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Phylogeny and biogeography of *Hydra* (Cnidaria: Hydridae) using mitochondrial and nuclear DNA sequences

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ABSTRACT

The polyp hydra is ubiquitous in freshwater and is highly variable, with many species names assigned to different strains. Types of hydra do fall into four morphologically recognizable groups but many of the species determinations are confusing. To assess the diversity of hydra we collected 101 strains from six continents and built a phylogeny using three genetic markers. Each of the four well-defined groups of species represents a clade in our phylogeny. The green hydra group diverged first, followed by the braueri group and finally the sister groups vulgaris and oligactis. Each of eight species easily definable by morphological criteria represents a distinct clade in our phylogeny. Hydra of two clades, the green and the vulgaris hydra, are found on all continents (except Antarctica) and many islands, whereas hydra of the other two groups (braueri and oligactis) are restricted to the Northern Hemisphere. Our best estimate of the time of origin of hydra is about 60 Ma, long after the breakage of Pangea into northern and southern landmasses. Hydra appear to have diversified in the Northern Hemisphere, and their current diversity is greatest here. Two species were then able to disperse to the Southern Hemisphere, perhaps due to their thermal tolerance.

1. Introduction

Since Abraham Trembley's experimental studies of the unusual properties of the freshwater *Hydra* (Trembley, 1744), the polyp has been used in many different fields of biology and the genome of one species has been recently assembled (Chapman et al., 2010). Yet, the taxonomy and phylogenetic relationships of the species within the genus *Hydra* have always been uncertain (Ewer, 1948) and the distributions of species of hydra throughout the world are not well known. Although the individual species themselves have been difficult to define, hydra clearly fall into four groups, a notion initiated by Schulze (1917) and refined by Semal-van Gansen (1954) and Campbell (1987). These groups can be distinguished morphologically on the basis of the nematocysts (stinging organelles) alone, as well as on the basis of the embryothecae alone. Hydra of the viridissima group ("green hydra") are green due to intracellular symbiotic algae, have the smallest nematocysts of any hydra (stenoteles 10 μm) and embryothecae covered with a cobbled surface. The braueri group ("gracile hydra") includes hydra with pyriform (broadly oval) holotrichous isorhiza nematocysts and embryothecae flattened against a substratum. Hydra of the oligactis group ("stalked hydra") have stenotele nematocysts of uniform size and smooth, spherical embryothecae. The vulgaris group ("common hydra") includes hydra with stenotele nematocysts variable in size, usually about 14–20 μm, holotrich isorhizas narrowly oval and embyothecae with spines. A number of described species fit into each of these four groups although it is unclear how many of these are valid species because some are synonymous and species boundaries are uncertain (Ewer, 1948; Slobodkin and Bossert, 1991).

The taxonomy of hydra is centered on Eurasian and North American specimens (Chen and Wang, 2008; Forrest, 1963; Holstein, 1995). Hydra are reported from all continents except Antarctica (Jankowski et al., 2008). But of the dozen or so species described from Africa, Oceania and South America, most are indistinguishable and based on single collections with little information about wider distributions. Given that so little is known of hydra in the Southern Hemisphere it is of interest to know their relationships to those of the Northern Hemisphere. Since hydra are strictly freshwater, presumably limiting dispersal across oceans, a strong phylogeographical structuring within species is expected. However, hydra might also be expected to disperse as humans transport fish and aquatic plants around the world.

Hydra from the braueri, oligactis, and vulgaris groups generally do not carry algal endosymbionts and they are referred to as brown hydra. Previous phylogenetic studies (Collins et al., 2005, 2006;

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Collins, 2000; Hemmrich et al., 2007) suggest that brown and green hydra are sister groups but too few species were sampled for clear conclusions.

In this study, we sequenced three genetic markers from hydra collected from around the world and constructed molecular phylogenies to investigate the following questions: (1) Is the existence of four morphological groups supported by the molecular data? (2) How are the four groups related? (3) Do green and brown hydra represent sister taxa? (4) Can we distinguish between species within each group? (5) What is the global distribution of groups and species? (6) Is there evidence of anthropogenic dispersal of hydra? (7) What is the time of divergence between major groups?

2. Materials and methods

2.1. Biological material and morphological analyses

Most hydra were collected in natural areas by the authors. Typically they were brought live into the laboratory and cultured for a few weeks before processing. Some were fixed in 95% alcohol in the field. Some strains of hydra were provided by other investigators (Supplementary material S1).

2.2. DNA extraction, PCR amplification and sequencing

DNA was typically extracted from 8 to 15 live individuals (clonally derived from a single hydra) using the Qiagen DNeasy tissue kit following the manufacture's protocol for animal tissues with a single final elution with 150 μl of buffer AE. Live animals were starved for at least 3 days before DNA extraction to ensure that food had been digested. When only alcohol-fixed animals were available, DNA extractions were performed using a single individual and the Qiagen Dneasy tissue kit. In a few cases DNA was extracted from 1 or 2 live animals using the following protocol: incubation at 60 °C for 1 h in lysis buffer (50 mM KCl, 2.5 mM $MgCl_2$, 10 mM, Tris–HCl pH 8.3, 0.45% TWEEN 20, 0.01% gelatin, 1 mg/ml proteinase K) and at 94 °C for 15 min to inactivate proteinase K.

The complete internal transcribed spacer 1 (ITS1), 5.8S rDNA, and ITS2 region of the nuclear ribosomal DNA was amplified using primers designed to anneal to the 18S (5′-CACCGCCCGTCGCTAC TACCGATTGAATGG-3′) and 28S (5′-CCGCTTCACTCGCCGTTACTAG GGGAATCC-3′) ribosomal genes. A fragment of approximately 589-bp of the mitochondrial large ribosomal RNA (16S rDNA) gene was amplified using the following primers: forward, 5′-TCGAC TGTTTACCAAAAACATAGC-3′; reverse, 5′-ACGGAATGAACTCAAATC ATGTAAG-3′. A 655-bp fragment of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene was amplified using the following primers: forward, 5′-GGTCAACAAATCATAAAGATATTGGAAC-3′ (CO1.Dawson.F.LCOjf); reverse, 5′-TAAACTTCAGGGTGACCAAAAA ATCA-3′ (CO1.Folmer.R.HC02198).

All PCR reactions were performed in a final volume of 50 μl using a Promega Master PCR mix (10× PCR buffer, 10 mM dNTP, 50 mM Mg_2Cl) with 0.5 μM primers and 0.02 unit/μl Taq DNA polymerase. PCR for ITS1-5.8S-ITS2 and 16S rDNA amplifications included an initial step of denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 1 min at 55 °C (47 °C for 16S rDNA), and extension for 1 min 30 s at 72 °C (1 min for 16S rDNA). A final extension step was carried out for 10 min at 72 °C. PCR for CO1 amplifications included an initial cycle of denaturation at 94 °C for 5 min, annealing for 2 min at 45 °C, and extension for 2 min at 72 °C, followed by a cycle of denaturation at 94 °C for 3 min, annealing for 2 min at 46 °C, and extension for 2 min at 72 °C, followed by 33 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 47 °C, and extension for 1 min 30 s at 72 °C. A final extension step was carried out for 5 min at 72 °C.

PCR products that produced a well-defined band on a 1% agarose gel were directly sequenced in both directions without purification using the PCR primers as sequencing primers. Typically, however, PCR products were ligated into pGEM T® easy vector (Promega, Madison, WI) and used to transform 50 μl of competent *Escherichia coli* (DH5α). Transformed cells were plated on LB/Ampicillin/Xgal plates and grown overnight at 37 °C for a blue–white screening. A few selected white colonies were grown overnight in 3 ml LB liquid medium. Plasmids from these cultures were purified using a QIAprep Spin Miniprep Kit (Qiagen). The length of the cloned fragments was verified by restriction digest with EcoR1 and 1% agarose electrophoresis. Only one clone per hydra strain was sequenced in both directions using T7 and Sp6 primers (Promega). Multiple copy genes like the ITS region of nuclear ribosomal DNA may exhibit high levels of intra-individual variation (e.g. Márquez et al., 2003). This variation has been eliminated from our study by our decision to sequence a single clone per PCR product for each hydra strain. However, in several cases in which we sequenced PCR products directly, no evidence of intra-individual variation was observed. Thus, we believe that the level of intra-individual variation for ITS in hydra is unlikely to be of sufficient magnitude to affect our results.

2.3. Phylogenetic analysis

All sequences were assembled and edited using Sequencher 4.6 (Gene Codes Corporation). Edited sequences of the ITS1-5.8S-ITS2 region were aligned using ClustalX (Thompson et al., 1997, 1994) with Multiple Alignment Parameters as follows: Gap Opening Penalty: 10.00, Gap Extension Penalty: 0.20 and equal weight for transitions and transversions. This alignment was further improved by eye using the software SeAl (Rambaut, 2002). Edited 16S and CO1 sequences were unambiguously aligned by eye using SeAl.

Phylogenetic analyses (Maximum Parsimony and Neighbor Joining) were conducted using PAUP* (Version 4.0b8, Swofford, 2002). Bootstrap values were calculated as follows: Maximum Parsimony: full heuristic search, 1000 replicates, stepwise-addition random with 10 replicates, branch swapping TBR; and Neighbor-Joining: 1000 replicates. Maximum Likelihood (ML) was implemented using GARLI (Zwickl, 2006). The appropriate ML model (corresponding to a GTR+I+G model) was selected using the AIC (Akaike Information Criteria) implemented by jModeltest (Guindon and Gascuel, 2003; Posada, 2008) and the estimated parameters were used for calculating branch lengths. Bootstrap values were calculated based on 1000 replicates.

The hypothesis of monophyly of green and brown hydra was assessed using a Maximum Likelihood phylogeny of 16S rDNA and CO1 sequences. *Ectopleura larynx* (Tubulariidae), *Ralpharia gorgoniae* (Tubulariidae) and *Corymorpha intermedia* (Corymorphidae), and *Candelabrum cocksii* (Candelabridae) were used as outgroups. The selection of these outgroups was based on a study by Collins et al. (2006) that suggests that Hydridae are closely related to species of Tubulariidae, Corymorphidae, and Candelabridae. These four taxa constitute the clade Aplanulata characterized by direct development from egg to polyp without a ciliated planula larva. This 16S-CO1 phylogeny was also used to calculate the divergence times between the four major groups of hydra. The appropriate ML model (corresponding to a GTR+I+G model) was selected using the AIC (Akaike Information Criteria) implemented by jModeltest (Guindon and Gascuel, 2003; Posada, 2008) and the estimated parameters were used for calculating branch lengths. Starting branch lengths were obtained using the Roger–Swofford approximation method and branch-length optimization was done using the Newton–Raphson method with a maximum of 20 smoothing

passes and a threshold (delta) of 10^{-6}. To calculate the earliest possible divergence time between two groups, we selected from each group the hydra strain with the longest branch to the node representing the common ancestor between the two groups being considered. We also estimated the most recent divergence times using the strains with the shortest branches to the ancestor node. Substitution rates for 16S + CO1 calculated for the marine hydroid *Obelia geniculata* and based on the comparison between North Atlantic and Japanese populations (Govindarajan et al., 2005) were used for our calculations. Two values calculated for *O. geniculata* and based on two estimates (3.1 and 4.1 Ma) for the opening of the Bering Strait, were used: a minimum of 4.63×10^{-9} substitutions site^{-1} year^{-1}, and a maximum of 6.13×10^{-9} substitutions site^{-1} year^{-1}.

3. Results

One hundred and one strains of hydra from six continents were identified as representing all four groups of species and eight major species (Supplementary material S1). All green hydra were identified as *H. viridissima* and all hydra of the vulgaris group were considered to be *H. vulgaris* because published descriptions do not permit further distinction. The other species studied were *H. oligactis*, *H. oxycnida* and *H. canadensis* of the oligactis group, and *H. circumcincta*, *H. hymanae* and *H. utahensis* of the braueri group. The hydra taxa sampled for this study likely represent the extant breadth of hydra diversity.

The four groups of species were found widely distributed in Eurasia and North America. Only two groups of species (viridissima and vulgaris) were found in Africa, Oceania and South America, and these two groups were widely distributed throughout these regions.

Maximum Parsimony, Neighbor-Joining (NJ), Maximum Likelihood (ML) supported the monophyly of each of the four groups of hydra and also the monophyly of each of the eight morphologically defined species (Fig. 1). Our analyses recover several distinct clades of *H. viridissima*. Within the braueri group, there are four different clades: one corresponding to all *H. hymanae*, another to all *H. utahensis* (both North American species) and two to *H. circumcincta* (Eurasian). *H. hymanae* is basal to *H. utahensis* and *H. circumcincta*. Interestingly, both clades of *H. circumcincta* include representatives from North America (Alaska).

Within the oligactis group, *H. oligactis* is widely distributed both in Eurasia and North America and is basal to both *H. oxycnida* from Europe and *H. canadensis* from North America, which are sister taxa.

Many species have been described in the vulgaris group but these are not distinct morphologically. Our study resolves several clades of *H. vulgaris* that correspond to geographical locations: Eurasia, North America, South America, Oceania (two) and South Africa. Even though each of these clades is well supported, the order of divergence between them is not clear since most deep nodes do not have strong support. European and Asian hydra of the vulgaris group form one clade with relatively little genetic variation. Two distinct groups with relatively high support are apparent within the North American clade and three groups are apparent in the South American clade. Several strains appear in clades that do not match their geographic origin. In these cases, the strains are virtually identical to other strains within the clades and probably represent recent dispersal events including anthropogenic introductions.

A subset of the strains representing each of the eight morphologically defined species of hydra was used to assess the monophyly of green and brown hydra and to estimate the times of divergence between the major groups of hydra. Maximum Parsimony, Neighbor-Joining, and Maximum Likelihood using mitochondrial CO1 and 16S data support the monophyly of each of two major hydra groups (Fig. 2). The estimated range of divergence times between green and brown hydra was 64–45 Ma (Table 1). These estimates were based on substitution rates for 16S + CO1 calculated for the marine hydroid *O. geniculata* (Govindarajan et al., 2005). The substitution rates in *Obelia* are comparable to the ones determined for other invertebrates and higher than the rates reported for Anthozoans (Govindarajan et al., 2005, and references therein).

4. Discussion

The taxonomy of hydra is confusing due to the large number of species that have been described, many before dependable taxonomic characters were established (Holstein et al., 1990). Over 80 species names have been proposed. Grayson (1971) considered 27 species as distinct, but as the variability of hydra is becoming increasingly clear, a recent estimate of valid species is 12–15 (Jankowski et al., 2008). Uncertainty in the number of species depends almost entirely on how much variation one accepts within species of the vulgaris group. Species do cluster into four species groups, however, and these are readily recognized morphologically. Our results provide support for this grouping since each group appears as a highly supported clade. The topology of our tree (Fig. 2) indicates that the viridissima (green hydra) group diverged first and that the brown hydra groups share a common ancestor. Within the brown hydra, the braueri group (with broadly oval holotrichous isorhizas) is a sister taxon to the clade formed by oligactis and vulgaris groups (both with narrowly oval holotrichous isorhizas).

The green hydra form a long-recognized group, set apart by Schulze (1914) in the genus *Chlorohydra*. They not only have a special color imparted by the intracellular algae, but they also have a distinctive cobbled surface to the embryotheca. Green hydra also have conspicuously small cells, nematocysts, and genome. Four species of green hydra have been described: *H. viridissima* (Pallas, 1766) from Europe, *H. hadleyi* (Forrest, 1959) from North America, *H. plagiodesmica* (Dioni, 1968) from South America and *H. sinensis* (Wang et al., 2009) from China. Their descriptions do not distinguish clearly between them and the validity of the species has been questioned (Campbell, 1987; Grayson, 1971). We find several distinct clades of green hydra and the relation of these to taxonomic species remains to be determined.

The braueri group was recognized by Hansen-Melander (1948) and Semal-van Gansen (1954). This distinctive group includes hydra that have broad holotrichous isorhiza nematocysts and flattened embryothecae. Our analysis reveals four distinct clades within the braueri group. The most basal clade represents the North American species *H. hymanae* (Hadley and Forrest, 1948). Another clade corresponds to *H. utahensis* (Hyman, 1931b), also North American. The two remaining clades are assigned to *H. circumcincta* (Schulze, 1914), a species that has only been reported from Eurasia (Holstein, 1995). Interestingly, both of these clades of *H. circumcincta* included representatives from the northwestern tip of North America (Alaska) in addition to the European strains. Thus, *H. circumcinta* should be considered a circumboreal rather than Eurasian species. Whether or not these two clades of *H. circumcincta* correspond to two distinct species requires further investigation. Numerous other species of the braueri group have been described from North America and Eurasia, but these are not clearly distinct from the species listed here.

Hydra of the oligactis group, for which Schulze established the genus *Pelmatohydra*, are distinguished from other non-green hydra by their smooth spherical embryothecae and by the fact that the stenotele nematocysts are of uniform size. Our study showed three distinct clades within this group corresponding to *H. oligactis* (Pallas, 1766) (the most basal), *H. oxycnida* (Schulze, 1914) and *H.*

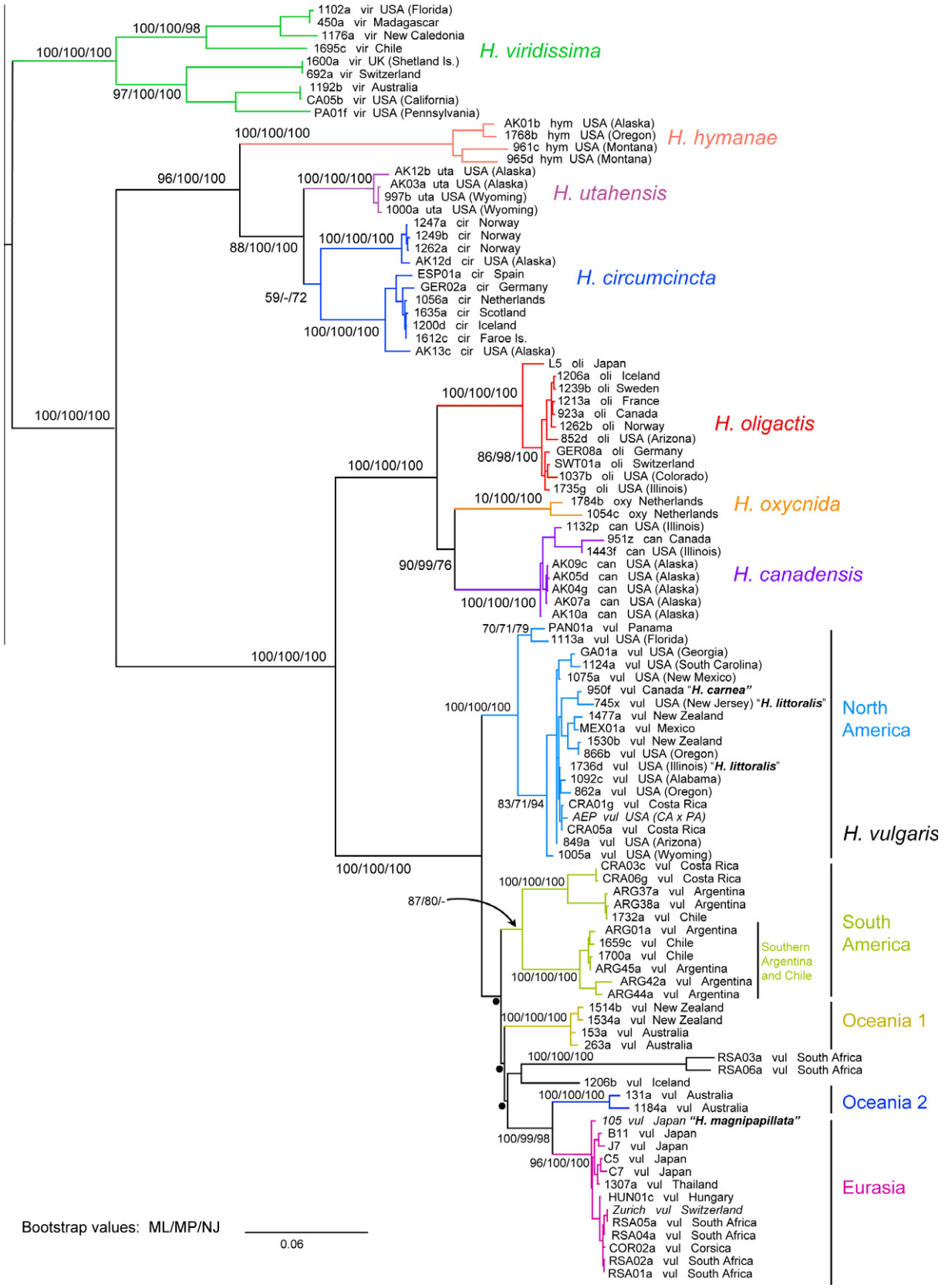

Fig. 1. Maximum Likelihood unrooted phylogram of 101 strains of hydra based on ITS1-5.8S-ITS2 region of rDNA, mitochondrial 16S rRNA gene and mitochondrial CO1 gene (GenBank accession numbers in Supplementary material S1). Strain names followed by species assignment based on morphology followed by country of origin. *can*: *Hydra canadensis*; *cir*: *H. circumcincta*; *hym*: *H. hymanae*; *oli*: *H. oligactis*; *oxy*: *H. oxycnida*; *uta*: *H. utahensis*; *vir*: *Hydra viridissima*; and vul: *H. vulgaris.* Numbers at nodes are bootstrap values for Maximum Likelihood (ML), Maximum Parsimony (MP) and Neighbor Joining (NJ). Black circles identify deep nodes without support.

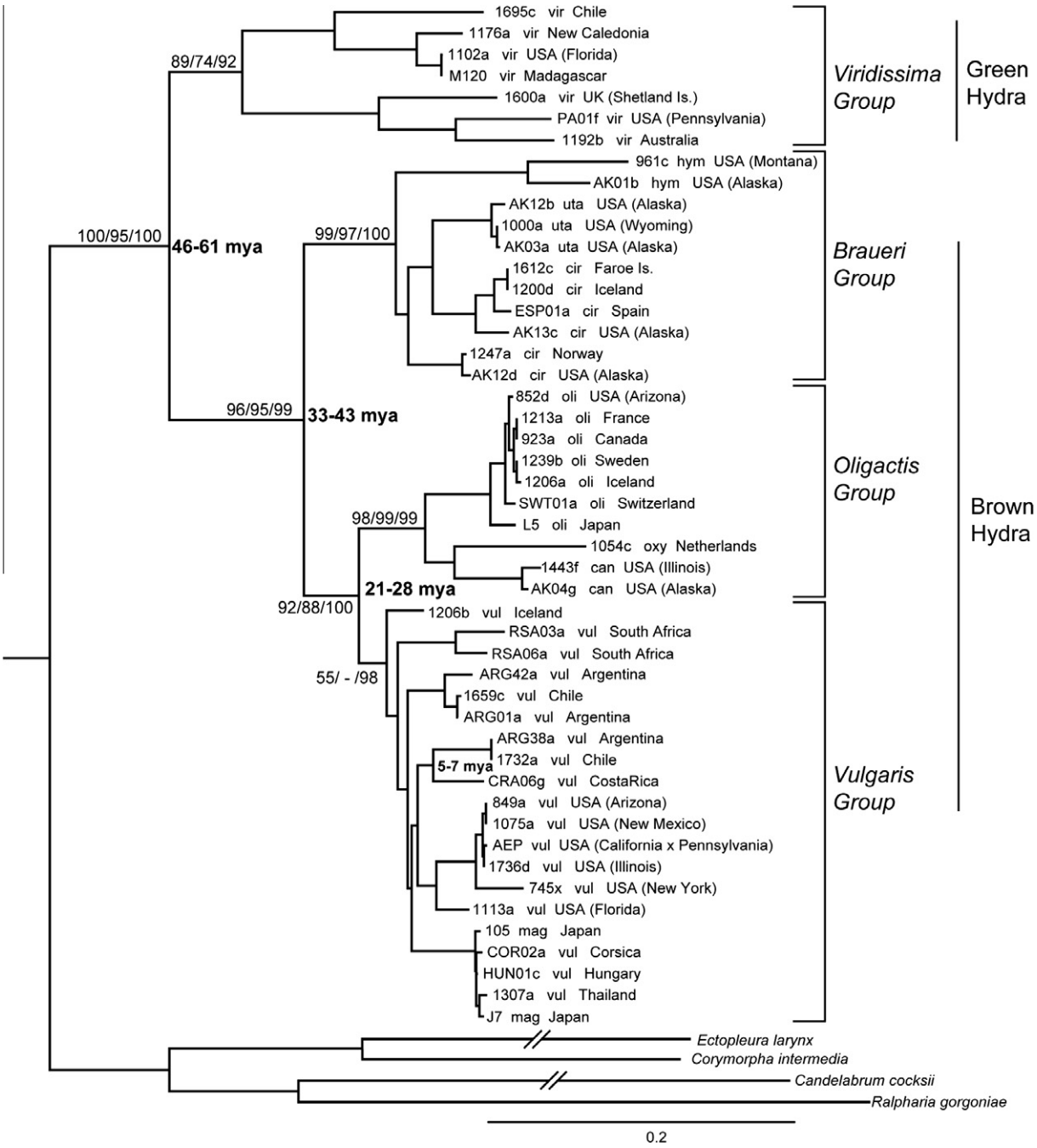

Fig. 2. Maximum Likelihood phylogram of 48 strains of hydra based mitochondrial 16S rRNA gene and mitochondrial CO1 gene. Tree rooted using *Ralpharia gorgoniae* (Voucher: KUNHM2778; 16S: EU305482; COI: GU812437); *Corymorpha intermedia* (Collins et al., 2006; 16S: AY512526; CO1: GU812436) (Collins et al., 2006; 16S: AY512526; CO1: GU812436); *Candelabrum cocksii* (Voucher: MHNGINVE29591; 16S: EU876529; CO1: GU812438); and *Ectopleura larynx* (Collins et al., 2006; 16S: AY512523; CO1: GU812435). Numbers at nodes are bootstrap values for Maximum Likelihood, Maximum Parsimony and Neighbor Joining. Divergence times (in boldface) between major groups of hydra species were calculated using substitution rates estimated for 16S + CO1 for the marine hydroid *Obelia geniculata* (Table 1).

canadensis (Rowan, 1930). *H. oligactis* is widely distributed both in Eurasia and North America. Very little sequence divergence was observed between the European and North American strains which appear intermixed in our tree (Fig. 1). The single Asian strain included in the analysis appears as a sister taxon of the European–North American group. The European species *H. oxycnida* and the North American species *H. canadensis* were recovered as sister taxa by our analyses. Both species comprise large hydra without the distinct stalk that is typical of *H. oligactis*.

The vulgaris group is a distinctive set of hydra with conspicuous spines on the embryothecae and mainly slender holotrichous isorhizas. A large number of species from around the world have been described. These hydra are variable and on the basis of their descriptions we cannot distinguish them from the species that has taxonomic priority, *H. vulgaris* (Pallas, 1766). In our tree they comprise several well-supported clades that correspond to geographic areas: North America, South America, Oceania, South Africa and Eurasia. The order of divergence of the lineages within the vulgaris group remains indeterminate because several of the deeper nodes of our tree lack support (Fig. 1).

North American hydra of the vulgaris group have been described as five species: *H. americana* (Hyman, 1929), *H. carnea*

Table 1
Divergence times between major groups of *Hydra* species calculated using substitution rates estimated for 16S + CO1 for the marine hydroid *Obelia geniculata*.

Groups	Strains	Time of divergence[A] (Ma)
Green–Brown	1192b-961c (M120-1206b)	61–46 (38–29)
Braueri–Oligactis/Vulgaris	961c-1054c (1247a-1206b)	43–33 (20–15)
Oligactis–Vulgaris	1054c-745x (852d -1206b)	28–21 (15–12)

[A] Calculated from strain pairs with the longest (and shortest) branch lengths.

(Agassiz, 1850), *H. cauliculata* (Hyman, 1938), *H. littoralis* (Hyman, 1931b), *H. rutgersensis* (Forrest, 1963). These species are not clearly distinguishable on the basis of their descriptions. Two species, *H. littoralis* and *H. carnea*, are reliably obtained on the basis of their stable populations at the type localities (Hyman, 1931a,b). They represent two extremes of morphological variability within the vulgaris group. Strain 950f in our tree is *H. carnea* and strains 745x and 1736d are *H. littoralis*. They appear tightly clustered in the tree, suggesting that the morphological differences in the vulgaris group do not correspond to taxonomic speciation.

Our study indentified two distinct clades within the North American vulgaris but they do not correspond to described species. One of the clades includes two strains, one from Florida and another from Panama. The other clade shows very little genetic differentiation and includes several strains from the United States and Mexico including the strains identified as *H. carnea* and *H. littoralis* mentioned above. This clade also includes strain AEP (produced by a cross between hydra from Pennsylvania and California which morphologically resembled *H. littoralis* and *H. carnea*, respectively) that is currently used to generate transgenic hydra (Wittlieb et al., 2006). Based on these results we propose that most or all North American hydra of the vulgaris group represent a single, variable, widespread species.

One of the North American vulgaris clades includes hydra from Costa Rica. These strains are genetically almost identical to strains from the US and are likely to represent North American hydra introduced to Central America.

The vulgaris hydra within the South American clade are grouped according to geographical regions: Costa Rican hydra form a clade with hydra from central Argentina and Chile. Another clade includes hydra from the Patagonian regions of Argentina and Chile. Our results show the Patagonian clade as basal, which suggests that the Costa Rican hydra may have originated by dispersal from South America. The estimated time of divergence of the Costa Rican hydra from their sister taxa in Argentina and Chile is 5.8–7.7 Ma (Fig 2). This date is consistent with the emergence of the Panama Isthmus that occurred 3.0–3.1 Ma (Cronin and Dowsett, 1996). One would expect hydra from northern South America to be more closely related to Costa Rican hydra and thus reflect divergence dates closer to the formation of the Panama Isthmus. Additional sampling in northern South America would help to clarify this point.

Vulgaris hydra from Oceania appear in two well-supported clades that do not group together. One clade has clear affinities with Eurasian hydra and includes hydra from Australia. The other clade has no clear affinities and includes both Australian and New Zealand hydra. In addition, there are two strains of hydra from New Zealand that appear deeply nested within the North American clade. This is likely to be the result of an unintended introduction of North American hydra to New Zealand, perhaps in conjunction with the introduction of trout. North American rainbow trout eggs were introduced into the North Island of New Zealand in 1883 reaching the South Island by 1888 (Crowl et al., 1992). The main source of rainbow trout eggs at that time was a hatchery on the McCloud River, California, that was run by the US Fish Commission. Under this scenario, one would expect the introduced New Zealand strains to resemble North American hydra from the western United States. In fact, these New Zealand strains cluster together with hydra from Oregon and Northwestern Mexico.

A well-supported clade includes all vulgaris hydra from Eurasia. Hydra from this clade were first described as *H. vulgaris* (Pallas, 1766), and have often been called *H. vulgaris* if collected in Europe (Holstein, 1995), *H. orientalis* if collected in India (Annandale, 1906), and *H. magnipapillata* if collected in Japan (Itô, 1947) as well as a host of other names. The group includes strains that have been used as experimental model organisms for many years: the Japanese strain 105 was used for the hydra genome project (Chapman et al., 2010) and the European strain "Zurich" has been a workhorse for modern experimental work. Our results indicate that there is very little genetic diversity within this group (e.g. the proportion of sites differing for the ITS region between the 105 and Zurich strains is 9/980 = 0.0092).

Four strains of hydra collected from South Africa also appear in the Eurasian clade. Given that these hydra are virtually identical to European hydra for the three markers studied here, these strains may represent hydra introduced to South Africa from Europe. Interestingly, three of these four African strains were collected from man-made ponds. Two additional strains collected from two South African rivers form a unique, well-supported clade with unclear affinities. These strains probably represent native African hydra. The grouping of an Icelandic strain with two South African hydra has no support and is likely to be an artifact due to long branch attraction.

The estimated range of divergence times between green and brown hydra, 64–45 Ma (Table 1), puts the root of the hydra radiation at the Paleocene (65–55 Ma). By this time the continents of the Southern Hemisphere had separated from the northern Laurasian supercontinent. Europe was still connected to Greenland, and North America was intermittently connected to Asia and beginning to separate from Greenland (Milne, 2006). The notable absence of two of the four hydra groups from continents of the Southern Hemisphere suggests a possible biogeographic scenario for the origin and evolution of hydra. The braueri and the oligactis groups are represented in both North America and Eurasia but absent from South America, Africa and Oceania. Furthermore, both the braueri and oligactis groups have species restricted to either North America (*H. hymanae*, *H. utahensis*, *H. canadensis*) or Eurasia (*H. oxycnida*). The distribution of these species is consistent with a scenario in which hydra originated and diverged in Laurasia and only two of the four groups were able to disperse into the continents of the Southern Hemisphere. Dispersal and successful colonization of new habitats from the Northern to the Southern Hemisphere would involve transit through warm Equatorial areas. Interestingly, *H. oligactis* differs from *H. vulgaris* in that *H. oligactis* shows little tolerance for thermal stress (Bosch et al., 1988) and it is considered as a cold water species. Furthermore, unlike *H. vulgaris*, *H. oligactis* does not produce detectable levels of new proteins, including heat shock protein HSP70, in response to thermal stresses or other stresses that would be expected to trigger the heat shock response (Bosch et al., 1988; Brennecke et al., 1998; Gellner et al., 1992). Thus, *H. oligactis* may be ill prepared for dispersal across the tropics. The tropical Paleocene was 5 °C warmer and wetter than the tropics today (Heat et al., 2009; Zachos et al., 2003). An alternative hypothesis to the evolution of hydra in Laurasia is that all hydra groups were initially distributed in all continents. This hypothesis requires a much earlier radiation of the hydra and an explanation for the extinction of the braueri and the oligactis groups from the continents of the Southern Hemisphere. We cannot think of any obvious incidents in the history of Earth that could explain such extinctions. Since their initial break-up from Pangea, which begun by the Late Triassic approximately 235 Ma, the continents of the Southern Hemisphere, with

the exception of Antarctica, have occupied low and intermediate latitudes with relatively mild climates. The Quaternary (2–0 Ma) glaciations that covered large portions of North America, Europe, and Siberia, for example, were restricted to high altitudes in the Southern Continents (Mercer, 1983). In the Northern Hemisphere Quaternary glaciations were responsible for the extinction of many species and shaped the distribution of circumboreal floras that were forced to take refuge in warmer areas (e.g. Iberian and Italian peninsulas in Europe). Quaternary glaciations are unlikely to explain the extinction of hydra from the Southern Hemisphere, however. Given our results, the simplest explanation is that hydra originated and diversified in Laurasia and only viridissima and vulgaris hydra dispersed into the continents of the Southern Hemisphere probably due to their thermal tolerance.

Our study indentified several strains of hydra that appear in clades that do not match their geographic origin and show very little molecular divergence. These cases are likely to be the result of transport by humans. Many fish, aquatic invertebrates and plants are moved around the world for agricultural, sport and aquarial purposes and it is not surprising that hydra could ride undetected. Interestingly, all the potential cases of anthropogenic dispersal between the Northern and the Southern Hemisphere detected by our analysis involve either viridissima or vulgaris hydra. This could represent further evidence that viridissima and vulgaris hydra are better equipped for dispersal.

There is little evidence of hydra species occupying small geographical territories, so our wide ranging collecting of hydra has probably uncovered most types. Fig. 1 thus gives a good general picture of the relations between most hydra. The probable time of origin of hydra is late relative to the origin of the metazoans, so hydra, though simple, are not particularly old. Yet at over 60 million years of age they are old enough that their distribution has been affected by continental drift. It appears that hydra arose and diversified in the Northern Hemisphere and then two of the four groups of species managed to expand into the continents of the Southern Hemisphere. This study provides a framework that can guide future taxonomic work in hydra.

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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.2010.06.016.

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