

earlier end of the most commonly cited genetic coalescence dates (21–23) may suggest the presence of modern humans in India at the time of the YTT event. This interpretation would be consistent with a southern route of dispersal of modern humans from the Horn of Africa (24); the latter, however, will remain speculative until other Middle Paleolithic sites in the Indian subcontinent and Arabian Peninsula (25) are excavated and dated.

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SOM Text

Figs. S1 to S14

Tables S1 to S6

References

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Buddenbrockia Is a Cnidarian Worm

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A major evolutionary divide occurs in the animal kingdom between the so-called radially symmetric animals, which includes the cnidarians, and the bilaterally symmetric animals, which includes all worm phyla. *Buddenbrockia plumatellae* is an active, muscular, parasitic worm that belongs to the phylum Myxozoa, a group of morphologically simplified microscopic endoparasites that has proved difficult to place phylogenetically. Phylogenetic analyses of multiple protein-coding genes demonstrate that *Buddenbrockia* is a cnidarian. This active muscular worm increases the known diversity in cnidarian body plans and demonstrates that a muscular, wormlike form can evolve in the absence of overt bilateral symmetry.

Most metazoans (true animals), including arthropods, annelids, mollusks, chordates, and all worm phyla, belong to the Bilateria. This clade excludes cnidarians, ctenophores, sponges, and placozoans. Myxozoa were originally placed outside the Metazoa, despite the presence of characters such as multicellularity of spores, septate junctions, and putative nematocysts (1–3). Sequencing of 18S ribosomal DNA (rDNA) confirmed that they are highly modified metazoans (4). However, precisely placing them in the animal kingdom has proven difficult. Most myxozoans are microscopic aquatic endoparasites with either plasmodial or sac-shaped bodies, with no gross similarity to other animals. There are two classes of myxozoans, the clades Myxosporidia, with over 2000 species, and the Malacosporea, with two

described species and two others recently identified by rDNA comparisons (5). Myxozoans parasitize a wide range of hosts, including fish, annelids, and (for malacosporeans) bryozoans. Myxozoans form complex spores containing polar capsules similar to the stinging organelles (nematocysts) of cnidarians, which they use to attach to a new host. Polar capsules differ from typical nematocysts of cnidarians in lacking chemo- and/or mechanosensory structures and neural connections that modulate discharge (6).

If polar capsules and nematocysts are homologous, then myxozoans could be cnidarians or the sister group to cnidarians. Alternatively, nematocyst-like structures may have evolved before the divergence of cnidarians and bilaterians, or they could have arisen independently. Some analyses of myxozoan 18S rDNA sequences have also suggested that myxozoans are related to cnidarians, most notably, when the highly divergent rDNA sequence of the endoparasitic cnidarian *Polypodium hydriforme* is included (3). In contrast, other rDNA analyses suggest myxozoans are bilaterians (7, 8). These contradictory phylogenetic results may be a consequence of the highly divergent (long-branch) rDNA sequences of myxozoans (9), making placement difficult.

The report of bilaterian-like Hox genes in two myxozoan species (10) and the surprising finding

that a rare endoparasitic worm that infects freshwater bryozoans, *Buddenbrockia plumatellae* (11) (Fig. 1), is actually a myxozoan (7, 12) have further confounded the placement of the myxozoans. *Buddenbrockia* worms are highly active, with continuous and vigorous sinuous writhing within the body cavity of bryozoan hosts (12, 13). The worms escape from their bryozoan hosts, probably through the vestibular pore, and undergo repeated coiling and straightening (13). The vermiform (wormlike) body plan of *Buddenbrockia* is reminiscent of bilaterian taxa, although *Buddenbrockia* lacks a recognizable nervous system, gut, and external

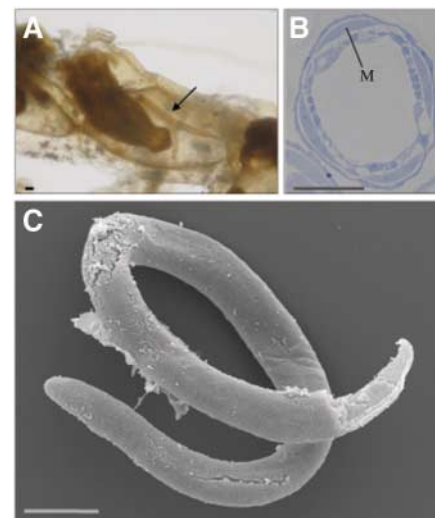


Fig. 1. (A) A zooid of the bryozoan *Plumatella* with *Buddenbrockia* worms (arrow) in the body cavity. Scale bar, 40 μ m. (B) Cross section of an immature *Buddenbrockia plumatellae* worm. Note the presence of four longitudinal muscle blocks (M) and absence of gut. Scale bar, 20 μ m. (C) Scanning electron microscopy image of a *Buddenbrockia plumatellae* worm. Scale bar, 100 μ m.

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sense organs (9, 11, 12) (Fig. 1). The sinuous movements of the body are bilaterian-like and quite unlike those of elongate cnidarians. For instance, planula larvae creep along the substratum, burrowing cerianthids are only capable of retraction, burrowing anemones undergo peristalsis (14), and the swimming narcomedusan *Tetraplatia* uses specialized locomotory flaps (15). In contrast, the four well-defined blocks of longitudinal muscles running the length of the body in *Buddenbrockia* (Fig. 1B) are more comparable to those of nematodes and nematomorphs. Although some cnidarians have four longitudinal muscles that run the length of the individual (Stauromedusae, for example), these animals are not vermiform.

Despite its gross dissimilarity to all other members of the phylum, there is strong evidence that this strange vermiform animal is indeed a true myxozoan. First, ultrastructural analyses revealed that *Buddenbrockia* has polar capsules similar to those of malacosporean myxozoans; these are found in infective spores and also in the epidermis of the worm (12). Additionally, both malacosporeans and the *Buddenbrockia* worm have an unusual form of cell junction in the body wall and use freshwater bryozoans as hosts (12). The 18S rDNA sequence of vermiform *Buddenbrockia plumatellae* is similar to that of the malacosporean myxozoan, *Tetracapsula bryozoides* [now revised to *B. plumatellae* (13)], which suggests that they are at least congeneric (7).

To investigate the phylogenetic affinities of myxozoans, we tested the veracity of the four Hox gene sequences previously reported from the myxozoans *Tetracapsula bryozoides* and *Myxidium lieberkuehni* (10). Surprisingly, polymerase chain reaction (PCR) with gene-specific primers amplified three of the genes *Myx1*, *Myx2*, and *Myx3*, from uninfected bryozoans (*Cristatella mucedo*). These bryozoans were collected from Littoistenjärvi, Finland, where myxozoan infection has not been observed, and were shown to be parasite-free by PCR amplification and sequencing of rDNA. The *Myx4* gene was amplified from Northern pike (*Esox lucius*), a natural host of the myxozoan *M. lieberkuehni*. All amplified sequences were verified by cloning and sequencing. These genes did not amplify in any of our myxozoan samples with the same gene-specific primers, which indicated that the Hox gene sequences reported from myxozoans derive from host DNA. We then turned to the phylogenetic analysis of orthologous nuclear protein-coding genes (16). To ensure that these were cloned from myxozoan tissue, and not contaminated by host tissue, we initially used universal PCR primers targeted to 18S rDNA (17) to screen a range of adult and spore samples from four species of myxozoan. Each amplified PCR band was cloned and sequenced to assess levels of contamination in each sample. A myxozoan sample free from contamination was obtained by dissecting infected bryozoan colonies and collecting individual *Buddenbrockia* worms released from the disrupted body cavity. Each worm is around 2 mm in length (range 0.05 to 3.6 mm). These samples yielded only myxozoan rDNA sequences after PCR amplification with universal primers (50/50 clones sequenced).

From a sample of 10 *Buddenbrockia* worms not contaminated by host DNA, we cloned a total of 50 different protein-coding genes that previously had been identified as unequivocal single-copy genes (18). Orthology was confirmed by phylogenetic analysis of each gene. We aligned 129 proteins (29,773 unambiguously aligned amino acid positions) from a wide diversity of animal species (47 animals and 13 outgroups), including *Buddenbrockia*, three sponges, five cnidarians, 14 ecdysozoans, 15 lophotrochozoans, and nine deuterostomes, choosing taxa from each group on the basis of the shortest branch lengths (tables S1 to S3, see SOM text). A Bayesian tree inferred with a WAG+ Γ model (16) (Fig. 2) was in agreement with the current view of animal evolution (19). In this phylogeny, *Buddenbrockia* is placed within the Cnidaria, forming a clade with Medusozoa (Hydrozoa plus Scyphozoa), to the exclusion of Anthozoa (with a posterior probability of 0.97). To further evaluate the robustness of this result, we analyzed the 115 possible positions of *Buddenbrockia* in a backbone tree lacking *Buddenbrockia* (fig. S1). This phylogenetic placement was favored over all others. However, the bootstrap support for this node was only 70%, and five alternative posi-

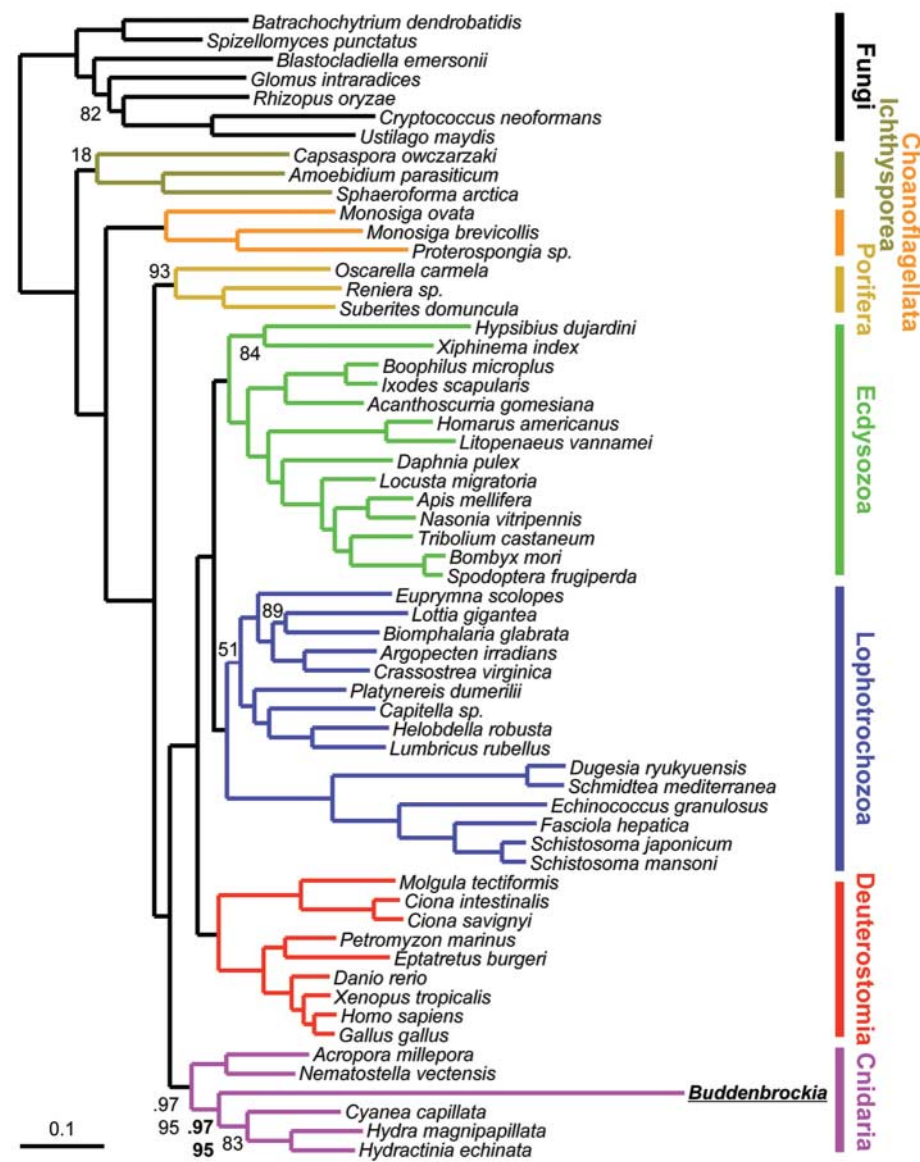


Fig. 2. Phylogenetic analyses of genomic data strongly support the grouping of *Buddenbrockia* and Medusozoa. Bayesian tree obtained from the analysis of 31,092 aligned amino acid positions. Posterior probabilities are equal to 1 except for the two nodes where 0.97 values are indicated. Bootstrap values obtained with the CAT model are indicated when <98% (see text and SOM). Scale bar indicates number of changes per site.

tions cannot be rejected by the approximately unbiased (AU) multiscale bootstrap test (20) at the 5% level (fig. S2). This uncertainty may relate to the fact that *Buddenbrockia* genes have undergone rapid sequence evolution, which can either cause artifactual groupings or reduce the support for the correct grouping (21, 22). This is not expected to be the cause of the grouping between *Buddenbrockia* and Medusozoa, because the branches of both the Hydrozoa and Scyphozoa species are short and should not act as a long-branch attractor. When trees were inferred by parsimony, a method highly susceptible to long-branch attraction (23), *Buddenbrockia* was grouped with an artifactual clade of long-branch platyhelminths and nematodes, not Medusozoa (fig. S3). To circumvent the long-branch attraction effect (24, 25), we reanalyzed the data under the CAT model, which explicitly handles the heterogeneity of the substitution process across positions (26). The CAT tree (fig. S4) was identical to the Bayesian tree except for the relative placement of some nonmetazoan branches. It is noteworthy that less phylogenetic resolution was observed within the *Buddenbrockia* + Medusozoa clade, as these results suggest that *Buddenbrockia* is either an outgroup to Scyphozoa plus Hydrozoa (83% CAT) or sister to Hydrozoa (17% CAT). On the basis of these data, we conclude that the *Buddenbrockia* worm is a cnidarian. This conclusion can be extrapolated to all Myxozoa, because previous work has established that *Buddenbrockia* is a member of this clade (7, 9). Therefore, the taxon Myxozoa should be placed within the phylum Cnidaria, on the medusozoan lineage.

Our data also show that, not only has anatomical simplification occurred in myxozoan evolution, but so has evolution of a muscular vermiform body. We infer that active, motile worms are not restricted to the bilaterian animals, but can be found among the cnidarians. One interpretation is that the common ancestor of cnidarians and bilaterians had a muscular worm-shaped body plan. However, this does not seem compatible with the ultrastructure of *Buddenbrockia* or the phylogenetic distribution of vermiform animals. Instead, we hypothesize that the muscular, motile worm form evolved independently within cnidarians, by means of a loss of the opening to the gastrovascular cavity and subsequent acquisition of a hydrostatic skeleton. Parallel evolution of the vermiform body may have exploited a conserved developmental system for patterning an ancestral mesodermal layer homologous between Bilateria and Cnidaria. (27)

Ultrastructural studies reveal that the four blocks of well-defined longitudinal muscles in *Buddenbrockia* are radially distributed (Fig. 1) (12). Hence, *Buddenbrockia* is a tetradial worm with two axes of symmetry across a transverse section, not a bilaterally symmetrical worm with one axis of symmetry. Bilateral symmetry was long thought to be associated with the evolution

of directed locomotion, perhaps in an ancestral bilaterian. This view is challenged by the existence of subtle bilateral symmetry in sessile anthozoan cnidarians (28, 29); hence, it has been suggested that bilateral symmetry arose through selection for effective internal circulation not directed locomotion (30). The finding that an active muscular worm evolved within the Cnidaria, yet retained radial symmetry, is consistent with this view, because it further dissociates locomotion from symmetry. *Buddenbrockia* is a worm, but not as we know it.

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Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References

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Genetic Properties Influencing the Evolvability of Gene Expression

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Identifying the properties of gene networks that influence their evolution is a fundamental research goal. However, modes of evolution cannot be inferred solely from the distribution of natural variation, because selection interacts with demography and mutation rates to shape polymorphism and divergence. We estimated the effects of naturally occurring mutations on gene expression while minimizing the effect of natural selection. We demonstrate that sensitivity of gene expression to mutations increases with both increasing trans-mutational target size and the presence of a TATA box. Genes with greater sensitivity to mutations are also more sensitive to systematic environmental perturbations and stochastic noise. These results provide a mechanistic basis for gene expression evolvability that can serve as a foundation for realistic models of regulatory evolution.

Regulatory variation underlies much of phenotypic diversity, and gene expression is the first step in making ecologically and evolutionarily relevant phenotypes. Differences among genes both in standing genetic variation and in interspecies divergence in gene expression have been linked to their particular roles in biological networks (1–4) and may reflect a history of selection. However, the influence of specific evolutionary forces cannot

be inferred solely from the distribution of natural variation, because selection interacts with demography and mutation to shape polymorphism and divergence (5). Measuring the effects of spontaneous mutations without the confounding effect of natural selection makes it possible to isolate the contribution of mutation to natural variation and is a fundamental step toward building models for the evolution of gene expression. The relationship between divergence and muta-