COLLECTION, PRESERVATION AND IDENTIFICATION OF OPISTHOBRANCH MOLLUSCS

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ABSTRACT

The Opisthobranchia probably comprise about 5-6000 species of which 15-40% may be undescribed. Opisthobranchs are best collected by handpicking them off suitable substrates, *e.g.* sponges, bryozoans, cnidarians and seaweeds. Many opisthobranchs are conspicuously coloured; this disappears on preservation. Most opisthobranchs have soft bodies without a shell or with a thin, reduced, internal shell. Hence shell collections are inadequate for this group. Opisthobranchs should be relaxed prior to fixation. Most tropical species relax when placed on ice for a short period of time; ice crystals must not form. Standard fixation is in 4% neutral formaldehyde, and ethanol is used for permanent storage. The high proportion of undescribed species, and the lack of identification keys for the tropical Indo-Pacific region make identification of opisthobranchs difficult. Construction of a pictorial identification key, at least to family level, is in progress, and diagnoses of opisthobranch orders are given here.

INTRODUCTION

The gastropod subclass Opisthobranchia probably comprise about 5-6000 species of which 15-40% may be undescribed (Gosliner & Draheim 1996). Most of these animals have soft bodies without a shell or with a reduced, thin, internal shell. Opisthobranchs rarely constitute a conspicuous part of the benthic fauna. In fact, opisthobranchs are usually considered rare animals. However, there are exceptions, especially during breeding season of larger species such as sea hares. In Hong Kong waters benthic surveys list 3 species of opisthobranchs among the 10 most widely distributed gastropods (Leung & Morton 1997). Also, some species have irruptive populations (Taylor & Jensen 1992). Most opisthobranchs are diet and habitat specialists. Thus collecting requires some knowledge of habitat requirements. The high proportion of undescribed species as well as the lack of suitable identification keys for opisthobranch molluscs make it highly desirable to disseminate to local marine scientists in SE-Asia a basic knowledge of collecting, preserving and identifying these animals, and also to outline the classification of the group. In the present study notes on collecting and preservation techniques are given, with a brief outline of the current classification and diagnoses of opisthobranch orders.

COLLECTING

Opisthobranchs may be collected accidentally in trawls or dredges, but usually have to be collected by SCUBA-diving, snorkelling or intertidal wading. Sponges, bryozoans, cnidarians and seaweeds are commonly used as food and substrate, and searching such organisms will often reveal several opisthobranchs. The small species are best collected by collecting potential food material, shaking it in a bucket of seawater to dislodge animals, or leaving it in sorting trays to let the animals crawl out. The commonly used method of washing algae in fresh-water to dislodge epifauna is absolutely not suitable for soft-bodied opisthobranchs; these animals swell and/or break into more or less unrecognisable pieces. Species feeding on finely branching

hydroids or algae can be collected by shaking clumps of food and collecting the dislodged animals with a suction-bottle (Clark 1971). Many opisthobranchs are conspicuously coloured and should be photographed as soon after collection as possible; detailed notes on colour patterns are a necessary supplement to the photographs.

PRESERVATION

Most tropical opisthobranchs are best relaxed by placing them in the freezing compartment of a household refrigerator for a short time (ice-crystals must not form). For field relaxation placing animals in petri-dishes on crushed ice usually works with tropical species. Another cheap relaxant is $MgCl_2.6H_2O$. A 7% solution of this chemical added to seawater will be isotonic, but many species need large amounts of relaxant, and this may cause certain glandular structures to swell. For general descriptive, anatomical studies, and also for museum specimens, preservation in 4% neutral formaldehyde is most convenient; neutralizing is usually done with borax added till saturation. Relaxed specimens are dropped into this solution and kept in it for at least 24h. Then animals may be transferred to ethanol, preferably 80% because water content of these soft-bodied animals is high. For histology animals should be preserved in Bouin's solution. For electron microscopy tissues should be preserved in 2.5% glutaraldehyde in a suitable buffer (different people prefer different buffers, e.g. phosphate, cacodylate, or just filtered seawater). Glutaraldehyde is a rather weak and unstable preservative, and specimens (and preservative) should be kept refrigerated. Note that glutaraldehyde and cacodylate-buffer are very poisonous.
Labelling of specimens is very important. Even specimens which cannot immediately be identified should have labels containing all relevant information, i.e. at least collecting site, depth, date, collector, but preferably also substrate, collecting method/gear, and a reference number to field notes, photos etc.

IDENTIFICATION

The shell, where present, is usually without any species-specific characteristics, and thus is not useful for identification. Colouration of opisthobranch bodies disappears upon preservation. Hence it is important for later identification to have detailed descriptions of colours and colour-patterns, and also to keep colour pictures with voucher specimens in museum collections. Most opisthobranchs are dietary specialists, and also "partial predators", i.e. they use their food as a substrate. Hence information on substrate may be important for identification. Although colouration is conspicuous, animals are often cryptic when on their food/substrate. Many opisthobranchs survive well in the laboratory, and important observations on feeding and reproduction can be made.

There are at present 8 recognized orders of opisthobranchs, i.e. Cephalaspidea, Anaspi-dea, Sacoglossa, Acochlidiacea, Notaspidea, Nudibranchia, Thecosomata and Gymnosomata. However, several of these are suspected of not being monophyletic, and even the subclass Opisthobranchia may not be monophyletic (Ponder & Lindberg 1997). This means that many taxonomic changes are to be expected in the near future. A pictorial identification key to families of Opisthobranchia from the South China Sea is in preparation by the present author.

The order Cephalaspidea (Fig. 1), commonly known as "bubble-shells", is definitely not monophyletic; some families may not even be opisthobranchs (Mikkelsen 1996; Ponder & Lindberg 1997). The order contains species with a thick, spirally coiled shell and an operculum (*e.g.* family Acteonidae), characters shared with the prosobranchs. Other species have a thin, inflated shell with an involute spire and no operculum (*e. g.* family Atyidae). Many species have an internal shell, i.e. the shell has been completely overgrown by the soft parts (*e. g.* family Philinidae); an internal shell is

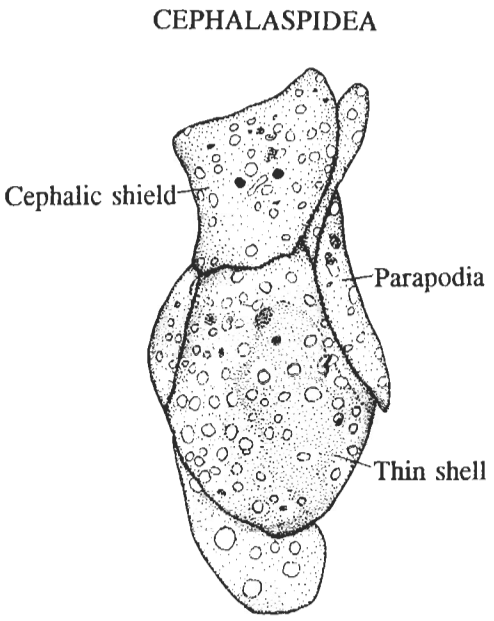

Fig. 1. *Haminaea* sp. from Phuket Thailand; drawn from colour slide by K.R. Jensen. Length of specimen approximately 15 mm. This is a rare species occuring intertidally in sandy sediments.

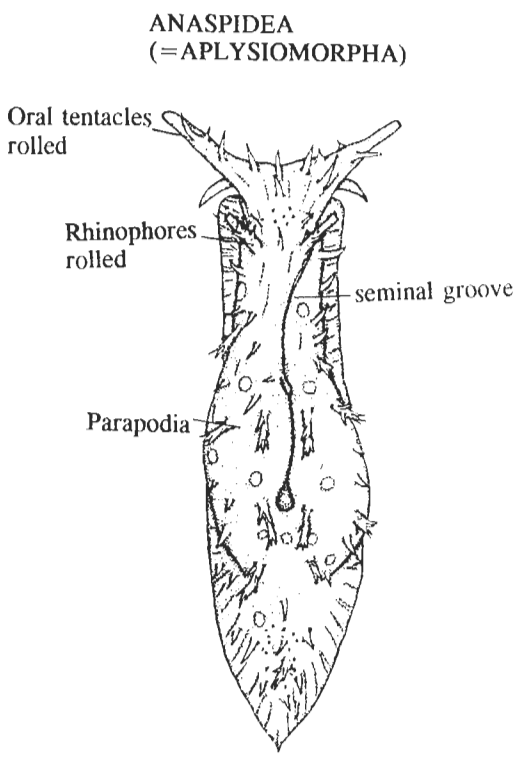

Fig. 2. *Bursatella leachi* (Blainville, 1817) from Hong Kong; drawn from live specimen by K.R. Jensen. Length of specimen approximately 110 mm. This is a very common species, forming breeding aggregations in shallow water in the spring.

usually thin, inflated and with an involute spire. Some species have lost the shell completely. Cephalaspideans have a cephalic shield and chemosensory structures, Hancock's organ, laterally on the head. Usually they have a strongly folded (plicate) gill and a monaulic reproductive system, that is a common genital aperture in the mantle cavity, an external sperm groove and a cephalic penis. Most species are relatively small (<3 cm), burrowing in soft sediments. Many are specialist carnivores.

Anaspidea (sea-hares) contain the shelled family Akeridae with only one genus *Akera*, and the Aplysiidae (Fig. 2) with a reduced, internal shell or without a shell. This order is almost certainly monophyletic. *Akera* is externally similar to the Cephalaspidea with a reduced cephalic shield and parapodia partly covering the shell, which has a sutural slit. The true sea-hares (fam. Aplysiidae) have two pairs of rolled cephalic tentacles, i.e. long, anterior oral tentacles and short, dorsal rhinophores behind the eyes. The visceral hump is usually large, and the small mantle cavity is covered by parapodia which may be partially fused. There is a conspicuous external seminal groove running from the anterior end of the mantle cavity to the penial opening immediately behind the right oral tentacle. In the mantle cavity a defensive gland, the purple gland, may secrete a strongly purple fluid which is supposed to deter predators. The sea-hares may reach considerable body size and often gather in shallow water during the breeding season. They are herbivores, grazing on various macroalgae.

The order Sacoglossa (Fig. 3) is also almost certainly monophyletic. There is some

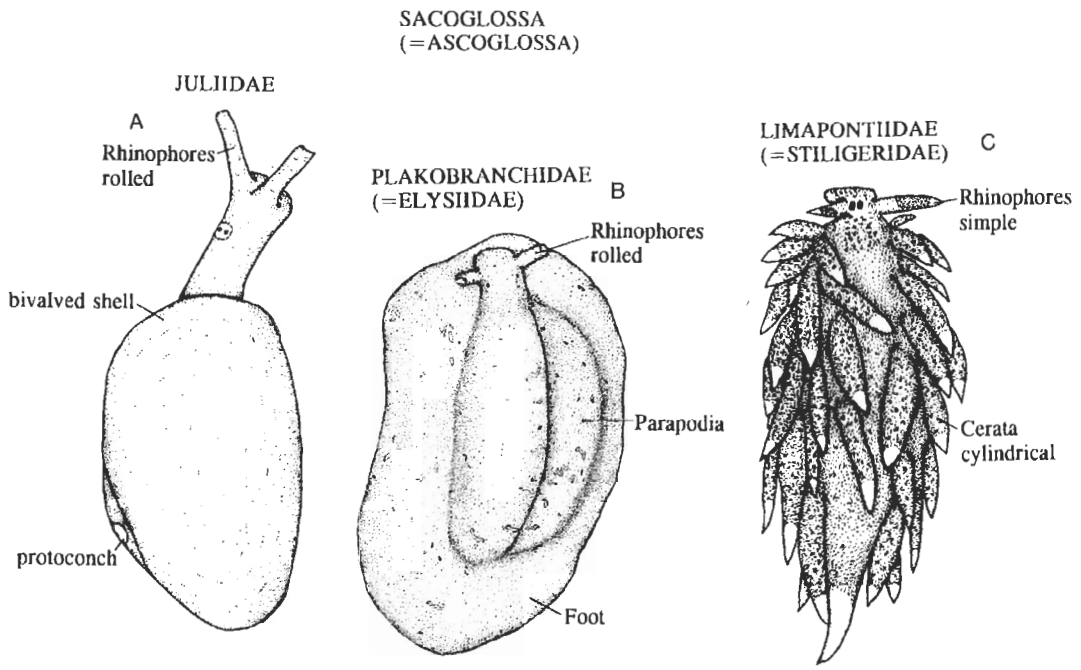

Fig. 3. Sacoglossa. (A) *Berthelinia* sp. from Cottesloe, Western Australia; drawn from colour slide by K.R. Jensen. Length of specimen approximately 10 mm. Lives on the green alga *Caulerpa*. (B) *Elysiella pusilla* Bergh, 1872, from Phuket, Thailand (see Jensen, in press). Lives on the green, calcareous alga *Halimeda*. (C) *Costasiella* sp. from Phuket, Thailand; drawn from colour slide by K.R. Jensen. Length of specimen approximately 4 mm. Lives on the green alga *Avrainvillea*. The 3 genera of Sacoglossa illustrated are those collected during the 9th TMMP workshop; *Berthelinia* and *Costasiella* at Kuta Beach, Lombok and *Elysiella pusilla* on Komodo Island.

debate whether the monogeneric family Cylindrobullidae should be included in this order or in the Cephalaspidea (Mikkelsen 1996, 1998; Jensen 1996a,b). The Sacoglossa contains species with a comparatively large shell (family Volvatellidae), a reduced shell more or less covered by parapodia (family Oxynoidae), a bivalved shell (family Juliidae; Fig. 3A), and shell-less species with winglike parapodia (family Plakobran-chidae; =Elysiidae; Fig. 3B) or dorso-lateral cerata (family Limapontiidae; =Stiligeridae; Fig. 3C). Most species are small (<3 cm). The Sacoglossa are suctorial feeders and, with the exception of 3 egg-eating species, they are all herbivores, feeding on the cytoplasm of marine plants.

The order Acochlidiacea consists of small, shell-less, often interstitially living species. They have a distinct visceral sac separated from the foot. They may have rhinophores and oral tentacles, but one or both of these paired tentacles may be missing. Many species have separate sexes. The only freshwater opisthobranchs are found in this order. It has been claimed that this group may be the sister-group of the Sacoglossa (Gosliner & Ghiselin 1984).

The Notaspidea (Fig. 4) is usually considered monophyletic and the sister group of the Nudibranchia (Schmekel 1985; Willan 1987), but recent molecular studies have shown a possible paraphyly (Thollesson, 1999). There are two suborders, Umbraculacea with a large, limpet-like shell, and Pleurobranchacea with a small, reduced, internal shell or without a shell (Willan

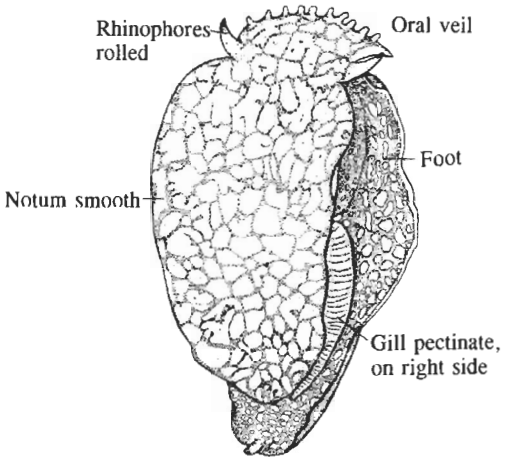

Fig. 4. *Pleurobranchaea brockii* Bergh, 1897, from Hong Kong (see Jensen 1997a). This large, subtidal species may be very abundant in certain habitats; it is a generalist carnivore showing pronounced cannibalism (Jensen 1997a).

1987). They have a conspicuous lateral bipectinate gill on the right side of the body. The anus and genital openings are also on the right side of the body. The rhinophores are rolled, and there is usually a large veil along the anterior margin of the head. This group contains some very large species (>20 cm) which may be very abundant in some habitats. They are mostly generalist predators; cannibalism has been described in several species (Jensen 1997a; Battle & Nybakken 1998).

The order Nudibranchia (Fig. 5) may be paraphyletic (Thollesson, 1999). There are 4 suborders, 3 of which, based on molecular data, may form a monophyletic group. A shell is absent. They are carnivores, mostly highly stenophagous.

Suborder Doridacea (Fig. 5A): Usually with a circlet of plumose gills surrounding the dorsal anus; families Phyllidiidae and Corambidae have posteroventral gills and anus. The dorsal mantle is often warty and may contain various spicules. The rhinophores are lamellate and can be retracted.

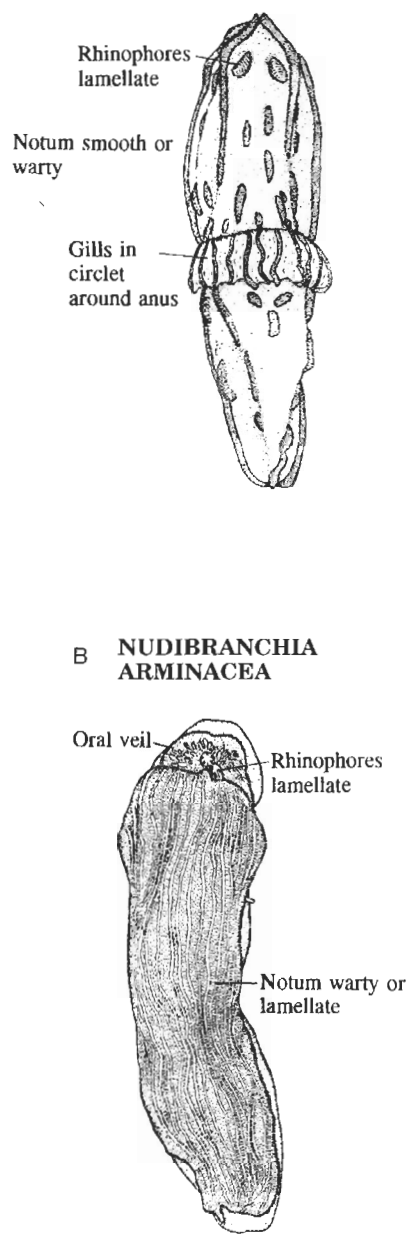

Fig. 5A and 5B. Nudibranchia. (A) *Gymnodoris (Analogium) striata* (Eliot, 1908), from Phuket, Thailand (see Jensen, 1998). (B) *Armina papillata* Baba, 1933, from Hong Kong (see Jensen 1997b). These species are relatively rare.

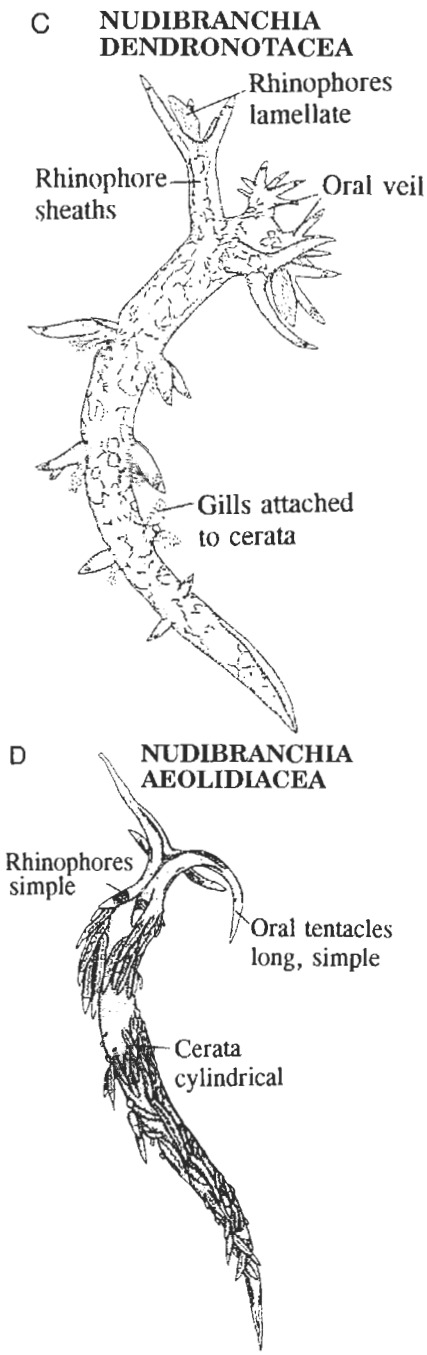

Figure 5C and 5D. (C) *Bornella stellifer* (Adams & Reeve in Adams, 1848), from Phuket, Thailand (see Jensen, 1998). (D) *Phidiana militaris* from Hong Kong; drawn from colour slide by K.R. Jensen. Length of specimen approximately 50 mm. These species are relatively rare.

The genital openings are on the right side of the body. Many species are very colourful (*e. g.* fam. Chromodorididae). Most are sponge-feeders, but many feed on bryozoans or ascidians. Some species are very big, but most are about 3-5 cm long. Indo-Pacific species of Chromodorididae have been reviewed in a series of papers including colour photos of the species studied (*e.g.* Rudman 1984, 1986, 1988). The Phyllidiidae have been reviewed by Brunckhorst (1993).

Suborder Arminacea (Fig. 5B): With ridged or warty notum (fam. Arminidae) or with cerata (superfam. Metarminoidea). The Arminidae have gills on the ventral surface of the notum, a large head veil, retractable rhinophores with vertical lamellae. The pharynx has conspicuous chitinous jaws. They are carnivores, mostly feeding on octocorals.

Suborder Dendronotacea (Fig. 5C): Elongate animals with dorsolateral cerata-like processes, often with gills attached to the bases. The base of the rhinophores is enclosed in a rhinophore-sheath which may bear conspicuous processes. Dendronotaceans have large chitinous jaws. They are carnivores, mostly feeding on cnidarians.

Suborder Aeolidiacea (Fig. 5D): Elongate animals with club-like cerata containing branches of the digestive gland and often a cnidosac at the tip, containing nematocysts from the cnidarian food. The anus may be latero-dorsal, between clusters of cerata, or located below the cerata on the right side. Usually with strong chitinous jaws.

The orders Thecosomata and Gymnosomata are holoplanktonic. The thin shells of the Thecosomata may form distinct deposits of bottom sediment. The Thecosomata feed by secreting a mucus net and capturing small plankton organisms. The Gymnosomata do not have a shell. They are voracious carnivores.

DISCUSSION

In recent years cladistic analyses have resulted in some drastic rearrangements of

gastropod classification (Ponder & Warén 1988; Ponder & Lindberg 1997). However, few opisthobranchs have been included in these studies, and it must be expected that future studies will also change the higher classification of opisthobranchs.

The rarity of most species of opisthobranchs, and also the high proportion of undescribed species, make it highly desirable that non-specialist marine scientists are trained to collect, preserve, and adequately label and photograph specimens to be sent to specialists for proper identification or description. Collaboration between local scientists and reference collections on one hand and experienced taxonomic specialists in large museums on the other should be encouraged in the future.

Extensive biodiversity monitoring programmes invariably include a high number of rare organisms, many of which may be opisthobranch molluscs. Correctly identifying habitat requirements of these species will be extremely important for decisions on habitat conservation and management.

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