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Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation

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Abstract

Five potential key characters which might have enhanced species radiation in the Opisthobranchia (Gastropoda) are discussed. These are: 3–4 cuticular plates in the gizzard of Cephalaspidea s.str., kleptoplasty in Sacoglossa, kleptocnides in Aeolidoidea, a symbiotic relationship with unicellular algae in *Phyllodesmium* Ehrenberg, 1831, and mantle dermal formations in Chromodorididae. Interpretation of the characters as key innovations is based on phylogeny and/or comparison of species numbers in subgroups. Possible adaptive zones are discussed, and alternative interpretations indicated.
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Keywords: Adaptive radiation; Key characters; Kleptoplasty; Kleptocnides; MDFs; Zooxanthellate symbiosis

Introduction

Adaptive radiation has been investigated mainly in vertebrates, insects and plants (for examples see Schluter 2001). It has been proposed that we do not know enough about the biology and phylogeny for many marine invertebrate organisms to suggest hypotheses on the "evolution of ecological diversity within a rapidly multiplying lineage" (Schluter 2001, p. 1). In the present study, five characters are discussed that could have been cues for the adaptive radiation of some marine gastropods belonging to the Opisthobranchia.

Adaptive radiation is a term that was and is applied in different ways, a fact that was described by Skelton (1993, p. 46) as "a sea of sloppy usage." According to him adaptive radiation includes a significantly higher cladogenesis over extinction in a certain episode, and an adaptive divergence that is enhanced by ecological "stimuli". Four cues are relevant for adaptive radiation,

with three extrinsic factors (primarily or secondarily unoccupied habitats or co-evolution), and one intrinsic one: "The evolution of features that prove to be preadaptive for the penetration of previously inaccessible adaptive zones" (Skelton 1993, p. 47).

Analysing species diversity along cladograms and comparing these to models of cladograms with random speciation and random extinction can test adaptive radiation. It would be desirable to have a comparison of two or more independent sister taxa relationships. One branch in these cladograms should show the character that is hypothesized to lead to a higher speciation rate, and the sister branch should lack it. Only if the clade with the character in question shows a higher species number in all independent cladograms, can the character be addressed as a key character that has lead to adaptive radiation (Skelton 1993). Skelton (1993) addressed some problems in these tests. We usually have no idea about the extinction rate after speciation. It could be higher in the sister taxon without the assumed key character and therefore lead to incorrect

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interpretation. Furthermore, one of the characters discussed below (kleptocnides) seems to have evolved only once, therefore a test as suggested by Skelton is not possible. Stratigraphical methods cannot be used for Opisthobranchia, because palaeontological evidence is extremely sparse and only available for a few shelled opisthobranchs, but it is lacking completely in shell-less forms, which comprise about 70% of all known species. Another problem that has become apparent during the present study is correct estimation of species. Especially in the Opisthobranchia, synonymy is extremely high in some genera and can only be resolved after thorough studies, which so far have been undertaken in only few cases. For example, Wägele (1990a, 1993) synonymized more than 14 species of the Antarctic genus *Austrodoris* Odhner, 1926 with *Austrodoris kerguelenensis* (Bergh, 1884). After their respective first description, many species are subsequently assigned to different genera, often without a thorough analysis. An extreme case are the 148 chromodorid species regularly listed under two different generic names, *Chromodoris* Alder and Hancock, 1855 and *Glossodoris* Ehrenberg, 1831. In addition, about 30 of these species appear under the name *Hypselodoris* Stimpson, 1855. This is probably the reason why the present author's counts of up to 530 species within the Chromodorididae are lower than Gosliner's estimations of "more than 600" (2001: 165). On the other hand, many species are not yet described, but detailed information on them is already available on the internet. For example, for several of the 26 distinguishable species of *Phyllodesmium* Ehrenberg, 1831 the formal descriptions are still in preparation, while illustrations and biological data on them are already available from Rudman (2003; http://www.sea-slugforum.net). In the present study, these undescribed species are not taken into consideration when mentioning species numbers. Species numbers for opisthobranch genera, including valid names, are not readily available from the literature. Although the present author has compiled an extensive file on the species of Opisthobranchia, it is very likely that some species and synonymizations are not yet represented.

In spite of these gaps of knowledge on the Opisthobranchia, formulating hypotheses on potential key characters for evolution in several subgroups can be a stimulant for opisthobranch researchers to have a closer look at biological data than has been done in the past.

Fig. 1 presents a cladogram of the Opisthobranchia which is a manual combination of molecular and morphological trees (Mikkelsen 1996, 2002; Wägele and Willan 2000; Valdés 2002; Wägele et al. 2003; Vonnemann et al. in press). Comparing species numbers (see Fig. 1), it becomes quite obvious that some taxa far outnumber others. Recent morphological investigations on the phylogeny of the major opisthobranch taxa (Willan 1987; Jensen 1996; Mikkelsen 1996, 2002;

Wägele and Willan 2000) now allow the formulation of hypotheses on a few key characters that have likely enhanced radiation of certain taxa. These characters are: the few gizzard plates in Cephalaspidea, kleptocnides in Aeolidoidea, and MDFs in Chromodorididae. Two special characters are assumed to be key characters due to their supposed impact on the animals' biology, although the phylogeny and sister taxa relationships of the taxa in question have not been resolved. These characters are the uptake of functional kleptoplastids in Sacoglossa, and symbiosis with zooxanthellae in cladobranch genera, especially in *Phyllodesmium*.

Potential key characters

Gizzard plates in Cephalaspidea s.str

According to recent results on the phylogeny of basal opisthobranch taxa (Mikkelsen 1996, 2002; Wägele et al. 2003; Vonnemann et al. in press) the Cephalaspidea s.str., as described by Mikkelsen (1996), are monophyletic (Fig. 1). Apomorphic characters are related to the nervous system (nerve ring preoesophageal, genital ganglion on visceral loop), the mantle cavity (the ciliated stripes, responsible for water current within the cavity, are flexed), and the digestive system (three cuticular plates in a specialized part of the oesophagus, the so-called gizzard).

Following the Nudibranchia (more than 2700 species), the Cephalaspidea s.str. are the second largest taxon within the Opisthobranchia with at least 840 extant species. Sister taxon to the Cephalaspidea s.str. is the monophyletic Anaspidea with approximately 75 species (Fig. 1). According to recent investigations by Vonnemann et al. (in press), the genus *Runcina* Forbes and Hanley, 1851 is the most basal taxon of the Cephalaspidea s.str. (Fig. 1). This genus is characterized by four plates in the gizzard. The Anaspidea forage on green and red algae, Runcinidae and most families of the Bulloidea forage on algae, whereas the Bullidae are omnivorous (Paula Mikkelsen, pers. comm.). Since the major food of these groups are algae, the common ancestor of the Anaspidea and Cephalaspidea s.str. is assumed to have been herbivorous. The Anaspidea have a gizzard with many small and at least ten larger plates. The smaller plates function as a transporting system, whereas the larger ones work like a grinding mill (Howells 1942). The herbivorous cephalaspideans (Runcinidae and Bulloidea) also have a gizzard with two different kinds and sizes of teeth. But contrary to the Anaspidea, the Runcinidae are characterized by the possession of four large plates, and the Bulloidea, as well as all other cephalaspideans, have three large plates. I assume that evolution of three large plates forming an
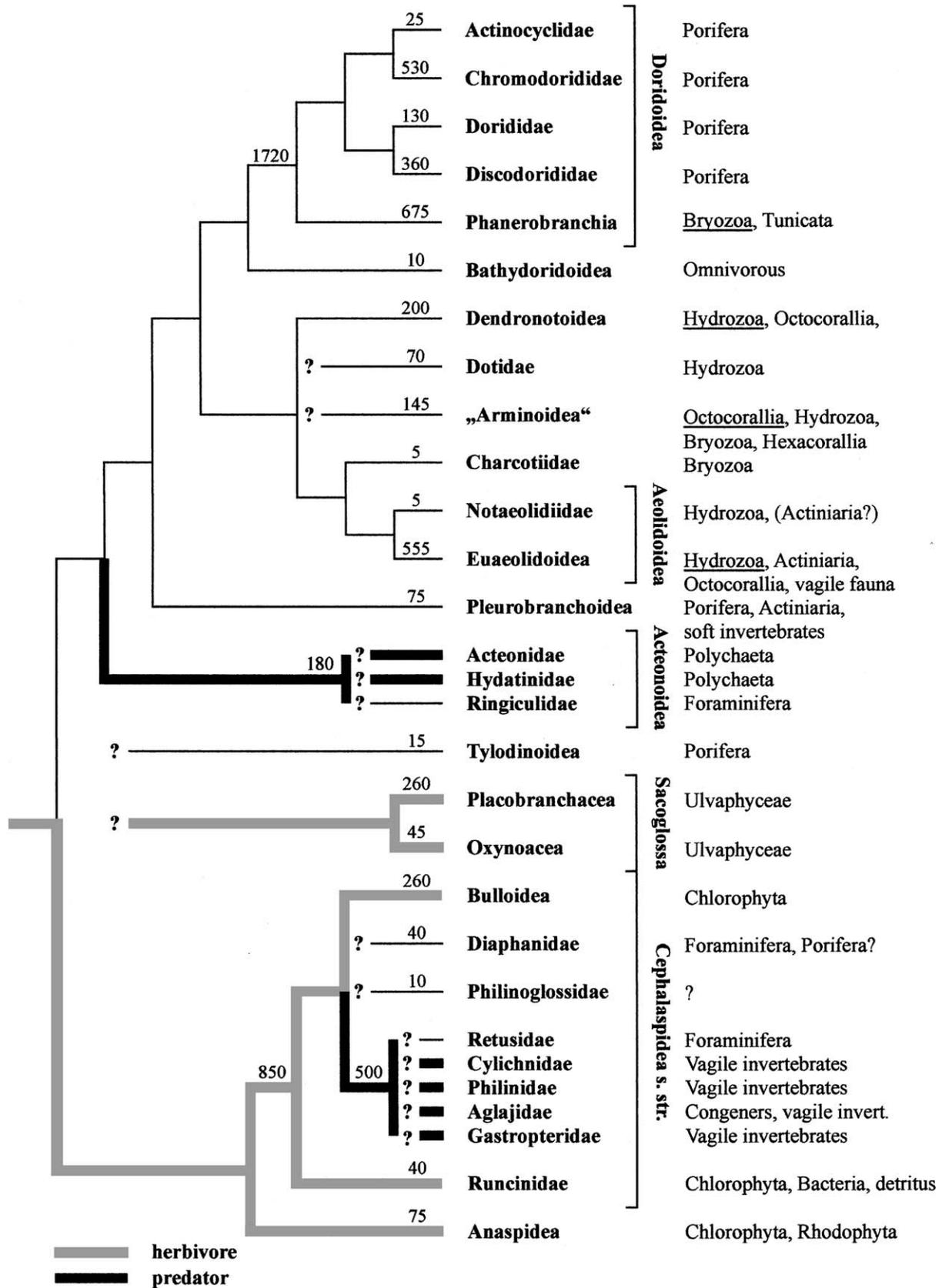
Fig. 1. Working hypothesis on the phylogeny of the Opisthobranchia based on Mikkelsen (1996, 2002), Wägele and Willan (2000), Valdés (2002), and unpublished data. Numbers indicate the approximate species numbers of the subgroups, based on a species file compiled by the author. Dendrodorididae and Phyllidiidae (Doridoidea), Acochlidiacea, Thecosomata and Gymnosomata are not included due to lack of sufficient data. Taxon names to the right of major groups indicate the preferred food of that taxon. Herbivorous slugs are indicated by wide grey bars, predators on vagile invertebrates by wide black bars.

efficient triturating structure in the digestive system (Fig. 2A) allowed a higher diversification by feeding on different kinds of food. Fig. 1 shows the respective major food items for each of the terminal taxa in the cladogram. The numbers of known species per group also given clearly demonstrate a much higher diversity for the Bulloidea than in the Anaspidea. It has to be checked in future analyses, whether the three gizzard plates enhance foraging on more algae species, as well as on other food items (e.g. foraminiferans). Possession of three to four plates might have enabled a more effective crushing of food with harder or calcified cell membranes. In addition, the switch to vagile carnivorous prey (e.g. Bivalvia, Gastropoda, Polychaeta) opened an adaptive zone that probably could only be explored to these extents by evolution of the three gizzard plates. Evidence for this is given by the high species number of predatory cephalaspids (500, see Fig. 1). Some cephalaspid taxa (Aglajidae) show secondary reductions of all hard structures in the digestive system (radula, jaws and gizzard). These animals feed on soft congeners, shell-less nudibranchs or polychaetes, and the digestive enzymes alone are sufficient for degrading the prey.

Comparing the cladogram of Cephalaspidea s.str. with that of three other families formerly included in the Cephalaspidea (Acteonidae, Hydatinidae, Ringiculidae), the hypothesis can be tested whether or not the presence of three gizzard plates is a key character. Mikkelsen
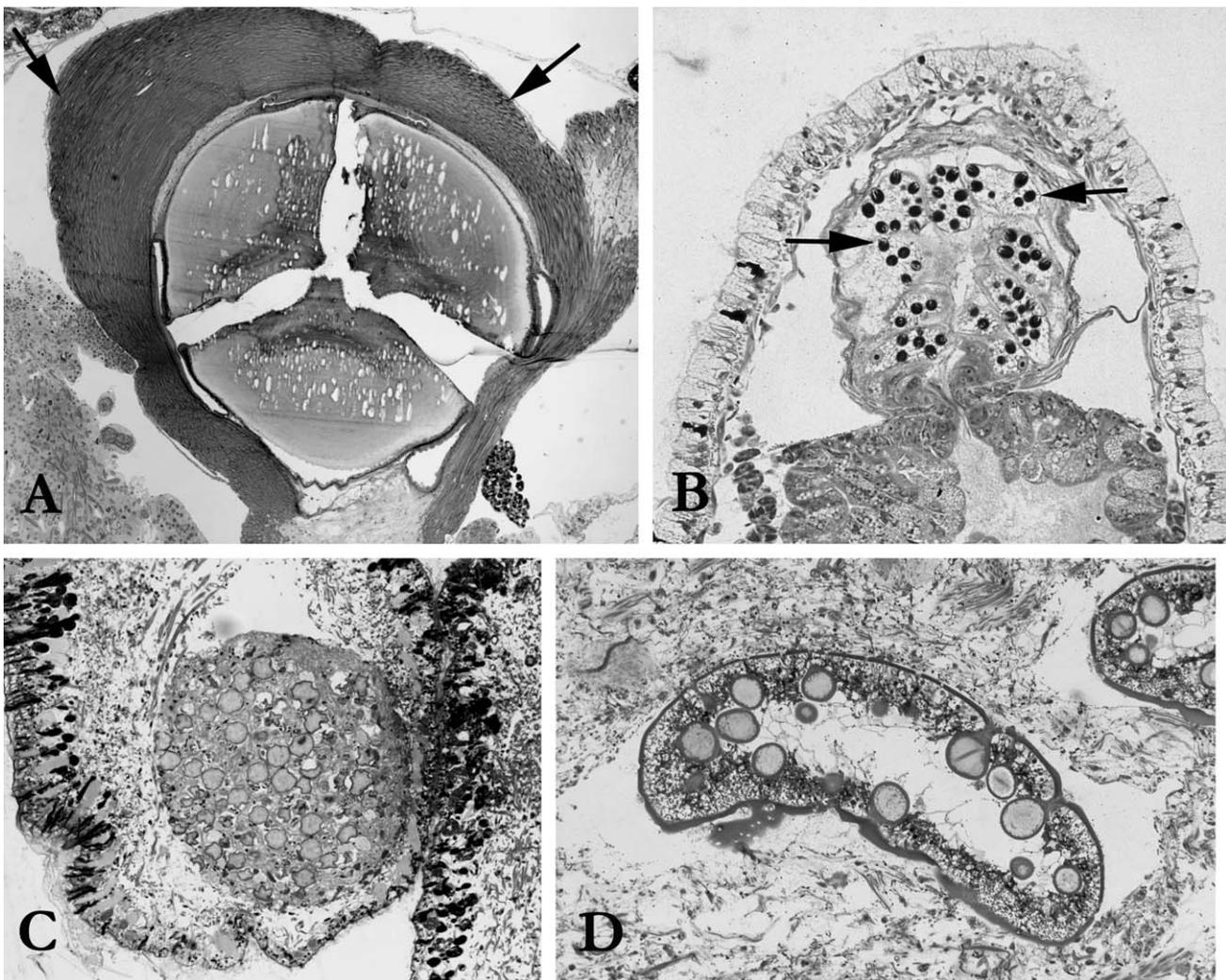

Fig. 2. (A) Cross section of the stomach of *Haminoea callidegenita* Gibson and Chia, 1989 (Cephalaspidea, Haminoeidae), showing the three gizzard plates working like a triturating system. The plates touch each other when the thick muscle layer (arrows) around the stomach contracts. (B) Cnidosac of *Cuthona* sp. (Nudibranchia, Aeolidoidea, Cuthonidae) with several kleptocnides (arrows) in the cells of the sac. (C) Mantle dermal formation (MDF) of *Placobranchus ocellatus* van Hasselt, 1824 (Sacoglossa, Placobranchacea, Placobranchidae), probably storing secondary metabolites of the algal food items. (D) MDF of *Chromodoris westralensis* (O'Donoghue, 1924) (Nudibranchia, Doridoidea, Chromodorididae), probably storing secondary metabolites of the poriferan prey and used as a defensive system.

(2002) considered the Acteonoidea, comprising the above three families, not to be monophyletic. In the analyses of Vonnemann et al. (pers. comm., Fig. 1), at least Acteonidae and Hydatinidae are sistergroups, the Ringiculidae are not considered. Acteonidae and Hydatinidae prey on vagile invertebrates, especially Polychaeta. Foraging strategies of the Ringiculidae are little known, only Foraminifera are indicated as potential food. Nearly 100 living species of Acteonidae, 25 of Hydatinidae, and 55 of Ringiculidae are known. Fossil records for the Acteonidae and Hydatinidae go back to the Lower Jurassic (180 myr), as do those for members of the Cephalaspidea s.str. (Diaphanidae: 160 myr, Cylichnidae: 150 myr) and for the most basal taxon of the Anaspidea (*Akera* Müller, 1777: 160 myr) (Wenz and Zilch 1959–1960; Tracey et al. 1993). Because of the groups' similar ages, habitats and the identical geographic distribution, the higher species numbers for the Cephalaspidea s.str. compared to the Acteonidae and Hydatinidae (about 840 versus 180) indicate the presence of a key character as a driving force for higher speciation rates. The acquisition of a highly efficient gizzard with three plates is the only morphological character or ecological trait known to date that can explain the higher diversity in Cephalaspidea s.str.

Kleptoplasty in Sacoglossa

Jensen (1996, 1997) presented a thorough phylogenetic analysis of the taxon Sacoglossa with an a posteriori analysis of its evolution. Sacoglossa comprises approximately 300 species, its monophyly is supported by several autapomorphies (Jensen 1996; Mikkelsen 1996). Probably the most important was the evolution of a uniseriate radula with just one median tooth per row. Algal cells are cut open with these teeth, then the cellular contents of the alga can be sucked out. Two major lines within the Sacoglossa can be observed, the shelled Oxynoacea and its sister taxon, the shell-less Placobranchacea (Fig. 1). The latter comprises the families Placobranchidae, Polybranchidae and Limapontiidae. Jensen (1997) developed a plausible scenario integrating her phylogenetic analysis on Sacoglossa, a hypothesis on the evolution of green algae, and correlation of food and tooth shape. She showed that the evolution of the Sacoglossa was initialized after cell-membrane composition in derived taxa of the Ulvophyceae, namely Caulerpales, had changed. Members of the Oxynoacea and Placobranchidae were only able to feed on Caulerpaceae (a subgroup of the Caulerpales) after the evolution of mannan or xylan as structural cell wall polysaccharides within the Caulerpaceae, and after the fiber arrangement in the cell walls had changed. A further specialization of the teeth enabled a switch to other algae using mannan or xylan only in one phase of

their life, and cellulose in another (Deserbiales). This step probably was a prerequisite for the switch to algae, which are using cellulose as cell wall polysaccharides and therefore show the plesiomorphic situation within the Ulvaphyceae (for a discussion of green algal phylogeny see Jensen 1997). It is only the highest-evolved genera within the three Placobranchacea families — e.g. *Ercolania* Trinchese, 1872 and *Limapontia* Johnston, 1836 within the Limapontiidae; *Aplysiopsis* Deshayes, 1864 within the Polybranchidae; *Elysia* Risso, 1818 and *Thuridilla* Bergh, 1872 within the Placobranchidae — which were then able to exploit the filamentous Cladophorales with exclusively cellulose walls. The only members known to feed on the most basal Ulvophyceae, namely the Ulvales, belong to the highly derived genus *Elysia*.

The Sacoglossa are known for the retention of chloroplasts for camouflage plus energy supplement. In several species, sequestered chloroplasts are functional for a very long time (see Williams and Walker 1999; Rumpho et al. 2000; Wägele and Johnson 2001). In cases of long-term storage, an exchange of metabolites and therefore a 'mutualistic symbiosis' is assumed. But long-term storage has been measured in only very few species (Trench et al. 1970, 1973, 1974; Clark et al., 1981).

Whereas the evolution of a special tooth for cutting algal cell walls was essential for the radiation of Sacoglossa, it is still unknown whether the uptake and retention of chloroplasts was a key character. Discussed benefits are a better survival during winter with no algal growth in boreal or temperate areas, or independence of cyclic calcification in algae. Furthermore, chloroplast-retaining sacoglossans are able to search for new food sources and may save rare food items (Jensen 1997; Rumpho et al. 2000). This opens a new adaptive zone. Morphological adaptations, especially the branched digestive gland with many ramifications housing the chloroplasts, are present in many sacoglossans without functional kleptoplastids. Even short-term storage of chloroplasts already renders the animals green and cryptic on their food substrate. In several analyses it has become obvious that the chloroplasts loose their ability to transfer electrons due to degradation (see Clark and Busacca 1978; Clark et al. 1990), but degradation time differs considerably among species. All the species showing long-term storage for more than five days belong to the family Placobranchidae, and particularly the placobranch genus *Elysia* has many species with a very high efficiency in chloroplast retention (see Table 1). *Elysia* is the most species-rich genus, its 120 nominal species represent nearly 40% of the known Sacoglossa. Marín and Ros (1992) showed that *Elysia timida* (Risso, 1818) is able to retain chloroplasts from its food *Acetabularia acetabulum* (Linné) for more than 45 days, after which the algae start to calcify and the

Table 1. Species of Sacoglossa, their experimentally verified long-term storage of chloroplasts, and preferred food. Boldface indicates food algae for which long-term storage of chloroplasts was recognized

Taxon	Storage duration	Reference on storage data	Preferred food	Reference on food data
Placobranchus ocellatus	> 70 days	Burghardt and Wägele (unpublished data)	*Udotea* (Udoteaceae), *Chlorodesmis* (Udoteaceae)	Jensen (1993)
Tridachia crispata	> 50 days	Clark and Busacca (1978)	*Caulerpa* (Caulerpaceae), *Halimeda* (Udoteaceae)	Jensen (1993)
Elysia viridis	> 41 days > 90 days	Hinde and Smith (1972)	**Codium** (Codiaceae), *Bryopsis* (Bryopsidaceae), *Chaetomorpha* (Cladophoraceae)	Hinde and Smith (1972), Jensen (1993)
Elysia chlorotica	> 8 months	Mujer et al. (1996)	**Vaucheria** (Xanthophyceae)	Mujer et al. (1996)
Elysia timida	> 45 days	Marín and Ros (1992)	**Acetabularia** (Dasycladales)	Marín and Ros (1992)
Costasiella lilianae	65 days	Clark et al. (1981)	**Avrainvillea** (Udoteaceae)	Clark et al. (1981)

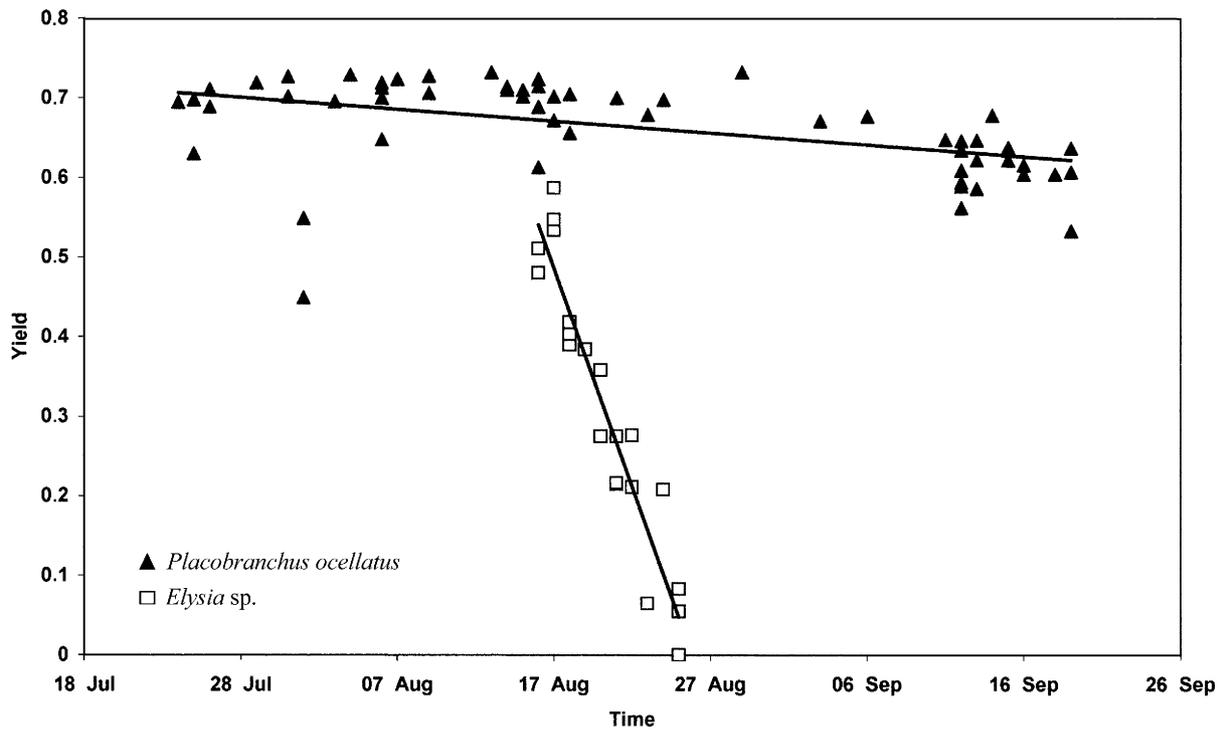Boldface indicates food algae for which long-term storage of chloroplasts was recognized

Fig. 3. Photosynthetic activity of chloroplasts from food measured in living sacoglossans, shown as maximum quantum yield of fluorescence of photosystem II in darkness over time. High yields indicate well-functioning reactivity centres of photosystem II. High yields after several days of starvation indicate a well-functioning symbiotic system (*P. ocellatus*), whereas a drop in yield values indicates degradation (digestion) of chloroplasts (*Elysia* sp.).

slug is no longer able to feed on that species. During normal feeding on the uncalcified *Acetabularia*, the chloroplasts are replaced in the slug nearly every second day. Recent long-term investigations with a pulse amplitude modulated fluorometer (PAM; for the method see Wägele and Johnson 2001) show that the efficiency of photosynthetic activity and the retention of chloroplasts varies considerably between species (Fig 3; Burghardt and Wägele, unpublished data). Chloroplast retention in *Placobranchus ocellatus* van

Hasselt, 1824 exceeded 70 days, whereas in an *Elysia* species photosynthetic activity was high only within the first days, then dropped to near zero by the 9th day of cultivation. Although in both tests the individuals had been collected on the intertidal flat and were measured in the first 2 days in similar light intensities, the electron transfer rate was always considerably lower in *Elysia* sp.

Jensen (1993) distinguished different types of chloroplasts. Their distribution does not show a simple correlation with the classification of higher algal taxa, instead the type can vary even within one genus. Chloroplasts that are maintained in the slug's body for a longer period seem to belong to the advanced type. The latter is characterized by the lack of a pyrenoid, the thylakoids are stalked, and a thylakoid organizing body is present. This implies that physiology not only of the slug but also of the chloroplasts could be essential for the effective relationship. Rumpho et al. (2001) assumed two prerequisites for the evolution of a symbiotic relationship of chloroplasts within *Elysia chlorotica* Gould, 1870: structural and functional stability of the *Vaucheria* plastids and a change in membrane organization. This may hold true for many other kleptoplastids. But there still is the problem of sustained activity of algal chloroplasts in the absence of the algal nucleus, as outlined by Mujer et al. (1996) for *E. chlorotica* which is able to retain chloroplasts for more than 8 months. A mechanism supplied by the slug also has to be assumed for *Placobranchus ocellatus*, considering the results on this species in long-term experiments (see above).

Many more long-term measurements of slugs and their food are needed. Many sacoglossan species seem to be polyphagous, therefore a detected long-term storage has to be correlated to the respective food species. In a next step the relevance of this specific food item to long-term retention has to be tested. Waugh and Clark (1986) investigated the seasonality of kleptoplastid uptake in *Elysia tuca* Marcus and Marcus, 1967 and emphasized the importance of environmental factors. Especially these findings lead to the conclusion that kleptoplasty with an efficient mutualism is much more widely distributed than so far considered in the literature (Williams and Walker 1999).

Nevertheless, we need robust phylogenetic hypotheses on the species involved. Then conclusions can be drawn, whether chloroplast retention and the mutualistic relationship was a key innovation in the Sacoglossa.

Kleptocnides in Aeolidoidea

According to recent analyses of the Cladobranchia, the Aeolidoidea are monophyletic with the Antarctic Charcotiidae as their sister taxon (Fig. 1; Wägele and Willan 2000). Considering relationships of the Aeolidoidea within the Cladobranchia, the molecular data are incongruent when using different genes (Wägele et al. 2003). Because morphology-based cladograms are not available, the overall number of higher cladobranch taxa are compared with the number of aeolidoidean species without respect to relationship. It is evident that the latter (at least 560 species) outnumber all other taxa of the Cladobranchia, namely the Dendronotoidea (including Dotidae) with roughly 270 species and the paraphyletic "Arminoidea" with about 145 species. Wägele and Willan (2000) mentioned the possession of cnidocysts, stored in a cnidosac (Fig. 2B) and used as defensive devices, as an autapomorphy for the Aeolidoidea. Other autapomorphies, e.g. the presence of additional oral tentacles, are less likely to be key characters than the novelty of kleptocnides, a character that is unique within gastropods. For example, additional oral tentacles also occur in the Anaspidea, a taxon with only about 75 extant species. According to Wägele and Willan (2000), the acquisition of a kind of terminal sac in the terminal branches of the digestive glands in the aeolidoidean sister taxon, the Charcotiidae (see Wägele 1991), was a prerequisite for a cnidosac. Charcotiidae, known only from five species, feed on bryozoans. An exception is the recently included *Leminda* Griffiths, 1985 which is assumed to feed on octocorals. Therefore, the possibility to store cnidocysts and to use them for defence seems to be the driving force for a higher speciation rate within the Aeolidoidea. The process of uptake, selection and storage of nematocysts is still little known (see Greenwood 1988).

The adaptive zone in which the aeolids could have evolved after becoming able to foster cnidocysts probably included a much higher number of food sources, because the defensive mechanism of using the functional kleptocnides in combination with aposematic colours allowed the exploration of cnidarian colonies in full visibility of potential predators. Comparing food sources of Aeolidoidea with other cladobranch taxa (Dendronotoidea, "Arminoidea"; see Table 2), it becomes obvious that food range in the latter two is as wide as within the former, and that hydrozoan species seem to be the predominant food item, especially in the more basal taxa. The Antarctic family Notaeolidiidae probably comprises less than five species the members of which seem to feed on hydrozoans (Barnes and Bullough 1996). Wägele (1990b) indicated actiniarians as food, although confirmation is needed. Her results are mainly based on the presence of a certain type of cnidocysts in the digestive tract of notaeolidians, namely spirocysts, known only from actiniarians (Fautin and Mariscal 1991). The most basal group within the Euaeolidoidea (Aeolidoidea excluding Notaeolidiidae) is the aeolid genus *Flabellina* Voigt, 1834. Nearly all of the 80–90 described species feed on different types of hydrozoans. This suggests an exploration of a large adaptive zone in temperate and tropical waters made

Table 2. Food sources of Cladobranchia

Taxon	Species number	Food items
AEOLIDOIDEA		
Aeolidiidae	70	Hexacorallia (Actiniaria)
Calmidae	2	Fish eggs, molluscan eggs
Cuthonidae	100	Hydrozoa
Eubranchidae	40	Hydrozoa
Facelinidae: Facelininae	82	Hydrozoa, Scyphozoa
Facelinidae: **Favorininae**	142	Hydrozoa, Octocorallia (Gorgonaria, Alcyonaria), Hexacorallia (Actiniaria), Nudibranchia, other invertebrates, eggs of molluscs
Fionidae	4	Velellina, Cirripedia
Flabellinidae	90	Hydrozoa, rarely on Actiniaria
Glaucidae	5	Hydrozoa
Goniaeolididae		?
Nossidae	1	?
Notaeolidiidae	3	Hydrozoa, ?Actiniaria
Piseinotecidae	5	Hydrozoa
Protaeolidiidae	2	Hydrozoa
Pseudovermidae	16	Hydrozoa
Pteraeolidiidae	3	Hydrozoa, Xeniidae (octocorals)
DENDRONOTOIDEA		
Embletoniidae		Hydrozoa
Bornellidae	10	Hydrozoa
Dendronotidae	20	Hydrozoa
Dotidae	67	Hydrozoa
Hancockiidae	8	Hydrozoa
Lomanotidae	5	Hydrozoa
Marianinidae	1	Hydrozoen
Phylliroidae	5	Appendicularia, Hydrozoa
Scyllaeidae	15	Vellelina, *Physalia*
Tethyidae	33	Crustacea, Mollusca, Pisces, plankton, detritus
Tritoniidae	101	Alcyonaria, Xeniidae
"ARMINOIDEA"		
Arminidae	92	Pennatularia, Alcyonaria,
Charcotiidae	5	Bryozoa, ?Alcyonaria, ?Gorgonaria
Dironidae	5	Hydrozoa, Bryozoa
Doridomorphidae	1	Heliopora
Heroidae	2	Hydrozoa
Heterodorididae	3	Gorgonaria
Madrellidae	7	Bryozoa
Pinufiidae	1	Porites
Zephyrinidae	31	Bryozoa

Information mainly taken from website by McDonald and Nybakken (2003): (http://people.ucsc.edu/~mcduck/nudifood.htm). Additional literature used: Willan (1981), Willan and Coleman (1984), Cattaneo Vietti and Boero (1989), Barnes and Bullough (1996). Species numbers compiled from the author's species file and from website by Long, 2003: (http://www.seaslug.com). Taxa with members feeding on Octocorallia are shown in boldface. Where more than one food item is mentioned, the respective major prey is underlined.

accessible by the acquisition of the key character. Physiological properties of the hydrozoan cnidom could have facilitated the uptake and storage of kleptocnides in the cnidosac. Interestingly, there are a few taxa lacking cnidocysts in their sacs, e.g. all investigated members of the genus *Phyllodesmium*, which are the only aeolids feeding on octocorals (see below), the genus *Phestilla* Bergh, 1874 feeding on scleractiniarian corals,

and, naturally, all species feeding on fish or molluscan eggs. This supports the hypothesis of Greenwood (1988) that selective retention is based on chemical or physical differences in the nematocysts themselves.

There seem to be other traits or characters that enabled a higher speciation rate in certain genera, e.g. *Cuthona* Alder and Hancock, 1855 with about 75 species also feeding on hydrozoans, but we do not know enough

about the phylogeny of these groups to speculate on further potential key characters.

Zooxanthellae in Aeolidoidea

Several members of the Cladobranchia are able to store unicellular algae of the genus *Symbiodinium* Freudenthal from their cnidarian food. A few of these species are known to have a high mutualistic relationship with these zooxanthellae, the slugs using the metabolites of the symbionts mainly for their own reproductive efforts (Hoegh-Guldberg and Hinde 1986). Mutualism with zooxanthellae was a driving force for the evolution of hermatypic corals and probably also other taxa, e.g. Foraminifera (Schlichter 1998). It was therefore an intriguing idea, whether a similar relationship with algae might have been a key character in nudibranch evolution. In the literature a mutualistic relationship is mainly deduced from the detection of the zooxanthellae by histological methods in short-term experiments (for a review see Wägele and Johnson 2001), but exchange of metabolites has been actually investigated only for the aeolidoidean species *Pteraeolidia ianthina* (Angas, 1864) (see Hoegh-Guldberg and Hinde 1986). Rudman (1981a, b, 1982a, b, 1991) was the first to investigate adaptations of the morphology of the digestive glandular system for more efficient housing of zooxanthellae in the genus *Phyllodesmium* Ehrenberg, 1831, which belongs to the same family (Facelinidae) as *Pteraeolidia* Bergh, 1876. He assumed that the uptake and use of algae in a mutualistic relationship evolved twice within *Phyllodesmium*. His evolutionary scenario of the genus with 12 known species at that time was based only on characters of the digestive system and their adaptive variability, not on a phylogeny deduced from independent characters. Nevertheless his investigations clearly indicated differences between the various species housing zooxanthellae. Rudman assumed that low branching of the digestive glandular system within the dorsal processes, e.g. in *P. serratum* (Baba, 1949), is less adapted to the symbiosis than high branching, e.g. in *P. magnum* Rudman 1991 and *P. longicirrum* (Bergh, 1905).

We know nothing about the probable sister taxon of the genus *Phyllodesmium*. Therefore, the assumption that storing zooxanthellae and using their metabolites for self-benefit is a key character for *Phyllodesmium* is based on the observed higher number of species in that genus compared to many other genera of the family Facelinidae or even within the Aeolidoidea. Facelinidae comprises approximately 33 genera, 13 of them being monotypic. The largest genus is *Facelina* Alder and Hancock, 1855 (36 species), followed by *Phyllodesmium* (16 described and at least 11 undescribed species). Food is known for about 25 genera: in 15 of them, including the species-rich genus *Facelina*, members feed exclusively on Hydrozoa. Feeding on octocorals is known to occur in only three genera: *Phyllodesmium*, the monotypic *Pauleo* Millen and Hamann 1992 (members feed on the gorgonacean *Plexaurella*; Millen and Hamann 1992), and the monotypic *Pteraeolidia* (food includes a variety of hydrozoans and octocorals; Kempf 1984; Willan and Coleman 1984; Rudman 2003: http://www.seaslugforum.net). If we assume that food change from hydrozoans to octocorals could trigger a higher speciation rate, then we have to ask why the other two genera foraging on octocorals do not show a trend of higher radiation. It cannot be ruled out that the switch to a certain kind of octocoral facilitated uptake of zooxanthellae (due to different algal species involved?), and then enabled adaptive radiation by exploring smaller and rarer food sources. However, information on feeding biology and systematic relationships of these two genera is far too insufficient for further conclusions. Studies by Burghardt and Wägele (Fig. 4; unpublished data) have shown that in long-term starvation experiments the photosynthetic activity in *P. ianthina* is constant over 70 days, and in different light intensities the involved zooxanthellae are more efficient than e.g. in the soft coral *Briareum*. Since the animal had been collected from sandy substrate, it was impossible to determine its prior feeding habits. *P. ianthina*, having a much broader spectrum of prey, occupies a broader ecological niche, which may not enhance speciation. Future phylogenetic analyses will show whether *Pteraeolidia* is phylogenetically closely related to the *Phyllodesmium* clade.

In many aeolidoidean species the range of food items is very limited, in some to only one kind. A shift of preference to a different, new food source in a population would thus suffice to separate the members of that particular species by ecological features.

Data available mainly from Rudman's (2003) website "Sea Slug Forum" indicate stenophagy within the 26 different species of *Phyllodesmium*. This genus seems to be appropriate for the investigation of the evolution of symbiosis, because many transitional forms from nearly no digestive glandular branches in the dorsal appendages up to highly branched systems are known from different species. From what we know about morphology of the digestive glandular system in the dorsal processes, a stepwise evolution can be assumed. It could have started with an uptake of zooxanthellae by feeding on octocorals containing algae, without derived benefits. The next step would have been short-term storage, rendering the slugs more cryptic by camouflage. The final step has led to a mutualistic relationship between slug and algae, including an exchange of metabolites. But it is not yet known, whether the acquisition of mutualism depended on the kind of food, on the kind of zooxanthellae (different species of *Symbiodinium*, or
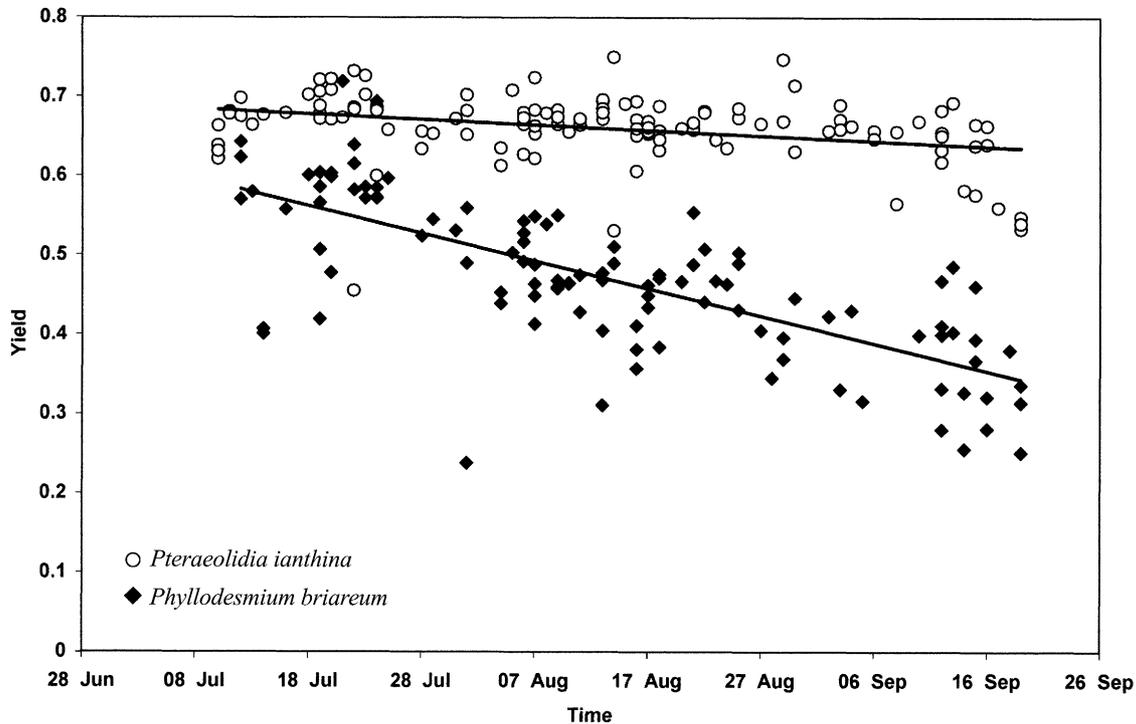
Fig. 4. Photosynthetic activity of zooxanthellae from food (octocorals) measured in living Aeolidoidea, shown by maximum quantum yield of fluorescence of photosystem II in darkness over time. High yields generally indicate well-functioning reactivity centres of photosystem II. High yields maintained after 70 days of starvation indicate a well-functioning symbiotic system in *P. ianthina*, whereas the slow drop of yield values at the same time in *P. briareum* indicates a certain degradation of chloroplasts in the zooxanthellae. Because the slope is only slightly negative (compared to *Elysia* sp., Fig. 3), a symbiotic relationship can also be assumed for *P. briareum*.

different ecotypes of the same species), or both. Recent investigations (Fig. 4; Burghardt and Wägele, unpublished data) show that adult *P. briareum* are able to survive for 70 days without any food if kept under illumination, but the 'health' of the zooxanthellae seems to decrease. This can be deduced from the yield values decreasing over time, indicating reduced efficiency of photosystem II in the chloroplasts of the zooxanthellae (for methods see Wägele and Johnson 2001).

Mantle dermal formations in Opisthobranchia

Only recently Cimino and Ghiselin (1999) discussed the evolutionary pathways of food metabolites in becoming major defensive tools in opisthobranch sea slugs, which consequently allowed the reduction of a heavy protective shell. This combination of traits probably was an important aspect of opisthobranch evolution. The authors assume, e.g., that the ability to exploit alkaloid-defended food in Phanerobranchia enabled species of this clade to switch from Bryozoa to Ascidiacea as food. Storage of the biochemicals is usually restricted to the epidermis and subepithelial structures, although histochemical investigations are

lacking (Avila and Durfort 1996). Broad surveys of chemical substances are available, for the slugs as well as their major food sources. These show the high efficiency of using biochemicals in feeding assays with possible slug predators, e.g. fish, crabs and echinoderms (Avila 1995; Avila and Paul 1997; Avila et al. 2000). One family of the Doridoidea (Nudibranchia), namely the Chromodorididae with more than 500 species, is considered to be extremely efficient by storing secondary metabolites from their sponge prey in special organs (mantle dermal formations — MDFs) lying beneath the mantle rim (Fig. 2C). Avila and Durfort (1996) separately investigated the digestive tract, gonad, dorsal mantle tissue, and mantle rim for presence of the metabolites. The results make it seem very likely that the compounds are located in the MDFs, although histochemical investigations are still lacking.

Rudman (1984), tentatively, and then Gosliner and Johnsen (1994, 1999) in a phylogenetic analysis have specified the presence of MDFs as an apomorphy of the family Chromodorididae. Monophyly of the family was confirmed by molecular systematics (Wägele et al. 2003). Gosliner (2001, p. 165) considered the "evolution of defensive mantle glands a key innovation that has contributed to the extensive radiation and speciation" of

the Chromodorididae. Further evidence that the MDFs as storage organs of toxic substances were an important cue for the evolution of the Chromodorididae are the bright aposematic patterns and colours in members of this family. Other Cryptobranchia families are generally cryptic.

Apart from defensive strategies, the ability to store toxic metabolites from food in special organs might have additional advantages, one being better 'control' over toxic substances in the body. On the other hand, storage in special organs might have allowed preying on even more toxic sponges, which consequently could have opened a new adaptive zone.

In traditional terms, the Doridoidea are divided into two groups, the Phanerobranchia and the Cryptobranchia (Fig. 1). The latter was considered monophyletic by Wägele and Willan (2000). Recently, Valdés (2002) presented a phylogenetic analysis of the Cryptobranchia. According to his and Gosliner and Johnsen's (1994) analysis, the sister taxon of the Chromodorididae is the genus *Actinocyclus* Ehrenberg, 1831, comprising only 16 known species. This sister-taxon relationship is based on only one synapomorphy: bursa copulatrix and receptaculum seminis in a semiserial arrangement. In former times the other cryptobranch species were assigned to a varying number of families. According to the phylogenetic analysis by Valdés (2002), these families are now amalgamated into two large families that are sister taxa, the Dorididae (with about 130 species) and the Discodorididae (with about 360 species). Based on Valdés' cladogram, the MDFs can be considered as a key character, provided that the sister taxon relationship with *Actinocyclus* is correct. If it is not, then Dorididae + Discodorididae would be the sister taxon of Chromodorididae. Similar species numbers for both branches would then imply that the MDFs are not a key innovation. This example shows the importance of a reliable phylogenetic analysis for the evaluation of key characters.

Furthermore, a survey by the present author covering many opisthobranch taxa has shown that the presence of so-called MDFs is not restricted to the Chromodorididae but can also be found in one member of the doridoidean Polyceridae, *Limacia clavigera* (Müller, 1771) (see Wägele 1997), which feeds on bryozoans, and in one sacoglossan belonging to the family Placobranchidae, *Placobranchus ocellatus*, which feeds on algae (Fig. 2D). These two species are the only members of their groups with MDFs, and under that aspect the hypothesis that MDFs are a key character actually would have to be rejected. However, one has to consider that *L. clavigera* sucks living zooids from more than 20 different bryozoan species in the northern Atlantic and Mediterranean, where the number of available bryozoans is not very high (probably less than 200 species). Species of Chromodorididae on the other hand have a high food specificity, very often focused on a single poriferan species, and they have their main distribution in the tropical Pacific with a high sponge diversity (about 3000 species; Gosliner et al. 1996). Therefore, the 'lack of an adaptive zone' could also explain the lack of adaptive radiation in species with the same key characters. Another reason might be the young evolutionary history of *L. clavigera* and the associated lack of time for further speciation. Future phylogenetic analyses are needed to clarify this problem.

Discussion

All potential key characters examined above are morphological characters, related to feeding and assumed to be triggers for exploring new food sources. Diet was already discussed by several authors as essential in the evolution of opisthobranch taxa (e.g. Thompson 1976; Cimino and Ghiselin 1999; Mikkelsen 1996, 2002). It has to be emphasized, however, that our knowledge of opisthobranch evolution is rather sketchy, and many more key characters could be identified in the future. Moreover, it is difficult to decide, whether the switch to a new food source was the key innovation, followed by a morphological adaptation facilitating radiation, or vice versa.

Considering the fact that the ability to use whole organisms (algae) or even organelles (cnidocysts, chloroplasts) seems to have evolved several times in opisthobranch foraging or defensive strategies, the question arises whether Opisthobranchia have acquired physiological properties that allow the evolution of these symbioses more readily than in other animal groups. Perhaps the lack of certain enzymes recognizing cellular or organelle membranes enhances the length of stay of these structures within digestive glandular cells. This would explain that, e.g., not all kinds of cnidocysts are stored (no slugs are known to house kleptocnides from octocorals), not all kinds of chloroplasts are stored (stability of isolated chloroplasts could be important; Jensen 1997; Rumpho et al. 2000), and that probably only different ecotypes of *Symbiodinium* species are incorporated (a hypothesis still to be proven). Biochemical recognition needs to be studied for a better understanding of the different symbiotic systems known from Opisthobranchia.

When discussing a key character in separate evolutionary lines, many other factors have to be evaluated. Although opisthobranch larval morphology is much less diverse than adult morphology, ecological or other traits of larvae cannot be ruled out as unimportant for radiation. The age of ecosystems, therefore availability of new resources that characterize the adaptive zone, has to be taken into consideration (addressed here for

L. clavigera, living in the younger and less diverse Atlantic Ocean than the Chromodorididae in the Pacific), as well as competitors that might have occupied the adaptive zone in one geographic area, but not in the other. Another problem is the fossil record, which is lacking for soft-bodied animals. A comparative study in two separate evolutionary lines is therefore problematic, because the age of the two compared groups may not be the same. Another problem in discussing key innovations especially in Opisthobranchia is the lack of reliable phylogenies in many subgroups, because hypotheses on phylogeny not reflecting evolution lead to false deductions of key characters.

Directions for future investigations

Up to now, only few studies have tried to explain hypotheses on opisthobranch phylogeny in the light of evolution and adaptive radiation, as was performed for the Sacoglossa by Jensen (1997). This is probably due to the lack of well-supported hypotheses of phylogenetic relationships within Opisthobranchia and its subgroups. Nevertheless, our knowledge of anatomy, biology and phylogeny increases, and even preliminary hypotheses on evolutionary processes are likely to enlighten our understanding of the evolution of Opisthobranchia, or might show incongruencies between phylogenetic hypotheses and available biological data. Here, hypotheses on key characters are presented, which are meant to encourage researchers to look more closely into the functioning of structures, and to put these findings in the context of existing phylogenies. However, it is also shown that not only knowledge of phylogeny and species numbers in related clades is important (see kleptoplasty and MDFs), but many other objects have to be considered when discussing key characters. These can include function (gizzard plates), physiology (uptake of chloroplasts) and biogeography (MDFs), as well as the function, physiology, biogeography and phylogeny of the involved food organisms (algae, cnidarians) or symbiotic partners (chloroplasts, zooxanthellae).

Acknowledgements

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