Life-history traits display strong associations to genome size in annelids

Nicholas Beaudreau[1], Gloria Massamba N'Siala[1,2], Claude Belzile, Piero Calosi[1], France Dufresne[1*]

[1]Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, QC, Canada

[2]Centre d'Écologie Fonctionnelle et Évolutive (CEFE- CNRS), UMR 5175, Montpellier Cedex 5, France

*Corresponding author: France_Dufresne@uqar.ca,

The last two authors have contributed equally

418-723-1986, 418-724-1849

Acknowledgements

We would like to thank Dr Valentina Grandi, Dr. Maria Pia Martino, Prof. Daniela Prevedelli, and Prof. Roberto Simonini and of the University of Modena and Reggio Emilia (Italy) for giving us access to the PhD thesis containing the life-history data of the *Ophryotrocha* species. The authors would like to thank Marie-Hélène Carignan for her help with the preparation of samples, Francis Beaudet for his help with the annelid cultures, and Astrid Tempestini for sharing DNA sequences to build our phylogenies. We also thank the Natural Sciences and Engineering Research Council of Canada (NSERC : RGPIN-2015-06500 and RGPIN- 06349), the Programme Établissement de Nouveaux Chercheurs Universitaires of the Fonds de Recherche du Québec - Nature et Technologies (FRQNT : No. 199173), BORÉAS UQAR, and the European Union through the

Marie Skłodowska-Curie Post-doctoral Fellowship (Proposal Number: 659359) for funding different members of this team (see Funding section).

Abstract

Genome size, known also as the C-value, has been proposed as an important determinant of life-history variation in numerous animal taxa. We assessed the relationships between genome size and fitness related life-history traits in six species of interstitial marine annelids of the genus *Ophryotrocha*. Life-history traits and genome-size data obtained from 18 additional annelid species was included in our analyses to have a broader phylogenetic scope. Unexpectedly, genome sizes assessed here by flow cytometry in four *Ophryotrocha* species were three times larger than previously reported values obtained using Feulgen densitometry. This has implications for the hypothesis that harsh interstitial habitats select for small genomes in meiofaunal annelids. Within the genus *Ophryotrocha*, significant and positive relationships were found between genome size and nucleus size, and between genome size, age at first egg mass deposition, body size, and lifespan. These relationships held up in the broader phylogenetic comparison. Our study provides evidence to the important role played by genome size in the evolution of life-history traits in annelids.

Keywords: *C-value; flow cytometry; Ophryotrocha; body size; developmental rate; lifespan*

Introduction

One longstanding and unresolved puzzle in evolutionary biology is the tremendous variation in genome size among eukaryotes. Genome size, here defined as the haploid nuclear content (or the C-value in pg DNA cell $^{-1}$), varies some 7000-fold among animals (0.02 – 132.83) (Gregory, 2020) with no apparent relationship with neither organismal complexity nor number of genes (Cavalier–Smith, 1985). Instead, genome size is known to correlate to non-coding DNA, more specifically transposable elements (Lynch & Conery, 2003). The C-value enigma (Gregory, 2005) refers to unresolved questions regarding the origin of the non-coding DNA, the phenotypic effects of non-coding DNA, and how it varies so greatly among taxa. The sheer amount of DNA in a genome can affect organismal phenotype through its nucleotypic effects. Several life-history traits, such as body size in species with determinate growth, have been found to correlate with genome size through the associated effects of nuclear DNA content on cell size (Hessen & Persson, 2009; Dufresne & Jeffery, 2011). Similarly, significant associations between genome size and life-history traits and developmental rate (Wyngaard et al., 2005) suggest that genome size could co-evolve with life history. These genome size – life-history traits relationships suggest that certain environments and lifestyles may be associated with larger genomes (Leiva et al., 2019). However, opposing evidence exists regarding the impact of deep-sea environment on genome size selection in amphipods (Ritchie et al., 2017). Non-adaptive theories suggest that mutations and genetic drift are the major drivers of genome size variation (Lynch & Conery, 2003). The *Mutational Hazard hypothesis* stipulates that larger genomes evolve in lineages with smaller long-term effective population size because this allows mildly deleterious insertions of non-coding DNA to accumulate by drift, rather than being eliminated by purifying selection (Lynch & Conery 2003). This has recently been shown in subterranean isopods (Lefébure et al., 2017). Hence under this hypothesis, the evolution of genome size is controlled by the opposing forces of mutations generating large scale insertions and their removal by selection or their fixation by drift.

Annelids are significantly underrepresented in the existing genome size database, and show a remarkable range of genome size (0.06 - 7.64 pg) (Gregory, 2020). Interstitial species, those that live among grains of sediment, are reported to have particularly small genomes relative to macrobenthic epifaunal species (Gambi et al., 1997). This is potentially a result of the evolution

of their ecological strategies, notably their small body size and *r* reproductive strategy (Gambi et al., 1997). Among interstitial annelid species, those belonging to the *Ophryotrocha* genus (Dorvilleidae, Annelida) are particularly known in the literature, thanks to the easy with which some of them have been cultured in the laboratory for a wide array of biological, eco-toxicological, and eco-evolutionary investigations (e.g. Thornhill et al., 2009; Prevedelli et al., 2006). The genus *Ophryotrocha* is a widely distributed group of benthic annelids occupying diverse habitats and including more than 70 known species (Thornhill et al., 2009), ten of which have recorded genome size showing a threefold variation (Sella et al. 1993). Moreover, information on life history traits of many *Ophryotrocha* species is available, making them ideal models to explore genome size – life-history traits relationships.

We used flow cytometry to measure the genome size (C-value) and nucleus size (forward light scatter) to explore their relationships to key life-history traits in six *Ophryotrocha* species (*Ophryotrocha robusta*, *O. labronica*, *O. diadema*, *O. puerilis*, *O. adherens*, *O. japonica*) (Paavo et al., 2000; Paxton & Åkesson, 2007, 2010, 2011). We report that the *de-novo* genome size measured in the six *Ophryotrocha* species has been greatly underestimated in the past, and that these interstitial species in fact have large genomes. We show that body size, lifespan, and age at first deposition (a proxy for developmental rate) increase with genome size in the interstitial annelid assemblage investigated. The relationships of genome size to life-history traits were then tested on a broader phylogenetic scale, with regressions run using an additional 18 annelid species for which body size, age at first deposition, lifespan and/or fecundity could be found in the literature. We show that genome size – life-history relationships remain significantly positive for body size, age at first deposition and lifespan at this broader phylogenetic scale.

Material and Methods

Ophryotrocha species rearing and genome size determination

Specimens of the six *Ophryotrocha* species investigated in our study came from laboratory strains established from individuals collected in Italy (La Spezia, 44°06'N; 09°49'E, and Porto Empedocle 37°18'N; 13°32'E) and kept under control laboratory conditions (salinity: 32-35; temperature: 22-24 °C; pH_{NBS} = 8.1; photoperiod L:D of 12:12 h) for approx. 20 to 60

generations prior to genome size estimation. Thirty mature individuals were ground in 1 mL of Galbraith buffer (Galbraith et al., 1983) in each flow cytometry run. Three to seven runs were performed for each species. *Daphnia pulex* Leydig 1869 was used as standard for analyses (Vergilino et al., 2009). The mixture of nuclei (*Ophryotrocha* - *Daphnia*) was co-stained using 20 µL of propidium iodide (1.0 mg mL^{-1}) for 45 min. and analyzed on a CytoFLEX flow cytometer (see Supplementary Figure 1). Nuclear DNA content of all annelid species was calculated using the following equation: nuclear DNA = *Ophryotrocha* fluorescence / (*Daphnia* fluorescence x 0.45 pg), where the nuclear DNA content is pg DNA and 0.45 pg corresponds to the nuclear DNA content of *D. pulex* (Vergilino et al., 2009).

Flow cytometry data also yields information on particle size through forward light scatter. In general, forward light scatter correlates closely with particle size (e.g., Figure 3 in Belzile and Gosselin 2015). Forward light scatter has been used previously as an index of nucleus size in *Daphnia* (Jalal et al., 2013) and will be henceforth referred to as such. Mean forward scatter of the *nuclei* was thus recorded in order to assess the relationship between this measure and genome size, and data was analyzed using CytExpert Software v.2.3 (Beckman Coulter).

Life-history traits and species selection

Life-history traits for the six laboratory *Ophryotrocha* species were obtained from studies that used comparable rearing conditions (Simonini and Prevedelli 2003; Grandi 2009; Martino, 2012; Paxton and Åkesson 2010) : body size (mm), growth rate (chaetigers . day $^{-1}$), age at first deposition (d), egg size (µm), fecundity (eggs . clutch $^{-1}$), lifetime fecundity (eggs . individual $^{-1}$), and lifespan (d). Age at first deposition is considered here as a developmental proxy. Body size and fecundity were measured as the maximum body length (mm) recorded in the species and the average number of eggs laid per clutch, respectively. Growth rates were measured as number of chaetigers (segments bearing bristles, Massamba-N'Siala et al., 2011) added daily until reaching the maximum body size (measured as number of chaetigers). Lifetime fecundity referred to the total amount of eggs produced by an individual during its lifetime. Finally, egg size was measured as the arithmetic mean between the longer and the shorter axes (Simonini and Prevedelli, 2003). Life-history data of the additional annelid species was obtained from the literature (see Supplementary Tables I & II). Four life-history traits were considered for all species: body size (mm), fecundity (eggs . clutch $^{-1}$), lifespan (d), and age at first deposition (d).

Growth rates, lifetime fecundity, and egg size were traits available only for the six *Ophryotrocha* species.

Species for which genome size was available in the literature were selected for the final analysis based on the availability of COI and 16S sequences and the reliability of genome size measures (species reported by Sella et al. 1993 were omitted due to considerable discrepancies between their study and ours). In addition, deep-sea and vent species were removed due to signs of gigantism (> 1000 mm body lengths seen in *Tevnia jerichonana* and *Riftia pachyptila*, for example). Finally, the catworm *Nephtys incisa* was not included in fecundity analysis because of its disproportionate higher reproductive output (250 000 eggs size . clutch $^{-1}$) compared to the other annelid species (1 – 2000 eggs . clutch $^{-1}$).

Maximum likelihood phylogenies

Two maximum likelihood (ML) phylogenies were constructed with COI and 16S sequences. The first phylogenetic tree was comprised exclusively of sequences obtained from laboratory specimens of the six *Ophryotrocha* species (Tempestini *et al*., in press.). The second phylogenetic tree was constructed by adding the sequences of 18 annelid species collected from GenBank to the original six *Opryotrocha* species. The marine nemertean worm *Cerebratulus lacteus* served as outgroup for both phylogenies (Struck et al., 2011). Accession numbers are provided below (section *Data availability*). Multiple sequence alignments were performed with MUSCLE (Edgar 2004) using the software MEGAX (Kumar et al., 2018) with default parameters and concatenated in MEGAX. The alignments were run through RAxML-HPC2 (Stamatakis, 2014) using default parameters as well. In R (R v3.4.2 and RStudio v1.1.383), packages *ape* and *phytools* were used to import and transform the resulting tree as well as the phenotypic data. Final phylogenetic trees were produced using FigTree v1.4.4 (Figure 1).

Statistical analyses

A one-way analysis of variance (ANOVA) test with species as fixed factor and flow cytometry runs as replication units was first performed to determine if the six *Ophryotrocha* species differed in genome size. Pairwise comparisons were subsequently performed using Tukey's HSD test. Linear regressions models were conducted to test for significant relationships between genome size and single life-history traits in six species of *Ophrytrocha*. Furthermore, the

relationships between genome size and four life-history traits was tested in the expanded data set containing 18 additional annelid species. Life-history traits and genome size values were corrected for phylogenetic relatedness using phylogenetically independent contrasts (*pic* function in *ape*) for both phylogenies, and these analyses were run through the origin. Significant and marginally significant relationships were plotted in R for both phylogenetically-corrected and non corrected data. Body size was tested as a covariate alongside other life history traits in all linear models, before being removed from the model once deemed non-significant. Normality of residuals, tested with a Shapiro-Wilks test, was rejected for the ANOVA test, which was corrected with a $\log_{10}$ transformation of genome size data. Normality of residuals was also rejected in four instances for the regression models: the relationship between phylogenetically corrected genome size and nucleus size in the six *Ophryotrocha* species, the relationship between raw and phylogenetically corrected genome size and fecundity in the enlarged dataset, and the relationship between raw genome size and lifespan in the enlarged dataset. In all cases except the first one, a logarithmic transformation of the raw values was sufficient to meet the assumption of normality.

Statistical analyses were conducted using R (R v3.4.2).

Results

The mean genome size was 1.47, 1.23, 1.04, 1.45, 0.80 and 1.40 pg for *O. robusta*, *O. labronica*, *O. diadema*, *O. puerilis* , *O. adherens* and *O. japonica* respectively. Significant differences in $\log_{10}$ transformed mean genome size were found among species ($F_{(5, 20)} = 76.2$; $P = 2.51 \cdot 10^{-12}$). *Ophryotrocha japonica* and *O. puerilis* had the largest genome sizes that differed significantly from the ones of *O. adherens* and *O. diadema*. The genome size of *O. labronica* was significantly smaller than that of *O. puerilis* and significantly larger one than that of *O. adherens* (Supplemental Table 1). All life-history trait regression results for *Ophryotrocha* are summarized in Table I. Body size (mm), age at first deposition (d), fecundity (eggs . clutch $^{-1}$) and lifespan (d) regression results for the expanded annelid dataset are summarized in Table 2.

Our analysis indicates that *Ophryotrocha* species possessing larger genome size displayed larger nucleus sizes estimated through forward scatter; a significant positive relationship was found between these two traits after phylogenetic correction ($R^2 = 0.764$; $F_{(1,4)} = 12.97$; $P = 0.023$;

Figure 2). Species with larger genome sizes were found to have larger body sizes and nucleus sizes. These traits show a significant positive relationship in *Ophryotrocha* after phylogenetic correction (Figures 3A). Similarly, there was a significant increase in age at first deposition (d) in *Ophryotrocha* species with larger genome sizes after phylogenetic correction (Figures 3B). Fecundity did not differ significantly in *Ophryotrocha* species with small and large genomes (Figures 3C). The relationship between lifespan and genome size was significant in *Ophryotrocha* after phylogenetic correction (Figure 3D). No significant relationships were detected between genome size and growth rate, genome size and egg size, and genome size and lifetime fecundity in the *Ophryotrocha* group (Table 1).

Further analysis of genome size and life-history on a broader phylogenetic scale revealed similar patterns for three of the four significant traits mentioned above. Annelid species with larger genome sizes displayed a significantly larger body size (Figure 3E), a later age at first deposition (Figure 3F) and an increased lifespan (Figure 3H) after phylogenetic correction. There was no significant relationship between fecundity and genome size in extant annelid species (Figure 3G).

Discussion

Our study reveals that a number of important life history traits positively correlate to genome size in a set of species from the marine annelid *Ophryotrocha*. Age at first deposition, body size, and lifespan were positively associated to genome size whereas no significant associations were found for egg size, fecundity, and growth rate. Those patterns held up on a broader phylogenetic scale using additional annelid species for which genome size and life-history data were available. We also report that genome size estimates measured here in flow cytometry contradict previous estimates using Feulgen densitometry, with implications for downstream genomic applications.

The six *Ophryotrocha* species investigated here exhibit a three-fold difference in body size, which significantly increases with genome size. The relationship remained significant among the additional annelid species tested here with body sizes varying ten-fold. It was initially suggested by Gambi *et al*. (1997) that harsh interstitial habitats select for small genomes in meiofaunal annelids *via* the genome size - body size relationship. This was apparent when considering the

reported genome size range of 0.07 to 1.16 pg in interstitial species and 0.4 to 7.2 pg in macrobenthic species. However, this hypothesis does not appear to hold for *Ophryptrocha* species, as the genome sizes in this group are considerably large (0.80 - 1.47), while they possess fairly small body sizes (2 to 7 mm). Positive relationships between body size and genome size have been reported in numerous invertebrates (Hessen & Persson, 2009; Jeffery et al., 2017; Lefébure et al., 2017) but are not ubiquitous. These relationships are most often found in species where growth occurs largely as a result of increase in cell volumes, rather than by increasing cell numbers. The strong relationship between genome size and nucleus size found in *Ophryotrocha* potentially contributes to the positive relationship between genome size and body size, which suggests that cell volume influences whole-organism body size in this genus.

Genome size was strongly correlated with age at first deposition in *Ophryotrocha* as well as in the larger annelid dataset. This relationship has been described in different groups, with genome size impacting different proxies for developmental rate/time, such as voltinism in Lepidoptera (Miller, 2014), maturation rates in copepods (Wyngaard et al., 2005), embryonic development in salamanders (Jockusch, 1997), and age at sexual maturity and hatching time in birds (Yu et al., 2020). The relationship is overall apparent in pancrustaceans (i.e. insects and crustaceans), where species possessing smaller genomes show a faster development (Alfsnes et al., 2017). Since *Ophryotrocha* species have a direct development, we hypothesize that genome size could be less constraining in this group than in taxa possessing complex life-history strategies with multiple larval stages. In contrast to age at first deposition that is a proxy of growth, growth rate did not show an association with genome size. We expected that *Ophryotrocha* species with smaller genomes would have a higher growth rate due to their potentially faster cell divisions. It could be that our proxy for growth rate 'number of chaetigers deposited per day' is not precise enough in this small dataset. Surprisingly, lifespan was positively associated with genome size both in *Ophryotrocha* and in the annelid dataset. Genome size is not known to be correlated to lifespan (or longevity) in reptiles (Olmo, 2003), birds (Gregory, 2002; Yu et al., 2020) nor in fish species (Gregory 2004; Hickey & Clements, 2005). This positive relationship between genome size and longevity in annelids may be mediated by age at first deposition and warrants further studies. Genome size increase in *Ophryotrocha* was not significantly associated with fecundity nor with egg size. The relationship between egg size and genome size depends on the group investigated.

247 For example, egg size is positively associated with genome size in fish (Hardie & Hebert, 2011)
248 and in rotifer (Stelzer et al., 2011) but not in in salamanders (Jockusch, 1997).

249 In addition, we show here that the genome size of *O. robusta* (0.47 instead of 0.37 pg), *O.*
250 *puerilis* (1.45 instead of 0.46 pg) *O. labronica* (1.23 instead of 0,44 pg), and *O. diadema* (1.04
251 instead of 0.44 pg), are 2.4 to 4 times larger than previously reported (Sella et al. 1993; Soldi et
252 al. 1994). These previous estimates were assessed through Feulgen densitometry and are
253 compared to those measured in flow cytometry (Supplementary Figure 1). Artefacts associated
254 with Feulgen technique such as sample size limitation, staining issues (comparison of different
255 cell types with different levels of DNA compaction and stain uptake), conditions of slide fixation
256 may have biased these past estimates (Hardie et al., 2002). *Ophryotrocha labronica* has
257 historically been used for the investigation of life-history traits ecology and evolution (Simonini
258 & Prevedelli, 2003; Prevedelli et al., 2006; Rodríguez-Romero et al., 2016) and is emerging as a
259 model organism for the investigation of transgenerational responses of marine invertebrates to
260 global change drivers (Chakravarti et al., 2016; Rodríguez-Romero et al., 2016; Gibbin et al.,
261 2017a, 2017b; Jarrold et al., 2019). As it will be part of a foreseeable sequencing endeavour for
262 the development of –omics approaches, it would have been misleading to assume that its genome
263 size was 2.5-fold smaller than expected (1.04 *vs*. 0.40 pg). Considering that nearly 20 % of
264 annelid genome size in the database reference these two studies, it is likely that inferences based
265 on this data should be reconsidered.

266 In conclusion, our study provides strong evidence of the determinant role played by genome size
267 in the evolution of life-history traits, validated at both the genus and phylum level. Annelids
268 being characterised by an overwhelming biodiversity in marine environments represent a very
269 promising group to delve deeper into the c-value paradox.

270 **Declarations**

271 **Compliance with Ethical Standards**

272 All applicable international, national, and/or institutional ethics guidelines for sampling, care and
273 experimental use of organisms have been followed in this study.

274 **Data Availability**

We have deposited the primary data underlying these analyses as follows:

Sampling locations, morphological data, and microsatellite genotypes: Dryad

DNA sequences: Genbank accessions *Branchiura sowerbyi* (LN810299.1, KY636792.1), *Cerebratulus lacteus* (KC698905.1, KX261740.1), *Erpobdella obscura* (AF003273.1, JQ821464.1), *Hirudo medicinalis* (EF446704.1, AF315058.1), *Laeonereis culveri* (MH235843, MH264663.1), *Limnodrilus hoffmeisteri* (LN810304.1, AY885613.1), *Limnodrilus udekemianus* (LN810320.1, KY636789.1), *Lumbriculus variegatus* (FJ639308.1, AY521550.1), *Myxicola infundibulum* (HQ024104.1, HM800977.1), *Neanthes acuminata* (KJ539071.1, KJ538996.1), *Nephtys incisa* (KT307667.1, GU179356.1), *Ophidonais serpentina* (LN810257.1, DQ459939.1), *Ophryotrocha adherens* (MK933737, xxxxxx), *Ophryotrocha japonica* (MK933739, xxxxxx), *Ophrytrocha diadema* (MK933738, xxxxxx), *Ophrytrocha labronica* (MK933740,xxxxx), *Ophrytrocha puerilis* (MK933741, xxxxxx), *Ophrytrocha robusta* (MK933742,xxxxxx), *Platynereis dumerilii* (KP127954.1, KP640622.1), *Polygordius appendiculatus* (KF808170.1, MG603472.1), *Scalibregma inflatum* (GU672569.1, KF511816.1), *Spirosperma ferox* (KY636947.1, KY636799.1), *Syllis prolifera* (JF903780.1, JF903739.1), *Tubifex tubifex* (HM138034.1, AF326005.1)

Data citation

The main dataset has been assembled and presented here as supplementary material.

Author contributions

The experimental design and work have been conceived and planned by NB, GMN, PC and FD. Life-history data was extracted from the literature by NB and GMN. NB conducted genome size measurements under the supervision of CB and FD. NB conducted statistical analyses and results interpretation supervised by GMC, PC and FD. NB wrote the first draft of this manuscript supported by PC and FD. All authors contributed to the final version of this manuscript.

Competing interests

We have no competing interests.

Funding

This work was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant (RGPIN- 2020-05627), Programme Établissement de Nouveaux Chercheurs Universitaires of the Fonds de Recherche du Québec - Nature et Technologies (FRQNT, No.199173) awarded to PC, an NSERC grant (RGPIN- 06349) awarded to FD, a BORÉAS UQAR Grant awarded to PC, FD and GMN, and by the European Union through the Marie Skłodowska-Curie Post-doctoral Fellowship (EVOLMARIN project - Proposal Number: 659359) awarded to GMN.

Consent to participate (Not appropriate)

Consent for publication (Not appropriate)

Code availability (Not appropriate)

References

Adreani, L., C. Bonacina, G. Bonomi, & C. Monti, 1984. Cohort cultures of Psammoryctides barbatus (Grube) and Spirosperma ferox Eisen: a tool for a better understanding of demographic strategies in Tubificidae. Hydrobiologia 115: 113–119.

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.

Belzile, C., & M. Gosselin, 2015. Free-living stage of the unicellular algae Coccomyxa sp. parasite of the blue mussel (Mytilus edulis): Low-light adaptation, capacity for growth at a very wide salinity range and tolerance to low pH. Journal of Invertebrate Pathology 132: 201–207.

Block, E. M., G. Moreno, & C. J. Goodnight, 1981. Observations on the life history of Limnodrilus hoffmeisteri (Annelida, Tubificidae) from the Little Calumet River in temperate North America. International Journal of Invertebrate Reproduction 4: 239–247.

Bonacina, C., A. Pasteris, G. Bonomi, & D. Marzuoli, 1994. Quantitative observations on the population ecology of Branchiura sowerbyi (Oligochaeta, Tubificidae). Hydrobiologia 278: 267–274.

Cavalier–Smith, T. (ed), 1985. The Evolution of Genome Size. Wiley-Blackwell, Chichester West Sussex ; New York.

331 Chakravarti, L. J., M. D. Jarrold, E. M. Gibbin, F. Christen, G. Massamba- N'Siala, P. U. Blier,
332 & P. Calosi, 2016. Can trans-generational experiments be used to enhance species resilience to
333 ocean warming and acidification?. Evolutionary Applications 9: 1133–1146.

334 Dean, D., S. R. Chapman, & C. S. Chapman, 1987. Reproduction and development of the
335 sabellid polychaete Myxicola infundibulum. Journal of the Marine Biological Association of the
336 United Kingdom 67: 431–439.

337 Ducrot, V., A. R. R. Péry, H. Quéau, R. Mons, M. Lafont, & J. Garric, 2007. Rearing and
338 estimation of life-cycle parameters of the tubicifid worm Branchiura sowerbyi: application to
339 ecotoxicity testing. The Science of the Total Environment 384: 252–263.

340 Dufresne, F., & N. Jeffery, 2011. A guided tour of large genome size in animals: what we know
341 and where we are heading. Chromosome Research 19: 925–938.

342 Fernández, J., & G. S. Stent, 1982. Embryonic development of the hirudinid leech Hirudo
343 medicinalis: structure, development and segmentation of the germinal plate. Journal of
344 Embryology and Experimental Morphology 72: 71–96.

345 Fischer, A., & A. Dorresteijn, 2004. The polychaete Platynereis dumerilii (Annelida): a
346 laboratory animal with spiralian cleavage, lifelong segment proliferation and a mixed
347 benthic/pelagic life cycle. BioEssays: News and Reviews in Molecular, Cellular and
348 Developmental Biology 26: 314–325.

349 Fraipont, J., 1887. Le genre Polygordius. Engelmann.

350 Galbraith, D. W., K. R. Harkins, J. M. Maddox, N. M. Ayres, D. P. Sharma, & E. Firoozabady,
351 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220: 1049–
352 1051.

353 Gambi, M. C., L. Ramella, G. Sella, P. Protto, & E. Aldieri, 1997. Variation in Genome Size in
354 Benthic Polychaetes: Systematic and Ecological Relationships. Journal of the Marine Biological
355 Association of the United Kingdom 77: 1045–1057.

356 Gibbin, E. M., L. J. Chakravarti, M. D. Jarrold, F. Christen, V. Turpin, G. M. N'Siala, P. U.
357 Blier, & P. Calosi, 2017a. Can multi-generational exposure to ocean warming and acidification
358 lead to the adaptation of life history and physiology in a marine metazoan?. Journal of
359 Experimental Biology 220: 551–563.

360 Gibbin, E. M., G. Massamba N'Siala, L. J. Chakravarti, M. D. Jarrold, & P. Calosi, 2017b. The
361 evolution of phenotypic plasticity under global change. Scientific Reports 7: 1–8.

362 Grandi, V., 2009. Nicchia ecologica, storie vitali e morfologia. PhD Thesis, XXII cycle,
363 Università di Modena e Reggio Emilia.

364 Gregory, T. R., 2002. Genome size and developmental parameters in the homeothermic
365 vertebrates. Genome 45: 833–838.

366 Gregory, T. R., 2005. The C-value enigma in plants and animals: a review of parallels and an
367 appeal for partnership. Annals of Botany 95: 133–146.

368 Gregory, T. R., 2020. Animal Genome Size Database. Animal Genome Size Database. ,
369 http://www.genomesize.com.

370 Griffith, O. L., G. E. E. Moodie, & A. Civetta, 2003. Genome size and longevity in fish.
371 Experimental Gerontology 38: 333–337.

372 Hardie, D. C., T. R. Gregory, & P. D. N. Hebert, 2002. From pixels to picograms: a beginners'
373 guide to genome quantification by Feulgen image analysis densitometry. The Journal of
374 Histochemistry and Cytochemistry 50: 735–749.

375 Hardie, D. C., & P. D. Hebert, 2011. Genome-size evolution in fishes. Canadian Journal of
376 Fisheries and Aquatic Sciences 61:1636-1646

377 Hessen, D. O., & J. Persson, 2009. Genome size as a determinant of growth and life-history traits
378 in crustaceans. Biological Journal of the Linnean Society 98: 393–399.

379 Hickey, A. J. R., & K. D. Clements, 2005. Genome Size Evolution in New Zealand Triplefin
380 Fishes. Journal of Heredity 96: 356–362.

381 Jalal, M., M. W. Wojewodzic, C. M. M. Laane, & D. O. Hessen, 2013. Larger Daphnia at lower
382 temperature: a role for cell size and genome configuration?. Genome 56: 511–519.

383 Jarrold, M. D., L. J. Chakravarti, E. M. Gibbin, F. Christen, G. Massamba-N'Siala, P. U. Blier,
384 & P. Calosi, 2019. Life-history trade-offs and limitations associated with phenotypic adaptation
385 under future ocean warming and elevated salinity. Philosophical Transactions of the Royal
386 Society B: Biological Sciences 374: 20180428.

387 Jeffery, N. W., E. A. Ellis, T. H. Oakley, & T. R. Gregory, 2017. The Genome Sizes of Ostracod
388 Crustaceans Correlate with Body Size and Evolutionary History, but not Environment. The
389 Journal of Heredity 108: 701–706.

390 Jockusch, E. L., 1997. An evolutionary correlate of genome size change in plethodontid
391 salamanders. Proceedings of the Royal Society B: Biological Sciences 264: 597.

392 Kennedy, C. R., 1966. The Life History of Limnodrilus Udekemianus Clap. (Oligochaeta:
393 Tubificidae). Oikos 17: 10–18.

394 Lefébure, T., C. Morvan, F. Malard, C. François, L. Konecny-Dupré, L. Guéguen, M. Weiss-
395 Gayet, A. Seguin-Orlando, L. Ermini, C. D. Sarkissian, N. P. Charrier, D. Eme, F. Mermillod-
396 Blondin, L. Duret, C. Vieira, L. Orlando, & C. J. Douady, 2017. Less effective selection leads to
397 larger genomes. Genome Research 27: 1016–1028.

398 Leiva, F. P., P. Calosi, & W. C. E. P. Verberk, 2019. Scaling of thermal tolerance with body
399 mass and genome size in ectotherms: a comparison between water- and air-breathers.
400 Philosophical Transactions of the Royal Society B: Biological Sciences 374: 20190035.

401 Linton, D. L., & G. L. Taghon, 2000. Feeding, growth, and fecundity of Capitella sp. I in relation
402 to sediment organic concentration. Marine Ecology Progress Series 205: 229–240.

403 Lynch, M., & J. S. Conery, 2003. The origins of genome complexity. Science 302: 1401–1404.

404 Mackie, A. S. Y., 1991. Scalibregma Celticum New Species (Polychaeta: Scalibregmatidae)
405 from Europe, with a Redescription of Scalibregma Inflatum Rathke, 1843 and Comments on the
406 Genus Sclerobregma Hartman, 1965. Bulletin of Marine Science University of Miami -
407 Rosenstiel School of Marine and Atmospheric Science 48: 268-276(9).

408 Martin, J., & R. Bastida, 2006a. Population structure, growth and production of Laeonereis
409 culveri (Nereididae: Polychaeta) in tidal £ats of R|¤ o de la Plata estuary, Argentina. Journal of
410 the Marine Biological Association of the UK 86: 235–244.

411 Martin, J. P., & R. Bastida, 2006b. Life history and production of Capitella capitata (Polychaeta:
412 Capitellidae) in Río de la Plata Estuary (Argentina). Thalassas 22: 25–38.

413 Matisoff, G., X. Wang, & P. L. McCall, 1999. Biological Redistribution of Lake Sediments by
414 Tubificid Oligochaetes: Branchiura sowerbyi and Limnodrilus hoffmeisteri/Tubifex tubifex.
415 Journal of Great Lakes Research 25: 205–219.

416 Mazurkiewicz, M., 1975. Larval development and habits of Laeonereis culveri (Webster)
417 (Polychaeta: Nereidae). The Biological Bulletin 149: 186–204.

418 Miller, W. E., 2014. Phenotypic Correlates of Genome Size in Lepidoptera. The Journal of the
419 Lepidopterists' Society 68: 203–210.

420 Monaghan, P., & N. B. Metcalfe, 2000. Genome size and longevity. Trends in Genetics 16: 331–
421 332.

422 Nascimento, H. L. S., & R. G. Alves, 2009. The effect of temperature on the reproduction of
423 Limnodrilus hoffmeisteri (Oligochaeta: Tubificidae). Zoologia (Curitiba) 26: 191–193.

424 Ohtaka, A., & T. Iwakuma, 1993. Redescription of Ophidonais serpentina (Müller,
425 1773)(Naididae, Oligochaeta) from Lake Yunoko, Central Japan, with Record of the Oligochaete
426 Composition in the Lake. Japanese Journal of Limnology (Rikusuigaku Zasshi) 54: 251–259.

427 Olmo, E., 2003. Reptiles: a group of transition in the evolution of genome size and of the
428 nucleotypic effect. Cytogenetic and Genome Research 101: 166–171.

429 Paavo, B., J. H. Bailey-Brock, B. Åkesson, & A. Nylund, 2000. Morphology and life history of
430 Ophryotrocha adherens sp. nov. (Polychaeta, Dorvilleidae). Sarsia 85: 251–264.

431 Paxton, H., & B. Åkesson, 2007. Redescription of Ophryotrocha puerilis and O. labronica
432 (Annelida, Dorvilleidae). Marine Biology Research 3: 3–19.

433 Paxton, H., & B. Åkesson, 2010. The Ophryotrocha labronica group (Annelida: Dorvilleidae) —
434 with the description of seven new species. Zootaxa 2713: 1–24.

435 Paxton, H., & B. Åkesson, 2011. The Ophryotrocha diadema group (Annelida: Dorvilleidae),
436 with the description of two new species. Zootaxa 3092: 43–59.

437 Prevedelli, D., G. M. N'siala, & R. Simonini, 2006. Gonochorism vs. hermaphroditism:
438 relationship between life history and fitness in three species of Ophryotrocha (Polychaeta:
439 Dorvilleidae) with different forms of sexuality. Journal of Animal Ecology 75: 203–212.

440 Reish, D. J., 1957. The life history of the polychaetous annelid Neanthes caudata (Delle Chiaje),
441 including a summary of development in the family Nereidae. Pacific Science 216–288.

442 Ritchie, H., A. J. Jamieson, & S. B. Piertney, 2017. Genome size variation in deep-sea
443 amphipods. Royal Society Open Science 4: 170862.

444 Rodríguez-Romero, A., M. D. Jarrold, G. Massamba-N'Siala, J. I. Spicer, & P. Calosi, 2016.
445 Multi-generational responses of a marine polychaete to a rapid change in seawater pCO2.
446 Evolutionary Applications 9: 1082–1095.

447 Simonini, R., & D. Prevedelli, 2003. Life history and demography of three populations of
448 Ophryotrocha japonica (Polychaeta: Dorvilleidae). Marine Ecology Progress Series 258: 171–
449 180.

450 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
451 large phylogenies. Bioinformatics 30: 1312–1313.

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

454 Struck, T. H., C. Paul, N. Hill, S. Hartmann, C. Hösel, M. Kube, B. Lieb, A. Meyer, R.
455 Tiedemann, G. Purschke, & C. Bleidorn, 2011. Phylogenomic analyses unravel annelid
456 evolution. Nature 471: 95–98.

457 Timm, T., 2020. Observations on the life cycles of aquatic Oligochaeta in aquaria. Zoosymposia
458 17: 102–120.

459 Tsutsumi, H., & T. Kikuchi, 1984. Study of the life history of Capitella capitata (Polychaeta:
460 Capitellidae) in Amakusa, South Japan including a comparison with other geographical regions.
461 Marine Biology 80: 315–321.

462 Vergilino, R., C. Belzile, & F. Dufresne, 2009. Genome size evolution and polyploidy in the
463 Daphnia pulex complex (Cladocera: Daphniidae). Biological Journal of the Linnean Society 97:
464 68–79.

465 Wyngaard, G. A., E. M. Rasch, N. M. Manning, K. Gasser, & R. Domangue, 2005. The
466 relationship between genome size, development rate, and body size in copepods. Hydrobiologia
467 532: 123–137.

468 Yu, J. P., W. Liu, C. L. Mai, & W. B. Liao, 2020. Genome size variation is associated with life-
469 history traits in birds. Journal of Zoology 310: 255–260.

Zajac, R. N., & R. B. Whitlatch, 1988. Population ecology of the polychaete Nephtys incisa in Long Island sound and the effects of disturbance. Estuaries 11: 117–133.

Figure Captions

Figure 1: Maximum likelihood phylogenies of cytochrome oxidase I and 16S sequences in six *Ophryotrocha* species (A) and 23 annelid species (B) with outgroup *Cerebratulus lacteus*, produced using RAxML-HPC2 and plotted in FigTree v1.4.4.

Figure 2: Relationship between genome size (C-value expressed in pg) and nucleus size estimated from forward light scatter in flow cytometry. Forward light scatter correlates closely to particle size and has previously been used as an index of nucleus size in Daphnia. The data points were corrected by phylogenetically independent contrasts applied using a cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper.

Figure 3: Relationships between genome size (C-value expressed in pg) and (A) body size (mm), (B) age at first deposition (d), (C) fecundity (eggs . clutch -1) and (D) lifespan (d) for the six laboratory Ophryotrocha species. The same relationships were plotted for (E) body size (mm) in 16 total species, for (F) age at first deposition (d) in 12 total species, for (G) log 10 transformed fecundity (eggs . clutch -1) in 13 total species and for (H) lifespan (d) in 13 total species. The data points were corrected by phylogenetically independent contrasts applied using two cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogenies detailed in the present paper.

Figure S1: Frequency histograms of isolated nuclei propidium iodide fluorescence; (A) *O. diadema* and (B) *O. labronica*. *Daphnia pulex* nuclei peaks are blue and *Ophryotrocha* nuclei peaks in red. The black arrow indicates where the supposed *Ophryotrocha* peaks would be found according to the genome size values reported by Sella *et al*., (1993).

Table Captions

Table 1. Regression test results carried out between genome size and seven life-history traits. The data points were corrected by phylogenetically independent contrasts applied using a

cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper. Significant relationships are indicated by stars.

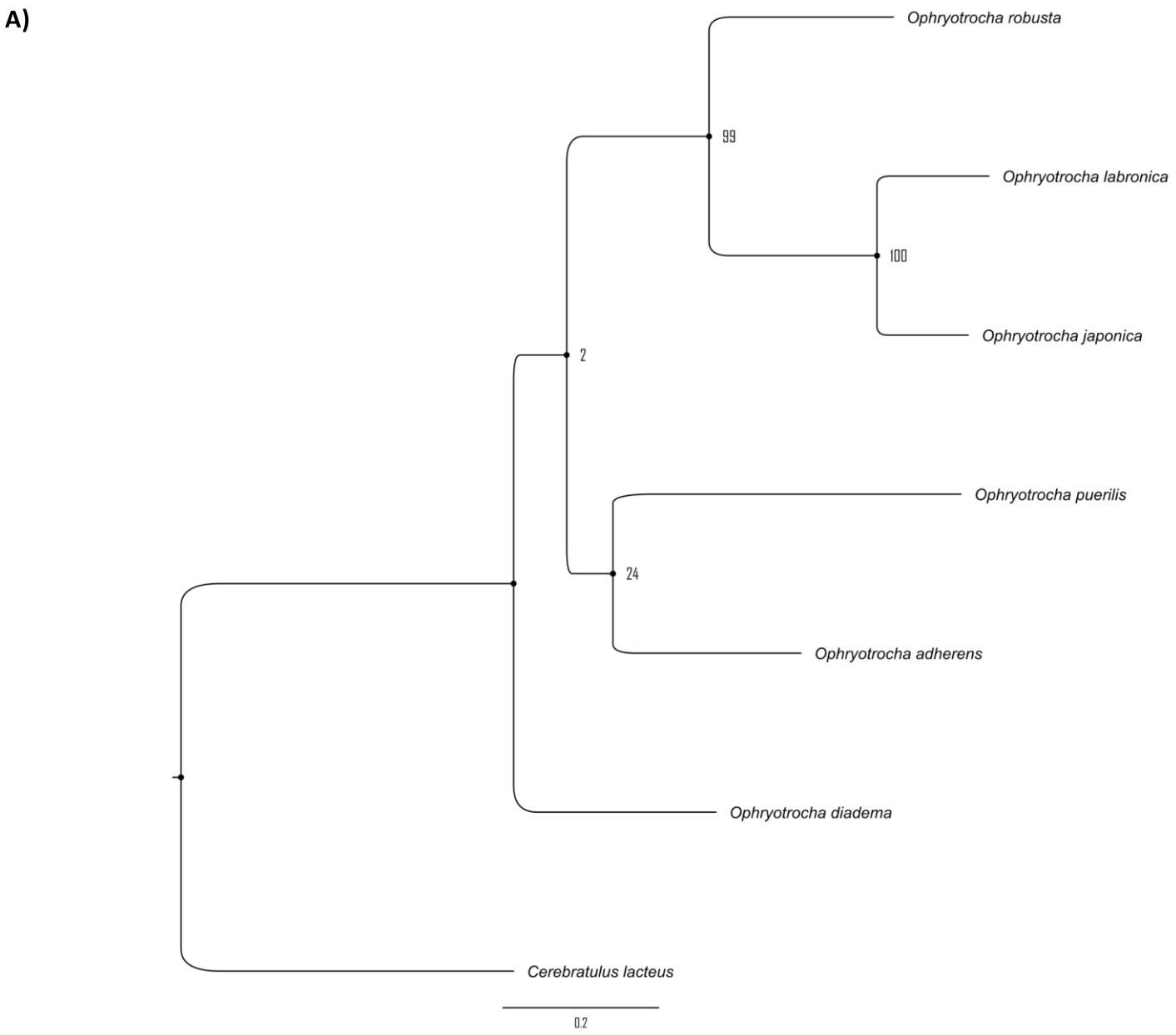
Table 2. Regression test results carried out between genome size and body size (mm), age at first deposition (d), log 10 transformed fecundity (eggs . clutch -1), log 10 transformed lifespan (raw) and lifespan (PIC) (d) in 16, 12, 13 and 13 annelid species respectively, before (raw) and after (PIC) phylogenetic correction. The data points were corrected by phylogenetically independent contrasts applied using a cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper.

Table SI: Mean life-history traits and genome size (C-value in picograms) for six *Ophryotrocha* species reared in laboratory.

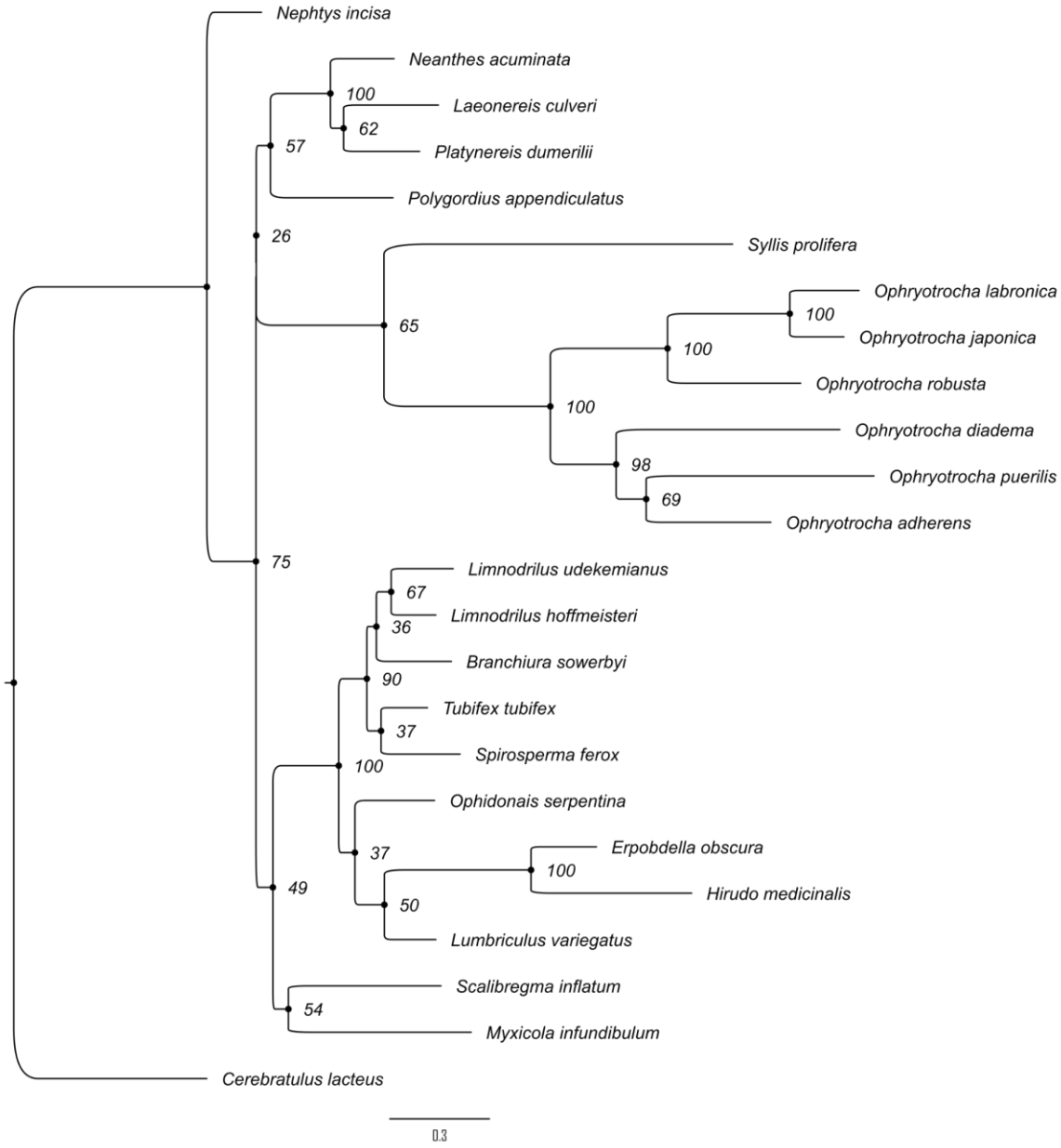
Table SII: Life-history traits collected from the literature and genome size (C-value in picograms) from the Animal Genome Size Database (Gregory, 2020) for ten additional annelid species.

Figure

Click here to access/download;Figure;Figure1final.docx



B)


Nephtys incisa
Neanthes acuminata
100
Laeonereis culveri
62
Platynereis dumerilii
57
Polygordius appendiculatus
26
Syllis prolifera
Ophryotrocha labronica
100
65
Ophryotrocha japonica
100
Ophryotrocha robusta
100
Ophryotrocha diadema
98
Ophryotrocha puerilis
69
Ophryotrocha adherens
75
Limnodrilus udekemianus
67
Limnodrilus hoffmeisteri
36
Branchiura sowerbyi
90
Tubifex tubifex
37
Spirosperma ferox
100
Ophidonais serpentina
37
Erpobdella obscura
100
Hirudo medicinalis
49
50
Lumbriculus variegatus
Scalibregma inflatum
54
Myxicola infundibulum
Cerebratulus lacteus
0.3

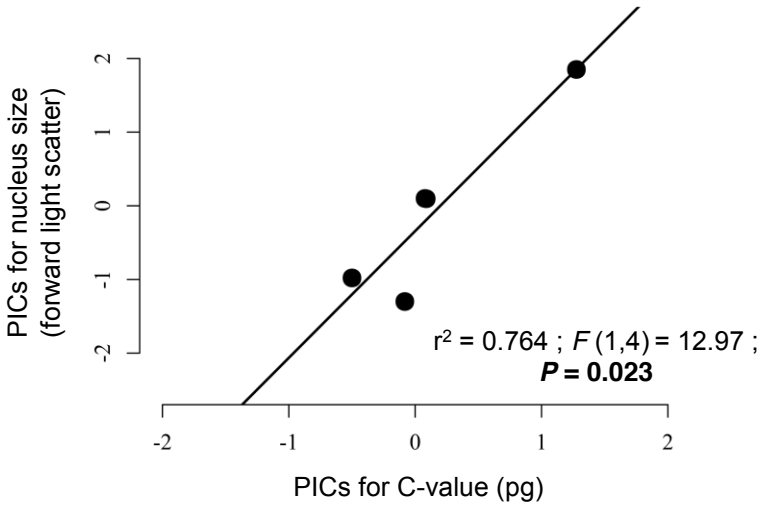


Figure 2. Positive linear relationship between genome size (C-value in picograms) and nucleus size estimated from forward light scatter in flow cytometry. Forward light scatter correlates closely to particle size and has previously been used as an index of nucleus size in *Daphnia*. The data points were corrected by phylogenetically independent contrasts applied using a cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper.

Figure

Click here to access/download;Figure;Figure3final.docx

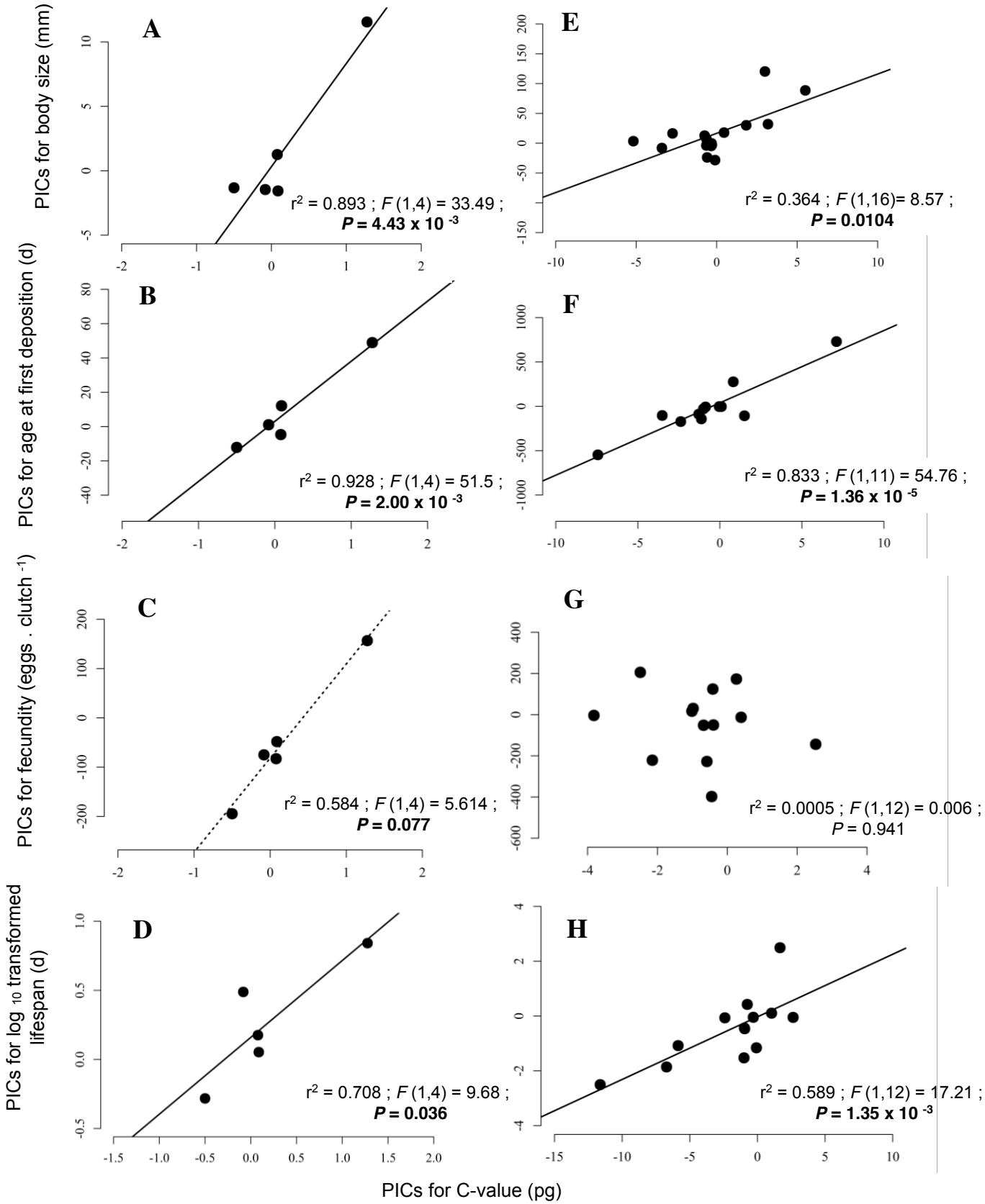


Figure 3. Relationships between genome size (C-value expressed in pg) and (A) body size (mm), (B) age at first deposition (d), (C) fecundity (eggs . clutch $^{-1}$) and (D) log $_{10}$ transformed lifespan (d) for the six laboratory *Ophryotrocha* species. The same relationships were plotted for (E) body size (mm) in 18 total species, for (F) age at first deposition (d) in 13 total species, for (F) fecundity (eggs . clutch $^{-1}$) in 14 total species and for (G) log $_{10}$ transformed lifespan (d) in 14 total species. The data points were corrected by phylogenetically independent contrasts applied using two cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogenies detailed in the present paper.

Table I: Results for the regression test carried out for seven life-history traits with genome size before and after phylogenetic correction. The data points were corrected by phylogenetically independent contrasts applied using a cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper. Significant relationships are indicated with stars.

Trait	pre-PIC			PIC		
	r^2	*F* (1,4)	*P*-value	r^2	*F* (1,4)	*P*-value
Egg size (µm)	0.520	0.219	0.664	0.108	0.486	0.524
Fecundity (eggs. clutch^{-1})	0.574	5.379	0.081	0.584	5.614	0.077
Lifetime fecundity (eggs)	0.416	2.852	0.167	0.398	2.644	0.179
Body size (mm)	0.612	6.312	0.066	0.893	33.49	4.43×10^{-3} **
Log_{10} lifespan (d)	0.587	5.692	0.076	0.708	9.68	0.036 *
Age at first deposition (d)	0.886	30.93	0.005 **	0.928	51.5	2.00×10^{-3} **
Growth rate (chaetigers . d^{-1})	0.476	2.724	0.197	0.516	4.258	0.108

Table 2. Results for the regression tests carried out for body size (mm), fecundity and age at first deposition (d) with genome size in 18, 14 and 13 annelid species respectively, before (left) and after (right) phylogenetic correction. The data points were corrected by phylogenetically independent contrasts applied using a cytochrome oxidase I and 16S maximum likelihood (RAxML-HPC2) phylogeny detailed in the present paper.

Trait	pre-PIC			PIC		
	r^2	F (1,16)	P-value	r^2	F (1,16)	P-value
Body size (mm)	0.476	13.64	2.17×10^{-3} **	0.364	8.57	0.0104 *
Fecundity (eggs . clutch^{-1})	0.002	F (1, 12) / 0.020	0.889	0.0005	F (1, 12) / 0.006	0.941
Log $_{10}$ maximum lifespan (d)	0.256	F (1, 12) / 4.127	0.065	0.589	F (1, 12) / 17.21	1.35×10^{-3} **
Age at first deposition (d)	0.650	F (1, 11) / 20.4	8.76×10^{-4} **	0.833	F (1, 11) / 54.76	1.36×10^{-5} ***