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Molecular phylogenetics in *Hydra*, a classical model in evolutionary developmental biology

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Abstract

Among the earliest diverging animal phyla are the Cnidaria. Freshwater polyps of the genus *Hydra* (Cnidaria, Hydrozoa) have long been of general interest because different species of *Hydra* reveal fundamental principles that underlie development, differentiation, regeneration and also symbiosis. The phylogenetic relationships among the *Hydra* species most commonly used in current research are not resolved yet. Here we estimate the phylogenetic relations among eight scientifically important members of the genus *Hydra* with molecular data from two nuclear (18S rDNA, 28S rDNA) and two mitochondrial (16S rRNA, cytochrome oxidase subunit I (COI)) genes. The phylogenetic trees obtained by maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods were generally compatible with present morphological classification patterns. However, the present analysis also bears on several long-standing questions about *Hydra* systematics and reveals some characteristics of the phylogenetic relationships of this genus that were unknown so far. It indicates that *Hydra viridissima*, the only species in *Hydra*, which contains symbiotic algae, might be considered as the sister group to all other species within this genus. Analyses of both nuclear and mitochondrial sequences support the view that *Hydra oligactis* and *Hydra circumcincta* are sisters to all other *Hydra* species. Unexpectedly, we also find that in contrast to its initial description, the strain used for making transgenic *Hydra*, *Hydra vulgaris* (strain AEP) is more closely related to *Hydra carnea* than to other species of *Hydra*.

Keywords: Molecular phylogeny; Hydrozoa; *Hydra*; 18S rRNA; 28S rRNA; 16S rRNA; COI; Mitochondrial DNA

1. Introduction

Among the basal metazoa, Cnidaria, the sister group of the Bilateria (Peterson and Eernisse, 2001), are among the first taxa in evolution that have a defined body plan, stem cells, a nervous system and a tissue layer construction. Cnidaria are of monophyletic origin (Bridge et al., 1995; Collins, 2002) and exhibit considerable diversity in morphology. Symbiosis with photosynthetic algae contributes to the success of many cnidarians (Geller and Walton, 2001; Habetha et al., 2003). Cnidarians such as the freshwater polyp *Hydra* have a long history as model systems in developmental biology because of the remarkable plasticity in their differentiation capacity and their ability to regenerate missing body parts (Bosch and Fujisawa, 2001; Steele, 2002; Bosch and Khalturin, 2002; Bode, 2003; Holstein et al., 2003). Hydra's regeneration capacity and the underlying mechanism responsible for specification of positional information present excellent opportunities for understanding how gradients of morphogens could be set up and maintained to control local developmental processes (Wolpert et al., 1972; Wolpert, 1973; Meinhardt and Gierer, 2000). By application of quantitative cellular techniques much has been learned about hydras cell populations, and the mechanisms controlling pluripotency, lineage commitment and position dependent cell differentiation (for reviews see Bosch and David, 1991; Bode, 1996; Bosch, 2006). Moreover, there is a good understanding of the

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cellular and metabolic aspects of the symbiotic relationships between *Hydra viridissima* (also called *Hydra viridis*) and the intracellular green algae of the *Chlorella* group (Habetha et al., 2003; Habetha and Bosch, 2005).

An impressive accumulation of gene sequences, novel tools and the development of genomic resources over the past few years has brought a new perspective on research in *Hydra* (for reviews see Steele, 2002; Holstein et al., 2003; Bode, 2003; Galliot et al., 2006). A National Science Foundation-funded Hydra EST Project (www.hydrabase.org) resulted in 170,000 ESTs. A National Human Genome Research Institute-funded Hydra genome project at the J. Craig Venter Institute currently provides 6× coverage of the *Hydra magnipapillata* genome sequence with an assembled draft genome sequence appearing later this year. The genome sizes and corresponding karyotypes for five *Hydra* species have been determined (Zacharias et al., 2004). *Hydra* became also amenable to reverse genetics through RNAi experiments, further expanding the capabilities of this model organism (Lohmann et al., 1999; Takahashi et al., 2005; Cardenas and Salgado, 2003; Chera et al., 2006; Amimoto et al., 2006). Suppression subtractive hybridization procedures (Genikhovich et al., 2006), phylogenetic footprinting (Siebert et al., 2005) and a comparative genomics online platform (www.compagen.org) complement the analytical tools. Finally, transgenic *Hydra* (Wittlieb et al., 2006) now pave the way for many important scientific and technological applications making resources and methods available to fully explore the biological opportunities that the polyp provides.

Different species of *Hydra* are currently used for different analytical purposes. *H. vulgaris* and *H. magnipapillata* for example, are used for most of the developmental approaches (Takahashi et al., 2005; Guder et al., 2006; Augustin et al., 2006). Certain aspects of axial patterning such as foot specific differentiation rely on analysis of *Hydra oligactis* and *Hydra robusta* (also called *Pelmatohydra robusta*) (Kobayakawa and Kodama, 2002; Amimoto et al., 2006). *H. viridissima* is used to gain insight in symbiosis (Rahat and Reich, 1984; Habetha et al., 2003; Habetha and Bosch, 2005). The gamete differentiation pathway and the mechanisms underlying sex determination are mostly studied in *H. oligactis* (Littlefield, 1985; Littlefield et al., 1991; Nishimiya-Fujisawa and Sugiyama, 1993, 1995). Most of the ESTs come from *H. magnipapillata* with some of them from *H. vulgaris* and others from *H. oligactis*. The genome project is currently done in *H. magnipapillata*. And transgenic polyps have been produced by injecting constructs into embryos of a *Hydra* strain described originally (Martin et al., 1997) as *H. vulgaris* (strain AEP).

Unfortunately, despite the observation (Collins, 2000; Collins et al., 2006) that *H. viridissima* (synonymous: "*Chlorohydra*") when compared to *Hydra littoralis* and *Hydra circumcincta* forms a phylogenetically distinctive group, the taxonomy within the Hydridae ("hydroids") is controversially discussed and not resolved (Holstein, 1995; Stepanjants et al., 2000; Anokhin, 2004). Up to this point, a vague number of 30 *Hydra* species are described (Anokhin, 2004) but there is no clear evidence on the exact number of species and genera within the Hydridae. The first *Hydra* species was taxonomically described in 1758 by Carl Linné as *Hydra polypus*. In the following years several more species were identified and described belonging to one genus *Hydra* (Pallas, 1766; Linné, 1767). At the beginning of the 20th century, the first classification of *Hydra* species into different genera was established by Schulze (1914, 1917). According to general morphological differences in the body plan (body shape, stalk, symbiotic algae), different modes of tentacle formation during budding and differences in specific types of nematocytes, Schulze proposed the three genera *Hydra*, *Pelmatohydra*, and *Chlorohydra* (Schulze, 1914, 1917). However, only few scientists use this taxonomy (Kramp, 1935; Hyman, 1940; Leloup, 1952; Stepanjants et al., 2000; Anokhin, 2002). The most popular view is still Linné's one-genus taxonomy (Campbell, 1987; Holstein, 1995). In 1987 Campbell placed all known *Hydra* species under one genus but into four different "groups": the "oligactis group" (stalked hydras), the "vulgaris group" (common hydras), the "viridissima group" (green hydras) and the "braueri group" (gracile hydras) (Campbell, 1987; Holstein, 1995). As basis for this grouping served all known morphological differences between the species. No molecular data were included. This grouping did not resolve the taxonomical controversy about the number of genera present, nor did it discern which *Hydra* species should be considered the most basal one.

Our recent focus on using transgenic *Hydra* to fully understand the function of developmentally important genes (Wittlieb et al., 2006) and our long-term goal to understand how taxon specific features are encoded (Bosch and Khalturin, 2002) has intensified our attempts towards elucidation of the phylogenetic relationships among the *Hydra* species frequently used in various laboratories. Here, we have used data from nuclear (18S rDNA SSU, 28S rDNA LSU) and mitochondrial (16S rRNA; cytochrome oxidase I (COI)) markers in maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses to reconstruct the evolutionary history of the eight *Hydra* species most commonly used in current research.

2. Materials and methods

2.1. Specimens and selection of outgroups

Molecular phylogenetic analyses were carried out with *H. magnipapillata*, *H. vulgaris* (strain Basel), *H. carnea*, *H. oligactis*, *H. robusta*, *H. circumcincta*, *H. viridissima* and *H. vulgaris* strain AEP (Martin et al., 1997; Technau et al., 2003). The animals were cultured according to standard conditions at 18°C. The marine hydrozoans *Obelia geniculata* and *Podocoryne carnea* and the anthozoan *Nematostella vectensis* served as outgroup in the phylogenetic analyses.

2.2. DNA isolation, PCR amplification, cloning and sequencing

Genomic DNA from 100 animals of each species was isolated using a standard Phenol/Chlorophorm nucleic acid extraction protocol. The target genes (partial sequences) were PCR amplified using heterologous primers designed for *H. magnipapillata*. LSU/28s primers: 28SrRNA_F 5′-GCTAAGCTTTGACGAGTAGG-3′, 28SrRNA_R 5′-CTGCCACAAGCCAGTTATC-3′; SSU/18s primers: 18SrRNA_F 5′-GATCCTGCCAGTAGTCATATG-3′, 18SrRNA_R 5′-GAGTCAAATTAAGCCGCAGG-3′; 16s primers: 16SrRNA_F 5′-GGATGCAGTAACTCTGACTG-3′, 16SrRNA_R 5′-CCTGTTATCCCTAAGGTAGC-3′; CO1 primers: CO1_F 5′-GGATGCAGTAACTCTGACTG-3′, CO1_R 5′-CTATCAGTTAGTAGCATAGTTAT-3′. Resulting PCR fragments were cloned into pGEM-T vector (Promega, Madison, Wisconsin) and transformed into electrocompetent DH10B *Escherichia coli* cells (Invitrogen, Karlsruhe, Germany). Plasmids were sequenced using a LI-COR 4300 DNA Analyzer plate sequencer (LI-COR Biosciences, Lincoln, Nebraska). All sequences have been submitted to GenBank (Accession Nos. EF059926–EF059957).

2.3. Cytophotometric determination of genome size for *H. magnipapillata*

Recently, genome sizes of five *Hydra* species have been reported and found to exhibit marked variation in nuclear DNA content (Zacharias et al., 2004). To include the genome size of the *Hydra* species which recently was selected for complete genome sequencing, we have determined the genome size of *H. magnipapillata* (strain 105). The diploid nuclear DNA content was determined using interphase nuclei from both small interstitial cells and epithelial cells. Chicken erythrocytes were used as a reference. The genome size of approximately 1290 Mbp is remarkable similar to the one of 1250 Mbp in *H. vulgaris* (Zacharias et al., 2004) supporting the view that both species are closely related.

2.4. Phylogenetic analysis

Sequence alignments were generated using ClustalW (Thompson et al., 1994) included into the BioEdit v.7.053 sequence analysis software package (Hall, 1998). Alignments were optimized by hand and converted into required file-formats (.nex, .phy). FindModel (Tao, 2005) was used to estimate the best-fit substitution models for further phylogenetic analyses. To infer phylogenetic relationships among the taxa, we conducted three different analytical methods. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods were used for the dataset of each single gene and for the combined (by concatenation) datasets of nuclear and mitochondrial genes, respectively. Trees were drawn using TreeView 1.6.6 (Page, 1996) and MEGA.

2.4.1. Maximum parsimony (MP) analyses

Maximum parsimony (MP) analyses were performed using the MEGA 3.1 software package (Kumar et al., 2004). A bootstrap test with 100.000 replicates and random seed was conducted to each analyzed dataset. Gaps were set to complete deletion. For the CO1 genes all three codon positions plus noncoding characters were included. The datasets were tested using the Close-neighbour-interchange (CNI) method with search level 1. Initial trees for CNI searches were build using the Minimal-Mini Heuristic method with a search factor of 100.

2.4.2. Maximum likelihood (ML) analyses

Maximum likelihood (ML) analyses were performed using the quartett-puzzling method implemented in Tree-Puzzle 5.2 (Schmidt et al., 2002). The analyses included 100.000 puzzling steps. Exact analysis parameters were estimated from each dataset using quartet sampling and NJ trees. Nuclear genes were tested using the Tamura–Nei substitution model. For testing mitochondrial genes the GTR (General Time Reversible) and the HYK (Hasegawa–Kishino–Yano-85) substitution models were used for CO1 and 16s, respectively.

2.4.3. Bayesian inference analyses

Bayesian inference analyses were carried out using Mr. Bayes v.3.0 (Huelsenbeck and Ronquist, 2001). All analyses were run for 100.000 generations and a sample frequency of 100. Trees were inferred at a burn-in of 250. The datasets were tested using the General Time Reversible (GTR) substitution model with 6 substitution types and gamma-shaped rate variation with a proportion of invariable sites. The gamma distribution was approximated using 4 discrete categories.

2.5. Cell types and microscopy

Microscopic differential interference contrast (Nomarski) images of nematocysts were taken using a Zeiss Axioskop 2 and a Zeiss AxioCam HR with an 100× objective.

3. Results

To determine the phylogenetic affinities of the species and strains of *Hydra* most frequently used in research, we conducted maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses of two nuclear and two mitochondrial DNA sequence data sets.

3.1. Phylogenetic inference using mitochondrial genes

For the mitochondrial DNA, the data sets included 401 base pairs (bp) of the mitochondrial (mtDNA) 16S ribosomal RNA (rRNA) gene as well as 573 bp of the cytochrome oxidase I (COI) gene. mtDNA sequences of the marine hydrozoans *Obelia geniculata* and the anthozoan *Nematostella vectensis* available on GenBank were included as outgroup. As shown in Fig. 1, both single-gene maximum likelihood analyses recovered *Hydra viridissima* as the sister group to all other *Hydra* species. *H. circumcincta* invariably resolved as the sister group to the other six *Hydra* species examined. The two members of the "oligactis" group (*H. oligactis* and *H. robusta*) are sister group to the remaining four species. Unexpectedly, all analyses of both mitochondrial genes strongly suggest that *H. vulgaris* (strain AEP) is most closely related to *Hydra carnea* and not to *H. vulgaris* (srain Basel) or *H. magnipapillata*. There were no conflicts between the MP, ML and BI analyses since results from the MP and BI analysis support all of the affinities recovered in the ML analysis (see Suppl. Figs. 1 and 2). We also performed analyses on the combined data sets of both mtDNA genes. Fig. 2 shows that as with the individual gene analyses, *H. viridissima* is strongly supported as basal species and *H. circumcincta* is the sister taxon to the "oligactis" and "vulgaris" group. *H. vulgaris* (strain AEP) and *H. carnea* form a monophyletic group.

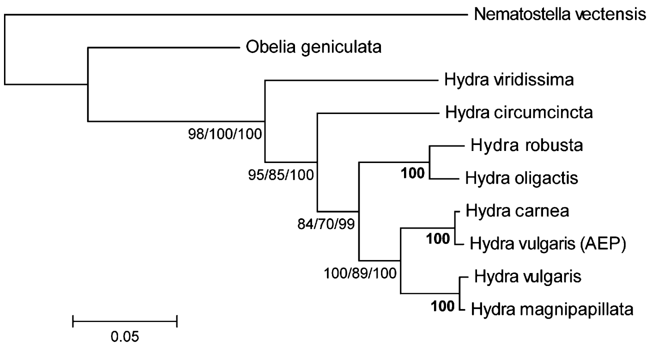

Fig. 2. Maximum likelihood phylogenetic analysis of the combined mitochondrial dataset, containing 16s and CO1 genes. Numbers near the nodes indicate bootstrap values of the ML and MP analyses as well as the Bayesian posterior probabilities (order = ML/MP/BI). Single values in bold letters indicate the identical result in all three analyses. The branch length indicator displays 0.05 substitutions per site.

3.2. Phylogenetic inference using nuclear genes

Two nuclear genes were used to provide an independent estimate of the evolutionary relationships among the *Hydra* species. The data sets included 1053 bp of the 18S small ribosomal subunit rRNA gene and 1275 bp of the 28S large ribosomal subunit rRNA gene. Corresponding sequences of the marine hydrozoan *Podocoryne carnea* available on GenBank were included as outgroup. As shown in Fig. 3, both single-gene maximum likelihood analyses recovered *H. viridissima* as the most basal group. *H. circumcincta* was recovered as the sister group to the other six *Hydra* species examined. The two members of the "oligactis" group (*H. oligactis* and *H. robusta*) are sister group to the remaining 4 species. The only difference between the trees shown in Fig. 3 A and B is in the position of *H. circumcincta*, as in the 18S rRNA tree it clusters with the "vulgaris" group, while in the the 28S rRNA tree—similar to the trees of mtDNA sequences (see Figs. 1 and 2)—it is recovered as the sister species to the "oligactis" and "vulgaris" group. Similar to the analyses of

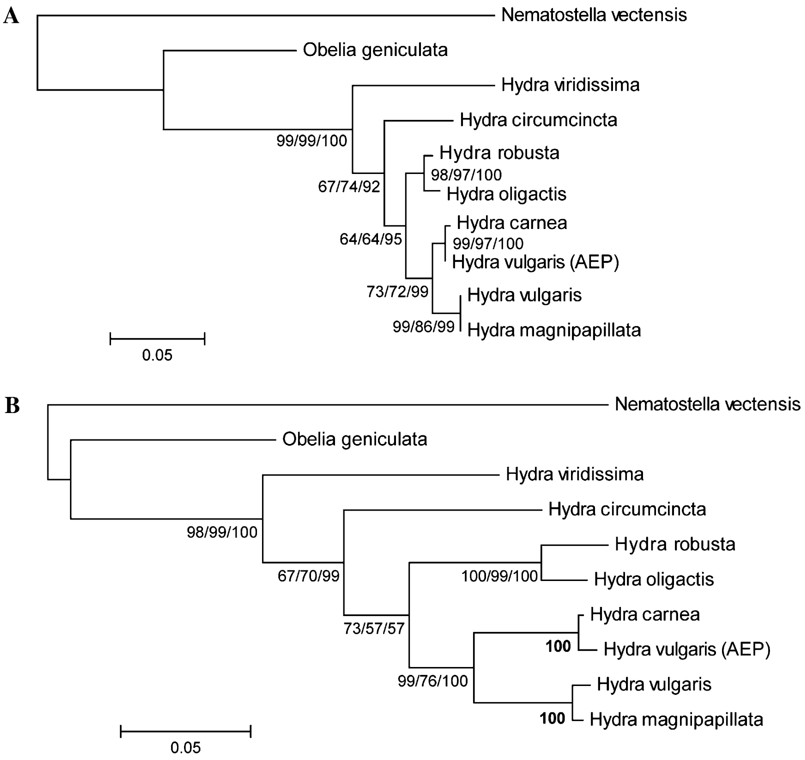

Fig. 1. Maximum likelihood phylogenetic trees inferred of the (A) mitochondrial 16s rRNA gene and (B) mitochondrial CO1 gene. Bootstrap values for ML and MP criteria and Bayesian posterior probabilities (BI) are depicted at the corresponding nodes (order = ML/MP/BI). Single values in bold letters indicate the identical result in all three analyses. Branch lengths are scaled to the expected number of substitutions (0.05 substitutions per site).

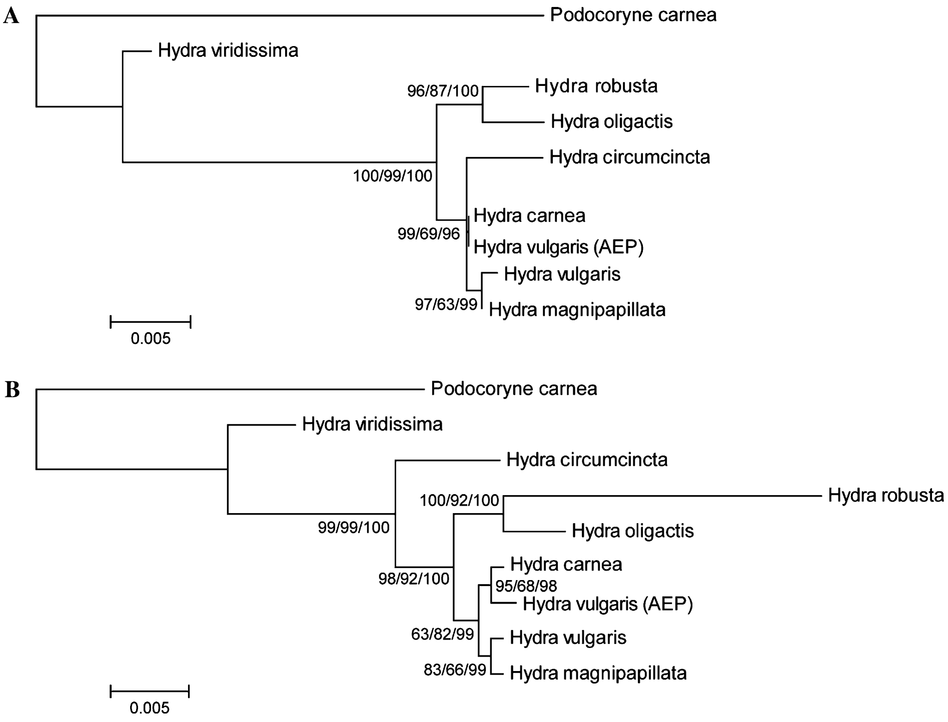

Fig. 3. Maximum likelihood phylogenetic trees inferred of the (A) nuclear 18s rRNA and (B) nuclear 28s rRNA gene. Bootstrap values for ML and MP criteria and Bayesian posterior probabilities (BI) are depicted at the corresponding nodes (order = ML/MP/BI). Branch lengths are scaled to the expected number of substitutions (0.005 substitutions per site).

mtDNA, phylogenetic trees of both nuclear genes strongly suggest that *H. vulgaris* (strain AEP) and *H. carnea* form a monophyletic group. Results from the MP and BI analysis support all of the affinities recovered in the ML analysis of the two nuclear genes (see Suppl. Figs. 3 and 4). The results of the ML analysis on the combined data sets including the 18S rRNA and the 28S rRNA genes is shown in Fig. 4 and indicates that *H. circumcincta* should be considered as sister species to *H. oligactis* and *H. robusta*. Taken together, in all trees *Hydra viridissima* was significantly differentiated from all the remaining species and recovered as the most basal species. *H. circumcincta* invariably resolved as the sister taxon to *H. oligactis*, *H. robusta*, *H. carnea* and *H. vulgaris* (Fig. 4). *H. vulgaris* strain AEP clusters with *H. carnea* rather than with *H. vulgaris* (Basel strain).

3.3. Phylogenetic inference using morphological characteristics fails to group H. vulgaris (strain AEP)

The compelling and surprising molecular evidence that *H. vulgaris* (strain AEP) is most closely related to *H. carnea* and not to *H. vulgaris* or *H. magnipapillata* prompted us to re-examine morphological characteristics traditionally used for identification purposes within the genus *Hydra*. Beside characters such as general morphology and the order in which tentacles arise on young buds, one of the few diagnostic and reliable features used to classify *Hydra* species is the shape and size of nematocysts (Campbell, 1983). We, therefore, examined the nematocysts in *H. vulgaris* (strain AEP) and compared them to the nematocysts in *Hydra carnea* and the other frequently used species. As shown in

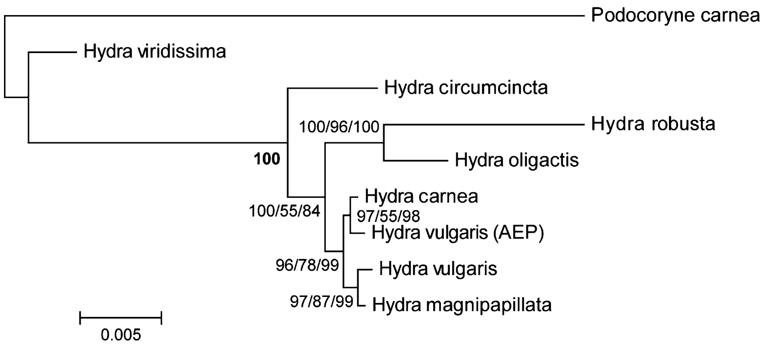

Fig. 4. Maximum likelihood phylogenetic analysis of the combined nuclear dataset, containing 18s and 28s rRNA genes. Numbers near the nodes indicate bootstrap values of the ML and MP analyses as well as the Bayesian posterior probabilities (order = ML/MP/BI). Single values in bold letters indicate the identical result in all three analyses. The branch length indicator displays 0.005 substitutions per site.

Fig. 5, on the basis of the size and shape of the nematocysts it is impossible to distinguish *H. vulgaris* (strain AEP) from the other three species of the "vulgaris" group (*H. vulgaris*, *H. magnipapillata*, *H. carnea*). Other characters such as body form, the order in which tentacles arise, pigments in the epithelium, the mode of sexual reproduction (hermaphroditic versus dioecious), and the genome size also do not allow to assign *H. vulgaris* (AEP) to either *H. vulgaris* or

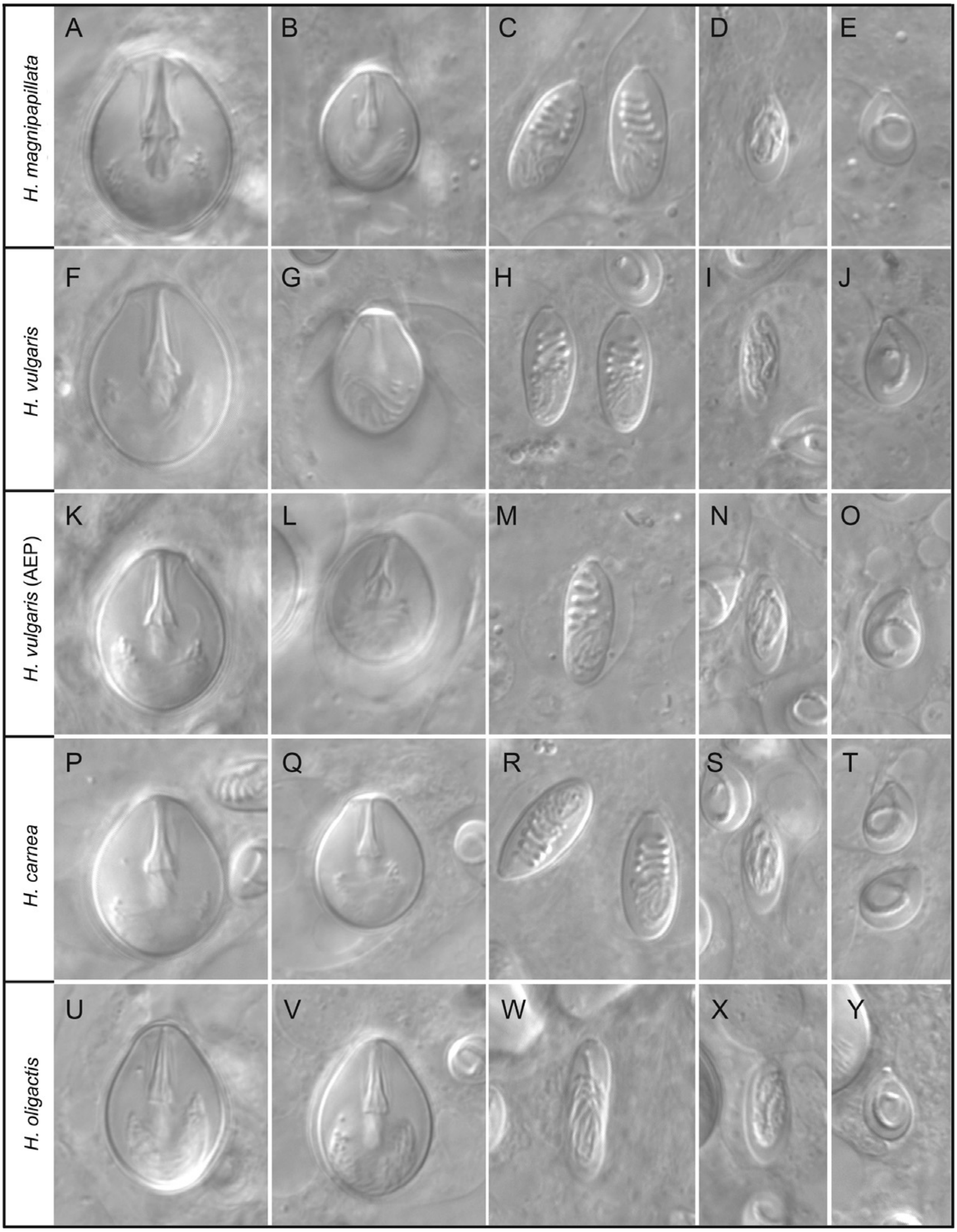

Fig. 5. Nematocysts of different species/strains of the "vulgaris-group" and *Hydra oligactis*. (A–E) *Hydra magnipapillata*; (F–J) *Hydra vulgaris*; (K–O) *Hydra vulgaris* (AEP); (P–T) *Hydra carnea*; (U–Y) *Hydra oligactis*; (A,B,F,G,K,L,P,Q,U and V) = stenotels; (C,H,M,R and W) = holotrichous isorhizas; (D,I,N,S and X) = atrichous isorhizas; (E,J,O,T and Y) = desmonemes.

H. carnea. Thus, while morphological evidence is not informative to infer the phylogenetic position of *H. vulgaris* (strain AEP), molecular evidence strongly suggests, that it is most closely related to *H. carnea*. The initial description of this new strain as a strain of the *H. vulgaris* species (Martin et al., 1997; Technau et al., 2003) obviously was affected by the lack of molecular data.

4. Discussion

Cnidaria branched off the metazoan tree before Urbilateria came into existence. Surprisingly, they appear to share a large part of their gene repertoire with vertebrates (Kortschak et al., 2003; Technau et al., 2005). For that reason, Cnidaria are a good choice for comparative evolutionary research. Several species of the freshwater cnidarian genus *Hydra* are classical model systems for evolutionary developmental biology. Since little was known about the phylogenetic affinities of these *Hydra* species, and since the advent of genomics and transgenics requires such information, we were determining the phylogenetic history of this group. We were not covering all species of *Hydra*, but focused on those species and strains that are most widely used in research. The close similarity of the trees obtained using three different methods—maximum likelihood, maximum parsimony and Bayesian inference—and using both two mitochondrial and two nuclear genes strongly suggests that our phylogenetic estimates are robust. Fig. 6 shows the reconstructed phylogeny of commonly used *Hydra* species and strains, which is concordant with morphological characters previously used for distinguishing between the *Hydra* species.

presence of symbiotic algae	+	-	-	-	-	-	-	-
presence of stalk	-	-	+	+	-	-	-	-
tentacle length/ body length	similar or shorter	similar or shorter	much longer	much longer	longer	longer	longer	longer
tentacle formation in buds	synchronously	synchronously	asynchronously	asynchronously	synchronously	synchronously	synchronously	synchronously
mode of sexual reproduction	hermaphroditic	hermaphroditic	dioecious	dioecious	dioecious	dioecious	dioecious or hermaphroditic	dioecious
genome size (Mbp)	380	1150	n.d.	1450	1350	1100	1250	1290

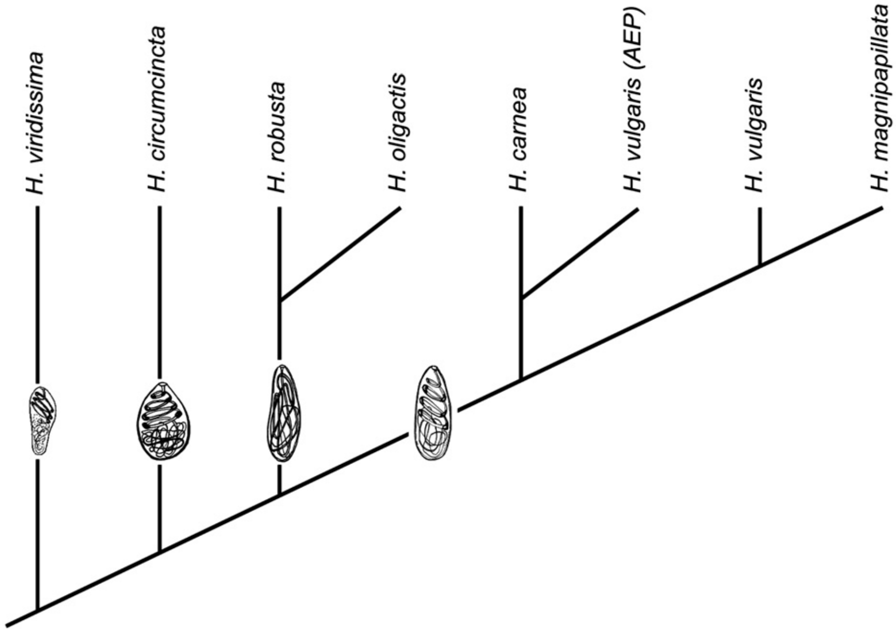

Fig. 6. Summary of phylogenetic relations within the genus *Hydra* including molecular and morphological data. Schematically depicted in the branches are holotrichous isorhizas of the different groups.

4.1. *Hydra viridissima*, the phylogenetic oldest extant *Hydra* species

Our data strongly support a basal position of *H. viridissima*. Two major characters distinguish this species from the other *Hydra* species: (1) *H. viridissima* has the smallest genome (380 Mbp) of all *Hydra* species examined so far (Zacharias et al., 2004). For comparison, *H. oligactis* possesses a large genome of 1450 Mbp, followed by a similar 1350 Mbp genome in *H. carnea*, 1250 Mpb in *H. vulgaris* (strain Basel) and 1150 Mbp in *H. circumcincta*. Interestingly, while the number of chromosomes is identical in all investigated *Hydra* species examined so far ($2n = 30$), the size of the chromosomes is strictly correlated to the size of the genome with *H. viridissima* having conspicuously small chromosomes (Zacharias et al., 2004). Since there is experimental evidence that within the "vulgaris group" some gene families are much more complex than in *H. viridissima* (Thomsen and Bosch, 2006), it is tempting to speculate that the evolutionary history of *Hydra* included one or more genome duplication events.

(2) *Hydra viridissima* is the only *Hydra* species known to form permanent and stable symbiotic associations with photosynthetic unicellular *Chlorella* algae. The symbionts are located in endodermal epithelial cells enclosed by an individual vacuolar membrane (Muscatine et al., 1975) resembling a plastid of eukaryotic origin. The pioneering studies by Rahat and Reich (1984, 1985, 1986) showed that there is a great deal of adaptation and specificity in this symbiotic relationship. We recently proposed, that an ascorbate peroxidase encoding gene, HvAPX1, which is expressed in *H. viridissima* exclusively during oogenesis, has been translocated during metazoan evolution from a plant symbiont to the *Hydra* genome (Habetha and Bosch, 2005). We have suggested (Habetha and Bosch, 2005) that the assumed gene transfer happened early in evolution from an ancient symbiont, which got lost and was replaced in *H. viridissima* by *Chlorella*. A symbiotic relationship with algae is under natural conditions observed only within *H. viridissima*; transient symbiosis, however, can experimentally be induced also in non-symbiotic *Hydra* (Rahat and Reich, 1984, 1985, 1986) indicating that the ability to form a symbiosis is a common feature of the *Hydra* group. Moreover, the APX gene is present and expressed not only in green but also in non-symbiotic species and there may have taken on a different function (Habetha and Bosch, 2005). Assuming the placement of *H. viridissima* in our molecular phylogenetic analyses at the base of the *Hydra* species is correct, a symbiotic polyp, therefore, may best represent a common ancestor. Whether the other species of *Hydra* have lost the intimate interaction with the symbiotic algae or were never involved in such an association is not known.

4.2. The one-genus/four-groups issue

Hydra taxonomy is in an unsettled state (Campbell, 1987, 1989). There are four Hydra "groups" to which polyps generally are referred to. Each "group" contains several species. While some authors suggest that there is only one genus *Hydra* with four groups (Campbell, 1987; Holstein, 1995), others proposed that the four groups should be considered as minimum three independent genera (Stepanjants et al., 2000; Anokhin, 2002, 2004). In general, our molecular analysis supports the current taxonomy (*sensu* Campbell, 1987) and presence of four distinct groups of *Hydra* species. The molecular data set, however, gives little support for the suggestion that these groups should be considered as independent genera. Addition of all related taxa will be required to determine whether the species within one "group" actually do form a monophyletic group and thus resolve the question of whether these groups represent independent genera or not.

4.3. Phylogenetic placement of *Hydra vulgaris* (strain AEP)

To exploit the genomic resources available in *Hydra* for functional studies we recently succeeded in producing transgenic *Hydra* (Wittlieb et al., 2006). The method involved microinjection of plasmid DNA containing a GFP gene driven by a *H. vulgaris* promoter in embryos of a *Hydra* strain described by Martin et al. (1997) as *H. vulgaris* (strain AEP). This strain differs from all other *Hydra* species by the continuous production of male and female gametes. This method meanwhile is widely used for in vivo tracking of labelled cells but also for functional analysis of various developmental control genes. For correct interpretation of the results obtained using this strain it is crucial to know the phylogenetic affinity between the transgenic host and the species in which the gene originally was identified and taken from. To our surprise, the molecular data presented here provide compelling evidence that *H. vulgaris* strain AEP is most closely related to *H. carnea* and may actually belong to this species. We note that previous Southern blot hybridizations carried out with DNA from various *Hydra* species using a probe specific for the PPOD gene family (see Fig. 3 in Thomsen and Bosch, 2006) already indicated that *H. vulgaris* (strain AEP) is closely related to *H. carnea* and significantly different from *H. vulgaris* . The fact that morphological characters do not allow to place this strain undoubtedly in one of the species of the "vulgaris" group highlights the benefits of molecular phylogenetics as a tool to determine the affinities between closely related species.

5. Conclusion

The results presented in this study represent a preliminary phylogenetic analysis of the *Hydra* species most commonly used in current research. Although the work clarifies some of the evolutionary relationships and establishes a solid foundation for future investigations, data from other *Hydra* species are needed to fully understand the evolutionary history and speciation of this fascinating group of basal metazoans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.10.031

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