

Symbiogenetic Origins of Cnidarian Cnidocysts

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Abstract

Cnidarian cnidocysts may have been derived through symbiogenesis from organelles in prototistans. In order to trace the course of cnidocyst evolution back to ordinary types, we have assembled a cnidocyst database from information in the systematic literature. That database is now available to the public on the World Wide Web at <http://www.pitt.edu/~sshostak/cnidocyst.html>. It contains the cnidomes, or census of cnidocysts, for 809 species and measurements on lengths and widths of cnidocyst capsules. The present analysis of cnidomes in the database suggests that cnidocysts belong to no less than two families. Members of one family of cnidocysts are found in Anthozoa, whereas members of both families are found in Medusozoa. Competition among cnidocyst families in the microcosm of organisms, in addition to the natural selection of cnidarians in their external environments, may have contributed to the evolution and diversification of cnidarian cnidocysts.

Keywords: evolution, nematocysts, organelles, symbiogenesis

1. Introduction

Cnidocysts are a class of membrane-enclosed cellular organelles, consisting of a capsule and an eversible tubule (Slautterback and Fawcett, 1959; Wood, 1988).

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Like other cellular organelles, such as mitochondria and chloroplasts (Margulis, 1991; Gillham, 1994), cnidocysts may have originated symbiotically (Lom, 1990; Shostak, 1993; Buss and Seilacher, 1994). Unlike other cellular organelles, however, cnidocysts are conspicuously diverse, appearing in at least twenty-seven morphologically distinct types (Weill, 1934; Werner, 1965; Mariscal, 1974). The cnidome, or census of cnidocysts in a species, contains up to seven different types of cnidocysts, and any of these types may differ in size even within the same body region (Carlgren, 1940, 1945, 1949; Schmidt, 1974).

The question of how cnidocysts originated will not be satisfactorily answered until molecular data on the sequence of amino acids in cnidocyst proteins from different types of cnidocysts and from different species is available, but the analysis of cnidocyst diversification and the possibility of their evolution within the Cnidaria can be advanced with data already available in the systematic literature. For more than a century, cnidarian systematists have considered the cnidome an important systematic character and have recorded cnidomes and information on the size and distribution of cnidocysts as part of a species' description. Moreover, systematists reclassified cnidocysts from the older literature for consistency with Weill's nomenclature (e.g., Carlgren, 1949; Stephenson, 1949; Ito and Inoue, 1962; Werner, 1965; Russell, 1970; Bouillon, 1985).

By tapping this systematic literature, we compiled a database on cnidocysts which we now make available to the public on the World Wide Web. The present report begins the statistical characterization of data in this database and the cladistic analysis of these data. Ultimately, we would like to trace extant cnidocysts back to the originary types and thereby make educated guesses about the sources of precnidarian cnidocysts. The present paper attempts to sort through the myriad pathways of cnidocyst diversification in extant Cnidaria in order to trace their evolution retrospectively.

2. Method

Data on cnidomes were assembled using Microsoft® Excel Version 4.0 for Macintosh® and converted to hypertext markup language (html) by the XL2HTML.XLSv1.21 program created by Jordan Evans. The database containing the data analyzed in this report is accessible to the public at <http://www.pitt.edu/~sshostak/cnidocyst.html>. To use the file server, log in as "anonymous" and enter an e-mail address when prompted for a password. The overall structure of directories appears in a README file at the root directory. Although the files are locked, corrections and additions are welcome

and will be made promptly in updated versions of the database. Correspondence should be addressed to the first author or via e-mail to sshostak+@pitt.edu.

The sample of 809 cnidomes was collected from the systematic literature (Carlgren, 1940, 1945, 1949; Ito and Inoue, 1962; Mackie and Mackie, 1963; Russell, 1970; Schmidt, 1974; Bouillon, 1985; Östman, 1979, 1982, 1983, 1987, 1988; Östman et al., 1987; Gravier-Bonnet, 1987; Hessinger and Ford, 1988; Purcell and Mills, 1988; Skaer, 1988) consists of 258 species of Anthozoa, 524 species of Hydrozoa, 26 species of Scyphozoa, and one species of Cubozoa. This sample comprises data from all the literature available to us.

The classification of cnidocysts follows Weill's (1934) general usage, and a recent convention on nomenclature (Watson and Wood, 1988) except that we refer to the thin portion of tubules as thread-like. Cnidocysts, the general term for the cnidarian cellular organelles, are divided into two categories, spirocysts (Sp) and nematocysts, to which a third category, ptychocysts (Pt), has since been added (Mariscal et al., 1977). Nematocysts are subdivided into about 30 varieties of which 25 are recognized below. Data listed in the tables were rounded to the nearest even number.

The MacClade program version 3.04 for the analysis of phylogeny and character evolution (Maddison and Maddison, 1992) is used to trace cnidocyst diversification. This program is a parsimony-based computer program capable of generating phyletic trees of minimum length and suitable for tracing character change in hypothetical ancestors. Rooted or unrooted trees are machine generated and easily driven in the direction of lower branch length with the help of various tools supplied in a pop-up menu. The qualities of characters, such as weights, are readily formatted and altered. The MacClade program also allows one to visualize changes in unordered characters retrospectively against a background of prescribed phylogenies (see below, Figs. 1 and 2).

The phylogenies used here as a background to trace cnidocyst evolution are those of Schmidt (1974) and of Petersen (1979). For the purpose of tracing character diversity, no weights were applied to cnidocysts. The MacClade algorithm assigned one of three labels to hypothetical branches of the prescribed phylogenies based on the presence or absence of the cnidocyst of interest in equally parsimonious trees: closed bars indicate that the cnidocyst identified is present in the hypothetical ancestor; open bars or "no" indicate that the cnidocyst is absent; stippled bars or "equivocal" indicate that the cnidocyst is present or absent in equally parsimonious trees.

3. Results

The results are presented in two sections: (1) Characterizing cnidomes; (2) Cnidocyst type diversity. The first section is intended to test the possibility that cnidomes evolved from the diversification of more than one originary type, and hence by competition among cnidocysts. The second section is intended to draw out hypothetical courses of cnidocyst evolution and diversification.

Characterizing cnidomes

Number of cnidomes, their population of cnidocyst types, and their distribution. All the cnidomes identified in the sampled literature are listed in Table 1 in columns entitled "cnidomes." The cnidomes are identified by the abbreviated names of cnidocysts separated by commas and recorded alphabetically. Columns entitled "no. species" give the number of species having the cnidome in the column toward the right.

The cnidomic number, or number of cnidocyst-types comprising a cnidome, is ascertained from Table 1 by counting the number of cnidocyst-types in a cnidome. Cnidomes consisting of only one cnidocyst type, or monocnidic cnidomes, two cnidocyst types, or dicnidic cnidomes, and three cnidocyst types, or tricnidic cnidomes, occur toward the bottom of the table, while cnidomes consisting of more than three cnidocyst types, or polycnidic cnidomes, occur toward the top.

The columns are arranged from left to right according to the number of species having given cnidomes: the first column of cnidomes is found in only one species (i.e., these are species-specific cnidomes); the middle column of cnidomes is found in two to five species (i.e., moderately species-general cnidomes); the third column of cnidomes is found in more than five species (i.e., broadly species-general cnidomes). Groups with the same cnidomic number are arranged in ascending order within each column, while cnidomes are listed in descending alphabetical order within the groups having the same cnidomic number.

The complete data set contains 114 cnidomes. Weighted for number of species, the mean cnidomic number is 2.44 ± 1.15 cnidocyst types, while the unweighted mean cnidomic number is 3.11 ± 1.27 cnidocyst types. Cnidomic populations differ significantly ($F = 2.05$; $F_{\text{cri}} = 1.52$). Moreover, about half of the species sampled (433/809 species) are clustered in the ten most common cnidomes. Thus, the distributions of cnidomes in species and of cnidocyst types in cnidomes are not random.

Regression of cnidomic population. The slope for the linear regression of cnidomic population as a function of number of species in the sample as a whole is -0.24 cnidocysts per species and is statistically highly significant according to the F statistic. As shown in Table 1, thirty-nine cnidomes are species-

specific (5% of all species); 43 cnidomes are moderately species-general, (125 species or about 15% of all species); 32 cnidomes are broadly species-general (645 species or 80% of all species). The population of cnidocyst types in species-specific cnidomes ranges from one to seven cnidocyst types (mean = 3.6 ± 1.4); in moderately species-general cnidomes from one to five cnidocyst types (mean = 3.04 ± 1.09); in broadly species-general cnidomes from one to six cnidocyst types (mean = 2.53 ± 1.16). Thus, the population of species-specific cnidomes tends to be slightly larger, while the population of species-general cnidomes tends to be slightly smaller than the mean population or that of moderately species-general cnidomes.

The inverse relationship of cnidomic population and number of species is partially explained by the distribution of relatively rare cnidocysts (apotrighous isorhizas [ApI], aspiroteles [AS], birhopaloides [BiR]; heterotrighous anisorhizas [HeA], homotrighous isorhizas [HoA], macrobasic mastigophores [MaM], merotrighous isorhizas [MeI], rhopalonemes [R including acrophores and anacrophores], semiophoric microbasic euryteles [SeMiE], spiroteles [S]). Table 1 shows that these cnidocysts tend to predominate in species-specific cnidomes and in moderately species-general cnidomes. The evolution of a rare cnidocyst in a moderately or broadly species general cnidome would make it larger and species-specific. For example, the addition of relatively rare spiroteles (S), changes a moderately species-general cnidome of four (namely, atrichous isorhizas [A], basitrichous isorhizas [BI], desmonemes [De] and stenoteles [St]), into a species-specific cnidome of five (AI, BI, De, S, St).

Rare cnidocysts are not always associated with large cnidomic populations, however. The smallest species-specific cnidomes (i.e., monocnidic cnidomes of only one cnidocyst) consist of rare cnidocysts (e.g., MeI, S, and SeMiE). The acquisition of these rare cnidocysts might have accompanied the loss of more common cnidocysts.

Cnidomic population. In an effort to see how cnidomes might have evolved as a function of cnidomic population, we calculated the mean and standard deviation for the number of cnidocyst-types associated with each cnidocyst in cnidomes and the numbers of cnidomes containing a given cnidocyst type. These data are listed in Table 2.

Individual cnidocysts occur in cnidomes with a broad range of mean cnidomic population (from 1 to 6; second column from left). If cnidomes evolved purely by natural selection in the Cnidaria, the most common cnidocysts, and hence the most general, would occur in smaller cnidomes. The data listed in Table 2 show, however, that cnidocyst types appearing in relatively large numbers of cnidomes (i.e., 10 or more; fourth column from left) also occur over a broad range of mean cnidomic populations (from 3.1 to 4.8 cnidocyst types). These cnidocyst

Table 1. Cnidomes clustered by number of species

Species-specific		Moderately species-general		Broadly species-general	
No.	Cnidomes	No.	Cnidomes	No.	Cnidomes
	species		species		species
1	AI, BI, HI, MaAm, MiAm, pRH, Sp	2	A, De, MiE, MiM, St	10	AI, bRh, HI, pRh, Pt, Sp
1	AI, BI, bRh, HI, MiAm, pRh, Sp	2	AI, BI, bRh, pRh, Sp	9	AI, BI, pRh, Sp
1	AI, BI, HI, MaAm, MiAm, Sp	2	AI, BI, De, MiE, St	7	AI, De, MiE, St
1	A, AI, BI, MeI, MiM	2	AI, BI, MiAm, pRh, Sp	17	BI, bRh, pRh, Sp
1	A, AI, De, MiE, St	3	AI, bRh, HI, pRh, Sp	11	BI, De, MiE, St
1	A, BI, De, S, St	4	A, BI, De, St	21	BI, MiAm, pRh, Sp
1	AI, MaE, MaM, MiE, St	4	A, De, MiE, St	10	bRh, HI, pRh, Sp
1	BI, bRh, HI, pRh, Sp	2	AI, BI, De, St	8	AI, BI, MiM
1	BI, De, HeA, MiE, St	4	AI, De, HI, St	7	AI, De, St
1	BI, HI, MaAm, MiAm, Sp	2	AI, De, MiM, R	8	AI, HI, MiE
1	A, AI, AS, St	3	AI, HI, pRh, Sp	9	BI, De, St
1	AI, AS, De, St	4	De, MiE, MiM, St	94	BI, pRh, Sp
1	AI, HI, MiE, MiM	3	MaAm, MiAm, pRh, Sp	7	bRh, pRh, Sp
1	AI, MaE, MaM, St	2	A, AI, St	7	De, MaE, MiE
1	AI, MiAm, pRh, Sp	3	AI, BI, MeI	16	De, MiE, St
1	BI, bRh, MiAm, Sp	4	AI, De, R	8	AI, BI
1	De, MaM, MiE, St	3	AI, HI, MiM	17	AI, MiE
1	MaE, MaM, MiE, St	2	AI, MaE, St	23	AI, MiM
1	A, AI, HI	2	AI, MiE, MiM	8	AI, St
1	A, BI, St	4	AI, MiE, St	10	BI, MiM
1	A, De, MiE	2	BI, MiAm, Sp	9	BI, Sp
1	A, De, St	3	De, MaM, MiE	81	De, MiE
1	A, MaE, MiE	5	De, MaM, St	36	De, St
1	A, MiM, R	7	De, MiE, MaM	10	AI, MiE

Table 1. Continued

Species-specific		Moderately species-general		Broadly species-general	
No.	Cnidomes	No.	Cnidomes	No.	Cnidomes
species		species		species	
1	AA, BiR, HoA	2	De, MiM, St	12	MiE, MiM
1	Al, Bl, Sp	3	bRh, Hl, Sp	14	MiE, St
1	Al, De, MiM	3	Hl, pRh, Sp	8	Al
1	Al, MaM, St	4	MaAm, MiAm, Sp	7	Bl
1	Bl, Hl, Sp	4	MaE, (pSt), St	50	MiE
1	Bl, MaE, MiE	3	MaE, MiE, St	54	MiM
1	bRh, Hl, pRh	3	MiAm, pRh, Sp	27	Sp
1	De, MaE, MaM	2	Al, Apl	30	St
1	MaE, MiE, MiM	2	Bl, MaM		
1	MaE, MiM, pSt	5	Bl, St		
1	Al, Sp	2	bRh, Sp		
1	De, MaE	3	Hl, Sp		
1	MeI	5	MaE, MiE		
1	S	2	MaM, MiM		
1	SeMiE	2	MaM, St		
		3	pRh, Sp		
		2	Apl		
		4	bRh		
		2	De		

Abbreviations: A = anisorhizas; AA = atrichous anisorhizas; Al = atrichous isorhizas; AS = aspiroteles; BI = basitrichous isorhizas; BiR = birhopaloids; bRh = b-rhabdoides; De = desmonemes; HeA = heterotrichous anisorhizas; HI = holotrichous isorhizas; HoA = homotrichous anisorhizas; MaAm = macrobasic amastigophores; MaE = macrobasic euryteles; MaM = macrobasic mastigophores; MeI = merotrichous isorhizas; MiAm = microbasic amastigophores; MiE = microbasic euryteles; MiM = microbasic mastigophores; pRh = p-rhabdoides; Pt = ptychocysts; pSt = pseudostenoteles; R = rhopalonemes; S = spiroteles; SeMiE = semiophoric microbasic euryteles; Sp = spirocysts; St = stenoteles.

Table 2. Cnidomic populations associated with individual cnidocysts

Cnidocyst*	Mean cnidomic population size containing cnidocyst	+/- (Standard deviation)	No. cnidomes containing cnidocyst
SeMiE	1		1
ApI	1.5	0.5	2
AA	3		1
BiR	3		1
HoA	3		1
MeI	3	1.2	3
pSt	3		2
S	3	2	2
MiM	3.1	0.3	17
MaE	3.1	0.3	14
MaM	3.2	0.3	11
MiE	3.3	0.2	31
R	3.3	0.3	3
St	3.5	0.2	37
De	3.5	0.2	31
AI	3.7	0.2	43
Sp	3.8	0.3	32
A	3.8	0.2	14
BI	3.8	0.2	33
bRh	4	0.4	13
AS	4		2
HI	4.1	0.3	20
pRh	4.3	0.3	18
MiAm	4.8	0.5	10
HeA	5		1
MaAm	5	0.7	5
Pt	6		1

* Abbreviations as in Table 1.

types predominate at the center of the range of cnidomic populations, while cnidocyst types occurring in relatively rare cnidomes (between 1 and 3) tend to occur at the top and bottom of the range of cnidomic populations. This distribution of rare and common cnidocyst types suggests that relatively rare cnidocyst types evolved in large cnidomes and either added to the cnidome (bottom of list) or supplanted previously existing cnidocyst types (top of list).

In order to determine if this quantitative association of cnidocyst types to cnidomic population reflected qualitative associations among cnidocyst types, we calculated the frequencies, weighted for species, with which the ten most common cnidocyst types are paired in cnidomes. These data are listed in Table 3.

Table 3. Frequencies of cnidocyst pairs*

If/ then	AI	BI	b-Rh	De	HI	MiE	MiM	p-Rh	Sp	St
AI	0.05	0.23	0.26	0.18	0.52	0.25	0.24	0.18	0.20	0.26
BI	0.17	0.03	0.36	0.11	0.09	0.06	0.20	0.61	0.77	0.13
b-Rh	0.09	0.09	0.06	0	0.47	0	0	0.84	0.92	0
De	0.14	0.12	0	0.01	0.02	0.64	0.06	0	0	0.52
HI	0.21	0.02	0.42	0.06	0	0.28	0.06	0.46	0.59	0.06
MiE	0.16	0.05	0	0.51	0.07	0.04	0.10	0	0	0.41
MiM	0.47	0.08	0	0.15	0.04	0.32	0.03	0	0	0.09
p-Rh	0.12	0.61	0.22	0	0.13	0	0	0	1.00	0
Sp	0.12	0.62	0.19	0	0.14	0	0	0.80	0.09	0
St	0.19	0.13	0	0.49	0.02	0.48	0.03	0	0	0.13

*AI = atrichous isorhizas; BI = basitrichous isorhizas; b-Rh = b-type rhabdoides; HI = holotrichous isorhizas; MiE = microbasic euryteles; MiM = microbasic mastigophores (unspecified); p-Rh = p-type rhabdoides; Sp = spiroteles; St = stenoteles.

The table can be read as an "if - then -" narrative for each row and column: if a cnidocyst listed in the column at the extreme left occurs in a cnidome, then a cnidocyst listed in the row at the top is present with the frequency given at the intersection of the given row and column. Monocnidic cnidomes (i.e., having only one type of cnidocyst) may be thought of as cnidomes in which the "if" cnidocyst type and the "then" cnidocyst type are the same. Monocnidic cnidomes are listed diagonally in Table 3 proceeding downward from top left to bottom right.

None of the monocnidic cnidomes is common and all but one are restricted to members of one class (see below). The exceptional cnidome is MiE which occurs in both Hydrozoa and Scyphozoa (Rhizostomeae). Monocnidic cnidomes of AI, BI, De, MiM, and St occur exclusively in Hydrozoa, while monocnidic cnidomes of b-Rh and Sp occur exclusively in Anthozoa.

The average ratio for pairs of the ten most common cnidocyst types is 0.19 ± 0.07 . Ratios of zero are consequences of the absence of cnidocyst types in some classes, and ratios in the vicinity of the mean could have resulted from random pairing. Some ratios, however, exceed the mean by factors of between two and five. We consider these ratios skewed and nonrandom.

Species scored for De have a high proportion of MiE (frequency = 0.64) and St (frequency = 0.52). Likewise, species scored for MiE, have De (frequency = 0.51) and St (frequency = 0.41), and species scored for St have De (frequency = 0.49) and MiE (frequency = 0.48).

Species scored for AI have a high proportion of HI (frequency = 0.52); species scored for BI have *p*-Rh (frequency = 0.61) and Sp (frequency = 0.77); species scored for MiM have AI (frequency = 0.47). Within Anthozoa (since Sp occur exclusively in Anthozoa) species scored for *p*-Rh have BI (frequency = 0.61) and Sp (frequency = 1.00 [rounded upward from 0.996]); species scored for Sp have BI (frequency = 0.62) and *p*-Rh (frequency = 0.80).

On the basis of these high ratios, we identify two families of linked cnidocyst types: (1) a family consisting of desmonemes (De), microbasic euryteles (MiE) and stenoteles (St); (2) a family consisting of atrichous isorhizas (AI), basitrichous isorhizas (BI), holotrichous isorhizas (HI), microbasic mastigophores (MiM or rhabdoides [Rh]) of the *b*-type (*b*-Rh) and *p*-type (*p*-Rh) and spirocysts (Sp). Members of the first family occur exclusively in the subphylum Medusozoa (containing classes Cubozoa, Hydrozoa, Scyphozoa), while members of the second family occur in both subphyla Medusozoa and Anthozoa.

Cnidomic distribution. Individual cnidocyst types (e.g., AI and MiM; Werner, 1965; Mariscal, 1974) cross subphylum lines, but no cnidome occurs in both subphyla of Cnidaria (i.e., Anthozoa and Medusozoa). Possibly no phylum-wide cnidome existed at the time the subphyla separated, or, possibly, cnidomes are too plastic and insufficiently stable to have survived to the present.

Three cnidomes occur throughout the classes of Medusozoa. These cnidomes always have microbasic euryteles (MiE) and may have, in addition, atrichous isorhizas (AI) or holotrichous isorhizas (HI). The monocnidic cnidome MiE occurs widely in the Hydrozoa (49 species) with the exception of the Capitata and in one species of Rhizostomeae among the Scyphozoa. The dicnidic cnidome AI+MiE occurs in polyps and medusae of the Filifera and in limnopolypae-medusae among the Hydrozoa (7 species) and in Rhizostomeae and Semaestomeae among the Scyphozoa (10 species). The dicnidic cnidome HI+MiE appears among medusae of the limnopolypae-medusae of the Hydrozoa (3 species) and Coronatae among the Scyphozoa (7 species). Tricnidic cnidomes containing MiE and isorhizas of some form are also common

in the Medusozoa. For example, AI+HI+MiE is common in Semaestomeae (8 species), and the tricnidic cnidome consisting of the pair AI+MiE with an atrichous anisorhizas (A) occurs in polyps of Capitata hydrozoans (one species). Furthermore, the polycnidic cnidome AI+HI+MiE+MiM constitutes the cnidome for the sole species of Cubozoa in the sample.

Cnidocyst type diversity

Ten cnidocyst types account for 92% of all cnidocysts in the total sample. These ten cnidocyst types are the atrichous isorhizas (AI), representing 9% of all cnidocysts, basitrichous isorhizas (BI), 12%, desmonemes (De), 11%, holotrichous isorhizas (HI), 4%, microbasic euryteles (MiE), 14%, microbasic mastigophores (MiM or rhabdoides of unspecified type), 7%, *b*-type rhabdoides (*b*-Rh), 4%, *p*-type rhabdoides (*p*-Rh), 10%, spirocysts (Sp), 13%, and stenoteles (St), 10%. The remaining 17 cnidocyst types, comprising 8% of the cnidocysts in the sample, are considered rare.

The distribution of the ten common cnidocyst types in suborders of Anthozoa and the main superfamilies of three orders of Hydrozoa are shown in Figs. 1a-f and 2a-g. The figures reproduce published phylogenies (Schmidt, 1974; Petersen, 1979) and show the distribution of cnidocyst types in hypothetical ancestors (i.e., branches and roots) as evaluated by the MacClade algorithm. Additional data on other Hydrozoa are summarized in Table 4.

Four of the ten common nematocysts, AI, MiM (either unspecified or of *b*-Rh and/or *p*-Rh types), HI and BI, are recognized in both Anthozoa and Hydrozoa. AI (Figs. 1a and 2a) are widespread in the Hydrozoa and lower Anthozoa, and bottom hypothetical ancestors in both classes are assigned equivocal labels by the MacClade program. Similarly, MiM (unspecified) or of either the *b*-Rh or *p*-Rh types (Figs. 1e-f and 2d) are widespread in Hydrozoa and Anthozoa. The MacClade program assigns *b*-Rh a positive label in the bottom hypothetical root of the Anthozoa, while *p*-Rh is assigned an equivocal label, and MiM is assigned an equivocal label in the bottom hypothetical root of Hydrozoa. AI also occur in Cubozoa and in Rhizostomeae and Semaestomeae but not the Coronatae among the Scyphozoa, and MiM occur in Cubozoa but not Scyphozoa.

HI are widespread in Anthozoa and have equivocal status in the bottom hypothetical ancestors of Hexacorallia and Octocorallia (Fig. 1b). HI also occur in Cubozoa and in the Coronatae and Semaestomeae but not Rhizostomeae among the Scyphozoa. HI are only sparsely distributed in Hydrozoa, however, and only uncertainly identified in the Olindiasidae (Table 4). HI receive a negative label in the bottom hypothetical ancestors of Hydrozoa (Fig. 2b). Similarly, BI are present only in higher clades of

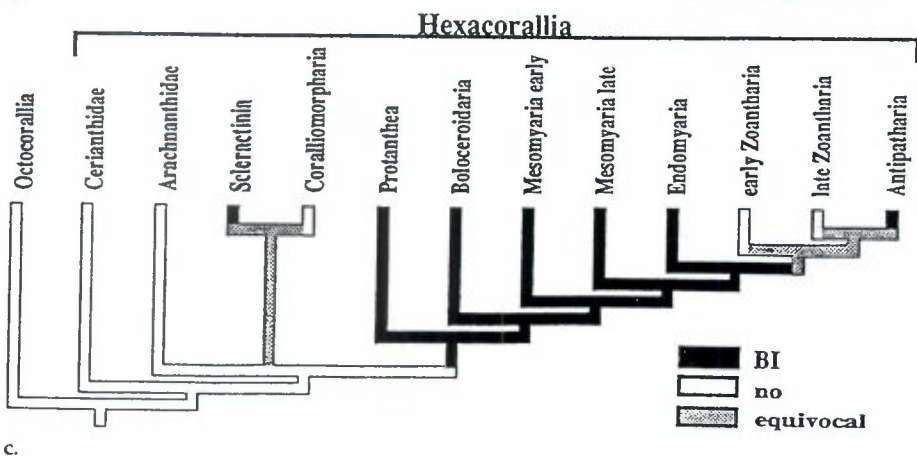
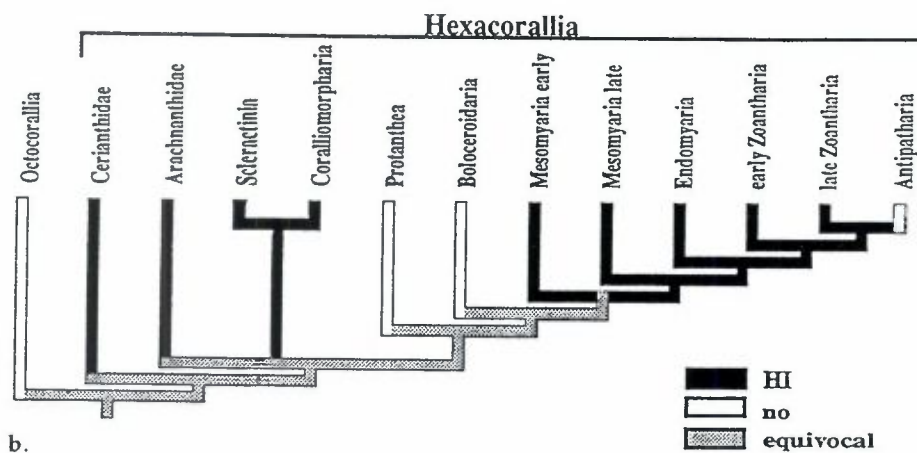
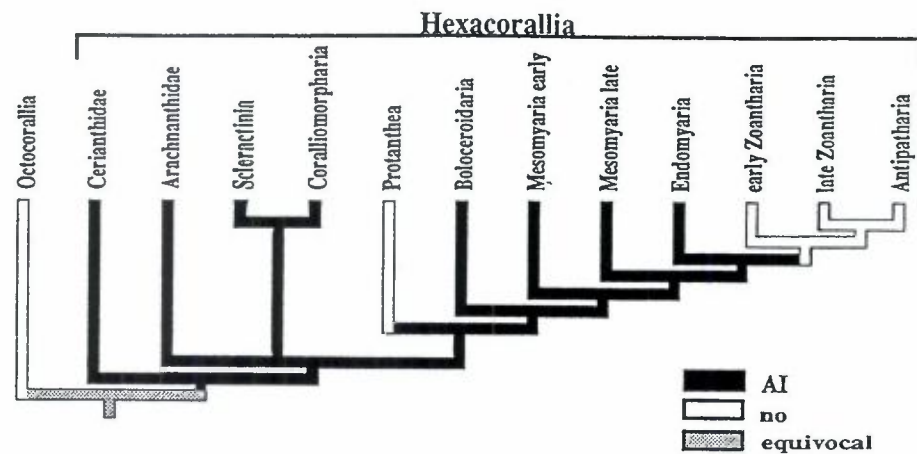


Figure 1. Anthozoa. a: atrichous isorhizas (AI); b: holotrichous isorhizas (HI); c: basitrichous isorhizas (BI).

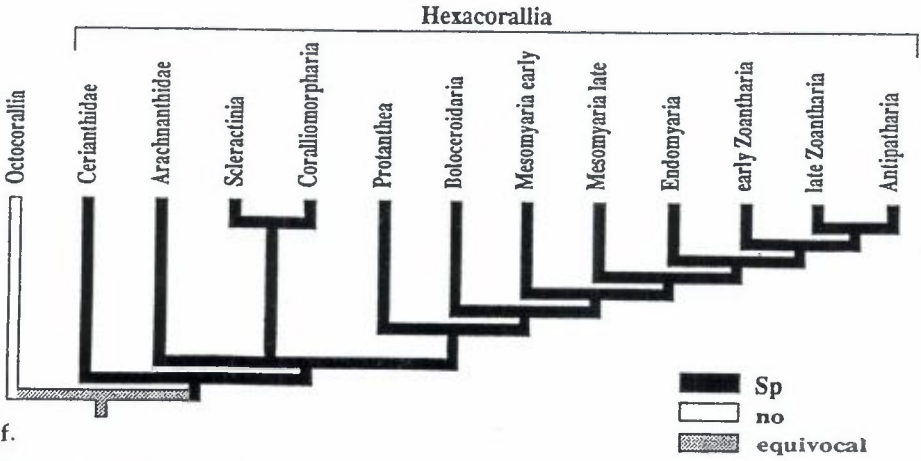
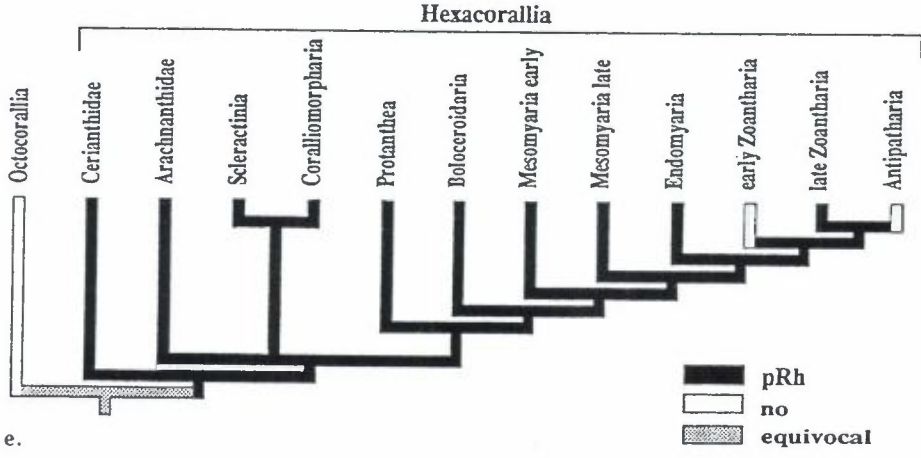
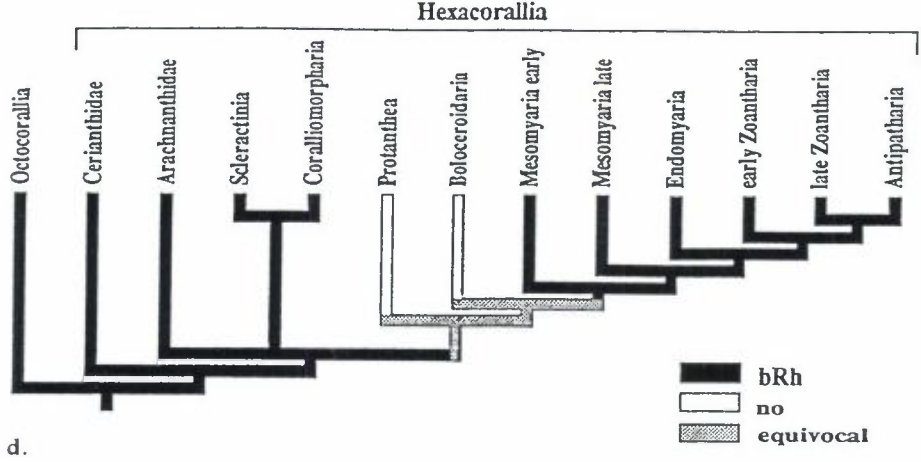


Figure 1. Anthozoa. d: b-rhabdoides (bRh); e: p-rhabdoides (pRh) f: spirocysts (Sp).

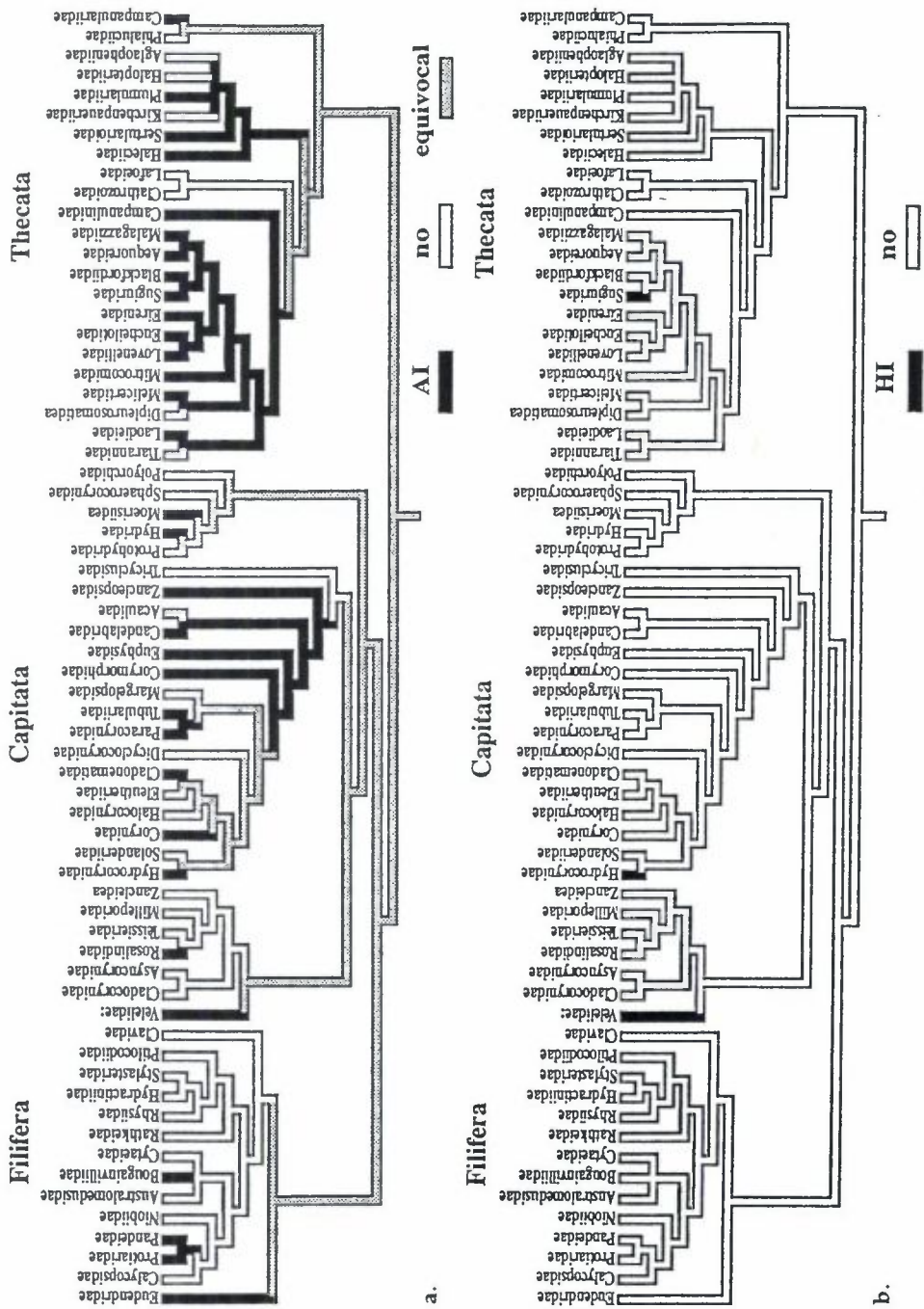


Figure 2. Hydrozoa. a: atrichous isorhizas (AI); b: holotrichous isorhizas (HI).

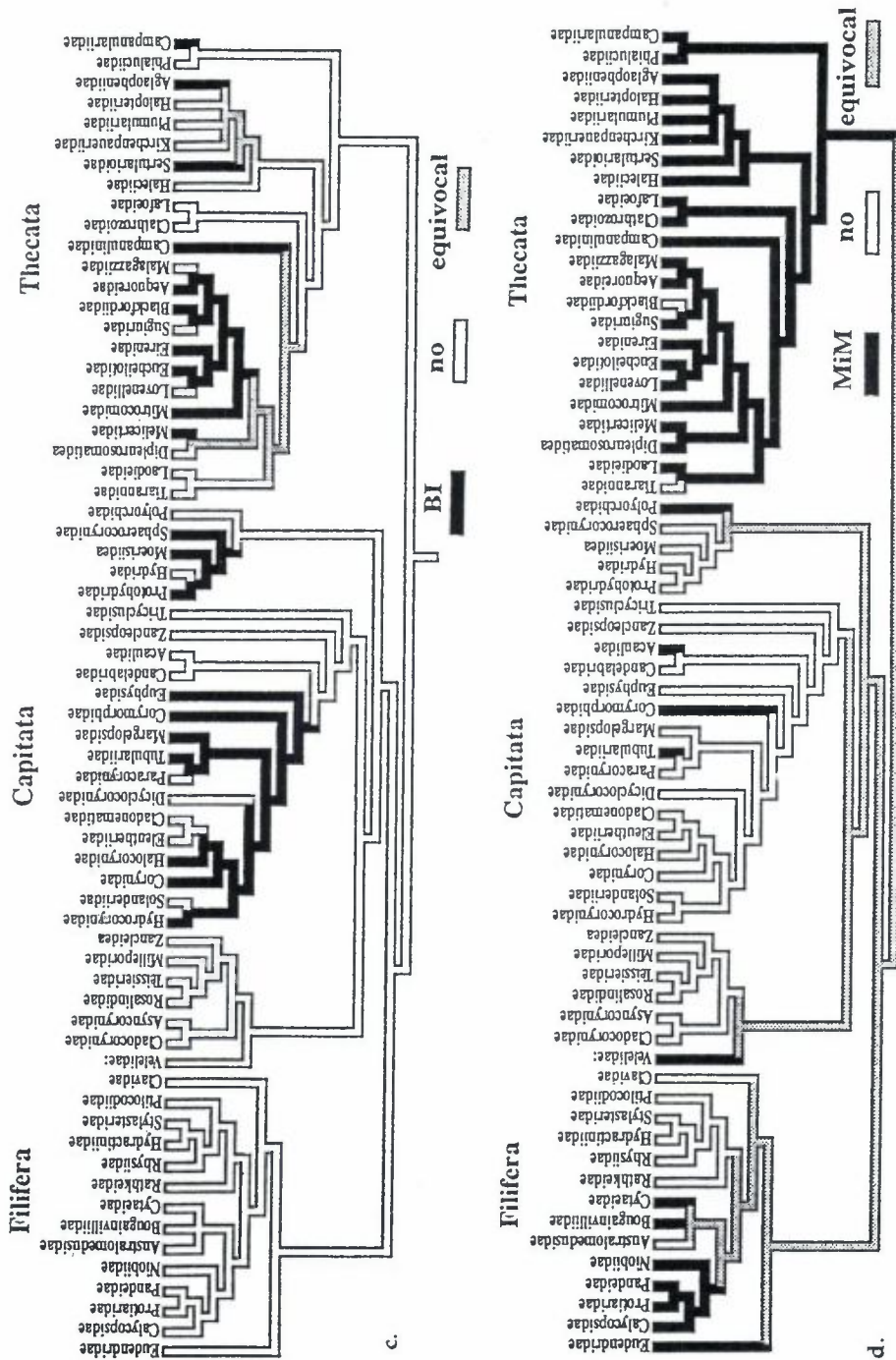


Figure 2. Hydrozoa. c: basitrichous isorhizas (BI); d: microbasic mastigophores (MiM = b- + pRh).

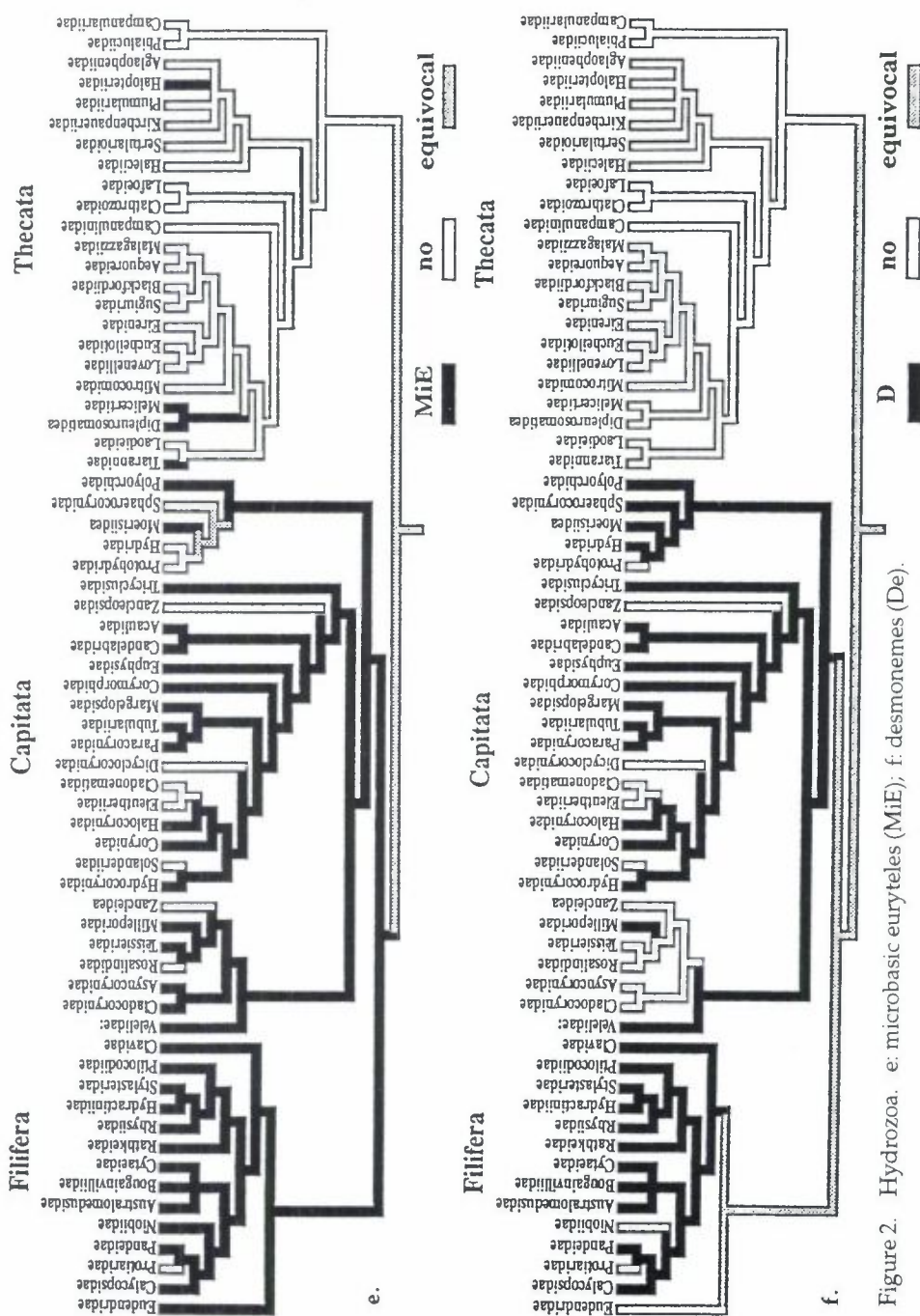


Figure 2. Hydrozoa. e: microbasic euryteles (MiE); f: desmonemes (De).

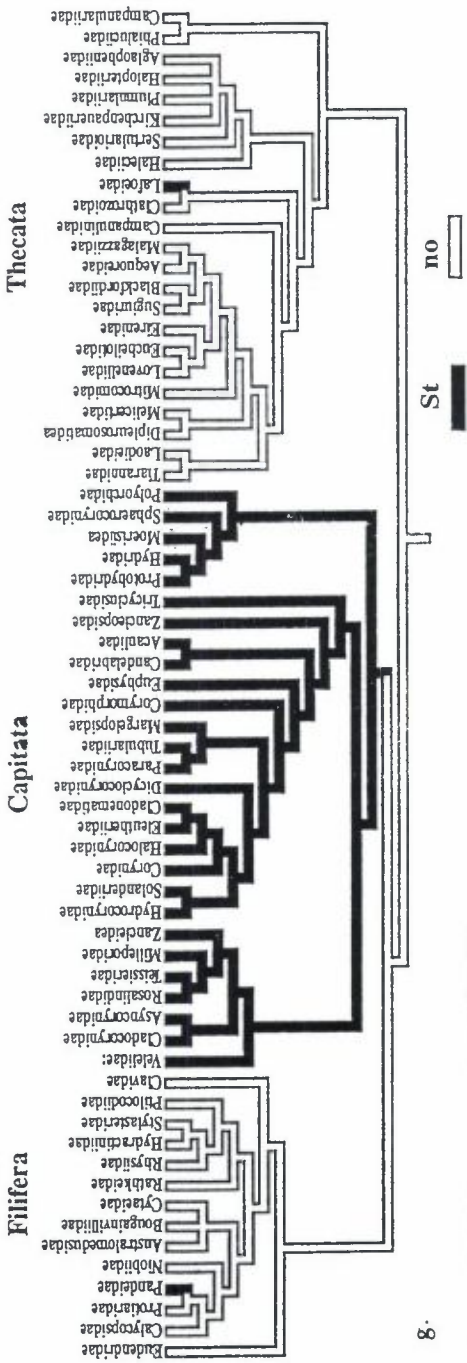


Figure 2. Hydrozoa. g: stenoteles (St).

Table 4. Continued

Subclass/ family/morph	AI	ApI	AS	BI	De	HeA	HI	MaE	MaM	MiE	MiM	S	SeMiE	St
Trachymedusae														
Geryoniidae	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Halicreatidae	(+)	-	-	-	-	-	-	-	-	(+)	-	-	-	+
Petasidae	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Ptychogastridae	+	-	-	-	-	-	-	-	-	+	-	-	-	+
Rhopalonematidae	(+)	-	-	-	-	-	-	-	-	(+)	-	-	-	(+)
Actinulidae														
Halammohydridae	+	-	+	-	+	+	-	-	-	-	+	-	-	+
Otohydridae	-	-	-	-	-	-	-	-	-	-	-	+	-	+

* + = present; - = absent; (+) = rare; ? = doubtful.
 Abbreviations: AI = atrichous isorhizas; ApI = apotrichous isorhizas; AS = aspiroteles; BI = basitrichous isorhizas; De = desmonemes; HI = holotrichous isorhizas; HeA = heterotrichous anisorhizas; MaE = macrobasic euryteles; MaM = macrobasic mastigophores; MiE = microbasic euryteles; MiM = microbasic mastigophores; S = spiroteles; SeMiE = semiophoric microbasic euryteles; St = stenoteles.

Anthozoa (Fig. 1c) and Hydrozoa (Fig. 2c), and hypothetical ancestors are labeled negative for BI.

None of the remaining common cnidocyst types occur in both Anthozoa and Hydrozoa. Sp are apomorphic for Hexacorallia (Fig. 1f) in the Anthozoa, and MiE are apomorphic for the Medusozoa. In the Hydrozoa, MiE are concentrated in the athecates (Filifera and Capitata, Fig. 2c), and the bottom hypothetical ancestors of Hydrozoa are assigned an equivocal label for MiE by the MacClade program. MiE are also present in the Limnopolypae-medusae and Probosidactylidae (Table 4) and in all orders of Scyphozoa.

The last two of the common cnidocyst types are present only in Hydrozoa. De (Fig. 2f), like MiE, are concentrated in the athecates and are present more or less coextensively with MiE in Probosidactylidae (Table 4). Aside from differences, such as the presence of De and the absence of MiE in the Otohrydidae (Table 4), MiE and De share the greater part of their distribution (compare Figs. 2e and f), suggesting the origin of De from MiE or from a common ancestor.

St are characteristic of the Capitata (Fig. 2g) and frequently occur paired with De and/or MiE in Capitata cnidomes (Table 1, see above). Stenoteles are also widespread in Trachymedusae (Table 4), however, where they appear in the absence of desmonemes and largely independently of microbasic euryteles. Stenoteles cannot be forced into a single clade with microbasic euryteles and desmonemes using the MacClade program (trees not shown).

4. Discussion

The systematic study of cnidocysts began on anemones with the work of Möbius (1866), Bedot (1896) and Will (1909) and on trachymedusans with the work of Iwanzoff (1895). Weill (1934), however, made a singular contribution that consolidates the field by specifying criteria and rational names for different cnidocyst types and by surveying the cnidocyst in 119 species across the Cnidaria. Weill (1934) also posed one of the great mysteries surrounding cnidocysts, namely, how it so happens that these cellular organelles in Cnidaria resemble cellular organelles in other organisms. Indeed, Weill dedicated his seminal 1934 paper in part to Edouard Chatton, his mentor and a leading exponent of the idea of extended homology, conceptually a forerunner of symbiogenesis.

Symbiogenesis

Despite Weill's authority, until recently, the possibility of homology

between cnidarian cnidocysts and protoctistan "cnidocysts" was generally dismissed as convergence (Pantin, 1951; Hand, 1959, 1961; Picken and Skaer, 1966; Hansen, 1977; Robson, 1985). Two arguments against homologizing these cellular organelles seemed insuperable: (1) that evolution proceeds monophyletically, that is, that one taxon could not be derived from more than one taxon; (2) that unicellular organisms possessing "cnidocysts" are unlikely sources of free-living, multicellular animals. The first obstacle is lowered if not quite breached, however, by arguments for the importance of hybridization in the evolution of animals (e.g., Williamson, 1992), and evidence for horizontal gene transfer, introgression, and para-sexual processes that permit the maintenance of symbiotic partnerships during reproduction despite genomic separation or incomplete genomic incorporation (see Margulis, 1991). The second obstacle becomes less formidable, at least in the case of myxozoans containing "cnidocysts," with findings that the base sequence of myxozoans small subunit rRNA resembles that of metazoans (Smothers et al., 1994). Myxozoans are, therefore, already animals and need not evolve into them. Thus, the possibility of homology among cnidarian cnidocysts and protoctistan "cnidocysts" is elevated to the level of hypothesis (Lom, 1990; Shostak, 1993; Buss and Seilacher, 1994).

Historically, microscopists have reported similarities between cnidocysts in Cnidaria and "cnidocysts" in phyla as remote as dinoflagellates (Martin, 1914; Chatton, 1914; also see Hovasse, 1951) and "Sporozoa" (see Lom, 1990). Electron microscopists have added extrusomes in *Polykrikos* (Westfall et al., 1983; Vickerman et al., 1991), pansporoblasts in Myxosporidia (Lom 1990), and the extrusion apparatus of Microsporidia (Perkins, 1991) to the list of protoctistan organelles with similarities to cnidarian cnidocysts (Westfall, 1966; Tardent 1988; Wood, 1988). In both protoctistans and cnidarians, tubules mature with their outside facing inward (Lom, 1964, 1969; Thomas and Edwards, 1991), and in both, tubules extend with their inside facing outward. In the case of the parasitic "sporozoans," eversion creates a tubule through which a naked amoebula enters the host cell, while in the case of cnidarians, the everted tubule of different types of cnidocysts is swollen at various levels, especially near the base, or narrow in a thread-like portion. Similarities in the mechanisms of tubular eversion in myxosporidians and cnidarians (Lom, 1964), and in the development of cnidocysts and of polar capsules in the sporoblasts of myxosporidians, reveal similarities "perhaps too close to be considered only a convergence phenomenon" (Lom, 1969, p. 435).

The issue of cnidocyst origins will not be decided until the sequencing of nuclear and extranuclear DNA is completed for each of the protoctistan candidates for sources of cnidarian cnidocysts. The present study indicates that the sequencing of nuclear and extranuclear DNA will also have to be completed

in several cnidarians in order to cover cnidocyst types belonging to members of two families of cnidocyst types: the De-MiE-St family and the AI-BI-HI-MiM-Sp family. Hypothetically, anthozoan ancestors would have been infected with a precursor of the AI-BI-HI-MiM-Sp family, while medusozoan ancestors would have been infected with a precursor of the De-MiE-St as well as with a member of the AI-BI-HI-MiM-Sp family.

Cnidocysts and cnidomes

The 27 types of cnidocysts discussed here represent the distillate of cnidocyst types identified in the systematic literature on Cnidaria. Originally, criteria for identifying cnidocyst types were tailored for systematists working in the field. The criteria proposed by Weill (1934) have been enormously influential, but they quickly began to change and expanded greatly with the advent of scanning electron microscopy and improvements in visualizing techniques. Microbasic mastigophores, which had early expanded to *b*- and *p*-types (Carlgren, 1940; Cutress, 1955) on the basis of the shape of the shaft, are identified in the present literature in no fewer than four categories (A-D) and, on the basis of shape of spines, more than 30 subtypes (Schmidt, 1974). Cnidocysts formerly identified simply as holotrichous isorhizas are now classified into types I and II (Schmidt, 1974), and atrichous isorhizas are classified into subtypes that frequently reach species-specificity (Östman, 1979, 1982, 1983, 1987, 1988, Östman et al., 1987).

Despite these improvements and changes, the number of basic types of cnidocysts has altered only slightly. One fundamental criterion used by Weill, whether the tubule was open distally or not, has fallen into disuse, but the other criteria remain more or less intact. Ptychocysts have been added as a third category of cnidocysts (Mariscal et al., 1977), although ptychocysts are probably derived from nematocysts (Mariscal, 1984), and two pseudo-, or transitional types, have been added to the list of nematocysts (e.g., Bouillon et al., 1986; Östman, 1982-1987). Basitrichous isorhizas recognized at the level of ordinary light microscopy may be indistinguishable from rhabdoides at the level of scanning electron microscopy, and macrobasic and microbasic amastigophores seem to be merely rhabdoides with incompletely everted tubules (Schmidt, 1974). Thus, Weill's criteria have been largely validated, and his categories of cnidocyst types and types of nematocysts are not only practically functional for the cnidarian systematists but fundamentally challenging to evolutionary theorists.

Evolution of cnidocyst types. Cnidaria is divided into two subphyla, Anthozoa and Medusozoa, on the basis of a variety of characteristics (Werner, 1973; Petersen, 1979) and evidence for different types of mitochondrial DNA in

members of the subphyla (Bridge et al., 1992). Of the twenty-seven cnidocyst types identified on the basis of morphological criteria, only four types occur in both subphyla (atrichous isorhizas [AI], basitrichous isorhizas [BI], holotrichous isorhizas [HI] and microbasic mastigophores [MiM]). Data on the distribution of these cnidocyst types (above) and evidence from electron microscopy raise doubts, however, about the identities of these cnidocyst types throughout the phylum.

The case for homology among HI is undermined by discontinuities in the distribution of HI and by the failure of the MacClade program for the analysis of phylogeny and character evolution to locate HI in the bottom hypothetical root of Hydrozoa. The relative rarity of HI in the Hydrozoa and the high frequency with which HI and AI are codistributed (with a frequency of 0.52 in Hydrozoa) suggest that, in the Hydrozoa at least, HI arose independently and several times from AI.

Historically, AI and MiM have often been confused. On the basis of observations made with the light microscope, AI were originally identified in the Octocorallia (Carlgren, 1940), but on the basis of observations made with the scanning electron microscope, AI are now considered absent (Schmidt, 1974) or only present in a rare and unique variety in the Octocorallia (Fautin and Mariscal, 1991). Scanning electron micrographs also indicate that MiM of the *b*-Rh type are the preponderant if not the sole cnidocyst in the Octocorallia (Schmidt, 1974), and the MacClade program assigns *b*-Rh a positive status in the bottom hypothetical root of Anthozoa. However, the complexity of spines on the tubule of octocorallian *b*-Rh suggests that these nematocysts are highly derived and unlikely candidates for a plesiomorphic character (Schmidt, 1974). Furthermore, in Hydrozoa, nematocysts previously identified as microbasic *b*-mastigophores (Mi *b*-M) on the basis of thick coils of proximal spines, now appear to lack a shaft and are considered an intermediate type dubbed pseudo-microbasic *b*-mastigophores (Östman, 1982, 1983, 1987).

BI were once considered widespread in Anthozoa (Carlgren, 1940, 1945, 1949), but they may be indistinguishable from *b*-Rh and *p*-Rh (Schmidt, 1974). Similarly, in the Hydrozoa, while nematocysts continue to be scored as BI, they are admittedly similar to rhabdoides (Purcell and Mills, 1988).

The dilemma posed by AI, BI, HI and MiM would be resolved if, as suggested above on the basis of the frequencies of cnidocyst pairing, these cnidocyst types belong to a family (the AI-BI-HI-MiM-Sp family). This family, including spirocysts (Sp), would be characterized by a tubule with a considerable thread-like portion, sometimes reinforced and thicker proximally but lacking any conspicuous swelling. A variety of less common nematocysts might be derived from primitive members of this family (e.g., apotrichous isorhizas [ApI], several types of anisorhizas, such as atrichous anisorhizas [A], heterotrichous

anisorhizas [HeA], homotrichous anisorhizas [HoA], macrobasic amastigophores [MaAm], macrobasic mastigophores [MaM], merotrichous isorhizas [MeI], microbasic amastigophores [MiAm]). The AI-BI-HI-MiM-Sp family, rather than the individual cnidocyst types, would thus be plesiomorphic and representative of both subphyla of Cnidaria.

The other family of nematocysts defined above on the basis of the frequencies of pairing (the De-MiE-St family) is confined to the Medusozoa. This family would be characterized by a tubule that is relatively thick, at least in part, sometimes with one or more than one conspicuous swelling, and sometimes coiled. Although De, with a coiled, thick tubule, might seem very different from St and MiE with swellings in and long thread-like portions of their tubule, morphological evidence of homology might still be found in the spines present on the concave side of the De tubule and throughout the thread-like portions of MiE and St tubules (Weill, 1934; Werner, 1965). The broad distribution of microbasic euryteles (MiE) in this family suggests that stenoteles (St) and desmonemes (De) are derived from MiE rhopalonemes (R) and a variety of less common euryteles (birhopaloids [BiR], various microbasic and macrobasic euryteles, and semiophoric microbasic euryteles [SeMiE], see Table 1) might also be derived from primitive members of this family.

The argument for an evolutionary relationship between microbasic euryteles and stenoteles is strengthened by pseudostenoteles (pSt), nematocysts of an intermediate type resembling both St and MiE. Pseudostenoteles are recognized specifically in the Thecata, in the halecid genus *Nemalecius* (Bouillon et al., 1986), and, in a smaller version, in the lafoeid genus *Zygophylas* (Gravier-Bonnet, 1987). The case of the pSt is not quite closed, however, since nematocysts of this type are also similar to microbasic mastigophores (MiM), putative members of the other family of cnidocyst types.

Evolution of cnidomes. Given that Cnidaria has 9,000–10,000 species (Hyman, 1940; Barnes and Harrison, 1991) and accepting the limits that no cnidome represents any more than 15% of the total, the present sample of 809 species is sufficient for sampling attributes at the 95% confidence level with 2–3% reliability. Moreover, the 114 cnidomes identified in the complete data set represent a statistically significant sample of cnidarian cnidomes.

Several observations concatenate to suggest that these cnidomes and the types of cnidocysts present in them are not derived randomly: (1) The 114 cnidomes found in the sample are a small fraction of the possible permutations of 27 cnidocyst types taken in groups of one to seven (even when limited to cnidocyst types present in members of the two subphyla). (2) Differences in cnidomic population and the slope of cnidomic population as a function of species number are statistically significant. (3) Particular cnidocyst types are associated with cnidomes of different populations. (4) Particular cnidocyst

types are preferentially coupled in cnidomes. These observations are likely to flow from consequences of evolution and selection.

The simplest explanation for the evolution of cnidomic populations is that mononidic cnidomes gave rise to compound cnidomes. Microbasic euryteles (MiE) are the best candidates for a cnidocyst present in a mononidic cnidome that could play the role of source for cnidocyst types in compound cnidomes. MiE appear in mononidic cnidomes in both Hydrozoa and Scyphozoa and in compound cnidomes throughout the Medusozoa. No other mononidic cnidome crosses class lines.

Alternatively, mononidic cnidomes may be explained by the loss of cnidocyst types from originally compound cnidomes. The possibility that cnidomic populations in compound cnidomes evolved toward reduction is supported by the tendency of compound cnidomes to be smaller when present in greater numbers of species (i.e., broadly species-general cnidomes) compared to compound cnidomes present in modest numbers of species (i.e., moderately species-general cnidomes) and in single species (i.e., species-specific cnidomes). Such an evolutionary trend would seem to be greater in the Medusozoa than in the Anthozoa, since all but two (*b*-Rh and Sp) of the mononidic cnidomes (see Table 1 and Table 3) occur in the Medusozoa. Greater reduction in cnidocyst number in the Medusozoa compared to the Anthozoa might have resulted from competitive exclusion among cnidocyst types belonging to the De-MiE-St family and AI-BI-HI-MiM-Sp family.

Finally, another of the enigmas surrounding cnidocysts cited by Weill (1934) would seem to be illuminated by the present analysis. This enigma is the greater complexity of cnidomes in Hydrozoa, which can now be enlarged to all of Medusozoa, compared to cnidomes in Anthozoa. In part, this complexity is due to the greater number of cnidocyst-types, especially exclusive cnidocyst types, in the Medusozoa compared to the Anthozoa: medusozoans possess 24 types of nematocysts and have monopolies on 18, whereas anthozoans possess only eight types of cnidocysts and have monopolies on two nematocysts, spirocysts and ptychocysts. The present report suggests that the evolution of cnidomes in Anthozoa was limited to mechanisms for selection among variants of the AI-BI-HI-MiM-Sp family. The evolution of cnidomes in Medusozoa, however, could have included character displacement coupled to competitive exclusion among members of the De-MiE-St family and AI-BI-HI-MiM-Sp family as well as mechanisms for selection among variants of both families.

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