SCI. MAR., 64 (Supl. 1): 117-122 SCIENTIA MARINA 2000

TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Sarsia marii n. sp. (Hydrozoa, Anthomedusae) and the use of 16S rDNA sequences for unpuzzling systematic relationships in Hydrozoa*

BERND SCHIERWATER[1,2] and ANDREA ENDER[1]

[1]Zoologisches Institut der J. W. Goethe-Universität, Siesmayerstr. 70, D-60054 Frankfurt, Germany,
E-mail: bernd.schierwater@ecolevol.de
[2]JTZ, Ecology and Evolution, Ti Ho Hannover, Bünteweg 17d, D-30559 Hannover, Germany.

SUMMARY: A new hydrozoan species, *Sarsia marii,* is described by using morphological and molecular characters. Both morphological and 16S rDNA data place the new species together with other *Sarsia* species near the base of a clade that developed, "walking" tentacles in the medusa stage. The molecular data also suggest that the family Cladonematidae (*Cladonema, Eleutheria, Staurocladia*) is monophyletic. The taxonomic embedding of *Sarsia marii* n. sp. demonstrates the usefulness of 16S rDNA sequences for reconstructing phylogenetic relationships in Hydrozoa.

Key words: *Sarsia marii* n. sp., Cnidaria, Hydrozoa, Corynidae, 16S rDNA, systematics, phylogeny.

INTRODUCTION

Molecular data and parsimony analysis have become powerful tools for the study of phylogenetic relationships among extant animal taxa. In particular, DNA sequence data have added important and surprising information on the phylogenetic relationships at almost all taxonomic levels in a variety of animal groups (for references see Avise, 1994; DeSalle and Schierwater, 1998). We may not forget, however, that inferring phylogenetic relationships from molecular data is neither trivial nor a final solution to systematics, since the molecular option can be prone to a large number of pitfalls and misunderstandings (for refs. see Miyamoto and Cracraft, 1991; Schierwater *et al*., 1994; Swofford *et al*., 1996). Nevertheless molecular data are especially useful or even indispensable in groups where other characters, like morphological data, are limited or hard to interpret.

One classical problem to animal phylogeny is the evolution of cnidarians and the groups therein (Hyman, 1940; Brusca and Brusca, 1990; Bridge *et al*., 1992, 1995; Schuchert, 1993; Schierwater, 1994; Odorico and Miller, 1997). Particularly the systematic relationships within the Hydrozoa represent one of the most difficult and long-standing problems to evolutionary biologists. In lack of fossil records and a sufficient number of unambiguous morphological characters, neither the phylogenetic relationships between nor within the orders have been understood and are still subject of controversial debates (see for

*Received February 17, 1999. Accepted May 10, 1999.

example, Petersen, 1979, 1990; Cunningham and Buss, 1993; Schuchert, 1996). Historically, hydroid systematics has been hindered by problems of deciding the importance of polyp versus medusoid characters (Rees, 1957; Naumov, 1960; Boero, 1980; Bouillon, 1981; Boero and Bouillon, 1987). Since in most hydroids the medusa stage has been reduced to sessile gonophores, reconstructions of phylogenetic relationships have mainly been based upon morphological characters of the hydroid stage and the assumption of a progressive reduction of the medusoid stage (Werner, 1984; Boero and Sarà, 1987). Here, the arrangement of tentacles and the inventory of nematocysts have been two of the most important characters. The morphological simplicity and plasticity of hydroids, however, makes morphological homoplasy likely to be common in this group. We suggest that molecular characters must be added to morphological and life cycle characters in order to resolve hydrozoan systematics.

By applying molecular data to previously undescribed species we might in many cases be able to resolve their relative taxonomic position even in the absence of morphological or developmental data. This approach promises valuable results if molecular data from closely related taxa are available for comparative analyses. We present an example from a new athecate hydroid species which we assign to the genus *Sarsia* based on both morphological and molecular data.

MATERIALS AND METHODS

Animal material

Based on morphological characters we used the following closely related species of the infra-order Capitata (Werner, 1984) for embedding the new species into a taxonomic framework: *Stauridiosarsia producta* Wright (1858), *Sarsia tubulosa* Sars (1835), *Sarsia reesi* Vannucci (1956) (all members of the family Corynidae), *Staurocladia bilateralis* Edmondson (1930), *Staurocladia oahuensis* Edmondson (1930), *Cladonema radiatum* Dujardin (1843) (Cladonematidae), and *Eleutheria dichotoma* Quatrefages (1842) (Eleutheriidae). The Filifera *Thecocodium quadratum* Werner (1965) served as an outgroup. Animal material of *Staurocladia bilateralis* and *S. oahuensis* were provided by Yayoi Hirano (Awagun, Japan), of *Stauridiosarsia producta* and *Sarsia tubulosa* by Gerhard Jarms (Hamburg, Germany), of *Sarsia reesi* by Alvaro Esteves Migotto (São Sebastião, Brazil), and of *Thecocodium quadratum* by Stefan Berking (Cologne, Germany).

Hydroids of *Eleutheria dichotoma*, *Cladonema radiatum* and *Sarsia marii* n. sp. were collected by the authors from the green alga *Ulva lactuca,* obtained 10-50 cm under the water surface at the rocky shore of the Laboratoire Arago, Banyuls sur Mer, France. Single polyps were isolated from the algae and grown in small finger bowls in the lab using our standard culturing conditions for hydrozoans (Schierwater *et al*., 1992; Schierwater and Hadrys, 1998). Polyps were maintained in artificial seawater (30‰ salinity) at 19-21°C and fed *ad libitum* two times weekly on 3-4 day old brine shrimp larvae (*Artemia salina*). Under these conditions polyps of all three species, including *Sarsia marii*, grew into colonies of polyps connected by a horizontal net of stolons (the latter grew attached to the glass surface of the finger bowl). While medusae of *Eleutheria dichotoma* and *Cladonema radiatum* are found regularly year round, *Sarsia marii* medusae were found only once in spring 1998. Since the latter stayed alive just for a few days, morphological traits could be observed on subadult medusae only.

The original polyp was found on a thallus of the green alga *Ulva lactuca* collected on 5 April 1997 at the Laboratoire Arago, Banyuls sur Mer, France, 42°29'N, 3°08'E.

DNA extraction, amplification and sequencing

Small samples of hydroid tissue were homogenized in HOM buffer (100mM Tris-HCL, 10mM EDTA, 100mM NaCl, 0.5% SDS, pH 8.0), and extracted once with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated by addition of two volumes ethanol and 1/10 volume 5M NH_4Ac, and after a single centrifugation and washing step resuspended in TE buffer (1mM Tris-HCL, 0.1mM EDTA, pH 7.5) (for details see Schierwater and Ender, 1993). For PCR amplification of part of the 16S rRNA mitochondrial large subunit, hydrozoan specific primers were used (Cunningham and Buss, 1993). The amplified region corresponds to the 5´-end of the 16S rRNA gene and revealed between 588 bp (*Eleutheria dichotoma*) and 595 bp (*Cladonema radiatum*). Amplification reactions were carried out with 10-20ng of DNA in a total volume of 25 μL using a 9.600 thermocycler from Perkin Elmer and the following temperature profile:

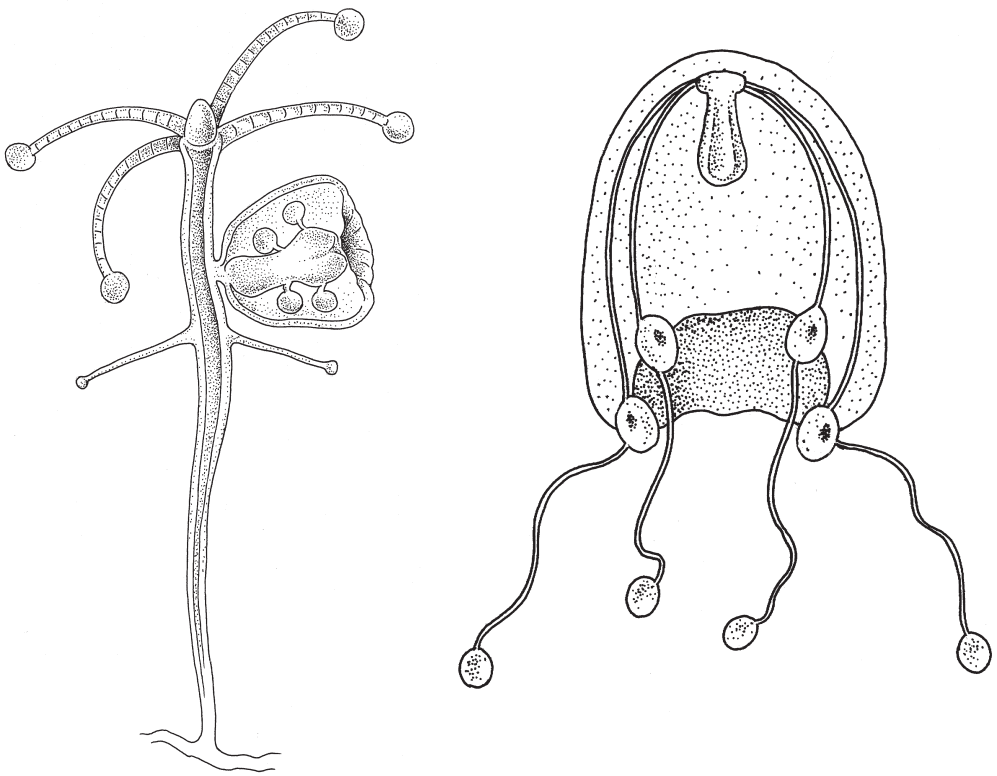

FIG. 1. – Drawings of live specimens of *Sarsia marii* n. sp. raised in the laboratory. A polyp with a well developed medusa bud above the aboral tentacle whorl and a four-day-old medusa are shown. Explanations are given in the text.

10 cycles (92°C/50 s, 45°C/50 s, ramp 3 s/1°C, 72°C/1 min) followed by 40 cycles (92°C/50 s, 50°C/1 min, ramp 3 s/1°C, 72°C/1 min); finally fragments were elongated at 72°C for 5 min. DNA cloning and sequencing were performed as described in Ender *et al*. (1996).

DNA sequence alignment and phylogenetic analyses

16S rDNA sequences were aligned with the aid of CLUSTAL (Higgins and Sharp, 1989) and ambiguous regions (often surrounding variable loops) were deleted. Since computer based alignment programs did not reveal consistent alignments, sequences were controlled by eye and compared to a secondary structure model of the *Eleutheria dichotoma* 16S rRNA molecule (Ender, 1997). A detailed description of the 16S rDNA data collection and analyses will be given elsewhere (together with a larger hydrozoan tree; Ender and Schierwater, in prep.). For tree inference by maximum parsimony or neighbor-joining data were analyzed using PAUP 3.1 (Swofford, 1993) and PHYLIP 3.75c (Felsenstein, 1995), respectively.

RESULTS

Sarsia marii n. sp.

The morphology of *Sarsia marii* polyps and medusae (Fig. 1) suggests relationships to the Cladonematidae and Corynidae (cf. Werner, 1984; Petersen, 1990). The polyp of *S. marii* looks rather similar to a *Cladonema radiatum* polyp and exhibits in addition to the oral whorl of capitate tentacles a second whorl of 4 filiform sensory tentacles. The number of capitate tentacles was always four, with each 10-11 endodermal cells (Table 1). The medusa looks like a typical *Sarsia* medusa and lacks any *Cladonema* typical tentacle branching patterns, suggesting that *Sarsia marii* is related to the genus *Sarsia*. Here the new species appears to be very similar to *S. reesi* (synonym: *Dipurena reesi* Vannucci, 1956; Brinkmann-Voss and Petersen, 1960), both in the polyp and medusa generation (Table 1). The typical long manubrium of *S. reesi* medusae, however, was not found in the immature medusae of *S. marii*. The most striking difference between *S. marii* and *S. reesi* medusae is found in the catching tentacles, which are capitate in *S. marii* and filiform in *S. reesi*.

TABLE 1 – Distinguishing morphological traits for *Cladonema radiatum*, *Sarsia reesi* (Brinkmann-Voss and Petersen, 1960; Schuchert, 1996) and *Sarsia marii* n. sp.

Characters	*Cladonema radiatum*	*Sarsia marii*	*Sarsia reesi*
Hydroid			
number of endodermal cells in capitate tentacles	7-8	10-11	18-20
number of capitate tentacles	4-5	4	3-7
number of filiform (sensory) tentacles	4-5	4	4-6
location of filiform tentacle	0.3/0.7	1/1	1/1
knob of capitate tentacles	cone shaped	cone shaped	button shaped
Medusa			
number of radial canals	7-10	4	4
length of manubrium	short	short	long
tentacle branching	present	absent	absent
type of defense tentacle	filiform	capitate	filiform
number of cnidocyst clusters of defense tentacle	50-100	1	up to 100

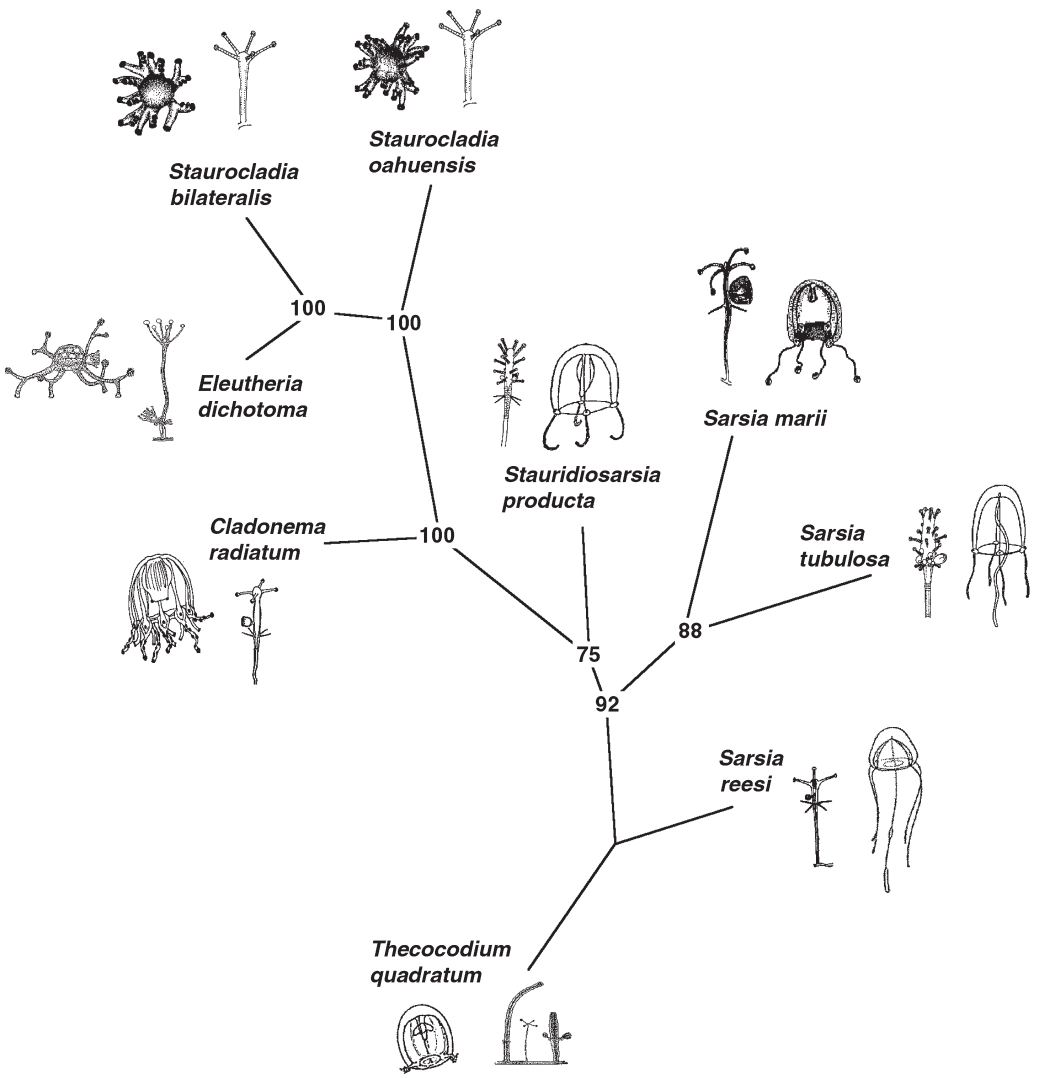

FIG. 2. – Most parsimonious 16S rDNA phylogram for *Sarsia marii* n. sp. and 7 related species of capitate athecates. The tree is rooted to the outgroup *Thecocodium quadratum* (Athecata, Filifera). Shown is the single most parsimonious tree (PAUP, Branch-and-Bound search, 500 bootstrap replicates, reweighting of characters RC=0.734, CI=0.847, RI=0.867). Note, the shown clade is a monophyletic clade within a more comprehensive tree that includes 27 hydrozoan species (Ender and Schierwater, in prep.). The number on nodes corresponds to the bootstrap value and branch lengths are drawn proportionally to the number of changes on each branch. The tree supports a close relationship between known Corynidae and the new species *Sarsia marii*, which appears within the *Sarsia* group. Note, that the Corynidae form a paraphyletic clade which varies according to the outgroup used. Please also note that the *Staurocladia* polyp shown is the drawing of a *Staurocladia wellingtoni* polyp (Schuchert, 1996) since polyps of *Staurocladia bilateralis* and *S. oahuensis* have not been found yet.

While it cannot be excluded from organismal observations alone that the observed differences between *S. reesi* and *S. marii* medusae are the result of phenotypic plasticity (we have not seen any mature *S. marii* medusae yet), 16S rDNA sequence data unambiguously identify them as different species.

Taxonomic placement

The 16S rDNA phylogram shown in Figure 2 supports the placement of *S. marii* within the genus *Sarsia*. The given branching patterns are the result of a branch and bound search using character reweighting according to the rescaled consistency index, and using bootstrap analyses with 500 resampling replicates. In the single most parsimonious tree (Fig. 2) *S. marii* clusters as a sister species to *S. tubulosa*. The genus *Sarsia* forms a paraphyletic group, depending on the outgroup used. The latter reflects the diversity - and consequently also the classification difficulties - of genera within the Corynidae, which are well known for their considerable morphological variability (including the number, placement and type of tentacles of hydroids; cf. Boero and Bouillon, 1987). For instance, the genera *Sarsia* and *Dipurena* were distinguished by a single character (gonads in one ring or gonads in two or more rings; Kramp (1961), and the genus *Stauridiosarsia* from *Sarsia* solely by the presence or absence, respectively, of aboral filiform tentacles (Bouillon, 1985).

The genus *Sarsia* appears near the base of a clade that gave rise to the invention of walking (Fig. 2). The derived genera *Cladonema*, *Staurocladia*, and *Eleutheria* all exhibit branched tentacles with specialized tentacles for attaching the medusae temporarily to a substrate. The tree supports a placement of *Stauridiosarsia* close to the family Corynidae. It is noteworthy that the above conclusions are supported by high bootstrap values and also neighbor-joining analysis (not shown), which revealed the same branching patterns as shown in Figure 2.

DISCUSSION

Hydroid taxonomists have traditionally faced difficulties many other taxonomists have not, including controversies over the relative importance of polypoid versus medusoid characters and the lack of one of the two bauplans in many groups. Furthermore, the independent gains and losses of morphological features in any one generation may produce high levels of morphological homoplasy (convergence), especially since the number of morphological characters in Hydrozoa is severely limited (cf. Boero, 1980; Boero and Bouillon, 1987; Petersen, 1979, 1990). The main advantages of adding molecular data to phylogenetic analyses of Hydrozoa include that molecular characters are independent of any developmental or life cycle stage and can be generated at *cum grano salis* unlimited numbers. Our example, in which we describe a new species, *Sarsia marii*, demonstrates the usefulness of 16S rDNA sequence data for verifying the taxonomic placement for a new species and simultaneously resolving its phylogenetic relationships to closely related species.

Both the morphology and the 16S rDNA sequence data provide unambiguous evidence that *Sarsia marii* n. sp. must be regarded as a member of the Corynidae, closely related to *Sarsia tubulosa*. Based on morphological data alone this taxonomic placement would be less clear, especially since we are yet lacking knowledge of the morphology of sexually mature medusae. However, it was the morphology which allowed us to identify closely related species for comparison in a molecular tree. The tree itself is widely congruent with our expectations from morphological data, and seems to be well supported by the high bootstrap values, and the independence of the tree topology from the tree building algorithm used. Furthermore, the clade discussed here also remains stable in a larger scale phylogeny, which includes a total of 27 hydrozoan species (Ender and Schierwater, in prep.). Nonetheless we would like to note that any molecular phylogeny is sensitive for example to the selection of taxa, to the choice of the outgroup, to the algorithm used for tree reconstruction, and in case of DNA sequence data, particularly also to the alignment of putative homologous nucleotide positions (for refs. see DeSalle and Schierwater, 1998). While future progress is to be expected – and needed – with respect to finding the most robust means for analyzing DNA sequence data, it seems clear that 16S rDNA data provide informative characters for unpuzzling phylogenetic relationships in Hydrozoa at different taxonomic levels.

ACKNOWLEDGEMENTS

We are grateful to Stefan Berking, Yayoi Hirano, Gerhard Jarms, and Alvaro Esteves Migotto for providing animal material, and to Ellen Mostafawy for

preparing the drawings of *Sarsia marii*. We acknowledge technical assistance from Margret Ruppert, critical comments from Werner Schroth and Heike Hadrys, and financial support from the Deutsche Forschungsgemeinschaft (DFG Schi-277/10-1, Schi-277/15-1).

REFERENCES

Avise, J.C. – 1994. *Molecular markers, natural history and evolution*. Chapman and Hall, New York, London.

Boero, F. – 1980. Life cycles of hydroids and hydromedusae: some cases of difficult interpretation. *Memoire Biol. mar. Oceanogr.* 10: 141-147.

Boero, F. and J. Bouillon. – 1987. Inconsistent evolution and paedomorphosis among the hydroids and medusae of the Athecatae/Anthomedusae and the Thecatae/Leptomedusae (Cnidaria, Hydrozoa). In: J. Bouillon, T. Boero, F. Cicogna and P.F.S. Cornelius (eds.) *Modern trends in the systematics, ecology, and evolution of Hydroids and Hydromedusae*, pp. 229-250. Clarendon Press, Oxford.

Boero, F. and M. Sarà. – 1987. Motile sexual stages and evolution of Leptomedusae (Cnidaria). *Boll. Zool.* 54: 131-139.

Bouillon, J. – 1981. Origine et phylogénèse des Cnidaires et des Hydropolypes-Hydroméduses. *Ann. Soc. R. Zool. Belg.* 111: 45-56.

Bouillon, J. – 1985. Esai de classification des Hydropolypes-Hydroméduses (Hydrozoa-Cnidaria). *Indo-Malayan Zool.* 2: 29-243.

Bridge, D., C.W. Cunningham, R. DeSalle and L.W. Buss. – 1995. Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. *Mol. Biol. Evol.* 12: 679-689.

Bridge, D., C.W. Cunningham, B. Schierwater, R. DeSalle and L.W. Buss. – 1992. Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proc. Natl. Acad. Sci. USA* 89: 8750-8753.

Brinkmann-Voss, A. and K.W. Petersen. – 1960. On some distinguishing characters of *Dipurena reesi* Vannucci, 1956 and *Cladonema radiatum* Dujardin, 1843. *Pubbl. Stn. Zool. Napoli.* 31: 386-392.

Brusca, R.C. and G.J. Brusca. – 1990. *Invertebrates*. Sinauer Associates, Sunderland, Mass.

Cunningham, C.W. and L.W. Buss. – 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. *Biochem. Syst. Ecol.* 21: 57-69.

DeSalle, R. and B. Schierwater (eds). – 1998. *Molecular approaches to ecology and evolution*. Birkhäuser, Basel-Boston.

Ender, A. – 1997. Untersuchungen zur Evolutionsgenetik des athekaten Hydrozoons *Eleutheria dichotoma* (Quatrefages 1842). Ph. D. thesis, J.W. Goethe University Frankfurt, Germany.

Ender, A., K. Schwenk, T. Städler, B. Streit, and B. Schierwater. – 1996. RAPD identification of microsatellites in *Daphnia*. *Mol. Ecol.* 5: 437-441.

Felsenstein, J. - 1995. PHYLIP: Phylogeny Inference Package. Version 3.57c. Department of Genetics, University o Washington, Seattle.

Higgins, D.G. and P.M. Sharp. – 1989. CLUSTAL. Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS* 5: 151-153.

Hyman, L.H. – 1940. *The Invertebrates, Vol. 1, Protozoa through Ctenophora*. McGraw-Hill, New York.

Kramp, P.L. – 1961. Synopsis of the medusae of the World. *J. Mar. Biol. Ass. UK* 40: 5-469.

Miyamoto, M.M. and J. Cracraft (eds). – 1991. *Phylogenetic Analysis of DNA Sequences*. Oxford University Press, Oxford.

Naumov, D.V. – 1960. *Hydroids and Hydromedusae of the marine brackish and freshwater basins of the USSR*. Israel Program for Scientific Translations, Jerusalem 1969 cat. no. 5108.

Odorico, D.M. and D.J. Miller. – 1997. Internal and external relationships of the Cnidaria: implications of primary and predicted secondary structure of the 5´-end of the 23-like rDNA. *Proc. R. Soc. Lond. B* 264: 77-82.

Petersen, K.W. – 1979. Development of coloniality in Hydrozoa. In: G. Larwood and B.R. Rosen (eds.) *Biology and systematics of colonial organisms*, pp. 105-139. Academic Press, New York.

Petersen, K.W. – 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). *Zool. J. Linn. Soc.* 100: 101-231.

Rees, W.J. – 1957. Evolutionary trends in the classification of capitate hydroids and medusae. *Bull. Brit. Mus. Nat. Hist. Zool.* 4: 455-534.

Schierwater, B., B. Piekos and L.W. Buss. – 1992. Hydroid stolonal contractions mediated by contractile vacuoles. *J. Exp. Biol.* 162: 1-21.

Schierwater, B. and A. Ender. – 1993. Different thermostable DNA polymerases may amplify different RAPD products. *Nucl. Acids Res.* 21: 4647-4648.

Schierwater, B. – 1994. Die Bedeutung von DNA-Merkmalen für die Analyse phylogenetischer Beziehungen innerhalb der Cnidaria. In: Gutman W.F., D. Mollenhauer and D.S. Peters (eds.) *Morphologie and evolution*. Symp 175 J Senck Naturf Ges. Kramer Verlag, Frankfurt/M: 435-442.

Schierwater, B., B. Streit, G.P. Wagner and R. DeSalle (eds). – 1994. *Molecular ecology and evolution: approaches and applications*. Birkhäuser, Basel-Boston.

Schierwater, B. and H. Hadrys. – 1998. Environmental factors and metagenesis in the hydroid *Eleutheria dichotoma*. *Inv. Reprod. Dev.* 34: 139-148.

Schuchert, P. – 1993. Phylogenetic analysis of the Cnidaria. *Z. zool. Syst. Evolut.-forsch.* 31: 161-173.

Schuchert, P. – 1996. *The marine fauna of New Zealand: Athecate Hydroids and their Medusae (Cnidaria: Hydrozoa)*. New Zealand Oceanographic Institute Memoir, Wellington, Auckland.

Swofford, D.L. – 1993. PAUP: *Phylogenetic analysis using parsimony*. Version 3.1. Illinois Natural History Survey, Champaign, III.

Swofford, D.L., G.J. Olsen, P.J. Waddell and D.M. Hillis. – 1996. Phylogenetic inference. In: D.M. Hillis, C. Moritz and B.K. Mable (eds.) *Molecular Systematics*, pp. 407-514. Sinauer Associates, Sunderland, Mass.

Vannucci, M. – 1956. Biological notes and description of a new species of *Dipurena* (Hydrozoa, Corynidae). *Proc. Zool. Soc. London* 127: 479-487.

Werner, B. – 1984. Stamm Cnidaria, Nesseltiere. In: H.E. Gruner (ed) *Lehrbuch der Speziellen Zoologie*, Band I, 2. Teil, pp. 11-305. Gustav Fischer Verlag, Jena.