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New chromosome counts and other karyological data for members of the *Stemonaceae*

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Abstract Chromosome numbers and other karyological data for ten *Stemona* species and for *Stichoneuron caudatum* are presented, including first reports for *Stemona burkillii*, *S. involuta*, *S. mairei* and *S. phyllantha*. All investigated taxa of *Stemona* exhibit $n = x = 7$ ($2n = 14$) chromosomes. For *Stichoneuron caudatum* an earlier count revealing $2n = 18$ is confirmed. The observed chromosome lengths range between 0.9 and 6.9 µm (largest chromosome in *Stichoneuron caudatum*). Additionally, the genome sizes of seven *Stemona* species and of *Stichoneuron caudatum* are reported. The obtained results are compared with literature data and discussed.

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INTRODUCTION

The small monocotyledonous family *Stemonaceae* is placed in the *Pandanales* (APG 2016) and comprises four genera (*Croomia* Torr., *Pentastemona* Steenis, *Stemona* Lour., *Stichoneuron* Hook.f.; Rudall & Bateman 2006) with 36 (The Plant List 2013) or 37 (Christenhusz & Byng 2016) species. To date, most of the studies on the family deal with the phytochemical composition of the genera *Stemona* (e.g., Kongkiatpaiboon et al. 2011, Chen et al. 2017a, 2018) and *Stichoneuron* (e.g., Schinnerl et al. 2005, Ramli et al. 2014) and with floral characters (Chen et al. 2017b). Phylogenetic studies on the family are rare. Only selected species were analysed so far in this respect (Li et al. 2008, Wang et al. 2017, Lu et al. 2018). However, a comprehensive study concerning the phylogeny/biogeography of the family has been published recently (Chen et al. 2021).

Cytological information for the *Stemonaceae* was last summarized and discussed by Hartl & Kiehn (2004). According to their survey, the four genera of this plant family show three different basic numbers: $x = 7$ (*Stemona*, *Pentastemona*), $x = 9$ (*Stichoneuron*) and $x = 12$ (*Croomia*). $x = 7$ is suggested as basic number of the family.

The present paper provides chromosome counts and other karyological data for the *Stemona* species *S. aphylla* Craib, *S. burkillii* Prain, *S. collinsae* Craib, *S. curtisii* Hook.f., *S. involuta* Inthachub, *S. japonica* (Blume) Miq., *S. kerrii* Craib, *S. mairei* (H.Lév.) K.Krause, *S. phyllantha* Gagnep., *S. tuberosa* Lour., as well as for *Stichoneuron caudatum* Ridl., and includes the results of first studies on the four species *S. burkillii*, *S. involuta*, *S. mairei* and *S. phyllantha*. For *S. curtisii*, the exact chromosome number is determined for the first time. Earlier reports for *S. collinsae*, *S. japonica* and *Stichoneuron caudatum* are confirmed. The paper also contains DNA amount measurements for seven *Stemona*-species and for *Stichoneuron caudatum*. Based on the discussions by Hanson et al. (2001) and Hartl & Kiehn (2004), implications of the karyological characters are evaluated.

MATERIAL AND METHODS

Plants were collected during field trips in Thailand by S. Vajrodaya and H. Greger or obtained in the context of research collaborations and were subsequently cultivated in the Botanical Garden of the University of Vienna (HBV). Origins and voucher specimen numbers of the investigated taxa are given in Table 1. Voucher specimens are deposited at the Herbarium of the University of Vienna (WU) and digital images and additional collection data may be found at the international herbarium database system JACQ (https://herbarium.univie.ac.at/database).

Chromosome counts

For chromosome counts and studies of other karyological characters flower buds, shoot apices or root tips from cultivated individuals were fixated with a fresh mixture of ethanol or methanol (96 %) and glacial acetic acid (3 : 1). The fixated material was stored at 4 °C. For chromosome staining Feulgen reagent was used (see Kiehn et al. 1991 for details on staining procedures). Permanent slides for all counts are deposited in the collection of M. Kiehn.

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Table 1 Chromosome numbers of studied *Stemona* species and *Stichoneuron caudatum*.

Taxon	ID	*n*/2*n*	tissue	origin / herbarium voucher
Stemona aphylla	STEM 37/5	–/14	fi	TH, Sukhothai; WU 0041593
S. burkillii	STEM 45	–/14	ap	TH, Kanchanaburi; WU 0041587
S. collinsae	STEM 13	7/14	PMC/ov	TH, Chon Buri WU 0041576
S. curtisii	STEM 50/2	–/14	rt	TH, Narathiwat; WU 0107064
	20357	–/14	fi, ov	TH, Chumphon; WU 0107067
	120113	–/14	fi, ov	TH, Chumphon; WU 0107063
S. involuta	STEM 51	–/14	rt	TH, Nakhon Ratchasima; WU 0041595
S. japonica	STEM 61	–/14	rt	CN, Hongkong; WU 0041594
S. mairei	–	–/14	rt	CN, Yunnan; WU 0107061
S. phyllantha	STEM 09	–/14	rt	TH, Bangkok; WU 0041556
	STEM 24	–/14	rt	ID, Bali; WU 0041552
S. tuberosa	STEM 62	–/14	rt	TH, Loei; WU 0041557
Stichoneuron caudatum	STEM 43	–/18	fi	TH, Yala; WU 0041539

Abbreviations: n = haplophasic chromosome number; 2n = diplophasic chromosome number (according Greilhuber et al. 2005, Greilhuber & Doležel 2009); investigated plant tissue: ov = young ovaries, fi = young filaments, PMC = pollen mother cells, rt = root tips, ap = shoot apex. CN = China, ID = Indonesia, TH = Thailand.

Genome size measurements

Following the chopping method of Galbraith et al. (1983), about 25 mg of fresh leaves from each plant sample were co-chopped in Otto's buffer I (Otto et al. 1981) together with *Pisum sativum* L. (1C (= DNA-amount in the haploid genome) = 4.42 pg; Greilhuber & Ebert 1994) or *Solanum pseudocapsicum* L. (1C = 1.29 pg; Temsch et al. 2010) as the internal standard organisms. The resulting isolated nuclei were filtered through a 30 µm nylon mesh and subsequently incubated with RNase A (Sigma, final concentration 0.15 mg/mL) for 30 minutes at 37 °C in the water bath for digestion of double-stranded RNA. The nuclei were then stained in Otto's buffer II (Otto et al. 1981) containing the fluorochrome propidium iodide (PI, 50 mg/L) for at least one hour or overnight in the refrigerator. For measurement, a CyFlow ML flow cytometer or a PAII (both Partec, Münster, Germany) were used. Light sources were for the CyFlow ML a green laser (532 nm, Cobolt Samba, Cobolt AB, Stockholm, Sweden) and for the PAII a mercury lamp. A single preparation was made per individual and from this at least three measurement runs were performed, with 5 000 measured particles per run. Usually, the coefficient of variation (CV) was less than 3 %, but whenever higher CVs occurred, additional runs were carried out. For each run, the 1C-value was calculated according to the formula: mean fluorescence intensity of the sample organism's G1 nuclei population divided by mean fluorescence intensity of the standard's G1 nuclei population multiplied by the 1C-value of the standard organism. The resulting sample values are shown in Table 2.

RESULTS AND DISCUSSION

Chromosome numbers of Stemona

n = 7 or 2*n* = 14 could be established with certainty for all investigated *Stemona* accessions, representing nine of the 24 accepted *Stemona* species (e.g., The Plant List 2013). This includes three accessions of *S. curtisii*, for which only a range of 2*n* = 13–16 had been reported by Hartl & Kiehn (2004). In the accession of *S. phyllantha* collected in Bali, Indonesia (WU 0041552), some counts seemed to reveal 28 chromosomes. At closer look, all these could be assigned to early anaphase stages with already well-separated chromatids. The present findings are in accordance with the former literature reports on the genus (see survey in Hartl & Kiehn 2004). They corroborate *x* = 7 as basic number for the genus.

Chromosome number of Stichoneuron

The result of 2*n* = 18 for *Stichoneuron caudatum* confirms the number reported by Duyfjes (1991).

Table 2 Estimated genome sizes from seven *Stemona* species and *Stichoneuron caudatum*.

Taxon	ID	1C (SD; CV)	origin / herbarium voucher
S. aphylla	STEM 37	1.11 (0.0028; 0.2540)	see Table 1
S. burkillii	STEM 45	1.17 (0.0015; 0.1306)	see Table 1
S. collinsae[1]	STEM 53	1.13 (0.0043; 0.3793)	TH, Si Racha; HG860
S. curtisii	STEM 33	1.11 (0.0018; 0.1639)	TH, Krabi; WU 0041566
	STEM 50/2	1.12 (0.0015; 0.1379)	see Table 1
	20357	1.12 (0.0024; 0.2094)	see Table 1
	120113	1.07 (0.0031; 0.2857)	see Table 1
S. kerrii[1]	STEM 36	1.19 (0.0025; 0.2073)	TH, Tak; WU 0041575
S. mairei	–	1.14 (0.0024; 0.2109)	see Table 1
S. phyllantha[2]	100095	0.89 (0.0039; 0.4334)	see Table 1
	120099	0.90 (0.0089; 0.9880)	see Table 1
Stichoneuron caudatum	STEM 43	6.23/6.33	see Table 1

1C values are given in pg and the variation coefficient is given in %. Leaves of *Pisum sativum* 'Kleine Rheinlaenderin' or *Solanum pseudocapsicum* were used as standards.

[1] The chromosome number (2*n* = 14) was already established by Hartl & Kiehn (2004).

[2] Seedlings of STEM 24 (originating from Bali, Indonesia). SD = standard deviation; CV = coefficient of variation.

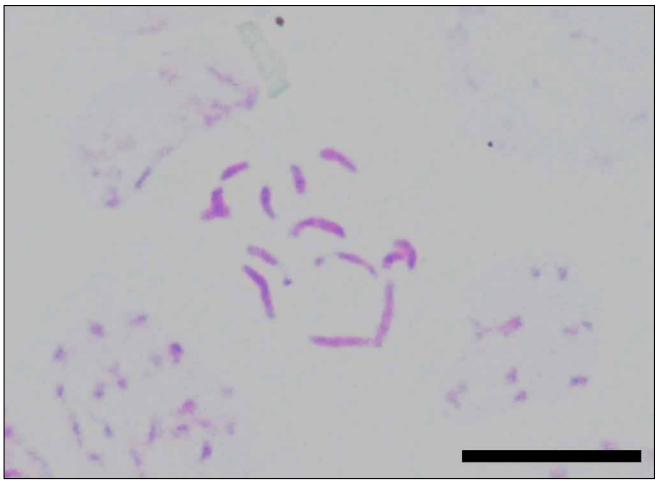

Fig. 1 *Stemona phyllantha*. (STEM 09): 2n = 14. (Pro)metaphase with two clearly visible satellites. — Scale bar = 10 µm.

Genome sizes

Within the order *Pandanales*, only eight DNA amount estimates with 1C values ranging between 0.4–1.5 pg have been reported so far (Leitch et al. 2010). The only DNA amount measurement reported previously for a *Stemonaceae* species relates to *S. tuberosa* (4C = 2.92 pg; Hanson et al. 2001). All new estimates for *Stemona* species presented here show a higher 1C value (Table 2). Amongst those, 1C-values of the six *Stemona* species are very similar. Only the two investigated accessions of *S. phyllantha* exhibit a lower 1C DNA amount of 0.89 and 0.90 pg, but even these values are higher than the reported 1C value of *S. tuberosa* (0.73 pg). Interestingly, the group of *Stemona* species exhibiting similar DNA amounts, including the Chinese endemic species *S. mairei*, also shows similarities in their alkaloid composition (e.g., Chen et al. 2018), but differ clearly from *S. phyllantha* and *S. tuberosa* (e.g., Chen et al. 2017a), and also from *Stichoneuron caudatum* (Schinnerl et al. 2005). Taking the close relationship of *S. phyllantha* with *S. tuberosa* into account (Chen et al. 2021) all reported DNA amounts in *Stemona* seem to be plausible.

With 6.23–6.33 pg (Table 2), the 1C-value established for *Stichoneuron caudatum* is around six-fold higher than in the *Stemona* species, and more than 4 times higher than the highest known value in the *Pandanales*. The assumption of Leitch et al. (2010) that "larger genomes may be found in *Stemonaceae*" is corroborated by this finding. Compared to the DNA amounts of other monocots (Bennett & Leitch 1995, 1997, Hanson et al. 2001), the estimated values are located in the lower range. According to Vinogradov (2001) the DNA amounts of the studied species are fitting well with perennial monocots, but due to the insufficient data no further conclusion can be drawn at the current stage.

Chromosome lengths and morphology

For most of the counted accessions, chromosome lengths could be assessed. Table 3 summarizes the obtained results and the current knowledge about chromosome lengths in the *Stemonaceae*. Only cells which appeared to exhibit fully condensed chromosomes were analysed. In each case, at least three different preparations were studied. The absolute lengths and the ranges for *Stemona* species reported by Duyfjes (1991), Oginuma et al. (2001), Hartl & Kiehn (2004) and of the present study are similar. For *Stichoneuron caudatum*, however, the data presented herein are approximately two-fold higher than the results published by Duyfjes (1991). The reason for this discrepancy is not known. Two satellites could be observed in all studied taxa. These satellites (Fig. 1) are likely to explain the range of 2*n* = 13–16 as reported by Hartl & Kiehn (2004) for *S. curtisii*. The differences in the chromosome lengths in *S. sessilifolia* and *S. japonica* were described as gradual by Oginuma et al. (2001). This also holds true for most *Stemona* taxa studied here. *Stemona collinsae*, however, has one pair of chromosomes (c. 2.3 µm) clearly smaller than all other chromosomes. This has already been reported for this species by Hartl & Kiehn (2004). There is no obvious correlation between chromosome lengths and DNA-amounts. Further karyological studies are required to quantify these observations and to elucidate potential implications of observed differences in karyological traits for the infrageneric classification in *Stemona*.

In view of the data presented here, of the molecular phylogenies of the *Pandanales* (e.g., Caddick et al. 2000, APG 2016), and

Table 3 Chromosome lengths reported for members of the *Stemonaceae*.

Taxon	ID	Lengths (µm)	References
Croomia heterosepala (Backer) Okuyama	–	2.3–4.4	Oginuma et al. 2001
Croomia japonica Miq.	–	1.7–4.6	Oginuma et al. 2001
Croomia pauciflora Torr. ex Torr. & A.Gray	–	1–1.5	Duyfjes 1991
Pentastemona egregia (Schott) Steenis	–	3–5	Duyfjes 1991
Stemona aphylla	STEM 37/5	2.6–3.9	this study
S. collinsae	–	2.5–6.5	Hartl & Kiehn 2004
	STEM 13	2.3–5.7	this study
S. curtisii	–	4–6.7	Hartl & Kiehn 2004
	STEM 120113	2.9–5.6	this study
	STEM 20357	2.4–6.2	this study
S. involuta	STEM 51	1.2–4.3	this study
S. japonica (Blume) Miq.	–	2.9–6.3	Oginuma et al. 2001
S. kerrii	–	4.2–7.5	Hartl & Kiehn 2004
S. mairei	–	2.1–4.6	this study
S. phyllantha	STEM 24	0.9–1.9	this study
	STEM 58	1.3–1.8	this study
	STEM 09	1.8–4.3	this study
S. sessilifolia (Miq.) Miq.	–	2.9–6.3	Oginuma et al. 2001
S. tuberosa	STEM 62	2.7–4.0	this study
Stichoneuron caudatum	–	2–4	Duyfjes 1991
	STEM 43	6.0–6.9	this study

of morphological studies (Rudall & Bateman 2006), $x = 7$ is further corroborated as original basic number for the whole family, with the two basal genera (*Pentastemona* and *Stemona*) characterized by the possession of this number.

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