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can help to establish a wiring diagram and guide rigorous theoretical models. The ability to examine a complex sensorimotor integration center down to the level of individual synaptic connections is incredibly powerful.

In conclusion, the central complex is a fascinating sensorimotor hub that offers the tantalizing hope of fully understanding the neural workings of aspects of navigation that are relevant across species. It takes in sensory and motor input, tracks heading, possibly with an attractor network, and generates predictive motor outputs. Learning, memory, and context also help shape the activity in this network, adding further flexibility to the workings of this region. It is no wonder that this powerful set of neurons is conserved across insects and crustaceans, and it will be fascinating to learn how the structure has evolved to meet each species' specific needs.

FURTHER READING

el Jundi, B., Warrant, E.J., Byrne, M.J., Khaldy, L., Baird, E., Smolka, J., and Dacke, M. (2015). Neural coding underlying the cue preference for celestial orientation. Proc. Natl. Acad. Sci. USA *112*, 11395–11400.

Harley, C.M., and Ritzmann, R.E. (2010). Electrolytic lesions within the central complex neuropils of the cockroach brain affect negotiation of barriers. J. Exp. Biol. *213*, 2851–2864.

Heinze, S., and Homberg, U. (2007). Maplike representation of celestial E-vector orientations in the brain of an insect. Science *315*, 995–997.

Loesel, R., Nässel, D.R., and Strausfeld, N.J. (2002). Common design in a unique midline neuropil in the brain of arthropods. Arthropod Struct. Dev. *31*, 77–91.

Martin, J.P., Guo, P., Mu, L., Harley, C.M., and Ritzmann, R.E. (2015). Central-complex control of movement in the freely walking cockroach. Curr. Biol. *25*, 2795–2803.

Müller, M., Homberg, U., and Kühn, A. (1997). Neuroarchitecture of the lower division of the central body in the brain of the locust. Cell Tissue Res. *288*, 159–176.

Neuser, K., Triphan, T., Mronz, M., Poeck, B., and Strauss, R. (2008). Analysis of a spatial orientation memory in Drosophila. Nature *453*, 1244–1247.

Ofstad, T.A., Zucker, C., and Reiser, M. (2011). Visual place learning in Drosophila melanogaster. Nature *474*, 204–207.

Pfeiffer, K., and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. Annu. Rev. Entomol. *59*, 165–184.

Seelig, J.D., and Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. Nature *521*, 186–191.

Strausfeld, N. (2012). The Brain within the Brain in Arthropod Brains (Cambridge, MA: The Belknap Press).

Wolff, T., Iyer, N., and Rubin, G. (2014). Neuroarchitecture and neuroanatomy of the Drosophila central complex: A GAL4-based dissection of protocerebral bridge neurons and circuits. J. Comp. Neurol. *523*, 997–1037.

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Dendrogramma is a siphonophore

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Dendrogramma was the iconic deep-sea animal of 2014, voted among the top-ten new species described that year [1]. The two species described are mushroom shaped animals, diploblastic, with an apparent gastrovascular system that extends from the base of the stalk to bifurcating canals that radiate through the flat disc [2]. The authors could not assign the new genus to any known animal group with certainty, leading to numerous media reports that it belonged to an entirely new phylum. Here we use phylogenomic data from newly collected specimens to show that *Dendrogramma* is a cnidarian, specifically a benthic siphonophore in the family Rhodaliidae. Although an entire *Dendrogramma* colony has not been found, we hypothesise that the mushroom-like bodies are bracts, possibly used to aid buoyancy or as defensive appendages to protect feeding gastrozooids or gonads.

In November 2015, an expedition by the Australian research vessel R/V Investigator to the continental slope off South Australia recovered 85 new specimens of *Dendrogramma* (Figure 1A,B). We preserved some specimens in RNALater and after the voyage successfully sequenced a partial transcriptome from extracted RNA. These are the first genetic data available for these species, as the original specimens were collected and preserved in formalin in 1986, which hindered extraction of DNA [2]. We targeted exons used in a recent metazoan phylogenomic study [3] as well as the ribosomal RNA genes (18S, 28S and 16S) used in older siphonophore-focused investigations [4,5]. Phylogenetic analyses of the exon data placed *Dendrogramma* as sister to two physonect siphonophores (*Agalma elegans* and *Nanomia bijuga*, 100% bootstrap support; Figure 1C, Supplemental information). The ribosomal BLAST and phylogenetic analyses identified the benthic siphonophore *Stephalia dilata* (family Rhodaliidae) as the nearest sequenced taxon to *Dendrogramma* (16S d_{xy} = 0.013; Supplemental information).

Siphonophores are bizarre pelagic colonial cnidarians in the class Hydrozoa. They are complex elongate or spherical organisms with specialised locomotive and feeding zooids, and a net of tentacles that can be extended to catch prey or attach to the seafloor [6]. There are 175 described species, living in a range of habitats from the sea surface (e.g., *Physalia physalis*, the Portuguese Man O'War) to the deep-sea [6]. Larger, more delicate species have been found mainly in the non-turbulent mesopelagic (300–1000 m) or bathypelagic zones (1000–3000 m).

A typical siphonophore has a pneumatophore for floatation, a short nectosome with nectophores (swimming bells) used for propulsion, and a siphosomal stem with repeating units called cormidia. Cormidial units have gastrozooids with long feeding tentacles, gonodendra for reproduction and bracts used for floatation or protection [6,7].

We hypothesise that the mushroom-shaped *Dendrogramma* is a cormidial bract. These can take various shapes, but notably rhodaliid genera such as *Tridensia*, *Arancialia* and *Archangelopsis* have detachable mushroom-like bracts with a central canal up the stalk that can branch through the disc on some species [7,8] (Supplemental information). *Dromalia* has quite complex branching, although the bracts are elongate [7]. While bract morphology is only available for ten of the fourteen species of the Rhodaliidae, bracts are typically small appendages that reach 2–6 mm in size. In contrast our largest specimen of *Dendrogramma* is 20 mm in diameter with bracteal canals that can branch at least five times, almost to the edge of the rounded disc. The canals are a pink or orange colour in life (Supplemental information). The 'mouth' of *Dendrogramma* is the attachment surface that articulates with a triangular bracteal lamella on the cormidial stem [8].

We also found an orange gas-filled object at two separate sites (Supplemental information), which is similar in appearance to a rhodaliid pneumatophore and the base of the siphosomal stem, denuded of all nectophores and cormidial units. Ribosomal DNA (16S, 28S) from one of these objects was identical to that found from the *Dendrogramma* bracts from the same site.

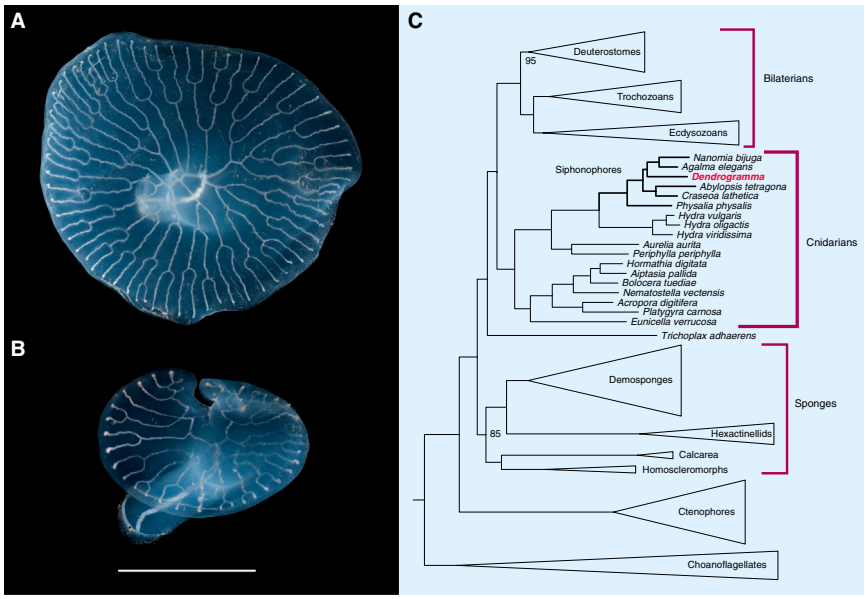

Figure 1. *Dendrogramma* in the tree of animal life.
Dendrogramma bracts showing the (A) 'discoides' and (B) 'enigmatica' morphologies (scale bar = 10 mm). (C) Simplified phylogenomic tree of the Metazoa, predominantly derived from Whelan *et al.* 2015 [3], showing the position of *Dendrogramma*. Bootstrap values are 100% unless otherwise indicated. The full tree is available in Supplemental Figure S1A.

Based on the phylogenetic analyses, available morphology, and the benthic environment from which specimens were collected, we conclude that *Dendrogramma* is a siphonophore in the family Rhodaliidae and that the Dendrogrammatidae is a junior synomym of that family. However, we have insufficient genetic or morphological evidence to identify *Dendrogramma* as a known rhodaliid genus or species. No described rhodaliid bracts are as large or with such complex canal branching patterns as *Dendrogramma*.

Additionally, we have evidence that there is only one, not two, species of *Dendrogramma*. Specimens with notched (*D. enigmatica*) or rounded (*D. discoides*) discs and long (*D. enigmatica*) or short (*D. discoides*) stalks (Figure 1A,B) were found in the same collection sample, with identical 16S sequences, and overall, collection site explains the low level of 16S variation ($\pi = 0.0015$) better than morpho-type (Figure S1C). Rhodaliid bract morphology is known to vary with ontology and location on the siphonosome. New bracts can replace older ones that become detached [7]. On this basis, we consider the two nominal species of *Dendrogramma* to be synonyms. The species *D. enigmatica* is the senior (and more appropriate) name as it was described first and nominated as the type species of the genus. *Dendrogramma enigmatica* is now known to occur around southern Australia from 34–42°S and 129–150°E at depths between 400 and 2900 m. All specimens have been collected with devices that sample surficial sediments and 0.5 m above the sediment, suggesting it lives attached to or just above the seafloor.

Genetic evidence has been used to shed light on many of the mysteries of evolution. Enigmatic deep-sea creatures such as vestimentiferans and pognophorans, at one point placed in their own phyla, have subsequently been found to be in the same family of polychaete worms [9]. On the other hand the simple bag-like *Xenoturbella* has been found to be a distinct bilaterian lineage [10]. Voyages of discovery continue to find new and engrossing animals in the deep-sea, including from the use of modern submersible technologies [4,10]. The description of life on our blue planet is far from complete.

SUPPLEMENTAL INFORMATION

Supplemental information includes two figures, experimental procedures and author contributions and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.04.051.

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REFERENCES

1. International Institute for Species Exploration (2015). The ESF Top 10 New Species for 2015. http://www.esf.edu/species/ Last assessed 18th Jan 2016.
2. Just, J., Kristensen, R.M., and Olesen, J. (2014). *Dendrogramma*, new genus, with two new non-Bilaterian species from the marine bathyal of southeastern Australia (Animalia, Metazoa *incertae sedis*) – with similarities to some medusoids from the Precambrian Ediacara. PLoS One *9*, e102976.
3. Whelan, N.V., Kocot, K.M., Moroz, L.L., and Halanych, K.M. (2015). Error, signal, and the placement of Ctenophora sister to all other animals. Proc. Natl. Acad. Sci. USA *112*, 5773–5778.
4. Dunn, C.W., Pugh, P.R., and Haddock, S.H. (2005). Molecular phylogenetics of the Siphonophora (Cnidaria), with implications for the evolution of functional specialization. Syst. Biol. *54*, 916–935.
5. Cartwright, P., Evans, N.M., Dunn, C.W., Marques, A.C., Miglietta, M.P., Schuchert, P., and Collins, A.G. (2008). Phylogenetics of Hydroidolina (Cnidaria, Hydrozoa). J. Mar. Biol. Assoc. UK *88*, 1663–1672.
6. Mapstone, G.M. (2014). Global diversity and review of Siphonophorae (Cnidaria: Hydrozoa). PLoS One *9*, e87737.
7. Pugh, P.R. (1983). Benthic siphonophores: A review of the family Rhodaliidae (Siphonophora, Physonectae). Philos.Trans. R. Soc. Lond. B *301*, 165–300.
8. Hissmann, K. (2005). In situ observations on benthic siphonophores (Physonectae: Rhodaliidae) and descriptions of three new species from Indonesia and South Africa. Systematics Biodiversity *2*, 223–249.
9. Halanych, K.M., Lutz, R.A., and Vrijenhoek, R.C. (1998). Evolutionary origins and age of vestimentiferan tube-worms. Cahiers de Biologie Marine *39*, 355–358.
10. Rouse, G.W., Wilson, N.G., Carvajal, J.I., and Vrijenhoek, R.C. (2016). New deep-sea species of *Xenoturbella* and the position of Xenacoelomorpha. Nature *530*, 94–97.

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Supplemental Information: *Dendrogramma* is a siphonophore

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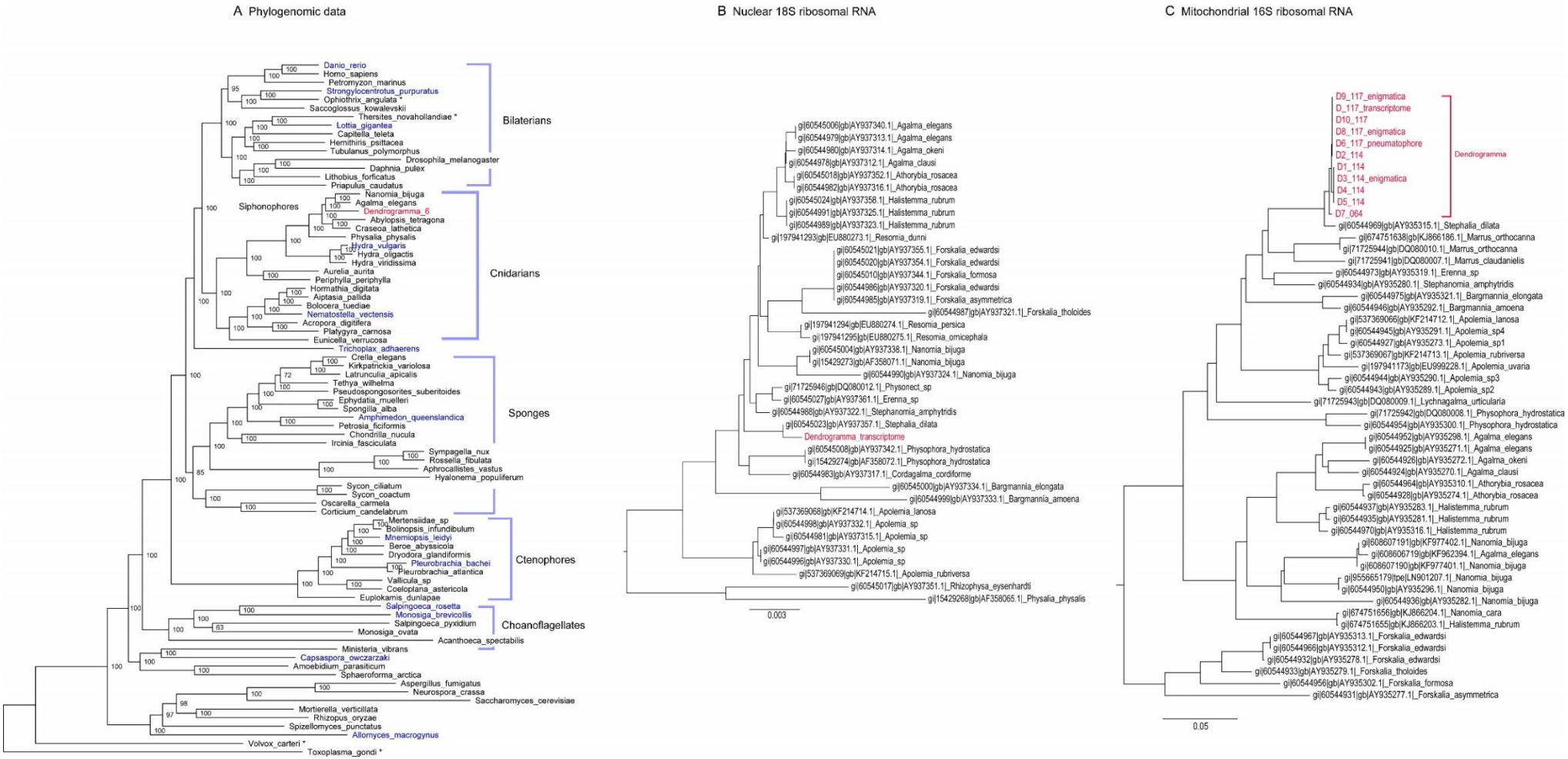
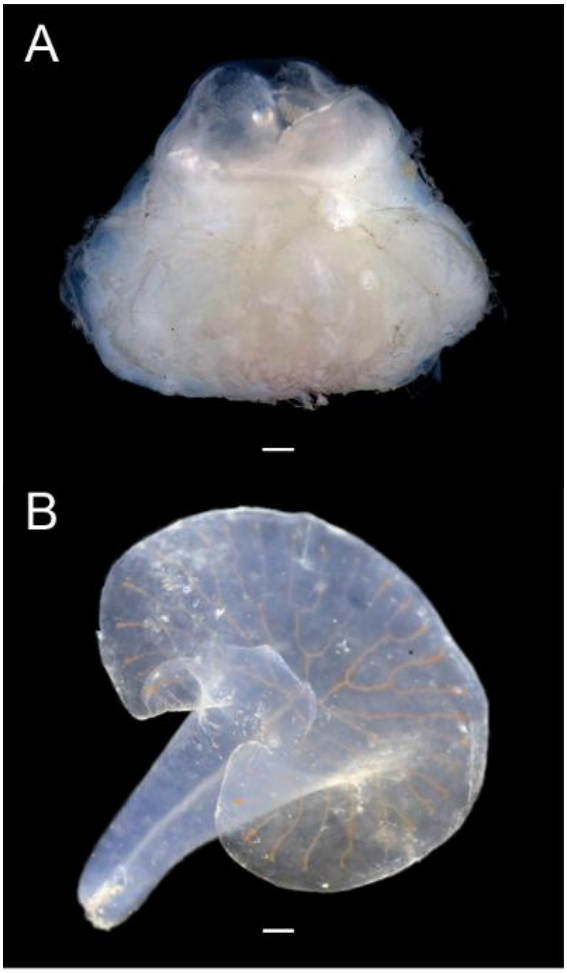
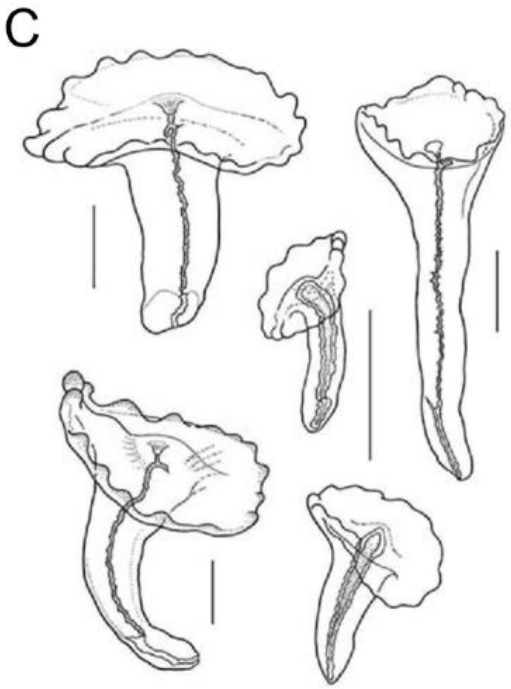


Figure S1 (A). RAxML tree of the Metazoa derived from Whelan et al 2015 [S6] with label names and bootstrap values. Taxa additional to Whelan et al. include *Dendrogramma* (red) and four others (indicated by asterisk). Taxa used as proteome references indicated in blue. (B) 18S and (C) 16S p-distance neighbour-joining trees of NCBI Physonectae taxa and *Dendrogramma* samples, midpoint rooted. The 16S *Dendrogramma* sample labels include the sample code and survey station number: D6 is a pneumatophore; D3, 8 and 9 are bracts with *D. enigmatica* morphology, the rest are similar to *D. discoides*. 16S nucleotide diversity (π) within *Dendrogramma* = 0.0015, distance (d_{yx}) to *Stephalia dilata* = 0.13.

Figure S2 (A) *Dendrogramma* preserved pneumatophore and corm; (B) Living *Dendrogramma* showing the orange coloured canals; C) Line drawings of detached bracts of *Arancialia captonia* (Rhodaliidae) (with permission from Hissmann 2005, fig. 13 [S10]). Scale bars = 1 mm





Supplemental Experimental Procedures

Samples and sequencing

The new *Dendrogramma* material was collected on the 13th and 24th November 2015 by benthic beam trawl on the MNF Investigator from four sites between 1000 and 2800 m in the Great Australian Bight, off southern Australia (see associated DRYAD repository). The material was hand-picked from the cod-end and pieces and promptly preserved in RNALater (Biomatrica), 95% ethanol or formalin. The specimens have been archived in the natural history collection of Museum Victoria (Melbourne, Australia) which also contains the type material of both described species of *Dendrogramma*, (preserved in formalin) as well as additional unreported material from a 1988 expedition (also preserved in formalin).

RNA was extracted from one of the RNALater-preserved specimens using a Qiagen RNeasy kit (Qiagen, Victoria, Australia) and subsequent high-throughput sequencing was done via Australian Genomic Research Facility (AGRF) (http:// http://www.agrf.org.au/) using Illumina TruSeq mRNA Libraries and a MiSeq sequencer, returning 15.4 million paired-end reads. In addition we extracted genomic DNA from ten 95% ethanol preserved specimens using a Qiagen DNeasy Blood & Tissue Kit and PCR-amplified and sequenced fragments of the ribosomal genes 16S and 28S.

Transcriptome assembly

Adapters and low quality read regions were removed using Trimmomatic-0.33 [S1]. Trinity assembly (v20140717) [S2], using trimmed reads and min_kmer_cov of 3, resulted in 41K contigs. The assembly was screened for nuclear and mitochondrial targets (28S/18S/16S rRNA and COI) to provide a general 'barcode' scan against the NCBI nr/nt database. In the transcriptome, large subunit rRNA contigs included only *Dendrogramma* and laboratory contaminant bacterial *E. coli*, while mitochondrial contigs also included low-level echinoderm contamination (ophiuroid and holothurian; probably due to deep-sea trawl capture).

'Barcode' sequence analyses

The NCBI BLASTn [S3] 'barcode' searches indicated siphonophores as closest taxa. Subsequently, all Physonectae 18S (38) and 16S (43) gene sequences were downloaded from NCBI, aligned with the *Dendrogramma* transcriptome and PCR sequences (MAFFT [S4]), trimmed to a major common length (18S = 1740 sites; 16S = 619 sites) and simple p-distance neighbour-joining trees generated (PAUP [S5]) to indicate the distances among sequences (Figure S1B & C).

In addition to the transcriptome sample 10 other samples were sequenced by PCR for sections of 16S and 28S. Together the 11 samples covered three station sites, both *Dendrogramma* (*enigmatica* and *discoides*) morphologies, and an unidentified pneumatophore fragment. All 28S sequences were identical (750 bases in common), while the 16S sequences ranged from 0-3 base differences.

Phylogenomic analysis

Our project goal was not to redraw the Tree of Life but to identify the likely phylogenetic position of *Dendrogramma*. Consequently, we decided to use an established data-matrix, leveraging the work already done in selecting loci and sequences, and enhancing likely ortholog selection in additional taxa. We chose the 251 gene Whelan et al. [S6] matrix as potentially able to resolve any major lineage within the Metazoa. Our broad strategy was to 1) use multi-reference reciprocal BLAST to identify the *Dendrogramma* contigs that were orthologous to gene sequences in the Whelan et al. dataset, 2) align and append the contigs to the Whelan et al. dataset, and 3) final manual check of the aligned data-matrix guided by individual locus trees. UNIX 'bash' shell scripts were developed to perform or assist in each of these stages. All transcriptome and proteome sequence matching was done using BLAST (v2.2.3) [S3] retaining the single best match (and matching amino acid sequence block) per locus with maximum e-value 10^{-15}.

Major issues with field-sampled partial transcriptomes are false-orthologs (wrong gene is present but right one is not) and contaminants (right gene but wrong organism), and particularly so at very large phylogenetic scales, where tree-based orthology methods have limited utility [S7, S8]. Hence we used a genome-based reference set of 13 phylogenetically-divergent taxa included by Whelan et al. (indicated on Figure S1A).

The Whelan matrix is a modified and trimmed alignment. Hence we constructed our reference dataset from the original genome-based proteomes, by extracting sequences for the 13 representative taxa from the Whelan matrix (script TEFORM3), and using BLAST [S3] to locate the proteome equivalent (script COLLATE2). The number of reference sequences we obtained for each locus ranged from 3-12 (median 8). Being based on proteomes, these references should be relatively free of false-orthologs and contaminant problems, while being spread across the phylogenetic range of the major lineages should aid paralog and in-group contaminant exclusion.

Dendrogramma contigs were then screened for matches to our reference dataset (via BLAST) and candidates retained only if the best matching contig was also the one that was identified by the majority of the references (script COLLATE3a). This eliminated on average 6% of candidates. These candidate sequences were then matched against the entire reference proteomes (reciprocal BLAST) and discarded if more than one-fifth of the reference matches was not the previously identified reference target (script COLLATE3b). This helped exclude paralogs, and removed a further 7% of candidate contigs.

The new sample sequences, of more than 50 amino-acids in length, were then added to the original Whelan gene alignments using MAFFT (v7.245: -add, L-INS-I, max-iteration 100) [S4], keeping the original alignment length (i.e. removing novel global inserts) (scripts COLLATE3c, BULK5). A new matrix of all 251 genes was then assembled, and in conjunction with the gene trees, the matrix was manually scanned for spurious sequences (confirmed by NCBI BLAST searches; typically protozoan or bacterial contaminants; 1% removed) and implausible alignments (e.g. highly fragmented end-sections), including a number (258 = 2%) of sequences in the original Whelan et al. dataset.

We identified 155 of the target 251 genes from the *Dendrogramma* transcriptome. In addition, two other transcriptomes and two protozoan proteomes were included (indicated on Figure S1A) to check pipeline performance and aid contaminant detection. The final matrix of 251 genes (81,008 sites) for 81 taxa (58% data-complete) was subjected to RAxML fast-

bootstrap phylogenetic analysis (v8.2.4 via CIPRES) [S9] using a single PROTGAMMALG model, adequate for the purposes here [S8]: The resulting tree (Figure S1A) is essentially the same (all but one node) as that published by Whelan et al. [S6].

Accession Numbers

Phylogenomic data, a list of specimens, and scripts have been deposited at DRYAD (http://dx.doi.org/10.5061/dryad.7qs68). Ribosomal RNA gene sequences deposited in GenBank with accession numbers KU716053-KU716075.

Author Contributions

T. O'H., A.F.H. and A.M. conceived the study. A.W. and H.M. designed and conducted the sampling. A.M. and K.N. performed the laboratory work. A.F.H. developed the bioinformatics pipelines and performed the phylogenomic analyses. T. O'H., A.F.H. and H.M. took the lead in writing the manuscript.

Supplemental References

S1. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics *30*, 2114-2120.

S2. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol *29*, 644–652.

S3. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res *25*, 3389-3402.

S4. Katoh, K., and Standley, D.M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol *30*, 772-280.

S5. Swofford, D.L. (2003). PAUP*. Phylogenetic Analysis using Parsimony (* and other methods). Version 4. (Sunderland, Massachusetts: Sinauer Associates).

S6. Whelan, N.V., Kocot, K.M., Moroz, L.L., and Halanych, K.M. (2015). Error, signal, and the placement of Ctenophora sister to all other animals. Proc Natl Acad Sci USA *112*, 5773-5778.

S7. Philippe, H., Brinkmann, H., Lavrov, D.V., Littlewood, D.T.J., Manuel, M., Worheide, G., and Baurain, D. (2011). Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol *9*, e1000602.

S8. O'Hara, T.D., Hugall, A.F., Thuy, B., and Moussalli, A. (2014). Phylogenomic resolution of the Class Ophiuroidea unlocks a global microfossil record. Curr Biol *24*, 1874-1879.

S9. Stamatakis, A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics *30*, 1312-1313

S10. Hissmann, K. (2005). In situ observations on benthic siphonophores (Physonectae: Rhodaliidae) and descriptions of three new species from Indonesia and South Africa. Syst Biodivers *2*, 223-249.