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Genome size and lifestyle in gnesiotrochan rotifers

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Abstract Gnesiotrochan rotifers display a variety of life styles ranging from taxa with free-swimming larval and sessile adult stages to those with motile adult stages and colonial habits. Several explanations for the *C*-value enigma posit that genome size is correlated with lifestyle. To investigate this, 13 gnesiotrochan species representing nine genera were measured by flow cytometry. Genome sizes (1C) within Gnesiotrocha ranged from 0.05 pg (*Hexarthra mira* and *Hexarthra fennica*) to 0.25 pg (*Sinantherina ariprepes*). Genome sizes varied within genera and species; e.g., the *H. fennica* (El Huérfano, Mexico) genome was estimated to be 15% larger than that of *H. mira* and *H. fennica* (Keystone Wetland, TX, USA). Gnesiotrochan genome sizes are similar to those reported within Ploima, which range from 0.06 pg (*Brachionus rotundiformis*, *B. dimidiatus*) to 0.46 pg (*B. asplanchnoidis*). Within Gnesiotrocha, genome size was found to be significantly smaller in sessile versus motile species as well as in solitary versus colonial species. To account for phylogenetic background, linear mixed models with hierarchical taxonomic ranks showed that there is a taxonomic component underlying genome size. This study provides the first estimates of genome size within the superorder, providing a baseline for genomic and evolutionary studies within the group.

Keywords *C*-value · Coloniality · Flow cytometry · Free-swimming · Sessile

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Introduction

The relationship of haploid genome size, or *C*-value, to a variety of biological phenomena is an ongoing topic of debate and is referred to as the C-value enigma (Elliott & Gregory, 2015). The *C*-value enigma encompasses several features of genome size including: (1) mechanisms driving growth or diminution, (2) differences among disparate taxa, and (3) biological consequences of genomic content. Factors driving genome size are multi-level and complex; at the proximate level, molecular mechanisms and population genetics likely drive genome size variation. At an evolutionary level, phylogenetic considerations and adaptation may be more important determinants of genome size (Alfsnes et al., 2017). One of the primary mechanisms of genome size expansion is the

accumulation of transposable elements (TEs) (Gregory, 2005; Lynch, 2007; Canapa et al., 2015). Evidence for this includes the linear relationship between the number of TEs and genome size in eukaryotes (Kidwell, 2002) and that the loss of TEs can contribute to genome size compaction (Kapusta et al., 2017). Effective population size influences the maintenance of both TEs and duplicated DNA. In large populations, purifying selection may remove excess DNA, while in those populations with small effective sizes this constraint is lifted, and selection may even maintain additional DNA (Lynch & Conery, 2003). This model provides an explanation of genome size variation among taxa.

Genome size is related to several morphological and ecological traits, such as cell size, development time, and, in some taxa, metabolic rate (Hughes & Hughes, 1995; Gregory, 2005; Wright et al., 2014). Thus, genome size should be correlated with life history attributes. Alternatively, if TEs and effective population sizes are driving genome size evolution, then it should be independent of lifestyle or correlated only with phylogeny. For instance, independence of genome size from taxonomic rank at lower phylogenetic levels has been suggested to be the result of adaptation in arthropods (Alfsnes et al., 2017).

C-value is negatively correlated with metabolism through what appears to be a nucleotypic effect in some groups (Gregory, 2001a). For instance, genome size is relatively small in vertebrates with powered flight: bats, birds, and extinct pterosaurs (Hughes & Hughes, 1995; Organ & Shedlock, 2009; Wright et al., 2014). Additionally, these groups have relatively small cell sizes (Gregory, 2001a). The metabolic rate hypothesis is a potential explanation of the observed relationship between genome size and flight (Hughes & Hughes, 1995; Gregory, 2001b). However, this link between metabolism and genomic content does not hold for all vertebrates. For example, in amphibians and ray-finned fishes, considerations such as egg size and development time may be more important adaptive drivers of genome size (Hardie & Hebert 2003; Smith & Gregory, 2009). Our understanding of where metabolism does and does not apply to genome size is less clear for invertebrate taxa (Gregory & Hebert 2003; Alfsnes et al., 2017). Invertebrate groups that display lifestyles with differing metabolic demands such as flying and flightless insects, and sessile and swimming aquatic species are ideal targets to determine whether the metabolic rate hypothesis applies more broadly. Rotifers display a variety of lifestyles that may impose different metabolic costs; e.g., planktonic, sessile, solitary, colonial, free-living, and parasitic lifestyles (Epp & Lewis 1984; Wallace, 1987; May, 1989; Vadstein et al., 2012).

Genome size in rotifers has been determined for several bdelloid and monogonont species (Pagani et al., 1993; Mark Welch & Meselson 1998a, 2003; Stelzer, 2011; Stelzer et al., 2011; Flot et al., 2013; Riss et al., 2017; Kim et al., 2018; Nowell et al., 2018). Bdelloid rotifers are likely degenerate tetraploids whereas seisonids, acanthocephalans, and most monogonont rotifers are diploid (Mark Welch & Meselson, 2001; Mark Welch et al., 2008; Hur et al., 2009). This difference in ploidy makes bdelloid genomes poor representatives of genome sizes for the phylum. Feulgen densitometry, static cell fluorometry, hybridization techniques, and full genome sequencing have been used to measure genomic DNA content in the Bdelloidea. Estimates of 1C genome size within this group range from 0.18 pg in *Rotaria magnacalcarata* (Parsons, 1892) to 1.22 pg in *Philodina roseola* Ehrenberg, 1832 (Mark Welch & Meselson 1998a; Nowell et al., 2018). Sequencing techniques have yielded smaller estimates than fluorometric techniques in the bdelloid *Adineta vaga* (Davis, 1873) (0.25 pg vs 0.36 pg, respectively) (Mark Welch & Meselson, 2003; Flot et al., 2013). Underestimates of genome size by sequencing arise due to the presence of heterochromatin and repeated regions (Bennett et al., 2003; Nishibuchi & Déjardin, 2017). Genome sizes in monogononts, as measured by flow cytometry, range from 0.06 in *Brachionus rotundiformis* Tschugunoff, 1921 and *Brachionus dimidiatus* Bryce, 1931 to 0.42 in some strains of *Brachionus asplanchnoidis* Charin, 1947 (Stelzer, 2011; Stelzer et al., 2011). Recently, an estimate of genome size in *Brachionus calyciflorus* Pallus 1766 was determined based on whole genome sequencing, 0.13 pg (Kim et al., 2018). Genome size has been well studied in the *Brachionus plicatilis* species complex (Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017). Within this complex, there is a positive relationship of genome size with body volume and egg size (Stelzer et al., 2011). This correlation may be related to the cell size, and thus potentially confirming the relationship between cell size and genome size found in other animals (Gregory, 2001b, 2005) and in accordance with the *C*-value enigma (Stelzer et al., 2011).

To date, there are no measurements of genome size within the Superorder Gnesiotrocha. Gnesiotrochan rotifers are comprised of ~ 217 species classified in two orders, the Collothecaceae and the Flosculariaceae (Segers, 2007). Gnesiotrochan rotifers possess a wide array of lifestyles, including: (1) free swimming forms as in the ploimids and bdelloids, (2) facultative sessility in some taxa, (3) sessile taxa in both orders, and (4) colonial taxa within the Flosculariaceae (Wallace, 1987; Young et al., 2018). Several families of gnesiotrochans (e.g., Hexarthridae, Testudinellidae, Trochosphaeridae) are free-swimming and unattached as adults. Colonies may either be sessile or planktonic, or facultatively planktonic as in some species of *Lacinularia* and *Sinantherina*. Unfortunately, the position of these families within the larger gnesiotrochan phylogeny is not well resolved. Given this, the evolution of lifestyle within the group cannot be disentangled from phylogenetic considerations at this point.

To determine how the genome sizes of gnesiotrochan rotifers compare to other rotifers, we measured the average genome sizes of 13 gnesiotrochan rotifers representing nine genera, along with one ploimid outgroup. To determine whether there is a relationship between lifestyle and genome size, we compared the genome sizes of gnesiotrochan rotifers including sessile, motile, solitary, and colonial representatives using non-parametric pairwise tests and linear mixed models with hierarchical taxonomic ranks. Because genome size has not been reported for any gnesiotrochan rotifer, we also compared our measurements to the known genome sizes of other rotifers.

Materials and methods

Rotifer collection and culture

Rotifers were collected opportunistically from the USA and Mexico (Table 1) using two methods: (1) a plankton net (64 µm mesh) to obtain planktonic rotifers, and (2) submerged macrophyte collection to obtain littoral and sessile species. In cases where waterbodies were small, a filter (20 µm mesh) was pulled through the water to concentrate plankton. Rotifers from Australia were hatched from rehydrated sediments. All rotifers were isolated, cultured in artificial hardwater (modified MBL; Stemberger, 1981), and fed a mixture of *Chlamydomonas reinhardtii* Dangeard, 1888 (Culture Collection of Algae at The University of Texas at Austin (UTEX) strain 90) and *Chlorella vulgaris* Berijerinck, 1890 (UTEX strain 30). For samples containing tube-building rotifers, carmine powder was added to provide suspended materials to aid in tube construction. Samples were cultured at room temperature (~ 21°C) under ambient lighting. Long-term cultures of some species are maintained in the laboratory. For instance, *Sinantherina socialis* (Linnaeus, 1758) is maintained and fed weekly with either *Rhodomonas minuta* Skutja 1948 or *Cryptomonas erosa* Ehrenberg 1832, depending on availability of the algal cultures.

Estimation of genome size by cytometry

To prepare rotifer cells for flow cytometry with propidium iodide (PI) stain, a detergent trypsin method was used following a protocol modified from Vindeløv et al., (1983). This method has been used successfully for some members of the *Brachionus plicatilis* species complex and other Ploima (Stelzer et al., 2011; Riss et al., 2017). Rotifers were not fed for 24–36 h to clear their guts of visible food. They were then cleaned by rinsing in MBL medium through serial transfer of the animals through fresh medium. Approximately, 100 to 800 rotifers were collected on a 20 µm mesh filter or placed directly into a 1 ml Dounce tissue homogenizer and re-suspended in MBL. Excess MBL was then removed and rotifers were lysed on ice with 15 strokes. The homogenate was filtered through a 20 µm mesh sieve to remove large particulates. Next, 0.003% trypsin (dissolved in stock buffer: 3.4 mM tri-sodium citrate dihydrate, IGEPAL® at 0.1% v/v, 1.5 mM spermine tetrahydrochloride, and 0.5 mM Tris(hydroxymethyl)aminomethane at pH 7.6) was added and samples were incubated at room temperature for 15 min. Trypsin inhibitor (0.05%) and 0.01% RNAse A, both dissolved in stock buffer, were subsequently added and samples were incubated at room temperature for 15 min. Samples were then stained by addition of 0.04% PI and 0.1% spermine tetrahydrochloride dissolved in stock buffer. PI-stained samples were incubated in the dark overnight at 4°C. All chemicals were obtained from MilliporeSigma. Following incubation, samples were subjected to flow cytometry on a Gallios flow cytometer (Beckman Coulter

Table 1 Summary of gnesiotrochan genome sizes (1C) determined by propidium iodide staining and lifestyle characterization

Species	Site; GPS coordinates	*N*	Genome size (pg)	SD	Lifestyle
Conochilus hippocrepis	Nockamixon State Park Fishing Pond, Bucks Co., PA 40.472833, − 75.224111	1	0.1265	n/a	Planktonic colonies
Collotheca ferox	Poza Azul, Coahuila, Mexico 26.922671, − 102.122589	3	0.1410	0.0012	Sessile Solitary
C. ornata	La Mancha Wetland, Doña Ana Co., NM 31.303271, − 106.553814	2	0.0616	0.0049	Sessile Solitary
Cupelopagis vorax	Moon Lake, Marquette Co., WI 43.806367, − 89.366509	3	0.1765	0.0018	Sessile solitary
C. vorax	Staring Lake, Hennepin Co., MN 44.836781, − 93.456119	1	0.1470	n/a	Sessile solitary
C. vorax	Turtle Basking Pond, Hennipin Co., MN 44.84506, − 93.369538	4	0.1572	0.0089	Sessile solitary
Filinia longiseta	Ojo de la Casa, Chihuahua, Mexico 31.366033, − 106.532085	2	0.0707	0.0093	Free-swimming solitary
F. longiseta	Behind Ranch House playa, Hueco Tanks State Park and Historic Site, El Paso Co., TX 31.923966, − 106.041668	3	0.0701	0.00028	Free-swimming solitary
Hexarthra fennica	El Huérfano Pond, Chihuahua, Mexico 31.294850, − 106.511633	1	0.0564	n/a	Free-swimming solitary
H. fennica	Keystone Heritage Park Wetland, El Paso Co., TX 31.822169, − 106.563532	1	0.0469	n/a	Free-swimming solitary
H. mira	Ojo de la Punta, Chihuahua, Mexico 31.385967, − 106.602017	1	0.0477	n/a	Free-swimming solitary
Hexarthra sp.	Stacia Hueco, Hueco Tanks State Park and Historic Site, El Paso Co., TX 31.92469, − 106.0426	3	0.0464	0.0010	Free-swimming Solitary
Lacinularia flosculosa	Laguna Prieta, Hueco Tanks State Park and Historic Site, El Paso Co., TX 31.924903, − 106.046654	3	0.1332	0.0101	Sessile colonies
L. flosculosa	Ryan's 2 Billabong, Wodonga, Australia − 36.11072222, 146.96664444	2	0.1571	0.0031	Sessile colonies
Limnias melicerta	Ryan's 2 Billabong, Wodonga, Australia − 36.11072222, 146.96664444	3	0.1182	0.0039	Sessile colonies
Sinantherina ariprepes	Murphy Lake, Scott Co., MN 44.712908, − 93.34506	2	0.2535	0.0076	Sessile colonies
S. socialis	Hyland Park Pond, Hennepin Co., MN 44.824466, − 93.37267	2	0.2022	0.0078	Sessile colonies
S. socialis	Naticook Lake, Hillsborough Co., NH 42.8200, − 71.5257	1	0.1617	n/a	Sessile colonies
S. socialis	Ryan's 2 Billabong, Wodonga, Australia − 36.11072222, 146.96664444	3	0.1227	0.0033	Sessile colonies
S. socialis	Boxford Pond, Essex Co., MA 42.700575, − 71.053345	1	0.1760	n/a	Sessile colonies
Testudinella patina	Hyland Park Pond, Hennepin Co., MN 44.824466, − 93.37267	1	0.0764	n/a	Free-swimming solitary

Table 1 continued

Species	Site; GPS coordinates	*N*	Genome size (pg)	SD	Lifestyle
T. patina	La Mancha Wetland, Doña Ana Co., NM 31.303271, − 106.553814	1	0.0866	n/a	Free-swimming solitary
T. patina	Miller Ranch Wetland, Jeff Davis Co., TX 30.623845, − 104.674005	1	0.0749	n/a	Free-swimming solitary
Plationus patulus	La Mancha Wetland, Doña Ana Co., NM 31.303271, − 106.553814	3	0.2535	0.0043	Free-swimming solitary

Plationus patulus, a plomid rotifer, is included as an outgroup

Site refers to the location from which the population was obtained

n sample size; *SD* standard deviation, *n/a* not applicable

Diagnostics) at 488 nm and subsequent fluorescence captured on a detector equipped with a 620/30 filter and analyzed with Kaluza version 1.3 (Beckman Coulter Diagnostics).

To estimate genome size, mean fluorescent intensity of rotifer cell populations were compared to those of *Drosophila melanogaster* Meigen, 1830, and approximated based on a ratio that includes the known genome size of the Oregon-R strain of *D. melanogaster*, 0.18 pg (Tavares et al., 2014). Several early cytometry runs used an available Canton-S strain. But because of the known variability in genome size among strains, Oregon-R flies were used to corroborate these results; and then used for all subsequent runs (Bosco et al., 2007). For rotifers with genome sizes nearly the same size of *D. melanogaster,* we used *Hexarthra* sp. from Hueco Tanks State Park and Historic Site (HTSPHS) as an internal standard.

Mean genome size per species was used for all analyses. For the *Brachionus plicatilis* complex, species were delineated as in Mills et al. (2017). To compare genome sizes of rotifers with sessile and motile adult forms, a Wilcoxon rank-sum test was used (R 3.5.0, R Core Team, 2018). Additionally, to account for the potential influence of phylogeny, linear mixed models with hierarchical taxonomic ranks as random effects were fitted to log-transformed genome size, lifestyle, and taxonomic rank (Bdelloida + Monogononta: superorder, order, genus; Monogononta: order). Only taxonomic ranks found to contribute to the variation in genome size were included in these models. To determine which model best explained the variance in genome size, an ANOVA was used and the model with the lowest Akaike's Information Criterion (AIC) was selected. Further, the genome sizes of gnesiotrochan rotifers were compared to those of ploimid (Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017, Kim et al., 2018) and bdelloid rotifers (Pagani et al., 1993; Mark Welch & Meselson 1998a, 2003; Flot et al. 2013; Nowell et al., 2018) using a Kruskal–Wallis test for stochastic dominance followed by Dunn's test implemented in R (R 3.5.0, R Core Team, 2018).

Results

We estimated genome size for 13 gnesiotrochan rotifer species and one ploimid species, resulting in values ranging from 0.05 pg in *Hexarthra* species to 0.25 pg in *Sinatherina ariprepes* Edmonson, 1939 and the ploimid *Plationus patulus* (Müller, 1786) (Table 1). Genome size measurements for rotifers were not normally distributed, thus non-parametric tests were used. The use of either reference standard, *Hexarthra* sp. or *D. melanogaster*, did not have a significant impact on genome size estimates for target species (Paired Wilcoxon rank-sum test, $V = 5$, $P = 0.5$). We could not distinguish fluorescent peaks for Canton-S and Oregon-R flies from each other when run together on the flow cytometer (data not shown). This indicates that the genome sizes of these strains are likely very close to one another or equivalent.

The mean genome sizes of motile and sessile gnesiotrochan rotifers, 0.07 pg and 0.15 pg,

Table 2 Results of linear mixed models with hierarchical taxonomic ranks, with log-transformed genome size as the response variable

Model	df	AIC	χ^2	*P*
Monogononta				
Taxonomic rank	3	50.1	n/a	n/a
Sessility + taxonomic rank	4	48.1	1.75	0.045
Coloniality + taxonomic rank	4	45.7	2.01	< 2e−16
Coloniality + sessility + taxonomic rank	5	45.3	2.35	0.123
Monogononta + Bdelloidea				
Taxonomic rank	5	74.0	n/a	n/a
Sessility + taxonomic rank	6	73.1	2.39	0.122
Coloniality + taxonomic rank	6	72.9	0.71	< 2e−16
Coloniality + sessility + taxonomic rank	7	73.5	1.43	0.233

Models for solely Monogononta and Monogononta + Bdelloidea were investigated separately

Taxonomic ranks as random variables, with superorder, order, and genus for Monogononta + Bdelloidea, and solely genus used for the Monogononta only analysis. In other models, superorder, order, family, and genus were used. Lifestyles sessility and coloniality were the fixed variables in all models

respectively, were significantly different (Wilcoxon rank-sum test, $W = 38$, $P = 0.014$). Mean genome sizes of colonial (0.18) species were significantly greater than solitary species (0.09) (Wilcoxon rank-sum test, $W = 33$, $P = 0.02$). When comparing lifestyles among all monogonont rotifers for which there is an estimate of genome size, we found no significant differences between comparisons of sessile versus free swimming or solitary versus colonial lifestyles. Linear mixed models with hierarchical taxonomic ranks showed that for Monogononta, the best model included sessility, coloniality, and taxonomic rank (genus) as predictors of genome size but was not significant (AIC = 45.3, $P = 0.123$). However, when the model was simplified to sessility + taxonomic rank or coloniality + taxonomic rank, results were significant (AIC = 48.12, 45.5; $P = 0.045$, < 0.001, respectively) (Table 2). When the model was expanded to include Monogononta + Bdelloidea, the best predictor of genome size was coloniality + taxonomic rank (superorder, order, and genus) (AIC = 72.9, $P \leq 0.001$) (Table 2).

We found a significant difference among the mean genome sizes of Ploima, Gnesiotrocha, and Bdelloidea (Kruskal–Wallis rank sum test, $\chi^2 = 18.7$, $P < 0.001$). Pairwise comparisons of genome size showed that gnesiotrochan genomes were not significantly different from those of ploimids but were significantly smaller than those of bdelloids (Dunn test, $P = 0.0002$) (Fig. 1).

Discussion

The range of genome sizes in the gnesiotrochan rotifers we investigated is similar to those found in ploimid rotifers (Mark Welch & Meselson 1998a; Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017) but smaller than those reported for bdelloid rotifers (Pagani et al., 1993; Mark Welch & Meselson 1998a, 2003). The genome of *P. patulus* (0.25 pg) was among the largest ploimid genome sizes, but still within the range of known genome sizes of populations of *B. asplanchnoidis* (0.22 to 0.42 pg) (Stelzer et al., 2011; Riss et al., 2017). The smallest genome sizes measured in gnesiotrochans were 0.05 for *H. mira*, *H. fennica*, and *Hexarthra* sp. (HTSPHS), which are comparable to the smallest genomes known for ploimid rotifers, 0.06 pg in *B. rotundiformis* and *B. dimidiatus* (Stelzer, 2011; Stelzer et al., 2011). The consistency of the low estimate among rotifers and other taxa may indicate a lower bound on genome size in free-living bilaterians. For instance, in the animal genome size database (Gregory, 2009), the only clades with representatives with genome sizes smaller than 0.06 pg are gastrotrichs (0.05 pg), tardigrades (0.05 pg), placozoans (0.04 pg), sponges (0.04 pg),

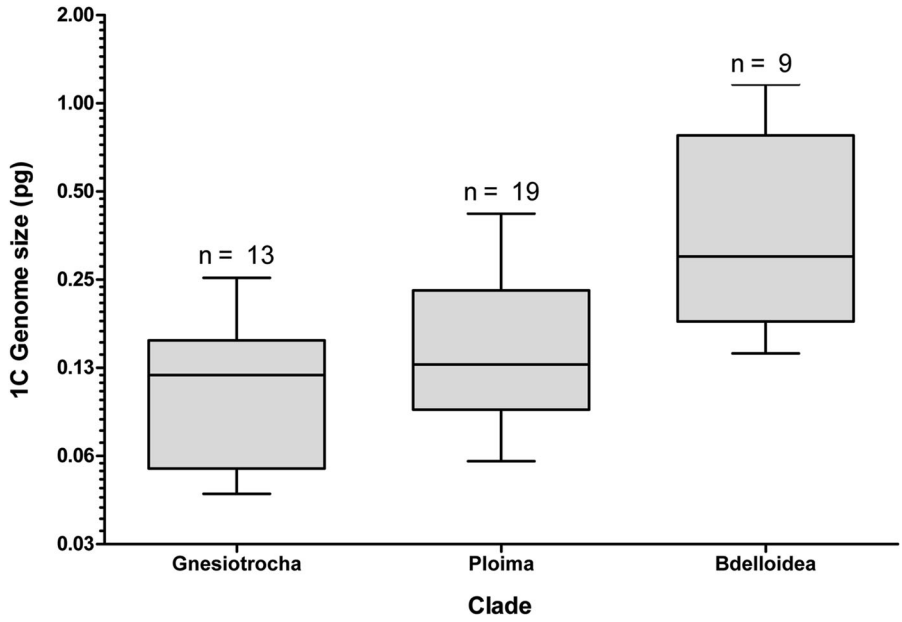

Fig. 1 Estimated genome sizes of gnesiotrochan, ploimid, and bdelloid rotifers. Values for Gnesiotrocha and *Plationus patulus* were obtained in this study; values for the Ploima and Bdelloidea are from past studies (Pagani et al., 1993; Mark Welch & Meselson 1998a, b, 2003; Stelzer 2011; Stelzer et al., 2011; Flot et al., 2013; Riss et al., 2017; Kim et al., 2018; Nowell et al., 2018)

and nematodes (as low as 0.02 pg in parasitic species) (Gregory, 2009). Additionally, several phyla have their smallest genome sizes at 0.06 pg; including platyhelminths, annelids, and chordates (Gregory, 2009). If genome size is constrained by selection for faster development in these groups, perhaps this represents a threshold value below which development cannot proceed more quickly or genome size reduction no longer appreciably speeds up development time. Non-bilaterian animals and parasitic species appear to exceptions to this phenomenon. To our knowledge, there are no genome size estimates for parasitic acanthocephalans or seisonids.

Our results are consistent with those of Stelzer et al. (2011) in that body size and genome size are correlated in rotifers. *Collotheca ornata* (Ehrenberg, 1830) was both the physically smallest of the collothecid rotifers measured and possessed the smallest genome size (0.06 pg). The largest genome size measured was that of *S. ariprepes* (0.25 pg), a moderately large gnesiotrochan with an expansive corona (Wallace & Starkweather, 1985). Given this, it is predicted that the largest known rotifer, *Pentatrocha gigantea* Segers & Shiel, 2008, will have a genome size equal to or larger than that of *S. ariprepes* (Segers & Shiel, 2008). In contrast, the *Hexarthra* species investigated in this study are rather large, yet yielded the smallest estimates of genome size ($\sim$ 0.05). This could be due to their requirement for rapid development in habitats with short hydroperiods (Schröder & Walsh, 2007), as larger genome sizes are correlated with increased development time (Gregory, 2005).

Several of the gnesiotrochan rotifers included in this study showed variation among populations. For instance, *S. socialis* hatched from Australian billabong sediments had a markedly smaller genome size than conspecifics from the US populations. *S. socialis* also showed variability among the US populations (Mean $\pm$ SE: 0.18 $\pm$ 0.02). Australian and US populations of *Lacinularia flosculosa* (Müller, 1773) had a similar pattern, with genome sizes of 0.16 and 0.13, respectively. *Cupelopagis vorax* (Leidy, 1857) displayed variability in its genome size (0.16 $\pm$ 0.02) among populations isolated from different lakes as well. These differences may either be the result of intraspecific variation or the presence of cryptic species complexes. While cryptic species complexes are relatively common in rotifers, high levels of intraspecific variation in genome size have been found in *Brachionus asplanchnoidis* so this alternative

cannot be discounted. The nature of these species as possible complexes warrants further investigation, particularly between species occurring on different continents.

We found that a sessile adult lifestyle is associated with a larger genome size as compared to a motile lifestyle. Motion by ciliary action in rotifers is metabolically costly, accounting for up to 62% of total metabolic costs in the free-swimming solitary species *Brachionus plicatilis* Müller, 1786 and *Asplanchna sieboldii* (Leydig, 1854) (Epp & Lewis, 1984). Additionally, metabolic measurements of attached and swimming rotifers suggest that attached rotifers expend somewhere between 1/3 and 1/5 the energy used by their swimming counterparts (Vadstein et al., 2012). This potential relationship between lifestyle and genome size fits the pattern of what is seen in some vertebrates (Hughes & Hughes, 1995; Organ & Shedlock, 2009; Zhang & Edwards, 2012; Wright et al., 2014; Kapusta et al., 2017) and follows from the predictions of the metabolic rate hypothesis of genome size (Hughes & Hughes, 1995). Two members of the Collothecaceae, *Cupelopagis vorax* and *Collotheca ferox* (Penard, 1914), had among the largest genome sizes of the gnesiotrochan rotifers. Rotifers in this clade do not generate current by ciliary action to suspension feed as in the Flosculariaceae, but rather either wait for food to approach their setae and then either sweep prey towards their mastax as in *C. ornata* or engulf prey in their infundibulum by muscular action as in *C. vorax* (Vasisht & Dawar, 1969; Koste, 1973; Bevington et al., 1995). *C. ferox* is a sit-and-wait ambush predator and, like *C. vorax*, the opening of its infundibulum is aligned with the substratum and it consumes metazoans smaller than itself such as *Lepadella* spp. (Meksuwan et al., 2013; pers. obs). This ambush predator lifestyle may represent a low metabolic alternative to suspension feeding by ciliary action. However, further study into differences in metabolism between swimming and sessile adult gnesiotrochan rotifers is necessary to test the metabolic rate hypothesis.

We found that colonial lifestyle was related to an increased genome size. This may support the energetic advantage hypothesis of rotiferan coloniality. The adaptive origin of coloniality in the Gnesiotrocha has been hypothesized to be either due to predator avoidance or an increase in feeding efficacy. Clearance rates for colonial versus non-colonial rotifers do not appear to differ (Wallace & Starkweather, 1985). Despite this lack of increase, there are reports of colonies of *S. socialis* where individuals within the colony orient their coronae in the same direction and form discrete incurrent and excurrent chimneys (Wallace, 1987). If colonial rotifers are in fact working together to gather food, they may be using a smaller percentage of their total metabolic costs in feeding processes. Assuming sessile rotifers have lower metabolism, we would expect them to follow the pattern found in other organisms; i.e., that they would have larger genome sizes than rotifers with free-swimming adult stages.

The importance of taxonomic rank in predicting genome size implies that there is phylogenetic underpinning influencing lifestyle and genomic content. Unfortunately, the phylogeny of the Gnesiotrocha is not well resolved and thus cannot serve as a reliable tree for more sophisticated analyses. The most recent tree for the group had low support for the placement of several families used in this study (i.e., Hexarthridae, Testudinellidae, and Trochosphaeridae), all of which are free-swimming solitary taxa within a superorder otherwise dominated by sessility (Meksuwan et al., 2015). Due to this uncertainty, it is unclear whether the relationship found between sessile and motile taxa is an artifact of phylogenetic signal or a true relationship. For example, if motile taxa are interspersed throughout the gnesiotrochan phylogeny it would imply that genome size and lifestyle are correlated, whereas if they are sister groups or stem lineages it may mean that the relationship observed is due to common ancestry or phylogenetic inertia.

Polyploidy is a possible mechanism of genome size expansion in rotifers. Polyploidy occurs in both bdelloid and ploimid species. For example, bdelloid rotifers are degenerate tetraploids as evidenced by sequencing (Mark Welch et al., 2008; Hur et al., 2009), while in monogononts, polyploidy occurs in certain populations of the ploimid rotifer *Euchlanis dilatata* Ehrenberg, 1830 (Walsh & Zhang, 1992). Whether or not polyploidy is an important factor driving genome size likely depends on the age of the event, as over time purifying selection should diminish genome size in the absence of other factors (Lynch & Conery, 2003). Consequently, ancient chromosomal duplications have less influence on current genome sizes. The genome sizes of sessile rotifers we measured were roughly twice that of the motile

rotifers, implying ploidy events may have occurred during evolution of the Gnesiotrocha. To our knowledge, there are no karyotypes for this group. Chromosome analysis would offer strong support either for or against polyploidy as a mechanism of genome size evolution within the group. It should be noted that many of these mechanisms can act in concert on populations to determine genome size, resulting in antagonistic or synergistic outcomes depending on a variety of factors. For example, polyploidy along with accumulation of TEs is antagonistic to decreases in genome size brought about by selection for faster metabolic rates. Sessile organisms may be more vulnerable to genome size increases due to these mechanisms, since their potentially lower energetic costs may lessen selection for high metabolic rates.

Increased sampling within the Gnesiotrocha may reveal the degree of variability in genome size among populations and clades. To investigate potential relationships between genome size and colonial lifestyle, increased sampling of taxa within the Flosculariaceae is needed. Rotifers to target for future genome size research include species with different colony recruitment strategies such as allorecruitive (*Floscularia,* some *Limnias,* some *Ptygura*), autorecruitive (some *Limnias, Octotrocha,* some *Ptygura,* some *Sinantherina*) and those rotifers in which colony-forming species are closely related to solitary forms (e.g., multiple species within the genera *Limnias* and *Floscularia*) (Wallace, 1987, 2002). There are several gnesiotrochans that have planktonic habits in otherwise sessile genera such as *Sinantherina spinosa* (Thorpe, 1983), *Ptygura libera* Myers, 1934, and *Collotheca libera* (Zacharias, 1894). These species along with the genus *Conochilus* either form colonies (e.g., *S. spinosa* and *Conochilus* spp.) or gelatinous tubes (e.g., *C. libera* and *P. libera*), which may serve as sources of drag to increase feeding efficiency as in other zooplankters (Kiørboe, 2011). If the metabolic rate hypothesis of genome size holds true for these taxa we would expect them to have genome sizes intermediate between sessile and free-swimming rotifers. Our genome size estimation for *Conochilus hippocrepis* (Schrank, 1803) of 0.127 pg cautiously follows this pattern. Additional observations are needed to substantiate this hypothesis. Rotifers with other lifestyles including facultatively sessile (e.g., *Brachionus rubens* Ehrenberg, 1838, *Philodina megalotrocha* Ehrenberg, 1832) (Gilbert, 2018; Wallace, 1987), ectoparasitic (e.g., seisonids) and endoparasitic (e.g., *Albertia* spp., *Drilophaga* spp.) and the wholly parasitic Acanthocephala are interesting candidates for genome size measurement because these lifestyles have diverse metabolic demands (May, 1989). To test whether the sessile gnesiotrochan rotifers possess a metabolic advantage over free-swimming species, investigations using proxies of metabolism, such as respiration rates and the production of metabolites, should be conducted.

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References

Alfsnes, K., H. P. Leinaas & D. O. Hessen, 2017. Genome size in arthropods; different roles of phylogeny, habitat and life history in insects and crustaceans. Ecology and Evolution 7: 5939–5947.

Bennett, M. D., I. J. Leitch, H. J. Price & J. S. Johnston, 2003. Comparisons with *Caenorhabditis* (approximately 100 Mb) and *Drosophila* (approximately 175 Mb) using flow cytometry show genome size in *Arabidopsis* to be approximately 157 Mb and thus approximately 25% larger than the *Arabidopsis* genome initiative estimate of approximately 125 Mb. Annals of Botany 91: 547–557.

Bevington, D. J., C. White & R. L. Wallace, 1995. Predatory behavior of *Cupelopagis vorax* (Rotifera; Collothecea; Atrochidae) on protozoan prey. Hydrobiologia 313(314): 213–217.

Bosco, G., P. Campbell, J. T. Leiva-Neto & T. A. Markow, 2007. Analysis of *Drosophila* species genome size and satellite DNA content reveals significant differences

among strains as well as between species. Genetics 177: 1277–1290.
Canapa, A., M. Barucca, M. A. Biscotti, M. Forconi & E. Olmo, 2015. Transposons, genome size, and evolutionary insights in animals. Cytogenetic and genome research 147: 217–239.
Elliott, T. A. & T. R. Gregory, 2015. What's in a genome? The *C*-value enigma and the evolution of eukaryotic genome content. Philosophical Transactions of the Royal Society B 370: 20140331.
Epp, R. W. & W. M. Lewis, 1984. Cost and speed of locomotion for rotifers. Oecologia 16: 289–292.
Flot, J. F., B. Hespeels, X. Li, B. Noel, I. Archipova, E. G. J. Danchin, A. Ejnol, B. Henrissat, R. Koszul, et al., 2013. Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. Nature 500: 453–457.
Gilbert, J. J., 2018. Attachment behavior in the rotifer *Brachionus rubens*: induction by Asplanchna and effect on sexual reproduction. Hydrobiologia. https://doi.org/10.1007/s10750-018-3805-7.
Gregory, T. R., 2001a. Coincidence, coevolution, or causation? DNA content, cell size, and the *C*-value enigma. Biological Reviews of the Cambridge Philosophical Society 76: 65–101.
Gregory, T. R., 2001b. The bigger the *C*-value, the larger the cell: genome size and red blood cell size in vertebrates. Blood Cells, Molecules and Disease 27: 830–843.
Gregory, T. R., 2005. Genome size evolution in animals. The evolution of the genome. Elsevier, San Diego, CA: 3–87.
Gregory, T. R., 2009. Animal Genome Size Database. [available on internet at http://www.genomesize.com].
Gregory, T. R. & P. D. Hebert, 2003. Genome size variation in lepidopteran insects. Canadian Journal of Zoology 81: 1399–1405.
Hardie, D. C. & P. D. N. Hebert, 2003. The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. Genome 46: 683–706.
Hughes, A. L. & M. K. Hughes, 1995. Small genomes for better flyers. Nature 377: 391.
Hur, J. H., K. Van Doninck, M. L. Mandigo & M. Meselson, 2009. Degenerate tetraploidy was established before bdelloid rotifer families diverged. Molecular Biology and Evolution 26: 375–383.
Kapusta, A., A. Suh & C. Feschotte, 2017. Dynamics of genome size evolution in birds and mammals. Proceedings of the National Academy of Sciences of the United States of America 114: 1460–1469.
Kidwell, M. G., 2002. Transposable elements and the evolution of genome size in eukaryotes. Genetica 115: 49–63.
Kim, H. S., B. Y. Lee, J. Han, C. B. Jeong, D. S. Hwang, M. C. Lee, H. M. Kang, D. H. Kim, H. J. Kim, S. Papakostas & S. A. Declerck, 2018. The genome of the freshwater monogonont rotifer *Brachionus calyciflorus*. Molecular ecology resources 18: 646–655.
Kiørboe, T., 2011. How zooplankton feed: mechanisms, traits and trade-offs. Biological Reviews of the Cambridge Philosophical Society 86: 311–339.
Koste, W., 1973. Das Rädertier-Porträt. Ein merkwürdiges festsitzendes Rädertier: Cupelopagis vorax. Mikrokosmos 62: 101–106. **In German**.
Lynch, M., 2007. The origins of genome architecture. Sinauer Associates Inc, Sunderland.
Lynch, M. & J. S. Conery, 2003. The origins of genome complexity. Science 302: 1401–1404.
Mark Welch, D. M. & M. Meselson, 1998a. Measurements of the genome size of the monogonont rotifer *Brachionus plicatilis* and the bdelloid rotifers *Philodina roseola* and *Habrotrocha constricta*. Hydrobiologia 387(388): 395–402.
Mark Welch, J. L. & M. Meselson, 1998b. Karyotypes of bdelloid rotifers from three families. Hydrobiologia 387: 403–407
Mark Welch, D. B. & M. Meselson, 2001. Rates of nucleotide substitution in sexual and anciently asexual rotifers. Proceedings of the National Academy of Sciences of the United States of America 98: 6720–6724.
Mark Welch, D. B. & M. Meselson, 2003. Oocyte nuclear DNA content and GC proportion in rotifers of the anciently asexual Class Bdelloidea. Biological Journal of the Linnean Society 79: 85–91.
Mark Welch, D. B., J. L. Mark Welch & M. Meselson, 2008. Evidence for degenerate tetraploidy in bdelloid rotifers. Proceedings of the National Academy of Sciences of the United States of America 105: 5145–5149.
May, L., 1989. Epizoic and parasitic rotifers. Hydrobiologia 186/187: 59–67.
Meksuwan, P., P. Pholpunthin & H. Segers, 2013. The Collothecidae (Rotifera, Collothecacea) of Thailand, with the description of a new species and an illustrated key to the Southeast Asian fauna. ZooKeys 315: 1–16.
Meksuwan, P., P. Pholpunthin & H. H. Segers, 2015. Molecular phylogeny confirms Conochilidae as ingroup of Flosculariidae (Rotifera, Gnesiotrocha). Zoologica Scripta 44: 562–573.
Mills, S., J. A. Alcantara-Rodriguez, J. Ciros-Pérez, A. Gómez, A. Hagiwara, K. Hinson Galindo, C. D. Jersabek, R. Malekzadeh-Viayeh, F. Leasi, J.-S. Lee, D. B. Mark Welch, S. Papakostas, S. Riss, H. Segers, M. Serra, R. Shiel, R. Smolak, T. W. Snell, C. P. Stelzer, C. Q. Tang, R. L. Wallace, D. Fontaneto & E. J. Walsh, 2017. Fifteen species in one: deciphering the *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy. Hydrobiologia 796: 39–58.
Nishibuchi, G. & J. Déjardin, 2017. The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals. Chromosome Research 25: 77–87.
Nowell, R. W., P. Almeida, C. G. Wilson, T. P. Smith, D. Fontaneto, A. Crisp, G. Micklem, A. Tunnacliffe, C. Boschetti & T. G. Barraclough, 2018. Comparative genomics of bdelloid rotifers: insights from desiccating and nondesiccating species. PLoS biology 16: e2004830.
Organ, C. L. & A. M. Shedlock, 2009. Palaeogenomics of pterosaurs and the evolution of small genome size in flying vertebrates. Biology Letters 5: 47–50.
Pagani, M., C. Ricci & C. A. Redi, 1993. Oogenesis in *Macrotrachela quadricornifera* (Rotifera, Bdelloidea)—I. Germarium eutely, karyotype and DNA content. Hydrobiologia 255: 225–230.

R Core Team, 2018. R: a language and environment for statistical computing. R Core Team. R Foundation for Statistical Computing, Vienna.

Riss, S., W. Arthofer, F. M. Steiner, B. C. Schlick-Steiner, M. Pichler, P. Stadler & C. P. Stelzer, 2017. Do genome size differences within *Brachionus asplanchnoidis* (Rotifera, Monogononta) cause reproductive barriers among geographic populations? Hydrobiologia 796: 59–75.

Schröder, T. & E. J. Walsh, 2007. Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of new species. Hydrobiologia 593: 129–140.

Segers, H., 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. Zootaxa 1564: 1–104.

Segers, H. & R. J. Shiel, 2008. Diversity of cryptic Metazoa in Australian freshwaters: a new genus and two new species of sessile rotifer (Rotifera, Monogononta, Gnesiotrocha, Flosculariidae). Zootaxa 1750: 19–31.

Smith, E. M. & T. R. Gregory, 2009. Patterns of genome size diversity in the ray-finned fishes. Hydrobiologia 625: 1–25.

Stelzer, C. P., 2011. A first assessment of genome size diversity in monogonont rotifers. Hydrobiologia 662: 77–82.

Stelzer, C. P., S. Riss & P. Stadler, 2011. Genome size evolution at the speciation level: the cryptic species complex *Brachionus plicatilis* (Rotifera). BMC Evolutionary Biology 11: 90–100.

Stemberger, R. S., 1981. A general approach to the culture of planktonic rotifers. Canadian Journal of Fisheries and Aquatic Sciences 38: 721–724.

Tavares, S., A. P. Ramos, A. S. Pires, H. G. Azinheira, P. Caldeirinha, T. Link, R. Abranches, M. D. C. Silva, R. T. Voegele, J. Loureiro & P. Talhinhas, 2014. Genome size analyses of Pucciniales reveal the largest fungal genomes. Frontiers in Plant Science 5: 1–11.

Vadstein, O., L. M. Olsen, & T. Andersen, 2012. Prey-predator dynamics in rotifers: density-dependent consequences of spatial heterogeneity due to surface attachment. Ecology 93: 1795–1801.

Vasisht, H. S. & B. L. Dawar, 1969. Anatomy and histology of the rotifer *Cupelopagis vorax* Leidy. Research Bulletin (N.S.) Panjab University 20: 207–221.

Vindeløv, L. L., I. J. Christensen & N. I. Nissen, 1983. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry: The Journal of the International Society for Analytical Cytology 3: 323–327.

Wallace, R. L., 1987. Coloniality in the phylum Rotifera. Hydrobiologia 147: 141–155.

Wallace, R. L., 2002. Rotifers: exquisite metazoans. Integrative and Comparative Biology 42: 660–667.

Wallace, R. L. & P. L. Starkweather, 1985. Clearance rates of sessile rotifers: in vitro determinations. Hydrobiologia 121: 139–144.

Walsh, E. J. & L. Zhang, 1992. Polyploidy in a natural population of the rotifer *Euchlanis dilatata*. Journal of Evolutionary Biology 5: 345–353.

Wright, N. A., T. R. Gregory & C. C. Witt, 2014. Metabolic "engines" of flight drive genome size reduction in birds. Proceedings of the Royal Society B 281: 20132780.

Young, A. N., R. Hochberg, E. J. Walsh & R. L. Wallace, 2018. Modeling the life history of sessile rotifers: larval substratum selection through reproduction. Hydrobiologia. https://doi.org/10.1007/s10750-018-3802-x.

Zhang, Q. & S. V. Edwards, 2012. The evolution of intron size in amniotes: a role for powered flight? Genome Biology and Evolution 4: 1033–1043.