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Unusual features of non-dividing somatic macronuclei in the ciliate class Karyorelictea

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Abstract

Genome structure and nuclear organization have been intensely studied in model ciliates such as *Tetrahymena* and *Paramecium*, yet few studies have focused on nuclear features of other ciliate clades including the class Karyorelictea. In most ciliates, both the somatic macronuclei and germline micronuclei divide during cell division and macronuclear development only occurs after conjugation. However, the macronuclei of Karyorelictea are non-dividing, i.e. division minus (Div –) and develop anew from micronuclei during each asexual division. As macronuclei age within Karyorelictea, they undergo changes in morphology and DNA content until they are eventually degraded and replaced by newly developed macronuclei. No less than two macronuclei and one micronucleus are present in karyorelictid species, which suggests that a mature macronucleus 1) might be needed to sustain the cell while a new macronucleus is developing and 2) likely plays a role in guiding the development of the new macronucleus. Here we use a phylogenetic framework to compile information on the morphology and development of nuclei in Karyorelictea, largely relying on the work of Dr. Igor Raikov (1932–1998). We synthesize data to speculate on the functional implications of key features of Karyorelictea including the presence of at least two macronuclei in each cell and the inability for macronuclei to divide.

Keywords

nuclear architecture; Karyorelictea; non-dividing macronuclei

Introduction

Ciliates are a clade of single-celled eukaryotes defined by the presence of cilia and dimorphic nuclei (Kovaleva and Raikov, 1992; Prescott, 1994; Raikov, 1982; Raikov, 1985). Numerous key discoveries have been made through studies of ciliates, including the discovery of self-splicing RNA (Cech, 1986; Kruger et al., 1982) and telomeres/telomerases

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(Greider and Blackburn, 1985). Yet the bulk of the work on ciliates has predominately focused on only a few lineages, such as *Paramecium* and *Tetrahymena* (Class Oligohymenophorea; Aury et al., 2006; Chalker, 2008; Eisen et al., 2006; Mochizuki and Gorovsky, 2004; Yao, 2008) and *Oxytricha* and *Stylonychia* (Class Spirotrichea; Chen et al., 2014; Nowacki et al., 2008).

As described in detail below, the monophyletic class Karyorelictea, the focus of this manuscript, appears unique among ciliates in that their somatic macronuclei cannot divide (Table 1; Raikov, 1982). Instead, Karyorelictea differentiate new macronuclei from micronuclei with each cell division and an individual macronucleus only persists for a few generations before degrading (Table 1; Raikov, 1985). In all other ciliates, the macronuclei divide by amitosis (i.e. dividing without centromeres and metaphase plates) during vegetative growth and only differentiate from zygotic nuclei following conjugation (e.g. Gao et al., 2016; Raikov, 1982). Throughout the remainder of this manuscript, we use the abbreviation Div– to describe the absence of macronuclear division in Karyorelictea; all remaining classes of ciliates are Div+ as they contain macronuclei that divide through amitosis (Table 1).

The class Karyorelictea is composed of ~170 ciliate morphospecies divided into six families (Loxodidae, Trachelocercidae, Kentrophoridae, Geleidae, Cryptopharyngidae and Wilbertomorphidae; Fig. 1; Lynn, 2008). However, extremely limited molecular data are available in karyorelictids; for example, as we write this manuscript, only eight protein-coding genes have been released on GenBank for the class Karyorelictea. The current phylogeny of Karyorelictea has largely relied on small subunit rDNA sequences (e.g. Andreoli et al., 2009; Campello-Nunes et al., 2015; Gao et al., 2016; Xu et al., 2015; Yan et al., 2016), with the exception of the family Cryptopharyngidae for which there are currently no molecular data. Phylogenetic analyses indicate that both the class Karyorelictea and its five families with molecular data are monophyletic and fall sister to the class Heterotrichea, the other member of the subphylum Postciliodesmatophora (Campello-Nunes et al., 2015; Gao and Katz, 2014; Gao et al., 2016; Xu et al., 2015; Yan et al., 2016). Most karyorelictids are specialized to interstitial marine environments such as sand, and recently they were also found to be epibionts of benthic harpacticoid copepods in the deep sea (Sedlacek et al., 2013); the one exception is the genus *Loxodes*, which is found in low oxygen freshwater environments (Fenchel, 1967, 1968; Finlay and Fenchel, 1986). Additionally, while none of the rest of Karyorelictea are reported as cultivable, the genus *Loxodes* has been cultivated successfully in a few labs. (e.g. Bobyleva, 1981; Buonanno et al., 2005; Finlay et al., 1986), likely accounting for the limited data on this clade.

Here we describe some unusual features within the class Karyorelictea, focusing on their somatic macronuclei. We divide the manuscript into three parts: 1) a description of the contributions from Dr. Igor Raikov, who generated the bulk of data on members of the class Karyorelictea; 2) insights into the cell biology of the Karyorelictea based on microscopic analyses; and 3) knowledge of life cycles, and particularly macronuclear development, in diverse lineages within the class Karyorelictea. Finally, we end with hypotheses on the evolutionary significance of Div– macronuclei.

Raikov and Karyorelictea

Dr. Igor B. Raikov (1932–1998) was the leading researcher in the study of Karyorelictea, and most information about these ciliates stems from his work and the work of his collaborators. Raikov characterized in detail the fine structure of nuclei for more than 20 karyorelictid species, including representatives from four of the six families in Karyorelictea (e.g. Kovaleva and Raikov, 1992; Raikov, 1982; Raikov, 1985; Raikov and Karadzhan, 1985; Raikov and Kovaleva, 1990). Using an impulse cytofluorimeter, Raikov isolated individual nuclei and measured their DNA content. From these data on the morphology and DNA content of karyorelictid nuclei, Raikov and his collaborator, Dr. Valentine G. Kovaleva, described the Div– macronuclei as “paradiploid”, implying that mature macronuclear are nearly diploid and similar to micronuclei in DNA content (Kovaleva and Raikov, 1978; Ovchinnikova et al., 1965), which is unusual as the macronuclei in other ciliate species can be highly polyploid (for example 45 in *Tetrahymena*, 800 in *Paramecium* and ~13150 in *Spirostomum*; Duret et al., 2008; Ovchinnikova et al., 1965; Woodard et al., 1972). Raikov’s many other contributions are highlighted in some of the work we review below.

Part I: Nuclear structure

Insights from Light Microscopy

As in all other ciliates, karyorelictids have both somatic macronuclei and germline micronuclei in every individual cell. The majority of macronuclei and micronuclei in Karyorelictea are spherical or elliptical and are usually 5–10 µm (macronuclei) and 2– 4 µm (micronuclei) in diameter or length (Raikov, 1985). Karyorelictean ciliates with only one macronucleus have not yet been found, while this arrangement of nuclei is common in all other ciliate classes (Lynn, 2008; Raikov, 1982). This suggests that each karyorelictean cell requires a minimum of two macronuclei and one micronucleus to survive (Andreoli et al., 2009; Lynn, 2008; Raikov, 1982).

Nuclei in karyorelictids can be distributed throughout the cell body or clustered together as “nuclear groups” or “nuclear complexes” (Fig. 1). Nuclear groups can either be nuclei enclosed by a fused membrane or nuclei that are closely associated with one another. A unit of two macronuclei and one micronucleus is the smallest and most frequent grouping, though groups of four macronuclei and two micronuclei are also commonly described in karyorelictid species (Raikov 1994). Many karyorelictids possess multiple groups of either 2+1 or 4+2 units (e.g. *Tracheloraphis oligostriata* and *T. colubis*; Fig. 1; Al-Rasheid, 2001; Xu et al., 2011b). Free nuclei, which are common in other multinucleate ciliates, are also often found in multinucleate karyorelictids (e.g. *Kentrophoros flavus* and *K. gracilis*; Fig. 1), and in many cases, these free nuclei form a longitudinal line along the cell meridian (Fig. 1; Xu et al., 2011a).

In karyorelictids, nuclear grouping and number of nuclei may relate to body shape and size in some, but not all lineages (Table 2). In *Loxodes* and *Remanella* (Loxodidae), larger cells tend to have more macronuclei and micronuclei, but the same pattern is not found in Trachelocercidae (Table 2). For example, both *Trachelocerca sagitta* and *Trachelocerca stephani* have single nuclear groups with 4 macronuclei and 2 micronuclei; however, the size

of *T. sagitta* is ~5 fold that of *T. stephani* (Table 2). There is also considerable variation within species. For example, the length of *Prototrichelocerca fasciolata* varies between 1000 and 3000 μm with 10 to 24 macronuclei and 3 to 7 micronuclei, and *Loxodes magnus* can have 8 to 31 macronuclei and 5 to 32 micronuclei (Bobyleva et al., 1980).

Insights from Electron Microscopy

Fine structure analyses of karyorelictid nuclei reveal variability in compositions of macronuclei while micronuclei have more canonical eukaryotic features (Raikov, 1985, 1994). In contrast to other ciliates, the somatic macronuclei of Karyorelictea are relatively small, ranging from 5–10 μm in diameter (Raikov, 1982; Raikov, 1985). Karyorelictean macronuclei are also described as the “vesicular type” due to their shape, and they often include nucleoli, chromocenters (i.e. areas of condensed chromatin), and “nuclear bodies” (Raikov, 1985, 1989). Nucleoli in karyorelictids have a similar ultrastructure to other eukaryotes and are described as having a classical fibrillar core and granular cortex (Raikov, 1985). Nucleoli serve as the location for the generation of rRNAs (Raikov, 1982). The number of nucleoli varies among karyorelictid species from one to over 100 (Fig. 2A–D; Raikov, 1985).

Chromocenters and chromatin bodies are two types of densely staining regions of chromatin varying by size and number (Fig. 2A). Chromatin bodies are usually smaller and more numerous than chromocenters, and chromocenters may be formed by the fusion of chromatin bodies (Fig. 2A; Raikov, 1979, 1990; Raikov, 1994). Chromatin bodies may represent lowly expressed heterochromatin regions, but the functional implications of chromocenters are as yet unknown.

Another unusual aspect of the fine structure of some karyorelictean macronuclei is the presence of nuclear bodies, which are either classified as spheres or crystalloids (Fig. 2A, B; Table 3; Raikov, 1979, 1984, 1985, 1990). These nuclear bodies are of unknown function and are not present in all Karyorelictea: for example, they have not been described in the genus *Loxodes* (Table 3; Raikov, 1979, 1990). Crystalloids are typically composed of some type of proteinaceous filaments, packed tightly together and parallel, and are usually less electron dense than the fibrillar core of nucleoli (Raikov, 1979, 1985, 1990, 1994). In spheres, filaments are tangled and are also typically less electron dense than the fibrillar core of nucleoli (Raikov, 1990). No consistent association between nuclear bodies (spheres or crystalloids) and other nuclear structures has been found in karyorelictean macronuclei (Table 3; Raikov, 1985, 1990).

Like in other ciliates, no nucleoli nor evidence of RNA synthesis has been found in karyorelictean micronuclei during vegetative stages (Raikov, 1982). Compared to macronuclei, micronuclei stain intensely and homogeneously with Feulgen indicating that they are packed with condensed chromatin (Raikov, 1985). In *Remanella multinucleata* and *Geleia orbis*, small nuclear bodies that appear to be crystalloids are sometimes found in the micronuclear periphery (i.e. the space between the chromatin mass and the nuclear envelope; Kovaleva and Raikov, 1978; Raikov, 1979, 1984).

Part II: Development of Macronuclei

Nuclear reorganization

The common ancestor of Karyorelictea evolved a mechanism to segregate nuclei between generations by differentiating at least one new macronucleus from a micronucleus with each division (Orias, 1991a). This contrasts with all other ciliates in which macronuclei divide through amitosis either with microtubules placed inside or outside the macronuclear envelope (subphylum Intramacronucleata and the class Heterotrichea, respectively; Lynn, 2008).

Division patterns of *Loxodes* and lineages with two other of the most common nuclear group types in karyorelictids exemplify the diversity of processes within this class. In the simplest case of two macronuclei and one micronucleus in each cell (e.g. *Loxodes rostrum*; Fig. 3A–D), the two parental macronuclei are separated into the daughter cells while the micronucleus undergoes mitosis twice (Fig. 3A–C) and two micronuclei from the second mitosis differentiate into new macronuclei (Fig. 3C–D; Raikov, 1957a). In species with two nuclear groups, each with one macronucleus and one micronucleus (e.g. *Loxodes striatus*), each micronucleus divides once (Fig. 3E–F), with one of the daughter micronuclei developing into the new macronucleus while the other divides again to reset micronuclear number of the daughter cells (Fig. 3G–H; Raikov, 1957a). In species with four macronuclei and two micronuclei in a nuclear group (e.g. *Tracheloraphis coluber*), each nuclear group is divided in half and then each half (two macronuclei and one micronucleus) undergoes similar developmental processes to form a complete nuclear group (four macronuclei and two micronuclei) after micronuclei divide twice (Fig. 3I–L; Raikov, 1969). Instead of generating a completely new nuclear group in one of the daughter cells, karyorelictean species that divide by the third method effectively decrease the age difference of nuclei between daughter cells, i.e. if a completely new nuclear group is generated and passed on to one of the daughter cells while the other one receives the parental nuclear group, there is a one-generation age difference between the offspring and this age difference would increase each division.

Development of macronuclei

Given the inability of karyorelictean macronuclei to divide (i.e they are Div–), nearly half of the macronuclei in each cell are newly differentiated from micronuclei following cell division. As described below, the general pattern of macronuclear development in karyorelictean species appears similar to other ciliates except that no evidence for genomic amplification during development has been recorded (Raikov, 1957a, 1959; Raikov, 1982). Macronuclear development begins with creation of vacuoles in the developing macronucleus (Fig. 4A), which is followed by the formation of chromatin granules (Fig. 4B). In some species the size and number of chromatin granules decrease during development (e.g. *Tracheloraphis margaritatus*; Fig. 4C; Raikov, 1957a), suggesting that DNA elimination may be occurring (Raikov, 1994). DNA elimination is a common aspect of macronuclear development in other ciliates where regions of germline DNA are selectively excised or deleted (Prescott, 1994). Later, the emergence of nucleoli indicates the maturation of macronuclei (Fig. 4D; Raikov, 1985). The few studies focusing on conjugation in

Karyorelictea show differences compared to dividing cells; for instance, macronuclear development is blocked during conjugation and resumes only after cells separate (Raikov, 1990).

Aging of macronuclei

Since karyorelictean macronuclei cannot divide, they age, and the macronuclei within a cell are always at different ages (Raikov, 1982). As macronuclei age, changes occur in their size, shape, staining properties, and fine structure (Raikov, 1985). The size of young macronuclei is often between that of micronuclei and mature macronuclei; for example, in *Trachelocerca multinucleata*, micronuclei are measured at 5–6 µm in diameter, and young macronuclei are 5–7 µm while mature macronuclei are 8–10 µm in diameter (Raikov, 1989). Young macronuclei also stain more poorly with Feulgen than mature or old macronuclei, and possess smaller and more scattered chromatin bodies (Bobyleva et al., 1980; Kovaleva and Raikov, 1978; Raikov and Kovaleva, 1996). In contrast, mature macronuclei stain darker and have larger chromocenters (Bobyleva et al., 1980; Kovaleva and Raikov, 1978; Raikov and Kovaleva, 1996). Mature macronuclei tend to be relatively more abundant in multinuclear cells (Raikov, 1985). Old macronuclei stain darker still and have more irregular shapes with a higher presence of spheres in some species (e.g. *Kentrophoros tubiformis* and *Kovalevaia (Trachelonema) sulcata*; Bobyleva et al., 1980; Kovaleva and Raikov, 1978; Raikov and Kovaleva, 1996).

DNA accumulation in macronuclei accompanies the morphological changes of aging. For example, the increasing staining darkness of macronuclei indicates DNA synthesis (i.e. likely replication of certain genes; Bobyleva et al., 1980; Kovaleva and Raikov, 1978; Raikov, 1994; Raikov et al., 1963). DNA accumulation also generates macronuclei with a wide distribution of DNA contents even within the same cell (Raikov, 1994). For example, in *L. magnus*, DNA content of young macronuclei is ~4C while aging macronuclei reach an average of 5–6C; in *Kovalevaia sulcata*, old macronuclei vary in DNA content from 2C to 12C (Bobyleva et al., 1980; Kovaleva and Raikov, 1978; Raikov et al., 1963). The functional implications of changing DNA content are unknown.

The Div– macronuclei in karyorelictids also have a relatively short life span. They persist only through 3–7 cell divisions as estimated from the percentage of degenerating macronuclei per cell in *Loxodes magnus*, while macronuclei appear to exist longer in *Kentrophoros fistulosum* (Raikov, 1985, 1994; Raikov and Kovaleva, 1996). In contrast, macronuclei in other ciliates divide by amitosis for many generations until conjugation occurs. Macronuclear amitosis is estimated to occur for 40–60 cell divisions in *Tetrahymena thermophila* (Class Oligomenophorea), and for more than 100 in *Dileptus anser* (Class Litostomatea; Lynn and Doerder 2012; Yudin and Uspenskaya 2007). The short life span of Div– macronuclei in Karyorelictea might function as a mechanism to avoid the accumulation of deleterious mutations in Div– nuclei.

Implications of unusual nuclear features in Karyorelictea

Since the 1950's, karyorelictean nuclei have been investigated primarily using cytochemical and microscopy methods, including Feulgen staining. Here we speculate on the implications

of the patterns described above. We propose two possible explanations for the maintenance of a minimum of two macronuclei within each cell. One possibility is that a mature macronucleus is necessary to sustain the cell while the new macronucleus is developing, so every karyorelictid cell has at least one old and one newly-developed macronucleus. In *Paramecium tetraurelia* (Oligohymenophorea), parental macronuclei generate 80% of the total RNA during the first two cell divisions following conjugation and as the new macronucleus develops (Berger, 1973; Lepere et al., 2008). Another possible explanation is that the old macronucleus plays an important role in the development of the new macronucleus through epigenetic processes, perhaps by enabling RNA-mediated rearrangements. Such phenomena have been documented in several model ciliates including *Tetrahymena* (e.g. Mochizuki, 2010), *Paramecium* (e.g. Garnier et al., 2004) and *Oxytricha* (e.g. Yerlici and Landweber, 2014). These two possible scenarios are not mutually exclusive.

The Div[−] macronuclei are unique to Karyorelictea. Several explanations have been proposed for the evolution of this feature. One hypothesis is that Div[−] macronuclei are the ancestral state in ciliates and Div⁺ macronuclei have evolved twice in the Heterotrichea and Intramacronucleata (i.e. all remaining ciliate classes; Katz, 2001; Orias, 1991a, 1991b). Alternatively, Karyorelictea may have lost the ability to divide their macronuclei, either stochastically or due to selection. In the latter case, macronuclear division may have been lost to avoid the cost of high mutational loads in polyploid macronuclei (Katz, 2001). A similar argument has been made for the evolution of sex in eukaryotes as a means for resetting ploidy and removing deleterious mutations (Katz and Kovner, 2010; Kondrashov, 1997; Normark et al., 2003). With the emerging tools of single-cell ‘omics’, we will be able to further explore both the nature and implication of nuclear architecture within Karyorelictea, and gain more insights into the evolution of genome features in ciliates.

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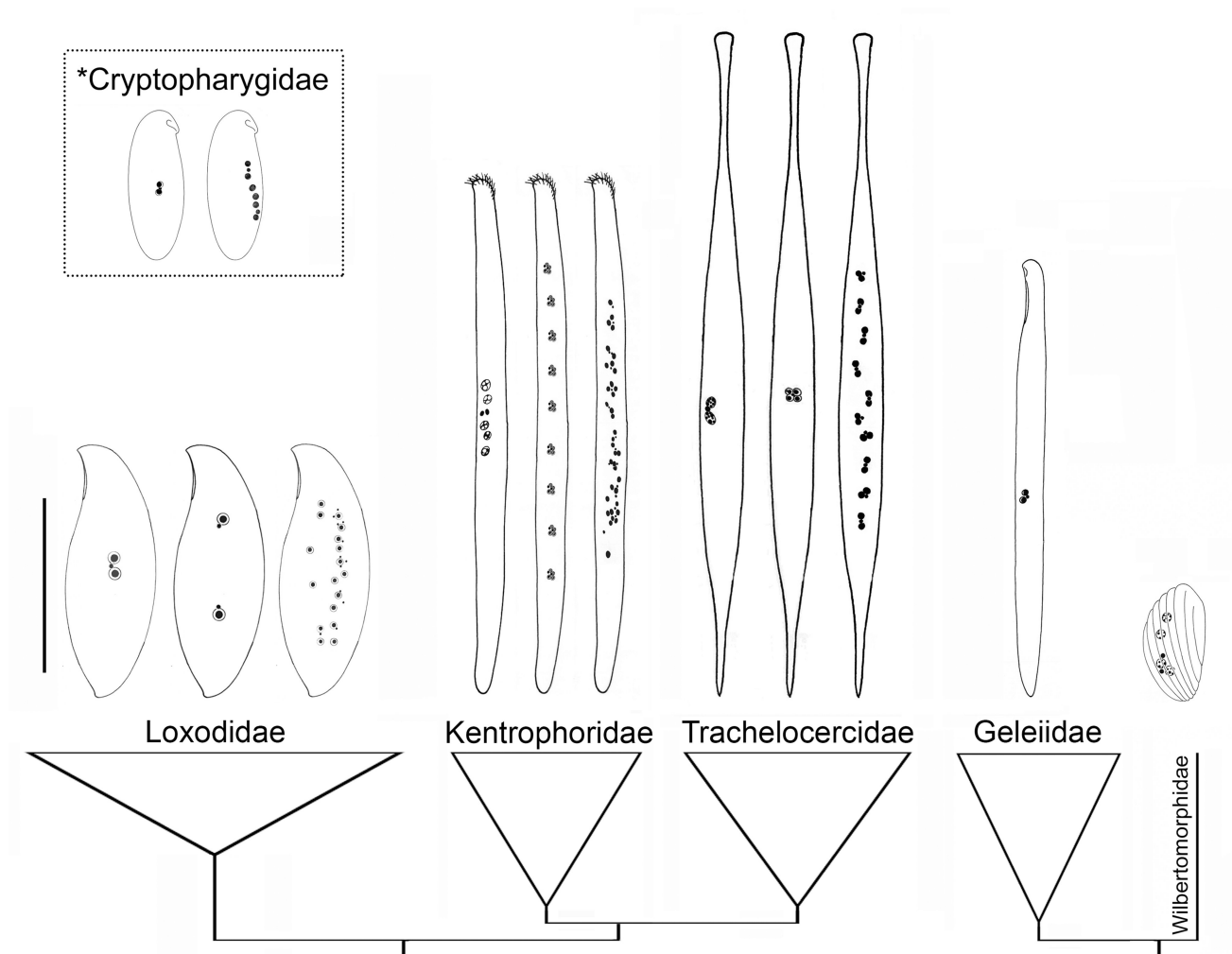
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**Fig. 1.**

A cartoon tree of Karyorelictea showing main diversity of nuclear clustering within and among families based on small subunit rDNA (SSU-rDNA) maximum likelihood tree. Cell sizes are drawn roughly to scale. There are currently no molecular data on Cryptopharyngidae (*); thus it shows as an orphan family.

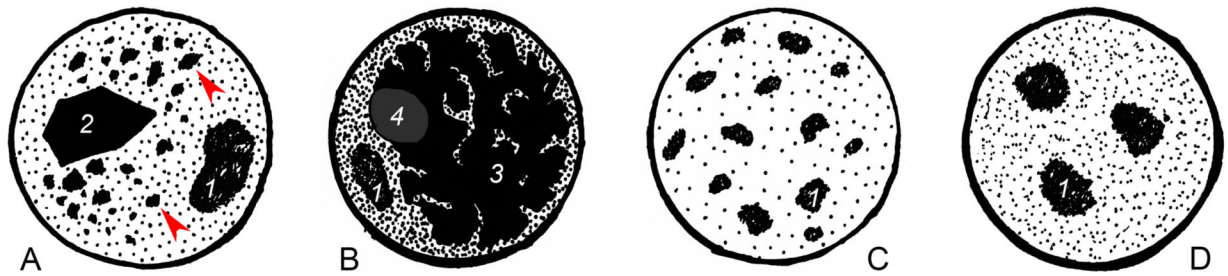


Fig. 2.

Examples of karyorelictid macronuclei showing different composition of nucleoli (1), crystalloid bodies (2), chromocenters (3) and unknown spheres (4): A. macronucleus with a single nucleolus and crystalloid plus numerous chromatin bodies (arrowheads) from *Tracheloraphis caudatus* (modified from Raikov 1994). B. macronucleus with a large chromocenter, nucleolus and sphere found in *Trachelocerca multinucleata* (modified from Raikov 1989). C. macronucleus with a large number of small nucleoli in *Geleia orbis* (modified from Raikov 1984). D. simple macronucleus with multiple nucleoli *Remanella granulosa* (modified from SEM in Raikov and Kovaleva, 1990).

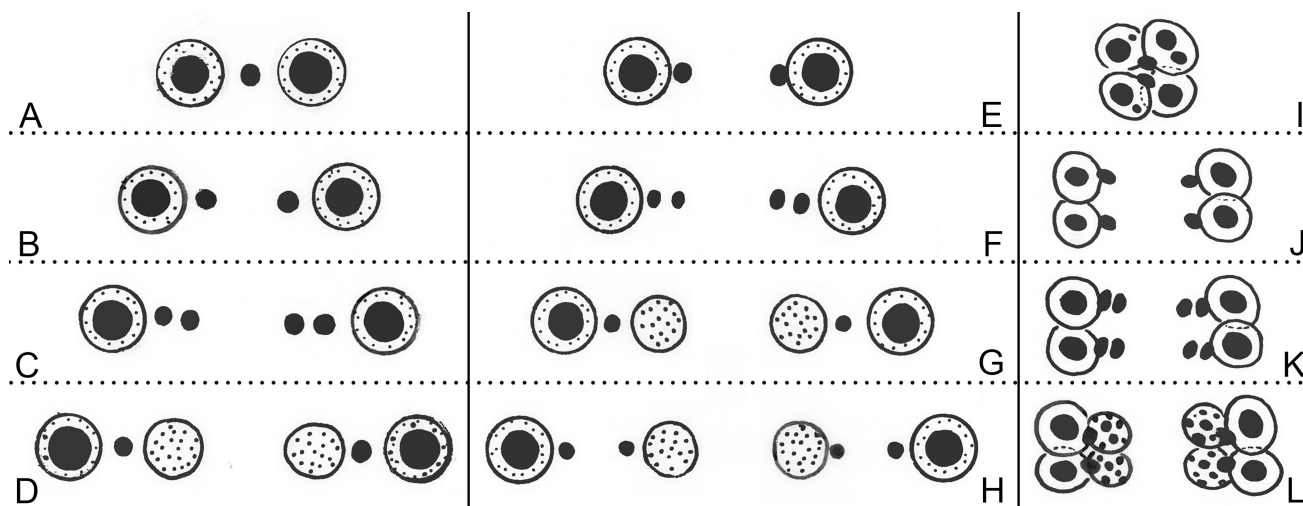


Fig. 3.

Nuclear reorganization during cell division in karyorelictids. A–D. Pattern of nuclear group with two macronuclei and one micronucleus (modified from *Loxodes rostrum*, Raikov 1957b): two parental macronuclei are segregated into daughter cells and each daughter cell receives a newly differentiated macronucleus. E–H. Pattern for a nuclear group with two macronuclei and two micronuclei (modified from *L. striatus*, Raikov 1957b): similar to the first pattern with an additional micronuclear division. I–L. Pattern for nuclear group with four macronuclei and two micronuclei (modified from *Trachelocerca coluber*, Raikov 1969): the parental nuclear group is separated into two halves, and the full complement of nuclei is restored by nuclear division and differentiation.

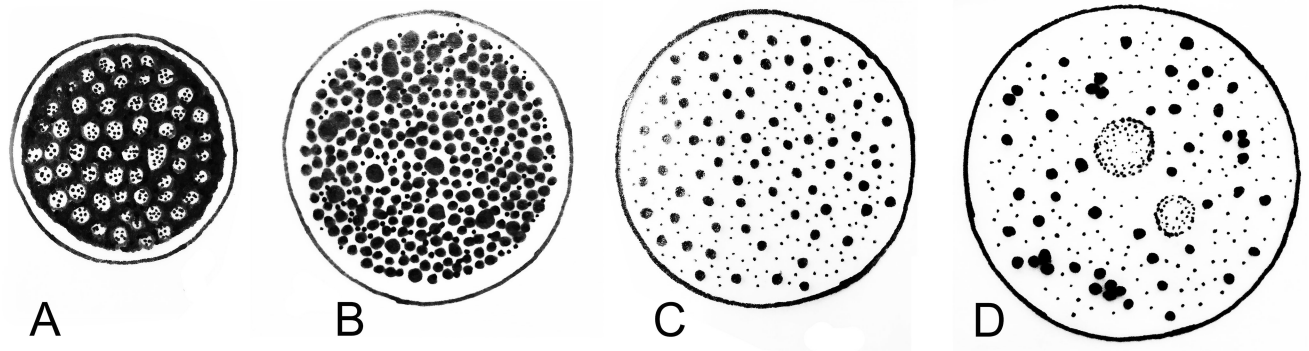


Fig. 4. Macronuclear development in *Tracheloraphis margaritatus* (Feulgen staining, modified from Raikov 1957). A. vacuolization stage from a macronuclear anlagen. B. macronuclear anlagen forming numerous chromatin granules. C. dechromatinization stage. D. appearance of nucleoli.

Table 1

Unusual nuclear features in karyorelictids in comparisons with other major ciliates classes.

Subphylum	Class	Mac Div ^a	Mac differentiation ^b post conjugation	Mac differentiation during asexual division	Mac aging ^c
	Heterotricha	Div+	+	-	-
	Litostomatea	Div+	+	-	-
	Armophorea	Div+	+	-	-
	Spirotrichea	Div+	+	-	-
	Colpodea	Div+	+	-	-
	Nassophorea	Div+	+	-	-
	Phyllopharyngea	Div+	+	-	-
	Prostomatea	Div+	+	-	-
	Plagiopylea	Div+	+	-	-
	Oligohymenophorea	Div+	+	-	-

^aAbility of macronuclear division.

^bMacronuclear differentiation.

^cMacronuclei age as they persist through several generations without dividing (see text for more detail).

Table 2

Summary table of cell size and number of nuclei/nuclear group in diverse karyorelictid species. Mac, macronucleus. Mic, micronucleus.

Family	Taxa name	Cell length	Cell width	Mac diameter/ μm	Mic diameter/ μm	# of nuclei/nuclear group	References
Wilbertomorphidae	<i>Wilbertomorphia colpoda</i>	45	15	2–3	1.5–2*	3–5 mac + 1–3 mic	Xu et al. (2013)
Cryptopharyngidae	<i>Apocryptopharynx hippocampoides</i>	50–90	30–40	5–6	4	2 mac + 1 mic	Foissner (1996a)
Loxodidae	<i>Loxodes striatus</i>	209–250	41–50	6.6–9.3	2.6–2.9	2 mac + 2 mic	Foissner and Rieder (1983)
	<i>Loxodes magnus</i>	340–440	80–84	7–8	2.8–3.6	18–26 mac + 10–16 mic	Foissner and Rieder (1983)
	<i>Remanella multinucleata</i>	500–1000	40–80	10×8	5×4.5	16 mac + 6 mic	Foissner (1996b)
	<i>Apotrachelocerca arenicola</i>	300–600	10–20	3–5	2*	2–3 mac + 1 mic	Xu et al. (2011b)
	<i>Kovalevaia sulcata</i>	800–1250	25	5–12	3–5	17–39 mac + 5–12 mic	Yan et al. (2013)
Trachelocercidae	<i>Prototrachelocerca caudata</i>	800–1500	–	6×4	3×2	(4 mac + 2 mic) × 5–37	Foissner (1996c)
	<i>Prototrachelocerca fasciolata</i>	1000–3000	50	12.5×9	6×5	10–24 mac + 3–7 mic	Foissner (1996c)
	<i>Trachelocerca stephani</i>	180	62.7	8×6	2.2	4 mac + 2 mic	Dragesco (2002)
	<i>Trachelocerca incaudata</i>	340	87	7.3×5.5	3.6	10 mac + 3 mic	Dragesco (2002)
	<i>Trachelocerca ditis</i>	437	90	7.9×6.3	2.2	4 mac + 2 mic	Dragesco (2002)
	<i>Trachelocerca ditis</i>	800–1000	40–50	10×4	4×3	4 mac + 2 mic	Foissner and Dragesco (1996)
	<i>Trachelocerca sagitta</i>	1000	30	8×5	2	4 mac + 2 mic	Foissner and Dragesco (1996)
	<i>Trachelocerca obscura</i>	1500–2000	75–100	10	6	7–14 mac + 2 mic	Raikov and Volkonitin (1989)
	<i>Trachelotolophos binucleatus</i>	700	30	7–10	3–6	2–3 mac + 1 mic	Yan et al. (2016)
	<i>Trachelotolophos quadrinucleatus</i>	1300	35	7–10	2–4	3–4 mac + 2 mic	Yan et al. (2016)
	<i>Tracheloraphis haloetes</i>	285–500	62–75	5–6	2–3	15–43 mac + 3–9 mic	Borror (1973)
	<i>Tracheloraphis oligostriata</i>	400–500	15	5–8	2–3	(2 mac + 1 mic) × 6–12	Al-Rasheid (2001)
	<i>Tracheloraphis primitarium</i>	400–900	40–60	8×5	2	3 mac + 2–3 mic	Epstein (1994)
	<i>Tracheloraphis phoenicopteris</i>	1000–1500	30–50	6–8	4	6–12 mac + 2 mic	Foissner and Dragesco (1996)
	<i>Tracheloraphis aragoi</i>	1100–2300	50–60	~10	2–2.6	5–12 mac + 2 mic	Foissner and Dragesco (1996)
Kentrophoridae	<i>Kentrophoros fistulosus</i>	500–2000	20–30	4×3.5	2	(4 mac + 2 mic) × 11–54	Foissner (1995)

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* number is estimated from figure.
- not available

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Table 3

Summary table of macronuclear inclusions in diverse karyorelictid species.

Taxon	Inclusion	Material	Diameter	Fiber width	Associations with aging/other structures	References
<i>Loxodes</i>	none	N/A	N/A	N/A	N/A	Raikov (1985)
<i>Remanella multinucleata</i>	sphere	Protein RNA	–	Not observable	none	Raikov (1979)
<i>Tracheloraphis phoenicopterus</i>	crystal /sphere	Protein	–	7–8 nm	In contact with chromocenters and chromatin bodies	Raikov and Kovaleva (1978)
<i>Tracheloraphis dogieli</i>	sphere	Protein	–	7–8 nm	Sometimes chromocenters	Raikov and Kovaleva (1980)
<i>Tracheloraphis crassus</i>	sphere	Protein RNA	–	10–12 nm	Sometimes nucleoli, occasionally chromocenters	Raikov and Karadzhan (1985)
<i>Trachelocerca obscura</i>	sphere	Protein RNA	–	10–12 nm	–	Raikov et al. (1989)
<i>Trachelocerca geopetiti</i>	sphere	Protein	–	7–8 nm	Sometimes nucleoli	Bobyleva et al. (1981)
<i>Kentrophoros tubiformis</i>	sphere	–	2 µm	7 nm	Always in aging macronuclei, continuous with matrix	Raikov and Kovaleva (1996)
<i>Geleia orbis</i>	sphere	Protein RNA	2–3 µm, up to 8 µm	10–12 nm	Always in contact with nucleoli, continuous with matrix	Raikov (1984)

N/A, not applicable;
–, not available.