RECENT ADVANCES IN RESEARCH ON OCTOCORALS

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Until the beginning of the present decade, the procedures and instruments for research on octocorals had not changed for more than a century. The use of skeletal sclerites as an important taxonomic character, first proposed in 1855 by Valenciennes (1314) and effectively put into practice by Kölliker ten years later (603), was the first major advance in the classification of octocorals since the beginning of Linnean taxonomy. It was made possible by improvements in the design and quality of microscopes. Even though the existence of sclerites in octocoral tissues was recognized by John Ellis as early as 1755, even before the publication of the 10th edition of Linnaeus's Systema Naturae, their usefulness in taxonomy was not suspected – perhaps because of the crude microscopes of that time. Little more than a powerful hand-lens, Ellis's microscope evidently revealed to him the sclerites of *Corallium* in a remarkably accurate manner, as shown by the small but clear drawings (Fig. 1) that he published in his *Essay* (266).

One of the greatest obstacles to a sound classification was the exclusive reliance upon gross external form in describing and discriminating between species prior to 1855. This makes it impossible to recognize accurately or define in modern terms any of the approximately 200 species described by the classic writers before Valenciennes without a reexamination of the original specimens. Even after the publication of Valenciennes' and Kölliker's papers, not all authors made use of the principles they established. Notably, J.E. Gray and Duchassaing & Michelotti all but ignored sclerites in writing their descriptions. Perhaps because of added tedium and increased expense, the observation and illustration of sclerites was slow to be adopted and was not completely accepted until after the beginning of the present century. Even the most competent investigators, including Sidney J. Hickson, Willy Kükenthal, J. Arthur Thomson, and many others, established genera and species by the score without any illustrations. The illustrations that were published were of very inconsistent quality and accuracy, and many are next to useless.

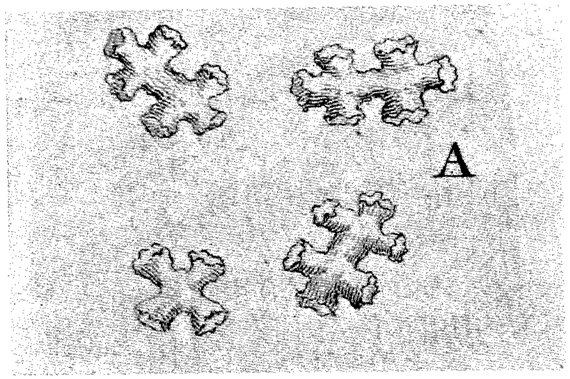

Fig. 1 – Sclerites of *Corallium rubrum* (Linnaeus) as illustrated in 1755 by John Ellis (266). Somewhat larger than original size.

Kölliker's hand-colored engravings published in 1865, the first taxonomic illustrations of octocoral sclerites, are still among the best that have ever been published (603).

Illustrations made by professional scientific artists rather than by the scientist personally, such as those in the world-renowned "Challenger" report on octocorals, often bear little resemblance to reality and are of limited scientific value. On the other hand, illustrations of good quality are of the greatest usefulness. These range, in various publications, from color lithographs to simple line drawings. Although widely different in aspect, they share accurate observations carefully drawn and skillfully rendered. Unfortunately, some of the most experienced investigators of this century were illustrators of indifferent or even mediocre skill, and the poor quality of their drawings limits the usefulness of their works. Furthermore, the widely different quality of drawing and method of rendering used by the various 20th century authors makes the comparative use of their works and the interpretation of their drawings very difficult.

Recognizing the importance of illustrations in descriptive biology, it has been my personal policy to illustrate as fully as possible every species that I report, whether or not it is new, but I have never been fully satisfied with ink drawings for this purpose. About 20 years ago, when working with a genus of gorgonians having sclerites with very subtle sculptural differences, I tried several techniques in an effort to find the most accurate way of depicting the essential characters. The resulting drawings each required many hours of work, and in consequence the paper was never finished.

Since that time, the observation and illustration of octocoral sclerites have been revolutionized by the development of the scanning electron microscope (S.E.M.). Today, it is possible in less than 5 minutes to photograph the electronic image of even the smallest sclerites with an accuracy that could not be equalled by many hours of the most meticulous drawing. Compare the result with the drawing of the same kind of sclerite, which took 3 or 4 hours to prepare, and with some earlier interpretations published by previous authors (Fig. 2). Because of the rapidity with which S.E.M. photographs can be made, it is possible to observe and record variation much more adequately than was possible when drawing was the only method of illustration available. A standard control on the stage of the microscope makes it possible to take stereoscopic pictures that reveal surface relief with unprecedented realism (Fig. 3). Because sculpturing is an important feature of octocoral sclerites, three-dimensional S.E.M. can be used to illustrate them with a degree of accuracy heretofore impossible.

The Smithsonian Institution maintains a Scanning Electron Microscopy Laboratory containing three instruments operated by a staff of three highly skilled electron microscopists. The original instrument in this laboratory is a Cambridge Stereoscan mark II-a, acquired in 1970 and modified with a Lanthanum hexaboride electron gun. The second is a Cambridge Stereoscan model S4-10, acquired by the U.S. Geological Survey in 1974 and shared with the staff of the Smithsonian Institution. This instrument was originally equipped with the Lanthanum hexaboride gun. The third is a Coates and Welter Cwikscan 106 with field-emission electron gun, which provides much higher resolution – about 60 Angstroms – than can be obtained with the Lanthanum hexaboride guns of the Cambridge instruments.

Because of the very heavy usage to which these instruments are put by our scientific staff, they are operated only by the S.E.M. laboratory staff, not by the scientists personally. Observations which the researcher wishes to record are exposed twice, once on type 52 polaroid film to obtain an immediate study print,

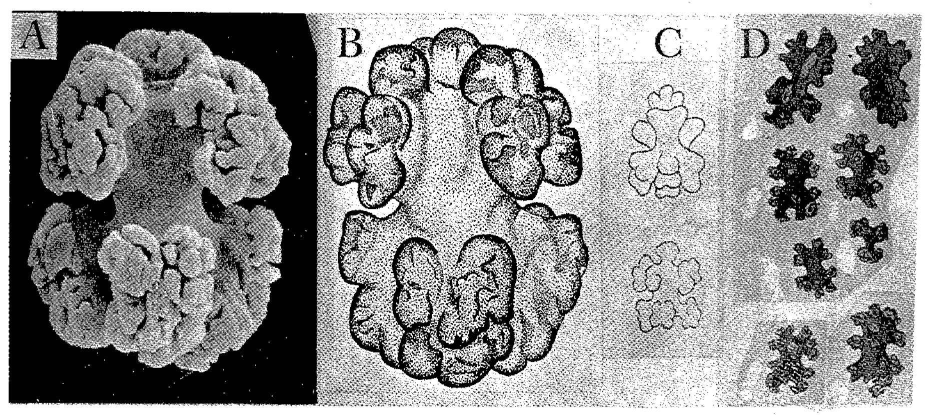

Fig. 2 – Sclerites of *Paragorgia arborea* (Linnaeus). A, Scanning electron micrograph by W.R. Brown, Smithsonian Institution, x590; B, stipple drawing by the author, light microscope and camera lucida, oil immersion, x590; C, line drawings by Deichmann (231), approximately original printed size, x300; D, shaded drawings by Stiasny (1098), slightly smaller than original printed size, here approx. x215. Discrepancies in size of figured sclerites due partly to variation in specimens, partly to authors' errors in magnification and measurement.

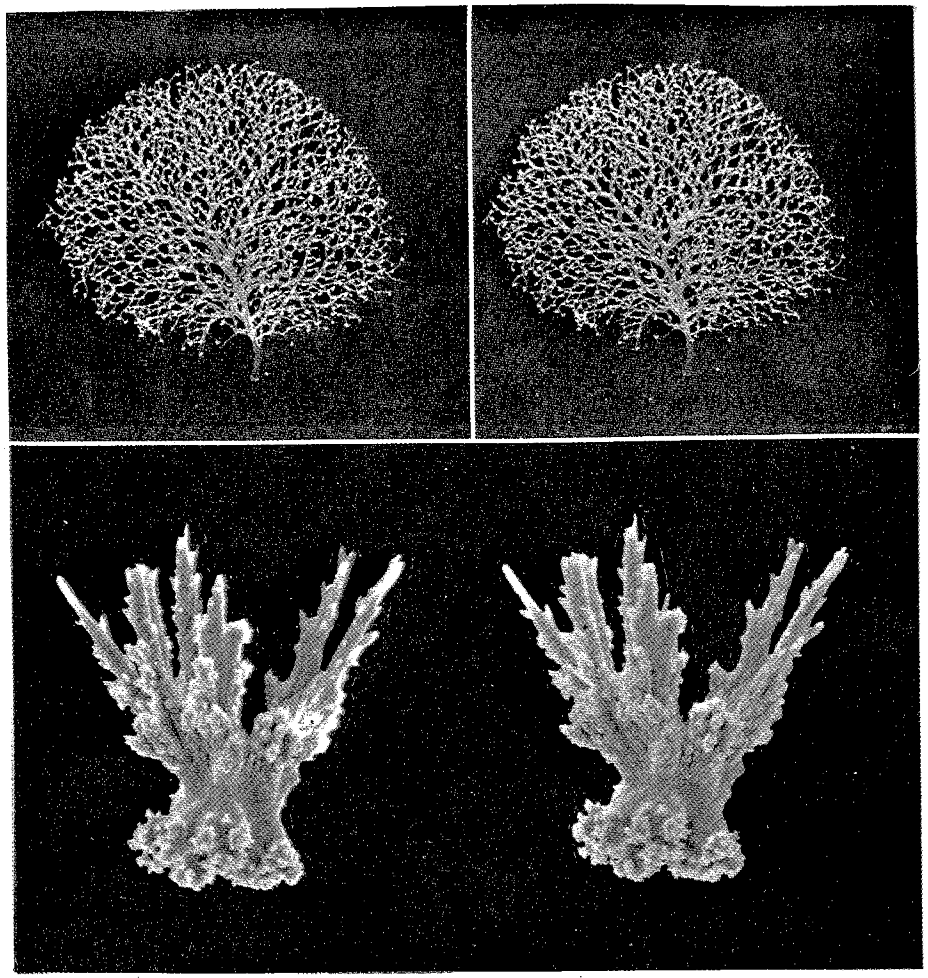

Fig. 3 – Top, colony of *Chrysogorgia desbonni* Duch. & Mich., stereoscopic photograph, x0.5. Bottom, sclerite of *Bebryce hicksoni* Thoms. & Henderson (*sensu* Nutting), S.E.M., stereoscopic pair x500, by W.R. Brown, Smithsonian Institution.

and once on sheet film which provides a negative for subsequent printing, with or without enlargement.

The principal advantage of examining sclerites by the scanning electron microscope is the rapidity with which extraordinarily accurate pictures can be made. This permits examining a larger number of sclerites with a permanent graphic record for comparative studies, than would be practical if all the sclerites had to be drawn by hand. The most serious procedural limitation is the time required for preparing sclerites, which must be meticulously cleaned for electron microscopy and positioned in such a way as to minimize search-time on the instrument and to provide uniform illumination (Fig. 4). The number of sclerites that can be

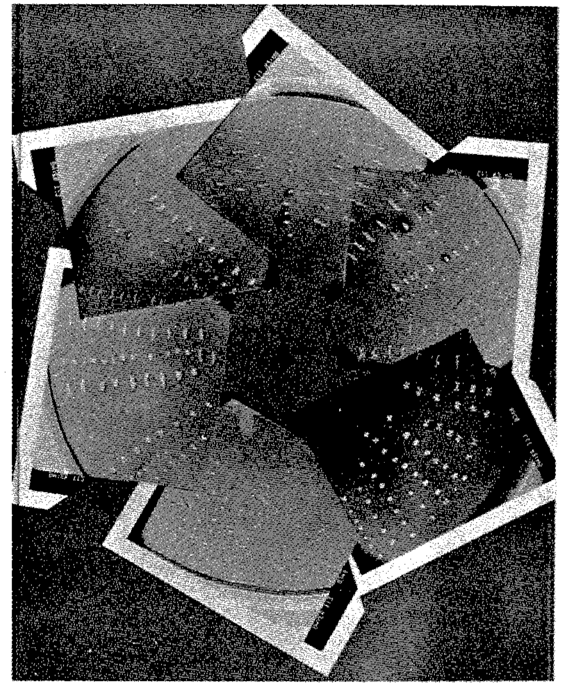

Fig. 4 – Composite photo of entire S.E.M. preparation, showing sclerites arranged for greatest ease of manipulation in the instrument and for uniform illumination. Specimens surrounded by a dark shadow are inadequately grounded and show the typical "charging" effects; these cannot be examined satisfactorily.

examined depends largely on the amount of time the scientist is willing to devote to the task of preparation. However, the sample must be sufficient to demonstrate the range of variation present and to provide alternate specimens to compensate for those broken ones that were not detected in the course of preparation, and for those that do not acquire adequate electrical grounding during the metal coating process that is necessary to prepare specimens for examination.

In general, the scanning electron microscope has not revealed any taxonomically useful characters that were not visible with light microscopy. It has shown for the first time the crystalline structure of sclerites that was observed optically by Schmidt, and it has shown that the details of this crystalline structure differ among the higher taxa within the Octocorallia.

All sclerites are composed of tightly packed crystals of calcite containing a variable amount of magnesium (Fig. 5). In some families, these crystals are extremely small – about two-tenths to 4 tenths of a micron in width – and projecting at the surface in a "hound's-tooth pattern" as seen in the families Coralliidae and Paragorgiidae. In the families Anthothelidae and Briareidae, the

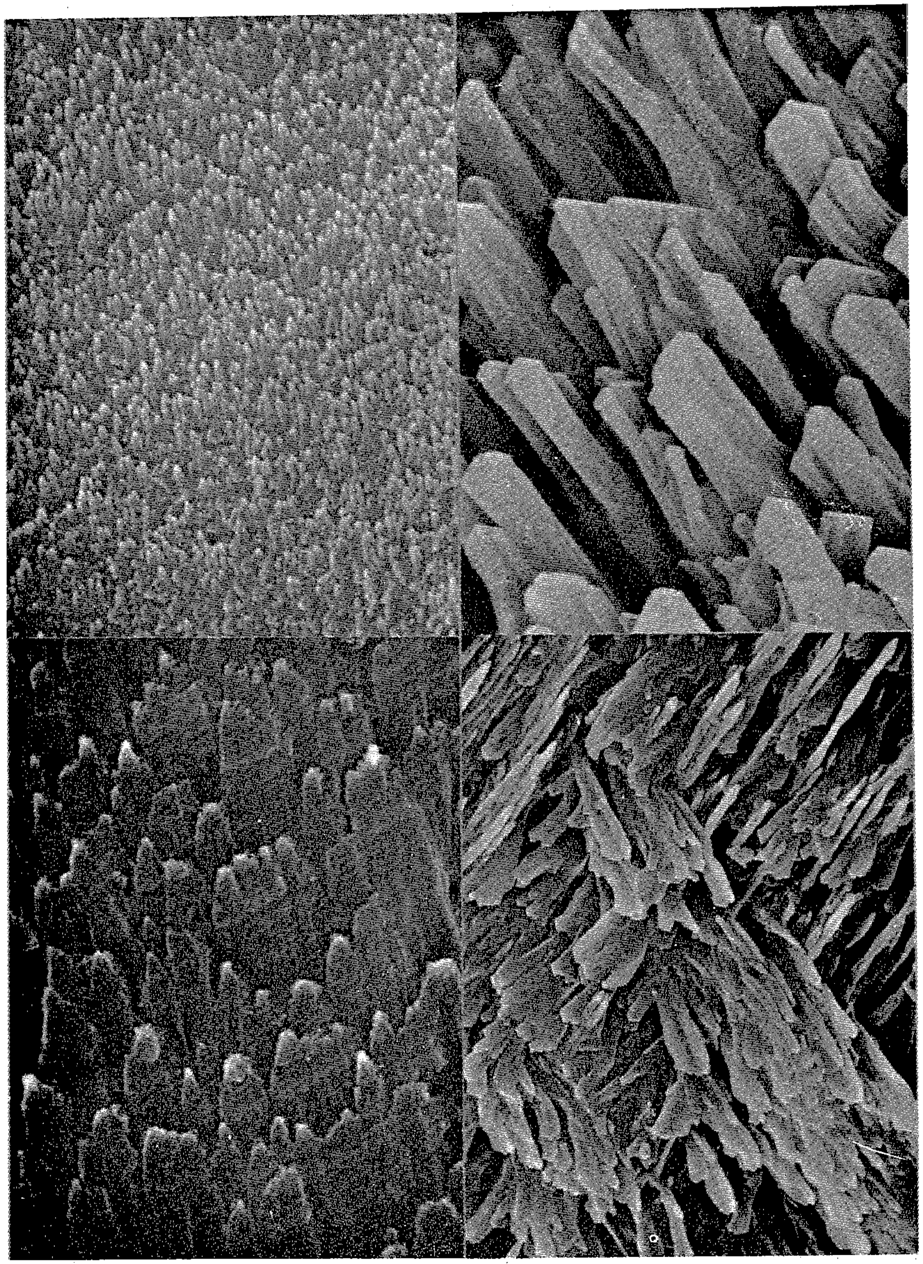

Fig. 5 – Ultrastructure of octocoral sclerites. Upper left, *Paragorgia arborea* (Linnaeus); upper right, *Muricea atlantica* (Kükenthal); lower left, *Plexaura homomalla* (Esper); lower right, *Chrysogorgia elegans* (Verrill); x15000 except lower right, which is x7500. Scanning electron micrographs by W.R. Brown, Smithsonian Institution.

crystals are similarly small or even smaller and appear at the surface only as minute granules without any evidence of crystal faces.

In contrast, the crystals of the sclerites of many plexaurids and paramuriceids are larger, from 0.8 – 1.0 μ., often projecting conspicuously and showing their crystal faces distinctly. Fine transverse striations that can be interpreted as lines of crystal growth are visible in many cases, as in *Plexaura homomalla*, whose crystals show projecting "hound's tooth" structure rather than regular crystal faces.

In forms such as Primnoids having flattened, scale-like sclerites, the crystals may be correspondingly flattened and shingle-like. If the sclerite has an expanded flattened blade arising from a root-like base, the crystals of the blade may be flattened whereas those of the base are not. Although it should be tempting to conclude from this that the shape of the component crystals determines the shape of the sclerite, there are too many exceptions to make this conclusion tenable. Certainly the gross form of the sclerite and the nature of its crystal structure are intimately related, and it is possible to predict from the form of a sclerite of a given species the kind of crystals that it will have, but the significance of this relationship remains to be discovered.

Another aspect of octocoral structure that the scanning microscope has made accessible for study is the axial skeleton of gorgonaceans (Fig. 6). In the families Ellisellidae, Primnoidae, Ainigmaptilidae, Chrysogorgidae, Isididae and some Plexauridae, the axis is composed of a scleroprotein, gorgonin, more or less densely permeated by calcareous deposits. Although it was known that the mineral does not occur in the form of sclerites, its structure has not heretofore been examined. The purely calcareous axial internodes of isidids can be examined directly with the scanning microscope, and the axis of the other families can be examined after the removal of their organic component by etching under partial vacuum in an oxygen plasma.

Where the calcareous internodes of the isidid axis join with the flexible proteinaceous nodes, the mineral has a crystalline structure closely corresponding to that of the sclerites, though this is not apparent in surface view. In the case of the other families I mentioned, the mineral is in crystalline form either concentrated in fusiform strands embedded in gorgonin, or densely permeated by fine reticulating strands of gorgonin. It appears that the gorgonin gives the axis flexibility and the mineral gives it strength.

The meager literature that mentions the mineralogy of octocorals (190, 748) reports that without exception the skeleton (sclerites) of octocorals other than *Heliopora* is magnesium-calcite, and that the non-spicular *Heliopora* is aragonite. While a study of the mineralogy in the various families by-x-ray diffraction shows that the sclerites are, indeed, calcite, we have found that the axial calcium may be either calcite, or calcite with an admixture of aragonite, or aragonite alone, as first noticed by Lowenstam (749). Although our survey is not sufficiently complete to show the distribution of aragonite among the families and genera, it occurs chiefly in certain genera of Primnoida and Chrysogorgidae.

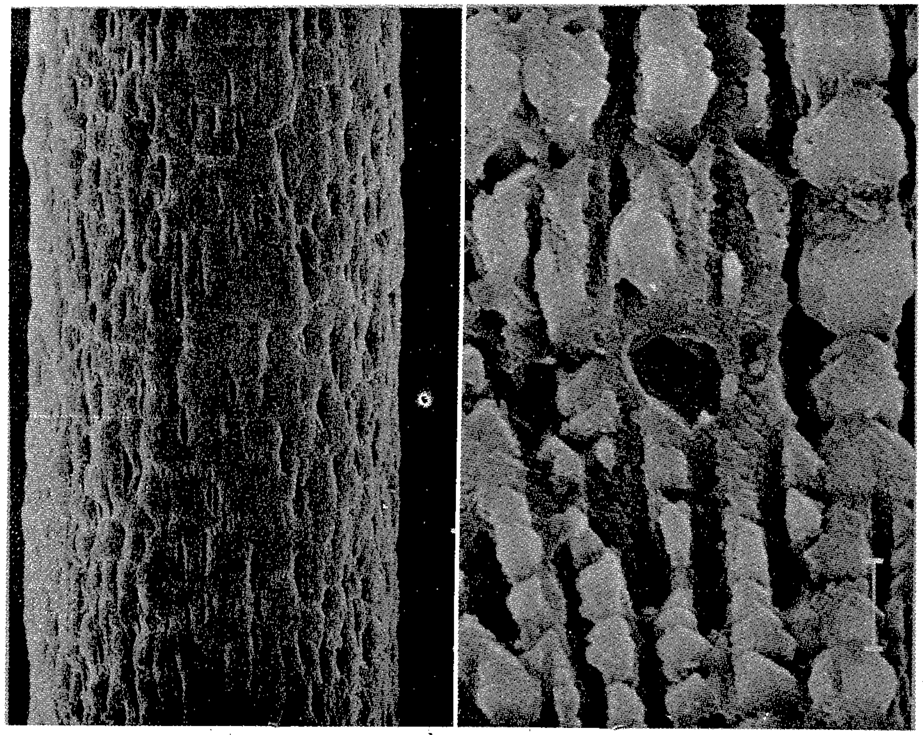

Fig. 6 – *Ctenocella pectinata* (Pallas). Structure of axis. Left, surface detail x95; right, ultrastructure x15000, scale represents 1 μ. S.E.M. micrographs by W.R. Brown, Smithsonian Institution.

The purely aragonitic, non-spicular skeleton of the Helioporacea is unique among the living Octocorallia. Its structure of hexagonal crystals of aragonite is spectacularly shown by S.E.M. (Fig. 7). Recent S.E.M. study of an inconspicuous Caribbean octocoral has shown that its skeleton is wholly crystalline (Fig. 7), not spicular, and x-ray diffraction has shown that it is composed of aragonite, not calcite. Because of these features, as well as several structural similarities with the Blue Coral *Heliopora*, my colleague Katherine Muzik and I have placed this curious coral in the Helioporacea, the second Recent species of the order known to science and the first in the Atlantic Ocean (74). I expect that the paper describing this coral, called *Lithotelesto micropora*, may be published about now in Washington, and this is the first public announcement anywhere. As the original locality of *Lithotelesto* is Barbados, it is not impossible that the species will be found also in Brazilian waters.

Another technique, very useful for the study of octocoral anatomy in relation to skeletal structure, is sectioning after embedment in epoxy resin. We have used this procedure to study the gross anatomy of a new solitary octocoral from New Zealand, and have also applied it to *Lithotelesto* and *Heliopora*. In the near future

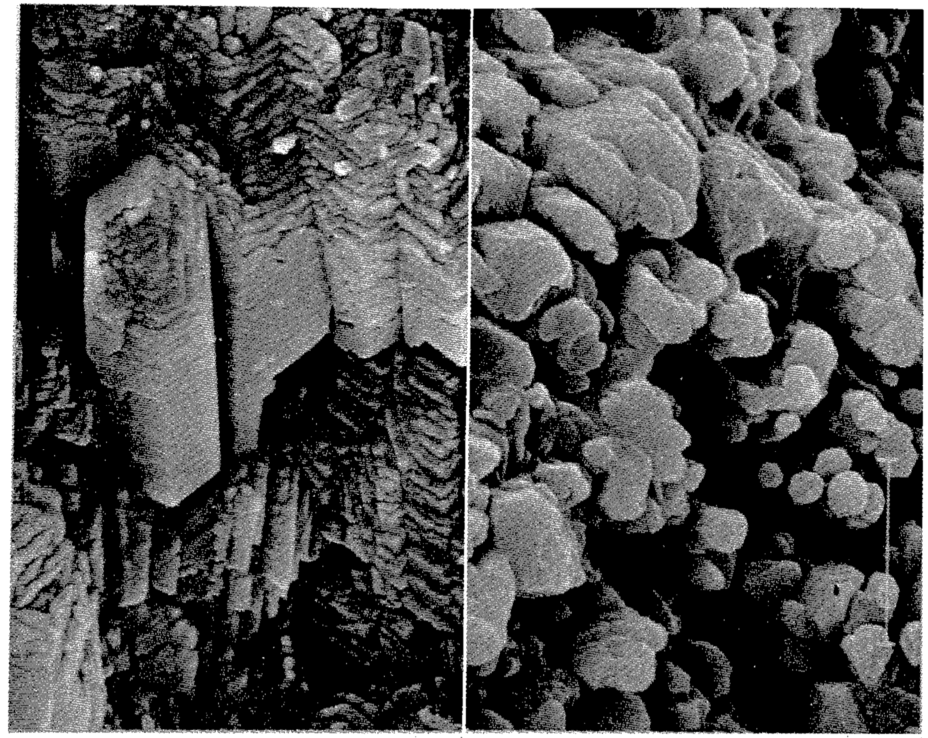

Fig. 7 – Ultrastructure of aragonitic octocoral skeleton. Left, *Lithotelesto micropora* Bayer & Muzik; right, *Heliopora coerulea* (Pallas). Both x15000; scale represents 2 μ. S.E.M. Micrographs by W.R. Brown, Smithsonian Institution.

we will publish some observations on their comparative anatomy in further support of our view that the two are closely related.

This technique, which is not at all difficult, is merely an adaptation of a standard geological and paleontological method to biological material. After dehydration and transfer to propylene oxide, the specimen is impregnated with epoxy resin in vacuo. The propylene oxide is boiled away by the vacuum, which also removes any trapped air bubbles. After appropriate curing, the embedded specimen is treated as if it were a geological specimen, and is cut on a diamond saw and polished on a lapidary wheel. After polishing, the surface can be lightly etched with acetic acid, and then replicated on an acetate slide. Such replicas ("peels") show skeletal structures clearly because of the etching, as well as many details of the soft anatomy – especially if the viewing is done with phase-contrast or Nomarski interference optics. By successive polishing and replication, serial peels through a specimen can be made up to the exact point where a section of actual tissue is desired. The polished surface is then cemented to a standard glass slide and the superfluous embedded tissue cut away with the diamond saw. By manipulation as a geological thin-section, the specimen may be cut as thin as about 10 micra. After staining, this kind of preparation reveals anatomical and histological detail in essentially natural relationship with mineral structures, a result that can be achieved in no other way known to me.

From a systematic point of view, the most important outcome of studies of octocorals by the scanning electron microscope has been a revision of the classification, which has resulted rather from an examination of a wide range of taxa than from the discovery of any unexpected new information. Of course, the new findings about crystal structure are valuable in that they tend to support the general conclusions reached on other grounds.

The original classification used by Linnaeus (743) and Pallas (900) was very simple. The octocorals were included in five genera of the class Zoophyta: *Alcyonium, Gorgonia, Isis, Tubipora* and *Pennatula.* This arrangement was elaborated somewhat in 1816 by Lamouroux, who introduced several new genera and divided the "flexible Corallines" into four classes, of which the first contained mostly hydroids, the second the algae (together with *Telesto*), the third the gorgonians, antipatharians and some algae, and the fourth the soft corals and zoanthids (710).

Milne Edwards & Haime in 1857 treated the octocorals as an order with a total of 50 genera distributed among three families, seven subfamilies, and four "agèles" (803). This general scheme was somewhat modified by Kölliker on the basis of spicular characters, but remains essentially the same in overall features (603).

The general classification developed by Kükenthal during more than three decades of leadership in this field and accepted by most workers was ultimately much more complex. In that classification as modified by me in 1956, the octocorals are a subclass of coelenterates divided into 7 orders, one of which is a fossil of doubtful affinity (56). In 1973, Utinomi & Harada (1311) established still another order, Gastraxonacea, for the peculiar genus *Pseudogorgia* described by Kölliker in 1870.

The objection to this system lies in the difficulty of assigning many species to their proper orders. For example, Verseveldt in 1940 showed that *Paragorgia* should be considered an alcyonacean, not a gorgonacean (1368). Already in 1939, Stiasny proposed a classification of only 4 orders, in which *Paragorgia*, along with several other "traditional" Gorgonacean genera, was placed in the Alcyonacea instead (111).

Similar difficulties exist in separating the "traditional" suborders of Gorgonacea – Holaxonia and Scleraxonia – and in assigning genera to the proper families. The result of my broad taxonomic survey of the octocoral genera confirms these difficulties, and attempts to solve the problem by a return to simplicity. I can concur in the main with Stiasny's four orders, but with the addition of a fifth order for solitary forms as originally proposed by Hickson. Definitions of families will not agree exactly with those of Stiasny, and therefore the assignment of genera to them will differ somewhat. In general, however, the scheme that will result from new S.E.M. studies and anatomical investigations will be similarly simple.

BIBLIOGRAPHY OF OCTOCORALLIA*
1469-1977

CLASSIFIED INDEX TO BIBLIOGRAPHY OF OCTOCORALLIA

General Works

Textbooks, handbooks, general treatises, cruise narratives and similar works not of strictly taxonomic nature but containing taxonomic, morphological, and distributional data, or information about procedures and techniques.
3, 112, 193, 212, 213, 219, 220, 221, 222, 236, 324, 423, 439, 446, 447, 462, 475, 478a, 524, 584, 680, 788a, 797, 811, 812, 848, 868a, 895, 943, 984, 1002, 1056, 1067a, 1067b, 1067c, 1186, 1268a, 1432, 1436, 1437.

Taxonomic: World Wide

Works including species from several major geographical regions; comprehensive taxonomic reviews and monographs.
56, 84, 88, 102, 145, 218, 261, 274, 275, 309, 365, 367, 380, 384, 385, 386, 455, 461, 497, 513, 514, 520, 532, 603, 607, 611, 612, 636, 637, 638, 639, 642, 643, 649, 650, 651, 652, 653, 656, 659, 671, 677, 681, 683, 689, 705, 706, 707, 709, 710, 711, 712, 714, 743, 744, 756, 771, 787, 803, 838, 840, 846, 847, 856, 900, 902, 903, 904, 971, 989, 994, 1065, 1073, 1094, 1098, 1100, 1106, 1109, 1135, 1137, 1138, 1148, 1157, 1236, 1237, 1238, 1239, 1240, 1241, 1246, 1247, 1248, 1262, 1263, 1292, 1295, 1317, 1325, 1335, 1337, 1338, 1438.

Taxonomic: North Atlantic Ocean and adjacent arctic waters, including Scandinavia

24, 25, 121, 122, 123, 124, 125, 126, 127, 128, 131, 132, 133, 136, 141, 142, 224, 225, 226, 227, 245, 392, 399, 400, 401, 402, 403, 404, 405, 406, 420, 421, 425, 542, 545, 546, 547, 548, 549, 614, 615, 617, 618, 619, 626, 627, 628, 648, 658, 722, 735, 736, 737, 762, 763, 764, 770, 775, 785, 788, 810, 813, 854, 881, 927, 1031, 1034, 1035, 1036, 1037, 1039, 1040, 1143, 1144, 1193, 1297, 1324 1353, 1365, 1368, 1375, 1414.

*Because this is the first attempt to compile a comprehensive bibliography of the world literature on Octocorallia, errors of both omission and commission certainly will be found. I hope that no significant taxonomic references have been overlooked, but the coverage of the palaeontological, biological and biochemical literature inevitably will contain more gaps. Likewise, I surely will have overlooked some titles among the 16th and 17th Century literature.

Although I have seen most of the works personally, some titles have been taken exclusively from reliable published sources so the exact bibliographic details have not been verified for them. I shall appreciate receiving any additions and corrections to this list that may come to the notice of users everywhere.