

## A SIMPLE TEST: EVALUATING EXPLANATIONS FOR THE RELATIVE SIMPLICITY OF THE EDWARDSIIDAE (CNIDARIA: ANTHOZOA)

MARYMEGAN DALY,<sup>1,2,3</sup> DIANA L. LIPSCOMB,<sup>1</sup> AND MARC W. ALLARD<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, The George Washington University, Washington D.C. 20052

<sup>2</sup>Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045

<sup>3</sup>E-mail: dalym@ku.edu

**Abstract.**—Many members of the cnidarian subclass Zoantharia (sea anemones, corals, and their allies) pass through a larval stage with eight complete mesenteries and without posterior musculature. This larva is usually transient, developing into an adult with 12 or more mesenteries. The adults of one family of sea anemones, the Edwardsiidae, bear the larval number and arrangement of mesenteries and lack the pedal disc seen in other sea anemones. The morphology of the Edwardsiidae has been interpreted in a number of ways: (1) the Edwardsiidae are the most basal extant zoantharian, having diverged before the evolution of additional mesenteries and basal musculature; (2) they are relatively advanced sea anemones that have secondarily simplified because they burrow in sand or mud rather than attaching to a hard substrate; or (3) edwardsiids are derived anemones that have retained a juvenile morphology through paedomorphosis. Phylogenetic analyses of small subunit ribosomal gene sequences reveal that the Edwardsiidae are derived zoantharians, nested within sea anemones. None of the proposed explanations fully explain the edwardsiid's body plan; edwardsiid anatomy is a mosaic of retained primitive and derived features. The results of the present study provide insight into zoantharian phylogeny and illustrate how phylogenetic tests can be used to study the evolution of cnidarian body plans.

**Key words.**—Adaptation, *Cnidaria*, comparative method, heterochrony.

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The evolution of organisms with relatively simple bodies present intriguing puzzles for evolutionary biologists because there can be many explanations for the absence of features. If the simplified organism diverged from its more complex sister group prior to the evolution of more complex traits, then the relatively simple body form is the primitive state. Alternatively, relative simplicity can be due to secondary evolutionary events and thus represent a derived condition. Adaptation to a specialized ecological niche may lead to the reduction or loss of features that are no longer needed or act as an impediment in the new habitat. Loss of limbs and other appendages in burrowing animals (e.g., Gans 1975; Rieppel 1979) or reduction of pigment and eyes in cave-dwelling animals (e.g., Culver et al. 1995) are well-known cases of secondary simplification due to ecological adaptation. Another possibility exists, however. If complex features appear late in the developmental process, then paedomorphosis (changes in the appearance or rate of development so that there is a retention of ancestral juvenile characters by descendant adults) will result in secondary simplification of the body (e.g., Wake 1969; Mishler 1988). The challenge is to determine whether simple body form is due to inheritance of the primitive condition or a secondary modification, perhaps due to ecological adaptation or paedomorphosis.

An interesting case of relatively simple body form in lower invertebrates is found in the edwardsiid sea anemones (Edwardsiidae: Anthozoa: Cnidaria). These anemones include many cryptic and poorly known animals, including two species listed as endangered or threatened (Williams 1983). In contrast to more familiar sea anemones, which have stout bodies, edwardsiids are long and vermiform. The difference in gross anatomy is reflected in the internal anatomy. For example, all anemones have sheets of tissue (mesenteries) extending radially from the body wall into the coelenteron. Mesenteries that span the entire gastrovascular cavity, con-

necting the body wall to the pharynx, are called complete mesenteries. Adults in the Edwardsiidae have fewer complete mesenteries than the adults of any other actiniarian family. Additionally, edwardsiids lack marginal sphincter muscles in the upper column and basilar muscles in the aboral end.

The distinctness of the Edwardsiidae has been explained by three different evolutionary scenarios. First, the absence of basilar muscles and relatively fewer mesenteries has been considered a primitive absence, indicating that the Edwardsiidae split from the rest of the Zoantharia before the evolution of these features. The Edwardsiidae may thus represent the primitive type for the Zoantharia (McMurrich 1891; Stephenson 1921; Hyman 1940). Alternatively, the muscles and mesenteries may have been lost in response to a habitat switch, from sessile attachment to active burrowing (Carlgrén and Stephenson 1928). Because edwardsiids burrow rather than attach to a hard substrate, basilar musculature may be unnecessary and could then be secondarily lost. The third explanation proposes that musculature and mesenteries are secondarily lost through paedomorphosis (Hand 1966). The paedomorphosis explanation attempts to account for the similarity between adult edwardsiids and the juvenile form of all other anemones and corals. Many members of the subclass Zoantharia (sea anemones, corallimorpharians, black and stony corals) have a larval stage with eight complete mesenteries and without posterior musculature. Most zoantharians pass through this “edwardsia” stage and develop into adults with 12 or more complete mesenteries; edwardsiids do not add any additional complete mesenteries after this stage. Changes in developmental timing such that the ancestral edwardsiid reached maturity while still in the larval body form would result in an adult with only eight complete mesenteries and without pedal musculature—precisely the body form seen in the Edwardsiidae.

Each explanation implies different evolutionary relation-

ships between the Edwardsiidae and the other Zoantharia. The alternative explanations can be evaluated with reference to the pattern of phylogenetic relationships recovered through analysis of independent data (Fink 1988; Brooks and McLennan 1991; Harvey and Pagel 1991; Wake 1996). Although consistency between the best phylogenetic hypothesis and the topology predicted by an evolutionary scenario cannot be equated with proof for that scenario, discordance between the predicted tree and the most robust phylogenetic hypothesis falsifies the evolutionary scenario underlying the predicted tree (Coddington 1988; Wenzel and Carpenter 1994). In other words, the evolutionary explanation must at least be consistent with the phylogenetic evidence to be considered a viable hypothesis. In the case of the Edwardsiidae, a well-supported phylogenetic tree can indicate whether the Edwardsiidae diverged basally or is sister to a more derived taxon from which it differs in ecological habit or in the timing of developmental events.

Evaluating these hypotheses and addressing the nature of the character distribution within the Edwardsiidae requires understanding relationships among the Zoantharia. Growth mode, habitat, and anatomy exhibit great diversity within the Zoantharia, making compatible morphological data difficult to obtain and difficult to interpret. Orders within the Zoantharia have been defined either by the presence of a few traits not found in any other taxa or by the absence of another order's autapomorphic features. For example, the Actiniaria are recognized almost exclusively by the features they lack: They have neither a protein (Antipatharia) nor mineral (Scleractinia) skeleton, they lack labial tentacles (Ceriantharia), and they lack mesogleal canals (Zoanthidea). Previous workers have instead used 16S (Romano and Palumbi 1996; Romano and Cairns 2000), 28S (Chen et al. 1995; Romano and Cairns 2000), or 18S (Song and Wong 1997; Berntson et al. 1999) rDNA sequences to investigate relationships among zoantharians. Ordinal diversity is best represented by the 18S rDNA sequences, and this gene has more variable and informative sites than either 16S or 28S. The nine species we have sequenced include three actiniarian families not previously represented in the 18S sequence matrix. The addition of these taxa facilitates exploration of the competing explanations for the unusual morphology of the Edwardsiidae.

#### MATERIALS AND METHODS

Six edwardsiids (representing the geographical, taxonomic, and morphological diversity within the Edwardsiidae, including Genbank accession numbers: *Edwardsia andresi*, Norway, AF25434; *Edwardsia elegans*, Maine, AF254376; *Edwardsia gilbertensis*, Guam, AF254377; *Edwardsia tuberculata*, Norway, AF254381; *Edwardsiella lineata*, Massachusetts, AF254378; *Nematostella vectensis*, Maryland, AF254382) and three nonedwardsiid, burrowing anemones (*Halcampa duodecimcirrata*, Maine, AF254375; *Halcampoides purpurea*, Ireland, AF254380; *Haloclava producta*, Massachusetts, AF254379) were collected from the North Atlantic and Pacific Oceans. Specimens were identified and fixed in 95% ethanol. Voucher specimens for each sequence are deposited at the American Museum of Natural History (AMNH 3609–3617). The small subunit ribosomal DNA gene

was amplified from whole genome preparations in three pieces, using previously described internal and external primers (Medlin et al. 1988; Apakupakul et al. 1999). The thermal profile of the polymerase chain reaction was optimized for each species and fragment (30 cycles: 30 sec at 94°C, 60 sec at 37–72°C, 90 sec at 72°C, 1.5–4.0 mM MgCl). ABI Prism BigDye dye-terminator chemistry and an Applied Biosystems Prism 377 automated sequencer with Sequencing Analysis Version 3.3 software (Perkin-Elmer Applied Biosystems, Foster City, CA) were used to sequence the 1750–1800 bp gene.

The nine new sequences are here combined with 39 zoantharian sequences from Genbank. The Genbank sequences include representatives of all zoantharian orders, with all currently available sequences included: Actiniaria: *Anthopleura kurogane*, Z21671; *Anthopleura midori*, Z86098; *Bunodosoma granulifera*, U52974; *Condylactis gigantea*, U52976; *Dactylanthus antarcticus*, AF052896; *Epiactis japonica*, Z292904; *Flosmaris mutsuensis*, Z292905; *Haliplanella luciae* (= *lineata*), Z86097; *Haloclava* sp., AF052891; *Hormathiidae* sp., AF052890; *Metridium senile*, U19550; *Nynanthae* sp., AF052880; *Stoichactis helianthus*, U52977; *Stomphia* sp., AF052888; Antipatharia: *Cirripathes lutkeni*, AF052902; *Antipathes lata*, Z92908; *Antipathes galapagensis*, AF100943; *Antipathes fiordensis*, AF052900; *Stichopathes speissi*, AF052899; *Bathypathes* sp., AF052901; Ceriantharia: *Ceriantheopsis americana*, AF052898; *Cerianthus borealis*, AF052897; *Cerianthus* sp., U19544; Corallimorpharia: *Corynactis californica*, AF052895; *Discosoma* sp., AF052894; Scleractinia: *Balanophylla elegans*, U52973; *Ceratotrochus magnahii*, AF052886; *Enallopsammia rostrata*, AF052885; *Fungia scutaria*, AF052884; *Pavona varians*, AF052883; *Phyllangia mouchezi*, AF052887; *Rhizopsammia minuta*, Z92907; *Tubastrea aurea*, Z92906; Zoanthidea: *Parazoanthus axinellae*, U42453; *Palythoa variabilis*, AF052892. (Note: The Z292904 sequence probably does not represent the actiniarian *E. japonica*. This sequence has greatest similarity to the scleractinian *T. aurea*. It is possible that this sequence is the result of contamination by scleractinian DNA or that the animal from which the DNA was extracted was misidentified.) A summary of the distinguishing features of each order is given in Table 1. The sequences we have used encompass greater taxonomic diversity than any previous study. The extent to which these sequences represent the phylogenetic diversity of the Zoantharia cannot be ascertained, as relationships within each zoantharian order are unknown.

Three members of the Octocorallia (*Alcyonium gracilimum*, Z92902; *Renilla muelleri*, U19552; *Dendrobranchia paucispina*, AF052903), the sister group of the Zoantharia (Bridge et al. 1995; France et al. 1996) are used as outgroups. Each species represents a different lineage within the Octocorallia (France et al. 1996; Berntson et al. 1999). The *Renilla* sequence is incomplete, as are a few of the actiniarian sequences. Although incomplete sequences do not contain all of the information present in complete sequences, some information is still present, and the use of partial sequences increases the taxonomic diversity of the study sample. The sequence alignment algorithms of DAPSA (Harley 1996) were used to align the sequences; corrections and adjustments

TABLE 1. Summary of distinguishing features of the six zoantharian orders.

Order	Common name	Solitary/colonial	Skeleton	Distinguishing characteristics
Actiniaria	sea anemones	solitary	absent	polyps with single circlet of tentacles, no skeleton, not colonial
Antipatharia	black corals	colonial	protein	proteinaceous skeleton, weak, almost amuscular pulps
Ceriantharia	tube anemones	solitary	absent	polyps fabricate tubes, have oral and marginal tentacles
Corallimorpharia	corallimorpharians (jewel anemones)	solitary and colonial	absent	polyps which short, often knobbed tentacles, no skeleton
Scleractinia	stony corals	primarily colonial	mineralic	calcium carbonate skeleton
Zoanthidea	zoanthids (mat anemones)	primarily colonial	absent	matlike attachment between polyps, network of canals through mesoglea

were made by eye (the alignment is available via <http://www.gwu.edu/~clade/cnidaria.html>).

Phylogenetic relationships were reconstructed using parsimony methods. Most parsimonious trees were found using a branch-and-bound search in Hennig86 (Farris 1988). Alignment gaps are treated as ambiguous (?) rather than as a fifth state. The differences in sequence length between incomplete and complete sequences necessitate this approach. Three character weighting schemes were explored. First, all data were weighted equally. Because transitions are more common than transversions in the 18S rDNA matrix and because transitions may be more prone to homoplasy than transversions (Brown et al. 1982; Swofford et al. 1996), two additional analyses were conducted in which transversions were a priori weighted twice as heavily as transitions (2:1), and in which transitions were omitted (1:0). If transitions show high levels of homoplasy, decreasing their relative weight in phylogenetic analysis should improve the phylogenetic signal.

The three explanations for the distinctiveness of the Edwardsiidae are evaluated through comparison to the most robust phylogenetic tree. In the case of the primitive simplicity hypothesis, the hypothesized placement of the Edwardsiidae relative to all other zoantharians is clear—the Edwardsiidae are expected to be the most basal lineage. Evaluating the other two explanations requires mapping the distribution of certain traits such as burrowing, basilar muscles, and number of mesenteries onto the tree. Characters of interest are mapped using Winclada (Nixon 1999). We have explored all equally parsimonious optimizations of mesentery number, basilar musculature, and burrowing. The concentrated changes test assesses whether traits that repeatedly coexist are independent (Maddison 1990). Burrowing and the absence of basilar muscles are commonly associated in the Actiniaria; the concentrated changes test evaluates whether these two traits actually coexist more frequently than would be expected, given the null hypothesis of characters being randomly distributed throughout the tree. This test is implemented through MacClade (Maddison and Maddison 1995).

## RESULTS

The alignment contains 305 variable, parsimony-informative positions. In all analyses, the Edwardsiidae is nested within sea anemones (order Actiniaria). The equally weighted data matrix recovers 12 most parsimonious trees of length

(L) 1134, with an ensemble consistency index (CI) of 0.43 and an ensemble retention index (RI) of 0.60. The strict consensus of these trees is shown in Figure 1.

Analysis of the 2:1 weighted matrix yields 324 trees (Fig. 2A; L = 1597, CI = 0.40, RI = 0.59). Phylogenetic analysis of the 1:0 weighted matrix (121 informative sites) recovers 342 equally parsimonious trees (Fig. 2B; L = 448, CI = 0.36, RI = 0.56). Comparison between Figures 1 and 2 demonstrates that the primary difference between the strict consensus of the differentially weighted trees and of the equally weighted trees is the degree of resolution: The equally weighted consensus tree includes more resolved clades than the 2:1 consensus tree, and the 2:1 consensus tree includes more resolved clades than the 1:0 consensus tree. Differences in resolution are especially pronounced in the Actiniaria.

Differential character weighting does not seem to amplify the phylogenetic signal in these data. The differentially weighted data are less internally congruent than the equally weighted data; there are more trees and greater disagreement among those trees in the 1:0 and 2:1 weighted data, compared to the equally weighted data. Decreasing the weight given to transitions effectively decreases the phylogenetic signal in the 18S data. Our findings are congruent with other recent studies (Davis et al. 1998; Källersjö et al. 1999; Broughton et al. 2000) that have discovered that much of the phylogenetic structure resides in molecular characters previously hypothesized to be highly homoplastic and too variable to be informative. Transitions provide useful information about relationships among the Zoantharia and their inclusion improves congruence and resolution. We therefore prefer the equally weighted matrix over the weighted matrices; the strict consensus of the equally weighted trees (Fig. 1) will serve as the basis for further discussion.

Although burrowing and the absence of muscles share a similar distribution within the Actiniaria (Fig. 3), the concentrated changes test fails to find a significant association between the two ( $P$ -values range from 0.1285 to 0.1372). This finding is contingent on the taxon sample and tree and does not mean that these two features are never linked. The absence of a correlation between burrowing and basilar muscles is due to the inclusion of two species in this analysis: *D. antarcticus*, whose members lack basilar muscles but do not burrow, and *F. mutsuensis*, whose members burrow but have basilar muscles.

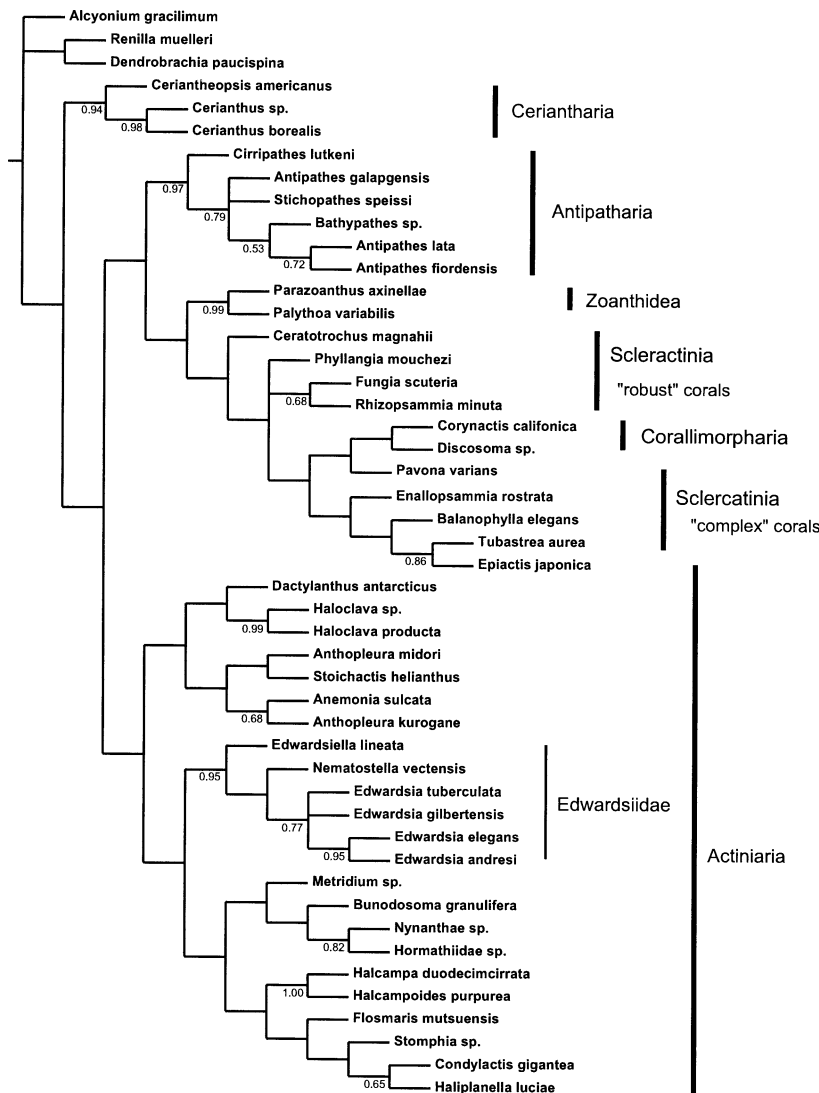


FIG. 1. Strict consensus of 12 equally parsimonious trees ( $L = 1134$ ,  $CI = 0.43$ ,  $RI = 0.60$ ), with major clades labeled. All sites were weighted equally. The numbers below the branches are jackknife values for 10,000 replicates (Farris et al. 1996); jackknife values below 0.50 are not reported. Ordinal characteristics are given in Table 1; ordinal classification follows Dunn (1982). The designation of scleractinians as robust or complex follows Romano and Cairns (2000).

## DISCUSSION

### *Evaluating Explanations*

The explanations for the edwardsiids' relative simplicity try to account for the reduction of two features: mesenteries and muscles. The paucity of mesenteries in adult edwardsiids is reminiscent of the condition seen in coral, corallimorpharian, and anemone larvae. Additionally, the eight-mesentery condition is seen in the Octocorallia, the sister group to the Zoantharia. The similarity in mesenterial number between edwardsiid adults, zoantharian larvae, and octocorals and the absence of actiniarian features such as basilar muscles led to the proposal that the Edwardsiidae is the most basal group within the Zoantharia (McMurrich 1891; Stephenson 1921; Hyman 1940). The absence of basilar muscles and the presence of relatively fewer complete mesenteries are construed as primitive absences; edwardsiids have fewer mes-

enteries and lack basilar muscles because they diverged from the rest of the Zoantharia before the evolution of these traits. The relationships reconstructed by 18S rDNA are incompatible with the underlying premise of this scenario. The Edwardsiidae is nested within the Actiniaria. The Edwardsiidae is not the sister group to the remaining Zoantharia.

The ecological hypothesis suggests that the simplicity of the Edwardsiidae is secondary and associated with the adoption of burrowing (Carlgren and Stephenson 1928; Hand 1966). The relatively simple edwardsiid morphology is interpreted as an adaptation to infaunal life. The ecological explanation presumes that the edwardsiid lineage has undergone a habitat shift, from sessile attachment to burrowing, and that this shift led to a reduction in musculature and in number of mesenteries.

The distribution of burrowing within the Actiniaria (Fig. 3) suggests that the Edwardsiidae may not have adopted bur-

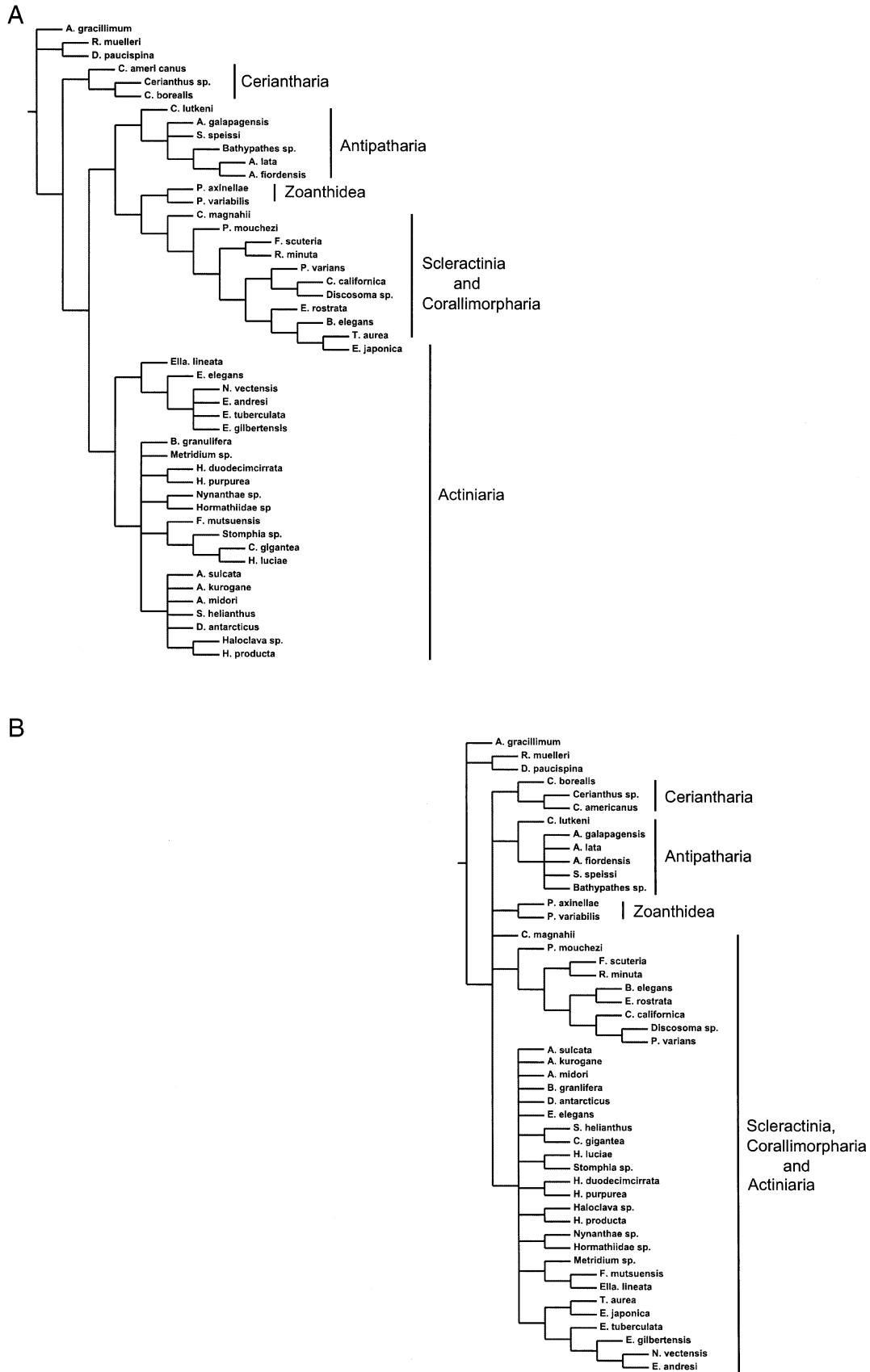


FIG. 2. Strict consensus of trees recovered by differentially weighted data. (A) Strict consensus of 324 equally parsimonious trees ( $L = 1597$ ;  $CI = 0.40$ ;  $RI = 0.59$ ) recovered by 2:1 weighted data. (B) Strict consensus of 342 equally parsimonious trees ( $L = 448$ ;  $CI = 0.36$ ;  $RI = 0.56$ ) recovered by transversions alone (1:0 weighting).

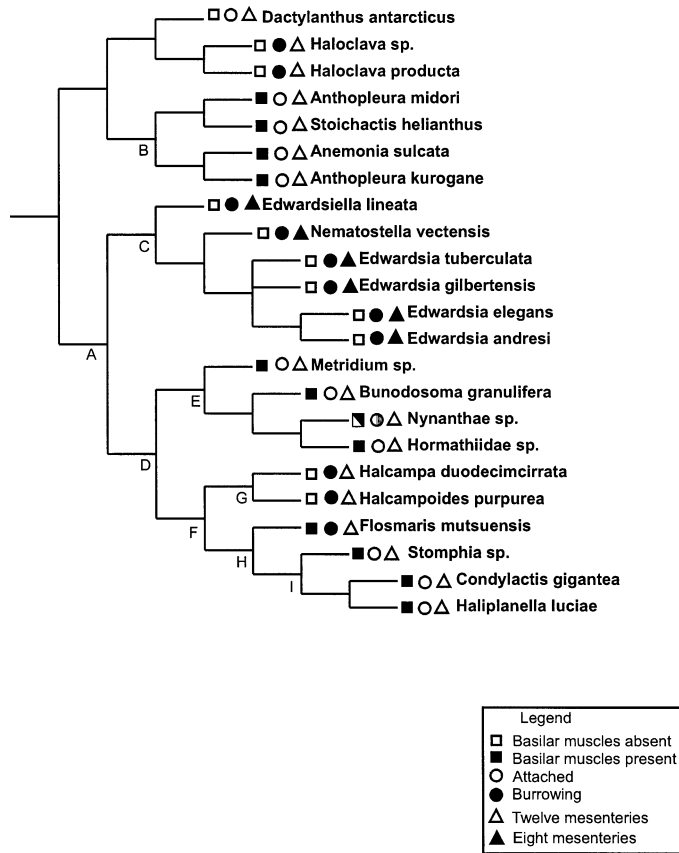


FIG. 3. The Actiniaria clade of Figure 1, with key nodes labeled and the distribution of basilar muscles, burrowing, and mesenteries shown. Squares indicate the distribution of basilar muscles, circles indicate the distribution of burrowing, and triangles indicate the distribution of mesenteries. Because the *Nynanthae* includes species that bear basilar muscles and those that do not, the symbols associated with the unidentified *Nynanthae* specimen have been partially shaded. The ambiguity of optimization in this specimen does not affect optimization of burrowing or muscles within the Actiniaria.

rowing independent of all other burrowing anemones. Optimization of habitat at the base of the Actiniaria is ambiguous. Optimization of burrowing within the Actiniaria actually determines the interpretation of this trait within the Zoantharia (see below). Within the Actiniaria, burrowing can be reconstructed several ways. If burrowing is assumed to be primitive for anemones, attachment was independently acquired four times: in *Dactylanthus*, in the *Anthopleura* clade (node B, Fig. 3), in the *Metridium* clade (node E), and in the *Stomphia* clade (node I). There are two optimizations of burrowing under the assumption that the primitive condition is attachment. The shift to burrowing may have occurred three times: along the branch leading to *Haloclava*; in the Edwardsiidae (node C, Fig. 3); and at the base of the clade containing *F. mutsuensis* and *Halcampa* and *Halcampoides* (node F, Fig. 3). This scenario requires a switch back to attachment at node I. Burrowing may have been adopted independently in *Haloclava* and in the larger clade containing the Edwardsiidae, *Flosmaris*, and *Halcampa* and *Halcampoides* (node A, Fig. 3), with attachment readopted at nodes E and I (Fig. 3). Alternatively, each clade that includes bur-

rowers may have switched from attachment to burrowing independently. This scenario entails four acquisitions of burrowing: in *Haloclava*, in the Edwardsiidae, in *Halcampa* and *Halcampoides*, and in *F. mutsuensis*. Only this last scenario supports the hypothesis that the Edwardsiidae have switched to an infaunal mode of life.

Hypotheses of natural selection minimally require that the change in habitat be coupled to a change in morphology (Coddington 1988). In the case of the Edwardsiidae, if simplification precedes the adoption of burrowing, then burrowing cannot be the selective cause of these changes. The non-existence of basilar muscles in Edwardsiidae need not be explained by the edwardsiid's habitat. The simplest explanation for the observed distribution of basilar muscles is that basilar muscles have arisen three times independently (at nodes B, E, and H; Fig. 3), or that they arose twice (at nodes B and D; Fig. 3) and were subsequently lost in the lineage leading to *Halcampa* and *Halcampoides* (node G, Fig. 3). In either case, basilar muscles have not been lost in the edwardsiid clade. Adaptation to burrowing has also been used to explain the edwardsiids' relatively streamlined vermiform body. A reduction in mesenterial number coincides with the adoption of burrowing in only one case: if burrowing is adopted independently in the Edwardsiidae, in *Halcampa* and *Halcampoides*, in *Haloclava*, and in *Flosmaris*.

The paedomorphosis hypothesis proposes that the simple body form of edwardsiids, which is reminiscent of the juvenile morphology of other zoantharians, is due to heterochrony (Hand 1966). Paedomorphosis is a general term describing the retention of juvenile features in the adult of a descendant species (Gould 1977), and, like adaptation, must be tested with reference to a phylogenetic hypothesis (Fink 1988). The paedomorphosis hypothesis predicts that the presence of eight mesenteries in adult edwardsiids is a derived condition and that the attainment of sexual maturity, and thus the end of development, occurs prior to the differentiation of the mesenteries and muscles seen in other anemones.

The paedomorphosis hypothesis predicts that the family Edwardsiidae is nested within anemones, and that there is truncation of the ontogeny of the Edwardsiidae, relative to its sister group, resulting in a reduction in musculature and in the number of mesenteries. Our most parsimonious trees place the Edwardsiidae within the Actiniaria (Fig. 1). Among actiniarians, sexual maturity is usually achieved after the completion of six mesenterial pairs, or 12 mesenteries (Mergner 1971). The presence of four pairs of mesenteries in the Edwardsiidae is thus a decrease in number from the ancestral condition. However, the paedomorphosis hypothesis predicts that basilar muscles are not seen in edwardsiid adults because these muscles normally develop after the edwardsia mesenteries. Although the morphology of the Edwardsiidae conforms to the expectations of the paedomorphosis hypothesis (because they, in fact, have no basilar muscles), the distribution of basilar muscles within the Actiniaria suggests this condition is not a reduction, but the retention of the primitive state.

The rejection of a particular explanation for the absence of mesenteries or muscles in the Edwardsiidae is contingent on the choice of optimization criterion and the choice of topology. Different optimization criteria favor different in-

interpretations of burrowing. Accelerated (ACCTRAN) optimization interprets burrowing as the primitive state for the Actiniaria and thus falsifies the ecological hypothesis; delayed (DELTRAN) optimization interprets burrowing as independently acquired by the Edwardsiidae, *Haloclava*, and the *Halcampa*, *Halcampoides*, and *Flosmaris* clade, leaving open the possibility that reduction in mesenterial number in the Edwardsiidae is related to the adoption of burrowing. Because we have no external information that would allow us to choose between the alternative optimizations, we consider hypotheses consistent with either optimization criterion potential explanations.

The degree to which the data favor one topology over another indicates the strength of support for a particular interpretation of evolutionary history (Bremer 1988). The trees on which we have based our conclusions are nine steps shorter than the shortest tree that would permit the interpretation of eight mesenteries as primitive. The shortest tree that reconstructs basilar muscles as present in the Actiniaria and subsequently lost in the Edwardsiidae is five steps longer than the most parsimonious tree.

*Halcampa duodecimcirrata* and *Halcampoides purpurea* represent the Halcampidae and the Halcampoididae, all of whose members burrow and lack basilar muscles. These two taxa are pivotal in interpreting the evolution of burrowing and of basilar muscles on the 18S tree. Some optimizations of burrowing interpret the *Halcampa* and *Halcampoides* lineage (node G, Fig. 3) as having undergone a shift in habitat. Furthermore, because basilar muscles are present in the sister group to *Halcampa* and *Halcampoides* (node H, Fig. 3) and outside the clade containing *Halcampa* and *Halcampoides* (node E, Fig. 3), these genera may have had a shift in morphology concomitant with a shift in habitat. Denser sampling in this subset of the actinarian tree may eliminate ambiguity in the interpretation of these traits and identify whether burrowing has led to a reduction in musculature within any actinarian lineage.

#### *Zoantharian Relationships*

The tree used to evaluate explanations of the relative simplicity of the Edwardsiidae has more general implications for our understanding of zoantharian evolution. The Zoantharia appear as a monophyletic group, with the hexacorals (non-cerianthid zoantharians) split into two groups. One clade includes all of the colonial and primarily skeletonized animals (corals, black corals, corallimorpharians, and zoanths) and the other includes the skeletonless, solitary anemones. A monophyletic Zoantharia has been found using 16S (France et al. 1996) and 18S sequence data (Song and Wong 1997; Berntson et al. 1999); anatomical and nematocyst features (e.g., Hyman 1940; Schmidt 1974) further support zoantharian monophyly. The grouping of all of the colonial and skeletonized animals is novel.

The optimization of burrowing on the 18S tree influences interpretations of edwardsiid morphology and influences the interpretation of zoantharian life-history evolution. The most basal clade within the Zoantharia is the tube anemones, or Ceriantharia. Cerianthids are large, solitary, anemone-like polyps that live in soft sediments in tubes constructed of

sediments, detritus, and exploded cnidae. Because cerianthids are burrowers, when burrowing is the primitive condition for anemones, burrowing is inferred as the ancestral condition for the Zoantharia. This interpretation of zoantharian evolution contradicts Hand's (1966) scenario for the evolution of anemones, which hypothesizes that anemones arose from skeletalized ancestors. If burrowing is ancestral, then skeletalization and attachment are evolutionary novelties, rather than a potentially shared common state for anemones and corals.

The results of this analysis bear on the monophyly of several zoantharian orders. The Scleractinia is polyphyletic, because one subset of corals is the sister group to the skeletonless corallimorpharians (*Corynactis californica* and *Discosoma*), rather than to the remaining scleractinians. A close relationship between scleractinians and corallimorpharians has long been suspected; animals in these orders have similar types of nematocysts, similar musculature, and similar histology (Duerden 1898; den Hartog 1980; Pires and Castro 1997). Molecular sequence data from mitochondrial and other nuclear genes (Chen et al. 1995; Romano and Cairns 2000) unite the Scleractinia and Corallimorpharia, but precise relationships are equivocal. The 18S data do not contradict the division of Scleractinians into "robust" (*Phyllangia*, *Fungia*, *Rhizopsammia*) and "complex" (*Pavona*, *Enallopsammia*, *Balanophyllia*, *Tabastrea*) clades (Fig. 1). Additionally, the placement of the corallimorpharians near the split between the two scleractinian clades may bolster arguments that the skeleton is not homologous between the "robust" and "complex" corals (Romano and Palumbi 1996; Romano and Cairns 2000).

The 18S sequences support the monophyly of the Actiniaria. Anemone monophyly has been controversial because there is currently no morphological evidence for it, and less densely sampled molecular analyses have generally not found a monophyletic Actiniaria (Chen et al. 1995; France et al. 1996; Song and Wong 1997). However, a DNA-DNA hybridization study (Fautin and Lowenstein 1992) and a recent maximum-likelihood analysis of Anthozoan phylogeny (Berntson et al. 1999) recovered a monophyletic Actiniaria. The absence of morphological characters corroborating actinarian monophyly is less troubling than it might appear, given that there have been only two previous analyses of actinarian relationships (Stephenson 1921; Schmidt 1974). As this analysis demonstrates with the particular case of basilar muscles, the interpretation of morphological characters within the Actiniaria is problematic. Seemingly identical structures have been given different names (e.g., hollow column vesicles called acrorhagi, marginal spherules, pseudo-spherules, and pseudoacrorhagi by different authors), and morphologically distinct structures have been given the same name (e.g., "cinclide" refers to all thin spots in the column, although they form in several ways).

#### *Conclusions*

No one hypothesis explains all aspects of the edwardsiid's morphology. Paedomorphosis may be the mechanism by which the Edwardsiidae came to bear only eight complete mesenteries, but it is not the reason that edwardsiids lack

basilar muscles. Primitive simplicity fails as an explanation for the presence of eight mesenteries in adult edwardsiids, but it is an adequate description for the absence of basilar muscles in the Edwardsiidae. The ecological hypothesis is not required to explain the absence of basilar muscles in edwardsiids, but may be relevant to understanding the loss of mesenteries. The ecological hypothesis has been applied to many taxa within the Actiniaria as a way to account for the general association between burrowing and absence of basilar muscles (Carlgren 1899; Hand 1966). The distribution of basilar muscles within the Zoantharia implies that, in general, such an explanation is unnecessary, but the 18S rDNA sequences suggest at least one particular case (*Halcampa* and *Halcampoides*) where the ecological explanation might pertain.

The 18S rDNA sequences suggest new hypotheses and explanations may be required to understand zoantharian evolution; suggest novel phylogenetic relationships, such as the coral, corallimorpharian, zoanthid, and antipatharian clade; and provide additional support for previously recovered but still controversial groups, such as the Corallimorpharia and Scleractinia clade, or a monophyletic Actiniaria. Additional data and denser sampling are needed to test these results and to explore the implications of these relationships on unsolved problems such as the evolution of coloniality, skeletalization, reproductive strategies, and symbiont acquisition.

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Corresponding Editor: M. Foote