Helgol Mar Res (2002) 56:203–210
DOI 10.1007/s10152-002-0113-3

ORIGINAL ARTICLE

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Cultivation of polyps and medusae of Coronatae (Cnidaria, Scyphozoa) with a brief review of important characters

Received: 17 September 2001 / Revised: 20 May 2002 / Accepted: 25 May 2002 / Published online: 26 July 2002

Abstract This work is a concise guide to the methods, techniques and equipment needed for the collection and transport of specimens, for arranging, maintaining and controlling cultures, for handling polyps, ephyrae, medusae and/or planuloids, and for standardising species description on the basis of life-cycle studies of Scyphozoa Coronatae. Objective characteristics meaningful to systematics are listed and illustrated. Suggestions for important literature sources are given, mainly on the rearing of metagenetic cnidarians in the laboratory.

Keywords Cnidaria · Scyphozoa · Coronatae · Rearing techniques

Introduction

Since there have been different groups of researchers working on medusae and polyps, respectively, non-consistent classification systems have been introduced for the two generations of metagenetic cnidarians. To unify these systems for phylogenetic systematics it is essential to study life cycles. Since Naumov (1960), who tried to unify the systems of the Hydrozoa, numerous studies on life cycles have been done on this group, e.g. by Boero (1980a, b, 1987), Boero and Bouillon (1989, 1993), Boero et al. (1991, 1992, 1995, 1997), Edwards (1972, 1973a, b, c), Kubota (1978a, b, 1979, 1980–82, 1981, 1988, 1991, 1993), Kubota and Horita (1995), Migotto (1996, 1998), Migotto and Marques (1999a, b) and many others. Life cycle studies on Cubozoa have been done by only a few researchers (Arneson and Cutress 1976; Hartwick 1991a, b; Okada 1927; Werner 1973a, 1975; Werner et al. 1971; Yamaguchi and Hartwick 1980). Rearing experiments with Scyphozoa, except coronates, have been done by Calder (1973, 1982), Gohar and Eisawy (1960), Kikinger (1992), Rippingale and Kelly (1995), Spangenberg (1968), Spangenberg et al. (1997), Thiel (1962, 1963) and others. Living polyps of scyphozoan coronates have been studied by Metschnikoff (1886), Komai (1935, 1936), Komai and Tokuoka (1939), Werner (1966, 1967, 1970, 1971, 1973b, 1974, 1983, 1984), Chapman and Werner (1972), Kawaguti and Matsuno (1981), Werner and Hentschel (1983), Ortiz-Corps et al. (1987), Jarms (1990, 1991, 1997), da Silveira and Morandini (1997, 1998a, b), Bumann and Jarms (1997), Jarms et al. (1999), Sötje and Jarms (1999), Morandini and da Silveira (2001a, b). Detailed information about the cultivation of hydrozoans and scyphozoans has been given, among others, by Cargo (1975), Cornelius (1995) and Tardent (1978, pp 191–198).

In this paper we compile information from previous papers and add our own experiences to present a manual for cultivating coronates and elucidating their life cycles. This may help in collecting relevant data for cladistic research.

Collecting specimens

The coronates have traditionally been considered as deep-sea animals. Many medusae have been found in mid- or deep waters, such as the genera *Atolla*, *Periphylla*, *Paraphyllina* and some species of *Nausithoe*. There are polyps from depths down to 7,000 m in the collection of Copenhagen (Kramp 1959, 1962). However, polyps with zooxanthellae have been described which can live only in the light-zone of shallow waters (*Linuche unguiculata*, some *Nausithoe* species). We are also aware of three species from marine caves in shallow waters: *Nausithoe eumedusoides*, *N. planulophora* and *Thecoscyphus zibrowii*.

Communicated by H.-D. Franke

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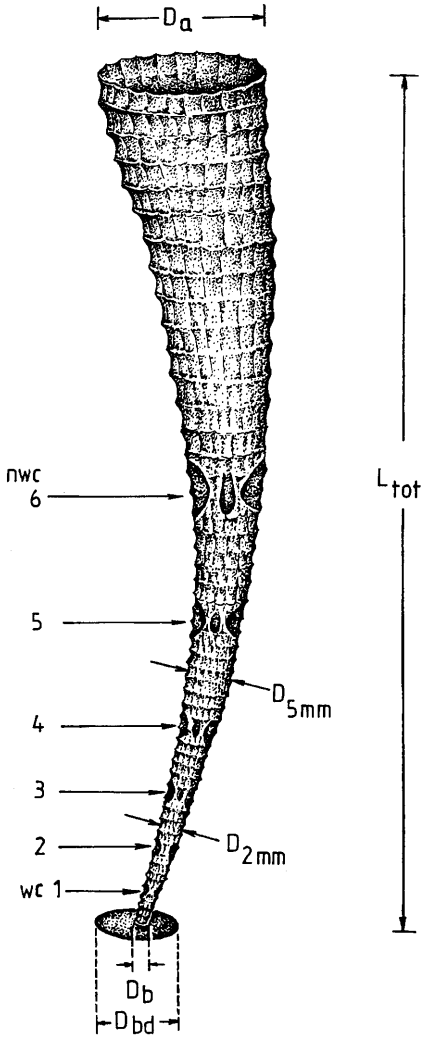

Fig. 1 Peridermal tube of a solitary coronate polyp (after Jarms 1991). D_a diameter aperture, D_{bd} diameter of the basal disc, D_b diameter of base of the tube, D_{2mm} D_{5mm} diameter in 2 and 5 mm height, L_{tot} total length, *nwc* number of whorl of internal cusps, *wc* whorl of internal cusps

We need to acknowledge that coronates live in all zones of the ocean.

The polyps of coronates need hard substrates to attach to. Mostly they are very small, from a few millimetres up to 1–2 cm. Usually they are found by careful inspection of rocks, biogenic hard substrate, such as shells, corals and gravels of these materials collected by dredges or box corers. For examination, these substrates are placed in seawater and inspected under a stereomicroscope. Normally many tubes are present on the collected substrates. Most of these are worm tubes, some may represent hydroid perisarc (for example tubulariids which may even have annulated tubes) or certain algae that may be confusingly similar to the coronate exoskeletons. However, coronates can easily be distinguished by a striking pattern on the external surface of the tube (Fig. 1). Specimens are collected, together with a small piece of substrate, and isolated in containers with fresh filtered seawater of the same temperature and salinity as at the sampling locality. Specimens collected from great depths have no problems with changing pressure, but they are stenoterm and have only a little tolerance for changing salinity. So it is important to ensure that temperature and salinity are the same as at the sampling place. If possible, water from the same station and depth should be used.

The best results in collecting specimens of shallow-water species are obtained by SCUBA diving. However, great experience with identifying marine animals is required because of the small size of the polyps and their often hidden habitats. Some species live in sponges, sticking their tentacles out of the oscula only when active. Sponges known to host *Nausithoe punctata* are, among others, *Cacospongia*, *Dysidea*, *Reniera*, *Suberites*, *Esperia*, *Myxilla*, *Spongelia* (Schulze 1877; Uriz et al. 1992) and *Mycale fistulifera* (Meroz and Ilan 1995). *Nausithoe racemosa* was found in association with Demospongiae species of the genera *Reniera*, *Suberites*, *Chalina*, and *Euspongia* (Komai 1935). In a special biogenetic formation in the Mediterranean, the so-called "coralligène", *Nausithoe marginata* lives hidden in the spaces of this hard substrate. Special substrates such as the skeletons of dead corals or rubbles can be found for example in the São Sebastião Channel (4–10 m depth) and in the Abrolhos region, both in Brazil. In these places *Nausithoe aurea* and *Linuche unguiculata* also occur on the buried surface of the substrate, so collected material has to be inspected carefully from the underside as well. The cave-dwelling species are mostly attached to the rocky walls and special tools (hammer, chisel, or at least a diver's knife) are needed to remove them from the substrate.

Transport of specimens

The transport of ship-collected specimens to the laboratory has to be well organised. This is essential for the development of the polyps and successful rearing of the species through the complete life cycle. At first the collected specimens have to be cleaned, i.e. all debris has to be removed from the surface of the tubes and, if possible, also the material which may plug the aperture of the tubes. Polyps are able to push some material out of their tubes, but bigger particles may block the aperture completely. Not only the polyps have to be cleaned, but also the small pieces of substrate they are attached to. In particular, living tissues of sponges as well as polychaetes have to be removed because after their death they may cause a rapid decrease in water quality. Of course, there will be a lot of bacteria left, but this is favourable for cultivation. Suitable containers for transporting cold-water species in warm regions or vice versa are Dewar containers. For longer transport it may be necessary to cool (for example by cooling elements or dry ice) or to heat. Filtered seawater of the original temperature and salinity should be used. The animals should not be fed for 1 day before transport because the remains of the meal may af-

fect the water quality. The transport container should be filled up completely with water to avoid turbulence, which may damage the specimens. Aeration is not recommended, as air bubbles may be fatal for the polyps. The animals should be transported in a large amount of water. In 250 ml of water about 30 polyps of 1 cm length can survive for at least 1 week at adequate temperature. During long-term transport, suitable water should be taken along in order to be able to change the medium and, ultimately, to start the first culture set.

Arranging cultures

The initial culture should be set up with the original seawater. Later, seawater from other localities may be used if the salinity is made equivalent by using distilled water or aquaristic sea salt. Our experience is that species cultivated only in artificial seawater sometimes do not develop normally and may have difficulties in propagation. All species without zooxanthellae can be kept in darkness in closed chambers (incubators) at a constant temperature depending on the animals' origin. Species with zooxanthellae, however, need light and have to be kept open in the rearing room or under a controlled light regime inside the incubators. The intensity of artificial light should be similar to daylight. It is not easy to find the most favourable illumination period because, on one hand, zooxanthellae need light (and polyps need zooxanthellae for their development, at least for their propagation) and, on the other hand, light makes algae grow in the cultures and on the tubes of the polyps. The growth of algae can be so strong that they overgrow the openings of the tubes and the polyps are no longer able to feed. In solitary species this may cause the animals' death, while in colonial species new polyps can grow from the scyphorhiza.

Long-term cultures can be kept in different containers. A small number of animals can be kept in 15 ml Petri dishes (da Silveira and Morandini 1997). These containers have the disadvantage that debris and mucus sticks easily to them if they are made of plastic, and that observation of specimens near the edge of the dish is nearly impossible. The advantage of Petri dishes is that the cultures can be easily covered. It is important to cover the containers because otherwise very soon a bacterial film will develop on the surface, which reduces gas exchange with the air. In some cases, however, it is recommended to have no air under the cover of the culture vessel (see below).

A very well established method is to cultivate polyps in an ordinary dessert glass bowl of 150 or 250 ml capacity with a glass cover (Werner 1968; Jarms 1978). These are easy to clean and all specimens can be seen clearly under a stereo microscope because there is no edge where specimens are difficult to observe. Debris accumulates in the rim of the glass bottom and can easily be removed with a pipette. A special substrate is not recommended but during propagation watch glasses can be helpful (see below).

Maintenance and control of cultures

The maintenance of polyp cultures depends mostly on their origin. The feeding intervals are longer the colder the culture is kept. For a normal development, species in 4–8°C water need food only once a fortnight. Specimens kept at 22–26°C need food every week, and if they are being encouraged to propagate feeding is recommended every 2–3 days.

Coronates are carnivorous. For normal maintenance, living crustaceans, such as the nauplii of *Artemia* spp. or the marine harpacticoid *Tisbe holothuriae*, are good food. The production of *Artemia* nauplii from commercially available dry cysts is easy and well established. The nauplii should serve as food only 2–3 days after hatching, when their colour has faded (i.e. when most of the yolk has been used up), because otherwise overfeeding of the polyps may result. *Artemia* nauplii are added to the culture vessels and cultures are kept in darkness in order to obtain an even distribution of food in the water. The nauplii of *Artemia* are positively phototactic and would normally assemble where light intensity is maximal, i.e. somewhere on the surface where the polyps cannot capture them. Between 20 min and 1 h after nauplii have been added, the medium needs to be changed. This because left-over nauplii, especially in warm water cultures, would die and decay very fast, causing oxygen deficiency for the polyps. The water for exchange has to be stored within the same culture chamber and has to be taken out of the chamber at the same time as the cultures, so that both change their temperature simultaneously and a temperature shock is avoided. If the polyps are too small to swallow 2–3-day-old nauplii, just hatched deep orange nauplii can be used. These contain a very high amount of lipids, and thus care has to be taken that only one or two nauplii are swallowed by each polyp. In this procedure, too, the medium has to be changed after a short while.

The culture of *Tisbe holothuriae* has been described by Werner (1971) and Jarms (1978). In a one-litre glass bowl with slight aeration *Tisbe* can be cultivated in normal sea-water at room temperature if this is not higher than 24°C. Sea water from the polyp cultures can be reused after filtration. *Tisbe* cultures have to be renewed every week: aeration is stopped, and particles settled on the bottom are removed by using a pipette. Because the water quality is worst near the bottom, *Tisbe* assembles mostly at the surface and can be poured through a 40 µm mesh net. The culture vessels are then cleaned with pure water and a brush, seawater is refilled and the animals from the sieve are added. Food is added and the aeration is started again. Boiled, 110°C dried (storable in a closed glass container for months) and minced mussels (*Mytilus edulis* or similar species) serve as food. Small harpacticoids stay alive for more than 14 days in the polyp cultures and can be used both in low- and high-temperature cultures. *Tisbe* should be added only after the water has been changed and only to cultures kept in bigger containers in order to avoid oxygen deficiencies. At room

temperature *Tisbe* has a 14-day life cycle, so that in the cultures there are always all developmental stages of the species available. Using a sieve set with mesh sizes of 125, 80 and 40 µm, respectively, will provide three different fractions (roughly adult stages, copepodits, and nauplii) for all sizes of polyps. Using this animal has the advantage of having a permanent supply of living food available. Furthermore, *Tisbe* cleans the tubes of the polyps from debris, bacteria and algae.

Propagation of coronates depends on several different factors. Besides the annual cycles known in two solitary species (*Nausithoe eumedusoides, N. hagenbecki*) and one colonial species (*N. racemosa*), propagation takes place mainly when the polyps are well fed and have a surplus of energy stored in the base (Werner 1979). Thus food rich in energy is likely to induce strobilation. The hepatopancreas of *Mytilus edulis* or similar species is a suitable food. The organ has to be removed from the animal, transferred to fresh seawater, and is cut into very small pieces. This mass must be rinsed several times with seawater to remove most of the mucus. When the water stays clear and separate small tissue pieces can be identified, the latter can be used as food. Single pieces have to be put directly into the tentacles of the polyps, because they are normally used to catching moving prey. Care has to be taken not to use pieces which are bigger than the aperture of the tube, because these may block the tubes and cause serious damage to the polyps. After pieces of the *Mytilus* hepatopancreas have been swallowed and can be seen deep in the gastric cavity of the polyps, the water has to be renewed. Tiny polyps can also be fed with homogenised gonads of mussels such as *Perna perna* or similar species (see below).

What to do when polyps propagate

Well kept polyps can start to strobilate. At first the tentacles shorten, then the whole head with collar, mouth plate and resorbed tentacles is retracted into the tube. The uppermost tissue forms a plate, closing the aperture of the tube. Sometimes it produces a cover of a thin periderm layer. Feeding is no longer necessary, but since not all polyps in a culture will strobilate at the same time it is better to isolate those polyps. To have a single polyp in a single jar is recommended when collect stephanoscyphistomae (Jarms 1991), because we do not know in advance what the result of the strobilation will be. Normally ephyrae are produced but also eumedusoids (*Nausithoe racemosa, N. eumedusoides*) or planuloids (*N. planulophora*) may be the outcome of a strobilation. Even an irregular fragmentation as in *Linuche unguiculata* (da Silveira and Morandini 1998b) can occur. The ephyrae have to be handled in a special way. Some species such as *N. thieli* (Jarms 1990) tend to swim to the surface for some days and then may adhere to the bacterial film at the surface and die. Also in *N. aurea, N. punctata, N. marginata*, species with very small and delicate ephyrae, this can happen accidentally. The only way to avoid this is to fill up the jar with seawater so that no air remains between water surface and cover.

First feeding of ephyrae requires special food and special methods. One method is to feed the small ephyrae with homogenised gonads of *Perna perna* or similar species (da Silveira and Morandini 1997). The gonad is extracted from live or deep-frozen mussels and macerated with filtered seawater in a glass homogeniser. A drop of this fluid is added to a Petri dish with seawater and the ephyrae are transferred into it. Each ephyra is pushed towards the food settled at the bottom of the dish. The ephyrae stay under observation with a stereomicroscope until the gastric cavity is filled up with food. Then they are transferred to a culture jar with fresh seawater. For normal growth, daily feeding is recommended. To feed more than once a day, however, causes the death of most ephyrae by overfeeding. Another method of feeding very small medusae (*Atorella vanhoeffeni*) is to use the eggs of *Tisbe holuthuriae*. Adult *Tisbe* are isolated by a sieve with 125 µm mesh size. After concentrating the animals, the eggs can be squeezed out gently. Each ephyra has to be fed by hand, using a very fine needle or hairs of a paintbrush.

Large ephyrae just after liberation can be fed with nauplii of *Artemia* but care has to be taken that they only get one each. An additional food for some species is the first nauplius stage of *Tisbe*, which can be obtained by sieving or from hatching eggs. This first stage is not useful for delicate ephyrae of some species because they can recover inside the gastric cavity after being swallowed and then can eat the ephyra from the inside. The bigger the young medusae grow, the more they may be fed with *Artemia* nauplii and, after a change of water, small *Tisbe* may also be added. Later when medusae are more than 1 cm in diameter, feeding every other day can be sufficient. Medusae become mature after between 40 days (*N. aurea*) and 3 months (*N. werneri*).

Planuloids and fragments are not to be fed but have to be transferred into a glass jar with a watch glass or cover slides inside. These substrates should have been exposed for at least 2 weeks to the seawater for the cultures to develop a bacterial film. This is needed for settlement of planuloids and fragments. After they have settled, young polyps can easily be transferred with their substrate to new containers with fresh filtered seawater. In this way they can stay settled on the substrate and are easy to handle.

Protocols

To describe the whole life cycle of a reared species continuous observation is needed. It is sufficient to observe the polyps whenever they are fed; they should not be exposed too long to temperatures much higher or lower than in the cultivating chambers. From the beginning of strobilation daily observation is needed; during the critical phase of liberation of ephyrae or planuloids, and the

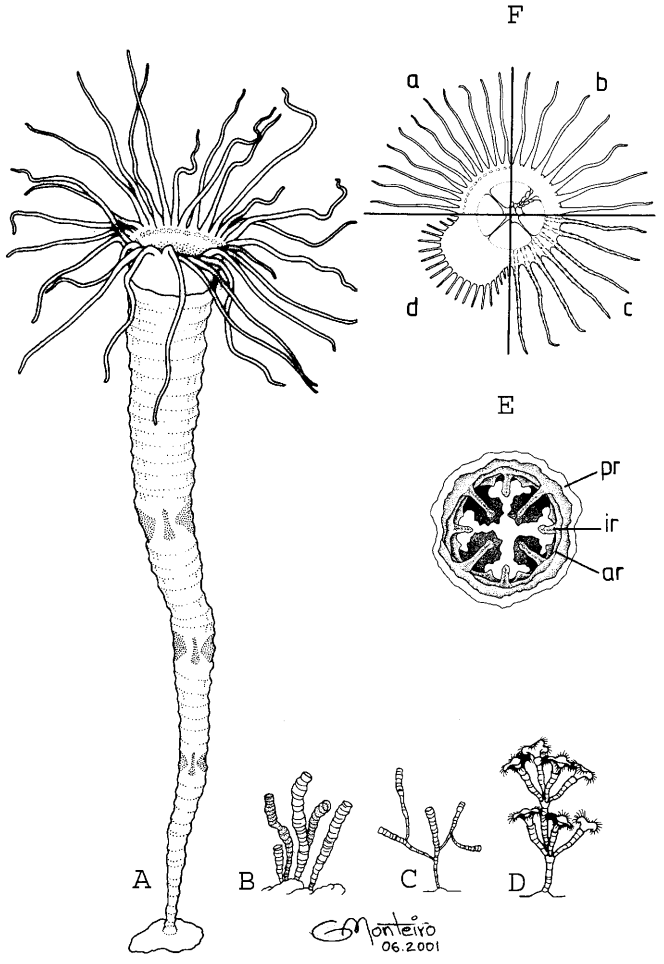

Fig. 2a–f Polyps of Coronatae. **A** General habit of a solitary coronatae polyp. The basal disc attaches the tube to the substrate. Inside the tube there are whorls of internal cusps. At the apical pole the collar with its crown of tentacles above the rim of the skeleton. **B** Stolonial colony of *Linuche unguiculata*. **C** Sympodial irregular colony of *Nausithoe punctata*. **D** Monopodial regular branched colony of *Nausithoe racemosa*. **E** Transect of the coronate tube showing a whorl of internal cusps; *ar* adradius, *ir* interradius, *pr* perradius. **F** Top view of the mouth plate and tentacle crown of a coronate polyp. Combined figure from (*a*) *Nausithoe planulophora* with white pigment spots near the tentacle bases, (*b*) *Nausithoe hagenbecki* with special mouth lips, (*c*) *Linuche unguiculata* with rows of zooxanthellae along the tentacles, (*d*) *Nausithoe racemosa* with a lobed collar and short tentacles

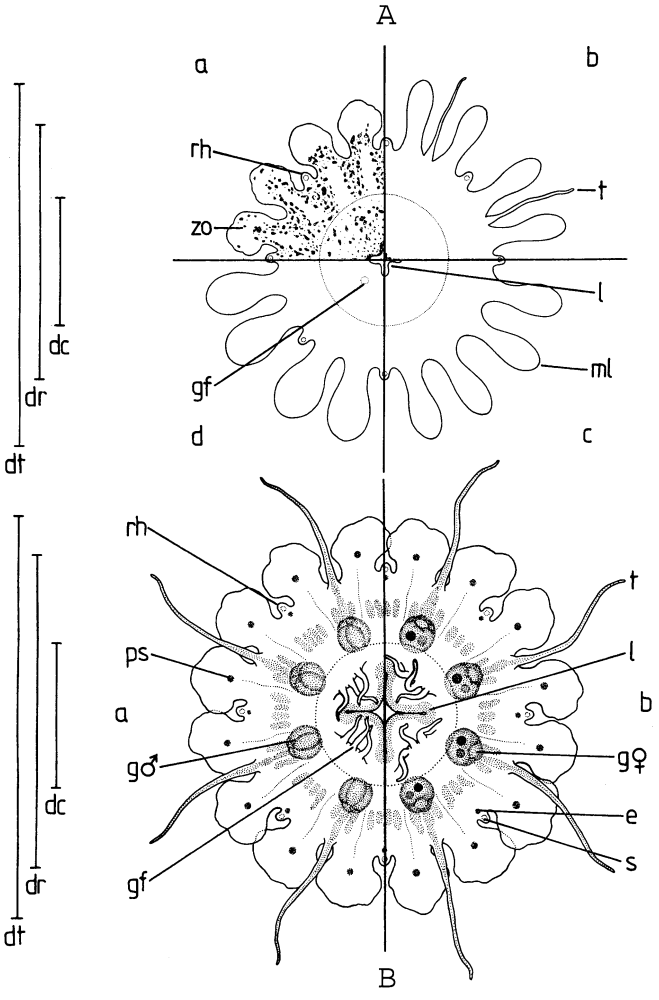

Fig. 3a, b Ephyra and medusa of Coronatae. **A** Just released ephyra of the Nausithoidae (Coronatae). Combined figure from (*a*) *Linuche unguiculata* with short lappets and zooxanthellae, (*b*) *Nausithoe marginata* with slender marginal lappets, rhopalia, and tentacles but without mouth lips, (*c*) *Nausithoe punctata* without tentacles and rhopalia, and (*d*) *Nausithoe werneri* with broad marginal lappets and buds of gastric filaments. *dc* Diameter of the coronal furrow, *dr* diameter between rhopalia, *dt* total diameter, *gf* bud of gastric filament, *l* lips around mouth, *ml* marginal lappets, *rh* rhopalium, *t* tentacle, *zo* zooxanthellae. **B** Adult medusa of *Nausithoe aurea*. Combined figure of a male (*left*) and female (*right*) specimen. *dc* Diameter of the coronal furrow, *dr* diameter between rhopalia, *dt* total diameter, *e* eyespot, *g* male and female gonads, *gf* gastric filament, *l* lips around mouth, *ps* pigment spot, *rh* rhopalium, *s* statolith, *t* tentacle

settlement of planuloids, observation may be needed every hour. To compare characters and measurements recorded by different researchers it is necessary to collect data by using a standard method.

There are solitary (Fig. 2A) and colonial species. We have found three different forms of colonies: stolonial (Fig. 2B), irregular sympodial (Fig. 2C), and monopodial racemous (Fig. 2D). The tubes of the polyps show several characters (see Jarms 1991), which are not only useful for determination but also for phylogenetic analysis. Very stable characters are the diameter just above the basal disc (Fig. 1), the number of the outer transversal structures per length unit; the number, shape, and arrangement of internal cusps (Fig. 2E), and the values of the formquotients D/L_{2mm} or D/L_{5mm} (see Jarms 1991). The diameter of the basal disc, colour of tubes, total length, and terminal diameter, are variable or age-dependent characters. Stable characters of the soft body are special shapes of the collar (Fig. 2F-d) and the mouth lips (Fig. 2F-b), and pigment spots at the tentacle bases (Fig. 2F-a). The number of tentacles is age-dependent but in adult specimens it is a good character, although the number decreases just after strobilation before it increases again. Zooxanthellae (Fig. 2F-c) are good for de-

termination but not for analysis. Stable characters of the just liberated ephyrae are the shape of marginal lappets (Fig. 3A), presence of rhopalia with or without eye spots (Fig. 3A-a, -b, -d), number and presence of tentacles (Fig. 3A-b), absence of all marginal organs (Fig. 3A-c), absence of mouthlips (Fig. 3A-b), presence of gastric filament buds (Fig. 3A-d), and shape of the central disc. The different combinations of these characters are complemented by measurements which are variable to a certain extent, such as the diameter of the coronal furrow, diameter between rhopalia, and total diameter (Fig. 3A). Pigments and zooxanthellae (Fig. 3A-a) again are useful for determination but less for analysis, which is also true for the mature medusae. Stable characters of medusae are the number of marginal organs such as lappets, tentacles, and rhopalia; the shape of the central disc; the number, size, and shape of gonads and their relative position to the coronal furrow; and the arrangement and number of gastric filaments (Fig. 3B). An important feature is the presence of pigmented eyespots in the rhopalia. Variable to a certain extent are length of tentacles, shape of marginal lappets, and all diameters.

The careful collection of all these data is the basis of all further work on systematics. For cladistics, characters have to be evaluated. This is the point where expertise about the group is needed and where subjectivity comes into the result. This subjectivity is also present with choice of an outgroup and cannot be avoided by using apparently objective computer programs.

Acknowledgements This work was supported by CAPES (PROAP/2000) and FAPESP (99/12433-0). We thank the Director and staff of CEBIMar, at São Sebastião, SP, for their support. We are deeply grateful to Mr. G.R.C. Monteiro (FITO) for the drawings. We are indebted to Brigitte Hilbig for her comments on the manuscript and revision of the English.

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