previously grouped with the flatworms. Several lines of molecular evidence have suggested that this association is incorrect and that they diverged early from all other triploblasts. However, each one of these analyses may have been affected by the rapid evolution of the acoelomorph sequences which would tend systematically to attract them artefactually towards the base of the tree.

Finally, two independent phylogenomic analyses have shown that the chaetognaths are certainly protostomes and not deuterostomes although there is no consensus as to whether they are lophotrochozoans or diverged before the lophotrochozoan/ecdysozoan split. Two analyses of their complete mitochondrial genomes were similarly in disagreement but their protostomian *nad5* signature at least is unambiguous.

Trends and prospects

A complete understanding of the relationships of the animals is a very laudable goal in itself but the animal phylogeny also functions as a framework to further our understanding of the evolution of the animals. At least three active fields of research depend upon the new animal phylogeny; evo-devo which attempts to find the basis of changes in morphology plotted over the phylogeny; molecular clocks and the timing of divergences within the animals; and the reconstruction of features of long extinct common ancestors through the identification of homologous characters in its descendants. The most prominent 'Ur-animal' in this last category is Urbilateria, the last common ancestor of the protostomes and the deuterostomes. The logic is simply that any homologous character found in both protostomes and deuterostomes must have been inherited from Urbilateria. The problem then is to identify homologous characters and this involves the search for complex similarities, usually the involvement of orthologous patterning genes in the ontogeny of the character of interest. Likely characteristics of Urbilateria identified to date are an antero-posterior axis patterned by *Hox* genes, photoreceptors patterned by a *pax6* ortholog and, more controversially, a pumping heart and a segmented body.

Finally, one thing that is obvious from the above discussion is that many nodes of the tree are unresolved. The relationships of the diploblasts, and those within the Ecdysozoa and Lophotrochozoa are very poorly understood. One important reason for this is that most of these phyla are very poorly sampled in terms of molecular characters and so the use of phylogenomic scale datasets is a very encouraging trend in this respect. As a note of caution, however, the continued failure to resolve the position of the chaetognaths with two respectably sized EST data sets shows that this approach is not a guarantee of success.

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Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians

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Invertebrate–dinoflagellate symbioses are responsible for the high productivity and structural complexity of the coral reef ecosystem. Coral reefs around the world are in decline with much of the mortality attributed to coral bleaching — the loss of photosynthetic algal symbionts — resulting from global warming [1–3]. These algae are essential to a host's survival, but many cnidarians must acquire their symbionts, members of the genus *Symbiodinium* referred to as zooxanthellae, anew at each generation. The presence of zooxanthellate corals on reefs, and the rapid acquisition of *Symbiodinium* by newly settled polyps ('recruits') in the field [4], imply the existence of an external supply of *Symbiodinium*. '*Symbiodinium*-like' dinoflagellates have been isolated from both sand and the water column [5–8], but neither the location(s) nor the dynamics of this symbiont reservoir are known. To understand how corals may respond to current threats on local and global scales, such as overfishing and global warming, respectively, and to successfully manage and protect potential symbiont sources that may repopulate reef corals, we need to identify the location of *Symbiodinium* in the environment and, more

importantly, demonstrate that these populations are capable of establishing symbioses. Here, we confirm the presence of *Symbiodinium* within the water column and the benthos, and show that these environmentally derived isolates are able to infect cnidarian (octocoral) recruits.

Symbiont change has been proposed to occur in stressful environments [9,10], but it is unclear whether these repopulating symbionts are from a residual population within the host or from an exogenous source. '*Symbiodinium*-like' dinoflagellates have been isolated from the water column [5–7] and benthic environments [8], and phylogenetic analyses of a dinoflagellate isolated from sand on Oahu, Hawaii [8] show that this isolate (*Symbiodinium* HA3-5) is allied with Clade A, but lays outside the members of the clade known to establish symbioses with cnidarians (Figure 1). Although these 'free-living' forms fall within the genus *Symbiodinium*, or are closely allied, it remains to be established whether they are capable of a symbiotic lifestyle, an essential criterion for identifying the source of potentially repopulating symbionts.

To this end, we sampled *Symbiodinium* from the water column 20 metres above the reef using asymbiotic octocoral recruits (*Briareum* sp.) as 'symbiont sampler arrays'. *Symbiodinium* were detected within all polyps, establishing their presence in the water column. *Symbiodinium* Clades A, B and C were identified in 7%, 63% and 2% of the polyps, respectively; an additional 28% of the polyps hosted multiple clades simultaneously (for example, A+B, 23%; B+C, 5%).

We also established that *Symbiodinium* resides in benthic habitats by culturing '*Symbiodinium*-like' dinoflagellates from reef rubble and from substrates placed at several sites in the Middle Florida Keys (see Table S1 in the Supplemental data available online). Over 380 cultures were established and molecular analyses of those containing dinoflagellate-like microorganisms confirmed their relationship to *Symbiodinium* (Table S2). Phylogenetic analyses of internal transcribed spacer (ITS) rDNA sequences from a subset of these cultures placed many within *Symbiodinium* (Figure 1); three of the isolates grouped within established *Symbiodinium* taxa (Figure 1): isolate 5196 had an identical sequence to AY074983 (Bermuda *Cassiopeia* [11], *Symbiodinium* type A1), while isolate 4562 grouped closely with *Symbiodinium* type A1 and *Symbiodinium* of Bermuda *Cassiopeia* (0.2% difference from 5196, Table S5). Additionally, isolate 4404 belonged to *Symbiodinium* Clade B, being most similar to type B1 (0.8% difference, Table S6).

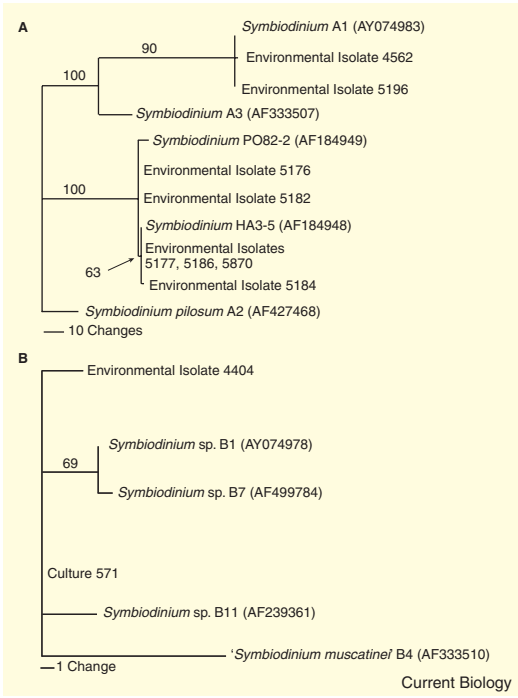

Figure 1. Phylogenetic analysis of a subset of *Symbiodinium* spp. isolated from the benthos based on complete ITS sequences.

Samples acquired from GenBank have accession numbers following the label in parenthesis (further information regarding symbiont host and literature references are provided in Table S4). (A) Clade A tree with the symbiont of *Zoanthus sociatus* (*S. pilosum*) as the outgroup and (B) Clade B tree with *S. muscantinei* as the outgroup.

Our study only identified members of *Symbiodinium* Clades A and B, and probably does not represent the true diversity of environmental populations. The inability to bring many *Symbiodinium* into culture is most likely due to these dinoflagellates being notoriously difficult to culture [12]. However, other clades have been detected in the environment, although their ability to establish symbioses has not been tested (I. Porto and J. Sánchez, personal communication). Our sampling and culturing methods also recovered potentially non-symbiotic, '*Symbiodinium*-like' isolates (5176, 5177, 5182, 5184, 5186 and 5870); phylogenetic analysis placed these within *Symbiodinium* Clade A along with types that have previously been characterized as 'free-living' (Figure 1A). Specifically, isolates 5177, 5186 and 5870 possessed identical ITS sequences to *Symbiodinium* HA3-5 (AF184948), the 'free-living' isolate from Hawaii [8].

We verified that these environmental populations of '*Symbiodinium*-like' dinoflagellates are capable of establishing a symbiosis with cnidarians by exposing asymbiotic recruits to the environmental isolates and recovering the specific isolate used to inoculate the polyp in six of eight treatments (Table S3). Phylogenetic analysis of ITS sequences from a subset of these polyps verified that the *Symbiodinium* recovered from the polyps were identical to that of the cultures (eight cases) while in a single case, the isolate differed by a single nucleotide (Figure 2). Based

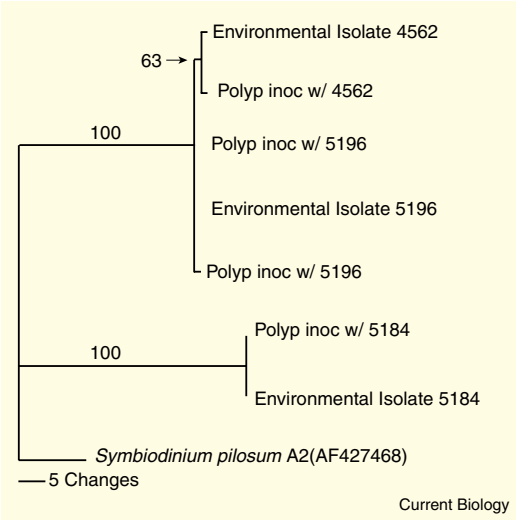

Figure 2. Comparison of *Symbiodinium* spp. isolated from the two infection experiments with the culture used to inoculate the polyps, based on complete ITS sequences.

Briareum spp. polyps were inoculated with environmental isolates 4562 or 5196, while *Pseudopterogorgia elisabethae* polyps were inoculated with isolate 5184. All symbionts belong to Clade A and the symbiont of *Zoanthus sociatus* (*S. pilosum*) is used as the outgroup. Symbiont host and literature references are provided in Table S4.

on this, three isolates (4404, 4562 and 5196) successfully infected recruits, establishing that these environmental isolates, phylogenetically similar to *Symbiodinium*, are indeed capable of initiating a symbiosis (Figure 2). In two treatments, the dominant clade recovered did not correspond to the environmental isolate used as an inoculum (isolates 5177 and 5184, Table S3), although isolate 5184 was detected in one polyp following analysis of ITS sequences (Figure 2). These observations suggest that isolates allied with the 'free-living' *Symbiodinium* (Figure 1A) may not readily form symbioses.

Our results show that a reservoir of *Symbiodinium* exists in the water column and the benthos. Recovering *Symbiodinium* from benthic substrates clarifies the mystery as to where these important symbionts reside outside of the host. Given the tendency of laboratory cultures of *Symbiodinium* to populate the bottom and sides of culture containers and undergo periodic motility into the 'water column' of the flask [13], we propose that the intrinsic diurnal motility of the cells results in the movement of *Symbiodinium* from the benthos into the water column and ensures encounters with competent hosts.

The environmental source of *Symbiodinium* that we have shown can infect cnidarian recruits is likely to be available to all reef-dwelling cnidarians and may prove important in the recovery of reef-building corals following bleaching events. Our results also show that not all *Symbiodinium* spp. isolated from the environment are capable of establishing symbioses with cnidarians. Future research should examine if these 'free-living' *Symbiodinium* can form symbioses with other, non-cnidarian hosts, determine whether environmental isolates that infect cnidarians are able to maintain the symbiosis in the field and test their competitive ability with other *Symbiodinium* types. Additional work is also needed to determine the physiological tolerances of these potential symbionts and if they represent a symbiont pool that might repopulate reef corals following temperature-induced (or other stress related) bleaching.

Acknowledgments

We thank the FKNMS, Keys Marine Lab, Niagara Aquarium, Sherwood, L. Bright, M. Dorner, A. Hannes, L. Holland, N. Kirk, H. Lasker, G. May, M. Medina, A. Monteiro, T. Naeher, D. Poland, T. Shearer, A. Siegel, B. Spataro, J. Stamos and D. Taylor. This research was supported by the National Science Foundation (M.A.C.)

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/16/23/R985/DC1

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