

Molecular Evidence for Multiple Episodes of Paedomorphosis in the Family Hydractiniidae

CLIFFORD W. CUNNINGHAM* and LEO W. BUSST†

*Zoology Department, University of Texas, Austin, TX 78712, U.S.A.;

†Biology Department, Yale University, New Haven, CT 06511, U.S.A.

Key Word Index—Hyroid; medusae reduction; paedomorphosis; phylogeny; mitochondrial DNA.

Abstract—Reduction of complex life cycles in the Hydrozoa is commonly achieved by the heterochronic reduction of the medusa from a free-living to a sessile stage. Two competing traditions of hydroid taxonomy dating from the last century disagree about whether the degree of medusa reduction is an appropriate generic character. A phylogeny based on a fragment of the sequence encoding mitochondrial large ribosomal subunit RNA is constructed for thirteen representatives of three hydractiniid genera, each defined by its degree of medusa reduction. The minimum length trees found by parsimony and distance methods are not consistent with a monophyletic origin for all three genera. Tree topologies constrained to hold the genera *Stylactus* and *Hydractinia* monophyletic were shown by a statistical test to be significantly worse than the minimum length trees. These results support the hypothesis that medusa reduction has happened repeatedly in the family Hydractiniidae, thereby calling into question the tradition of hydroid taxonomy which defines genera based on degree of medusa reduction.

Introduction

The reduction of complex life cycles by the heterochronic elimination of late ontogenetic stages has been important to the evolutionary radiation of groups ranging from fungi and aphids to hydroids (Saville, 1976; Hille Ris Lambers, 1980; Moran, 1986; Moran and Whitham, 1988; Boero, 1986, 1987). The most obvious example of hydrozoan heterochrony is the paedomorphic reduction of the sexual medusoid stage from a free-living state to an attached, sessile condition (Fig. 1), so that sexual maturity is reached at a truncated point in medusoid development. These episodes of medusa

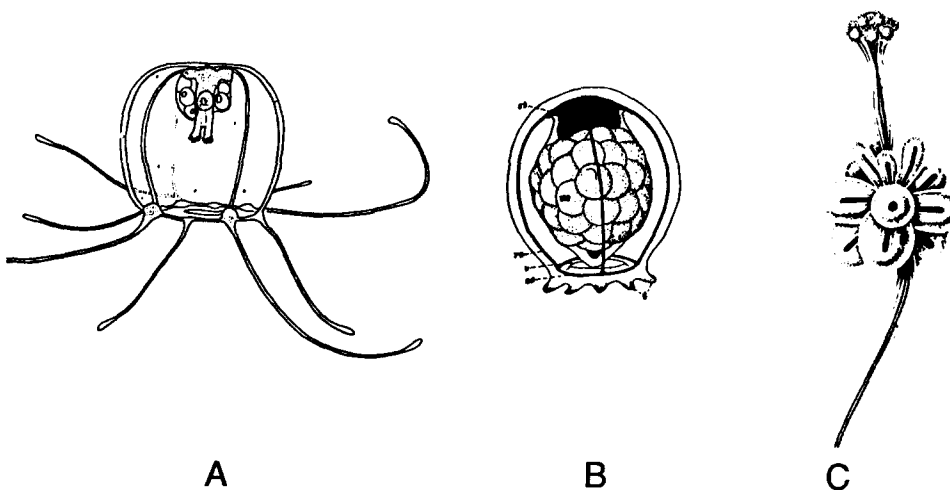


FIG. 1. MEDUSA REDUCTION IN THE HYDRACTINIIDAE. A, Free living medusoid (*P. selena*, after Mills, 1976). B, Detachable eumedusoid (*S. hooperi*, after Sigerfoos, 1899). C, Sessile sporosac (*H. echinata*, after Allman, 1872).

(Received 15 December 1991)

reduction are often followed by extensive radiation of the paedomorphic sessile lineage (Allman, 1864; Broch, 1916; Kramp, 1949; Naumov, 1960; Alberch *et al.*, 1979; Petersen, 1979, 1990; Boero, 1986, 1987; Boero and Boullion, 1987; Boero and Sará, 1987).

Exploring the history of medusa reduction is hampered by a system of taxonomic classification which is often based on reduction of the medusoid stage. The convention of defining hydroid genera by degree of medusa reduction was begun by Allmann (1864), and has been upheld by some hydroid taxonomists (Stechow, 1919; Rees, 1957; Millard, 1975). Conversely, a second school has argued that the degree of medusa reduction is not an appropriate generic character (Levinsen, 1893; Broch, 1916; Kramp, 1949). Both traditions of hydroid taxonomy are currently being practiced. Boero and Sará (1987) have proposed phylogenies of the thecate families Haleciidae and Campanulariidae based on the assumption of a progressive reduction of the medusoid stage. By contrast, Petersen (1979, 1990) has used a cladistic analysis of morphological characters of the athecate Order Captitata to argue that paedomorphic medusa reduction has occurred repeatedly within as well as between established genera.

We investigated the evolutionary history of paedomorphosis and medusa reduction in the Family Hydractiniidae. Allman (1864) first divided the Family Hydractiniidae into the genera *Podocoryne* (free-living medusae) and *Hydractinia* (reduced, sessile sporosacs). A third hydractiniid genus, *Stylactis* has come to include species with transitional stages of medusa reduction (Hirohito, 1988). Although Broch (1916) proposed that the genus *Podocoryne* be subsumed under the genus *Hydractinia*, the generic status of hydractiniids has continued to be defined by their degree of medusoid reduction (e.g. Rees, 1957; Naumov, 1960; Hirohito, 1988).

To distinguish between the two traditions of hydroid taxonomy with respect to the Family Hydractiniidae, a phylogeny has been constructed based on a region of the DNA sequences encoding mitochondrial large ribosomal subunit RNA (MLRS DNA). Sequences have been obtained from thirteen representatives of the hydractiniid genera *Podocoryne*, *Stylactis*, and *Hydractinia*, as well as for three outgroup taxa. The Boero–Sará hypothesis of gradual, progressive medusa reduction is supported if each genus is found to be monophyletic, and if the two genera with reduced medusae are sister groups. The Petersen hypothesis is supported if the monophyly of any or all of the genera is not supported, indicating that medusa reduction has taken place more than once.

Materials and Methods

Study species. Table 1 lists the major host types, degree of medusoid reduction, collection localities, and systematic references used for species identification of the 13 hydractiniid species used in this study. The only species not previously reported from the collection locality is the Monterey Bay population of *Stylactis hooperi*, which has previously only been reported from the Atlantic Coast of North America (Sigerfoos, 1899; Nutting 1901; Cunningham *et al.*, 1991).

DNA extraction. Hydroid tissue was homogenized in buffer (4M EDTA, 10mM Tris–HCl, 2% Sodium Sarkosyl, pH 9.4), extracted once with phenol–chloroform–isoamyl alcohol (25:24:1), precipitated by addition of an equal volume of isopropyl alcohol and 1/10 volume of 4.4 M NH₄Ac, resuspended in water, and further purified by the GENE CLEAN (BIO 101) procedure.

Amplification using the polymerase chain reaction (PCR). The primers used in this study were designed by searching the *Hydra vulgaris* (syn. *H. attenuata*) MLRS DNA sequence (G. A. Pont, C. G. Vassort, R. Okimoto, R. Warrior and D. R. Wolstenholme, unpublished) for sequences corresponding to the opposing primers 16SAR and 16SBR, which were developed for use in other metazoans (Palumbi *et al.*, 1991). The sequence for these primers (5'–3') are:

TCGACTGTTTACCAAAAACATAGC (primer 1), and ACGGAATGAACTCAAATCATGTAAG (primer 2). These primers enclose a 641 base pair fragment in the *H. vulgaris* MLRS DNA sequence, and consistently amplified a single similarly sized fragment from all cnidarian DNA attempted. A third, internal primer (GTCGCCCCAAC-TAAACTACCAAACCTT: primer 3) was constructed for sequencing purposes. The PCR (Saiki *et al.*, 1988) was carried out for single and double-stranded amplifications directly from whole genomic DNA as described by Palumbi *et al.* (1991). A Perkin–Elmer CTS machine was programmed for 10 cycles (94°C, 1 min; 40°C, 1.5 min;

TABLE 1. COLLECTION INFORMATION AND SYSTEMATIC REFERENCES FOR HYDRACTINIID SPECIES USED IN STUDY

Hydractiniid species	Major hosts	Gonophore type	Collection locality
<i>Hydractinia symbiolongicarpus</i>	Hermit crabs ¹	Sessile sporosac ¹	Old Quarry Harbor, Guilford, Connecticut
<i>Hydractinia symbiolongicarpus</i>	Hermit crabs ¹	Sessile sporosac ¹	Woods Hole, Massachusetts
<i>Hydractinia polyclina</i>	Hermit crabs ¹	Sessile sporosac ¹	Boothbay Harbor, Maine
<i>Hydractinia echinata</i>	Hermit crabs ¹	Sessile sporosac ¹	North Sea, F.R.G.
<i>Hydractinia</i> [GM]	Hermit crabs ^{2,7}	Sessile sporosac ^{1,8}	Dickerson Bay, Wakulla, Florida
<i>Hydractinia californica</i>	Rocks ³	Sessile sporosac ³	Catalina Island, California
<i>Hydractinia milleri</i>	Rocks ^{3,4}	Sessile sporosac ³	Bodega Bay, California
<i>Hydractinia serrata</i>	Hermit crabs, Gastropods ^{2,5}	Sessile sporosac ⁵	Bering Sea
<i>Hydractinia allmani</i>	Hermit crabs ¹	Sessile Cryptomedusoid ⁶	Bering Sea
<i>Stylactis inabai</i>	Hermit crabs, Gastropods ⁵	Detachable Eumedusoid ⁵	Hokkaido Bay, Japan
<i>Stylactis hooperi</i>	Gastropods ¹¹	Detachable Eumedusoid ^{2,10}	Monterey Bay, California
<i>Podocoryne carnea</i>	Hermit crabs, Gastropods ⁸	Free-living Medusoid ⁹	Old Quarry Harbor, Guilford, Connecticut
<i>Podocoryne selena</i>	Hermit crabs, Snails ⁸	Free-living Medusoid ⁹	Dickerson Bay, Wakulla, Florida

Systematic references: ¹Buss and Yund, 1989; ²Cunningham *et al.*, 1991 and personal observation; ³Fraser, 1937; ⁴Grosberg, personal communication; ⁵Hirohito, 1988; ⁶Kramp, 1943; ⁷Mercando and Lytle, 1980; ⁸Mills, 1976; ⁹Naumov, 1960; ¹⁰Sigerfoos, 1899, Nutting, 1901; ¹¹Watanabe, personal communication.

72°C, 2.5 min) to be followed immediately by 40 cycles (94°C, 1 min; 52°C, 1.5 min; 72°C, 2.5 min) and finally to a 4°C soak cycle until samples could be retrieved.

Sequencing of PCR products. All DNA sequencing was carried out by the dideoxy chain termination process (Sanger *et al.*, 1977) using T7 DNA polymerase (Sequenase Version 2.0, United States Biochemical) and radioactive labelling with S₃₅ (Amersham). Sequencing was achieved by one of three methods: (1) direct sequencing of double-stranded products, (2) direct sequencing of single-stranded products, and (3) sequencing of supercoiled plasmid DNA carrying the fragment of interest.

(1) PCR samples were prepared for direct double-stranded sequencing by removing the primers using the GENE CLEAN procedure, and resuspending in 21 µl of water. Seven µl of the resuspended DNA and 1 µl of 100 µM primer solution were boiled together in 1X Sequenase reaction buffer for 2 min and placed immediately on an ice slurry to minimize reassociation of double-stranded DNA. DNA sequencing was carried out by the preferred method of Tonequzzo *et al.* (1988). Double stranded sequencing was carried out for all species in Table 1 except for *S. hooperi* (see below).

(2) PCR samples were prepared for direct single-stranded sequencing and sequenced as described by Palumbi *et al.* (1991). Single-stranded amplification with primer 1 as the limiting primer was carried out for two species, *H. polyclina* and *H. echinata*, and sequenced using primers 1 and 2. The results of single-stranded sequencing were compared to those from double-stranded sequences for evidence of compression artifacts.

(3) PCR samples were prepared for cloning by removing the primers using the GENE CLEAN procedure. Cloning was carried out as directed by the TA Cloning Version 1.0 instruction manual (Invitrogen). This cloning system uses a PUC vector (PCR 2000), and requires no restriction sites to be added to the primers. Amplification of cloned DNA was carried out as described by Tonnequzzo *et al.* (1988), except that the initial volumes were increased tenfold to increase DNA yield, cultures were grown in 50 µg/µl of Kanamycin, and the GENE CLEAN procedure was used in the place of RNAase as a final step of purification before sequencing. All sequencing steps were carried out according to the recommended procedure in Tonnequzzo *et al.* (1988). The cloning procedure was carried out for fragments amplified from *P. carnea*, *P. selena* and *S. hooperi*, as well as two outgroup species, *Obelia nichotoma* and *Bougainvillea* sp. At least three clones of each species were sequenced to check for errors. The *P. selena* sequence obtained in this way was compared against the corresponding double-stranded sequence for evidence of compression in the latter, and none was found.

Sequencing reactions for all three methods were then run on 6% acrylamide 8M urea denaturing sequencing gels, washed, dried, and visualized as described in Palumbi *et al.* (1991).

DNA sequence alignment and phylogenetic analysis. All data analysis was carried out on a Macintosh II microcomputer. Sequences were aligned using the CLUSTAL program (Higgins and Sharp, 1989). Phylogenetic analysis of the aligned sequences were carried out using PAUP 3.0 (Swofford, 1990) and MACPHYLP 3.3, a Macintosh compatible version of Joseph Felsenstein's PHYLIP 3.3, (Felsenstein, 1990; available from Willem Ellis, Instituut voor Taxonomische Zoologie, Zoologisch Museum, Universiteit van Amsterdam, Plantage Middenlaan 64, 1018DH Amsterdam, Netherlands).

Results

Analysis and alignment of DNA sequences

Each of the 13 hydractiniid DNA sequences were aligned individually to three

outgroups, *Hydra vulgaris* (G. A. Pont, C. G. Vassort, R. Okimoto, R. Warrior, and D. R. Wolstenholme, unpublished), *Obelia dichotoma* and *Bouganvilla* sp. using the CLUSTAL program (Higgins and Sharp, 1989) with gaps given a weight of ten and transitions unweighted. Regions of DNA sequence where the individual alignments disagreed in the placement of gaps were deleted from the phylogenetic analysis. The MLRS DNA sequences are shown in Table 2, with deleted sections underlined. This approach of alignment to outgroup is suggested by Lake (1991) to identify and delete regions of uncertain homology, as well as to avoid the bias which can result from the order in which sequences are aligned. Inclusion of these sequences added homoplasy without affecting the conclusions of the study (Cunningham, 1991 and unpublished). Three species, *H. echinata*, *H. symbiopollicaris*, and *H. polyclina* (Table 1) had identical sequences. For simplicity, only the *H. polyclina* sequence is shown in Table 2 and in all subsequent figures.

Phylogenetic analysis

With *Hydra vulgaris*, *Bouganvilla* sp., and *Obelia dichotoma* as well as the designated outgroups one most parsimonious tree 220 steps long was found using Paup 3.0, with 67 phylogenetically informative positions (Fig. 2). Modified Kimura (1980) distances (taking into account unequal base-pair composition, Felsenstein, 1990) were calculated with the PHYLIP 3.3 program DNADIST program, and a minimum length least-squares tree was found using the PHYLIP 3.3 FITCH program (221 steps long, Fig. 2). The results of 1000 bootstrap replications (Felsenstein, 1985) are shown on both trees (Fig. 2). The two minimum length trees are shown in Fig. 3 with branch lengths in modified Kimura distance units calculated by the FITCH program.

Testing for multiple events of medusa reduction

Both the minimum length trees require multiple events of medusa reduction with the Family Hydractinidae. The character distribution of stages of medusoid reduction in both minimum length trees is shown in Fig. 4. The constraints option of Paup 3.0 was used to find the most parsimonious trees supporting the Boero–Sarà hypothesis, such that each stage of medusa reduction has only happened once, and in a progressive manner. One tree 231 steps long was found with all three genera monophyletic and with the pedomorphic genera *Hydractinia* and *Stylactis* as sister taxa (Fig. 5). This tree only requires two events of pedomorphic medusa reduction (Fig. 5).

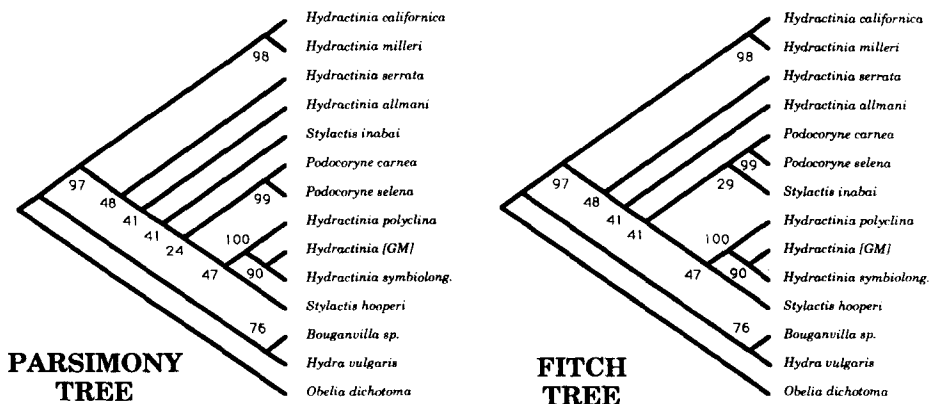


FIG. 2. MINIMUM LENGTH PHYLOGENETIC TREES FOR MLRS DNA SEQUENCES. Numbers refer to percentage of times that node was supported in 1000 bootstrap pseudoreplicates (Felsenstein, 1985, PAUP 3.0, Swofford, 1990). Parsimony tree: 220 steps in length (PAUP 3.0, Swofford, 1990). Consistency Index (excluding uninformative characters) = 0.649. Sum of squares = 0.624 (FITCH, PHYLIP 3.3, Felsenstein 1990). FITCH tree; 221 steps in length (PAUP 3.0, Swofford, 1990). Consistency Index (excluding uninformative characters) = 0.627. Sum of squares = 0.518 (FITCH, PHYLIP 3.3, Felsenstein 1990).

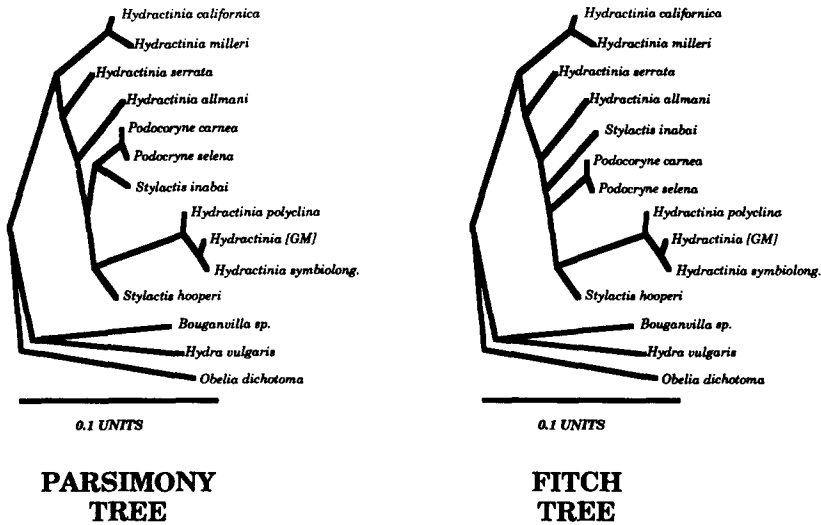


FIG. 3. MINIMUM LENGTH PHYLOGENETIC TREES WITH BRANCH LENGTHS IN MODIFIED KIMURA DISTANCES AS CALCULATED BY THE DNADIST AND FITCH PROGRAMS (PHYLIP 3.3, Felsenstein 1990).

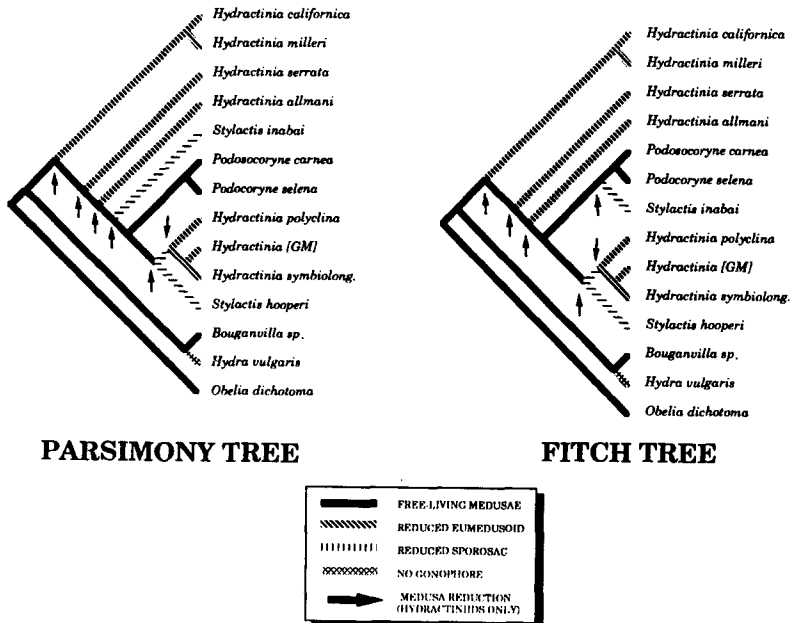


FIG. 4. NUMBER OF PAEDOMORPHIC MEDUSA REDUCTION EVENTS REQUIRED WITHIN THE FAMILY HYDRACTINIIDAE ON BOTH MINIMUM LENGTH TREES. See Fig. 1 for descriptions of stages of hydractiniid medusoid reduction.

TABLE 2. ALIGNED HYDRACTINIID MLRS SEQUENCES

<i>Obelia dichotoma</i>	A A A T G A A A A T	A A A T T T A A A G	G A C G C G G T A T	C I X T G A C C C G T	G A T A A T G T A G	C A T A A T C A C T
<i>Bougainvillea</i> sp.	. T T . T T . A G .	. T . T . T A . C
<i>Hydra vulgaris</i>	. T T . T A . T . A . T .	. T . A . T . A . T C A T . G .
<i>Stylactis hooperi</i>	T T T A T T A G .	A . A . T C .	T	T	G T .
<i>Stylactis inabai</i>	T T T A T T A .	A . A . C .	T	T T .
<i>Podocoryne carnea</i>	T T T A T T A G .	A . A . C .	T	T	G T .
<i>Podocoryne selena</i>	T T T A T T A G .	A . A . C .	T	T	G T .
<i>Hydractinia milleri</i>	T T T A T T A G .	A . A . C .	T	T	G T .
<i>Hydractinia californica</i>	? ? ? ? ? ? ? ?	? G
<i>Hydractinia allmani</i>	T T T T T T A .	A . A . C .	T	T	G T .
<i>Hydractinia serrata</i>	. T T A . . G A .	. C T .
<i>Hydractinia symbiolongicarpus</i>	T T T A . . T A G X .	. A . A . C .	T	T	G T .
<i>Hydractinia</i> [GM]	T T T T . . T A G X .	. A T .
<i>Hydractinia polycilina</i>	T T T T . . T A G X .	. A T .
<i>Obelia dichotoma</i>	C G C C A T T T A A	T T A A T X G G A T	A G T A T G A A T G	G T T A A A C G A A	T A T A A C A C T G	T C C A A A T A A A
<i>Bougainvillea</i> sp.	. T . T G T . T T
<i>Hydra vulgaris</i>	. T . T G A T . T T
<i>Stylactis hooperi</i> G T C T T T
<i>Stylactis hooperi</i> G T C T T T
<i>Podocoryne selena</i> G T C T T T
<i>Podocoryne carnea</i> G T C T T T
<i>Hydractinia milleri</i> G T T T T
<i>Hydractinia californica</i> G T T T T
<i>Hydractinia allmani</i> G T C T T T
<i>Hydractinia serrata</i> G T C T T T
<i>Hydractinia symbiolongicarpus</i> G T C T T T
<i>Hydractinia</i> [GM] G T C T T T
<i>Hydractinia polycilina</i> G T C T T T
<i>Obelia dichotoma</i>	A X X A T C T A T A	A A A T T G A A T	A A T A G T A A A G	A T G C T A T T T A	A A A T T G T A A G	A C G A G A A G A C
<i>Bougainvillea</i> sp.	. G A T C T C T
<i>Hydra vulgaris</i>	. A T . T . T T
<i>Stylactis hooperi</i>	. G A . A T C . G A T
<i>Stylactis inabai</i>	. G A . T C . G
<i>Podocoryne selena</i>	. G A . A T C . G T C T
<i>Podocoryne carnea</i>	. G A . A T C . G T C T
<i>Hydractinia milleri</i>	. G G T A T C . G A
<i>Hydractinia californica</i>	. G A T A T C . G A
<i>Hydractinia allmani</i>	. G A . A T C . G A T

<i>Obelia dichotoma</i>	A A G A T A T C C T	G A A A
<i>Bougainvillea</i> sp.	A . T .
<i>Hydra vulgaris</i>	A . T .
<i>Stylactis hooperi</i> T .
<i>Stylactis inabai</i> T .
<i>Podocoryne selena</i> T .
<i>Podocoryne carnea</i> T .
<i>Hydractinia milleri</i> T .
<i>Hydractinia californica</i>	A . T .
<i>Hydractinia allmani</i>	A . T .
<i>Hydractinia serrata</i> T .
<i>Hydractinia symbiolongicarpus</i> T .
<i>Hydractinia</i> [GM]	A G T .
<i>Hydractinia polyclina</i>	A G T .

Sequences are aligned as described in text. Dots denote identity to first base in column, X = gaps, and ? = missing data. Regions of uncertain homology which were deleted prior to phylogenetic analysis are underlined. Gaps within underlined sections are placed arbitrarily.

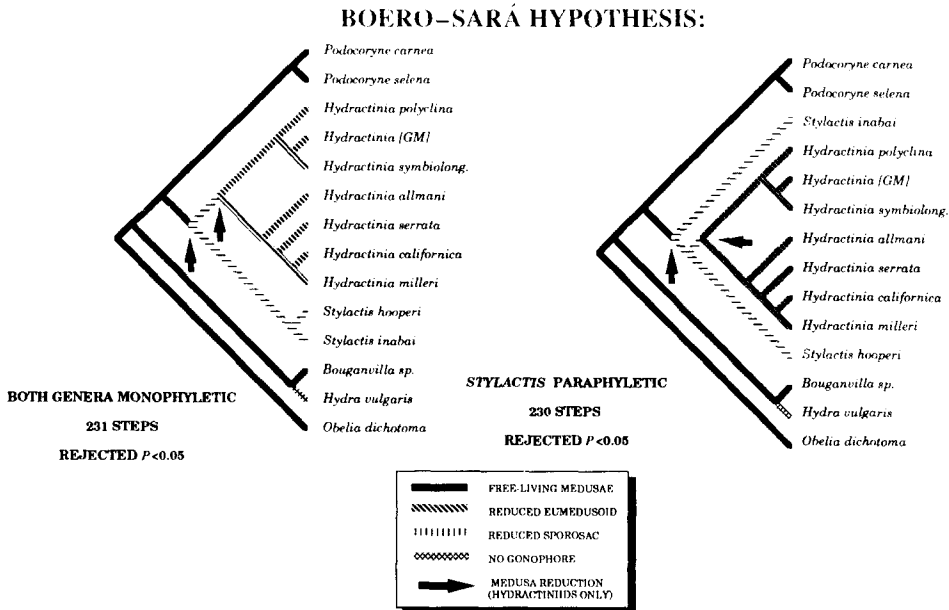


FIG. 5. THE MOST PARSIMONIOUS TREES SATISFYING THE BOERO-SARÁ HYPOTHESIS OF GRADUAL, PROGRESSIVE MEDUSA REDUCTION CONSTRAINED SO THAT EACH STAGE OF MEDUSOID REDUCTION MAY ONLY HAPPEN ONCE (PAUP 3.0, Swofford, 1990). Both trees are significantly worse than either minimum length trees (Figs 2 and 3) according to Kishino and Hasegawa's (1989) version of Templeton's (1983) test (DNAPARS program, PHYLIP 3.3, Felsenstein 1990).

A second tree 230 steps long was found in which the intermediately pedomorphic genus *Stylactis* was allowed to be paraphyletic, but which also only required two events of pedomorphic medusa reduction (Fig. 5). The DNAPARS program of PHYLIP 3.3 was used to perform Kishino and Hasegawa's (1989) version of Templeton's (1983) nonparametric test (Felsenstein, 1990) to ask whether the number of character positions favouring the trees in Fig. 5 were significantly lower than the number supporting the minimum length trees. Both trees in Fig. 5 were found to be significantly worse than either the minimum length parsimony or least-squares minimum length trees ($P < 0.05$). This result is consistent with a conclusion drawn by considering the length distribution of most parsimonious trees, which reveals that 4643 more parsimonious trees must be considered before finding a tree consistent with the Boero-Sará hypothesis (Fig. 6).

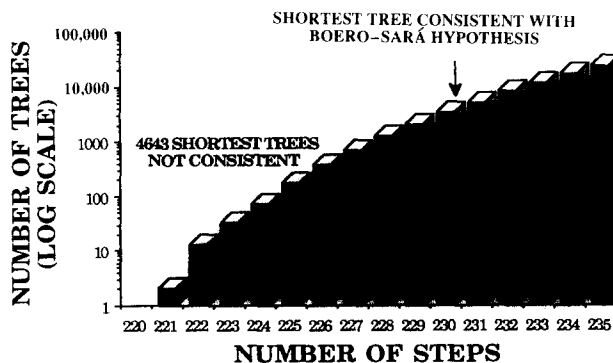


FIG. 6. TREE LENGTH DISTRIBUTION FOR ALL TREES WITHIN 15 STEPS OF THE MOST PARSIMONIOUS TREE, SHOWING SHORTEST TREE CONSISTENT WITH BOERO-SARÁ HYPOTHESIS (PAUP 3.0, Swofford, 1990).

But what of the monophyly of each of the three genera taken separately? The two representatives of the genus *Podocoryne* are monophyletic in 98% of 1000 bootstrap replicates. The most parsimonious trees constraining either *Stylactis* or *Hydractinia* to be monophyletic cannot be rejected using the statistical tests described above (see Fig. 7). But both of these trees require multiple events of medusa reduction within the Hydractiniidae, and hence are not consistent with the Boero–Sarà hypothesis.

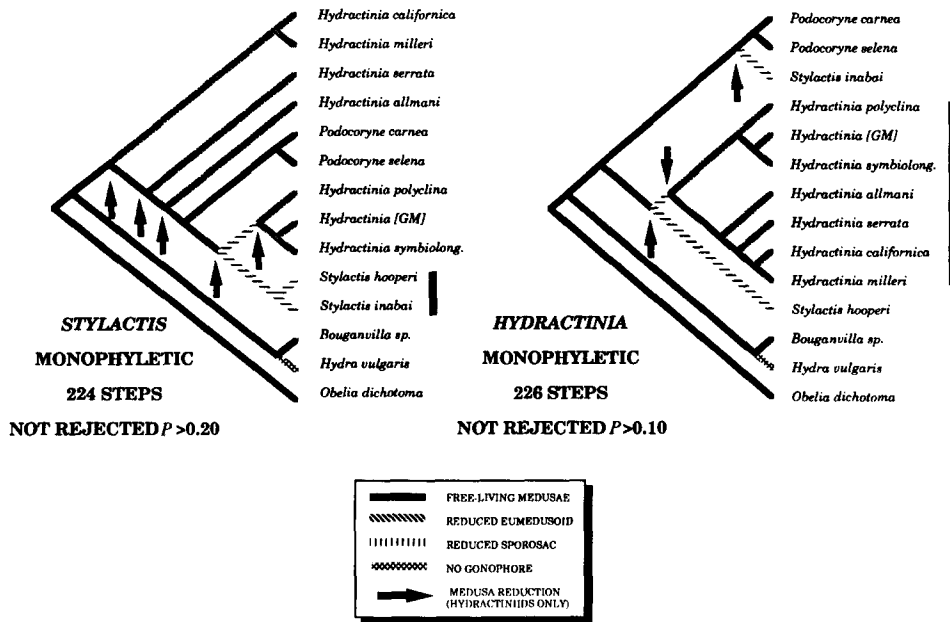


FIG. 7. THE MOST PARSIMONIOUS TREES CONSTRAINED TO SATISFY MONOPHYLY OF THE GENUS *STYLACTIS*, AND MONOPHYLY OF GENUS *HYDRACTINIA* (PAUP 3.0, Swofford, 1990). Neither tree is significantly worse than the minimum length trees (Figs 2 and 3) according to Felsenstein's version of Templeton's (1983) test (DNAPARS program, PHYLIP 3.3, Felsenstein 1990). Number of medusae reduction events as shown.

Discussion

Determining the progression of morphological evolution from phylogenetic information is one of the most important and most difficult tasks in systematics (reviewed by Donaghue, 1989). Molecular systematists in particular often find themselves in the position of rejecting long and dearly held hypotheses about the progress of morphological evolution (e.g. Raff *et al.*, 1988; but see Smith, 1989). To avoid doing more harm than good, it is critical to eliminate Type I errors, the incorrect rejection of a correct hypothesis (Sokal and Rohlf, 1981). If every phylogenetic algorithm for a molecular data set gives the same result, and bootstrapping analysis supports most or all nodes with 95% probability Type I errors are minimized. It is only necessary to map the characters of interest onto the minimum length tree and proceed from there. Morphologically-based hypotheses which do not agree with this tree can then be rejected. Such a high degree of resolution throughout a phylogeny is only rarely achieved in molecular systematics. How much attention should be given to the most parsimonious topology if it is contradicted by trees only a few steps longer which are not significantly worse? A reasonable goal for molecular systematics may be to find the tree not statistically worse (e.g. Templeton, 1983; Kishino and Hasegawa, 1989) than the minimum length tree which requires invoking the least amount of morphological homoplasy.

The hypothesis requiring the least homoplasy with respect to medusa reduction is

the Boero–Sarà hypothesis of progressive medusa reduction. This hypothesis predicts that each stage of medusa reduction, (i) free living to detachable eumedusoid, and (ii) reduction of the eumedusoid to the strictly sessile condition, will each have happened only once in the evolutionary history of the hydractiniid lineage. Since hydractiniid genera have been based on their degree of medusa reduction, the Boero–Sarà hypothesis is supported if each genus is found to be monophyletic. The minimum length PARS and FITCH trees are at variance with these predictions. The free-living medusoid condition is present in two of the three outgroups, but is lost six times within the Hydractiniidae whether the PARS or FITCH tree is considered, shown in Fig. 4.

As described in the results, the most parsimonious trees consistent with the Boero–Sarà hypothesis are 10 or more steps longer than the most parsimonious tree, and both are significantly worse than either the PARS or FITCH trees (Fig. 5, $P < 0.05$). Furthermore, 4643 more parsimonious trees must be considered before finding a tree consistent with the Boero–Sarà hypothesis (Fig. 6). These tests strongly support the conclusion that there has been more than one episode of medusa reduction from the free-living to the eumedusoid condition, even though most nodes on the minimum length phylogenetic trees are only poorly supported by bootstrap analysis (Fig. 2).

These results allow us to reject the Allman/Boero–Sarà tradition of a hydroid taxonomy based on the degree of medusa reduction for the Family Hydractiniidae (Allman, 1864; Boero and Sarà, 1987), insofar as taxonomy should reflect phylogeny. Like Petersen's (1990) cladistic analysis of morphological characters of the Capitate hydroids, our molecular study finds that paedomorphic medusa reduction is a flexible character, and can happen repeatedly within monophyletic groups like the Hydractiniidae. While it can be concluded with confidence that all three genera are not monophyletic (Fig. 5), the present study does not provide sufficient resolution to reject the monophyly of one or the other of the two paedomorphic genera, *Hydractinia* and *Stylactis* (Fig. 7). More data are necessary before undertaking a taxonomic revision of the Hydractiniidae.

Acknowledgements—We would like to thank N. Blackstone, D. Bridge, M. Dick, W. Hartman, B. Schierwater, and E. Vrba, for comments; D. Bridge and C. Wray for assistance in the laboratory, and J. Taschner for technical support. N. Blackstone, R. Grosberg, S. Kubota, J. Marks, P. Yund, J. Watanabe, and R. Otto and the crew of the NMFS research vessel "Alaska" are to be thanked for assistance in collecting tissues. This work was supported by grants from Sigma Xi (grants in aid of research), ONR (N00014-89-5-3046), NSF (OCE-9018396, BSR-8805961), and a training fellowship from the NIH.

References

- Alberch, P., Gould, S. J., Oster, G. F. and Wake, D. B. (1979) Size and shape in ontogeny and phylogeny. *Paleobiology* **5**, 296–317.
- Allman, G. J. (1864) On the construction and limitation of genera among the Hydroida. *A. Mag. Nat. Hist. Series* **3** **13**, 345–380.
- Allman, G. J. (1872) *A Monograph of the Gymnoblasic or Tubularian Hydroids, Part II*, Ray Society, London.
- Boero, F. (1986) Ecology and evolution: contributions from the study of hydromedusae (Cnidaria). *Nov. Thalass.* **8**, 471–476.
- Boero, F. (1987) Evolutionary implications of habitat selection in the hydroids of *Posidonia oceanica* meadows. In *Modern Trends in the Systematics, Ecology, and Evolution of Hydroids and Hydromedusae* (Boullion, J., Boero, F., Cicogna, F. and Cornelius, P. F. S., eds), pp. 251–256. Clarendon Press, Oxford.
- Boero, F. and Boullion, J. (1987) Inconsistent evolution and paedomorphosis among the hydroids and medusae of the Athecatae/Anthomedusae and the Thecatae/Leptomedusae (Cnidaria, Hydrozoa). In *Modern Trends in the Systematics, Ecology, and Evolution of Hydroids and Hydromedusae* (Boullion, J., Boero, F., Cicogna, F. and Cornelius, P. F. S., eds), pp. 229–250. Clarendon Press, Oxford.
- Boero, F. and Sarà, M. (1987) Motile sexual stages and evolution of Leptomedusae (Cnidaria). *Boll. Zool.* **54**, 131–139.
- Broch, H. (1916) Hydroida. (Part 1) *Dan. Ingolf Exp.* **5**, 1–66.
- Buss, L. W. and Yund, P. O. (1989) A sibling species group of *Hydractinia* in the Northeastern United States. *J. Mar. Biol. Ass. U.K.* **60**, 857–875.
- Cunningham, C. W., Buss, L. W. and Andersen, C. A. (1991) Molecular and geologic evidence of shared history between hermit crabs and the symbiotic genus *Hydractinia*. *Evolution* **45**, 1301–1316.

- Cunningham, C. W. (1991) *The Ecology and Evolution of the Family Hydractinidae and its Anomuran Hosts*. Phd. Thesis, Yale University, New Haven, Connecticut.
- Donoghue, M. J. (1989) Phylogenies and evolutionary sequences, with examples from seed plants. *Evolution* **43**, 1137–1157.
- Felsenstein, J. (1985) Confident limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1990) *PHYLIP Manual, Version 3.3*. University Herbarium, University of California, Berkeley, CA.
- Fraser, C. M. (1937) *Hydroids of the Pacific Coast of Canada and the United States*. University of Toronto Press, Toronto.
- Higgins, D. G. and Sharp, P. M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS* **5**, 151–153.
- Hille Ris Lambers, D. (1980) Aphids as botanists? *Symp. Bot. Upsal.* **22**, 11–119.
- Hirohito, Emperor of Japan (1988) *The Hydroids of Sagami Bay*. Biological Laboratory, Imperial Household, Tokyo.
- Kimura, M. (1980) A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Molec. Evol.* **16**, 111–120.
- Kishino, H. and Hasegama, M. (1989) DNA sequence data and the branching order in Hominoidea. *J. Molec. Evol.* **29**, 170–179.
- Kramp, P. L. (1943) The zoology of East Greenland, Hydroida. *Medd. Grønland* **121**, 1–52.
- Kramp, P. L. (1949) Origin of the hydroid family Corimorphidae. *Vid. Medd. Dansk Naturh. For.* **111**, 183–215.
- Lake, J. A. (1991) The order of sequence alignment can bias the selection of tree topology. *Molec. Biol. Evol.* **8**, 378–385.
- Levinson, G. J. R. (1893) Meduser, Ctenophorer og Hydroider fra Grønlands Vestkyst tilligemed bemærkninger om Hydroidernes systematik. *Vid. Medd. Dansk Naturh. For.* **44**, 143–220.
- Mercando, N. A. and Lytle, C. F. (1980) Specificity in the association between *Hydractinia echinata* and sympatric species of hermit crabs. *Biol. Bull.* **159**, 337–348.
- Millard, N. A. H. (1975) Monograph on the Hydroida of southern Africa. *Ann. South. Af. Mus.* **68**, 1–513.
- Mills, C. E. (1976) *Podocoryne selenae*, a new species of hydroid from the Gulf of Mexico, and a comparison with *Hydractinia echinata*. *Biol. Bull.* **151**, 214–224.
- Moran, N. A. (1986) Benefits of host plant specificity in *Uroleucon* (Homoptera: Aphididae). *Ecology* **67**, 108–115.
- Moran, N. A. and Whitham, T. J. (1988) Evolutionary reduction of complex life cycles: loss of host-alternation in *Pemphigus* (Homoptera: Aphididae). *Evolution* **42**, 717–728.
- Naumov, D. (1960) *Hydroids and Hydromedusae of the Marine Brackish and Freshwater Basins of the U.S.S.R.* Israel Program for Scientific Translations, 1969 cat. no. 5108.
- Nutting, C. C. (1901) The hydroids of the Woods-Hole region. *Bull. U.S. Fish. Comm.* **1899**, **19**, 325–386.
- Palumbi, S., Kessing, B. and Martin, A. (1991) *The Simple Fool's Guide to PCR, Version 2.0*. Zoology Department, Honolulu, Hawaii.
- Petersen, K. W. (1979) Development of coloniality in Hydrozoa. In *Biology and Systematics of Colonial Organisms. Systematic Association Special Volume No. 11* (Larwood, G. and Rosen, B. R., eds), pp. 105–139. Academic Press, London.
- Petersen, K. W. (1990) Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). *Zool. J. Linn. Soc.* **100**, 101–231.
- Raff, R. A., Field, K. G., Ghiselin, M. T., Lane, D. J., Olsen, G. J., Page, N. R., Parks, A. L., Par, B. A. and Raff, E. C. (1988) Molecular analysis of distant phylogenetic relationships in echinoderms. In *Echinoderm Phylogeny and Evolutionary Biology* (Paul, C. R. C. and Smith, A. B. eds), pp. 29–42. Clarendon Press, Oxford.
- Rees, W. J. (1957) Evolutionary trends in the classification of capitate hydroids and medusae. *Bull. Brit. Mus. (Nat. Hist.)*, *Zool.* **4**, 455–534.
- Saiki, R. K., Gelf, D. H., Stoffe, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.
- Sanger, F., Nicklen, S. and Coulson, A. R. (1977) DNA sequencing with chain-termination inhibitors. *Proc. Natn. Acad. Sci. U.S.A.* **74**, 5463–5467.
- Saville, D. B. O. (1976) Evolution of the rust fungi (Uredinales) as reflected by their ecological problems. *Evol. Biol.* **9**, 137–207.
- Sigerfoos, C. P. (1899) A new hydroid from Long Island Sound (*Stylactis hooperi*). *Am. Nat.* **33**, 801–807.
- Smith, A. B. (1989) RNA sequence data in phylogenetic reconstruction: testing the limits of its resolution. *Cladistics* **5**, 321–344.
- Sokal, R. R. and Rohlf, F. (1981) *Biometry, Second edition*. Freeman, San Francisco, California.
- Stechow, E. (1919) Zur Kenntnis der Hydroidenfauna des Mittelmeeres, Amerikas und anderer Gebeite, nebst Angaben über edrige Kriechenpauer'sche typen vol Plumulariden. *Zool. Jahrb. (Syst.)* **42**, 1–172.
- Swofford, D. L. (1990) *PAUP: Phylogenetic Analysis Using Parsimony, version 3.0*. Illinois Natural History Survey, Champagne, Illinois.
- Templeton, A. R. (1983) Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**, 221–244.
- Toneuzzo, F., Glynn, S., Levi, E., Mjolsness, S. and Hayday, A. (1988) Use of a chemically modified T7 DNA polymerase for manual and automated sequencing of supercoiled DNA. *Biotechniques* **6**, 460–469.