Phylogeny of the Nudibranchia

HEIKE WÄGELE*

Lehrstuhl für Spezielle Zoologie, Ruhr-Universität Bochum, 44780 Bochum, Germany

RICHARD C. WILLAN

Museum & Art Gallery of the Northern Territory, GPO Box 4646, Darwin, Northern Territory 0801, Australia

More than 100 morphological, anatomical and histological characters pertaining to the Nudibranchia are discussed in the course of a phylogenetic analysis. Based on our own investigations on anatomy, histology and published literature, the polarity of each character is assessed by comparing its expression with outgroups, chiefly the Pleurobranchoidea, but also with other opisthobranch taxa (Cephalaspidea, Anaspidea, Sacoglossa), and ultimately with the Pulmonata, Vetigastropoda and Caenogastropoda. By extracting 46 applicable characters expressed in 30 taxa, a phylogenetic analysis of the major groups of the Nudibranchia is performed by using the computer program PAUP. **Nudipleura new taxon** (containing the Pleurobranchoidea plus Nudibranchia) is defined. The monophyly of the Nudibranchia, the Anthobranchia (containing the Bathydoridoidea plus Doridoidea), and the Cladobranchia (containing the Dendronotoidea plus Aeolidoidea and 'Arminoidea') are confirmed and autapomorphies are highlighted. The paraphyly of the 'Arminoidea' is demonstrated. Incongruities at lower taxonomic level are discussed.

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ADDITIONAL KEY WORDS:—Mollusca – Gastropoda – Nudibranchia – Anthobranchia – Cladobranchia – phylogeny – anatomy – histology – character analysis.

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* Corresponding author. E-mail: Heike.Waegele@ruhr-uni-bochum.de

INTRODUCTION

"The nudibranches (including Pleurobranchacea) constitute a homogeneous whole from the evolutive point of view, and a clearly definite and natural order" (Tardy, 1970a: 301).

The Nudibranchia stands at the pinnacle of the assemblage of 'sea slugs' known variously as the Opisthobranchia, Euthyneura or Heterobranchia. Nudibranchs are diagnosed by loss of the shell (and operculum) and simultaneous expansion of the notum over the dorsal body surface during metamorphosis, presence of papillae on the notum, detorsion so extensive as to result in virtually complete (external) bilateral symmetry, head distinct from foot, paired gustatory oral tentacles/veil, paired dorsal chemosensory tentacles (rhinophores), multiple bilaterally symmetrical gills, hermaphroditic reproductive system with simultaneous maturation of gametes, obligate cross-fertilization involving copulation, semelparity, and 13 haploid chromosomes (Thompson, 1976; Boss, 1982; Todd *et al.*, 1997, pers. obs.). The soft body is protected by elaborate defences—morphological (spicules and kleptoplasty of cnidarian nematocysts), chemical (direct utilization of toxins from prey and *in situ* synthesis of toxins as repugnatory fluids), and behavioural (autotomy, crypsis). The group is exclusively free living and marine (except for the freshwater *Ancylodoris baicalensis* Dybowski, 1900), occurring in all habitats—even the plankton and neuston. Diets consist of many major animal groups (Porifera, Cnidaria, Bryozoa, Crustacea, Mollusca, Ascidiacea), with individual species often displaying great specificity. Willan & Coleman (1984) estimated there are 3000 species of nudibranchs worldwide. Nudibranchs range in size from *Vayssierea cinnabarea* (Ralph, 1944) with an adult length of 4 mm (Powell, 1979) to *Hexabranchus sanguineus* (Rüppell & Leuckart, 1830) with an adult length of 600 mm (Marshall & Willan, 1999), though most species are under 30 mm.

Several evolutionary trends are apparent within the Nudibranchia. The most pervasive is for 'aeolidization'; both externally (i.e. reduction in size, development of dorsal papillae [and subsequent amalgamation into clusters] and propodial tentacles), and internally (i.e. reduction in the number of lateral rows in the radula, branching of the digestive gland, reduction in the length of the optic nerve, shortening of the pedal commissure) (Willan, 1988). This trend for 'aeolidization' is apparent in many families of the Nudibranchia as well as Sacoglossa and Acochlidiacea. Undoubtedly steps in the process have occurred independently in numerous taxa of opisthobranchs, but the final 'aeolid' is disarmingly similar regardless of that from which it was derived. Another trend is for dietary switching. These convergences and parallelisms were only recognized as significant quite recently (Odhner, 1936; Tardy, 1970a; Minichev, 1970) and they are viewed by some workers as hindrances

to a phylogenetic understanding of opisthobranchs as a whole (Gosliner, 1981, 1991b; Gosliner & Ghiselin, 1984). They have probably thwarted the recognition of monophyletic taxa to this day.

Historical summary and previous classifications

The Nudibranchia entered zoological classification by the hand of Cuvier (1817) who erected it from the Linnean 'Vermes' (Table 1). Cuvier appreciated that nudibranchs were not worms, but molluscs related to snails with shells. He introduced the name (as an order) for those marine slugs with distinct external and uncovered gills. Lamarck (1819) maintained Cuvier's taxon but renamed it Les Tritoniens. Milne-Edwards (1848) placed it as a family of his Opisthobranchiata. Férussac (1822) introduced two suborders, Anthobranches and Polybranches, with three families, Les Doris, Les Tritoniens and Les Glauques. Blainville (1825) renamed Férussac's two suborders Cyclobranchiata and Polybranchiata. Sars (1841) was the first to view the metamorphosis of nudibranchs from shelled larvae—indeed this was the first documented instance of the phenomenon of metamorphosis in any mollusc.

Quatrefages (1844) amalgamated several opisthobranch genera into a new group, Phlebentera, because of the supposed assumption of the functions of other (atrophied) organ systems, notably the circulatory system, by the branches of the stomach (this hypothesis of multipurpose vessels he termed phlebenterism).

Several anatomists clamoured against phlebenterism, notably Souleyet (1844) and Alder & Hancock. In a monograph that extended to seven published volumes, Alder & Hancock (1845–1855) treated many British nudibranchs while soundly debunking phlebenterism, which we suggest was a primary goal of the entire work. Their combined anatomical and artistic skills amply demonstrated that nudibranchs did indeed have all the normal organ systems and that each maintained its customary function. The volumes by Alder & Hancock are magnificent works, even by today's standards; they present the first real leap forward in nudibranch research by illuminating the diversity, adaptation and specialization that are the hallmarks of the Nudibranchia. Taxonomically, Alder and Hancock were conservative in following Férussac's system of classification. They actually said (Alder & Hancock, 1845: 39): "The plan of the work would not allow any excessive alterations, and happily such were not required." Their classification was as follows:

Subkingdom Mollusca
Class Gastropoda
Order Nudibranchia
  Family Dorididae
    Subfamily Doridinae
    Subfamily Polycerinae
  Family Tritoniadae
  Family Eolididae
    Subfamily Dendronotinae
    Subfamily Melibaeinae
    Subfamily Hermaeinae
    Subfamily Proctonotinae

The arbitrary division of the Gastropoda into Tectibranchia and Nudibranchia, to

TABLE 1. Historical summary of divisions of the Nudibranchia as represented by names for major clades

Author	Major clades				Remarks
Cuvier, 1817		NUDIBRANCHIA			Sacoglossa included
Lamarck, 1819		LES TRITONIENS			
Férussac, 1822	Anthobranches Les Doris	Les Tritoniens	Polybranches	Les Glauques	
Blainville, 1825	Cyclobranchiata		Polybranchiata		
Alder & Hancock, 1845	Dorididae	NUDIBRANCHIA Tritoniadae		Eolididae	Sacoglossa included
Von Ihering, 1876		NUDIBRANCHIA			Sacoglossa excluded
Bergh, 1877		NUDIBRANCHIA			Sacoglossa excluded
Bergh, 1890, 1902, 1906	Holohepatica		Cladohepatica		
Pelseneer, 1894	Doridoidea	Tritonioidea		Aeolidoidea	Sacoglossa included
Eliot, 1910	Holohepatica (Dorididae, Tritoniidae)		Cladohepatica		Sacoglossa excluded
Thiele, 1931	Doridacea (=Holohepatica) (Dorididae, Tritoniidae)		Aeolidacea (= Cladohepatica)		Rhodopacea included in Nudibranchia
Odhner, 1934	Doridacea (with Gnathodoridacea and Eudoridacea)	NUDIBRANCHIA Dendronotacea	Arminacea	Aeolidacea	Sacoglossa excluded
Boettger, 1955		NUDIBRANCHIA			Tylodinoidea and Pleurobranchoidea included
Odhner in Franc, 1968	Doridacea	Dendronotacea	Arminacea	Aeolidacea	Rhodopidae included in Doridoidea
Minichev, 1970	Anthobranchia		Nudibranchia		
Tardy, 1970a	Euctenidiacea		Actenidiacea		
Schmekel & Portmann, 1982	Ctenidiacea		Actenidiacea		
Willan & Morton, 1984	Anthobranchia		Cladobranchia		
Wägele, 1989b	Ctenididacea		Actenidiacea		*Doridoxa* excluded from Anthobranchia
Salvini-Plawen, 1990, 1991	Anthobranchia		Nudibranchia		
Salvini-Plawen & Steiner, 1996		'ACOELA'			
Wägele & Willan, present study	Anthobranchia	NUDIBRANCHIA	Cladobranchia		Position of *Doridoxa* not resolved

which Alder and Hancock and their predecessors adhered, was not so much based on the presence or absence, respectively, of a shell but the presence or absence of a single 'well-developed ctenidium' on the right side (Eliot, 1910). The Nudibranchia encompassed those forms with multiple bilaterally symmetrical gills, whereas the tectibranchs encompassed all the rest. Exceptions, and there were many already recognized by the middle of the nineteenth century, like the Elysiidae, 'Pteropoda', Hedylidae (now called Acochlidea) and Onchidiidae (or Soleolifera) (now included in the higher group Gymnomorpha; Salvini-Plawen, 1990, 1991), were simply forced into one group or the other.

In an article discussing the evolutionary relationships of molluscs, von Ihering (1876) made the second major leap forward. He recognized a group (his third order, named Sacoglossa) containing shelled (Lophocercidae, now Oxynidae) plus unshelled (Elysiadae, Plakobranchidae, Limapontiadae, Hermaeadae and Phyllobranchidae) members within the Ichnopoda. Thus von Ihering clearly segregated the Sacoglossa from the Nudibranchia indicating that he understood that the shell-less members of the former group were more closely related to the shelled members than to the latter group. In doing so he made the first real progress towards a natural (i.e. monophyletic) grouping for the Nudibranchia.

Exactly this same conclusion was published by Bergh one year later (Bergh, 1877) (although Bergh mentioned neither von Ihering nor Sacoglossa, using Ascoglossa for the identical group). [Von Ihering had apparently sent the manuscript for this article to Bergh who hastily replied that "he had just reached the same conclusion" (Jensen, 1991). Von Ihering mentioned Bergh's reply in a postscript to his article].

Bergh (1890, 1902, 1906) split the nudibranchs into two lineages according to the form of the digestive gland, the character he considered paramount and, in his view, certainly transcending the form of the foregut and radula. Those nudibranchs with a compact digestive gland were grouped as the (tribe) Holohepatica and the rest with a branched (or even merely subdivided) digestive gland were grouped as the (tribe) Cladohepatica. Throughout his long career as the pre-eminent specialist on opisthobranchs, Bergh (1890, 1906) interpreted nudibranchs as having a monophyletic origin, so his formal scheme (Bergh, 1892) was quite incongruous. There he envisaged two independent evolutionary lineages of opisthobranchs: Cephalaspidea–'Ascoglossa'–Aeolidoidea and Pleurobranchoidea–Tritonioida, thus raising the possibility of polyphyly within the Nudibranchia for the first time.

Pelseneer (1894) was another advocate for polyphyly, even though he retained a single group, the (order) Nudibranchia. The subgroups (tribes) of Pelseneer were Tritonioidea, Doridoidea, Aeolidoidea (all three derived independently from the [family] Tritonia) and Elysioidea (derived from the aeolid stem).

In his supplement to Alder and Hancock's monograph, Eliot (1910) dismissed Pelseneer's quadripartite opisthobranch scheme by claiming that the groups were not "equally important and distinct" and he returned to the Berghian bipartite system (two tribes). Nevertheless, by this time there were even more problematic taxa to deal with than fifty years previously because of discoveries made during the epoch of nationalistic expeditions around the turn of the century. Eliot's solution was, however, not different to those of his predecessors; forceful inclusion was used even when that meant degrading natural clades. For example, Eliot forced the Tritoniidae into the Holohepatica admitting that one genus in that family, *Tritonia*, was "to some extent the connecting link" between the Holohepatica and Cladohepatica.

In the *Handbuch der Systematischen Weichtierkunde*, Thiele (1931) made some advances.

Following von Ihering and Bergh, he segregated the sacoglossans from the nudibranchs as independent taxa yet, following Guiart (1900), he also combined the Nudibranchia and Notaspidea into a single group Acoela. Furthermore, he included the enigmatic Rhodopidae as a tribe within the Nudibranchia, a group with a checkered history, and whose position within the Gastropoda is still not clarified (Haszprunar & Künz, 1996).

Bergh's status as the senior figure in opisthobranch research devolved to Odhner in the first half of the present century. Odhner was dissatisfied with existing evolutionary schemes for nudibranchs as unnatural, so he devised a new one. It first appeared in 1934 in his account of the material taken during the British Antarctic 'Terra Nova' Expedition. Although he fully understood the importance of the form of the digestive gland (and continued to consider it as highly significant throughout his lifetime), he downgraded its use as the major criterion for subdividing the Nudibranchia. In an effort to achieve more 'natural groupings', Odhner erected a new taxon, Arminacea, because most species possessed an oral veil (or oral tentacles derived from such a veil) and resurrected an old taxon, Dendronotacea (equivalent to Pelseneer's Tritonioidea), wherein all species possessed a sheath around the rhinophores. These new groups were equivalent in rank (as suborders) to the Doridacea and Aeolidacea. In his subsequent monographs (1936 on the Dendronotacea, 1939, 1944), Odhner reiterated his scheme forcefully and at length, with the addition of subgroups (ranked as tribes) for the Arminacea and Aeolidacea, these being, respectively, Euarminacea, Pachygnatha, Leptognatha (the latter two united as Metarminacea) and Pleuroprocta, Acleioprocta, Cleioprocta. Such was the status of Odhner that his scheme rapidly gained worldwide acceptance and it has remained in general use to the present day (e.g. Taylor & Sohl, 1962; Boss, 1982; Vaught, 1989; Ruppert & Barnes, 1994; Beesley et al., 1998).

Additional acts attributable to Odhner were firstly separation of the Doridacea into sections for members with non-retractile gills, Phanerobranchia (a new name for Bergh's Suctoria) and for the rest with retractile gills, Cryptobranchia (for Bergh's Nonsuctoria) (Odhner, 1934: 232), and secondly segregating the Bathydorididae and Doridoxidae into a new taxon, Gnathodoridacea, equivalent in rank to the remaining dorids, Eudoridacea (Odhner, 1934: 233), and lastly, dismemberment of the Acoela (Odhner, 1939: 13).

Franc (1968) assembled notes supplied by Odhner and incorporated material of his own for the systematic section in the *Traité de Zoologie*. Odhner's four major taxa were recognized, but all four were accorded ordinal rank, thus removing any possibility of monophyly for the Nudibranchia (Odhner himself would probably not have agreed) and the dendrodorids and phyllidiids were kept together in the Porodoridacea (=Porostomata Bergh, 1892). The only proponents of this quadripartite classification were Marcus & Burch (1965) and Marcus & Marcus (1967).

As evidenced by his comments as early as 1926, Odhner was evaluating characters (rather than group taxa) as primitive or advanced (Odhner, 1926: 4, 1934: 230, 1939: 26), so in using phylogenetic logic he was really actually ahead of his time. He clearly recognized multiple major groups within the Nudibranchia. However, his contribution was lessened by his devaluing the importance of the form of the digestive gland because the two major groups recognized on that basis, the Holohepatica and Cladohepatica, could not be reconciled with his concept of the Arminacea and Dendronotacea, which he perceived as natural. Oddly, even though the concept of parallel evolution was well understood by Odhner (e.g. Odhner,

1936: 1060), it was never functionally incorporated into his phylogenetic scheme. In our view, recognition of the pervasiveness of parallel and convergent evolution marks the third leap forward in the history of nudibranch research.

Workers since Odhner have refocused on two major clades within the Nudibranchia. Minichev (1970) investigated relationships based on the notal complex, circulatory system and form of the heart, and concluded there are only two main taxa groups (ranked as orders) stemming independently from the Cephalaspidea (Minichev, 1970: 176). Minichev called his clades Anthobranchia (for the dorids) and Nudibranchia (renamed Cladobranchia by Willan & Morton, 1984) for the aeolids, arminids and dendronotaceans. This scheme was adopted by Willan & Morton (1984) and Willan & Coleman (1984) with lower rankings under the belief that 'taxonomic inflation' obscured the underlying similarity of the nudibranchs. Minichev interpreted the circum-anal gills of dorids as the end product of a series of polymerizations (species within the genus *Bathydoris* show several stages in this transition) rather than deformation of a single ctenidium into a circle. Independently, Tardy (1970a) reached the same conclusion as Minichev on the existence of two major nudibranch groups through his investigations of embryology but, contrary to Minichev, Tardy envisaged a common stem for both taxa within the pleurobranchs (Tardy, 1970a: 365). Tardy called the lineages Euctenidiacea (for the dorids) (renamed Ctenidiacea by Schmekel & Portmann, 1982) and Actenidiacea (for the aeolids, arminaceans and dendronotaceans).

In the latter half of the present century several authors have again strived for a 'natural scheme' of relationships within the Nudibranchia and, in turn, between the Nudibranchia and other groups of the Opisthobranchia (Boettger, 1955; Schmekel, 1985). Their analyses were based on only a few characters, and these were interrelated. Different authors emphasized different organ systems in their studies. For instance, Boettger (1955) used the nervous system, Ghiselin (1966) used the reproductive system, and Minichev (1970) and Baranetz & Minichev (1995) used the gills.

Under the interpretation, first noted by Evans (1914), that the dorid gill is homologous with the prosobranch ctenidium [The alternate beliefs are that either the tectibranch gill was homologous with the prosobranch ctenidium and that nudibranch gills were all secondary, or that all opisthobranch gills were secondary and had independent origins within each major clade (Morton, 1972: 345, 1979)], Baranetz & Minichev (1995) argued that the group of the Doridoidea was 'heterogeneous' and split it into four clades (called orders), Doridoxida, Corambida, Phyllidiida, Doridida, corresponding to the four major types of branchial arrangement, with each presumably derived independently.

Phylogenies generated from cladistic principles

Since the methodology of Hennig (1966) has spread through the scientific community, a few malacologists have attempted to construct phylogenies within the Opisthobranchia according to its principles of outgroup comparison, polarity and parsimony. And there have also been some attempts at phylogenetic reconstruction within the Nudibranchia. Usually only a few characters were employed in these analyses because those were all for which adequate comparative data existed. Wägele (1989b) re-interpreted *Bathydoris* and concluded that the Bathydorididae, minus *Doridoxa*, was the sister-group (Gnathodoridacea) to the rest of the Doridoidea. Schmekel (1985), Haszprunar (1985), Salvini-Plawen (1990, 1991) and Salvini-Plawen & Steiner (1996) used characters derived from the chromosomes, nervous

and reproductive systems, respectively, to test Odhner's (1934) phylogenetic scheme for the Nudibranchia.

Within the Nudibranchia, some of the most penetrating cladistic discussions have been presented in the context of systematic studies within the Doridoidea and Aeolidoidea. Willan (1987b) discussed aeolids in relation to the genus *Godiva*, Wägele (1989b) discussed the Doridoidea in relation to *Bathydoris* and *Doridoxa*, Millen & Nybakken (1991) presented a phylogenetic tree for the suctorial Phanerobranchia, Gosliner & Kuzirian (1990) and Gosliner & Willan (1991) discussed aeolids in relation to *Flabellina*, Brunckhorst (1993) assessed the phylogeny of the Phyllidiidae, Gosliner & Johnson (1994) discussed the Doridoidea in relation to *Hallaxa*, and Gosliner (1996) discussed the phylogeny of *Ceratosoma* in relation to other members of the Chromodorididae. No similar studies have been conducted within the Dendronotoidea or Arminoidea.

The conclusions of Martynov's studies on the enigmatic Corambidae are far-reaching phylogenetically because they add another dimension to the possibilities for nudibranch evolution. Martynov (1995) postulated that the Corambidae had retained some characters of the primitive nudibranch like mid-ventral anal location, asymmetry of the notal lobes and smooth rhinophores, and had therefore been derived from the Onchidorididae by paedomorphosis. Besides possessing some characters only found in juvenile onchidorids, like asymmetry of the notal lobes and smooth rhinophores, Martynov argued that the Corambidae had acquired unique characters like cuticular shedding and the form of the radular teeth.

Phylogenetic analyses of other major clades of opisthobranchs, which are all potential outgroups to the Nudibranchia, have also been undertaken; Gosliner (1981) and Mikkelsen (1996) for the Cephalaspidea, Willan (1987a) for the Notaspidea, and Jensen (1996) for the Sacoglossa.

Aims of the present study

Following Odhner (1934), the classification of the Nudibranchia (and sometimes the Nudibranchia plus Pleurobranchidae or Notaspidea) as a single clade (at ordinal level) has been widely accepted (for example by Tardy (1970a)—see quotation at start of Introduction—and Beesley *et al.*, 1998), even though the assumption that underlies it, namely the monophyly of the clade, has not been tested cladistically. Interestingly, when one reviews past studies, one actually finds more advocates for polyphyly (Bergh, 1892; Pelseneer, 1894; Franc, 1968; Minichev, 1970; Thompson, 1976) than monophyly.

The aim of this study is to test the assumption of monophyly for the Nudibranchia and their contained higher taxa by generating a phylogenetic hypothesis. The Nudibranchia is very diverse as regards external, as well as internal, morphology. The expressions ('states') for many of the important characters are already known, but these states have not been evaluated and their polarities have not been established. Therefore an extensive character analysis was necessary and each character needed thoughtful evaluation prior to its inclusion in a phylogenetic analysis. We could not hope to include all the genera (approximately 190) and families (approximately 60) that have been described in a phylogenetic computer analysis because we still lack so much basic information. Nevertheless our thorough character analysis helps us to recognize possible synapomorphies which characterize certain groups, without knowledge of every detail of every species.

Considering the fact that even nowadays many papers dealing with new species are published which contain little information about general anatomy, we think that our summary and assessment of characters might be useful as a kind of manual pointing to improvements for future taxonomic descriptions. We really do hope that this paper inspires taxonomists to have a closer look at characters which might not be significant for identifying lower taxa *per se*, but which might serve the 'higher' cause of elaborating relationships between opisthobranch molluscs.

Composition and names of groups under study

We use a stem-based nomenclature for the higher groups without any rankings. Ponder & Lindberg (1997) made the valid point that there is no objective way to assign ranks to groups, and the process is unnecessary in a phylogenetics context. We must, however, explain the exact groups we have used in terms of composition and nomenclature to avoid confusion. The use of the suffix -oidea by us in this study does not imply any ranking in terms of the conventional zoological hierachy.

To avoid repetition of long series of names of genera that constitute the higher groups (listed in Table 2), conventional higher group names have been used in this work where they already exist. Of course, their use does not imply *a priori* that we support their monophyly. As explained above, testing the monophyly of these groups of nudibranchs is the chief object of this work.

Outgroups

CEPHALASPIDEA is defined according to Mikkelsen (1996: 416). It is used for the 'bubble shells' instead of the alternatives Bullomorpha or Bulloidea.

SACOGLOSSA is defined according to Jensen (1991, 1996: 118) and incorporates the nomenclatural changes included therein except for the spelling of Plakobranchidae (from Placobranchidae) which was emended subsequently (Jensen, 1997: 180–181). It is used for the 'sap-sucking slugs' plus Cylindrobullidae (Mikkelsen, 1998) instead of the alternatives Saccoglossa (sic), Ascoglossa, or Monostichoglossata.

ANASPIDEA is defined according to Mikkelsen (1996: 416). It is used for the 'sea hares' plus Akeridae instead of the synonymous Aplysiomorpha.

Notaspidea is paraphyletic as defined by Schmekel (1985: 254) in that it is only rendered monophyletic by inclusion of the Nudibranchia. TYLODINIDOIDEA is used for the Tylodinidae plus Umbraculidae instead of the alternatives Umbraculoidea (*sensu* Willan, 1987a: 238) or Umbraculomorpha. [The choice of the name is based on historical precedent; Tylodinidae Gray, 1847 predates Umbraculidae Dall, 1889.] PLEUROBRANCHOIDEA is used for the Pleurobranchidae instead of Pleuro-branchomorpha or Pleurobranchacea.

Ingroup

NUDIBRANCHIA is defined according to Odhner (1934 *et seq.*).

ANTHOBRANCHIA is defined for that major group of nudibranchs that share a more recent common ancestor with *Doris* than with *Armina* (i.e. the 'dorids'). All Anthobranchia have a true ctenidium (=primary gill) and the anus and excretory pore lie in the dorsal midline. *Doridoxa* is excluded from the group (Wägele, 1989b). Anthobranchia is used instead of the alternatives Doridoidea (*sensu* Pelsencer, 1894), Holohepatica, Ctenidiacea, Euctenidiacea or Doridida (*sensu* Baranetz & Minichev,

TABLE 2. List of genera and families mentioned in this work and their systematic position within the Opisthobranchia. [1] Position of Doridoxidae not yet resolved; [2] Embletoniidae is held by Miller & Willan (1991) to belong to the Dendronotoidea. * At least one species of this genus was examined partially or completely by histological means. Genera appearing in **bold** type are those chosen for the computer analysis

Higher category	Family	* Genus
INCERTAE SEDIS	Acteonidae	*Acteon* Montfort, 1810
	Hydatinidae	*Hydatina* Schuhmacher, 1817
CEPHALASPIDEA	Aglajidae	*Aglaja* Renier, 1804
	Haminoeidae	*Haminoea* Turton, 1830
	Philinidae	*Philine* Ascanius, 1772
	Cylichnidae	*Scaphander* Montfort, 1810
SACOGLOSSA	Plakobranchidae	* *Elysia* Risso, 1818
ANASPIDEA	Akeridae	*Akera* Müller, 1776
	Aplysiidae	* *Aplysia* Linné, 1767
TYLODINOIDEA	Tylodinidae	* *Tylodina* Rafinesque, 1819
	Umbraculidae	*Umbraculum* Schuhmacher, 1817
PLEUROBRANCHOIDEA	Pleurobranchidae	* ***Bathyberthella*** Willan, 1983
		* *Berthella* Blainville, 1824
		Berthellina Gardiner, 1936
		Euselenops Pilsbry, 1896
		Pleurobranchaea Meckel in Leue, 1813
		Pleurobranchella Thiele, 1925
		Pleurobranchus Cuvier, 1804
		* ***Tomthompsonia*** Wägele & Hain, 1991
NUDIBRANCHIA		*Doridoxa* Bergh, 1899
ANTHOBRANCHIA	Doridoxidae[1]	
Bathydoridoidea	Bathydorididae	* ***Bathydoris*** Bergh, 1884
Doridoidea	Hexabranchidae	***Hexabranchus*** Ehrenberg, 1931
(Phanerobranchia)	Onchidorididae	* *Acanthodoris* M.E. Gray, 1850
		* *Adalaria* Bergh, 1878
		* *Onchidoris* Blainville, 1816
	Goniodorididae	*Akiodoris* Bergh, 1879
		Ancula Lovén, 1846
		Ancylodoris Dybowski, 1900
		Armodoris Minichev, 1972
		Goniodoris Forbes & Goodsir, 1839
		Hopkinsia MacFarland, 1905
		Okenia Brown, 1822
		Teshia Edmunds, 1966
		* ***Trapania*** Pruvot-Fol, 1931
	Corambidae	* *Corambe* Bergh, 1869
	Gymnodorididae	*Analogium* Risbec, 1928
		* *Gymnodoris* Stimpson, 1855
	Polyceridae	* *Nembrotha* Bergh, 1877
		* ***Polycera*** Cuvier, 1817
		* ***Roboastra*** Bergh, 1877
		Tambja Burn, 1962
		* *Thecacera* Fleming, 1828
	Triophidae	*Crimora* Alder & Hancock, 1862
		Holoplocamus Odhner, 1926
		Kalinga Alder & Hancock, 1864
		Kaloplocamus Bergh, 1892
		* *Limacia* Müller, 1781
		Plocamopherus Rüppell & Leuckart, 1828
		Triopha Bergh, 1880
	Aegiridae	* ***Aegires*** Lovén, 1844
		* *Notodoris* Bergh, 1875

TABLE 2—*continued*

Higher category	Family	* Genus
(Cryptobranchia)	Dorididae	*Actinocyclus* Ehrenberg, 1831
		Aldisa Bergh, 1878
		Alloiodoris Bergh, 1904
		* *Archidoris* Bergh, 1878
		Asteronotus Ehrenberg, 1831
		* *Austrodoris* Odhner, 1926
		Baptodoris Bergh, 1884
		Carminodoris Bergh, 1889
		Diaulula Bergh, 1878
		* *Discodoris* Bergh, 1877
		Geitodoris Bergh, 1891
		Halgerda Bergh, 1880
		Hallaxa Eliot, 1909
		Homoiodoris Bergh, 1881
		* *Jorunna* Bergh, 1876
		Kentrodoris Bergh, 1876
		Miamira Bergh, 1875
		Paradoris Bergh, 1884
		Platydoris Bergh, 1877
		* *Rostanga* Bergh, 1879
		Sclerodoris Eliot, 1903
		Sebadoris Er. & Ev. Marcus, 1960
		Siraius Er. Marcus, 1955
		Taringa Er. Marcus, 1955
		Thordisa Bergh, 1877
		Trippa Bergh, 1877
	Chromodorididae	*Ardeadoris* Rudman, 1984
		* *Cadlina* Bergh, 1878
		Cadlinella Thiele, 1931
		Ceratosoma A. Adams & Reeve, 1848
		* *Chromodoris* Alder & Hancock, 1855
		Durvilledoris Rudman, 1984
		* *Glossodoris* Ehrenberg, 1831
		* *Hypselodoris* Stimpson, 1855
		Mexichromis Bertsch, 1977
		Noumea Risbec, 1928
		Risbecia Odhner, 1934
		Thorunna Bergh, 1878
		Verconia Pruvot-Fol, 1931
	Conualeviidae	*Conualevia* Collier & Farmer, 1964
	Dendrodorididae	* *Dendrodoris* Ehrenberg, 1831
	Phyllidiidae	* *Phyllidia* Cuvier, 1797
		Ceratophyllidia Eliot, 1903
CLADOBRANCHIA		
Dendronotoidea	Tritoniidae	* *Marionia* Vayssière, 1877
		Marianina Pruvot-Fol, 1930
		Marioniopsis Odhner, 1934
		Tochuina Odhner, 1963
		* *Tritonia* Cuvier, 1797
		* *Tritoniella* Eliot, 1907
	Dendronotidae	* *Dendronotus* Alder & Hancock, 1845
	Lomanotidae	* *Lomanotus* Verany, 1844
	Dotidae	* *Doto* Oken, 1815
	Bornellidae	*Bornella* A. Adams & Reeve, 1848
	Hancockiidae	*Hancockia* Gosse, 1877
	Phylliroidae	*Phylliroe* Péron & Lesueur, 1810
	Tethydidae	*Melibe* Rang, 1829

TABLE 2—*continued*

Higher category	Family	* Genus
	Scyllaeidae	*Scyllaea* Linné, 1758
		Crosslandia Eliot, 1902
		Notobryon Odhner, 1902
Arminoidea	Arminidae	* ***Armina*** Rafinesque, 1814
		* ***Dermatobranchus*** van Hasselt, 1824
	Goniaeolididae	*Goniaeolis* Sars, 1859
	Heterodorididae	*Heterodoris* Verrill & Emerton in Verrill, 1882
	Charcotiidae	* ***Charcotia*** Vayssière, 1906
		* ***Pseudotritonia*** Thiele, 1912
	Doridomorphidae	*Doridomorpha* Eliot, 1906
	Lemindidae	*Leminda* Griffiths, 1985
	Dironidae	*Dirona* Eliot in Cockerell & Eliot, 1905
	Zephyrinidae	*Caldukia* Burn & Miller, 1962
		Galeojanolus Miller, 1971
		* *Janolus* Bergh, 1884
		Proctonotus Alder, 1844
	Madrellidae	*Madrella* Alder & Hancock, 1864
	Pinufiidae	*Pinufius* Er. Marcus, 1959
Aeolidoidea	Notaeolidiidae	* ***Notaeolidia*** Eliot, 1905
	Flabellinidae	* ***Flabellina*** Voigt, 1834
		Samla Bergh, 1905
	Calmidae	*Calma* Alder & Hancock, 1855
	Aeolidiidae	* *Aeolidia* Cuvier, 1798
		Aeolidiopsis Pruvot-Fol, 1956
		Cerberilla Bergh, 1873
		Baeolidia Bergh, 1888
		Berghia Trinchese, 1877
		* ***Protaeolidiella*** Baba, 1955
		Spurilla Bergh, 1864
	Facelinidae	*Anetarca* Gosliner, 1991
		Babakina Roller, 1973
		Bajaeolis Gosliner & Behrens, 1986
		Cratena Bergh, 1864
		Dicata Schmekel, 1967
		Facelina Alder & Hancock, 1855
		Favorinus M.E. Gray, 1850
		Godiva Macnae, 1954
		Jason Miller, 1974
		Learchis Bergh, 1896
		Moridilla Bergh, 1889
		* *Phestilla* Bergh, 1874
		* ***Phyllodesmium*** Ehrenberg, 1831
		Pruvotfolia Tardy, 1970
	Embletoniidae[2]	*Embletonia* Alder & Hancock, 1851
	Fionidae	*Fiona* Alder & Hancock, 1855
	Glaucidae	* *Glaucus* Forster, 1777
	Pseudovermidae	*Pseudovermis* Periaslavzev, 1891
	Eubranchidae	*Cumanotus* Odhner, 1907
		* ***Eubranchus*** Forbes, 1838
	Tergipedidae	* ***Cuthona*** Alder & Hancock, 1855
		Catriona Winckworth, 1941
		* ***Tergipes*** Cuvier, 1805

1995). Two subgroups constitute the Anthobranchia—the Doridoidea and the Bathydoridoidea.

DORIDOIDEA is defined according to Wägele (1989b) for all the 'dorids' excluding the Bathydorididae. Though familial classification within the Anthobranchia is far

from resolved, families are defined according to the conservative classification scheme adopted by Burn (1975).

BATHYDORIDOIDEA is defined according to Wägele (1989b, 1993) as the name for the group containing only the Bathydorididae. It is used instead of the alternative Gnathodoridacea.

CLADOBRANCHIA (*sensu* Willan & Morton, 1984) is herein defined for that major group of nudibranchs that share a more recent common ancestor with *Aeolidia* than with *Tylodina*, *Umbraculum* and *Doris*. All Cladobranchia have the anus and excretory pore on the right side and none possesses a true ctenidium. Cladobranchia is used instead of the alternatives Cladohepatica, Aeolidacea (*sensu* Thiele, 1931), Actenidiacea, Nudibranchia (*sensu* Minichev, 1970).

DENDRONOTOIDEA is defined according to Odhner (1936). It is used instead of the alternatives Tritonioidea or Dendronotacea.

ARMINOIDEA is defined according to Odhner (1934).

AEOLIDOIDEA is defined according to Odhner (1934). It is used for the 'aeolids' instead of the alternatives Aeolidacea or Eolidacea. The composition of the families within this group is considerably more resolved than that of the Anthobranchia, and we reiterate the distinction between two families here. Miller (1974) enlarged the Facelinidae (*sensu* Odhner, 1939) by attaching the Glaucidae, but we follow Gosliner (1980) and Willan (1987b: 82), who argued on cladistic principles, that Glaucidae merited restriction to just the genera *Glaucus* and *Glaucilla* because of the multitude of autapomorphies they share.

Nomenclature at the species-level adopted in this work

Throughout the text we have updated species-level names according to the most recent published literature rather than citing them in the form they appeared in an original work (i.e. *Pellibranchus cinnabareus* has been corrected to *Vayssierea cinnabarea* and *Gymnodoris striata* to *Analogium striatum*, etc).

MATERIAL AND METHODS

Many taxa of the Nudibranchia and representatives of the Cephalaspidea, Anaspidea, Sacoglossa and Pleurobranchoidea have been investigated very thoroughly by us over many years, by studying living animals and by dissecting chemically fixed ones. No type material was re-examined for this particular study. The senior author has had extensive experience using histological methodology (see Wägele, 1998). The 'Kulzer Method' was employed for histological preparations as outlined in the instructions from Cambridge Chemicals Ltd (i.e. the specimen was embedded in hydroxyethylmethacrylate, sections were cut at 2.5 μm, and sections were stained with toluidine blue). This histological methodology allowed us to include several new characters (Wägele, 1998) and to evaluate characters previously mentioned in the literature (unpublished data of senior author), rather than simply reviewing the published literature. The histological preparations are in the collection of the senior author in Germany.

When discussing and deducing the polarity of characters, we chiefly used the Pleurobranchoidea (especially the Pleurobranchinae) as an outgroup, but we also always considered all other taxa within the Opisthobranchia (Cephalaspidea *sensu*

lato, Anaspidea, Sacoglossa, Tylodinoidea). This was to ensure that characters which are derived in the pleurobranchs were not coded as plesiomorphic in our main outgroup. In some cases it was also necessary to consider the distribution of character states within some 'prosobranch' and pulmonate groups. Table 2 lists all genera (and their systematic position) considered in the character and phylogenetic analysis, so readers not familiar with the genera/genus under discussion can find out where they(it) lie(s) with respect to all the genera.

It is impossible to cite all the literature that was consulted for determining the states and polarity of characters except for the major/most significant works. The significant citations are given in the chapters dealing with character analysis.

Extensive examples (usually at genus or family level) are given in the character analysis, but absolute completeness of coverage was not our aim. In other words there are many more nudibranch genera than those listed in Table 2.

We selected the 30 taxa listed in Table 4 for the phylogenetic analysis because they are representative of groups typically given high taxonomic rank, and for which adequate morphological data exist. Furthermore, representatives of all these genera (except *Hexabranchus* and *Dirona*) collected by the authors or their colleagues were reinvestigated completely by histological means. Therefore the data in the data matrix are derived both from the literature and from our own observations, several of which are not published. Unfortunately some of the nudibranch genera regarded as intermediate or whose relationships are unknown (i.e. *Doridoxa, Heterodoris, Rhodope*) could not be included in this analysis due to lack of data.

In our study the data set was subject to a computational analysis by including those characters which were relevant for the 30 selected taxa (Table 3). For ease of location, these characters are numbered sequentially through the text, and those that could not be used in this analysis, because they were autapomorphies or absent from the taxa considered here, are unnumbered. The cladograms were obtained by using PAUP version 3.1 (Swofford, 1991) executing the data matrix in Table 4. Unknown states were scored '?' and inapplicable ones '—' (Table 4). The heuristic search and bootstrap analyses were performed using different algorithms by choosing different options. Both the heuristic search options ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation) assumptions were applied. The heuristic search options 'general' and 'branch swapping' options were compared with different assessments. The 'steepest descent' option was generally used. Branch swapping methods were employed using the Tree-bisection-reconnection (TBR), as well as the Nearest-neighbour interchange (NNI). All characters were initially treated as unordered and unweighted with the different options mentioned above. Following Haszprunar (1996), who argued that non-weighting of characters is actually the most incorrect form of weighting, we subsequently used weights for those character states where homology hypotheses were highest (applied weighting = 2 for characters 15, 16, 19, 24, 35, 42, see Table 3). This is important when using multistate characters, which have unequal influence than binary characters. Furthermore, character 30 (see Table 3) was treated as ordered.

To avoid the coding of autapomophies as plesiomorphics by using a real taxon as an outgroup, a hypothetical ancestor was used as the outgroup (all zero outgroup), and the two species of the Pleurobranchoidea (*Tomthompsonia antarctica* and *Bathyberthella antarctica*) were treated as if they were members of the ingroup.

Bootstrap analyses (100 replicates) were performed using the heuristic search algorithms. Because there were no major differences in the results when applying

TABLE 3. Character analysis. Only those characters that were used for the phylogenetic analysis are listed. Column 2 lists the plesiomorphic and apomorphic state(s) for each character. Column 3 indicates the weighting (W) that was applied through successive analyses. Only character 30 was treated as ordered in many analyses

Character	Plesiomorphic/apomorphic state(s)	W
1	Anterior notum in relation to head: 0 - notum free from head and ending posterior to head; 1 - notum extending over head	1
2	Anterior notum in relation to head: 0 - notum free from head and ending posterior to head; 1 - lateral and frontal notal margin fused with head	1
3	Notum in relation to foot: 0 - notum same size as foot, or slightly smaller; 1 - notum considerably larger than foot; 2 - notum considerably smaller than foot	1
4	Notal margin: 0 - free; 1 - fused with foot	1
5	Notal margin: 0 - smooth; 1 - papillae situated on notal margin and containing digestive diverticula	1
6	Notal edge: 0 - simple notal margin, or papillae present at margin; 1 - dichotomous or bushy gills at notal margin	1
7	Arrangement of papillae on notum: 0 - in transverse rows; 1 - in oblique rows	1
8	Margin of oral veil: 0 - smooth; 1 - bearing frontal processes	1
9	Oral tentacles: 0 - oral veil without lateral tentacles; 1 - oral veil bearing lateral tentacles	1
10	Propodial tentacles: 0 - absent; 1 - present	1
11	Rhinophores: 0 - hollow and enrolled; 1 - solid	1
12	Rhinophoral sheaths: 0 - absent; 1 - sheaths formed by frontal notal margin; 2 - sheaths formed by elevation of rim of notal pocket	1
13	Anal gills: 0 - present; 1 - absent	1
14	Number of anal gills: 0 - one to three; 1 - five or more	1
15	Retractability of anal gills: 0 - not retractile; 1 - retractile into sub-notal cavity	2
16	Position of anus: 0 - lateral on right side near middle of body; 1 - medio-dorsal towards rear of body	2
17	Shell: 0 - present; 1 - absent	1
18	Oral glands: 0 - consisting of glandular layer only; 1 - distinct oral glands with two ducts; 2 - distinct oral glands with one duct	1
19	Buccal pouch: 0 - absent; 1 - present	2
20	Composition of jaw: 0 - jaw composed of platelets; 1 - aliform jaw	1
21	Masticatory border of aliform jaws: 0 - with several rows of denticles; 1 - with one row of denticles; 2 - without denticles	1
22	Number of lateral teeth: 0 - multidenticulate radula (many laterals); 1 - one to three laterals; 2 - laterals absent	1
23	Rachidian tooth: 0 - present; 1 - absent	1
24	First (inner) lateral tooth: 0 - not differentiated from succeeding laterals; 1 - first or second lateral larger and relatively broader than succeeding laterals	1
25	First lateral: 0 - present; 1 - absent	1
26	Form of outer laterals: 0 - not differentiated from remaining laterals, hook-shaped; 1 - differentiated from remaining laterals, plate-like	1
27	Cuticle in oesophagus: 0 - present; 1 - restricted to a small portion within proximal oesophagus; 2 - completely absent	1
28	Interior of stomach: 0 - without cuticular lining; 1 - lined with cuticle	1

TABLE 3—*continued*

Character	Plesiomorphic/apomorphic state(s)	w
29	Caecum (right digestive gland): 0 - with glandular epithelium; 1 - differentiated into caecum; 2 - caecum absent	1
30	Terminal sacs and cnidosacs: 0 - both absent; 1 - terminal sacs present; 2 - cnidosacs present, containing kleptocnides; 3 - cnidosacs present, but without kleptocnides	2
31	Typhlosole: 0 - present; 1 - absent	1
32	Statocyst: 0 - containing multiple otoconia; 1 - containing one otolith	1
33	Position of gonad: 0 - discrete, located in front of digestive gland; 1 - spreading over digestive gland	1
34	Gonadial acini: 0 - spermatogonia and oogonia located in same follicle; 1 - spermatogonia and oogonia in separate follicles	1
35	Genital system: 0 - diaulic; 1 - triaulic	2
36	Bursa copulatrix and receptaculum seminis: 0 - both present; 1 - bursa copulatrix absent	1
37	Penial hooks: 0 - absent; 1 - present	1
38	Orientation of pericardial complex: 0 - transverse orientation; 1 - orientation parallel to longitudinal axis	1
39	Location of pericardial complex: 0 - anterior or median within body cavity; 1 - posterior within body cavity	1
40	Blood gland: 0 - present; 1 - absent	1
41	Location of blood gland: 0 - adjacent to heart; 1 - between heart and nervous system, or on top of nervous system	1
42	Marginal glands: 0 - absent; 1 - present	2
43	Glandular stripe on side of notum: 0 - restricted to right side of body; 1 - present on both right and left sides of notum	1
44	Location of follicles of glandular stripe: 0 - present in lateral notum; 1 - follicles within papillae	1
45	Gill glands: 0 - absent; 1 - present	1
46	Glandular stripe: 0 - present; 1 - absent	1
47	Specialized vacuolated epithelium: 0 - absent; 1 - present	1

the different options mentioned above in the heuristic search options, the following algorithms were applied for the bootstrap analyses: ACCTRAN; Branch swapping (with trees retained which are two steps longer than the shortest tree); TBR; steepest descent and MULPARS option in effect. Again, the Pleurobranchoidea was included in the ingroup and a hypothetical ancestor (all zero) used as outgroup. Characters were weighted by treating weightings as repeated counts.

CHARACTER ANALYSIS

In the following analysis, the number(s) of the characters used in the phylogenetic analysis (see characters listed in Table 3 and data matrix in Table 4) is (are) given in the subheading of the particular character.

TABLE 4. Character matrix for cladistic analysis of the Nudibranchia. ? denotes missing data, '–' denotes that the character is not applicable for the taxon in question

Taxon	1 1234567890	2 1234567890	3 1234567890	4 1234567890	1234567
All zero outgroup	0000000000	0000000000	0000000000	0000000000	0000000
Bathyberthella	000000–000	0000000000	0010000000	0001000000	00?–010
Tomthompsonia	000000–000	0000000000	0010000000	0001000000	00?–010
Bathydoris	100000–010	1000011001	2000000010	1000000110	00--011
Aegires	100000–010	1200011000	0010002010	1010101110	10--111
Hexabranchus	100000–0?0	1?01011000	00100020?0	?0??100110	10--111
Hypselodoris	100000–010	1201111000	0010002010	1011100110	10--111
Jorunna	101000–010	1201111000	0010002010	1010100110	10--111
Archidoris	101000–010	120111100–	0010002010	1010100110	10--111
Onchidoris	101000–000	1201011010	0011012020	0010101110	10--111
Polycera	102100–000	1000011001	00110120?0	1011101110	10--111
Trapania	102100–011	1000011010	01111–2010	0011101110	10--111
Roboastra	102100–010	1200011000	0001112020	1011101110	10--111
Tritonia	010001–110	111–001001	0000001100	0011010101	–0--011
Dendronotus	010001–110	111–001001	0000001100	001?010101	–0?00?1
Doto	010111–000	111–001001	2100001000	?010010101	–011001
Lomanotus	010000–110	111–001001	0110001100	0011010101	–000001
Armina	000000–001	101–001201	0000001000	0010010101	–1--011
Dermatobranchus	000000–000	101–001201	0000001000	0010010101	–1--011
Charcotia	010000–000	101–001001	0100001001	0010010101	–00–001
Pseudotritonia	010000–000	101–001001	0100001001	001?010101	–00–001
Dirona	0100?00000	101–001001	200001000?	?01?010101	–010001
Notaeolidia	0100100010	101–001001	2000001002	0010010101	–000001
Flabellina falklandica	0100100010	101–001?01	0100001002	?010010101	–000001
F. pedata	0101101011	101–001101	0100001002	0010010101	–000001
F. affinis	0101101011	101–001101	0100001002	0010010101	–000001
Cuthona	010110–010	101–001101	120---1002	011?010101	–011001
Tergipes	010110–010	101–001001	1100001002	0111010101	–011001
Eubranchus	010110–010	101–001001	1100001002	0111010101	–0?1001
Phyllodesmium	0101101010	101–001001	120---1001	001?010101	–0--011
Protaeolidiella	0101101010	101–001001	220---1003	0011010101	–0--011

External characters

1–4. Notum in relation to other organs

(1) In nudibranchs, the notum is separated from the foot by way of a distinct notal margin (or brim) all round. The notum is as large as, or somewhat smaller than, the foot (also in outgroups Pleurobranchoidea, Anaspidea and Cephalaspidea). The head is also distinct and generally not covered by the notum. The rhinophores arise near the front of the notum. The following characters can be observed within the Nudibranchia regarding the notum's relationship to other external organs.

The rhinophores and head are clearly separate from the distinct anterior notal brim in *Armina*, *Dermatobranchus*, *Heterodoris* and *Pseudotritonia*. This situation is also found in the outgroups (Tylodinoidea, Pleurobranchoidea), and is therefore considered to be the plesiomorphic state (Fig. 1A,B).

Two different types of notal extension can occur: The notum can have an anterior extension (medially and laterally) over the head, covering the oral veil and surrounding the rhinophores in *Doridoxa*, Bathydoridoidea, Doridoidea (e.g. *Ceratosoma* Fig. 1H), *Doridomorpha*). This situation is unique within the opisthobranchs and is therefore considered to be apomorphic. In *Goniodoris*, the notum does not extend completely
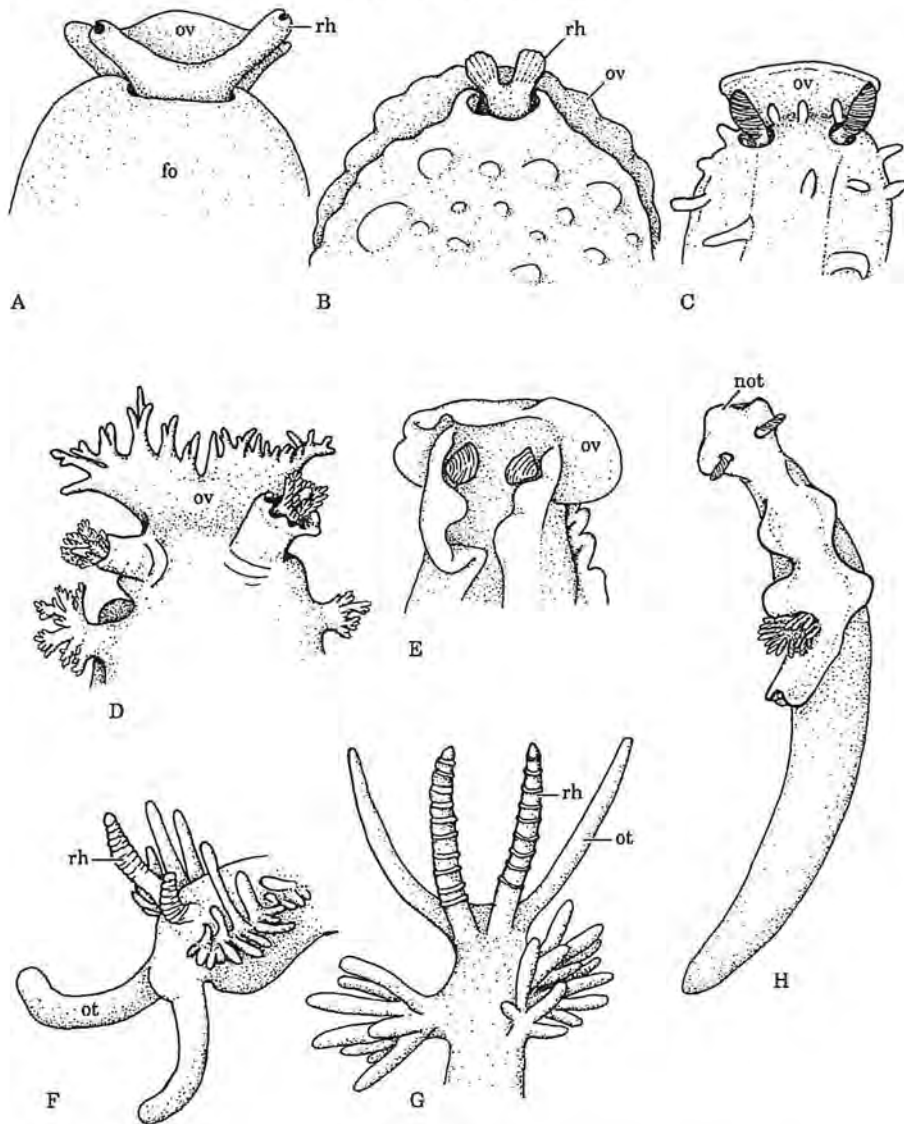
Figure 1. Dorsal or dorsolateral view of head region of selected Opisthobranchia: A, *Berthella aurantiaca*; B, *Armina maculata*; C, *Heterodoris antipodes* (redrawn after Willan, 1981); D, *Marionia blainvillea*; E, *Pseudotritonia gracilidens* (redrawn after Wägele, 1991); F, *Notaeolidia gigas*; G, *Flabellina ischitana*; H, *Ceratosoma tenue*. Abbreviations: fo - foot; not - notum; ot - oral tentacle; ov - oral veil; rh - rhinophore.

over the oral veil, a situation which can be interpreted either as the beginning of overgrowth or as a secondary reduction following overgrowth.

(2) Fusion of the anterio-lateral part of the notum with the head can occur laterofrontal to the rhinophores in the Dendronotoidea (e.g. *Marionia* Fig. 1D), Charcotiidae, *Dirona* and *Notaeolidia* (Fig. 1E,F). In those species with processes on
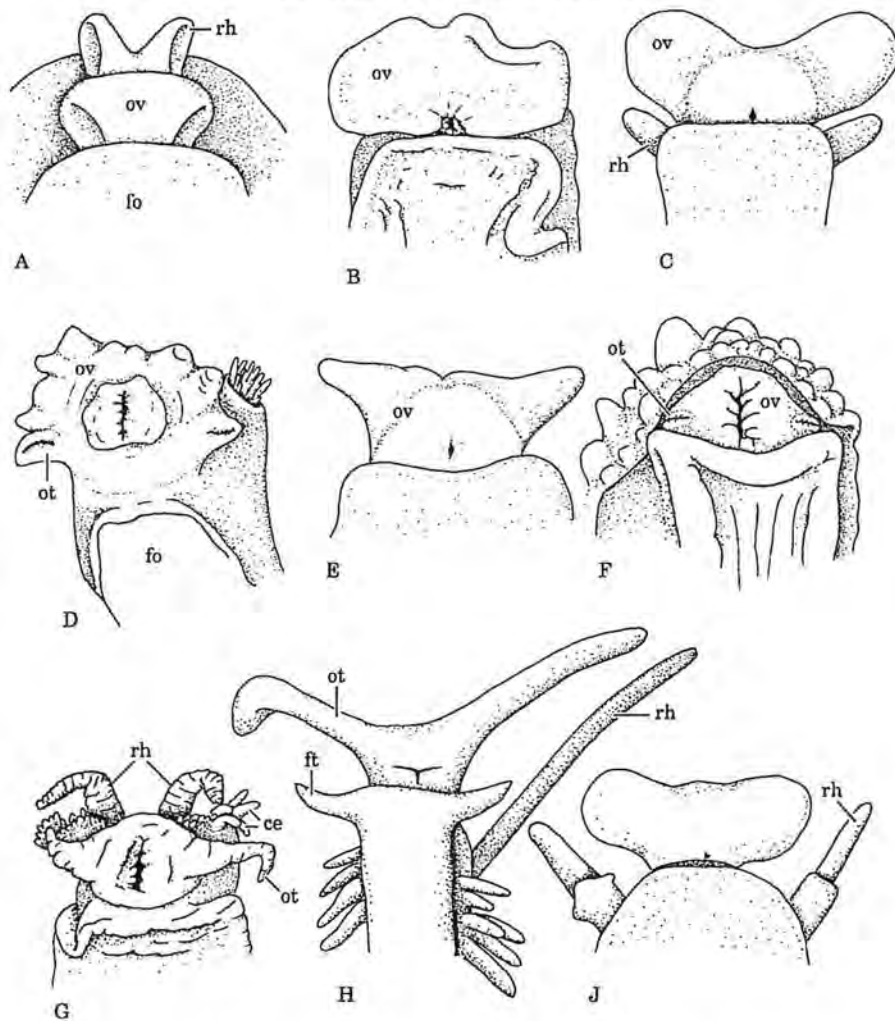
Figure 2. Ventral view of head region of selected Opisthobranchia: A, *Tomthompsonia antarctica* (redrawn after Wägele & Hain, 1991); B, *Pseudotritonia antarctica* (redrawn after Wägele, 1991); C, *Embletonia pulchra* (redrawn after Schmekel & Portmann, 1982); D, *Tritonia antarctica* (redrawn after Wägele, 1995); E, *Goniodoris castanea* (redrawn after Schmekel & Portmann, 1982); F, *Aegires albus*; G, *Notaeolidia schmekelae* (redrawn after Wägele, 1990a); H, *Learchis evelinae* (redrawn after Edmunds & Just, 1983); J, *Doto floridicola* (redrawn after Thompson *et al.*, 1990). Abbreviations: ce - ceratapapillae; fo - foot; ft - propodial tentacle; ot - oral tentacle; ov - oral veil; rh - rhinophore.

the notal margin (*Dirona*, *Notaeolidia*), these processes come to lie in front of, or alongside, the rhinophores. This is also considered to be an apomorphic trait.

Two apomorphic trends can be deduced as derivatives of the latter situation. In some groups (Zephyrinidae) the lateral notal margin elongates in front of the head, then covers the oral veil, with the processes of the notal margin surrounding the whole animal. Within many Aeolidoidea, the papillae are confined to that part lying

posteriorly to the rhinophores. This trend can be observed within the genus *Flabellina* and is considered to be an apomorphic trait (Fig. 1G).

(3) The situation of the notum equal in size to the foot, or a little smaller, can be found within several taxa of the Opisthobranchia (Sacoglossa, *Tylodina*, many Pleurobranchoidea) as well as the Nudibranchia (Bathydoridoidea, Chromodorididae, Dendronotoidea). We consider that this is the plesiomorphic state. Two different trends can be observed within the Nudibranchia, which are both considered as separate apomorphies; enlargement of the notum so that it covers the foot completely (e.g. *Doridoxa*, Cryptobranchia, *Kalinga*, *Doridomorpha*), or a decrease in size of the notum so only half the body, or even less, is covered (e.g. *Notodoris*, *Goniodoris*, *Okenia*, *Gymnodoris*, Polyceridae, *Ceratosoma*).

(4) A distinct notal margin is present in some taxa of the Opisthobranchia (Anaspidea, Tylodinoidea, Pleurobranchoidea) and many nudibranch taxa (Bathydoridoidea, Cryptobranchia, Aegiridae, Tritoniidae, Arminidae, Charcotiidae, Notaeolidiidae, etc.). This is considered to be the plesiomorphic state.

Fusion of the lateral notal margin with the foot, so as to leave no vestige of a brim is the apomorphic state and it can be observed in several taxa within the nudibranchs (Polyceridae, Gymnodorididae, Dotidae, several taxa of the Aeolidoidea). The notal margin itself can be regularly lacerated (*Hexabranchus* - pers. obs.), or extended into flattened triangular papillae (*Verconia* - Rudman, 1984).

A separation between the rear end of the foot (the 'tail') and the posterior notum is present in many opisthobranchs (see also above, free notal edge): Tylodinoidea, Pleurobranchoidea, Bathydoridoidea, many Doridoidea, *Tochuina* and *Marioniopsis*. In these groups the foot is usually larger than the free notal edge. In the Tritoniidae, *Lomanotus*, *Hancockia*, *Crosslandia*, *Scyllaea*, *Notobryon*, *Heterodoris* and *Notaeolidia*, the notal margin is connected to the tail all the way to the tip, but it is never fused with it. A free notal edge above the tail is considered to be the plesiomorphic state. Complete fusion of the posterior notum margin with the foot as observed in many Aeolidoidea, *Polycera*, *Tambja*, *Roboastra*, *Thecacera*, *Aegires* (except *A. albus*), *Dendronotus*, etc. is considered to be the apomorphic state. It should be noted that the latter character state very often coincides with fused lateral notal margins. This state represents the most derived situation, i.e. the complete fusion of the entire notum edge with the foot, as in *Bornella*, *Doto*, *Marianina*, many members of the Aeolidoidea, etc. Because this character can vary within genera (e.g. *Aegires*), it was not used in this analysis.

1–2. Notum in relation to head

Polarity: overgrowth of head by the notum during ontogeny (or the fusion of the anterio-lateral notum with the head) is considered to be apomorphic.

1. *Coding*: 0 - notum free from head and ending posterior to head; 1 - notum extending over head.

2. *Coding*: 0 - notum free from head and ending posterior to head; 1 - notum and head fused between rhinophores.

3. Notum in relation to foot

Polarity: Notum larger than, or considerably smaller than, the foot are considered to be two independent apomorphic states and are treated as unordered.

Coding: 0 - notum only slightly smaller than, or same size as, foot; 1 - notum larger than foot; 2 - notum considerably smaller than foot (about 2/3 its size).

4. Notal margin

Polarity: A free notal margin around the whole foot is considered to be the plesiomorphic state, whereas the complete fusion of the notum with the foot is the apomorphic state.

Coding: 0 - free notal margin; 1 - notal margin fused with foot.

5–7. Papillae on the notum

Traditionally, several terms have been used to denote the elaborations/appendages arising from the notum or notal margin. These terms include 'cerata', 'processes', 'gill-like structures', 'papillae', 'tubercles' and 'pustules'. Because all these elaborations are derivatives of the notum, and our adoption of any particular one of them might be misconstrued as implying homology for these elaborations, we will use the neutral term 'papilla' for all the extensions off the dorsal notum. After clarifying the phylogeny it might be more appropriate to use more particular terms that are specific for homologous structures. Distinct papillae at the notal margin, in the form of digitiform processes, are present only within the Sacoglossa and the Nudibranchia.

The following arrangements can be observed within the Nudibranchia:

(a) Papillae (without internal digestive diverticulum) arising over the whole dorsal part of the notum (many Doridoidea, *Heterodoris, Charcotia*) (Fig. 3A,B).

(b) Papillae (without internal digestive diverticulum) present in a single row around the notal margin (some species of *Okenia, Teshia, Plocamopherus, Kaloplocamus*).

(c) One row of papillae (containing a diverticulum of the digestive gland) is present in some dendronotoid and aeolid genera (*Lomanotus, Hancockia, Dendronotus, Eubranchus*) (Fig. 3C).

(d) Branched processes arising from the notal margin are present in (most of) the Tritoniidae and also (a few) Doridoidea (Fig. 1D).

(e) Many Dendronotoidea have an outer row of papillae containing a digestive diverticulum and an inner row of branched processes without digestive glandular branches (Bornellidae, Dotidae).

(f) Zephyrinidae have several rows of papillae on the notal margin, most of which contain digestive glandular branches (*Janolus, Galeojanolus, Proctonotus, Caldukia*).

(g) Many Aeolidoidea bear papillae in several more or less distinct longitudinal rows on the notal margin, all containing a digestive diverticulum (e.g. *Notaeolidia, Flabellina, Protaeolidiella, Babakina*).

Sometimes the papillae themselves possess characters which allow us to postulate homologies. Therefore, using them at the genus or family level is phylogenetically informative.

The presence of a cnidosac (a region for storing undischarged nematocysts derived from the food for that individual's own defense) at the apex of digitiform papillae within the Aeolidoidea is unique. We consider this character as an autapomorphy for all Aeolidoidea, including *Notaeolidia*. The plesiomorphic state is having papillae without cnidosacs. For coding see character 30.

The shape of the papillae themselves can be very distinctive (e.g. *Eubranchus, Phyllodesmium*) and shape can be used to indicate relationship at least within family or genera level.

Within the monotypic Bathydorididae, the notum is completely covered by club-shaped papillae which autotomize very easily. The papillae are filled by loosely

arranged connective tissue (Fig. 3D), and at their base a small muscular ring probably facilitates autotomy. This character is considered to be an autapomorphy of *Bathydoris*. In the zephyrinid genus *Galeojanolus* the papillae have a similar distribution to *Bathydoris* and they can also be autotomized easily. Whether they also possess muscles at the base has not yet been investigated. They differ from the papillae of the Bathydorididae by the incorporation of digestive diverticula although Gosliner (1981) described a certain extent of intraindividual variability. One genus of the Phyllidiidae (*Ceratophyllidia*) is also characterized by stalked papillae, which can be detached easily. Nothing is known about their structure. This character (autotomy of papillae) is not coded because *Ceratophyllidia* and *Galeojanolus* are not included in this analysis due to lack of information.

A specialized type of papilla is the so-called caryophyllidium that is present in some Doridoidea (*Jorunna*, *Rostanga*, *Kentrodoris*, *Taringa*) (Fig. 3B). Caryophyllidia are papillae with a sensory knob at the core and spicules which surround the knob and emerge through the notal epidermis (Marcus, 1976; Kress, 1981). In accordance with Foale & Willan (1987) and Gosliner (1994), we consider the possession of such a complex structure as a good synapomorphy for the genera mentioned above. Unfortunately tubercles with protruding spicules may easily be misinterpreted as caryophyllidia, and a thorough investigation (preferably with SEM or histology) is always needed to confirm the exact situation. Having simple papillae with non-emergent spicules and without a sensory knob is the plesiomorphic state. In the genera used for this analysis only *Jorunna* is characterized by caryophyllidia, therefore, being a trivial character, this character is not coded.

Due to the limited selection of species in the phylogenetic analysis, only a few characters discussed above are included here:

(5) According to outgroup comparison a smooth notal margin is considered to be the plesiomorphic state, whereas the elaboration of papillae at the notal margin with digestive glandular branches reaching far into these papillae is a derived state, which is considered to be apomorphic.

(6) Dichotomously branched or bushy processes at the notal margin, without any accompanying papillae, are known only from a few members of the Nudibranchia (Tritoniidae, *Kalinga*, *Kaloplocamus*, *Plocamopherus*). In several other groups, the branched processes are located on the inner side of simple papillae lying at the notal margin. Possession of branched papillae is considered to be the apomorphic state, whereas a smooth notal edge or simple papillae are considered to be the plesiomorphic state.

(7) The increase of one row of papillae on the notal margin to several rows is easy to explain. As the notum grows outward at its edge, new papillae form on this edge and the older (larger) ones shift progressively inwards. Therefore, the smallest papillae are always found at the very edge of the notum (Fig. 1F). An irregular arrangement of the papillae in longitudinal rows along the notum edge is considered to be the plesiomorphic state. It can be observed in the Zephyrinidae, several species of *Flabellina*, etc.). Within the Aeolidoidea, the papillae can also occur in more precise arrangements such as oblique rows or arches which represent the apomorphic state. Because in some species both arrangements (rows and arches) can be observed, we did not code these specific states for this phylogenetic analysis separately.

5. Papillae on the notum

Polarity: A smooth notal margin is considered to be the plesiomorphic state. Papillae

on the notal margin containing digestive glandular branches is considered to be the derived state.

Coding: 0 - simple notal margin; 1 - papillae (containing digestive diverticulum) situated on notal margin.

6. Branched gills on the notum or notal papillae

Polarity: Branched or bushy gills located on the notal edge or alongside the notal papillae are only known in a few nudibranch taxa and their presence is therefore considered to be the derived state.

Coding: 0 - simple notal edge, or papillae at edge; 1 - branched or bushy gills at notal edge.

7. Arrangement of papillae

Polarity: Arrangement of the papillae in rows across the notum is considered to be the plesiomorphic state, and the arrangement of the papillae in oblique rows is the derived state. This character cannot be applied for those genera without papillae. *Coding*: 0 - papillae in simple rows; 1 - papillae in oblique rows; '–' not applicable.

8-9. Oral veil

Within the Opisthobranchia, only *Tylodina*, the Pleurobranchoidea and (some of) the Nudibranchia possess a sail-like, trapezoidal structure surrounding the mouth that is separate from the anterior notal margin above and the margin of the propodium. This structure is called an 'oral veil'. In some members of the Sacoglossa (i.e. the Polybranchiidae), a structure in front of, and ventral to, the rhinophores can be observed during ontogeny, which can be homologized with the oral veil (Jensen, 1996). This structure is fused dorsally with the notal area but probably never with the foot. This structure might be homologous with the cephalic shield (or part of it) of the Cephalaspidea, but at this time it is difficult to deduce its polarity. For this reason we prefer not to consider the polarity within the Opisthobranchia (separate oral veil versus fused oral veil). But within the Pleurobranchoidea and several nudibranch taxa, the oral veil is clearly a distinct structure, especially in those taxa where no further elaboration of the oral veil occurs, so we consider the free oral veil to be the plesiomorphic state within the Nudibranchia and the fusion of the oral veil with the notum as the apomorphic state.

(8) An oral veil with a smooth anterior margin is present in all members of the Tylodinoidea (except *Umbraculum* where there is no oral veil), Pleurobranchoidea (except *Pleurobranchella*, *Pleurobranchaea* and *Euselenops* where compound papillae are present along the anterior margin of the oral veil), and some Nudibranchia (i.e. *Okenia*, *Heterodoris*, Charcotiidae, Arminidae, *Dirona*, *Madrella*, *Embletonia*) (Fig. 2A–C). We consider this as the plesiomorphic state. Digitiform processes on the anterior edge of the oral veil are only present in members of the Dendronotoidea (e.g. Tritoniidae (Fig. 2D), *Dendronotus*, *Bornella* and *Hancockia*). All the frontal processes present in the other taxa (*Kalinga*, *Triopha*, *Plocamopherus*, Zephyrinidae) are derivations directly off the anterior margin of the notum and therefore not homologous with the frontal processes of the Dendronotoidea.

(9) Gosliner (1994) described oral tentacles in the Aplysiidae, Pleurobranchoidea and Nudibranchia, without assessing any possible homology. Many Pleurobranchoidea do

not have distinct oral tentacles, but they still possess an oral veil with lateral grooves (Fig. 2A). A similar situation can be found in the Tritoniidae (Fig. 2D), and to a certain extent in other dendronotoid genera. But usually, the oral veil is partially absent in the latter, so that only the oral tentacles and several frontal processes arise from the head (e.g. *Bornella*, *Dendronotus*). A reduction of the oral veil with elaboration of the oral tentacles can be observed within the Anthobranchia. *Bathydoris* has both a rather large oral veil, albeit overgrown by the anterior notum, and also thick oral tentacles (Wägele, 1989a). A distinct oral veil is present in *Goniodoris* (Fig. 2E), but the lateral edges are tentacular. Unfortunately we do not know whether they possess grooves. Other members of the Doridoidea with a considerably reduced oral veil are reported, which do have oral tentacles with grooves (*Paradoris*, *Kalinga*, *Triopha*, *Glossodoris*, *Aegires*, Phyllidiidae—see Hoffmann, 1939; Brunckhorst, 1993; Miller, 1995) (Fig. 2F). The absence of lateral grooves in a distinct oral veil is confirmed for the Dotidae, many arminoid genera (*Armina*, Charcotiidae, *Dirona*) and *Embletonia* (Fig. 2B,C,J).

In many cladobranch groups the oral veil is lost at the expense of the oral tentacles. This occurs several times within the different groups (e.g. some Zephyrinidae and Aeolidoidea (Fig. 2G,H)). No grooves are reported from the oral tentacles in these groups, and so the evolution from an oral veil without grooves is therefore most probable.

The Tethydidae is characterized by an enlarged, cowl-shaped head which is used to capture active crustacean prey. It is not clear whether this cowl is derived from the oral veil or from the anterior edge of the notum. Nevertheless, such a highly derived and unique character can be used for phylogenetic analysis.

8. Anterior processes on the oral veil

Polarity: A smooth oral veil without any anterior processes (independent from absence or presence of oral tentacles) is considered to be the plesiomorphic state, and the presence of such processes is the apomorphic state.

Coding: 0 - smooth anterior margin to oral veil; 1 - oral veil with processes arising from anterior margin.

9. Oral tentacles

Polarity: The absence of lateral tentacles on the oral veil is considered to be plesiomorphic, and the presence, independent of the reduction of the rest of the oral veil, apomophic. The presence or absence of grooves is not used here due to the lack of reliable data.

Coding: 0 - oral veil without lateral tentacles; 1 - oral veil bearing lateral tentacles.

10. Anterior foot border

(10) In many opisthobranch taxa (Tylodinoidea, Pleurobranchoidea, Bathydoridoidea, many Doridoidea, Dendronotoidea, *Heterodoris*, Charcotiidae, *Dermatobranchus*, *Embletonia*, *Notaeolidia*, *Flabellina athadona*, *Cuthona*, *Eubranchus*, etc.) the anterior margin of the foot is rounded and devoid of fronto-lateral (propodial) tentacles. This situation is also present in many members of the Cephalaspidea and Sacoglossa. We consider it to be the plesiomorphic state.

Enlarged propodial tentacles (Fig. 2H) are present in many taxa of the Aeolidoidea (e.g. *Flabellina* (except *F. amabilis* and *F. trilineata* with angulate foot corners), Facelinidae). Propodial tentacles are present, though smaller, in some *Armina* and *Janolus*

species and in the doridoideans *Trapania* and *Mexichromis*. Small subtentaculate foot corners are also present in some cephalaspid and sacoglossan species. We do not know anything about the innervation of these elaborations. Nevertheless, we assume that they represent another sensory organ, probably mechanical, for investigating the substrate. The presence of propodial tentacles is considered to be an apomorphy.

The anterior foot border of many opisthobranchs is more or less divided horizontally into two lips and, at least in the Nudibranchia and Pleurobranchidae, this area usually has a thick layer of subepidermal glands. Because the presence of a divided anterior foot border is apparently widespread throughout the Opisthobranchia, it is not used in this study.

In some nudibranchs a vertical notch in the upper lip of the anterior foot border (termed a *philtrum* by analogy with the similar cleft in the upper lip of the Carnivora), is present. The philtrum might be a specialization related to a particular food type or a structure related to the intake of food—with a philtrum, the mouth and the oral tube have more space to protrude for grasping food. Because a philtrum is absent in *Tylodina*, Pleurobranchoidea, Bathydoridoidea, many Doridoidea (*Aegires*, *Austrodoris*, *Hexabranchus*), and all other nudibranch groups, we consider its absence represents the plesiomorphic state. At the moment, the presence of a philtrum is confirmed for members of the cryptobranch Anthobranchia (e.g. *Jorunna*, *Halgerda*, *Rostanga*, *Geitodoris*, *Carminodoris*, *Kentrodoris*, *Paradoris*, *Dendrodoris*).

10. Propodial tentacles

Polarity: The presence of propodial tentacles is considered to be the apomorphic state, though outgroup comparison suggests that convergent evolution occurs.
Coding: 0 - anterior foot border without fronto-lateral (propodial) tentacles; 1 - propodial tentacles present.

11–12. Rhinophores

(11) Whereas the rhinophores are essentially rolled sheets containing (chemo)sensory tissue in all opisthobranchs (except Cephalaspidea), they are solid columns in the Nudibranchia. Gosliner (1994) argued for the independent evolution of rhinophores in several opisthobranch lineages. We assume that the rhinophores are homologous at least in the Pleurobranchoidea and Nudibranchia, because they are innervated by a thick rhinophoral nerve emerging at the anterior side of the cerebral ganglia in both groups. This nerve is usually provided with a rhinophoral ganglion. And in addition, in nearly all opisthobranch taxa and the basal pulmonate groups, the eyes lie at the base of the rhinophores (Basommatophora, Gymnomorpha, Anaspidea, Sacoglossa, Pleurobranchoidea, many groups of the Nudibranchia), a fact that points to the possible homology of the rhinophores across the entire Opisthobranchia, or even the Euthyneura.

Within the Nudibranchia, the rhinophores are solid in construction and circular in cross section. In this group there are many different kinds of rhinophoral ornamentation (see Hoffmann, 1939; Gosliner, 1994). Within the different taxa of nudibranchs, the lamellate (=perfoliate) type is most widespread (*Doridoxa*, Bathydoridoidea, nearly all Doridoidea, many Zephyrinidae, many Aeolidoidea). A lamellate type without a smooth proximal section ('stalk') (*Doridoxa*, Bathydoridoidea, *Notaeolidia*) is less elaborate compared to the type with proximal stalk and distal, enlarged, club-like, generally ornamented section ('clavus') that may or may not be orientated in the same axis as

the stalk. We therefore assume that the lamellate type of rhinophore without a stalk has evolved in the common ancestor of the Nudibranchia, and is therefore an autapomorphy of the entire group. This is in accord with the conclusions of Willan (1987b) for the Aeolidoidea. Because no outgroup comparison was possible, we decided not to use this character in our phylogenetic analysis.

The deviation of the lamellate type of rhinophore into types with other ornamentation needs to be considered when dealing with family-level and subordinate taxa (i.e. genus and even species level).

The following substructures can be found on the rhinophores in nudibranchs:
(a) Lamellate with distinct stalk (many Doridoidea, *Heterodoris*) (Fig. 1C);
(b) Smooth (Aegiridae, Corambidae, *Conualevia*, Dotidae, *Pseudotritonia*, several members of the Aeolidoidea) (Fig. 2C,H,J);
(c) Tall papillae confined to the posterior face, or all the way round (*Madrella*, *Baeolidia*, *Berghia*, some *Flabellina* species, *Noumeaella*);
(d) Plume-like structures arising from the clavus (the palmate condition of the Tritoniidae) (Fig. 1D);
(e) Vertical ribs (Arminidae and, to some extent, *Pseudotritonia quadrangularis*) (Fig. 1B);
(f) Annulate (some Aeolidoidea). But note that this ornamentation is often very hard to recognize in preserved specimens and not very consistent even within the same genus (see *Learchis* species with annulate or smooth rhinophores) (Fig. 1G);
(g) With flanges on the clavus (*Favorinus*);
(h) With regular swellings ('bulbs') on the clavus (*Favorinus*);
(i) Rhinophores completely absent (in some *Pseudovermis* species).

(12) In two unrelated groups of the Nudibranchia, one finds tubular elevations of skin surrounding the rhinophores and presumably affording extra protection to the rhinophores. 'Rhinophoral sheaths', as these elevations are termed, are formed either by the growth of the frontal notal margin around the rhinophores (Dendronotoidea), or by elevation of the rim of the rhinophoral pocket (some Cryptobranchia). Usually the rhinophores can be withdrawn completely into these sheaths. Rhinophoral sheaths are absent in most taxa of the Nudibranchia (many Phanerobranchia, Arminoidea, Aeolidoidea) and all other opisthobranch groups, so their presence is considered to be the apomorphic state.

The situation in *Tochuina* is not clear. It seems that in this (monotypic) genus the notal margin incompletely fused around the rhinophores. This situation would present the plesiomorphic state at least within the Tritoniidae, a taxon usually possessing complete rhinophoral sheaths.

Willan (1989) described an interesting situation in *Thecacera boyla*, where the rhinophoral sheaths are further elaborated into long mobile 'tentacles' that probably serve as sensory appendages, apparently in lieu of velar tentacles.

11. Rhinophores
Polarity: The presence of enrolled rhinophores is considered to be plesiomorphic, and the presence of solid rhinophores is apomorphic.
Coding: 0 - rhinophores hollow and enrolled; 1 - rhinophores solid.

12. Rhinophoral sheaths
Polarity: The absence of any sheath is considered to be the plesiomorphic state. The formation of sheaths occurs in two different ways, which are coded separately.

Coding: 0 - rhinophoral sheaths absent; 1 - rhinophoral sheaths formed by frontal edge of notum; 2 - sheaths formed by elevation of the rim of the notal pocket.

13–15. Respiratory structures

(13) Whether or not the gills of the Anthobranchia are homologous with the ctenidium of the Cephalaspidea, Anaspidea, Tylodinoidea and Pleurobranchoidea has been a contentious issue (pro: Evans, 1914; Pruvot-Fol, 1939; Minichev, 1970; Tardy, 1970a; Gosliner, 1981; Schmekel & Portmann, 1982; contra: Morton, 1972; Brace, 1977). Nonetheless, the close juxtaposition between the auricle of the heart, the efferent branchial vessel of the gill (which enters directly into the auricle), the anus, and the nephroproct cannot be denied. This situation, with all four organs lying next to each other, occurs in all opisthobranchs (except some groups of the Nudibranchia). This juxtaposition represents the plesiomorphic state as seen in the reduced pallial cavity in the 'Prosobranchia' (see discussion by Jonas, 1985, 1986). Because it seems very improbable for a slug with no shell to evolve secondary gills around the anus, we assume that the *possession* of gills next to the anus presents the plesiomorphic state for the Nudibranchia, whereas the absence of such a gill is the apomorphic state.

The possession of gills next to the anus does not imply that the entire circle of anal gills in the Anthobranchia is homologous with the plicatidium of the pleurobranchs and other opisthobranch taxa, as was assumed by Pruvot-Fol (1939). Baranetz & Minichev's (1995) proposal of a polymerized ctenidium seems to be a more plausible alternative.

To avoid the criticism of *a priori* reasoning that would be implicit in our use of the term 'primary gills', be they a polymerized ctenidium or a novel respiratory structure, we use the phylogenetically neutral term 'anal gills' to denote the gills of the Anthobranchia throughout the remainder of this work. Similarly, we will use 'dichotomously branched' or 'bushy' gills to denote those (probably secondary) respiratory structures of the Dendronotoidea (see below).

Anal gills are present in Bathydoridoidea and Doridoidea.

(14) We believe that only a few anal gills (2 or 3) with an asymmetrical arrangement on one side of the anus (as in *Bathydoris clavigera*) represents the more primitive condition and that a circle comprising more numerous gills (3 to 30) (Cryptobranchia, many Phanerobranchia) represents the more derived condition. In *Teshia* there are three separate branchial stalks, each supporting six or seven unipinnate gills (Edmunds, 1966), in *Hexabranchus* there are many separate gills. The most extreme type of branchial elaboration occurs in *Risbecia* and *Glossodoris* where one finds subsidiary spirals within the gill circle itself (Rudman, 1984).

The absence of anal gills represents the apomorphic state and this state occurs in all other nudibranch taxa (Cladobranchia) and *Doridoxa*, and (obviously secondarily) in some anthobranch taxa (e.g. *Vayssierea, Murphydoris, Gymnodoris* (a few species only)).

(15) Nearly all nudibranchs that bear anal gills have (at least some) muscle fibres lying within, or at the base of, the gill rachis. Contraction of these muscles merely causes a contraction of the gills into a tight bundle close to the notum (*Bathydoris, Aegires, Goniodoris, Limacia, Trapania, Roboastra, Hexabranchus*). Within the Cryptobranchia and some other Doridoidea (including *Dendrodoris, Onchidoris*), a sub-notal cavity ('branchial pocket') has evolved, into which the gills can be more or less completely retracted

when danger threatens. The capacity for complete retraction of the gills into a branchial pocket is considered to be derived. Actually this cavity is invariably combined with the presence of an enlarged retractor muscle. Because no species is known to possess a strong retractor muscle but lack a branchial cavity, these two characters are considered to be a functional entity and are coded as one character. We consider this character of higher significance, since more than one anatomical detail is included in its manifestation (i.e. retractor muscles plus branchial cavity). As with the rhinophores, the notum surrounding the rim of the branchial pocket can be elevated into a tubular sheath, we assume for additional protection of the gills, in some Cryptobranchia (*Kentrodoris*, *Hypselodoris* (a few species only)).

Structures have apparently evolved repeatedly within different groups to reinforce simple epidermal oxygen uptake. They have partly been described in the section 'Papillae on the notum' above, but they need to be elaborated again here for completeness.

(a) Dichotomously branching or bushy structures (see above) are present only in the Dendronotoidea. Within the family Tritoniidae, they are located directly on the edge of the notum, but in all other families of the Dendronotoidea they lie inside the papillae at the very edge of the notum. Because these structures are not present in any other opisthobranch or nudibranch group, we consider them apomorphic. Their absence is plesiomorphic (see character 6).

(b) Permanent undulations along the notal margin unaccompanied by papillae are present in *Glossodoris* (most species; becoming more pronounced with age), *Lomanotus* (some species), *Tritoniella* and *Leminda*.

(c) Papillae along the notal edge (usually termed 'cerata' in the literature—see also character 'papillae') have evolved several times independently within the Opisthobranchia, for example within the Sacoglossa (Stiligeridae, Polybranchiidae), the Doridoidea, the Dendronotoidea (but only in *Marianina* within the Tritoniidae), the Zephyrinidae and the Aeolidoidea. Very often these enlargements of the area for gaseous exchange are connected with an enlargement of the digestive epithelia by penetration of diverticula of the digestive gland (see character 'digestive gland').

(d) In a few species, papillae are dispersed all over the notum and they sometimes bear a rich network of lacunae (e.g. Bathydoridoidea, many cryptobranch Doridoidea, *Charcotia*) (Fig. 3A,D).

(e) The posterior branchial lamellae between the notum and the foot in the Corambidae can be derived from anal gills. Their circulatory system is more like that of an anthobranch (Wägele, 1984; García & García-Gómez, 1990a; Martynov, 1995) than a phyllidiid or an arminid. Nevertheless, the position and arrangement of these corambid gills are unique and can be considered as autapomorphies of this family.

(f) Multiple branchial lamellae lying in the space between the notum and the foot are present in the Phyllidiidae. These undoubtedly represent secondary respiratory structures because of the different circulatory system compared to other Anthobranchia (Wägele, 1984). The presence of these 'lamellate gills' can be considered to be an autapomorphy for the Phyllidiidae.

In the Arminidae (except *Dermatobranchus* and *Pleurophyllidiella*), the respiratory structures are also represented as lamellae in the space between the notum and the foot, but they are orientated longitudinally in one row along the anterior third of the body, not all the way round the body as in the Phyllidiidae. The ventral position of these lamellae in the Arminidae and Phyllidiidae must be considered as the result

of convergence, because the arrangement differs so fundamentally. Additionally, *Armina* has ventrally lying hyponotal lamellae, which are arranged longitudinally or obliquely, and into which digestive gland diverticula penetrate. Lamellate gills and hyponotal lamellae are synapomorphies for certain taxa within the family Arminidae (Kolb & Wägele, 1998). Using only the arminid members *Armina* and *Dermatobranchus* in this analysis, both features would represent autapomorphies of *Armina* and are therefore not coded here.

13. Occurrence of anal gills
Polarity: The occurrence of gills next to the anus is considered to be the plesiomorphic state, and the absence is the apomorphic state.
Coding: 0 - anal gills present; 1 - anal gills absent.

14. Gill number
Polarity: The presence of only one, or two (*Bathydoris*), or three (*Trapania*, *Aegires*, *Polycera*, *Roboastra*) anal gills is considered to be the plesiomorphic state, and the polymerization of anal gills with five and more represents the apomorphic state. This character is coded as inapplicable for those genera that lack anal gills.
Coding: 0 - one to three anal gills; 1 - five or more anal gills; '–' not applicable because anal gills absent.

15. Retractility of gills into a branchial pocket
Polarity: Anal gills, which can contract to a certain degree, but which do not retract completely into a cavity is considered to be the plesiomorphic state. Alternatively anal gills with a strong retractor muscle accompanied by a branchial pocket into which they can be completely withdrawn represents the apomorphic state. Weighting 2 was applied through successive analyses.
*Coding*: 0 - gills non-retractile; 1 - gills retractile into branchial pocket beneath notum.

16. Anus
In many opisthobranch taxa the anus opens on the anterior right side of the body beneath the notal margin (Cephalaspidea, Anaspidea, many Sacoglossa, *Tylodina*, Pleurobranchoidea). This site also occurs in many nudibranch taxa (Dendronotoidea, Arminoidea (except Zephyrinidae), Aeolidoidea) and is therefore considered to be the plesiomorphic state. Two different anal positions can be observed within the nudibranchs. Firstly the anus is located towards the posterior part of the body, but still on the right side and still beneath the notum (*Doridoxa*, *Heterodoris*). This shifting is most prominent in *Fryeria* and *Corambe* where the anus opens in the midline at the rear end between the notum and the foot. This process is paralleled in *Umbraculum*, where the anus opens on an anal tube in the posterior midline well behind the rear end of the gill's basement membrane.
Second is the migration of the anus, during ontogeny, onto the dorsal surface (as represented by acleioproctic forms of Aeolidoidea—see Schmekel & Portmann, 1982). The shift is paralleled in some Cephalaspidea (see Mikkelsen, 1996) and Sacoglossa (Jensen, 1996). This apomorphic state is not used separately in this analysis because of difficulties in coding in some aeolidoideans.
(16) In some nudibranch groups a combination of both evolutionary shifts can be deduced, so that the anus comes to lie in the midline posteriorly *and* dorsally
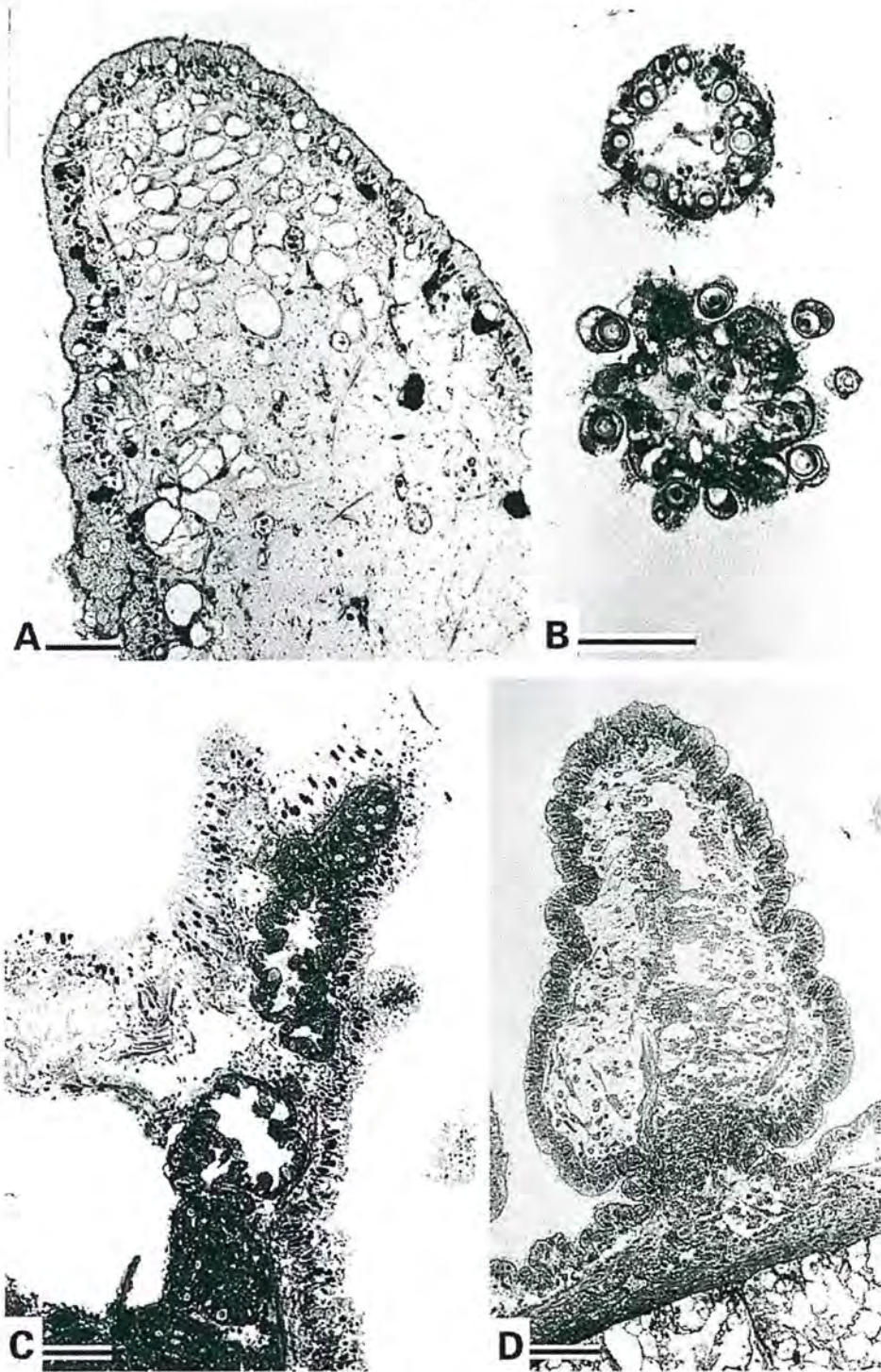
(Bathydoridoidea, Doridoidea, Zephyrinidae). This position certainly represents an apomorphy, because it is clearly distinct from the plesiomorphic site. Therefore only this character state (i.e. anus dorsal *and* posterior) is used in our analysis. We consider it to be of great significance because movement of several organ systems is involved (anus plus gills with adjacent vessels plus nephroproct). We do not code the shift of the different orifices of the organ systems separately, because they seem to be correlated in the evolution of the whole mantle complex in the Gastropoda (Haszpru-nar, 1988; Ponder & Lindberg, 1997). Probably the genetic background of the shift of the entire complex is not very complicated.

In nearly all taxa of the Opisthobranchia, a close juxtaposition between the anus and the pericardium is present, even if there is a trend for the complex to shift posteriorly as a whole (e.g. in all Anthobranchia). This close juxtaposition between the pericardium and the anus is considered to be the plesiomorphic state. The separation between the anus and the heart, including the nephroproct, is considered to be the apomorphic state and is represented by *Janolus*, wherein the anus has shifted posteriorly but the heart still remains in the middle region of the notum.

16. Position of anus
Polarity: The lateral position of the anus on the right side in the middle of the body is considered to be the plesiomorphic state, and the location in the posterior and dorsal part of the body is the apomorphic state. Because of the complexity of the structures involved, weighting 2 was applied through successive analyses.
Coding: 0 - anus lateral on right side near middle of body; 1 - anus medio-dorsal, towards rear of body.

Position of nephroproct
The nephroproct is usually situated close to the anus and when the anus shifts posteriorly, or dorsally, this close juxtaposition is maintained. The Notacolidiidae is the only family where the nephroproct opens in front of the genital aperture, and where it is clearly disconnected from the anus. This position is considered to be an autapomorphy for the monotypic family (Wägele, 1990a), and is therefore not applied in this analysis.

17. Shell
(17) A shell is present in adults in (most) Cephalaspidea, (most) Anaspidea, (all) Tylodinoidea, (some) Sacoglossa and all pleurobranchid genera (except *Pleuro-branchella, Pleurobranchaea* and *Euselenops*). The presence of a shell is undisputedly the plesiomorphic state (Gosliner, 1994; Mikkelsen, 1996), whereas the absence of a shell represents the apomorphic state. Although the loss of the shell has occurred many times within opisthobranch taxa (most lineages have shell-less taxa at their ultimate branches), we consider the absence of a shell as a synapomorphy for the Nudibranchia as a whole.

Figure 3. Histology of notal structures: A, longitudinal section through papilla of *Charcotia granulosa*; B, cross section through basal and apical part of caryophyllidia in *Jorunna tomentosa*; C, longitudinal section through papilla of *Dendronotus frondosus* with digestive gland; D, longitudinal section through papilla of juvenile *Bathydoris hodgsoni*. Scale bars: A,C,D = 100 μm, B = 50 μm.

With the loss of the shell, the adductor muscle, which is present in shelled Pleurobranchidae (e.g. *Bathyberthella antarctica* - Wägele & Willan, 1994), must have become lost too. Because this character is linked to the presence or absence of the shell itself, it is not independent and is not used here.

17. Presence or absence of a shell
Polarity: Presence of a shell is the plesiomorphic state, and its absence is the apomorphic state.
Coding: 0 - shell present; 1 - shell absent.

Cuticle covering the notum

A cuticular covering to the upper surface of the notum is present only in some species of the Corambidae and in *Vayssierea*. A cuticle is also present in the Onchidiida, but an histological investigation has revealed that the cuticles differ in several respects between these taxa and convergent evolution seems highly probable (see Weiß & Wägele, 1998, unpubl. data). The absence of a cuticle is the plesiomorphic state, and its presence is the apomorphic state.

Digestive system

Although the digestive system of nudibranchs takes numerous forms depending on the diet and feeding habits, many morphological features of the system can be used for clarifying phylogeny.

Because some terms relating to certain parts of the digestive system are used in different ways by different authors, we will discuss and define those we have used here (Fig. 4A,B).

18. Oral tube

The oral tube is that part of the foregut between the mouth and the labial disc. According to the literature and our own investigations, there is apparently no cuticular lining in the oral tube. In all the nudibranchs that we investigated histologically, the oral tube is underlain by a more or less thick layer of glandular follicles which, according to their staining properties, contain mucopolysaccharides.

Oral glands with separate ducts leading into the oral tube have been described for several nudibranchs. The terms used in this literature for these glands differ. Gosliner (1994) used the term 'oral glands' for all glands entering the oral tube and 'ptyaline glands' for the glands entering the oral tube in the Phyllidiidae and Dendrodorididae. Bergh (1892 *et seq.*), Eliot (1909) and Odhner (1929 *et seq.*) used the term 'ptyaline glands' for all glands entering the oral tube. But this latter term has also been used to denote glands entering the pharyngeal cavity (usually called 'salivary glands', see below). Usually the term oral gland is restricted to those glands which enter the oral tube in front of the labial disc, and salivary glands to those that enter the foregut behind the labial disc, that is in the pharyngeal cavity. We follow these definitions in this work, without regards to their probable homeoplaseous evolution.

A glandular layer near to the area of the mouth and oral tube can be observed in nearly all nudibranchs. This layer is usually composed of subepidermal follicles
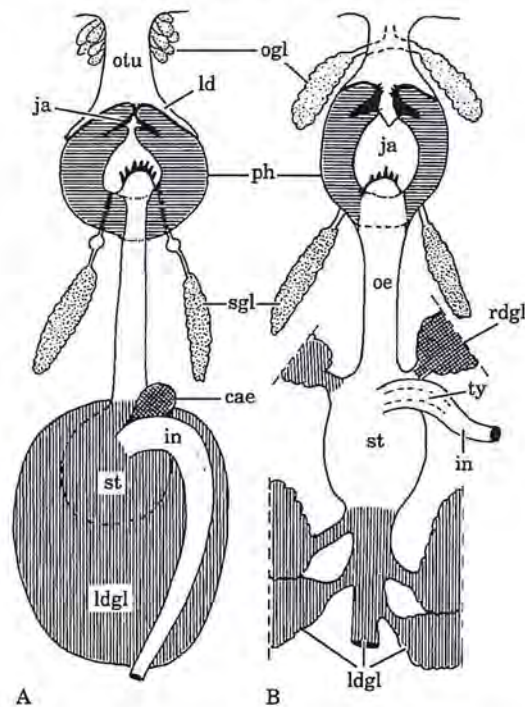
Figure 4. Schematic diagrams of the digestive system: A, holohepatic digestive system of Anthobranchia; B, cladohepatic system of Cladobranchia. Abbreviations: cae - caecum; in - intestine; ja - jaws; ld - labial disc; ldgl - left digestive gland; oe - oesophagus; ogl - oral gland; otu - oral tube; ph - pharynx; rdgl - right digestive gland; sgl - salivary gland; st - stomach; ty - typhlosole.

(see above) which individually reach to the surface. This glandular layer forms a distinct gland with an efferent duct leading into the oral tube in a few taxa. Only these glands will be considered here.

Oral glands (with efferent ducts) are present in some taxa of the Opisthobranchia such as the Pleurobranchoidea (except *Tomthompsonia*, some species of *Berthella* and *Pleurobranchus*). Here one observes a single gland, which can be either unbranched (*Bathyberthella*) or highly branched to the extent it ramifies between all the viscera (*Pleurobranchaea*). It inserts via a single duct on the dorsal midline just in front of the transition of the oral tube into the pharyngeal bulb. This gland produces acids (Thompson, 1976). Histologically it contains very large cells with a single large vacuole, but with no staining contents. This gland is unique to the Pleurobranchidae and therefore an autapomorphy of that family (Fig. 5A). A single oral gland is present in *Hydatina* (Rudman, 1972a; Mikkelsen, 1996). According to Hoffmann (1939), the Sacoglossa also have oral glands, but in this case they lie laterally and open laterally into the distal part of the oral tube. Jensen (1996) did not mention them in her extensive phylogenetic analysis of the Sacoglossa. No oral glands are described for any member of the Anaspidea.

(18) Oral glands are present in a few groups of the Nudibranchia. When present,
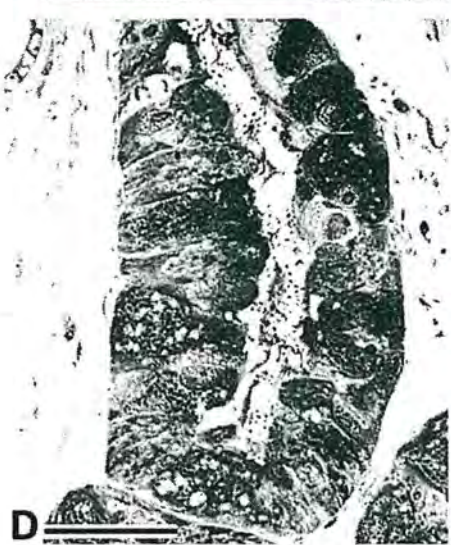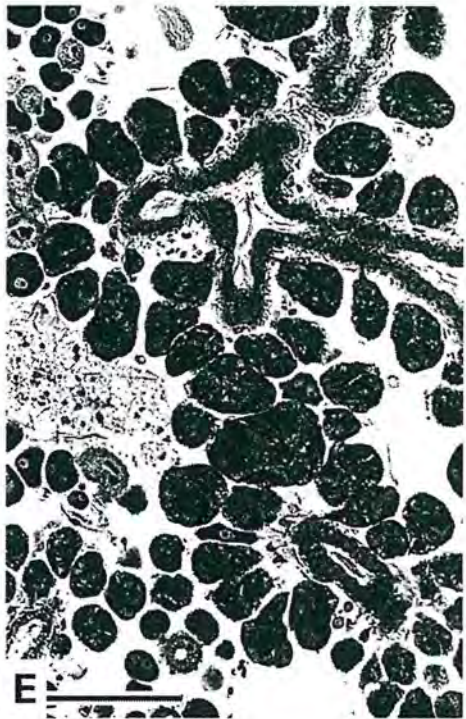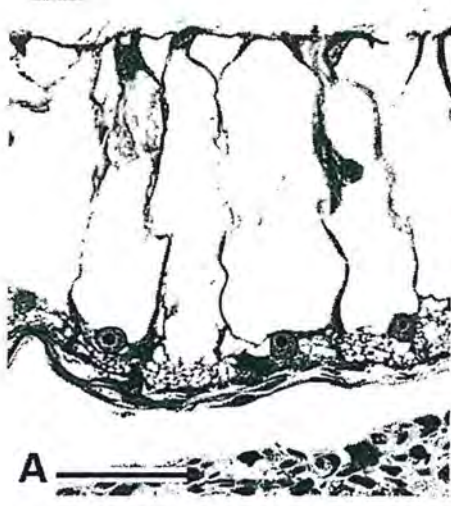
they are paired, with either a common duct entering the oral tube from the ventral side (*Dendrodoris* Fig. 5B, *Armina*, *Dermatobranchus*, *Calma*, *Spurilla*, *Berghia*, *Facelina* - Dreyer, 1913; Hoffmann, 1939), or separate ducts (mainly Aeolidoidea, e.g. *Cuthona* - Fig. 5C, *Cerberilla*, *Pinufius*, *Phestilla*, *Flabellina* - Fig. 5E, and also other nudibranch taxa, e.g. *Trippa* - Hoffmann, 1939; Baba & Hamatani, 1964; Schulze & Wägele, 1998). Whether those glands with only one duct evolved by fusion of two separate ducts is difficult to decide *a priori*. Many authors have described oral glands in species of nudibranchs where the oral glandular follicles are so dense that they reach into the visceral cavity, although no separate ducts can be detected. This is the case for *Doto coronata* and an undescribed *Doto* sp., and for *Eubranchus exiguus* (unpublished data). All three species belong to genera which are said to possess oral glands. Baba (1971) even mentioned a second pair of oral glands in some species of *Doto*, but he did not mention any salivary glands. Because the salivary glands lie in the head in all the species of *Doto* we have examined (pers. obs.), it can be safely assumed that Baba misinterpreted the salivary glands as the second pair of oral glands. Therefore we emphasize that the form of the 'oral glands' needs to be thoroughly investigated before they can be used in a phylogenetic analysis. Moreover, in some nudibranchs (e.g. *Cuthona* Fig. 5C) the oral glands are microscopic structures lying partly embedded in the connective tissue and therefore barely visible in dissections. *Dendrodoris* stands apart from the rest of the Doridoidea because of the histology of its oral gland (Wägele *et al.*, 1999). The situation within the Phyllidiidae is also rather complicated and the form of the oral glands can only be used for phylogenetic analysis within that group (see Brunckhorst, 1993). According to Hoffmann (1939), *Hancockia* has one pair of oral glands which reach into the rhinophores. The glands have a common duct on the ventral side which enters ventrally into the oral tube. Additional to these paired oral glands, *Hancockia* has an unpaired gland which lies ventrally, reaches to the posterior end of the body, and opens into the common duct of the paired oral gland. This system needs to be reinvestigated by histological methodology.

18. Oral glands

Polarity: The presence of diffuse, subepidermal, glandular follicles that open into the proximal oral tube, is considered to be the plesiomorphic state. The presence of distinct oral glands with efferent ducts (recognizable in histological sections at least) is considered to be the apomorphic state. The character states are treated as unordered.

Coding: 0 - oral gland consisting of glandular layer without ducts; 1 - distinct oral glands with two separate efferent ducts opening into oral tube; 2 - oral glands with one common efferent duct.

19. Pharynx

In the opisthobranch literature the terms pharynx and buccal bulb are used interchangeably for the same section of the foregut. The pharynx is the part that

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Figure 5. Histology of foregut glands (figures showing cross sections): A, oral gland of *Bathyberthella antarctica*; lumen of glandular duct in upper part of illustration; B, oral gland of *Dendrodoris nigra*; dark staining glandular cells surrounding smaller ducts, muscle cells surrounding exiting ducts (left and below); C, oral gland of *Cuthona* sp.; D, salivary gland of *Cuthona* sp.; E, oral gland of *Flabellina affinis*. Scale bars: A,B = 100 μm, C,D = 50 μm, E = 100 μm.

follows the oral tube and is completely covered by a more or less thick cuticular lining. It starts at the labial disc (inclusive) and ends at the transition into the oesophagus where the ducts from the salivary glands usually enter the pharyngeal musculature (not the pharyngeal lumen!). The term buccal bulb does not accurately prescribe the anterior limit because it could also include the oral tube. So we prefer to use the term pharynx or pharyngeal bulb. Special features of the pharynx are the buccal pumps.

Mikkelsen (1996) applied the term 'oral' to all the structures related to the pharynx (e.g. 'oral cuticle') which means the cuticular lining within the pharynx. We prefer to use the term 'pharyngeal cuticle', which starts as the labial disc and continues to the oesophagus. Special areas of differentiation of this pharyngeal cuticle are the jaws and the radula, which are described separately below.

The labial disc is that part of the pharynx which protrudes as a papilla into the oral tube (Fig. 4A,B). It seems to be covered by a cuticle in all the Nudibranchia, even in *Dendrodoris* which lacks jaws and a radula (Wägele *et al.*, 1999), but not in *Phyllidia* (pers. obs.). The situation within the Tethydidae (which also lacks a radula) has yet to be clarified.

Many authors consider the jaw-like structures of the Doridoidea simply as a part of the labial cuticle (e.g. Hoffmann, 1939), but we consider them as real jaws (see below).

The presence of paired salivary glands is an almost ubiquitous feature of opisthobranchs and is considered to be a plesiomorphy within the nudibranchs. These glands insert close to the transition of the pharynx into the oesophagus, but they are actually part of the pharynx, because their ducts open into the pharyngeal cavity (Fig. 4A,B). Salivary glands are absent in only a few genera (i.e. *Fiona* see Bergh, 1879). Their absence is considered to be the apomorphic state. All the genera used for this analysis possess salivary glands, and therefore this character was not applied here.

Salivary glands are usually long and ribbon-like (Fig. 5D), and they extend posteriorly parallel to (sometimes annexed onto) the oesophagus in (many) Cephalaspidea, Anaspidea, (many) Nudibranchia (e.g. *Austrodoris, Hexabranchus, Siraius, Pseudotritonia, Notaeolidia*). Therefore we consider this form to be plesiomorphic. In *Dendrodoris*, the salivary glands consist of two, small, spherical structures which have similar histological appearance to oral glands (Wägele *et al.*, 1999). The salivary glands are considerably reduced in some species of *Melibe* too. Reduction in the size of the salivary glands would appear to be an evolutionary trend that has occurred convergently in both the Dendrodorididae and Tethydidae, which represents autapomorphies (therefore trivial characters) for both families.

In some taxa, the usual ribbon-like form of the salivary glands is not obvious and the gland does not extend to the posterior part, but it spreads over the oesophagus (*Bathydoris, Heterodoris, Armina maculata*).

Within the Pleurobranchoidea, the salivary ducts have become exceptionally long, and the glandular tissue forms a cushion next to the digestive gland.

Because the configuration of the salivary glands themselves is unknown in many groups, we cannot use this character for further analysis.

In some groups of opisthobranchs the ducts of the salivary glands possess a small swelling ('salivary bulb') situated next to the insertion into the pharynx (Fig. 4A). At present we cannot postulate the polarity of this character for want of comparative data. Salivary bulbs are definitely present in the Pleurobranchoideae (including

Tomthompsonia) and in *Bathydoris*, but they are absent in *Austrodoris*, Tritoniidae, Charcotiidae and *Notaeolidia*.

(19) Muscular extensions of the pharynx functioning as a sucking pump are described for a few taxa within the Opisthobranchia. The Sacoglossa possess two pouches, one lying on either side of the pharynx (Jensen, 1996). Within the Nudibranchia, only the doridoidean families Onchidorididae, Corambidae and Goniodorididae have a buccal pouch which lies on the dorsal side of the pharynx. Martynov (1995) considered a sucking pump to have developed independently in two doridoidean lineages. One, represented by *Acanthodoris*, *Onchidoris*, *Okenia* and *Goniodoris*, has the buccal pump with a peripheral muscle that divides it into two halves. In the other, represented by *Ancula*, *Trapania*, *Akiodoris* and *Armodoris*, the peripheral muscle is absent and no division into halves is discernible. It is conceivable that the more elaborate buccal pump of the former group could have evolved from the latter group. Because the possession of a single dorsal buccal pouch is unique within the Opisthobranchia, we consider its presence as an apomorphic feature and consider this character of high significance because of its complexity.

19. Presence of a buccal pouch
Polarity: The absence of a buccal pouch on the pharynx is considered to be the plesiomorphic state, and its presence is the apomorphic state. Weighting 2 was applied through successive analyses.
Coding: 0 - buccal pouch absent; 1 - buccal pouch present.

20–21. Jaws
   Two separate jaws are present in many opisthobranch taxa (Cephalaspidea, Thecosomata, Anaspidea, nearly all Nudibranchia, e.g. Bathydoridoidea, many Doridoidea, Dendronotoidea except Tethydidae, Arminoidea, Aeolidoidea). Therefore their presence is considered to be the plesiomorphic state and their absence (*Crimora*, many Cryptobranchia, e.g. *Doris*, *Archidoris*, *Austrodoris* and *Sclerodoris*, Dendrodorididae, Phyllidiidae) is the apomorphic state. This is in agreement with Hoffmann (1939) and Gosliner (1994). The loss of the jaw elements does not necessarily involve loss of the pharyngeal cuticle, as has been shown in *Dendrodoris nigra* (Wägele *et al.*, 1999). Since *Archidoris* is the only genus to lack jaws in this analysis, the character is not used here.

(20) Usually the opisthobranch jaws are composed of single elements which form rodlets (because of the apcial denticles often called 'rods'), or more or less polygonal platelets (Fig. 7B) (see Eliot, 1906; Hoffmann, 1939; Marcus & Marcus, 1957; Gosliner, 1994; Mikkelsen, 1996). According to Mikkelsen (1996), these jaws are structures that originate in generative grooves and only these structures should be called jaws (Fig. 6A–D). Within the Nudibranchia, we find jaws composed of distinct elements formed in generative grooves in many Doridoidea (Fig. 6B,C) and, like Gosliner (1994), we consider this state to be plesiomorphic. The fusion of these platelets into a solid jaw with only a few rows of platelets remaining on the masticatory border or masticatory process (Figs 6D, 7C, 8A–C), or even no platelets at all, can be observed in several groups of nudibranchs (*Bathydoris*, Polyceridae, Tritoniidae, Lomanotidae, Scyllaeidae, Tethyidae, *Doridomorpha*, Arminidae, Charcotiidae, Flabellinidae, and other aeolidoideans), and this is considered to be
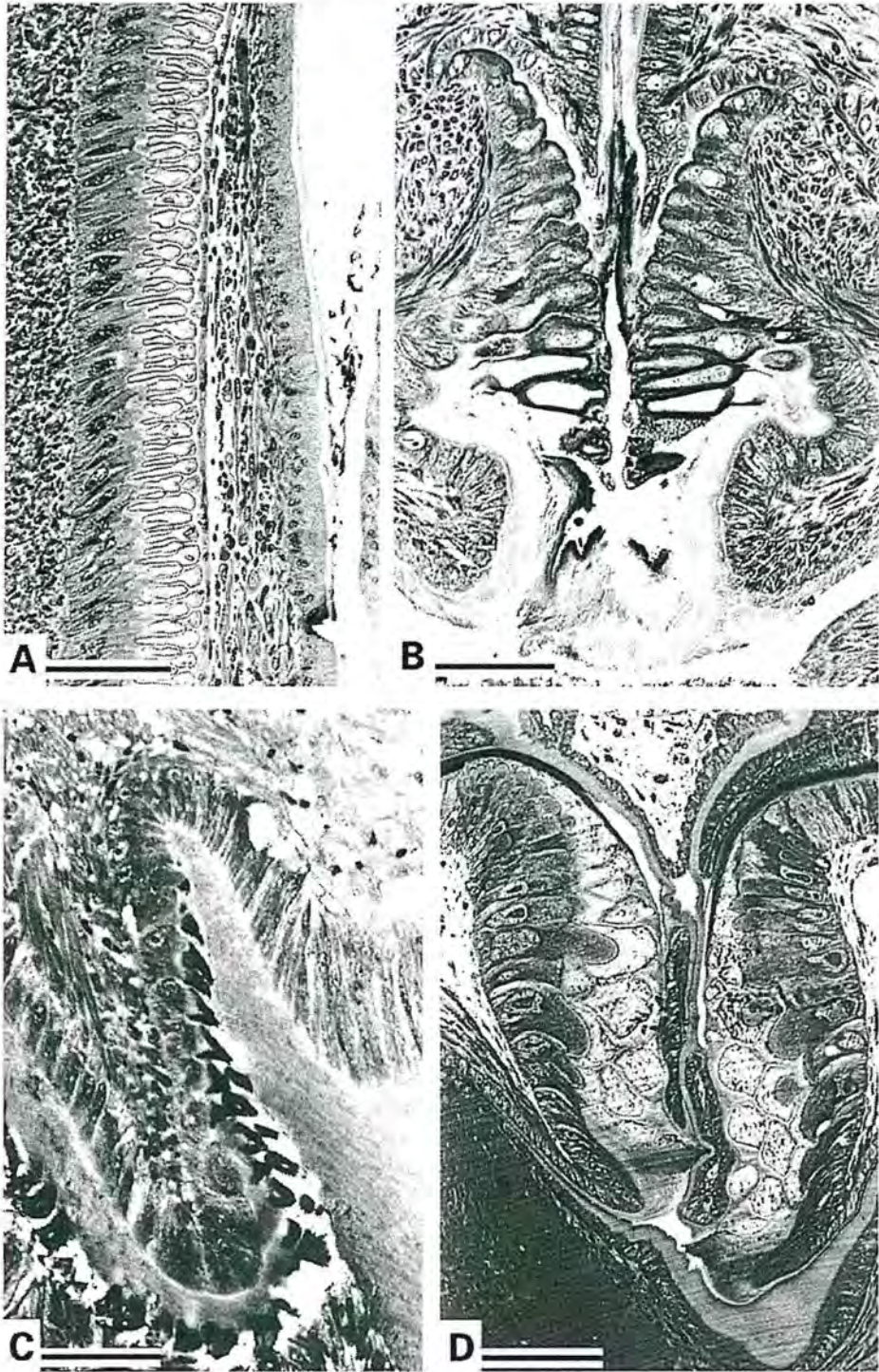
the apomorphic state. We use the term 'aliform' for this kind of solid jaws with no platelets, or just a few rows of platelets. One cannot discount the possibility that the solid part of the jaw in the Cladobranchia has been the result of *de novo* thickening of the pharyngeal cuticle which has fused with the primary jaw composed of rodlets. But because this scenario would yield the apomorphic state anyway, the character 'aliform jaws' is used in this study irrespective of its homology.

(21) In some nudibranch taxa, there can be several rows of denticle-bearing platelets at the margin of the aliform jaws (Fig. 7C). Presumably these platelets are homologous with, and represent a reduction from, the rodlets constituting the entire solid jaw just described. The presence of several rows of these denticles (e.g. *Tritonia*, (some) *Flabellina, Babakina, Bajaeolis, Favorinus*) represents the plesiomorphic state, and the reduction to just a single row (e.g. *Eubranchus, Cuthona, Tergipes, Embletonia, Glaucus*) or their complete absence (e.g. *Bathydoris, Jason, Protaeolidiella, Dicata*), represents the apomorphic states. But we must be mindful of Colgan's (1914) interesting record of ontogenetic variation in this character; Colgan noted denticles on the edge of the jaws of juvenile *Dendronotus frondosus*, but the denticles were lost as individuals grew to maturity. Such a case of ontogenetic variation in jaw development has never been reported again, and information is still too sketchy to reach a final conclusion. Gosliner (1980) recorded an unusual variation in a population of *Berghia major* from Hawai'i; whereas this species is known elsewhere to have a smooth jaw edge, the specimens from Hawai'i had 50 to 110 denticles on their cutting edge.

The jaw elements themselves differ in appearance between the two types of jaws. In many Cephalaspidea, Anaspidea, and nearly all Pleurobranchoidea, the elements bear polyfid (i.e. multidenticulate) apices (Fig. 7B). Elements with polyfid apices can also be present in the jaws of *Tritonia antarctica* (Wägele, 1995) and some members of the Chromodorididae and Actinocyclidae. But usually within the Nudibranchia, the elements bear unicuspid (Tritoniidae, Flabellinidae) or (sometimes) bifid apices (*Pinufius* - Marcus & Marcus, 1959), or sometimes no apices are discernible at all. This is very often the case in the more distal rows of the aliform jaws in the Flabellinidae. Because the type of apex can vary intraspecifically to a considerable extent (see *Tritonia antarctica* - Wägele, 1995), we do not consider this character as very valuable for phylogenetic analyses.

Aliform jaws of the shape shown in Figure 8A and B are widespread within different nudibranch groups (Bathydoridoidea, *Doridoxa*, Tritoniidae, *Heterodoris, Goniaeolis*, Arminidae) and probably represent the plesiomorphic state. But configurations differing from this type occur in several taxa. For instance, a shortening of the cutting edge together with simultaneous perpendicular elongation of the longitudinal axis, can be observed in the Dendronotidae, Charcotiidae, Zephyrinidae, and in aeolids (to a lesser extent in *Notaeolidia* Fig. 8C). There are several other configurations of the jaws which might be of phylogenetic significance at lower levels (e.g. particular shape, presence or absence of a masticatory process, etc). Such

Figure 6. Histology of the jaws: A, generative groove of jaw in *Bathyberthella antarctica*; cavity on right side represents the pharyngeal lumen; B, jaws and generative groove in *Trapania maculata*; cavity below jaws represents the oral tube; C, generative groove of *Acanthodoris pilosa*; D, generative zone of jaw in *Tritonia antarctica*, showing detail of area where rodlets are formed. Scale bars: A,D = 100 μm, B,C = 50 μm.
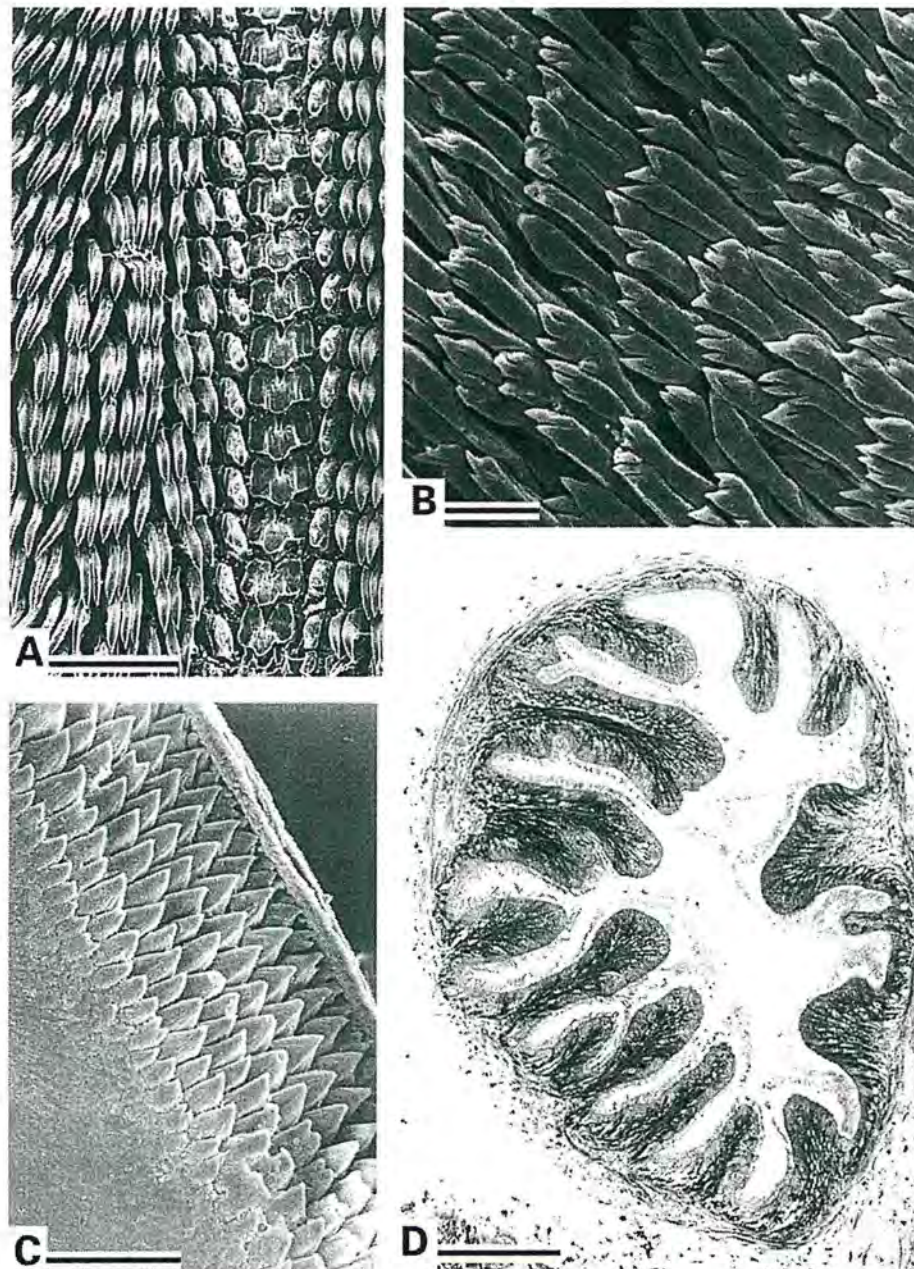
Figure 7. Scanning electron micrographs of cuticular structures in the pharynx (A–C); D, histology of caecum (D): A, radula of *Tritonia antarctica*; B, mandibular elements of *Bathyberthella antarctica*; C, masticatory border of jaws of *Tritonia antarctica*; D, cross section of caecum of *Trapania maculata*. Scale bars: A = 500 μm, B = 50 μm, C,D = 100 μm.
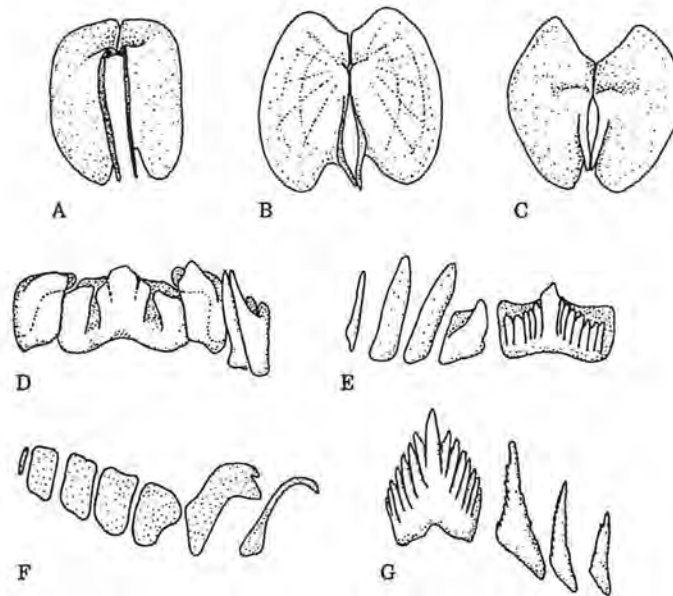
Figure 8. Cuticularized structures in the pharynx: A, jaws of *Tritonia antarctica* (redrawn after Wägele, 1995); B, jaws of *Armina neapolitana* (redrawn after Kolb, 1998); C, jaws of *Notaeolidia depressa* (redrawn after Wägele, 1990a); D, rachidian tooth and innermost laterals of *Tritonia antarctica* (redrawn after Wägele, 1995); E, half row of radula of *Armina neapolitana* (redrawn after Kolb, 1998); F, half row of radula of *Limacia clavigera* (redrawn after Thompson & Brown, 1984); G, half row of radula of *Notaeolidia depressa* (redrawn after Wägele, 1990a).

configurations have to be thoroughly analysed for taxa at higher levels and cannot be discussed here in full (see also Hoffmann [1939] for further information).

Different kinds of specialized jaw structure can be observed within several taxa of Nudibranchia and they certainly represent apomorphic traits, which can be used for phylogenetic analysis at lower taxonomic levels (e.g. the highly cuticularized denticulate anterior margin of some species of *Janolus*, or the two-winged jaws in *Pinufius* and *Caldukia*).

20. Composition of jaws

Polarity: Jaws composed of numerous platelets are considered to be plesiomorphic, whereas those being compact (aliform) with only few rows of platelets, or none at all, are apomorphic. The character is coded inapplicable in *Archidoris* because the members of this genus have no jaws.

Coding: 0 - jaws composed of platelets; 1 - aliform jaws; '–' not applicable.

21. Form of the masticatory border

Polarity: Aliform jaws with several rows of denticles on the masticatory border is considered to be plesiomorphic, whereas those with only one row of denticles, or no denticles are apomorphic. This character is coded 0 in those genera with non-aliform jaws fully composed of denticles. Character states are treated as unordered.

Coding: 0 - several rows of denticles; 1 - one row of denticles; 2 - no denticles at all.

22–26. Radula

Although the presence of a radula is a symplesiomorphy for all gastropods (Ponder & Lindberg, 1997), this organ is absent in several opisthobranch groups (Retusidae, (most) Aglajidae, and (some) Nudibranchia (i.e. Phyllidiidae, Dendrodorididae, Tethydidae)). No species of Tylodinoidea or Pleurobranchoidea lacks a radula. The absence of the radula therefore represents the apomorphic state within the Nudibranchia.

(22) In agreement with Hoffmann (1939) and Gosliner (1994), we consider the broad, many toothed ('multidenticulate') radula as the plesiomorphic type. This type of radula is present in (most) Anaspidea, Tylodinoidea, Pleurobranchoidea and Nudibranchia (i.e. Cryptobranchia, *Scyllaea*, Tritoniidae, *Heterodoris*, Arminidae, etc.) (Fig. 7A). A relatively smaller number of lateral teeth are present within different taxa of Nudibranchia; a reduction of the number of laterals down to about four per side (*Notaeolidia*), or just a single lateral ((some) *Doto*, *Madrella*, *Hancockia*, *Flabellina*, *Pseudovermis*, *Cumanotus*, *Eubranchus*), or ultimately the complete loss of all the lateral teeth (most Aeolidoidea). The loss of the lateral teeth can also be observed within other opisthobranch taxa (see Gosliner, 1994) and the Caenogastropoda (Ponder & Lindberg, 1997). The possession of only one to three lateral teeth is coded here as the apomorphic state along with the complete loss. However, this does not necessarily imply a strict evolutionary sequence; the evolution of a radula composed solely of a row of rachidian teeth could theoretically have occurred directly from a multidenticulate ancestral type by loss of all the lateral teeth simultaneously.

(23) In many opisthobranch groups with a multidenticulate radula (Anaspidea, Tylodinoidea, Pleurobranchoidea), a central tooth ('rachidian') is present in the midline. Although a rachidian is present in the Bathydoridoidea, *Nembrotha*, *Tambja*, *Roboastra*, (many) Chromodorididae and (some) *Onchidoris* species, its absence is very widespread. By contrast, most of the Cladobranchia possess a rachidian (exceptions are *Lomanotus* and *Notobryon*). Although Verrill (1882) and Eliot & Evans (1908) recorded the absence of a rachidian in *Heterodoris*, Odhner (1926) and Willan (1981) more recently, have recognized the existence of this tooth in *H. robusta* and *H. antipodes*, respectively.

Probably the primitive form of the rachidian within the Opisthobranchia is a broad tooth with a (principal) cusp extending somewhat in advance of the denticles (i.e. the cusp is protracted) and numerous denticles on either side ((many) Cephalaspidea, Anaspidea and Nudibranchia: *Bathydoris*, *Tritonia*, *Armina*, *Heterodoris*, *Goniaeolis*, some *Janolus*, *Notaeolidia*, *Flabellina*) (Fig. 8D,E,G). Nevertheless, some additional character states can be observed which certainly represent apomorphies and can be used for phylogenetic analysis at lower levels.

In some taxa of the Aeolidoidea, the cusp is not protracted, but retracted below the level of the denticles (e.g. *Cratena*, (some) *Flabellina* species). This is considered to be the apomorphic state, but is not included in the phylogenetic analysis because in the species considered only *Flabellina affinis* possesses it.

The Aeolidiidae is characterized by a so-called 'pectinate' rachidian, where the tooth forms a broad (or less often frequently, acute) arch crowned with numerous denticles of uniform size. This form of rachidian is unique within the Nudibranchia and considered to be an apomorphy.

Usually the cusp is larger than the denticles (see above), but in some taxa the

cusp is relatively small or even absent. We consider the latter to represent the apomorphic trait (*Cadlina*, *Cadlinella*).

According to the ubiquity of denticles on the rachidian, we consider their presence as a symplesiomorphy of the Opisthobranchia. The precise form of the denticles is very variable and the recent description of *Phyllodesmium guamense*, an aeolid with two rows of denticles on the rachidian (Avila *et al.*, 1998) adds another dimension to this variation. The complete absence of denticles is observed in a few otherwise widely separated species (*Heterodoris antipodes*, *Pseudotritonia antarctica*, *Favorinus tsuruganus*, *Janolus rebeccae*), but because the absence of denticles apparently varies within genera, it can probably only be used at the species level.

A character which might ultimately have phylogenetic significance is the shape of the base of the rachidian itself. Two extreme shapes of basal plate can be distinguished, arched (e.g. (nearly all) Aeolidoidea) or truncate (*Cadlina*). This character is not used here because we do not have enough information about the distribution of these types within the Nudibranchia and therefore the polarity is not clear.

The lateral teeth of many Cephalaspidea, Pleurobranchoidea and Nudibranchia are similar in size and shape across the entire row, being uniformly hook-shaped (uncinate or hamate), with or without denticles on the outer face, and without any further differentiation (for examples and figures see Gosliner, 1994) (Fig. 7A). As such, they resemble those in other euthyneuran groups (Pulmonata, Gymnomorpha). We consider the uncinate shape as the plesiomorphic state for the lateral teeth.

We will not use the character 'smooth versus denticulate laterals', because there are a great many different forms and outgroup comparison does not help due to the considerable variation in the form of the denticulation among the outgroups.

(24) In some groups the lateral teeth are morphologically separable across rows, with the inner laterals quite different in form to the outer laterals, a character which we consider as the apomorphic trait. When this occurs, the outermost teeth are called 'marginals', for example the feather-like outer rows of *Jorunna*, *Rostanga* and *Aldisa*, which represent a possible synapomorphy. But since only *Jorunna* is included in this phylogenetic analysis, this character is not applied. Gosliner (1994) indicated the non-homology of the opisthobranch 'marginals' with those of the 'prosobranchs'. We follow his suggestions and use the terms outer and inner laterals. The simplified plate-like laterals (Fig. 8F) which are typical of the Polyceridae (*Polycera*, *Roboastra*) have been used as a character in our phylogenetic analysis. They do not have any cusps and their configuration is unique within the Nudibranchia.

(25, 26) A similar differentiation across rows in combination with a reduction in the number of lateral teeth is present in some taxa of the Phanerobranchia. Within the Polyceridae, the radula is characterized by a reduced rachidian (or none at all), a small innermost (first) lateral, a rather large and broad second lateral, followed by a few non-hooked laterals, and finally several plate-like outer laterals (Fig. 8F). Within the Goniodorididae, many taxa have a similar radula to that of the Polyceridae, but it is the innermost lateral that is enlarged and the succeeding laterals are few in number. Considering that, in general, the radula of the Goniodorididae is not as broad as in the Polyceridae, but identical dental morphology which is rather unique within the Nudibranchia occurs in both families, we postulate a scenario in which the true first lateral of the Polyceridae has been lost in the Goniodorididae. In other words, the innermost lateral of the Goniodorididae actually represents the

second lateral of the Polyceridae. The shape of these homologous laterals is very characteristic.

22. Number of lateral teeth
Polarity: The multidenticulate radula is considered to represent the plesiomorphic state, whereas the paucidenticulate radula (or one with no lateral teeth at all) is the apomorphic state. Character states are considered as unordered.
Coding: 0 - many lateral teeth; 1 - one to three lateral teeth only; 2 - no lateral teeth.

23. Rachidian tooth
Polarity: The presence of a rachidian tooth is considered as the plesiomorphic state, and its absence is the apomorphic state.
Coding: 0 - rachidian tooth present; 1 - rachidian tooth absent.

24. Form of innermost and second lateral teeth
Polarity: Undifferentiated inner lateral teeth are considered plesiomorphic. The innermost lateral tooth of the Onchidorididae is considered to be homologous with the second lateral of the Polyceridae and the true first lateral of the Polyceridae is considered to have been lost. This is coded separately (25). The character is coded inapplicable in those genera where there are no laterals at all.
Coding: 0 - inner lateral not morphologically separable from succeeding laterals; 1 - first or second lateral larger and relatively broader than succeeding laterals; '–' not applicable.

25. Absence of first lateral tooth
Polarity: We assume that the true first lateral tooth in *Onchidoris* and *Trapania* has been lost (see character 24). The absence of the first lateral in *Cuthona*, *Phyllodesmium* and *Protaeolidella* is here coded as inapplicable, because this character has already been partly applied as state 2 in character 22.
Coding: 0 - first lateral present; 1 - (true) first lateral absent; '–' not applicable.

26. Form of outer lateral teeth
Polarity: Outer laterals not differentiated from the inner laterals is considered to be plesiomorphic, whereas the simplification of the outer laterals into plates ('marginals') is the apomorphic state. For those genera without multiple lateral teeth (e.g. *Trapania* with only one inner lateral), the character is coded as inapplicable.
Coding: 0 - outer laterals hook-shaped; 1 - outer laterals plate-like; '–' inapplicable.

27–28. *Oesophagus and stomach*
  Usually the gastropod stomach is a sac-like structure, with the oesophagus entering anteriorly and the intestine and two digestive glandular ducts opening into it separately. It is often difficult to detect such an enlargement in the midgut by macroscopical investigation and even with the help of histological methods it can sometimes be hard to distinguish the stomach from the remainder of the midgut, as for example in *Phyllidia*. We will apply the term 'stomach' to that region of the midgut where the intestine originates and where the main openings into the digestive glands are situated (Fig. 4A and B).
  A cuticular lining to the oesophagus, at least its posterior section, is present in

the Cephalaspidea (Gosliner, 1994; Mikkelsen, 1996; taken from Gosliner, 1981). This region of the oesophagus is sometimes specialized as a crop. According to Gosliner (1981), a cuticle is also present in the oesophagus of the Tylodinoidea. Whereas a cuticle is lacking in *Tomthompsonia* (see Wägele & Hain, 1991), Wägele & Willan (1994) showed one is definitely present in the oesophagus of *Bathyberthella antarctica*. Ev. Marcus (1985) described cuticularized plates in the stomach of *Umbraculum*, rather than in the oesophagus. According to the drawings of *Tylodina fungina* by MacFarland (1966: pl. 11), the cuticularized structures definitely lie in front of the openings of the digestive glands and the intestine. Therefore we consider this region as the oesophagus in agreement with Gosliner (1981). The cuticularized structures are homologous with the crop of the Cephalaspidea. A cuticularized region, also termed the crop, is present in the Anaspidea.

Bathydoris is the only nudibranch known to possess a cuticular lining to the oesophagus. Here the entire oesophagus is lined with a cuticle which is very thin anteriorly but becomes thickened towards the posterior part where the cuticularized longitudinal ridges also bear small spines (see Wägele, 1989a). Hoffmann (1939) interpreted this region as the remnant of the crop of the Cephalaspidea. According to Haefelfinger & Stamm (1959), *Baeolidia nodosa* apparently also has a cuticle within the oesophagus, but this claim requires verification with new material. In Cladobranchia there is a cuticularized epithelium lining the anterior end of the oesophagus and this is followed by a vacuolated non-cuticularized epithelium.

(27) We consider a cuticular lining to the entire oesophagus to represent the plesiomorphic state, whereas the presence of a short cuticularized region or the complete absence of a cuticular lining (in all Doridoidea) is the apomorphic state. Reduction and/or absence of the cuticle does not only occur within the Nudibranchia, but also within the Sacoglossa and the Pleurobranchoidea.

The possession of spines in the posterior region of the oesophagus in *Bathydoris*, akin to those within the crop of the Anaspidea (see Guiart, 1900), is probably a plesiomorphic feature for the Bathydoridoidea.

(28) According to embryological studies, a cuticle is always present in the stomach of nudibranch larvae (Bickell *et al.*, 1981; Bickell & Kempf, 1983), but it is usually lost during metamorphosis. The stomach of the adult *Doridella steinbergae* is formed by the uncuticularized vestibule of the larval digestive tract (Bickell *et al.*, 1981), therefore the primary absence of a cuticle in the stomach in adult nudibranchs is highly probable. Some dendronotoid genera apparently retain the cuticle of the larval stomach into adulthood (Tritoniidae, *Bornella*, *Scyllaea*, *Melibe*, *Dendronotus*). The retention of this cuticle in the stomach is a unique character within adult Nudibranchia. A thin cuticle in the stomach is recorded for members of the Acteonidae and Cephalaspidea *s.s.* (Rudman, 1972b,c), and thick plates in the posterior part of the stomach are known in *Ringicula* (Fretter, 1960). According to outgroup comparison (Pleurobranchoidea) and the ontogenetic formation of the stomach during metamorphosis, the presence of the cuticle is considered as apomorphic, and its absence is plesiomorphic. But taking into consideration the presence of a cuticle in the stomach in some members of the Opisthobranchia, the character is coded as unordered. Marcus (1955, 1957) described a cuticle with broad spines in the posterior stomach of *Goniodoris mimula* and *Thecacera pennigera*, but this observation has not been repeated subsequently. It seems probable to us that this observation actually

represents the misidentification of a cuticularized oesophagus, and further investigations are warranted.

No other opisthobranch groups are reported to possess a cuticular lining within the stomach.

Some genera of the Dendronotoidea lack a cuticle in the stomach as adults (e.g. *Tritonia*, *Lomanotus* and *Doto*) and this has now been confirmed histologically. According to Gosliner (1994), this absence is probably secondary. This polarity definitely seems correct for the family Tritoniidae; whereas all genera of that family possess at least a cuticular lining, or cuticular plates, *Tritonia* alone seems to lack any cuticular structures within its stomach (confirmed for *Tritonia antarctica* and *Tritonia vorax* - Wägele, 1995). Willan (1988) has already noted the impossibility of separating genera within the Tritoniidae according to existing criteria and data relating to the presence or absence of a cuticle in the stomach may well assist in the resolution of the genera in the future. Indeed all dendronotoid families are in need of analysis, genus by genus, to determine whether the cuticle is primarily absent or whether it has been secondarily lost.

The cuticle is represented in the stomach only as a thin lining in some dendronotoid genera (*Tritoniella*), whereas in others it is thickened into plates (*Melibe*, *Marionia*, *Marionopsis*) or rodlets (*Bornella*). Further research is needed on the form and distribution of such cuticular elaborations.

A stomach, completely separated from the digestive gland, is present in all gastropods during their ontogeny. Two ducts, one from the right and the other from the left digestive gland, enter the stomach. This holds true for all molluscs (with a few exceptions) and has been confirmed for nudibranchs by Thompson (1958), Schmekel & Portmann (1982) and Thompson & Brown (1984). This condition is interpreted as plesiomorphic within both the Opisthobranchia and the Nudibranchia. In some groups, the distinction between the stomach and the digestive glandular chamber is blurred. Here fusion of the lumen of the stomach with the central cavity of the left digestive gland has occurred (e.g. in *Dendrodoris*, *Phyllidia*, *Armina*, *Flabellina*). In these instances, the location of the original stomach can only be recognized histologically (i.e. by its ciliated epithelial lining), whereas the digestive glandular part is characterized by glandular cells. In several taxa, principally the Dotidae and members of the Aeolidoidea, the ciliated areas have expanded into the main channels of the digestive gland. Unfortunately too little is known about this situation within the various nudibranch groups to use these characters in our analysis.

In some Aeolidoidea, the ducts leading into the digestive gland are rather long, especially in those taxa where digestive glandular tissue is confined to the diverticula within the papillae (e.g. *Doto*, *Flabellina pedata*, *F. affinis*). In these instances the epithelium lining these ducts is similar to that of the oesophagus, stomach and intestine. Therefore the precise determination of these regions is also fraught with difficulty.

27. Cuticle in the oesophagus
Polarity: A cuticle within the oesophagus is widespread among the Opisthobranchia and is therefore considered to be the plesiomorphic state, although it is only seldom present within the Nudibranchia. The restriction to a very short cuticularized section within the oesophagus, or the complete absence of the cuticle represents the apomorphic states. Character states are considered as unordered.
Coding: 0 - oesophagus lined completely with cuticle; 1 - cuticle restricted to a small section within proximal oesophagus; 2 - complete absence of oesophageal cuticle.

28. Cuticle in the stomach

Polarity: The absence of a cuticle within the stomach is considered to be the plesiomorphic state, and its presence is the apomorphic state.

Coding: 0 - stomach without a cuticular lining; 1 - stomach lined with cuticle.

29-30. Digestive gland

Across the major gastropod taxa (including Caenogastropoda, Pulmonata, Cephalaspidea, Tylodinoidea and Pleurobranchoidea), one generally finds a 'holohepatic' digestive gland, that is a compact and rather solid organ. Therefore, we consider this type of digestive gland as plesiomorphic within the Opisthobranchia and Nudibranchia. Such a holohepatic digestive gland is present in *Doridoxa*, Bathydoridoidea, Doridoidea and Tritoniidae amongst the Nudibranchia (Fig. 4A). However, a trend exists for opening up of the digestive glandular mass into a floccular, less compact organ and ultimately into a series of tubes (see Introduction). This situation where the gland consists of a series of tubes is termed the 'cladohepatic' type of digestive gland (Fig. 4B). A floccular digestive gland, but still with holohepatic contours can be observed in the Anaspidea, *Heterodoris* and *Goniaeolis*. A still more floccular appearance with diverticula extending into the notum can be found within the Charcotiidae and *Leminda*. A variation in the extent of branching occurs in *Janolus mokohinau* where some diverticula just reach the papillae and others extend well into them (Miller & Willan, 1986). In all Aeolidoidea and in several groups of (derived) Sacoglossa, the ultimate diverticula of the digestive gland always extend to the very tips of the notal papillae. Even more extreme is the extension of the diverticula into the rhinophores or rhinophoral sheaths (*Dendronotus*, *Hancockia*, some *Lomanotus* species), oral veil (*Pseudotritonia*), and/or foot (*Charcotia*) (see MacFarland, 1966; Wägele, 1991; Wägele *et al.*, 1995). We interpret the branching of the digestive gland as an adaptation to increase the surface area for digestion. Extensive branching of the digestive gland is often also correlated with dietary specialization (storage of cnidocysts, zooxanthellae, chloroplasts). Although we consider branching of the digestive gland as the apomorphic state, we do not use it here because its distribution is variable within the ingroup taxa and more details on certain taxa are needed for better understanding of this complex character.

In those cases where the glandular tissues of the digestive gland have been transferred into the peripheral diverticula, no glandular tissue can be detected histologically in the digestive glandular tubes in the visceral cavity. Such glandular tissue is only detectable peripherally (e.g. in the lateral notal wings (*Pseudotritonia*) or in the dorsal papillae (Aeolidoidea) or in the ventral lamellae (*Armina*)). Although we consider this state to be derived, it should be useful for future studies on phylogeny at lower taxonomic levels.

During its ontogeny, the opisthobranch digestive gland develops from two separate evaginations of the midgut, a larger left one and a smaller right one (Hamatani, 1960, 1961; Bickell *et al.*, 1981). Usually in the Nudibranchia the right half is considerably smaller than the left (Schmekel & Portmann, 1982). This holds true for Doridoidea (see below) and also for the cladohepatic digestive gland of Dendronotoidea, Arminoidea, and Aeolidoidea, where the right digestive gland is confined to the anterior right section of the visceral cavity. According to Schmekel (1985), the digestive gland of the Sacoglossa is bilaterally symmetrical with both

halves equal in size. We do not have enough information on this character yet for it to be phylogenetically informative.

Primitively the digestive gland of opisthobranchs is paired, with two separate openings from the stomach (Fischer, 1892; Schmekel & Portmann, 1982). This arrangement can be observed during ontogeny in all major opisthobranch clades. Therefore we consider two openings from the stomach in adult specimens as the plesiomorphic state (Bathydoridoidea, many Doridoidea, Tritoniidae, *Caldukia*) (see Odhner, 1934; Miller, 1970; Wägele, 1989a). More than two openings (usually three), can be observed in nearly all members of the Dendronotoidea, Arminoidea and Aeolidoidea (e.g. in *Dendronotus, Bornella, Doto, Armina, Janolus, Leminda, Notaeolidia, Embletonia* - Bergh, 1885; MacFarland, 1966; Griffiths, 1985; Miller & Willan, 1986; Wägele, 1990a) (Fig. 4B). Usually two of these openings are situated at the anterior edge of the stomach (right and left) and the third is in the midline posteriorly. There are also three openings in the Onchidiida, but the two anterior ones lie one above the third. The presence of three openings is considered to be the apomorphic state. This character was not used in this phylogenetic analysis because of the variability within especially the dendronotoid species and lack of data for other groups.

(29) Schmekel & Portmann (1982) concluded that, in the Doridoidea, the right digestive gland formed a glandular diverticulum, the so-called 'stomach caecum', which opens directly into the stomach, independently of the opening of the intestine. Not only does this caecum appear as a small bulb-like structure considerably smaller in size than the left digestive gland, but it also has a different epithelial lining to that of the left digestive gland, with relatively undifferentiated ciliated cells and no mucous cells (Fig. 7D). The function of the caecum is not known, although some authors have postulated that it might store spicules derived from the food (Hoffmann, 1939). A caecum is present in the Bathydoridoidea, *Doridoxa* and (many) Doridoidea (e.g. *Hypselodoris, Aegires, Jorunna, Archidoris, Paradoris, Siraius, Plocamopherus* - e.g. Miller, 1995). Wägele (1989b) concluded that a caecum represented a synapomorphy for the three groups mentioned above (*Doridoxa*, Bathydoridoidea, and Doridoidea). However, we have recently discovered that some Doridoidea (*Dendrodoris, Onchidoris, Roboastra*) definitely do not possess a caecum (pers. obs.). In these cases it is unclear, whether the right digestive gland has been completely lost or whether it has been integrated into the left digestive glandular mass. Only ontogenetic investigations can reveal the fate of the right digestive gland in these taxa. The presence or absence of a caecum apparently varies within some genera (*Glossodoris, Paradoris* - see Ortea, 1995). Brodie *et al.* (1997) described a 'caecum' in two Indo-Pacific *Dendrodoris* species and noted that it apparently displayed considerable intraspecific variation. However, histological examination of this organ in one of these species has revealed it is not homologous with the caecum formed from the right digestive gland as in other caecate nudibranchs, but a separate outpocketing of the intestine (Wägele *et al.*, 1999).

The right digestive gland was depicted schematically for the Arminoidea by Schmekel & Portmann (1982: fig. 4.2) as a small, solid and spherical organ. We do not know the source of their data because, according to the literature and to our investigations, the right digestive gland is never reduced to the extent of becoming a caecum in any arminoidean taxon, although it is certainly quite small in some of the constituent genera. Salvini-Plawen (1990) interpreted the Schmekel & Portmann diagram as indicating that a caecum was present in the Arminoidea, and thus he

employed it to falsify Wägele's (1989b) hypothesis that *Doridoxa* was the sister-group to the Bathydoridoidea plus Doridoidea. Wägele had advocated this relationship because both the latter groups shared the derived state of reduction of the right digestive gland into a caecum. This matter needs reinvestigation because nothing is known about the histology of the structure called a 'caecum' in *Doridoxa*.

(30) Sac-like structures at the terminations of the diverticula in the branching type of digestive gland (Fig. 9B) have been described in several groups of nudibranchs (Wägele, 1991; Wägele *et al.*, 1995). These so-called 'terminal sacs' consist of greatly enlarged cells containing very large vacuoles. These cells do not contain any cnidocytes, but sometimes their vacuoles stain bluish, especially when the digestive glandular lumen is filled with nutritive fluid. The separation of the terminal sac from the glandular part of the digestive glandular branch is not distinct, as in the cnidosac where there is a narrow ciliated duct separating the different regions. We believe that the terminal sac probably serves an excretory function in that it rids the diverticula of substances no longer needed for digestion. Terminal sacs are present in some arminoid and aeolid genera (*Pseudotritonia*, *Charcotia*, *Phestilla* - Harris, 1973; Wägele, 1991). According to Miller & Willan (1991), *Embletonia gracile* also has terminal sacs. The presence of terminal sacs is considered to be the apomorphic state.

In the Aeolidoidea, the terminal region of the digestive diverticula within the dorsal papillae possesses another specialized sac-like structure which is similar histologically to the terminal sac, but which is usually separated from the digestive diverticulum by a sphincter muscle and a more or less distinct duct (Conklin & Mariscal, 1977 and Fig. 9A,C). The epithelial cells of these sacs are very large and they contain vacuoles that enclose functional cnidocytes (termed 'kleptocnides') derived from the cnidarian prey and used for the nudibranch's own defense. These very special elaborations are called cnidosacs. Because of the overall histological similarity between terminal sacs and cnidosacs, we consider them as homologous structures, and furthermore, we consider the elaboration of specialized terminal sacs into cnidosacs as a prerequisite for the evolution of kleptoplasty. Therefore cnidosacs are coded here as apomorphic.

Rudman (1990) recorded the absence of kleptocnides in the acolid *Protaeolidiella*, but he assumed it was an artefact. We can confirm there are no kleptocnides in the papillae in this genus, but there is definitely a sac-like structure at the tip of each papilla. This sac is connected to the digestive gland by a duct and it thus resembles the cnidosac of other aeolids. Therefore, even though *Protaeolidiella* definitely feeds on hydrozoans (*Protaeolidiella* is an obligate associate of the athecate genus *Seratella* - Willan & Coleman, 1984), it presumably does not store the cnidocytes. Harris (1973) described and figured sac-like structures which look very similar to those of the Charcotiidae in another aeolid genus, *Phestilla*. Their fine structure needs to be clarified to decide whether they are actually cnidosacs without kleptocnides or terminal sacs. Some other genera of the Aeolidoidea do not store cnidocytes either (confirmed here for *Phyllodesmium*), even though they feed on cnidarians and possess cnidosacs. And whether *Fiona*, *Favorinus*, *Aeolidiopsis* and *Calma* lack the entire cnidosac, as claimed by Baba (1949), Marcus (1961) and Pruvot-Fol (1956), needs to be investigated histologically.

Thompson (1972) confirmed the presence of cnidosacs in the papillae of the dendronotoidean *Hancockia burni*. Our investigation of one specimen of *Hancockia*
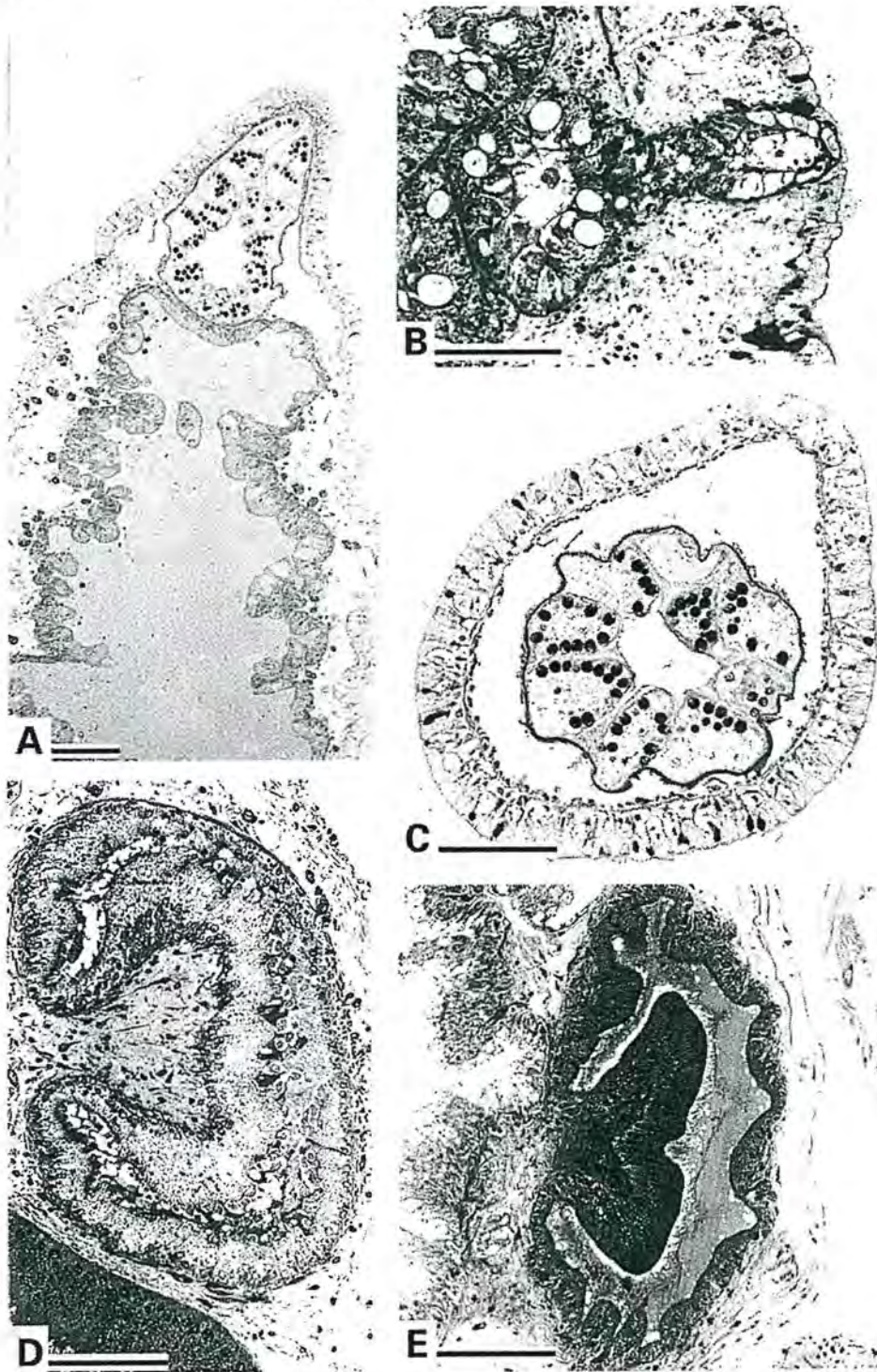
uncinata did not permit any more definitive conclusions to be drawn because the structures described as kleptocnides in these cnidosacs are extremely small, actually considerably smaller than in any aeolid. New investigations, especially ultrastructural ones, are required before we can substantiate any claim of kleptoplasty in the Dendronotoidea.

29. Caecum (right digestive gland)

Polarity: The presence of a right digestive gland with a glandular epithelial lining is considered to be the plesiomorphic state. The presence of a caecum, opening into the stomach next to the openings of the oesophagus and intestine, and with a ciliated non-glandular epithelium, is considered to be the derived state. The absence of both a caecum and the right digestive gland is considered to be the apomorphic state. The latter two states are treated as unordered.

Coding: 0 - right digestive gland present with glandular epithelial lining; 1 - right digestive gland differentiated into caecum; 2 - both caecum and right digestive gland absent.

30. Terminal sacs and cnidosacs

Polarity: The absence of both these structures is the plesiomorphic state. The acquisition of a terminal sac containing exceptionally large vacuoles is considered to be the prerequisite for a cnidosac which is completely separate from the digestive gland and communicates with the latter by a duct. Therefore we consider this character initially as unordered and subsequently as ordered. The presence of a sac with a duct into the digestive gland is considered to represent a cnidosac without any kleptocnides. There is a high probability of these structures being homologous because of their similar configurations and therefore a higher significance is assumed and weighting 2 was applied in successive analyses.

Coding: 0 - terminal sacs and cnidosacs absent; 1 - terminal sacs present; 2 - cnidosacs containing kleptocnides; 3 - cnidosacs present but lacking kleptocnides.

31. Intestine

Usually the intestine originates from the anterior border of the stomach in nudibranchs (Fig. 4A,B) and we consider this as the plesiomorphic state. In *Tylodina*, the intestine was described as originating from the posterior part of the stomach (MacFarland, 1966), but a thorough re-investigation of the relationship of the stomach and oesophagus needs to be undertaken. The larger section of the anterior digestive tract was apparently misinterpreted as the stomach, although it is really the posterior section of the oesophagus.

In a few nudibranch taxa there is a departure from this anterior position for the origin of the intestine, which may ultimately be useful for phylogenetic analyses:
(a) In the Bathydoridoidea the intestine originates ventrally on the left side of the stomach opposite the point of entry of the oesophagus (Wägele, 1989a).

Figure 9. Histology of the digestive system: A, longitudinal section of cnidosac in *Cuthona* sp. and adjacent digestive gland; B, cross section of terminal sac of *Charcotia granulosa* with adjacent digestive gland; C, cross section of papilla with cnidosac in *Cuthona* sp.; D, cross section of intestine with typhlosole in *Trapania maculata*; E, cross section of typhlosole in *Armina neapolitana*. Scale bars: A–E = 100 µm.

(b) In *Tritoniella* the intestine originates on the right side of the stomach (Wägele, 1989c).

These character states are not used here because of lack of data.

(31) In nudibranchs the typhlosole is a thickened fold within the intestine commencing at the beginning of the intestine where it emerges from the stomach and usually ending long before it terminates at the anus. Whether this relatively short typhlosole is homologous with the much longer structure seen in 'prosobranchs' is not certain, but such an homology cannot be discounted because it has already been demonstrated in the Cephalaspidea (see Mikkelsen, 1996). According to Mikkelsen (1996), a short typhlosole is the plesiomorphic state for the Cephalaspidea. The function of the typhlosole in opisthobranchs is not completely understood. Mikkelsen (1996) suggested it produced a secretion that consolidated faecal pellets. Another possible function for the typhlosole could be the alignment of elongate hard structures derived from the food such as alcyonarian or sponge spicules into a longitudinal direction for ease of discharge.

In the Nudibranchia, the presence of a typhlosole is considered to be plesiomorphic, and its absence is apomorphic. There is no typhlosole in the Anaspidea (Mikkelsen, 1996), many pleurobranchs (*Bathyberthella*, *Tomthompsonia*), Bathydoridoidea, and many Doridoidea (confirmed histologically in *Austrodoris*, *Jorunna*, *Dendrodoris*, *Aegires*, and *Phyllidia*). According to Hoffmann (1939), a typhlosole is generally absent in Doridoidea and Arminoidea, but this claim could not be confirmed, because some dorids (e.g. *Trapania*, *Onchidoris*; Fig. 9D), and all the Arminoidea and Aeolidoidea we have investigated (e.g. *Dendronotus*, *Lomanotus*, *Armina*, *Cuthona*, *Flabellina*) (Fig. 9E) did possess a typhlosole.

Anal glands are annexed to the hindgut at the point where the intestine opens to the exterior. Because anal glands are not very widespread within the Opisthobranchia (Hoffmann, 1939), we consider their absence as plesiomorphic, and their presence as apomorphic. Within the Nudibranchia anal glands are only known within the genera *Janolus* (present probably only in *J. barbarensis*, *J. capensis*, *J. cristatus*, *J. longidentatus* and *J. rebeccae*) and *Vayssierea* (Baba, 1931; Gosliner, 1981; Schrödl, 1996), so they are not relevant for the present phylogenetic analysis.

31. Typhlosole
Polarity: A typhlosole starting at the transition from the stomach into the intestine is considered to be the plesiomorphic state, and the absence of a typhlosole is the apomorphic state.
Coding: 0 - typhlosole present; 1 - typhlosole absent.

Nervous system

Position of circumoesophageal nervous system
According to Mikkelsen (1996), the circumoesophageal central nervous system was primitively located behind the pharynx, but during the course of evolution (after the splitting of Anaspidea and Cephalaspidea) it shifted from the postpharyngeal to a prepharyngeal position. A postpharyngeal position is also found in the Tylodinoidea (prepharyngeal in Pleurobranchoidea). In order to clarify the polarity Gosliner (1994) compared the situation with additional outgroups, namely the Mathildidae,
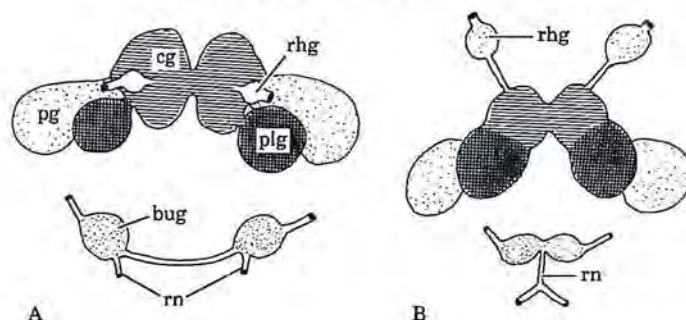
Figure 10. Schematic diagrams of nervous system: A, *Bathydoris* (redrawn after Wägele, 1989a); B, *Notaeolidia* (redrawn after Wägele, 1990a). Abbreviations: bug - buccal ganglion; cg - cerebral ganglion; pg - pedal ganglion; plg - pleural ganglion; rhg - rhinophoral ganglion; rn - radular nerve.

Basommatophora and some members of the Vetigastropoda and Heterobranchia. He concluded that the prepharyngeal position was the plesiomorphic one. This hypothesis necessitated the conclusion that postpharyngeal position was an apomorphic trait that evolved repeatedly and independently within different opisthobranch clades (also within the Pulmonata). All the Nudibranchia we have investigated have the nerve ring situated around the anterior oesophagus (that is at the *rear* of the pharynx). Although our chief outgroup, the Pleurobranchoidea, have a prepharyngeal configuration, we cannot exclude the possibility that this situation presents the derived situation within the Opisthobranchia. At the moment we cannot choose between the opposing hypotheses as regards the polarity of this character as it relates to our ingroup, the Nudibranchia (discretion being the better part of valour!) and so we have not used it for our phylogenetic analysis.

Fusion of ganglia

Several authors have already outlined the different types of fusion of ganglia of the visceral loop within the Opisthobranchia (Boettger, 1955; Schmekel & Portmann, 1982). Within the Nudibranchia, nearly all the ganglia are fused apart from the visceral ganglion in some taxa. Primitively the cerebral and pleural ganglia were also separated, a condition observed in the Cephalaspidea, Anaspidea, Tylodinoidea, Bathydoridoidea (Fig. 10A), and to a greater extent in *Actinocyclus, Gymnodoris, Corambe* and *Plocamopherus* (some species only) (Vayssière, 1912; Hoffmann, 1939; Schmekel, 1985; Wägele, 1989a). But we have to emphasize here that good descriptions of the nervous system are rare, and new comprehensive investigations are urgently needed.

In (many) Sacoglossa, (all) Pleurobranchoidea (including *Tomthompsonia*), and (nearly all) Nudibranchia (except of those just mentioned), the pleural ganglion is fused with the cerebral ganglion (Fig. 10B). We consider the separation of these ganglia is the plesiomorphic state, and their fusion is the apomorphic state.

Martynov (1995) considered the separate cerebral and pleural ganglia in *Corambe* as a product of paedomorphosis. Because the Corambidae display other characters which can be explained convincingly with the help of paedomorphosis (i.e. position of gills and anus), the presence of separate ganglia may also be a result of this developmental process. Whether or not the process might be operating in cases where one finds separation of the pleural and cerebral ganglia within the Nudibranchia needs

to be assessed on a case-by-case basis. Whilst such speculation is interesting, this character is not used here.

As with the case of unfused pleural ganglia, a free visceral ganglion on the visceral loop is considered to be the plesiomorphic state. It has been described in general for Pleurobranchoidea and Doridoidea (Schmekel, 1985), but here the same problem exists in that only a few detailed descriptions on the nervous system are available and even these few seldom contain information about the visceral ganglion. According to Wägele (1990b) the position, and even the presence, of the visceral ganglion can vary considerably within one species (for example *Austrodoris kerguelenensis*). The position can also vary in *Onchidella* (Weiß & Wägele, 1998). Therefore this character is not included in our phylogenetic analysis.

Position of ganglia

Usually in the Opisthobranchia, a small ganglion lies at the base, or basal part, of each rhinophore and it is connected with the cerebral ganglion by a more or less long nerve, the rhinophoral nerve (Tylodinoidea, Pleurobranchoidea, (several) Doridoidea, e.g. *Hexabranchus*, Cladobranchia). We consider this position as the plesiomorphic state, and a position near the cerebral ganglion as the apomorphic state. The latter position is found in the Doridoidea, Bathydoridoidea and (a few) Aeolidoidea (e.g. *Samla, Cratena* - Hoffmann, 1939).

Sometimes no rhinophoral ganglia can be observed (*Tritoniella, Pseudotritonia* - Wägele, 1989c, 1991). In these instances it is not clear whether the rhinophoral ganglion is fused with the cerebral ganglion, or whether it is not present at all, or (just) difficult to detect. Because of these problems with interpretation we did not use the presence or absence of rhinophoral ganglia in our analysis.

A few species of nudibranchs seem to have two ganglia per rhinophore, one annexed to the cerebral ganglion and the other situated distally at the base of the rhinophore (e.g. *Polycera quadrilineata, Gymnodoris* - see Hoffmann, 1939). We do not know enough about this condition to use it for phylogenetic analyses.

The buccal ganglia are situated on the ventral side of the oesophagus next to its origin from the pharynx. Two different situations can be observed; either the two buccal ganglia are located very close together (Fig. 10B) (as in many members of the Opisthobranchia), or they are separated from each other (Fig. 10A). In the Bathydoridoidea and *Analogium striatum*, the connective between the right and left buccal ganglion is very long (Hoffmann, 1939; Wägele, 1989a), whereas in other taxa the connective is shorter, although the two ganglia are still separate (*Tomthompsonia, Gymnodoris, Ancula, Corambe, Janolus*). No evaluation of this character is possible at present.

Two separate nerves, one each from the two buccal ganglia, innervate the radular area (Sacoglossa, *Bathyberthella, Berthella, Pleurobranchaea*, Bathydoridoidea, (possibly all) Doridoidea, *Melibe, Phylliroe, Goniaeolis, Heterodoris, Dirona, Fiona, Glaucus, Cratena*) (Fig. 10A). In several taxa these two nerves are united and they originate from the extremely short connective between the two ganglia (*Haminoea, Scaphander, Aglaja, Akera*, Aplysiidae, *Tylodina, Pleurobranchus, Tritoniidae, Armina, Notaeolidia*) (Fig. 10B). We assume that the separate radular nerves represent the plesiomorphic state and fusion is the apomorphic state. This character is not used in this phylogenetic analysis because we do not know the situation in a few of the genera used here.

32. Sensory organs

Virtually all opisthobranchs have eyes and this represents the plesiomorphic state. Eyes are absent only in some deep sea ((many) Bathydoridoidea, *Heterodoris antipodes* - Willan, 1981; Wägele, 1989a) and interstitial species ((some) *Pseudovermis* - Challis, 1969). The absence of eyes is apomorphic and it can be interpreted as an adaptation to the unavailability of light in particular habitats.

The eyes are usually located at the base of the rhinophores and they are connected with the cerebral ganglion by relatively long optic nerves, that is the nerves are several times longer than the diameter of the eye. This is the case in most species of Heterobranchia, Cephalaspidea (see Mikkelsen, 1996), basal Basommatophora, Anaspidea, Sacoglossa, Tylodinoidea, *Bathyberthella, Euselenops, Pleurobranchaea*, Tritoniidae, Bathydoridoidea, (many) Doridoidea (e.g. *Austrodoris*), Dendronotoidea, and Arminoidea. We consider this state (relatively long optic nerves) as plesiomorphic. Short, but still distinct optic nerves with a length less than three times the diameter of the eye are present in several opisthobranch genera. This represents an apomorphic state. The following examples with short optic nerves have been illustrated in the literature: *Scaphander, Toledonia, Diaphana, Haminoea, Philine, Berthella, Pleurobranchus, Corambe, Glossodoris, Archidoris, Hancockia, Notaeolidia schmekelae, Flabellina* (Bergh, 1898; Vayssière, 1898; Hoffmann, 1939; Wägele, 1990a; Mikkelsen, 1996). There is a trend for shortening the optic nerve to the point of complete loss, so that the eye sits directly on top of the cerebropleural complex (i.e. it is sessile). This reduction can be explained by the simultaneous development of more elaborate chemical and mechanical sensory organs such as the rhinophores, oral tentacles and propodial tentacles. Sessile eyes is the final stage in this series of reductions, and we consider this as the apomorphic state, compared to the presence of long optic nerves. Sessile eyes are present in *Gymnodoris, Holoplocamus, Plocamopherus, Aegires, Polycera, Goniodoris, Okenia, Hallaxa, Asteronotus, Phyllirhoe, Melibe, Notaeolidia depressa, Samla, Calma, Facelina, Aeolidia* (for examples, see Hoffmann, 1939). We have not used the length of the optic nerve in our study for two reasons. Firstly authors seldom specify the real length of the long optic nerve; if located close to the central nervous system it could be truly short or it could actually be shortened due to contraction during fixation. Secondly, the length of the nerve can, and does, vary interspecifically within one genus (e.g. *Notaeolidia*). A colleague has even observed great variation in this character within the same species (*Janolus rebeccae* - Schrödl, pers. comm.). Nevertheless, more information on this character might help to solve relationships at lower taxonomic levels.

According to Hoffmann (1939), statocysts seem to be present in all opisthobranchs. They lie next to the pedal ganglia and in the Nudibranchia, because of the apposition of cerebropleural and pedal ganglia, they actually lie between these two ganglia.

(32) Several 'otoconia' (small, calcareous, spherical bodies) lie in the lumen of each statocyst (Fig. 11A,B) in many opisthobranch taxa. In others, only a single body has been described, in which case it is called an 'otolith' (Fig. 11C). Hoffmann (1939) cautioned that (some of) the otoconia could be dissolved during fixation and preparation with chemicals. Therefore, especially older literature has to be read with caution. Nevertheless, it seems that nearly all Cephalaspidea, Anaspidea, Tylodinoidea and Pleurobranchoidea (including *Tomthompsonia*) do have several otoconia, and we therefore consider the presence of several otoconia to be the plesiomorphic state within the Nudibranchia, and the presence of just one otolith as the apomorphic state. Sacoglossa in general and some genera or species of the
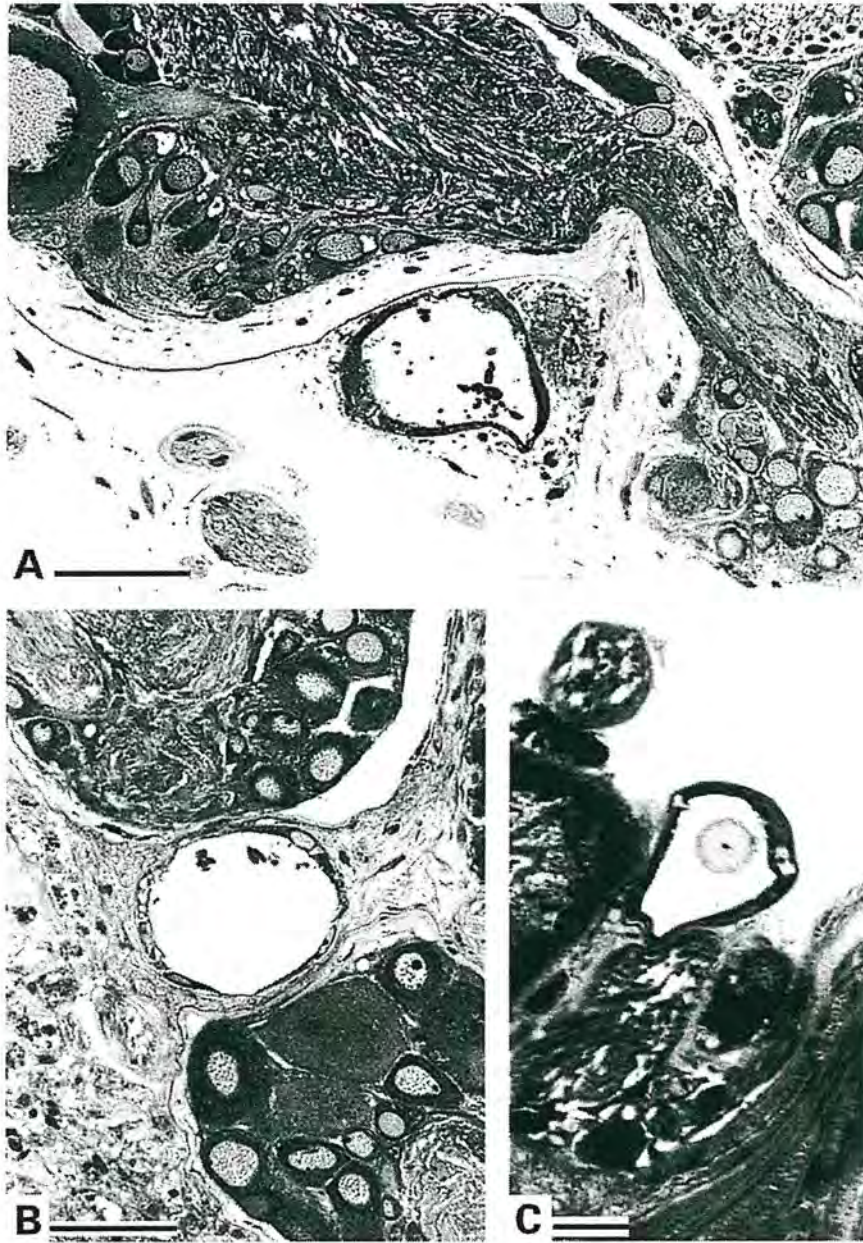
Figure 11. Histology of the statocyst: A, statocyst with many otoconia in *Flabellina affinis*; B, statocyst with many otoconia in *Trapania maculata*; C, statocyst containing one otolith in *Tergipes tergipes*. Scale bars: A,B = 50 µm, C = 10 µm.

Nudibranchia in particular definitely have only one otolith within the statocyst (*Okadaia, Eubranchus, Tergipes, Cuthona, Fiona, Calma, Pseudovermis, Embletonia* - Bergh, 1886; Pelseneer, 1894; Baba, 1937; Delamare-Deboutteville, 1960). Some species of *Goniodoris* and *Gymnodoris* are described as having one large otolith and several small otoconia (Pelseneer, 1894; Risbec, 1928).

32. Statocyst
Polarity: The presence of otoconia in the statocysts is considered to be the plesiomorphic state, and the presence of only an otolith is the apomorphic state.
*Coding*: 0 - statocyst containing multiple otoconia; 1 - statocyst containing only an otolith.

Genital system

33–34. Gonad
(33) The gonad is completely separated from the digestive gland in most taxa of the Opisthobranchia. Both organs are quite distinct even in the Pleurobranchoidea where the gonad lies immediately in front of, and tightly pressed against, the digestive gland. This state where each organ maintains its integrity, though adpressed, can also be observed in a few nudibranch genera (e.g. *Bathydoris, Alloiodoris, Gymnodoris, Heterodoris* - Eliot, 1908; Macnae, 1958; Wägele, 1989a). This state is considered to be plesiomorphic. But in many nudibranchs, the gonad spreads over the digestive gland dorsally and is more or less strongly connected to it (Medina *et al.*, 1986). In some genera (e.g. *Dendrodoris*), the gonad actually intermingles with the digestive gland and kidney (Wägele *et al.*, 1999). The state where the gonad envelopes the digestive gland is considered to be apomorphic. It can be observed in (most) Doridoidea, Tritoniidae, (nearly all) Arminoidea and (many) Aeolidoidea (*Notaeolidia, Flabellina, Protaeolidiella*). In some derived nudibranch taxa the rather uniform layer of gonadial follicles can be dispersed and the follicles are completely separate (*Scyllaea* - Risbec, 1928). Usually this situation is correlated with a branched digestive gland and it is therefore not used here as a separate character. A similar situation can be observed in the Sacoglossa.

(34) All opisthobranchs (except some of the Acochlidioidea) are hermaphrodite, the gonad being an ovotestis where male and female gametes lie more or less side by side. For the Nudibranchia at least, sperm probably start maturing first (protandry) but this male phase is very short in duration, and it is outlasted by the simultaneous production of both sperm and eggs (simultaneous hermaphroditism). Within the different taxa of the Opisthobranchia the arrangement of the spermatogonia and oogonia differ to a certain extent (Henneguy, 1925). For instance, in many nudibranch genera (*Aegires, Jorunna, Acanthodoris, Tritonia, Doto, Charcotia, Dermatobranchus, Notaeolidia, Flabellina*), the male and female gametes are located in one and the same follicle, but usually spermatogonia are concentrated in the medullary part of the follicle, whereas oogonia are cortical (Fig. 12A,B). Mikkelsen (1996) considered this situation as plesiomorphic and we agree with her arguments. Within many taxa of the Nudibranchia, separation between the sperm- and egg-producing areas can be observed, although there never seems to be a complete separation. Usually oogonia are concentrated in separate follicles, several arranged around one male follicle (Fig.
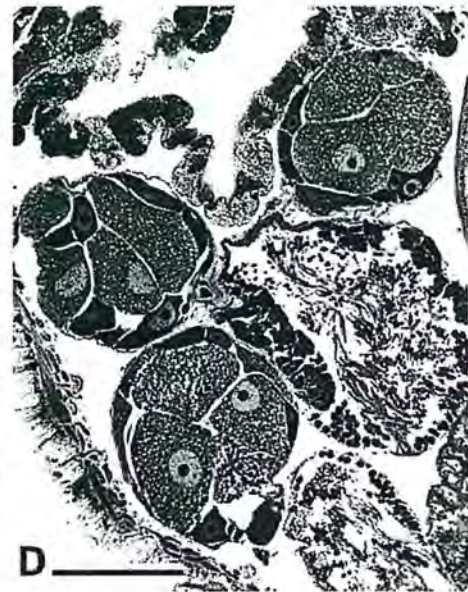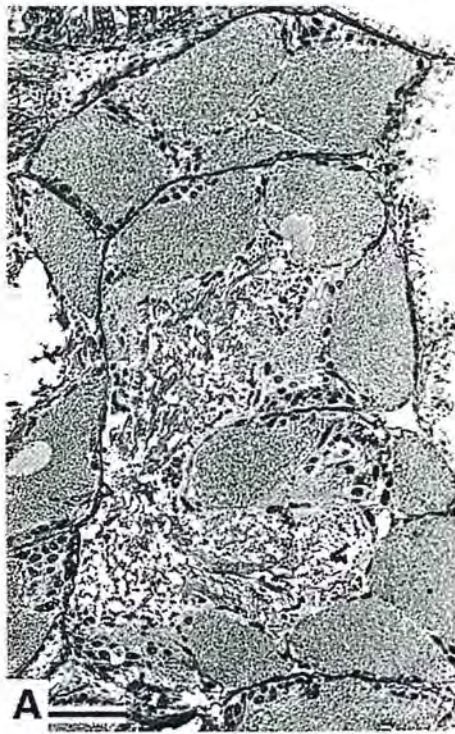
12C,D). The female follicles are still connected to the male follicle and the eggs actually have to pass the male part in order to reach the hermaphrodite duct. This situation, which we consider is the apomorphic state, can be observed in *Hypselodoris*, *Trapania*, *Tergipes* and *Eubranchus*. Gosliner (1994) has already recognized this feature (female follicles arranged like petals around the male follicles) as a synapomorphy for the families Eubranchidae and Tergipedidae.

We assume that an ampulla (the distal section of the hermaphroditic duct, usually filled with autosperm in sexually mature individuals) is present in all nudibranch species, but sometimes it is barely distinguishable from the remainder of the hermaphrodite duct. Although a great amount of data about the size and shape of the ampulla exists for nudibranchs, the use of this character for phylogenetic analysis is difficult because its shape and length are so dependant on the state of maturity of the animal and its phase in the sexual cycle (all nudibranchs are semelparous, but insemination can occur at a very young age - Todd *et al.*, 1997). Nevertheless, some authors use the number of coils and the relative length of the ampulla as the basis of taxonomic discrimination between species (e.g. *Dendrodoris* - Valdés *et al.*, 1996).

The ampulla is typically sausage-shaped, but sometimes it appears as a bulge on one side of the hermaphroditic duct (Phyllidiidae, *Culhona*, *Eubranchus*, *Learchis evelinae* - Schmekel & Portmann, 1982; Edmunds & Just, 1983; Brunckhorst, 1993). Because there are not sufficient reliable data concerning presence or absence of the ampulla or its shape, this character is not used in this analysis.

Schmekel (1970, 1971) reported a ciliated tract within the ampulla in some nudibranchs and a similar tract has also been mentioned for several shelled opisthobranchs (see Ghiselin, 1966; Gosliner, 1994; Mikkelsen, 1996). Mikkelsen has astutely pointed out the difficulties associated with using this character for phylogenetic analysis. The presence of a ciliated tract could not always be confirmed in our material, and because the detection of cilia can be difficult with histological methods when preservation is suboptimal, we have not used this character.

33. Position of gonad
Polarity: A gonad lying in front of the digestive gland is considered to be the plesiomorphic state, and the gonad spreading over the dorsal part of the digestive gland is the apomorphic state.
Coding: 0 - gonad discrete, located in front of digestive gland; 1 - gonad spreading over digestive gland.

34. Gonadal acini
Polarity: The production of spermatogonia and oogonia within the same follicle is considered to be the plesiomorphic state, and the separation of spermatogonia and oogonia into distinct follicles is the apomorphic state.
Coding: 0 - spermatogonia and oogonia located in same follicle; 1 - spermatogonia and oogonia located in separate follicles.

Figure 12. Histology of the gonad: A, gonad of *Charcotia granulosa*; B, gonad of *Flabellina affinis*; C, gonad of *Trapania maculata*; D, gonad of *Tergipes tergipes*. Scale bars: A–D = 100 μm.

am

pr

nigl

rec
buc

pen

A

B

nigl

rec
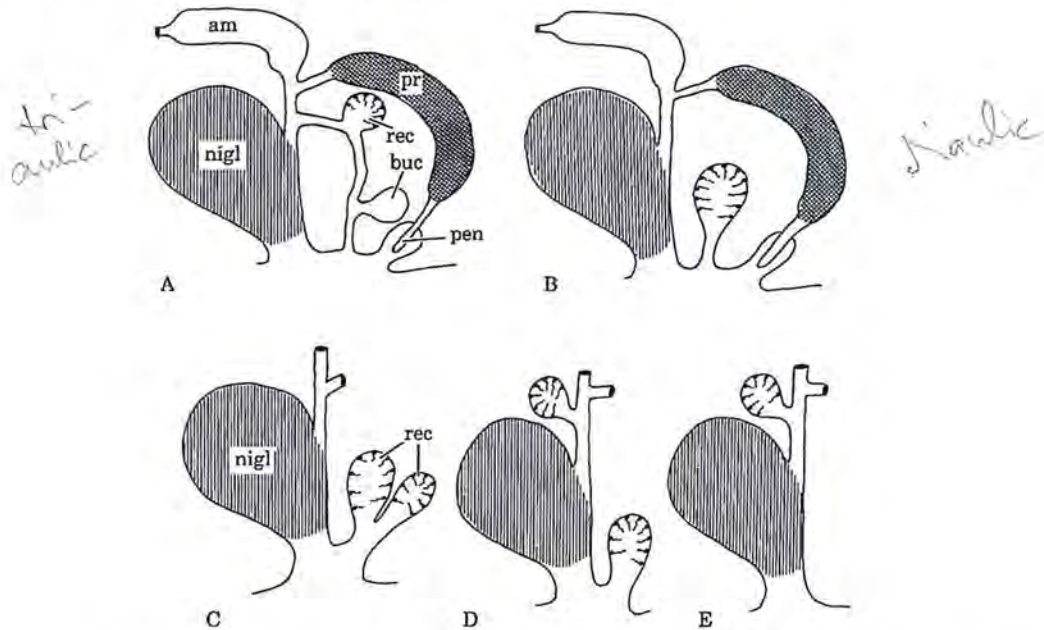
C

D

E

*tri-
aulic*

diaulic

Figure 13. Schematic diagrams of the reproductive system: A, triaulic genital system; B, diaulic genital system; C, female part of genital system of *Flabellina affinis* (after Geiger, 1993); D, female part of *F. ischitana* (after Hirano & Thompson, 1990); E, female part of *F. babai* (after Schmekel & Portmann, 1982). Abbreviations: am - ampulla; buc - bursa copulatrix; nigl - nidamental glands; pen - penis; pr - prostate; rec - receptaculum seminis.

35–37. Distal genital system

(35) In his discourse on reproductive function and phylogeny in the Opisthobranchia, Ghiselin (1966) hypothesized the evolution of diaulic and triaulic genital systems from an ancestral monaulic type. Because only androdiaulic and triaulic systems are present in the Pleurobranchoidea and Nudibranchia, only these systems are discussed here. In the androdiaulic system, the autosperm canal (vas deferens) is separate from the oviduct and allosperm-receiving duct, whereas in the triaulic system the allosperm-receiving duct (vagina) with its annexed organs (bursa copulatrix and receptaculum seminis) is separated from the oviduct. A triaulic system can be observed in some pleurobranchids, which Willan (1987a) considered to be the apomorphic condition within the Pleurobranchoidea. We follow this assumption that the complete separation of canals for sperm and eggs is more derived and therefore consider the triaulic system as apomorphic, and the diaulic system as plesiomorphic. A triaulic genital system (Fig. 13A) is present in pleurobranchs of the genera *Bathyberthella*, *Berthella*, and (some) *Pleurobranchus* species, nearly all Doridoidea, *Lomanotus*, and (some) *Janolus* species (Schmekel, 1970; Gosliner, 1982; Schmekel & Portmann, 1982). Diauly occurs in *Bathydoris* and in nearly all Cladobranchia (*Heterodoris*, *Armina*, Charcotiidae, *Flabellina*, etc.). Because of the complexity of this character, it is accorded a higher significance.

(36) According to Ghiselin (1966), Gosliner (1981), Schmekel (1985) and Mikkelsen

(1996), the presence of both a proximal receptaculum seminis (allosperm-storing organ, originating near the gonad and common hermaphroditic duct) and a distal bursa copulatrix (gametolytic gland, originating near the common genital atrium) represents the plesiomorphic state within the Opisthobranchia (Fig. 13A). The terms for these allosperm vesicles have been clarified and they are clearly discernible thanks to Schmekel's (1971) investigation of their ultrastructure. The usually folded wall of the receptaculum seminis has rather indistinct cells, is surrounded by a more or less thick muscular layer, and the allosperm are oriented perpendicular to the wall. The bursa copulatrix is thin walled because its wall is usually composed of apocrin-secreting cells rather than muscles, and the contents consist of more or less degraded gametes and secretions (Medina *et al.*, 1988). Mikkelsen (1996) has already indicated that the so-called 'bursa' in the Caenogastropoda is not homologous with the organ described above. In some nudibranch taxa (e.g. *Armina*), a receptaculum can be observed with typical histological characteristics and sperm orientation as to indicate a sperm storage function, but a mass of degraded sperm can be observed within its lumen. Gosliner (1994: 320) mentioned that in some Cladobranchia the receptaculum is "a bursa copulatrix, rather than a receptaculum seminis". We do not share this conclusion, but consider those vesicles with a muscular layer and sperm oriented perpendicular to the walls to be a receptaculum seminis *sensu* Schmekel (1971).

Both vesicles (bursa copulatrix and receptaculum seminis), as defined histologically by Schmekel (1971), are only present in the Doridoidea, where they are usually attached to the vagina. In many other taxa of the Nudibranchia, only one vesicle can be observed, which seems to function chiefly as a receptaculum. This is considered to be the apomorphic trait (Fig. 13B).

Within the Aeolidoidea, especially the Flabellinidae (Fig. 13C–E), additional vesicles can sometimes be observed, usually lying proximally (see also next section). These vesicles appear to have similar histological features to the receptaculum seminis (pers. obs.). Because comparative data relating to these additional vesicles are lacking, no statements about possible homologies can be made and we do not discuss this character further. Gosliner (1994) noted the presence of a bursa in some aeolids, but this was not confirmed histologically.

Wägele (1989a) recorded only a bursa copulatrix in *Bathydoris*. This state is unique within the Nudibranchia and, because it represents an autapomorphy for this genus, it is not used in the present phylogenetic analysis.

In some nudibranch taxa, the bursa copulatrix is enveloped by the prostate gland (e.g. *Bathydoris*, *Thecacera darwini*, some species of *Halgerda*, *Plocamopherus* - Wägele, 1989b; Willan & Brodie, 1989; Carlson & Hoff, 1993). We still have not enough information about this character to use it in phylogenetic analysis.

The plesiomorphic position of the receptaculum seminis is proximal (Anaspidea), but in many pleurobranch and nudibranch taxa, excluding the Doridoidea, it lies distally, very often with a separate duct and not entering the distal oviduct (*Tomthompsonia*, *Berthellina*, *Berthella*, *Tritonia*, *Doto*, *Charcotia*, *Armina*, *Notaeolidia*, *Tergipes*, *Protaeolidiella*, etc.). Nevertheless, the position can vary considerably within one genus, for example in *Flabellina* two receptacula may insert distally (*F. affinis* Fig. 13C), or one distally and one proximally (*F. nobilis*, *F. ischitana*, *F. capensis* - Fig. 13D), or only one proximal (*F. babai*, *F. baetica* - Fig. 13E), or two proximal (*F. pedata*, *F. bertschi*) (Schmekel & Portmann, 1982; Gosliner & Kuzirian, 1990; Hirano & Thompson, 1990). A thorough investigation is needed to clarify the histological properties of

these receptacula before a phylogenetic analysis can be undertaken (see also above).

The following scenarios could explain the different situations which can be observed within the Cladobranchia:

(a) One receptaculum lying proximally. This would represent the plesiomorphic situation.

(b) Two receptacula, one proximal and one distal. The second receptaculum could have evolved independently with the same function as the original one, or the distal bursa could have switched its function into that of a sperm-nourishing receptaculum.

(c) A single receptaculum close to the vaginal opening (=distal). This type could have arisen from the second type by the loss of the proximal receptaculum, or the single receptaculum described in type one above could have shifted distally.

Because we do not know enough about this intriguing character and its states, the matter is not pursued further in this analysis.

The vagina itself can possess folds and/or pigmentation on the walls distally and/ or glands centrally (e.g. *Halgerda aurantiomaculata* - Willan & Brodie, 1989). Although such characters are extremely important for recognition at the species level, they are probably too variable to be useful at higher levels.

A cuticularized lining to the vagina or cuticular armature (styles, etc) is recorded in several nudibranch genera (e.g. *Platydoris, Asteronotus, Kentrodoris, Jorunna, Gargamella* - Edmunds, 1971; Schrödl, 1997; reviewed by Gosliner, 1994). Because no such structures are present in any of the outgroups or the majority of nudibranchs, we consider the presence as the apomorphic state and the absence the plesiomorpic state. The problem arises as to whether such cuticular armature should be assigned to the vaginal duct or to the vas deferens because at copulation the genital atrium is completely everted. For instance Gosliner (1994) referred to the armature in *Jorunna* as a part of the vagina, referring to Ev. Marcus (1976) who described it as part of the vestibular gland. But according to Ev. Marcus' actual drawings of different *Jorunna* species (1976: figs 27, 31, 39) no clear assignment is possible.

Accessory glands can be present besides the opening of either the vagina or the oviduct (e.g. many Doridoidea). Such glands were usually considered as vaginal, but as with the problem of armature within the vagina mentioned above, we consider such conclusions to be premature. The accessory glands very often open into the common genital atrium, and therefore assignment to the vagina, the oviduct, or even sometimes to the vas deferens, is impossible without a knowledge of reproductive physiology.

Glands annexed to the female apertures ('vestibular glands') are present in several genera of the Doridoidea, but absent in all Pleurobranchoidea and Cladobranchia (Gosliner, 1994). The presence of such glands can apparently vary within one genus (e.g. *Dendrodoris, Discodoris, Aegires*). Gosliner assumed that the vestibular glands had arisen more than once, not only within the Doridoidea, but even within families (Chromodorididae). Glandular structures of this type are reported for the following taxa: *Ceratosoma, Gargamella immaculata, Thordisa, Discodoris indecora, Aegires sublaevis, Okenia impexa, Durvilledoris, Hypselodoris, Thorunna, Rostanga muscula, Paradoris, Phyllidiopsis berghi, Flabellina athadona* (Schmekel, 1970; Schmekel & Portmann, 1982; Rudman, 1984; Baba, 1987; Gosliner, 1994; Valdés & Ortea, 1996; Schrödl, 1997). A vestibular gland is absent in many species of *Aegires, Chromodoris, Doris, Archidoris, Rostanga* (except *R. muscula*), *Discodoris* and *Onchidoris*. So although the presence or absence of a vestibular gland varies within genera, we are inclined to consider the presence as

the apomorphic trait, but before using the character for phylogenetic analyses, the homologies need to be clarified histologically.

In nudibranchs, the vas deferens is a tube that conducts autosperm to the penis. Usually the sperm are surrounded by a greater or lesser volume of secretion which is produced in a glandular region, the prostate. In many opisthobranch taxa this prostate is a thickened, tubular organ with a secretory epithelium. Because this prostatic region is sometimes hardly any thicker than the non-secreting regions of the vas deferens, it is often said to be absent in anatomical descriptions. However, most significantly, our extensive histological survey has shown that a prostatic section is actually always present (pers. obs). Because the prostate gland is generally sausage-shaped, we consider that shape as the plesiomorphic state. In some genera, however, the prostate is subspherical or bulb-like (*Pleurobranchaea, Homoiodoris, Polycera, Melibe*). This shape is considered to represent the apomorphic state, but is not used here, because all genera included possess a tube-like prostate. Several authors have mentioned a branched prostate gland in *Plocamopherus* (Alder & Hancock, 1864; Eliot, 1906), a unique condition within the Nudibranchia, but typical of some Sacoglossa (e.g. *Elysia*).

Although a simple penis appears plesiomorphic, it often bears superficial ornamentation in both the Nudibranchia (e.g. *Marioniopsis* - Willan, 1988) and Pleurobranchoidea (i.e. *Euselenops* - Willan, 1987a), but such ornamentation is probably species-specific. One of the most bizarre elaborations occurs in *Pruvotfolia* where, despite a simple penis, three sets of specialized papillae surround the genital aperture in mature individuals. These papillae dilate at arousal and appear to assist sperm transfer (Tardy, 1970b).

In all members of the Nudibranchia plus the outgroups (except *Umbraculum*) the penis can be retracted after copulation and the number and arrangement of retractor muscles may have phylogenetic significance (Miller, pers. comm.). *Umbraculum* is unique in possessing a permanently protracted penis in a vertical cleft in the anterior midline of the voluminous foot (Willan, 1987a).

(37) In many nudibranch taxa, the penis is simple but armed with cuticularized hooks arranged in lines or rows (e.g. several species of *Dendrodoris, Phyllidia, Acanthodoris, Aegires, Baptodoris, Gargamella, Cadlina, Nembrotha, Platydoris*, many phanerobranch taxa - Kay & Young, 1969; Rudman, 1984; Wägele, 1987; Brunckhorst, 1993; Valdés *et al.*, 1996). Cuticularized hooks are also present in some Anaspidea, but they are absent in Tylodinoidea and Pleurobranchoidea. Therefore like Gosliner (1994), we consider the presence of spines as the apomorphic state, and the absence of such spines as the plesiomorphic state.

In some genera of the Aeolidoidea, the penis bears a single, prominent, apical, cuticular spine (e.g. *Eubranchus, Anetarca, Catriona, Glaucus, Godiva, Phestilla, Embletonia* - Burn, 1966; Edmunds & Kress, 1969; Miller, 1974; Willan, 1987b; Gosliner, 1991a). Gosliner (1994) postulated that such a spine could have evolved several times, because of the different morphology observed (hollow or solid). Only a detailed analysis of the ultrastructure of the spines in different taxa can show whether they are homologous or not.

Although not widespread, a few groups possess a bulb-like structure connected to the penial sheath and opening into the penis. This is the so-called 'penial gland' (e.g. *Berthellina, Tomthompsonia, Cuthona, Eubranchus, Cratena, Phestilla*, (some species of) *Embletonia* - Chambers, 1934; Marcus & Marcus, 1955; Burn, 1961; Millen &

Hadfield, 1986; Willan, 1987a; Wägele & Hain, 1991). At present it is difficult to reach any conclusions about either the function—which is supposedly glandular—or the homology of this structure. Penial 'bulbs' are also described for some gymnomorphs (*Onchidium*, Rathousiidae, Veronicellidae - see Tillier, 1984), but they certainly represent cases of convergent evolution with those of the opisthobranchs just mentioned. Nevertheless, such structures can be considered as apomorphies for the opisthobranch taxa in our analyses.

35. Triauly

Polarity: A diaulic genital system is considered plesiomorphic within the Nudibranchia, and a triaulic system is apomorphic. Weighting 2 was applied through successive analyses.
Coding: 0 - diaulic; 1 - triaulic.

36. Bursa copulatrix and receptaculum seminis

Polarity: The presence of both a bursa copulatrix and a receptaculum seminis is considered to be the plesiomorphic state. The absence of a bursa copulatrix, as well as the absence of a receptaculum seminis are both interpreted as separate apomorphic states. Only the former condition is coded here, because the latter represents an autapomorphy for the Bathydoridoidea.
Coding: 0 - bursa copulatrix and receptaculum seminis both present; 1 - bursa copulatrix absent.

37. Penial hooks

Polarity: The absence of penial hooks within the vas deferens is considered as the plesiomorphic state, and the presence of several hooks in lines or rows is the apomorphic state.
Coding: 0 - penial hooks absent; 1 - penial hooks present.

Morphology of sperm

Healy & Willan (1991a) investigated sperm ultrastructure in 27 species of Nudibranchia and found some indicators of relationships between and within families, especially in acrosomal and nuclear features. These authors concluded that there were not enough data available for a cladistic analysis. We consider sperm morphology to be a very valuable source of characters and expect that future research will contribute directly to knowledge of nudibranch phylogeny.

The ultrastructure of sperm has been described for six species of Pleurobranchoidea and one species of Tylodinoidea (Thompson, 1973; Healy & Willan, 1984, 1991b). Instances of periodic banding in the acrosomal pedestal (hitherto unreported in the Euthyneura) were recorded for the Pleurobranchoidea and two character states were shared. These authors found spermatological support for the separation of the Pleurobranchoidea and Tylodinoidea. The sperm of the single tylodinid investigated, *Umbraculum umbraculum*, is highly derived in possessing an extremely long nucleus which is coiled around the axoneme and the anterior portion of the mitochondrial derivative.

The only instances of the sperm being united into a spermatophore are those summarized by Tardy (1965).
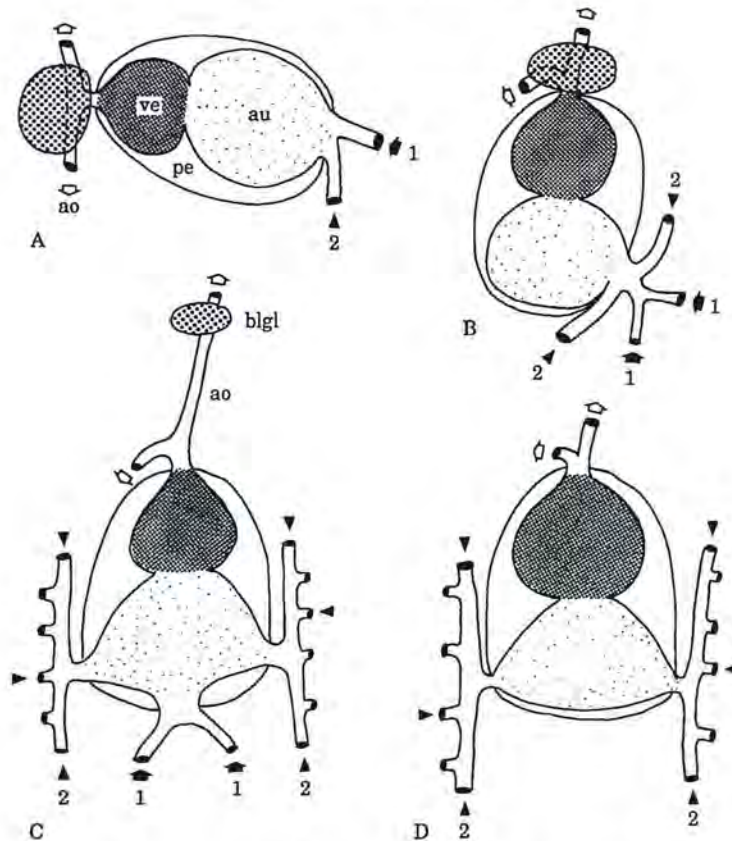
Figure 14. Schematic diagrams of the pericardial complex (partly redrawn after Wägele, 1998): A, Pleurobranchoidea; B, *Bathydoris*; C, Doridoidea; D, Cladobranchia. Abbreviations: ao - aorta; au - auricle; blgl - blood gland; pe - pericardium; ve - ventricle; 1 - oxygenated haemolymph coming from gills; 2 - oxygenated haemolymph coming from notum.

Circulatory system

38-39. Pericardial complex

(38) The orientation of the pericardial complex with the ventricle and auricle (atrium) aligned transversely across the longitudinal axis of the body is the plesiomorphic state, as can be seen in the Cephalaspidea, Anaspidea and Pleurobranchoidea (Fig. 14A). The auricle lies on the right side of the ventricle, and oxygenated blood enters the auricle on the right side. We consider this transverse orientation as the plesiomorphic state within the Opisthobranchia. All nudibranchs have the heart complex orientated longitudinally, with the auricle lying posteriorly to the ventricle (Fig. 14B-D). This state is not observed elsewhere in the Opisthobranchia, except for some Sacoglossa (Jensen, 1996). The longitudinal orientation of the pericardial complex is considered to be a synapomorphy of the nudibranch taxa.

(39) Within the Pleurobranchoidea, the pericardium usually lies in the central or

anterior part of the visceral cavity (*Berthella*, *Bathyberthella* - Lacaze-Duthiers, 1859; Wägele & Willan, 1994). The pericardium always lies in the anterior half of the body in the Dendronotoidea, Arminoidea and Aeolidoidea. We consider this anterior location as the plesiomorphic state. A location in the posterior part of the visceral cavity is only found within the Bathydoridoidea and Doridoidea. At first glance, some anthobranchs seem to have the pericardial complex in the anterior part, when one is assessing the overall length of the animal (e.g. *Kaloplocamus*, *Ceratosoma*). But one has to be aware that most of the 'body' in these species is actually the foot and not the notum with the visceral cavity beneath (see also character 3). The posterior location of the pericardium within the visceral cavity is considered to be an apomorphy.

Baba (1931) recorded the absence of a heart in *Vayssierea elegans*, but this was denied in subsequent investigations on this and other *Vayssierea* species (Baba, 1937; Ralph, 1944). However, Haszprunar & Künz (1996) definitely recorded the absence of a heart in *Rhodope*. The problem here is one of taxonomic placement; these authors assigned this enigmatic, microscopic 'slug' *Rhodope* to the Doridoidea, although there were no synapomorphies advanced to sustain this placement. If *Rhodope* really belonged to the Doridoidea, it would be the only nudibranch that definitely lacked a heart.

38. Orientation of pericardial complex

Polarity: The orientation of the pericardial complex across the longitudinal axis of the body (auricle on the right side of ventricle) is considered plesiomorphic, and the orientation along the longitudinal axis (auricle behind ventricle) is apomorphic.
Coding: 0 - orientation of pericardial complex transverse; 1 - orientation parallel to longitudinal axis.

39. Location of pericardial complex

Polarity: Location of the pericardial complex in the anterior or median part of body is considered the plesiomorphic state, and its shift to the posterior part is the apomorphic state.
Coding: 0 - pericardium anterior or median within body cavity; 1 - pericardium posterior within body cavity.

Auricle with connected sinus and vessels

Information about the circulatory systems of opisthobranch taxa is still very scarce. In those taxa with gills next to the anus one finds an efferent branchial vessel transporting oxygenated blood from the gills to the auricle and a lateral sinus transporting oxygenated blood from both sides of the body into the auricle (Fig. 14A).

According to Lacaze-Duthiers (1859), the genus *Berthella* also has a sinus coming from the notum which supplies blood into the auricle and lies next to the opening of the efferent branchial vessel. The presence of an efferent branchial vessel and a lateral sinus is considered to be the plesiomorphic state.

In *Bathydoris clavigera*, the lateral sinuses and the efferent branchial vessels share the same opening into the auricle (see Evans, 1914; García & García-Gómez, 1990b). This seems to be the primitive situation in the primary gill-bearing Nudibranchia (Fig. 14B). It somewhat resembles the situation described above for *Berthella* and a

similar configuration has been described for *Goniodoris castanea* (see Schmekel & Portmann, 1982; Jonas, 1985). In nearly all dorids, the auricle is arranged symmetrically on top of the viscera and the two lateral sinuses enter symmetrically, one on either side, whereas the efferent branchial vessel enters the posterior end in the middle of the auricle (see Jonas, 1985) (Fig. 14C). We consider the latter state with three openings in the auricle as apomorphic, and the state in *Bathydoris* with one opening as plesiomorphic. Alder & Hancock (1864) described four sinuses entering the auricle in *Bornella*. This observation has never been confirmed and additional investigations are needed before any phylogenetic conclusions can be drawn.

The separation of the efferent branchial vessel from the lateral sinus and its shift to the posterior middle part of the auricle is also a derived state, considering the more primitive state in *Bathydoris*. But this state is connected with the position of the gills, and is therefore not an independent character.

With the loss of the anal gills, the mediocaudal opening of the efferent branchial vessel (presence considered plesiomorphic) was also lost, and only the lateral orifices, providing oxygenated blood from the notum or notal appendages remained (Fig. 14D). This situation has been described for a few nudibranchs, e.g. *Phyllidia* (with secondary gills lying beneath the notum and foot, see Wägele, 1984), Tritoniidae (pers. obs.) and *Armina* (García & García-Gómez, 1990a). The absence of efferent branchial vessels is the apomorphic state, but because it is dependent on the loss of the anal gills, we prefer not to use it in our analysis.

The division of the single efferent branchial sinus into two sinuses in the Corambidae is an autapomorphy for this family and it can be explained by the gills' location within this family. According to Martynov (1995), the Corambidae originated from the Onchidorididae by paedomorphosis. The gills have come to lie ventrally and become polymerized in the more advanced corambids. Their distribution up to the medioventral area between the foot and the notum probably warranted better and shorter connections to the pericardial complex. Members of the Corambidae are not included in the phylogenetic analysis, therefore this character complex is not used here.

40–41. Blood gland

(40) A thin-walled organ (Fig. 15A) of unknown function is present dorsally in the anterior part of the visceral cavity in the Pleurobranchoidea (*Berthella*, *Bathyberthella*), Bathydoridoidea, and Doridoidea. Because of a location near the pericardium and because blood lacunae ramify through it, the organ is generally termed a 'blood gland'. Although carrying the same name, that part of the kidney called a 'blood gland' in the Trochidae (Fretter & Graham, 1962) is definitely not homologous with this opisthobranch organ. Wägele (1998) has described the structure of the opisthobranch blood gland histologically; the cells seem to be loosely connected by threads of connective tissue. Schmekel & Wechsler (1973) speculated that it might store or produce haemocyanin because they measured high concentrations of copper.

A blood gland is not known in any member of the Cephalaspidea, Anaspidea or Sacoglossa, and its absence has been confirmed by our histological investigations in a few members of these groups. Because this organ's structure is rather complicated, it is assumed that the blood gland could have evolved only once within the Opisthobranchia and its presence is probably a synapomorphy of the Pleurobranchoidea and Nudibranchia. The presence of a blood gland is the plesiomorphic
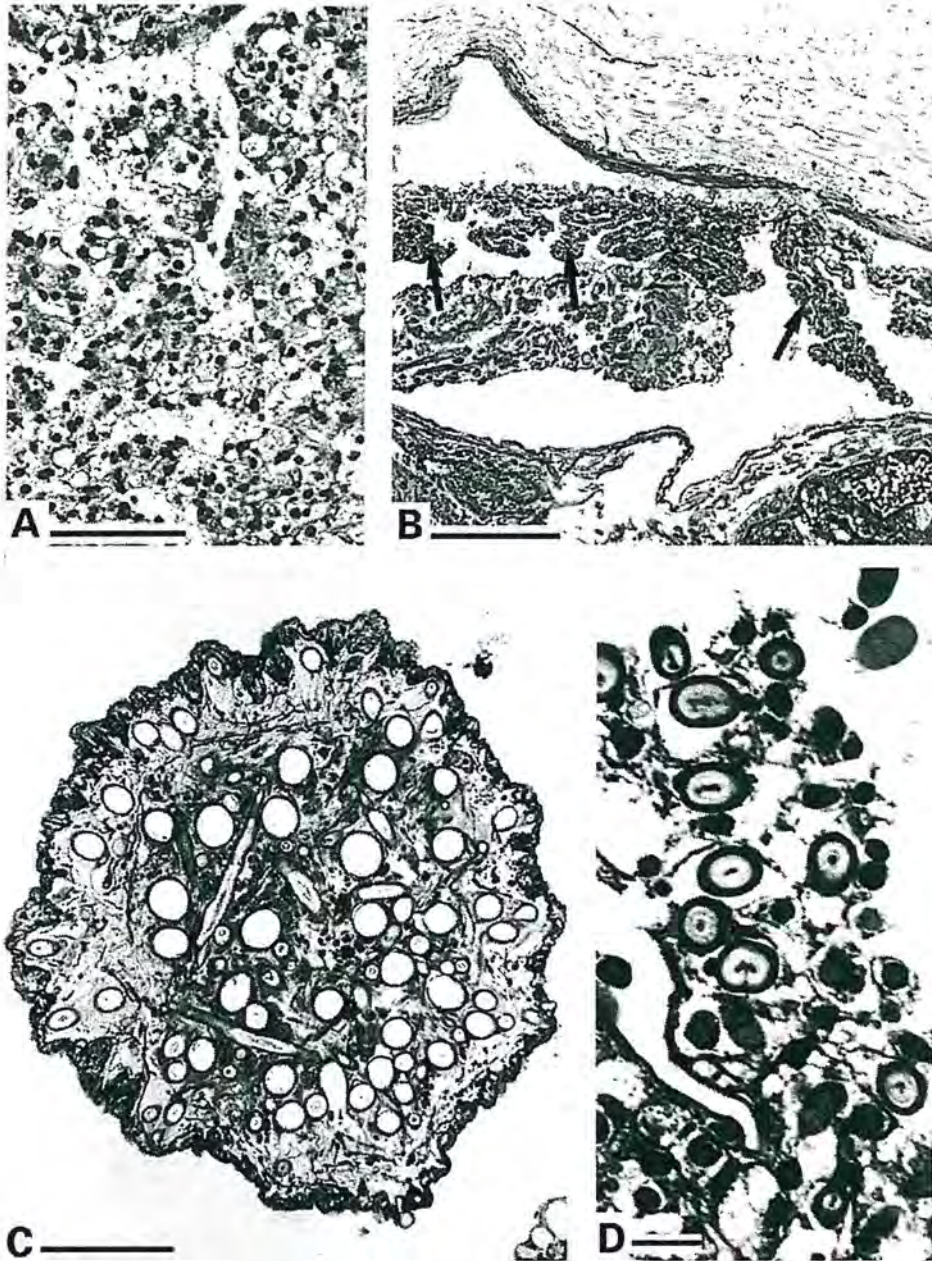
Figure 15. Histology of the circulatory system and notal structures: A, blood gland of *Discodoris atromaculata*; B, pericardial glands of *Dendrodoris nigra* (arrowed) above ventricle within pericardium; C, cross section of papilla of *Archidoris pseudoargus* with spicules; D, nodule cells of mantle rim organ of *Jorunna tomentosa*. Scale bars: A = 50 μm, B,C = 100 μm, D = 10 μm.

state within the Nudibanchia. The blood gland is absent (apomorphic state) in the Dendronotoidea, Arminoidea and Aeolidoidea.

(41) Wägele (1998) has investigated the position of the blood gland as a potential character for phylogenetic analysis. We consider the location of this organ adjacent to the heart as the plesiomophic state (Fig. 14A,B) (see also Minichev, 1970), because this same position occurs in the Pleurobranchoidea and in a few nudibranch taxa. Alternative positions—between the heart and the central nervous system, or on top of the central nervous system (Fig. 13C)—represent apomorphic states. The latter location is known for the cryptobranchs and some phanerobranchs (e.g. *Polycera*).

40. Blood gland
Polarity: The presence of a blood gland is the plesiomorphic state, and the absence is the apomorphic state.
Coding: 0 - blood gland present; 1 - blood gland absent.

41. Location of blood gland
Polarity: The position of the blood gland next to the heart is considered plesiomorphic. The alternative positions—between the heart and the nervous system, or on top of nervous system—are not coded separately because the distinction between these two configurations can be difficult to determine, particularly in poorly fixed specimens. The character is coded inapplicable in those forms without a blood gland.
Coding: 0 - blood gland adjacent to heart; 1 - blood gland located between heart and nervous system or on top of the latter; '—' not applicable.

Pericardial glands
In several taxa of the Anthobranchia, the dorsal pericardial wall forms folds that intrude into the lumen of the pericardium (Fig. 15B). These so-called 'pericardial glands' are usually located in front of the ventricle. Contrary to the term 'gland', which is generally applied in the literature (Grobben, 1891; Eliot, 1907; Hoffmann, 1939), these folds are not glandular (pers. obs.). In fact, no glandular cells have been present in any genus for which this character has been investigated (i.e. *Dendrodoris*, *Phyllidia*, *Limacia*, *Polycera*). Neither could we find these structures in *Hypselodoris tricolor* nor *Jorunna tomentosa* (but they are recorded for *Jorunna spazzola* - Ev. Marcus, 1976). More investigations are needed before this character can be used for phylogenetic analysis.

Further characters within the pericardial complex
Wägele (1989a) found glandular follicles within the dorsal wall of the auricle in *Bathydoris hodgsoni*, but they were absent in *B. clavigera*. These glands seem to be an autapomorphy for *B. hodgsoni*, but too little is known about the histology of the pericardial system to draw any final conclusions.

Excretory system

General outline
Only meagre information is available concerning the anatomy of the excretory system in the Nudibranchia. As far as can be deduced from the literature and our

own observations, the excretory system starts with the syrinx opening into the pericardium. This opening lies ventrally on the right side and usually consists of a pear- or tube-shaped organ with strong internal ciliated folds. The syrinx leads by a relatively short duct into an extensively folded or branched renal chamber. The length of the ureter leading from the chamber to the nephroproct differs intraspecifically. At the moment there are apparently no characters related to this general outline which can be used for phylogenetic analyses.

Glands annexed to the excretory system

Glands annexed to the ureter have been described in only two nudibranch species. Baba (1937) described a gland next to the nephroproct in *Vayssierea elegans*. A similar gland was described in *Bathydoris clavigera*, but it was apparently absent in other species of the same genus (Wägele, 1989a). Because these glands were only observed in these two species, their presence is considered as a trivial character and is not used here.

Glands and other structures in the notal tissue

Spicules within the notum

Rod-like spicules lying within the notum are known for the many taxa of the Doridoidea (Fig. 15C), where they are conspicuous and impart the characteristic rough texture like that of sandpaper to the notum. Spicules are absent in *Doridoxa*, *Bathydoris*, *Hexabranchus*, Corambidae (except *Corambe thompsoni* - see Millen & Nybakken, 1991), Vayssiereidae, *Miamira*, *Hallaxa* and many Chromodorididae, and they are also absent in nearly all Cladobranchia with just a few exceptions (*Pseudovermis mortoni*, *Armina formosa*, *Embletonia gracile* - Bergh, 1869; Risbec, 1928; Challis, 1969). At the moment, we lean toward the view that the presence of these spicules in Nudibranchia is plesiomorphic because they also occur in our chief outgroup, the Pleurobranchoidea, even though they are stellate in form. Their absence is considered to be apomorphic. But because the intrageneric variability is apparently high for this character (e.g. *Armina*, pers. obs.) and not enough data are known, the character is not used in our phylogenetic analysis.

A very precise arrangement of spicules (some emergent) within notal tubercles in some Dorididae (Fig. 3B), described above under the name of 'caryophyllidia', can potentially be used in phylogenetic analyses. But it is not used in our analysis because caryophyllidia only occur in *Jorunna*, and as such is a trivial character. Also the arrangement of spicules in the form of a network (see Gosliner, 1994) is an apomorphic trait which might be useful within lower level taxa, but more data are needed before it could be used here.

42–45. Glandular structures in the notal tissue

Glandular structures of various types are present within the notal tissue in several nudibranch taxa, usually being located near the notal edge. These glandular structures, described below, are not connected to the digestive gland (in contrast to the terminal sacs described above).

(42) Within the Arminidae, glandular sacs termed 'marginal glands' are located

near the notal margin. These glands (Fig. 16A) are often conspicuous because of their spherical shape and the fact that the contents are extruded when animals are preserved. Previously, they were considered as cnidosacs (e.g. MacFarland, 1966, but contradicted by Hoffmann, 1939). Histological investigations reveal them to be composed of large cells filled with acid mucopolysaccharides and that they open via a pore to the exterior (Kolb, pers. comm.). Because marginal glands with similar cellular structure have never been observed in any group of nudibranchs other than the Arminidae, Kolb & Wägele (1998) considered their presence as an autapomorphy for that family.

García-Gómez *et al.* (1990, 1991) and Avila & Durfort (1996) investigated another type of glandular structure that is present in the notal margin of members of the Chromodorididae and coined the term 'mantle dermal formations' (MDF) for them. MDFs are composed of large cells completely occupied by a single vacuole which does not stain homogeneously with toluidine blue and therefore does not contain acid mucopolysaccharides (Fig. 16B,C). MDFs are surrounded by a more or less thick layer of muscles. Gosliner & Johnson (1994) considered the presence of MDFs an autapomorphy of the Chromodorididae, but Wägele (1998) has recently described identical MDFs in the triophid *Limacia clavigera* (Fig. 16D). Because similar structures have not been observed in any other taxon of the Opisthobranchia, the presence of MDFs is considered to be apomorphic, and the absence plesiomorphic. In our phylogenetic analysis MDFs were present only in *Hypselodoris*, and therefore the character was not used in the phylogenetic analysis. The branched notal glands described in *Ardeadoris* by Rudman (1984) and some species of *Chromodoris*, *Miamira* and *Ceratosoma* (Gosliner, pers. comm.) probably present an apomorphic state as compared to the (plesiomorphic) spherical MDFs of other members of the Chromodorididae. Further investigations are needed here.

Foale & Willan (1987) described cellular aggregates near the notal margin of some Doridoidea (*Rostanga arbutus*, *Jorunna* sp.) (Fig. 15D) which they called 'mantle rim organs' (MRO). These structures are composed of cells with a translucent cytoplasm and they contain an electron-dense, rod-shaped structure. The presence of these structures in *Jorunna tomentosa* and their absence in other nudibranchs has been confirmed by Wägele (1998). The absence of MROs is considered to be a plesiomorphic feature and their presence an autapomorphy for *Jorunna* in this analysis (therefore not included).

(43) Wägele (1998) has discussed the distribution of states of the glandular stripe amongst various opisthobranch taxa. She has speculated about the possible homology of the glandular stripe with the gill glands in the Doridoidea where no such stripe is ever present. She could present transitional stages from the rather compact glandular stripe lying on the lateral side in the notum (as in *Flabellina falklandica*), to a displacement into the papillae (as in *Flabellina pedata*), to scattered single glands in *Flabellina affinis*. Because the primary position of the glandular tissue seems to be on the right side (*Aplysia*, *Lomanotus*, Charcotiidae, *Notaeolidia*, *Flabellina*) commencing behind the genital opening, all other positions can be considered as apomorphic (*Doto*, *Cuthona*, *Eubranchus*, see Wägele, 1998).

(44) Shifting of the glandular tissue into the lateral papillae (*Doto*, *Eubranchus*, *Cuthona*) is considered to be apomorphic and is coded separately from the further extension onto the left side. This is despite the fact that the presence of glandular follicles within the papillae is always correlated with the presence of the gland on both sides,
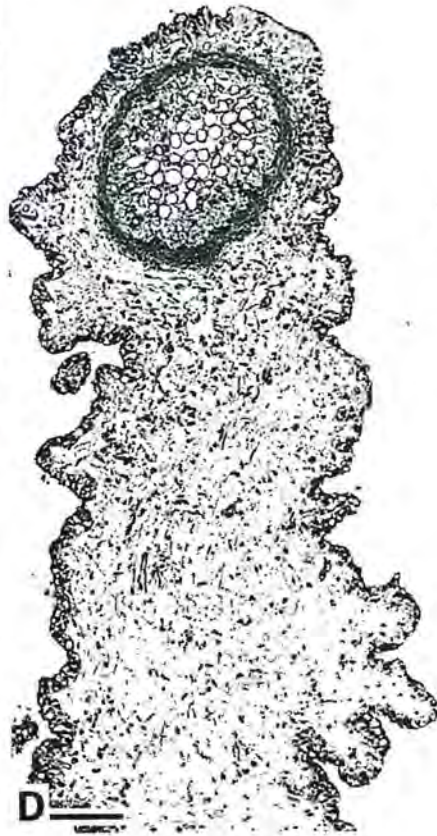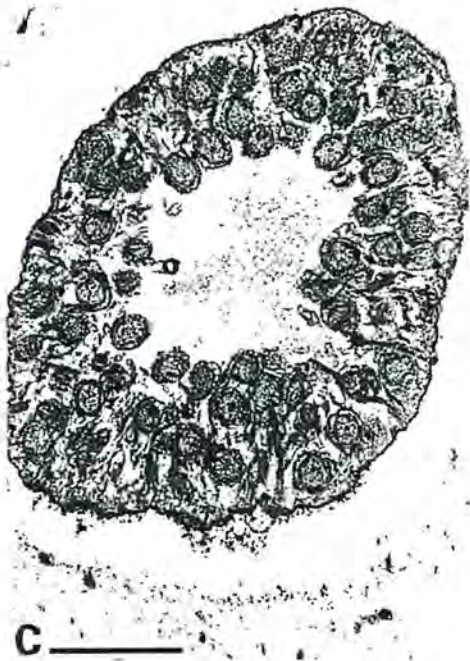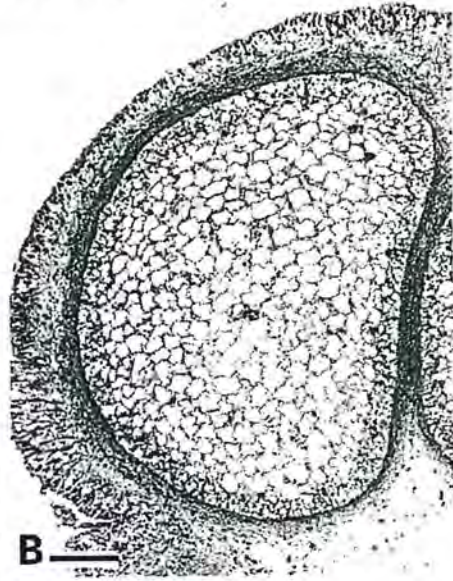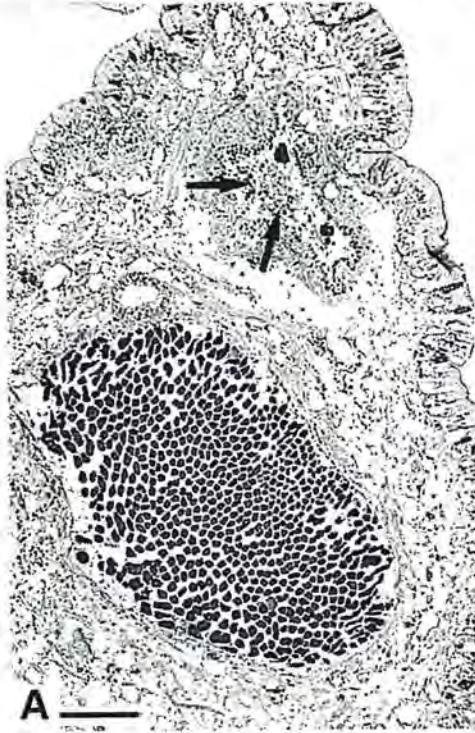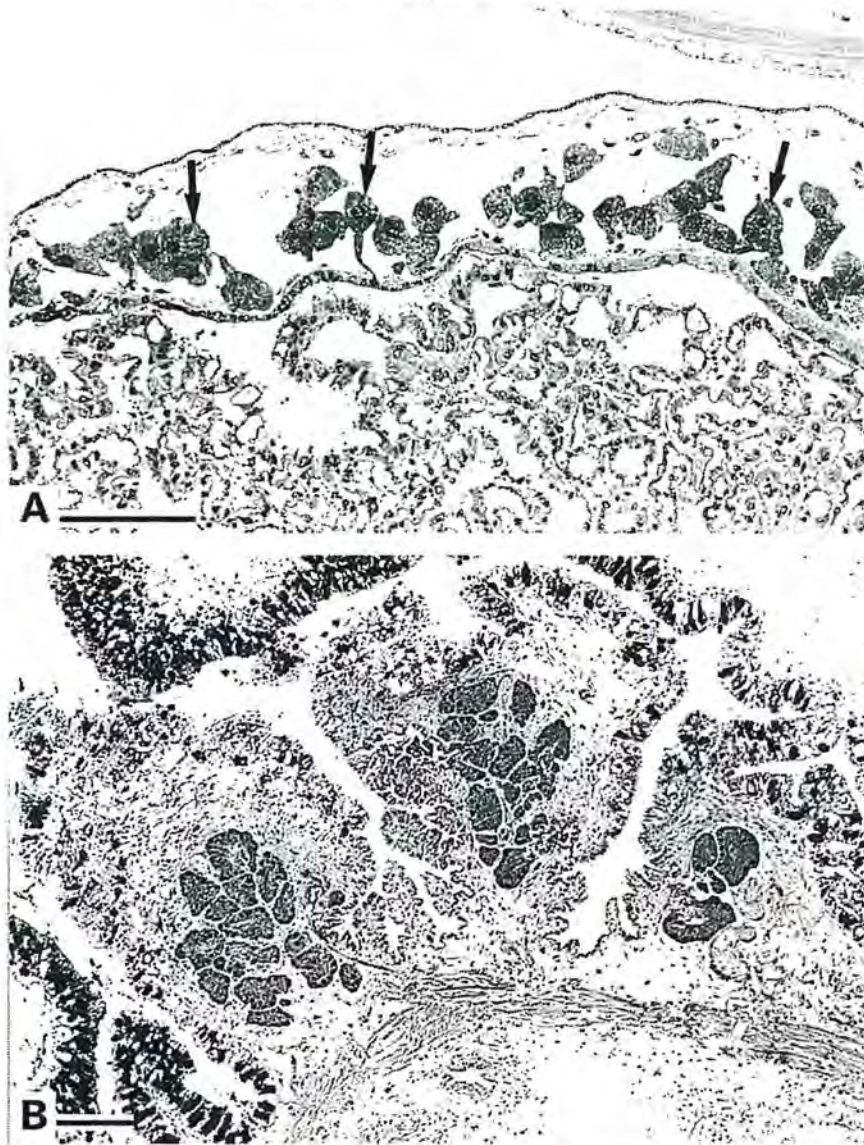
Figure 17. Glandular structures in the notum: A, glandular stripe (opaline gland) in *Aplysia punctata* (arrows indicate glandular follicles); B, gill glands in *Roboastra gracilis*. Scale bars: A,B = 100 µm.

Figure 16. Glandular structures in the notum: A, marginal gland of *Dermatobranchus ornatus*. Note reduced marginal gland with contents expelled (arrowed); B, mantle dermal formation of *Hypselodoris tricolor*; C, mantle dermal formation of *Cadlina laevis*; D, longitudinal section through papilla of *Limacia clavigera* with mantle dermal formation. Scale bars: A–D = 100 µm.

but not vice versa (for instance *Dirona* has a stripe on both sides in the lateral notum (MacFarland, 1912)).

(45) In all Doridoidea, similar-looking glandular follicles ('gill glands') are located between the bases of the gill raches (mainly Cryptobranchia) or within the gill rachis itself (Phanerobranchia) (Fig. 17B). Even considering the possibility that the gill glands in the Doridoidea could be homologous with the glandular stripe discussed above, the position close to the gills is unique and therefore available as a character in this analysis. This position close to the gills is here considered to be the apomorphic state. Even if the gill glands were not homologous with the glandular stripe, the presence of the glands could still be interpreted as an apomorphy. Therefore, regardless of our state of knowledge, we can use the presence or absence of gill glands as a character in our analysis.

(46) The presence of a glandular stripe (see Wägele, 1991; Wägele *et al.*, 1995) has now been confirmed for many representatives of the Cladobranchia, for *Aplysia punctata* (Wägele, 1998) (Fig. 17A) plus some other members of the Anaspidea, and for *Elysia viridis* (unpubl. data). Although the glandular stripe could not be found in the few members of the Pleurobranchoidea that we examined, its presence is still considered to be plesiomorphic and its absence apomorphic due to the situation in other members of the Opisthobranchia.

42. Marginal glands
Polarity: The absence of marginal glands is considered the plesiomorphic state, and the presence is the apomorphic state.
Coding: 0 - marginal glands absent; 1 - marginal glands present.

43. Glandular stripe on side of notum
Polarity: Glandular follicles restricted to the right side of the notum represents the plesiomorphic state, and follicles on both sides represents the apomorphic state. This character is coded as inapplicable in those genera where there is no glandular stripe.
Coding: 0 - glandular stripe present on right side only; 1 - glandular stripe present on both sides of notum; '–' not applicable.

44. Location of glandular stripe
Polarity: Glandular follicles restricted to the lateral notal tissue represents the plesiomorphic state, and the shifting of the follicles into the papillae is the apomorphic state. This character is coded as inapplicable in those genera where there is neither a stripe within the papillae nor any papillae at all.
Coding: 0 - glandular follicles present in lateral notum; 1 - glandular follicles within papillae; '–' not applicable.

45. Gill glands
Polarity: The absence of gill glands is considered to be the plesiomorphic state, and the presence of gill glands is the apomorphic state.
Coding: 0 - gill glands absent; 1 - gill glands present.

46. Presence or absence of glandular stripe

Polarity: Due to outgroup comparison the presence of a glandular stripe is considered to be the plesiomorphic state, the absence the apomorphic state.
Coding: 0 - glandular stripe present; 1 - glandular stripe absent.

47. *Specialized vacuolated epithelium*

(47) Henneguy (1925), and more recently Schmekel & Wechsler (1967), Storch & Welsch (1969) and Schmekel (1982), described a specialized type of cell within the epithelium of some nudibranchs which Schmekel & Wechsler (1967) called 'Vakuolenkörper'. These cells are elongate to cylindrical with a basal nucleus. The apical part of the cell has a reticulate appearance because of copious vacuoles. These vacuoles do not stain with toluidine blue, but a rod-shaped structure can be detected by ultrastructural methods within all of them. Schmekel (1982) suggested that these cells represented an apomorphy of the Nudibranchia because they were not present in pleurobranchs and that suggestion has now been confirmed by Wägele (1998) through investigation of representatives belonging to other opisthobranch taxa. This type of vacuolated epithelial cell is very distinctive and is not known in any other major group of the Gastropoda. Therefore higher weighting was applied through successive analyses.

47. Specialized vacuolated epithelium

Polarity: The absence of a specialized vacuolated epithelium is considered plesiomorphic, and the presence is apomorphic. Weighting 2 was applied through successive analyses.
Coding: 0 - specialized vacuolated epithelium absent; 1 - specialized vacuolated epithelium present.

Additional characters not elaborated here, but probably applicable for phylogenetic analyses

Allomones and pigmentation

Some nudibranchs are brightly coloured with vivid contrasting patterns. Although few rigorous field trials have been carried out, it is assumed these colours and patterns are either aposematic in function or involved in (Batesian and/or Müllerian) mimetic relationships (Gosliner & Behrens, 1990, reviewed by Edmunds, 1987, 1991), rather than simply being a means of disposing of chemical byproducts from the food or metabolism of the food. Undoubtedly some nudibranchs can, and do, incorporate toxic substances from their foods for their own protection (see Faulkner & Ghiselin, 1983, reviewed by Avila, 1995). Harris (1973) has demonstrated that many pigments are taken up with the food and incorporated into the notum or the digestive gland. Even more significantly, Cimino *et al.* (1983) have demonstrated that *Dendrodoris limbata* can actually synthesize new toxins ('allomones') from raw materials derived from its food. Whether there is any phylogenetic relationship between pigment (colours and patterns) or the use (or synthesis) of toxic chemicals and taxonomic groups is pure speculation at the moment.

Behaviour

According to Hennig (1966) and other phylogeneticists, behavioural characters could be used to supplement anatomical characters in phylogenetic analyses. Hickman (1996: 188–190) strove to do exactly this in formulating her data set for the

Trochoidea. Her rationale was that a structural basis has to exist for all the behaviours one observes in nature. Hickman also understood that it would require considerable effort to provide such structural proof for behaviour because the explanation might lie deep at the ultrastructural level. This approach is reasonable in terms of general behaviour (swimming, mantle movements, etc) of nudibranchs and it could certainly be coded. For example, Hickman coded the speed of burrowing. However, more than half the behavioural characters Hickman coded were trivial and this would definitely also be the case with nudibranchs.

Data on behavioural characters are actually rather rare for nudibranchs and they are widely scattered throughout the literature. We have not incorporated any behavioural characters in this phylogenetic analysis, but several are so conspicuous they could have great potential for future analyses: mantle flapping; 'waving' the tentacles on the head; motility of the gills; swimming; burrowing; autotomy of body parts; luminiscence (Bergh, 1875; Pace, 1901; Eliot, 1908; Thompson & Slinn, 1959; Farmer, 1970; Harris, 1973; Thompson, 1976; Rudman, 1984; Willan, 1984; Picton & Morrow, 1994).

Diet

Opisthobranchs feed on a great variety of food items. Nevertheless, their diets are broadly conservative (McDonald & Nybakken, 1978, 1991). The Sacoglossa generally feed on siphonaceous algae, by puncturing just one cell and then sucking out the contents (reviewed by Jensen, 1997). The Anaspidea also specialize in algae, but they graze whole portions of the thallus. Grazing as the method of food uptake is also represented in the Doridoidea, although their prey consists of Porifera, Bryozoa and Tunicata. Data pertaining to food items and feeding strategy might serve as an independent test for the results of our phylogenetic analyses, especially on subordinate levels. (Data on diet are available from Miller, 1967; Young, 1969; Edmunds, 1975; Nybakken & McDonald, 1981; McDonald, 1983; McDonald & Nybakken, 1991; and many others.)

It is obvious from the literature that grazing is the most widespread strategy for food uptake by opisthobranchs and so it is arguably the plesiomorphic state. However, because food uptake and food handling is undertaken by the radula and jaws, these characters cannot be considered as completely independent (Nybakken & McDonald, 1981). And neither can consequential modifications of the rest of the gut. Switches of diet inevitably link in with significant changes to the whole digestive system. For these reasons we do not agree with Hickman (1996) in extending behaviour to diet (Hickman, 1996, coded sponge and coelenterate feeding in the Trochoidea), or to including food and/or feeding strategy in this analysis.

Spawn and development

The structure of the spawn mass (Hurst, 1967) is not included in our phylogenetic analysis because there are insufficient comparative data on the fine structure of the egg clutches themselves. Neither are data available that conclusively demonstrate environmental factors (like habitat or diet) may not have a greater influence on the form of the spawn than phylogenetic affinity.

Neither are we including developmental characters in this analysis. Although many morphological data relating to larval development are available in the literature for nudibranchs (for example shell type - Thompson, 1961), a thorough study on

the relationship of ecological factors to such developmental differences does not exist. Furthermore, it is quite probable that larval and adult morphologies of opisthobranchs have not evolved in parallel. This belief stems from the fact that since larval and adult forms are adapted for very different life styles today, they must have been subjected to independent selection pressures during historical time.

Chromosome number

Chromosome numbers are now known from about 120 opisthobranchs and they have proven to be rather uniform. Cephalaspidea, Sacoglossa and Anaspidea usually have a haploid number of 17 (Ghiselin, 1966; Jensen, 1996; Mikkelsen, 1996). In contrast, nudibranchs usually have 13 (see Schmekel, 1985; Salvini-Plawen, 1990). Chromosome number has already been used to test the monophyly of opisthobranch groups (Schmekel, 1985; Haszprunar, 1985; Salvini-Plawen, 1990). However, Wägele & Stanjek (1996) have shown that the chromosome number of the Pleurobranchoidea cannot be used to support the monophyly of the Notaspidea, or even the Pleuro-branchoidea, because all four species that have been investigated so far belong to the Pleurobranchoidea and their chromosome numbers vary between 12 and 13. Nothing is known about the chromosome numbers of the Tylodinoidea.

Because nothing is known about the homology of the different chromosomes in those species with chromosome numbers outside those mentioned above (e.g. *Platydoris argo, Discodoris atromaculata, Tethys leporina, Phidiana lottini* - Vitturi *et al.*, 1985; Wägele & Stanjek, 1996; Godoy *et al.*, 1997), evaluation of chromosome numbers is problematic. Therefore characters relating to the chromosomes are not used in this analysis.

Molecular evidence

Until now only two phylogenetic analyses based solely on molecular data had been undertaken within the Opisthobranchia. Thollesson (1999) presented a phylogenetic analysis based on the mitochondrial gene 16S rDNA, which rendered the Nu-dibranchia monophyletic while using distance methods but paraphyletic while using a maximum parsimony method. *Berthella sideralis*, the only member of the Pleurobranchoidea used in the analysis consistently clustered within the Antho-branchia and not as the sister-group to the Nudibranchia. According to Tholleson's findings, the Anthobranchia and Doridoidea were both monophyletic, therefore supporting (some of) our conclusions presented below.

Wollscheid & Wägele (1999) compared the complete sequences of the nuclear gene 18S rDNA within 20 species of Nudibranchia against those of five opisthobranch taxa, one gymnomorph and one pulmonate as outgroups. Their analysis was performed with the help of distance, parsimony and statistical methods using the computer programs MEGA (Kumar *et al.*, 1993), SPLITSTREE (Huson, 1998) and PAUP. Their analysis confirmed the monophyly of the Nudibranchia, the Doridoidea, and the Cladobranchia. In addition, the monophyly of the Aeolidoidea was supported by a high phylogenetic signal.

PHYLOGENETIC ANALYSIS

In this analysis, the position of the Nudibranchia within the Opisthobranchia, and the monophyly of the Nudibranchia, Anthobranchia, Cladobranchia, and their
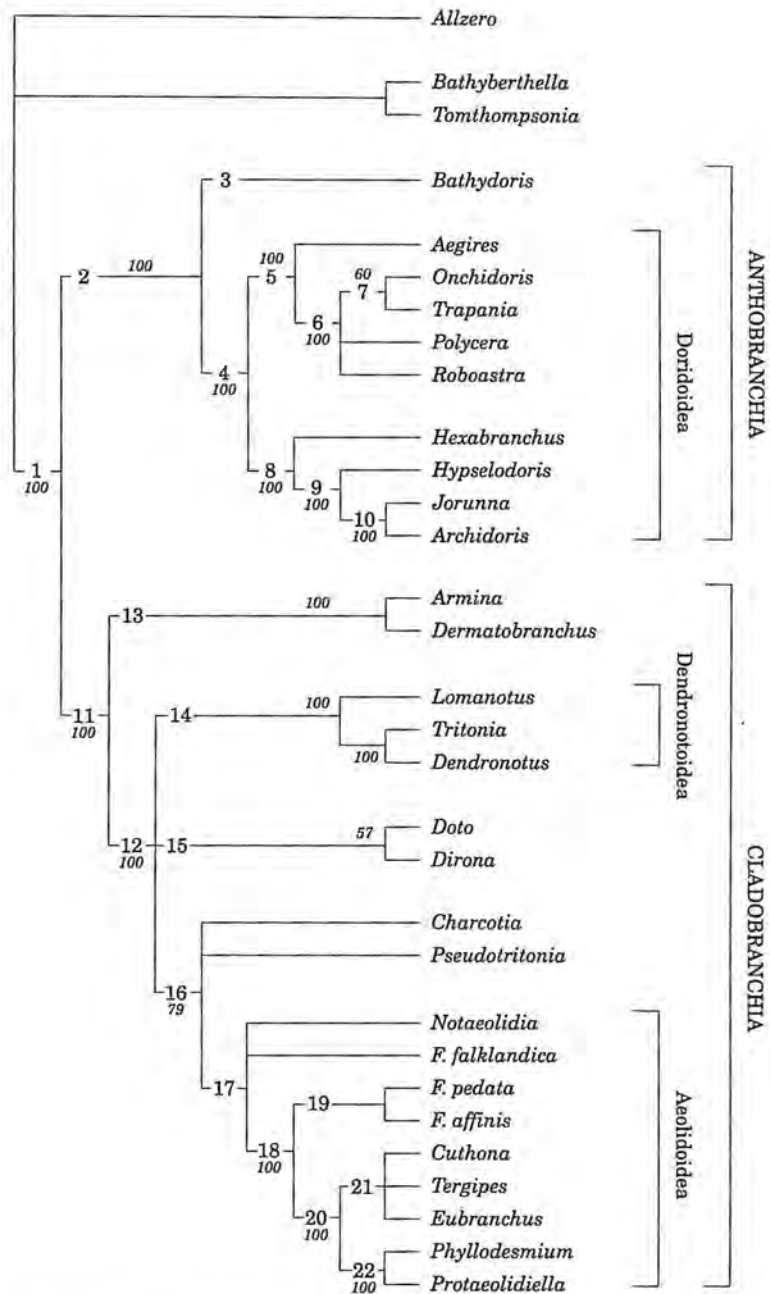
Figure 18. Computer created dendrogram (50% majority-rule consensus tree, CI = 0.583, HI = 0.417, RI = 0.850) of the phylogenetic relationship of the Nudibranchia performed with the data matrix in Table 4. Several characters weighted (Table 3) and character 30 ordered. Numbers in italics represent the percentage level of support. The other numbers represent the nodes. Numbers in parentheses in the legend refer to the synapomorphies in Tables 2 and 3. Character reversals are indicated by an asterisk: **1** Nudibranchia (11, 17, 20, 23*, 33, 34, 38, 47); **2** Anthobranchia (1, 9, 16, 29, 31, 39, 45);

constituent taxa are evaluated. Not all the characters that were discussed in the first part of this study could be used to assess these higher-level questions. Table 3 presents all the characters used for the computer analysis together with their deduced polarities, with the numbers in that table corresponding to the sections in the character analysis above. The resulting character matrix is presented in Table 4.

Results

The data set in Table 4 was analysed with the computer program PAUP as explained in Methods. PAUP has no option to ignore inapplicable characters in the data matrix but it uses them as if their state were unknown. Of course, inapplicability of any particular character can be due either to the transformation of that character into a second state or the character may not even exist in the plesiomorphic state as discussed. The unknown state was applied to all inapplicable characters, even if the character was transformed into another character state. This is an allowable procedure (Swofford, 1991) because the alternative state has been evaluated in another character.

Figure 18 presents a 50% majority-rule consensus tree (gaps coded as unknown state, ACCTRAN; branch swapping, TBR, characters 15, 16, 19, 30, 35, 42, 47 with weighting 2 applied; character 30 ordered) of the heuristic search option. With these options the length of the shortest trees are 115 steps and 420 trees of equally shortest length are retained. Consistency, homoplasy and retention indices of the trees are 0.583, 0.417 and 0.850 respectively. Figure 19 presents the strict consensus tree with the same settings as in Figure 18. There are some minor differences in the topologies regarding the arrangement of the higher groups within the Cladobranchia according to the different algorithms and options outlined in the Methods. When no weightings were applied and character 30 was treated as unordered (Fig. 20: 50% majority rule consensus tree, CI 0.571, HI 0.429, RI 0.846; 79 shortest trees with 98 steps), the Charcotiidae, containing *Charcotia* and *Pseudotritonia*, appear as the sister-group to the Dendronotoidea and Aeolidoidea. *Cuthona* appears as the sister-group to the combination *Tergipes* plus *Eubranchus*. *Doto* appears within the Aeolidoidea.

The application of the option Nearest-Neighbour Interchange (NNI, in combination with ACCTRAN as well as DELTRAN) results in a lower resolution of the cladobranch groups, with a polytomy between Dendronotoidea, Arminidae, and the (monophyletic) remainder of the cladobranch groups.

Bootstrap analyses were performed with the options mentioned in the Methods section. Figure 21 presents the 50% majority-rule consensus tree when the following options were applied: character 30 ordered and characters 15, 16, 19, 30, 35, 42 and 47 weighted with 2 (treated as repeated counts). The consistency index is 0.486, the retention index is 0.788 and the homoplasy index is 0.514. High bootstrap values (95 or above) are produced for the groups Nudibranchia (96), Anthobranchia

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3 Bathydoridoidea (21, 33*); 4 Doridoidea (12, 20*, 23, 27, 35, 41); 5 (37); 6 Phanerobranchia (3, 24, 26, 29); 7 (9, 14, 19, 31); 8 (14); 9 Cryptobranchia (15); 10 (3); 11 Cladobranchia (13, 27, 36, 40); 12 (2, 22); 13 Arminidae (18, 42, 46); 14 Dendronotoidea (8, 9, 12, 28, 34); 15 (21, 43); 16 (22, 30); 17 Aeolidoidea (5, 9, 30); 18 (4, 7); 19 (10, 18); 20 (21, 22, 34, 43, 44); 21 Tergipedidae (32); 22 (46).
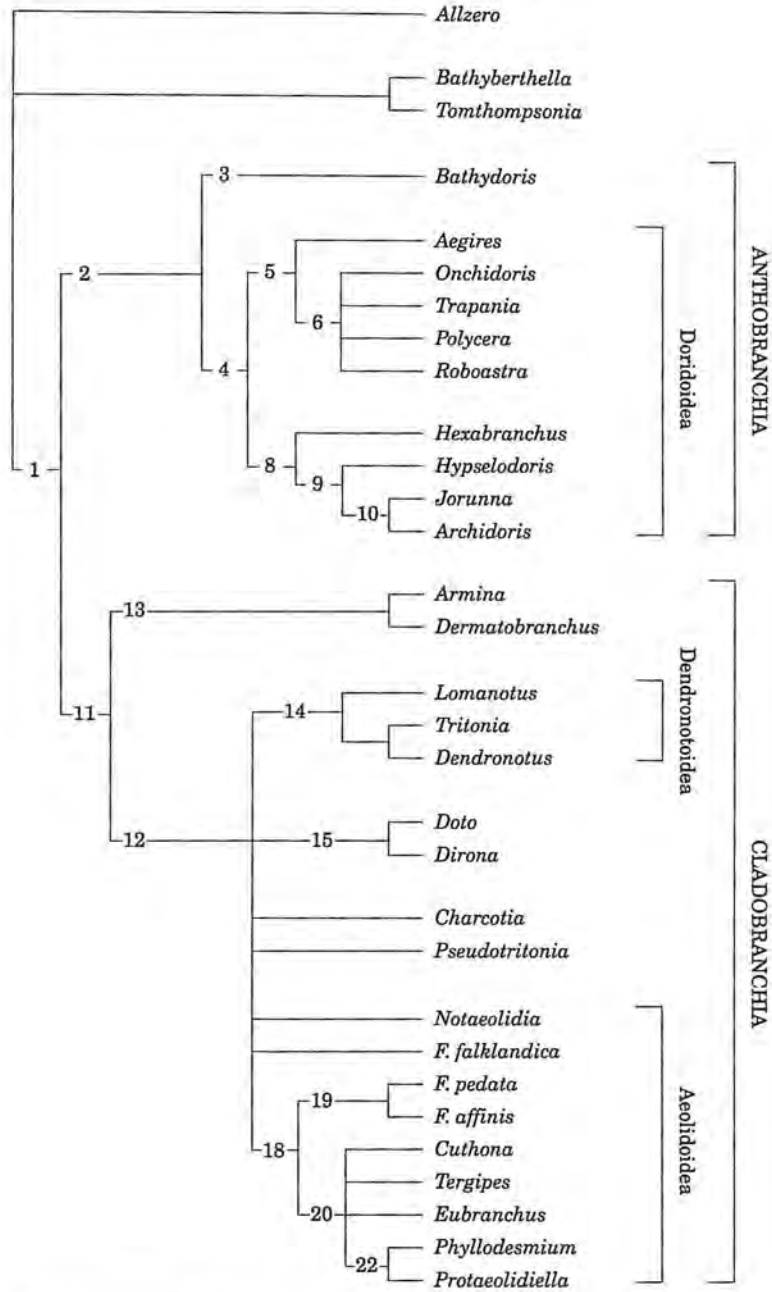
Figure 19. Computer-created dendrogram (strict consensus tree) with the same settings as in Figure 18. The numbers refer to the nodes. See legend of Figure 18 for characters.

Figure 20. Computer-created dendrogram (50% majority rule consensus tree, CI=0.571, HI=0.429, RI=0.846) with no weighting applied and character 30 unordered. Numbers in italics represent the percentage level of support.

Figure 21. Bootstrap analysis performed with the data matrix in Table 4 and same settings as in Figure 18 (50% majority-rule consensus tree). The numbers indicate bootstrap values.

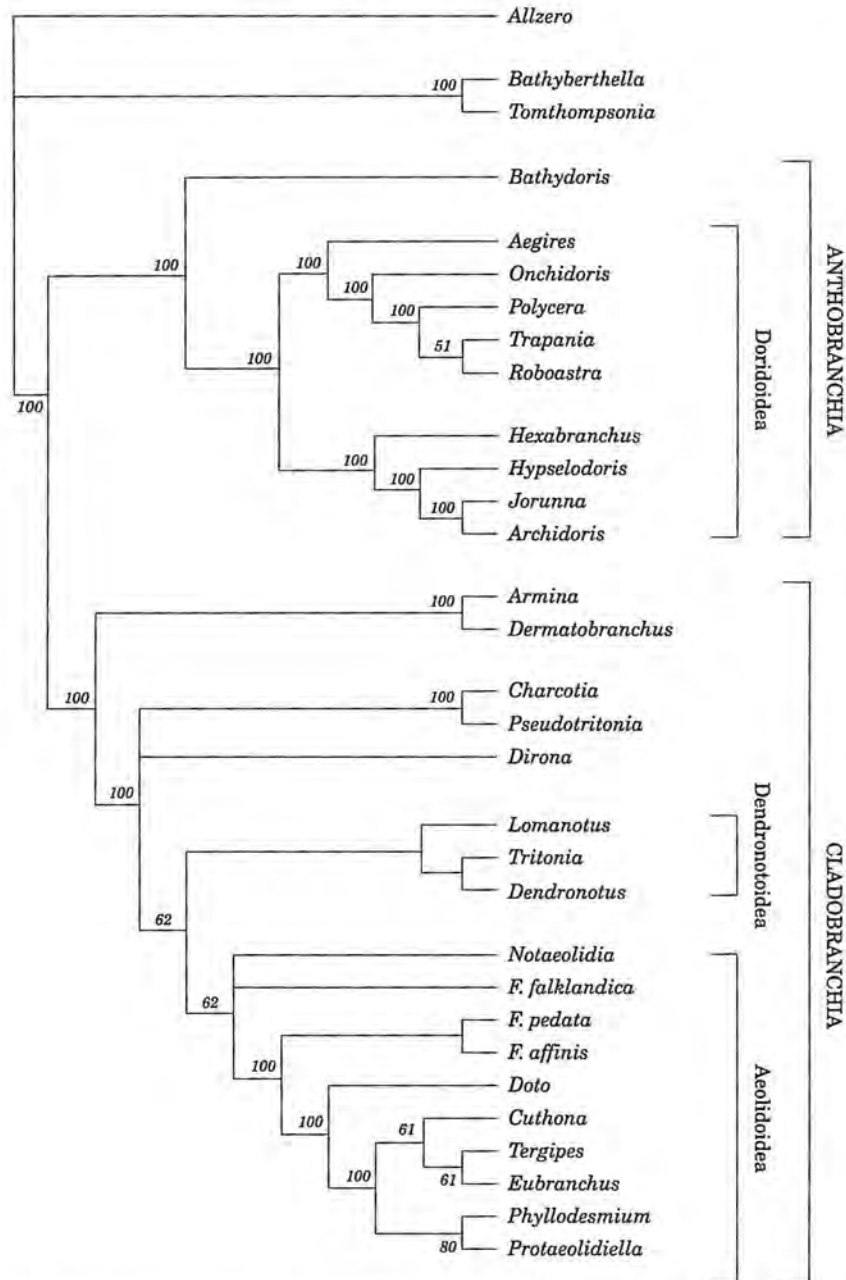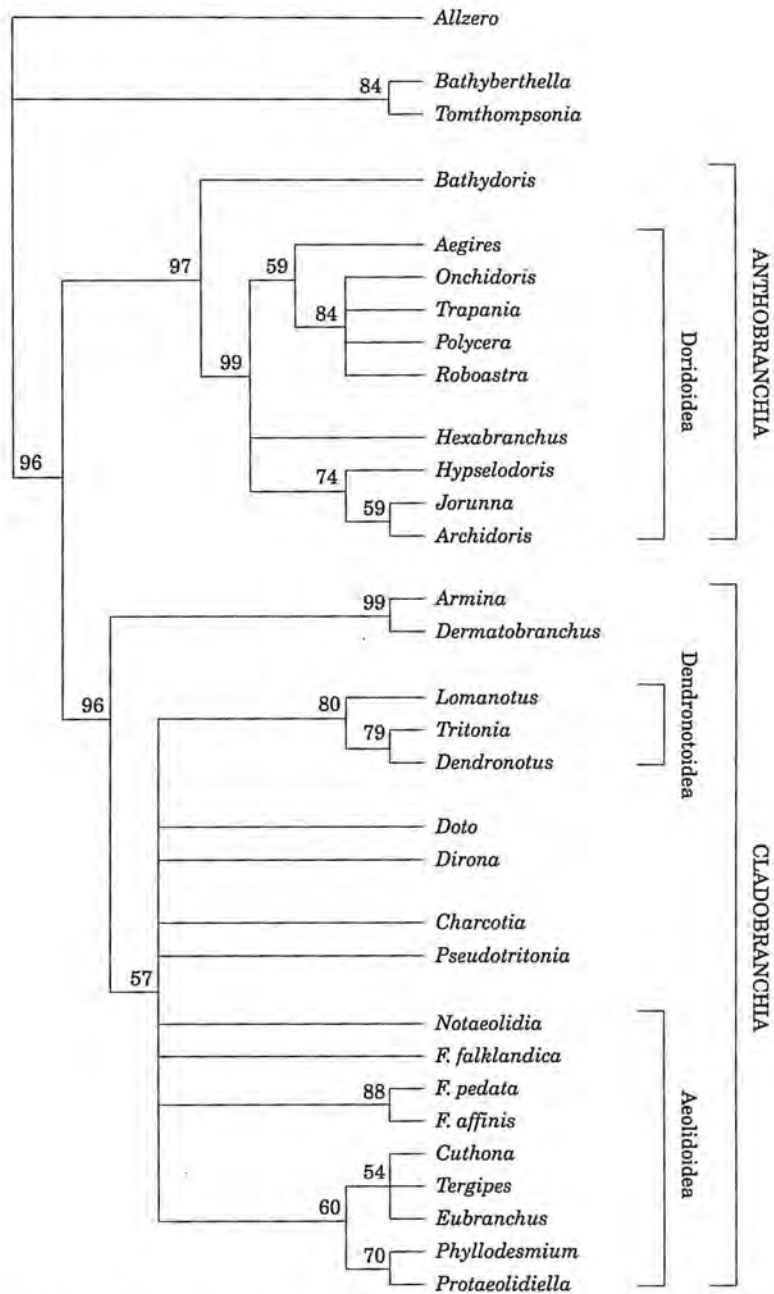(97), Doridoidea (99), Cladobranchia (96), and Arminidae (99). These results support those already obtained with the heuristic search algorithms of PAUP. But contrary to the heuristic search options, the bootstrap analysis does not reveal the monophyly of the Aeolidoidea. Resolution within the Cladobranchia is particularly low. Lower bootstrap values (between 70 and 90) are produced for the Pleurobranchoidea (84), Cryptobranchia (minus *Hexabranchus*) (74), Phanerobranchia (84), Dendronotoidea (80), the combination *Tritonia* plus *Dendronotus* (79), the combination *Flabellina pedata* plus *F. affinis* (88), and the combination *Phyllodesmium* plus *Protaeolidiella* (70).

The Bathydoridoidea and the Doridoidea always cluster as sister-groups. The Cryptobranchia and the Phanerobranchia seem to be monophyletic groups. Whereas the former is characterized by a strong synapomorphy (retractility of gills into a sub-notal pocket), no equally strong synapomorphy could be advanced for the Phanerobranchia. The Dendronotoidea (minus *Doto*) always turns out to be monophyletic. *Doto* itself is consistently excluded from the Dendronotoidea in all the analyses. The Aeolidoidea is monophyletic, as is the Tergipedidae. This latter taxon is characterized chiefly by the presence of a pair of oral glands with separate ducts. *Eubranchus* clusters as the sister-group to the Tergipedidae due to the presence of only a single otolith in the statocyst. In almost all our analyses *Flabellina* turns out to be paraphyletic. The various 'arminoid' groups (i.e. Arminidae, Charcotiidae, *Dirona*) never cluster on one branch, clearly demonstrating paraphyly.

Discussion

In this analysis the presence of penial spines and the absence of a caecum unite the phanerobranch groups. But penial spines also occur in other taxa not considered here (see character 31 in analysis above). According to the analysis, *Onchidoris* shows two character reversals (typhlosole present, male and female gametes produced in the same follicle) and the apomorphic traits observed within the Cryptobranchia (notum larger than foot, notal margin free from foot). This might be due to the lack of information on characters and/or the exclusion of important taxa.

Although our analyses revealed the monophyly of the Dendronotoidea, they consistently excluded *Doto* from that group. This is despite the fact that all members of that genus do possess rhinophoral sheaths, a character state unique to the Dendronotoidea, within the Cladobranchia. So this character state could perhaps have evolved twice in parallel (as with the case of convergence in those cryptobranchs that also possess rhinophoral sheaths). *Doto* also lacks the processes of the oral veil, the tentacular grooves, and the cuticle lining the stomach that are present in all the rest of the Dendronotoidea. The fact that this genus is clustered with *Dirona* (characters 21 and 43) is due to the lack of other taxa which might be more closely related (e.g. *Embletonia*).

In almost all our analyses *Flabellina* turned out to be paraphyletic. *Flabellina* is a very large genus and its members are very heterogeneous compared to those of other aeolidoidean genera. Gosliner & Kuzirian (1990) and Gosliner & Willan (1991) presented phylogenetic analyses of the genus using more than 30 species and they were able to recognize monophyletic subgroups, but they did not include other taxa of the Aeolidoidea. Many species of *Flabellina* possess plesiomorphic characters compared to the rest of the Aeolidoidea (i.e. papillae in rows along the notal edge, pleuroproctic position of the anus, glandular stripe confined to the right side and

lying laterally in the notum, e.g. *F. falklandica*) and other species are very derived by possessing apomorphic traits (flattened motile oral tentacles, oral glands, papillae on peduncles, rachis with retracted main cusp, e.g. *F. affinis*). We believe that several aeolid families could have evolved from the flabellinid stem, but only a thorough analysis of the different aeolid families in combination with the different *Flabellina* species will clarify this complicated situation.

The sister-group relationship of *Phyllodesmium* and *Protaeolidiella* is only based on the joint absence of the glandular stripe, so consideration of further aeolidoidean taxa may reveal synapomorphies which support stronger relationships with other genera within the Aeolidoidea.

When we evaluated the character polarities applied by the computer program, the following incongruities in our character analysis became apparent. Such anomalies may have affected tree length but they would not have affected the branching patterns. Following the apomorphic list for the heuristic search, the program assumes that the gonad of the Nudibranchia enveloping the digestive gland and the position in front of the digestive gland is a reversal and therefore an apomorphic state in *Bathydoris*. However, this could simply be explained by the fact that *Bathydoris* is the only genus with the gonad in this configuration in this analysis. We assume that the enlargement of the gonad to spread over the digestive gland occurred twice independently within the Nudibranchia, once within the Doridoidea and once within the Cladobranchia. The same logic probably applies to the character states 'aliform jaws' and 'loss of the rachidian tooth'; the computer program interpreted both states as synapomorphies for all the Nudibranchia.

In conclusion, in spite of applying very different assumptions for the characters (weighting, coding inapplicable characters) as well as different search algorithms, there are no major differences in the results of the phylograms. Therefore the phylogenetic reconstruction, as presented in the Figures 18 and 19, is most likely to be the correct one, especially for the higher taxa.

The major clades and their autapomorphies

The sister-group relationship of the Pleurobranchoidea and Nudibranchia

The Pleurobranchoidea has traditionally been assigned to the Notaspidea, comprising the Pleurobranchidae plus Tylodinidae (Odhner, 1939; Ev. Marcus, 1984, 1985; Willan, 1987a). Not only the flattened shell, but more particularly the scroll-like form of rhinophores, the presence of a ctenidium on the right side of the body and its precisely identical morphology, are characters which were advanced to unite both taxa into the one group (Willan, 1983a, 1987a). Schmekel (1985) was first to realize that the pleurobranchs shared some derived characters with the nudibranchs that were absent in other groups of opisthobranchs such as the androdiaulic genital system and the lack of an albumen gland. The latter is difficult to evaluate because we know so little about the homology of the different glandular parts of the nidamental glands within the Opisthobranchia.

Salvini-Plawen (1990, 1991) added four more characters that supposedly reinforced this relationship between pleurobranchs and nudibranchs—loss of the gizzard, loss of the osphradium, location of the blood gland close to the heart, and a haploid chromosome number of 12. Because a gizzard is also present in *Bathydoris*, we consider this character as invalid for use at this level. An osphradium is present in

Tylodina, Anaspidea and Cephalaspidea (MacFarland, 1966; Mikkelsen, 1996), but is not described for any pleurobranch or nudibranch. Therefore, its absence (through loss) represents a synapomorphy of the Pleurobranchoidea and Nudibranchia. There are no other records of blood glands outside the Pleurobranchoidea and Nudibranchia (Wägele, 1998), so this gland does indeed represent a novel character evolving in the common line of both taxa. Its original position was next to the heart. Therefore, that position, applied in the sense of Salvini-Plawen (1990), is not a valid character, but becomes a valuable feature within the Doridoidea. According to the literature relating to chromosome numbers in Opisthobranchia (see Burch, 1965; Wägele & Stanjek, 1996), there has been a reduction from the primary number of 17 haploid chromosomes to 12 or 13. But we know absolutely nothing about the homology of these chromosomes, so the character is not applicable. And besides, there are no data for chromosome numbers within the Tylodinoidea.

Nudipleura new taxon

Therefore, on the strength of the following synapomorphies we recognize and formally name a new monophyletic higher group encompassing the Pleurobranchoidea plus Nudibranchia which we call Nudipleura new taxon. The characters defining this group are the possession of a blood gland, androdiaulic reproductive system, and absence (through loss) of the osphradium. The Nudipleura consists of two monophyletic sister- groups: the Pleurobranchoidea and the Nudibranchia.

The monophyly of the Nudibranchia

The monophyly of the Nudibranchia has been questioned, overtly or tacitly, by several malacologists (see Introduction), most recently by Minichev (1970), who envisaged the origin of the Anthobranchia within the Cephalaspidea and the Cladobranchia within the Pleurobranchoidea stem. Schmekel (1985) and Salvini-Plawen (1990) discussed two characters in this context; the absence (through loss) of the shell (although this character has occurred many times in parallel within the higher gastropods (e.g. Pulmonata, Sacoglossa)), and the position of the visceral ganglion on the right side. The latter character is considered to be weak because migration of the visceral ganglion towards the right side can be also observed in several Pleurobranchoidea (Hoffmann, 1939). The presence of a specialized vacuolated epithelium is unique within the gastropods and therefore a very good additional autapomorphy for the Nudibranchia. Another unique feature is the longitudinal orientation of the pericardial complex. A similar arrangement seems to have evolved independently in some clades of the Sacoglossa. Schmekel (1985) included the shape of the rhinophores, which are not enrolled as in the Anaspidea, Sacoglossa, Tylodinoidea and Pleurobranchoidea. In the Nudibranchia the rhinophores are solid digitiform structures. Salvini-Plawen (1990) mentioned the smaller size of the right digestive gland in the Nudibranchia, but virtually no comparative data exists relating to the shape of the digestive gland in the Pleurobranchoidea. According to Wägele & Hain (1991), the right digestive gland of *Tomthompsonia antarctica* is somewhat larger than the left. In *Bathyberthella antarctica*, the digestive gland is a single compact mass and differentiation of the right and left portions is impossible. Considering our present knowledge, this character might be another synapomorphy, but further investigations of other opisthobranchs are needed.

In summary, we recognize the following autapomorphies for the Nudibranchia: (1) rhinophores solid (character 11); (2) absence (through loss) of the shell (character 17); (3) pericardial complex orientated longitudinally (character 31); (4) presence of specialized vacuolated epithelium (character 47).

The monophyly of the Anthobranchia

The monophyly of the Anthobranchia has never been in any contention. Nevertheless, some of the characters which have been used to support this hypothesis are not valid in a rigorous phylogenetic context. Amongst others, Schmekel (1985) mentioned the absence of jaws, presence of a blood gland (synapomorphy of the Nudibranchia and Pleurobranchoidea, see above), and specialized vacuolated epithelium in the rhinophores. The latter is problematic because we do not know anything about the distribution of the epithelial types in the ancestor of the nudibranchs. Histological investigations of embryos of *Bathydoris* (pers. obs.) have revealed the presence of specialized vacuolated cells in the notal epithelium.

Salvini-Plawen (1990) noted the significance of branchial symmetry. Whilst this is indeed true for all the Doridoidea, it is not correct for all species within the Bathydoridoidea. For instance, *Bathydoris clavigera* usually possesses an asymmetrical branchial arrangement, with one gill in front of the anus and the other on the right side of it. But we agree with others (Schmekel, 1985; Salvini-Plawen, 1990) that the medio-dorsal position of the gills and the anus is an autapomorphy for the group.

Wägele (1989b) considered that the presence of a caecum was a synapomorphy of the Anthobranchia. This was contested by Salvini-Plawen (1990), who argued that a caecum was also present in *Philinoglossa* and *Marionia*. However, as we have explained in the relevant section above, a caecum which is only lined by ciliated epithelium (not by digestive glandular epithelium) is present in the Anthobranchia. Therefore, this caecum (not merely the digestive gland reduced in size) is still considered to be an autapomorphy of the Anthobranchia. A new character is the elongation of the anterior notum, which extends during ontogeny to enclose the rhinophores and ultimately overgrows the head. This leads to a reduction of the oral veil to merely a pair of small oral tentacles. Within the Cladobranchia there are several groups which still have a free head, so that the rhinophores stand in front of the notum (Arminidae) or the notum starts laterally, but the oral veil is still free (Charcotiidae, *Dirona*, etc.). Another character typical of the Anthobranchia is the shifting of the pericardial complex to the posterior third of the body cavity.

In summary, we recognize the following autapomorphies for the Anthobranchia: (1) notum overgrowing head and enclosing rhinophores during ontogeny (character 1); (2) postero-median site of anus, nephroproct and anal gills (character 16); (3) presence of a caecum lined with ciliated epithelium (character 29).

The monophyly of the Bathydoridoidea

Wägele (1989b) excluded the monotypic genus *Bathydoris* from the Doridoidea on cladistic grounds. She recognized the following characters for the Bathydoridoidea: club-shaped papillae and absence (through loss) of the bursa copulatrix. These characters are still valid, although the former is weak, because club-shaped papillae are also present in *Ceratophyllidia* (Phyllidiidae) (and also convergently in *Galeojanolus* (Zephyrinidae)). An additional character is the presence of compact jaws. The

Cladobranchia also have compact jaws, but they still possess a masticatory border (and/or process) composed of singular elements which are homologous with the individual elements in the jaws of the Cephalaspidea, Pleurobranchoidea and Doridoidea. The development of compact aliform jaws must have occurred independently at least twice within the Nudibranchia.

In summary, we recognize the following autapomorphies for the Bathydoridoidea: (1) aliform jaws without any denticles (character 21); (2) club-shaped papillae that autotomize very easily (trivial character); (3) receptaculum seminis absent (trivial character).

The monophyly of the Doridoidea

Previously several characters and character states were advocated in support of doridoidean monophyly and several of these remain valid in the light of our phylogenetic analysis (e.g. the triaulic genital system - Schmekel, 1985; Wägele 1989b). Wägele (1989b) included the 'absence of jaws' as one such character state, but that is no longer valid. The enlargement of the gonad by overgrowing the digestive gland also occurs in the Cladobranchia. Besides, we still lack information about the position of the gonad in some basal dorids. Two new characters recognized here that support the monophyly of the Doridoidea are the complete loss of the cuticular lining in the oesophagus and the presence of gill glands. The former is paralleled in the Cladobranchia, but there one usually observes a thin cuticle at least in the most proximal section of the oesophagus. The glands at the base of the anal gills are apparently without parallel in the Opisthobranchia.

In summary, we recognize the following autapomorphies for the Doridoidea: (1) oesophagus without any cuticular lining (character 27); (2) triaulic reproductive system (character 35); (3) blood gland situated next to genital system or on top of cerebro-pleural complex (character 41); (4) presence of gill glands (character 45).

The monophyly of the Cladobranchia

Besides the characters used here to support the hypothesis of the monophyly for the Cladobranchia, Schmekel (1985) also mentioned the position of the anus on the right side and the presence of vacuolated epithelium in the digestive system. The former represents a plesiomorphy within the Nudibranchia, as evidenced by outgroup comparison, and the latter involves the same inherent difficulties as already discussed for the Anthobranchia. We do not know whether all ectodermal epithelia primarily had vacuolated epithelium, or whether the presence in the digestive system came about secondarily.

Wägele (1989b) and Salvini-Plawen (1990) mentioned the fusion of the cerebral and pleural ganglia in the context of phylogenetic discussions about the Cladobranchia. Within the Nudibranchia, only a few species have separate cerebral and pleural ganglia (e.g. Bathydoridoidea). Therefore this fusion has occurred at least twice within the Nudibranchia (in both the Doridoidea and in the Cladobranchia).

Salvini-Plawen (1990: 26) considered another character relating to the (clado-branch) state of the digestive gland within the Cladobranchia ("left midgut gland subdivided ('Cladobranchia')"). The 'cladobranch' situation applies also to the right digestive gland when the digestive gland is branched. But there are several Cladobranchia which still have a compact digestive gland and the right and left lobes have only one opening each into the stomach (Tritoniidae).

In summary, we recognize the following autapomorphies for the Cladobranchia: (1) absence (through loss) of the primary gills (ctenidium) (character 13); (2) aliform jaws (character 20); (3) absence (through loss) of the bursa copulatrix (character 36); (4) absence (through loss) of the blood gland (character 40). We hasten to add, however, that not one of these character states is unique to the Cladobranchia. Three of the four synapomorphies represent losses and aliform jaws have also evolved convergently in the Bathydoridoidea.

The monophyly of the Dendronotoidea

No phylogenetic analysis or assessment of characters exists for the Dendronotoidea. Odhner (1934) advocated the rhinophoral sheath as the principal character by which the group could be characterized. In our analysis another character has turned out to be equally significant in delimiting this group, that is the possession of a cuticle lining the stomach. We cannot deny that in some genera this cuticle could be secondarily lost and indeed the trend for reduction has been clearly set in place in the Tritoniidae.

An additional character supposedly unique to the Dendronotoidea is the presence of tentacular extensions on the oral veil, but this is debatable because they are absent in the Dotidae. Actually the inclusion of the Dotidae in this group is still very much open to debate (see computer analysis). Interestingly, Healy & Willan (1991a) identified such wide variation in sperm morphology within the Dendronotoidea that they questioned its monophyly, and even then they did not examine the sperm of *Doto*.

In summary, we recognize the following autapomorphies for the Dendronotoidea: (1) presence of tentacular extensions on the oral veil (character 9); (2) presence of a rhinophoral sheath (character 12); (3) possession of a cuticle lining the stomach (character 28).

The monophyly of the Aeolidoidea

The Aeolidoidea is recognized by the synapomorpies 'presence of a cnidosac' (character 30) (though this structure is secondarily lost in *Phyllodesmium*) and elaboration of the oral veil into oral tentacles (character 9).

The paraphyly of the Arminoidea

Several families of nudibranchs have been united under the name Arminoidea for over sixty years because most species possessed an oral veil or oral tentacles derived from such a veil (Odhner, 1934). Our phylogenetic analysis has revealed that this group represents an amalgam of heterogeneous families. No synapomorphy is known which unites all the families and therefore the status of each needs to be reinvestigated before the relationships of the families to the other two (monophyletic) groups of the Cladobranchia can be understood. This undertaking should encompass all the present genera within the 'Arminoidea' plus other 'problematic' cladobranch genera like *Embletonia*, *Hancockia*, *Hero* and *Doto*.

We believe the Arminidae could represent the most basal group within the Cladobranchia because it possesses several plesiomorphic character states (e.g. head and foot completely separate from notum). Furthermore, this family has a notopodial spur which is similar in structure and position with that occurring in sexually mature

specimens of (some species of) the Pleurobranchoidea. Therefore, homology is quite evident and this spur probably represents a symplesiomorphy within the Nudibranchia. Because this spur is unknown elsewhere in the Nudipleura, we assume that it has been lost in all other Cladobranchia and Anthobranchia.

The Charcotiidae is the sister-group to the Aeolidoidea due to the possession of the terminal sacs which we assume to be the precursor to the cnidosac (see character 30).

Considering all the other data sets that we mentioned above, but which were excluded from our analysis because the self-imposed limits of taxa and/or because of insufficient information, only those relating to molecular data can be mapped onto our cladogram. The results of a study on 18S rDNA are congruent with the cladogram in many aspects (Wollscheid & Wägele, 1999), and the same applies to Tholleson's 1999 analysis on 16S rDNA, at least as regards the subordinate taxa.

Postscript

Any phylogenetic analysis can be compromised by the limitations of computerization. According to the actual principles of Hennig (1966), phylogenies can be analysed and dendrograms can be created by hand giving investigators the opportunity to step back and view a heterogeneous group more broadly than they could do with a computer-derived analysis. However, 'purist' cladists object to the premise on which such hand-derived analyses rests, that of *a priori* judgement of characters and polarities.

Two examples from our data set demonstrate the limitations of computer-derived analyses. The first shows that the conclusions the computer program reaches are only as good as the size of the data set. Firstly consider character 47 (specialized vacuolated epithelium). We are convinced that the polarity goes from absence to presence of this cell type in the Nudibranchia because of our experience with many opisthobranch taxa beyond those considered here. Indeed this conviction is actually based on many more taxa than were included in this analysis so it would have been supported even more strongly by the inclusion of a larger data set. Secondly consider character 29 (caecum). If we had included additional doridoidean taxa known by us to lack a caecum (e.g. *Dendrodoris*, (some) *Glossodoris*, *Paradoris*), those taxa lacking a caecum would have outnumbered those possessing it, so the computer program would have misinterpreted the polarity. Exactly the same situation would have applied to character 46 (glandular stripe). Inclusion of more aeolidoidean taxa might have disproved the *Phyllodesmium-Protaeolidiella* sister-group relationship supported by the sole synapomorphy 'absence of the glandular stripe'. Hand-derived analyses can prevent such clustering of nonsense or poorly supported groups on the basis of similarities/convergences perceived by the computer program (see examples above and also the clustering of *Doto* and *Dirona* on the shared lack of oral tentacles).

We did perform a hand-derived phylogenetic analysis on more taxa than those listed in Table 4. This phylogeny served as a 'commonsense' standard against which the computer-generated dendrogram could be compared (J.W. Wägele, 1994) since they were both based on the same character analysis. Our hand-derived cladogram had a high congruence with the PAUP analysis except for those few branches just discussed where there was no resolution due to lack of synapomorphies.

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