

Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives

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Abstract Despite most of the cytogenetic research is focused on monocentric chromosomes, chromosomes with kinetochoric activity localized in a single centromere, several studies have been centered on holocentric chromosomes which have diffuse kinetochoric activity along the chromosomes. The eukaryotic organisms that present this type of chromosomes have been relatively understudied despite they constitute rather diversified species lineages. On the one hand, holocentric chromosomes may present intrinsic benefits (chromosome mutations such as fissions and fusions are potentially neutral in holocentrics). On the other hand, they present restrictions to the spatial separation of the functions of recombination and segregation during meiotic divisions (functions that may interfere), separation that is found in monocentric chromosomes. In this study, we compare the diversification rates of all known holocentric lineages in animals and plants with their most related monocentric lineages in order to elucidate whether

holocentric chromosomes constitute an evolutionary advantage in terms of diversification and species richness. The results showed that null hypothesis of equal mean diversification rates cannot be rejected, leading us to surmise that shifts in diversification rates between holocentric and monocentric lineages might be due to other factors, such as the idiosyncrasy of each lineage or the interplay of evolutionary selections with the benefits of having either monocentric or holocentric chromosomes.

Keywords Centromere · chromosome · diversification rates · holocentric · phylogeny

Abbreviations

CenH3 Centromere-specific histone H3 variant
%GC Percentage of guanine-cytosine content in the DNA

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Introduction

Chromosomal evolution constitutes one of the main drivers of speciation, affecting diversification rates in eukaryotic organisms (Coghlan et al. 2005). Chromosomal evolution could be caused by several phenomena: whole genome duplication (polyploidy), partial genome duplications, or deletions, fusions, fissions, and translocations (Faulkner 1972; Luceño 1993; Luceño and Guerra 1996; Mola and Papeschi 2006; Hipp et al. 2013). The consequences of some of these events are

dissimilar depending on the location of the kinetochores in the chromosomes. In monocentric chromosomes, kinetochores are concentrated in a single locus, the centromere. In holocentric chromosomes, the kinetochore activity is widely distributed along the chromosomes, the diffuse centromere (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013).

In holocentric chromosomes, chromosome fragments (due to fissions) and fused or translocated chromosomes are correctly segregated during the anaphase. In monocentric chromosomes, chromosome fragments that lack kinetochore activity are lost during cell divisions, and fused chromosomes form dicentric chromosomes that also fail in correct segregation during cell divisions (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013). Nonetheless, holocentric chromosomes could present structural restrictions during the meiosis. The functions of segregation (kinetochore function) and recombination are not spatially separated. This is probably the reason that explains that holocentric chromosomes usually have one or two chiasmata during meiotic divisions (Nijalingappa 1975; Nokkala et al. 2004; Bigliardo et al. 2011; Luceño, pers. obs.; for *Rhynchospora* species in Cyperaceae) (Nijalingappa 1975; Nokkala et al. 2004) and it has been reported that the presence of only three chiasmata already hindered the process of segregation during meiotic divisions (Nokkala et al. 2004). As a result, organisms with this type of chromosomes have developed different mechanisms to correctly segregate during metaphase I (Mola and Papeschi 2006; Melters et al. 2012): (i) limiting the loci that present kinetochore activity to the ends of the chromosomes (restriction of kinetochore activity); (ii) splitting sister chromatids during meiosis I and homologous chromosomes during meiosis II, known as inverted meiosis; and (iii) reducing considerably the number of cross-over events or do not develop chiasmata (achiasmate meiosis). It is worth mentioning that some authors have questioned the presence of inverted meiosis in some groups (see Nokkala et al. 2006).

Holocentric chromosomes are shared by a reduced number of unrelated eukaryotic lineages comprised in Rhizaria, Archaeplastida, and Opisthokonta superclades [reviewed by Mola and Papeschi (2006), Melters et al. (2012), and Escudero et al. (2016)]. Reviews about the presence of these peculiar chromosomes in the Tree of Life showed that within the Rhizaria superclade, these are exhibited by the genera *Aulacantha* (i.e., *A. scolymantha*, Aulacanthidae, order Phaeocystida), *Spongospora*,

Sorosphaera, and *Plasmodiophora* (Plasmodiophoraceae, order Plasmodiophorales). In the Opisthokonta superclade, there are 12 holocentric lineages: in Nematoda orders Ascaridida, Rhabditida, and Tylenchida and in the genus *Epiperipatus* (Peripatidae, order Euonychophora) and several Arthropoda lineages such as families Dysderidae and Segestriidae (order Araneae), superorder Acariformes and genus *Rhipicephalus* (Ixodidae, Parasitiformes), superfamily Buthoidea (order Scorpiones), class Chilopoda (orders Lithobiomorpha and Scutigermorpha), and Hexapoda orders Odonata, Zoraptera, Dermaptera, Psocoptera, Phthiraptera, Thysanoptera, Hemiptera, Trichoptera, and Lepidoptera. Finally, in the Archaeplastida superclade, holocentric chromosomes appear in three eudicotyledonous angiosperms *Myristica* (i.e., *M. fragrans*, Myristicaceae, order Magnoliales), *Drosera* (Droseraceae, order Caryophyllales), and *Cuscuta* (Convolvulaceae, order Solanales) genera and in two monocotyledonous lineages Melanthiaceae (order Liliales) and Cyperaceae + Juncaceae clade (order Poales). Although holocentric chromosomes had been reported in some families of the order Zingiberales (Chakravorti 1948a, b), these results were rejected by later studies (see revision of Mahanty 1970). Holocentricity has been also rejected in the order Palpigradi (Kral et al. 2008).

Melters et al. (2012) also stated that holocentricity has undergone four reversion processes to monocentricity in insects, but Escudero et al.'s (2016) results pointed out five separate origins of holocentricity, which is supported by the four independent losses of CenH3 (centromere-specific histone H3 variant) (Drinnenberg et al. 2014). One fascinating lineage of insects with holocentric chromosomes is the order Lepidoptera that constitutes a remarkable case of the variability that this trait confers on the chromosome number [families Lycaenidae, $2n = 20\text{--}268$ (Lukhtanov et al. 2005; Kandul et al. 2007), and Nymphalidae, $2n = 10\text{--}240$ (Dincă et al. 2011)]. Two other lineages with remarkable chromosome variability are the genera *Cyperus* and *Carex* from the plant family Cyperaceae ($2n = 10\text{--}224$ and $12\text{--}124$; Roalson 2008).

The commonly accepted hypothesis of holocentricity as independently arisen from monocentricity, although probable, remains currently unclear (Escudero et al. 2016). Despite the theoretical advantages presented by holocentric chromosomes, monocentric chromosomes could be also advantageous because of some evolutionary benefits. For example, the spatial separation between the functions of segregation and recombination

may ease the correct segregation of chromosomes during meiosis. At present, there are no studies that disentangle whether or not there are differences in diversification rates between these lineages (Escudero et al.'s (2016) results were not satisfactory on this issue because of the low percentage of sampling in the current phylogeny of eukaryotes). Thus, the aim of this study is to elucidate whether or not there are significant differences in diversification rates between lineages with holocentric chromosomes and their monocentric counterpart sister clades. Due to the better knowledge and the availability of literature about species number and age estimations, here, we focus our study on Archaeplastida and Opisthokonta superclades.

Materials and methods

Evolutionary relationships and chromosomal study revision

Although scarce, cytological studies of holocentricity have promoted the search of these peculiar chromosomes to confirm their occurrence or to reject it in doubtful lineages (e.g., monocentricity in Zingiberales and Palpigradi; Mahanty 1970; Král et al. 2008). Studies on systematics of most holocentric lineages have been carried out throughout the years since Melters et al. (2012) published the first representation of how holocentric chromosomes are phylogenetically distributed along eukaryotic organisms. However, these advances do not affect Melters et al.'s main conclusions (see also Escudero et al. 2016). Here, we display main changes and gaps in the current information available about organisms with holocentric chromosomes and how we treated them in this study.

Droseraceae (order *Caryophyllales*)

The holokinetic sundew genus *Drosera* represents an unusual case because of the presence of monocentric chromosomes in some of its species, concretely *Drosera regia* (Shirakawa et al. 2011) and maybe *Drosera aliciae* and *Drosera binate* [stated by Demidov et al. (2014) and questioned by Veleba et al. (2017)]. Other separate *Drosera* species and genus *Aldrovanda* are holocentric (Sheikh et al. 1995; Sheikh and Kondo 1995; Shirakawa et al. 2011). However, the monotypic genus *Dionaena* (Droseraceae) seems to present monocentric chromosomes

(see Shirakawa et al. 2011). In this study, we compared all *Droseraceae* species (which include some monocentric species) with its sister monocentric clade which is composed of the families *Nepenthaceae*, *Drosophyllaceae*, *Dioncophyllaceae*, and *Ancistrocladaceae* (Veleba et al. 2017).

Myristicaceae (order *Magnoliales*)

The main problem in this family is that holocentricity has only been inferred in *Myristica fragrans* (Flach 1966), and it has not been studied in other *Myristicaceae* species. The presence of holocentric chromosomes is unclear in this lineage. Waiting for further detailed information about the phylogeny and cytological data, here, we compared the entire family *Myristicaceae* with its sister clade which is constituted by the families *Magnoliaceae*, *Degeneriaceae*, *Himantandraceae*, *Eupomatiaceae*, and *Annonaceae* in the order *Magnoliales* (Sauquet et al. 2003).

Melanthiaceae (order *Liliales*)

Holokinetic activity is only known in the genus *Chionographis* (eight spp., Wu et al. 2016) and its related monotypic genus *Chamaelirium* (Kim et al. 2016), although some studies pointed them as monocentric (Tamura 1998; Tanaka 2003). Kinetochoic activity has not been confirmed yet and remains to be thought as holocentric (see Bureš et al. 2013). Nevertheless, monocentric chromosomes have been reported in the tribe *Heloniadeae*, sister to *Chionographis* and *Chamaelirium* (e.g., genus *Ypsilandra*, Hsu et al. 2011). We have compared *Chionographis* and *Chamaelirium* against genera *Helonias*, *Ypsilandra*, and *Heloniopsis*.

Cuscuta (*Convolvulaceae*, order *Solanales*)

It is worth mentioning that, in this genus, holocentricism has been reported mostly in the subgenus *Cuscuta*, as well as in one species (*C. parodiana*) of the monocentric subgenus *Grammica*, whereas monocentric chromosomes have been confirmed in the species of the subgenus *Monogynella* (Pazy and Plitmann 1995; Guerra et al. 2010; Bureš et al. 2013). Meanwhile, the kinetochore distribution of the endemic South African subgenus *Pachystigma* (Costea et al. 2015), with just five species and evolutionary placed between subgenera

Cuscuta and *Grammica* (García et al. 2014; Costea et al. 2015), has not been yet studied. Nevertheless, due to the large amount of monocentric chromosome records in *Grammica*, this type of chromosomes might be expected in this subgenus. Nonetheless, whether or not monocentrism could occur within *Pachystigma* and *Grammica* must be confirmed by thorough cytological studies. In any case, holocentricity would constitute a paraphyletic character presented in subgenera *Cuscuta* and *Grammica* but not in the subgenus *Pachystigma* nor the rest of *Grammica* (Guerra et al. 2010; Bureš et al. 2013). Most of the species of the subgenus *Grammica* could be monocentric based on indirect data (different genome sizes, see Bureš et al. 2013). Thus, we compared the subgenus *Cuscuta* with the presumably monocentric subgenera *Grammica* and *Pachystigma*.

Cyperaceae and *Juncaceae* (order *Poales*)

Cyperaceae and Juncaceae constitute two sister families which kinetochore activity has been well studied, leaving no doubt regarding the presence of holocentric chromosomes in these species lineages (e.g., Malheiros and De Castro 1947; Greilhuber 1995; Håkansson 2010). Unlike these taxa, the clade sister family Thurniaceae has not been so well studied. Whether monocentric or holocentric chromosomes are presented in this lineage is yet unclear. Nonetheless, we treat this family as holocentric because other studies infer so (see Kubitzki 1998) and have been supported due to its low percentage of guanine-cytosine content in the DNA (%GC) and genome size, similar to Cyperaceae and Juncaceae (Šmarda et al. 2014). Also, further cytological studies of the related taxa to these families are yet to be done, although we can assume the families of its sister clade could be monocentric (e.g., Poaceae, see Electronic Supplementary Material S1). We compared Cyperaceae, Juncaceae, and Thurniaceae against Poaceae, Ecdociaceae, Joinvilleaceae, Flagellariaceae, Restionaceae, Xyridaceae, Eriocaulaceae, and Mayacaceae families.

Insecta (Arthropoda)

In Opisthokonta, most of systematic changes occurred in arthropods, in which phylogenomic studies have resolved the backbone of subphylum Hexapoda (Misof et al. 2014), showing orders Zoraptera and Dermaptera as the most ancient monophyletic lineage in the superorder Polyneoptera. Moreover, Thysanoptera and

Hemiptera orders (superorder Condylgnatha) constitute a sister clade to several monocentric (belonging to Holometabola superorder) and holocentric (i.e., Psocodea superorder and clade Trichoptera + Lepidoptera in Holometabola) orders. On the other hand, the superorder Palaeoptera, which diverged early in time and includes every holocentric lineage of Hexapoda and most of monocentric lineages, comprises both holocentric and monocentric taxa (Odonata and Ephemeroptera, respectively; Kiauta and Mol 1977; Soldán and Putz 2000). Holocentric and monocentric chromosomes have been only reported in just a few species of these lineages (see Drinnenberg et al.'s (2014) revision of holocentric insects) if the high species richness of insects is taken into account. However, kinetochore structure is accepted to remain stable along the lineages (although there exist some exceptions: i.e., hemipteran genus *Ranatra*; Desai and Deshpande 1969), showing well-documented cases of the holocentric chromosome appearance (Drinnenberg et al. 2014). This well-documented stability of holocentric chromosomes allowed us to consider it as apomorphic in the different lineages.

Thus, we compared five insect lineages: (i) Odonata against Ephemeroptera, (ii) Zoraptera and Dermaptera (Polyneoptera) against remaining monocentric Polyneoptera (Plecoptera, Orthoptera, Mantophasmatodea, Grylloblattodea, Embioptera, Phasmatodea, Mantodea, and Blattodea), (iii) Condylgnatha against Psocodea and Holometabola, (iv) Psocodea against Holometabola, and (v) holocentric Holometabola (Trichoptera and Lepidoptera) against remaining Holometabola (Siphonaptera, Mecoptera, and Diptera).

Centipedes (class *Chilopoda*, *Arthropoda*)

Holocentrism has been reported in the centipede orders Lithobiomorpha and Scutigermorpha. In the first case, holocentric chromosomes were proposed and confirmed for some species of the family Henicopidae but not for its sister families Lithobiidae and Ethopolyidae, which were inferred as monocentric (Ogawa 1953, 1955; Battaglia and Boyes 1955; Colmagro et al. 1986). In the second order, kinetochore nature has been scarcely studied. Most of the studies are in Scutigeridae family which has been stated to present holocentric chromosomes (Ogawa 1953; Colmagro et al. 1986). However, its sister family Scutigerinidae and the family Pselliodidae (sister to Scutigeridae and Scutigerinidae)

have not been karyologically studied. Due to the probable independent nature of holocentricity in Chilopoda and in the light of the studies in Lithobiomorpha, we assume holocentric chromosomes occur within family level until more cytological studies are made. Other closely related families as Geophilomorpha and Scolopendromorpha have been reported as monocentric whereas Craterostigmomorpha has not been yet studied (Ogawa 1953; Colmagro et al. 1986).

Scutigermorpha family Scutigerae and family Scutigerae were analyzed as holocentric and monocentric paired lineages, respectively. Lithobiomorpha families Henicopidae and Lithobiidae were analyzed as holocentric and monocentric lineages, respectively.

Acariformes and Parasitiformes superorders (Arthropoda)

There are few studies regarding the mite superorder Acariformes, but here, we assumed it is entirely holocentric. Holocentricity has been proved for most of the studied species, especially for many species from Prostigmata and Oribatida suborders (e.g., Oliver 1972; Oliver et al. 1974; Oliver 1977; Eroğlu and Per 2016). Nonetheless, Oribatida also includes species with a localized centromere (Heethoff et al. 2007) which could be pointing to a separate origin of holocentricity in Acariformes. Further studies in order to clarify these origins would be required. On the other hand, thick superorder Parasitiformes presents holocentric chromosomes in the family Ixodidae. In this family, they only appear to be present in some species of the genus *Rhipicephalus* (Oliver 1977; Hill et al. 2009) but it has not been reported in other lineages of this superorder. We treated the holocentric *Rhipicephalus* as a sister group of *Amblyomma* (Jeyaprakash and Hoy 2009).

Superorder Acariformes (Sarcoptiformes and Trombidiformes) were analyzed against Parasitiformes (Holothyrida, Ixodida, and Mesostigmata). On the other hand, the Ixodidae holocentric genus *Rhipicephalus* was compared to the sister genus *Amblyomma*.

Dysderoidea (order Araneae, Arthropoda)

Six-eyed spider lineages in which holocentrism was first documented (Dysderidae and Segestriidae, see Král et al. 2006) appear now included in a clade with Orsolobidae and Oonopidae families (Garrison et al. 2016; Wheeler et al. 2016). In this clade, the presence

of a diffuse kinetochore has been recently reported (Král et al. 2006). This suggests holocentricity as an ancestral character in the superfamily Dysderoidea. However, cytological reports informing whether the sister clade conformed by Caponiidae and Trogloraptoridae presents monocentric chromosomes were not found and we decided to treat them as the closest monocentric related lineage. Thus, we treated Dysderoidea as a sister to families Caponiidae and Trogloraptoridae.

Buthoidea (order Scorpiones, Arthropoda)

Recent studies have shed light into the phylogenetic relationships of the order Scorpiones, classifying it into two parvorders (Buthida and Iurida; Sharma et al. 2015). Holocentricity has been confirmed in Buthida (Shanahan 1989a; Schneider et al. 2009; Mattos et al. 2013; Adilardi et al. 2016), specifically in the Buthidae family. However, there are no cytological studies for the sister families Chaerilidae and Pseudochactidae. Nevertheless, studies of Iurida families confirm monocentricity in some members of that parvorder (Shanahan 1989b; Schneider et al. 2009; Kovařík et al. 2017). Thus, we decided to compare both parvorders that are mostly monocentric and holocentric (Iurida against Buthida), respectively.

Peripatidae family (Onychophora)

A single species of the genus *Epiperipatus* (family Peripatidae) has been reported as holocentric (i.e., *E. biolleyi*; Mora et al. 1996) while other species of Peripatidae and its sister family Peripatopsidae have been documented of presenting localized centromeres (see Rowell et al. 1995; Jeffery et al. 2012; Oliveira et al. 2012a, b). The analysis of genus *Epiperipatus* is problematic due to the unresolved evolutionary history of the velvet worms, especially in this genus whose species appear separate in the latest phylogenies (Oliveira et al. 2012a; Murienne et al. 2013), some of which are monocentric (see Jeffery et al. 2012). Further studies on cytogenetic and systematics of the onychophorans are required. Taking into account the uncertainty concerning this lineage (see Oliveira et al.'s (2012b) revision) and with awareness of the problematic that represents a lineage with both monocentric and holocentric species, we compared the family Peripatidae (holocentric and, probably, also monocentric) with the family Peripatopsidae (only monocentric).

Phylum Nematoda

Phylogenetic relationships among the lineages of this phylum have been recently well established (Blaxter 2011; Rota-Stabelli et al. 2013), although cytogenetic studies have not been widely performed. Chromosomes with holocentromeres have been reported in several species of classes Secernentea and Chromadorea (see revision of Melters et al. 2012 and literature therein), whereas in the sister lineage constituted by the remaining Nematoda class Enoplea (Rota-Stabelli et al. 2013), monocentric chromosomes have been reported (Mutafova et al. 1982; Špakulová et al. 1994). Thus, we compared the first two classes with the third one.

Studied lineages

Literature of the lineages (a total of 18 holocentric lineages) was checked in Archaeplastida and Opisthokonta (Table S1). Phylogenetic relationship between holocentric lineages and their monocentric sister lineages, species richness, and stem node ages (when divergence between both lineages occurred) were collected. For *Cuscuta*, stem node was obtained from a secondary calibration of previous studies (unpublished data). Diversification rates for holocentric lineages and their sister monocentric lineages were estimated with the R package geiger (Harmon et al. 2008), under two scenarios: with no extinction presented ($\mu / \lambda = 0$) and with a higher rate of extinction ($\mu / \lambda = 0.9$). μ depicts the extinction rate and λ represents the speciation rate.

Because some of monocentric sister groups include holocentric taxa (i.e., sister clades of Thysanoptera + Hemiptera, Psocoptera + Phthiraptera, and Trichoptera + Lepidoptera) and because some of the holocentric clades include some monocentric species (i.e., Acariformes superorder, Hemiptera order, and families Droseraceae and Peripatidae), we created two datasets. The first one including all lineages (18 pair of lineages) whereas the second was only constituted for those holocentric and monocentric lineages that did not contain any monocentric or holocentric species, respectively (12 pure pair of lineages). We conducted the same analyses in both datasets.

Comparison tests

The Shapiro-Wilk tests were carried out in the R package stats (R Development Core Team 2016) in order to detect

the normality of the data. Because normality was not inferred in some occasions, we realized both parametric and non-parametric tests. In non-parametric analyses, comparison of means was assisted with the Wilcoxon signed-rank test (package stats, R Development Core Team 2016). For parametric analyses, data was normalized using the square root of the values according to the transformation suggested by the R package cars (function powerTransform, Fox and Weisberg 2011), for an ulterior Bayesian paired test implemented in the R package BEST (Kruschke 2013).

Results

Our results showed different species richness according to the divergence ages of the related holocentric-monocentric lineages (Table S1), resulting in datasets for which diversification rates followed a logarithmic distribution for both pairs of lineages and both high and none extinction rate scenarios (Table S2, Fig. 1). Mean values of the complete dataset are 0.04121267 and 0.04581069 net speciation events per million years, for holocentric and monocentric lineages under no extinction scenario, and 0.02444473 and 0.02880966 net speciation events per million years in high extinction scenario, for holocentric and monocentric lineages, respectively (Fig. 1). Moreover, diversification rates of monocentric lineages were subtracted from the holocentric ones, depicting a roughly gradual continuum among lineages, except for *Cuscuta* lineage, which difference in diversification rates is the highest (Fig. 2).

The Shapiro-Wilk tests for detect normalization of the data revealed that diversification rates with high extinction rate were normally distributed. However, diversification rates for holocentric chromosomes with no extinction were not normally distributed (diversification rates of monocentric lineages were normal). In case of the subset of pure pair of lineages, all the data followed a normal distribution (Table S3).

On the first analyses, all data were treated as non-normal and the Wilcoxon signed-rank test for paired groups showed that equal means could not be rejected for both high and none extinction scenarios (p value = 0.154 and 0.1815, respectively; Table S3). This analysis was not performed in the pure dataset because it was normally distributed. Finally, after square root transformation (Fox and Weisberg 2011) of the values of the complete dataset, implementation of the BEST

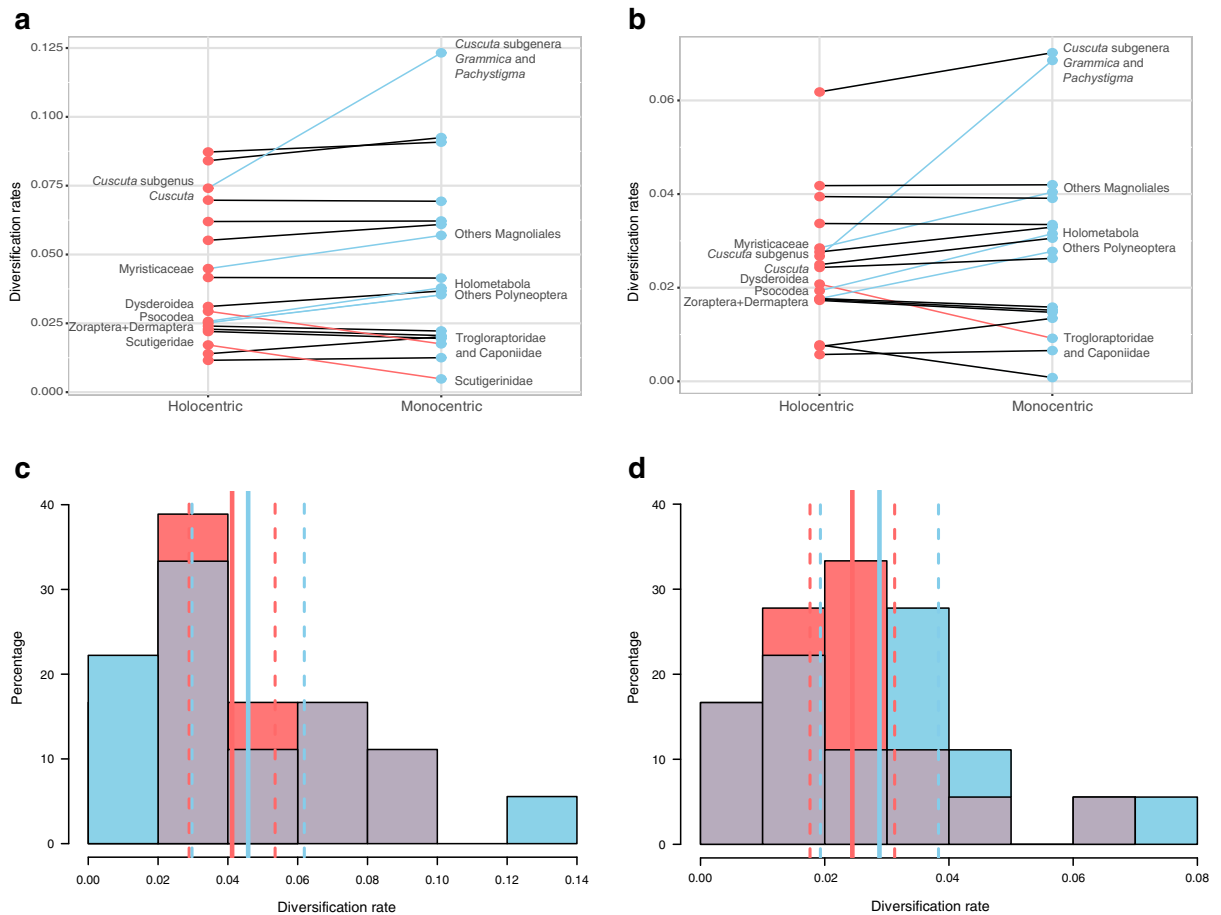


Fig. 1 Diversification rates of related holocentric and monocentric lineages for absent (a) and high (b) extinction rate scenarios. Taxa names are depicted for those lineages with the highest diversification rate differences (connected by red or blue

lines). Histograms of holocentric (red) and monocentric (blue) lineages; gray area depicts overlapping. Mean and 95% interval of confidence (dashed lines) for absent (c) and high (d) extinction rate scenarios

package (Kruschke 2013) also revealed that differences between means could not be rejected with a 95% of the highest density interval (Fig. 3, Table S3–S4). Furthermore, identical result was also obtained from the BEST package (Kruschke 2013) analysis of the pure lineages (Table S3, Table S5, Figure S1).

Discussion

No holocentric vs. monocentric shifts in diversification rates and methodological caveats

Diversification rates could represent a quantifiable estimation of the evolutionary success of a lineage in comparison with its most closely related lineage. Despite for

some groups (butterflies and sedges) holocentric chromosomes may present intrinsic benefits by providing karyological variability and, thus, increasing speciation (Lukhtanov et al. 2005; Kandul et al. 2007; Hipp 2007; Roalson 2008; Dincă et al. 2011), our results did not support that statement when comparing monocentric lineages with related holocentric ones (Fig. 1, Table S2–S3). In fact, our analyses proved how diversification rates are relatively balanced among these sister lineages (Fig. 1a, b), with most of values below a rate of ca. 0.04 net speciation event per million years in scenarios of absent or high extinction levels, overlapping in their distributions (Fig. 1c, d, Table S2). Moreover, the differences of means are quite gradual among the lineages, from *Cuscuta* genus, with the higher score in monocentric, to Scutigeromorpha, with higher value in the holocentric lineages (Fig. 2).

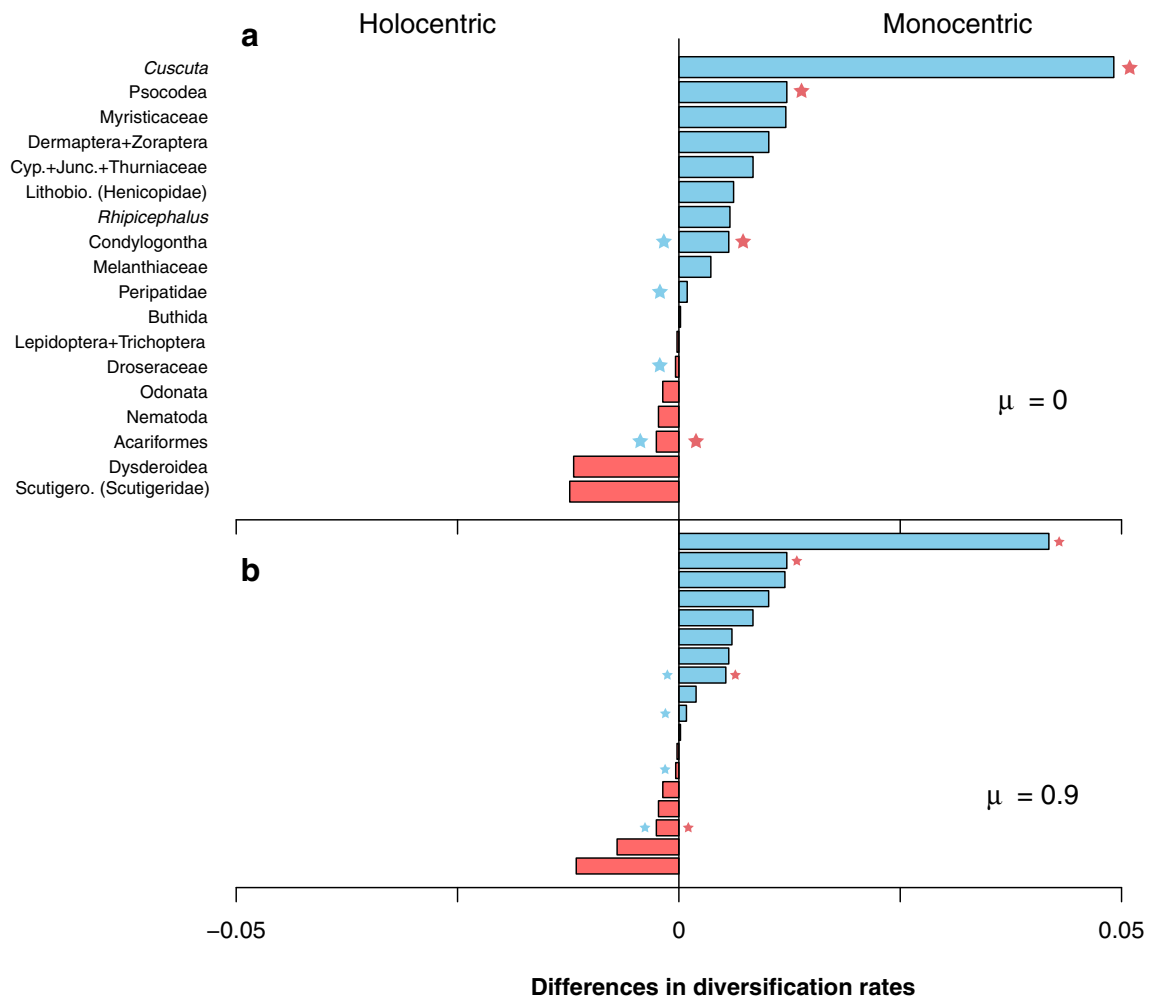


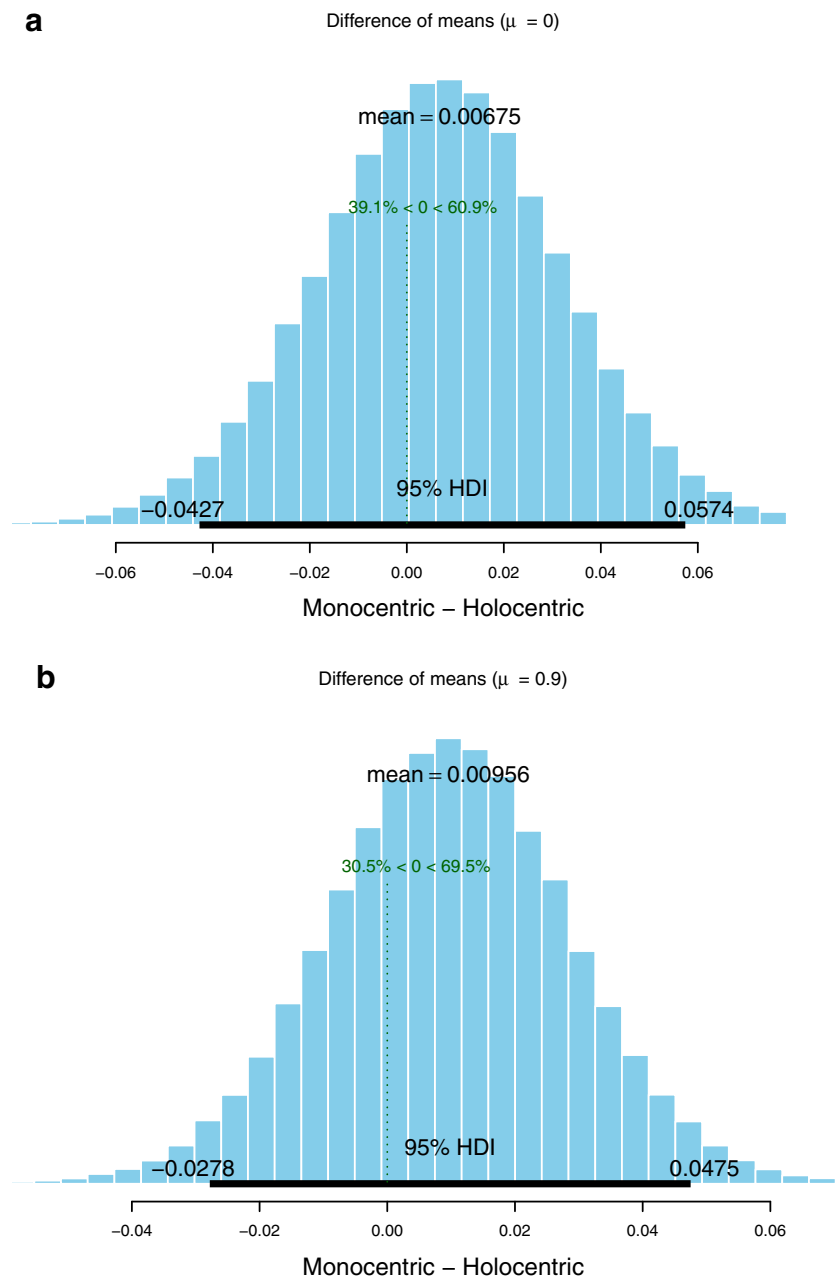
Fig. 2 Subtraction of holocentric diversification rates minus their monocentric sister lineages for absent (a) and high (b) extinction rate scenarios. Stars represent those holocentric lineages that contain monocentric species (blue) and vice versa (red)

These results did not depict an abrupt offset in favor of the eukaryotic organism that present holocentric chromosomes.

Nonetheless, as stated above (see “[Materials and methods](#)”), due to the lack of studies regarding cytological information about the presence of primary constrictions of some of the lineages and the different knowledge of species richness for each one, we made assumptions that might though be somewhat biasing the results. However, we consider that our findings are not compromised by cytogenetic uncertainty. First, holocentric vs. monocentric chromosomes are usually conservative trait states in the lineages with rare cases of fine-scale reversions. Second, statistical results of the differences between holocentric and monocentric means in diversification rates support the

actual results even when we exclude the lineages with the high uncertainty as a result mix pattern of monocentricity and holocentricity. (This is supported by our congruent results based on all data and only pure lineages; [Figure S1](#)). Third, this pattern of no difference in diversification rates holds even if we would vary extremely patterns of species richness in the lineages with the highest uncertainty. In this way, one of the most remarkable cases is the genus *Cuscuta*, in which we assumed monocentricity in the subgenus *Pachystigma* and that presents the highest monocentric diversification rate under both scenarios (Fig. 1a, b). However, in these scenarios (the subgenus *Pachystigma* either having localized centromeres or not), monocentricity would present a higher diversification rate due to the small number of species appearing in the mostly

Fig. 3 Bayesian estimate for the difference of diversification rate means of the complete dataset and its 95% high density intervals (HDI) for absent (**a**) and high (**b**) extinction rate scenarios



monocentric subgenus *Grammica* (153 spp., although some clade might be holocentric; Guerra et al. 2010; Bureš et al. 2013) as well as the low species number of the subgenus *Pachystigma* itself (five spp.; see Table S1). Other problematic lineage would be Myristicaceae, for which further cytological studies are required at family level. Nonetheless, whether Myristicaceae was removed from the analyses (see Electronic Supplementary

Material S1) or the genus *Myristica* was the only holocentric genus in Myristiceae, there would certainly not be any meaningful modification in our study results. In the latter case, *Myristica* would be compared to its sister genus *Knema* (Sauquet et al. 2003). Other instances in which kinetochoric activity must be examined are Buthidae—that would predominate as holocentric when facing its two poorly diversified sister families Chaerilidae

and Pseudochactidae—likewise Dysderoidea and its sister cytologically understudied families Caponiidae and Trogloraptoridae, genus *Drosera*, family Peripatidae, order Hemiptera, and superorder Acariformes; these four latter lineages were presenting confirmed monocentric chromosomes (Desai and Deshpande 1969; Heethoff et al. 2007; Shirakawa et al. 2011; Jeffery et al. 2012). As remarked before, despite this cytogenetic uncertainty, both datasets (full dataset and pure dataset, which excludes doubtful lineages) leave no doubt in this regard: holocentric and monocentric lineages do not have significant differences in mean diversification rates (Figs. 2 and 3, Electronic Supplementary Material S1).

Karyotypic diversity within holocentric lineages does not drive higher diversification rates

As stated before, holocentric and monocentric lineages do not have significant differences in mean diversification rates (Table S3–S5). The question here is whether monocentric vs. holocentric chromosomes are neutral in terms of evolutionary success or both have an evolutionary meaning being more evolutionarily successful depending on the specific situation. The restriction of having holocentric chromosomes in comparison of having monocentric chromosomes is the non-spatial separation of the functions of recombination and segregation which may result in a problematic segregation of the chromosomes during meiosis I, when chiasmata are formed (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013). Holocentric organisms have developed several mechanisms to elude it. The best documented case is the restriction of kinetochore activity to a small region of the chromosome, presented in the model species *Caenorhabditis elegans* (Albertson and Thomson 1993; Monen et al. 2005) or some hemipterans (e.g., *Oncopeltus fasciatus*; Comings and Okada 1972). In these cases, chromosomes act as monocentric in meiosis with the kinetochoric plate located in the chromosomes termini, which leads to a monocentric-like meiosis (further information in Melters et al. 2012). In the so-called “inverted meiosis,” a frequent mechanism in organisms having holocentric chromosomes, homologous chromatids are auto-oriented in meiosis I, whereas they are cooriented in monocentric organisms (Faulkner 1972; Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013). Inverted meiosis has been observed

in several organisms such as sedges, rushes, arachnids, hemipterans, dragonflies, and damselflies (Bongiorni et al. 2004; Mola 2004; Viera et al. 2009; Davies 2010; Östergren 2010; Mola et al. 2011). However, inverted meiosis has been recently questioned in several lineages such as Juncaceae and Homoptera, specifically in *Luzula purpurea* and *Cacopsylla mali* (Nokkala et al. 2006). Other strategy is the reduction the number of chiasmata or avoidance of the chiasmata. Achiasmatic meiosis has been observed in both sexes of buthids (scorpions and bed bugs) (Shanahan and Hayman 1990; Nokkala et al. 2004; Schneider et al. 2009; Bigliardo et al. 2011) and suggested in monovalents in *Carex* meiosis (Faulkner 1972; Escudero et al. 2012, 2013). The reduced number of chiasmata has been observed in Homoptera and Cyperaceae (Nijalingappa 1975; Nokkala et al. 2004; Luceño, pers. obs.; for *Rhynchospora* species in Cyperaceae). In some instances, such as *Rhynchospora tenuis*, achiasmatic and inverted meiosis have been reported (Cabral et al. 2014). Also, it is worth mentioning that in the hemipteran suborder Heteroptera, inverted meiosis (Viera et al. 2009), restricted kinetochoric activity (Comings and Okada 1972), and monocentric chromosomes have been reported (i.e., *Ranatra*; Desai and Deshpande 1969), the first two mechanisms even coexisting in the same cell (Melters et al. 2012).

The diffuse kinetochoric plate seems to lead into a karyotypic diversity (Cook 2000; Lukhtanov et al. 2005; Kandul et al. 2007; Hipp 2007; Roalson 2008; Schneider et al. 2009; Dincă et al. 2011; de Almeida et al. 2017), but our results suggest that there is no correlation between diffuse centromere and diversification rates or species richness, at least at high evolutionary scales. Thus, holocentricity per se will not always constitute an evolutionary advantage in comparison to monocentricity.

A possible scenario in which localized centromeres could be clearly a benefit would be one in which evolutionary innovation (high recombination rates as result of a high number of chiasmata) constitutes an advantage. The higher chiasmata number (higher recombination rate) in monocentric organisms increases the diversity and facilitates adaptation processes and, eventually, speciation. Contrarily, absent or low recombination rates would imply limited adaptation abilities and, thus, higher extinction when evolutionary innovation is required. That is to say, whereas in monocentric

chromosomes extra cross-over events are allowed, increasing more exchanges of pairs of loci (Butlin 2005), holocentric chromosomes only permit a restricted number of cross-over events in each chromosome—by a reduced set of mechanisms—(Bigliardo et al. 2011; Escudero et al. 2012), promoting linkage disequilibrium of genes. Nevertheless, despite this limited number of cross-over events per chromosome, higher recombination rates could be viable in these latter taxa if they present a higher number of chromosomes (e.g., Escudero et al. 2012) that could potentially result in a similar rate of recombination per meiotic cell. Moreover, fission and fusion in holocentrics could modify the linkage groups and, thus, the allele frequencies, making possible further diversification based on the evolutionary suitability of the changes in the allele frequency in the different populations of the species. Thus, holocentric organisms with higher chromosome numbers would present higher recombination rates, compared to other holocentric taxa with fewer chromosomes which could ameliorate their potential limitation in comparison with monocentric organisms. However, at least at higher evolutionary scales, holocentric lineages that present such features that increase recombination rates (e.g., inverted meiosis in Cyperaceae Bureš et al. 2013) do not result in a significant increase in diversification rates (see Figs. 1 and 2).

Thus, evolutionary repercussion at higher scales of this particular meiotic feature, holocentricity and its real effects on diversification, will require further studies due to the uncertainty in some aspects of the meiosis process (Lenormand et al. 2016). In any case, both monocentric and holocentric chromosomes seem to present their own evolutionary benefits, which explains the several reversions from one state to the other along the tree of life.

Conclusions

Despite the assumptions and inferences made in some lineages, the analyses carried out in this study exposed clearly that there is no significant difference in mean diversification rates between holocentric lineages compared with their most closely related monocentric lineages. We have inferred a gradient of rates of diversification that is only disrupted in some exceptional instances (e.g., genus *Cuscuta*). This lead us to think of how higher or lower diversification rates in Opisthokonta and Archaeplastida might be related with

either monocentric vs. holocentric benefits or adaptations of the organisms unrelated with their chromosome behavior. Both types of chromosomes present different evolutionary advantages that result in shifts from one to another state in the tree of life, even within recent lineages.

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Conflict of interest The authors declare that they have no conflict of interest.

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