LETTER

Independent evolution of striated muscles in cnidarians and bilaterians

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Striated muscles are present in bilaterian animals (for example, vertebrates, insects and annelids) and some non-bilaterian eumetazoans (that is, cnidarians and ctenophores). The considerable ultrastructural similarity of striated muscles between these animal groups is thought to reflect a common evolutionary origin1–2. Here we show that a muscle protein core set, including a type II myosin heavy chain (MyHC) motor protein characteristic of striated muscles in vertebrates, was already present in unicellular organisms before the origin of multicellular animals. Furthermore, ‘striated muscle’ and ‘non-muscle’ myhc orthologues are expressed differentially in two sponges, compatible with a functional diversification before the origin of true muscles and the subsequent use of striated muscle MyHC in fast-contracting smooth and striated muscle. Cnidarians and ctenophores possess striated muscle myhc orthologues but lack crucial components of bilaterian striated muscles, such as genes that code for titin and the troponin complex, suggesting the convergent evolution of components of bilaterian striated muscles, such as genes that code for titin and the troponin complex, suggesting the convergent evolution of muscle contraction by coupling regulatory light chain phosphorylation to increased cytoplasmic Ca2+ concentrations in muscle and non-muscle cells6–10 (Supplementary Fig. 1b, d). Notably, all associated regulatory components, except caldesmon, are present in all animals (Fig. 1a and Supplementary Fig. 1b). Hence, of the different known modes of muscle-contraction regulation6, myosin light chain kinase-dependent regulatory light chain phosphorylation seems to be the most ancient. A third major finding is that not one of the 47 structural or regulatory proteins we analysed is shared uniquely between cnidarians and bilaterians; that is, no protein correlates with the evolutionary origin of muscle. These observations suggest that the core contractile apparatus in eumetazoan muscles antedates the origin of the animal kingdom and that lineage-specific innovations underlie muscle evolution in cnidarians and bilaterians.

Previous studies suggested that a gene-duplication event gave rise to two distinct phylogenetic groups of myhc orthologues in bilaterian animals, each having a distinct function and pattern of expression11–13. Bilaterian ‘non-muscle’ (NM) orthologues function during common cellular processes (for example, cell division or migration) and during vertebrate smooth-muscle contraction14, whereas bilaterian ‘muscle’ orthologues (hereafter termed ST MyHC) function specifically in vertebrate striated muscles and in both smooth and striated muscles of protostomes15. Counter-intuitively, our analyses demonstrate that the gene duplication that generated the two MyHC orthology groups occurred much earlier than the origin of muscle cells (Fig. 2 and Supplementary Fig. 2). Bilaterians, cnidarians, ctenophores, placozoans and sponges (the latter two lacking muscles) each possess at least one of each ST and NM MyHC orthologues with specific coiled-coil domain structures, whereas the unicellular organisms Capasaspora owczarzaki and Sphaeroforma arctica possess a clear member of the NM MyHC group, characterized by a specific coiled-coil structure (Fig. 2 and Supplementary Fig. 2). The tree topology indicates strongly that the ST and NM myhc genes had already separated in the last common ancestor of all animals and the aforementioned protists having lost ST myhc (Fig. 2 and Supplementary Figs 1d and 2).

To address how ST and NM myhc are used in non-bilaterian animals, we investigated their expression in two sponges, generally considered to lack muscles, as well as two cnidian species harbouring striated and smooth muscles (Fig. 3 and Supplementary Figs 3–6). In Tethya wilhelma, a demosponge, NM myhc expression was detected in a wide variety of cell types (Supplementary Fig. 3a–g), including the pinacocytes, primarily responsible for the peristalsis-like contractions of the adult sponge16 (Supplementary Fig. 3e). By contrast, T. wilhelma ST myhc expression is restricted to the outlet pore (apopyle) of the current-producing choanocyte chambers (Fig. 3a, c and Supplementary Fig. 3h–k), the site of a sieve-like cell type (Fig. 3b, c and Supplementary Fig. 3i–k).

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Figure 1 | Complex phylogenomic distribution of contractile machinery and Z-disc interactome components. a, b. Rows: gene names of vertebrate and/or D. melanogaster contractile machinery (a) or Z-disc complex components (b). Columns: species and their phylogenetic relationship29,30. Only a preliminary assembly without gene predictions was available for Supplementary Fig. 4) proposed to regulate water flow17. In larvae of the demosponge Amphimedon queenslandica, the myhc orthologues were found to be differentially expressed in regions of presumptive cell shape change or migration18, with NM myhc more broadly expressed than ST myhc (Supplementary Fig. 5). We conclude that the segregation of a ‘general-function’ NM myhc orthologue and a more specialized ST myhc orthologue had already occurred in the last common ancestor of demosponges and all other animals, accounting for their evolutionary retention over long time periods.

In cnidarians, we found ST myhc orthologues expressed prominently in fast-contracting muscle cells, but also in a few non-muscle cell types. In the sea anemone Nemastoma vectensis (with smooth muscles), ST myhc (formerly termed myhc1 (ref. 19)) is strongly expressed in the tentacle and body column retractor muscles that contract orally–abnormally during escape response (Fig. 3d–j). Conversely, N. vectensis NM myhc is expressed broadly in the whole endoderm, hence in all endodermal epitheliomuscular cells (Supplementary Fig. 6a–c). In the hydrozoan Clytia hemisphaerica, ST myhc expression is detected in the developing striated ring muscles of the velum and the subumbrella (Fig. 3k–p and Supplementary Fig. 6g–k) that propel the medusa by fast contractions. It is also detected in the tentacles and the mouth tube ectoderm (manubrium; Fig. 3m, q), which both include longitudinal smooth muscles. C. hemisphaerica NM myhc, like in N. vectensis, is expressed broadly in the endoderm including the smooth-muscle-rich gastrovascular system, and in non-muscle cells of the tentacle bulb nematocyte precursor region (Supplementary Fig. 6d–f). Thus, in both cnidarian species examined, ST myhc is expressed predominantly in fast-contracting muscles whereas the broad NM myhc expression includes smooth and non-muscle cell types. A clear segregation of function between a muscle MyHC and a more general-purpose MyHC is thus observed, albeit less pronounced than in bilaterians.

ST MyHC is the predominant motor protein in all striated muscles investigated so far, but its function in smooth retractor muscles in N. vectensis shows that it is not sufficient to confer striation. We therefore examined the presence of other striated muscle components in cnidarians. A hallmark of striated muscle regulation and formation in bilaterians is the troponin complex, comprising troponin I, C and T20. To our surprise, none of the corresponding genes could be found in the genome sequences of the non-bilaterian species, the cnidarians N. vectensis, Acropora digitifera or Hydra magnipapillata, the ctenophore Mnemiopsis leidyi, the placozoan Trichoplax adhaerens, or in the deeply sequenced transcriptome of C. hemisphaerica medusae (Fig. 1a and Supplementary Fig. 1a, d), indicating that muscle striation or the regulation of contraction are not troponin dependent in these organisms.
Concerning components of the Z-disc, a marked lack of conservation was discovered even within the Bilateria. Nearly half of the known vertebrate (13 out of 28) and one-quarter of the known protist phyla. Diagrams, MyHC representations (Fig. 1b) and schematic representations (c, h-j, o-q) of ST myhc expression in the adult demospone T. wilhelma (Tw, a, c), the anzoan N. vectensis (Nv, d-j) and the hydrozoan C. hemisphaerica (Ch; k-q). Scanning electron microscopy image (b) and schematic representation (c) of a sectioned choanoocyte chamber of T. wilhelma. T. wilhelma ST myhc-expressing multi-porous cells (b, white arrows, inlet; c, red) are probably involved in water flow (blue dotted arrows) regulation through choanoocyte chambers (within dotted white lines). N. Velum of a young medusa was lifted. Developmental stages: d, e, h, i, 4-day-old planula; f, j, 9-day-old primary polyps; k, l, o, p, medusal buds; m, n, q, young medusae; a–c, g, adults. a, g, Cross-sections of stained animals; d–g, k, whole-mount micrographs. Views: d, f, h, j, k, o, q, lateral; e, i, l–n, p, oral. Aboral towards top (d, h, q) or right (f, j, k, o). Asterisks denote the mouth. In Clytia hemisphaerica, two identically expressed paralogues of ST myhc exist, ST myhc-a and -b. Ap, apopyle; cc, choanocyte chamber; cc, excurrent channel; inc, incurrent channel; mh, mesohyl; pp, prosopyle; rc, ring canal; rm, retractor muscle; su, subumbrella; tb, tentacle bulb; tm, tentacle muscle; v, velum. Scale bar, 10 μm.

seems to be absent in non-bilarians, as immunoglobulin/fibronectin type III-domain super repeats, characteristic of the giant titin protein, were not identified in any predicted proteins from non-bilarian genomes (Fig. 1b and Supplementary Fig. 8b, c).

Proteins found in the Z-discs of D. melanogaster but so far not in the vertebrate Z-discs seem to be absent outside Bilateria. Among

**Figure 2 | Ancient myhc gene duplication predated animal radiation.**

Maximum-likelihood phylogenetic tree of MyHC type II proteins with nodes collapsed if they diverged between neighbour-joining (NJ), maximumlikelihood (ML) or Bayesian inference. The nesting of protist MyHcs within the NM MyHC orthology group supports a myhc duplication event in the common ancestor of Metazoa, Choanozoa, Filasterida and Ichthyosporea, but also assumes secondary losses of ST myhc genes in protist phyla. Diagrams, MyHC domain structures. Final alignment length, 1,730 amino acids (a.a.). Scale bar, 0.2 changes per site. Coloured numbers represent positions of non-canonical coiled-coil domains. Sequence accession and protein model numbers are provided in Supplementary Table 1.

**Figure 3 | Expression of ST myhc in a demosponge and in anthozoan and hydrozoan cnidarians.** a–q. In situ hybridizations (a, d–g, k–n) and schematic representations (c, h–j, o–q) of ST myhc expression in the adult demospone T. wilhelma (Tw, a, c), the anzoan N. vectensis (Nv, d–j) and the hydrozoan C. hemisphaerica (Ch; k–q). Scanning electron microscopy image (b) and schematic representation (c) of a sectioned choanoocyte chamber of T. wilhelma. T. wilhelma ST myhc-expressing multi-porous cells (b, white arrows, inlet; c, red) are probably involved in water flow (blue dotted arrows) regulation through choanoocyte chambers (within dotted white lines). N. Velum of a young medusa was lifted. Developmental stages: d, e, h, i, 4-day-old planula; f, j, 9-day-old primary polyps; k, l, o, p, medusal buds; m, n, q, young medusae; a–c, g, adults. a, g, Cross-sections of stained animals; d–g, k, whole-mount micrographs. Views: d, f, h, j, k, o, q, lateral; e, i, l–n, p, oral. Aboral towards top (d, h, q) or right (f, j, k, o). Asterisks denote the mouth. In Clytia hemisphaerica, two identically expressed paralogues of ST myhc exist, ST myhc-a and -b. Ap, apopyle; cc, choanocyte chamber; cc, excurrent channel; inc, incurrent channel; mh, mesohyl; pp, prosopyle; rc, ring canal; rm, retractor muscle; su, subumbrella; tb, tentacle bulb; tm, tentacle muscle; v, velum. Scale bar, 10 μm.

**Figure 4 | Absence of Clytia hemisphaerica muscleLIM and ldb3 expression in striated muscles.** In situ hybridization (a–d) and schematic representation (e, f) of muscleLIM (a, b) and ldb3 (c, d) expression, mainly restricted to the developing radial canal endoderm (a–f). ST myhc-positive subumbrella striated muscle precursor cells (arrows, compare with Fig. 3i) do not show muscleLIM or ldb3 expression. a, c and e represent the medusal bud stage and b, d and f represent the young medusa stage. Views: oral (a–e) and lateral (f).
vertebrate Z-disc components not detected in *D. melanogaster* Z-discs, many have general roles in the cytoskeleton (*capZ-a, capZ-b* and *lap*), signal transduction (*calcineurin A* and *calcineurin B*) or protein degradation (*trim9* and *trim67* ubiquitin ligases). Orthologues of these genes are expressed ubiquitously in *C. hemisphaerica* (Supplementary Fig. 9g) and in the smooth muscle-forming *N. vectensis* endoderm (Supplementary Fig. 11kk–mm), suggesting a more general role in cnidarians.

To conclude, we have shown that cnidarians lack all molecular hallmarks of bilaterian striated muscles except ST myhc expression, and thus striated muscles in Bilateria and Hydrozoa are very likely to have evolved convergently from cells with ancient contractile machinery (Supplementary Fig. 1d). This may also apply to the striated muscles of the clonophore *Euplokamis* sp.4,6, as suggested by their isolated occurrence within the clonophores. We suggest that the observed correlation between *ST myhc* expression and striated muscles in bilaterians and hydrozoan jellyfish is due to functional constraints: *ST myhc*-based ‘bipolar’ thick filaments (as found in vertebrate and protostome striated muscles) may favour a faster contraction and re-iteration of the actomyosin machinery when compared with NM MyHC-based ‘side-polar’ thick filaments (as found in vertebrate smooth muscle and non-muscle cells)25. Our work showed that the origin of many components integral to muscle cell function (notably ST *myhc*) pre-dates that of muscle cells, whereas others (such as the troponin complex, paramyosin or titin) were acquired progressively during muscle specializations in different animal groups (Supplementary Fig. 1d). A similar scenario may also apply to other complex cell types. Our analysis of striated muscle evolution therefore highlights that ultrastructural similarity alone is not a reliable indication of common evolutionary origin, but can be achieved independently by different sets of proteins.

**METHODS SUMMARY**

Details of animal husbandry, genome mining, protein domain, phylogenetic and expression analyses, cloning, microscopy and image processing are found in Methods.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Seipel, K. & Schmid, V. Evolution of striated muscle: jellyfish and the origin of triploblasty. *Dev. Biol.* 282, 14–26 (2005).
2. Schuchert, P., Reber-Müller, S. & Schmid, V. Life stage specific expression of a myosin heavy chain in the hydrozoan *Podocoryne carnea*. *Differentiation* 54, 11–18 (1993).
3. Chapman, D. M. in Coelenterate Biology (eds Muscatine, L. & Lenhoff, H. M.) Ch. 1 (Academic, 1974).
4. Burton, P. M. Insights from diploblasts: the evolution of mesoderm and muscle. *J. Exp. Zool.* 308B, 1–10 (2007).
5. Schmidt-Rhaesa, A. *The Evolution of Organ Systems* 1st edn (Oxford Univ. Press, 2007).
6. Mackie, G. O., Mills, C. E. & Singla, C. L. Structure and function of the prehensile tentilla of *Euplokamis* (Clcnoherida, Cymaddipida), *Zoolog. J. Linn. Soc.* 107, 319–337 (1988).
7. Boekestein, U. An electron microscopic study of early developmental stages, myogenesis, oogenesis, and chordogenesis in the anemoheda, *Podocoryne carnea* M. *Sars. J. Morphol.* 154, 259–289 (1977).
8. Squire, J. M., Al-Khayat, H. A., Knuopp, C. & Luther, P. K. Molecular architecture of muscle contractile assemblies. *Adv. Protein Chem.* 71, 17–87 (2005).
9. Hooper, S. L., Hobbs, K. H. & Thuma, J. B. Invertebrate muscles: thin and thick filament structure; molecular basis of contraction and its regulation, catch and asynchronous muscle. *Prog. Neurobiol.* 86, 72–127 (2008).
10. Kamm, K. E. & Stull, J. T. Dedicated myosin light chain kinases with diverse cellular functions. *J. Biol. Chem.* 276, 4527–4530 (2001).
11. Korn, E. D. Coevolution of head, neck, and tail domains of myosin heavy chains. *Proc. Natl Acad. Sci. USA* 97, 12559–12564 (2000).
METHODS

Animal culture and collection. N. vectensis was cultured and gametogenesis induced as described\(^\text{41}\). C. hemisphaerica was cultured as described previously\(^\text{32}\) but using artificial sea salt (Red Sea). Adult specimens of A. queenslandica were collected on Heron Island Reef, Great Barrier Reef as previously described\(^\text{32}\). Adult specimens of T. wilhelma were collected from aquaria of the Zoologisch- Botanischen Garten Wilhelm\(^\text{a}\) and cultured as previously described\(^\text{30,43}\).

Basic local alignment search tool (BLAST) searches. A reference set of 47 muscle proteins from mouse, human or D. melanogaster was compiled from in-depth literature searches and searched in the publicly available, fully sequenced genome of Drosophila melanogaster, C. elegans, D. maria, H. magnipapillata\(^\text{44}\), M. leidyi\(^\text{45}\), T. adhaerens\(^\text{46}\), A. queenslandica\(^\text{47}\), Monosiga brevicollis\(^\text{48}\), Salpingoa rosetta\(^\text{49}\), C. owczarzaki\(^\text{50}\), Allomyces macrognys\(^\text{51}\), Spizellomyces punctatus\(^\text{52}\), Thecamonas trahanii\(^\text{53}\), Chlamydomonas reinhardtii\(^\text{54}\), Selaginella moellendorffii\(^\text{55}\), Dictyostelium discoideum\(^\text{56}\), Naegleria gruberi\(^\text{57}\), Phanerochaete chrysosporium\(^\text{58}\), Saccharomyces cerevisiae and Schizosaccharomyces pombe\(^\text{59}\) (for confirmation of gene absence in Helobdella robusta and Lottia gigantea) by reciprocal BLASTP and TBLASTN using the web-interface BLAST pages of the Joint Genome Institute (JGI) (http://www.jgi-psf.org/) or http://www.phytozome.net/) (for N. vectensis and A. queenslandica geneome assembly 1.0, the A. queenslandica geneome assembly or C. hemisphaerica and T. wilhelma transcriptomics data (see Supplementary Table 1).

Whole-mount in situ hybridization. Whole-mount in situ hybridization (WMISH) was performed as previously described for N. vectensis\(^\text{216}\) and A. queenslandica\(^\text{217,218}\). For C. hemisphaerica, the N. vectensis protocol was modified using 10 min of 0.1 μg/ml proteinase K (Ambion) at 37 °C and hybridized at 60 °C (lb3 and muscleLIM) or 63 °C (all other genes). As several splice variants of C. hemisphaerica lb3 were found, a 5′ rapid amplification of cDNA ends (RACE) fragment containing the amino-terminal PDZ domain, a crucial α-actinin-binding motif also found in all vertebrate Ldb3 isoforms, was chosen to perform in situ hybridizations.

For T. wilhelma, sponges were removed from the culture mesh, settled in a glass dish (1–2 weeks) and prevented from merging with each other. Just before fixation, the dish containing sponges was removed from the aquarium and sponge contraction was inhibited by incubation for 15 min at 25 °C in 10% Listerine mouthwash (Johnson & Johnson). Sponges were scrapped off the dish, rapidly transferred into a six-well plate containing fixative and fixed as previously described\(^\text{63}\), with a fixative change after 5 min, and transferred to screw-cap tubes of 70% ethanol for –20 °C storage. For WMISH, the A. queenslandica protocol\(^\text{64}\) was modified by skipping proteinase K digestion, acetylation and post-fixation. For paraffin embedding, WMISH samples were dehydrated in a seven-step series of increasing ethanol concentrations and transferred into Histo-Clear (National Diagnostics) through a intermediate step of ethanol/Histo-Clear (1:1). After several changes of Histo-Clear at room temperature (23 °C), specimens were incubated in Histo-Clear/Paraplast (1:1) at 42 °C for 1 h, followed by 48 h in Paraplast at 58 °C with regular total replacement of the Paraplast every 12 h. Samples were subsequently embedded into Paraplast at room temperature and sectioned at 7 μm after hardening using a Microm HM360. Serial sections were transferred to slides, cleared from Paraplast using Histo-Clear and mounted in Euparal (Anachemia). Light and confocal microscopy. All light microscopy pictures were done with Nikon eclipse E80 and an Olympus BH2, both equipped with differential interference contrast optics and a Zeiss AxioCam. All confocal microscopy pictures were done with a Leica TCS SP5 X.

Scanning electron microscopy. The T. wilhelma sample in Fig. 3 was fixed in a 0.45 M sodium acetate buffer (pH 6.4 in filtered aquarium sea water) with 2% OsO\textsubscript{4}, 2% glutaraldehyde and 0.29 M sucrose immediately after sampling, desilified in 5% hydrofluoric acid for 1 h and then embedded in styrene-

matrixate\(^\text{66}\). After semi-thin sectioning on a Microm HM360, the remaining plastic was removed using xylene treatment and the samples dehydrated in increasing acetone concentrations and transferred into Histo-Clear (National Diagnostics) through an intermediate step of ethanol/Histo-Clear (1:1). After several changes of Histo-Clear at room temperature (23 °C), specimens were incubated in Histo-Clear/Paraplast (1:1) at 42 °C for 1 h, followed by 48 h in Paraplast at 58 °C with regular total replacement of the Paraplast every 12 h. Samples were subsequently embedded into Paraplast at room temperature and sectioned at 7 μm after hardening using a Microm HM360. Serial sections were transferred to slides, cleared from Paraplast using Histo-Clear and mounted in Euparal (Anachemia).

Image-processing software. WMISH images were cropped and adjusted for levels, brightness, contrast and colour balance using Adobe Photoshop CS2 (Adobe). Stacks of light microscopy focal planes were assembled with Helicon Focus Software. All schematics and panels were designed with FreeHand MX (Adobe).

31. Fritzennwanker, J. H. & Technau, U. Induction of gametogenesis in the basal cnidian Nematostella vectensis. Dev. Genes Evol. 212, 99–103 (2002).
32. Chavvai, S., Martin, A., Leclerc, L., Arniel, A. & Houliston, E. Polarised expression of FoxB and FoxO2 genes during development of the hydrozoan Clytha hemisphaerica. Dev. Genes Evol. 216, 709–726 (2006).
33. Leys, S. P. & Degnan, B. M. Cytological basis of photoresponsive behavior in a sponge larva. Biol. Bull. 201, 323–338 (2001).
34. Sará, M., Sará, A., Nickel, M. & Brümmer, F. Three new species of Tethya (Porifera: Demospongiae) from German aquaria. Stutt. Beitr. Naturkd. A 631, 1–15 (2001).
35. Nickel, M. Kinetics and rhythm of body contractions in the sponge Tethya wilhelma (Porifera: Demospongiae). J. Exp. Biol. 207, 4515–4524 (2004).
36. Putnam, N. H. et al. The amphioxus genome and the evolution of the chordate karyotype. Nature 453, 1064–1071 (2008).
37. Putnam, N. H. et al. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317, 86–94 (2007).
38. Chapman, J. A. et al. The dynamic genome of Hydra. Nature 464, 592–596 (2010).
39. Ryan, J. F. et al. The homeobox domain complement of the ctenophore Mnemiopsis leidyi suggests that Ctenophora and Porifera diverged prior to the ParaHoxozoa. Mol. Biol. Evol. 19, 207, 4515–4524 (2004).
40. Srivastava, M. et al. The Trichoplax genome and the nature of placozoans. Nature 454, 955–960 (2008).
41. Srivastava, M. et al. The Amphimedon queenslandica genome and the evolution of animal complexity. Nature 466, 720–726 (2010).
42. King, N. et al. The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans. Nature 451, 783–788 (2008).
43. Ruiz-Trillo, I. et al. The origins of multicellularity: a multi-taxon genome initiative. Trends Genet. 23, 113–118 (2007).
44. Merchant, S. S. et al. The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science 318, 245–250 (2007).
45. Banks, J. A. et al. The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science 332, 960–963 (2011).
46. Eichinger, L. et al. The genome of the social amoeba Dictyostelium discoideum. Nature 435, 43–57 (2005).
47. Fritz-Laylin, L. K. et al. The genome of Naegleria gruberi illuminates early eukaryotic versatility. Cell 140, 631–642 (2010).
48. Fernandez-Fueyo, E. et al. Comparative genomics of Cerioporiopsis subvermispora and Phanerochaete chrysosporium provide insight into selective ligninolysis. Proc. Natl. Acad. Sci. USA 109, 5458–5463 (2012).
49. Wood, V. et al. The genome sequence of Schizosaccharomyces pombe. Nature 415, 871–880 (2002).
50. Burge, C. & Karlin, S. Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. 268, 78–94 (1997).
51. Shinzato, C. et al. Using the Acropora digitifera genome to understand coral responses to environmental change. Nature 476, 320–323 (2011).
52. Hunter, S. et al. InterPro: the integrative protein signature database. Nucleic Acids Res. 37, D211–D215 (2009).
53. Finn, R. D. et al. The Pfam protein families database. Nucleic Acids Res. 38, D211–D222 (2009).
54. Lupas, A., Van Dyke, M. & Stock, J. Predicting coiled coils from protein sequences. Science 252, 1162–1164 (1991).
55. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797 (2004).
56. Castresana, J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552 (2000).
57. Abascal, F., Zardoya, R. & Posada, D. ProtTest: selection of best-fit models of protein evolution. Bioinformatics 21, 2104–2105 (2005).
58. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882 (1997).
59. Guindon, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321 (2010).
60. Altekar, G., Dwarkadas, S., Hueslenbeck, J. P. & Ronquist, F. Parallel Metropolis-coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20, 407–415 (2004).
61. Ronquist, F. & Hueslenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574 (2003).
62. Genkinovich, G. & Technau, U. In situ hybridization of starlet sea anemone (Nemanotella vectensis) embryos, larvae, and polyps. Cold Spring Harb. Protoc. 2009, pdb.prot5282 (2009).
63. Larroux, C. et al. Developmental expression of transcription factor genes in a demosponge: insights into the origin of metazoan multicellularity. Evol. Dev. 8, 150–173 (2006).
64. Larroux, C. et al. Whole-mount in situ hybridization in Amphimedon. Cold Spring Harb. Protoc. 2008, pdb.prot5096 (2008).
65. Hammel, J. U., Herzen, J., Beckmann, F. & Nickel, M. Sponge budding is a spatiotemporal morphological patterning process: insights from synchrotron radiation-based x-ray microtomography into the axialsexual reproduction of Tethya wilhelma. Front. Zool. 6, 19 (2009).
66. Weissentals, N. Scanning electron microscope histology of spongy Ephydatia fluviatilis. Microsc. Acta 85, 345–350 (1982).
67. Nickel, M., Donath, T., Schweikert, M. & Beckmann, F. Functional morphology of Tethya species (Porifera): 1. Quantitative 3D-analysis of Tethya wilhelma by synchrotron radiation based X-ray microtomography. Zoomorphology 125, 209–223 (2006).