



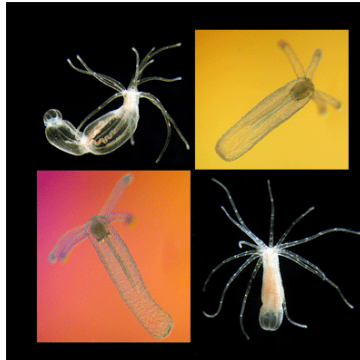
# ENZYMES OF THE SHIKIMIC ACID PATHWAY ENCODED IN THE GENOME OF A BASAL METAZOAN, THE SEA ANEMONE *NEMATOSTELLA VECTENSIS*, HAVE MICROBIAL ORIGINS

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## Background

- The shikimic acid pathway is responsible for the biosynthesis of many aromatic compounds by a broad range of organisms, including bacteria, fungi, plants and some protozoans.
- Animals are considered to lack this pathway as evinced by their dietary requirement for shikimate-derived aromatic amino acids.
- We challenge the universality of this traditional view in this novel report of genes encoding enzymes for the shikimate pathway in an animal, the starlet sea anemone *Nematostella vectensis*.

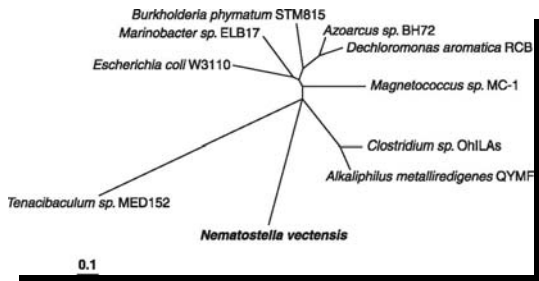


## Methods

The DNA sequence of the *N. vectensis* genome was downloaded from StellaBase version 1.0 (<http://evodevo.bu.edu/stellabase/>) and translated into all six reading frames using Transeq (<http://www.ebi.ac.uk/emboss/transeq/>). For profile analyses, HMMER version 2.3.2 (<http://hmmerr.janelia.org/>) and release 20 of the Pfam database (<http://www.sanger.ac.uk/Software/Pfam/>) were used. Similarity searches used the BLAST service at NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>).

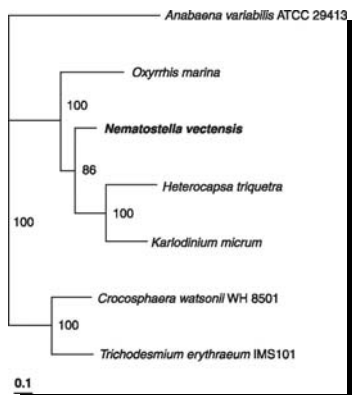
## Results 1

Phylogenetic tree showing the relationship of the predicted protein sequence of the *N. vectensis* *aroA*-like gene to the predicted *murA* protein sequences of the seven best hits in a BLAST analysis, as well as to those in *E. coli* and *Tenacibaculum*. Distances were calculated from a CLUSTAL W alignment using the Jones-Taylor-Thornton matrix and the tree was constructed using the neighbor-joining algorithm in programs of the PHYLIP package (version 3.63). The distance is proportion of amino acid substitutions.



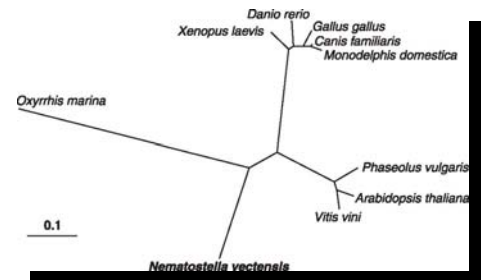
## Results 2

Phylogenetic tree showing the relationship of the deduced protein sequence of the *aroB* part of the AroB-O-methylase protein of *N. vectensis* to homologous dinoflagellate proteins. Sequences were aligned with CLUSTALW and the tree constructed using the neighbor-joining algorithm with distances derived from the Jones-Taylor-Thornton model (using PHYLIP version 3.63). The tree was rooted using *Anabaena variabilis* as an out group. The distances are the proportion of amino acid substitutions, and the bootstrap values based on 100 samples are shown.



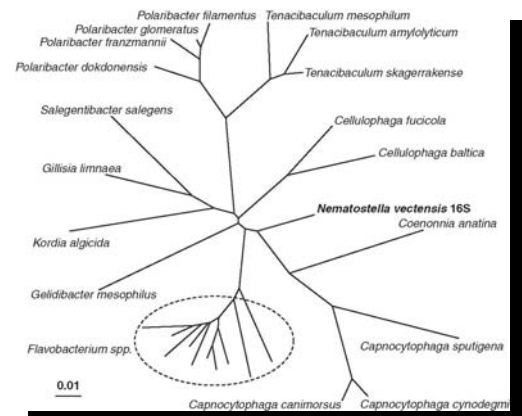
## Results 3

Phylogenetic tree of PCNA protein sequences. The sequence of the PCNA protein of *O. marina* was used for a BLAST search against the translated genomic sequences of *N. vectensis*. The BLAST alignments were used to assemble the protein sequence from *N. vectensis*. The sequences from the two species were used for BLAST searches of GenBank and a selection of the best hits for each species was used to construct a phylogenetic tree using the neighbor-joining algorithm in programs of the PHYLIP package (version 3.63). The distance is proportion of nucleotide substitutions.



## Results 4

Phylogenetic tree showing the relationship of the 16S rRNA gene sequence found in the *N. vectensis* genome sequence (720 bp fragment in entry c429301624.Contig1 of StellaBase; <http://evodevo.bu.edu/stellabase/>, supplementary material, Table 8a) to the sequences of the closest type strains in Ribosomal Data Base Project II (release 9.52, <http://rdp.cme.msu.edu/>). Distances were calculated from a CLUSTAL W alignment using the F84 model and the tree was constructed as in Results 3.



## Discussion

- Molecular evidence establishes, for the first time, horizontal transfer of ancestral genes of the shikimic acid pathway into the *N. vectensis* genome from both bacterial and eukaryotic (dinoflagellate) donors.
- Bioinformatic analysis also reveals four genes that are closely related to those of *Tenacibaculum* sp. MED152, raising speculation for the existence of a previously unsuspected bacterial symbiont. Indeed, the genome of the holobiont (i.e., the entity consisting of the host and its symbionts) comprises a high content of *Tenacibaculum*-like gene orthologs, including a 16S rRNA sequence that establishes the phylogenetic position of this associate to be within the family Flavobacteriaceae.

## Significance

- These results provide a complementary view for the biogenesis of shikimate-related metabolites in marine Cnidaria as a "shared metabolic adaptation" between the partners.
- The results could also have wider implications for future development of the shikimic acid pathway as a 'druggable' target for the discovery of new antibiotics and for the treatment of parasitic infections, including malaria.

## Acknowledgments

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