



COMMENT

Do Micrognathozoa have micro-genomes?

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Based on the extremely small sizes of their jaw components, I predict that members of the Micrognathozoa will have some of the smallest nuclear genomes of any metazoans or, possibly, even of any free-living (non-parasitic) eukaryotes. Micrognathozoan jaws may also be enervated by anucleate neurons. Consistent with the prediction of small genomes, micrognathozoan jaw parts have remarkably small cell nuclei. Identical arguments may apply to other members of the Gnathifera, namely Rotifera and Gnathostomulida. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **112**, 640–644.

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An important tradition in evolutionary biology, going back at least to Georges Cuvier, is that form tells us something about function, and vice versa (Russell, 1916), a tradition that is still active (Vogel, 1988, 2012). There is an equally important, albeit less well-emphasized, tradition in using macro-form (morphology) to tell us something about micro-form (anatomy), and vice versa. In that vein, there is a well-accepted notion that chromosomes take up a lot of space. A cell nucleus is supposedly no larger than it absolutely needs to be. Double the nuclear genome size via polyploidy (i.e. whole genome duplication; see Gorelick & Olson, 2013) and the nucleus will be roughly twice the volume. Polyploid cells are larger than their diploid progenitors (Lomax *et al.*, 2009). This is how Masterson (1994) inferred polyploidy in fossil flowering plants by measuring the size of stomatal guard cells. This is why diploid and polyploid amphibian red blood cells are larger than mature mammalian red blood cells, which lack nuclei. Cells with large genomes also have slower rates of mitotic division – it takes lots of time and energy to replicate chromosomes – consequently, they

have smaller numbers of cells throughout the body (Fankhauser, 1945; Nurse, 1985; Henery, Bard & Kaufman, 1992; Roth, Nishikawa & Wake, 1997; Conlon & Raff, 1999). The converse is that animals with very small genomes should have lots of small cells that undergo frequent mitotic divisions. '[T]he size of a cell is proportional to its ploidy. . . [but] the relationship between ploidy size does not extend to the size of the whole animal or its organs, as polyploid animals usually compensate and have correspondingly fewer cells than the corresponding diploid animals' (Conlon & Raff, 1999: 236) (citing Fankhauser, 1952; Nurse, 1985; Henery *et al.*, 1992). [Nematodes, rotifers, tardigrades, gastrotrichs and dicyemids are often considered eutelic, i.e. have a finite number of mitotic divisions per sexual generation, and hence cannot grow larger by increasing the number of their mitotic divisions (van Cleave, 1932). However, it is not altogether certain whether these taxa are truly eutelic (e.g. Rusin & Malakhov, 1998; Cunha *et al.*, 1999).] Because genomes of eubacteria and archaea are typically so tiny, they typically have cell sizes orders of magnitude smaller than those of eukaryotes. Similarly, the ophisthokonts with the smallest cells also have the smallest genomes, namely members of the Microsporidia. The

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microsporidian *Encephalitozoon (Septata) intestinalis* has a C-value of 0.0023 pg (Vivares, 1999; Gregory, 2014) and microsporidian cells that infect mammals are only 1.0–3.0 $\mu\text{m} \times 1.5$ –4.0 μm in diameter (Vávra & Larsson, 1999). Typical animals have cell diameters in the range 10–20 μm (Guertin & Sabatini, 2006) and nuclei with diameters in the range 5–7 μm (Alberts *et al.*, 2002). Animal genomes (C-values) range from 0.1 to 100.0 pg (Gregory, 2014).

Members of Micrognathozoa seem to have outrageously small cells and incredibly rich architectures, especially in their mouthparts (Kristensen & Funch, 2000; Sørensen, 2003). Mature specimens of the only known member of the Micrognathozoa, *Limnognathia maerski*, are only 150 μm long (Kristensen & Funch, 2000). However, it is the micrognathozoan jaw apparatus, for which they are named, that is most amazing. The entire jaw apparatus is 15 μm in diameter (roughly twice the length of a mature human red blood cell; Turgeon, 2004), composed of many adjacent structural elements, called sclerites, that are only 2–10 μm long and often less than 0.25 μm in diameter (Sørensen, 2003). Furthermore, micrognathozoan sclerites are attached to a multitude of tiny muscles and ligaments (tendons) and to a small ganglion by nerve cells. How can metazoan cells be that small? Sørensen (2003) contains gorgeous pictures of micrognathozoan jaws. Illustrated papers on jaws of the closely related rotifers can be found at the ‘rotifer trophi web page’ (http://www.rotifera.hausdernatur.at/Rotifer_data/trophi/start.html). So-called ‘minor phyla’ are those that contain small animals (sometimes with many cryptic species, e.g. Kinorhyncha and Tardigrada; Piper, 2013). Micrognathozoa and other ‘minor phyla’, especially other members of the Gnathifera (Rotifera and Gnatostomulida), can provide crucial insights into metazoan evolution, especially because ‘these clearly show that small and simple animals do not necessarily represent ancestral or primitive taxa’ (Garey & Schmidt-Rhaesa, 1998: 907), but often have elaborate highly derived architectures.

Are micrognathozoan sclerites, and accompanying muscles, ligaments and nerves, not cells, but just parts of cells? An analogous case exists with peristome teeth on some mosses, which are composed of cell walls rather than whole cells (arthrodontous versus nematodontous peristome teeth; Tyshing & Gibson, 2006; Shaw, Szövényi & Shaw, 2011). Maybe it is not so ironic that micrognathozoans were first found on mosses. Tiny complex structures need not be whole cells, but can even be organelles, as with cnidocysts in Cnidaria and the possibly homologous apicoplasts in Apicomplexa (Slautterback & Fawcett, 1959; Shostak, 1993). Micrognathozoan sclerites are extracellular cuticles that are dead when func-

tional, much like vascular plant tracheids and vessels or non-vascular plant hydroids (Evert, 2006; Vanderpoorten & Goffinet, 2009). However, it seems highly unlikely that accompanying muscles and nerves are anything but whole cells.

If micrognathozoan jaw muscles and nerves are each individual cells, is it possible that these cells lack nuclei, such as we see in plant phloem sieve cells? The smallest flying insects are 170 μm long – about the same size as *Limnognathia maerski* – and lack nuclei in the neurons of mature insects. These parasitic wasps have nuclei in cell bodies during larval stages, but their neuronal nuclei and cell bodies lyse during metamorphosis (Polilov, 2012). Insects that do this in the genus *Megaphragma* have physically smaller adults than larvae. Micrognathozoans, however, are not known to have large larvae. All metazoan muscle cells seem to contain nuclei. Several muscle cells can fuse to form a multinucleate muscle fibre, a syncytium (Daubenmire, 1936), but this probably does not happen in micrognathozoan jaws, whose muscle fibres are probably uninucleate cells. Muscle cells in most animals are also notoriously endopolyploid (Anatskaya & Vinogradov, 2004), which would make muscle cells even larger. I propose that micrognathozoan jaws are enervated by anucleate sensory neurons with jaw muscles that are uninucleate and not aggregated in a syncytium. There does not appear to currently be any microscopic evidence either for or against micrognathozoan jaw neurons lacking nuclei (Martin Sørensen, pers. comm.).

Because of their muscle fibres in small spaces amongst sclerites, I predict that micrognathozoans have extremely small genomes. Supporting this prediction, many parasitic nematodes are very small animals with many cells, albeit not as diminutive as micrognathozoan adults. Nematodes have the smallest known metazoan genomes, as small as 0.02 pg in several species (Leroy *et al.*, 2007; Gregory, 2014). Although nematodes do not have the smallest eukaryotic genomes, I suspect that the elaborate architecture of micrognathozoan jaws with their numerous muscle fibres indicates that they have an even smaller genome than most nematodes.

Rotifera trophi are composed of sclerites, which are small, intricate, hard, extracellular cuticles that originated from multiple cells and thus are very similar to Micrognathozoan sclerites (Markevich & Kutikova, 1989; Kristensen & Funch, 2000; Segers, 2004). Rotifer sclerites are extracellular chitinous cuticles (Klusemann, Kleinow & Peters, 1990; Segers, 2004) and thus do not grow once functional (Fontaneto, Melone & Wallace, 2003), which probably also applies to Micrognathozoa and other related taxa. Rotifer muscles are usually unicellular and uninucleate, with a few bicellular exceptions (Clément & Amsellem,

1989). Rotifers (including Acanthocephala and Seisonidae) and Gnathostomulida are closely related to Micrognathozoa, forming the clade Gnathifera (Sørensen *et al.*, 2000), with a broad consensus growing that members of Gnathifera are closely related to Gastrotricha and Platyhelminthes in the superphylum Platyzoa (Giribet *et al.*, 2000; Peterson & Eernisse, 2001; Edgecombe *et al.*, 2011). Gnathifera was 'founded on the basis of a special ultrastructure of the pharyngeal hardparts', which are complex (Ahlrichs, 1997: 41; Sørensen, 2002). Therefore, rotifers and gnathostomulids may also have small genomes and anucleate neurons enervating their jaws. However, C-values for rotifers are in the range of 0.25 pg (*Adineta vaga*) to 1.22 pg (*Philodena roseola*) (Mark Welch & Meselson, 1998; Mark Welch & Meselson, 2003; Gregory, 2014), which are small, but not ultra-small. Wulfken & Ahlrichs (2012) compared the ultrastructure of the jaw apparatus (mastax, including sclerites) of the small-genome species *Adineta vaga* with that of other rotifers, but noted nothing special. Genome content has not been measured in gnatostomulids.

It is not only the members of Platyzoa that have small jaws. So do the phylogenetically distant Tardigrada, Kinorhyncha and Cyclophora (Edgecombe *et al.*, 2011), which are all small animals (Piper, 2013), begging for a grand comparative study that is beyond the scope of this article. Of these three non-platyzoan phyla, C-values only exist for Tardigrada, which are between 0.08 pg (*Hybysibius dujardini* and *Isohybysibius monoicus*) and 0.82 pg (*Amphibolus volubilis* and *A. weglarskae*), i.e. C-values that are relatively small and probably smaller than for rotifers (Redi & Garagna, 1987; Garagna, Rebecchi & Guidi, 1996; Gabriel *et al.*, 2007; Gregory, 2014).

Just by measuring the sizes of animals and their parts, it is possible to infer that Micrognathozoa and other gnathiferans (rotifers and gnathostomulids) have extraordinarily small genomes. Although this is just a hypothesis, it follows from simple well-established patterns throughout eukaryotes. Ophisthokonts with ultra-small genomes all seem to be parasites, such as microsporidians and nematodes (Gregory, 2014). Parasitic organisms are not just developmentally and morphologically degenerate, but also do not need to perform all the metabolic functions that are required by their non-parasitic sister taxa. Therefore, parasites can have smaller genomes. We even see this pattern of smaller genomes with plastids in parasitic/saprophytic flowering plants that, over evolutionary time, have lost most of their chloroplast genome (Barrett & Davis, 2012). The smallest nuclear plant genomes are in species of the two closely related carnivorous plant genera *Genlisea*

and *Utricularia*, with C-values as small as 0.015 pg (Greilhuber *et al.*, 2006). By contrast, micrognathozoa are probably free-living (non-parasitic) seemingly normal heterotrophs, i.e. normal apart from their size, especially of their intricate jaws. Micro-genomes seem to be the most likely prediction arising from small jaws. However – because rotifers have modestly small (not ultra-small) genomes – micrognathozoans, rotifers and gnathostomulids might simply keep their muscle and nerve nuclei in parts of their cells outside of the small intricate jaw apparatus, have uninucleate muscle fibres and/or have mature pharyngeal neurons that lack nuclei. These would provide alternative ways to keep their jaws small.

Figures 10 and 11 in Kristensen & Funch (2000) show several nuclei in the jaw apparatus of Micrognathozoa. These nuclei may be associated with cells whose cuticles form sclerites; the nuclei do not appear to be in nerve or muscle cells. Although Kristensen & Funch (2000) do not list the size of the pictured nuclei, they appear to be between 2.1 and 2.3 µm in diameter. These nuclear diameters must be fairly typical for the entire animal, except for midgut and egg cells: 'The midgut consists of cells with . . . giant nuclei. These nuclei can reach a diameter of 4 µm and are the second largest nuclei in the animal. Only the nucleus of the oocyte may be larger' (Kristensen & Funch, 2000: 25). Most metazoans have cell nuclei in the range 5–7 µm in diameter, except for endoploid cells (e.g. giant salivary gland cells) and egg cells (Alberts *et al.*, 2002). Eukaryotes with ultra-small genomes have nuclei between 0.5 and 1.0 µm in diameter, such as the apicomplexan *Plasmodium*, the excavate *Leishmania* and the microsporidian fungus *Encephalitozoon* (Raikov, 1982 [1978]; Xu *et al.*, 2006), all of which are intracellular parasites. For free-living protists, the smallest nuclei are in the range 1–2 µm, but these may all be in dinoflagellates (Raikov, 1982 [1978]). Based on the information provided in Kristensen & Funch (2000), micrognathozoan nuclei are at the very low end of the size spectrum for nuclei of free-living eukaryotes, which is consistent with the size of their small jaws. Moreover, very small nuclei almost invariably translate into very small genomes, as we should expect in the Micrognathozoa.

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