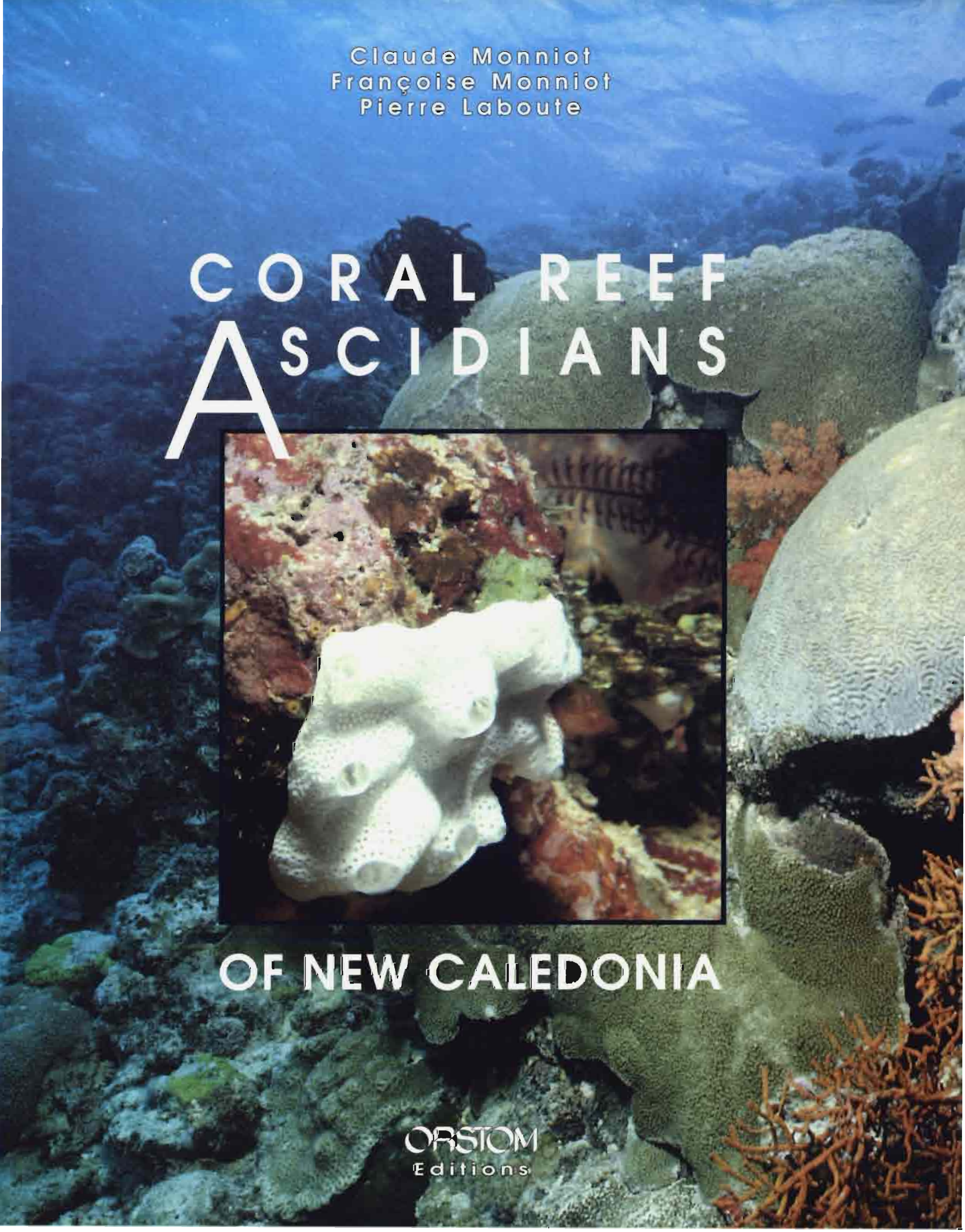
Claude Monniot
Françoise Monniot
Pierre Laboute

CORAL REEF ASCIDIANS OF NEW CALEDONIA



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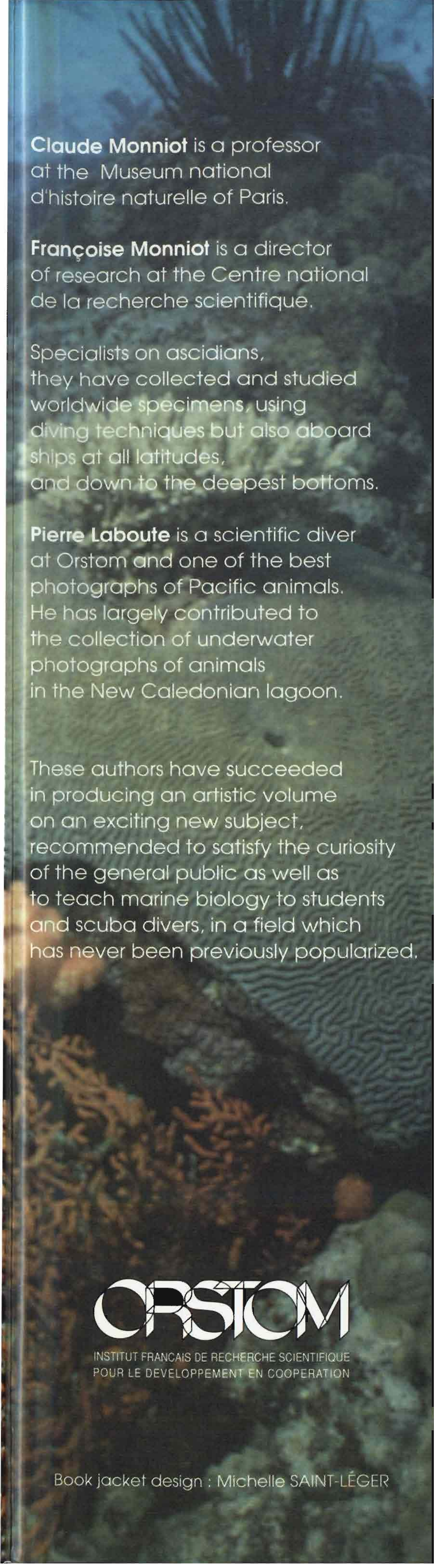

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Specialists on ascidians, they have collected and studied worldwide specimens, using diving techniques but also aboard ships at all latitudes, and down to the deepest bottoms.

Pierre Laboute is a scientific diver at Orstom and one of the best photographs of Pacific animals. He has largely contributed to the collection of underwater photographs of animals in the New Caledonian lagoon.

These authors have succeeded in producing an artistic volume on an exciting new subject, recommended to satisfy the curiosity of the general public as well as to teach marine biology to students and scuba divers, in a field which has never been previously popularized.

ORSTOM
INSTITUT FRANCAIS DE RECHERCHE SCIENTIFIQUE
POUR LE DEVELOPPEMENT EN COOPERATION

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OF NEW CALEDONIA

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Claude Monniot
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Éditions de l'ORSTOM
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Collection Faune tropicale nº XXX
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We gratefully acknowledge Orstom facilities
and the "Vauban" and "Dawa" 's crew
for their help in collecting the animals.
We are also grateful to G. Bargiban,
J. L. Menou and A. J. Bruce
who graciously gave us some pictures
in addition to P. Laboute's illustration.
Special thanks also go to A. Crosnier
whose continuing interest in our book
has largely contributed to its production.
We are much indebted to Liz and Todd Newberry
for their skillfull and creative revision of the english.

La collection *Faune tropicale* a pour vocation de diffuser les connaissances les plus récentes sur la systématique des invertébrés et des vertébrés des régions chaudes, connaissances indispensables aux recherches orientées vers le développement.
Le contenu des ouvrages varie de la mise au point taxonomique sur un groupe zoologique au guide faunistique de terrain ou à la faune régionale illustrée, touchant ainsi un public plus ou moins large : systématiciens, écologistes, enseignants, étudiants ou simples amateurs d'histoire naturelle.

Pierre Le Loeuff
Directeur de collection

Derniers volumes parus dans cette collection :

XXIX. *Les Coléoptères Scarabaeoidea de Nouvelle-Calédonie* - Renaud Paulian.

XXVIII. *Faune des poissons d'eaux douces et saumâtres de l'Afrique de l'Ouest* (tome 1) - Christian Lévêque, Didier Paugy, Guy G. Teugels.

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XXV. *Guide des étoiles de mer, oursins et autres échinodermes du lagon de Nouvelle-Calédonie* - Alain Guille, Pierre Laboute et Jean-Louis Menou.

INTRODUCTION

The natural world, although often unfamiliar, is waiting for us to acquaint ourselves with it and to understand it through close, patient observations. While we expect to encounter the unknown when we venture on hikes and dives, we often come upon it in disconcertingly intimate ways. For example, we generally know what we eat. But seafoods have a way of surprising us - and even leave us puzzled after our meal has been "identified". You may on occasion have eaten a "violet", an "oya", or a "piure", but did you realize you were eating an ascidian, a so-called sea squirt? On scuba or snorkeling trips you surely have seen ascidians, probably without knowing it and perhaps without realizing that you were looking at an animal at all. In fact, ascidians are our closest relatives among the invertebrates. Even so, except for a few specialists, naturalists rarely have more than a passing acquaintance with these inconspicuous creatures. Ascidians lack the external skeletons or shells that draw our attention to animals like molluscs, crustaceans, and echinoderms. Nor do they move about. Instead, ascidians usually form small, soft, immobile masses, often quite drab in color, that resemble sponges or even stones. But what a remarkable variety of shapes, patterns, and colors they reveal to the practiced eye!

Ascidians have been recognized as a coherent group only since the end of the 19th century. Aristotle mentioned ascidians but did not try to classify them among other animals. Names like stony or soft corals, zoophytes, molluscs without shells, or molluscoids all suggest how difficult it has been for zoologists since antiquity to interpret the structures of these strange creatures. In the late 19th century, however, when embryology was transforming the ideas of systematists, the Russian biologist Kowalevsky found

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similarities between ascidian larvae and frog tadpoles. With this discovery, he theorized a link between invertebrates and vertebrates. The group Protochordata was born, and ascidians became the topics of lively debates about evolution.

During the 20th century, much of the earlier interest aroused by ascidians has waned. No one now doubts that they are among the precursors of vertebrates, but the difficulties inherent in studying "soft" organisms have often discouraged research on the biology of ascidians. This decline in attention has been unfortunate, because we find so many extraordinary traits in these organisms. First of all, ascidian adults are in many ways simpler animals than their own larvae - a situation unique among animals. Moreover, the tunic that envelops ascidian bodies contains large amounts of a substance very much like plant cellulose - again, an extremely rare occurrence in the animal kingdom. Third, the ascidian heart and the body's entire circulatory system reverse the direction of their bloodflow every few minutes. And finally, many of these protochordates not only breed sexually but also replicate their bodies asexually by growing new ones from tissue fragments.

The current revival of interest in ascidians, however, has little to do with such odd traits as these. Instead, it grows out of recent recognition of the ascidian's possible medicinal value, as pharmacological research has turned to natural products of marine origin. General screenings of organisms for possible medicinal use have consistently singled out two groups, sponges and ascidians, both of which are sedentary filter-feeders.

Ascidians may also play a role in another major concern of our times - the quality of our environment. Their coastal marine habitats are particularly affected by pollution. Since ascidians concentrate a number of toxic elements, including heavy metals and hydrocarbons, they are turning out to be very good indicators of water quality.

So ascidians are once again in fashion. Their abundance and diversity in reef habitats are now arousing the attention of scientists and divers alike. Of course, the current scarcity of specialists in ascidian biology, and even of naturalists generally, limits our comprehension of ascidian biology. But international efforts now devoted to studying reef systems - their biota and ecology - will certainly lead to a better understanding of these still mysterious creatures.

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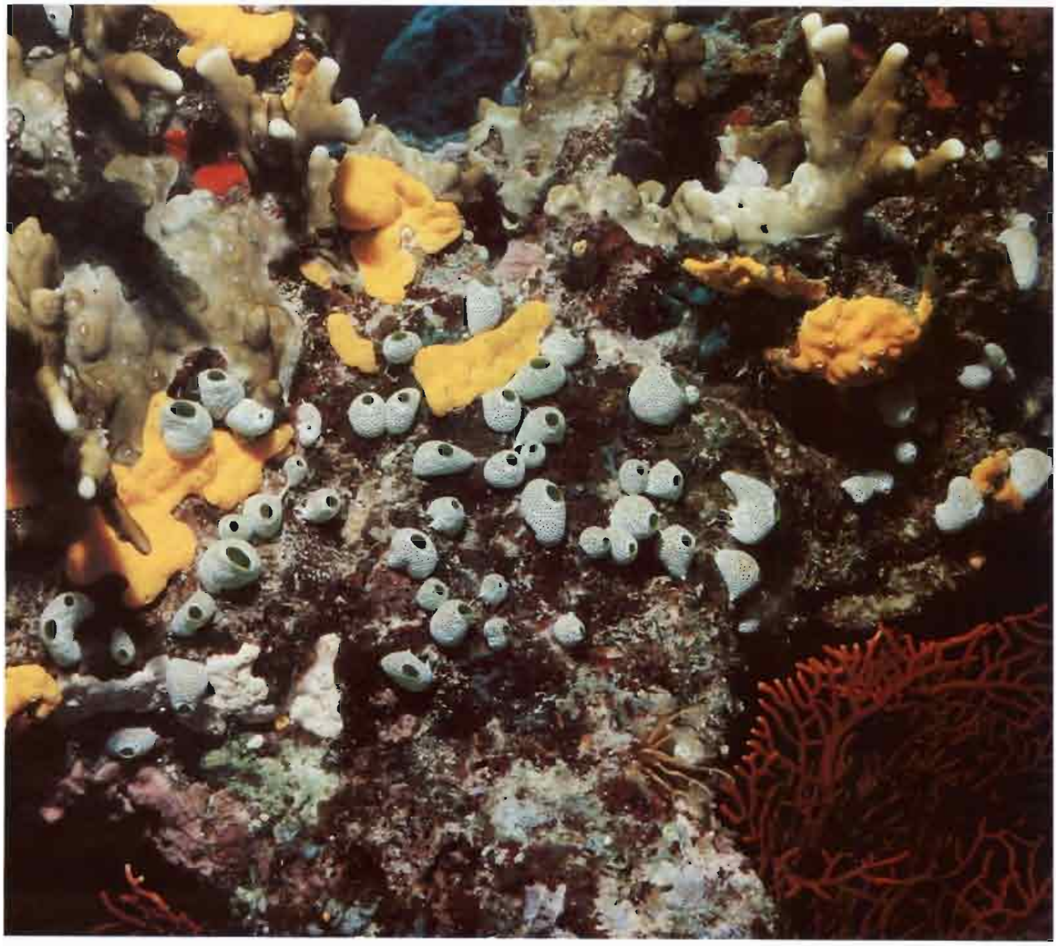
General organisation



# of an ascidian

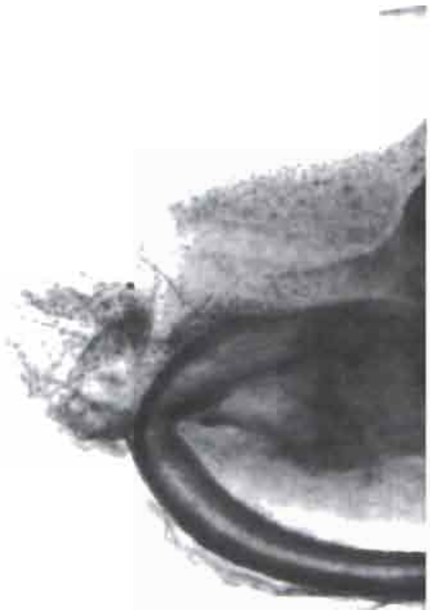
FROM THE EGG TO METAMORPHOSIS

Ascidians are hermaphrodites: they have independent male and female gonads (even if these gonads are tightly conjoined) in the same body.

They all release sperm into the sea. Mature eggs may also be released as gametes, to be fertilized in the open sea (oviparous species), or they may be held and fertilized inside the parental body. In the latter case, the embryos are brooded until they complete their pre-larval development (ovoviviparous species). In either case, the eggs, which are almost always less than 1 mm in diameter, undergo total cleavage and develop into swimming larvae. The larvae are called tadpoles because they have ovoid trunks and motile tails, features that recall frog tadpoles. Whether they develop from freely spawned eggs or emerge as active tadpoles from the parent's body, ascidian larvae do not feed. In ovoviviparous species, the tadpole's free-swimming stage is extremely short, with metamorphosis occurring just minutes or, at most, a few hours after its release.

Tadpole Structures and their Protochordate Characteristics

The larva is divided into a trunk and a tail, and its whole body is covered by a delicate tunic. A notochord, consisting of a column of about 40 large cells, extends from the posterior part of the trunk to the tip of the tail. Along each side of the tail are bundles of striated muscle fibers.

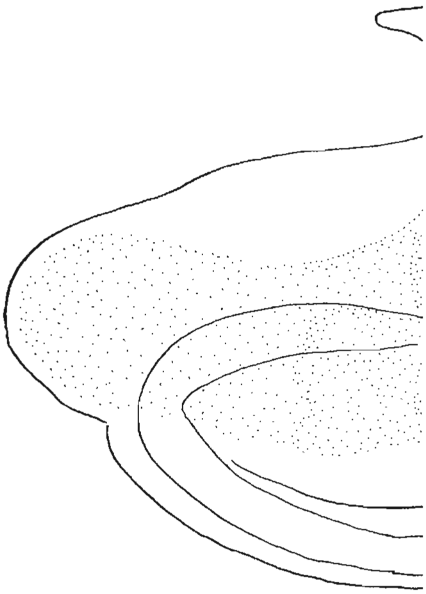


Ascidian tadpole:
the tail, containing the notochord,
encircles the trunk.
Three anterior adhesive papillae
can be seen inside
a ring of epidermal papillae.
The sensory vesicle
contains black granules,
the otolith and ocellus,
and is located near
the still-closed oral siphon.
The branchial sac and the gut
have begun their development.

The tadpole's nervous system is most highly developed in the trunk, where it forms an anterior ganglion and a large sensory vesicle. It, too, runs the length of the tail, as a hollow, dorsal neural tube above the notochord. Both in formation and position, the notochord and the dorsal nerve are homologous to those of vertebrates. The trunk's sensory vesicle, a sort of larval "brain", combines a balance organ, the statocyst, with a light-sensitive structure, the ocellus.

The tadpole's digestive tract consists of an enlarged and perforated pharynx leading into a rather uniform tubular gut. Since the gut lacks an open mouth and ends blindly, it is not yet functional in the tadpole. Even so, the outlines of the adult's incurrent and excurrent water siphons are already visible on the dorsal part of the trunk. Adhesive papillae (either two or three, depending on the species) project from the anterior part of the trunk. These papillae will attach the tadpole to the substrate when it settles and metamorphoses. At this stage, the trunk also contains the rudiment of a heart and masses of undifferentiated cells.

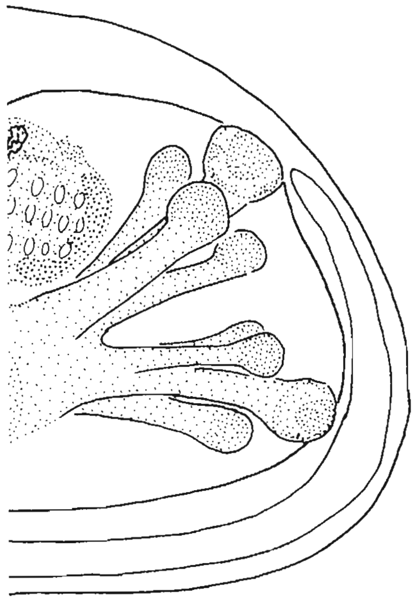
The tadpole swims by beating its tail sporadically but vigorously from side to side. It tends to swim upward and toward light.



Metamorphosis

Suddenly, within a span of just a few minutes, the tadpole turns away from the light and seeks the substrate. When it encounters a suitable surface, it affixes itself with its adhesive papillae. Immediately, the tail collapses and is resorbed into the trunk, as larval tissues break up and disappear. The animal then rotates

90° within the tunic, so that the oral siphon now points away from the substrate; it then opens. The sensory vesicle and the rest of the larval nervous system disappear, while a new neural ganglion forms. The animal begins to feed. It grows rapidly, its body wall develops an array of smooth muscles, and the tunic thickens. The young ascidian has now lost all trace of its larval notochord and neural tube. It will not show any signs of cephalization and, with rare exceptions, will remain fixed in place, immobile.



ORGANS OF THE ADULT ASCIDIAN

Solitary or colonial, all ascidians have the general body plan depicted in the figure. Since the larva's nervous system is dorsal, the neural ganglion, situated between the siphons, is now used to define the dorsal surface of the adult.

The oral siphon is taken to mark the body's anterior end. The posterior end is more arbitrarily designated but usually is considered to lie beyond the gut-loop, as far as possible from the oral siphon. Thus the cloacal siphon is roughly "mid-dorsal", and the endostyle of the branchial sac marks the body's mid-ventral line.

Solitary individuals, the largest of which may reach 30 cm from base to apex, are generally much larger than the component bodies of colonies, whose zooids are usually measured in millimeters. Some colonies, however, may spread over an enormous surface, as large as a square meter or more.

The adult body is hollow, a complex sac open to the outside through two tubular projections, the siphons. One of these openings allows water to enter the body and be filtered, and the other conducts filtered water out, along with digestive wastes and sexual products.

The Tunic, an Animal Cellulose

The adult body is entirely embedded within a tunic, which forms a somewhat flexible exoskeleton. The tunic's composition is very unusual, since it consists largely of polysaccharides much like the constituents of plant cellulose. Various proteins also occur in the tunic, as do some blood cells. The tunic is secreted in part by the cells it contains and in part by the external epithelium of the body wall.

The consistency of the tunic varies enormously. In some didemnids it can be very soft, even slimy. Some pyurids and styelids, on the other hand, have such hard, thick tunics that they feel like leather and look like small stones. Between these extremes, the tunic ranges from soft and yielding to tough and cartilaginous. The tunic's appearance varies so widely, in fact, that it may be of little use in identifying species in the field. Transparent as glass or densely pigmented, drab or brilliant in color, the tunic can easily

A *Diplosoma* colony's slimy tunic, is so transparent that the zooids are easily seen.

lead one to confuse ascidians with other organisms, especially sponges or algae.

The tunic has several functions. First, it anchors the animal to the substrate. In some species, it produces long filaments that help the animal anchor itself on soft sediments. The tunic can also retain all sorts of animal, mineral, and plant elements on its surface or within its matrix. These inclusions tend to increase the tunic's firmness, to isolate the animal from environmental extremes, and to provide some protection against predators. Some tunics produce papillae or spines in various arrangements, scales, and other short outgrowths, and some contain calcareous spicules with shapes characteristic of the particular species.

A firm tunic, the most common type, also maintains the animal's shape, preventing the collapse of its internal cavities. In fact, the tunic acts antagonistically to the musculature of the body wall. When the animal is disturbed, these muscles contract, closing the siphons and reducing the body's internal cavities; the body regains its full volume and its open apertures only when the muscles relax and the elastic support of the tunic can reassert itself.

The tunic is by no means an inert structure. Its activity is implied by its many blood sinuses, and especially by the variety of blood cells dispersed within it. It is also a route by which to eliminate

Phallusia julinea (Ascidiidae) has a cartilaginous and clear tunic and often grows, as here, among madreporarian corals.

Polycarpa pigmentata is a solitary ascidian of the family Styelidae. It has a thick, very hard, leathery tunic. Only its siphons reveal the animal here.

some metabolic wastes and to accumulate others, perhaps for later reuse.

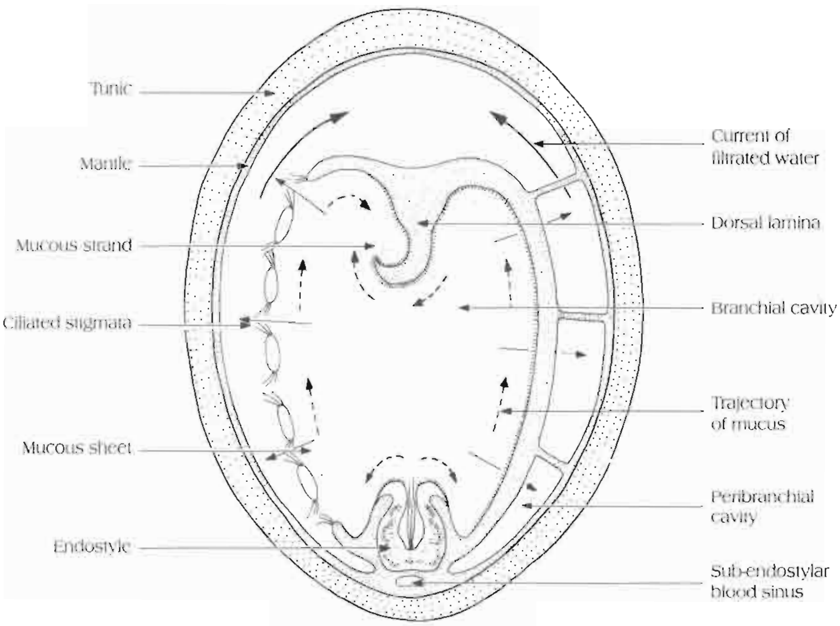
A variety of epibionts - sponges, algae, bryozoans, barnacles, polychaetes - may settle and live on some hard tunics. Some bivalved molluscs and amphipods may even dig pits in the tunic and live there.

The Mantle

The body wall, or mantle, lines the enveloping tunic. It comprises two epithelial sheets, an external one lying against the tunic and an internal one delimiting the peribranchial cavity. Between these two layers lie mesenchymatous tissues, the dorsal neural complex, muscular fibers, and extensive circulatory sinuses. The mantle's musculature consists of smooth muscle and shows two arrangements: on the one hand, a fine, felt-like coating of short fibers, variously distributed within the mantle, around the gut, and elsewhere; and, on the other hand, juxtaposed circular and longitudinal bundles of long fibers.

Strong sphincters encircle the siphons; elsewhere, circular muscles may wrap the entire body. The bundles of longitudinal fibers generally lie beneath the circular muscle-bands, at sharp angles to them, and radiate from the siphons or the intersiphonal dorsal area. They may in some cases reach all the way to the posterior part of the body.

These long bundles of muscle fibers enable either siphon to close and can contract the body itself, either partially or completely. Such contractions can be very sudden, producing the squirt



Diagrammatic transverse section
of an ascidian
showing the filtration process
through the branchial tissue.

of "sea squirts". But these contractions exhaust the ascidian's repertory of movements; ascidians are truly sedentary creatures.

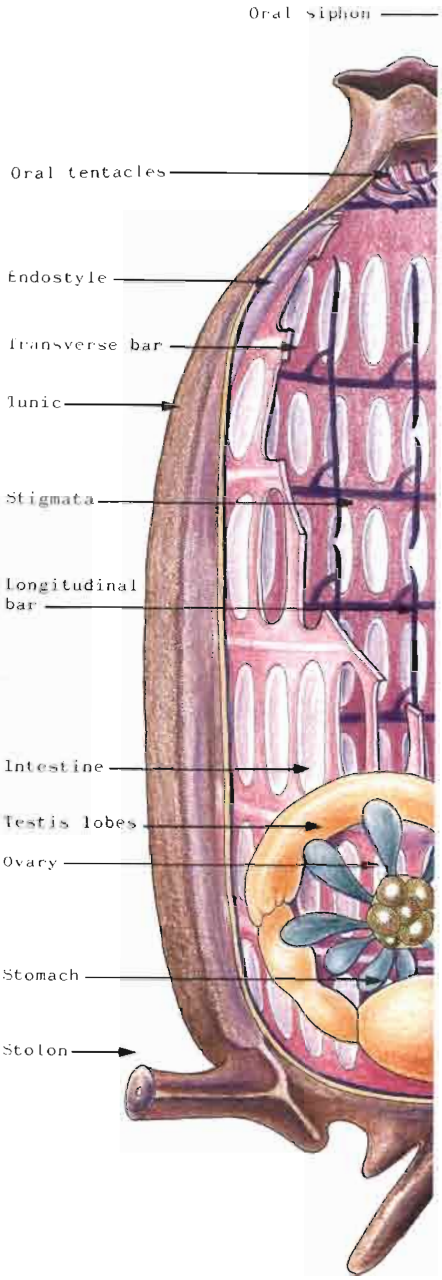
The Branchial Sac

Within the ascidian body is suspended a pharynx, or branchial sac, surrounded by a peribranchial cavity. The pharynx is attached anteriorly to the body wall (or mantle) at the base of the oral aperture, slightly below a circle of thread-like tentacles, and it is also attached along the body's mid-ventral line. At the bottom of the pharynx, a narrow opening marks the entrance to the esophagus.

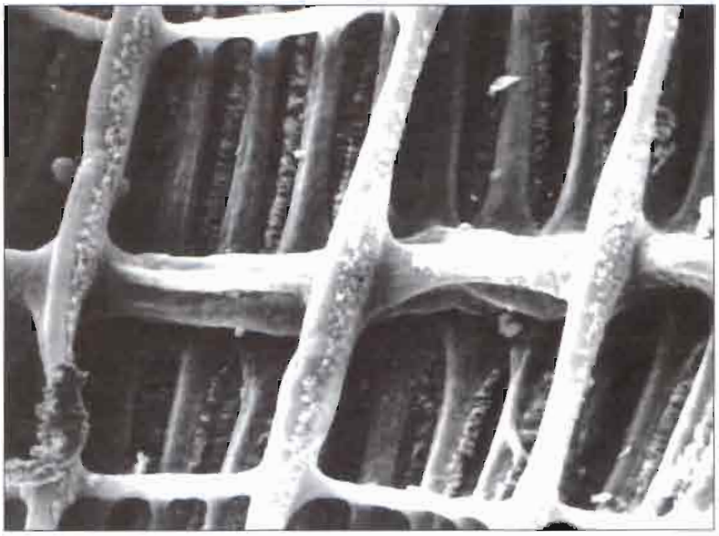
The branchial sac is held open within the peribranchial cavity by vascular struts, which connect the outer epithelium of the branchial sac to the inner epithelium of the mantle. Blood flows back and forth through these struts, between the sinuses of the mantle and those of the pharyngeal wall. The branchial wall is perforated by many slits, called stigmata, which are usually arranged in very regular patterns. The stigmata are bordered by cilia. Their strong beat draws a current of water into the oral aperture and forces it through the stigmata, into the peribranchial cavity, and then out the cloacal aperture. Sea water thus bathes the entire branchial sac and assures gas-exchange at the pharyngeal wall.

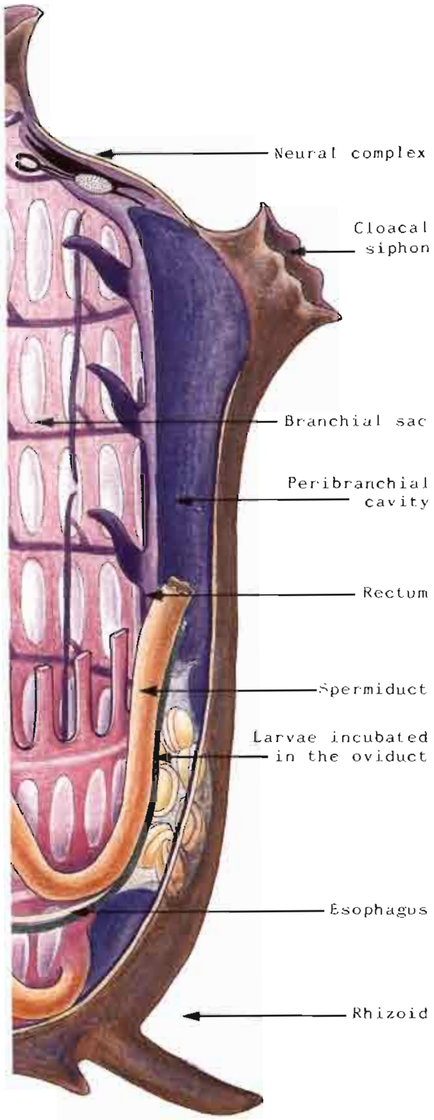
The branchial sac is also attached to the mantle along the mid-ventral, antero-posterior line, where the wall of the sac carries a complex groove, the endostyle. The walls groove of the endostyle have alternating longitudinal bands of glandular and ciliated cells; these glandular strips secrete mucus and enzymes. Along

Ascidian
general structure.



Ciliated longitudinal perforations (stigmata) seen from within the branchial sac. Transverse and longitudinal bars delimit "meshes" of the pharyngeal wall (scanning electron microscopy after critical-point drying).





the bottom of the endostyle runs a line of cells bearing long flagella. The endostyle presents us with still another ascidian trait that has intriguing vertebrate parallels. Both in its structure and its physiological activities, such as iodine-fixation and thyroxin-production, the ascidian endostyle closely resembles that of Cephalochordata (lancelets) and even of the ammocoete larva of the cyclostome fishes (lampreys). Opposite the endostyle, along the branchial sac's dorsal line, either a row of tentacular dorsal languets or else a continuous membrane (called the dorsal lamina) projects into the lumen of the sac.

In the simplest ascidians, the branchial sac has a flat surface, perforated by a large number of stigmata arranged in transverse rows. In more complex forms, the branchial surface develops internal projecting elements: papillae and internal longitudinal and transverse bars. At its most complex, the wall of the pharynx greatly increases its surface by forming longitudinal pleats. As we shall see, these levels of branchial complexity are important in interpreting major taxonomic relationships among the ascidians.

Filtration

Each branchial perforation, or stigma, is bordered by a ring of seven parallel rows of ciliated cells. All the cilia of a stigma beat synchronously, forcing water from the branchial sac to the peribranchial cavity. This current can be brisk: a solitary ascidian 8 cm long can move three or four liters of water per hour across its pharyngeal wall.

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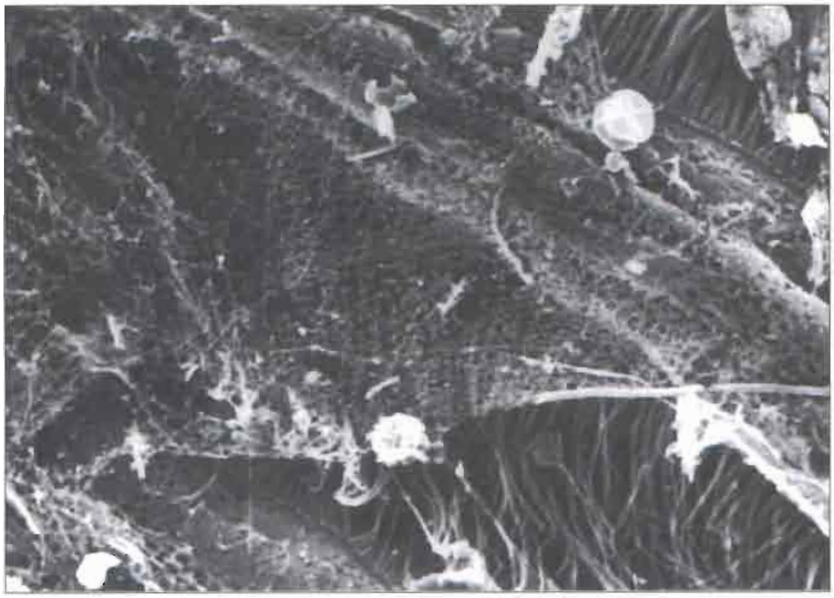


Mucous web covering a few stigmata. Note the tiny size of the web's openings compared to the size of the stigmata cilia.

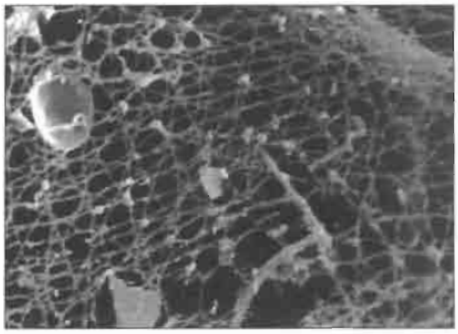
The cilia drive water but they do not filter food-particles from it. That task is accomplished by very fine mucous webs, that are secreted along the entire length of the endostyle and that glide transversely upon the pharyngeal wall, toward the branchial sac's mid-dorsal line. These webs are carried along by cilia located on the pharyngeal wall or on the sac's internal branchial papillae or bars. Along the dorsal line, these webs are rolled into a rope-like strand. Particles suspended in the current of sea water are caught and held on the sticky mucous webs, whose pores range in size from 0.1 to 0.3 µm. This particulate food gets mixed into the dorsal mucous strand, which moves along the dorsal languets or lamina to the esophagus, where strand and food together are ingested.

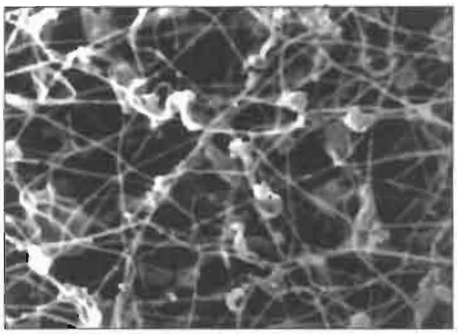
Filtration by ascidians is not selective; particles are not actively sorted. Of course, the oral tentacles, inside the oral siphon, do block very large objects. And when undesirable particles are engulfed, or too much sediment enters the branchial sac, the ascidian can contract suddenly and violently, ejecting the sac's contents back through the oral siphon. This behavior prevents clogging of the animal's respiratory and food filtration apparatus.

The Digestive Tract

The rather simple ascidian digestive tract forms a loop that brings the rectum back close to the esophagus. Starting at the bottom of the branchial sac, we find, in the following order: a cylindrical esophagus, a more or less globular stomach, and an intestine that occasionally is divided by constrictions into separate

Details of two different kinds
of mucous adhesive secretions
responsible for branchial filter-feeding.



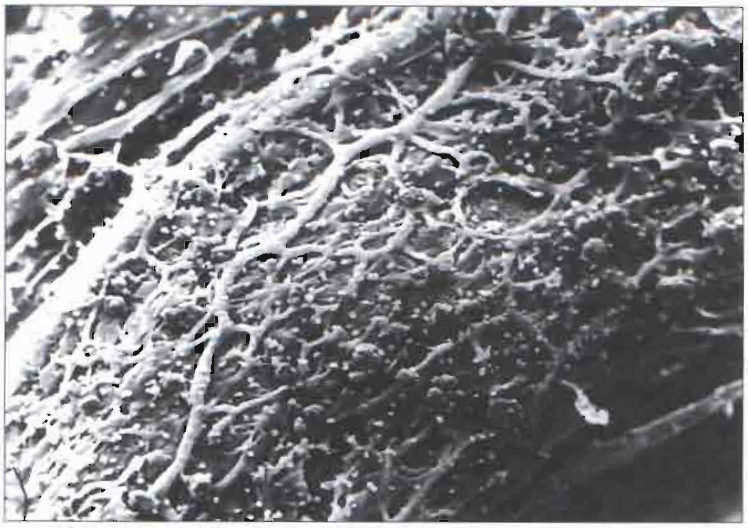


regions. The digestive epithelium is ciliated along its whole length. It also includes mucus-secreting cells, glandular cells, endocrine cells, and of course food-absorbing cells. There are no elaborate digestive diverticula or accessory structures. In some families, near where it joins the intestine, the stomach does contain a "caecum", but this small, finger-like sac has no special histological characteristics; its function is unknown. The stomach wall may be creased by longitudinal folds or ornamented by tubercles, which correspond to cellular differentiation of the stomach wall. In the families Pyuridae and Molgulidae, bunches of glandular cells within diverticula of the stomach wall form a so-called liver.

The posterior intestine is surrounded by a network of anastomosing tubules whose tips lie against the internal digestive epithelium. These tubules converge into thin ducts that run along the gut-loop. The small ducts join in turn to form a common pyloric duct, sometimes dilated into an ampulla, that penetrates the wall of the stomach. Various activities, excretory or digestive, have been attributed to this "pyloric gland", which is found in all ascidians, but its actual role remains unknown.

Food moves continuously through the gut. Feces form either sausage-like shapes or, more often, oval pellets. They are ejected into the peribranchial cavity, which functions as a cloacal cavity, and pass out of the body with the water current pouring through the cloacal siphon.

Depending on the family, the gut-loop may be located below the branchial sac or beside it, generally on the left (in one family, the Corellidae, it lies on the right). The gut-tract is always separated from the peribranchial cavity by the mantle's inner epithelium.



Tubules of the pyloric gland covering the external wall of the intestine (scanning electron microscopy after critical-point drying). The internal epithelium of the mantle has been removed to show the outside of the intestine itself.

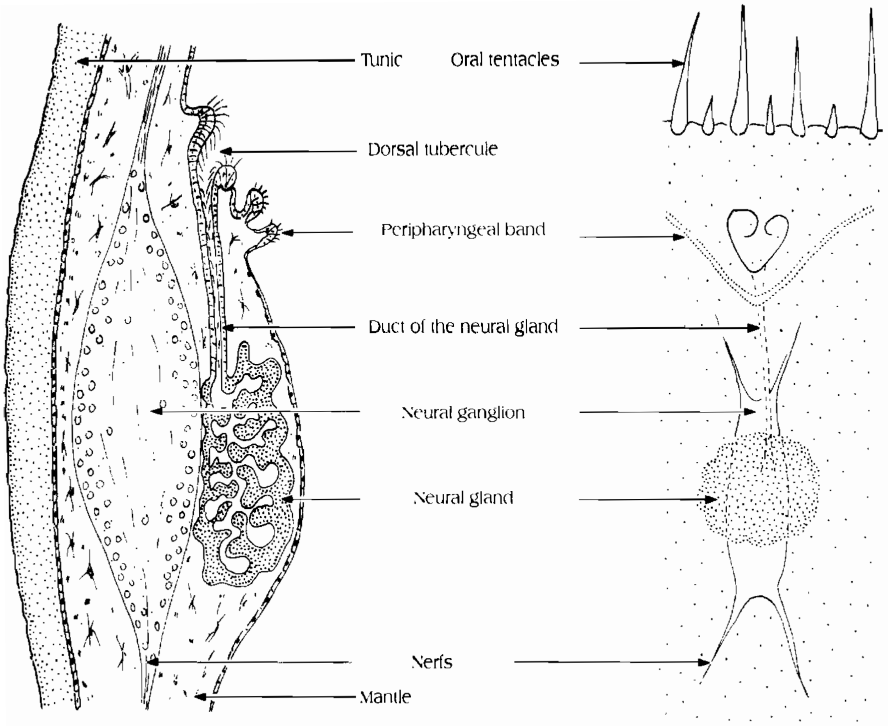
If the gut projects very far into the peribranchial cavity, it is connected to the body wall only by intermittent trabeculae. In many kinds of ascidians, the distal part of the rectum, and its sometimes rather elaborately frilled anus, lie completely free of the cloacal cavity's wall, near the base of the cloacal siphon.

The "Kidney"

There is no specialized excretory organ in ascidians. Excretion is carried out instead by specialized blood cells, the nephrocytes. Even so, in the Molgulidae, and only in this family, a closed, bean-shaped vesicle called a "kidney" or "renal sac", lies on the right side of the body. This vesicle contains a liquid isotonic with sea water, nitrogenous concretions (especially uric acid), and symbiotic bacteria and fungi. The actual role of this organ remains unclear; it may be a nitrogen-storage organ rather than an excretory one.

The Nervous System

The nervous system comprises nerves, a single ganglion, and a neural gland that is tightly associated with this ganglion. The ganglion lies dorsally inside the mantle, between the two siphons. Its cells are arranged in an external cortex around a fibrous medulla. The ganglion is elongated, and its anterior and posterior corners extend into nerves that quickly ramify through the mantle toward the siphons and over the flanks of the body. A visceral nerve



SAGITTAL SECTION

INTERNAL SIDE

Nervous system.

penetrates ventrally, as well, toward the dorsal part of the pharynx. It is important to note that neither the ascidian's heartbeat nor the movement of its branchial cilia is neurogenic at all, let alone controlled by the neural ganglion.

We still know very little about the sensory organs of these creatures. Sudden changes in light, vibrations, and chemical and thermal stimuli may cause ascidians to contract. Sensory cells have been described in the internal epithelium of the mantle, near the siphonal apertures, but no one has yet studied the behavioral traits and sensory physiology of ascidians very thoroughly. The little one can say is that stimuli provoke both direct and crossed reflexes. Some experiments indicate that the neural ganglion is involved only in crossed reflexes and that it thereby provides a certain amount of muscular coordination.

The neural ganglion is always associated with a round neural gland, made up of clumps of cells forming an intricately convoluted surface that surrounds a central lumen. A ciliated duct leads from this gland to a dorsal opening at the base of the oral siphon, just ahead of the branchial sac, between the oral tentacles and the beginning of the dorsal lamina. This opening is variously called the ciliated funnel, the ciliated slit, or the vibratile organ; its site is called the dorsal tubercle. The neural gland shows cyclic cellular activity to which different roles have been attributed: excretory, neurosecretory, and especially endocrine. Some investigators have shown that the neural gland secretes hormones resembling those of the posterior lobe of the vertebrate hypophysis. Others believe that the neural gland induces spawning of eggs or sperm when an individual detects certain chemical stimuli, especially other individuals' sexual products, in the sea water around it.

Blood and the Circulatory System

Ascidian blood is a colorless plasma, isotonic with sea water, that carries a large array of circulatory cells. Again we know too little about ascidians to assign roles confidently to these structural elements. The ascidian circulatory system is not closed; such vessels as exist are not lined by an endothelial vascular wall, except near the heart. Rather, blood circulates among lacunae in the loose mesenchyme ("connective tissue") of the mantle, the pharyngeal wall, and around the gut and gonads. These spaces sometimes form veritable channels or sinuses that resemble real vessels. They do so alongside the endostyle and the branchial sac's dorsal line, and in the branchial sac's longitudinal and transverse bars. Indeed, these branchial sinuses are often called vessels. In many ascidians, still other "vessels" penetrate widely into the tunic.

The ascidian heart is extraordinary. It entirely reverses its blood flow every few minutes. Lined by a simple epithelium, it forms a V-shaped tube of striated muscle fibers arranged spirally around its long axis. Waves of contractions, spaced several seconds apart, move blood through the heart in one direction for a few minutes. Then the heart pauses before waves of contractions begin again, driving blood through in the opposite direction.

Among the blood cells, undifferentiated hemoblasts are probably the source of all the other types of blood cells: lymphocytes, amoebocytes, nephrocytes, phagocytes, pigment cells, and vacuolated cells (which include granulocytes, signet-ring cells, compartment cells, and morula cells). Some types of vacuolated cells can store metals - in some species large amounts of vanadium, an ability unique to ascidians. The role of these metal-storing cells, called vanadocytes or (if they concentrate iron) ferrocytes, is still poorly understood. Recent studies indicate that the metals are associated with "tunichrome", an animal polyphenol unique to ascidians. Some vacuolar cells are well known, too, for their strong acidity. Still other ascidian blood cells promote coagulation by secreting lectin.

Blood seems to play a minor role in ascidian respiration. It carries no respiratory pigments, either in its plasma or its cells. It serves primarily to store and transport metabolic products. In many kinds of ascidians, too, the circulatory system plays an important role in processes essential to the growth of colonies.

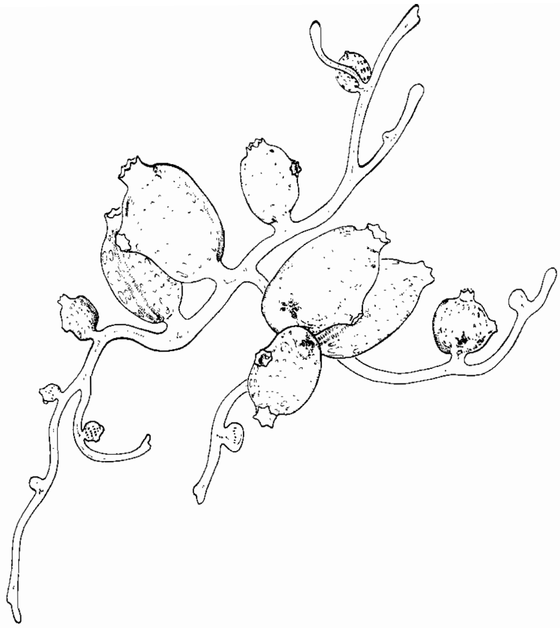
Sexual Organs

Ascidian ovaries and testes are independent of each other even though they are simultaneously present in the same individual. They are often juxtaposed to form a single gonad, but their gonoducts remain separate. There may be one or many gonads in an ascidian body, depending on the species, and they may mature

either simultaneously or successively or even in such a way that male and female phases alternate during the body's adulthood. Both mature sperm and eggs are expelled from the gonoducts into the peribranchial cavity - sperm to be swept out the cloacal siphon, eggs to be spawned or retained. Cross-fertilization is the rule, but self-fertilization may also occur.

The location of gonads in the body varies among the different major groups of ascidians. In the entirely colonial aplousobranchs, whose bodies are divided into two or three parts, the gonads are found in the abdomen or post-abdomen, below the thoracic branchial sac. In phlebobranchs, they adhere to the gut, usually within the intestinal loop. Among stolidobranchs, the gonads may be distributed anywhere in the peribranchial wall of the mantle, depending on the species.

Ascidians lack accessory sexual organs, but ovoviviparous forms may have structures to shelter their offspring through embryogenesis. Thus, some ovoviviparous species brood their young in the peribranchial cavity, but others have evolved an incubatory pouch formed by a dilation of the distal part of the oviduct. This pouch may hold one or several embryos at various stages of development. Some colonial ascidians - the Didemnidae, for example - hold their embryos in chambers inside the colony's common tunic until they are released as active tadpoles.



Example of a stolonial colony. In this species, *Ecteinascidia ndouae*, the zooids are far from each other, linked by their stolons, so the colony occupies a large surface of the substrate, which is made here mostly of calcareous algae.

BUDDING AND REGENERATION: ASEXUAL REPRODUCTION

All ascidians reproduce sexually. Many also replicate their bodies (reproduce asexually) by strobilation or budding to form colonies. The first individual of a colony, an oozooid, begins to bud very soon after it settles. Depending on the order and family it belongs to, an ascidian may bud one or more individuals, called blastozooids. The blastozooids will bud more individuals, all of them genetically identical to the parent zooid. The oozooid generally has the same anatomy as its budded offspring. It may persist in the colony or disappear once colony growth is underway.

Among the three orders of ascidians, aplousobranchs are always colonial. Phlebobranchs and stolidobranchs have both solitary and colonial genera. At least twelve modes of asexual reproduction have been described so far, varying from family to family and especially from order to order. We have greatly condensed them into the following groups.

Stolonic Budding

Stolonic budding appears to be the simplest mode of asexual multiplication. The family Perophoridae shows stolonic budding very clearly. A stolon protrudes from the basal or posterior part of

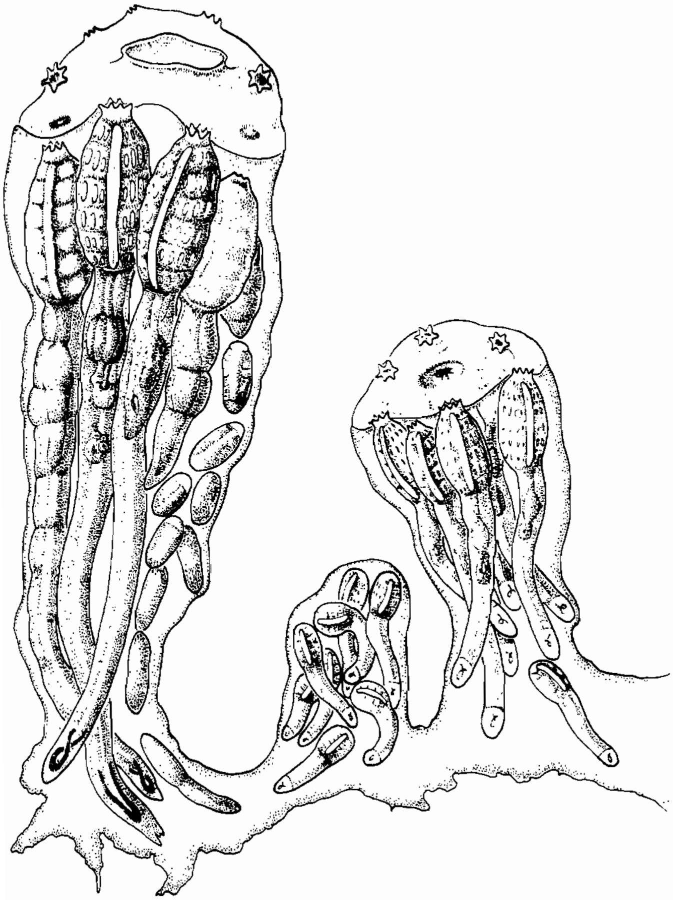
C. Monniot, F. Monniot
P. Laboute

In this species of *Aplidium* one colony is massive, while the other's buds have organized into circular systems, mostly on separate lobes.

the zooid. It is an evagination of the mantle, containing mesenchymatous cells and blood, that elongates, all the while secreting tunic and adhering to the substrate. Hernias develop either at its tip or along its length. Some of them may form branches of the stolon, but most of these swellings enlarge, their tissues become denser, and organogenesis produces complete and functioning new zooids. All these blastozooids remain connected by stolons. Depending on the species, perophorid stolons may be long and thread-like, with the individuals well separated, or they may be short, in which case the colony resembles a bunch of grapes.

Budding by Strobilation

Strobilation, universal in the Polyclinidae and widespread among the Polycitoridae, involves a segmentation of the elongated body into two or more parts. Each part undergoes partial or complete disorganization, then regrows and reorganizes to reconstitute an entire zooid. In the Polyclinidae, whose zooids are quite long, the process is very clear. Zooids have three parts: a thorax with the branchial sac, an abdomen with the gut-loop, and a post-abdomen with the gonads and heart. The zooid first shows a series of constrictions along its whole length. Next the body divides at these constrictions. The tissues of the resultant vesicles (strobilae) dedifferentiate. Then their reorganization begins. Each section of the parental body retains its original polarity as the new individuals grow. In other words, the anterior part of each strobila buds a thorax, and the posterior part buds an abdomen and a post-abdomen. Each bud's growth brings it to the colony's surface, where it opens and begins to feed. In those Polycitoridae that can strobilate, only the abdomen does so, and only in a few places.



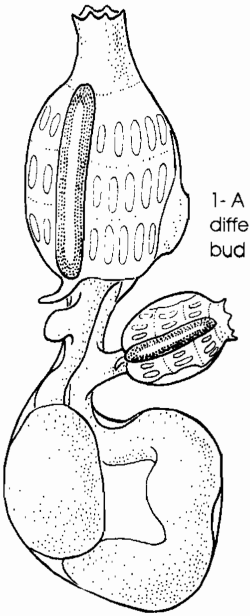
Schematic structure
of a polyclinid colony,
showing, on the left,
the splitting of zooids into strobilae
following dedifferentiation.
On the right, each strobila
reorganizes to constitute a new zooid,
and groups of zooids
together reconstitute "systems".

Esophageal-rectal Budding in the Didemnidae

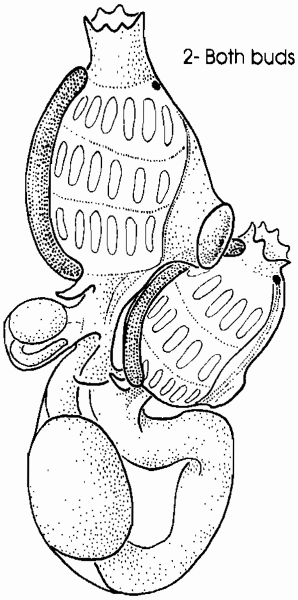
In this family, zooids are tiny, about 1 mm long on average. They have a thorax containing the branchial sac, and an abdomen that is connected to the thorax by a thin waist. As the figure shows, two different buds appear at this waist; one protrudes a new abdomen near the parental thorax; the other, a new thorax near the parental abdomen. For a little while, the result is a bizarre double-body with two thoraxes and two abdomens. But as they grow and adjust the proportions of their new parts, the two newly forming zooids eventually separate. In fact, thoracic and abdominal protrusions are not always simultaneous; the new thorax generally appears first. So vigorously budding didemnid colonies may well contain scattered zooids with two thoraxes and only one abdomen. This type of budding is esophageal-rectal, because the esophagus and rectum are the sites where the new body parts actually appear, as the figure shows.

Budding by strobilation interrupts sexual reproduction, since

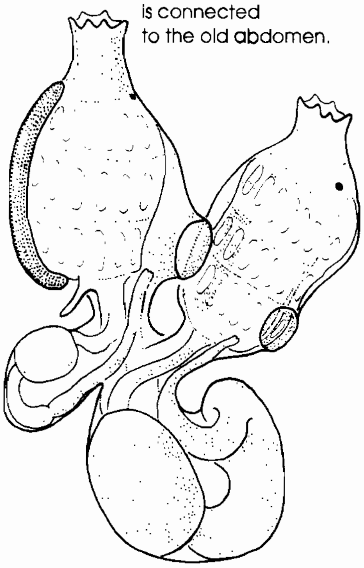
Esophageal-rectal budding in the Didemnidae:



1- A thoracic bud has already differentiated while the abdominal bud remains rudimentary.



2- Both buds organize.



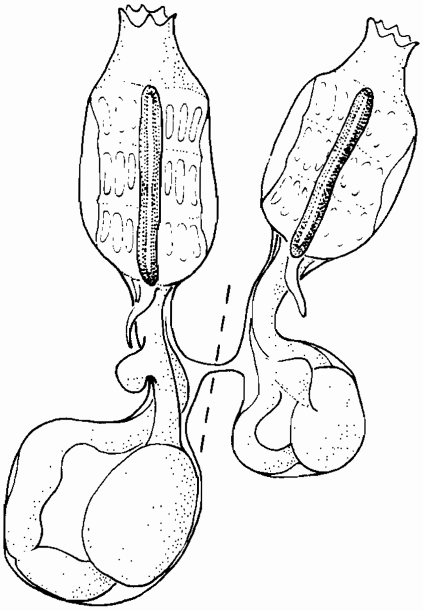
3- The old thorax and newly formed abdomen join, while the new thorax is connected to the old abdomen.

each strobila's tissues must be reorganized. But budding in didemnids can go on during the maturation of the gonads, which are not disturbed in the parental abdomen. Most often, however, periods of colony growth and bouts of sexual reproduction alternate with each other, probably as the colony's energy budget is directed toward one activity or the other.

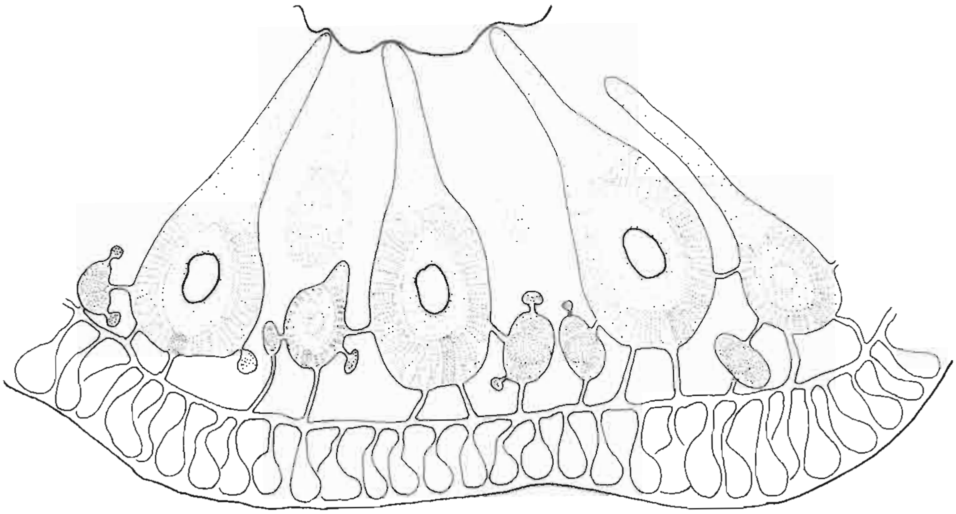
Peribranchial, or Pallial, Budding

Among stolidobanchs, the family Styelidae is the only one with colonial representatives. Several colonial styelid genera show stolonic budding much like that of the Perophoridae or Cionidae among the phlebobranchs. But in *Botryllus* and "polystyelids", budding proceeds in still another way. The mantle on each side of the body swells into the tunic as a hernia. In *Botryllus*, this hernia becomes a bud, which itself forms new buds before it completes its own development. When the first buds reach the colony's surface, the parent zooid collapses and disintegrates. Thus, the zooids of a botryllid colony proliferate extremely rapidly in the spaces between their parents. Various polystyelids have a comparable mode of budding, although their zooids do not show *Botryllus*'s highly organized schedule of zooidal development and disintegration.

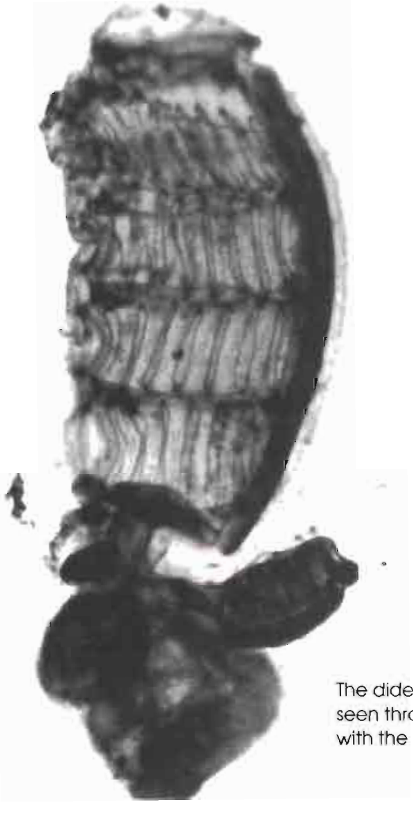
There are, as well, other modes of budding that appear to be derived from the major types we have described. This brief survey merely suggests how extensively ascidians have exploited budding and colony formation as part of their growth strategies. Each of the four main modes of budding gives rise to colonies of very different shapes. Derivative modes increase the array of colony forms still further.



4- The two individuals progressively separate.

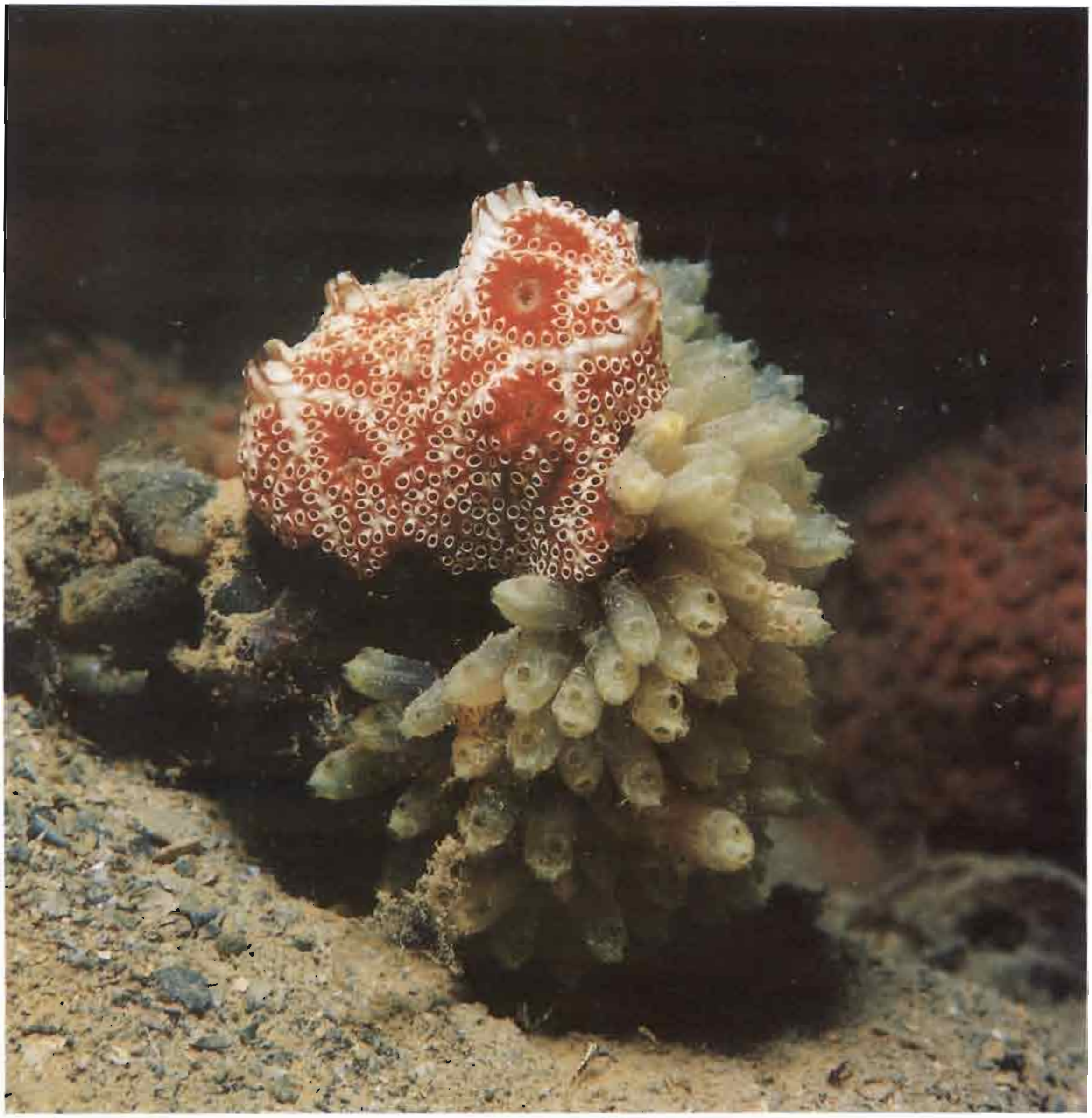


Palleal budding of *Botryllus*.
The colony develops numerous vascular ampullae in the common tunic on its external edge.
Each zooid buds a new individual on each side, and these in turn bud as the parent zooid disorganizes and disappears.



The didemnid budding stage-2 seen through a microscope, with the tunic removed.

Different kinds of budding
produce different shapes of colonies.
The white and red *Botryllus leptus*
has all its zooids deeply embedded
in a common mass of tunic,
but the yellow individuals of
Euherdmania claviformis share only
a common basal sheet of tunic
to create a colony
with the appearance of a bouquet.



FROM THE INDIVIDUAL TO THE COLONY: ASCIDIAN DIVERSITY

Solitary Ascidians

During a dive, an observer may have a hard time singling out these solitary animals, attached as they are to the substrate and often colored like the rock itself or the other animals and algae that grow on it. Furthermore, ascidians may be hidden in crevices, under stones, or among the branches of coral. Most solitary ascidians are small, on the order of several centimeters or less, and even this size is reached only by the oldest animals. Growth is continuous but always slows down after the settled animal's first few months. The life span of a solitary ascidian has been reckoned only from animals living in aquariums. It seems to range from several months to two or three years.

The shape of a solitary ascidian depends heavily on the consistency of its tunic. If its tunic is thick and hard, the animal will often have a globular, erect shape and may carry a fairly dense coating of epibiotic organisms. This appearance is especially common among stolidobranch ascidians, such as pyurids and styelids, some of which are even stalked. In New Caledonia, *Polycarpa clavata* is a striking example of this body form. As the animal grows, the body grows away from the substrate by secreting a flexible stalk of especially firm tunic. The animal sways back and forth on its stalk.

C. Monniot, F. Monniot
P. Laboute

With its large size (30 cm)
and bright color,
Polycarpa clavata is one of the most
spectacular ascidians of New Caledonia.
The animal sways atop its stalk;
the oral and cloacal apertures
are almost opposite each other,
and their gaping allows water
to pass easily through the body.

The tunic of small solitary phlebobranch ascidians is generally clean and often transparent and soft, but it may become opaque and rather cartilaginous in large species. These animals often lie broadly attached to the substrate, sometimes adhering to it by almost their entire left sides. Perhaps because their tunics are relatively fragile, many of these animals prefer less exposed habitats - crevices and the gaps among coral branches, for example - where they find some protection from predators. Consequently, except in quiet waters, phlebobranchs can rarely be found just by swimming above the bottom; instead, they have to be searched out patiently in recessed, half-hidden, often dark places. In some solitary ascidians, the tunic is encrusted with sediment, and the animals are ovoid, with their two siphons close together. These forms live in silty habitats, where they can hide in the sediment. Their tunics may hold onto sand or mud with adhesive papillae or with hair-like processes, or sediment may even be incorporated during growth into the tunic's matrix.

Most solitary ascidians live as isolated individuals, although their distribution and density can vary greatly. Their camouflage can be so perfect that an experienced diver senses the presence of an ascidian more than he actually sees it. It is only by touching the animal that he can be sure it is really there! Large ones may betray themselves by their gaping siphons, which may be pale or brightly colored internally and which shut abruptly at the slightest disturbance.

In areas rich in organic matter or in quiet places like ports, some kinds of solitary ascidians settle next to or even on each other. The individuals, which may be of various ages, often adhere so tightly to each other that the resultant mass resembles a colony.

Polycarpa nigricans, *Styela canopus*, and *Pyura confragosa* aggregate in this way. These aggregations generally shelter an epifauna that lives in the small cavities between the ascidians' bodies. This entire living substrate may itself now become a favorable place for other kinds of ascidians to settle. Thus, aggregations may be monospecific or be made up of several species. In most cases, one species dominates numerically or by sheer mass. This aggregative behavior eases cross-fertilization among the grouped parents. Aggregative behavior is probably enhanced by the secretion of substances that attract later larvae to settle and join the crowd.

Aggregations are not limited to solitary species. The larvae of different species of aplousobranchs may settle near each other. Their subsequent colonies pack together and may even partially cover one another. This situation is common where solid substrates are rare and also densely populated - on harbor floats and pilings, for example.

Polycarpa cryptocarpa here reveals only its white apertures. The tunic is covered with sediment.

The presence of one individual of the solitary *Polycarpa nigricans* attracts other larvae of the same species, which settle close to it. The aggregated bodies may even fuse their tunics together, to resemble members of a colony.

Polycarpa aurita's hard tunic is covered with many epibionts. When this animal's yellow siphons are closed, it can hardly be seen.

Each element
of this *Eusynstyela* colony
is linked to neighbors
by thin transparent stolons
which are almost invisible
on the irregular substrate.

By extending budding stolons, *Symplegma alterna* can form a colony that exploits a thin, linear substrate such as a small, branching seaweed.

Colonial Ascidians

STOLONIC COLONIES

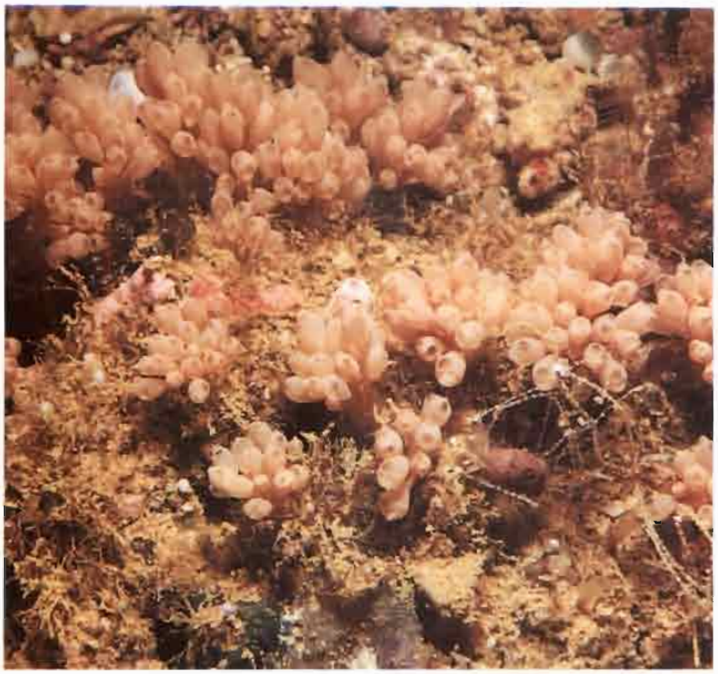
Colonies of this type occur among the Perophoridae and the Styelidae. They result from budding by stolonic outgrowths of the mantle, as we have already described. The settled larva metamorphoses into the colony's founding zooid, or oozooid. It puts out stolons in several directions; then blastozooids develop from side-or end-pockets of these stolons. These zooids in turn put out new stolons, which produce still more buds. If the growing colony encounters favorable conditions, zooids develop quickly and bud, so the whole colony grows in this direction. If, on the other hand, a stolon grows into a less favorable area, its zooids differentiate more slowly or not at all. And if conditions deteriorate generally, part of the colony may degenerate or die. Thus, stolonic budding allows a colony a certain choice of habitat, since the effect of these patterns of growth and regression is to shift the whole colony's position on the substrate.

All colonies that can put out stolons in a particular direction are potentially mobile as long as the substrate can accommodate their displacement. In such colonies, regression occurs on the side away from active budding, and material from those degenerating zooids is broken down and reused by the growing parts of the colony. In species where stolons are long and individuals widely spaced, colonial movement over the substrate may be quite rapid, even though each individual zooid remains immobile during its lifetime.

Very short stolons, by contrast, produce colonies with individuals joined closely together (the stolidobranch genera *Amphicarpa* and *Eusynstyela* are examples). Zooidal contact can be so tight that the tunic around zooids may actually fuse. The colony then forms a virtually continuous crust, and the boundaries between individual zooids appear to be mere grooves.

BOUQUET-LIKE COLONIES

A colony of this sort has a shallow but usually sheet-like tunic that unites the colony only at the substrate. Either the zooids produce short basal stolons (e. g., *Perophora*) or they strobilate (as in *Euherdmania*). After strobilation, each piece regenerates separately, grows within a newly secreted tunic, and is joined to the other zooids only by the thin basal sheet of tunic. In the clavelinids (Polycitoridae), too, zooids are embedded in a common tunic only along their posterior parts, while almost all the rest of each zooid remains anatomically isolated from the others. The attachment surface of the colony may be very small in relation to the entire surface and volume of the clustered zooids that make up the colony. Colonies of this design have several advantages: diverse orientation of the zooids, fixation onto restricted surfaces, and the projection of zooid siphons away from the substrate. This arrangement minimizes potential damage to the colony by predators, because one destroyed zooid need not endanger the health of the rest of the colony. In fact, damaged parts of the colony heal quickly. Colonies whose zooids form clustered "bouquets" adapt well to frequent and abrupt changes in bottom currents; since zooids' orientations vary, some zooids can close their siphons while others keep theirs open.



Clavelina flava (Polycitoridae) makes bouquet-like colonies in which the zooids are united only by their basal tunic. Their thoraxes remain independent of each other.

In almost all colonial ascidians, the varying ages that result from continuous budding spread out gametogenesis over time. Consequently, these species enjoy extended breeding seasons and thus enhanced chances for a broad distribution. But when a colony is large, or when ecological conditions become less favorable, budding slows down throughout the colony, and the formation of gametes begins. Sexual maturity thus may occur simultaneously over the whole colony, shortening an otherwise extended breeding period.

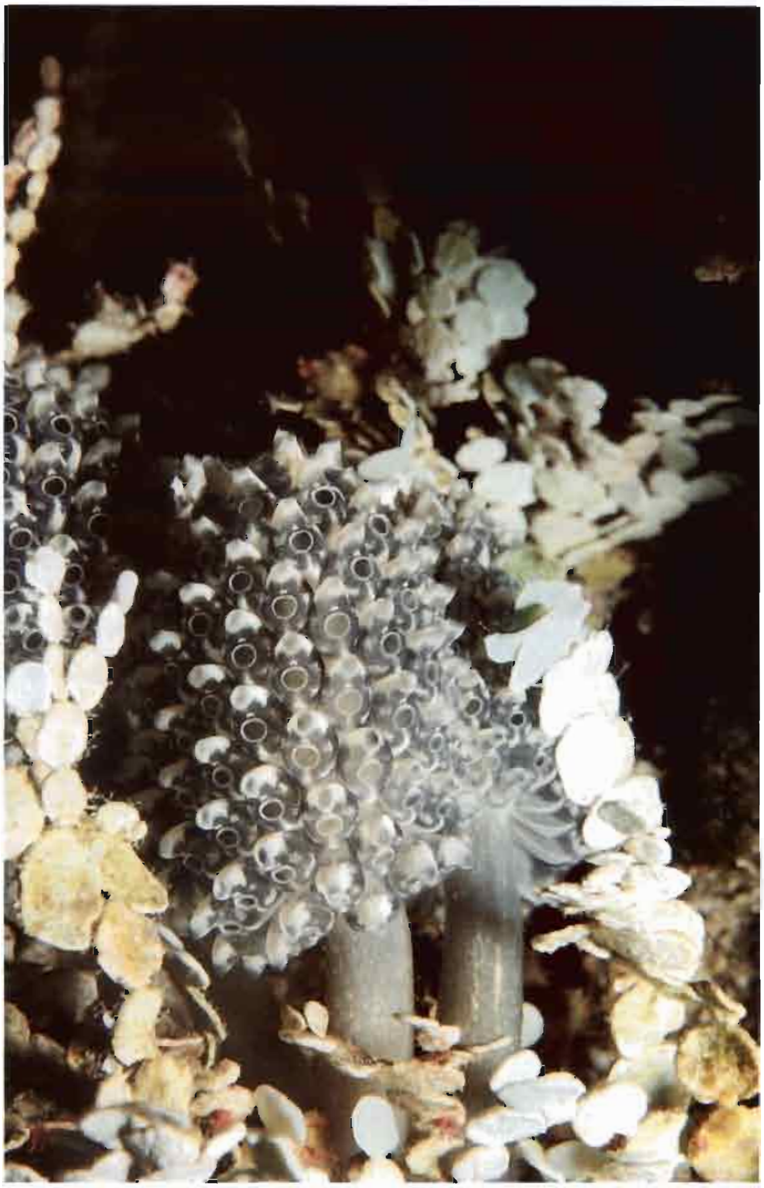
COLONIES WITH ZOOIDS PARTIALLY EMBEDDED IN THE COMMON TUNIC

There are many encrusting or pedunculate colonies in which the abdomens of the individual zooids are enclosed within a dense mass of tunic, itself tightly attached to the substrate, while the zooids' thoraxes project more or less independently from this common mass. *Oxycorynia fascicularis* is a dramatic example. This arrangement is intermediate between colonies whose zooids are grouped in "bouquets", only basally connected, and ones whose zooids are fully embedded in a common tunic. In these intermediately thick colonies, zooids show no orderly arrangement in relation to each other. They simply are held close together. The zooids' exposed thoraxes are less protected from predation than their more embedded abdomens; it is in their abdomens that active reproduction, whether sexual or asexual, occurs.

COLONIES WITH ZOOIDS ENTIRELY EMBEDDED IN A COMMON TUNIC

These colonies have diverse shapes. Some encrust the substrate in thick or thin sheets. Others form small cushions or even balls that are attached to the substrate by only a small part of the tunic. There are, as well, massive colonies that grow away from the substrate on stout peduncles or stalks ("clavate" forms) and thereby project their zooids well out into the water. The surfaces of these colonies, whatever their forms, may be smooth and even, or contorted into folds, lobes, or finger-like projections that greatly increase the colony's surface for water intake. Depending on the environment in which it grows, even a single species (of *Polyclinum*, for example) may either grow erect, pedunculate lobes or spread out as an encrusting sheet.

A colony's zooids are arranged quite independently of the colony's overall shape. In all cases, every zooid's oral siphon opens at the surface of the colony's tunic. In the most simply organized type of colony, each zooid opens independently to the outside through its cloacal siphon, as well. Occasionally, all the zooids orient their siphons in the same way, giving the colony an orderly appearance. This regular arrangement of zooids is especially visible in *Citorclinum laboutei*, where each siphon is encircled by a pigmented white ring. In other species (some species of *Eudistoma*, for example), the zooids form "systems" in which they are arranged spoke-like in circles. Their separate cloacal siphons lie close together toward the center of the circle, and their oral siphons are on the periphery.



In the polycitorid *Oxycorynia fascicularis*, the abdomens of the zooids are packed together in a dense common tunic and form the stalks of the colonies. In the colonies's "heads", the thoraxes are partially isolated, each in its own thin tunic, and are easily seen, thanks to their pigmentation.

At a more complex level of organization, the zooids form even more thoroughly integrated systems, in which the common tunic has one or more cavities into which the cloacal apertures of a few or many individual zooids empty; each cavity itself opens to the outside by a common cloacal aperture. This type of organization, simple when the common cloacal cavities are themselves simple, becomes complicated in a variety of ways as these cavities ramify. A mere pouch-like invagination may penetrate the tunic below the colony's surface and spread out at the level of the zooids' thoraxes to create channels. These ducts may branch

A thin encrusting colony where the small (1 mm) individuals lie along the paths of meandering cloacal channels.

in turn, always on the same thoracic plane, and develop a pattern that resembles a river system. In opaque colonies of this sort, only the star-like openings of the zooids' oral siphons are visible, along with, here and there, the distinctively larger and sometimes raised openings of the common cloacal apertures. In colonies with transparent tunics or richly pigmented zooids, by contrast, it is easy to follow the course of common cloacal channels. This arrangement, with the channels spreading in one plane only at the level of the thorax, is found in such widely separated genera as the polyclinid *Aplidium* and the styelid *Botryllus*.

In many Didemnidae, too, common cloacal cavities spread as channels at the level of the zooids' thoraxes. In other didemnids, however, the channels also penetrate farther toward the substrate, even beyond the zooids' abdomens. From the colony's surface, nothing is visible except the zooids' oral apertures and the systems' common cloacal apertures. But inside the colony, little groups of zooids lying in the common tunic are surrounded by large common cloacal channels. In some species, these small, pillar-like masses of zooids and tunic join one another and embed the zooids' abdomens in a thick mass of common basal tunic. In others, the pillars thin out below the zooids' abdomens, connecting them only by thread-like elements to a thin basal sheet of tunic lying well below. In the latter case, the colony becomes very hollow and fragile, especially when, as in the genus *Diplosoma*, it contains no calcareous spicules. Colonies with very extensive, even labyrinthine, common cloacal channels are characteristic of the Didemnidae. The tadpoles of some didemnids are incubated next to the zooids, within the tunic that enve-

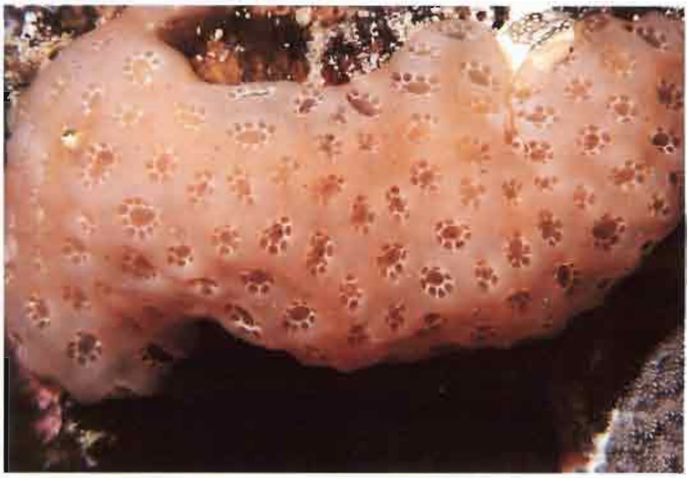
ome didemnid colonies
ike *Didemnum spongioides* increase
heir surface by making lobes and folds,
chieving an almost sponge-like final structure.
icroscopic oral apertures pierce
he entire external surface.
arge, branched cloacal channels
ermeate the colony,
pening by large, osculum-like apertures
t the tops of the lobes.

lops their abdomens. But in other didemnids with very extensive common cloacal channels, the larvae develop inside the basal sheet of the tunic before being released through these channels to the sea.

Colonies that have large common cloacal cavities sometimes take a fairly globular shape, with a single, apical, common cloacal aperture. Such colonies show two different types of structure. The central cavity may be very large, the result of an extreme dilation of a cloacal pouch; the didemnid *Atriolum robustum* provides a good example. Or the center of the colony may be solid and surrounded by extensive, complicated channels, as described above. In this case, instead of spreading out over the substrate, the basal layer of tunic may invaginate deeply into the colony and fill its center. *Didemnum molle*, even though it resembles *A. robustum*, is an example of this different type of colonial structure.

In this species, *Citorclinum laboutei*, oral and cloacal apertures open independently. There are no systems or common cloacal cavities, but the colony does show an orderly pattern of zooids : all have the same orientation in regular rows.

In *Aplidium flavolineatum*,
as in many other polyclinids,
the zooids form "rosettes".
The oral apertures are arranged in circles
around a common central cloacal cavity
and its aperture.
This colony shows many such circular "systems".



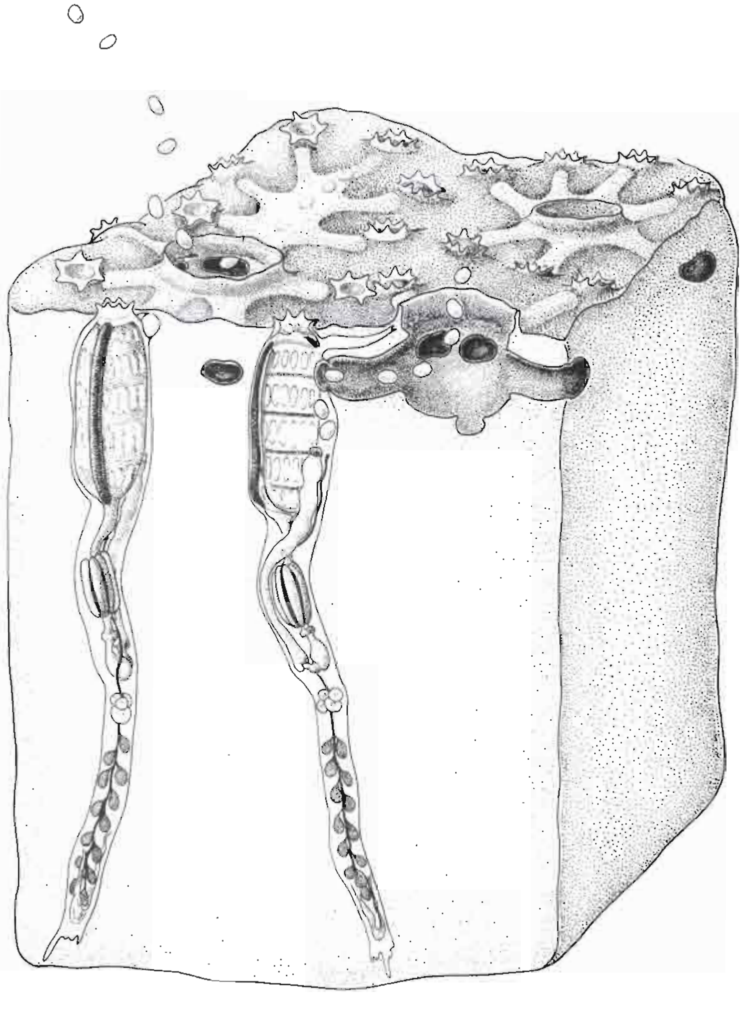
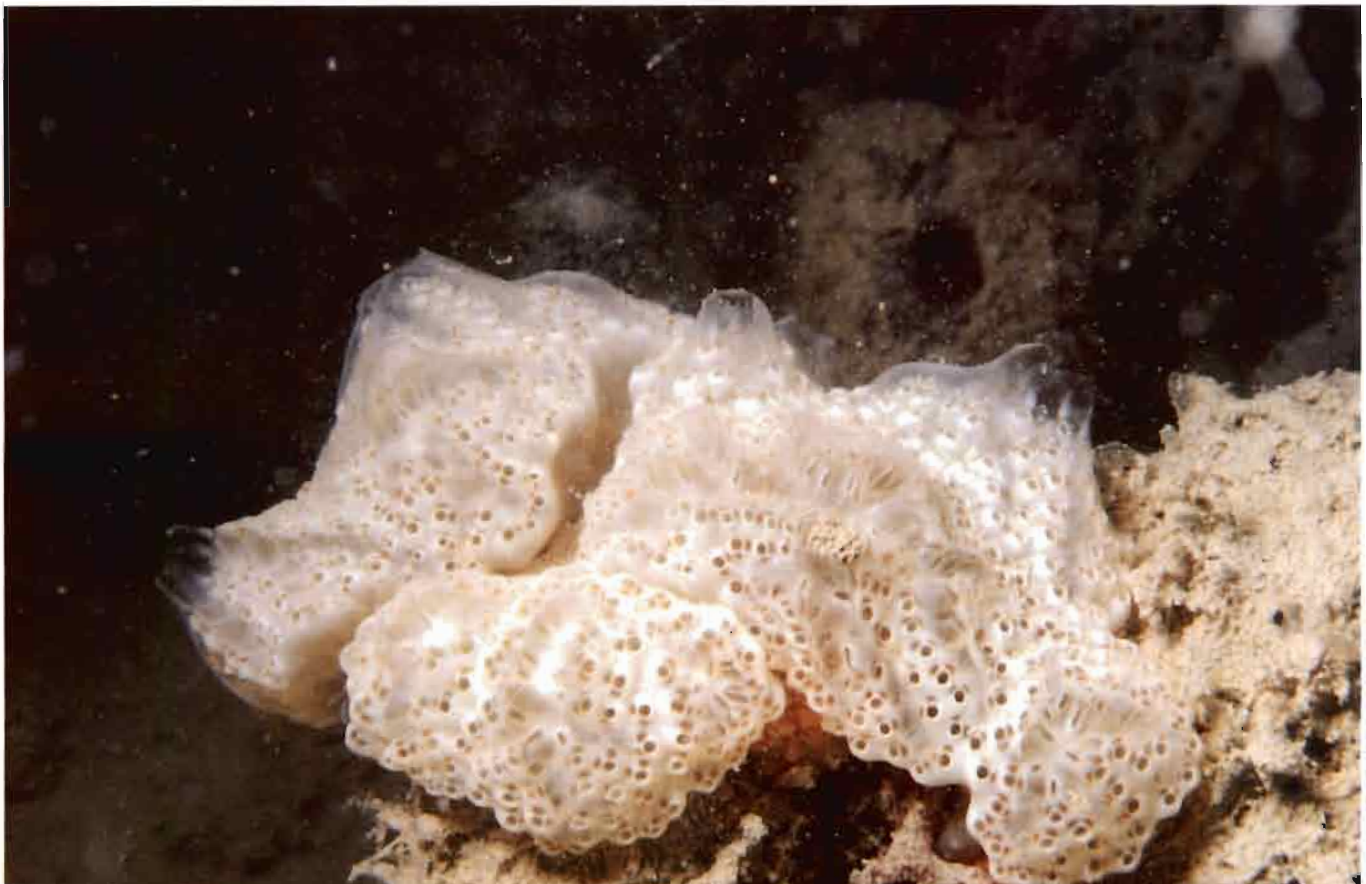
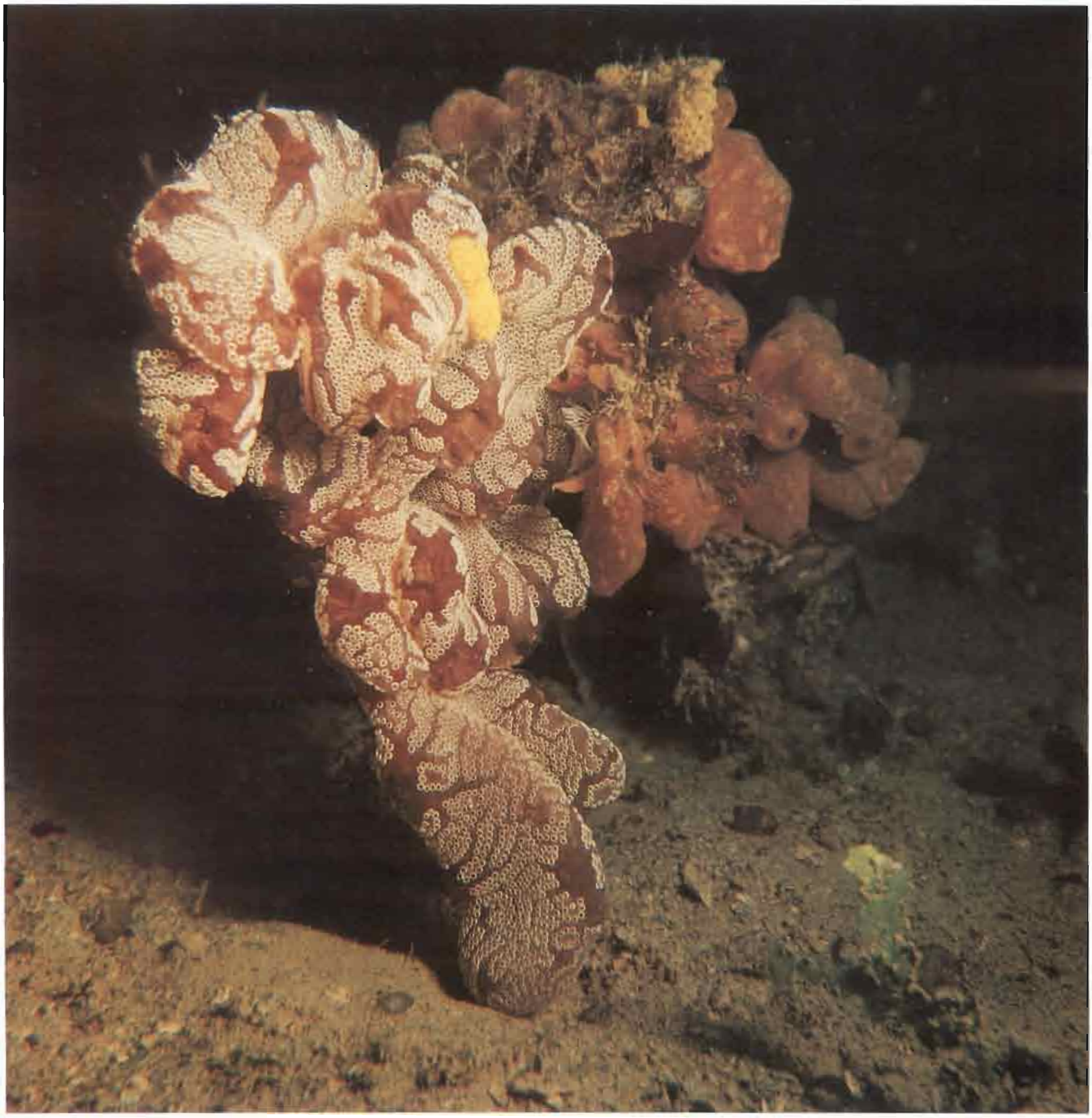


Diagram showing the zooids' arrangment
into "systems" in a polyclinid colony.
In a system, the cloacal siphons of several zooids
empty into cloacal channels that converge
on a common cloacal cavity.
The cavity then empties to the outside
through an exhalant orifice,
the common cloacal aperture.

On the surface of this well-inflated,
encrusting colony, each zooid shows its oral siphon,
marked by two brown spots.
These zooids are arranged along internal channels
that converge and empty through
common cloacal apertures
raised on transparent chimneys.

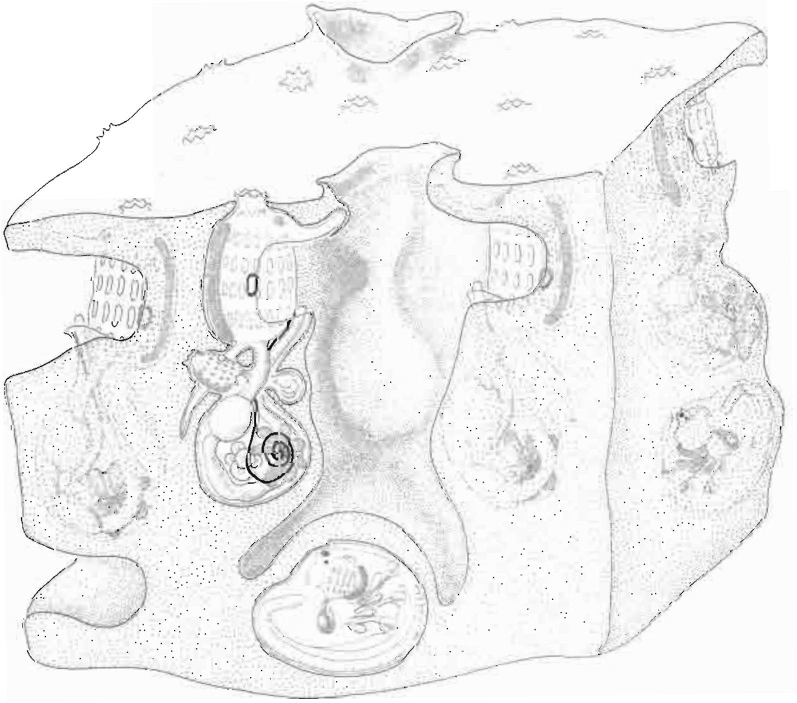


Botryllus leptus's bright pigmentation
reveals the zooids' distribution
in the common tunic.
Here the yellow oral siphons
make sinuous designs in a brown-red tunic.
This species occurs in other striking colors, as well.



The abundant *Didemnum molle*
forms urn-shaped, independent systems.
The zooids are scattered beneath the surface,
under a layer of calcareous spicules.
There is only one common aperture
on the top of each colony.
Unicellular symbiotic algae live in each system's
large common cloacal cavity.

Diagram of an encrusting didemnid colony.
The zooids are embedded in the tunic
in small groups.
The large, individual cloacal apertures
open into extensive cavities
that are not limited to the zooids' thorax level
but rather go well into the depths of the colony.
Larvae develop in the colony's basal tunic.



Budding of Colonies: Lobulation

Rapid growth may produce didemnid colonies of great size, extending over more than a square meter when the substrate is large enough. In such immense colonies, there may be many juxtaposed or even imbricated systems, each with its common cloacal aperture. In other species or circumstances, colonies may consist of only one or two systems. As soon as budding has produced a sufficient number of zooids, they organize around a new common cloacal aperture to form a new system. In doing so, the systems actually move apart on the substrate. At first, the new systems are united in a common colony, but soon only a thin mass of tunic links them. Eventually, even this neck narrows and then breaks, and the two systems form completely separate colonies. This budding of whole colonies, called "lobulation", allows a mobility of sorts for some didemnids. While lobulation is the rule for some species, in others it is simply an occasional way of adapting to a particular substrate. For example, when *Lissoclinum voeltzkowi* settles on the seaweed *Halimeda* or on other sturdy seaweeds of the reef flats, it forms lobulating colonies. This is adaptively beneficial, because the seaweed's segments present a fragmented, flexible substrate that precludes the formation of massive colonies. Lobulation allows the offspring of a single larva of this species to exploit a large surface, but in bits and pieces. Another example of the way lobulation lets colonies adapt to a variety of substrates is found in one species of *Polysyncraton*. When the substrate is large and even enough, or free of other encrusting organisms, this ascidian

Colonies themselves may be able to bud.
This multiplication process is called lobulation.
Various stages of lobulation can be seen here;
each system has a white common cloacal
aperture in the center of a yellow mass.
As a new system forms, the two pull apart

In this didemnid species,
young colonies are round and older
ones more elongate.
When they become too large, they divide.
This allows the animal to distribute its colony
extensively over the substrate.
Lobulation has permitted this didemnid
to exploit the irregular surface
of a ramified seaweed.
The ascidian is green
because it contains symbiotic algae.

can form a sort of pavement in which each block-like system has a central common cloacal aperture. But when this species encounters territorial competition from other animals or simply grows on restricted surfaces like coral branches, its systems separate from each other by lobulation and become independent colonies.

POLYCHROMATISM

Ascidians come in a astonishing array of colors and in every shade from pure white to jet black. While it is true that the largest solitary ascidians are often drab or covered by epibionts, some of the commonest solitary species are exceptions to this rule. *Phallusia julinea*, for example, is a beautiful sulfurous yellow, *Polycarpa clavata* is vivid orange, and *Pyura momus* has a bright red tunic. Overall, however, colonial species tend to be much more colorful than solitary ones and are less often obscured by epibionts.

Pigments either cover ascidian bodies uniformly or are concentrated into lines or patches. Often, concentrations of pigment in the tissues accentuate the contours or parts of bodies, making them visible through a translucent or transparent tunic. Colored patches in the tunic itself, however, usually bear no particular relationship to the body inside.

This *Polysyncraton* (Didemnidae) has a design resembling pavement that incorporates several systems. In a different habitat, the same species may be found with isolated systems.

Pigments

Pigments within ascidian bodies are carried in a particular type of blood cell. They are made of various compounds, including carotenoids, flavins, and melanins. In addition to true pigments, structural colors occur in some excretory products, especially in metallic salts that are stored in vacuolar blood cells. Finally, some excretory blood cells, called nephrocytes, may be colored. These cells concentrate microcrystals of nitrogenous products such as purines, pterins, and urates. Cells that store these crystals become opaque and form white, yellow, orange, or red patterns, dull or shiny according to the substances involved. These cells accumulate most often in the branchial sac and in certain parts of the mantle, especially on or near the siphons and around the abdomen.

Pigments occur, as well, in other ascidian tissues, including the tunic. Their density varies greatly from one organ to another and from one tissue to another. Pigment cells often delineate large blood sinuses or sinusoidal ampullae within the tunic; this is the case, for example, in *Phallusia julinea* and *Polycarpa aurita*. Unless it is covered by epibionts, the tunic of *P. aurita* looks uniformly golden yellow to the naked eye. But a hand-lens reveals a multitude of yellow spheres in the tunic, each one made up of tiny yellow dots-pigmented blood cells.

Pigments may be of a single type, like the uniform white of *Aplidium cellis*, or of several sorts in a single animal. In *Cystodytes fuscus*'s tunic, there are both brown and white pigments, variously distributed depending on the colony. These pigments make the tunic completely opaque, so that even its large, calcareous spicules cannot be seen from the outside. The range of browns,

Didemnid species often are snow-white.

from light to dark, and the wide variety of white patterns can make these colonies look so different from each other that one might hesitate even to call them members of the same species. And yet such variations can distinguish not just separate colonies of a species but even parts of a single colony!

As a group, the Didemnidae present both the most brilliant and the most delicate ascidian colors. Yet their colors provide almost no clues to their identification. Whether the colony is lemon yellow, deep orange, bright pink, mauve, gray, or blue, color alone will not tell us whether we are looking at a *Didemnum*, a *Trididemnum*, a *Lissoclinum*, or a *Leptoclinides*. Exact identification, even to genus, requires a close look at the zooid's anatomy. Even worse, didemnid colors may differ from colony to colony within a single species and within a single habitat. Such variability is especially characteristic of didemnid ascidians, but it also occurs in other families. Colors are independent of a colony's age or stage of sexual maturity; instead, they are generally a matter of individual variation. However, when didemnid colonies lobulate to form new colonies nearby, all these related colonies show the same general coloring. Among the urn-shaped colonies of *Didemnum molle*, for example, some show a reddish brown pigment that does not occur in others - a slight difference that may distinguish one lobulated cluster of colonies from another.

Intraspecific color variations are confusingly common, but even

The tunic of *Archidistoma rubripunctum* (Polycitoridae) is so transparent that only the scattered orange spots made by accumulations of pigment cells in the blood and mantle make the animal visible. The colony lives near a sponge of the same color, seen to one side in this picture.

The polyclinid *Aplidium cellis* has protruding white lobules separated by less pigmented furrows in which the oral apertures of the zooids open.

In *Clavelina detorta*, the glassy tunic lets us see the blue transverse bars of the branchial sacs and yellow cells in the zooids' other internal structures.

Cystodytes fuscus (Polycitoridae) is always dark brown, but sometimes with irregular patches of white pigment.

Leptoclinides dubius is a fleshy didemnid that is a more or less dark pink, according to the density of the calcareous spicules in the surface layer of the tunic.

All colors can be found
in the family Didemnidae.
Here are a yellow and a red *Didemnum*,
the pale violet *Lissoclinum japonicum*,
and a blue *Tridídemnum cerebriforme*.

many didemnid species have "characteristic" colors. Then a species' color may provide a helpful, if risky, clue to its identity, especially when the colony's size, shape, and consistency are also taken into account. A particularly striking example is *Lissoclinum vareau*, which forms soft, encrusting colonies. Sea water filters sunlight so that this species appears to be an intense blue at, say, 20 meters' depth, but it turns out to be a beautiful, deep mauve pink at the surface. This surprise occurs whether the colonies are encountered in New Caledonia, Tahiti, or the Maldives.

Apart from the mostly tropical Didemnidae, the colonial ascidians that show the widest color variations, both between and within species, are certainly the botryllids, which abound worldwide, again with very confusing representatives. The botryllids are colonial styelids that have been used in many studies of ascidian pigments and their genetic control. In the past, many European botryllid species were named and described solely on the basis of their colors. Since then, experimental studies have shown that these colors vary within one species, *Botryllus schlosseri*, according to fairly simple genetic combinations. Carefully controlled crossings in the laboratory have duplicated all the colors and patterns that this species shows in nature. In New Caledonia, enormous color variations are characteristic of the commonest botryllid, *B. leptus*, and they, too, almost certainly depend on genetic mechanisms.

In the port of Noumea, *B. niger* forms large, orange colonies on the quay walls, just as it does in the harbor of Papeete in Tahiti.

At 20 m depth,
without artificial light,
the characteristic violet
of *Lissoclinum vareau* would appear
to be deep blue.

But along the coasts of the tropical Atlantic - in the Antilles, for example - this very abundant species is sometimes orange, sometimes strikingly polychromatic. We can speculate that *B. niger* is native to the western Atlantic and was carried to the Pacific as a fouling organism on ships' hulls. If so, then the species established itself in Pacific ports from the offspring of only a few individuals, necessarily of a greatly reduced genetic range. A history of this sort could explain the otherwise perplexing presence in the Pacific of solely orange *B. niger* colonies.

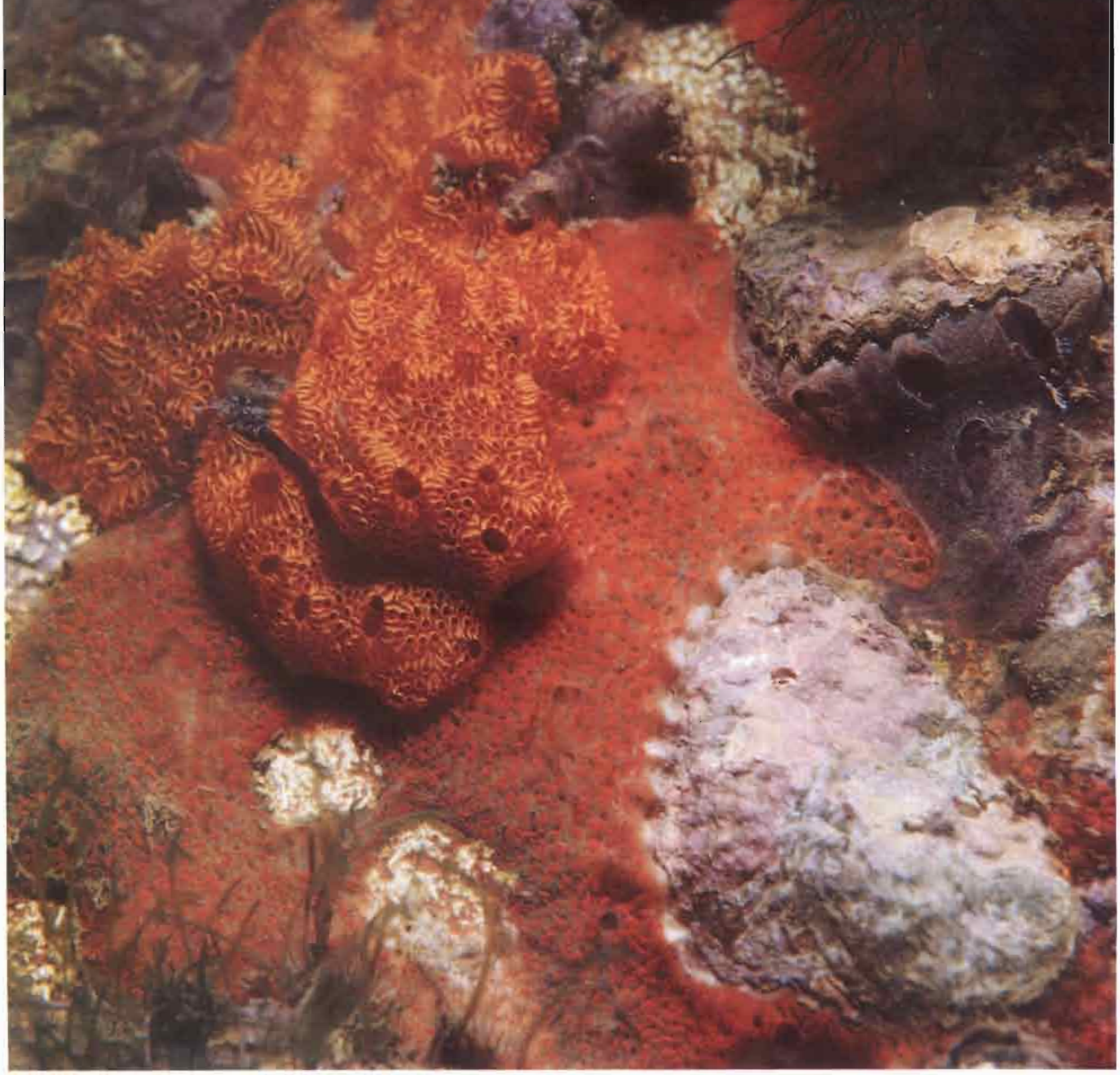
Only living ascidians come in bright colors. In fact, an unwary naturalist who collects these animals without separating them from each other, fixes them, and then looks at them several weeks later is in for some unpleasant surprises. Where, among these dark specimens, is that splendid, golden yellow colony that he remembers collecting? Is the label a mistake, or is it now this black colony? In fact, the very name *B. niger* is based on fixed material, since, regardless of its original color, this species always rapidly turns black when it dies. Such chromatic surprises occur in all ascidian families. For example, colonies of the polycitorid *Eudistoma album*, which form small, chalky white, pedunculate masses on the reef flat, also turn black very soon after they die. Unfortunately, handsome ascidians can be very disappointing objects after they are collected. No matter what the species, they lose their color within mere hours or, at best, during several months of even the most careful preservation in a jar. Like all organisms, ascidians are at their best alive.

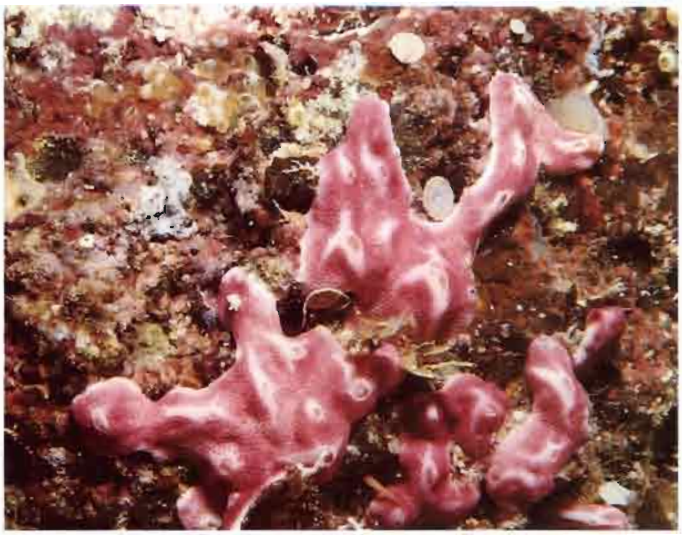
Two different colors,
one cream and the other white,
of the same didemnid species.
Notice that lobulation
of the cream-colored colony
has produced daughter colonies
of the same color.

Diversity of zooid patterns, colors, and tunic consistency in the species *Botryllus leptus.*

The diversity of *Botryllus leptus*.

Living *Botryllus niger* has bright orange colonies, but its name was given to it because it turns black after it dies.





Spicules tend to accumulate around the common cloacal apertures of this *Didemnum*, where they cover the colony's pink pigmentation.

Coloration by Spicules

Among colonial ascidians, most Didemnidae and some Polycitoridae contain calcareous spicules in their common tunics. These spicules are composed of aragonite, a form of calcium carbonate, and are always white. When the tunic lacks pigment, the spicules color the colonies white with an intensity that varies with their density. When a colony is uniformly pigmented and its spicules are abundant, it takes on even, pastel shades like those created by painters when they mix white with colors. Marbled patterns, often very decorative, result when the tunic contains abundant spicules and irregularly dispersed pigments. Conversely, among even uniformly pigmented didemnid colonies, spicules may concentrate around the common cloacal apertures, whitening them by obscuring the pigment there more completely.

Coloration due to Symbiotic Algae

In warm waters, ascidians often shelter microscopic, unicellular algae. These organisms may live on the colony's surface, in their common cloacal cavities, or inside the matrix of the tunic.

The symbiotic alga that lives on the surface of ascidians is *Prochloron*. For many colonies, this association is facultative - that is, the ascidians survive quite well in the absence of any *Prochloron*. Furthermore, the association appears to be non-specific: the same kind of *Prochloron* coats many kinds of ascidians,

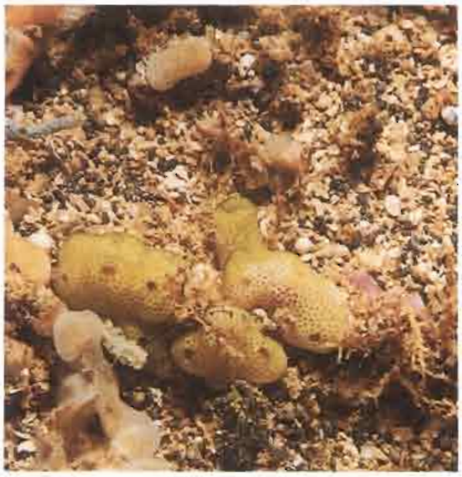
Difficult to recognize, this *Leptoclinides dubius* is normally pink; here it appears greenish yellow because the symbiotic algae on its surface have completely obscured the ascidian's own pigment.

especially among the didemnids. Colonies that are densely coated with *Prochloron* may live side by side with ones even of the same species that harbor only patches of the alga or have no *Prochloron* at all.

A single didemnid colony may have a continuous range of color - from pink to yellow-green, for example. In this case, the ascidian's red pigments and its tunic's white spicules interact to appear pink, but an uneven distribution of symbiotic *Prochloron* adds green tints in places. If you rub the colony surface with a finger, removing the surface film of algae, the colony's pink color appears.

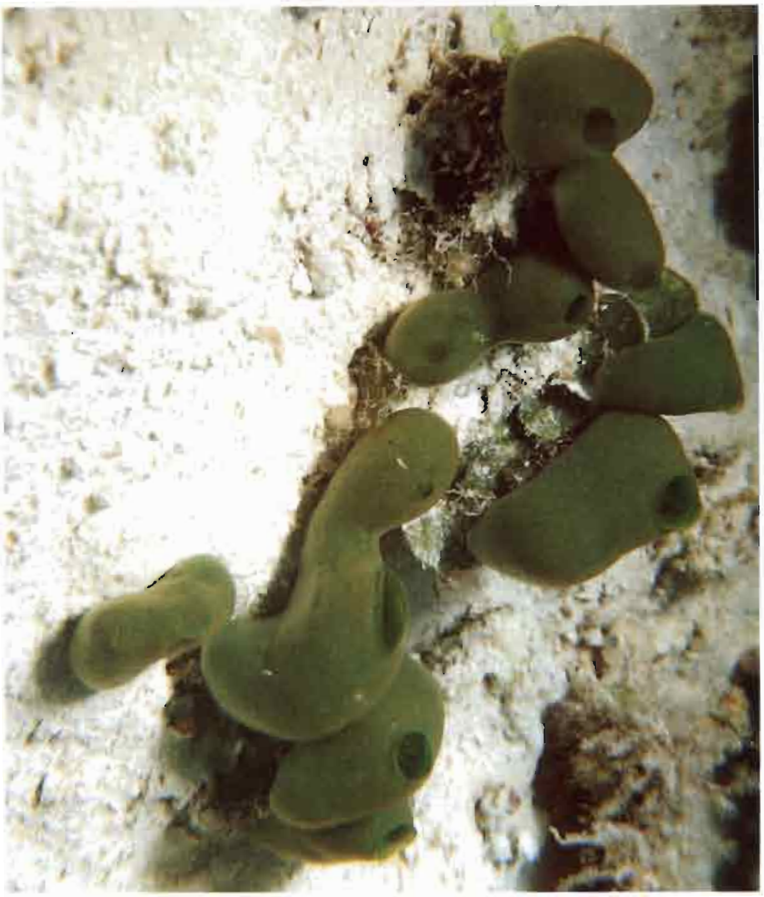
Prochloron lives abundantly in the common cloacal cavities of certain species; its color ranges from yellow-green to blue-green. Again, the apparent color of the ascidian depends on the density and depth of spicules in its tunic, since these spicules obscure algal colors as effectively as they mask the ascidian's own colors. For example, in *Didemnum molle*, some colonies look from the outside like white urns but have dark green internal surfaces and dark green edges to their siphons, where their spicules are sparse. Other *D. molle* colonies are dark green all over, because their abundant algal symbionts are hidden by only a few, widely scattered spicules.

The unicellular symbiotic alga most often associated with ascidians is the green-pigmented *Prochloron*. But other algae that are symbiotic in ascidians belong to a genus, *Synechocysris*, which produces red compounds. These algae color colonies pink with



The variable density of *Prochloron* (symbiotic algae) living on this white didemnid's colony spreads more or less dark green patches over its surface

Symbiotic algae
give diverse colors
to the didemnids they inhabit.



algal, not ascidian, pigments. This is the case in some colonies of *Lissoclinum bistratum*.

The didemnid *Diplosoma similis* contains no spicules at all and has a very thin, transparent tunic. This species contains abundant *Prochloron*, but the colony's green color is extremely dark, much more intense than *Prochloron*'s, due to the zooids' own dark pigment; this ascidian also has a blue iridescence of unknown origin.

Structural Colors

Other ascidians of diverse families have clear, transparent tunics that often appear brilliantly colored - blue, white, greenish yellow. Yet neither spicules nor pigments nor symbionts have generated these hues in these animals. Instead, the color is the result of light scattering off microcrystals contained in cells or off the clear, very fine, fibrillar structure of the tunic. The blue or greenish colors suggest a kind of fluorescence, but they are nothing of the sort. Rather, they are the colors of diffracted light. Through really transparent tunics, we can sometimes see, as well, the iridescence of light being scattered by the cilia of the branchial sac's stigmata. The inner surface of many pyurids' gaping siphons may also appear iridescent when the light is right. Again, light is being scattered, in this case by the innumerable tiny spines with which the tunic inside the siphons fairly bristles.

The scattering of light on the branchial cilia
can be seen through a glassy tunic.
It can give almost the same color
to a didemnid colony as to a polyclinid.

SPICULES

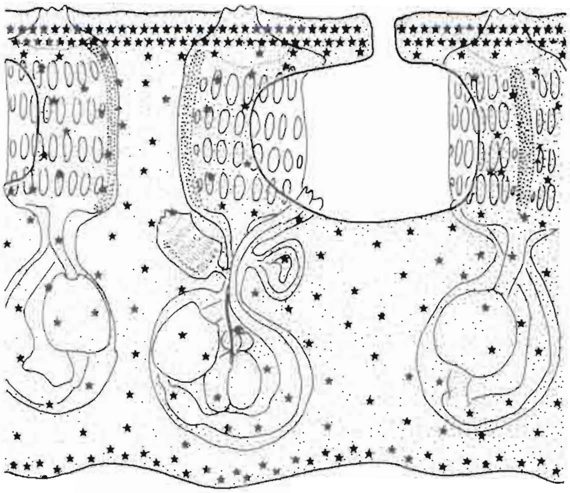
We have already had a good deal to say about spicules as they affect ascidian colors. The chemical composition of ascidian spicules is simple: they are made of calcium carbonate, crystallized as aragonite. Not all ascidians have spicules, but they occur in many aplousobranchs (Didemnidae and Polycitoridae) and in some stolidobranchs (Pyuridae). Their shape and structure depend on their location: spicules lodged in the tunic are completely different from those found in the body's mesenchyme.

Didemnid Spicules

The crystals that make up didemnid spicules form needles or double pyramids with a hexagonal base. These crystals are almost always tightly arranged into spheres or star-shaped combinations whose average diameter is about 40 µm. Didemnid spicules rarely reach 100 µm. The shapes and sizes of spicules differ from one species to another, and several shapes may be found within a single species and in some cases even within one colony.

LOCATION OF THE SPICULES

Spicules never occur inside the bodies of didemnid zooids. They lie in the tunic, sometimes in close contact with zooids but more often scattered through the tunic's matrix.



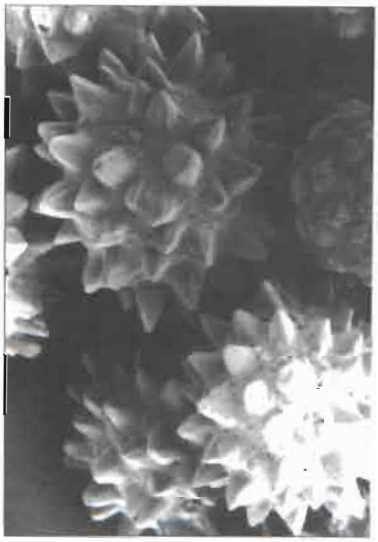
Diagrammatic sections
through a didemnid species
with stellate spicules

Diversity in spicule shapes
in four species of Didemnidae.
1 and 2: homogeneous shapes in each species.
3 and 4: diverse shapes in each species.
Each photograph shows spicules
from a single colony of its species.

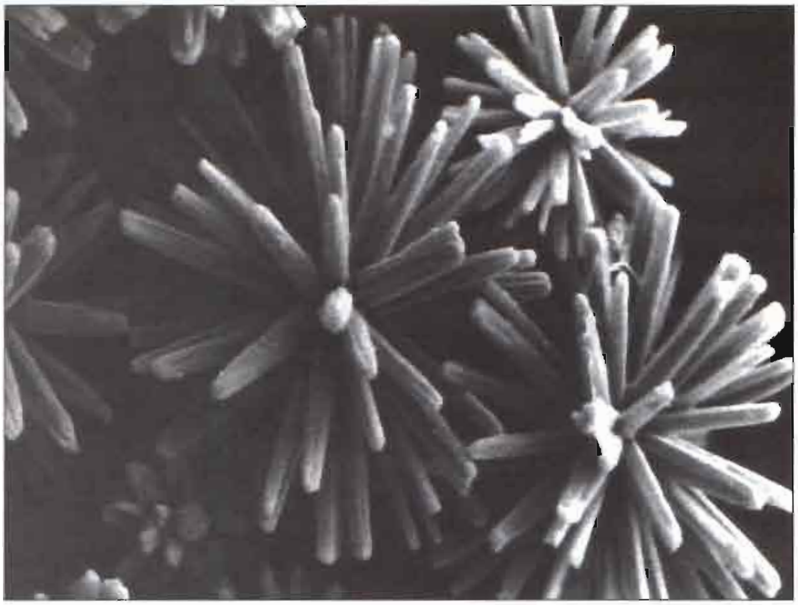
1. *Didemnum* sp.
Scale bar: 10 µ.

The density of spicules varies greatly among the Didemnidae. The genus *Diplosoma* is distinguished from the genus *Lissoclinum*, for example, only by *Diplosoma*'s complete lack of spicules. But some species of *Lissoclinum* and even *Didemnum* have so few spicules that only a careful search by microscopy reveals their presence. The colonies of other species, especially of the genus *Trididemnum* and *Didemnum*, may be so densely spiculated that they become hard and brittle.

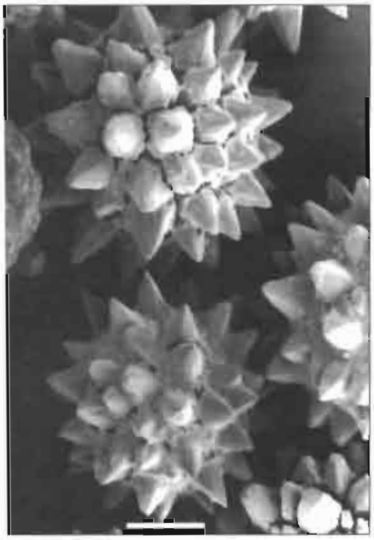
Species not only have more or fewer spicules, they also may show a wide variation in the distribution of spicules in the colony. A sagittal section through a spiculate colony generally reveals several superposed layers of varying spicule density. The most superficial layer, a thin cuticle, usually contains no spicules. Below this is a densely spiculated layer that encloses the zooids' oral siphons and their anterior thoraxes; this layer lies just above the colony's network of common cloacal channels. Below this in turn, at the thoracic level, spicules are generally less dense, and the tunic itself, widely penetrated by common cloacal channels, is flimsier. Deeper still, around the zooids' abdomens, either the spicules remain sparse, or else they form a dense layer comparable to the one just below the colony's upper surface. Where a spiculate colony meets the substrate, there is always at least a thin spiculate layer of tunic. This succession of layers describes an "ideal" didemnid arrangement. But the didemnids are a family of such contradictions that many other patterns also occur - among them, a cup-shaped layer of spicules surrounding just part of the abdomen, or spicules arranged only around the oral siphons, or just one densely spiculated surface layer with no spicules below. Striking differences in the distribution of spicules



3. *Polysyncraton* sp.
Scale bar: 10 µ.



2. *Lissoclinum fragile.*



4. *Leptoclinides dubius.*
Scale bar: 10 μ.

occur too, from one colony to another within a species. Even within a single colony, there may be significant differences from one part to another.

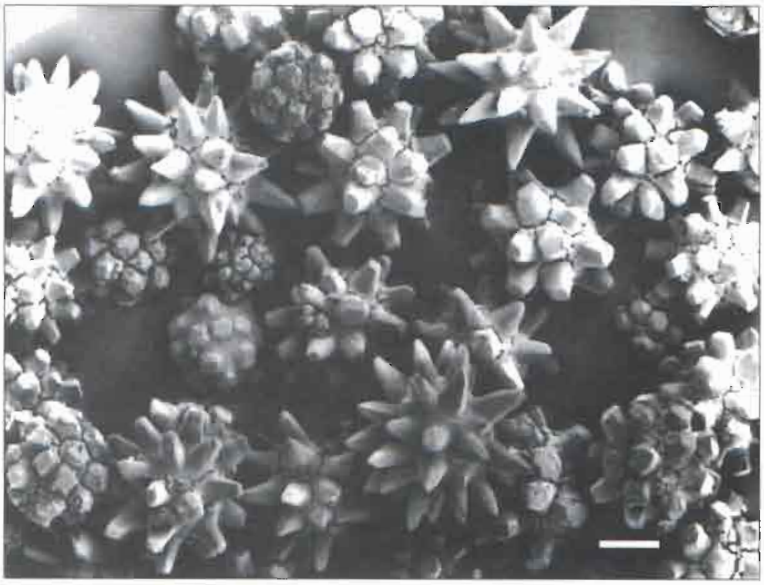
THE SHAPES OF DIDEMNID SPICULES

Photographs taken with a scanning electron microscope provide the clearest idea of these remarkable objects. All the spicule designs we encounter share one basic form: elongate crystals radiating from a central point. When crystals of equal length project evenly in all directions from that center, they form spicules that are spheres or globular star-bursts. Unequal rays or restricted patterns of projection, however, can produce many derivative spicule shapes. Needle-or stick-like rays produce spicules that look like pompons or pincushions. Or the rays may taper into pointed or bevelled or truncated or even twinned tips of crystals whose fused bases form a central mass. Needle-like or rhomboid crystals may occur in different lengths within a single spicule, complicating its geometric form. And, of course, differing numbers of crystalline rays also generate different overall spicule forms.

THE ORIGIN OF SPICULES

Among the Didemnidae, the presence of spicules is usually - but not always - linked to the existence on the zooid's thorax of two structures, of the mantle, called the lateral thoracic organs. These organs may protrude as odd, ear-shaped diverticula, or they may form dimples in the thoracic mantle. Their location varies greatly in relation to the zooid's cloacal opening, its ventral

1. *Polysyncraton thallomorpha.*
Scale bar: 10 μ.



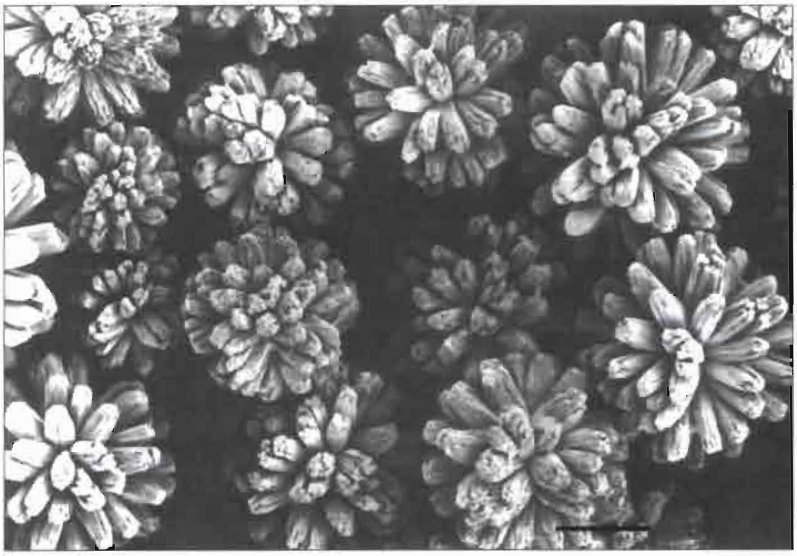
Various kinds of spicule crystals :
stars with pointed rays,
pompons, dense spheres,
and stars with wedge-shaped rays.

line, and the peduncular "waist" between its thorax and abdomen, but their location is distinctive in any species. These lateral organs are not differentiated glands; their cells are ordinary epithelial cells. We usually find tiny spicules in the tunic in close contact with these lateral thoracic organs. As a result, it was long thought that the spicules are secreted within or at least on these organs, later migrating into the rest of the tunic. But tiny spicules are also found in contact with other parts of the zooid - on the oral siphon and around the abdomen, for example - and also throughout the thickness of the tunic.

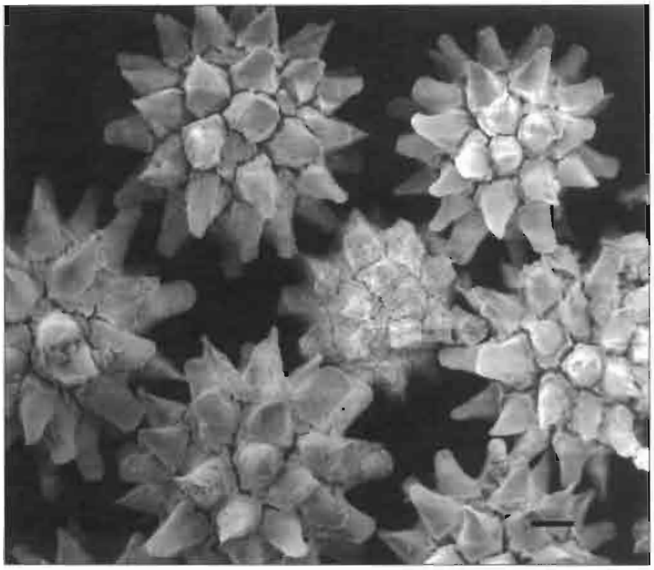
In 1925 the great French biologist M. Prenant suggested that spicules arise when calcium carbonate precipitates during a reaction of sea water with the serum of ascidian blood in the tunic. He had earlier been unable to demonstrate any intracellular formation of spicules, such as occurs in sponges and some other invertebrates. After several attempts, he succeeded in producing spicules in a test tube in which he had placed solid agar between two solutions of calcium and sodium salts. We repeated this experiment in 1970 and showed by scanning electron microscopy that, at carefully controlled levels of acidity, the crystals thus formed have shapes and sizes very much like those of didemnid spicules.

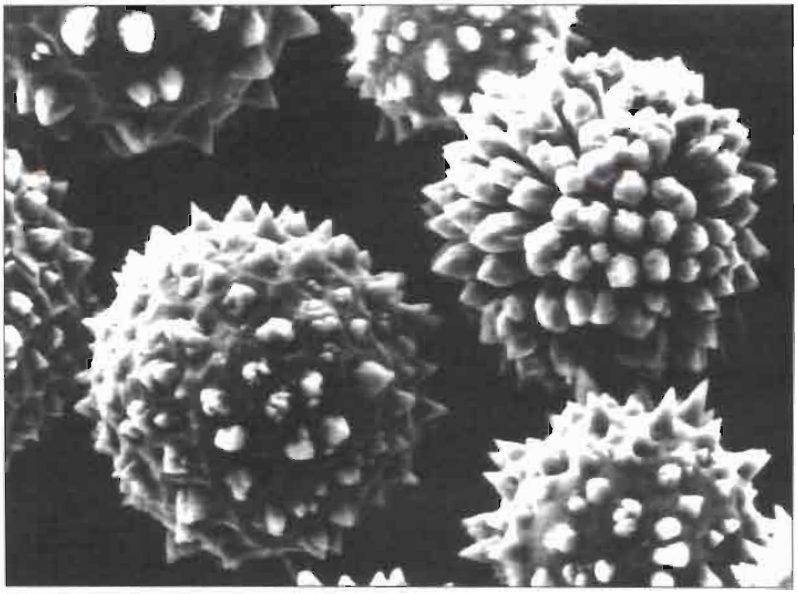
Blood serum leaks out of the mantle into the tunic. Tiny spicules will form in greatest numbers where the mantle and the tunic adhere most tightly: around the oral siphon, next to the lateral organs, and around the thorax-abdomen "waist" and the anterior part of the abdomen. Once begun this way, these minute spicules will grow simply by further crystallographic precipitation, molded by the tunic's fibrous fine structure as they disperse into

2. *Lissoclinum japonicum.*
Scale bar: 10 μ.



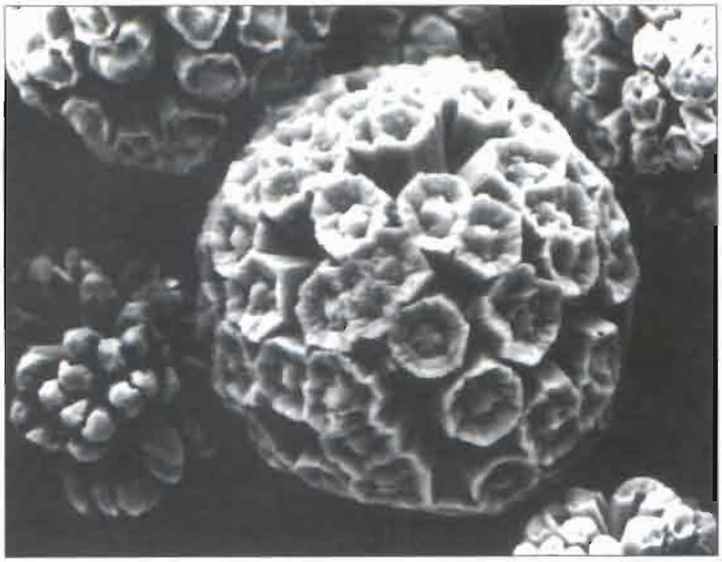
4. *Atriolum marsupialis.*
Scale bar: 10 μ.





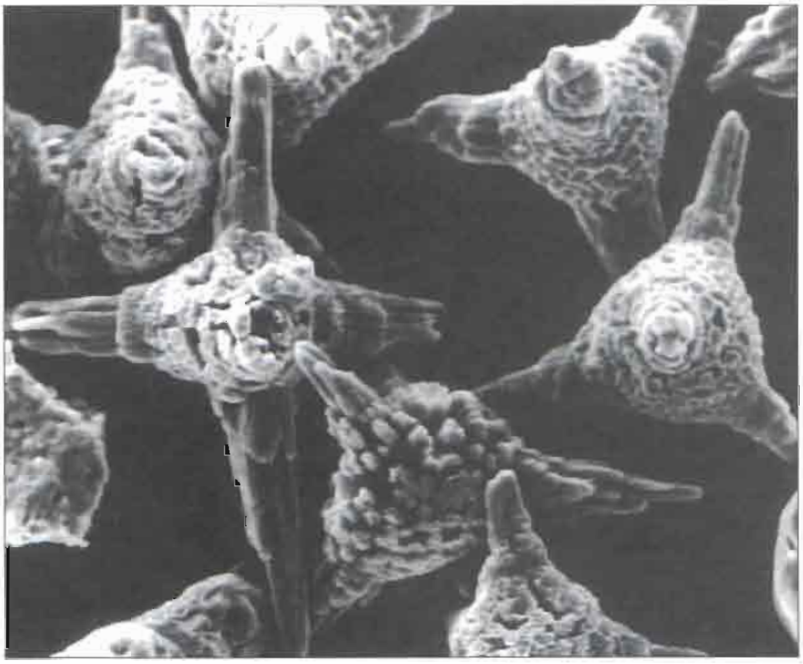
3. *Didemnum ligulum.*

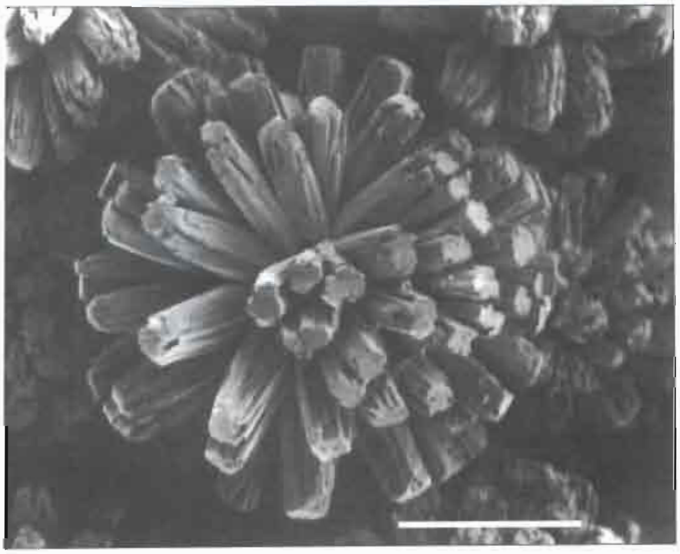
Several spicule shapes
resulting from irregular crystallization.



1. Equal rays
with peculiar tops
(*Lissoclinum bistratum*).

2. Rays that are unequal
in length and diameter
(*Lissoclinum tuheiavae*).





3. Stars with truncated rays
(*Lissoclinum japonicum*).
Scale bar: 10 µ.

4. Artificial spicules obtained in agar, showing hexagonal rays issuing from a dense central mass.

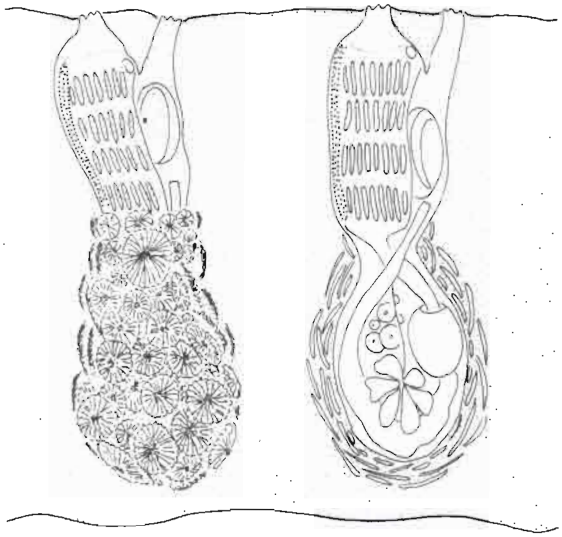
the tunic farther from the zooids. Thus, experimental evidence, observations of the tunic's fine structure, and the regular growth of crystals all strongly support the idea of an extracellular origin of didemnid spicules. The same sort of origin appears to be the case in the Polycitoridae.

Spicules in the Polycitoridae

Two genera of Polycitoridae, *Cystodytes* and *Polycitorella*, also have spicules. The spicules of *Polycitorella* closely resemble in size and shape those of the Didemnidae, and they are just as variable. They are dense in all parts of the tunic, so that the tunic itself is brittle.

In the thickly encrusting and mound-like colonies of *Cystodytes*, spicules take two different forms. Some species contain small calcareous "pincushions" like those of the Didemnidae; in New Caledonia, *C. luteus* has such spicules. But spicules in this genus are far more commonly shaped like flat discs or plates, made of calcareous needles that, instead of developing in all directions to form a sphere, grow and fuse in a single plane. These disc-like spicules pile on top of one another around the zooid, encapsulating its abdomen. Each zooid's capsule is surrounded in turn by a particularly fibrous jacket of tunic. Besides this orderly arrangement around the zooids' abdomens, spicules also concentrate more irregularly in the deep part of the colony's thick tunic.

In *C. punctatus*, the white capsule of spicules around each zooid's abdomen is visible from the outside, through this species'



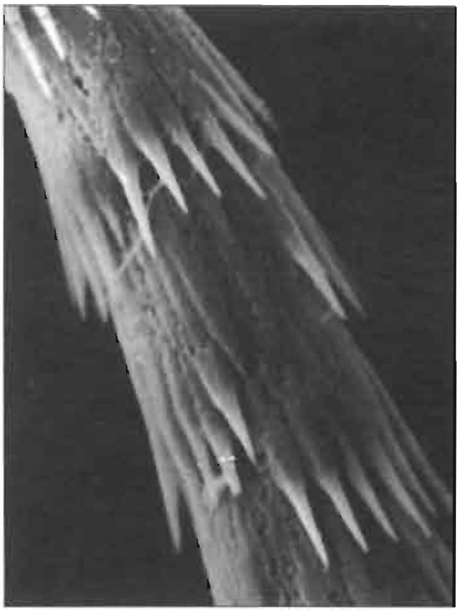
Diagrammatic section through a polycitorid, *Cystodytes*, a genus with discoid spicules.

extremely transparent tunic. The whole colony appears glassy and white-spotted. But this is unusual among *Cystodytes*: in most species, pigment cells make the tunic opaque, and so their spicules, which almost never lie in the colony's superficial layer, cannot be seen except by cutting the colony open for a very close look.

The edges of discoid, polycitorid spicules may be thick or thin, plain or irregularly indented, or even finely toothed. Some discs are made up of crystalline needles so perfectly fused that the discs' surfaces are smooth; in others, the component rays are distinctly visible. Many discs are flat, but in others the center may be raised conically or it may even be perforated centrally by a secondary dissolution of the disc.

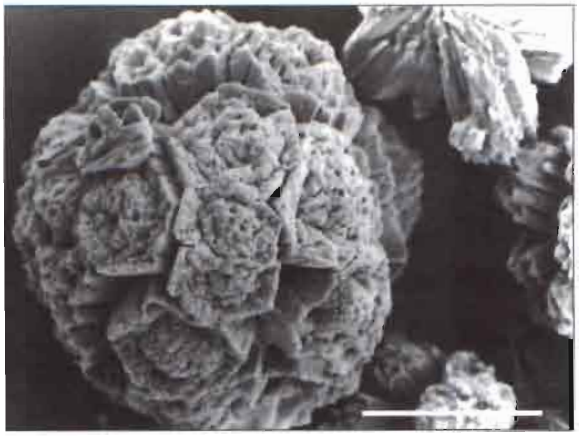
Along with discoid spicules, some polycitorids have star-shaped spicules much like those we have already described in connection with the Didemnidae. This is particularly apparent in some species of *Polycitorella*. Whatever their shape, polycitorid spicules vary greatly in size, with the largest ones in *Cystodytes* reaching a diameter of 1 mm. They form in the tunic alongside each zooid's abdomen and move out into the surrounding tunic as they grow. Their developing shapes seem to be influenced by the tunic's fibrous fine structure, just as didemnid spicules are.

Part of a pyurid spicule, in which tiny spines form verticils along the spicule's whole length.



Pyurid Spicules

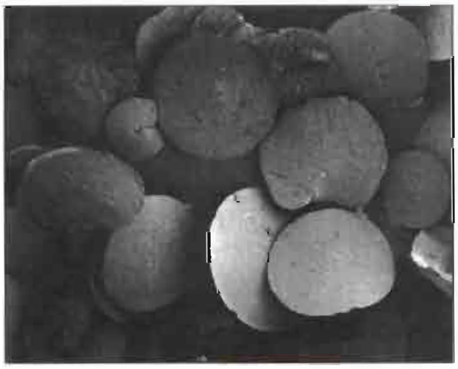
The Pyuridae have spicules that differ considerably from those we have described so far. They can be seen in one of the commonest solitary ascidians of New Caledonia, *Pyura momus*.



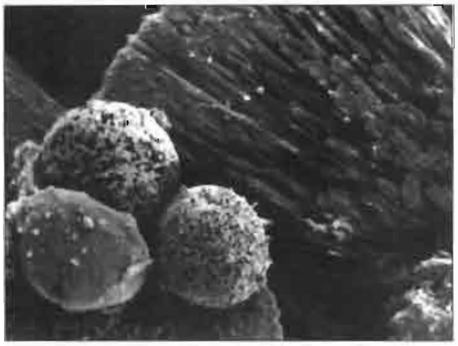
1-2. *Polycitorella mariae.*
Scale bar: 10 µ.

Spicules in the family Polycitoridae.

3. *Cystodytes punctatus.*



These spicules occur in the body's blood sinuses and lacunae, especially in the branchial sac and around the gut-tract and gonads. Pyurid spicules are transparent and elongate; their surfaces show successive, overlapping whorls of tiny spines. In both composition and formation, these stolidobranch spicules differ from those of the aplousobranchs. They are made of calcium carbonate crystallized as vaterite, not aragonite. And, in contrast to those of the Polycitoridae and Didemnidae, pyurid spicules form intracellularly. A pyurid spicule remains within a fibrous sheath and grows with the animal; some eventually approach 2.5 mm. In addition, small spicules form in the blood sinuses that penetrate the tunic. These spicules, still inside their fibrous sheaths, may then migrate out into the tunic's matrix, later protrude from the surface of the tunic, and finally be expelled into the sea. Their role in the animal's life is puzzling; could they serve to ward off possible predators ?



4. *Cystodytes violatinctus.*

Spicules of Fossil Ascidians

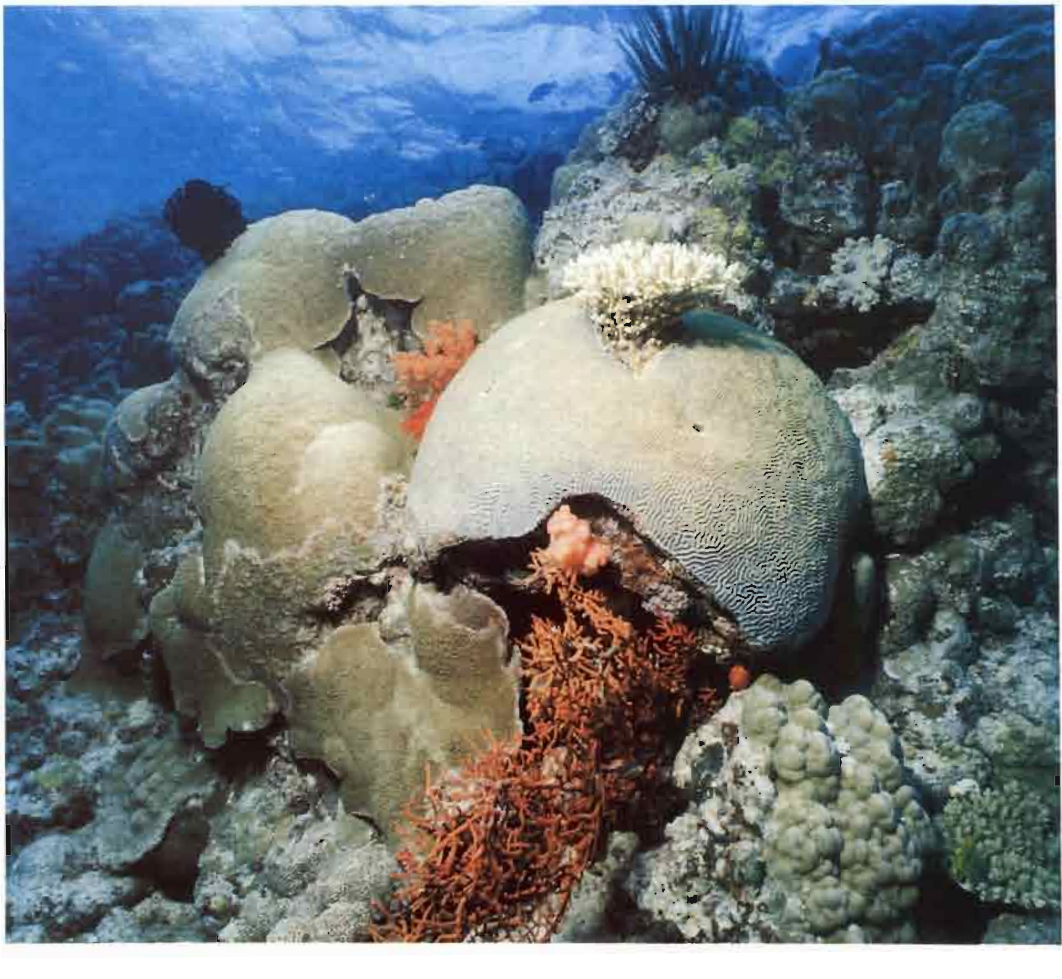
Aside from a few forms with tough tunics, ascidians are mostly soft animals, and so they are very unlikely to be fossilized. Some fossil imprints have been interpreted - but debatably - as belonging to whole ascidian bodies.

Spicules, on the other hand, may persist as fossils if the habitat is not too acidic. As a result, ascidian spicules have aroused the interest of paleontologists, and fossil spicules have been described from all parts of the world. Those ascribed to the didemnids are always very small and star-shaped; their various forms, named *Micrascidites* by paleontologists, have been found primarily in early or late Tertiary deposits. First discovered in french sediments, they have been recorded from the Balcombian formation in Australia. The oldest form, *M. irregularis*, is found in the Toarcian formation (lower Jurassic) in southwestern France.

Fossil polycitorid spicules from *Cystodytes* have been found along with *Micrascidites* in Tertiary deposits. These spicules are disc-shaped, with edges that are thin and toothed or somewhat thickened, and thus are just as diverse as those in living species of this genus.

Fossil ascidian spicules have not provided information about the evolution of ascidian groups. So far they have been used only to help identify sediments as coming from shallow-water marine communities. Consequently, their occurrence, while of paleontological value, has not evoked much interest among biologists. But these spicules do show that a widespread and diverse, well-evolved ascidian fauna probably existed as long ago as Mesozoic times.

Ecology



ECOLOGICAL REQUIREMENTS

Larval Life

The ecological requirements of ascidian adults differ tremendously from those of their larvae. After all, the larvae swim and do not feed, while the adults are sessile and filter-feeding.

Patterns of sexual reproduction vary with the family and sometimes with the species. The great majority of solitary ascidians are oviparous. They release both eggs and sperm directly into the sea, where both fertilization and development then take place. In many tropical species, gametes are released throughout the year; these ascidians' gonads appear as soon as the animals reach a competent size, and their gonads remain functional throughout their lives. But it remains uncertain whether gametes actually are emitted continuously; perhaps they are released sporadically in some species, or in a regular pattern alternating between eggs and sperm in others.

Fertilized eggs often float on the water's surface and are simply carried along by currents. No doubt, large numbers of them are lost to predators as they drift about. But cleavage of the egg and its further development into a swimming tadpole take only a short time - from several hours to several days.

Colonial species, by contrast, are all ovoviviparous. Sperm are shed to the sea, but not eggs. Fertilization takes place either inside the mother's oviduct or in the peribranchial cavity. Development of the embryo into a swimming tadpole also goes on inside the mother. Then tadpoles are released into the sea. They swim freely for only a very brief time.

Neither solitary nor colonial ascidians have feeding larvae. From its earliest development, the embryo is wholly enveloped by a tunic that persists intact until metamorphosis. The swimming tadpole's siphons remain closed within this sheath of tunic, and whatever organogenesis occurs before metamorphosis does so at the expense of the egg's original stores of yolk.

Except in the family Styelidae, there are great differences between the ways colonial and solitary ascidians undergo metamorphosis. In colonial species other than styelids, organogenesis is well advanced in the larva; the branchial sac is tiny but fully developed, and the stigmata are present, often even in their final number. As soon as the tadpole settles, its siphons open and it begins to feed. By contrast, all oviparous solitary ascidians, as well as colonial, ovoviviparous styelids, develop somewhat differently: at metamorphosis the tadpole's organs are still rudimentary, and the adult stigmata begin to differentiate only after larval settlement and metamorphosis. These forms cannot filter water and feed right after metamorphosis; they must undergo considerable further reorganization to achieve fully "juvenile" status.

A single solitary ascidian releases many more eggs than does a zooid in a colony, but the number of eggs produced and brooded by the whole large colony may well be equivalent to those spawned by a small solitary form. In addition, among colonial ascidians, fewer embryos are lost, because the free-living larval period is much shorter, reducing the chances of being eaten by predators or swept away by currents. The briefer its planktonic life, the better the chance that a tadpole on the verge of metamorphosing will find a habitat like that of its parents - a habitat that will probably be favorable for it, too. Many colonies, especially in favorable habitats, tend to go on growing, at the apparent

Didemnum molle,
having symbiotic algae,
tolerates light well as an adult.
Nevertheless, the larvae prefer
to settle on the dark part
of the substrate.

expense of sexual activity. By contrast, a sexually mature solitary ascidian remains so all its life. Thus, according to their design, different ascidians balance growth and sex in different ways to achieve maximum eventual reproductive potential.

Regardless of the environmental conditions, the tadpole eventually undergoes metamorphosis. If it has not yet found a favorable habitat for settling, it quickly dies. Metamorphosis is a critical time, influenced by many internal and environmental factors. These factors may, for example, accelerate or retard settling on a substrate, resorption of the tail and other larval structures, rotation of the body to its adult posture, opening of the siphons, development of the branchial sac, and formation of the adult nervous system.

Light and probably also the color of the substrate play roles in larval settling. Most settling ascidians avoid light. In experiments with submerged artificial substrates, metamorphosed individuals occur in greatest numbers on those surfaces with the least illumination. Moreover, if illumination is equal, dark surfaces are more attractive than pale ones to many species. But some ascidian species are actually attracted to light at metamorphosis, and there are others for which light levels seem to make no difference.

Several as yet unidentified chemicals appear to be influential both on spawning and on metamorphosis. Some of these compounds originate in the parent zooids and are released into the sea, provoking the simultaneous expulsion of both eggs and sperm in a population of solitary ascidians. Other metabolites attract larvae, which may then settle on or near their parents. The larvae themselves may produce some chemical mediators. In lab experiments, tadpoles in the presence of metamorphosing individuals settle more rapidly than do isolated controls.

Some inorganic substances, too, may repel or attract tadpoles that are ready to settle. Among metals, for example, copper repels tadpoles and iron tends to attract them. But we know very little about how these factors work, because so few studies have focussed on these chemical cues and their effects.

Finally, it appears likely that biological rhythms play important roles in the reproduction of ascidians. Most strikingly, spawning and the release of larvae often take place around sunrise or sunset. This rhythm, of course, can be attributed to the effects of changing light levels on animals in the field; but the same rhythms persist for a while in the lab in permanently lit aquaria, indicating some rhythmic pattern of internal control over reproductive events.

Ecological Constraints on Adults

Although chemicals in the water, light, the kind of substrate, and nearby organisms all influence larval settling and metamorphosis, these factors have little or no effect once the ascidian has become a functioning sessile animal. Adults have other environmental requirements, however, most of them involving feeding behavior.

UNSTABLE SEDIMENTS

Unstable substrates can create severe difficulties for ascidians, especially when waves and currents lift sediments into suspension. Ascidian filter-feeding, which involves very little selection of

To protect itself from abrasion by water-born sediment particles, *Aplidium lobatum* (Polyclinidae) incorporates sand grains in its tunic.

particles, is greatly hampered if mineral particles accumulate in the branchial sac and the digestive tract. Large sand grains are usually too heavy to be drawn in by the ascidian's own feeding current, and ones that occasionally do get into the branchial sac can be rejected by sudden contractions, which expel these irritants back through the oral siphon. But if silt enters continuously, it may well clog the pharynx and suffocate the animal. While ascidians can close their siphons for fairly long periods, sooner or later they must circulate enough water through the branchial sac and across unencumbered stigmata to maintain respiration.

Abrasion is another problem caused by sediments suspended by moving water; soft tunics are especially vulnerable to damage. Some ascidians reinforce the tunic against abrasion by incorporating sand into its upper layer or even throughout its thickness.

EXPOSED HABITATS

Most soft and fragile ascidians are excluded from exposed, projecting sites, where they are especially vulnerable to predators and to disruptive currents. This sort of habitat is also subject to much more sudden changes in the water itself than are grottoes, crevices, and the sides or undersides of rocks and coral. Solitary ascidians often survive the hardships posed by exposed sites better than do colonial species. In such places, they are often covered by epibionts that exploit the tunic as a substrate while providing their ascidian host with camouflage and perhaps some physical protection.

BRACKISH HABITATS

Although ascidians tolerate decreasing salinity very poorly, they may establish themselves in places that are subject to just such changes: in mangroves, in harbors, or at the mouths of rivers whose flow is highly variable. During dry periods, they may grow abundantly on floats, on pilings, on the roots of mangrove trees, or along the edges of subtidal channels worn by small streams. But heavy rains will leave a layer of light fresh water on top of the heavy sea water. These layers mix very slowly in calm habitats, and so a brackish surface layer of water may persist for a surprisingly long time. When this happens, all the ascidians disappear. Many other sessile marine animals, too, are killed by osmotic stress if these conditions are severe or prolonged enough.

Their intolerance of reduced salinities keeps ascidians and their relatives thoroughly marine. Nowhere in the world have tunicates, whether planktonic or benthic, succeeded in colonizing fresh or persistently brackish water.

SUSPENDED ORGANIC PARTICLES

Ascidians exist at all latitudes and in all sorts of truly marine environments. But the diversity and density of their populations may vary tremendously. The diversity of ascidians species depends primarily on a habitat's diversity of hard substrates. A population's density, on the other hand, depends on the availability of food - that is, on organic particles suspended in the water.

On the outer slope of New Caledonia's barrier reef, where phytoplankton is less abundant than in the lagoon, the sea's lack of organic matter limits both the number of individuals and their size. It probably also reduces their reproductive capacity. Colonial species dominate here, since, by their ovoviviparous habits and very brief larval stages, they protect the young more effectively than viviparous species can. In the lagoon, by contrast, ascidians profit both from more abundant plankton and from the suspended detritus of nearby organisms. And in the harbors, human activity enriches the water with particulate and dissolved organic matter, supporting often immense ascidian populations. Here, solitary ascidians greatly dominate over colonial species, both in biomass and in the rather reduced diversity that exploits in harbors.

DEEP-WATER ASCIDIANS

A peculiar ascidian fauna lives in deep water on the continental slope off New Caledonia. These regions support few individuals but many species, which are adapted in various ways to abyssal life. They must cope with such hazards as unstable sedimentary substrates and scarce food. Many ascidians that inhabit these depths are very small: most of their bodies' tissues develop slowly, but their gonads appear early, and energy is devoted to the production of gametes that can assure the persistence of these dwarf species.

Other abyssal species have peduncles that raise them off the substrate. Their siphons flare remarkably widely, so that water currents flow through passively, and their branchial walls no longer have cilia that create water currents. Passive exploitation of the habitat's own water flow seems to represent a real economy of energy, since the solitary ascidians that practice it can grow to be several centimeters across, which is large for any ascidian. This structural adaptation has occurred independently in many families among all three orders of ascidians.

Deep-sea species of the phlebobranch family Octacnemidae have abandoned filter-feeding and instead have become carnivores on relatively large prey. Their immense, gaping, oral siphons act as traps that capture whatever small animals venture onto the inner surface. As strange as this design may be, it seems just as surprising that shallow-water ascidians have not evolved anything similar.

FINDING, CLAIMING, AND OCCUPYING SUBSTRATES

Finding a Place to Settle

The ascidian's tadpole larva rarely exceeds a millimeter in length. It settles on a site that will support the animal for the rest of its life. The tunic, which is already well developed around the settled larvae of both solitary and colonial ascidians, protects the young animal as soon as it settles. A solitary species or an erect colony will occupy only a relatively small surface compared to an encrusting species. But in all cases the settling larva and then the growing juvenile immediately interact with other sessile organisms; clean, inert surfaces exist only momentarily in natural habitats. Very little is known about how an ascidian larva chooses where to settle. We have already reviewed salient features of this critical time in the animal's life, but it is important to note, as well, that success at this juncture merely sets the stage for further ecological interactions, which we will consider next.

Occupation of the Substrate

SOLITARY SPECIES

A solitary ascidian is not a fragile animal. Predators aside, only a few aspects of the substrate - for example, its extent, its stability, and its consistency - influence the animal's growth. But these influences can be both subtle and significant.

If a solitary ascidian settles on a substrate with a very limited surface - a pebble, say - it will rapidly grow more bulky than its support. The animal can then be rolled, scraped, or tossed about and can no longer feed in an orderly way. Some species manage to overcome these hazards by growing rhizoids that grip nearby surfaces and thereby consolidate the sediment.

But ascidians that settle on mud or other soft substrates can easily be torn away by water currents. And, of course, ascidians that settle on other organisms - for example, on algae, sponges, or

The upright position of
Polycarpa mytiligera has permitted algae
to grow on the ascidian's hard tunic
as if it were a rocky substrate.

Many epibionts live on the hard tunic of *Polycarpa aurita*.

Polycarpa cryptocarpa is another ascidian whose tunic seems as solid as the rock around it.

soft corals - may find themselves carried to sea simply by the death of their host.

The tunic clearly provides solitary ascidians with very effective protection. Even when its tunic is deformed by epibionts, the animal's body is rarely affected. In addition, the tunic is quite resilient; it recovers quickly from small wounds by regenerating its losses or forming scars.

Many solitary ascidians live atop one another in aggregations. Despite some advantages, this habit can spell trouble. Each body's attachment to the others or to the underlying substrate is small in comparison to the bulk of the aggregated bodies. These tightly clumped populations can be torn free by turbulence or simply break loose by their own sheer weight. Such masses are often strewn in the debris that the sea throws upon the shore during storms.

COLONIAL SPECIES

In a colony, individuals begin to multiply immediately after metamorphosis, sometimes even sooner (e. g., *Diplosoma*). Ideally, the colony should be able to grow equally in all directions. Actually, of course, the colony's form is greatly affected by the substrate.

The substrate's features influence encrusting colonies more than others; in fact, pedunculate colonies grow with an independence comparable to that of solitary species. On hard, flat surfaces, encrusting colonies grow into round, flat masses. This shape is especially common among, say, small colonies of didemnids. But colonies with surfaces more than a few centimeters across

This didemnid colony
has an irregular outline.
It follows the shape of the substrate,
but its growth is stopped or slowed
when it meets other,
already settled organisms.

rarely have such regular contours. More often, they break apart into separate masses or at least have very erratic borders. Growth may come to a halt where the colony confronts other sessile animals, including other ascidians; it continues vigorously elsewhere in the same colony, further distorting the colony's shape.

On narrow, linear substrates, such as gorgonians or worm tubes, colonies often spread entirely around the axis of support until the two growing edges meet. One of several outcomes may follow: the two edges can simply stop growing, leaving a distinct fissure; or they may fuse to form a virtually cylindrical colony; or the colony may grow out into the water as a fleshy lobe with the joined basal surfaces of the colony each serving as the substrate for the other. These patterns also appear where ascidian colonies grow on algae or plants - for example, in colonies growing on the little seaweed Halimeda or on the marine seed-plant *Thalassia*.

Competition for Space

Competition to occupy the substrate at all - to lay claim to any part of it - must occur only at settlement and at the beginning of sessile growth. Thereafter, what competition there may be involves exploiting these claims.

Once settled, solitary ascidians stay in place and grow without much competition for space. Some species accept epibionts, which are usually the same organisms that are growing on the substrate nearby. In this situation, the tunic acts simply as a benign extension of neighboring surfaces. In other cases, the tunic re-

A restricted substrate has forced this colony of *Leptoclinides robiginis* to grow out into fleshy lobes. This species very often shelters amphipods that dig small, blister-like pits into the tunic's surface (the largest holes in the colony are, of course, common cloacal apertures).

mains clean, often because its surface exudes mucus or in some other way presents an unattractive consistency to potential epibionts. In some species, the ascidian secretes metabolites that actively repel settlers.

Colonial ascidians have very active periods of growth, when they may overgrow all kinds of other sessile organisms, from algae, sponges, bryozoans, and corals, to barnacles and even molluscs. But the opposite almost never happens: colonial ascidians are almost never massively overgrown by the animals around them.

Ascidians settle only on dead parts of coral, but they may overgrow areas that still contain living polyps, which then starve and die. The rapid spread of a colony's tunic can also starve or suffocate bryozoans, barnacles, and bivalved molluscs, whose valves are blocked and whose orifices are overgrown. *Botryllus* colonies are particularly destructive this way. Some species form lobes that hold onto the substrate only by strands of tunic; as they grow, these colonies virtually stride across the substrate, smothering some organisms in the process and sheltering others.

The chemical interactions between ascidians and other sessile organisms are still poorly understood. We know that some species with acidic surfaces prevent the close approach of other species. On the other hand, where bivalved molluscs are cultivated

This *Didemnum* probably settled first on a dead piece of coral, but now the colony has reached the coral's living branches, which it overgrows. The didemnid itself does not tolerate any epibionts.

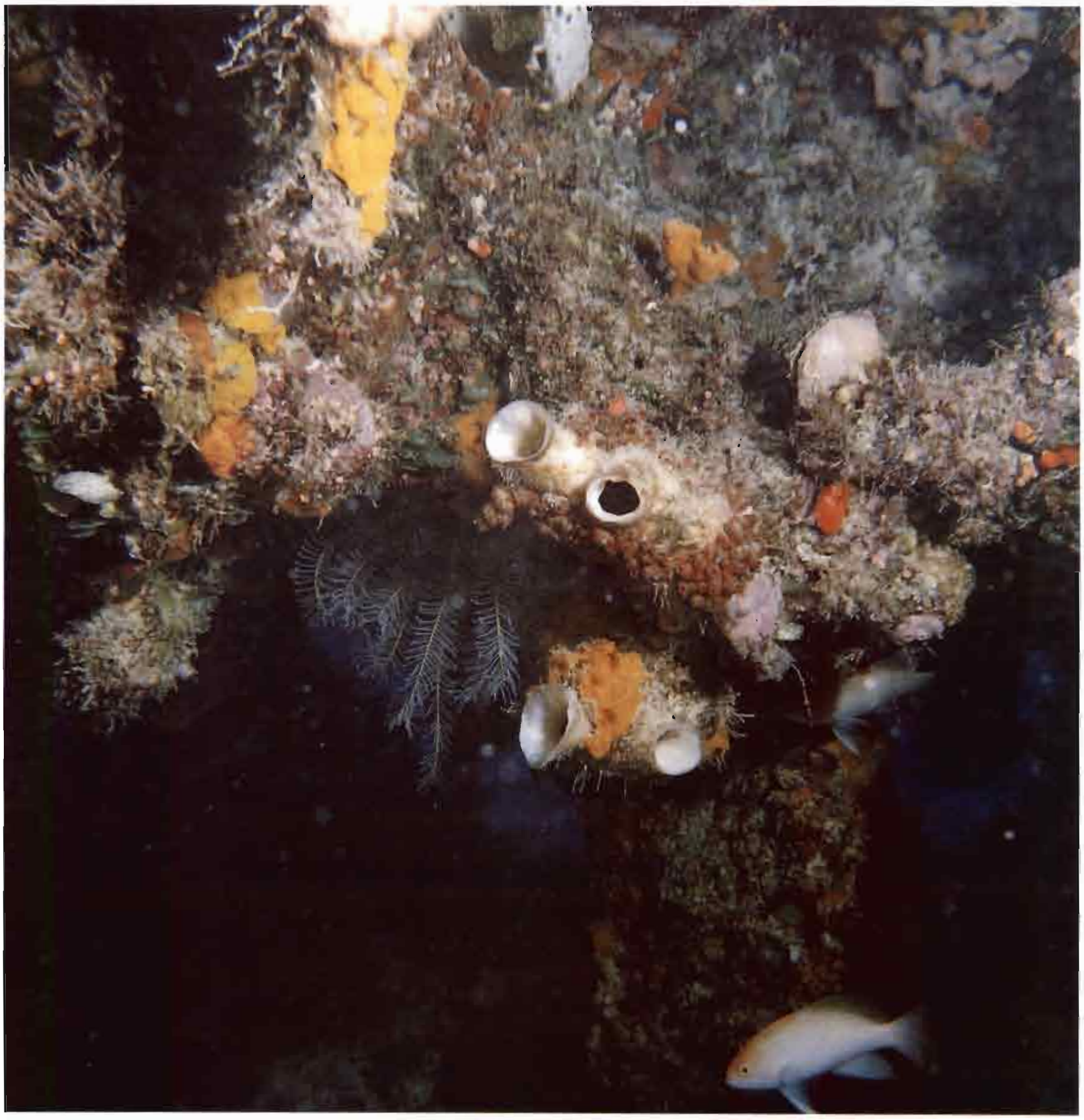
as food, the presence of ascidians seems to promote the settling of molluscan larvae - an intriguing observation that has not yet been followed up by analytic experiments.

EPIBIONTS ON ASCIDIANS

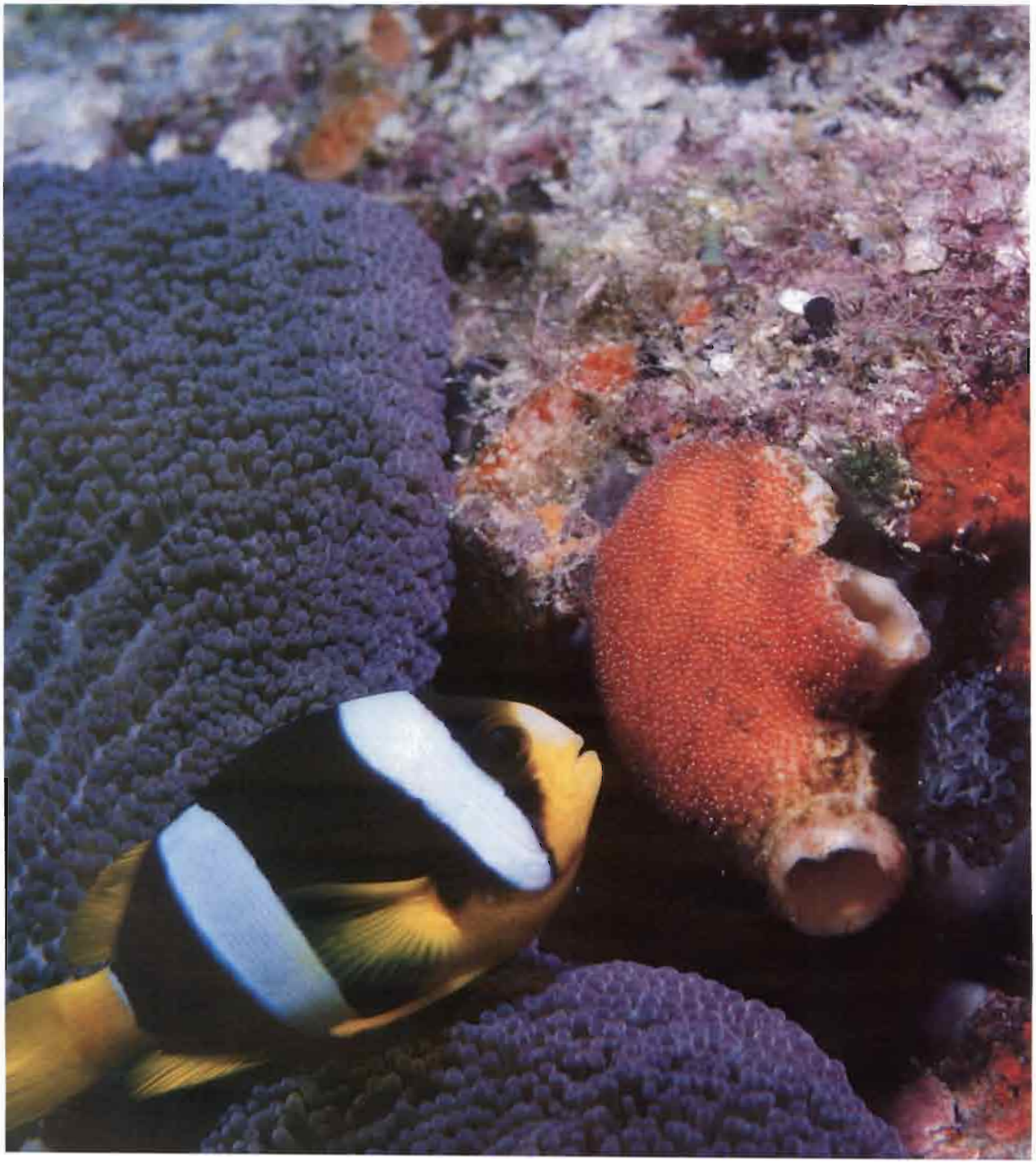
Ascidians with hard, thick tests and long life spans - large solitary species, in particular - support the most luxuriant growth of epibionts. It is no exaggeration to say that virtually every group of marine organisms, from protists to vertebrates, is represented among the epibionts on ascidians; even some fish lay their eggs on tunics. For these fish, the tunic provides a rough surface to which eggs can be solidly attached and which also is raised away from the surrounding substrate, into water that is more likely to be constantly renewed. The ascidian adds its own gentle current to this circulating water. Moreover, the parent fish can keep a close watch on the restricted area where their developing eggs are concentrated. Molluscs, too, often lay their eggs on the tunic, which may then grow around the egg capsules, providing extra protection.

For most other epibionts, however, the ascidian tunic does not seem to offer special advantages apart from its location in circulating water.

Polycarpa cryptocarpa is densely covered with many kinds of epibionts and even with colonial ascidians.



At 20 m depth, the fish *Amphiprion clarki* has placed its red eggs on the ascidian *Polycarpa cryptocarpa* and stays close to them. The ascidian apparently is not disturbed by this fish's persistent activity around it.

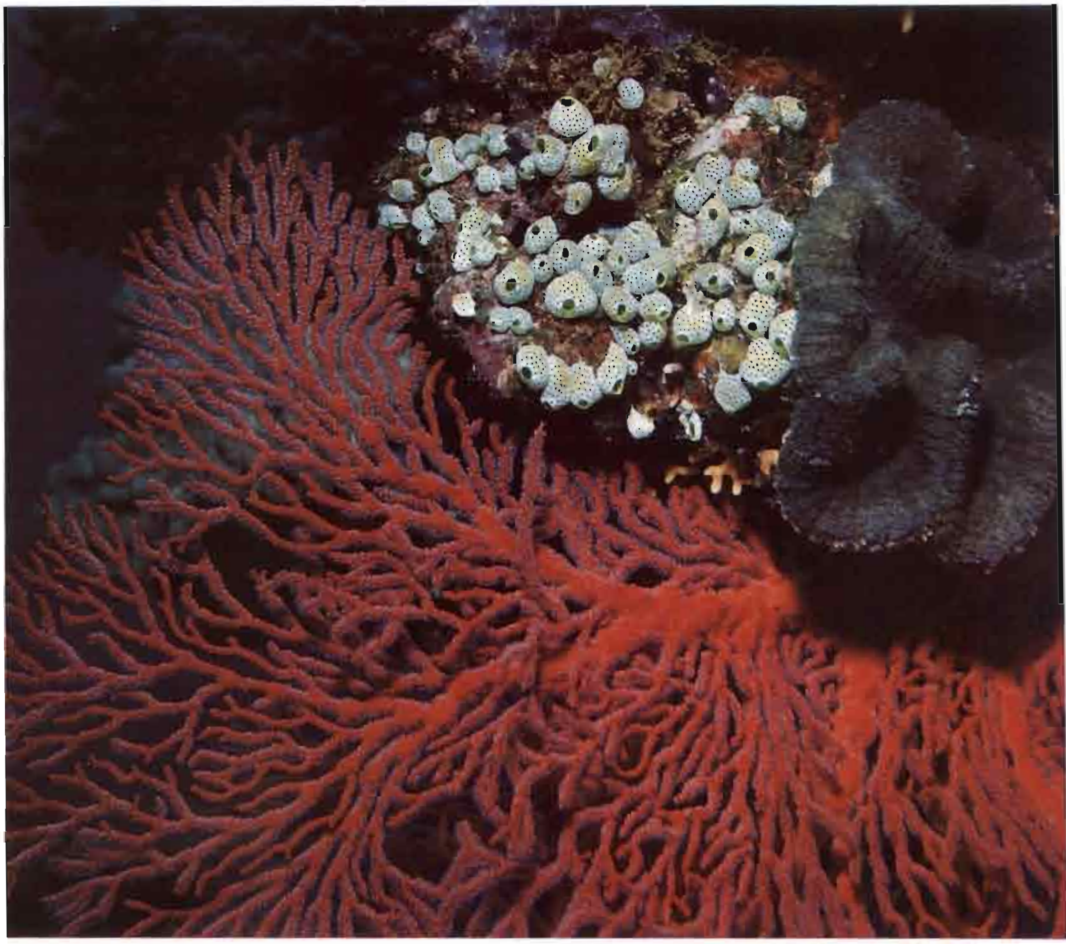


Does a densely covered ascidian suffer or benefit from its epibionts? It is difficult to say. They certainly reduce the tunic's flexibility. At the edges of the siphons, the tunic is always clean, since the epibionts would impede the extension and contraction of these vital structures. On the rest of the tunic, the camouflage afforded by epibionts probably benefits ascidians, in the same way it seems to protect decorator crabs.

As we have seen, what grows on ascidians generally reflects what grows on the surrounding substrate: various algae, sponges, bryozoans, small cnidarians, tubicolous polychaete worms, and tanaid crustaceans, as well as some other solitary and colonial ascidians. These fixed epibionts in turn shelter many tiny mobile animals, including worms, copepods, ostracods, amphipods, isopods and acarians. In fact, the small world of minute creatures that develops on some ascidians inspired the name of one genus, *Microcosmus*.

The colonial *Pseudodistoma fragilis* has settled on a large pyurid ascidian, which provides a living substrate raised away from the bottom. Note that the growth of the colony stops abruptly at the solitary ascidian's siphon, which remains.

Symbionts, parasites, and predators



Symbiotic *Prochloron* algae are unevenly scattered on the smooth surface of this thickly encrusting white *Didemnum*. The resulting color is pale green.

Ascidians shelter mainly symbiotic and parasitic organisms, including protozoans, algae, and animals. Animals living in ascidians reside where water circulates through large cavities - the branchial sac, the peribranchial cavity, and, in some forms, the cloacal channels that permeate the tunic. Or they may invade such organs as the tunic, the digestive tract, and the heart.

Long-term associations of ascidians and other organisms fall into three categories: the epibiotic or commensal ones that we have discussed above, in which no metabolic exchanges take place; symbiotic ones, in which at least one of the partners profits benignly from products of the other; and parasitic ones, in which one organism benefits at the expense of the other.

SYMBIOTIC ALGAE

In coral reef habitats, nearly all the major kinds of invertebrates harbor symbiotic algae. The association is often obligate - as in madreporarian corals, for example - and the symbionts' ecological impact is impressive: symbiotic algae can be responsible for at least 50 % of a reef's primary production. The symbiotic dinoflagellate *Symbiodinium microadriaticum* lives in virtually all groups of tropical invertebrates; in fact, ascidians are unusual in that they do not harbor this unicellular alga. Instead, they are host to two genera of unicellular algae: the procyanophyte *Synechocystis* and the prochlorophyte *Prochloron*.

Zoologists long ago noticed symbiotic associations of some tropical ascidians with unicellular photosynthetic algae, but the algae's precise identity and activity remained unclear. In 1975, biologists working in two different parts of the world - in the Gulf of California (Lewin) and on Australia's Great Barrier Reef (Newcomb and Pugh) - simultaneously reported the unique nature of some of these symbionts. These algae are spherical cells about 30 µm in diameter, whose outer surface is smooth and plain. They multiply by dividing into two equal daughter cells. Since the cells lack nuclei, they can be considered prokaryotes. And yet they contain more than one tylacoid (a primitive chloroplast), without phycobilin but with both chlorophyll a and b.

When they were first described, these symbionts were thought to be a very odd *Synechocystis*. But in 1976, because of their unusual traits, Lewin proposed they be put in an entirely new algal division, the Prochlorophyta, and he placed these symbiotic cells in the new division's single genus, *Prochloron*.

Location of Symbiotic Algae in Ascidians

There appear to be three sorts of alga-ascidian symbiosis, corresponding primarily to the location of the algae in or on their ascidian hosts, and, secondarily, to how restricted taxonomically the symbiosis may be. These three different combinations of

Symbiotic algae invade all the common cloacal cavities of *Lissoclinum bistratum*. They appear bluish when viewed through the colony's layer of spicules. Other algae of a grass-green color are scattered on the outer surface of the colony.

ascidian and alga occur widely; some ascidian colonies may even show all three types of symbiosis at once.

The first *Prochloron* described, *P. didemni* (Lewin, 1975), lives only on the surface of didemnid colonies. These algal cells adhere so loosely to their hosts that they can be scattered by simply rubbing the colony. Usually they cover the colony's surface in a fairly thin, irregular layer. If the host colony is white, *Prochloron*'s presence turns it yellow-green or blue-green; if the host is colored, the green of the alga combines with the colony's pigments to produce still other hues.

Since they were first described from didemnids, surface-dwelling *Prochloron* cells have been found not only on ascidians of other families, but also on other invertebrates as widely divergent as sponges and holothurians. Recently, they have even been found abundantly as free-living cells in the plankton. *Prochloron*'s surface symbiosis appears to be facultative for both the alga and the ascidian.

Prochloron algal cells abound, too, in the common cloacal cavities of many didemnids, including species of the genera *Trididemnum*, *Didemnum*, *Lissoclinum*, *Diplosoma*, *Leptoclinides*, and *Atriolum*. This kind of symbiosis seems to be obligate for the ascidian, whose mucus helps retain the algae, but a little pressure on the colony will force out some of the algae. When algal symbionts live in its common cloacal cavities, an ascidian colony's colors depend primarily on the presence of both spicules and pigments in various parts of the colony. Thus, *Diplosoma similis* and *D. virens*, which do not have spicules, are deep green. Species that have few spicules, or at least few spicules in the tunic's superficial layer, are more often various

The surface of this *Didemnum* colony is raised into small papillae that probably help the colony to retain its coating of greenish symbiotic *Prochloron* algae. Hints of this species' reddish zooids can be seen through the common cloacal openings.

shades of leaf-green. Other colors result when the ascidian hosts have brown pigments, like *Didemnum molle's*, or red ones, like *Atriolum robustum's*.[2]

Symbiotic algae included inside the tissues of the host or in the tunic are either *Prochloron* or *Synechocystis*. Some didemnids show this most intimate of endosymbioses. Here again, the relationship between the symbiotic partners seems to be obligate.

The urn-shaped colony of *Atriolum robustum* has only one common cloacal aperture. The unicellular symbiotic algae *Prochloron* live on its surface.

The Geographic Distribution of Symbiotic Algae

Studies of symbiotic unicellular algae living on or in ascidians are all so recent that no one can yet draw a precise map of their geographic distribution. The lack of sufficient morphological criteria has so far hobbled efforts to establish a detailed taxonomy of symbiotic algae, and so we are not yet able to say whether certain forms are linked to geographic areas, to ascidian species, or even to types of symbiosis. Still, a few generalizations seem justified. Prochloron *algae* - apparently all members of the single species already described - live on the surface of ascidian hosts in all the tropical oceans. By contrast, *Prochloron* living inside the common cloacal cavities or in the tissues or tunics of didemnid colonies, while widely distributed, has not yet been recorded from the Atlantic or the eastern Pacific. Moreover, no one has yet determined whether this cloacal or endosymbiotic *Prochloron* is the same species as that which coats external surfaces. The other algal symbiont of ascidians, *Synechocystis*, has been found in all the tropical oceans and also in the Gulf of Mexico and among the islands of the Caribbean Sea.

All colonies of this *Lissoclinum* have symbiotic algae. This association is obligatory and probably implies metabolic exchanges.

The dark green color of *Diplosoma similis* comes from the abundant unicellular algae living inside the colony's cloacal cavities; the algal pigment is not obscured, because spicules do not occur in this genus.

Bathymetric Distribution

Symbiotic algae are found in didemnids from the surface of the sea down to depths of 60 m, perhaps deeper, but are most abundant in the uppermost few meters. Of course, sunlight is essential for the algae's photosynthesis, but exposure to too strong sunlight is debilitating. In didemnid colonies that live less than 2 m deep in direct sunlight, spicules lying very close to the tunic's surface form a partial sunscreen. In colonies of the same species living in the shade of large algae or coral outcrops, the superficial spicules are less abundant, and so the ascidians appear greener. In New Caledonia, for example, this correlation between habitat and sun-screening spicules in the colony is typical for *Lissoclinum voeltskowi*. In fact, where this didemnid grows on oyster shells, colonies living in the sun on the upper valves are gray, while those in the shade beneath the lower shells are green.

No research has yet been done to determine the depth limit of symbiotic algae living with ascidians. These algae evidently survive in very dim light, since *Didemnum molle* and *Atriolum robustum* with their symbionts have been found by divers at 60 m in New Caledonia. So far, however, no ascidian with symbiotic algae has been found in samples taken by dredge from 90 m in these same waters. Other, non-symbiotic algae, however, live not only at this depth but much deeper, down even to 300 m.

Metabolic Exchanges between Algae and Ascidians

Several attempts to grow ascidians and their symbiotic algae in isolation from each other have been unsuccessful. These results, however, need not mean that metabolic exchanges are essential for the survival of the symbionts. For one thing, it is hard in any case to maintain healthy ascidians in aquaria; it is even harder to rid their tunics and cloacal cavities entirely of all unicellular algae. On the other hand, obtaining a suspension of cloacal cavity algae by gently pressing the ascidians is relatively easy. The problems, then, of culturing these algae in isolation may well indicate that they do need products supplied by an ascidian host, while the results as they pertain to the ascidian hosts themselves remain more problematic.

Experiments comparing the metabolism of ascidians with and without algal symbionts have all been conducted in darkness, on the assumption that darkness inactivates or even kills the algae. The results are hard to assess, since the algae can withstand conditions of very dim light. If other marine alga-invertebrate symbioses are any guide, however, we may speculate that algal photosynthesis provides the host ascidian with oxygen, while the algae get carbon dioxide and nitrogenous compounds from the ascidian's metabolism. The algae, in turn, may also release metabolites of their own that are useful to their hosts.

Research on *Prochloron*-ascidian symbioses is still so new that we cannot yet expect any certainty about such subtle matters as the means of transfer of metabolites from one organism to the other. Although some researchers have proposed that *Prochloron* cells are directly phagocytized by ascidian cells, this remains only an intriguing idea that has not been clearly demonstrated.

The Transfer of Symbionts from One Host to Another

Ascidian tadpoles provide the means by which intratissular symbiotic algae pass from one generation to the next. Symbionts that live inside ascidian tissues or in the tunic lodge in the superficial layer of the tadpole's own tunic. Since didemnid tadpoles remain within the parent colony throughout their development, the transfer of these sorts of symbionts is thus virtually direct from the colony of one generation to that of the next.

Prochloron living outside tissues but in the common cloacal cavities of didemnids are also transmitted by the tadpoles. A tadpole ready to be released develops a tuft of filaments (named the rastrum by P. Kott) at the base of its tail and maintains a cushion of accumulated algal cells there. The tadpole swims very briefly, and the new colony it forms upon settling is immediately invaded by its algae. It is also possible that algal cells may move from one colony to another freely in the plankton; they may be ejected into the water by the muscular contractions of one host ascidian colony and then enter another through its siphons. *Prochloron* cells that live as facultative symbionts on the surface of ascidian tunics almost certainly move from host to host in the plankton.

PARASITES AND ANIMAL SYMBIONTS

Gregarines are the common protozoan parasites in the ascidian gut-tracts. They form cysts between the cells of the digestive epithelium. These parasites then live in the host's intestine, until, at the appropriate stage in their life-cycle, they are expelled into the surrounding water. There, in most cases, they reproduce sexually. Sometimes, however, the cysts burst through the gut wall and into their host's body, and the parasites' subsequent stages invade all the ascidian's tissue. Gregarines seem to do little damage to their ascidian hosts, at least if they are confined to the gut. These parasites are common in New Caledonia, as they are everywhere in the world. Those forms that invade ascidians have not been studied, but the cysts are easily visible in ascidian colonies, appearing as white dots in the zooids' gut-tracts.

Suctorian ciliates frequently attach themselves to the oral tentacles and to the anterior parts of the branchial sac of ascidian hosts. Whether as commensals or as parasites, they feed on the hosts' fluids. These microscopic protozoans have not yet been studied, despite their widespread abundance.

The tunics of aplousobranchs and large stolidobranchs like *Polycarpa* and *Pyura* often harbor bivalved molluscs of the genus *Musculus*. These bivalves vary in size from a few mm to 4 cm. The host's tunic completely envelops the shells of the mollusc, whose siphons project through a slit it maintains in the tunic. To burrow into its host's tunic, the bivalve first fixes itself by byssus threads to the ascidian's surface. Then, by steady repetitions of pressure, it creates a depression in the yielding tunic and gradually sinks into it. Eventually, the tunic thickens around the intruder, as it will around any irritant, inert or living. The result is a cushion with a slit-like pocket holding the bivalve, which, securely nestled, now uses its byssus threads to maintain its hold and to keep the margins of its little burrow from gaping.

Amphipods, which are very common in didemnid, polyclinid, and polycitorid colonies, have the same sort of symbiotic association with the ascidians that molluscs do. They dig into the tunic with their pereiopods (thoracic legs). Once they are well inside the tunic's superficial layer, they hold themselves in place with their third pereiopods, keeping the burrow open with their other four pairs. Only their antennae and mouthparts project, beating to filter the water. While both molluscs and amphipods

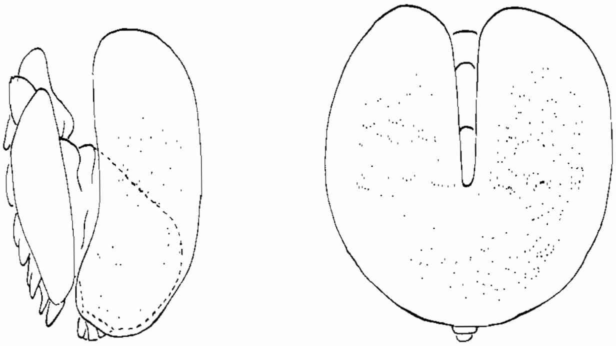
Pontonia sibogae is a well-camouflaged shrimp living in the branchial sac of *Pyura momus*.

gain shelter by lodging in ascidians, they do not, as far as we know, provide any benefit to their hosts in return.

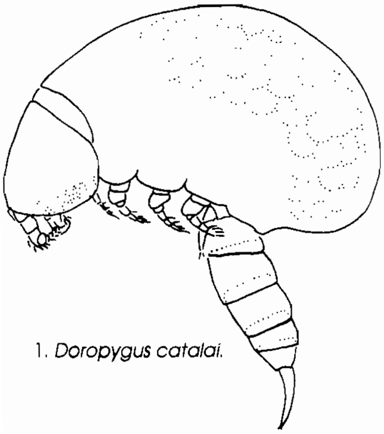
Crustaceans - and, among them, mostly copepods, amphipods, and decapod shrimp - have evolved symbionts and parasites that inhabit ascidians' internal cavities. Amphipods may live in the branchial sacs or cloacal cavities of solitary ascidians and also in the common cloacal cavities of colonial species. This association, while symbiotic, is neither obligate nor specific; these amphipods simply exploit the ascidians' water currents to filter out food particles. Some shrimp of the decapod family Pontoniidae, however, are more closely linked to their ascidian hosts. They often live in pairs, insuring sexual reproduction, and have evolved their colors to match those of their hosts. Thus, shrimp that live inside the common cloacal channels of very transparent colonies - for example, inside some *Diplosoma* colonies - are also transparent, with only their eyes showing color; hence, they are almost invisible. In contrast, certain other shrimp that inhabit pigmented didemnids have marbled patterns resembling those of their hosts.

By far the most abundant and diverse crustacean commensals with ascidians are copepods. About 80 copepod genera and several hundred species belonging to four different families are known only from members that live in ascidians. Extremely abundant in New Caledonia, commensal copepods are found in at least a third of the region's ascidian species, but only a few of these cryptic crustaceans have been studied. Most such copepods live in their host's branchial sac, where they can move about and eat food that has been trapped on the sac's mucous

Didemnids often shelter shrimps in their common cloacal cavities. Here a *Periclimenes* can hardly be seen on its ascidian host, from which it has briefly emerged.



2. *Apodelphys lamellipes,* after Illg, 1970.



1. *Doropygus catalai.*

Parasitic copepods are common and diverse
in the branchial sac or digestive tract
of many kinds of ascidians.
The commonest of these copepods
belong to the family Notodelphyidae.
Their legs are reduced and most of the body
is occupied by a large brood pouch.

sheet. Most of these pharynx-inhabiting copepods live in solitary ascidians, but some tiny forms can be found in didemnid zooids that are less than 1 mm long.

Ascidicolous copepods are all cyclopoid copepods. Some, like *Doropygus catalai*, resemble free-living copepods, for they are only slightly modified anatomically for their life within ascidians; others, such as *Apodelphys lamellipes*, have taken on astonishing and bizarre shapes. In New Caledonia, these two species both live in *Ascidia sydneiensis*. Other species of copepods have adapted to parasitic life in the stomachs or intestines of many species of colonial ascidians. The presence of the parasite distends the walls of the digestive tract and seems to encumber the zooid. But the zooid, no doubt deriving some nutritive support from other zooids in the colony, nonetheless survives and can still mature sexually.

Some highly modified copepods of the family Notodelphyidae live inside the common tunic of certain didemnids. As adults, these parasites are sausage-shaped: their legs are reduced or even absent, and, oddly, they are considerably larger than the zooids of the host colony. Still another example of copepod-ascidian parasitism, found in New Caledonia, involves the copepod *Sphaerothylacus polycarpa*, which forms cysts in the branchial blood vessels of *Polycarpa cryptocarpa*. The host encapsulates the parasite in a mass of cells, some of which migrate into the cyst and are eaten by the copepod dwelling there.

Several other parasites that are often found in ascidians elsewhere in the world, including decapod crabs of the genus *Pilumnus* and some nemertean worms, have not yet been found in New Caledonia.

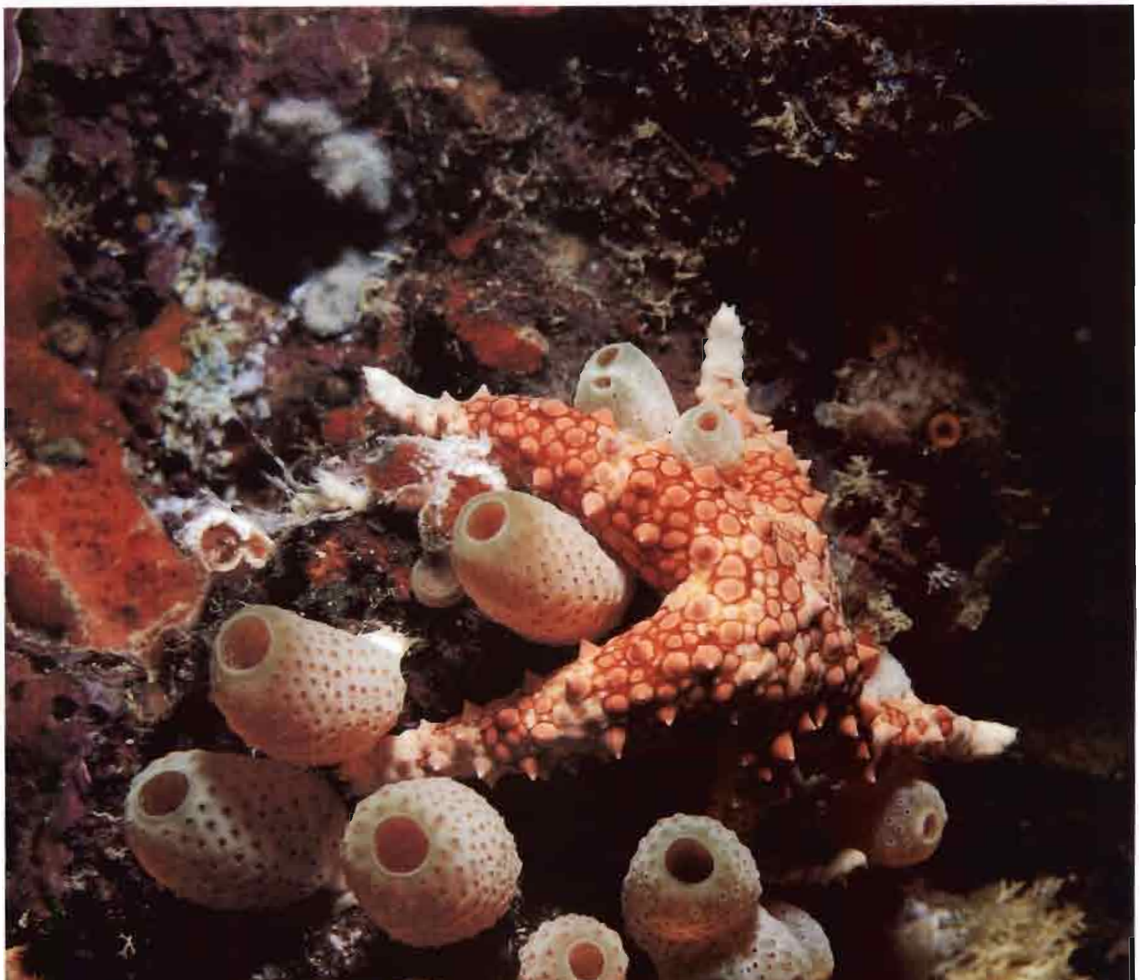
PREDATORS

While it is easy to find many kinds of animals in and on colonial and solitary ascidians, it can be much harder to determine whether any of them are predators. Proof of predation requires finding an identifiable piece of an ascidian in the alleged predator's stomach - not an easy task, especially if the animal feeds by sucking! Such clear evidence has been found among fish that graze on didemnid colonies: pieces of spiculated tunic have been recovered from the fishes' stomachs. Getting comparable material evidence that a snail or crab actually has preyed on ascidians is much harder, although close behavioral observations may occasionally catch them in the act, and a trail of destroyed zooids may then confirm our suspicions.

Predators on ascidians are still only little known in New Caledonia, but they are numerous and varied in other parts of the world. Ascidians have been found even in the stomachs of some mammals: in seals in Scotland (Rae, 1968) and in cetaceans in the Antarctic (Robineau and Duhamel, 1984). Some fish eat ascidians, though never exclusively.

Among invertebrates, some echinoderms, especially sea stars, include ascidians in their diets. In Chile, sea stars (and people) eat the big *Pyura chilensis*. In New Caledonia, the sea star *Gomophia egyptiaca* has been seen eating the didemnid *Atriolum robustum*. I. Goodbody reported from the Caribbean in 1962 that polychaete worms fed on newly metamorphosed ascidians. New Caledonian didemnids fall prey to other invertebrates, too: *Polysyncraton thallomorpha* is taken by a flatworm of the genus

The common sea star *Gomophia egyptiaca* eats colonies of *Atriolum robustum* in the lagoon in New Caledonia.



Planarians such as this black and white *Pseudoceros* often feed on several species of ascidians, here on the didemnid *Polysyncraton thallomorpha.*

Pseudoceros, and *A. robustum* by the snail *Gyrineum gyrinum*. More extensive observations will certainly lengthen the list of predators on ascidians; our knowledge of them at this point is spotty and mostly anecdotal or has been gathered incidentally during studies of other aspects of the taxa or the habitats involved.

The gastropod *Gyrineum gyrinum* tastes the ascidian *Atriolum robustum* and does not seem disturbed by the spicules scattered in the tunic.

Ascidians

and people

ASCIDIANS AS FOOD

New Caledonians do not eat ascidians, perhaps because large pyurids are scarce in the lagoon. In parts of the world where ascidians are treated as seafood, the species eaten are all large - in fact, more than 10 cm long - and are easily collected in large quantities. Chilean "piures" (*Pyura chilensis* and now also *Pyura praeputialis*, the latter introduced from Australia) abound in the intertidal zone along more than 1,000 km of Chile's coastline and may reach lengths of 30 cm. They are sold there commercially, both fresh and canned. In southern Australia, fishermen use *P. praeputialis*, which form immense mats of "cunjevoi" in the low-tide zone of rocky shores, merely as bait. In Europe, "violets" (*Microcosmus*) are eaten raw and are sold in markets all over Mediterranean France, Spain, Italy, and Greece. The Japanese cultivate and cook bright red "oyas" (*Halocynthia roretzi*), and occasionally they eat the stalked *Styela clava*, which is also much appreciated in Korea.

ASCIDIANS AS FOULING ORGANISMS

Along all the world's coasts, "fouling organisms" quickly cover new man-made structures, both those set firmly in place and mobile surfaces like the hulls of ships and buoys. Fouling, of such immense economic importance, has been extensively studied, and a good deal about it is well known. The phenomenon

The styelid *Polyandrocarpa rollandi* abounds in Noumea Harbor. The animals photographed here are growing under the Quai des Scientifiques.

also plays a major role in the biogeography of many species of animals and the ecology of their habitats. Marine fouling organisms include both algae and fixed animals, as well as a whole fauna of tiny motile creatures that live among fixed organisms or as epibionts on them. And, of course, predators complete the trophic picture. Harbor structures provide many good substrates for larvae to settle on and favorable conditions for subsequent growth. But this fouling fauna shows a distinct compositional bias: many species that are abundant in ports are often rare or even absent from nearby shores or lagoons, even though they may enjoy a cosmopolitan geographic distribution.

Throughout the world's ports and harbors, wherever the water is salty enough, ascidians make up a significant group of fouling organisms. For ascidians, these places provide a great variety of permanently submerged structures that offer good surfaces for larval attachment: quays and jetties, floating docks, wharf and pier pilings, buoys, ships' hulls, anchor chains, and more. The substrates themselves also vary: local rocks, other sorts of rock brought in from a distance for construction, concrete, iron, wood, rope, and all kinds of plastics. All of these materials can be colonized, often within a few months. At Noumea, for example, photographs taken in February 1987 show a rich fauna, including ascidians, already well established on the Quai des Scientifiques, even though this site had been built only the year before. To combat fouling, anti-fouling paints - some of them evidently extremely toxic - are generally used on ships' hulls; they do have some ability to repel settlers of many kinds, but they are effective for only a short time.

Harbor waters are nutritionally rich waters. Their abundant organic matter comes from a variety of sources, including sewage

Diplosoma listerianum, forms a gray, jelly-like tunic that can adhere everywhere in harbors. Very soft and fragile, these colonies are often torn apart, but they quickly regenerate. This species endures pollution well. In this photograph *Diplosoma* contrasts sharply with another, much whiter didemnid.

Botryllus niger's name
refers to the black color
this animal takes when it dies.
But living colonies are vividly pigmented.

Didemnum perlucidum is a typical
harbor ascidian in all tropical seas.
Very thin, it may cover extensive surfaces
on all sorts of substrates.

and other wastes from vessels and from nearby towns and local industries. Pollution though it may be, this influx sustains an intense proliferation of bacteria, which constitute food for filter-feeding invertebrates like ascidians. Harbors are, almost by definition, protected places. Even so, within their normally shallow basins, the movements of boats and ships constantly force the water to circulate and mix, both horizontally and vertically. Such water circulation also favors filter-feeders, because it keeps food particles in suspension and oxygenates even highly eutrophic habitats.

For ascidians, which generally prefer to avoid excessive sunlight, harbors provide many shaded substrates: vertical walls, pilings, chains, the undersides of floating docks and buoys, and of course boats' hulls. The turbidity of harbor water accentuates this sheltering from the sun. The semi-enclosed and protected waters of harbors also favor the survival of ascidian larvae and their successful settlement. Even though the ascidian tadpole swims only briefly, in the open sea it can be carried far from an appropriate substrate for attachment. Oviparous species, whose gametes must find each other in the water, also benefit by both the confined dimensions of ports and by the constant mixing of water there.

Once the larvae settle, the abundant food of harbor waters provokes a rapid rate of individual growth and the formation of dense populations. Solitary ascidians reach sexual maturity quickly, while colonial species bud actively - in fact, in tropical waters, they may double their mass in four days. At sexual maturity, ascidians gain still another, reproductive benefit from harbor conditions: as we have noted above, both spawning and the release

Species	Nouméa New Caledonia	Papeete Tahiti	Pointe à Pitre Guadeloupe
Polyclinum constellatum	X	X	X
Didemnum perlucidum	X	X	X
Diplosoma listerianum	X	X	X
Lissoclinum fragile		X	X
Perophora viridis		X	X
Ascidia sydneiensis	X	X	X
Phallusia julinea	X		
Botrylloides nigrum	X	X	X
Symplegma oceania	X	X	
Metandrocarpa manina	X	X	
Polyandrocarpa rollandi	X		
Cnemidocarpa valborg	X	X	X
Polycarpa maculata	X	X	
Styela canopus	X	X	X
Pyura confragosa	X		
Pyura momus	X	X	X
Pyura sacciformis	X	X	
Pyura scortea	X		
Microcosmus exasperatus	X	X	X

Occurrence of ascidians
in three tropical ports.

of tadpoles by individuals of the same species are evidently stimulated by chemical cues secreted into the water. The abundance and density of harbor populations and their confined surroundings most likely enhance this effect of reproductive "signaling".

That the conditions of port habitats are special is evident if we compare the ascidian fauna of ports with that of nearby but more natural habitats like rocky shores or, around Noumea, the lagoon. While we might expect to find many of the same species in both habitats, this is rarely the case; only a few species of ascidians occur both in ports and in nearby natural habitats. And for those few that do, there is no relationship between a species' abundance in the port and its much smaller populations outside; that is, the sheer force of a species' number in port does not lead to its spread to nearby, natural habitats.

THE SPREAD OF ASCIDIANS BY SHIPS

Worldwide shipping has introduced exotic species into many ports. In the port of Noumea, one finds several cosmopolitan species that also live in Papeete, for example, and even in the West Indies (Table). The circumstances that lead to this wide

A buoy in Noumea harbor, shows a rich fauna, partly covered by algae along the vertical sides. Underneath, in the shadow, ascidians largely dominate.

distribution in ports are fairly straightforward. Tolerant species of ascidians arrive in a port on ships' hulls that have not been recently cleaned. There, if conditions permit, they reproduce, forming populations of a few hardy species. From there, the tadpoles of offspring may settle on the hulls of still other ships and so be carried into yet other ports, where sooner or later they again find favorable conditions. Many ascidians tolerate considerable temperature variations, if the changes are not brusque; they do so simply by slowing their already rather slow ways and, in effect, estivating or hibernating. Then, when they regain more favorable conditions, they grow and reproduce anew. For many resistant species, predators and pollution seem to pose only slight threats; over generations, these ascidians can travel from port to port around the world. The result is a history of continual re-infusions of these species into the populations of the ports where they already occur, and of repeated forays into new places.

By contrast, these same species, once established in a new harbor, may find it virtually impossible to spread out into neighboring natural habitats. Many factors - currents, reduced availability of food, competition for substrates - may come into play. The result is that exotic fouling organisms, widespread globally and hardy though they may be, typically are confined to disrupted habitats like ports, which are fairly uniform around the world, while native species, whose adaptive advantages have evolved slowly amidst unique mixes of local selective pressures, continue to prevail in more natural marine environs.

TOLERANCE OF POLLUTION AND PURIFICATION OF WATER

The very resistance of ascidians to many pollutants explains why they make up such an important part of the fouling fauna of ports. The consequence of their activities, however, is a considerable cleansing of these waters by these animals.

Ascidians purify water by two means, one purely mechanical and the other biological. Mechanically, they capture particles suspended in the water, embed them in a mucous strand, pass them through the gut, and finally return them to the habitat as dense fecal pellets that fall to the bottom to be recycled or to become part of the sediment. An ascidian a few cm long may filter 2 to 3 liters of water per hour. The ascidian biomass of Noumea's harbor amounts to tons; it follows that all the water in the port may well pass through ascidians several times a year!

Biological purification of water involves the digestion and elimination of those organic particles, especially bacteria, that have been mechanically trapped. Ascidians are also able to absorb dissolved organic materials from the water and can take up, as well, various metals (most famously vanadium) and some toxic substances - heavy metals and hydrocarbons - that are stored in specialized blood cells. Of course, one might well wonder about eating animals that show ascidians' remarkable water-cleansing, toxin-concentrating activities. And our next comments, about ascidians as sources of powerful, medicinal cytotoxins, can scarcely reassure the gourmet.

NATURAL PRODUCTS FROM ASCIDIANS

Over the past two decades, a systematic screening of all kinds of marine organisms has been undertaken to discover natural compounds that might have pharmacological uses. Among marine invertebrates, sponges and ascidians have turned out to be especially interesting as sources for such natural products. Research in several countries has shown that compounds isolated from ascidians are highly cytotoxic and hence have a potential medicinal value if their effects can be directed at target phenomena (such as tumors) and their side-effects controlled. Interest in ascidians has been particularly keen because of a major American campaign which has sought to assay all the major groups of marine organisms in the Caribbean. A breakthrough in this effort occurred in 1981, when Rinehart and his colleagues discovered a group of compounds called cyclodepsipeptides - then called "didemnines" - which they extracted from the didemnid species *Trididemnum solidum*. These compounds are effective anti-cancer agents and also have anti-viral properties, but they are also extremely toxic - obviously a serious drawback but not necessarily an insurmountable one if synthetic didemnines can be modified to narrow their scope of action. Since this early discovery, ascidians have been collected intensively, especially in the tropics, in the search for still other biologically active compounds.

Many unusual compounds, most of them nitrogenous, have now been isolated from ascidians. The presence in New Caledonia of a major research laboratory and a team of professional divers has let us add significantly to the list of marine natural products, and chemical laboratories in France have pursued analyses of these New Caledonian finds. For many other marine invertebrates than ascidians, the local, traditional knowledge of animals with toxic or medical properties can often guide the selection of animals for further analysis; ascidians, however, have been so largely ignored by folk medicine that the search must proceed without such help.

The first cytotoxic products to be identified from ascidians were non-nitrogenous metabolites. In 1979 Howard and Clarkson isolated prenylhydroquinones from *Aplidium californicum*, a polyclinid species that lives in the eastern and western Pacific. These compounds showed interesting activity *in vivo* against mouse leukemia and also against the bacterium *Salmonella typhimurinum*. Pentenones, which are also active against mouse leukemia, have been isolated from Fijian samples of the didemnid

This colonial polyclinid, *Aplidium californicum*, makes large, soft, rather transparent cushions. It is distributed along both western and eastern Pacific coasts. It contains toxic quinones.

C. Monniot, F. Monniot
P. Laboute

Lissoclinum voeltzkowi may be very abundant at low tide on algae such as *Cymodocea* or *Halimeda*, and on shells. The greenish brown color is the result of a mixture of brown ascidian pigment cells and green symbiotic algal cells. This species shows promising pharmacological properties.

Lissoclinum voeltzkowi. Other anti-cancer agents from this didemnid, which also grows abundantly on the reef flats of New Caledonia, are under study now in France.

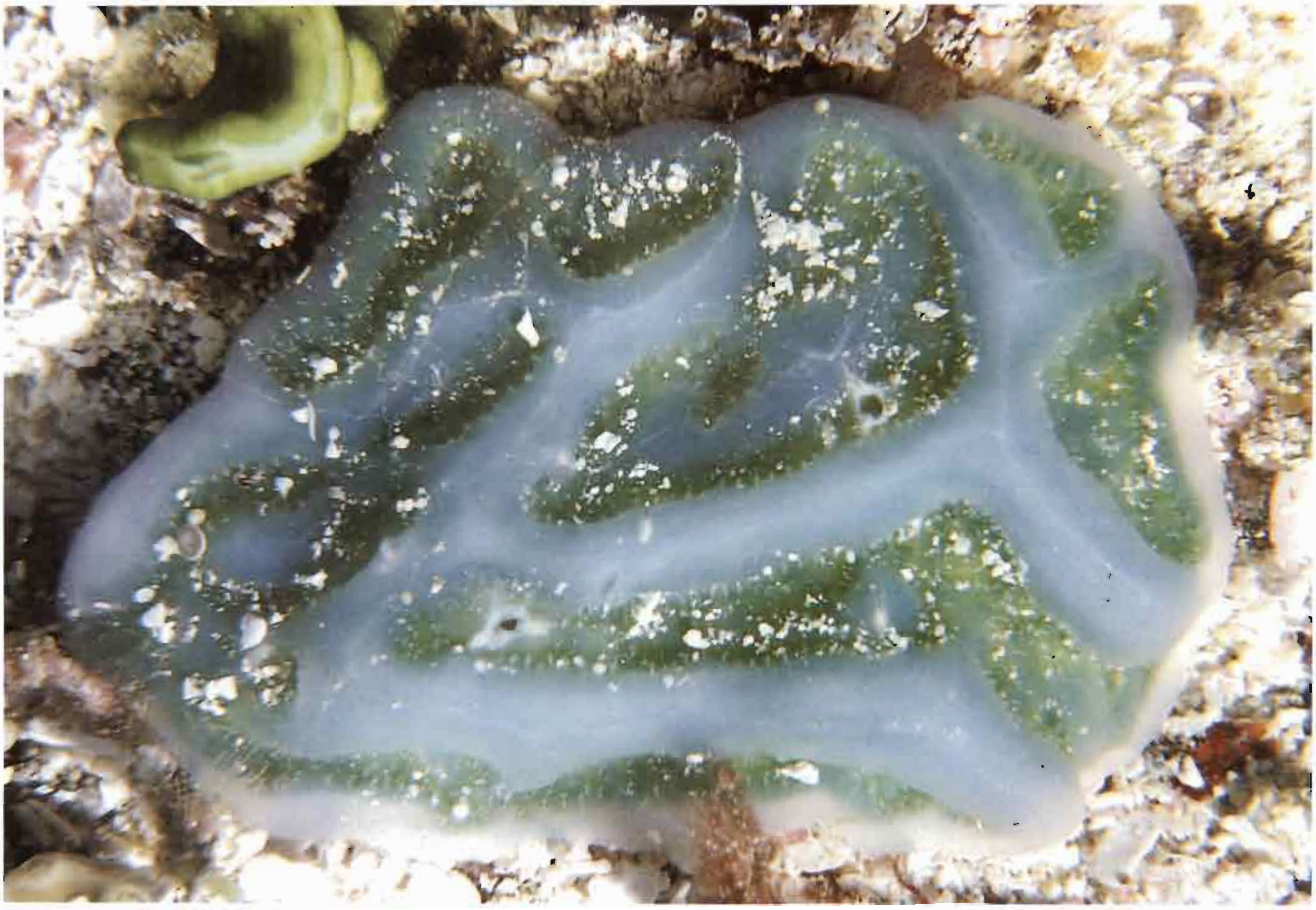
Most of the active marine natural products recorded so far are derivatives of amino acids: alkaloids and peptides. Among the alkaloids, some anti-cancer *alpha*-carbolines, including one named "dendrodoine", have been isolated from a species of the styelid genus *Dendrodoa* that grows in the decidedly non-tropical English Channel; these are now under study at the Museum of Natural History in Paris. And some *beta*-carbolines called "eudistomines" have been isolated from the Caribbean polycitorid *Eudistoma olivaceum*. A closely allied compound described under the name "woodinine" has been isolated from *Eudistoma fragum*, an abundant species in the lagoon around southern New Caledonia. The eudistomines show a weak anti-bacterial action but significant activity against the *Herpes simplex* virus. Woodinine, however, appears to be more strictly anti-bacterial.

Ascidian peptides seem to have very promising prospects. The first ones were found in 1980 in the didemnid *Lissoclinum patella* that was collected in Palau. Since then, twelve products, all cyclic and none with terrestrial equivalents, have been found in this widespread species, which also lives in New Caledonia. These products are of four sorts: ulithiacyclamide, patellamides, ascidiacyclamides, and lissoclamides. The first of these, ulithiacyclamide, is a powerful anti-cancer agent *in vitro*; it is active *in vivo* against mouse leukemia.

Bistramines, isolated from *Lissoclinum bistratum* that was collected in New Caledonia, are turning out to be especially interesting. These compounds are polyethers with two carboxamide func-

As big as fists, *Eudistoma fragum* colonies live at about 30 m on substrates subject to strong water currents.

The symbiotic algae of *Lissoclinum patella* are located in the colony's common cloacal chambers. The colony surface is raised into very characteristic transparent folds. The pharmacological properties of this species are being studied extensively.



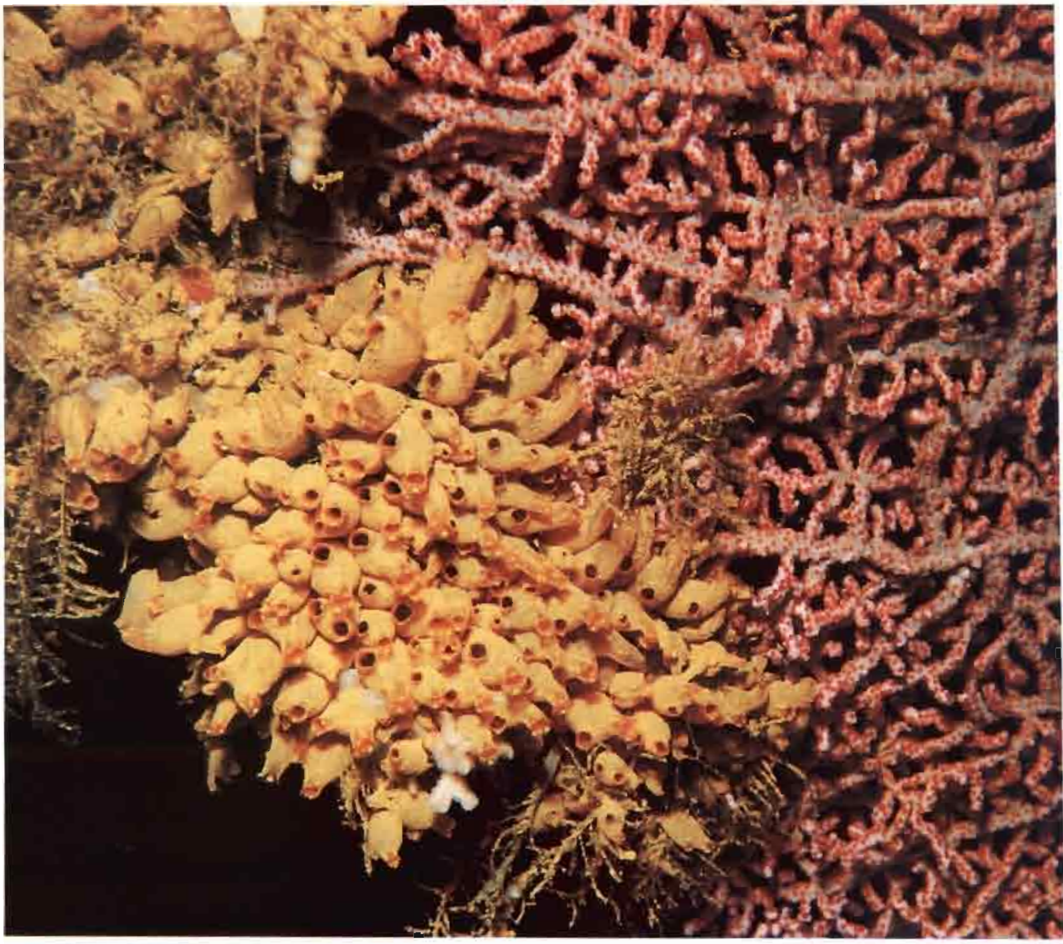
Bistramides are extracted from *Lissoclinum bistratum*, a didemnid with symbiotic algae.

tions. At the Faculté de Pharmacie in Nantes, where these products have been studied, a lyophilized powder of *L. bistratum* seriously poisoned two investigators, causing paralysis of the mouth and heaviness in the legs. Extracts tested in mice brought on paralysis, respiratory failure, and death. These extracts also proved extremely toxic to brine shrimp (*Artemia salina*) and to the fish *Gambusia affinis*. The active agent appears to act on the nervous system, and its effects are clearly much stronger and faster than those of many other marine products. The toxin's structure is now being determined.

L. bistratum contains abundant symbiotic *Prochloron* algae, and it is quite possible that these *Prochloron* cells, not the ascidian itself, are the source of the active agent in toxins extracted from the entire ascidian colony, algae and all. In fact, a good deal of current pharmacological interest focusses on didemnids that contain symbiotic algae, suggesting a relationship between natural products and symbiosis. As the distribution of ascidian alkaloids shows, however, it is important to realize that species in all ascidian families may contain metabolites with toxic properties or with pharmacological promise. So far, the tropics have been the primary area of the search for these products, for that is where we find the greatest species diversity and can collect large numbers of individuals. But since our knowledge of ascidians is still so fragmentary, we can hope to discover many species with new products elsewhere, as well, and with uses in aquaculture as well as in medicine.

For the ascidians themselves, these toxic compounds may play a role in warding off predators, and perhaps they also repel fouling organisms. These products are likely to be consequential in the lives of their producers, though what we know of their action in nature remains very speculative.

Systematics



So far in this book, we have surveyed the diversity of ascidian forms and colors, colonial patterns, life histories, ecological relationships, and other features that apply across the board. Now we return to taxonomic diversity - the diversity of orders, families, genera, and species of ascidians. For the field naturalist, one practical problem arises right away: the external appearance of an ascidian reveals virtually nothing about those internal traits that indicate its family or other taxonomic affiliations. Identifying a puzzling ascidian even to the broad level of its family usually depends at least on removing its tunic. How often have specialists made this first step and discovered that they actually had collected not an ascidian at all but rather a soft coral or even a sponge! But fortunately, many well known ascidians do have consistent and striking traits that identify them in the field. We will concentrate our account on these more distinctive forms.

Systematics involves much more than arranging identified specimens into designated groups according to some "official rules". True, identifying kinds of organisms is a key part of the whole job of systematics. But the science goes well beyond this, for it seeks to understand organisms' relationships at different levels - relationships among families, say, or genera.

All classifications reflect practicalities, and so different groups of things, including organisms, are bound to be classified in different, even non-equivalent ways, according to what traits are available at all to assess and compare. This part of systematics is rather like organizing the inventory of a supermarket: different departments must be arranged by different criteria. Detergents may be sorted out according to whether they are liquid or powder, or for dishes or laundry, or for hand-wash or machines. None of these traits - but, instead, many others - let us sort out the hardware department's nails, screws, and bolts. Aisle by aisle, the diversity of the supermarket falls into place according to classificatory decisions adjusted to the objects themselves. Systematics proceeds in much the same way to organize biodiversity, using some traits in this group, others in that, according to what is available and "works". But here enters a crucial difference: in systematics, what works practically to arrange taxa conveniently is only a preliminary kind of success. More importantly, classifications of living things should reflect theories about how that diversity itself came to be; they organize taxa in ways that express our ideas about their various levels of natural, evolutionary affiliation.

At the start of the 19th century, all the known species of solitary ascidians were classified into only four genera, on the basis of their tunics' consistency and transparency and their posture - whether erect or prostrate - on the substrate. Colonial ascidians that lacked spicules were then considered "soft corals", and those with spicules were grouped with "stony corals"! Clearly, too few traits were being used to interpret adequately the memberships of major groups, and the theoretical basis of interpreting their affinities was equally poor. Nowadays, biological classifications try to express ideas about the evolutionary relationships of taxa. All available information is drawn together toward this goal: fossil forms, adult anatomy, developmental traits from egg to larva and beyond, chemical composition, responses to environmental factors, and much more. As a result, biological classifications are not fixed, static statements but rather are flexible, interpretive systems that are revised as we learn more about a group's entire range of diversity.

Recent work with ascidians provides a good example of how modern systematics works. Deep-sea explorations have brought up hitherto unknown, highly unusual ascidians whose traits do not coincide with those of currently recognized genera or families. These finds have not been shoehorned into rigidly characterized groups. Rather, they have forced revisions of the criteria by which we define these very genera and families and recognize their members. Thus, these new finds have been seized as opportunities, from new evidence, to reconsider various traits as clues to how ascidian taxa have evolved and to rethink our ideas about how existing families and their genera are related.

Another example - indeed, a test - of the flexibility of modern systematics is its use of contributions from molecular biology. Nucleic acid sequences seem to differ more between two taxa the longer ago those taxa diverged in evolution. If the "molecular difference" between two taxa does measure their evolutionary proximity, then we should be able to tell if two taxa that appear similar really are closely related or, instead, stand well apart in evolution, one persistently primitive and the other derivative but with enough regressive traits to give it a confusing similarity to the more persistently conservative taxon. Our current classifications will have to be reexamined at every level, and many of them revised, as we learn how to assess the evolutionary implications of molecular attributes, which appear to provide valuable tests of interpretations based on morphological data.

Ascidian systematics is the domain of specialists, but a keen observer can recognize plenty of well-described species if some documentary guidance is available. Underwater photographs are especially useful aids for identifying many distinctive ascidians. Photographs can depict traits that are hard to describe, and show details that may be of minor biological importance but nonetheless distinguish one species from another. In brief, a pictorial approach can help us stress fieldmarks that identify New Caledonia's commoner and more striking ascidians. In this section of our book, we first introduce the three great orders of ascidians and each family's diagnostic features. Then, as we consider each family in turn, we will point out how to recognize its more prominent shallow-water species and some deep-sea forms.

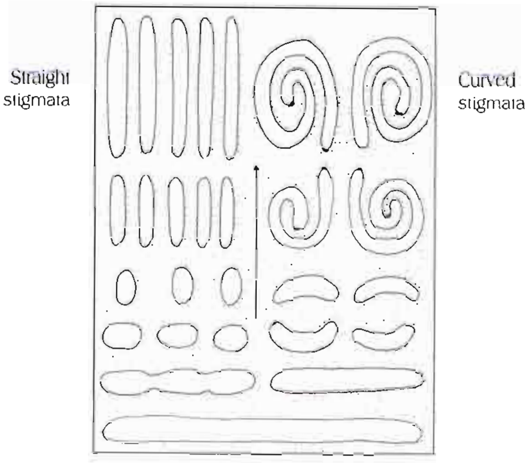
CLASSIFICATION OF THE CLASS ASCIDIACEA

Ascidians constitute a class - the Ascidiacea - of the chordate sub-phylum Urochordata. All tunicates are urochordates, but many tunicates fall into other classes than the Ascidiacea. The tunicates comprise four classes. Two classes are planktonic: the Thaliacea (salps, doliolids, pyrosomes) and the Appendicularia (or Larvacea). Two classes are benthic: the exclusively abyssal Sorberacea, whose pharynx is not developed into an elaborate branchial sac; and the Ascidiacea, whose diverse biology we have discussed so far and whose New Caledonian representatives we will survey in the accounts of families in this section of our book.

The Orders

A century ago, Lahille proposed that the structure of the adult's branchial sac be used to divide the class Ascidiacea into three orders: Aplousobranchiata, Phlebobranchiata, and Stolidobranchiata. We still use these ascidian orders today, for they have withstood the tests of all our more recent taxonomic findings. All the families of benthic tunicates that have been proposed since Lahille's day fit comfortably into one or another of his orders. Of course, there are alternatives. For example, one can divide ascidians into just two groups, based on the relative locations of their gonads and gut-loop - a somewhat looser but arguable approach. In the Enterogona, the gonads lie within or below the gut-loop; in the Pleurogona, the gonads lie in the internal wall of the thoracic mantle with little clear relationship to the gut-loop. The aplousobranchs and the phlebobranchs are enterogonid orders; the stolidobranchs are pleurogonid ascidians. These two classifications are not contradictory; they are based on the structure of different organs: branchial sac, digestive tract, and gonads - which evolved independently. Either system arrives at essentially the same results, confirming the validity of the three orders.

In its development from larval metamorphosis to adult, the branchial sac changes through stages of complexity that characterize the ascidian orders and, within them, the families. While it is not baldly true that "ontogeny recapitulates phylogeny", everyone agrees that development provides important evidence by which to interpret the evolutionary relationships of taxa. In principle, if simple traits generally precede their complex counterparts in evolution, we can take the simplest branchial anatomy to be primitive.



Division of a protostigma into straight stigmata, on the left, and curved stigmata, on the right.

Phlebobranch branchial sac (*Ecteinascidia*).

Of course, even this can be a risky interpretation. Evolution is not linear, and numerous examples of evolutionary regression exist. The regressed forms are often small-sized species, and they occur in all orders and families. Nevertheless, the branchial sac's structure is one of the major guides in the interpretative effort and the taxonomy that expresses it.

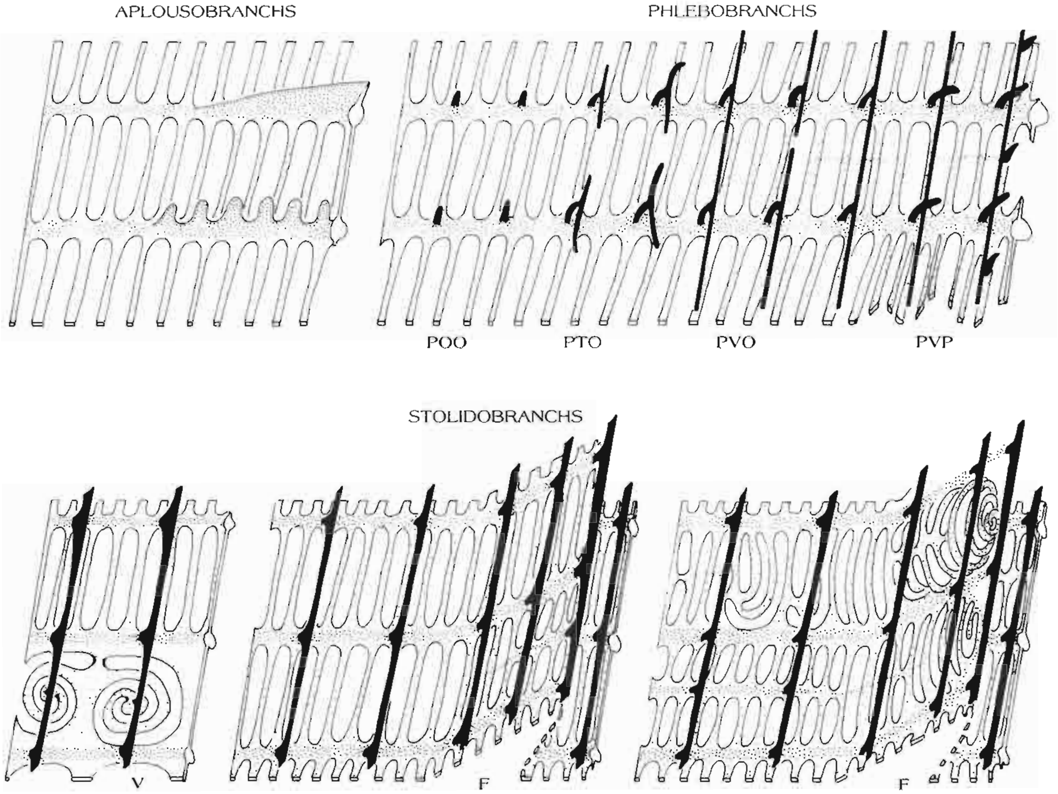
ORDER APLOUSOBRANCHIATA
(*APLOUSO* = SIMPLE IN GREEK)

The simplest branchial condition, very rare in adults, is one in which the pharyngeal wall has only "protostigmata", large, ciliated perforations elongated transversely (that is, dorso-ventrally). Usually, each protostigma breaks up in development to form a transverse row of longitudinally oriented stigmata that assume simple shapes and various sizes as they grow. The internal pharyngeal wall remains flat except between successive rows of stigmata, where the branchial epithelium produces an even-edged or scalloped membranous flap (figure). These flaps are called transverse vessels, since they contain blood channels. This simple branchial arrangement - transverse rows of longitudinally oriented stigmata separated by transverse vessels - typifies the aplousobranchs. The order comprises three large families: Polyclinidae, Polycitoridae, and Didemnidae.

ORDER PHLEBOBRANCHIATA
(*PHLEBO* = VESSEL IN GREEK)

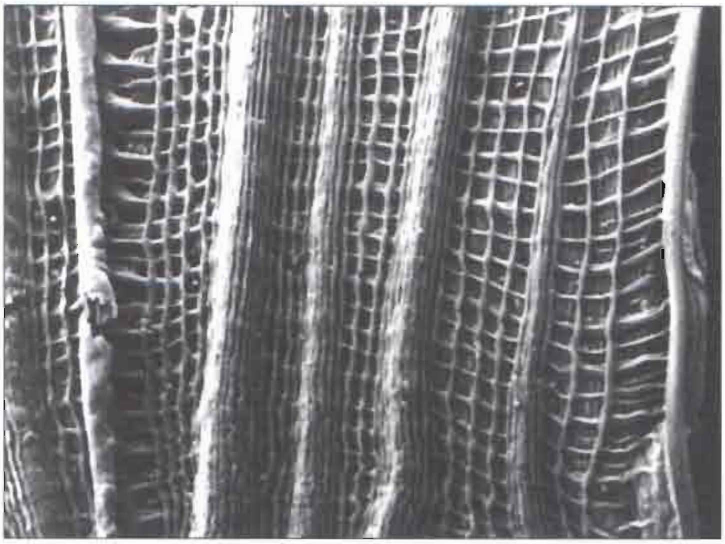
The stigmata may be small and straight or take complex spiral shapes. Independently of this variation, protuberances appear on

Aplousobranch branchial sac (*Clavelina*). The structure to the right is a dorsal languet. Scale bar = 100 µm.



Stages in the formation of bars, papillae, and folds in the branchial wall.

Stolidobranch branchial sac (*Styela*). The endostyle is to the right, and the dorsal lamina is to the left.



the pharyngeal wall's transverse vessels during the branchial sac's development. At first, finger-like papillae project from these vessels (figure). The tip of these papillae may then branch into T's or crosses. The papillae may remain separate from one another (figure), or they may join their arm-tips from stigmatal row to row to form a set of longitudinally oriented bridges, which, since they carry blood channels, are called internal longitudinal vessels. Distinct papillae and internal longitudinal vessels characterize the phlebobranch branchial sac.

Depending on the family or the genus, the stigmata take diverse shapes, from round holes to elongated slits aligned side by side or curved into spiral complexes. Stigmata of any shape may be entirely open or interrupted by thin "parastigmatic" vessels that both brace the openings and elaborate even further the pharyngeal wall's circulatory system. In most phlebobranchs the branchial sac is extensively perforated by stigmata, but in some forms stigmata may be scanty or completely absent from large areas of the pharyngeal wall. Seven families make up the order: Cionidae, Perophoridae, Ascidiidae, Corellidae, Agnesiidae, Plurellidae, and Octacnemidae.

ORDER STOLIDOBRANCHIATA
(*STOLIDO* = FOLDED, PLEATED IN GREEK)

The internal longitudinal vessels are numerous; where they particularly crowd one another, the branchial wall is thrown into internal longitudinal folds (figure). This elaboration, too, of the branchial sac's wall is independent of variations in the stigmata's shapes. Folds occur both in solitary and colonial stolidobranchs. The absence of folds in some species is probably a secondary loss.

Thus some small colonial species of styelids have folds, and some of the largest molgulids lack them. This order includes many prominent species but comprises only three families: Styelidae, Pyuridae, and Molgulidae.

Techniques for Anatomical Observations

Anatomical study of ascidians requires that the specimens be fixed, preferably in formalin. The simplest way to proceed is to place the living animals in sea water, then to anaesthetize them by adding some crystals of menthol (or some drops, if the menthol is dissolved). This anaesthetization takes about two hours; it prevents troublesome contractions of the specimens when they are fixed. To fix these animals, place them in a jar with enough sea water to barely cover them, and add full-strength, commercial, buffered formalin. The quantity of formalin to add should be 1/10 the volume of the jar's entire ascidian-and-water contents that you are about to fix. Thus, the fixative will be "10 % formalin". The specimens must then harden in this fixative for at least 48 hours before they are dissected.

To dissect a solitary ascidian or well-isolated zooids of a colonial species, remove the tunic by cutting it from one siphon to the other along the mid-ventral line - in other words, along the longest possible distance between the siphons. Be careful not to pierce the mantle. Gently extract the animal from its tunic. Now cut the mantle and the branchial sac along the mid-ventral line, from the oral aperture to the cloacal siphon. The endostyle, which marks this line, can usually be seen, even if indistinctly, at least in silhouette. Since the endostyle is where the branchial sac is most extensively attached to the mantle, this cut will open that sac along with the mantle. All of the body's organs are now accessible for study, although they may be hidden behind the branchial sac. Pin the specimen open in a shallow dissecting dish (for example, in a Petri dish with some paraffin coating its bottom), covered by water.

To enhance the visibility of the body's tissues, add a small amount of "hemalum" stain. This is a mixture of 0.2 grams of hematein in 100 ml of potassium alum, which has been boiled for 3 minutes, then filtered. After only a few minutes of staining, rinse the dissection in tap water. Examine the branchial sac for various diagnostic traits, and then remove it by very carefully cutting the trabeculae that hold the sac to the mantle wall of the peribranchial space. The operation reveals the gut-tract and the gonads and other structures in the mantle.

To examine colonial ascidians whose zooids are embedded in a common tunic, cut off a piece of the colony and carefully extract some zooids. By very gently tearing the tunic and successively slicing sections of the colony, zooids can be located and removed for study. The isolated zooids can then be stained with hemalum. This stain usually renders their major organs visible. Reflected light will show some structures best, while transmitted illumination will reveal others, when specimens are examined closely. Sometimes one must open the branchial sac to count the rows of stigmata or to examine transverse vessels - a very delicate operation. For accurate identification, several zooids must be examined, to take account of their variability.

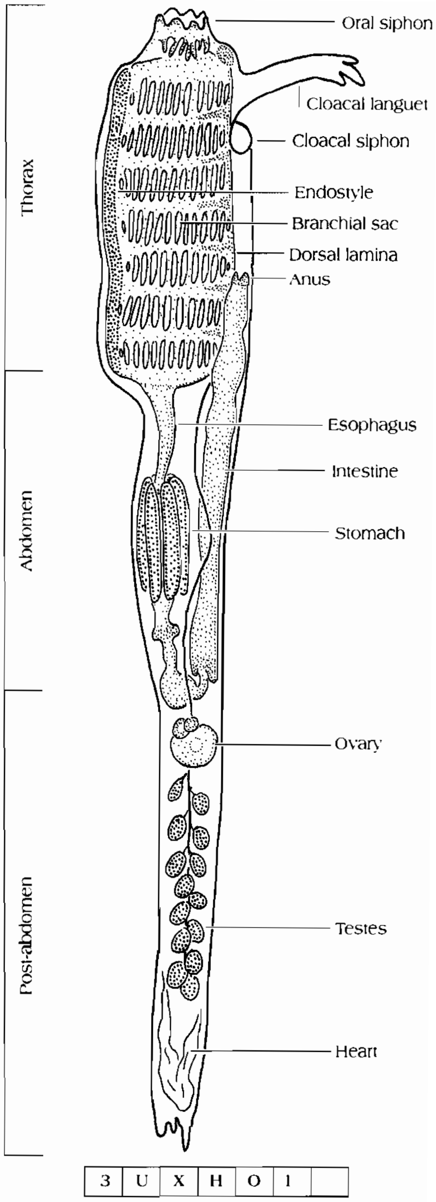
Dissections and parts of dissected specimens can be preserved either in 10 % formalin, or, if small, they can be dehydrated and mounted in resin on slides.

Keys to the Families and Genera of Ascidians

All the families and a remarkably large proportion of the genera of the world's ascidians occur in New Caledonia, living at all depths, from shallow lagoon and reef habitats to the deep bottom at the base of the great geological platform that supports the islands. The survey of New Caledonian invertebrates on which this book and its series is based began in the southern part of Grande Terre and is far from complete, but it already reveals not only the region's vast faunal diversity but also the presence of some highly unusual forms. This richness and singularity applies to ascidians as much as it does to other major taxa, and many more species doubtless will be found that are as yet unrecorded from the region or wholly new to science. This sense of inventorial incompleteness persuades us to provide keys that take account of all the world's known genera, not just ones recorded locally so far.

Tabular keys such as we use in this book have advantages over the more familiar dichotomous keys. They bring together simultaneously a large set of characters, and so they do not force the user to sort through traits just pair by pair in a particular order. With tabular keys, even when some data are lacking (because a specimen is damaged or there are other observational obs-

Schematic organization
of a polyclinid zooid.

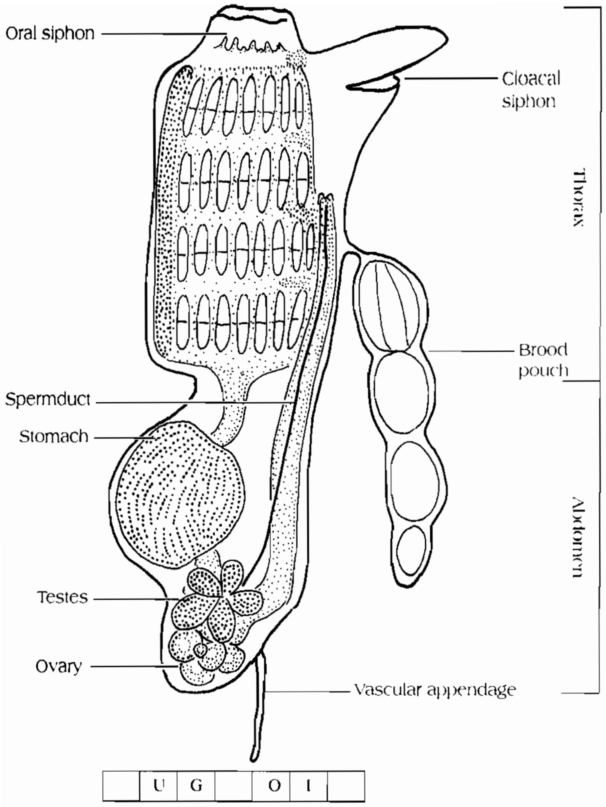


tacles), progress toward a satisfactory identification may still be possible by turning to other clues among all those presented at once in the tables.

A multiplicity of traits helps confirm identifications, just as it provides a rich context in which to place and assess new and surprising finds. But our goal in this book is not a formal biosystematic one; a growing body of technical papers addresses that aim. Rather, by our keys as well as our general text and pictures, we wish simply to indicate the diversity of the world's ascidians and of New Caledonia's share of them. With this in mind we stress the fewest and simplest characters essential fir identifying the more easily recognizable ascidians known to occur in the region.

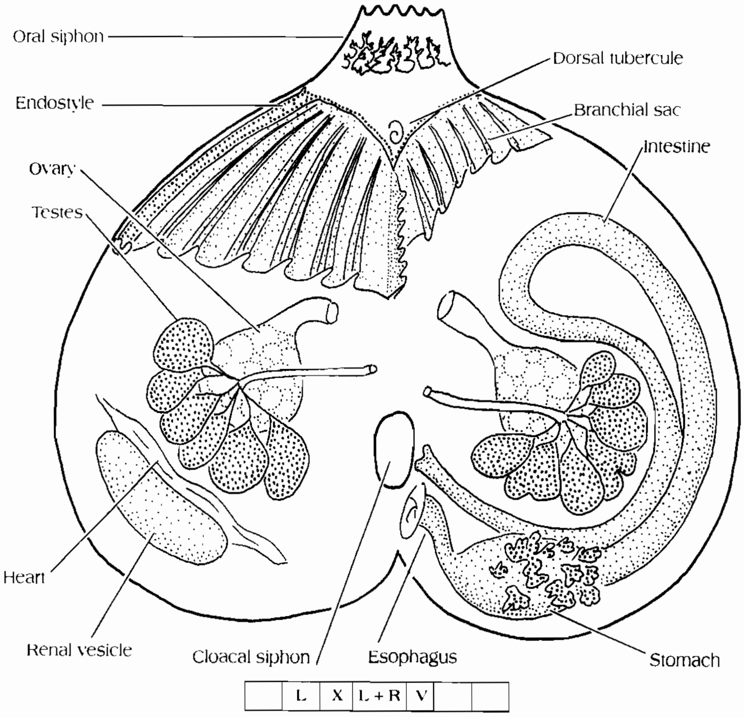
In each tabular key, traits are aligned from left to right in an order that ought to help the user eliminate combinations of traits that do not correspond with the specimen at hand. This process of elimination quickly limits possible alternative identifications. An effective complementary procedure is to use the organizational format of the key's columns - the traits they use and choices they permit - to devise the "key formula" of the specimen at hand. One can compare this summary with the tabular keys. By combining these methods so that they reinforce each other, one can decide at least the family to which a specimen belongs. Our accounts and keys for the families themselves then provide the means to make a more precise identification to genus.

In our remarks about each family, we concern ourselves with those genera that occur in reef and lagoon habitats around New Caledonia and mention abyssal forms mostly in passing. This is, after all, a book for divers and field naturalists, for whom the accessible sea-bottom is relatively shallow. Many shallow-water



Schematic organization of a polycitorid zooid in a genus having an incubatory pouch.

Schematic organization of a stolidobranch ascidian, opened along the ventral line's endostyle. The branchial sac is partly removed to show structures on the internal wall of the mantle.



1	2	3	4	5	6	7	Families
3	U	X	H	0	I	C	Polyclinidae
3	U	X		0	I	C	Polycitoridae (part)
3	U	G		P V	I	S C	Cionidae (part)
2	U	G		0	I	C	Polycitoridae
2	U	G	B	0	I	C	Didemnidae
2	U	G		P V	I	S C	Cionidae (part)
1	U	G		P V	I	S C	Cionidae (part)
1	U	G		0	X	S C	Octacnemidae
1	L	G		V	I	C	Perophoridae
1	L U	X G	T	V	I	S C	Plurellidae
1	L	G		V	I	S	Ascidiidae
1	L U	G		0 P V	C	S	Agnesiidae
1	R	G		V	I C	S	Corellidae
1	L	X		V F	I	S C	Styelidae
1	L	X	L	P V F	I C	S	Pyuridae
1	L	G X	R + L	0 P V F	C	S	Molgulidae

Families of Ascidiacea.

species live hidden from easy view, in cavities among corals or under rocks and rubble. We have included some of them in our commentary, but we concentrate on those species that one can expect to encounter without unduly disrupting the habitat. Even within these constraints, however, we introduce many species; anyone who gains an acquaintance with them is well along toward a substantial knowledge of New Caledonia's ascidian fauna.

FAMILIES OF ASCIDIACEA

1 - Body divisions (tunic removed):
  - 1 - *One* part: gut beside or just under the branchial sac.
  - 2 - *Two* parts: thorax and abdomen: gut well under the branchial sac.
  - 3 - *Three* parts: thorax with branchial sac, abdomen with gut, post-abdomen with gonads.

2 - Location of the gut in relation to the branchial sac:
  - L - On the *left* side.
  - R - On the *right* side.
  - U - *Under* the branchial sac (when the gut is not clearly on one side, use U).

3 - Location of the gonads in relation to the gut loop:
  - G - Gonads inside the *gut* loop exclusively.
  - X - Gonads outside the gut loop (when several, may also exist inside the loop).

4 - Peculiar characters:
  - H - *Heart* located at the end of the post-abdomen.
  - B - *Buds* at the junction between thorax and abdomen.
  - T - Male and female gonads separated and located inside the *tunic*.

L - *Liver*: hepatic diverticula on the stomach wall.
R - *Renal* sac on the right side.

5 - Internal wall of the branchial sac:
O - Without protruding diverticula.
P - With *papillae*.
V - With longitudinal *vessels*.
F - With vessels grouped into *folds*.

6 - Shape of the stigmata when they are present:
I - Straight stigmata.
C - *Curled* stigmata.
X - Round stigmata without cilia.

7 - *Solitary* S or *colonial* C.

ORDER APLOUSOBRANCHIA

Family Polyclinidae

All members of this family are colonial. The zooids lie in a common tunic that thickly encrusts the substrate, or they are embedded in finger-like lobes, or in a few cases they rise as separate elements atop a thin and irregular base of shared tunic. Some species have clean surfaces, while in others the tunic typically is encrusted or impregnated with sediment. The tunic consistency

1	2	3	4	5	6	7	Genus		Distribution
S	I	D	PP	P	N	S	*Euherdmania* Ritter, 1904	S	Widely distributed
S	I	D	PP	S	N	S	*Dumus* Brewin, 1952		New Zealand
S	I	D	PP	P	N	0	*Monniotus* Millar, 1988		Madagascar
E	I	D	PP	A P	N	S	*Ritterella* Harant, 1931	S D	Widely distributed
E	I	D	PT	S	N	S	*Placentella* Redikorzev, 1927		North Pacific
E	I	D	PP	S	N	S	*Homoeodistoma* Redikorzev, 1927	S	Pacific
E	I	D	PP	P S	3	S	*Pseudodistoma* Michaelsen, 1924	S	Widely distributed
E	I	D	PT	S	3	0	*Citorclinum* Monniot & Millar, 1990*	S	New Caledonia
E	C	D	PP	P	N	S	*Aplidium* Savigny, 1816	S	Widely distributed
E	C	D	PP	A S	N	S	*Synoicum* Phipps, 1774	S	Widely distributed
E	C	D	PP	T	N	S	*Atopogaster* Herdman, 1886		Indonesia
E	C	D	PP	P	0	S	*Pharyngodictyon* Herdman, 1886	D	Deep sea
E	C	D	PP	S	N	0	*Protopolyclinum* Millar, 1860		Australia N. Zealand
E	C	N	PP	S	N	0	*Polyclinum* Savigny, 1816	S	Widely distributed
E	C	N	PP	S	N	S	*Aplidiopsis* Lahille, 1890	S	Widely distributed
E	C	N	PT	S	N	S	*Sidneioides* Kesteven, 1909		Australia Japan

Families Polyclinidae.

varies from species to species - loose and jelly-like in one, more fibrous in another. Polyclinid tunics never contain spicules.

The elongate polyclinid zooid has three body regions. Its thorax extends from the siphons to the entrance of the esophagus and includes the branchial sac and the peribranchial cavity. All polyclinids are viviparous, brooding embryos in the oviduct, in the peribranchial cavity, or in special cavities developed for this role. A polyclinid's abdomen encloses the gut-loop. The post-abdomen contains the gonads and, at its tip, the zooid's V-shaped tubular heart. During colony growth, zooids replicate by transversely pinching off a series of block-like fragments, each of which can form a new zooid.

GENERA OF POLYCLINIDAE

1 - Structure of the colony:
  S - Zooids *separate* for a part of their length.
  E - Zooids wholly *embedded* in a common test.
2 - Cloacal siphons:
  I - Directly and *individually* opening on the surface of the colony.
  C - Opening into *common* cloacal cavities.
3 - Connection of the post-abdomen with abdomen:
  D - Post-abdomen in *direct* continuation with abdomen.
  N - Post-abdomen sac-like, connected with abdomen by a slender *neck*.
4 - Position of gonads:
  PP - Both male and female gonads in *post-abdomen*.
  PT - Testis in *post-abdomen*, ovary in the thorax.

5 - External surface of stomach wall:
  - P - Longitudinally *plicated*.
  - A - *Areolated*.
  - S - *Smooth*.
  - T - *Transversely* plicated.

6 - Number of rows of stigmata:
  - 3 - Three.
  - N - More than three.
  - O - No true stigmata.

7 - Transverse vessels:
  - U - *Undulated* edge.
  - S - *Straight* edge.

* Heart located close to the gut-loop; intermediate in structure between Polyclinidae and Polycitoridae.

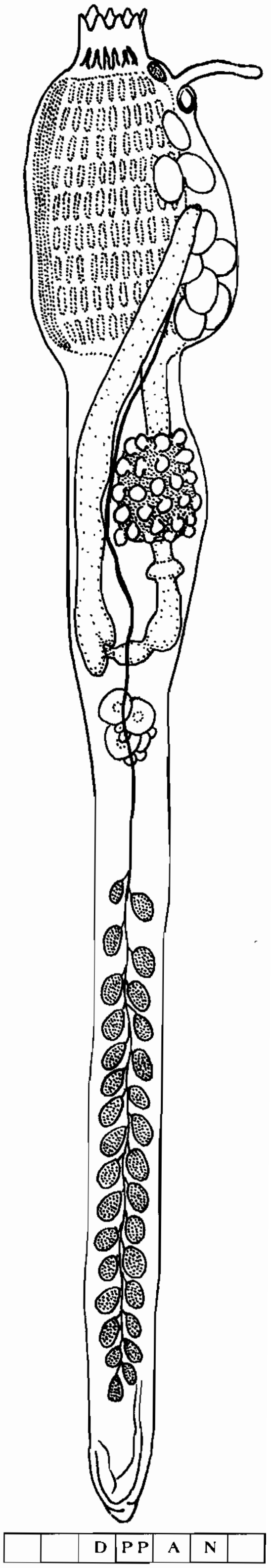
Polyclinid colonies show two sorts of organization. In some genera each zooid's oral and cloacal siphons open independently at the colony's surface; in others the zooid's cloacal siphons open into common cloacal cavities that are simple pockets or ramifying channels within the tunic.

POLYCLINIDS WITHOUT COMMON CLOACAL CAVITIES

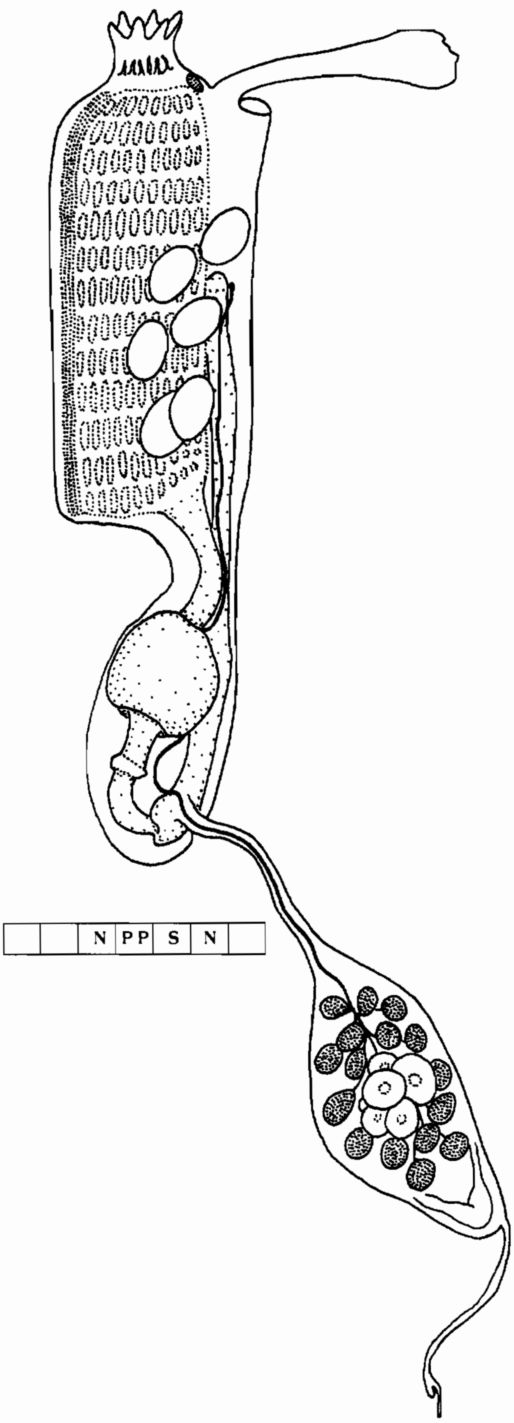
In some polyclinids that lack common cloacal cavities, the zooids' thoraxes are relatively independent of one another, rising individually above a shallow colonial base to give the impression of aggregated solitary bodies. This design is characteristic of the genus *Euherdmania*. The species *E. clavifomis* is common in the lagoon that surrounds the island of New Caledonia. Its colonies form tight bouquets of pale yellow zooids 1 to 2 cm long, with a generally clean tunic. These colonies resemble some species of the polycitorid aplousobranch *Clavelina* or even the perophorid phlebobranch *Ecteinascidia*. But the presence of post-abdomens in the colony's basal tunic quickly identifies these polyclinids as such. A much less conspicuous species of *Euherdmania*, *E. dumosa*, has sediment-covered zooids that appear separated from one another but are in fact connected by a meandering and branching colonial base of tunic.

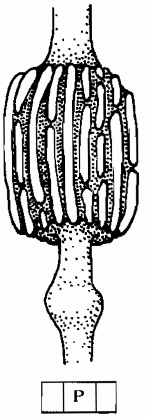
In most polyclinids whose cloacal siphons open independently at the colony's surface, the zooids are fully embedded in a thick common tunic. These colonies grow as erect masses or form sheets or cushions with smooth or lobed surfaces. These polyclinid genera are very hard to distinguish by external appearances alone. Instead, zooids must be removed from the colony and examined closely for their diagnostic traits - details of the branchial sac, for example.

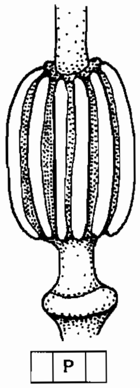
Members of the genus *Pseudodistoma* have only three rows of branchial stigmata, in contrast to the many rows in most other polyclinids. Two New Caledonian species are fairly easy to recognize. Brown, purplish, or yellow colonies of *P. arborescens* have numerous lobes that look like thick pins up to a cm long topped by swollen pinheads. These soft, clean-surfaced lobes protrude from a sandy, often pedunculate base. Each "pinhead" contains the thoraxes of several zooids whose abdomens and post-abdomens are aligned below in the "shank" of the lobe. This species is common on the barrier reef below a depth of 10 m. It lives beneath overhangs and in the fissures in the coral, where it is

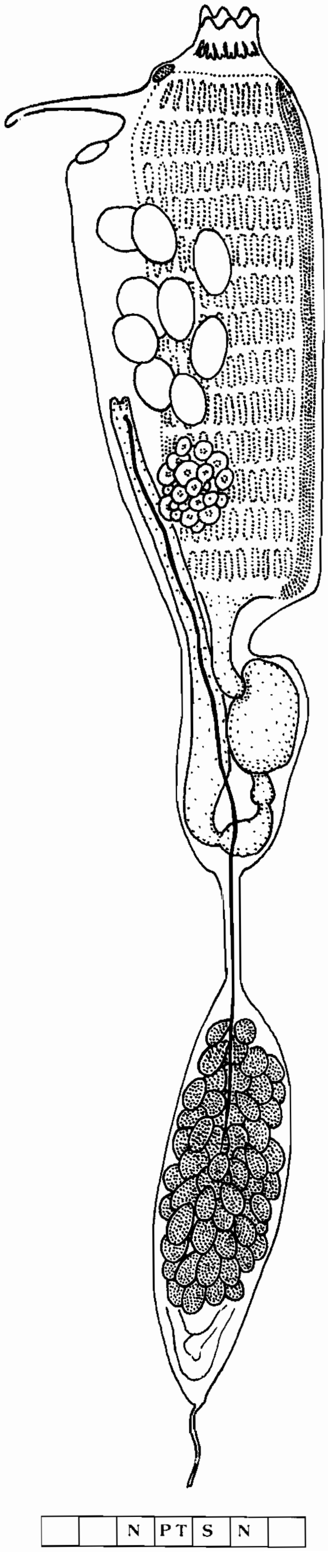


Several kinds of polyclinid zooids
with the corresponding key formulae.

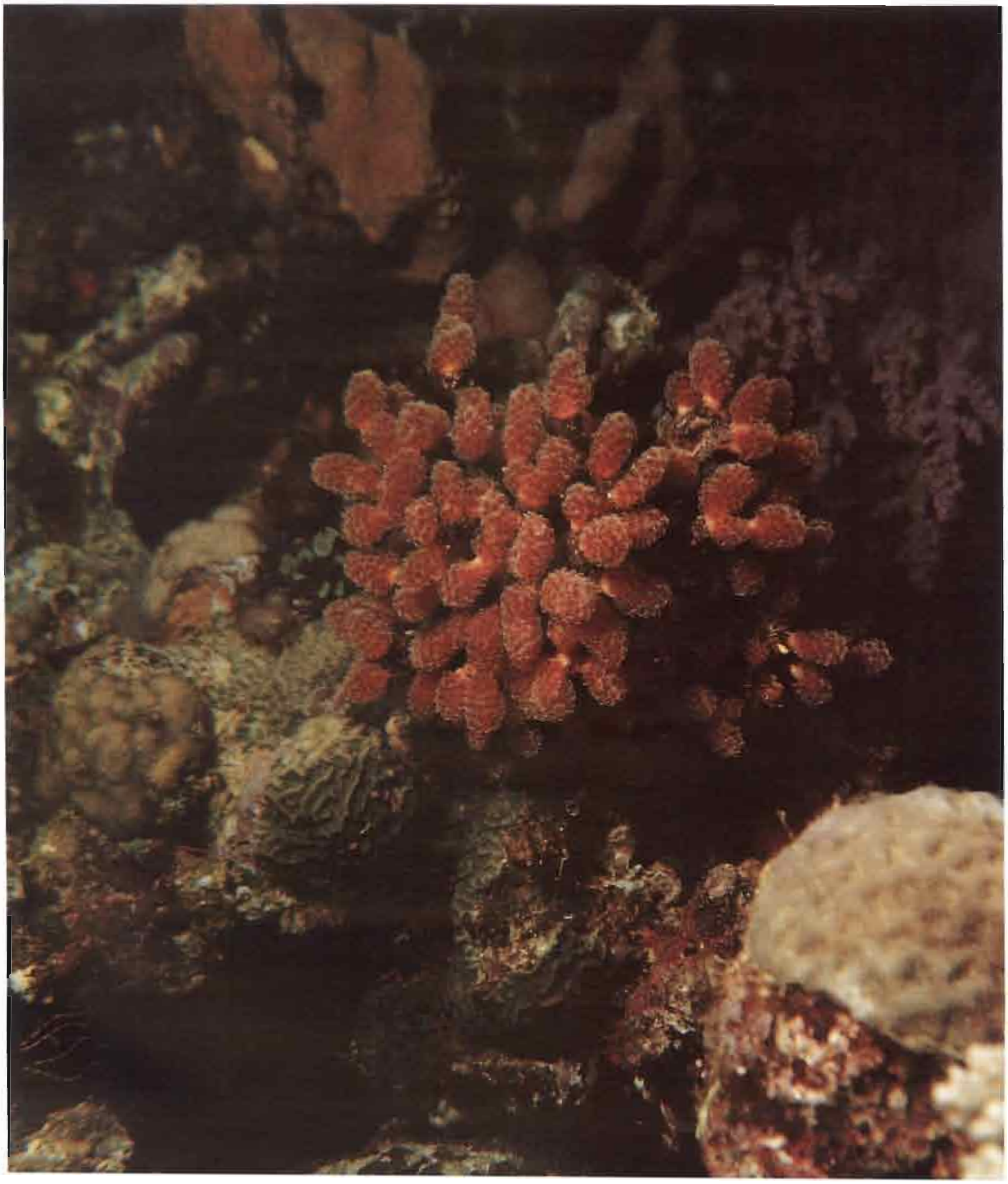








Pseudodistoma arborescens
is made of numerous "heads"
held on peduncles united at their bases.
Each head contains the thoraxes
of many zooids. The color of the species
may be red, violet, or yellow.



Very transparent and gelatinous, *Pseudodistoma fragilis* is well named. Each zooid opens both its siphons independently at the colony's surface.

The large (2 cm long) zooids of *Euherdmania claviformis* share a common base which contains their post-abdomens.

easily visible, so there is no need to break coral blocks or overturn rocks to find it. Colonies of *P. fragilis* live on the sides of islets in the lagoon. The colonies are like little crystal balls, more transparent even than the famously clear polycitorid *Polycitor crystallinus*, which they resemble.

Homoeodistoma has many rows of branchial stigmata and a smooth-walled stomach, a combination that is diagnostic for the genus. New Caledonia's one species, *H. omasum*, forms massive colonies more than 10 cm across that are completely impregnated with sand. The colony's zooids form wavy ridges on a common base, to give the impression of aggregated worm-tubes. This animal has been found on sediments more than 30 m deep.

The genus *Ritterella* is represented in the northern lagoon by *R. circularis*. This species shows all the generic characters well: each zooid sends both its siphons directly to the colony's surface, the branchial sac has many rows of stigmata, and the stomach's external surface has prominent pleats. *R. circularis* forms a thick, opaque yellow crust on whose surface the zooids are grouped into regular, circular systems, their oral siphons toward the periphery and their cloacal siphons toward the center.

POLYCLINIDS WITH COMMON CLOACAL CAVITIES

All other New Caledonian polyclinids form massive colonies with zooids organized into systems with common cloacal cavities. Again, external appearances are not enough to identify these colonies even to genus, since the distinctive traits are ones of the zooid anatomy. Both *Synoicum* and *Aplidium* have a wide connection between the abdomen and the post-abdomen, but they can be told apart by the shape of the cloacal siphon and the character of the stomach. Both *Polyclinum* and *Aplidiopsis* have a narrow constriction between the abdomen and the post-abdomen, and they can be told apart only by the presence (in *Polyclinum*) or lack (in *Aplidiopsis*) of scalloping along the free edge of the branchial sac's transverse vessels - a trait that can be detected only by microscopic study of prepared material.

Synoicum is a widely encountered genus, but it is a rare animal in New Caledonia, where only one colony of a single species, *S. partitionis*, has yet been found. This specimen, from 20 m, is divided into sandy lobes 2 or 3 cm across. Synoicum zooids have an externally papillose ("areolated") stomach, unlike the evenly grooved stomach of *Aplidium*, a genus we describe below.

The widespread genus *Aplidiopsis* also has only a single shallow-water New Caledonian species, *A. gelidus*, which grows at a few meters' depth in the lagoon as thick, gelatinous, lobate, rosy sheets to some 7 cm across. This genus occurs in deep water, too, with a close relative, the sediment-encrusted *A. parvastigma*.

In contrast to these meagerly represented genera, *Polyclinum* and *Aplidium* are very common in New Caledonia, as they are around the world. The region's two most common species of *Polyclinum* are widely distributed in the tropics and are easily confused with each other. *P. constellatum* forms mounds several cm across that are sometimes coated with sand; cleaned colonies range from grey to dark green, sometimes with a rusty tint. Its zooids form systems along ramifying common cloacal channels. From the colony's surface these zooids look like little stars in intricate constellations dispersed about the tunic - hence the spe-

The six-lobed siphons
of *Ritterella circularis* appear
very clearly on the colony surface.
Each siphon is marked by white spots.
The green patches on the animal
are due to *Prochloron* algae.

Aplidiopsis gelidum
is a massive, soft polyclinid
with a rather translucent tunic.

Blue common cloacal channels appear clearly in *Aplidium caelestis*. In each colony they converge into a large central common cloacal cavity. The zooids' oral siphons are the very numerous, minute, dark perforations piercing the tunic's sandy surface.

cies' name. This species is a common fouling organism on hulls, buoys, and harbor structures, but it is rare in less polluted places, except for some very shallow habitats (for example, the sea-grass beds at Mont-Dore beach). Where it does occur outside of ports, this species often grows with *P. macrophyllum*. Colonies of these two species are hard to tell apart: those of *P. macrophyllum* tend to have a more convoluted surface - too slight a difference to help when both species are sand-encrusted, as they often are.

Aplidium is New Caledonia's most diverse polyclinid genus, with a dozen shallow-water and three deep-sea species recorded so far and surely more to be found. Only subtle anatomical differences distinguish most shallow-water species of *Aplidium*, but, despite their varied shapes and colors, a few spectacular species can be recognized at once. For example, *A. caelestis* cannot be confused with any other ascidian. Its colonies, forming sheets at least 5 mm thick and several cm across, have patches of sandy tunic separated by clean, smooth, sky-blue channels. This species often grows with *A. nadaense*, whose colonies closely resemble the sandy parts of *A. caelestis* but lack that species' striking, blue common cloacal channels. Colonies of *A. lobatum*, a cosmopolitan tropical species, also have a sandy, mounding surface but no sharp furrows. Many species of *Aplidium* have a clean, slightly transparent tunic - for example, *A. flavolineatum*, *A. longithorax*, and *A. cellis*. In the last species the tunic bulges distinctively into tightly packed, round or polygonal, smooth, white mounds. The zooids' oral apertures open along the mounds' edges, and their cloacal apertures open into common cloacal channels running along deep, gray grooves between the mounds.

An *Aplidium longithorax* colony
has a cushion shape.
Its long zooids go deep inside
the colony's tunic. They are arranged
in regular "rosettes" with their oral apertures
surrounding a common cloacal orifice.

Deep-water species of Aplidium, dredged from 230 to 650 m, are densely coated with sediment. Compared to relatives from lesser depths, their branchial sacs have very few stigmata.

Pharyngodictyon is a deep-water polyclinid with three New Caledonian species. Their branchial sacs are simplified into nets of large, gaping holes with unciliated borders and a few transverse vessels. How these animals gather food is still a puzzle, since they seem to have no means by which to generate a filter-feeding current; perhaps they simply let the slow flow of the bottom currents carry water through their wide-open branchial sacs. The impressive species *P. cauliflos*, which has been taken from about 500 m on banks south of Grande Terre, has exactly the shape, size, and color of a head of cauliflower. Its zooids lie in the apical surface of stout lobes that branch out from a massive stalk.

When *Aplidium lobatum*
is not heavily coated with sediment,
its zooids' siphons
and the common cloacal openings
of zooidal systems can be seen
all over the encrusting colony.

Family Polycitoridae

Like polyclinids, polycitorids are all colonial. But their zooids lack a post-abdomen; the gonads lie in or next to the posterior curve of the abdominal gut-loop, and the heart is nearby.

GENERA OF POLYCITORIDAE

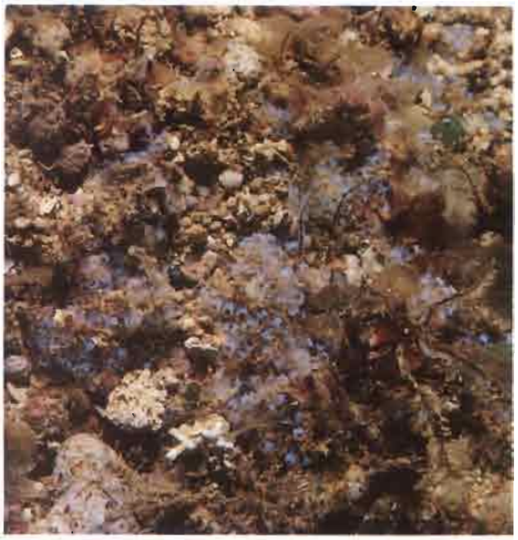
1 - Structure of the colony:
    I - Zooids entirely *isolated*.
    P - Zooids *partly* isolated.
    E - Zooids completely *embedded* in the common test.
2 - Number of rows of stigmata: *0, 2, 3, 4*, or *N*.

1	2	3	4	5	6	7		Genus		Distribution
I	3	0	0	P	0	0		*Millarus* Monniot, 1989		New Zealand
I	2	0	0	S	0	0		*Archiascidia* Julin, 1904		Mediterranean
P	3	0	0	S	0	0		*Archidistoma* Garstang, 1891	S	Widely distributed
P	3-N	0	0	S P	0	0		*Clavelina* Savigny, 1816	S	Widely distributed
E	0	0	0	S P	0	0		*Protoholozoa* Kott, 1969	D	Deep sea
E	3	0	0	S	0	0		*Eudistoma* Caullery, 1900	S	Widely distributed
E	3	0	0	S	0	0	2	*Cyathocormus* Oka, 1912		Japan
E	3	0	0	S	0	0	3	*Hypodistoma* Tokioka, 1967	S	Africa Malaysia
E	3	0	0	S	0	0	4	*Atapozoa* Brewin, 1956		Australia N.Z.
E	4	V	P	S P	0	0		*Distaplia* Della Valle, 1891	S	Widely distributed
E	4	V	P	P	0	0		*Hypsistozoa* Brewin, 1953		N.Z. Kerguelen Peru
E	4	0	0	S	0	S 0		*Cystodytes* Drasche, 1883	S	Widely distributed
E	4	0	P	S	0	0		*Sycozoa* Lesson, 1830	S	Antiboreal
E	3	0	0	S	0	0	5	*Citorclinum* Monniot & Millar, 1990	S	New Caledonia
E	N	0	0	S	0	S		*Polycitorella* Michaelsen, 1924	S	Widely distributed
E	N	0	0	S	0	0		*Oxycorynia* Drasche, 1882	S	West Pacific
E	N	0	0	P	0	0	1	*Stomozoa* Kott, 1957	S	Widely distributed
E	N	0	0	P	0	0		*Polycitor* Renier, 1778	S	Widely distributed

Families of Polycitoridae.

3 - Parastigmatic vessels:
  V - Present.
  0 - Absent.
4 - Incubatory *pouch*:
  P - Expanded into a sac-like diverticulum external to the zooid.
  0 - *Other* modes of incubation.
5 - External surface of stomach wall:
  S - *Smooth*.
  P - Longitudinally *plicated*.
6 - *Common* cloacal cavities:
  C - Present.
  0 - No common cloacal cavities; cloacal apertures opend individually on the surface of the colony.
7 - Calcareous *spicules*:
  S - Present.
  0 - Absent.
8 - Peculiar characteristics:
  1 - Siphons with 2 lips.
  2 - Axis of the colony hollow (as in Pyrosoma).
  3 - Tubular cloacal siphons issue from the posterior part of the thorax.
  4 - Incubatory pouch on thoracic peduncle.
  5 - Testes in a post-abdomen.

Except for one deep-sea genus, *Millarus*, found near New Zealand, the Polycitoridae consists of two groups with distinctly different appearance. On the one hand are some taxa in which thinly sheathed zooids protrude individually from a basal sheet or bed of tunic shared by the whole colony; *Clavelina*'s bouquet-like



This small species, *Archidistoma richeri*,
with zooids about half a centimeter long,
is apparently very fragile.
Nevertheless, it lives in strong currents.
The colony's common base,
covered with sediment,
is firmly attached to the substrate.
The zooids' three rows of stigmata,
with their blue color, can be seen
through the thoracic tunics -
but only by looking closely !

colonies are of this design. On the other hand, zooids of most polycitorid taxa are completely embedded in a common tunic, protruding not at all or only around their relaxed siphons. Both these designs occur around New Caledonia.

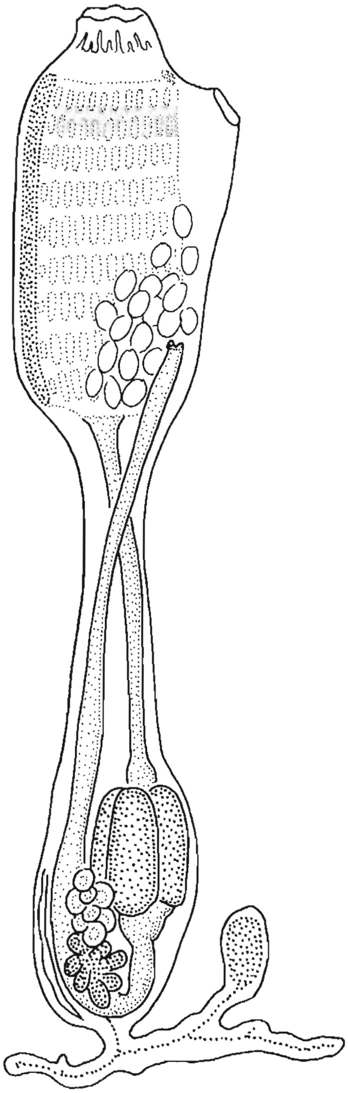
GENERA WITH PARTIALLY ISOLATED ZOOIDS

The genus *Archidistoma* is distinctive among polycitorids of this "clavelinid" type in having only three rows of branchial stigmata and a smooth stomach. In contrast, New Caledonian species of the genus *Clavelina* have many more than three rows of stigmata and an externally pleated (that is, plicated) or ornamented stomach. Polycitorids with colonies of this type look quite similar, but their colors, which are often very bright, vary widely.

A. richeri projects its long zooids' thoraxes and upper abdomens about 0.5 cm above the colony's basal tunic. These animals would be almost invisible were it not for the extraordinary bright blue of their thoraxes. They live 30 m deep where the lagoon is swept by strong currents. Between 20 and 30 m on the barrier reef's outer slope, another member of this genus, the more conspicuous *A. rubripunctum*, holds almost the entire 2 cm length of its zooids above the colony's common tunic. These zooids have a thin abdomen but a swollen, globular thorax that is transparent and colorless but richly speckled with red and orange dots.

Species of *Clavelina* can be told apart in life by their colors, but these quickly fade when they die. The zooids' shapes are also clues to their identity, as is the number of rows of stigmata in their branchial sacs. *C. fecunda* seems to be widely distributed in

A polycitorid zooid
with its key formula.

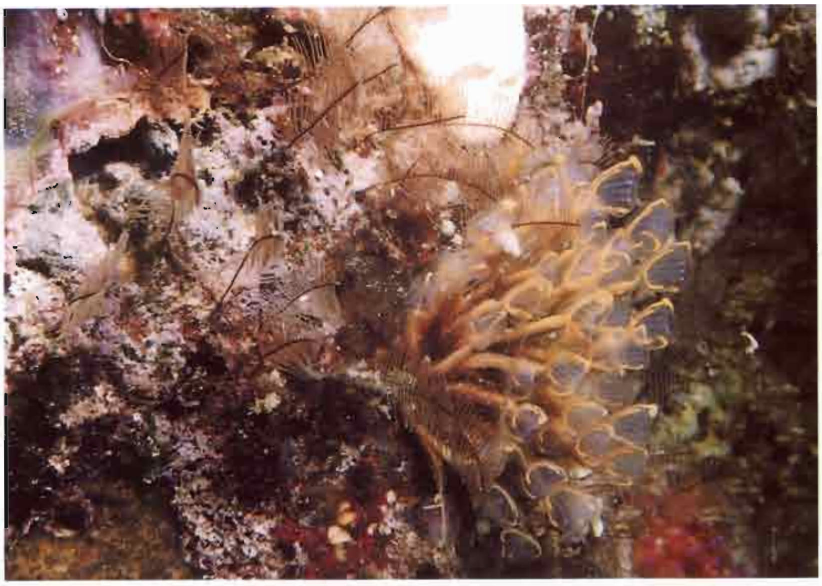


I	N	O	O	P	O	

Raised by their abdomens
above a hard substrate,
the zooids of *Archidistoma rubripunctum*
display beautiful flecks
of orange pigment cells.

Clavelina fecunda is a large species
growing by stolons on hard bottoms.
It is widely distributed
in the tropical west Pacific.
A dappled blue color and white stripes
are characteristic of the species.

Clavelina flava is not transparent. It may be bright yellow, as here, or almost white.



Clavelina detorta lives in bouquets, preferring strong currents.
The basket-like branchial sac is clearly visible, with its transverse bars blue from diffracted light ;
the rest of the body shows heavy concentrations of yellow cells

the tropical western Pacific but has not been found in New Caledonia. It does occur in the Chesterfield Islands. This species has big, intensely blue zooids that are decorated by lines along the endostyle and around the siphons. The magnificent *C. detorta* lives only on the outer reef slope, but it is abundant there. Its zooids rise above the colony's common tunic for their whole 2 cm length, forming dense bouquets. Their transparent tunics reveal the zooids' opaque yellowish gut-tracts and the brilliant blue lines of their transverse branchial vessels. Earning its name, the species' zooids are oddly shaped: the thorax is bent at right angles to the abdomen, and the zooid's oral aperture is closer to its abdomen than is its cloacal aperture. But, as our photograph shows, the zooids' bent posture sets these siphons all at about the same level in the colony as a whole. Both on the outer reef slope and in the lagoon, one frequently encounters a little species, *C. clava*, known only from New Caledonia. Its opaque, white to golden yellow zooids project their clean thoraxes, about 0.5 cm long, in little bouquets that emerge from stubby branches of the colony's rather thick and sandy common tunic.

GENERA WITH ZOOIDS COMPLETELY EMBEDDED IN THE COMMON TUNIC

Most polycitorid genera form sheets or stalked lobes in which the colony's zooids are completely submerged. These genera are difficult to tell apart and can even be mistaken for some polyclinids or didemnids. We must depend for identifications on internal attributes: branchial traits, the character of the stomach, and, at times, the presence of spicules in the tunic. Unfortunately, most of these polycitorids have a very strong muscle, running the length of the zooid, that contracts the disturbed zooid with great force and persistence. As a result, the zooids of many preserved colonies, despite the best attempts at anaesthetization prior to fixation, are grotesquely distorted and consequently very difficult to identify.

Genera with three rows of branchial stigmata

In a New Caledonian polycitorid, a branchial sac with three rows of stigmata generally points to members of the genus *Eudistoma*. Other genera with so few stigmatal rows - *Hypodistoma*, *Atapozoa*, *Cyathocormus* - are rare, have few species, and lack any known New Caledonian representatives.

Eudistoma has many species, of diverse forms - thin or thick sheets, mounds, ball-like cushions, erect lobes. Some species have clean tunics, and others are densely covered or impregnated with sediment. Some *Eudistoma* show no signs at all of colonial organization into zooidal systems; in many others, zooids form systems but ones in which their cloacal siphons, though close together, still open independently to the colony's surface. The colors of living *Eudistoma* colonies sometimes provide clues to their identity, but these colors often change drastically upon the death of the colony. The internal, zooidal traits of this genus *per se* are three rows of branchial stigmata, an elongate abdomen in which a smooth-walled stomach lies at the posterior extremity, a lack of spicules in the tunic, and incubation of the larvae in the peribranchial cavity. Traits that distinguish one species of *Eudistoma* from another have to do with the precise form of the gut-tract (hard to detect when the zooid's muscles contract), gonadal traits, and larval structure.

Common at low tide, *Eudistoma album* forms white cushions, sometimes with pink and blue iridescence. When it dies, this animal immediately turns black.

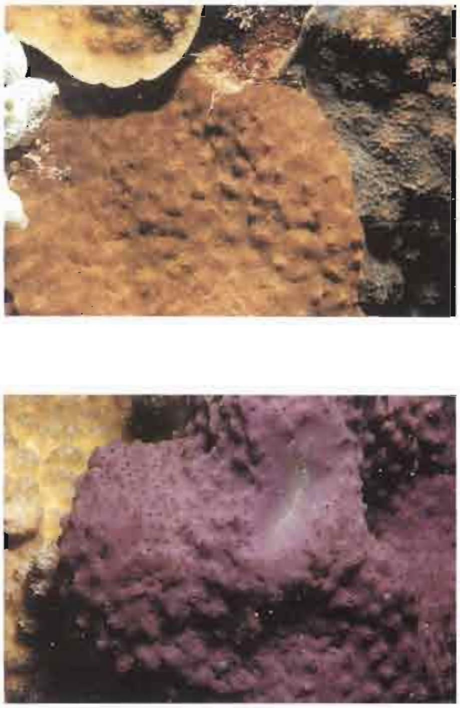
E. album forms cushion-like colonies, several cm across, that are chalky white, sometimes with blue or pink reflections in life; they turn black at death. This species lives at the lowest tidal levels on the reef flat and in shallow parts of the lagoon. *E. fragum* colonies red and speckled with the lighter dots of siphonal openings resemble big strawberries. Soft, even slightly gelatinous, they form ball-like colonies or very thick cushions several cm across in lagoon habitats, but only below 30 m and where currents prevail. This species is exceptional in that, unlike most ascidians, its zooids - in fact, its entire colonies - are wholly male or female, not hermaphroditic. The colonies differ somewhat in color according to their sex, if only because the incubated larvae give females a particularly rich redness.

Both *E. stellatum* and *E. vulgare* form thick, heavily sand-impregnated sheets at shallow and moderate depths in the lagoon; *E. stellatum* has been collected, as well, on the outer reef slope. These species are hard to tell apart except by small contrasts: *E. stellatum*'s dark brown color where its siphons project or bits of its tunic are visible amidst the sand, its zooids' two stomach-folds (*E. vulgare* has no stomach folds), and dorsal clusters of small papillae on its tadpole. *E. laysani*, a widely dispersed tropical Pacific species with a clean, gray-green tunic, forms mounded sheets in the lagoon. About 20 m deep around the Chesterfield Islands, the violet or beige *E. hospitale* grows as sheets 3 cm thick that can span a meter. All the zooids of this species' colonies harbor a coiled, filamentous symbiont in their abdomens; the activity and even the identity of this symbiotic partner remain unknown.

Genera with four rows of branchial stigmata

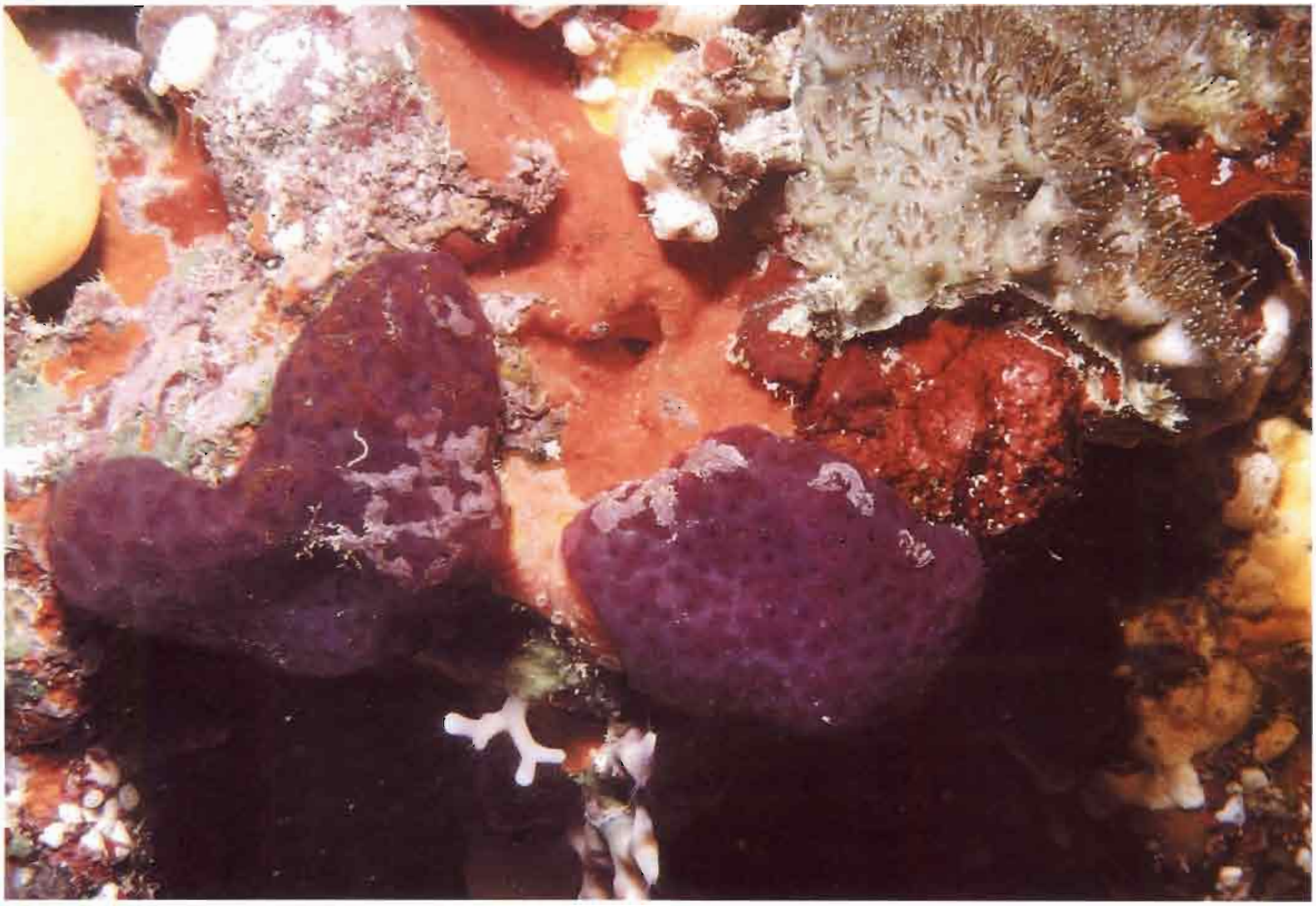
The distinction between three and four rows of stigmata may seem a slight one to depend on, and it is often a hard one to detect. But the elongate zooids of New Caledonian *Eudistoma* species (with three rows) differ enough in other ways, as well, from the stubby zooids of *Cystodytes* and *Distaplia* (four rows) to make this distinction a;practical one in our account. Among polycitorid genera with four stigmatal rows, the presence of spicules in the tunic immediately identifies *Cystodytes*, while genera without spicules (of which only *Distaplia* is likely to be encountered) can be told apart by characteristics of their stomachs and branchial sacs.

As we have discussed earlier in this book, species of the genus *Cystodytes* have spicules dispersed irregularly in the tunic or concentrated into capsules around the zooids. Eight species have been found around New Caledonia - a significant diversity. Their colonies all form thickly encrusting sheets, but these differ greatly in consistency and colors. We will comment first on one series of four species that can cause confusion in the field. *C. violatinctus* is slightly opaque, and of a lovely violet hue; it encrusts corals both in the lagoon and on the outer reef slope. *C. solitus* is more grayish brown, with a somewhat purplish tint; it has been found only below 30 m in the Woodin Strait, at the south end of New Caledonia. Another species of mixed violet and brown, *C. multipapillatum*, lives only in very shallow parts of the lagoon. These three species thus differ in their ecological preferences and consequent distribution; unfortunately, the gray to brown *C. aucklandicus* intrudes all too frequently, to confuse matters. It occurs widely at depths of 3 to 35 m in both the northern



Eudistoma hospitale forms very large encrusting colonies on coral in the Chesterfield Islands. The colonies are various shades purple or brown. The cloacal apertures of the zooids are very close together in the centers of round systems, surrounded by their oral apertures.

Cystodytes violatinctus
is more translucent than transparent.
The masses of spicules around
the zooids' abdomens cannot be seen
from the surface of the colony.
The species often lives
in very shallow water on corals
that fringe small islets in lagoons.



The tunic of *Cystodytes punctatus* is colorless and transparent, allowing one to see the zooids encapsulated by masses of white spicules.

and the southern parts of the lagoon and also on the outer reef slope. Separating these four species involves close inspection in the lab, with particular attention to the shape and arrangement of the spicules, to the structure of the larvae, to incubatory modes, and to whether or how the zooids and their siphons are arranged into systems.

New Caledonia's four other species of *Cystodytes* are easier to recognize, for each presents clear fieldmarks. *C. luteus* forms distinctive, translucent, orange sheets throughout the lagoon and on the outer reef slope. The northern lagoon's *C. fuscus* is a deep maroon or even black, sometimes with white spots; its opaque tunic all but hides the orderly rosettes of its zooids' systems. The southern lagoon's spectacular *C. punctatus* has a colorless, gelatinous tunic in which the spicules are all concentrated into tiny (2 mm) but easily visible, white capsules around each zooid . And on the barrier reef's outer slope lives *C. mucosus*, whose name aptly suggests the almost runny quality of its milky white tunic.

Polycitorids with four stigmatal rows but without spicules in the tunic, sometimes grouped into the sub-family Holozoinae, are widespread in cold waters but much less diverse in tropical regions. Of these genera - *Distaplia*, *Sycozoa*, and *Hypsistozoa* - only one shallow-water species, *Distaplia stylifera*, has been found so far in New Caledonia. It is a common lagoon species, forming inconspicuous, colorless or gray-green cushions about a cm across. Beyond these shallow-water habitats, the transparent, viscous colonies of *D. progressa* have been found in the colder waters that prevail at depths of 200 to 500 m.

Genera with more than four rows of branchial stigmata

Clavelina, which we have reviewed already, has many rows of branchial stigmata, but its zooids are largely separate from one another in bouquet-like colonies. Four other genera, whose zooids are entirely embedded in a common tunic - *Stomozoa*, *Polycitor*, *Oxycorynia*, and *Polycitorella* - have representative apiece in the New Caledonia region.

Stomozoa murrayi has been found only in New Caledonia's northern lagoon, on vertical walls at a few meters' depth. Its colonies form rather hard, opaque, creamy white cushions. Zooids have a very large complement of branchial stigmata - at least 24 rows - and the thoracic mantle is prominently marked by a characteristic arrangement of strong muscle bundles. The siphons, which open individually to the colony's surface, have fringed lobes but close as if by two lips, a behavior that gives even contracted colonies a recognizable feature. All the other traits of this ascidian are similar to those of *Polycitor*, to which *Stomozoa* is clearly closely related.

A colony of *Polycitor crystallinus* is a large (sometimes exceeding even 5 cm), transparent, colorless ball, fixed to the substrate by a short, thick stalk. This species has been found between 20 and 30 m deep in the southern lagoon. The zooids are invisible, for their siphons, thoraxes and upper abdomens, in the clarity of

Even though both siphons of each zooid open independently to the surface, *Polycitorella mariae* is easily confused with a didemnid ascidian, because its tunic is densely packed with small, stellate spicules. Also, adding to the confusion, the surface of the colony is frequently covered with symbiotic algae, unlike the very clean colony shown here.

the ball, are crystal-clear, while their colored lower abdomens are in the stumpy stalk below the ball. This species could be confused only with the similarly crystal-clear polyclinid *Pseudodistoma fragilis*.

In the Chesterfield Islands, the impressive *Oxycorynia fascicularis* forms colonies, occasionally branched, that resemble large morel mushrooms. The transparent tunic reveals zooids of a deep blue-violet arranged in tight, regular rows in the swollen lobes atop the colony's thick stalks. The erect elements of a colony can stand 8 cm high. The species, which lives at Bampton Island at depths of about 45 m, has been found widely in the western Pacific - in northern Australia, Indonesia, and the Philippines.

Although New Caledonia itself has no known *Polycitorella*, the Chesterfield Islands do harbor, at about 20 m depth, one shallow-water species of this genus, *P. mariae*. It also occurs in New Zealand. The pale yellow colonies, their tunics densely impregnated with stellate spicules, closely resemble a didemnid, but the zooids are those of a typical polycitorid. One can tell this species from the polycitorid genus *Cystodytes* by *Polycitorella*'s more elongate zooids and by its more numerous stigmatal rows.

A polycitorid genus without any stigmata: *Protoholozoa*

This genus contains only deep-sea species. At depths of 300 m around New Caledonia, *P. anthos* forms little bouquet-like lobular colonies about a cm across. Deeper still, from 66 to 2 000 m

This curious species, *Citorclinum laboutei*, forms cushions several centimeters across. Very characteristically, the zooids' apertures are each lined by a circle of white pigment. This animal's zooidal structure is peculiarly intermediate between a polyclinid and a polycitorid design.

down, *P. lilium* has been dredged from these waters, as it has been from comparable depths in other oceans.

The perplexing genus *Citorclinum*

It is only with reservations that we place the sole species of this genus, *C. laboutei*, in the family Polycitoridae. This species, known only from New Caledonia, forms transparent, ball-like colonies several cm in diameter. The zooids' siphons open independently at the colony's surface, often in a regularly aligned pattern that is accentuated by a little ring of opaque white pigment around each siphon. The species is frequently encountered at moderate depths - 6 to 35 m - on the outer reef slope and on lagoon bottoms that are swept by fairly strong currents. This animal has a puzzling mix of polycitorid and polyclinid traits. Like a polycitorid, it has its heart and its ovary in the abdomen. But, like a polyclinid, it has a prominent, muscled post-abdomen in which all its testis-lobes reside. The branchial sac has three rows of stigmata. *C. laboutei* seems to mix some traits of the polyclinid genus *Pseudodistoma* with other traits of the polycitorid genus *Eudistoma*.

Family Didemnidae

This is the dominant family of ascidians in shallow-water tropical habitats, including those of New Caledonia. Some didemnid species are well known, but others are still under study, and many

1	2	3	4	5	6	7	8	Genus		Distribution
3	C	1	0 T	6	N	S 0	3	*Trididemnum* Della Valle, 1881	S	Widely distributed
4	C	1 2	0 L	6	N	S	3	*Didemnum* Savigny, 1816	S	Widely distributed
4	C	N	0 L	6	N	S	3	*Polysyncraton* Nott, 1892	S	Widely distributed
4	C	1 N	T	6	N	S	4	*Leptoclinides* Bjerkan, 1905	S	Widely distributed
4	C	1	T	5 6	1 N	S	4	*Atriolum* Kott, 1983*	S	Indian Ocean N.Caledonia
4	C	N	5	6	1	S	4	*Askonides* Kott, 1962		Australia
?	C	?	?	5	1	S	?	*Coelocormus* Herdman, 1886		Deep sea
4	S	1 N	0	6	N	S	4	*Lissoclinum* Verrill, 1871	S	Widely distributed
4	S	1 N	0	6	N	0	4	*Diplosoma* Macdonald, 1859	S	Widely distributed

Families Didemnidae.

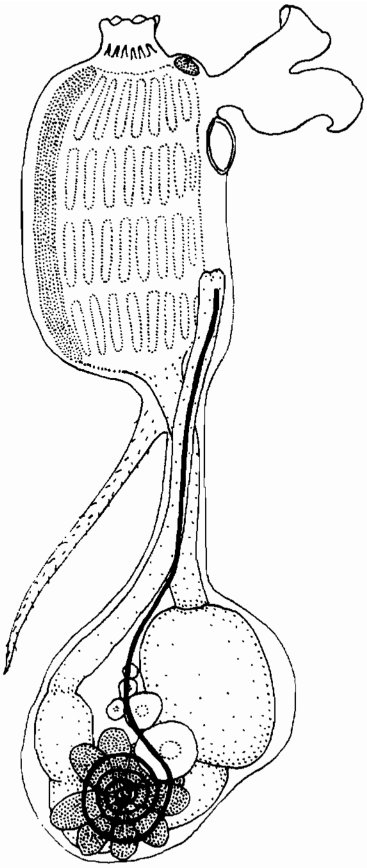
didemnid species have never been collected at all. Identifying an ascidian to its family as "a didemnid" is usually feasible, but - a few well-marked species aside - identifying it more precisely than that can be exceptionally difficult. Success requires not only microscopic study after dissection of the colony but also specimens with both clearly defined gonads and mature larvae. But very often, a colony's zooids are all at the same developmental stage: all budding actively but lacking sufficiently developed gonads, all with mature testes but without larvae, all with mature ovaries and larvae but with testes in regression. Only a specialist can hope to identify most didemnids to species - and then sometimes with considerable trepidation!

All didemnids are colonial, with minute zooids that never exceed lengths of 2 mm. Common cloacal channels that can become immensely complex receive the waterflow from these zooids. The consistency of a colony's tunic varies greatly according to species; some are so insubstantial that they come apart merely when touched, while others forms such hard, brittle crusts that they can be mistaken for sponges or thin corals or even calcareous algae. These variations reflect differences in the tunic's own structure as well as the density of its complement of calcareous spicules. The appearance of the colony - its consistency, its encrusting or globular form, its colors - will help identify only a few of the most distinctive didemnids, and most of these only tentatively; for the others, verification in the lab by close anatomical inspection is essential.

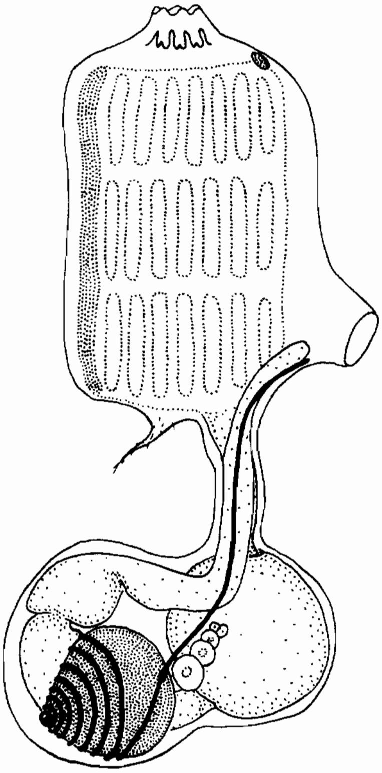
1 - Number of rows of stigmata: *3* or *4*.
2 - Shape of the sperm duct:
  S - *Straight*.
  C - *Curved*.
3 - Number of testes: *1*, *2* or *N*.
4 - Form of cloacal apertures:
  T - *Tubular*.
  L - With a *languet*.
5 - With 5 lobes.
  H - Simple *hole*, no process or tube.
  5 - Number of lobes of branchial aperture: *5* or *6*.
6 - Number of common cloacal openings:
  1 - One opening only, in the upper part of a single large common cloacal cavity.
  N - *Numerous* common cloacal openings.
  0 - No common cloacal cavities; each zooid opening to surface separately.
7 - Calcareous *spicules*:
  S - Present.
  0 - Absent.
8 - Number of rows of stigmata in the larval oozooid: *3* or *4*.

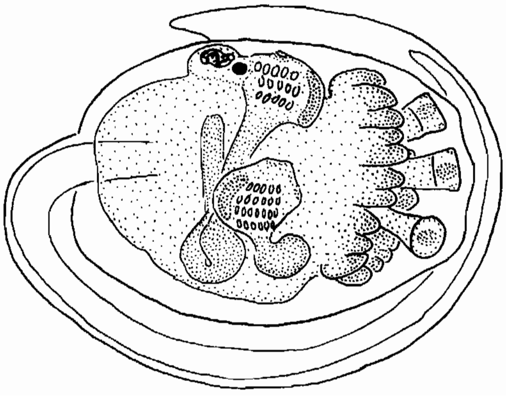
* Pedunculated incubatory pouch.

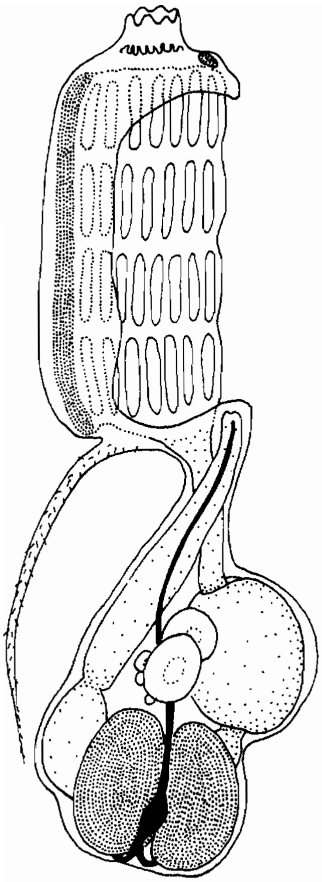
Didemnids are identified to genus first by counting the number of rows of branchial stigmata: only *Trididemnum* has three, all the other genera have four. These other genera can then be separated mainly by the number of testis lobes, the form of the sperm



Three different schematic zooids of the family Didemnidae, and one larva, showing variously associated characters.







4	S		H	6	

duct, the shape of the zooids' cloacal siphons or extensive cloacal apertures, and the presence or absence of spicules in the tunic.

DIDEMNIDS WITH THREE ROWS OF STIGMATA: THE GENUS *TRIDIDEMNUM*

Trididemnum not only has three rows of stigmata, but also a testis that - with rare exceptions - consists of just one vesicle - surrounded by several spiral turns of a coiled sperm duct. The zooid's cloacal siphon varies: usually it is a complete tube, though often a very short one, but in a few species the cloacal aperture is actually a cut-out portion of the mantle's dorsal surface. The tunic is more or less densely spiculated, again according to species. Species also vary in the size of their mature larvae - 400 µm to 2 mm. In some species these larvae are "simple", but in others they begin to bud even while still being incubated in the mother colony.

Two species of *Trididemnum* found in New Caledonia contain internal symbiotic algae. *T. cyclops* forms irregular lettuce-green crusts on the barrier reef, on the reef flat, and in the lagoon. Even the biggest colonies rarely span 3 cm. Concentrations of spicules give them a distinctly white border. The species is common at shallow depths on all sorts of substrates - shells, rigid algae such as *Halimeda*, and corals. *T. cyclops*' elongate zooid has a long oral siphon whose dorsal lobe is much shorter than the others around the oral aperture; the cloacal aperture is a narrow transverse slit in the dorsal mantle, not a protruding siphon at all. Another alga-containing trididemnid, *T. miniatum*, forms very small green colonies that have the consistency of mucus. Its zooids are tiny even for didemnids - only 0.5 mm long!

Trididemnum cerebriforme
forms a typical encrusting didemnid
colony, although it is rather thick.
It has an irregular outline,
only a few large common cloacal openings,
and an immense number of zooids,
whose oral siphons are evenly
distributed over the colony's surface.
This species is often gray,
sometimes greenish or blue.

Other species of *Trididemnum* are variously colored, but shades of gray dominate. For example, *T. cerebriforme* forms hard and brittle, fairly extensive crusts at least 2 mm thick. The zooids' black oral siphons dot the colony's surface; the rest of the colony's gray color is more or less somber according to the distribution of the tunic's white calcareous spicules. Pale to dark gray colonies of *T. banneri* can cover several 2 cm in very shallow places and have a velvety appearance, due to the absence of both spicules and pigments in the tunic's surface layer. Still other Trididemnum that occur in New Caledonia are simply white, or occasionally pink.

DIDEMNIDS WITH FOUR ROWS OF STIGMATA

Didemnid genera with a coiled sperm duct

The genus *Didemnum* is by far the most diversified in the family Didemnidae. There are 35 species of *Didemnum* already recorded from New Caledonia alone, very few of them known beyond the acquaintance needed simply to identify them. The settled larva and resultant oozooid has only three rows of branchial stigmata, but all subsequent zooids in a colony have four rows. The cloacal aperture may have a languet,or it may be just a broad opening of the dorsal mantle. The testis consists of one or, at most, two vesicles, surrounded by the spiral turns of a coiled sperm duct. The genera *Didemnum* and *Polysyncraton* are almost, if not actually, identical. They differ only in that *Polysyncraton* has both a cloacal languet and two or more testis lobes, traits that *Didemnum* does not combine in any one species - a weak reed on which to hang a genus, and not one we can break or buttress here (but see F. Monniot, 1984 for a discussion of this matter).

The name of *Didemnum spongioides*, a large species, reflects its startling resemblance to a sponge.

A few of New Caledonia's many species of *Didemnum* are easy to recognize in the field. Below depths of 20 m in the lagoon, *D. spongioides* grows into large, many-lobed, white colonies that look like bath sponges. Equally distinctive but totally different (if one recognizes it as an ascidian at all!), *D. perlucidum* forms very fine, soft, white films over all sorts of supports close to the water's surface. This cosmopolitan tropical species adheres easily to shells, polychaete tubes, and other hard natural bases and also fouls buoys, boat hulls, and wharves. *D. granulatum* grows widely as thin, extensive, orange or red sheets; its tiny zooid's cloacal aperture is relatively enormous, exposing most of the branchial sac's peribranchial surface to the common cloacal channel. Less striking but no less curious, the thin, gray-beige crusts of *D. psammathodes* gain their color from the deposition of fecal pellets in the mostly spicule-free tunic. The urn-shaped colonies of *D. molle* are hard to confuse with any other didemnid except perhaps *Atriolum robustum*. *D. molle*'s colonies, which often live in dense concentrations, can grow to more than 3 cm long and come in colors ranging from white to dark green and

Didemnum granulatum
comes in different bright colors,
from yellow to deep red.
Here it covers a worm tube,
but it may encrust any kind of substrate,
forming flat sheets or rounded lobes.

The white *Didemnum perlucidum* is always
very thin, no more than 2 mm thick,
and covers all kinds of substrates -
coral, dead shells, seaweeds,
sponges, solitary ascidians,
and all kinds of artificial substrates.

Very easy to identify,
with its urn-shaped colonies
and abundant mucus, *Didemnum molle*
shelters a large amount of symbiotic algae.
It also has a brown pigment.
The balance between the green algae,
the brown pigment, and the colony's white
spicules can produce many different colors.

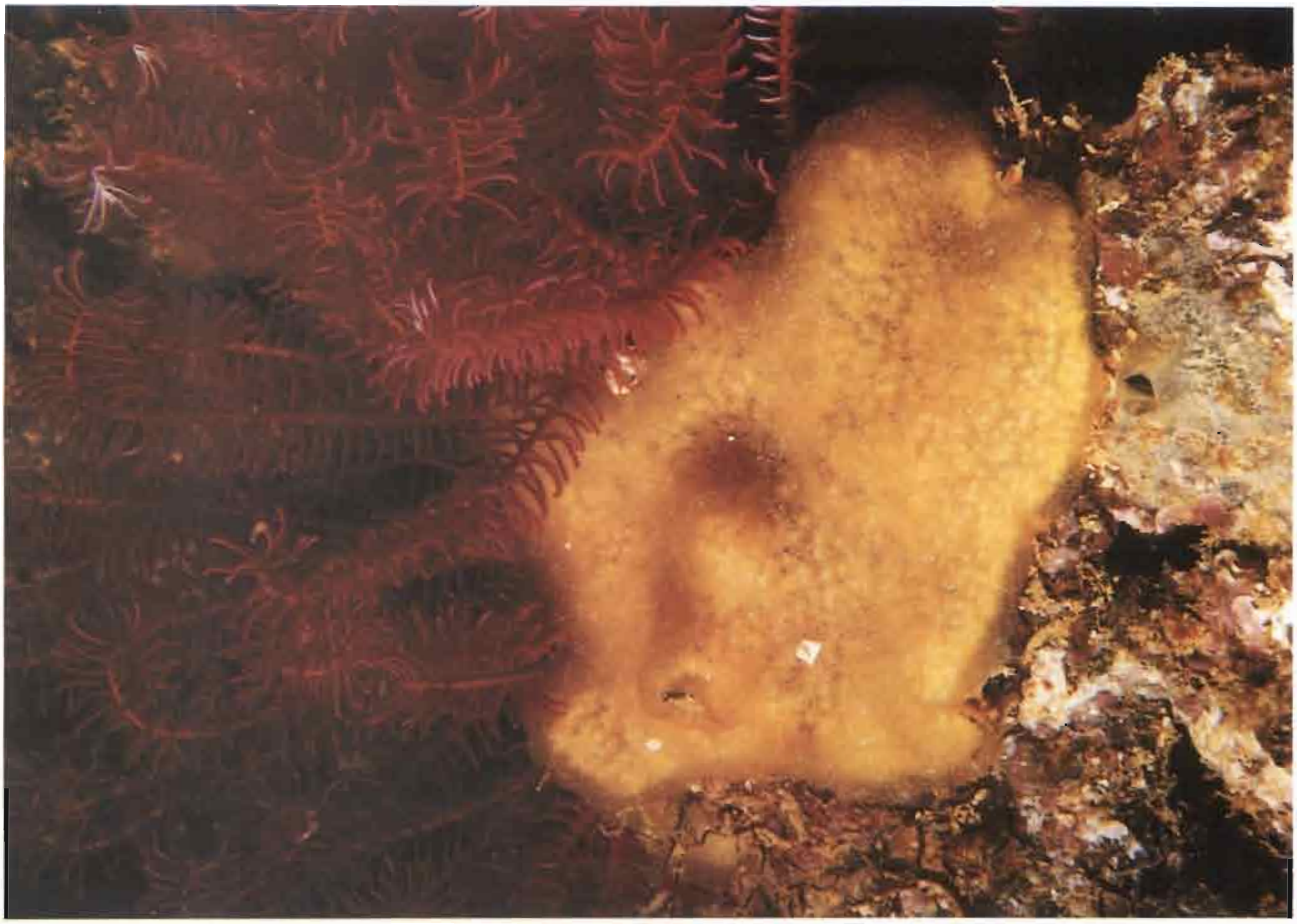
sometimes brown. They harbor large quantities of the symbiotic alga *Prochloron*. This species is also famous for its habit of secreting an abundance of mucus, so sticky that any diver who touches it cannot mistake it!

The many other species of *Didemnum*, taken together, seem to exploit virtually all available solid supports, but especially places sheltered from direct sunlight and from possible predators. Whenever we examine surfaces beneath overhangs, or turn over coral blocks and rubble, or peer into narrow fissures in the reef, we are likely to encounter the brilliant encrustations of orange, pink, maroon, gray, yellow, and white *Didemnum* colonies. Even where the lagoon's bottom is soft, some species will grow as epibionts on hydroids, gorgonians, tubicolous polychaetes, algae, or any other sufficiently rigid substrate, including other ascidians. As epibionts on such small sites, these colonies may grow into unusual shapes - jackets, say, or lobes projecting into the water. Such exposed colonies show just how sensitively contractile these animals can be. Before one approaches too closely, the zooids' oral siphons are open and the colony appears thick and swollen. As soon as its zooids detect vibrations, the whole colony contracts. It relaxes to its former, inflated state only a long while later. But not all intruders evoke this contractile response; the activities of some fishes and shrimps are tolerated without any such reaction.

A few didemnids assigned to the genus *Polysyncraton* occur in New Caledonia. As its name implies, *P. thallomorpha* can easily be mistaken for an alga. Colonies form thin, irregularly shaped, fan-like or leafy crusts that stand out from the substrate. One surface of the colony is gray or beige and carries the zooids' oral

This *Didemnum* forms thin crusts
that make it difficult to remove colonies
undamaged from the substrate.

Some *Didemnum* species, as here, have a transparent superficial layer of gelatinous tunic. Zooids lie under a layer of spicules, which can be seen through the transparent overlying tunic.



On soft bottoms, species of *Didemnum* grow on algae or invertebrates. Where the substrate is very broken up, the ascidian colonies fold upon themselves and rise in finger-like lobes.

siphons; the other surface, is white form a layer of densely packed, fine spicules and with concentric ridges that look like growth-rings, exactly resembles a calcareous alga. Even if a colony of this species lies flat on a rock, its attachment is so slight that the colony is surprisingly easy to remove. Another species of *Polysyncraton* paves surfaces with juxtaposed polygonal systems of zooids. Its yellow or brown-violet colonies are only 2 or 3 mm thick but often extend over 10 cm of substrate.

Like *Didemnum*, the genus *Leptoclinides* has four rows of stigmata, a coiled sperm duct, and a spiculated tunic. But the cloacal siphons of almost all the zooids in a *Leptoclinides* colony are real tubes, not mere languets. Also, in *Leptoclinides* even the oozooid has four rows of stigmata, not just three. The testis may have only one or two vesicles, as in *Didemnum*, or many lobes, according to species. *Leptoclinides* colonies are as variously

In this red *Didemnum*
the common cloacal openings
are particularly abundant
and raised on small domes.

Rather soft, this *Polysyncraton* species
is able to grow on a loose bottom.
The species' dark orange pigment
is most clearly seen near the common cloacal
openings, where spicules are less dense.

Polysyncraton thallomorpha is a brittle species
that grows in large sheets and takes an "algal"
shape. The colony's upper surface
is irregularly marked with brown or gray patches.
Its undersurface is very smooth and white,
with concentric low ridges
that simulate the marks left
on some organisms by cyclic growth.

colored as *Didemnum* colonies, but usually are thicker. Several species of the genus occur in New Caledonia.

Didemnid genera with a straight sperm duct

On the outer reef slope to depths of 80 m and on soft lagoon bottoms 10 to 35 m deep, *Leptoclinides dubium* forms cushions several cm across whose large common cloacal apertures are pale peach-colored to bright pinkish violet. The symbiotic alga *Prochloron* that coats many of these colonies disappears with the slightest rubbing. A zooid of this ascidian can be recognized by its numerous testis lobes, which are bunched tightly together and surrounded by one complete coil of the sperm duct. *L. robiginis* is another species with fairly reliable fieldmarks: not only its rusty color but also its habit of growing into finger-like lobes that easily attain lengths of 10 cm. It lives 20 to 35 m deep on sandy bottoms that are swept by strong currents, and it also occurs 4 to 5 m deep in wave-swept habitats just outside the barrier reef. *L. apertus* might be confused with *L. robiginis*, but its color is iron gray, and its colonies' surfaces show orange rings around the common cloacal openings. *L. apertus* forms rather flat colonies on various surfaces 5 to 35 m deep in the southern lagoon. Dredging has recovered colonies of *L. duminus* outside the barrier reef, from depths down to 300 m. These colonies are white urns with a single, apical common cloacal opening.

From *Leptoclinides* to the closely related genus *Atriolum* is a matter mainly of adding a pedunculate incubatory pouch to the zooid's thorax. Two species live around New Caledonia. The aptly named *A. marsupialis* forms orange-streaked, gray encrusting colonies where strong currents prevail in the lagoon and in

C. Monniot, F. Monniot
P. Laboute

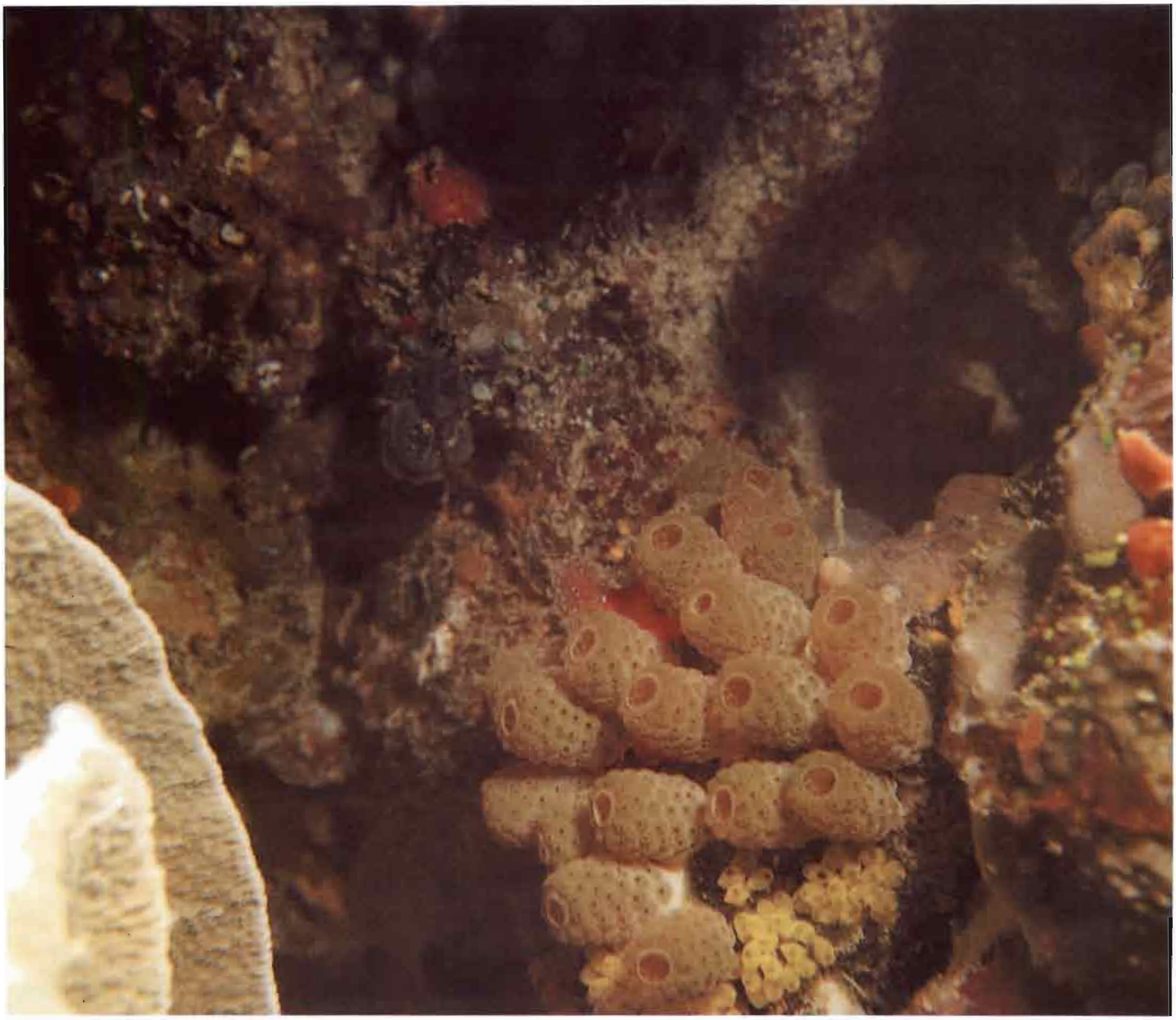
Leptoclinides dubius makes fleshy cushions with few but large common cloacal openings. Its color depends not only on the density of surface spicules, but also on the quantity of the green *Prochloron* algae that grow facultatively on the colony surface.

areas of heavy wave-action on the barrier reef. It is not as common as the urn-shaped *A. robustum*, which is reminiscent of *Didemnum molle*. Cream-colored colonies of *A. robustum* are tinted reddish by their red-pigmented zooids. Like *D. molle*, *A. robustum* harbors symbiotic algae. It has a vast geographical range, from Madagascar to Australia to many parts of the western Pacific, and lives from shallow waters to depths of 300 m. In New Caledonia it is common only below 40 m.

Lissoclinum and *Diplosoma*, like all other didemnids except *Trididemnum*, have four rows of branchial stigmata. But unlike other didemnids, these two genera lack the oddity of a coiled sperm duct; instead, that gonoduct goes directly from the testis to its gonopore in the peribranchial cavity. These two genera are probably closely related; their distinction turns on the character of the tunic. *Lissoclinum* has calcareous spicules in its tunic, even if sometimes they are very sparsey, but *Diplosoma* never has spicules. In the field, some heavily spiculated species of *Lissoclinum* can be mistaken for colonies of *Didemnum*, while lightly spiculated ones can as easily be confused with *Diplosoma*.

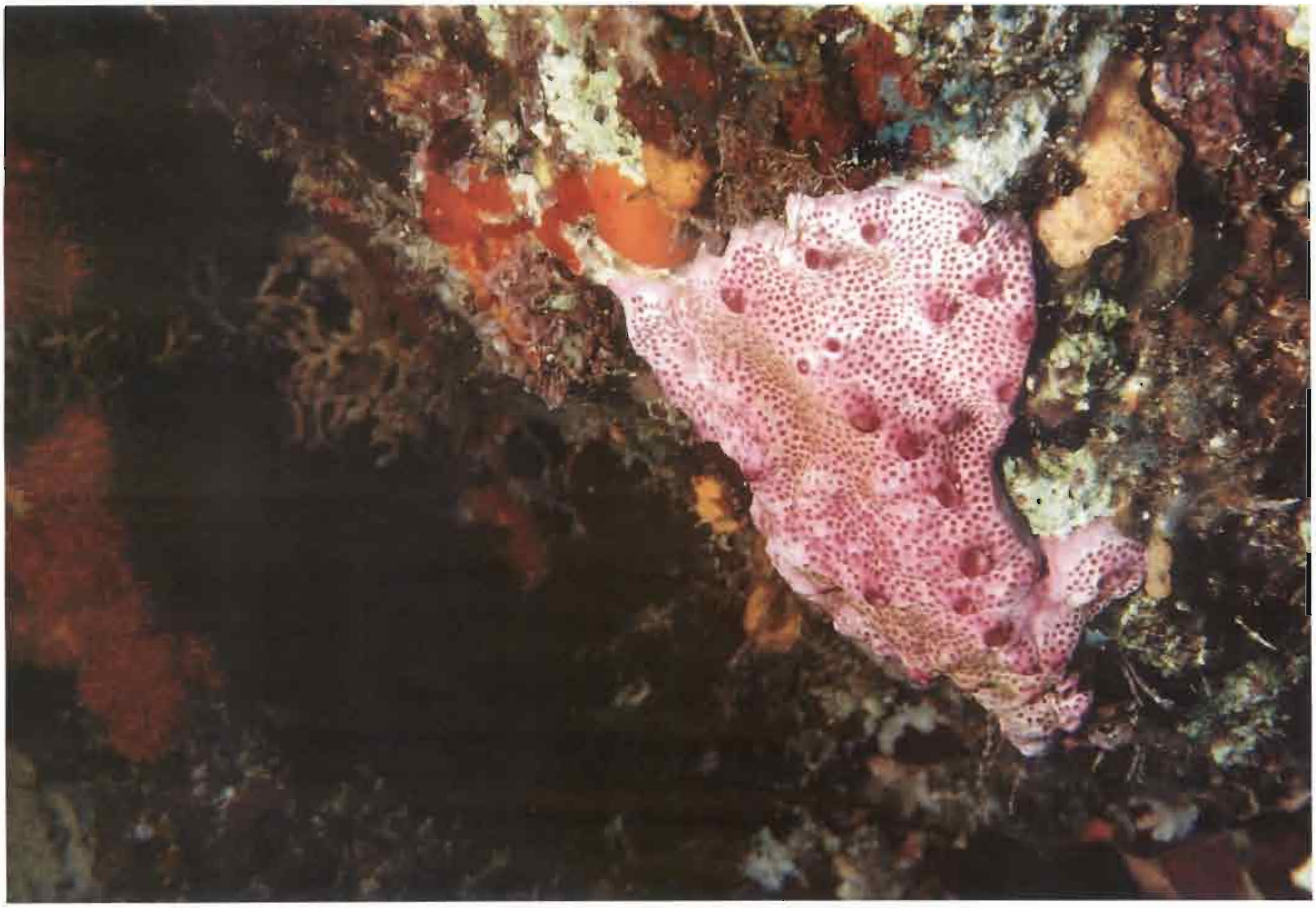
L. japonicum has abundant spicules and forms solid, thickly encrusting colonies of a pale rose-purple color that may span several cm. *L. vareau* also grows as an encrusting colony, but is very soft and fragile; it is violet-purple in clear sunlight, but when encountered even at modest depths, appears to be a brilliant blue. Several other heavily spiculated species of this genus are simply white. The expansion of the common cloacal channels around the zooids' thoraxes often inflates *Lissoclinum* colonies to a thicker appearance than that of many *Didemnum* species and also makes them more fragile. Thus *L. fragile* is well named; it is a delicate form with white colonies whose zooids contain pale brown pigment cells.

Atriolum rubustum makes urn-shaped colonies, resembling *Didemnum molle.* But the colony's outer surface is lumpier in *Atriolum*, and the oral siphons are much sparser than in *D. molle.* Also in *Atriolum*, both the internal side of the large, central, common cloacal cavity and the small oral siphons on the colony's outer surface show the red color of the species' zooids. The greenish color of the colonies is due to the presence of *Prochloron* algal cells on their surfaces. A yellow perophorid colony is growing next to the didemnid.



Lissoclinum mereti is very closely related to *Lissoclinum punctatum* but has no symbiotic algae. The zooids live nestled in capsules of spicules, easy to see through the glassy tunic.

Lissoclinum vareau has a very characteristic purple-violet pigment, which appears deep blue when seen without artificial light at much depth. The colonies are so fragile that they cannot be taken off the substrate undamaged. Here, too, some *Prochloron* algae make greenish patches on the surface.



The fleshy colonies of *Diplosoma versicolor* have very large common cloacal cavities that may be inhabited by a commensal shrimp of the genus *Periclimenes*.
The cream-colored patches are made by pigment cells, not by spicules.

This *Diplosoma*, thick when undisturbed, becomes very thin when touched and very difficult to remove from the substrate.
The almost slimy consistency of its tunic makes matters worse!

Symbiotic *Prochloron* algae color colonies of several species of *Lissoclinum*, such as *L. patella* and *L. voeltzkowi*, a lively green, either uniformly or patchily. *L. bistratum* can be green or pink; the latter color is probably due to a different algal symbiont.

Several species of *Lissoclinum* provide a sort of transition to the spicule-free genus *Diplosoma*. *L. mereti* and *L. punctatum* have only a few spicules, and these are concentrated around the zooids, while *L. verrilli*'s meager allotment of spicules is more dispersed throughout the tunic. *L. verrilli*'s spicules are odd and characteristic: they have only a few, long rays, like children's jacks, and provide a useful indicator of the species when examined under a strong hand-lens.

Diplosoma colonies can take on three different forms. Some species, such as *D. versicolor*, have massive, lobate colonies marbled white or pink or brown; in species like this, it can be very hard to decide whether spicules are present or absent at all. Other species, in contrast, have thin, glassy tunics, so transparent and often so insubstantial that they can scarcely be distinguished from their substrate. In *D. listerianum*, for example, only the darkly pigmented zooids are visible in their loose, clear matrix - and sometimes even the zooids are missing from considerable parts of the tunic. This cosmopolitan species lives in New Caledonia on the barrier reef, in the lagoon, and in ports. *D. listerianum* and other species with such flimsy colonies are inflated with water when relaxed but contract into the thinnest of films when disturbed. *Diplosoma* takes on a third appearance in those species that harbor *Prochloron*. The spicule-free tunics of these species cannot mask *Prochloron*'s green color, and may even add their own blues and greens. Thus *Diplosoma virens* forms dense populations of round, grass-green pads 1 to 2 cm across on the reef flat. And *D. similis*, avoiding the direct sunlight, penetrates extensively into the interstices of coral formations with its intensely blue-green sheets and films.

ORDER PHLEBOBRANCHIA

Family Cionidae

Often considered to be of primitive design, the family Cionidae comprises 12 genera, five of them wholly abyssal or bathyal and two with some deep-sea representatives. This family does not have many shallow-water species, and in the tropics these species are always rare. Of New Caledonia's five cionid genera, *Pterygascidia* and *Araneum* are strictly deep-sea forms. The three shallow-water genera - *Ciona*, *Rhopalaea*, and *Diazona* - have only a single species apiece in New Caledonia

GENERA OF CIONIDAE

1 - Position of gonads in relation to body divisions:

   1 - No narrowing of body between the branchial sac and the gut, gonads in the gut-loop.

1	2	3	4	Genus		Distribution
3	PV0	C	S	*Pseudodiazona* Millar, 1963		Australia & Deep sea
3 2	P00	C	S	*Tylobranchion* Herdman, 1886		Antarctic & Deep sea
2	PV0	S P	S	*Rhopalaea* Philippi, 1843	S	Widely distributed
2	PV0	S P	S	*Rhopalopsis* Herdman, 1880**		West Pacific
2	PV0	C	S	*Diazona* Savigny, 1816	S D	Widely distributed
2	PVP	C	S	*Syndiazona* Oka, 1927		Japan China
1	000	S	0	*Tantillulum* Monniot & Monniot, 1982		Deep sea
1	P00	S	0 S	*Araneum* Monniot & Monniot, 1973	D	Deep sea
1	PTU	S	S 0	*Pterygascidia* Sluiter, 1904	D	Deep sea
1	PV0	S	0	*Dimeatus* Monniot & Monniot, 1982*		Deep sea
1	PVP	S	S	*Ciona* Fleming, 1822	S	Widely distributed
1	PVV	S	S	*Mysterascidia* Monniot & Monniot, 1982		Deep sea

Families Cionidae.

  2 - Body divided into *two* distinct parts, most of the gut under the branchial sac, gonads in the gut-loop.
  3 - The gonads extend into a part of the body posterior to the gut-loop.

2 - Structure of papillae of the branchial wall:
  000 - No papillae.
  P00 - Finger-like *papillae* only.
  PTU - *T*-like *papillae* more or less *united* by longitudinal vessels
  PVP - *Papillae projecting* from complete longitudinal *vessels*.
  PVV - *Papillae* with two longitudinal *vessels*.

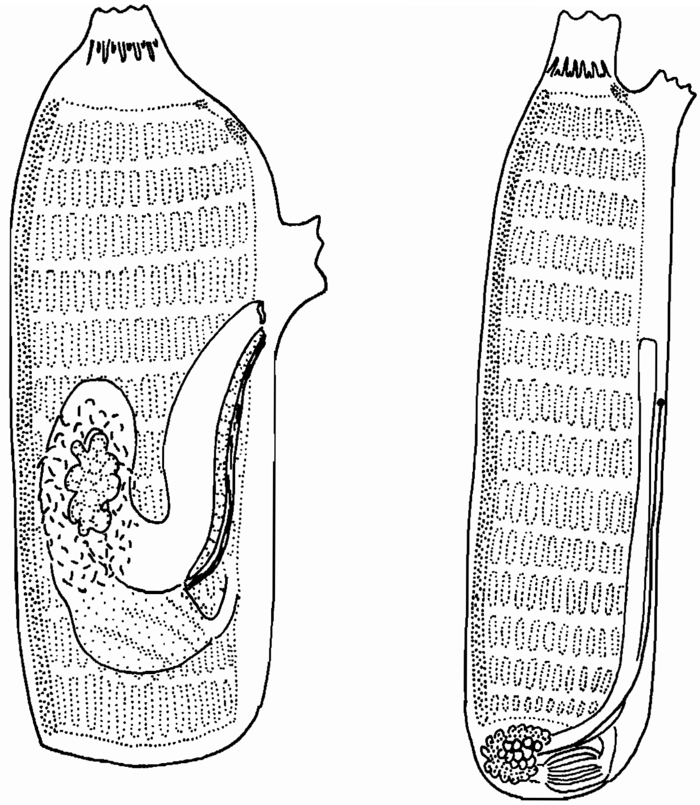
3 - General structure:
  S - *Solitary* ascidians.
  C - *Colonial* ascidians, completely embedded in common test.
  P - *Partially* embedded in common test.
  (When a solitary Cionidae is found without gonads, it may be an oozooid of a colonial species.)

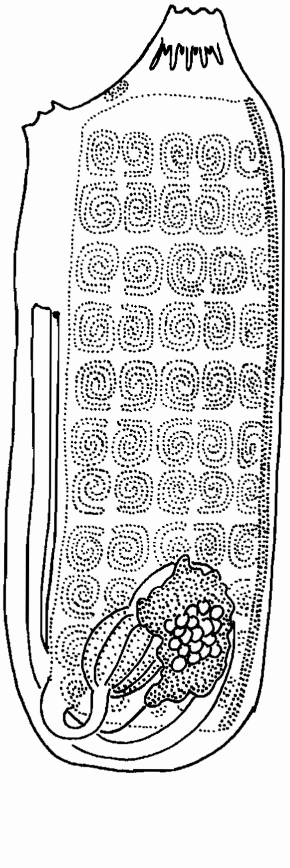
4 - Stigmata:
  S - Ciliated *stigmata*.
  0 - No true stigmata.

* Two cloacal siphons.

** *Rhopalopsis* has minute plications in the branchial sac.

Both *Ciona intestinalis* of the North Atlantic and *C. savignyi* of Japan are common cool-water species that have spread widely as fouling organisms and as a result are known as introduced animals in many temperate-zone ports in both hemispheres. In the tropics, however, *Ciona*'s very presence has been a point of debate. For example, material described as *Ciona* from Indonesia has turned out, on careful re-examination, to be species of

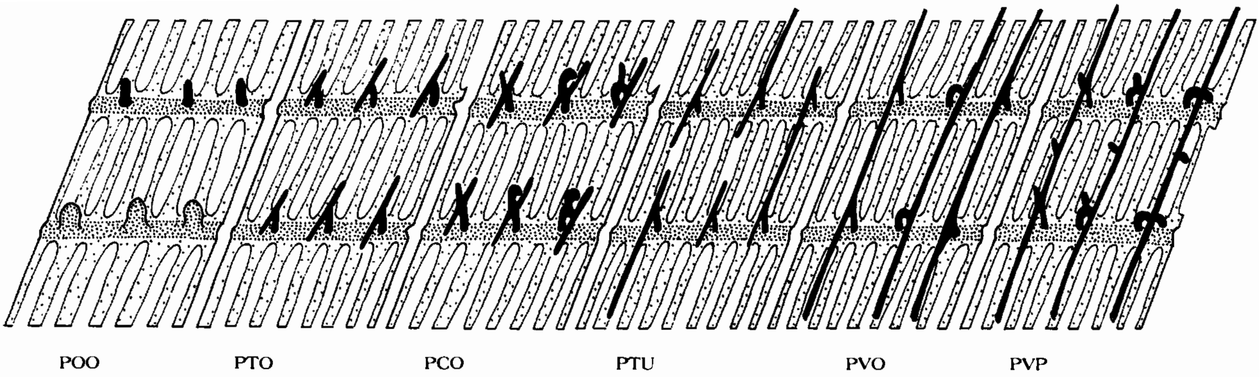


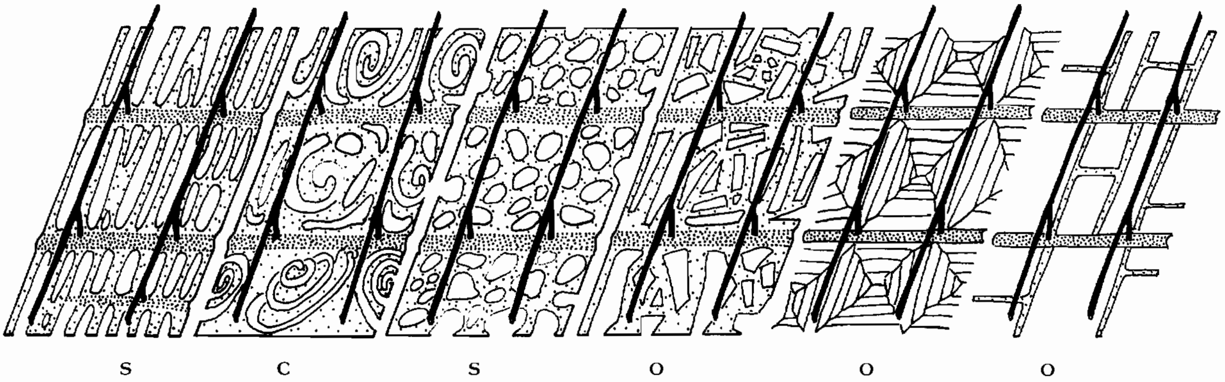


Schemata of the anatomical possibilities of the whole body and of the branchial wall in Phlebobranchs, with key formulae.

Rhopalaea. But recently a true *Ciona* specimen has been found in southern New Caledonia, near the island of Uatio: *Ciona hoshinoi*.

During a cruise of the research vessel *Alis*, a survey of the shallow summit of the Gemini seamount, south of the Loyalty Islands, revealed a population of *Rhopalaea rescipiens*. Solitary or in little bouquet-like colonies of only two zooids, this species looks like a member of the polycitorid genus *Clavelina*, but its branchial sac is quite different. Also, even in the field, *Rhopalaea*'s prominent mantle musculature distinguishes it from *Clavelina*, which has very fine mantle musculature. *R. rescipiens*'s oral siphon is encircled by a brightly pigmented ring, easily visible through the colorless, transparent tunic. This tunic is so clear that one can see the structural (not pigmented) bluish color of the branchial sac's beating cilia.


POO
PTO
PCO
PTU
PVO
PVP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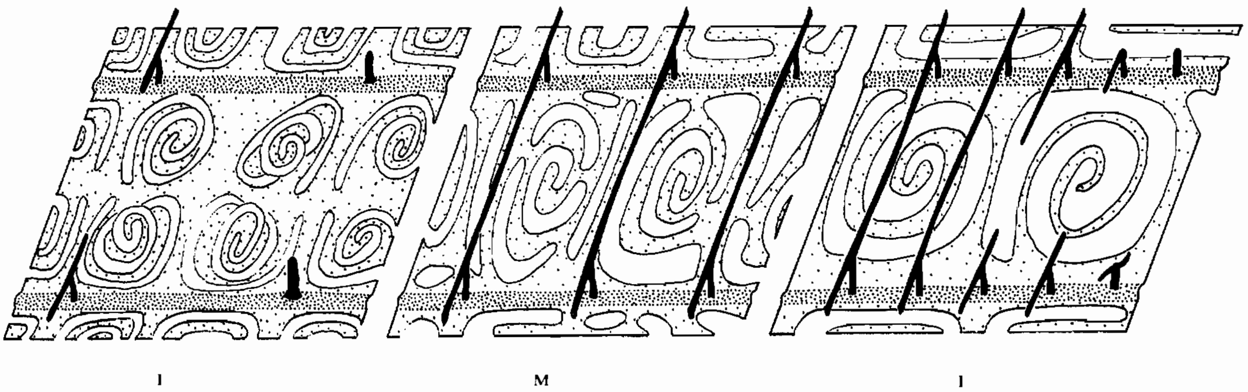
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Rhopalaea respiciens may grow as solitary individuals or form colonies of only a few zooids.

Diazona textura was first described from the waters between Grande Terre and Ile des Pins, where it lives about 50 to 60 m deep. It has also been dredged from depths below 40 m on the seaward slopes of the Loyalty Islands, from the Norfolk Ridge south of New Caledonia, and around the Chesterfield Islands. These are all sites tantalizingly near or beyond the limits of most divers, and so this animal has not yet been photographed or even observed in place. Solitary individuals are not rare, especially at greater depths, but this species is mostly colonial. Its colonies might be mistaken for polyclinid colonies, but *D. textura*'s soft, colorless tunic reveals that its zooids are 2 to 3 cm wide, much bigger than those of most polyclinids. Confirming this identification requires examining the branchial sac's wall. This close scrutiny will separate both *Diazona* and *Rhopalaea* from polycitorids, as well, since it will reveal typically phlebobranch internal longitudinal vessels in these cionids, in contrast to their absence in aplousobranchs.

Family Perophoridae

This is a family of small, sometimes brightly colored phlebobranchs that all form stolonic or bouquet-like colonies. There are only two perophorid genera, each of which has only a small number of species. *Perophora*'s zooids have only 4 or 5 rows of stigmata, depending on how completely the anterior row has divided into two rows during development. *Ecteinascidia*'s somewhat larger zooids always have more than 5 stigmatal rows. Many species of *Perophora* and almost all

	Genus		Distribution
F	*Perophora* Wiegman, 1835	S	Warm and temperate seas
>5	*Ecteinascidia* Herdman, 1880	S	Warm seas

Families Perophoridae.

those of *Ecteinascidia* live in warm shallow waters. In New Caledonia this is a common and diverse family, but the zooids and even whole colonies are often so little that they escape notice.

GENERA OF PEROPHORIDAE

Number of rows of stigmata:

F - *Few*, 3 or 4, and the upper row may be incompletely divided into two, forming a fifth row.

>5 - More than 5 complete stigmata rows.

Perophora zooids are spheres no more than 4 or 5 mm across. Only one local species is easy to see: *P. modificata*, which forms golden yellow sheaths around the axes of gorgonians or colorful clusters on dead corals. This species lives only in the clear waters of the barrier reef, from the reef-flat to depths of about 50 m. In the lagoon, *P. hutchinsoni* grows in little whitish bunches on solid surfaces. Two other shallow-water species, *P. viridis* and *P. multiclathrata*, are pale green or colorless, with zooids well separated from one another along the colony's stolons. Both these species range widely in the tropics.

Species of *Ecteinascidia* are both larger and usually rarer (but more noticeable) than those of *Perophora*. The commonest species on the outer reef slope is *E. jacerens*. Its greenish zooids, 5 or 6 mm long, adhere broadly to the substrate and are linked to one another by a network of light green stolons. In the lagoon, *E. aequale* lives beneath rocks near Noumea. Its zooids can be a cm long and may lie flat, attached ventrally to the substrate, or stand erect, attached posteriorly. Colonies usually contain only a

Perophora modificata has rather short stolons, and its zooids are crowded together.

Ecteinascidia jacerens
may have long stolons, separating
the zooids considerably from each other.
The zooids' numerous rows of stigmata
can be seen through their transparent tunics.

few zooids. Living zooids are olive-green with pigmented lines marking the branchial sac's transverse vessels. The animal's color disappears immediately upon its death. The almost transparent zooids of *E. koumaci* are larger, reaching 1.5 cm, and have orange dots scattered dorsally on their bodies. Colonies of this species have been found only in the Koumac Passage; some isolated individuals have been collected around Noumea.

Several other perophorids live in New Caledonia, but they are too rare or too small to be identified easily. One must examine these obscure forms microscopically to discern the diagnostic details of their musculature and of their gonadal anatomy.

Family Ascidiidae

Among the order Phlebobranchiata, three families of solitary ascidians are closely related. The same anatomical characters are used to isolate genera.

GENERA OF ASCIDIIDAE, CORELLIDAE AND AGNESIIDAE

1 - Position of the gut:
    L - The greater part on the *left*.
    R - The greater part on the *right*.
    U - *Under* the branchial sac.
2 - Shape of stigmata:
    S - *Straight* or ovoid-round.
    C - *Curved* stigmata.

O - No ciliated stigmata, or an irregular network of angular stigmata that resembles a spider's web.

3 - Relationship between spirals and meshes of branchial wall.
  M - One spiral per *mesh*.
  I - Spirals *Independent* of meshes.
4 - Structure of the branchial wall:
  OOO - No papillae.
  POO - Finger-like *papillae* only.
  PTO - *T*-like *papillae* only.
  PCO - *Cross*-like *papillae* only.
  PTU - *T*-like *papillae* more or less *united* by longitudinal vessels
  PVO - *Papillae* all united by longitudinal *vessels*.
  PVP - *Papillae projecting* from complete longitudinal *vessels*.
5 - Structure of dorsal lamina:
  L - Divided into a series of *languets*.
  D - Continuous dorsal lamina with free edge *dentate*.
6 - Incubation:
  I - *Incubation* of embryos.
  O - *Oviparous*.
7 - Special peculiarities:
  V - Flap-like *valves* covering both siphons.
  A - Long neural duct with *accessory* openings into cloacal cavity (visible only in large specimens).

In the family Ascidiidae, the order's traits reach their fullest expression, least modified by specialization. This is also the most diverse phlebobranch family, with as many species as there are in all the order's other families combined. The ascidiid branchial sac has rows of straight stigmata and internal longitudinal vessels with accessory papillae, and the gut-loop lies in the mantle on the left side of the body.

1	2	3	4	5	6		Genus		Distribution
							Family ASCIDIIDAE		
L	S		PV0	D	0		*Ascidiella* Roule, 1883		North Atlantic N.Z.
L	S		PVP	D	0		*Ascidia* Linné, 1767	S D	Widely distributed
L	S		PVP	D	0	A	*Phallusia* Savigny, 1816	S	Widely distributed
L	S		PVP	D	I		*Psammascidia* Monniot, 1963		English Channel
							Family CORELLIDAE		
R	S		P00	L	0		*Xenobranchion* Ärnbäck, 1950		Antarctic & Deep sea
R	S		PV0	D	0		*Abyssascidia* Herdman, 1880		Deep sea
R	S		PV0-PVP	D	0	V	*Rhodosoma* Ehrenberg, 1828	S	Warm seas
R	S		PVP	L	I		*Dextrogaster* Monniot, 1962		Scandinavia
R	C	M	PV0	L	0		*Corella* Alder & Hancock, 1870	S	Widely distributed
R	C	M	PT0-PVU	L	0		*Chelyosoma* Broderip & Sowerby, 1830		North hemisphere
R	C	M	PC0	L	0		*Corelloides* Oka, 1926		North Pacific
R	0		PV0	L	0		*Clatripes* Monniot & Monniot, 1974		Deep sea Argentina
							Family AGNESIIDAE		
L	C	I	000	L	0		*Proagnesia* Monniot & Monniot, 1973		Deep sea
L	C	I	P00	L	0 I		*Agnesia* Michaelsen, 1898		Arctic Antarctic Deep sea
L	C	I	PT0	L	0		*Adagnesia* Kott, 1963	S D	Widely distributed
L	C	I	PT0	D	0 I		*Caenagnesia* Ärnbäck, 1938		Antarctic Deep sea
U R	C 0	I	PT0-PV0	L	0		*Corynascidia* Herdman, 1882	D	Deep sea

Families Ascidiidae, Corellidae and Agnesiidae.

New Caledonia's ascidiids, all species of the genera *Ascidia* and *Phallusia*, share several fieldmarks: a translucent tunic that in some species contains brightly colored elements; gaping siphons that shut tight when disturbed but usually do not retract as far into the body as do those of other solitary ascidians; broad attachment to the substrate by the body's left or left-posterior region; and, fairly often, a mud-filled gut-loop, visible through the tunic if one detaches the animal from the substrate. Eleven ascidiid species have been found in New Caledonia. Of these, only 5 are readily recognizable or common; the others are smaller (1 to 2 cm), rarer, and inconspicuous, and live inaccessibly beneath rocks and rubble or in fissures or among shells or coral branches. Identifying these small species requires dissection and microscopic examination.

New Caledonia's most common and spectacular big ascidiid is *Phallusia julinea*, which can reach a length of 20 cm. Specimens from deep water are almost colorless, but most animals from shallower habitats have a characteristic sulfurous yellow color. The color is imparted by pigmented blood cells that accumulate in the extensive circulatory channels of the animal's clear tunic. This yellow fresh blood oxidizes to a greenish black; an injured specimen will blacken one's hands. The tunic is clean, never obscured by epibionts. *P. julinea* has been dredged from bottoms more than 100 m deep on the outer reef slope, but the species avoids the barrier reef's more turbulent shallow waters. It also lives among patch corals in quieter parts of the lagoon, and it prospers under the docks in Noumea harbor and in slightly muddy places in Dumbea Bay.

Less striking but still prominent, *Ascidia sydneiensis*, a cosmopolitan warm-water species, flourishes not only on buoys in ports but also on broken shells in sheltered lagoon waters. This animal grows to some 12 cm. The drab, transparent tunic around its gray or whitish body is often wholly obscured by epibionts. Its rather elongate, distinctly furrowed siphons sometimes have traces of purple. *A. sydneiensis's* mantle musculature is very odd: its right side has only a fringe of short muscle fibers that are set perpendicular to the dorsal and ventral midlines, while the central part of the right mantle completely lacks muscles. The gut-tract of this species is also extraordinary: even in bodies only 0.5 cm long and even more in larger ones, the intestine is grossly swollen by an enormous mass of mud; only a few other species of *Ascidia* show this bizarre intestinal dilatation. *A. munda*, a smaller (2 to 5 cm) and rarer species than *A. sydneiensis*, has the same unusual right-mantle musculature, but it lacks any marked intestinal swelling. Mature specimens of *A. munda* are recognizable, too, by their bright pink eggs.

Two other fairly common species of moderate size (2 to 6 cm) are *A. glabra* and *A. gemmata*. They live hidden among corals and under rocks in calm lagoon waters and behind the barrier reef. *A. glabra* is smooth-surfaced; *A. gemmata's* tunic feels grainy. Both species vary from white to partly or entirely red. A net-

Phallusia julinea is the most brightly colored member of the family Acidiidae in New Caledonia. Without epibionts, it is often deeply inserted in the furrows of brain corals.

Unobtrusive with its drab color and transparency, *Ascidia sydneiensis* (Ascidiidae) is living here attached to some dead coral. The darker brownish patch is the digestive tract, which is full of mud.

Ascidia munda,
colorless and transparent,
often inhabits dead shells.

work of muscle strands invests the entire right mantle in both these animals. The species can be told apart by their intestines: *A. gemmata*'s is unexceptional, but *A. glabra*'s is swollen and mud-packed like that of *A. sydneiensis*.

A third ascidiid in this size range, *A. melanostoma*, is rare and restricted to places behind the barrier reef. As its name suggest, *A. melanostoma*' siphons vary from light brown to black, apparently according to how sunny its attachment site is. Finally, the little, whitish *A. alterna*, which lives on the underwater banks south of the Chesterfield Islands, provides a good example of an ascidian-crustacean symbiotic partnership; its branchial sac almost always houses the decapod shrimp *Periclimenes monnioti*.

Family Corellidae

This family has only a few species and is never abundant in the tropics even though some of its species are widespread there. Corellids are unusual in having their gut-loop in the right mantle, where its course differs distinctly from that of gut-loops that lie in the left mantle. The corellid branchial sac may have rows of magnificent spiral stigmata (as in the genus *Corella*) or of short, straight stigmata (as in *Rhodosoma*). Both these genera are represented in New Caledonia.

The little (1 cm)) *Corella minuta* lives in very shallow water under rocks, in fissures, on on the reef-flats, and on the fringing reefs of some islets in the lagoon. The animal attaches broadly to the substrate, like a drop of gelatin, revealing its brown gut-tract through its transparent, colorless tunic. Its spiral stigmata must

Rhodosoma turcicum has both siphons hidden in a dorsal groove and covered by a 'lid' of tunic.

be artificially stained to be seen. This animal, though widely distributed in tropical waters, is never abundant and lives only in high-energy, even wave-battered habitats. It is almost always found with *Ascidia archaia*, which shares both its geographic spread and ecological preference.

Rhodosoma turcicum grows to a length of 2 to 5 cm and, with its flat top, is easy to recognize. The animal lies on its side under rocks, in dead shells, and among coral branches. Its translucent tunic is light ochre or rather colorless, and tough or even hard. The animal's two siphons, close to each other, lie on a flat platform that is creased so that half of it acts as a flap that can close over the other half, exactly like a lid or the valve of an oyster. Powerful, specialized muscles control this flap to expose or hide the siphons. Though generally uncommon, *R. turcicum* is a cosmopolitan species in warm water, occurring even in the Mediterranean Sea. It was the very first ascidian to be reported from New Caledonia - from the Bellona Reef in 1862!

Family Agnesiidae

In the agnesiids, all solitary ascidians, branchial stigmata form spirals. But unlike corellids, in which each such spiral neatly fills a mesh between transverse and internal longitudinal vessels, agnesiids have stigmata independent of the vessels and papillae arrangement. The agnesiid gut-loop lies in the left mantle or, cionid-like, under the gut, sometimes then spilling over a little onto the right side.

This small family is most diverse in the deep sea. Deep dredging

	Genus		Distribution
S	*Microgastra* Kott, 1985	D	Indo-Pacific
C	*Plurella* Kott, 1973		Australia

Family Plurellidae.

on the slopes and banks around New Caledonia have brought up two kinds of agnesiids: the very soft, transparent and fairly large (3 to 4 cm) *Corynascidia alata* and a species of *Adagnesia* that is, like many abyssal members of this genus, a tiny animal only a few mm across.

Recently, a minute (2 mm) species, *Adagnesia cautis* has been found living interstitially in a gravel bottom near the M'Ba reef. It is the first species of its family to be found in shallow tropical waters.

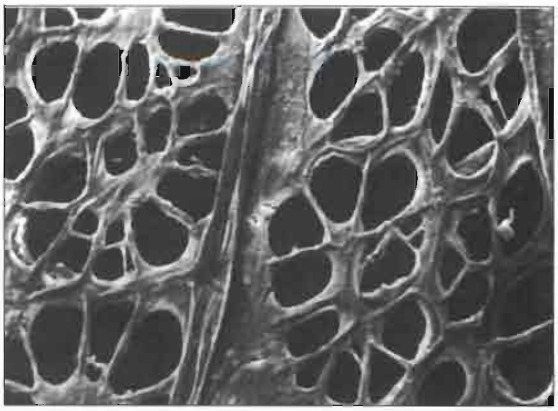
Family Plurellidae

Structure of the body:
S - *Solitary*, single ovary.
C - *Colonial*, several ovaries.

This curious family has only two described species assigned to two very different genera. The colonial *Plurella elongata* lives only in Australia. The solitary *Microgastra granosa* lives widely in the western Pacific and in the Indian Ocean. It inhabits submarine banks south of New Caledonia and the slopes around the Loyalty Islands; it should be watched for in shallower waters, too. New Caledonian specimens are 3 to 5 cm across and of a rather squat shape, broadly attached to the substrate. *M. granosa*'s tunic is so thoroughly impregnated with sand that living animals are very brittle; it encases an extremely fragile body. Very peculiar, plurellids protrude their gonads from the body into pockets in the surrounding tunic.

1	2	3	Genus		Distribution
0	F	S	*Cryptia* Monniot & Monniot, 1983		Atlantic
0	C	S	*Hypobythius* Moseley, 1879*		Atlantic
?	C	S	*Benthascidia* Ritter, 1907*		California
2	C	S	*Dicopia Sluiter*, 1905	D	Widely distributed
2	F	S	*Situla* Vinogradova, 1967	D	Widely distributed
2	0	S	*Cibacapsa* Monniot & Monniot, 1985		Antarctic
8	C	S	*Octacnemus* Moseley, 1879	D	Widely distributed
8	C	C	*Polyoctacnemus* Ihle, 1935		Chile

Family Octacnemidae.



Part of the branchial sac of a species of *Dicopia* (Octacnemidae). This view shows the endostyle and the adjacent left and right branchial walls.

Family Octacnemidae

This is an exclusively abyssal family of truly extraordinary ascidians. Their bodies take very different shapes from other members of the class. Unlike any other ascidians, octacnemids do not filter-feed. Instead, they gather relatively large prey. The octacnemid oral siphon has been transformed into a trap that shuts on small animals that swim too close. A branchial sac with stigmata persists in some octacnemid genera, but in others these perforations are reduced to mere rounded holes, arranged roughly in circles, that lack any cilia to create a branchial water-current.

GENERA OF OCTACNEMIDAE

1 - Structure of the oral aperture:
  0 - Plain edged.
  2 - With *two* lips.
  8 - With *eight* lobes.
2 - Shape of the branchial sac:
  C - *Cone* shaped.
  F - *Flat*, surrounding the esophagus entry.
  0 - Without stigmata.
3 - General structure:
  S - *Solitary* ascidian.
  C - *Colonial* ascidian.

In deep water around New Caledonia - on the outer slopes and in the basin of the Loyalty Islands - this odd family is well represented by three genera and six species. In fact, the New Caledonian

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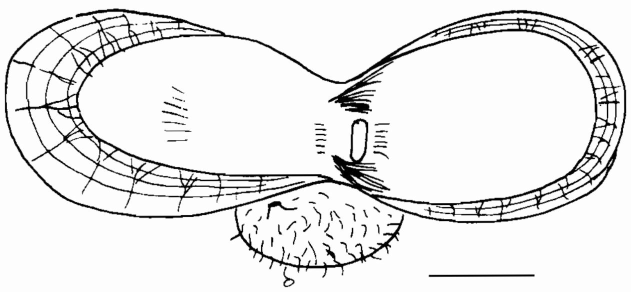

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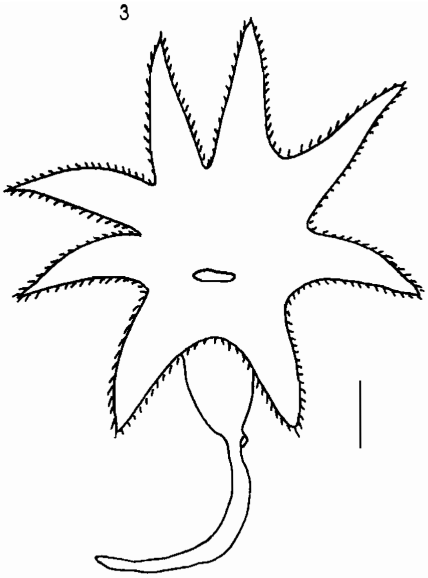

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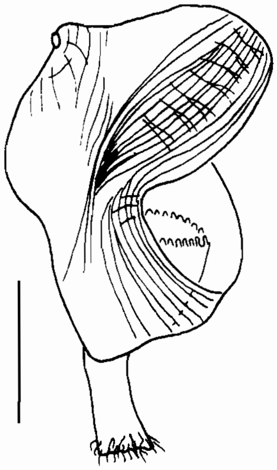


The Octacnemidae
are animals of bizarre shapes.
These are not filter-feeders;
they capture relatively large prey,
often small crustaceans,
by trapping them with their lips or lobes.
The three species *Dicopia fimbriata* (1),
Situla galeata (2), and *Octacnemus ingolfi* (3)
live in the Loyalty Basin.
Scale = 1 cm.

region has the world's greatest diversity of octacnemid ascidians. It is a pity they live so far beyond the limits of access by Scuba; perhaps deep submersibles may let us observe these creatures some day in their natural surroundings.

ORDER STOLIDOBRANCHIA

Family Styelidae

It is virtually impossible to summarize a set of traits that adequately characterizes the family Styelidae. Styelid structures and appearances have by far the greatest range among ascidians families, and styelids can resemble in one way to another species of almost any other family. Both solitary and colonial styelids attain their greatest diversity in the tropics. Sizes range from the colonial *Chorizocarpa*'s round zooids, a mere 1 to 2 mm diameter, which one can confuse at first glance with those of a *Perophora*, to the large solitary *Polycarpa pigmentata*, 15 cm long and 10 cm across, which, hidden by abundant epibionts, can be confused at the first glance with a rock. To the styelids, too, belongs the immense *Polycarpa clavata*, the largest New Caledonian ascidian.

1	2	3	4	5	6	7	8	9	10	Genus		Distribution
C	V3	U	B	B	0	0	1	G	L	*Botryllus* Savigny, 1816	S	Widely distributed
C	V3	U	R L	L	0	0	1	G R	L	*Chorizocarpa* Michaelsen, 1904	S	Australia Indonesia
C	V3	M	R	0	B	E	1	G	T	*Protostyela* Millar, 1954		Great Britain
C	V3-4	R	0	0	B	N	1	G	L	*Symplegma* Herdman, 1886	S	Widely distributed
C	V3-8	M	B	0	B	E	1	G	L	*Polyzoa* Lesson, 1830		Widely distributed
C	V4	B	0	0	L	P	1	G	L	*Kukenthalia* Hartmeyer, 1903		Atlantic Arctic
C	V4	U	L	L	0	0	1	G	T	*Berrillia* Brewin, 1952		New Zealand
C	V4-10	M U	B	B	B	E	1	G	L	*Metandrocarpa* Michaelsen, 1904(3)	S	Widely distributed
C	V5-n	U	L	R B	0	0	1-n	G	L	*Alloeocarpa* Michaelsen, 1900		Antiboreal
C	Vn	M	B	B	B	E	1	G	L	*Theodorella* Michaelsen, 1922		New Zealand
C	Vn	U	B	L	0	0	1	G	L	*Okamia* Brewin, 1948		New Zealand
C	F	B	0	0	B	N	1	G	T	*Tibitin* Monniot, 1983		Widely distributed
C	F	U	L	R	0	0	n	G E	L	*Oligocarpa* Hartmeyer, 1911		Kerguelen I. South Africa
C	F	U	B	0	0	0	1	G	L	*Arnbackia* Brewin, 1950		New Zealand
C	F	B	0	0	R	E	1	E	L	*Oculinaria* Gray, 1868	S	Australia New Caledonia
C	F	B	0	0	R	N	1	G	L	*Gynandrocarpa* Michaelsen, 1900(2)		South Africa
C	F	B	0	0	R	N	1	G	L	*Dextrocarpa* Millar, 1955(2)		South Africa
C	F	U	L	R	0	0	1	G	L	*Distomus* Gaertner, 1774	D	Atlantic
C	F	M	B	0	L R	I	1	G	L	*Stolonica* Lacaze-Duthiers, 1892	S	Widely distributed
C	F	M	B	0	B	I	1	G	L	*Amphicarpa* Michaelsen, 1922	S	Widely distributed
C	F	B	0	0	B	E	1	G	L	*Polyandrocarpa* Michaelsen, 1904(1)	S	Widely distributed
S	V3	B	0	0	B	E	1	G	L	*Dicarpa* Millar, 1955		Antarctic Deep sea
S	V	B	0	0	B	N	1	E	L	*Pelonaia* Goodsir & Forbes, 1841		Atlantic Boreal
S	V	B	0	0	B	E	1	G	L	*Monobotryllus* Oka, 1915		India
S	V	B	0	0	B	E	1	G E	T	*Bathystyeloides* Seeliger, 1904	D	Deep sea
S	V F	B	0	0	B	E	1	E	0	*Bathyoncus* Herdman, 1882	D	Deep sea
S	F	B	0	0	B	E	1	G	L	*Polycarpa* Heller, 1877	S D	Widely distributed
S	F	B	0	0	L	E	?	G	L	*Skaiostyela* Sluiter, 1904		Indonesia
S	F	B	0	0	B	P	1	G	L	*Seriocarpa* Diehl, 1969(4)		Widely distributed
S	F	B	0	0	B	E	1	E	L	*Cnemidocarpa* Hunstman, 1912	S D	Widely distributed
S	F	B	0	0	B	E	1	R	L	*Asterocarpa* Brewin, 1946		Antiboreal
S	F	B	0	0	B	N	1	E R	L	*Styela* Fleming, 1822	S D	Widely distributed
S	F	B	0	0	R	E	n	E R	L	*Dendrodoa* Mac Leay, 1825		Boreal
S C?	F	B	0	0	R	E	n	R	L	*Syndendrodoa* Tokioka, 1951		Japan

Family Styelidae.

GENERA OF STYELIDAE

1 - General structure:
- C - *Colonial* ascidian.
- S - *Solitary* ascidian.

2 - Structure of the branchial wall:
- V - Only with longitudinal *vessels* (3, 4, ... n), no folds.
- F - With at least one longitudinal *fold*.

3 - Sexual composition of gonads:
- B - *Bisexual* (hermaphroditic) gonads.
- U - *Unisexual* gonads.
- M - In each body both unisexual and hermaphroditic gonads.

4 - Disposition of unisexual male gonads:
- R - On the *right*.
- L - On the *left*.
- B - On *both* sides.
- 0 - No male gonads.

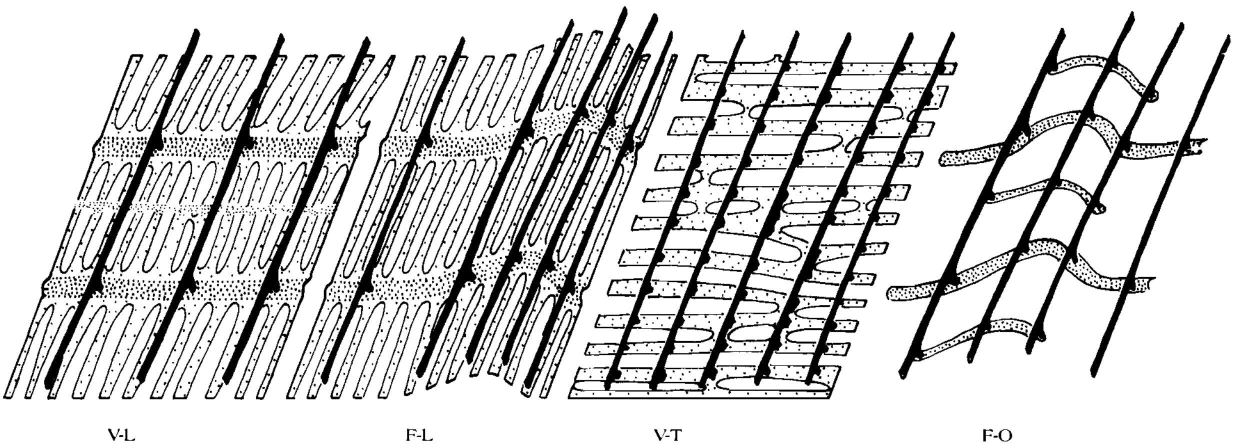
5 - Disposition of unisexual female gonads: R, L, B, O.
6 - Disposition of bisexual (hermaphroditic) gonads: R, L, B, O.
7 - Disposition of male and female glands in bisexual gonads:
  E - Male and female glands *enclosed* in a massive gonad.
  N - Male and female glands *not* enclosed in a common envelope, sperm ducts running upon the inner side of the ovary.
  P - Gonads *protruding* through the external wall of the body and inserted into the tunic.
  I - *Independent* male and female ducts.
8 - Number of sperm ducts per gonad: *1*, *N*.
9 - Shape of the ovary:
  G - *Globular*.
  E - *Elongated* (length more than three times the width).
  R - *Ramified*.
10 - Shape of stigmata:
  T - Only *transverse* stigmata, but they may be interrupted.
  L - *Longitudinal* stigmata, at least in the anterior part of the branchial wall.
  O - No stigmata, branchial sac with only transverse and longitudinal vessels.

(1) - Each gonad with 1 testis: *Monandrocarpa* Michaelsen, 1904. Each gonad with 2 testes: *Eusynstyela* Michaelsen, 1904. Each gonad with n testes: *Polyandrocarpa* Michaelsen, 1904 *sensu stricto*.

(2) - Genital ducts opening in the branchial cavity by a permanent aperture: *Gynandrocarpa*. No permanent connection between the brood-pouch and the branchial cavity: *Dextrocarpa*.

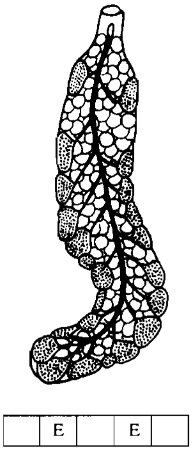
(3) - Numerous variations in the gonads' distribution: sometimes male and female gonads in separate zooids.

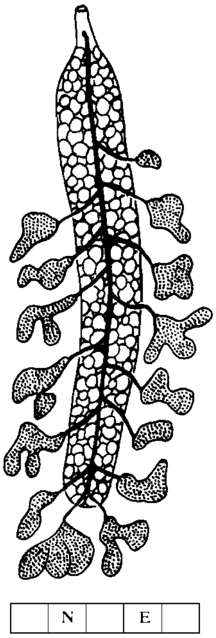
(4) - *Seriocarpa* has testes with the structure of *Eusynstyela* but included in the tunic. The type species of this genus may cut its body in two parts, giving two immediately isolated zooids.



THE COLONIAL STYELIDAE

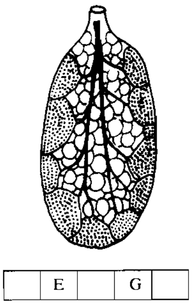
Genera of colonial styelids differ greatly in appearance, not only because their zooids are larger or smaller but also because they differ in their manner of budding (as we have discussed in sections "Budding and Regeneration: Asexual Reproduction" and "From the Individual to the Colony: Ascidians Diversity".





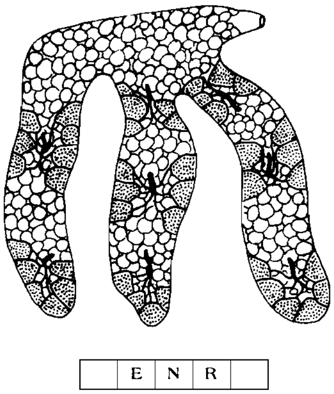
Colonies with common cloacal cavities

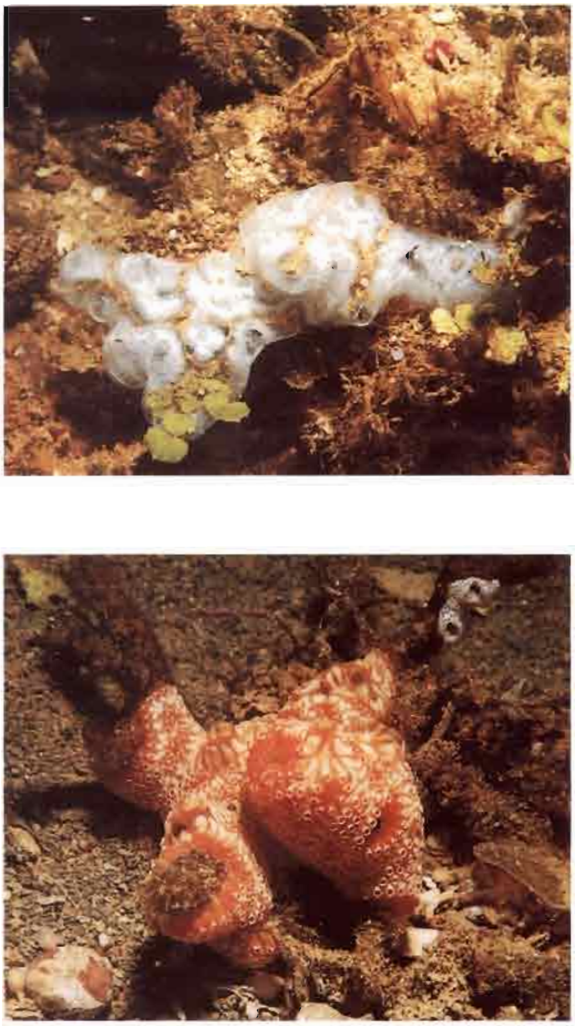
The genus *Botryllus* has the most complex colonies. Stubby, small (1 to 4 mm) zooids orient their long axes perpendicularly to the colony's surface. They arrange themselves around lacunae in the tunic that may be simple common cloacal cavities or long, meandering common cloacal channels. Living animals are brightly colored, often with several colors creating regular patterns on each zooid. These colors and banded patterns are genetically controlled; their wide variations are the results of differing combinations of interacting alleles. Blood cells that circulate through vessels and terminal ampullae in the tunic, and the walls of these ampullae themselves, are also colorful and in different hues than the zooids.



Botryllid tunics also vary in consistency. Many species grow as thin crusts, but some others form thick, even lobate, sheets closely resembling polyclinid or polycitorid colonies. Of the seven species of *Botryllus* living in New Caledonia, three are very rare and usually inconspicuous and form only small colonies (less than 1 to 2 cm across). We will comment only on the four more regularly encountered species.

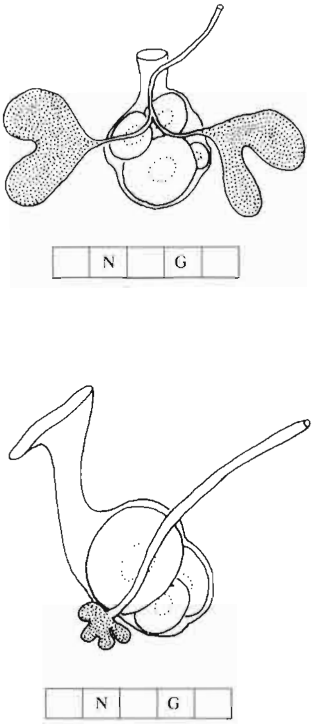
The most common botryllid is *B. leptus*, and it is also the most variable. It abounds on the bottom of the lagoon on all sorts of





The botryllids have small zooids, from 1 to 4 mm long, wholly embedded in the colony's tunic along common cloacal channels. Here *Botryllus leptus*, abundant in New Caledonia, shows some of the confusing variability of its colonies.

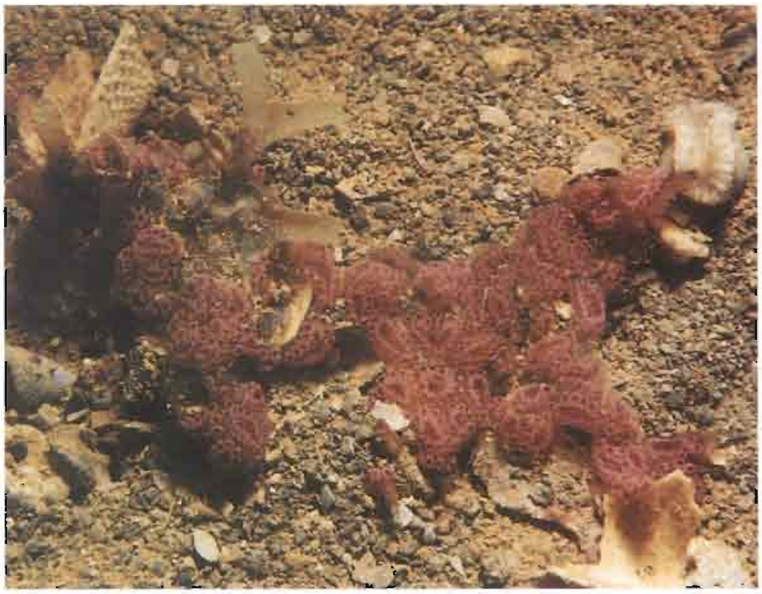
Diagrams of branchial walls and gonads in some members of the family Styelidae, with key formulae.



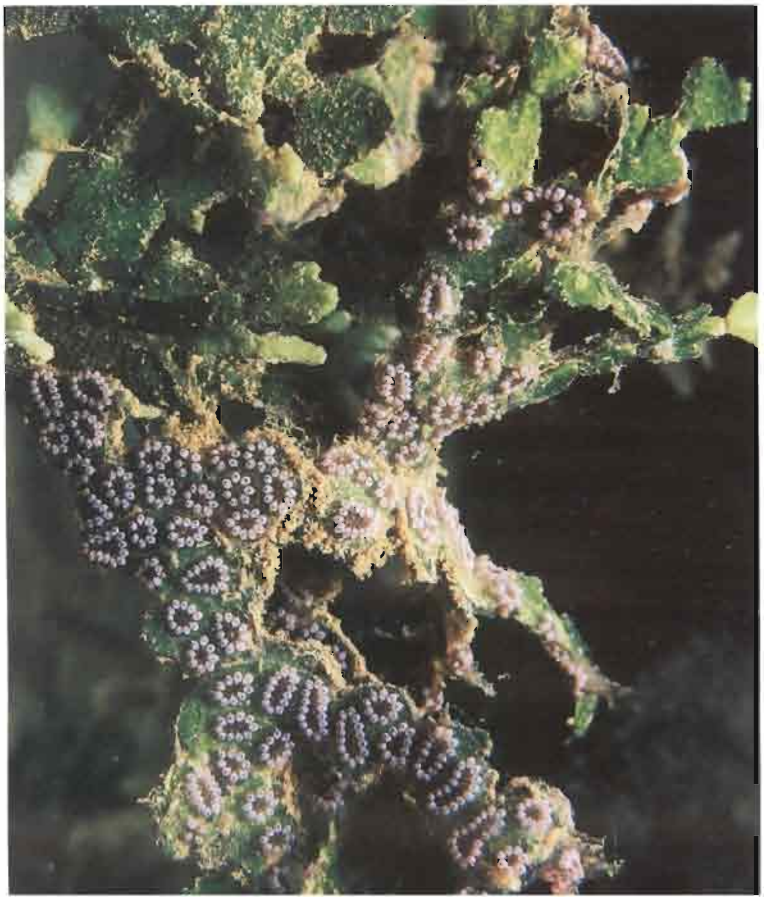
substrates, where it forms large colonies of often sumptuous colors. The zooidal systems may be circular or meandering. This species also lives in Australia.

Rarer but as recognizable, *B. tuberatus* inhabits sheltered parts of the lagoon. Zooids have only four rows of branchial stigmata. New Caledonian colonies have violet or green zooids that form circular systems, visible through the colorless tunic. In other parts of its immense range through the tropical Pacific and Atlantic, this species may be quite differently colored. In the Caribbean, for example, *B. tuberatus* is deep green, almost black.

B. niger, a magnificent species, forms thin, bright orange sheets that turn pitch black in death. In New Caledonia this species has



Botryllus tuberatus can be identified by its almost circular systems. Colonies come in a great range of colors, from deep purple to rose to light green.



been found only in the port of Noumea, under wharfs and buoys - the classic habitat of fouling organisms. *B. niger* probably originated in the western tropical Atlantic, where it shows a very wide polychromatism, and has been carried by ships into western Pacific ports like Noumea and Papeete, where it appears in only its orange form.

The completely sand-covered *B. arenaceus* attaches to bits of solid material on the sandy bottom of the Woodin Strait and on the outer reef slope southeast of New Caledonia. Each colony's lobe forms a squat little cylinder about a cm in diameter and a cm long with a single, large, dark common cloacal aperture at its center. In superficial appearance it is so much like an actinian or zoantharian polyp that it fools many divers.

Botryllus arenaceus is difficult to detect on a littered bottom, as its colony is encrusted with sand. It is living here among the didemnids *Leptoclinides* and *Polysyncraton*.

Colonies whose zooids have independent cloacal siphons

The genus *Chorizocarpa* has a very reduced design. Its branchial sac has only three internal longitudinal vessels on each side, and its zooids and even its entire colonies are entirely male or female, not hermaphroditic. Colonies are not thick, botryllid-like sheets but rather bunches of distinctly separate zooids connected by stolons. *C. guttata*'s little (2 to 3 mm) zooids are bright yellow with a white ring around each siphon. This very inconspicuous species is known only from New Caledonia and from Port Jackson, Australia.

Two very different New Caledonian species of *Symplegma* form encrusting colonies whose tiny, ovoid zooids (a few mm long) have four internal longitudinal branchial vessels per side, not *Chorizocarpa*'s three. The most common *Symplegma*, *S. alterna*, is especially abundant in protected parts of the lagoon. Its colonies' tightly packed zooids may stand rather erect on the substrate or lie flat and broadly attached by their ventral surfaces; in either case their two little siphons are clearly visible. Colonies, which may span many cm, vary from sheets embedding all the zooids to thin films with distinctly separate zooids. The delicate tunic is rendered virtually opaque by yellow, pink-white, or white pigments held in blood cells throughout the tunic's extensive vascular system. The color fades rapidly when a colony dies. Colonies are bordered by numerous vascular ampullae the same color as the zooids. *S. alterna* has an unusual sexual cycle, the inspiration for its name. A colony is first male, with well developed testes but with some very small oocytes lodged near their sperm ducts; then the testes regress and persist only as tiny remnants while the ovaries mature in their turn.

In New Caledonia, *S. oceania* lives only in the port at Noumea. Its zooids are simultaneously hermaphroditic, and the tunic is quite transparent. Zooids have bluish spots on their siphons and some color in their meshes of transverse and internal longitudinal branchial vessels. *S. oceania* is another species that has arrived in New Caledonia by ship. By chance, T. Tokioka in 1961 described this species from material taken from the hull of an old boat in Noumea harbor. The specioes is now known from ports and marecultural facilities all over the tropical Pacific; there is no way to tell what its original home may have been.

The other colonial genera, *Metandrocarpa* and *Eusynstyela*, have rounded zooids ventrally attached to the substrate and linked by stolons within a fairly thin, opaque tunic. Although these genera look alike externally, they have significantly different branchial structures. *Metandrocarpa's* branchial sac has, besides transverse

Symplegma alterna forms encrusting colonies of numerous, densely packed zooids.

vessels, only a series of regularly spaced internal longitudinal vessels; in contrast, *Eusynstyela* has well developed branchial folds, each carrying many internal longitudinal vessels. Each genus has a few New Caledonian species.

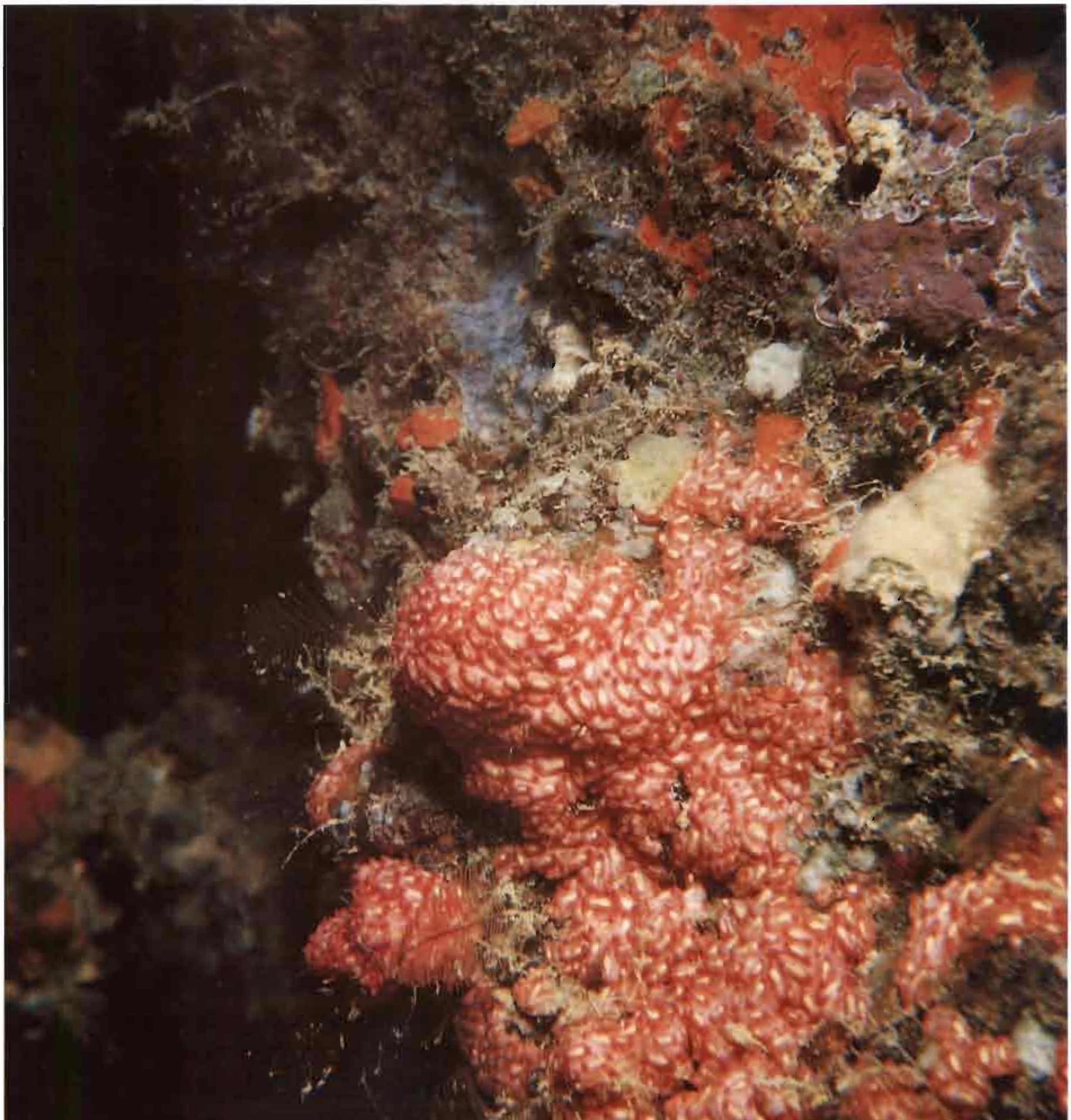
M. manina has zooids 4 to 5 mm across that are barely linked to each other by fine strands of tunic. It lives both in the port of Noumea and on the outer reef slope. *M. reducta*'s tiny, colorless zooids (2 mm) form colonies that often nestle in sponges or around clumps of hydroids.

Even a single species of *Eusynstyela* may have colonies of well separated zooids or tightly packed ones, so colonial pattern is not reliable taxonomic clue in this genus. The blood-red species are *E. latericius* and *E. misakiensis*. The later has a distinctive, white band between the siphons. The two species differ, as well, by their gonadal arrangement aligned in a single rows on each side of the endostyle in *E. latericius*, but mostly in double rows in *E. misakiensis*. Despite their bright color and the size (6 mm to 1 cm) of their zooids, these species are not generally to be seen when one is diving, for they lurk in such inaccessible places as the reef's deeper hollows and inside the valves of dead molluscs.

Genera with erect zooids linked by fine stolons

Two genera, *Amphicarpa* and *Stolonica*, have somewhat bigger zooids (1.5 to 2 cm high) that stand erect on the substrate. The differences between these genera are not obvious. Both have branchial folds. Both genera also have many compact, little gonads, but they differ in the way these are arranged in the internal mantle wall of the peribranchial cavity. *Amphicarpa* has testes and hermaphroditic gonads on both sides of the body - some-

In *Metandrocarpa manina* the zooids are well separated from each other, interconnected by scarcely visible vessels that traverse the colony's transparent and thinly encrusting tunic.



Eusynstyela misakiensis
forms a crust of tightly linked,
bright red zooids, each with a white stripe
between its siphons.

The beautiful *Stolonica variata* is not common in New Caledonia. Its deep red zooids are erect, united by stolons within the colony's thin tunic.

times more than a hundred - set in lines along both sides of the endostyle and in irregular groups in the mantle. *Stolonica* has a row of gonads on each side of the endostyle, one composed entirely of testes and the other on hermaphroditic organs. A third genus, *Distomus*, lacks hermaphroditic gonads and instead has only testes in the right mantle wall and only ovaries in the left. No shallow-water species of *Distomus* is known from New Caledonia, but one species, *D. pacificus*, lives on the summit of a deep-sea plateau not far to the south.

The rare *S. variata* forms large patches of a lovely red with white dorsal spots (hence its name). It has been found only three times - in New Caledonia at the islet Surprise and the Ile des Pins, and in Australia at Marion Reef.

Three species of *Amphicarpa* live in New Caledonia's lagoon. *A. duploplicata* has delicate, light brown tunic somewhat obscu-

Amphicarpa duploplicata has a thin tunic coated with sediment. The border of each siphon has four yellow spots, characteristic of the species. This colony has another colonial styelid, *Symplegma alterna*, growing on it.

red by sediment, but distinctively marked by four orange (or occasionally green) spots on each siphon. The species forms dense, extensive masses of hundreds of even thousands of zooids, doubtless of many colonies intermixed, where strong currents wash the bottom of the lagoon. In the Woodin Strait, colonies of this animals abound not only directly on the bottom sediment but also on the dead parts of gorgonians.

The pale brown *A. laboutei* closely resembles *A. duploplicata* but has slightly smaller zooids (about 1.5 cm), and its siphons have four red marks. Again, accumulated sediment often hides the zooids' color. The species are best told apart by their branchial folds - two on each side in *A. duploplicata*, three on each side in *A. laboutei*. A third species of this genus, *A. agnata*, has been found only in the northern lagoon. It has many fewer gonads than the other New Caledonian *Amphicarpa*, and its branchial sac has three folds on the left side and four on the right.

Colonies with large, tightly united zooids: *Polyandrocarpa*

The genus *Polyandrocarpa* does not differ in any anatomical traits from the genus *Polycarpa*. The sole difference between these two genera is that *Polyandrocarpa* forms buds and *Polycarpa* does not bud - at least not habitually. *Polyandrocarpa*'s buds usually emerge from often very short stolons to produce colonies in which the closely packed zooids are linked by fine vascular connections in the common tunic. A bizarre mode of budding involving evisceration occurs in some species of *Polyandrocarpa* and has its counterpart in the behavior of some species of *Polycarpa*. In both genera, all the gonads are hermaphroditic.

P. rollandi grows almost everywhere, often in very shallow water, in the lagoon and in the port of Noumea. Its colonies form patches that span many cm, so heavily impregnated with sediment that they can be confused with the bottom itself if their tightly packed zooids have contracted. The grayish zooids, 2 to 3 cm long, have black siphons that project in many directions as stiff tubules from the encrusting colony's mass. The zooids are exceedingly difficult to remove undamaged from the colonial tunic for individual examination.

P. glandulosa is one of those *Polyandrocarpa* in which budding involves evisceration. Near the gut-tract, a mass of dense tissue projects posteriorly from the left wall of each zooid's mantle into the tunic. At sexual maturity, the zooid expels its gut-tract, its branchial sac, and its sexual products. Only the mantle remains after this evisceration. Then this remnant mantle, on the one hand, and the small left-posterior mass of projected tissue, on the other, each forms a new zooid in the common mass of tunic. To the extent that these double regenerations occur throughout the colony, they double the colony's complement of zooids. *P. glandulosa's* reddish colonies have been found at about 40 m depth on the outer reef slope near Noumea and on the reef at Ua. As one might expect, their mode of budding leads to intricate colonies in which zooids of many different sizes, 5 mm to almost 5 cm, abut and pile up on one another within a thick, soft tunic that is filled with lacunae.


Polyandrocarpa rollandi
is colonial but its zooids are quite big, 2 to 3 cm long. The colony forms a thick, sturdy incrustation.

SOLITARY STYELIDAE WITH BRANCHIAL FOLDS

The three genera of this sort that are represented in New Caledonia - *Polycarpa*, *Cnemidocarpa*, and *Styela* - are also the three that have the greatest diversity of species worldwide.

The genus *Polycarpa*

This immensely varied genus contains species that suggest how closely *Polycarpa*'s solitary forms approach *Polyandrocarpa*'s colonial ones. Some species are capable of evisceration and, after the primary body has completely degenerated, subsequent regeneration. This regeneration forms one, two, or even several bodies, which may separate completely from one another or may lie together within the remnant tunic that marks where the primary zooid had been. In some species (e. g. *Polycarpa papillata*) the parent zooid may protrude a basal tissue mass like *Polyandrocarpa glandulosa*'s. The parallel eviscerative and regenerative habits of some members of these two genera are bound to raise intriguing evolutionary questions as well as taxonomic misgivings.

In five species of *Polycarpa* that the assiduous searcher is likely to find around New Caledonia, the gonads lie well inside the mantle and scarcely bulge toward the peribranchial cavity even when mature; usually, only the papilla-tips of their gonoducts reveal their presence unless the mantle itself is searched. In contrast, some other species of the genus have gonads that protrude into the peribranchial cavity, occasionally even to the point of appearing merely suspended by a mesentery from the internal mantle wall. We will review first the species whose gonads are deeply included in the mantle.

P. papillata lives either as isolated individuals or in little heaps of aggregated bodies. In the latter case even the keenest observer cannot tell whether the mass has been formed by successive eviscerations and regenerations or by aggregative settlings of larvae. The erect individuals have their siphons close together; their yellowish tunics are free of epibionts. *P. papillata* lives in the deepest parts of the lagoon and down to almost 500 m on the outer reef slope.

P. nigricans also grows both as separate bodies and in masses that show every sign of evisceration and regeneration. It coexists in many places with *P. papillata*. Its bodies grow to lengths of 2 to 4 cm and are encased in a opaque, black tunic with siphons that are even blacker, both inside and out.

On the reef, in the lagoon, and especially near the shore, *P. cryptocarpa* forms large populations of tightly packed bodies. It grows into big, globular bodies, 5 to 10 cm across. Its flexible, brownish gray tunic is sometimes completely covered by epibionts, so that only the white interior of its siphons reveals the animal's presence. This species, too, can eviscerate and regenerate; but it does not appear to do so often, and it does not seem to be able to reconstitute several bodies that way.

P. mytiligera is very similar to *P. cryptocarpa*, but its tunic is very dark on the outside, and the interior surfaces of its siphons are black. This is predominantly a species of the lagoon, less commonly found on the reef. Removed from its tunic and cut open, the animal shows a black mantle that is thickened by large swellings that abut one another along long grooves in the peribranchial cavity's wall; the gonads lie in this swollen sections of the mantle. Like *P. cryptocarpa*, *P. mytiligera* grows on hard substrates like rocks and fissures among corals; but it can also inhabit loose bottoms by extending a network of rhizoids to bind the sand particles into an effectively solid support.

P. pigmentata lives in fairly restricted habitats on the outer reef slope and around islets in the lagoon. This is a very big, stout

The erect bodies of *Polycarpa papillata* project their clean tunics above the bottom.

The internal surface of each siphon
is black in *Polycarpa nigricans*,
and the whole tunic's outer surface
is quite dark and somber.
The species may live in aggregates
or as isolated individuals.

In sharp contrast to *Polycarpa nigricans*, *Polycarpa cryptocarpa* has a pale tunic and white internal siphonal surfaces. It lives in large populations in shallow habitats.

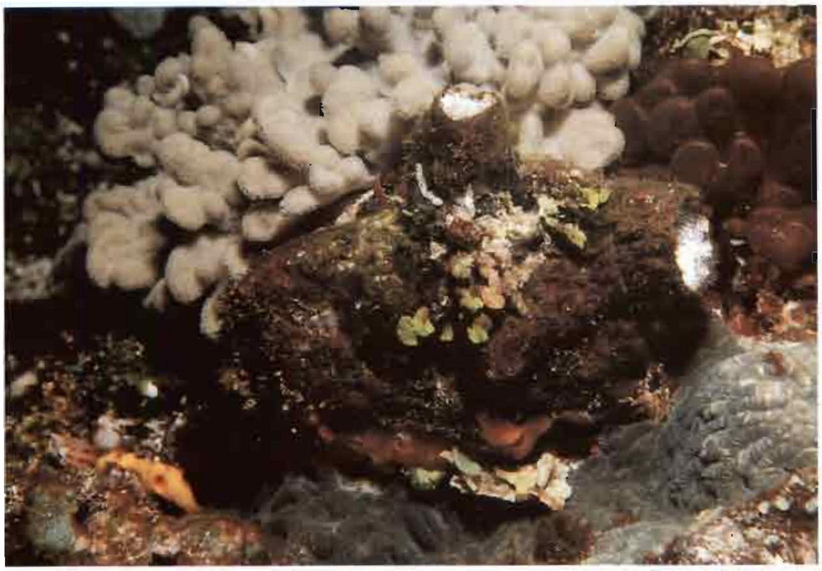
Polycarpa mytiligera is black inside and black outside. But its leathery surface is almost entirely hidden beneath epibionts. The species is sometimes able to live on soft substrates.

animal, reaching lengths of 15 cm, but it can be surprisingly hard to find, because the entirely black or deep brown tunic is covered by epibionts that make the body blend in well with its surroundings. If the siphons are open, their creamy white internal surfaces are easily seen, but the animal responds to the slightest vibration by shutting them.

Like *P. pigmentata*, *P. aurita* lives on the outer reef slope and around islets in the lagoon and is heavily covered by epibionts. And its siphons, too, close at the slightest alarm. But their interior surfaces, visible when the animal is relaxed, are orange to red, and the animal's external tunic is not black but rather yellowish or brown. If its epibionts are removed, the tunic shows a dense network of superficial blood channels that carry yellow cells.

The most impressive ascidian of New Caledonia is certainly *P. clavata* - so striking it has even been depicted on a postage stamp! This is a big animal, 25 to 30 cm high when its long stalk is included, and colored a brilliant yellow or orange. It is an altogether stunning sight as it sways gracefully in the water. This species is usually encountered at least 25 m deep, and prefers the outer reef slope and the sides of lagoon islets. Even more drastically than the stalked polycitorid *Clavelina detorta*, this stalked styelid is "inverted" on its stalk, with its oral siphon close to the peduncle and its cloacal siphon more distal. Individuals in groups are often oriented parallel to one another, with their oral siphons pointed into the current. The tunic is clean around the body but carries some epibionts on its stalk, a consequence of molting, as Catala showed for the first time in *Carnaval sous la mer* (1964). Periodically the body, but not most of the stalk, undergoes a series of abrupt contractions that slough off the

The internal surfaces
of *Polycarpa aurita*'s siphons
are bright orange, helping to reveal
and identify the species
even when it is covered with epibionts.



The externally visible combinations multiply: *Polycarpa pigmentata* has snow-white internal surfaces in its siphons, but the outer surface of its tunic is a rich, dark brown.

outer layer of its tunic and reveal the dazzling new surface just beneath. The animal's swaying movements, which carry it regularly through arcs of over 90 °, are generated by the animal itself, not just by the waters around it. They are quite out of synchrony with the swayings of neighboring seaweeds and gorgonians, for example. The ascidian's own movements are provoked by contractions of sinuses in the mantle near the top of the stalk.

There are other New Caledonian *Polycarpa*, but they are hard to see, uncommon, and small, and they mostly lack good fieldmarks for identification. Only *P. richeri*, dredged from the loose sediments it inhabits in the eastern lagoon, has a distinctive external trait: the entire posterior tunic of this small (1 cm), spherical animal carries a beard of rhizoids with which to bind the sediment around it.

The genus *Cnemidocarpa*

Cnemidocarpa differs from *Polycarpa* only by having much more elongated hermaphroditic gonads. New Caledonia has only one species, *C. valborg*, which is also distributed through all the Indo-Pacific and in the Caribbean. It is occasionally found in the shallower waters of the lagoon. This species has few external traits to distinguish it from many other solitary styelids and pyurids of moderate size, brownish-red color, and generally rugged appearance. Only a look inside - for several traits matching the key formulae for the family Styelidae, and then for the compact, elongate gonads matching the key formulae for this genus within the family can identify this animal as a species of *Cnemidocarpa*.

The genus *Styela*

This immense genus is represented in New Caledonia by only one species - *S. canopus* (until recently known widely as *S. partita*). A drab red-brown and inconspicuous, rarely exceeding 2 cm and usually sequestered among other ascidians, it occupies shallow habitats that are rich in organic matter; thus, it abounds as a fouling organism in the port of Noumea. Some very small individuals (less than 5 mm across), clean or covered by sediment, can be found on the reef flat. As with *Cnemidocarpa*, one must look inside to establish *S. canopus*'s identity with any assurance. Among solitary styelids this species has the most immense range of all: in shallow habitats in all the tropical seas and well into colder waters in many places, even to the Arctic Circle in the Atlantic.

ABYSSAL STYELIDAE

The family Styelidae has the largest number of species among shallow-water solitary ascidians and is comparably significant at greater depths. Around New Caledonia each of the family's three great genera of solitary forms -- *Polycarpa*, *Cnemidocarpa* and

Polycarpa clavata is the largest species of the family Styelidae in New Caledonia. The individuals often live in small groups, in which they all have the same orientation in the water current. This suggests there is some ingenious engineering involved, when the species presents itself like this for a diver's contemplation.

Styela - has a representative species. These deep waters hold, as well, two other styelid genera that are very specialized and uniquely abyssal. *Bathystyeloides* is a genus in which the branchial sac has, on each side, only internal longitudinal vessels, which cross long, transversely oriented stigmata that may extend over the entire width of the sac. In *Bathyoncus* true stigmata are lost altogether, and the branchial sac is reduced to an open mesh of transverse and longitudinal vessels, the latter sometimes bunched in folds.

Family Pyuridae

The pyurid body is the most complex among ascidians. Its oral tentacles are frilled or elaborate by feathered. In contrast to a styelid's filiform tentacles. The filtration efficiency of its branchial sac is enhanced by the pharyngeal wall's greatly increased surface, which has been augmented by at least six longitudinal folds on each side, compared to no more than four folds in styelids. Its gut-tract has a digestive gland that is large and well differentiated into ramifying and arborescent elements. Most pyurids have stout, globular bodies inside a thick, tough tunic that often supports a rich array of epibionts, so much that one genus is called *Microcosmus*, "little world".

GENERA OF PYURIDAE

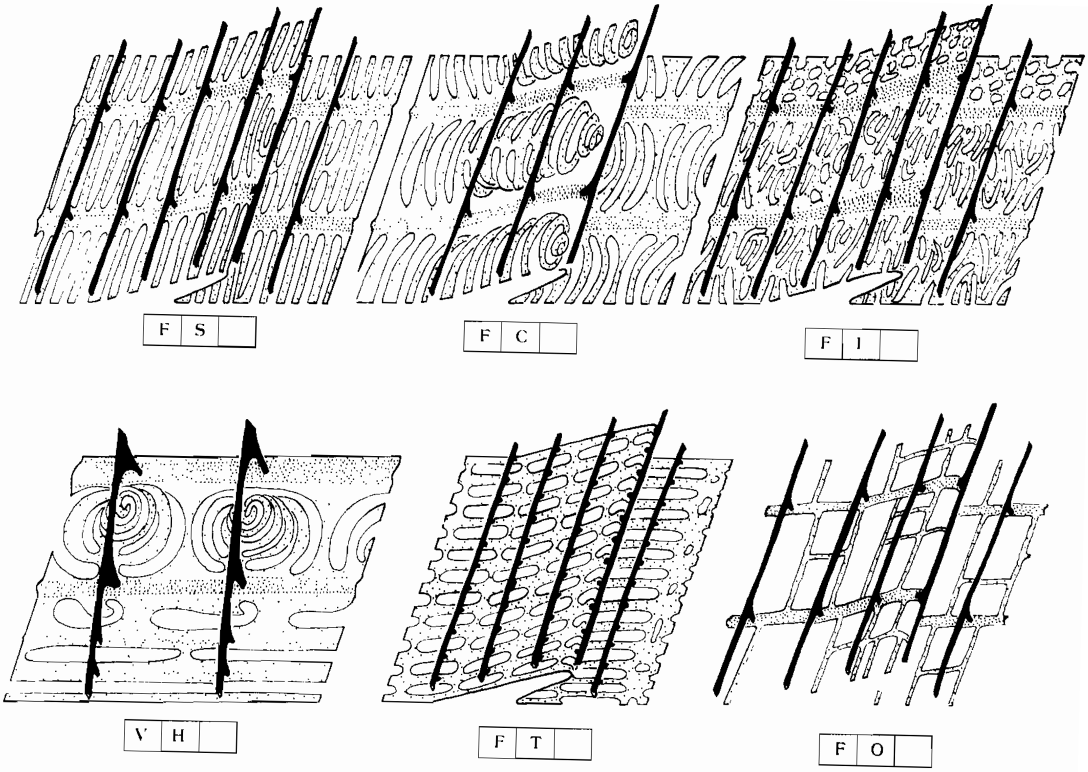
1 - Structure of the branchial wall:
  V - Longitudinal *vessels* only (less than 7).
  F - Longitudinal *folds*.

1	2	3	4	Genus		Distribution
V	T	S	0	*Bathypyura* Monniot, 1971	D	Deep sea
V	H	S	0	*Heterostigma* Ärnbäck, 1924		Europe
F	0	L	P E	*Culeolus* Herdman, 1881	D	Deep sea
S	I	S	P	*Bathypera* Michaelsen, 1904		Widely distributed
F	T	L	P	*Boltenia* Savigny, 1816	D	Widely distributed
F	H	L	E	*Bolteniopsis* Harant, 1927	S	North Atlant. New Caled.
F	C	S	E 0	*Cratostigma* Monniot C. & F., 1961		Nort Atlant. Mediterranea
F	S	S	P M	*Microcosmus* Heller, 1877	S D	Widely distributed
F	F	S	M	*Hartmeyeria* Ritter, 1913		Widely distributed
F	S	L	P	*Pyura* Molina, 1782	S	Widely distributed
F	F	L	P	*Ctenyura* Van Name, 1918		West Pacific
F	S	L	H	*Hartmeyeria* Verrill, 1879	S	Widely distributed

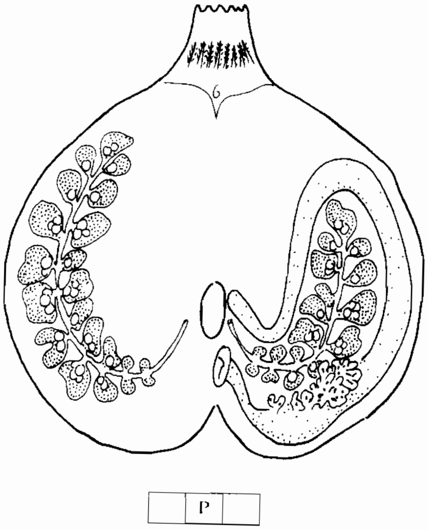
Family Pyuridae.

2 - Shape of stigmata:
  T - Only *transverse*.
  C - *Curved* in spirals under longitudinal vessels or folds on the whole branchial wall.
  H - Curved in spirals on the anterior part of the branchial wall, transverse in the posterior part (*heterogeneous* stigmata).
  F - Stigmata straight between the folds, in spirals of several turns at the top of the *folds*.
  S - Stigmata *straight* between the folds, sometimes spirals on the upper part of the fold (1 turn).
  I - No typical stigmata, *irregular* perforations.
  O - No stigmata, open meshes with longitudinal and transverse vessels only.
3 - Structure of dorsal lamina:
  S - Dorsal lamina with a plain *smooth* free edge.
  L - Dorsal lamina divided into a series of *languets*.
4 - Type and disposition of left gonad:
  P - Left gonad (one or several) in the intestinal loop (*Pyura* type).
  M - Left gonad in the intestinal loop with distal end lying across the intestine (*Microcosmus* type).
  H - Left gonads flask-shaped or tubular, lying across the intestine, their ventral ends connected, the dorsal ends free (*Halocynthia* type).
  E - Left gonad (one or several) *external* to the intestinal loop.
  O - No left gonad.

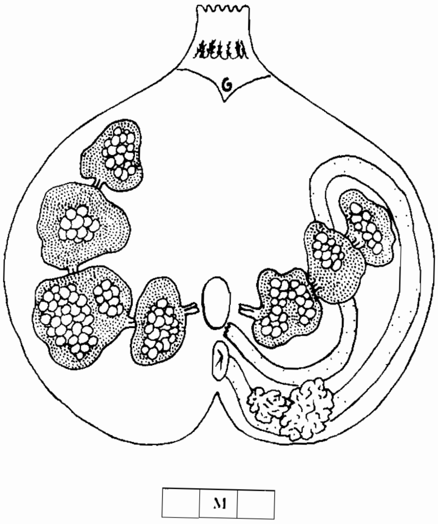
All pyurids are solitary ascidians, although they sometimes aggregate into very dense masses. The species in these masses

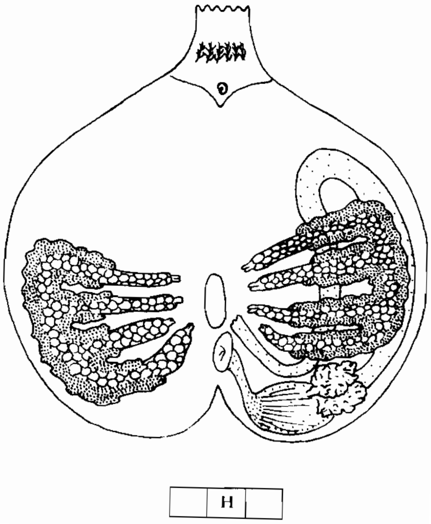


Shape and distribution of stigmata related to the branchial bars and folds, and of gonads in the family Pyuridae, illustrating the key's formulae.



Shapes of gonads in the family Pyuridae.





This picture suggests how hard it can be to detect pyurids on the sea bottom! Especially when they are covered by other ascidians as here by *Symplegma oceania*, a colonial styelid

Pyura confragosa has extended its erect siphons outside of the crevice where it hides when it contracts.

can bewilder a hasty observer. For example, in the port of Noumea, 9 of New Caledonia's 11 littoral species of pyurids live together, often piled atop each other. Epibionts so completely cover most of these masses that only the ascidians' siphons are visible. Under such circumstances field identifications become notoriously unreliable. Trying to distinguish species by the consistency of their tunics - very soft (*P. momus*), soft (*P. sacciformis*), fairly tough (*Microcosmus exasperatus*), tough (*P. confragosa*), and very hard (*P. vittata*) - leads, we have found, to about 20 % to 50 % mistakes!

Pyurids are not common in the lagoon or on the reefs. And despite their obvious internal contrasts with styelids, pyurids show no fieldmarks that let a diver tell them apart from that family. Only an internal examination will provide the needed clues. But its tough tunic and soft and muscular body make a pyurid an impossible animal simply to cut open alive for a quick look inside. Anyone who has opened a *Microcosmus* or *Halocynthia* to eat knows how misshapen the strongly contracted bodies of these animals can get. Careful anaesthetization and fixation in formalin are essential preparations for even cursory dissections. Just as styelid-pyurid distinctions depend on internal traits, so do identifications of most pyurid genera themselves. Among New Caledonian shallow water pyurids, only *Halocynthia*, whose siphons bristle with prominent spines, and occasionally a clean *P. momus* are exceptions to this rule.

P. momus (once *Herdmania momus*) lives on the reef and in most parts of the lagoon. It is often lightly coated with silt. When a relaxed, sediment-free animal's siphons are gaping, its reddish tunic contrasts with the blue-tinted reflections of the siphons' internal surfaces. This species' tunic is thin and easily torn. The

The red color of *Pyura momus*'s tunic
is often obscured by epibionts.

body's mantle is mixed red and white, and its ripe gonads are a rosy pink. All the animal's tissues contain large, needle-shaped spicules, visible with a hand-lens.

Halocynthia hispida has a distinctive appearance: its tunic has many mound-like tubercles, each crowned with a ring of 5 to 8 spines. These tubercles are larger on the siphons, where they carry several prominent whorls of spines. In New Caledonia *H. hispida* is small (2 to 3 cm) and very rare. It grows larger elsewhere in the Pacific: Australia, the South American coast, and the Galapagos Islands, where its length can exceed 10 cm. The species is closely related to Japan's edible *H. roretzi*.

Some pyurids are very small. In gravel beds of the southern New Caledonia lagoon, the tiny, spherical *Bolteniopsis pacificus* lives among the sand grains. It does not exceed a diameter of 3 mm, including even the sandy coating on its tunic that makes it totally invisible. This interstitial species is the only known shallow-water member of its otherwise deep-sea genus.

Deep-sea pyurids are well represented by 10 species around New Caledonia. Some are very similar to shallow-water forms. For example, *P. pennata* closely resembles *P. momus*, even to having spicules in its tissues; But this abyssal species, which lives on the outermost slopes, is a small (1 cm), spherical, sand-covered animal. *Microcosmus longicloa*, which inhabits submarine banks, also shares many traits with littoral species of the genus. But some other pyurids belong to genera that are very much adapted to abyssal life. The four species of *Culeolus* living between 300 and 3 000 m are truly spectacular. A globular or cylindrical body several cm across and up to 10 cm long perches atop a fairly stiff stalk that is only a few mm wide but may be a meter long. The body's oral aperture opens close to this stalk, while the cloacal aperture opens farther up the cylindrical mass. The branchial sac lacks any ciliated stigmata; it is instead a network of transverse and longitudinal vessels, complicated by rather slight folds, delimiting wide-open holes that can exceed 4 mm^2. Attached to the bottom, a , looks like an anchored buoy or an airport wind-sock, riding downstream from its tether, revealing the direction of the current around it.

Family Molgulidae

The Molgulidae are often considered the most highly evolved of stolidobranch ascidians, because their branchial structure is often so complex and their organs are so well differentiated. This family is unique in having, in its right mantle, a vesicle that accumulates nitrogenous products and stores them as uric acid. Also, molgulids have freed themselves from dependency on hard substrates for larval settlement and subsequent growth; most species inhabit mud or sand. These animals typically are drab little spheres that are coated with sand or silt or even buried in the bottom, with only their siphons exposed. The family is diverse in the cold waters of high latitudes, but it is sparsely represented in the tropics, and then usually by very small and inconspicuous species.

1	2	3	4	5	6	7	Genus		Distribution
P	T	0	0	0	B R	S	*Minipera* Monniot C. & F., 1973	D	Deep sea
V	T	0	0	0	R	0	*Protomolgula* Monniot F., 1971	D	Deep sea
V	C	0	0	0	B	S	*Paramolgula* Traustedt, 1885		South America
V	C	I	L	T	B	P	*Eugyrioides* Seeliger, 1906		Widely distributed
V	C	I	Z	L	B L	P	*Bostrichobranchus* Traustedt, 1882		Atlantic North America
V	C	I	L	T	L	P	*Eugyra* Alder & Hancock, 1870		Widely distributed
V	C	I	L	T	R	0	*Gamaster* Pizon, 1896		Widely distributed
V	C	I	Z	N	B	P S	*Pareugyrioides* Hartmeyer, 1914		Widely distributed
F	C	I 0	L Z	N	B	S	*Molgula* Forbes, 1848	S D	Widely distributed
F	C	I 0	L	N	B	P	*Molguloides* Huntsman, 1922	D	Widely distributed
F	C	I	Z	T N	L	P	*Rhizomolgula* Ritter, 1901		Arctic
F	C	I	Z	N	R	0	*Anomopera* Hartmeyer, 1923		Deep sea
F V	0	0	0	B	S	C	*Fungulus* Herdman, 1881	D	Deep sea

Family Molgulidae.

GENERA OF MOLGULIDAE

1 - Structure of the branchial sac:
- P - *Papillae* only.
- V - *Vessels* only (no folds).
- F - Vessels making several *folds*.

2 - Shape of stigmata:
- T - *Transverse* only.
- C - *Curved* in spirals, at least on the top of the folds.
- O - No stigmata, only longitudinal and transverse vessels.

3 - Infundibula:
- I - Stigmata placed on finger-like invaginations of the branchial wall: *infundibula*.
- O - No infundibula: stigmata in one level.

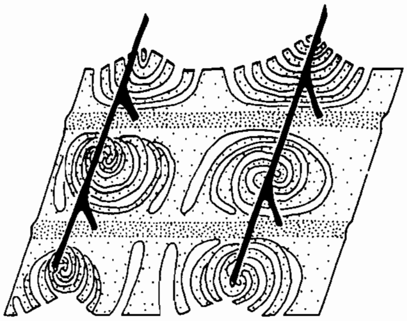
4 - Disposition of the infundibula:
- L - Infundibula only along anteroposterior *lines*.
- Z - Infundibula irregularly placed.

5 - Disposition of stigmata on the infundibula:
- T - *Two* uninterrupted stigmata coiled in a double spiral.
- N - Stigmata interrupted several times along the spirals.

6 - Position of gonads:
- B - On *both* sides of the body.
- L - On the *left* only.
- R - On the *right* only.

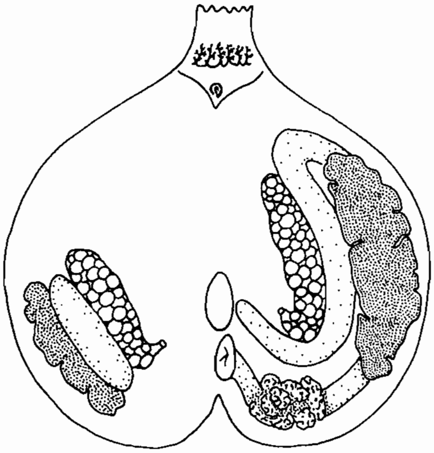
7 - Position of left gonad in relation to intestinal loop:
- P - Blind end of the ovary in the *primary* intestinal loop.
- S - Ovary in the *secondary* intestinal loop.

One shallow-water molgulid lives in New Caledonia: *Molgula bisinus*, a spherical, often sand-encrusted animal a cm across that



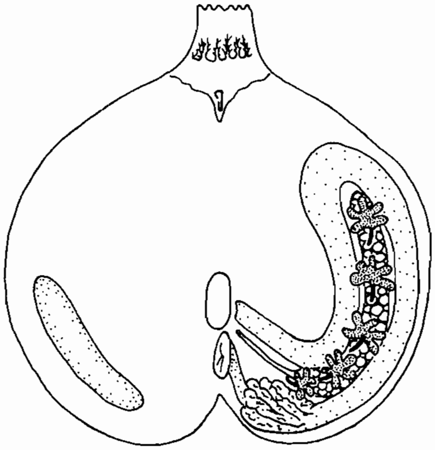
V C I L T

F C I I T



B O

Schematic design of the stigmata in part of a molgulid branchial wall. Examples of molgulid gonads' shapes and locations, with key formulae.



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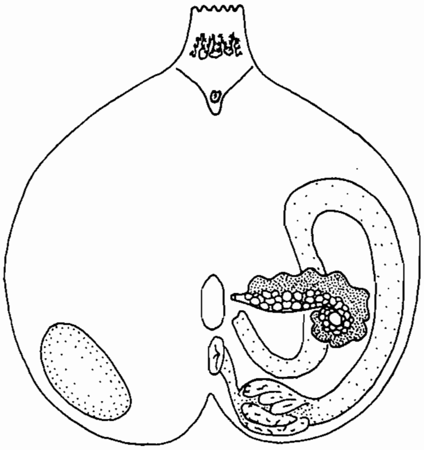

L P

may host small epibionts. Throughout the lagoon it lives buried in the sediment, where it can be found only by sifting the sand itself. Or it may attach itself to bits of shell, algae, and other ascidians, especially to *Polyclinum*.

In deep waters around New Caledonia, the Molgulidae are represented by 8 species in 5 genera. One, *M. delicata*, is a big animal (5 to 6 cm in diameter) but so fragile that its body's own contraction can tear its tunic. Four species of the genus *Molguloides*, all little spheres 1 to 2 cm across, inhabit the region's depths, as do species of *Bathypera* and *Fungulus*, the latter a molgulid of Culeolus-like design. The exclusively abyssal genus *Minipera* lives here, too. Its species are the smallest known solitary ascidians, less than 1 mm in diameter, and also the simplest, in striking contrast to their larger molgulid relatives. *Minipera*'s branchial sac, for example, has been reduced to a minute cavity with only two perforations on each side. Even so, diminutive and reduced, this is as splendid a beast as all its cousins - "every inch an ascidian"!

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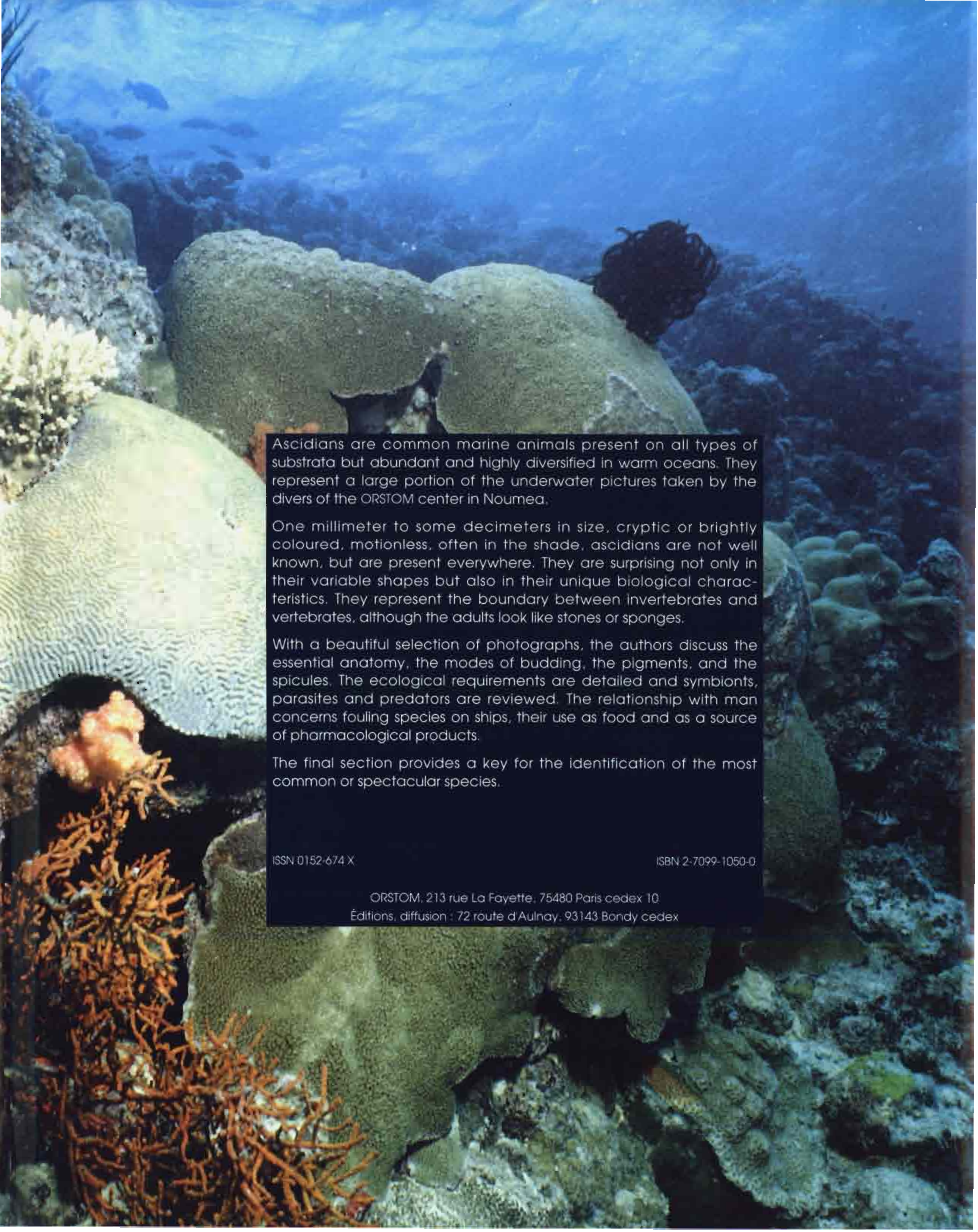

Ascidians are common marine animals present on all types of substrata but abundant and highly diversified in warm oceans. They represent a large portion of the underwater pictures taken by the divers of the ORSTOM center in Noumea.

One millimeter to some decimeters in size, cryptic or brightly coloured, motionless, often in the shade, ascidians are not well known, but are present everywhere. They are surprising not only in their variable shapes but also in their unique biological characteristics. They represent the boundary between invertebrates and vertebrates, although the adults look like stones or sponges.

With a beautiful selection of photographs, the authors discuss the essential anatomy, the modes of budding, the pigments, and the spicules. The ecological requirements are detailed and symbionts, parasites and predators are reviewed. The relationship with man concerns fouling species on ships, their use as food and as a source of pharmacological products.

The final section provides a key for the identification of the most common or spectacular species.

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