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# Miscellaneous notes

## AMARYLLIDACEAE

### CHROMOSOME STUDIES ON AFRICAN PLANTS. 20. KARYOTYPES OF SOME *CYRTANTHUS* SPECIES

#### INTRODUCTION

The genus *Cyrtanthus* Aiton (Amaryllidaceae) is endemic to sub-Saharan Africa. The centre of distribution is the southeastern Cape with smaller centres in other parts of the Western and Eastern Cape, Gauteng, Mpumalanga and KwaZulu-Natal Provinces (Du Plessis & Duncan 1989; Meerow & Snijman 1998; Snijman & Archer 2003). *Cyrtanthus* is the largest amaryllid genus in southern Africa and currently consists of 56 species, one subspecies and seven varieties (Snijman & Archer 2003, 2006).

There are two major trends characterizing amaryllidaceous karyotype evolution (Meerow 1995). Certain genera, e.g., *Crinum* L. and *Hippeastrum* Herb., show great karyotypic stability, with a low frequency of polyploidy. Similar chromosome morphology among the species of these genera is characteristic. Their polyploids have a tendency to be autoploid in origin. A genus may also exhibit great variation in both chromosome number and morphology, e.g. *Hymenocallis* Salisb. and *Lycoris* Herb. Both allopolyploidy and Robertsonian changes have been implicated as important factors in speciation in such genera.

Chromosomes in *Cyrtanthus* are large, as in the rest of the Amaryllidaceae (Wilsenach 1963). The genus has base numbers of x = 6, 8 and 11 (Meerow 1995). Most *Cyrtanthus* species studied (Figures 1, 2) have a somatic

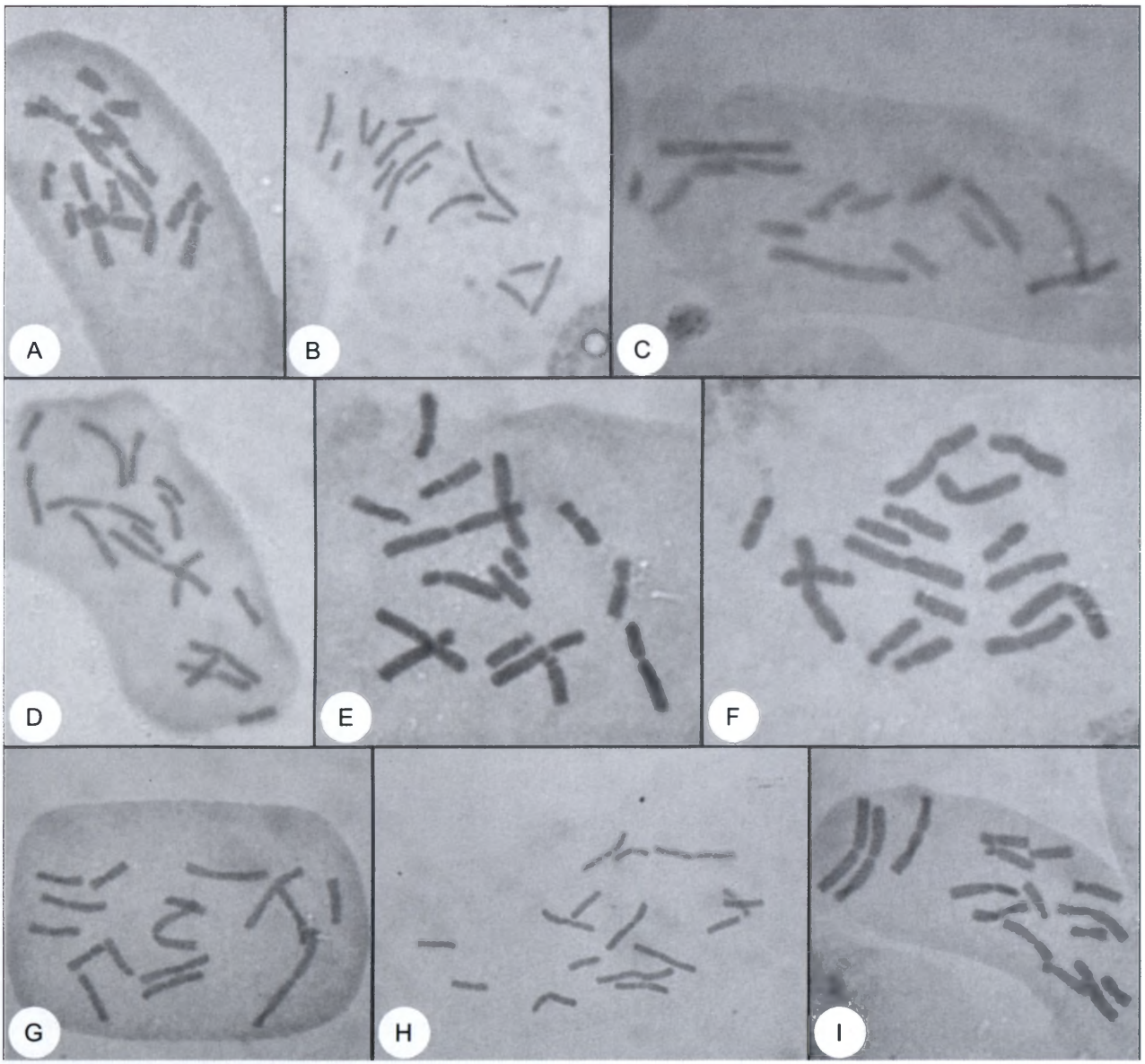


FIGURE 1.—Mitotic chromosomes in various *Cyrtanthus* species with 2n = 16. A, *C. bicolor*, *Spies 7185*; B, *C. brachyscyphus*, *Spies 7186*; C, *C. breviflorus*, *Spies 7189*; D, *C. elatus*, *Spies 7193*; E, *C. epiphyticus*, *Spies 7200*; F, *C. eucallus*, *Spies 7262*; G, *C. falcatus*, *Spies 7208*; H, *C. herrei*, *Spies 7267*; I, *C. loddigesianus*, *Spies 7203*.


FIGURE 2.—Mitotic chromosomes in various *Cyrtanthus* species. A, *C. mackenii* var. *cooperi*, *Spies 7211*; B, C, *C. mackenii* var. *mackenii* (diploid); D, tetraploid, *Spies 7268, 7274, 7370*; E, F, *C. macowanii*, *Spies 7201*; G, H, *C. montanus*, *Spies 7275*; I, *C. obliquus*, *Spies 7276*; J, *C. obrienii*, *Spies 7376*; K, *C. sanguineus*, *Spies 7216*; L, *C. tuckii* var. *viridilobus*, *Spies 7377*.

chromosome number of 2n = 16 (Taylor 1925; Gouws 1949; Tjio & Levan 1950; Flory 1955; Ising 1962, 1966, 1970; Wilsenach 1963; Bose 1965; Nandi 1973; Venkateswarlu & Lakshmi 1976; Laksmi 1980) or 2n = 32 (Ising 1966), but 2n = 14 and 22 were recorded by Bose (1965) and Satô (1938, 1942), respectively for *C. obliquus*, and Mookerjea (1955) and Bose (1965) recorded 2n = 18 for *C. sanguineus*. Variations in karyotypes have been described (Wilsenach 1963; Ising 1966).

The aim of this study was to determine the chromosome numbers of more species and to compare their karyotypes.

## MATERIALS AND METHODS

Bulbs were collected in the field and grown in greenhouses at ARC-Roodeplaat, Pretoria. Herbarium specimens are stored in the Geo Potts Herbarium (BLFU), Bloemfontein (Appendix 1).

Root tips were collected between 08:00 and 10:00 in glass bottles filled with tap water (4°C) and refrigerated for 24 hours. Subsequent to the cold pre-treatment, the water was replaced with Pienaar's fixative (Pienaar 1955). The root tips were macerated in 1N HCl for 7 minutes at 60°C and stained with 2% propionic-carmine,

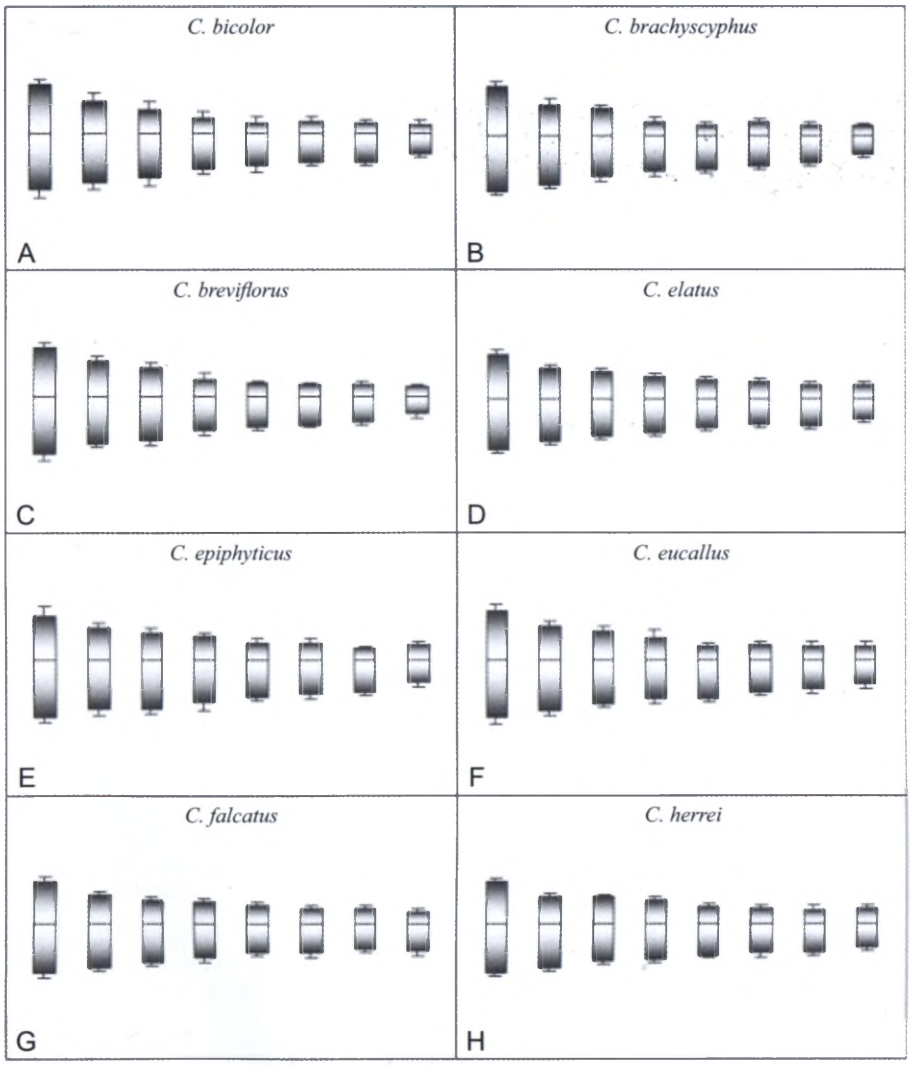


FIGURE 3.—Ideograms of some *Cyrtanthus* species. A, *C. bicolor*; B, *C. brachyscyphus*; C, *C. breviflorus*; D, *C. elatus*; E, *C. epiphyticus*; F, *C. eucallus*; G, *C. falcatus*; H, *C. herrei*.

to which a trace of ferric-acetate was added. Permanent slides were analysed under a Nikon Microphot FXA microscope, chromosome counts were made and cells with well-spread chromosomes were photographed with a Cool Pix digital camera.

All chromosomes in a minimum of 20 well-spread metaphase cells were measured to determine the karyotypes. The following parameters were used for each metaphase plate: 1, chromosome length of each individual chromosome (CL); 2, length of the short arm (p); 3, total chromosome length (TCL); 4, relative chromosome length ($CL_x$/TCL); 5, relative short arm length ($p_x$/TCL). The arithmetic averages of the relative chromosome lengths and relative short arm lengths were used to draw the ideograms and to indicate the standard deviation from the mean for each chromosome pair.

## RESULTS AND DISCUSSION

Sixty-two specimens, representing 19 species were studied. A somatic chromosome number of 2n = 2x = 16 was observed in all specimens (Figures 1, 2), except two *C. mackenii* var. *mackenii* specimens, *Spies 7274* (Figure 2D) and *7372*, which were both tetraploid with 2n = 4x = 32 (Appendix 1). This is the first report on a natural occurring tetraploid in *C. mackenii* and these somatic chromosome numbers indicate a basic chromosome number of x = 8 (Figures 1, 2).

Reported chromosome numbers for *Cyrtanthus* indicate 2n = 22 as the previous highest somatic chromosome number (Satô 1938, 1942). This report further supports the chromosome numbers observed by Taylor (1925), Gouws (1949), Tjio & Levan (1950), Flory (1955), Ising (1962, 1966, 1970), Wilsenach (1963), Bose (1965), Nandi (1973), Venkateswarlu & Lakshmi (1976) and Laksmi (1980). The apparent different basic chromosome numbers described in the literature, such as x = 6 and 11 in *Cyrtanthus*, are most probably the results of incorrect identifications or incorrect interpretation of metaphase plates. In the Satô report (1938), the material was embedded in paraffin and cut in cross sections. This method does not enable researchers to examine single layers of large cells in their totality and thus may result in miscounts of chromosomes (Darling & La Cour 1942). No vouchers were cited in this report and therefore they cannot be consulted for accuracy of chromosome acounts. The Bose report (1965) is also a miscount, given ample evidence from recent counts that *C. sanguineus* has 2n = 16. Ising (1970) already commented on this issue, concluding that the secondary constrictions occurring sometimes, could also be a reason

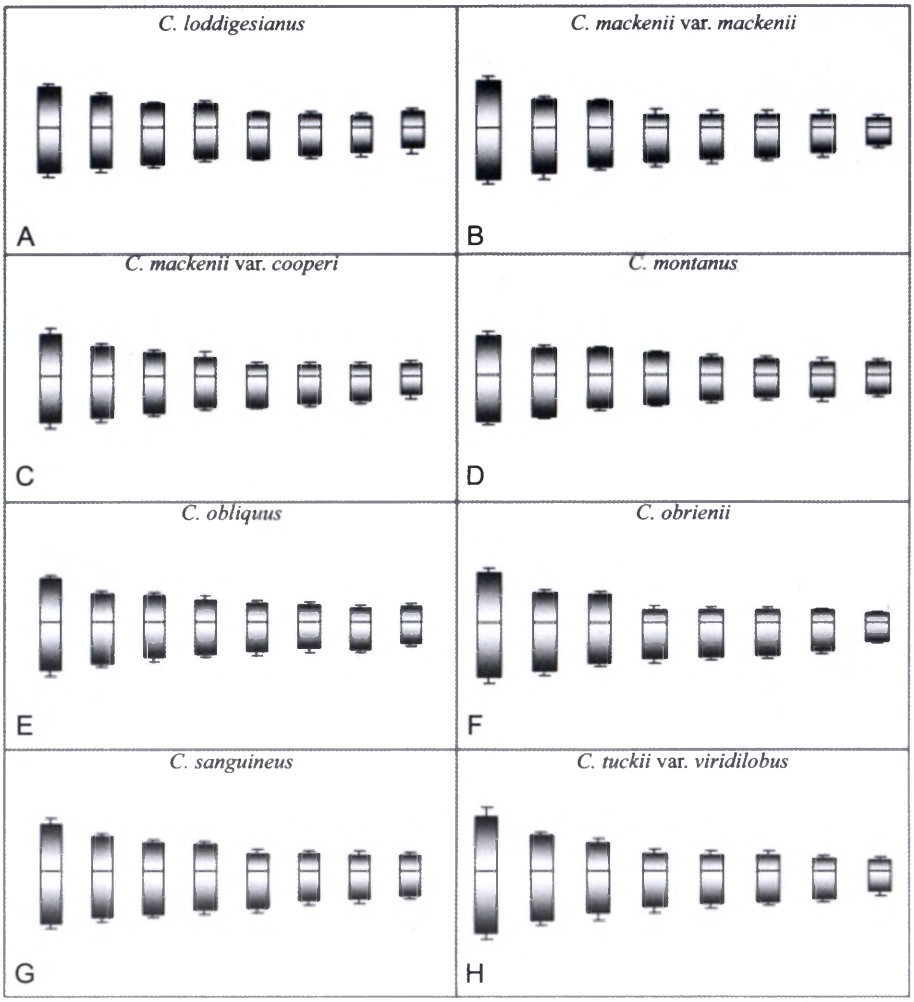


FIGURE 4.—Ideograms of some *Cyrtanthus* species. A, *C. loddigesianus*; B, *C. mackenii* var. *mackenii*; C, *C. mackenii* var. *cooperi*; D, *C. montanus*; E, *C. obliquus*; F, *C. obrienii*; G, *C. sanguineus*; H, *C. tuckii* var. *viridilobus*.

for incorrect counts. The real base number is x = 8, as supported by this study. In this study, the somatic chromosome number of 2n = 32 indicates a doubling of the somatic chromosome number of 2n = 16.

Previous studies indicated that ideograms vary among the different *Cyrtanthus* species and may be used in the identification of certain species (Ising 1970). This variability is not supported by this study. Chromosome contraction varies greatly between different chromosomes and in a single root tip, different ideograms can be obtained from different cells, varying slightly depending upon the time the cell entered mitosis. The ideograms of 16 diploid taxa are shown in Figures 3 and 4. Each taxon has eight meta- to submetacentric chromosomes, arranged in the ideograms from the longest to the shortest. There is a significant difference in length between the longest and shortest chromosomes in each of these taxa. However, when a large number of metaphase cells are measured, and the average length of a chromosome pair in all cells are used, the variation in ideograms is minimal and cannot be used for identification purposes.

This study clearly indicated that *Cyrtanthus* has a chromosome complement of 2n = 2x = 16. Although ideograms provided additional information, they proved to be relatively unreliable for identification purposes.

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APPENDIX 1.—Specimens of *Cyrtanthus* taxa studied with their voucher numbers, localities or author and somatic chromosome numbers

| Taxon | Voucher | Locality/author | 2n | Figure |
|---|---|---|---|---|
| *C. angustifolius* (L.f.) Aiton | | Flory 1955. | 16 | |
| *C. bicolor* R.A.Dyer | *Spies 7185* | Locality unknown, grown from seeds. | 16 | 1A; 3A |
| *C. brachyscyphus* Baker (= *C. parviflorus* Baker) | *Spies 7186* | KWAZULU-NATAL.—2930 (Pietermaritzburg): near Durban, (–DD). | 16 | 1B; 3B |
| | *Spies 7187, 7204, 7272* | Localities unknown, grown from seeds. | 16 | |
| | | Taylor 1925; Ising 1962, 1966, 1970; Wilsenach 1963. | 16 | |
| *C. breviflorus* Harv. (= *Anoiganthus breviflorus* (Harv.) Baker.; *A. luteus* (Baker) Baker; *C. luteus* Baker) | *Spies 7188* | GAUTENG.—2528 (Pretoria): near Bronkhorstspruit, (–DC). | 16 | |
| | *Spies 7189* | MPUMALANGA.—2529(Witbank): near Witbank, (–CC). | 16 | 1C; 3C |
| | *Spies 7278, 7366* | Localities unknown, grown from seeds. | 16 | |
| | | Ising 1970. | 16 | |
| | | Ising 1970. | 32 | |
| *C. carneus* Lindl. | | Wilsenach 1963. | 16 | |
| *C. clavatus* (L'Hér.) R.A.Dyer | | Ising 1970. | 16 | |
| *C. contractus* N.E.Br. | *Spies 7190* | MPUMALANGA.—2730 (Vryheid): on Farm Bergplaas, Piet Retief District, (–BB). | 16 | |
| | | Wilsenach 1963; Ising 1970. | 16 | |
| *C. elatus* (Jacq.) Traub (= *Valotta speciosa* T.Durand & Schinz) | *Spies 7194* | MPUMALANGA.—2530 (Lydenburg): near Nelspruit, (–BD). | 16 | |
| | *Spies 7193* | WESTERN CAPE.—3322 (Oudtshoorn): near Ruitersbos, (–CC). | 16 | 1D; 3D |
| | *Spies 7197* | WESTERN CAPE.—3421 (Riversdale): near Stilbaai, (–AD). | 16 | |
| | *Spies 7192, 7195, 7202* | WESTERN CAPE.—3322 (Oudtshoorn): near George, (–CD). | 16 | |
| | *Spies 7196, 7198, 7367* | Localities unknown, grown from seeds. | 16 | |
| | | Ising 1970. | 16 | |
| *C. epiphyticus* J.M.Wood | *Spies 7200* | EASTERN CAPE.—3128 (Umtata): near Maclear, (–AB). | 16 | 1E; 3E |
| | *Spies 7368* | Locality unknown, grown from seeds. | 16 | |
| | | Ising 1970. | 16 | |
| *C. erubescens* Killick | | Wilsenach 1963; Ising 1970. | 16 | |
| *C. eucallus* R.A.Dyer | *Spies 7218* | MPUMALANGA.—2530 (Lydenburg): near Nelspruit, (–BD). | 16 | |
| | *Spies 7262* | Locality unknown, grown from seeds. | 16 | 1F; 3F |
| | | Ising 1970. | 16 | |
| *C. falcatus* R.A.Dyer | *Spies 7208, 7263, 7264* | Localities unknown, grown from seeds. | 16 | 1G; 3G |
| | | Flory 1955; Ising 1962; Wilsenach 1963. | 16 | |
| *C. flanaganii* Baker | | Ising 1970. | 16 | |
| *C. galpinii* Baker | *Spies 7265* | KWAZULU-NATAL.—2731 (Louwsburg): near Pongola, (–BC). | 16 | |
| | | Wilsenach 1963. | 16 | |
| *C. helictus* Lehm. | | Wilsenach 1963. | 16 | |
| *C. herrei* (F.M.Leight.) R.A.Dyer (= *Cryptostephanus herrei* F.M.Leight.) | *Spies 7194, 7267* | Localities unknown, grown from seeds. | 16 | 1H; 3H |
| | | Wilsenach 1963; Ising 1970. | 16 | |

APPENDIX 1.—Specimens of *Cyrtanthus* taxa studied with their voucher numbers, localities or author and somatic chromosome numbers (cont.)

| Taxon | Voucher | Locality/author | 2n | Figure |
|---|---|---|---|---|
| *C. huttonii* Baker | | Wilsenach 1963; Ising 1970. | 16 | |
| *C. loddigesianus* (Herb.) R.A.Dyer (= *C.affinis* R.A.Dyer) | *Spies 7203* | EASTERN CAPE.—3325 (Port Elizabeth): near Port Elizabeth, (–DC). | 16 | 1I; 4A |
| *C. mackenii* Hook.f. var. *cooperi* (Baker) R.A.Dyer | *Spies 7273* | KWAZULU-NATAL.—2930 (Pietermaritzburg): near Durban, (–DD). | 16 | |
| | *Spies 7211, 7373* | Localities unknown, grown from seeds. | 16 | 2A; 4C |
| | | Ising 1966. | 16 | |
| *C. mackenii* Hook.f. var. *mackenii* | *Spies 7270* | NAMIBIA.—1917 (Tsumeb): near Otavi, (–CB). | 16 | |
| | *Spies 7269* | KWAZULU-NATAL.—2930 (Pietermaritzburg): near Durban, (–DD). | 16 | |
| | *Spies 7268, 7271,7272, 7369,7370, 7371, 7374* | Localities unknown, grown from seeds. | 16 | 2B, C; 4B |
| | | Flory 1955; Ising 1962, 1966; Wilsenach 1963; Bose 1965; Nandi 1973; Venkateswarlu & Lakshmi 1976; Laksmi 1980. | 16 | |
| | *Spies 7274, 7372* | Localities unknown, grown from seeds. | 32 | 2D |
| *C. macowanii* Baker | *Spies 7201* | MPUMALANGA.—2530 (Lydenburg): near Nelspruit, (–BD). | 16 | 2E, F |
| *C. montanus* R.A.Dyer | *Spies 7209, 7275, 7375* | Localities unknown, grown from seeds. | 16 | 2G, H; 4D |
| *C. obliquus* (L.f.) Aiton | *Spies 7276, 7277, 7278* | Localities unknown, grown from seeds. | 16 | 2I; 4E |
| | | Bose 1965. | 14 | |
| | | Ising 1962; Wilsenach 1963. | 16 | |
| | | Satô 1938, 1942. | 22 | |
| *C. obrienii* Baker | *Spies 7193, 7215,7279, 7376* | Localities unknown, grown from seeds. | 16 | 2J; 4F |
| | | Flory 1955; Ising 1970. | 16 | |
| *C. ochroleucus* (Herb.) Burch. ex Steud. (= *C. lutescens* Herb.) | | Tjio & Levan 1950; Flory 1955; Ising 1962; Wilsenach 1963. | 16 | |
| *C. rotundilobus* N.E.Br. | | Ising 1970. | 16 | |
| *C. sanguineus* (Lindl.) Walp. | *Spies 7281* | KWAZULU-NATAL.—2930 (Pietermaritzburg): near Durban, (–DD). | 16 | |
| | *Spies 7216, 7280, 7282* | Localities unknown, grown from seeds. | 16 | 2K; 4G |
| | | Flory 1955; Ising 1962; Wilsenach 1963; Bose 1965; Nandi 1973. | 16 | |
| | | Mookerjea 1955; Bose 1965. | 18 | |
| *C. smithiae* Watt ex Harv. | | Wilsenach 1963. | 16 | |
| *C. staadensis* Schönland | | Ising 1970. | 16 | |
| *C. stenanthus* Baker | | Ising 1970. | 16 | |
| *C. stenanthus* Baker var. *stenanthus* | *Spies 7283* | Locality unknown, grown from seeds. | 16 | |
| *C. thorncroftii* C.H.Wright | | Wilsenach 1963. | 16 | |
| *C. tuckii* Baker var. *transvaalensis* I.Verd. | | Gouws 1949. | 16 | |
| *C. tuckii* Baker var. *tuckii* | | Ising 1970. | 16 | |
| *C. tuckii* Baker var. *viridilobus* I.Verd. | *Spies 7377* | Locality unknown, grown from seeds. | 16 | 2L; 4H |