The most complex seaweeds are composed by holdfast(s), stipe(s) and frond(s). A typical example of this morphology is the genus *Sargassum* (Fig. 41A). The function of the holdfast is solely attachment (as opposed to roots in higher plants which also play a role in extracting water and nutrients from the soil). It can be rhizoids (thin filamentous structures: *Caulerpa* spp., Fig. 41B). In *Avrainvillea erecta* (Fig. 41C) and *Halimeda macroloba*, these filamentous structures get intricated and hold large amounts of sand, resulting in a 'bulbous holdfast' which is completely sunken in the soft substratum. Attachment can also be performed by a disc (most *Sargassum* spp., most red algae, Fig. 41D). The stem-like portion (stipe) of the thallus can be cylindrical or compressed, unbranched or branched, supple or rigid. It bears one or several blades (the genus *Sargassum*, Fig. 41G) which are wider than the stipe and are the main photosynthetic part of the seaweed. At the basis of the stipe, horizontally spread branches can be present (stolons or rhizomes, Figs 19D, 41E, F), spreading across the substratum, possibly attaching to the substratum again and giving rise to new uprights. In some species (*Sargassum*) the uprights bear air bladders (Fig. 41H) as 'floaters', to keep the plant upright and optimalize the surface for photosynthesis.

Fig. 41. Seaweed morphology: holdfasts, leaf-like structures, air bladders. A. *Sargassum* sp.: a thallus with holdfasts, stipes and blades; B. *Caulerpa sertularioides* with prostrate rhizomes attached by numerous rhizoids; C. *Avrainvillea amadelpha* with a bulbous holdfast composed of intertwined filaments; D. *Halymenia durvillei*: discoid holdfast; E. *Turbinaria* sp. with stolons; F. *Gracilaria corticata*, with basal stolons; G. *Sargassum crassifolium* with leaf-like blades on the stipes; H. *Sargassum crassifolium* with air bladders (aerocysts).

The growth direction of seaweeds can vary: most are erect (*Dermonema virens*, Fig. 42A), at least when they are submerged. Others grow horizontally and mostly have numerous attachment points to the substratum (*Dictyota* sp., Fig. 42B): they are prostrate. Some are horizontally spread in the basal portion, but upwardly curved towards their apices (*Halimeda gracilis*, Fig. 42C): they are ascending, or downwardly curved: they are arcuate (*Valoniopsis pachynema*, Fig. 42D). Others again are horizontally spread from a vertical wall (*Peyssonnelia* spp., some *Halimeda* spp. Fig. 42E): they are resupinate. Finally some seaweeds hang down from vertical or overhanging walls (some *Halimeda* spp., Fig. 42F): they are pendulous.

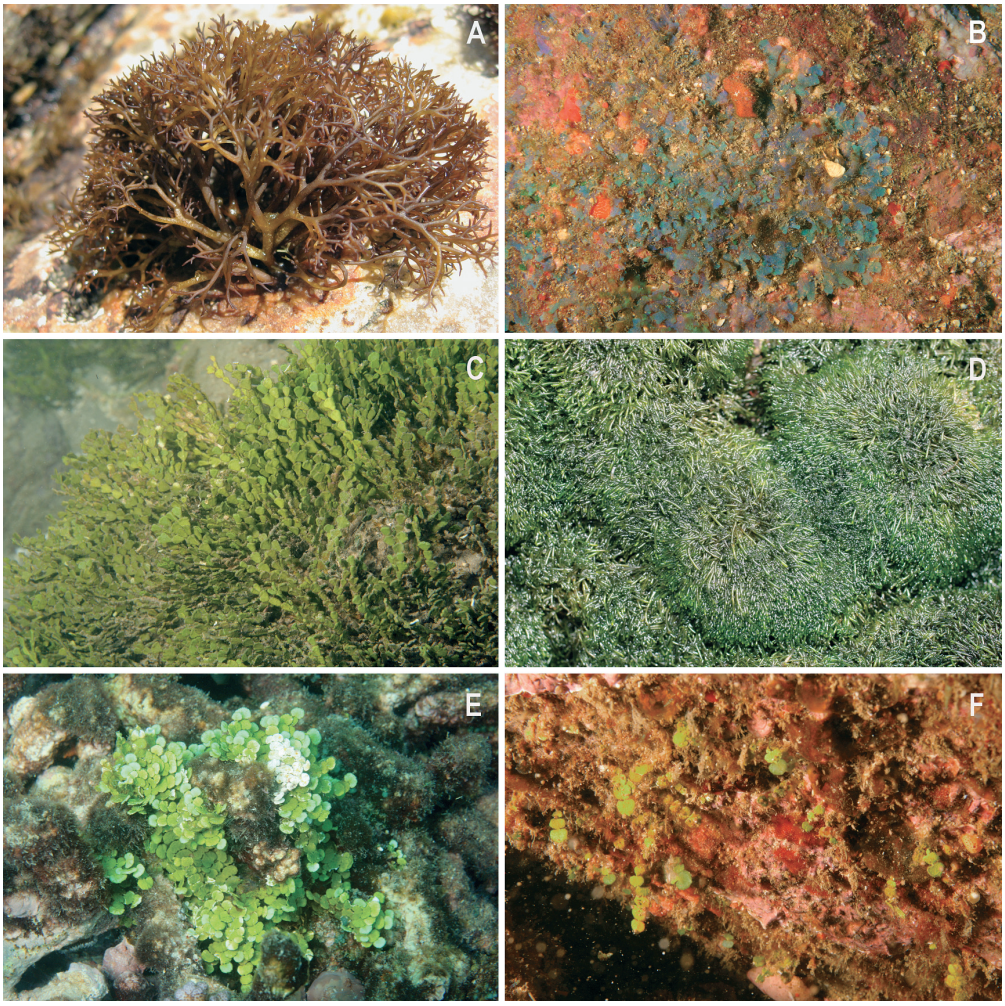

Fig. 42. Growth forms. A. Erect: *Dermonema virens*; B. Prostrate: *Dictyota*; C. Ascending: *Halimeda gracilis*; D. Arched branches of *Valoniopsis pachynema*; E. Resupinate: *Halimeda* sp.; F. Pendulous: *Halimeda* sp. hanging down from an overhang.

Another vegetative character that can be used in some groups of seaweeds is the way of cell division. In most cases, the apical cell undergoes a transverse division, the daughter cells grow longitudinally, elongating the main axes. A successive inclined division at the apical pole results in a lateral branch. If this cell division process is repeated, the result is an acropetal organization of the thallus: the side branches are progressively longer from the apex to the basis (Fig. 43A). In other taxa, intercalary cell divisions occur: older cells undergo cell divisions. This results in a non acropetal organization of the thallus: longer side branches alternate with shorter ones (Fig. 43B). In other green algae, the formation of a transverse wall at the basis of the side branch is delayed (*Cladophoropsis sundanensis*, Fig. 43C). In some green algae (the genera *Siphonocladus*, *Struvea*, *Dictyosphaeria*) a special kind of cell division occurs, called segregative cell division. A multinucleate protoplast divides into several, rounded daughter protoplasts within the mother cell (Figs 43D, E), which subsequently become surrounded by a wall (Fig. 43F). The newly formed cells are either released after rupture of the mother cell (*Valonia ventricosa*), remain *in situ* and form parenchymatic thalli (*Dictyosphaeria* spp.), or rupture old parental walls and form branches (*Struvea* spp., *Siphonocladus* spp., Figs 43G-H). In the genera *Ernodesmis* (Fig. 43I) and *Valonia*, small, lens-like cells are formed at the apex of the mature cells, growing out to new cells.

A major problem in describing or identifying seaweeds is their morphological plasticity. Depending on the ecological conditions, the same species can become larger (in a sheltered lagoon) or smaller (on the seaward, surf-exposed rock wall), less or more densely branched, plane or spirally twisted, without or with hook-like branches. An extreme example is the *Caulerpa racemosa*-complex, where on the same stolon (thus the same individual) the erect branches can have a different morphology from the proximal to the distal part of that stolon. Sometimes the side branchlets of a single upright can be different from the basis to the tip, being cylindrical at the basis, club-shaped higher up, becoming turbinate or even peltate at the tip. As the morphology of these side branchlets has been used in the past to describe taxa (species, varieties or forms), the presence of a mixture of morphologies creates major identification problems. Other seaweeds change their morphology by ageing or show sexual dimorphism (the genus *Sargassum*, *Boodlea composita-Phyllodictyon anastomosans* complex).

On the other hand, molecular systematics frequently points out to ‘cryptic diversity’: seaweeds with a similar morphology appear to belong to different taxa, based on the DNA-information. As a result, new species will have to be described, preferably with (at least) a distinguishing morphological or anatomical character or a different geographical distribution (the different taxa being present in different oceans).

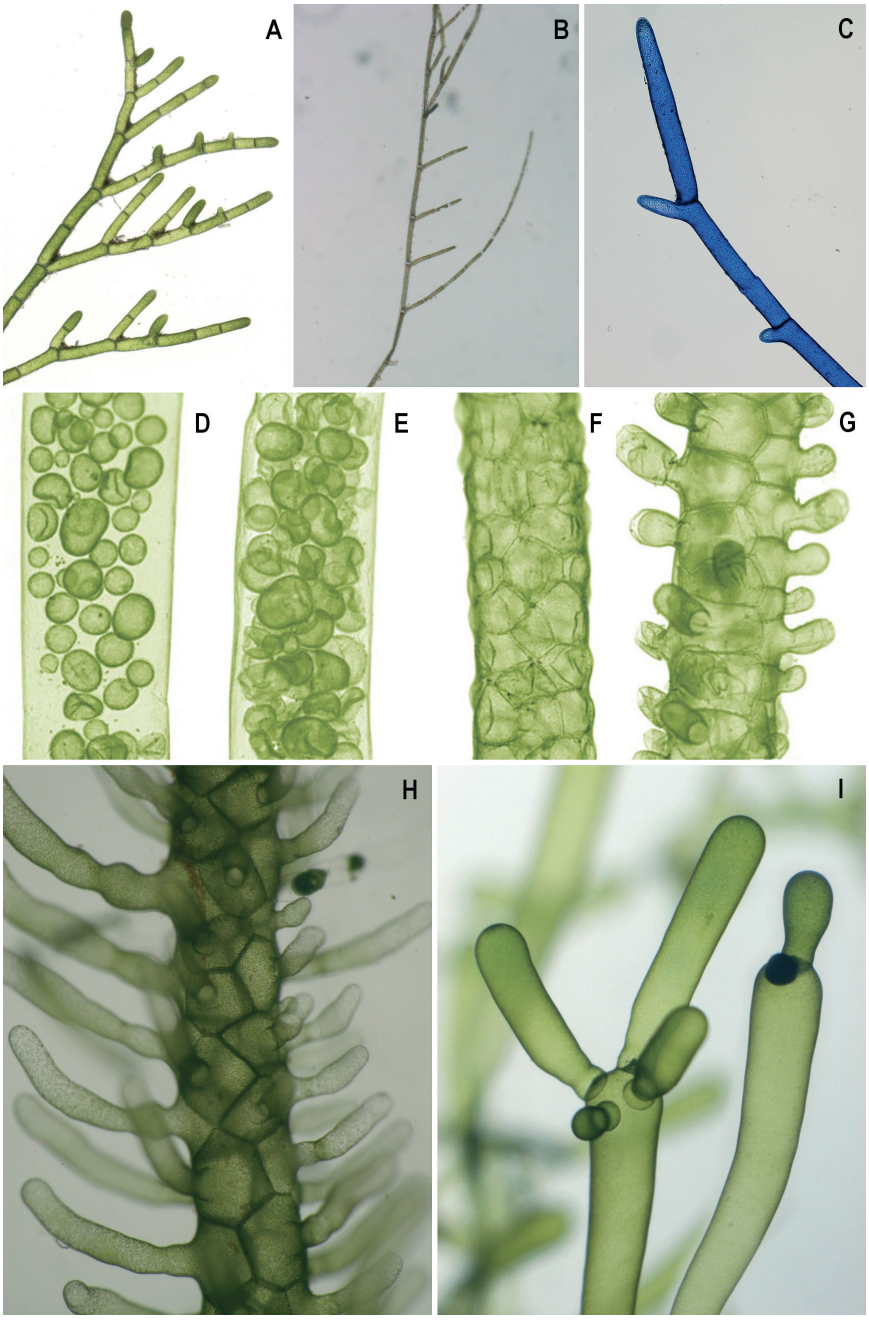

Fig. 43. Cell division. A. Acropetal organization: the branchlets gradually become more developed proximally; B. Non acropetal organization, with short branchlets alternating with longer ones (*Cladophora sericea*); C. Postponed transverse cell wall formation after the formation of a lateral branch (*Cladophoropsis sundanensis*); D-G. Segregative cell division in *Siphonocladus* sp.; H. Final stage of segregative cell division: numerous side branchlets growing out of the parental cell; I. Formation of apical lenticular cells from where new cells grow out (*Ernodesmis* sp.).

8.4. Life histories and reproduction

Life histories in seaweeds are complex; moreover they vary among and even within groups. Therefore only a general scheme can be given here, although characters of the reproductive structures can be critical for the identification on species or even on genus level. In most green and brown algae there is an alternation of two generations: the haploid gametophytes and the diploid sporophytes. The gametophytes produce gametes in gametangia, specialized structures which, in general, can only be observed by microscope. In several brown algae however, where reproductive structures are often grouped in sori (*Dictyota* sp., Figs 44A-D) or in receptacles with gametangia in *Sargassum* sp. (Fig. 44E) and *Turbinaria* sp. (Fig. 44F) which can be observed with the naked eye. The male and female gametangia are mostly produced on different plants, but in some cases they are both present on the same plant. The gametes will fuse and produce a diploid zygote which germinates into a diploid sporophyte. On the sporophyte, meiosis takes place and haploid spores are produced (*Dictyota* spp., Figs 44G, H), developing into new gametophytes. In some rare cases (*Codium* spp., *Caulerpa* spp.), the life cycle is reduced to a single diplont generation, the only haploid stages being the gametes. Moreover, in the genera *Halimeda, Caulerpa* and other green algae, the whole cytoplasmic content of the thallus is being transformed to gametes (= holocarpy, Fig. 44I).

In red algae, the life history is even more complex by the addition of a third generation: fertilisation of the female gamete (carpogonium) attached on a carpogonial branch, is performed by a male gamete (spermatium), produced in a spermatangium (Fig. 45A); spermatangia can be grouped in sori (Fig. 45B). The diploid zygote remains attached to the haploid female gametophyte and develops in a diploid carposporophyte. This part of the life history (a generation) usually has only small dimensions, but generally visible with the naked eye, as globular structures, called gonimoblasts (Fig. 45C) or as lateral, ball-like structures, called cystocarps (Figs 45D-F). In some cases, the cystocarps are embedded in the thallus and therefore more difficult to see in the field. The carposporophytes produce diploid carpospores which germinate after liberation into tetrasporophytes in which meiosis takes place with the production of haploid tetraspores (Fig. 45G) which in some cases can be grouped in stichidia (Fig. 45H) or in sori (Fig. 45I). The tetraspores germinate into haploid gametophytes. In most of the red algae, the life cycle thus consists of three generations, of which the gametophyte and the tetrasporophyte are often (almost) identical. In some cases (*Asparagopsis taxiformis*), the tetrasporophyte (*Falkenbergia hildenbrandii*) is markedly different from the gametophyte (Fig. 45J). In the past, both generations of that seaweed have been described as different algae, placed in different genera, as phycologists then were unaware of the fact that they represent two phases of the same seaweed. It is only after culture experiments in aquaria that this was discovered. In some brown (*Sargassum*) and red algal genera (*Liagora*) one of the phases can be microscopic or crustose.

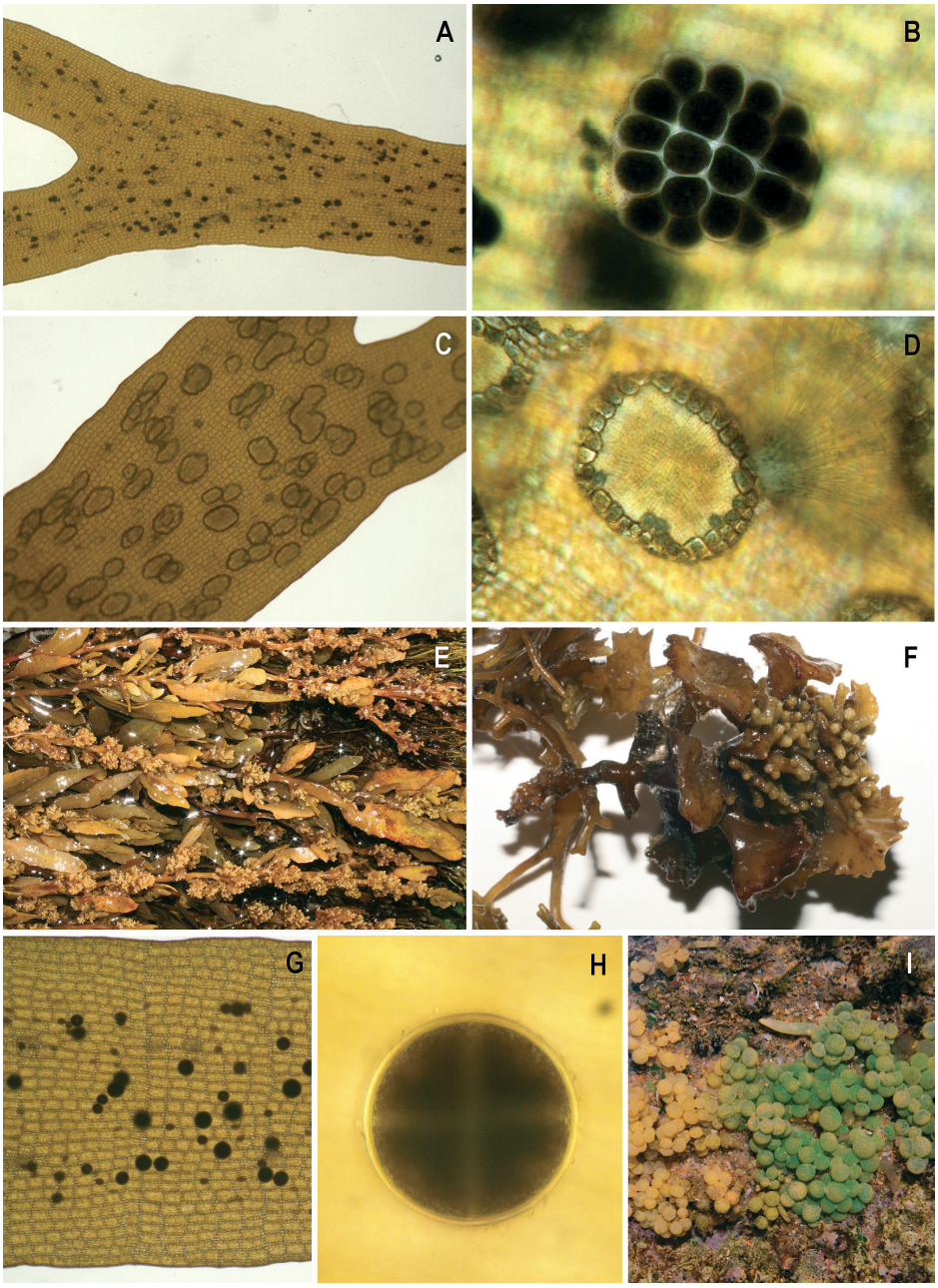

Fig. 44. Reproduction structures in brown and green algae. A. Sori of female gametangia (oogonia) on the haploid gametophyte of a *Dictyota*; B. Detail of a sorus of oogonia of a *Dictyota*; C. Sori of male gametangia (spermatangia) on the haploid gametophyte of a *Dictyota*; D. Detail of a sorus of spermatangia of a *Dictyota*; E. Receptacles of a *Sargassum*, containing the gametangia; F. Receptacles of a *Turbinaria*, containing the gametangia; G. Tetrasporangia on the diploid sporophyte of a *Dictyota*; H. Detail of a cruciately divided tetrasporangium of a *Dictyota*; I. A *Caulerpa*-plant in which the formation of gametes is taking place (the yellowish part of the thallus).

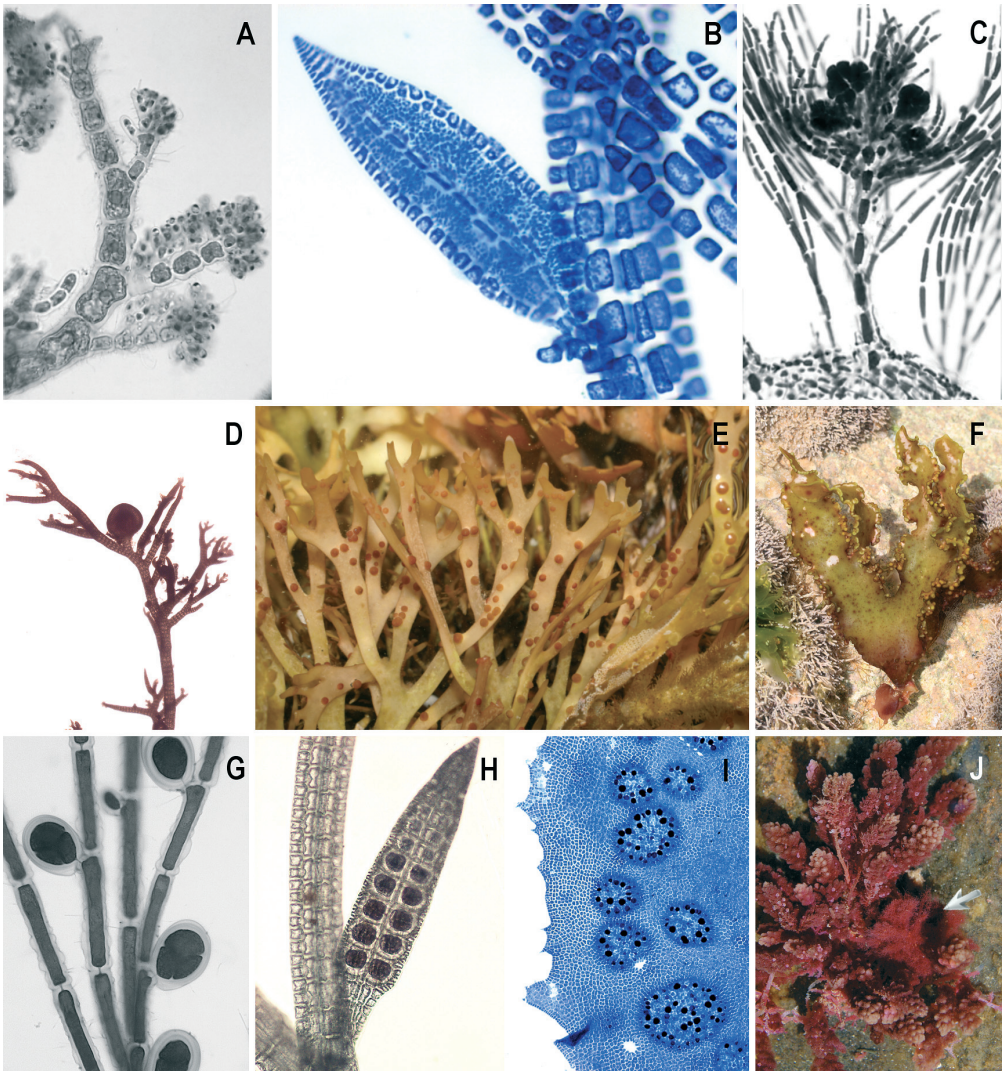

Fig. 45. Reproduction structures in red algae (mainly shown by African examples). A. Spermatangia in *Sciurothamnion stegengae*; B. A sorus of spermatangia in *Platysiphonia delicata*; C. Gonimoblasts (groups of diploid carpospores) in *Sciurothamnion stegengae*; D. A cystocarp on *Rhodomelopsis africana*; E. Cystocarps as wart-like protrusions on *Gracilaria corticata*; F. Cystocarps (mainly) on the margin of the female blade of *Sarcodia montagneana*; G. Tetraspores in *Sciurothamnion stegengae*, produced after meiosis in tetrasorangia on the diploid sporophyte; H. Tetrasporangia in a stichidium of *Platysiphonia delicata*; I. Sori of tetrasporangia in *Augophyllum marginifructum*; J. *Asparagopsis taxiformis*: the large gametophyte with cystocarps and the filamentous tetrasporophyte (*Falkenbergia hildenbrandii*) in the centre (arrow).

Reproductive structures, or even the presence of a particular life history phase, are generally seasonal. It is therefore imperative to carry out collecting in different seasons as reproductive characters are mostly needed for correct identification (as flowers are in higher plants).

Many seaweeds also reproduce asexually (without formation of gametes), by the production of asexual spores. Some even multiply vegetatively, by fragmentation (some branches break off, stay alive, attach to the substratum and go on growing to new plants) or by production of propagules (*Sphacelaria* spp.: branchlets with a special morphology, detaching from the mother plant and each of them producing a new juvenile; Fig. 104). Others again, growing in soft substratum, can produce underground, horizontally growing bundles of rhizoids from which new erect plants develop (genera like *Udotea*, *Halimeda*, …).

8.5. Biodiversity of seaweeds

In most biodiversity studies the algae are omitted, probably because they are 'invisible' as a result of their submersed habitat. The total number of species of algae is difficult to assess: the important environmentally induced morphological plasticity and variability results in major identification problems: some entities are classified on different taxonomic levels, depending on the author (species, variety, form). The total number of Algae [including (freshwater) microalgae] would be approximately 350 000 spp. (Brodie & Lewis, 2007).

Some areas in the world are more species rich than others. In the Pacific Ocean, species-rich areas are the Philippines and Japan; in the Atlantic Ocean: Europe (N-Spain, France, United Kingdom), the Caribbean Sea. The Red Sea and the Indian Ocean are still understudied, but South Africa and southern Australia seem to have a high seaweed diversity. In South Africa this could be the result of the presence of different climate zones.

Maximum seaweed endemism is present in Antarctica, southern Australia and New Zealand.

Based on data from the literature, Silva *et al*. (1996) mention 455 taxa belonging to 410 species and 161 genera for Sri Lanka. However, as already mentioned in the chapter on the history of seaweed research in Sri Lanka, this island is absolutely understudied. Historical collections from sublittoral biotopes are sporadic, and recent ones are still under study. A study by Mallikarachchi (2004) shows that a large percentage of the known seaweed flora of the island is present along a limited SW-shoreline. This conclusion is partly biased as this part of Sri Lanka has been most frequently visited by phycologists, whereas collections from the N and East coast are scarce and therefore even more fragmentary.

Smaller species (such as turf-forming algae and epiphytes) are more numerous, adding to the species diversity, but they are not readily observable/identifiable and therefore most of these are not included in this Field Guide.

8.6. Nomenclature, taxonomy and classification of seaweeds

The nomenclature of algae (giving scientific names to organisms and groups to which they belong), similarly to higher plants, follows the International Code of Botanical Nomenclature (ICBN, 2006). Macroscopic seaweeds belong to 4 Divisions (or Phyla) if the blue-greens are included: Cyanophyta (Cyanobacteria) - Blue-green algae (prokaryotic), Chlorophyta - Green algae, Phaeophyceae - Brown algae, Rhodophyta - Red algae. The divisions are subdivided in classes, which names end on -phyceae (exclusively for Algae). The classes contain orders, ending on -ales, subdivided in

families, ending on -aceae. The nomenclature in botany (including flowering plants, ferns, mosses, algae and fungi) is binomial, meaning that the name is composed of two words: the genus name (e.g. *Rhodymenia*), with a capital initial followed by the species epithet (e.g. *Rhodymenia tripinnata*) written in lower case. The genus and species names are usually written in italics. They are followed by the name of the author who described the species (e.g. *Rhodymenia tripinnata* Hering). Sometimes, further research shows that the original author placed the species in a wrong genus. In this case, the name of the first author is placed between brackets and the name of the author who makes the new combination (putting the species in the correct genus) is added (e.g. *Portieria tripinnata* (Hering) De Clerck). In some species, subentities (infraspecific taxa) are distinguished which are called varieties (var.) or forms (f.), the names of which are again usually written in italics.

When proposing a new species, a type specimen is designated after which the species is described. For seaweeds this is generally a herbarium specimen, which is then deposited in a registered herbarium. Quite frequently isotype specimens are deposited in other important herbaria. These isotype specimens were collected at the same type locality (place where the type specimen is coming from), on the same day as the type specimen and were regarded as 'duplicates' by the original author (= form part of a single collection). Type specimens are extremely important for subsequent studies of the species (checking for new characters, for DNA-analysis, …). Preferably, several specimens should be mounted on a 'type sheet', with the indication of the real type specimen, the holotype, as to show the morphological variation of the species (sometimes gametophytes and sporophytes are (slightly) different, or different ecological situations induce a morphological change).

The description of a new species of seaweed has to include the reference to the type specimen as well as a diagnosis in Latin (what are the characters of this species, distinguishing it from other species of the same genus). Illustrations also have to be added.

Subsequent analysis sometimes indicate that two 'species', each with their own name, described from different areas (even from different oceans) are identical. Only a single name can be applied for that species, and the name from the oldest description has to be chosen; the other name then becoming a synonym. Opposite to this, molecular analysis sometimes proves that a species, present in different oceans (with a similar morphology at the different locations), belongs to different species according to the locality (cryptic species). The specimens from the type locality then keep the original name, whereas the other ones have to be described as new species. A thorough study of morphological and anatomical characters then 'hopefully' leads to discriminating characters for each species. All this means that names of seaweeds change in time and that the same taxon can have different names in different books, depending on the time of publication. If one is compiling a species list from a region, he should be aware of these synonymies for not including the same species several times under different names.

As a result of ongoing molecular research, the higher rank classification of seaweeds also changes on a regular basis. We here follow the Index Nominum Algarum (http://ucjeps.berkeley.edu/INA.html) and Guiry & Guiry (2009; www.algaebase.org) which are both excellent sources for keeping up with recent taxonomic revisions as they are

continuously updated (see remark at introduction of Rhodophyta). Silva *et al*. (1996, http://ucjeps.berkeley.edu/rlmoe/tioc/ioctoc.html) is also an excellent basis to find synonymies, taxonomic remarks, and a systematic classification of the seaweeds of the Indian Ocean. Be aware, however, that since 1996 a surprisingly large number of names have changed already.

As opposed to terrestrial plants, seaweeds rarely have common (vernacular) names. Moreover, they sometimes induce confusion, such as 'Ceylon Moss' which is not a moss at all, but a red alga, *Hydropuntia edulis* (not included in the present book).

8.7. Identification of seaweeds

If possible, one should start by following a training course where specialists can introduce you to the most common genera and species of the area. If this is not possible, Field Guides on the area (such as this book) or from the same ocean or from an adjacent tropical region should be used. They are becoming more numerous nowadays. Useful recent guides for Sri Lanka are: Huisman (2000) on Marine Plants of Australia, Littler & Littler (2003) on South Pacific reef plants, De Clerck *et al.* (2005a) on the seaweeds of Kwazulu-Natal, Oliveira *et al*. (2005) on Marine Plants of Tanzania, Huisman *et al*. (2007) on Hawaiian Reef Plants, Ohba *et al*. (2007) on Marine Plants of Palau, Skelton & South (2007) on Samoan Benthic Marine Algae. For the identification of red turf algae, Price & Scott (1992) is very useful. Anyway, one should remain cautious with identifying organisms solely based on field guides: as opposed to a real 'Flora' they only contain the dominant species! The possibility that a different, closely related species was collected cannot be excluded. Therefore, the next step is the use of (preferably recent) monographs of a group (e.g. De Clerck, 2003 on the genus *Dictyota* in the Indian Ocean, or Leliaert & Coppejans, 2006 on the genus *Cladophoropsis*) or detailed regional publications (e.g. Van den Heede & Coppejans, 1995 on the genus *Codium* from Kenya, Tanzania and the Seychelles; Kraft, 2007 on the marine green algae of the Lord Howe Island area), as well as comparison with specimens from existing herbaria with trustworthy identifications.

Anyway, for the identification of macroalgae on species-level, morphological and anatomical characters are needed (e.g. in the genus *Codium*, measurements of utricles have to be made; in *Ulva* spp., the number of pyrenoids per cell have to be counted, …). In brown and red algae, quite often the analysis of reproductive structures is important for identification on genus and/or species level (just like flowers in higher plants!). The analysis of these characters can only be carried out in a laboratory, with the use of a microscope with a calibration plate. Sterile specimens therefore frequently remain unidentified because critical characters for species (or even genus) distinction are absent.

8.8. Seaweed resources from Sri Lanka

Natural populations of the red alga Ceylon Moss (*Hydropuntia edulis* (S.G. Gmelin) Gurgel et Fredericq) were harvested in the past for extraction of agar from its cell walls. This seaweed is quite abundant in Puttalam lagoon, but it is not collected anymore and not included in this guide.

9. Survey methods for seaweeds

For this chapter we also refer to Leliaert & Coppejans (2004); http://www.persga.org/.

9.1. Qualitative assessment of the macroalgal flora of an area

Qualitative assessment of the marine flora of a coastal area implies general collecting in a specific area, resulting in a more or less complete list of species. Depending on the study, the coastal area can vary from a small area (e.g. a coastal strip of 10 m, a rock outcrop, etc.) to a large area (e.g. one to several km of coastline, a small offshore island, etc.). When comparing species numbers or biodiversity indices of different coastal areas, these areas should be of comparable size. The resulting species list is important for calculating biodiversity indices of an area. A major disadvantage of qualitative collection data is that species abundance is not taken into account. This can partially be corrected by making the sampling method semi-quantitative. This implies that each species is ranked based on its abundance, evaluated by visual observations. An example of such a ranking is the Tansley scale (Table 1 in Appendix). The growth form (sociability) of the seaweeds can also be taken into account; here the Braun-Blanquet's sociability scale can be used for each species (Table 2 in Appendix). As a matter of fact, Braun-Blanquet's cover-abundance scale is most used (Table 3 in Appendix). These data can be added on the herbarium labels.

9.1.1. Getting ready for fieldwork

It is evident that adapted clothing (protection against the sun/rain) is needed. In this respect good footwear is extremely important. The use of booties (tight, ankle-high, rubber boots with a thick sole and a zipper) is advisable, as well on rocky as on sandy or even muddy substratum, because they completely protect the feet against sharp obstacles (barnacles, oysters, coral fragments, …). If snorkeling is planned, a (thin) rubber wetsuit is useful for protection against sharp walls or irritating animals (jelly fishes, siphonophores, …), or at least knee pads. The availability of a towel also comes out handy.

The value of a report/publication on the biodiversity of an area largely depends on the presence of reference (voucher) specimens which allow ulterior control of the identifications. On its turn, the value of these specimens depends on the field data which are added to them. Therefore, a notebook (intertidal work) or a white plexiglass plate (in the subtidal and in intertidal pools) and a pencil are indispensable (Fig. 46A). Collecting gear includes a bucket, plastic vials, plastic bags, prenumbered labels on hard paper. Many algae and some seagrasses can be removed by hand, but a scraper or a stout knife may be handy or even necessary. Some thick encrusting algae can be removed with a knife, but many (especially the crustose coralline algae) must be collected along with the substratum. This can only be done by use of a heavy instrument such as a hammer and a chisel.

Fig. 46. Field work. A. Using a plexiglass plate and a pencil for taking notes in the water; B. Collecting by wading at low tide; C. Even with a seemingly smooth sea, a sudden big wave can emerge; D. Fully equipped for SCUBA-diving and underwater photography; E. Putting specimens in zip-lock bags during SCUBA-diving; F, G. Sorting out specimens on the field.

If available, a camera, a map, and a Global Positioning System (GPS) can be extremely useful. Be careful in this wet environment: put them in a watertight camerabox or (ziplock) bags!

Intertidal habitats can be sampled by wading (Fig. 46B) during (extreme) low tide or by snorkeling at high tide. Therefore, check the time of low tide as to get organized for the sampling. If snorkeling is planned (deep intertidal pools or subtidal) mask, snorkel, fins, mesh bag, plastic collecting bags and labels shouldn't be forgotten. For

extensive collecting or observation in the subtidal, SCUBA diving is advisable. Next to the snorkeling gear, a rubber suit, a belt, weights, the full air-cylinder, regulator, diving watch, depth gauge, inflatable backpack (= BCD, buoyancy control device), and a buoy should be brought or hired at a diving center (Figs 46D, E). These generally check the diving license, so don't forget to bring it.

Freshly collected specimens should be processed as soon as possible to minimize decay. If the way back to the laboratory is long, the specimens might decay under way. It then is preferable to prepare the collected specimens in the field (Figs 46F, G) or to store them in a cool box. If specimens are sorted at the collecting site, bring sorting and preparation trays, a floater, herbarium paper, a plant press with straps, card board, newspaper and fleeces, jars (Eppendorfs) and silicagel, formalin, zip-lock bags and hermetically closed jars (e.g. ice cream boxes).

9.1.2. Arriving in the field

Note the date, the locality (name of the closest town or village + eventually local name of the collecting site). If you have a GPS-system: add the GPS and longitude-latitude coordinates.

Make a general description of the site: is it a peninsula (Fig. 6B), a straight coastline, eventually with a beachrock platform (Fig. 4), a wide bay (Fig. 3B), an enclosed bay (Fig. 3C), isolated rocky outcrops (Figs 7C, D), an island (Figs 7B, C), a lagoon (Fig. 3D)? Describe the substratum: solid rock (Fig. 6A), boulders (Fig. 6C), sand, mud. Rate the general coast inclination: overhanging, vertical, sloping, subhorizontal. Give a general description of the biotope(s): seaweed vegetation (Fig. 6C), seagrasses (Figs 12A, B), mangrove (Figs 13A-C), coral reef (Fig. 7E).

Eventually add pictures.

9.1.3. Field collecting

Extensive and well-prepared collections are the basis of diversity based studies of (marine) organisms. The importance of good collections for taxonomic studies is evident, but it is equally important that representative collections - often referred to as voucher specimens - be kept of each species recorded in ecological surveys. Without such specimens, there is little or no possibility of later checking on the basis of names used in publications. Such specimens should be numbered, labeled and be deposited in a recognized herbarium (Womersley, 1984).

Take the time of low tide into account, certainly if you want to collect by wading. If it is already low tide upon arrival, go to the lowermost part first and come up with the tide. Take care not to get encircled by water. If the tide is still going down, go down with the tide and do the uppermost parts on your way back.

Collecting can be done by species (a single species and label per bag: numerous bags will be needed, but sorting out will be much easier); note the field identification of each number. Sometimes preference is given to collecting by biotope (a pool, a rock wall, a phorophyte: several species in a single bag, but with a single label). In species-rich areas or time shortage the latter method is being used. Always add ample seawater in the bags as to avoid decay by temperature rise or desiccation. Also add a label, which corresponds with a number in your note book (plexiglass plate) where you add: the detailed ecology of the collecting site (air-exposed/submerged at low tide;

pool: vertical/overhanging/sloping wall/(sand-covered) bottom; epilithic/epiphytic (on what?); the level relative to the tides (above high tide level (supralittoral); between high and low tide (intertidal (high -, mid -, low -)); under low water mark (subtidal)). Make notes on morphological characters which will be lost after processing the specimens, such as growth form (isolated plants, individual tufts, gregarious, forming intricated cushions); growth direction (erect, ascendant, prostrate, pendulous); *in situ* colour: some seaweeds are iridescent when alive; some seaweeds change colour upon drying; consistency: membranous, gelatinous, cartilaginous, stiff, brittle; eventually, presence of reproductive structures.

ALWAYS collect several specimens as to illustrate morphological variability and to be able to look for fertile specimens. ALWAYS collect complete specimens, including the holdfast as this might be a character needed for identification: presence of a disc, haptera, rhizoids, a bulbous structure.

While collecting, be aware of possible danger: even with a seemingly smooth sea, a sudden big wave can emerge (Fig. 46C).

9.1.4. Coming back from the field

If the laboratory is far from the sea and not provided with seawater, collect a (plastic) drum with seawater for sorting out the specimens, or … sort out in the field! (Figs 46F, G).

9.1.5. Sorting out the specimens

If the species have been collected individually, put them in separate trays (vials) and add the field number. If the collecting was made by collecting site, put the collection of one bag and its label in a large tray and sort out the different species in smaller trays (vials) giving them each a subnumber (e.g. collection from site 3: species 3a, 3b, 3c, 3d) (Fig. 47A).

9.1.6. Finally numbering and labelling the species

Copy the data from your field notebook or from the plexiglass plate on the computer or in the final notebook: date, place, general description of the site.
Each species gets a final serial number, preferably preceded by the collector's initials (e.g. HEC = Herbarium Eric Coppejans). Start with 0001 and go on all of your life: e.g. day 1: HEC 0001-0024, day 2 HEC 0025 – 0056 and so on). Add the detailed ecological data from the field as well as the morphological data (eventually add observations carried out in the laboratory). A HERBARIUM SPECIMEN WITHOUT A (complete) LABEL IS SCIENTIFICALLY (almost) USELESS!

Individual labels are printed out and added to the herbarium specimens. All these label data are introduced in a database. This way data can be retrieved by: collector, place, period, genus or species level (over different regions, oceans); herbarium, formalin preserved, silicagel, culture specimens.

The final label

Number: HEC 16128 (eventually +F, +S, +L; see further)
Name: *Caulerpa racemosa* (Forsskål) J. Agardh
Locality: Sri Lanka, Galle, in front of the lighthouse
Collection date: 15 August 2008
Ecology: on the sand-covered bottom of a low intertidal pool
Morphology: thallus fleshy, dark green, with starlike, slightly iridescent light green stripes; well attached to the substratum by numerous rhizoids; stolons prostrate, intricated; uprights with short rachis and densely set vesicular branchlets, resulting in a grape-like appearance
Collector: Eric Coppejans
Identification: W.F. Prud'homme van Reine (+ date of identification)

9.1.7. Preparation of a herbarium specimen

- Take a tray and fill it with clean SEAwater;

- Put a (cork)floater in the water (or an inclined smooth surface; Fig. 47B);
take a bristol card (or strong drawing paper) of the size adapted to that of the specimen that you want to prepare;

- Write the serial number IN PENCIL in the right down corner;

- Put the bristol paper and the seaweed in the tray, on the floater (Fig. 47C);

- Choose (a) nice, complete specimen(s) (with holdfast; eventually fertile);

- Arrange the specimen(s) in an optimal way, by pushing the floater under water (Figs 47D, E); filamentous, supple specimens can be spread by a small brush;

- Take the floater, bristol card and specimen slightly inclined out of the water and let the surplus of water run off (Fig. 47F);

- Put the bristol card + specimen on a newspaper on a horizontal surface and let it air-dry somewhat (Fig. 47G); don't leave it in the sun and don't wait too long: the specimens should not shrivel!

- Put the air-dried specimens between newspapers, covered by a fleece (Figs 48A-E), preferably regularly alternated by corrugated cardboard (for aeration);

- Close the plantpress with belts or put weights on them (Fig. 48F);

- Keeping the plant press in the sun or adding a ventilator directed on the press increases the drying speed, avoiding molding of the specimens; NEVER put the plant press in an oven (unless it is a drying oven with ventilation)!

- Change the newspapers (not the flieces) daily until the specimens are dry;

- Mount the herbarium specimen on standard dimension sheets (eventually stick the loose plants with glued paper strips, never directly with glue; certainly don't plastify them!!!) together with the label (Figs 49A, G);

- Store in a dry room, sheltered from direct sunlight (Figs 49B-F).

Fig. 47. Preparing herbarium specimens. A. Sorting out specimens in trays filled with seawater; B. The cork floater in the tray filled with seawater; C. The numbered bristol card on the floater; D. Arranging the specimens of the bristol card; E. Specimens arranged on the bristol card, on the floater, still in the tray with seawater; F. Taking the floater and the bristol card with specimens out of the water, letting drip off most of the water; G. The bristol card with specimens is (shortly) air dried.

Fig. 48. Preparing herbarium specimens. A. In the plant press a corrugated cardboard and dry newspaper is placed; B. Placing the bristol card with specimens on the newspaper; C. Putting a fleece on the specimens; D. Adding a newspaper on the fleece; E. Adding a corrugated cardboard on top; F. Closing the plant press.

Fig. 49. Storing specimens. A. Example of a seaweed herbarium specimen (*Grateloupia lithophila*) and label in the ring binder at the GENT herbarium; B. The National Herbarium of Sri Lanka in Peradeniya; C. The inside of the National Herbarium of Sri Lanka; D. The cupboards where the specimens are kept in the National Herbarium of Sri Lanka; E. Cupboard with the large herbarium specimens at the GENT herbarium; F. Cupboard with smaller herbarium specimens classified in ring binders at the GENT herbarium; G. A mounted specimen of *Acrosorium ciliolatum* (Harvey) Kylin, with field identification on the full label and final identification on the 'Determinavit-label'.

9.1.8. Formalin-preserved specimens

Most herbarium specimens can be resoaked for anatomical analysis, but most of the time cells remain shrivelled and cytological details (e.g. plasts) difficult to observe. Therefore it is better to keep (part of) a specimen in 4% formaldehyde (pure formalin = 40%, so 1 part of formalin + 9 parts of SEAwater; the concentration is not critical and even half the above will usually give good preservation). Add the same label (number) as the serial number of the herbarium specimen and add '+F' on the herbarium label and in your data set as to indicate that there is a formalin-preserved specimen.

Formalin is a strong irritant and carcinogenic and therefore should be handled with care, avoiding inhalation or direct contact with the skin. Store the formalin preserved specimens in hermetically closed vials, out of the light, in a (preferably cool) room with ventilation and NEVER in a room where persons are working on a regular basis (separate store room)!!!

9.1.9. Silica-preserved specimens

Fragments of most herbarium specimens can be used for molecular analysis (in as far as they have not been previously stored in formalin), but most of the time results are (much) better when fragments are immediately dried in silicagel. Therefore, Eppendorfs are being used, (almost) filled with fine-grained silicagel. The Eppendorfs should be kept closed at all times, otherwise the silicagel would attract air humidity. Only a small fragment (a few mm only) of an apical part of the specimen should be cut off and cleaned and dried with a paper tissue. The young apices are less epiphytized but still have to be cleaned as to remove the eventual single-celled epiphytes (e.g. diatoms). The fragment is put in the Eppendorf and a tiny label with the same serial number as the herbarium specimen is added (Fig. 50A). The Eppendorf should be closed immediately (Fig. 50B) and somewhat shaked, as to completely surround the fragment by silicagel: the quicker the drying process, the better the molecular extraction will proceed. Some scientists prefer to dry two fragments for the case that the DNA-extraction on the first fragment didn't succeed. It is useful to indicate on the top of the Eppendorf that it has been used (Fig. 50C). On the herbarium label and in the data set '+S' should be added as to indicate that there is a silicagel-preserved portion. Of course this should be deleted from the data set as soon as the fragment(s) have been used.

Fig. 50. Silicagel dried specimens. A. Putting a specimen in a labeled Eppendorf; B. Closing the Eppendorf; C. Indicating that the Eppendorf has been used.

Molecular techniques are outside the scope of this field guide. For details we refer to Hillis & Moritz (1996).