

Miscues misplace sponges

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In contrast to several recent publications (1–5), Pisani et al. (6) claim that (i) genomic data do not support ctenophores as the sister group to other animals and (ii) independent evolution of complex features (e.g., neurons, muscles) in ctenophores is not supported. These claims are based on selective interpretation, subjective criteria, and improper assumptions about original analyses.

Pisani et al.'s (6) conclusions about evolution improperly treat complex traits as single characters. Features such as neuromuscular systems possess several independently evolving cell lineages and mechanisms. Given that underlying components of these systems use remarkably different molecular machinery (1), whether Porifera or Ctenophora is sister to other animals is irrelevant to interpreting evolutionary origins of neuromuscular systems (1). Pisani et al. (6) also ignore placozoan placement in their interpretations. Evolution of integrative systems is more complex than simple gains or losses, and no information is provided supporting a single origin of these complex systems.

Pisani et al. (6) misrepresent studies (1, 2) by implying that a single homogenous partition was used rather than partitioned analyses. For example, the Whelan-16 dataset modeled 89 data partitions, not 1, as implied by Pisani et al. (6). Thus, claims from model validation tests are unsupported because approaches used by others (1, 2) were not examined. Moreover, rationalizations for superiority of CAT (category) models rely on assumptions about the "true tree," including sponge placement (7), making arguments about model superiority circular. Furthermore, discussion of Bayesian analyses is meaningless if Markov chains do not reach convergence. However, incomplete analyses are extensively discussed to support Pisani et al.'s (6) conclusions.

Perhaps most concerning is the assumption that using only closely related outgroups yields the correct result. Whelan et al. (2) used objective criteria to examine many sources of systematic error, including long-branch attraction and outgroup choice, as well as explicit hypothesis-testing approaches (table 1 in ref. 2). No such objective approaches were used by Pisani et al. (6). Since Whelan et al. (2), we have further explored amino acid composition as a source of error. Compared with other taxa, choanoflagellates and select sponge lineages (e.g., *Hyalonema*) show high amino acid compositional heterogeneity, potentially causing artifacts. Using only choanoflagellates may exacerbate artifacts arising from amino acid composition biases, and Pisani et al. (6) do not examine datasets that controlled for such compositional heterogeneity (as in ref. 2). Pisani et al. (6) examine two datasets from Whelan et al. (2) that were filtered for select sources of systematic error, but not base compositional heterogeneity like others from Whelan et al. Analyses of these datasets yielded unconventional bilaterian relationships with protostomes nested in a paraphyletic Deuterostomia (2, 6). Furthermore, these datasets were small, and larger datasets, with arguably more phylogenetic signal, were casually dismissed. Additionally, a single gene, opsin, was arbitrarily used to bolster their argument over results from hundreds of other genes. Finally, use of gene content for interphyletic relationships is questionable because gene content varies greatly within phyla, and taxon sampling was limited.

The definitive tone of Pisani et al. (6) is not warranted. They recover Ctenophora-sister in multiple analyses but dismiss these findings without objective criteria. Thus, the comment "we found no support for Ctenophora-sister" (6) is incorrect.

- 1 Moroz LL, et al. (2014) The ctenophore genome and the evolutionary origins of neural systems. *Nature* 510(7503):109–114.
- 2 Whelan NV, Kocot KM, Moroz LL, Halanych KM (2015) Error, signal, and the placement of Ctenophora sister to all other animals. *Proc Natl Acad Sci USA* 112(18):5773–5778.
- 3 Ryan JF, et al.; NISC Comparative Sequencing Program (2013) The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* 342(6164):1242592.

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- 4 Borowiec ML, Lee EK, Chiu JC, Plachetzki DC (2015) Extracting phylogenetic signal and accounting for bias in whole-genome data sets supports the Ctenophora as sister to remaining Metazoa. *BMC Genomics* 16(1):987.
- 5 Chang ES, et al. (2015) Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. *Proc Natl Acad Sci USA* 112(48):14912–14917.
- 6 Pisani D, et al. (2015) Genomic data do not support comb jellies as the sister group to all other animals. *Proc Natl Acad Sci USA* 112(50):15402–15407.
- 7 Philippe H, et al. (2011) Resolving difficult phylogenetic questions: Why more sequences are not enough. *PLoS Biol* 9(3):e1000602.