

Genome Size Estimation of the  
Protist Symbionts of *Coptotermes formosanus*

by

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## ABSTRACT

Parabasalia is a phylum of flagellated protists with a large range of cell sizes, spanning from as little as 7  $\mu\text{m}$  in length (e.g. *Pentatrichomonas hominis*) to well over 300  $\mu\text{m}$  (e.g. *Pseudotrichonympha grassii*). Many Parabasalia are associated with animals in mutualistic, parasitic, or commensal relationships. The largest Parabasalia species are obligate mutualists of termites, which help to digest lignocellulose. While the specific digestive roles of different protist species are mostly unknown, Parabasalia with different cell sizes are known to inhabit different regions of the termite hindgut. It is currently unclear whether these size differences are driven by selection or drift, but it is well known that cell size correlates with genome size in eukaryotes. Therefore, in order to gain insight into possible selection pressures or mechanisms for cell size increase, genome sizes were estimated for the five Parabasalia species that inhabit the hindgut of *Coptotermes formosanus* Shiraki. The cell volumes and C-values for the five protist species are 89,190  $\mu\text{m}^3$  and 147 pg in *Pseudotrichonympha grassii*, 26,679  $\mu\text{m}^3$  and 56 pg in *Holomastigotoides hartmanni*, 8,985  $\mu\text{m}^3$  and 29 pg in *Holomastigotoides minor*, 1,996  $\mu\text{m}^3$  and 12 pg in *Cononympha leidy*, and 386  $\mu\text{m}^3$  and 6 pg in *Cononympha koidzumii*. The positive correlation between genome size and cell size was maintained in this group ( $R^2 = 0.76$ ). These genome sizes are much larger than the previously estimated genome sizes of non-termite associated Parabasalia, which spanned 2-fold ranging from 0.088 pg (in *Tetratrichomonas gallinarum*) to 0.181 pg (in *Trichomonas foetus*). With these new estimates, the range now spans over 1,500-fold from 0.088 pg to 147 pg in *P.*

*grassii*, implying potential differences in the level of selective pressures for genome size in termite-associated Parabasalia compared to other protists.

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## 1. INTRODUCTION

Genome size has large variation across eukaryotes, most of which is unexplained. It has long been noted that some organisms considered less complex than humans had larger genome sizes; this disconnect between perceived organismal complexity and genome size is known as the C-value enigma (Gregory 2002). Many attempts have been made to correlate genome size to cell characteristics such as metabolic rate, rate of development, and body size which have occasionally revealed weak lineage-specific relationships. The only characteristic that has a consistent correlation to genome size across eukaryotes is cell size (Gregory 2001; Cavalier-Smith 1980). Despite knowing these relationships exist and many of the factors that can contribute to genome changes, the evolutionary forces behind genome expansion and reduction are highly debated. These forces are generally explained through ‘mutational pressure’ or ‘optimal DNA’ types of theories. In mutational theories, genome size is explained by the balance between constant mutational pressure to accrue insertions and selective pressures to keep the genome size in check (Pagel and Johnstone 1992). Under this view, any correlation between C-value and cell size or other cell characteristics is merely coincidental. Examples of mutational pressure theories include the junk DNA theory, in which non-genic regions or transposable elements (TE) accumulate over time until replication is strained, and selfish DNA theories, in which transposable elements propagate themselves within the DNA sequence (Doolittle and Sapienza 1980; Orgel and Crick 1980; Gregory 2001). Conversely, optimal DNA theories consider the quantity of DNA to affect an organism’s fitness, such that genome sizes and other cell characteristics, such as cell volume or metabolic rate can coevolve (Gregory 2001; Cavalier-Smith 1980). In the



nucleo-skeletal optimal DNA theory, cell volume may be the primary target of selection but changes in nucleus volume accompany those changes and modulation of DNA content is necessary to alter the nucleus envelope size (Cavalier-Smith 1978).

This study aims to estimate the genome size of *Coptotermes formosanus* symbionts and place their cell and genome sizes in context of both the evolution of Parabasalia as well as the generalized context of the evolution of eukaryotes as a whole. Regardless of the explanation as to why, this study recognizes the positive correlation that cell size has with DNA content and hypothesizes that due to the relatively large size of *C. formosanus* symbiont protists they are expected to display relatively large genome sizes. Of the *C. formosanus* symbionts, the smallest is similar in size to and the largest is about 30 times larger than *Trichomonas vaginalis*, a well-studied model parabasalid (Jasso-Selles et al. 2020). Although the protist community of *C. formosanus* has recently been characterized by newly identifying two species using molecular and phylogenetic means and discovering species specific functional roles, there is still a lot that needs to be done to get a better understanding of their role in the symbiotic relationship with termites and their evolution (Jasso-Selles et al. 2020; Nishimura et al. 2020).

In this study, C-value and genome size both refer to the amount of DNA in the nucleus of the dominant life cycle stage, for example the soma of an animal or the vegetative phase of a protist. This can be further specified as 2C for predominantly diploid organisms or 1C for predominantly haploid organisms (or the gametes of diploid organisms). Genome size is usually given in the units of mega- or giga-bases (Mbp, Gbp) while C-value is given in picograms (pg); 1 pg = 978 Mbp (Doležel et al. 2003).

Through karyotype testing, it has recently been determined that trichomonads are haploid (1C). This paper will calculate the genome sizes of the *C. formosanus* symbionts as if they were haploid as well (Zubáčová et al. 2008).

### **1.1 Parabasalia**

Recent advances have brought relative stability to the eukaryotic tree of life, resulting in a number of super-kingdom level lineages called supergroups. While most of the supergroups remain relatively stable in the tree, the Metamonada, to which Parabasalia belong, have uncertain placement. Metamonada was previously groups with Discoba and *Malawimonas* in the supergroup Excavata but the phylogenetic support has been mixed (Hampl et al. 2009; Brown et al. 2018). Within Metamonada are the phyla Preaxostyla, Parabasalia, and Fornicata, which mainly include species that form symbiotic relationships with animals. Symbiosis refers to the relationships between two different organisms that is either parasitic, commensal, or mutualistic (Paracer and Ahmadjian 2000). Parasitic symbiosis occurs when one organism benefits from the relationship at the cost of the other. This describes the relationship between pathogens and their hosts, such as *T. vaginalis* mentioned earlier. Commensal symbiosis occurs when one organism benefits while the other neither benefits nor is harmed by the presence of the other. A large portion of Metamonada species fall into this category. Finally, mutualistic symbiosis describes the relationship between two organisms when both benefit from the presence of the other. Symbiont refers to the organisms within such relationships, in this paper it refers to the group of Parabasalia protists that reside in the hindgut of *C. formosanus*.

Of the three Metamonada phyla, Preaxostyla and Parabasalia have species known to reside in certain termites and wood-eating roaches in the genus, *Cryptocercus*. Within Preaxostyla, the order Oxymonadida, which is comprised of roughly 140 species, contains all of the termite and *Cryptocercus*-associated species of Preaxostyla (Hampl 2016). The order also contains some species that can be found elsewhere besides the termite or cockroach gut, such as *Monocercomonoides* which can be found in a wide variety of terrestrial animals, such as guinea pigs, crickets, and lizards (Hampl 2016).

There are approximately 450 described Parabasalia species grouped into six classes: Trichomonadea, which is divided into two orders (Trichomonadida and Honigbergiellida) and four families; Tritrichomonadea, which has one order (Tritrichomonadida) and four families; Hypotrichomonadea, which has one order (Hypotrichomonadida) and one family; Cristamonadea, which has one order (Cristamonadida) and one family; Spirotrichonymphea, which has one order (Spirotrichonymphida) and one family; and Trichonymphea, which has two orders (Trichonymphida and Lophomonadida) and a total of six families (Figure 1; Čepička et al. 2017). Parabasalia are mostly anaerobic and symbiotic, having parasitic, mutualistic, or commensal relationships. The orders Trichonymphida, Spirotrichonymphida, and Cristamonadida are only found in termites and *Cryptocercus* and have an obligate mutualistic relationship to their hosts (Cleveland 1925; Čepička et al. 2010). Members of the other four orders are often found in the digestive tracts of various animals although some have been found outside their hosts digestive tract, such as *T. vaginalis* which is a

human sexually transmitted infection. Some are host specific while others, such as *Trichomitus batrachorum* and *Pentatrichomonas hominis*, can infect multiple host species (Honigberg 1953; Čepička et al. 2005; Smejkalová et al. 2011). A few species are free-living, such as *Pseudotrichomonas keilini* and *Monotrichomonas carabina* (Honigbergiellidae), and can be found in freshwater, brackish, and marine anoxic or microoxic environments (Bishop 1935; Bishop 1939; Bernard et al. 2000; Yubuki et al. 2010). Across these classes there is a lot of variability in cell size and complexity as well as number of flagella. Parabasalia is informally divided into two groups based on these character differences: trichomonads are mostly small cells with few flagella while hypermastigotes tend to be large and have many flagella, from around 20 to more than ten thousand.

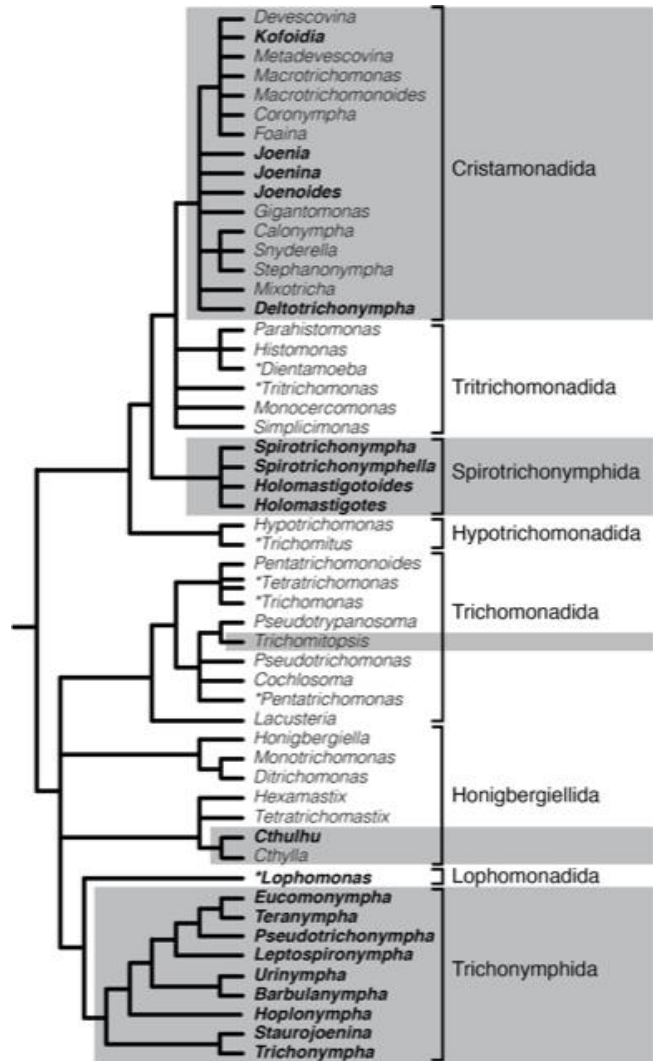
The informal term “trichomonad” describes the smaller pleisomorphic species belonging to Trichomonadida, Honigbergiellida, Tritrichomonadida, and Hypotrichomonadida as well as some larger uni-nucleate species belonging to Cristamonadida (Čepička et al. 2010; Gile and Slamovits 2012; James et al. 2013). They range from 10 – 100  $\mu\text{m}$ ; those found in vertebrates tend to be closer to 10  $\mu\text{m}$  while those that inhabit termites tend to be closer to the 100  $\mu\text{m}$  end of the range. Trichomonads are primarily uninucleate, although two lineages of Cristamonadida have developed multiple nuclei (Cleveland et al. 1964; Brugerolle 2004; Harper et al. 2009; Gile et al. 2011). They have up to six flagella per nucleus including a recurrent flagellum that runs posteriorly along the cell, (Kirby 1942; Hollande and Valentin 1969; Brugerolle and Lee 2000). Hypermastigotes include all species from the orders Trichonymphida,

Lophomonadida, and Spirotrichonymphida and a few species from Cristamonadida and Honigbergiellida (Čepička et al. 2010; Gile and Slamovits 2012; James et al. 2013).

Although they share many of the same cell structures as the trichomonads, the cells tend to be larger, measuring in the hundreds of  $\mu\text{m}$ , and can have more than ten thousand flagella. The Trichonymphida hypermastigotes have an anteriorly positioned rostrum while Spirotrichonymphida hypermastigotes lack a rostrum.

Parabasalids and oxymonads share many characteristics, such as anaerobic metabolism and ecto- and endosymbiotic relationships with bacteria. They differ in certain aspects as well, like in mitochondria and Golgi (Čepička et al. 2017). Both groups are anaerobic, being intolerant of oxygen, which allows them to survive in the guts of animals. Both groups also experience symbiotic relationships with bacteria and protists inside and outside their cell such as with spirochetes which are attached to the outside of the cell of some species (Ohkuma et al. 2015; Čepička et al. 2017). There is also a trend of larger species of oxymonads that reside in the gut of termites to increase in flagella number, though not as drastic an increase as in Parabasalia (Čepička et al. 2017). In contrast, *Preaxostyla* have a range of mitochondria types from present to reduced to absent. Reduced mitochondria called hydrogenosomes are present in *Trimastix* and *Paratrimastix* (similar to Parabasalia) while mitochondria tend to be absent, with no homologous or modified organelle, in Oxymonadida (Tachezy 2008; Karnkowska et al. 2016; Čepička et al. 2017). Oxymonads also differ in their Golgi complex. In Parabasalia, there is a distinctive and characteristic Golgi complex that is part of their parabasal body. In contrast, the Golgi complex in Oxymonadida is not identified

(Karnkowska et al. 2016). There are Golgi specific genes in oxymonads indicating its presence but is too discreet to identify in electron microscopic images (Karnkowska et al. 2016).



**Figure 1.** Schematic phylogeny of Parabasalia, including about half of the approximately 100 described genera, modified from Čepička et al. 2017. Gray boxes indicate exclusively termite-associated genera. Asterisks indicate genera for which at least one species has been reported to inhabit or infect humans. Bold type indicates hypermastigotes.

## 1.2 The Termite *Coptotermes formosanus* Shiraki

With the help of their gut endosymbionts, termites are one of the few groups of animals that can break down the cellulose in wood. This ability makes them both ecologically important and economically destructive, as they are major contributors to plant decomposition but can also severely damage human-made wooden structures (Rust and Su 2011). Termites form a clade along with the *Cryptocercus* wood roaches within the cockroach class Blattodea. Termites are divided into two groups informally referred to as “lower” and “higher” termites. Gut protists were acquired in the ancestor of this clade and helped establish eusocial behavior along with shifts from parental to alloparental brood rearing in the termites (Nalepa 2015). In later-diverging host lineages these protists were lost, and this loss is what distinguishes “lower” and “higher” termites. “Lower” termites include the deeper-branching, more plesiomorphic termites who have retained gut protist communities while “higher” termites, which comprise the majority of termite species, have a more recent origin and do not harbor specialized wood-feeding protist symbionts (Chouvenc et al. 2021). The loss of protists and acquisition of new bacteria and archaea to replace and perform new digestion-related function in “higher” termites allowed for increased diversity and specialization of those termites (Chouvenc et al. 2021). Although it is not clear how diversification effected the loss of protists or vice versa, it is known that there is an increase in diversity of feeding modes and other colony characteristics in “higher” termites. Rather than feeding strictly on wood, the “higher” termites can feed on wood, soil, leaf litter, and fungi (Bignell 2016). The change in

symbiont community composition also accompanied increased complexity of nesting patterns and caste differentiation.

*Coptotermes formosanus* Shiraki (Blattodea: Rhinotermitidae) is a subterranean “lower” termite (Krishna et al. 2013). It lives in warm to temperate subtropical climates and is native to China and Taiwan but has also been found in the United States, specifically in Florida, Louisiana, and Hawaii (Li et al. 2009; Chouvenc, Li, et al. 2016). *Coptotermes formosanus* was most likely first established in Florida in the 1970s. By the 2000s, it was causing minimal localized damage within the South Florida Metropolitan Area and it is projected that by 2040 roughly 50% of the South Florida Metropolitan Area will experience termite infestation (Chouvenc, Scheffrahn, et al. 2016). As of 2010, the annual global cost of termite pest management and damage repair was estimated to be \$40 billion. Of the termite species considered pests, subterranean termites (belonging to the “lower” termite family Rhinotermitidae) contribute to about 80% of the costs (Rust and Su 2011).

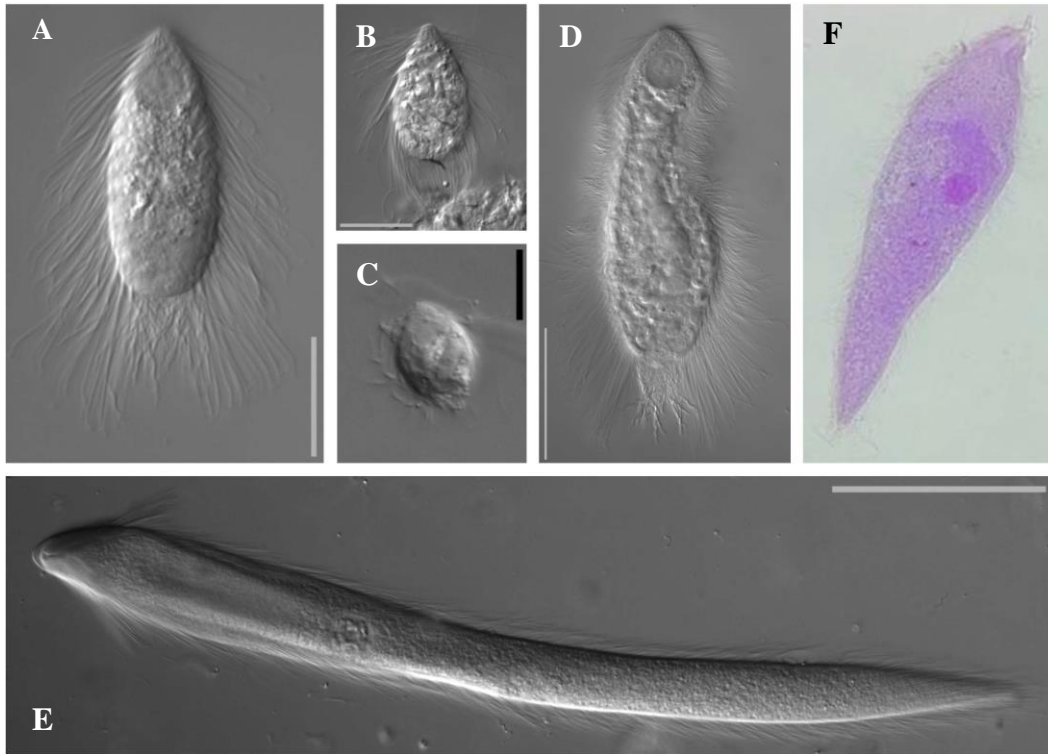
All “lower” termites possess a protist community hosted in their gut which aid in the digestion of wood particles. Protist identification from selected termite species hindguts have been performed through morphological and molecular methods. Morphological identification methods have tended to under-estimate the number of species compared to molecular methods although the use of both methods is preferable (Harper et al. 2009; Duarte et al. 2018). Initial investigations into the *C. formosanus* hindgut microbiota using morphologic identifiers revealed three protist species: *Pseudotrichonympha grassii*, *Holomastigotoides hartmanni*, and *Cononympha*



*leidyi* (previously named *Spirotrichonympha leidyi*) (Koidzumi 1921). Recent studies using 18S rRNA gene sequence and single-cell PCR have identified five parabasal species within the *C. formosanus* hindgut, adding *Holomastigotoides minor* and *Cononympha koidzumii* (Jasso-Selles et al. 2020; Nishimura et al. 2020) (Figure 2).

*Cononympha leidyi* cells are small to medium, ranging about 15-60  $\mu\text{m}$  long, cone shaped, have a centrally positioned nucleus, and spiral flagellar bands that cover the entire cell (Koidzumi 1921; Jasso-Selles et al. 2020). *Cononympha koidzumii* has a shorter, more blunt, apex and rounder posterior due to spiral flagellar bands extending only half way down the cell body (Jasso-Selles et al. 2020). They are small cells that range from about 10-35  $\mu\text{m}$  long (Jasso-Selles et al. 2020). *Holomastigotoides hartmanni* are large cells, measuring 50-170  $\mu\text{m}$  in length and 30-105  $\mu\text{m}$  in width, with a somewhat irregular oval shape (Koidzumi 1921; Jasso-Selles et al. 2020). They have tight spiral flagellar bands that cover the entire cell and spirochetes at the posterior of the cell (Koidzumi 1921; Jasso-Selles et al. 2020). The recently described *Holomastigotoides minor* cells are medium in size, measuring from 23-117  $\mu\text{m}$  in length. They tend to have a flattened bell shape from a front view or a narrow ovoid shape in side view with spiral flagellar bands that cover the entire cell and spirochetes at the posterior (Jasso-Selles et al. 2020; Nishimura et al. 2020). *Pseudotrichonympha grassii* cells are long and narrow, covered in flagella, and measure 150-400  $\mu\text{m}$  long with

distinctive an apical rostrum with a nose cone and longer flagella around the base of the rostrum (Grassi and Foà 1911; Koidzumi 1921).



**Figure 2.** Symbiont community of *C. formosanus*. (A-E) Differential interference contrast light microscopy of live cells; (F) Light microscopy of fixed and Feulgen stained cells. (A) *Holomastigotoides minor* Nishimura, (B) *Cononympha leidy* Koidzumi, (C) *Cononympha koidzumii* Jasso-Selles & Gile, (D) *Holomastigotoides hartmanni* Koidzumi, (E,F) *Pseudotriconympha grassii* Koidzumi. Scale bars: (A,B) 20  $\mu\text{m}$ , (C) 10  $\mu\text{m}$ , (D) 50  $\mu\text{m}$ , (E) 100  $\mu\text{m}$ .

### 1.3 Symbiotic Digestion in “Lower” Termite Hindguts

The “lower” termites’ ability to digest lignocellulose comes from their symbiotic relationships with the microbiota of their hindgut which includes diverse protists and bacteria (Cleveland 1923; Cleveland 1924). The wood-eating protists all belong to the phylum Parabasalia or the order Oxymonadida (phylum Preaxostyla). They are vertically inherited from parent to offspring in newly established colonies and later between the

workers, soldiers, and reproductives of established colonies through proctodeal trophallaxis (Eggleton 2010; Bourguignon et al. 2015; Nalepa 2015). Due to this vertical inheritance, the host termites and their hindgut symbionts have co-evolved together into an obligate mutualistic association. Certain symbiont genera are restricted to specific termite families and each of the lower termite species contains a unique assortment of protists within its hindgut which, to a certain degree, can be used to identify the termite species (Kirby 1949). The various protist species tend to reside in specific areas of the hindgut; the regional differentiation of the protists may be related to each species' oxygen requirements (Lai et al. 1983). In *C. formosanus*, *Cononympha* often reside in the more posterior portions of the hindgut, *Holomastigotoides* reside in the middle, and *Pseudotriconympha* in the anterior hindgut regions (Lai et al. 1983; Tsunoda 2016). Different protists also perform differentiated enzymatic activities to aid in lignocellulose digestion. For example, in *C. formosanus*, *Cononympha leidy* has been found to perform functions related to chitin degradation and *Pseudotriconympha grassii* has been found to produce enzymes that aid in the initial degradation of cellulose at the beginning of the hindgut (Watanabe et al. 2002; Nishimura et al. 2020).

#### **1.4 Genome Size Variation in Eukaryotes**

Genome size across eukaryotes span 200,000-fold. Some groups such as birds and echinoderms have relatively low variation with a C-values spanning 2-fold and 8-fold respectively while other taxa have very large variation such as angiosperms which can span over 1,000-fold (Gregory 2001; Gregory 2005). C-values have been found to correlate with several characteristics in specific lineages. For example, there is a

negative correlation between rate of development and genome size in some arthropods and between metabolic rate and genome size of birds (Wyngaard et al. 2005; Ji and DeWoody 2017). Across all eukaryotes, only cell size consistently correlates with genome size (Gregory 2001; Gregory 2002; Gregory 2005). The variation in genome size is a result of mutations that can drive genome expansion or reduction. The primary drivers of this change include mobile elements and whole genome duplication events although there are smaller factors such as intron expansion and pseudogene accumulation that can also contribute to genome expansion (Gregory 2004).

Mobile elements have been linked to genome expansion throughout eukaryotes (Zubáčová et al. 2008; Hebert et al. 2019). Mobile elements, sometimes referred to as TEs or selfish DNA, are DNA segments that can insert into other DNA regions. They are divided into two main classes: class I retrotransposable elements that use RNA as an intermediate template before reverse transcribing into DNA that can then be inserted at DNA nicks, and class II transposable elements that use copy-and-paste mechanisms to insert directly into the DNA. Mobile elements mostly insert randomly into the genome and can change gene expression if inserted into regulatory regions or genes, causing frame shift mutations or gene truncation (Bennetzen 2000; Bennetzen et al. 2005; Grover et al. 2008). The proliferation of mobile elements is not constant but happens in bursts that make periodic quantum gains in genome size possible (Neumann et al. 2006; Piegu et al. 2006; Hawkins et al. 2007; Vitte et al. 2007). Proliferation rates are controlled by recombination rates, insertion location, epigenetics, and population genetics.

Recombination rate has been shown to positively correlate with TE removal and can

therefore aid in genome size reduction (Wright et al. 2003; Tian et al. 2009). While many TEs insert randomly, certain TE families have a bias to insert themselves into pericentromeric or centromeric regions which experience lower recombination rates (Choo 1998; Jiang et al. 2003). RNA interference (RNAi) can check uncontrolled proliferation of TEs: as TE families increase in the cell small interfering RNAs that target the TEs also increase, upregulating the RNAi process and enhancing the silencing of the TEs (Hollister and Gaut 2009). However, in small populations or populations experiencing bottlenecks, selection to purge mobile elements based on environmental pressures is expected to be less effective so genome size can expand more rapidly in those populations (Lynch 2007; Grover et al. 2008; Lockton et al. 2008; Ross-Ibarra et al. 2008). Recently it has been observed that within the largest known gypsy moth species, *Lymantria dispar*, the subspecies *L. dispar asiatica* and *L. dispar japonica* experienced TE proliferation associated with their genome expansion. Their genomes range between 865 and 999 Mbp with 60% of their total genomes being comprised of TEs and sequence repeats (Hebert et al. 2019).

In contrast to mobile element proliferation, whole genome duplication (WGD) can cause rapid quantum shifts in the quantity of DNA by replicating entire chromosomes or genomes. Whole genome duplication is often associated with quick bursts of genomic expansion between closely related species, although such expansions have also been found to be caused by mobile element proliferation (Blommaert et al. 2019). Although WGD can occur in any eukaryote, it is most common in plants, which can have multiple duplications (Woodhouse et al. 2009). Recent WGD events will result in

polyploidization, although over time the duplication may be reduced with some of the duplication being lost while others are retained in the genome. Those genome sections that remain may evolve novel functions, thus it can be difficult to detect past WGD events as they can be obscured through deletions or rearrangements, but long colinear duplication spans of genes or chromosomes are often thought to be indicators of past WGD events (Lynch 2007; Gerstein and Otto 2009; Marburger et al. 2018). Post-duplication TE proliferation can occur due to the WGD event interrupting the RNAi's ability to silence the mobile elements resulting in even more drastic genomic shifts (Oliver and Greene 2009).

### **1.5 Feulgen Densitometry as Best Method for Estimating Genome Sizes in Mixed Protist Samples**

Genome size data are valuable when considering future genome sequencing projects and can provide comparisons for genome structure and evolution studies. There are several genome databases for eukaryotes but there is currently not a detailed database for protists, which is an under-sequenced group compared to other eukaryotic groups (Kullman et al. 2005; Leitch et al. 2019; Gregory 2021). Genome sizes can be quantified through several methods including fluorometry, Feulgen densitometry, and k-mer analysis. Fluorometry measures fluorescence to detect, measure, and identify molecule concentrations. The most common fluorometry technique used to estimate genome size is flow cytometry in which extracted and stained nuclei (usually DAPI or propidium iodide) from samples of interest are suspended in fluid that flows through a laser beam of a specific wavelength. The stimulated light emission by the fluorochrome is measured

and the genome size can be inferred by comparing the unknown sample to a known standard (Hardie et al. 2002). This process can provide rapid and accurate results but requires expensive equipment that may not be readily available and large quantities of sample nuclei which can be difficult to obtain if the samples are not able to be cultured.

Genome sizes can also be estimated using bioinformatic approaches, such as k-mer counting. K-mers are strings of unique DNA sub-sequences of a specific length. Any sequence, even unassembled short reads, can be broken down into its k-mer units. K-mers of about 20 nucleotides in length are usually best for estimating genome size. Traditionally, this method has worked best with sequence coverage greater than 10X but newer methods can get decent estimations with coverage as low as 0.5X (Hozza et al. 2015). In a given sequence, all unique k-mers are identified and it is determined how many times each unique k-mer appeared in the sequence. From there, a k-mer frequency distribution is generated and the peak should represent the average sequencing depth for the genome sequence (Li and Waterman 2003). These distributions are then analyzed using varying models that can determine genomic characteristics such as size, coverage, or heterozygosity (Hozza et al. 2015). Compared to flow cytometry, k-mer counting tends to underestimate genome sizes; in most cases it is only a slight underestimation although there is an instance where the reported k-mer genome size was half that of the flow cytometry reported size (Pflug et al. 2020).

Densitometry is the measurement of optical density (OD) using microscopy in the quantification of genome size. The DNA is stained and OD calculations are performed comparing the stained DNA to background. Feulgen densitometry is one of the most

common staining procedures for quantifying DNA. In this process, fixed tissue preparations or cells are stained through stages of hydrolysis which depurinates the DNA and creates open aldehyde groups. Schiff's reagent, which contains fuchsin decolorized by  $\text{SO}_2$ , can interact with the DNA and the fuchsin will bind to the aldehyde groups, releasing the  $\text{SO}_2$  and resulting in a pink coloration of the DNA (Hardie et al. 2002). It is important to note that this is not a true stain but rather a series of chemical reactions. The quantity of the stain bound to the DNA should be proportional to the amount of DNA present in the nucleus. The stain quantity is determined by its OD in the nucleus, since OD is the amount of light absorbed it cannot be measured directly but must be measured indirectly through transmittance (the difference between the light intensity of the target nuclei from an empty space on the slide). As DNA content is not consistent throughout the nucleus, many point measurements must be made throughout the nucleus to get a good estimation of the DNA content. In the past, such point measurements have been performed using a flying spot or scanning microscopy and would be very time consuming. With modern image analysis techniques, the entire microscope field can be captured with a microscope mounted camera connected to a computer. In this case, each pixel in the image could act as a point measurement. The target nuclei can be outlined using microscope image software and the mean light intensity for all the pixels in the selected area can be automatically calculated. This drastically reduces the time and effort to compute the OD and makes this method a rapid, cost effective, and user-friendly method to estimate genome size. Comparisons between Feulgen densitometry image



analysis and flow cytometry have shown that flow cytometry using propidium iodide provides comparable results to Feulgen densitometry (Doležel et al. 1998).

Feulgen densitometry has been the most common method for estimating genome size, being first implemented in the 1950s (Hardie et al. 2002). As technology advanced, flow cytometry has become more common and is widely used in genome size estimation. Feulgen densitometry is still used quite frequently for tissue samples where it is more difficult to isolate single cells and extract nuclei, such as is the case with these samples. All five of the protist species reside together in the same guts, and as of yet, these species have not been able to be cultured singly or together. Due to the benefits, flow cytometry would have been the preferable method but deciphering the results of a mixed-species flow run would be difficult. Since flow cytometry uses extracted nuclei, multiple peaks that represent genome size would be present but there would be no way to directly confirm which peak corresponds to which species. Due to these issues, this study used Feulgen densitometry image analysis because it allowed morphological identification of the cells as their genome sizes were calculated, which is not possible using flow cytometry.

## **1.6 Objectives**

Recognizing the known positive correlation cell size has with genome size and the relatively large cell size of the *C. formosanus* protist symbionts, this study aimed to estimate the C-values of *P. grassii*, *H. hartmanni*, *H. minor*, *C. leidy*, and *C. koidzumii* to see if they also have relatively large genome sizes. This was accomplished through Feulgen densitometry image analysis to estimate the C-value in picograms for each of the

five species. The study also aimed to place their cell and genome sizes in context of the evolution of Parabasalia and Eukaryotes. This was achieved through comparisons of these findings to other Parabasalia with known genome sizes, specifically certain trichomonas belonging to the Tritrichomonadida, Hypotrichomonadida, and Tritrichomonadida, and by comparison of the Parabasalia genome size range to those of other other eukaryotic taxa.

## 2. METHODS AND MATERIALS

### 2.1 Termite Collection and Care

The *C. formosanus* termites are from a lab colony originating from the University of Florida, Ft. Lauderdale Research and Education Center Subterranean Termite Laboratory, Broward County, Florida, USA (26.10, -80.17). Termite alate collection and laboratory colony rearing conditions followed the protocol previously described (Chouvenc and Su 2017; Jasso-Selles et al. 2020).

### 2.2 Genome Size Estimation

#### 2.2.1 Reagent Preparation

Fixation Solution (500  $\mu$ L): in a 1.5 mL tube, 418  $\mu$ L milliQ water, 50  $\mu$ L 10x PBS, and 32  $\mu$ L 32% paraformaldehyde were combined and vortexed for 30 seconds to mix.

Schiff's Reagent (30 mL): 0.55 g basic fuchsin was dissolved in 5 mL 1.0 N HCl in a glass bottle. In a separate beaker, 0.5 g sodium bisulfite ( $\text{Na}_2\text{S}_2\text{O}_5$ ) was dissolved in 50 mL dH<sub>2</sub>O then combined with the fuchsin and HCl solution. The reagent was a dark

purple to red color. The bottle was tightly capped and stored in a room temperature dark area for 72 hours, mixed every eight hours to ensure no precipitate formed. The solution was decolorized through a series of incorporating and then filtering out activated charcoal. Activated charcoal (1.75 g) was added in 0.25 g increments to the solution, shaken for two minutes, and filtered through 8  $\mu\text{m}$  pore size filter paper using a Buchner funnel. The resulting solution was a clear light pink and the final volume was 30 mL. Reagent was stored at 4 °C in the dark. Schiff's reagent was used within a month of preparation and discarded once white precipitate formed. The same batch was used on all samples being compared.

Bisulfite solution (16 mL): 750  $\mu\text{L}$  of 10% sodium metabisulfite solution was combined with 15 mL dH<sub>2</sub>O then 750  $\mu\text{L}$  1.0 N HCl was added and the solution was vortexed for 30 seconds to mix. Solution was prepared fresh for every use.

### **2.2.2 Feulgen Densitometry Image Analysis**

Feulgen densitometry uses a series of chemical reactions to “stain” the nucleus. There is a proportional relationship between the amount of DNA and the amount of stain bound to the nucleus which allows the C-value to be quantified. This study followed a protocol modified from “From pixels to picograms: a beginners' guide to genome quantification by Feulgen image analysis densitometry” as follows (Hardie et al. 2002).

Roughly 200 *C. formosanus* termites were removed from their colony and placed in a separate container with a damp paper towel and placed in an incubator at 28 °C for three days to allow the protists to digest ingested wood particles that can interfere with staining and image analysis. One hundred whole guts from the *C. formosanus* starved

workers were extracted using fine-tipped forceps. To fix protist cells, 10 guts were placed in each 1.5 mL tube with 500  $\mu$ L fixation solution (milliQ water, 10x PBS, 32% paraformaldehyde) and opened using the forceps to release the gut contents into the solution. Fixed samples were stored in the fridge for 24 to 48 hours at 4 °C. The samples were then stained using Feulgen reactions and Schiff's reagent.

First, the samples were rinsed three times by adding 400  $\mu$ L tepid water, inverting the tube ~20 times to mix, centrifuging at 4,400 rcf for 5 minutes, and discarding the supernatant. Samples were hydrolyzed with 400  $\mu$ L 5.0 N HCl for 15 minutes at room temperature, then rinsed three times with 400  $\mu$ L 0.1 N HCl, inverting the tube ~20 times to mix, centrifuging at 4,400 rcf for 5 minutes, and discarding the supernatant each time. Schiff's reagent (fuchsin, 1.0 N HCl, sodium metabisulfite ( $\text{Na}_2\text{S}_2\text{O}_5$ ), dH<sub>2</sub>O) was prepared and 400  $\mu$ L was added to the pellet to stain the sample nuclei. Each sample with Schiff's reagent was placed in the dark for 90 minutes and inverted 10x every fifteen minutes to ensure the contents were well mixed. The Schiff's reagent was rinsed with 400  $\mu$ L of the bisulfite solution (1.0 N HCl, sodium metabisulfite ( $\text{Na}_2\text{S}_2\text{O}_5$ ), dH<sub>2</sub>O) three times. Samples were then rinsed with 400  $\mu$ L tepid water and resuspended in 300  $\mu$ L water. Samples were stored in the fridge at 4 °C and used within a week. Trout nuclei (BioSure Controls TEN Cytometry Control) standard was prepared similarly but with a hydrolysis and stain time of 120 minutes. Trout was chosen as the standard because it is a commonly and reliably used standard in genome estimation and had the largest genome size of all readily available nuclei standards.

The stained samples were mounted on a slide and Kohler illumination was established using an AxioImager upright compound light microscope with an AxioCam 503 monochrome camera (Zeiss). The Zeiss AxioCam 503 camera response was tested for linearity before image data were gathered. Using the camera and software, brightfield images of the stained protists were taken at a magnification of 400x and exposure of 1/125 seconds. The nuclei were outlined and area ( $\mu\text{m}^2$  and pixel) and foreground intensity mean (IF) measurements for the nuclei were taken. Background intensity mean (IB) measurements were also taken from an empty space on the slide for comparison. For each cell, an integrated optical density (IOD) was calculated using the following equation where  $n$  = the total number of pixels within the nucleus:

$$IOD = \sum_{i=1}^n -\log_{10} \left( \frac{IF_i}{IB_i} \right)$$

and the C-values (CV) for the protists were calculated using the equation:

$$CV_u = CV_s \times \left( \frac{IOD_u}{IOD_s} \right)$$

where the subscript  $u$  indicates the unknown and  $s$  indicates the standard (Hardie et al. 2002). The  $IOD_s$  used to calculate the  $CV_u$  was the mean of all trout nucleus IODs. Cells were then identified as one of five protist species (*C. leidyi*, *C. koidzumii*, *H. hartmanni*, *H. minor*, and *P. grassii*) according to morphological criteria including flagella type and location, cell size and shape, and nucleus location (Jasso-Selles et al. 2020). A total of 586 protists were imaged, 66 *P. grassii*, 41 *H. hartmanni*, 158 *H. minor*, 119 *C. leidyi*, and 202 *C. koidzumii*.

### 2.3 Cell and Nucleus Volume Estimates and Their Correlation to Genome Size

The measurements of the cells and nuclei were taken from digital micrographs of the stained cells from the AxioImager upright compound light microscope fitted with an AxioCam 503 camera. The nuclei were outlined and length (anterior to posterior) and width (longest measurement perpendicular to the length) measurements ( $\mu\text{m}$ ) were taken for each of the cells and nuclei. The five species fall under three shape categories which were used to approximate their cell volume: cone-like, ellipsoid, or cylindrical. *C. koidzumii*, *H. hartmanni*, *H. minor*, and all the nuclei are all ellipsoid, *C. leidyi* is cone-shaped, and *P. grassii* is cylindrical. The nucleus and cell volumes ( $V$ ) were approximated using the following equations:

$$V_e = \frac{3}{32}\pi ab^2$$

$$V_{cs} = \frac{1}{6}\pi ab^2$$

$$V_c = \frac{1}{4}\pi ab^2$$

where the subscript  $e$  indicates ellipsoid,  $cs$  indicates cone-shaped, and  $c$  indicates cylindrical and “ $a$ ” is the cell or nucleus length and “ $b$ ” is the cell or nucleus width. The mean, median, and interquartile range (IQR) were calculated for each of the species and outliers were determined as any value that falls outside  $Q1 - 1.5 \times IQR$  or  $Q3 + 1.5 \times IQR$ . Linear regression analyses were completed to test for relationships between cell volume and genome size and nucleus volume and genome size.

### 3. DATA ANALYSES AND RESULTS

#### 3.1 Cell and Nucleus Sizes

The cell and nucleus volumes were calculated from the imaged cell measurements and approximate cell shape (Table 1). All species had large ranges for cell volumes, often having large outliers that inflated the mean to be quite larger than the median. The ranges for the nucleus volume were narrower across the five species and their mean and median values were closer together, but the median values were still considerably lower than the mean due to large outliers. There was a similar pattern with the distribution of genome sizes (Figure 3). Because of this, the median and IQR are better descriptors of the data. The cell shapes for these five species were approximated as either cone-like, ellipsoid, or cylindrical, and their volumes were calculated based on that descriptor. *Cononympha koidzumii* is a small ellipsoid cell with a median cell volume of  $386 \mu\text{m}^3$  and nucleus volume of  $5 \mu\text{m}^3$ . *Cononympha leidyi* is a small to medium cone-shaped cell with a median cell volume of  $1,996 \mu\text{m}^3$ , about five times larger than *C. koidzumii*, and nucleus volume of  $13 \mu\text{m}^3$ . *Holomastigotoides minor* is a medium ellipsoid cell and has a median cell volume of  $8,985 \mu\text{m}^3$  and nucleus volume of  $54 \mu\text{m}^3$ . *Holomastigotoides hartmanni* is a large irregular ellipsoid shaped cell with a median cell volume about three times larger than the *H. minor* cells,  $26,679 \mu\text{m}^3$ , and nucleus volume of  $178 \mu\text{m}^3$ . *Pseudotrichonympha grassii* is the largest species with a cylindrical cell shape and a median cell volume of  $89,190 \mu\text{m}^3$  which is over 200 times larger than the smallest symbiont, *C. koidzumii*, and nucleus volume of  $378 \mu\text{m}^3$  which is over 70 times larger than *C. koidzumii*.

### 3.2 Genome Sizes

A total of 586 cells from the five protist species and 105 extracted trout nuclei were imaged. All measurement data collected for the five protist species are recorded in Appendix A and summarized in Table 1 and Figure 3. All measurement data for the standard IOD (IOD<sub>s</sub>) is recorded in Appendix B. The optical densities from Feulgen staining, which correspond to DNA content, revealed large C-values compared to other Parabasalia. The largest C-values were found in *P. grassii*. From 66 *P. grassii* nuclei, the mean C-value was determined to be 153±50 pg with a median value of 147 pg and a single outlier of 330 pg. The C-values of the *Holomastigotoides* species were the second largest. From 41 *H. hartmanni* nuclei, the mean C-value was determined to be 81±51 pg with a median value of 56 pg. For *H. minor*, the mean and median values were 34±22 pg and 29 pg (n=158 nuclei). In this case there were 12 outliers ranging between 72 and 140 pg. The *Cononympha* species had the smallest C-values, around ten times smaller than that of *P. grassii*. *Cononympha leidy* was measured (n=119 nuclei) and its mean was 12±5 pg and the median was 12 pg with four outliers ranging between 25 and 30 pg. *Cononympha koidzumii* is the smallest of the cells, 202 cells were collected and had a mean C-value of 9±7 pg and median value of 6 pg with eight outliers ranging from 24 to 39 pg.



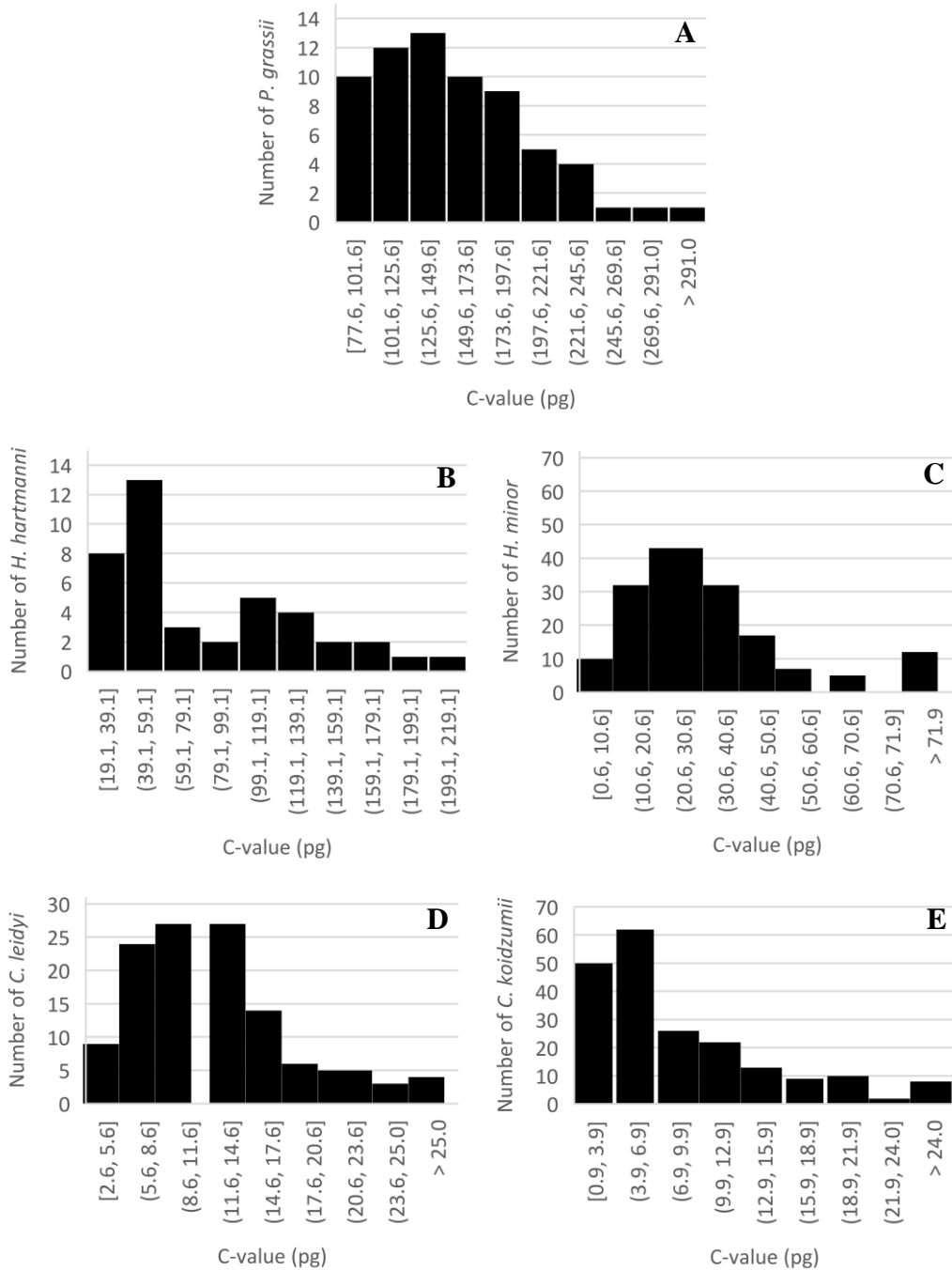
**Table 1.** Cell and Nucleus Volumes and C-values of *C. formosanus* Symbiont Species

	Cell			
	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Volume <sup>a,b,c</sup> ( $\mu\text{m}^3$ )	Volume ( $\mu\text{m}^3$ )
	mean $\pm$ S.D. (n)	mean $\pm$ S.D.	mean $\pm$ S.D.	median (Q1, Q3)
<i>Cononympha koidzumii</i>	16.96 $\pm$ 7.97 (199)	11.64 $\pm$ 5.91	1195.28 $\pm$ 1662.09	385.70 (155.77, 1679.82)
<i>Cononympha leidyi</i>	25.61 $\pm$ 9.13 (119)	13.78 $\pm$ 4.99	3463.96 $\pm$ 3726.56	1996.44 (976.49, 4804.30)
<i>Holomastigotoides minor</i>	45.99 $\pm$ 9.76 (151)	27.07 $\pm$ 7.48	11240.21 $\pm$ 7949.28	8985.09 (5507.92, 14308.12)
<i>Holomastigotoides hartmanni</i>	67.37 $\pm$ 12.62 (39)	37.71 $\pm$ 8.59	30912.83 $\pm$ 18546.16	26679.03 (16745.49, 37722.20)
<i>Pseudotriconympha grassii</i>	170.34 $\pm$ 46.99 (48)	28.09 $\pm$ 9.20	113697.95 $\pm$ 78616.45	89189.72 (59313.53, 141760.81)
	Nucleus			
	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Volume <sup>a</sup> ( $\mu\text{m}^3$ )	Volume ( $\mu\text{m}^3$ )
	mean $\pm$ S.D. (n)	mean $\pm$ S.D.	mean $\pm$ S.D.	median (Q1, Q3)
<i>Cononympha koidzumii</i>	2.88 $\pm$ 1.03 (202)	2.98 $\pm$ 10.63	10.63 $\pm$ 12.35	5.04 (2.65, 14.89)
<i>Cononympha leidyi</i>	3.77 $\pm$ 1.02 (119)	3.49 $\pm$ 0.90	15.63 $\pm$ 11.46	12.93 (8.49, 19.94)
<i>Holomastigotoides minor</i>	5.47 $\pm$ 1.75 (158)	6.26 $\pm$ 2.12	77.50 $\pm$ 74.15	53.73 (29.64, 87.25)
<i>Holomastigotoides hartmanni</i>	7.31 $\pm$ 2.25 (41)	9.84 $\pm$ 3.74	280.55 $\pm$ 279.29	177.66 (77.34, 392.23)
<i>Pseudotriconympha grassii</i>	11.05 $\pm$ 1.92 (66)	11.03 $\pm$ 2.49	430.18 $\pm$ 231.03	377.92 (246.92, 571.32)
	Genome Size			
	C-value (pg)	C-value (pg)		
	mean $\pm$ S.D. (n)	median (Q1, Q3)		
<i>Cononympha koidzumii</i>	8.76 $\pm$ 6.94 (202)	6.32 (3.99, 12.03)		
<i>Cononympha leidyi</i>	12.36 $\pm$ 5.51 (119)	11.57 (8.35, 15.07)		
<i>Holomastigotoides minor</i>	34.35 $\pm$ 22.19 (158)	28.56 (20.40, 41.01)		
<i>Holomastigotoides hartmanni</i>	80.59 $\pm$ 51.45 (41)	56.12 (39.99, 106.52)		
<i>Pseudotriconympha grassii</i>	152.88 $\pm$ 50.08 (66)	146.77 (113.50, 184.51)		

<sup>a</sup> All nucleus and cell volumes for *C. koidzumii*, *H. hartmanni*, and *H. minor* were calculated using the formula  $V=(3/32)\pi ab^2$

<sup>b</sup> Cell volumes for *C. leidyi* were calculated using the formula  $V=(1/6)\pi ab^2$

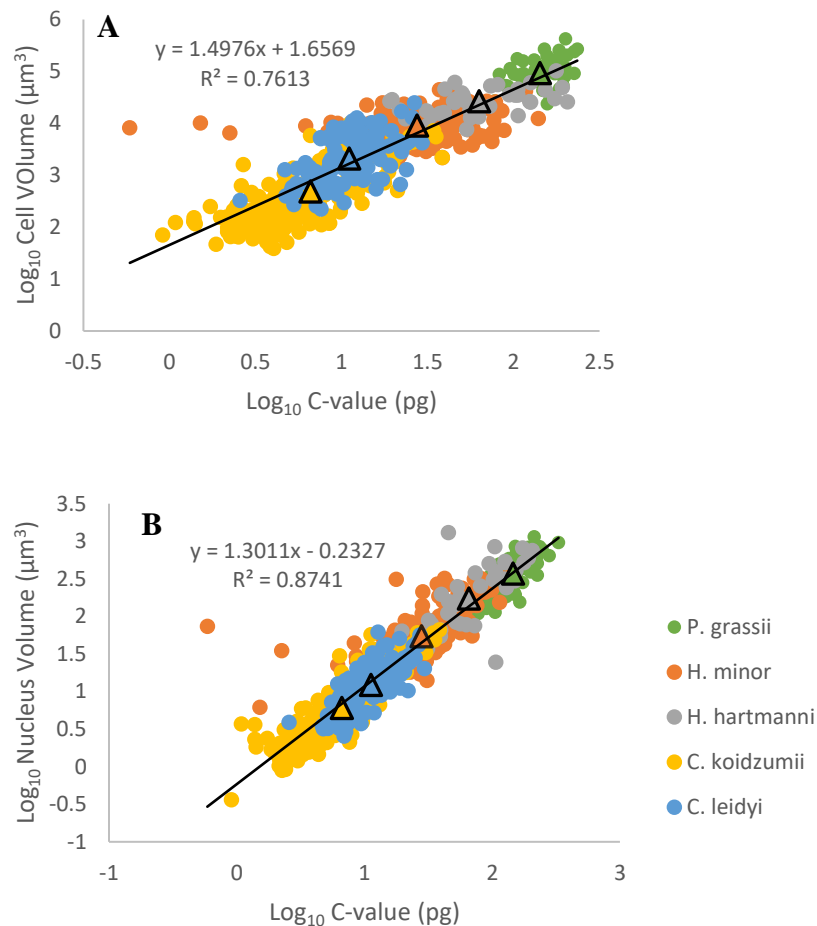
<sup>c</sup> Cell volumes for *P. grassii* were calculated using the formula  $V=(1/4)\pi ab^2$ ; "a" is the length and "b" is the width.



**Figure 3.** Distribution histogram of the C-values for the five *C. formosanus* Parabasalia symbiont species. (A) Distribution of 66 *P. grassii*, median: 147 pg, (B) Distribution of 41 *H. hartmanni*, median: 56 pg, (C) Distribution of 158 *H. minor*, median: 29 pg, (D) Distribution of 119 *C. leidyi*, median: 12 pg, (E) Distribution of 202 *C. koidzumii*, median: 6 pg. Bin size: (A) 24 pg, (B) 20 pg, (C) 10 pg, (D,E) 3 pg; last bin contains all the outliers for each species.

### 3.3 Correlation Between Cell and Nuclei Sizes and Genome Sizes

The cells with the largest cell, nucleus, and genome sizes were represented by *P. grassii* (Table 1). Those with the smallest genome sizes, *C. koidzumii*, also had the smallest cell and nucleus volumes. The calculated log C-values were positively correlated with both the log cell volume ( $R^2 = 0.76$ ) and the log nucleus volume ( $R^2 = 0.87$ ), with a stronger correlation to nucleus volume (Figure 4).



**Figure 4.** Correlation between log C-value and log cell volume and log C-value and log nucleus size of *C. formosanus* protist symbiont species. The dots represent individual measurements from each species and the triangles represent the median values for each species. (A) Positive correlation between log C-value and log cell volume,  $R^2=0.76$ . (B) Positive correlation between C-value and nucleus volume,  $R^2=0.87$ .

## 4. DISCUSSION

### 4.1 Sources of Error

Compared to other studies estimating genome sizes in plants, crustaceans, Oomycota, and insects using Feulgen densitometry, these data showed larger C-value ranges with larger standard deviation relative to mean C-value (Michaelson et al. 1991; Voglmayr and Greilhuber 1998; Jeffery et al. 2016; Kelly 2018). Genome size of each individual cell within a species should be fairly consistent but our results show an abnormal amount of variation. There are several potential sources of error related to Feulgen densitometry, especially in relation to these symbiont samples which could be causing this level of variation.

Size and shape of these nuclei could be a cause of potential error. Along with there being large variation with the genome size, there is also large variation of the nucleus volumes for each species. Since the size of the nucleus goes into the calculation for the genome size, it seems reasonable to think if there is variation in one of the calculation components there would also be variation in the calculation results. There may also be issues getting accurate outlines of the nucleus. Due to the large size of these nuclei, small deviations outlining the nuclei can lead to larger deviation in the calculated nucleus area which would affect the C-value calculations. C-values at the extremes of the ranges are potentially caused by anomalies in the nucleus, such as folding or improper nucleus orientation, or minor staining abnormalities (Hardie et al. 2002).

Some of the variation may be caused by the cell exterior still being intact. Since the cell exterior was required in order to identify the cells to determine which genome

sizes corresponded to which species, the protist samples estimated were whole cells. However, the standard used was extracted nuclei which may have biased the genome size estimation. This may have resulted in the nucleus area of the symbiont samples to have greater light absorption due to flagella, cell membrane, and cytoplasm being in the light path. This could potentially result in higher IOD and C-values.

Another possible source of error is the large range of genome sizes across the species. When performing genome size estimations in which a standard is used for comparison, it is best practice to use a standard with a genome size no more than three times smaller or larger than the genome size of the sample (Vindeløv et al. 1983; Doležel and Bartos 2005). In this case, one standard was used as comparison for all the symbiont cells. The standard was chosen because it is one of the larger standards available and is considered a reliable reference for genome size estimation. C-value for the standard (5.2 pg) was close to the *Cononympha* species but much smaller than the *Holomastigotoides* and *Pseudotrichonympha* species. This would have made the species with C-values closer to the trout value more likely to be accurate. Regardless of the potential sources of error, there remains support for the large range of genome sizes across these five species, the ratios of the genome sizes of the symbiont species relative to each other, and the large difference between these species compared to other Parabasalia.

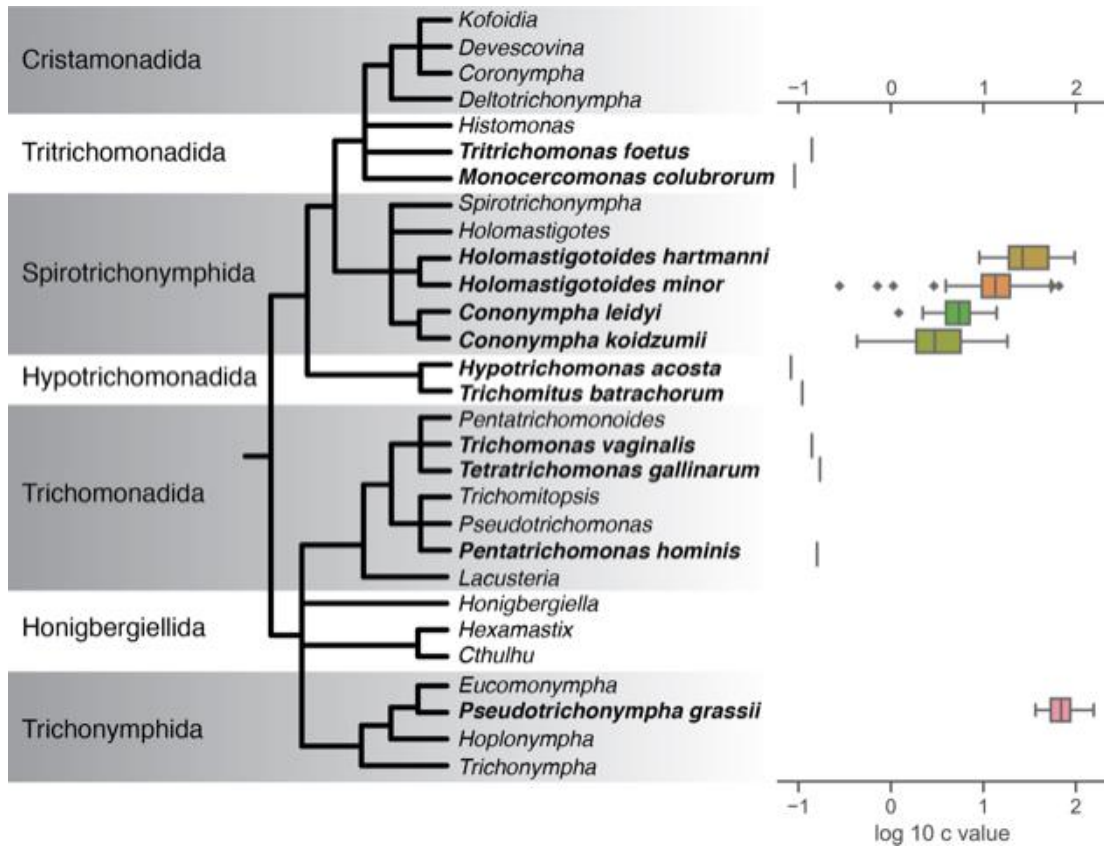
#### **4.2 *C. formosanus* Symbionts in Relation to Parabasalia and Eukaryotes**

Genome sizes have been estimated for several Parabasalia species across five orders. Two have been estimated from the order Tritrichomonadida, four from Spirotrichonymphida, two from Hypotrichomonadida, three from Trichomonadida, and

one from Trichonymphida (Figure 5). The *Holomastigotoides* and *Cononympha* species estimated in this paper belong to Spirotrichonymphida and the *Pseudotriconympha grassii* belongs to Trichonymphida. The species whose genomes sizes were previously estimated are in the orders Tritrichomonadida, Hypotrichomonadida, and Trichomonadida. These species are all parasites or commensals of vertebrates and all have estimated genome sizes less than 0.2 pg (Zubáčová et al. 2008). This highlights a difference in genome size between Parabasalia that are parasitic or commensal and those that are mutualistic in termites, which leads to the enquiry: can the relationship between protists and their hosts have some sort of effect on their genomes' tendency to expand, compress, or maintain size? To explore this notion further, more genomes across Parabasalia need to be estimated, including those that are free-living, to compare those with different host – protist relationships and to those outside of such relationships.

Prior to this study, Parabasalia had a narrow range of variation of known genome sizes, having a 2-fold difference from 0.088 pg found in *Tetratrichomonas gallinarum* to 0.181 pg found in *Tritrichomonas foetus* (Zubáčová et al. 2008). These genome sizes were estimated using flow cytometry. The reference standard used was *T. vaginalis* and the nuclei were stained with Hoechst 33342 for flow cytometric size estimation (Zubáčová et al. 2008). The genome size of the reference standard used was estimated from sequence data (~160 Mbp  $\approx$  0.16 pg), but the assembly was highly fragmented due to large amounts of repeat sequences and its genome size was most likely under-reported (Carlton et al. 2007). This would cause the other trichomonad genome size estimations to be smaller than the actual size. The study showed consistent genome sizes of different

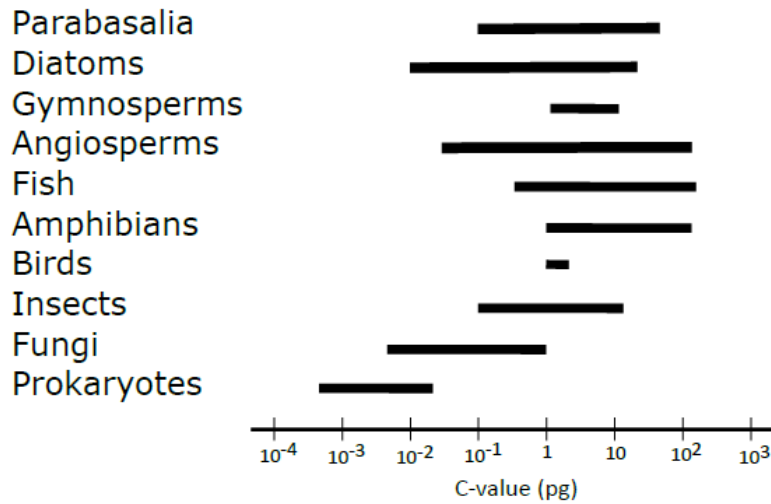
strains of the same species, even between the sequenced reference *T. vaginalis* strain and the additional *T. vaginalis* sample strain, indicating the methods used were most likely effective (Zubáčová et al. 2008).



**Figure 5.** Phylogenetic relationships between Parabasalia with corresponding log c-value. The taxa used in this genome size comparison are in bold and those whose genome sizes were estimated in this study have their full species name given and a box plot of their log c-values (pg).

Although this range seems narrow, it is similar to the range found in birds (Gregory 2005). With these new genome size estimates, Parabasalia now spans over 15,000-fold range with the largest known Parabasalia genome size now being 146.8 pg in *P. grassii*. This large range is not uncommon as similar ranges can be found in such

differing groups of eukaryotes as diatoms and angiosperms (Figure 6). More studies are needed to assess whether these *C. formosanus* symbiont species are representative of all Parabasalia – termite symbionts or if they are uncommonly large for Parabasalia symbionts.



**Figure 6.** Overview of C-value variation across groups of organisms. Parabasalia genome sizes measured so far span three orders of magnitude. Adapted from several publications, (Connolly et al. 2008; Zubáčová et al. 2008; Gregory et al. 2004).

### 4.3 Adaptive Role of Variation

As previously mentioned, mobile elements and whole genome duplication events can have a large impact on the variation of genome sizes. Whether the genomes experience expansion or compaction has a lot to do with whether there is active selection occurring or not. There are two types of theories that explain the governing forces of genomic change: mutational pressure theories and optimal DNA theories (Gregory 2001). Mutational pressure theories rely on the natural tendency of genomes to accumulate mutations, resulting in genome expansion, and require selective pressures to reduce



genome size (Pagel and Johnstone 1992). Optimal DNA theories assert that genome size is a selectable trait which can be governed by environmental pressures by having a direct link to traits such as cell and nucleus sizes (Orgel and Crick 1980; Cavalier-Smith 1982). Looking at the genome size expansion in the *C. formosanus* symbionts through the optimal DNA lens it can be hypothesized that their genome expansion could be due to selective pressures favoring larger cells in order to digest large wood particle, which according to nucleo-skeleton theories would require larger nuclei and genomes to support the larger cells. The location differentiation in which small cell species tend to be at one end of the hindgut and larger species at the other suggests there may be something about the environments at the different gut pouches that may apply selective pressure towards cells of a certain size.

On the other hand, bottleneck effects can reduce the efficacy of selection and have been shown to allow species to undergo and maintain genome expansions (Lynch 2007; Gerstein and Otto 2009). Looking through the mutational pressure lens it can be argued that since these species experience repeated bottlenecks as part of their relationship with termites, they may have had the opportunity to expand their genomes with weak selective pressure for compaction. This has also been suggested as a possible contributing factor to the genome size increase in *T. vaginalis* compared to other parasitic protists as well as to the genome size expansion in *Helianthus* hybrid species (Baack et al. 2005; Carlton et al. 2007).

Now that the genome sizes for the *C. formosanus* Parabasalia symbionts have been determined, the next step in understanding the symbiotic relationship between the

termites and their symbionts is to estimate the genome sizes of the symbionts of other termite species to see if they follow the same pattern. It would also be interesting to examine protists within and outside of Parabasalia that have either mutualistic, parasitic, or commensal symbiotic relationships to animals to see if certain relationships are more likely to result in genome expansion or compaction of symbionts.

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APPENDIX A

SAMPLE DATA COLLECTED FOR THE FIVE *C. FORMOSANUS* SYMBIONT

SPECIES

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
1	H. minor	30.09	27.78	6839.32	5.73	5.13	44.37	1728	7441.52	9798.39	206.48	8.311
2	H. minor	42.15	28.53	10104.20	2.84	2.71	6.14	511	4520.77	5356.75	37.66	1.516
3	C. leidyi	15.91	9.19	703.91	3.24	2.76	7.26	517	2481.96	4965.68	155.71	6.267
4	C. koidzumii	8.05	5.49	71.37	2.69	1.97	3.08	367	2564.92	4371.64	84.99	3.421
5	C. koidzumii	10.10	7.16	152.29	2.20	2.37	3.62	321	4153.73	5318.85	34.47	1.387
6	C. koidzumii	7.55	6.04	80.96	3.10	2.57	6.02	568	2569.46	3640.53	85.95	3.459
7	C. koidzumii	10.00	6.58	127.27	1.84	2.07	2.32	284	2886.98	3813.84	34.34	1.382
8	H. minor	35.77	24.87	6515.63	4.66	5.04	34.92	1460	3742.40	4087.15	55.87	2.249
9	C. koidzumii	11.71	8.70	260.63	2.06	1.95	2.30	269	2367.94	4003.03	61.34	2.469
10	C. koidzumii	8.60	5.08	65.28	1.41	1.46	0.89	150	3073.84	7303.37	56.38	2.269
11	C. leidyi	17.39	8.54	663.64	2.56	3.53	9.37	578	2830.82	7839.34	255.69	10.291
12	C. koidzumii	14.19	9.53	379.27	2.96	3.00	7.83	569	5502.04	14600.43	241.17	9.707
13	H. hartmanni	59.20	38.90	26377.32	6.82	12.48	312.42	5670	2719.49	9547.12	3092.33	124.462
14	C. koidzumii	10.85	6.26	124.97	1.76	1.67	1.45	202	3960.96	10046.36	81.65	3.286
15	C. leidyi	26.87	13.42	2535.12	5.34	3.62	20.64	1159	4874.37	10899.94	405.08	16.304
16	C. leidyi	28.98	15.43	3612.58	2.90	3.60	11.04	720	3788.03	11836.65	356.27	14.339
17	P. grassii	159.46	40.51	205544.70	14.44	11.10	524.00	10643	2904.68	8740.98	5092.27	204.956
18	P. grassii	134.59	33.71	120105.31	9.29	13.63	508.14	8233	2901.40	8740.98	3943.21	158.708
19	C. leidyi	33.63	16.17	4600.99	6.57	3.77	27.45	1592	5268.28	11372.30	532.01	21.413
20	P. grassii	163.03	36.73	172784.39	11.52	13.02	574.80	9595	3260.17	9500.64	4457.00	179.387
21	P. grassii	118.22	17.53	28527.27	12.00	12.76	575.17	9816	3782.39	9773.74	4047.08	162.889
22	P. grassii	221.64	38.22	254297.51	13.96	13.33	730.09	11771	3992.76	9775.69	4577.44	184.235
23	H. minor	47.80	24.28	8296.48	6.73	6.10	73.80	2504	5098.15	10911.65	827.52	33.306
24	P. grassii	193.74	39.81	241105.02	11.33	13.79	633.83	10630	2752.73	9248.65	5594.72	225.179
25	P. grassii	95.70	24.00	43288.25	8.69	9.08	211.20	5057	1776.32	9484.20	3678.87	148.069
26	P. grassii	133.51	63.46	422339.61	10.36	15.17	701.80	10381	3541.32	10707.74	4988.40	200.775
27	P. grassii	134.59	33.71	120105.31	10.91	16.02	825.01	11279	3192.77	8747.24	4936.85	198.700

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
28	<i>P. grassii</i>	134.59	33.71	120105.31	11.13	15.92	830.75	10610	3126.77	8743.66	4738.40	190.713
29	<i>C. leidyi</i>	14.62	6.22	296.41	2.87	2.49	5.24	511	3635.71	11599.46	257.47	10.363
30	<i>P. grassii</i>	178.27	31.21	136395.99	12.69	11.39	485.10	9504	4379.95	11098.45	3837.65	154.459
31	<i>H. minor</i>	35.53	19.34	3915.22	2.96	5.36	25.08	1163	2457.00	9991.29	708.52	28.517
32	<i>C. koidzumii</i>	8.77	4.99	64.32	2.20	1.46	1.39	207	5357.19	10757.10	62.67	2.522
33	<i>C. leidyi</i>	19.21	12.29	1520.16	4.51	3.55	16.74	1139	3707.79	9755.36	478.53	19.260
34	<i>C. koidzumii</i>	11.00	6.75	147.37	2.01	1.90	2.15	270	4717.62	13106.14	119.81	4.822
35	<i>H. minor</i>	26.87	15.27	1845.82	3.55	3.26	11.07	773	4157.09	11149.93	331.22	13.331
36	<i>C. koidzumii</i>	10.79	6.85	149.13	1.37	1.89	1.45	187	5482.55	11733.79	61.80	2.487
37	<i>C. koidzumii</i>	9.77	7.61	166.47	1.62	1.75	1.46	204	3580.32	12190.86	108.55	4.369
38	<i>H. minor</i>	47.13	33.34	15429.75	5.21	6.31	61.12	2470	7862.66	11662.36	422.91	17.021
39	<i>C. leidyi</i>	35.09	18.60	6357.08	4.44	3.91	20.00	1106	5843.55	10926.60	300.62	12.099
40	<i>C. koidzumii</i>	14.69	6.88	204.94	2.02	2.09	2.59	295	3516.49	11008.12	146.20	5.884
41	<i>C. koidzumii</i>	17.51	11.13	638.73	2.06	2.23	3.01	316	5162.39	11759.03	112.98	4.547
42	<i>H. hartmanni</i>	62.62	29.05	15564.02	5.69	6.28	66.03	2321	4366.02	11594.07	984.45	39.623
43	<i>C. koidzumii</i>	9.49	7.72	166.61	2.18	2.02	2.62	291	3120.36	11882.46	168.98	6.801
44	<i>H. minor</i>	58.56	27.14	12705.48	5.10	5.28	41.90	1647	3962.32	10959.13	727.69	29.288
45	<i>H. hartmanni</i>	73.80	35.04	26679.03	4.03	5.67	38.14	1436	5547.55	11890.70	475.47	19.137
46	<i>C. koidzumii</i>	7.08	5.83	70.81	0.88	1.18	0.36	78	5300.72	10346.15	22.65	0.912
47	<i>C. koidzumii</i>	12.27	7.93	227.29	2.29	1.98	2.64	315	5337.97	11441.38	104.30	4.198
48	<i>C. koidzumii</i>	9.61	6.28	111.67	1.81	2.00	2.12	235	2069.62	13330.17	190.10	7.651
49	<i>H. minor</i>	55.51	29.15	13889.09	5.52	5.64	51.75	2117	6582.90	11751.62	532.81	21.445
50	<i>C. leidyi</i>	16.57	10.57	969.40	3.09	3.09	8.67	610	5229.38	10989.09	196.73	7.918
51	<i>C. koidzumii</i>	14.57	7.22	223.62	2.04	2.15	2.77	310	3962.05	11145.59	139.25	5.604
52	<i>H. minor</i>	20.83	23.11	3274.58	4.25	3.95	19.56	1158	4627.34	11457.26	455.96	18.352
53	<i>H. minor</i>	27.26	15.28	1873.46	3.98	4.18	20.52	962	3455.26	12084.48	523.08	21.053
54	<i>C. koidzumii</i>	8.80	5.92	90.94	2.01	1.58	1.48	209	5747.69	11583.01	63.60	2.560

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
55	<i>C. koidzumii</i>	16.10	12.75	770.46	4.00	3.23	12.25	952	5307.68	11422.36	316.87	12.754
56	<i>H. hartmanni</i>	62.62	29.05	15564.02	5.84	5.98	61.50	2252	4487.32	11594.39	928.41	37.367
57	<i>H. minor</i>	59.25	30.66	16399.60	5.72	6.23	65.29	2128	5338.57	11454.04	705.50	28.395
58	<i>C. koidzumii</i>	8.73	6.18	98.16	1.75	1.99	2.04	181	5470.12	10895.37	54.16	2.180
59	<i>C. koidzumii</i>	6.58	5.90	67.42	1.26	1.57	0.92	142	4280.61	11336.91	60.06	2.418
60	<i>C. koidzumii</i>	8.09	4.65	51.41	2.01	1.62	1.55	224	4431.48	11318.70	91.22	3.672
61	<i>C. koidzumii</i>	5.33	4.96	38.66	1.98	2.05	2.45	320	5502.39	11318.70	100.24	4.034
62	<i>C. koidzumii</i>	9.50	6.01	100.98	5.09	1.80	4.87	261	3841.31	10884.06	118.05	4.751
63	<i>C. koidzumii</i>	9.76	5.79	96.49	2.76	2.52	5.16	454	6365.85	10747.96	103.27	4.157
64	<i>C. koidzumii</i>	11.82	7.47	193.97	2.44	2.69	5.20	433	5799.09	11314.43	125.69	5.059
65	<i>C. koidzumii</i>	11.08	8.05	211.42	1.72	2.47	3.10	268	6844.88	11304.43	58.39	2.350
66	<i>H. minor</i>	NA	NA	NA	5.60	4.75	37.28	1699	3152.02	10474.00	886.07	35.663
67	<i>C. leidyi</i>	12.56	8.36	459.88	2.28	2.66	4.75	405	3230.66	10457.64	206.61	8.316
68	<i>C. koidzumii</i>	10.25	7.51	170.43	2.09	1.99	2.44	285	4391.86	10990.65	113.54	4.570
69	<i>C. leidyi</i>	14.47	10.44	826.26	3.74	3.97	17.36	1005	4515.98	11354.86	402.43	16.197
70	<i>C. leidyi</i>	21.43	14.04	2211.22	3.79	3.85	16.57	1004	6613.49	11532.19	242.45	9.758
71	<i>H. minor</i>	59.02	28.02	13651.04	5.78	5.32	48.19	1960	4783.20	11813.14	769.59	30.975
72	<i>C. leidyi</i>	15.88	10.84	976.49	1.92	2.50	3.54	325	3519.69	11527.67	167.45	6.740
73	<i>C. leidyi</i>	NA	NA	NA	4.57	4.22	23.94	1296	4759.63	11574.88	500.18	20.132
74	<i>C. koidzumii</i>	6.88	6.32	80.99	1.23	1.84	1.22	158	5269.53	11579.17	54.02	2.174
75	<i>H. minor</i>	36.76	25.22	6884.68	4.67	5.56	42.51	1672	2592.18	11080.49	1054.85	42.456
76	<i>C. leidyi</i>	21.47	14.38	2325.46	4.28	5.06	32.36	1360	6775.15	11521.45	313.60	12.622
77	<i>P. grassii</i>	250.00	21.54	91066.78	9.56	11.14	349.43	6779	4656.28	12173.91	2829.49	113.883
78	<i>C. koidzumii</i>	13.15	10.31	412.00	2.08	2.99	5.48	400	5203.28	11538.96	138.36	5.569
79	<i>H. minor</i>	44.49	24.87	8104.12	4.47	4.24	23.71	1300	3506.56	11706.73	680.62	27.394
80	<i>H. minor</i>	49.79	26.85	10573.60	4.28	4.56	26.20	1263	4629.21	11696.25	508.41	20.463
81	<i>H. minor</i>	40.54	16.87	3398.26	4.02	3.91	18.09	997	3143.15	11638.91	566.84	22.814

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
82	<i>C. koidzumii</i>	25.44	17.38	2264.68	3.32	3.90	14.87	870	4484.34	12239.66	379.38	15.270
83	<i>C. koidzumii</i>	18.23	8.81	416.45	2.44	2.95	6.23	466	4316.06	11510.66	198.52	7.990
84	<i>H. minor</i>	47.58	19.09	5104.33	6.28	7.58	106.29	3230	3725.12	10964.41	1514.37	60.951
85	<i>H. hartmanni</i>	51.65	29.52	13257.77	6.39	6.41	77.34	2792	3177.42	11534.39	1563.30	62.920
86	<i>H. minor</i>	59.02	28.02	13651.04	5.81	5.36	49.16	1990	4807.90	11816.22	777.14	31.279
87	<i>C. koidzumii</i>	10.40	6.22	118.64	3.05	2.89	7.53	576	4866.81	11298.99	210.70	8.480
88	<i>C. koidzumii</i>	9.05	7.64	155.77	1.11	2.18	1.55	194	5815.99	11324.28	56.14	2.260
89	<i>P. grassii</i>	220.77	33.94	199724.78	12.37	13.09	623.63	9504	4227.15	10847.09	3889.66	156.553
90	<i>C. koidzumii</i>	19.89	19.31	2183.57	4.30	4.29	23.32	1167	4499.76	10771.89	442.41	17.806
91	<i>C. koidzumii</i>	12.02	8.04	228.71	3.44	2.30	5.37	523	3848.54	11323.76	245.13	9.866
92	<i>C. koidzumii</i>	10.40	6.22	118.64	3.19	2.96	8.20	581	4876.61	11292.72	211.88	8.528
93	<i>C. koidzumii</i>	11.40	6.29	133.00	1.74	1.68	1.45	205	4565.10	10812.78	76.77	3.090
94	<i>C. leidy</i>	16.10	9.09	696.79	2.86	2.38	4.77	500	3888.07	11329.38	232.24	9.347
95	<i>C. koidzumii</i>	7.06	4.75	46.90	2.07	1.66	1.68	234	7205.57	11365.73	46.32	1.864
96	<i>C. leidy</i>	22.20	12.27	1749.98	3.54	3.48	12.63	789	7262.11	11361.53	153.36	6.173
97	<i>H. hartmanni</i>	68.15	38.05	29060.05	7.31	7.21	112.00	3203	5180.90	11791.97	1144.05	46.046
98	<i>C. leidy</i>	31.08	19.17	5977.39	5.87	3.39	19.88	1285	6047.59	12024.83	383.57	15.438
99	<i>C. koidzumii</i>	27.27	20.94	3520.10	3.10	5.11	23.86	1000	6289.40	11377.66	257.44	10.362
100	<i>C. leidy</i>	39.60	22.80	10775.79	6.16	5.83	61.73	2496	8469.22	11353.32	317.69	12.786
101	<i>C. koidzumii</i>	15.93	9.23	399.98	1.98	1.96	2.23	249	4190.62	10758.10	101.95	4.104
102	<i>C. koidzumii</i>	12.36	9.10	301.77	3.74	1.82	3.65	432	6645.31	10792.89	90.99	3.662
103	<i>C. koidzumii</i>	11.17	8.75	251.85	2.72	2.09	3.51	392	7191.28	12022.11	87.48	3.521
104	<i>C. koidzumii</i>	32.68	16.77	2708.10	4.97	4.19	25.71	1425	7158.83	12018.12	320.62	12.904
105	<i>C. koidzumii</i>	26.08	15.75	1904.79	4.58	2.97	11.89	845	7252.84	12003.16	184.88	7.441
106	<i>C. koidzumii</i>	25.68	21.01	3338.18	5.12	4.90	36.18	1665	7424.17	10803.11	271.23	10.917
107	<i>C. koidzumii</i>	8.66	7.33	136.77	2.46	2.15	3.35	351	5261.93	10807.01	109.71	4.416
108	<i>C. koidzumii</i>	15.93	9.23	399.98	1.70	2.07	2.14	249	4166.66	10757.07	102.56	4.128

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
109	<i>C. leidyi</i>	41.19	19.70	8368.66	4.43	3.78	18.63	1067	6752.26	10944.91	223.82	9.008
110	<i>C. koidzumii</i>	13.88	10.60	458.86	3.37	2.77	7.63	631	7953.49	11802.75	108.17	4.354
111	<i>C. koidzumii</i>	8.72	7.72	153.26	2.02	1.61	1.54	211	6889.94	11800.24	49.31	1.984
112	<i>C. koidzumii</i>	7.74	5.09	59.18	1.96	1.86	1.98	237	5629.63	11757.24	75.80	3.051
113	<i>C. koidzumii</i>	9.05	7.64	155.77	1.76	1.41	1.03	167	5428.14	11316.18	53.28	2.144
114	<i>C. koidzumii</i>	18.10	10.82	624.46	2.12	2.06	2.65	307	6989.23	11392.72	65.14	2.622
115	<i>C. koidzumii</i>	36.12	23.44	5844.00	4.83	3.57	18.08	1095	8086.42	11423.98	164.32	6.614
116	<i>C. koidzumii</i>	12.37	9.15	304.68	3.09	2.16	4.23	416	6614.36	11437.28	98.94	3.982
117	<i>C. koidzumii</i>	13.41	7.44	218.51	1.99	2.28	3.03	304	6099.89	10886.24	76.47	3.078
118	<i>C. koidzumii</i>	8.67	8.36	178.40	2.26	2.14	3.05	308	4415.88	10928.24	121.21	4.878
119	<i>H. minor</i>	42.65	27.63	9591.92	4.24	3.61	16.31	1026	5584.19	10519.43	282.18	11.357
120	<i>C. koidzumii</i>	12.34	5.74	119.86	3.36	1.39	1.92	344	5420.60	11443.66	111.63	4.493
121	<i>C. leidyi</i>	31.87	25.76	11072.16	3.47	3.71	14.11	845	5236.21	11464.47	287.58	11.575
122	<i>C. koidzumii</i>	21.56	22.30	3158.03	2.81	3.85	12.25	736	5387.57	11365.83	238.62	9.604
123	<i>H. minor</i>	47.60	26.70	9991.48	5.45	7.35	86.67	2519	5030.30	10923.69	848.34	34.144
124	<i>C. leidyi</i>	31.01	22.34	8102.98	3.49	4.87	24.32	1088	6458.00	10923.69	248.36	9.996
125	<i>C. koidzumii</i>	12.35	7.75	218.71	2.68	2.12	3.54	404	5491.32	10714.71	117.28	4.720
126	<i>H. minor</i>	45.41	28.33	10736.09	4.41	6.03	47.15	1831	4640.30	10479.83	647.82	26.074
127	<i>C. koidzumii</i>	11.23	8.67	248.46	2.30	1.87	2.37	311	8318.31	11440.32	43.04	1.732
128	<i>C. leidyi</i>	24.95	18.11	4284.65	4.69	3.76	19.54	1193	7474.36	11436.25	220.36	8.869
129	<i>C. koidzumii</i>	19.55	11.59	773.52	3.33	2.40	5.64	591	5836.21	10734.09	156.40	6.295
130	<i>C. leidyi</i>	22.59	10.64	1339.56	2.62	3.06	7.22	523	6034.81	11049.10	137.37	5.529
131	<i>C. koidzumii</i>	9.98	6.26	115.00	1.55	1.99	1.82	211	7445.84	10929.11	35.17	1.415
132	<i>C. leidyi</i>	24.87	12.38	1996.44	3.54	3.52	12.93	801	3295.06	10931.68	417.18	16.791
133	<i>C. koidzumii</i>	11.38	6.65	147.98	2.32	2.12	3.06	309	3530.80	11447.71	157.85	6.353
134	<i>C. koidzumii</i>	17.29	9.00	412.23	2.30	2.22	3.35	358	4703.03	10845.97	129.91	5.229
135	<i>C. leidyi</i>	17.72	8.25	630.71	3.30	2.62	6.68	571	4810.99	10902.01	202.86	8.165



Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
136	<i>C. leidyi</i>	16.25	10.06	860.85	3.56	4.18	18.36	1056	5685.95	10846.60	296.20	11.922
137	<i>C. koidzumii</i>	18.83	11.24	699.88	2.89	3.44	10.11	676	5297.72	10510.95	201.15	8.096
138	<i>C. koidzumii</i>	15.20	10.28	473.22	2.12	2.77	4.80	380	4959.54	10504.34	123.85	4.985
139	<i>C. koidzumii</i>	12.96	9.63	354.19	2.22	2.84	5.28	423	5227.54	10921.21	135.35	5.448
140	<i>C. leidyi</i>	26.04	14.97	3056.50	4.32	3.56	16.13	1017	5149.15	10475.31	313.67	12.625
141	<i>C. koidzumii</i>	15.05	11.37	573.04	3.25	2.98	8.51	696	5667.98	10929.19	198.47	7.988
142	<i>C. koidzumii</i>	29.61	25.41	5631.61	4.15	4.12	20.75	1124	6308.79	10925.36	268.06	10.789
143	<i>C. koidzumii</i>	8.67	6.20	98.20	2.04	1.39	1.15	199	5580.87	11431.78	61.97	2.494
144	<i>C. koidzumii</i>	11.48	8.85	264.92	2.81	1.93	3.08	371	3519.41	9951.90	167.48	6.741
145	<i>C. koidzumii</i>	14.70	11.78	600.76	2.46	3.52	8.99	570	3252.16	10877.16	298.88	12.029
146	<i>P. grassii</i>	141.10	30.57	103556.98	10.83	10.65	361.78	7313	3716.92	10518.89	3303.91	132.977
147	<i>H. minor</i>	37.23	22.27	5439.51	4.17	6.60	53.55	1217	4356.40	10509.90	465.47	18.734
148	<i>H. minor</i>	48.18	23.29	7694.13	3.44	5.73	33.32	1353	4262.23	10513.19	530.51	21.352
149	<i>H. minor</i>	38.24	28.25	8985.09	3.81	3.42	13.09	1134	5436.53	11269.07	358.99	14.449
150	<i>H. minor</i>	32.00	15.74	2333.49	4.40	3.83	19.02	1040	3164.54	10513.20	542.28	21.826
151	<i>C. leidyi</i>	24.96	17.80	4139.50	4.28	3.83	18.51	1088	3377.06	9974.33	511.74	20.597
152	<i>C. koidzumii</i>	19.51	21.00	2533.69	3.17	4.43	18.27	1029	3211.57	10044.48	509.57	20.509
153	<i>C. koidzumii</i>	12.89	6.87	179.03	2.25	1.70	1.90	269	4447.31	10462.59	99.94	4.023
154	<i>C. koidzumii</i>	23.13	20.47	2854.02	4.02	4.14	20.24	1094	4861.81	10483.14	365.06	14.693
155	<i>C. koidzumii</i>	7.82	7.08	115.49	2.13	2.13	2.84	302	2968.33	10253.77	162.59	6.544
156	<i>C. leidyi</i>	27.18	16.01	3649.25	4.88	5.93	50.50	1760	5350.90	9852.48	466.61	18.780
157	<i>C. leidyi</i>	21.89	13.31	2031.71	4.37	3.98	20.37	1088	6690.05	10588.15	216.94	8.731
158	<i>H. minor</i>	44.70	21.18	5903.46	4.90	4.21	25.50	1297	3679.14	10424.04	586.62	23.611
159	<i>C. koidzumii</i>	12.00	7.95	223.43	2.11	2.16	2.90	318	4256.15	9919.55	116.86	4.703
160	<i>C. koidzumii</i>	9.36	8.90	218.19	1.68	2.05	2.09	250	3276.81	10483.30	126.26	5.082
161	<i>C. leidyi</i>	13.80	7.85	445.31	2.16	2.79	4.95	404	3907.94	11348.45	187.05	7.528
162	<i>H. minor</i>	47.68	22.97	7409.65	4.35	4.07	21.21	1107	4195.32	11402.60	480.70	19.348

Cell #	Protist Species	Cell Length (μm)	Cell Width (μm)	Cell Vol. (μm <sup>3</sup> )	Nuc Length (μm)	Nuc Width (μm)	Nucleus Vol. (μm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
163	<i>C. koidzumii</i>	14.48	7.55	243.33	2.96	1.86	3.02	383	4261.75	10801.34	154.69	6.226
164	<i>C. leidyi</i>	15.55	11.19	1018.72	4.41	2.72	9.59	783	5957.18	10974.91	207.78	8.363
165	<i>C. koidzumii</i>	9.69	6.53	121.79	1.75	1.93	1.92	259	4534.76	10154.12	90.67	3.649
166	<i>C. koidzumii</i>	14.20	9.58	383.59	2.34	2.72	5.09	447	4504.57	10154.12	157.79	6.351
167	<i>C. koidzumii</i>	13.65	7.67	236.31	2.45	1.59	1.83	271	4405.10	10281.32	99.75	4.015
168	<i>C. koidzumii</i>	12.65	8.89	294.32	2.43	1.91	2.59	290	4668.59	10472.43	101.75	4.095
169	<i>C. leidyi</i>	17.48	7.56	523.32	3.00	2.34	4.83	450	5191.07	9974.43	127.63	5.137
170	<i>C. koidzumii</i>	14.48	9.51	385.70	3.32	1.55	2.35	315	6370.40	11624.88	82.28	3.312
171	<i>H. hartmanni</i>	52.86	32.22	16162.86	6.20	5.46	54.37	2079	3756.87	11119.58	979.75	39.434
172	<i>C. leidyi</i>	29.05	21.48	7015.14	4.73	4.52	28.44	1376	5286.21	10474.14	408.64	16.447
173	<i>H. hartmanni</i>	78.58	33.06	25299.83	11.52	12.36	518.28	8938	4968.64	11113.48	3124.83	125.770
174	<i>C. koidzumii</i>	18.30	11.37	696.41	2.47	2.40	4.18	394	5109.02	10847.26	128.83	5.185
175	<i>C. leidyi</i>	18.97	11.31	1269.97	2.68	2.10	3.47	362	5186.75	10874.72	116.39	4.685
176	<i>C. koidzumii</i>	7.31	4.86	50.89	2.08	2.68	4.40	356	4826.30	10451.38	119.46	4.808
177	<i>C. leidyi</i>	19.36	10.72	1164.70	3.94	3.48	14.01	881	6582.00	10441.50	176.56	7.106
178	<i>H. minor</i>	65.46	30.02	17376.17	9.97	8.51	212.55	5215	7960.14	10895.16	710.87	28.611
179	<i>H. minor</i>	60.85	39.92	28564.59	8.30	8.09	159.70	4153	6500.31	11451.89	1021.40	41.110
180	<i>C. leidyi</i>	38.00	22.68	10230.49	5.31	5.17	41.82	1678	6552.65	10940.94	373.59	15.036
181	<i>P. grassii</i>	94.85	41.19	126358.24	13.25	13.66	727.59	11471	5273.15	11447.54	3861.62	155.424
182	<i>P. grassii</i>	199.22	41.55	270053.27	12.86	15.00	852.79	11733	5468.65	11448.48	3764.74	151.525
183	<i>C. koidzumii</i>	28.10	19.60	3177.82	5.09	4.17	26.05	1520	7816.96	10910.60	220.11	8.859
184	<i>H. hartmanni</i>	51.74	22.41	7649.97	8.12	10.19	248.61	5234	89.13	159.71	1325.82	53.362
185	<i>H. hartmanni</i>	62.82	43.28	34653.45	7.35	12.42	334.12	5052	50.22	166.64	2631.75	105.924
186	<i>H. minor</i>	62.47	23.47	10134.89	4.50	3.84	19.51	1176	57.58	171.55	557.55	22.440
187	<i>C. leidyi</i>	28.13	17.58	4550.68	4.30	3.47	15.23	965	80.47	165.39	301.90	12.151
188	<i>C. leidyi</i>	31.97	17.38	5058.03	4.34	3.42	14.98	1001	115.54	178.28	188.56	7.589
189	<i>H. hartmanni</i>	72.83	41.39	36745.00	12.18	15.37	848.17	10000	97.77	178.11	2604.89	104.843

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
190	<i>C. koidzumii</i>	10.89	7.85	197.82	2.27	2.08	2.91	309	99.11	178.38	78.86	3.174
191	<i>C. koidzumii</i>	12.22	7.30	191.71	2.93	2.18	4.09	435	108.16	178.38	94.51	3.804
192	<i>H. minor</i>	54.08	52.64	44145.63	6.24	12.10	268.82	5248	124.12	185.96	921.33	37.082
193	<i>C. leidyi</i>	27.95	9.58	1344.00	3.83	1.97	4.38	501	66.93	185.48	221.77	8.926
194	<i>C. leidyi</i>	34.04	18.76	6274.70	4.51	4.85	31.25	1395	96.86	158.01	296.51	11.934
195	<i>P. grassii</i>	135.85	27.87	82854.09	10.67	11.06	384.31	7954	58.90	158.65	3422.50	137.750
196	<i>H. minor</i>	40.64	28.21	9526.26	5.12	5.99	54.13	2107	97.92	180.01	557.16	22.425
197	<i>C. leidyi</i>	24.00	11.94	1791.21	4.29	3.21	13.02	889	97.88	169.68	212.43	8.550
198	<i>C. koidzumii</i>	22.10	15.83	1630.31	3.34	3.26	10.48	782	97.52	169.60	187.93	7.564
199	<i>C. leidyi</i>	19.00	14.16	1995.69	4.17	4.10	20.64	1165	100.94	169.59	262.51	10.566
200	<i>C. leidyi</i>	30.43	13.22	2785.12	4.76	3.44	16.58	1046	82.57	168.16	323.12	13.005
201	<i>C. koidzumii</i>	11.99	9.09	291.57	2.17	3.28	6.88	503	79.30	168.16	164.21	6.609
202	<i>H. minor</i>	56.48	30.65	15628.15	4.57	5.35	38.47	1539	66.02	169.63	630.74	25.386
203	<i>P. grassii</i>	165.88	34.81	157855.29	10.22	12.84	496.06	8043	76.53	171.41	2816.77	113.370
204	<i>H. minor</i>	51.00	23.96	8622.20	4.82	4.84	33.23	1458	83.21	184.39	503.80	20.277
205	<i>C. koidzumii</i>	23.23	15.28	1597.48	2.11	2.20	3.00	287	107.99	184.39	66.68	2.684
206	<i>C. leidyi</i>	34.09	15.93	4528.17	4.09	4.84	28.23	1322	55.31	162.91	620.17	24.961
207	<i>C. leidyi</i>	33.82	17.61	5489.00	4.42	3.35	14.62	992	94.52	165.10	240.28	9.671
208	<i>P. grassii</i>	NA	NA	NA	11.98	13.49	641.94	10039	34.21	168.25	6944.82	279.518
209	<i>C. leidyi</i>	31.48	15.90	4167.56	2.66	4.43	15.35	820	67.58	160.75	308.58	12.420
210	<i>C. koidzumii</i>	18.78	11.32	708.56	4.20	4.15	21.29	1105	67.66	169.96	442.03	17.791
211	<i>H. hartmanni</i>	59.96	31.32	17328.13	5.10	4.97	37.07	1696	55.94	159.91	773.67	31.139
212	<i>P. grassii</i>	127.66	27.59	76297.20	9.68	10.86	336.40	6922	52.99	170.41	3511.48	141.332
213	<i>C. koidzumii</i>	8.92	5.61	82.80	1.60	2.05	1.97	221	79.65	171.09	73.38	2.953
214	<i>C. leidyi</i>	23.55	11.87	1738.54	4.98	3.75	20.56	1197	89.51	166.05	321.25	12.930
215	<i>C. leidyi</i>	35.64	20.80	8071.61	4.85	4.37	27.23	1253	98.21	173.08	308.36	12.411
216	<i>P. grassii</i>	NA	NA	NA	13.56	13.92	774.36	12427	48.61	151.39	6131.24	246.773

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
217	<i>P. grassii</i>	NA	NA	NA	11.74	10.62	390.19	7668	58.06	158.60	3346.20	134.679
218	<i>P. grassii</i>	NA	NA	NA	7.53	8.35	154.67	4275	18.98	171.95	4091.36	164.671
219	<i>H. minor</i>	38.33	22.36	5643.35	4.79	7.72	83.95	2410	37.74	181.39	1643.16	66.135
220	<i>C. leidyi</i>	23.88	9.01	1015.09	4.07	2.74	9.03	728	59.53	169.63	331.08	13.326
221	<i>C. koidzumii</i>	27.30	23.78	4546.87	3.79	5.26	30.95	1260	59.31	171.84	582.14	23.430
222	<i>C. koidzumii</i>	14.11	9.89	405.98	2.54	2.29	3.93	378	50.08	149.07	179.07	7.207
223	<i>C. koidzumii</i>	10.13	7.06	148.76	2.17	2.17	3.03	302	63.97	153.08	114.45	4.606
224	<i>C. koidzumii</i>	14.54	10.17	442.60	3.32	2.56	6.41	594	46.57	153.08	306.99	12.356
225	<i>H. minor</i>	37.82	34.54	13291.57	5.12	6.34	60.53	2003	33.66	169.97	1408.57	56.693
226	<i>C. leidyi</i>	17.63	10.79	1075.10	3.18	3.09	8.91	695	55.63	169.97	337.09	13.567
227	<i>C. koidzumii</i>	12.32	7.05	180.44	1.97	2.39	3.31	285	75.53	158.01	91.36	3.677
228	<i>C. koidzumii</i>	8.53	5.72	82.12	1.78	1.62	1.37	177	61.84	158.01	72.11	2.902
229	<i>C. koidzumii</i>	6.64	4.64	42.03	2.32	1.98	2.67	311	77.92	158.01	95.49	3.843
230	<i>C. koidzumii</i>	9.11	5.71	87.40	2.01	2.76	4.50	367	77.37	158.01	113.80	4.580
231	<i>H. hartmanni</i>	75.13	34.57	26440.49	6.57	7.53	109.88	3017	73.34	171.61	1113.85	44.831
232	<i>C. koidzumii</i>	33.16	22.78	5066.42	3.77	3.67	14.90	918	76.68	170.03	317.46	12.777
233	<i>C. koidzumii</i>	9.21	7.02	133.61	1.69	2.57	3.30	297	83.36	170.03	91.93	3.700
234	<i>C. koidzumii</i>	11.08	9.02	265.56	2.99	3.03	8.08	609	84.42	170.03	185.18	7.453
235	<i>C. koidzumii</i>	16.37	13.46	873.13	3.65	3.45	12.76	786	58.96	151.75	322.73	12.989
236	<i>P. grassii</i>	NA	NA	NA	14.82	14.82	958.08	14022	41.04	158.09	8212.86	330.555
237	<i>C. leidyi</i>	21.14	13.25	1943.88	3.28	3.35	10.83	738	65.18	159.44	286.71	11.540
238	<i>H. hartmanni</i>	60.80	50.42	45518.28	5.70	10.81	196.17	3715	86.52	160.16	993.49	39.986
239	<i>H. minor</i>	52.63	34.12	18043.57	5.74	6.74	76.87	2738	68.86	156.77	978.25	39.373
240	<i>C. koidzumii</i>	29.39	19.04	3137.34	4.59	4.09	22.60	1237	67.60	172.10	502.01	20.205
241	<i>C. koidzumii</i>	24.90	15.20	1694.88	4.23	4.43	24.42	1233	68.84	172.81	492.88	19.838
242	<i>C. leidyi</i>	20.54	11.61	1448.93	3.40	3.68	13.58	850	80.63	165.21	264.81	10.658
243	<i>C. koidzumii</i>	17.15	14.69	1089.07	3.77	4.31	20.66	989	81.30	162.88	298.46	12.012

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
244	<i>C. koidzumii</i>	26.48	17.55	2400.93	4.88	4.14	24.69	1295	62.80	159.40	523.90	21.086
245	<i>C. koidzumii</i>	17.12	9.76	480.47	3.79	3.80	16.09	936	74.69	159.40	308.17	12.403
246	<i>C. koidzumii</i>	11.87	6.50	147.42	2.37	2.91	5.90	471	70.01	159.40	168.30	6.774
247	<i>C. koidzumii</i>	18.98	10.29	591.62	3.17	3.02	8.52	637	93.03	162.92	155.03	6.240
248	<i>C. koidzumii</i>	17.20	11.50	669.80	1.98	3.36	6.59	419	63.50	157.70	165.54	6.663
249	<i>C. koidzumii</i>	9.93	8.38	205.28	2.17	2.22	3.16	292	65.09	162.21	115.81	4.661
250	<i>C. leidy</i>	21.98	16.02	2951.59	3.30	4.28	17.81	860	60.74	165.81	375.05	15.095
251	<i>C. koidzumii</i>	27.71	18.46	2780.73	4.76	4.39	27.02	1299	66.56	164.64	510.92	20.564
252	<i>H. minor</i>	52.63	34.73	18694.85	5.86	7.41	94.73	2855	79.31	173.27	968.91	38.997
253	<i>P. grassii</i>	NA	NA	NA	8.98	14.57	560.88	8557	40.91	149.49	4815.81	193.829
254	<i>H. hartmanni</i>	57.02	50.85	43420.26	9.68	11.95	406.87	6900	43.43	154.62	3805.10	153.149
255	<i>C. koidzumii</i>	29.28	25.42	5572.66	3.86	5.81	38.43	1551	64.51	162.10	620.66	24.981
256	<i>H. minor</i>	41.65	29.58	10732.51	6.28	6.38	75.18	2701	70.90	154.29	912.05	36.709
257	<i>C. koidzumii</i>	12.25	6.74	164.01	2.44	2.28	3.73	377	74.49	168.46	133.60	5.377
258	<i>H. minor</i>	47.43	38.23	20417.87	4.26	5.48	37.71	1478	40.43	168.46	916.07	36.871
259	<i>C. koidzumii</i>	15.70	8.95	370.18	2.80	2.18	3.91	426	46.21	168.05	238.86	9.614
260	<i>H. minor</i>	41.42	29.31	10480.04	6.68	6.48	82.45	2788	68.96	150.37	943.95	37.993
261	<i>H. minor</i>	27.80	28.41	6607.89	4.94	6.75	66.15	2183	53.36	117.52	748.53	30.127
262	<i>H. minor</i>	52.82	46.19	33187.49	7.59	10.88	264.60	4955	97.75	164.92	1125.54	45.301
263	<i>P. grassii</i>	NA	NA	NA	13.21	11.41	506.19	9768	41.05	153.73	5601.51	225.452
264	<i>H. minor</i>	65.97	30.68	18283.66	4.60	4.67	29.51	1479	62.22	174.04	660.69	26.592
265	<i>H. minor</i>	54.90	28.27	12921.52	4.80	4.52	28.90	1278	59.66	155.12	530.36	21.346
266	<i>C. koidzumii</i>	27.86	7.89	510.51	2.74	1.82	2.66	335	44.96	174.47	197.29	7.941
267	<i>C. koidzumii</i>	10.92	7.27	170.00	2.46	1.75	2.21	270	91.02	159.31	65.64	2.642
268	<i>C. koidzumii</i>	15.16	9.65	415.62	2.09	2.56	4.03	360	107.08	172.23	74.31	2.991
269	<i>C. koidzumii</i>	13.47	8.44	282.49	2.60	2.10	3.36	323	91.55	172.17	88.61	3.566
270	<i>H. minor</i>	53.74	26.82	11381.90	4.50	6.51	56.16	1927	72.61	163.04	676.99	27.248

Cell #	Protist Species	Cell Length (μm)	Cell Width (μm)	Cell Vol. (μm <sup>3</sup> )	Nuc Length (μm)	Nuc Width (μm)	Nucleus Vol. (μm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
271	H. minor	NA	NA	NA	6.54	4.56	39.99	1853	82.05	163.04	552.63	22.242
272	C. koidzumii	14.81	13.46	789.72	3.47	2.97	9.03	661	88.77	181.90	205.95	8.289
273	C. koidzumii	22.81	14.28	1368.88	2.92	3.35	9.65	630	106.85	171.98	130.22	5.241
274	C. koidzumii	11.47	7.48	189.16	2.50	2.82	5.84	462	98.48	172.62	112.62	4.533
275	C. koidzumii	7.54	7.37	120.64	2.78	1.53	1.91	268	94.94	172.62	69.58	2.801
276	C. koidzumii	8.59	6.98	123.18	1.96	2.53	3.70	319	142.10	172.62	26.96	1.085
277	H. minor	61.21	37.64	25541.29	6.88	6.27	79.59	2885	49.69	114.75	1048.66	42.207
278	H. hartmanni	79.91	40.55	38699.41	7.47	8.14	145.68	4000	88.48	180.87	1242.10	49.992
279	H. minor	39.48	20.97	5113.46	4.46	4.75	29.58	1412	127.39	180.74	214.51	8.634
280	P. grassii	187.09	19.81	57686.98	12.09	10.85	419.44	7721	60.60	150.76	3056.02	123.000
281	P. grassii	212.08	23.02	88283.55	9.38	8.54	201.25	5986	57.59	150.76	2501.87	100.696
282	H. minor	37.14	35.12	13489.93	4.67	8.92	109.37	2538	91.25	172.77	703.66	28.321
283	C. koidzumii	9.98	4.83	68.49	1.46	2.01	1.73	192	49.32	169.00	102.70	4.133
284	C. koidzumii	26.54	23.31	4246.07	2.86	5.71	27.44	1064	74.31	170.70	384.31	15.468
285	H. minor	58.87	23.53	9598.75	8.44	6.35	100.33	3507	90.02	168.28	952.85	38.351
286	C. koidzumii	23.59	15.48	1664.77	3.84	3.90	17.21	975	53.88	159.53	459.62	18.499
287	C. koidzumii	37.02	22.43	5486.92	5.50	5.48	48.66	1974	55.37	154.51	879.76	35.409
288	C. leidyi	26.26	10.00	1375.47	4.63	2.72	10.11	897	89.72	172.06	253.69	10.211
289	C. leidyi	25.95	10.23	1420.68	3.81	3.25	11.88	791	93.37	172.06	209.98	8.451
290	C. leidyi	30.60	9.70	1506.34	3.13	3.06	8.62	628	96.09	172.06	158.89	6.395
291	P. grassii	160.34	26.90	91152.41	11.56	10.19	353.15	7986	33.91	170.33	5598.02	225.311
292	C. koidzumii	9.67	6.23	110.41	2.78	2.68	5.89	458	116.00	173.21	79.74	3.209
293	C. leidyi	27.48	15.50	3454.99	3.60	3.61	13.83	798	47.83	157.60	413.27	16.633
294	C. leidyi	24.04	10.63	1421.52	3.30	2.32	5.21	486	41.48	168.24	295.55	11.895
295	C. leidyi	16.41	7.91	537.84	1.99	2.08	2.54	380	3928.51	11174.82	172.52	6.944
296	H. minor	52.79	23.46	8559.98	7.08	5.93	73.27	2637	3817.06	10098.78	1114.24	44.846
297	C. koidzumii	29.37	28.87	7210.97	4.55	6.68	59.69	1794	3493.86	10476.24	855.56	34.435

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
298	<i>C. leidyi</i>	15.25	7.35	431.16	2.36	2.32	3.73	385	2767.15	11186.15	233.56	9.400
299	<i>C. koidzumii</i>	32.77	23.75	5445.09	3.55	6.30	41.44	1550	4309.68	11180.88	641.75	25.829
300	<i>C. leidyi</i>	24.94	14.72	2828.01	5.19	4.47	30.52	1490	4534.02	10701.07	555.69	22.365
301	<i>C. leidyi</i>	21.61	10.58	1265.36	4.63	4.40	26.46	1330	3846.30	10724.11	592.27	23.838
302	<i>H. minor</i>	35.27	19.28	3859.83	3.73	7.58	63.04	2216	4561.02	10485.00	801.10	32.243
303	<i>H. minor</i>	46.21	33.10	14908.90	5.91	10.32	185.44	3736	5616.52	10075.82	948.25	38.165
304	<i>H. minor</i>	48.27	35.03	17439.88	7.28	11.06	262.23	5411	4932.83	12195.78	2127.14	85.614
305	<i>P. grassii</i>	142.74	28.35	90095.89	10.72	9.44	281.33	6666	2384.89	10587.74	4315.13	173.677
306	<i>C. leidyi</i>	29.93	16.25	4138.85	5.22	5.13	40.51	1738	5040.71	11136.40	598.31	24.081
307	<i>C. koidzumii</i>	9.10	5.48	80.34	2.25	2.28	3.45	338	4233.04	11056.23	140.93	5.672
308	<i>C. leidyi</i>	13.62	6.17	271.84	2.33	2.23	3.41	348	4394.33	10473.54	131.27	5.283
309	<i>H. hartmanni</i>	108.91	56.54	102530.96	7.77	15.08	520.14	7900	3147.66	11363.86	4404.55	177.276
310	<i>H. hartmanni</i>	92.72	47.30	61080.36	11.41	19.70	1304.80	2226	3419.46	11010.79	1130.50	45.501
311	<i>C. leidyi</i>	18.76	7.44	543.84	3.54	3.08	9.86	697	2876.68	10582.86	394.30	15.870
312	<i>C. leidyi</i>	50.23	12.63	4193.61	5.00	3.70	20.21	1219	2804.77	11198.42	732.94	29.500
313	<i>C. koidzumii</i>	NA	NA	NA	2.77	2.35	4.49	443	4220.84	11162.70	187.11	7.531
314	<i>H. hartmanni</i>	NA	NA	NA	7.14	10.73	242.05	4921	2485.19	11162.70	3210.51	129.218
315	<i>C. koidzumii</i>	11.75	9.30	299.25	1.95	2.00	2.29	256	4684.01	10368.82	88.35	3.556
316	<i>H. minor</i>	NA	NA	NA	6.18	5.79	60.95	2282	4399.91	10656.22	876.65	35.284
317	<i>H. hartmanni</i>	55.16	27.98	12721.13	6.44	6.49	79.71	2839	3730.30	11557.07	1394.24	56.116
318	<i>C. koidzumii</i>	17.75	7.42	288.04	2.83	2.83	6.68	515	2600.32	11201.06	326.63	13.146
319	<i>H. minor</i>	51.72	31.58	15185.34	5.54	5.17	43.63	1842	4845.32	11027.86	657.91	26.480
320	<i>H. minor</i>	NA	NA	NA	5.49	4.89	38.65	1663	2923.00	11027.86	958.99	38.598
321	<i>H. hartmanni</i>	83.06	32.60	26004.57	10.11	15.91	753.72	11062	4079.75	11837.07	5117.40	205.968
322	<i>C. koidzumii</i>	15.87	6.48	196.45	2.36	2.60	4.68	413	3030.82	11837.07	244.37	9.835
323	<i>H. minor</i>	69.45	35.46	25715.30	9.02	8.72	202.14	4888	4273.27	10698.99	1948.27	78.415
324	<i>P. grassii</i>	127.83	15.32	23573.30	9.85	10.51	320.21	6638	2759.75	10665.69	3897.30	156.860

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
325	H. minor	34.90	23.45	5650.95	5.76	5.64	53.98	1953	3223.21	10079.27	967.01	38.921
326	C. leidyi	13.98	5.92	256.09	2.64	1.94	2.93	347	3601.74	11707.63	177.65	7.150
327	C. leidyi	18.10	8.39	667.26	5.34	2.55	10.24	834	2422.85	10969.01	546.97	22.015
328	C. koidzumii	20.98	9.01	502.16	4.27	2.42	7.35	657	4190.58	10900.41	272.77	10.978
329	H. minor	52.04	19.81	6012.83	7.74	5.44	67.58	2928	3326.16	11223.29	1546.50	62.244
330	C. koidzumii	26.15	17.53	2365.87	4.15	3.98	19.34	1069	3878.84	10700.70	471.12	18.962
331	H. minor	44.16	19.98	5191.21	4.75	5.46	41.75	1688	3307.41	10034.61	813.64	32.748
332	H. minor	40.94	16.08	3115.43	5.00	4.15	25.30	1329	3045.80	9913.17	681.13	27.414
333	C. leidyi	23.66	11.18	1548.81	3.30	3.39	11.13	813	2794.40	10676.33	473.27	19.049
334	H. minor	58.45	46.30	36898.43	7.48	8.36	153.84	3871	2041.78	11033.00	2836.22	114.153
335	H. minor	56.46	40.09	26718.31	11.85	3.96	54.80	3220	3051.54	10549.63	1734.67	69.818
336	H. minor	38.36	17.85	3599.97	4.34	6.77	58.46	1859	5563.27	10140.93	484.73	19.510
337	C. leidyi	26.43	14.13	2762.42	3.80	3.86	16.66	965	4068.05	10605.39	401.58	16.163
338	C. koidzumii	23.61	23.36	3794.57	4.48	3.76	18.68	1035	3533.99	10879.56	505.44	20.343
339	C. koidzumii	11.49	7.99	215.91	2.07	2.05	2.56	272	6326.28	11211.18	67.59	2.721
340	C. koidzumii	35.76	17.45	3207.08	3.57	3.72	14.52	906	3951.60	10180.30	372.35	14.987
341	H. hartmanni	65.45	33.10	21119.32	5.77	6.64	74.90	2478	2224.44	11919.10	1806.52	72.709
342	H. minor	NA	NA	NA	13.73	7.45	224.62	5995	4115.69	10576.54	2457.35	98.905
343	H. minor	NA	NA	NA	7.83	7.45	127.96	3801	5209.11	10576.54	1169.11	47.055
344	H. hartmanni	59.48	44.52	34718.50	6.03	8.96	142.40	3480	4377.34	10118.74	1266.43	50.972
345	C. koidzumii	24.47	17.76	2272.75	2.46	2.82	5.76	450	3639.00	10118.74	199.87	8.044
346	H. minor	42.86	28.30	10111.55	4.80	4.82	32.87	1446	2568.91	10641.91	892.57	35.925
347	C. koidzumii	NA	NA	NA	5.40	4.32	29.70	306	3210.42	10641.91	159.26	6.410
348	H. minor	64.70	25.09	11997.24	3.89	6.15	43.31	1445	3557.26	10656.23	688.53	27.712
349	C. leidyi	11.60	6.05	222.11	2.72	2.20	3.88	385	3357.15	10396.67	189.01	7.607
350	C. koidzumii	NA	NA	NA	3.49	3.56	13.01	835	4092.89	10567.38	343.97	13.844
351	H. minor	39.49	29.27	9965.57	4.76	5.48	42.13	1667	3064.77	10567.38	896.13	36.068



Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
352	H. minor	57.32	20.05	6786.90	4.03	4.14	20.31	1102	3600.13	10431.88	509.17	20.493
353	H. minor	43.17	22.45	6407.06	3.81	3.67	15.11	991	4558.94	10430.18	356.19	14.336
354	C. koidzumii	26.76	13.67	1473.23	4.07	3.66	16.06	981	5154.99	9842.86	275.56	11.091
355	H. minor	58.93	32.62	18468.05	4.94	4.77	33.14	1594	4244.61	10667.57	637.96	25.677
356	C. leidy	13.72	9.03	585.69	1.93	2.38	3.21	320	4103.38	10339.07	128.43	5.169
357	C. leidy	34.47	16.67	5017.56	4.98	4.71	32.51	1343	6561.83	10740.82	287.42	11.568
358	C. koidzumii	23.83	11.77	971.88	3.71	3.20	11.22	793	4917.04	10817.65	271.55	10.929
359	P. grassii	180.96	24.09	82453.94	10.91	12.29	485.15	8511	2797.49	10146.64	4762.36	191.677
360	P. grassii	170.85	21.90	64333.09	10.15	9.63	276.94	6590	3220.83	10592.09	3407.12	137.131
361	P. grassii	210.83	22.05	80486.56	11.33	10.80	388.76	7778	2485.05	10733.22	4942.10	198.912
362	C. koidzumii	21.79	10.23	671.01	4.09	3.19	12.25	816	6919.24	11182.96	170.13	6.848
363	H. minor	41.91	19.82	4847.58	4.61	4.50	27.42	1371	4298.83	10750.55	545.77	21.966
364	H. minor	35.03	22.18	5076.82	4.51	8.05	86.07	2405	3653.88	10779.70	1129.99	45.481
365	C. koidzumii	32.64	16.69	2678.32	3.68	4.18	18.91	1160	3982.46	11242.48	522.82	21.043
366	C. leidy	23.94	10.63	1417.00	3.45	3.57	12.90	838	4116.32	10853.72	352.86	14.202
367	C. leidy	24.49	18.32	4304.66	3.72	5.06	27.99	1286	4379.06	10850.85	506.79	20.398
368	C. koidzumii	24.67	20.57	3075.83	3.16	3.67	12.55	785	5127.55	10211.46	234.85	9.453
369	H. hartmanni	50.21	24.99	9237.92	4.50	8.20	89.03	2434	5122.60	10821.28	790.53	31.818
370	C. leidy	33.13	16.86	4931.58	4.50	4.34	25.02	1283	5221.96	10653.69	397.30	15.991
371	H. minor	47.44	24.25	8218.58	5.19	7.56	87.40	2455	4528.62	10652.42	911.99	36.706
372	C. leidy	43.90	14.46	4804.30	5.09	3.39	17.18	1230	5736.22	9629.47	276.72	11.138
373	C. koidzumii	33.01	20.48	4076.89	2.04	7.23	31.31	1032	3966.53	9626.47	397.38	15.994
374	C. koidzumii	31.77	16.59	2574.94	3.24	3.02	8.68	666	4372.88	9724.31	231.16	9.304
375	H. minor	33.81	25.65	6550.75	3.16	5.17	24.85	1057	4597.40	11424.28	417.85	16.818
376	C. koidzumii	27.50	16.73	2267.05	5.60	3.20	16.88	1167	4615.99	10297.19	406.65	16.367
377	C. leidy	12.89	8.46	482.56	2.33	2.32	3.69	357	4773.73	11345.53	134.22	5.402
378	C. leidy	17.55	11.07	1126.58	2.51	2.09	3.23	356	3753.53	10434.09	158.07	6.362

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
379	<i>C. koidzumii</i>	37.10	22.11	5341.33	3.65	6.07	39.57	1335	3829.25	11508.97	638.03	25.680
380	<i>H. minor</i>	39.44	21.68	5460.35	4.69	4.27	25.17	1219	2584.48	10795.15	756.82	30.461
381	<i>H. minor</i>	55.11	26.97	11806.50	5.92	7.07	87.03	2496	4535.65	13807.35	1206.74	48.570
382	<i>C. koidzumii</i>	25.22	22.90	3896.57	2.86	7.18	43.29	1327	3452.50	9613.61	590.19	23.754
383	<i>C. leidyi</i>	19.96	8.93	834.25	4.20	3.25	13.07	844	3244.15	10490.08	430.17	17.314
384	<i>C. koidzumii</i>	18.12	7.87	330.19	2.21	2.12	2.91	303	3348.47	10311.30	148.01	5.957
385	<i>H. minor</i>	35.84	16.50	2873.80	3.88	3.50	14.00	907	1506.89	10610.76	768.83	30.944
386	<i>C. koidzumii</i>	20.34	12.48	933.33	4.06	4.88	28.50	1303	4815.00	10553.80	444.08	17.873
387	<i>H. minor</i>	37.92	19.96	4451.51	8.17	9.73	227.86	5010	4540.73	10699.46	1864.90	75.059
388	<i>H. minor</i>	35.37	19.08	3791.67	6.48	7.35	103.09	3087	3563.55	10384.02	1433.86	57.711
389	<i>H. minor</i>	35.43	18.23	3468.64	4.26	8.36	87.79	2375	3099.10	10192.61	1227.99	49.425
390	<i>H. hartmanni</i>	62.82	51.25	48603.94	10.18	14.13	598.36	9494	3240.82	10297.45	4766.70	191.852
391	<i>H. minor</i>	50.16	17.41	4479.28	4.98	5.95	51.82	2032	3335.27	10492.19	1011.40	40.707
392	<i>H. hartmanni</i>	72.58	46.08	45388.24	8.38	12.61	392.23	6487	4290.87	10910.01	2629.05	105.815
393	<i>P. grassii</i>	181.41	16.34	38042.13	5.76	8.88	133.84	4110	2453.51	10220.64	2546.93	102.510
394	<i>C. leidyi</i>	34.18	17.16	5266.25	4.88	3.59	18.55	1193	6001.36	10087.58	269.07	10.829
395	<i>C. koidzumii</i>	12.94	9.66	355.60	2.04	2.15	2.78	301	4485.80	10078.93	105.82	4.259
396	<i>C. koidzumii</i>	20.25	18.70	2085.03	3.49	5.93	36.23	1313	6013.60	10078.93	294.48	11.852
397	<i>H. minor</i>	61.60	32.03	18615.04	5.00	5.49	44.42	1795	4153.95	10041.26	688.07	27.694
398	<i>H. minor</i>	41.98	34.51	14727.15	4.70	7.94	87.33	2511	4514.98	10214.74	890.33	35.834
399	<i>H. minor</i>	45.14	18.95	4775.03	4.56	4.86	31.65	1487	6063.24	9587.76	295.93	11.911
400	<i>C. koidzumii</i>	36.52	30.53	10022.48	4.64	6.47	57.23	1998	6812.81	9399.58	279.28	11.241
401	<i>H. minor</i>	60.28	30.81	16849.74	8.55	4.29	46.28	2319	5023.64	10599.87	752.01	30.267
402	<i>C. koidzumii</i>	11.39	6.95	162.03	3.20	2.30	4.99	476	3461.88	9538.99	209.53	8.433
403	<i>P. grassii</i>	225.00	25.25	112648.75	11.01	9.54	295.25	6901	4319.34	9538.99	2374.53	95.571
404	<i>C. leidyi</i>	40.17	22.92	11047.66	4.24	4.22	22.22	1151	4688.27	10805.03	417.37	16.798
405	<i>C. koidzumii</i>	30.93	24.52	5478.07	4.16	5.85	41.84	1496	4781.60	10267.85	496.53	19.985

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
406	<i>C. leidyi</i>	NA	NA	NA	6.65	5.37	56.40	2236	5111.98	10267.85	677.26	27.259
407	<i>H. minor</i>	39.23	23.89	6597.06	4.88	7.63	83.50	2376	6447.46	11264.60	575.77	23.174
408	<i>C. leidyi</i>	27.94	12.43	2261.11	5.22	5.10	40.01	1765	6047.36	9530.32	348.66	14.033
409	<i>C. leidyi</i>	12.77	6.97	325.18	2.16	2.47	3.88	351	6674.52	10152.95	63.94	2.574
410	<i>H. minor</i>	40.20	18.83	4197.16	6.50	5.61	60.27	2429	5783.71	9954.57	572.80	23.054
411	<i>C. koidzumii</i>	22.17	17.45	1987.42	3.54	4.72	23.28	1089	5136.73	10623.41	343.66	13.832
412	<i>C. koidzumii</i>	26.52	17.09	2280.47	4.51	4.79	30.54	1387	6257.26	11634.94	373.63	15.038
413	<i>H. hartmanni</i>	54.50	33.05	17532.76	6.68	9.50	177.66	4120	4703.85	10191.23	1383.38	55.679
414	<i>H. minor</i>	44.05	19.45	4909.93	5.00	5.31	41.52	1687	4479.69	10200.63	602.90	24.266
415	<i>H. minor</i>	38.96	27.92	8944.17	4.33	8.28	87.35	2478	5864.47	9485.17	517.45	20.826
416	<i>H. minor</i>	43.13	26.38	8842.64	5.10	3.94	23.31	1275	4883.43	9576.64	372.92	15.010
417	<i>H. minor</i>	37.72	26.64	7885.28	5.17	4.34	28.61	1440	5037.05	9593.31	402.90	16.216
418	<i>H. minor</i>	NA	NA	NA	8.26	11.47	319.76	4704	6965.13	11646.93	1050.32	42.274
419	<i>P. grassii</i>	162.27	19.06	46313.82	13.11	12.02	557.68	10146	3441.14	9744.25	4586.47	184.598
420	<i>H. hartmanni</i>	72.32	40.81	35473.50	8.66	14.06	504.03	7845	5355.14	10662.19	2346.25	94.433
421	<i>C. leidyi</i>	19.15	7.92	628.15	2.66	2.62	5.38	447	4177.13	10662.19	181.91	7.322
422	<i>C. koidzumii</i>	15.51	11.92	649.01	3.47	3.16	10.21	710	4970.51	10662.19	235.33	9.472
423	<i>C. koidzumii</i>	20.94	17.97	1991.53	3.22	4.41	18.44	857	4561.69	10660.02	315.92	12.715
424	<i>C. koidzumii</i>	7.35	6.32	86.48	1.85	2.01	2.19	237	3949.11	10660.02	102.21	4.114
425	<i>C. leidyi</i>	15.40	9.67	754.72	3.00	2.92	7.52	582	4466.50	10660.02	219.87	8.850
426	<i>C. koidzumii</i>	9.33	7.04	136.17	2.12	2.45	3.73	346	4345.14	10660.02	134.85	5.428
427	<i>C. leidyi</i>	11.71	8.07	399.40	1.77	2.47	3.19	307	4131.10	10009.99	118.00	4.749
428	<i>C. koidzumii</i>	9.70	7.30	152.24	2.06	2.28	3.14	349	4851.00	10009.99	109.80	4.419
429	<i>C. leidyi</i>	15.60	9.20	690.60	2.49	2.35	4.04	399	4573.70	10009.99	135.73	5.463
430	<i>H. minor</i>	47.81	24.14	8206.19	8.54	7.97	159.58	4513	4484.12	9346.17	1439.45	57.936
431	<i>H. minor</i>	52.61	34.42	18357.07	7.42	12.03	316.29	5889	4502.29	10001.65	2041.35	82.161
432	<i>C. leidyi</i>	25.99	16.74	3812.83	3.79	2.74	8.37	705	3836.87	10001.65	293.35	11.807

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
433	<i>C. leidyi</i>	26.66	15.87	3514.11	3.71	3.20	11.20	730	3110.64	10039.89	371.48	14.952
434	<i>C. leidyi</i>	15.74	9.83	796.90	3.33	3.34	10.91	707	3390.59	10039.89	333.32	13.416
435	<i>H. minor</i>	53.90	25.38	10225.75	4.90	6.79	66.55	2305	3904.44	10235.61	964.77	38.830
436	<i>P. grassii</i>	165.79	21.44	59855.72	10.93	8.49	231.96	5986	3469.13	10235.76	2812.82	113.212
437	<i>C. leidyi</i>	14.63	11.79	1065.02	2.19	4.01	10.38	615	3836.88	10682.46	273.49	11.007
438	<i>P. grassii</i>	161.39	20.72	54408.60	12.55	12.97	621.98	10319	3657.18	10707.67	4814.32	193.769
439	<i>H. minor</i>	58.21	36.14	22391.17	7.53	7.95	140.11	4074	3737.94	10032.20	1746.78	70.305
440	<i>H. minor</i>	56.69	27.70	12813.44	5.26	7.16	79.40	2500	4831.25	10319.98	824.05	33.167
441	<i>H. minor</i>	44.64	29.79	11666.42	7.19	5.95	74.99	2806	5497.15	9586.04	677.65	27.275
442	<i>C. koidzumii</i>	19.93	10.74	676.79	3.15	3.34	10.34	712	5184.80	9585.69	190.03	7.648
443	<i>C. leidyi</i>	18.54	11.95	1386.95	2.90	2.77	6.54	526	4969.44	10158.90	163.34	6.574
444	<i>C. koidzumii</i>	17.24	11.40	659.38	2.08	2.21	2.99	293	4577.63	9581.96	94.00	3.783
445	<i>H. minor</i>	44.33	28.26	10427.09	3.98	4.63	25.12	1202	6240.55	9440.39	216.08	8.697
446	<i>C. koidzumii</i>	21.78	21.20	2881.55	5.81	3.53	21.28	1379	4526.78	9573.51	448.56	18.054
447	<i>C. koidzumii</i>	31.50	21.55	4308.36	3.14	4.95	22.65	1061	4986.45	10731.92	353.19	14.215
448	<i>C. leidyi</i>	35.85	17.46	5725.00	3.64	3.88	16.14	937	5913.31	10464.04	232.25	9.348
449	<i>H. minor</i>	44.69	31.89	13387.54	4.29	6.81	58.61	1823	5143.40	9554.93	490.35	19.736
450	<i>P. grassii</i>	225.00	25.11	111394.06	10.89	13.54	588.02	9601	4599.35	10290.78	3357.97	135.153
451	<i>C. koidzumii</i>	34.71	20.57	4327.64	4.48	6.79	60.72	2019	4783.72	10000.10	646.56	26.023
452	<i>C. leidyi</i>	14.15	9.93	730.35	2.63	2.38	4.39	420	4147.65	10739.40	173.53	6.985
453	<i>H. minor</i>	39.47	23.82	6593.48	6.51	8.50	138.59	3595	3016.88	10091.46	1885.20	75.876
454	<i>C. leidyi</i>	20.95	9.58	1005.86	2.65	2.67	5.56	464	3437.36	9609.05	207.16	8.338
455	<i>H. minor</i>	39.66	26.00	7896.64	3.51	5.95	36.60	1373	2676.48	9838.81	776.26	31.243
456	<i>C. leidyi</i>	22.42	9.18	988.84	4.26	2.69	9.07	783	4055.76	9537.52	290.78	11.703
457	<i>H. minor</i>	32.62	20.34	3973.67	3.36	5.07	25.38	1109	4036.65	9715.74	423.03	17.026
458	<i>C. leidyi</i>	23.27	12.30	1844.18	3.43	3.43	11.85	750	3381.52	9547.21	338.07	13.607
459	<i>H. hartmanni</i>	71.87	35.96	27365.63	5.60	7.11	83.33	2858	3354.48	9525.04	1295.37	52.136

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
460	H. minor	42.32	17.09	3639.74	4.69	4.69	30.29	1402	4526.73	9612.36	458.52	18.455
461	C. leidyi	31.53	20.92	7225.59	3.70	3.70	14.88	873	4061.76	9524.31	323.11	13.005
462	C. koidzumii	24.25	22.21	3521.44	2.32	5.21	18.60	726	3055.02	9842.50	368.87	14.847
463	H. minor	38.59	19.79	4452.76	5.18	5.87	52.58	1905	3652.27	10925.94	906.58	36.488
464	H. hartmanni	63.60	39.99	29951.40	12.09	15.08	810.02	10605	4063.67	10356.11	4308.58	173.414
465	H. hartmanni	50.09	30.74	13942.79	3.47	4.91	24.58	1119	13.76	3190.12	2646.65	106.523
466	H. hartmanni	NA	NA	NA	8.34	11.00	297.23	6471	2862.27	10816.20	3736.12	150.373
467	C. koidzumii	15.70	15.84	1159.68	3.17	3.45	11.10	806	3956.74	9548.91	308.39	12.412
468	C. koidzumii	21.10	15.46	1486.17	3.69	3.80	15.66	1012	4871.58	9548.91	295.79	11.905
469	C. koidzumii	15.94	18.05	1528.66	3.50	4.69	22.65	1048	4660.21	9260.51	312.54	12.579
470	H. minor	35.64	17.71	3294.04	6.68	6.41	80.81	2739	4703.61	9510.39	837.50	33.708
471	C. leidyi	32.58	16.17	4459.80	3.57	3.00	9.47	704	3542.15	9508.44	301.91	12.151
472	H. minor	55.22	37.81	23245.86	5.27	5.27	43.01	1771	4675.59	9843.39	572.58	23.045
473	H. minor	64.96	19.49	7270.06	8.92	8.92	209.10	5083	3764.16	10163.43	2192.67	88.251
474	P. grassii	133.01	22.33	52072.58	8.76	7.34	138.89	4363	2842.15	9595.94	2305.59	92.796
475	P. grassii	148.33	22.20	57405.74	9.48	8.32	193.32	4927	1733.74	10107.11	3772.32	151.830
476	H. minor	45.85	29.44	11704.27	2.88	5.23	23.24	983	2903.27	9505.50	506.33	20.379
477	C. leidyi	27.38	18.57	4941.79	3.64	3.64	14.19	846	4050.15	10125.12	336.65	13.550
478	H. minor	40.25	26.27	8178.93	6.15	6.37	73.59	30	3169.00	9561.51	14.63	0.589
479	C. koidzumii	22.60	12.66	1066.35	1.86	2.27	2.82	272	4060.57	10796.30	115.51	4.649
480	H. minor	38.43	25.79	7526.69	3.72	9.94	108.33	2563	3204.46	10796.30	1352.03	54.417
481	H. hartmanni	78.79	38.76	34863.07	7.46	11.86	308.95	5514	3602.17	10396.52	2538.23	102.160
482	H. minor	40.41	38.03	17216.81	8.32	11.25	309.89	631	2013.50	10025.25	439.90	17.705
483	P. grassii	134.22	22.41	52944.78	9.28	6.81	126.76	4194	2890.72	9872.58	2237.18	90.043
484	H. minor	44.47	20.47	5486.23	4.25	3.68	16.99	1040	2129.51	9542.45	677.44	27.266
485	C. leidyi	35.50	21.14	8309.22	3.33	3.33	10.83	706	4190.39	10019.23	267.28	10.757
486	C. koidzumii	13.05	7.13	195.48	1.96	1.96	2.23	246	3374.43	10019.23	116.27	4.680

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
487	H. minor	32.23	15.16	2182.22	6.60	6.60	84.56	2780	4297.35	9490.11	956.52	38.498
488	C. koidzumii	28.82	19.89	3357.69	4.18	4.18	21.54	1117	3811.68	9490.11	442.51	17.810
489	H. minor	41.67	25.01	7677.39	5.07	7.67	87.92	2336	3417.01	9510.78	1038.52	41.799
490	H. minor	57.84	31.37	16768.02	7.29	7.29	113.96	3392	4502.86	9984.68	1173.11	47.216
491	P. grassii	NA	NA	NA	9.87	9.87	282.84	6217	4087.83	10605.65	2574.12	103.604
492	H. minor	47.30	19.11	5087.17	3.99	7.21	61.09	1813	3844.56	10062.73	757.60	30.492
493	P. grassii	NA	NA	NA	12.69	9.94	369.32	7955	4832.46	9848.92	2459.85	99.005
494	H. hartmanni	90.07	44.65	52882.28	7.92	12.74	378.58	5902	4925.62	10048.97	1827.62	73.559
495	C. leidy	27.39	14.74	3116.42	3.09	3.09	8.71	611	4330.53	10215.27	227.73	9.166
496	C. leidy	20.11	9.43	935.90	2.89	2.25	4.32	444	3255.63	9098.56	198.17	7.976
497	C. koidzumii	30.60	16.07	2325.96	4.57	2.61	9.19	782	4346.68	9534.53	266.77	10.737
498	H. minor	41.24	26.04	8238.83	4.87	6.13	53.91	2442	4755.19	11120.22	900.97	36.262
499	C. koidzumii	16.42	7.61	280.34	2.16	2.16	2.97	298	4404.72	10813.54	116.23	4.678
500	H. minor	39.70	28.32	9377.69	4.51	4.51	26.95	1297	3510.06	10413.72	612.56	24.655
501	C. leidy	44.76	17.79	7417.22	3.81	3.81	16.29	927	5891.35	11358.62	264.30	10.638
502	H. minor	52.00	19.00	5529.61	4.66	4.66	29.84	1388	4954.02	9459.37	389.90	15.693
503	P. grassii	NA	NA	NA	18.10	14.69	1150.26	16334	4472.15	9449.60	5306.87	213.593
504	C. koidzumii	20.19	13.97	1160.40	3.91	3.91	17.65	978	3603.17	10808.59	466.59	18.779
505	C. koidzumii	9.05	6.60	116.26	1.96	1.96	2.23	246	3648.07	10808.59	116.04	4.670
506	C. koidzumii	16.92	11.25	630.48	3.32	3.32	10.73	702	3053.28	10081.06	364.16	14.657
507	H. minor	43.65	26.60	9095.96	3.21	5.24	25.97	1151	2958.92	10312.26	624.10	25.119
508	H. minor	39.49	31.33	11413.49	8.74	8.74	196.43	4876	4081.97	10054.15	1908.83	76.828
509	C. leidy	37.57	15.07	4466.93	3.32	3.32	10.78	704	3194.37	10108.11	352.20	14.176
510	C. koidzumii	34.12	19.90	3981.40	2.43	4.16	12.35	692	3471.00	10108.11	321.24	12.929
511	H. minor	41.78	31.65	12323.71	6.66	13.25	344.32	5728	2702.59	10871.88	3462.72	139.369
512	C. koidzumii	25.67	17.05	2197.41	5.16	6.59	65.94	2017	3642.93	10871.88	957.78	38.549
513	H. minor	52.67	41.15	26265.05	5.36	8.22	106.48	2888	3276.01	9794.93	1373.70	55.289

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
514	C. leidyi	43.54	27.21	16883.47	4.34	4.34	24.01	1201	3709.37	10785.52	556.71	22.407
515	H. minor	59.04	39.39	26977.11	4.09	9.40	106.51	2450	3511.50	10741.39	1189.64	47.881
516	C. koidzumii	33.87	24.31	5896.59	4.22	5.16	33.02	1377	2959.03	10350.13	748.81	30.138
517	H. minor	52.66	46.63	33716.92	8.74	5.18	69.02	2927	4174.24	9545.00	1051.37	42.316
518	H. minor	49.17	19.69	5611.67	3.84	3.75	15.92	1012	3949.60	10920.76	447.00	17.991
519	H. minor	74.27	43.61	41597.24	8.89	11.30	333.95	6549	3678.15	10251.81	2915.42	117.341
520	H. minor	50.89	28.57	12231.59	4.42	5.68	41.96	1756	2944.57	10400.63	962.35	38.733
521	H. minor	33.30	22.24	4852.35	5.59	6.35	66.43	2276	4076.89	10779.79	961.11	38.683
522	C. koidzumii	9.62	7.63	165.01	1.65	1.65	1.33	174	4062.04	10779.79	73.75	2.968
523	P. grassii	141.77	24.21	65280.53	9.57	8.08	183.82	4918	2183.14	9779.88	3202.86	128.910
524	P. grassii	161.90	14.60	27108.61	7.59	6.99	109.35	3544	3050.56	10681.21	1928.79	77.631
525	P. grassii	188.61	26.07	100684.60	10.09	12.10	434.78	8104	2849.63	9972.79	4408.81	177.448
526	P. grassii	124.13	19.69	37807.67	8.78	7.92	162.38	4788	2791.65	10490.68	2752.82	110.797
527	P. grassii	275.00	27.16	159300.87	10.45	10.98	370.99	7398	3299.18	9188.02	3290.75	132.447
528	P. grassii	300.00	33.68	267241.39	12.79	14.99	846.57	12256	3170.26	9486.14	5833.78	234.801
529	C. leidyi	50.63	30.51	24681.29	4.44	5.65	41.68	1685	4250.85	10467.14	659.43	26.541
530	P. grassii	NA	NA	NA	10.73	7.04	156.69	4770	3326.51	10467.14	2374.69	95.578
531	P. grassii	284.00	28.13	176476.11	11.72	5.74	113.73	5137	3034.00	9792.05	2614.01	105.210
532	P. grassii	NA	NA	NA	12.63	11.86	523.10	9370	3946.04	9647.67	3638.00	146.424
533	P. grassii	147.90	26.78	83281.68	11.55	7.36	184.16	5669	2854.71	9305.12	2909.10	117.087
534	P. grassii	NA	NA	NA	13.21	10.46	425.18	9365	3842.98	10122.93	3939.27	158.550
535	C. leidyi	46.27	23.02	12841.69	5.53	5.22	44.48	1827	4514.35	10122.93	640.75	25.789
536	H. minor	54.45	23.49	8850.75	3.76	4.51	22.55	1039	7478.28	10512.99	153.69	6.186
537	H. minor	44.80	27.45	9944.12	3.77	3.60	14.40	1016	5874.33	10043.88	236.67	9.526
538	H. hartmanni	69.52	37.33	28536.43	5.99	5.99	63.24	2290	6229.57	10188.53	489.27	19.692
539	H. minor	34.96	29.42	8909.98	4.67	6.32	54.94	1928	4436.51	10037.97	683.68	27.517
540	H. minor	37.10	32.76	11725.46	8.97	4.53	54.31	2968	4163.33	9961.23	1124.49	45.259

Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
541	<i>P. grassii</i>	NA	NA	NA	9.05	11.10	328.40	5882	4285.67	10638.86	2322.66	93.484
542	<i>H. minor</i>	54.40	34.39	18951.47	5.20	8.37	107.36	2645	4064.37	10019.30	1036.43	41.715
543	<i>H. minor</i>	57.56	20.56	7167.83	5.56	5.28	45.58	1971	5181.50	8824.80	455.79	18.345
544	<i>H. minor</i>	59.27	23.03	9254.89	5.69	5.69	54.14	2065	5231.65	8964.54	482.98	19.439
545	<i>C. leidyi</i>	37.61	19.08	7166.00	4.70	4.34	26.06	1358	4243.95	9669.92	485.69	19.548
546	<i>C. leidyi</i>	32.15	17.58	5204.23	5.01	2.79	11.51	949	6510.82	10620.29	201.66	8.117
547	<i>P. grassii</i>	91.90	47.89	165562.85	11.25	10.56	369.55	7949	3671.26	9486.96	3277.46	131.913
548	<i>C. koidzumii</i>	20.89	15.00	1384.57	2.97	3.60	11.34	696	5081.77	10343.81	214.83	8.647
549	<i>H. minor</i>	33.08	17.39	2947.02	4.04	2.79	9.27	725	3584.39	10489.78	338.10	13.608
550	<i>H. minor</i>	44.94	19.34	4951.44	3.78	3.78	15.84	910	5308.74	9993.51	250.00	10.062
551	<i>H. minor</i>	44.69	43.37	24762.24	4.52	7.00	65.22	2017	6718.09	10987.66	430.95	17.345
552	<i>H. minor</i>	49.75	39.10	22399.42	6.10	5.28	50.19	2016	6460.69	9625.82	349.09	14.050
553	<i>H. hartmanni</i>	63.31	36.75	25175.57	5.82	9.60	157.82	3642	4780.10	9764.83	1129.85	45.475
554	<i>C. koidzumii</i>	17.15	11.47	664.80	2.67	2.97	6.94	498	3074.54	9764.83	249.94	10.060
555	<i>H. hartmanni</i>	68.99	52.17	55299.03	7.09	10.99	252.27	5066	4022.94	10014.52	2006.58	80.762
556	<i>C. leidyi</i>	49.52	22.09	12652.08	4.04	3.56	15.09	1046	5061.46	9905.41	305.01	12.276
557	<i>P. grassii</i>	NA	NA	NA	11.17	11.18	411.02	8171	3548.21	9939.65	3655.39	147.124
558	<i>C. leidyi</i>	28.53	11.54	1990.04	3.83	3.04	10.43	764	4404.84	10474.88	287.43	11.569
559	<i>H. minor</i>	30.38	27.56	6795.14	3.14	6.33	37.11	1277	4970.23	9939.65	384.37	15.470
560	<i>C. koidzumii</i>	15.25	10.63	507.53	3.94	3.94	17.97	1023	3208.62	10474.88	525.65	21.157
561	<i>H. minor</i>	53.34	34.95	19185.68	7.44	10.38	236.22	5089	4967.05	9566.95	1448.71	58.308
562	<i>P. grassii</i>	153.13	26.65	85389.18	10.66	10.00	313.69	6768	4067.56	9949.38	2629.11	105.818
563	<i>H. hartmanni</i>	77.53	51.31	60119.83	11.35	12.69	537.98	9743	4880.54	10232.12	3132.36	126.072
564	<i>H. minor</i>	34.72	24.09	5931.88	4.68	6.07	50.73	1802	5019.91	9887.75	530.51	21.352
565	<i>C. leidyi</i>	28.20	18.92	5282.19	2.72	3.58	10.26	625	4720.57	9430.51	187.84	7.560
566	<i>P. grassii</i>	NA	NA	NA	9.76	11.23	362.69	7379	3173.29	9430.51	3490.45	140.485
567	<i>H. minor</i>	34.24	21.68	4738.22	3.80	3.97	17.63	921	3946.10	10130.96	377.13	15.179



Cell #	Protist Species	Cell Length (µm)	Cell Width (µm)	Cell Vol. (µm <sup>3</sup> )	Nuc Length (µm)	Nuc Width (µm)	Nucleus Vol. (µm <sup>3</sup> )	Nucleus Area (Pixels <sup>2</sup> )	IF	IB	IOD <sub>u</sub>	C-value (pg)
568	<i>C. koidzumii</i>	14.76	10.32	462.50	1.43	1.68	1.19	167	3622.83	10130.96	74.58	3.002
569	<i>C. leidyi</i>	26.77	12.87	2321.34	3.53	3.12	10.10	746	4960.88	10130.96	231.33	9.311
570	<i>C. koidzumii</i>	14.24	9.68	392.67	2.26	2.57	4.38	369	4988.85	10130.96	113.52	4.569
571	<i>H. minor</i>	55.10	34.23	19018.17	7.54	11.92	315.42	5875	5239.05	10605.78	1799.45	72.425
572	<i>H. minor</i>	46.52	42.25	24451.42	5.49	9.28	139.03	3220	6349.17	10522.63	706.49	28.435
573	<i>P. grassii</i>	NA	NA	NA	9.52	9.19	236.91	6046	3246.20	9660.77	2863.61	115.256
574	<i>H. minor</i>	37.89	15.58	2709.38	3.27	3.27	10.31	703	4804.47	10569.94	240.73	9.689
575	<i>H. minor</i>	35.68	17.82	3335.45	4.51	4.84	31.20	1481	3681.59	10083.41	648.04	26.083
576	<i>P. grassii</i>	183.00	21.55	66729.00	11.10	9.95	323.71	6792	3544.12	10110.70	3092.22	124.457
577	<i>C. leidyi</i>	33.58	18.64	6106.68	2.74	3.62	10.61	697	4924.24	10076.12	216.73	8.723
578	<i>H. minor</i>	37.78	30.03	10033.92	3.19	5.78	31.32	1213	4759.51	9466.04	362.21	14.578
579	<i>H. minor</i>	54.30	34.25	18756.42	7.45	9.50	198.08	4363	5228.66	10634.75	1345.27	54.145
580	<i>P. grassii</i>	NA	NA	NA	12.20	8.07	234.16	6746	4762.23	10634.75	2353.79	94.737
581	<i>P. grassii</i>	193.00	23.97	87122.07	9.54	7.70	166.50	4536	3839.43	10887.98	2053.36	82.645
582	<i>H. hartmanni</i>	56.96	26.97	12205.60	3.49	5.02	25.93	1161	3332.45	10624.97	584.64	23.531
583	<i>H. minor</i>	42.77	16.96	3621.91	4.30	4.30	23.35	1179	5622.17	9639.88	276.08	11.112
584	<i>C. koidzumii</i>	25.74	20.22	3099.14	4.28	3.18	12.71	950	4954.42	9640.61	274.66	11.054
585	<i>H. hartmanni</i>	63.28	31.22	18161.11	5.86	4.69	38.04	1857	5163.17	10072.64	538.95	21.692
586	<i>H. hartmanni</i>	66.61	26.97	14266.56	6.11	6.63	79.11	2476	4758.63	10310.49	831.44	33.464

APPENDIX B  
STANDARD DATA COLLECTED

<b>TENS</b>	<b>Nucleus Area (Pixels)</b>	<b>IF</b>	<b>IB</b>	<b>IOD<sub>s</sub></b>
1	881	7722.74	10988.16	134.93
2	868	7887.03	10987.10	124.97
3	916	7901.44	10991.06	131.29
4	985	8113.43	11020.74	131.01
5	681	7011.01	10951.77	131.91
6	666	6910.80	10954.33	133.24
7	688	7893.58	11024.89	99.83
8	603	8273.37	11597.41	88.45
9	521	7488.88	11570.63	98.44
10	645	8269.14	11559.72	93.84
11	640	8212.77	11605.59	96.11
12	571	7892.11	11603.71	95.59
13	573	7769.12	11616.86	100.11
14	665	5515.31	8780.47	134.30
15	616	5301.05	8783.32	135.09
16	628	6411.84	10529.76	135.29
17	590	6294.06	10479.82	130.64
18	514	5598.46	10589.89	142.29
19	487	5355.18	10587.58	144.16
20	755	5699.32	10752.69	208.15
21	781	6009.39	10789.07	198.49
22	742	7464.14	11631.87	142.96
23	405	5257.28	11559.82	138.59
24	527	6289.48	11553.93	139.19
25	484	5869.37	11553.93	142.36
26	508	5970.73	11550.81	145.59
27	464	5647.30	11550.81	144.20
28	478	6081.10	11415.90	130.75
29	484	5413.14	11415.95	156.85
30	503	5741.83	11214.30	146.23
31	525	5745.40	11414.96	156.53
32	546	5840.67	11428.66	159.18
33	521	7108.10	11928.98	117.15
34	508	6202.16	11928.95	144.30
35	565	6904.39	11941.71	134.44
36	387	5530.73	11941.71	129.37
37	477	6296.54	11930.82	132.40
38	530	6010.94	11391.85	147.16
39	561	7017.81	11391.85	118.03
40	527	5990.45	11412.84	147.53
41	553	6245.36	11299.63	142.40
42	452	6214.10	12443.12	136.30

<b>TENS</b>	<b>Nucleus Area (Pixels)</b>	<b>IF</b>	<b>IB</b>	<b>IOD<sub>s</sub></b>
43	499	6035.67	12443.12	156.79
44	721	7969.04	12414.63	138.81
45	650	6872.01	11315.73	140.79
46	581	6229.99	11315.73	150.59
47	536	5941.45	11344.18	150.55
48	566	6364.96	11344.18	142.05
49	514	6146.39	11344.18	136.80
50	561	5697.17	11270.29	166.21
51	569	6415.97	11270.29	139.22
52	560	6414.93	11380.42	139.42
53	511	5925.58	11380.42	144.83
54	570	7177.90	11380.42	114.09
55	418	5263.55	11338.98	139.32
56	573	6261.44	11386.99	148.83
57	448	5715.28	11396.01	134.27
58	463	5591.91	11398.62	143.20
59	383	4776.75	10767.05	135.19
60	532	5453.00	10767.05	157.19
61	448	5292.57	10767.05	138.18
62	400	4829.36	10682.79	137.92
63	439	5028.88	10682.79	143.65
64	374	4475.14	10682.79	141.33
65	649	6449.35	10725.35	143.36
66	763	6914.28	10728.01	145.56
67	665	6435.06	10728.01	147.61
68	547	6284.06	10726.23	127.02
69	653	6660.55	10726.23	135.13
70	537	6489.56	11736.61	138.18
71	483	5783.47	11736.61	148.45
72	572	6256.83	11744.14	156.42
73	456	5701.67	11747.98	143.17
74	519	6679.22	11902.09	130.22
75	661	7426.24	11902.09	135.41
76	591	6999.23	11902.09	136.27
77	533	6982.77	11902.09	123.44
78	497	6486.32	11902.09	131.02
79	580	7173.77	11902.09	127.53
80	547	6981.29	11902.09	126.73
81	669	7817.61	11902.09	122.13
82	566	6805.25	11902.09	137.41
83	675	7779.90	11444.38	113.14
84	574	7877.59	11444.38	93.10

<b>TENS</b>	<b>Nucleus Area (Pixels)</b>	<b>IF</b>	<b>IB</b>	<b>IOD<sub>s</sub></b>
85	527	7306.81	11444.38	102.69
86	542	5811.74	11162.94	153.64
87	534	6556.79	11162.94	123.40
88	447	6092.73	11162.94	117.55
89	547	6348.70	11162.94	134.07
90	551	6205.95	11162.94	140.49
91	426	3853.08	6275.18	90.23
92	408	3871.25	6277.89	85.66
93	571	4544.10	6718.57	96.97
94	434	4390.34	6720.68	80.25
95	625	3968.26	5708.25	98.69
96	618	3675.98	5773.20	121.16
97	401	3113.46	4573.10	66.95
98	410	2833.08	4563.66	84.89
99	444	3194.56	4563.39	68.77
100	483	3820.87	5838.25	88.93
101	466	3425.61	5830.19	107.62
102	449	2428.34	3824.53	88.57
103	422	2186.97	3842.26	103.28
104	389	2283.94	3842.40	87.88
105	406	2385.86	3839.94	83.91
			<b>AVERAGE</b>	<b>129.20</b>