

Phylogenetic Relationships Among Scleractinians, Actinians, and Corallimorpharians (Coelenterata: Anthozoa)

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Abstract. Using radioimmunoassay, with Ceriantharia as the outgroup, we tested whether corallimorpharians are so closely related to scleractinians that they should be considered members of that order. From analysis of distance data, we infer that the two lineages of corallimorpharians (tropical, plate-like zooxanthellate forms and largely temperate/deep sea, columnar, azooxanthellate ones) are as closely related to a lineage of deep-sea corals as they are to one another. We therefore concur with the minority opinion that Corallimorpharia does not constitute an order distinct from and equivalent in rank to Scleractinia. We further infer that Actiniaria, which branches from a different lineage of Scleractinia, may not represent a separate order, either. These data support morphological evidence that scleractinians are ancestral to corallimorpharians and actinians, contrary to the general paradigm. We speculate that both corallimorpharians and actinians may have had multiple origins from corals, and that other groups of non-skeletalized polyps, now extinct, may also have arisen from corals.

Introduction

Corallimorpharians are anthozoans morphologically intermediate between actinians (sea anemones) and scleractinians (hard corals). The most

current and generally accepted classification schemes recognize Corallimorpharia as an order of Class Anthozoa, Subclass Hexacorallia, equivalent in rank to Actiniaria and Scleractinia (Carlgren 1949; Wells and Hill 1956; Dunn 1982). Like actinians, corallimorpharians lack calcareous skeletons. Indeed, they are often popularly referred to as sea anemones, and were classed among actinians until early in the 20th century (e.g. Duerden 1900; see den Hartog [1980] for an historical summary of this taxon). However, their nematocysts and internal anatomy more closely resemble those of corals than of sea anemones *sensu stricto*. Taxonomists such as Stephenson (1921), Schmidt (1974), and den Hartog (1980), considering these features more significant than those shared with actinians, have advocated categorizing corallimorpharians with corals, the only difference between the two components of this new taxon being whether they form a skeleton.

Corallimorpharians are divided into three (Carlgren 1949) or four (den Hartog 1980) families. They are of two distinct morphologies. Most living in shallow tropical seas have delicate, plate-like oral discs bearing many short, often branched tentacles, and contain zooxanthellae; they constitute families Actinodiscidae and partly Corallimorphidae *sensu* Carlgren (1949) or Discosomatidae and Ricordeidae *sensu* den Hartog (1980). Robust polyps lacking zooxanthellae, with columns nearly as wide as oral

discs, and relatively few, long, unbranched, capitate tentacles, are mostly inhabitants of temperate seas, often at considerable depth; they include Sideractinidae and some members of Corallimorphidae (cf. Carlgren 1949; den Hartog 1980).

Because hexacorals lacking skeletons are virtually without a fossil record (the rare exceptions [e.g. Arai and McGugan 1968; Boyd 1974] lack critical taxonomic features), their evolutionary history must be deduced from extant species. Debate about the phylogenetic position of corallimorpharians relative to corals is unlikely to be resolved by further study of morphology. We therefore brought molecular features to bear on this problem. We first compared by radioimmunoassay (RIA) proteins of corallimorpharians of two families (one of each morphology) and two poritid corals, using sea anemones as the outgroup.

However, Actiniaria did not function as an outgroup in our analysis. We therefore expanded our investigation to the nature of the relationship among Actiniaria, Corallimorpharia, and Scleractinia. Members of these orders have paired mesenteries that are added in couples around the circumference of the animal during ontogeny (see Hand [1966] for a full explanation of this feature and discussion of its significance), a feature shared with only one of the other four hexacorall orders. This is the Ptychodactiaria, which contains three species of uncommon, poorly-studied, skeletonless "anemones" from high latitudes that are difficult to obtain and so were not studied. Mesenteries in other anthozoans are not paired and coupled. A cerianthid (Subclass Hexacorallia; Order Ceriantharia) formed our outgroup (non-anthozoan cnidarians proved unsatisfactory for this purpose, but produced interesting results themselves: Fautin and Lowenstein 1992).

Materials and Methods

Species examined

We studied specimens of the corals *Goniopora pandoraensis* Veron and Pichon, 1982, and *Porites cylindrica* Dana, 1846 (both members of family Poritidae), from the Great Barrier Reef near Townsville, Australia, and *Fungiacyathus marenzelleri* (Vaughan, 1906) (formerly questionably categorized in family Fungiidae [e.g. Wells 1956], but now placed in Fungiacyathidae [Chevalier and Beauvais 1987]), dredged at 4000 m off southern California. All were returned frozen to the laboratory. The corallimorpharians examined were *Rho-*

dactis sp. and *Corynactis californica* Carlgren, 1936. The former, of the plate-like morphology, belongs to family Discosomatidae (= Actinodiscidae *sensu* Carlgren). Our specimens of *Rhodactis*, which originated in the central tropical Indo-Pacific, were obtained from a tropical fish wholesaler through the Steinhart Aquarium of the California Academy of Sciences. The specimens of *Corynactis*, collected near Monterey, California, exemplify the robust form, and belong to family Corallimorphidae. The actinians *Epiactis prolifera* Verrill, 1868, *E. ritteri* Torrey, 1902, and *Anthopleura artemisia* (Pickering in Dana, 1846), members of family Actiniidae, and *Metridium giganteum* Fautin, Bucklin, and Hand, 1990, family Metridiidae, were collected near Bodega Bay, California. The latter species belongs to what Carlgren (1949) referred to as "subtribe" Acontiarina and the former three to "subtribe" Endomyaria; these higher taxa should more properly be termed infraorders or superfamilies. The outgroup was represented by the cerianthid *Pachycerianthus fimbriatus* McMurrich, 1910, from Monterey Bay, California.

Experimental protocol

Tissue of an individual or colony was ground in a Waring blender, and the soluble fraction was extracted with phosphate-buffered saline solution. In the case of corals, tissue was abraded from the skeleton by a strong stream of water from a WaterPik. The macerated tissue of zooxanthellate animals was immediately centrifuged; the plant material was concentrated in a pellet and discarded.

The resulting extract was lyophilized, which produced an antigenic powder that was dissolved in distilled water for tests and immunization. Antibodies were produced in New Zealand white rabbits by a series of four subcutaneous injections of about 0.5 mg antigen each. The first was given with 0.5 ml Freund's complete adjuvant; others, given at two-week intervals, were without adjuvant. One or two weeks after the final injection, the rabbits were euthanized with pentobarbital, and exsanguinated by cardiac puncture. This protocol has been approved by the Committee on Animal Care of the University of California, San Francisco.

The solid-phase radioimmunoassay proceeds as follows:

- 1) Antigenic solutions from target species are placed in wells of a 96-well polyvinyl microtiter plate. Protein binds to the plastic.

- 2) Unbound protein is washed from the wells with soy protein (0.25%), which also blocks remaining binding sites on the plastic.
- 3) Rabbit antisera are added in 0.02 ml aliquots to the wells for 24 h. The antibodies bind best to their homologs, but cross-react with related species in proportion to their degree of relatedness.
- 4) A second soy protein wash removes unbound antiserum.
- 5) Radioactive (^{125}I -labeled) goat anti-rabbit gamma globulin (GARGG) is placed in each well for 24 h, where it binds to the rabbit antibody.
- 6) Unbound GARGG is removed with running tap water.
- 7) Radioactivity of individual wells, cut from the plate, is measured in a scintillation counter. Total radioactivity in each well depends on the amount of bound antibody, which is a function of the species-specificity of the protein. ID (quantitative immunological distance) = $100 \log A/B$, where A and B are different antiserum concentrations that yield identical radioactivities for homologous and heterologous proteins, respectively. ID is linearly correlated with the amino acid sequence differences between compared proteins (Prager and Wilson 1971). Therefore, matrices of IDs can be used for constructing phylogenetic trees.

To assay replicability of our method and variability of animals with RIA, we ran second specimens of *E. prolifera* and *E. Ritteri* blind. These, being the most closely related of our subject taxa, had the potential to be the most difficult to distinguish.

Analysis

We used each protein mixture as both antibody and antigen, averaging the two values to construct the tree. Reactions are seldom symmetrical because, unlike with microcomplement fixation, there is no control over quantity (concentration) of antigen; averaging reciprocal values compensates for this.

Our consensus tree (Fig. 1) was built from at least half a dozen trees obtained through application of the Fitch-Margoliash and Kitsch programs from PHYLIP.

Results

Pachyercianthus fimbriatus functioned as an outgroup to the nine species of anthozoans with paired and coupled mesenteries (Fig. 1).

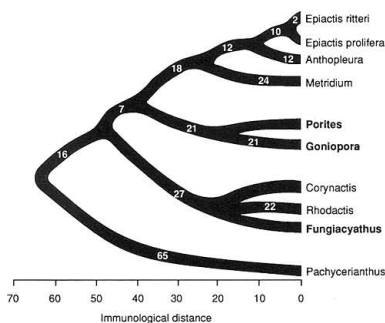


Fig. 1. Consensus tree of some hexacorallian cnidarians based on analysis of radioimmunoassay results. *Pachyercianthus*, which constitutes the outgroup, is a cerianthid. The other taxa in light typeface are actinians; the three boldface taxa are scleractinians.

Based on our analysis, we hypothesize that the corals we used belong to two lineages; sea anemones are derived from one lineage and corallimorpharians from the other. That is, we infer that corallimorpharians and actinians diverged from some corals more recently than those corals diverged from one another. The branching order of the two corallimorpharians and the deep-sea coral cannot be resolved.

The specimens of *Epiactis prolifera* and *E. Ritteri* that we ran blind produced unambiguous and accurate results. They reacted identically to the samples of those species used as standards, and were easily distinguished from one another.

Discussion

Phylogeny

The widely held assumption is that a "... group of anemones ... gave rise to the Scleractinia. This is usually regarded as the Corallimorpharia (or possibly the Actinaria)" (Scrutton and Clarkson 1989: 21). Ultimately, of course, corals presumably had a soft-bodied ancestor, but, as Wells (1956: F362) recognized, the ancestor may not have belonged to an extant group: "A large question in the evolution of anthozoan corals is ... whether they were ... derived from skeletonless anemone stocks—the scleractinians from the same stock that led to the living corallimorphs and actinarians." Hand (1966) argued, based on morphology, that actinians descended from scleractinians—contrary to pre-

vailing wisdom. The absence of paired mesenteries in hexacorals orders such as Ceriantharia demonstrates that even rather large skeletonless polyps can exist with only single mesenteries. Pairing, according to Hand, evolved in animals in which a calcareous septum existed between the members of a pair; it persists in descendants of corals that no longer possess a skeleton, and in which such anatomy serves no functional end. Indeed, it stretches credulity to imagine that paired mesenteries arose spontaneously in an anemone, which was thereby preadapted to have a calcareous septum deposited between the members of each pair. Our data support Hand's compelling argument.

Rhodactis, *Fungiacyathus*, and *Corynactis* constitute an unresolved trichotomy (Fig. 1). Corallimorpharians of the two sorts may therefore represent independent, albeit closely related, derivations from the lineage of corals that includes *Fungiacyathus*, or they may be, together, the sister group of *Fungiacyathus*. (The closeness of the corallimorpharians surprised us, considering how much they differ in habitus and ecology.) Regardless of the sequence of origin of these lineages, Hand's (1966: 139) conclusion that Corallimorpharia "is more recently derived from the corals and . . . represents a parallel to the origin of the Actiniaria" accords with our findings. Carlgren, who did not include corals in his analyses, also believed (1940) that Corallimorpharia and Actiniaria are independent lineages from a common ancestor, but our findings conflict with his (1942) conclusion that the former is more primitive and therefore diverged earlier.

The phylogeny of Actiniaria has never been satisfactorily resolved. Taxa possess few unique morphological features (autapomorphies), being generally distinguished by mosaics of characters. In 1942, Carlgren reviewed in detail anemone phylogeny based on anatomy, providing many examples of likely plesiomorphy; in his catalog (1949: 42), he was "doubtful whether the three [subtribes] include genera which are always genetically related to each other." Any alternative arrangement demanding monophyly would suffer from the same inconsistencies because of the mosaic nature of the characters. If we are correct in our hypothesis that at least two lineages of "sea anemones" (corallimorpharians and actinians) arose from corals, then, we assert, others may have done so as well. The lack of a coherent phylogenetic pattern would be understandable if the taxon Actiniaria consists of lineages that arose more than once from corals, at least some of them closely related to one another. Thus,

just as Corallimorpharia may not be monophyletic, Actiniaria may not be.

Hand (1966), in pointing out similarities in cnidae and growth form of members of Zoanthidea and Scleractinia, speculated that zoanthids represent another group independently descended from corals. Indeed, many other stocks of naked polyps, including Ptychodactaria, may also have arisen from corals; since they leave no fossil record, we can be certain only of those that persist today.

Our data do not support the *status quo* of three coequal major hexacorals orders: Actiniaria, Corallimorpharia, and Scleractinia. Nor are they consistent with the widely held alternative of two orders—Actiniaria and Corallimorpharia plus Scleractinia—as proposed by Stephenson (1921), Schmidt (1974), and den Hartog (1980), for some corals appear more closely related to actinians than they are to other corals. Although the actinians we tested cluster together on our tree, no characters of which we are aware uniquely distinguish Actiniaria from Corallimorpharia-Scleractinia. Indeed, as Yonge (1972: 12) observed, "the Actiniaria and the Scleractinia . . . differ only in the powers of calcification possessed by the latter." If Scleractinia is redefined to include some non-calcifying members, then nothing separates sea anemones from corals.

Mechanisms

We know of two mechanisms that could account for the evolutionary sequence we propose. Corals of at least some species can abandon their skeletons (Goreau and Goreau 1959; Sammarco [1981, 1982], who termed the process "polyp bail-out"). Although some may be able to secrete new skeletons (*Seriatopora hystrix*: Sammarco 1981, 1982), others cannot (*Cyanarina lacrimalis*: P. Alderslade, Northern Territory Museum, pers. comm.). This does not mean that a coral, having abandoned its skeleton, *ipso facto* transforms into another species. Skeleton abandonment may, however, allow an organism to occupy a new niche which, over time, could result in divergence from the parent population.

Alternatively, or in addition, corals may lose the ability to calcify. The skeleton of *Fungiacyathus* is so delicate that, without living tissue to bind it, it hardly holds together. Thus, it is nearly a corallimorpharian, and its close relationship to animals that may be only slightly more advanced down a similar path is not as surprising as it may seem at first. It has been suggested to us that, rather than corallimorpharians being corals, *Fungiacyathus* may really be a corallimorpharian, thereby restrict-

ing corals to a single branch of Figure 1. The only feature that consistently distinguishes a corallimorpharian from a coral is the latter's skeleton. Thus, operationally, corallimorpharians with skeletons would be classed as scleractinians; and such skeletons in the fossil record are considered to have been made by corals.

Chronology

Porites is believed to have arisen from *Goniopora* in the Eocene (the Eocene began about 58 mya) (Wells 1956). This allows us to calibrate our tree, recognizing that proportionality between ID and time diminishes with time (Lowenstein 1981), so RIA underestimates time of divergence for higher taxa. However, having calibrated our tree relatively far into the interval of interest, we believe it is reasonably accurate for these groups, assuming consistent evolutionary rates across taxa. We estimate from Figure 1 that actinians diverged from their common ancestor with poritid corals a minimum of 116 mya (= 42 ID), and the corallimorpharian-containing branch diverged from its common ancestor with actinians 135 mya (= 49 ID)—both early Cretaceous.

The earliest indubitable scleractinian fossils are from the mid-Triassic (Wells 1956). Because they are well diversified shortly after first appearance, corals certainly arose earlier. A mid-Ordovician "scleractiniomorph coral" is regarded by its authors as "an early and ultimately unsuccessful attempt at skeleton acquisition by the group of anemones that later gave rise to the Scleractinia" (Scrutton and Clarkson 1989: 21). Thus, our inference of an early Cretaceous origin of the Actiniaria supports Hand's reasoning from morphology: corals, having existed at the time that they and actinians diverged, represent the ancestral stock. Corallimorpharians, we infer, are the product of a similar event at about the same time as *Porites* arose. We speculate that divergence of the branches leading to the Actiniaria and Corallimorpharia were part of the burst of diversification in Scleractinia that occurred in the Jurassic and Cretaceous (Wells 1956).

Methodology and reliability

Our choice of species to test was dictated by opportunity. Having obtained frozen specimens of *Porites* and *Goniopora*, both members of family Poritidae, we sought corals representing other lineages. We took advantage of a rare opportunity to obtain living specimens of *Fungiacyathus*, which had the additional attribute of lacking zooanthel-

lae. Inferring from these admittedly preliminary data that the sea anemones we tested are derived from a different lineage of corals than are corallimorpharians, we are now testing corals of other families and suborders to determine which corals are likely to have given rise to contemporary actinians and corallimorpharians.

RIA is semi-quantitative, concentrations of antigen and antibody being unknown. Moreover, because we used whole animals, we do not know the identity of the antigens producing the patterns we observe. We believe, however, that our results are due to an additive effect of small differences in many proteins that might not manifest detectable dissimilarities if compared individually (e.g. Olsen-Stojkovich et al 1986; Price et al 1987; Price and Lowenstein 1989).

The distance between the two congeneric species of actinians was the least of all comparisons made, followed by that between the cofamilial anemones and corals. This conforms qualitatively to expectations, based on taxonomy, and therefore gives us confidence in our method.

The accuracy of our tests with specimens of *Epiactis* that we ran blind demonstrates that a single specimen is representative of a species, and that species of anthozoans (even closely related ones) are distinguishable with whole-animal RIA. (This testing of the system was analogous to that in which species of elephants were successfully discriminated [Lowenstein et al 1981].)

Finally, early in this research, when we were dealing with only a few species, each one we added shifted the topology of the resulting tree. The tree has been stable with the addition of the last several species, however, giving us additional confidence in the robustness of our results.

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