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ORIGINAL ARTICLE

Application of glycerol-preserved nuclei protocol for genome size estimation in the field conditions of a tropical rainforest

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Abstract

The extraordinary biodiversity of tropical regions has attracted many researchers. Despite an increasing number of genome size (GS) estimations, those of tropic flora remain poorly explored. In this study, we applied the glycerol-preserved nuclei protocol for GS estimation in the field conditions of a tropical rainforest in Brunei Darussalam, northern Borneo. Samples were prepared in the field following this protocol and subjected to the flow cytometry analysis in the laboratory approximately 1 month later. The glycerol-preserved nuclei protocol enabled us to perform GS estimations of thirty taxa of Euphorbiaceae s.l. (twenty taxa of Euphorbiaceae and ten taxa of Phyllanthaceae family, respectively), all representing first estimates of GS for respective taxa. We found 5.09-fold overall variation in GS, with the lowest value in *Croton* sp. ($2C = 0.97$ pg) and the highest value in *Aporosa elmeri* ($2C = 4.94$ pg). The vast majority of species presented very small GS (77%), while 23% of the species had small GS. Using available data of Euphorbiaceae s.l. from the Plant DNA *C*-values database, we also tested the correlations of GS with climate (tropical vs. temperate) and growth form (woody vs. herbaceous). Tropical taxa of Euphorbiaceae s.l. (incl. Euphorbiaceae s.s. and Phyllanthaceae) have significantly smaller GS values than temperate ones. No significant difference in GS between woody and herbaceous taxa was detected.

Keywords Borneo · Euphorbiaceae · Flow cytometry · Phyllanthaceae · Tropical · Woody

Introduction

The rise of the plant flow cytometry brings many benefits to the community of botanists and enables the expansion of its knowledge. Its development represents a huge step forward in this sense, especially due to its simplicity, speed, thriftiness to plants, accuracy and efficiency (e.g. Marie and Brown 1993; Doležel et al. 2007; Greilhuber et al. 2007; Bourge et al. 2018, and references therein). Nowadays, the FCM method is the most common way to estimation of DNA-ploidy level and nuclear genome size (GS), i.e. quantification of the DNA amount in cell nuclei (e.g. Doležel and Bartoš 2005; Doležel et al. 2007; Greilhuber et al. 2007). As a basic genome characteristic, GS associates with other biological traits of plant species. Over the years, GS variability has been shown to have significant consequences at different levels (see Leitch and Bennett 2007). For example, it may be helpful in species delimitation or hybrid identification (e.g. Mahelka et al. 2005; Prančl et al. 2018).

Although knowledge of the nuclear DNA amount of angiosperms has increased rapidly in the last decades, it remains underrepresented for tropical woody plants (cf. Leitch et al.

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2019). Estimation of GS for tropical woody plants is limited mainly by the prerequisite of fresh plant material for FCM analyses (Doležel and Bartoš 2005; Greilhuber et al. 2007), consequently hindering investigation of samples from distant and remote tropical localities. Additionally, well-developed FCM facilities are concentrated mainly in temperate climatic belt of the northern hemisphere (Bennett and Leitch 2005; Kolář et al. 2012; Šmarda et al. 2019), while tropical regions that are often distant, difficult to access and with considerably less developed FCM facilities remain insufficiently explored (cf. Leitch et al. 2019). Alternative approaches to substitute fresh samples include collection of seeds and growing plants close to the FCM facility (Suda et al. 2005), use of dormant seeds for DNA content estimation (Sliwinska et al. 2005), analysis of desiccated (Suda and Trávníček 2006a, b) or frozen plant tissue (e.g. Cires et al. 2009), and preservation of plant nuclei in ice-cold buffer with glycerol (Kolář et al. 2012). However, seeds of tropical woody plants are usually unavailable or are difficult to access. Silica-dried or frozen samples stained with propidium iodide yield histograms with much lower resolution due to degradation of DNA (Nsabimana and Van Staden 2006; Suda and Trávníček 2006a; Suda et al. 2007) and freezing also reduces the number of nuclei (Nsabimana and Van Staden 2006). Therefore, glycerol-treated samples seem to be a viable option for larger analyses. This rather neglected protocol introduced by Kolář et al. (2012) allows sample preparation from leaf tissue in field conditions and storage of samples for several weeks.

The aim of this study was to apply the glycerol-preserved nuclei protocol (Kolář et al. 2012) for GS estimation of selected tropical woods in Brunei Darussalam, northern Borneo. Specifically, we focused on tree species of Euphorbiaceae s.l. because: (1) it is one of the most common families at the studied locality (Hédl et al. 2009); (2) no previous GS values were available for the majority of tropical taxa of this family (Leitch et al. 2019); (3) variation of chromosome numbers including polyploidy was observed (Hans 1973); and finally, (4) most sampled species were represented by trees of lower heights allowing ease of leaf collection. Using our data and available records from the Plant DNA *C*-values database (Leitch et al. 2019), we tested whether (1) tropical species of Euphorbiaceae s.l. have smaller GS than temperate ones, and (2) woody species of Euphorbiaceae s.l. have smaller GS than herbaceous ones.

Materials and methods

Plant material

Plant material was collected in the lowland mixed dipterocarp tropical rainforest in the Temburong District of Brunei Darussalam (northern Borneo, Southeast Asia) in February 2015 at Kuala Belalong Field Studies Centre (KBFSC, 4° 32′ 48″ N, 115° 9′ 28″ E), a research field station of Universiti Brunei Darussalam (UBD). A total of 87 individuals (accessions) belonging to 34 taxa of the Euphorbiaceae and Phyllanthaceae families (21 and 13 species, respectively) were investigated (see Online Resource 3). The herbarium specimens are deposited in the Herbarium of the Palacký University in Olomouc (OL).

Flow cytometric standards (i.e., *Zea mays* L. 'CE-777', *Secale cereale* L. 'Daňkovské' and *Vicia faba* L. 'Inovec') were grown from seeds at the KBFSC. As their germination rate was rather low and the plants were rotting in hyperhumid conditions (up to 99% air humidity), *Musa borneensis* var. *flavida* (M. Hotta) Häkkinen & Meekiong growing in the area of KBFSC was also used as secondary reference standard.

Sample preparation and preservation

Samples were prepared following the simplified two-step protocol using Otto's buffers (Otto 1990; Doležel et al. 2007), modified for analysis of glycerol-preserved samples (Kolář et al. 2012). Briefly, ca. 0.5 cm^2 of fresh leaf tissue and an appropriate amount of the internal standard were chopped with a razor blade in a Petri dish (Galbraith et al. 1983) containing 500 µl of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween-20). *Zea mays* 'CE-777', $2C = 5.43$ pg (Lysák and Doležel 1998) served as a primary standard, GS values of the other references were calibrated against *Zea mays*, i.e. *Musa borneensis* var. *flavida* ($2C = 1.50$ pg), *Secale cereale* 'Daňkovské' ($2C = 15.43$ pg) and *Vicia faba* 'Inovec' ($2C = 25.85$ pg). The solution was filtered through a 42-µm nylon mesh, mixed with 500 µl of 85% glycerol, gently shaken and stored in a freezer at approximately − 18 °C for a week (except for a 1-day transport to the Czech Republic when reusable cooling Polar Packs were used). For each accession, samples were prepared at least three times, usually with two different standards, resulting in 285 samples. For *Musa*, six samples were prepared with *Zea mays* 'CE-777' to calculate its GS. Before FCM analysis, samples were centrifuged for 3 min at 1090×*g* and after the supernatant was removed, the pellet was re-suspended in 100 µl of ice-cold Otto I buffer. After the 15 min incubation at room temperature, 1 ml of fresh Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) was added. Then, the solution was supplemented with RNase and stained with propidium iodide (both at a concentration of 50 µg/ml; Sigma-Aldrich, St Louis, MO, USA). Finally, samples were run on the flow cytometer after 10 min of incubation at room temperature.

Genome size estimation

The absolute DNA content (genome size, GS; Greilhuber et al. 2005) was estimated by flow cytometry using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with a blue laser (488 nm, 20 mW, BD Accuri™; BD Biosciences, San Jose, CA, USA). The fluorescence intensity of 5000 particles was recorded. Gates were set at positions 40,000–70,000 (of the total of 16,000,000 channels available) in all histograms to remove background noise. Genome size (2*C*-value; Greilhuber et al. 2005) was calculated by multiplying the 2*C*-value of the standard with the mean sample peak position/mean standard peak position ratio. Percentual variation between genome sizes of one accession established by FCM analysis (see above) was calculated as the (highest 2*C*-value–lowest 2*C*-value)/mean of 2*C*-values of all samples × 100 (Online Resource 1). The conversion from picograms (pg) to base pairs (bp) followed Doležel et al. (2003), using formula 1 pg DNA = 978 Mbp.

Statistical analysis

Descriptive statistics of GS (i.e. mean and standard deviation of the mean) were calculated for each taxon. Because the data were not normally distributed, differences in GS between families were tested using a nonparametric Kruskal–Wallis test. In addition, using our data supplemented with available data of Euphorbiaceae and Phyllanthaceae from the Plant DNA *C*-values Database (Leitch et al. 2019; Online Resource 2), differences in the variation of GS for tropical versus temperate species (based on phytogeographical affiliation of species' primary range) and woody versus herbaceous species were also tested using a nonparametric Kruskal–Wallis test. All analyses were performed both with and without outliers using NCSS 9 (Hintze 2013). Outliers were defined as exceeding the formula boxplot edge ± 3* interquartile range.

Results

Quality and error rate of the method

Using the protocol of glycerol-preserved nuclei (Kolář et al. 2012), flow cytometric analyses yielded histograms with distinct peaks in 79.30%, i.e. in 226 out of 285 prepared samples (Online Resource 1), therefore, we were able to determine GS values for 73 out of 87 plant individuals representing 30 out of 34 collected species. With a few exceptions, the measurement quality, as given by the coefficients of variation (CVs) of G1 peaks, was good. CV values ranged from 0.81 to 6.89% (mean 2.40 ± 0.93%, median 2.28%) and from 0.87 to 5.82% (mean 2.82 ± 1.04%, median 2.69%) for the standard and sample peak, respectively (for illustrative histograms see Fig. 1). The differences between lowest and highest flow cytometry run for one accession ranged between 0.17% in *Hancea eucausta* (Airy Shaw) S.E.C.Sierra, Kulju & Welzen and 7.82% in *Elateriospermum tapos* Blume (mean 3.59 ± 2.86%, median 3.20%). For most of the taxa (often viscous samples), it was impossible to meet a between-day fluctuation of < 2% (Doležel et al. 2007) and thus a higher between-day fluctuation threshold was considered acceptable (< 5%). However, for a few samples this threshold has been slightly exceeded (Online Resource 1).

GS estimations of Euphorbiaceae s.l.

In total, GS estimates for 73 plant individuals belonging to 30 taxa of Euphorbiaceae s.l., 20 taxa of Euphorbiaceae and 10 taxa of Phyllanthaceae, were determined (Table 1). For all sampled species, these are the first estimation of GS as no previous *C*-values were available.

Among the investigated species, GS varied 5.09-fold with an average of 2.46 pg (Fig. 2). The lowest GS was obtained for *Croton* sp. (2*C* = 0.97 ± 0.01 pg) and the highest one was for *Aporosa elmeri* Merr. (2*C* = 4.94 ± 0.11 pg). According to the GS categories defined by Leitch et al. (1998), 76.67% of the taxa have a very small GS (i.e. ≤ 1.4 pg/1*C*) and 23.33% have a small GS (i.e. ≤ 3.5 pg/1*C*, Table 1). No species with intermediate, large or very large GS were detected. Also, no significant difference of GS among families ($\chi^2 = 1.716$, $P = 0.190$; without outliers: $\chi^2 = 2.371$, $P = 0.124$; Fig. 2) was found. Within a species which has more individuals sampled, the GS variation fell within a narrow range (0.59–6.46%). Only in *Macaranga hypoleuca* (Rchb.f. & Zoll.) Müll.Arg., the variation of 14.01% was detected (see Table 1; Online Resource 1).

No GS data were obtained for four species, namely *Aporosa falcifera* Hook.f., *Antidesma neurocarpum* Miq., *Antidesma* sp. and *Neoscortechinia kingii* Pax & K.Hoffm (for more details see Online Resource 1).

Differences in GS in tropical versus temperate and woody versus herbaceous species

To test the differences in GS based on climate (tropical vs. temperate) and growth form (woody vs. herbaceous), available data from the Plant DNA *C*-values Database (Leitch et al. 2019) were used (see Online Resource 2). The comparison of GS between tropical and temperate species of Euphorbiaceae s.l. showed a significant difference with tropical taxa having smaller GS values ($\chi^2 = 8.562$, $P = 0.003$; without outliers (not significant): $\chi^2 = 2.506$, $P = 0.113$; Fig. 3a). In contrast, no statistically significant differences were detected

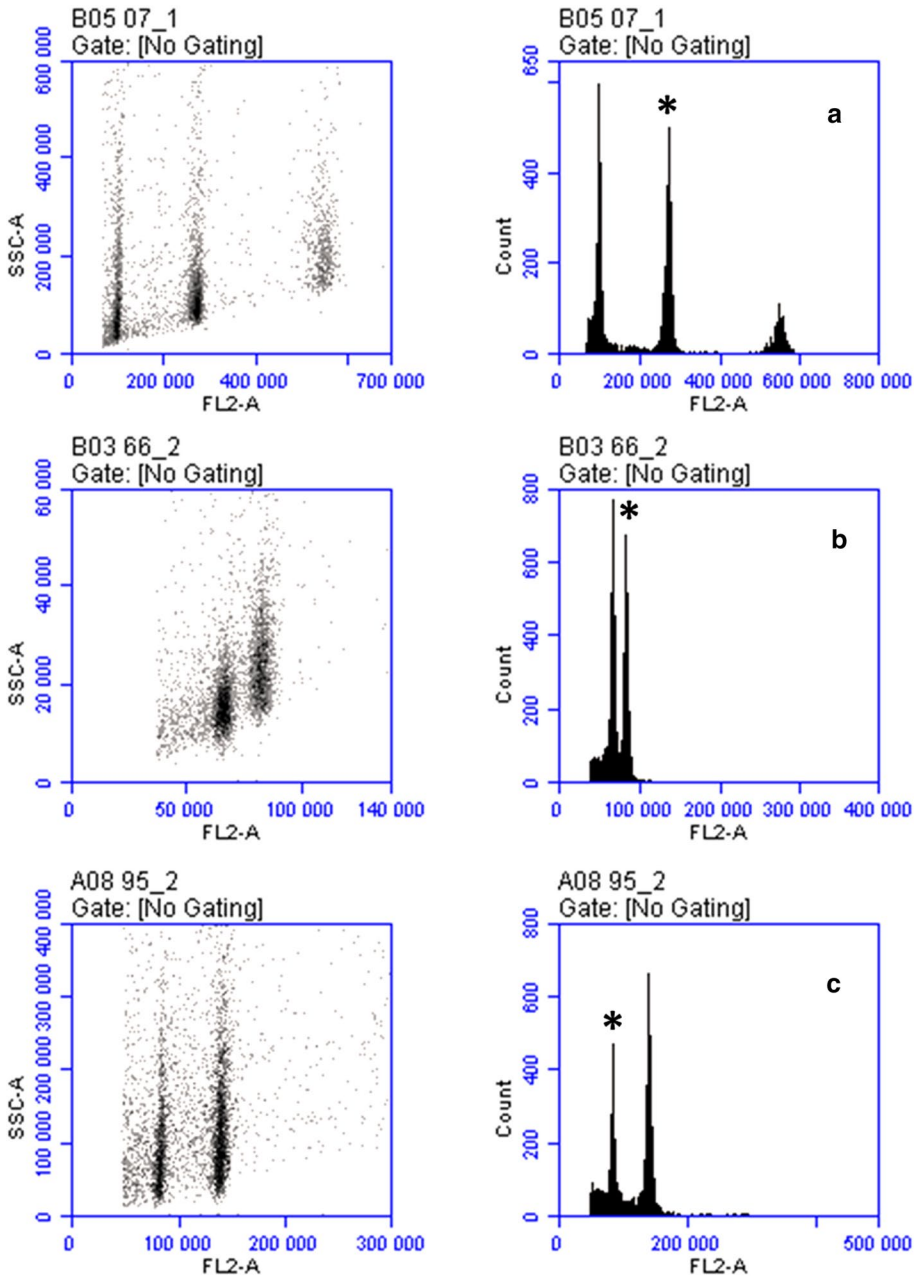

Fig. 1 Illustrative flow cytometric histograms and side scatter/fluorescence graphs of **a** *Aporosa grandistipula* Merr. (tree ID 07), **b** *Neoscortechinia sumatrensis* S.Moore (tree ID 66) and **c** *Macaranga bancana* Müll.Arg. (tree ID 95) analysed with *Musa borneensis* var. *flavida* (**b**, **c**) or *Zea mays* 'CE-777' (**a**), as the internal reference (marked as *). CVs (%) of G1 peaks of Sample/Standard are 1.68/1.23 (**a**), 5.08/3.02 (**b**) and 2.07/2.88 (**c**)

in GS between woody and herbaceous species ($\chi^2 = 2.029$, $P = 0.154$; without outliers: $\chi^2 = 2.300$, $P = 0.130$; Fig. 3b). Considering the phylogeny of investigated taxa, we were able to test the variation in GS only among genera of the family Euphorbiaceae as the family Phyllanthaceae is in data set represented only by tropical, woody species (Fig. 3a, b) and no data for temperate and herbaceous taxa were available (Leitch et al. 2019). Within the Euphorbiaceae s.s., tropical taxa have significantly smaller GS ($\chi^2 = 9.700$, $P = 0.002$; without outliers: $\chi^2 = 4.283$, $P = 0.038$; Fig. 3a), whereas there is no significant difference based on growth form ($\chi^2 = 2.417$, $P = 0.120$; without outliers $\chi^2 = 2.812$, $P = 0.094$; Fig. 3b).

Discussion

Application of glycerol-preserved nuclei protocol in tropical rainforest

In this study, we investigated the GS values of selected tropical woods using the preservation of nuclear suspensions in ice-cold buffer with glycerol solution (Kolář et al. 2012). This methodological approach of sample preparation can be quite easily performed in the field and additionally, it allows long-term sample storage in the frozen state. Importantly, it provides estimates of nuclear DNA content that are highly comparable to those obtained using fresh material (Kolář

Table 1 Summary of the nuclear DNA content in the studied tropical, woody taxa of Euphorbiaceae s.l

Family	Taxon	*N*	2*C* (pg) mean ± SD	1*C* (pg)	1*C* (Mbp)	GS category
Phyl	*Aporosa bullatissima* Airy Shaw	2	1.8423 ± 0.0608	0.9212	900.88	1
Phyl	*Aporosa elmeri* Merr.	1	4.9358	2.4679	2413.61	2
Phyl	*Aporosa grandistipula* Merr.	3	1.8787 ± 0.0581	0.9394	918.68	1
Phyl	*Aporosa nitida* Merr.	3	2.1015 ± 0.0459	1.0508	1027.63	1
Phyl	*Baccaurea deflexa* Müll.Arg.	1	2.2565	1.1282	1103.41	1
Phyl	*Baccaurea racemosa* Müll.Arg.	3	2.7794 ± 0.0084	1.3897	1359.15	1
Phyl	*Baccaurea tetrandra* Müll.Arg.	1	2.7443	1.3722	1341.98	1
Phyl	*Breynia coronata* Hook.f.	1	3.0491	1.5245	1491.01	2
Euph	*Croton* sp.	3	0.9756 ± 0.0087	0.4878	477.07	1
Euph	*Elateriospermum tapos* Blume	2	1.3629 ± 0.0301	0.6815	666.47	1
Euph	*Endospermum diadenum* (Miq.) Airy Shaw	5	4.5281 ± 0.0706	2.2640	2241.22	2
Phyl	*Glochidion glomerulatum* Boerl	1	2.2029	1.1014	1077.21	1
Phyl	*Glochidion superbum* Baill.	3	4.4446 ± 0.0654	2.2223	2173.40	2
Euph	*Hancea eucausta* (Airy Shaw) S.E.C.Sierra, Kulju & Welzen	3	3.9706 ± 0.0928	1.9853	1941.63	2
Euph	*Macaranga aëtheadenia* Airy Shaw	1	1.9965	0.9983	976.31	1
Euph	*Macaranga bancana* Müll.Arg.	3	2.4998 ± 0.0462	1.2499	1222.38	1
Euph	*Macaranga beccariana* Merr.	1	2.4254	1.2127	1186.04	1
Euph	*Macaranga hullettii* King ex Hook.f.	4	2.5050 ± 0.0111	1.2525	1224.92	1
Euph	*Macaranga hypoleuca* Müll.Arg.	1	2.0928	1.0464	1023.38	1
		2	2.4134 ± 0.0066	1.2067	1180.17	1
Euph	*Macaranga lowii* King ex Hook.f.	3	1.3738 ± 0.0239	0.6869	671.81	1
Euph	*Macaranga praestans* Airy Shaw	2	1.4431 ± 0.0192	0.7216	705.68	1
Euph	*Macaranga* sp.	1	2.5162	1.2581	12304.40	1
Euph	*Macaranga trachyphylla* Airy Shaw	4	2.5485 ± 0.0382	1.2743	1246.22	1
Euph	*Macaranga umbrosa* S.J.Davies	3	2.4817 ± 0.0296	1.2408	1213.54	1
Euph	*Mallotus korthalsii* Müll.Arg.	3	1.6077 ± 0.0225	0.8039	786.17	1
Euph	*Mallotus wrayi* King ex Hook.f.	3	2.0182 ± 0.0383	1.0091	986.90	1
Euph	*Moultonianthus leembruggianus* (Boerl. & Koord.) Steenis	3	1.1547 ± 0.0065	0.5773	564.64	1
Euph	*Neoscortechinia sumatrensis* S.Moore	3	1.1005 ± 0.0248	0.5503	538.17	1
Euph	*Pimeleodendron griffithianum* (Müll.Arg.) Hook.f.	1	3.4892	1.7446	1706.23	2
Euph	*Trigonostemon detritiferus* R.I.Milne	3	3.2220 ± 0.0154	1.6110	1575.53	2

The values are given as the mean and standard deviation of the GS (2*C*-value; Greilhuber et al. 2005) and holoploid GS (1*C*-value, 1 pg DNA = 978 Mbp; Doležel et al. (2003)). Family: *Euph* Euphorbiaceae, *Phyl* Phyllanthaceae, *N* total number of analysed individuals, GS category: genome size categories according to Leitch et al. (1998): *1* very small (≤ 1.4 pg/1*C*), *2* small (≤ 3.4 pg/1*C*)

et al. 2012). Although this method has been used for estimation of GS in laboratory conditions (Magauer et al. 2014; Frajman et al. 2015), it has only been field-tested by Kolář et al. (2012).

Several tropical plants from Papua New Guinea were also involved in the study of Kolář et al. (2012), including five species of the family Euphorbiaceae, with acceptable histograms using ice-cold glycerol solution in all but one species (*Macaranga fallacina* Pax & K.Hoffm.). In contrast, we obtained satisfactory results for all 10 *Macaranga* species sampled. However, several limitations of this approach for estimation of GS in the field remain. Firstly, a low temperature (− 18 °C) is required for samples in glycerol to remain as liquid (Kolář et al. 2012), which requires equipment (e.g. freezer) that is not always available in the field conditions. Second, sample preparation with internal standard requires prior knowledge about approximate GS to avoid overlapping of sample and standard peaks, and too long distance between sample and standard peaks (Doležel and Bartoš 2005; Doležel et al. 2007). Alternatively, samples can be prepared with several standards. Third, plants commonly used as internal standards in temperate belt (e.g. Doležel et al. 2007) could have considerably low germinability and growth in hyper-humid tropical climate as shown in our example. This problem can be solved by preparing a new set of standards for local non-laboratory conditions. Despite these limitations, the

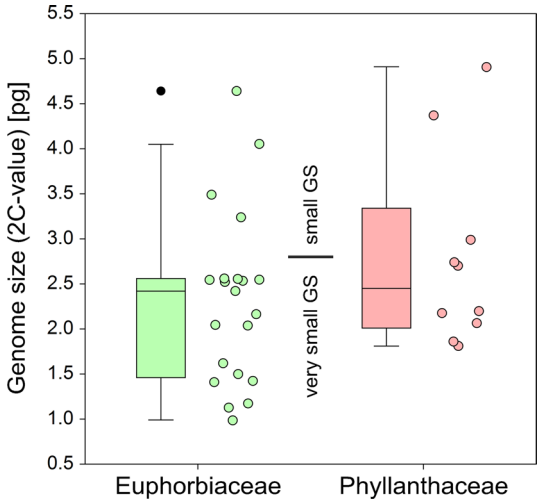

Fig. 2 Comparison of the genome size variation between the investigated species of Euphorbiaceae (20 taxa/54 individuals) and Phyllanthaceae (10 taxa/19 individuals) family, respectively. Dots within boxplots symbolize each individual species. Rectangles define the 25th and 75th percentiles, horizontal lines show median values, whiskers are 10–90 percentiles and black dots show extreme values

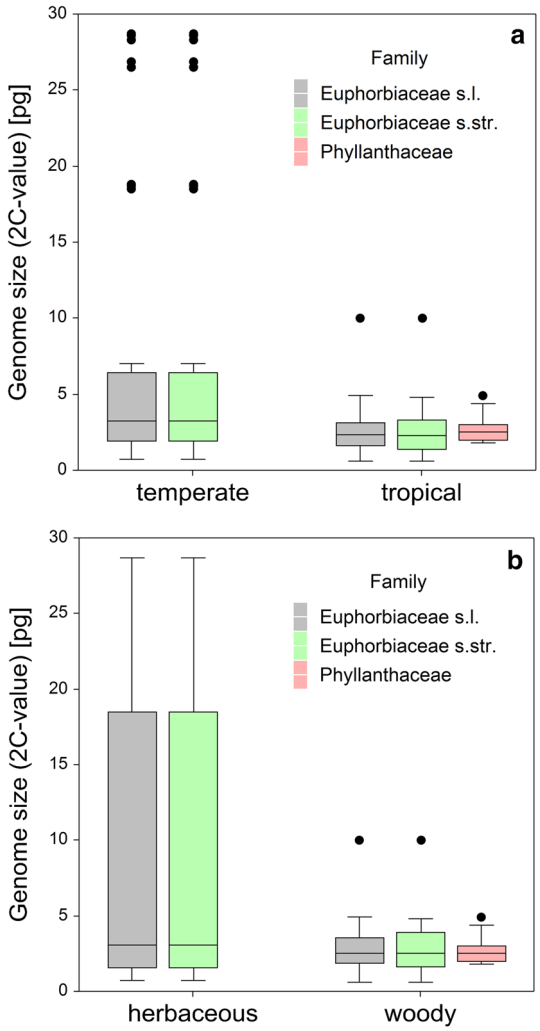

Fig. 3 Boxplots indicating variation of the genome size (**a**) among temperate (36 species: all Euphorbiaceae s.s.) and tropical (47 species: 32 Euphorbiaceae s.s. and 15 Phyllanthaceae), (**b**) among herbaceous (31 species: all Euphorbiaceae s.s.) and woody (53 species: 38 Euphorbiaceae s.s. and 15 Phyllanthaceae) taxa, respectively. Except our data, additional GS values (53 records) from the Plant DNA *C*-values database (Leitch et al. 2019) were also used (Online Resource 2). Rectangles define the 25th and 75th percentiles, horizontal lines show median values, whiskers are 10–90 percentiles and dots show extreme values

protocol proved to be efficient in our case and can be used for large scale studies of GS in the tropics.

Genome size and polyploidy in Euphorbiaceae s.l. from Brunei Darussalam

According to the GS categories defined by Leitch et al. (1998), all analysed species have a very small or small GS (Table 1). Similarly, very small or small GS were reported for other tropical taxa of Euphorbiaceae s.l., except of *Hura crepitans* L. with intermediate GS (Leitch et al. 2019; Online Resource 2). Combining our GS values together with data from the Plant *C*-values database (Leitch et al. 2019), we found tropical species to have significantly smaller GS than temperate ones (Fig. 3a). On the other hand, an assumed smaller GS among woody species compared to herbs were not confirmed (Fig. 3b). Generally, woody angiosperms have been predicted to possess a small GS with lower variance in comparison to herbaceous species (e.g. Ohri 2005; Beaulieu et al. 2008; Ohri 2015). Likewise, small GS varying more or less within a narrow range have been found within tropical hardwood (Ohri and Kumar 1986; Ohri 2002). In woody plants, larger GS may have a negative impact on the size of cambial cells and mechanical properties of woody tissues (Darlington 1937; Stebbins 1938, 1950) or on the size and density of stomata needed to transpiration and stomatal conductance (Beaulieu et al. 2008). Unfortunately, the knowledge of GS in woody angiosperms (and especially tropical ones) is still very sketchy (Leitch et al. 2019) and extensive studies except few (e.g. Chen et al. 2014) are almost lacking. An investigation of GS variation within the tree family Fagaceae revealed a small GS among its genera and detected larger GS within tropical groups in comparison to their temperate relatives (Chen et al. 2014) which is quite contrary to the situation we report here for Euphorbiaceae.

However, we cannot conclusively confirm the variation of GS within woody and herbaceous taxa since our results may be affected by the limited available dataset. We assume that a detailed study of the GS variation of both families (especially of the Euphorbiaceae s.s.) will lead to a better understanding of the GS evolution among various growth

forms (incl. lianas, climbers or succulents) and across biotic zones. Especially for tropical, woody plants, which are not so much explored, it would greatly improve our knowledge. For example, GS study of tropical genus *Dalbergia* L.f. detected higher DNA amount within shrubs and woody climbers in comparison to their relative tree species (Hiremath and Nagasampige 2004).

From a global perspective, the lowest polyploid frequencies are found in the tropical and subtropical biomes (especially in tropical and subtropical moist broadleaf forests; Rice et al. 2019). Moreover, polyploids are considerably less frequent among perennial woods, which can be explained by different ecological and historical factors (Stebbins 1971). Nevertheless, the frequency of polyploidy among woody taxa still remains largely unknown, especially among tropical species. For example, no evidence of polyploidy was detected within the tree family Fagaceae (Chen et al. 2014). However, even among tropical woody species the occurrence of polyploidy has been documented, e.g. in Dipterocarpaceae (Ng et al. 2016), Fabaceae (Tosso et al. 2016; Donkpegan et al. 2017), Melastomataceae (Renner 1989; Almeda 1993; Brito et al. 2016) and Myrtaceae (Costa and Forni-Martins 2006).

According to our GS data, the occurrence of polyploidy can be considered in two analysed genera, *Aporosa* Blume and *Glochidion* J.R.Forst. & G.Forst., both from Phyllanthaceae family. In *Aporosa*, four species were analysed with one of them possessing twice as high GS value (Table 2) than the others. Unfortunately, almost no chromosomal data were published for this genus. Only chromosome counts are available for two *Aporosa* species from India, both with $2n = 52$ (Mehra and Hans 1969; Elumalai 2013). For the family Phyllanthaceae, $x = 13$ appears to be the base chromosome number (e.g. Hans 1973; Webster 1994). Based on that, the published chromosome data correspond to tetraploids, which suggest the involvement of polyploidy in its evolution. Moreover, *Aporosa* belongs to the sister clade of the genus *Bischofia* Blume (Wurdack et al. 2004; Hoffmann et al. 2006), in which whole genome duplication (WGD) was discovered (Cai et al. 2017), indicating the possibility of polyploidy occurrence also in *Aporosa*. Similarly, in the genus *Glochidion*, one of the two analysed taxa possessed twice as high GS value as the other one (Table 2). For several Indian *Glochidion* species, chromosome numbers $2n = 52$ were counted (Mehra and Hans 1969), likewise corresponding to tetraploids. Additionally, the incidence of WGD was recorded within the genus *Sauropus* Blume (Cai et al. 2017), the sister clade of the genus *Glochidion* (Wurdack et al. 2004; Hoffmann et al. 2006; Kathriarachchi et al. 2006), thus polyploidy could be more frequent among the members of the *Sauropus* clade. In addition, for Chinese *G. puberum* (L.) Hutch., $2n = 64$ was reported (Hsu et al. 1994), indicating the occurrence of other chromosomal variations (aneuploidy, different basic chromosome number etc.). However, detailed study involving investigation of chromosome numbers is required for elucidation of GS evolution and possible polyploidy occurrence in Phyllanthaceae.

Based on the study of Hans (1970), polyploidy was also observed within the genus *Antidesma* L. belonging to the Phyllanthaceae family. Unfortunately, we were unable to obtain any information about the GS of two sampled species of *Antidesma*, since all measurements failed.

Furthermore, the GS values for more than one species were also acquired for *Baccaurea*, *Macaranga* and *Mallotus*. Within the *Baccaurea* and *Mallotus* genera, only minor differences in GS (0.54 pg and 0.43 pg, respectively) were detected between sampled species, corresponding to intraspecific variation (Šmarda and Bureš 2010). In *Macaranga*, all species had very similar GS (mean $2C = 2.43 \pm 0.20$ pg; Table 1), with the exception of *M. praestans* Airy Shaw and *M. lowii* King ex Hook.f., which possessed a lower GS value ($2C = 1.42$ pg and $2C = 1.49$ pg, respectively). These two species are the only studied taxa belonging to *Macaranga* sect. *Pseudorottlera* (Rchb.f. & Zoll. ex Zoll.) Pax & K.Hoffm., a basal sister lineage to the rest of *Macaranga* species (Kulju et al. 2007), suggesting the

Table 2 The genera with the expected occurrence of polyploidy based on obtained GS estimations

Taxon	2*C* (pg) mean ± SD	Estimated DNA-ploidy level*	1*Cx* (pg)**
Aporosa bullatissima	1.8423 ± 0.0430	2	0.92
Aporosa elmeri	4.9358	6	0.82
Aporosa grandistipula	1.8787 ± 0.0474	2	0.93
Aporosa nitida	2.1015 ± 0.0374	2	1.05
Glochidion glomerulatum	2.2029	2	1.10
Glochidion superbum	4.4446 ± 0.0533	4	1.11

For each taxon, the GS (2*C*-value), estimated DNA-ploidy level and the calculated monoploid GS (1*Cx* value; Greilhuber et al. 2005) are given

*Estimate based on 2*C*-value, whereas the lower value was assumed as diploid

**Calculated according to estimated ploidy level

possible different base chromosome number. Unfortunately, no published chromosomal data are available for sampled taxa. Only for several Indian and one Hawaiian species, identical diploid chromosome number $2n = 22$ was previously counted (Miller and Webster 1966; Mehra and Hans 1969; Devar 1981). In addition, in *Macaranga hypoleuca*, we detected the variation of 14% among analysed individuals, indicating the chromosome number variation.

Conclusion

In conclusion, this study shows the applicability of glycerol-treated nuclear suspension (Kolář et al. 2012) for GS estimation in field conditions. For the first time, we determined GS for 20 taxa of Euphorbiaceae and 10 taxa of Phyllanthaceae family, respectively. Very small or small GS were determined in all studied taxa. Compared with the available data in Leitch et al. (2019), tropical species of Euphorbiaceae possessed smaller GS than temperate ones. No significant difference was detected between woody and herbaceous species. A comprehensive study, including a chromosome number investigation, will lead to better understanding of GS evolution of this group, and it will provide further insight into the variation of GS between various growth forms and across biomes. With a great range of life forms and almost cosmopolitan distribution (Webster 2014), the family Euphorbiaceae best fits to such kind of study. Furthermore, it will also contribute to the knowledge of GS in tropical woody angiosperms as it is abundant in tropical regions (Webster 2014).

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Data availability The data reported here are archived as supplemental material in Plant Systematics and Evolution.

Compliance with ethical standards

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

Information on electronic supplementary material

Online Resource 1. Detailed results of genome size measurements using flow-cytometry. Genome size (2*C*), holoploid genome size (1*C*), percentual intraspecific genome size variation and genome size category according to Leitch et al. (1998) of investigated taxa.
Online Resource 2. List of the data taken from the Plant *C*-values Database (Leitch et al. 2019) with genome size (2*C*), holoploid genome size (1*C*), and original reference cited in the *C*-values Database to each record and growth form and biome to each taxon.
Online Resource 3. List of localities sampled of collected plant material. Tree no. indicate exact tree in ecological plot. Herbarium specimens are deposited in the Herbarium of the Palacký University in Olomouc (OL).

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