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

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Chromosome counts were obtained from six species of Costaceae from Asia. Our count of 2n = 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, 2n = 18 and 2n = 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 2n = 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.

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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 2n = 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 2n = 18, except for Cheilocostus speciosus (J.Koenig) C.D.Specht. This species shows counts varying between 2n = 13 and 2n = 72, with the majority being 2n = 36 (see Table 1). Most counts for the family are 2n = 18, which may suggest that x = 9 is the basic number in Costaceae, and 2n = 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 (2n = 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.

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| Species                                | Region                  | $2n$ | $n$ | 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   |     | Sato (1948, 1960), Miège (1962), Chattopadhyay & Sharma (1983), Lodh & Basu (2013)                                                          |
|                                        |                         | 18   |     | Simmonds (1954), Nagendra & Abraham (1981), Chattopadhyay & Sharma (1983), Lohd & Basu (2013)                                              |
|                                        |                         | 27   |     | Banerji (1940), Raghavan & Venkatasubban (1943), Chakravorti (1948), Sharma & Bhattacharyya (1959), Miège (1962), Nagendra & Abraham (1981), Chattopadhyay & Sharma (1983), Lohd & Basu (2013) |
|                                        |                         | 36   |     | Mahanty (1970, as *Costus deistelii* K.Schum.), Gill (1978)                                                                                  |
|                                        |                         | 72   |     | Chakravorti (1948), Sharma & Bhattacharyya (1959), Miège (1962), Nagendra & Abraham (1981), Chattopadhyay & Sharma (1983), Lohd & Basu (2013) |
| *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*         | 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     | 2n | 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.*                 | 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)                                                                |

* 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),...
| 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 Aj, 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-Ganforina 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 |                | 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 |                | 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                    |

* All collections made from living material at RBGE have been deposited at E.

* 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 $2n = 18$ and $2n = 36$; the former is a new number in the genus *Paracostus*, the only other count available so far being $2n = 36$ 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 Subrahmanyan & Khoshoo (1986) for *Costus malortieanus* H.Wendl. (1.3–2.5 μm, 2x) and *C. afer* Ker Gawl. (as *C. megalobractea* K.Schum.; 1.4–3.5 μm, 4x). In *Cheilocostus*, similar ranges were reported by Lodh & Basu (2013) for *C. speciosus*: 1.47–3.27 μm (2x) to 1.60–4.37 μm (4x). 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, $2n = 18$) and *C. speciosus* (19751812*A, $2n = 18$) 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 $2n = 18$). The Malaysian *Costus muluensis* (19773484*A), still to be combined in *Paracostus*, had a chromosome count of $2n = 36$, equal to its African relative *Paracostus englerianus*. *Paracostus* sp. from Borneo (20040947*A) and *Cheilocostus sopuensis* (20090617*A) both had $2n = 18$, 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 $2n = 13$ 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 2n = 36 (19773474*A); the arrow indicates a squashed chromosome. B, Prometaphase of *Paracostus* sp. with 2n = 18 (20040947*A). C, Prometaphase of *Cheilocostus borneensis* with 2n = 18 (20040728*A). D, Protometaphase of *Cheilocostus globosus* with 2n = 18 (20070757*A). E, Metaphase of *Cheilocostus sopuensis* with 2n = 18 (20090617*A); the arrows indicate possible secondary constrictions. F, Late prometaphase of *Cheilocostus speciosus* with 2n = 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.

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