Universidade Federal de Juiz de Fora
Pós-Graduação em Ciências Biológicas
Mestrado em Comportamento e Biologia Animal

Bianca Aline de Souza

EVOLUTIONARY ORIGIN AND HIDDEN GENETIC DIVERSITY OF *Paramecium* (CILIOPHORA: OLIGOHYMENOPHOREA)

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia): Comportamento e Biologia Animal, da Universidade Federal de Juiz de Fora, como requisito parcial para a obteção de grau de Mestre.

Orientador: Prof: Dr. Marcus Vinícius Xavier Senra
Co-Orientador: Prof: Dr. Roberto Junio Pedroso Dias

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RESUMO

Paramecium são seres vivos, microscópicos, que podem ser encontrados em vários ambientes aquáticos, tanto de água doce quanto salgada. Eles são seres unicelulares, ou seja, o seu corpo é formado apenas de uma célula, e é todo recoberto de cílios, alimentam-se de bactérias, algas, matéria em decomposição, pois eles não conseguem produzir seu próprio alimento. Estes seres vivos são muito abundantes, distribuídos entre 19 espécies válidas, e bastante utilizados como modelo de estudo em vários campos de pesquisa. Porém, questões sobre quando esses organismos surgiram, e como sua estrutura populacional se encontra ainda não estão muito claras para os pesquisadores. Então, os objetivos deste trabalho foram identificar quando estes seres vivos surgiram, e como essa estrutura populacional encontra-se organizada. Para isso, utilizamos sequencias de DNA, depositadas em bancos de dados próprios para esse fim, que juntamente com programas computacionais e fósseis datados previamente conhecidos, permitem estabelecer este surgimento. Encontramos que estes seres vivos surgiram há cerca de 300 milhões de anos atrás, e que cada uma de suas espécies, tiveram uma data distinta de surgimento. Além disso, certamente existam bem mais espécies do que é conhecido atualmente, e questões ambientais, bem como questões fisiológicas destes seres vivos, é o que provavelmente influencia na sua distribuição.

Palavras-chave: Ambiente; distribuição; *Paramecium;* surgimento

ABSTRACT

Members of the genus *Paramecium* (Ciliophora, Oligohymenophorea) are widespread ciliates able to colonize many aquatic brackish and freshwater environments. They are currently divided in 5 subgenera encompassing 19 valid species, including *Paramecium multimicronucleatum*, *Paramecium caudatum, Paramecium aurelia (complex)* and *Paramecium bursaria*, which are considered cosmopolitan organisms. Despite that many *Paramecium* species have been historically and extensively used as model organisms in research fields ranging from genomics to conservation biology, their origin and populational structure remains elusive. Here, two datasets (18S rDNA and cytochrome oxidase subunit I [COI]) including sequences from all 5 subgenera and from most of the accepted species, we used the 18S rDNA to reconstruct a time-calibrated tree of *Paramecium* to elucidate the evolutionary origin of extant accepted *Paramecium* species and to evaluate intra-genus phylogenetic relationships. The COI dataset was used to evaluate, through species delimitation algorithms (PTP and mPTP) the existence of independent evolutionary unities (IEUs) within accepted *Paramecium* species. Our data rises more evidences supporting the idea that most accepted *Paramecium* species may be, in fact complexes of species, and that the diversity of cryptic species may be higher than previously though; the identified IEUs are not spatially structured, actually, many of than co-occurs over vast geographical areas, indicating that intercontinental distances are not barriers for IEUs` dispersion and gene flow, and other eco-physiological features may be responsible to their distribution.

Keywords: Cryptic species; time tree, species delimitation; genetic diversity; species complex.

LISTA DE ABREVIATURAS E SIGLAS

COI	Gene Citrocomo Oxidase- I
18S	Gene 18S ribosomal RNA
PTP	Poisson Tree Processes
MPTP	Multi-rate PTP/ Multi-taxa PTP
MCMC	Markov Chain Monte Carlo
MAFFT	Multiple sequence alignment program
IEU	Independent evolutionary unities
Bold	Barcode of Life Data System
BEAST	Bayesian evolutionary analysis by sampling trees
ESS	Effective sample size
LPCA	Last *Paramecium* Common Ancestor
Ma	Millions of years
ML-	Maximum likelihood
WGD	Whole-genome duplication

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SUMÁRIO

1.INTRODUCTION

Among members of the phylum Ciliophora Doflein, 1901, unicellular microeukaryotes able to colonize a wide variety of environments, including soil, fresh and marine waters, and which have as synapomorphies: nuclear dimorphism (somatic and germinative nuclei within the same cell), presence of an infraciliature, and sexual reproduction through conjugation (Lynn, 2008), *Paramecium* Muller, 1786 (Oligohymenophorea) is possibly the best known and studied genus (Tarcz et al., 2012). These organisms are widespread and molecularly diverse (Krenek et al., 2015), exhibiting high reproduction rate (Grolière et al., 1990) and thly can be easily cultivate under laboratory conditions, amenable to genetic modification and have high sensitivity to environmental disturbances (Delmonte Conrrado et al., 2006; Tan et al, 2010).

These characteristics make *Paramecium* good model organisms and have been extensively used over the last 250 years in a vast array of studies, ranging from genomics to conservation biology (Mansano et al., 2016; Quispe et al., 2016) and have been applied for biomonitoring and bioremediation of impacted environments (Sleigh, 1988; Grolière et al., 1990; Sparagano and Groliere, 1991; da Silva-Paiva and Silva-Neto, 2004).

Moreover, many information on the function and functioning of microRNAs (catalytic RNAs), telometric repeats, histone epigenetic modifications came also from works using *Paramecium* as model organisms (Bass and Cech 1984; Greider and Blackburn 1985; Greider and Blackburn 1989 ; Gao et al., 2013; Wang and Sheng, et al., 2017; Zhao et al.,2017; Zheng et al., 2018).

Despite this long history of vast research, aspects about the origin and evolution still elusive (Krenek et al., 2015). Recently, Fernandes and Schrago (2019) stablished the origin of the genus *Paramecium* in 450-240 Ma. However, many questions remain unanswered, such as when was the emergence of the extant species and how it may have influenced their actual geographic distribution?

There are, currently more than 40 described species in the literature, however, only 19 are considered valid species, being this validation, the species based in description morphological, and most of the descriptions were not done correctly. (Fokin, 2002; Fokin, 2010; Krenek, 2015; Silva Paiva, 2016). The genus *Paramecium* can be divided into 5 subgenera: [1]*Paramecium* (Jankowski,1969) encompassing the species of *P. aurelia* complex (Sonneborn, 1975), *P. multimicronucleatum* (Powers and Mitchell, 1910), *P. caudatum* (Ehrenberg, 1833), *P. jenningsi* (Diller and Earl,1958), *P. schewiakoffi* (Fokin et al, 2004), *P. africanum* (Dragesco, 1970), *P. jankoswskii* (Dragesco, 1972), *P. wichtermani* (Mohammed and Nashed, 1969) and *P. ungandae* (Dragesco, 1972); [2]*Cypriostomum*, including *P. neprhridiatum* (Gelei, 1925), *P. woodruffi* (Wenrich, 1928) *P. polycaryum* (Woodruff and Spencer, 1923), *P. pseudotrichium* (Dragesco, 1970) and *P. calkinsi* (Woodruff, 1921); [3]*Viridoparamecium*, represented by the species *P. chorelligerum* (Kahl, 1935); [4]*Helianter* (Jankowski, 1969), comprising *P. putrinum* (Claparède and Lachmann, 1858), *P. duboscqui* (Chatton and Brachon, 1933) and *P. buetschlii* (Krenek, 2015); and [5]*Chloroparamecium*, represented by *P. bursaria* (Focke, 1836) Recently, a novel species was included to the list, *Paramecium grohmannae* (Paiva et al., 2016), which was described from an urban wastewater treatment plant in Rio de Janeiro (Brazil), highlighting the importance of more taxonomical studies within neotropics. (These accepted species and their geographical distribution are summarized in Table 1).

Despite these microeukaryotes can be found, in general in most freshwater (lakes, rivers, streams) (Sonneborn, 1975) and brackish environments all around the world (Fokin and Chivilev, 1999) with temperatures ranging from 3ºC to 28ºC (Landis, 1988; Krenek et al., 2015), their geographic distribution and the amount of genetic variability remain topics of intense debate (Catania et al., 2009). Among protistologists there are two competing schools, some authors supporting the “everything is everywhere” microbiology maxima (few species with widespread distribution) (Fenchel and Finlay, 2004). While others defend the view that, there is a great richness of protist species, and they tent, actually to be endemics (Foissner, 1999). In fact, data on the geographic distribution of some *Paramecium* species do not seems to support this cosmopolitan model of distribution (Fokin, 2010). According to Fokin (2010), although some species, such as *P. caudatum*, *P. multimicronucleatum, P. aurelia* (complex) and *P. bursaria* do appear to be cosmopolitan, others may be, in fact endemic to certain regions, such as *P. sonneborni* (North American), *P. schewiakoffi* and *P.*

wichtermani (Asia) (Table 1). Still, this author and others also defend the view that the assumption of cosmopolitanism in ciliates is likely associated with the lack of taxonomic specialists and largely unexplored areas, such as the tropics, which lead to misidentifications and poor taxonomical species characterizations (Foissner, 1999; Fokin et al., 2004, Fokin, 2010).

Table 1. Distribuition of *Paramecium* morphospecies

Species	**Locality**						**Reference**
	Europe	**Asia**	**North A**	**South A**	**Africa**	**Oceania**	
Paramecium caudatum	+	+	+	+	+	+	Ehrenberg 1838
Paramecium multimicronucleatum	+	+	+	+	+	+	Powers and Mitchell 1910
Paramecium shewiakoffi	-	+	-	-	-	-	Fokin et al.,2004
Paramecium sonneborni	+	-	+	-	-	-	Aufderheide et al., 1983
Paramecium jennignsi	+	+	+	-	+	-	Diller and Earl, 1958
Paramecium aurelia	+	+	+	+	+	+	Müller, 1773
Paramecium dubosqui	+	+	+	+	+	-	Chatton and Brachon, 1933
Paramecium putrinum	+	+	+	-	-	+	Claparede and Lachmann, 1858
Paramecium chlorelligerum	+	+	-	-	-	-	Kahl 1935; Kreutz et al. 2012
Paramecium woodruffi	+	+	+	-	-	-	Wenrich 1928
Paramecium calkinsi	+	+	+	-	-	-	Woodruff, 1921
Paramecium nephridiatum					-		Gelei 1925
Paramecium polycaryum	+	+	+	-	+	-	Woodruff and Spencer, 1923
Paramecium bursaria	+	+	+	+	+	+	Focke, 1836
Paramecium africanum	-	-	-	-	+	-	Dragesco 1970
Paramecium wichtermani	-	-	-	-	+	-	Mohammed and Nashed 1968-1969
Paramecium pseudotrichium	-	-	-	-	+	-	Dragesco 1970
Paramecium grohmannae	-	-	-	+	-	-	Silva-Paiva et al.; 2016

(+) Presence (-) Absence

Moreover, there are evidences suggesting that many accepted *Paramecium* species would be, in fact complexes of species (Catania et al., 2009;

Krenek et al., 2015; Przyboś et al., 2018). Among these, the best studied complex is the *P. aurelia* complex, a group of 15 sibling species, which are morphologically (Catania et al., 2009) identical, but are genetically and reproductively isolated, exhibiting great levels of genetic variability across siblings (Catania et al., 2009). Krenek et al. (2015) found genetic evidence for cryptic species in *P. caudatum* and *P. multimicronucleatum*. According to Prybós and Tarcz (2016), *Paramecium jenningsi* should be considered a complex of species, describing three isolated reproductive groups, and then proposing the binominal names: *P. primjenningsi*, *P*. *bijenningsi*, and *P. trijenningsi*. *P. bursaria*, the only species within the *Chloroparamecium* subgenera, is also divided into five syngens (cryptic species) (Greczek-Stachura et al., 2012; Zagata et al., 2015).

The widespread existence of cryptic species within the accepted *Paramecium* species represents an obstacle toward the better understanding on the systematics and ecology of this group of organisms. Two methods have been used to identify and characterize cryptic species within *Paramecium*: Crossing (mating tests) (Prybós and Tarcz, 2016) and molecular tests (Krenek et al., 2015). Mating tests may be inconvenient and more laborious, since one has to co-cultivate possible mates and evaluate the occurrence of conjugation (or not) and so, not efficient for large populational studies, while molecular methods could be efficiently applied to in large scale studies.

However, we still do not have a consensus on the boundaries between what can be considered intraspecific and interspecific variations. Therefore, the use of new molecular methodologies, which could efficiently identify cryptic species would be greatly desirable to improve our current understanding about the real species diversity, evolution and the spatial distribution of *Paramecium*.

In this view, in this present work, we used a 18S rDNA-based time calibrated phylogenetic reconstruction in order to evaluate the evolutionary origin and the intra-genus relations of the genus *Paramecium.* We applied (and compared) commonly used molecular species delimitation algorithms to evaluate the existence of Independent Evolutionary Units (IEUs) within a Cytochrome Oxidase I (COI)-based phylogenetic reconstruction, in which members of all *Paramecium* subgenera and sequences from most of accepted species were included into the analyses, aiming to identify new cryptic species within

Paramecium. Moreover, we compare whether the IEUS corroborate with the valid species.

2.MATERIAL AND METHODS

2.1.DATASET

Two datasets were generated and the sequences used are listed in Table S1 and S2. The first one includes all 18S rDNA sequences longer than 1200bp of *Paramecium* available at the curated ARB-SILVA database (Quast et al., 2013) plus 4 *Tetrahymena* (Oligohymenophorea, Hymenostomatida), 2 *Euplotes* (Spirotricha, Euplotida) and 1 Monoeuplotes (Spirotcricha, Euplotida) sequences, which were used as out group. This dataset was aligned using SINA v1.2.11 (WWW.arb-silve.de/aligner), manually inspected for unaligned nucleotides and then trimmed using SeaView v3 (Gouy and Gascuel, 2010), resulting in a matrix of 133 taxons and 1907 characters. The second dataset used in this work was constructed using 546 cytochrome oxidase subunit I (COI) sequences of the genus *Paramecium*, plus 8 *Tethrahymena* sequences, which were used as outgroup. All these sequences were retrieved from the Barcode of Life Data System (Bold) molecular database (www. barcodinglife.org). The Dataset was aligned using MAFFT v7 (https://mafft.cbrc.jp/alignment/software/) with default parameters, and then visually inspected and curated using Gblocks (Castresana, 2000), as implemented in SeaView v3 (Gouy and Gascuel, 2010) for removal of poorly aligned sites. From the initial 546 *Paramecium* COI sequences, 28 short (~320bp) *P. aurelia* sequences, and 1 *P. chlorelligerum* and 1 *P. bueschii* low quality sequences were removed from the final aligned dataset, resulting in a matrix of 638bp and 507 *Paramecium* taxa.

2.2.PHYLOGENETIC RECONSTRUCTION

Phylogenetic reconstructions were performed using a Bayesian (18S rDNA and COI datasets) and maximum likelihood (18S rDNA) frameworks. First, the best model for sequence evolution within this dataset was evaluated using jModelTest2 (Darriba, Taboada and Posada, 2012). Then, we used BEAST 2.4.8

(Bayesian Evolutionary Analysis Samples Trees) (Drumont et al., 2006) to infer phylogenies using GTR +G +I and combinations of strict and relaxed molecular clock models under Yule and Coalescence priors. The analyses were conducted for 20 million generations using Markov Chain Monte Carlo (MCMC), which were enough to ensure an effective sample size (ESS) greater than 200, as verified using the Tracer (Drumont and Rambaut, 2007). The resulting trees were summarized by a majority rule criterion using TreeAnnotator (Drumont et al.,2006 & Drumont et al., 2012). Since all trees generated presented the same overall topologies, the strict Yule tree was selected for further analyses. ML inference were conducted using phyML v3 (Guindon et al., 2010), applying GTR +G +I sequence evolution model and reliability of branches was accessed through nonparametric bootstrap method of 1000 pseudoreplicates.

2.3.TIME CALIBRATED TREE

A timetree was inferred using Reltime 2016 method as implemented in Mega X (Kumar et al., 2016) using as template the 18S rDNA BI inferred topology and branch lengths. As calibration constraint, we used the interval of 450-240 million years established by Fernandes and Schrago (2019) to the node representing the Last *Paramecium* Common Ancestor (LPCA).

2.4.GENETIC DIVERSITY WITHIN *PARAMECIUM*

Within subgenera and species genetic diversity was estimated using MEGA X (Kumar et al., 2016) from 18S rDNA and COI dataset by measuring p-distances between sequences.The genetic distances of the 18S rDNA of *P. buetschlii, P.schewiakoffi*, *P. woodruffi*, *P. polycaryum* and COI of *P. schewiakoffi*, *P. woodruffi* and *P. sonnerborni* have not been calculated, as they have only 1 sequence. And *P. sonnerborni* for not having an 18 rDNA sequence, and *P. buetschlii* COI available.

2.5.INDEPENDENT EVOLUTIONARY UNITS (IEUS) DELIMITATION

Two commonly used algorithms of computational species delimitation, the Poisson Tree Processes (PTP) (Zhang et al., 2013) and the multi-rate PTP (MPTP) (Kapli et al., 2017) were employed, using default parameters to evaluate the existence of potential cryptic species within the formally recognized species within both the 18S rDNA and COI BI phylogenetic trees. Both methods compute the ML delimitations from an inferred phylogenetic tree. But, while PTP uses a single exponential distribution for the delimitation process, the MPTP assumes a distinct exponential distribution for the branching events of each of the delimited species, which allows, according to the authors (Kapli et al., 2017) to fit to a wider range of empirical datasets.

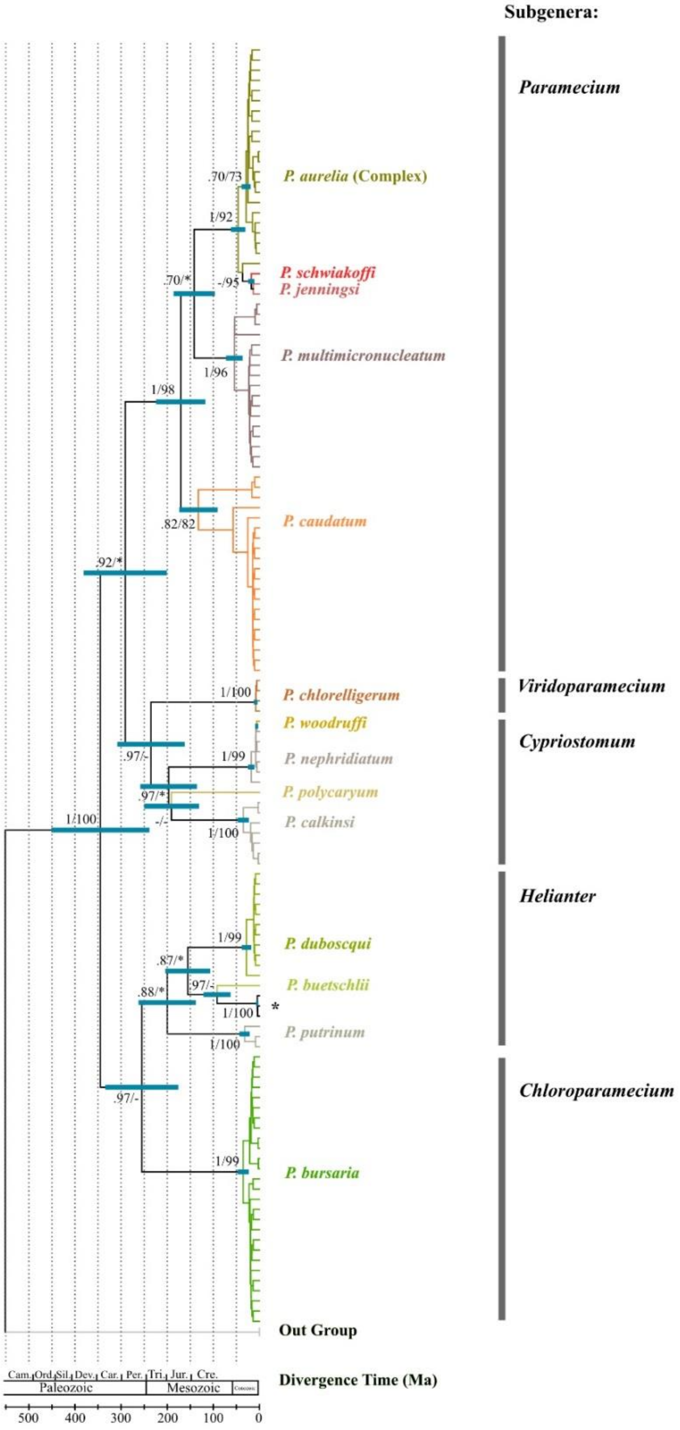
3.RESULTS

3.1.EVOLUTIONARY ORIGIN OF *PARAMECIUM*, SUBGENERA AND EXTANT SPECIES

All data generated on the origin and evolution of *Paramecium* are presented in Figure 1 and data summarized in Table 2. Accordingly, we found evidences that all extant *Paramecium* species irradiated from a last common ancestor during the transition from Devonian to Carboniferous. The first cladogenesis event (345 Ma) within the *Paramecium* phylogeny separated the genus in two main clades: in one side, *Paramecium*, *Cypriostomum* and *Viridoparamecium* subgenera; and *Chloroparamecium* and *Helianter* subgenera in the other. The next dichotomy (290.91 Ma), resulted in the split, during the late carboniferous of *Paramecium* from *Cypriostomum/Viridoparamecium* subgenera clade, which became independent groups 235.23 Ma during the Triassic. The emergence of *Helianter* and *Chloroparamecium* subgenera may have occurred within the transition Permian/Triassic (255.14 Ma), after split from a common ancestral.

Within the subgenus *Paramecium*, the first split, during the Jurassic period (170.57 Ma) separated *P. caudatum* from *P. aurelia* (complex), *P. schewiakoffi*, *P. jenningsi* and *P. multimicronucleatum* (Figure 1 and Table 2), which remained as a clade until the late Jurassic (141.82 Ma) when *P. multimicronucleatum* emerged as independent evolutionary lineage. *P. schewiakoffi* and *P. jenningsi* evolved from a common *P. aurelia* (complex) ancestral within the Paleogene (36.40 Ma), eventually splitting to become separated species in the mid Neogene (17.40 Ma).

Figure 1- Phylogenetic reconstruction based on data from 18S sequences of the genus *Paramecium* along with their divergence time. genus *Paramecium* along with their divergence time.



Fonte: O Autor

Table 2: Means and 95% credibility intervals of *Paramecium* subgenera and divergence times

Taxon*	Mean divergence time (Ma)	95% CI
Last *Paramecium* common ancestor	**345**	**240-450**
Last *Paramecium/Cypriostomum/Viridoparamecium* subgnera common ancestor	**290.91**	**202.37-379.45**
Last *Helianter/Chloroparamecium* subgenera common ancestor	**255.14**	**177.49-332.79**
Last *Cypriostomum/Viridoparamecium* subgenera common ancestor	**235.23**	**163.64-306.83**
Subgenus *Chloroparamecium*	**35.62**	**24.78-46.46**
P. bursaria	35.62	24.78-46.46
Subgenus *Helianter*	**199.85**	**139.03-260.68**
P.dubosqui	28.34	19.71-38.96
P. bueschlii	92.03	64.02-120.03
P.putrinum	31.99	22.25-41.72
Subgenus *Paramecium*	**170.57**	**118.65-222.48**
P.caudatum	132.53	92.19-172.86
P.multimicronucleatum	54.54	37.94-71.13
P.aurelia (complex)	46.07	32.05-60.09
P.jenningsi	17.40	9.46-17.74
P.schewiakffi	36.40	12.11-22.70
Subgenus *Cypriostomum*	**196.84**	**136.93-256.75**
P.calkinsi	35.23	24.51-45.95
P.polycaryum	190.63	132.61-248.65
P.nephridiatum	17.81	12.39-23.22
P.woodruffi	6.19	4.30-8.07
Subgenus *Viridoparamecium*	**8.14**	**5.67-10.62**
P.chlorelligerum	8.14	5.67-10.62

*Entities of high taxonomic levels are highlighted in bold
Fonte: O autor

The subgenus *Viridoparamecium* which has a single species, *P. chlorelligerum* and is a sister group of *Cypriostomum* may have arisen in the Carnian`s late Triassic (235.23 Ma). The first split within the subgenus *Cypriostomum* was at the Jurassic/Triassic transition (196.84 Ma) and separated the group in two clades: *P. nephridiatum* and *P. woodruffi* from *P. polycaryum* and *P. calkinsi*. The second split (190.63 Ma) separated *P. polycaryum* from *P. calkinsi*. And finally, within Neogene (6.19 Ma) *P. woodruffi* emerged from an *P. nephridiatum* ancestor as an independent lineage (Figure 1).

The subgenus *Chloroparamecium*, which has a single species, *P. bursaria* originated after a split of the clade composed by *Chloroparamecium* and *Helianter* subgenera within the Permian/Triassic transition (255.14 Ma). The second dichotomy within this ancestral clade separated the *Helianter* subgenus in *P. putrinum* and *P. duboscqui* and *P. bueschlii* during the Jurassic/Triassic transition (199.85 Ma). While the third, during the Jurassic/Cretaceous transition (155.44 Ma) gave rise to *P. duboscqui* and *P. bueschlii* (Figure 1).

3.2.GENETIC DIVERSITY WITHIN *PARAMECIUM*

The evolutionary origin of each accepted *Paramecium* species with available 18S rDNA sequence which were include in our phylogenetic reconstructions is presented in Table 2. Our reconstructions demonstrate that the species did not all appear at the same time. And since the emergence of each of them, species are in a constant process of speciation, may be exposed to different selective pressures and have been subjected to an independent diversification dynamic, resulting from the balance of (high / low) diversification and (high / low) extinction rate.

As shown in Table 3 the highest genetic distances, within the 18S rDNA dataset were observed for *Helianter* and *Cypriostomum* subgenera, 0.033 and 0.034, respectively, and within *P. caudatum* and *P. putrinum* species, 0.016 and 0.012, respectively. While the lowest genetic distances were found for *Viridoparamecium* and *Chloroparamecium* subgenera, 0.001 and 0.007, respectively and for *P. chlorelligerum* and *P. nephridiatum* species, 0.001 and 0.002, respectively.

On the other hand, within the COI dataset, the highest genetic distances were observed in *Paramecium* and *Cypriostomum* subgenera, 0.185 and 0.152, respectively, and within *P. aurelia* (complex) and *P. calkinsi*, 0.157 and 0.183, respectively. While the lowest, was in *Viridoparamecium* and *Chloroparamecium*, 0 and 0.058, respectively and in *P. nephridiatum* and *P. duboscqui*, 0.031 and 0.038 respectively (Table 3).

Table 3: 18S rDNA and COI genetic distance table of *Paramecium* lineages

Taxon	18S rDNA	COI
Paramecium	0.03	0.185
Helianter	0.033	0.109
Cyprostomum	0.034	0.152
Chloroparamecium	0.007	0.058
Viridoparamecium	0.001	0
P.aurelia	0.004	0.157
P.putrinum	0.012	0.062
P.calkinsi	0.006	0.183
P.caudatum	0.016	0.041
P.multimicronucleatum	0.008	0.066
P.bursaria	0.007	0.058
P.nephridiatum	0.002	0.031
P.chlorelligerum	0.001	0
P.buestchii	n/c	-
P.dubosqui	0.012	0.038
P.jenningsi	0.003	0.076
P.schewiakoffi	n/c	n/c
P.woodruffi	n/c	n/c
P.polycaryum	n/c	0.053
P.sonneborni	-	n/c

n/c Not calculated; (-) Sequence absent in the dataset
Fonte: O autor

3.3.DISTRIBUTION AND INDEPENDENT EVOLUTIONARY UNITS (IEUS) WITHIN *PARAMECIUM* ACCEPTED SPECIES

The high genetic diversity observed in some cosmopolitan species, such as *P. caudatum* and *P. multimicronucleatum* (Table 3) raises questions about the structure of their population across wide geographical areas and about the presence of cryptic species within these formally accepted *Paramecium* species. To shed some light into these long termed debated questionings we applied commonly used species delimitation the algorithms PTP (Zhang et al., 2013) and mPTP (Kapli et al., 2016) (as described in method section) to our phylogenetic reconstructions using both the 18S rDNA dataset and the population-level molecular marker (COI) dataset.

The results from these analyses varied across datasets (18S rDNA or COI) and algorithms (PTP or mPTP). In general, mPTP resulted in more conservative analyses, delimiting less IEUs within the COI dataset (69 IEUs) and within the 18S rDNA (16 IEUs), while PTP tended to divide Paramecium in more IEUs (COI = 82 and 18S rDNA = 19) (Table 4, Table S1 and in Figure S1). Concerning the 18S rDNA dataset, most of the accepted *Paramecium* morphospecies were assigned into unique IUEs. Both algorithms divided *P. caudatum* and *P. multimicronucleatum* into 2 and 3 IUEs, respectively. While, PTP also identified 2 IEUs within *P. duboscqui*, *P. putrinum* and *P. calkinsi* (Table 4, Table S1 and in Figure S1). However, there were two cases, in which different morphospecies were grouped in a same IUEs, such as *P. sonneborni*, *P. schewiakoffi* and *P. jenningsi* (PTP, IEU 14; mPTP, IEU 12) and *P. woodruffi* and *P. nephridiatum* (PTP, IEU 18; mPTP, IEU 15) (Table 4, Table S1 and in Figure S1). With this applied method also we could identify a clade composed by metagenome sequences isolated from Denmark, which is a sister group of P. buetschlli and was identified as a possible new species in Table 4 and marked with (*) in Figure 1). However, no morphological data is available what makes the proper characterization of these organisms unfeasible. In addition, two sequences of *Paramecium sp*, were delimited common with the clade of *P. bursaria*. Two sequences with a *P. caudatum* clade; Four sequences with a *P. multimicronucleatum* clade; 3 sequences to the aurelia complex, two sequences with *P. calkinsi* and a sequence of *P. nepridiatum*, reported that may be possible in these morphospecies.

Table 4: Number of Independent Evolutionary units (IEUs) identified within the *Paramecium* 18S rDNA and COI datasets using PTP and MPTP algorithms

	Number of IEUs			
	18S rDNA		COI	
Species	**PTP**	**MPTP**	**PTP**	**MPTP**
P.caudatum	2	2	6	6
P.multimicronucleatum	3	3	10	9
P. sonneborni	-	-	1	
P. schewiakoffi			1	1^{Θ}
P. jennignsi	1^{ψ}	1^{ψ}	4	4
P. aurelia			16	16
P. dubosqui	2	1	5	5
P. putrinum	2	1	10	6
P. chlorelligerum	1	**1**	1	1
P. calkinsi	2	1	8	8
P. woodruffi	1*	1*	1	1
P. nephridiatum			3	$1^{\S}$
P. polycaryum	1	1	4	3
P. buetschlii	1	1	-	-
P. Sp nov. (?)	1	1	-	-
Paramecium sp	1	1	2	1
P.bursaria	1	1	10	8
TOTAL	19	16	82	69

(*ψ*), (*) and (*Θ*) marked species which were delimited in the same IEU. (-) species absent within the dataset.(§) this species also apears twice in IEUs shared with *P. calkinsi*

Fonte: O autor

Considering the analyses of the COI dataset, the results were relatively similar for both algorithms (Table 4, Table S1 and in Figure S2), succeeding in most of the cases in discriminate valid species (PTP = 10/13 and mPTP = 11/13). However, the number of IEUs identified within *Paramecium* varied considerably, (82 using PTP and 69, using mPTP). Despite of this discrepancy, both methods congruently suggest that most morphospecies were subdivided into multiples IEUs. On the other hand, both algorithms had problems to discriminate some sequences of *P. nephridiatum* and *P. calkinsi* (mPTP: IEUs 54 and 56; PTP: IEUs 66) and some sequences of the *P. aurelia* (complex) (mPTP IEUs 24, 29, 32, 33 and 34; PTP IEUs 26, 32, 36, 37, 38) (Table S1 and Figure S2). Additionally, mPTP algorithm also failed to discriminate *P. schewiakoffi* and *P. sonneborni* (IEU 17) (Table S1 and Figure S2). In addition, the mPTP algorithm also failed to discriminate *P. schewiakoffi e P. sonneborni* (IEU 17) (Table S1 e Figure S2). These being two species are valid, indicating that this method is more conservative than PTP, it is not possible to recover the data obtained with the morphology. However, it is also worth remembering that only two sequences were available in the database (one from each), highlighting the need for more sequences of these species for analysis. This data has also increased evidence that as the species accept the *Paramecium* can be subdivided into IEUs, suggesting that these species, facts and complexes are identified. Or, many strings present in the databases may be identified as wrong, since there was irradiation of species (*P. calkinsi* and *P. nephridiatum*) in several points of the tree (never for its subgenus *Cypriostomum*).

We next compared the continent from which each *Paramecium* sequence was obtained with the data on the IEUs delimitation, aiming to stablish the geographical distribution of these IEUs (Table S1). Table 5 summarizes the data from the analysis of the COI dataset using the PTP algorithm (for complete data, please see Table S1 and Figures S1-2). *P. calkinsi*, *P. nephridiatum*, *P. aurelia* (complex), *P. schewiakoffi* and *P. sonneborni* and *P. woodruffi* were removed from this analysis because of the problems concerning assigning of IEUs, as mentioned above, which would have caused misleading interpretation of the data. In general, the morphospecies can be composed by IEUs with restricted and/or wide geographical distribution and different IEUs from the same morphospecies can occupied the same geographical area (Table 5). However, in none of the cases a clear populational structure correlation between geographic distribution and IEUs could be noticed, indicating that the distances between continents do not constitute significative barriers to their dispersion. This dispersion can occur through the intermediary of insects and migratory animals, whose habitat is associated with water and, consequently, introducing the species in different locations. This is possibly common in all species that can be considered as cosmopolitans.

Table 5: Geographic distribuition of IEU within the COI database using PTP algoritim

Species	Locality	Species/IEU	Locality
P.bursaria		***P. polycaryum***	
IEU I	Europe and Asia	IEU I	North America
IEU II	Asia	IEU II	Africa
IEU III	North America	IEU III	Europe and Asia
IEU IV	Asia	IEU IV	Europe, Asia and North America
IEU V	Asia	***P. jenningnsi***	
IEU VI	Asia	IEU I	Asia
IEU VII	Oceania, Europe and Asia	IEU II	Europe and Asia
IEU VIII	Asia	IEU III	Africa, Asia and Europe
IEU IX	Asia	IEU IV	Asia
IEU X	Asia	***P. multimicronucleatum***	
P.dubosqui		IEU I	North America
IEU I	Asia	IEU II	Asia
IEU II	Asia	IEU I	Europe, Asia and Oceania
IEU III	Asia	IEU II	Asia
IEU IV	Europe and Asia	IEU III	North America
IEU V	Europe and Asia	IEU VI	Europe and Asia
P. chlorelligerum		IEU V	Europe, Asia and South America
IEU I	Europe and Asia	IEU VI	Asia
P. putrinum		IEU VII	Europe and Asia
IEU I	Asia	IEU VIII	North America
IEU II	Asia	IEU IX	North America
IEU III	Asia	IEU X	Asia
IEU IV	Europe and Asia	***P. caudatum***	
IEU V	Europe and Asia	IEU I	Asia, North America and South America
IEU VI	Asia	IEU II	Asia
IEU VII	Asia	IEU III	Europe and Asia
IEU VIII	Asia	IEU IV	?
IEU IX	Europe and Asia	IEU V	Oceania
IEU X	Europe and Asia	IEU VI	Oceania

Fonte: O autor

4.DISCUSSION

The establishment of accurately time-calibrated phylogenetic trees is of great importance to the better understanding of the evolution of any living organism (Parfrey et al., 2011). The first attempt to investigate the origin of Ciliophora, one of the most diverse group within microeukaryotes was done by Wright and Lynn (1997) using, as secondary calibrations for their 18S rDNA-based phylogeny the fossil record of teleost fishes which are parasitized by the ciliate *Ichthyophthirius*. According to their data the origin and diversification of Ciliophora occurred in the Paleo-proterozoic (1980-2200 Ma). Later, Fernandes and Schrago (2019), using multi-gene phylogeny and primary calibrations, based of unambiguously identified ciliate taxa, proposed a substantially earlier emergence of Ciliophora, which may have occurred 1143 Ma, with a highest diversification rate within the Paleozoic. According to these present work the evolutionary origin of *Paramecium* may have occurred within the Carboniferous (345 Ma). Therefore, besides it was one of the firsts Intramacronucleata (Ciliophora) groups to irradiate (Fernandes and Schrago, 2019), it occurred relatively late within Eukaryote evolution. By this period, terrestrial animal life was already well stablished and major extant insect lineages were already flying through out Earth, que contribuiu para o seu processo de dispersão (Misof et al., 2014).

However, despite many works have applied molecular dating methods to study the evolution of many groups of Ciliophora (Wright and Lynn,1997; Vďačný 2015; Rajter and Vďačný 2016; Rataj and Vďačný 2018; Vďačny 2018; Jiang et al. 2019; and Fernandes and Schrago 2019) the origin and evolution of extant *Paramecium* species, which have been extensively studied for more than 250 years as model organisms in a wide variety of research fields (Krenek et al. 2015) have not been accessed in previous works, therefore remaining elusive. Our estimate on the timescale of *Paramecium* origins indicates that the subgenus *Paramecium* was the first to irradiates within the Carboniferous (290.91 Ma) after split from a common ancestor with *Cypriostomum* and *Viridoparamecium* subgenera. Following, *Chloroparamecium* and *Helianter* subgenera separated within the Permian (255.14). The last subgenera to start their irradiation were *Cypriostomun* and *Viridoparamecium*, which may have occurred within the

Triassic (235.23 Ma). Among all extant *Paramecium* species, the first species to emerge was *P. polycaryum* (*Cyprinostomum*) while the last was *P. chlorelligerum* (*Viridoparamecium*).

Among species with cosmopolitan distribution *P. caudatum* was the first to emerge (132.53 Ma, 95% = 92.19-172.86 Ma), possibly within the Jurassic (145-201 Ma). Therefore, concomitantly with the final breakup of Laurasia and Gondwana supercontinents (Ericson 2011), which may have contributed to its worldwide distribution. On the other hand, *P. multimicronucleatum*, *P. aurelia* and *P. bursaria*, also cosmopolitans may have originated after the split of the continents suggesting efficient dispersion mechanisms may act to disperse these fresh/brackish water *Paramecium* through 6 out 7 continents.

Unfortunately, not all accepted *Paramecium* species have their 18S rDNA sequence available at public repositories, such as *P. africanum*, *P. wichtermani*, *P. pseudotrichium*. *P. grohmannae.* However, this sequence was eliminated from the analyses because is shorter than our stablished length cut off value (>1200 bp). The inclusion of short sequences into the dataset results in phylogenetic inferences with lower resolution, which could lead to erroneous phylogenetic relationships and low branch support values (Dittmar et al. 2006). Nevertheless, members of all subgenera could be included into our dataset and their evolutionary origin could be established.

Since its emergence, the genus *Paramecium* has certainly been exposed the distinct selective pressures and has had plenty of time for accumulate changes in its genetic material. And as presented in this work and in previous data from the morphology and molecular (Fokin, 2010; Krenek, 2015), the genus *Paramecium* recorded in a monophyletic as a sister group of *Tetrahymena* sp. subdivided into 5 subgenera

As in Hoshina et al. (2006), in this work, recover the subgenus groups that reflect similarities in body size in the cytostome position, stomatognathic patterns, number of somatic kinetics and nuclear characteristics, according to data with the Fokin et al. (2004). The members of the genus Paramecium were divided into two large groups: on one side or subgenera *Chloroparamecium* (*P. bursaria*) and *Helianter* (*P. duboscqui* and *P. putrinum*), on the other side *Cypriostomum* (*P. calkinsi, P. polycaryum, P. nephridiatum*) *Viridoparamecium* (*P. chloregelligerum*) and subgenera *Paramecium* (*P. woodruffi*, *P. jenningsi*, *P. aurelia complex*, *P. schewiakoffi*, *P. multimicronucleatum* and *P. caudatum* clade).

The quantity and micronucleus, as well as the structure of the contractile vacuole (Fokin, 2010) are also important taxonomic morphological characters for distinguishing species. Regarding the a variations of the micronucleus of this genus, the following variations can be found: Micronucleus of the vesicular type, which includes the species of *P. polycaryum*, *P. aurelia*, *P. multimicronucleatum.* Endosomal micronucleus: *P. calkinsi*, *P. woodruffi*, *P. dubosqui*, *P. nephridiatum*; Chromosomal type micronucleus: *P. jenningsi*, *P. schewiakoffi* and Compact micronucleus, which includes the species: *P. caudatum*, *P. bursaria*, *P. putrinum* and probably *P. ungandae* and *P. wichtermani*. In our work, we did not recover clades that reflected the similarity in relation to the micronucleus, considering then the grouping of these then artificial subgenera. And the same can be said for the pore number character of a contractile vacuole. For example, *P. caudatum* and *P. aurelia complex* spp. always present one pore per contractile vacuole, whereas *P. bursaria* and *P. nephridiatum*, from 2 to 5.

Regarding distance genetics, according to Shirk et al., (2016) it can be understood as the distance between differences in genetic material between species of the same species or not. In this work, *P. calkinsi* is the one with the highest average intraspecific genetic distance within the COI genetic marker of the Paramecium species, corroborating with Barth (2006), where he is also showing the highest average intraspecific variability. *P. calkinsi* has a close relationship with *P. nefridiatum* and also *P. woodruffi*, showing a lot of cytological similarities (number and type of micronuclei).

Their differences are more related to the behavioral level of swimming and molecular analysis (Przybós et al., 2012). So, we believe that this distance is greater in our analysis, and it is due errors of identification of some sequences of P. calkinsi and P. nphridiatum, leading to several phylogenetic placements independent of these two species in our phylogenetic reconstruction used in IOC, generating results confused in the mean intraspecific variation estimate.

When eliminating these species from the analysis, cosmopolitan species, *P. aurelia* (0.157), *P. multimicronucleatum* (0.066), *P. bursaria* (0.058) and *P. caudatum* (0.041), are among the *Paramecium* species with the highest intraspecific genetic distances within the COI molecular marker. Catania et al.

(2006), demonstrated that the strains reproductively use *P. aurelia* presenting substantial genetic variability. Barth et al., (2006) demonstrated an intraspecific divergence in the following COI data strings from *P. caudatum* and *P. multimicronucleatum.* Zhao et al., (2013), also demonstrate a significant genetic variation in *P. bursaria*, which raises questionings about their genetic structure and existence of cryptic species within accepted *Paramecium* species. In fact, previous works have already identified that many *Paramecium* morphospecies consist of cryptic species, morphologically identical species but genetically and reproductively isolated.

The most well-known species complex is *P. aurelia*, composed by 15 sibling species (Aufderheide et al. 1983; Sonneborn 1975) which may have diverged as result of two consecutive rounds of whole-genome duplication (WGD) events (Aury, 2006; McGrath et al., 2014). According to McGrath et al (2014), the most recent WGD event leading to *P. sexaurelia-P. biaurelia/P.tetraurelia* speciation, occurred ~320 Ma. This estimate came out on the bases of the stablished per site per cell division mutation rate of *P. tetraurelia* (Sung et al. 2012), assuming that this organism divides 100 times per year in nature. According to our time-calibrated tree this speciation event may have, actually occurred ~28.83 Ma. Suggesting that their generation time or mutation rate in nature may be substantially higher than predicted (McGrath et al., 2014).

However, although in lower numbers, evidences for the existence of cryptic species have been described in many morphospecies across most subgenera, such as subgenera *Paramecium*: *P. multimicronucleatum* (Tarcz et al., 2012) *P. caudatum* (Krenek et al., 2015), *P. jenningsi* (Przyboś and Tarcz, 2013, 2016), *P. bursaria* (Greczek-Stachura et al., 2012; Zagata et al., 2015); Subgenera Helianter: *P. putrinum* (Tarcz et al., 2014), *P. duboscqui* (Boscaro et al., 2012); Subgenera *Cypriostomum*: *P. calkinsi* (Przyboś et al., 2012), *P. polycarium* (Przyboś et al., 2018) and *P. nephridiatum* (Fokin et al., 1999; Przyboś et al., 2018).

But it is worth mentioning that the current understanding of the population genetics of free-living single-celled eukaryotes is still limited and the amount of genetic variability in these organisms is still a matter of debate.

Our data on the diversity of independent evolutionary units (IEUs) within the 18S rDNA and COI datasets using both species delimitation algorithms (PTP and mPTP) suggest that the diversity of cryptic species within *Paramecium* may be higher than previously thought. As expected, more IEUs were suggested within the COI dataset than in the 18S rDNA dataset, given their differences sequence conservation. Our data on diversity of independent evolutionary units (IEUs) in the 18S rDNA and COI data sets using the species delimitation algorithms (PTP and mPTP) suggest that the diversity of enigmatic species in *Paramecium* may be greater than previously thought. As expected, more IEUs were suggested in the COI dataset than in the 18S rDNA dataset, given as differences in sequence conservation, as we know that there is a greater variation in mtDNA when compared to the 18S gene. For this reason, mtDNA sequences are often used to study intraspecific genetic variation between species (Barth et al., 2006). It is also worth mentioning that, in our work, we had more IEUs delimited due to the much greater number of COI sequences used (507 sequences), than the 18S gene (125 sequences). As expected, given the greater sequence variation and greater sequence numbers compared to the 18S rDNA data set, analysis of IEUs using the COI data set identified substantially higher numbers of IEUs in the *Paramecium* morphospecies. And between the two analyzes (PTP and mPTP) within the COI data set, we would tend to believe that mPTP can represent a more realistic scenario about the diversity of enigmatic species in *Paramecium* morphospecies, representing a more conservative estimate. This can be attributed to the distinct Poisson exponential distribution for the branching events used by mPTP, which may allow the use of this algorithm for a wider range of empirical data than its predecessor (PTP) for delimiting IEUs (Kapli et al., 2017).

Considering data from the more conservative analysis (mPTP) of the 18S rDNA dataset *P. caudatum* and *P. multimicronucleatum* was divided in 2 and 3 IEUs, respectively, correctly distinguishing previously identified cryptic species, such as *Paramecium* sp. GO1 ("*Eucandidatus Paramecium germanicus*" - KM091236) (Krenek et al. 2015) and *P. caudatum* pakistanicus (HE662763, HE662764, HE662765) (Shakoori et al., 2014) from other *P. caudatum* and have distinguished *Paramecium* sp. BR3 ("*Eucandidatus paramecium brazilianum*" – AJ548822) (Krenek et al., 2015) from other *P. multimicronucleatum*. In his work, Krenek (2015) uses 18S-rDNA and COI markers, which already clearly indicate which species are in question, if they are possible cryptic species.

However, this method underestimated the number of potential cryptic species in comparison with data in the literature (Przyboś et al., 2018 and references within) by assigning to most other species only one IEU, and by grouping, *P. schewiakoffi*, *P. jenningsi* and *P. aurelia*, and *P. calkinsi* and *P. nephridiatum* as single IEUs. With respect to the analysis using PTP algorithm, our data also corroborate. Boscaro et al. (2012), in his work used three molecular markers, 18S rDNA, COI and the ITS1-5.8S-ITS2 genes, which provided evidence of the presence of cryptic species within *P. dubosqui*. Here, we identified two IEUs within *P. duboscqui*, one formed by HE819871-79 plus AF100312 and the other by AM236094.

In any case, these data from these two analyses highlight the limited power of resolution of this molecular marker to distinguish close related species and indicating more variable molecular marker would be preferable for identification of IEUs within *Paramecium* morphospecies.

Nevertheless, since all the work done here are based solely in DNA sequences and computational analyses future works performed by taxonomists applying integrative taxonomy insights would be required to better evaluate the real cryptic species diversity within *Paramecium* lineages. In any case, we understand that computational methods for IEUs identification may greatly contribute to this field, given methodological impediments of mating test performance for cryptic species identification in large population studies by means of reverse taxonomy (Markmann and Tautz 2005), as a tool to indicate potential lineages to be tested in more details using classical methods.

As mentioned before, the complex species structure in addition to under-sampling of many *Paramecium* morphospecies (Fokin, 2010), may effectively interfere with in our understanding with phylogeographic patterns within *Paramecium* (Przyboś et al., 2018). To shed some more light into this problem, we compare the data on the localities, within each sequence within the COI dataset was sampled with the IEUs delimitation data. However, similar results were noticed for the mPTP analysis. Our data show that, every one of these morphospecies can be composed by IEUs with restricted or wide geographical distribution; two or more IEUs can have overlapped distribution; However, any structure, correlation between IEUs and geographical distribution could be noticed for any morphospecies.

A crucial fact for the discussion about the distribution of these species is also a subsampling that has these microeucaryotes. On the European, Asian, and North American continents, the knowledge about the occurrence of *Paramecium* species is much more robust. In contrast, there is a gap about which species occur in the climatic regions (South America, Africa, for example). So, make a correlation between endemicity and omnipresence is still something that requires many studies, mainly in the identification of species by specialized taxonomists, and in the correct sequence of their genetic materials in climatic regions.

It seems that the "Every gene is everywhere, except the environment you select" view of Low Becoming in the microbial world can be applied to some IEUs identified in this work *P. bursaria*, *P. duboscqui*, *P. chlorelligerum*, *P. putrinium, P. polycaryum*, *P. jenningsi*, *P. multimicronucleatum* and *P. caudatum*, However, further studies combining more molecular markers, correct species identification, is desirable for a better explanation of this scenario.

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APENDICE A- Sequências 18S rDNA *Paramecium*

Table S1. 18S rDNA *Paramecium* sequences used in this work

Taxon	Accession number	Locality	IEU (PTP/mPTP)	Reference
Paramecium biaurelia	KU729877.1	North America	12/14	Szokoli,F.et al.,2016
Paramecium biaurelia	KU729876.1	North America	12/14	Szokoli,F.et al.,2016
Paramecium biaurelia	KX712111.1	?	12/14	Szokoli, unpublished
Paramecium biaurelia	MG009435.1	Europe	12/14	Potekhin,A.et al.,2018
Paramecium biaurelia	MG009438.1	Europe	12/14	Potekhin,A.et al.,2018
Paramecium biaurelia	MG009437.1	Europe	12/14	Potekhin,A.et al.,2018
Paramecium buetschlii	KM091234.1	Europe	03/04	Krenek,S. et al.,2015
Paramecium bursaria	AB206537.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB206538.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	KC495065.1	?	01/01	Gong. J. unpublished
Paramecium bursaria	AB206539.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB206541.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB206543.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	KC495069.1	?	01/01	Gong. J.unpublished
Paramecium bursaria	KC495068.1	?	01/01	Gong. J.unpublished
Paramecium bursaria	AB206540.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB206542.1	Asia	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB219526.1	Oceania	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	KC495067.1	?	01/01	Gong. J. unpublished
Paramecium bursaria	KC495070.1	?	01/01	Gong. J. unpublished
Paramecium bursaria	KC495064.1	?	01/01	Gong. J. unpublished
Paramecium bursaria	KC495066.1	?	01/01	Gong. J. unpublished
Paramecium bursaria	AB206544.1	Europe	01/01	Hoshina,R. et al.,2005
Paramecium bursaria	AB206545.1	Europe	01/01	Hoshina,R.et al.,2005
Paramecium bursaria	AB252000.1	?	01/01	Hoshina,R.et al.,2006
Paramecium bursaria	AB252001.1	Europe	01/01	Hoshina,R. et al.,2006
Paramecium bursaria	LN869937.1	Europe	01/01	Rossi, A.et al.,2015
Paramecium bursaria	LN869940.1	Europe	01/01	Rossi, A.et al.,2015
Paramecium bursaria	AB252002.1	?	01/01	Hoshina,R.et al.,2006
Paramecium bursaria	LN869938.1	Europe	01/01	Rossi, A.et al.,2015
Paramecium bursaria	MG589318.1	Asia	01/01	Lanzoni, O. et al., 2018
Paramecium bursaria	MG589317.1	Asia	01/01	Lanzoni, O. et al.,2018
Paramecium bursaria	MG589316.1	Asia	01/01	Lanzoni, O. et al.,2018
Paramecium calkinsi	AF100301.1	North America	13/15	Struder-Kypke,M.C.et al.,2000
Paramecium calkinsi	AF100310.1	Europe	13/15	Struder-Kypke,M.C.et al.,2000
Paramecium calkinsi	MH819561.1	South America	13/15	Campello-Nunes,P.H. 2018
Paramecium calkinsi	KY996811.1	Europe	13/16	Sabaneyeva,E.et al.,2018
Paramecium calkinsi	MG018200.1	Europe	13/16	Sabaneyeva,E.et al.,2018

Paramecium caudatum	AB252003.1	Asia	06/08	Hoshina,R. et al.,2006
Paramecium caudatum	AB252005.1	Asia	06/08	Hoshina,R. et al.,2006
Paramecium caudatum	AF217655.1	North America	06/08	Struder-Kypke,M.C. et al.,2000
Paramecium caudatum	MG589311.1	Europe	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MG697233.1	Europe	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MG589309.1	Asia	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MG009439.1	Europe	06/08	Potekhin,A.et al.,2018
Paramecium caudatum	MG589314.1	Asia	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MG589310.1	Europe	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MH188278.1	South America	06/08	Castelli, M. et al.,2018
Paramecium caudatum	AB252004.1	Asia	06/08	Hoshina,R. et al.,2006
Paramecium caudatum	HE664171.1	Europe	06/08	Schrallhammer,M. et al.,2013
Paramecium caudatum	MG589312.1	Europe	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	MG589313.1	Asia	06/08	Lanzoni, O. et al.,2018
Paramecium caudatum	LT549004.1	Asia	06/08	Serra,V. 2017
Paramecium caudatum	HE662763.1	Asia	08/10	Shakoori et al., 2014
Paramecium caudatum	HE662765.1	Asia	08/10	Shakoori et al., 2014
Paramecium caudatum	HE662764.1	Asia	08/10	Shakoori et al., 2014
Paramecium chlorelligerum	KM091235.1	Europe	16/19	Krenek,S. et al.,2015
Paramecium chlorelligerum	KX669631.1	Asia	16/19	Lanzoni, O. et al.,2017
Paramecium chlorelligerum	KX669630.1	Asia	16/19	Lanzoni, O. et al.,2017
Paramecium chlorelligerum	KX669629.1	Asia	16/19	Lanzoni, O. et al.,2017
Paramecium duboscqui	AF100312.1	Europe	02/02	Struder-Kypke,M.C et al.,2000
Paramecium duboscqui	HE819879.1	Africa	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819874.1	Europe	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819871.1	Asia	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819873.1	North America	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819872.1	Asia	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819876.1	Europe	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819875.1	Europe	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819877.1	Europe	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	HE819878.1	Europe	02/02	Boscaro,V. et al.,2012
Paramecium duboscqui	AM236094.1	Asia	02/03	Schrallhammer,M. et al.,2006
Paramecium duboscqui	HM140398.1	?	15/18	Khan and Shin,unpublished
Paramecium jenningsi	AF100311.1	Asia	12/14	Struder-Kypke,M.C.et al.,2000
Paramecium jenningsi	HE662760.1	Asia	12/14	Shakoori et al., 2014
Paramecium multimicronuclatum	AB252006.1	Asia	09/11	Hoshina,S. et al.,2006
Paramecium multimicronuclatum	AF255361.1	North America	09/11	Struder-Kypke,M.C.et al.,2000
Paramecium multimicronuclatum	AB252007.1	Asia	09/11	Hoshina,S. et al.,2006

Paramecium multimicronuclatum	MG994988.1	Asia	10/12	Kaur,R. et al.,2018
Paramecium multimicronuclatum	HE650906.1	Asia	11/13	Shakoori et al., 2014
Paramecium multimicronuclatum	HE662762.1	Asia	11/13	Shakoori et al., 2014
Paramecium multimicronuclatum	HE650907.1	Asia	11/13	Shakoori et al., 2014
Paramecium multimicronuclatum	HG315606.1	Europe	11/13	Vanini,C.et al.,2014
Paramecium multimicronuclatum	HG315607.1	Europe	11/13	Vanini,C.et al.,2014
Paramecium multimicronuclatum	HE662761.1	Asia	11/13	Shakoori et al., 2014
Paramecium multimicronuclatum	HG315605.1	Europe	11/13	Vanini,C.et al.,2014
Paramecium multimicronuclatum	LT549006.1	Europe	11/13	Serra,V. 2017
Paramecium multimicronuclatum	LT549005.1	Europe	11/13	Serra,V. 2017
Paramecium nephridiatum	AF100316.1	Asia	15/18	Struder-Kypke,M.C.et al.,2000
Paramecium nephridiatum	HE978251.1	Europe	15/18	Boscaro,V. et al.,2013
Paramecium nephridiatum	AF100317.1	Asia	15/18	Struder-Kypke,M.C.et al.,2000
Paramecium nephridiatum	MG573198.1	Asia	15/18	Lanzoni, O. et al.,2018
Paramecium novaurelia	MG009440.1	Europe	12/14	Potekhin,A.et al.,2018
Paramecium pentaurelia	MG573197.1	Asia	12/14	Lanzoni, O. et al., 2018
Paramecium polycaryum	AF100313.1	Asia	14/17	Struder-Kypke,M.C.et al.,2000
Paramecium primaurelia	AF100315.1	Asia	12/14	Struder-Kypke,M.C.et al.,2000
Paramecium primaurelia	MG573195.1	Europe	12/14	Lanzoni, O. et al.,2018
Paramecium primaurelia	MG573196.1	Europe	12/14	Lanzoni, O. et al.,2018
Paramecium primaurelia	MG573194.1	Asia	12/14	Lanzoni, O. et al.,2018
Paramecium putrinum	AF255360.1	Asia	05/06	Struder-Kypke,M.C. et al.,2000
Paramecium putrinum	MG573199.1	Europe	05/07	Lanzoni, O. et al.,2018
Paramecium schewiakoffi	AJ548821.1	Asia	12/14	Fokin,S. et al.,2004
Paramecium tetraurelia	AB252008.1	North America	12/14	Hoshina,S. et al.,2006
Paramecium tetraurelia	AB252009.1	?	12/14	Hoshina,S. et al.,2006
Paramecium tetraurelia	X03772.1	?	12/14	Sogin & Elwood.,1986
Paramecium tetraurelia	AF149979.1	Asia	12/14	Preer,L.B et al.,1999
Paramecium tetraurelia	AY102613.1	Asia	12/14	Beier,C.L.et al.,2002
Paramecium tetraurelia	KY852452.1	?	12/14	Wang, unpublished
Paramecium tetraurelia	EF502045.1	Asia	12/14	Shuja,R.N.et al.,2009
Paramecium woodruffi	AF255362.1	Asia	15/18	Struder-Kypke,M.C.et al.,2000
Paramecium sp.	LN869981.1	Europe	06/08	Rossi, A.et al.,2015
Paramecium sp.	KM091236.1	Europe	07/09	Krenek,S. et al.,2015
Paramecium sp.	AJ548822.1	Asia	11/13	Michaux,unpublished
Paramecium sp.	FR869987.1	Asia	11/13	Shakoori et al., 2014

Paramecium sp.	LN869927.1	Europe	11/13	Rossi, A.et al.,2015
Paramecium sp.	LN869962.1	Europe	11/13	Rossi, A.et al.,2015
Paramecium sp.	LN869956.1	Europe	12/14	Rossi, A.et al.,2015
Paramecium sp.	LN869961.1	Europe	12/14	Rossi, A.et al.,2015
Paramecium sp.	MG009436.1	Europe	12/14	Potekhin,A.et al.,2018
Paramecium sp.	FJ875142.1	?	13/15	Zhang and Song unpublished
Paramecium sp.	FR869986.1	?	13/15	Zahid, unpublished
Paramecium sp.	KM091237.1	Europe	15/18	Krenek,S. et al.,2015
metagenome	OBAT01029766.1	Europe	15/18	Karst M,S. 2017
metagenome	OBEP011407967.1	Europe	15/18	Karst M,S. 2017
metagenome	OBRS01776142.1	Europe	15/18	Karst M,S. 2017
metagenome	OBEP011068739.1	Europe	05/06	Karst M,S. 2017
Tetrahymena rostrata	AF364042.1	North America		Struder-Kypke,M.C.et al.,2001
Tetrahymena pyriformis	EF070254.1	?		Chantangsi,unpublished
Tetrahymena tropicalis	EF070259.1	?		Chantangsi,unpublished
Terahymena thermophila	M10932.1	North America		Spangler and Blackburn,1985
Euplotes eurystomus	AF452707.1	?		Chen and Song,unpublished
Euplotes aediculatus	AF508756.1	North America		Hewitt,E.A.et al.,2003
Moneuplotes crassus	AF508761.1	North America		Hewitt,E. A. et al.,2003

APENDICE B- Sequências COI *Paramecium*

Table S2. COI *Paramecium* sequences used in this work

Taxon	Accession number	Locality	IEU (PTP/mPTP)	Reference
Paramecium biaurelia	JX661375.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	MF593920.1	Europe	38/34	Tarcz,S. et al., 2018
Paramecium biaurelia	JX661378.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	KX752228.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	HF969033.1	North America	38/34	Boscaro,V. et al.,2014
Paramecium biaurelia	MF593919.1	Oceania	38/34	Tarcz,S. et al., 2018
Paramecium biaurelia	KX008306.1	North America	38/34	Szokoli,F. et al., 2017
Paramecium biaurelia	KX008305.1	North America	38/34	Szokoli,F. et al., 2017
Paramecium biaurelia	KX752227.1	Europe	38/34	Tarcz,S. et al.,2017
Paramecium biaurelia	JX661383.1	North America	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661379.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661377.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661381.1	Asia	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661376.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661380.1	Europe	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	HF948010.1	North America	38/34	Boscaro, V. et al.,2013
Paramecium biaurelia	KX752226.1	Europe	38/34	Tarcz,S. et al., 2017
Paramecium biaurelia	JX661382.1	Asia	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	JX661374.1	North America	38/34	Tarcz,S. et al., 2013
Paramecium biaurelia	HF969032.1	Europe	38/34	Boscaro, V. et al.,2014
Paramecium biaurelia	FN421325.1	Europe	38/34	Barth & Berendonk, 2011
Paramecium biaurelia	JX661433.1	Asia	38/34	Tarcz,S. et al., 2013
Paramecium bursaria	JX082039.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082034.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082030.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082041.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082037.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082035.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082040.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082036.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082033.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082028.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082038.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082027.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082025.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082032.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082031.1	Asia	82/69	Zhao, Y. et al.,2013

Paramecium bursaria	JX082023.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082026.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082029.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082022.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082062.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082063.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082061.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082060.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JF708906.1	Asia	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JF708903.1	Asia	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JF708907.1	Asia	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JF708908.1	Asia	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701571.1	Asia	82/69	Zagata,P. et al.,2014
Paramecium bursaria	JF708910.1	Asia	82/69	Greczek-Stachura, M. et al.,2012
Paramecium bursaria	JF708909.1	Asia	82/69	Greczek-Stachura, M. et al.,2012
Paramecium bursaria	JF708902.1	Asia	82/69	Greczek-Stachura, M. et al.,2012
Paramecium bursaria	JX082058.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082059.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082057.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082015.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082017.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082056.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082019.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082014.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082045.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JF708891.1	Europe	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JX082012.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082048.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082050.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082046.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	KJ701573.1	Asia	82/69	Zagata,P. et al., 2014
Paramecium bursaria	JX082043.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082053.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082042.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082021.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082049.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082054.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082020.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082051.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082047.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082016.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	KJ701572.1	Asia	82/69	Zagata,P. et al., 2014
Paramecium bursaria	JX082013.1	Asia	82/69	Zhao, Y. et al.,2013

Paramecium bursaria	JX082055.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082052.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JX082018.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JF708894.1	Europe	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JX082044.1	Asia	82/69	Zhao, Y. et al.,2013
Paramecium bursaria	JF708901.1	Asia	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JF708892.1	Europe	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	JF708886.1	Europe	82/69	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701576.1	Asia	81/68	Zagata,P. et al., 2014
Paramecium bursaria	KJ701577.1	Asia	81/68	Zagata,P. et al., 2014
Paramecium bursaria	JF708905.1	Asia	81/68	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701575.1	North America	80/67	Zagata,P. et al.,2014
Paramecium bursaria	KJ701574.1	North America	80/67	Zagata,P. et al.,2014
Paramecium bursaria	JF708916.1	Asia	79/66	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701563.1	Asia	78/65	Zagata,P. et al.,2014
Paramecium bursaria	KJ701560.1	Asia	78/65	Zagata,P. et al.,2014
Paramecium bursaria	JF708913.1	Asia	77/65	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701564.1	Asia	77/65	Zagata,P. et al.,2014
Paramecium bursaria	KJ701570.1	Asia	77/65	Zagata,P. et al.,2014
Paramecium bursaria	KJ701561.1	Asia	77/65	Zagata,P. et al.,2014
Paramecium bursaria	KJ701569.1	Asia	77/65	Zagata,P. et al.,2014
Paramecium bursaria	KJ701568.1	Asia	77/65	Zagata,P. et al.,2014
Paramecium bursaria	JF708912.1	Asia	77/65	Tarcz,S. et al.,2012
Paramecium bursaria	JF708911.1	Asia	77/65	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701567.1	Asia	77/65	Zagata,P. et al., 2014
Paramecium bursaria	KJ701566.1	Asia	77/65	Zagata,P. et al., 2014
Paramecium bursaria	KJ701562.1	Asia	77/65	Zagata,P. et al., 2014
Paramecium bursaria	JF708904.1	Asia	77/65	Tarcz,S. et al.,2012
Paramecium bursaria	KJ701565.1	Asia	76/64	Zagata,P. et al.,2014
Paramecium bursaria	JF708915.1	Oceania	76/64	Tarcz,S. et al.,2012
Paramecium bursaria	JF708914.1	Oceania	76/64	Tarcz,S. et al.,2012
Paramecium bursaria	JF708898.1	Europe	76/64	Tarcz,S. et al.,2012
Paramecium bursaria	FN421334.1	Asia	76/64	Barth & Berendonk, 2011Tarcz,S. et al.,2012
Paramecium bursaria	KJ701558.1	Asia	75/63	Zagata,P. et al.,2014
Paramecium bursaria	KJ701556.1	Asia	74/63	Zagata,P. et al.,2014

Paramecium bursaria	KJ701559.1	Europe	73/62	Zagata,P. et al.,2014
Paramecium bursaria	JF708899.1	Europe	73/62	Tarcz,S. et al., 2012
Paramecium bursaria	JF708897.1	Europe	73/62	Tarcz,S. et al., 2012
Paramecium bursaria	JF708896.1	Europe	73/62	Tarcz,S. et al., 2012
Paramecium bursaria	JF708893.1	Europe	73/62	Tarcz,S. et al., 2012
Paramecium bursaria	KJ701557.1	Asia	73/62	Zagata,P. et al.,2014
Paramecium bursaria	JF708895.1	Europe	73/62	Tarcz,S. et al.,2012
Paramecium bursaria	FJ905152.1	North America	73/62	Strueder-Kypke & Lynn, 2016
Paramecium calkinsi	JF304178.1	Asia	68/58	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304173.1	Asia	66/56	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304174.1	Asia	66/56	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304175.1	Asia	62/54	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304180.1	Asia	61/53	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304181.1	Asia	61/53	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304179.1	Asia	61/53	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304177.1	Asia	60/52	Tarcz,S. et al., 2012
Paramecium calkinsi	JF304176.1	Asia	60/52	Tarcz,S. et al., 2012
Paramecium calkinsi	FN421329.1	Europe	58/50	Barth, D. & Berendonk, 2011
Paramecium calkinsi	MG953270.1	North America	57/49	Tarcz,S. et al., 2012
Paramecium calkinsi	MG953269.1	North America	57/49	Tarcz,S. et al., 2012
Paramecium calkinsi	FJ905147.1	Europe	56/48	Strueder-Kypke & Lynn, 2016
Paramecium caudatum	HQ149724.1	Oceania	14/14	Krenek,S. et al., 2012
Paramecium caudatum	HQ149723.1	Oceania	14/14	Krenek,S. et al., 2012
Paramecium caudatum	HQ149722.1	Oceania	13/13	Krenek,S. et al., 2012
Paramecium caudatum	FN256283.1	North America	12/12	Snoke,M.S. et al., 2016
Paramecium caudatum	HQ149725.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	FN256281.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256280.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256264.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256263.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256274.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256272.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256275.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256273.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256271.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256279.1	Europe	11/11	Snoke,M.S. et al., 2016

Paramecium caudatum	FN256265.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	HQ149721.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	HQ149720.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	FN256266.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	HQ149726.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	HQ149716.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	FN256270.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256269.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256268.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256267.1	Asia	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	AM072782.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	FN256278.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	FN256276.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	HQ149719.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	FN256277.1	Europe	11/11	Snoke,M.S. et al., 2016
Paramecium caudatum	AM407718.1	Europe	11/11	Krenek,S. et al., 2016
Paramecium caudatum	AM072781.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	HQ149717.1	Europe	11/11	Krenek,S. et al., 2012
Paramecium caudatum	AM072778.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	HQ149718.1	Asia	11/11	Krenek,S. et al., 2012
Paramecium caudatum	FJ905148.1	Asia	11/11	Strueder-Kypke & Lynn, 2016
Paramecium caudatum	AM072779.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	AM407720.1	Europe	11/11	Strueder-Kypke & Lynn, 2016
Paramecium caudatum	AM072777.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	AM407719.1	Europe	11/11	Krenek, S. et al., 2016
Paramecium caudatum	AM072780.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	JF304187.1	Europe	11/11	Przybos,E. et al., 2012
Paramecium caudatum	AM072776.1	Europe	11/11	Barth,D. et al., 2016
Paramecium caudatum	KY399443.1	Europe	10/10	Killeen,J. et al., 2017
Paramecium caudatum	FN256282.1	South America	09/09	Snoke,M.S. et al., 2016
Paramecium caudatum	FJ905142.1	South America	09/09	Strueder-Kypke & Lynn, 2016
Paramecium caudatum	AM072775.1	Oceania	09/09	Barth,D. et al., 2016
Paramecium caudatum	JX082083.1	Asia	09/09	Zhao, Y. et al., 2013

Paramecium caudatum	JX082096.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082101.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082080.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082079.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082097.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082100.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082095.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082099.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082078.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082094.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082081.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082103.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082077.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082102.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082098.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082076.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082069.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082086.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082088.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082084.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082093.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082068.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082090.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082073.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082092.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082089.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082072.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082074.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082071.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082070.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082087.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082082.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082091.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082085.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082066.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082067.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082075.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082065.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	JX082064.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium caudatum	AM072774.1	Asia	09/09	Zhao, Y. et al., 2013
Paramecium chlorelligerum	KX669633.1	Asia	55/47	Lanzoni, O. et al., 2017
Paramecium chlorelligerum	KX669632.1	Asia	55/47	Lanzoni, O. et al., 2017
Paramecium chlorelligerum	KX669634.1	Asia	55/47	Lanzoni, O. et al., 2017
Paramecium chlorelligerum	KM091239.1	Europe	55/47	Krenek, S. et al., 2015

Paramecium decaurelia	JX661435.1	Asia	32/29	Tarcz,S. et al.,2013
Paramecium decaurelia	JX661434.1	Asia	32/29	Tarcz,S. et al.,2013
Paramecium decaurelia	JX661437.1	Europe	26/24	Tarcz,S. et al.,2013
Paramecium decaurelia	JX661436.1	Europe	26/24	Tarcz,S. et al.,2013
Paramecium decaurelia	JX661419.1	Europe	37/33	Tarcz,S. et al.,2013
Paramecium dubosqui	HE819881.1	Asia	44/40	Boscaro,V. et al.,2012
Paramecium dubosqui	HE819880.1	Asia	43/39	Boscaro,V. et al.,2012
Paramecium dubosqui	JX082147.1	Asia	42/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082145.1	Asia	42/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082142.1	Asia	42/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082141.1	Asia	42/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082128.1	Asia	42/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082146.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082131.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082136.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082130.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082134.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082139.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082137.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082143.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082138.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082132.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082140.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082135.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082133.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082144.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082127.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082129.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	JX082126.1	Asia	41/38	Zhao, Y. et al.,2013
Paramecium dubosqui	HE819886.1	Asia	40/37	Boscaro,V. et al.,2012
Paramecium dubosqui	HE819883.1	Asia	40/37	Boscaro,V. et al.,2012
Paramecium dubosqui	FN421332.1	Asia	40/37	Barth & Berendonk, 2011
Paramecium dubosqui	HE819885.1	Asia	40/36	Boscaro,V. et al.,2012
Paramecium dubosqui	HE819882.1	Asia	40/36	Boscaro,V. et al.,2012
Paramecium dubosqui	HE819884.1	Asia	40/36	Boscaro,V. et al.,2012
Paramecium dubosqui	FJ905146.1	Asia	40/36	Strueder-Kypke & Lynn, 2016
Paramecium dubosqui	JF304185.1	South America	39/35	Przybos,E. et al., 2012
Paramecium jennignsi	KF110713.1	Asia	23/21	Przybos and Tarcz., 2014
Paramecium jennignsi	JF304188.1	Asia	23/21	Przybos,E. et al., 2012

Paramecium jennignsi	KF110712.1	Asia	22/20	Przybos and Tarcz., 2014
Paramecium jennignsi	FN421328.1	Europe	22/20	Barth and Berendonk, 2011
Paramecium jennignsi	KF110711.1	Africa	21/19	Przybos &Tarcz, 2014
Paramecium jennignsi	KY635386.1	Asia	21/19	Tarcz,S. et al., 2018
Paramecium jennignsi	KF110710.1	Africa	21/19	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110709.1	Asia	21/19	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110718.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110719.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110715.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110717.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110716.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	KF110714.1	Asia	20/18	Przybos &Tarcz, 2014
Paramecium jennignsi	FJ905139.1	Asia	20/18	Strueder-Kypke & Lynn, 2016
Paramecium multimicronucleatum Clade I	JF741271.1	North America	08/08	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	MH544200.1	Asia	07/07	Lu,X. et al.,2019
Paramecium multimicronucleatum Clade I	FJ905145.1	Asia	07/07	Strueder-Kypke & Lynn., 2016
Paramecium multimicronucleatum Clade I	JF741247.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741246.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741257.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741270.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741245.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	AM072771.1	Europe	07/07	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	JF741251.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741244.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741261.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741269.1	Asia	07/07	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	AM072770.1	Europe	07/07	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	AM072772.1	South America	06/06	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	MH544191.1	Asia	05/05	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544190.1	Asia	05/05	Lu,X. et al., 2019

Paramecium multimicronucleatum Clade I	JF741259.1	Asia	05/05	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741248.1	Asia	05/05	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	MH544199.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544197.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544195.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544193.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544192.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544194.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544189.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544188.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544185.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	JF741260.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741255.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	MH544196.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	AM072769.1	Asia	04/04	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	JF741242.1	Europe	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	MH544187.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	MH544186.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	JF741267.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741268.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741256.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	MH544198.1	Asia	04/04	Lu,X. et al., 2019
Paramecium multimicronucleatum Clade I	JF741266.1	Asia	04/04	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	AM072768.1	Asia	04/04	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	JF741273.1	North America	03/03	Tarcz,S. et al.; 2012
Paramecium multimicronucleatum Clade I	JF741272.1	North America	03/03	Tarcz,S. et al.; 2012
Paramecium multimicronucleatum Clade I	JF304189.1	North America	03/03	Tarcz,S. et al.; 2012
Paramecium multimicronucleatum Clade I	FJ905144.1	North America	02/02	Strueder-Kypke & Lynn, 2016
Paramecium multimicronucleatum Clade I	FJ905143.1	North America	02/02	Strueder-Kypke & Lynn, 2016
Paramecium multimicronucleatum Clade I	JF741249.1	Asia	01/01	Tarcz,S. et al., 2012

Paramecium multimicronucleatum Clade I	JF741254.1	Europe	01/01	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741243.1	Europe	01/01	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	JF741252.1	Europe	01/01	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	AM072767.1	Europe	01/01	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	JF741253.1	Europe	01/01	Tarcz,S. et al., 2012
Paramecium multimicronucleatum Clade I	AM072766.1	Oceania	01/01	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade I	JF741258.1	Asia	01/01	Barth,D. et al., 2016
Paramecium multimicronucleatum Clade II	JF741265.1	Asia	16/15	Tarcz,S.et al.,2012
Paramecium multimicronucleatum Clade II	JF741264.1	Asia	16/15	Tarcz,S.et al.,2012
Paramecium multimicronucleatum Clade II	JF741263.1	Asia	16/15	Tarcz,S.et al.,2012
Paramecium multimicronucleatum Clade II	JF741262.1	Asia	16/15	Tarcz,S.et al.,2012
Paramecium multimicronucleatum Clade II	JF741250.1	Asia	15/15	Tarcz,S.et al.,2012
Paramecium nephridiatum	MG953273.1	Asia	67/57	Tarcz,S. et al.,2018
Paramecium nephridiatum	MG953272.1	Asia	67/57	Tarcz,S. et al.,2018
Paramecium nephridiatum	MG953271.1	Asia	67/57	Tarcz,S. et al.,2018
Paramecium nephridiatum	JX082124.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082115.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082125.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082112.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082116.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082111.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082117.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082108.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082118.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082106.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082122.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082105.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082119.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082120.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082114.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082123.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082110.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082109.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082121.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082113.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082107.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	JX082104.1	Asia	67/57	Zhao & Song, 2013
Paramecium nephridiatum	FN421331.1	Europe	67/57	Barth, D. 2016
Paramecium nephridiatum	FJ905151.1	Asia	66/56	Strueder-Kypke & Lynn, 2016

Paramecium nephridiatum	MG953276.1	Asia	65/55	Tarcz,S. et al., 2018
Paramecium nephridiatum	MG953275.1	Asia	62/55	Tarcz,S. et al., 2018
Paramecium nephridiatum	MG953274.1	Asia	63/55	Tarcz,S. et al., 2018
Paramecium novaurelia	KX752244.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium novaurelia	KX752246.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium novaurelia	KX752245.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium novaurelia	KX752247.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium novaurelia	KX752243.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium novaurelia	JX661431.1	Asia	33/30	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661430.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661432.1	Asia	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661428.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661425.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	KP973727.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661426.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661427.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	JX661429.1	Europe	32/29	Tarcz,S. et al., 2013
Paramecium novaurelia	FN421327.1	Europe	32/29	Barth,D. & Berendonk, 2011
Paramecium novaurelia	JX661389.1	Europe	31/28	Tarcz,S. et al., 2013
Paramecium octaurelia	JX661424.1	Asia	37/33	Tarcz,S. et al.,2013
Paramecium octaurelia	JX661422.1	Europe	37/33	Tarcz,S. et al.,2013
Paramecium octaurelia	JX661421.1	Europe	37/33	Tarcz,S. et al.,2013
Paramecium octaurelia	JX661420.1	Europe	37/33	Tarcz,S. et al.,2013
Paramecium octaurelia	JX661418.1	Asia	37/33	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661405.1	Europe	32/29	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661402.1	Asia	32/29	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661401.1	Europe	29/27	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661406.1	Asia	29/27	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661404.1	Europe	29/27	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661400.1	Europe	29/27	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661403.1	Asia	29/27	Tarcz,S. et al.,2013
Paramecium pentaurelia	JX661399.1	Europe	29/27	Tarcz,S. et al.,2013
Paramecium polycaryum	MG953285.1	North America	72/61	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953278.1	Africa	71/61	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953284.1	Asia	70/60	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953283.1	Asia	70/60	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953277.1	Asia	70/60	Tarcz,S. et al., 2018
Paramecium polycaryum	FN421330.1	Europe	70/60	Barth, D. 2016
Paramecium polycaryum	MG953282.1	North America	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953280.1	Asia	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953281.1	Asia	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953279.1	Asia	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	MH432643.1	Asia	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	MG953286.1	North America	69/59	Tarcz,S. et al., 2018
Paramecium polycaryum	FJ905155.1	Asia	69/59	Strueder-Kypke & Lynn, 2016

Paramecium primaurelia	JX661373.1	Asia	28/26	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661370.1	Asia	27/25	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661368.1	Asia	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661367.1	Asia	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661372.1	Europe	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661366.1	Europe	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661369.1	Asia	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661365.1	Europe	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JX661371.1	North America	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	JF304182.1	Europe	26/24	Tarcz,S. et al., 2013
Paramecium primaurelia	FN421324.1	Asia	26/24	Barth & Berendonk, 2011
Paramecium primaurelia	FJ905141.1	Europe	26/24	Strueder-Kypke & Lynn, 2016
Paramecium primaurelia	KX752234.1	Asia	36/34	Tarcz,S. et al., 2017
Paramecium putrinum	KF649989.1	Asia	54/46	Tarcz,S. et al.,2014
Paramecium putrinum	KF649988.1	Asia	54/46	Tarcz,S. et al.,2014
Paramecium putrinum	KF649984.1	Asia	53/45	Tarcz,S. et al.,2014
Paramecium putrinum	KF649992.1	Asia	52/44	Tarcz,S. et al.,2014
Paramecium putrinum	KF649994.1	Asia	51/44	Tarcz,S. et al.,2014
Paramecium putrinum	KF649993.1	Europe	51/44	Tarcz,S. et al.,2014
Paramecium putrinum	KF649987.1	Asia	51/44	Tarcz,S. et al.,2014
Paramecium putrinum	KF649991.1	Asia	50/43	Tarcz,S. et al.,2014
Paramecium putrinum	KF649990.1	Asia	49/43	Tarcz,S. et al.,2014
Paramecium putrinum	KF649986.1	Asia	48/43	Tarcz,S. et al.,2014
Paramecium putrinum	KF649985.1	Asia	48/43	Tarcz,S. et al.,2014
Paramecium putrinum	KF649983.1	Asia	47/42	Tarcz,S. et al.,2014
Paramecium putrinum	KF649982.1	Asia	45/41	Tarcz,S. et al.,2014
Paramecium putrinum	FN421333.1	Asia	45/41	Barth & Berendonk, 2011
Paramecium putrinum	FJ905153.1	Asia	45/41	Strueder-Kypke & Lynn, 2016
Paramecium septaurelia	JX661417.1	Asia	37/33	Tarcz,S. et al., 2013
Paramecium septaurelia	JX661415.1	Asia	37/33	Tarcz,S. et al., 2013
Paramecium septaurelia	JX661416.1	Asia	37/33	Tarcz,S. et al., 2013
Paramecium septaurelia	JX661414.1	Asia	37/33	Tarcz,S. et al., 2013
Paramecium septaurelia	JX661396.1	Asia	36/32	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661413.1	Asia	25/23	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661410.1	Asia	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661409.1	Asia	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661411.1	Asia	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661408.1	Asia	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	KX752239.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752237.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752238.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752241.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752236.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752240.1	Europe	24/22	Tarcz,S. et al., 2017

Paramecium sexaurelia	JX661412.1	Asia	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	JX661407.1	Europe	24/22	Tarcz,S. et al., 2013
Paramecium sexaurelia	KX752242.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	KX752235.1	Europe	24/22	Tarcz,S. et al., 2017
Paramecium sexaurelia	FJ905154.1	Europe	24/22	Strueder-Kypke & Lynn, 2016
Paramecium sexaurelia	KY635387.1	Asia	23/21	Tarcz,S. et al., 2018
Paramecium sonneborni	KF650001.1	Europe	19/17	Przybos,E. et al.,2014
Paramecium shewiakoffi	AM072773.1	Asia	18/18	Barth,D. et al.,2006
Paramecium tedrecaurelia	JX661440.1	Asia	39/35	Tarcz,S. et al., 2013
Paramecium tedrecaurelia	JF304184.1	Europe	39/35	Przybos,E. et al., 2012
Paramecium tedrecaurelia	KX752225.1	Europe	38/34	Tarcz,S. et al.,2017
Paramecium tetraurelia	JX661392.1	Europe	36/32	Tarcz,S. et al. 2013
Paramecium tetraurelia	JX661394.1	Europe	36/32	Tarcz,S. et al. 2013
Paramecium tetraurelia	JX661393.1	Europe	36/32	Tarcz,S. et al. 2013
Paramecium tetraurelia	JF304183.1	Oceania	36/32	Przybos,E. et al., 2012
Paramecium tetraurelia	KX752233.1	Europe	36/32	Tarcz,S. et al., 2017
Paramecium tetraurelia	KX752232.1	Europe	36/32	Tarcz,S. et al., 2017
Paramecium tetraurelia	KX752231.1	Europe	36/32	Tarcz,S. et al., 2017
Paramecium tetraurelia	KX752229.1	Europe	36/32	Tarcz,S. et al., 2017
Paramecium tetraurelia	JX661395.1	Europe	36/32	Tarcz,S. et al.,2013
Paramecium tetraurelia	JX661398.1	Europe	36/32	Tarcz,S. et al.,2013
Paramecium tetraurelia	JX661397.1	Europe	36/32	Tarcz,S. et al.,2013
Paramecium tetraurelia	FN421326.1	Europe	36/32	Barth & Berendonk, 2011
Paramecium tetraurelia	JX661439.1	North America	35/31	Tarcz,S. et al.,2013
Paramecium triaurelia	JX661390.1	Asia	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661388.1	Europe	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661391.1	Asia	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661387.1	Europe	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661386.1	North America	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661385.1	North America	31/28	Tarcz,S. et al., 2013
Paramecium triaurelia	JX661384.1	North America	30/28	Tarcz,S. et al., 2013
Paramecium undecaurelia	JX661438.1	North America	34/31	Tarcz,S. et al., 2013
Paramecium undecaurelia	KX752248.1	Europe	33/30	Tarcz,S. et al., 2017
Paramecium woodruffi	FJ905150.1	Asia	59/51	Strueder-Kypke & Lynn, 2016
Paramecium sp.	KM091241.1	Europe	64/54	Krenek,S.et al.,2015
Paramecium sp.	JX082150.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082159.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082149.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082158.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082148.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082152.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082151.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082154.1	Asia	43/39	Zhao, Y. et al., 2013

Paramecium sp.	JX082153.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082156.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082155.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	JX082157.1	Asia	43/39	Zhao, Y. et al., 2013
Paramecium sp.	FJ905140.1	North America	26/24	Strueder-Kypke & Lynn, 2016
Paramecium sp.	KM091240.1	Europe	17/16	Krenek,S.et al.,2015
Paramecium sp.	FJ905149.1	Asia	11/11	Strueder-Kypke & Lynn, 2016
Tetrahymena thermophila	DQ411879.1	North America		Lynn & Strueder-Kypke,2006
Tetrahymena sp.	GU439294.1	North America		Kher,C.P.et al., 2011
Tetrahymena sp.	GU439293.1	North America		Kher,C.P.et al., 2011
Tetrahymena sp.	GU439292.1	North America		Kher,C.P.et al., 2011
Tetrahymena tropicalis	GU439311.1	North America		Kher,C.P.et al., 2011
Tetrahymena pyriformis	GU439229.1	North America		Kher,C.P.et al., 2011
Tetrahymena canadensis	GU439213.1	North America		Kher,C.P.et al., 2011
Tetrahymena hegewischi	GU439219.1	North America		Kher,C.P.et al., 2011