EDINBURGH JOURNAL OF BOTANY 78, Article 337: 1–13 (2021). https://doi.org/10.24823/EJB.2021.337

Published by the Royal Botanic Garden Edinburgh
ISSN (online): 1474-0036, ISSN (print): 0960-4286

OPEN ACCESS
PEER REVIEWED

NEW CHROMOSOME COUNTS OF ASIAN COSTACEAE AND INITIAL INSIGHTS INTO THE GENOME EVOLUTION OF THE FAMILY

P. H. van Caspel[1]*, A. D. Poulsen[1] & M. Möller[1]

Chromosome counts were obtained from six species of Costaceae from Asia. Our count of 2*n* = 18 for *Cheilocostus speciosus* confirms previous counts, and the other five counts have been made for the first time (*Cheilocostus borneensis*, *Cheilocostus globosus*, *Cheilocostus sopuensis*, *Costus muluensis* and *Paracostus* sp.). These chromosome counts reveal two somatic numbers, 2*n* = 18 and 2*n* = 36, of which the former is a new diploid number for the genus *Paracostus*. A comprehensive review of existing counts was conducted through literature and database searches. Mapping of these on a published comprehensive phylogenetic tree suggests that the diploid count of 2*n* = 18 is probably ancestral in the Costaceae, with repeated parallel evolution of tetraploidy and one case of octoploidy. The existence of triploid counts in several lineages harbouring polyploids suggests that diploids and tetraploids may exist in close proximity, and that crosses or meiotic irregularities may lead to triploid genotypes occurring frequently.

Keywords. Borneo, *Cheilocostus*, *Costus*, cytology, *Hellenia*, *Paracostus*.
Received 4 May 2020 Accepted 14 September 2020 Published 10 March 2021

Introduction

Relatively few cytological studies have focused on the tropical plant family Costaceae Nakai. So far, the majority of chromosome numbers obtained are of Neotropical members of the family, which show a predominant somatic number of 2*n* = 18 (Mahanty, 1970; Maas, 1972), and only four Asian species of the family have been counted (**Table 1**). All Asian Costaceae share the somatic number of 2*n* = 18, except for *Cheilocostus speciosus* (J.Koenig) C.D.Specht. This species shows counts varying between 2*n* = 13 and 2*n* = 72, with the majority being 2*n* = 36 (see **Table 1**). Most counts for the family are 2*n* = 18, which may suggest that *x* = 9 is the basic number in Costaceae, and 2*n* = 18 the ancestral state, but this has never been investigated for the family, only proposed for the genus *Costus* L. (Maas, 1972). Regarding *Paracostus* C.D.Specht, only one count (2*n* = 36) is known for the African *P. englerianus* (K.Schum.) C.D.Specht. This count has not been confirmed, and more studies are needed to investigate whether this is a tetraploid species or represents a tetraploid population (Mahanty, 1970).

In 2006, Specht published a generic recircumscription of Costaceae resulting in the restriction of *Costus* to Africa and the Neotropics. The Asian species formerly in *Costus* were instead placed in *Cheilocostus* C.D.Specht and *Paracostus*, although combinations in these genera were not made for all Asian species, for example *Costus tonkinensis* Gagnep.

[1] Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, UK.
* Corresponding author. E-mail: phcaspel@protonmail.com.

Table 1. Known chromosome numbers of Costaceae, with region for each species and reference for each count

Species	Region	2*n*	*n*	Reference(s)
Chamaecostus cuspidatus (Nees & Mart.) C.D.Specht & D.W.Stev.	Neotropical	18		Raghavan & Venkatasubban (1943), Maas (1972, as *Costus igneus* N.E.Br.)
Cheilocostus lacerus (Gagnep.) C.D.Specht	Indochina	18		Chen *et al.* (1986)
Cheilocostus speciosus (J.Koenig) C.D.Specht	Australasia and Indomalaya	13		Chattopadhyay & Sharma (1983)
		18		Sato (1948, 1960), Miège (1962), Chattopadhyay & Sharma (1983), Lodh & Basu (2013)
		27		Simmonds (1954), Nagendra & Abraham (1981), Chattopadhyay & Sharma (1983), Lohd & Basu (2013)
		36		Banerji (1940), Raghavan & Venkatasubban (1943), Chakravorti (1948), Sharma & Bhattacharyya (1959), Miège (1962), Nagendra & Abraham (1981), Chattopadhyay & Sharma (1983), Lohd & Basu (2013)
		72		Chattopadhyay & Sharma (1983)
Costus afer Ker Gawl.	Afrotropical	18		Miège (1962, as *Costus deistelii* K.Schum.), Gill (1978)
		36		Venkatasubban (1946), Subrahmanyam & Khoshoo (1986, as *Costus megalobractea* K.Schum.)
Costus arabicus L.	Neotropical	18		Mahanty (1970, as *Costus discolor* Roscoe), Maas (1972), Guerra (1988, as *Costus brasiliensis* K.Schum.)
		36		Venkatasubban (1946, as *Costus glabratus* Sw.)
Costus claviger Benoist	Neotropical	18		Simmonds (1954, as *Costus niveo-purpureus* Jacq.), Mahanty (1970, as *Costus niveus* G.Mey), Maas (1972)
Costus comosus (Jacq.) Roscoe	Neotropical	18		Simmonds (1954, as *Costus friedrichsenii* Petersen)
Costus curvibracteatus Maas	Neotropical	18		Guerra (1988)
Costus dhaninivatii K.Larsen[a]	Thailand	18		Larsen (1965)
Costus dinklagei K.Schum.	Afrotropical	18		Miège (1962)
Costus dirzoi García-Mend. & Ibarra-Manr.	Neotropical	27		Vovides & Lascurain (1995)
		28		Vovides & Lascurain (1995, 28 = probably $3x + 1$)
Costus dubius (Afzel.) K.Schum.	Afrotropical	18		Gill (1978, as *Costus zechii* K.Schum.)
		36		Chen *et al.* (1986), Mahanty (1970, as *Costus albus* A.Chev.)
Costus erythrocoryne K.Schum.	Neotropical	18		Guerra (1988)
Costus giganteus Welw. ex Ridl.	Afrotropical	18		Miège (1962)
Costus glaucus Maas	Neotropical	18		Guerra (1988)

Species	Region	2*n*	*n*	Reference(s)
Costus guanaiensis Rusby	Neotropical	18		Guerra (1988)
Costus guanaiensis var. *macrostrobilus* (K.Schum.) Maas	Neotropical	18		Mahanty (1970, as *Costus macrostrobilus* K.Schum.)
Costus lucanusianus J.Braun & K.Schum.	Afrotropical	18		Maas (1972), Edeoga & Okoli (2000)
		27		Bisson *et al.* (1968), Edeoga & Okoli (2000)
		36		Miège (1962)
Costus malortieanus H.Wendl.	Neotropical	18		Gregory (1936, assumed misspelled as *Costus malarotiensis*), Venkatasubban (1946, as *Costus elegans* Host. ex. Petersen), Miège (1962)
		27		Miège (1962), Ramachandran (1969, as *Costus elegans* Host. ex Petersen)
		38		Omanakumari & Mathew (1988)
Costus montanus Maas	Neotropical	18		Maas (1972)
Costus mosaicus W.Bull	Afrotropical	18		Venkatasubban (1946)
Costus pictus D.Don	Neotropical	18	9	Vovides & Lascurain (1995)
		36		Venkatasubban (1946)
Costus pulverulentus C.Presl	Neotropical	18		Maas (1972), Guerra (1988)
Costus scaber Ruiz & Pav.	Neotropical	18		Maas (1972), Vovides & Lascurain (1995)
Costus schlechteri H.J.P.Winkl.	Afrotropical	27		Mahanty (1970)
Costus spectabilis (Fenzl) K.Schum.	Afrotropical	18		Miège (1962)
		27		Miège (1962)
Costus spicatus (Jacq.) Sw.	Neotropical	16		Boehm (1931, as *Costus cylindricus* Jacq.)
		18		Simmonds (1954, as *Costus cylindricus* Jacq.)
Costus spiralis (Jacq.) Roscoe	Neotropical		19	Bhattacharyya (1968)
Costus talbotii Ridl.	Afrotropical	18		Miège (1962)
Costus tappenbeckianus J.Braun & K.Schum.	Afrotropical	18		Mahanty (1970)
Costus tonkinensis Gagnep.[a]	Indochina	18		Chen & Chen (1984)
Costus villosissimus Jacq.	Neotropical	18		Maas (1972)
Dimerocostus strobilaceus subsp. *gutierrezii* (Kuntze) Maas	Neotropical	28		Maas (1972)
Paracostus englerianus (K.Schum.) C.D.Specht	Afrotropical	36		Mahanty (1970, as *Costus englerianus* K.Schum.)
Tapeinochilos ananassae (Hassk.) K.Schum.	Australasian	18		Mahanty (1970)

[a] Species not yet combined in the appropriate genus.

(Specht & Stevenson, 2006). Choosing to ignore this recircumscription, Meekiong *et al.* (2006) subsequently published *Costus muluensis* Meekiong, Ipor & Tawan in *Costus* subg. *Paracostus* K.Schum. A paper is in preparation to make the necessary combination in *Paracostus*.

The name *Cheilocostus* is superfluous because an older name is available: *Hellenia* Retz. This name, however, is confusing, because *Hellenia* Willd. *nom. illeg.* has been used widely for species now placed in *Alpinia* Roxb. in Zingiberaceae, the sister family to Costaceae. A proposal to conserve the name *Cheilocostus*, or at least move away from the confusing *Hellenia*, has therefore been initiated (Leong-Škorničková & Šída, 2016). Because a decision has yet to be made, we adhere in this publication to the use of the generic name *Cheilocostus* so as not to confuse the situation further or to establish the use of *Hellenia*.

Chromosome counts in members of the Costaceae date back to 1931, when Boehm, relying on pollen mother cells, established a count of $x = 8$ for *Costus spicatus* (Jacq.) Sw. This number has been doubted by Mahanty (1970, p. 37), because of a later count in this species of $2n = 18$ (Simmonds, 1954). Early work relied on the paraffin-sectioning method, which may obscure chromosome details (Gregory, 1936; Raghavan & Venkatasubban, 1943; Venkatasubban, 1946; Sato, 1948, 1960; Mahanty, 1970; Maas, 1972). More recent work has used the root-tip squash method (e.g. Ramachandran, 1969; Mahanty, 1970; Subrahmanyam & Khoshoo, 1986). These later authors also employed Feulgen staining to overcome the problem, previously noted by Mahanty (1970), of stainability of Zingiberales mitotic chromosomes.

Although some doubtful counts may be attributable to methodological obstacles, some odd counts appear repeatedly, with $2n = 3x = 27$ for some species (e.g. Mahanty, 1970; Subrahmanyam & Khoshoo, 1986; Lohd & Basu, 2013) (see **Table 1**). These may represent triploids of crosses between diploid and tetraploid genotypes. Polyploids can basically arise in two forms: as autopolyploids possessing sets of identical chromosomes originating from meiotic replication errors and/or fusion of unreduced gametes forming multivalents at meiosis; and as allopolyploids having two different sets of chromosomes, each haploid set from a different species, with subsequent genome duplication forming bivalents at meiosis (Stebbins, 1971; Levin, 2002). Autopolyploidy is a common feature in vegetatively propagating plants (Mahanty, 1970; Meyers & Levin, 2006; Lohd & Basu, 2013).

The ecological advantage of polyploidy would be the ability to carry more than two alleles (fixed in allopolyploids) over diploids, which might result in superior genotypes (e.g. Otto, 2007; Alix *et al.*, 2017). Whole-genome duplication events (autopolyploidy) have occurred repeatedly throughout the evolution of the angiosperms, including several times in monocots (Weiss-Schneeweiss, 2013; Landis *et al.*, 2018), and it is estimated that about 70% of angiosperms have experienced increases in ploidy level (Meyers & Levin, 2006), largely because polyploidy is irreversible (Stebbins, 1971; Grant, 1981). Although diploid and polyploid counts in the family Costaceae have been published, genome evolution within the family is as yet unstudied.

We aimed in the present study to add to the scant counts of Asian members of the Costaceae and to fill gaps in our knowledge of this group, with a focus on Bornean representatives. At the same time, we hoped to supplement the existing counts in the family and study the evolution of their genomes, elucidating the basic number and ploidy level in a phylogenetic context in the family.

Materials and methods

The living collections at the Royal Botanic Garden Edinburgh (RBGE) enabled us to include the following Asian species in the present study: *Cheilocostus borneensis* A.D.Poulsen, *C. globosus* (Blume) C.D.Specht, *C. sopuensis* (Maas & H.Maas) C.D.Specht, *C. speciosus*, *Costus muluensis* and a species of *Paracostus* that is probably undescribed (**Table 2**). Eight accessions of the six species in the three genera were sampled, with two samples each for *Cheilocostus globosus* and *C. muluensis*. To increase the chances of cytological success, stem cuttings were taken and cultivated to produce fresh, actively dividing roots, which were harvested about 6 weeks after the cuttings were taken.

The cytological methods followed Jong's (1997) Feulgen squash technique, with slight alterations. Briefly, root tips were pretreated in either 8-hydroxyquinoline or paradichlorobenzene in the dark for 5 h at room temperature. The roots were fixed in Farmer's fluid (3:1, ethanol:glacial acetic acid) and hydrolysed in 5 M hydrochloric acid for 30 min. They were then stained in freshly prepared Feulgen reagent (Fox, 1969) and placed in the dark for up to 2 h. Softening of the roots was achieved by immersion in a 1:1 enzyme mixture of 4% pectinase and 4% cellulase at 36°C for 30 min. Root-tip meristems were then squashed in 0.05% acetocarmine counterstain to reduce fading of the Feulgen stain over time in permanent slides.

Permanent slides were prepared using a vapour exchange method (Bradley, 1948; Jong, 1997). Images were captured using AxioVision Rel. v. 4.7 and an AxioCam MRc 5 camera mounted on an AxioPhot brightfield microscope (all Zeiss, Welwyn Garden City, UK). Root-tip squash preparations were repeated until at least two confirmatory counts had been obtained (see **Table 2**). Several images were recorded, but only one per species is shown in this paper.

The initial root harvest of *Cheilocostus globosus* did not lead to satisfactory preparations. A second root harvest was carried out and given a slightly altered pretreatment: roots were placed in 8-hydroxyquinoline at room temperature for 6 h instead of 5 h.

To facilitate a discussion of the genome evolution of Costaceae, the phylogenetic tree of Specht (2006) was used to plot the chromosome numbers established for the family in this study, alongside counts from previous studies. The species identities of the accessions used for the counts were updated following the currently accepted synonymy. Furthermore, we believe Specht (2006) made a misidentification when including a sample of *Paracostus* from Borneo as *P. paradoxus* (K.Schum.) C.D.Specht. In the modified tree (see **Figure 2**),

Table 2. Species and samples included and chromosome counts made in the present study[a]

Species name used in the study	RBGE living accession no. and qualifier	RBGE collection no(s).	Cytology voucher no.	Field collection	Wild collection locality details	Count ($2n$)	No. of counts done	No. of roots squashed
Cheilocostus borneensis A.D.Poulsen	20040728*A	*Poulsen* 2596	2019-40	*Poulsen & Raymond* 1964 (holo SAR, iso AAU)	Malaysia. Sarawak, Batang Ai, Nanga Sumpa longhouse	18	9	2
Cheilocostus globosus (Blume) C.D.Specht	20070755*A	*Poulsen* 3202	2019-50	*Škorničková* 74757 (living only)	Indonesia. Eastern Kalimantan: Malinau regency, forest around Gongsolok Village	18	2	2
Cheilocostus globosus (Blume) C.D.Specht	20070757*A	*Newman & Poulsen* 2431	2019-51	*Škorničková* 74764 (living only)	Indonesia. East Kalimantan, Malinau regency, forest around Gongsolok village, on way to caves	18	5	6
Cheilocostus sopuensis (Maas & H.Maas) C.D.Specht	20090617*A	*Newman* 2695, *van Caspel* 3	2019-47	*Poulsen* 2736 (BO, E)	Indonesia. Sulawesi, Central Sulawesi Province, Kebunkopi	18	9	4
Cheilocostus speciosus (J.Koenig) C.D.Specht	19751812*A	*Poulsen & Sánchez-Ganfornina* 3200	2019-38	*Winters & Higgins s.n.* (living only)	Papua New Guinea. Hill below Oregenang village, 1280 m altitude	18	2	3
Costus (subg. *Paracostus*) *muluensis* Meekiong, Ipor & Tawan[b]	19773474*A	*van Caspel* 5	2019-32	*Kerby* 216 (living only)	Malaysia. Sarawak, Gunung Mulu National Park	36	9	3
Costus (subg. *Paracostus*) *muluensis* Meekiong, Ipor & Tawan[b]	19773484*A	*van Caspel* 6	2019-44	*Kerby* 226 (E)	Malaysia. Sarawak, Gunung Mulu National Park	36	2	1
Paracostus sp.	20040947*A	*Poulsen* 2466, 3027	2019-34	*Poulsen* et al. 2031 (AAU, SAR)	Malaysia. Sarawak, Kubah NP, Sungai Rayu	18	2	1

[a] All collections made from living material at RBGE have been deposited at E.
[b] Species not yet combined in the appropriate genus.

we have therefore labelled the branch as *Paracostus* spp. to represent *Paracostus* sp. and *Costus muluensis*, belonging to subgenus *Paracostus*, as explained above.

Results and discussion

In the present study, new counts of five species of Asian Costaceae were obtained and the count of *Cheilocostus speciosus* was confirmed. For two of the species, two accessions were examined and gave identical counts. One of the new counts is for an undescribed species of *Paracostus* (see **Table 2**, **Figure 1**). The chromosome counts revealed two somatic numbers among the samples, namely 2*n* = 18 and 2*n* = 36; the former is a new number in the genus *Paracostus*, the only other count available so far being 2*n* = 36 in *P. englerianus* (Mahanty, 1970).

Most root-tip cell preparations showed chromosomes in prometaphase, so they were difficult to measure (see **Figure 1**). The length range of the metaphase chromosomes of *Costus muluensis* was between 1.2 and 3.5 μm, and for *Cheilocostus sopuensis* between 0.9 and 3.1 μm. The variation may be influenced by the condensation level of the chromosomes in the preparations, although Mahanty (1970) gives a length range for *Costus guanaiensis* var. *macrostrobilus* (K.Schum.) Maas (as *C. macrostrobilus* K.Schum.) of 2.3–3.7 μm, which is well within the ranges of the species studied here. Similar ranges were reported by Subrahmanyam & Khoshoo (1986) for *Costus malortieanus* H.Wendl. (1.3–2.5 μm, 2*x*) and *C. afer* Ker Gawl. (as *C. megalobractea* K.Schum.; 1.4–3.5 μm, 4*x*). In *Cheilocostus*, similar ranges were reported by Lodh & Basu (2013) for *C. speciosus*: 1.47–3.27 μm (2*x*) to 1.60–4.37 μm (4*x*). The authors of these studies commented on the uniformly gradual series of longest to shortest chromosome, similar to the findings presented here.

Without exception, in the present study chromosome numbers were found to be consistent with a basic number of *x* = 9. The numbers found in *Cheilocostus globosus* (20070757*A, 2*n* = 18) and *C. speciosus* (19751812*A, 2*n* = 18) matched that of their presumed congeners in *Cheilocostus*: *Costus tonkinensis* (placed within the *Globosus* complex; Maas, 1979) and *Cheilocostus lacerus* (Gagnep.) C.D.Specht (each 2*n* = 18). The Malaysian *Costus muluensis* (19773484*A), still to be combined in *Paracostus*, had a chromosome count of 2*n* = 36, equal to its African relative *Paracostus englerianus*. *Paracostus* sp. from Borneo (20040947*A) and *Cheilocostus sopuensis* (20090617*A) both had 2*n* = 18, a number widespread in the family. Across all counts in the family, only *Dimerocostus* Kuntze deviated from the basic number of *x* = 9 and has previously been counted with *x* = 14 chromosomes (see **Table 1**) (Maas, 1972).

Infraspecific variation in chromosome numbers has previously been attributed to differences at the population level in several species (see **Table 1**). For instance, in *Cheilocostus speciosus*, a range of ploidy counts varying from diploid to octoploid including a triploid were determined for different populations (see **Table 1**), although no explanation was given for the odd count of 2*n* = 13 by Chattopadhyay & Sharma (1983). Others, however,

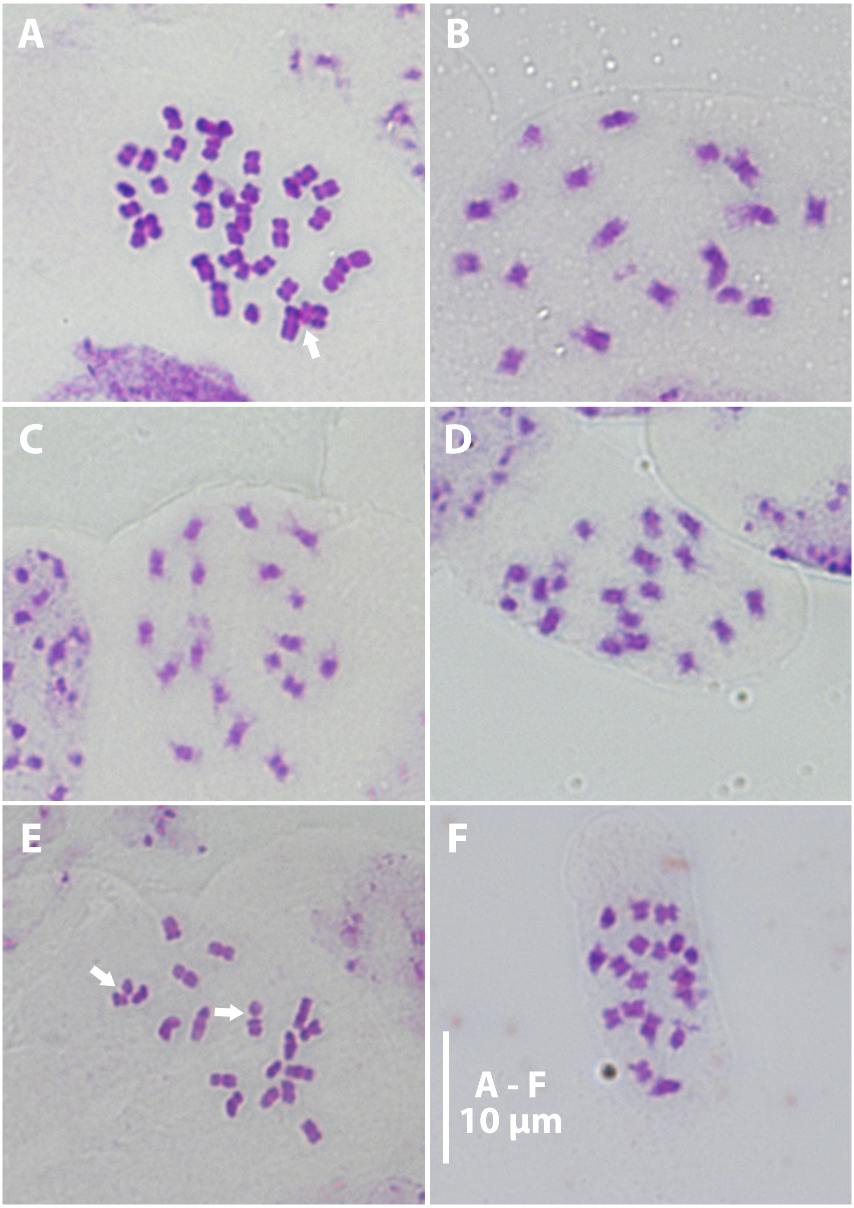

Figure 1. Root-tip chromosome squash preparations for members of Costaceae (garden accession numbers are in parentheses). A, Metaphase of *Costus muluensis* with 2*n* = 36 (19773474*A); the arrow indicates a squashed chromosome. B, Prometaphase of *Paracostus* sp. with 2*n* = 18 (20040947*A). C, Prometaphase of *Cheilocostus borneensis* with 2*n* = 18 (20040728*A). D, Protometaphase of *Cheilocostus globosus* with 2*n* = 18 (20070757*A). E, Metaphase of *Cheilocostus sopuensis* with 2*n* = 18 (20090617*A); the arrows indicate possible secondary constrictions. F, Late prometaphase of *Cheilocostus speciosus* with 2*n* = 18 (19751812*A). All images are at the same scale (scale bar, 10 µm).

considered *Cheilocostus speciosus* to represent a species complex, with several varieties currently placed within the species, based on the similar vegetative and floral morphology (Specht & Stevenson, 2006; Harrington & Zich, 2012). In fact, new species closely related to *Cheilocostus speciosus* have been described recently (Harrington & Zich, 2012; Kumar *et al.*, 2016), indicating that this species may be split up. Future molecular studies may reveal that some of the varieties currently described could be recognised at species level or sunk, depending on the results. Some of the cytological differences may be attributable to different species or varieties, or the fact that morphological differences between taxa are an expression of their different ploidy levels (e.g. Mahanty, 1970; Lohd & Basu, 2013; Weiss-Schneeweiss, 2013; Kolář *et al.*, 2017).

The existence of infraspecific ploidy level variation may indicate autopolyploidy as the mechanism for the increase in chromosome numbers, as suggested by Mahanty (1970) and Lohd & Basu (2013). Such an autopolyploid scenario has been proposed for *Costus lucanusianus* J.Braun & K.Schum., probably through the formation of polyploids from unreduced gametes (Edeoga & Okoli, 2000). Autopolyploidy would result in the formation of multivalents during meiosis, but in several independent studies of *Costus speciosus*, summarised by Subrahmanyam & Khoshoo (1986, p. 739, and references therein), only bivalents were found in triploid and tetraploid plants. This may suggest that allopolyploidy, that is, hybridisation between different species followed by genome duplication, is the source of the polyploids (Lohd & Basu, 2013). An alternative explanation could be diploidisation of autopolyploid plants, whereby duplicated genes are randomly lost over time until only two homologous genomes exist (e.g. Gatt *et al.*, 1998; Dodsworth *et al.*, 2016). Clearly, more work is required to understand the origin and nature of polyploidy in Costaceae.

Although we refrain from a formal character optimisation here, due to missing chromosome counts for a range of species included in the phylogenetic tree, some preliminary inferences can be made on genome evolution in the family. The diploid number of $2n = 18$ occurs across the phylogenetic tree of Costaceae (**Figure 2**), except for the clade on the basal-most lineage, and is probably the ancestral state in the family. To test this hypothesis, more counts are required in the *Dimerocostus* and *Chamaecostus* C.D.Specht & D.W.Stev. clades. The only representative of the former cytologically investigated is *Dimerocostus strobilaceus* subsp. *gutierrezii* (Kuntze) Maas, which resulted in a unique count of $2n = 28$ (see **Figure 2**) (Maas, 1972). At present, it is unclear whether this is an autapomorphy of the species or a characteristic of the genus or the clade, or a miscount of a triploid, as observed in several other species, due to the sectioning method used by Maas (1972).

Tetraploids with $2n = 4x = 36$ appear scattered in several different places across the family phylogeny and have very probably evolved independently from each other. This is supported by the fact that in four out of six instances of tetraploidisation, diploids were also found (see **Figure 2**). The case of *Paracostus* is unclear, because the branch leading to *Paracostus* spp. includes data obtained from two different species (see above; see **Figure 2**).

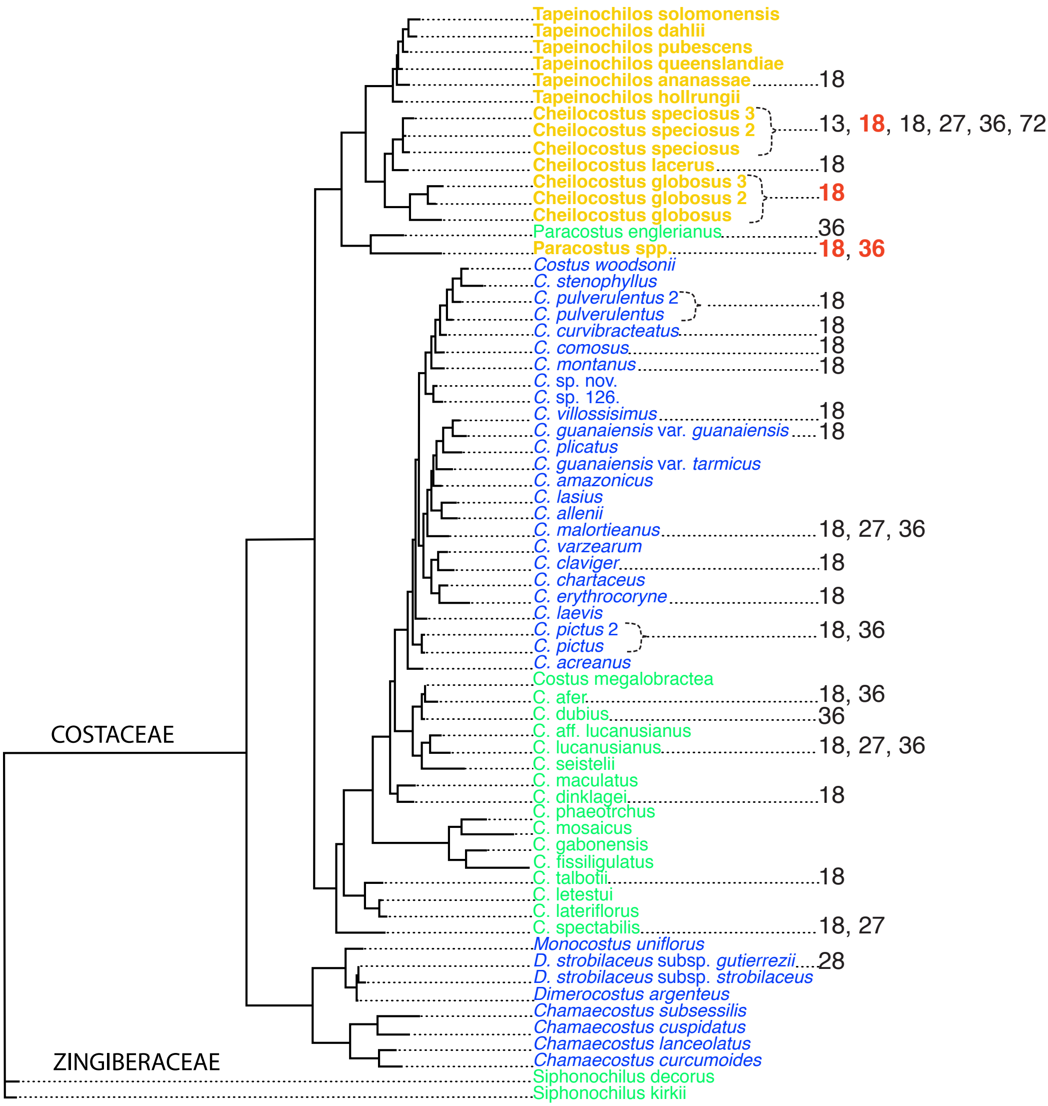

Figure 2. Phylogenetic tree of Costaceae, modified from Specht (2006), with chromosome numbers ($2n$) from previous studies in black (see **Table 1** for references) and counts made in the present study in red. African species are in green roman text, Neotropical in blue italic text and Asian in yellow bold text. In the work of Specht, the Bornean *Paracostus* sample included was identified as *P. paradoxus*, which we believe is a misidentification; the branch has therefore been labelled *Paracostus* spp. and represents the undescribed *Paracostus* ($2n$ = 18) and *Costus* (subg. *Paracostus*) *muluensis* ($2n$ = 36).

It is also interesting to note that in three out of seven polyploidisation events, triploids with $2n = 3x = 27$ were found (see **Figure 2**), suggesting crosses between diploid and tetraploid forms. Triploids or aneuploids also occur in many Zingiberaceae Martinov, such as *Curcuma* L. (Leong-Škorničková *et al.*, 2007). Families in the order Zingiberales may be prone to meiotic errors and unbalanced karyotypes, and the persistence of sterile triploids may result from their rhizome-forming vegetative reproductive strategy. This is an area in which further studies are necessary.

In summary, the chromosome numbers of the Asian Costaceae studied here ($2n = 2x = 18$ and $2n = 4x = 36$) are in line with those observed in other members of the family and share a common basic number of $x = 9$. In phylogenetic terms, diploidy seems to have been ancestral, and polyploids seem to have arisen on several occasions independently. The mechanisms by which the polyploids arose may perhaps be different; evidence of both allopolyploidy and autopolyploidy exists, and no single mechanism may exist for Costaceae. The vegetative reproduction of the plants aids the persistence of odd polyploids and may be one facet of the scenario. To fully understand the situation, each case would require detailed studies, including meiotic and molecular studies. Our new counts presented here fill gaps in our knowledge but more work is needed.

Acknowledgements

The authors would like to extend their gratitude to Stephen Willis, for taking great care in growing the stem cuttings and keeping the ginger collection at RBGE in great shape; the Sibbald Trust, for financial support for the write-up of this study; and of course our RBGE colleagues Edeline Gagnon and Thibauld Michel, who have provided moral support. The Royal Botanic Garden Edinburgh is supported by the Scottish Government's Rural and Environmental Science and Analytical Services Division.

References

Alix K, Gérard PR, Schwarzacher T, Heslop-Harrison JS. 2017. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. Annals of Botany. 120(2):183–194.

Banerji IA. 1940. A contribution to the life history of *Costus speciosus* Smith. Journal of the Indian Botanical Society. 19:181–196.

Bhattacharyya R. 1968. Systematic status of the family Zingiberaceae. Nucleus, Supplement. 39:41.

Bisson S, Guillemet S, Hamel J-L. 1968. Contribution à l'ètude caryo-taxonomique des Scitaminées. Mémoires du Muséum National d'Histoire Naturelle. 18:59–133.

Boehm K. 1931. Embryologische Untersuchungen an Zingiberaceen. Planta. 14(2):411–440.

Bradley MV. 1948. A method for making aceto-carmine squashes permanent without removal of the cover slip. Stain Technology. 23(1):41–44.

Chakravorti AK. 1948. Multiplication of chromosome numbers in relation to speciation of Zingiberaceae. Science and Culture. 14:137–140.

Chattopadhyay S, Sharma AK. 1983. Genetic diversity in *Costus speciosus* (Koen.) Sm. Cytologia. 48(1):209–214.

Chen Z, Chen S. 1984. A report on chromosome numbers of Chinese Zingiberaceae. Guihaia. 4:13–18.

Chen Z-Y, Chen S-J, Huang S-F. 1986. A report on chromosome numbers on Chinese Zingiberaceae (3). Acta Botanica Austro Sinica. 3:57–61.

Dodsworth S, Chase MW, Leitch AR. 2016. Is post-polyploidization diploidization the key to the evolutionary success of angiosperms? Botanical Journal of the Linnean Society. 180(1):1–5.

Edeoga HO, Okoli BE. 2000. Chromosome numbers of *Costus lucanusianus* (Costaceae) in Nigeria. Folia Geobotanica. 35(3):35–318.

Fox DP. 1969. Some characteristics of the cold hydrolysis technique for staining plant tissue by Feulgen reaction. Journal of Histochemistry and Cytochemistry. 17(4):266–272.

Gatt M, Ding H, Hammett K, Murray B. 1998. Polyploidy and evolution in wild and cultivated *Dahlia* species. Annals of Botany. 81(5):647–656.

Gill LS. 1978. Chromosome numbers of angiosperms in Tanzania: II. Adansonia. 18:19–24.

Grant V. 1981. Plant Speciation, 2nd edition. New York: Columbia University Press.

Gregory PJ. 1936. The floral morphology and cytology of *Elettaria cardamomum* Maton. Journal of the Linnean Society, Botany. 50(334):363–391.

Guerra MS. 1988. Characterization of different types of condensed chromatin in *Costus* (Zingiberaceae). Plant Systematics and Evolution. 158:107–115.

Harrington M, Zich F. 2012. A new combination in Australian Costaceae. Telopea. 14:9–10.

Jong K. 1997. Laboratory Manual of Plant Cytological Techniques. Edinburgh: Royal Botanic Garden Edinburgh.

Kolář F, Čertner M, Suda J, Schönswetter P, Husband BC. 2017. Mixed-ploidy species: progress and opportunities in polyploid research. Trends in Plant Science. 22(12):1041–1055.

Kumar R, Singh SK, Sinha BK, Sharma S. 2016. Description of two new species of *Hellenia* (Costaceae) from North-east India. Keanean Journal of Science. 5:3–8.

Landis JB, Soltis DE, Li Z, Marx HE, Barker MS, Tank DC, Soltis PS. 2018. Impact of whole-genome duplication events on diversification rates in angiosperms. American Journal of Botany. 105(3):348–363.

Larsen K. 1965. *Costus dhaninivatii*, a new species from S.E. Thailand. Southeast Asian Studies. 149–152.

Leong-Škorničková J, Šída O. 2016. Proposal to conserve the name *Cheilocostus* against *Hellenia* and *Tsiana* (*Costaceae*). Taxon. 65(6):1426–1427.

Leong-Škorničková J, Šída O, Jarolímoá V, Sabu M, Fér T, Trávnícek P, Suda J. 2007. Chromosome numbers and genome size variation in Indian species of *Curcuma* (Zingiberaceae). Annals of Botany. 100(3):505–526.

Levin DA. 2002. The Role of Chromosomal Change in Plant Evolution. New York: Oxford University Press.

Lodh D, Basu S. 2013. Karyomorphological analysis and cytotypic diversity in natural populations of *Costus speciosus* Koen. ex Retz. Nucleus. 56(3):155–162.

Maas PJM. 1972. *Costoideae (Zingiberaceae)*. Flora Neotropica, monograph 8. New York: Hafner.

Maas PJM. 1979. Notes on Asiatic and Australian Costoideae (Zingiberaceae). Blumea. 25(2):543–549.

Mahanty HK. 1970. A cytological study of the Zingiberales with special reference to their taxonomy. Cytologia. 35(1):13–49.

Meekiong K, Ipor IB, Tawan CS, Muliati M. 2006. Three new species from Sarawak, and notes on the cauliferous *Costus* Group (Costaceae, Subgenus *Paracostus*). Folia Malaysiana. 7(1–2):55–72.

Meyers LA, Levin DA. 2006. On the abundance of polyploids in flowering plants. Evolution. 60(6):1198–1206.

Miège J. 1962. Quatrieme liste de nombres chromosomiques d'especes d'Afrique Occidentale. Revue de Cytologie et de Biologie Végétales. 24:149–164.

Nagendra P, Abraham PZ. 1981. Polyploidy and speciation in *Costus speciosus* (Koen.) Sm. Current Science. 50:26–28.

Omanakumari N, Mathew PM. 1988. Karyomorphological studies on two species of *Costus* L. New Botanist: An International Quarterly Journal of Plant Science Research. 15:33–37.

Otto SP. 2007. The evolutionary consequences of polyploidy. Cell. 131(3):452–462.

Raghavan TS, Venkatasubban KR. 1943. Cytological studies in the family Zingiberaceae with special reference to chromosome numbers and cyto-taxonomy. Proceedings of the Indian Academy of Sciences, Section B. 17(4):118–132.

Ramachandran K. 1969. Chromosome numbers in Zingiberaceae. Cytologia. 34(2):213–221.

Sato D. 1948. The karyotypes and phylogeny in Zingiberales. Japanese Journal of Genetics. 23:44–45.

Sato D. 1960. The karyotype analysis in Zingiberales with special reference to the protokaryotype and stable karyotype. Scientific Papers of the College of General Education, University of Tokyo. 10:225–243.

Sharma AK, Bhattacharyya NK. 1959. Cytology of several members of Zingiberaceae. Cellule. 50:297–346.

Simmonds NW. 1954. Chromosome behaviour in some tropical plants. Heredity. 8:139–146.

Specht CD. 2006. Systematics and evolution of the tropical monocot family Costaceae (Zingiberales): a multiple dataset approach. Systematic Botany. 31(1):89–106.

Specht CD, Stevenson DW. 2006. A new phylogeny-based generic classification of Costaceae (Zingiberales). Taxon. 55(1):153–163.

Stebbins GL. 1971. Chromosomal Evolution in Higher Plants. London: Addison-Wesley.

Subrahmanyam GV, Khoshoo TN. 1986. Cytological studies in the genus *Costus* L. Cytologia. 51:737–748.

Venkatasubban KR. 1946. A preliminary survey of chromosome numbers in Scitamineae of Bentham and Hooker. Proceedings of the Indian Academy of Sciences, Section B. 23(6):281–300.

Vovides AP, Lascurain M. 1995. Numeros cromosomicos de cuatro especies de *Costus* (Costaceae), una de *Calathea*, una de *Maranta* y una de *Stromanthe* (Marantaceae). Acta Botanica Mexicana. 33:81–86.

Weiss-Schneeweiss H, Emadzade K, Jang T-S, Schneeweiss GM. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenetic and Genome Research. 140(2–4):137–150.