Evolution of the Sacoglossa (Mollusca, Opisthobranchia) and the ecological associations with their food plants

KATHE R. JENSEN[*]

Finnish Museum of Natural History, Zoological Museum, University of Helsinki, PO Box 17, FIN-00014 Helsinki, Finland

Summary

Evolution in the opisthobranch order Sacoglossa has been closely linked to their specialized suctorial her-bivorous habits. All shelled Sacoglossa (about 20% of the species) feed on one algal genus, *Caulerpa*. The non-shelled Sacoglossa have 'radiated' to other diets, mainly siphonalean or septate green algae (Class Ulvo-phyceae). Comparing the phylogeny of sacoglossan genera with the phylogeny of the Ulvophyceae indicates that co-speciation may have taken place at the basal node of the Sacoglossa, and that host switching has taken place several times in the two non-shelled clades. It is suggested that the most important evolutionary process has been speciation by 'resource-tracking'; the resource tracked is most probably cell wall composition of the algal prey. The fossil record of extant sacoglossan genera dates back to the Eocene and, based on the fossil record of siphonalean green algae, the Sacoglossa most likely appeared in the Cretaceous. It is hypothesized that the ancestral sacoglossan was epifaunal, suctorial and herbivorous, and the 'ancestral' food plant was not *Caulerpa*, but filamentous, calcified, now extinct, Udoteaceae.

Keywords: ecological associations; evolution; integrative biology; Opisthobranchia; resource tracking; Sacoglossa; Ulvophyceae

Introduction

Sacoglossan opisthobranchs are unique in that the entire order are suctorial feeders with a uni-seriate radula of distinct organization. Except for three species, all sacoglossans are herbivores, feeding on the cytoplasm of marine plants. Extreme stenophagy is the rule rather than the ex-ception. Furthermore, the Sacoglossa comprise shelled as well as non-shelled forms, and the shelled species all feed on a single algal genus, the green siphonalean *Caulerpa*. This has led to the conclusion that *Caulerpa* is the 'ancestral' food within the group, and that evolution in the non-shelled group has been closely linked with adaptive radiation to other diets (Kay, 1968; Clark and Busacca, 1978; Clark and DeFreese, 1987; Jensen, 1983, 1993a,c).

The order Sacoglossa comprises about 250–300 species of rather small animals. Most species have maximum body lengths of 1–3 cm, and as they are usually of the same colour as their algal food ('nutritional homochromy'; Clark *et al.*, 1990), they are not easily spotted in the field. Even when their diets and habitats are known, many species occur in extremely low densities, and thus they are a 'difficult' group for ecological and evolutionary studies.

The close associations between the Sacoglossa and their food plants, in particular the si-phonalean green algae, have intrigued malacologists for many years, and have invoked terms such as 'co-evolution' (Clark and DeFreese, 1987; Clark, 1992, 1994). However, no evidence for co-

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* Address all correspondence to Kathe R. Jensen, Zoological Museum, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark.

evolution has been presented. A number of recent studies have shown that most plant–herbivore associations involving marine invertebrates are of fairly recent origin (Steneck, 1992; Vermeij, 1992). The fact that all shelled sacoglossans feed on one algal genus, and that the majority of non-shelled species feed on siphonalean green algae, indicates that the association between the Sacoglossa and their food plants may be old.

Co-evolution has been defined as stepwise reciprocal adaptive responses between ecologically closely associated species (Ehrlich and Raven, 1964; Futuyma and Slatkin, 1983). A somewhat broader definition, encompassing both the 'stepwise, reciprocal' or phylogenetic part (= co-speciation) and the reciprocal adaptive responses (= co-adaptation), has been given by Brooks and McLennan (1991). Recent cladistic analysis of sacoglossan phylogeny to the generic level (Jensen, 1996a) has enabled preliminary study of the possible co-evolution of the Sacoglossa and their food plants. Unfortunately, a corresponding cladistic analysis of the genera of siphonalean green algae does not exist.

The present study analyses the ecological associations between the Sacoglossa and their food plants based on an integrative biological approach to information from the literature, including functional morphology, ecology, behaviour, physiology and the fossil record. Also, some previously unpublished observations are included. From these analyses, the evolution within the Sacoglossa and the possible co-evolution with food algae are evaluated.

Sacoglossan phylogeny

The Sacoglossa is a well-defined monophyletic group. Until recently, the shelled families were thought to be paraphyletic and the non-shelled ones diphyletic. This seemed to parallel the observed 'diet radiation' (Boettger, 1963; Clark and Busacca, 1978; Clark *et al.*, 1990; Clark, 1992; Jensen, 1993a). Preliminary character analysis revealed that the most 'primitive' non-shelled sacoglossans are not those feeding on *Caulerpa*; rather, the plesiomorphic species almost all feed on udoteacean algae, in particular the calcified, segmented *Halimeda* or the filamentous *Chlorodesmis* (Jensen, 1993a,d). This indicated that (1) the shelled sacoglossans might constitute a monophyletic group, and (2) the 'ancestral' diet of the (also monophyletic) non-shelled sacoglossans was not *Caulerpa*. The first of these was supported by detailed character analysis (Jensen, 1996a); the second will be examined further in the present study.

The suctorial feeding method of the Sacoglossa has resulted in the evolution of a characteristic buccal apparatus shared by all sacoglossans. This includes a barrel-shaped, muscular, suctorial pharynx and a uniseriate radula in which all teeth are stored throughout life (Fig. 1). Muscular pharyngeal pouches surround the pharynx postero-ventrally in many species (Jensen, 1993d). Pharyngeal pouches are best developed in species feeding on Caulerpales (Jensen, 1993c). Loss of pharyngeal pouches appears to be associated with a shift in radular function (Jensen, 1993d). The formation of a separate, epithelium-lined pocket on the ventral surface of the pharynx for storage of used teeth is a sacoglossan innovation (Jensen, 1996a). The radula is composed of a dorsal ascending limb, a ventral descending limb, which in most species is as long as the ascending limb, and an ascus containing the used teeth, either rolled up in a spiral or packed in a dense heap (Jensen, 1993c). The ascending limb is attached within the odontophore by several radula suspending muscles in a fan-shaped arrangement. The descending limb is surrounded by a layer of longitudinal muscles, the ascus-muscle (Jensen, 1993c,d). Primitively, the radular teeth have triangular, denticulate cusps. From this, blade-shaped teeth with or without lateral or medial denticles have evolved. The most advanced teeth are the sabot-shaped teeth. Observations on pharynx movements during feeding and the arrangement of radula suspending muscles indicate that the three types of radular teeth penetrate the cell wall of the food algae in different ways (Jensen,
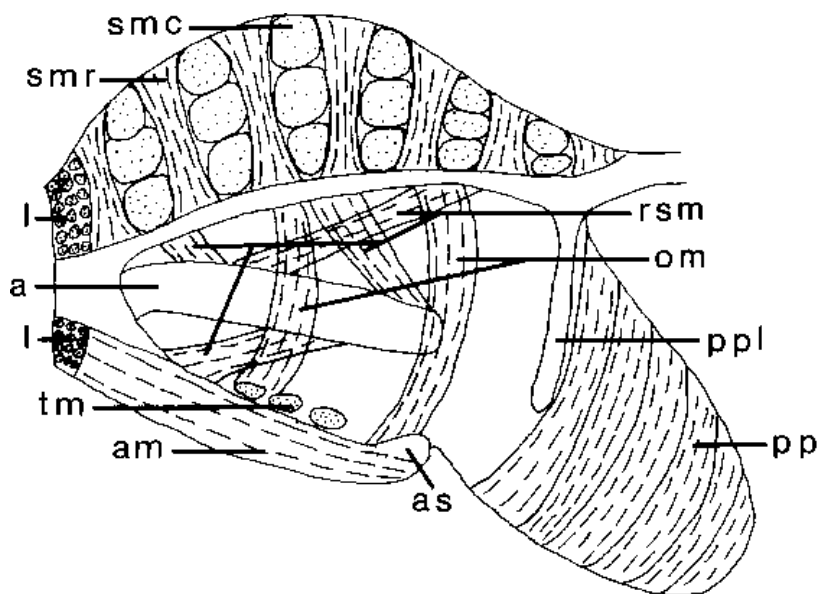
Figure 1. Schematic drawing of the sacoglossan pharynx. a, ascending limb; am, ascus-muscle; as, ascus; l, pharyngeal lips; om, odontophore muscles; pp, pharyngeal pouch; ppl, lumen of pharyngeal pouch; rsm, radula suspending muscles; smc, circular muscle-strands of dorsal septate muscle; smr, radial muscle-strands of dorsal septate muscle; tm, transverse muscles.

1993a,d). For detailed descriptions of apomorphies, parallellisms and functions, see Jensen (1991a, 1992, 1993a,c,d).

A phylogenetic tree based on recent cladistic analysis to the generic level (Jensen, 1996a) is presented in Fig. 2. This analysis was based on morphological characters. Only one-third of the characters used are from the buccal apparatus; another third are external characters, and the final third are other anatomical characters, mainly from the reproductive system. The consensus tree is not fully resolved, and the consistency index is low (0.39), indicating a high degree of homoplasy.

A number of characters of the buccal apparatus appear to have evolved in parallel in shelled and non-shelled sacoglossans. This is consistent with: (1) these characters are adaptations for enhancing feeding efficiency, and (2) there are constraints (phylogenetic, developmental, or other) limiting the ways in which efficiency can be increased. Feeding efficiency has apparently been enhanced by a separation of the ascending limb of the radula from the descending limb, either functionally by inserting a thick layer of transverse muscles between the two limbs, or morphologically by posterior separation of the ascus-muscle from the ventral pharynx wall. These separations have evolved in parallel in shelled and non-shelled Sacoglossa (Jensen, 1996a). A gradual narrowing of the radular teeth apparently also enhances feeding efficiency. Blade-shaped teeth evolved homoplasicly in all three major clades, whereas the specialized sabot-shaped teeth evolved only in the Limapontioidea (Jensen, 1993a,c, 1996a).

The shelled genera of the Sacoglossa form a monophyletic clade composed of three morphologically very different families, each containing two or three genera: (1) Volvatellidae with a 'large' (1–2 cm), thin, elastic, bulloid shell and white body (mantle green in some species of *Volvatella*); (2) Oxynoidae with a reduced, ± bulloid shell, large (often more than 3 cm) green body with parapodia and long 'tail'; (3) Juliidae with a bivalved shell and small (< 1.5 cm) green body. The bivalved Juliidae are more closely related to the Oxynoidae than to the Volvatellidae. The non-

Figure 2. Phylogenetic tree of sacoglossan genera based on cladistic analysis (Jensen, 1996a).

shelled genera also form a monophyletic clade; that is, the shell has only been lost once within the Sacoglossa. There are two monophyletic superfamilies within the non-shelled sacoglossans, the Placobranchoidea (= Elysioidea) and the Limapontioidea (= Stiligeroidea; = Polybranchoidea). In the parapodia-bearing family Placobranchidae, external morphology has been very conservative. The numerous species assigned to the genus *Elysia* reflect that external morphology cannot – or only with difficulty – be used to differentiate genera. Resolution in this part of the clade is poor, and most likely the genus *Elysia* will have to be subdivided. However, detailed anatomical descriptions exist for very few species, so a meaningful subdivision cannot be made at the present time. In the cerata-bearing Limapontioidea, morphology is more variable, though basically involving the formation of dorso-lateral cerata. This is reflected in the much higher number of supraspecific taxa introduced in this clade. The Polybranchiidae, which are most likely paraphyletic, are rather large animals (for sacoglossans that means more than 3 cm long), with flat, leaf-like cerata and bifid rhinophores, whereas the other families comprise mostly small species

(less than 1.5 cm), with more or less cylindrical cerata (Jensen, 1996a). Following more detailed redescriptions of some genera (e.g. *Stiliger* and *Hermaea*), many species previously assigned to these genera apparently have to be transferred to other, most likely new, genera (Jensen, 1993b). However, the phylogenetic relationships within the Limapontiidae are not fully resolved. A few species exist without a shell, parapodia and cerata; their placement has been based on apomorphic characters shared with one or the other non-shelled group. Table 1 shows the genera presently

Table 1. Families and genera of the Sacoglossa (numbers in parentheses denote the approximate number of species in the genus)

Family	Genus
Volvatellidae	*Ascobulla* (5–6)
	Volvatella (15–20)
Juliidae	*Julia* (5)
	Berthelinia (12–20)
Oxynoidae	*Roburnella* (1)
	Lobiger (4)
	Oxynoe (6–8)
Polybranchiidae	*Sohgenia* (1)
(= Caliphyllidae)	*Cyerce* (11)
	Mourgona (3–4)
	Polybranchia (8–10)
	Caliphylla (1–2)
Hermaeidae	*Hermaeopsis* (1)
	Hermaea (10–12)
	Aplysiopsis (= *Hermaeina*) (8–12)
Costasiellidae	*Costasiella* (13)
Limapontiidae	*Stiliger* (10–20)
(= Stiligeridae)	*Placida* (11–20)
	Calliopaea (2–3)
	Olea (1)
	Alderia (1–3)
	Alderiopsis (1)
	Ercolania (> 25)
	Limapontia (3)
Boselliidae	*Bosellia* (5)
Placobranchidae	*Placobranchus* (1–14)
(= Elysiidae)	*Thuridilla* (15)
	Elysiella (2)
	Tridachia (1)
	Tridachiella (1)
	Pattyclaya (2–3)
	Elysia (> 70)
Platyhedylidae	*Platyhedyle* (1)
	Gascoignella (1)

recognized, the approximate number of species in these genera, and their familial affiliations. Only the genera for which a diet has been identified and anatomical descriptions exist have been included in the present study.

Phylogeny of food plants

Current estimates of algal phylogeny are rather unstable. It is not even certain that 'algae' constitute a monophyletic group; on the contrary, there appears to be much evidence for a polyphyletic origin of the various algal divisions (see, e.g. Round, 1984). Green algae (Chlorophyta) and vascular plants, however, seem to form a monophyletic group (Pickett-Heaps, 1979). As the Sacoglossa mainly utilize this group, and almost exclusively those referred to the class Ulvophyceae *sensu* Floyd and O'Kelly (1989), this section will concentrate on these algae. In previous studies, I have used the classification of Womersley (1984) because it is based on characters of the macrothallus, which is what can be sensed by the sacoglossan herbivores. Other classifications, based on ultrastructural characters of motile cells, differ mainly in the taxonomic level assigned to various groups, and in nomenclature. The classification of Womersley (1984) is compared to that of O'Kelly and Floyd (1984) in Table 2. As the classification of Womersley (1984) separates the siphonalean groups at the ordinal level corresponding to their cell wall structure, this classification will also be used in the present study. However, it will be necessary in this section to refer to other classifications as well.

Phylogeny of the Ulvophyceae (Floyd and O'Kelly, 1989) places Ulotrichales at the base and Cladophorales at the top of the tree. The Dasycladales is placed as the sister group of the Cladophorales. This late separation is not supported by the fossil record, where Dasycladales were abundant before the Caulerpales (Elliott, 1981). No modern phylogeny exists of the taxa included in the Caulerpales *sensu* O'Kelly and Floyd (1984) (Table 2). Based on chloroplast morphology (Hori and Ueda, 1967; Calvert *et al.*, 1976), it is suggested that Derbesiales *sensu* Womersley (1984) is the most primitive taxon, having a large pyrenoid and no 'thylakoid organizing body' (TOB; Borowitzka and Larkum, 1974). Also, it is not heteroplastic. Codiales and Caulerpales both have 'tough' chloroplast envelopes (Giles and Sarafis, 1974; Trench and Ohlhorst, 1976); Codiales are not heteroplastic and the chloroplasts have no pyrenoid, nor TOB. Caulerpales have a TOB, are heteroplastic, and some have pyrenoids, others do not. This indicates that Caulerpales *sensu* Womersley (1984) are most highly derived. Figure 3 shows a phylogenetic tree based on that of Floyd and O'Kelly (1989), but with the above tentative assignment of those taxa which Floyd and O'Kelly (1989) included in the Caulerpales.

From this phylogeny, a number of inferences can be made about the ancestral thallus morphology and life history in the Ulvophyceae. Ulotrichales and Ulvales have uninucleate cells, primitively forming filamentous thalli (Womersley, 1984); this must be considered plesiomorphic. Ulotricales have heteromorphic life histories, with unicellular, microscopic sporophytes. Also, they are mostly haplontic – that is, the macrothallus is haploid (Womersley, 1984). Thus heteromorphic, haplontic life histories are most likely plesiomorphic. Ulvales and Cladophorales/Siphonocladales have diplohaplontic, isomorphic life histories – that is, haploid gametophytes are similar to diploid sporophytes. Cladophorales/Siphonocladales further have multinucleate, septate, primitively filamentous thalli; more complex thallus structures occur in the Siphonocladales. The cell walls of all of the above orders are composed of cellulose microfibrillae, in the Cladophorales/Siphonocladales organized in 'cross-fibrillar' layers (Womersley, 1984).

All of the large, siphonalean groups (i.e. Dasycladales, Codiales and Caulerpales) are diplontic (Womersley, 1984); that is, they have dispensed with the haploid thallus, and produce gametes directly from a diploid macrothallus, which must be considered apomorphic. Cell walls of Cau-

Table 2. Classifications of Ulvophyceae (taxa in parentheses are not known to be utilized by sacoglossans; ordinal level taxa not eaten by sacoglossans have been excluded)

Womersley (1984)	O'Kelly and Floyd (1984)
Ulotrichales	Ulotrichales
Ulotrichaceae	('*Eugomontia* group')
	('*Monostroma* group')
	('*Ulothrix* group')
	('*Chlorocystis* group')
	'*Acrosiphonia* group'
Ulvales	Ulvales
Ulvaceae	Ulvaceae
	(Ulvellaceae)
Cladophorales	Siphonocladales
Cladophoraceae	Cladophoraceae
(Anadyomenaceae)	(Anadyomenaceae)
Acrosiphoniales	(Siphonocladaceae)
Acrosiphonaceae	Valoniaceae
Siphonocladales	(Chaetosiphonaceae)
(Siphonocladaceae)	(Arnoldiellaceae)
Valoniaceae	
Codiales	Caulerpales
Codiaceae	Codiaceae
Caulerpales	Udoteaceae
Udoteaceae	Caulerpaceae
Caulerpaceae	Bryopsidaceae
Derbesiales	(Dichotomosiphonaceae)
Bryopsidaceae	(Ostreobiaceae)
Derbesiaceae	
Dasycladales	Dasycladales
Dasycladaceae	familial taxa not
Polyphysaceae	mentioned

lerpales are composed of xylan microfibrillae, whereas Codiales and Dasycladales have mannan cell walls, apparently without formation of microfibrillae (Womersley, 1984; Jensen, 1993a). In *Caulerpa*, thallus morphology is highly variable, but anatomy strictly monosiphonaceous. Filament rigidity of the often thick erect siphons is maintained by a network of internal trabeculae (Womersley, 1984). In the Udoteaceae, thalli usually consist of tightly interwowen, narrow siphons. In some Udoteaceae and the Codiales, thalli are pseudoparenchymatous (i.e. 1–3 orders of utricles cover central medullary filaments). This is also seen in some Dasycladales, and calcification is common in the Udoteaceae and Dasycladales (Womersley, 1984; Jensen, 1993a, 1994). Caulerpalean algae differ from all other algae by having rhizoids, which not only anchor the algal thallus on soft as well as on hard substrates (as do holdfasts of other algae), but also take up dissolved nutrients from the sediment, like the roots of angiosperms (Williams, 1984). The rhizoids also seem to have a sediment-stabilizing function. Also, many species have horizontal, more or less
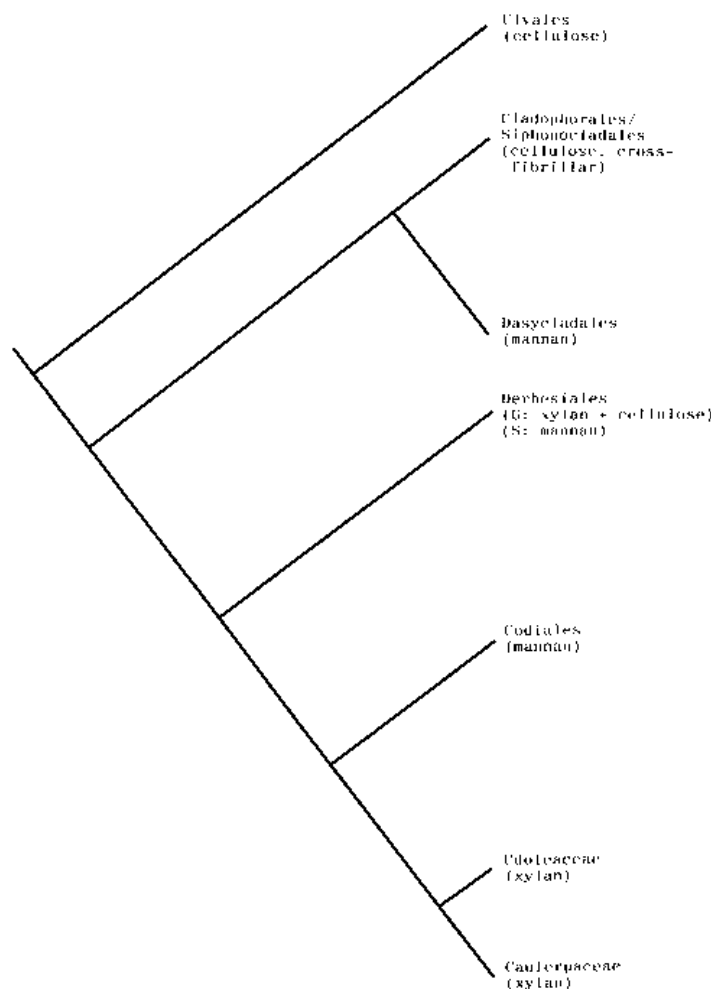
Figure 3. Phylogenetic tree of the Ulvophyceae. Based on Floyd and O'Kelly (1989), with addition of ordinal level taxa *sensu* Womersley (1984) (see text). The order Caulerpales *sensu* Womersley (1984) has been split into families Udoteaceae and Caulerpaceae. Cell wall compositions are indicated on the figure. G, gametophyte; S, sporophyte.

sediment-covered stolons. New, erect thalli are formed from the growing tips of these stolons. Other specializations are cytoplasmic streaming, indicating intra-thallus translocation of nutrients and photosynthates, viscous cytoplasm, and wound plug formation (also in Codiales, Derbesiales, Dasycladales and Siphonocladales) (Clark and DeFreese, 1987; Jensen, 1993a).

   The Derbesiales are difficult to categorize, both with respect to life history and cell wall composition. Their life histories are diplohaplontic and in most cases heteromorphic. The cell walls of the gametophytes are composed of xylan and cellulose, those of the sporophytes of mannan. However, the two families included differ with respect to size of the various phases: in Derbesiaceae, the gametophyte is microscopic and vesiculate, the sporophyte tufted and filamentous; in Bryopsidaceae, the gametophyte is branched and filamentous, whereas the sporophyte is a uninucleate, creeping filament (Womersley, 1984). The latter is similar to the plesiomorphic condition,

and the Derbesiaceae must be considered derived. From the above it would seem that the Dasycladales should be more closely related to the Codiales and Caulerpales, which is also supported by the absence of quadriflagellate zoospores in these three orders (O'Kelly and Floyd, 1984).

The following evolutionary trends in the Ulvophyceae seem certain: (1) from haplontic through diplohaplontic to diplontic; (2) from heteromorphic to isomorphic (or loss of haploid phase); (3) from uninucleate to multinucleate (septate or coenocytic); (4) from filamentous to pseudo-parenchymatous; and (5) from cellulose to xylan or mannan as structural cell wall polysaccharide. The latter character appears to have gone through a rather complex series of transformations, possibly involving homoplasies.

Ecological associations

The phylogenetic aspect of the association between the Sacoglossa and their food plants was noted early (Kay, 1968; Clark and Busacca, 1978). First, gradual radiation, from *Caulerpa* through other Caulerpales to other green algae and, in a few cases, more 'exotic' diets (e.g. diatoms, seagrasses, opisthobranch eggs), was assumed (Clark and Busacca, 1978; Jensen, 1980). This was supported by the fact that all shelled Sacoglossa feed exclusively on *Caulerpa*, and that several non-shelled species, from the Placobranchoidea as well as the Limapontioidea, feed on this algal genus. Later, two main radiations were hypothesized: (1) from *Caulerpa* to other Caulerpales (= Udoteaceae), and (2) from Udoteaceae through Derbesiales to either Codiales/Dasycladales or Cladophorales/Siphonocladales (Jensen, 1993a,c,d, 1994). This scenario was supported by the correlation noted between the shape of the radular teeth and cell wall composition of the food plants, with triangular teeth being almost exclusively associated with cell walls composed of xylan (Caulerpales), and sabot-shaped teeth being associated exclusively with cellulose cell walls and, with the exception of one species, of the 'cross-fibrillar' type (Cladophorales and Siphonocladales) (Jensen, 1993a). Derbesiales with heteromorphic life histories, in which cell wall composition differs between sporophyte and gametophyte, were thought to have been instrumental in the transition from Caulerpales to either Codiales/Dasycladales or Cladophorales/Siphonocladales (Jensen, 1993a,c, 1994). These findings indicate that 'resource tracking' of cell wall composition has been an important factor in the evolution of the Sacoglossa. This will be examined based on the recent phylogenetic analysis.

Sacoglossan diets were first reviewed by Jensen (1980), and again by Jensen (1993a). These reviews, and other publications (e.g. Jensen, 1981, 1983; Clark and DeFreese, 1987; Bleakney, 1989, 1990; Jensen and Wells, 1990; Trowbridge, 1991, 1993), have shown that most sacoglossans will eat several or all species of a particular algal genus. Some species are more euryphagous and include algae of two or more genera in their diets (Jensen, 1983, 1989b, 1991b, 1993a; Bleakney, 1989; Trowbridge, 1991). Thus the associations are not as strictly species to species as in insect–plant or host–parasite associations (Brooks and McLennan, 1991; Page, 1994). Considering the general stenophagy of individual species and the wide variety of diets among different species of Sacoglossa, it may be hypothesized that speciation has been related to changes in diet. This will be examined below.

The associations between the Sacoglossa and their food plants are illustrated in Fig. 4. Besides the two obvious conclusions that all shelled sacoglossans feed on *Caulerpa* and that the most plesiomorphic non-shelled sacoglossans feed on Udoteaceae, several other conclusions can be made: (1) most genera of Sacoglossa are restricted to one green algal order; (2) in the Placo-branchoidea, diet radiation is almost entirely restricted to the genus *Elysia*; (3) in the Lima-
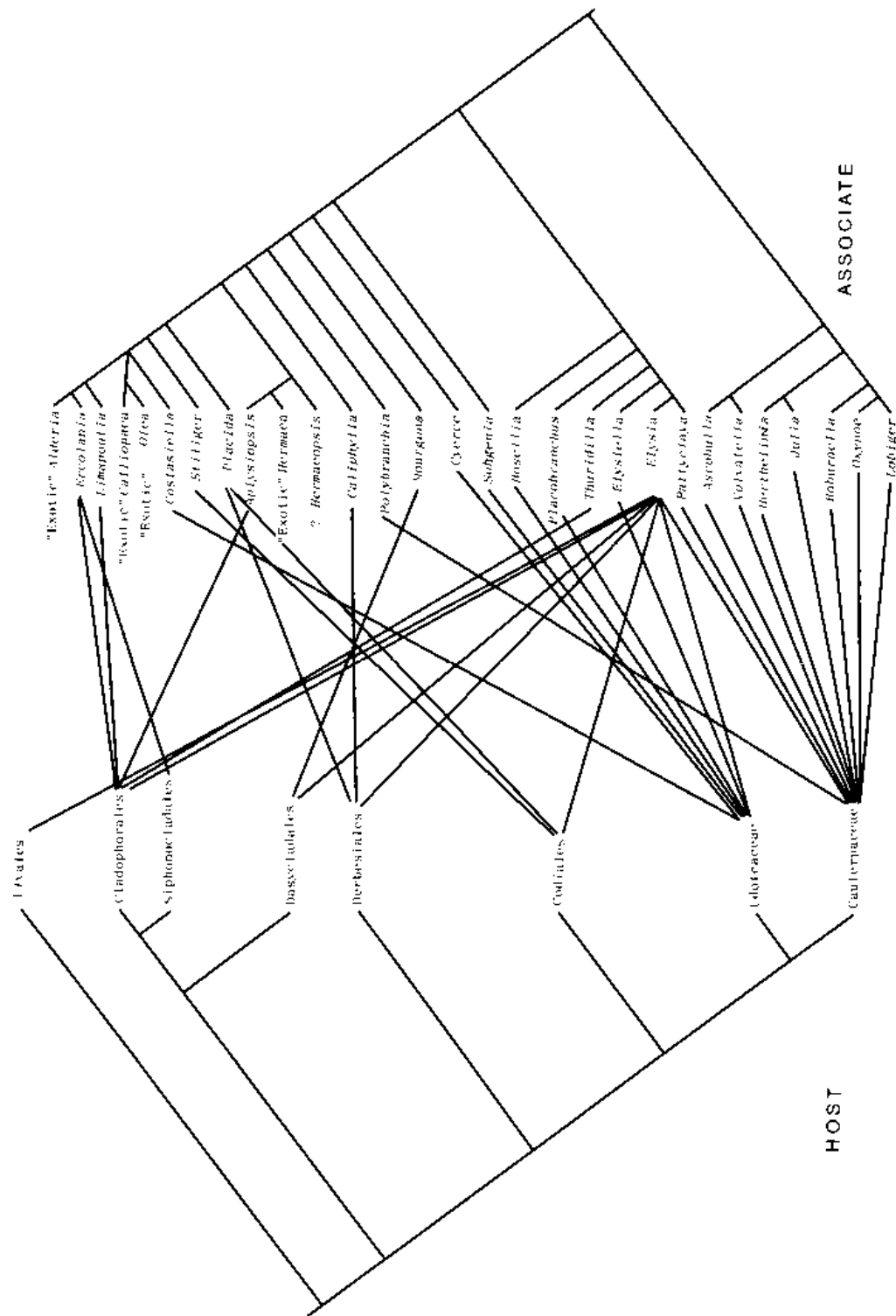
Figure 4. Associations between genera of the Sacoglossa and their diets (hosts) within the Ulvophyceae. 'Exotic' denotes a non-Ulvophycean diet (see text) and '?' an unknown diet. Information on diets from Jensen (1993a,b).

pontioidea, the majority of genera feed on non-caulerpalean diets; and (4) the non-shelled sacoglossans that feed on *Caulerpa* are highly derived.

Buccal regurgitation is a process whereby algal cytoplasm already sucked out is regurgitated into the algal siphon, probably mixed with saliva. This occurs in many non-shelled sacoglossans, but has not been recorded in any species feeding on Caulerpales. It is particularly common in species feeding on *Bryopsis* (Jensen, 1981; Gascoigne, 1983) (Table 3). The absence of buccal regurgitation in species feeding on Caulerpales indicates that this is not an adaptation to viscous cytoplasm or to prevent wound plug formation, but possibly to soft, thin, easily collapsing filaments.

Functional kleptoplasty (= *chloroplast symbiosis*)

The sacoglossan opisthobranchs are well known for their ability to take up functional chloroplasts from their food plants and maintain them in the cells of the digestive gland (Muscatine and Greene, 1973; Trench, 1975; Clark *et al.*, 1990). Kay (1968) speculated that functional kleptoplasty was what allowed the sacoglossans to radiate to other diets than *Caulerpa*. However, studies on the distribution of functional kleptoplasty have revealed that features of the chloroplasts as well as of the sacoglossans are important in determining whether sequestered chloroplasts will be functional over any length of time (Muscatine and Greene, 1973; Hinde and Smith, 1974; Clark *et al.*, 1981, 1990). Functional kleptoplasty occurs only in the non-shelled Sacoglossa. However, morphologically intact chloroplasts have been described from the digestive gland cells of some shelled species (Clark *et al.*, 1990). Acquisition of functional chloroplasts may enable the animals to survive periods of food shortage, and allow them to search for and test other food sources. Clark *et al.* (1990) suggested that functional kleptoplasty is plesiomorphic in the non-shelled Sacoglossa, and that a partial suppression of the immune recognition and/or transfer of a few plastid genes to the genome of the non-shelled sacoglossan ancestor might be responsible for the transition from non-functional to functional retention of chloroplasts. However, functional kleptoplasty has not been described from the most plesiomorphic Limapontioidea (Clark *et al.*, 1990).

Kleptoplasty is a sacoglossan 'innovation', though some nudibranchs are capable of taking up functional zooxanthellae from the coral polyps they feed on (Rudman, 1981). The capability of sequestering viable microorganisms or organelles seems to be widespread, and thus probably ancestral, in the molluscs: *Tridacna* harbouring zooxanthellae (Muscatine and Greene, 1973); *Thyasira*, sulphur-oxidizing bacteria (Dando and Southward, 1986); *Calyptogena*, chemoautotrophic bacteria (Cavanaugh, 1983); nudibranchs, nematocysts of cnidarian food, and in some cases zooxanthellae; and sacoglossans, chloroplasts from their food plants. Most recently, bacterial symbionts have been described from the epidermis of a monoplacophoran (Haszprunar *et al.*, 1995).

Table 3. Sacoglossans for which buccal regurgitation has been described

Species	Diet	Reference
Elysia ornata	*Bryopsis*	Jensen (1981)
Elysia filicauda	*Acetabularia*	Jensen and Wells (1990)
Caliphylla mediterranea	*Bryopsis*	Gascoigne (1983)
Hermaea bifida	*Griffithsia*	Gascoigne (1983)
Placida dendritica	*Bryopsis*	Brüel (1904)
Placida kingstoni	*Bryopsis*	Jensen (1981)
Ercolania funerea	*Chaetomorpha*	Jensen (1981)

Functional kleptoplasty is correlated with the morphology of the digestive gland. In the shelled sacoglossans, the digestive gland is a dense mass of fine tubules enclosed within the shell. Such a 'holohepatic' arrangement is also seen in the non-shelled *Cyerce*. In some species of the shelled genera *Volvatella* and *Berthelinia*, the digestive gland sends branches into the mantle fold (Jensen, 1991a; Clark, 1992); this is considered preadaptive for the acquisition of functional kleptoplasty. Limapontioids with long main ducts and rather wide lumina of the digestive gland tubules generally have short-term functional kleptoplasty, whereas most placobranchoids with short main ducts and a densely branching system of narrow-lumined digestive gland tubules have long-term functional kleptoplasty (Clark *et al*., 1990). This has been interpreted as the limapontioids being evolutionarily intermediate between the shelled sacoglossans and the placobranchoids (Muscatine and Greene, 1973). However, newly metamorphosed placobranchoids also have long main ducts and few wide-lumined digestive gland tubules; only after feeding for several days do the fine, narrow-lumined digestive gland tubules develop, possibly as a response to beginning retention of functional chloroplasts (Clark *et al*., 1979; Clark, 1992). Placobranchoids feeding on algae having 'unsuitable' chloroplasts do not have long-term functional kleptoplasty (Clark *et al*., 1990), though they do have short main ducts of the digestive gland. Cladistic analysis has shown that the Limapontioidea and Placobranchoidea are sister groups (Jensen, 1996a).

Functional kleptoplasty may also be correlated with another organ system found only in the non-shelled Sacoglossa, namely the system of dorsal branching vessels entering the reno-pericardium. The function of these vessels is unknown, but it has been proposed that they act as a 'negative gill', transporting photosynthetically produced oxygen away from, and supplying carbon dioxide to, chloroplast-harbouring tissues (Clark *et al*., 1981).

Noxious secondary metabolites

Siphonalean green algae are known to produce several noxious secondary metabolites, which act as feeding deterrents of generalist herbivores (Norris and Fenical, 1982; Paul and Fenical, 1986; Paul and Hay, 1986). Sacoglossans may sequester, concentrate and/or modify these metabolites (Paul and Van Alstyne, 1988; Hay *et al*., 1989, 1990; Cimino *et al*., 1990). This has led to speculation of an evolutionary 'arms race' in the sacoglossan-siphonalean associations (Clark, 1992). Unfortunately, very few algae and even fewer sacoglossans have been examined.

The most potent secondary metabolite of *Caulerpa* is the sesquiterpenoid caulerpenyne (Paul and Fenical, 1986). Caulerpin and caulerpicin occur in the highest concentrations in those species of *Caulerpa* which are most favoured by sacoglossans (Vest *et al*., 1983). Thus these substances may have evolved as a specialized defence against sacoglossan herbivores, though the sacoglossans have at the present time developed a tolerance for these substances. Although this relationship may count as an 'arms race', it does not appear to be related to phylogeny of the sacoglossans, and probably not of *Caulerpa* spp. either. It would be interesting to compare concentrations of these metabolites with density of sacoglossans, and to test the toxicity of caulerpin and caulerpicin on sacoglossans not usually feeding on *Caulerpa*.

Many sacoglossans are also capable of synthesizing *de novo* noxious metabolites, which may be more potent feeding deterrents than some of the algal metabolites; this also occurs in species feeding on algae which apparently are not chemically defended (Ireland and Faulkner, 1981; Dawe and Wright, 1986; Roussis *et al*., 1990; Di Marzo *et al*., 1991; Vardaro *et al*., 1992a,b). Many of these are polypropionate derivatives, which are also found as defensive metabolites in other opisthobranchs, herbivorous as well as carnivorous (Avila, 1992). Thus the metabolic pathways synthesizing polypropionates may be plesiomorphic within the Sacoglossa. The algal metabolites are mostly terpenoids or aromatics (Hay and Fenical, 1988).

Cerata in the Limapontioidea may have first evolved as defensive structures. In *Cyerce* (and the poorly known *Sohgenia*), the digestive gland does not extend into the cerata (Marcus, 1982; Jensen, 1996a). The cerata are easily autotomized when the animal is roughly handled, and in some species the cerata have been shown to store higher concentrations of defensive metabolites than the remaining body (Jensen, 1984; Di Marzo *et al.*, 1991). Cerata are quickly regenerated, and at least one of the secondary metabolites synthesized by a sacoglossan shows an enhancing effect on regeneration (Di Marzo *et al.*, 1991). Vardaro *et al.* (1992b) suggested that evolution within the Sacoglossa might be linked to their ability to synthesize polypropionates.

Co-evolution

Rigorous application of cladistic methodology in co-evolution analysis requires fully resolved phylogenies of the 'hosts' as well as the 'associates'. Although this prerequisite is not fulfilled for the associations between sacoglossan opisthobranchs and their food plants, an attempt will be made to evaluate the contributions of the processes responsible for co-evolution.

Comparing the cladograms for the siphonalean algae and the Sacoglossa (Fig. 4) clearly shows that co-speciation is not a very likely process in the evolution of either hosts or associates; the most primitive sacoglossans feed on the most advanced siphonalean algae, Caulerpales *sensu* Womersley (1984). Also, no matter how algal phylogeny is arranged, it is obvious that the sacoglossans with diets of Xanthophyta, Rhodophyta, diatoms, seagrasses or opisthobranch eggs cannot have co-evolved with their diet. These species most likely evolved through 'host switching' (Brooks and McLennan, 1991).

An attempt to 'reconcile' (Page, 1994) the phylogenetic trees of the sacoglossan herbivores and their algal 'hosts' results in one possible event of co-speciation, namely at the base of the saco-glossan phylogenetic tree, where the division into the shelled Oxynoacea and the non-shelled Placobranchacea may have coincided with the division of the Caulerpales into Caulerpaceae and Udoteaceae. The division of the two major non-shelled clades apparently was not accompanied by a switch in diet, at least not at the host family level; the most primitive members of both clades feed on Udoteaceae. Thus speciation at this node was associated with diet duplication (Page, 1994).

To reconstruct the evolutionary steps leading to the associations of present-day sacoglossans and their algal hosts, it is necessary to determine the most likely diets of the ancestors. This is done by mapping the diets of the terminal taxa onto the cladogram (Fig. 5), and then tracing the diets of the internal nodes (Page, 1994).

If diets are plotted on the cladogram of the non-shelled sacoglossan genera, it is seen that in most cases diet is similar within a particular genus. Hence 'host switching' appears to have been a rare event in speciation in extant genera. This probably also applied to previous speciation events (i.e. supraspecific taxa).

If the internal nodes of the Limapontioidea (Fig. 5) are labelled L1–L14, it is seen that the diet of ancestor L14 could have been Xanthophyta or Cladophorales/Siphonocladales; as ancestor L13 almost certainly fed on Cladophorales, this was most probably also the diet of ancestor L14.

Ancestor L12 ate opisthobranch eggs, whereas the diet of ancestor L11 is more difficult to determine. Also the diet of ancestor L10 is ambiguous. Ancestor L9 almost certainly ate Derbesiales, and if the principle of 'resource tracking' of cell wall composition is followed, then ancestors L10 and L11 most likely also fed on Derbesiales – that is, speciation was connected with duplication of diet. Within the genus *Stiliger*, host switching to *Codium* took place, and the genus *Costasiella* almost exclusively feeds on the udoteacean *Avrainvillea*. The latter is probably a sec-ondary reversal to Udoteaceae, or it may indicate that *Costasiella* should be placed closer to the
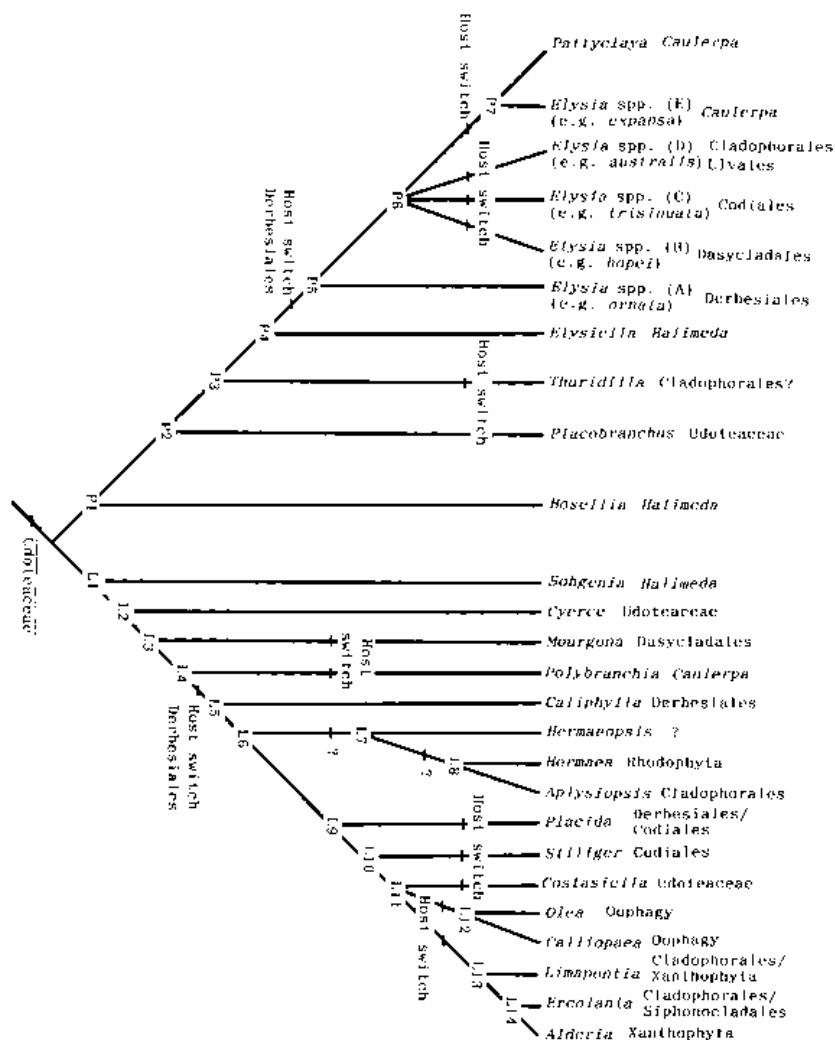
Figure 5. Cladogram indicating diets of extant genera of the Placobranchacea and derivation of ancestral diets. Nodes within the Placobranchoidea are marked P1–P7 and nodes within the Limapontioidea are marked L1–L14. Host switches at ordinal (family within Caulerpales) level have been indicated. '?' indicates questionable site of host switch.

Polybranchiidae. The latter may also be supported by some morphological characters; this node is weakly supported in the cladistic analysis.

Ancestor L6 most likely also fed on Derbesiales. Unfortunately the diet of *Hermaeopsis* is unknown, but host switching to Cladophorales could have happened either in ancestor L7 or L8. *Aplysiopsis* feeds on Cladophorales and *Urospora* (Gonor, 1961; Jensen, 1993a), and the majority of *Hermaea* species feed on red algae (Vogel, 1971; Schmekel and Portmann, 1982; Jensen, 1993a,c). Ancestor L5 ate Derbesiales, and *Caliphylla* duplicated this diet. Ancestors L3 and L4 could have eaten Udoteaceae, but they could also have eaten Derbesiales – still with xylan cell walls in the gametophyte. *Mourgona* then switched to Dasycladales with mannan cell walls, and *Poly-branchia* switched to *Caulerpa*, still with xylan cell walls. If ancestor L3 ate Udoteaceae, then

Mourgona switched directly to mannan cell walls, skipping the heteromorphic, intermediary Derbesiales. In this case, ancestor L4 most likely also fed on Udoteaceae.

To determine which of these ancestral diets were most likely, one can look at the accompanying morphological changes. In ancestor L3, the digestive gland sends branches into the cerata, the ascus-muscle separates from the pharynx posteriorly, and the anus moves to a lateral position, below the cerata (Jensen, 1996a). In ancestor L5, blade-shaped teeth develop; also buccal regurgitation and functional kleptoplasty occurs here (Jensen, 1981, 1996a; Clark *et al.*, 1990). Unfortunately, very few observations exist of feeding in *Mourgona* and *Polybranchia* (Jensen, 1981; Clark, 1994; Hamatani, 1994), so it cannot be completely ruled out that buccal regurgitation and functional kleptoplasty occur in these genera. It should be noted that in the Limapontioidea, pharyngeal pouches appear to have been lost subsequently to the evolution of blade-shaped teeth and buccal regurgitation.

The above scenario supports fairly well the hypothesis that the switch to Derbesiales occurred in ancestor L5 and that 'Stiliger' *smaragdinus*, which almost certainly is not a *Stiliger* (Jensen, 1996a), and *Costasiella* 'reverted' to Caulerpales. Host switching to Cladophorales has taken place in two places, L7 or L8 and L13, and host switching to algae with mannan cell walls has taken place in three genera, *Mourgona* to Dasycladales, and *Stiliger* and some species of *Placida* to *Codium*.

Labelling the internal nodes of the Placobranchoidea P1–P7 (Fig. 5) gives the result that ancestors P1–P4 ate Udoteaceae; speciation at these nodes was by duplication. Host switching has taken place within the genus *Thuridilla*, the Mediterranean *T. hopei* feeding on *Cladophora vagabunda* (Marin and Ros, 1988). Unfortunately, this is the only record of diet within the genus *Thuridilla*. Thus it cannot be unequivocally determined that host switching was directly from Udoteaceae to Cladophorales, or whether there have been intermediate transitions. However, of all the sacoglossans feeding on Cladophorales, *T. hopei* is the only species with triangular, denticulate teeth and a pharyngeal pouch. The position of the genus *Elysiella* within the Placobranchidae is somewhat ambiguous (Jensen, 1996a). Here it has been placed before the separation of the genus *Elysia*, because it has retained triangular teeth, and one species has a penial stylet. In the strict consensus tree of Jensen (1996a), it formed the sister taxon of ancestor P7 due to its posession of a long renal ridge, which also occurs homoplasicly in the limapontioid genus *Ercolania*; hence *Elysiella* has been moved in the present study.

Ancestor P5 switched to Derbesiales, and *Elysia ornata* duplicated this diet. Several host switches have taken place within the genus *Elysia*, thus possibly forming a true adaptive radiation. Only further resolution of the cladogram can elucidate whether switching to Codiales and Dasycladales was within one clade, and happened only once; the same is true for switching to Cladophorales. However, ancestor P6 (which is artificial because it gives rise to an unresolved polytomy) most likely still fed on Derbesiales. Ancestor P7 switched to *Caulerpa* and both descendants duplicated this diet.

The above scenario supports the earlier proposed hypothesis that host switching to Derbesiales took place at the base of the genus *Elysia*. Blade-shaped teeth and buccal regurgitation occurred in ancestor P5, thus coinciding with host switching to Derbesiales, as in the Limapontioidea. However, functional kleptoplasty has been described in *Bosellia* and *Placobranchus* (Muscatine and Greene, 1973; Clark *et al.*, 1990), and pharyngeal pouches were lost in ancestor P4 – that is, prior to the evolution of blade-shaped teeth (Jensen, 1996a).

The analysis of ancestral diets supports the key position of Derbesiales in the evolution of the Sacoglossa. The present-day Derbesiales is a rather small group of uncalcified, fine filamentous forms with heteromorphic life histories in which cell wall structure differs between gametophyte and sporophyte. This could be a response to sacoglossan predation. Heteromorphic life cycles are plesiomorphic within the Ulvophyceae, but differences in cell wall composition have not been

recorded outside the Derbesiales, though gametangia of some Dasycladales have cellulose cell walls and the vegetative parts mannan cell walls. No toxic secondary metabolites have been recorded from Derbesiales (see Hay and Fenical, 1988, for a review). It is significant that in some Derbesiales, the sporophyte forms the microthallus and in others it is the gametophyte which is microscopic. This could further 'confuse' sacoglossan predators. Very few present-day sacoglossans feed on Derbesiales, but apparently many ancestral species had this diet.

To test whether an elevated diversification rate ('adaptive radiation') has taken place in the non-shelled Sacoglossa following diet switch to non-udoteacean algae, the probability of trees being as skewed or more skewed than those obtained by cladistic analysis has been calculated (Slowinski and Guyer, 1989). According to this test, the probability of obtaining the configuration of any given tree corresponds to $2(n-1)^{-1}$, if the numbers of species on either side of the basal node are different. The cumulative probability of obtaining a tree equally or more skewed than a given configuration is: $P_{cum} = 2r(n-1)^{-1}$, where r is the number of species on the side of the basal node containing the smaller number of species. Although phylogenetic analysis has not been carried out to species level in the Sacoglossa, it has been assumed that species cluster within the analysed genera, and the numbers of species in the genera listed in Table 1 have been used in the calculations. P_{cum} values have been calculated for the branches emerging from node L6 in the Limapontioidea, and for the branches emerging from node P4 in the Placobranchoidea. These were then compared with the P_{cum} values for nodes without diet changes (i.e. the entire Limapontioidea versus the entire Placobranchoidea, and Volvatellidae versus the higher Oxynoacea). In the two non-shelled clades, the nodes with diet switches are also those in which blade-shaped teeth develop; the two shelled clades also represent the change to blade-shaped teeth, though with no diet switching. As seen in Table 4, P_{cum} values for nodes with diet switching are much lower than for nodes without diet switching, thus supporting the occurrence of adaptive radiation following diet switching. A Mann-Whitney U-test comparing P_{cum} values for nodes with and without diet switching gave borderline significance values ($U = 36.5$, $z = 1.614$, $P = 0.106$ for 'yes' plus 'yes?' against 'no' and 'no?' in Table 4; and $U = 37$, $z = 1.933$, $P = 0.053$ for 'yes' plus 'yes?' and 'no?' against 'no').

Origin of the Sacoglossa

Recent phylogenetic analysis has shown that the closest sister group of the Sacoglossa is the monogeneric Cylindrobullidae, and that there is a considerable morphological 'gap' between the Sacoglossa–Cylindrobullidae clade and the remaining Opisthobranchia; the relationship to other opisthobranchs remains unresolved (Jensen, 1996a,b).

Below the fossil record of the Sacoglossa and earlier, possibly related groups – and also the siphonalean green algae – will be summarized. Also, the 'primitive' Sacoglossa will be compared with the Cylindrobullidae to establish the origin of the group and the selective regime under which it evolved.

Fossil record

Fossils of opisthobranchs date back to the Carboniferous (Bandel, 1988; Bieler, 1992). These fossils are tall-spired *Acteon*-like species with rather solid shells. Thin-shelled opisthobranchs with flattened or even concave apical areas, as in the anaspidean (= Aplysiomorpha) *Akera*, are known from the Upper Triassic (Haas, 1953). One genus has actually been named *Cylindrobullina*. The organization of the columella and the patterns of breakage in these fossil shells, especially the subgenus *Euconactaeon* (Fig. 6), are very similar to those observed in *Akera*, *Cylindrobulla* and *Ascobulla*.
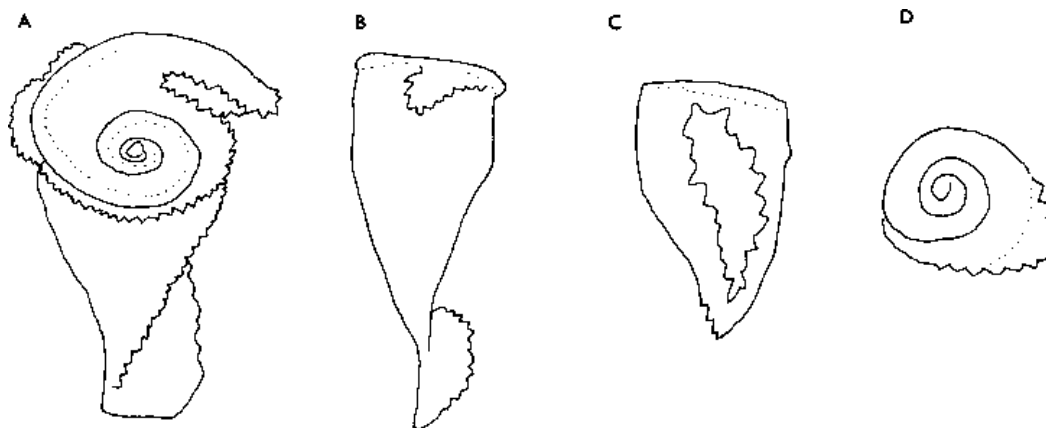
Figure 6. Outline of fossil opisthobranchs similar to Volvatellidae and Akeridae, from Upper Triassic. Drawings traced from plate in Haas (1953). (A, B) *Cylindrobullina* (*Euconactaeon*) *ninacacana*; (C, D) *C.* (*E.*) *tambosolensis*.

The first definite sacoglossan fossils are the shells of the bivalved *Berthelinia* from the Eocene of the Paris Basin (Keen and Smith, 1961). Other fossil bivalved sacoglossans are known from the Middle Eocene (the extinct *Ludovicia*), Upper Eocene (the extinct *Anomalomya*) and Miocene (the extant *Julia*) (Keen and Smith, 1961; Kay, 1968). Due to the thin shells of the Sacoglossa, the fossil record is very sparse. Also, the dominant habitat – shallow water with strong water movement and well-aerated, coarse sediment – is not conducive to fossilization. Finally, shelled species are only a fraction of the total number of species.

The occurrence in the Eocene of shells identifiable with those of an extant genus implies that at this time *Caulerpa* existed (Clark, 1992). Unfortunately, most species of *Caulerpa* are uncalcified and hence have not left a fossil record. Fossils of the udoteacean alga *Halimeda* date back to the Cretaceous (Hillis-Colinvaux, 1980), though 'halimediform' algae appeared in the Triassic (Elliott, 1981). By the Middle Jurassic, udoteacean algae (with a fossil record) occurred only in non-tropical areas, and in the Upper Jurassic they were rare (Elliott, 1965; Flügel, 1988). The other fossil udoteacean algae (e.g. *Bouenia*, *Leckhamptonella* and *Arabicodium*) disappeared at the latest in the early Tertiary (Elliott, 1965). Calcareous algae of the order Dasycladales have an even longer fossil history, though their successful dispersal was associated with the Tethyan transgression (Elliott, 1981; Flügel, 1988).

When did the Sacoglossa first appear?

The fossil record of siphonalean algae would indicate that the first real sacoglossans evolved at the latest in the Cretaceous – that is, when the genus *Halimeda* first appeared in the fossil record. However, they may have existed all through the Jurassic, if *Caulerpa* evolved before *Halimeda*. If the separation of the shelled and non-shelled sister groups of Sacoglossa coincided with the separation of Udoteaceae and Caulerpaceae, then the Sacoglossa probably appeared before the rather advanced udoteacean *Halimeda*. Unfortunately, it is not possible to determine whether the extinct udoteacean algae form a monophyletic clade with the extant Udoteaceae, or whether they speciated before the separation of the Caulerpaceae; it is also possible that the Caulerpaceae evolved from one of the extinct lines (Fig. 10).

What was the ancestral sacoglossan like?

To infer ancestral ecological associations, it is necessary to compare the least derived 'ingroup', here represented by the family Volvatellidae, with the closest 'outgroup', in this case the family Cylindrobullidae. Only four species are presently referred to the genus *Cylindrobulla* (Jensen, 1989a). Externally, *Cylindrobulla* is very similar to the sacoglossan genus *Ascobulla*, so much so that for many years they were considered one genus (Marcus, 1972; Jensen, 1989a). Conchologically they are almost identical and also the pallial organs share several synapomorphies, for example the long, lamellate gill on the surface of the kidney and the diagonal shell adductor muscle. It has been suggested that the Cylindrobullidae be included in the Sacoglossa (Thompson, 1976; Boss, 1982; Vaught, 1989; Clark, 1994). However, the buccal apparatus differs sufficiently from the sacoglossan system that this inclusion is not warranted (Jensen, 1996a,b). The pharynx of *Cylindrobulla* is small and not particularly muscular, and it does not have muscular pouches. The odontophore is long and attached posteriorly. Several teeth of the 'ascending limb' are free on the dorsal surface of the odontophore, and the teeth of the long 'descending limb' remain on the ventral surface of the odontophore; that is, they do not descend into a ventral, epithelium-lined pocket, as in the Sacoglossa (Jensen, 1996b). In most species of *Ascobulla* and *Volvatella*, the odontophore does not reach the pharyngeal opening, and the descending limb of the radula is rather short. In some species of *Volvatella*, it descends almost vertically into the short pocket (i.e. it is not on the ventral surface of the odontophore); in the Sacoglossa, the odontophore is attached to the pharyngeal wall laterally as well as ventrally (Jensen, 1996a). The radular teeth of *Cylindrobulla* are very small and not distinctively unicuspid (Jensen, 1989a). Articulation is less well developed than in the Sacoglossa, and the cusps are not interlocking. Although some used teeth are often found in a heap around the posterior part of the descending limb, a distinct ascus is not formed, and preradular teeth have never been observed (Jensen, 1996b). All shelled Sacoglossa have a long, rod-shaped preradular tooth, and the non-shelled Sacoglossa have several small preradular teeth without cusps (Jensen, 1996a).

Very little is known about the ecology of *Cylindrobulla*. It burrows shallowly in coarse, well-aerated sediment, often in association with the rhizoids of the siphonalean, calcareous green alga *Halimeda* (Jensen, 1989a; Clark, 1994). Feeding has not been observed, nor has copulation or spawning. It is highly unlikely that the short teeth of *Cylindrobulla* can be used to penetrate algal cell walls; if they should succeed in gradually scraping away a section of the cell wall, only a small amount of cytoplasm will be expelled before a wound plug is formed. Clark (1992) hypothesized that *Cylindrobulla* might feed on wound plugs.

The synapomorphies delimiting the Sacoglossa from the Cylindrobullidae are almost all connected to the anterior alimentary system (i.e. the pharyngeal musculature and radula) (Jensen, 1996a). It is unlikely that these synapomorphies developed independently, one after the other; in modern Sacoglossa, they form a functional entity (but as they have diversified separately in different sacoglossan clades, they have been treated as separate characters). Radula suspending muscles probably developed gradually as the size of the teeth, especially the cusps, increased, and it became necessary to position, pivot and retract the teeth with precision in the process of piercing the algal cell wall. It is apparently the acquisition of these 'key innovations' which have enabled the Sacoglossa to speciate profusely, whereas the Cylindrobullidae has remained a monogeneric group. P_{cum} (Slowinski and Guyer, 1989) for the Cylindrobullidae versus the Sacoglossa is as low as 0.0287, indicating a diversification pattern consistent with an adaptive radiation in the Sacoglossa.

From the above, some interesting questions arise, which I shall attempt to answer below: Was the ancestral sacoglossan burrowing? Was it suctorial? Was it stenophagous? Was *Caulerpa* the ancestral diet?

Was the ancestral sacoglossan burrowing?

Mapping habitats on a cladogram that includes the sister group of the Sacoglossa (Fig. 7), estimates that the common ancestor of the two non-shelled superfamilies was epifaunal; also, the common ancestor of the Juliidae and Oxynoidae is estimated to be epifaunal. Nutritional homochromy (i.e. green bodies) also occurred in this ancestor. The common ancestor of *Cylindrobulla* and the Sacoglossa, on the other hand, was most likely burrowing, as this is plesiomorphic in the Opisthobranchia (Brace, 1977). If the rhinophores of the higher Oxynoacea and the Placobranchacea are homologous, then they were probably present in the common ancestor. In this case, the common ancestor (node 2) is suggested to be epifaunal. In the Volvatellidae, rhinophores are absent, and *Ascobulla* and some species of *Volvatella* are burrowing (Thompson, 1979; DeFreese, 1987; K.R. Jensen, unpublished observation). *Ascobulla* even has a cephalic shield, as in *Cylindrobulla* and other, more distantly related, burrowing opisthobranchs. This indicates that the common ancestor (node 4) was burrowing. From the above, the common ancestor of the Volvatellidae and the higher Oxynoacea (node 3) was most likely epifaunal. The extensive rhizoids and horizontal stolons of most Caulerpales permit suctorial herbivores to feed while buried in the sediment. Several species of *Ascobulla* and *Volvatella* prefer feeding on the stolons and rhizoids of *Caulerpa* (Gascoigne and Sartory, 1974; Jensen, 1981; K.R. Jensen, unpublished observation). However, most species of *Volvatella*, including all those with green mantle folds, are epifaunal, and even *Ascobulla* ventures up among the assimilators of *Caulerpa* to feed (Jensen, 1981; Clark, 1992). *Cylindrobulla* is apparently associated with the non-photosynthetic, uncalcified rhizoids of *Halimeda* (Jensen, 1989a; Clark, 1994).

Was the ancestral sacoglossan suctorial?

The synapomorphies of the buccal apparatus accumulated in the ancestral sacoglossan (Jensen, 1996a) can all be interpreted as adaptations to suctorial feeding. It is unknown whether the now extinct udoteacean algae had xylan cell walls, as do the extant Caulerpales, though the phylogenetic position of Caulerpales (Fig. 3) indicates that this was the case. However, they were coenocytic, filamentous and calcified (Elliott, 1965). Cellulose cell walls are plesiomorphic within the Ulvophyceae, and cellulose also occurs in the cell walls of many red and brown algae (Parker,
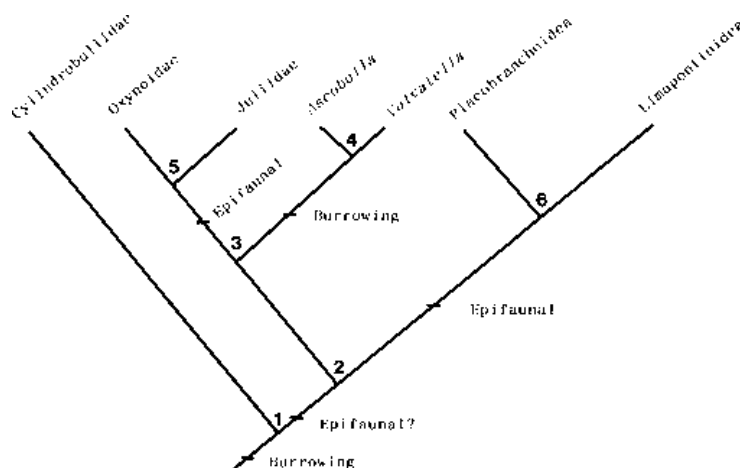

Figure 7. Cladogram outlining ancestral habitats at basal nodes of the Sacoglossa and its sister group, Cylindrobullidae. Internal nodes have been labelled 1–6.

1970). Cellulose may be broken down by symbiotic microorganisms in the digestive systems of herbivores. I have not seen any records of xylan- or mannan-degrading microorganisms in the digestive systems of marine herbivores. Thus the change in cell wall composition to xylan and mannan could be a response to predation by generalist macroherbivores (gastropods, echinoids, etc.). The first anti-predatory response may have been calcification. However, calcification alone could be overcome by increasing crushing capability of predator buccal apparatus, but if a major proportion of the plant tissue remains indigestible, the herbivore will probably find another diet. The specialization of a piercing-suctorial feeding mechanism would overcome the impediment of indigestible cell walls, and may have been the main impetus for the evolution of the Sacoglossa.

Though it is very likely that present-day volvatellids have further improved the ancestral sacoglossan buccal apparatus, there can be little doubt that the large, unicuspid, articulating and interlocking radular teeth have evolved as adaptations to piercing algal cell walls. The teeth of the ancestral sacoglossan may not have had as long cusps as those of present-day volvatellids (Fig. 8A); they may have been more similar to those of the non-shelled *Bosellia mimetica* (Fig. 8B), which feeds on *Halimeda*.

Was the ancestral sacoglossan stenophagous?

The phylogenetic analysis clearly indicates that the ancestral sacoglossan was restricted to feeding on Caulerpales, but one cannot say whether it was restricted to just one genus. The cladogram indicates stenophagy at the basal nodes of both major clades, hence stenophagy must be considered plesiomorphic. A possible paleoecological scenario is outlined below.

1. Several shelled sacoglossan-like species were feeding on more or less filamentous, more or less calcified udoteacean (= caulerpalean) algae.
2. The sacoglossan synapomorphies of the buccal apparatus probably developed as a response to increased calcification and viscous cytoplasm of algae. *Cylindrobulla* (or its ancestor) split off at this point; it neither developed piercing teeth nor a suctorial pharynx.
3. Decrease in population density (caused by climatic changes, interspecific competition, predation and/or other factors) – and eventually extinction – of most of the simply filamentous, but calcified udoteacean algae, coinciding with (or followed by) speciation and dispersal of the genus *Halimeda* and other complex Udoteaceae. *Caulerpa* most likely split off at this time.
4. Those ancestral sacoglossans which were able to feed on either *Caulerpa* or *Halimeda* (and other complex Udoteaceae) survived and speciated; the remaining species became extinct.

This scenario implies a number of extinct taxa within the hosts (algae) as well as associates (opisthobranchs). Three phylogenetic arrangements of extinct opisthobranch taxa are possible (Fig. 9):

1. Extinct taxa speciated before the separation of the Oxynoacea and Placobranchacea (Fig. 9A). These taxa may or may not have been classified as Sacoglossa, depending on the presence or absence of the synapomorphies of the buccal apparatus. In this arrangement, co-speciation is likely, and the extinct taxa probably became extinct simultaneously with the extinction of the 'primitive' udoteacean algae.
2. Extinct taxa speciated after the separation of the Oxynoacea and Placobranchacea, and would have been classified as Oxynoacea (i.e. they had one long, rod-shaped preradular tooth and an eversible oral tube). If the extinct taxa did not feed on *Caulerpa* (Fig. 9B), co-speciation has not taken place.
3. Extinct taxa speciated after the separation of the Oxynoacea and Placobranchacea, and would have been classified as Placobranchacea (Fig. 9C). In this case, co-speciation may have taken
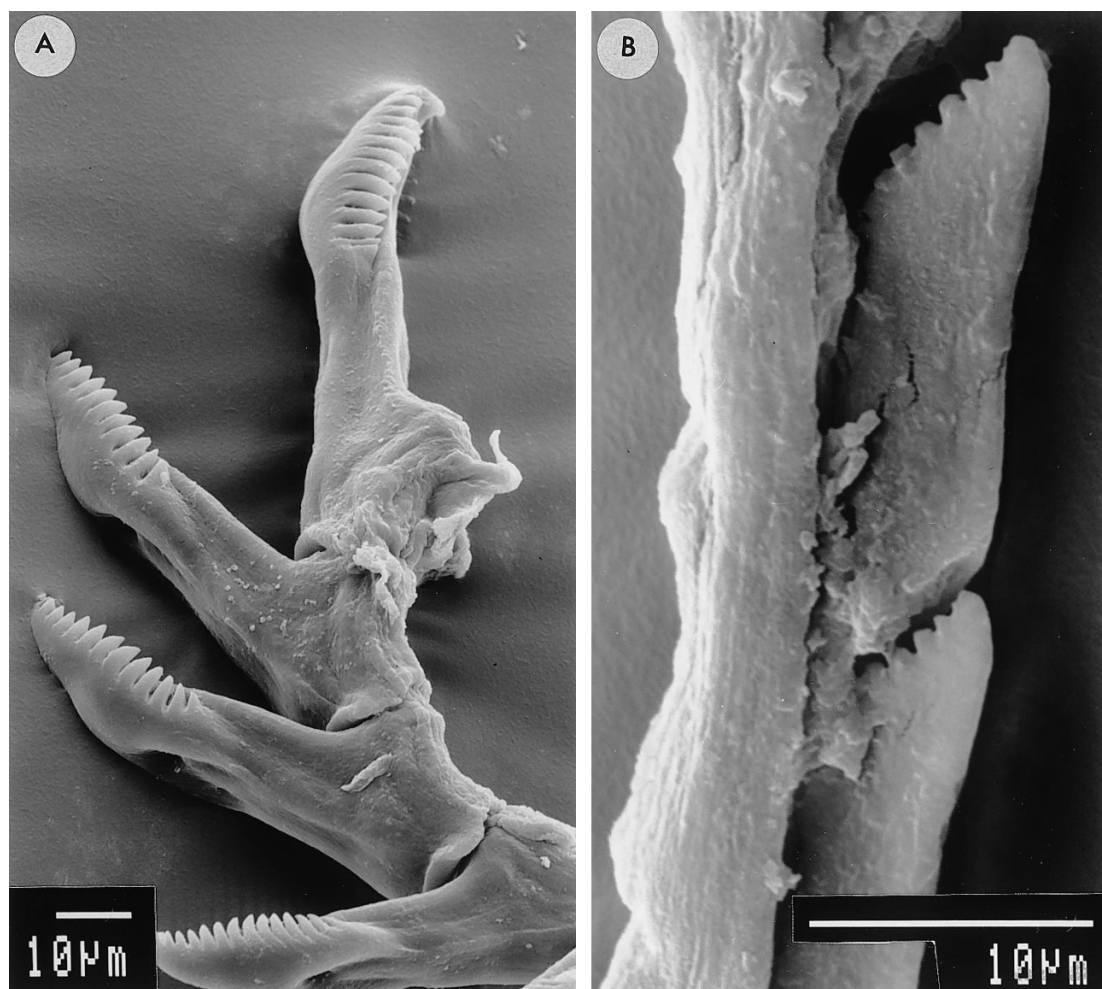
Figure 8. SEM photographs of radular teeth of (A) *Volvatella* sp. (Darwin Harbour, Northern Territory, Australia) and (B) *Bosellia mimetica* (St. Thomas, US Virgin Islands).

place. The extinct taxa may or may not have lost the shell. Also, extinction may not have been associated with the extinction of the 'primitive' udoteaceans.

It is not known how the extinct udoteaceans were related to extant taxa. Four arrangements seem possible (Fig. 10):

1. The extinct taxa formed a paraphyletic grade leading to the node separating Caulerpaceae from Udoteaceae (Fig. 10A). This would most likely have been associated with co-speciation.
2. The extinct taxa were part of a monophyletic Udoteaceae – that is, Caulerpaceae separated before the extinct taxa (Fig. 10B). This may have been associated with co-speciation, in which case the separation of the Oxynoacea and Placobranchacea probably was not associated with loss of the shell.
3. Some of the extinct taxa existed before the separation of the Caulerpaceae, others speciated after the separation (Fig. 10C). In this case, it is impossible to determine whether co-speciation has taken place.
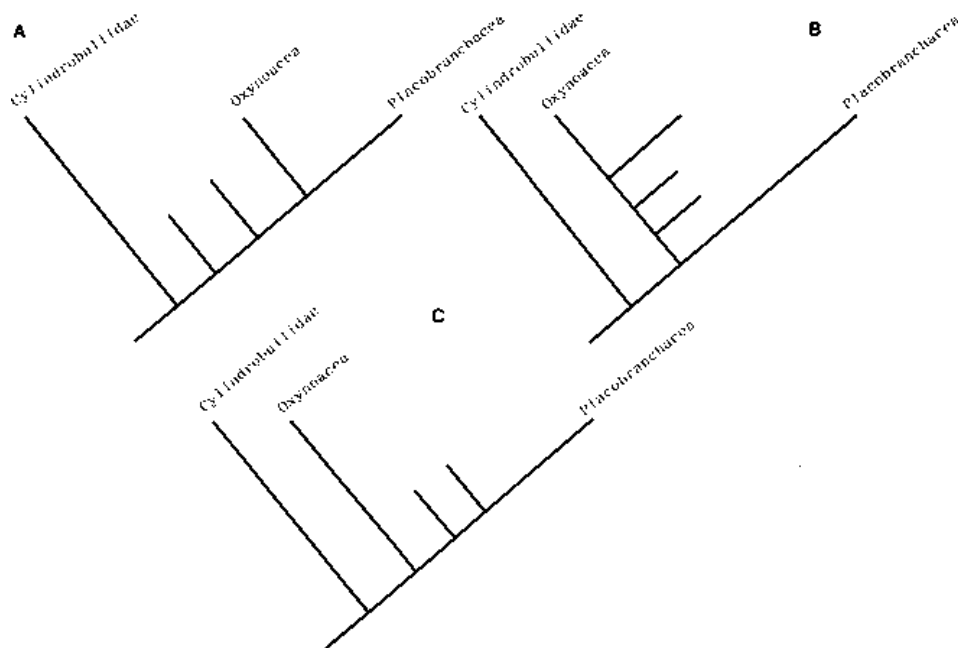
Figure 9. Hypothetical relationships of extinct and extant taxa in the initial evolution of the Sacoglossa. (A) Extinct taxa speciated before the split between extant suborders; (B) extinct taxa belonged to extant Oxynoacea, but speciated before extant families; (C) extinct taxa belonged to extant Placobranchacea, but speciated before extant families.

4. The extinct taxa may be diphyletic – that is, the sister group of the Caulerpaceae is extinct (Fig. 10D). If co-speciation took place in this scenario, it was not associated with host switching.

Was the ancestral diet Caulerpa?

The early fossilized siphonalean green algae had a simpler thallus structure than most present-day Udoteaceae; they were filamentous, much like *Chlorodesmis*, though calcified (Elliott, 1965). It is possible that the successful radiation of more complex, corticated forms, such as *Halimeda*, and a concurrent decrease in filamentous species could have occasioned the first dietary split in the Sacoglossa, the Oxynoacea switching to the morphologically variable but anatomically simple *Caulerpa*, and the placobranchacean ancestor specializing on *Halimeda* (i.e. co-speciation). Thus *Caulerpa* was almost certainly not the ancestral diet.

Loss of the shell

The shell has been lost in several opisthobranch clades (Gosliner, 1991). In most cases, this has happened in connection with a transition from burrowing to epifaunal habits. However, the estimated phylogeny indicates that the ancestral sacoglossan was epifaunal (Fig. 7). It has been speculated that the maintenance of an external shell for protection is energetically expensive, and that, provided protection could be obtained from other sources (e.g. sequestration of dietary secondary metabolites), the shell could be lost.
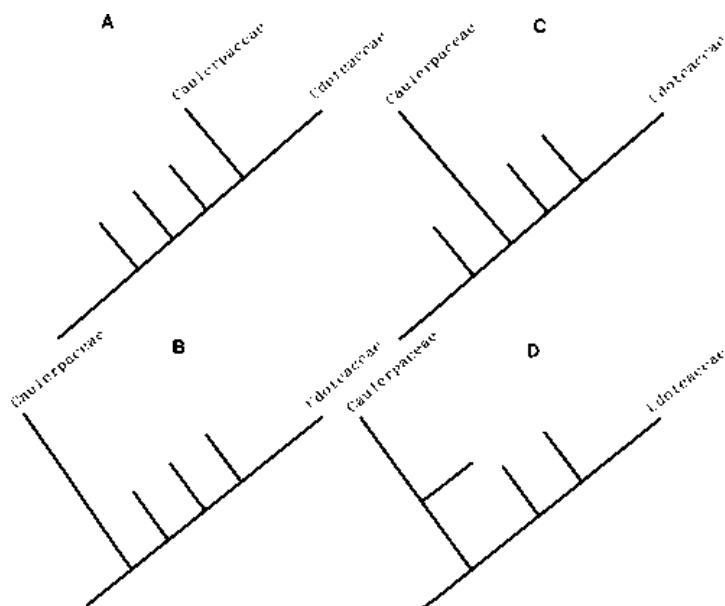
Figure 10. Hypothetical relationships of extinct and extant taxa of Caulerpales *sensu* Womersley (1984). (A) Extinct taxa speciated before split between Udoteaceae and Caulerpaceae; (B) extinct taxa belonged to extant Udoteaceae; (C) some extinct taxa speciated before Caulerpaceae, others after; (D) extinct taxa are diphyletic, and Caulerpaceae did not speciate directly from extant Udoteaceae.

The cladogram of extant sacoglossans (Fig. 2) indicates that loss of the shell was associated with the only possible co-speciation event – that is, the separation of the Oxynoacea and Placobranchacea. However, it is possible that the first, now extinct, placobranchaceans (having the plesiomorphic characters of two or more short preradular teeth and a non-eversible oral tube) were shelled. This could be the case if some of the extinct udoteaceans speciated after the separation of *Caulerpa* (Fig. 10C).

It is not known how the loss of the shell came about. During metamorphosis in the opisthobranchs, the axis of coiling is apparently shifted about 90°, a process called 'heterostrophy'. Also, growth changes from ascending the axis of coiling (hyperstrophic) to descending this axis (orthostrophic). This involves a reorganization of the mantle complex, which probably renders the metamorphosing larvae very vulnerable. The evolution of an ancestor without a shell was a major breakthrough in the evolution of the Sacoglossa. This was the event which allowed exploitation of functional chloroplasts derived from the food plant. Kleptoplasty may initially have developed to cope with secondary metabolites, but in non-shelled forms could provide extra nutrients for extended periods of time. This would allow the animals to try out other food sources, thus initiating an adaptive radiation.

The loss of the shell may have been accompanied by the formation of dorsal vessels. *Cyerce*, which still has a 'holohepatic' digestive gland, has dorsal vessels, but does not have functional kleptoplasty (Clark *et al.*, 1990). Dorsal vessels also occur in *Bosellia*, but not in the Platyhedylidae or *Placobranchus*, though functional kleptoplasty has been described in the latter genus (Muscatine and Greene, 1973; Jensen, 1996a).

Evolution in the Oxynoacea

The Oxynoacea appear to have undergone an initially rapid speciation; blade-shaped teeth occur at the second internal node. The three oxynoacean families radiated into widely different 'morpho-spaces' following their initial switch to *Caulerpa*: (1) bivalved, (2) extensive soft parts of body in part covering the shell, (3) a plesiomorphic, bulloid morphology. This indicates that environmental stress was eased, allowing 'deviating' forms to survive (Parsons, 1994). However, two questions remain: (1) Why have the shelled sacoglossans not exploited algae other than *Caulerpa*? (2) Why are there so few shelled sacoglossans at the present time (max. 20–25% of the total number of species)? Phylogenetic analysis cannot be used to answer these questions directly. However, the hypothesis that speciation rate has been higher in the non-shelled Sacoglossa, and thus that there are significantly fewer shelled species, can be tested.

Why have the shelled sacoglossans not exploited algae other than Caulerpa?

It could be because they have extremely specialized feeding methods: more or less eversible oral tubes, large pharyngeal pouches, fine lateral denticles and often bifid tips of radular teeth. Also, the radula of settling-competent larvae may already be too specialized: the long, rod-shaped pre-radular tooth of shelled sacoglossans is an important synapomorphy of the group. However, the explanation may be much simpler.

The shelled sacoglossans have not exploited other algae because *Caulerpa* has 'always' formed a widespread, stable food source. This is supported by the widely differing morphologies of the shelled families; evolution of highly deviating forms is usually correlated with low environmental stress (Parsons, 1994), for example low competition for food. Also, the wide variety of thallus morphology and the great number of species of *Caulerpa* may support the high age of this genus.

Why are there so few shelled sacoglossans?

First, we must examine whether there really are significantly fewer shelled sacoglossans. Various methods have been proposed to test the 'balance' of a phylogenetic tree. The main problem appears to be in establishing a 'null hypothesis': some tests have a completely unbalanced tree ('comb') as the configuration most likely to occur with natural selection; others have a perfectly balanced tree as the most likely outcome of random speciation (Slowinski, 1990; Guyer and Slowinski, 1993). Unfortunately, no confidence intervals have been given for the calculated probabilities of these tests.

If speciation comprising host switching imposes an evolutionary advantage causing increased speciation rates, and speciation including duplication of diet does not impose this advantage, then this could explain the higher number of terminal taxa in the non-shelled clade. In principle, only sister taxa can be compared (Slowinski, 1990); that is, Oxynoacea should be compared with Placobranchacea and Limapontioidea with Placobranchoidea. The former node was associated with host switching, possibly co-speciation, whereas the latter was not.

In Table 4, the various sister taxa within the Sacoglossa are compared (species numbers are taken from Table 1, and changes in diet are indicated). As blade-shaped teeth are apparently more efficient than triangular teeth, the taxa having these types of teeth have also been compared, though in the Limapontioidea the two groups are not sister taxa. The Oxynoacea comprise less than 20% of the total number of species. The sister taxa not separated by host switching are much less unbalanced, and the major division between the two non-shelled superfamilies is almost perfectly balanced. The major host switching event (i.e. from Udoteaceae to Derbesiales), however, is distinctly marked by a configuration consistent with adaptive radiation following this switch.

Table 4. Comparison of sacoglossan subclades with and without diet changes (numbers of species have been taken from Table 1)

Taxa compared	Diet change	% Species (*n*) in smallest taxon	P_{cum}
Oxynoacea/Placobranchacea	yes[a]	17 (288)	0.348
Volvatellidae/Juliidae + Oxynoidae	no	40 (50)	0.816
Oxynoidae/Juliidae	no	40 (30)	0.828
Julia/Berthelinia	no	28 (18)	0.588
Ascobulla/Volvatella	no	25 (20)	0.526
Placobranchoidea/Limapontioidea	no	44 (238)	0.886
Polybranchia/higher Limapontioidea	yes	8.5 (118)	0.157
Elysiella/higher Placobranchidae	yes	4.8 (84)	0.096
Hermaeidae/Limapontiidae	no?	24 (105)	0.481
Hermaea/Aplysiopsis	yes?	50 (24)	1.0
Node L7 (Fig. 5)	yes?	4 (25)	0.083
Node L11 (Fig. 5)	yes	32 (50)	0.653
Placobranchoidea: Triangular/blade-shaped teeth[b]	yes	24 (105)	0.481
Limapontioidea: Triangular/blade-shaped teeth[b]	yes	26 (95)	0.532

[a]No change in cell wall composition; [b]not sister taxa.
P_{cum} was calculated from the equation: $P_{cum} = 2r(n-1)^{-1}$, where n = total number of species and r = number of species in smallest taxon (Slowinski and Guyer, 1989).

It could also be speculated that the shell has been the constraint slowing down speciation rate. This is supported by a comparison of shelled and non-shelled sister taxa in other opisthobranch groups (Table 5). Due to taxonomic instability, this comparison can only be made at the generic level. In this comparison, internal, highly reduced shells are included in the non-shelled clade in most cases. The Notaspidea are usually considered the sister taxon of the Nudibranchia (Schmekel, 1985). The Cephalaspidea has been excluded from the present comparison because phylogeny is highly unresolved, and the group may not even be monophyletic (Salvini-Plawen, 1990; Mikkelsen, 1993). The comparison shows that the shelled taxa are consistently less speciose in opisthobranch sister taxa, indicating lower diversification rates.

Table 5. Comparison of shelled and non-shelled sister taxa of Opisthobranchia (genera with internal and highly reduced shells were included in the non-shelled group)

Taxa compared	% Shelled genera	*n*	P_{cum}
Sacoglossa	21	34	0.424
Anaspidea (= Aplysiomorpha)	10	10	0.222
Notaspidea	27	11	0.600
Notaspidea/Nudibranchia	6.2	178	0.124

Number of genera in various taxa have been taken from Thiele (1931), Willan (1987) and Table 1 of present study. P_{cum} was calculated as detailed in the footnotes to Table 4.

Evolution in the Placobranchacea

The most primitive non-shelled sacoglossans feed on Udoteaceae. Unfortunately, a phylogeny of the genera of the Udoteaceae does not exist, so it is not possible to determine whether co-speciation may have taken place within the placobranchacean genera. *Bosellia*, *Sohgenia* and some species of *Cyerce* feed on *Halimeda*, whereas other species of *Cyerce* feed on *Penicillus*, *Udotea* or the uncalcified *Chlorodesmis* (Jensen, 1980, 1993a). The natural diet of *Placobranchus* is unknown, but in the laboratory it feeds on *Udotea* and *Chlorodesmis* (Jensen, 1980).

Algae of the Udoteaceae all have cell walls composed of xylan. Host switching to algae of the Derbesiales will represent the least change in cell wall composition, the gametophytes having cell walls composed of xylan and cellulose. Host switching has apparently taken place in parallel in the two non-shelled clades. Diversification rates have been different in the two clades. In the Placobranchoidea, almost 50% of the species with known diets ($n = 35$) have retained a diet of Caulerpales, and external morphology is very uniform, indicating that diet radiation may be of recent origin. Only 15% of the Limapontioidea ($n = 42$) have caulerpalean diets, and 31% have 'exotic' diets (calculations based on information on diets in Jensen, 1993a,b); external morphology is more variable, indicating that diet radiation began much earlier in this clade. Both major clades have dispersed into temperate regions, but whether they dispersed after they had switched to non-udoteacean diets, or whether they switched diet after udoteacean algae disappeared from temperate zones, is unknown. Possibly, both processes have occurred at different speciation events.

Some species of *Placida* feed on *Codium* as well as Derbesiales. It is unknown whether these species are more derived anatomically than those feeding exclusively on Derbesiales, but this would be a good working hypothesis for future studies. Within the genus *Stiliger*, host switching to *Codium* has taken place. '*Stiliger*' *smaragdinus* feeds on *Caulerpa*. The switch to Cladophorales in the Limapontioidea is accompanied by the evolution of sabot-shaped teeth (Jensen, 1993a). This (nodes L8 and L11) has not resulted in an adaptive radiation (Table 4).

Future studies should aim to elucidate phylogenetic relationships between the species with different diets from the same region; if these are more closely related to one another than to species with the same diet from different regions, then 'host switching' has taken place locally. For example, the European *Elysia viridis*, which has a broad diet including *Codium*, *Bryopsis* and *Chaetomorpha* (Jensen, 1989b), seems to be more closely related to *E. australis*, which also has a broad diet including Ulvales, Cladophorales and possibly Dasycladales (Jensen, 1991b), than to *E. maoria* or *E. trisinuata*, which both feed exclusively on *Codium* (Jensen, 1990, 1993b). However, more anatomical characters need to be defined, or molecular data must be included, to clarify these relationships completely.

Host switching to 'exotic' diets

Host switching to 'exotic' diets (e.g. diatoms, Rhodophyta, seagrasses or opisthobranch eggs) has apparently occurred several times in both major clades of non-shelled sacoglossans (Fig. 5).

Only two sacoglossans feed on diatoms. One of these is the Caribbean '*Elysia*' *evelinae* (Jensen, 1981). Anatomically, this species appears to be more closely related to *Thuridilla* than to any species of *Elysia*: its radular teeth (Fig. 11A) are triangular, though smooth (Jensen, 1981); it has pharyngeal pouches, though thin-walled and apparently connecting to the lumen surrounding the descending limb of the radula (Jensen, 1991a), whereas the pharyngeal pouches of other sacoglossans connect to the pharyngeal cavity, close to the oesophageal opening (Jensen, 1991a, 1992); and its reproductive system has a large median ampulla and a dorsal vaginal opening (Marcus, 1957), as in *Thuridilla* (Jensen, 1992). Unfortunately, diet is only known for the Mediterranean *Thuridilla hopei*, which feeds on *Cladophora vagabunda* (Marin and Ros, 1988). The Indo-Pacific
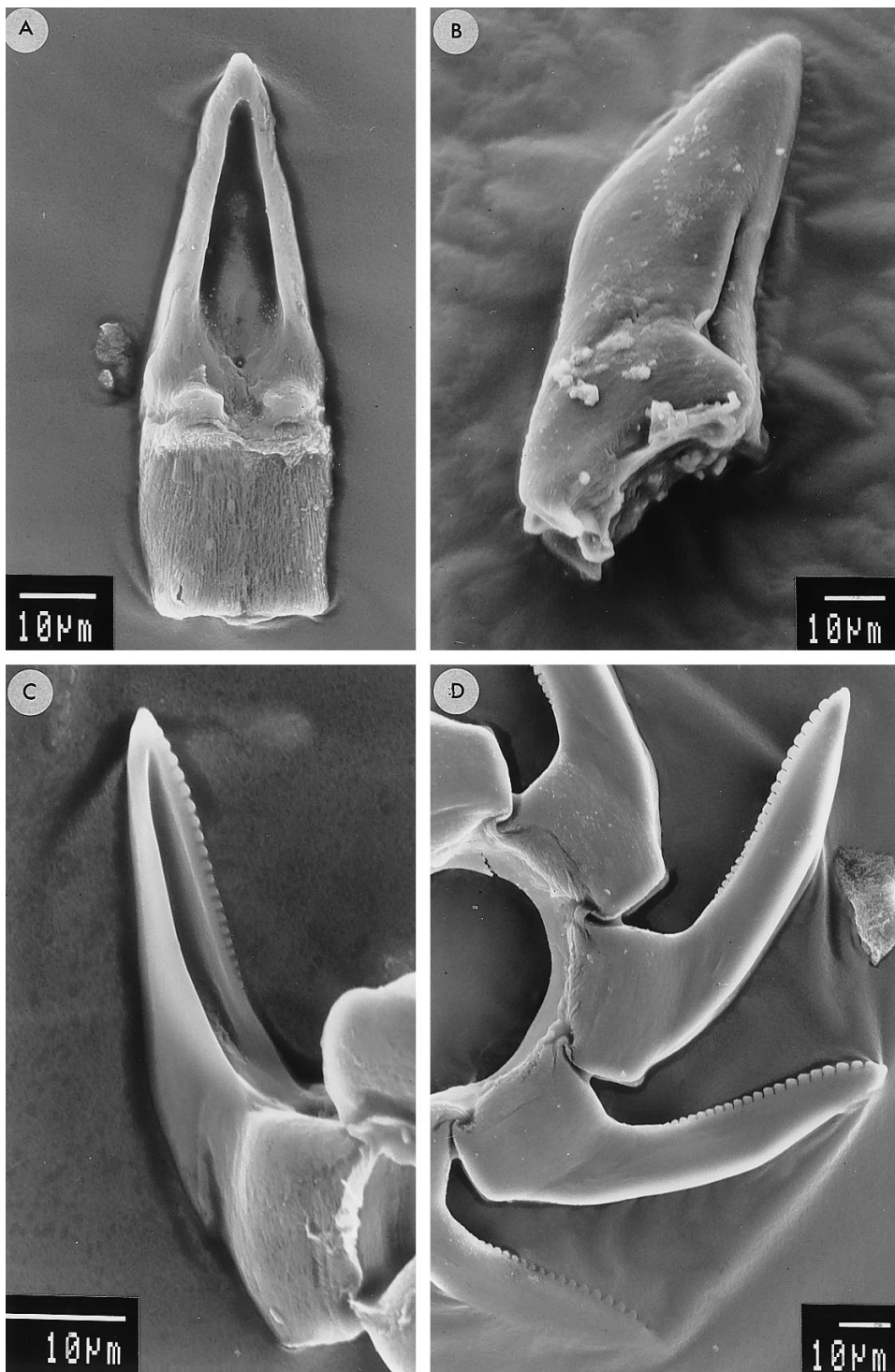
Figure 11. SEM photographs of radular teeth of sacoglossans with 'exotic' diets. (A) *Elysia eveline* (diet: diatoms) (Florida). (B) *Hermaea vancouverensis* (diet: diatoms) (San Juan Island, Washington, coll. J.S. Bleakney). (C, D) *Elysia serca* (diet: seagrasses) (Banana River, Florida).

species generally have been collected from dead coral without any obvious dietary source (K.R. Jensen, unpublished observation), and it is suggested that they might feed on filamentous algae encrusted in the coral rock. The identity of these algae has not been determined. Although *E. evelinae* has evolved from *Thuridilla*, it is unknown whether its ancestor fed on Cladophorales.

The other sacoglossan feeding on diatoms is *Hermaea vancouverensis* (Williams and Gosliner, 1973). Most species of *Hermaea* feed on red algae (Fig. 5). The teeth of *H. vancouverensis* (Fig. 11B) are shorter and stouter than those of other species of *Hermaea*, and they appear to have a small cavity near the base (i.e. they are triangular). It seems unlikely that the species feeding on red algae arose from the diatom-feeding species, because several species feeding on red algae have fine lateral denticles on the blade-shaped teeth, which is considered plesiomorphic (Jensen, 1993c). Hence another internal node may have to be inserted, either between L7 and L8 (Fig. 5), if host switching occurred in ancestor L7, or above L8, if host switching was in ancestor L8.

Two species presently referred to the genus *Elysia* – '*E.*' *catulus* and '*E.*' *serca* – feed on seagrasses (Clark, 1975; Jensen, 1982, 1993a). The superficial similarity between *Elysiella pusilla* and *Elysia catulus* was noted early on, and *E. catulus* was placed in *Elysiella* (Verrill, 1872). Unfortunately, *Elysiella pusilla* was for many years considered a species which would never be reidentified. However, after the synonymization of *E. pusilla* and *Elysia halimedae* and the anatomical description of the species (Jensen and Wells, 1990; Jensen, 1992), it is possible to compare this species with the two seagrass-feeding species. Both have triangular teeth, and in *E. serca* these may be unilaterally denticulate (Fig. 11C,D) (Jensen, 1982), as in *E. pusilla*. Also, the pharynx of *Elysia serca* is very tall, and in both species the ascus-muscle is thick (Marcus, 1980; Jensen, 1982). This is also similar to *Elysiella* (Jensen and Wells, 1990). Unfortunately, the reproductive systems of the two seagrass-feeding species have not been described in sufficient detail to compare them with that of *Elysiella*.

In the north-east Atlantic, *Limapontia depressa* and *Alderia modesta* feed on the xanthophyte *Vaucheria*. *A. modesta* occurs also in the north-west Atlantic and north-east Pacific. Both species have sabot-shaped teeth, and at least *L. depressa* speciated from Cladophorales-feeding species; its two congeners, *L. capitata* and *L. senestra* both feed on Cladophorales. The very wide distribution of *A. modesta* makes it difficult to trace its origin. Its sabot-shaped teeth, though with pointed tips (Bleakney, 1988), indicate that it evolved from Cladophorales-feeding ancestors. *Vaucheria* is also the diet of *Elysia chlorotica* from the north-west Atlantic. To trace the descent of *E. chlorotica*, its anatomy should be compared with that of Caribbean placobranchids, and also to that of the eastern Atlantic *E. viridis* and the north-eastern Pacific *E. hedgpethi*, both of which feed on *Codium*, though *E. viridis* also feeds on Cladophorales and *Bryopsis*. None of the Caribbean placobranchids feed on Cladophorales (Clark and DeFreese, 1987). Unfortunately, no description of the reproductive anatomy of *E. chlorotica* exists.

Elysia japonica and *E. furvacauda* have both been recorded as feeding on red algae (Brandley, 1984; Jensen, 1985). The two species may be synonymous, and both have rather broad diets, so red algae are not their main diet. *E. japonica* also feeds on Cladophorales, and the rounded tips of its radular teeth (Jensen, 1985) indicate that this is its usual diet. Red algae are the usual diet of most species of *Hermaea* (Fig. 5). Also, '*Stiliger*' *fuscovittatus* feeds on red algae (Lance, 1962). This species most likely needs to be transferred to a new, as yet undescribed, genus. Unfortunately, the internal anatomy of this species is unknown, except for the radular teeth, which are described as chisel-shaped (Lance, 1962).

Two or possibly three species of Sacoglossa feed on opisthobranch eggs. In the north-east Pacific, *Olea hansineensis* feeds on eggs (Crane, 1971). In the north-east Atlantic, *Calliopaea oophaga* feeds on eggs, mostly of other opisthobranchs, but sometimes including those of the prosobranch *Turritella communis* (Jensen, 1986). Oophagy has also been described in *Stiliger*

vesiculosus (Haefelfinger, 1962), but the identity of this species remains elusive. *Olea* and *Calliopaea* appear as sister taxa in the cladistic analysis, but their relationship to most other limapontiids remains unresolved. The highly specialized diet does not help clarify phylogenetic affinities.

Conclusions

Sacoglossans are not easily fossilized, but, based on fossils of bivalved sacoglossans from the Eocene and thin-shelled opisthobranchs with slightly concave apical areas from the Triassic, it is concluded that the Sacoglossa most likely evolved in the Jurassic or Cretaceous, probably sometime before the evolution of the calcareous, siphonalean alga *Halimeda* (Cretaceous). The three shelled families of Sacoglossa are very different in external morphology, indicating that environmental stress was released during this period of evolution. In the non-shelled, parapodia-bearing Placobranchidae, morphology has been very conservative, though speciation and diet radiation have been rampant, both indicating intense competition. In the non-shelled, cerata-bearing superfamily Limapontioidea, morphology is more variable. Most species have non-caulerpalean diets. However, many species are inadequately described, and phylogenetic relationships among genera remain unresolved.

The present study has shown that the diet radiations previously described for the Sacoglossa must be modified, not only in accordance with new data on phylogeny. First, *Caulerpa* does not seem to have been the ancestral diet; the ancestral sacoglossan probably fed on calcified, filamentous ancestors of *Halimeda* (i.e. Udoteaceae). The separation of the monophyletic shelled Sacoglossa, which all feed exclusively on *Caulerpa*, from the monophyletic non-shelled Sacoglossa, may have coincided with the split of *Caulerpa* from the Udoteaceae, thus supporting the hypothesis of co-speciation by descent. It may also have coincided with the appearance of *Halimeda*, which presumably constituted the food of the ancestral non-shelled sacoglossan. The diet radiation path outlined previously (Jensen, 1993a,c) – that is, Udoteaceae → Derbesiales → Codiales/Dascycla-dales or Cladophorales/Siphonocladales – has apparently been followed independently in the two major clades of non-shelled Sacoglossa. The evolutionary process responsible for this speciation pattern is most likely 'host switching by resource tracking' (Brooks and McLennan, 1991). The resource tracked has apparently been cell wall structure and composition, though chloroplast structure may also have been important. The species switching to 'exotic' diets (e.g. diatoms, red algae, seagrasses or opisthobranch eggs) may not have followed the resource-tracking path; though most of these species are related to species feeding on Cladophorales, some may have descended directly from species feeding on Udoteaceae.

With the resource-tracking hypothesis as a basis, new studies can be initiated, examining sets of congeners having similar external morphology but different diets. The working hypothesis would then be that the most plesiomorphic species have udoteacean diets, more advanced species feed on Derbesiales, and the most advanced species feed on either Codiales/Dascycladales or Cladophorales/Siphonocladales.

Acknowledgements

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