosliner (1980). This configuration of glands can also be proposed for the ground pattern of the Euthyneura since *Pyramidella sulcata* which is even more primitive (Haszprunar 1988), also has this arrangement of glands. Whether the albumen glands have been tubular or sac-like in the opisthobranch ancestor, cannot be conclusively decided at this point; but since most taxa within the Opisthobranchia investigated here have saccular albumen glands, and since in the outgroups (Pulmonata, Gymnomorpha, Pyramidelloidea) tubular albumen glands are present it is suggested that the tubular arrangement is ancestral, which complies with Ghiselin (1965), and saccular albumen glands have evolved within the Opisthobranchia. The membrane and mucous glands have most probably also been tubular in the hypothetical ancestor of the Opisthobranchia. An arrangement of alternating glandular and supporting cells, which has been found in all glandular parts in all taxa studied, represents the basic histological pattern. Ghiselin (1965), Gosliner (1980) and Schmekel (1985) postulated a monaulic hermaphroditic system to represent the primitive condition in all opisthobranchs. Ghiselin (1965) has tried to reconstruct the development of the primary monaulic hermaphroditic system, with all its inefficiencies to diaulic and triaulic systems on a functional basis. I agree with his argumentation that with regards to the construction of more efficient systems (by developing separate ducts for the transportation of the autogametes and allosperm) it would be implausible to first develop separate ducts and later fuse them to form one common duct again. Thus I consider the monaulic system as the most primitive condition. This contradicts Rudman (1978), who believed the oodiaulic system to be the most primitive within the opisthobranchs. He reconstructed the evolution of the reproductive systems within the Philinacea (a group of the "Cephalaspidea s. l.") and found the oodiaulic system to be the precursor for all other systems. Rudman believed that monaulic systems within these taxa have evolved more than once. I disagree with him. His findings of an oodiaulic system in *Chelidonura* are not congruent to the present investigation, where *Chelidonura inornata* was found to possess a

monaulic reproductive system. Rudman stated that *Philinopsis* presents a transitional stage with regards to the structure of the reproductive system. He did not further explain what is meant by "transitional". I found *Philinopsis gardineri* to have a monaulic system just like the one present in *Chelidonura inornata*.

Diaulic and triaulic systems seem to have evolved independently within the Opisthobranchia more than once.

Gosliner (1980) and Schmekel (1985) stated that the ancestor of the Opisthobranchia possessed a proximal receptaculum seminis and a distal bursa copulatrix as well as an open seminal groove. Mikkelsen (1996) believed that the bursa copulatrix has undergone a functional change to a gametolytic gland within the Opisthobranchia and that a bursa copulatrix has been developed secondarily within the Sacoglossa again. This hypothesis cannot be proven nor dismissed on the basis of the present findings, but at least seems to be questionable. Since histological and fine structural investigations of the receptacula and bursae copulatrices within the Opisthobranchia are sparse, it is assumed that more detailed studies of these organs are needed to throw more light on the question of their homologies. To summarize, the hypothetical ancestor of the Opisthobranchia possessed a monaulic reproductive system, with an albumen gland, a membrane gland and a mucous gland. A proximal receptaculum seminis and a distal bursa were present as well as an open seminal groove. This condition has been found to be present in many "cephalaspid" taxa (*Acteocina*, *Philine*, *Scaphander* and *Runcina*) here. Together with the diaulic forms *Acteon* and *Pupa* I consider these taxa as the "lower Cephalaspidea", because they all possess the ancestral arrangement of albumen, membrane and mucous gland. This arrangement is also present in the Sacoglossa and the Anaspidea. A major evolutionary change with regards to the structure and function of the nidamental glands has taken place within the Opisthobranchia. *Haminoea*, *Chelidonura* and *Philinopsis*, which I consider as the "derived Cephalaspidea", as well as the Tylodinoidea, Pleurobranchoidea and Nudibranchia do not have an albumen gland in the proximal part of the nidamental glands but possess a capsule gland instead. Because of the corresponding complexity of the albumen-capsule glands in all taxa, I assume that this change has taken place only once in the common ancestor of all "derived Cephalaspidea", Tylodinoidea, Pleurobranchoidea and Nudibranchia. Two hypotheses are possible to explain the evolution of the albumen-capsule glands:

1. The albumen gland has been reduced in the ancestor of the above named taxa and a "new" capsule gland has been developed.
2. The glandular cells in the albumen gland have undergone a structural change, resulting in the capsule gland, which now serves a different function. This would demand that the albumen and capsule glands are homologous structures, which has been hypothesized in chapter 4.2 already. This second explanation seems to be more probable, because of the likely homology of the two glands and because less evolutionary steps need to be assumed to lead to this functional change; hence the evolutionary scenario would be more conceivable and more parsimonious.

If we believe that this major functional change has occured within the Opisthobranchia, we need to ask what the evolutionary advantage of such a change might have been. As has been shown in the previous chapters (4.3 and 4.4), the change from an albumen gland to a capsule gland implicitly leads to a change in the structure of the egg masses. The albumen, present as a flocculent mass in those taxa possessing an albumen gland, forms a compact inner capsule layer adjacent to the outer membraneous layer in the taxa which possess a capsule gland. This inner layer seems to be dissolved and ingested by the veliger during intracapsular development, thus still serving a nutritive function. Hence the nutritive function of the albumen has not been lost in those taxa possessing a capsule gland. What evolutionary advantage could it have, to pack the nutritive fluid (albumen) into a compact layer? More stability for the egg capsule might be one advantage, but since the inner layer is dissolved sooner or later, this argument seems to be very weak. Another, and in my eyes more persuasive, advantage might be the fact that those embryos which are not surrounded by a rigid mass of albumen, but merely float in an intracapsular fluid (see also Schmekel 1971; Eyster 1986), can move more easily, can rotate more quickly and diffusion of respiratory gases and of waste materials can take place with higher efficiency. This would have a positive effect on the metabolism and development of the larva and be advantageous in contrast to those larvae which float in albumen. Another advantage might be a reduced intracapsular osmotic stress, because flocculent albumen molecules would have a higher osmolarity than coagulated albumen molecules. This argument, however, is very speculative, since we do not know anything about the exact biochemical composition

of the albumen fluid in these egg masses nor about the composition of the inner capsule layer.

Although the phylogenies of the major opisthobranch taxa (except for the Anaspidea) have been studied copiously in recent times (Willan 1987; Mikkelsen 1996; Jensen 1996; Wägele and Willan in press), the detailed structure of the nidamental glands has never been taken into consideration for these analyses. However, the results gained from the present investigation prove to yield phylogenetic implications. Figure 34 presents a dendrogram, based on data gathered from various phylogenetic analyses by different authors (Haszprunar 1985, 1988; Willan 1987; Salvini-Plawen 1990; Gosliner 1991; Jensen 1996; Mikkelsen 1996; Wägele & Willan in press) combined with the data gained in the present study. Apomorphies supporting a certain clade, which are taken from the literature, are put into the dendrogram just for the purpose of justification of this particular clade. The dendrogram is created to present a synthesis about the phylogeny of the Opisthobranchia, in order to verify, where the results of this study fit in. It is not the purpose of this thesis to analyse the different characters and their polarities to see whether they prove to be useful for resolution of the phylogeny of certain clades. Therefore the characters are merely mentioned in the dendrogram but are not discussed further.
The functional change within the nidamental glands, from an albumen gland to a capsule gland, is considered to have been a unique event in the evolution of the Opisthobranchia and hence, the possession of a capsule gland in the "derived Cephalaspidea", Tylodinoidea, Pleurobranchoidea and Nudibranchia is considered a synapomorphy of these taxa. A capsule gland does not occur in any other taxon outside these groups and it has been found in all representatives of these taxa, which have been investigated. Therefore they can be united as a monophyletic group on the basis of this shared character. The "loss" of the albumen gland, which has been shown to coincide with the presence of a capsule gland, has been proposed to be a synapomorphic character of the Pleurobranchoidea and Nudibranchia by Schmekel (1985) and Salvini-Plawen (1990). Their hypotheses are disproved here. The creation of a monophyletic group comprising the "derived Cephalaspidea", the Tylodinoidea, Pleurobranchoidea and the Nudibranchia has never been undertaken so far. I am well aware that the presence of a capsule gland and the consequently specific structure in the egg mass (additional inner capsule layer) as the

only characters to support this group do not form a sound base for this monophylum, but the present findings provide a first argument that these taxa might be closer related. Further phylogenetic studies will help to prove or falsify this hypothesis.

Another conclusion that can be drawn from the present data is the fact that the "Cephalaspidea s. l." are paraphyletic. This has been assumed for a long time and has already been proven by a detailed phylogenetic analysis performed by Mikkelsen (1996). Figure 35 shows the dendrogramm created by Mikkelsen. Only those taxa which have also been investigated in the present study, are shown. The synapomorphies of the various clades are stated in the dendrogramm but are not discussed further. In contrast to Mikkelsen, who did not include the Aglajidae in her analysis, the split within the "Cephalaspidea s. l.", which I found, is different from the split she found. Mikkelsen excluded the basal taxa *Acteon*, *Ringicula* and *Hydatina* from the "Cephalaspidea s str." and united the remaining taxa *Bulla*, *Haminoea*, *Smaragdinella*, *Cylichna*, *Retusa*, *Acteocina*, *Scaphander* and *Philine* (not including the Aglajidae) in the "Cephalaspidea s.str." on the base of the possession of three gizzard plates as the synapomorphic character of these taxa. The Anaspidea were found by Mikkelsen to be the sister taxon to the "Cephalaspidea s. l.". In accordance with Mikkelsen, my findings support the basal position of *Acteon*, but in contradiction to her dendrogramm (fig. 35B), *Acteocina*, *Philine*, *Scaphander* (as well as *Pupa* and *Runcina*, not investigated by Mikkelsen) also belong to the "lower Cephalaspidea". *Haminoea* (in accordance with Mikkelsen) and the Aglajidae (represented by *Chelidonura* and *Philinopsis*) are considered to be the "derived Cephalaspidea" in the present study. In the opinion of Mikkelsen, *Haminoea* shows a close relationship to *Bulla* (because of the presence of an exogeneous ciliated strip and a secondarily dorsolateral eye direction) and is more basal in position than for example *Acteocina*, *Scaphander* and *Philine*. This cannot be confirmed by the present data. The latter species possess an albumen gland, which is considered to be more primitive, while *Haminoea* possesses a capsule gland. In the dendrogram created here (fig. 34) the Anaspidea and Sacoglossa branch off from the tree before the "derived Cephalaspidea" do so but it cannot be resolved with the present data, whether the Anaspidea *or* the Sacoglossa branch off earlier. Mikkelsen (1996) believed the Sacoglossa to branch off earlier than the Anaspidea, because of the synapomorphic presence of gizzard plates in the latter and the "Cephalaspidea s.str.".

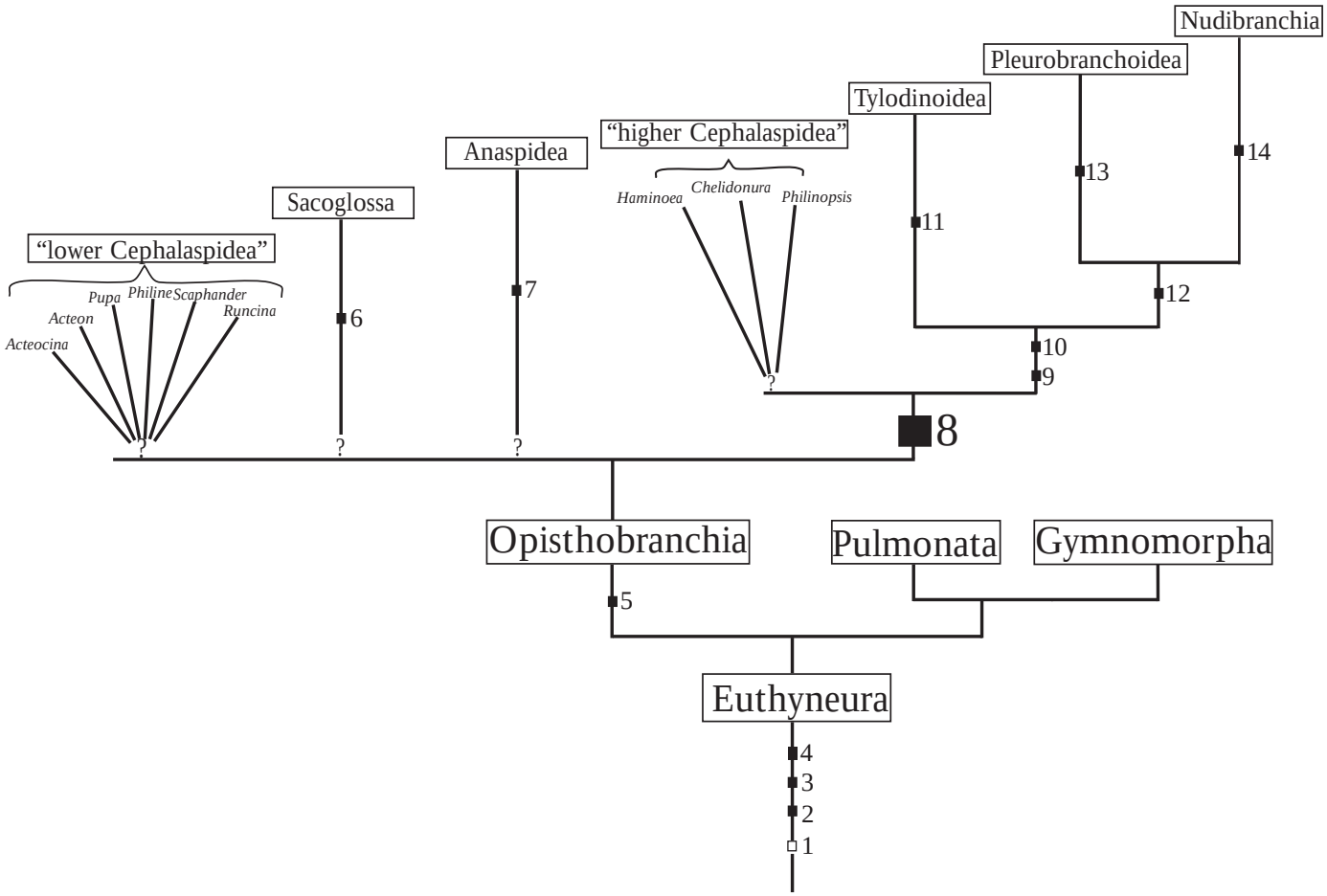

Figure 34: Dendrogram of the Euthyneura after several authors. New characters found in the present study are added accordingly (bold print). List of ■ synapomorphies and □ symplesiomorphies: 1: presence of albumen gland, membrane gland and mucous gland (this study; Ghiselin 1965; Gosliner 1980); 2: pallial caecum, repugnatorial glands, subcerebral commissure, similar shell muscle system (Haszprunar 1988); 3: two additional parietal ganglia (Haszprunar 1985); 4: plicatidium newly established at site of former left ctenidium, hermaphroditic monaulic reproductive system, open seminal groove, chromosome number = 17 (Salvini-Plawen 1990); 5: external branch of labio-tentacularis nerve newly established, Hancocks sense organs, giant nerve cells (Salvini-Plawen 1990); 6: presence of shell adductor muscle, absence of plicate gill (Jensen 1996); 7: atrial gland (this study); 8: functional change of albumen gland into capsule gland; coagulation of albumen to inner capsule layer (this study); 9: reduction of head shield and clypeo-capitis nerves, reduction of mantle cavity but plicatidium retained (Salvini-Plawen 1990); 10: association of visceral ganglion with right side of body (Gosliner 1991); 11: cuticularized labial ring (Willan 1987); 12: possession of blood gland, androdiaulic genital system, reduction of osphradium (Wägele & Willan in press); 13: presence of pedal gland, presence of median buccal gland, reduction of autospermal groove at penis, penis protrusible (Willan 1987); 14: special vacuolated epithelium, pericardial complex in longitudinal orientation, reduction of shell, rhinophores solid (Wägele & Willan in press).

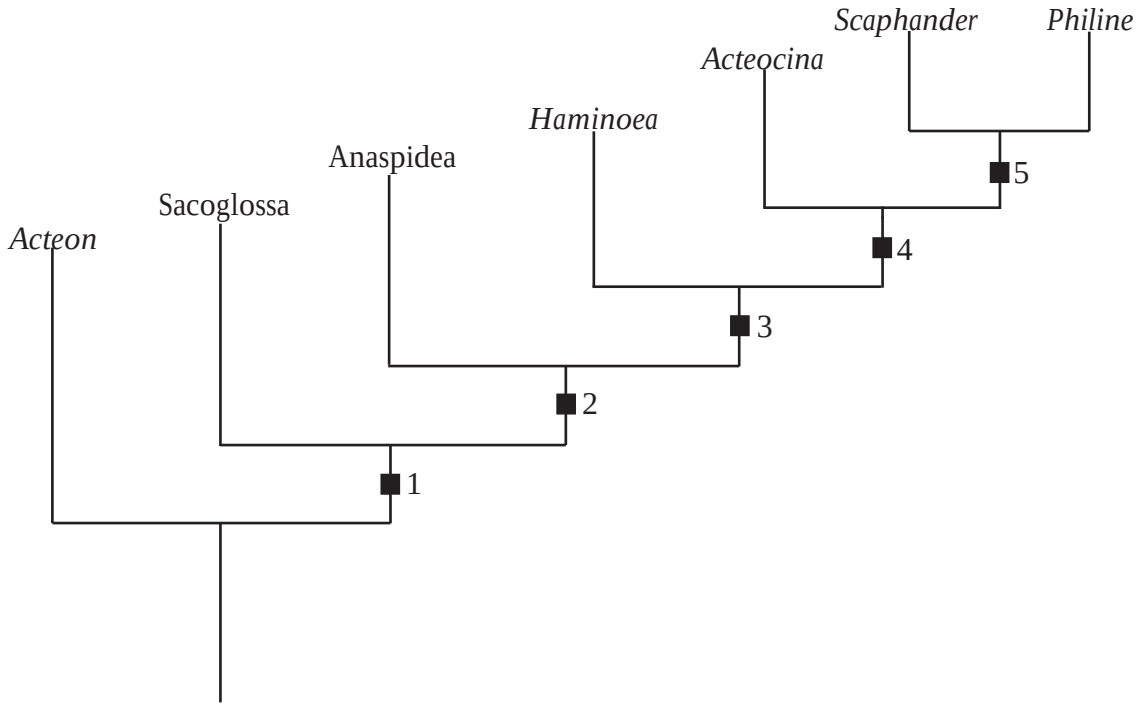

Figure 35: Dendrogramm after Mikkelsen (1996) of selected taxa (which have also been investigated in the present study) of the "Cephalaspidea s. l.". ■ Synapomorphies found by Mikkelsen: 1: stomach pouch lost, external ciliated sperm groove present; 2: oesophagus with numerous gizzard plates; 3: reduction of gizzard plates to number of 3; 4: gizzard plates calcified; 5: ejaculatroy duct present and continuous with external ciliated sperm groove, jaws absent.

However, since the results of this study have to be considered preliminary, and since Mikkelsen also stated that her results were preliminary, only more data about various organ systems in various taxa will elucidate the phylogenetic relationships of the different opisthobranch taxa better. The monophyly of the Anaspidea can be confirmed here, because of the synapomorphic presence of an atrial gland. This gland has not been observed in any other taxon of the Opisthobranchia, nor within the outgroups, and is therefore regarded to be unique to the Anaspidea. Gosliner (1991) affirmed the relationship of *Akera* with the Anaspidea due to the presence of an atrial gland in these taxa.

The results of this comparative study of the nidamental glands and egg masses have shown not only to improve our understanding of the homologies of the various glandular parts and structures and their functional morphology, but have also been valuable for unravelling certain aspects of opisthobranch evolution and phylogeny. Comparative morphology has proven once more to be an exciting and useful method for the biological systematic sciences!

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Appendix

List of chemicals used:

acetic acid (HAc)
aceton
acrolein
activated charcoal
alcian blue 8 GX
aniline blue Orange G
azocarmine
lead citrate
borax (sodium tetraborate, $Na_2B_4O_7$)
bromephenol blue
chloroform
dimethylarsinic acid
dimethyl sulfoxide
ethanol
formalin
glutaraldehyde
hydrochloric acid (HCl)
isopropanol (2-propanol)
lead citrate
lead nitrate ($Pb(NO_3)_2$)
mercuric chloride ($HgCl_2$)
methacrylate
osmiumtetroxide (OsO_4)
paraffin oil
paraformaldehyde
paraplast embedding media
pararosaniline
periodic acid
phosphothungstic acid
potassium disulfite ($K_2S_2O_5$)
propylene oxide
rotihistol
Schiff´s reagent
sodium chloride (NaCl)
sodium citrate ($Na_3(C_6H_5O_7)$ x 2 H_2O)
sucrose
tert-butanol
toluidine blue
uranyl acetate

List of recipes for staining solutions and protocols for staining processes:

1. Alcian blue (changed after Romeis 1989)**:**

Dissolve 0.1 g alcian blue 8 GX in 100 ml acetic acid with pH 2.5 or hydrochloric acid with pH 1.0, respectively. Filter.

Staining with alcian blue pH 2.5:

- place sections in acetic acid (pH 2.5) for 3 minutes
- stain with 0.1 % alcian blue (pH 2.5) for 60 minutes
- wash in acetic acid (pH 2.5)
- wash in distilled water
- dry sections and counterstain with 0.1% eosin in distilled water for 20 to 30 seconds
- dry sections and mount with Entellan or Eukitt

Staining with alcian blue pH 1.0:

- place sections in hydrochloric acid (pH 1.0) for 3 minutes
- stain with 0.1% alcian blue (pH 1.0) for 60 minutes
- wash in hydrochloric acid (pH 1.0)
- dry sections and counterstain with 0.1% eosin in distilled water for 20 to 30 seconds
- dry sections and mount with Entellan or Eukitt

2. Azan (Azocarmine-Aniline blue-Orange G staining) (after Heidenhain in Romeis 1989)**:**

Azocarmine staining solution:

Dissolve 0.1 g azocarmine G in 100 ml distilled water, heat unto boiling and cool off. Filter at room temperature. Last, add 1% concentrated acetic acid.

Aniline blue Orange G staining solution:

Dissolve 0.5 g aniline blue and 2 g Orange G in 100 ml distilled water and add 8 ml concentrated acetic acid. Bring to boiling, cool off and filter at room temperature.

Staining with azan:

- de-wax sections in rotihistol for 10 minutes
- repeat treatment with rotihistol for 5 minutes
- rinse in 100% isopropanol for 2-3 minutes
- rinse in 70% ethanol for 5 minutes
- wash in distilled water for 1-2 minutes
- stain with (preheated) azocarmine in oven (56°) for 10-15 minutes
- wash in distilled water for 1-2 minutes
- differentiate in aniline-alcohol (0.1 ml aniline oil in 100 ml absolute ethanol) for a few seconds

- wash off aniline with acetic alcohol (1 ml concentrated acetic acid in 100 ml absolute ethanol) for 30-60 seconds
- treat in 5% phosphothungstic acid for 1-3 hours
- wash in distilled water for 1-2 minutes
- stain with aniline blue orange G solution for 1-3 hours
- wash with distilled water for 1-2 minutes
- differentiate in absolute ethanol fot 20 seconds
- treat in 100% isopropanol for 5 minutes
- clear in rotihistol 3 times (5,5 and 10 minutes respectively)
- mount with eukitt

3. Bromephenol blue (after Mazia et al. 1953):

Dissolve 10 g of mercuric chloride ($HgCl_2$) and 0.1 g of bromephenol blue in 100 ml distilled water.

Staining with bromephenol blue:

- stain sections in bromphenol blue solution for 15 minutes
- wash in 0.5% acetic acid for 20 minutes
- wash in distilled water for 15 minutes
- treat in water or buffer of pH 6-7 for 3 minutes
- dry sections and mount with entellan or eukitt

4. Periodic acid-Schiff (PAS) (changed after Romeis 1989):

Periodic acid: Dissolve 0.4 g periodic acid in 100 ml distilled water.

Schiff´s reagent:

Dissolve 0.5 g pararosaniline in 15 ml 1N hydrochloric acid. Dissolve 0.5 g potassium disulfite in 85 ml distilled water. Mix both solutions and keep in darkness for 24 hours at room temperature or 36 hours in the refrigerator to allow chemical reaction. Mix reagent with 0.3 g activated charcoal powder for 2 minutes. Store solution in refrigerator.

Staining with PAS:

- oxidize sections in 0.4% periodic acid at 55° (in the oven) for 30 minutes
- rinse in tab water
- rinse 3 times in distilled water
- stain with Schiff´s reagent at room temperature for 15 minutes
- rinse in tab water
- rinse in distilled water
- dry sections and mount with entellan or eukitt

5. Toluidine blue (changed after Trump et al. 1961; taken from Romeis 1989):

Dissolve 1 g toluidine blue, 1 g borax and 20 g sucrose in 78 ml distilled water. Filtrate solution. Dilute 1:10 (staining solution to water) with distilled water.

Staining with toluidine blue:

- stain sections with toluidine blue solution for 20 seconds
- wash in tab water for 30 seconds
- wash twice in distilled water for 1 minute
- dry sections and mount with Entellan, DPX or Eukitt

List of recipes for fixatives and protocols for fixation and embedding methods for histology and electron microscopy:

1. Methacrylate histology:

a) Preservation of samples:
Preserve specimens or tissue samples in 4-6% formalin in seawater or freshwater, respectively. Exchange formalin with 70% ethanol at least 24 hours before processing for embedding.

b) Dehydration:
Dehydrate samples in a graded ethanol series as follows:

- 3x 80% ethanol
- 3x 90% ethanol
- 3x 96% ethanol

Each step should be performed for at least one hour.
After the last dehydration step exchange the ethanol with Technovit 7100 intermedium. This consists of methacrylate resin (100 ml) plus first hardener (1 g). Mix components well until hardener has dissolved completely.

c) Embedding:
After 24 to 48 hours (depending on the size of the sample) the intermedium has infiltrated the tissue. For embedding media mix intermedium (15 g or 7.5 g) with second hardener (1.0 ml or 0.8 ml respectively). Place samples in moulds and cover them with embedding media. It takes about 8-24 hours for the resin to polymerize.

d) Sectioning:
Glue blocks to metal stud. Methacrylate is sectioned with a thungsten carbide knife on a powered microtome. Serial sections of 2.5 to 3 µm are stained with toluidine blue and mounted with Entellan, DPX or Eukitt. These sections serve for reconstruction of the anatomy and histology of the organ systems. Single sections can be stained with other stains for histochemical studies.

2. Paraffin histology:

a) Preservation of samples:
The same preservation method as described above for methacrylate resin histology applies to paraffin histology.

b) Dehydration:
Tert-butanol (TBA) is used in increasing concentrations for dehydration as follows:

- 1x 85% TBA (in aqua dest.) 1-2 hours
- 1x 95% TBA 1-2 hours
- 1x absolute TBA over night (in the oven at 40°C)
- 1x wash with absolute TBA

- 1x absolute TBA / paraffin oil (1:1) 1-2 hours

c) Embedding:

Before the final embedding in paraplast the tissue samples have to go through paraffin oil and liquid paraffin. Before use, fresh paraffin has to be heated to 70°C and cooled off to room temperature again at least three times.

Treat tissue samples as follows:

- 1x paraffin oil 1-2 hours
- 3x pure paraplast (heated to 54-56°) 1-3 hours

Embedd samples in moulds placed on a glass slide. Place samples smoothly in liquid paraplast and stirr cautiously with a fine needle at surface of embedding media to reduce the speed of cooling off. Hard paraplast should have a grey color. Store blocks in refrigerator.

d) Sectioning:

Glue blocks to wooden stud. The paraplast is sectioned with a steal knife. Serial sections are 5-12 µm thick and can be stained with various stains. Before the tissue can be stained, though, the paraplast has to be dissolved. This can be done with rotihistol which is less harmful than xylene.

3. Transmission Electron Microscopy (TEM):

a) Preservation:
Fixation of fresh tissue samples is performed with a solution of glutaraldeyhyde, formalin, acrolein and dimethyl sulfoxide in cacodylate buffer. The recipes for the components are as follows (changed after Cloney & Florey 1968):

Sodium cacodylate buffer: Dissolve 3.21 g dimethylarsinic acid in 150 ml distilled water. Add 1M HCl until pH 7.4. Fill up to 300 ml with distilled water.
Add 2.7 g NaCl or 15 g sucrose to 150 ml of this buffer.

Fixative: Dissolve 9 g paraformaldehyde in 50 ml of the above buffer. Heat solution up to 63° C until paraformaldehyde has completely dissolved and solution is clear.
Mix 12 ml 25% glutaraldehyde, 11.1 ml 18% paraformaldehyde, 1.0 ml acrolein (this ingredient is very poisonous and can be omitted!) and 2.5 ml dimethyl sulfoxide. Fill up to 100 ml with cacodylate buffer.

Storage solution: Samples can be stored up to four weeks after first fixation step in the following solution:
Dissolve 6.9 g sucrose in 96 ml pure cacodylate buffer(no NaCl or sucrose added before) and add 4 ml 25% glutaraldehyde .

Postfixation follows in 2% OsO_4 in cacodylate buffer. Osmium tetroxide is very harmful and has a high steam pressure. Thus it has to be handled with extreme care.
The chemical is delivered in form of cristals which take at least 24 hours to dissolve.

The above recipes apply to marine animals. For freshwater samples cacodylate buffer without additional NaCl or sucrose was used. All other solutions were used in the same way.

A few samples were preserved in 3% glutaraldehyde in seawater and postfixed in 1% OsO_4 in seawater. The preservation of these samples, especially the nidamental glands was very poor compared to the fixation method described above.

Preservation protocol as follows:

- fixation in glutaraldehyde solution 3 hours
- wash samples in cacodylate buffer 6 times for ten minutes
- postfixation in OsO_4 3 hours
- wash samples 2 times in buffer or seawater

Before beginning with the dehydration process the resin for embedding has to be mixed according to instruction sheet:

Agar Resin 100:

b) Dehydration:
Dehydration of samples can be performed in a graded ethanol series followed by propylene oxide or aceton. The latter is less harmful and gives the same results and should be preferred. Thus aceton is listed in the following protocol:

place samples in:	for:
• 70% EtOH	10 minutes
• 90% EtOH	15 minutes
• 90% EtOH	15 minutes
• 96% EtOH	15 minutes
• 96% EtOH	15 minutes
• 96% EtOH	15 minutes
• 100% aceton	15 minutes
• 100% aceton	15 minutes
• aceton:resin (3:1)	120 minutes
• aceton:resin (2:1)	180 minutes
• aceton:resin (1:1)	over night

c) Embedding:
Before final embedding tissue samples have to be infiltrated in pure resin for at least five hours. New resin has to be mixed for this and the following processes.
For embedding freshly mixed resin is poured into a petri dish or into moulds and tissue samples are cautiously placed on resin surface. They will sink slowly to the ground of the moulds or petri dish and can be placed in the desired position with a fine needle. The resin takes 48 hours to polymerise at 60°C.

A few samples were embedded in Spurr´s resin. The whole dehydration process performed was slightly different from the one described above. For dehydration only ethanol was used. Samples were placed in increasing concentrations (70, 75, 80, 85, 90, 95 and 96% EtOH) for five minutes each. They were then treated with 96% EtOH:resin (1:1) for 3 hours. Treatment with EtOH:resin (1:2) followed for 3 hours, again followed by 100% resin overnight. The next day the samples were embedded in pure resin which polymerizes in 16 hours at 65-70°C.
Since Spurr´s resin is reported to be harmful it should be avoided and Agar Resin 100 or comparable resins should instead be used.

d) Sectioning:
Semi-thin sections (2.0 µm) were done with glass knives and stained with a concentrated solution of toluidine blue.
Ultra-thin sections (70-95 nm) were prepared with diamond knives on an ultra-cut microtome. The sections are fixed on copper grids which have a thin formvar support film. Sections are stained with uranyl acetate and lead citrate successively.

Uranyl acetate staining solution: Mix 5 % uranyl acetate in distilled water. Filter solution a few times and centrifuge a few times. Centrifuge once before usage.
Stain sections for 15 minutes and wash off uranyl actetate with distilled water three times.

Uranyl acetate can also be used in an alcoholic solution.

Lead citrate staining solution: Mix 1.33 g $Pb(NO_3)_2$, 1.76 g $Na_3(C_6H_5O_7)$ x $2H_2O$ and 30 ml bidest water. Occasionally shake flask for 1 minute. Add 8 ml 1N NaOH. Fill up to 50 ml with bidest water. Filter and centrifuge a few times. Centrifuge once at 5000 spins/minute before use. Stain sections for 10 minutes and wash off lead citrate twice with 0.02 N NaOH and three times with distilled water.

Scientists don´t immerse themselves in particulars only for the grandiose (or self-serving) reason that such studies lead to important generalities. We do it for fun. The pure joy of discovery transcendts import. And we do it for the adventure and for expansion.

Stephen Jay Gould

List of abbreviations

adr: adhesive region
agl: albumen gland
alb: albumen
amp: ampulla
atgl: atrial gland
avs: albumen vesicle
bac: bacteria
bc: bursa copulatrix
bl: basal lamina
cap: capsule
cgl: capsule gland
cm: cell membrane
cmat: capsule material
ci: cilia
ct: connective tissue
dct: duct
dov: distal oviduct
e: embryo
er: endoplasmic reticulum
ga: genital aperture
gc: golgi complex
icl: inner capsule layer
ivs. immature vesicle
lip: lipid droplet
mma: mucous matrix
me: membrane
megl: membrane gland
mi: mitochondrium
mst: mucous strand
mv: microvilli
mvs: mucous vesicle
muc: mucus
mugl: mucous gland
nuc: nucleus
omc: outer mucous cover
ov: oviduct
ovgl: oviducal gland
pe: penis
pov: proximal oviduct
rs: receptaculum seminis
sc: supporting cell
sgr: secretory granule
spgl: spermoviduct gland
spov: spermoviduct
sub: substrate
vag: vagina
vch: vaginal channel
vd: vas deferens
vest: vestibulum
vs: vesicle
vsh: veliger shell